

# Phytochemical Resources for Medicine and Agriculture



Edited by  
**Herbert N. Nigg**  
and  
**David Seigler**

# **Phytochemical Resources for Medicine and Agriculture**

# **Phytochemical Resources for Medicine and Agriculture**

**Edited by**

**Herbert N. Nigg**

*The University of Florida  
Lake Alfred, Florida*

**and**

**David Seigler**

*University of Illinois  
Urbana, Illinois*

**Springer Science+Business Media, LLC**

**Library of Congress Cataloging-in-Publication Data**

---

**Phytochemical resources for medicine and agriculture / edited by  
Herbert N. Nigg and David Seigler.**

p. cm.

"Proceedings based on an American Chemical Society Symposium on  
Plant Chemicals Useful to Humans, held September 10-15, 1989, in  
Miami, Florida"--T.p. verso.

Includes bibliographical references and index.

1. *Materia medica, Vegetable--Congresses.* 2. *Plant products--  
Congresses.* I. Nigg, Herbert N., 1941-. II. Seigler, David S.  
RM666.H33P485 1992  
615'.32--dc20

92-20152

CIP

---

**Proceedings based on an American Chemical Society Symposium on  
Plant Chemicals Useful to Humans, held September 10-15, 1989,  
in Miami, Florida**

ISBN 978-1-4899-2586-2      ISBN 978-1-4899-2584-8 (eBook)  
DOI 10.1007/978-1-4899-2584-8

© 1992 Springer Science+Business Media New York  
Originally published by Plenum Press, New York in 1992.

Softcover reprint of the hardcover 1st edition 1992  
All rights reserved

**No part of this book may be reproduced, stored in a retrieval system, or transmitted  
in any form or by any means, electronic, mechanical, photocopying, microfilming,  
recording, or otherwise, without written permission from the Publisher**

## PREFACE

This book was the result of a symposium held at the American Chemical Society meeting in Miami Beach, Florida, September 10-15, 1989. The symposium was jointly sponsored by The Society for Economic Botany and the American Chemical Society Food and Natural Product subdivision. There were five speakers.

During the social sessions (mostly over drinks in a hotel room), it became obvious that, regardless of the discipline, we were all speaking the same language. Yet, prior to the symposium, only a few of the participants knew one another. We decided to expand the symposium into a book. The book would, we hoped, accomplish for others what we had discovered in ourselves. That is, the field of Natural Products is broad, but similar in techniques and approach, ancient but modern, and has been and continues to be extremely valuable to humankind. We wanted the book to serve as an introductory text for courses and as a reference work for the future. We also determined to include the structure of every chemical in the chapter where it was mentioned so the reader would not have to find the structure somewhere else or to try and deduce the structure from the chemical name. Little did we know what an undertaking these goals would be or the time this would take.

We thank Cynthia Evans, Word Processing Supervisor, Citrus Research and Education Center, Lake Alfred, Florida, for her cooperation and enthusiasm in doing this book. We thank the typists at many organizations who provided rough drafts on disc. We especially thank Wilma Tomlinson and Barbara Thompson who keyed in, proofed, formatted, and worked long hours to make the chapters consistent, quality efforts. We express our appreciation to Margaret Kent at the University of Illinois, Urbana for expediting communication between the two editors. Without these diligent, faithful people, this book might never have appeared in print.

We believe this work is the finest anywhere and that readers will find all of the chapters interesting, intriguing, and educational.

The authors are responsible for opinions expressed. There was unequivocal academic freedom in this respect. The editors take responsibility for misspelled words and mistakes in structures. We would appreciate knowing about mistakes.

Enjoy the book and welcome to the field of natural products.

Herbert N. Nigg and David S. Seigler

## CONTENTS

An Historical Perspective of Ancient Poisons .....	1
H. G. Cutler	
Plants and Plant Products Used in Mummification .....	15
A. R. David	
Plants Used Medically by Indigenous Peoples .....	33
W. H. Lewis	
Plants as Sources of Medicinally and Pharmaceutically Important Compounds .....	75
A. D. Kinghorn	
Socio-Economic Poisons: Khat, the Natural Amphetamine .....	97
R. Brenneisen and M. A. ElSohly	
Antiparasitic Agents from Plants .....	117
R. P. Borris and J. M. Schaeffer	
Antifungal Compounds from Plants .....	159
J. Kuc	
Nematicidal Compounds from Plants .....	185
D. J. Chitwood	
Herbicidal Compounds from Higher Plants .....	205
H. G. Cutler	
Insecticidal Compounds from Plants .....	227
W. S. Bowers	
Natural Medicines are Natural Pesticides? .....	237
J. A. Duke	
Natural Toxicants in Foods .....	247
R. C. Beier and H. N. Nigg	
Future for Natural Products .....	369
H. N. Nigg and D. S. Seigler	

Taxonomic Index . . . . .	377
Chemical Index . . . . .	389
Subject Index . . . . .	415

## AN HISTORICAL PERSPECTIVE OF ANCIENT POISONS

Horace G. Cutler

USDA, ARS  
Richard B. Russell Center  
P. O. Box 5677  
Athens, GA 30613

### INTRODUCTION

When it comes to the use of poisons to extinguish life, human nature remains the same from century to century and the motives of revenge or greed are universal. These deeds often remain hidden, are sometimes very close to home, and become exposed accidentally. Perhaps a modern experience will serve to illustrate the point.

Almost 25 years ago, I lived in a small Southern agrarian community; and on my way to putting out experimental field plots, I would drive by three trailers parked near the highway in a semi-circle. Two daughters lived in the domiciles on either end, while their father resided in the middle. Externally, their cheek-by-jowl existence appeared cozy and tranquil but there were plans brewing that were to rock the community. For over a period of months, the two sisters had spoken to each other by telephone and plotted the early demise of their father. Considering the close proximity in which the sisters lived, this appeared to be an odd way to plan a murder; but had they met to discuss the plot it was a fair bet, it was later disclosed, that the old man would have seen the two talking and might have become suspicious. As their plan escalated, they went into minute detail as to the sort and flavor of cake they were going to bake in order to administer the poison and there was a minor point as to which toxic material they were going to use, either rat poison (warfarin), which seemed to be appropriate in this case, or strychnine, the white crystalline alkaloid derived from the seed of *Strychnos nux-vomica*, which could be purchased from the local drugstores and which had recently had a revival for use as a dog poison, both domestic and wild. Fortunately, telephone systems had not been automated to the extent that they are these days, especially in rural districts, and the telephone operator who had little to occupy her working hours listened in to the conspiratorial conversations. The sheriff was called and the plot was scotched before any real damage could be done. Both sisters claimed that they had been abused as children and that they planned to exact revenge and compensate themselves with the residual estate if, and when, their father died. For some reason, a case was never prosecuted and the three continued to live in tranquility side-by-side in their respective trailers.

It is not commonplace for murderers to proclaim their guilt, and if poison is employed it points to both a subtle and premeditated crime, rather it is the criminal

attempt to make the incident appear to be mere circumstance or even a neutral event. The only exceptions to this rule are the mentally unhinged, those who proclaim themselves to be gods or goddesses, and executioners who carry out the bidding of the state. These are the examples that find their place in history.

## Hemlock

One of the first historically well-documented cases of successful poisoning involved Socrates, the Greek philosopher, in 399 BC. His execution has been depicted by many artists and perhaps the most famous is "The Death of Socrates," painted in 1787 by Jacques Louis David, in which he is seen accepting the cup laced with hemlock in a resigned but defiant manner. He had been condemned because of impiety, specifically in a court of law, as Plato recorded in his *Apology*, "Socrates is guilty of corrupting the young and of not believing in the gods recognized by the city but rather in other supernatural beings of his own invention." By today's standards, the trial was somewhat messy and Socrates took an uncompromising stand so that he antagonized his accusers. There were alternatives to the death penalty but he was almost defiant in his refusal to suggest other punishment. Even later, in prison, his friend Crito arranged for a convenient escape which could have been easily carried out but Socrates refused the offer to be spirited away, saying only that his verdict had been rendered by a legitimate court and, therefore, he was subject to execution. Normally, the verdict of the court was carried out within 24 hours and the condemned were requested to "drink the hemlock." Socrates had to wait a month to partake of the cup because the Sacred Ship had been sent to Delos, which was an annual event, and no executions were allowed during that period. He died in the presence of his close friends, though Plato was conspicuously absent, and he is said to have described a numbness first in his legs, then in his lower torso before succumbing (Cooper, 1976).

The poison most probably used to execute Socrates was coniine (Fig. 1), the principle toxic natural product in poison hemlock, *Conium maculatum*, a biennial plant of the Umbelliferae which commonly grows in Europe along hedgerows, borders, and waste places (Schuyler-Mathews, 1912, p. 312). The plant is also found in the temperate parts of Asia, North America, and South America. Hemlock is derived from the Greek, "glory" and "leaves" (Pratt, ca. 1890). Normally, the plant grows 3 to 6 feet tall and has a taproot that is often mistaken for parsnip in Europe. The seeds, too, are often misidentified as caraway with drastic results. Commonly, the plant is called poisonous parsley but, in small amounts, the powdered dried leaves are used as a sedative and the weed as an analgesic in cases of arthritis, gout, and ulcers. It seems very probable that hemlock was mixed with the myrrh and vinegar offered to Christ on a sponge saturated with the solution at his crucifixion (Rose, 1976, p. 67). Myrrh is also a medicinal that is still used as a carminative.

Death by poison was the Greek custom by which murderers, political prisoners, and other undesirables were put to death, including Theramenes and Phocion, though the Socrates execution is the most famous. The poison acts by causing weakness, drowsiness, nausea, vomiting, labored respiration, paralysis, asphyxia, and death (Pratt, ca. 1890). While the method seems somewhat slower than other forms of modern execution, for example, the guillotine, electric chair, hanging, and gunfire, it appears to have had a refined non-violent quality about it which was very much in keeping with the Greek philosophy of life and death. It is of interest to note that the modern state executions are being carried out by lethal injection. By way of a footnote, one other society has used poison to execute criminals and that was in Hawaii. The bark, leaves, and roots from Akai (*Wikstroemia sandwicensis*) were made into a drink which, at the appointed time, was handed to the condemned person (Rose, 1976, p. 36). It is interesting that, in both cases, the ingestion of the poison has to be performed by

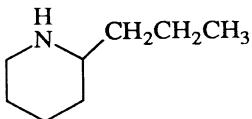


Figure 1. Structural formula of coniine.

the one undergoing execution thereby conferring the choice of the moment of death on the individual and, in a sense, giving dignity to the act.

### **Cantharidin**

While Spanish fly (*Lytta vesicatoria*) has been used as an aphrodisiac for at least two thousand years, it is nevertheless a poison and the threshold to induce physical damage and death is easily reached. The drug is one of the few mentioned by Hippocrates (460 to 377 BC) (Sollman, 1949). The insect from which the drug is obtained is about 1.2 to 1.8 cm long, and is a brilliantly colored beetle of the Meloidae family; its habitat is Southern Europe. When squeezed, cantharidin is secreted from the insect and acts as a skin vesicant causing nasty blisters (Arnett, 1976). Cantharidin (Fig. 2), the anhydride of cantharidic acid, has been shown to induce C-mitosis (Baggini et al., 1958), as does colchicine in onion root tips that have rapidly dividing cells; but this effect has not yet been reported in animal cells. It is of interest to note that colchicine ( $C_{22}H_{25}NO_6$ ), a major alkaloid of meadow saffron, *Colchicum autumnale*, is also a potent poison ( $LD_{50}$  mouse epidermis is 3.5 mg/kg) and has been used in a similar manner as cantharidin for antineoplastic purposes. Cantharidin has been used to control epithelial cancers in rabbit and man (Dubois and Ball, 1933). However, only in modern times are there reports of suicide by colchicine ingestion.

The most famous case of cantharidin poisoning appears to be that of Nero by his wife, Poppea Sabina. Unfortunately for history, the poisoning was not acute but the dose administered appears to have unhinged Nero sufficiently to account for his aberrant behavior including the conflagration of Rome, which lasted nine days, during which time he played upon the lyre (Gibbon, 1776-1788). It seems that Poppea may have felt slighted by the lack of attention paid to her by her husband and, in order to perk him up, she fed him too much Spanish fly. That this turn of events should happen is not surprising. Poppea was noted for her immorality and cruelty and it was at her request that Nero murdered his mother Agrippina in 58 AD and ordered Octavia, his wife when Poppea was his mistress, to be executed in 62 AD. In addition, Poppea had a fairly checkered history. Born to Titus Ollius, she married a praetorian prefect, Rufus Crispinus, by whom she was divorced, at which point she married Marcus Salvius Otho (a future, but short-lived emperor) but deserted him to marry Nero after being his mistress for some time (Poppea Sabina, 1976). In 65 AD, Nero killed her in a fit of temper (Nero, 1976).

Nero Claudius Caesar was not exactly a paragon of virtue either before or after his cantharidin poisoning and his encounter with the drug only served to exacerbate his propensity to evil. At the death of Nero's father, his mother, Julia Agrippina, married her uncle, Emperor Claudius, in 49 AD and he adopted Nero a year later. In order to doubly seal the bargain, Claudius also made Nero his son-in-law at the

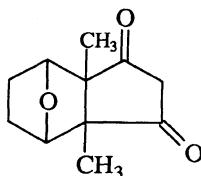


Figure 2. Structural formula of cantharidin.

instigation of Agrippina by marrying him to his daughter Octavia whom, as we have already seen, Nero later eliminated (Nero, 1976).

The use of poisons was commonplace in the family and they practiced the art consummately; their particular vehicle was the mushroom. Claudius was poisoned in 54 AD by Agrippina probably by the use of poisonous mushrooms, a topic covered in some detail in the book *Claudius the God* in historical novel form (Graves, 1962) and referred to by Roman authors. These include Seutonius, in *Claudius*, who states that Agrippina in probable collusion with Claudius' taster Halotus, a eunuch, prepared a dish of mushrooms which was fed to Claudius at a banquet with the priests in the capitol castle. However, the possibility exists that he was poisoned at home in the same manner by a dish prepared by Agrippina who recognized his weakness for mushrooms. He was known to be "greedy of these morsels" (Ramsbottom, 1945)—*Amanita caesarea*, and it would be easy to substitute another *Amanita*, possibly the species *phalloides*. The latter is extremely poisonous and there are no known antidotes even today. In the commentary by Seutonius, it is stated that Claudius "immediately lost the power of speech" (which was very convenient and signifies the extensive knowledge of poisons of which the murderer was aware) "spent the night in torment and died shortly before dawn." Again, there appear to be two schools of thought and, in the second version, Seutonius quotes another source in which Claudius, having eaten the poisonous mushrooms, fell into a deep sleep but later awoke and vomited, at which point he was fed gruel containing more poison. Tacitus, in his *Annals*, gives a slightly different version of Claudius' death. While he agrees that the victim was fed a dish of edible mushrooms and that Claudius had a great love of these fungi, he believes that the dish was doped with poison though the origin remains unclear. The immediate effects seem to have been a loosening of the bowels which served to void the poison at which time Agrippina called upon the services of Xenophon, with whom she had come to an agreement to dispose of Claudius, and a feather liberally laced with poison was administered to the back of Claudius' throat to induce vomiting; he may have anticipated being poisoned by ingestion and called for the feather himself to purge his system and never suspected such an intricate plot. Besides, feathers were commonly available at Roman banquets where purge followed binge in order to make room for more food and there is a vomit trough at the house of the Vettii brothers in Pompeii, somewhat resembling in size a large modern wash basin, for such purposes. Yet a third account by Dio Cassius, Book LXI, probably comes closer to the truth. That is, Claudius was fed a dish of mushrooms containing poison, most likely in the form of associated poisonous mushrooms, and was carried from the banquet in an apparent alcoholic stupor, a repeated event in his life; and during the night, he became speechless and died. Agrippina had employed the services of Locusta who was known to have had considerable knowledge about poisons and their administration. A chance remark by Nero, recorded by some author, indicates that Nero had a more than passing interest in the death of Claudius for

Agrippina was determined to make Nero emperor, though she must have had regrets later. Nero was heard to quip that mushrooms were the food of the gods and, indeed, it was a mushroom that had transformed Claudius into a god. Nero the artist, actor, poet, and musician could hardly have allowed such a moment of poetic irony to pass without taking full advantage. The symptoms suffered by Claudius are consonant with *Amanita* poisoning. For example,  $\alpha$ -amanitin (Fig. 3) has a latent phase from 6 to 24 hours following ingestion and this is followed by gastrointestinal disturbances that include pain, violent vomiting, diarrhea, and tenderness and enlargement of the liver. Then the symptoms apparently disappear only to return some time later. There follows hypoglycemia (also noted with carboxyatractylloside in cocklebur poisoning, *Xanthium strumarium*), headache, coma, mental confusion, and convulsions (note the similarity of these three symptoms with alcoholic poisoning). Renal failure and liver and intestinal damage take place, fluid collects in the lungs, and death is inevitable. The poison acts by inhibiting messenger RNA transcription from chromosomal DNA; and consequently, because messenger RNA is lacking, *de novo* protein synthesis shuts down (Blackwell, 1990).

Upon Claudius' death, Seneca wrote a satire in verse interspersed with prose titled "The Pumpkinification of Claudius" which had Claudius debating the gods while undergoing deification. Unfortunately, while this may have pleased Nero at the time, Seneca was ordered to commit suicide by Nero in 65 AD. Nero was probably also responsible for the death of Britannicus, the son of Claudius, by poisoning.

The breeding and environmental exposure seem to have been firmly in place for Nero to dabble in poison. Livia Drusilla, known generally as Livia (55 BC to 29 AD), was the grandmother of Claudius. She had been married to Tiberius Claudius Nero by whom she had two sons, Drusus and Tiberius; the latter became emperor and is mentioned prominently in the Bible. Her husband divorced her at the request of Augustus Caesar and she married Augustus, but they had no children. Livia is suspected of being a superb practitioner in the art of poisons and used her knowledge to promote her own ends. Marcellus, the nephew of Augustus, had been chosen by Augustus to succeed him as emperor upon his death. Mysteriously, he died by poison as did Augustus' grandsons, Gaius and Lucius Caesar, who were also in line as emperor; and all the evidence pointed to the hand of Livia. Indeed, it appears very possible that Livia may have poisoned Augustus. Her crimes were never seen to have caught up with her and she died at the old age of 74 under natural circumstances. Her life and death must have set a fine example for the younger family members (Livia Drusilla, 1957).

## Snake Venom

One of the most ritualistic forms of administering poison occurred in the Egyptian Empire. The Pharaoh was believed to be both man and god. Because of this elevated status, the Pharaoh personified the fundamentals of life that included fertility, the essence of being, born of the sun-god who breathed into plants the power of movement and growth. The Pharaoh was also the Perfect Victim who could offer his life for the good of the people when it was necessary so that the essentials of life could be perfectly manifested, albeit in an imperfect world. If a famine occurred in the land, then the divine ruler was expected to make the ultimate sacrifice so that the land would be fertile and yield a bountiful harvest (little wonder that Pharaoh elevated the slave Joseph to such an exalted position when Joseph interpreted Pharaoh's dream and thereby ensured a plentiful supply of grain during the 7 year famine). At the time of sacrifice, the Pharaoh would be approached by a priest of Anubis, the jackal-god whose likeness is captured on wall paintings and statues, who

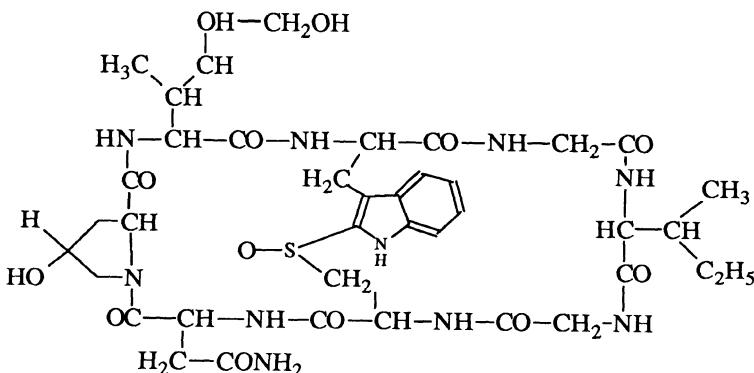


Figure 3. Structural formula of  $\alpha$ -amanitin.

would be dressed in the jackal mask. It is believed that the priest also handed the Pharaoh the instrument of death, a snake. While the ceremony is not overtly stated either in Egyptian cartouches or murals, there is sufficient indirect evidence to support the practice. In the early years of the Egyptian Empire, the horned viper "fu," later abbreviated to "f," was the ceremonial snake used and it is telling that the names of early kings were, for example, Khu-fu and Shepses-ka-f. Later, in the development of Egypt, the names changed to reflect the heliocentric origin of Pharaoh and the title Re was adopted by the kings to reflect their descent from the sun-god. Concurrently, the snake used in the death ceremony changed from the horned viper to the Egyptian hoodless cobra which, in later dynasties, decorates the Pharaoh's crown and the reasons for the change were of a purely practical nature. Viper venom is slow acting with a consequent effect of a lingering and painful death (Murray, 1959). Even gods must preserve their creature comforts while they are mortal. Conversely, cobra venom induces rapid symptoms with nerve paralysis being among the first effects. The passage from life to death is quick, relatively painless, and guaranteed.

It is not certain as to whether the choice of snake venom also ensured that the victim, or Perfect Sacrifice, remained relatively unblemished. Following transpiration, part of the ceremony included evisceration of the victim and, with the exception of the heart and lungs, the organs were placed in funeral jars (four canopic jars) prior to mummification. The heart and lungs were ploughed into the fields, the heart being the seat of life and the lungs representing the breath of life, so that a new spirit would be given to the soil.

The most illustrious case of snakebite death is that of Cleopatra VII Philopator (69 to 30 BC), the last member of the house of Ptolemy which had come on genetically hard times due to severe inbreeding. At the age of 18, she and her young brother, Ptolemy XIII, ascended to the throne at the wish of her father and together Cleopatra and Ptolemy XIII ruled Egypt. Because of her strong-willed ways and her insistence in being the primary ruler, she was exiled from Alexandria only 2 years after her ascendancy. However, Julius Caesar had designs on Egypt as a source of economic and agricultural wealth to bolster the Roman Empire and Cleopatra, following the successful conquest of Alexandria (47 BC) by Rome, supported Caesar, married her youngest brother, Ptolemy XIV, and again became co-ruler of Egypt. In this case, Ptolemy XIV was not very bright intellectually (the result of too much incestuous inbreeding) and Cleopatra was the leader (Peck, 1976).

But, as is often the case, history produced some curious turns of events. Cleopatra had formed both a political and physically intimate liaison with Julius Caesar and, in 46 BC, she gave birth to his son, Ptolemy Caesar. In one sense, hybrid vigor had returned to Rome with Julius Caesar, leaving her brother, Ptolemy XIV, on the throne, until that fateful day on the Ides of March, 44 BC, when Caesar was assassinated. She fled to Alexandria, had Ptolemy XIV liquidated by poison (snake venom?), and placed her Roman-Egyptian son on the throne as Ptolemy XV Philopator Philometor. Fortunately, she had previously the good sense to have her son recognized as the true offspring of the sun-god by the priests of Hermouthis (Peck, 1976).

The next set of historical events led inexorably to Cleopatra's death. The battle of Phillipi between the Republicans and Triumvirate, at which the Roman poet, Horace, threw down his shield and fled only to later write that "It is a sweet and noble thing to die for one's country.", left Mark Antony, Lepidius, and Octavian in power. Mark Antony invited the Eastern monarchs to Tarsus to give an accounting of their activities in the battle of Phillipi, and Cleopatra was included. She was found innocent of aiding Cassius (of the "lean and hungry look" in Shakespeare's play *Julius Caesar*) but the meeting cemented a powerful relationship between the two and they became lovers during the winter of 41 to 40 BC. They married 3 years later in Antioch but the ceremony was not recognized under Roman law because Antony was still married to Octavian's sister, Octavia. Cleopatra conceived and bore Antony's twins, a boy and girl, and later another boy so that Cleopatra's hold on Antony and his power was firm. But further developments took a downward path. Octavian declared war against Cleopatra in 32 BC; Antony sided with her. A decisive battle, for which Cleopatra devised Antony's naval plan, took place at Actium in 31 BC. With Antony's fleet defeated, Octavian won the day; Cleopatra fled to Alexandria; and Antony, believing that Cleopatra had killed herself, committed suicide. With Antony dispatched, Cleopatra felt that she could sue Octavian for the rights of her children but she lost her case and planned to immolate herself with her accumulated treasure in a mausoleum but, unfortunately, her plan was thwarted and she died a prisoner. The instrument of her death was an Egyptian cobra that was smuggled in to her in a basket of figs. It seems probable that Rome knew of her suicide plans and conveniently aided the plot (Peck, 1976).

The snake venom neurotoxins have been examined in detail and, of all animal toxins, they are the most understood in terms of chemistry and mode of action. They have been divided into several classes according to their origin and activities and these include the curarimimetic neurotoxins, crototoxins,  $\beta$ -bungarotoxins, notexins, and taipoxins. In the case of Cleopatra's death, the curarimimetic neurotoxins played the leading role; these are found in cobras, mambas, kraits, and sea-snakes (Karlsson, 1973). These proteins act by blocking nicotinic acetylcholine receptors at the myoneural synapses; and relatively, they behave like curare with the exception that they have a far greater affinity for the receptor and, therefore, are far more toxic. A rapid death is caused by asphyxiation due to paralysis accompanied by violent spasms in the terminal phases.

## Monkshood

The poisonous and medicinal properties of the genus, *Aconitum*, have been known since very early times. In Greek mythology, it is stated that the plant originated in the saliva drops that fell to the ground and rooted from the jaws of Cerberus. The latter being the three headed dog that guarded the entrance to Hades, but whether he kept intruders out or kept the inmates from escaping is a moot point. The nomenclature is also derived from the Greek for javelin and it is certain that both

spear and arrow points were dipped into the juice of the plant for quick kill of the quarry both in war and in hunts. The alternate name for the plant is Wolfsbane and this most probably originated in England, before the wolf was hunted to extinction, when the plant played a significant role for tipping arrows with the poison. The species name of one *Aconitum lycocotonum*, means wolf killer in Greek (Benn and Jacyno, 1983).

Monkshood has almost as an illustrious history as hemlock. Even though the reference is historically displaced, the Latin poet and historian, Ovid (43 BC to 17 AD?), writes that Medea (Greek mythology), a princess and sorceress (note the occupation), used monkshood to poison Theseus, who was responsible for slaying the Minotaur, by giving it to him in a cup of wine. While this may have been the case in that mythology may be based on factual events, it is more likely that Ovid was familiar with the customs of his own country and transferred them to those earlier protagonists. That is, Rome had a ban on the cultivation of *Aconitum* because, most probably, the use of the material to dispatch opponents had been rather abused. The emperor, Trojan, made the production of Monkshood punishable by death (Benn and Jacyno, 1983). However, Ovid does have two points in his favor to support the use of the plant by Medea. First, euthanasia was practiced on the Greek island of Khios, about 60 miles due west of Izmir, Turkey, and second, there was an alternate choice for poison hemlock available to condemned prisoners. In both cases, *Aconitum* was used. The plant is also referred to in Shakespeare's play *Henry IV*.

"Thou shalt prove a shelter to thy friends,  
A hoope of gold to binde thy brothers in,  
That the united vessels of their blood  
Shall never leave, though it do work as strong  
As Aconitum or rash gunpowder."

There are a number of species of *Aconitum*, all of which are poisonous. Some of these species are: *A. columbianum*, *A. falconeri*, *A. ferox*, *A. karakolicum*, *A. lycocotonum*, *A. napellus*, and *A. uncinatum*. They are slender plants requiring support and bear a strong resemblance to columbine. The flowers are generally purple or ultramarine-purple and have five sepals and two petals. The superior sepal is enlarged and has the shape of a hood, or capuche, similar to those worn by monks (Schuyler-Mathews, 1912, p. 148), hence the origin of the common name, Monkshood. The plant is found in woods and hedgerows and has wide geographical distribution. All parts of the plant are poisonous and records state that sniffing the leaves and blossoms has a narcotic effect. Pollen also causes swelling and pain; and, if skin lesions are present while gathering the plants, enough juice and cell fragments can enter the subdermal layer to induce pain and unconsciousness (Pratt, ca. 1890). Aconitine (Fig. 4), the drug, will induce cardiac arrhythmias, including flutters and fibrillation. Depending upon the test animal used, hypothermia and a drop in blood pressure have been observed; an extensive review of the literature has been published concerning the clinical symptoms of aconite (Benn and Jacyno, 1983).

Folklore claims that native *Aconitum* is an unreliable drug, but this is probably due to the different titres of active compounds which may depend on the ecosystem in which the plants are grown. The plant induces odd hallucinations and among those experiences recorded by people who have participated in the use of *Aconitum*, the most extraordinary is that of "flying" (Rose, 1976, p. 86). This may explain the use of the plant by witches on their broom handles to induce the broom, on which the witch is seated (and grasping the handle!), to fly. If this "flying" experience is real, and there is no reason to doubt the veracity of the reports, it is possible that the drug affects that part of the brain that controls our dream life. All of us have experienced a

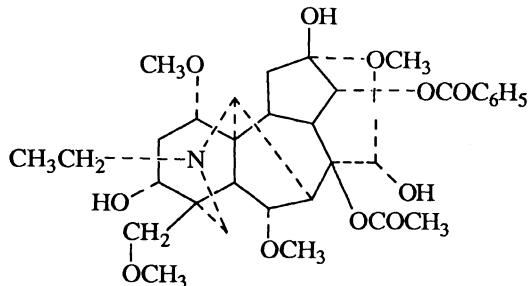


Figure 4. Structural formula of aconitine.

dream in which we fly with the apparent effortless ease of birds and the memory of those experiences, in which our supple bodies weave in and out of space somewhere between heaven and earth, remain with us during our waking hours. Certainly, the drug acts on the nervous system and produces delirium and hallucination (from the Latin, *hallucinari*, to "wander" in the mind). Similar flying experiences have been recorded from the use of Belladonna. The principal alkaloids of *Aconitum* sp. are aconitine and aconine, though others do occur, including mesaconitine. The lethal dose of aconite for man is 4 mg but the poison is curious in that the mind does not become fuddled and intelligence remains to the end (Aconite, 1957).

### Belladonna

*Atropa belladonna*, deadly nightshade, also appears in Greek mythology as an instrument of the gods, for its fruit was used by Atropos (hence the chemical name atropine) who controlled the thread of life. Precisely at the moment of death, those threads were severed by the goddess and mortals started their journey to Hades across the river Styx in Charon's ferry. The name of the plant is derived from the Greek for inflexible, inevitable, not to turn, which adequately describes death.

According to Plutarch (46? to 120? AD), Mark Antony's troops, after their bitter defeat, were so starved that on seeing the berries they ate them, thinking them to be edible, and died. The fruits are known to have no unpleasant scent and their taste is sweet, making them very dangerous for the uninitiated, and it seems probable that they may be mistaken for wild cherries. Macbeth, who was made notorious for his treachery by Shakespeare, reigned in Scotland from 1040 to 1057, defeated a potential invading Danish army (a common prayer for Ireland, Scotland, and England during that period was, "From the wrath of the Vikings defend us"\*) (Arbman, 1961) by inviting them to a banquet during a period of truce and liberally lacing the ale, bread, and wine with the juice of the berries (Pratt, ca. 1890). Consequently, that particular Viking contingent never survived to fight again. The Saxons, who controlled England prior to the Norman Conquest in 1066, called deadly nightshade Dwale and Banewort: Bana = murdering or killing and wort = weed.

---

\**A furore Normannorum libera nos.*

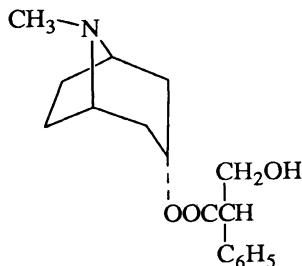


Figure 5. Structural formula of atropine.

The plant is found in waste ground, hedgerows, and woods, and is very common throughout Europe. The berries are particularly attractive, green when young, turning to red then purple at maturity, and measure approximately 8 mm long. Their growth habits make the berries attractive and readily accessible to children. Belladonna contains the alkaloid atropine (hyoscyamine) (Fig. 5) in all parts and scopolamine (Fig. 6) in the roots. The former also causes dilation of the pupil when instilled in the eye and, historically, the juice of the berries was used by Italian ladies to give the eyes a luminous appearance, hence the name belladonna or "beautiful lady." Atropine is used by optometrists as a mydriatic to dilate the pupils and the effect lasts for about 3 hours in temperate climates. In the tropics, the effect lasts for about 24 hours though no satisfactory explanation for this prolonged effect has been advanced (personal experiences of the author). Atropine is also used as an antidote to phosphate and carbamate poisoning. The berries have strong narcotic properties and three to four berries may induce death. The effects of belladonna poisoning are apparent within 15 minutes. Pupils are dilated, double vision, light-headedness, hallucinations, parched and sore throat which makes swallowing difficult, very pronounced thirst, nausea, and a rapid but weak pulse are some of the symptoms. The skin may also be flushed and there may be a resemblance to scarlatina (Atropine, 1957). A sensation of "flying," or floating may also occur. In very recent times, cases of belladonna poisoning have been recorded wherein two subjects ate the berries (Rose, 1976, p. 42). One had a pleasant relaxed floating experience that lasted about 2 hours. The other subject had an unpleasant experience and had to be hospitalized.

Scopolamine, also called hyoscine, has been used as a mydriatic, sedative, and as a "truth serum." It has been mixed with barbiturates or demerol for medicinal purposes; and until the mid-1950's, scopolamine was mixed with morphine (25:1) to induce seminarcosis or twilight sleep during which the patient experienced an analgesic and amnesiac state. The mixture was used for preoperative conditioning and during childbirth. Scopolamine also found use in controlling the quivering and rigidity of Parkinson's disease; and because of its effect as an antispasmodic and central nervous system depressant, it was the main ingredient in anti-seasick medicines (Scopolamine, 1957).

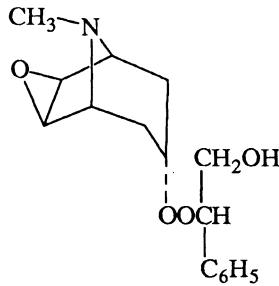


Figure 6. Structural formula of scopolamine.

## Henbane

*Hyoscyamus niger* also produces scopolamine and was considered by the ancients to be both a medicinal and poison. Pliny (23 to 79 AD), the Elder, who wrote *Historia Naturalis* and perished in the smoke and fumes that engulfed Pompeii, describes three types of henbane and says generally of the plant that "Henbane has some of the characteristics of wine and may both offend understanding and troubles the head." One has to assume that it induces severe hangover. In the post-christian era, it has been used by the officiating minister at Black Masses who smoked the weed to build up his fury and magical powers. The plant is alluded to in Shakespeare's *Hamlet*: "The juice of cursed Heberon in a phial." The main constituents are narcotic poisons and, in addition to scopolamine, atropine occurs.

## CONCLUSIONS

While there are many poisons of botanical origin, their nefarious ancient use is not well documented and one can only conjecture at their abuse. For example, English Yew, *Taxus baccata*, contains the poison, taxine, in the leaves; and because of its illustrious history, it is difficult to accept that superstitious country folk did not use the poison to eliminate their rivals. The almost magical properties of the yew are rightly attributed. It adorns every old English churchyard and many are well over 500 years old; it was in the churchyards that villagers cut the boughs to craft their bows. Originally, the longbow, which was borrowed from the Welsh and developed by Edward I, was used successfully against the Scots. French knights were slaughtered (1,542) when it was used in battle for the first time at Crécy in 1346 by the English and the battle is a paradigm in the slaughter of large numbers of troops by a singular weapon (the Church outlawed its use, unsuccessfully) with the exception, perhaps, of the atomic bomb. The yard long arrows were capable of penetrating protective armor and were deadly accurate up to 200 yards with a terminal range of 300 yards. A trained archer could fly 20 accurately aimed arrows a minute.

Caesar, Virgil, and Livy all speak of the poison of yew and Shakespeare writes of it in *Hamlet*:

"Upon my secure hour thy uncle stole,  
With juice of cursed hebona<sup>1)</sup> in a vial,  
And in the porches of mine ears did pour  
The lep'rons distilment; whose effect  
Holds such an enmity with blood of man  
That swift as quicksilver it courses through  
The natural gates and alleys of the body,  
And with a sudden vigour it doth posset<sup>2)</sup>  
And curd, like eager<sup>3)</sup> dropping's into milk,  
The thin and wholesome blood"

<sup>1)</sup> hebona = yew

<sup>2)</sup> posset = curdle

<sup>3)</sup> eager = acid

It is known that if the foliage is cut and fermented that the juice is more poisonous than the fresh material. However, there is no firm evidence that yew was used as a poison.

These historical examples point to one fact. With the exceptions of cantharidin and snake venom, the remainder of the poisons are all alkaloids which are nicely lethal. A good deal of trial and error must have taken place before they were customarily used to take a life and the knowledge to successfully employ these poisons must have evolved over a long period of time. For those who believe in Judgement Day, the revelation of the illicit use of organic poisons of natural origin should take up a fair bit of time.

## REFERENCES

- Aconite, 1957, p. 122, Encyclopedia Britannica, Volume 18.  
Arbman, H., 1961, Chapter 3, p. 81, in: "The Vikings," Frederick A. Praeger, New York, New York.  
Arnett, R. H., Jr., 1976, p. 81, Encyclopedia Americana, Volume 4, Blister bettle, Americana Corporation, New York.  
Atropine, 1957, p. 655, Encyclopedia Britannica, Volume 2.  
Baggini, A., Miradoli-Zatti, M., Pavan, M., and Valcurone, M. L., 1958, Effeci della cantaridina e della norcantaridina sulla mitosi, *Allium cepa*, Societe Medico-Chirurgicale di Pavia, 72:39-43.  
Benn, M. H., and Jacyno, J. M., 1983, The toxicology and pharmacology of diterpenoid alkaloids, p. 153-210, in: "Alkaloids: Chemical and Biological Perspectives," Volume 1, S. W. Pelletier, ed., J. Wiley and Sons, Inc., New York, New York.  
Blackwell, W. H., 1990, in: "Poisonous and Medicinal Plants," Prentice Hall, New Jersey.  
Cooper, J. M., 1976, p. 168, Encyclopedia Americana, Volume 25, Socrates, Americana Corporation, New York, New York.  
Dubois, R., and Ball, M. V., 1933, Traitemennt du caueer épithérial de la peau par la cantharidine, Bulletin de l'Academie Nationale de Médecine (Paris), 110:791-973.

- Gibbon, E., 1776-1788, Chapter XVI, p. 456, *in*: "Decline and Fall of the Roman Empire," Random House, Inc., Modern Library, New York, New York.
- Graves R., 1962, Three accounts of Claudius's death, p. 447-467, *in*: "Claudius the God," Random House, Inc., New York, New York.
- Karlsson E., 1973, Chemistry of some potent animal toxins, *Experientia*, 29:1319-1327.
- Livia Drusilla, 1957, p. 237, Encyclopedia Britannica, Volume 14.
- Murray, M. A., 1959, Chapter IV, p. 174-180, *in*: "The Splendour That Was Egypt," Philosophical Library, New York, New York.
- Nero, 1976, p. 104, Encyclopedia Americana, Volume 20, Americana Corporation, New York, New York.
- Peck, C. N., 1976, p. 51-52, Encyclopedia Americana, Volume 7, Cleopatra, Americana Corporation, New York, New York.
- Poppea Sabina, 1976, p. 364, Encyclopedia Americana, Volume 22, Americana Corporation, New York, New York.
- Pratt, A., ca. 1890, p. 208, *in* "The Poisonous, Noxious, and Suspected Plants of Our Fields and Woods," Society for Promoting Christian Knowledge, London.
- Ramsbottom, J., 1945, p. 5, *in*: "Poisonous Fungi," King Penguin Books, London.
- Rose, J., 1976, p. 36, 42, 67, 86, *in*: "Herbs and Things," Grosset and Dunlap Workman Publishing Co., New York, New York.
- Schuyler-Mathews, F., 1912, p. 148, 312, *in*: "Field Book of American Wild Flowers," G. P. Putnam and Sons, New York, New York.
- Scopolamine, 1957, p. 134, Encyclopedia Britannica, Volume 20.
- Sollman, T., 1949, *in*: "A Manual of Pharmacology," W. B. Saunders Co., Philadelphia, Pennsylvania.

## **PLANTS AND PLANT PRODUCTS USED IN MUMMIFICATION**

**A. Rosalie David**

The Manchester Museum  
University of Manchester  
Manchester M13 9PL  
England

### **TYPES OF MUMMIFICATION**

Today, the term 'mummy' is used to describe a naturally or artificially preserved body in which desiccation of the tissues has enabled it to resist putrefaction. Such examples have been discovered in a number of countries, although originally the use of the word 'mummy' (which is derived from the Persian or Arabic word 'mumia' meaning 'bitumen' or 'pitch')\* was reserved for the artificially preserved bodies of the ancient Egyptians.

Human remains, consisting of the skeleton and body tissues, can be preserved indefinitely in a number of ways. The first group consists of those preserved by natural circumstances. Environmental conditions, such as dryness provided by the sand in which the body is buried, heat or cold provided by the climate, and absence of air in the burial, are the major factors in this unintentional form of preservation. These factors occurred either singly or in combination and produced results with varying degrees of success in countries such as Egypt, Peru, Aleutia, and Alaska (Cockburn and Cockburn, 1980).

In northwest Europe, another method of unintentional preservation by natural means occurred (Fischer, 1980; Glob, 1969). Bodies were buried in bogs of three types: (a) raised bogs which were acid and contained peat moss (sphagnum) which, with its compressed layers, prevented oxygen from reaching the underlying layers; in these bogs, the bodies remained well-preserved and appeared little different from when they were first deposited there; (b) fens, which contained lime; and

\*'Mumia' was reputedly originally a substance that flowed down from the mountain tops and, mixing with the waters that carried it down, coagulated like mineral pitch. The 'Mummy Mountain' in Persia was famous for the black, bituminous material which oozed forth and was credited with medicinal properties. Because the preserved bodies of ancient Egypt often have a blackened appearance, they were likened to 'mumia' and credited with similar properties, thus leading to their use in medieval and later times as medicinal ingredients. The use of the term 'mummy' for these bodies, although erroneous, has continued.

(c) transitional bogs; in these areas, the soft tissues of the bodies have usually disappeared and only the skeleton or adipocere remain.

In some countries, intentional natural mummification was developed, whereby some of the naturally occurring environmental factors were deliberately enhanced. Thus, in some instances, the body would be dried thoroughly, still using natural heat sources such as sun, fire, or candle heat; in some cases, the bodies were smoked or cured by smoke. Other methods included stuffing the body cavities and surrounding the body with dry grass and natural materials. By ensuring that the body remained in a sealed environment, the process of preservation could be continued.

However, true mummification can be classified as a method of preservation which was not only intentional but which also involved a number of sophisticated techniques which incorporated the use of chemical and other agents. These processes were used either separately or in combination and had undoubtedly been introduced after a considerable period of experimentation. The ancient Egyptians provided the best evidence of this type of preservation (Fig. 1).

From earliest times, they had interred their dead in shallow pit-graves on the edges of the desert. The combination of the sun's heat and the dryness of the sand ensured that the body tissues became desiccated before decomposition set in, thus producing a remarkable degree of natural preservation. With advances in material prosperity and building techniques, more sophisticated tombs were introduced for royalty and the nobility by ca. 2900 BC. These had brick-lined, underground burial chambers, and thus the very environmental factors which had ensured the excellent natural preservation of the body in the pit-grave were now missing, for the bodies were no longer buried in the sand.

However, clearly defined religious beliefs were now firmly established and these required that the body should be preserved in as complete a state as possible. It was believed that the spirit of the deceased would return to the tomb to gain sustenance from food offerings left there and would need to recognize and reenter the preserved body to use it to obtain nourishment from the food.

There was a period of experimentation when the Egyptians sought to discover a method of retaining the deceased's physical likeness and of preserving the body by artificial means. In the earliest 'mummies', the body was encased in resin-soaked linen which was carefully molded to shape, and the details of the face and genitalia were painted on the outermost linen covering. An example was held in the Museum of the Royal College of Surgeons in London, England, until it was destroyed by enemy action in 1941. However, such methods were largely ineffectual because the body tissues were not preserved and they deteriorated and disintegrated beneath the linen wrappings.

The first definite evidence of true mummification occurs at the beginning of the 4th Dynasty (ca. 2600 BC) when the visceral remains of Queen Hetepheres (mother of Cheops who built the Great Pyramid at Gizeh) were placed in a chest and buried near Cheops' pyramid. Analysis of the packets containing the viscera indicated that they had been dehydrated by means of natron (Lucas, 1962, p. 271).

Mummification was used in Egypt from at least this date until the Christian era. Although by the period when Egypt was occupied by the Greeks and Romans (ca. 332 BC to 4th century AD) it had become available to a much wider social group, true mummification was never universally practiced in Egypt. The majority of the population, unable to afford these elaborate and costly funerary practices, continued to be interred in mass graves on the desert's edge (Figs. 2 and 3).



Figure 1. Mummified head of a man, showing the excellent state of preservation of the skin tissue. Egypt, Ptolemaic period, ca. 200 BC.

#### LITERARY SOURCES

There is no extant Egyptian account of the technical processes involved in mummification although there are scattered literary references to mummification and the associated rituals (Maspero, 1875). Again, there is no known visual record although two tombs at Thebes (No. 23 belonging to Thoy (Dawson, 1927, pl. xvii) and No. 41 to Amenemope (Dawson, 1927, pl. xviii)) have wall scenes that show some of the stages in preparation of the bandaging and decorating of the mummy; however, these supply interesting details rather than an accurate sequence of actions.

The earliest available detailed descriptions occur in the writings of the Greek historians, Herodotus (1946) (5th century BC) and Diodorus Siculus (1968) (1st century BC), and some information is supplied in other Classical authors (Pliny, 1957; Strabo, 1959). Herodotus provides the most complete account, but Diodorus provides additional information. Although these descriptions are not a totally reliable source, since they were probably based to some extent on hearsay and were written centuries after the process of mummification had passed its peak, they nevertheless provide a reasonable basis for understanding the process. Indeed, modern experiments carried out to assess their accuracy have produced encouraging results.

Herodotus describes three main methods which were available according to cost; it has been shown that the most expensive and elaborate method would have produced the best results (Garner, 1979). This involved the removal of the brain, partly through mechanical methods and partly through the use of substances of an unspecified nature, and the removal of the viscera and abdominal contents through an abdominal incision in the flank. The viscera were then cleansed with palm wine and spices, and the body cavity was filled with myrrh, cassia, and other unspecified aromatic substances. The incision was sewn up, the body was dried by means of natron, and it was washed and wrapped in bandages that were fastened together with gum.

In the second method, 'cedar-oil' was injected into the body *per anum* and it was then treated with natron; while in the third (and cheapest) method, an unspecified liquid was injected into the body *per anum* and it was subsequently treated with natron.

Diodorus probably based his account on that of Herodotus; it is not so detailed (for example, he mentions three grades of funerals but only describes the most expensive, involving the evisceration of the body), but he does supply some information which is not given by Herodotus.

## THE METHOD OF MUMMIFICATION

Apart from the literary evidence, we have the information supplied by the mummified remains; some of the materials connected with the process have also survived. Regarded as sacred because they had been in direct contact with the deceased's body, they were sometimes placed inside the coffin or packed into jars and put in the tomb or buried in a pit nearby and, occasionally, archaeologists have discovered this debris.

There is sufficient evidence to enable us to reconstruct the process of mummification with a degree of accuracy (Dawson, 1927; Dawson and Smith, 1924; Lauer and Iskander, 1955-56; Smith, 1912, 1914). There were two main stages: the evisceration of the body (although not all mummies underwent this process) and the essential procedure of desiccating the body which the Egyptians achieved by dehydrating the tissues with natron. In addition, the body was anointed with oils and unguents and, in some cases, it was coated with resin. There were two major developments in the long history of mummification: from perhaps as early as the Middle Kingdom (ca. 1900 BC), the brain was excerebrated, and this became a widespread procedure from the New Kingdom period (ca. 1550 BC) (Leek, 1969); during the 21st Dynasty (1087 to 945 BC), certain refinements were introduced to restore the shrunken body to a plumper and more lifelike appearance, and this involved packing the face, neck, and other areas with materials (including linen, sawdust, earth, sand, and butter) inserted through incisions made in the skin.

Although the main principle of mummification was desiccation and this was achieved by using the dehydrating agent natron, plants and plant products were also



Figure 2. Body coffin of a female; the scene painted on the center front shows the mummy on a bier, surrounded by the gods, awaiting his resurrection. Egypt, ca. 250 BC.

employed throughout the mummification process although here the physical evidence provided by the mummies does not always agree with the literary sources.

Nevertheless, we can attempt to reconstruct the stages in mummification as it was carried out in the New Kingdom (ca. 1550 to 1100 BC). Despite the fact that the evidence of the physical remains is best documented from this period, it must, however, be noted that the accounts of Herodotus and Diodorus were written hundreds of years later.



Figure 3. Mummy of a female; the cartonnage chest cover is painted with a scene showing Anubis (the jackal-headed god of embalming) preparing the mummy of the deceased in the presence of the gods. Egypt, ca. 250 BC.

## THE MUMMIFICATION PROCEDURE

At death, the body of the deceased was taken by his family from the house to the embalmer's workshop (Dawson, 1927, p. 40-41). Called *w'bt* ("The Pure Place"), this was probably a temporary structure or tent rather than a permanent building. For very important individuals, this would be erected adjacent to the tomb; but for others, mummification would have been carried out in a communal structure situated in the necropolis.

The procedure is stated to have lasted for 70 days although probably only 40 days were required to prepare the mummy (recent experiments have shown that this was the optimum period for the natron treatment and that there was no improvement in preservation of the tissues after this length of time) (Garner, 1979). Specific rituals would have occupied the rest of the time. The embalmers and their assistants probably impersonated gods (by wearing masks) who, according to the mythology, had been present at the embalming of Osiris, the god of death and resurrection. At least one funerary priest would have presided over the various stages of mummification and recited the relevant formula as each was completed. Egyptian texts supply references to such ritual recitations (Maspero, 1875).

At first, the body was stripped and placed on a board or platform. The brain was extracted, usually via a passage chiseled through (usually) the left nostril and the ethmoid bone into the cranial cavity. A metal hook was then inserted to reduce the brain tissue to fragments and assist its removal. Using a kind of spatula, the fragments were then extracted and the cranial cavity was perhaps washed out with a fluid to break down any remaining tissue. However, brain removal was often incomplete and some tissue was left behind. The extracted brain fragments were not preserved or kept; the cranial cavity was either left empty or, in one of the final stages of the procedure, it was filled with resin or resin-soaked linen. Other methods of excerebration included intervention through the base of the skull or through a trepanned orbit. The mouth was washed out and packed with resin-soaked linen, and the face was coated with a resinous paste. The eyes were not removed; they collapsed into the orbits and linen pads were inserted over the eyeballs, underneath the eyelids. In the 21st and 22nd Dynasties, to achieve a greater realism, artificial eyes of obsidian and other materials were placed over the eyeballs (Lucas, 1934).

Next, the body was eviscerated through an incision in the flank. Diodorus specifies the left flank; most of the evidence from the mummies confirms this although there are some instances where this was not followed. Some mummies were not eviscerated at all and, in others, there is no abdominal incision and the viscera had been removed *per anum*.

In most cases, however, the embalmer gained access to the abdominal cavity through the flank incision; inserting his hand, he cut the organs free with a special knife and removed them from the body. Then, making an additional incision in the diaphragm, he used this and the flank incision to insert his arm into the chest cavity and remove the thoracic organs. Only the heart was left *in situ* because, according to religious belief, it was the seat of the intellect and the emotions, the essential part of the person. However, evisceration was frequently imperfect and part of or even all of the heart was removed with the other organs. Diodorus also claimed that the kidneys were left in place although there is no known religious explanation for this retention and modern investigations of mummies have lent little support to this theory; some embalmers may have left the kidneys behind because they were physically difficult to remove.

The body cavities were then washed out with palm wine mixed with various unspecified spices (according to Diodorus) and were then filled with myrrh, cassia, and other aromatic substances. Herodotus claims that this stage was completed before the natron treatment although this has been disputed. Lucas proposed a feasible explanation for this (Lucas, 1962, p. 301): that a temporary packing was inserted at this stage to assist the process of dehydration, to prevent the collapse of the body wall during further treatment, and to lessen the odor of putrefaction. Probably consisting of dry natron, packets containing a natron and resin mixture, and linen impregnated with resin, this temporary stuffing would ultimately have been replaced with the final packing after the natron treatment was completed. In some cases, the thorax and abdomen were left empty.

The extracted viscera were then dehydrated, using the agent natron, and were wrapped into four parcels and placed under the protection of the demi-gods known as the 'Four Sons of Horus'. From the 4th Dynasty onwards (ca. 2600 BC), they were placed in canopic jars and kept in the tomb, sometimes inside a special chest. Each set consisted of four jars and some examples had stoppers that represented the heads of the four deities: human-headed Amset protected the liver, the baboon Hapy was responsible for the lungs, the jackal Duamutef cared for the stomach, and the falcon Qebehsenuef looked after the intestines. During the 21st and 22nd Dynasties, a new practice emerged of making the viscera into four parcels, each decorated with a wax image of the appropriate deity, which were replaced in the abdominal and thoracic cavities. In the 26th Dynasty (ca. 600 BC), canopic jars were reintroduced; but in later times, the viscera were wrapped into one large parcel, often impregnated with spices, and placed on the legs of the mummy.

Although these methods of evisceration were applied to the most expensive type of mummification, according to Herodotus, the viscera were also removed in the cheaper methods. In his 'second method', he claimed that the viscera were reduced to a fluid state for easier removal by the use of a 'cedar-oil' injection. The 'third method' again describes the use of an injection to clear out the intestines although here the type of fluid is not specified.

The next and most important stage was desiccation of the body. Modern methods of preservation include injection of preserving fluids into the blood vessels, deep freezing, or freeze-drying. The ancients could use heat (sun or fire) or a dehydrating agent. There is no conclusive evidence that Egyptian mummies were ever intentionally dried by fire-heat, and it is now generally accepted that natron was the main dehydrating agent, although the use of salt or lime as viable alternatives has been discussed (Lucas, 1914a, p. 119-158; 1932a, p. 62-66; 1932b, p. 125-140; 1962, p. 274-295).

Natron is a mixture of sodium carbonate and bicarbonate with impurities that include high proportions of salt and sodium sulfate; it occurs in natural deposits in Egypt and was used for a variety of cleansing purposes. An early translation of the Classical account led to a long-held misconception by modern scholars regarding the form in which natron was employed for mummification. There has consequently been much discussion about whether it was used in a solid state or in the form of a solution. Reexamination of the ancient Greek and evidence from the mummies have shown no real basis to infer that the bodies were steeped in a natron solution, and modern experiments have confirmed that natron was used in its natural dry state and that this method achieved the best results (Sandison, 1963, p. 259 ff).

Packed with dry natron, the dehydration of the body may have taken up to 40 days. It was then removed from the natron bed and washed with water to remove all traces of natron and other debris. Natron not only had the ability to preserve the body and to destroy the fat and grease, but it was also credited with spiritual cleansing and purifying properties. In the same way, lustration of the body with pure water was regarded as a spiritual as well as a physical cleansing process.

In its relatively pliable state, the body was now straightened out into the horizontal position so that it would ultimately fit into the coffin. During the 21st Dynasty, it was at this stage that the embalmers inserted the subcutaneous packing material.

The stages between the washing and the wrapping of the body are not clear, but there seem to have been two distinct processes of anointing the body. In the first, according to Diodorus, the body was anointed with 'cedar-oil' and 'precious ointments' (the ingredients of which are unknown although these may have included a paste made of animal fat and resin mixed with natron or salt). Then it was rubbed with myrrh, cinnamon, and other fragrant substances.

The flank incision prepared for evisceration was now closed; it was not usually sewn, but the edges were drawn together and covered with a metal or beeswax plate that embedded itself in the resinous coating that was now applied to the body. The cranial cavity was packed with pieces of linen impregnated with resin, and the nostrils were plugged with resin or wax. The body received a further coating of resinous paste before the limbs and the body were carefully wrapped in linen bandages and cloths; the arms were arranged in their final position, either across the chest or extended alongside the body (this varied from one period to another).

After the mummy had been prepared and wrapped, a second anointment seems to have taken place when, in a special ceremony, a liquid or semi-liquid resinous substance was poured over the mummy, the viscera if they were in a separate container, and the coffin. The family then received the mummy and the funerary goods, the burial ceremony could take place, and the deceased and his possessions would be placed in the tomb (Figs. 4 and 5).

## PLANTS AND PLANT REMAINS

From this brief survey, it is evident that plants and plant products were used in mummification in a number of ways: for cleansing (washing or injecting), as packing material, for perfuming, and anointing.

No complete ancient Egyptian Herbal has yet been discovered, although a few fragments have survived dating to the 2nd century AD. In his Greek Herbal, Dioscorides (1655) (1st century AD) incorporated many drugs that were known to the Egyptians; in addition, some examples derived from Egyptian medical papyri have survived in the Arab compilations (7th to 9th centuries AD) and in medieval medical manuscripts. Nevertheless, literary evidence regarding ancient Egyptian plants and their uses is limited (Germer, 1979, 1985; Manniche, 1989).

If we turn to the physical evidence, again there are problems. Studies carried out on mummified remains to identify the substances applied to the bodies and the bandages have been infrequent and often inadequate. In the investigation of the substances used to impregnate the bandages belonging to Mummy 1770 in the Manchester Museum collection (Benson et al., 1979), the opportunity was taken to evaluate the applicability of various current methods of analysis, especially chromatography, in identifying the natural products used in mummification. However, such relatively sophisticated studies are rare and, even in this instance, the authors acknowledged that it was likely that they had only identified those substances which are currently known to have been available to the ancient Egyptians, which were used in relatively large quantities, and which were sufficiently chemically stable to have survived storage for at least 2000 years. Therefore, in considering the plant remains used in mummification, it must be acknowledged that existing information is incomplete, literary sources and evidence from the mummies is sometimes at variance, and that modern analyses can provide only limited facts.

The use of 'cedar-oil' in mummification presents a special set of problems. Herodotus mentions that it was injected into the body *per anum* to reduce the viscera to a fluid state. (This method was also probably used for the mummification of the larger animals, especially the sacred bulls, although smaller animals, birds, and fish were not normally eviscerated but were preserved with natron and resin.) The use of a corrosive or astringent fluid thus injected may have arrested the decomposition prior to the desiccation of the body; but at first sight, 'cedar-oil' would not have had this effect. However, the true meaning of 'cedar-oil' in these texts is unclear. Diodorus also mentions it as an anointing fluid, and Lucas has suggested that possibly these were two distinct substances or that one or the other of the writers was mistaken

(Lucas, 1962, p. 309). He indicates that, in any case, neither would have been the present-day 'cedar-oil' which is obtained from the American juniper (*Juniperus virginiana*) by distillation, a process unknown at that date. The substance used for injection could have been impure oil of turpentine or pyroligneous acid containing admixed oil of turpentine and wood tar; for anointing, it is possible that ordinary oil was used, perfumed by volatile oil of juniper.

The body, its cavities, and the viscera were cleansed with palm wine. The date-palm (*Phoenix dactylifera* L.) (Lucas, 1962, p. 316) was cultivated in Egypt from earliest times; and in the Pharaonic and Classical periods, a wine was produced from the dates, which were steeped in water; a liquid was then pressed out and left to ferment. However, since traces of wine would not remain unaltered until the present day, tests to reveal its presence in mummies have been unsuccessful, although both Herodotus and Diodorus claim that it was utilized for its cleansing properties.

Various plant remains were used to pack the body cavities; materials found in mummies have included linen, linen and resin, sawdust, sawdust and resin, earth and natron, lichen, one or more onions, and peppercorns.

Lichen (*Parmelia furfuracea*) (Lucas, 1962, p. 312) has been discovered in abdominal cavities and notably in the mummies of kings, Siptah and Rameses IV. Black peppercorns (*Piper nigrum* L.) (ERC, 1985) were found in the nostrils and abdomen of the mummy of Rameses II. Sawdust (Lucas, 1962, p. 324) (both alone and mixed with resin) has also occurred in body cavities; in one instance, the body had been sprinkled with powdered aromatic wood or sawdust, and, amongst the debris of embalming materials, archaeologists have found bags of chaff and chopped straw. Fragrant juniper wood (*Juniperus phoenicea* L. or *Juniperus drupacea* L.) was probably used for the sawdust.

The onion (*Allium cepa* L.) (Lucas, 1962, p. 316) also occurs as a packing material. One or two were sometimes placed in the pelvis or the thoracic cavity, in the ear, or in front of the eyes; in some cases, they were inserted amongst the bandages or put in the coffin, and onion skins have also been found over the eyes. In particular, the embalmers of the 20th and 21st Dynasties made use of the onion.

The Classical writers refer to the use of spices (Lucas, 1962, p. 325). Herodotus mentions cassia and Diodorus relates that the body was rubbed with myrrh, cinnamon, and other spices to preserve it. The cassia and cinnamon they mention may in fact be the same material (Lucas, 1962, p. 308-309): both are derived from the dried bark of certain varieties of laurel—cassia from *Cinnamomum cassia* and cinnamon from *Cinnamomum zeylanicum*, which both grow in India, Ceylon, and China. In antiquity, not only the bark but also the flowerheads, twigs, and wood from the trees were used as spices. Cinnamon has been tentatively identified on mummies; in one example, the body is described as retaining a "thick layer of spicery covering" (Osburn, 1828, p. 6); but cassia, which was more pungent and astringent, may in fact have been more widely used.

The Classical writers also claim that myrrh (Lucas, 1962, p. 322-323) was employed in mummification. The Egyptians appear to have had access to both true resins and gum resins, and myrrh would have been obtained from *Commiphora pedunculata*; it was imported from or through the 'incense-land' of Punt, situated somewhere on the Red Sea coast, and was used for a variety of purposes, including temple rituals. However, it has never been identified with certainty in the body cavities of any of the mummies that have been examined.

Henna (Lucas, 1962, p. 309-310) may also have been employed in treating the body in a number of ways. The odiferous flowers of the henna plant (*Lawsonia alba*, *Lawsonia inermis*, a shrub widely cultivated in Egypt) may have been added to perfume some of the ointments. When alive, the Egyptians used henna to dye their



Figure 4. Mummy of a young man with elaborate studded diagonal bandaging. The panel portrait would have been painted when he was alive and then incorporated in the bandages. Egypt, ca. 150 BC.

hair and to stain the palms of their hands, the soles of their feet, and their toenails and fingernails, and it may also have been applied to the mummies for the same purposes.

As already stated, the use of 'cedar' in mummification has raised some problems of identification. The preservative qualities of this substance were highly regarded and the Greek herbalist, Dioscorides, stated that "... it is a preservative of [dead] bodies, hence some have called it 'the life of him that is dead'" (Dioscorides, 1655—Oxford, 1934, I. 105).

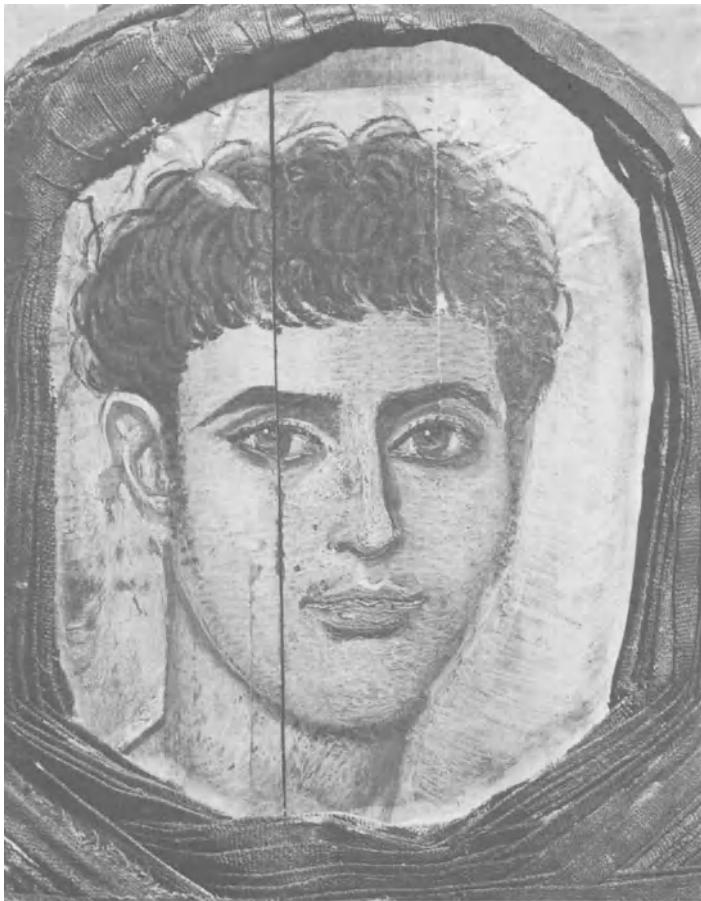


Figure 5. Detail of the panel portrait placed over the face of a mummy of a young man. Egypt, ca. 150 BC. Such portraits are believed to be true likenesses of the individual owners and are the earliest examples of oil or wax painting.

It has been suggested that the 'cedar-oil' used as an injection in mummification and that employed as an anointing agent were different substances and that the latter was perhaps ordinary oil which was scented with essential oil of juniper derived from juniper berries (*Juniperus phoenicea* L. or *Juniperus drupacea* L.). Also, the sawdust used to pack the body cavities was probably derived from fragrant juniper wood.

Juniper (Lucas, 1962, p. 310-312) was probably imported into Egypt from Asia Minor and, from earliest times, it appears to have had a religious significance (perhaps as a preservative and therefore life-giving agent) and to have been highly valued. Juniper berries were included amongst the funerary goods in the earliest graves, even before they were used in mummification, and the tradition continued for thousands of years when, even in the Christian cemeteries of the 5th century AD (Smith and Jones, 1910), they were mixed with the salt in which the bodies were placed to dehydrate.

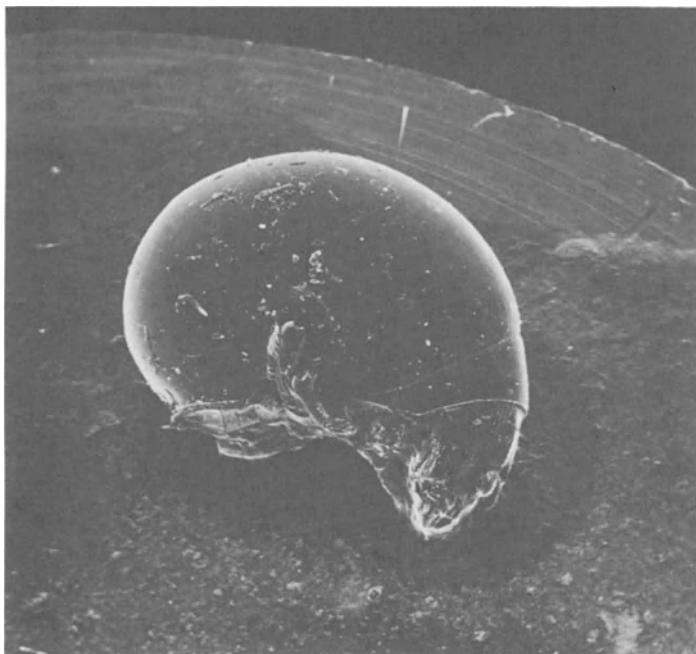


Figure 6. An adult hump spider beetle, *Gibbium psylloides*, x 36, found in the mummified remains of the Two Brothers in the Manchester Museum. A serious pest of vegetable products, this may have used the mummy bandages as a food source.

Apart from natron, however, the major preservative agent in mummification appears to have been resin (Lucas, 1962, p. 316-324); this was used in a number of ways: as part of the packing of the body cavities, the cranial cavity, the mouth, and the nostrils; as a paste applied to the face and body surfaces; and, in a molten form, it was finally poured over the mummy, the viscera, and the coffin. However, the extent to which resin was used in mummification has been much discussed; some have argued that although many mummies have a blackened appearance, this is because the tissue becomes so changed with age that it takes on the appearance associated with resin treatment and behaves with solvents in the same way as resin.

The source of the resin which the Classical accounts claim was used in mummification has also been the subject of debate. The Egyptians had access to gum-resins and true resins; gum-resins such as myrrh may well have been employed in the initial anointment of the body, but the true resins were also probably employed at some stage. They were imported into Egypt; their specific botanical sources are unknown, but they were probably derived from coniferous trees found in the eastern Mediterranean: the Cilician fir (*Abies cilicia*), the Aleppo Pine (*Pinus halepensis*), and the Stone or Umbrella Pine (*Pinus pinea*) (Lucas, 1962, p. 321).

Another vexed question is whether or not the Egyptians used bitumen (Lucas, 1914b) in mummification. Natural bitumen (pitch) is a mixture of hydrocarbons and other substances such as wood tar and asphalt; it was not found in Egypt but occurs in the Dead Sea region and this, some scholars maintain, was the source that the Egyptians used. Both Strabo (1959, xvi; ii, 45) and Diodorus (Siculus, 1968, xix, 6)

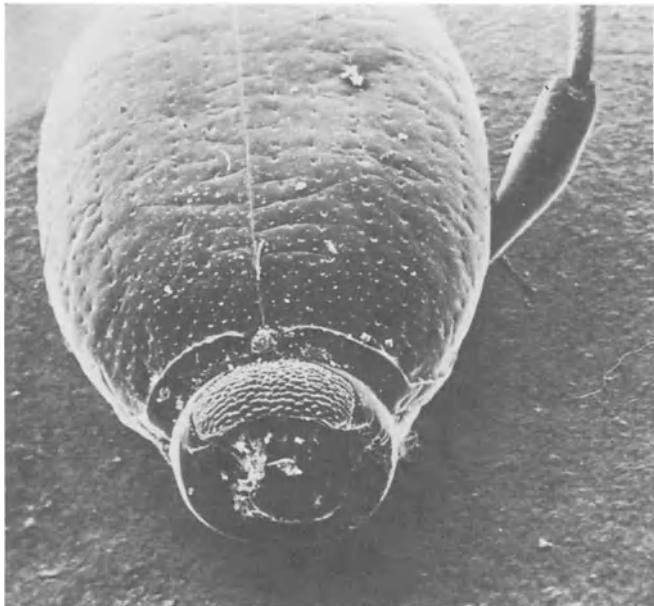


Figure 7. Remains of a beetle, *Mesostenopa* sp., x 30, found in the mummified remains of the Two Brothers in the Manchester Museum. These are pests of stored food and may have invaded the body at the time of death, during the process of mummification, or subsequently in the tomb.

state that the Egyptians employed bitumen; according to the latter, "... they transport the pitch (i.e. bitumen) into Egypt and there sell it for the use of the embalming of the dead; for if they do not mix this with other aromatic spices, the bodies cannot be preserved long from putrefaction."

However, widely divergent opinions have been expressed: Lucas (1962, p. 303-308) queried the use of bitumen in mummification, suggesting that the bituminous appearance of many mummies was caused not by the application of bitumen but by a form of slow spontaneous combustion; Ruffer (1914) found no evidence of bitumen in the mummies he examined; Dawson (1927, p. 46) claimed that it was never used until the Ptolemaic Period and that even then, in many cases, the molten resin that was applied often took on the appearance of pitch or bitumen but should not be confused with them. However, Spielmann (1932) and Zaki and Iskander (1943) suggested that Dead Sea bitumen was used to prepare mummy wrappings; while in the Manchester investigation (Benson et al., 1979), although the evidence for the presence of bitumen in the wrappings was incomplete, there was a strong indication that it had been used in the layers of bandaging nearest to the body. This analysis found that the bandages had been impregnated with a mixture that contained beeswax, galbanum, a water soluble substance or substances, possibly tamarind extract, and bitumen (except in the outer bandage layers).

In general, a systematic examination, using modern analytical methods, of many more examples of mummified tissue and bandage would help to elucidate this problem.

Other plant products known to the Classical writers and probably used in the mummification process include wood pitch and wood tar (Lucas, 1962, p. 325) (these were probably imported from areas where coniferous trees grew), and 'cedar juice'



Figure 8. Body coffins of the Two Brothers, Khnum-Nakht (left) and Nekht-Ankh, now in the Manchester Museum. From an undisturbed tomb at Rifeh, Egypt, excavated in 1905 to 1906, which dates to ca. 1900 BC. Their almost skeletal mummies have been the subject of intensive scientific studies.

(*Cedri succus*) (Lucus, 1962, p. 309) which is mentioned by Pliny (1957, xxiv, 11). This again was obtained from a coniferous tree (but not the cedar), and the juniper tree was probably the main source for these substances.

## CONCLUSION

A variety of plant remains were thus employed in mummification to assist the preservation process, to cleanse the tissues, to reduce or mask the odor of putrefaction, and to enhance the suppleness of the skin tissue.

However, it is doubtful that they made any significant contribution to the process. Preservation of the body was primarily achieved through desiccation of the tissues. The spices and scented oils may have been intended to have some insect repellent properties but, again, it is unlikely that they were effective. Most Egyptian mummies show some evidence of insect infestation, either on the body or within the wrappings, which could have occurred before the body was mummified, during the mummification process, or before the wrapping of the body. However, studies (Garner, 1986) have shown that even the natron treatment of the body, which provided an unnatural and hostile environment for the growth and reproduction of insects, could not be relied upon to kill or inhibit the insects that had gained access to the body before mummification (Figs. 6, 7, and 8).

Again, the unguents and resins applied to the skin surface probably had little effect in strengthening and preserving the suppleness of the tissues.

The use of perfumed oils, unguents, and spices may have had one practical benefit: to conceal to some extent the pungent and unpleasant odors that the mummification process produced.

However, despite the limitations of these applications, their religious significance would have been more important than any practical benefit. Plants were symbols of life and rebirth, and the Egyptians believed that their magical and life-giving properties would assist the deceased in his passage from this world to the next and would enhance his chances of resurrection and eternal life.

## REFERENCES

- Benson, G. G., Hemingway, S., and Leach, F. N., 1979, The analysis of the wrappings of Mummy 1770, p. 119-132, in: "The Manchester Museum Mummy Project: Multidisciplinary Research on Egyptian Mummified Remains," A. R. David, ed., Manchester Museum, Manchester, England.
- Cockburn, A., and Cockburn, E. (eds.), 1980, Mummies, disease and ancient cultures, Cambridge University Press, Cambridge, England.
- Dawson, W. R., 1927, Making a mummy, Journal of Egyptian Archaeology, 13:40-49, pls. xvii-xviii.
- Dawson, W. R., and Smith, G. E., 1924, Egyptian mummies, Allen and Unwin, London, England.
- Dioscorides, 1655, The Greek Herbal (Translated by J. Goodyer (1655), ed. by R. T. Gunther, Oxford University Press, Oxford, 1934).
- ERC (Éditions Recherche sur les Civilisations), 1985, La momie de Ramsès II. A.D.F.P., Paris, France, p. 173-175.
- Fischer, C., 1980, Bog bodies of Denmark, p. 177-193, in: "Mummies, Disease and Ancient Cultures," A. Cockburn and E. Cockburn, eds., Cambridge University Press, Cambridge, England.

- Garner, R., 1979, Experimental mummification, p. 19-24, *in: "The Manchester Museum Mummy Project: Multidisciplinary Research on Egyptian Mummified Remains,"* A. R. David, ed., Manchester Museum, Manchester, England.
- Garner, R., 1986, Insects and mummification, p. 97-100, *in: "Science in Egyptology: Proceedings of the 'Science in Egyptology' Symposia,"* A. R. David, ed., Manchester University Press, Manchester, England.
- Germer, R., 1979, Untersuchung über Arzneimittelpflanzen im alten Ägypten, Ph.D. Thesis, University of Hamburg, Germany.
- Germer, R., 1985, Flora des pharaonischen Ägypten, Philipp von Zabern, Mainz.
- Glob, P. V., 1969, "The Bog People," Faber and Faber, London, England, p. 177-193.
- Herodotus, 1946, "The Histories," Book II, Para. 86-88 (Translated by A. D. Godley, Loeb Classical Library), Cambridge, Massachusetts.
- Lauer, J.-P., and Iskander, Z., 1955-56, Donnée nouvelles sur la momification dans l'Égypte ancienne, *Annales du Service des Antiquités de l'Égypte*, 53:167-194.
- Leek, F. F., 1969, The problem of brain removal during embalming by the ancient Egyptians, *Journal of Egyptian Archaeology*, 55:112-116.
- Lucas, A., 1914a, The use of natron by the ancient Egyptians in mummification, *Journal of Egyptian Archaeology*, 1:119-158.
- Lucas, A., 1914b, The use of bitumen by the ancient Egyptians in mummification, *Journal of Egyptian Archaeology*, 1:241-245.
- Lucas, A., 1932a, The occurrence of natron in ancient Egypt, *Journal of Egyptian Archaeology*, 18:62-66.
- Lucas, A., 1932b, The use of natron in mummification, *Journal of Egyptian Archaeology*, 18:125-140.
- Lucas, A., 1934, Artificial eyes in ancient Egypt, *Ancient Egypt*, 2:84-99.
- Lucas, A., 1962, Chapter 12: Mummification, p. 272-326, *in: "Ancient Egyptian Materials and Industries,"* (4th ed. revised and enlarged by J. R. Harris), Edward Arnold, London, England.
- Manniche, L., 1989, "An Ancient Egyptian Herbal," British Museum Publications, London and University of Texas Press, Texas.
- Maspero, G. (publisher), 1875, The ritual of embalming, p. 10-15, *in: Mémoire sur quelques papyrus du Louvre*, Paris, France.
- Osburn, W., 1828, Account of an Egyptian mummy presented to the museum of the Leeds Philosophical and Literary Society, The Leeds Philosophical and Literary Society, Leeds, England, p. 6.
- Pliny, 1957, "Natural History" (Translated by D. E. Eichholz, W. H. S. Jones, and H. Rackham, Loeb Classical Library) Cambridge, Massachusetts.
- Ruffer, M. A., 1914, *Mémoirés de l'Institut Égyptien*, 7:6, footnote.
- Sandison, A. T., 1963, The use of natron in mummification in ancient Egypt, *Journal of Near Eastern Studies*, 22:259 ff.
- Siculus, Diodorus, 1968, History, Book I, para. 91 (Translated by C. H. Oldfather, Loeb Classical Library), Cambridge, Massachusetts.
- Smith, G. E., 1912, "The Royal Mummies," Cairo Museum, Cairo, Egypt.
- Smith, G. E., 1914, Egyptian mummies, *Journal of Egyptian Archaeology*, 1:189-206.
- Smith, G. E., and Jones, F. W., 1910, Archaeological survey of Nubia, 1907-1908, Bulletin 2, p. 218, Cairo, Egypt.
- Spielmann, P. E., 1932, To what extent did the ancient Egyptians employ bitumen for embalming?, *Journal of Egyptian Archaeology*, 18:177-180.
- Strabo, 1959, The Geography of Strabo, Book VIII (Translated by H. L. Jones, Loeb Classical Library), Cambridge, Massachusetts.
- Zaki, A., and Iskander, Z., 1943, Materials and method used for mummifying the body of Amentefnekht, Saqqara, 1941, *Annales du Service des Antiquités de l'Égypte*, 42:223-250.

## **PLANTS USED MEDICALLY BY INDIGENOUS PEOPLES\***

Walter H. Lewis

Department of Biology  
Washington University  
St. Louis, MO 63130

### **INTRODUCTION**

Indigenous peoples traditionally use a wide range of plants to maintain their health. Many such plants have been selected empirically for generations, a continuing experimental process still under way. Modern medicine has benefited from anecdotal results of these experiments by selecting needed candidates for a currently inadequate pharmacopeia to treat large numbers of diseases and symptoms. When the rapid destruction of tropical vegetation—where the majority of cultured peoples using traditional medicines still live and where the richness of the flora implies great numbers of medically valuable plants—is considered in relation to the recent upsurge of interest in finding antiviral and antineoplastic agents, there is ample reason to justify learning what plants indigenous peoples are using, how they are using them, and under what circumstances they are proving efficacious. These often ignored ethnobotanical findings set the stage for targeting plant materials which can then be meaningfully analyzed for activity using appropriate bioassays and, when these are significant, for chemical isolation and characterization of active principles.

The importance of plants during the long history of mankind is immense. Prehistorically, the detection of plants as sources of food and for construction, fuel, fabric, and medicine attested to human's curiosity driven in large part by a quest to survive and flourish. Early searches for edible plants, for example, must have turned up those that besides being nonedible were also toxic and, between these extremes, a large number of interesting plants which relieved pain or fought symptoms of disease when used in small amounts. From these early findings, refined and extended as they were passed from generation to generation, sprang industries and technologies based on products isolated from these plants and required by the developing needs of the civilized world. The use of these products and their analogs and semisynthetics reached a zenith in the late 19th century during a period of unprecedented growth and diversity of industrial phytochemistry, only to recede under competition with

\*Supported in part by a grant from the National Science Foundation (BSR-8508075). I thank Jennifer L. Barry who typed the manuscript so efficiently, Ruth Lewis of the biology library who helped with reference materials, and Cathy M. Crandall who assisted in compiling Table 2.

synthetic organic chemistry during the 20th century. In many ways, humans nurtured in the European culture had become antithetic to their prehistory by becoming increasingly independent of plants in everyday life, and as a consequence, they found little need to study them in any serious and rigorous way.

Only in recent years has this trend, certainly as it pertains to medicine, been forestalled. In 1964 appeared *Green Medicine* by Margaret B. Kreig, and in 1977 alone, three books were published which focused on plants in relation to human health (*Nature's Healing Arts: From Folk Medicine to Modern Drugs*, Aikman; *Medical Botany: Plants Affecting Man's Health*, Lewis and Elvin-Lewis; *Major Medicinal Plants: Botany, Culture and Uses*, Morton). These works were responding to a rekindled interest in the use of natural plant products for improved health, particularly among laymen. Their impact among professionals was more gradual, but even here, there has been a renewed consideration of natural products in teaching and research about medicine and health in the final decades of the 20th century, in essence, reversing a neglect during the past 100 years.

Assuming that this renewed interest in plants will continue and expand into the 21st century, what resources are there to satisfy such a demand and what approaches should be used to provide a meaningful interpretation of these resources? Unfortunately, new demand coincides with an accelerated destruction of tropical rain forests, where the majority of species grow, so that the annual loss of 20,000 species suggested by some individuals, over and above normal rates of extinction, is clearly shrinking forever the world's diversity of potentially useful plants (Hedberg, 1987; Plotkin, 1988; Soejarto and Farnsworth, 1989). The loss of these forests is obviously reducing the numbers of species available for current and future exploration, so much so that governments, agencies, groups, and private citizens on a worldwide basis point to this problem as one of the most significant facing civilization in the latter part of the 20th century.

Given that even in the worst scenario some important species will remain, how do we learn about their properties and potentialities? Random sampling of thousands for a particular purpose is one way, but this can be a very time-consuming and costly procedure deemed less valuable an approach than folklore preselection, at least for predicting antitumor activity (Spjut and Perdue, 1976). This information may be limited to fragments which have survived in printed works or recollections of a few people, but whenever possible, ethnobotanical research ought to be undertaken. To ignore practices of people, particularly of culturally intact humans, is to dismiss the value of empirical methodology which has been functioning for thousands of years. Unfortunately, like the rain forests, peoples with their unique cultures are decreasing rapidly, but where they exist, their knowledge is essential in establishing a baseline of traditional use of plants as medicines.

How valuable is this initial approach in discerning what plants to bioassay and chemically analyze? Is there some degree of reliability as to efficacy and active principles or is historic and current ethnomedicine meaningless from a modern Western medical view? I shall try to answer these questions by example using both past and present records of human uses of medically valued plants.

## HISTORY OF SELECTED MEDICINAL PLANTS

### *Ephedra*

Although the Chinese long prized *Ephedra* (Ephedraceae) as a medicinal plant, there has been little data of its early use elsewhere. However, there now exists strong indirect ethnobotanical evidence of value attributed to these plants from a

Neanderthal cave in the highlands of Iraq. Here, 60,000 years ago, a man was buried surrounded by eight plant species of which seven were cited by Al-Rawi and Chakravarty (1964) as having herbal and medicinal properties. All coincidence? Not likely, given the fact that seven of eight plants available to be added around the body are notable medicinal plants today, not necessarily known for their beauty now, and very probably not so then. At least one plant, *Ephedra*, was certainly not included because of its showy flowers, for it has none and no sexual parts of special notice, though it does have important medicinal properties. Six species of the genus are now indigenous to the northern area of Iraq, where several species are used to treat asthma and edema and as cardiac stimulants. Some of these contain ephedrine, in particular *E. alata* Dcne. (Solecki, 1971, 1975).

Since many believe that the Neanderthals, who became extinct perhaps 30,000 years ago, were in modern humanity's direct line of evolution, it is reasonable to speculate that, if they did possess knowledge of medicinal plants, it would have been passed to modern *Homo sapiens* who would have continued to accumulate such knowledge by empirical methodology, a process still in progress by indigenous peoples (e.g., Amazonia, Lewis et al., 1991).

If this discovery were restricted to an area where data of its traditional medical use did not exist, such indirect evidence might be viewed with skepticism. Yet this is not the case. Pliny [The Elder] wrote, shortly before his death by asphyxiation following the eruption of Vesuvius in 79 AD (or of a heart attack, Majno, 1975), of a plant "some call ephedron...The Greeks hold various views about this plant...assuring us that so wonderful is its nature, its mere touch stanches a patient's bleeding...its juice kept in the nostrils checks hemorrhage...and taken in sweet wine it cures cough." As Majno (1975) contends, one cannot help but be startled by the association of a plant named *ephedron* stopping hemorrhages and curing coughs, for these are the two main effects of the powerful alkaloid ephedrine extracted from *Ephedra*. He notes that a surgical incision in skin injected with ephedrine is almost bloodless, and a spell of asthmatic cough can be relieved by it as if by a miracle. A similar text was written by a contemporary of Pliny, Dioscorides in the 1st century (but a drawing dates from 510 AD, Fig. 1). They were undoubtedly referring to *E. major* Host native to the Mediterranean region with sufficient ephedrine to treat cough, asthma, and colic.

On the other side of Old World, *Ephedra* and its product ephedrine were thought to be a unique discovery of the Chinese when introduced into Western medicine in 1924. Certainly, the use of *Ephedra* dated from several thousand years earlier, but not until 1083 did Thang Shen-Wei produce a clear illustration of the plant (Fig. 2) which cured feverish chills (malaria), arrested coughing, and dispersed obstructions in the bowels. In his dispensatory of 1596, Li Shih-Chen claimed it improved circulation, caused sweating, eased coughing, and reduced fever.

Even though an ancient medical history existed in both the Mediterranean basin and China, the introduction of the plant into Western medicine is a classic episode in the recent history of botany and medicine. Between 1922 and 1924, the American, Carl F. Schmidt, while working at Peking Union Medical College, tested some of the most popular Chinese traditional plants in the hope of discovering new active principles (Majno, 1975). Of the five he selected, *Ephedra* extracts gave significant results when injected into dogs: a spectacular rise in blood pressure. He had found a naturally occurring sympathomimetic drug with an action similar to epinephrine (Fig. 7) and norepinephrine (Fig. 7), except for its much longer duration of action and effectiveness after oral administration. In addition to such vasopressor effects, ephedrine acts on the heart, with the heart rate usually increasing, dilates the bronchi and thus useful in treating asthma, acts locally and systemically as a nasal decongestant by inducing vasoconstriction of the nasal mucosa, and these same



Figure 1. *Ephedra major* Host, Dioscorides (1st century), illustration by a Byzantine in 512 AD; IV, 46.

properties allow treatment of vascular collapse in various allergic and anaphylactic conditions.

At least five species of *Ephedra* are sources of ephedrine (Fig. 7), *E. major* Host, *E. gerardiana* Wall., *E. intermedia* Schrenk & Mayer, *E. sinica* Stapf, and *E. equisetina* Bunge (*mahuang* in China). Supplies from China could be irregular in the 1920s, and this led to the development of potent CNS stimulants, like the amphetamines, and a host of further derivates related to ephedrine and the adrenergic stimulants. It took a long time for Western medicine to recognize the value of *Ephedra*, but advocates of traditional medicine had been using it for thousands of years for well-being.

### Colchicum

A plant also native to the Mediterranean region and known to Dioscorides was the meadow saffron or autumn crocus, *Colchicum autumnale* L. (Liliaceae) (Fig. 3). Gerard (1597, 1633) wrote: "The roots of all the sorts of meadow saffrons are very hurtful to the stomach, and being eaten they kill by choking, as mushrooms do, according to Dioscorides; whereupon some have called it *Colchicum strangulatorium*." This poisonous reputation undoubtedly deterred the use of *Colchicum* by some; but in 1552, Bock described that the root had been employed by Arabian physicians in cases of gout and rheumatism. A few years later, Gerard again wrote that roots "stamped, and mixed with the whites of eggs, barley meal, and crumbs of bread, and applied plasterwise, ease the pain of the Gout, swellings and aches about the joints." Even though gout was of serious concern throughout the 17th and 18th centuries, European medicine was extremely slow in investigating this folk remedy; but finally in the early 19th century, Sir Edward Howe successfully treated many patients in London and his favorable results brought wide acceptance of the root as a valued



Figure 2. *Ephedra sinesis* Stapf. (*mahuang*), Thang Shen-Wei (1083). From Needham (1986).

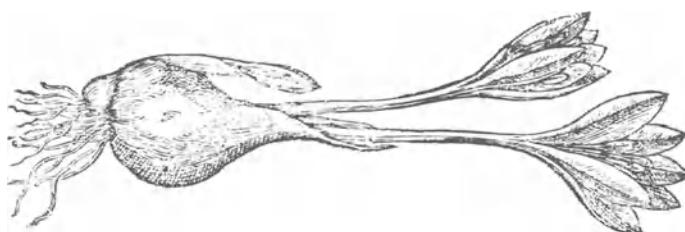


Figure 3. *Colchicum autumnale* L., Gerard (1633, p. 163).

remedy. Colchicine (Fig. 7) was extracted in 1820 and it, along with allopurinol (Fig. 7), continues as the drug of choice for treating gout (Copeman, 1964, 1970).

These two instances of plants used in traditional medicine, eventually finding acceptance in modern medicine once ethnobotanical data were seriously considered, can be illustrated many more times. They show the value in many instances of knowing what plants are used by indigenous peoples and how they use them.

## Psychoactives

Of great interest today, and also illustrating the need to understand ethnobotany and phytogeography, is a trilogy of plants with long histories of human uses; in two instances so much so that wild populations of the species are unknown. These two originated in the Old World and a third is native to South America: the opium poppy, *Papaver somniferum* L., marijuana, *Cannabis sativa* L., and coca, *Erythroxylon coca* L. (wild populations still known, Plowman, 1984) and *E. novogranatense* (Morris) Hieron, all of significant medical use, but also producing major psychoactive drugs of abuse.

**Opium Poppy (Fig. 4).** Because of long domestication, some of the oldest plants used by humans have no wild populations; and still others, like the opium poppy, have been selected to such an extent that they have lost the ability to survive under natural conditions and are now known only in agricultural fields or environments close to farmlands and areas disturbed by humans. Merlin (1984) provided convincing evidence from archaeological records of domestication of the opium poppy and its use as a medicine and psychoactive drug for ritualistic or recreational purposes in Europe during the Neolithic (Stone) and Bronze Age. Use became particularly widespread by the Late Bronze Age (ca. 1600 BC) in the eastern Mediterranean region from Greece to southern Egypt and eastern Syria. "The earliest



Figure 4. *Papaver somniferum* L., Dioscorides (1st century), illustration by a Byzantine in 512 AD; IV, 65.

drug use of the opium poppy may very well even reach back past the Greeks into the early Neolithic, Mesolithic or upper Paleolithic periods of cultural evolution;" and as humans have always engaged in a search for greater comfort, a high priority was surely given to the development of drugs to relieve pain. By 350 BC, Hippocrates mentioned poppy juice as a cathartic, hypnotic, narcotic, and astringent/hemostat; and in the 2nd century, Galen stated that opium is the strongest of drugs to numb the senses and induce a deadening sleep (Duke, 1973). In the 15th century, Schöffer (1484) provided a summary of a plant which has stood the test of time: The plant's juice helps those with coughs, diarrhea, headaches, and earaches; and it also causes sleep and soothes pain, whether from boils, overheated livers, or other bodily ailments.

We now know that opium poppy exudes a white latex from shallow cuts in green capsules which forms the crude drug opium. From it, morphine, codeine, thebaine (dimethylmorphine) (Fig. 6), and many other alkaloids are extracted having hypnotic/analgesic and antitussive properties in particular, and semisynthetics and derivate like oxymorphone, hydromorphone, and perhaps most important of all diacetylmorphine or heroin (Fig. 6) are produced. This illustrates that a plant product may have major medical value as well as be subject to abuse with ramifications equalling or even overshadowing benefits (Table 1).

**Marijuana (Fig. 5).** A second plant with psychoactive properties long cultivated by humans is hemp or marijuana. Pollen evidence shows that it was cultivated in western Europe two thousand years ago; and earlier, Herodotus, ca. 500 BC, described how in the Middle East Scythians and Thracians intoxicated themselves with fumes given off by roasting the seeds on white-hot stones (Grinspoon, 1977). Chinese and Indians of Asia smoked hemp as a euphoric during the same period and probably had done so for centuries.

By the Middle Ages in Europe, however, knowledge of the psychoactive properties of the species appears to have been lost. For instance, Schöffer (1474) detailed wide medicinal uses for marijuana, including clearing scales from the head,



Figure 5. *Cannabis sativa* L., Dioscorides (1st century), illustration by a Byzantine in 512 AD; III, 165.

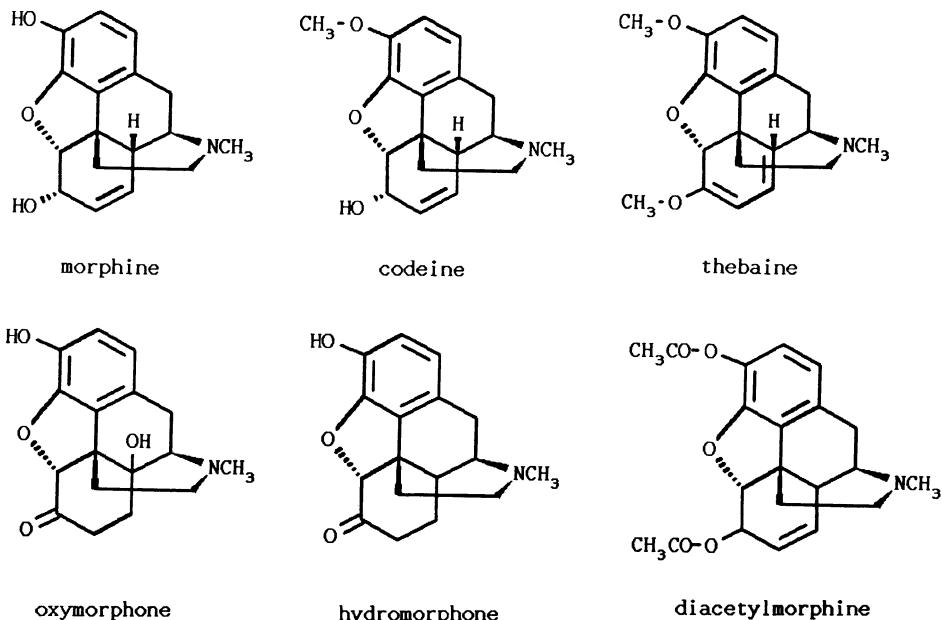


Figure 6. Structural formulas of morphine, codeine, thebaine, oxymorphone, hydromorphone, and diacetylmorphine.

growing hair, aiding digestion; treating erisipelas, painful wounds, and dropsy; drying moisture in the ears, forming plasters for abscesses; as an analgesic, relieving flatulence, curing migraines, and drying up the sperm. A number of these uses were confirmed by Gerard a century later (1597) but, again, without indicating psychoactive potential.

Marijuana was introduced to the American colonies in 1611 near Jamestown. This early crop served to produce bagging, webbing, twine, marine rope, ship sails, and, by 1630, half of the winter clothing of Americans and nearly all of their summer clothing (Grinspoon, 1977). In parts of the Midwest prior to the Civil War, marijuana was a major cash crop, as illustrated at Lexington, Missouri, by the Missouri River east of Kansas City when, at the time of the Civil War, the town was shipping 20,000 tons annually to all parts of the country and world.

In the 20th century, the active principles, tetrahydrocannabinols (Fig. 7), and whole plant extracts are used by cancer patients who are less nauseated if they smoke or ingest marijuana before receiving cytotoxic drugs. Persons suffering from glaucoma, multiple sclerosis, and AIDS may also use prescribed marijuana (Randall, 1990). Central and cardiovascular side effects can occur following high use; however, an analog, nabilone (Fig. 7), greatly reduces these effects and it is active orally. Even considering these medical uses, however, a crop estimated in excess of \$10 billion in the U.S. during 1982 and a total production of 7,850 tons from Mexico, Colombia, and Jamaica alone (Table 1) must be used otherwise—its value clearly relates to hallucinogenic and euphoric recreational abuse.

**Coca (Fig. 8).** Since pre-Columbian times, Amerindians have chewed coca leaves as a CNS stimulant; shortly after the Conquest, leaves were introduced to Europe by explorers returning from Peru. Gardeke, in 1855, was the first to extract

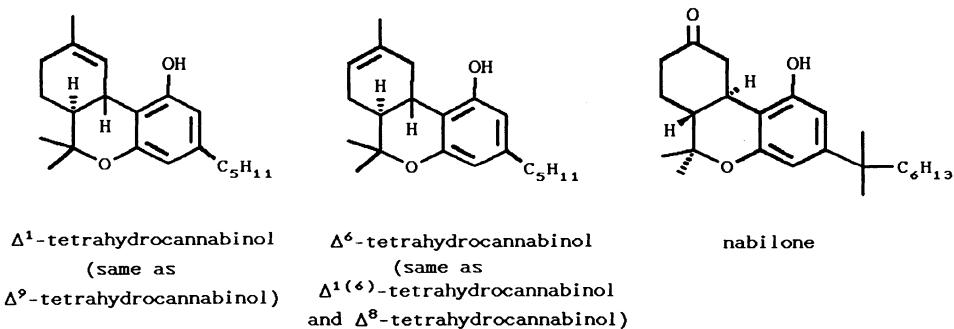
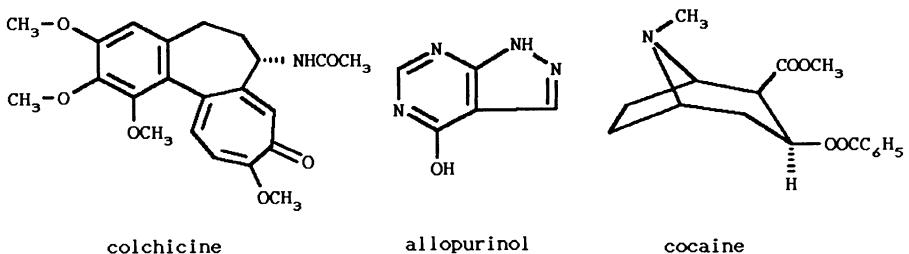
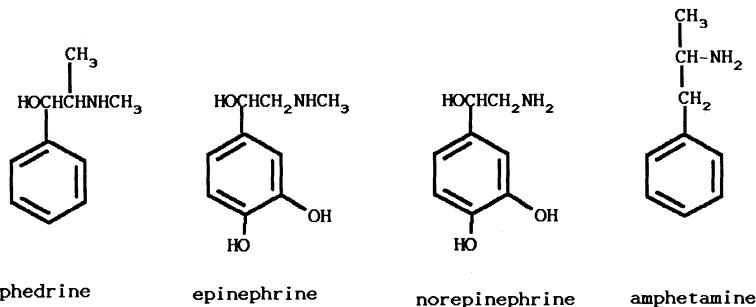


Figure 7. Structural formulas of ephedrine, epinephrine, norepinephrine, amphetamine, colchicine, allopurinol, cocaine, tetrahydrocannabinol isomers, and nabilone.

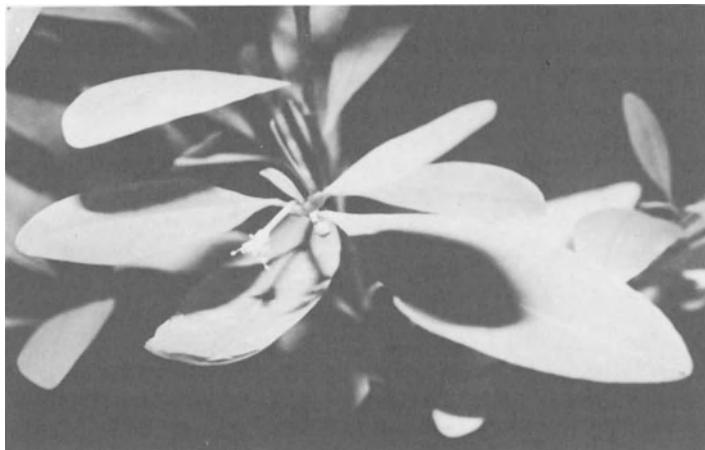


Figure 8. *Erythroxylon novogranatense* (Morris) Hieron.

the active ingredient, calling it erythroxylon, and 5 years later, Niemann isolated the alkaloid, naming it cocaine (Fig. 7) (Van Dyke and Byck, 1977).

The earliest archaeological evidence indicating coca use is found in the Valdivia Culture of southwestern Ecuador. These small ceramic lime containers thought to be used in coca chewing were found that date from about 2100 BC, and small, decorated lime pots can be found from 1000 to 300 BC. A ceramic figurine showing the prominent cheek bulge of a coca chewer from this area dates from 1600 to 1500 BC, the earliest known example of a long Ecuadorian tradition of making such figurines (Rury and Plowman, 1984).

Similarly, coca chewing paraphernalia have been found along the Peruvian coast dating from 2500 to 1800 BC. In fact, lime pots, lime dippers, and ceramic, coca-chewing human figurines, as well as occasional preserved leaves of coca, have all been found throughout the Peruvian coast from the early ceramic period to Incan times. In Colombia, the same type of artifacts are often in gold. During the Incan Empire, this social drug attained a high ritualistic significance, as well as use as a respected medical panacea, prescribed for altitude sickness, asthma, bronchitis, colds, menstrual irregularities, pneumonia, and sore muscles (Weil, 1986).

Despite its early introduction into Europe, the plant was essentially ignored until the 19th century. It was known to allay fatigue of Andean Indians who chewed its leaves, but little else was appreciated about coca until those who tasted the extracted crystals of cocaine after its isolation in 1860 experienced a numbing of the tongue. From this property arose the first local anesthetic during the 1880s when cocaine was introduced into ophthalmology in particular and otolaryngology in general throughout Europe and North America. Other medical uses are summarized by Grinspoon and Bakalar (1981).

In the latter part of the 20th century, cocaine has become perhaps the major drug of abuse, certainly in the U.S. and Europe. In Peru, Bolivia, and Colombia, 200,000 tons of leaves are harvested annually (Table 1); and 80% of the extracted cocaine makes its way illegally to the U.S. at a cost to our culture of tens of billions of dollars annually. Here is an example of major abuse of a plant whose long traditional use in the Andes was to help functioning at high altitudes and for various medicinal and religious purposes, which led ultimately to a valued drug in Western

Table 1. 1989 production of three major psychoactive drugs.

Species	Country	Metric tons
<i>Cannabis sativa</i> L. plants	Mexico	4,750
	Colombia	2,700
	Jamaica	400
<i>Erythroxylon coca</i> Lam. and <i>E. novogranatense</i> (Morris) Hieron. leaves	Peru	110,000
	Bolivia	70,000
	Colombia	20,000
<i>Papaver somniferum</i> L. latex	Burma	1,300
	Afghanistan	750
	Iran	300
	Laos	250

medicine and finally to the current recreational emphasis with disastrous social, health, and economic consequences.

## PLANTS OF MODERN MEDICINE

Based on the total percentage of natural products represented in U.S. prescriptions, Farnsworth (1973) found that, in 1967, 42.4% (excluding vitamins) contained one or more natural products as active ingredients. That figure had not changed appreciably from data reported in 1962, suggesting that natural products form a relatively stable group of medicinal agents (Gosselin, 1962). Using Farnsworth's therapeutic category outline and amplified by data from Morton (1977), Leung (1980), Farnsworth and Soejarto (1985), Sneider (1985), Tyler (1987), and Der Marderosian (1991), examples of uses and origins are provided for a broad spectrum of plants which continue to enrich pharmacopeias for human benefit. These examples (excluding antibiotics), supplemented by those in Table 2, show a dependence on and a quest for natural products that is accelerating as the 21st century approaches (Tyler, 1986).

### Allergies

Cromolyn (as a disodium salt), a synthetic derived from khellin (Fig. 9), a chromone extracted from seeds of *Ammi visnaga* (L.) Lam. (Apiaceae), was introduced in the 1970s primarily as a preventive for bronchial asthma and other allergic conditions (Lewis and Elvin-Lewis, 1983). Aside from its prophylactic use in asthma, it is also given as eye drops to prevent allergic conjunctivitis and nasal sprays in preventing allergic rhinitis. The plant had been used for centuries in traditional medicine of the eastern Mediterranean region as an antispasmodic, for relieving pains of renal colic, dental caries, and angina pectoris, and for treating bronchial congestion (Quimby, 1953).

### Analgesics

The opium alkaloids, morphine and codeine (Fig. 6), widely prescribed analgesics/hypnotics from *Papaver somniferum* L., have already been described and

Table 2. Plants as sources of useful drugs or products in medical and pharmaceutical preparations.

Species	Family	Drug/product/use
<i>Abies balsamea</i> (L.) Miller	Pinaceae	Canada balsam oil (antiseptic, hemorrhoidal prep)
<i>Acacia senegal</i> (L.) Willd.	Fabaceae	Gum arabic (pharmaceutical aid)
<i>Achillea millefolium</i> L.	Asteraceae	Yarrow oil (diaphoretic)
<i>Acokanthera oppositifolia</i> (Lam.) Codd, <i>A. schimperi</i> Bentham	Apocynaceae	Ouabain (cardiotonic)
<i>Aconitum napellus</i> L.	Ranunculaceae	Aconitine (heart treatment), liniment
<i>Agathosma betulina</i> (Berg.) Pill.	Rutaceae	Buchu oil (diuretic prep)
<i>Aletris farinosa</i> L.	Liliaceae	Proprietary prep (female disorders, laxative, antiflatulent)
<i>Allium sativum</i> L., etc.	Liliaceae	Allicin (antibiotic), garlic (digestive aid <sup>a</sup> ), garlic oil (anthelmintic, rubefacient)
<i>Aloe ferox</i> Miller, <i>A. perryi</i> Baker, <i>A. vera</i> (L.) Burm.f.	Liliaceae	Leaf juice (purgative <sup>a</sup> ), aloe gel (emollient)
<i>Althaea officinalis</i> L.	Malvaceae	Althaea root syrup (cough prep)
<i>Ammi majus</i> L.	Apiaceae	Xanthotoxin
<i>A. visnaga</i> (L.) Lam.	Apiaceae	Bronchial asthma
<i>Anamirta cocculus</i> (L.) W. & A.	Menispermaceae	Picrotoxin (barbiturate antidote)
<i>Ananas comosus</i> (L.) Merr.	Bromeliaceae	Bromelain (anti-inflammatory, digestive aid)
<i>Anethum graveolens</i> L.	Apiaceae	Dill oil (aromatic, carminative)
<i>Angelica archangelica</i> L.	Apiaceae	Bronchial ailments <sup>b</sup>
<i>Aralia racemosa</i> L.	Araliaceae	Compound syrup of spikenard
<i>Arctium lappa</i> L.	Asteraceae	Root (diuretic and laxative prep, skin aid)
<i>Arctostaphylos urva-ursi</i> (L.) Sprengel	Ericaceae	Plant diuretic and laxative prep, urinary disinfectant <sup>b</sup>
<i>Ardisia japonica</i> Blume	Myrsinaceae	Bergemin (chronic bronchitis)
<i>Areca catechu</i> L.	Arecaceae	Arecoline (anthelmintic)
<i>Arnica</i> spp.	Asteraceae	Anti-inflammatory analgesic
<i>Artemisia absinthium</i> L.	Asteraceae	Absinthium oil (liniment)
<i>A. maritima</i> L.	Asteraceae	Santonin (anthelmintic)
<i>Asparagus officinalis</i> L.	Liliaceae	Roots diuretic
<i>Astragalus gummifer</i> Lobill.	Fabaceae	Gum sarcocolla, gum tragacanth (pharmaceutical prep)
<i>Atropa belladonna</i> L., <i>A. acuminata</i> Royle ex Lindl.	Solanaceae	Belladonna extract (anticholinergic)
<i>Avena sativa</i> L.	Poaceae	Oatmeal concentrate, bath for itching <sup>a</sup> , dermatological aid
<i>Azadirachta indica</i> A. Juss.	Meliaceae	Leaves antibiotic, insecticide; seed oil toothpaste, soap, etc.
<i>Berberis vulgaris</i> L.	Berberidaceae	Berberine (eye drops, eyewashes, tonics)
<i>Betula lenta</i> L.	Betulaceae	Birch oil, methyl salicylate (counterirritant)
<i>Brassica nigra</i> (L.) Koch	Brassicaceae	Allyl isothiocyanate (counterirritant, nasal decongestant <sup>a</sup> )
<i>Calendula officinalis</i> L.	Asteraceae	Topical analgesic
<i>Camellia sinensis</i> (L.) Kuntze	Theaceae	Caffeine, theophylline (stimulants)
<i>Cannabis sativa</i> L.	Cannabaceae	Cannabis resin
<i>Capsicum annuum</i> L., <i>C. baccatum</i> L., <i>C. chinense</i> Jacq., <i>C. frutescens</i> L.	Solanaceae	Capsicum oleoresin
<i>Carica papaya</i> L.	Caricaceae	Papain, chymopapain
<i>Carthamus tinctorius</i> L.	Asteraceae	Dietary suppl. in hyper-cholesterolemia
<i>Carum carvi</i> L.	Apiaceae	Carminative
<i>Cassia italica</i> (Miller) Sprengel, <i>C. senna</i> L., <i>C. angustifolia</i> Vahl	Fabaceae	Senna leaves, pods (laxatives)
<i>Catharanthus roseus</i> (L.) G. Don	Apocynaceae	Vincristine, vinblastine (antineukemic)

Species	Family	Drug/product/use
<i>Caulophyllum thalictroides</i> (L.) Michx.	Berberidaceae	Root/rhizome (diuretic, uterine antispasmodic, emmenagogue)
<i>Cephaelis ipecacuanha</i> (Brot.) Tussac	Rubiaceae	Ipecac (emetic <sup>a</sup> )
<i>Cephalosporium</i> spp.	[Fungus]	Cephalosporin (reduce transplant rejections)
<i>Cephalotaxus harringtonia</i> (D. Don) C. Koch	Cephalotaxaceae	Homoharringtonine (antileukemic <sup>b</sup> )
<i>Chamaemelum nobile</i> (L.) All.	Asteraceae	Anticonjunctivitis, carminative, antispasmodic, dermatological aid
<i>Chenopodium ambrosioides</i> L.	Chenopodiaceae	Chenopodium oil, wormseed oil (anthelmintics)
<i>Chondrodendron tomentosum</i> R.&P.	Menispermaceae	Tubocurarine (muscle relaxant)
<i>Cimicifuga racemosa</i> (L.) Nutt.	Ranunculaceae	Analgesic and tonic prep
<i>Cinchona calisaya</i> Wedd., <i>C. officinalis</i> L.	Rubiaceae	Quinine (antimalarial), quinidine (antiarrhythmic)
<i>Cinnamomum camphora</i> J. Presl	Lauraceae	Camphor (acne <sup>a</sup> , nasal decongestant <sup>a</sup> , rubefacient, wart remover <sup>a</sup> )
<i>C. verum</i> J. Presl	Lauraceae	Cinnamon (carminative)
<i>Citrullus colocynthis</i> (L.) Shrader	Cucurbitaceae	Colocynth (stimulant laxative <sup>a</sup> )
<i>Citrus aurantium</i> L.	Rutaceae	Orange oil (expectorant)
<i>C. limon</i> (L.) Burm.f.	Rutaceae	Pectin (antidiarrheal)
<i>C. sinensis</i> (L.) Osbeck	Rutaceae	Citrus bioflavonoids (hemostat)
<i>Claviceps purpurea</i> Tul.	Clavicipitaceae	Ergot alkaloids (vasoconstrictor, oxytocic), extract (dermatological aid)
<i>Coffea arabica</i> L., <i>C. canephora</i> Pierre ex Froehner, <i>C. liberica</i> W. Bull ex Hiern	[Fungus]	Caffeine (stimulant)
<i>Colchicum autumnale</i> L.	Liliaceae	Colchicine (gout)
<i>Coleus barbatus</i> (Andr.) Bentham	Lamiaceae	Forskolin (glaucoma <sup>c</sup> )
<i>Commiphora abyssinica</i> (Berg.) Engl., <i>C. opobalsamum</i> Engl.	Burseraceae	Myrrh (mouthwash)
<i>Copaifera officinalis</i> L.	Fabaceae	Copaiba oil and oleoresin (cough prep, diuretic)
<i>Cornus florida</i> L.	Cornaceae	Cornine (astringent bitter)
<i>Crataegus laevigata</i> (Poiret) DC., <i>C. monogyna</i> Jacq.	Rosaceae	Cardiotonic <sup>b</sup>
<i>Croton tiglium</i> L.	Euphorbiaceae	Croton oil (counterirritant, laxative)
<i>Curcuma longa</i> L.	Zingiberaceae	Circumin (choleretic, antibacterial)
<i>Cymopsis tetragonolobus</i> (L.) Taubert	Fabaceae	Guar gum (bulk laxative <sup>a</sup> )
<i>Cymbopogon nardus</i> (L.) Rendle	Poaceae	Insect repellent
<i>Cynara scolymus</i> L.	Asteraceae	Cynarin (hyperlipidemia <sup>b</sup> )
<i>Cytisus scoparium</i> (L.) Link	Fabaceae	Branches (diuretic, laxative, tonic prep), sparteine (oxytocic)
<i>Daphne genkwa</i> Siebold & Zucc.	Thymelaeaceae	Yuanhuacine (abortifacient)
<i>Datura stramonium</i> L.	Solanaceae	Stramonium (anti-asthmatic)
<i>Digitalis lanata</i> Ehrh.	Scrophulariaceae	Digoxin, lanatoside C, acetylglitoxin (cardiotonics)
<i>D. purpurea</i> L.	Scrophulariaceae	Digitoxin, whole leaf (cardiotonics)
<i>Dionaea muscipula</i> Ellis	Droseraceae	Cytostatic <sup>b</sup>
<i>Dryopteris filix-mas</i> (L.) Schott	Aspleniaceae	Oleoresin filicin (anthelmintic)
<i>Duboisia myoporoides</i> R.Br.	Solanaceae	Atropine, scopolamine (anticholinergic, motion sickness, mydriatic)
<i>Ecballium elaterium</i> (L.) A.Rich.	Cucurbitaceae	Elaterin resin (stimulant laxative <sup>a</sup> )

(Continued)

Table 2. Plants as sources of useful drugs or products in medical and pharmaceutical preparations.  
(Continued)

Species	Family	Drug/product/use
<i>Echinacea angustifolia</i> DC., <i>E. purpurea</i> (L.) Moench	Asteraceae	Immunostimulant <sup>c</sup> , topical analgesic
<i>Elettaria cardamomum</i> (L.) Maton	Zingiberaceae	Cardamom (carminative)
<i>Ephedra gerardiana</i> Wall., <i>E. sinica</i> Stapf	Ephedraceae	Ephedrine (bronchodilator, stimulant)
<i>Eriodictyon californicum</i> Greene	Hydrophyllaceae	Yerba santa syrup
<i>Erythroxylon coca</i> Lam., <i>E. novogranatense</i> (Morris) Hieron.	Erythroxylaceae	Cocaine (stimulant, topical anesthetic)
<i>Eucalyptus globulus</i> Labill., etc.	Myrtaceae	Eucalyptol, eucalyptus oil (anthelmintic, expectorant, local antiseptic, nasal decongestant <sup>a</sup> )
<i>Eupatorium cannabinum</i> L., <i>E. perfoliatum</i> L.	Asteraceae	Proprietary medicine (eupatorin)
<i>Fagopyrum esculentum</i> Moench	Polygonaceae	Rutin (decrease capillary fragility)
<i>Ferula assa-foetida</i> L., <i>F. galbaniflua</i> Boiss. & Buhse	Apiaceae	Asafetida, gum galbanum (antispasmodic, carminative, expectorant)
<i>Foeniculum vulgare</i> Miller	Apiaceae	Fennel oil, syrup (carminative)
<i>Fraxinus rhynchophylla</i> Hance	Oleaceae	Aesculetin (dysentery)
<i>Galipea officinalis</i> Hancock	Rutaceae	Angostura bark (bitter tonic)
<i>Garcinia hanburyi</i> Hook.f.	Clusiaceae	Pipe & Siam gamboge (stimulant laxative <sup>a</sup> )
<i>Gaultheria procumbens</i> L.	Ericaceae	Wintergreen oil (corn/callus remover <sup>a</sup> )
<i>Gaurea rusbyi</i> (Britton) Rusby	Meliaceae	Cocillana extract with rusbyine (expectorant)
<i>Gelidium amansii</i> Lamour., <i>G. cartilagineum</i> (L.) Gaill., <i>Gracilaria confervoides</i> (L.) Grev.	[Algae]	Agar bulk laxative, emulsions, gels, etc.
<i>Gelsemium sempervirens</i> (L.) St. Hil.	Loganiaceae	Gelsemine (central stimulant)
<i>Gentiana lutea</i> L.	Gentianaceae	Glycoside bitters (tonic)
<i>Ginkgo biloba</i> L.	Ginkgoaceae	Circulatory stimulant <sup>c</sup>
<i>Glycyrrhiza glabra</i> L.	Fabaceae	Licorice extract (pharmaceutical aid), glycyrrhizin (anti-ulcer)
<i>Gossypium arboreum</i> L.	Malvaceae	Gossypol (male contraceptive <sup>c</sup> )
<i>G. hirsutum</i> L.	Malvaceae	Cottonseed oil (emollient laxative)
<i>Hamamelis virginiana</i> L.	Hamamelidaceae	Witch hazel extract (antihemorrhoidal, astringent, dermatological aid, hemostatic)
<i>Hedeoma pulegioides</i> (L.) Pers.	Lamiaceae	Pennyroyal oil (carminative, diaphoretic)
<i>Humulus lupulus</i> L.	Cannabaceae	Sedative
<i>Hydrastis canadensis</i> L.	Ranunculaceae	Hydrastine (eyewash)
<i>Hypericum perforatum</i> L.	Clusiaceae	Hypericin (antiviral and antidepressant <sup>c</sup> )
<i>Hyoscyamus niger</i> L.	Solanaceae	Hyoscyamine (anticholinergic), hyoscyamus oil (smooth muscle relaxant, sedative)
<i>Hyssopus officinalis</i> L.	Lamiaceae	Expectorant, coughs and colds
<i>Illicium verum</i> Hook.f.	Illiciaceae	Star anise, anise oil (carminative, expectorant)
<i>Ipomoea orizabensis</i> Ledenois., <i>I. purga</i> (Wender.) Hayne	Convolvulaceae	Jalap, purging resin <sup>a</sup>
<i>Juniperus mexicana</i> Spreng., <i>J. virginiana</i> L., etc.	Cupressaceae	Cedarwood oil (insect repellent), cone (diuretic)
<i>J. sabina</i> L.	Krameriaceae	Savin oil (anthelmintic, emmenagogue)
<i>Krameria triandra</i> R. & P.	Lamiaceae	Rhatany root
<i>Lavandula angustifolia</i> Miller, <i>L. stoechas</i> L.	Lamiaceae	Lavender oil (aromatic, carminative)
<i>Laminaria, Macrocystis</i> , etc.	[Algae]	Algin (bulk laxative, demulcent)
<i>Levisticum officinale</i> Koch.	Apiaceae	Diuretic, carminative

Species	Family	Drug/product/use
<i>Linum usitatissimum</i> L.	Linaceae	Linseed oil (emollient)
<i>Liquidambar styraciflua</i> L., <i>L. orientalis</i> Miller	Hamamelidaceae	Compound tincture benzoin
<i>Lobelia inflata</i> L.	Campanulaceae	Lobelaine (respiratory stimulant)
<i>Lycopodium clavatum</i> L.	Lycopodiaceae	Spores absorbent
<i>Malpighia puniceifolia</i> L.	Malpighiaceae	Vitamin C (richest source)
<i>Malus domestica</i> Borkh.	Rosaceae	Pectin (antidiarrheal)
<i>Marrubium vulgare</i> L.	Lamiaceae	Expectorant, coughs and colds
<i>Melaleuca cajuputi</i> Powell	Myrtaceae	Cajeput oil (expectorant, topical parasiticide, counterirritant)
<i>M. quinquenervia</i> (Cav.) S. T. Blake	Lamiaceae	Niaouli oil (anthelmintic)
<i>Melissa officinalis</i> L.	Lamiaceae	Balm, melissa oil
<i>Mentha arvensis</i> L., <i>M. x gentilis</i> L., <i>M. x piperita</i> L., <i>M. x spicata</i> L.	Lamiaceae	Menthol, peppermint oil, spearmint oil (nasal decongestant <sup>a</sup> , dandruff prep <sup>a</sup> , psoriasis <sup>a</sup> , digestive aid <sup>a</sup> )
<i>Myrica cerifera</i> L.	Myricaceae	Root bark (indolent ulcers, colds/chills)
<i>Myristica fragrans</i> Houtt.	Myristicaceae	Nutmeg oil (rubefacient)
<i>Myroxylon balsamum</i> (L.) Harms	Fabaceae	Balsam of Tolu (expectorant)
<i>M. balsamum</i> var. <i>pereirae</i> (Royle) Harms		Balsam of Peru (scabicide, skin ulcer therapy)
<i>Olea europaea</i> L.	Oleaceae	Olive oil (emollient)
<i>Panax ginseng</i> C. Meyer, <i>P. quinquefolium</i> L.	Araliaceae	Adaptogen <sup>c</sup>
<i>Papaver bracteatum</i> Lindl.	Papaveraceae	Codeine
<i>P. somniferum</i> L.	Papaveraceae	Opium, codeine (antitussive), morphine, heroin (analgesic)
<i>Passiflora incarnata</i> L.	Passifloraceae	Mild sedative <sup>b</sup>
<i>Pausinystalia johimbe</i> (K. Sch.) Pierre ex Beille	Rubiaceae	Yohimbine (adrenergic blocker)
<i>Persea americana</i> Miller	Lauraceae	Avocado oil (skin healing, sclerosis <sup>b</sup> )
<i>Petroselinum crispum</i> (Miller) A. W. Hill	Apiaceae	Digestive aid, diuretic
<i>Peumus boldus</i> Molina	Monimiaceae	Boldo leaves (tonic and diuretic prep)
<i>Physostigma venenosum</i> Balf.	Fabaceae	Physostigmine (cholinergic, miotic)
<i>Picrasma excelsa</i> (Sw.) Planchon	Simaroubaceae	Quassia bitters (anthelmintic)
<i>Pilocarpus jaborandi</i> Holmes, <i>P. pinnatifolius</i> Lem.	Rutaceae	Pilocarpine (antiglaucoma, cholinergic-ophthalmic)
<i>Pimenta dioica</i> (L.) Merr.	Myrtaceae	Allspice oil
<i>Pimpinella anisum</i> L.	Apiaceae	Anise oil (cough mixtures and lozenges, carminative)
<i>Pinus mugo</i> Turra, <i>P. strobus</i> L., <i>P. sylvestris</i> L.	Pinaceae	Bark and needle oil (cough/cold prep), turpentine oil (inhalation expectorant)
<i>Piper cubeba</i> L.	Piperaceae	Cubeb oil (urinary antiseptic)
<i>P. methysticum</i> Forster f.	Piperaceae	Kawain (sedative)
<i>Plantago afra</i> L. ( <i>P. psyllium</i> ) <i>P. ovata</i> Forssk.	Plantaginaceae	Psyllium (bulk laxative)
<i>Podophyllum peltatum</i> L.	Berberidaceae	Podophyllin, podophyllotoxin (etoposide, antineoplastic <sup>c</sup> ), resin (stimulant laxative <sup>a</sup> )
<i>Polygala senega</i> L.	Polygalaceae	Senega fluid extract (expectorant, cough syrups <sup>b</sup> )
<i>Polygonum multiflorum</i> Thung	Polygonaceae	Laxative
<i>Populus balsamifera</i> L., <i>P. nigra</i> L.	Salicaceae	Poplar buds (balm of Gilead, cough prep), salicin (analgesic)

(Continued)

Table 2. Plants as sources of useful drugs or products in medical and pharmaceutical preparations.  
(Continued)

Species	Family	Drug/product/use
<i>Prunus amygdalus</i> Batsch	Rosaceae	Sweet and bitter almond oil (emollient, laxative) Laetrile (disproved antineoplastic)
<i>P. armeniaca</i> L.		Prune prep (stimulant laxative <sup>a</sup> )
<i>P. domestica</i> L.		Wild cherry bark (cough prep)
<i>P. virginiana</i> L.		Bitters, anthelmintic
<i>Quassia amara</i> L.	Simaroubaceae	Glaucarubin (amoebicide)
<i>Q. simarouba</i> L.f.	Simaroubaceae	Tannic acid galls (astringent)
<i>Quercus infectoria</i> Olivier	Fagaceae	Reserpine, ajmaline (antihypertensive, tranquilizer)
<i>Rauvolfia serpentina</i> (L.) Kurz	Apocynaceae	Cuprea bark (quinine, quinidine)
<i>R. vomitoria</i> Afzel.		
<i>Remijia pedunculata</i> Flueck., <i>R. purdieana</i> Wedd.	Rubiaceae	Frangula bark, cascara (stimulant laxative <sup>a</sup> )
<i>Rhamnus frangula</i> L., <i>R. purshiana</i> DC.	Rhamnaceae	
<i>Rheum officinale</i> Baillon	Polygonaceae	Stimulant laxative <sup>a</sup>
<i>Rhododendron molle</i> G. Don	Ericaceae	Rhomitoxin (antihypertensive)
<i>Rhus cortaria</i> L.	Anacardiaceae	Tannins (astringent)
<i>Ricinus communis</i> L.	Euphorbiaceae	Castor oil (wart remover <sup>a</sup> ), ricin (lectin)
<i>Rosa gallica</i> L.	Rosaceae	Rose petal infusion
<i>Rosmarinus officinalis</i> L.	Lamiaceae	Rosemary oil (stomachache, tonic)
<i>Rubus idaeus</i> L.	Rosaceae	Pharmaceutical prep (diarrhea)
<i>Rumex crispus</i> L., <i>R. hymenosepulus</i> Torrey	Polygonaceae	Astringent, laxative tonic, high tannin
<i>Ruscus aculeatus</i> L.	Liliaceae	Vasoconstrictor (circulation of legs), anti-inflammatory
<i>Ruta graveolens</i> L.	Rutaceae	Antispasmodic
<i>Salix alba</i> L.	Salicaceae	Salicin, saligenin (analgesic)
<i>Salvia officinalis</i> L.	Lamiaceae	Sage oil (astringent, antiseptic)
<i>Sanguinaria canadensis</i> L.	Papaveraceae	Sanguinaria root (cough remedies)
<i>Santalum album</i> L.	Santalaceae	Sandalwood oil (urinary anti-infective)
<i>Sassafras albidum</i> (Nutt.) Nees	Lauraceae	Sassafras oil (antispasmodic, anti-infective, tonic)
<i>Serenoa repens</i> (Bartr.) Small	Arecaceae	Saw palmetto berries (prostate products <sup>b</sup> )
<i>Sesamum indicum</i> L.	Pedaliaceae	Sesame oil (pharmaceutical solvent)
<i>Silybum marianum</i> (L.) Gaertn.	Asteraceae	Antihepatitis <sup>b</sup> , <i>Amanita</i> poisoning antidote <sup>b</sup>
<i>Smilax aristolochiifolia</i> Mill., <i>S. febrifuga</i> Kunth, etc.	Liliaceae	Tonic, diuretic
<i>Spigelia marilandica</i> L.	Loganiaceae	Rhizome/root anthelmintic
<i>Stachys officinalis</i> (L.) Trev.	Lamiaceae	Astringent (diarrhea)
<i>Sterculia urens</i> Roxb.	Sterculiaceae	Karaya gum, Indian tragacanth (laxative)
<i>Strophanthus gratus</i> (Hooker) Franchet, <i>S. kombe</i> Oliver	Apocynaceae	Ouabain, strophanthin (cardiotonic)
<i>Strychnos nux-vomica</i> L.	Loganiaceae	Strychnine (central stimulant)
<i>Styrax benzoin</i> Dryander, <i>S. officinalis</i> L.	Styracaceae	Benzoin gum in friar's balsam (topical protectant)
<i>Symphtum officinale</i> L.	Boraginaceae	Allantoin (external healing, skin protectant)
<i>Syzygium aromaticum</i> (L.) Merr. & Perry	Myrtaceae	Clove oil (eugenol, local analgesic/ anesthetic <sup>a</sup> , carminative)
<i>Tabernanthe iboga</i> Baill.	Apocynaceae	Ibogaine (antidepressant), tabernanthine (analgesic, serotonin antagonist)
<i>Tanacetum cinerariifolium</i> (Trev.) Schultz-Bip.	Asteraceae	Pyrethrin insecticide
<i>Taxus brevifolia</i> Nutt.	Taxaceae	Taxol (antineoplastic <sup>c</sup> )

Table 2. (Continued)

Species	Family	Drug/product/use
<i>Theobroma cacao</i> L.	Sterculiaceae	Theobromine (stimulant), oil (dermatological aid)
<i>Thuja occidentalis</i> L.	Cupressaceae	Cedar leaf oil (counterirritant)
<i>Thymus vulgaris</i> L.	Lamiaceae	Thymol (acne <sup>a</sup> , antiseptic, boils <sup>a</sup> , carminative, counterirritant)
<i>Trichosanthes kirilovii</i> Maxim.	Cucurbitaceae	Trichosanthin (abortifacient, anti-AIDS <sup>c</sup> )
<i>Trigonella foenum-graecum</i> L.	Fabaceae	Antidiabetic <sup>c</sup>
<i>Triticum aestivum</i> L.	Poaceae	Wheat germ oil (vitamin E <sup>a</sup> , $\beta$ -sitosterol)
<i>Ulmus rubra</i> Muhl.	Ulmaceae	Bark mucilage (sore throats and coughs)
<i>Uncaria gambir</i> (Hunter) Roxb.	Rubiaceae	Astringent
<i>Urginea maritima</i> (L.) Baker	Liliaceae	Squill extract (cardiotonic, diuretic, emetic, expectorant)
<i>Urtica dioica</i> L.	Urticaceae	Diuretic prep <sup>b</sup>
<i>Usnea</i> spp.	[Lichen]	Antibacterial, antifungal creams <sup>c</sup>
<i>Valeriana officinalis</i> L.	Valerianaceae	Valerian oil (sedative <sup>b</sup> )
<i>Vanilla planifolia</i> Andr.	Orchidaceae	Vanillin (pharmaceutical aid)
<i>Veratrum album</i> L.	Liliaceae	Antihypertensive <sup>b</sup>
<i>Verbascum thapsus</i> L.	Scrophulariaceae	Cough remedy <sup>b</sup>
<i>Viburnum prunifolium</i> L., <i>V. opulus</i> L.	Caprifoliaceae	Root bark (tonics, uterine sedative and antispasmodic, diuretic)
<i>Viscum album</i> L.	Viscaceae	Antihypertensive <sup>b</sup>
<i>Yucca aloifolia</i> L., etc.	Agavaceae	Anti-arthritis <sup>c</sup>
<i>Zea mays</i> L.	Poaceae	Cornsilk (allantoin, diuretic), corn oil (pharmaceutical aid, $\beta$ -sitosterol = antihyperlipoproteinemic)
<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Ginger oleoresin and oil (motion sickness, mouthwash)

<sup>a</sup>Over-the-counter (OTC) products/drugs proposed for removal as "ineffective" by FDA (HerbalGram 23:32-35, 1990).

<sup>b</sup>Used largely in Europe.

<sup>c</sup>Research phase.

their long use in traditional medicine exemplified. Many synthetics have been derived from these opiate alkaloids; and these, together with ipecac, the tropane alkaloids, and methyl salicylate, are also used as analgesics.

### Anesthetics

In the 1880s, cocaine (Fig. 7) was introduced into medicine as a topical anesthetic in ophthalmology, the first effective use of local anesthesia. Since that time, numerous compounds have been prepared by various means using this natural product as a model compound (Farnsworth, 1973).

With the introduction in the late 1930s of (+)-tubocurarine (Fig. 10) from *Chondrodendron tomentosum* R. & P. (Menispermaceae) long used to tip blow-gun darts in South America to affect skeletal muscle relaxation, Western medicine obtained a highly valuable natural product of immense medical significance. This new kind of neuromuscular blocker, acting as a competitive antagonist of acetylcholine (Fig. 9), allowed the paralysis of respiratory musculature which made anesthesia safer by permitting lower doses of anesthetic to attain full muscular flaccidity (Sneader,

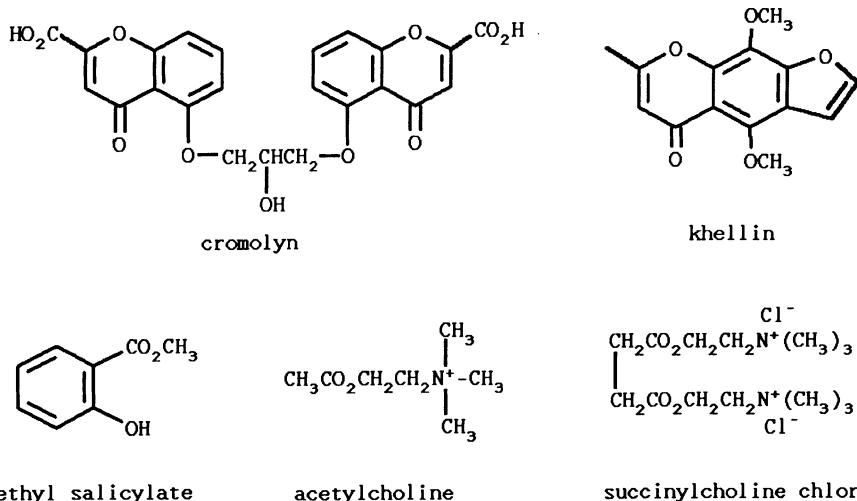


Figure 9. Structural formulas of cromolyn, khellin, methyl salicylate, acetylcholine, and succinylcholine chloride.

1985). It rapidly came into wide use and later provided the model for similarly acting synthetic compounds, as well as those acting by depolarizing the muscle end plate so as to prevent it from responding to acetylcholine, e.g., succinylcholine (Fig. 9). Even with the production of these synthetics, tubocurarine and its semisynthetics enjoyed great popularity until the 1970s; but with the recent syntheses of vecuronium and atracurium (Fig. 10) (Stenlake et al., 1983), these efficient neuromuscular blocking agents, together with succinylcholine, have become drugs of choice of most anesthetists. Few now use the natural product a half century after its introduction, but tubocurarine remains an important example of a plant product used by indigenous peoples whose introduction revolutionized two fields of Western medicine, anesthesia and surgery.

### Antidiarrheal Preparations

Besides using the tropane alkaloids obtained from members of the Solanaceae, other important plant producers in this category are pectin from *Citrus limon* (L.) Burm.f. (Rutaceae), lemon peel being the source of about one-half of the pectin made in the U.S., and *Malus 'domestica'* Borkh. or the common apple. Opiate alkaloids as paregoric, usually a tincture in combination with tropane alkaloids or pectin, are also commonly prescribed. All have a long history of use in domestic medicine for the symptomatic treatment of diarrhea.

### Antineoplastics

Many anticancer preparations include vincristine or vinblastine (Fig. 10) extracted from leaves of the Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don (Apocynaceae). Remission rate and cure, especially using combination chemotherapy, can be spectacular for treating gestational choriocarcinoma tumors, Burkitt's lymphoma, testicular tumors, Wilm's tumor, acute lymphocytic leukemia, and Hodgkin's disease (Lewis, 1982). Etoposide (Fig. 11), a semisynthetic of

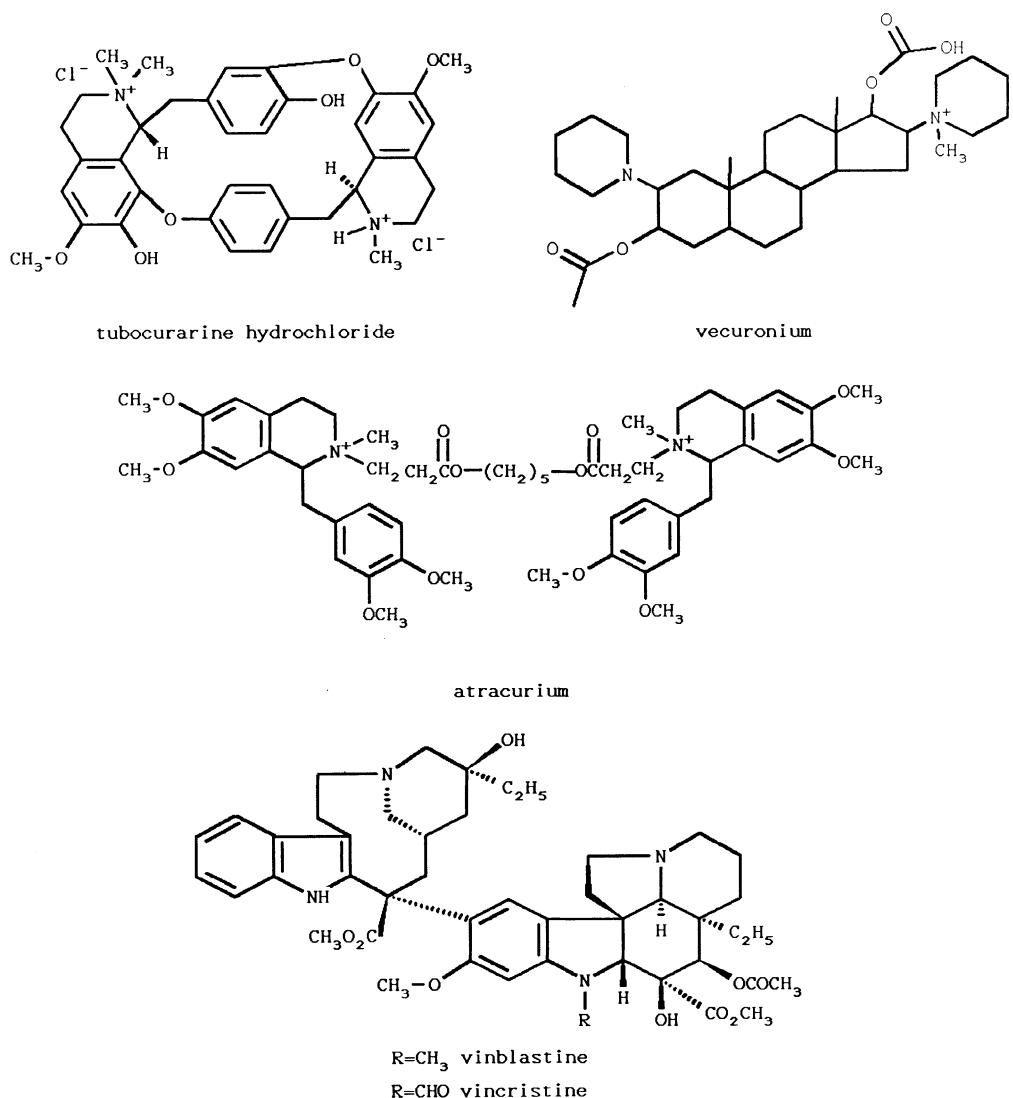


Figure 10. Structural formulas of tubocurarine, vecuronium, atracurium, vinblastine, and vincristine.

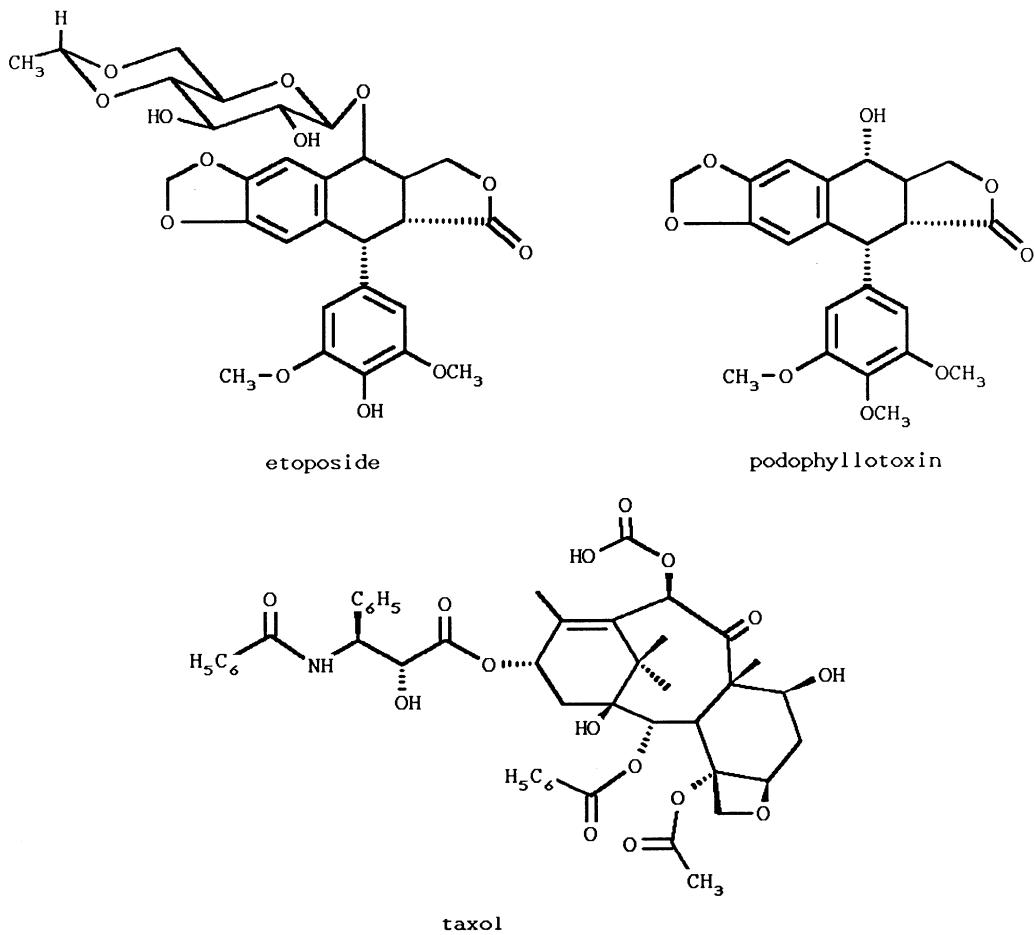
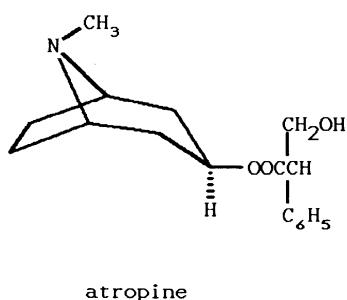


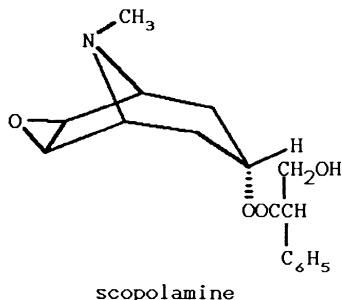
Figure 11. Structural formulas of etoposide, podophyllotoxin, and taxol.

podophyllotoxin (Fig. 11) extracted from *Podophyllum peltatum* L. (Berberidaceae) roots, has recently been introduced to treat testicular and small cell lung cancers. Other drugs of plant origin are in various phases of clinical trials as, for example, the diterpene taxol (Fig. 11) extracted from *Taxus brevifolia* Nutt. (Taxaceae) bark and foliage for treating ovarian and breast cancers.

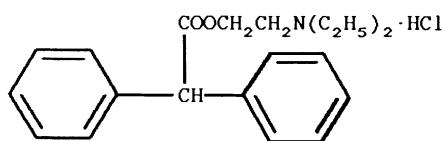
The diagnosis of many forms of cancer by indigenous peoples is impossible, so traditional cures of cultured populations are often limited. Nevertheless, Hartwell (1967-1971) compiled an impressive list of plants used by often accultured peoples to treat neoplasms, though few have proved efficacious in the screening program used by the National Cancer Institute. In fact, the discovery of the value of periwinkle was quite serendipitous, for its chief use in traditional medicine had been as an antidiabetic (which proved valueless), though perhaps without this traditional use, the plant may never have been brought into the laboratory for screening (Lewis and Elvin-Lewis, 1977).



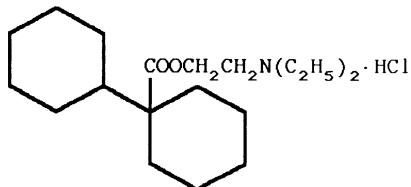
atropine



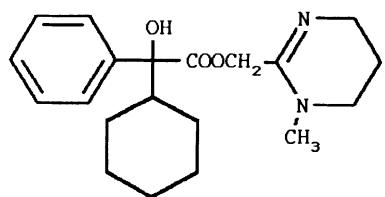
scopolamine



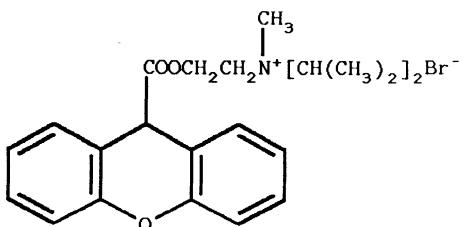
adiphenine hydrochloride



dicyclomine hydrochloride



oxyphencyclimine



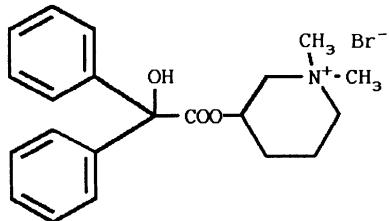
propantheline bromide

Figure 12. Structural formulas of atropine, scopolamine, adiphenine, dicyclomine, oxyphencyclimine, and propantheline.

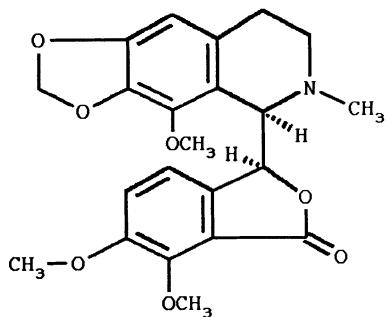
### Antispasmodics

Tropane alkaloids are commonly found in antispasmodic preparations. Although atropine (Fig. 12) is synthesized, most of the commercial alkaloid and also scopolamine (Fig. 12) are obtained from *Duboisia* spp. (Solanaceae) native to Australia. Alkaloid mixtures and tinctures involving other sources in the family, such as *Atropa* and *Hyoscyamus*, are also prescribed.

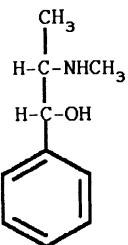
The Aborigines of Australia used *Duboisia myoporoides* R.Br. as a narcotic. Holes were made in the trunk of trees and filled with water which, when drunk the following day, produced stupor. Some people experienced giddiness and nausea when staying in closed rooms containing branches of the species (Lassak and McCarthy, 1983). In 16th and 17th century Europe, *Atropa belladonna* L., *Datura metel* L., *Hyoscyamus niger* L., and *Mandragora officinarum* L. were used as narcotics/sedatives to cause sleep and offset pain.



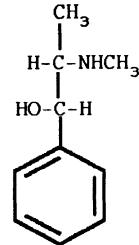
mepenzolate bromide



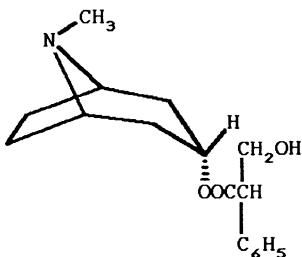
noscapine



D(-)-ephedrine (*1R,2S*)



L(+)-pseudoephedrine (*1S,2S*)



hyoscyamine

Figure 13. Structural formulas of mepenzolate, noscapine, ephedrine, pseudoephedrine, and hyoscyamine.

Synthetic antispasmodics are mostly structurally related to major tropane bases and include adiphenine, dicyclomine, oxyphencyclamine, propantheline (Fig. 12), and mepenzolate (Fig. 13).

### **Antitussives and Decongestants**

These therapeutic categories include narcotic, antihistamine, and antitussive preparations, oral decongestants, and products used for treating the common cold. Major plant products include codeine (Fig. 6) and noscapine (Fig. 13) from *Papaver somniferum* L., ephedrine and pseudoephedrine (Fig. 13) from *Ephedra* spp. (Ephedraceae), ipecac extract from *Cephaelis ipecacuanha* (Brot.) A.Rich. (Rubiaceae), and mixtures of atropine (Fig. 12), scopolamine (Fig. 12), and hyoscyamine (Fig. 13). Minor products in this category are opium, morphine derivates, papaverine (Fig. 18), wild cherry bark, and cephaeline (Fig. 14).

These plants all possess long histories of traditional medical use, as discussed for *Papaver* and *Ephedra*. Plants producing tropane alkaloids, e.g., *Hyoscyamus albus* L. (Solanaceae) seeds mixed with water and honey to treat coughs, and juice of the roots of *Mandragora autumnalis* Bertol. and *M. officinarum* L. (Solanaceae) "taken in small quantities purgeth the belly exceedingly from phlegm," were used in Europe prior to 1597 (Gerard, 1597, 1633).

### **Bronchodilators**

Ephedrine (Fig. 13) from *Ephedra* spp. (Ephedraceae) is the major product of this category, being present in 42% of all such prescriptions (Farnsworth, 1973). Theophylline (Fig. 14), from synthetic sources but modeled from that found in tea, *Camellia sinensis* (L.) Kuntze (Theaceae), and theobromine (Fig. 14) from *Theobroma cacao* L. (Sterculiaceae) have minor uses as bronchodilators.

The long prehistoric and historic use of *Ephedra* to help treat colds and coughs has been discussed. Tea has a long history in the Orient as a stimulating beverage.

### **Cardiovascular Drugs**

The fourth most frequently prescribed therapeutic category is cardiovascular. For treating hypotension, reserpine (Fig. 14) and its derivates, alone or in combination, are present in 61% of products prescribed, mostly obtained from roots of *Rauvolfia serpentina* (L.) Benth. ex Kurz and secondarily from *R. canescens* L. and *R. tetraphylla* L. This adrenergic antagonist, together with the more recently introduced chlorpromazine (Fig. 14), opened the entire field of psychopharmacology, for, heretofore, there had been no effective therapeutic agent available for the treatment of major mental diseases, especially schizophrenia (Lewis and Elvin-Lewis, 1983). A vast improvement in the management of many psychotic patients became possible because of central tranquilizing effects of reserpine, and even though this first tranquilizer introduced to modern medicine in 1952 is not used today for this purpose, its synthetic models are.

For centuries, *Rauvolfia* had been used in India for its calming effect. Holy men chewed it while meditating, village medicine men employed it to treat highly agitated mental patients, and it was even used to calm fretful babies. But all of this was ignored by Western medicine, even though in recent times such personages as Mahatma Gandhi sipped a tea brewed from its leaves or roots whenever he needed to induce a state of philosophical detachment or succeed in a hunger strike and, thus, achieve his desired tranquility and lowered metabolic rate.

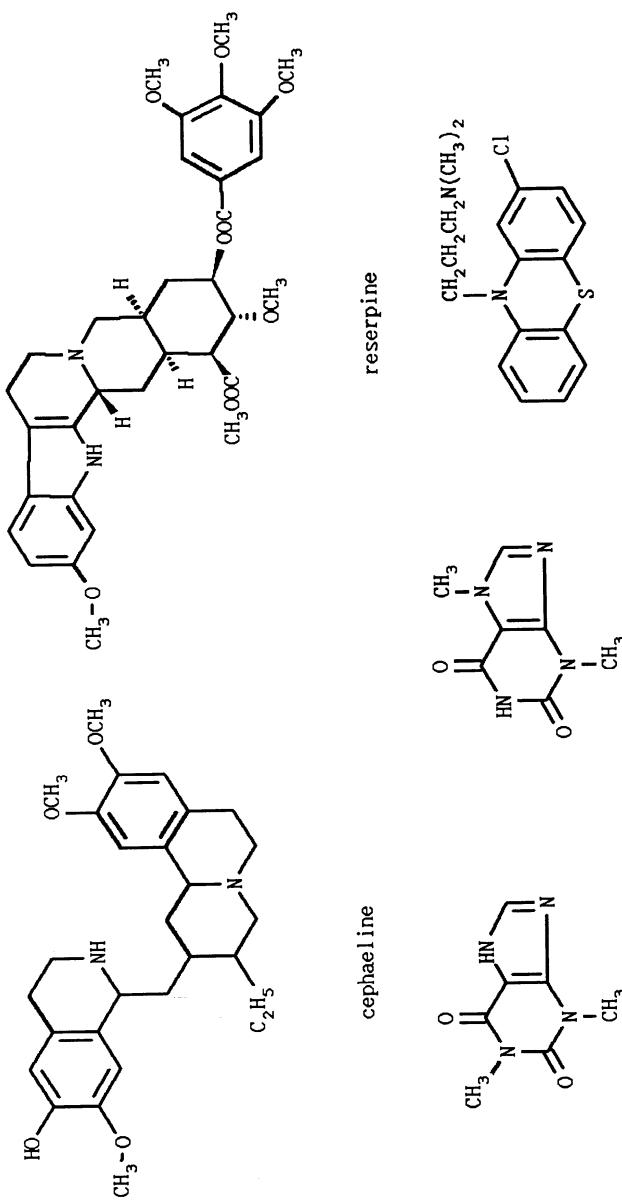


Figure 14. Structural formulas of cephaeline, reserpine, theobromine, theophylline, and chlorpromazine.

Dried rhizomes and roots of *Veratrum album* L. and *V. viride* Ait. (Liliaceae) also yield alkaloids for use as hypotensives. Protoveratrine A and B (Fig. 15), for example, are the active alkaloids of *V. album*. Considered a strong medicine in traditional use, even so it could be more safely "given unto Country people which feed grossly, and have hard, tough, and strong bodies" to treat the falling sickness, phrenesies, and dropsies in medieval Europe (Gerard, 1597, 1633).

Papaverine, originally isolated from *Papaver somniferum* L. (Papaveraceae), though now synthesized, is occasionally used as papaverine hydrochloride, sometimes in combination with codeine, to affect coronary smooth muscle relaxation. It occurs naturally in opium, up to 1%, and thus gives the same results as the more concentrated morphine and codeine when used in traditional medicine.

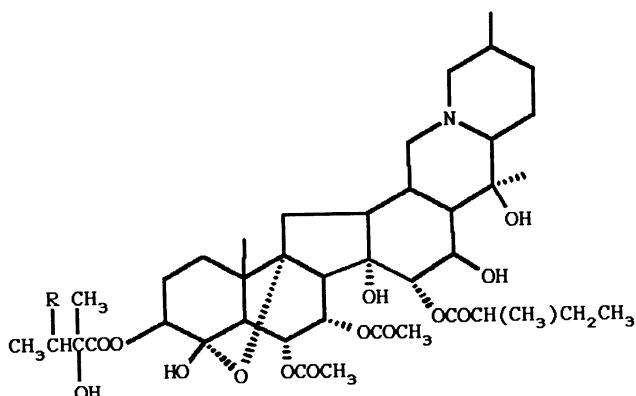
The most widely used cardiovascular drug is the *Digitalis lanata* Ehrh. (Scrophulariaceae) glycoside digoxin (Fig. 15), though lanatoside A-C (Fig. 16), digitoxin (Fig. 15), gitalin (amorphans glycoside mixture), and whole leaf of *D. purpurea* L. are often prescribed to treat congestive heart failure, atrial flutter, and fibrillation. They act by increasing cardiac output and relieving pulmonary congestion and peripheral edema. These foxglove species are indigenous to Europe and were used for centuries in a recipe of about 20 herbs to treat edema. William Withering who selected *Digitalis* as the probable one affecting heart and circulation published his monumental account describing the diuretic effect of *Digitalis*, its relief of edema, and associating it with a remarkable power over the heart (Withering, 1785). This is one of the most striking examples of an English folk remedy being thoroughly studied in the 18th century and remaining in many ways unaltered to the present.

In Africa, seed extracts of *Strophanthus gratus* Franch. (Apocynaceae), *S. kombe* Oliver, and other species were traditionally and effectively used as arrow-poisons. Their stimulating action on the heart was thus well known to indigenous African tribes, information which served as the basis for introducing the additional fast-acting cardioactive glycosides ouabain (Fig. 17) and its aglycone G-strophanthin into Western medicine. These drugs are suitable only for intravenous because of poor absorption from the gastrointestinal tract, suggesting that the traditional arrow-poison use of injecting crude extract directly into a wound, and hence into circulation and the heart, would be a highly effective weapon.

Quinidine as a sulfate and gluconate (Fig. 18) are cardiac depressants used particularly to inhibit auricular fibrillation. They are extracted from the bark of several *Cinchona* spp. (Rubiaceae) and also the closely allied (sometimes congeneric) *Remijia*, particularly *R. pedunculata* Fleck. and *R. purdieana* Wedd. Observations of lowered antiarrhythmias among persons routinely taking bark extracts of *Cinchona* led to the use of quinidine, but there are no known data regarding its similar use in traditional medicine.

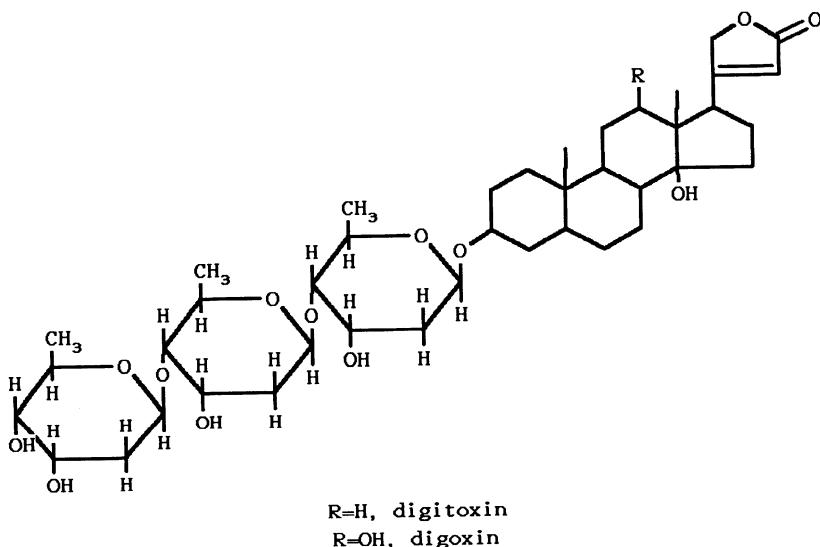
### Dermatological Preparations

A small number of miscellaneous compounds and extracts of natural and semisynthetic origin are used in dermatology, including opium, hydrocortisone (Fig. 18), ergot extract, benzoin (Fig. 18), oatmeal, theobroma oil, hamamelis extract, and chamomile extract. However, the topical treatment of dermatological diseases has been revolutionized by the introduction of the anti-inflammatory corticosteroids, as indeed has the use of many synthetic analogs for internal use as well (e.g., prednisone and prednisolone (Fig. 18)). Vitamin A synthetics, such as etretinate for treatment of psoriasis, and tretinoin and isotretinoin (Fig. 18) as anti-acne drugs, are modeled from the vitamin.



R=H, protoveratrine A

R=OH, protoveratrine B



R=H, digitoxin

R=OH, digoxin

Figure 15. Structural formulas of protoveratrine A and B, digitoxin, and digoxin.

## Enzymes

Bromelain extracted from the pineapple, *Ananas comosus* (L.) Merr. (Bromeliaceae), and papain from the papaya, *Carica papaya* L. (Caricaceae), are protein digestants having actions similar to that of pepsin. They may be used to relieve the symptoms of episiotomy and also in tenderizing meats. In traditional medicine, they are widely employed as anthelmintics.

A second protease, chymopapain, obtained from the unripe fruit of papaya is injected into intervertebral discs of patients having herniated disc syndrome and liquifies that part of the disc pinching the spinal nerve, thus, relieving lower back pain in 77% of patients (Ford, 1990). It was approved for use in the U.S. in 1982, but it had been widely used in Canada and Europe prior to this approval. There is no evidence for such a use in traditional medicine.

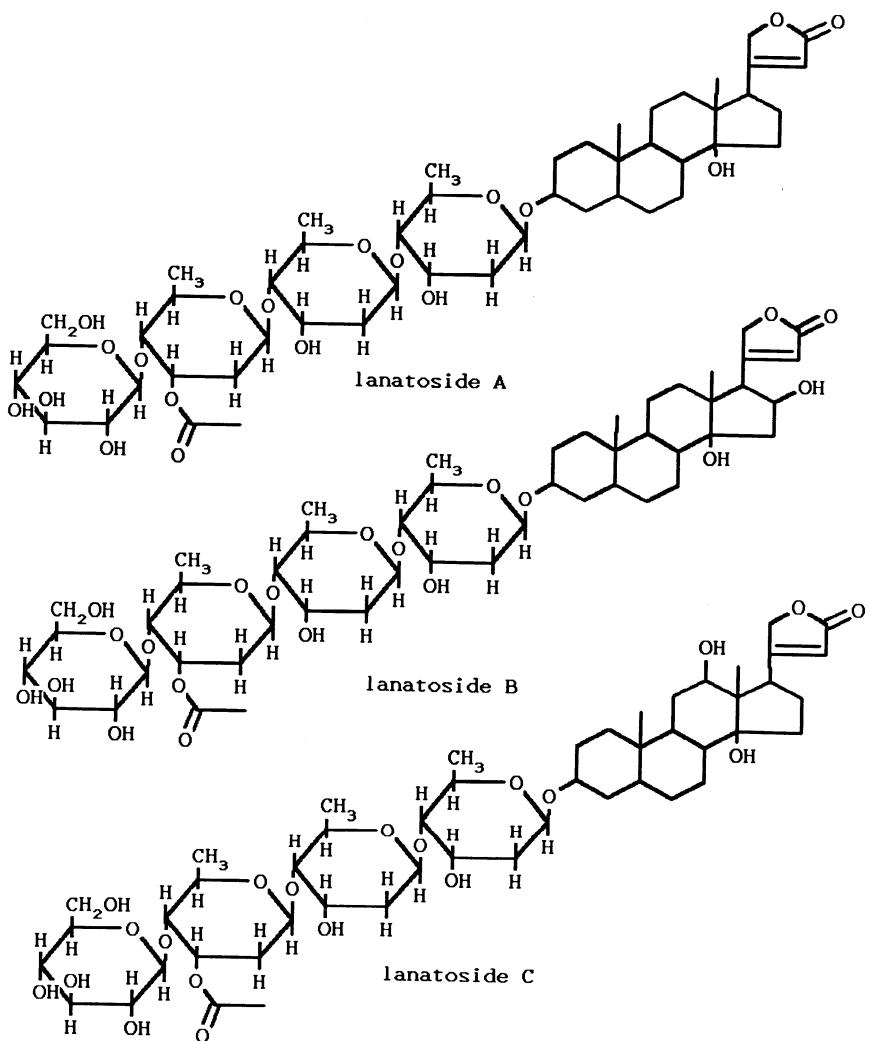


Figure 16. Structural formulas of lanatosides A, B, and C.

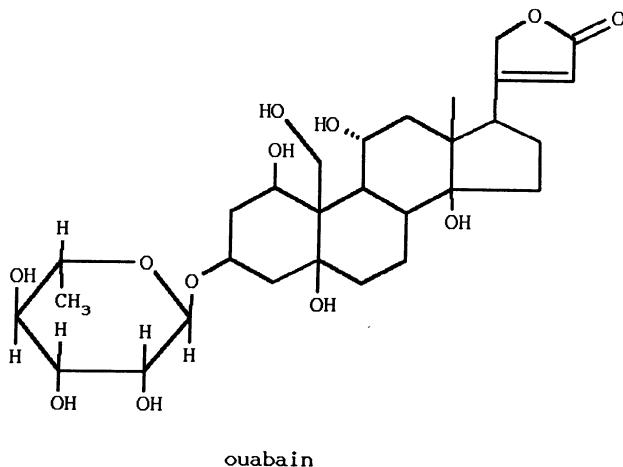


Figure 17. Structural formula of ouabain.

## Gout

Colchicine (Fig. 7) obtained from *Colchicum autumnale* L. (Liliaceae) is specific in the diagnosis and treatment of gout, and its historic use in Europe as a traditional medicine and introduction to Western medicine to treat this metabolic disorder have been discussed.

## Hemorrhoidal Preparations

A mixture of belladonna extract and opium, various preparations containing astringents, and particularly the semisynthetic hydrocortisone (Fig. 18) are all used to treat hemorrhoids. The use of astringents has a long historical application in domestic medicine, e.g., Gerard (1597, 1633) noted that pounded seeds of *Rhus cortaria* L. (Anacardiaceae) "mixed with honie and the powder of Oken coles, healeth the Hemorrhoides." Both sumac and oak are common sources of astringent tannins.

## Hemostatics

Citrus bioflavonoids are used as hemostats, as well as vitamin Ks (Fig. 19) available synthetically.

## Hormones

The second largest therapeutic category, after microbial-derived antibiotics which are not included in this review, is the hormones. These are almost all prepared by conversion from plant-derived steroidal saponins, particularly diosgenin (Fig. 19) from several *Dioscorea* spp. of Mexico and elsewhere. These roots are rarely used in traditional medicine and then not for affecting birth control nor as topical preparations as extract derivatives are used in modern medicine.

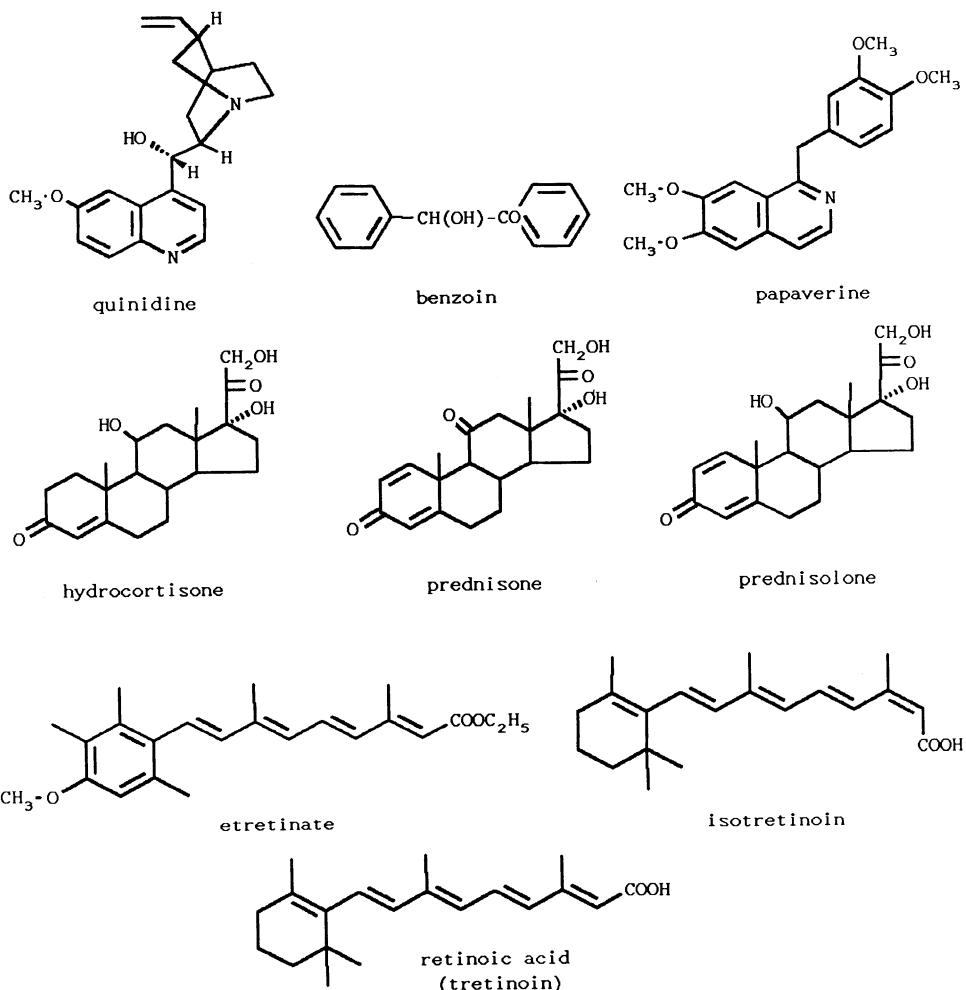


Figure 18. Structural formulas of quinidine, benzoin, papaverine, hydrocortisone, prednisone, prednisolone, etretinate, isotretinoin, and retinoic acid (tretinoin).

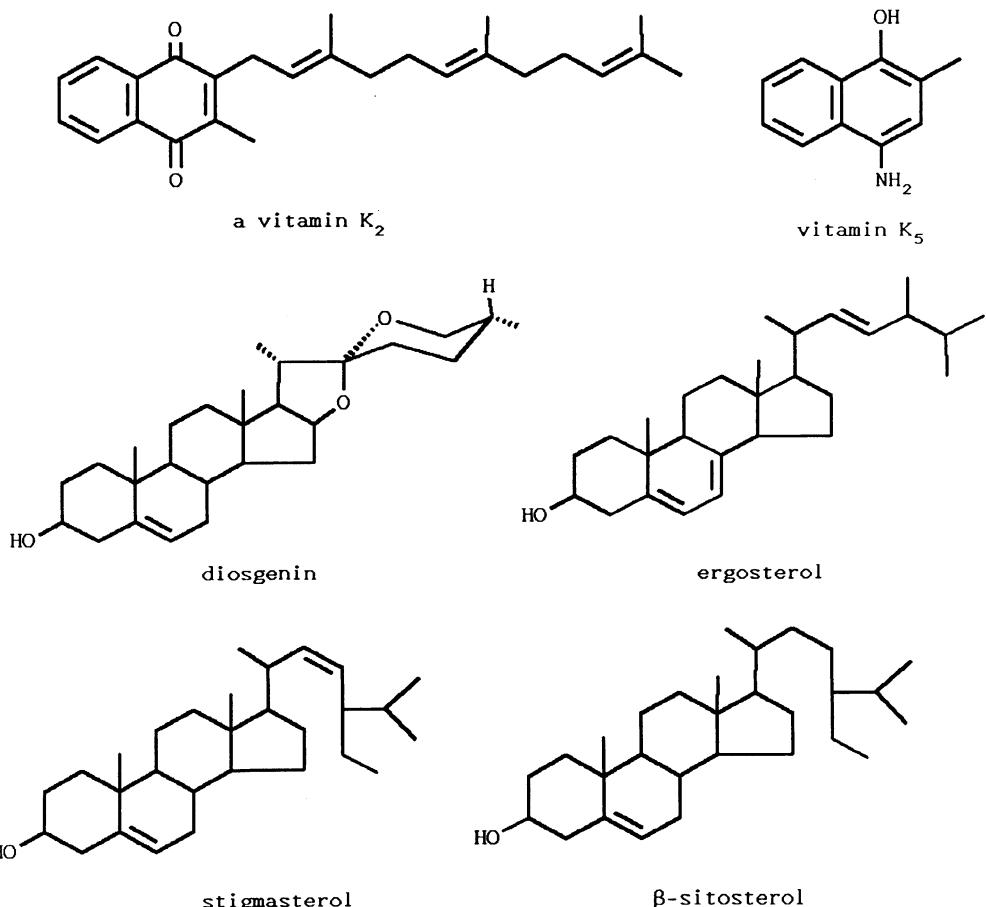


Figure 19. Structural formulas of vitamin K, diosgenin, ergosterol, stigmasterol, and  $\beta$ -sitosterol.

### Hypocholesteremics

Used in the treatment of atherosclerosis to reduce cholesterol absorption, ergosterol from fungi, stigmasterol from calabar beans (*Physostigma venenosum* Balf., Fabaceae) and soybean oil (*Glycine max* (L.) Merr., Fabaceae), and the ubiquitous  $\beta$ -sitosterol obtained from wheat germ oil, rye germ oil, corn oil, cottonseed oil, and other seed oils may be of value (Fig. 19). Derivations of some of these are now being marketed.

### Laxatives

Laxatives may be divided into groups based on their mode of action. Plant products are widely used in two groupings of OTC preparations: as stimulant cathartics—cascara sagrada (sacred bark) from *Rhamnus purshiana* DC. (Rhamnaceae), senna from *Cassia* spp. (Fabaceae), castor oil from *Ricinus communis* L., aloin (Fig. 20) from *Aloe vera* and others, and rhein anthrones (Fig. 20) from rhubarb, *Rheum emodi* Wall. and *R. officinale* Baill. (Polygonaceae), and as bulk-forming

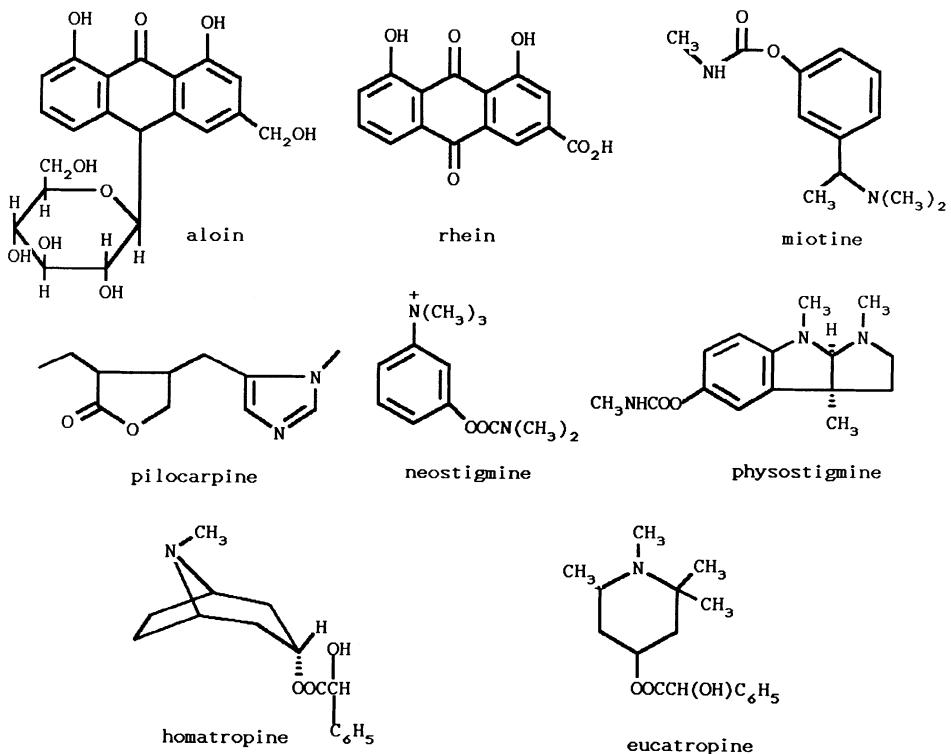


Figure 20. Structural formulas of aloin, rhein, miotine, pilocarpine, neostigmine, physostigmine, homatropine, and eucatropine.

cathartics—represented by psyllium seeds from *Plantago afra* L., *P. indica* L., and *P. ovata* Forsk. All are widely used without prescription, and stimulant cathartics in particular have a long history of use in traditional medicine throughout the world.

### Miotics

Pilocarpine (Fig. 20) from *Pilocarpus jaborandi* Holmes and *P. pinnatifolius* Lem. (Rutaceae), in particular, is frequently employed to mimic the action of acetylcholine on the eye and to decrease the intraocular pressure in glaucoma. Farnsworth (1973) found that as many as 75.4% of prescriptions in this category contained pilocarpine. Fewer than 1% contained physostigmine (Fig. 20), an anticholinesterase alkaloid extracted from the seeds of *Physostigma venenosum* Balf. (Fabaceae). Derivatives, such as miotine and neostigmine (Fig. 20), are more widely used miotic agents. Effects of *Pilocarpus* were observed in Brazil in the 19th century with the excessive salivation of Indians chewing leaves. Leaf preparations were then used to induce profuse sweating (diaphoresis).

Trees of *Pilocarpus* species have become very rare in parts of the Brazilian rain forest where they are cut for leaves as the source of pilocarpine. There are no commercial plantations of these endangered species.

## **Mydriatics**

Atropine (Fig. 12), or the preferred derivates homatropine and eucatropine (Fig. 20) because of their more rapid mydriatic action, and scopolamine (Fig. 12) represent almost all compounds used in the class.

A search for the drug of choice to dilate pupils was short, for Linnaeus' specific epithet of the European *Atropa belladonna* L. (Solanaceae) originated from *bella* = beautiful and *donna* = lady. Large eyes (pupils) were considered beautiful among Renaissance courts, so extracts from the plant were dropped on ladies' pupils to make them more attractive, if temporarily blind (Mattioli, 1579).

## **Oxytocics**

To stimulate the motility of the uterus and thus induce labor, the drug of choice is oxytocin of animal origin. But for use postpartum to firm uterine contractions and to decrease postpartum uterine bleeding, the ergot alkaloids ergonovine (Fig. 21) and the semisynthetic methylergonovine (Fig. 21) from the fungus *Claviceps purpurea* Tul. (Clavicipitaceae) are preferred over oxytocin because they combine low toxicity, rapid onset, and sustained duration of action. In addition, sparteine (Fig. 21) from *Cytisus scoparius* (L.) Link (Fabaceae) is occasionally used.

During the Dark and Middle Ages, and undoubtedly earlier, thousands died in Europe after eating bread made from rye contaminated with ergot. Known as St. Anthony's Fire, outbreaks often reached epidemic proportions in France and Germany with death ensuing after gangrene of the limbs had set in. Such epidemics have only been sporadic since the cause of the affliction became generally appreciated during the 17th century. Lonicer (1582) in his *Kreuterbüch* noted that ergot was used by midwives to hasten labor, and the dose he recommended is consistent with contemporary usage (Sneader, 1985). By early in the 19th century, orthodox practitioners were paying heed to the therapeutic potential of ergot in obstetrics.

## **Parasiticidal Agents**

Antimalarials, amoebicides, antitrypanosomals, and anthelmintics are included in this group. These are significant in Western medicine, not because of *in situ* concerns, but because travelling to the tropics has become more common by those living in temperate zones, thereby increasing exposure rates. The most important concern in Western medicine, however, relates to the antimalarials. Virtually all products used consist of natural quinine (Fig. 21) from *Cinchona* spp. (Rubiaceae), involving up to 1,000 tons consumed per year (Table 3), and its synthetic models quinacrine, amodiaquine (Fig. 21), chloroquine, hydroxychloroquine, and primaquine (Fig. 22). Some strains of malarial parasites are becoming increasingly resistant to the synthetic antimalarials, yet often can be controlled by the natural product quinine. Nevertheless, new chemical nuclei must be incorporated into Western medicines' therapeutic armament if malaria is to be fully controlled.

Malaria is still the most prevalent tropical disease in the world, its incidence having changed little during the past 15 years. Transmissions still occur in 102 countries or areas, mostly tropical, where 2.7 billion people live. In 1985, 4.8 million cases of malaria were reported to the WHO, but this figure is incomplete, especially from countries of the subSahara Africa, so this incidence is an underestimate of the true situation. The frequency of malaria-related mortality is virtually unknown, but an estimate in the early 1970s placed the figure at 1 million deaths in subSahara Africa alone.

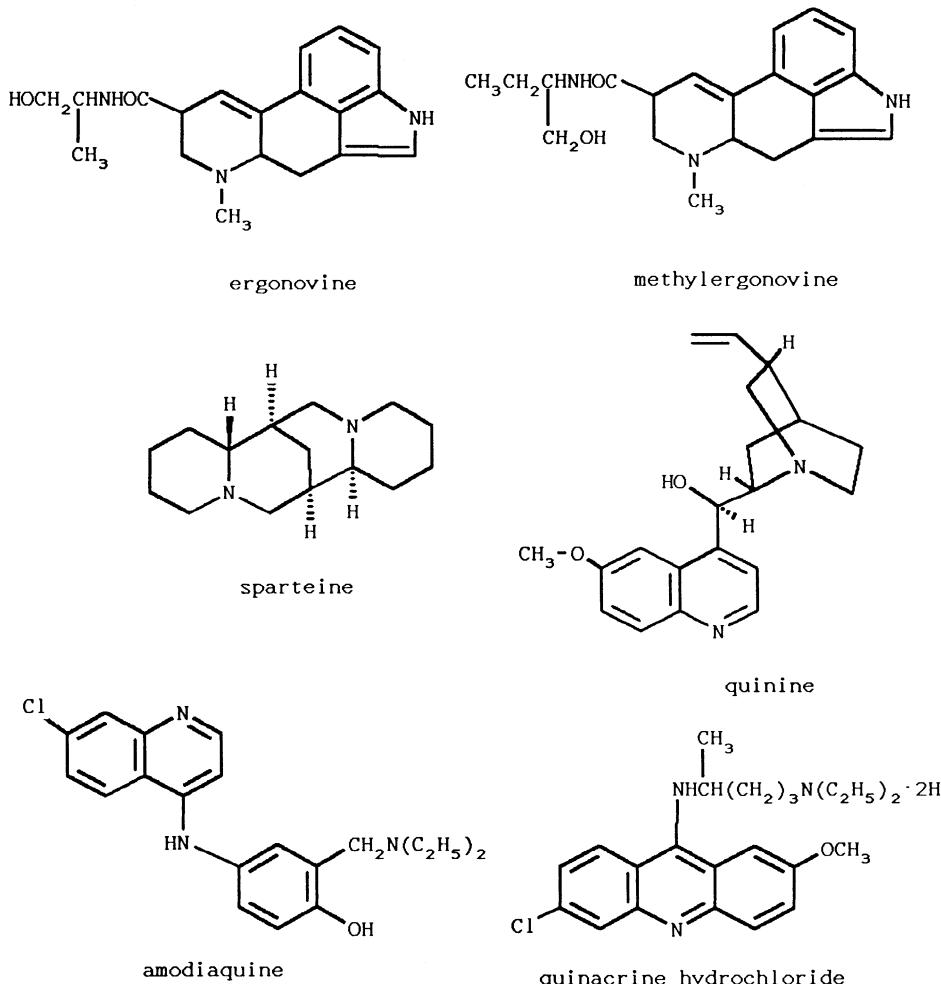


Figure 21. Structural formulas of ergonovine, methylergonovine, sparteine, quinine, amodiaquine, and quinacrine.

The earliest report of the use of extracts of *qinghao*, of which *huanghuahao* was considered a variety, was in the *Prescriptions for 32 Kinds of Diseases* found in the Mawangdui Han Dynasty Tomb (168 BC) and recommended to treat hemorrhoids. Treatment for fever, including malaria, was first recorded in the *Zhou Hou Bei Ji Fang* by Ge Hong (340 AD) and later by Li Shih-Chen (1596). These plants of ancient Chinese traditional medicine consist of two species, *qinghao* being *Artemisia apiacea* and *huanghuahao*, *A. annua*, although only the latter contains appreciable amounts of the active antimalarial principle artemisinin (Fig. 22).

Artemisinin is found in the leaves and flowering tops of the plant, not the roots. Yields in China range from 0.01% to 0.5% (w/w), with genotypes growing in Sichuan apparently yielding the highest amounts. An adventive population of the Eurasian *A. annua* in Washington, D.C. yielded 0.06% (w/w) (Klayman et al., 1984). Artemisinin is a sesquiterpene lactone whose peroxide linkage is essential for antimalarial activity. It has been synthesized by several methods, but such syntheses

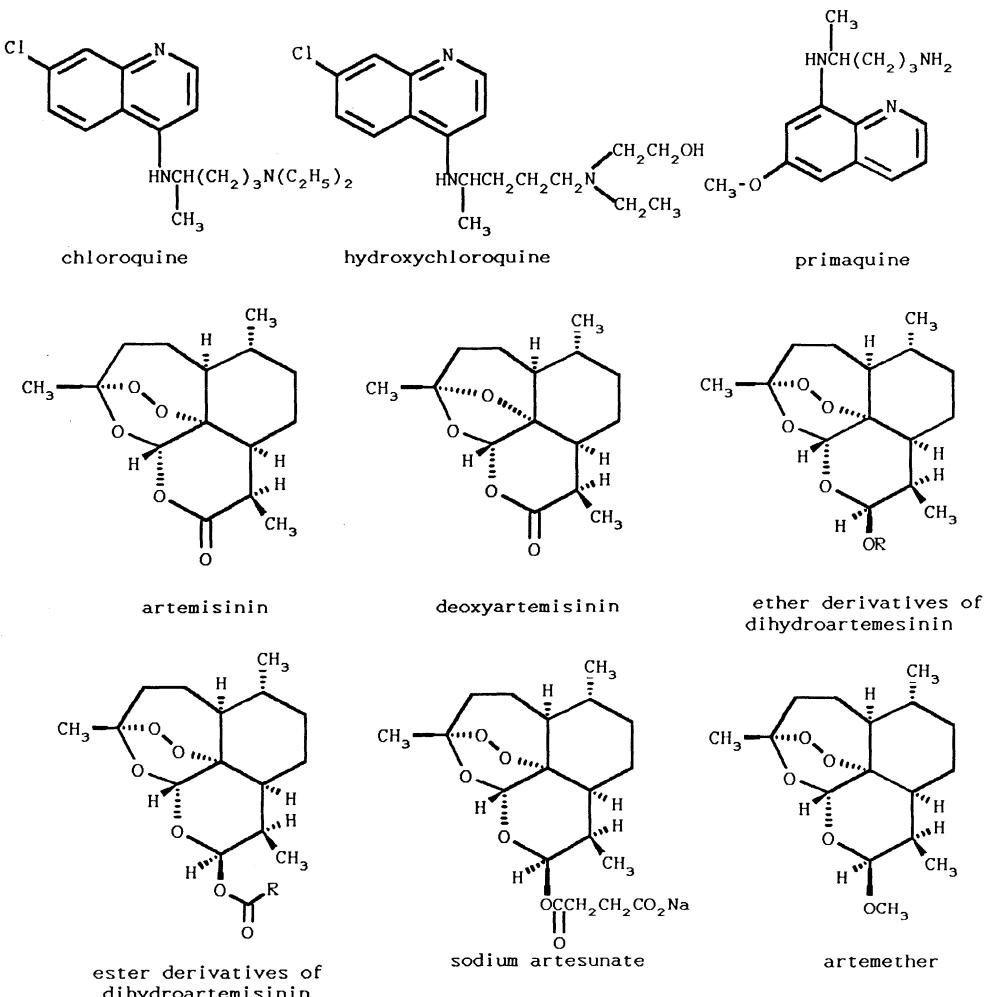


Figure 22. Structural formulas of chloroquine, hydroxychloroquine, primaquine, artemisinin, deoxyartemisinin, ether and ester derivatives of dihydroartemisinin, artesunate, and artemether.

may not be economical for the large scale production of artemisinin needed. Reduction of the metabolite to deoxyartemisinin (Fig. 22) lacking the peroxide bridge is devoid of antimalarial activity, whereas reduction to dihydroartemisinin (Fig. 22) does show antimalarial properties even more active than artemisinin itself. Synthetic derivatives of dihydroartemisinin, however, showed the presence of the peroxide bridge correlated with antimalarial activity. Other derivatives of artemisinin have even greater antimalarial efficacy, such as sodium artesunate (Fig. 22) and artemether (Fig. 22), yet most are more unstable than the parent compound (Trigg, 1989).

The first major trial was conducted in southern China during 1973 to 1978. Artemisinin was used to treat 2,099 malaria patients, of whom 588 were infected with *Plasmodium falciparum* and 1,511 with *P. vivax*, using various formulations (e.g., tablets orally, total of 0.5 to 0.9 g). All patients were clinically cured with the time

Table 3. A selection of medicinal plants, supplying countries, and annual consumption throughout the world (Modified from Sivarajan, 1988).

Species	Suppliers	Annual consumption (metric tons)
<i>Cassia angustifolia</i> Vahl	S. India, Sudan, Egypt	5,000-6,000
<i>Cinchona officinalis</i> L.	India, Africa, S. America	500-1,000
<i>Digitalis lanata</i> Ehrh.	Europe, India	1,000
<i>Dioscorea floribunda</i> M. Martens & Galeotti	Guatemala, China, India	800
<i>Glycyrrhiza glabra</i> L.	Europe, W. Asia	500 (Europe alone)
<i>Panax ginseng</i> C. Meyer	China, USSR	2,000
<i>P. quinquefolium</i> L.	USA	1,163 <sup>a</sup>
<i>Plantago ovata</i> Forssk.	India	15,000
<i>Rauvolfia serpentina</i> (L.) Kurz	Thailand, India, Sri Lanka, Burma	11

<sup>a</sup>U.S. supply in 1989 (Foster, 1991). Figure for Canada not known.

required for a decline in fever, 24 to 46 hours in falciparum patients and 20 to 30 hours in vivax patients. Clearance of *P. falciparum* parasites took 26 to 35 hours, and that of *P. vivax* parasites 24 to 40 hours.

Important for the enrichment of modern pharmacopeias is the discovery of novel structures and modes of action, and the artemisinin series of compounds fulfils these criteria. Such an addition is relevant today when resistance to the 4-aminoquinolines, particularly chloroquine, has spread to most of the major endemic areas of the world and has now been detected in over 60 countries. As the number of effective drugs for the treatment of malarial infections is small, the need for new antimalarial drugs is obvious.

#### TRADITIONAL USES OF PLANTS BY AN INDIGENOUS PEOPLE, THE JÍVARO

We\* chose to study a tribe in South America known as the Jívaro with a culture still largely intact. Theirs is an oral tradition in which skills are learned by the young from older members of the community who have become noteworthy for performing certain tasks. Existence and survival of the Jívaro, therefore, depend on an understanding of uses of plants and animals in their ecosystem for food, clothing, shelter, fuel, medicines, and for many other purposes as well.

The inventory field research phase (1982 to 1988) is essentially complete during which time over 5,500 specimens representing about 1,000 species used in various ways were collected. A hallmark of the study was to collect plants from several tribal groups (Achuar, Huambisa, Mayna, and Shuar), a nearby Candoshi tribe, and Mestizo populations in more or less accultured communities beyond the Jívaro heartland. By so doing, we obtained an "epidemiological" sample of medicinal uses broad enough

\*Field research was conducted with Dr. Memory P. Elvin-Lewis, Daniel Fast, and Maurizio Gnerre whose collaboration is greatly appreciated.

to indicate what species are most widely used and considered of greatest value by the largest number of persons among similar and different groups. From these selections, research is being conducted in search of novel compounds for potential uses but also to show the significance of Jívaroan plant medicines as a whole in treating diseases and infections recognized by them and in their preventative medicine. The role of empirical methodology relative to medical ethnobotany can then be judged for a major people who survive today as they did years ago, and as humans once did worldwide, by utilizing plants selected by trial and error for particular purposes.

What do Jívaros recognize as disease states or other conditions needing treatment? Have they selected plants with efficacious principles? The first can be answered by using some examples from the field and the second by preliminary data now giving clues to an indigenous peoples' prowess in medicine.

The Jívaro recognize a broad spectrum of medically significant features, and they utilize plants to treat these conditions. Plants are usually recognized by a unique name for rapid and efficient recall (Lewis et al., 1988). If more than one plant is used for treatment, these are usually ranked from the best to the least preferred, depending on the severity of a condition and plant availability.

They recognize plants for: treating broken bones, wounds and cuts, tumors, bleeding, snake and insect bites, headache, conjunctivitis, hemorrhoids, pain, toothache, earache, body aches, arthritis/rheumatism, inflammation and swelling, itching, and diarrhea; treating infections, such as malaria, leishmaniasis, hepatitis, and bacterial and fungal skin problems; treating infestations, like worms, lice, and mites; preventing plaque formation and caries, and pregnancies; extracting teeth; stimulating emesis; having psychoactive effects using stimulants, depressants (beer), and hallucinogens; tonics to enhance well-being, as aphrodisiacs, to improve circulation, and children's walking ability. They also recognize a wide range of dangerous plants that even in small amounts can be seriously toxic.

In a Jívaro village, how many plants are used over a period of time? As mentioned above, the number of species used in the region approximates 1,000; but only a third of these are preferred and sought with any degree of regularity in a single community.

The main source of nutrition is cassava (*Manihot esculenta* Crantz), mostly drunk as a masticated beer of low alcoholic content (perhaps 3%) which is consumed all day long. An adult male drinks an average of 7.57 L (2 gallons) daily. There is one main meal a day for adults consisting of baked or boiled vegetables such as taro, plantain, cassava with the root bark removed, and yams; fresh fruits like pineapple, banana, and custard apple; and supplemented when available by baked fish in leaf wrap, grilled/smoked game, or roasted birds. Traditionally domestic animals are absent but chickens are now raised as a cash crop and occasionally eaten. Protein of animal origin is thus limited and oil ingestion is low. No food is fried. Snacking occurs throughout the day, mostly on fresh fruits, seeds, and grubs as found when traveling in the rain forest or working in and around the villages.

Traditional male clothing consists of cotton weaved from fibers of the indigenous species; but even among the Jívaro, such clothing has largely disappeared. In only remote villages can one find the *itip*, a wraparound skirt worn by men as their only garment. The cotton thread may be dyed from extracts of crushed leaves and roots, often mordanted to remain fast. Headbands, also weaved from cotton, coronets, and other regalia (often adorned with toucan feathers), purses, beads, ear decorations, and so forth are all made from parts of the palm, grass, sedge, and cotton families, in particular.

To construct huts, trees are selected for their strength and durability and for antipest properties. Most huts include struts, sidings, bed stands, flooring, palm leaf roofing, and binding cord; and all are made from plants. Many plant families provide

these materials, although those from the palm family supply the roof, siding, and floor if used, and often the bed slats. Furniture is limited, usually to a stool. Firewood is highly selected, consisting of slow-burning logs as the basic component of the fire, with fast-burning and resinous woods used as kindling and for hot, fast fires.

Blowguns and darts are made from palms with dart tips painted with curare extracts. Using this reversible muscle relaxant as an aid in hunting has proved extremely efficient, an ingenious method of incapacitating treed mammals and birds. All potentially poisonous alkaloids are metabolized by the animal and what may remain is destroyed by cooking.

As with hunting, fishing is distinctive. Leaves and stems of *Clibadium* (Asteraceae) are crushed and swirled in a basket within a stream. A potent neurotoxin is released which stuns and disorients the fish, and as they swim erratically on or near the surface, they can be easily plucked from the water.

Transportation is supplied by dug-out canoes fashioned from trunks of particular trees or occasionally rafts. There are no animals of burden or wheels, so there are no carts.

Utensils include baskets weaved from largely aerial roots and gourds from various fruits which serve for drinking and sometimes eating. Clay pots are made and decorated using plant dyes.

What of plants used for health and well-being? Most cultures begin the day by drinking a stimulant, and the Jívaro are no exception. Before dawn, men drink large quantities, usually over 2 L (2.1 quarts) of *wayus*, a decoction prepared from leaves of the native holly *Ilex guayusa* Loes. These leaves contain moderate to high amounts of xanthine alkaloids, largely caffeine (Fig. 23), but also small or trace amounts of theophylline and theobromine (Fig. 14) (Lewis et al., 1991). Caffeine concentration varies strikingly from plant to plant, but men prefer *wayus* made from plants having 1.7 to 3.5% (dw/w). When caffeine reaches about 4% or greater, such plants are avoided, for even after emesis, drinking of these decoctions may result in disorientation, irritability, and even mild hallucinations (e.g., branches on the jungle floor appear to move as snakes). Emesis is practiced by all participants in the *wayus* ceremony, occurring between 1/2 and 3/4 hour after drinking begins, which prevents the complete absorption of xanthine alkaloids from the gastrointestinal tract but enough to stimulate without unwanted hyperstimulation and other effects.

Because of their life-style, wounds and cuts are an everyday occurrence for the Jívaro, and it is perhaps no surprise that they harvest a plant product reputed to have wound healing properties. Sap from spurge trees (*Croton* spp.) is smeared on a wound site, and if it is large or deep it may be dressed. Sap is added periodically, and healing is supposed to be accelerated. Initial experiments using an *in vivo* assay support their contention, for cell influx and tissue regrowth were greater and more rapid in wounded areas using the sap when compared to controls. Experiments are now under way using two *in vivo* model systems and the aporphine alkaloid taspine (Fig. 23) extracted from the sap in order to provide a more precise description of biological activity.

One of the few viral diseases recognized by the Jívaro is hepatitis. Within this complex, they distinguish between the yellow jaundice causing mild illness and the one that can kill. The first experiments, using plant extracts they use to treat the "killing jaundice," show remarkably high inhibition of hepatitis B replication *in vitro*.

In our culture, there is always a concern regarding skin blemishes and more serious skin afflictions such as acne, staph, etc. The Indians, as well, are concerned about their appearance, and although their skin is by and large healthy, they can be troubled by periodic boils and pimples. To eliminate some blemishes, they rub affected areas with a freshly cut amaryllid bulb of *Eucharis*. Initial data using cut pieces of the bulb, together with other bulbs known to possess antibiotic activity

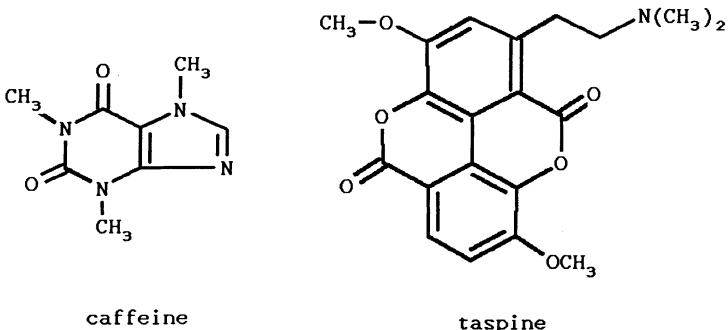


Figure 23. Structural formulas of caffeine and taspine.

(onion and narcissus), show the broadest spectrum of activity against a wide range of bacteria by the amaryllid. As topical antibiotics are infrequent in our pharmacopeia, further studies are in progress to elucidate what is involved.

Jívaro women seem to control family size by determining the time they wish to become pregnant. They do this by ingesting infusions of underground parts of sedges (*Cyperus* spp.) at specified times. Experiments with mice have yet to confirm the presence of antifertility principles in these plants, but experiments with other animals are required. Apart from using contraceptives, women also employ a fungus growing apically on these sedges for oxytocic purposes. When in labor, they ingest a decoction made from *Balansia cyperi* to aid in parturition or postpartum contractions and to reduce bleeding following birth (Lewis and Elvin-Lewis, 1990). This fungus is closely related to ergot, so circumstantial evidence strongly suggested the presence of alkaloids similar to ergot alkaloids in *B. cyperi*, a suggestion which has just been confirmed (Plowman et al., 1991). Thus, the use by Jívaro women of a plant parasite to aid in obstetrics is yet another sophisticated example of human ingenuity using empirical methodology in the selection of natural products for medical purposes.

## CONCLUSION

There is great scope for new drug discoveries based on traditional medicinal plant use throughout the world (e.g., Cox et al., 1989; Moerman, 1991; Phillipson and Anderson, 1989; Schultes and Raffauf, 1990; Turner and Herbda, 1990), a theme made eminently clear throughout this review of plants utilized medically by indigenous peoples. First, several examples of plant uses are described within an historic context; second, current uses in modern medicine and pharmacy of hundreds of plants are outlined by medical categories, illustrating recent selections of natural products and their incorporation in some way into modern pharmacopeias; and, finally, uses of plants are given of an intact people (Jívaros) as used now and as they have been for perhaps thousands of years and as cultures once did worldwide for survival on a daily basis. Serious dangers exist for the survival of such peoples and their cultures, and the ecosystems which nurture them and provide Western and traditional medicines with novel plant products for human well-being everywhere. In this race against ecosystem destruction, researchers in many disciplines must rally to provide the impetus to save global diversity while, at the same time, accelerating studies of ethnomedicine in consort with biomedical and chemical teams for developing new natural products and drugs needed by humans into the next century.

## LITERATURE

- Aikman, L., 1977, "Nature's Healing Arts: From Folk Medicine to Modern Drugs," National Geographic Society, Washington, D.C., 200 p.
- Al-Rawi, A., and Chakravarty, H. L., 1964, "Medicinal Plants of Iraq," Iraq Ministry of Agriculture, Baghdad, 109 p.
- Bock [Tragus], H., 1552, "Kreuter Buch," Strassburg.
- Copeman, W. S. C., 1964, "A Short History of the Gout and the Rheumatic Diseases," University of California Press, Berkeley, 236 p.
- Copeman, W. S., 1970, Historic aspects of gout, Clinical Orthopaedics, 71:14-22.
- Cox, P. A., Sperry, R., Tuominen, M., and Bohlin, L., 1989, Pharmacological activity of the Samoan ethnopharmacopoeia, Economic Botany, 43:487-497.
- Der Marderosian, A. H., 1991, The need for cooperation between modern and traditional medicine, HerbalGram, 24:30-37.
- Dioscorides, 1st Century, see Gunther, R. T. (1934) "The Greek Herbal of Dioscorides" (Illustrated by a Byzantine 512 AD, Englished by John Goodyer 1655 AD), Hafner, London and New York, New York.
- Duke, J. A., 1973, Utilization of *Papaver*, Economic Botany, 27:390-400.
- Farnsworth, N. R., 1973, Importance of secondary plant constituents as drugs, p. 351-380, in: "Phytochemistry: Volume 3, Inorganic Elements and Special Groups of Chemicals," L. P. Miller, ed., Van Nostrand, New York, New York.
- Farnsworth, N. R., and Soejarto, D. D., 1985, Potential consequence of plant extinction in the United States on the current and future availability of prescription drugs, Economic Botany, 39:231-240.
- Ford, L. T., 1990, An update on chymopapain for treating lumbar disc ruptures, Missouri Medicine, 87:152-154.
- Foster, S., 1991, Harvesting medicinals in the wild, HerbalGram, 24:10-16.
- Galen, 2nd century, Claudii Galeni Opera Omnia, p. 1821-1833, in: "Lipsiae," C. G. Kühn, ed., 22 volumes.
- Ge Hong, 340 AD, "Zhou Hou Bei Ji Fang" [Handbook of Prescriptions for Emergency Treatment].
- Gerard, J., 1597, "The Herball or Generall Historie of Plantes," London (imprinted by John Norton).
- Gerard, J., 1633, "The Herball or Generall Historie of Plantes...Very much Enlarged and Amended" by Thomas Johnson, London (printed by Adam Islip, Joice Norton, and Richard Whitakers).
- Gosselin, R. A., 1962, The status of natural products in the American pharmaceutical market, Lloydia, 25:241-243.
- Grinspoon, L., 1977, "Marihuana Revisited," 2nd Edition, Harvard University Press, Cambridge, Massachusetts, 474 p.
- Grinspoon, L., and Bakalar, J. B., 1981, Coca and cocaine as medicines: An historic review, Journal of Ethnopharmacology, 3:149-159.
- Hartwell, J. L., 1967-1971, Plants used against cancer: A survey, Lloydia 30-34 [see Lewis and Elvin-Lewis (1977:145) for detailed references].
- Hedberg, I., 1987, Research on medicinal and poisonous plants of the tropics--past, present and future, p. 9-15, in: "Medicinal and Poisonous Plants of the Tropics," A. J. M. Leeuwenberg, compiler, Pudoc Wageningen, The Netherlands.
- Herodotus, 5th century BC, Translated AD, Godley, Loeb Classical Library, Harvard University Press, Cambridge, Heinemann (1961-1966, 4 volumes).
- Hippocrates, ca. 350 BC, See Littré (1839-1861).
- Klayman, D. L., Lin, A. J., Acton, N., Scovill, J. P., Hoch, J. M., Milhous, W. K., Theoharides, A. D., and Dobeck, A. S., 1984, Isolation of artemisinin

- (*ginghaosu*) from *Artemisia annua* growing in the United States, *Journal of Natural Products*, 47:715-717.
- Kreig, M. B., 1964, "Green Medicine," Rand McNally, Chicago, Illinois.
- Lassak, E. V., and McCarthy, T., 1983, "Australian Medicinal Plants," Methuen Australia, North Ryde, 240 p.
- Leung, A. Y., 1980, "Encyclopedia of Common Natural Ingredients," Wiley & Sons, New York, New York, 409 p.
- Lewis, W. H., 1982, Plants for man: Their potential in human health, *Canadian Journal of Botany*, 60:310-315.
- Lewis, W. H., and Elvin-Lewis, M. P. F., 1977, "Medical Botany: Plants Affecting Man's Health," Wiley & Sons, New York, New York, 515 p.
- Lewis, W. H., and Elvin-Lewis, M. P. F., 1983, Contributions of herbology to modern medicine and dentistry, p. 785-815, in: "Handbook of Natural Toxins, Volume 1: Plant and Fungal Toxins," R. F. Keeler and A. T. Tu, eds., Marcel Dekker, New York, New York.
- Lewis, W. H., Elvin-Lewis, M. P. F., and Gnero, M. C., 1987, Introduction to ethnobotanical pharmacopeia of the Amazonian Jívaro of Peru, p. 96-103, in: "Medicinal and Poisonous Plants of the Tropics," A. J. M. Leeuwenberg, compiler, Pudoc Wageningen, The Netherlands.
- Lewis, W. H., Elvin-Lewis, M., Gnero, M. C., and D. Fast W., 1988, Role of systematics when studying medical ethnobotany of the tropical Peruvian Jívaro, in: "Systematic Botany—A Key Science for Tropical Research and Documentation," I. Hedberg, ed., *Symbolae Botanicae Upsalienses*, 28(3):189-196.
- Lewis, W. H., and Elvin-Lewis, M. P., 1990, Obstetrical use of the parasitic fungus *Balansia cyperi* by Amazonian Jívaro women, *Economic Botany*, 44:131-133.
- Lewis, W. H., Elvin-Lewis, M. P., and D. Fast W., 1991, *Pentagonia gigantifolia* (Rubiaceae) as a snakebite remedy: Empirical methodology functioning in Amazonian traditional medicine, *Economic Botany*, 45:137-138.
- Lewis, W. H., Kennelly, E. J., Bass, G. N., Wedner, H. J., Elvin-Lewis, M. P., and D. Fast W., 1991, Ritualistic use of the holly *Ilex guayusa* by Amazonian Jívaro Indians, *Journal of Ethnopharmacology*, 33:25-30.
- Li Shih-Chen, 1596, "Pén Tshao Kang Mu" [The Great Pharmacopoeia; or, The Pandects of Natural History].
- Littré, E., 1839-1861, "Oeuvres Complètes d'Hippocrate," Baillière, Paris (10 volumes).
- Lonicer, A. [Lonitzer], 1582, "Rösskin Kreuterbüch," Franckfurt am Meyn.
- Majno, G., 1975, "The Healing Hand: Man and Wound in the Ancient World," Harvard University Press, Cambridge, Massachusetts, 571 p.
- Mattioli, P., 1579, "Commentaires," Lyons.
- Merlin, M. D., 1984, "On the Trail of the Ancient Opium Poppy," Fairleigh Dickinson University Press, Cranbury, New Jersey, 324 p.
- Moerman, D. E., 1991, The medicinal flora of native North America: An analysis, *Journal of Ethnopharmacology*, 31:1-42.
- Morton, J. F., 1977, "Major Medicinal Plants: Botany, Culture and Uses," C. C. Thomas, Springfield, Illinois, 431 p.
- Needham, J., 1986, "Science and Civilisation in China, Volume 6, Biology and Biological Technology, Part I: Botany," Cambridge University Press, Cambridge, Massachusetts, 718 p.
- Phillipson, J. D., and Anderson, L. A., 1989, Ethnopharmacology and western medicine, *Journal of Ethnopharmacology*, 25:61-72.

- Pliny [The Elder] (Caius Plinius Secundus), "Natural History," Translated by H. Rackham, W. H. S. Jones, and D. E. Eichholz, Harvard University Press, Cambridge, Massachusetts, and London, Heinemann (1956-66, 10 volumes).
- Plotkin, M. J., 1988, Conservation, ethnobotany, and the search for new jungle medicines: Pharmacognosy comes of age...again, *Pharmacotherapy*, 8:257-262.
- Plowman, T., 1984, The ethnobotany of coca (*Erythroxylon* spp., Erythroxylaceae), *Advances in Economic Botany*, 1:62-111.
- Plowman, T. C., Leuchtmann, A., Blaney, C., and Clay, K., 1990 (issued 1991), Significance of the fungus *Balansia cyperi* infecting medicinal species of *Cyperus* (Cyperaceae) from Amazonia, *Economic Botany*, 44:452-462.
- Quimby, M. W., 1953, *Ammi visnaga* Lam.—a medicinal plant, *Economic Botany*, 7:89-92.
- Randall, R. C., ed., 1990, "Cancer Treatment and Marijuana Therapy," Galen Press, Washington, D.C., 365 p.
- Rury, P. M., and Plowman, T., 1983 (issued 1984), Morphological studies of archaeological and recent coca leaves (*Erythroxylon* spp.), *Botanical Museum Leaflets*, Harvard University, 29:297-341.
- Schöffer, P., 1484, "The Latin Herbarius," Mainz.
- Schlutes, R. E., and Raffauf, R. F., 1990, "The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia," Dioscorides Press, Portland, Oregon, 484 p.
- Sivarajan, V. V., 1988, Indian medicine and medicinal plants: A taxonomic dilemma, *in:* "Systematic Botany—A Key Science for Tropical Research and Documentation," I. Hedberg, ed., *Symbolae Botanicae Upsalienses*, 28(3):197-206.
- Sneader, W., 1985, "Drug Discovery: The Evolution of Modern Medicines," John Wiley & Sons, New York, New York, 435 p.
- Soejarto, D. D., and Farnsworth, N. R., 1989, Tropical rainforests: Potential sources of new drugs?, *Perspectives in Biology and Medicine*, 32:244-256.
- Solecki, R. S., 1971, "Shanidar: The First Flower People," Knopf, New York.
- Solecki, R. S., 1975, Shanidar IV, a Neanderthal flower burial in northern Iraq, *Science*, 190:880-881.
- Spjut, R. W., and Perdue, R. E., Jr., 1976, Plant folklore: A tool for predicting sources of antitumor activity?, *Cancer Treatment Reports*, 60:979-985.
- Stenlake, J. B., Waigh, R. D., Urwin, J., Dewar, G. H., and Coker, G. G., 1983, Atracurium: Conception and inception, *British Journal of Anaesthesia*, 55:3S-10S.
- Thang Shen-Wei, 1083, "Chêng Lei Pên Tshao" [Classified Pharmaceutical Natural History].
- Trigg, P. I., 1989, Qinghaosu (artemisinin) as an antimalarial drug, p. 19-55, *in:* "Economic and Medicinal Plant Research, Volume 3," H. Wagner, H. Hikino, and N. R. Farnsworth, eds., Academic Press, London/San Diego, California.
- Turner, N. J., and Herbda, R. J., 1990, Contemporary use of bark for medicine by two Salishan native elders of southeast Vancouver Island, Canada, *Journal of Ethnopharmacology*, 29:59-72.
- Tyler, V. E., 1986, Plant drugs in the twenty-first century, *Economic Botany*, 40:279-288.
- Tyler, V. E., 1987, "The New Honest Herbal," 2nd Edition, G. F. Stickley, Philadelphia, Pennsylvania, 254 p.
- Van Dyke, C., and Byck, R., 1977, Cocaine: 1884-1974, p. 1-30, *in:* "Cocaine and Other Stimulants," E. H. Ellinwood, Jr., and M. M. Kilbey, eds., Plenum Press, New York, New York.

Weil, J., 1986, Beyond the mystique of cocaine: Coca in Andean cultural perspective, p. 306-328, in: "Plants in Indigenous Medicine & Diet: Biobehavioral Approaches," N. Etkin, ed., Redgrave Publishing Company, Bedford Hills, New York.

Withering, W., 1785, "An Account of the Foxglove, and Some of its Medical Uses, with Practical Remarks on Dropsy, and Other Diseases," M. Swinney, Birmingham, Alabama, Reprinted, Medical Classics, 5(4):303-443, 1937.

## **PLANTS AS SOURCES OF MEDICINALLY AND PHARMACEUTICALLY IMPORTANT COMPOUNDS**

**A. Douglas Kinghorn**

Program for Collaborative Research in the  
Pharmaceutical Sciences and Department of  
Medicinal Chemistry and Pharmacognosy  
College of Pharmacy  
University of Illinois at Chicago  
Chicago, IL 60612

### **INTRODUCTION**

Mankind has profitably used extracts of plants for the treatment of human diseases for centuries. The beginning of the 19th century heralded an era in which the active secondary-metabolite principles of medicinal plants began to be purified, with such pure constituents then introduced into therapy, as evidenced in turn by morphine, quinine, atropine, papaverine, cocaine, and pilocarpine. Important plant-derived drugs have continued to be incorporated into the physician's armamentarium in the 20th century, such as digitoxin, digoxin, ergometrine, ergotamine, reserpine, tubocurarine, vinblastine, and vincristine (Baerheim Svendsen and Scheffer, 1982; Tyler et al., 1988). The current importance and/or the potential of plants as sources of drug substances per se, as lead compounds for synthetic modification, and as excipients in pharmaceutical formulations has been addressed by others in recent years (Baerheim Svendsen and Scheffer, 1982; Balandrin and Klocke, 1988; Balandrin et al., 1985; Farnsworth, 1984; Galeffi and Marini-Bettolo, 1988; Hosler and Mikita, 1987; Phillipson and Anderson, 1987, 1989; Steiner, 1986; Tyler et al., 1988).

In the present chapter, an overview will be provided of the prominence that naturally occurring drugs from higher plants have gained in western medicine and pharmacy with mention being made of the manner in which such compounds have been discovered. A brief description will be provided of the role of plant-derived constituents as biologically active template molecules for the synthesis of new drugs with better therapeutic efficacy and/or less toxicity than the original natural products. Recent progress will be described on the discovery of plant secondary metabolites in five categories of biological activity, namely, compounds possessing antineoplastic, antimalarial, antiviral (with particular emphasis on the compounds with potential anti-AIDS activity), fertility-regulating, and sweetening effects. Finally, prospects for the discovery of additional medicinally and pharmaceutically useful compounds from plants will be discussed.

## OVERVIEW OF THE IMPORTANCE OF PLANT CONSTITUENTS IN MEDICINE AND PHARMACY

Plant organic constituents may be classified as primary or secondary metabolites with the former being involved in basic cellular metabolism and the latter being of more limited taxonomic distribution without apparent metabolic functions. Secondary metabolites, which are biosynthetically derived from primary metabolites like amino acids and sugars, appear to have ecological roles, such as serving to ward off animal or plant predators or to attract insect pollinators. It has been found on numerous occasions that a structurally complex plant secondary metabolite as, for example, an alkaloid or a terpenoid, occurs naturally in exactly the right chiral form to exhibit a particular type of biological activity (Balandrin and Klocke, 1988; Balandrin et al., 1985; Tyler et al., 1988). Thus, it is perhaps not surprising that almost every pharmacological class of drug possesses a natural product prototype (Dohadwalla, 1985; Farnsworth, 1977; Farnsworth and Bingel, 1977). There are an estimated 200,000 to 500,000 higher plants (gymnosperms and angiosperms) and 200,000 or more lower plants (algae, fungi, lichens, bryophytes, and pteridophytes) on the earth (Schultes, 1972). Given that it has been projected that about 90% of the world's flora has not been subjected to any form of scientific phytochemical or biological screening study, then it is apparent that plants represent a virtual treasure trove of structurally diverse potentially bioactive organic molecules (Baerheim Svendsen and Scheffer, 1982; Balandrin and Klocke, 1988; Balandrin et al., 1985; Hosler and Mikita, 1987; Phillipson and Anderson, 1987; Schultes, 1972).

The plant-derived natural products that are used as therapeutic agents in western medicine are primarily representatives of various structural types of alkaloids, in addition to certain steroidal glycosides (Table 1). It may be pointed out that such glycosides normally are inherently quite water soluble while the alkaloids can be rendered water soluble by salt formation, thereby facilitating both their original laboratory evaluation and therapeutic administration. In addition, plant constituents serve as pharmaceutical aids that are useful in the manufacturing or compounding of medicinal agents. Such compounds include the terpenoid and phenylpropanoid volatile oils (primarily used as flavoring agents), fats and waxes (used to prepare ointment and suppository bases), fixed oils (used as emollients in ointments and liniments), gums and mucilages (hydrocolloids used as emulsifying and suspending agents), and polysaccharides (used in dusting powders and certain diagnostic products) (Tyler et al., 1988).

It is considered that some four-fifths of the world's population place reliance on traditional medicine for their health care and, as such, use medicinal plants as a major part of their drug therapy (Farnsworth et al., 1985). There has been an attempt to estimate the number of higher plant-derived purified constituents that are used as drugs in one or more countries of the world. Thus, Farnsworth and collaborators have shown that 119 drugs of known structure are in use at the present time and are obtained from 91 species of higher plants and represent some 60 therapeutic categories (Farnsworth, 1987; Farnsworth et al., 1985). However, somewhat less than half of these are currently utilized in western countries (Farnsworth, 1984). It is interesting to note that, of the 45 or so plant-derived drugs used in industrialized countries, only some of the more structurally simple (e.g., caffeine, theophylline, and papaverine) are manufactured synthetically with the rest produced more economically by cultivation and extraction (Farnsworth and Morris, 1976). Classifications of plant natural product drugs by therapeutic category have been provided for the United States and the United Kingdom, respectively, by Farnsworth (1973) and Phillipson and Anderson (1989).

Table 1. Examples of therapeutic agents derived from higher plants that are used in the United States.<sup>a</sup>

Drug name	Compound class	Pharmacological activity
Atropine	Tropane alkaloid	Anticholinergic
Codeine	Isoquinoline alkaloid	Analgesic; Antitussive
Digoxin	Steroidal glycoside	Cardiotonic
Emetine	Isoquinoline alkaloid	Antiamoebic; Emetic
Morphine	Isoquinoline alkaloid	Analgesic
Physostigmine	Indole alkaloid	Cholinesterase inhibitor
Pilocarpine	Imidazole alkaloid	Parasympathomimetic
Quinine	Quinoline alkaloid	Antimalarial
Reserpine	Indole alkaloid	Antihypertensive; Psychotropic
Tubocurarine	Isoquinoline alkaloid	Skeletal muscle relaxant
Vincristine	Indole alkaloid	Antineoplastic

<sup>a</sup>Taken from Tyler et al. (1988) and Farnsworth (1977).

The use of plant products in prescriptions dispensed in community pharmacies in the United States appears to represent a very stable market (Farnsworth, 1987; Farnsworth and Morris, 1976; Farnsworth et al., 1985; Principe, 1989). Thus, in 1980, it was calculated that 25% of all prescriptions in the U.S. contained an extractive (e.g., belladonna, ipecac, and rauvolfia) or a pure constituent obtained from a higher plant (Farnsworth, 1987; Farnsworth et al., 1985; Principe, 1989). The total number of such prescriptions has been placed at 1 billion in 1980, at an average price per prescription item of about \$8.00. Therefore, the total value of prescriptions containing products obtained from higher plants has been estimated as \$8 billion for that year (Farnsworth, 1987; Farnsworth et al., 1985; Principe, 1989).

## ORIGIN OF PLANT-DERIVED DRUGS

Throughout history, mankind has passed on information about efficacious and non-toxic medicinal plants by word-of-mouth and through various writings. As a result of this continual refinement of knowledge, about 20,000 plant species are now used for medicinal purposes around the world (Phillipson and Anderson, 1989). Crude plant drugs became used as aqueous or alcoholic extracts, and chemical work resulting in the isolation of their active principles began in earnest in the 19th century (Baerheim Svendsen and Scheffer, 1982; Midgley, 1988; Tyler et al., 1988). The activity-directed isolation of plant principles continues in many academic, governmental, and laboratories today wherein extracts exhibiting a particular biological activity of interest are purified chromatographically, guided by periodic evaluation with one or more bioassay systems, resulting in the eventual isolation of one or more biologically active constituents.

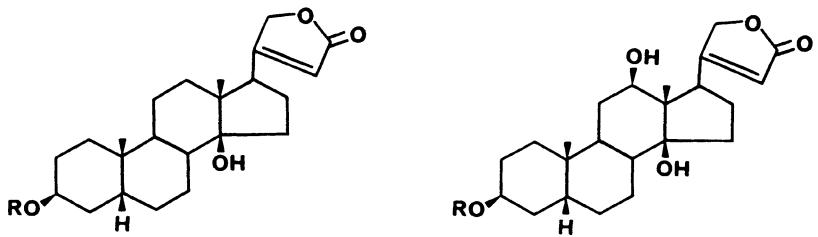
Observations made by local populations have played a large part in the development of plant-derived drugs. Of the previously mentioned 119 important plant-derived drugs reported by Farnsworth and colleagues (Farnsworth, 1987; Farnsworth et al., 1985), 88 (74%) were judged as having been discovered because their plant of origin was used in traditional medicine (Farnsworth et al., 1985). A good example of this is digitoxin, which is a principal cardiac glycoside constituent of *Digitalis purpurea* L. (purple foxglove), and remains an important drug for the treatment of atrial fibrillation. The propensity of *D. purpurea* in treating dropsy

(edema), mediated by heart failure, was first documented in 1776 by an English physician, William Withering, after he had observed the folkloric use of the plant for this same disease state. Withering then successfully experimented clinically with preparations made from *D. purpurea* leaves over a period of several years (Aronson, 1987). During the 19th century, partial purification of the *D. purpurea* cardiac glycoside constituents occurred until digitoxin (Fig. 1 [1]) and other cardiac glycosides were purified and structurally determined in the early part of the present century (Aronson, 1987). The ethnomedical use of *D. purpurea* and the successful isolation of therapeutically useful compounds from this plant source also stimulated the discovery of digoxin (Fig. 1 [2]) and several additional cardiac glycosides from *Digitalis lanata* Ehrhart, since it has been found that taxonomically related plants frequently biosynthesize chemically similar secondary metabolites. *D. lanata* has no history of use in traditional medicine for the treatment of either heart disease or diuresis (Farnsworth et al., 1985). The "contemptuous attitude" of "some chemists and pharmacologists in advanced nations" towards folk remedies, as noted by Schultes (1972), seems to have moderated in recent years judging by many publications that advocate an ethnopharmacological approach toward natural product drug development (e.g., Farnsworth and Kaas, 1981; Farnsworth et al., 1985; Hosler and Mikita, 1987; Labadie, 1986; Phillipson and Anderson, 1989; Spjut and Perdue, 1976; Steiner, 1986).

The important antineoplastic agent, vincristine (leurocristine), which is recommended for the treatment of acute leukemia and is used in combination therapy for Hodgkin's disease and other human cancers, is an example of a plant-derived drug that was discovered without a direct ethnopharmacological connection. This dimeric indole alkaloid (Fig. 2 [3]) was isolated by Svoboda as an active compound present in an extract of *Catharanthus roseus* (L.) G. Don (syn. *Vinca rosea* L., Madagascan periwinkle) that proved to be responsible for the prolongation of the life span observed for DBA/2 mice infected with P-1534 leukemia (Svoboda, 1961). The only incidence of the ethnomedical use of *C. roseus* in the treatment of human cancer was recorded subsequent to the discovery of the antileukemic effects of vincristine (Farnsworth and Kaas, 1981). It is interesting to note that vinblastine (vincaleukoblastine), an analog of vincristine, that is now used in cancer chemotherapy to treat several types of neoplasms, was discovered prior to vincristine in a study by Noble, Beer, and Cutts designed to investigate the oral hypoglycemic effects of *C. roseus* as employed in folk medicine in the West Indies (Noble et al., 1958). Vinblastine (Fig. 2 [4]) was obtained as a *C. roseus* isolate that produced severe leukopenia in rats (Noble et al., 1958) and was subsequently determined to have *in vivo* antileukemic activity in mice by Svoboda et al. (1959).

It is germane to consider the contribution of the world's tropical rain-forests toward drug discovery since great concern has been expressed recently about the acute need to conserve the genetic diversity of the tropics (Elliott and Brimacombe, 1988; Peters et al., 1988; Plotkin, 1988; Soejarto and Farnsworth, 1989). The tropical forests of South and Central America, Africa, and Southeast Asia cover less than 10% of the world's surface yet contain perhaps 50% of its total flora (Plotkin, 1988). Unfortunately, the present global extinction rate of plants has been estimated as 400 times greater than in the recent geological past (Plotkin, 1988). Since most of the endangered species are in the tropics, efforts to investigate rain-forest species for the occurrence of potential drug substances are urgent. It has been determined by Farnsworth and Soejarto that over 20 drugs used in the United States originate from tropical regions (Soejarto and Farnsworth, 1989), and a selection of the more important compounds is provided in Table 2.

In the People's Republic of China (PRC), great credence is still placed on traditional medicine in which herbal remedies play an integral role and represent some 30 to 50% of the total consumption of medicines in that country (Chen and



**1** ( $R = \beta\text{-D-dgx}^4\text{-}\beta\text{-D-dgx}^4\text{-}\beta\text{-D-dgx}$ )      **2** ( $R = \beta\text{-D-dgx}^4\text{-}\beta\text{-D-dgx}^4\text{-}\beta\text{-D-dgx}$ )

Figure 1. Structures of digitoxin [1] and digoxin [2]. (Dgx = digitoxose).

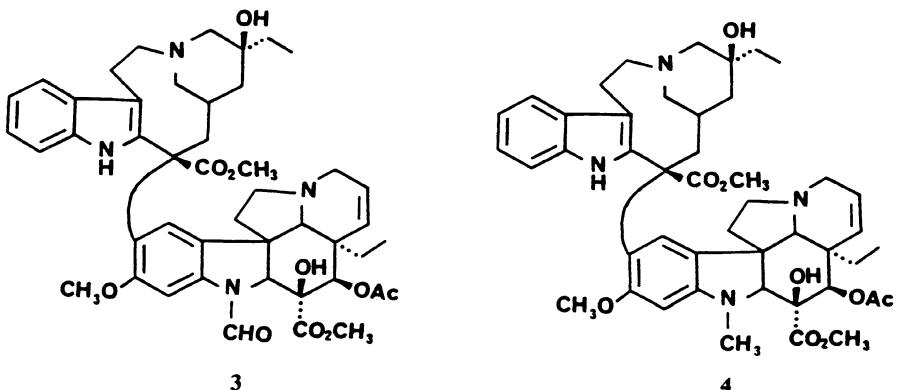


Figure 2. Structures of vincristine [3] and vinblastine [4].

Shen, 1986; Xiao and Chen, 1987). Nearly 6,000 medicinal plants have had some utilization in the PRC, and 500 species are in common usage (Wang and Liu, 1985; Xiao and Fu, 1987). Phytochemical work on traditional Chinese plant medicines, both in the PRC and in other countries, has now resulted in the isolation of 250 pharmacologically active compounds from which approximately 60 naturally occurring and semi-synthetic drugs have been derived (Han, 1988; Wang and Liu, 1985; Xiao and Chen, 1987, 1988; Xiao and Fu, 1987). Examples of useful plant-derived drugs utilized in the PRC include the tropane alkaloid, anisodamine (Fig. 3 [5]), which is a mild centrally acting anticholinergic agent employed for the treatment of septic shock resulting from toxic bacillary dysentery; the structurally related compound, anisodine (Fig. 3 [6]), which possesses effectiveness in the treatment of migraine headaches and is used to treat certain types of poisoning; the isoquinoline alkaloid, *dl*-tetrahydropalmatine (Fig. 3 [7]), which acts as a non-addictive sedative and tranquilizer; and the indole alkaloid, indirubin (Fig. 3 [8]), which is an active compound in the treatment of chronic myelocytic leukemia (Han, 1988; Wang and Liu, 1985; Xiao and Chen, 1987, 1988; Xiao and Fu, 1987). Many of the plant-derived drugs used in the PRC seem worthy of investigation for their potential use in western medicine. Further examples of compounds that have been discovered as a result of their occurrence in plants used in Chinese traditional medicine will be mentioned later in this chapter.

Table 2. Some drugs from tropical plants used in the United States.<sup>a</sup>

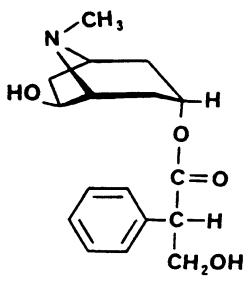
Drug name	Species of origin	Clinical use
Castor oil	<i>Ricinus communis</i> L.	Cathartic
Cocaine	<i>Erythroxylon coca</i> Lamk.	Local anesthetic
Deserpidine	<i>Rauvolfia canescens</i> L.	Antihypertensive; tranquillizer
Emetine	<i>Cephaelis ipecacuanha</i> (Brot.) A. Richard	Antiamoebic; emetic
Ouabain	<i>Strophanthus gratus</i> Baill.	Cardiotonic
Papain	<i>Carica papaya</i> L.	Proteolytic
Physostigmine	<i>Physostigma venenosum</i> Balf.	Cholinesterase inhibitor
Pilocarpine	<i>Pilocarpus jaborandi</i> Holmes	Parasympathomimetic
Quinidine	<i>Cinchona ledgeriana</i> Moens ex Trimen	Antiarrhythmic
Quinine	<i>C. ledgeriana</i> Moens ex Trimen	Antimalarial
Rescinnamine	<i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz	Antihypertensive
Reserpine	<i>R. serpentina</i> (L.) Benth. ex Kurz	Antihypertensive
Scopolamine	<i>Datura metel</i> L.	Sedative
Tubocurarine	<i>Chondrodendron tomentosum</i> R. & P.	Skeletal muscle relaxant
Vinblastine	<i>Catharanthus roseus</i> (L.) G. Don	Antineoplastic
Vincristine	<i>C. roseus</i> (L.) G. Don	Antineoplastic

<sup>a</sup>Taken from Soejarto and Farnsworth (1989).

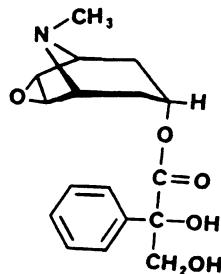
## PLANT CONSTITUENTS AS LEAD COMPOUNDS FOR SYNTHETIC MODIFICATION

In addition to their use as drugs in a chemically unmodified form, plant constituents may serve as raw materials for synthetic modification, such as in the production of anabolic, corticosteroid, and oral contraceptive steroids from *Dioscorea* species (Mexican yams) and plant sterols (Baerheim Svendsen and Scheffer, 1982; Balandrin and Klocke, 1988; Farnsworth, 1973; Tyler et al., 1988). However, plant constituents and other natural products are widely acknowledged as being very important as structurally varied template or "lead" molecules for the design and generation of completely new drug substances (Baldwin, 1987; de Souza et al., 1982; Midgley, 1988; Spilker, 1989). A lead compound may be defined as a new compound with an interesting pharmacological or other biological activity (Freter, 1987). Such substances have been discovered by one of several procedures, including random screening, directed screening, and drug design, although, to date, serendipity has also played an important role (Baldwin, 1987; Freter, 1987). Frequently, the analogs of a lead compound may exhibit unanticipated pharmacological effects and, therefore, can be structurally modified themselves. Thus, one lead compound may lead to a host of useful drugs (Midgley, 1988). According to Midgley, 21 of 151 lead substances that have been modified to produce 1,300 drugs in the current British National Formulary are plant products (Midgley, 1988).

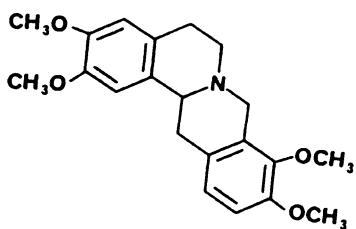
The opium poppy (*Papaver somniferum* L.) is quite remarkable in being the source of four drugs that are used in the United States, i.e., morphine (analgesic), codeine (analgesic and antitussive), noscapine (antitussive), and papaverine (smooth muscle relaxant) (Tyler et al., 1988). On top of this, morphine is perhaps the plant constituent that has been used as a lead compound to the widest extent in attempts to produce derivatives that produce similar narcotic and pain-relieving properties but do not have the habit-forming tendencies of this parent molecule (Hite, 1988;



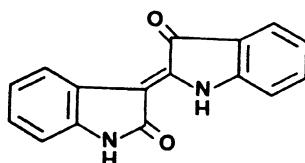
5



6

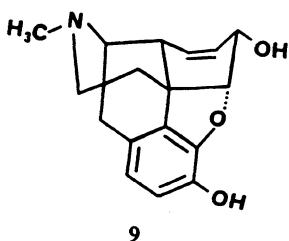


7

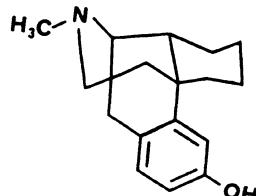


8

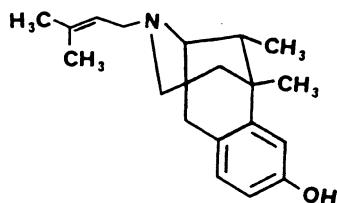
Figure 3. Structures of four plant-derived drugs used in Chinese traditional medicine: anisodamine [5]; anisodine [6]; *dl*-tetrahydropalmatine [7]; and indirubrin [8].



9



10



11

Figure 4. Structures of the plant constituent, morphine [9], and two synthetic analgesic derivatives, levophanol [10] and pentazocine [11], based on this lead compound.

Nogrady, 1985; Tyler et al., 1988). Two examples of the many clinically used analgesics that are modeled on the molecule of morphine (Fig. 4 [9]) are levophanol (Levo-Dromoran<sup>TM</sup>) and pentazocine (Talwin<sup>TM</sup>; Fortral<sup>TM</sup>). Levophanol (Fig. 4 [10]) is a morphinan derivative in which the oxygen bridge (furan ring) has been removed and is a more potent analgesic than morphine and also causes less emesis and less constipation. The benzomorphan derivative, pentazocine (Fig. 4 [11]), has neither the furan ring nor the ring C of morphine and is a shorter-acting analgesic with a lower dependence tendency (Baldwin, 1987; Spilker, 1989). There are numerous other examples of the use of plant constituents as lead compounds, and they include cocaine as the prototype local anesthetic that led to the development of procaine (Sneader, 1985a); salicin, a phenolic glycosidic constituent of willow bark that led to the discovery of aspirin (Nogrady, 1985); and the unstable isoquinoline alkaloid, petaline, which assisted in the development of atracurium besylate, a new neuromuscular blocking agent that rapidly degrades under physiological conditions to nontoxic products (Midgley, 1988; Sneader, 1985b).

## PLANT CONSTITUENTS WITH POTENTIAL MEDICAL OR PHARMACEUTICAL USES

### Antineoplastic Activity

The advances in the treatment of human neoplasms since the advent of cancer chemotherapy nearly 50 years ago have been made mainly against highly proliferative tumors. Thus, as a consequence of the introduction of cyclic combination chemotherapy, very high cure rates can now be obtained for acute childhood leukemias, Hodgkin's disease, and metastatic testicular cancer, among others. However, little or no prolongation of life is yet possible for more common human carcinomas such as breast cancer, colorectal cancer, and lung cancer (Krakoff, 1986).

Of the nearly 40 anticancer drugs on the market in the United States, two are plant constituents [vincristine (Fig. 2 [3]) and vinblastine (Fig. 2 [4])] and one is a semisynthetic derivative [etoposide (Fig. 5 [12])] based on a plant secondary metabolite prototype [podophyllotoxin (Fig. 5 [13])]. The success of vincristine (Oncovin<sup>TM</sup>) and vinblastine (Velban<sup>TM</sup>) in the treatment of leukemias and other types of cancer (Gerzon, 1980) has already been mentioned in this chapter. Podophyllotoxin is a constituent of *Podophyllum peltatum* L. (American mandrake), which has a folkloric reputation in the treatment of cancer (Hartwell, 1968). Etoposide (4'-demethylepipodophyllotoxin-β-D-ethylidene glucoside, VePesid<sup>TM</sup>), a less toxic and more potent antineoplastic agent than podophyllotoxin, is used clinically in the United States for the treatment of testicular cancer and small-cell lung carcinoma (Hussar, 1984, 1987; Jardine, 1980).

The clinical success of vincristine and vinblastine, in particular, has stimulated the search for additional antineoplastic agents of plant origin. At the forefront of this effort has been the National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland, which sponsored the screening of over 120,000 extracts of 35,000 plant species over a 25-year period (Spjut, 1985). Recently, the NCI has initiated another natural products acquisition program, part of which will involve the collection of several thousand rain-forest plants from three continents (Booth, 1987). Extracts of these plants are currently being tested against panels of tumor cell lines representing 11 types of human cancer (carcinomas of the breast, colon, head and neck, kidney, lung, ovary, and prostate; glioma; leukemia; melanoma; and sarcoma) (Alley et al., 1988). In this manner, it is hoped that the discovery of natural products with selective cytotoxicity will result; such compounds would then be evaluated in

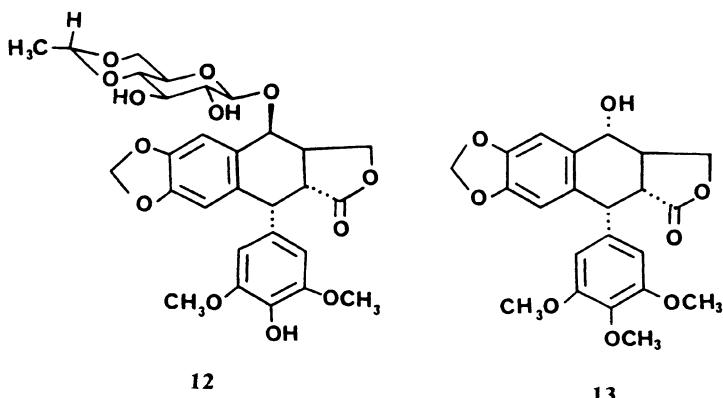
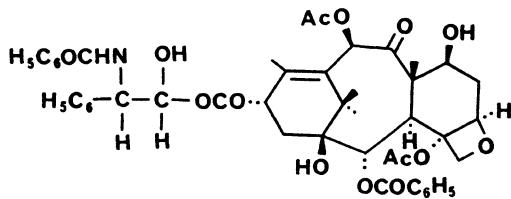


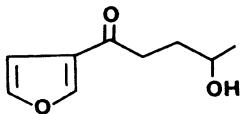
Figure 5. Structures of the semi-synthetic antineoplastic agent, etoposide [12], and the prototype lignan, podophyllotoxin [13].

athymic mice bearing the same type of tumor for which *in vitro*-active data were obtained (Alley, 1988; Cragg and Suffness, 1988).

Among the plant-derived compounds that show particular potential for future use as cancer chemotherapeutic agents are taxol (Fig. 6 [14]), 4-ipomeanol (Fig. 6 [15]), and 20(*RS*)-9-aminocamptothecin (Fig. 6 [16]). Taxol is an antileukemic nitrogen-containing diterpenoid that was isolated from the bark of the western yew, *Taxus brevifolia* Nutt., by Wani and Wall and colleagues (Wani et al., 1970). This compound has shown significant *in vivo* activity against a number of human tumor xenograft animal models (Cragg and Suffness, 1988) and recently was found to produce a good remission rate in patients with advanced ovarian cancer in a phase II clinical trial (Blume, 1989). Difficulties in obtaining adequate amounts of taxol by cultivation are presently hindering the further clinical evaluation of this most promising drug (Blume 1989; Booth, 1987). 4-Ipomeanol, a furanoid pulmonary toxin, was first isolated by Boyd and Wilson from sweet potatoes (*Ipomoea batatas* (L.) Lam.) infested with the mold, *Fusarium solani* (Boyd and Wilson, 1972). The compound is actually a stress metabolite produced by *I. batatas*, rather than a fungal metabolite (Cragg and Suffness, 1988). 4-Ipomeanol requires metabolic activation within the Clara bronchiolar cells to elicit its toxic effects in the mammalian lung and has undergone preclinical development at the National Cancer Institute with the intention of evaluation in clinical trials as an antineoplastic agent for lung cancer (Christian et al., 1989). The quinoline alkaloid, camptothecin (Fig. 6 [17]), was first isolated by Wall and Wani from a Chinese tree, *Camptotheca acuminata* Decne., and found to exhibit potent life prolongation in mice implanted with L1210 leukemia (Wall et al., 1966). The sodium salt of camptothecin briefly entered clinical trials in the United States but caused excessive leukopenia and hemorrhagic cystitis (Giovanella et al., 1989). Interest in camptothecin and its analogs as potential anticancer drugs has been aroused for two reasons. First, the parent compound has been found to be most unusual in interacting specifically with DNA topoisomerase I, an enzyme that acts by relaxing supercoiled DNA (Giovanella et al., 1989). Second, the 20(*RS*)-9-amino- derivative of camptothecin has been found recently to be effective in treating immunodeficient mice carrying three different lines of human colon cancer and did not show any toxic effects (Giovanella et al., 1989).



14



15

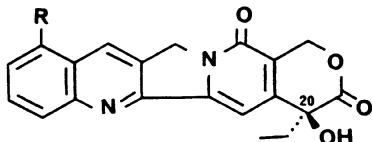
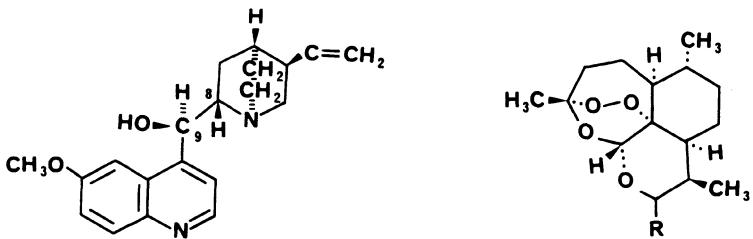
16 ( $R = \text{NH}_2$ )17 ( $R = \text{H}$ )

Figure 6. Structures of the antineoplastic agents, taxol [14], 4-ipomeanol [15], 9-aminocamptothecin [16] (shown as the 20S-stereoisomer), and camptothecin [17].

### Antimalarial Activity

Human malaria, the most prevalent tropical disease known, is caused by several species of the genus *Plasmodium*, a blood parasite that is transmitted by the *Anopheles* mosquito. *Plasmodium falciparum* is the most deadly of the human malarias and is responsible for more deaths in Africa than any other parasitic disease (Shuler, 1985). Transmission of malaria still occurs in 102 countries of the world in which there are some 2,700 million inhabitants, and the disease affects up to 100 million people per year (Trigg, 1989). Despite extensive eradication efforts to wipe out the insect vector using insecticides and the development of several types of synthetic antimalarials, the incidence of the disease is still increasing due to shortages in funding for control measures and the evolution of drug-resistant strains of *P. falciparum* (Shuler, 1985; Trigg, 1989).

Plant constituents have had, and will continue to have, an important role in the control of malaria. The oldest antimalarial drug in the western world is quinine (Fig. 7 [18]), a quinoline alkaloid obtained from the bark of *Cinchona ledgeriana* Moens ex Trimen and other *Cinchona* species (Bruce-Chwatt, 1988). Quinine remains an essential drug in treating severe manifestations of falciparum malaria, and the recent annual world demand for this compound as an antimalarial agent was placed at about 40,000 kg (Bruce-Chwatt, 1988). Quinine served as a lead compound for the development of the 8-aminoquinoline (e.g., primaquine) and the 4-aminoquinoline (e.g., chloroquine) classes of synthetic antimalarial drugs (Roche et al., 1988). Recently, a further promising plant compound has emerged as an excellent antimalarial substance, namely, qinghaosu (artemisinin) (Fig. 7 [19]). This compound, a sesquiterpene containing an endoperoxide functionality, is extracted from the herb, *Artemisia annua* L., a plant that has a tradition of several centuries of use in the People's Republic of China for the treatment of malaria and fevers (Klayman, 1985; Trigg, 1989). Qinghaosu was isolated and characterized by Chinese scientists in 1972.



**18**

**19 (R = O)**

**20 (R = β-OOCCH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Na)**

**21 (R = β-OCH<sub>3</sub>)**

**22 (R = β-OCH<sub>2</sub>CH<sub>3</sub>)**

Figure 7. Structures of the antimalarial agents, quinine [18], qinghaosu (artemisinin) [19], sodium artesunate [20], artemether [21], and arteether [22].

and has been shown to successfully treat several thousand patients infected with either *P. falciparum* or *P. vivax* (Klayman, 1985; Trigg, 1989). Two derivatives of this compound, sodium artesunate (which is water-soluble) (Fig. 7 [20]) and artemether (Fig. 7 [21]), are more potent drugs than qinghaosu itself and are now being produced as antimalarial drugs in the People's Republic of China. In the United States, arteether (Fig. 7 [22]), a crystalline and stable compound, has been chosen for preclinical toxicity trials in which it will be administered by formulation in sesame oil (Brossi et al., 1988).

In addition to quinine and qinghaosu, several other classes of plant secondary metabolites have been found to exhibit antimalarial activity, including various alkaloids, limonoids, and quassinoids (O'Neill and Phillipson, 1989). Since effective bioassay procedures are available to guide activity-monitored fractionation procedures (O'Neill and Phillipson, 1989), it may be anticipated that further useful antimalarials will be discovered from plant sources.

#### Antiviral Activity (with Emphasis on Anti-HIV Activity)

During the 1980s, acquired immunodeficiency syndrome (AIDS) has emerged as a severe threat to our public health, and this rapidly spreading disease has been traced to a retrovirus, which is now known as human immunodeficiency virus (HIV-1) (Piot et al., 1988). The alarming spread of AIDS has necessitated an urgent need for the discovery of therapeutic agents to combat the virus since only one drug, 3'-azido-2',3'-dideoxythymidine (AZT) has so far demonstrated an ability to prolong the life span of those suffering from this infectious disease (De Clercq, 1989).

Since many plant secondary metabolites are thought to be defense substances, it is perhaps not too surprising that there are frequent literature reports on plant extracts and isolates with antiviral activity. According to Vanden Berghe and colleagues (Vanden Berghe et al., 1986), the results of about 25 antiviral screening studies on over 900 plants in 150 families have appeared in the literature since 1958. Some 30% of the extracts were regarded as exhibiting some degree of *in vitro* or *in vivo* activity against one or more plant or animal viruses (Vanden Berghe et al., 1986). Not all of these leads have been followed up by activity-guided fractionation,

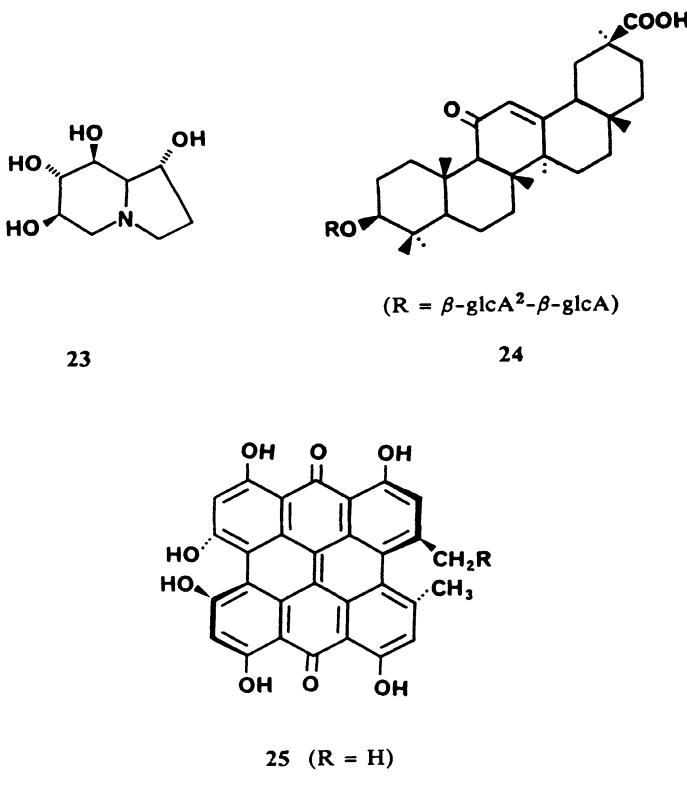
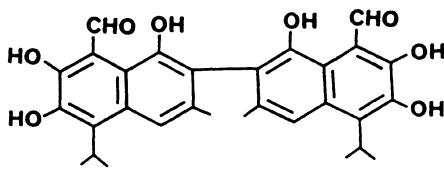


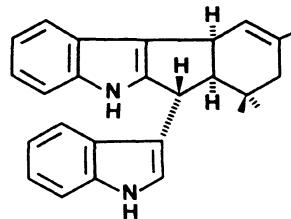
Figure 8. Structures of four plant constituents with activity against HIV-1: castanospermine [23], glycyrrhizin [24], hypericin [25], and pseudohypericin [26] (GlcA = glucuronic acid).

but of the plant-derived compound classes that have emerged as possessing antiviral activity against at least one type of virus, are alkaloids (especially those of the benzophenanthridine, indole, isoquinoline, purine, quinoline, and tropane types), amino acids, carbohydrates, cardiotonic glycosides, flavonoids, lignans, phenols, tannins, and terpenoids (Vanden Berghe et al., 1986).

Although AIDS has been recognized as a human disease condition only very recently, there has been an increasing interest from many scientific groups in the discovery of potential natural product anti-AIDS agents. Of great significance is the National Cancer Institute's program to screen extracts of plants, marine organisms, and microorganisms for anti-HIV activity, using a tetrazolium-based microculture assay (Booth, 1987; Gustafson et al., 1989). Among the pure substances from plants that have already been found to specifically inhibit HIV-1 are the alkaloid, castanospermine (Fig. 8 [23]), an indolizidine alkaloid first isolated by Bell and coworkers from *Castanosperma australe* A. Cunn. (Hohenschutz et al., 1981), that acts against HIV-1 as a glucosidase inhibitor (Tims and Taylor, 1988); the oleanane-type triterpene glycoside, glycyrrhizin (Fig. 8 [24]), which appears to inhibit HIV-1 cell binding (Ito et al., 1987); hypericin (Fig. 8 [25]) and pseudohypericin (Fig. 8 [26]), which are aromatic polycyclic diones from the herb, *Hypericum triquetrifolium* Turra, and affect the retroviral infection and replication cycle at two or more points (Lavie et al., 1989); and GLQ223, a purified preparation of the ribosome-inactivating



27



28

Figure 9. Structures of the plant-derived antifertility agents, gossypol [27] and yuehchukene [28].

protein, trichosanthin, isolated from tubers of the Chinese plant, *Trichosanthes kirilovii* Maxim., which reduces HIV-1 cellular protein and RNA levels (McGrath et al., 1989). The diversity of structure and mechanism of action of just these few examples is indicative of the great potentiality that the future systematic investigation of plant constituents as anti-AIDS compounds could well afford.

### Fertility-Regulating Activity

Although current indications are that the world population will eventually stabilize, there are now about 90 million more human births than deaths each year, and it is generally recognized that a continuing international effort is necessary to lower the global birth rate to an acceptable level (Potts, 1989). The investigation of plant constituents with antifertility effects represents a potential alternative approach to birth control than presently available methods such as the use of intrauterine devices and steroid oral contraceptives. There is an abundance of information available on the fertility-regulating properties of medicinal plants, both in terms of their ethnomedical uses and the laboratory investigation of their crude extracts (Soejarto et al., 1978), and many purified plant constituents are known to have antifertility properties (Bingel and Fong, 1988; Farnsworth et al., 1983; Kong et al., 1985; Waller et al., 1985). In addition, both *in vitro* and *in vivo* bioassays have been established that permit the activity-guided fractionation of antifertility agents from plant extracts as exemplified by the estrogenized guinea pig uterine strip method and the 22-day pregnant guinea pig assay, respectively (Bingel and Fong, 1988). Among the plant secondary metabolites that have produced a great deal of interest as antifertility agents are gossypol (Fig. 9 [27]) and yuehchukene (Fig. 9 [28]). Gossypol is a phenolic sesquiterpene dimeric constituent of the cotton plant (*Gossypium* species), and its propensity as a male contraceptive agent in humans was discovered in the People's Republic of China and reported in 1978 (Waller et al., 1985). This compound causes a decrease in the sperm count in males and produces infertility by one or more of several potential mechanisms (Waller et al., 1985). Despite its apparent effectiveness, several side effects have been noted in the clinical literature on gossypol, most particularly hypokalemia, lassitude, and occasional paralysis (Bingel and Fong, 1988; Waller et al., 1985). Yuehchukene, an unstable indole alkaloid obtained as a minor constituent of *Murraya paniculata* (L.) Jack roots, was reported as the first non-steroidal substance to demonstrate anti-implantation effects in pregnant rats at potentially non-estrogenic dose levels (Kong et al., 1985). This compound has not yet been tested in humans but, if it were found to be active, would probably require precise timing when administered (Bingel and Fong, 1988).

## Sweetening Activity

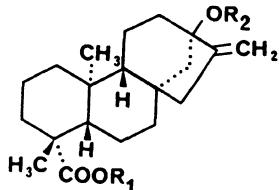
While the four types of biological activity that have been chosen hitherto in this section of the chapter refer to potential new plant-derived drugs, recent progress has also been made in the development of pharmaceutically significant plant constituents that can assist in the formulation of drugs. For example, many medicinal agents are bitter tasting, but their unpleasant taste can be masked with sweetening agents in order to improve their palatability. Unfortunately, it has been found that medicines sweetened with sucrose or fructose syrups tend to promote dental caries, especially in chronically sick children (Rekola, 1989). Thus, noncariogenic sweet substances have been sought to replace the use of fermentable carbohydrates in medicines.

Many naturally occurring and synthetic organic compounds have been found to exhibit a sweet taste, and the so-called high-intensity sweeteners are some 50 to 100 or more times sweeter than sucrose. Although a number of synthetic highly sweet compounds such as acesulfame K, aspartame, cyclamate, and saccharin are on the market in one or more countries as noncaloric sweeteners, each of these compounds is a less-than-perfect sweetener, either in terms of its cost of production, quality of taste, perceived safety, or chemical stability (Farber, 1990; Kinghorn and Compadre, 1985; O'Brien Nabors and Gelardi, 1986). Of the fifty or so of highly sweet substances that are known to be biosynthesized by plants, the diterpene glycosides stevioside (Fig. 10 [29]) and rebaudioside A (Fig. 10 [30]) hold considerable promise as sucrose substitutes (Kinghorn and Soejarto, 1989). These highly sweet *ent*-kaurene glycosides are constituents of a plant native to Paraguay, namely, *Stevia rebaudiana* (Bertoni) Bertoni, and are very unusual for secondary metabolites by occurring in very high yield in the leaves of the plant (typically over 8% w/w combined yield) (Kinghorn and Soejarto, 1985). It is pertinent to note that extracts and crystalline products made from *S. rebaudiana* now occupy over 40% of the high-intensity sweetener market in Japan despite having been introduced there less than 20 years ago (Anonymous, 1988). However, prior to the approval of the *S. rebaudiana* products for sweetening foods, beverages, and medicines in countries in North America and Europe, their safety needs to be more fully demonstrated than is the case at present (Farber, 1990; Kinghorn and Soejarto, 1989; Pezzuto, 1986).

## FUTURE PROSPECTS

The few examples of biologically active constituents of plants that have been chosen in this chapter can be seen already to profoundly affect the lives of humans or else have the possibility of doing so in the future. It can be confidently predicted that the scientific study of medicinal plants will continue to yield a dazzling array of structurally novel organic compounds which will be attractive candidates for inclusion in drug discovery programs in industrial, academic, and governmental settings. Providing that the approaches to this type of endeavor are multidisciplinary in nature and encourage the cooperation of botanists, chemists, and biologists, there is every reason to suppose that many new drugs, lead compounds, and biological tools (probes for investigating biochemical mechanisms) will continue to be discovered from plants.

There are several recent developments in the last few years that, when combined, offer better chances for discovering new plant drugs than ever before. For instance, there have been significant advances in biological test methodology so that less reliance needs to be placed on the somewhat cumbersome *in vivo* assay procedures formerly employed in the investigation of the biological activities of natural products. Thus, plant extracts and their purified components can be included in screens in which a large amount of biochemical information can be rapidly



	$R_1$	$R_2$
29	$\beta\text{-glc}$	$\beta\text{-glc}^2\text{-}\beta\text{-glc}$
30	$\beta\text{-glc}$	$\beta\text{-glc}^2\text{-}\beta\text{-glc}$ $\beta\text{-glc}$

Figure 10. Structures of the plant-derived sweetening agents, stevioside [29] and rebaudioside A [30] (Glc = glucose).

obtained, utilizing, for example, bioengineered or synthetic enzymes, substrates, inhibitors, and receptors. In addition, such data may be obtained with robotics and can be routinely handled with computers (Freter, 1987). Over the last few years, there has been a continual improvement in the sensitivity and capability of analytical methods used to characterize natural products. The refinements in nuclear magnetic resonance spectroscopy, in particular, permit the unambiguous structural identification of extremely complex plant constituents with just a few milligrams of material (Derome, 1989). The rational selection of plant material for investigation may be aided by computerized literature surveillance. For example, the NAPRALERT™ database (Loub et al., 1985) has been utilized to prioritize several hundred plants with fertility-regulating activity based on both ethnomedical reports and on the quality of existing scientific literature describing observations made in the laboratory by groups around the world (Farnsworth et al., 1983; Soejarto et al., 1978). The wealth of experience in the judicious use of herbal remedies in Chinese traditional medicine (Chen and Shen, 1986; Han, 1988; Klayman, 1985; Trigg, 1989; Wall et al., 1966; Waller et al., 1985; Wang and Liu, 1985; Xiao and Chen, 1987, 1988; Xiao and Fu, 1987) augurs well for the ultimate development of many useful substances in western medicine.

Despite the successful introduction of many plant-derived drugs into therapy, there are problems that serve to restrict their development. The investigation of biologically active natural compounds (from all sources) is often limited by their low concentration levels in the producing organism and also such substances may be thermally or hydrolytically unstable or have unfavorable solubility properties. Biological active plant constituents of interest can only be obtained in quantity on large-scale recollection of the plant part of origin. Frequently, due to biological variation, such recollection efforts do not result in the presence of the original biological activity and, hence, re-isolation studies for the compound of interest would prove fruitless. In addition, political unrest in the country of origin of a plant of interest may impede the further supply of source material. Alternatively, the species of origin of a promising natural product may not be available in large quantity without

disturbing the natural environment of the plant. These types of uncertainty are not appealing to pharmaceutical companies, where, typically, natural product drug development efforts tend to focus on microbial products, since the producing organisms of the latter can be cultured reliably in quantity by fermentation (Tyler et al., 1988). It is to be hoped that advances in plant tissue culture (Verpoorte et al., 1987) will be sufficient in the future to overcome the problem of supply of promising plant-derived compounds. However, the problem of lack of investment in plant drug discovery programs by the pharmaceutical industry is long-standing (Farnsworth, 1977) and will probably be overcome only after promising compounds are obtained in laboratories elsewhere. The threatened extinction of over 10% of the world's flora in the next few years, particularly in tropical rain forests (Principe, 1989), along with the loss of folkloric information as primitive cultures change their way of life (Plotkin, 1988) are both of extreme concern in the context of the development of plant-derived drugs. Conservation efforts are urgently necessary so that endangered genetic resources can be preserved for future generations of scientists to investigate more thoroughly than is possible at present (Balandrin and Klocke, 1988).

Despite these types of problems, enthusiasm remains high among those involved in the discovery of new biologically active natural products from plants and other sources. Not only is this type of multidisciplinary study of considerable inherent interest, but there exists a very real opportunity to discover chemical entities that may cure some of the most threatening forms of disease known to mankind.

## REFERENCES

- Alley, M. C., Scudiero, D. A., Monks, A., Mursey, M. L., Czerwinski, M. C., Fine, D. L., Abbott, B. J., Mayo, J. G., Shoemaker, R. H., and Boyd, M. R., 1988, Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay, *Cancer Research*, 48:589-601.
- Anonymous, 1988, High sweeteners - market size 7.2 billion yen. Stevia occupies 41%, but future gains will be made by aspartame, *Food Chemicals* (Tokyo), June issue, p. 19-26.
- Aronson, J. K., 1987, The discovery of the foxglove as a therapeutic agent, *Chemistry in Britain*, 23:33-36.
- Baerheim Svendsen, A., and Scheffer, J. J. C., 1982, Natural products in therapy. Prospects, goals and means in modern research, *Pharmaceutisch Weekblad Scientific Edition*, 4:93-103.
- Balandrin, M. F., and Klocke, J. A., 1988, Medicinal, aromatic and industrial materials from plants, p. 3-36, in: "Biotechnology in Agriculture and Forestry 4. Medicinal and Aromatic Plants I," Y. P. S. Bajaj, ed., Springer-Verlag, Berlin.
- Balandrin, M. F., Klocke, J. A., Wurtele, E. S., and Bollinger, W. H., 1985, Natural plant chemicals: Sources of industrial and medicinal materials, *Science*, 228:1154-1160.
- Baldwin, J. J., 1987, Drug design, p. 33-71, in: "Drug Discovery and Development," M. Williams and J. B. Malick, eds., Humana Press, Clifton, New Jersey.
- Bingel, A. S., and Fong, H. H. S., 1988, Potential fertility-regulating agents from plants, p. 73-118, in: "Economic and Medicinal Plants Research," Volume 2, H. Wagner, H. Hikino, and N. R. Farnsworth, eds., Academic Press, London, United Kingdom.
- Blume, E., 1989, Investigators seek to increase taxol supply, *Journal of the National Cancer Institute*, 81:1122-1123.
- Booth, W., 1987, Combing the earth for cures to cancer, *AIDS, Science*, 237:969-970.

- Boyd, M. R., and Wilson, B. J., 1972, Isolation and characterization of 4-ipomeanol, a lung-toxic furanoterpenoid produced by sweet potatoes (*Ipomea batatas*), Journal of Agricultural and Food Chemistry, 20:428-430.
- Brossi, A., Venugopalan, B., Dominguez Gerpe, L., Yeh, H. J. C., Flippen-Anderson, J. L., Buchs, P., Luo, X. D., Milhous, W., and Peters, W., 1988, Arteether, a new antimalarial drug: Synthesis and antimalarial properties, Journal of Medicinal Chemistry, 31:645-650.
- Bruce-Chwatt, L. J., 1988, Cinchona and its alkaloids: 350 years, New York State Journal of Medicine, 88:318-322.
- Chen, H., and Shen, C., 1986, Health care in China: A unique relationship between ancient and modern medicine, Impact of Science on Society, 143:275-286.
- Christian, M. C., Witles, R. E., Leyland-Jones, B., McLemore, T. L., Smith, A. C., Grieshaber, C. K., Chabner, B. A., and Boyd, M. R., 1989, 4-Ipomeanol: A novel investigational new drug for lung cancer, Journal of the National Cancer Institute, 15:1133-1143.
- Cragg, G., and Suffness, M., 1988, Metabolism of plant-derived anticancer agents, Pharmacology and Therapeutics, 37:425-461.
- De Clercq, E., 1989, New acquisitions in the development of anti-AIDS agents, Antiviral Research, 12:1-19.
- Derome, A. E., 1989, The use of N. M. R. spectroscopy in the structure determination of natural products: Two dimensional methods, Natural Products Reports, 6:111-141.
- de Souza, N. J., Ganguli, B. N., and Reden, J., 1982, Strategies in the discovery of drugs from natural sources, Annual Reports in Medicinal Chemistry, 17:301-310.
- Dohadwalla, A. N., 1985, Natural product pharmacology: Strategies in search of leads for new drug designs, Trends in Pharmacological Sciences, 6:49-53.
- Elliott, S., and Brimacombe, J., 1988, Tropical forests - nature's pharmacy, Manufacturing Chemist, October issue, p. 25-26.
- Farber, S. A., 1990, The price of sweetness, Technology Review, January issue, p. 46-53.
- Farnsworth, N. R., 1973, Importance of secondary plant constituents as drugs, p. 351-380, in: "Phytochemistry - III," L. P. Miller, ed., Van Nostrand Reinhold, New York, New York.
- Farnsworth, N. R., 1977, The current importance of plants as a source of drugs, p. 61-73, in: "Crop Resources," D. S. Seigler, ed., Academic Press, New York, New York.
- Farnsworth, N. R., 1984, The role of medicinal plants in drug development, p. 17-30, in: "Natural Products and Drug Development: Alfred Benzon Symposium 20," P. Krosgaard-Larsen, S. Brogger-Christensen, and H. Kofod, eds., Munksgaard, Copenhagen, Denmark.
- Farnsworth, N. R., 1987, International perspectives regarding the use of food/natural products as drugs, Drug Information Journal, 21:245-250.
- Farnsworth, N. R., and Bingel, A. S., 1977, Problems and prospects of discovering new drugs from higher plants by pharmacological screening, p. 1-22, in: "New Natural Products and Plant Drugs with Pharmacological, Biological, or Therapeutical Activity," H. Wagner and P. Wolff, eds., Springer-Verlag, New York, New York.
- Farnsworth, N. R., and Kaas, C. J., 1981, An approach utilizing information from traditional medicine to identify tumor-inhibiting plants, Journal of Ethnopharmacology, 3:85-89.
- Farnsworth, N. R., and Morris, R. W., 1976, Higher plants - the sleeping giant of drug development, American Journal of Pharmacy, 147:46-52.

- Farnsworth, N. R., Fong, H. H. S., and Diczfalusy, E., 1983, New fertility regulating agents from plants, p. 776-809, in: "Research on the Regulation of Human Fertility," Volume 2, E. Diczfalusy and A. Diczfalusy, eds., Scriptor, Copenhagen, Denmark.
- Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., and Guo, Z., 1985, Medicinal plants in therapy, Bulletin of the World Health Organization, 63:965-981.
- Freter, K. R., 1987, Drug discovery - today and tomorrow: The role of medicinal chemistry, Pharmaceutical Research, 5:397-400.
- Galeffi, C., and Marini-Bettolo, G. B., 1988, New approaches to the utilization of plants in the preparation of pharmaceuticals and pesticides, Fitoterapia, 54:179-205.
- Gerzon, K., 1980, Dimeric *Catharanthus* alkaloids, p. 271-317, in: "Anticancer Agents Based on Natural Product Models," J. M. Cassady and J. D. Douros, eds., Academic Press, New York, New York.
- Giovanella, B. C., Stehlin, J. S., Wall, M. E., Wani, M. C., Nicholas, A. W., Liu, L. F., Silber, R., and Potmesil, M., 1989, DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts, Science, 246:1046-1048.
- Gustafson, K. R., Cardellina, II, J. H., Fuller, R. W., Weislow, O. S., Kiser, R. F., Snader, K. M., Patterson, G. M. L., and Boyd, M. R., 1989, AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae), Journal of the National Cancer Institute, 81:1254-1258.
- Han, J., 1988, Traditional Chinese medicine and the search for new antineoplastic drugs, Journal of Ethnopharmacology, 24:1-17.
- Hartwell, J. L., 1968, Plants used against cancer. A survey, Lloydia, 31:71-74.
- Hite, G. J., 1988, Analgesics, p. 239-275, in: "Principles of Medicinal Chemistry," 3rd Edition, W. O. Foye, ed., Lea & Febiger, Philadelphia, Pennsylvania.
- Hohenschutz, L. D., Bell, E. A., Jewess, P. J., Leworthy, D. P., Pryce, P. J., Arnold, E., and Clardy, J., 1981, Castanospermine, a 1,6,7,8-tetrahydroxyoctahydroindolizidine alkaloid, from the seeds of *Castanosperma australe*, Phytochemistry, 20:811-814.
- Hosler, D. M., and Mikita, M. A., 1987, Ethnobotany: The chemist's source for the identification of useful natural products, Journal of Chemical Education, 64:328-332.
- Hussar, D. A., 1984, New drugs of 1983, American Pharmacist, NS24 (No. 3), p. 23-40.
- Hussar, D. A., 1987, New drugs of 1986, American Pharmacist, NS27 (No. 3), p. 26-61.
- Ito, M., Nakashima, H., Baba, M., Pauwels, R., De Clercq, E., Shiget, S., and Yamamoto, N., 1987, Inhibitory effect of glycyrrhizin on the *in vitro* infectivity and cytopathic activity of the human immunodeficiency virus [HIV (HTLV-III/LAV)], Antiviral Research, 7:127-137.
- Jardine, I., 1980, Podophyllotoxins, p. 319-351, in: "Anticancer Agents Based on Natural Product Models," J. M. Cassady and J. D. Douros, eds., Academic Press, New York, New York.
- Kinghorn, A. D., and Compadre, C. M., 1985, Naturally occurring intense sweeteners, Pharmaceutical International, 6:201-204.
- Kinghorn, A. D., and Soejarto, D. D., 1985, Current status of stevioside as a sweetening agent for human use, p. 1-52, in: "Medicinal and Economic Plant Research," Volume 1, H. Wagner, H. Hikino, and N. R. Farnsworth, eds., Academic Press, London, United Kingdom.
- Kinghorn, A. D., and Soejarto, D. D., 1989, Intensely sweet compounds of natural origin, Medicinal Research Reviews, 9:91-115.
- Klayman, D. L., 1985, *Qinghaosu* (Artemisinin): An antimalarial drug from China, Science, 228:1049-1055.

- Kong, Y. C., Cheng, K.-F., Cambie, R. C., and Waterman, P. G., 1985, Yuehchukene: A novel indole alkaloid with anti-implantation activity, *Journal of the Chemical Society, Chemical Communications*, p. 47-48.
- Krakoff, I. H., 1986, Cancer chemotherapy: Where are we today?, p. 17-23, in: "Cancer Chemotherapy: Challenges for the Future," K. Kimura, K. Yamada, I. H. Krakoff, and S. K. Carter, eds., *Excerpta Medica*, Amsterdam, The Netherlands.
- Labadie, R. P., 1986, Problems and possibilities in the use of traditional drugs, *Journal of Ethnopharmacology*, 15:221-230.
- Lavie, G., Valentine, F., Levin, B., Mazur, Y., Gallo, G., Lavie, D., Weiner, D., and Meruelo, D., 1989, Studies on the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin, *Proceedings of the National Academy of Sciences of the United States of America*, 86:5963-5967.
- Loub, W. D., Farnsworth, N. R., Soejarto, D. D., and Quinn, M. L., 1985, NAPRALERT: Computer handling of natural product research data, *Journal of Chemical Information and Computer Science*, 25:99-103.
- McGrath, M. S., Hwang, K. M., Caldwell, S. E., Galston, I., Luk, K.-C., Wu, P., Ng, V. L., Crowe, S., Daniels, J., Marsh, J., Deinhart, T., Lekas, P. V., Vennari, J. C., Yeung, H. W., and Lifson, J. D., 1989, GLQ223: An inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage, *Proceedings of the National Academy of Sciences of the United States of America*, 86:2844-2848.
- Midgley, J. M., 1988, Drug development: From sorcery to science, *Pharmaceutical Journal*, 241:358-365.
- Noble, R. L., Beer, C. T., and Cutts, J. H., 1958, Role of chance observations in chemotherapy: *Vinca rosea*, *Annals of the New York Academy of Sciences*, 76:882-894.
- Nogradi, T., 1985, "Medicinal Chemistry - A Biochemical Approach," Oxford University Press, New York, New York, p. 375-394.
- O'Brien Nabors, L., and Gelardi R. C., eds., 1986, "Alternative Sweeteners," Marcel Dekker, New York, New York, 355 p.
- O'Neill, M. J., and Phillipson, J. D., 1989, Plants as sources of antimalarial compounds, *Revista Latinoamericana de Quimica*, 20-23:111-118.
- Peters, C. M., Gentry, A. H., and Mendelsohn, R. O., 1988, Valuation of an Amazonian rainforest, *Nature*, 339:655-656.
- Pezzuto, J. M., 1986, Chemistry, metabolism and biological activity of steviol (ent-13-hydroxykaurene-16-en-19-oic acid), the aglycone of stevioside, p. 371-386, in: "New Trends in Natural Products Chemistry 1986," Atta-ur-Rahman and P. W. Le Quesne, eds., Elsevier Scientific Publishers, Amsterdam, The Netherlands.
- Phillipson, J. D., and Anderson, L. A., 1987, Plants as sources of new medicines, *Pharmaceutical Journal*, 239:662-666.
- Phillipson, J. D., and Anderson, L. A., 1989, Ethnopharmacology and western medicine, *Journal of Ethnopharmacology*, 25:61-72.
- Piot, P., Plummer, F. A., Mhalu, F. S., Lamboray, J.-L., Chin, J., and Mann, J. M., 1988, AIDS: An international perspective, *Science*, 239:573-579.
- Plotkin, M. J., 1988, Conservation, ethnobotany, and the search for new jungle medicines: Pharmacognosy comes of age...again, *Pharmacotherapy*, 8:257-262.
- Potts, M., 1989, Guest editorial, *Contraception*, 40:v-vi.
- Principe, P. P., 1989, The economic significance of plants and their constituents as drugs, p. 1-17, in: "Economic and Medicinal Plant Research," Volume 3, H. Wagner, H. Hikino, and N. R. Farnsworth, eds., Academic Press, London, United Kingdom.

- Rekola, M., 1989, *In vivo* acid production from medicines in syrup form, *Caries Research*, 23:412-416.
- Roche, E. B., Kier, L. B., and Foye, W. O., 1988, Parasite chemotherapy, p. 717-738, *in: "Principles of Medicinal Chemistry,"* 3rd Edition, W. O. Foye, ed., Lea & Febiger, Philadelphia, Pennsylvania.
- Schultes, R. E., 1972, The future of plants as sources of new biodynamic compounds, p. 103-124, *in: "Plants in the Development of Modern Medicine,"* T. Swain, ed., Harvard University Press, Cambridge, Massachusetts.
- Shuler, A. V., 1985, "Malaria, Meeting the Global Challenge," Agency for International Development, Oelgeschlager, Gunn & Hain, Boston, Massachusetts, 110 p.
- Sneader, W., 1985a, "Drug Discovery: The Evolution of Modern Medicines," John Wiley & Sons, Chichester, United Kingdom, p. 48-57.
- Sneader, W., 1985b, "Drug Discovery: The Evolution of Modern Medicines," John Wiley & Sons, Chichester, United Kingdom, p. 127-135.
- Soejarto, D. D., and Farnsworth, N. R., 1989, Tropical rain forests: Potential source of new drugs?, *Perspectives in Biology and Medicine*, 32:244-256.
- Soejarto, D. D., Bingel, A. S., Slaytor, M., and Farnsworth, N. R., 1978, Fertility regulating agents from plants, *Bulletin of the World Health Organization*, 56:343-352.
- Spilker, B., 1989, "Multinational Drug Companies: Issues in Drug Discovery and Development," Raven Press, New York, New York, p. 27-76.
- Spjut, R. W., 1985, Limitations of a random screen: Search for new anticancer agents in higher plants, *Economic Botany*, 39:266-288.
- Spjut, R. W., and Perdue, R. E., Jr., 1976, Plant folklore: A tool for predicting sources of antitumor activity?, *Cancer Treatment Reports*, 60:979-985.
- Steiner, R. P., ed., 1986, "Folk Medicine: The Art and the Science," American Chemical Society, Washington, D.C., 223 p.
- Svoboda, G. H., 1961, Alkaloids of *Vinca rosea* (*Catharanthus roseus*). IX. Extraction and characterization of leurosidine and leurocristine, *Journal of Pharmaceutical Sciences*, 24:173-178.
- Svoboda, G. H., Neuss, N., and Gorman, M., 1959, Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don.). V. Preparation and characterization of alkaloids, *Journal of American Pharmaceutical Association Scientific Edition*, 48:659-666.
- Tims, A. S., and Taylor, D. L., 1988, Activity of glucosidase inhibitors against HIV infections, *Journal of Antimicrobial Chemotherapy*, 22:271-274.
- Trigg, P. I., 1989, Qinghaosu (artemisinin) as an antimalarial drug, p. 19-55, *in: "Economic and Medicinal Plant Research,"* Volume 3, H. Wagner, H. Hikino, and N. R. Farnsworth, eds., Academic Press, London, United Kingdom.
- Tyler, V. E., Brady, L. R., and Robbers, J. E., 1988, "Pharmacognosy," 9th Edition, Lea & Febiger, Philadelphia, Pennsylvania, 519 p.
- Vanden Berghe, D. A., Vlietinck, A. J., and Van Hoof, L., 1986, Plant products as potential antiviral agents, *Bulletin del'Institut Pasteur*, 84:101-147.
- Verpoorte, R., Harkes, P. A. A., and ten Hoopen, H. J. G., 1987, Plant cell cultures as a tool in the production of secondary metabolites. Prospects and problems, p. 263-281, *in: "Topics in Pharmaceutical Sciences 1987,"* D. D. Breimer and P. Speiser, eds., Elsevier Science Publishers, Amsterdam, The Netherlands.
- Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T., and Sim, G. A., 1966, Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*, *Journal of the American Chemical Society*, 88:3888-3890.

- Waller, D. P., Zaneveld, L. J. D., and Farnsworth, N. R., 1985, Gossypol: Pharmacology and current status as a male contraceptive, p. 87-112, in: "Economic and Medicinal Plants Research," Volume 1, H. Wagner, H. Hikino, and N. R. Farnsworth, eds., Academic Press, London, United Kingdom.
- Wang, Z.-G., and Liu, G.-Z., 1985, Advances in natural products in China, Trends in Pharmacological Sciences, 6:423-426.
- Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P., and McPhail, A. T., 1970, Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*, Journal of the American Chemical Society, 93:2325-2327.
- Xiao, P., and Chen, K., 1987, Recent advances in clinical studies of Chinese medicinal herbs. 1. Drugs affecting the cardiovascular system. Phytotherapy Research, 1:53-57.
- Xiao, P.-G., and Chen, K., 1988, Recent advances in clinical studies of Chinese medicinal herbs. 2. Clinical trials of Chinese herbs in a number of chronic conditions, Phytotherapy Research, 2:55-62.
- Xiao, P.-G., and Fu, S.-L., 1987, Pharmacologically active substances of Chinese traditional and herbal medicines, p. 1-55, in: "Herbs, Spices, and Medicinal Plants: Recent Advances in Botany, Horticulture, and Pharmacology," Volume 2, L. E. Craker and J. E. Simon, Oryx Press, Phoenix, Arizona.

## SOCIO-ECONOMIC POISONS: KHAT, THE NATURAL AMPHETAMINE

Rudolf Brenneisen

Institute of Pharmacy  
University of Berne  
Baltzerstrasse 5, CH-3012 Berne  
Switzerland

Mahmoud A. ElSohly

Research Institute of Pharmaceutical Sciences  
School of Pharmacy  
University of Mississippi  
University, MS 38677, and  
ElSohly Laboratories, Incorporated  
1215 1/2 Jackson Avenue  
Oxford, MS 38655

### INTRODUCTION

Natural products have long been used in medicine, and plants have been a major and essential source for medicinal agents. Examination of not-too-distant and well-respected pharmacopoeias, such as the British Pharmacopoeia (B.P.) and the United States Pharmacopoeia (U.S.P.), reveals the use of many crude plant extracts for the treatment of a wide range of ailments. Pure natural products have been isolated, purified, and standardized as part of our contemporary armamentum against disease. The list of naturally occurring substances used in medicine today is a long and well-respected one and encompasses such agents as quinine (antimalarial), vinblastine and vincristine (anticancer), reserpine (hypotensive), and digitoxin (cardiac glycoside), to name a few, in addition to the extensive list of antibiotics isolated from fermentation broth. Although interest in natural products declined in the last few years with much attention being given to totally synthetic drug molecules, natural products are still an important source for new prototype drugs. Two of the most recent examples are the antimalarial agent artemisinin (qinghaosu), isolated from *Artemisia annua* and the anticancer agent taxol, which showed significant activity against ovarian cancer in clinical trials.

Because of the potential for discovery of new prototype chemical structures from plants, natural products are regaining their position as an important source for truly useful medicinal agents. With all that natural products have to offer for the benefit of humanity, some natural products possess certain characteristics which make

them true socio-economic poisons when used beyond prescribed limits. These are the naturally occurring "drugs of abuse" represented by marijuana, cocaine, opium (and derivatives thereof), and khat, which are used widely, not because of their medicinal values but because of their mind-altering (psychological) properties.

Since much is already known about marijuana, cocaine, and heroin, this chapter will focus on an old traditional drug, khat, which is slowly but surely gaining acceptance in the Western culture. Discussions will focus on the history, chemistry, pharmacology, and social and economic impact of the drug.

## HISTORY

Khat is commonly known as the leaves, tender twigs, young shoots, or stem tips of *Catha edulis* Forsk., an evergreen shrub or tree of the Celastraceae family. The family of Celastraceae includes 80 to 90 genera, mainly shrubs or trees, with over 850 species distributed in both hemispheres. The psychoactive fresh drug is traditionally chewed mainly by people living near cultivation areas to attain a state of euphoria and stimulation. Khat is regarded as a Muslim drug and alcohol substitute (Krikorian, 1984). Although Muslims have always been and still are the most desirous users of khat, the chewing of this drug now cuts through many faiths, social levels, and age groups (Krikorian, 1984).

The khat habit has, especially in Yemen, a deep-rooted social and cultural function (Weir, 1985) and is an appropriate tool for enhancing social interaction. The khat session also plays an important role at weddings and other family events (Schopen, 1978). In East Africa (Ethiopia, Djibouti, and Kenya), the psychosocial benefits of khat consumption are of secondary importance. It is rather the pharmacological action that induces the use of this drug (Kalix, 1988). Khat is frequently used in these countries during work by craftsmen, laborers, taxi and bus drivers, and especially by farmers in order to reduce physical fatigue (Getahun and Krikorian, 1973).

Although already known in the 14th century, the earliest scientific report about khat was in the 18th century by the Swedish botanist and physician Peter Forskal. His description was published posthumously 1775 in "Flora Aegyptiaco-Arabica" (Forskal, 1775; Krikorian, 1984). Most taxonomists consider that the genus *Catha* consists of the single species *Catha edulis*. However, several other *Catha* species have been mentioned in the literature: *Catha spinosa* Forsk. (Forskal, 1775; Revri, 1983), *Catha transvaalensis* Codd (Syn.: *Catha cassinoides* N.K.B. Robson) (Begley et al., 1990; Crombie et al., 1989; Demisew, 1984), and *Catha abbotii* Van Wyk & Prins (Van Wyk and Prins, 1987). More botanical and chemotaxonomic studies are necessary to clarify the status of these little known species.

Whereas the local names for khat in countries with Muslim tradition are variants of the word "cat," "qat," or "kat," black Africans have their own names. The Kenyan people, for example, call it "mirra," "mira," "mirungi," or "muirungi" (Krikorian, 1984); in Tanzania, khat is known as "muhulo" and in Uganda, as "musitate" (Geissbüsl, 1988). The plant itself is known by many names depending on the geographical area. The most common term is the Arabic name, "khat," sometimes also spelled "chat," the way it is pronounced in most of Ethiopia. In some literature, khat is also called Abyssinian, Arabian, or Somali tea (Getahun and Krikorian, 1973) but today ingestion by chewing is more popular.

The distribution of khat extends from the Arabian Peninsula (Yemen) to East Africa (Ethiopia, Somalia, Kenya, and Tanzania) to South Africa and the Cape. It was introduced into northern Madagascar and apparently occurs in Afghanistan and Turkestan (Krikorian, 1984). The main areas of commercial cultivation have

traditionally been in the Hararghe province of Ethiopia, the slopes of Taizz in Yemen, and the highlands in the Meru district in Kenya. Khat is quite adaptable to varying ecological conditions. It grows well under a wide range of soil types and climatic conditions; for example, on fairly moist slopes and hillsides at altitudes of 1500 to 2500 m above sea level, the plantations often terraced like South American coca. In Ethiopia, sorghum [*Sorghum bicolor* (L.) Moench], corn, and sweet potatoes are generally intercropped with khat. Even though the plant flowers and bears fruit, the seeds are not used for propagation. The breeding of khat is done vegetatively from suckers or branches arising near the ground level (Getahun and Krikorian, 1973). The first harvest usually begins 5 to 8 years after planting. *Catha edulis* is very polymorphic, especially concerning the shape, size, and color of its leaves (Schorno, 1982). In Ethiopia, three dominant types referred to as "white," "intermediate," and "red" are distinguished (Al-Meshal et al., 1985; Assefa, 1983; Geisshüsler and Brenneisen, 1987; Getahun and Krikorian, 1973). In other regions, additional types may be found (Krikorian, 1984). Usually, these types also differ in quality and psychotropic potency (Geisshüsler and Brenneisen, 1987). The shoots at the tips of the branches are cut in the early morning, bundled, and then usually wrapped in leaves of banana (*Musa x paradisiaca* L.) leaves of false banana (*Ensete ventricosum* (Welw.) Cheesm.), damp papers, or plastic to avoid drying and wilting.

Most authors believe khat to be of Ethiopian origin (Getahun and Krikorian, 1973; Schopen, 1978) and that khat was known and used on the Ethiopian uplands in very ancient times (Elmi, 1983). According to an old story, khat originated in Yemen. Its use was discovered by a herder who noticed the effect of the leaves of khat on his goats and tried them himself (Getahun and Krikorian, 1973). A fascinating, but obviously weak, hypothesis identified in khat the magic smoke that inspired the Delphic pythoness, Homer's "nepenthe" offered by Helen to Telemacus, an energetic medicine that Alexander the Great used to cure his army (Elmi, 1983). Nonetheless, chewing of khat leaves has been practiced for many centuries (Krikorian, 1984) and it is well accepted that khat drinking and chewing is at least older than coffee drinking (Schorno, 1982).

In recent years, khat has made its appearance on local khat markets in regions far from the areas of cultivation, mainly due to the possibilities of air transportation. Shipments of khat have been observed by custom authorities in France, Italy, Switzerland, Great Britain, and the United States (Kalix and Braenden, 1985). Fresh khat is, for example, sold in London, where a legally non-restricted market and distribution network exists, and New York (Gough and Cookson, 1984, 1987; Kalix et al., 1991; Mayberry et al., 1984; McLaren, 1987). Its use is largely confined to those ethnic communities accustomed to its traditional use.

## Chemistry

The chemistry of khat has been an intriguing puzzle to both plant chemists and pharmacologists for more than a hundred years. The plant is alkaloid-containing; but in contrast to the situation with other alkaloid-containing stimulant plants, our knowledge of khat has progressed slowly and its interpretation has remained fairly controversial until about 10 to 15 years ago. Despite the progress achieved since the first attempt to characterize the active constituents, the actual active principle either was overlooked or simply escaped isolation and characterization for various reasons, including poor quality of the starting material, inadequate isolation procedures, and insufficient purity of the final product. Thus, until recently, the characteristic stimulant activity of the fresh plant material could not be fully explained in terms of the then known khat components.

The chemical study of khat goes back to the year 1887 when Flückiger and Gerock (1887), searching for caffeine as the possible stimulating principle, found no traces of it but discovered instead an alkaloid they named katin. Mosso (1891) extracted from the plant a basic fraction with stimulant-like properties and called it celastrine. However, the first comprehensive study on khat was carried out by Beitter (1900, 1901), who obtained crystalline salts of a substance he concluded was identical to both Flückiger's katin and Mosso's celastrine. Beitter additionally mentioned the presence of an essential oil as well as tannins, sugars, and latex-like compounds. The chemical composition of khat was next studied by Stockman (1912a,b), who described the three distinct alkaloids, cathine, cathinone, and cathidine, without characterizing them structurally. Although the cathine he described was in all probability identical to the substance found by former workers, neither cathinone nor cathidine was later identified as an individual substance. Nevertheless, they have frequently been mentioned in the literature. An important step forward was the contribution of Wolfes (1930), who, using a technique similar to Beitter's, detected the presence of (+)-norpseudoephedrine (Fig. 1 [3]) in khat and concluded that this substance corresponds to katin. This alkaloid is also sometimes referred to as "cathine" (Kalix and Braenden, 1985; Schorno et al., 1982). He also observed the presence of water-insoluble base which, like Stockman's cathidine, can be regarded as an impure representative of the polyester-type khat alkaloids. In subsequent studies, it was repeatedly stated that (+)-norpseudoephedrine was the main if not the only phenylalkylamine-type constituent present in the plant. Whereas Alles et al. (1961) and Winterfeld and Bernsmann (1960) concluded that cathine was the only extractable base present in substantial amounts in the plant, several authors were able to demonstrate the presence of other alkaloidal compounds. Depending upon the extraction and chromatographic procedures, Paris and Moyse (1957, 1958) detected three to six alkaloids. They suggested that one of the components might be ephedrine, a suggestion which was repeated by Ristic and Thomas (1962), Karawya et al. (1968), and Elkley et al. (1968), but which could never be substantiated by unequivocal isolation and characterization procedures. Karawya and his coworkers separated three alkaloidal products in addition to cathine and ephedrine: cathinone, cathidine, and eduline. However, no structures were proposed for them. Later Rücker et al. (1973), using gas-liquid chromatography/mass spectrometry (GLC/MS), indicated the absence of ephedrine but the presence of seven nitrogen-containing substances in the basic fraction. Again, with the exception of cathine, none was adequately characterized. As mentioned earlier, most authors concluded that the characteristic stimulating effects of khat could be satisfactorily explained by its cathine content. The first to question this conclusion and suggest that the chemistry of khat might be more complex than expected was Von Brücke (1941). On the basis of simple pharmacological experiments, he felt that the stimulating effect of cathine was too small to be alone responsible for the effect of the fresh plant. He suggested the possible presence of a substance with a more powerful stimulating action. His suggestion was partially supported by Alles et al. (1961) and by the fact that consumers show preference and pay a higher price for fresh khat. Most of the earlier chemical and pharmacological studies were performed on dried plant material of varying quality. The first serious attempt to resolve this question was undertaken by Brilla (1962) and Friebel and Brilla (1963), who searched for a specific substance in the fresh plant that might have a greater activity than cathine. Using a combination of chemical and pharmacological methodology, they compared the effect on locomotor

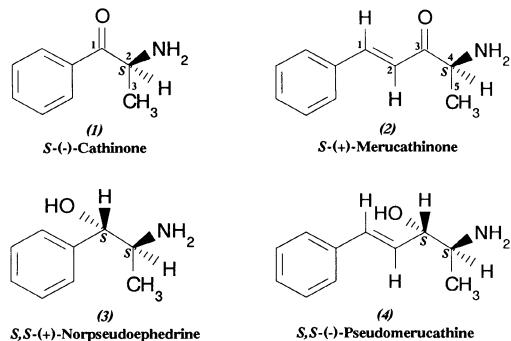


Figure 1. Structural formulas of cathinone [1], *S*-(+)-merucathinone [2], *S,S*-(+)-norpseudoephedrine [3], and *S,S*-(-)-pseudomerucathine [4].

activity of synthetic (+)-norpseudoephedrine oxalate with that of the oxalates prepared from freeze-dried and air- and sun-dried khat samples. The three preparations had qualitatively similar effects, but the oxalate from the freeze-dried plant sample showed a stronger effect on the locomotor activity. Differences were also found in the physical and chemical characteristics of the samples, and the authors concluded that the substance isolated from the freeze-dried plant was a cathine-like compound, possibly a labile precursor of cathine, for which no correct structure could be proposed on the basis of the data available. The earlier indications of the presence of water-insoluble alkaloids in khat, such as cathidine (Stockman, 1912a), were checked by Cais et al. (1964), who carried out extensive extraction and separation experiments on a weakly basic khat alkaloid fraction they described as a mixture of closely related alkaloids. It seems evident now that Stockman's cathidine was the earliest finding of a second class of *N*-containing khat constituents: weakly basic, fairly lipophilic polyester alkaloids that have repeatedly been overlooked by most investigators because of their non-typical alkaloid nature. One of these compounds, named cathidine D, was isolated in crystalline form and a partial structure proposed for it. It was suggested that this alkaloid, because of its high molecular weight and complicated nature, might be related to alkaloids like evonine and maytoline originating from the same plant family. Unfortunately, no alkaloid structure of this type was known at that time, mainly because of the lack of adequate techniques for structure determination. A new area in this field began in 1970 with the determination of the chemical structures of maytoline and evonine. These structures served as keys for a rapidly increasing number of related structures from various plants of the Celastraceae family. On this basis, Cais et al. (1975) proposed two tentative structures for cathidine D. As a result of joint effort between the Chemistry Department of the Nottingham University (Baxter et al., 1976a,b, 1979a,b; Crombie 1980; Crombie et al., 1979) and Szendrei at the UN Narcotics Laboratory (UN Document, 1976, 1977), a series of new polyester alkaloids named cathedulins K1 (Fig. 4 [13]), K2, K5, K12 (Fig. 4 [14]), K15, E2, E3 (Fig. 5 [15]), E4, E5 (Fig. 5 [16]), E6, and E8—polyesters or lactones of sesquiterpene polyols with molecular weights in

the range of 600 to 1200 have been isolated. Until now, no adequate pharmacological testing has been done on these cathedulin-type alkaloids due to their extremely poor water solubility and lack of sufficient amounts of pure material.

Chemical research on khat was taken up again by several groups in the early seventies. The UN Narcotic Laboratory in Geneva, on the recommendation of WHO and the UN Commission on Narcotic Drugs, initiated a research project on the chemical composition of khat involving several major classes of secondary plant products present in the fresh or well-preserved material. A simple thin-layer chromatography (TLC) analysis of the methanolic crude extract clearly showed that, besides varying amounts of (+)-norpseudoephedrine and other minor amine-type components, an unidentified compound was present in large quantities. This was separated and rapidly identified as (-)- $\alpha$ -aminopropiophenone. For this new compound, the trivial name cathinone (Fig. 1 [1]) was proposed (UN Document, 1974, 1975). Independent from the UN group around Szendrei, research work on khat was started by Schorno. The pharmacognostical and phytochemical thesis, finished in 1979, brought forward important new knowledge about the analysis, stereochemistry, and synthesis of the khatamines (Schorno, 1979; Schorno and Steinegger, 1979). The three main khat phenylpropylamines were isolated and identified as *S*(-)- $\alpha$ -aminopropiophenone (cathinone) (Fig. 1 [1]), *S,S*(+)-norpseudoephedrine (norpseudoephedrine) (Fig. 1 [3]), and *R,S*(-)-norephedrine (norephedrine) (Fig. 2 [5]). The *S* configuration at C-2 of the side chain is also true for all other known naturally occurring khat phenylalkylamines as well as the natural *R,S*(-)-ephedrine (Fig. 3 [9]) and the synthetic *S*(+)-amphetamine (Fig. 2 [8]). The presence of the respective *N*-methylated derivatives, e.g. ephedrine, postulated by Ristic and Thomas (1962) and Karawya et al. (1968), could not be confirmed. Synthetic racemic cathinone and its optically pure enantiomers have been known for almost 100 years (Al-Meshal et al., 1987; Berrang et al., 1982; Gabriel, 1908; McClure et al., 1981; Wolf and Pfander, 1986). Related synthetic products, for example diethylpropione (Fig. 3 [10]), the *N,N*-diethyl derivative of cathinone, are still used as stimulants of the central nervous system (CNS) or anorectic drugs. Cathinone as a ketoamine base is extremely unstable. Withering, drying, and cleanup of the plant material result in various degradation products or artifacts. Enzymatic reduction transforms cathinone into the less active norpseudoephedrine and norephedrine. Thus, a fresh drug may contain one hundred times more cathinone than dried material, which in turn shows an increased content of norpseudoephedrine and norephedrine. The oxidation product 1-phenyl-1,2-propandione (Fig. 3 [11]) can even be detected in the essential oil from fresh plants.

It is also possible that this diketone is formed by enzymatic desamination or by photolysis. The cathinone dimers 3,6-dimethyl-2,5-diphenylpyrazine (Fig. 3 [12]) and its dihydro derivatives, however, are purely artifacts of the isolation. The instability of the khat principle cathinone explains why about 100 years of chemical research were needed to identify this rather simple component and why khat users insist on fresh drug material. Due to the easy enolization of the keto group, cathinone racemizes quickly, particularly as a free base and in polar solvents. In contrast, its salts (e.g., oxalates) are stable in solid form.

Further analytic studies of the CNS-active khatamines from khat samples of varying origin indicated the presence of other amines in addition to the known phenylpropylamines, cathinone, norpseudoephedrine, and norephedrine (Schorno et al., 1982). This was deduced from chromatographic and spectroscopic data. Three new phenylpentenylamines were subsequently isolated from fresh plant material which

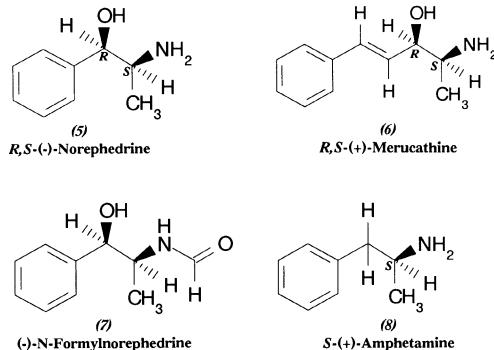


Figure 2. Structural formulas of *R,S*-(-)-norephedrine [5], *R,S*-(+)-merucathine [6], (-)-*N*-formylnorephedrine [7], and *S*-(+)-amphetamine [8].

was cultivated in the Meru area, North Kenya, sold in Nairobi's khat street market, and identified as *S*-(+)-merucathinone (merucathinone) (Fig. 1 [2]), *S,S*-(+)-pseudomerucathine (pseudomerucathine) (Fig. 1 [4]), and *R,S*-(+)-merucathine (merucathine) (Fig. 2 [6]) (Brenneisen and Geissbüsl, 1987; Brenneisen et al., 1984; Geissbüsl, 1988). Merucathine, the *trans*-olefinic cathinone analog, has already been postulated by Szendrei (1980) as cinnamoylethylamine, based on GLC/MS data. (-)-*N*-Formylnorephedrine (Fig. 2 [7]) was identified in Saudi Arabian khat (Al-Meshal et al., 1986).

The nitrogen-free khat fraction is mainly composed of polyphenols and terpenes. The tannins were found to be of flavonoid nature: glycosides of kaempferol, quercetin, and myricetin (El Sissi and Abd Alla, 1966). Qédan (1972) analyzed the volatile components of khat by TLC and gas-liquid chromatography (GLC) and found about 40 components in the essential oil distilled from the plant. Eleven of these compounds were identified as monoterpenes (ocimene,  $\beta$ -phellandrene, terpinolene,  $\alpha$ -/ $\beta$ -pinene, nerol, linalool,  $\alpha$ -terpineol,  $\alpha$ -/ $\beta$ -thujone, fenchone, etc.).

High-performance liquid chromatography (HPLC) is the analytical method of choice for determination of khat phenylalkylamines in biological matrix (plant, body fluids) (Brenneisen and Geissbüsl, 1985; Brenneisen et al., 1986, 1991; Schorno et al., 1982), whereas TLC may be used for rapid screening and preliminary identification of khat samples (Lehmann et al., 1990). GLC and GLC/MS on capillary columns have been especially used for creating qualitative khatamine profiles and for structure elucidation but require prior derivatization of the khat extracts (Brenneisen et al., 1985; Geissbüsl, 1988; Szendrei, 1980).

Khat samples from the most important markets of Ethiopia, Kenya, North Yemen, and Madagascar were analyzed by HPLC for their khatamine content (Brenneisen and Geissbüsl, 1985; Geissbüsl and Brenneisen, 1987; Schorno et al., 1982). The aim of these studies was not only to screen for psychoactive alkaloids but also to investigate its distribution in the plant tissue and the influence of origin, type, age, and time of harvesting on the variation of the phenylalkylamine profiles. Another point was to look for a possible correlation between cathinone content and khat quality—meaning "psychotropic potency" and price—as estimated by dealers and

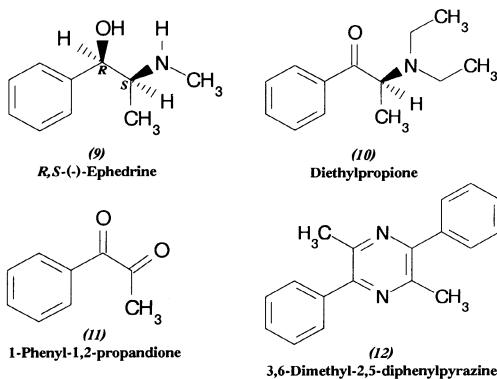


Figure 3. Structural formulas of *R,S*-(-)-ephedrine [9], diethylpropione [10], 1-phenyl-1,2-propandione [11], and 3,6-dimethyl-2,5-diphenylpyrazine [12].

consumers. Analyses made on different parts of Kenyan khat trees showed that the absolute content and the percentage (relative to the total khatamine content) of cathinone varied with the age of the respective plant part and with its metabolic state. The highest concentration of phenylpropylamines (cathinone + norpseudoephedrine + norephedrine: 290 mg/100 g plant material, calculated per dry weight) was found in young shoots already carrying leaves. In young shoot tops with a high cell differentiation rate, the total khatamine content was lower (190 mg/100 g) but the cathinone percentage was significantly higher (51%). In the fully developed leaves, the content was still 170 mg/100 g, the cathinone percentage however reduced to 2.4%. Stem and bark of older branches showed only traces of cathinone, whereas in the bark of the trunk and in the roots, no khatamines could be detected. A considerable variation in the khatamine content, depending on the origin, could be observed. Khat bundles collected from trees cultivated in the Meru region (Kenya) and sold in Nairobi's street market contained, for example, a higher amount of phenylalkylamines and of cathinone than commercial khat samples from Madagascar collected from shrubs. The total amine content varied from 20 to 96 mg/100 g khat, the cathinone content from 9 to 330 mg/100 g. Norpseudoephedrine and norephedrine varied in the range of 5 to 750 and 0.7 to 80 mg/100 g, respectively. The amount of the three phenylpentenylamines, merucathinone, merucathine, and pseudomerucathine, together varied between < 0.1 and 56 mg/100 g. With a cathinone content up to 330 mg/100 g and a very high percentage of cathinone (mean: 48%, relative to the total khatamine content), Kenyan khat was the most potent of all samples analyzed. Although the percentage of cathinone in Ethiopian khat was low (mean: 18%), a cathinone content of 30 to 190 mg/100 g showed its high potency. Much higher norpseudoephedrine percentages (up to 89%) were found than in khat from Kenya so that norpseudoephedrine might participate in the CNS-activity of the drug. The cathinone content of khat originating from North Yemen and Madagascar was significantly lower than that of Kenya or Ethiopia. It was not at all surprising that the quality estimation, especially of Ethiopian and Kenyan consumers, and the price

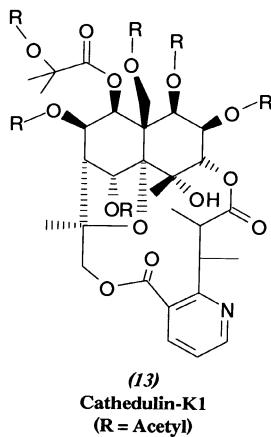
paid for the khat samples correlated nicely with the analytically determined content of the total phenylalkylamines and of cathinone. It should be noted that although some dealers claim to sell "first quality," real top quality does not appear on the market as it is consumed by the khat producers themselves. In addition, prices fluctuate in accordance with drug supply, which depends on season, meteorological conditions, distance from the cultivation area to the market, and efficiency of the transportation facilities.

## Pharmacology

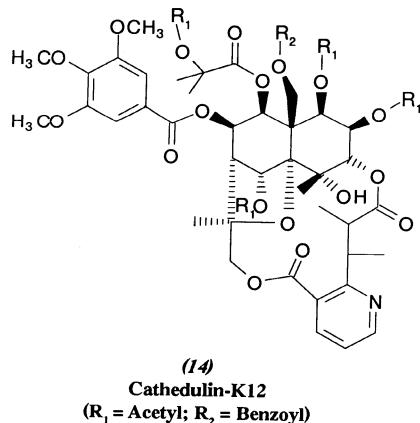
For consumption, freshly harvested twigs are rapidly transported to the markets where they are sold in bundles that are wrapped in order to preserve freshness. Khat is generally accepted as fresh for up to 4 days (Krikorian, 1984). Habitual khat users prefer the tender leaves from the tips of the branches and those of young shoots since these are the most potent ones (Geisshäuser and Brenneisen, 1987; Schorno et al., 1982). The size of a portion of khat varies within 50 to 200 g. Excessive daily doses of five 500 g khat bundles are not uncommon (Krikorian, 1984). The leaves are removed from the branches one by one and a large wad is placed in the mouth. After thoroughly masticating the leaves for about 10 minutes, the juice is swallowed with the saliva, whereas the residue of leaves is stored in the cheek as a bolus of macerated material for further extraction, making a characteristic bulge in the cheek of the chewer. In Ethiopia, swallowing of the mass is the usual practice, whereas in Yemen, the residue is finally spit out. Often, cigarettes are smoked while chewing (Getahun and Krikorian, 1973). Most people trying khat for the first time do not appreciate the bitter taste and do not experience the typical and pleasurable stimulating effects of khat (Krikorian, 1984).

The pleasurable as well as the unwanted effects of khat chewing are very similar to those induced by amphetamine consumption, the differences rather being quantitative than qualitative (Eddy et al., 1965; Hodgkinson, 1962; World Health Organization, 1964). Self-experiments of Alles et al. (1961) and Hughes (1973) have shown that the effects of one khat portion were similar to that of 5 to 10 mg amphetamine. The syndrome induced by khat chewing is mainly characterized by stimulating effects on the central nervous system (CNS) and various peripheral sympathomimetic effects. The social environment in which khat is consumed, for example the setting of a typical khat session, plays a certain role in the development and type of the psychoactivity of this drug (Kalix and Braenden, 1985). The CNS action of khat gives, particularly to habitual users, the subjective and euphorogenic feeling of increased energy, of well-being, mental alertness, and self-confidence. It improves the ability to communicate and enhances the imaginative ability and capacity to associate ideas (Elmi, 1983; Kalix, 1988; Kalix and Braenden, 1985). Objectively, khat induces a state of mild euphoria and excitement characterized by loquacity, sometimes hyperactivity (Kalix, 1988; Kalix and Braenden, 1985), and hypomania (Laurent, 1962; Margetts, 1967).

Late and unwanted effects of khat consumption are sleeplessness with following disruption of day-night cycle (Kalix, 1988), nervousness, and nightmares (Elmi, 1983; Kennedy, 1987; Pantelis et al., 1989). In exceptional cases, khat may induce toxic psychosis, probably by enhancement of a subacute prepsychotic or psychotic condition (Kalix and Braenden, 1985). Several case reports of khat-induced toxic psychosis describe the observed symptoms as manic-like, schizophreniform, and paranoid (Ardouin and Gendron, 1976; Carothers, 1945; Critchlow and Seifert, 1987;



(13)  
Cathedulin-K1  
(R = Acetyl)



(14)  
Cathedulin-K12  
(R<sub>1</sub> = Acetyl; R<sub>2</sub> = Benzoyl)

Figure 4. Structural formulas of cathedulin-K1 [13] and cathedulin-K12 [14].

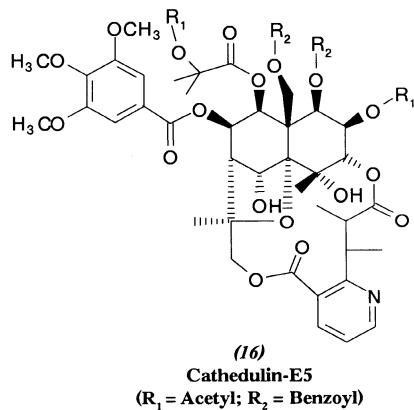
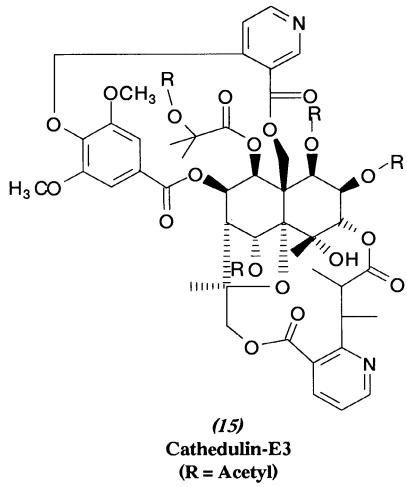


Figure 5. Structural formulas of cathedulin-E3 [15] and cathedulin-E5 [16].

Dhadphale et al., 1981; Giannini and Castellani, 1982; Gough and Cookson, 1984; Heisch, 1945; McLaren, 1987). Other serious psychic effects are reactive depression, anxiety, and irritation (Elmi, 1983).

Khat induces moderate but often psychic dependence but, in contrast to amphetamine abuse, no physical dependence or tolerance to the CNS-effects (Eddy et al., 1965). The ingestion and absorption of the active khat alkaloids are limited by the bulkiness of the plant material to be chewed. The physical limits on the amount of khat that can be chewed make an increase of the dose difficult, and this probably explains why tolerance to the CNS effects of this drug does not seem to occur. Heavy khat chewers experience true, but not physical, withdrawal symptoms. They consist of lethargy, mild depression, slight trembling, and recurrent bad dreams (Giannini et al., 1986; Halbach, 1972; Kennedy, 1987; Kennedy et al., 1980; Luqman and Danowski, 1976). In 1973, the WHO Expert Committee on Drug Dependence classified khat as a "dependence-producing drug."

Peripheral side effects of khat include cardiovascular effects of the sympathomimetic type, like arrhythmia, tachycardia (Elmi, 1983), and dose-dependent increase of blood pressure (Halbach, 1972; Kalix and Braenden, 1985; Nencini et al., 1984). Changes in pulse rate and blood pressure appear to be less pronounced in chronic users, which would indicate the development of tolerance to the sympathomimetic effects of khat (Nencini et al., 1984). Another sympathomimetic effect of khat is mydriasis (Halbach, 1972; Margetts, 1967; Nencini et al., 1984) which, along with a staring look and the brownish staining of the teeth, is characteristic for the khat habit (Luqman and Danowski, 1976).

Side effects of khat on the digestive tract are mainly due to the high tannin content. Inflammations of mouth, gastric disturbances, and constipation resulting often in high laxative use (Pantelis et al., 1989) are common among khat users (Elmi, 1983; Getahun and Krikorian, 1973; Halbach, 1972; Kalix and Braenden, 1985; Kennedy et al., 1983; Luqman and Danowski, 1976; Maresova, 1967). That the polyphenolic tannins increase the probability of oesophageal cancer is not proven (Kalix, 1987; Morton, 1980). Respiratory problems often result from associated heavy smoking during khat sessions (Kennedy et al., 1983). Furthermore, khat consumption may cause spermatorrhea, impair male sexual function, and after long-term use, lead to impotence (Halbach, 1972; Laurent, 1962; Le Bras and Frétilière, 1965; Margetts, 1967). Reduced birth weight of babies (Kalix, 1987) and inhibition of lactation (Luqman and Danowski, 1976) have been observed in khat-chewing pregnant women. As amphetamines, khat also increases the adrenocorticotrophic hormone and the growth hormone (Nencini et al., 1983).

The somatic, behavioral, CNS, and cellular effects of isolated or synthesized khat alkaloids have recently been reviewed in detail by Kalix (1988, 1990). The stimulating effect of khat was originally attributed to the phenylalkylamine norpseudoephedrine (Fig. 1 [3]). The detection of cathinone (Fig. 1 [1]) about 15 years ago initiated a series of pharmacological *in vitro* and *in vivo* studies with the synthesized substance. Table 1 demonstrates clearly the similarity of the pharmacological profiles of cathinone (Fig. 1 [1]) in animals and khat in humans. Furthermore, it shows cathinone as a potent amphetamine-like compound with indirect sympathomimetic action. In fact, from several *in vitro* experiments, it could be concluded that cathinone has the same mechanism of action as amphetamine, inducing the release of neurotransmitters (dopamine and noradrenaline) from storage sites in the central and peripheral nervous system. Animal studies indicated that cathinone is the dependence-producing constituent of khat. Among the three

phenylpropylamines (Fig. 1 [1,3] and Fig. 2 [5]), cathinone is the most lipophilic and CNS-potent alkaloid. At peripheral sites, however, it is equipotent with norpseudoephedrine and norephedrine, meaning that the latter two alkaloids, with considerably higher concentrations in khat compared to cathinone, are mainly responsible for the sympathomimetic side effects of khat on the cardiovascular system. The recently found khat alkaloids of the phenylpentenylamine type (Fig. 1 [2,4] and Fig. 2 [6]) play only a minor role concerning the psychoactive effects of khat. Kalix et al. (1987a,b) have shown with *in vitro* experiments a very low effect on the release of catecholamines from central and peripheral presynaptic storage sites. The structurally more complex cathedulins (sesquiterpene ester alkaloids) have not yet been pharmacologically tested.

### Social and Economic Impact

It can be estimated that each day 2 to 8 million khat portions are chewed worldwide (personal communication). The average price of a bundle with 50 g fresh, chewable material is about \$10 (U.S.) (Al-Meshal et al., 1985) and may climb up to \$60 (U.S.) depending on quality as well as supply and demand. The result of the diversion of income are often serious economic balance of payment problems, followed by neglect of the family needs, disintegration of the family, and sometimes divorce (Elmi, 1983; Kalix, 1987; Pantelis et al., 1989). Other profound social and economic consequences of khat consumption are due to absenteeism and decreased productivity, frequently leading to unemployment (Kalix and Braenden, 1985). Khat use has also an indirect negative effect on the public health system. Not all khat users have recreational motives since, particularly among the rural population, the drug is also used for improving work performance and to lessen the feeling of hunger (Kalix and Braenden, 1985). The loss of appetite leads to malnutrition, resulting in acute and chronic diseases, infective and/or deficiency disease. The rise of morbidity incriminates the public health system and increases the social assistance costs (Al-Meshal et al., 1985).

However, khat consumption undoubtedly shows certain beneficial consequences at the social level. It enhances the inter-individual communication and structures social life in rural and urban societies. It plays an important role, not only in family events (celebrations and marriages), but also in business matters and political meetings. Thus, prohibition of khat would imply the risk of interfering with a basic social and cultural institution of a country. In several cases, riots have followed such unpopular government decisions, illustrating that the ban of khat may be desirable from the socio-economic point of view but, at least in some countries, politically not feasible (Kalix, 1987).

Today, khat circulates freely in most East African countries and Yemen and, in some countries, khat chewing is allowed or tolerated. In others, the use is officially banned, but law enforcement is completely lacking. The prohibition of khat is difficult to enforce and has little effect. Decisions regarding its restriction have largely turned from physiological to psychosocial and, particularly, economic considerations (Krikorian, 1984). Better chances of success are the reduction of availability by restricting the trading of khat, like in North Yemen, where khat markets have been moved to the periphery of cities (Kalix and Braenden, 1985). In South Yemen, it is allowed to sell khat only on holidays. On recommendation of WHO, cathinone, the psychoactive principle of khat, has recently been put under international control and included in schedule I of the UN Convention on Psychotropic Substances. In

Table 1. Effects of khat and cathinone (Kalix, 1988).

Effects of khat chewing in humans	Effects of cathinone in animals
Anorexia	Anorexia (rat, monkey)
Insomnia, lack of fatigue	Restlessness (monkey)
Hyperactivity	Hypermotility (mouse, rat)
Excitation	Stereotyped oral activity (mouse, rat, rabbit)
Euphoria	
Logorrhea	
Hyperthermia	Hyperthermia (rabbit)
Increased respiration	Increased oxygen consumption (rat)
Mydriasis	Mydriasis (monkey)
Arrhythmias	Positive inotropic and chronotropic effect (guinea pig atrium)
Hypertension	Hypertension (cat)
Constipation (probably due to tannins)	Cathinone self-administration (monkey)
Compulsive khat consumption	

Switzerland, for example, cathinone since 1986 has been an illicit substance and can only be used for research purposes by special authorization of the Federal Health Authorities.

In North Yemen, the khat habit is a socially sanctioned, deep-rooted cultural tradition. Khat is tolerated by the Yemeni religious authorities, while alcohol is not (Kalix, 1987). Fifty percent of male adults are daily khat consumers and attend the famous afternoon khat sessions. Women's sessions are less formal and less frequent (Kalix and Braenden, 1985). About 25% of the daily earnings is spent for purchasing khat (Pantelis et al., 1989). A substantial part of the resources is diverted from backflow money of emigrant workers in Saudi Arabia (Kalix, 1987, 1988). More than 60% of the limited farmland is used for khat cultivation and not for crop production (Alhubaishi and Abdel-Kader, 1991). The gross agricultural income from khat is about \$1 billion (U.S.) (Alhubaishi and Abdel-Kader, 1991).

In Ethiopia, where khat is native, no legal restrictions for khat trade and consumption exist. Khat plantations occupy scarce arable land competing, especially in the Hararghe Province, with coffee for well-irrigated terraces (Getahun and Krikorian, 1973) as well as with other agronomical crops like sorghum and corn. In the Harrar district, khat is a high-cash income crop, providing 30 to 50% of the total annual cash income of a family (Getahun and Krikorian, 1973). Khat consumption is practiced by high school and university students as well as the general population (Kalix and Braenden, 1985). In Awedai, one of the most important khat markets of Ethiopia, 85% of the people are khat consumers (Assefa, 1983). From the nearby airport at Dire Dawa, khat is exported each day to Djibouti by a special aircraft service, contributing about \$20 million (U.S.) per year to the national economy (Kalix and Braenden, 1985).

Khat consumption in Djibouti is a legally non-restricted luxury habit since the arid climate does not allow its cultivation. Khat import from Ethiopia and khat trade are controlled by a private corporation. A special sales tax, initially introduced to

discourage consumption, now accounts for more than 10% of the government budget. The khat use is ubiquitous, 90% of men and 10% of women are daily or occasional users. Thirty percent of all wages is spent on khat purchases (Kalix and Braenden, 1985).

In Saudi Arabia, khat production, trade, and consumption were banned some 30 years ago by a royal decree, the ban still today strictly enforced (Kalix and Braenden, 1985). In Somalia, khat trade and use were prohibited in 1983. In Sudan, where khat grows in a small area in the south, cultivation, trade, and use are strictly forbidden. Several other Arab countries, like Kuwait, Egypt, and Morocco, have preventively banned this drug. From Israel, sporadic khat cultivation has been reported since the plant had been introduced by exiles from Yemen (Hes, 1970). In Kenya, the British authorities tried, in the early 1930s, to control khat use; but in 1977, President Kenyatta suspended the Miraa Prohibition Act (Krikorian, 1984). Although later the demand for a repressive legislation did not stop, khat is today extensively cultivated around Mount Kenya and sold daily on the important Nairobi khat market in the center of the city.

## REFERENCES

- Alhubaishi, A. A. A., and Abdel-Kader, M. I. A., 1991, Phyllosphere and phylloplane fungi of qat in Sana'a, Yemen Arab Republic, *Journal of Basic Microbiology* 31(2):83-89.
- Alles, G. A., Fairchild, M. D., and Jensen, M., 1961, Chemical pharmacology of *Catha edulis*, *Journal of Medicinal and Pharmaceutical Chemistry*, 3:323-352.
- Al-Meshal, I. A., Ageel, A. M., Parmar, N. S., and Tariq, M., 1985, *Catha edulis* (khat): Use, abuse and current status of scientific knowledge, *Fitoterapia*, 56:131-152.
- Al-Meshal, I. A., Nasir, M., and El-Ferally, F. S., 1986, (-)-N-Formylnorephedrine from *Catha edulis*, *Phytochemistry*, 25:2241-2242.
- Al-Meshal, I. A., Al-Rashood, K. A., Nasir, M., and El-Ferally, F., 1987, (-)-Cathinone: Improved synthesis and carbon-13 NMR assignments, *Journal of Natural Products*, 50:1138-1140.
- Ardouin, C., and Gendron, Y., 1976, Manifestations psychiatriques de l'intoxication au khat, *Médecine des Armées*, 4:407-410.
- Assefa, M., 1983, Socio economic aspects of khat in the Harrarghe Administrative Region (Ethiopia), p. 72-77, in: "The Health and Socio-Economic Aspects of Khat Use," B. Shahandeh, R. Geadah, A. Tongue, E. Tongue, and J. Rolli, eds., *Proceedings of First International Conference on Khat* (Antananarivo, Madagascar), International Council on Alcohol and Addictions, Lausanne, Switzerland.
- Baxter, R. L., Crombie, L., Simmonds, D. J., and Whiting, D. A., 1976a, Extractives of *Catha edulis* (Khat): Occurrence of Celastraceous alkaloids having mono- and bis-macrolide bridges, *Journal of the Chemical Society Chemical Communications*, 12:463-465.
- Baxter, R. L., Crombie, L., Simmonds, D. J., and Whiting, D. A., 1976b, Structures of cathedulin-2 and cathedulin-8, new sesquiterpene alkaloids from *Catha edulis*, *Journal of the Chemical Society Chemical Communications*, 12:465-466.
- Baxter, R. L., Crombie, L., Simmonds, D. J., Whiting, D. A., Braenden, O. J., and Szendrei, K., 1979a, The alkaloids of *Catha edulis* (khat). Part 1. Isolation and

- characterization of eleven new alkaloids with sesquiterpene cores (cathedulins) from Ethiopian, Kenyan, and Yemeni khat; identification of the quinone-methide root pigments, Journal of the Chemical Society Perkin Transactions I, 12:2965-2971.
- Baxter, R. L., Crombie, W. M. L., Crombie, L., Simmonds, D. J., Whiting, D. A., and Szendrei, K., 1979b, Alkaloids of *Catha edulis*. Part 4. Structures of cathaedulins E3, E4, E5, E6 and K12, Novel sesquiterpene alkaloids with mono- and bis-macrolide bridges, Journal of the Chemical Society Perkin Transactions I, p. 2982-2989.
- Begley, M. J., Crombie, L., Crombie, W. M. L., Toplis, D., and Whiting, D. A., 1990, The dihydroagarofuran esters of *Catha transvaalensis*, Planta Medica, 56:524-525.
- Beitter, A., 1900, Pharmakognostisch-chemische Untersuchung der *Catha edulis*, Thesis, Strasbourg.
- Beitter, A., 1901, Pharmakognostisch-chemische Untersuchung der *Catha edulis*, Archiv der Pharmazie, 239:17-33.
- Berrang, B. D., Lewin, A. H., and Carroll, F. I., 1982, Enantiomeric  $\alpha$ -aminopropiophenones (cathinone): Preparation and investigation, Journal of Organic Chemistry, 47:2643-2647.
- Brenneisen, R., and Geisshüsler, S., 1985, Psychotropic drugs III: Analytical and chemical aspects of *Catha edulis* Forsk, Pharmaceutica Acta Helveticae, 60:290-301.
- Brenneisen, R., and Geisshüsler, S., 1987, Phenylpentenylamines from *Catha edulis*, Journal of Natural Products, 50:1188-1189.
- Brenneisen, R., Geisshüsler, S., and Schorno, X., 1984, Merucathine, a new phenylalkylamine from *Catha edulis*, Planta Medica, 50:531.
- Brenneisen, R., Geisshüsler, S., and Schorno, X., 1986, Metabolism of cathinone to (-)-norephedrine and (-)-norpseudoephedrine, Journal of Pharmacy and Pharmacology, 38:298-300.
- Brenneisen, R., Mathys, K., Geisshüsler, S., Fisch, H. U., Koelbing, U., and Kalix, P., 1991, Determination of *S*-(*-*)cathinone and its main metabolite *R,S*-(*-*)norephedrine in human plasma by high-performance liquid chromatography and photodiode array detection, Journal of Liquid Chromatography, 14:271-286.
- Brilla, R., 1962, Ueber den zentralerregenden Wirkstoff der frischen Blätter von *Catha edulis* Forskal, Dissertation, University of Bonn, Germany.
- Cais, M., Ginsburg, D., and Mandelbaum, A., 1964, Abstracts of IUPAC Symposium on the Chemistry of Natural Products, Kyoto, p. 95.
- Cais, M., Ginsburg, D., and Mandelbaum, A., 1975, Constituents of *Catha edulis*. Isolation and structure of cathidine D, Tetrahedron, 31:2727-2731.
- Carothers, J., 1945, Miraa as a cause of insanity, East African Medical Journal, 22:4-6.
- Critchlow, S., and Seifert, R., 1987, Khat-induced paranoid psychosis, British Journal of Psychiatry, 150:247-249.
- Crombie, L., 1980, The cathedulin alkaloids, Bulletin on Narcotics, 32:37-50.
- Crombie, L., Crombie, W. M. L., Whiting, D. A., and Szendrei, K., 1979, Alkaloids of *Catha edulis*. Part 3. Structures of cathedulins K1, K2, K6 and K15; new macrolide-bridged polyesters of euonyminol, Journal of the Chemical Society Perkin Transactions I, p. 2976-2981.
- Crombie, L., Fleming, R. A., Toplis, D., and Whiting, D. A., 1989, Vaalens sesquiterpenes of *Catha transvaalensis*; structure of vaalens-5 using the COLOC n.m.r. pulse sequence, Journal of the Chemical Society Perkin Transactions I, p. 1700-1702.
- Demisew, S., 1984, Botanical aspects of "khat" (*Catha edulis*, Celastraceae), Proceedings of the International Symposium on Khat, Addis Ababa, p. 5-11.

- Dhadphale, M., Mengech, A., and Chege, S., 1981, Miraa (*Catha edulis*) as a cause of psychosis, East African Medical Journal, 58:130-135.
- Eddy, N., Halbach, H., Isbell, H., and Seevers, M., 1965, Drug dependence: Its significance and characteristics, Bulletin of the World Health Organization, 32:721-733.
- Elkiey, M. A., Karawya, M. S., and Ghourab, M. G., 1968, Estimation of the alkaloids of *Catha edulis* Forsk. growing in Egypt, Journal of the Pharmaceutical Sciences of the United Arab Republic, 9:159-169.
- Elmi, A., 1983, The chewing of khat in Somalia, Journal of Ethnopharmacology, 8:163-176.
- El Sissi, H. I., and Abd Alla, M. F., 1966, Polyphenolics of leaves of *Catha edulis*, Planta Medica, 14:76-83.
- Flückiger, F. A., and Gerock, J. E., 1887, Contributions to the knowledge of *Catha* leaves, Pharmaceutical Journal of Transvaal, 18:221-232.
- Forskal, P., 1775, Flora Aegyptiaco-Arabica, Carsten Niebuhr, ed., Den Haag, Holland, p. 63.
- Friebel, H., and Brilla, R., 1963, Ueber den zentralerregenden Wirkstoff der frischen Blätter und Zweigspitzen von *Catha edulis* Forsk., Naturwissenschaften, 50:354-355.
- Gabriel, S., 1908, Wandlungen der Aminoketone, Berichte der Deutschen Chemischen Gesellschaft, 41a:1127-1156.
- Geisshüsler, S., 1988, Zur Chemie, Analytik und Pharmakologie von Phenylalkylaminen aus *Catha edulis* Forsk. (Celastraceae), Dissertation, University of Berne, Switzerland.
- Geisshüsler, S., and Brenneisen, R., 1987, The content of psychoactive phenylpropyl and phenylpentenyl khatamines in *Catha edulis* Forsk. of different origin, Journal of Ethnopharmacology, 19:269-277.
- Getahun, A., and Krikorian, A. D., 1973, Chat: Coffee's rival from Harar, Ethiopia. I. Botany, cultivation and use, Economic Botany, 27:353-377.
- Giannini, A., Burge, H., Shaheen, J., and Price, W., 1986, Khat: Another drug of abuse?, Journal of Psychoactive Drugs, 18:155-158.
- Giannini, A., and Castellani, S., 1982, A manic-like psychosis due to khat, Journal of Toxicology and Clinical Toxicology, 19:455-459.
- Gough, S., and Cookson, I., 1984, Khat-induced schizophreniform psychosis in UK, Lancet, 8374:455.
- Gough, S., and Cookson, I., 1987, Khat-induced paranoid psychosis, British Journal of Psychiatry, 150:875-876.
- Halbach, H., 1972, Medical aspects of the chewing of khat leaves, Bulletin of the World Health Organization, 47:21-29.
- Heisch, R., 1945, A case of poisoning by *Catha edulis*, East African Medical Journal, 22:7-9.
- Hes, J., 1970, On the use of *Catha edulis*, Journal of the Israel Medical Association, 78:283-284.
- Hodgkinson, R., 1962, A study of the clinical effects of *Catha edulis* (khat, miraa) in Kenya, Medical Journal of Australia, 49:884-886.
- Hughes, P., 1973, Khat chewing in Yemen, Fourth International Institute on the Prevention and Treatment of Drug Dependence, International Council on Alcoholism and Addictions, ed., Lausanne, Switzerland, p. 32-46.
- Kalix, P., 1987, Khat: Scientific knowledge and policy issues, British Journal of Addiction, 82:47-53.
- Kalix, P., 1988, Khat: A plant with amphetamine effects, Journal of Substance Abuse Treatment, 5:163-169.

- Kalix, P., 1990, Pharmacological properties of the stimulant khat, *Pharmacology and Therapeutics*, 48:397-416.
- Kalix, P., and Braenden, O., 1985, Pharmacological aspects of the chewing of khat leaves, *Pharmacological Reviews*, 37:149-164.
- Kalix, P., Geissbüsl, S., and Brenneisen, R., 1987a, The effect of phenylpentenylkhatamines on the release of radioactivity from rat striatal tissue prelabelled with  $^3\text{H}$ -dopamine, *Journal of Pharmacy and Pharmacology*, 39:135-137.
- Kalix, P., Geissbüsl, S., and Brenneisen, R., 1987b, Differential effect of phenylpropyl- and phenylpentenyl-khatamines on the release of radioactivity from rabbit atria prelabelled with  $^3\text{H}$ -noradrenaline, *Pharmaceutica Acta Helveticae*, 62:332-334.
- Kalix, P., Geissbüsl, S., Brenneisen, R., Koelbing, U., and Fisch, H.-U., 1991, Cathinone, a phenylpropylamine alkaloid from khat leaves that has amphetamine effects in humans, *NIDA Research Monograph Series* (in press).
- Karawya, M. S., Elkay, M. A., and Ghourab, M. G., 1968, A study of the alkaloids of *Catha edulis* Forsk. growing in Egypt, *Journal of the Pharmaceutical Sciences of the United Arab Republic*, 9:147-157.
- Kennedy, J., 1987, The flower of paradise - the institutionalized use of the drug qat in North Yemen, D. Reidel, Dordrecht, The Netherlands.
- Kennedy, J., Teague, J., and Fairbanks, L., 1980, Qat use in North Yemen and the problem of addiction: A study in medical anthropology, *Culture, Medicine and Psychiatry*, 4:311-344.
- Kennedy, J., Teague, J., Rokaw, W., and Conney, E., 1983, A medical evaluation of the use of qat in North Yemen, *Society Science and Medicine*, 17:783-793.
- Krikorian, A. D., 1984, Kat and its use: An historical perspective, *Journal of Ethnopharmacology*, 12:115-178.
- Laurent, J.-M., 1962, Toxique et toxicomanie peu connus, le cath, *Annales Médico-Psychologiques*, 120:649-657.
- Le Bras, M., and Frétilière, Y., 1965, Les aspects médicaux de la consommation habituelle du cath, *Médecine Tropicale*, 25:720-732.
- Lehmann, T., Geissbüsl, S., and Brenneisen, R., 1990, Rapid TLC identification test for khat (*Catha edulis*), *Forensic Science International*, 45:47-51.
- Luqman, W., and Danowski, T., 1976, The use of khat in Yemen: Social and medical observations, *Annals of Internal Medicine*, 85:246-249.
- Maresova, Z., 1967, Kat, a little known stimulant, *Vnitri Lekarstvi*, 13:753-759.
- Margetts, E., 1967, Miraa and myrrh in East Africa - clinical notes about *Catha edulis*, *Economic Botany*, 21:358-362.
- Mayberry, J., Morgan, G., and Perkin, E., 1984, Khat-induced schizophreniform psychosis in UK, *Lancet*, 8374:455.
- McClure, D. E., Arison, B. H., Jones, J. H., and Baldwin, J. J., 1981, Chiral  $\alpha$ -amino ketones from the Friedel-Crafts reaction of protected amino acids, *Journal of Organic Chemistry*, 46:2431-2433.
- McLaren, P., 1987, Khat psychosis, *British Journal of Psychiatry*, 150:712-713.
- Morton, J., 1980, Search for carcinogenic principles, *Recent Advances in Phytochemistry*, 14:53-73.
- Mosso, U., 1891, Azione fisiologica del principio attivo de *Celastrus edulis*, *Revista clinica*, 30:65-79.
- Nencini, P., Anania, M., Abdullahi, M., Amiconi, G., and Elmi, A., 1983, Physiological and neuroendocrine effects of khat chewing in man, p. 148-152, in: "The Health and Socio-Economic Aspects of Khat Use," B. Shahandeh, R. Geadah, A. Tongue, E. Tongue, and J. Rolli, eds., *Proceedings of First International*

- Conference on Khat (Antananarivo, Madagascar), International Council on Alcohol and Addictions, Lausanne, Switzerland.
- Nencini, P., Ahmed, A., Amiconi, G., and Elmi, A., 1984, Tolerance develops to the sympathetic effects of khat in humans, *Pharmacology*, 28:150-154.
- Pantelis, C., Hindler, C. G., and Taylor, J. C., 1989, Use and abuse of khat (*Catha edulis*): A review of the distribution, pharmacology, side effects and a description of psychosis attributed to khat chewing, *Psychological Medicine*, 19:657-668.
- Paris, M. R., and Moyse, H., 1957, Essai de caractérisation du Kat ou thé des Abyssins (*Catha edulis* Forsk., Celastracées), drogue récemment inscrite au tableau B, *Annales Pharmaceutiques Françaises*, 15:89-97.
- Paris, M. R., and Moyse, H., 1958, Abyssinian tea (*Catha edulis* Forsk., Celastraceae), *Bulletin on Narcotics*, 10:29-34.
- Qédan, S., 1972, *Catha edulis*, eine wenig bekannte Rausch- und Genussdroge, *Planta Medica*, 21:410-415.
- Revri, R., 1983, *Catha edulis* Forsk.: Geographical dispersal, botanical, ecological and agronomical aspects with special reference to Yemen Arab Republic, Dissertation, University of Hohenheim, Germany.
- Ristic, S., and Thomas, A., 1962, Ueber die Inhaltsstoffe von *Catha edulis*, *Archiv der Pharmazie*, 295:524-525.
- Rücker, G., Kröger, H., Schikarski, M., and Quédan, S., 1973, Ueber die Alkaloide aus *Catha edulis*, *Planta Medica*, 24:61-65.
- Schopen, A., 1978, Das Qat, Geschichte und Gebrauch des Genussmittels *Catha edulis* Forsk. in der arabischen Republik Jemen, Research reports of the Department of Ethnology, Volume 8, University of Frankfurt, F. Steiner Verlag, E. Haberland, A. Kronenberg, W. Lindig, and K. E. Müller, eds., Wiesbaden, Germany.
- Schorno, X., 1979, Zur Pharmakognosie von *Catha edulis* Forsk. unter besonderer Berücksichtigung der ZNS-aktiven Phenylalkylamine, Dissertation, University of Berne, Switzerland.
- Schorno, X., 1982, Khat, Suchtdroge des Islams, *Pharmazie in unserer Zeit*, 11:65-73.
- Schorno, X., Brenneisen, R., and Steinegger, E., 1982, Qualitative und quantitative Untersuchungen über das Vorkommen ZNS-aktiver Phenylpropylamine in Handelsdrogen und über deren Verteilung in verschiedenen Organen von *Catha edulis* Forsk. (Celastraceae), *Pharmaceutica Acta Helveticae*, 57:168-176.
- Schorno, X., and Steinegger, E., 1979, CNS-active phenylpropylamines of *Catha edulis* Forsk. (Celastraceae) of Kenyan origin, *Experientia*, 35:572-574.
- Stockman, R., 1912a, The active principles of *Catha edulis*, *The Pharmaceutical Journal and Pharmacist*, 89:676-678.
- Stockman, R., 1912b, The pharmacological action of *Catha edulis* and its alkaloids, *The Journal of Pharmacy and Experimental Therapeutics*, 4:251-262.
- Szendrei, K., 1980, The chemistry of khat, *Bulletin on Narcotics*, 32:5-35.
- United Nations Document, 1974, Studies on the chemical composition of khat. I. Extraction, screening investigations and solvent separation of khat components, MNAR, 10/75.
- United Nations Document, 1975, Studies on the chemical composition of khat. III. Investigations on the phenylalkylamine fraction, MNAR, 11/75.
- United Nations Document, 1976, Studies on the chemical composition of khat. IV. Evonine-type polyester alkaloids from khat, MNAR, 5/76.
- United Nations Document, 1977, Studies on the chemical composition of khat. V. On the structure of the polyester alkaloids from khat, MNAR, 2/77.
- Van Wyk, A. E., and Prins, M., 1987, A new species of *Catha* (Celastraceae) from southern Natal and Pondoland, *South African Journal of Botany*, 53:202-205.

- Von Brücke, F. T., 1941, Ueber die zentral erregende Wirkung des Alkaloides Cathin, Naunyn Schmiedeberg's Archiv für Experimentelle Pathologie und Pharmakologie, 198:100-106.
- Weir, S., 1985, Qat in Yemen: Consumption and social change, British Museum Publications, Dorset, UK.
- Winterfeld, K., and Bernsmann, G., 1960, Zur Kenntnis der Inhaltsstoffe von *Catha edulis* Forskal, Archiv der Pharmazie, 63:991-1000.
- Wolf, J.-P., and Pfander, H., 1986, Synthese und Strukturaufklärung von Merucathinon und Synthese von Cathinon, Inhaltsstoffe von *Catha edulis* Forsk, Helvetica Chimica Acta, 69:1498-1504.
- Wolfes, O., 1930, Ueber das Vorkommen von *d*-Norisoephedrin in *Catha edulis*, Archiv der Pharmazie, 268:81-83.
- World Health Organization, 1964, WHO Expert Committee on Addiction-Producing Drugs: Khat, World Health Organization, Technical Report Series No. 273, p. 10-11.

## ANTIPARASITIC AGENTS FROM PLANTS

Robert P. Borris and James M. Schaeffer

Merck Sharp and Dohme Research Laboratories  
Rahway, NJ 07065

### INTRODUCTION

Parasites are generally grouped into one of three broad classes: protozoa (single-celled organisms), helminths (including nematodes, trematodes, and cestodes), and ectoparasites. Parasitic diseases inflict tremendous damage and suffering to plants, animals, and humans. The scope of parasitism is difficult to imagine; it has been estimated that one billion people suffer from intestinal nematode infections alone, and as many as 150,000 avoidable deaths occur each year due to helminthiasis (Bundy, 1990).

The control of parasitism has been primarily via chemotherapeutic intervention and, to a lesser extent, vaccines and biologicals. The history of antiparasitic treatments dates back as far as such diseases have been recognized. The efficacy of ancient medicines was not impressive, although Alexander of Tralles (525 to 605 AD), apparently an accomplished helminthologist of his day, listed the following as anthelmintics: celery, leek, parsley, garlic, mint, pomegranate pips, cress, castor oil, walnuts, cabbage seeds in olive oil with rue and portulaca, fern root, wormwood, chenopodium, and santonin (Leake, 1975). This may be an eclectic sampling, but the last four plant substances have been established as modern-day anthelmintics. Traditional medicine continues to be practiced in many parts of the world today and important chemical entities have been realized, particularly emanating from China (recently reviewed by Xiao and Fu, 1986).

The purpose of this chapter is to present an up-to-date review of plant substances which have demonstrated parasiticidal activity. It is intended as a guide into the literature rather than an encyclopedic treatise.

### ANTIPROTOZOAL AGENTS

A wide range of protozoans are known to cause disease in man or domestic animals. These include, among others, the intestinal amoebae, e.g., *Entamoeba* (dysentery), *Naegleria* (primary amoebic meningoencephalitis), *Giardia* (diarrhea), *Trichomonas* (urogenital infections), *Plasmodium* (malaria), *Trypanosoma* (African sleeping sickness, Chagas disease), *Leishmania* (Oriental sore, kala azar), *Toxoplasma*

(toxoplasmosis), *Eimeria* (coccidiosis), and *Pneumocystis* (pneumonia). Some of these organisms are difficult to grow in culture, making *in vitro* screening difficult, if not impossible. It is perhaps not surprising that natural product screening programs for the discovery of antiprotozoal agents in general and phytochemical screening programs in particular have focused on the most common and most deadly of the protozoal diseases: malaria, trypanosomiasis, amoebiasis, and leishmaniasis with few reports of compounds active against the other organisms.

The use of naturally occurring compounds as antiprotozoal agents has been the subject of a few specific reviews in recent years (Pei-Gen and Shan-Lin, 1986; Phillipson and O'Neill, 1989) and has also been discussed in less detail in reviews of a more general nature (Pei-Gen and Keji, 1988; Werbel and Worth, 1980). Reviews of antimalarial agents obtained from plants (Ding, 1988; O'Neill and Phillipson, 1989; Vasanth et al., 1990; Xihe, 1979; Xu, 1982, 1983) have appeared more frequently, reflecting the greater amount of attention this disease has drawn from the phytochemical community.

## Alkaloids

**Quinine and related compounds.** No discussion of natural antiparasitic agents would be complete without at least passing mention of quinine (Fig. 1 [1]), the main antimalarial alkaloid from the bark of *Cinchona* and *Remijia* species (Rubiaceae). An in-depth review of the chemistry and pharmacology of this famous compound would fill volumes and is clearly beyond the scope of the present chapter. Only a few points of interest will be mentioned.

While quinine is the most important antimalarial alkaloid from *Cinchona* bark, it is not the only one. During World War II, a crude mixture of *Cinchona* alkaloids called Totaquine, of which 70 to 80% consisted of quinine, quinidine, cinchonidine, and cinchonine, was used for the treatment of malaria. Using an avian model, Seeler et al. (1943) demonstrated that Totaquine was approximately equipotent as pure quinine and that the four major alkaloids all had approximately the same level of activity. More recently, the use of quinidine (Fig. 1 [2]) has been advocated as an alternative treatment for falciparum malaria in adults (Phillips et al., 1985; White et al., 1981), although its cardiac effects preclude recommendation for use in children (Sabchareon et al., 1988).

A receptor common to both quinine and chloroquine has been partially characterized (Chou et al., 1980) and evidence suggests that haemin is an essential component of that receptor. Spectroscopic studies (Warhurst, 1981), using purified haemin and various antimalarial agents, show that quinine forms a lipophilic coordination complex with haemin while the antimalarially inactive 9-epiquinine does not. It has been postulated that the lipophilicity of this complex may be responsible in part for the toxicity of these compounds to the parasite. The formation of lipophilic aliphatic esters of the hydroxyl at C-9 of quinine failed to increase the activity of that compound against *Plasmodium falciparum* (Waddell et al., 1984). The quinoline antimalarials are preferentially toxic to the mature stages of the parasite (Geary et al., 1989).

Drug resistance continues to plague the chemotherapy of malaria, and reports of resistant strains of *Plasmodium* continue to appear (Brandicourt et al., 1986; Glew et al., 1978; Smrkovski et al., 1985, *inter alia*). It is noteworthy that a mixture of equal parts of quinine, quinidine, and cinchonine referred to as LA40221 has been found to be as effective as quinine alone in sensitive strains of *P. falciparum* but is 2 to 10 times as effective as quinine in resistant strains *in vitro* (Druilhe et al., 1988). Clinical evaluation of this mixture administered orally or by intravenous injection at 12 mg/kg every 8 hours for 22 doses cured all of 14 cases of chloroquine resistant falciparum

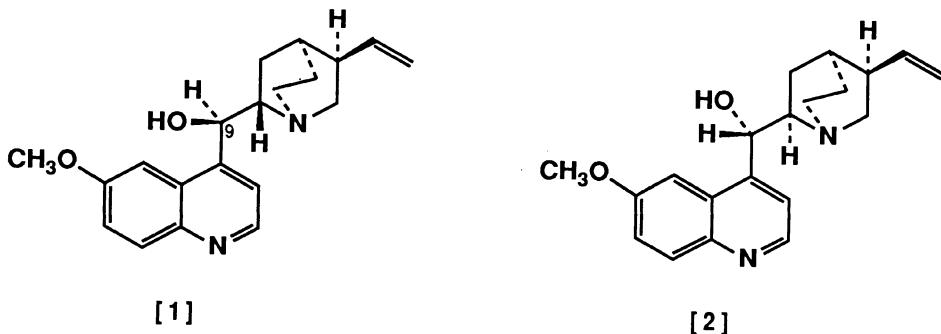


Figure 1. Structural formulas of quinine [1] and quinidine [2].

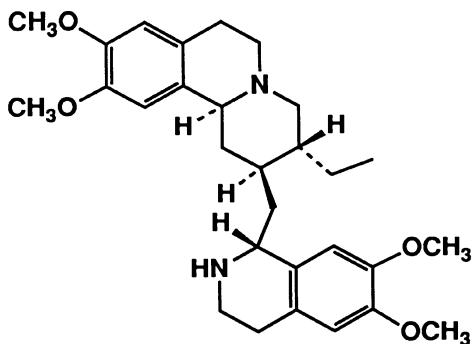
malaria (Bunnag et al., 1987). Additional double-blind studies (Bunnag et al., 1989) failed to show an advantage over quinine by itself.

While the antimalarial activity of quinine and its relatives is well-known, it must be noted that this group of compounds possesses antiamoebic activity also. Quinine and quinidine are amoebicidal with  $IC_{50}$  values of 14.8  $\mu\text{g}/\text{ml}$  and 16.6  $\mu\text{g}/\text{ml}$ , respectively. Interestingly, quinidinone, the analog of quinidine in which the C-9 hydroxyl has been oxidized to a ketone, is not an antimalarial but is a more potent amoebicide ( $IC_{50} = 7.4 \mu\text{g}/\text{ml}$ ) than either quinine or quinidine and is more cytotoxic (Keene et al., 1986).

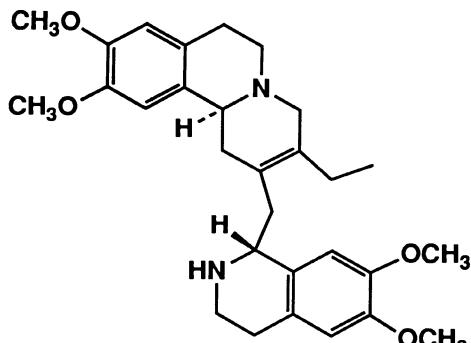
**Emetine and related compounds.** Since its discovery in the early 19th century, emetine (Fig. 2 [3]), the active constituent of *Cephaelis ipecacuanha*, has been a mainstay in the chemotherapy of amoebic dysentery, only recently being supplanted by newer, safer drugs. While its use as an antiamoebic has been limited by toxicity, it still finds application in the treatment of severe ulcerative amoebiasis. In addition to its amoebicidal effects on such organisms as *Entamoeba histolytica* (Dutta and Yadava, 1972; Keene et al., 1986; Prasad et al., 1984, *inter alia*) and *Acanthamoeba* sp. (Pfaffman and Klein, 1966), emetine has been shown to be a clinically effective antimalarial (James, 1985) and an antileishmanial in animals (Neal, 1964, 1970) and man (Sinderson, 1924). Neal and Croft (1984) have shown, using *Leishmania donovani* amastigotes in cultured mouse peritoneal macrophages, that 2,3-dehydroemetine (Fig. 2 [4]) was unable to clear the macrophages of the parasite at nontoxic concentrations.

That emetine is toxic to mammalian cells as well as protozoans is not surprising. It is a potent inhibitor of protein and DNA synthesis (Grollman, 1966), acting at the 40-S ribosomal subunit (Gupta and Siminovitch, 1977; Rao and Grollman, 1967) preventing ribosomal translocation along mRNA, thereby inhibiting peptide synthesis (Grollman and Jarkovsky, 1974).

Emetine has been found to inhibit *Entamoeba histolytica* *in vitro* with an  $IC_{50}$  of 70 ng/ml (Keene et al., 1987). In the same study, its cytotoxicity against cultured guinea pig keratinocytes had an  $IC_{50}$  of 20 ng/ml. 2,3-Dehydroemetine had cytotoxicity comparable to that of the parent compound but was 2 to 3 times less potent as an amoebicide. That 2,3-dehydroemetine is less toxic *in vivo* has been attributed to its faster rates of metabolism and excretion (Grollman and Jarkovsky, 1974). Removal of the methoxy groups from the "upper half" of emetine results in a 52-fold loss of amoebicidal potency but a 270-fold loss of cytotoxicity (Keene et al., 1987). These results clearly support the importance of these substituents to the mechanism of action of emetine (Gupta et al., 1980) and suggest the possibility of the



[3]



[4]

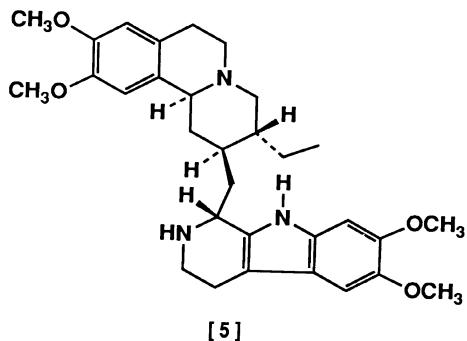
Figure 2. Structural formulas of emetine [3] and 2,3-dehydroemetine [4].

development of a synthetic analog with potent antiamoebic activity but with a better therapeutic index. That 9,10-demethoxyisoemetine, the C-1'  $\alpha$ -epimer of 9,10-demethoxyemetine, is 10-fold more cytotoxic than its  $\beta$ -epimer indicates the importance of stereochemistry at this center. Unfortunately, insufficient quantities of this compound precluded its amoebicidal evaluation (Keene et al., 1987).

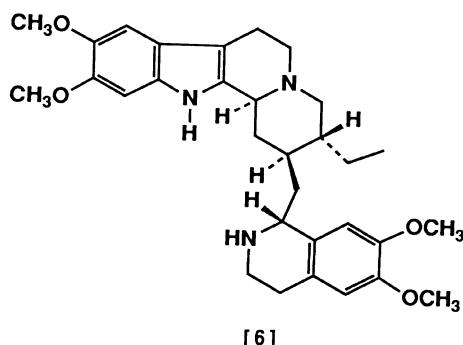
The emetine molecule may be considered to be composed of two halves: an upper tricyclic system elaborated from a tetrahydroisoquinoline ring system and a lower bicyclic system also comprised of a tetrahydroisoquinoline moiety. Replacement of the lower half with a tetrahydro- $\beta$ -carboline moiety gives rise to the tubulosine group of alkaloids (e.g., tubulosine (Fig. 3 [5])). Replacement of the upper half of the emetine molecule with a tetracyclic system based on the tetrahydro- $\beta$ -carboline ring system gives rise to the pseudotubulosines (Fig. 3 [6]). Replacement of both tetrahydroisoquinoline moieties with tetrahydro- $\beta$ -carboline systems affords the ochrolifuanine alkaloids (Fig. 3 [7]) and the cinchophylline alkaloids (Fig. 3 [8]).

While tubulosine and pseudotubulosine, isolated from *Alangium lamarckii* (Alangiaceae) and *Pogonopsis tubulosa* (Rubiaceae), have each replaced half of the emetine structure with a tetrahydro- $\beta$ -carboline system, both retain its overall stereochemistry. Tubulosine retains most of the cytotoxicity of emetine but shows a 23-fold loss of amoebicidal activity, while pseudotubulosine shows dramatic reductions in both activities, 115-fold and 619-fold, respectively (Keene et al., 1987). The antiamoebic activity of tubulosine was originally predicted from mechanistic considerations and its structural relationship to emetine and then confirmed experimentally (Grollman, 1967).

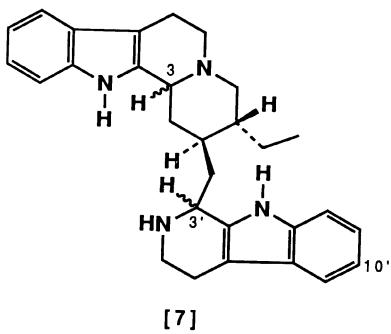
The ochrolifuanines, from *Ochrosia* and *Dyera* species (Apocynaceae), and cinchophyllines, from *Cinchona ledgeriana*, differ amongst themselves in substitution on the aromatic rings, unsaturation in the side chain, and stereochemistry at C-3 and C-3'. Ochrolifuanine A, which lacks substitution on both aromatic rings, has a saturated side chain, has C-3 $\alpha$ , C-3' $\beta$  stereochemistry, is amoebicidal with an IC<sub>50</sub> value of 1.3  $\mu$ g/ml, and is cytotoxic with an IC<sub>50</sub> value of 1.9  $\mu$ g/ml. Hydroxylation, methylation, or methylation at C-10' increases amoebicidal activity 2- to 3-fold without significantly changing cytotoxicity. Methylation at N-4' increases amoebicidal activity 3-fold while slightly increasing cytotoxicity. 3 $\alpha$ ,17 $\beta$ -Cinchophylline (note: a



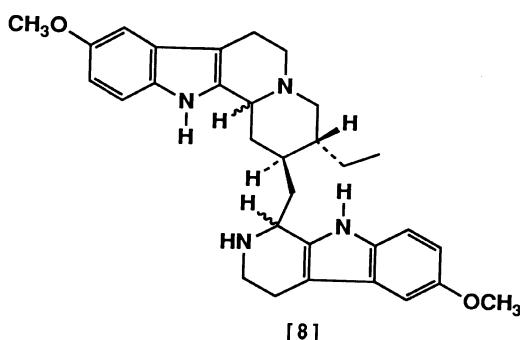
[5]



[6]



[7]



[8]

Figure 3. Structural formulas of tubulosine [5], pseudotubulosine [6], ochrolifuanine A [7], and cinchophylline [8].

different numbering system is used to describe the cinchophyllines) has methoxyl groups at C-10 and C-10', an unsaturated vinyl side chain, and C-3 $\alpha$ , C-3' $\beta$  stereochemistry. While it is a slightly more potent amoebicide than ochrolifuanine A, it is 24-fold more cytotoxic. This marked increase in cytotoxicity is lost in the C-3 $\beta$ , C-3' $\beta$ -isomer (with slight improvement in amoebicidal activity), while both activities are decreased in the C-3 $\alpha$ , C-3' $\alpha$ -isomer (Keene et al., 1983, 1987).

While it is tantalizing to start outlining structure-activity relationships for the ochrolifuanines and cinchophyllines, one must bear in mind that the tested compounds differ at more than one site and that groups of compounds differing at only one site need be compared to truly define the significance of that site. Similarly, while it is interesting to speculate on the importance of the skeletal changes among the five groups of alkaloids related to emetine, it is important to keep the gross substitution patterns constant when making the comparisons. Thus, while tubulosine may be significantly less potent than emetine when compared to the alkaloid with comparable substitution, 9,10-demethoxyemetine, it is 2.3-fold more potent as an amoebicide and 135-fold more cytotoxic. Pseudotubulosine is 12-fold less potent as an amoebicide and only 2-fold more cytotoxic. Appropriately substituted members of the ochrolifuanines and cinchophyllines were not available for testing. As the amoebicidal activity and

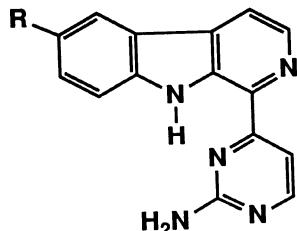
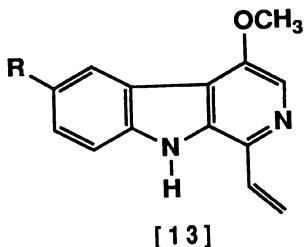
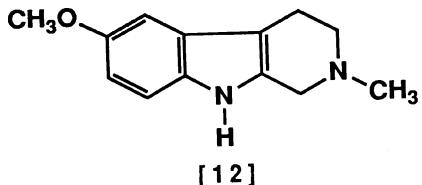
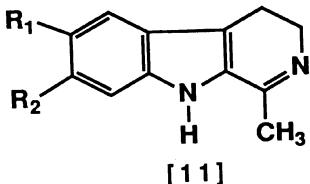
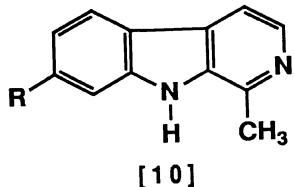
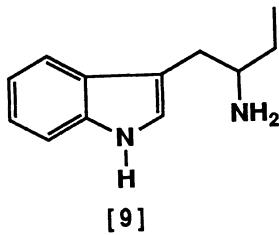
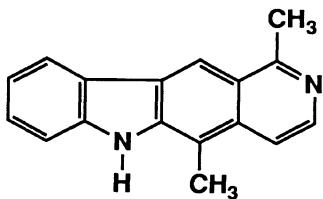


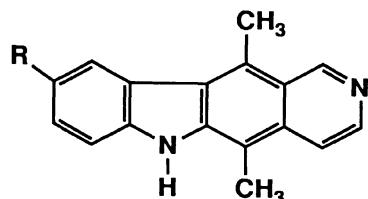
Figure 4. Structural formulas of  $\alpha$ -ethyltryptamine [9], harman [10, R = H], harmine [10, R = OCH<sub>3</sub>], harmol [10, R = OH], harmaline [11, R<sub>1</sub> = H, R<sub>2</sub> = OCH<sub>3</sub>], harmalol [11, R<sub>1</sub> = OH, R<sub>2</sub> = OH], 6-methoxyharmalan [11, R<sub>1</sub> = OCH<sub>3</sub>, R<sub>2</sub> = H], 6-methoxy-*N*-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline [12], 4-methoxy-1-vinyl- $\beta$ -carboline [13, R = H], 4-methoxy-6-hydroxy-1-vinyl- $\beta$ -carboline [13, R = OH], annomontine [14, R = H], and methoxyannomontine [14, R = OCH<sub>3</sub>].

cytotoxicity both changed markedly in response to structural modifications in this group of compounds and the changes in the two activities are not parallel, it seems likely that the medicinal chemist would be able to maximize amoebicidal activity while reducing cytotoxicity in some member of the series, perhaps resulting in the discovery of a new, clinically relevant amoebicide.

**Indole alkaloids.** A number of relatively simple indole alkaloids have been demonstrated to possess antiprotozoal activity. *N*-methyltryptamine has been shown to inhibit the growth of the trypanosome *Crithidia fasciculata* *in vitro* (Dos Santos Filho and Gilbert, 1975), while the synthetic compound  $\alpha$ -ethyltryptamine (Fig. 4 [9]) was found to be active against *Leishmania mexicana amazonensis* both *in vitro* and *in vivo* in mice (Evans and Croft, 1987). Substituted  $\beta$ -carboline derivatives have demonstrated a wide range of antiprotozoal activities with harman (Fig. 4 [10, R = H]), harmine (Fig. 4 [10, R = OCH<sub>3</sub>]), harmol (Fig. 4 [10, R = OH]), and harmaline (Fig. 4 [11, R<sub>1</sub> = H, R<sub>2</sub> = OCH<sub>3</sub>]) being active against the epimastigotes of *Trypanosoma cruzi*, while harmalol (Fig. 4 [11, R<sub>1</sub> = H, R<sub>2</sub> = OH]) and 6-methoxyharmalan (Fig. 4 [11, R<sub>1</sub> = OCH<sub>3</sub>, R<sub>2</sub> = H]) were inactive (Cavin et al.,



[15]



[16]

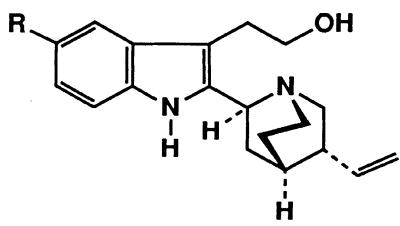
Figure 5. Structural formulas of olivacine [15], ellipticine [16, R = H], and 9-hydroxyellipticine [16, R = OH].

1987; Hopp et al., 1976). Harmaline also shows activity against *Leishmania mexicana amazonensis* (Evans and Croft, 1987), while 6-methoxy-N-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline (Fig. 4 [12]) from *Nectandra megapotamica* (Lauraceae) inhibits the growth of *Critidium fasciculata* (Dos Santos Filho and Gilbert, 1975) and 4-methoxy-1-vinyl- $\beta$ -carboline (Fig. 4 [13, R = H]) and its 6-hydroxy derivative (Fig. 4 [13, R = OH]) from *Picrasma javanica* (Simaroubaceae) both inhibit multidrug resistant *Plasmodium falciparum* *in vitro* (Pavanand et al., 1988). The unusual aminopyrimidinyl substituted  $\beta$ -carbolines, annomontine (Fig. 4 [14, R = H]) and methoxyannomontine (Fig. 4 [14, R = OCH<sub>3</sub>]) from *Annona montana* (Annonaceae) are weakly amoebicidal (Lebouef et al., 1982).

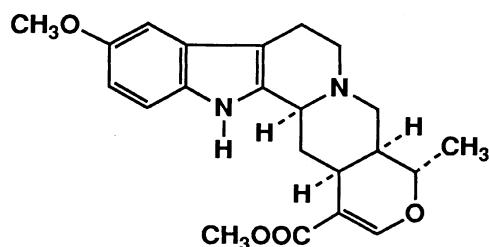
The antitumor pyridocarbazole alkaloids, olivacine (Fig. 5 [15]) and ellipticine (Fig. 5 [16, R = H]), isolated from *Aspidosperma* and *Ochrosia* species (Apocynaceae), and their derivatives are known intercalators of DNA (LePecq et al., 1974). This series of compounds has been shown to possess trypanocidal activity against *Trypanosoma cruzi* *in vitro* (Benard and Riou, 1977; Benard et al., 1975; Cruz et al., 1975). The mechanism of the trypanocidal activity of 9-hydroxyellipticine (Fig. 5 [16, R = OH]) appears to be inhibition of the replication and transcription of kinetoplast DNA (Benard and Riou, 1977). This inhibition occurs at the template DNA via intercalation, rather than at the polymerase enzyme. Initiation of transcription is more sensitive to the drug than is chain elongation. In addition to inhibiting nucleic acid synthesis, olivacine also inhibits lipid and protein synthesis and reduces cellular respiration and oxidative metabolism of *T. cruzi* *in vitro*. Unfortunately, the compound was not effective *in vivo* (Leon et al., 1978).

The quinuclidinylindole alkaloids, cinchonamine (Fig. 6 [17, R = H]) and 10-methoxycinchonamine (Fig. 6 [17, R = OCH<sub>3</sub>]), from *Remijia purdieana* both have amoebicidal activity against *Entamoeba histolytica* as does aricine (Fig. 6 [18]) from *Cinchona pelletierana*, various *Rauvolfia* (Apocynaceae) and *Aspidosperma* species, and its demethoxytetrahydro quaternary analog, alstonine (Fig. 6 [19]) (Keene et al., 1986). Aricine is remarkable in that it possesses marked amoebicidal activity ( $IC_{50} = 3.6 \mu\text{g/ml}$ ) but very low cytotoxicity ( $IC_{50} > 100 \mu\text{g/ml}$ ). Isocorynantheol (Fig. 6 [20]) from the leaves of *Cinchona ledgeriana* has comparable amoebicidal activity but is much more cytotoxic ( $IC_{50} = 5.4 \mu\text{g/ml}$ ) (Keene et al., 1987).

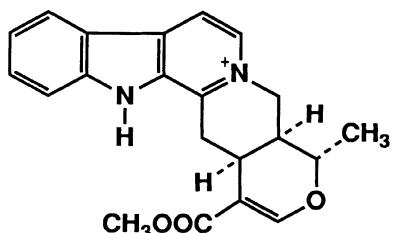
**Isoquinoline alkaloids.** Berberine (Fig. 7 [21]) from *Berberis* and *Mahonia* species (Berberidaceae) has been used for the treatment of cutaneous leishmaniasis in man (DasGupta, 1930; DasGupta and Dikshit, 1929; Devi, 1929) and domestic animals (Bennett, 1935) since the early part of the 20th century. It has been stated that the treatment of oriental sore by direct injection of berberine is the only practical therapeutic use of the compound (Hahn and Ciak, 1975). Intraperitoneal



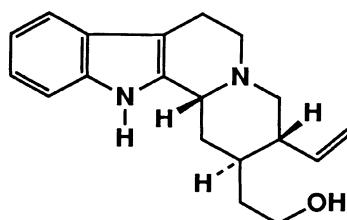
[17]



[18]



[19]



[20]

Figure 6. Structural formulas of cinchonamine [17, R = H], 10-methoxycinchonamine [17, R = OCH<sub>3</sub>], aricine [18], and alstonine [19], and isocorynantheol [20].

administration of the compound to rats infected with *Trypanosoma equiperdum* gave rise to a statistically significant prolongation of life (Seery and Bieter, 1940). Berberine, the protoberberine alkaloids, palmatine (Fig. 7 [22, R = CH<sub>3</sub>]) and jatrorrhizine (Fig. 7 [22, R = H]), from *Enantia chlorantha* (Annonaceae) and a number of synthetic analogs have been evaluated as antimalarial agents. While all three of the natural alkaloids displayed a potency comparable to quinine against *Plasmodium falciparum* *in vitro*, none was active against *P. berghei* in mice (Vennerstrom and Klayman, 1988). Berberine sulphate was shown to be weakly amoebicidal against *Entamoeba histolytica* *in vitro* (Subbaiah and Amin, 1967) and in rats (Kulkarni et al., 1972). Solutions containing 0.1 to 0.5% sanguinarine (Fig. 7 [23]), a metabolite of *Sanguinaria* and *Chelidonium* species (Papaveraceae), have been shown to inhibit *Trichomonas vaginalis* *in vitro* (Bodalski et al., 1958; Vichkanova et al., 1969), while dilute aqueous solutions inhibit *Trypanosoma lewisi* (Hopp et al., 1976).

While definitive experiments on the mechanism of antiparasitic action of berberine are lacking, studies have been performed on the mechanism of its antibacterial activity. The alkaloid appears to act as a DNA intercalating agent, interfering with RNA transcription at the ribosome, leading to inhibition of RNA and protein synthesis (Hahn and Ciak, 1975; Wolfe et al., 1972). The similarity between this mechanism of action and that of the trypanocidal activity of the pyridocarbazole alkaloids (*vide supra*) is obvious.

When given subcutaneously as a solution in DMSO, the phthalide isoquinoline alkaloid, capnoidine (Fig. 8 [24]), and the spirobenzylisoquinoline alkaloid, corpaine

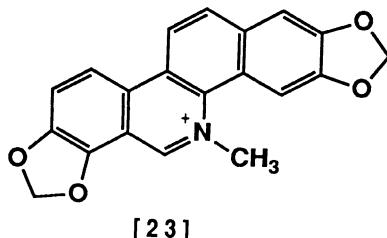
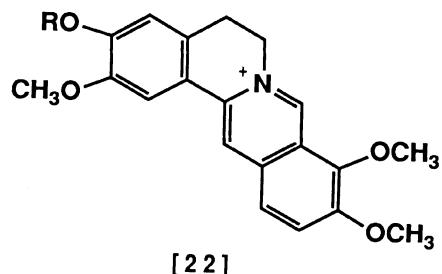
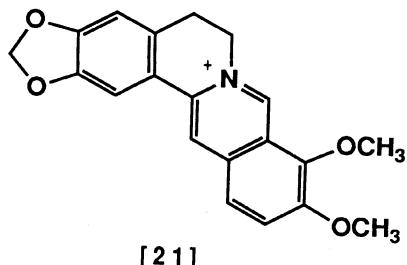
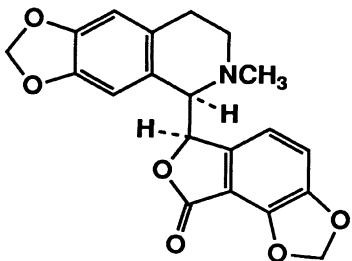


Figure 7. Structural formulas of berberine [21], palmatine [22, R = CH<sub>3</sub>], jatrorrhizine [22, R = H], and sanguinarine [23].

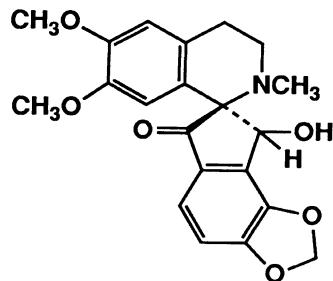
(Fig. 8 [25]), both from *Corydalis* species (Fumariaceae), show slight activity against *Trypanosoma brucei brucei* infections in mice at a dose of 200 mg/kg. Activity was not observed when these compounds were administered as a dispersion in carboxymethyl cellulose (Dreyfuss et al., 1987). The benzophenanthridine alkaloid, coraline (Fig. 8 [26]), a known antitumor agent, has been shown to possess activity against *Trypanosoma rhodesiense* infections in mice (Kinnamon et al., 1979). Coraline and other antitumor agents were selected for evaluation because of the metabolic similarities seen in African trypanosomes and some tumor cells (Borst, 1977).

A number of dimeric bisbenzyltetrahydroisoquinoline alkaloids have been found to possess antiparasitic activity. Gyrocarpine (Fig. 9 [27]) from *Gyrocarpus americanus* (Gyrocarpaceae), daphnandrine (Fig. 9 [28, R<sub>1</sub> = H, R<sub>2</sub> = OH]) from *Daphnandra micrantha* (Atherospermataceae), and obaberine (Fig. 9 [28, R<sub>1</sub> = CH<sub>3</sub>, R<sub>2</sub> = OCH<sub>3</sub>]) from *Berberis* and *Mahonia* species (Berberidaceae) all demonstrated interesting levels of activity against both the epimastigotes of *Trypanosoma cruzi* *in vitro* (Fournet et al., 1988a) and the promastigotes of three strains of *Leishmania* *in vitro* (Fournet et al., 1988b) with IC<sub>50</sub> values less than 50 µg/ml in all cases.

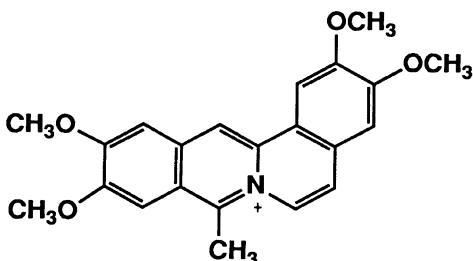
Pycnamine (Fig. 10 [29, R = H]) was found to inhibit a multi-drug resistant strain (K1) of *Plasmodium falciparum* with an IC<sub>50</sub> of 150 ng/ml while its methyl analog, phaeanthine (Fig. 10 [29, R = CH<sub>3</sub>]), was 10-fold less active (Partridge et al., 1988). Aromoline (Fig. 10 [30]) was also quite active against this organism (IC<sub>50</sub> = 670 ng/ml) while the dibenzo-1,4-dioxin alkaloids, cocsuline (Fig. 10 [31]) and trigilletimine (Fig. 10 [32]), were 25- to 30-fold less active (Partridge et al., 1988). All five alkaloids were isolated from *Triclisia patens* (Menispermaceae). Nor-tiliacorinine A (Fig. 11 [33, R = H, H<sub>1</sub>S]) and tiliacorine (Fig. 11 [33, R = CH<sub>3</sub>, H<sub>1</sub>R]) from *Tiliacora triandra* are both potent inhibitors of *Plasmodium falciparum* *in vitro* with



[ 24 ]



[ 25 ]



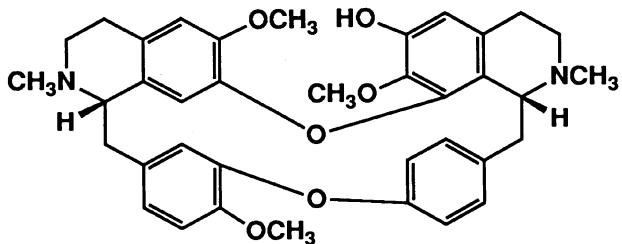
[ 26 ]

Figure 8. Structural formulas of capnoidine [24], corpaine [25], and coraline [26].

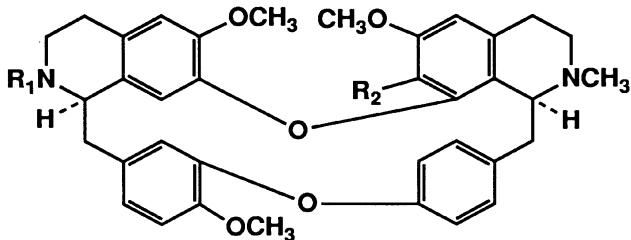
$IC_{50}$  values between 500 and 700 ng/ml, while tiliacorinine (Fig. 11 [33, R = CH<sub>3</sub>, H<sub>1</sub>S]) from the same plant was 5- to 6-fold less active (Pavanand et al., 1989a).

Tetrandrine, the dextrorotatory form of phaeanthine found in the Menispermaceous plant *Stephania tetrandra*, a verapamil-like calcium channel blocking agent, has been shown to be a more potent inhibitor of chloroquine-resistant *Plasmodium falciparum* than of chloroquine-sensitive strains (Ye and Van Dyke, 1989). Further, this inhibitory activity is synergistic with the activities of other antimalarials such as chloroquine and artemisinin (= qinghaosu) (Ye et al., 1989). The calcium channel blocker, verapamil (Fig. 11 [34]), while not an antimalarial, has been shown to potentiate the activity of chloroquine against both chloroquine-resistant and chloroquine-sensitive strains of *Plasmodium chabaudi* (Tanabe et al., 1990). Verapamil and other calcium channel blockers appear to be able to reverse chloroquine resistance in this organism, making it possible to treat the disease with markedly lower doses of chloroquine or other antimalarial agents (Tanabe et al., 1990).

**Phenanthroquinolizidine and phenanthroindolizidine alkaloids.** Tylophorine (Fig. 12 [35, R<sub>1</sub> = R<sub>2</sub> = H]), a phenanthroindolizidine alkaloid, has been shown to be the active antiamoebic agent from the Indian medicinal plant, *Tylophora asthmaticus* (Chopra and Chakerbury, 1935). Examination of a series of natural and synthetic phenanthroindolizidines and other known antiamoebic agents indicates that tylophorine is twice as potent against cultured *Entamoeba histolytica* than is emetine. The 4-methoxy-14-hydroxy analog of tylophorine (Fig. 12 [35, R<sub>1</sub> = OCH<sub>3</sub>, R<sub>2</sub> = OH]) is twice as potent as the parent compound while septicine (Fig. 12 [36]), the



[ 27 ]



[ 28 ]

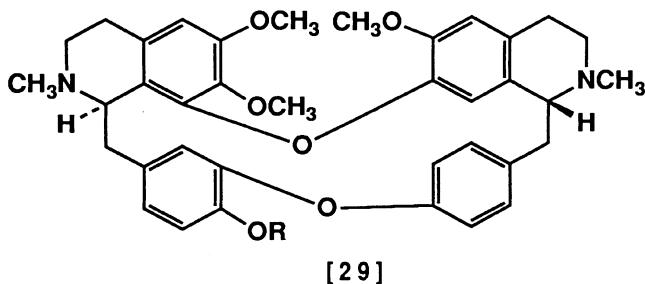
Figure 9. Structural formulas of gyrocarpine [27], daphnandrine [28,  $R_1 = H$ ,  $R_2 = OH$ ], and obaberine [28,  $R_1 = CH_3$ ,  $R_2 = OCH_3$ ].

B-ring-*seco* analog, also found in *T. asthmaticus*, is 32-fold less active (Bhutani et al., 1987). The phenanthroindolizidine alkaloids, phenanthroquinolizidine alkaloids such as cryptopleurine (Fig. 12 [37]), and the emetine family of alkaloids all appear to possess the same mechanism of action (Gupta and Siminovitch, 1977; Gupta et al., 1980).

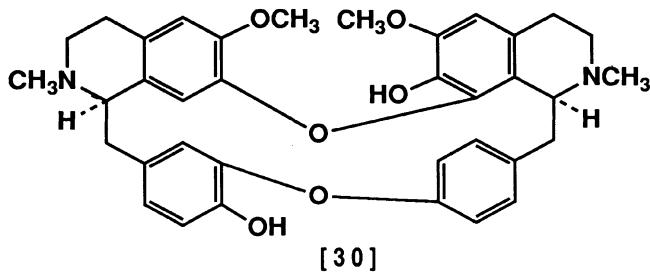
**Acridone alkaloids.** A group of 30 acridone alkaloids isolated from Rutaceous plants of the genera, *Citrus*, *Glycosmis*, or *Severinia*, have been evaluated for antimalarial activity against *Plasmodium yoelii* *in vitro*. Seven compounds, including simple acridones such as glycocitrine I (Fig. 13 [38]), angular pyranoacridones such as atalaphillinine (Fig. 13 [39]), and the dimeric pyranoacridone, glycobismine A (Fig. 13 [40]), showed antimalarial potencies comparable to chloroquine while honyumine (Fig. 13 [41]), the only linear pyranoacridone tested, was devoid of activity (Fujioka et al., 1989). Atalaphillinine completely suppressed *P. berghei* and *P. vinckeii* in mice at a daily intraperitoneal dose of 50 mg/kg/day. None of the tested acridone alkaloids showed any amoebicidal activity.

**Steroidal alkaloids.** *Holarrhena antidysenterica* (Apocynaceae), an Indian medicinal plant used for the treatment of dysentery, has afforded the steroidal alkaloid connessine (Fig. 14 [42]) as the main antiamoebic constituent (Bhandari and Mukerji, 1959). The same active constituent has been found in *Wrightia tomentosa* (Apocynaceae), a common adulterant of *H. antidysenterica* (Jayaswal, 1976). While the compound had been used clinically in Africa for the treatment of amoebiasis, this use was discontinued due to neurotoxicity (Woolfe, 1963).

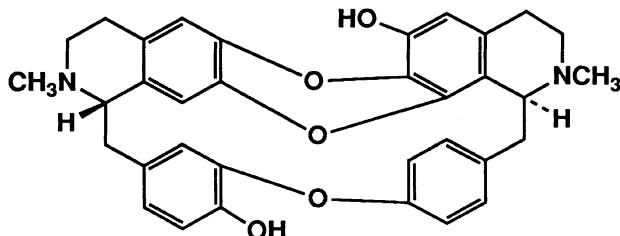
In a large scale screening program for new antiparasitic agents from plants, only one of over 2500 extracts of plants indigenous to India showed promising activity



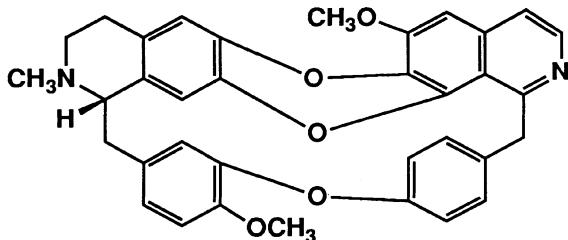
[ 29 ]



[ 30 ]

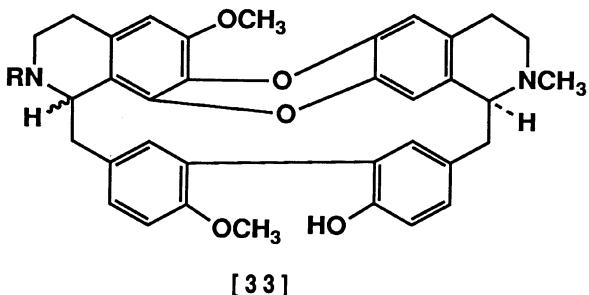


[ 31 ]

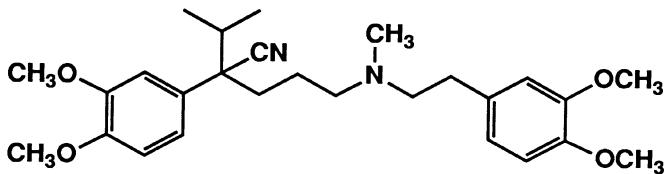


[ 32 ]

Figure 10. Structural formulas of pycnamine [29,  $\text{R} = \text{H}$ ], phaeanthine [29,  $\text{R} = \text{CH}_3$ ], aromoline [30], cocculine [31], and trigilletimine [32].



[ 33 ]



[ 34 ]

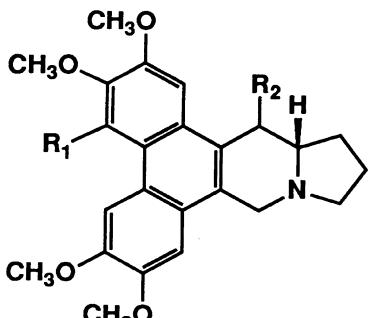
Figure 11. Structural formulas of nor-tiliacorinine A [33, R = H, H<sub>1</sub>S], tiliacorine [33, R = CH<sub>3</sub>, H<sub>1</sub>R], tiliacorine [33, R = CH<sub>3</sub>, H<sub>1</sub>S], and verapamil [34].

against *Entamoeba histolytica* *in vivo*. The extract of the roots of *Chonemorpha fragrans* (Apocynaceae) afforded the steroidal alkaloid chonemorphine (Fig. 14 [43]) as the active constituent, having an MIC of 25 µg/ml *in vitro*, and ED<sub>50</sub> values of 66 mg/kg/day (x 4) against hepatic amoebiasis in golden hamsters and 122.5 mg/kg/day (x 4) against intestinal amoebiasis in weanling rats (Chatterjee et al., 1987).

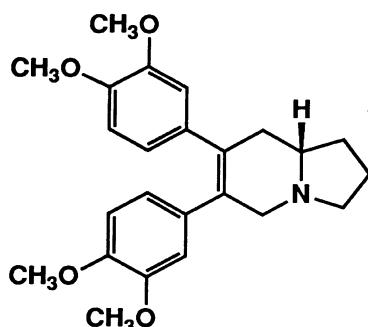
**Miscellaneous alkaloids.** The well-known antimitotic alkaloid, colchicine (Fig. 15 [44]), from *Colchicum autumnale* (Liliaceae) slows the growth and/or division of cultured *Trypanosoma cruzi* while stimulating differentiation from the epimastigote to trypomastigote forms (Astolfi Filho et al., 1978). Anuclear cells comprised up to 25% of the culture population following treatment with colchicine and, together with the appearance of polyploid forms, suggest that interference with microtubule function is the likely mechanism of action of this agent (Astolfi Filho et al., 1978; Williamson and Scott-Finnegan, 1978). A similar mechanism has been proposed for the trypanocidal activity of the antileukemic dimeric indole alkaloid, vinblastine (Williamson and Scott-Finnegan, 1978).

Homoharringtonine (Fig. 15 [45]), an antineoplastic agent obtained from *Cephalotaxus harringtonia* (Cephalotaxaceae), has been shown to be an inhibitor of chloroquine-resistant *Plasmodium falciparum* *in vitro* and of *P. yoelii* infection in mice (Whaun and Brown, 1990).

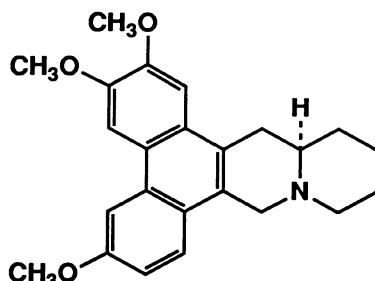
In the late 1940s, several groups isolated a potent antimalarial compound from the roots and leaves of *Dichroa febrifuga* (Hydrangeaceae) and the leaves of *Hydrangea* species (Ablondi et al., 1952; Henderson et al., 1949; Koepfli et al., 1947, 1949; Kuehl et al., 1948). This alkaloid, febrifugine (Fig. 15 [46]), was found to have 100 times the potency of quinine, but its use as an antimalarial was limited due to toxicity. Febrifugine and some of its derivatives have been shown to be effective coccidiostats (Johne, 1986 and references therein).



[35]



[36]



[37]

Figure 12. Structural formulas of tylophorine [35, R<sub>1</sub> = R<sub>2</sub> = H], 4-methoxy-14-hydroxytylophorine [35, R<sub>1</sub> = OCH<sub>3</sub>, R<sub>2</sub> = OH], septicine [36], and cryptopleurine [37].

## Terpenoids

**Sesquiterpenes.** Without question, the most exciting natural antimarial agent discovered since World War II is the sesquiterpene endoperoxide, artemisinin (also called qinghaosu and arteannuin) (Fig. 16 [47]). This compound, isolated from *Artemisia annua* (Asteraceae) by Chinese workers (Coordinating Group for Research on the Structure of Qing Hau Sau, 1977), was identified by spectroscopic and chemical means (Liu et al., 1979) and its structure was confirmed by X-ray crystallography (Academia Sinica, 1980). Initial studies (Qinghaosu Antimalaria Coordinating Research Group, 1979) indicated that it was a potent and relatively nontoxic antimarial agent acting on the erythrocytic stages of the parasite. The chemistry and pharmacology of artemisinin have been the subject of two excellent recent reviews (Klayman, 1985; Luo and Shen, 1987), and the interested reader is directed to these papers for a more in-depth discussion of this compound.

Numerous attempts have been made to improve the solubility (in aqueous and lipophilic milieu) and stability of artemisinin (Lin et al., 1987, 1989, 1990 and references therein). Reduction of the lactone to a lactol afforded dihydroartemisinin (Fig. 16 [48, R = H]), which retained the potency of the parent compound but improved water-solubility. The lactol could then be esterified with succinic acid forming the succinate half ester, artesunate (Fig. 16 [48, R = COCH<sub>2</sub>CH<sub>2</sub>COOH]).

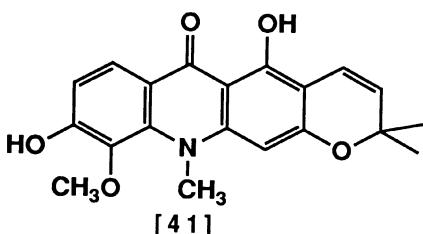
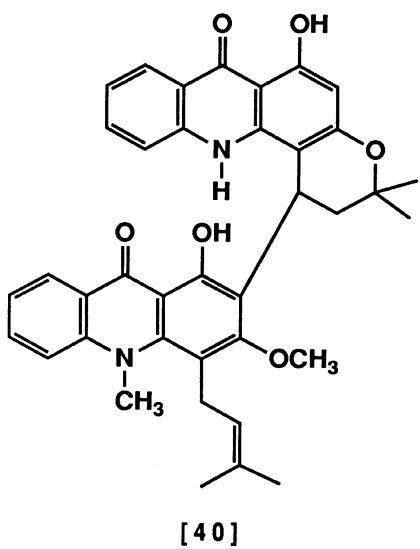
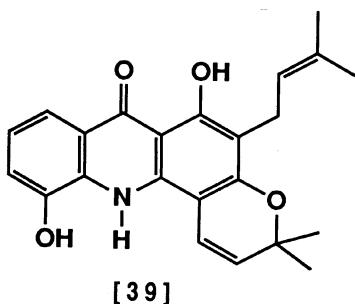
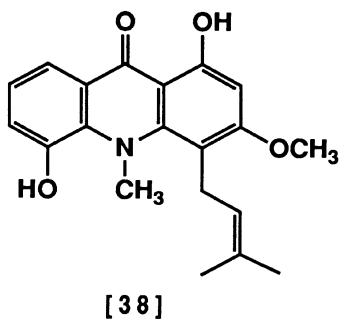


Figure 13. Structural formulas of glycocitrine I [38], atalaphillinine [39], glycobismine A [40], and honyumine [41].

usually used as the sodium salt. Sodium artesunate is water-soluble and can be administered by intravenous injection, but its utilization is impaired by the lability of the ester linkage. Lipophilicity of dihydroartemisinin was enhanced by formation of short chain aliphatic ethers of the lactol hydroxyl affording artemether (Fig. 16 [48, R = CH<sub>3</sub>]) and arteether (Fig. 16 [48, R = CH<sub>2</sub>CH<sub>3</sub>]), both with antimalarial activities superior to the parent compound. Arteether has been shown to be effective against *Plasmodium knowlesi* in monkeys (Bajpai et al., 1989), *P. cynomolgi* in monkeys (Dutta et al., 1989a), and a multiple drug-resistant strain of *P. yoelii* in mice (Dutta et al., 1989b); and while artemether has been shown effective in the treatment of cerebral malaria (Myint et al., 1989; Shwe et al., 1989) and uncomplicated falciparum malaria (Naing et al., 1988), arteether is likely to replace its lower homolog in these applications due to the potential methanol toxicity of artemether. Development of stable water-soluble derivatives required creation of a hybrid compound containing a polar functionality joined to the molecule by a stable ether linkage. Artelinic acid

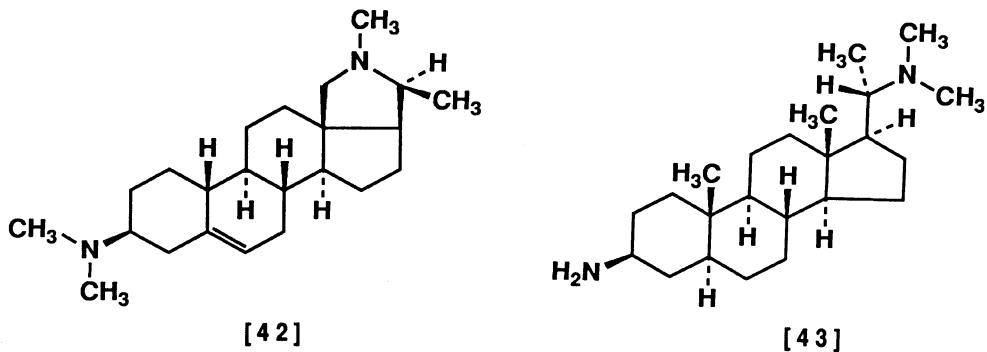


Figure 14. Structural formulas of connessine [42] and chonemorphine [43].

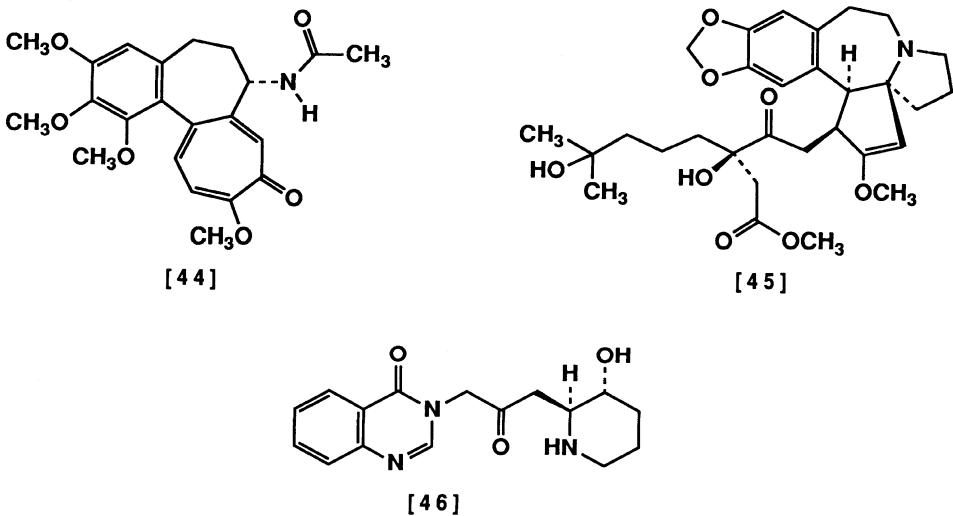
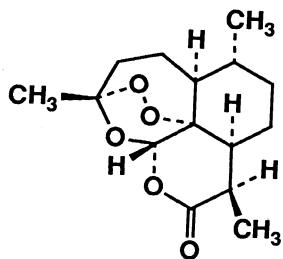


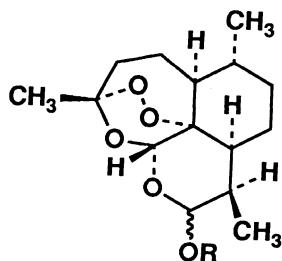
Figure 15. Structural formulas of colchicine [44], homoharringtonine [45], and febrifugine [46].

(Fig. 16 [49]) possesses the desired water-solubility plus the stability and high potency of artemether and arteether (Lin et al., 1987). Recently, a series of derivatives in which the lactol hydroxyl has been converted to a secondary aromatic amine (Fig. 16 [50]) have been prepared (Lin et al., 1990). While these compounds were several-fold more potent than artemisinin against *Plasmodium falciparum* *in vitro*, no activity was detected against this organism *in vivo*.

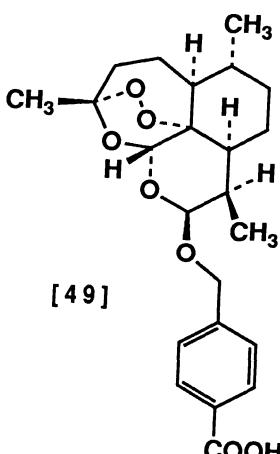
Recent studies on the mechanism of antimalarial activity of these compounds suggest that artemisinin and related compounds increase the level of lipid peroxidation in infected erythrocytes to a greater degree than in uninfected cells. Addition of a free radical scavenger to infected cells treated with sodium artesunate effectively antagonized the trypanocidal activity of the drug, suggesting a free radical mechanism



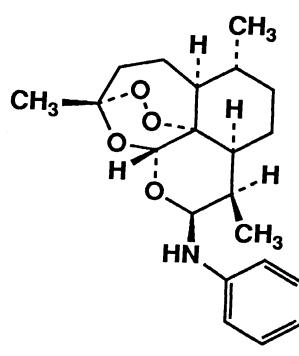
[47]



[48]



[49]



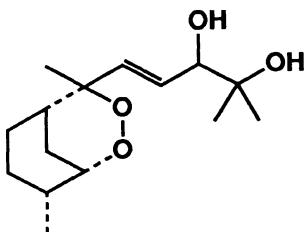
[50]

Figure 16. Structural formulas of artemisinin [47], dihydroartemisinin [48, R = H], artesunate [48, R = COCH<sub>2</sub>CH<sub>2</sub>COOH], artemether [48, R = CH<sub>3</sub>], arteether [48, R = CH<sub>2</sub>CH<sub>3</sub>], artelinic acid [49], and an aromatic amino analog of dihydroartemisinin [50].

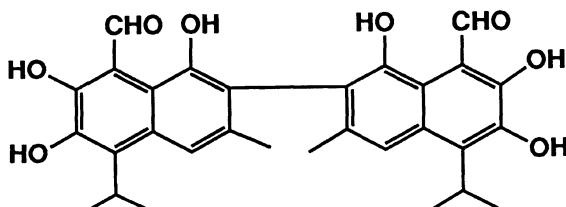
(Lin and Pan, 1989; Meshnick et al., 1989). This hypothesis gains some support from the finding that diets deficient in free radical scavengers enhance the activity of artemisinin while diets rich in fish oil, vitamin E, or selenium decrease the activity of the drug (Levander et al., 1989). It has been demonstrated that the activity of these compounds is not due to a direct effect on the lipid structure of the parasites' cell membrane (Browning and Bisby, 1989) nor is it due to intercalation of nucleic acids (Hassan, 1989).

Another sesquiterpene endoperoxide, Yingzhaosu A (Fig. 17 [51]), isolated from the Chinese medicinal plant, *Artobotrys uncinatus* (Annonaceae), has been shown to be an antimalarial agent (Pei-Gen and Shan-Lin, 1986; Xihe, 1979; and references therein). Attempts to synthetically incorporate an endoperoxide moiety into  $\alpha$ -santonin have succeeded in forming such a structure but failed to produce antimalarial activity (Tani et al., 1985).

Gossypol (Fig. 17 [52]), a symmetrical dimer comprised of two cadinane sesquiterpene units from *Gossypium* species (Malvaceae), under investigation as a male antifertility agent, has been shown to inhibit *Plasmodium falciparum* (Heidrich



[51]



[52]

Figure 17. Structural formulas of Yingzhaosu A [51] and gossypol [52].

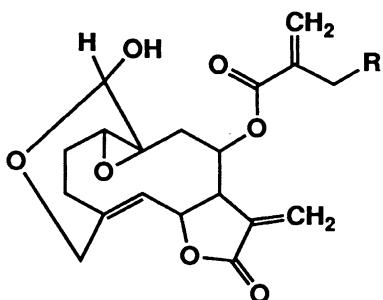
et al., 1983) and *Trypanosoma cruzi* *in vitro* (Blanco et al., 1983). It is a potent inhibitor of NAD-linked enzymes such as lactate dehydrogenase and malate dehydrogenase (Heidrich et al., 1983; Montamat et al., 1982) and NADP-dependent enzymes such as glutamate dehydrogenase and glucose-6-phosphate dehydrogenase (Gerez de Burgos et al., 1984). Addition of serum albumin completely inactivates the parasiticidal activity of the compound (Rovai et al., 1990) suggesting the formation of an inactive protein-drug complex which renders the drug of little use as a clinical entity.

The lactol bridged germacrenolides, vernolide (Fig. 18 [53, R = H]) and hydroxyveranolide (Fig. 18 [53, R = OH]), from *Vernonia colorata* (Asteraceae) have been shown to inhibit *Entamoeba histolytica* *in vitro* and *in vivo*. The *in vitro* activity of vernolide is 5 to 10 times that of its hydroxy analog and is comparable to that of metronidazole (Gasquet et al., 1985). Similarly, parthenin (Fig. 18 [54]), a pseudoguaianolide from *Parthenium hysterophorus* (Asteraceae), has been found to have *in vitro* activity against *E. histolytica* comparable to metronidazole and to be effective in treatment of hepatic amoebiasis in hamsters (Sharma and Bhutani, 1988). A series of 82 sesquiterpene lactones have been evaluated by Soviet scientists for activity *in vitro* against *E. histolytica* and *Trichomonas vaginalis* (Rubinchik et al., 1976). The guaianolides (Fig. 18 [55,56]) were the most active members of the series.

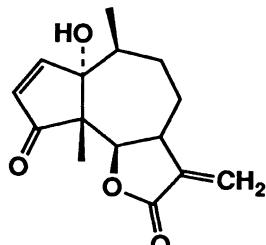
**Diterpenes.** A study of *Tetradenia riparia* (Lamiaceae), a medicinal plant used in Rwanda against a number of diseases, has afforded a diterpenediol with weak antitrichomonal activity. The compound, 8(14),15-sandaracopimaradiene-7 $\alpha$ ,18-diol (Fig. 19 [57]), had an MIC of 20 to 40  $\mu$ g/ml against *T. vaginalis* *in vitro* (Hakizamungu et al., 1988).

The experimental antitumor agent, taxol (Fig. 19 [58]), from *Taxus brevifolia* (Taxaceae) has been shown to stabilize the microtubular cytoskeleton of *Trypanosoma cruzi*, allowing the multiplication of various cellular organelles but inhibiting cell division. This results in motile organisms with extra organelles which are unable to replicate (Baum et al., 1981). A similar phenomenon has been observed for the alkaloid, colchicine (*vide supra*).

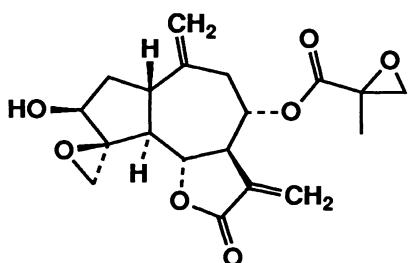
**Triterpenes.** The phytochemical literature contains numerous reports of quassinoids from plants in the Simaroubaceae having antimalarial activity (Bray et al., 1985, 1987a,b; Chan et al., 1986, 1989; Fandeur et al., 1985; Guru et al., 1983; Monjour et al., 1987; O'Neill et al., 1985, 1986, 1987a,b, 1988; Pavanand et al., 1986; Trager and Polonsky, 1981, *inter alia*). O'Neill et al. (1986) have reported a study comparing the antimalarial, antimoebic, antileukemic, and cytotoxic properties of a series of quassinoids and in so doing have formulated an informal set of structure-activity relationships for *in vitro* antimalarial activity. The presence of an



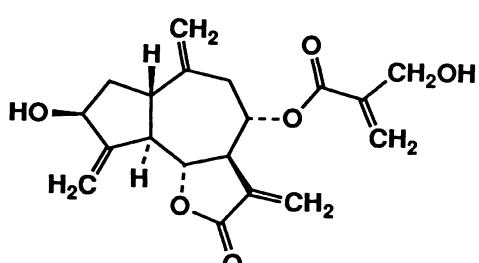
[53]



[54]

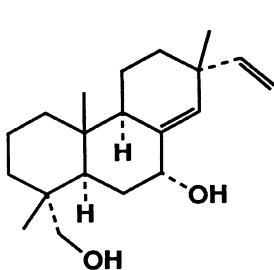


[55]

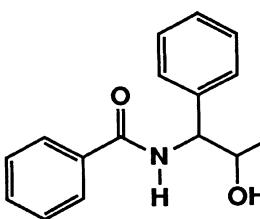


[56]

Figure 18. Structural formulas of vernolide [53, R = H], hydroxyvernolide [53, R = OH], parthenin [54], and guaianolides [55,56].



[57]



[58]

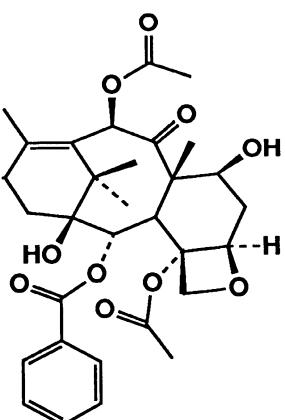


Figure 19. Structural formulas of 8(14),15-sandaracopimaradiene-7 $\alpha$ ,18-diol [57] and taxol [58].

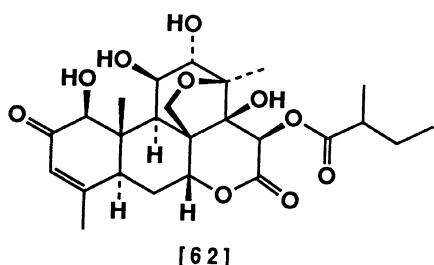
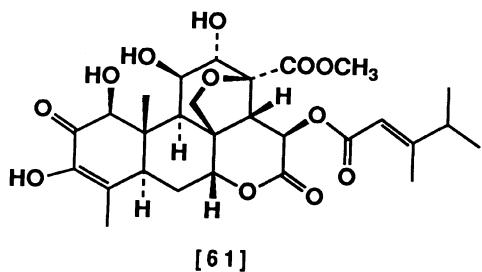
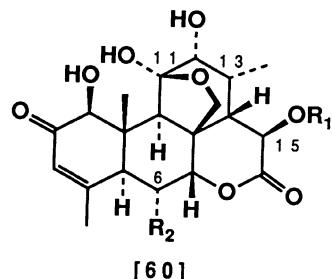
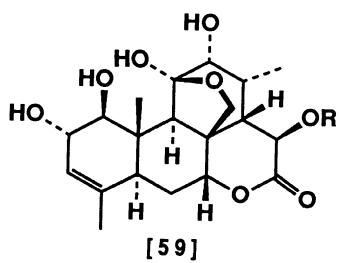


Figure 20. Structural formulas of glaucarubin [59, R =  $\text{COC}(\text{CH}_3)(\text{OH})\text{CH}_2\text{CH}_3$ ], glaucarubol [59, R = H], glaucarubinone [60, R<sub>1</sub> =  $\text{COCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$ , R<sub>2</sub> = H], holacanthone [60, R<sub>1</sub> =  $\text{COCH}_3$ , R<sub>2</sub> = H], undulatone [60, R<sub>1</sub> =  $\text{COCH}_3$ , R<sub>2</sub> =  $\text{OCOC}(\text{CH}_3)=\text{CHCH}_3$ ], bruceantin [61], and simalikalactone D [62].

ester function at C-15 is important for activity as glaucarubin (Fig. 20 [59, R =  $\text{COC}(\text{CH}_3)(\text{OH})\text{CH}_2\text{CH}_3$ ]) is eight times as potent as glaucarubol (Fig. 20 [59, R = H]). Changes in the ester function can make marked changes in the biological activity, as glaucarubinone (Fig. 20 [60, R<sub>1</sub> =  $\text{COCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$ , R<sub>2</sub> = H]) is twice as potent as holacanthone (Fig. 20 [60, R<sub>1</sub> =  $\text{COCH}_3$ , R<sub>2</sub> = H]). An ester at C-15 improves activity over a similar ester at C-6, and esterification at both sites holds little advantage over esterification at C-6 alone as undulatone (Fig. 20 [60, R<sub>1</sub> =  $\text{COCH}_3$ , R<sub>2</sub> =  $\text{OCOC}(\text{CH}_3)=\text{CHCH}_3$ ]) is only slightly more active than holacanthone. The A-ring substitution pattern is critical to activity as glaucarubinone with an  $\alpha, \beta$ -unsaturated ketone moiety is over 10 times as active as glaucarubin which has an allylic alcohol. Attachment of the oxygen bridge from C-20 to C-11 or C-13 makes no obvious difference. In this study, bruceantin (Fig. 20 [61]) and simalikalactone D (Fig. 20 [62]) were the most potent antimalarials with IC<sub>50</sub> values of 0.8 ng/ml and 0.9 ng/ml, respectively, against *Plasmodium falciparum* and were also the most potent compounds in terms of antiamoebic, antileukemic, and cytotoxic activity. It is thought that quassinoids act by inhibiting protein synthesis at the level of the ribosome rather than acting on nucleic acid synthesis (Kirby et al., 1989).

There are few reports of the antimalarial activity of quassinoids *in vivo*. Sergeolide from *Picrolemma pseudocoffea* (Simaroubaceae) and brusatol and bruceines A, B, and D from *Brucea javanica* of the same family all inhibited *Plasmodium berghei* infections in mice with oral ED<sub>50</sub> values from 0.2 to 4 mg/kg/day (Fandeur et al., 1985; O'Neill et al., 1987b). Toxicity appears to preclude the use of these agents in man.

Seven of twelve quassinoids tested have also been demonstrated to have antileishmanial activity (Robert-Gero et al., 1985) with simalikalactone D again being one of the most potent. Antiamoebic activity has also been described in a number of these compounds (Calzado-Flores et al., 1983; Gillin et al., 1982; Wright et al., 1988).

The neem tree, *Azadirachta indica* (Meliaceae), has provided a number of biologically active triterpenoids. Nimbolide (Fig. 21 [63]) inhibits *Plasmodium falciparum* *in vitro* ( $EC_{50} = 0.95 \mu\text{g/ml}$ ) but is inactive against the same organism in mice (Rochanakij et al., 1985). Gedunin (Fig. 21 [64]) shares a similar activity profile (Bray et al., 1990; Khalid et al., 1986, 1989). A curious and perhaps more interesting situation exists for the known insecticide, azadirachtin (Fig. 21 [65]). This compound, when fed to the arthropod vector of Chagas disease, *Rhodnius prolixus*, as part of a blood meal, prevents subsequent parasitic infestation of the arthropod by *Trypanosoma cruzi*. Although azadirachtin was not directly toxic to the trypanosome, exposure of infected *R. prolixus* to the compound suppressed the infection (Rembold and Garcia, 1989). The mechanism of this phenomenon has not been elucidated.

The quinonoid triterpene, tingenone (Fig. 22 [66]), a metabolite of various members of the Celastraceae, has been shown to inhibit *Trypanosoma cruzi* *in vitro* with complete inhibition of the parasite at  $30 \mu\text{g/ml}$  and also to inhibit the nonpathogenic *Critchidia fasciculata* at higher concentrations (Goijman et al., 1985). The related compound, pristimerin (Fig. 22 [67]), from *Celastrus paniculatus* (Celastraceae) showed weak activity against multidrug resistant *Plasmodium falciparum* *in vitro* (Pavanand et al., 1989b). It has been speculated (Goijman et al., 1985) that interaction with the DNA of the parasite is the likely site of action of these compounds.

## Miscellaneous Natural Products

The phenylpropenoids, eugenol (Fig. 23 [68]) and isoeugenol (Fig. 23 [69]), from clove oil (*Eugenia caryophyllata* (Myrtaceae)) were found to inhibit *Trichomonas vaginalis* with  $IC_{50}$  values of  $15 \mu\text{g/ml}$  and  $10 \mu\text{g/ml}$ , respectively (Zemek et al., 1987). Egyptian workers have advocated the use of eugenol containing vaginal douches as a low cost method of controlling *T. vaginalis* infections (Salem, 1980) though the irritant nature of the compound is likely to limit its acceptability.

The prenylated hydroxynaphthoquinone, lapachol (Fig. 24 [70]), found in *Tecoma* species (Bignoniaceae) shows weak activity against amastigotes of *Leishmania donovani* cultured in mouse peritoneal macrophages (Neal and Croft, 1984). This compound also shows very weak inhibition of the erythrocytic stages of *Plasmodium falciparum* *in vitro* (Carvalho et al., 1988) as well as *Trypanosoma cruzi* and *Critchidia fasciculata* (Lopes et al., 1978).  $\beta$ -lapachone (Fig. 24 [71]), a 1,2-naphthoquinone formed by the angular cyclization of lapachol, is significantly more active against the latter two organisms while  $\alpha$ -lapachone (Fig. 24 [72]), the linear analog, is less active (Boveris et al., 1977; Lopes et al., 1978). It is thought (Boveris et al., 1978; Cruz et al., 1978) that the generation of superoxide ion and hydrogen peroxide within the parasite is responsible for the toxic effects of these quinones.

The flavonol, quercetin (Fig. 25 [73]), isolated from species of the Ericaceous genus *Rhododendron*, is a potent inhibitor of the membrane ATPase responsible for the high rate of aerobic glycolysis seen in some tumor cells. Quercetin markedly suppresses the infectivity of African trypanosomes which also show a high rate of aerobic glycolysis *in vitro* when grown in serum-free media. Activity is quickly lost upon addition of serum as the drug is strongly protein bound (Williamson and Scott-Finnegan, 1978). Two polymethoxyflavones from *Artemisia annua*, casticin (Fig. 25 [74], R =  $\text{CH}_3$ ) and artemetin (Fig. 25 [74], R = H), while inactive against *Plasmodium* species alone, were found to selectively enhance the activity of

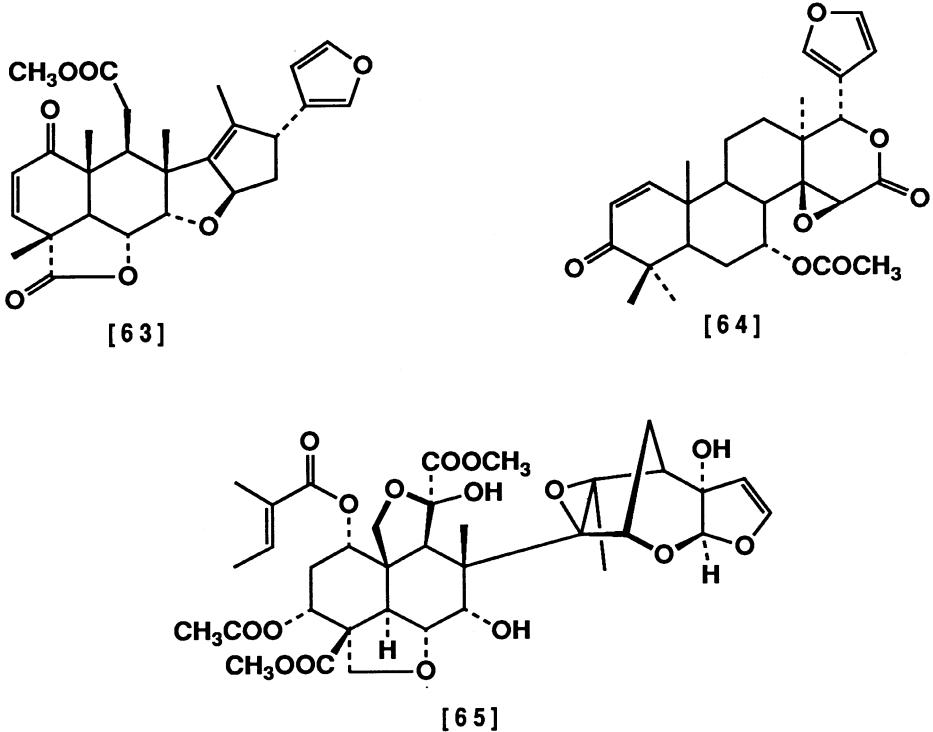


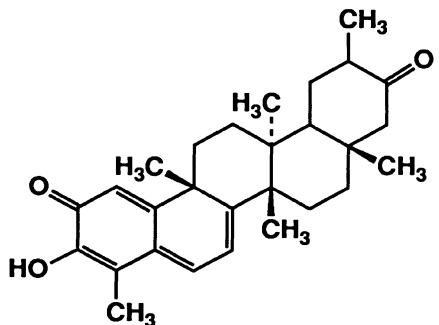
Figure 21. Structural formulas of nimbolide [63], gedunin [64], and azadirachtin [65].

artemisinin against *Plasmodium falciparum*. Similar experiments using chloroquine showed little if any potentiation (Elford et al., 1987). While the mechanism of the enhancement of artemisinin activity has not been elucidated, the phenomenon suggests that artemisinin and chloroquine act via different biochemical mechanisms. It is interesting to note that these flavonoids co-occur with artemisinin in *A. annua* and that crude extracts of the plant may indeed offer a therapeutic advantage over the purified sesquiterpene.

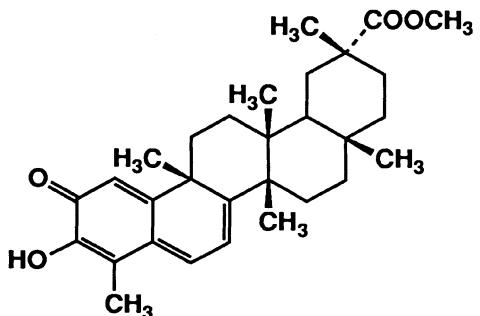
Chinese workers have reported that a crystalline phloroglucinol derivative, japonicine A (Fig. 26 [75]), isolated from *Hypericum japonicum* (Clusiaceae), possesses antimalarial activity (Gu et al., 1984). Robustadial A (Fig. 26 [76]) isolated from *Eucalyptus robusta* (Myrtaceae), a grossly similar compound, has activity against *Plasmodium berghei*. The robustadials A and B (Fig. 26 [77]), differing in stereochemistry at C-7, from the same plant are reported to be more potent antimalarials, but assay data have not been reported (Xu et al., 1984).

$\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC) (Fig. 26 [78]), the well-known biologically active constituent of *Cannabis sativa* (Cannabidaceae), has been evaluated as a chemotherapeutic for treatment of *Naegleria fowleri* infections. Selected because it accumulates in the brain, the site of *N. fowleri* infection,  $\Delta^9$ -THC was found to be amoebastatic at 5 to 50  $\mu\text{g}/\text{ml}$  *in vitro* (Pringle et al., 1979).

The cyclohexene epoxide, (-)-pipoxide (Fig. 27 [79]), from *Uvaria ferruginea* (Annonaceae) has been shown to possess weak activity against *Plasmodium falciparum* *in vitro* (Nkunya et al., 1987) while the related compounds, (+)-pandoxide (Fig. 27 [80,

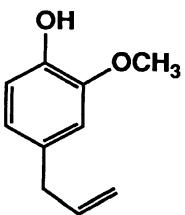


[ 66 ]

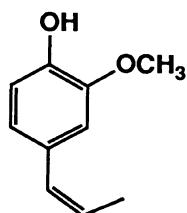


[ 67 ]

Figure 22. Structural formulas of tingenone [66] and pristimerin [67].

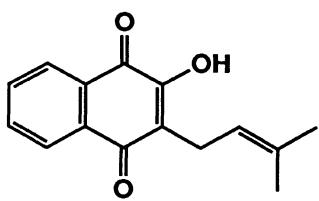


[ 68 ]

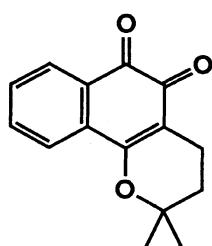


[ 69 ]

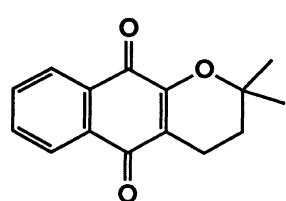
Figure 23. Structural formulas of eugenol [68] and isoeugenol [69].



[ 70 ]

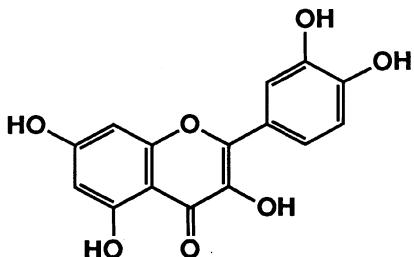


[ 71 ]

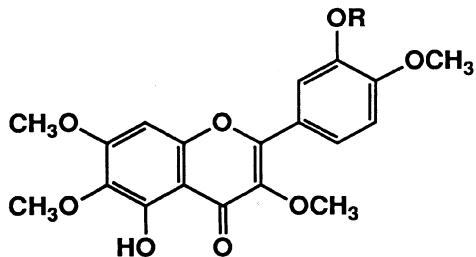


[ 72 ]

Figure 24. Structural formulas of lapachol [70],  $\beta$ -lapachone [71], and  $\alpha$ -lapachone [72].



[73]



[74]

Figure 25. Structural formulas of quercetin [73], casticin [74, R = CH<sub>3</sub>], and artemetin [74, R = H].

R = acetyl, R' = CH<sub>3</sub>]) and (+)-β-senepoxide (Fig. 27 [80, R = R' = acetyl]), from the same plant were inactive.

Allicin (Fig. 28 [81]), a sulfinothioc acid ester responsible for the odor of garlic (*Allium sativum* (Alliaceae)), has been shown to irreversibly inhibit the growth of *Entamoeba histolytica* *in vitro* at 30 µg/ml (Mirelman et al., 1987). Lower concentrations inhibited growth but trophozoite lethality was incomplete, allowing regrowth to occur after 48 hours. Inactivation of sulhydryl containing enzymes is presumed to be the mode of action of this compound.

The polyacetylene, 1-phenyl-1,3,5-heptatriyne (Fig. 28 [82]) from *Bidens pilosa* (Asteraceae) has been found to inhibit *Trichomonas vaginalis* *in vitro* (MIC = 10 µg/ml) and *Plasmodium falciparum* *in vitro* (IC<sub>50</sub> = 45 ng/ml). The compound was inactive against *P. berghei* in mice (N'dounga et al., 1983).

Saturated fatty acids of 10, 12, and 16 carbons have been found to inhibit the motility of *Leishmania donovani*, *L. tropica* promastigotes, and *Trypanosoma cruzi* epimastigotes and trypomastigotes at levels below 100 µg/ml (Cunningham et al., 1972). It is thought that these compounds destabilize the parasitic cell membrane leading to lysis of the organisms.

## ANTHELMINTHIC AGENTS

Natural products have been used for centuries for the treatment of helminth diseases with varying amounts of success. Today, the search for novel, safe, and efficacious compounds continues. However, there are virtually no commercially available anthelmintic agents derived from plant sources. The most widely used anthelmintic agents, the avermectins/milbemycins and the benzimidazoles, are either natural and semisynthetic products isolated from bacterial sources, or synthetic compounds. This does not preclude the possibility that important new anthelmintics or new leads will be identified from plant sources. A major impediment in the development of a commercial product from plants is the difficulty in obtaining large amounts of material grown under constant conditions (in contradistinction to bacterial sources which may be grown in large scale fermentors under controlled conditions). With this caveat in place, it should be emphasized that plant-derived anthelmintics have been isolated and widely used; and many laboratories continue to search for better anthelmintic agents from such sources.

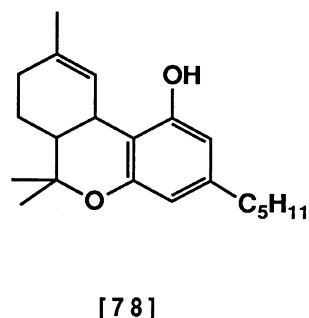
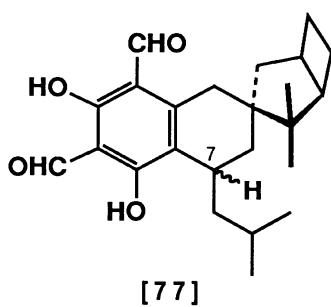
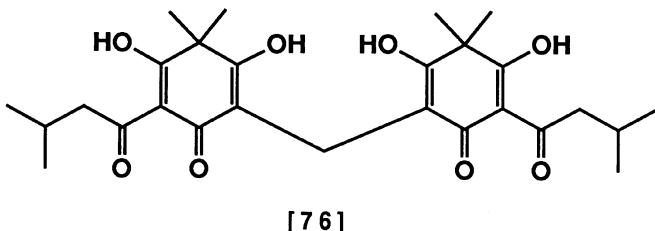
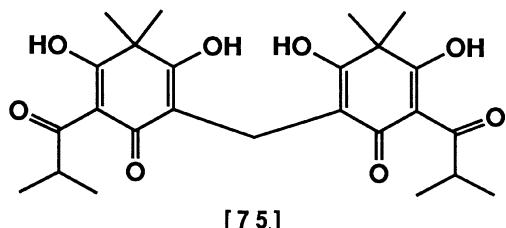


Figure 26. Structural formulas of japonicine A [75], robustao A[76], robustadials A and B [77], and  $\Delta^9$ -tetrahydrocannabinol [78].

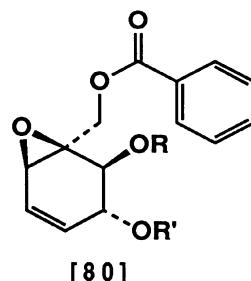
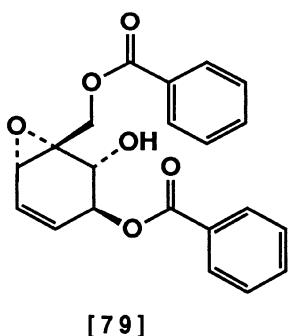


Figure 27. Structural formulas of (-)-pipoxide [79], (+)-pandoxide [80, R = acetyl, R' = CH<sub>3</sub>], and (+)- $\beta$ -senepoxide [80, R = R' = acetyl].

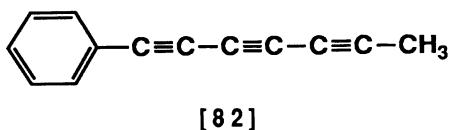
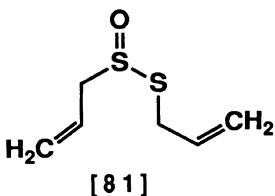


Figure 28. Structural formulas of allicin [81] and 1-phenyl-1,3,5-heptatriyne [82].

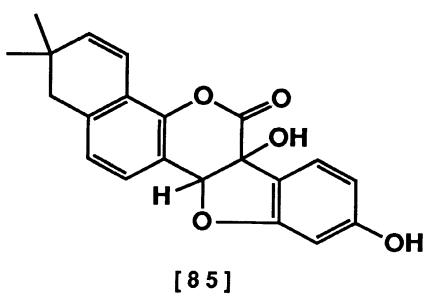
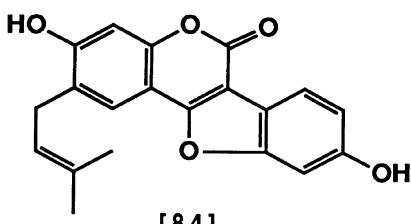
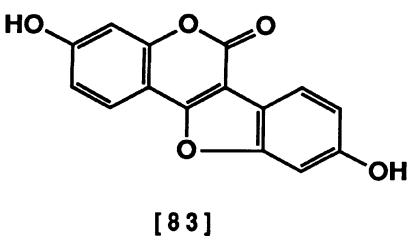


Figure 29. Structural formulas of coumestrol [83], psoralidin [84], and glyceollin [85].

## Nematicidal Agents

**Phytoalexins.** Phytoalexin derives from the Greek term meaning "warding-off agent in plants." The phytoalexin theory suggests that plants synthesize chemoprotectants in response to various pathogens. Hundreds of compounds have been isolated from plants and identified as phytoalexins. The phytoalexin response can be elicited by a variety of agents, including viruses, bacteria, and nematodes (O'Neill, 1986). When resistant plants are infected by nematodes, phytoalexins with anthelmintic activity are produced (Veech, 1982). An example of such a response is the resistant lima bean (*Phaseolus lunatus* (Fabaceae)). When the roots were inoculated with the nematode *Pratylenchus scribneri*, the plant accumulated coumestans such as coumestrol (Fig. 29 [83]) and psoralidin (Fig. 29 [84]) at the sites of nematode attack (Rich et al., 1977). Coumestrol inhibits *P. scribneri* motility *in vitro* with an ED<sub>50</sub> of 10 to 15 µg/ml; however, it is not efficacious against a broad spectrum of nematodes. Glyceollin (Fig. 29 [85]) is reported to be a soybean phytoalexin. When

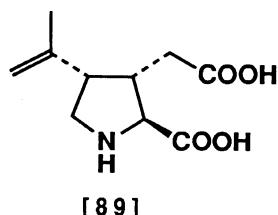
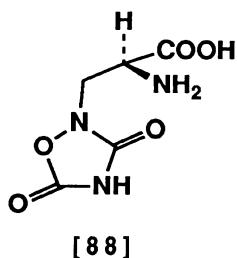
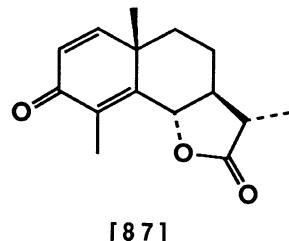
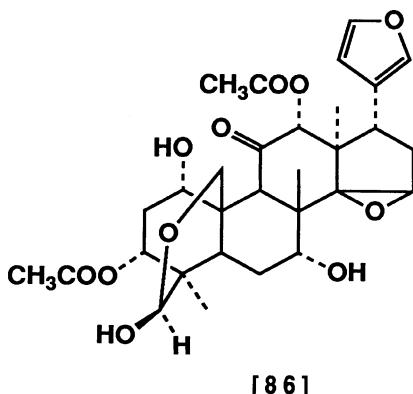


Figure 30. Structural formulas of chuanliansu [86], santonin [87], quisqualic acid [88], and  $\alpha$ -kainic acid [89].

resistant soybean cultivars were infected with *Meloidogyne incognita*, the levels of glyceollin increased dramatically, particularly in the stеле. Under *in vitro* conditions, it was demonstrated that glyceollin is a potent cidal agent against *M. incognita* and ineffective against *M. javanica* (Kaplan et al., 1980a). The mode of action of glyceollin is reported to be due to inhibition of the electron transport system (Kaplan et al., 1980b). A phytoalexin-type mechanism of resistance of cotton to the root-knot nematode has also been reported (Veech, 1979; Veech and McClure, 1977). Cotton (*Gossypium hirsutum* (Malvaceae)) may produce gossypol (Fig. 17 [52]) and related terpenoids in root tips upon infection of some nematodes (i.e., *M. incognita*). Gossypol is known to inhibit the motility of *M. incognita*.

A critical issue arising from these studies is whether a phytoalexin with nematocidal activity against a specific plant nematode will provide a useful lead for the development of compounds for the treatment of nematodal infections in animals.

**Ascaricidal agents.** The bark of *Melia toosendan* (a cultivar of *M. azedarach* (Meliaceae)) is used to expel ascarids in China. Its active principal is chuanliansu, the chemical structure of which has been defined as 28-deacetyl-sandanin (Fig. 30 [86]) (Shu and Liang, 1980). The anthelmintic effect of chuanliansu is similar to that of santonin (Fig. 30 [87]), another natural anthelmintic derived from the wormwood, *Artemisia maritima* (Asteraceae) (Guru et al., 1982). Santonin usually paralyzes the worms but does not always expel them efficiently, so it requires coadministration with a cathartic agent. Chuanliansu is less toxic and does not need to be administered with a cathartic.

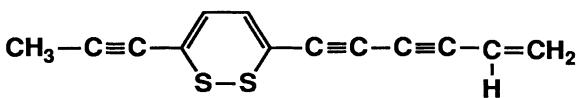
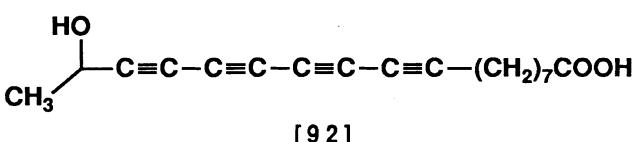
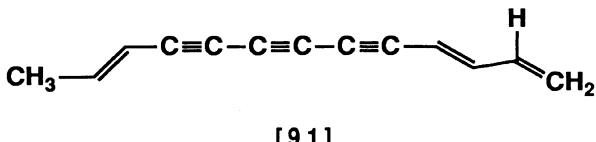
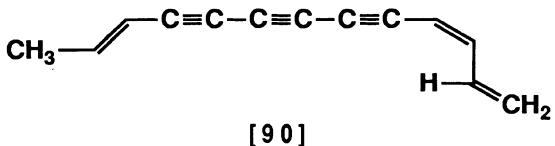


Figure 31. Structural formulas of *Carthamus tinctorius* polyacetylenes [90,91], minquartynoic acid [92], and thiarubrine A [93].

The seeds of the "Rangoon creeper," *Quisqualis indica* (Combretaceae) are also commonly used for ascariasis in China. The active component is quisqualic acid (Fig. 30 [88]), whose structure was elucidated as L- $\beta$ -(3,5-dioxo-1,2,4-oxadiazolidine-2-yl)-alanine (Pan et al., 1976). In clinical trials, potassium quisqualate at doses of 125 mg was strongly anthelmintic; the proportion of patients who excreted ascarids approached that of patients treated with santonin and was greater than that of patients who received the raw or baked seeds (Duan, 1983). The only drawback of using potassium quisqualate seems to be the common side effect of hiccoughs.

*Caloglossa leprieurii* (Asteraceae) is also used as an anthelmintic in China. Its active ingredient has been identified as  $\alpha$ -kainic acid (Fig. 30 [89]) (Liu, 1979).

Both quisqualate and kainate bind to glutamate receptors in mammalian brain tissue (Krogsgaard-Larsen and Honore, 1983) and increase the influx of monovalent cations. It was recently reported that nematodes contain specific high affinity glutamate binding sites (Rohrer et al., 1990; Schaeffer et al., 1990) suggesting that the ascaricidal activity of quisqualate and kainate may be mediated via a glutamate receptor interaction.

**Polyacetylene nematocidal agents.** Two polyacetylenes with nematocidal activity were isolated from *Carthamus tinctorius* (Asteraceae) (Fig. 31 [90,91]) (Kogiso et al., 1976). The biological activity was assessed by monitoring *in vitro* motility of *Aphelenchooides besseyi*. Compound [91] (Fig. 31) was 5- to 10-fold more active than



[94]

Figure 32. Structural formula of palasonin [94].

[90] (Fig. 31). Additional information, such as toxicity and breadth of spectrum, was not reported.

The stem bark of *Minquartia guianensis* (Oleaceae) is used by the Quijos Quichua people of Ecuador's Amazonian lowlands in an infusion drunk to treat intestinal parasitic infections. A pure compound was isolated from an aqueous extract of this bark using the brine shrimp larvicidal bioassay to monitor biological activity. The novel cytotoxic polyacetylene, (-)-17-hydroxy-9,11,13,15-octadecatetraynoic acid (minquartyoic acid) (Fig. 31 [92]), was isolated (Marles et al., 1989). The determination of whether this compound is the agent responsible for the anthelmintic activity was not reported.

Wild chimpanzees selectively pick and swallow entire leaves of *Aspilia mossambicensis* Oliv., *A. pluriseta* O. Hoffm., and *A. rufis* Oliv. (Asteraceae) without chewing them. It was suggested that this feeding behavior may be related to a special pharmacological effect. Rodriguez et al. (1985) isolated thiarubrine A (Fig. 31 [93]) as a bioactive constituent of *Aspilia*. Thiarubrine A has nematocidal activity in a *Caenorhabditis elegans* (a free-living nematode) motility assay and it is suggested that consumption of these leaves may be beneficial to the chimpanzee.

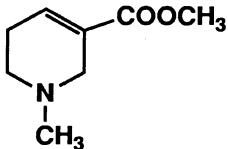
The mode of action of the polyacetylenes is not known; however, it has been suggested (Rodriguez et al., 1985) that these compounds may be photosensitizing agents like  $\alpha$ -terthienyl (Kagan et al., 1989).

**Other nematocidal agents.** Raj and Kurup (1967) reported that the seeds of *Butea frondos* (Fabaceae), an Indian tree, contain substances with nematocidal properties. The compound was isolated and named palasonin (Fig. 32 [94]). The active component was later identified (Bochis and Fisher, 1968) as *exo-cis*-3,6-epoxy-1-methylhexahydrophthalic anhydride.

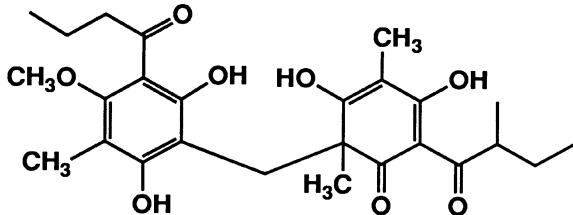
### Anticestodal Agents

The cestofugal properties of betel nut (*Areca catechu* (Arecaceae)) extracts have long been known. The active compound is the alkaloid, arecoline (Fig. 33 [95]), which has been widely used in veterinary medicine since 1921 (Watkins, 1958).

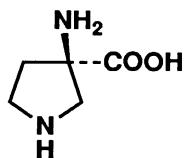
The powdered root sprout of Agrimony (*Agrimonia pilosa* (Rosaceae)) is widely used in China to expel tapeworms. Agrimophol (Fig. 33 [96]) isolated from the buds has a confirmed effect on *Taenia solium* *in vitro*, and the efficacy of this compound as a taeniafuge has been confirmed in clinical trials (Xiao and Fu, 1986). Pumpkin seeds (*Cucurbita moschata* (Cucurbitaceae)) are also used in folk medicine to expel cestodes. It has now been demonstrated that the active component is an unusual amino acid, cucurbitine (Fig. 33 [97]). Cucurbitine effectively expels *T. hydatigena*, *T. pisiformis*, and *Diphyllobothrium mansoni* with no signs of toxicity at therapeutic doses (Chen, 1980).



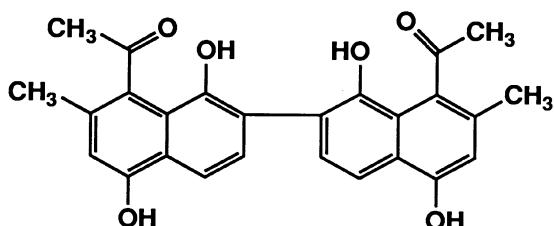
[95]



[96]



[97]



[98]

Figure 33. Structural formulas of arecoline [95], agrimophol [96], cucurbitine [97], and hemerocallin [98].

*Vernonia colorata* (Asteraceae) leaves have been traditionally used as a medication to treat intestinal nematode and cestode infections in the Ivory Coast. Two sesquiterpene lactones, vernolide and hydroxyvernolide (Fig. 18 [53, R = H, OH, respectively]) have been identified as the active components of this plant (Gasquet et al., 1985). Vernolide is 5- to 10-fold more active than hydroxyvernolide *in vitro*; however, only hydroxyvernolide is active *in vivo*. The potency of hydroxyvernolide is not impressive, requiring 500 mg/kg/day for 4 days in order to be 60% effective against *Syphacia obvelata*.

### Antitrematodal Agents

A series of chemical derivatives of artemisinin (qinghaosu) have been synthesized in order to enhance the antimarial activity and solubility properties (*vide supra*). During the evaluation of these compounds, it was determined that one of these semisynthetic compounds, artemether (Fig. 16 [48, R = CH<sub>3</sub>]) as well as artemisinin, was effective in the treatment of *Schistosoma japonicum* infections (Le et al., 1982, 1983). *Schistosoma mansoni*-infected mice were used as a model to determine the potential usefulness of these drugs in the treatment of *S. mansoni* infections in man. *In vivo*, artemether was 98% effective at high doses (200 mg/kg/day, six doses) against 2- to 3-week-old liver-stage parasites but ineffective against the adult stages of the parasite (Xiao and Catto, 1989).

In addition to their effects on *Taenia*, both agrimophol (Fig. 33 [96]) and cucurbitine (Fig. 33 [97]) are active against *Schistosoma japonicum* (You et al., 1982). When pairs of adult worms isolated from rabbits were transferred to a culture medium containing 4 µg/ml of agrimophol, a stimulation of worm activity appeared within 4 minutes followed by a spasmodic contraction of the schistosome musculature. Even

at a concentration of 0.5 to 20 µg/ml, agrimophol markedly reduced the survival time of worms in this culture medium. In tests using experimentally infected mice, oral administration of agrimophol at 300 mg/kg/day combined with oral niridazole at 75 mg/kg/day for 5 days caused a hepatic shift of the worms. When the worms were perfused out of the liver and maintained in a drug free medium, they could not recover their normal activity. In contrast, cucurbitine appears to exert a protective effect against *S. japonicum*. When mice, each infected with cercariae, were given daily oral cucurbitine at 400 mg/kg for 28 days, the development of schistosomula was greatly retarded. Similarly, when mice were infected with cercariae and treated 4 weeks later with cucurbitine at 250 mg/kg for 7 days followed by 500 mg/kg for 21 days, the resulting adult worms were small and had degenerate reproductive organs. Small pathological lesions associated with cucurbitine treatment were observed in these mice (Chen, 1980).

Hemerocallin (Fig. 33 [98]) isolated from the day lily, *Hemerocallis minor* (Liliaceae), is a potent anti-schistosomal compound when evaluated in mice experimentally infected with *S. japonicum* (Chen et al., 1962). However, the toxicity of hemerocallin precludes use as a therapeutic tool.

## CONCLUSION

The Plant Kingdom is a vast but poorly utilized resource for the discovery and development of antiparasitic agents. Few known antiparasitic phytochemicals have the combination of potency, efficacy, and safety necessary for development and use in today's Western medical practice. The widespread use of plants as antiparasitic agents in the traditional medical practices of indigenous people worldwide and the large numbers of species left unstudied in the laboratory suggest that a substantial number of potentially interesting active compounds may still remain to be discovered, representing a fertile area for investigation in years to come.

## ACKNOWLEDGMENTS

The authors wish to thank Dr. J. Ravindra Babu for helpful discussions during preparation of this manuscript and Professor Norman R. Farnsworth, Program for Collaborative Research in the Pharmaceutical Sciences, University of Illinois at Chicago, for access to the NaprAlert database.

## REFERENCES

- Ablondi, F., Gordon, S., Morton, J., II, and Williams, J. H., 1952, An antimalarial alkaloid from Hydrangea. II. Isolation, Journal of Organic Chemistry, 17:14-18.
- Academia Sinica, 1980, Crystal structure and absolute configuration of qinghaosu, Scientia Sinica (English Edition), 23(3):380-396.
- Astolfi Filho, S., Periera de Almeida, E. R., and Ganderm, E. S., 1978, The influence of hydroxyurea and colchicine on growth and morphology of *Trypanosoma cruzi*, Acta Tropica, 35:229-237.
- Bajpai, R., Dutta, G. P., and Vishwakarma, R. A., 1989, Blood schizontocidal activity of a new antimalarial drug, arteether ( $\alpha/\beta$ ), against *Plasmodium knowlesi* in rhesus monkeys, Transactions of the Royal Society of Tropical Medicine and Hygiene, 83:484.

- Baum, S. G., Wittner, M., Nadler, J. P., Horwitz, S. B., Dennis, J. E., Schiff, P. B., and Tanowitz, H. B., 1981, Taxol, a microtubule stabilizing agent, blocks the replication of *Trypanosoma cruzi*, Proceedings of the National Academy of Sciences U.S.A., 78(7):4571-4575.
- Benard, J., Dat-Xuong, N., and Riou, G., 1975, Activite trypanocide de quelques derives de l'ellipticine sur *Trypanosoma cruzi* cultive *in vitro*, Comptes Rendus de l'Academie des Sciences (Paris) Serie D, 280(9):1177-1180.
- Benard, J., and Riou, G., 1977, Effects of 9-hydroxy ellipticine on *in vitro* transcription of *Trypanosoma cruzi* DNAs, Biochemical and Biophysical Research Communications, 77(4):1189-1195.
- Bennett, S. C. J., 1935, Equine cutaneous leishmaniasis: Treatment with berberine sulphate, Journal of Comparative Pathology and Therapeutics, 48:241-243.
- Bhandari, P. R., and Mukerji, B., 1959, *Holarrhena antidysenterica* Wall (Kurchi), The Pharmaceutist, 1959:31-35.
- Bhutani, K. K., Sharma, G. L., and Ali, M., 1987, Plant based antiamoebic drugs. Part I. Antiamoebic activity of phenanthroindolizidine alkaloids: Common structural determinants of activity with emetine, Planta Medica, 53(6):532-536.
- Blanco, A., Aoki, A., Montamat, E. E., and Rovai, E., 1983, Effect of gossypol upon motility and ultrastructure of *Trypanosoma cruzi*, Journal of Protozoology, 30(4):648-651.
- Bochis, R. J., and Fisher, M. H., 1968, The structure of palasonin, Tetrahedron Letters, 16:1971-1974.
- Bodalski, T., Pelczarska, H., and Ujec, M., 1958, Dzialanie sangwinarny i chelerytryny na *Trichomonas vaginalis* *in vitro*, Archiwum Immunologii i Terapii Doswiadczennej, 6:705-711.
- Borst, P., 1977, Metabolism and chemotherapy of African trypanosomes, Transactions of the Royal Society of Tropical Medicine and Hygiene, 71:3-4.
- Boveris, A., Docampo, R., Turrens, J. F., and Stoppani, A. O. M., 1977, Accion de  $\beta$  y  $\alpha$ -lapachona sobre la produccion de  $H_2O_2$  y el crecimiento de *Trypanosoma cruzi*, Revista de la Asociacion Argentina de Microbiologia, 9(2):54-61.
- Boveris, A., Stoppani, A. O. M., Docampo, R., and Cruz, F. S., 1978, Superoxide anion production and trypanocidal action of naphthoquinones on *Trypanosoma cruzi*, Comparative Biochemistry and Physiology, 61C:327-329.
- Brandicourt, O., Druilhe, P., Diouf, F., Brasseur, P., Turk, P., and Danis, M., 1986, Decreased sensitivity to chloroquine and quinine of some *Plasmodium falciparum* strains from Senegal in September 1984, American Journal of Tropical Medicine and Hygiene, 35(4):717-721.
- Bray, D. H., O'Neill, M. J., Boardman, P., Phillipson, J. D., and Warhurst, D. C., 1985, Structure related *in vitro* antimalarial activities of some quassinoids, Journal of Pharmacy and Pharmacology (Supplement), 37:142P.
- Bray, D. H., O'Neill, M. J., Phillipson, J. D., and Warhurst, D. C., 1987a, *In vivo* antimalarial activity of quassinoids, Journal of Pharmacy and Pharmacology (Supplement), 39:85P.
- Bray, D. H., Boardman, P., O'Neill, M. J., Chan, K. L., Phillipson, J. D., Warhurst, D. C., and Suffness, M., 1987b, Plants as a source of antimalarial drugs. 5. Activities of *Ailanthus altissima* stem constituents and of some related quassinoids, Phytotherapy Research, 1(1):22-24.
- Bray, D. H., Warhurst, D. C., Connolly, J. D., O'Neill, M. J., and Phillipson, J. D., 1990, Plants as sources of antimalarial drugs. Part 7. Activity of some species of Meliaceae plants and their constituent limonoids, Phytotherapy Research, 4(1):29-35.

- Browning, P. M., and Bisby, R. H., 1989, Qinghaosu does not affect the major thermotropic phase transition in model membranes of dipalmitoylphosphatidylcholine, *Molecular and Biochemical Parasitology*, 32(1):57-60.
- Bundy, D. A. P., 1990, New initiatives in the control of helminths, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 84:467-468.
- Bunnag, D., Harinasuta, T., Vanijanonta, S., Looareesuwan, S., Chittamas, S., Pannavut, W., Berthe, J., and Druilhe, P., 1987, Treatment of chloroquine resistant falciparum malaria with a combination of quinine, quinidine, and cinchonine (LA 40221) in adults by oral and intravenous administration, *Acta Leidensia*, 55:139-149.
- Bunnag, D., Harinasuta, T., Looareesuwan, S., Chittamas, S., Pannavut, W., Berthe, J., and Mondesir, J. M., 1989, A combination of quinine, quinidine and cinchonine (LA 40221) in the treatment of chloroquine resistant falciparum malaria in Thailand: Two double-blind trials, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83:66.
- Calzado-Flores, C. C., Segura, J. J., Rodriguez, V. M., and Dominguez, X. A., 1983, A new amoebicide agent from *Castela texana*, *Proceedings of the Western Pharmacology Society*, 26:431-434.
- Carvalho, L. H., Rocha, E. M. M., Raslan, D. S., Oliveira, A. B., and Krettli, A. U., 1988, *In vitro* activity of natural and synthetic naphthoquinones against erythrocytic stages of *Plasmodium falciparum*, *Brazilian Journal of Medical and Biological Research*, 21:485-487.
- Cavin, J. C., Krassner, S. M., and Rodriguez, E., 1987, Plant-derived alkaloids active against *Trypanosoma cruzi*, *Journal of Ethnopharmacology*, 19:89-94.
- Chan, K. L., O'Neill, M. J., Phillipson, J. D., and Warhurst, D. C., 1986, Plants as sources of antimalarial drugs. Part 3. *Eurycoma longifolia*, *Planta Medica*, 52(2):105-107.
- Chan, K. L., Lee, S. P., Sam, T. W., and Han, B. H., 1989, A quassinoid glycoside from the roots of *Eurycoma longifolia*, *Phytochemistry*, 28(10):2857-2859.
- Chatterjee, D. K., Iyer, N., and Ganguli, B. N., 1987, Antiamoebic activity of chonemorphine, a steroid alkaloid, in experimental models, *Parasitology Research*, 74:30-33.
- Chen, C., Huang, N. Y., and Hsueh, A. J., 1962, Clinical evaluation of hemerocallin as an antischistosomal drug, *Acta Pharmaceutica Sinica*, 9:579-586.
- Chen, J. H., 1980, Anticestodal activity of cucurbitine, *Chinese Traditional Herbal Drugs*, 11:1-14.
- Chopra, R. N., and Chakerbury, M., 1935, The pharmacological action of tylophorine: The alkaloid occurring in *Tylophora asthmaticus*, *Indian Journal of Medical Research*, 23:263-269.
- Chou, A. C., Chevli, R., and Fitch, C. D., 1980, Ferriprotoporphyrin IX fulfills the criteria for identification as the chloroquine receptor of malaria parasites, *Biochemistry*, 19:1543-1549.
- Coordinating Group for Research on the Structure of Qing Hau Sau, 1977, A new type of sesquiterpene lactone - Qing hau sau, *K'o Hsueh T'ung Pao*, 22(3):142.
- Cruz, F. S., Vasconcellos, M. E., and Leon, W., 1975, Inhibition of different strains of *Trypanosoma cruzi* by olivacine and olivacine pamoate, *Journal of Protozoology*, 22:86A-87A (Abstract).
- Cruz, F. S., Docampo, R., and DeSouza, W., 1978, Effect of  $\beta$ -lapachone on hydrogen peroxide production in *Trypanosoma cruzi*, *Acta Tropica*, 35:35-40.
- Cunningham, L. V., Kazan, B. M., and Kuwahara, S. S., 1972, Effect of long-chain fatty acids on some trypanosomatid flagellates, *Journal of General Microbiology*, 70:491-496.

- DasGupta, B. M., 1930, The treatment of oriental sore with berberine acid sulphate, Indian Medical Gazette, 65:683-685.
- DasGupta, B. M., and Dikshit, B. B., 1929, Berberine in the treatment of oriental sore, Indian Medical Gazette, 64:67-70.
- Devi, A. L., 1929, Berberine sulphate in oriental sore, Indian Medical Gazette, 64:139-140.
- Ding, G. S., 1988, Recent studies on antimalarials in China: A review of literature since 1980, International Journal of Experimental and Clinical Chemotherapy, 1(2):9-21.
- Dos Santos Filho, D., and Gilbert, B., 1975, The alkaloids of *Nectandra megapotamica*, Phytochemistry, 14:821-822.
- Dreyfuss, G., Allais, D. P., Guinaudeau, H., and Bruneton, J., 1987, Recherche de l'activite trypanocide d'alcaloides isoquinoliques chez la souris, Annales Pharmaceutiques Francaises, 45(3):243-248.
- Druilhe, P., Brandicourt, O., Chongsuphajaisiddhi, T., and Berthe, J., 1988, Activity of a combination of three *Cinchona* bark alkaloids against *Plasmodium falciparum* *in vitro*, Antimicrobial Agents and Chemotherapy, 32(2):250-254.
- Duan, M. F., 1983, Anthelmintic properties of *Quisqualis indica*, p. 1024-1030, in: "The Pharmacology and Usage of Traditional Chinese Drugs," Y. S. Wang, ed., People's Medical Publishing House, Beijing, China.
- Dutta, G. P., and Yadava, J. N. S., 1972, Direct amoebicidal action of known antiamoebic drugs against axenically grown *Entamoeba histolytica*, Indian Journal of Medical Research, 60:1156-1163.
- Dutta, G. P., Bajpai, R., and Vishwakarma, R. A., 1989a, Comparison of antimalarial efficacy of artemisinin (qinghaosu) and arteether against *Plasmodium cynomolgi* B infection in monkeys, Transactions of the Royal Society of Tropical Medicine and Hygiene, 83:56-57.
- Dutta, G. P., Bajpai, R., and Vishwakarma, R. A., 1989b, Antimalarial efficacy of arteether against multiple drug resistant strain of *Plasmodium yoelii nigeriensis*, Pharmacology Research, 21(4):415-419.
- Elford, B. C., Roberts, M. F., Phillipson, J. D., and Wilson, J. M., 1987, Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones, Transactions of the Royal Society of Tropical Medicine and Hygiene, 81:434-436.
- Evans, A. T., and Croft, S. L., 1987, Antileishmanial activity of harmaline and other tryptamine derivatives, Phytotherapy Research, 1(1):25-27.
- Fandeur, T., Moretti, C., and Polonsky, J., 1985, *In vitro* and *in vivo* assessment of antimalarial activity of sergeolide, Planta Medica, 51(1):20-23.
- Fournet, A., Manjon, A. M., Munoz, V., Angelo, A., Bruneton, J., Hocquemiller, R., Cortes, D., and Cave, A., 1988a, Activite antiparasitaire d'alcaloides bisbenzylisoquinoleiques. II. Activite *in vitro* sur des epimastigotes de trois souches typifiees de *Trypanosoma cruzi*, Journal of Ethnopharmacology, 24:337-343.
- Fournet, A., Munoz, V., Manjon, A. M., Angelo, A., Hocquemiller, R., Cortes, D., Cave, A., and Bruneton, J., 1988b, Activite antiparasitaire d'alcaloides bisbenzylisoquinoleiques. I. Activite *in vitro* sur des promastigotes de trois souches de *Leishmania*, Journal of Ethnopharmacology, 24:327-335.
- Fujioka, H., Nishiyama, Y., Furukawa, H., and Kumada, N., 1989, *In vitro* and *in vivo* activities of atalaphillinine and related acridone alkaloids against rodent malaria, Antimicrobial Agents and Chemotherapy, 33(1):6-9.
- Gasquet, M., Bamba, D., Babadjamian, A., Balansard, G., Timon-David, P., and Metzger, J., 1985, Action amoebicide et anthelminthique du vernalide et de l'hydroxyvernalide isolés des feuilles de *Vernonia colorata* (Willd.) Drake, European Journal of Medicinal Chemistry, 20(2):111-115.

- Geary, T. G., Divo, A. A., and Jensen, J. B., 1989, Stage specific actions of antimalarial drugs on *Plasmodium falciparum* in culture, American Journal of Tropical Medicine and Hygiene, 40(3):240-244.
- Gerez de Burgos, N. M., Burgos, C., Montamat, E. E., Rovai, L. E., and Blanco, A., 1984, Inhibition by gossypol of oxidoreductases from *Trypanosoma cruzi*, Biochemical Pharmacology, 33(7):955-959.
- Gillin, F. D., Reiner, D. S., and Suffness, M., 1982, Bruceantin, a potent amoebicide from a plant, *Brucea antidyserterica*, Antimicrobial Agents and Chemotherapy, 22(2):342-345.
- Glew, R. H., Collins, W. E., and Miller, L. H., 1978, Selection of increased quinine resistance in *Plasmodium falciparum* in Aotus monkeys, American Journal of Tropical Medicine and Hygiene, 27(1):9-13.
- Goijman, S. G., Turrens, J. F., Marini-Bettolo, G. B., and Stoppani, A. O. M., 1985, Effect of tingenone, a quinonoid triterpene, on growth and macromolecule biosynthesis in *Trypanosoma cruzi*, Experientia, 41:646-648.
- Grollman, A. P., 1966, Structural basis for inhibition of protein synthesis by emetine and cycloheximide based on an analogy between ipecac alkaloids and glutarimide antibiotics, Proceedings of the National Academy of Sciences U.S.A., 56:1867-1874.
- Grollman, A. P., 1967, Structural basis for the inhibition of protein biosynthesis: Mode of action of tubulosine, Science, 157:84-85.
- Grollman, A. P., and Jarkovsky, Z., 1974, Emetine and related alkaloids, Antibiotics, 3:420-435.
- Gu, G., Feng, S., Wang, X., Zhou, Y., and Li, G., 1984, Studies on the active principles of Di-er Cao. The isolation and structure of japonicaine A, Kexue Tongbao, 29(4):548-549.
- Gupta, R. S., and Siminovitch, L., 1977, Mutants of CHO cells resistant to the protein synthesis inhibitors, cryptopleurine and tylocrebrine: Genetic and biochemical evidence for a common site of action of emetine, cryptopleurine, tylocrebrine and tubulosine, Biochemistry, 16(14):3209-3214.
- Gupta, R. S., Krepinski, J. J., and Siminovitch, L., 1980, Structural determinants responsible for the biological activity of (-)-emetine, (-)-cryptopleurine, and (-)-tylocrebrine: Structure-activity relationship among related compounds, Molecular Pharmacology, 18:136-143.
- Guru, P. Y., Warhurst, D. C., Harris, A., and Phillipson, J. D., 1982, Antimalarial activity of bruceantin *in vitro*, Annals of Tropical Medicine and Parasitology, 77(4):433-435.
- Hahn, F. E., and Ciak, J., 1975, Berberine, Antibiotics, 3:577-584.
- Hakizamungu, E., Van Puyvelde, L., Wery, M., De Kimpe, N., and Schamp, N., 1988, Active principles of *Tetradenia riparia*. III. Anti-Trichomonas activity of 8(14),15-sandaracopimaradiene-7 $\alpha$ ,18-diol, Phytotherapy Research, 2(4):207-208.
- Hassan, Y. A., 1989, Evidence that the antimalarial activity of artemisinin is not mediated via intercalation with nucleotides, Drug Design and Delivery, 4(2):129-133.
- Heidrich, J. E., Hunsaker, L. A., and Vander Jagt, D. L., 1983, Gossypol, an antifertility agent, exhibits antimalarial activity *in vitro*, IRCS Medical Science, 11:304.
- Henderson, F. G., Rose, C. L., Harris, P. N., and Chen, K. K., 1949,  $\gamma$ -Dichroine, the antimalarial alkaloid of Ch'ang Shan, Journal of Pharmacology and Experimental Therapeutics, 95:191-200.
- Hopp, K. H., Cunningham, L. V., Bromel, M. C., Schermeister, L. J., and Wahba Khalil, S. K., 1976, *In vitro* antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi*, Lloydia, 39(5):375-377.

- James, R. F., 1985, Malaria treated with emetine or metronidazole, *Lancet*, 8453:498.
- Jayaswal, S. B., 1976, Amoebicidal activity of steroid alkaloids of *Wrightia tomentosa* *in vitro*, *Indian Journal of Pharmacy*, 38(4):112-113.
- Johne, S., 1986, Quinazoline alkaloids, p. 99-140, in: "The Alkaloids," Volume 29, A. Brossi, ed., Academic Press, New York, New York.
- Kagan, J., Bazin, M., and Santus, R., 1989, Photosensitization with  $\alpha$ -terthienyl: The formation of superoxide ion in aqueous media, *Journal of Photochemistry and Photobiology, B: Biology*, 3:165-174.
- Kaplan, D. T., Keen, N. T., and Thomason, I. J., 1980a, Association of glyceollin with the incompatible response of soybean roots to *Meloidogyne incognita*, *Physiology and Plant Pathology*, 16:309-318.
- Kaplan, D. T., Keen, N. T., and Thomason, I. J., 1980b, Studies on the mode of action of glyceollin in soybean incompatibility to the root-knot nematode, *Meloidogyne incognita*, *Physiology and Plant Pathology*, 16:319-325.
- Keene, A. T., Anderson, L. A., Phillipson, J. D., and Warhurst, D. C., 1983, Anti-amoebic and cytotoxic activities of cinchophylline alkaloids, *Journal of Pharmacy and Pharmacology (Supplement)*, 35:20P.
- Keene, A. T., Harris, A., Phillipson, J. D., and Warhurst, D. C., 1986, *In vitro* amoebicidal testing of natural products. Part 1. Methodology, *Planta Medica*, 52:278-285.
- Keene, A. T., Phillipson, J. D., Warhurst, D. C., Koch, M., and Seguin, E., 1987, *In vitro* amoebicidal testing of natural products. Part 2. Alkaloids related to emetine, *Planta Medica*, 53:201-206.
- Khalid, S. A., Farouk, A., Geary, T. G., and Jensen, J. B., 1986, Potential antimalarial candidates from African plants: An *in vitro* approach using *Plasmodium falciparum*, *Journal of Ethnopharmacology*, 15:201-209.
- Khalid, S. A., Duddeck, H., and Gonzalez-Sierra, M., 1989, Isolation and characterization of an antimalarial agent of the neem tree *Azadirachta indica*, *Journal of Natural Products*, 52(5):922-927.
- Kinnaman, K. E., Steck, E. A., and Rane, D. S., 1979, Activity of antitumor drugs against African trypanosomes, *Antimicrobial Agents and Chemotherapy*, 15(2):157-160.
- Kirby, G. C., O'Neill, M. J., Phillipson, J. D., and Warhurst, D. C., 1989, *In vitro* studies on the mode of action of quassinoids with activity against chloroquine-resistant *Plasmodium falciparum*, *Biochemical Pharmacology*, 38(24):4367-4374.
- Klayman, D. L., 1985, Qinghaosu (artemisinin): An antimalarial drug from China, *Science*, 228:1049-1055.
- Koepfli, J. B., Mead, J. F., and Brockman, J. A., Jr., 1947, An alkaloid with high antimalarial activity from *Dichroa febrifuga*, *Journal of the American Chemical Society*, 69:1837.
- Koepfli, J. B., Mead, J. F., and Brockman, J. A., Jr., 1949, Alkaloids of *Dichroa febrifuga*. I. Isolation and degradative studies, *Journal of the American Chemical Society*, 71:1048-1054.
- Kogiso, S., Wada, K., and Munakata, K., 1976, Nematocidal polyacetylenes, 3Z,11E- and 3E,11E-trideca-1,3,11-triene from *Carthamus tinctorius* L., *Tetrahedron Letters*, 2:109-110.
- Krogsgaard-Larsen, P., and Honore, T., 1983, Glutamate receptors and new glutamate agonists, *Trends in Pharmacological Sciences*, 31:33-36.
- Kuehl, F. A., Spencer, C. F., and Folkers, K., 1948, Alkaloids of *Dichroa febrifuga* Lour., *Journal of the American Chemical Society*, 70:2091-2093.

- Kulkarni, S. K., Dandiya, P. C., and Varandani, N. L., 1972, Pharmacological investigations of berberine sulphate, Japanese Journal of Pharmacology, 22:11-16.
- Le, W. J., You, J. Q., and Mei, J. Y., 1983, Chemotherapeutic effect of artesunate in experimental schistosomiasis, Acta Pharmacologica Sinica, 18:619-621.
- Le, W. J., You, J. Q., Yang, Y. Q., Mei, J. Y., Guo, H. F., Yang, H. Z., and Zhang, W. W., 1982, Studies on the efficacy of artemether in experimental schistosomiasis, Acta Pharmacologica Sinica, 17:187-193.
- Leake, C. D., 1975, An historical account of pharmacology to the 20th century, Charles C. Thomas, Springfield, Illinois, 210 p.
- Lebouef, M., Cave, A., Forgacs, P., Tiberghien, R., Provost, J., Touche, A., and Jacquemin, H., 1982, Alcaloides des annonacees XL: Etude chimique et pharmacologique des alcaloides de l'*Annona montana* Macf, Plantes Medicinales et Phytotherapie, 16(3):169-184.
- Leon, L., Vasconcellos, M. E., Leon, W., Cruz, F. S., Docampo, R., and de Souza, W., 1978, *Trypanosoma cruzi*: Effect of olivacine on macromolecular synthesis, ultrastructure, and respiration of epimastigotes, Experimental Parasitology, 45:151-159.
- LePecq, J. B., Dat-Xuong, N., Gosse, C., and Paoletti, C., 1974, New antitumoral agent, 9-hydroxy-ellipticine; possibility of a rational design of anticancerous drugs in the series of DNA intercalating drugs, Proceedings of the National Academy of Sciences U.S.A., 71:5078-5082.
- Levander, O. A., Ager, A. L., Jr., Morris, V. C., and May, R. G., 1989, Qinghaosu, dietary vitamin E, selenium, and cod-liver oil: Effect on the susceptibility of mice to the malarial parasite *Plasmodium yoelii*, American Journal of Clinical Nutrition, 50(2):346-352.
- Lin, A. J., Klayman, D. L., and Milhous, W. K., 1987, Antimalarial activity of new water-soluble dihydroartemisinin derivatives, Journal of Medicinal Chemistry, 30:2147-2150.
- Lin, A. J., Lee, M., and Klayman, D. L., 1989, Antimalarial activity of new water-soluble dihydroartemisinin derivatives. 2. Stereospecificity of the ether side chain, Journal of Medicinal Chemistry, 32:1249-1252.
- Lin, A. J., Li, L. Q., Klayman, D. L., George, C. F., and Flippens-Anderson, J. L., 1990, Antimalarial activity of new water-soluble dihydroartemisinin derivatives. 3. Aromatic amine analogs, Journal of Medicinal Chemistry, 33(9):2610-2614.
- Lin, F., and Pan, H., 1989, Peroxidative antimalarial mechanism of sodium artesunate, Zhongguo Yixue Kexueyuan Xuebao, 11(3):180-184.
- Liu, J. M., Ni, M. Y., Fan, J. F., Tu, Y. Y., Wu, Z. H., Wu, Y. L., and Chou, W. S., 1979, Structure and reaction of arteannuin, Hua Hsueh Hsueh Pao, 37(2):129-143.
- Liu, J. S., 1979, Identification of kainic acid in extracts from *Caloglossa liprieurii*, Yaoxue Tongboa, 14:256-257.
- Lopes, J. N., Cruz, F. S., Docampo, R., Vasconcellos, M. E., Sampaio, M. C. R., Pinto, A. V., and Gilbert, B., 1978, *In vitro* and *in vivo* evaluation of the toxicity of 1,4-naphthoquinone and 1,2-naphthoquinone derivatives against *Trypanosoma cruzi*, Annals of Tropical Medicine and Parasitology, 72(6):523-531.
- Luo, X. D., and Shen, C. C., 1987, The chemistry, pharmacology and clinical applications of qinghaosu (artemisinin) and its derivatives, Medicinal Research Reviews, 7(1):29-52.
- Marles, R. J., Farnsworth, N. R., and Neill, D. A., 1989, Isolation of a novel cytotoxic polyacetylene from a traditional anthelmintic medicinal plant, *Minquartia guianensis*, Journal of Natural Products, 52:261-266.

- Meshnick, S. R., Tsang, T. W., Lin, F. B., Pan, H. Z., Chang, C. N., Kuypers, F., Chiu, D., and Lubin, B., 1989, Activated oxygen mediates the antimalarial activity of qinghaosu, *Progress in Clinical Biology Research*, 313:95-104.
- Mirelman, D., Monheit, D., and Varon, S., 1987, Inhibition of growth of *Entamoeba histolytica* by allicin, the active principle of garlic extract (*Allium sativum*), *Journal of Infectious Diseases*, 156(1):243-244.
- Monjour, L., Rouquier, F., Alfred, C., and Polonsky, J., 1987, Essais de traitement du paludisme murin experimental par un quassinoide, la glaucarubinone, *Comptes Rendus de l'Academie des Sciences (Paris)*, Serie III, 304(6):129-132.
- Montamat, E. E., Burgos, C., Gerez de Burgos, N. M., Rovai, L. E., Blanco, A., and Segura, E. L., 1982, Inhibitory action of gossypol on enzymes and growth of *Trypanosoma cruzi*, *Science*, 218:288-289.
- Myint, P. T., Shwe, T., Soe, L., Htut, Y., and Myint, W., 1989, Clinical study of the treatment of cerebral malaria with artemether (qinghaosu derivative), *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83:72.
- N'dounga, M., Balansard, G., Babadjamian, A., Timon-David, P., Gasquet, M., and Boudon, G., 1983, Contribution a l'etude de *Bidens pilosa* L. Identification et activite antiparasitaire de la phenyl-1 heptatriyne-1,3,5, *Plantes Medicinales et Phytotherapie*, 17(2):64-75.
- Naing, U. T., Win, U. H., Nwe, D. Y. Y., Myint, U. P. T., and Shwe, U. T., 1988, The combined use of artemether, sulfadoxine and pyrimethamine in the treatment of uncomplicated falciparum malaria, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 82:530-531.
- Neal, R. A., 1964, Chemotherapy of cutaneous leishmaniasis: *Leishmania tropica* infections in mice, *Annals of Tropical Medicine and Parasitology*, 58:420.
- Neal, R. A., 1970, Effect of emetine and related compounds on experimental cutaneous leishmaniasis, *Annals of Tropical Medicine and Parasitology*, 64(2):159-165.
- Neal, R. A., and Croft, S. L., 1984, An *in vitro* system for determining the activity of compounds against the intracellular amastigote form of *Leishmania donovani*, *Journal of Antimicrobial Chemotherapy*, 14:463-475.
- Nkunya, M. H. H., Weenen, H., Koyi, N. J., Thijss, L., and Zwanenburg, B., 1987, Cyclohexene epoxides, (+)-pandoxide, (+)- $\beta$ -senepoxide and (-)-pipoxide, from *Uvaria pandensis*, *Phytochemistry*, 26(9):2563-2565.
- O'Neill, M. J., 1986, Phytoalexins: Antiparasitics of higher plants, *Parasitology Today*, 2:358-359.
- O'Neill, M. J., and Phillipson, J. D., 1989, Plants as sources of antimalarial compounds, *Revista Latinoamericana de Quimica*, 20(3-4):111-118.
- O'Neill, M. J., Boardman, P., Chan, K. L., Bray, D. H., Phillipson, J. D., and Warhurst, D. C., 1985, Antimalarial activity of *Brucea javanica* fruits, *Journal of Pharmacy and Pharmacology* (Supplement), 37:49 p.
- O'Neill, M. J., Bray, D. H., Boardman, P., Phillipson, J. D., Warhurst, D. C., Peters, W., and Suffness, M., 1986, Plants as sources of antimalarial drugs: *In vitro* antimalarial activities of some quassinoids, *Antimicrobial Agents and Chemotherapy*, 30(1):101-104.
- O'Neill, M. J., Bray, D. H., Boardman, P., Wright, C. W., Phillipson, J. D., Warhurst, D. C., Gupta, M. P., Correya M., and Solis, P., 1987a, The activity of *Simarouba amara* against chloroquine-resistant *Plasmodium falciparum* *in vitro*, *Journal of Pharmacy and Pharmacology* (Supplement), 39:80 p.
- O'Neill, M. J., Bray, D. H., Boardman, P., Chan, K. L., and Phillipson, J. D., 1987b, Plants as sources of antimalarial drugs. Part 4. Activity of *Brucea javanica* fruits against chloroquine-resistant *Plasmodium falciparum* *in vitro* and against *Plasmodium berghei* *in vivo*, *Journal of Natural Products*, 50(1):41-48.

- O'Neill, M. J., Bray, D. H., Boardman, P., Wright, C. W., Phillipson, J. D., Warhurst, D. C., Gupta, M. P., Correya, M., and Solis, P., 1988, Plants as sources of antimalarial drugs. Part 6. Activities of *Simarouba amara* fruits, Journal of Ethnopharmacology, 22:183-190.
- Pan, P. C., Fang, S. D., and Tsai, C. C., 1976, Chemical synthesis of quisqualic acid, Scientia Sinica, 19:691-701.
- Partridge, S. J., Russell, P. F., Kirby, G. C., Bray, D. H., Warhurst, D. C., Phillipson, J. D., O'Neill, M. J., and Schiff, P. L., 1988, *In vitro* antimalarial activity of *Trichilia patens* and of some of its constituent alkaloids, Journal of Pharmacy and Pharmacology (Supplement), 40:53 p.
- Pavanand, K., Nutakul, W., Dechatiwongse, T., Yoshihira, K., Yongvanitchit, K., Scovill, J. P., Flippen-Anderson, J. L., Gilardi, R., George, C., Kanchanapee, P., and Webster, H. K., 1986, *In vitro* antimalarial activity of *Brucea javanica* against multi-drug resistant *Plasmodium falciparum*, Planta Medica, 52(2):108-111.
- Pavanand, K., Yongvanitchit, K., Webster, H. K., Dechatiwongse, T., Nutakul, W., Jewwachdamrongkul, Y., and Bansiddhi, J., 1988, *In vitro* antimalarial activity of a Thai medicinal plant *Picrasma javanica* Bl., Phytotherapy Research, 2(1):33-36.
- Pavanand, K., Webster, H. K., Yongvanitchit, K., and Dechatiwongse, T., 1989a, Antimalarial activity of *Tiliacora triandra* Diels against *Plasmodium falciparum* *in vitro*, Phytotherapy Research, 3(5):215-217.
- Pavanand, K., Webster, H. K., Yongvanitchit, K., Kun-anake, A., Dechatiwongse, T., Nutakul, W., and Bansiddhi, J., 1989b, Schizontocidal activity of *Celastrus paniculatus* Willd. against *Plasmodium falciparum* *in vitro*, Phytotherapy Research, 3(4):136-139.
- Pei-Gen, X., and Keji, C., 1988, Recent advances in clinical studies of Chinese medicinal herbs. 2. Clinical trials of Chinese herbs in a number of chronic conditions, Phytotherapy Research, 1(2):55-62.
- Pei-Gen, X., and Shan-Lin, F., 1986, Traditional antiparasitic drugs in China, Parasitology Today, 2(12):353-355.
- Pfaffman, M. A., and Klein, R. L., 1966, Effects of amoebicides on growth of *Acanthamoeba* sp. (30824), Proceedings of the Society of Experimental Biology and Medicine, 121:539-541.
- Phillips, R. E., Warrell, D. A., White, N. J., Looareesuwan, S., and Karbwang, J., 1985, Intravenous quinidine for the treatment of severe falciparum malaria: Clinical and pharmacokinetic studies, New England Journal of Medicine, 312(20):1273-1278.
- Phillipson, J. D., and O'Neill, M. J., 1989, New leads to the treatment of protozoal infections based on natural product molecules, Acta Pharmaceutica Nordica, 1(3):131-144.
- Prasad, B. N. K., Bansal, I., Das, P., and Srivastava, R., 1984, Antiamoebic action of drugs and synthetic compounds against trophozoites of *Entamoeba histolytica* under axenic and polyxenic culture conditions and in the infected rat caecum, Current Science, 53(15):778-781.
- Pringle, H. L., Bradley, S. G., and Harris, L. S., 1979, Susceptibility of *Naegleria fowleri* to  $\Delta^9$ -tetrahydrocannabinol, Antimicrobial Agents and Chemotherapy, 16(5):674-679.
- Qinghaosu Antimalaria Coordinating Research Group, 1979, Antimalaria studies on Qinghaosu, Chinese Medical Journal (Peking, English Edition), 92(12):811-816.
- Raj, R. K., and Kurup, P. A., 1967, Isolation of palasonin from the seeds of *Butea frondosa*, Indian Journal of Chemistry, 5:86-89.

- Rao, S. S., and Grossman, A. P., 1967, Cycloheximide resistance in yeast: A property of the 60s ribosomal subunit, Biochemical and Biophysical Research Communications, 29(5):696-704.
- Rembold, H., and Garcia, E. S., 1989, Azadirachtin inhibits *Trypanosoma cruzi* infection of its triatomine insect host, *Rhodnius prolixus*, Naturwissenschaften, 76(2):77-78.
- Rich, J. R., Keen, N. T., and Thomason, I. J., 1977, Association of coumestans with the hypersensitivity of lima bean roots to *Pratylenchus scribneri*, Physiology and Plant Pathology, 10:105-116.
- Robert-Gero, M., Bachrach, U., Bhatnagar, S., and Polonsky, J., 1985, Inhibition *in vitro* de la croissance des promastigotes de *Leishmania donovani* par des quassinoides, Comptes Rendus de l'Academie des Sciences (Paris), Serie II, 300(16):803-806.
- Rochanakij, S., Thebtaranonth, Y., Yenjai, C., and Yuthavong, Y., 1985, Nimbolide, a constituent of *Azadirachta indica*, inhibits *Plasmodium falciparum* in culture, Southeast Asian Journal of Tropical Medicine and Public Health, 16(1):66-72.
- Rodriguez, E., Arellano, T., Nishida, T., Uehara, S., Wrangham, R., Abramowski, Z., Finlayson, A., and Towers, G. H. N., 1985, Thiarubrine A, a bioactive constituent of *Aspilia* (Asteraceae) consumed by wild chimpanzees, Experientia, 41:419-420.
- Rohrer, S. P., Evans, D. V., and Bergstrom, A. R., 1990, A membrane associated glutamate binding protein from *Caenorhabditis elegans* and *Haemonchus contortus*, Comparative Biochemistry and Physiology, 95C:223-228.
- Rovai, L. E., Aoki, A., Gerez de Burgos, N. M., and Blanco, A., 1990, Effect of gossypol on tryptomastigotes and amastigotes of *Trypanosoma cruzi*, Journal of Protozoology, 37(4):280-286.
- Rubinchik, M. A., Rybalko, K. S., Evstratova, R. I., and Konovalova, O. A., 1976, Sesquiterpene lactones of higher plants as a possible source of new antiprotozoal drugs, Rastitelnye Resursy, 12:170-181.
- Sabchareon, A., Chongsuphajaisiddhi, T., Sinhasivanon, V., Chanthavanich, P., and Attanath, P., 1988, *In vivo* and *in vitro* responses to quinine and quinidine of *Plasmodium falciparum*, Bulletin of the World Health Organization, 66(3):347-352.
- Salem, F. S., 1980, Evaluation of clove oil and some of its derivatives as trichomonacidal agents, Journal of Drug Research in Egypt, 12(1-2):115-119.
- Schaeffer, J. M., White, T., Bergstrom, A. R., Wilson, K. E., and Turner, M. J., 1990, Identification of glutamate-binding sites in *Caenorhabditis elegans*, Pesticide Biochemistry and Physiology, 36:220-228.
- Seeler, A. O., Dusenberry, E., and Malanga, C., 1943, The comparative activity of quinine, quinidine, cinchonine, cinchonidine and quinidine against *Plasmodium lophurae* infections in Pekin ducklings, Journal of Pharmacology and Experimental Therapeutics, 78:159-163.
- Seery, T. M., and Bieter, R. N., 1940, A contribution to the pharmacology of berberine, Journal of Pharmacology and Experimental Therapeutics, 69:64-67.
- Sharma, G. L., and Bhutani, K. K., 1988, Plant based antiamoebic drugs. Part II. Amoebicidal activity of parthenin isolated from *Parthenium hysterophorus*, Planta Medica, 54(2):120-122.
- Shu, G. X. and Liang, X. T., 1980, Identification of 28-deacetyl-sendaenol as the active component of *Melia toosendan*, Acta Chimica Sinica, 38:196-197.
- Shwe, T., Myint, P. T., Myint, W., Htut, Y., Soe, L., and Thwe, M., 1989, Clinical studies on treatment of cerebral malaria with artemether and mefloquine, Transactions of the Royal Society of Tropical Medicine and Hygiene, 83:489.

- Sinderson, H. C., 1924, Emetine hydrochloride in the treatment of oriental sore, Transactions of the Royal Society of Tropical Medicine and Hygiene, 18:108.
- Smrkovski, L. L., Buck, R. L., Alcantara, A. K., Rodriguez, C. S., and Uylangco, C. V., 1985, Studies of resistance to chloroquine, quinine, amodiaquine and mefloquine among Philippine strains of *Plasmodium falciparum*, Transactions of the Royal Society of Tropical Medicine and Hygiene, 79:37-41.
- Subbaiah, T. V., and Amin, A. H., 1967, Effect of berberine sulphate on *Entamoeba histolytica*, Nature, 215:527-528.
- Tanabe, K., Kato, M., Izumo, A., Hagiwara, A., and Doi, S., 1990, *Plasmodium chabaudi*: In vivo effects of calcium antagonists on chloroquine-resistant and chloroquine-sensitive parasites, Experimental Parasitology, 70(4):419-426.
- Tani, S., Fukamiya, N., Kiyokawa, H., Musallam, H. A., Pick, R. O., and Lee, K. H., 1985, Antimalarial agents. 1.  $\alpha$ -Santonin-derived cyclic peroxide as potential antimalarial agent, Journal of Medicinal Chemistry, 28:1743-1744.
- Trager, W., and Polonsky, J., 1981, Antimalarial activity of quassinoids against chloroquine-resistant *Plasmodium falciparum* in vitro, American Journal of Tropical Medicine and Hygiene, 30(3):531-537.
- Vasanth, S., Gopal, R. H., and Rao, R. B., 1990, Plant antimalarial agents, Journal of Scientific and Industrial Research, 49(2):68-77.
- Veech, J. A., 1979, Histochemical localization and nematotoxicity of terpenoid aldehydes in cotton, Journal of Nematology, 11:240-246.
- Veech, J. A., 1982, Phytoalexins and their role in the resistance of plants to nematodes, Journal of Nematology, 14:2-9.
- Veech, J. A., and McClure, M. A., 1977, Terpenoid aldehydes in cotton roots susceptible and resistant to the root knot nematode, *Meloidogyne incognita*, Journal of Nematology, 9:225-229.
- Vennerstrom, J. L., and Klayman, D. L., 1988, Protoberberine alkaloids as antimalarials, Journal of Medicinal Chemistry, 31:1084-1087.
- Vichkanova, S. A., Rubinchik, M. A., Adgina, V. V., and Fedorchenko, T. S., 1969, Chemotherapeutic action of sanguinarine, Farmakologiya i Toksikologiya, 32:325-328.
- Waddell, T. G., Woods, L. A., Harrison, W., and Meyer, G. M., 1984, Aliphatic esters of quinine: Screening for antiplasmodial activity, Journal of the Tennessee Academy of Science, 59(3):48-50.
- Warhurst, D. C., 1981, The quinine-haemin interaction and its relationship to antimalarial activity, Biochemical Pharmacology, 30(24):3323-3327.
- Watkins, T. I., 1958, The chemotherapy of helminthiasis, Journal of Pharmacy and Pharmacology, 10:209-227.
- Werbel, L. M., and Worth, D. F., 1980, Antiparasitic agents, Annual Reports in Medicinal Chemistry, 15:120-129.
- Whaun, J. M., and Brown, N. D., 1990, Treatment of chloroquine resistant malaria with esters of cephalotaxine: Homoharringtonine, Annals of Tropical Medicine and Parasitology, 84(3):229-237.
- White, N., Looareesuwan, S., Warrell, D. A., Chongsuphajaisiddhi, T., Bunnag, D., and Harinasuta, T., 1981, Quinidine in falciparum malaria, Lancet, 2:1069-1071.
- Williamson, J., and Scott-Finnigan, T. J., 1978, Trypanocidal activity of antitumor antibiotics and other metabolic inhibitors, Antimicrobial Agents and Chemotherapy, 13(5):735-744.
- Wolfe, A. D., Allison, R. G., and Hahn, F. E., 1972, Labilizing action of intercalating drugs and dyes on bacterial ribosomes, Biochemistry, 11(9):1569-1572.
- Woolfe, G., 1963, Chemotherapy of amoebiasis, p. 355-443, in: "Experimental Chemotherapy," Volume 1, R. J. Schnitzer and F. Hawking, eds., Academic Press, New York, New York.

- Wright, C. W., O'Neill, M. J., Phillipson, J. D., and Warhurst, D. C., 1988, Use of microdilution to assess *in vitro* antiamoebic activities of *Brucea javanica* fruits, *Simarouba amara* stem, and a number of quassinoids, *Antimicrobial Agents and Chemotherapy*, 32(11):1725-1729.
- Xiao, P.-G., and Fu, S.-L., 1986, Traditional antiparasitic drugs in China, *Parasitology Today*, 2:333-335.
- Xiao, S., and Catto, B. A., 1989, *In vitro* and *in vivo* studies of the effect of artemether on *Schistosoma mansoni*, *Antimicrobial Agents and Chemotherapy*, 33:1557-1562.
- Xihe, T., 1979, Development of natural products as antimalarial agents, *Proceedings of the U.S.-China Pharmacology Symposium*, October 29-31, 1979, National Academy of Sciences, Washington, D.C., p. 137-141.
- Xu, Q. C., 1982, Advances in the study of antimalarial agents from plants, *Yao Hsueh T'ung Pao*, 17(9):544-547.
- Xu, Q. C., 1983, A distributive survey of antimalaria constituents in plants, *Chung Ts'ao Yao*, 14(2):93-95.
- Xu, R.-S., Snyder, J. K., and Nakanishi, K., 1984, Robustadials A and B from *Eucalyptus robusta*, *Journal of the American Chemical Society*, 106(3):734-736.
- Ye, Z., and Van Dyke, K., 1989, Selective antimalarial activity of tetrandrine against chloroquine resistant *Plasmodium falciparum*, *Biochemical and Biophysical Research Communications*, 159(1):242-248.
- Ye, Z., Van Dyke, K., and Castranova, V., 1989, The potentiating action of tetrandrine in combination with chloroquine or qinghaosu against chloroquine-sensitive and resistant falciparum malaria, *Biochemical and Biophysical Research Communications*, 165(2):758-765.
- You, J. Q., Le, W. J., and Mei, J. Y., 1982, Studies on the efficacy of agrimophol and cucurbitine on experimental schistosomiasis, *Acta Pharmaceutica Sinica*, 17:663-666.
- Zemek, J., Valent, M., Podova, M., Kosikova, B., and Joniak, D., 1987, Antimicrobial properties of aromatic compounds of plant origin, *Folia Microbiologia*, 32:421-425.

## ANTIFUNGAL COMPOUNDS FROM PLANTS

Joseph Kuc

Department of Plant Pathology  
University of Kentucky  
Lexington, KY 40546

### INTRODUCTION

Plants are an extremely rich source of diverse organic compounds which are antifungal. In general, the interest in such antifungal compounds depends upon the concentration required for activity and the biological spectrum of activity. Some of the compounds are preformed and are located in external plant tissues, i.e., bark, peel, and cuticle; others are located throughout the plant, often in vacuoles; and still others are produced by plants in response to physiological stress or infection. In the latter group are the phytoalexins, low molecular weight organic compounds produced by plants in response to infection or stress and localized at the site of infection or stress. This group of compounds is usually lipophilic and includes compounds as diverse as simple phenols, flavonoids, isoflavonoids, coumarins, isocoumarins, sesquiterpenoids, polyenes, stilbenes, furanoterpenoids, and derivatives of these compounds. The levels of still another group of antifungal compounds are enhanced locally and systemically after infection or stress and a second infection often markedly enhances their levels. These are high molecular weight and include chitinases,  $\beta$ -1,3-glucanases, and thionins. A group of compounds, not directly antifungal, are the structural biopolymers which are localized at sites of infection or stress and which restrict fungal development in plants by forming barriers to fungal development, e.g., lignin, callose, and hydroxyproline-rich glycoproteins. Even compounds in vacuoles, which would not contact fungi in intact plant cells, can be liberated when cells collapse. The released compounds may be active per se or they may be modified by liberated enzymes, esterases, glycosidases, phenoloxidases, and peroxidases, to antifungal compounds.

It is tempting, and with some of the compounds justified, to assign them a role in plant defense against infectious agents. Some of the compounds, or the regulation of genes leading to their controlled synthesis, may find application for plant disease control; others may find direct application in medicine or industry or function as models for the synthesis of biologically active compounds of medicinal or industrial value.

It is impossible to exhaustively consider the broad subject of plant antifungal compounds in this chapter. I will emphasize selected preformed or response compounds which are often associated with plant defense against fungi. Comprehensive reviews on different aspects of the subject are available (Bell, 1981;

Coxon, 1982; Dixon and Lamb, 1990; Ebel, 1986; Ingham, 1982; Kuc, 1982; Overeem, 1976; Rao and Kuc, 1991; Stoessl, 1982, 1983).

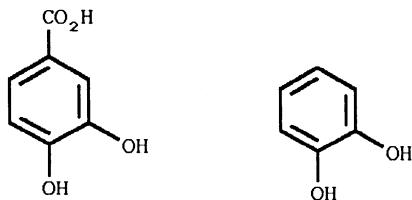
## PREFORMED COMPOUNDS

Preformed antifungal compounds are often located in external plant tissues or are released from cells after cell damage. At best, they provide a general defense against a broad spectrum of potential pathogens. They do not, however, explain race specificity in plant-pathogen interaction nor do they provide an absolute defense against disease. Analogous to these plant compounds and protective plant polymers, e.g., cutin, suberin, lignin, and callose, are skin and the antimicrobial compounds associated with skin and membranes protecting exposed tissues. A response phase to the immune system is important for both plants and animals. Unequivocal evidence is not available for the presence of a single or group of related preformed compounds being sufficient to completely explain resistance of a plant to disease, and such an explanation would likely be too simplistic and incompatible with survival of a species under the selection pressure of evolution.

### Phenolics

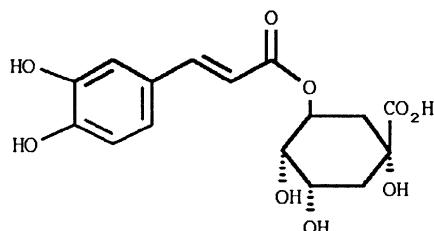
An early approach to explain disease resistance in plants on a chemical level is found in the reports of the protective effects of protocatechuic acid (Fig. 1 (1)) and catechol (Fig. 1 (2)), found in the outer scales of colored onion bulbs, against the smudge disease caused by *Colletotrichum circinans* (Angell et al., 1930; Link et al., 1929; Link and Walker, 1933). The compounds are associated with outer onion scales, and they diffuse into water droplets on the surface of onion bulbs. Thus, they encounter spores of the pathogen during a period when spores will or have started to germinate due to the presence of available water.

Johnson and Schall (1952, 1955) presented evidence that endogenous phenols in potato peel and those produced at the site of injury were important in the resistance of tubers to scab, and they reported a positive correlation between the content of chlorogenic acid (Fig. 2 (3)) and resistance to potato scab. They further reported that caffeic acid (Fig. 3 (4a)) and oxidation products of chlorogenic and caffeic acids were produced after infection or tissue injury and that these products were even more fungitoxic, though transitory, than the parent phenols. Kuc (1957) also reported the accumulation of the acids and their oxidation products in slices inoculated with several nonpathogens of potato. Both chlorogenic and caffeic acid are widely distributed in plants. Other widely distributed phenolics with antifungal properties include ferulic acid (Fig. 3 (4b)), p-coumaric acid (Fig. 3 (4c)), umbelliferone (Fig. 4 (5a)), aesculetin (Fig. 4 (5b)), and scopoletin (Fig. 4 (5c)). The latter three compounds are most frequently found as their glycosides which are hydrolyzed after tissue damage. The hydroxycinnamic acids are also found as glycosides or sugar esters. Though the phenols discussed in this section are not notably antifungal ( $EC_{50}$  ca  $10^{-2}$  to  $10^{-4}$  M for fungal growth), they can be found in tissues or liberated after tissue damage at levels which may contribute to the restriction of some fungi. Though preformed, the phenolics discussed also generally increase around sites of plant injury and infection. With some of the coumarin derivatives, the increase may be systemic.



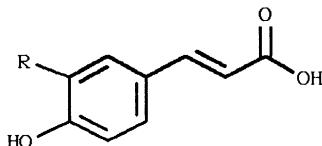
1. Protocatechuic acid 2. Catechol

Figure 1. Structural formulas of protocatechuic acid (1) and catechol (2).



3. Chlorogenic acid

Figure 2. Structural formula of chlorogenic acid (3).

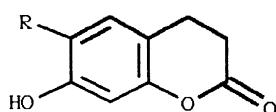


4a Caffeic acid ( $R = OH$ )

4b Ferulic acid ( $R = OCH_3$ )

4c *p*-coumaric acid ( $R = H$ )

Figure 3. Structural formulas of caffeic (4a), ferulic (4b), and *p*-coumaric (4c) acids.



5a Umbelliferone ( $R = H$ )

5b Esculetin ( $R = OH$ )

5c Scopoletin ( $R = OCH_3$ )

Figure 4. Structural formulas of umbelliferone (5a), esculetin (5b), and scopoletin (5c).

## Steroid Glycoalkaloids

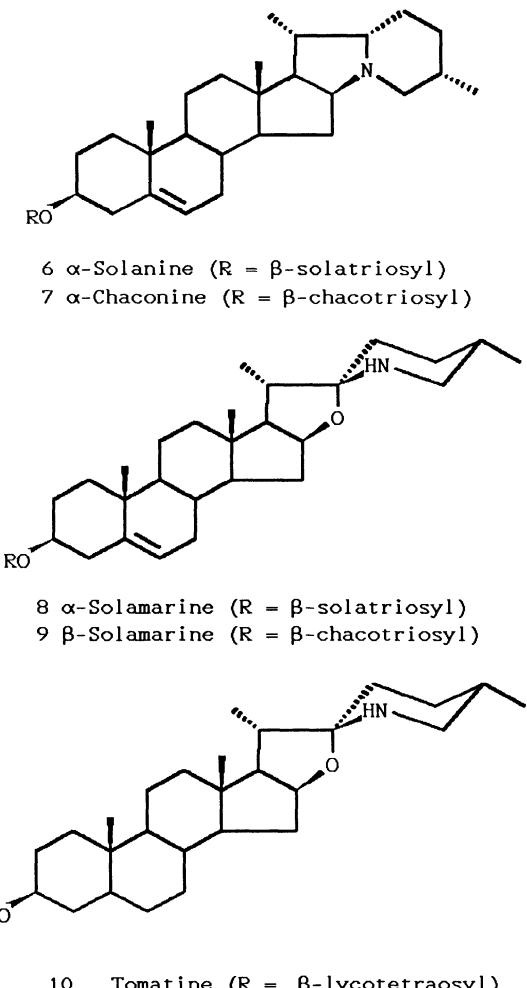
Along with the reports concerning chlorogenic and caffeic acid in potato appeared reports on the possible role of steroid glycoalkaloids, found in peel or produced around sites of injury in tubers, in disease resistance (McKee, 1955, 1959).  $\alpha$ -solanine (Fig. 5 (6)) and  $\alpha$ -chaconine (Fig. 5 (7)) are the main steroid glycoalkaloids in potato. They are found in healthy tubers as well as foliage (Shih, 1972) and their concentration increases under stress conditions in tubers (Locci and Kuc, 1967);  $\alpha$ -solamarine (Fig. 5 (8)) and  $\beta$ -solamarine (Fig. 5 (9)) have been reported as additional steroid glycoalkaloids in the cultivar Kennebec (Shih and Kuc, 1974). Tubers of the wild potato species, *S. acaule*, contain appreciable quantities of demissine and tomatine (Fig. 5 (10)). The steroid glycoalkaloids are largely localized in the peel below the primary periderm of healthy tubers (outer 2 mm of tissue), but their concentration increases markedly around sites of injury to levels which may reach or surpass that in the peel. They are the major extractable fungitoxic compounds in the peel and in tuber tissue around sites of mechanical injury (Allen and Kuc, 1968; McKee, 1955). Analyses of four cultivars indicated an average of 0.55 and 0.03 mg/g fresh weight in the peel and peeled tubers, respectively. Flowers, fruit, sprouts, and foliage contain a considerable higher content of steroid glycoalkaloids than do whole tubers (Shih, 1972). The accumulation of the glycoalkaloids in tubers is suppressed by inoculation with various pathogens and nonpathogens or treatment with elicitors from *P. infestans*, including arachidonic acid (Ishizaka and Tomiyama, 1972; Shih and Kuc, 1973; Shih et al., 1973; Zook and Kuc, 1990). The suppression is concomitant with an increase in the accumulation of norsesqui- and sesquiterpenoid phytoalexins, e.g., rishitin, lubimin, solavetivone, and phytuberin. With all fungi tested,  $\alpha$ -chaconine was more fungitoxic than  $\alpha$ -solanine (Allen and Kuc, 1968; McKee, 1959). An interesting aspect of the fungitoxicity of the steroid glycoalkaloids is that the protonated forms (approximately pH 5.5 or lower) are less active than the free bases (Allen and Kuc, 1968; McKee, 1959). The difference is greater than 100-fold between pH 5.5 and 7.4.

The contribution of steroid glycoalkaloids in disease resistance of the potato is uncertain. Locci and Kuc (1967) and Allen and Kuc (1968) suggested that the compounds are part of a general mechanism for the disease resistance of potato but that the mechanism for resistance is multicomponent. The evidence is strong that the steroid glycoalkaloids and norsesqui- and sesquiterpenoids are synthesized via the acetate-mevalonate pathways (Shih and Kuc, 1973; Stoessl, 1982, 1983; Zook and Kuc, 1990).

A strong case has been reported for the role of tomatine (Fig. 5 (10)) in the resistance of young tomato fruit to *Fusarium solani* (Defago, 1982). As with the other antifungal steroid glycoalkaloids, a possible mechanism for antifungal activity is based on complexing with membrane sterols.

## Duvatrienol

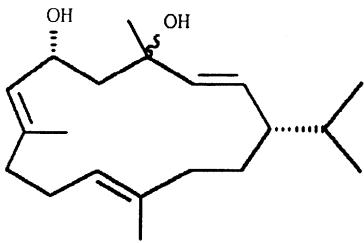
Cuticular components of some tobacco genotypes have been reported to have a role in the resistance of tobacco to some insects (Severson et al., 1985). Although the role of these compounds, notably  $\alpha$ - and  $\beta$ -4,8,13-duvatriene-1,3-diols (DVT) (Fig. 6 (11,12)) in resistance to some insects is established, their role in resistance to fungal pathogens still remains uncertain. Cruickshank et al. (1977) reported DVT inhibited spore germination of *Peronospora tabacina* with an EC<sub>50</sub> of 20  $\mu$ g/ml. The compounds are synthesized in glandular trichomes present on leaf surfaces and they may be released from the trichomes by injury (Keene and Wagner, 1985). Reuveni et al. (1986a) observed that dipping leaf strips of tobacco in acetone for 1 second



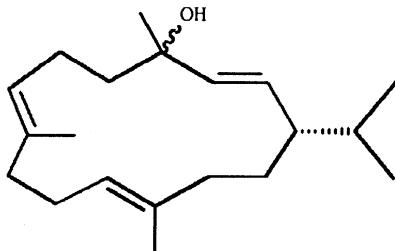
10. Tomatine ( $R = \beta$ -lycotetraosyl)

Figure 5. Structural formulas of  $\alpha$ -solanine (6),  $\alpha$ -chaconine (7),  $\alpha$ -solamarine (8),  $\beta$ -solamarine (9), and tomatine (10).

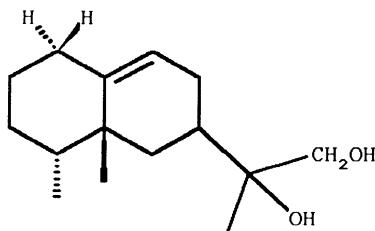
increased their susceptibility to blue mold, and the increase in susceptibility was accounted for by the removal of DVT from the leaf surface. Washing leaves with water solubilized DVT and also increased susceptibility to *P. tabacina*. As tobacco ages, resistance to *P. tabacina* markedly increases and, in greenhouse tests, this was closely associated in the cv. Ky 14 with increased levels of DVT (Reuveni et al., 1986a,b). Field tests, however, demonstrated that environmental factors, including rainfall, influenced resistance to *P. tabacina* and DVT content and that resistance related to age in tobacco was complex and DVT was only one of the contributors (Rao et al., 1989a,b; Tuzun et al., 1989). The cuticular component debneyol (Fig. 6 (13)), though not a dufvane, is highly fungitoxic to spores of *P. tabacina* and was isolated from *Nicotiana debneyi* (Burden et al., 1985).



11.  $\alpha$ - and  $\beta$ -4,8,13-Duvatrien-1,3-diols



12.  $\alpha$ - and  $\beta$ -4,8,13-Duvatrien-1-ol



13. Debneyol

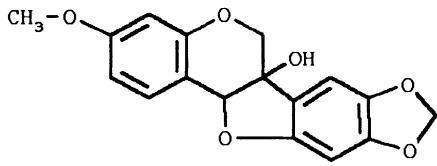
Figure 6. Structural formulas of duvatrien-1-ols (11,12) and debneyol (13).

## Other Compounds

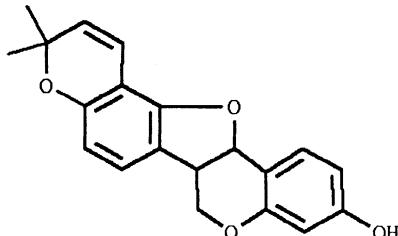
Other naturally occurring antifungal compounds include pinosylvin, thujaplicins, mansonones, 2-benzoxazolinones, hordatine derivatives, and avenacin. These compounds are well-reviewed in the chapter written by J. C. Overeem (1976).

## PHYTOALEXINS

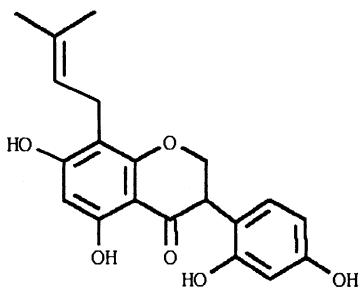
The immune system in plants includes numerous mechanisms we know of and probably many of which we are still unaware. One mechanism for disease resistance in plants is the rapid accumulation of low molecular antimicrobial substances (phytoalexins) at and immediately around sites of infection. The accumulation of phytoalexins has been demonstrated in at least 25 plant families (Bell, 1981; Coxon, 1982; Ingham, 1982; Kuc, 1982; Rao and Kuc, 1991; Stoessl, 1982, 1983). Similarities



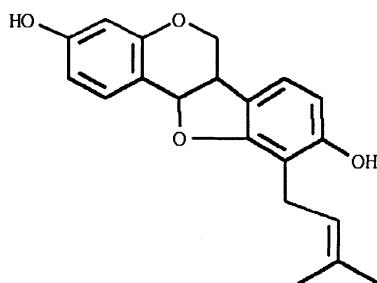
14. Pisatin  
Pea (*Pisum sativum*)



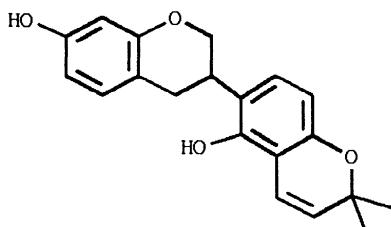
15. Phaseollin  
Green bean (*Phaseolus vulgaris*)



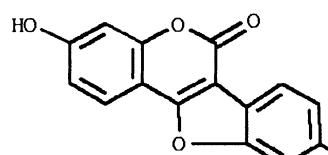
16. Kievitone  
Green bean



17. Phaseollidin  
Green bean



18. Phaseollolinisoflavan  
Green bean

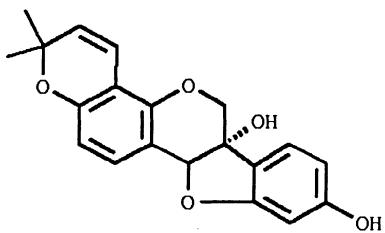


19. Coumestrol  
Green bean

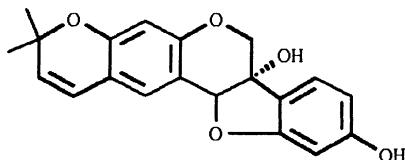
Figure 7. Structural formulas of pisatin (14), phaseollin (15), kievitone (16), phaseollidin (17), phaseollolinisoflavan (18), and coumestrol (19).

are evident among phytoalexins from plants within a family. Plants from the Leguminosae, Solanaceae, Compositae, and Convolvulaceae produce predominantly isoflavonoids, carbocyclic sesquiterpenoids, polyacetylenes, and furano-sesquiterpenoids, respectively. Though some phytoalexins are distributed among many families (e.g., caffeic acid derivatives accumulate in potato, carrot, and sweet potato), plants in the Leguminosae have not been reported to produce sesquiterpenoid phytoalexins and those in the Solanaceae have not been reported to produce isoflavonoids. Phytoalexins exert their protective effect in plants by inhibiting fungal growth once the fungus has penetrated into the plant. Depending upon the phytoalexin and bioassay employed, they inhibit growth of fungi at  $10^{-3}$  to  $10^{-5}$  M.

In all cases reported to date, phytoalexin accumulation as a factor for disease resistance is not determined by the presence or absence of genetic information for the requisite biosynthetic pathways (Dean and Kuc, 1987; Kuc, 1972, 1982, 1983, 1987,

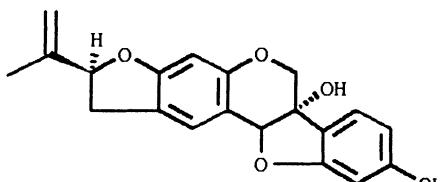


20. Glyceollin I

Soybean (*Glycine max*)

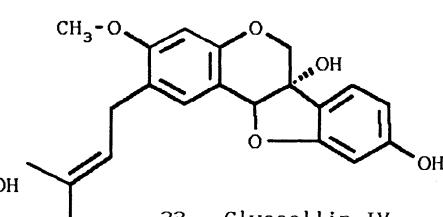
21. Glyceollin II

Soybean



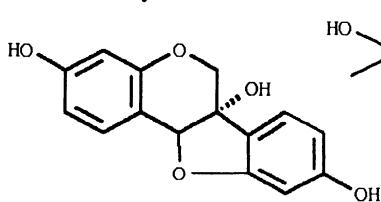
22. Glyceollin III

Soybean



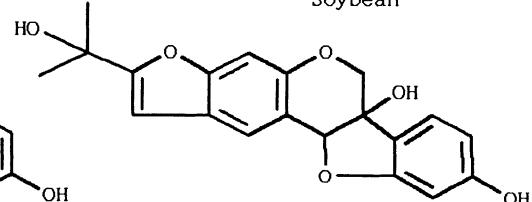
23. Glyceollin IV

Soybean



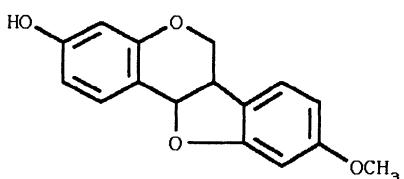
24. Glycinol

Soybean



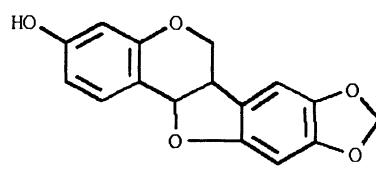
25. Glycefuran

Soybean



26. Medicarpin

(Demethylhomopterocarpin)

Red clover (*Trifolium pratense*)White clover (*Trifolium repens*)Alfalfa (*Medicago sativa*)

27. Maackiaain

(Inermin: Demethylpterocarpin)

Red clover, white clover, pea

Figure 8. Structural formulas of glyceollin I (20), II (21), III (22), IV (23), glycinol (24), glycefuran (25), medicarpin (26), and maackiaain (27).

1990a,b; Kuc and Caruso, 1977; Kuc and Preisig, 1984; Kuc and Rush, 1985; Rao and Kuc, 1991). Thus, phytoalexins as factors in disease resistance would be compatible with the hypothesis that all plants contain the genetic potential for resistance mechanisms.

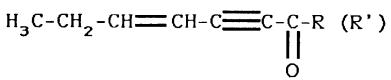
Phytoalexins are degraded by host and microbial enzymes (Smith, 1982; VanEtten et al., 1989). Thus, accumulation is a function of both rate of synthesis and degradation. They are not notably active as antibiotic agents though the quantity that accumulates at sites of infection is sufficient to markedly inhibit the growth of some fungi and bacteria *in vitro*. Phytoalexins have a broad spectrum of activity against the pathogens and nonpathogens of a host; and a broad range of pathogens, nonpathogens, and physiological stresses elicit accumulation of the same phytoalexin. A case for the involvement of phytoalexins in disease resistance depends upon the speed and magnitude with which they are produced and not selective toxicity per se or selectivity of elicitation (Kuc, 1972, 1987, 1990a,b; Kuc and Preisig, 1984; Kuc and Rush, 1985; Rao and Kuc, 1991; Smith, 1982). Unlike protein antibodies in animals, the phytoalexins are low molecular weight, generally lipophilic substances that are products of secondary metabolism, and they are not translocated. Some phytoalexins are phytotoxic at concentrations that inhibit the development of microorganisms. Attempts to protect plants against disease by eliciting and maintaining high levels of phytoalexins systemically in foliage proved unsuccessful. The frequent application of fungus-derived glucan elicitors to the foliage of greenbean and soybean plants caused severe necrotization and stunting of growth.

Though protecting plants by directly eliciting phytoalexin accumulation has not been successful, sensitizing plants to rapidly mobilize defense mechanisms and accumulate phytoalexins after infection has proven successful in laboratory and field experiments. The sensitization has been accomplished by limited infection with fungi, bacteria, or viruses; products from immunized plants; or synthetic chemicals (Doubrava et al., 1988; Farih et al., 1981; Gottstein and Kuc, 1989; Kuc, 1982, 1983, 1987, 1990a,b; Kuc and Caruso, 1977; Kuc and Tuzun, 1983; Langcake, 1981; Sutton, 1979).

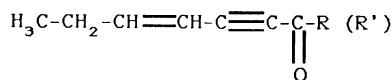
Though infection with fungi, bacteria, and viruses elicits phytoalexin accumulation, other stress-inducing factors, such as mercuric or cupric chloride, ethylene, UV radiation, metabolic inhibitors, cell wall components of fungi, bacteria and plants and their derivatives, microbial metabolites, and insect and nematode damage, are also active elicitors (Dean and Kuc, 1987; Dixon and Lamb, 1990; Ebel, 1986; Kuc, 1982, 1983, 1987, 1990a; Rao and Kuc, 1991). This is especially true for plants in the Leguminosae. This rather low level of specificity as related to elicitation neither supports nor detracts from the possible contribution of phytoalexins in disease resistance. A role for phytoalexins in the resistance of plants to virus diseases is unclear.

In some susceptible reactions between fungi and plants, phytoalexins accumulate to higher levels than in resistant interactions. This is often evident when biotrophic growth terminates, sporulation occurs, and lesions develop. In this case, phytoalexin accumulation probably occurred too late to contain the fungus and restrict disease development.

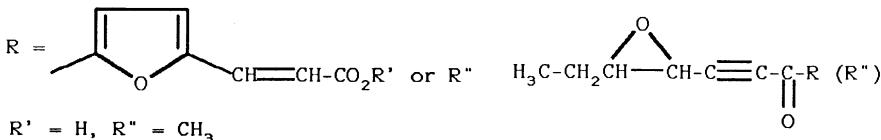
Two distinct aspects of phytoalexin elicitation are apparent. The first is necrotization or damage to plant cells by chemical or physical agents, which releases a nonphytotoxic substance. The second is migration of the nonphytotoxic substance to healthy cells where it conditions enhanced phytoalexin synthesis. The phytoalexins are then transported out of the producer cells and accumulate in the necrotized or damaged tissues. The actual elicitor of phytoalexin synthesis and accumulation may not be the fungal glucan, toxic chemical, or UV radiation but rather the substance



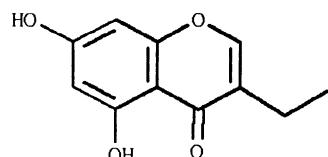
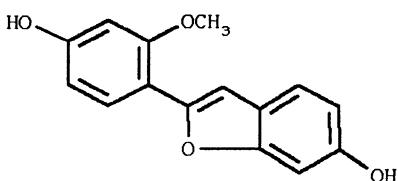
28. Wyerone Acid  
Broad bean (*Vicia faba*)



29. Wyerone  
Broad bean

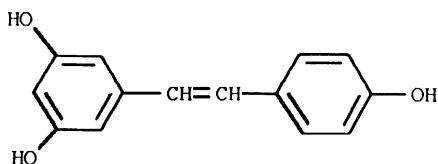


30. Wyerone Epoxide  
Broad bean



31. 6-Demethylvignafuran  
*Anthyllis vulneraria*  
*Coronilla eumurus*  
*Tetragonolobus maritimus*

32. Lathodoratin  
*Lathyrus hirsutus*  
*Lathyrus odoratus*



33. *cis*- and *trans*-Resveratrol  
Peanut (*Arachis hypogaea*)

Figure 9. Structural formulas of wyerone acid (28), wyerone (29), wyerone epoxide (30), 6-demethylvignafuran (31), lathodoratin (32), and *cis*- and *trans*-resveratrol (33).

released by the damaged tissues. Thus, a major emphasis should be placed on why many fungal cell wall glucans are highly phytotoxic rather than on how they elicit phytoalexin accumulation. Many studies support the hypothesis that infection and physiological stress cause the release of plant cell wall fragments containing galacturonic acid residues, and these fragments elicit the accumulation of phytoalexins (Dean and Kuc, 1987; Dixon and Lamb, 1990; Ebel, 1986; Kuc, 1982, 1983, 1987, 1990a; Rao and Kuc, 1991). It is possible that phytotoxic elicitors from microbial cell walls damage plant cells, bringing plant hydrolases in contact with plant cell walls, and cause the release of galacturonic acid-containing oligosaccharides and polysaccharides. These fragments may be the actual elicitors of phytoalexin accumulation. Fungal or bacterial hydrolases active on plant cell walls could also act as the plant enzymes. Alternatively, the microbial cell walls and products from plant

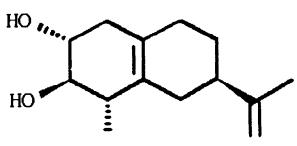
cell walls may directly elicit phytoalexin accumulation. It is not unlikely that endogenous elicitors are present in all plants reported to accumulate phytoalexins. The release of the endogenous elicitors may be analogous to the release of alarm signals that activate resistance mechanisms. Mechanical wounding or short-term injury generally are not adequate for appreciable accumulation of phytoalexins. It appears that a low-level persistent stress, perhaps caused by the continued presence of the infectious agent or elicitor, is required for the accumulation of high levels of phytoalexins. This is consistent with the observation that, when the infectious agent is contained or, in the case of fungi and bacteria, perhaps killed, phytoalexin levels drop to levels that approach those in uninfected plants. Similarly, the hydrolysis of glucan elicitors and dilution or sequestering of inorganic toxicants would, with time, remove the source of stress and phytoalexin levels would drop unless the elicitors or toxicants caused the release of additional elicitors of host origin.

It is generally conceded, however, that phytoalexin accumulation is only one component of the plant's mechanisms for disease resistance; and evidence is lacking that phytoalexins have a role in plant resistance to diseases caused by viruses. Other components of the disease-resistance complex include the presence and accumulation after infection of peroxidases, chitinases,  $\beta$ -1,3-glucanases, proteases, hydroxyproline-rich glycoproteins, lignin, callose, thionins, and phenolic cross-linked cell wall polymers (Kuc, 1987, 1990a; Rao and Kuc, 1991). Some of these components, e.g., chitinases,  $\beta$ -1,3-glucanases can hydrolyze the walls of fungi which contain chitin and glucans. Other components, e.g., lignin, hydroxyproline-rich glycoproteins, callose and phenolic cross-linked cell wall polymers and are barriers to the development of infectious agents. The thionins are antifungal proteins, and the peroxidases can be involved in the generation of oxidation products of phenols,  $O_2^-$ ,  $OH^-$ , and  $H_2O_2$ , which are antifungal.

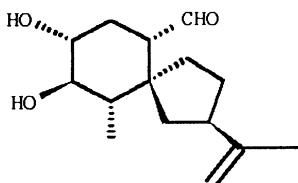
Disease resistance in plants likely depends upon the activation of coordinated, multicomponent defense mechanisms. A single mechanism or compound with a single metabolic site of action would be unsatisfactory, as illustrated by the rapid development of resistant strains of pathogens to single-site-dependent systemic fungicides and antibiotics. The mechanisms for resistance probably include those that exclude pathogens, those that are general and nonspecific responses to wounding or metabolic insult, and those directed, with various degrees of specificity, against particular groups of pathogens. Undoubtedly, each different mechanism contributes to the overall outcome of the interaction. This layering of mechanisms, an apparent duplication of effort under some circumstances, provides depth and hence stability to resistance.

### **Leguminosae**

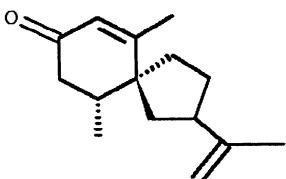
The chemical characterization of phytoalexins by Cruickshank and coworkers in pea and green bean and their concomitant biological studies were critical to the recognition of phytoalexins as a component of a plant's defense against fungal and bacterial disease (Cruickshank, 1963). More research on phytoalexins and more phytoalexins have been reported in the Leguminosae than any other plant family. The great majority of the characterized phytoalexins in the Leguminosae are isoflavanoid derivatives (Figs. 7 and 8 (14-27)), but furanoacetylenes (Fig. 9 (28-30)), benzofurans (Fig. 9 (31)), chromones (Fig. 9 (32)), and stilbenes (Fig. 9 (33)) have also been reported. The phytoalexins in the Leguminosae, as in other plant families, are produced in response to infection by fungi bacteria or virus or in response to a broad spectrum of chemical toxicants, including simple compounds such as cupric chloride and mercuric chloride, oligogalacturonates, cell wall oligosaccharides derived from plants and fungi and chitin degradation products from fungal cell walls. All of the



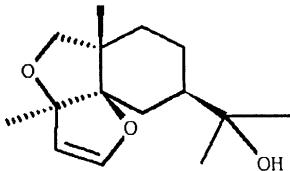
34. Rishitin  
Potato (*Solanum tuberosum*)  
Tobacco (*Nicotiana tabacum*)  
Tomato (*Lycopersicon esculentum*)



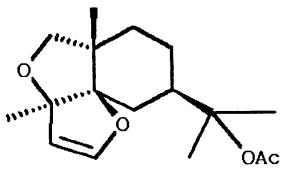
35. Lubimin  
Potato, Tobacco, Eggplant  
(*Solanum melongena*), Jimsonseed  
(*Datura stramonium*)



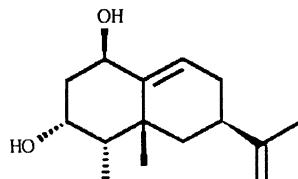
36. Solavetivone  
Potato



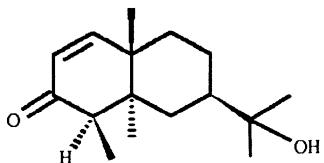
37. Phytuberol  
Potato



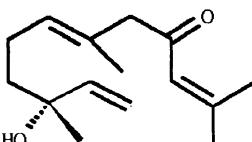
38. Phytuberin  
Potato, Tobacco



39. Capsidiol  
Pepper, Tobacco, Jimsonweed



40. Aubergenone  
Eggplant



41. 9-Oxonerolidol  
Eggplant

Figure 10. Structural formulas of rishitin (34), lubimin (35), solavetivone (36), phytuberol (37), phytuberin (38), capsidiol (39), aubergenone (40), and 9-oxonerolidol (41).

isoflavanoid phytoalexins are synthesized via the joint participation of the malonate and shikimate pathways. Some also require precursors from the mevalonate pathway (Ebel, 1986; Stoessl, 1982, 1983). Though early research with pea and green bean used opened pods inoculated on the exposed seed cavities, there is considerable evidence that roots, stems, and leaves produce phytoalexins. This may be true for most phytoalexins in most legumes. An excellent review of phytoalexins in the Leguminosae is provided in the chapter by J. L. Ingham (1982). The chapter contains

a thorough literature review for the phytoalexins presented here as well as information concerning source, isolation, and chemical characterization. The listing of phytoalexins in this chapter is limited to those from major crop plants; and, in some instances, a phytoalexin may be found in different crop plants within the family, e.g., medicarpin (Fig. 8 (26)) in alfalfa, red clover, and white clover, but an element of specificity also exists. Thus, phaseollin (Fig. 7 (15)) is found in green bean but not pea, pisatin (Fig. 7 (14)) is found in pea and not green bean, and neither of the phytoalexins is found in soybean.

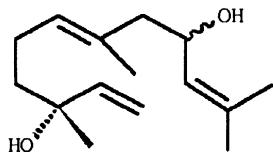
### Solanaceae

The Leguminosae, Solanaceae, and Gramineae include the major food crops. Most phytoalexin research has been conducted with plants in the Leguminosae and Solanaceae though the number of phytoalexins reported in the Gramineae is increasing. Three main classes of phytoalexins have been reported in the Solanaceae: the phenylpropanoid phytoalexins derived from the shikimate pathway (Fig. 2 (3), Fig. 3 (4a,b,c), and Fig. 4 (5a,b,c)), the terpenoid phytoalexins derived from the mevalonate pathway (Fig. 10 (34-41) and Fig. 11 (42-44, 48)), and the acetylenes and polyacetylenes derived from the malonate pathway (Fig. 11 (45-47)). Isoflavonoid- and furan-containing phytoalexins have not been reported in the Solanaceae.

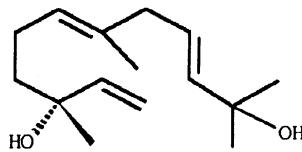
The major phytoalexins isolated from potato are the norsesterpenoid and sesquiterpenoid stress metabolites (SSM) rishitin (Fig. 10 (34)), lubimin (Fig. 10 (35)), solavetivone (Fig. 10 (36)), phytuberol (Fig. 10 (37)), and phytuberin (Fig. 10 (38)) (Kuc, 1982; Kuc and Rush, 1985). Much of the research with SSM in potato has been with R-gene resistance in potato to the late blight pathogen, *Phytophthora infestans*. In resistant reactions with the fungus, the phytoalexins accumulate more rapidly than in susceptible reactions. Factors other than phytoalexin accumulation, e.g., suberization, lignification, and activities of chitinases,  $\beta$ -1,3-glucanases, and peroxidases, may also be important; and their levels are also enhanced earlier in resistant reactions.

Two C<sub>20</sub> polyunsaturated fatty acids, arachidonic and eicosapentaenoic, are potent elicitors of SSM in potato tubers (Bostock et al., 1981, 1982). Both are found in *P. infestans* but not potato. In the presence of glucan fractions from *P. infestans*, they elicit SSM accumulation at picomole concentrations (Preisig and Kuc, 1985); and the accumulation of SSM is concomitant with the inhibition of steroid glycoalkaloid accumulation, activation of sesquiterpenoid cyclase, and inhibition of squalene synthetase (Tjamos and Kuc, 1982; Zook and Kuc, 1990). The elicitation of SSM in potato is under tighter metabolic control than is the elicitation of isoflavonoid phytoalexins in legumes. Mercuric chloride, cupric chloride, and other toxicants elicit little or no SSM accumulation. The relationship between phytoalexins and disease resistance in potato is complex and may involve elicitors, suppressers, enhancers, active oxygen, and Ca<sup>2+</sup> (Doke, 1983, 1985; Doke et al., 1982; Kuc and Preisig, 1984; Preisig and Kuc, 1987; Zook et al., 1987). At least 16 additional sesquiterpenoids have been reported in potato tubers in lesser quantities than the SSM discussed (Stoessl, 1982, 1983).

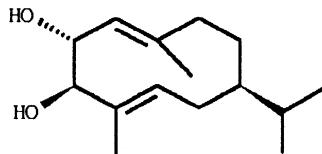
The major sesquiterpenoid phytoalexin found in tobacco and pepper is capsidiol (Fig. 10 (39)), though rishitin (Fig. 10 (34)), lubimin (Fig. 10 (35)), and phytuberin (Fig. 10 (38)) also accumulate in infected tobacco. The infection of tobacco with local lesion viruses or fungi also causes the systemic accumulation of many phenolics including chlorogenic acid (Fig. 2 (3)), 1-caffeooyl, feruloyl, and 0-coumaryl esters of glucose (Kuc, 1982). Tomato phytoalexins include rishitin (Fig. 10 (34)), falcarindiol (Fig. 11 (45)), falcarinol (Fig. 11 (46)), and *cis*-tetradeca-6-ene-1,3-dyne-5,8-diol (Fig. 11 (47)). Phytoalexins in eggplant include lubimin (Fig. 10 (35)), aubergenone



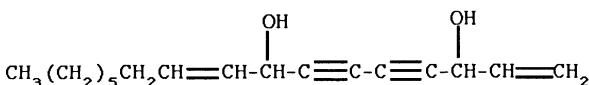
42. 9-Hydroxynerolidol  
Eggplant



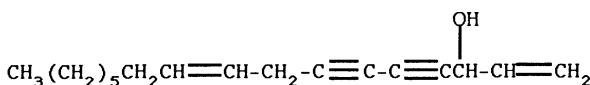
43. 11-Hydroxy-9,10-dehydronerolidol  
Eggplant



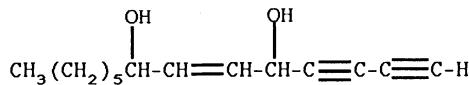
44. 2,3-Dihydroxygermacrene  
Eggplant



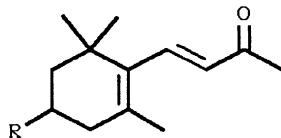
45. Falcarindiol Tomato



46. Falcarinol Tomato



47. *cis*-Tetradeca-6-ene-1,3-dyne-5,8-diol Tomato



48a  $\beta$ -Ionone, R = H

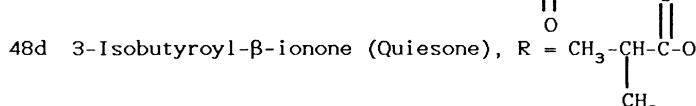
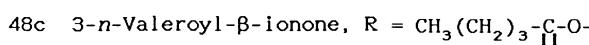


Figure 11. Structural formulas of 9-hydroxynerolidol (42), 11-hydroxy-9,10-dehydronerolidol (43), 2,3-dihydroxygermacrene (44), falcarindiol (45), falcarinol (46), *cis*-tetradeca-6-ene-1,3-dyne-5,8-diol (47), and  $\beta$ -ionones (48a,b,c,d).

(Fig. 10 (40)), 9-oxonerolidol (Fig. 10 (41)), 9-hydroxynerolidol (Fig. 11 (42)), and 11-hydroxy-9,10-dehydronegerolidol (Fig. 11 (43)), and lubimin (Fig. 10 (35)), 3-hydroxylubimin, capsidiol, and 2,3-dihydroxygermacrene (Fig. 11 (44)) are found in jimson weed (Kuc, 1982; Stoessl, 1982, 1983).

Salt et al. (1986) reported that tobacco stems injected with spores of *Peronospora tabacina*, the blue mold pathogen, systemically induced resistance to blue mold in tobacco in the greenhouse and field and caused a 50- to 600-fold increase in  $\beta$ -ionone (Fig. 11 (48a)). The injected tobacco also had increased fresh and dry weight even in the absence of blue mold. Fatty acid derivatives of 3-hydroxy- $\beta$ -ionone also increase in infected tobacco; and 3-n-butyroyl- $\beta$ -ionone (Fig. 11 (48b)) and 3-n-valeroyl- $\beta$ -ionone (Fig. 11 (48c)) were active in inhibiting spore germination at 6 and 25 parts per trillion, respectively (Salt et al., 1988). When sprayed on young tobacco plants, 3-n-butyroyl- $\beta$ -ionone induced systemic resistance to blue mold. Though highly antifungal, the fatty acid esters of 3-hydroxy- $\beta$ -ionone may also function as immunity signals activating or sensitizing for activation of multiple mechanisms for disease resistance, or release such signals, in addition to functioning as phytoalexins. The isobutyroyl derivative of 3-hydroxy- $\beta$ -ionone, quiesone (Fig. 11 (48d)), inhibited spore germination of *P. tabacina* at parts per billion (Leppik et al., 1972).

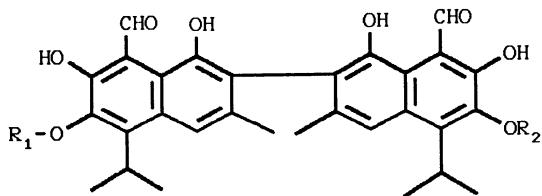
### Malvaceae

Gossypol (Fig. 12 (49)) and a wide range of gossypol-related compounds (Fig. 12 (50-56)) have been isolated from cotton plants (*Gossypium* spp.) infected with *Verticillium* spp. (Bell, 1981). Gossypol is a natural pigment found in pigment glands of certain cotton plants and the accumulation of gossypol, its derivatives, and oxidation products of the compounds increases after infection or treatment of plant tissues with toxicants such as cupric or mercuric chloride. The increase of gossypol and derivatives occurs in cotton plants with or without the glands for the accumulation of free gossypol. The EC<sub>50</sub> for gossypol and its derivatives for fungal spore germination ranges from 1 to 100  $\mu\text{g}/\text{ml}$ . 6-Methoxygossypol (Fig. 12 (50)) and 6,6'-dimethoxy-gossypol (Fig. 12 (51)) are antifungal compounds normally present in high concentration in the roots of healthy cotton plants.

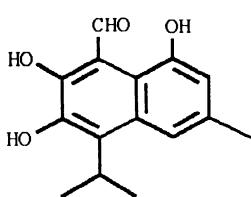
More recently, two sesquiterpenoid naphthols, 2,7-dihydroxycadalene (DHC) (Fig. 12 (57)) and 2-hydroxy-7-methoxycadalene (Fig. 12 (58)), and their optically active oxidation products, lacinilene C (Fig. 12 (59)) and lacinilene C 7-methyl ether (Fig. 12 (60)), have been isolated from cotton leaves and cotyledons inoculated with incompatible bacteria (Essenberg et al., 1990). When irradiated by the sun or cool-white fluorescent lamps (300 to 700 nm), DHC markedly increased in antibacterial activity and the photoactivated compound also inactivated virions of cauliflower mosaic virus (Tzeli et al., 1988, 1989). It appears likely that DHC, its derivatives, and their photoactivated products are antifungal. Light activated DHC was bactericidal at 0.1 Mm to *Xanthomonas campestris* pv. *malvacearum*.

### Umbelliferae

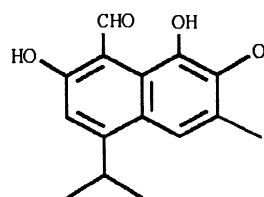
One of the earliest phytoalexins was the compound 6-methoxymellein (Fig. 13 (61)) isolated from carrot slices inoculated with the fungus *Ceratocystis fimbriata* (Condon and Kuc, 1960, 1962). Simple toxicants, ethylene, and other pathogens also induced accumulation of 6-methoxymellein (Chalutz et al., 1969; Condon et al., 1963; Coxon et al., 1973). Other phytoalexins produced in carrot are falcarinol (Fig. 11 (46)) and *p*-hydroxybenzoic acid (Harding and Heale, 1980). Falcarindiol (Fig. 11 (45)) is a preformed polyacetylene in carrot. The EC<sub>50</sub> values for growth of *Botrytis cinerea* of 6-methoxymellein (Fig. 13 (61)), *p*-hydroxybenzoic



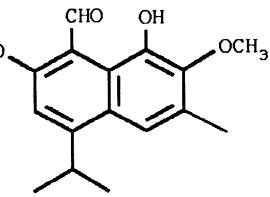
49. Gossypol ( $R^1 = R^2 = H$ )  
 50. 6-Methoxygossypol ( $R^1 = CH_3$ ,  $R^2 = H$ )  
 51. 6,6'-Dimethoxygossypol ( $R^1 = R^2 = CH_3$ )



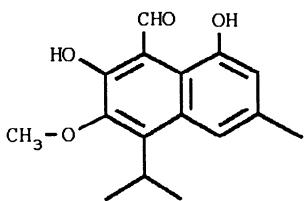
52. Hemigossypol



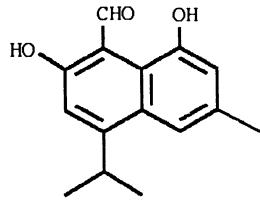
53. Isohemigossypol



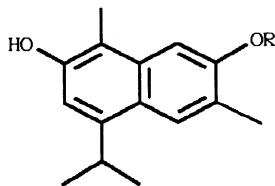
54. Gossyvertin



55. 6-Methoxyhemigossypol



56. 6-Deoxyhemigossypol



57. 2,7-Dihydroxycadalene  $R = H$   
 58. 2-Hydroxy-7-methoxycadalene  $R = CH_3$   
 59. Lacinilene C  $R = H$   
 60. Lacinilene C 7-methylether  
 $R = CH_3$

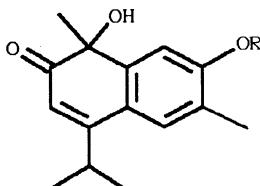
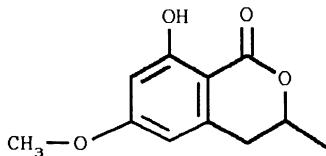
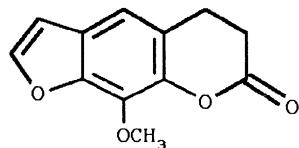


Figure 12. Structural formulas of gossypol (49), 6-methoxygossypol (50), 6,6'-dimethoxygossypol (51), hemigossypol (52), isohemigossypol (53), gossyvertin (54), 6-methoxyhemigossypol (55), 6-deoxyhemigossypol (56), 2,7-dihydroxycadalene  $R = H$  (57), 2-hydroxy-7-methoxycadalene  $R = CH_3$  (58), lacinilene C  $R = H$  (59), and lacinilene C 7-methyl ether  $R = CH_3$  (60).

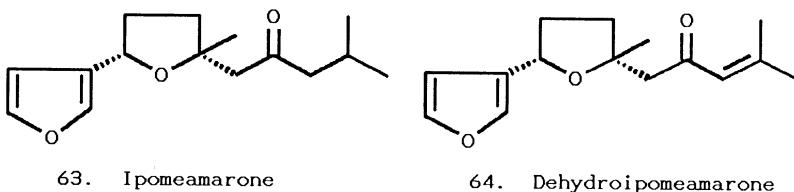


61. 6-Methoxymellein



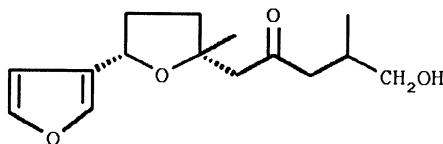
62. Xanthotoxin

Figure 13. Structural formulas of 6-methoxymellein (61) and xanthotoxin (62).



63. Ipomeamarone

64. Dehydroipomeamarone



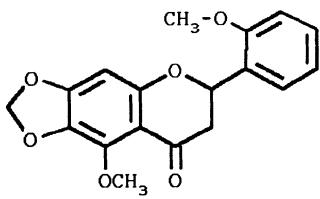
65. Ipomeamaronol

Figure 14. Structural formulas of ipomeamarone (63), dehydroipomeamarone (64), and ipomeamaronol (65).

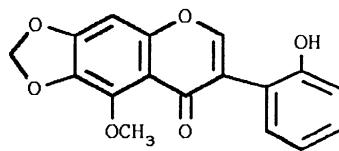
acid, and falcarinol are 104, 607, and 9.2  $\mu\text{g}/\text{ml}$ , respectively. The furanocoumarin, xanthotoxin (Fig. 13 (62)), has been identified as a phytoalexin in parsnip (Johnson et al., 1973). The  $\text{EC}_{50}$  for growth of *C. fimbriata* is 21.6  $\mu\text{g}/\text{ml}$ .

### Convolvulaceae

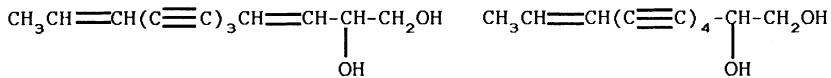
Ipomeamarone (Fig. 14 (63)), an antifungal furanosesquiterpene, was isolated from infected sweet potato roots or roots treated with chemical toxicants. Together with pisatin from pea, phaseolin from green bean, and 6-methoxymellein from carrot, it was one of the early phytoalexins studied (Akazawa, 1960; Akazawa and Wada, 1961; Uritani et al., 1960). Many additional furanosesquiterpene phytoalexins have



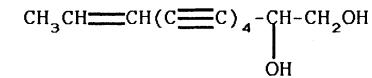
66. Betagarin



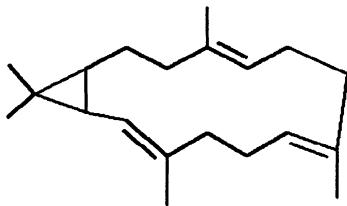
67. Betavulgarin



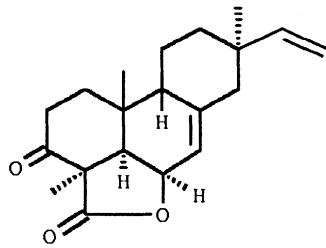
68. Safynol



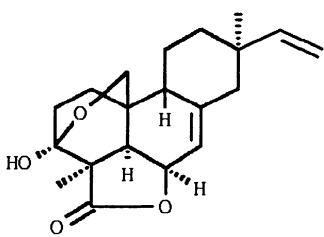
69. Dehydrosafynol



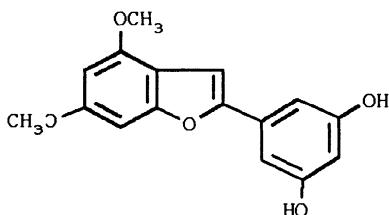
70. Casbene



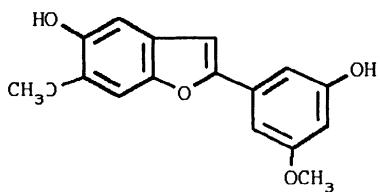
71. Momilactone A



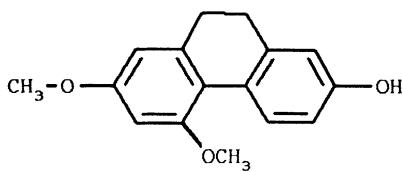
72. Momilactone B



73. Moracin A

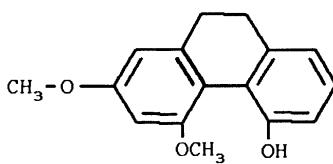


74. Moracin B

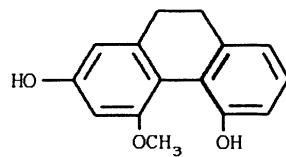


75. Orchinol

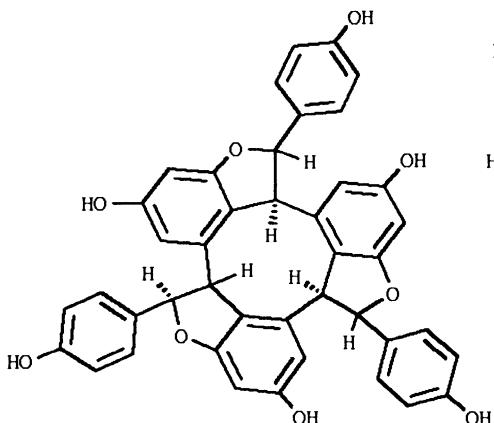
Figure 15. Structural formulas of betagarin (66), betavulgarin (67), safynol (68), dehydrosafynol (69), casbene (70), momilactone A (71), momilactone B (72), moracin A (73), moracin B (74), and orchinol (75).



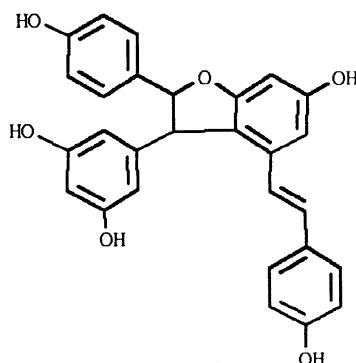
76. Loroglossol



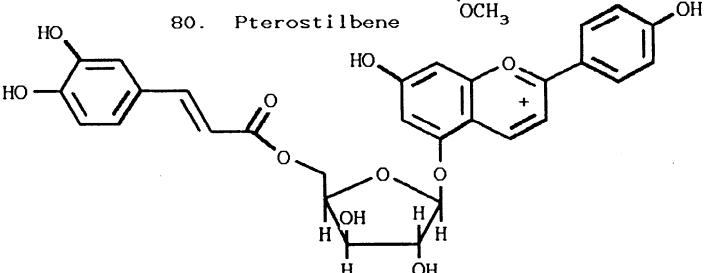
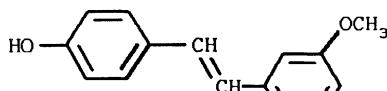
77. Hircinol



78.  $\alpha$ -Viniferin



79.  $\epsilon$ -Viniferin



81. Caffeic acid ester of arabinosyl 5-O-apigeninidin

Figure 16. Structural formulas of loroglossol (76), hircinol (77),  $\alpha$ -viniferin (78),  $\epsilon$ -viniferin (79), pterostilbene (80), and caffeic acid ester of arabinosyl-5-O-apigeninidin (81).

been isolated from sweet potato including dehydroipomeamarone (Fig. 14 (64)) and ipomeamaronol (Fig. 14 (65)). The furanoterpenes have mammalian toxicity.

#### **Chenopodiaceae, Compositae, Euphorbiaceae, Gramineae, Moraceae, Orchidaceae, and Vitaceae**

Though varied in structure, depending on the plant family from which they arise, these phytoalexins have EC<sub>50</sub> values for growth or spore germination of fungi of 1 to 200 µg/ml. They include: a flavonoid, betagarin (Fig. 15 (66)), and an isoflavonoid, betavulgarin (Fig. 15 (67)), from sugar beets in the Chenopodiaceae (Geigert et al., 1973); the polyacetylenes, safynol (Fig. 15 (68)) and dehydrosafynol (Fig. 15 (69)), from safflower of the Compositae (Allen and Thomas, 1971a,b,c); the diterpene hydrocarbon, casbene (Fig. 15 (70)), from castor bean in the Euphorbiaceae (Sutton and West, 1975); the diterpenoids, momilactone A (Fig. 15 (71)) and momilactone B (Fig. 15 (72)), from rice in the Gramineae (Cartwright et al., 1981); the benzofurans, moracin A (Fig. 15 (73)) and moracin B (Fig. 15 (74)), from mulberry in the Moraceae (Takasugi et al., 1978); the dihydrophenanthrenes, orchinol (Fig. 15 (75)), loroglossol (Fig. 16 (76)), and hircinol (Fig. 16 (77)), from the Orchidaceae (Hardegger et al., 1963; Urech et al., 1963); the oligomers of *trans*-resveratrol,  $\alpha$ - and  $\beta$ -viniferin (Fig. 16 (78,79)) (Langcake and Pryce, 1977a,b), and the stilbene, *trans*-pterostilbene (Fig. 16 (80)) (Langcake et al., 1979), from the Vitaceae; and the flavonoid apigeninidin (Fig. 16 (81)) and the caffeic acid ester of arabinosyl-5-O-apigeninidin (Fig. 16 (81)) from sorghum in the Gramineae (Hipskind et al., 1990). Apigeninidin and the caffeic acid ester of arabinosyl-5-O-apigeninidin were toxic to *Colletotrichum graminicola* at less than 10 µM.

#### **DISCUSSION**

In this chapter, I have emphasized antifungal compounds in plants for which some association has been made with plant disease resistance. This, of course, excludes many compounds which may have antifungal activity and have not been tested for such activity and compounds of medicinal or industrial value which have not been investigated for a role in disease resistance. The chapter does bring to the reader compounds of known antifungal activity and, hopefully, introduces, to those not in the plant field, compounds which might have otherwise escaped their attention. This is especially true for the phytoalexins, a broad spectrum of diverse compounds, which are not preformed but which accumulate at sites of infection or stress in plants and only relatively recently appeared in the literature. Though logic has its place in science, so does trial and error. There are likely to be many chemically characterized compounds in the literature that have interest for biological activity other than their antifungal properties. A general screening of such compounds is not out of place. Naturally occurring compounds may also serve as models for metabolically active centers and derivatives of such compounds may find medicinal or industrial use.

Since the expression of genes for the gene products contributing to disease resistance is regulated either directly or indirectly by compounds (elicitors) of plant and microbial origin, and gene regulation - not the presence or absence of genes for resistance mechanisms - is likely to be the determinant of disease resistance in plants, it is not surprising that all of the defense compounds which are part of the response mechanism can be produced equally well by resistant and susceptible plants. The speed, magnitude, and timing of different elements of the response and the activity of the gene products as influenced by the environment determine resistance. The induction of systemic resistance (immunization) in plants, including plants reported

to lack genes for resistance to a specific pathogen, further supports the importance of gene expression (Dean and Kuc, 1987; Kuc, 1983, 1987, 1990a,b; Kuc and Caruso, 1977; Kuc and Preisig, 1984; Kuc and Rush, 1985, Kuc and Tuzun, 1983; Preisig and Kuc, 1987; Rao and Kuc, 1991). Immunization is possible by restricted inoculation with pathogens, attenuated pathogens, selected nonpathogens, and treatment with signals produced by immunized plants or chemicals which release such signals (Doubrava et al., 1988; Gottstein and Kuc, 1989; Kuc, 1987, 1990a). Immunization is effective against fungi, bacteria, and viruses and it has been successfully tested in the laboratory and field (Kuc, 1987, 1990a; Tuzun and Kuc, 1989; Tuzun et al., 1986).

In considering antifungal compounds, it is also important to consider elicitors which induce the synthesis of such compounds, as well as immunity or alarm signals which activate or sensitize for activation multiple defense mechanisms and compounds which release elicitors or immunity signals. Dixon and Lamb (1990) have written a thought-provoking review on molecular communication between plants and pathogens.

As we create technology to solve problems, we create new problems. The survival of our planet depends upon our ability to anticipate the problems we create and to meet the challenge of their solution. With increasing concerns for the environment and human health resulting from pesticide use, it becomes not only important to develop new technologies for pest control, but also to integrate for maximum effectiveness the existing technologies including plant breeding for resistance, agronomic practices, biocontrol, genetic engineering, immunization, and pesticide use.

## REFERENCES

- Akazawa, T., 1960, Chromatographic isolation of pure ipomeamarone and reinvestigation of its chemical properties, *Archives Biochemistry Acta*, 90:82-89.
- Akazawa, T., and Wada, K., 1961, Analytical study of ipomeamarone and chlorogenic acid alterations in sweet potato roots infected by *Ceratocystis fimbriata*, *Plant Physiology*, 36:139-144.
- Allen, E., and Kuc, J., 1968,  $\alpha$ -Solanine and  $\alpha$ -chaconine as fungitoxic compounds in extracts of Irish potato tubers, *Phytopathology*, 58:776-781.
- Allen, E., and Thomas, C., 1971a, *Trans-trans*-3,11-tridecadiene-5,7,9-triyne-1,2-diol, an antifungal polyacetylene from diseased safflower (*Carthamus tinctorius*), *Phytochemistry*, 10:1579-1582.
- Allen, E., and Thomas, C., 1971b, Time course of safynol accumulation in resistant and susceptible safflower infected with *Phytophthora drechsleri*, *Physiological Plant Pathology*, 1:235-240.
- Allen, E., and Thomas C., 1971c, A second antifungal polyacetylene from *Phytophthora*-infected safflower, *Phytopathology*, 6:1107-1109.
- Angell, H. R., Walker, J. C., and Link, K. P., 1930, The relation of protocatechuic acid to disease resistance in onion, *Phytopathology*, 20:431-438.
- Bell, A. A., 1981, Biochemical mechanisms of disease resistance, *Annual Review of Plant Physiology*, 32:21-81.
- Bostock, R. M., Kuc, J., and Laine, R. A., 1981, Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in potato, *Science*, 212:67-69.
- Bostock, R. M., Laine, R. A., and Kuc, J., 1982, Factors affecting elicitation of sesquiterpenoid phytoalexin accumulation by eicosapentaenoic and arachidonic acids in potato, *Plant Physiology*, 70:1417-1424.

- Burden, R. S., Rowell, P. M., and Bailey, J. A., 1985, Debneyol, a fungicidal sesquiterpene from TNV-infected *Nicotiana debneyi*, *Phytochemistry*, 24:2191-2194.
- Cartwright, D., Langcake, P., Price, R., Leworthy, D., and Ride, J., 1981, Isolation and characterization of phytoalexins from rice as momilactones A and B, *Phytochemistry*, 20:535-537.
- Chalutz, E., De Vay, J., and Maxie, E., 1969, Ethylene-induced isocoumarin formation in carrot root tissue, *Plant Physiology*, 44:235-241.
- Condon, P., and Kuc, J., 1960, Isolation of a fungitoxic compound from carrot root tissue inoculated with *Ceratocystis fimbriata*, *Phytopathology*, 50:267-270.
- Condon, P., and Kuc, J., 1962, Confirmation of the identity of a fungitoxic compound produced by carrot root tissue, *Phytopathology*, 52:182-183.
- Condon, P., Kuc, J., and Draudt, H., 1963, Production of 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin by carrot root tissue, *Phytopathology*, 53:1244-1250.
- Coxon, D., Curtis, R., Price, K., and Levett, G., 1973, Abnormal metabolites produced by *Daucus carota* roots stored under conditions of stress, *Phytochemistry*, 12:1881-1885.
- Coxon, D.T., 1982, Phytoalexins from other families, p. 106-132, in: "Phytoalexins," J. A. Bailey and J. W. Mansfield, eds., Blackie, Glasgow, London.
- Cruickshank, I. A. M., 1963, Phytoalexins, *Annual Review of Phytopathology*, 1:351-374.
- Cruickshank, I., Perrin, D., and Mandryk, M., 1977, Fungitoxicity of duvatrienediols associated with the cuticular wax of tobacco leaves, *Phytopathology Zeitschrift*, 90:243-249.
- Dean, R., and Kuc, J., 1987, Immunization against disease: The plant fights back, p. 383-410, in: "Fungal Infection of Plants," G. F. Pegg and P. G. Ayres, eds., Cambridge University Press, Cambridge, Massachusetts.
- Defago, G., 1982, Genetic analysis of tomatin resistance and pathogenicity of *Fusarium solani* mutants, p. 324-325, in: "Active Defense Mechanisms in Plants," R. K. S. Wood, ed., Plenum Press, New York, London.
- Dixon, R. and Lamb, C., 1990, Molecular communication in interactions between plants and microbial pathogens, *Annual Review of Plant Physiology*, 41:339-367.
- Doke, N., 1983, Involvement of superoxide anion generation in the hypersensitive response of potato tubers inoculated with *Phytophthora infestans* and to the hyphal wall components, *Physiological Plant Pathology*, 23:345-357.
- Doke, N., 1985, NADPH-dependent O<sub>2</sub><sup>-</sup> generation in membrane fractions isolated from wounded potato tubers inoculated with *Phytophthora infestans*, *Physiological Plant Pathology*, 27:311-322.
- Doke, N., Tomiyama, N., and Furuichi, N., 1982, Elicitation and suppression of the hypersensitive response in host-parasite specificity, p. 79-96, in: "Plant Infection: The Physiological and Biochemical Basis," Y. Asada, W. Bushnell, S. Ouchi, and C. Vance, eds., Japan Scientific Society Press and Springer-Verlag, Tokyo, Berlin.
- Doubrava, N., Dean, R., and Kuc, J., 1988, Induction of systemic resistance to anthracnose caused by *Colletotrichum lagenarium* in cucumber by oxalate and extracts from spinach and rhubarb leaves, *Physiological Molecular Plant Pathology*, 33:69-80.
- Ebel, J., 1986, Phytoalexin synthesis: The biochemical analysis of the induction process, *Annual Review of Phytopathology*, 24:235-264.
- Essenberg, M., Grover, P., and Cover, E., 1990, Accumulation of antibacterial sesquiterpenoids in bacterially inoculated *Gossypium* leaves and cotyledons, *Phytochemistry* (in press).

- Farih, A., Tsao, P., and Menge, J., 1981, Fungitoxic activity of efosite aluminum on growth, sporulation and germination of *Phytophthora parasitica* and *P. citrophthora*, *Phytopathology*, 71:934-936.
- Geigert, J., Stermitz, F., Johnson, G., Maag, D., and Johnson, D., 1973, Two phytoalexins from sugar beet (*Beta vulgaris*) leaves, *Tetrahedron*, 29:2703-2706.
- Gottstein, D. and Kuc, J., 1989, Induction of systemic resistance to anthracnose in cucumber by phosphates, *Phytopathology*, 79:176-179.
- Hardegger, E., Biland, H., and Corrodi, H., 1963, Synthese von 2,4-dimethoxy-6-hydroxyphenanthren und konstitution des orchinols, *Helvetica Chimica Acta*, 46:1354-1360.
- Harding, V., and Heale, J., 1980, Isolation and identification of the antifungal compound accumulating in the induced resistance response of carrot slices to *Botrytis cinerea*, *Physiological Plant Pathology*, 17:277-289.
- Hipskind, J., Hanau, R., Leite, B., and Nicholson, R., 1990, Phytoalexin accumulation in sorghum: Identification of an apigeninidin acyl ester, *Physiological Molecular Plant Pathology*, 36:381-396.
- Ingham, J. L., 1982, Phytoalexins from the Leguminosae, p. 21-80, *in:* "Phytoalexins," J. A. Bailey and J. W. Mansfield, eds., Blackie, Glasgow, London.
- Ishizaka, N., and Tomiyama, K., 1972, Effect of wounding or infection by *Phytophthora infestans* on the contents of terpenoids in potato tubers, *Plant and Cell Physiology*, 13:1053-1063.
- Johnson, C., Brannon, D., and Kuc, J., 1973, Xanthotoxin: A phytoalexin of *Pastinica sativa* root, *Phytochemistry*, 12:2961-2962.
- Johnson, G., and Schaal, L., 1952, Relation of chlorogenic acid to scab resistance in potatoes, *Science*, 115:627-629.
- Johnson, G., and Schaal, L., 1955, The inhibitory effect of phenolic compounds on growth of *Streptomyces scabies* as related to the mechanisms of scab resistance, *Phytopathology*, 45:626-628.
- Keene, C. K., and Wagner, G. J., 1985, Direct demonstration of dufatrienediol biosynthesis in glandular heads of tobacco trichomes, *Plant Physiology*, 79:1026-1032.
- Kuc, J., 1957, A biochemical study of the resistance of potato tuber to attack by various fungi, *Phytopathology*, 47:676-680.
- Kuc, J., 1972, Phytoalexins, *Annual Review of Phytopathology*, 10:207-232.
- Kuc, J., 1982, Phytoalexins from the Solanaceae, p. 81-105, *in:* "Phytoalexins," J. A. Bailey and J. W. Mansfield, eds., Blackie, Glasgow, London.
- Kuc, J., 1983, Induced systemic resistance in plants to diseases caused by fungi and bacteria, p. 191-221, *in:* "The Dynamics of Host Defense," J. Bailey and B. Deverall, eds., Academic Press, Sydney, Australia.
- Kuc, J., 1987, Plant immunization and its applicability for disease control, p. 255-274, *in:* "Innovative Approaches to Plant Disease Control," I. Chet, ed., John Wiley and Son, New York, New York.
- Kuc, J., 1990a, Immunization for the control of plant disease, p. 355-373, *in:* "Biological Control of Soil Borne Plant Pathogens," D. Hornby, ed., CAB International, Wallingford, United Kingdom.
- Kuc, J., 1990b, A case for self defense in plants, *Phytoparasitica*, 18(1):3-7.
- Kuc, J., and Caruso, F., 1977, Activated coordinated chemical defense against disease in plants, p. 78-89, *in:* "Host Resistance to Pests," P. Hedin, ed., American Chemical Society Press, Washington, D.C.
- Kuc, J., and Preisig, C., 1984, Fungal regulation of disease resistance mechanisms in plants, *Mycologia*, 76(5):767-784.
- Kuc, J., and Rush, J., 1985, Phytoalexins, *Archives of Biochemistry and Biophysics*, 236:379-389.

- Kuc, J., and Tuzun, S., 1983, Immunization for disease control in tobacco, *Recent Advances in Tobacco Science*, 9:179-213.
- Langcake, P., 1981, Alternative chemical agents for controlling plant diseases, *Philosophical Transactions of the Royal Society of London B Biological Sciences* 295:83-101.
- Langcake, P., Cornford, C., and Pryce, R., 1979, Identification of pterostilbene as a phytoalexin from *Vitis vinifera* leaves, *Phytochemistry*, 18:1025-1027.
- Langcake, P., and Pryce, R., 1977a, A new class of phytoalexins from grapevines, *Experientia*, 33:151-152.
- Langcake, P., and Pryce, R., 1977b, The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiation, *Phytochemistry*, 16:1193-1196.
- Leppik, R., Holloman, D., and Bottomly, W., 1972, Quiesone: An inhibitor of germination of *Peronospora tabacina* conidia, *Phytochemistry*, 11:2055-2063.
- Link, K. P., Dickson, A. D. and Walker, J. C., 1929, Further observations on the occurrence of protocatechuic acid in pigmented onion scales and its relation to disease resistance in the onion, *Journal of Biological Chemistry*, 84:719-725.
- Link, K. P., and Walker, J. C., 1933, The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions, *Journal of Biological Chemistry*, 100:379-383.
- Locci, R., and Kuc, J., 1967, Steroid glycoalkaloids as compounds produced by potato tubers under stress, *Phytopathology*, 57:1272-1273.
- McKee, R., 1955, Host-parasite relationship in the dry rot disease of potatoes, *Annals of Applied Biology*, 43:147-148.
- McKee, R., 1959, Factors affecting the toxicity of solanine and related alkaloids to *Fusarium caeruleum*, *Journal of General Microbiology*, 20:686-696.
- Overeem, J. C., 1976, Pre-existing antimicrobial substances in plants and their role in disease resistance, p. 195-206, in: "Biochemical Aspects of Plant-Parasite Relationships," J. Friend and D. R. Threlfall, eds., Academic Press, London, England.
- Preisig, C., and Kuc, J., 1985, Arachidonic acid-related elicitors of the hypersensitive response in potato and enhancement of their activities by glucans from *Phytophthora infestans*, *Archives of Biochemistry and Biophysics*, 236:379-389.
- Preisig, C. L., and Kuc, J., 1987, Phytoalexins, elicitors, enhancers, suppressors and other considerations in the regulation of R-gene resistance to *Phytophthora infestans* in potato, p. 203-221, in: "Biochemical and Molecular Determinants of Plant Diseases," S. Nishimura, C. Vance, and N. Doke, eds., Japan Scientific Society Press, Tokyo.
- Rao, N., and Kuc, J., 1991, Induced systemic resistance in plants, p. 347-362, in: "The Fungal Spore and Disease Initiation in Plants and Animals," G. T. Cole and H. C. Hoch, eds., Plenum Press, New York, New York.
- Rao, M. N., Siegel, M. R., Ferriss, R. S., Nesmith, W. C., Wiglesworth, M. D., Burton, H. R., Reuveni, M., Tuzun, S., and Kuc, J., 1989a, Relationships between susceptibility of field-grown burley tobacco to blue mold and contents of duvatrienediols, *Phytopathology*, 79:267-270.
- Rao, M. N., Siegel, M. R., Nielsen, M. T., Wiglesworth, M. D., Burton, H. R., and Kuc, J., 1989b, Evaluation and induction of resistance to blue mold in tobacco genotypes differing in contents of duvatrienediols, *Phytopathology*, 79:271-275.
- Reuveni, M., Tuzun, S., Cole, J., Siegel, M., and Kuc, J., 1986a, Removal of duvatrienediols from the surfaces of tobacco leaves increases their susceptibility to blue mold, *Physiological Molecular Plant Pathology*, 30:44-51.

- Reuveni, M., Tuzun, S., Cole, J., Siegel, M., and Kuc, J., 1986b, The effects of plant age and leaf position on the susceptibility of tobacco to blue mold caused by *Peronospora tabacina*, *Phytopathology*, 76:455-458.
- Salt, S., Tuzun, S., and Kuc, J., 1986, Effects of  $\beta$ -ionone and abscisic acid on the growth of tobacco and resistance to blue mold, *Physiological and Molecular Plant Pathology*, 28:287-297.
- Salt, S., Reuveni, M., and Kuc, J., 1988, Inhibition of *Peronospora tabacina* (blue mold of tobacco) and related plant pathogens *in vitro* and *in vivo* by esters of 3(R)-hydroxy- $\beta$ -ionone, 42nd Tobacco Chemists Conference Proceedings, Tobacco Chemists Society, Lexington, Kentucky, p. 22.
- Severson, R. F., Johnson, A. W., and Jackson, D. M., 1985, Cuticular constituents of tobacco: Factors affecting their production and their role in insect and disease resistance and smoke quality, *Recent Advances in Tobacco Science*, 11:105-174.
- Shih, M. J., 1972, The accumulation of isoprenoids and phenols and their control as related to the interaction of potato (*Solanum tuberosum*) with *Phytophthora infestans*, Ph.D. Thesis, Purdue University, Lafayette, Indiana.
- Shih, M., and Kuc, J., 1973, Incorporation of  $^{14}\text{C}$  from acetate and mevalonate into rishitin and steroid glycoalkaloids by potato slices inoculated with *Phytophthora infestans*, *Phytopathology*, 63:826-829.
- Shih, M., and Kuc, J., 1974,  $\alpha$ - and  $\beta$ -Solarmarine in Kennebec *Solanum tuberosum* leaves and aged tuber slices, *Phytopathology*, 13:997-1000.
- Shih, M., Kuc, J., and Williams, E. B., 1973, Suppression of steroid glycoalkaloid accumulation as related to rishitin accumulation in potato tubers, *Phytopathology*, 63:821-826.
- Sitton, D., and West, C., 1975, Casbane: An antifungal diterpene produced in cell-free extracts of *Ricinus communis* seedlings, *Phytopathology*, 14:1921-1925.
- Smith, D., 1982, Toxicity of phytoalexins, p. 218-252, *in: "Phytoalexins,"* J. A. Bailey, and J. W. Mansfield, eds., Blackie, Glasgow, London.
- Stoessl, A., 1982, Biosynthesis of phytoalexins, p. 133-180, *in: "Phytoalexins,"* J. A. Bailey and J. W. Mansfield, eds., Blackie, Glasgow, London.
- Stoessl, A., 1983, Secondary plant metabolites in preinfectional and postinfectional resistance, p. 71-122, *in: "The Dynamics of Host Defense,"* J. A. Bailey and B. J. Deverall, eds., Academic Press, Sydney, Australia.
- Sutton, D., 1979, Systemic cross protection in bean against *Colletotrichum lindemuthianum*, *Australasia Plant Pathology*, 8:4-5.
- Takasugi, M., Nagao, S., Masamune, T., Shirata, A., and Takahashi, K., 1978, Structure of moracin A and B, new phytoalexins from diseased mulberry, *Tetrahedron Letters*, 34:797-798.
- Tjamos, E. C., and Kuc, J., 1982, Inhibition of steroid glycoalkaloid accumulation by arachidonic and eicosapentaenoic acids in potato, *Science*, 217:542-544.
- Tuzun, S., and Kuc, J., 1989, Induced systemic resistance to blue mold, p. 177-200, *in: "Blue Mold of Tobacco,"* W. E. McKeen, ed., American Phytopathological Society, St. Paul, Minnesota.
- Tuzun, S., Nesmith, W., Ferriss, R., and Kuc, J., 1986, Effects of stem injections with *Peronospora tabacina* on growth of tobacco and protection against blue mold in the field, *Phytopathology*, 76:938-941.
- Tuzun, S., Reuveni, M., Siegel, M., and Kuc, J., 1989, The effect of removing leaf surface components with acetone from immunized and nonimmunized resistant tobacco plants on their susceptibility to blue mold, *Phytopathology*, 79:1024-1027.
- Tzeli, J., Melcher, U., and Essenberg, M., 1988, Inactivation of cauliflower mosaic virus by a photoactivatable cotton phytoalexin, *Physiological Molecular Plant Pathology*, 33:115-126.

- Tzeli, J., Essenberg, M., and Melcher, U., 1989, Photoactivated DNA nicking, enzyme inactivation and bacterial inhibition by sesquiterpenoid phytoalexins from cotton, *Molecular Plant-Microbe Interactions*, 2:139-147.
- Urech, J., Fesctig, B., Nuesch, J., and Vischer, E., 1963, Hircinol, eine antifungisch wirksame substanz ans knollen von *Loroglossum hircinum* (L.) Rich, *Helvetica Chimica Acta*, 46:2758-2766.
- Uritani, I., Uritani, M., and Yamada, H., 1960, Similar metabolic alterations induced in sweet potato by poisonous chemicals and by *Ceratocystis fimbriata*, *Phytopathology*, 50:30-34.
- VanEtten, H., Matthews, D., and Matthews, P., 1989, Phytoalexin detoxification: Importance for pathogenicity and practical implications, *Annual Review of Phytopathology*, 27:143-164.
- Zook, M., and Kuc, J., 1990, Elicitation of sesquiterpenoid cyclase and suppression of squalene synthetase activity in potato tuber tissue, *Phytopathology*, 80:1009 (Abstract).
- Zook, M., Rush, J., and Kuc, J., 1987, A role for  $\text{Ca}^{2+}$  in the elicitation of rishitin and lubimin accumulation in potato tuber tissue, *Plant Physiology*, 84:520-525.

## NEMATICIDAL COMPOUNDS FROM PLANTS

David J. Chitwood

Nematology Laboratory, USDA-ARS  
Building 467, BARC-East  
Beltsville, MD 20705

### INTRODUCTION

Current chemicals available for control of plant-parasitic nematodes are far from perfect with respect to environmental safety, mammalian toxicity, or expense (Hague and Gowen, 1987; Johnson and Feldmesser, 1987; Thomason, 1987). During the past decade, several agriculturally important nematicides have been deregistered or restricted in use because of concern about groundwater contamination or adverse effects on human health. Other nematicides face the possibility of similar restrictions. Because annual losses to agricultural productivity induced by plant-parasitic nematodes are at least 6 billion dollars in the U.S. and 77 billion dollars in the world (Sasser and Freckman, 1987), the need to develop safe chemical control agents is acute. Unfortunately, only about 0.2% of the latter figure is being spent on plant nematology research, teaching, and extension (Sasser and Freckman, 1987).

Most agrichemical firms involved in the development of new insecticides routinely screen compounds for nematicidal activity (Morton, 1987). The development of agronomically viable nematicides is complicated by the fact that such compounds must be either capable of rapid movement within the rhizosphere without deactivation or else be systemic in plants (Feldmesser et al., 1985). These are obstacles not faced in development of new compounds active against animal-parasitic nematodes. Certainly, one of the most frustrating aspects of preparing this review was the frequent encounter of investigations in which the primary objective was development of new anthelmintics. This review will be limited to scrutiny of reports of compounds from higher plants with biological activity against plant-parasitic nematodes. A few exceptions will be made for compounds with demonstrated activity against free-living nematodes, provided that the researchers clearly indicated that they were using free-living nematodes as model organisms for determining activity against plant parasites. In addition, although direct toxicity is the most common biological activity reported for many of these compounds, many of them do not cause death; indeed, most agronomically useful nematicides are effective in the field at sublethal concentrations (Bunt, 1987; Wright, 1981).

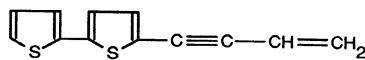
In agreement with the rest of this volume, this review will not focus on investigations in which crude plant extracts were examined for activity against phytoparasitic nematodes without identification of the active components. Such

studies can have substantial value for development of control strategies based on incorporation of plant extracts or residues into soil; readers with further curiosity can consult several references (Chang and Rohde, 1969; Husain and Masood, 1975a,b; Lal et al., 1977; Miller et al., 1973; Nandal and Bhatti, 1983; Pillai and Desai, 1975; Ram Nath et al., 1982; Sukul et al., 1974; Taylor and Murant, 1966; Ueno and Iyatomi, 1978). Also, this review will only briefly address postinfectational compounds in plants that have nematicidal activity, even though these compounds have aroused the curiosity of many nematologists and generated substantial controversy. Postinfectational compounds and their possible roles in mechanisms of resistance of plants to nematodes have been reviewed (Giebel, 1982; Gommers, 1981; Gommers and Bakker, 1988; Huang, 1985; Kaplan and Davis, 1987; Kaplan and Keen, 1980; O'Neill, 1986; Rohde, 1972; Veech, 1981, 1982). Although the author's research interest includes the existence and function of insect molting or juvenile hormones in nematodes and although these compounds occur in higher plants and have been reported to have biological activity when applied to nematodes, this review will exclude these compounds because plant-parasitic nematodes have not been the focus of these investigations. Readers with curiosity about this research front can refer to recent reviews (Barker et al., 1990; Chitwood, 1987). The precise focus of this review, i.e., the identification of specific, naturally occurring compounds in plants with biological activity against phytoparasitic nematodes, has been the subject of or included in previous reviews (Gommers, 1981; Gommers and Bakker, 1988; Huang, 1985; Kaplan and Keen, 1980; Munakata, 1978, 1983; Veech, 1981). This review is organized by phytochemical structure, although some overlap of categories is unavoidable.

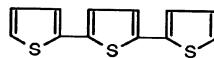
## POLYTHIENYLS

Because of the frequently suppressive effect of marigolds on populations of soil nematodes, marigolds (*Tagetes* spp., Asteraceae) were among the first plants to be rigorously examined for naturally occurring nematicidal principles. The first such compound isolated from *Tagetes erecta plena* roots was  $\alpha$ -terthienyl (Fig. 1) (Uhlenbroek and Bijloo, 1958). It was nematicidal in 6-day tests *in vitro* against the stem and bulb nematode, *Ditylenchus dipsaci*, at 5  $\mu\text{g}/\text{ml}$ ; the wheat seed gall nematode, *Anguina tritici*, at 0.5  $\mu\text{g}/\text{ml}$ ; and the potato cyst nematode, *Globodera rostochiensis*, at 0.1 to 0.2  $\mu\text{g}/\text{ml}$ . Uhlenbroek and Bijloo (1959) next isolated 5-(3-buten-1-ynyl)-2,2'-bithienyl (Fig. 1) as another nematicidal polythienyl. Although nematotoxicity of the native compound was not evaluated, its hydrogenated derivative (5-butyl-2,2'-bithienyl) was nematotoxic against the same three nematode species.

Gommers and voor in 't Holt (1976) performed a thorough survey of 110 different Asteraceae, for suppressive effects on populations of the root lesion nematode, *Pratylenchus penetrans*, in greenhouse experiments; over 40 species suppressed nematode population levels and at least 15 contained one of the thiens. Since these pioneering studies on marigold thiens, a substantial body of work has accumulated on the nematotoxicity of these compounds and synthetic analogs. Readers are referred to reviews by Gommers (1981) and Gommers and Bakker (1988) for discussion in greater detail of the suppressive effects of marigolds on soil populations, the weak nematicidal activity of marigold thiens or synthetic analogs when incorporated into soil, and the mode of action of the compounds, which require light, peroxidase, or other activators to release the singlet oxygen that kills nematodes. Tetrachlorothiophene, a rather simple analog, was a registered nematicide in the U.S. but was evaluated as obsolete several years ago (Feldmesser et al., 1985).



5-(3-buten-1-ynyl)-2,2'-bithienyl



$\alpha$ -Terthienyl

Figure 1. Structural formulas of  $\alpha$ -terthienyl and 5-(3-buten-1-ynyl)-2,2'-bithienyl.

## ALKALOIDS

Bijloo (1965) reported that *D. dipsaci* was immobilized by 1000  $\mu\text{g}/\text{ml}$  of physostigmine (Fig. 2), an alkaloid originally isolated from the Calabar bean, *Physostigma venenosum* (Fabaceae). The compound was not nematicidal, however, in that nematodes rapidly regained motility and infectivity upon transfer to water. Interestingly, pretreatment of pea seedlings with much smaller quantities (e.g., 30  $\mu\text{g}/\text{ml}$ ) of physostigmine sulfate significantly protected the seedlings against subsequent infection.

Onda et al. (1965, 1970) identified three tetracyclic alkaloids in *Bocconia cordata* (Papaveraceae): chelerythrine, sanguinarine, and the previously unknown bocconine (Fig. 3). Although activity against phytoparasitic nematodes was not determined, the three compounds possessed substantial nematicidal activity at 50 to 100  $\mu\text{g}/\text{ml}$  against the free-living nematodes *Rhabditis* sp. and *Panagrolaimus* sp.

Another alkaloid, monocrotaline (from *Crotalaria spectabilis*, Fabaceae) (Fig. 2), inhibited movement of juveniles of the root-knot nematode, *Meloidogyne incognita*, at concentrations as low as 10  $\mu\text{g}/\text{ml}$  (Fassuliotis and Skucas, 1969). Exposure of infective juveniles to monocrotaline solutions did not prevent infection, however, nor was there any correlation between monocrotaline content of various species of *Crotalaria* or *Cynoglossum* and resistance to *M. incognita*.

Allen and Feldmesser (1970) investigated the effects of the solanaceous steroidal glycoalkaloid  $\alpha$ -tomatine (Fig. 4) on the free-living nematode, *Panagrellus redivivus*. As would be expected for many nitrogenous compounds, the ED<sub>50</sub> varied with pH and was as low as 50  $\mu\text{g}/\text{ml}$ . Similar results were obtained with the structurally related alkaloid,  $\alpha$ -chaconine (Fig. 4), with the most effective ED<sub>50</sub> (85  $\mu\text{g}/\text{ml}$ ) occurring at pH 6.7 (Allen and Feldmesser, 1971). In both cases, maximal activity was associated with a nonprotonated nitrogen atom.

Matsuda et al. (1989) isolated from methanolic extracts of *Sophora flavescens* (Fabaceae) two alkaloids (*N*-methylcytisine and anagyrine) (Fig. 5) that possessed nematicidal activity against the pinewood nematode, *Bursaphelenchus xylophilus*. The compounds were effective when applied in cotton balls in amounts of 3 to 6  $\mu\text{g}$  to cultures of *B. xylophilus* reproducing in culture dishes containing the fungus, *Botrytis cinerea*. Two other naturally occurring alkaloids, nicotine and cytisine (Fig. 5), had slightly greater activity.

## ACETYLENES

There have been several reports of nematotoxic polyacetylenes (Fig. 6) from higher plants, nearly all of them Asteraceae. Gommers (1973) (described in

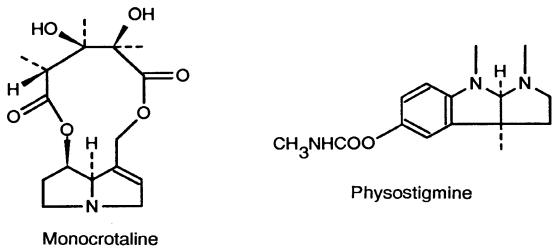


Figure 2. Structural formulas of monocrotaline and physostigmine.



Figure 3. Structural formulas of sanguinarine, chelerythrine and bocconine.

Gommers, 1981) first characterized a *Helenium* sp. nematicidal polyacetylene (tridec-1-en-3,5,7,9, 11-pentayne) active against *Pratylenchus penetrans*. Kogiso et al. (1976a,b) isolated 3-cis, 11-trans- and 3-trans,11-trans-trideca-1,3,11- triene-5,7,9-triyne from flowers of *Carthamus tinctorius*. Nematicidal activity of the latter compound occurred at 1.0  $\mu\text{g}/\text{ml}$  against the rice white tip nematode, *Aphelenchoides besseyi*. Kawazu et al. (1980) isolated two nematicidal acetylenes (tridec-1-en-3,5,7,9,11-pentayne and 9,10-epoxyheptadec-16-en-4,6-diyne-8-ol) (Fig. 7) from roots of *Cirsium japonicum*. These completely inhibited reproduction of *Bursaphelenchus xylophilus* when applied in cotton balls in amounts of 16 or 250  $\mu\text{g}$ , respectively, to cultures reproducing on *Botrytis cinerea* in petri dishes. Consequently, the researchers examined three additional naturally occurring acetylenes: 1-phenylhepta-1,3,5-triyne and 2-phenyl-5-(1'-propynyl)-thiophene (Fig. 7) from *Coreopsis lanceolata* and *cis*-dehydromatricaria ester from the goldenrod, *Solidago canadensis* (= *S. altissima*). These three compounds completely inhibited nematode reproduction at 110  $\mu\text{g}/\text{ball}$ .

Kimura et al. (1981b) purified from roots of yet another member of the Asteraceae, *Erigeron philadelphicus*, two polyacetylenes: methyl 2-trans,8-cis-deca-2,8-diene-4,6-diynoate (2-trans,8-cis-matricaria ester) and 2-cis,8-cis-deca-2,8-diene-4,6-diynoate (2-cis,8-cis-matricaria ester) (Fig. 8). Both induced at least 50% mortality in the root lesion nematode, *Pratylenchus coffeae*, at concentrations as low as 3.0  $\mu\text{g}/\text{ml}$ . Four additional naturally occurring polyacetylenes (*cis*- and *trans*-dehydromatricaria (Fig. 9) and *cis*- and *trans*-lachnophyllum esters) and four synthetic analogs (lachnophylic acid, dehydromatricarianol, dehydromatricarianyl acetate, and lachnophyllol) (Fig. 10) were also active. The lack of activity of three monoynes indicated that a conjugated diyne system may be required for nematotoxicity. Munakata (1983) identified heptadeca-1,9-diene-4,6-diyne-3,8-diol (Fig. 6) from roots

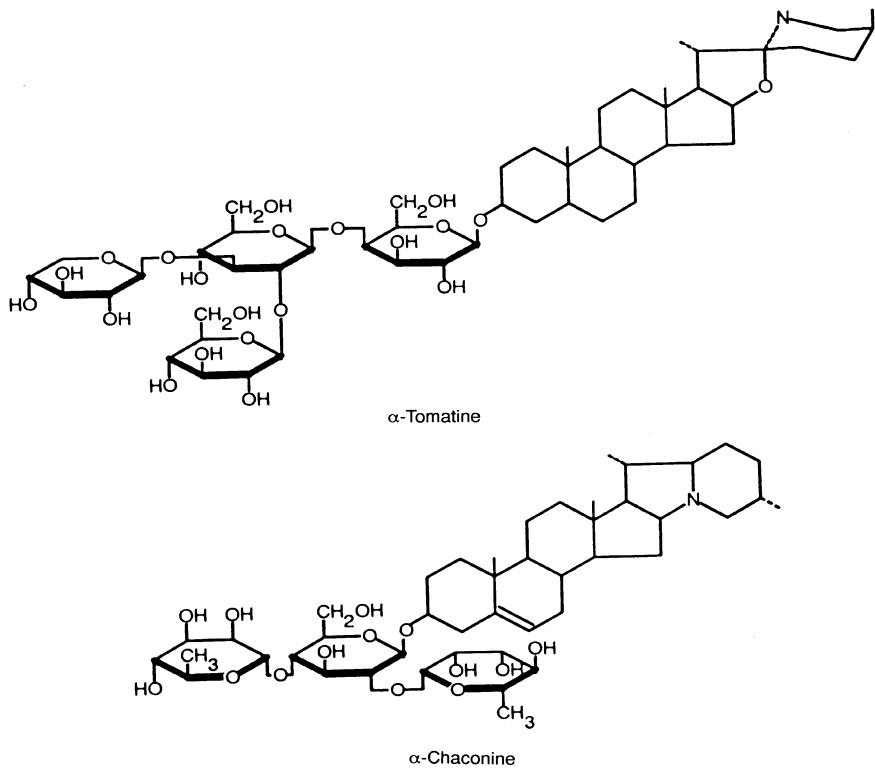


Figure 4. Structural formulas of  $\alpha$ -tomatine and  $\alpha$ -chaconine.

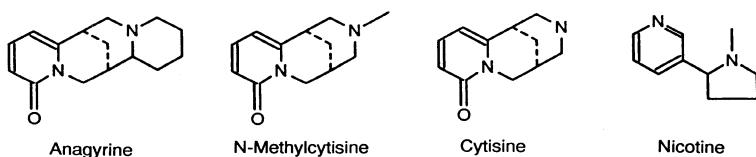


Figure 5. Structural formulas of anagyrine, *N*-methylcytisine, cytisine, and nicotine.

of *Angelica pubescens* (Apiaceae) and demonstrated its nematicidal activity against *A. besseyi*. As a result of the substantial investigation of polyacetylenes, Mori et al. (1982) investigated the activity against *P. coffeae* of 28 different synthetic acetylenes, none with more than one triple bond. Several were active at concentrations of less than 1.0  $\mu\text{g}/\text{ml}$ . Activity was greatest when an aryl, ester, or ketone functional group was conjugated to the triple bond.

Lastly, the nematotoxicity of several polyacetylenes against the free-living nematode, *Caenorhabditis elegans* (as well as insecticidal activity against mosquito and blackfly larvae), was promoted by exposure of treated animals to UV irradiation (Wat et al., 1981).

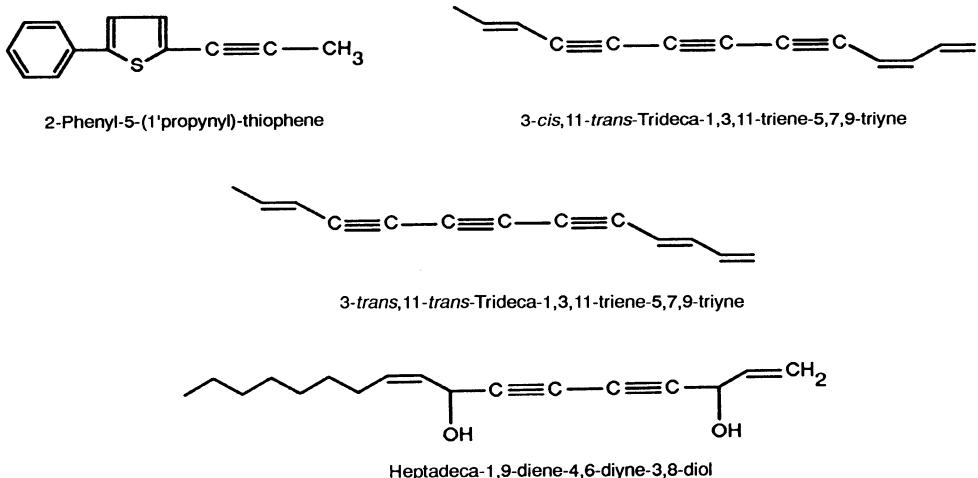


Figure 6. Structural formulas of 2-phenyl-5-(1'-propynyl)-thiophene, 3-*cis*-11-*trans*-trideca-1,3,11-triene-5,7,9-triyne, 3-*trans*-11-*trans*-trideca-1,3,11-triene-5,7,9-triyne, and heptadeca-1,9-diene-4,6-diyne-3,8-diol.

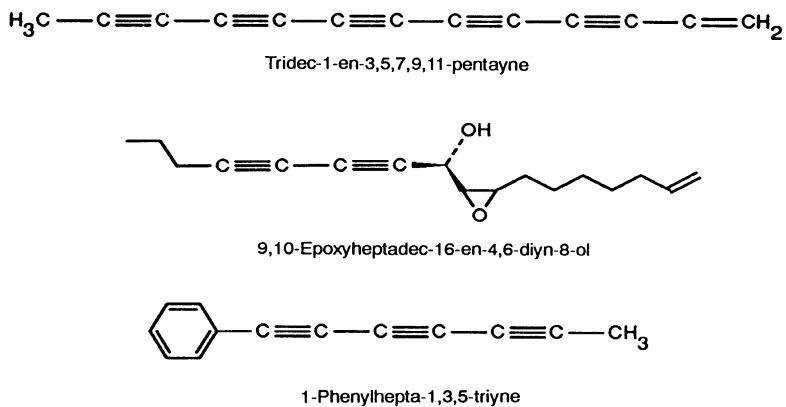


Figure 7. Structural formulas of tridec-1-en-3,5,7,9,11-pentayne, 9,10-epoxyheptadec-16-en-4,6-diyn-8-ol, and 1-phenylhepta-1,3,5-triyne.

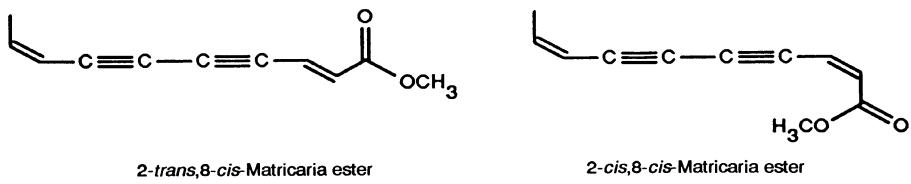
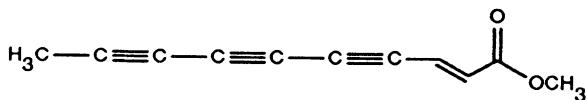
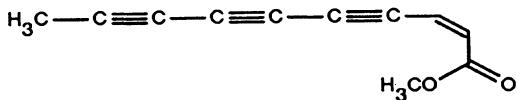


Figure 8. Structural formulas of 2-*trans*-8-*cis*-matricaria ester and 2-*cis*-8-*cis*-matricaria ester.

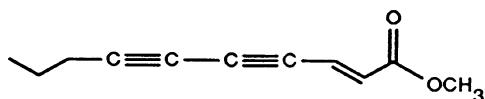


*trans*-Dehydromatricaria ester

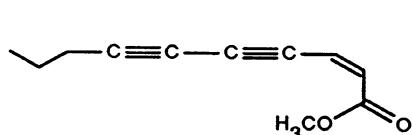


*cis*-Dehydromatricaria ester

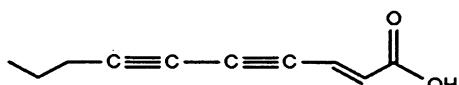
Figure 9. Structural formulas of *trans*-dehydromatricaria ester and *cis*-dehydromatricaria ester.



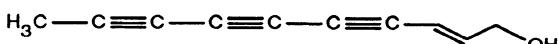
*trans*-Lachnophyllum ester



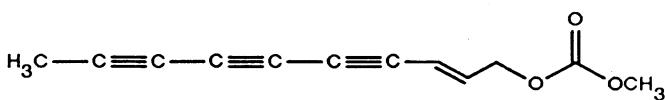
*cis*-Lachnophyllum ester



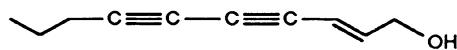
*trans*-Lachnophyllic acid



*trans*-Dehydromatricarianol



*trans*-Dehydromatricarianyl acetate



*trans*-Lachnophyllol

Figure 10. Structural formulas of *trans*-lachnophyllum ester, *cis*-lachnophyllum ester, *trans*-lachnophyllic acid, *trans*-dehydromatricarianol, *trans*-dehydromatricarianyl acetate, and *trans*-lachnophyllol.

## QUASSINOIDS

Prot and Kornprobst (1983) obtained an extract of seeds of *Hannoia undulata* (Simaroubaceae) containing three known polycyclic lactones: chaparrinone, klaineanone, and glaucarubolone (Fig. 11). Penetration of tomato roots by infective juveniles of the root-knot nematode, *Meloidogyne javanica*, was inhibited by 1 µg/ml of the mixed quassinooids and completely inhibited by 5 µg/ml. Because the latter concentration also disrupted movement in a sodium chloride gradient and because concentrations of 100 µg/ml were not nematotoxic, the primary effect of the compounds seemed to be on motility.

## FATTY ACIDS AND DERIVATIVES

Sayre et al. (1965) identified butyric acid (Fig. 12) in decomposing rye (*Secale cereale*) and timothy (*Phleum pratense*) and demonstrated its activity at 880 µg/ml in immersion bioassays against *M. incognita* and *P. penetrans*. The compound was inactive against the free-living nematodes, *Rhabditis*, *Cephalobus*, and *Plectus*. The pH-dependency of nematicidal activity indicated that the dissociated acid was not nematicidal. This report was not the first demonstration of the nematicidal activity of fatty acids, as Tarjan and Cheo (1956) had discovered such activity in most of the 41 fatty acids or their salts evaluated at 1000 µg/ml against *Panagrellus redivivus* and most of the 13 tested versus the tobacco cyst nematode, *Globodera tabacum*.

Munakata (1983) detected activity against *Aphelenchoïdes besseyi* in benzene extracts of *Iris japonica* roots. Subsequent fractionation with HPLC yielded three fatty acids with strong nematicidal activity: myristic, palmitic, and linoleic acids (Fig. 12). Consequently, activity of other simple aliphatic acids was investigated with 2-undecylenic acid having the strongest activity (70 to 80% mortality at 10 µg/ml). During an investigation of the components of the peanut, *Arachis hypogaea* (Fabaceae), Kimura et al. (1981a) found that di-n-butyl succinate (Fig. 12) was artificially produced but possessed significant nematicidal activity (90% mortality at 100 µg/ml) against the root lesion nematode, *Pratylenchus coffeae*. Seventeen other dialkyl succinates were synthesized and 11 of these were nematicidal. More recently, Saleh et al. (1987) isolated a nematicidal triglyceride (*sn*-glycerol-1-eicosa-9,12-dienoate-2-palmitoleate-3-linoleate) (Fig. 12) from seeds of *Argemone mexicana* (Papaveraceae). The ED<sub>50</sub> of the compound *in vitro* was 90 µg/ml; treatment of infective juveniles of *Meloidogyne incognita* with 100 µg/ml concentrations prevented subsequent infection of tomato plants.

## MISCELLANEOUS PHENOLICS

Elevated levels of phenolics have been correlated (or not correlated) with resistance or response of plants to nematode infection (Brueske and Dropkin, 1973; Kaplan and Davis, 1987; Rohde, 1972; Singh and Choudhury, 1973). Except for postinfectionally synthesized phytoalexins (described later), few of these compounds have been isolated from plant roots, identified, and then examined for nematicidal activity. Scheffer et al. (1962) reported that pyrocatechol (Fig. 13) isolated from *Eragrostis curvula* (Gramineae) was toxic to root-knot nematode juveniles.

Application of the widely distributed plant phenolic, quercetin (Fig. 13), as a soil drench at 400 µg/ml inhibited reproduction of *M. javanica* (Osman and Viglierchio, 1988).

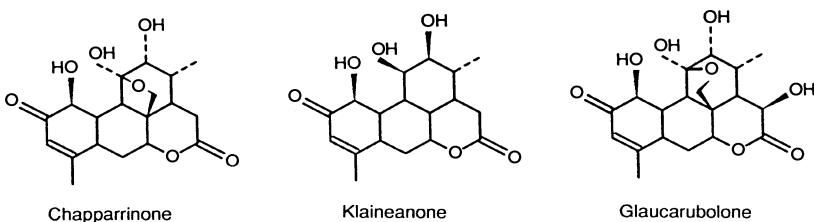


Figure 11. Structural formulas of chapparinone, klaineanone, and glaucarubolone.

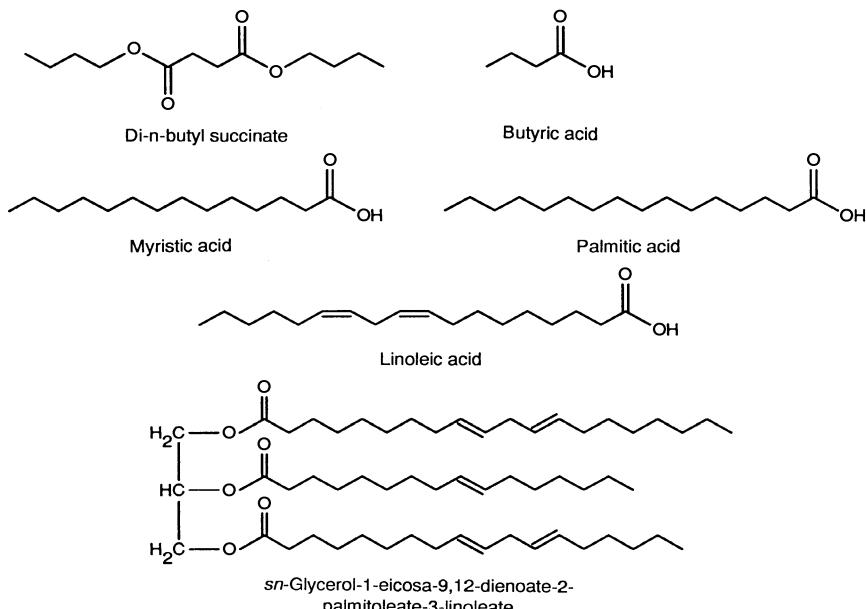


Figure 12. Structural formulas of di-n-butyl succinate, butyric acid, myristic acid, palmitic acid, linoleic acid, and *sn*-glycerol-1-eicosa-9,12-dienoate-2-palmitoleate-3-linoleate.

## TERPENOIDS

Osman and Viglierchio (1988) performed an interesting *in vivo* investigation of 20 nontraditional chemical agents applied as either root dips or soil drenches to tomato plants for control of *M. javanica*. Two terpenoids, citral and geraniol (Fig. 14), inhibited nematode reproduction by 52% and 86%, respectively, when applied as 100 µg/ml soil drenches. Limonene (Fig. 14), a component of citrus oil and an inhibitor of insect neurotransmission, reduced population development of the cyst nematode, *Heterodera schachtii*, to 3% of that of controls when applied as a drench at 100 µg/ml to sugar beet plants growing in sand (Viglierchio and Wu, 1989).

Malik et al. (1987) and Sangwan et al. (1990) investigated steam-distilled essential oils of three Labiateae (basil, *Ocimum basilicum*; tulsi, *O. sanctum*; and peppermint, *Mentha piperatum*), two Myrtaceae (bottle brush, *Callistemon lanceolatus*,

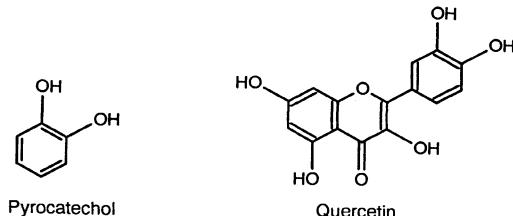


Figure 13. Structural formulas of pyrocatechol and quercetin.

and clove, *Eugenia caryophyllata*), and kachi grass (*Cymbopogon caesius*, Gramineae), as well as five synthetic terpenoid constituents identified in the essential oils: linalool, eugenol, menthol, cineole, and geraniol for activity against second-stage juveniles of four phytoparasitic nematodes. Most of the oils and all of the terpenoids exhibited some nematicidal activity with clove oil, geraniol, linalool, and eugenol (Fig. 14) having the broadest activity. The toxic effects were apparently irreversible, as treated juveniles did not recover when removed from terpenoid solutions. Higher levels were phytotoxic.

## POSTINFECTIONAL COMPOUNDS

As indicated in the introduction to this article, the literature on the possible involvement in plant resistance of compounds synthesized by plants as a result of nematode infection is too complex and controversial to be adequately addressed in this review. However, brief reference to some of these compounds and their biological activity is warranted.

Rich et al. (1977) identified coumestrol (Fig. 15) and, tentatively, psoralidin (Fig. 16) as compounds produced by lima beans in response to infection by the lesion nematode, *Pratylenchus scribneri*. Coumestrol inhibited the motility of *P. scribneri* at 5 to 25 µg/ml; interestingly, it did not inhibit motility of *M. javanica*. Four other structurally related compounds (daidzein, 2',4',7-trihydroxyisoflavone, 4',5,7-trihydroxyisoflavone, and rotenone) and the phenolic chlorogenic acid did not inhibit motility of *P. scribneri* at the concentrations tested. Similarly, Veech and McClure (1977) demonstrated that postinfectational production of terpenoid aldehydes in a resistant variety of cotton (*Gossypium hirsutum*, Malvaceae) was associated with resistance to *M. incognita*. The compounds included gossypol, hemigossypol (Fig. 16), 6-methoxygossypol, and 6-methoxyhemigossypol (Fig. 17). Terpenoid aldehyde concentration decreased in roots of a susceptible variety. A crude terpenoid aldehyde extract from cotton inhibited movement of *M. incognita* juveniles at 50 µg/ml, as did gossypol at 125 µg/ml (Veech, 1979).

Accumulation of the phytoalexin, glyceollin (Fig. 15), in soybean roots was associated with an incompatible response to root-knot nematodes (Kaplan et al., 1980a,b). At 15 µg/ml, the compound strongly inhibited movement of *M. incognita* *in vitro*; nematodes recovered upon removal from glyceollin solutions. Zinovieva and Chalova (1987) identified rishitin (Fig. 15) in potato tuber discs infected with either the potato rot nematode, *Ditylenchus destructor*, or the stem nematode, *D. dipsaci*. The compound stopped movement of 50% of the juveniles of *D. dipsaci* in a motility assay at 100 µg/ml. Interestingly, there was a positive correlation between the resistance of various potato cultivars to *D. destructor* and the amount of rishitin produced in response to infection.

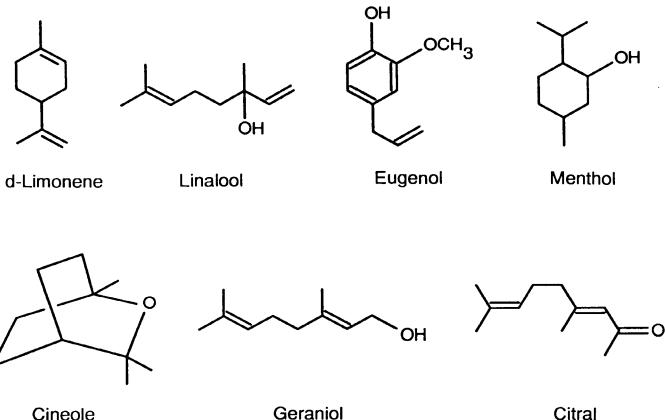


Figure 14. Structural formulas of *d*-limonene, linalool, eugenol, menthol, cineole, geraniol, and citral.

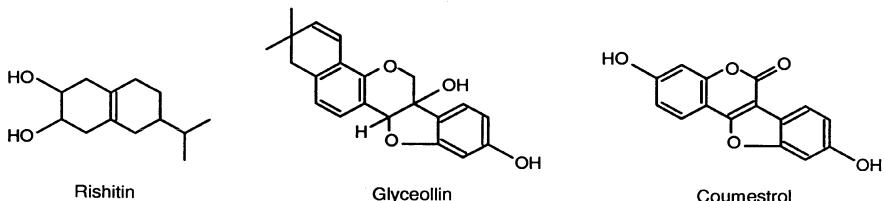


Figure 15. Structural formulas of rishitin, glyceollin, and coumestrol.

## MISCELLANEOUS

Among the earliest investigations of chemically mediated interactions between plants and nematodes were investigations on inhibition of hatching of eggs of *Globodera rostochiensis* by mustard seedlings. Allyl isothiocyanate (Fig. 18), a component of black mustard seed oil (*Brassica nigra*, Cruciferae), inhibited egg hatch *in vitro* at 50 µg/ml and significantly improved yield of potatoes in experiments when incorporated into field soils (Ellenby, 1951). Various isothiocyanates or related compounds have been evaluated as preplant soil fumigants, including the commercially useful sodium methyldithiocarbamate which degrades in soil to yield methyl isothiocyanate (Johnson and Feldmesser, 1987).

Concurrently with the efforts of Uhlenbroek and Bijloo (1958) to identify the first nematicidal phytochemical, Rohde and Jenkins (1958) were determining the nature of resistance of asparagus (*Asparagus officinalis*, Liliaceae) to various phytoparasitic nematodes, particularly the stubby-root nematode, *Paratrichodorus christiei*. They clearly demonstrated that resistance was due to a preformed chemical substance in roots and root exudates. Characterization of the compound by hydrolysis, paper chromatography, and a number of analytical tests indicated that it was a glycoside with a low molecular weight aglycone. At concentrations of 100 µg/ml, the compound stopped movement of four phytoparasitic nematodes, and root drenches or foliar sprays of the compound reduced populations of *M. incognita* on tomato. Unfortunately, the compound was not characterized completely. From 30 kg of

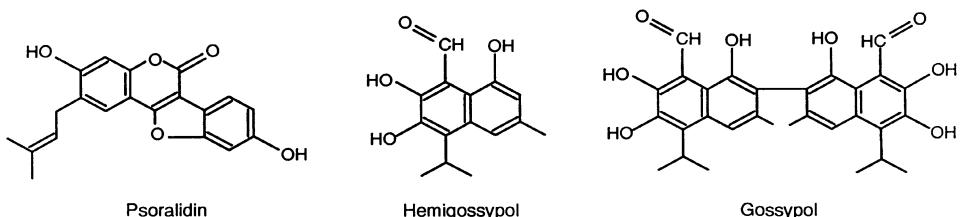


Figure 16. Structural formulas of psoralidin, hemigossypol, and gossypol.

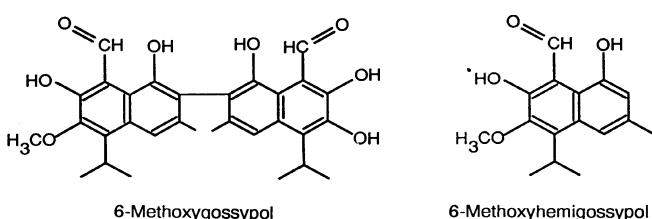


Figure 17. Structural formulas of 6-methoxygossypol and 6-methoxyhemigossypol.

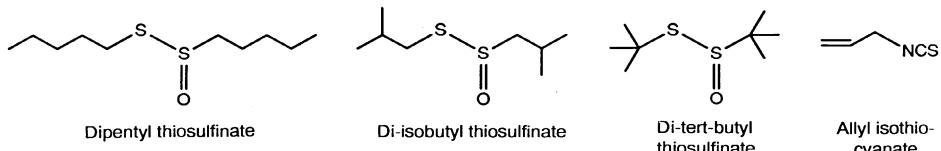


Figure 18. Structural formulas of dipentyl thiosulfinate, di-isobutyl thiosulfinate, di-*tert*-butyl thiosulfinate, and allyl isothiocyanate.

asparagus roots, Takasugi et al. (1975) identified asparagusic acid (Fig. 19) as a nematicide, as it inhibited hatch of the soybean cyst nematode, *Heterodera glycines*, and *Globodera rostochiensis* at 50 µg/ml, even in the presence of hatching stimulants. The same concentration induced 80 to 99% mortality in *G. rostochiensis*, the root-knot nematode *Meloidogyne hapla*, *Pratylenchus penetrans*, and the pin nematode *Paratylenchus curvitatus*. The compound occurred in roots at a level of at least 35 µg/ml and migrated on paper chromatography identically to the glycoside of Rohde and Jenkins (1958), but Takasugi et al. (1975) did not speculate upon this fact.

Meher et al. (1988) also investigated the nematicidal activity of glycosides, some from plants related to garden asparagus. In this case, eight saponins were evaluated in motility assays with *M. incognita*. Four of the saponins (asparanin I and asparanin B (Fig. 20) from seed of *Asparagus adscendens*, Liliaceae; albichinin II from *Albizia chinensis*, Fabaceae; and sonunin III from *Acacia concinna*, Fabaceae) were active at concentrations as low as 200 µg/ml.

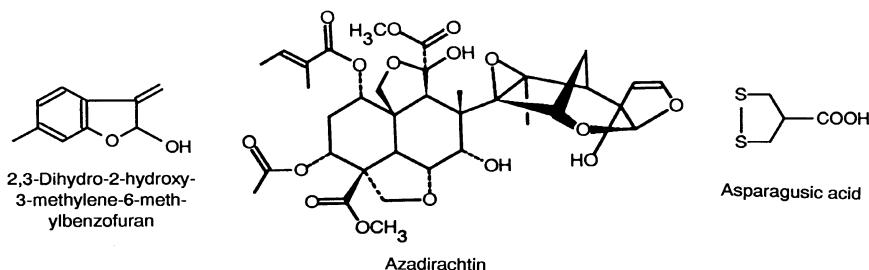


Figure 19. Structural formulas of 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran, azadirachtin, and asparagusic acid.

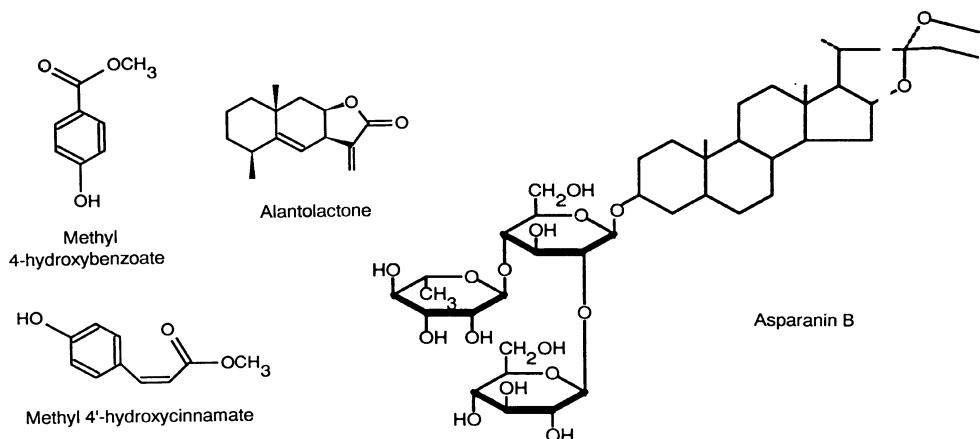


Figure 20. Structural formulas of methyl 4-hydroxybenzoate, alantolactone, methyl 4'-hydroxycinnamate, and asparanin B.

Gommers (1971) identified 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran (Fig. 19) from *Helenium* sp. (Asteraceae) and described it as having strong nematicidal activity in an undescribed bioassay.

The insecticidal properties of the neem tree, *Azadirachta indica* (Meliaceae), have been the focus of much research. One compound from neem, azadirachtin (Fig. 19), is an insect antifeedant and growth and molt inhibitor (for references, refer to Smith and Mitchell, 1988). Exudates of neem roots inhibited egg hatch of six species of phytoparasitic nematodes (Alam et al., 1975), and lipid-containing extracts of neem seeds inhibited hatch and were nematicidal to juveniles of *M. incognita* (Devakumar et al., 1985). Aqueous leaf extracts were directly toxic to the lesion nematode, *Pratylenchus brachyurus*, *in vitro* and also provided control and yield increases in microplot experiments with *Zea mays* (Egunjobi and Afolami, 1976). Aqueous extracts of neem oilcakes inhibited motility of several phytoparasitic nematodes and hatch of *M. incognita* (Khan et al., 1974). A neem seed extract applied to tomato plants as a root drench at 125 µg/ml inhibited reproduction of *M. javanica* (Osman and Viglierchio, 1988). Because azadirachtin at 10 µg/ml inhibits microfilarial release in the animal-parasitic nematode, *Brugia pahangi* (Barker et al., 1989), it is also probably at least one of the components in neem active against phytoparasitic nematodes, possibly by a similar mode of action to that in insects.

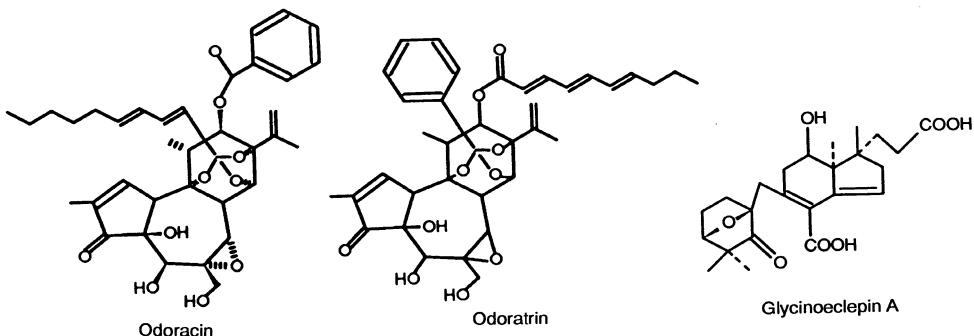


Figure 21. Structural formulas of odoracin, odoratrin, and glycinoeclepin A.

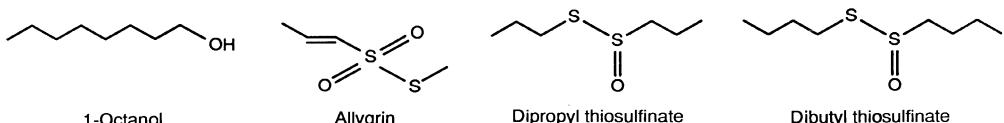


Figure 22. Structural formulas of 1-octanol, allygrin, dipropyl thiosulfinate, and dibutyl thiosulfinate.

Mahajan et al. (1986) discovered nematicidal activity at 1100 µg/ml *in vitro* against *M. incognita* in most of 18 synthetic sesquiterpenoid lactones, including alantolactone (Fig. 20), a therapeutically utilized, naturally occurring compound from *Inula helenium*, Asteraceae.

Kogiso et al. (1976c) discovered nematicidal activity of benzene extracts of roots of *Daphne odora* (Thymelaeaceae) in immersion tests against *Aphelenchoides besseyi*. After a rather complex analysis of 2 kg of dried roots, 102 mg of the active constituent was identified and named odoracin (Fig. 21); the purified component caused 100% mortality of *A. besseyi* at 5.0 µg/ml. A related compound, odoratrin (Fig. 21), was isolated subsequently and possessed greater activity (Munakata, 1983).

Tada et al. (1988) isolated four compounds from methanol extracts of entire plants of *Allium grayi* (Liliaceae) that were active *in vitro* against *M. incognita*: 1-octanol (Fig. 22), methyl 4-hydroxybenzoate, methyl 4'-hydroxycinnamate (Fig. 20), and allygrin (Fig. 22). An ether extract of *A. fistulosum* var. *caespitosum* contained five thiosulfonates (Figs. 18 and 22), all of which had greater activity than the four compounds from *A. grayi*. Several related thiosulfonates and thiosulfonates were synthesized and possessed similar nematicidal (and antibacterial) activity.

Although hatching stimulants are not nematicidal, they could theoretically be used in field situations to induce hatch in the absence of host plants. Approximately 1.2 mg of a hatching stimulant for *Heterodera glycines* was isolated from 1058 kg (from 10 ha) of dried roots of kidney bean (*Phaseolus vulgaris*, Fabaceae) and was named glycinoeclepin A (Fig. 21) (Fukuzawa et al., 1985a; Masamune et al., 1982). Two related nortriterpenoids were also isolated but did not stimulate hatch (Fukuzawa et al., 1985b). Miwa et al. (1987) designed two simpler analogs that stimulated hatch, although higher concentrations were required than for glycinoeclepin A.

Recent research of the involvement of plant lectins in plant-nematode recognition events has been intense (Davis et al., 1989; Kaplan and Davis, 1987). Application of concanavalin A, a lectin from the jackbean, *Canavalia ensiformis*

(Fabaceae), resulted in substantial control of *M. incognita* on tomato in growth chamber, greenhouse, and microplot experiments (Marban-Mendoza et al., 1987). One suggestion for the mode of action of concanavalin A was its binding to nematode chemoreceptors. Some stimulation of hypersensitivity of soybean to infection by *M. incognita* resulted from treatment of infective juveniles with concanavalin A, wheat germ agglutinin, or soybean agglutinin (Davis et al., 1989).

## SUMMARY

The most conspicuous aspect of these investigations of nematicidal phytochemicals is that attention has centered upon few botanical families, primarily the Asteraceae and Fabaceae. Obviously, a broader range of plant taxa needs to be included in future studies. The few investigations of phytochemicals with biological activity against nematodes have yielded a wide variety of structurally diverse compounds. Although it may be tempting to speculate that such compounds are functionally active in higher plants as antinematodal substances, most previous reviewers have pointed out that inadequate attention has been given in most cases to the concentrations of many of these compounds within plants or their cellular or subcellular locations. (Indeed, in a few cases, the concentrations evaluated *in vitro* have been so large that physiological activity would be contraindicated.) The difficulty in comparing *in vitro* results with endogenous concentrations prevents a precise functional analysis of most such compounds. Of course, the agricultural chemist is concerned primarily not with physiological function within plants but rather with development of environmentally safe, inexpensive, agronomically useful compounds. From this perspective, development of nematicidal phytochemicals is in its infancy, as most of this research has been of a basic, descriptive nature. In only a few of the investigations discussed in this review have chemists synthesized potentially nematicidal compounds based upon naturally occurring models. Except for the compounds from *Tagetes*, the nematicidal mode of action of these compounds is unknown except for the few cases in which high mammalian toxicity is known or suspected. The multibillion dollar losses inflicted by phytoparasitic nematodes and the lack of excellent chemical management tools warrants that research of natural products with activity against nematodes be intensified.

## ACKNOWLEDGMENT

The author thanks Patricia Oakley for preparation of the figures.

## REFERENCES

- Alam, M. M., Masood, A., and Husain, S. I., 1975, Effect of margosa and marigold root-exudates on mortality and larval hatch of certain nematodes, Indian Journal of Experimental Biology, 13:412-414.
- Allen, E. H., and Feldmesser J., 1970, Nematicidal effect of alpha-tomatine on *Panagrellus redivivus*, Phytopathology, 60:1013 (Abstract).
- Allen, E. H., and Feldmesser, J., 1971, Nematicidal activity of  $\alpha$ -chaconine: Effect of hydrogen-ion concentration, Journal of Nematology, 3:58-61.
- Barker, G. C., Chitwood, D. J., and Rees, H. H., 1990, Ecdysteroids in helminths and annelids, Invertebrate Reproduction and Development, 18:1-11.

- Barker, G. C., Mercer, J. G., Svoboda, J. A., Thompson, M. J., Rees, H. H., and Howells, R. E., 1989, Effects of potential inhibitors on *Brugia pahangi* *in vitro*: Macrofilaricidal action and inhibition of microfilarial production, Parasitology, 99:409-416.
- Bijloo, J. D., 1965, The "Pisum" test: A simple method for the screening of substances on their therapeutic nematicidal activity, Nematologica, 11:643-644.
- Brueske, C. H., and Dropkin, V. H., 1973, Free phenols and root necrosis in Nematex tomato infected with the root knot nematode, Phytopathology, 63:329-334.
- Bunt, J. A., 1987, Mode of action of nematicides, p. 461-468, *in: "Vistas on Nematology,"* J. A. Veech and D. W. Dickson, eds., Society of Nematologists, Hyattsville, Maryland.
- Chang, L., and Rohde, R. A., 1969, The repellent effect of necrotic tissues on the nematode *Pratylenchus penetrans*, Phytopathology, 59:398 (Abstract).
- Chitwood, D. J., 1987, Inhibition of steroid or hormone metabolism or action in nematodes, p. 122-130, *in: "Vistas on Nematology,"* J. A. Veech and D. W. Dickson, eds., Society of Nematologists, Hyattsville, Maryland.
- Davis, E. L., Kaplan, D. T., Dickson, D. W., and Mitchell, D. J., 1989, Root tissue response of two related soybean cultivars to infection by lectin-treated *Meloidogyne* spp., Journal of Nematology, 21:219-228.
- Devakumar, C., Goswami, B. K., and Mukerjee, S. K., 1985, Nematicidal principles from neem (*Azadirachta indica* A. Juss). Part I. Screening of neem kernel fractions against *Meloidogyne incognita*, Indian Journal of Nematology, 15:121-124.
- Egunjobi, O. A., and Afolami, S. O., 1976, Effects of neem (*Azadirachta indica*) leaf extracts on populations of *Pratylenchus brachyurus* and on the growth and yield of maize, Nematologica, 11:125-132.
- Ellenby, C., 1951, Mustard oils and control of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber: Further field and laboratory experiments, Annals of Applied Biology, 38:859-875.
- Fassuliotis, G., and Skucas, G. P., 1969, The effect of a pyrrolizidine alkaloid ester and plants containing pyrrolizidine on *Meloidogyne incognita acrita*, Journal of Nematology, 1:287-288 (Abstract).
- Feldmesser, J., Kochansky, J., Jaffe, H., and Chitwood, D., 1985, Future chemicals for control of nematodes, p. 327-344, *in: "Agricultural Chemicals of the Future,"* J. L. Hilton, ed., Rowman and Allanheld, Totowa, New Jersey.
- Fukuzawa, A., Furusaki, A., Ikura, M., and Masamune, T., 1985a, Glycinoeclepin A, a natural hatching stimulus for the soybean cyst nematode, Journal of the Chemical Society, Chemical Communications, 1985:222-224, 748.
- Fukuzawa, A., Matsue, H., Ikura, M., and Masamune, T., 1985b, Glycinoeclepins B and C, nortriterpenes related to glycinoeclepin A, Tetrahedron Letters, 26:5539-5542.
- Giebel, J., 1982, Mechanism of resistance to plant nematodes, Annual Review of Phytopathology, 20:257-279.
- Gommers, F. J., 1971, A nematicidal principle from the roots of a *Helenium* hybrid, Phytochemistry, 10:1945-1946.
- Gommers, F. J., 1973, Nematicidal principles in Compositae, Dissertation, Agricultural University, Wageningen, The Netherlands, 73 p.
- Gommers, F. J., 1981, Biochemical interactions between nematodes and plants and their relevance to control, Helminthological Abstracts, 50B:9-24.
- Gommers, F. J., and Bakker, J., 1988, Physiological diseases induced by plant responses or products, p. 3-22, *in: "Diseases of Nematodes,"* Volume I, G. O. Poinar and H.-B. Jansson, eds., CRC Press, Boca Raton, Florida.

- Gommers, F. J., and voor in 't Holt, D. J. M., 1976, Chemotaxonomy of Compositae related to their host suitability for *Pratylenchus penetrans*, Netherlands Journal of Plant Pathology, 82:1-8.
- Hague, N. G. M., and Gowen, S. R., 1987, Chemical control of nematodes, p. 131-178, in: "Principles and Practice of Nematode Control in Crops," R. H. Brown and B. R. Kerry, eds., Academic Press, Sydney.
- Huang, J.-S., 1985, Mechanisms of resistance to root-knot nematodes, p. 165-174, in: "An Advanced Treatise on *Meloidogyne*, Volume I: Biology and Control," J. N. Sasser and C. C. Carter, eds., North Carolina State University Graphics, Raleigh, North Carolina.
- Husain, S. I., and Masood, A., 1975a, Nematicidal action of plant extracts on plant parasitic nematodes, Geobios, 2:74-76.
- Husain, S. I., and Masood, A., 1975b, Effect of some plant extracts on larval hatching of *Meloidogyne incognita* (Kofoid & White) Chitwood, Acta Botanica Indica, 3:142-146.
- Johnson, A. W., and Feldmesser, J., 1987, Nematicides—a historical review, p.448-454, in: "Vistas on Nematology," J. A. Veech and D. W. Dickson, eds., Society of Nematologists, Hyattsville, Maryland.
- Kaplan, D. T., and Davis, E. L., 1987, Mechanisms of plant incompatibility with nematodes, p. 267-276 in: "Vistas on Nematology," J. A. Veech and D. W. Dickson, eds., Society of Nematologists, Hyattsville, Maryland.
- Kaplan, D. T., and Keen, N. T., 1980, Mechanisms conferring plant incompatibility to nematodes, Revue de Nématologie, 3:123-134.
- Kaplan, D. T., Keen, N. T., and Thomason, I. J., 1980a, Association of glyceollin with the incompatible response of soybean roots to *Meloidogyne incognita*, Physiological Plant Pathology, 16:309-318.
- Kaplan, D. T., Keen, N. T., and Thomason, I. J., 1980b, Studies on the mode of action of glyceollin in soybean incompatibility to the root-knot nematode, *Meloidogyne incognita*, Physiological Plant Pathology, 16:319-325.
- Kawazu, K., Nishii, Y., and Nakajima, S., 1980, Two nematicidal substances from roots of *Cirsium japonicum*, Agricultural and Biological Chemistry, 44:903-906.
- Khan, M. W., Alam, M. A., Khan, A. M., and Saxena, S. K., 1974, Effect of water soluble fractions of oil-cakes and bitter principles of neem on some fungi and nematodes, Acta Botanica Indica, 2:120-128.
- Kimura, Y., Mori, M., Hyeon, S.-B., Suzuki, A., and Mitsui, Y., 1981a, A rapid and simple method for assay of nematicidal activity and its application to measuring the activities of dicarboxylic acids, Agricultural and Biological Chemistry, 45:249-251.
- Kimura, Y., Mori, M., Suzuki, A., and Kobayashi, A., 1981b, Isolation and identification of two nematicidal substances from roots of *Erigeron philadelphicus* L. and nematicidal activities of their related compounds, Agricultural and Biological Chemistry, 45:2915-2917.
- Kogiso, S., Wada, K., and Munakata, K., 1976a, Isolation of nematicidal polyacetylenes from *Carthamus tinctorius* L., Agricultural and Biological Chemistry, 40:2085-2089.
- Kogiso, S., Wada, K., and Munakata, K., 1976b, Nematicidal polyacetylenes, 3Z,11E- and 3E,11E-trideca-1,3,11-triene-5,7,9-triyne from *Carthamus tinctorius* L., Tetrahedron Letters, 2:109-110.
- Kogiso, S., Wada, K., and Munakata, K., 1976c, Odoracin, a nematicidal constituent from *Daphne odora*, Agricultural and Biological Chemistry, 40:2119-2120.
- Lal, A., Yadav, B. S., and Nandwana, R. P., 1977, Effect of chopped leaves of various plants and sewage on the plant growth and reniform nematode, *Rotylenchulus reniformis*, Indian Journal of Mycology and Plant Pathology, 7:68-69.

- Mahajan, R., Singh, P., Bajaj, K. L., and Kalsi, P. S., 1986. Nematicidal activity of some sesquiterpenoids against rootknot nematode (*Meloidogyne incognita*), *Nematologica*, 32:119-123.
- Malik, M. S., Sangwan, N. K., Dhindsa, K. S., Verma, K. K., and Bhatti, D. S., 1987, Nematicidal efficacy of some monoterpenes and related derivatives, *Pesticides*, 21(5):30-32.
- Marban-Mendoza, N., Jeyaprakash, A., Jansson, H.-B., Damon, R. A., and Zuckerman, B. M., 1987, Control of root-knot nematodes on tomato by lectins, *Journal of Nematology*, 19:331-335.
- Masamune, T., Anetai, M., Takasugi, M., and Katsui, N., 1982, Isolation of a natural hatching stimulus, glycinoeclepin A, for the soybean cyst nematode, *Nature*, 297:495-496.
- Matsuda, K., Kimura, M., Komai, K., and Hamada, M., 1989, Nematicidal activities of (-)-N-methylcytisine and (-)-anagyrine from *Sophora flavescens* against pine wood nematodes, *Agricultural and Biological Chemistry*, 53:2287-2288.
- Meher, H. C., Walia, S., and Sethi, C. L., 1988, Effect of steroidal and triterpenic saponins on the mobility of juveniles of *Meloidogyne incognita*, *Indian Journal of Nematology*, 18:244-247.
- Miller, P. M., Turner, N. C., and Tomlinson, H., 1973, Toxicity of stem and leaf extracts to *Tylenchorhynchus dubius*, *Journal of Nematology*, 5:173-177.
- Miwa, A., Nii, Y., Okawara, H., and Sakakibara, M., 1987, Synthetic study on hatching stimuli for the soybean cyst nematode, *Agricultural and Biological Chemistry*, 51:3459-3461.
- Mori, M., Hyeon, S.-B., Kimura, Y., and Suzuki, A., 1982, The nematicidal activity of acetylene compounds, *Agricultural and Biological Chemistry*, 46:309-311.
- Morton, H. V., 1987, Industry perspectives in nematology, p. 47-51, in: "Vistas on Nematology," J. A. Veech and D. W. Dickson, eds., Society of Nematologists, Hyattsville, Maryland.
- Munakata, K., 1978, Nematicidal substances from plants, p. 295-302, in: "Advances in Pesticide Chemistry," H. Geissbühler, G. T. Brooks, and P. C. Kearney, eds., *Symposia Papers of the 4th International Congress of Pesticide Chemistry*, Zurich.
- Munakata, K., 1983, Nematocidal natural products, p. 299-310, in: "Natural Products for Innovative Pest Management," D. L. Whitehead and W. S. Bowers, eds., Pergamon Press, Oxford.
- Nandal, S. N., and Bhatti, D. S., 1983, Preliminary screening of some weedy shrubs for their nematicidal activity against *Meloidogyne javanica*, *Indian Journal of Nematology*, 13:123-127.
- Onda, M., Takiguchi, K., Hirakura, M., Fukushima, H., Akagawa, M., and Naoi, F., 1965, Studies on the constituents of *Bocconia cordata*. Part I. On nematocidal alkaloids, *Nippon Nageikagaku Kaishi*, 39:168-170.
- Onda, M., Abe, K., Yonezawa, K., Esumi, N., and Suzuki, T., 1970, Studies on the constituents of *Bocconia cordata*. II. Bocconine, *Chemical and Pharmaceutical Bulletin*, 18:435-439.
- O'Neill, M. J., 1986, Phytoalexins: Antiparasitics of higher plants, *Parasitology Today*, 2:358-359.
- Osman, A. A., and Viglierchio, D. R., 1988, Efficacy of biologically active agents as nontraditional nematicides for *Meloidogyne javanica*, *Revue de Nématologie*, 11:93-98.
- Pillai, S. N., and Desai, M. V., 1975, Antihelminthic property of 'Marotti' cake (*Hydnocarpus laurifolia*), *Pesticides*, 9(4):37-39.
- Prot, J.-C., and Kornprobst, J.-M., 1983, Étude préliminaire de l'action des

- quassinoïdes extraits de *Hannoa undulata* sur les juvéniles du nématode *Meloidogyne javanica*, Comptes Rendus de l'Académie des Sciences Série III, 296:555-557.
- Ram Nath, M. N., Khan, R. S., Kamalwanshi, R. S., and Dwivedi, R. P., 1982, Effect of *Argemone mexicana* on *Meloidogyne javanica* in okra (*Abelmoschus esculentus*), Indian Journal of Nematology, 12:205-208.
- Rich, J. R., Keen, N. T., and Thomason, I. J., 1977, Association of coumestans with the hypersensitivity of lima bean roots to *Pratylenchus scribneri*, Physiological Plant Pathology, 10:105-116.
- Rohde, R. A., 1972, Expression of resistance in plants to nematodes, Annual Review of Phytopathology, 10:233-252.
- Rohde, R. A., and Jenkins, W. R., 1958, Basis for resistance of *Asparagus officinalis* var. *utilis* L. to the stubby-root nematode *Trichodorus christiei* Allen 1957, University of Maryland Agricultural Experiment Station Bulletin A-97, 19 p.
- Saleh, M. A., Abdel Rahman, F. H., Ibrahim, N. A., and Taha, N. M., 1987, Isolation and structure determination of new nematicidal triglyceride from *Argemone mexicana*, Journal of Chemical Ecology, 13:1361-1370.
- Sangwan, N. K., Verma, B. S., Verma, K. K., and Dhindsa, K. S., 1990, Nematicidal activity of some essential plant oils, Pesticide Science, 28:331-335.
- Sasser, J. N., and Freckman, D. W., 1987, A world perspective on nematology: The role of the Society, p. 7-14, in: "Vistas on Nematology," J. A. Veech and D. W. Dickson, eds., Society of Nematologists, Hyattsville, Maryland.
- Sayre, R. M., Patrick, Z. A., and Thorpe, H. J., 1965, Identification of a selective nematicidal component in extracts of plant residues decomposing in soil, Nematologica, 11:263-268.
- Scheffer, F., Kickuth, R., and Visser, J. H., 1962, Die Wurzelausscheidungen von *Eragrostis curvula* (Schrad.) Nees und ihr Einfluss auf Wurzelknoten-Nematoden, Zeitschrift für Pflanzenährung und Bodenkunde, 98:114-120.
- Singh, B., and Choudhury, B., 1973, The chemical characteristics of tomato cultivars resistant to root-knot nematodes (*Meloidogyne* spp.), Nematologica, 19:443-448.
- Smith, S. L., and Mitchell, M. J., 1988, Effects of azadirachtin on insect cytochrome P-450 dependent ecdysone 20-monooxygenase activity, Biochemical and Biophysical Research Communications, 154:559-563.
- Sukul, N. C., Das, P. K., and De, G. C., 1974, Nematicidal action of some edible crops, Nematologica, 20:187-191.
- Tada, M., Hiroe, Y., Kiyohara, S., and Suzuki, S., 1988, Nematicidal and antimicrobial constituents from *Allium grayi* Regel and *Allium fistulosum* L. var. *caespitosum*, Agricultural and Biological Chemistry, 52:2383-2385.
- Takasugi, M., Yachida, Y., Anetai, M., Masamune, T., and Kegasawa, K., 1975, Identification of asparagusic acid as a nematicide occurring naturally in the roots of asparagus, Chemistry Letters, 1975:43-44.
- Tarjan, A. C., and Cheo, P. C., 1956, Nematicidal value of some fatty acids, University of Rhode Island Agricultural Experiment Station Bulletin 332, 41 p.
- Taylor, C. E., and Murant, A. F., 1966, Nematicidal activity of aqueous extracts from raspberry canes and roots, Nematologica, 12:488-494.
- Thomason, I. J., 1987, Challenges facing nematology: Environmental risks with nematicides and the need for new approaches, p. 469-476, in: "Vistas on Nematology," J. A. Veech and D. W. Dickson, eds., Society of Nematologists, Hyattsville, Maryland.
- Ueno, Y., and Iyatomi, K., 1978, Plants screened for nematicidal root exudates, (Preliminary report), Science Reports of the Faculty of Agriculture, Meijo University, 14:7-18.

- Uhlenbroek, J. H., and Bijloo, J. D., 1958, Investigations on nematicides. I. Isolation and structure of a nematicidal principle occurring in *Tagetes* roots, Recueil des Travaux Chimiques des Pays-Bas, 77:1004-1009.
- Uhlenbroek, J. H., and Bijloo, J. D., 1959, Investigations on nematicides. II. Structure of a second nematicidal principle isolated from *Tagetes* roots, Recueil des Travaux Chimiques Pays-Bas, 78:382-390.
- Veech, J. A., 1979, Histochemical localization and nematotoxicity of terpenoid aldehydes in cotton, Journal of Nematology, 11:240-246.
- Veech, J. A., 1981, Plant resistance to nematodes, p. 377-403, in: "Plant Parasitic Nematodes," Volume III, B. M. Zuckerman and R. A. Rohde, eds., Academic Press, New York, New York.
- Veech, J. A., 1982, Phytoalexins and their role in the resistance of plants to nematodes, Journal of Nematology, 14:2-9.
- Veech, J. A., and McClure, M. A., 1977, Terpenoid aldehydes in cotton roots susceptible and resistant to the root-knot nematode, *Meloidogyne incognita*, Journal of Nematology, 9:225-229.
- Viglierchio, D. R., and Wu, F. F., 1989, Selected biological inhibitors for *Heterodera schachtii* control, Nematropica, 19:75-79.
- Wat, C.-K., Prasad, S. K., Graham, E. A., Partington, S., Arnason, T., Towers, G. H. N., and Lamb, J., 1981, Photosensitization of invertebrates by natural polyacetylenes, Biochemical Systematics and Ecology, 9:59-62.
- Wright, D. J., 1981, Nematicides: Mode of action and new approaches to chemical control, p. 421-449, in: "Plant Parasitic Nematodes," Volume III, B. M. Zuckerman and R. A. Rohde, eds., Academic Press, New York, New York.
- Zinovieva, S. V., and Chalova, L. I., 1987, Phytoalexins of potato and their role in the resistance to stem nematodes, Helminthologia, 24:303-309.

## HERBICIDAL COMPOUNDS FROM HIGHER PLANTS

Horace G. Cutler

USDA, ARS  
Richard B. Russell Center  
P. O. Box 5677  
Athens, GA 30613

### INTRODUCTION

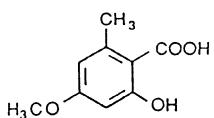
Higher plants may have a future in agriculture as a renewable resource for natural products; and one of the best examples of large scale crop production as a source of agrochemicals is that of the *Pyrethrum* daisy to produce the insecticide commonly known as pyrethrin, a mixture of two esters with molecular formulae  $C_{21}H_{28}O_3$  and  $C_{22}H_{28}O_5$ . Before the chemistry was elucidated, the insecticide was sold as a powder made from pulverized flowerheads and consisted of two lots: one known as Persian insect powder, which had its origin in *Chrysanthemum coccinuum* and *C. onethifolium*, the other was commonly called Dalmatian insect powder and came from *C. cinerariaefolium*. Later, the pyrethrin molecules became templates from which the highly potent pyrethroids were derived. It is difficult to give a single example where higher plants have been grown either on a small or large scale to produce compounds that directly affect plant growth and development and yet the literature abounds with allelochemical references (Cutler, 1988; Rice, 1974; Waller, 1987). In fact, no such example exists for herbicide production on a commercial scale. However, using the history of the development of the pyrethrin-pyrethroid insecticides as a paradigm, it is possible that we have bypassed the middle step wherein the crude, powdered material has been used and the approach is now to isolate the active components, identify them, and synthesize the parent molecule with a view to elaborating it in order to change not only the specific activity but also the target specificity. This systematic approach is undoubtedly the way in which the technically advanced countries will seek new molecules in order to develop biodegradable, environmentally safe agrochemicals, but the question arises as to how the underdeveloped countries can maintain a stabilized agriculture in order to progress technologically without importing high cost agricultural chemicals. Perhaps the answer lies in harvesting cultivated plants and forest resources to obtain powders or crude extracts for local use.

The purpose of this review is to point out the structural diversity of natural products from higher plant sources that may have potential as plant growth inhibitors and herbicides. In certain cases, it will be seen that congeners, which differ by only minor changes, elicit altered biological responses and that synthetic derivatization of the parent molecule has changed the specific activity.

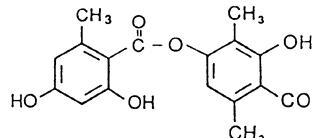
## The Lichens

While natural products from lichens have been known and used for centuries as dyes and indicators, for example, in the cottage industries of Scotland to produce earthtones for Harris tweeds and in the classroom as the well-known litmus test, other uses as basic chemicals have been virtually ignored. One notable exception is usnic acid, an antibacterial and antitumor natural product from *Usnea barbata* (Merck, 1976). In their natural habitat, the lichens are among the first in succession and are grown in inhospitable places: on rocks, in crevices, and on barren ground. In some cases, it is obvious that they stand alone with no competition from other plants in their immediate vicinity, at least during part of their life cycle, implying that they may produce compounds that inhibit the growth of alien species. Morphologically, the lichens fall into three categories: crustose, foliose, and fruticose; and these organisms are a composite of two organisms: a fungus, usually an ascomycete, but sometimes a basidiomycete, in which is embedded a green or blue green alga. Approximately eighteen thousand species of lichens have been classified (Kimball, 1978). The symbiotic relationship is such that it may be the prototype of a biotransformational system. That is, a donor organism may be giving preformed molecules to the recipient and these are transformed into elaborated products. At the present, the production of plant growth regulatory chemicals in the lichens is poorly understood and it is not clear, with the exception of a few cases, whether the compounds are of fungal or algal origin, or both.

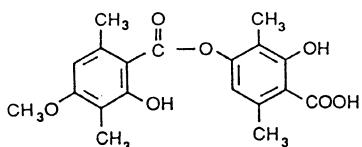
It has been observed in Japan that the rapid growth of lichens, such as *Usnea* and *Parmelia* species on trees, induces growth inhibition which may eventually lead to their death. Of these, *Usnea longissima*, which grows on pine trees in eastern and central Hokkaido in damp, foggy locations, has been examined for plant growth inhibitors. Earlier work has shown the presence of barbatic acid, (+)-usnic acid, diffractic acid, protocetraric acid, evernic acid, atranorin, fumarprotocetraric acid, and salazinic acid in *U. longissima* (Asahina, 1936, 1937, 1956; Dhar et al., 1959); and some of these metabolites have been assayed against higher plants (Huneck and Schreiber, 1972). The natural products isolated in a later study were 4-*O*-methylorsellinic acid, barbatic acid, evernic acid, diffractic acid,  $\beta$ -orcinolcarboxylic acid, 4-*O*-demethylbarbatic acid, orsellinic acid, lecanoric acid, 4-*O*-methylorsellinic acid ethyl ester, and 3- $\alpha$ -hydroxydiffractic acid (Fig. 1). With the exception of lecanoric acid, all the metabolites were tested from  $5 \times 10^{-3}$  to  $5 \times 10^{-4}$  M in a lettuce bioassay system that consisted of incorporating the materials in agar by dissolving the natural products in acetone (125  $\mu$ l), adding the mixture to hot agar solution (15 ml), and allowing the matrix to cool in petri dishes. Fifteen imbibed lettuce seeds were added to the agar surface and allowed to germinate for 7 days at 22°C under a 14-hour-day length. Measurements were then made of the radicles and hypocotyls. To a greater or lesser degree, all the metabolites were inhibitory to either radicle or hypocotyl growth and indicated some tissue selectivity, except 4-*O*-methylorsellinic acid ethyl ester (Nishitoba et al., 1987). Concerning structure-activity functions, it was seen that both the positioning and numbers of methyl and hydroxyl groups played a major role. This was especially so with the  $\beta$ -orcinol type depsides which had greater activity than the orcinol depsides. The 3- $\alpha$ -hydroxydiffractic acid congener, which was only tested at  $4 \times 10^{-4}$  M, inhibited radicle extension 90% and hypocotyl growth 60%. Of the other metabolites tested, their ascending order of activity against lettuce radicles was (in percent) orsellinic acid (0) < barbatic acid (20) < evernic acid (40) <  $\beta$ -orcinolcarboxylic acid (60) < diffractic acid (70) < demethylbarbatic acid (90) = 4-*O*-methylorsellinic acid (90). The effects on hypocotyls were not in identical sequence and were orsellinic acid (20) < barbatic acid (25) < evernic acid (30) < diffractic acid (70).



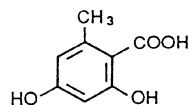
4-O-Methylorsellinic Acid



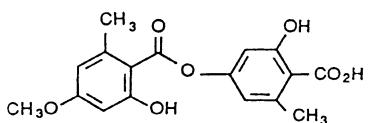
4-O-Demethylbarbatic Acid



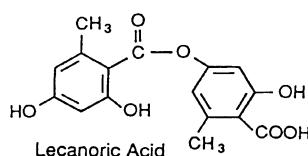
Barbatic Acid



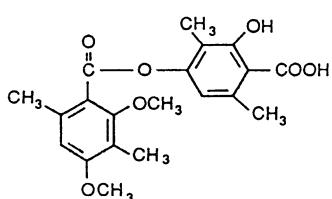
Orsellinic Acid



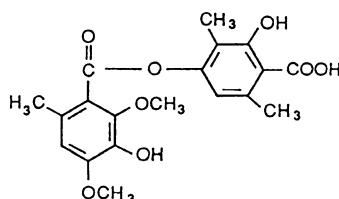
Evernic Acid



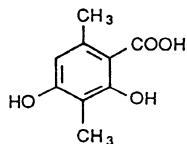
Lecanoric Acid



Diffractaic Acid



3- $\alpha$ -Hydroxydiffractaic Acid  
(*Usnea longissima*)



$\beta$ -Orcinolcarboxylic Acid

Figure 1. Structural formulas of methylorsellinic acid, demethylbarbatic acid, barbatic acid, orsellinic acid, evernic acid, lecanoric acid, diffractaic acid, hydroxydiffractaic acid, and orcinolcarboxylic acid.

< demethylbarbatic acid (80) <  $\beta$ -orcinolcarboxylic acid (90) = 4-O-methylorsellinic acid (90) (Nishitoba et al., 1987).

Each of these compounds has functional groups that lend themselves to derivatization and includes, as the authors suggest (Nishitoba et al., 1987), oxidation of the C<sub>1</sub> substitution at the 3 position and alkylation of the hydroxyl groups. While a great deal of chemical work was accomplished at the turn of the century with the lichens to determine the characteristics of natural dyes, it may now be time to return to lichen natural products as a source of useful agricultural chemicals including, possibly, fungicides. It is also an interesting observation that insects do not generally appear to be a problem in lichens.

## The Liverworts

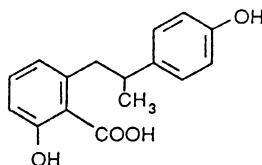
In the evolutionary classification, the liverworts are an intermediate life form between the algae and vascular plants. They lack vascular tissue and grow in damp, boggy areas, on rocks in streams, and in ponds. As with the lichens, very limited work has been published concerning the chemistry of the liverworts though, as we shall see, their chemistry is closely allied with that of the higher plants.

Lunularic acid (Fig. 2) was isolated from the liverwort, *Lunularia cruciata* L. Dum., 30 years ago as a result of some astute observations. The strain of *Lunularia* found in Israel was very sensitive to changes in daylength so that, under long days and high temperatures, it was seen to go into a dormant state. The vegetative thalli have the ability to remain dormant for years under dry conditions and will resume normal growth when rehydrated. However, if in the dormant and dry state the thalli are exposed to short day conditions, they die. Earlier experiments had shown that the daylength response was controlled by phytochrome and these implied that dormancy was controlled by an endogenous growth inhibitor (Wilson and Schwabe, 1964). The first experiments to determine the presence of an inhibitor used gemmalings. These were obtained from gemmae cups which are asexual reproductive structures that resemble miniature birds' nests and are found on the thalli of liverworts. In the gemmae are groups of cells, the gemmalings, which are splashed out of the cups during rainstorms and, upon distribution in a suitable environment, develop into new offspring. The gemmalings were grown in either continuous light or short days in culture medium; and the precaution was taken not to confuse the lack of plant development, because of nutrient deficiency, by replacing the culture medium every 3 days. While the inhibitor was present in both long and short day culture medium that supported gemmaling growth, it was present in far greater quantity in the long day (continuous light) solutions. Subsequently, these were extracted and the extracted material was added to fresh culture medium to which new gemmalings had been transferred and 5 to 10 days later the growth was measured. Gemmaling growth was inhibited. Following purification, lunularic acid was found to be the active substance and was bioassayed against gemmae at concentrations ranging from 0.1 to 10 ppm; this induced a 12 to 33% inhibition (Valio et al., 1969). It is a subtle but important point that, even at the highest concentration, gemmalings were only inhibited but not killed, especially in light of the fact that 0.1 ppm induced a 12% inhibition; but as we shall shortly see, the effects on other plant species are different and this suggests the possibility that lunularic acid may play a dual role. First, it is a self inhibitor in *Lunularia* and protects that species against the exigencies of the environment such as drought and, at the same time, serves as a defense mechanism against other competing species.

As with the lichen natural products, the structure of lunularic acid has several possibilities for the production of potential biologically active analogs. Both the hydroxyl and carboxyl groups are readily available for exploitation and certainly with a little imagination other interesting derivatives, both chemically and biologically, can be conceived.

### *Hydrangea macrophylla*: Stilbene Derivatives

During the workup of lunularic acid, it became evident that a model compound was necessary to elucidate the structure of the then unknown inhibitor and attention was drawn to the physical data of the dihydroisocoumarin, hydrangenol, and the isomeric stilbene, hydrangeic acid, which had been extracted from *Hydrangea* (Asahina and Asano, 1929, 1930a,b; Asahina and Miyake, 1916). The similarity between



Lunularic Acid  
 ( Lunularia cruciata )

Figure 2. Structural formula of lunularic acid.

lunularic acid and dihydrohydrangeic acid soon became apparent when Valio et al. (1969) followed the procedure of Ibrahim and Towers (1960, 1962) to isolate hydrangenol from the roots of *Hydrangea macrophylla*, followed by reduction with sodium and ethanol to produce dihydrohydrangeic acid. The physical data for both compounds could be superimposed (Valio et al., 1969).

After the passage of a number of years, interest again surfaced in the plant growth regulatory properties of the natural products of *Hydrangea macrophylla* subsp. *serrata* and more specifically in the stilbene synthetic derivatives thereof. It should come as no surprise to pharmacognosists that *Hydrangea* contains many physiologically interesting substances, for example, phyllodulcin, from *H. macrophylla* var. *thunbergii*, is one thousand times sweeter than sucrose and is used, in addition, as a refrigerant (Yamato et al., 1972). On the other hand, plant physiologists and plant pathologists may be surprised to learn that the self inhibitor, lunularic acid, and the stilbenes, which are receiving considerable attention as naturally induced fungicides in wounded plants, are chemically close relatives. Three novel dihydroisocoumarin glucosides designated macrophyllisosides A, B, and C were extracted from the dried leaves of *H. macrophylla* subsp. *serrata* and the structures were: (3S)-3',4',5'-trimethoxyphenyl-8-β-D-glucopyranosyl dihydroisocoumarin and (3R)- and (3S)-3',5'-dimethoxy-4'-hydroxyphenyl-8-β-D-glucopyranosyl dihydroisocoumarin, respectively. In addition, a mixture of the known compounds (3R)- and (3S)-hydrangenol-8-β-glucoside was isolated. When a mixture of macrophyllisosides B and C was hydrolysed with 1N H<sub>2</sub>SO<sub>4</sub>, D-glucose and an aglycone resulted. Alkaline hydrolysis of the aglycone gave a stilbene derivative (Fig. 3) and reduction of the exocyclic double bond in this structure, using sodium borohydride and palladium chloride, yielded the dihydrostilbene derivative (Fig. 4). When lunularic acid, the derivatized stilbene, and the dihydrostilbene derivative were bioassayed against germinating rice seed at 500 ppm, there was 100% growth inhibition and, furthermore, elongation of the second coleoptile of rice seedlings was inhibited ~70% (Hashimoto et al., 1987). It also transpires that stilbene glucosides I and II have been isolated from *H. macrophylla* var. *thunbergii* (Asahina and Asano, 1930a,b, 1931; Suzuki et al., 1977; Yagi et al., 1972). These resemble lunularic acid with the exception that, in these stilbenes, the methyl group of lunularic acid is replaced by an hydroxyl and, also, there is a glucose residue on the carboxyl group. Furthermore, the substituents on the non-benzoic acid ring are hydrogen, or hydroxyl, or O-methyl. It does not appear that these natural derivatives have been bioassayed against plants or fungi. Even though the stilbenes are notoriously unstable to light and heat, it may be that suitable substitution may stabilize them without the loss in plant growth regulatory or fungicidal activity and much work remains to be done to produce derivatives and formulations of this class of compounds.

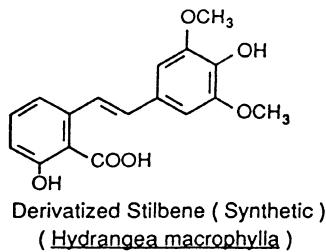


Figure 3. Structural formula of derivatized stilbene.

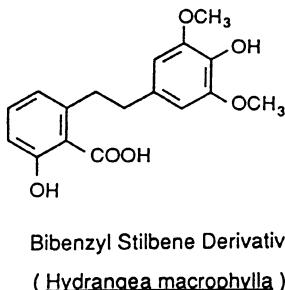
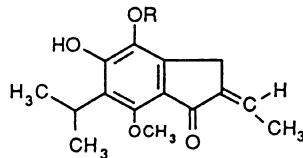


Figure 4. Structural formula of bibenzyl stilbene derivative.

### *Eremochloa ophiurooides:* The Ophiuroloidins

Centipede grass, *Eremochloa ophiurooides*, a native of China that was introduced into the United States in 1919, is a vigorous grower and withstands the droughts and high temperatures of the southeastern United States where it is used for lawns. It produces phenolic compounds of which two novel structures have been recently elucidated (Gueldner et al., 1988). These are ophiuroloidin B and C which differ structurally by a single methyl group (Fig. 5). In bioassays utilizing the etiolated wheat coleoptile (*Triticum aestivum* L. cv. Wakeland) (Cutler, 1984), it was found that both compounds significantly ( $P < 0.01$ ) inhibited extension equally at  $10^{-3}$  and  $10^{-4}$  M. That is, 100 and 42%, respectively, relative to controls (Fig. 6). This led to greenhouse experiments where week-old corn plants (*Zea mays* L. cv. Norfolk Market White) were treated by introducing 100  $\mu$ l of test solution at  $10^{-2}$  to  $10^{-4}$  M into the leaf sheath of each plant. Within 48 hours, there was localized necrosis of the leaves treated with ophiuroloidin B, but not C, from which the plants recovered 2 weeks later. Bean seedlings (*Phaseolus vulgaris* L. cv. Black Valentine) were unaffected by either metabolite. Germinating radish seed radicles were strongly inhibited by ophiuroloidin B at  $10^{-2}$  M in petri dish assays. Further tests, using disk bioassays, revealed that both metabolites inhibited the growth of the bacteria, *Bacillus subtilis*, *B. cereus*, and *Mycobacterium thermospectum*, while *Escherichia coli* was only slightly inhibited and *Citrobacter freundii* was not inhibited. Thus, both metabolites had certain selective properties and biological activity over a broad range of biological systems. The structure of ophiuroloidin A has yet to be determined. As with the previously discussed compounds, the functional groups and their derivatization should lead to substances exhibiting interesting structure-activity relationships in diverse biological systems.



Ophiuroidin B : R = H  
 Ophiuroidin C : R = CH<sub>3</sub>

( *Eremochloa ophioides* )

Figure 5. Structural formulas of ophiuroidins B and C.

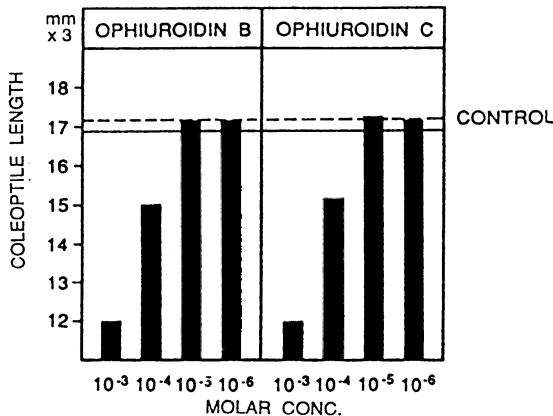
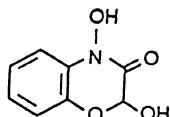


Figure 6. Effect of ophiuroidins B and C on oat coleoptile.

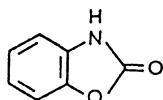
#### *Secale cereale, Acanthus mollis, and Nitrogen Containing Compounds*

During recent years, the allelochemic properties of winter rye (*Secale cereale* L.) have been explained. It had been observed in no-till management that when rye crop residues remained undisturbed, there was poor germination of weed seeds especially at and near the soil surface (Barnes and Putnam, 1983). An investigation of aqueous extracts of rye and rye residues led to the finding that many plant species were killed by the natural products present (Barnes and Putnam, 1985). A concerted effort was subsequently made to isolate the phytotoxic metabolites of rye (*Secale cereale* L. cv. Wheeler) from shoots using, for bioassay system, cress (*Lepidium sativum* L. cv. Curly) and noting the effects of separated fractions on germination and seedling growth (Barnes et al., 1987). Extraction, isolation, and purification of the bioactive metabolites finally yielded two compounds: 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA) and 2(3H)-benzoxazolinone (BOA) (Fig. 7). DIBOA was inhibitory to cress roots at concentrations greater than  $0.4 \times 10^{-3}$  M and shoots required greater than  $0.5 \times 10^{-3}$  M to induce inhibition. Barnyardgrass (*Echinochloa crus-galli* L.) was also inhibited with DIBOA at 10.5, 1.0, 1.5, and  $2.0 \times 10^{-3}$  M by 63, 97, 100, and 100%, respectively, relative to controls. However, BOA at the same concentrations was only active at 1.5 and  $2.0 \times 10^{-3}$  M and induced only a 10 and 45% inhibition, respectively (Barnes et al., 1987).



2,4-Dihydroxy-1,4(2H)-benzoxazin-3-one

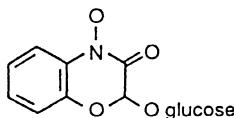
( Secale cereale )



2(3H)-benzoxazolinone

( Secale cereale )

Figure 7. Structural formulas of 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one and 2(3H)-benzoxazolinone.



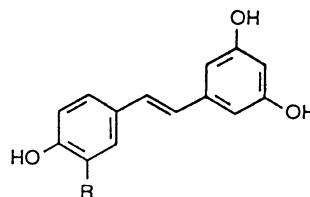
2,4-Dihydroxy-1,4-benzoxazin-3-one Glucoside

( Acanthus mollis )

Figure 8. Structural formula of 2,4-dihydroxy-1,4-benzoxazin-3-one glucoside.

An earlier work (Wolf et al., 1985) had shown the presence of 2,4,dihydroxy-1,4-benzoxazin-3-one glucoside (Fig. 8) in bear's breech (*Acanthus mollis*) seed and this compound, which comprised 4% of the dry weight, gave DIBOA on enzymatic hydrolysis, which degraded to BOA. The DIBOA glucoside was inactive against germinating velvetleaf seed (*Abutilon theophrasti*) at 5 mM but it did inhibit vegetative growth. DIBOA, at 2 mM, caused complete cessation of germination, even after 2 weeks, and lower concentrations slightly inhibited germination; vegetative growth was also inhibited. By contrast, BOA produced only transient effects and, paradoxically, at 0.5 mM germination was promoted. These compounds have also been found in corn, wheat, wild rye (*Elymus gayanus* L.), Job's tears (*Coix lacryma-jobi* L.) and bamboo (*Chusquea cumingii*), and giant reed (*Arundo donax* L.) (Tang et al., 1975; Tipton et al., 1967; Zuniga et al., 1983). As may be anticipated from looking at the structure, DIBOA and BOA possess fungicidal properties and are active against *Fusarium nivale*, *Sclerotinia trifoliorum*, and *Helminthosporium maydis* race T (Dawe, 1973; Virtanen and Hietala, 1955; Wahlroos and Virtanen, 1958; 1959).

The plant growth promotory properties of BOA are of interest, for BOA may be viewed as an indole analog. That is, an oxygen replaces a carbon in the pyrrole ring. It is also important to remember that, in the 1960's, it was thought by certain groups that indole-3-acetic acid was not biologically active *per se* as a plant growth regulator but that a transformed species was the active form and, possibly, it was transient oxindole. If one examines BOA in the light of indole chemistry, many synthetic possibilities come to mind and also the potential production of a herbicide.



Resveratrol : R = H

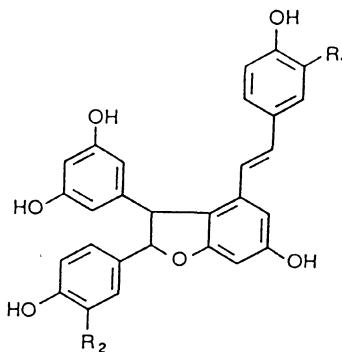
Piceatannol : R = OH

( *Scirpus maritimus* )

Figure 9. Structural formulas of resveratrol and piceatannol.

### *Scirpus maritimus*: The Stilbene Metabolites

While some of the stilbene compounds have already been described, those of *Scirpus maritimus* L. have not. The plant, a member of the Cyperaceae, grows in estuarine marshes and prairie saline wetlands (Ewing, 1983) and also Africa, Eurasia, and North America. A successful effort (Powell et al., 1987) has been undertaken to isolate and identify the biologically natural products present in *S. maritimus* because, in addition to possible agriculturally useful chemicals that may be contained therein, there may also be medicinals. The roots of the plant have been used in Chinese medicine as a diuretic and astringent (Chopra et al., 1956). A series of five compounds were isolated: resveratrol (Fig. 9), piceatannol, e-viniferin, scirpusin A (Fig. 10), and scirpusin B. These were tested, with the exception of e-viniferin which was obtained only in very small quantity, in a variety of biological systems that included brine shrimp (*Artemia salina*), 3PS leukemia in mice, crown gall tumors on potato tuber disks (*Solanum tuberosum*), fall armyworm (*Spodoptera frugiperda*) antifeedant assays, and duckweed (*Lemna minor*). The most active of these metabolites in the duckweed assay, which has been used for almost 50 years as an indicator of herbicide potential (Fromm, 1951), were piceatannol (Fig. 9) and scirpusin B (Fig. 10) at 333 ppm (Powell et al., 1987). The remainder of the compounds were inactive and this raises several important points. First, resveratrol and piceatannol differ by one oxygen atom; that is, the oxygenated species is the most active in the duckweed bioassay. However, it has been reported that both compounds inhibit photosynthesis (Gorham and Coughlan, 1980) and the question then arises as to why, if this is the case, both compounds were not active (even unequally) in the assay. Second, the obvious structural relationship between the two compounds and lunularic acid (Fig. 2) and the evolutionary development of plant species intuitively leads to thoughts about genetic conservation. Of the scirpusins, scirpusin B was active and, again, the activity difference between A and B appears to be attributable to the singular difference of an oxygen atom. Also, the inhibition figures reported by Powell et al. (1987) for piceatannol and scirpusin B in duckweed are almost identical for each chemical species; yet because of the increase in the number of oxygens in scirpusin B and other structural features, it might be anticipated that scirpusin B would be the more active. Derivatization and biological testing of each of these compounds should answer a number of questions posed by their anomalous behavior. It is important to remember that they are all members of the stilbene family and, therefore, deserve to be tested for fungicidal properties.



Scirpusin A : R<sub>1</sub> = H; R<sub>2</sub> = OH

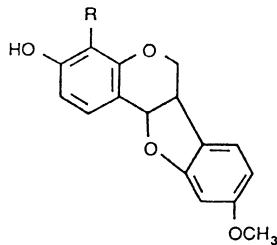
Scirpusin B : R<sub>1</sub> = R<sub>2</sub> = OH

( Scirpus maritimus )

Figure 10. Structural formulas of scirpusin A and B.

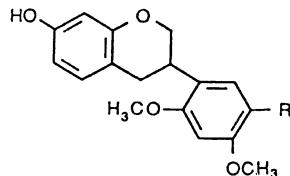
### ***Medicago sativa*: The Medicarpins**

It has long been known that alfalfa (*Medicago sativa* L.) has two growth regulatory problems. One is that the seed requires scarification in order to improve germination though it is not clear whether this is due to a self inhibitor in the seed coat, that either has to be leached out or destroyed as is the case of scarification with concentrated H<sub>2</sub>SO<sub>4</sub>, or not. The other is that fields which have supported alfalfa growth in one season became thrifty if a second alfalfa crop is attempted during the same year or the following year (Miller, 1983). In both cases, there is a self-inhibitory or autotoxic effect. Recent examination of the natural products in alfalfa has led to the isolation of medicarpin, 4-methoxymedicarpin (Fig. 11), sativan, and 5'-methoxysativan (Fig. 12) (Dornbos et al., 1990; Miller et al., 1988), all of which are legume phytoalexins and are produced as a result of wounding, either mechanically or disease induced, and stress (Ingham, 1982). In one report, only medicarpin and 4-methoxymedicarpin (Fig. 11) were assayed against lettuce seed (*Lactuca sativa*) and alfalfa seed (cv. Vernal). Medicarpin significantly inhibited lettuce seed germination at  $1 \times 10^{-3}$  M and 4-methoxymedicarpin very significantly inhibited lettuce seed at  $0.8 \times 10^{-3}$  M. In the case of alfalfa seed germination,  $10^{-1}$  M medicarpin had inhibited 59% after 6 days and  $1.7 \times 10^{-3}$  M of 4-methoxymedicarpin had inhibited 55% after 5 days (Miller et al., 1988). Later, all four compounds were bioassayed for germination inhibition activity in a unique agar system that allowed both lipophilic and hydrophilic substances to be tested (Dornbos and Spencer, 1990) using alfalfa and velvetleaf (*Abutilon theophrasti* L.) (Dornbos et al., 1990). However, none of these compounds inhibited either alfalfa or velvetleaf germination in the agar systems in direct contrast to the effects that medicarpin produced on both lettuce and alfalfa seed germinated on filter paper (*vide supra*). But when medicarpin was applied exogenously at  $1 \times 10^{-7}$  mole/seed, to seed grown on agar, vegetative growth, as opposed to germination, was inhibited in both species 39% after 72 hours (Dornbos et al., 1990). The different responses obtained in germination assays between the two systems are puzzling. There is a possibility that certain compounds may become bound to the agar thereby making them unavailable though we have found this to be a rare occurrence (Cutler, unpublished).



Medicarpin : R = H  
 4 - Methoxymedicarpin : R = OCH<sub>3</sub>  
 (Medicago sativa)

Figure 11. Structural formulas of medicarpin and 4-methoxymedicarpin.



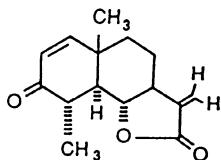
Sativan : R = H  
 5' - Methoxysativan : R = OCH<sub>3</sub>  
 (Medicago sativa)

Figure 12. Structural formulas of sativan and 5'-methoxysativan.

The pterocarpans, medicarpin and 4-methoxymedicarpin, have been isolated from tropical heartwood (Harper et al., 1965a,b) and a pterocarpan has been shown to be a seed inhibitor in the case of "clover sickness" (Chang et al., 1969; Tamura et al., 1969). But whether these compounds can be developed as preemergence herbicides is a matter of conjecture and depends to a large extent on their stability, especially in the presence of soil microorganisms.

#### ***Sonchus tuberifer*: Tuberiferin and Synthetic Precursors**

In an earlier review (Cutler, 1991), we noted that many biologically active natural products of microbial origin contain the  $\alpha$ ,  $\beta$ -unsaturated ketonic function. One such compound isolated from the roots of a compositae, *Sonchus tuberifer*, is tuberiferine (Fig. 13), the isolation and structure of which was very briefly reported in 1967 (Barrera et al., 1967); but no biological data were given. There followed, almost 10 years later, publication of the total synthesis of ( $\pm$ )-tuberiferine and again no biological data were forthcoming; but in the acknowledgments, the National Cancer Institute, the Shell Development Company, and the Alfred P. Sloan



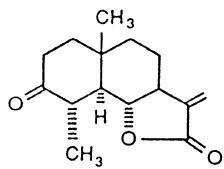
( + ) - Tuberiferin  
 ( Sonchus tuberifer. )

Figure 13. Structural formula of (+)-tuberiferin.

Foundation were thanked for their support, thereby implying that the compound may have useful biological properties (Grieco and Nishizawa, 1976). Eventually, a publication (Ando et al., 1987) discussed the fact that sesquiterpene lactones possessing  $\alpha$ -methylene- $\gamma$ -lactonic functions have diverse biological activity including allergenic, cytotoxic, antineoplastic, antimitotic, and antihistosomal properties and tuberiferin (note the change in spelling) was a member of this family. There are some 900 members presently known in this class of natural products (Fisher et al., 1979). The case in point revealed the synthesis of (+)-tuberiferin and both the final product (tuberiferin) and the synthetic precursors, which had been derived from the starting material 1- $\alpha$ -santonin, were assayed against the seed of three plant species, Japanese millet (*Echinochloa frumentacea*), brown mustard (*Brassica juncea*), and cucumber (*Cucumis sativus*), and the effects on germination and seedling growth noted. Additionally, the compounds were tested against the plant pathogens: *Pyricularia oryzae*, *Rhizoctonia solani*, *Erysiphe graminis*, *Pythium aphanidermatum*, *Plasmopora viticola*, *Phytophthora infestans*, and *Venturia inequalis*. The compounds assayed were: 3,3-(ethylenedioxy) eudesm-11(12)-eno-13,6  $\alpha$ -lactone; (11S)-3-oxoeudesm-11(12)-eno-13,6  $\alpha$ -lactone; 2  $\alpha$ -bromo-3-oxoeudesm-11(12)-eno-13,6  $\alpha$ -lactone; and (+)-tuberiferin. The most active of these was (+)-tuberiferin which, at 1000 ppm, was extremely inhibitory to seed germination of all the species tested. At 100 ppm, activity was only manifested in *E. frumentacea* and *C. sativus* and that activity was mild. In both cases, stems and roots were inhibited. The next most active compound was (11S)-3-oxoeudesm-11(12)-eno-13,6  $\alpha$ -lactone (Fig. 14), but shoots of cucumber were only slightly inhibited at 1000 ppm; while at 100 ppm, activities were approximately the same as those obtained with tuberiferin except that *B. juncea* roots were slightly inhibited (Ando et al., 1987). In concentrations that ranged from 50 to 500 ppm, all compounds containing the bromine residue were only active against *R. solani*, *P. viticola*, and *V. inequalis*. It was satisfying to read that all the synthetic precursors to tuberiferin were tested in a series of unrelated bioassays that included seed, plants, fungi, and the P388 lymphocytic leukemia test. Generally, this does not happen with synthetic precursors and there is a good possibility that a number of valuable herbicides and antimicrobials have been lost as a result.

#### *Rabdosia eriocalyx*: Maoecrystal I and J

Another series of compounds that have herbicidal activity are maoecrystal I and J (Fig. 15), *ent*-kaurene diterpenoids whose activity appears to be dependent upon the  $\alpha$ -methylene-cyclopentanone functions (Shen et al., 1989). These natural products have been isolated from the Chinese medicinal herb, *Rabdosia eriocalyx* Hara, which has its habitat in the Yunnan province where the plant is used to reduce swellings. Each



(11S)-3-Oxoeudesm-11(12)-eno-13,6 $\alpha$ -lactone

Figure 14. Structural formula of (11S)-3-oxoeudesm-11(12)-eno-13,6 $\alpha$ -lactone.

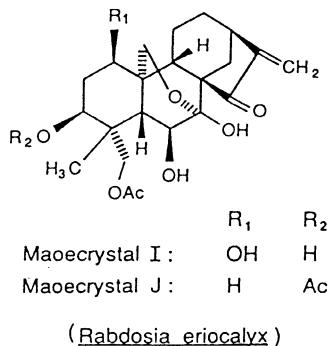
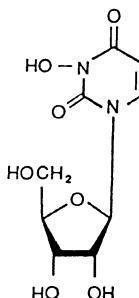


Figure 15. Structural formulas of maoecrystal I and J.

purified product was tested against lettuce seedlings grown hydroponically in Hoagland's solution at 1, 2, 5, 10, 50, 100, 200, 500, and 1000 ppm. The minimum inhibitory concentration was less than 200 ppm for maoecrystal I and 20 ppm for maoecrystal J (Shen et al., 1989). The mode of action of these metabolites is attributed to the  $\alpha$ -methylene cyclopentanone moiety which binds to the sulfhydryl of the enzyme.

#### *Baillonella toxisperma:* 3-Hydroxyuridine

Purine and pyrimidine structures have fascinated plant physiologists since the discovery of the cytokinin, kinetin, in 1955 (Miller et al., 1955). While cytokinins have been utilized in plant tissue culture, where they play a significant role, there has not been a clear-cut case for either the purines or pyrimidines inducing visible effects on crops when applied exogenously. However, derivatives of these templates may have practical agricultural use and the next example of a natural product pyrimidine, which has selective herbicidal activity, holds great promise for the future of these compounds. *Baillonella toxisperma* is a tree that grows in the tropical rain forests of Cameroon and achieves a height of 50 m and a diameter of 1.9 m at 1.5 m above the ground. Saplings have great difficulty becoming established under the umbrella of the tree (30 m) and reach a height of only 1.6 m, and this suggests the presence of growth inhibitory substances that are being released from the tree (Ohigashi et al., 1989). Extraction of both the aerial and root portions of the tree with methanol, followed by chromatography and purification, led to the recovery of a colorless resin which was determined to be 3-hydroxyuridine (Fig. 16). The metabolite was tested on cucumber and radish seed and rice seedlings for growth regulatory properties at 50, 100, 150,



3 - Hydroxyuridine

( Baillonella toxisperma )

Figure 16. Structural formula of 3-hydroxyuridine.

and 200  $\mu\text{M}$ . In cucumber, the 50% inhibitory concentration ( $I_{50}$ ) for both hypocotyl and root growth was approximately 40  $\mu\text{m}$ . The  $I_{50}$  in radish was 300  $\mu\text{M}$  for hypocotyls and 200  $\mu\text{M}$  for roots. The compound was completely inactive against the second sheath leaf of rice with rates as high as 700  $\mu\text{M}$  though there was some reduction of root weight. Other tests included spraying monocotyledonous and dicotyledonous weeds with concentrations of 3-hydroxyuridine up to 30 mg. The species challenged were: *Abutilon avicennae*, *Cassia tora*, *Echinochloa crus-galli*, *Pharbitis purpurea*, *Setaria viridis*, and the cultivated plant, *Zea mays*. The weeds were either killed or strongly inhibited, but *Zea mays* was not affected.

Significantly, 3-hydroxyuracil possessed almost the same activity against root growth as 3-hydroxyuridine and the  $I_{50}$ s for cucumber, radish, and rice were 20, 60, and 60  $\mu\text{M}$ , respectively; additionally, 3-hydroxyuracil was also inactive against second sheath leaves of rice. It was concluded that oxidation at N-3 confers plant growth inhibitory activity on the molecule (Ohigashi et al., 1989).

The chemical opportunities afforded by the discovery of the herbicidal properties of 3-hydroxyuridine are numerous and one is tempted to inquire as to whether the pyrimidine and purine structures have been worked or not. The fact that these structures are part of the fundamental mechanisms that control life, DNA and RNA, supposes that they must have been extensively examined. But, history reiterates that the obvious is very often overlooked because it is commonplace and the fallacious attitude prevails that everyone has had the same ideas—and has carried them through to completion. Possible homologs include ribosyl alloxan and ribosyl thiouracil for testing in plants.

#### *Eucalyptus citriodora*: Mentane-3,8-diols [and Menthol Derivatives]

Eucalyptus trees are fast growing and, although native to Australia, are now found in many parts of the world. Consequently, they are a renewable resource and have attracted the attention of scientists as a source of biologically active natural products that are, specifically, plant growth regulators. Crude acetone fractions of fresh eucalyptus leaves were inhibitory to seed of *Setaria viridis*, *Amaranthus retroflexus*, and *Panicum crus-galli*; and fractionation led to the separation, purification, and identification of ( $\pm$ )-*p*-menthane-3,8-*cis*-diol and ( $\pm$ )-*p*-menthane-3,8-*trans*-diol; the former inhibited lettuce seed germination 96% and the latter 27% at rates of 200 ppm (Nishimura et al., 1982). As a result of the obvious fact that the *cis*-form was almost

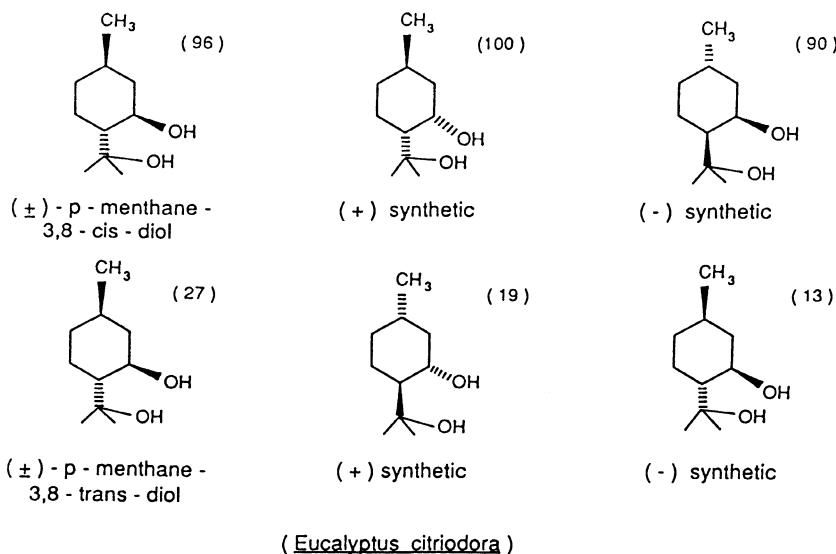


Figure 17. Structural formulas of mentane 3,8 diols.

four times more active than the *trans*-isomer, experiments were undertaken to produce the (+)- and (-)-*cis*- and *trans*-isomers by synthetic means. It transpired, in lettuce seed germination assays at 200 ppm, that the (+)-*cis*-synthetic *p*-methane-3,8-diol inhibited 100% while the (-)-*cis*-synthetic isomer inhibited 90%. The *trans*-synthetic isomers were considerably less active and the (+)-synthetic inhibited 19%; the (-)-synthetic compound inhibited only 13% (Nishimura et al., 1982) (Fig. 17).

Simple molecules such as *p*-methane-3,8-diol which exhibit biological activity are always attractive candidates for synthetic manipulation. Menthol, a common natural product, has been derivatized to produce a series of bioactive products. Treatment of menthol with *meta*-chlorobenzoic acid (MCPBA) produces hydroxylated menthol (Fig. 18). Treating rice seed with 1-menthol reduced second coleoptile growth 24% at 62.5 ppm and 52% at 250 ppm, whereas roots were inhibited 30 and 82% at the respective concentrations relative to controls. Hydroxylated menthol inhibited coleoptiles 12% and roots 32% at 62.5 ppm; while at 250 ppm, they were inhibited 61 and 100%, respectively.

Starting with 1-methyl acetate, one of the reaction products, upon treatment with MCPBA, was 4-hydroxy methyl acetate (Fig. 18) and this compound, tested only at 250 ppm, inhibited second rice coleoptiles 66%; continued reaction with MCPBA also gave the 1-hydroxymethyl acetate derivative (Fig. 18) which inhibited coleoptiles 67% at the same concentration. That is, both chemical species were equally as active in rice assays. Iso-menthol (Fig. 18), which inhibited rice coleoptiles 71% at 250 ppm when it was reacted with MCPBA, gave 1 $\alpha$ -hydroxy-1-methyl acetate that inhibited coleoptiles 57% at 250 ppm. Dry ozonization of neo-menthol (a tricky procedure because of the danger of explosion) gave, after several transitory compounds, hydroxylated neo-menthol (Fig. 18) which inhibited rice coleoptiles 69% at 250 ppm. Thus, the activity of the derivatives in descending order of magnitude was: iso-menthol > hydroxylated neo-menthol > 1-hydroxylated menthol acetate = 4-hydroxy methyl acetate > hydroxymenthol > 1 $\alpha$ -hydroxy-1-methyl acetate > 1-menthol > menthol acetate (the inhibition range is 71 to 36% inhibition of rice second coleoptiles) (Asakawa et al., 1988). Hence, in

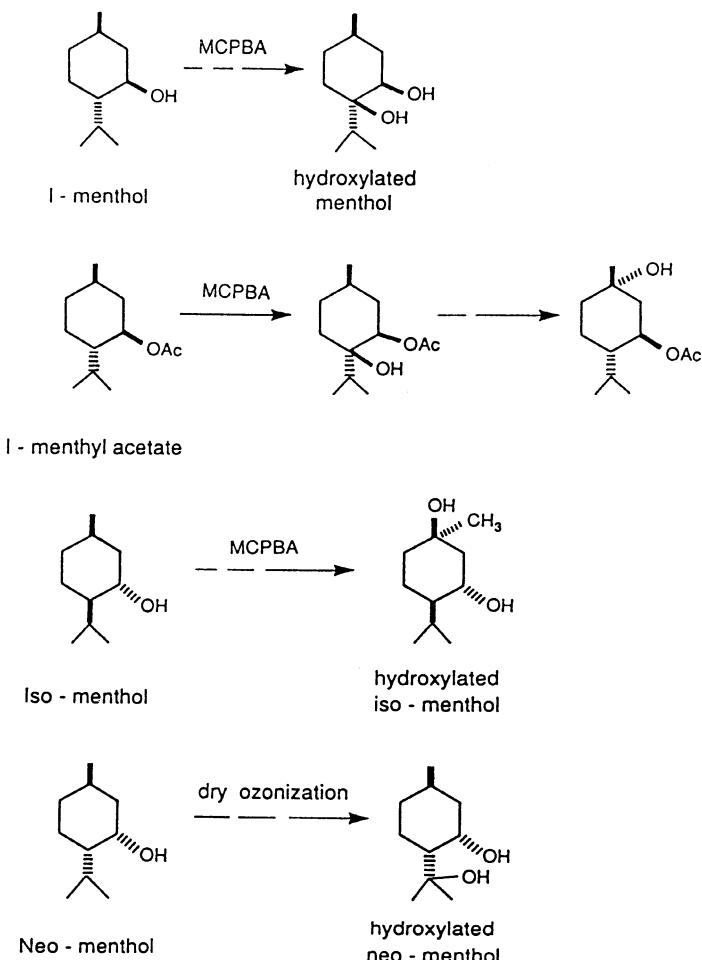


Figure 18. Structural formulas of menthol, iso-menthol, neo-menthol, and derivatives.

this example, the biological activity of a natural product was improved by synthetic elaboration, but whether this activity translates across a variety of bioassays has yet to be determined with these specific compounds. This statement should not be misconstrued; however, the detailed efforts of Asakawa and his colleagues is to be lauded and their message is that forest products may have a place in future agrochemical developments.

#### *Pimpinella* species: The Phenylpropenoids

A number of phenylpropenoids have been isolated from different species of *Pimpinella* that include: *P. anisum*, *P. diversifolia*, *P. tragium* (Fig. 19A); *P. saxifraga*-3 (Fig. 19B); *P. diversifolia*, *P. peregrina*, *P. saxifraga*-1 and -2 (Fig. 19C); *P. major* (Fig. 19D); *P. major* (Fig. 19E) (Kleiman et al., 1988); and these are relatively potent inhibitors of seed germination. Phenylpropenoids A-D, inclusive, are epoxides and it is a general rule that the epoxide function may confer biological activity on molecules (the trichothecenes and, to a certain extent, the cytochalasins are but two examples).

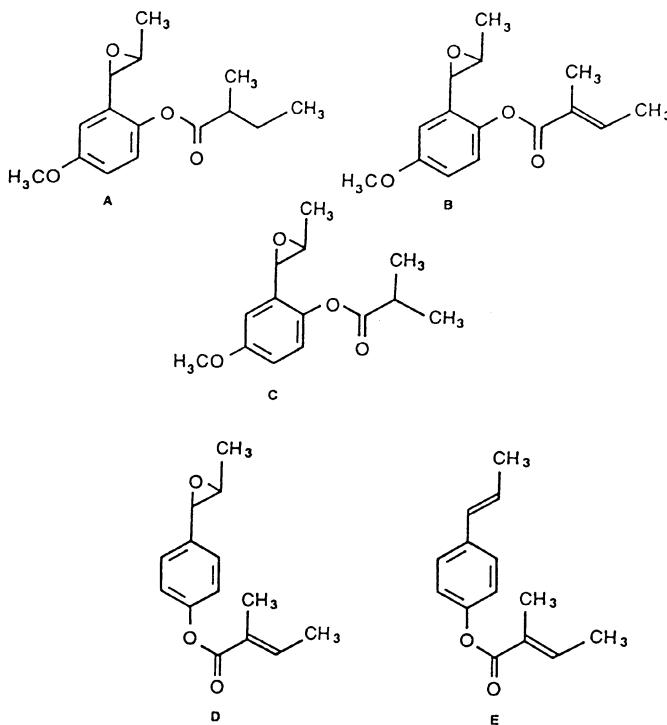
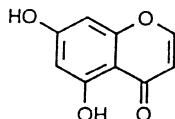


Figure 19. Structural formulas of phenylpropenoids.

Compound E does not contain an epoxide ring and is relatively less active. Compounds were tested on carrot, cucumber, lettuce, radish, ryegrass, tomato, and velvetleaf at rates of  $10^{-2}$  to  $10^4$  M. Compound A was strongly inhibitory to ryegrass and velvetleaf; mildly inhibitory to carrot, lettuce, and tomato; while radish was unaffected. Compound B, which differed from A by two hydrogen atoms (Fig. 19B), was strongly inhibitory to all seed except radish. Compound C, which contained one less carbon than A or B, strongly inhibited carrot, lettuce, and ryegrass; it was mildly inhibitory to cucumber, tomato, and velvetleaf; and radish was unaffected. Compound D, which closely resembled compound B except that there is a C1 substitution (the epoxide is *para* instead of an *ortho* one and the *meta*-methoxy is missing), strongly inhibited carrot, lettuce, ryegrass, and tomato; yet cucumber, radish, and velvetleaf were unaffected. Compound E, which resembles D except for the epoxide, which is replaced by a double bond, is only mildly active against carrot and lettuce; and radish was not affected. There is a common denominator with all the phenylpropenoids tested—they do not inhibit the growth of radish seed (Kleiman et al., 1988). It would have been enlightening to have hydrogenated the double bond in E to see the effect on biological activity. However, the possibility exists for making selective preemergence herbicides with these compounds.



5,7-Dihydroxychromone  
 ( Polygonum lapathifolium L. )  
 ( P. persicaria L.)  
 ( Arachis hypogaea L.)

Figure 20. Structural formula of 5,7-dihydroxychromone.

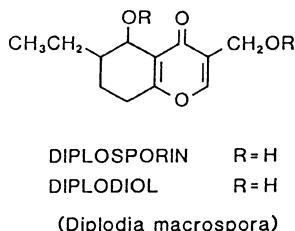


Figure 21. Structural formulas of diplosporin and diplodiol.

### *Polygonum lapathifolium:* 5,7-Dihydroxychromone

It has been shown that 5,7-dihydroxychromone (Fig. 20), a flavonoid decomposition product, has fairly potent antigermination activity against velvetleaf (*Abutilon theophrasti* Medic) seed (Spencer and Tjarks, 1985). While this compound has been isolated from both peanut hulls (*Arachis hypogaea*) (Pendse et al., 1973) and *Polygonum persicaria* L. seed (Romussi and Ciarallo, 1974), the biological activity was not reported until 1985. Concentrations of 6, 4, 2, and 1 mM inhibited velvetleaf germination 100, 85, 70, and 23%, respectively.

Chromone structures have also been found in fungi. A previous report disclosed the presence of a metabolite toxic to day-old cockerels in *Diplodia macrospora* that had LD<sub>50</sub> of 88.4 mg/kg and was given the trivial name diplodiol (Cutler et al., 1980) = diplosporin (Chalmers et al., 1978). It had structure *trans*-6-ethyl-5-hydroxy-3-hydroxymethyl-5,6,7,8-tetrahydrochromone (Fig. 21). While diplodiol did not inhibit the growth of etiolated wheat coleoptiles (*Triticum aestivum* L. cv. Wakeland), it was observed that the synthetic diacetate significantly inhibited their growth at 1 mM by 19%, relative to controls, and this was repeatable from assay to assay. The chromone structure lends itself to several synthetic possibilities and it is possible that more information will be forthcoming concerning this class of structures relative to plant growth regulation. In addition, these compounds offer interesting chemical evolutionary links between fungi and higher plants.

## CONCLUSION

Higher plants are sources of unique biologically active compounds that may be useful for agricultural applications and, furthermore, they are a renewable resource. During the course of the next two decades, plants may be grown as crops solely for the isolation of these compounds, especially in third world countries where crude extracts may be used as herbicides, or may be farmed in tissue culture to produce the necessary metabolites. These products may be used, after purification, in their native state or they may be elaborated by synthetic or biotransformational means for use as selective pre- or postemergence herbicides.

## REFERENCES

- Ando, M., Wada, T., Kusaka, H., Takase, K., Hirata, N., and Yanagi, Y., 1987, Studies on the syntheses of sesquiterpene lactones. 10. Improved syntheses of (+)-tuberiferin and the related  $\alpha$ -methylene- $\gamma$ -lactones and their biological activities, *Journal of Organic Chemistry*, 52:4792-4796.
- Asahina, Y., 1936, Microchemischer Nachweis der Flechtenstoffe. II. Über das Erythrin, *Journal of Japanese Botany*, 12:859-872.
- Asahina, Y., 1937, Über den Taxonomischen Wert der Flechtenstoffe, *Botanical Magazine Tokyo*, 51:759-764.
- Asahina, Y., 1956, Lichens of Japan, Volume III, Genus *Usnea*. Research Institute for Natural Resources, Shinjuku, Tokyo, Japan. 129 p. XXIV plates.
- Asahina, Y., and Asano, J., 1929, Über die Konstitution von Hydrangenol und Phyllodulcin, *Chemische Berichte*, 62:171-177.
- Asahina, Y., and Asano, J., 1930a, Über die Konstitution von Hydrangenol und Phyllodulcin. II, *Yakugaku Zasshi*, 50:573-588.
- Asahina, Y., and Asano, J., 1930b, Constitution of hydrangenol and phyllodulcin, *Chemische Berichte*, 63:429-437.
- Asahina, Y., and Asano, J., 1931, Synthese des Phyllodulcindimethyläthers (Über die Konstitution von Hydrangenol und Phyllodulcin. IV), *Yakugaku Zasshi*, 51:749-753.
- Asahina, Y., and Miyake, K., 1916, Hydrangenol, a chemical constituent of *Hydrangea hortensis*, *Chemical Abstracts*, 10:1523-1524.
- Asakawa, Y., Matsuda, R., Tori, M., and Hashimoto, T., 1988, Preparation of biologically active substances and animal and microbial metabolites from menthols, cineoles and kauranes, *Phytochemistry*, 27:3861-3869.
- Barnes, J. P., and Putnam, A. R., 1983, Rye residues contribute to weed suppression in no-tillage cropping systems, *Journal of Chemical Ecology*, 9:1045-1057.
- Barnes, J. P., and Putnam, A. R., 1985, Evidence for allelopathy by residues and aqueous extracts of rye (*Secale cereale*), *Weed Science*, 34:384-390.
- Barnes, J. P., Putnam, A. R., Burke, B. A., and Aasen, A. J., 1987, Isolation and characterization of allelochemicals in rye herbage, *Phytochemistry*, 26:1385-1390.
- Barrera, J. B., Breton, J. L., Fajardo, M., and Gonzalez, A. G., 1967, Terpenoids from the Sonchus. VI. Tuberiferine from *Sonchus tuberifer* Svent, *Tetrahedron Letters*, 36:3475-3476.
- Chalmers, A. A., Gorst-Allman, C. P., Kriek, N. P. J., Marasas, W. F. O., Steyn, P. S., and Vleggar, R., 1978, Diplosporin, a new mycotoxin from *Diplodia macrospora* Earle, *South African Journal of Chemistry*, 31:111-114.

- Chang, C.-F., Suzuki, A., Kumai, S., and Tamura, S., 1969, Chemical studies on "clover sickness." Part II. Biological functions of isoflavonoids and their related compounds, *Agricultural and Biological Chemistry*, 33:398-408.
- Chopra, R. N., Nayar, S. M., and Chopra, I. C., eds., 1956, p. 224, in: "Glossary of Indian Medicinal Plants," New Delhi, Council of Science and Industrial Research, India, 329 p.
- Cutler, H. G., 1984, A fresh look at the wheat coleoptile bioassay, *Proceedings of the 11th Annual Meeting of the Plant Growth Regulator Society of America*, p. 1-9.
- Cutler, H. G., 1988, Biologically Active Natural Products: Potential Use in Agriculture, *American Chemical Society Symposium Series* 380, Washington, D.C., 483 p.
- Cutler, H. G., 1991, Effects of natural products from microorganisms on higher plants, p. 113-139, in: "Plant Biochemical Regulators," H. W. Gausman, ed., Marcel Dekker, Inc., New York, New York.
- Cutler, H. G., Crumley, F. G., Cox, R. H., Cole, R. J., Dorner, J. W., Lattrell, R. M., and Rossi, A. E., 1980, Diplodiol: A new toxin from *Diplodia macrospora*, *Journal of Agricultural and Food Chemistry*, 28:135-138.
- Dawe, D. H., 1973, The relation of 1,4-benzoxazolinones and related compounds in *Zea mays* to *Helminthosporium maydis* resistance. *Dissertation Abstracts International*, B34:3031-3032.
- Dhar, M. L., Neelakantan, S., Ramanujam, S., and Seshadri, T. R., 1959, Chemical investigation of Indian lichens: Part XXII, *Journal of Scientific and Industrial Research*, 18B:111-113.
- Dornbos, D. L., Jr., and Spencer, G. F., 1990, Natural products phytotoxicity: A bioassay for small quantities of slightly water-soluble compounds, *Journal of Chemical Ecology*, 16:339-352.
- Dornbos, D. L., Jr., Spencer, G. F., and Miller, R. W., 1990, Medicarpin delays alfalfa seed germination and seedling growth, *Crop Science*, 30:162-166.
- Ewing, K., 1983, Environmental controls in Pacific Northwest intertidal marsh plant communities, *Canadian Journal of Botany*, 61:1105-1116.
- Fischer, H. D., Fischer, N. H., Franck, R. W., and Olivier, E. J., 1979, The biogenesis and chemistry of sesquiterpene lactones, p. 214-223, in: "Progress in the Chemistry of Organic Natural Products," Volume 38, W. Herz, H. Grisebach, and G. W. Kirby, eds., Springer-Verlag, Vienna/New York, New York.
- Fromm, F., 1951, A quantitative evaluation of the *Lemna* test for herbicides, *Botanical Gazette*, 113:86-89.
- Gorham, J., and Coughlan, S. J., 1980, Inhibition of phytosynthesis by stilbenoids, *Phytochemistry*, 19:2059-2064.
- Grieco, P. A., and Nishizawa, M., 1976, Application of organoselenium chemistry to the total synthesis of ( $\pm$ )-tuberiferine, *Journal of the Chemical Society, Chemical Communications*, 582-583.
- Gueldner, R. C., Cutler, H. G., Arrendale, R. F., Himmelsbach, D. S., and Van Halbeek, H., 1988, Identification and biological activity of phenolic compounds from centipede grass roots, *Bulletin de Liason N°14 du Groupe Polyphenols*, p. 197-200.
- Harper, S. H., Kemp, A. D., and Underwood, W. G. E., 1965a, Heartwood constituents of *Swartzia madagascariensis*, *Chemistry and Industry*, 13:562-563.
- Harper, S. H., Kemp, A. D., and Underwood, W. G. E., 1965b, Heartwood constituents of *Swartzia madagascariensis*, *Journal of the Chemical Society, Chemical Communications*, 14:309-310.
- Hashimoto, T., Tori, M., and Asakawa, Y., 1987, Three dihydroisocoumarin glucosides from *Hydrangea macrophylla* subsp. *serrata*, *Phytochemistry*, 26:3323-3330.

- Huneck, S., and Schreiber, K., 1972, Wachstumsregulatorische Eigenschaften von Fletchen-und Moos-inhaltsstoffen, *Phytochemistry*, 11:2429-2434.
- Ibrahim, R. K., and Towers, G. H. N., 1960, Studies of hydrangenol in *Hydrangea macrophylla* Ser. I. Isolation, identification, and biosynthesis from  $^{14}\text{C}$ -labelled compounds, *Canadian Journal of Biochemistry and Physiology*, 38:627-634.
- Ibrahim, R. K., and Towers, G. H. N., 1962, Studies of hydrangenol in *Hydrangea macrophylla* Ser. II. Biosynthesis of hydrangenol from  $^{14}\text{C}$ -labelled compounds, *Canadian Journal of Biochemistry and Physiology*, 40:449-453.
- Ingham, J. L., 1982, Phytoalexins from the Leguminosae, p. 21-80, in: "Phytoalexins," J. A. Bailey and J. W. Mansfield, eds., Blackie and Son, Glasgow.
- Kimball, J. W., 1978, "Biology," Addison-Wesley Publishing Company, Reading, Massachusetts, 659 p.
- Kleiman, R., Plattner, R. D., and Weisleder, D., 1988, Antigermination activity of phenylpropenooids from the genus *Pimpinella*, *Journal of Natural Products*, 51:249-256.
- "Merck Index," 1976, Monograph number: 9556, M. Windholz, ed., Merck and Co., Inc., Rahway, New Jersey, 1270 p.
- Miller, C. O., Skoog, F., VonSaltza, M. H., and Strong, F. M., 1955, Kinetin, a cell division factor from deoxyribonucleic acid, *Journal of the American Chemical Society*, 77:1392.
- Miller, D. A., 1983, Allelopathic effects of alfalfa (*Medicago sativa*), *Journal of Chemical Ecology*, 8:1059-1072.
- Miller, R. W., Kleiman, R., and Powell, R. G., 1988, Germination and growth inhibitors of alfalfa, *Journal of Natural Products*, 51:328-330.
- Nishimura, H., Kaku, K., Nakamura, T., Fukazawa, Y., and Mizutani, J., 1982, Allelopathic substances, ( $\pm$ )-*p*-menthane-3,8-diols isolated from *Eucalyptus citriodora* Hook, *Agricultural and Biological Chemistry*, 46:319-320.
- Nishitoba, Y., Nishimura, H., Nishiyama, T., and Mizutani, J., 1987, Lichen acids, plant growth inhibitors from *Usnea longissima*, *Phytochemistry*, 26:3181-3185.
- Ohigashi, H., Kaji, M., Sakaki, M., and Koshimizu, K., 1989, 3-Hydroxyuridine, an allelopathic factor of an African tree, *Baillonella toxisperma*, *Phytochemistry*, 28:1365-1368.
- Pendse, R., Rama Rao, A. V., and Venkataraman, K., 1973, 5,7-Dihydroxychromone from *Arachis hypogaea* shells, *Phytochemistry*, 12:2033-2034.
- Powell, R. G., Bajaj, R., and McLaughlin, J. L., 1987, Bioactive stilbenes of *Scirpus maritimus*, *Journal of Natural Products*, 50:293-296.
- Rice, E. L., 1974, "Pest Control with Nature's Chemicals," University of Oklahoma Press, 224 p.
- Romussi, G., and Ciarallo, G., 1974, 5,7-Dihydroxychromone from *Polygonum persicaria* seeds, *Phytochemistry*, 13:2890-2891.
- Shen, X., Isogai, A., Furihata, K., Kaniwa, H., Sun, H., and Suzuki, A., 1989, *Ent*-kaurene diterpenoids from *Rabdodia eriocalyx*, *Phytochemistry*, 28:855-858.
- Spencer, G. F., and Tjarks, L. W., 1985, Germination inhibition by 5,7-dihydroxychromone, a flavanoid decomposition product, *Journal of Plant Growth Regulation*, 4:177-180.
- Suzuki, H., Ikeda, T., Masumoto, T., and Noguchi, M., 1977, Isolation and identification of a new glycoside, phyllodulcin-8- $\beta$ -D-glucose from the cultured cells and fresh leaves of amacha (*Hydrangea macrophylla* Seringe var. *thunbergii* Makino), *Agricultural and Biological Chemistry*, 41:1815-1817.
- Tamura, S., Chang, C.-F., Suzuki, A., and Kumai, S., 1969, Chemical studies on "Clover Sickness." Part I. Isolation and structural elucidation of two new isoflavonoids in red clover, *Agricultural and Biological Chemistry*, 33:391-397.

- Tang, C.-S., Chang, S. H., Hoo, D., and Yanagihara, K. H., 1975, Gas chromatographic determination of 2(3)-benzoxazolinones from cereal plants, *Phytochemistry*, 14:2077-2079.
- Tipton, C. L., Klun, J. A., Husted, R. R., and Pierson, M. D., 1967, Cyclic hydroxamic acids and related compounds from maize. Isolation and characterization, *Biochemistry*, 6:2866-2870.
- Valio, I. F. M., Burdon, R. S., and Schwabe, W. W., 1969, New natural growth inhibitor in the liverwort *Lunaria cruciata* (L.) Dum, *Nature*, 223:1177-1178.
- Virtanen, A. I., and Hietala, P. K., 1955, 2(3)-Benzoxazolinone, an anti-Fusarium factor in rye seedlings, *Acta Chemica Scandinavica*, 9:1543-1544.
- Wahlroos, O., and Virtanen, A. I., 1958, On the antifungal effect of benzoxazolinone and 6-methoxybenzoxazolinone, respectively, on *Fusarium nivale*, *Acta Chemica Scandinavica*, 12:124-128.
- Wahlroos, O., and Virtanen, A. I., 1959, The precursors of 6-methoxybenzoxazolinone in maize and wheat plants, their isolation and some of their properties, *Acta Chemica Scandinavica*, 13:1906-1908.
- Waller, G. R., 1987, Allelochemicals: Role in Agriculture and Forestry, American Chemical Society Symposium Series 330, Washington, D.C., 606 p.
- Wilson, J. R., and Schwabe, W. W., 1964, Growth and dormancy in *Lunularia cruciata* (L.) Dum. III. The wavelengths of light effective in photoperiodic control, *Journal of Experimental Botany*, 15:368-380.
- Wolf, R. B., Spencer, G. F., and Plattner, R. D., 1985, Benzoxazolinone, 2,4-dihydroxy-1,4-benzoxazin-3-one, and its glucoside from *Acanthus mollis* seeds inhibit velvetleaf germination and growth, *Journal of Natural Products*, 48:59-63.
- Yagi, A., Washida, Y., Takata, N., and Nishioka, I., 1972, Studies on *Hydrangea* species. I. Phenolic components of *Hydrangea serrata* Seringe var. *thunbergii* Sugimoto, *Chemical and Pharmaceutical Bulletin (Tokyo)*, 20:1755-1761.
- Yamato, M., Kitamura, T., Hashigaki, K., Kuwano, Y., Yoshida, N., and Koyama, T., 1972, Synthesis of biologically active isocoumarins. I. Chemical structure and sweet taste of 3,4-dihydroisocoumarins, *Yakugaku Zasshi*, 92:367-370.
- Zuniga, G. E., Argandona, V. H., Niemeyer, H. M., and Corcuera, L. J., 1983, Hydroxamic acid content in wild and cultivated Gramineae, *Phytochemistry*, 22:2665-2668.

## INSECTICIDAL COMPOUNDS FROM PLANTS

William S. Bowers

Department of Entomology  
The University of Arizona  
Tucson, AZ 85721

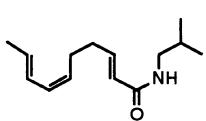
### INTRODUCTION

Plants possess limited movement and cannot escape in space from their enemies. Required to stand and fight to preserve their substance and progeny, they have evolved superlative defensive mechanisms. Dense bark, hard wood, spines, and thorns are classical morphological features of many plants. Less apparent, but probably more important in suppressing predation and disease, is the complex secondary chemistry of plants. Just as flowers elaborate a variety of alluring scents and tasty nectaries to attract birds, bees, and other insects to assist in cross pollination, many plants produce repellents, poisons, antifeedants, growth regulators, and antibiotics to reduce exploitation by herbivores and pathogens. Because insects compose more than 90% of all planetary species and are clearly the dominant herbivores, plants have evolved an extensive variety of defensive strategies targeted to limit their exploitation by pests. The earliest insecticides used by man were simple powders or aqueous extracts of poisonous plants. Hundreds of toxic plants have been employed by primitive cultures for purposes of insect control and most of these have been evaluated as prospective insecticides (Fig. 1), but only a scant few have been found to possess useful commercial insecticidal properties.

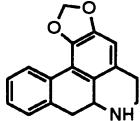
While a few plant compounds like nicotine, rotenone, and pyrethrum continue to find use in specialty applications, most plant defensive chemicals are intrinsically labile, difficult or expensive to produce synthetically, and lack the activity spectrum required for commercial production, formulation, and application. However, the discovery of biologically active natural products with insecticidal or growth regulant activity has opened simultaneously many new biochemical/physiological target sites for insect control and yielded innovative lead chemistry for the development of new methods of insect control.

Botanical insecticides have been an important resource of prototypic chemistry that has been vastly improved through biologically guided synthetic optimization efforts. The natural pyrethrins exemplify the opportunities intrinsic within biological chemistry (Fig. 2).

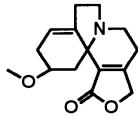
While modification of the pyrethrins seems to have proceeded from the sublime to the ridiculous, nevertheless, insecticidal analogs with vastly superior activity against a broad spectrum of insect pests have resulted.



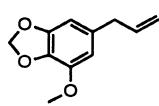
Afinin  
Ex. *Helianthus longipes*



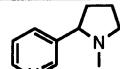
Anonane  
Ex. *Annona reticulata*



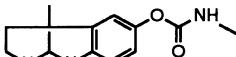
Cocculolidine  
Ex. *Coccus trilobus*



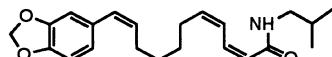
Myristicin  
Ex. *Pastinaca sativa*



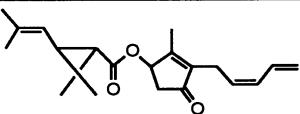
Nicotine  
Ex. *Nicotiana tabacum*



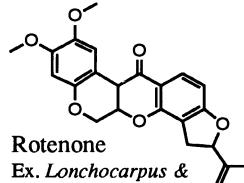
Physostigmine  
Ex. *Physostigma venenosum*



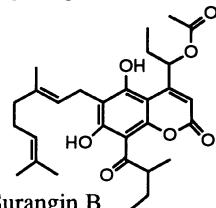
Pipericide  
Ex. *Piper nigrum*



Pyrethrin I  
Ex. *Chrysanthemum cinerariaefolium*



Rotenone  
Ex. *Lonchocarpus & Derris spp.*



Surangin B  
Ex. *Mammea longifolia*

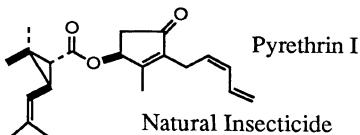
Figure 1. Botanical insecticides. Shaded compounds are commercial products.

Plants have provided not only insecticidal models from their defensive repertoire but have also furnished the lead chemistry for the development of insecticide adjuvants. Sesame oil was discovered to improve and extend significantly the biological activity of many drugs and insecticides including the natural pyrethrins. Two compounds, entirely lacking insecticidal activity but significantly synergising pyrethrin toxicity, were isolated from sesame seed oil and identified as sesamin and sesamolin. Insects treated with pyrethrins are rapidly knocked down but often recover unless a synergist is included in the formulation. The synergists prevent oxidative decomposition of the pyrethrins at labile sites in the molecule, especially the allyl, carbomethoxy, isobutetyl, and pentadienyl moieties (Casida and Maddrell, 1971; Yamamoto and Casida, 1966; Yamamoto et al., 1969). Although natural synergists were used extensively in pyrethrum formulation, synthetic efforts soon capitalized on their chemistry and provided improved synergists such as piperonyl butoxide (Fig. 3).

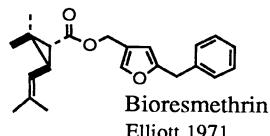
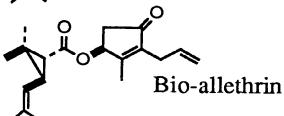
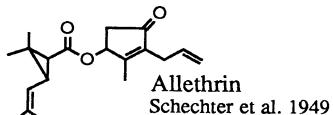
Another botanical model leading to important insecticides was the drug physostigmine from the calabar bean, *Physostigma venenosum*. A few of the commercial products derived from this phytochemical lead are shown in Figure 4.

## INSECT GROWTH REGULATORS

Industry has traditionally placed heavy reliance on seeking nerve poisons, i.e., pyrethroids, carbamate, and organophosphate insecticides that are directly toxic to insects and can rapidly translate into commercial products. Other modalities of insect control have been discovered from academic investigations of insect physiology, biochemistry and chemical ecology, and from studies of non-poisonous secondary chemical defensive systems in plants targeted to the disruption of insect specific processes.



Early Optimization Efforts



Current Synthetic Directions

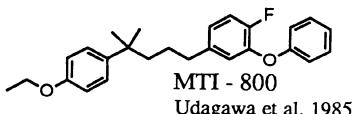
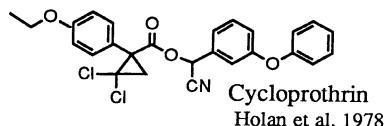
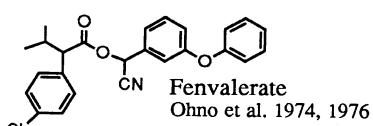
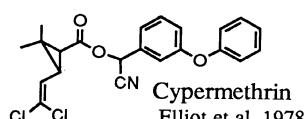
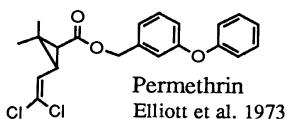


Figure 2. Synthetic optimization of pyrethrin I to pyrethroid insecticides.

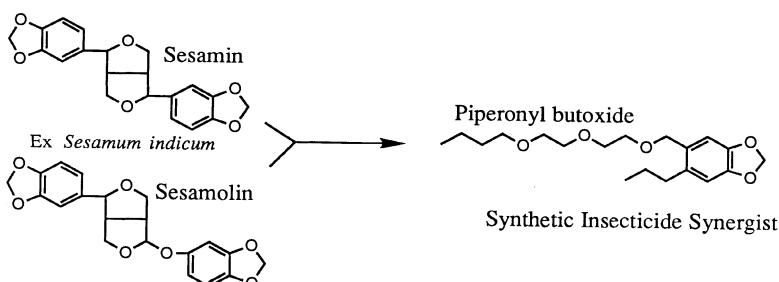
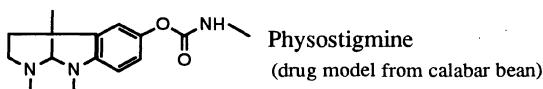


Figure 3. Natural and synthetic synergists.



### Carbamate Insecticides

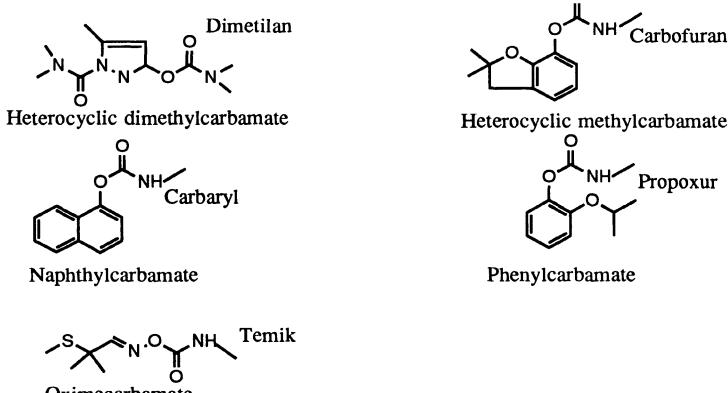


Figure 4. Optimization of physostigmine to carbamate insecticides.

### **Juvenile Hormone**

The discovery of hormones in insects controlling metamorphosis and reproduction, called the juvenile hormones (JH), gave birth to a new class of insect control agents called Insect Growth Regulators (IGRs). Although JH is present during certain critical periods of insect embryogenesis and throughout their immature growth stages, it must be absent during those periods of differentiation called metamorphosis when the immature insect initiates development to the adult stage. If an exogenous source of JH is supplied to insects undergoing adult differentiation, many tissues remain immature resulting in a lethal developmental derangement. Several plant chemicals have been found to mimic natural insect hormones and to interfere with hormonal mediated processes of development, reproduction, and embryogenesis. Since JH has no insecticidal action on the developing immature stages, its original use in insect control was targeted largely to insects that are of economic importance during the adult stage such as mosquitoes, mature breeding flies, fleas, etc. Recent studies, however, have opened up vast new markets for JH analogs when it was revealed that caste differentiation and reproduction in social insect pests, like the imported fire ant and termites, can be interrupted by hormone-containing baits. The loss of "hard" pesticides, like dieldrin and chlordane that were formerly used in fire ant and structural pest control, may be offset by the development of the intrinsically non-toxic insect growth regulators. The chemistry of the natural insect JH seems an ideal model for optimization. The natural JHs are all related to sesquiterpenoids (Fig. 4).

### **Juvenile Hormone Analogs**

The simple structure of the natural JHs has permitted extensive synthetic innovation. The earliest commercial analog, methoprene, was developed directly from

JH III (Fig. 5). It continues as a successful product for animal protection and urban pest control. A second generation of JH analogs has capitalized on increasing aromatic substitution for improved stability and efficacy. The discovery of several cyclic and especially aromatic plant JH mimics gave new insights into the structural liberties that might be taken in optimization efforts and the polyaromatic phenoxyphenoxy analogs, Fenoxycarb and Sumilarv, are several thousand times more active than their natural insect and plant parents. Optimization of the JHs resulted in the first "Biorational" chemicals for insect control (Fig. 6).

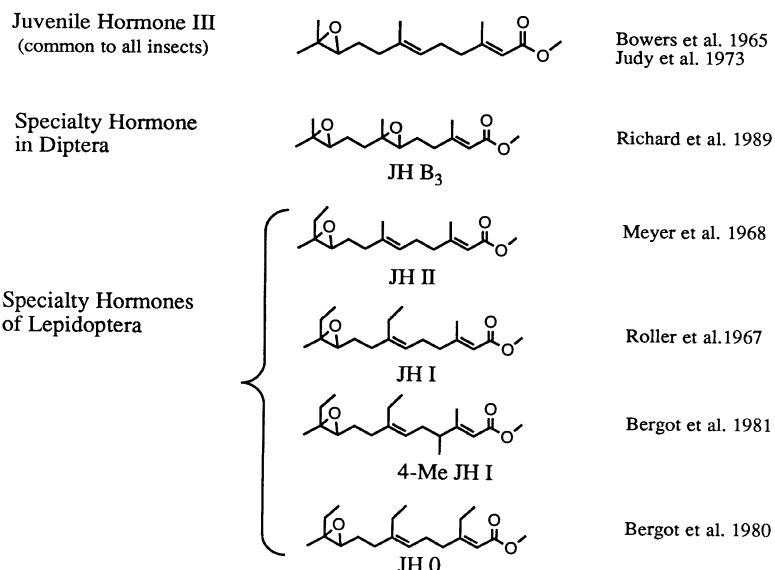
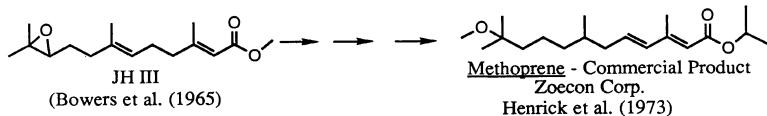


Figure 5. Natural juvenile hormones of insects.

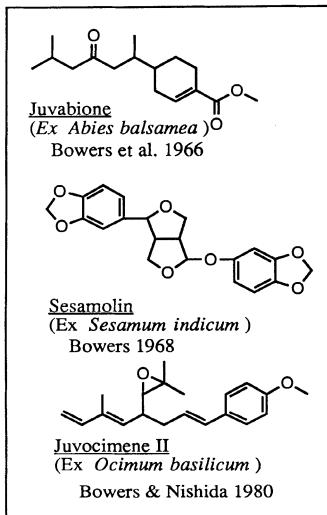
### Antijuvenile Hormones

The principal limitation of JH analogs is their lack of effect on the immature stages of insects. The juvenile stages are the most voracious feeding stages and, therefore, are responsible for most agricultural crop losses. JH analogs have no effect on them since these growing stages require juvenile hormone to sustain their immature status. Basic studies have established that microsurgical removal of the glands that secrete JH causes them to stop feeding and develop into diminutive sterile adults. A chemical method of preventing JH secretion would be an ideal IGR. The investigation of plants as a possible resource of chemicals utilizing antijuvenile hormones as a defensive tactic was the first to demonstrate that JH secretion could

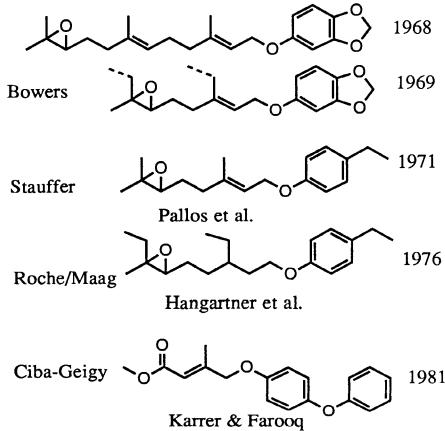
### Natural Juvenile Hormone



### Phyto-Juvenile Hormone Models



### Evolution of Aromatic Analogs



### Commercial Products

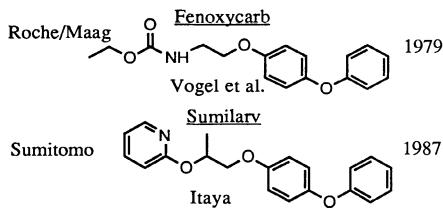
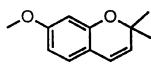
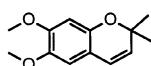


Figure 6. Phytochemical contribution to the evolution of juvenile hormone analogs.

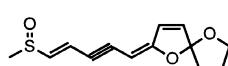


Precocene I  
(Ex *Ageratum houstonianum*)

Bowers 1976, Bowers et al., 1976)



Precocene II



Compound C.  
(Ex *Chrysanthemum coronarium*)

Bowers and Aregullin 1987

Figure 7. Antijuvenile hormones from plants.

be chemically terminated. The first anti-JH compounds were discovered in the plant *Ageratum houstonianum*, a member of the family Compositae. The active compounds were called precocene I and II because of their substituted chromene structure and biological effects that included the induction of precocious metamorphosis and sterilization in insects (Fig. 7). Optimization of precocene chemistry has resulted in some augmentation of activity but compounds of industrial utility remain to be developed. Recently, another compound with anti-JH activity was discovered, isolated, and identified from *Chrysanthemum coronarium*, another plant in the family Compositae (Fig. 7).

Numerous research efforts have explored the possibility of synthesizing rational inhibitors of JH biosynthesis with some success (see review, Staal, 1986) but none has succeeded in discovering compounds of commercial utility. No doubt an investment of effort equivalent to that devoted to the optimization and commercialization of the juvenile hormones will produce successful antihormonal products.

Despite years of investigation, the origin, evolution, and utility of plant secondary chemistry remains largely a mystery. Compounds with direct poisonous consequences to vertebrate and invertebrate herbivores are probably correctly interpreted to be defensive while those with repellent and/or attractant actions that relate to defense or assist in pollination can easily be seen as components in the success of plant survival. In addition to direct toxicants, plants contain a vast repertoire of secondary/defensive chemicals which are now being discovered as basic investigations of insect/plant interactions reveal their chemical ecological roles. Thus, plant chemicals are being discovered that interfere with or otherwise control insect communication (Pheromones), oviposition, feeding (antifeedants, repellents, and antimetabolites), molting, metamorphosis, and reproduction (juvenile hormone analogs and antijuvenile hormones). While not insecticides in the conventional sense, their selective action promises to create new products of increased safety and efficacy. Since plant secondary chemicals possess a genetic basis, they exist as a resource base of natural resistance mechanisms to the molecular biologist/plant breeder for recombinant genetic improvement of pest susceptible commercial plant cultivars. The mystery and the challenge is to understand the meaning and utility of the vast numbers of secondary compounds whose participation in plant biology is not readily apparent. Compounds now seen to possess hormone and antihormone activity, antifeedant, repellency, etc. could only be recognized in their biological context from the basic studies of insect/plant interactions. Many other phytochemical resources await chemical optimization for product development and/or genetic incorporation into commercial cultivars by conventional and recombinant breeding techniques. The much vaunted biotechnology will find its true meaning and success when the natural defensive mechanisms of plants and animals are understood and engineered into the creative service of agriculture and medicine.

## REFERENCES

- Bergot, B. J., Jamieson, G. C., Ratcliff, M. A., and Schooley, D. A., 1980, JH zero: New naturally occurring insect juvenile hormone from developing embryos of the tobacco hornworm, *Science*, 210:336-338.
- Bergot, B. J., Baker, F. C., Cerf, D. C., Jamieson, G. C., and Schooley, D. A., 1981, Qualitative and quantitative aspects of juvenile hormone titers in developing embryos of several insect species: Discovery of a new JH-like substance extracted from eggs of *Manduca sexta*, p. 33-45, in: "Juvenile Hormone Biochemistry: Action, Agonism and Antagonism," G. E. Pratt and G. T. Brooks, eds., Elsevier/North Holland, Amsterdam.
- Bowers, W. S., 1968, Juvenile hormone: Activity of natural and synthetic synergists, *Science*, 161:895-897.
- Bowers, W. S., 1969, Juvenile hormone: Activity of aromatic terpenoid ethers, *Science*, 164:323-325.
- Bowers, W. S., 1976, Discovery of insect antiallatotropins, p. 394-408, in: "The Juvenile Hormones," L. I. Gilbert, ed., Plenum Press, New York, New York.
- Bowers, W. S., and Aregullin, M., 1987, Discovery and identification of an antijuvenile hormone from *Chrysanthemum coronarium*, p. 51-54, in: "Memoria do Instituto Oswaldo Cruz," Supplement III, Volume 82, E. Garcia, ed., Rio de Janeiro, Brazil.
- Bowers, W. S., Fales, H. M., Thompson, M. J., and Uebel, E., 1966, Juvenile hormone: Identification of an active compound from balsam fir, *Science*, 154:1020-1022.
- Bowers, W. S., and Nishida, R., 1980, Juvocimenes: Potent juvenile hormone mimics from sweet basil, *Science*, 209:1030-1032.
- Bowers, W. S., Ohta, T., Cleere, J. S., and Marsella, P. A., 1976, Antiallatotropins: Inhibition of corpus allatum development, *Science*, 193:542-547.
- Bowers, W. S., Thompson, M. J., and Uebel, E. C., 1965, Juvenile and gonadotropic hormone activity of 10,11-epoxyfarnesenic acid methyl ester, *Life Science*, 4:2323-2331.
- Casida, J. E., and Maddrell, S. H. P., 1971, Diuretic hormone release on poisoning *Rhodnius* with insecticide chemicals, *Pesticide Biochemistry and Physiology*, 1:71-83.
- Elliott, M., 1971, The relationship between the structure and activity of pyrethroids, *Bulletin of the World Health Organization*, 44:315-327.
- Elliott, M., Farnham, A. W., Janes, N. F., Needham, P. H., Pulman, D. A., and Stevenson, J. H., 1973, A photostable pyrethroid, *Nature (London)*, 246:169-170.
- Elliott, M., Farnham, A. W., Janes, N. F., and Soderlund, D. M., 1978, Insecticidal activity of the pyrethrins and related compounds. Part XI. Relative potencies of isomeric cyano-substituted 3-phenoxybenzyl esters, *Pesticide Science*, 9:112-116.
- Hangartner, W. W., Suchy, M., Wipf, H. K., and Zurflueh, R. C., 1976, Synthesis and laboratory and field evaluations of a new highly active and stable insect growth regulator, *Journal of Agricultural and Food Chemistry*, 24:169-175.
- Henrick, C. A., Staal, G. B., and Siddall, J. B., 1973, Alkyl 3,7,11-trimethyl-2,4-dodecadienoates, a new class of potent insect growth regulators with juvenile hormone activity, *Journal of Agricultural and Food Chemistry*, 21:354-359.
- Holan, G., O'Keefe, D. F., Virgona, C., and Walser, R., 1978, Structural and biological link between pyrethroids and DDT in new insecticides, *Nature (London)*, 272:734-736.

- Judy, K. J., Schooley, D. A., Dunham, L. L., Hall, M. S., Bergot, B. J., and Siddall, J. B., 1973, Isolation, structure, and absolute configuration of a new natural insect juvenile hormone from *Manduca sexta*, Proceedings of the National Academy of Sciences of the United States of America, 70:1509-1513.
- Karrer, F., and Farooq, S., 1981, Some insect growth regulators with aromatic rings: Their development and biological properties, Scientific Papers of the Wroclaw Technical University, 22:289-302.
- Meyer, A. S., Schneiderman, H. A., Hanzmann, E., and Ko, J. H., 1968, The two juvenile hormones from the *Cecropia* silkworm, Proceedings of the National Academy of Sciences of the United States of America, 60:853-860.
- Ohno, N., Fujimoto, K., Okuno, Y., Mizutani, T., Hirano, M., Itaya, N., Honda, T., and Yoshioka, H., 1974, A new class of pyrethroidal insecticides; -substituted phenylacetic acid esters, Agricultural and Biological Chemistry, 38:881-883.
- Ohno, N., Fujimoto, K., Okuno, Y., Mizutani, T., Hirano, M., Itaya, N., Honda, T., and Yoshioka, H., 1976, 2-Arylalkanoates, a new group of synthetic pyrethroid esters not containing cyclopropanecarboxylates, Pesticide Science, 7:241-246.
- Pallos, F. M., Menn, J. J., Letchworth, P. E., and Miaullis, J. B., 1971, Synthetic mimics of insect juvenile hormone, Nature (London), 232:486-487.
- Richard, D. S., Applebaum, S. W., Sliter, T. S., Baker, F. C., Schooley, D. A., Reuter, C. C., Henrich, V. C., and Gilbert, L. I., 1989, Juvenile hormone bisepoxide biosynthesis *in vitro* by the ring gland of *Drosophila melanogaster*: A putative juvenile hormone in the higher Diptera, Proceedings of the National Academy of Sciences of the United States of America, 86:1421-1424.
- Roller, H., Dahm, K. H., Sweely, C. C., and Trost, B., 1967, Die struktur des juvenilhormons, Angewandte Chemie International Edition in English, 6:179-180.
- Schechter, M. S., Green, N., and La Forge, F. B., 1949, Constituents of pyrethrum Flowers XXIII. Cinerolone and the synthesis of related cyclopentenolones, Journal of the American Chemical Society, 71:3165-3174.
- Staal, G. B., 1986, Anti-juvenile hormone agents, Annual Review of Entomology 31:391-429.
- Udagawa, T., Numata, S., Oda, K., Shiraishi, S., Kodaka, K., and Nakatani, K., 1985, A new type of synthetic pyrethroid insecticide, p. 192-204, *in:* "Recent Advances in the Chemistry of Insect Control," N. F. Janes, ed., The Royal Society of Chemistry, Burlington House, London, England.
- Yamamoto, I., and Casida, J. E., 1966, *O*-Demethyl pyrethrins II analogues from oxidation of pyrethrin I, allethrin, dimethrin, and phthalathrin by a housefly enzyme system, Journal of Economic Entomology, 59:1542-1543.
- Yamamoto, I., Kimmel, E. C., and Casida, J. E., 1969, Oxidative metabolism of pyrethrins in houseflies, Journal of Agricultural and Food Chemistry, 17:1227-1236.

## NATURAL MEDICINES ARE NATURAL PESTICIDES?

J. A. Duke

B-001, R-133  
ARS, USDA  
Beltsville, MD 20705

"Most of the species suggested to have pronounced chemical effects on themselves or other species have been demonstrated subsequently to have such effects...Many, widely used in medicine and...known to have powerful medicinal effects, have pronounced allelopathic effects also." (Rice, 1984)

### INTRODUCTION

I did not believe Bruce Ames (1983) when he said we consumed 10,000 times more natural pesticides than synthetic pesticide residues, so I set out trying to disprove him. But then I learned that tannins were viricidal and essential oils were antiseptic and that tannins and essential oils may constitute up to 10% of the dry weight of such culinary herbs as oregano (Duke, 1992c) meaning that many herbs and spices contain 100,000 parts per million (ppm) natural pesticides or biocides. Spices from *Allium* (chives, garlic, leek, onion, etc.) to *Zingiber* (ginger) are well-endowed with phytochemicals which have proven medicinal and pesticidal properties.

### METHODS

The Father Nature's Farmacy (FNF) database, which includes many pharmaceutically interesting plants, lists all the Generally Recognized as Safe (GRAS) and many Generally Recognized as Food (GRAF) higher plant species and most of the world's most important medicinal species (Duke, 1992a, *ined.*). For each species, I compiled the compounds and elements reported as constituents. Where available, I also calculated and recorded quantities, in parts per million (ppm), with a coded indication of the source(s) of the data. The other database, called the Biologically Active Phytochemicals (BAP) database, lists the biological activities for the compounds and, where available, the Effective Concentrations (ECs), Inhibitory Concentrations (ICs), for various activities and occasionally the lethal doses ( $LD_{50}s$ ), again with cryptic references to the sources for the data. Readers interested in the coded references will find them fully explained in the upcoming database publications (Duke, 1992b, *ined.*).

Though I have been involved in construction of these databases for 5 years, first at home, then at the office, they have always been part-time activities. The databases were moved to the USDA and copied to the NCI as well as to the Herb Research Foundation (HRF) and the Food and Drug Administration (FDA) in early 1990. NCI was interested in the chemopreventive (cancer-preventing) compounds in the database, HRF in the medicinal and toxic compounds, and FDA more in the toxic compounds. In many cases, the FDA was looking for the harmful constituents in herbs, the HRF for the healthful and/or medicinal constituents. For the NCI, I augmented my databases on conventional foods and herbs that they deemed most promising as cancer preventives, including several representatives of the cabbage, carrot, citrus, garlic, mint, and potato families. At the same time, Dr. Chris Beecher was preparing NAPRALERT (Natural Products Alert Database, representing more than 50 persons/year's compilation) printouts on these same species. It was clear, in aggregating data from NAPRALERT with the FNF databases, that NAPRALERT was much stronger in pharmacological compounds while FNF was often stronger in nutritional compounds and elements and in quantitative data. On average, NAPRALERT seemed to augment FNF by about 40% and FNF seemed to augment NAPRALERT by about 40%. NAPRALERT is mostly a primary compilation, while FNF suffers from being a secondary compilation. For FNF, I exhaustively searched my most promising compilations, all of which will be cited in Duke, 1992a, *ined*.

## RESULTS

As of September 1991, the FNF database treats about 1,000 species in ca. 1.5 megabytes (ca. 900 text pages) of a WordPerfect database. The BAP database treats about 3,000 compounds in about 350,000 bytes (ca. 200 text pages). In preparation for a West Virginia workshop (Duke, 1991a,b), I combed the BAP database, asterisking entries that could be deemed pesticidal, e.g., antiseptic, bacteristat, molluscicide, etc., but not necessarily flagging those compounds termed cytotoxic in NCI reports. About half of the entries now bear the asterisk. Many of the data and conclusions have been derived and updated from the West Virginia workshop.

Here, I reiterate FNF entries for pennyroyal, *Hedeoma pulegioides*, and mountain mint, *Pycnanthemum muticum*, two aromatic West Virginia weeds that may prove useful in repelling ticks. Although the malodorous garlic (*Allium sativum*) has been reported to repel both the dog-tick and the deer-tick genera, respectively the carriers of Rocky Mountain Spotted Fever and Lyme Disease, the pennyroyal was not mentioned as a tick repellent by Grainge and Ahmed (1988). Garlic smells bad to and repels some people as well, apparently, as some ticks. Pennyroyal smells good to some humans, rather like corn mint (*Mentha arvensis*), which also repels ticks and which contains up to 25,000 ppm pulegone, one of the dominant aroma compounds reported in pennyroyal (Table 1).

Numbers indicate the quantity of the individual compounds in ppm. Ranges were often calculated by multiplying low percentages for essential oil by the lowest report of the percentage of the individual compound and the highest by the highest. Note that the highest pulegone figure is 27,600 ppm (equals up to 2.76% of the biomass). Pennyroyal is not a bulky plant, but it is the most frequently used natural insect repellent in rural Pendleton County, West Virginia, at least among my acquaintances there. Often growing in the same forest is the much bulkier mountain mint, some chemical constituents of which are tabulated in Table 2.

This more advanced printout from the database shows not only the ppm of most chemicals but the plant parts (SH = Shoots) and the source (BML = Lawrence,

Table 1. Compounds reported from American Pennyroyal (For sources of data, see Duke, 1992a, *ined.*).

Acetic acid	Menthol 6-30
Butyric acid	(-)-Menthone 36-420
Caryophyllene 30-150	Methylcyclohexanone
1,8-Cineole 6-30	Methyl salicylate
Decyclic acid	Myrcene 30-150
Diosmin 10,000	3-Octanol 18-90
Dipentene	1-Octen-3-ol 48-240
Essential oil 6,000-30,000	3-Octyl acetate 18-90
<i>trans</i> - $\beta$ -farnesene	Octylic acid
Formic acid	$\alpha$ -pinene 24-210
Germacrene-d 36-180	$\beta$ -pinene 12-180
$\alpha$ -humulene 30-150	Piperitone 12-270
Isoheptylic acid	Piperitenone 1-1,410
Isomenthone 48-9,300	Pulegone 3,678-27,600
Limonene 36-570	Sabinene 18-90
Linalyl acetate 36-180	Salicylic acid
Menthofuran 1	Tannin

1981). Note that mountain mint, which probably produces 10 times the biomass of pennyroyal, also has a higher percentage of pulegone. Unlike the ephemeral annual pennyroyal, mountain mint is a prolific perennial. Under management, mountain mint could produce a conservatively estimated five MT biomass containing 1 to 4% pulegone or 50 to 200 kilograms pulegone per hectare.

While it is difficult for me to believe that any of the named compounds are inert, I do not have reported activities for all of them in the BAP database. Some (14) of the BAPs in various pennyroyal species are listed in Table 3.

The asterisks, as mentioned, indicate that the compound has some pesticidal activity. In this case, only 2 of the 14 bioactives in pennyroyal did not have reported pesticidal (or pheromonal) activity. Stated differently, if the pennyroyal compounds are average for the whole database, then 12 of 14 (86%) of the compounds are pesticidal. Conversely, 13 of 14 (93%) of the bioactive compounds have medicinal activity and 12 of 24 (86%) have both pesticidal and medicinal activities. I'll venture to predict that, if all the compounds were intensively screened for many activities, 100% would show pesticidal and medicinal activities.

Tables 1 and 2 are derived from the FNF database and may be searched mechanically. Table 3 is derived from the BAP database which may also be searched mechanically. With Macro programs in WordPerfect, one can ask the FNF database to list all species with quantitative data for any element, like boron, or any compound, like salicylic acid. Strange revelations often follow with corn salad (*Valerianella*) turning up highest quantitatively for boron, coffee beans (*Coffea*) for bactericidal chlorogenic acid, caraway for insecticidal (and cancer-preventing) limonene, dandelion flowers (*Taraxacum*) for lecithin, evening-primrose seed (*Oenothera*) for tryptophan, pears (*Pyrus*) for caffeic acid, pansy (*Viola*) and pagoda tree (*Sophora*) for rutin, self-heal (*Prunella*) for antiherpetic rosmarinic acid, etc. Of course, these data are no better than the sources from which I have compiled. Supposed superlatives should be checked out to verify that they are indeed uncommonly high in the element or compound of interest.

Table 2. Compounds reported from mountain mint.

<i>trans</i> - $\alpha$ -Bergamotene 29-200 SH BML	Menthone 2,320-11,300 SH BML
Borneol 58-1,350 SH BML	D-menthone 2,320-4,000 SH BML
Camphepane 1-50 SH BML	Myrcene 29-200 SH BML
Caryophyllene 29-300 SH BML	Neoisomenthol 1-50 SH BML
1,8-Cineole 1-50 SH BML	Neomenthol 29-100 SH BML
<i>p</i> -Cymene SH BML	<i>cis</i> -Ocimene 1-50 SH BML
Essential oil 29,000-50,000 SH BML	1-Octen-3-ol 29-900 SH BML
Germacrene-D 1-350 SH BML	$\alpha$ -Pinene 29-200 SH BML
Isomenthone 29-2,100 SH BML	$\beta$ -Pinene 29-150 SH BML
<i>cis</i> -Isopulegone 29-750 SH BML	Piperitone 1-200 SH BML
<i>trans</i> -Isopulegone 116-200 SH BML	Piperitonene 27-950 SH BML
Limonene 87-900 SH BML	<i>trans</i> -Piperitol 1-100 SH BML
Menthol 87-450 SH BML	Pulegone 17,980-40,250 SH BML
D-menthol 4,350-7,500 SH BML	Sabinene 1-50 SH BML

Table 3. Biologically active phytochemicals in pennyroyal.

*Acetic acid: Antivaginitic; bactericide (5,000 ppm); osteolytic; verrucolytic; LD <sub>50</sub> = 3,310 (oral, rat).
*Caryophyllene: 30 to 150 ppm. Insectifuge; spasmolytic; termitifuge.
*1,8-Cineole: 6 to 30 ppm. Anesthetic; antibronchitic; antilaryngitic; antipharyngitic; antirhinitic; antiseptic; antitussive; choleretic; CNS-stimulant; hepatotonic; herbicide (IC <sub>50</sub> = 3 to 180 mM); hypotensive; insectifuge; LD <sub>50</sub> = 2,480 (oral, rat).
Diosmin: 10,000 ppm. Anticapillary-fragility; antihemorrhoidal; antimetrorrhagic.
*Formic acid: Antiseptic; antisyncopic; astringent; counterirritant. LD <sub>50</sub> = 1,100 (oral, mus). LD <sub>50</sub> = 1,210 (oral, rat).
*Germacrene-D: 36 to 180 ppm. Pheromonal.
*Limonene: 36 to 570 ppm. Anticancer; cancer-preventive; insecticide; insect repellent. LD <sub>10</sub> = 4,600 (oral, rat).
*Menthol: 6 to 30 ppm. Anesthetic; anti-inflammatory; antipruritic; CNS depressant; counterirritant; rubefacient; vibriocide. LD <sub>50</sub> = 3,180 (oral, rat).
Methyl salicylate: Analgesic; anti-inflammatory; antipyretic; antirheumatic. LD <sub>10</sub> = 170 (oral, hmn); LD <sub>50</sub> = 887 (oral, rat); LD <sub>50</sub> = 1,110 (oral, mus).
*Myrcene: 30 to 150 ppm. Bactericide; insectifuge; spasmolytic.
*Pinene: 12 to 390 ppm. Antiseptic; expectorant; herbicide (IC <sub>50</sub> = 30 mM); insect repellent.
*Piperitone: 12 to 270 ppm. Antiasthmatic; herbicide (IC <sub>50</sub> = 30 mM).
*Pulegone: 3,678 to 27,600 ppm. Antipyretic; avifuge; herbicide (IC <sub>50</sub> = 1.5 mM); insecticide; pulifuge. LD <sub>50</sub> = 150 (ipr, mus).
*Salicylic acid: Analgesic; antidermatotic; antieczemic; antineuralgic; antiperiodic; antipodagric; antipsoriac; antipyretic; antirheumatic; antiseborrheic; bactericide; febrifuge; fungicide; keratolytic; tineacide; ulcerogenic. LD <sub>10</sub> = 450 (oral, dog). LD <sub>50</sub> = 891 (oral, rat).

\*Indicates that the compound has some pesticidal activity.

There are thousands of interesting compounds tabulated in my databases. Here are entries for limonene and *d*-limonene.

\*Limonene: AChE-inhibitor; anticancer; antilithic; bactericide; cancer-preventive; herbicide; insectifuge; insecticide; irritant; sedative ED = 1 to 32 mg/kg; viricide; LD<sub>50</sub> = 4,600 (oral, rat);

\**d*-Limonene: Anticancer (breast); antitumor-promoter; cancer-preventive; hypocholesterolemic; insecticide; insectifuge; litholytic; LD<sub>10</sub> = 4,600 (oral, rat).

The above entries from BAP suggest that a beverage rich in limonene might help in cancer-prevention or reversion, especially in breast cancer, if orally ingested limonene can be proved effective. Weiner (1991) summarizes about 10 studies showing that dietary intake of the insecticide *d*-limonene can lower the incidence of chemically induced cancers as well as delay their appearance. It even "is effective in reversing preformed tumors, as evidenced by an increase in the tumor regression rate...dietary *d*-limonene was effective in reducing the numbers of chemically induced mammary tumors in rats when provided either during the initiation phase or during the promotion/progression phase...Up to 90% of tumors completely disappeared in mice fed this compound." Further, "limonene is a potent, natural cholesterol-lowering compound...Limonene is also a powerful agent for dissolving gallstones (Weiner, 1991).

It would not be wise of me to suggest that "limonenade," like soybean milk, might prevent breast cancer, even though the NCI may make the latter suggestion. The USDA does not encourage its personnel to encourage dietary modification to prevent disease. My main theme is: Moderation in all things except variety. Echoing the Surgeon General and the USDA, I recommend an increase in the proportional quantity and variety of fruits, vegetables, grains, and, to a lesser degree, nuts.

I prepared Table 4, not for potential sufferers of breast cancer, gallstones, hypercholesterolemia, or insomnia, but for potential sufferers of bug bites. You see, limonene is an insect repellent. The USDA still studies natural repellent, if not disease-preventive, activities of plants. Table 4 could be considered a "formula" that contains high-limonene plants that might repel insects. It is a list of the GRAS and GRAF herbs and foods richest (over 5,000 ppm on a dry weight basis according to Duke, 1992a, *ined.*) in limonene.

I enjoy beverages combining any of these limonene-containing herbs that I have on hand. I will increase my intake of such fruits and herbs, even if I am not targeted for breast cancer. There are a lot of bugs at my home, Herbal Vineyard (organic), and where I work, USDA (relatively "inorganic").

Because of recent USDA interest in controlling the zebra mussel, I also searched the BAP database for molluscicidal compounds. The results are shown in Table 5.

Of the estimated 2,000 pesticidal compounds in the BAP database, 36 (1.8%) were reported as molluscicides. Of these molluscicidal compounds, 12 (33%) had medicinal activities that were not necessarily related to their pesticidal properties, but closer to 66% were medicinal in the broader sense, including the key words antiseptic, bactericide, candidicide, and fungicide. If molluscicides prevent schistosomiasis, all (100%) might be viewed as medicinal in this even broader sense.

The database can be searched mechanically for any key word therein and is proving very useful at addressing questions posed by the taxpaying public as well as by my USDA peers and superiors.

Table 4. Leading sources of limonene (Source, Duke, 1992a,b, *ined.*).

Potential "limonenade" ingredients		
<i>Apium graveolens</i>	Celery	530 to 24,700 SD
<i>Carum carvi</i>	Caraway	7,860 to 30,180 SD
<i>Citrus aurantifolia</i>	Lime	2,795 to 6,400 FR
<i>Citrus aurantium</i>	Sour orange	1,000 to 8,000 FR
<i>Citrus limon</i>	Lemon	2,796 to 8,000 EO
<i>Citrus reticulata</i>	Tangerine	6,500 to 9,400 FR
<i>Citrus sinensis</i>	Orange	8,300 to 9,700 FR
<i>Elettaria cardamomum</i>	Cardamon	595 to 9,480 FR
<i>Foeniculum vulgare</i>	Fennel	200 to 9,420 FR
<i>Illicium verum</i>	Star-anise	100 to 5,220 FR
<i>Mentha spicata</i>	Spearmint	200 to 5,725 FR
<i>Myristica fragrans</i>	Nutmeg	720 to 5,760 FR
<i>Pycnanthemum</i> spp.	Mountain mint	100 to 14,500 PL (not GRAS)
<i>Thymus vulgaris</i>	Thyme	15 to 5,200 PL

Table 6 shows an entry for berberine from the BAP database (Duke, 1992b, *ined.*). Berberine also illustrates the fact that natural medicinal compounds are often also natural pesticides. Giardia is one of the pests against which berberine is reportedly effective, at 10 mg/kg body weight. With me, weighing 100 kg, I figure it would take a gram ( $100 \times 10 \text{ mg} = 1,000 \text{ mg} = 1 \text{ gram}$ ) of berberine to treat me for giardia. The LD<sub>50</sub> (lethal dose at which half of an experimental population is killed) indicates that, were I an experimental 100 kg rat, 10 grams might kill me. That is a rather narrow therapeutic dose.

Berberine, in addition to reported medicinal activities as anti-inflammatory, anticancer, antiulcer, astringent, cardiodepressant, carminative, choleric, colyrial, fever-reducing, hemostatic, hypotensive, immunostimulant, pain killing, respirostimulant, spasmolytic, uterotonic, and vasoconstrictor, also has a reputation against a wide array of pests: amoeba, bacteria, candida, cholera (*Vibrio*), dysentery, fungi, giardia, leishmania, malaria, protozoa, trichomonads, trypanads, and viruses. Thus, the compound, berberine, presumably evolved because it extends a selective advantage in protecting its producer from pests, may also protect us from a variety of pests and diseases. Like so many phytochemicals, well over 1,000 in my database, medicinal phytochemicals are also pesticidal.

## CONCLUSIONS

My 5 painful years in the construction of these databases has led me to several, still somewhat speculative, conclusions.

(1) Most biologically active compounds have several bioactivities, some medicinal, some pesticidal. Often, the dosage determines whether these compounds will be medicinally or pesticidally active in experimental subjects. Minute doses may actually stimulate the immune system through hormesis. Higher doses may be medicinal or bioregulatory. Still higher doses may be homocidal or pesticidal, if you choose to treat these as two rather than one category.

(2) Often a plant contains a suite of similar compounds with rather similar bioactivities. These frequently prove to be synergistic. In such cases, whole plant

Table 5. Phytomolluscicides in BAP database.

- \*Allodesacetylconfertiflorin: molluscicide.  
\*Anacardic acid: antitumor; bactericide; molluscicide (< 10 ppm); nematicide; schistosomicide.  
\*Balanitin: antifeedant; antiseptic; molluscicide.  
\*Bayogenin (glycoside): Fungicide (7.5 to 25 ppm); molluscicide (7.5 to 25 ppm).  
\*Bergapten: Antiaperfif; anticonvulsant; antihistaminic; anti-inflammatory; antipsoriac; antitumor; hypotensive; insecticide; molluscicide; spasmolytic.  
\*Cantalasaponin-2: Molluscicide; schistosomicide.  
\*Cardanol: Molluscicide (ED = 80 to 100).  
\*Carol: Molluscicide (ED = 7 to 15 ppm).  
\*Chalepensin: Molluscicide.  
\*Confertiflorin: Molluscicide.  
\*Cytisine: Anti-inflammatory; molluscicide; psychoactive; LD<sub>50</sub> = 101 (oral, mus).  
\*Damnacathin: Molluscicide.  
\*Dodonoside: Molluscicide.  
\*Hederagenin (glycoside): Fungicide (7.5 to 25 ppm); molluscicide (7.5 to 25 ppm).  
\*Imperatorin: Anticonvulsant; anti-inflammatory; antileukodermic; hepatotoxic; molluscicide; antiviligic; LD<sub>10</sub> = 600 (par, mus).  
\*Isopimpinellin: Antiaffpetant; antifeedant; anti-inflammatory (100 ppm); diuretic (125 mg/kg); insecticide; molluscicide; mutagenic.  
\*Isotenulin: Molluscicide.  
\*Lemmatoxin: Antifertility; helicicide (LD<sub>90</sub> = 1.5 mg/L); molluscicide (LD<sub>90</sub> = 1.5 mg/L); spermicide.  
\*Lupanine: Hypoglycemic; molluscicide.  
\*Medicogenic acid: Aphicide; candidicide; fungicide (7.5 to 25 ppm); molluscicide (7.5 to 25 ppm).  
\*2-Methylantraquinone: Antifeedant; molluscicide (10 ppm).  
\*7-Methyljuglone: Allelochemic (IC<sub>95</sub> = 8 mM); fungicide; molluscicide (5 ppm); termitecide.  
\*Mukaadial: Molluscicide.  
\*Naphthoquinone: Antifeedant; fungicide; molluscicide; rubefacient.  
\*Oleanolic acid-3-O-glucoside: Molluscicide.  
\*Oruwacin: Molluscicide (1 to 3 ppm).  
\*Oruwal: Molluscicide (10 ppm).  
\*Phebalosin: Antitumor; crustacicide (47 ppm); fungicide; molluscicide.  
\*Polygodial: Antifeedant; candidicide; helicicide; molluscicide; piscicide.  
\*Primulic acid: Molluscicide.  
\*Solamargine: Fungicide; molluscicide.  
\*Solasoline: Cardiotonic; molluscicide.  
\*Soranjidiol- $\alpha$ -acetate: Molluscicide (10 ppm).  
\*Sparteine: Anti-inflammatory; cathartic; diuretic; hypoglycemic; molluscicide; oxytocic; LD<sub>10</sub> = 30 (ivn, rbt).  
\*Tomatine: Anti-inflammatory; bactericide; fungicide; molluscicide; LD<sub>10</sub> = 800 (oral, rat).  
\*Warburganal: Antifeedant (0.1 to 10); candidicide (3 to 12 ppm); cytotoxic (10 ppb); fungicide (3 to 100); helicicide; molluscicide (5 to 10); piscicide.  
\*Xanthotoxin: Antifeedant; antihistaminic; anti-inflammatory (20 mg/man/day); antipsoriac; antispasmodic; antiviligic (20 mg/man/day); herbicide; insecticide; molluscicide; spasmolytic.

\*Indicates that the compound has some pesticidal activity.

Table 6. Printout for berberine (Source, Duke, 1992b, *ined.*).

\*Berberine: Amoebicide 414/; analgesic; anticholeric 150 mg/man/day MAR; anticonvulsant 411/; antidiarrheic MAR; antidyserteric HG24:37; antigiardial (10 mg/kg/day) 414/; anti-inflammatory HG23:17; antileishmanic 10  $\mu$ g/mL (PR4(4):132); antimarial 50  $\mu$ M (PR4(4):132); antitrachomic MAR; antitubercular 414/; antitumor; antiulcer; astringent; bactericide 411/; candidicide 414/; cardiodepressant; carminative; choleric 411/; collyrium; febrifuge M11; fungicide; hemostat; hypotensive 411/; immunostimulant; myocardiodepressant; protisticide; respirodepressant MAR; RNA-depressant; sedative 411/; stomachic; trichomonicide M29; trypanocide 411/; uterotonics 411/; vasoconstrictor 414/; viricide; MLD = 24.3 (ipr, mus) 52/; LD<sub>50</sub> = 100 mg/kg; (for code to references, see Duke, 1992a, *ined.*)

\*Indicates that the compound has some pesticidal activity.

extracts might be both more economical and more effective than solitary isolates. Ames et al. (1990) state "Dozens of mammalian metabolites are commonly produced from any reasonably complex molecules." From this we might infer, rightly or wrongly, that if a plant has 10,000 complex molecules, there might be more than 100,000 metabolites in mammals who ingested the plant. If differences between individual herbs and herbivores are genetic, and genetics is based upon chemically different genes and reactions controlled by these genes, there are about 5 billion chemically distinct humans whose chemical profiles vary with ecological, temporal (diurnal, lunar, seasonal, and annual, at least), and psychological conditions to react differently to the metabolites resulting from the ingestion of foods coming from millions of individuals of hundreds of food species. Small wonder that pharmaceutical and pesticidal industries prefer to conduct their research on pure compounds instead of highly variable whole-plant concoctions.

(3) Contrary to my previous unlearned expectations, semisynthetic derivatives of natural compounds are not necessarily more toxic to humans or experimental animals than the natural compound from which they were derived. In the first four data sources I perused, hoping to prove that synthetics were more dangerous, the converse proved true; more often than not, semisynthetic derivatives were *less* active than the natural toxin from which they were derived. Rationalizing, I suspect that easy modifications of the molecules might have already occurred in nature, being selected against, if they were less toxic. So, when the chemist makes the same semisynthetic modification in the lab, it proves less toxic in his experiments.

(4) Oregano contains 100,000 times more natural pesticides on a ppm basis than synthetic pesticide residues, even more than the 10,000 Ames (1983) laments (Duke, 1992c). I suspect that the 6-magnitude difference observed in oregano applies to most spices, whereas Ames's 5-magnitude difference applies to most conventional foods normally consumed. Some people argue that the naturals are less harmful because we have co-evolved with them. The immune system is a huge complex of rapidly evolving cells which, when healthy, are quick to learn to recognize a new alien, friend or foe, synthetic or natural.

(5) Industry is talented at accidentally or intentionally removing fibers, minerals, and/or nutrients from foods. Might they just channel these talents to removing natural pesticides from the food chain, putting them into recyclable pesticide containers, leaving the synthetics, like the relatively harmless Alar, in the minds of man instead of the mouths of babes? *If* natural pesticides are more rapidly degradable than synthetics, as some scientists maintain, environmental and health benefits might accrue from using food-derived natural pesticides rather than synthetics.

(6) All plants contain phytobiocides (PBCs; that makes them sound dangerous), and all plants contain medicinal compounds. All plants contain toxins and antitoxins, oxidants and antioxidants, nutrients and antinutrients. All plants contain vitamins, minerals, etc. Until we have firm quantitative data, the statement that a plant contains an acaricidal compound or a phytoalexin is relatively meaningless. We need to know how much it contains and how much it takes to do the job. If you define a biocidal or medicinal plant as one that contains a biocidal or medicinal compound, all plants are biocidal and medicinal.

(7) More formal databases than a WordPerfect file are better for massive movement of data, for updating, and for cross-tabulations.

WordPerfect 5.1 with macros can be very useful. All tables in this paper were generated in less than 5 minutes each from the database.

Early entries in the database (like *Hedeoma* above) were based on a dozen major sources not individually referenced. Sources of data for every compound and

every biological activity should be individually documented for fact-checking purposes. I can backtrack through my methodology, but others cannot.

## ACKNOWLEDGMENTS

Since 1986, I have been accumulating, into user-friendly databases, a catalog of the published phytochemical constituents (with their biological activities) of all the GRAS (generally recognized as safe) and many of the GRAF (generally recognized as food) herbs. In early 1990, I was invited to collaborate with Dr. Herbert Pierson's National Cancer Institute's (NCI) Designer Food Program. Then I moved the database from a computer system at home (connected to the University of Maryland's Botany Department) to a USDA COMPAQ 386/20 Deskpro, moving the data from a Star database to a Word Perfect system manipulable by Macro programs. Thanks are extended to Dr. J. Reveal and Dr. R. Broome for 4 years of cooperation in maintaining and manipulating the Star Database and to Dr. Broome for restructuring the database to make it more user friendly. Thanks are also extended to Dr. Herbert Pierson for encouraging this expansion of my database to include those food species the NCI is reviewing for their potential to prevent cancer. Thanks also to Dr. Allan Stoner for encouraging this collaboration, albeit in the mistaken belief that the USDA and I would be supporting the NCI program for a 5-year program.

## REFERENCES

- Ames, B. N., 1983, Dietary carcinogens and anticarcinogens, *Science*, 221:1256-1262.
- Ames, B. N., Profet, M., and Gold, L. S., 1990, Nature's chemicals and synthetic chemicals: Comparative toxicology, *Proceedings of the National Academy of Science, USA*, 87:7782-7786.
- Duke, J. A., 1991a, Natural plant compounds as pesticides, p. 291-298, *in:* "Biological Control of Postharvest Diseases of Fruits and Vegetables (Workshop Proceedings)," C. Wilson and E. Chalutz, eds., USDA, ARS-92, June 1991, 324 p.
- Duke, J. A., 1991b, What's happening with natural compounds, p. 299-318. *in:* "Biological Control of Postharvest Diseases of Fruits and Vegetables (Workshop Proceedings)," C. Wilson and E. Chalutz, eds., USDA, ARS-92, June 1991, 324 p.
- Duke, J. A., 1992a, *ined.*, "CRC Handbook of Biologically Active Phytochemicals in 1,000 GRAS Herbs, Foods and Medicinal Plants," Ca. 900 pages, to be published as hard copy and as computer diskette (WordPerfect 5.1).
- Duke, J. A., 1992b, *ined.*, "CRC Handbook of Biologically Active Phytochemicals and Their Biological Activities," Ca. 200 pages, to be published as hard copy and as computer diskette (WordPerfect 5.1).
- Duke, J. A., 1992c, *ined.*, "Biting the Biocide Bullet, Proceedings of the Third International Symposium on Poisonous Plants," Iowa State University Press, Ames, Iowa.
- Grainge, M., and Ahmed, S., 1988, "Handbook of Plants with Pest-Control Properties," John Wiley and Sons, New York, New York, 470 p.
- Lawrence, B. M., 1981, "Essential Oils 1979-1980," Allured Publishing Corporation, Wheaton, Illinois, 291 p.
- Rice, E. L., 1984, "Allelopathy," 2nd Edition, Academic Press, Inc., Orlando, Florida, 422 p.
- Weiner, M. A., 1991, Orange peel oil studies as cancer fighting agent, *Herbal Healthline*, 2(2):12.

## NATURAL TOXICANTS IN FOODS

Ross C. Beier

Food Animal Protection Research Laboratory

U.S. Department of Agriculture\*

Agricultural Research Service

Route 5, Box 810

College Station, TX 77845

Herbert N. Nigg

Citrus Research and Education Center

University of Florida, IFAS

700 Experiment Station Road

Lake Alfred, FL 33850

### INTRODUCTION

The purpose of exploring the potential naturally occurring toxic hazards of food plants is not to suggest an irrational avoidance of these common foods. However, it is important to identify, define, and investigate the natural toxicants in our foods and to provide some perspective on these chemicals and to show clearly that their toxicology is unknown in most cases. Many natural toxicants have functions in a manner similar to synthetic pesticides or other biohazardous chemicals. Humans apply synthetic pesticides to food and ornamental plants to prevent insect, fungal, and other pest damage. However, plants produce natural toxicants to protect themselves from pathogens and pests. The natural pesticide concentration in our foods may be as much as 10,000 times higher than that of synthetic pesticide residues (Ames, 1983). Because of the protection they provide to plants, these natural chemicals are prime candidates to be bred into plants by plant producers and plant breeders (Barz et al., 1990).

The main consideration of the Committee on Food Protection, National Research Council (1973), when reviewing natural toxicants in foods, was "the hope that it may contribute to a more informed, realistic, and sensible attitude on the part of the public toward the food supply." Today, as was the case in 1966, natural toxic components in foods have received little study (Committee on Food Protection, National Research Council, 1966). Most people routinely accept that plants eaten in their "pristine" state are not only safe for one's health but are better than plants "manipulated" by man (e.g., pesticide treated or fertilized with manufactured

\*Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

nutrients). Many people believe that if the food is natural, then it is naturally good for you; but consider one case in point. The plant family *Solanaceae* includes species that are both highly poisonous and also used for some common medicinal drugs. For example, *Solanum nigrum* L. and *Atropa belladonna* L. are extracted for their bioactive drugs, including atropine, scopolamine, and hyoscyamine. Tobacco is also related to these plants, as are common food plants such as eggplant, garden peppers, tomato, and white potato. Livestock have died after ingesting potato vines, green potatoes, or tomato vines; and human poisoning episodes and fatalities also have been reported (Hansen, 1925; Kingsbury, 1964; McMillan and Thompson, 1979; Morris and Lee, 1984; Willmott, 1933; Wilson, 1959).

There are a number of points in the human food chain through which natural toxicants can gain entry. Some of these are: meat, milk, eggs, fish, grains, fruits, herbs, vegetables, and liquids (beer, water, wine, etc.). Our first example of the occurrence of a naturally occurring toxicant appearing in our food chain involves milk. This will be followed by notable examples of natural toxins in food plants.

### Milk Sickness

The disease in humans referred to as "milk sickness," was first noted in North Carolina by the time of the American Revolution and remains the classical example of milk poisoning. In animals, the disease is called "trembles," which is based on the signs of muscle trembling of poisoned animals. White snakeroot (*Eupatorium rugosum* Houtt) was the etiologic agent responsible for milk sickness in humans and trembles in animals; but over a century went by before the plant was connected with the disease. Milk sickness in humans was caused by the use of milk or milk products from animals consuming this plant. Trembles in animals was caused by directly ingesting the plant or, in young animals, by utilizing milk from poisoned mothers. A thorough description of the plant, habitat, historical aspects, and isolation of components are presented by Beier and Norman (1990). White snakeroot may be found in damp open areas of the woods, shaded areas, along rivers, and in steep canyons. Figure 1 indicates the distribution of white snakeroot throughout the United States (Committee Report, 1981; Hardin, 1973; Harvill et al., 1986; Jones and Coile, 1988; Ownbey and Morley, 1992; Sperry et al., 1964; Stotts, 1984).

The first written description of the disease milk sickness was in 1809 by Dr. Thomas Barbee (Niederhofer, 1985). The first published name for the disease, 'Sick Stomach', was coined by an anonymous author in 1811.

Abraham Lincoln's mother, Nancy Hanks Lincoln, died during an epidemic of the disease in 1818 at Pigeon Creek, Illinois (Christensen, 1965). Nancy's great aunt and great uncle, as well as two neighbors, died within a few weeks of each other during the same epidemic.

The disease progresses slowly in humans and is characterized by restlessness with vague pains, vomiting, loss of appetite, constipation, acetone breath, severe acidosis, coma, and death. Recovery from an attack is slow and may never be complete (Couch, 1927).

The literature on white snakeroot is very vast, as it has been written about since 1809. Unfortunately, information is so diverse and inconsistent that it is very difficult to follow the true story surrounding white snakeroot poisoning. It is interesting to note that an article by Molyneux and James (1990) incorrectly gives Drake credit for establishing the causal relationship for the disease. In fact, Drake's outstanding reputation in the scientific community prompted the acceptance of his incorrect theory and stopped research into the real cause of milk sickness (Niederhofer, 1985).

The suspected causitive agent in white snakeroot poisoning is tremetone (Fig. 2)



Figure 1. The dotted area indicates the distribution of white snakeroot throughout the U.S.

(Bonner and DeGraw, 1962; Bonner et al., 1961). There were three main ketones isolated from white snakeroot: dehydrotremetone, tremetone, and hydroxytremetone (Fig. 2). However, synthetic tremetone was not active in animal tests (Bowen et al., 1963), and again there was a lull in research on the cause of milk sickness.

The availability of a good bioassay seemed to be the main stumbling block. Many bioassays were evaluated for possible success in showing toxic activity to components from white snakeroot and a microsomal activation assay was selected (Beier et al., 1987). The assay allowed the isolation and identification of the primary activatable toxic component in white snakeroot which is indeed tremetone (Fig. 2). Crude extracts of rayless goldenrod (*Isocoma wrightii* (jimmyweed)), which cause trembles and milk sickness in the Southwestern United States (Snively and Furbee, 1966), also are positive in the microsomal activation assay.

### Phytoalexins

Bell (1974) has reviewed ways in which plants may express resistance to pathogens. The production of phytoalexins (toxic chemicals) is a major mechanism of plant defense. There have been many definitions for the term phytoalexin; and certainly none seem to cover the complexity of biosynthesis or the range of biological activities of these compounds. A working definition of the term phytoalexin is "low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms" (Paxton, 1980). Phytoalexins exhibit toxicity across much of the biological spectrum; however, their activity is not just confined to microorganisms (Smith, 1982). Various chemical groups that comprise some of these phytoalexins are discussed in reviews by Bailey and Mansfield (1982), Bell (1981), and Grisebach and Ebel (1978). These chemical groups include the coumarins, glycoalkaloids, isocoumarins, isoflavonoids, linear furanocoumarins,

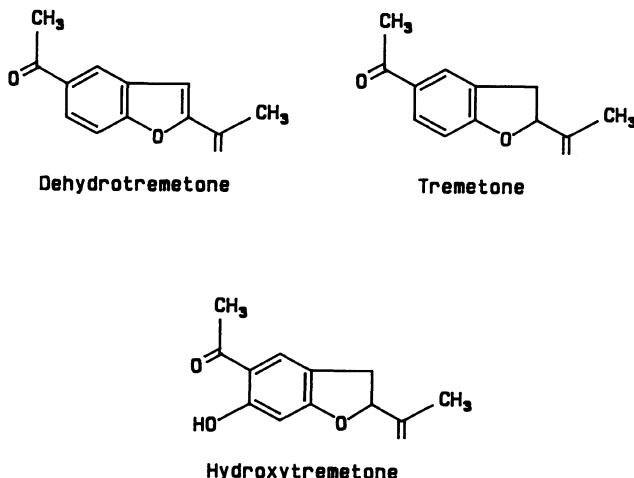


Figure 2. Three main ketones isolated from white snakeroot (Bonner and DeGraw, 1962).

stilbenes, and terpenoids. An abbreviated list of phytoalexins found in some common food plants is presented in Table 1.

Induction of phytoalexin synthesis can result from a plant's exposure to many kinds of stimuli, e.g., bacterial or viral infection (Lord et al., 1988; Ryan, 1973), exposure to cell wall fragments (Tietjen and Matern, 1984; Ryan, 1988; Ryan et al., 1986), cold, UV light, heavy metal salts (Beier and Oertli, 1983), antibiotics, fungicides (Grisebach and Ebel, 1978), herbicides (Kömives and Casida, 1983), at feeding sites of nematodes (Kaplan et al., 1980; Rich et al., 1977; Veech, 1979), and acidic fog can stimulate the phytoalexin response in celery (Derckx et al., 1990). A single stimulus like the herbicide acifluorfen can increase the production of phytoalexins and stress metabolites in crops as diverse as bean, celery, cotton, pea, soybean, and spinach (Kömives and Casida, 1983). Since plants have the ability to increase the levels of phytoalexins in response to external stimuli, it is important to know the foods in which such natural chemicals may be a potential problem for humans. It also is important to understand how the chemical content of our foods can be unfavorably altered by various treatments during production, handling, processing, shipping, and marketing.

In a hypothetical society that does not use synthetic pesticides, an environmental health scientist would be well-advised to take a close look at the food consumed by the inhabitants of that society. Reasons for doing so include (Rodericks, 1978):

1. The number and types of natural-occurring compounds present in foods.
2. The immense number of chemically uncharacterized compounds present in foods.
3. The unknown toxic effects of these chemicals.
4. The level and frequency of human consumption of the compounds in foods.

These four reasons for scientific investigation of the food consumed in a hypothetical society do not differ from the real society in which we live. Thus, the chemical and toxicological study of food deserves serious attention. To help focus on naturally occurring pesticides as potential human toxicants, a Thanksgiving Day dinner

Table 1. Phytoalexins in some food plants.

Plant	Phytoalexins
Alfalfa	Vesitol, sativan, medicarpin
Pea	Pisatin, cinnamylphenols, 2'-methoxychalcone
Soybean	Glyceollin
Bean	Phaseollin
Broadbean	Wyerone
Grapes	Viniferin, resveratrol
Cotton	Gossypol, cadalenes, lacinilenes, hemigossypol
Peanut	Resveratrol
Celery	Furanocoumarins
Parsley	Furanocoumarins
Parsnip	Furanocoumarins
Rice	Momilatones, oryzalexins
Castor bean	Casbene
Potato	Rishitin, hydroxylubimin, phytuberin, $\alpha$ -solanine, $\alpha$ -chaconine, lubimin, solavetivone, phytuberol
Pepper	Capsidiol
Sweet potato	Ipomeamarone
Carrot	6-Methoxymellein, falcarinol
Tomato	$\alpha$ -Tomatine, rishitin, falcarindiol, falcarinol
Lima bean	5-Deoxykievitol, 8,2'-dihydroxygenistein
Tobacco	Rishitin, lubimin, phytuberin, phytuberol, solavetivone, capsidiol, glutinosone
Eggplant	Lubimin

menu developed by the American Council on Science and Health (1987) is presented in Table 2, and the potential toxicants are listed. The health effects of these toxicants include blood pressure elevation from tyramine (wine) and antithyroid activity from glucosinolates (broccoli). The dinner also includes a variety of mutagens and carcinogens like hydrazine (mushrooms) and eugenol (cranberry sauce).

In the 1978 review, "Phytoalexins and Human Health," phytoalexins of garden pea (pisatin), green bean (phaseolin), and carrot (chlorogenic acid and myristicin) (Fig. 3) were discussed (Surak, 1978). A 400 mg dose of myristicin administered to humans will produce cerebral excitations, and larger doses may produce hallucinations (Truitt et al., 1961). Myristicin is found in carrots at 0.6  $\mu\text{g/g}$  (Yates and England, 1982), which means it would require about 1,468 pounds of carrots to produce hallucinations. On the other hand, the common spice, nutmeg, has been a drug of abuse due to its narcotic-like effects. Myristicin was shown to be converted in the rat liver to 3-methoxy-4,5-methylenedioxymphetamine (MDMA) (Braun and Kalbhen, 1973). Nutmeg contains approximately 2.5% myristicin (Archer, 1988). A dose of 16 to 20 g of nutmeg would be expected to cause a narcotic or hallucinogenic effect. Carrot also contains the acetylenes: carotatoxin (*trans*-1,10-heptadecadiene-5,7-dien-3-ol), 10  $\mu\text{g/g}$  (Crosby and Aharonson, 1967); falcarinol, 18.2  $\mu\text{g/g}$ ; falcarindiol, 41.6  $\mu\text{g/g}$ ; acetylfalcarindiol, and falcarinolone (Fig. 4). Falcarindiol has antifungal activity and is possibly a phytoalexin (Yates and England, 1982). Carotatoxin is neurotoxic to mice with an  $LD_{50}$  of about 100  $\mu\text{g/g}$  and is toxic to *Daphnia magna* Straus (Crosby and Aharonson, 1967).

The consumption of oxalate-rich food plants may lead to calcium deficiency as well as poor absorption of iron, magnesium, and copper. Acute toxic effects from oxalate may include muscle cramps, cardiovascular collapse, and renal insufficiency. The tomato may contain a mean oxalate concentration of 15,000  $\mu\text{g/g}$ , and spinach

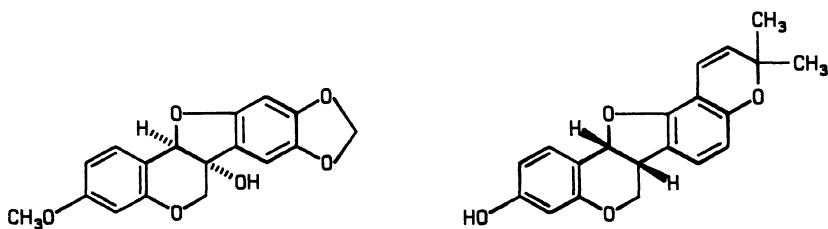
Table 2. Thanksgiving dinner menu.<sup>a</sup>

Course	Chemical composition includes
<b>Appetizer</b>	
Cream of Mushroom Soup	Hydrazines
Fresh vegetable tray	
Carrots	Carotatoxin, myristicin, isoflavones, nitrate
Radishes	Glucosinolates, nitrate
Cherry tomatoes	Hydrogen peroxide, nitrate, quercetin glycoside, tomatine
Celery	Nitrate, psoralens
<b>Entree</b>	
Roast turkey	Heterocyclic amines, malonaldehyde
Bread stuffing w/onions, celery, black pepper, mushrooms	Benzo[a]pyrene, di- and tri-sulfides, ethyl carbamate, furan derivatives, hydrazines, psoralens, safrole
Cranberry sauce	Eugenol, furan derivatives
<b>Choice of vegetable</b>	
Lima beans	Cyanogenetic glycosides
Broccoli spears	Allyl isothiocyanate, glucosinolates, goitrin, nitrate
Baked potato	Amylase inhibitors, arsenic, chaconine, isoflavones, nitrate, oxalic acid, solanine
Sweet potato	Cyanogenetic glycosides, furan derivatives, nitrate
Rolls with butter	Amylase inhibitors, benzo[a]pyrene, ethyl carbamate, furan derivatives, diacetyl
<b>Dessert</b>	
Pumpkin pie with cinnamon and nutmeg	Myristicin, nitrate, safrole
Apple pie with cinnamon	Acetaldehyde, isoflavones, phlorizin, quercetin glycosides, safrole
<b>Beverages</b>	
Coffee	Benzo[a]pyrene, caffeine, chlorogenic acid, hydrogen peroxide, methylglyoxal, tannins
Tea	Benzo[a]pyrene, caffeine, quercetin glycosides, tannins
Red wine	Alcohol, ethyl carbamate, methylglyoxal, tannins, tyramine
Water available upon request	Nitrate
<b>Assorted nuts</b>	
Mixed nuts	Aflatoxins

<sup>a</sup> American Council on Science and Health (1987).

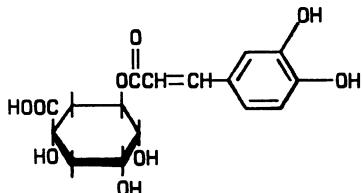
leaf may contain a mean of 68,500 µg/g fresh weight. The lethal dose of oxalic acid for humans varies from 2 to 30 g (Libert and Franceschi, 1987).

A number of food toxicants are also described in part A and B of the book entitled "Food Toxicology" by J. M. Concon (1988a,b). Theobromine (Fig. 5) in tea and cocoa powder (2%) may be a potential health hazard. Theobromine can produce testicular atrophy and spermatogenic cell abnormalities in rats (Ames, 1983). Alfalfa sprouts fed to monkeys cause a severe lupus erythematosus-like syndrome (Malinow et al., 1982). Burned and browned materials produced from cooking protein are mutagenic, and oils used for preparing foods or making salad dressings might also have adverse biological activities. There has been an increased use of cottonseed



Pisatin

Phaseolin



Chlorogenic acid



Myristicin

Figure 3. Phytoalexins from various vegetables. Large doses of myristicin can produce hallucinations (Truitt et al., 1961).

oil. Crude cottonseed oil contains gossypol (Fig. 5), even when it is obtained from glandless cotton (Fisher et al., 1988), and this compound causes reversible sterility in males at an oral dose of about 10 mg per day (Ames, 1983). Hydrogenated oils used in margarine having a *cis-trans* isomerization of lipids may play a role in cancer and aging. These are a few examples of the many naturally occurring potential toxicants in foods.

As scientists, we have more than the simple responsibility of advancing the production rates and postharvest quality of our foods; we are also responsible for the wholesomeness and the subtle toxicological effects that foods may have on humans. It has been estimated that as much as 35% of all cancer might be related to diet (Doll and Peto, 1981). Cancer is only one ailment that may be related to the presence of natural toxicants in our diet. There are many more subtle problems that potentially may be diet related, including the ubiquitous condition, arthritis.

## EXAMPLES OF TOXINS IN FOOD PLANTS

### CRUCIFERS (CRUCIFERAE; *BRASSICA*): BROCCOLI, BRUSSELS SPROUTS, CABBAGE, AND CAULIFLOWER

Cruciferous vegetables contain natural compounds that exhibit a variety of biological activities. In ancient times, these crops were cultivated primarily for medicinal purposes (Fenwick et al., 1983). The first adverse biological activity investigated was their goitrogenic activity. Plants that contain natural goitrogens and belong to this group of vegetables are listed in Table 3.

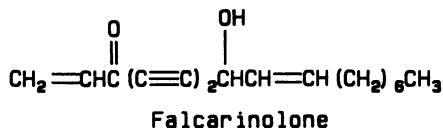
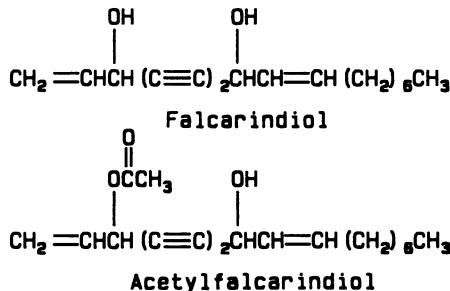
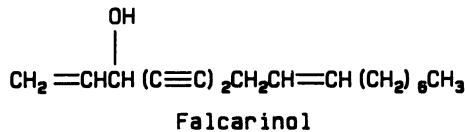
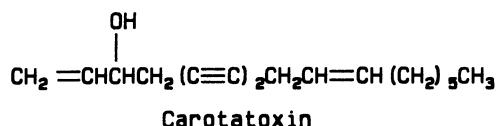
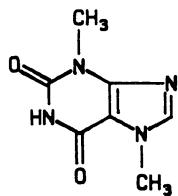


Figure 4. Acetylenes from carrot.

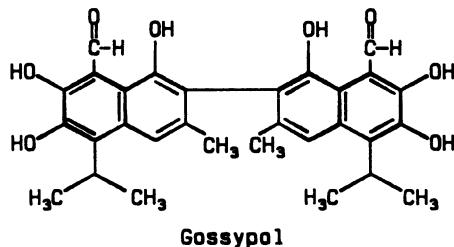
## Goitrogens

As early as 1928, laboratory animals fed cabbage were induced to develop goiters (Chesney et al., 1928; Webster et al., 1931). Lambs were also lost from ewes that were fed plants containing goitrogenic components. The exact cause of the goiter induced during these early investigations was not known (Langer and Greer, 1977; Matovinovic, 1983).

Experiments with rats and guinea pigs in 1964 showed that cabbage has a marked goitrogenic capacity (Langer et al., 1964). The first goitrogen isolated from cabbage was thiocyanate, but its effect at twice the concentration found in cabbage was less than the total observed effects from cabbage itself (Langer and Štolc, 1964). The goitrogenicity of cabbage and other cruciferous plants can be explained by the combined action of thiocyanate, goitrin, and allyl isothiocyanate (Langer, 1966). These compounds are enzymatically hydrolyzed from various glucosinolates. McDowell et al. (1988) reviewed the chemical and biological properties of indole glucosinolates. The range of glucosinolate concentrations found in various cruciferous vegetables is listed in Table 4 (Fenwick et al., 1983). Brussels sprouts have the highest observed levels of glucosinolates, 1,430 to 1,760 µg/g of fresh sprouts. The types of glucosinolates and their quantities have been determined in 22 different varieties and various head sizes of cabbage (VanEtten et al., 1976). These glucosinolates, and their concentrations in medium sized hybrid cabbage, are listed in Table 5. The total glucosinolate concentration was 663 µg/g of fresh cabbage. The



Theobromine



Gossypol

Figure 5. Theobromine from tea and cocoa powder, and gossypol from cottonseed.

Table 3. Plants containing goitrogenic compounds.<sup>a</sup>

Latin name	Common name
<i>Beta vulgaris</i> var. <i>ciela</i>	Chard
<i>Brassica carinifera</i>	Kohlrabi
<i>Brassica hirta</i>	White mustard seed
<i>Brassica napus</i>	Rape seed or meal
<i>Brassica nigra</i>	Black mustard seed
<i>Brassica oleracea</i> var. <i>acephala</i>	Kale
var. <i>botrytis</i>	Broccoli
var. <i>capitata</i>	Cabbage
var. <i>gemmifera</i>	Brussels sprouts
var. <i>napobrassica</i>	Rutabaga
<i>Brassica pekinensis</i>	Chinese cabbage
<i>Brassica rapa</i>	Turnip root
<i>Glycine max</i>	Soybean
<i>Linum usitatissimum</i>	Flax
<i>Juglans regia</i> <sup>b</sup>	Walnut
<i>Arachis hypogaea</i> <sup>b</sup>	Peanut

<sup>a</sup>From Kingsbury (1964).

<sup>b</sup>From Matovinovic (1983).

Table 4. Glucosinolate content of cruciferous vegetables.

Vegetable	Concentration ( $\mu\text{g/g}$ )		Reference
	Mean	Range	
Broccoli	740	450 - 1,480	Mullin and Sahasrabudhe, 1977
Brussels sprouts	1,590	1,430 - 1,760	Mullin and Sahasrabudhe, 1977
	2,000	600 - 3,900	Heaney and Fenwick, 1980
Cauliflower	480	270 - 830	Mullin and Sahasrabudhe, 1977
Red cabbage	770	470 - 1,240	VanEtten et al., 1980
	320	160 - 460	Mullin and Sahasrabudhe 1977
White cabbage (kraut)	610	430 - 760	VanEtten et al., 1980
	890	670 - 1,020	VanEtten et al., 1976
White cabbage (market)	530	260 - 1,060	VanEtten et al., 1980
	650	300 - 1,070	VanEtten et al., 1976

Table 5. Types of glucosinolates found and their concentrations in medium size hybrid cabbage.<sup>a</sup>

Glucosinolates of the following chemicals	Concentration ( $\mu\text{g/g}$ )
Allyl isothiocyanate	41
3-Methylthiopropyl isothiocyanate	1
3-Methylsulfinylpropyl isothiocyanate	55
Butenyl isothiocyanate	4
5-Vinyloxazolidinethion (goitrin)	7
4-Methylthiobutyl isothiocyanate	0.2
4-Methylsulfinylbutyl isothiocyanate	12
4-Methylsulfonylbutyl isothiocyanate	4
Benzyl isothiocyanate	1
Phenylethyl isothiocyanate	2
3-Indolylmethyl and <i>N</i> -methoxy-3-indolylmethyl isothiocyanate	29
Total glucosinolate content	663

<sup>a</sup> From VanEtten et al. (1976).

highest total glucosinolate concentrations found in four cabbage varieties, Red Hollander, Savoy Perfected Drumhead, Wisconsin Hollander, and Stonehead, were 1,203, 1,288, 1,014, and 1,065  $\mu\text{g/g}$  of fresh cabbage, respectively (VanEtten et al., 1976).

### Carcinogenicity Modulation

Rabbits fed cabbage leaves survived a lethal dose of uranium (Eisner, 1931). This led to the epidemiological conclusion in the 1970s and 1980s that cruciferous vegetables can provide protection from cancer. Indoles in vegetables of the *Brassica*

genus were known to inhibit carcinogenesis in experimental animals. 3-Substituted indoles, indole-3-carbinol (I3C) or 3-indolemethanol, 3-indoleacetonitrile or 3-indolylacetonitrile, and 3,3'-diindolylmethane (Fig. 6) are inhibitors of induced cancer (Bradfield and Bjeldanes, 1987b). These three indoles are produced by enzymatic hydrolysis of indolylmethylglucosinolate (glucobrassicin) (Fig. 6) by the pH-dependent plant enzyme, myrosinase, following disruption of plant material (Loub et al., 1975; Virtanen, 1965).

Nearly 80 naturally occurring glucosinolates have been described. Isothiocyanates or nitriles formed from the glucosinolates are dependent on the type of plant material and the treatment of the material prior to and during hydrolysis (VanEtten and Tookey, 1983). Seven isothiocyanates were tested for mutagenicity on *Salmonella typhimurium* TA100, and all tested positive with allyl isothiocyanate having the highest potency. Allyl isothiocyanate glucoside (sinigrin) showed an equivalent mutagenicity potency to allyl isothiocyanate itself. Thiocyanates were found to be non-mutagenic, while isocyanates showed mutagenicity on *S. typhimurium* TA100 even without activation (Yamaguchi, 1980). Sedation, ataxia, loss of righting reflex, and sleep were induced in rats by 3-indolylacetonitrile and I3C. Phenylpropyl isothiocyanate and allyl isothiocyanate were not teratogenic to rat fetuses, but they did cause embryonal death and decreased fetal weight (Nishie and Daxenbichler, 1980). Animals fed diets high in cruciferous vegetables and then exposed to various carcinogens expressed lower tumor yields and increased survival rates (Boyd et al., 1982; Stoewsand et al., 1978; Wattenberg, 1983).

It is readily known that many drugs and other chemicals induce metabolizing enzymes. This ability, when possessed by a chemical component of food, does not differ from the inducing effects of other chemicals. Animal feeding studies have demonstrated induction of mixed-function oxidases (MFO) in rats fed Brussels sprouts or cabbage (Wattenberg, 1971) and cauliflower (Babish and Stoewsand, 1975), and the indoles present in these vegetables were shown to be the cause of the induction (Loub et al., 1975; Pantuck et al., 1976). Especially noted is induction of the intestinal aryl hydrocarbon hydroxylase (AHH) system (Babish and Stoewsand, 1978; Wattenberg, 1971). I3C isolated from *Brassica oleracea*, var. *gemmaifera*, cv. Jade Cross, and was a significant inducer of hepatic and intestinal MFOs (Loub et al., 1975). I3C was shown to enhance the activities of rat intestinal AHH and ethoxycoumarin *O*-deethylase (ECD), which are capable of metabolizing benzo[ $\alpha$ ]pyrene and other xenobiotics. Additionally, both hepatic and intestinal glutathione *S*-transferase (GST) and microsomal epoxide hydrolase (EH) of the small intestine were induced by I3C. Hepatic cytochrome P-450 was increased by Brussels sprouts and I3C (Bradfield and Bjeldanes, 1984).

A diet containing Brussels sprouts and cabbage increases the apparent metabolic clearance rate of antipyrine and phenacetin (Pantuck et al., 1979) and acetaminophen, while also enhancing its glucuronide conjugation in humans (Pantuck et al., 1984). I3C administered orally to humans increased estradiol 2-hydroxylation (Michnovicz and Bradlow, 1990). The effect of I3C on estradiol 2-hydroxylation is similar to that caused by smoking.

Epidemiological studies indicate that colon cancer risk is higher in individuals who ate fewer cruciferous vegetables (Graham et al., 1978). Epidemiological studies of people who had gastric cancer also suggested that those who ate cruciferous vegetables were protected (Graham et al., 1972; Hirayama, 1977). Other epidemiological studies indicated a lower incidence of breast cancer (Armstrong and Doll, 1975; Phillips, 1975) and prostatic cancer (Correa, 1981; Phillips, 1975) in vegetable-consuming populations. Evidence from an epidemiological case-control study of diet and cancer also suggested that consumption of cruciferous vegetables was associated with a decreased incidence of cancer (Graham, 1983).

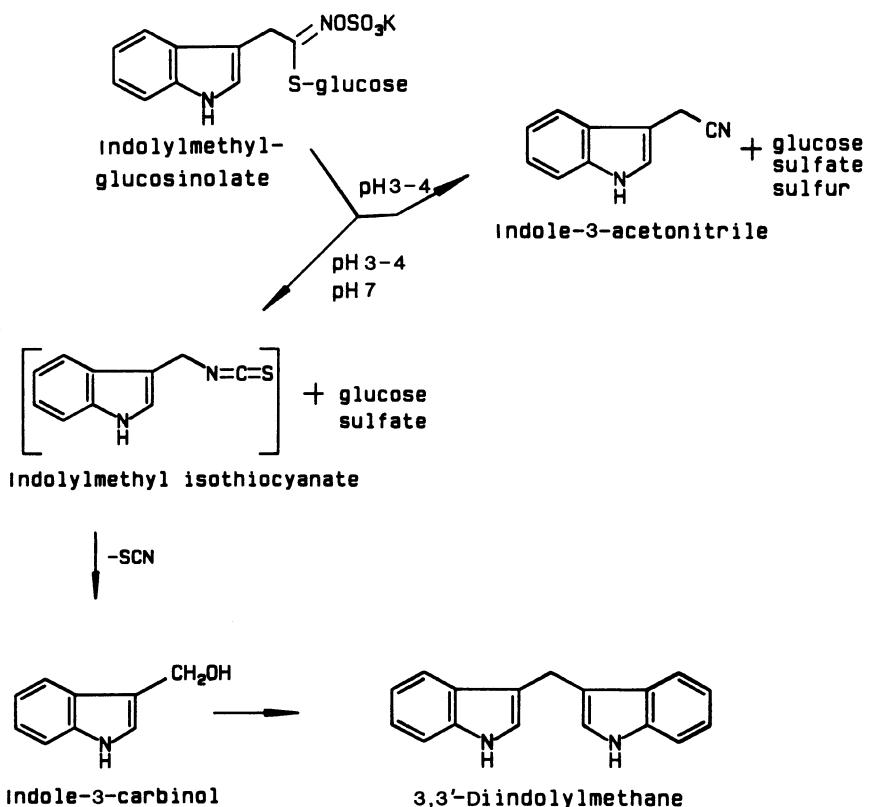


Figure 6. Enzymatic hydrolysis of indolylmethylglucosinolate. Adapted from Loub et al., 1975.

The public has been advised to include more cruciferous vegetables, such as cabbage, broccoli, Brussels sprouts, kohlrabi, and cauliflower, in their diets (American Cancer Society, 1984; National Research Council, 1982). Today, physicians are making the same recommendation.

Unfortunately, the early feeding experiments generally used only one type of protocol; cruciferous vegetables or I3C was given prior to or during administration of a carcinogen. When this protocol was changed so that a carcinogen was given before consumption of cruciferous vegetables or I3C, higher cancer rates were obtained in laboratory animals treated with cruciferous vegetables or I3C. When I3C was given to trout before aflatoxin B<sub>1</sub>, the trout were protected against liver carcinogenesis (Bailey et al., 1985). However, when aflatoxin B<sub>1</sub> was given before I3C, hepatocarcinogenesis was strongly promoted (Bailey et al., 1987). This promoter effect was also observed with 1,2-dimethylhydrazine enhancement of colon cancer in rats (Pence et al., 1986). Indole-3-carbinol was most responsible for tumor morbidity in mice and appears to promote tumorigenesis by inducing AHH activity. Colon tumor incidence increased where 1,2-dimethylhydrazine was administered to mice fed a diet containing cabbage (Temple and El-Khatib, 1987). Feeding cabbage to hamsters elevated the incidence of gallbladder adenocarcinoma, plus feeding high fat diets elevated pancreatic ductular carcinoma in hamsters administered N-nitroso-*bis*-(2-oxopropyl) amine (BOP). Skin papilloma, initiated by

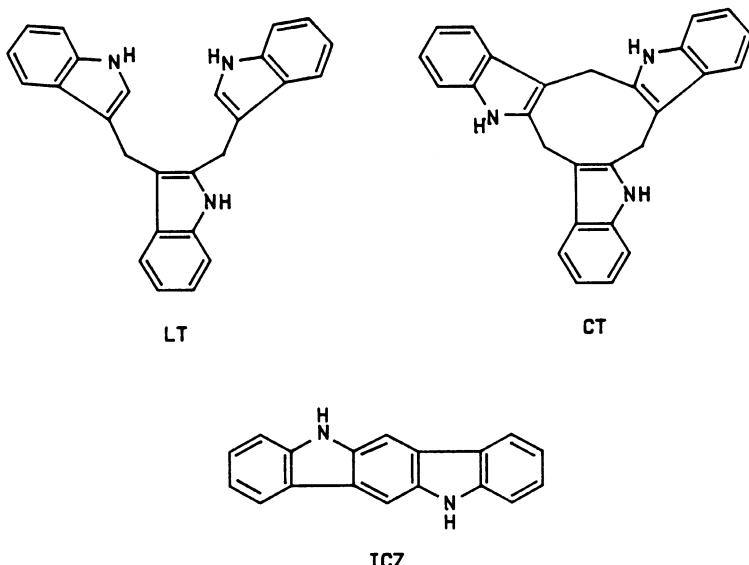


Figure 7. Condensation products of indole-3-carbinol in acidic conditions similar to the stomach.

7,12-dimethylbenz[a]anthracene (DMBA) and promoted by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), increased when mice were fed diets containing 10% dried cabbage (Birt et al., 1987).

Studies of the possible biochemical mechanisms behind the promotional effects of I3C have been initiated. Indole-3-carbinol, given intraperitoneally, does not induce hepatic ethoxresorufin *O*-deethylase activity (EROD). But, given orally, it does induce EROD (Bradfield and Bjeldanes, 1987b). Acid treatment of I3C, under conditions similar to stomach acid conditions, produced a reaction mixture that induced EROD by either the intraperitoneal or oral route. Chromatographic separation of the acid reaction mixture suggests that there are at least four I3C condensation products that will induce EROD (Bradfield and Bjeldanes, 1987a). The mechanism by which I3C and its analogs induce cytochrome P-448 dependent monooxygenases is mediated via condensation products generated in the acidic conditions of the stomach (Bradfield and Bjeldanes, 1987b).

Glucobrassicin (Gmelin and Virtanen, 1961) and neoglucobrassicin (Gmelin and Virtanen, 1962) are proposed as the sources of 3-indolylacetonitrile, I3C, and other simple indolic compounds. The glucobrassicin content of fresh Brussels sprouts varies from 220 to 1,110 µg/g (Heaney and Fenwick, 1980). The pH-dependent enzymatic hydrolysis of these indole glucosinolates by myrosinase produces I3C and a series of simple indoles (Loub et al., 1975; Virtanen, 1965) (Fig. 6). Indole-3-carbinol, 3-indolylacetonitrile, and the well-known MFO inducer, 3,3'-diindolylmethane (Fig. 6), from cabbage and cauliflower were all demonstrated to induce the AHH receptor (Loub et al., 1975). Since compounds such as indolo[3,2-*b*]carbazole (ICZ) (Fig. 7) can be generated from 3,3'-diindolylmethane in the presence of acid, air, and light (Bergman, 1970), it was postulated that the carbazole may be present (Bradfield and Bjeldanes, 1987b). ICZ was also the most active of all the indoles studied for inducing the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) receptor (Gillner et al., 1985).

The three most prevalent UV-absorbing compounds in the reaction mixture of acid treated I3C are 2-(indol-3-ylmethyl)-3,3'-diindolylmethane (LT), 5,6,11,12,17,18-hexahydrocyclonona[1,2-*b*:4,5-*b'*:7,8-*b''*]triindole (CT) (Fig. 7), and 3,3'-diindolylmethane (Fig. 6) (Bradfield and Bjeldanes, 1991). Molar yields of the three were in the range of 2 to 6% of the original amount of I3C. The presence of LT and 3,3'-diindolylmethane was consistent with previous findings (Amat-Guerri et al., 1984). Upon further analysis, ICZ was shown to be present in the acid condensation reaction mixture (Bjeldanes et al., 1991). ICZ is produced from I3C in yields on the order of 0.01% *in vitro* and, after oral intubation, *in vivo*. The receptor-binding affinity of ICZ is  $3.7 \times 10^2$  times that of the binding affinity of TCDD. The most potent AHH receptor agonist identified in the acid condensation reaction mixture is ICZ. ICZ and related condensation products appear responsible for the inducing effects of dietary I3C. Due to the higher yields of the weaker binding oligomers, ICZ appears of roughly equal importance to other oligomers in inducing activity of the mixture. TCDD has well-known and established activities as both an anti-initiator and as a promotor of carcinogenesis. Similar effects are observed for the cancer modulating activity of I3C. I3C or TCDD given before a carcinogen protects against carcinogenesis; whereas, when either are given after a carcinogen, they both strongly promote carcinogenesis. The AHH-receptor binding of ICZ and TCDD are similar in all respects. A 100 g portion of Brussels sprouts could provide a dose of from 0.256 to 1.28 µg ICZ. This dose is considerably in excess of the maximum acceptable daily human dose for TCDD established by the U.S. Environmental Protection Agency—which is at 400 fg/70kg person. However, there may be a number of factors that could lower the relative hazard or benefit and the half-life of ICZ compared to TCDD. Bjeldanes et al. (1991) concluded that it appears unlikely that normal levels of ICZ in the diet are a significant hazard compared with the benefit of the micronutrients in *Brassica* vegetables.

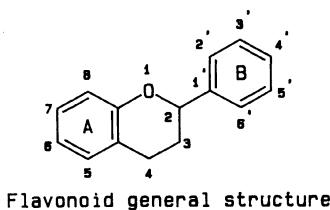
## FRUITS AND VEGETABLES

### Flavonoids

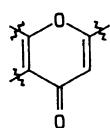
The flavonoid literature is enormous. We refer the reader to several books for a general view of flavonoid chemistry and biochemistry (Cody et al., 1986, 1988; Das, 1989; Harborne, 1988; Harborne et al., 1975a,b) (Fig. 8 for general structure).

Flavonoids are widespread in the plant kingdom. *The Flavonoids*, 1988, edited by Harborne, contains extensive distributional data. McClure (1975) reviewed the function and physiology of flavonoids. Plant flavonoid content may be influenced by light, water, temperature, sugars, mineral nutrition, mechanical damage, pathogens, plant growth regulators, and various other chemicals (de Riera et al., 1988; Downum et al., 1991; McClure, 1975). Flavonoids may be localized in plant tissues and cells and secreted in various exudates. They may function as antioxidants, enzyme inhibitors, pigments for light absorbance, and visual attractants for pollinators, light screens, promoters or inhibitors of plant growth, plant growth regulators, legume *Rhizobium* root nodule gene inducers (Hungria et al., 1991a,b), phytoalexins, and morphogenic and sex determination agents (McClure, 1975).

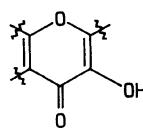
**Flavonoid Metabolism.** Booth et al. (1956) found 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid), and *meta*-hydroxyphenylacetic acid (*m*-HPAA) in rabbit, rat, guinea pig, and human after oral doses of quercetin. These compounds were presumptively identified from urine by paper chromatography. The proposed metabolic sequence is presented in Figure 9. When quercetin was given i.p. (species not specified), bypassing gut metabolism,



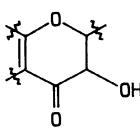
Flavonoid general structure



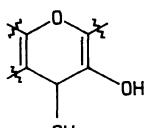
Flavones



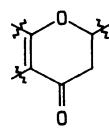
Flavonols



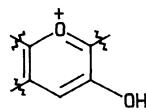
Flavanonols



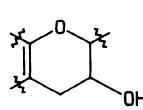
Leucoanthocyanins



Flavanones



Anthocyanins



Catechins

Figure 8. Flavonoid general structures.

homovanillic acid was found in urine but not 3,4-dihydroxy or *meta*-hydroxyphenylacetic acid. Conjugates were not found and metabolites were not found in intestinal contents (rabbit only) (Booth et al., 1956). Hesperidin, eriodictyol, homoeriodictyol, and diosmin (Fig. 10) were metabolized to *meta*-hydroxyphenyl propionic acid (Fig. 11) and other metabolites in the rabbit (Booth et al., 1958). In the rat, with hesperetin (aglycone of hesperidin), diosmetin, eriodictyol, and homoeriodictyol, *m*-HPPA was a major metabolite. Homoeriodictyol was also excreted unchanged. Humans consuming hesperetin or hesperidin excreted 3-hydroxy-4-methoxyphenylhydrylic acid and a small amount of glucuronic acid conjugate (Booth et al., 1958). In the rat, (+)-catechin was excreted as *m*-hydroxyphenyl propionic acid, *m*-hydroxyhippuric acid, and (+)-catechin (Griffiths, 1964). Administration of antibiotics eliminated detection of these metabolites from the urine of (+)-catechin fed rats. When 20 mg of (+)-catechin was given i.p. to rats, no phenolic metabolites were found in urine; (+)-catechin was excreted unchanged for 24 hours after injection. Catechin was not excreted in feces (Griffiths, 1964).

Intestinal microflora have been implicated in metabolism of flavonoids to phenolic acids. Phenylvalerolactones were excreted in the urine of the guinea pig, rat, rabbit, and man (Griffiths, 1975). Rats bred under germ-free conditions and maintained on germ-free diet did not excrete phenolic-type metabolites when fed (+)-catechin, apigenin, naringin, myricetin, hesperidin, and rutin. Aglycone and ring

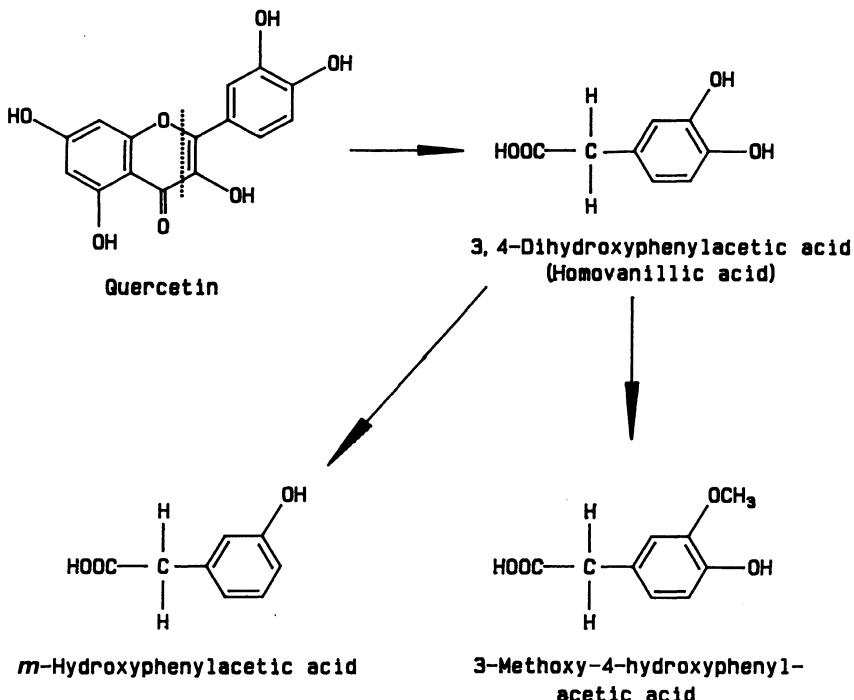


Figure 9. Metabolism of quercetin in rabbit, rat, guinea pig, and human (Booth et al., 1956).

fission were suppressed in the intestine and only conjugates of the hydroxyflavan molecule were found in urine.

Takács and Gábor (1975) showed that perfused rat liver is capable of metabolizing rutin and apigenin. Intermediate flavonoids were formed as well as *p*-hydroxybenzoic, cinnamic, phenylacetic, and phenylpropionic acids. Förster (1975) showed with perfused rat small intestine, blood sampling, and thin layer chromatography that rutosides were absorbed into the blood and increased in concentration over the 3-hour period of perfusion experiments. Kühnau (1976), Hackett (1986), and Adzet and Camarasa (1986) reviewed flavonoid metabolism. Less hydroxylated or methoxylated flavonoids are biologically more active and are more resistant to intestinal microflora metabolism. An exception is the biflavan, cyanidin, 5, 7, 3', 4'-hydroxylated flavonoid which is resistant to gut microflora (Fig. 12). The flavonoids are metabolized to phenolic acids or lactones in the gut, absorbed by the gut as aglycones, may be excreted unchanged in bile, and may be metabolized by the liver. They may be oxidized, reduced, methylated, and conjugated. Although most of these experiments have been in small animals, human experiments suggest similar processes (Hackett, 1986). Epicatechin was excreted rapidly in urine in humans after an oral dose (Géro, 1946). Clark and MacKay (1950) fed 50 mg/kg of rutin, esculetin, and quercetin to humans and recovered virtually no parent compound in urine. Gugler et al. (1975) injected quercetin i.v. into humans. The elimination half-life from plasma was about 2.5 hours. Plasma proteins bound over 98% of the quercetin. About 0.7% of the i.v. dose was excreted unchanged; 7.4% was excreted as conjugates in the urine. In humans, only 1% of a single 4 g oral dose of quercetin appeared in plasma. Oral administration of hydroxyethyl rutosides to humans yielded a peak plasma concentration of rutosides in 6 to 10 hours. Nine male human

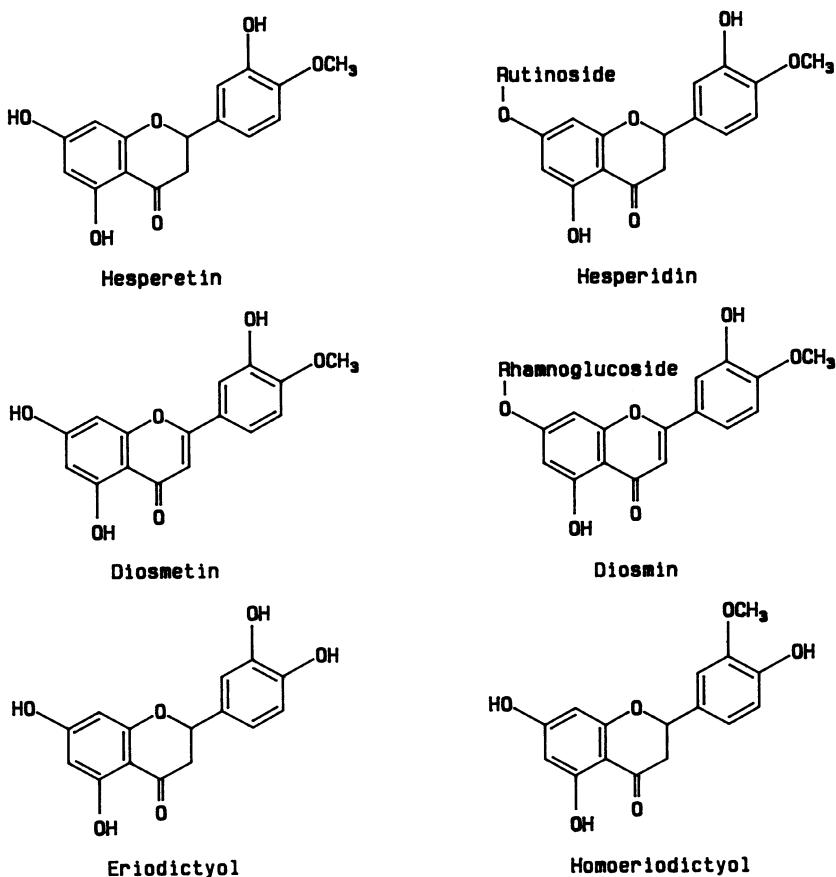


Figure 10. Structural formulas of hesperetin, hesperidin, diosmetin, diosmin, eriodictyol, and homoeriodictyol.

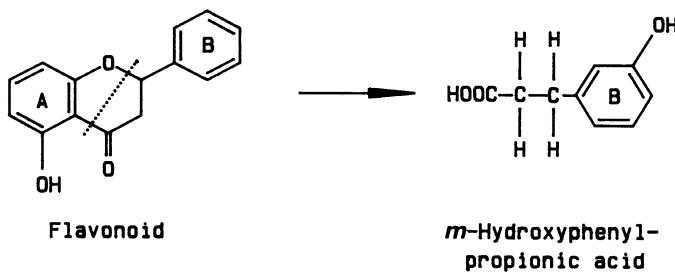
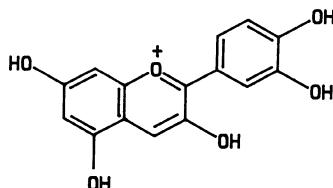


Figure 11. Metabolism of flavonoid to *meta*-hydroxyphenylacetic acid (*m*-HPPA).



**Cyanidin**

Figure 12. Structural formula of cyanidin.

volunteers each took one 314 mg capsule daily of a phosphatidylcholine complex (IdB 1016) of silybin, an antihepatotoxic agent, for 8 consecutive days. This was equivalent to 120 mg silybin per day. Peak plasma silybin levels were reached in about 2 hours and the elimination half-life was about 2 hours. On the eighth day, the plasma peak occurred in about 1 hour. Only about 3% of the dose was recovered in urine over the 12 hours after dosing. Most of the silybin found in plasma was the sulphate or glucuronide conjugate. Peak concentrations of total silybin were about 0.45 µg/ml plasma (Barzaghi et al., 1990). See Figures 13, 14, 15, and 16 for the structural complexity of silybin compounds.

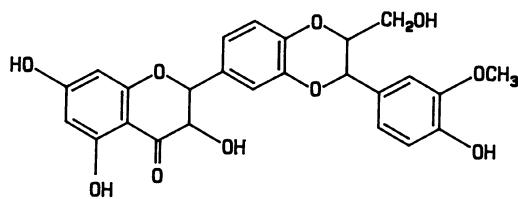
Overall, the absorption, metabolism, and excretion of flavonoids in any animal, particularly humans, have been poorly studied. Radiochemical purity of starting materials is usually not determined, and the form of the radiolabel recovered is, in most cases, not determined. No study has accounted for the entire dose and there are no mass-balance studies. Elimination half-lives are helpful but are not necessarily related to dose. The pharmacokinetics of absorption, metabolism, and excretion of flavonoids is critical for estimating benefits and risks.

### Dietary Flavonoids

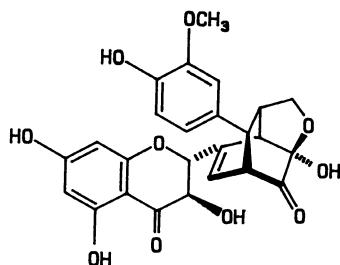
Flavonoids, usually conjugated to a sugar, are widespread in the plant kingdom (Harborne, 1986a, 1988). The occurrence of the flavonoid aglycone occurrence is less pronounced compared with the carbohydrate moiety of the conjugates and appears to be associated with secretory structures and lipophilic plant products (Wollenweber and Dietz, 1981 for a review).

Pierpoint (1985, 1986, 1990) lists several difficulties in estimating flavonoid intake. There are many compounds involved. Flavonoid content varies by season, plant cultivar, plant maturity, and plant condition. National diets vary, so an estimate for the United States may not apply to other countries and cultures. Kühnau (1976) published an estimate of the total United States flavonoid daily intake per person of 1,020 mg/day (winter) and 1,070 mg/day (summer). Of these totals, 41% (winter) and 39% (summer) are from cocoa, cola, coffee, beer, and wine. Fruit juices contribute an additional 26% (winter) and 29% (summer) followed by spices at 16% (winter) and 15% (summer). These three food groups contribute 83% of the total daily United States intake per person of flavonoids winter and summer.

The occurrence of some specific flavonoids in fruits and vegetables are reported in Tables 6, 7, and 8. These are not the only flavonoids which occur in food. Anthocyanins in fruit and vegetables have been reviewed (Timberlake, 1981). Tables 6, 7, and 8 illustrate several points about determination of flavonoids (or any natural product) in the human diet. Varietal differences occur. The stages of maturity differ.

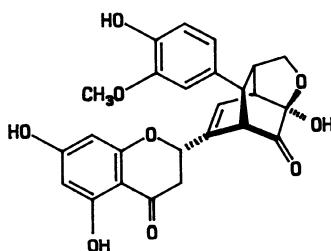


Silybin

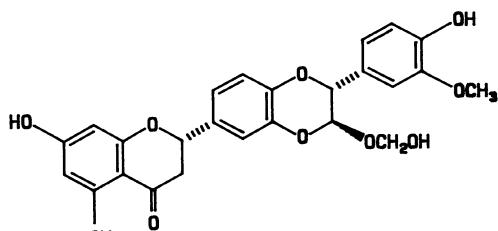


Silydianin

Figure 13. Structural formulas of silybin and silydianin.



Silymonin



Silandrin

Figure 14. Structural formulas of silymonin and silandrin.

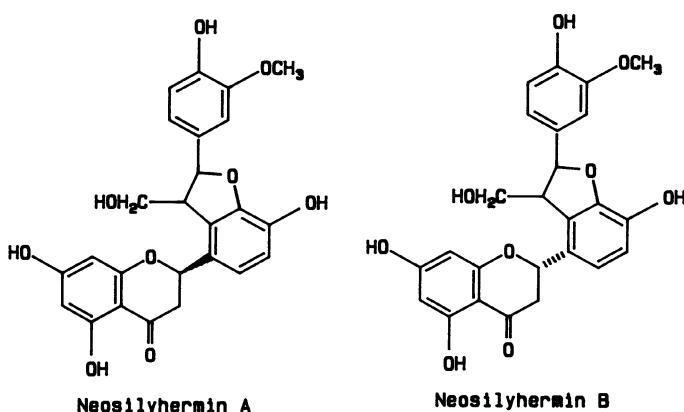
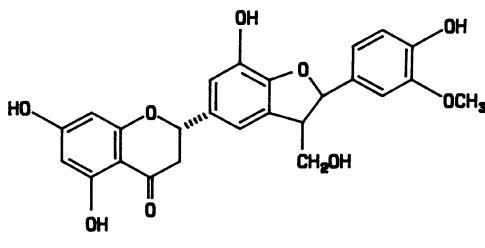


Figure 15. Structural formulas of silyhermin, neosilyhermin A, and neosilyhermin B.

Light conditions affect flavonoid content. Disease status affects flavonoid content. A recent example is a study by Omidiji and Ehimidu (1990). Mature, healthy purple onions (*Allium cepa* L cv. Red Creole) were challenged with *Pseudomonas cepacia*, *P. marginalis*, and *P. fluorescens*. *P. cepacia* (pathogenic) at the highest inoculation level induced a 305% (about 560 µg/g) increase in phenols above controls compared to a 42% increase with wounding. This peak occurred 5 days after inoculation. *P. marginalis* and *P. fluorescens*, both non-pathogenic, produced levels of phenols similar to wounding, about 280 µg/g. The flavonoids were identified as isorhamnetin glucoside and quercetin glucoside (Omidiji and Ehimidu, 1990). Processing, shipping, and handling may also affect final flavonoid levels.

Pierpoint (1986, 1990) discussed culture differences for flavonoid intake. In the U.K., for instance, the average tea consumption of 4.7 cups daily would provide about 900 mg flavonoids; whereas, the total estimated United States daily consumption from all sources is about 1,000 mg (Kühnau, 1976). Dependent on diet, total flavonoid content in some cultures may be 2,000 to 3,000 mg/day (Pierpoint, 1990).

Some of human flavonoid consumption is undoubtedly from food contamination. Commercial grain may be contaminated with toxic weed seeds, sicklepod (*Cassia obtusifolia*), jimson weed (*Datura stramonium*), velvetleaf (*Abutilon theophrasti*), and morning glory (*Ipomoea* spp.) (Friedman and Henika, 1991). Sicklepod seeds contain anthraquinone derivatives, β-sitosterol and flavonoids, UV quenching compounds, and fluorescent blue compounds of unknown structure (Crawford et al., 1990). Jimson weed contains tropane alkaloids, velvet bean contains delphinidin, quercetin, catechin, myricetin, (-)-epicatechin, and cyanidin (Paszkowski and Kremer, 1988). Morning glory seeds contain chlorogenic acid (Friedman and

Table 6. Quercetin and kaempferol content of vegetables ( $\mu\text{g/g}$ ).

Vegetable	Quercetin	Kaempferol	Reference
Bell pepper	63	0	Herrmann, 1976
Kohlrabi	7	6	Herrmann, 1976
Small radish	0	27	Herrmann, 1976
Horseradish	0	77.5	Herrmann, 1976
Potato, green	47.2	66	Herrmann, 1976
Lettuce, 'Blanco', open air			
Outer leaves	60 (48)		Herrmann, 1976
Inner leaves	3.4 (52)		Herrmann, 1976
Lettuce, Valentine, open air			
Outer leaves	462 (59)		Herrmann, 1976
Inner leaves	7.6 (41)		Herrmann, 1976
Lettuce, Valentine, glasshouse			
Outer leaves	10.8 (55)		Herrmann, 1976
Inner leaves	> 1 (45)		Herrmann, 1976
Endive, open air			
Outer leaves		258 (58)	Herrmann, 1976
Inner leaves		5.7 (42)	Herrmann, 1976
Cabbage, Savoy (all leaves)	5	29	Herrmann, 1976
Cabbage, red	6	< 0.1	Herrmann, 1976
Broccoli	6	30	Herrmann, 1976
China Cabbage	3	11	Herrmann, 1976
Leek, 9 varieties	10-25	90-200	Herrmann, 1976
Chive	300	10	Herrmann, 1976
Garlic	Trace		Herrmann, 1976
Onion, white	10	—	Herrmann, 1976
Onion, colored, outer skin	25,000-65,000		Herrmann, 1976
Broad bean			
Pod	1,340	800	Herrmann, 1976
Seed	19	5	Herrmann, 1976
Broad bean, pod only			
Immature	47	230	Tomas-Barberan et al., 1991
Mature	332	220	Tomas-Barberan et al., 1991
Endive			
Green	9,400 total		Goupy et al., 1990
Etiolated	1,730 total		Goupy et al., 1990
Leaf lettuce, 4 varieties	24	0.5	Bilyk and Sapers, 1985
Chive			
Green leaves	55	9	Bilyk and Sapers, 1985
White leaves	ND	16	Bilyk and Sapers, 1985
Garlic chive, green leaves	4	6	Bilyk and Sapers, 1985
Leek			
Green leaves	ND	20	Bilyk and Sapers, 1985
White leaves	ND	ND	Bilyk and Sapers, 1985
Kale			
Dwarf Siberian	7	30	Bilyk and Sapers, 1985
Vates blue curled dwarf	20	13	Bilyk and Sapers, 1985
Red cabbage, whole	2	ND	Bilyk and Sapers, 1985
Red radish, whole	ND	4	Bilyk and Sapers, 1985

Table 7. Quercetin and kaempferol content of fruits ( $\mu\text{g/g}$ ).

Fruit	Quercetin	Kaempferol	Reference
Apple, Weisser Klarapfel	98	< 1	Herrmann, 1976
Gravensteiner	58	2	Herrmann, 1976
Cox Orangen Renette	263	7	Herrmann, 1976
Pear, Williams Christ	28	12	Herrmann, 1976
Quince, Portugiesische Quitte	180	210	Herrmann, 1976
Sweet Cherry			
Buttners role Knorpelkirsche	6	6	Herrmann, 1976
Badoconer Reisen	24	0	Herrmann, 1976
Sour Cherry			
Schattenmorelle (1971)	80	17	Herrmann, 1976
Schattenmorelle (1974)	23	5	Herrmann, 1976
Plum, The Czar Fruhwetsche	15	2	Herrmann, 1976
Wangenheims	3	2	Herrmann, 1976
Mirabelle von Nancy	< 0.1	< 0.1	Herrmann, 1976
Peach, Red Haven	0	0	Herrmann, 1976
Mangipam	< 0.1	< 0.1	Herrmann, 1976
Fruher	4	2	Herrmann, 1976
Apricot	53	2	Herrmann, 1976
Raspberry Schonenmann	29	< 0.1	Herrmann, 1976
Blackberry Theodor Reimers	33	14	Herrmann, 1976
Black Currant			
Rosenthal long traubige Schwarze (1971)	33	< 0.1	Herrmann, 1976
Rosenthal long traubige Schwarze (1974)	33	6	Herrmann, 1976
Silvergieters Schwarze (1973)	68	10	Herrmann, 1976
Silvergieters Schwarze (1974)	41	10	Herrmann, 1976
Red Currant	27	2	Herrmann, 1976
Rote Heros	2	0.1	Herrmann, 1976
Heinemanns rote Spatlese	11	2	Herrmann, 1976
White Currant			
Weisse Versailler	7	1	Herrmann, 1976
Weisse aus Juterborg	3	0.2	
Gooseberry			
Weisse Triumph	< 0.1	< 0.1	Herrmann, 1976
Rote Triumph	< 0.1	0	Herrmann, 1976
Bilberry, wild	32	0	Herrmann, 1976
Bilberry, cultivated			
Clon 98	160	6	Herrmann, 1976
Heerma I	105	0	Herrmann, 1976
Heerma II	159	0	Herrmann, 1976
Elderberry (1973)	237	0	Herrmann, 1976
Elderberry (1974)	105	0	Herrmann, 1976
Cranberry			
Stevens, dark	250	16	Bilyk and Sapers, 1986 <sup>a</sup>
Early Black, dark	177	1.8	Bilyk and Sapers, 1986 <sup>a</sup>
Ben Lear, dark	157	2.7	Bilyk and Sapers, 1986 <sup>a</sup>
Franklin, dark	175	ND	Bilyk and Sapers, 1986 <sup>a</sup>
McFarlin, dark	169	ND	Bilyk and Sapers, 1986 <sup>a</sup>
Howes, dark	112	ND	Bilyk and Sapers, 1986 <sup>a</sup>

Table 7. Quercetin and kaempferol content of fruits ( $\mu\text{g/g}$ ). (Continued)

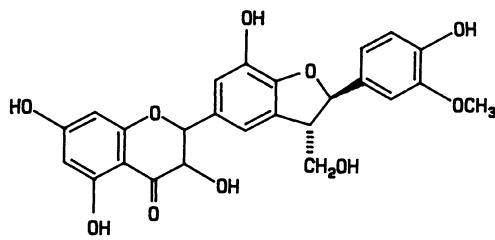
Fruit	Quercetin	Kaempferol	Reference
<b>Highblush Blueberry, ripe</b>			
Earliblue	29	ND	Bilyk and Sapers, 1986 <sup>a</sup>
Weymouth	26	ND	Bilyk and Sapers, 1986 <sup>a</sup>
Coville	25	ND	Bilyk and Sapers, 1986 <sup>a</sup>
Bluetta	24	ND	Bilyk and Sapers, 1986 <sup>a</sup>
<b>Blackberry, Thornless</b>			
Smoothstem, black	19	2.1	Bilyk and Sapers, 1986 <sup>a</sup>
Black satin, black	13	0.9	Bilyk and Sapers, 1986 <sup>a</sup>
Dirksen thornless, black	10	0.9	Bilyk and Sapers, 1986 <sup>a</sup>
Hull thornless, black	7.3	1.2	Bilyk and Sapers, 1986 <sup>a</sup>
C-33, black	35	2.6	Bilyk and Sapers, 1986 <sup>a</sup>
C-55, black	18	2.6	Bilyk and Sapers, 1986 <sup>a</sup>
C-60, black	8.3	1.9	Bilyk and Sapers, 1986 <sup>a</sup>
C-57, black	8.2	2.2	Bilyk and Sapers, 1986 <sup>a</sup>
C-62, black	5.2	0.6	Bilyk and Sapers, 1986 <sup>a</sup>
C-58, red	17	2.2	Bilyk and Sapers, 1986 <sup>a</sup>
C-52, red	11	0.5	Bilyk and Sapers, 1986 <sup>a</sup>
Plum, dry	22,000		Raynal et al., 1989
Grape juice	12.1		Spanos and Wrolstad, 1990

<sup>a</sup>Myricetin not detected in cranberry, blueberry, and blackberry.

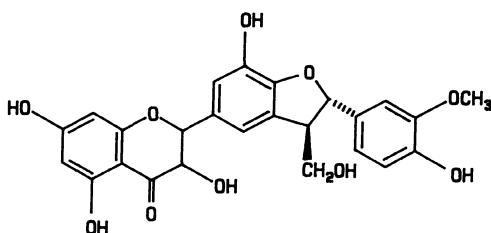
Henika, 1991). Range plants may also contain flavonoids. *Gutierrezia microcephala* A. Gray (broomweed, perennial snakeweed, broom snakeweed, stinkweed, turpentine weed) consumption by cattle in the American southwest is associated with abortion with placental retention, hemorrhage, and non-surviving weak offspring (Dollahite and Anthony, 1957; Mathews, 1936; Roitman and James, 1985). Over 20 oxygenated flavonol methyl ethers were isolated from this *Gutierrezia* species (Roitman and James, 1985). Soybeans contain daidzein (aglycone of daidzin) and genistein (aglycone of genistin) (Fig. 17) (Matsuura et al., 1989). These two compounds increase due to  $\beta$ -glucosidases in soybean during the processing of soy milk. On a dry soybean basis, these compounds increased from about 50  $\mu\text{g/g}$  to 350  $\mu\text{g/g}$  at pH 6.0 (close to pH of soy milk) and 20°C in the soak step of processing (Matsuura et al., 1989). In the soybean plant, daidzein, genistein, and coumestrol (Fig. 17) increased in the roots over a 12-day period after transplanting and inoculation with *Bradyrhizobium japonicum*. Nitrogen application decreased the isoflavone (Fig. 18) concentration in roots (Cho and Harper, 1991a,b). Conjugates of daidzein and genistein are selectively excreted into root and seed exudates at a level of 1 to 10  $\mu\text{M}$ . Their levels in the seed may be > 1,000  $\mu\text{g/g}$  seed tissue and it is postulated they may act as signal molecules in chemoattraction of nodulation-forming bacteria (Graham, 1991). Subterranean clover (*Trifolium subterraneum* L.) is a forage legume widely grown in Mediterranean climates (Smith et al., 1986). Identified isoflavones in subterranean clover include biochanin A, formononetin, genistein, and daidzein (Fig. 17). Of four cultivars, only one showed an isoflavone content difference based on harvest date. Total isoflavones were almost unchanged comparing fresh to frozen samples, but dried samples showed a 30 to 50% decrease (Smith et al., 1986). Production of isoflavones was induced in bean (*Phaseolus vulgaris* L.) by ozone,  $\text{SO}_2$ , and several herbicides (Rubin et al., 1983).

Table 8. Miscellaneous flavonoid content of fruits and vegetables ( $\mu\text{g/g}$ ).

	Flavonoid	Content	Reference
<b>Fruit</b>			
Apple, peel			
Spartan	Phloridzin	Not quantified	Dick et al., 1987
Cox Orangen Renette	Catechin (total)	64	Mosel and Herrmann, 1974
James Grieve	Catechin (total)	34.5	Mosel and Herrmann, 1974
Jonathan (Ruthe)	Catechin (total)	105	Mosel and Herrmann, 1974
Jonathan (Stuttgart)	Catechin (total)	163	Mosel and Herrmann, 1974
Golden Delicious (Stuttgart)	Catechin (total)	61	Mosel and Herrmann, 1974
Golden Delicious (Ruthe)	Catechin (total)	47	Mosel and Herrmann, 1974
Schoner von Boskoop (Stuttgart)	Catechin (total)	78	Mosel and Herrmann, 1974
Schoner von Boskoop (Ruthe)	Catechin (total)	83	Mosel and Herrmann, 1974
Pear			
Conference (Avg. 2 determinations)	Catechin (total)	11.1	
Alexander Lucas (Avg. 2 determinations)	Catechin (total)	14	
Sweet cherry	Catechin (total)	65.3	Mosel and Herrmann, 1974
Buttners Rote Knorpelkirche (Avg. 4 determinations)			
Sour cherry			
Schattenmorelle (Avg. 2 determinations)	Catechin (total)	186.5	Mosel and Herrmann, 1974
Peach	Catechin (total)	66	Mosel and Herrmann, 1974
Apricot	Catechin (total)	246	Mosel and Herrmann, 1974
Plum (Avg. 3 varieties)	Catechin (total)	36.7	Mosel and Herrmann, 1974
Rough lime			
Peel and membrane	Naringin	517	Yusof et al., 1990
Seed	Naringin	29.2	Yusof et al., 1990
Juice	Naringin	98.4	Yusof et al., 1990
Musk lime	Naringin	ND	Yusof et al., 1990
Mexican lime	Naringin	ND	Yusof et al., 1990
Mandarin orange	Naringin	ND	Yusof et al., 1990
Pummelo			
Peel and membrane	Naringin	3,910	Yusof et al., 1990
Juice	Naringin	220	Yusof et al., 1990
<b>Vegetable</b>			
Peanut, hull			
Yellow	5,7-Dihydroxychromone	0	Daigle et al., 1988
Black	5,7-Dihydroxychromone	1,488	Daigle et al., 1988
Yellow	Eriodictyol	0	Daigle et al., 1988
Black	Eriodictyol	3,765	Daigle et al., 1988
Yellow	Luteolin	0	Daigle et al., 1988
Black	Luteolin	6,009	Daigle et al., 1988
Carrot, 4 var.			
Without leaves	Apigenin	< 1	Herrmann, 1976
Without leaves	Luteolin	< 1	Herrmann, 1976
Leaves	Apigenin	350-800	Herrmann, 1976
Leaves	Luteolin	400-1,500	Herrmann, 1976



**Silychristin**



**Isosilychristin**

Figure 16. Structural formulas of silychristin and isosilychristin.

### Biological Effects of Flavonoids

This is a controversial subject. There are as many, perhaps more, good health claims for flavonoids as there are adverse effects. Humans have more experience with the healthy aspects of citrus, pome fruits, and vegetables. The United States Surgeon General (1988) and the National Academy of Sciences (1989) have strongly recommended consuming more fruits and vegetables. The National Research Council (1989) flatly stated "Every day eat five or more servings of a combination of fruits and vegetables, especially green and yellow vegetables and citrus fruit."

Kühnau (1976), Singleton (1981), and Attaway (1992) reviewed the nutritional effects of flavonoids. Flavonoids are considered "semi-essential" food components and not harmful. Flavonoids are antioxidants. Quercetin (Fig. 9), myricetin (Fig. 19), quercetagetin, and gossypetin are the best antioxidants; catechin has some activity. Hesperidin is inactive. Daidzin, genistin, malonyl-daidzin, and malonyl-genistin were antioxidants (Fleury and Magnolato, 1990). Quercetin, morin, myricetin, and fisetin were the most active inhibitors of induced lipid peroxidation in the presence of ferrous ions (Das and Ratty, 1986). Diosmetin, apigenin, hesperetin, naringenin, and 4',5,7-trihydroxyflavone (all lack the 3-OH group) were least active. With ascorbic acid as the inducer, 3-hydroxy-flavone, fisetin, taxifolin, (+)-catechin, quercetin, myricetin, and morin were the most effective inhibitors of lipid peroxidation (Das and Ratty, 1986). These all have a 3-OH group. Isovitexin, a glycosyl flavonoid from rice hull, is a better antioxidant than BHA (butylated hydroxyanisole) and  $\alpha$ -tocopherol (Ramarathnam et al., 1989). This compound is absent in rice seeds which do not store well, i.e., deteriorate or rot. Only long-lived rice seed hulls contain isovitexin. Epicatechin 3-*O*-gallate and other procyanidins from grape seeds were found to be potent, oxygen-free, radical scavengers. The best scavenger was procyanidin B<sub>2</sub> 3'-*O*-gallate (da Silva et al., 1991).

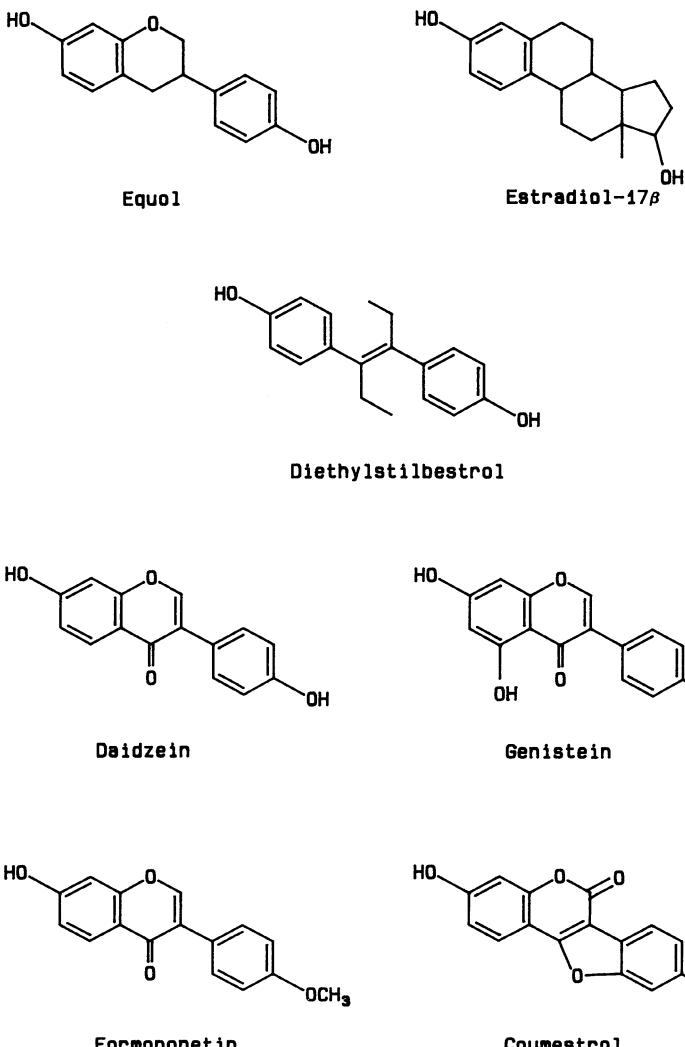
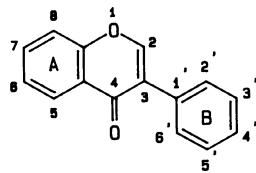


Figure 17. Structural formulas of equol, estradiol-17 $\beta$ , diethylstilbestrol, daidzein, genistein, formononetin, and coumestrol.

Some flavonoids can chelate metal ions, particularly copper (Kühnau, 1976; MacGregor, 1984). Copper flavonoid complexes inhibit hyaluronidases, stabilize structural proteins, and strengthen fragile membranes (Kühnau, 1976). Flavonoids hydroxylated in the 5, 3', 4'- or 5, 3', 4', 5'-positions may be competitive antagonists to catecholamines like epinephrine and norepinephrine and extend the effect of these compounds. Flavonoids also extend the action of Vitamin C and, at one time, were referred to as Vitamin P (Robbins, 1980). The term Vitamin P was replaced by bioflavonoid. Vitamin P was a mixture of eriodictyol and hesperidin. The interaction between Vitamin C and flavonoids is considered to be an antioxidant effect (for reviews, see Huet, 1982; Middleton, 1988).



**Isoflavonoid general structure**

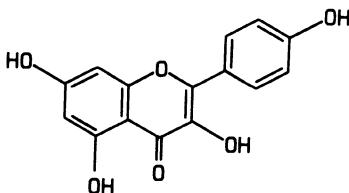
Figure 18. Isoflavonoid general structure.

Flavonoids inhibit a variety of enzymes *in vitro* (Middleton, 1988; Nikaido et al., 1989), which are required for normal physiological function. Some of these enzymes are involved in axonal transport, basophil-mast cell secretion, cell locomotion/chemotaxis, DNA synthesis, endo/exocytosis, insulin secretion, intestinal chloride ion secretion, membrane phosphorylation, microtubular dissociation, mitogenesis, neurotransmitter release, platelet function, and smooth muscle contraction (Middleton, 1988).

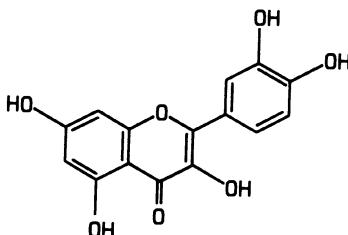
Flavonoids reportedly affect lymphocyte and fibroblast glucose uptake and other immune functions, act as smooth muscle antispasmodics, and anti-inflammatory agents. They inhibit platelet aggregation, act as antiviral agents, are synergistic with other antiviral agents, and are bacteriostatic. Flavonoids also are potent multifunction oxidase inducers (thus increasing metabolism of xenobiotics), correct abnormal capillary permeability and fragility after x-radiation and a variety of diseases, and act as anticancer and antimutagenic agents. Quercetin (Fig. 19) is the principal flavonoid tested for these effects (Attaway, 1992; Kühnau, 1976; MacGregor, 1984; Middleton, 1988; Robbins, 1980).

Quercetin at 25,000 µg/g in laboratory chow or 70 µg/g in water prevented cataracts in the degu (*Octodon degus*) possibly through inhibition of lens aldose reductase (Varma et al., 1975, 1977).

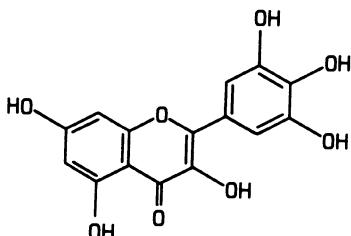
In 1977, Bjeldanes and Chang reported that quercetin, a large fraction of the total flavonoids in the daily diet (Brown and Dietrich, 1978), was mutagenic without cytochrome P-450 activation but was more mutagenic with activation (Bjeldanes and Chang, 1977). In further studies, quercetin and myricetin were mutagenic without activation; kaempferol was mutagenic only with activation (Hardigree and Epler, 1978; MacGregor and Jurd, 1978) (Fig. 19). Quercetin was mutagenic to mouse cell lines (Meltz and MacGregor, 1981). Flavonoid content correlated with the mutagenicity of nutritional supplements and tobacco (snuff) containing rutin (Hardigree and Epler, 1978). The literature since 1978 has been reviewed by Woo et al. (1988) and MacGregor (1984). Of the 70 naturally occurring and synthetic flavonoids tested, 33 were positive in the Ames assay (Woo et al., 1988). All active flavonoids have maximum active response toward strain TA98 and a highly significant response toward strain TA100 (MacGregor, 1984; Woo et al., 1988). The flavonoid structural features essential for mutagenic activity in *S. typhimurium* strains TA100 and TA98 were determined, and only the flavonols (3-hydroxyflavones) appeared to be mutagenic (MacGregor and Jurd, 1978). Structural requirements for mutagenic activity appear to be: 1) a free 3-hydroxyl group, 2) a 2, 3 double bond, and 3) a 4-keto group. However, there are flavonoids without the 3-OH group which are mutagenic toward the TA100 strain, but not strain TA98 (MacGregor, 1984). Quercetin is the most active flavonoid mutagen with strain TA98. Oxygen, tyrosinase, and alkaline pH irreversibly inactivate the mutagenicity of quercetin with strain TA98 (Friedman and Smith, 1984). Norwogonin and sexangularetin are more mutagenic than quercetin



Kaempferol



Quercetin



Myricetin

Figure 19. Mutagenic flavonols from fruits and vegetables.

with strain TA100. This second class of flavonoid mutagens requires activation with the S9 or cytosol cell fraction, and has hydroxy- or methoxy-substitution at 5, 7, and 8 of the A ring. The B ring and 2, 3 positions are apparently not involved in this second class of flavonoid mutagens (MacGregor, 1984). Flavonoids may be responsible for the mutagenicity of grain contaminated with weed seeds (Freidman and Henika, 1991). The positive results with quercetin probably prompted inclusion in the National Toxicology Program's testing regimen. Quercetin was mutagenic in *Salmonella* assays and cytogenic in Chinese hamster ovary cells (National Toxicology Program, 1989). Quercetin was subsequently shown to exhibit some carcinogenicity in male mice because of increased renal tubular cell adenomas when fed at 40,000 ppm (National Toxicology Program, 1991).

Cancer studies on flavonoids have been reviewed (Lai and Woo, 1987; MacGregor, 1984; Woo et al., 1988). Quercetin (Fig. 19) was positive for intestinal tumors, bile duct tumors, bladder tumors, hepatomas, and liver preneoplastic foci in three studies. In 10 other studies, there was no increase in carcinomas. Rutin, kaempferol (Fig. 19), tiliroside, and catechin were negative (MacGregor 1984; Woo et al., 1988). In chromosomal aberration tests, quercetin has been both positive and negative (Hayashi et al., 1988; MacGregor, 1984; Woo et al., 1988). Flavan-3-ols,

(+)-catechin, (-)-epicatechin, (+)-gallocatechin, and (-)-epigallocatechin-3-O-gallate, and procyanidin B-1 and C-1 break double stranded DNA in the presence of cupric ion (Shirahata et al., 1989). Quercetin at 0.1% in the diet reduced the life span of 'shorter-living' male mice (Jones and Hughes, 1982).

Flavone acetic acid, a synthetic compound, is used as an anticancer drug (Futami et al., 1991; Pratesi et al., 1991). Various flavonoids are antimutagenic agents (Huang et al., 1983; Stich and Rosin, 1984; Wall et al., 1988). Quercetin reduced mortality and the cytotoxic effects of T-2 mycotoxin in mice (Markham et al., 1987). Various flavonoids inhibited the mutagenicity of aflatoxin B<sub>1</sub> towards TA100 and TA98 *Salmonella typhimurium* (Francis et al., 1989).

Canada et al. (1989) challenged isolated guinea pig enterocytes with kaempferol, quercetin, and myricetin (Fig. 19). All three compounds produced cellular damage at 450 µM. Quercetin and myricetin appeared to be more toxic than kaempferol. These authors suggested that flavonoids might exacerbate or cause inflammatory bowel diseases (Canada et al., 1989).

Flavonoids may affect reproduction. Kumar et al. (1989) administered kaempferol orally at 250 mg/kg/day for 60 days to male rats. Spermatids were reduced by 73.7%. Mature and immature leydig cells decreased by 39.2% and 46.6%, respectively. Testicular cholesterol increased and androgen dependent sialic acid and protein declined in the testes, epididymides, and sex accessory glands (Kumar et al., 1989). Shore and Lytle (1986) studied the inhibition of rat uterine peroxidase, an enzyme which increases in the uterus in response to estrogen. Diethylstilbestrol, genistein (isoflavone) (Fig. 17), zearalenone (Fig. 38), and zearalenol (Fig. 42) were competitive inhibitors; coumesterol (isoflavone) was a non-competitive inhibitor. Coumesterol was 2 to 6 times as inhibitory to peroxidase as diethylstilbestrol, whereas genistein was 25 times less active than diethylstilbestrol (Shore and Lytle, 1986). Flavonoids have been associated with abortion in cattle in the American southwest from consumption of the genus *Gutierrezia* (Roitman and James, 1985). The isoflavones of subterranean clover, formononetin, biochanin A, genistein, and daidzein are associated with an infertility syndrome in sheep (Bennets et al., 1946; Morley et al., 1964; Moule et al., 1963). A reduction in fallopian tube sperm in ewes grazing on subterranean clover has been reported (Lightfoot et al., 1967). The isoflavone infertility problem has prompted immunization attempts against genistein and equol with temporary success (Cox, 1984). Equol has been identified in the urine of pregnant mares (Marrian and Haslewood, 1932), goat (Klyne and Wright, 1957), cow (Klyne and Wright, 1959), hen (Common and Ainsworth, 1961; MacRae et al., 1960), sheep (Braden et al., 1967; Shutt and Braden, 1968), humans and rats (Axelson et al., 1982). The metabolism of formononetin and biochanin A in bovine rumen fluid has been studied (Dickinson et al., 1988). Analytical methods for isoflavones in soy protein (Barbush et al., 1989) and in human urine (Adlercreutz et al., 1991) are available.

Equol and other phytoestrogens possess weak estrogenic activity (Kitts, 1987; Newsome and Kitts, 1980; Shutt and Cox, 1972; Tang and Adams, 1980). Estrogenic responses to isoflavones vary by species and, for bioassays using mice, by strain (Farmakalidis and Murphy, 1984). Human volunteers excreted large quantities of equol, a phytoestrogen, after consuming 40 g of textured soya for 5 days. Fecal flora were also incubated with textured soya and intestinal microbes produced equol. Equol excretion exceeded 6.0 mg/day in one subject after a 40 g soya meal. Estrone-glucuronide, the principal urinary estrogen in the follicular phase of women, is excreted at 2 to 27 µg/day (Setchell et al., 1984). Urinary excretion of equol in humans not consuming soy products is about 80 µg/day (Setchell et al., 1984). This study is important for several reasons. It showed conversion in man to a biologically

active flavonoid from a dietary source. Equol crossed into the blood stream in quantity and was excreted as a glucuronide.

Phytoestrogens apparently compete for the same estrone binding sites on  $\alpha$ -fetoprotein in rats and in humans (Garreau et al., 1991). Phytoestrogens have a wide range of biological activities from anticancer to antiestrogen effects (Garreau et al., 1991 and references therein). Flavones and isoflavones including genistein, biochanin A, prunetin, kaemferol, and quercetin, inhibit tyrosine protein kinase which is necessary for retrovirus carcinogenicity (Ogawara et al., 1989). Genistein induces mouse erythroleukemia cells synergistically with mitomycin C (Watanabe et al., 1989). Immunological data on genistein suggest it is a powerful immunosuppressant (Atluru and Atluru, 1991). Genistein is also associated with plant resistance to *Cytospora persoonii* induced bark canker (Geibel et al., 1990).

Perhaps other flavonoids in the diet are converted to biologically active molecules which have yet to be isolated. Increased dietary fat has been linked to prostate cancer and increased plasma levels of male hormones (Howie and Shultz, 1985). Low fat diets can influence plasma levels and excretion of estrogens and influence the incidence of breast cancer (Goldin and Gorback, 1988; Goldin et al., 1986; Hagerty et al., 1988; Shultz et al., 1987). We suggest that a better understanding of the changes in hormone levels caused by bioactive flavonoids and flavonoid metabolites in the vegetarian diet would be a good starting point in searching for "anticancer nutrients."

## HERBS

The use of herbs and herbal preparations have been reviewed with a historical perspective (Dubick, 1986). Many people believe that plant remedies are naturally superior to synthetic drugs and that when herbal preparations are used, they cannot be harmful to human beings. In 1977, the Consumer Response Corporation reported that a survey showed that the most convincing sales claim to put on a food or beverage label is "Natural" and that 42% of the consumers surveyed believed that natural products have no adverse effects and are more healthful and safer (Stephenson, 1978). In the past two decades, "Natural" foods and herbal medicines have gained substantial popularity in the United States (Hogan, 1983). In 1978, the sale of herbs and other related commodities in health food stores alone amounted to \$1.1 billion, and this figure was projected to triple by the year 1990 (Dubick, 1986).

There is no doubt that some plants do contain biologically active compounds that are medicinally useful. More than 20% of the commercially prepared drugs originate from plants, but these plants also contain many active ingredients that can provoke adverse reactions. Many people, including physicians, are not aware of the side effects; and dangerous and sometimes fatal adverse reactions may occur with their use (Saxe, 1987).

## Onion and Garlic

We are learning more about the potential good and bad aspects associated with herbs and herbal preparations that are obtainable in health food stores today. Historically, people have taken onion and garlic juices as a remedy for a long list of ailments. Both onion and garlic juice can prevent the rise of serum cholesterol after a fatty meal (Bordia et al., 1975). Garlic inhibits lipid synthesis; reverses cholesterol-induced atherosclerosis in rabbits; decreases serum cholesterol, triglycerides, low density lipids (LDL), and very low density lipids (VLDL); and

increases high density lipid (HDL) levels (Dubick, 1986). However, in high doses, wild garlic can cause gastroenteritis, diarrhea, rash, and leukocytosis. Long-term ingestion of wild garlic or onion will also block iodine uptake by the thyroid (Saxe, 1987).

### **Yarrow, Oregano, Basil, Sassafras, and Chamomile**

Herbs and natural herbal preparations, in most cases, do not have just a single active component; rather, they have an elaborate array of biologically active components. For example, over 40 indigenous naturally occurring chemical components of the herb yarrow (*Achillea millefolium*) and their biological activities are listed in Table 9. Yarrow is reportedly a hemostatic herb, but it also contains coumarins which are anticoagulants (Duke, 1987a). Thus, varied biological activities can be manifested from crude plant preparations when the proportions of indigenous chemicals change as a result from variable growing conditions, crop treatments during and after the growing period, and processing. The biological activities and LD<sub>50</sub>s of some compounds from the common herb oregano (*Origanum vulgare*) are listed in Table 10. Compounds in basil (*Ocimum basilicum*) compiled by Duke (1988a) are listed in Table 11.

Herbal teas are used by many people for medicinal purposes as well as for enjoyment. Sassafras root bark continues to be freely available in health food stores despite evidence indicating its carcinogenicity, and despite legal restrictions prohibiting the use of safrole in foods. Safrole is hepatocarcinogenic and is a major constituent of the oil of sassafras root bark (Segelman et al., 1976).

Chamomile tea is a herbal drink commonly sold in supermarkets, and people may have allergic or anaphylactic reactions to it. Allergens from chamomile flower heads cross-react with ragweed, chrysanthemums, or other species of the family Compositae (Casterline, 1980). The Mexican-American population in Southern Colorado commonly treat childhood illnesses with the tea. However, chamomile is low in sodium, and its continued use without other food intake can cause water intoxication with subsequent hyponatremic seizures (Lipsitz, 1984). Excessive use of chamomile also can cause diarrhea (Saxe, 1987).

### **Rosemary and Sage**

Rosemary and sage are commonly used herbs that contain bioactive components. Extracts from both plants are toxic to yeasts such as *Candida albicans*, causing cell wall destruction and impairment of metabolism. Eucalyptol appears to be the fungicidal constituent in these herbs (Steinmetz et al., 1987). Compounds found in rosemary (*Rosmarinus officinalis*) are listed in Table 12 (Duke, 1987b). There are also known allergic symptoms from ingesting sage (*Salvia officinalis*). Small amounts (like those used in dressings) can cause various symptoms including severe headache, nausea, and vomiting that may be reduced by taking caffeine soon after the onset of symptoms. Compounds found in sage are listed in Table 13 (Duke, 1987b).

**Abortifacients.** Sage, along with rue (*Ruta graveolens* L.), apiol, cohosh, and pennyroyal oil, is used by women in the United States as herbal abortifacients. The reasoning for turning to herbal substances in part is thought to be a movement away from conventional medical services. The following are three reasons for not using herbal abortifacients (Gold and Cates, 1980): 1) There is a chance of toxic reactions with increased dosages of herbal preparations; 2) Herbal preparations, if unsuccessful, could have a teratogenic effect on the developing child; and 3) By delaying an abortion, through failure of a herbal remedy, a woman could significantly increase her risks to a later, induced abortion.

Table 9. Natural chemicals in the herb yarrow (*Achillea millefolium*) and their biological activities.<sup>a</sup>

Chemical	Concentration ( $\mu\text{g/g}$ dry wt)	Activity
Achilletin		Hemocoagulant
Achilleine		Hemostat
Apigenin		Antihistaminic, antispasmodic
Azulene	0 - 140	Anti-inflammatory, antipyretic
Betaine		Antimyoatrophic, emmenagogue
Borneol	255	
Bornyl acetate	210	Insectifuge
5-Cadinene	8	
Caffeic acid		Antitumor, choleric, hepatotropic
Camphene	602	
Camphor	1,779	(LDLo <sup>b</sup> 990 mg ipr in rats) analgesic, anesthetic, antiseptic, antipruritic, carminative, deliriant, emetic, rubefacient, stimulant
Caryophyllene	159	
Chamazulene		Anodyne, anti-inflammatory, antiseptic, antispasmodic
Choline		(LD <sub>50</sub> 400 mg ipr in rats) lipotropic, hypotensive
1,8-Cineole	959	(LD <sub>50</sub> 2,480 mg orally in rats) antibronchitic, antilaryngitic, antipharyngitic, antirhinitic, expectorant, insectifuge
Copaene	59	
Coumarins		Anticoagulant
Cuminaldehyde	11	(LD <sub>50</sub> 1,390 mg orally in rats)
p-Cymene	369	(LD <sub>50</sub> 4,750 mg orally in rats) fungitoxic, insectifuge
Essential oil	1,000 - 14,000	
Eugenol		(LD <sub>50</sub> 3,000 mg orally in mice) analgesic, anesthetic, antiseptic, fungicide, larvicide
Humulene	22	
Isoartemisia ketone	860	
Limonene	171	
Luteolin		Anti-inflammatory, antispasmodic, antitussive
Menthol		(LD <sub>50</sub> 3,180 mg orally in rats) analgesic, anesthetic, counterirritant, antipruritic
Myrcene	22	
$\alpha$ -Pinene	941	Allelochemic, beetle-attractant, expectorant
$\beta$ -Pinene	713	Expectorant, insectifuge
Quercetin		(LD <sub>50</sub> 161 mg/kg orally in rats) anti-inflammatory, antispasmodic
Rutin		(LD <sub>50</sub> 950 mg/kg ivn in mice) antiatherogenic, antiedemic, anti-inflammatory, antithrombogenic, hypotensive, spasmolytic, vasopressor
Sabinene	1,235	
Salicylic acid		(LD <sub>50</sub> 891 mg/kg orally in rats) analgesic, antipyretic, antirheumatic
$\beta$ -Sitosterol		Antihypercholesterolemic, antiprostatic, antiprostatadenomic, antitumor, aphrodisiac, estrogenic
Stachydrine		Cardiotonic
Tannins		Antidiarrhetic, bactericide, viricide
$\alpha$ -Terpinene	131	Insectifuge
$\gamma$ -Terpinene	371	
Terpinen-4-ol	431	Antiallergenic, antiasthmatic, antiseptic, antitussive, bactericide, expectorant, fungicide, insectifuge
Terpinolene	48	
Thujone abortifacient		(LDLo <sup>b</sup> 120 mg/kg ipr in rats)
Tricyclene	27	
Triganelline		(LDLo <sup>b</sup> 5,000 mg/kg subcutaneously in rats) hypoglycemic

<sup>a</sup>From Duke (1987a,b).<sup>b</sup>LDLo = Lowest dose proven lethal in experimental animals.

Table 10. Biologically active compounds in oregano (*Origanum vulgare*).<sup>a,b</sup>

Compound	LD <sub>50</sub> (mg/kg)	Activities
Anethole	2,090	Carminative, expectorant, gastrostimulant, insecticide, lactagogue
Apigenin		Antiallergic, antioxidant, antispasmodic, choleric
Borneol	(LDLo) <sup>c</sup>	Analgesic, anti-inflammatory, antipyretic, hepatoprotective, spasmolytic
Caffeic acid		Antitumor, bactericide, choleric, antihistaminic
Camphor	(ip) <sup>d</sup> 3,000	Analgesic, anesthetic, antiseptic, antipruritic, carminative, deliriant, ecbolic, emetic, CNS-stimulant, rubefacient, stimulant, respiroinhibitor
Carene	4,800	
Carvacrol	810	Antiseptic, fungicide, spasmolytic, tracheorelaxant, vermifuge
Caryophyllene		Spasmolytic
Cineole	2,480	Antibronchitic, antilaryngitic, antipharyngitic, antirhininitic, choleric, expectorant, hepatotonic, insectifuge
Copaene		Insect-attractant
Cymene	4,750	Antiflu, viricide
Diosmetin		Capillary-fortificant
Essential oil	1,850	Choleretic, expectorant, spasmolytic
Farnesene		Pheromone
Geraniol	3,600	Antiasthmatic, candidicide, fungicide, insectifuge, insect-attractant
Hexanol	4,890	Antiseptic
Kaempferol		Anti-inflammatory, antifertility, choleric, diuretic, natriuretic
Limonene	(LDLo) <sup>c</sup> 4,600	Insecticide, dermatogenic
Linalool	2,790	Antiseptic, spasmolytic, perfumery
Luteolin		Anti-inflammatory, antispasmodic, choleric, diuretic
Myrcene		Bactericide, insectifuge, spasmolytic, perfumery
Naringenin		
Nerylacetate		Viricide
Oleanolic acid		Anticariogenic, antifertility, antihepatotoxic, cardiotonic, uterotonic, antisarcoma
Pentyl alcohol		Mucoirritant, narcotic
Pinene		Expectorant, insect-attractant, insectifuge
Rosmarinic acid		Antioxidant
Tannins		Antidiarrheic, antitumor, bactericide, viricide
Terpinen-4-ol		Antiseptic, fungicide, insectifuge, spermicide
Terpineol	4,300	Antiallergic, antiasthmatic, antitussive, bactericide, expectorant
Thujone	(ip) <sup>d</sup>	Cerebrodepressant, convulsant, epileptogenic, larvicide, insecticide, respiroinhibitor
Thymol	980	Anesthetic, anthelmintic, antibronchitic, antineuritic, antiseptic, bactericide, fungicide, tracheorelaxant
Ursolic acid		Anticariogenic, antitumor, CNS-depressant, diuretic

<sup>a</sup> From Duke (1985).

<sup>b</sup> From Duke (1988a,c).

<sup>c</sup> LDLo = Lowest dose proven lethal in experimental animals.

<sup>d</sup> ip = Intraperitoneal.

Table 11. Compounds found in basil (*Ocimum basilicum*).<sup>a</sup>

Compound	Concentration ( $\mu\text{g/g}$ dry wt)	Compound	Concentration ( $\mu\text{g/g}$ dry wt)
cis-Allo-ocimene	1	$\alpha$ -Guaiene	
trans-Allo-ocimene	1	$\delta$ -Guaiene	
$\alpha$ -Amorphene		$\gamma$ -Gurjunene	
cis-, trans-Anethol		cis-3-Hexanol	40
Ascorbic acid	612	$\alpha$ -Humulene	
Benzyl alcohol		Isocaryophyllene	
Benzyl acetate	163	Isoeugenol	95
cis- $\alpha$ -Bergamotene		Juvocimene	
trans- $\alpha$ -Bergamotene		Ledene	
Bicyclogermacene		Limonene	934
Bicycloelemene		Linalyl acetate	240
Bisabolene		Linalool	116
Borneol	435	Menthol	32
Bornyl acetate		Menthone	1
$\beta$ -Bourbonene		Methionine	20
$\epsilon$ -Bulgarene		Methylcatichol	780
$\alpha$ - $\gamma$ -Cadinene		Methyl cinnamate	800
10-Cadinol		Methyleugenol	400
Calamene		$\alpha$ -Murolene	
Camphepane	400	Myrcene	80
Camphor	143	Nerol	
$\delta$ -3-Carene	33	Nerolidol	
Caryophyllene	3,196	$\beta$ -Ocimene	435
$\beta$ -Caryophyllene	0 - 113	cis-Ocimene	252
$\beta$ -Cedrene		trans-Ocimene	50
2-Epi- $\alpha$ -cedrene		Octanol	
1,8-Cineole	143	3-Octanone	50
Citronellol	364	1-Octen-3-ol	
Copaene	20	Phenylethyl alcohol	136
$\alpha$ -Cubebene	1	Phytosterol	60
$\beta$ -Cubebene		$\alpha$ -Pinene	132
Cyclosativene		$\beta$ -Pinene	83
p-Cymene	16	Rosmarinic acid	1,000
Cystine	590	Sabinene	
$\alpha$ - <i>p</i> -Dimethylstyrene	1	trans-Sabinene hydrate	1
$\beta$ -Elemene		cis-Sabinene hydrate	
$\alpha$ -Elemene		Safrole	
1-Epibicyclosesquiphellandrene		$\alpha$ -Selinene	
Essential oil	1,500 - 10,000	$\beta$ -Selinene delta	
Estragole		Sesquithujene	
Eugenol	920	$\alpha$ -Terpinene	10
$\alpha$ -Farnesene		$\gamma$ -Terpinene	10
Farnesol	70	$\alpha$ -Terpineol	190
Fenclyl acetate	60	Terpinoline	22
Fenclyl alcohol	951	Terpinen-4-ol	120
Fenchone		$\alpha$ -Terpinyl acetate	
Furfural		$\alpha$ -Thujone	1
Geraniol	163	$\beta$ -Thujone	1
Geranyl acetate	84		

<sup>a</sup>From Duke (1988a,c).

Table 12. Compounds in rosemary (*Rosmarinus officinalis*).<sup>a</sup>

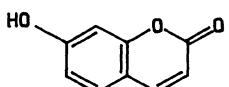
Compound	Concentration ( $\mu\text{g/g}$ dry wt)	Activity
Borneol	120 - 470	(LD <sub>50</sub> ) <sup>b</sup> 2,000 mg/kg orally in rabbits)
Camphor	539 - 2,910	Carminative, deliriant, analgesic, anesthetic, antiseptic, antipruritic, rubefacient, stimulant
Carvacrol		(LD <sub>50</sub> 810 mg/kg orally in rats) anthelmintic, antiseptic, fungicide, tracheorelaxant
Carvone		(LD <sub>50</sub> 1,640 mg/kg orally in rats) carminative, CNS-stimulant, insecticide
1,8-Cineole	852 - 5,120	(LD <sub>50</sub> 2,480 mg/kg orally in rats) antibronchitic, antilaryngitic, antipharyngitic, antirhinitic, expectorant, insectifuge
Essential oil	4,000 - 19,000	Has pesticidal and medicinal properties
Epirosmanol		Antioxidant
Glycolic acid		Diuretic
Isorosmanol		Antioxidant
Linalool	40 - 120	(LD <sub>50</sub> 2,790 mg/kg orally in rats) anticonvulsant, antiseptic
$\alpha$ -Pinene	1,030 - 3,226	Allelochemic, beetle-attractant, expectorant
Rosmarinic acid		Antioxidant
Rosmanol		
Safrole	32 - 95	(LD <sub>50</sub> 1,950 mg/kg orally in rats) anesthetic, antiseptic
Terpinen-4-ol	10 - 520	(LD <sub>50</sub> 4,300 mg/kg orally in rats) antiallergenic, antiasthmatic, antiseptic, antitussive, bactericide, expectorant, fungicide, insectifuge
Thymol		(LD <sub>50</sub> 980 mg/kg orally in rats) bactericide, fungicide, larvicide, tracheorelaxant, vermicide
Ursolic acid	39,000	Antitumor (ED <sub>50</sub> = 50 mg/kg), diuretic

<sup>a</sup>From Duke (1987b).<sup>b</sup>LD<sub>50</sub> = Lowest dose proven lethal in experimental animals.

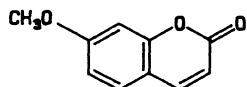
Individuals of Spanish and Mexican descent in New Mexico have used a number of plants other than those mentioned above for emmenagogues and abortifacients (Conway and Slocumb, 1979). Some of these plants are cotton root bark (*Gossypium* sp.), inmortal (*Asclepias capricornu* Woodson), poleo chino (*Hedeoma oblongifolia* (Gray) Heller), and wormseed (*Chenopodium ambrosioides* L.). When used as an abortifacient, cotton root bark seems to exhibit the lowest toxicity. Two to four ounces of fresh root bark is boiled in one quart of water for one-half hour. The entire decoction is drunk the first thing in the morning, and the abortion occurs from two to 6 days later with about a 30% success rate (Conway and Slocumb, 1979). The attention of the medical profession was attracted to cotton root bark in 1840 because it was a popular abortifacient among slaves in the United States. It was then dispensed as a pharmaceutical drug having a weak stimulating effect upon the uterus, as well as a vasoconstrictor action (Osol and Farrar, 1947).

### Bishop's Weed Seed

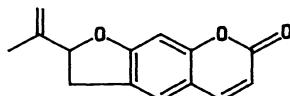
Bishop's weed (*Ammi majus* L.) is an annual plant that is used for the cut-flower trade and for medicinal purposes (Duke, 1988b). The chemical contents of the herb's seed are shown in Table 14 (Duke, 1988c). Ammirin, umbelliferone, herniarin, umbelliferone-(3'-hydroxymethyl-1t.-buten-1'-yl)-ether, and umbelliferone-(3'-methyl-but-1t.3-dien-1'-yl)-ether (Fig. 20) have been isolated from cell suspension



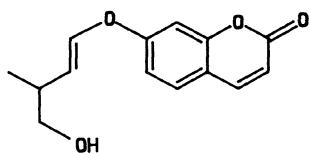
Umbelliferone



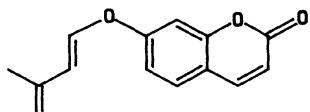
Herniarin



Ammirin



Umbelliferone-(3'-hydroxymethyl-1-buten-1'-yl)-ether



Umbelliferone-(3'-methyl-buta-1,3-dien-1'-yl)-ether

Figure 20. Coumarins isolated from Bishop's weed cell suspension cultures.

cultures of Bishop's weed (*Ammi majus* L.) (Hamerski et al., 1990). Bishop's weed has been used to treat skin depigmentation in the Middle East for centuries, and this weed also induces phototoxic responses in livestock and poultry (Ivie, 1978). The compounds in Bishop's weed can cause cataract formation in both humans and animals (Lerman, 1986). See section entitled "Parsleys..." for a discussion of biological properties and mode of action of furanocoumarins found in Bishop's weed and other similar plants.

### Bay Leaf

At least one herb, bay leaf, exhibits biological effects because of its physical size and shape. Bay leaf can become physically stuck in the pharyngeal pouch (Johns, 1980), becoming lodged in the mucos, and blocking the esophagus. It also can rupture meckel's diverticulum resulting in severe rectal pain (Panzer, 1983). Panzer suggests that bay leaf complications may be a more important source of morbidity than the present literature suggests.

Table 13. Compounds in sage (*Salvia officinalis*).<sup>a</sup>

Compound	Concentration ( $\mu\text{g/g}$ dry wt)	Activity
Borneol	140 - 2,636	(LDLo <sup>b</sup> 2,000 mg/kg orally in rabbits)
Bornyl acetate	57 - 656	Insectifuge
Camphor	28 - 1,410	(LDLo 900 mg/kg ipr in rats) analgesic anesthetic, antiseptic, antipruritic, carminative, deliriant, emetic, rubefacient, stimulant
Carnosic acid		Antioxidant
1,8-Cineole	550 - 5,410	(LD <sub>50</sub> 2,480 mg/kg orally in rats) antibronchitic, antilaryngitic, antipharyngitic, antirhinitic, expectorant, insectifuge
p-Cymene	81 - 324	(LD <sub>50</sub> 4,750 mg/kg orally in mice) fungicide, insectifuge
Essential oil	7,000 - 20,000	Has pesticidal and medicinal properties
Labiatic acid		Antioxidant
Linalool	1,191 - 3,500	(LD <sub>50</sub> 2,790 mg/kg orally in rats) anticonvulsant, antiseptic
$\alpha$ -Pinene	30 - 856	Allelochemic, beetle-attractant, expectorant
Rosmarinic acid	2,000	Antioxidant
Salvin		Bactericide
Terpinen-4-ol	29 - 1,018	(LD <sub>50</sub> 4,300 mg/kg orally in rats) antiallergenic, antiasthmatic, antiseptic, antitussive, bactericide, expectorant, fungicide, insectifuge
Thymol		(LD <sub>50</sub> 980 mg/kg orally in rats) bactericide, fungicide, larvicide, tracheorelaxant, vermicide
Ursolic acid	21,000	Antitumor (ED <sub>50</sub> = 50 mg/kg), diuretic

<sup>a</sup>From Duke (1987b).<sup>b</sup>LDLo = Lowest dose proven lethal in experimental animals.

### Asian Medicinal Herbs

Throughout history, infectious diseases have been treated with herbal medications, and scientists at present continually attempt to evaluate and identify their active principles. A review of various biologically active plants and active ingredients in medicinal plants were evaluated from Aztec derived Mexican folk medicines to Chinese herbal preparations (Steiner, 1986).

One hundred seventy-eight traditional Chinese medicinal herbs were investigated for an anti-*Bacteroides fragilis* substance. *B. fragilis* is found predominantly in fecal material and produces butyric acid. The bacterium is often obtained from soft-tissue infections. Only one of these herbs, rhubarb root (*Rheum officinale*), was found to possess the anti-*B. fragilis* activity. The active substance was subsequently isolated and shown to be 1,8-dihydroxyanthraquinone (Cyong et al., 1987). Eighteen herbs were also evaluated against 10 microbial pathogens. Eleven of the preparations were active against at least one pathogen, six were active against at least three pathogens, and two were active against five pathogens (Franzblau and Cross, 1986).

Chinese medicinal herbs, first used more than 2,000 years ago, are still used today to treat heart problems (Keji, 1981). When Chinese and Western medicines were combined in the treatment of coronary heart disease in China, a decrease in the mortality rate from 20 to 30% to 10 to 15% was observed. Extracts of 11 out of 27 Chinese medicinal herbs were active against the human immunodeficiency virus (HIV). Chinese medicinal herbs appear to be a rich source of drugs for the treatment of HIV (Chang and Yeung, 1988). Immunomodulatory activity was documented in fractions of *Astragalus membranaceus*. Fractions from this Chinese medicinal herb

Table 14. Compounds in Bishop's weed (*Ammi majus* L.) seed.<sup>a</sup>

Compound	Concentration ( $\mu\text{g/g}$ seed)
Alloimperatorin	1
Bergapten	400 - 3,100
Heracleinin	100 - 8,000
Isoimperatorin <sup>b</sup>	< 100
Isopimpinellin <sup>b</sup>	2,300
Marmesin	400 - 3,300
Marmesinin	100 - 1,450
Oxypeucedanin	3,000
Oxypeucedanin hydrate	< 100
Pabulenol	< 100
Saxalin	< 100
Xanthotoxin	2,300 - 10,000
Total psoralens	15,000 - 20,000

<sup>a</sup>From Duke (1988c).<sup>b</sup>From Ivie (1978).

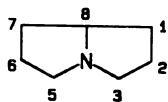
fully corrected an *in vitro* T-cell function deficiency in cancer patients (Chu et al., 1988).

When 10 Korean medicinal herbs were evaluated for mutagenicity by the Ames test, false-negative reactions were obtained. The substances that inhibited the production of positive reactions were removed through solvent fractionation which improved the reliability of the results (Lee et al., 1987). Morimoto et al. (1982), while examining 104 medicinal herbs, also found that medicinal herbs contained cytotoxic materials that can limit the applicability of the Ames test. Ten Pakistani medicinal herbs or mixtures used in treating children were tested with the Ames test. Extracts of Peshwar (mixture of unknown herbs), *Saussurea lappa*, *Swertia chiraita*, and *Skimmia laureda* were mutagenic. The addition of liver microsomal enzymes increased the activities of two extracts (Riazuddin et al., 1987).

There is no doubt that herbal medicines contain effective drugs for specific illnesses. Unfortunately, the herbal medications usually are not prescribed by individuals with the scientific knowledge of their contents, and they may contain many biologically active components. At times, herbal preparations are laced with drugs to improve their effectiveness (Saxe, 1987). The manufacturer of some Chinese herbal medicines for the treatment of arthritis and back pain have adulterated these herbal products with aminopyrine and phenylbutazone (probably to promote the commercial potential of their products). Both compounds are well-known causes of agranulocytosis and have caused many fatalities. Aminopyrine was removed from over-the-counter sale in the United States in 1938 (Ries and Sahud, 1975).

Because of the increased popularity of natural foods, health foods, and herbal products, the public needs to be aware and be concerned about the potential dangers associated with extensive use of these herbal products (Casterline, 1980). The biological activities found in a single herb can be truly diverse. A given herb will usually contain many components having quite varied, and often opposite, biological activities. At least 25 psychoactive substances have been identified in herbal preparations, and a number of intoxications have resulted from their use. Plants used

(a)



(b)

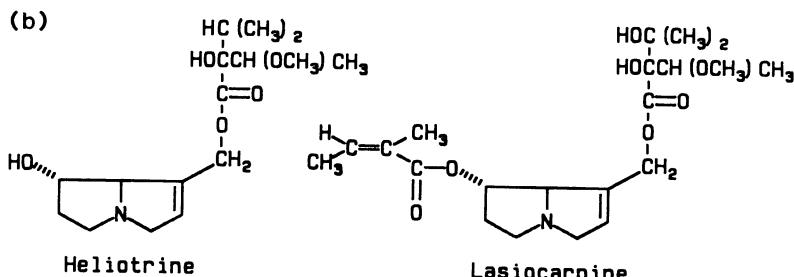


Figure 21. Basic structural moiety of the pyrrolizidine alkaloids (a) and two toxic members of that family, heliotrine and lasiocarpine (b).

in herbal preparations that contain psychoactive substances include broom, California poppy, catnip, cinnamon, hops, hydrangea, juniper, kola nut, nutmeg, periwinkle, thorn apple, and wild lettuce (Siegel, 1976). Natural pesticides and other biologically active materials in health foods and herbal products constitute a pharmacopoeia of uncontrolled substances in our nation's health food stores.

**Pyrrolizidine alkaloids.** Consumption of herbal medicines that contain pyrrolizidine alkaloids may contribute to the high incidence of chronic liver diseases in Asia and Africa. In one study, 3 of 50 medicinal plant species from Sri Lanka (*Crotalaria verrucosa* L., *Holarrhena antidysenterica* (L.) Br., and *Cassia auriculata* L.) contained pyrrolizidine alkaloids (Arsecularatne et al., 1981). In another study of 75 medicinal plants from Sri Lanka, only *Crotalaria juncea* L. contained pyrrolizidine alkaloids. Three other plant species not containing pyrrolizidine alkaloids produced hepatic lesions in rats, and two produced marked renal lesions (Arsecularatne et al., 1985).

Pyrrolizidine alkaloids can cause cirrhosis of the liver and occur in at least eight plant families (Cordell, 1981). Pneumotoxicities also may result from pyrrolizidine alkaloids (Schoental, 1968). A single i.v. 3.5 mg/kg dose of monocrotaline pyrrole produced delayed pulmonary microvascular leak, interstitial inflammation, and pulmonary hypertension in 14 days in rats (Reindel et al., 1990). These alkaloids contain a basic moiety consisting of one nitrogen at the bridgehead of two 5-membered rings (Fig. 21a). Cordell (1981) has reviewed the chemistry of these highly biologically active alkaloids. Indian herbal teas caused hepatic veno-occlusive disease in people who had consumed them (McLean, 1970). The herbs, identified as *Heliotropium lasiocarpum* Fisch. and Mey., contained pyrrolizidine alkaloids. The total alkaloid content of this herbal mixture was 0.47%, dry weight. The major compounds identified were heliotrine and lasiocarpine (Fig. 21b), and the minor constituents were europine and heleurine (Culvenor et al., 1986). The LD<sub>50</sub> of heliotrine is 300 mg/kg, and lasiocarpine is 72 mg/kg to rats (McLean, 1970). Comfrey (*Symphytum* spp.) is

Table 15. Phytoalexins in stressed lima bean.<sup>a</sup>

Compound	Concentration ( $\mu\text{g/g}$ fresh wt)
8-Hydroxygenistein	3,600
Genistein	2,300
8,2'-Dihydroxygenistein	5,800
2'-Hydroxydaidzein	2,300
2'-Hydroxygenistein	5,100
2'-Methoxygenistein	5,100
2,3-Dehydrokievitone	900
2,3-Dehydrokievitol	800
Luteone	200
Cyclo-2,3-dehydrokievitone hydrate	2,100
Isoferreirin	1,100
5-Deoxykievitone	2,300
Kievitone	1,300
5-Deoxykievitol	33,000
Kievitol	1,900
Kievitone hydrate	9,300
3'-( $\gamma,\gamma$ -Dimethylallyl)kievitone	9,700
1'',2''-Dehydrocyclokievitone	5,600
Cyclokievitone hydrate	500
Phaseollidin	4,700
4-( $\gamma,\gamma$ -Dimethylallyl)phaseollidin	400
2,10-Di( $\gamma,\gamma$ -dimethylallyl)glycinol	500
Coumestrol	1,200

<sup>a</sup> From Harborne (1986b).

a herb used as a green vegetable, beverage, or remedy. The leaves and roots of a species from Japan (*Symphytum officinale*) were hepatocarcinogenic in rats; this species contains at least eight pyrrolizidine alkaloids. Large differences in alkaloid concentrations occur between young and old comfrey leaves. The large, mature leaves have the lowest concentrations (Mattocks, 1980). The alkaloid contents of dried leaves are 0.003 to 0.2%, and those of dried roots are 0.2 to 0.4%. The amount of alkaloid consumed in a cup of comfrey root tea is 12 to 36 mg. A gelatinous residue forms during the process of making the tea; if it is consumed, as much as 26 mg of alkaloids could be consumed. Reliable data on effects of comfrey on humans are scarce, but available data indicate that the use of comfrey root tea could have significant human health consequences (Roitman, 1981).

### LIMA BEANS (*PHASEOLUS LUNATUS*)

Lima bean produces various phytoalexins to external stimulus (Rich et al., 1977) that are listed with relative concentrations in Table 15 (Harborne, 1986b). Most of these chemicals have measurable fungitoxicities (O'Neill et al., 1983). The variety of inducible chemicals identified from lima bean (Table 15) is much greater than only those suggested as the major plant chemicals presented in Table 1. For example, many plants like lima bean may contain a large variety of synthetic pathways that undergo increased chemical production in a phytoalexin response.

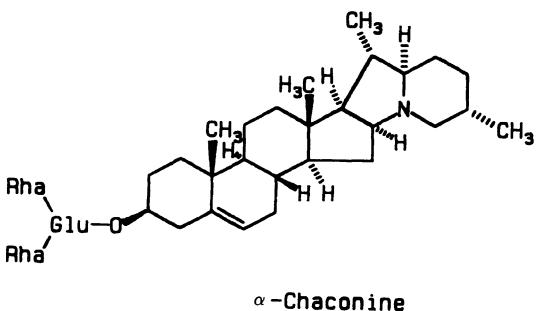
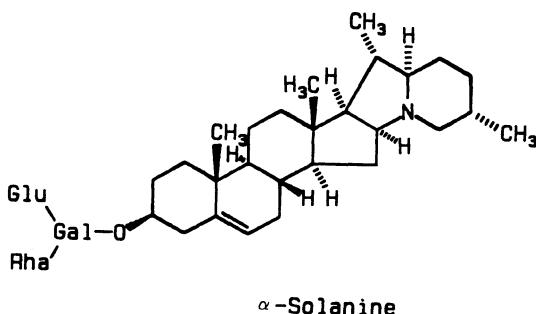


Figure 22. The major glycoalkaloids in potato,  $\alpha$ -solanine and  $\alpha$ -chaconine.

## NIGHTSHADES (SOLANACEAE): EGGPLANT, GARDEN PEPPERS, TOMATOES, AND WHITE POTATOES

### White Potatoes

The white potato, a member of the nightshade family, originally came from the highlands of Peru and was brought to this country by the Irish in 1719. Scottish highlanders ate large amounts of potatoes during the famine of 1782 and noticed a high incidence of dropsy (accumulation of fluid in the joints) (Salaman, 1985). Livestock also have died after ingesting potato vines, sprouts, peels, or green and cull potatoes. Severe human illness (McMillan and Thompson, 1979; Wood and Young, 1974) and fatalities have been caused by eating greened potatoes (Hansen, 1925; Kingsbury, 1964; Morris and Lee, 1984). Forsyth (1954) stated "Fatality in human beings and innumerable instances of severe illness have been reported from all over the world, after people have eaten greened or sprouted potatoes." Consumers have the notion that most of the potato's nutrients are in the peel and are unaware that toxic compounds are also in the peel (Mondy and Gosselin, 1988).

Phytoalexins (natural pesticides) like hydroxylubimin and rhisitin (Sato et al., 1978), lubimin and phytuberin (Grisebach and Ebel, 1978), and constitutive antibiotics like caffeic acid, chlorogenic acid, scopolin, and solanidine (Kuć, 1972) are present in potato. Two major constitutive glycoside alkaloids,  $\alpha$ -solanine and  $\alpha$ -chaconine (Fig. 22), are fungitoxic and are synthesized at cut (wound) surfaces (Allen and

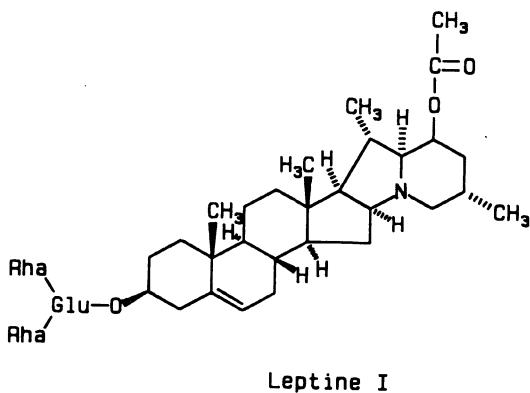


Figure 23. The potato glycoalkaloid, leptine I, is one of the most inhibitory natural products to human plasma cholinesterase (Orgell, 1963).

Kuć, 1968; Locci and Kuć, 1967). The synthesis of  $\alpha$ -solanine and  $\alpha$ -chaconine is also stimulated in tubers by mechanical injury and aging (Locci and Kuć, 1967; McKee, 1955; Sinden, 1972). Table 16 lists various glycoalkaloids found in potato (Jadhav et al., 1981). Compounds found in stressed potato have been reviewed by Stoessl et al. (1976). These include hydroxycinnamic acid, esculin, umbelliferone, rishitinol, anhydro- $\beta$ -rotunol, solavetivone, veticpiranes, and desacetylphytuberin. Glycosides of tomatidenol are also induced in specific varieties by slicing (Shih and Kuć, 1974). Any general disturbance of plant metabolism may trigger phytoalexin production in the potato (Stoessl et al., 1976).

$\alpha$ -Solanine (Fig. 22) is a weak to moderate inhibitor of both specific and non-specific cholinesterases (Patil et al., 1972), and  $\alpha$ -chaconine (Fig. 22) is a potent inhibitor of the cholinesterase isoenzymes (Alozie et al., 1979). When extracts of potato were used to inhibit human plasma cholinesterase, extracts from peel were 10 to 40 times more active than those from the innermost flesh, showing the accumulation of alkaloids in the peel (Orgell et al., 1958a,b). The potato glycoalkaloids, leptine I (Fig. 23) and demissidine (Table 16), were among the most inhibitory natural products to human plasma cholinesterase (Orgell, 1963). Cholinesterase inhibition was used to categorize 24 potato varieties (Orgell and Hibbs, 1963). No carcinogenicity data exist for the cholinesterase inhibitors found in potato (Ames et al., 1987).

Potato breeders, in general, have attempted to keep the  $\alpha$ -solanine content of commercial potatoes below 200  $\mu\text{g/g}$  fresh weight (Smith, 1977). The USDA potato breeding program has an accepted guideline of 200  $\mu\text{g/g}$  tuber for the total glycoalkaloid (TGA) content of parents and offspring of potential potato varieties. Although this is not a mandated or regulated TGA limit, it is accepted as being appropriate for insuring that growers and the public have high quality potatoes (Sinden, personal communication; Sinden and Webb, 1972). The  $\alpha$ -solanine content of 32 varieties of potatoes grown in Wisconsin ranged from 20 to 130  $\mu\text{g/g}$  of tuber (Wolf and Duggar, 1946). These glycoalkaloids are usually present at about 75  $\mu\text{g/g}$  of potato or 15,000  $\mu\text{g}$  per 200 g serving of potatoes (Jadhav et al., 1981). In comparison, malathion, the main synthetic cholinesterase inhibitor used as an insecticide, is present in our total diet at about 17  $\mu\text{g/day}$  (Ames et al., 1987).

Under certain weather conditions, potato tubers may synthesize considerable amounts of  $\alpha$ -solanine. Exposure to light in the field or in the marketplace can

Table 16. Potato glycoalkaloids.<sup>a</sup>

Compound	Formula
$\alpha$ -Solanine	C <sub>45</sub> H <sub>73</sub> NO <sub>15</sub>
$\beta$ -Solanine	C <sub>39</sub> H <sub>63</sub> NO <sub>11</sub>
$\gamma$ -Solanine	C <sub>33</sub> H <sub>53</sub> NO <sub>6</sub>
$\alpha$ -Chaconine	C <sub>45</sub> H <sub>73</sub> NO <sub>14</sub>
$\beta$ -Chaconine	C <sub>39</sub> H <sub>63</sub> NO <sub>10</sub>
$\gamma$ -Chaconine	C <sub>33</sub> H <sub>53</sub> NO <sub>6</sub>
Solanidine	C <sub>27</sub> H <sub>43</sub> NO
Demissine	C <sub>50</sub> H <sub>83</sub> NO <sub>20</sub>
Commersonine	C <sub>49</sub> H <sub>81</sub> NO <sub>19</sub>
Demissidine	C <sub>27</sub> H <sub>45</sub> NO
5 $\beta$ -Solanidan-3 $\alpha$ -ol	C <sub>27</sub> H <sub>45</sub> NO
Leptidine	C <sub>29</sub> H <sub>45</sub> NO <sub>3</sub> <sup>b</sup>
Leptine I	C <sub>47</sub> H <sub>75</sub> NO <sub>16</sub> <sup>b</sup>
Leptine II	C <sub>47</sub> H <sub>75</sub> NO <sub>17</sub>
O(23)-Acetylleptinidine	C <sub>29</sub> H <sub>45</sub> NO <sub>3</sub>
Leptinine I	C <sub>45</sub> H <sub>73</sub> NO <sub>15</sub>
Leptinine II	C <sub>45</sub> H <sub>73</sub> NO <sub>16</sub>
Leptinidine	C <sub>27</sub> H <sub>43</sub> NO <sub>2</sub>
$\alpha$ -Solamarine	C <sub>45</sub> H <sub>73</sub> NO <sub>16</sub>
$\beta$ -Solamarine	C <sub>43</sub> H <sub>73</sub> NO <sub>15</sub>
Tomatidenol	C <sub>27</sub> H <sub>43</sub> NO <sub>2</sub>

<sup>a</sup>From Jadhav et al. (1981).<sup>b</sup>From Morris and Lee (1984).

increase the  $\alpha$ -solanine content to dangerous levels (Griebel, 1924). The glycoalkaloid content can also increase during potato growth as well as after harvest (Sinden and Webb, 1972). The amounts of  $\alpha$ -solanine and  $\alpha$ -chaconine increase with light- or wound-induction (Wu and Salunkhe, 1978). Several outbreaks of illness have been traced to the use of potatoes with  $\alpha$ -solanine contents ranging from 100 to 400  $\mu\text{g/g}$  (Alfa and Heyl, 1923). Acute illnesses induced by glycoalkaloid poisoning probably are more prevalent than is indicated by the few recorded medical cases (Wood and Young, 1974).

The TGA content of potato tissues is given in Table 17 (Wood and Young, 1974). Bruising can dramatically increase the TGA content at various temperatures (Jadhav et al., 1981), significantly enhance the phenolics, and decrease ascorbic acid levels. In fresh Katahdin potatoes, the greatest increase of phenols from about 210  $\mu\text{g/g}$  to 1,720  $\mu\text{g/g}$  occurred at 5°C, and an increase in TGA content from about 50  $\mu\text{g/g}$  to 220  $\mu\text{g/g}$  occurred at 20°C (Mondy et al., 1987). At 10 days after wounding, Juliver variety tubers held in the dark had  $\alpha$ -chaconine and  $\alpha$ -solanine concentrations of 685.4  $\mu\text{g/g}$  and 499.6  $\mu\text{g/g}$ , respectively; unwounded control tubers contained 371.7  $\mu\text{g/g}$  and 271.6  $\mu\text{g/g}$ , respectively. Final concentrations of glycoalkaloids in wounded potatoes were usually higher in the light. Potato chips produced from unwounded tubers had  $\alpha$ -chaconine and  $\alpha$ -solanine concentrations of 107.1  $\mu\text{g/g}$  and 69.2  $\mu\text{g/g}$ , respectively, and those from wounded tubers had 245.7  $\mu\text{g/g}$  and 197.6  $\mu\text{g/g}$ , respectively (Ahmed and Müller, 1978). Potatoes that contain more than 200  $\mu\text{g/g}$  TGA per tuber are considered unfit for consumption (Smith, 1977; Wood and Young, 1974). Average TGA contents of potato tubers from Lenape, Kennebec, Russet Burbank, Katahdin, Irish Cobbler, and Red Pontiac potato

Table 17. Total glycoalkaloids in potato tuber tissues.<sup>a</sup>

Potato tissue	Total glycoalkaloids ( $\mu\text{g/g}$ fresh wt)
Skin, 2-3% of tuber	300-600
Peel, 10-15% of tuber	150-300
Peel and eye, 1/8-in. (3-mm) disk	300-500
Flesh	12-50
Whole tuber	75
Peels from bitter tubers	1,500-2,200
Bitter whole tubers	250-800

<sup>a</sup>From Wood and Young (1974).

varieties were 293, 97, 79, 79, 62, and 43  $\mu\text{g/g}$  potato, respectively (Sinden and Webb, 1972).

Consumption of potato peels in restaurants has increased (Mondy and Gosselin, 1988). At least one company is producing a potato chip made from potato peels. Fried potato peels are a source of large quantities of glycoalkaloids. In one study, quantities of  $\alpha$ -solanine plus  $\alpha$ -chaconine in fried peels ranged from 1,390 to 1,450  $\mu\text{g/g}$  (Bushway and Ponnampalam, 1981). These quantities are more than seven times the recommended upper safety limit of 200  $\mu\text{g/g}$  of potato for potato glycoalkaloids. In another study, cooked peels had glycoalkaloid levels from two to eight times that of the upper safety limit (Bushway et al., 1983). Table 18 lists  $\alpha$ -solanine and  $\alpha$ -chaconine (Fig. 22) concentrations in baked/fried peel of commercial potato varieties.

$\alpha$ -Solanine and  $\alpha$ -chaconine contents of potatoes are not affected by baking, boiling, or microwaving and are only slightly reduced by frying (Bushway and Ponnampalam, 1981). During cooking, glycoalkaloids move into the cortex region; whereas, phenols migrate from the peel into both the cortex and internal tissues. When potatoes were peeled before cooking, their phenolic contents were decreased, but the TGA contents in the internal tissues were unchanged (Mondy and Gosselin, 1988). Symptoms of TGA poisoning can be easily mistaken for gastroenteritis. Common symptoms are nausea, diarrhea, vomiting, stomach cramps, headaches, and dizziness (Wood and Young, 1974). Potato glycoalkaloids have a saponin-like activity and can lyse cells (König, 1953). Syrian hamsters receiving dried potato sprouts or alkaloid extracts had severe gastric and intestinal mucosal necrosis. Lesions observed in intestinal epithelium of hamsters are similar to those caused by alkaloids. These lesions are consistent with the gastroenteritis observed in humans and animals suspected of being intoxicated by potatoes (Baker et al., 1987).

Bitterness, indicative of high TGA content in potato tubers, can readily be detected by chewing a small piece of the raw peel. TGA levels higher than about 100  $\mu\text{g/g}$  of tuber cause a slow developing, hot burning, persistent irritation of the sides of the tongue and back of the mouth. Potatoes that contain more than 200  $\mu\text{g/g}$  give an immediate burning sensation (Wood and Young, 1974).

Potato varieties have been investigated for toxic glycoalkaloids that could be utilized against the Colorado potato beetle (CPB), a devastating potato pest (Sinden et al., 1980). The potato glycoalkaloid, leptine I (Fig. 23), was a better pest deterrent than  $\alpha$ -solanine or  $\alpha$ -chaconine (Sinden et al., 1986). Its biological activity may be due to its cholinesterase-inhibiting effects. Leptine I is the most inhibitory to human

Table 18. Combined  $\alpha$ -solanine and  $\alpha$ -chaconine concentrations in baked-fried peels of commercial potato varieties.<sup>a</sup>

Variety	$\alpha$ -solanine + $\alpha$ -chaconine range ( $\mu\text{g/g}$ )
Russet Burbank	558.6 - 1,256.8
Kennebec	1,364.0 - 1,557.4
Katahdin	537.0 - 1,041.4
Superior	289.2 - 495.4
Allagash Russet	368.1 - 414.0
Round White	252.4 - 314.1
Green Mountain	341.4 - 411.0
Round White	215.6 - 230.2
Bel Rus	113.0 - 187.0
Russet	41.7 - 136.8

<sup>a</sup>From Bushway et al. (1983).

plasma cholinesterase among the natural products tested (Orgell, 1963). Kuhn and Löw (1961) did not find leptines in tubers of potato varieties that contained high concentrations in foliar tissues. Leptines were not observed in tubers of three *Solanum* clones with high leptine foliar concentrations (Sinden et al., 1986). But leptinines, which are obtained from leptines by the loss of an acetyl group, were found in tubers in concentrations ranging from 20  $\mu\text{g/g}$  to 390  $\mu\text{g/g}$  fresh tuber. Leptinines are weak deterrents of pest feeding. Also, beetles resistant to DDT are less sensitive to leptines or leptinines than normal beetles (Stürckow and Löw, 1961). New potato cultivars produced by cell fusion, a genetic engineering technique, contained high foliar leptine concentrations but contained no measurable amounts in their tubers (Berberich, 1988). These varieties have good resistance against CPBs without increasing the tuber leptine content.

High TGA concentrations in foliar tissues can be a significant factor in the defense of the potato plant against pests (Tingey et al., 1978). Breeding can produce pest resistant potato varieties without elevating the glycoalkaloid levels in their tubers (Sinden, personal communication). However, plant breeder efforts to produce more pest resistant potatoes can result in a potato with high levels of glycoalkaloids. High  $\alpha$ -solanine content (Lepper, 1949) or other glycoalkaloids appear to be a varietal characteristic. High levels of steroid glycoalkaloids in the potato cultivar "Lenape," caused its quick withdrawal from the marketplace because of acute toxicity to humans (Jadhav et al., 1981). This is exactly the same situation as recently observed with celery in which human toxicity problems resulted from increased levels of linear furanocoumarins inadvertently bred into celery to obtain better pest resistance (Berkley et al., 1986; Seligman et al., 1987).

Nitrates can react with secondary and tertiary amines to form carcinogenic and mutagenic *N*-nitroso compounds (Walters et al., 1979). Nitrate also can easily be reduced to nitrite under physiological conditions. It is estimated that 80% of our nitrate intake is from vegetables, and less than 20% comes from cured meats. A list of vegetables and their average nitrate levels are shown in Table 19.

Potatoes contribute approximately 14% of the per capita ingestion of nitrate in the United States. The average nitrate level in potatoes is 119  $\mu\text{g/g}$  fresh weight. The highest average levels of nitrate found in vegetables are in beets, celery, and spinach: 2,760, 2,340, and 1,860  $\mu\text{g/g}$  of fresh weight, respectively (White, 1975). The

Table 19. Nitrate content of vegetables.<sup>a</sup>

Vegetable	Concentration ( $\mu\text{g/g}$ fresh wt)
Potatoes	119
Tomatoes	62
Lettuce	850
Melons	433
Corn	45
Onions	134
Bean, snap	253
Pickles	59
Carrots	119
Cabbage	635
Beans, dry	13
Peas	28
Celery	2,340
Sweet potatoes	53
Cucumbers	24
Sweet peppers	125
Spinach	1,860
Beets	2,760
Sauerkraut	191
Broccoli	783
Cauliflower	547
Asparagus	21
Bean, lima	54
Pumpkin	413
Eggplant	302

<sup>a</sup> From White (1975).

of solanidine, the alkaloid aglycone of  $\alpha$ -solanine and  $\alpha$ -chaconine (Fig. 22), is known nitrate content of potatoes is influenced by variety, type, and amount of nitrogen fertilizer, climate, moisture stress, and storage conditions. Improper irrigation along with high nitrogen fertilizer rates can dramatically increase nitrate-nitrogen levels in tubers. Tubers have been found with nitrate-nitrogen levels over 1,200  $\mu\text{g/g}$  (Augustin et al., 1977; Carter and Bosma, 1974). In a study of Katahdin and Kennebec potatoes, the application of the auxin (naturally occurring hormone), indoleacetic acid (IAA), significantly decreased the TGA and nitrate-nitrogen content of both varieties (Ponnampalam and Mondy, 1986).

The second edition of the book, "A Diet to Stop Arthritis: The Nightshades and Ill Health," contains over 200 non-scientifically controlled case histories of people who removed nightshades (glycoalkaloids) from their diets and reportedly recovered to varying degrees from arthritis symptoms—from just feeling better to becoming ambulatory (Childers, 1981).

**Teratogens.** Many common commercial food products contain potatoes; there is even a potato ice cream. We serve them in many ways in our homes, and potatoes are also used in baby foods. It is difficult for an adult or child to avoid large exposures to potatoes or potato products. Because of this fact, it would be very important to breed potato varieties with low levels of glycoalkaloids in their tubers.

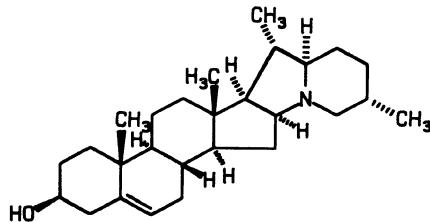
The glycoalkaloids in potato may be possible teratogens (Ames, 1983). Renwick (1972) put forth the hypothesis that potato avoidance by pregnant women would reduce the incidence of human anencephaly and congenital spina bifida (ASB)

by 95% in certain geographical areas. Severity of potato late-blight (which causes an increase in glycoalkaloid content of potatoes) correlates with the incidence of ASB in man. Ireland has highly suitable weather for the blight fungus and also has the world's highest incidence for ASB (Penrose, 1957). Similar correlations between late-blight and ASB exist in potato growing regions around the world. Areas that have changed to potato varieties (having more blight resistance) with greater glycoalkaloid content have also undergone as much as a doubling in the frequency of anencephaly (Renwick, 1972).

Late-blight or other fungi causing elevated natural plant chemicals in potato, which might result in toxicity problems, is not a new phenomenon. Many plant poisoning syndromes are known to occur as a result of fungal invasion with subsequent production of natural toxic chemicals. One of these, the increase of phototoxic linear furanocoumarins in celery infected with *Sclerotinia sclerotiorum* (Floss et al., 1969; Scheel et al., 1963; Wu et al., 1972), causes photodermatitis in celery workers and handlers and is due to an increase of plant phytoalexins (linear furanocoumarins) as a result of the fungal attack (Beier and Oertli, 1983). Another is the salivary syndrome caused by slaframine and characterized by excessive salivation. Legume forages parasitized by the fungus *Rhizoctonia leguminicola*, when consumed by small animals and cattle, result in salivary episodes ranging in duration from 6 hours to 3 days (Smalley, 1978). Neurologic diseases of cattle that are characterized by sustained tremors can be produced by at least six tremorgenic mycotoxins. These mycotoxins are produced by several species of fungi belonging to the genera *Aspergillus* and *Penicillium* (Buck and Cysewski, 1986b). A problem of both animals and man is the nervous ergotism syndrome. This problem arises from alkaloids produced by fungi on rye, wheat, or barley. These alkaloids stimulate smooth muscle (Buck and Cysewski, 1986a). Toxic problems that are either caused directly by a fungus or caused by a plant in response to fungal invasion are well-known. A requirement for onset of toxic episodes is to have the appropriate weather conditions for fungal growth.

Initial reports cast doubt on the epidemiologic relationships that formed the basis of Renwick's hypothesis that potato avoidance by pregnant women would reduce the incidence of human anencephaly and congenital spina bifida (Elwood and MacKenzie, 1973; Emanuel and Sever, 1973; Field and Kerr, 1973; Kinlen and Hewitt, 1973; McMahon et al., 1973; Nevin and Merrett, 1975; Spiers et al., 1974). Keeler (1986) also was unable to cause teratogenic effects in animals during 1973 to 1975. Then, both pure  $\alpha$ -solanine and glycoalkaloid extracts from blighted potatoes were shown to produce teratogenic effects in chick embryos (Jelinek et al., 1976; Mun et al., 1975), and teratogenic effects were also produced in NAW/pr mice by  $\alpha$ -chaconine but not by  $\alpha$ -solanine (Pierro et al., 1977). Neural tube defects were caused in Syrian hamsters by  $\alpha$ -chaconine and, to a lesser extent, by  $\alpha$ -solanine (Renwick, 1982a).

Keeler et al. (1978) showed that the new sprouts of all potato varieties tested (Kennebec, Nampa, Norchip, Pioneer, Russet, Sebago, and Targhee) were teratogenic in Simonson hamsters. The type of deformities in offspring were mainly cranial blebs and exencephalics, with some microphthalmics and spina bifidas. The tubers and peels of Kennebec potatoes were not teratogenic. Sprouts from the British potato cultivar, Arran Pilot, also were teratogenic and caused cranial bleb, encephalocele, exencephaly, and spina bifida (Renwick et al., 1984). Most of the teratogenic activity was traced to  $\alpha$ -chaconine and, at higher doses,  $\alpha$ -solanine. Women who had not passed the reproductive age were discouraged from eating desprouted potatoes (Renwick, 1982b). Nevin and Merrett (1975) emphasized that some mothers on potato avoidance diets during pregnancy still gave birth to ASB children and claimed that this result refuted Renwick's hypothesis. Only limited information on the effects



**Solanidine**

Figure 24. Solanidine, potential teratogen that is stored in the body for prolonged periods of time (Claringbold et al., 1982).

Zitnak (1961) reasoned that solanidine (Fig. 24) may be the most active participant in toxic episodes and suggested that the physiological effects of solanidine need to be better understood. Kinetic and retention studies of solanidine (Fig. 24) in man and verification of this alkaloid in postmortem liver samples by mass spectrometry suggested that solanidine is stored in the body for prolonged periods of time (Claringbold et al., 1982). Claringbold et al. (1982) concluded that, in times of metabolic stress (pregnancy, starvation, or illness), stored solanidine might be mobilized. The evidence for human teratogenic risk from consumption of potatoes is still only circumstantial; however, in a recent review of the toxicity and teratogenicity of Solanaceae glycoalkaloids, Morris and Lee (1984) suggested that green or damaged potato tubers especially should be avoided by women likely to become pregnant.

A testing program for toxic components was suggested for new potato varieties (Jadhav et al., 1981). A rapid analytical method was developed, based on high-performance liquid chromatography (HPLC), to evaluate the concentrations of  $\alpha$ -solanine and  $\alpha$ -chaconine in fresh potatoes (Carman et al., 1986). The sum of the  $\alpha$ -solanine and  $\alpha$ -chaconine content was termed the total glycoalkaloids without considering other potentially present glycoalkaloids. As of this writing, there remains no compliance levels set for potato glycoalkaloids.

### Eggplant

The main phytoalexin identified in eggplant is lubimin (Fig. 25) (Stoessl et al., 1974; Ward et al., 1975), but other sesquiterpenes also have been identified after inoculation of eggplant with various fungi (Stoessl et al., 1976; Ward et al., 1975). The spirosolane alkaloid, solasodine (Fig. 25), occurs in eggplant as well as other food plants (Schriener, 1968). Solasodine produced teratogenic effects in hamsters during the primitive streak/neural plate stage. The deformities were cranial bleb, exencephaly, and spina bifida (Keeler et al., 1976a,b). Solasodine was not teratogenic in rats (Keeler, 1973). However, Russian workers found solasodine to be embryotoxic and teratogenic in rats at 5 to 10 mg/animal/day and also that solanine (Fig. 22) had no effect at 5 mg/animal/day (Seifulla and Ryzhova, 1972).

### Green Peppers

Green peppers contain the sesquiterpene phytoalexin capsidiol (Fig. 26). About half of the extractable material from pepper is capsidiol (Stoessl et al., 1972).

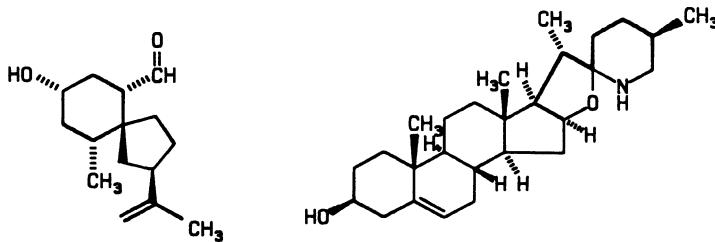


Figure 25. The phytoalexin, lubimin, and the teratogenic solasodine.

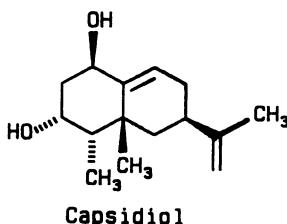


Figure 26. The phytoalexin, capsidiol.

Phenolic compounds also accumulate in peppers after fungal or viral infection or chilling injury (Stoessl et al., 1976).

### **Tomatoes**

Tomato produces  $\alpha$ -tomatine (Fig. 27) as its primary toxic agent, a steroidal glycoalkaloid, and its distribution throughout the plant was reported by Jadhav et al. (1981). The  $\alpha$ -tomatine content is responsible for making tomato plants unpalatable to livestock. Consumption of green tomato plants by pigs resulted in illness or death (Forsyth, 1954).

$\alpha$ -Tomatine (Fig. 27) has antibiotic properties inhibiting cultures of several organisms (Irving et al., 1946), and it is toxic to a wide range of living organisms (Jadhav et al., 1981). The aglycone of  $\alpha$ -tomatine is tomatidine. In mice, intravenous injections of tomatidine were slightly more toxic than those of the parent glycoside (Wilson et al., 1961). There are about 360  $\mu\text{g/g}$  of  $\alpha$ -tomatine in red tomato fruit, 450  $\mu\text{g/g}$  in yellow fruit, and 870  $\mu\text{g/g}$  in green fruit (Jadhav et al., 1981).  $\alpha$ -Tomatine and tomatidine have not been tested in other toxicity or cancer bioassays.

### **PARSLEYS (UMBELLIFERAE) AND CITRUS (RUTACEAE) FAMILIES**

Linear furanocoumarins are potent photosensitizing toxins that also act as phytoalexins in celery (Beier and Oertli, 1983; Beier et al., 1983a), parsley (Knogge et al., 1987; Tietjen et al., 1983), parsnip (Johnson et al., 1973), citrus, and fig leaves (Zaynoun et al., 1984). Contact dermatitis and photodermatitis has been described in children handling limes (Gross et al., 1987; Maryland Department of Health and Mental Hygiene, 1984); in individuals who handle figs (Zaynoun et al., 1984); in parsley pickers and cutters (Smith, 1985; Stransky and Tsankov, 1980); and in celery

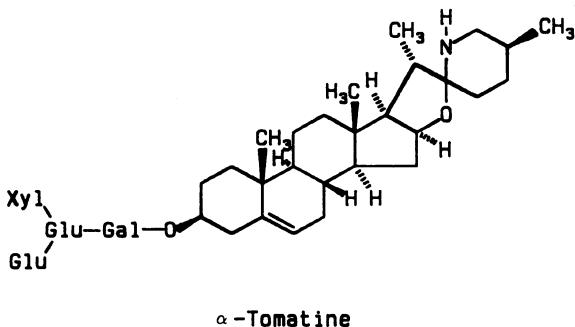
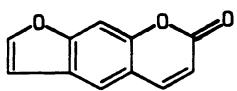


Figure 27. The steroidal glycoalkaloid,  $\alpha$ -tomatine.

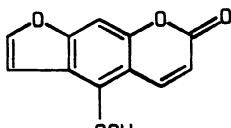
handlers, field workers, and processors (Birmingham et al., 1961; Legrain and Barthe, 1926). Celery dermatitis of the fingers, hands, and forearms is known to be caused by photosensitizing linear furanocoumarins (Scheel et al., 1963). It was initially thought that only diseased celery, especially celery infected with *Sclerotinia sclerotiorium*, contained these compounds and caused the photosensitization in humans (Scheel et al., 1963; Wu et al., 1972). However, grocery workers have experienced phytophotodermatitis caused by apparently healthy celery; incidents documented by the Centers for Disease Control (CDC) (Berkley et al., 1986; Seligman et al., 1987).

Biological activities of linear furanocoumarins are expressed in a broad spectrum of living things, from fungi to man. This diverse activity is due to intercalation of linear furanocoumarins into DNA (Belogurov and Zavilgelsky, 1981; Cassier and Moustacchi, 1981; Grekin and Epstein, 1981; Parsons, 1980; Scott et al., 1976) and RNA (Talib and Banerjee, 1982) where they form covalent bonds in the presence of long-wave UV light resulting in both mono- and di-adducts (i.e., cross-links). An excellent discussion of the photochemistry of psoralens and their DNA and RNA adducts is presented by Hearst (1989), and their toxicological, environmental, and co-evolutionary significance is presented by Ivie (1987).

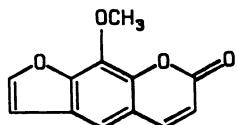
Because linear furanocoumarins are potent photoactive compounds, they have been used clinically to treat skin depigmentation (vitiligo, a type of leukoderma) (Pathak et al., 1962; Scott et al., 1976), psoriasis (McEvoy and Stern, 1987; Van Scott, 1976), and prurigo nodularis lesions (Hann et al., 1990). Recently, a patent application was made for 5-methoxysoralen (bergapten) (Fig. 28) and related linear furanocoumarins to be used for suppressing or preventing jet lag (Forlot, 1990). An oral or topical dose of psoralen (Fig. 28) followed by exposure to long wavelength (320 to 400 nm) ultraviolet light (UVA), is known as PUVA (psoralen + UVA). PUVA appears to still be the best available treatment for vitiligo (Plott and Wagner, 1990), and sequential combined treatment of UVB irradiation and topical PUVA appears to be the best treatment of generalized prurigo nodularis and prevention of new lesions (Hann et al., 1990). The medicinal use of these compounds has caused concern (National Institute of Environmental Health Sciences, 1982). PUVA caused mice to develop papillomas, keratoacanthomas, and squamous cell carcinomas (Hannuksela et al., 1986). Stern et al. (1979) suggested, following a study of PUVA patients, that PUVA treatment poses an increased carcinogenic risk. An animal study indicated that PUVA therapy may initiate tumors (Young, 1986). PUVA therapy unequivocally can cause cataract formation in both animals and humans (Lerman, 1986). An 8-year follow-up of patients failed to show an increase of tumors from PUVA treatments (Henseler et al., 1987) unless patients were previously exposed to



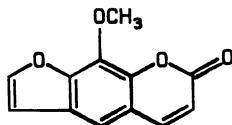
Psoralen



Bergapten



Xanthotoxin



Isopimpinellin

Figure 28. The four major linear furanocoumarins in celery.

carcinogens (Tanew et al., 1986). However, in a 12.3 year prospective study of 892 men that received PUVA treatment for psoriasis resulted in the incidence of invasive squamous-cell carcinoma on the genitalia was 286 times that of the general population (Stern et al., 1990). Stern et al. (1990) concluded that "the strongly dose-dependent increase in the risk of genital tumors associated with exposure to PUVA and ultraviolet B radiation that we observed makes it prudent for men to use genital protection whenever they are exposed to PUVA or other forms of ultraviolet radiation for therapeutic, recreational, or cosmetic reasons." The advent of tanning booths can be a definite health hazard to those individuals taking psoralen treatments or those exposed to celery juice or other produce containing too high of a level of linear furanocoumarins. A 45-year-old woman died from burns she received in a tanning booth while she was taking psoralen (Anonymous, 1989), produce workers were badly burned when they visited a tanning parlor after work [CG Toby Mathias (NIOSH), personal communication], and a 65-year-old woman developed a severe generalized phototoxic reaction following a visit to a suntan parlor resulting in erythema, edema, and blistering, which was restricted to UV-exposed skin. The woman had eaten a celery root weighing approximately 450 g one hour prior to visiting the suntan parlor; she also drank the juice in which the celery was cooked. The total amount of psoralens in the consumed root was approximately 45 mg, an equivalent dosage to PUVA therapy (Ljunggren, 1990).

## Celery

Celery contains at least four linear furanocoumarins: psoralen, bergapten, xanthotoxin, and isopimpinellin (Fig. 28) (Beier et al., 1983b). Isopimpinellin is not a photosensitizer (Ashwood-Smith et al., 1983; Hudson et al., 1987) and, therefore, has not been quantified in many studies. Scheel et al. (1963) described another linear furanocoumarin, 4,5',8-trimethylpsoralen (TMP) as being the causal agent for photosensitized skin reactions in farm workers. To our knowledge, no one else has been able to demonstrate the presence of TMP in celery or from any other natural source. A number of researchers have stated that they were not able to observe TMP in fresh or diseased celery samples (Ashwood-Smith et al., 1985; Austad and Kavli,

Table 20. Volatile compounds in fresh celery.<sup>a</sup>

3-Hexanol	$\beta$ -Caryophyllene
Hexanol	$\alpha$ -Selinene
$\beta$ -Pinene	Butylphthalide
2-Pentylfuran	<i>trans</i> -3n-Butylidene phthalide
<i>p</i> -Cymene	Sedanenolide
Limonene	<i>cis</i> -Ligustilide
$\gamma$ -Terpinene	Sedanolide
Camphor	Psoralen
Pentylbenzene	Bergapten
<i>cis</i> -Verbenone	Xanthotoxin
<i>trans</i> -Carveol	Isopimpinellin
Linoleic acid	

<sup>a</sup>From Tang et al. (1990).

1983; Beier and Oertli, 1983; Beier et al., 1983b; Chaudhary et al., 1985; Wu et al., 1972). Recently, a report of photoactive furanocoumarins in diseased celery included in the data table the observation of TMP made by Scheel et al. (1963) (Karasawa et al., 1990). The observation by Sheel et al. (1963) appears likely to be in error and it would be in the best interest of other researchers in the field to discontinue using the Scheel et al. (1963) observation of TMP unless the presence of TMP in celery can be demonstrated by another laboratory.

In a study of the volatile compounds present in fresh celery, the components listed in Table 20 were identified and quantified (Tang et al., 1990). However, all compounds were quantified according to the single standard, methyl decanoate. If the flame ionization detector response factor varied for any component from methyl decanoate, then the determined concentration of that constituent would be in error. Therefore, since the concentrations of bergapten and xanthotoxin are usually observed to be larger than that of psoralen in fresh celery, and those same concentrations are usually at least 10-fold greater than that published by Tang et al. (1990), the determined concentrations by Tang et al. (1990) are not listed here.

Linear furanocoumarins are phytoalexins in celery (Beier and Oertli, 1983). Their phytoalexin behavior results in increased levels in celery as a response to general elicitors including copper sulfate,  $Hg^{++}$  and  $Cu^{++}$  ions, UV light, cold, fungicides, herbicides, polyamines, and fungal cell wall fragments. It is quite understandable that acidic fog at pH levels similar to that experienced in commercial celery production near major population centers in California would stimulate the phytoalexin response of the linear furanocoumarins in celery (Dercks et al., 1990). A single 4-hour acidic fog resembling air pollution near major population centers stimulated the production of psoralen, bergapten, xanthotoxin, and isopimpinellin (Fig. 28) in celery foliage within 24 hours. Concentrations of photoactive linear furanocoumarins in some common celery cultivars (Beier et al., 1983b) are compared (Table 21) with those of the celery cultivar implicated in photophytodermatitis of grocery store workers by CDC studies in 1984 (Berkley et al., 1986) and 1986 (Seligman et al., 1987). The average quantity of linear furanocoumarins in the implicated cultivar was 14 times higher than that in other cultivars; the concentration of the most photoactive linear furanocoumarin, psoralen, was 19 times higher in the implicated cultivar. In both CDC studies, the same celery cultivar was determined to be the causative agent of the photophytodermatitis. Linear furanocoumarin concentrations from about 8 to 10  $\mu g/g$  fresh weight of the implicated cultivar were

Table 21. Concentrations of linear furanocoumarins in celery cultivars.

Celery cultivar	State grown	Mean concentration $\pm$ SD <sup>a</sup> ( $\mu\text{g/g}$ fresh wt)			
		Psoralen	5-MOP	8-MOP	Total
Tall Utah 5270-R <sup>b</sup>	CA	0.15 $\pm$ 0.06	0.14 $\pm$ 0.04	0.61 $\pm$ 0.14	0.9 $\pm$ 0.24
Florida 2192 <sup>b</sup>	FL8	< 0.03	0.04 $\pm$ 0.03	0.04 $\pm$ 0.06	0.11 $\pm$ 0.09
Florida 683 <sup>b</sup>	MI	0.07 $\pm$ 0.03	0.35 $\pm$ 0.05	0.47 $\pm$ 0.05	0.89 $\pm$ 0.13
Implicated Brand, <sup>c</sup> 1984	-- <sup>d</sup>	2.50 $\pm$ 1.90	0.82 $\pm$ 0.35 <sup>e</sup>	6.35 $\pm$ 2.73 <sup>e</sup>	9.67 $\pm$ 4.98
Implicated Brand, <sup>f</sup> 1986	-- <sup>d</sup>	1.70 $\pm$ 0.20	0.70 $\pm$ 0.10	5.50 $\pm$ 0.50	7.90 $\pm$ 0.80

<sup>a</sup>Standard deviation.<sup>b</sup>From Beier et al. (1983b).<sup>c</sup>From Berkley et al. (1986).<sup>d</sup>The state grown, grower, and cultivar are undisclosed by the CDC.<sup>e</sup>Values were previously unreported.<sup>f</sup>From Seligman et al. (1987).

responsible for the grocery workers photophytodermatitis. These workers could be expected to have multiple exposures to trimmed celery; whereas, concentrations as low as 12.5  $\mu\text{g/g}$  fresh weight are known to cause contact dermatitis (Austad and Kavli, 1983). It is interesting to note that the amount of linear furanocoumarins observed in diseased celery was about 25 times that in healthy celery (Ashwood-Smith et al., 1985). In a number of studies designed to demonstrate the phytoalexin behavior of celery,  $\text{CuSO}_4$  treatments elevated the levels of linear furanocoumarins from 23 to 26 times that observed in the controls (Beier and Oertli, unpublished data).

Photophytodermatitis in grocery store workers was a direct result of plant breeding to obtain a more pest resistant variety of celery. All celery varieties contain photoactive linear furanocoumarins, and humans have coexisted well with them. However, when chemical concentrations were increased 10- to 15-fold in this new more pest-resistant variety, the toxicity threshold was exceeded and human illness resulted. Thus, the often related theory, "humans have evolved with the common food plants, and are not susceptible to any of nature's chemicals that are found in those plants," is not always true. The FDA has been concerned that new plant varieties may contain larger amounts of natural toxicants (Hanson, 1974). This concern has now become reality in both celery and potato (see the section entitled "Nightshades...").

Recently, a celery breeding project evaluated *Apium* species for resistance to a key insect pest of celery, *Liriomyza trifolii* (Burgess) (Trumble and Quiros, 1988). *L. trifolii* is a leaf miner in celery and reduces yield and marketability. The celery cultivar *Apium nodiflorum* had the lowest levels of linear furanocoumarins and also the best resistance against *L. trifolii*. No significant correlation could be observed between the foliar content of linear furanocoumarins and *L. trifolii* adult production (Trumble et al., 1990). These results suggest that it might be possible to obtain resistance to other insect pests of celery without raising the indigenous levels of linear furanocoumarins.

We are proposing a new program strategy for breeders of human food plants containing known toxic compounds to be called the Integrated Breeding and Environmental Chemicals (IBEC) strategy. The IBEC strategy involves, in the

Table 22. Concentrations of linear furanocoumarins in parsnip root.

	Mean concentration $\pm$ SD <sup>a</sup> ( $\mu\text{g/g}$ fresh wt)			
	Psoralen	5-MOP	8-MOP	Total
Whole <sup>b</sup>	7.1 $\pm$ 1.4	7.3 $\pm$ 0.7	48.0 $\pm$ 6.5	62.4 $\pm$ 8.6
Peel <sup>b</sup>	9.4 $\pm$ 2.9	22.5 $\pm$ 4.4	154.4 $\pm$ 30.9	186.3 $\pm$ 38.2
Whole diseased <sup>b</sup>	537.0 $\pm$ 220.0	90.8 $\pm$ 15.8	1,109.0 $\pm$ 185.0	1,736.8 $\pm$ 420.8
Raw <sup>c</sup>	10.5 $\pm$ 0.5	3.2 $\pm$ 0.3	26.1 $\pm$ 1.8	39.8 $\pm$ 2.6
Boiled <sup>c</sup>	11.8 $\pm$ 1.8	4.1 $\pm$ 0.4	28.8 $\pm$ 2.4	44.7 $\pm$ 4.6
Microwaved <sup>c</sup>	10.7 $\pm$ 0.6	3.3 $\pm$ 0.5	27.9 $\pm$ 1.6	41.9 $\pm$ 2.7

<sup>a</sup>Standard deviation.<sup>b</sup>From Ceska et al. (1986).<sup>c</sup>From Ivie et al. (1981).

breeding process, evaluation of levels of the toxic compound(s) in all prospective new varieties. In celery, the toxic compounds would be psoralen, bergapten, and xanthotoxin. The variety with the lowest levels would generally be used in the marketplace, assuming the variety meets other desired characteristics needed for a marketable, quality product.

### Parsley and Parsnips

Mixed crystals of linear furanocoumarins were detected on surfaces of parsnip roots by scanning electron microscopy (SEM) and concentrations of the compounds were determined (Ceska et al., 1986) (Table 22). The concentrations of linear furanocoumarins also have been determined in raw, boiled, and microwaved parsnip root (Ivie et al., 1981) (Table 22) and in fresh and dried parsley (Beier and Ivie, 1985; Chaudhary et al., 1986) (Table 23). Ivie et al. (1981) pointed out that consumption of moderate quantities of parsnip root could result in ingestion of appreciable amounts of linear furanocoumarins. Their study also demonstrated that these chemicals are stable to cooking. Concentrations of linear furanocoumarins found in parsley by Chaudhary et al. (1986) and by Beier and Ivie (1985) are compared in Table 23. There is considerable difference between the two data sets describing the quantities of linear furanocoumarins found in parsley. Linear furanocoumarins are more concentrated in parsley (Table 23) than in celery (Table 21). Parsley also contains the linear furanocoumarins, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, and graveolone (Fig. 29) (Beier and Ivie, 1985; Chaudhary et al., 1986; Tietjen et al., 1983), in addition to those found in celery (Fig. 28). The linear furanocoumarin, oxypeucedanin, is weakly mutagenic in the dark with the Ames test (Ivie et al., 1980) and it also causes photodermatitis. In Table 23, the brand of commercial parsley flakes marked "high" contained over 300  $\mu\text{g/g}$  dry weight of the phototoxic linear furanocoumarins: psoralen, bergapten (5-methoxysoralen or 5-MOP), and xanthotoxin (8-methoxysoralen or 8-MOP). Double curled parsley had 112  $\mu\text{g/g}$  of total active linear furanocoumarins on a fresh weight basis, which is about 175-fold higher than that found in celery.

Table 23. Concentrations of linear furanocoumarins in parsley.

	Mean concentration $\pm$ SD <sup>a</sup> ( $\mu\text{g/g}$ ) <sup>b</sup>				
	Leaves of <sup>c</sup> curled parsley	Parsley <sup>c</sup> flakes	Double <sup>d</sup> curled parsley	Parsley <sup>d</sup> flakes (high)	Parsley <sup>d</sup> flakes (low)
Psoralen	2.5 $\pm$ 0.6	1.6 $\pm$ 1.6	32.7 $\pm$ 6.2	104.7 $\pm$ 4.2	32.3 $\pm$ 1.8
5-MOP	10.2 $\pm$ 1.7	6.6 $\pm$ 1.8	63.9 $\pm$ 13.4	146.7 $\pm$ 19.4	56.7 $\pm$ 6.5
8-MOP	3.6 $\pm$ 0.8	2.3 $\pm$ 0.6	15.4 $\pm$ 5.1	53.0 $\pm$ 5.3	5.3 $\pm$ 2.9
Total	16.3 $\pm$ 3.1	10.5 $\pm$ 4.0	112.0 $\pm$ 24.7	304.6 $\pm$ 28.9	94.3 $\pm$ 11.2
Imperatorin	0.5 $\pm$ 0.1	2.5 $\pm$ 0.8			
Isopimpinellin	1.8 $\pm$ 0.4	3.1 $\pm$ 0.1			
Oxypeucedanin	102.9 $\pm$ 14.0	88.6 $\pm$ 17.9			

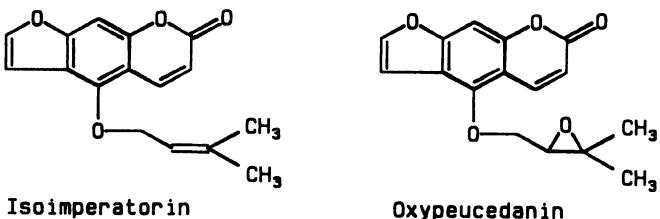
<sup>a</sup>Standard deviation.<sup>b</sup>Values are given for fresh weight except for parsley flakes which were dry.<sup>c</sup>From Chaudhary et al. (1986).<sup>d</sup>From Beier and Ivie (1985).

### Citrus and Figs

The total number of coumarins reported from natural sources was over 600 in 1977 (Gray and Waterman, 1978), and that number increased by the year 1982 (Murray et al., 1982). Coumarins are naturally occurring chemicals that are distributed throughout the citrus species. The coumarin content of cold-pressed lime oil is about 7% by weight, but that of orange oil is less than 0.5% (Stanley and Jurd, 1971). Citrus oils are pressed from the peel and used for flavoring candies, soft drinks, and baked goods. Solids recovered by column chromatography of cold-pressed citrus peel oils are shown in Table 24. These solids reflect the coumarin content of the citrus oils, except for bitter orange oil, which consists primarily of flavonoids. The coumarins and linear furanocoumarins present in citrus peel are nonvolatile and are not found in distillates. But, the best quality oils are pressed directly from the peel without distillation.

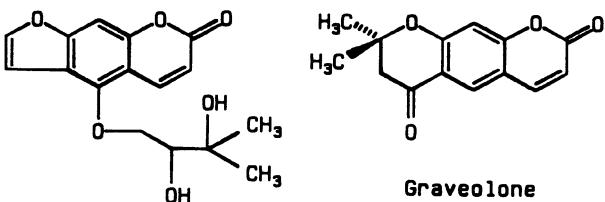
*d*-Limonene (Fig. 30) in citrus juice is the main aromatic component and is present at 70 to 95% of the total volatile substances (Massaldi and King, 1974). Table 25 shows the *d*-limonene content of various orange juices and a grapefruit juice obtained by different techniques. These data led to the understanding that the retention behavior of citrus volatiles during the freeze drying process is based on physico-chemical and biological characteristics of the food (Massaldi and King, 1974). The flavor of *d*-limonene was very dependent upon other nonvolatile interfering constituents including acids, pectins, and sugars (Ahmed et al., 1978).

7-Geranoxycoumarin is present in grapefruit, isopimpinellin (Fig. 28) is present in lime oil, but neither occur in lemon oil (Stanley and Jurd, 1971). Using such differences, reliable methods have been developed for detecting cross contamination of these citrus oils (Vannier and Stanley, 1958). With few exceptions, the derivatives found in the citrus family are derived from psoralen (Fig. 28) and coumarin (Fig. 31) (Stanley and Jurd, 1971). The linear furanocoumarins found in citrus are: psoralen (Tatum and Berry, 1979), bergaptol, bergapten, and bergamottin in grapefruit;



Isoimperatorin

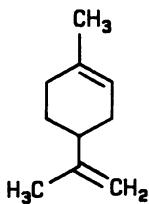
Oxypeucedanin



Oxypeucedanin  
hydrate

Graveolone

Figure 29. Three additional linear furanocoumarins and graveolone in parsley.



d-Limonene

Figure 30. d-Limonene.

phellopterin, 8-geranoxypsoralen, and bergamottin in lemon; and bergaptol and bergapten in orange (Fisher and Trama, 1979). For an extensive review of the chemical constituents in the family Rutaceae, to which the commercial citrus varieties belong, see Gray and Waterman (1978) and Stanley and Jurd (1971). The biogenesis, structural diversity, and distribution of simple furano- and pyranocoumarins in the family Rutaceae are described by Gray and Waterman (1978). Herniarin and 7-ethoxycoumarin (Fig. 31) were the most active coumarins against yeasts, molds, and bacteria (Stanley and Jurd, 1971).

Fig (*Ficus carica*) phytophotodermatitis occurs in some individuals who handle figs, and 10% of all the individuals who handle figs in Turkey contract it. The skin reaction results from contact with the milky sap (latex) followed by exposure to the sun. The major photoactive compounds isolated from fig leaf are psoralen and bergapten (Fig. 28). Higher levels of these photoactive compounds are produced in the spring and summer and are responsible for the increased incidence of fig dermatitis during these times. The peak psoralen concentration measured in fig leaf is 1,650 µg/g fresh weight and in fig leaf sap is 2,090 µg/ml. The peak bergapten concentration measured in fig leaf is 480 µg/g fresh weight and in fig leaf sap is

Table 24. Solids recovered from cold pressed citrus peel oils by column chromatography on silicic acid.<sup>a</sup>

Citrus oil	Weight %
Lime, Mexican	6.67
Grapefruit	1.37
Bergamot	0.56
Lemon	0.47
Bitter orange	0.23

<sup>a</sup>From Stanley and Jurd (1971).

Table 25. *d*-Limonene concentrations in citrus juices obtained by different processes.<sup>a</sup>

Variety	Fruit	Type <sup>b</sup>	<i>d</i> -Limonene(ppm)
Navel (2) <sup>c</sup>	Orange	Fresh squeezed	53.8 <sup>d</sup>
Valencia	Orange	Fresh squeezed	41.4
Brands (2) <sup>c</sup>	Orange	Commercial pasteurized	109.0 <sup>d</sup>
Brands (3) <sup>c</sup>	Orange	Commercial frozen, reconstituted	161.0 <sup>d</sup>
Brand	Grapefruit	Commercial frozen, reconstituted	176.0

<sup>a</sup>From Massaldi and King (1974).

<sup>b</sup>The type of process used to produce the citrus juice.

<sup>c</sup>These values are the number of observations of *d*-limonene values obtained from similarly processed citrus juice, and were used to provide the mean value presented in this table.

<sup>d</sup>Mean value.

620 µg/ml. Photoactive furanocoumarins were not detected in any part of the fruits (Zaynoun et al., 1984). (Note added in proof: This laboratory has investigated fig fruits for linear furanocoumarins, and these photoactive compounds were not detected at a 0.03 µg/g detection limit.)

Figs can also be found contaminated with the mycotoxin, aflatoxin (see "mycotoxins"). Aflatoxin-contaminated dried figs were observed at a rate of about 1 in 100 fruits and contained aflatoxin B<sub>1</sub> and G<sub>1</sub> at < 0.2 ng/g to over 10,000 ng/g; most contaminated figs could be removed by observing their fluorescence (Steiner et al., 1988).

**Chromatography.** There are various high performance liquid chromatographic (HPLC) methods for quantifying the amounts of linear furanocoumarins in plants (Beier, 1985; Spencer et al., 1987). Most HPLC separation methods for linear furanocoumarins use normal phase conditions because all compounds will not separate on most reverse phase columns.

A new method of assessing quantities of phototoxic linear furanocoumarins may be applicable for monitoring skin patches or skin surfaces. A portable fiber optics luminoscope developed for monitoring skin contamination in various settings (Vo-Dinh, 1987; Vo-Dinh and Gammage, 1981) has been applied to the measurement of linear furanocoumarins (psoralens) in vegetable products (Vo-Dinh et al., 1988).

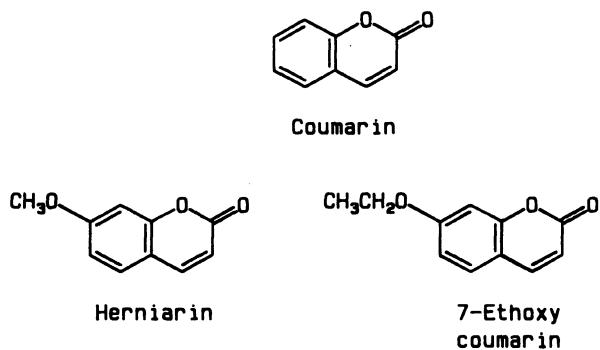
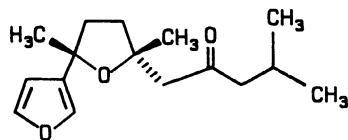


Figure 31. Herniarin and 7-ethoxycoumarin were the two most active coumarins tested against yeasts, molds, and bacteria (Stanley and Jurd, 1971).



Ipomeamarone

Figure 32. The first phytoalexin isolated from sweet potatoes.

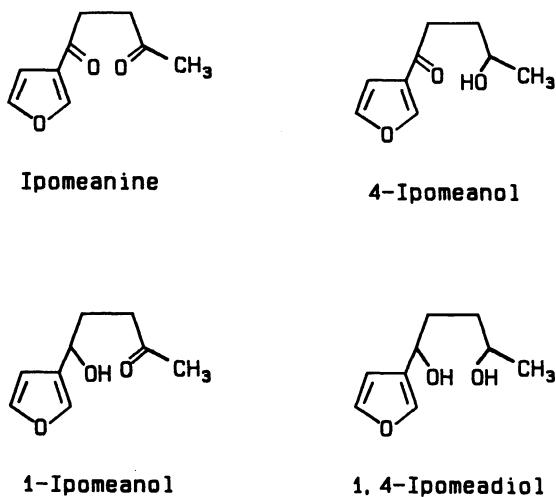
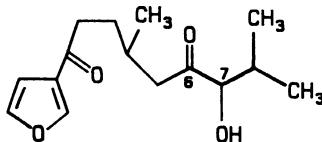
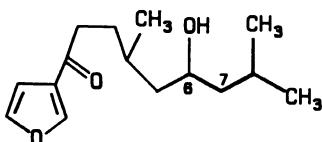


Figure 33. Closely related lung toxins isolated from sweet potatoes.



**7-Hydroxymyoporone**



**6-Myoporol**

Figure 34. Hepatotoxic compounds.

### SWEET POTATOES (*IPOMOEA BATATAS*)

Sweet potatoes are widely used as human food. A large proportion of the total production (about 35%) is lost by spoilage (Steinbauer and Cushman, 1971). Sweet potatoes produce numerous natural pesticides or phytoalexins. The first phytoalexin isolated from infected sweet potatoes was the furanosesquiterpene, ipomeamarone (Fig. 32), which is hepatotoxic (Takeuchi, 1946) as well as toxic to fungi, bacteria, and yeasts (Taira and Fukagawa, 1958). Other chemicals (furanoterpenes and eudesmanes) isolated from fungal-infected sweet potatoes are listed in Table 26. Sweet potatoes stressed by  $HgCl_2$  respond by producing the phytoalexins or toxic chemicals shown in Table 27.

Mold-damaged sweet potatoes can have catastrophic effects in cattle; in one case, 69 out of 275 exposed cattle died of lung edema (Peckham et al., 1972). Phytoalexins are apparently responsible for the toxicity of sweet potatoes to animals (Wilson and Burka, 1983). A scheme for the biogenesis of sweet potato stress metabolites was presented by Schneider et al. (1984).

Four closely related 1,4-dioxygenated-1-(3-furyl)pentanes: ipomeanine, 4-ipomeanol, 1-ipomeanol, and 1,4-ipomeadiol (Fig. 33) have been suggested by Boyd et al. (1973) as being responsible for the observed lung endema caused by sweet potato. The  $LD_{50}$  values of these chemicals, and ipomeamarone, in mice are presented in Table 28. Each of the lung toxins produces identical reactions when given orally, intraperitoneally, or by intravenous injection (Wilson et al., 1971). At  $LD_{50}$  concentrations, 1-ipomeanol and 1,4-ipomeadiol are toxic also to the kidneys. Tubular nephrosis and accumulation of intratubular debris occur in mice (Boyd et al., 1973).

High concentrations of some toxic furanoterpenoids can accumulate in infected sweet potatoes (Table 29). Intraperitoneal injections of 200 to 250  $\mu g/g$  body weight of the stress metabolite, 7-hydroxymyoporone (Fig. 34), is toxic to the liver as well as the lungs of mice. This compound is equivalent in hepatotoxicity to ipomeamarone (Fig. 32) (Burka et al., 1974). 6-Myoporol (Fig. 34) is two to three times more hepatotoxic than ipomeamarone (Burka and Iles, 1979).

Table 26. Chemicals isolated from sweet potatoes infected with *Ceratocystis fimbriata* and other pathogens.

Chemical	Concentration ( $\mu\text{g/g}$ )
7-Hydroxymyoporone <sup>a</sup>	20
7-Hydroxycostal <sup>b</sup>	125
7-Hydroxycostol <sup>b</sup>	1,120
9-Hydroxyfarnesoic acid <sup>c</sup>	
6-Oxodendrolasinolide <sup>c</sup>	
Ipomeatetrahydrofuran <sup>c</sup>	
(Z)-1,6-Dioxoisodendrolasin <sup>c</sup>	
(E)-1,6-Dioxoisodendrolasin <sup>c</sup>	
10-Hydroxyipomeabifuran <sup>c</sup>	
Ipomeamaronolide <sup>c</sup>	
4-Hydroxymyoporonal <sup>c</sup>	
4-Hydroxymyoporonal ketal <sup>c</sup>	6,724
Ipomeararone <sup>d</sup>	
4-Ipomeanol <sup>e,f</sup>	
1-Ipomeanol <sup>e,g</sup>	
Ipomeanine <sup>e,g</sup>	
1,4-Ipomeadiol <sup>e,g</sup>	
Chlorogenic acid <sup>h,i</sup>	
Caffeic acid <sup>h,i</sup>	
Isochlorogenic acid <sup>h,i</sup>	
Pseudochlorogenic acid <sup>h,i</sup>	
Dehydroipomeamarone <sup>j,k</sup>	
Ipomeamaronol <sup>j,k</sup>	
4-Hydroxydehydromyoporone <sup>j,k</sup>	
4-Hydroxymyoporone <sup>g,j</sup>	

<sup>a</sup>From Burka et al. (1974).

<sup>b</sup>From Schneider and Nakanishi (1983).

<sup>c</sup>From Schneider et al. (1984).

<sup>d</sup>From Clark et al. (1981).

<sup>e</sup>From Boyd et al. (1973).

<sup>f</sup>From Wilson et al. (1971).

<sup>g</sup>Sweet potatoes were infected with *Fusarium solani*.

<sup>h</sup>From Uritani and Miyano (1955).

<sup>i</sup>Sweet potatoes were infected with *Ceratostomella fimbriata*.

<sup>j</sup>From Inoue and Uritani (1979).

<sup>k</sup>Sweet potatoes were infected.

Blemished, damaged, or infected sweet potatoes from U.S. (Boyd and Wilson, 1971) and U.K. (Coxon et al., 1975) food stores have been analyzed for ipomeamarone (Fig. 32). An average concentration for the sweet potatoes in U.S. stores was 1,832  $\mu\text{g/g}$ , while a maximum of 900  $\mu\text{g/g}$  was found in those from the U.K. Samples that apparently were free from damage contained a total of 68 to 328  $\mu\text{g/g}$  ipomeamarone and showed the presence of the lung-edema toxin, ipomeanine (Fig. 33) (Coxon et al., 1975). Lung-edema toxins occur in blemished and diseased areas of sweet potatoes (Catalono et al., 1979), and toxic sweet potatoes may show only slightly darkened areas without significant color changes (Wilson et al., 1970). The actual levels of toxins are not predictable by visual examination (Coxon et al., 1975), and normal baking or boiling does not affect the toxin levels (Wilson et al., 1970).

Table 27. Phytoalexins isolated from sweet potatoes stressed with HgCl<sub>2</sub>.

Chemical	Concentration ( $\mu\text{g/g}$ fresh wt)
Myoporone <sup>a</sup>	39.0
6-Hydroxymyoporol <sup>a</sup>	109.0
1-Hydroxymyoporol <sup>a</sup>	53.0
6-Oxodendrolasin <sup>b</sup>	8.5
6-Hydroxydendrolasin <sup>b</sup>	1.2
9-Oxofarnesol <sup>b</sup>	0.7
9-Hydroxyfarnesol <sup>b</sup>	12.6
6-Dihydro-7-hydroxymyoporone <sup>c</sup>	26.0

<sup>a</sup>From Burka and Iles (1979).<sup>b</sup>From Burka et al. (1981).<sup>c</sup>From Burka (1978).Table 28. LD<sub>50</sub> values for chemicals from sweet potatoes in mice.

Chemical	Concentration ( $\mu\text{g/g}$ )		
	Oral	Intraperitoneal	Intravenous
Ipomeamarone <sup>a</sup>	230	--	--
4-Ipomeanol <sup>b</sup>	38	36	21
4-Ipomeanol <sup>c</sup>	--	--	24 <sup>d</sup>
1-Ipomeanol <sup>b</sup>	79	49	34
Ipomeanine <sup>b</sup>	26	25	14
1,4-Ipomeadiol <sup>b</sup>	104	67	68

<sup>a</sup>From Taira and Fukagawa (1958).<sup>b</sup>From Boyd et al. (1973).<sup>c</sup>From Boyd and Burka (1978).<sup>d</sup>The LD<sub>50</sub> was obtained in the rat.

The lung-edema toxin, 4-ipomeanol (Fig. 33), is oxidatively metabolized by lung clara cells in hamsters, mice, and rats (Boyd, 1977). Boyd (1976) suggested that the xenobiotic-metabolizing clara cells, containing the cytochrome P-450 dependent MFO system, may be a prime site for bronchogenic cancers (Boyd, 1977). The toxicity of 4-ipomeanol to rats is apparently due to a reactive metabolite. The cytochrome P-450 inhibitors, pyrazole, piperonyl butoxide, and cobaltous chloride, decreased the pulmonary alkylation and the toxicity although SKF-525A had no effect. Radiolabeled 4-ipomeanol administered by intraperitoneal injection bound more to lung tissue than to any other site. Liver tissue only bound 20% of the 4-ipomeanol and was second in binding potential to lung tissue (Boyd and Burka, 1978). Repeated intraperitoneal injections of 4-ipomeanol in rats resulted in extensive clara cell necrosis (Doster et al., 1983), and in mice it caused interstitial edema (Durham et al., 1985). When mice having viral pneumonia were given 4-ipomeanol, the severity of pneumonia increased with increasing doses of the lung toxin (Durham et al., 1987).

In studies of chronic lung disease in New Guinea populations, Woolcock et al. (1970) point out that men and women suffer from chronic respiratory disease of unknown etiology. They suggest that it may be an environmental factor. It was suggested by Wilson (1973) that humans may be affected adversely by sweet potato consumption. Wilson (1973) reports that natives of that area raise and consume large quantities of sweet potatoes. Dawson and Mitchell (1990) state that the Maori and Pacific Island children have a higher mortality rate from asthma than European children. They also state that the cause of the high rates of asthma and asthma related deaths is not known, and continued research in New Zealand and other countries is needed to identify the causative agent(s). Although the death rate among young people (aged 5 to 34 years) due to asthma in New Zealand has decreased from over 4.0 per 100,000 in 1980, it is still persistently high as shown in the death rate of 1988 at 2.6 per 100,000 (Sears, 1991).

## MUSHROOMS

About 50 volatile compounds have been identified from each of eight species of edible fresh mushrooms, including *Cantharellus*, *Cibarius*, *Gyromitra esculenta*, *Boletus edulis*, *Lactarius trivialis*, *Lactarius torminosus*, *Lactarius rufus*, and *Agaricus bisporus*. The main volatile compound in most fresh wild mushrooms is 1-octen-3-ol. In the commonly cultivated *Agaricus bisporus*, benzyl alcohol is the most abundant; and 1-octen-3-ol is the second most abundant volatile (Pyytalo, 1976). Two edible mushroom species contain hydrazine analogs: *Agaricus bisporus* contains  $\beta$ -N-[ $\gamma$ -L(+)-glutamyl]-4-hydroxymethylphenylhydrazine (agaritine, GPH) and *Gyromitra esculenta* or (wild false morel) contains acetaldehyde N-methyl-N-formylhydrazone (gyromitrin, acetaldehyde MFM). Gyromitrin readily yields various hydrazines that are toxic and hepatocarcinogenic (Toth, 1979).

### *Agaricus bisporus*

*Agaricus bisporus* is the main mushroom of commerce in the United States (Levenberg, 1961), and the hydrazine analogs isolated from it are shown in Table 30. Fresh *A. bisporus* contains as much as 700  $\mu\text{g/g}$  of agaritine, and fresh frozen mushrooms contain as much as 300  $\mu\text{g/g}$ . Cooking or canning with water destroys the agaritine, but cooking in olive oil at 300°C for 7 minutes left 300  $\mu\text{g/g}$  of agaritine (Ross et al., 1982). Agaritine does not induce tumors in mice (Toth and Sornson, 1984; Toth et al., 1981) but forms 4-hydroxymethylphenylhydrazine as a breakdown product which can be transformed into 4-methylphenylhydrazine (4-MPH) *in vitro* (Gigliotti and Levenberg, 1964; Kelly et al., 1962) (Fig. 35). Soft-tissue tumors developed at injection sites after 4-MPH was administered to mice (Toth and Nagel, 1981). 4-MPH was the first diazonium compound discovered to be carcinogenic (Toth et al., 1980). The *N*<sup>1</sup>-acetyl derivative of 4-hydroxymethylphenylhydrazine is an enzymatic product of agaritine. When it was given to Swiss albino mice, the lung tumor incidence rose 25% in females and 26% in males; and the incidence of blood vessel tumors rose 24% in females and 25% in males (Toth, 1983). Subcutaneous injections of 4-MPH into Swiss mice induced a 36% incidence of lung tumors in females and a 44% incidence in males; whereas, intragastric treatments caused a 40% incidence of lung tumors in females (Toth, 1983).

Table 29. Maximum concentrations of chemicals observed in sweet potatoes produced by infection with one of 13 different sweet potato pathogens.<sup>a</sup>

Chemical	Concentration ( $\mu\text{g/g}$ )
Ipomeamarone	23,346 <sup>b</sup>
4-Ipomeanol	236 <sup>c</sup>
1,4-Ipomeadiol	469 <sup>c</sup>
Total furanoterpenoids	106,429 <sup>d</sup>

<sup>a</sup>From Clark et al. (1981).

<sup>b</sup>Maximum concentration from *Sclerotium rolfsii* inoculated sweet potatoes.

<sup>c</sup>Maximum concentration from *Fusarium solani* inoculated sweet potatoes.

<sup>d</sup>Maximum concentration from *Diaporthe batatas* inoculated sweet potatoes.

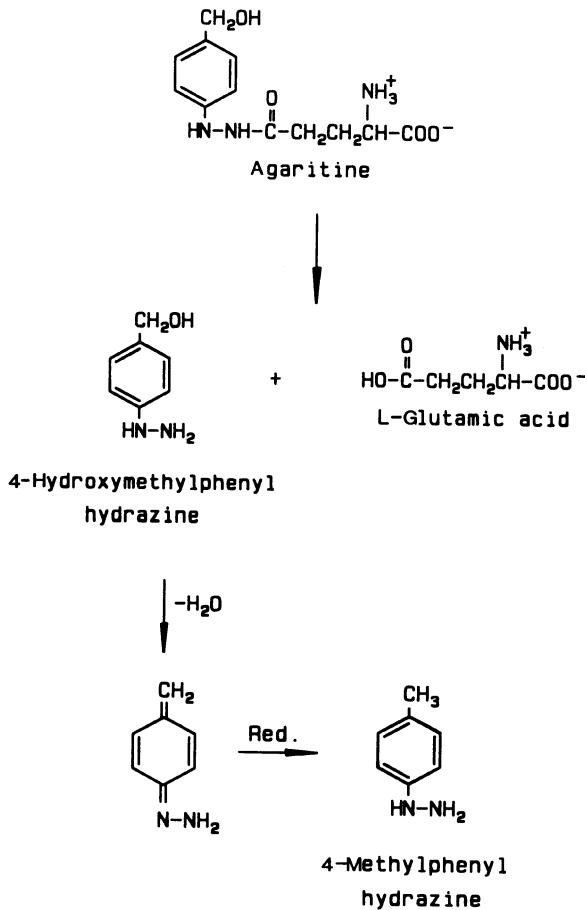


Figure 35. Hydrolysis products of agaritine ( $\beta$ -N-[ $\gamma$ -L-(+)-glutamyl]-4-hydroxymethylphenylhydrazine).

Table 30. Hydrazine compounds isolated from fresh *Agaricus bisporus* mushrooms.

Compounds	Concentration ( $\mu\text{g/g}$ fresh wt)
<i>p</i> -Hydrazinobenzoic acid (HB)	10.7 <sup>a</sup>
$N^2$ -[ $\gamma$ -L-(+)-Glutamyl]-4-carboxyphenylhydrazine (GCPH)	42.0 <sup>b</sup>
$N^2$ -[ $\gamma$ -L-(+)-Glutamyl]-4-(hydroxymethyl)	400 <sup>c</sup>
Phenylhydrazine (agaritine, GHPH)	700 <sup>d</sup>

<sup>a</sup>From Chauhan et al. (1984).<sup>b</sup>From Chauhan et al. (1985).<sup>c</sup>From Kelly et al. (1962).<sup>d</sup>From Ross et al. (1982).Table 31. Compounds and concentrations in false morel.<sup>a</sup>

Compounds	Concentration ( $\mu\text{g/g}$ fresh wt)
<i>N</i> -methyl- <i>N</i> -formylhydrazine (MFH) (dry weight) <sup>b</sup>	500.0
Methylhydrazine <sup>b</sup>	14.0
<i>N</i> -methyl- <i>N</i> -formylhydrazone (MFHO)	
Acetaldehyde ( <i>gyromitrin</i> )	49.9
Propanol	1.0
Butanol	0.6
3-Methylbutanol	2.2
Pentanal	0.8
Hexanal	1.4
Octanal	0.2
<i>trans</i> -2-Octenal	0.6
<i>cis</i> -2-Octenal	0.3

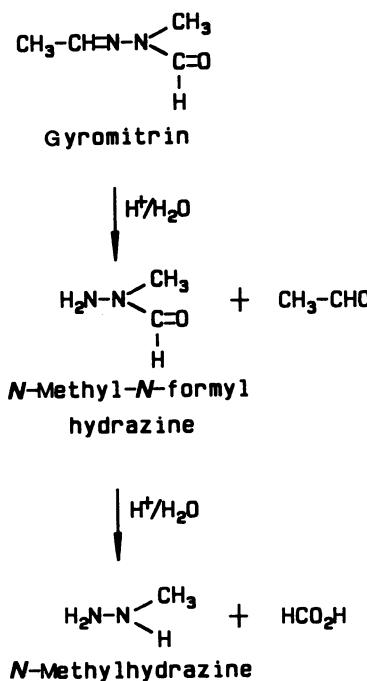
<sup>a</sup>From Pyysalo and Niskanen (1977).<sup>b</sup>From Toth (1983).

### *Gyromitra esculenta*

*Gyromitra esculenta* (false morel) is highly poisonous fresh but edible when cooked by boiling (Franke et al., 1967). Toxicity is caused by the volatile indigenous hydrazone, gyromitrin (Pyysalo and Niskanen, 1977). Concentrations of hydrazone analogs isolated from *Gyromitra esculenta* are shown in Table 31. During cooking, care must be taken because highly toxic vapors also volatilize from the mushrooms (Franke et al., 1967). Boiling for 10 minutes is required to reduce hydrazone concentrations below 1  $\mu\text{g/g}$ . A minimum of 3 liters of water are recommended per kilogram of mushrooms. False morels are used as food after cooking or drying (Schmidlin-Mészáros, 1975). The structure of gyromitrin was reported in 1967 (List and Luft, 1967) (Fig. 36). Mass spectrometry analysis of gyromitrin from *Helvella esculenta* was made by McClusky et al. (1978).

Table 32. LD<sub>50</sub> of hydrazine and the hydrolysis products of gyromitrin in various animals.

Animal	Gyromitrin	LD <sub>50</sub> concentration ( $\mu\text{g/g}$ )		
		N-methyl-N-formylhydrazine	N-methylhydrazine	Hydrazine
Dog <sup>a</sup>	--	--	12	25
Mice <sup>b</sup>	344	159	124	(low toxicity)
Rabbit <sup>c</sup>	70	--	--	--
Rat <sup>a</sup>	320 <sup>c</sup>	--	33	55

<sup>a</sup>From Witkin (1956).<sup>b</sup>From Wright et al. (1978).<sup>c</sup>From Makinen et al. (1977).Figure 36. Chemical structure of gyromitrin and its hydrolysis products produced *in vitro* and *in vivo* (Braun et al., 1980).

The LD<sub>50</sub> of hydrazine and hydrolysis products of gyromitrin in various animals are presented in Table 32 (Fig. 36). Hydrazine and *N*-methylhydrazine are toxic to *Escherichia coli*, and *N*-methyl-*N*-formylhydrazine is not, but *N*-methyl-*N*-formylhydrazine is more toxic than hydrazine to mice (Wright et al., 1978). Rabbits are more susceptible than rats or mice to gyromitrin, and dogs are more susceptible than rats or mice to *N*-methylhydrazine or hydrazine. The route of administration (intraperitoneal, intravenous, or oral) has essentially no influence on the toxicity of these compounds (Witkin, 1956). The difference between the no-effect amount and

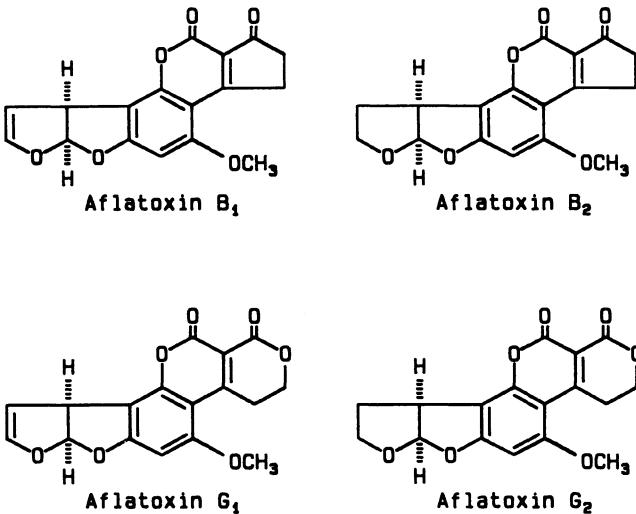
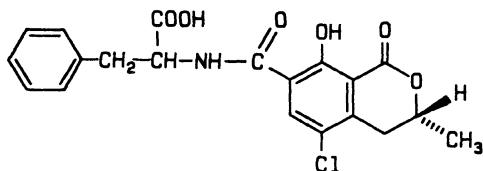


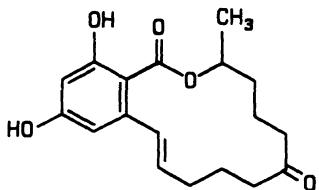
Figure 37. The major aflatoxins.

a lethal dosage of *N*-methylhydrazine is small in apes (Mäkinen et al., 1977), but there is a large variation in individual human tolerances as indicated by case histories (Franke et al., 1967). Calculated lethal doses for children and human adults, based on reports of false morel intoxication, are 10 to 30 µg/g and 20 to 50 µg/g, respectively (Schmidlin-Mészáros, 1974, 1975). *N*-methylhydrazine caused a decisive increase of lung tumors in Swiss mice; whereas, it caused malignant histiocytomas and tumors of the cecum in hamsters (Toth, 1983).

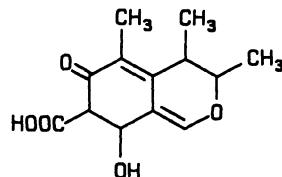
Gyromitrin can cause serious liver injury. When given by intragastric administration to Swiss mice, it also caused tumors of the lungs, preputial glands, forestomach, and clitoral glands (Toth, 1983). Gyromitrin forms *N*-methyl-*N*-formylhydrazine (MFH) and *N*-methylhydrazine upon hydrolysis (Braun et al., 1980); and Swiss mice treated with MFH produced tumors of the liver, lungs, gallbladder, and bile duct. MFH-treated hamsters developed liver cell tumors, malignant histiocytomas, and tumors of the gallbladder and bile ducts (Toth, 1983). Both gyromitrin and *N*-methylhydrazine are mutagenic and bactericidal. As much as 20 to 30% of the hydrazones in the false morel decompose during cooking to *N*-methylhydrazine, which is present in the steam (Pyysalo et al., 1979). However, the hepatotoxic and carcinogenic MFH is more mutagenic after metabolic transformation (Von Der Hude and Braun, 1983) into the highly reactive *N*-nitroso-*N*-methylformamide (NMFA) by liver microsomal monooxygenase (Braun et al., 1980). NMFA is unstable and can spontaneously decompose to molecular nitrogen, formic acid, and a methyl cation alkylating agent (Huisgen and Reimlinger, 1956). Cytochrome P-450 is required for the biological activity of gyromitrin or other hydrazines, which result in the destruction of cytochrome P-450 during their activation. Hydrazine exposures that decrease liver cytochrome P-450 levels may cause subsequent xenobiotic exposures to be toxic. Pretreatment with the cytochrome P-450 inhibitor, SKF-525A, abolishes the loss of cytochrome P-450 by *N*-methylformylhydrazine. Braun et al. (1980) suggest that the methyl cation formation from nitrosamide could be responsible for the hepatocarcinogenicity of MFH, but hydrazine carcinogens may also act by producing oxygen radicals (Ames, 1983).



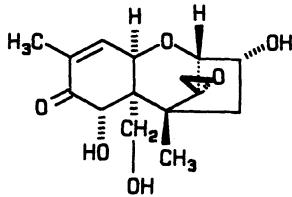
Ochratoxin A



Zearalenone



Citrinin



Deoxynivalenol  
(Vomitoxin)

Figure 38. Worldwide important mycotoxins.

## MYCOTOXINS

The term, mycotoxin, is derived from the Greek word "mykes" meaning fungus and the Latin word "toxicum" meaning poison (Goldblatt, 1972). Mycotoxins are toxic compounds produced by fungi on grains and other foods and can be health hazards to both animals and humans. Mycotoxicosis is the toxicity syndrome resulting from the intake of mycotoxin contaminated foods.

### A Global Perspective

The outbreak of Turkey X disease in England during 1960 was caused by the mycotoxin, aflatoxin. This led to the realization that low levels of mold metabolites in feeds and foods could cause disease in animals and humans (Bullerman, 1979). Despite impressions that mycotoxins are recent causes of diseases, they were recorded as early as 5000 years ago in China (Hesseltine, 1979). Schoental (1984) discussed how mycotoxins might have played an important part in the biblical account of the 10 plagues of Egypt. During the 16th century, ergot poisoning caused what is recognized as the first reported mycotoxicosis of man (Rutledge, 1977). However, an

Table 33. Metabolites isolated from cultures of *Fusarium* species.<sup>a</sup>

Apotrichothecene	Isotrichodermin
3-Hydroxy	7-Hydroxy
Butenolide	8-Hydroxy
	3-Deacetyl
Calonectrin	Fumonisin B <sub>1</sub> <sup>b</sup>
3-Deacetyl	Fumonisin B <sub>2</sub> <sup>b</sup>
15-Deacetyl	Fumonisin B <sub>3</sub> <sup>c</sup>
7-Hydroxy	Fusarin C <sup>d</sup>
8-Hydroxy	Sambucinol
7,8-Dihydroxy	3-Deoxy
3,15-Dideacetyl	Sambucoin
8-Keto	
8-Keto,15-deacetyl	Zearalenone
Cumorin	
1-Keto	
Deoxynivalenol	
3-Acetyl	
15-Acetyl	
3,15-Diacetyl	

<sup>a</sup> From Greenhalgh et al. (1985).<sup>b</sup> From Bezuidenhout et al. (1988).<sup>c</sup> From Plattner et al. (in press).<sup>d</sup> From Gelderblom et al. (1984).

epidemic apparently resulting from ergotism was reported in 430 BC in Sparta (Linsell, 1977). As late as 1962, mycotoxicosis was described by Hayes (1980) as the "neglected disease."

Widespread public interest in mycotoxins was aroused by the discovery in the 1970s that "yellow rain" in Southeast Asia and Afghanistan contained mycotoxins. By 1982, over 100 mycotoxins were described (Pollock, 1982); now over 200 mycotoxins are structurally characterized (Harvey et al., 1988a). Mycotoxins are non-antigenic small molecules that usually have a molecular weight below 500 (Hamilton, 1982). Molds that produce mycotoxins are quite ubiquitous. Problems in the United States resulting from mycotoxins are very similar to those of the rest of the world as similar mycotoxins occur worldwide. Since molds know no borders, the same fungal species that produce aflatoxin in India or Africa will produce aflatoxin in the United States. The major mycotoxins, ranked in declining order of their relative worldwide importance based on an opinion poll of researchers in 30 countries (Hesseltine, 1985), are: aflatoxins (Fig. 37); ochratoxin A, trichothecenes, zearalenone, deoxynivalenol, and citrinin (Fig. 38); sterigmatocystin and patulin (Fig. 39); cyclopiazonic acid, nivalenol, stachybotrys toxin, diplodia toxin, ergot, and phomopsin. Mycotoxin metabolites isolated from *Fusarium* species grown in liquid culture are listed in Table 33. We must now add the fumonisins (Figs. 40 and 41) to the above list of major mycotoxins of worldwide importance. Based on projected relative importance, they must be listed close to aflatoxin. Schlatter (1988) describes various mycotoxicosis resulting from contaminated foods and thoroughly examines these events.

Humans are exposed to mycotoxins through several different avenues including: 1) cereal crops, 2) meat products, 3) milk and eggs, 4) peanuts (Hayes, 1980), and

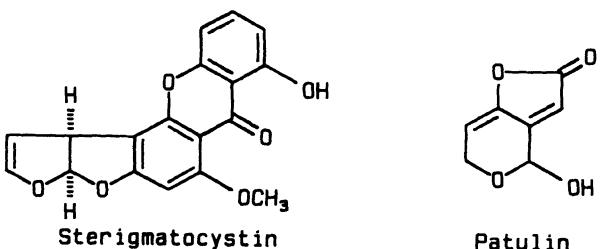


Figure 39. The mycotoxins, sterigmatocystin and patulin.

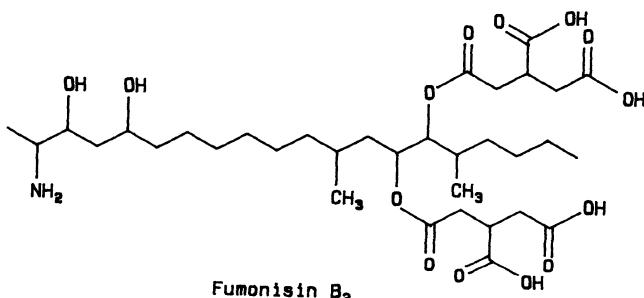
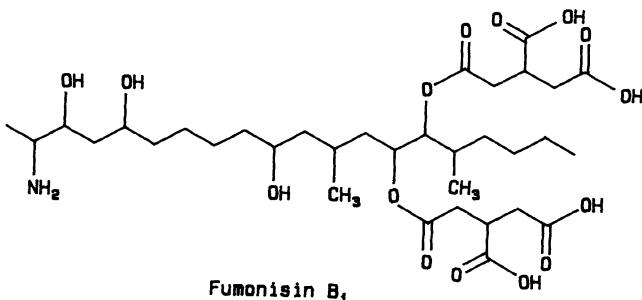


Figure 40. Fumonisins B<sub>1</sub> and B<sub>2</sub>, cancer-promoting mycotoxins from *F. moniliforme*.

5) occupational exposure (Sorenson, 1990). Many mycotoxins were first studied as potential antibiotics in the 1930s and 1940s although they were almost invariably too toxic to higher life forms to be of value. In addition to acute toxicities, aflatoxin B<sub>1</sub>, sterigmatocystin, patulin, and penicillic acid are also potential carcinogens (Bullerman, 1979). In fact, aflatoxin B<sub>1</sub> is one of, if not the most potent carcinogen found in nature (Bullerman, 1979; Fu-Sun and Kong-Nien, 1986). The major aflatoxin-producing fungi are *Aspergillus flavus* and *A. parasiticus*, and the major aflatoxins are aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> (Fig. 37) (World Health Organization, 1987). The discovery of naturally occurring carcinogens in common human foodstuffs such as corn, milk, and peanuts was so surprising that, briefly, the animal toxicoses were nearly forgotten (Hamilton, 1982). Corn and peanuts are far more easily contaminated by mycotoxin producing fungi than are other foodstuffs, and Fu-Sun and Kong-Nien (1986) suggested that corn is more important in China because it is a

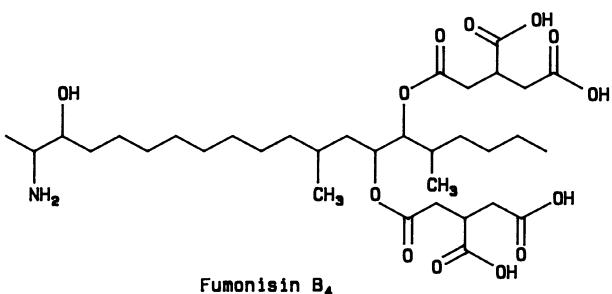
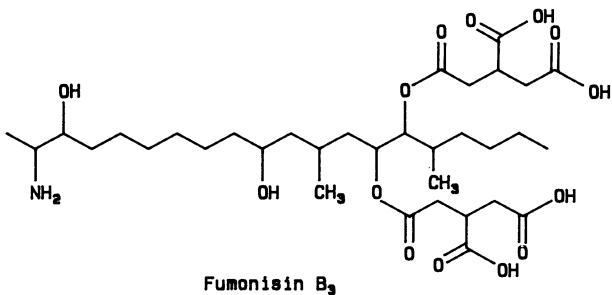


Figure 41. Fumonisins B<sub>3</sub> and B<sub>4</sub> are the newest members in the fumonisin mycotoxin family.

staple food; but others feel that, on a worldwide scale, peanuts are consumed in the highest quantity. With few exceptions, the percentages of corn in Chinese staple foods correlated well with the observed mortality rates from liver cancer (Fu-Sun and Kong-Nien, 1986). However, as in Sri Lanka, the consumption of herbal medicines containing hepatotoxic substances also could be of importance in chronic liver disease. If aflatoxin indeed is the most significant cause of liver disease in China, consumption of herbs may contribute synergistically to the development of liver disease (Arseculeratne et al., 1981). Commodities that have mycotoxin contamination problems are: barley, cheese, corn, cottonseed, milk, peanuts, rice, sorghum, tree nuts, and wheat. The UN Food and Agriculture Organization (FAO) estimated that 25% of the world's food crops are contaminated by mycotoxins. This includes 10 to 50% of the grain crops in Africa and the Far East (Mannon and Johnson, 1985). Conditions which lead to mycotoxin contamination of feeds and foods have been reviewed by Hesseltinge (1976). Extensive surveys have demonstrated a measurable incidence of aflatoxin residues in United States grain mills and of ochratoxin residues in Danish pork (Hamilton, 1982).

### **Food Safety and Public Health Hazard**

The significant involvement of mycotoxins in human health was recognized by Wilson (1978). Human illnesses caused by mycotoxins may be a larger problem than anyone realizes because long periods of time elapse before an illness is recognized unless large amounts of mycotoxins are consumed resulting in acute symptomatology (Hesseltinge, 1985). Mycotoxins also are relatively stable to cooking and processing (Rutledge, 1976); therefore, food preparation procedures cannot be expected to

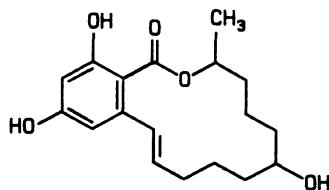
remove mycotoxins. Small intake of mycotoxins for long periods of time can be detrimental; thus, endemic nephropathy and primary liver-cell carcinoma may take decades to become evident. Endemic nephropathy or chronic kidney disease took at least 15 to 20 years to present manifestations in people who moved into a region in Romania that was known for the disease. The age group of individuals primarily affected by the disease in that region was from 30 to 50 years old. In Bulgaria, it was noticed that none of the children from age 4 to 14 years were affected by endemic nephropathy (Akkmeteli, 1977). Because of the long-term effects, a direct relationship of human health to mycotoxins as the etiological agents of disease syndromes in man is difficult to show. A similar case exists in understanding the role that natural-occurring pesticides play in human diseases. Do long-term effects occur from natural pesticides, and how do they manifest themselves?

Conventional analytical methods including biological assay, thin-layer, liquid, and gas chromatography, and mass spectrometry have been used for the past 25 years to investigate mycotoxins. These methods often require extensive cleanup of samples containing the complex backgrounds from the many different kinds of foods evaluated. Immunochemical assays have been proposed in the past because they are specific; therefore, extensive cleanup of samples may not be needed. Radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISAs) have been developed for aflatoxin B<sub>1</sub> and M<sub>1</sub>, deoxynivalenol, T-2 toxin, and zearalenone. ELISAs can take as little as 10 minutes per sample to analyze for a mycotoxin. Although ELISAs need to be supported by existing analytical methods for conformation purposes, their speed and ease of use will help assure an increased level of safety in human and animal food (Pestka, 1988).

### **Mycotoxins and Disease**

Based on animal studies, epidemiological data and reports of human diseases, toxicologic problems caused by mycotoxins can be divided into three types: acute toxicogenic, chronic toxicogenic, and carcinogenic (Bullerman, 1979). Twenty different mycotoxins have been compared for embryotoxic, teratogenic, mutagenic, and carcinogenic effects; and only aflatoxin B<sub>1</sub> has all four activities (Hayes, 1980). Aflatoxin B<sub>1</sub> is a potent hepatocarcinogen in every studied mammalian species (World Health Organization, 1987). Besides aflatoxins being hepatotoxic to fish, mammals, and poultry, they also attack the immune system (Coppock and Swanson, 1986). Acute effects of aflatoxin ingestion and the possible roles of long-term exposures in human disease have been reviewed in detail (Linsell, 1977). Over 100 people died out of nearly 400 people that acquired acute aflatoxicosis associated with a localized 1975 famine in India (Linsell, 1977).

The liver and kidneys are important in the metabolism of chemical neurotransmitters that control brain function. In Southern Georgia, consumption of foods that are potential sources of high levels of aflatoxins were significantly related to mental retardation of the local children (Caster et al., 1986). There also is a reported problem with mental retardation in a region of East Africa known to have high concentrations of aflatoxins in the food supply (Brown, 1981). Aflatoxins have been suggested as being involved in Reye's Syndrome (Caster et al., 1986). A review of this disease and its possible link with aflatoxin is explored by Denning (1987). Although the data linking aflatoxin to Reye's Syndrome is preliminary, there is evidence in favor of a role for aflatoxin in some cases. Reye's Syndrome occurs in epidemic proportions in children in Northeast Thailand and is characterized by vomiting, hypoglycemia, convulsions, and coma followed by death (Bourgeois et al., 1971). Autopsy reports of victims in Thailand have shown aflatoxin B<sub>1</sub> in human tissue specimens from 22 of 23 cases (Shank et al., 1971). Results showed that



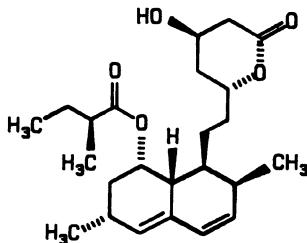
**Zearalenol**

Figure 42. The estrogenic mycotoxin, zearalenol.

aflatoxin B<sub>1</sub> appears to be linked to Reye's Syndrome and supports the suggestion that aflatoxin B<sub>1</sub> may be involved in its etiology (Hayes, 1980). Denning (1987) concluded that the data linking aflatoxin exposure to Reye's syndrome is preliminary.

**Estrogenic agents.** In Canada during 1980, there were epidemic proportions of fungi causing mycotoxin contamination of human foods and animal feeds. The predominant mycotoxin was identified as 4-deoxynivalenol (vomitoxin or DON) (Fig. 33). Metabolites isolated from liquid cultures of the involved *Fusarium* species are listed in Table 33 (Greenhalgh et al., 1985). Livestock in Northern Belgium fed moldy feeds containing mycotoxins such as the estrogenic zearalenone (Fig. 38), and zearalenol (Fig. 42) may produce milk and milk products that contain these estrogenic substances (Schoental, 1977a). Estrogenic agents can increase the plasma levels of cholesterol and triglycerides in females, and an association between oral-estrogen use and myocardial infarction and stroke has been described (Wallace et al., 1977). Patients treated for prostatic cancer with the estrogen diethylstilbestrol suffered increased mortality rates from cardiovascular disease (Blackard et al., 1970). Excessive amounts of estrogens, regardless of their structure, are known to affect conception, induce thromboembolic disease, and cause liver hepatomas (Schoental, 1976). However, there is a conflict in the understanding of risks and benefits of estrogen replacement in postmenopausal women. A study by Sullivan et al. (1990) indicates that the survival rate of estrogen replacement in postmenopausal women is increased. Whereas, others have reported increased mortality from myocardial infarction and cerebrovascular accidents in women using estrogen replacement (Barrett-Connor and Bush, 1989; Ernster et al., 1988). Because barley used for beer production can contain *Fusarium* species, Schoental (1977a) suggests that it also may be appropriate to not only monitor milk for these estrogenic substances but to also monitor beer, since many *Fusarium* species will produce estrogenic substances. Zearalenol (Fig. 42), though less active than other estrogens, affects the same target organs as do some commonly known estrogens (Schoental, 1981). Livestock fodder containing zearalenone can produce estrogenism including abortions, sterility, and vulvovaginitis in pigs and cattle (Mirocha et al., 1977). The ability of the *Fusarium* species to produce estrogenic metabolites may explain the variations in symptomatology observed in mycotoxicosis. A common fungal contaminant of barley is *Aspergillus terreus* (Tuite, 1977) which produces the hypocholesterolemic agent, mevinolin (Fig. 43), as a metabolic product (Vederas, 1985). Thus, products prepared from barley might contain this compound.

Epidemiological studies indicate that environmental contaminants such as mycotoxins, independently or in combination with Hepatitis B virus, may be important factors in primary hepatocellular carcinoma (Harris and Tsung-Tang, 1986). Human liver cancer is most heavily concentrated in areas where climate and food storage methods favor growth of *Aspergillus flavus* (Rutledge, 1977). About one-quarter of a



**Mevinolin**

Figure 43. The hypocholesterolemic mycotoxin, mevinolin.

million people die from hepatocellular cancer each year; this disease is one of the major malignant diseases worldwide. Symptoms of liver cirrhosis also are evident in about 80% of the cases. In low hepatocellular cancer risk populations, alcoholic cirrhosis is a more important disease (Kew, 1986). There would appear to be the possibility that some alcoholic cirrhosis cases may be due in part to aflatoxins in alcoholic beverages. There is a growing belief that high rates of liver cancer, like those observed in adult males in some countries of tropical Africa, may have multiple causes: food contaminants, herbal teas, and environmental chemicals, among others (Bababunmi, 1978). Epidemiological studies have revealed a positive correlation between esophageal cancer in China and foodstuffs contaminated with fungi. *F. moniliforme* is reported to be one of the most predominant fungi associated with foodstuffs in the high cancer area of the Chinese county of Linxian (Li et al., 1980; Lin and Tang, 1980; Yang, 1980). Feed contaminated with *F. moniliforme* is acutely toxic to pigs (Harrison et al., 1990; Kriek et al., 1981b), horses, sheep, baboons (Kriek et al., 1981a), ducklings (Engelhardt et al., 1989; Jeschke et al., 1987; Kriek et al., 1981a), chickens (Engelhardt et al., 1989; Fritz et al., 1973; Maruanovic et al., 1991; Sharby et al., 1973), turkey poult (Engelhardt et al., 1989), rats (Gelderblom et al., 1988; Jaskiewicz et al., 1987b; Kriek et al., 1981a; Marasas et al., 1984a; Norred et al., 1989; van Rensburg et al., 1982; Voss et al., 1989; Wilson et al., 1985a), and monkeys (Jaskiewicz et al., 1987a). Since primates fed diets containing corn inoculated with *F. moniliforme* strain MRC 826 developed hepatitis (Jaskiewicz et al., 1987a), it then follows that *F. moniliforme* may play a role in the etiology of human liver disease. As suggested in this review, aflatoxins may play a role in alcoholic cirrhosis of the liver in beer drinkers; however, *F. moniliforme* mycotoxins may play an even larger role in that same disease.

Leukemia may in part be caused by mycotoxins. Houses in which there have been more than one case of leukemia in Cracow, Poland, are damper than other houses; and molds isolated from such houses produce carcinogens (Aleksandrowicz and Smyk, 1973). Similar observations have been made in Texas (Wray and O'Steen, 1975).

The carcinogenicity potential of mycotoxins has been thoroughly reviewed by Hayes (1980). However, a report by Schoental (1981) indicates that the role of mycotoxins in human abnormalities has yet to be appropriately investigated. Some mycotoxins are also potent teratogens; the first indication of such effects were shown for aflatoxin in 1964. Hamsters are the most susceptible test animal, and malformations develop in the head region when aflatoxin is administered on the eighth day of gestation. A number of mycotoxins can induce teratogenic effects when administered to pregnant experimental animals (Table 34). Rubratoxin B is also teratogenic when administered during organogenesis (Abramovici, 1977).

Table 34. Mycotoxin induction of fetal abnormalities in animals.<sup>a</sup>

Mycotoxin	Animal	Dosage (mg/kg)
Aflatoxin B <sub>1</sub>	Hamster, rat	1-1.5
Ochratoxin A	Rat	1-5
	Hamster	7-20
	Mouse	5
	Chick embryo	0.5-5 <sup>b</sup>
Patulin	Chick embryo	2-68 <sup>b</sup>
T-2 toxin	Mouse	1-1.5
Zearalenone	Rat	5-10

<sup>a</sup>From Schoental (1981).<sup>b</sup>The dosage units are in µg/egg.

Ochratoxin A (Fig. 38) is the only known mycotoxin that can induce fetal abnormalities in a large number of animal species including chick embryos, hamsters, mice, and rats. It is also nephrotoxic. Zearalenone (Fig. 38) is teratogenic in rats and may be teratogenic in humans since other estrogenic agents are known to be teratogenic in both rats and humans (Henderson et al., 1979; Schoental, 1976, 1977a,b).

When moldy brewers' grains containing T-2 toxin (Fig. 44) were added to feeds in the United States, Japan, Scotland, and England, animal deaths occurred (Schoental, 1981). An outbreak of cardiomyopathy that killed at least 40 beer drinkers in Quebec and others in the United States and Belgium during 1967 (Morin and Daniel, 1967) was probably caused by moldy grain used to prepare the beer (Schoental, 1980). Acute and chronic lesions observed in these humans were reproduced in experimental animals administered T-2 toxin (Ueno, 1977).

T-2 toxin (Fig. 44) exerts immunosuppressive effects in mice (Corrier and Ziprin, 1986b; Holt and DeLoach, 1988a), exerting a differential effect on infection in mice which are dependent on the infective agent and route of inoculation (Ziprin and McMurray, 1988). Lymphoid cells may be affected (Corrier and Ziprin, 1986a) in a dose-dependent manner (Holt et al., 1988; Holt and DeLoach, 1988b). Concentrations of T-2 toxin that caused lymphoid cell depletion affected the *in vivo* macrophage phagocytic activity in antigenically sensitized mice and was a function of the time between toxin treatment and antigenic stimulation (Corrier et al., 1987). T-2 toxin interactions with erythrocytes suggest that one of its effects is on cell membranes (DeLoach and Kachatourians, 1988). T-2 toxin hemolyses mammalian erythrocytes at different rates. Mouse, dog, man, guinea pig, pig, rabbit, rat, and horse erythrocytes are hemolyzed by T-2 toxin in decreasing order of susceptibility (DeLoach et al., 1989).

The nephrotoxin, citrinin (Fig. 38), is eliminated from normal rats primarily by the kidney. However, after the animals received nephrotoxic doses of citrinin, hepatic elimination played a prominent role in its removal (Hayes, 1980). Nephrotoxicity and mycotoxins have been extensively reviewed by Krogh (1976).

Patulin (Fig. 39) is produced by *Penicillium patulum* and *Aspergillus clavatus* and is commonly found in apples and barley. Apricots, bananas, grapes, peaches, pears, pineapples, plums, and sweet cherries also may be contaminated with patulin (McKinley and Carlton, 1980b). Patulin inhibits respiration of higher plant cells (Polacco and Sands, 1977) and has a neurotoxic effect on animals (Abramovici, 1977). It can cause edema of the lungs and brain, congestion of visceral organs, and hepatic

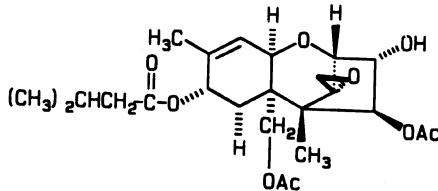


Figure 44. The immunosuppressive mycotoxin, T-2 toxin.

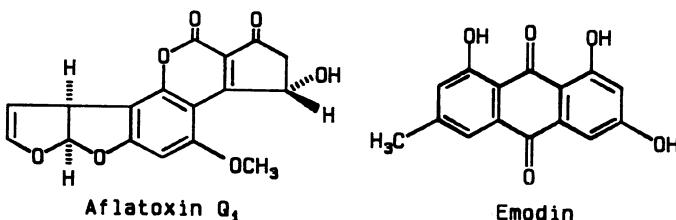


Figure 45. Aflatoxin Q<sub>1</sub>, the mammalian metabolite of aflatoxin B<sub>1</sub>, and emodin are both mutagens.

and renal necrosis in both rats and mice. Its LD<sub>50</sub> in chickens is 170 mg/kg body weight. The LD<sub>50</sub> for patulin administered orally, SC, and IP in hamsters were 31.5, 23.0, and 10.0 mg/kg body weight, respectively. Sarcomas developed at the SC injection sites on hamsters (McKinley and Carlton, 1980a). The LD<sub>50</sub> for patulin administered orally, SC, and IP to the mouse was 48.0, 10.0, and 7.5 mg/kg body weight, respectively (McKinley and Carlton, 1980b). It can induce vomiting in experimental animals (Hayes, 1980) and mutations in a mammalian cell line (Umeda et al., 1977).

Aflatoxin Q<sub>1</sub> (Fig. 45) is a mammalian metabolite of aflatoxin B<sub>1</sub>; and 52.9% of the recoverable metabolites from aflatoxin B<sub>1</sub> metabolism was aflatoxin Q<sub>1</sub> and was detected in chickens, monkeys, and rats administered aflatoxin B<sub>1</sub> (Masri, 1984). Aflatoxin Q<sub>1</sub> is mutagenic to *Salmonella typhimurium* strain TA100 and is also mutagenic in the presence or absence of liver microsomes. Non-activated aflatoxin Q<sub>1</sub> mutagenesis was nearly equal to that obtained when it was activated. It is mutagenic to rainbow trout (Yourtee and Kirk-Yourtee, 1986).

Pure aflatoxin B<sub>1</sub> given to lactating rats resulted in a toxic substance in the milk (van der Linde et al., 1964). The "milk toxin" resulted from aflatoxin B<sub>1</sub> conversion in the liver, and the trivial name "aflatoxin M" was suggested (Allcroft et al., 1966). The Council for Scientific and Industrial Research Laboratories in Pretoria, South Africa, confirmed its presence and determined the structure of aflatoxins M<sub>1</sub> and M<sub>2</sub> (Fig. 46), corresponding to aflatoxin B<sub>1</sub> and B<sub>2</sub> (Fig. 37) (Holzapfel et al., 1966). Aflatoxin M<sub>1</sub> is one of the oxidation products of aflatoxin B<sub>1</sub>, and the reduction product is aflatoxicol (Fig. 46). Acute toxicity of aflatoxin M<sub>1</sub> in the day-old duckling was found to be approximately the same as that of aflatoxin B<sub>1</sub> (Purchase, 1967). Acute toxicities in the rat also were similar (Wogan and Paglialunga, 1974). But the carcinogenic potency of aflatoxin M<sub>1</sub> was lower than that of B<sub>1</sub> in the rat (Wogan and Paglialunga, 1974) and rainbow trout (Canton et al., 1975; Sinnhuber et al., 1970).

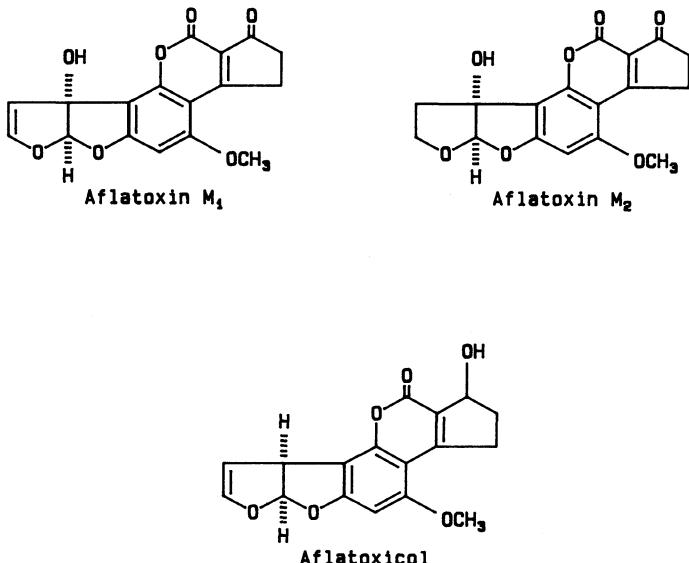


Figure 46. Aflatoxin M<sub>1</sub>, aflatoxin M<sub>2</sub>, and aflatoxicol are metabolic products of aflatoxin.

The relative mutagenicity of the major aflatoxins as determined by the Ames test are as follows: aflatoxin B<sub>1</sub> (Fig. 37), aflatoxicol, aflatoxin M<sub>1</sub> (Fig. 46), and aflatoxin G<sub>1</sub> (Fig. 37); 100, 23, 3, and 3, respectively (Stoloff, 1980). An aflatoxin B<sub>1</sub> level of 300 ng/g in the feed of a cow could result in a 1 ng/ml level of aflatoxin M<sub>1</sub> in the milk (Stoloff, 1980). Like many mycotoxins, aflatoxin M<sub>1</sub> is stable in raw and processed milk and is unaffected by pasteurization.

The mycotoxin, emodin (Fig. 45), is a diarrheagenic toxicant and is produced by various fungi including *Cladosporium fulvum* Cooke, *Penicillium rugulosum* Thom, *Aspergillus ochraceus* Wilhelm (Wehner et al., 1979), and *Aspergillus wentii* Wehmer (Wells et al., 1975). Emodin is also produced in plants (Robinson, 1991). Products formed from emodin by metabolic activation act as frame shift mutagens in the Ames test (Wehner et al., 1979).

Anatoxin A is produced by toxigenic strains of freshwater blue-green algae. Anatoxin A is a potent neurotoxin and has caused death in pets, livestock, and wildlife which drink water contaminated with these strains of algae. Anatoxin A has an oral LD<sub>50</sub> of about 16 mg/kg to mice (Stevens and Kreiger, 1991).

Linear furanocoumarins have been considered as mycotoxins produced by *Sclerotinia sclerotiorum* in diseased celery (Busby and Wogan, 1981; Hayes, 1980; Richards, 1972). However, linear furanocoumarins have since been shown to be produced by the celery plant itself and function as phytoalexins in celery (Beier and Oertli, 1983). It is extremely unlikely that these compounds are produced by fungi (see section entitled "Parsleys..." for further information on linear furanocoumarins).

### Fumonisins

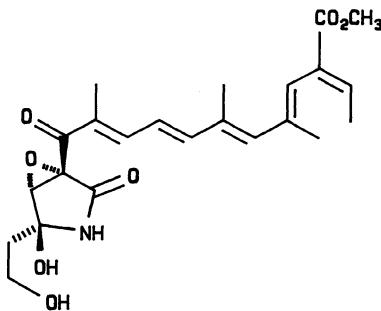
*Fusarium moniliforme* Sheldon is a phytopathogenic fungus that occurs worldwide on a variety of plant hosts including wheat, barley, soybeans, rice, and oats (Maruanovic et al., 1991). It is one of the most important ear rot pathogens of maize (*Zea mays L.*) (Booth, 1971; Marasas et al., 1984b).

Marasas et al. (1979) observed that two known *Fusarium* mycotoxins, deoxynivalenol and zearalenone (Fig. 38), were at high levels in naturally contaminated corn kernels in the Republic of Transkei, Africa. The main staple food of that Republic is corn. Transkei has a high incidence of esophageal and liver cancer (Rose and Fellingham, 1981). It was suggested that *Fusarium* mycotoxins may play a role in the development of tumors of the digestive tract (Schoental, 1977b; Schoental and Joffe, 1974; Schoental et al., 1976, 1978). It was concluded that *F. moniliforme*-contaminated food is positively associated with the esophageal cancer risk in Transkei (Marasas, 1982; Marasas et al., 1981). A positive correlation was found between the incidence of *F. moniliforme* in homegrown maize and human esophageal cancer (Marasas et al., 1984a).

In 1971, several species of fungi obtained from an outbreak of equine leukoencephalomalacia (ELEM) were isolated and grown-out on autoclaved corn and fed to donkeys. The corn infected with *F. moniliforme* Sheldon produced clinical signs and lesions characteristic of ELEM (Wilson and Maronpot, 1971; Wilson et al., 1973). *F. moniliforme* was shown to produce ELEM under other experimental conditions (Kellerman et al., 1972; Kriek et al., 1981a; Pienaar et al., 1981) and was consistently isolated from feed corn in all episodes of ELEM investigated (Buck et al., 1979). However, commercial pelleted and nonpelleted horse rations also have been implicated as a source of *F. moniliforme* (Wilson et al., 1985b). ELEM is a fatal neurotoxic syndrome of horses and other equines (Kriek et al., 1981a) and is often characterized pathologically by liquification necrosis of the white matter of one or both cerebral hemispheres (Wilson et al., 1985b). ELEM is a disease that has been reported in the United States since the early 1900s and is known in Egypt, Africa, China, Japan, and European countries (Buck et al., 1979; Haliburton and Buck, 1986; Marasas et al., 1988; McCue, 1989). Outbreaks of ELEM which end in acute deaths are characterized by various neurological signs including ataxia, head pressing, circling, and blindness of 1 to 2 days duration (Wilson et al., 1990), perivascular hemorrhage, satellitosis, and neuronophagia (Buck et al., 1979). Corn obtained from an outbreak of ELEM was fed to male Fisher 344 rats, and all rats had multiple hepatic nodules (Wilson et al., 1985a). In another study, corn samples associated with ELEM was fed to male Sprague-Dawley rats in a short-term bioassay and caused hepatotoxicity and renal toxicity (Voss et al., 1989).

An isolate of *F. moniliforme* strain MRC 826 was obtained from corn collected in Transkei, southern Africa, where the esophageal cancer rate is high (Marasas et al., 1979). MRC 826 was found to be both highly toxic and to produce ELEM (Kriek et al., 1981b) and was also mutagenic (Gelderblom et al., 1983). The isolate was shown to be hepatocarcinogenic to rats (Jaskiewicz et al., 1987b; Marasas et al., 1984a). The main mutagen was isolated from strain MRC 826 and shown to be fusarin C (Fig. 47) (Gelderblom et al., 1984). Although fusarin C is a potent mutagen, it failed to exhibit carcinogenicity (Gelderblom et al., 1986) which makes it an unlikely candidate to be involved in the carcinogenic effects of the fungus.

Culture material from MRC 826 was fed to a variety of animals and caused ELEM in horses; pulmonary edema in pigs; acute nephrosis and hepatosis in sheep; and cirrhosis, intraventricular cardiac thrombosis, and nephrosis in rats (Kriek et al., 1981a). A lifelong feeding experiment of MRC 826 in rats demonstrated that the culture material was not only highly hepatotoxic, but it also was hepatocarcinogenic at low dietary concentrations (Marasas et al., 1984a). At dietary concentrations as low as 2%, the culture material caused hepatocellular carcinoma in 80% and ductular carcinoma of the liver in 63% of the surviving rats. Many of the rats also had hyperplasia of the basal cells (Marasas et al., 1984a). MRC 826 also causes significant enhancement of nitrosamine-induced esophageal carcinoma in rats (van Rensburg et al., 1982). Male white leghorn chickens fed diets containing varying levels of



**Fusarin C**

Figure 47. Fusarin C is the main mutagen isolated from *F. moniliforme* strain MRC 826.

MRC 826 culture material had decreased weights of the bursae and were immunosuppressed in both primary and secondary responses (Maruanovic et al., 1991). Culture material from this same strain was highly toxic to vervet monkeys (*Ceropithecus pygerythus*) and caused acute, subacute, and chronic toxic hepatitis (Jaskiewicz et al., 1987a). A mammalian cell line of MDCK dog kidney epithelial cells was sensitive to fumonisins B<sub>1</sub> and B<sub>2</sub> ( $\text{IC}_{50} = 2.5$  and 2, respectively) (Shier et al., 1991). MDCK cells exhibited morphological changes within 24 hours of treatment.

Utilizing a short-term cancer initiation-promotion bioassay, the cancer-promoting activity in cultures of *F. moniliforme* strain MRC 826 were isolated (Gelderblom et al., 1988) and chemically characterized (Bezuidenhout et al., 1988). The cancer-promoting effect of fumonisin B<sub>1</sub> in rats was associated with a toxic effect, and the principal pathological change was an insidious and progressive toxic hepatitis similar to that of the culture material of *F. moniliforme* strain MRC 826 (Gelderblom et al., 1988). Four fumonisins were isolated and characterized, fumonisins A<sub>1</sub> and A<sub>2</sub> and fumonisins B<sub>1</sub> and B<sub>2</sub> (Fig. 40). Fumonisins A<sub>1</sub> and A<sub>2</sub> are amides. Since Plattner et al. (1992) were unable to observe A<sub>1</sub> and A<sub>2</sub> in extracts, it was thought that they may be artifacts as a result of the NH<sub>2</sub> group on fumonisins B<sub>1</sub> and B<sub>2</sub> reacting with acetic acid from the chromatography solvent system. However, fumonisins A<sub>1</sub> and A<sub>2</sub> were isolated by Cawood et al. (1991) and their findings confirmed that there was no indication that either fumonisins B<sub>1</sub> or B<sub>2</sub> were converted into these compounds. This work confirms the work by Bezuidenhout et al. (1988) showing that fumonisins A<sub>1</sub> and A<sub>2</sub> are metabolites produced by *F. moniliforme*.

The first experimental evidence that fumonisin B<sub>1</sub> isolated from *F. moniliforme* caused ELEM was presented by Marasas et al. (1988). A horse was injected intravenously seven times over a period of 9 days and on day 10 was euthanized. The principal lesions were severe edema of the brain and early, bilaterally symmetrical, focal necrosis in the medulla oblongata (Marasas et al., 1988). Fumonisins B<sub>1</sub> and B<sub>2</sub> were first identified by Norred et al. (1989) and Voss et al. (1989) in naturally contaminated corn screenings that caused field cases of ELEM and were hepatotoxic to rats. Fumonisin B<sub>1</sub> was also identified in moldy homegrown corn collected from an area of Transkei, southern Africa, that has a high incidence of esophageal and liver cancer in humans (Sydenham et al., 1990). Outbreaks of porcine pulmonary edema syndrome (PPE) have been noted to overlap at the same places and times that ELEM outbreaks have occurred. PPE has now been reproduced by IV injection of fumonisin B<sub>1</sub> in swine (Harrison et al., 1990).

Fumonisins B<sub>1</sub> and B<sub>2</sub> levels were evaluated in feeds associated with 13 horses that died during an outbreak of ELEM (Wilson et al., 1990). Levels ranged from 37 to 122 µg/g fumonisin B<sub>1</sub> and from 2 to 23 µg/g fumonisin B<sub>2</sub>. Feeds associated with 44 cases of ELEM and 42 cases of PPE were analyzed for fumonisin B<sub>1</sub> (Ross et al., 1991a). The feeds associated with ELEM contained fumonisin B<sub>1</sub> levels ranging from less than 1 µg/g up to 126 µg/g with 75% of the cases on feeds containing levels above 10 µg/g (Ross et al., 1991a,b). The feeds associated with the PPE had fumonisin B<sub>1</sub> levels ranging from less than 1 µg/g up to 330 µg/g with 71% of the cases on feeds above 10 µg/g (Ross et al., 1991a).

Three separation methods are described for fumonisin B<sub>1</sub> but were not fully developed analytical procedures for the mycotoxin (Sydenham et al., 1990). The methods consisted of TLC, reverse phase HPLC, and capillary GC analysis of various derivatives of fumonisin B<sub>1</sub>. Another study (Wilson et al., 1990) also used TLC, HPLC, and GC/MS to determine levels of fumonisins B<sub>1</sub> and B<sub>2</sub> in feed samples. The methods used were similar to those of Sydenham et al. (1990). An analytical method was presented by Plattner et al. (1990) for the detection of fumonisins by hydrolysis and silylation followed by GC/MS quantification. The hydrolysis removes the ester moieties at C14 and C15 leaving a C1 to C20 backbone typical for each fumonisin. A comparative analytical study utilizing a fluorescamine procedure with four laboratories resulted in acceptable agreement with fumonisin concentrations ranging from 4 to 1,800 µg/g (Plattner et al., 1991). Korfmacher et al. (1991) makes a comparison of mass spectrometry characterization methods of fumonisin B<sub>1</sub> using thermospray, FAB, and electrospray.

Recently, the fifth and sixth members of the fumonisin mycotoxin family, fumonisins B<sub>3</sub> and B<sub>4</sub> (Fig. 41), were characterized (Gelderblom et al., 1992; Plattner et al., 1992). Fumonisin B<sub>3</sub> has one less hydroxyl group than fumonisin B<sub>1</sub>, and fumonisin B<sub>4</sub> has one less hydroxyl group than fumonisin B<sub>2</sub>. Methodology for the quantitative purification of fumonisins B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> from corn cultures of *F. moniliforme* strain MRC 826 is described by Cawood et al. (1991).

Primarily, isolate MRC 826 has been used in studies by most researchers up until now. Most isolates of *F. moniliforme* examined by Plattner et al. (1992) predominantly produce fumonisin B<sub>1</sub>; typically about 70% of the total fumonisins detected. However, *F. subglutinans* appears only to make low, if any, amounts of fumonisin.

#### Aflatoxin Absorption and Removal from Feeds and Foods

There are numerous approaches that attempt to counteract the aflatoxin problem and some of them include: physical separations, thermal inactivation, irradiation, microbial degradation, and chemical treatments. These should be supplemented, whenever appropriate, by basic preventive measures such as proper harvesting, drying and storage of grains, proper processing and storage of feeds and feedstuffs, and routine clean out of feeding equipment and bins. Two chemical treatment methods offer more promise than others. They are: 1) the ammoniation of grains and feedstuffs to destroy aflatoxin and 2) the addition of sorbents to feeds to chemically bind aflatoxin, thereby removing this toxin from the food chain (Harvey et al., 1988a).

The ammoniation process converts aflatoxin B<sub>1</sub> to aflatoxin D<sub>1</sub> (Fig. 48). The mutagenicity (Ames test-TA100) and toxicity (chick embryo test) of aflatoxin D<sub>1</sub> compared to aflatoxin B<sub>1</sub> are reduced by 450-fold and 18-fold, respectively (Lee et al., 1981; Park et al., 1988). This process effectively decontaminates aflatoxin-containing feeds. There are two superior ammoniation processes: 1) a high temperature and high pressure treatment for about 1 hour used in commercial treatment plants and

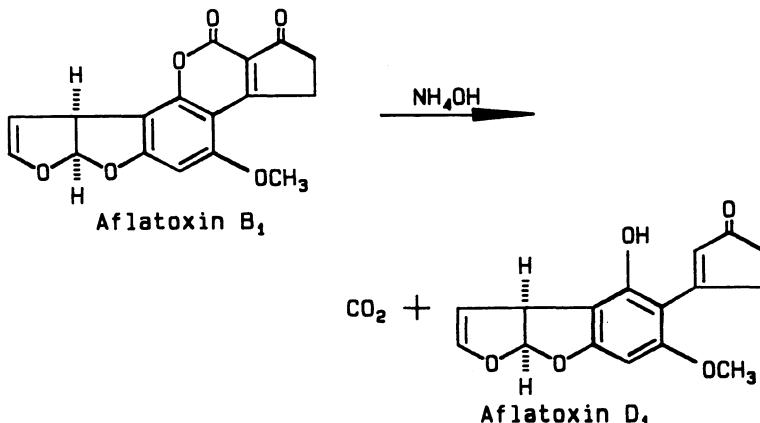


Figure 48. Aflatoxin B<sub>1</sub> is converted to aflatoxin D<sub>1</sub> by the ammoniation process.

2) a method used at farm sites that takes 2 to 3 weeks at ambient temperature and atmospheric pressure. Either process can remove up to 90% of aflatoxin B<sub>1</sub> contamination (Park et al., 1988). Ammonia was the only satisfactory chemical method for detoxification of any mycotoxin (Hesseltine, 1985) prior to detoxification by sorbent technology.

A number of sorbents including aluminas, aluminosilicates, and silicas have been investigated for their ability to bind aflatoxin (Phillips et al., 1988b). The chemically selective hydrated sodium calcium aluminosilicate (NovaSil®) has a high affinity for aflatoxin. NovaSil® is currently used as an anti-caking agent for feeds and is considered GRAS (generally recognized as safe). When chicks were fed diets containing 7.5 ppm aflatoxin B<sub>1</sub>, the addition of NovaSil® (0.5%) offered 55 to 100% protection from aflatoxicosis (Kubena et al., 1987; Phillips et al., 1988a). NovaSil® (0.5%) also protected chicks fed a mixture of aflatoxins on *A. flavus* fermented rice (Kubena et al., 1988). When barrows were fed diets containing 3 ppm aflatoxin and 0.5% or 2.0% NovaSil®, both NovaSil® treatments prevented toxicosis and death, which are caused by aflatoxin alone (Harvey et al., 1988b,c). NovaSil® treatments decreased aflatoxin M<sub>1</sub> in goat's milk by 60%, and in cows fed 200 ppm and 100 ppm aflatoxin by 27% and 43%, respectively (Harvey et al., 1988c). Aflatoxins are thought to be bound by NovaSil® in the upper gastrointestinal tract reducing their bioavailability (Davidson et al., 1987).

### Inhibition of Aflatoxin Production

It was thought that the single best postharvest control measure for aflatoxins was to reduce the water content of grains (Hamilton, 1982). However, the effectiveness of this control measure is limited by the fact that *A. flavus* grows better than most organisms at a reduced moisture content. A number of natural products, typically phenolics, inhibit growth of *Aspergillus* species and/or the production of aflatoxin (Zaika and Buchanan, 1987). A common inhibitor of molds and yeasts is the food additive sorbate (sorbic acid). Sorbic acid was first isolated from pressed unripened berries of rowan or mountain ash trees in 1859. Sorbate has been commercially used to preserve baked goods, berries, dairy products, fish, fruits, meat products, and vegetables (Liewen and Marth, 1985). Sorbic acid, as well as acetic, benzoic, citric, lactic, or propionic acids, will partially or completely inhibit aflatoxin

production. Pyridazinone herbicides cause variable changes in aflatoxin production by *Aspergillus* species. *A. flavus* produced lower levels of aflatoxin, but *A. parasiticus* produced higher levels of aflatoxin when the herbicide was placed in the media (Bean and Southall, 1983). Aflatoxin production is also inhibited, unaffected, or stimulated by various synthetic insecticides (Draughon and Ayres, 1981). Natural components of some herbs and spices also inhibit aflatoxin production (Rusul and Marth, 1988). Caffeine inhibits growth and aflatoxin production by *A. parasiticus* (Buchanan et al., 1983); it also inhibits glucose transport (Buchanan and Lewis, 1984). Mold growth and aflatoxin production is inhibited by cinnamon which is not used as a food preservative but could be an added safety factor against mold production (Bullerman, 1974).

### Chemical Inhibitors of Fungal Melanin and Aflatoxin Biosynthesis

Aflatoxin production is limited to the species, *Aspergillus flavus* and *A. parasiticus*. Diener and Davis (1969) calculated that 60% of the known strains were aflatoxigenic; more recently, 56% of 3,343 isolates were aflatoxigenic (Bennett, 1982). Aflatoxin-deficient mutants of *A. parasiticus* have been made by ultraviolet light or nitrosoguanidine treatments (Bennett, 1985); cultures of these strains accumulate intermediates in the aflatoxin biosynthetic pathways. Insight into the biosynthetic pathway of aflatoxins can be gained by studying blockers of aflatoxin biosynthesis and melanin biosynthesis in fungi.

Fungal melanins occur primarily in cell walls and are formed either enzymatically or autooxidatively and are thought to be produced mainly from 1,8-dihydroxynaphthalene (DHN). The melanins are important for survival of dormant propagules and for virulence in some fungal species. The pentaketide biosynthetic route for melanin synthesis from acetate via scytalone and DHN is shown in Figure 49. The reductase [H] steps can be inhibited by a number of inhibitors but are shown here as a potential site for tricyclazole (t) inhibition (Bell and Wheeler, 1986). Tricyclazole (Fig. 50) is commercially used to prevent rice blast disease caused by *Pyricularia oryzae* (Bell and Wheeler, 1986; Wheeler and Bell, 1988). Chlobenthiazole (Fig. 50), another fungicide that prevents rice blast disease, inhibits the melanin pathway at the same enzymatic steps as does tricyclazole (Wheeler and Bell, 1988). Chlobenthiazole also is a strong inhibitor of aflatoxin synthesis by *A. flavus*. Aflatoxin B<sub>1</sub> quantities in *A. flavus* cultures were decreased by 90 and 99% when chlobenthiazole was added at 1 and 4 µg/ml, respectively (Wheeler et al., 1988). At these concentrations, chlobenthiazole inhibited the mycelial dry weight of *A. flavus* by only 8 and 14%, respectively. Tricyclazole also inhibits the production of aflatoxin by *A. flavus* without strongly affecting mycelial growth, but it is not as efficient as chlobenthiazole (Wheeler, et al., 1989, 1991). Blastin, tetrachlorophthalide, and pyroquilon showed significant inhibitory effect on the accumulations of aflatoxins B<sub>1</sub>, B<sub>2</sub>, and B<sub>2a</sub> in culture (Wheeler and Bhatnagar, 1991). Blastin and tetrachlorophthalide are slightly weaker inhibitors of aflatoxin synthesis than chlobenthiazole, but are stronger inhibitors than pyroquilon or tricyclazole.

In summary of the above, it is clear that there are a number of natural-occurring and synthetic compounds (typically phenolics) that inhibit aflatoxin synthesis and/or the growth of the *Aspergillus* species. Knowledge of these and related compounds might lead to the identification of an innocuous inhibitor which might possibly be genetically engineered into crops providing them with protection from fungal growth and, ultimately, aflatoxin production (Wheeler, personal communication).

There is probably no other recognized group of chemicals that has caused such a high number of recorded human or animal deaths as mycotoxins. Even now, victims

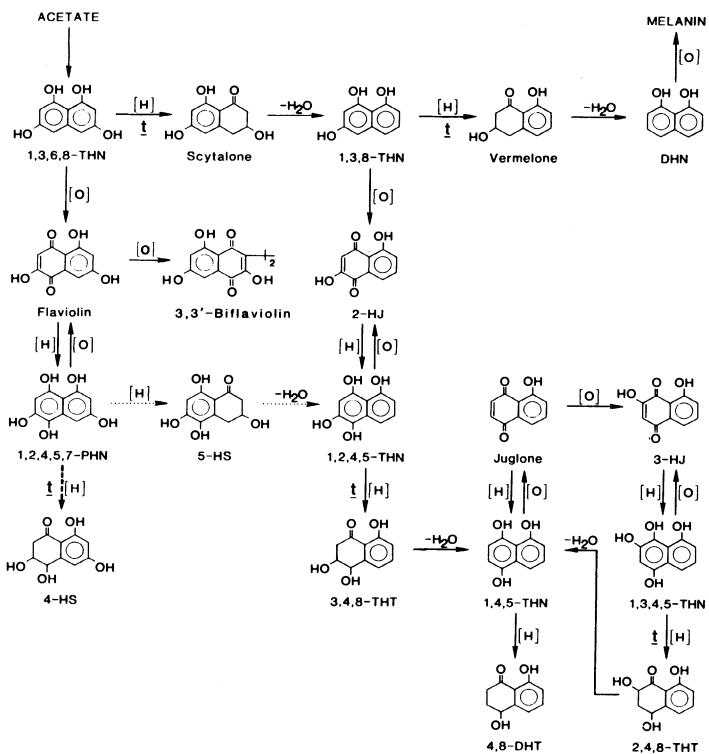


Figure 49. Biosynthesis pathway of DHN melanin. The reductase steps [H] and their inhibition by tricyclazole (t) are shown. Adapted from Wheeler and Bell (1988).

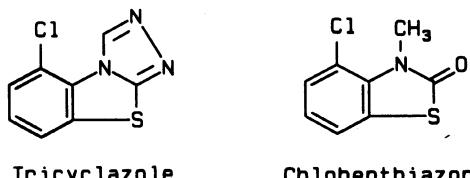


Figure 50. Tricyclazole and chlobenthiazone are inhibitors of the reductase [H] steps in the DHN melanin biosynthetic pathway.

of mycotoxin exposure are probably more frequent than victims of other chemicals such as synthetic pesticides and industrial chemicals (Schlatter, 1988). Despite the notion that diet is a major environmental source of cancer, only a few dietary carcinogens such as aflatoxin have been recognized (Balducci et al., 1986). There is no doubt that aflatoxin is a major natural disease agent. However, many other mycotoxins are also potentially very important in human diseases. These are produced by a number of different fungi and include the fumonisins, ochratoxin A, trichothecenes, citrinin, patulin, as well as a number of other toxic compounds. These naturally occurring chemicals may have long-term subtle toxic effects on man which can make them difficult to identify. The fast, obvious reactivity of aflatoxins has made them the focal point of many research investigations. Aflatoxins are regulated at

20 ppb in most food stuffs (0.5 ppb in whole milk), and Deoxynivalenol is regulated in finished wheat products at 1,000 ppb (Van Egmond, 1989).

## SUMMARY

When we discuss toxicants in general, and particularly toxicants in food, we should remember a general principle of toxicology which originated with Paracelsus.

What is there that is not poison? All things are poison and nothing (is) without poison. Solely the dose determines that a thing is not poison.

-- Paracelsus, 1492-1541  
(Deichmann et al., 1986)

Regardless of the toxicity, mode of action, biological targets, or the end result of a chemical compound, there is a dose-response relationship.

Synthetic pesticides have taught us many lessons about how chemicals interact with nature. Biomagnification, resistance, enzyme induction, and cholinesterase inhibition are well-known phenomena in pesticide toxicology and in many other branches of science. The study of these phenomena was driven by the appearance of unanticipated effects on wildlife and humans alike. We have subsequently regulated the appearance of synthetic pesticides in our foods; that is, we have regulated the dose to which the U.S. population is exposed.

For naturally occurring mixtures of chemicals, the reduction of exposure by reducing levels will be infinitely more complex.

Examples: Crucifers, including broccoli, Brussels sprouts, cabbage, and cauliflower contain naturally occurring components that are goitrogenic resulting from the combined action of allyl isothiocyanate, goitrin, and thiocyanate. Although crucifers may provide some protection from cancer when taken prior to a carcinogen, crucifers act as promoters of carcinogenesis in animals when taken after a carcinogen. The acid-condensed mixture of indole-3-carbinol (an enzyme released component of crucifers) binds to the TCDD receptor and causes responses similar to those of TCDD.

Flavonoids may act as 'vitamins', but other compounds in this class may cause abortion.

Herbs contain many biologically active components with more than 20% of the commercially prepared human drugs coming from these plants. Onion and garlic juices can help to prevent the rise of serum cholesterol. Most herbs used in treatments may have many natural constituents that act oppositely from their intended use. Some herbs, like bishop's weed seed, contain carcinogens; and many contain pyrrolizidine alkaloids that can cause cirrhosis of the liver.

The general phytoalexin response in plants (including potatoes, tomatoes, peppers, eggplant, celery, and sweet potatoes) induced by external stimuli can increase the concentrations of toxic chemical constituents in those plants. In potatoes, two major indigenous compounds are  $\alpha$ -solanine and  $\alpha$ -chaconine which are human plasma cholinesterase inhibitors and teratogens in animals. Because of its toxicity to humans, the potato variety, Lenape, was withdrawn from the market. Plants like celery, parsley, and parsnips contain the linear furanocoumarin phytoalexins, psoralen, bergapten, and xanthotoxin, which can cause photosensitization and which also are photomutagenic and photocarcinogenic. Celery field workers and handlers incur photosensitization problems as a result of these indigenous celery furanocoumarins. Following the introduction of a new celery cultivar (a result of plant breeding to

produce a more pest resistant variety) that reached the market, two CDC studies showed that this variety was responsible for outbreaks of photophytodermatitis of grocery store workers.

Sweet potatoes contain phytoalexins that can cause lung edema. At least one of these compounds, 4-ipomeanol, can cause extensive lung clara cell necrosis and increase the severity of pneumonia in mice. Some of the phytoalexins in sweet potatoes are hepatotoxic to mice and are also toxic to mouse kidneys. It is interesting to speculate if these lung toxins can exacerbate asthma conditions. The possibility of this is now being pursued (J. D. Mann, personal communication).

A commonly cultivated mushroom, *Agaricus bisporus*, contains benzyl alcohol as its most abundant volatile, and *A. bisporus* and *Gyromitra esculenta* both contain hydrazine analogs. As much as 20 to 30% of the hydrazone in *G. esculenta* decompose during cooking to *N*-methylhydrazine, causing the steam to be toxic. *N*-methylhydrazine is metabolized to a reactive species that decomposes to produce a methyl cation alkylating agent.

Mycotoxins are a worldwide problem. Mycotoxins are commonly found in corn, cottonseed, fruits, grains, grain sorghums, and nuts (especially peanuts). Consequently, they also occur in apple juice, bread, peanut butter, and other products made from contaminated starting materials. Mycotoxins have many biological activities resulting in toxicity, mutagenicity, or carcinogenicity. The aflatoxins are the most often cited offenders. They are linked to leukemia, liver cancer, mental retardation, and Reye's syndrome. The four major aflatoxins found on peanuts are B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>. Aflatoxin contaminated dried figs were observed to contain aflatoxin B<sub>1</sub> and G<sub>1</sub>. A carcinogenic metabolite of aflatoxin B<sub>1</sub>, aflatoxin M<sub>1</sub>, is present in milk and milk products produced from animals fed aflatoxin-contaminated feed. Aflatoxins are hepatotoxic to fish, mammals, and poultry; and they also attack the immune system. Fumonisin B<sub>1</sub> causes equine leukoencephalomalacia, is toxic to a wide range of animals including monkeys, is hepatotoxic to rats, causes porcine pulmonary edema syndrome, is toxic to cultured mammalian cells, and has been identified in moldy homegrown corn collected from southern Africa in an area that has a high incidence of esophageal and liver cancer in humans.

The mycotoxins, zearalenone and zearalenol, are estrogenic agents; and it is known that estrogenic agents can cause an increase in plasma levels of cholesterol and triglycerides. Fungal species that produce these estrogenic agents can be a contaminant of barley and, as a result, these agents could conceivably occur in beer or other barley containing products. Some mycotoxins (ochratoxin A and zearalenone) have been shown to be teratogens. Patulin, the mycotoxin commonly found in apples and barley, is neurotoxic to animals and can cause edema of the lungs and brain.

The plant species discussed here are by no means the only producers of toxic chemicals. Most plants probably either contain high concentrations of toxic chemicals and/or produce phytoalexins in response to disease, foreign chemicals, or injury. A foreign chemical may be something as common as a herbicide; thus, the herbicide acifluorfen increases the production of phytoalexins and stress metabolites in crops as diverse as bean, celery, cotton, pea, soybean, and spinach. A foreign chemical may be as common as acidic air pollution. Celery was shown to increase its levels of phototoxic psoralens in a phytoalexin response to acidic fog at pH levels similar to that experienced by commercial celery production near major population centers.

Besides containing toxic natural products (natural pesticides) and serving as a substrate for the growth of mycotoxin producing fungi, plants also absorb and translocate toxic chemicals from the soil. Endive and lettuce absorb and translocate selenium and mutagens from fly ash. Other food plants known to actively translocate

chemicals include: cantaloupes, carrots, celery, coffee, green beans, herbal plants used for teas, mushrooms, peppers, radishes, rhubarb, and spinach (Shane et al., 1988).

If we understood the intake of natural chemicals, i.e., the dose, we could begin to estimate risks. An article by Ames and Gold (1989) speaks directly to the Alar issue and asks the question, "what risks might we incur by banning Alar?" They point out what function Alar had in the apple industry and what natural risks may be elevated due to its banning. For instance, Alar can play a role in reducing pesticide use in some apples. It helps produce firmer apples and results in fewer apples falling to the ground, hence a potential for less pesticide use in the following year and also possibly less mycotoxin (patulin) production in apples.

We take risks each day. Life is a trade-off of risks and, hopefully, we will be able to choose between risks. There appears to be little direct evidence that our normal exposure to toxic chemicals in foods (with certain exceptions) poses significant risks. But there has yet to be a risk assessment study of naturally occurring toxicants (except aflatoxin) in foods. About half (27/52) of the few plant toxins that have been tested in animal cancer bioassays are rodent carcinogens, and these chemicals are present in many common foods (Ames et al., 1990a). Ames et al (1990a) concluded that both natural and synthetic chemicals are equally likely to be positive in animal cancer tests; about half of both natural and synthetic chemicals tested in animal cancer tests at the maximum tolerated dose (MTD) are carcinogens. In studies conducted at the MTD, a high proportion of both natural and synthetic chemicals are either carcinogens, mutagens, teratogens, or clastogens (30 to 50% for each group). It has been concluded that these statistics undermine the current regulatory efforts to protect public health by monitoring for synthetic chemicals based only on these tests (Ames et al., 1990b). Animal cancer tests carried out at the MTD of a particular chemical is a near-toxic dose which can cause chronic mitogenesis (Ames et al., 1990a), and mitogenesis is known to increase mutagenesis (Ames and Gold, 1990a,b). Chronic mitogenesis appears to be an important factor for many, if not most, of the known causes of human cancer (Ames and Gold, 1990a,b). Ames and Gold (1990a,b) agree that "the idea that mitogenesis increases mutagenesis helps explain promotion of carcinogenesis." However, humans have developed numerous defense systems that are active against natural as well as synthetic toxicants (Ames and Gold, 1989). Animal defenses are mostly of a general type and not only can offer protection from natural toxicants, but they also offer protection from synthetic toxicants as well (Ames et al., 1990b). In most cases, it requires concentration levels far above what any individual would obtain in a normal diet to observe biological effects from natural toxicants in foods. There are, however, a few natural pesticides that cause adverse biological activities near the present levels of human consumption.

Humans have been worried about synthetic pesticide residues in their foods for decades. It is appropriate that the United States has aggressively pursued the regulation of pesticides in the environment and in foods. The United States Food and Drug Administration (FDA) has found, in a "total diet" study of eight population groups during 1982 to 1984, that none of the pesticide residue levels measured approached the tolerances set by the U.S. Environmental Protection Agency (EPA). The intakes of the persistent chlorinated pesticides have also steadily declined (Gunderson, 1988). FDA's monitoring for pesticides in 14,492 samples from October 1, 1986 to September 30, 1987 showed that the United States' populations' exposure to pesticide residues is consistently below established limits. Less than 1% of the samples contained pesticide residues that exceeded regulatory limits (Food and Drug Administration Pesticide Program, 1988). During 1987, the California Department of Food and Agriculture (CDFA) analyzed 7,010 samples from marketplaces; only 1.5% of these samples had detectable levels of illegal pesticides,

and only 0.3% of the samples had pesticide residues in excess of tolerance limits for specific foods (Anonymous, 1988). Ames et al. (1990a) concluded "that at the low doses of most human exposures the comparative hazards of synthetic pesticide residues are insignificant."

The Committee on Food Protection, National Research Council (1973), suggested that plant breeders attempting to develop higher yielding or disease-resistant crop varieties must be alerted to the possible production of undesirable components. In fact, that suspicion has now become reality in at least two plant species, celery and potatoes. Phytoalexins and other naturally occurring pesticides produced by plants represent 99.99% of all the toxic chemicals we consume (Ames and Gold, 1989; Ames et al., 1990a). If Doll and Peto's (1981) estimate that as much as 35% of all cancer might be related to diet is correct, then it follows that chemicals other than synthetic pesticides are likely responsible for cancer and other disease syndromes in man. This is, therefore, a potentially enormous risk to humans; and extensive basic research on the problem of natural toxicants in human foods should receive increased emphasis.

A critical area of interest and question is the genetic engineering of new food plant varieties. We may make mistakes with genetic engineering. We do not well understand the levels and toxicology of chemicals in our present foods. Without this basic scientific knowledge, we cannot know what we have produced or might produce with genetic engineering. However, the concern should not be centered on genetic engineering *per se* but rather on the results, which might be the production of varieties with larger quantities of toxicants. Older, more established methods of breeding may arrive at essentially the same result, only more slowly. Increasing the toxicants in a crop may prove to be devastating and only hinder the progress of genetic engineering. However, the genetic engineering of aflatoxin inhibitors into crops like corn and peanuts, protecting them from fungal growth or mycotoxin production, might be a practical and attainable goal.

With very few exceptions (e.g., aflatoxins), there are no guidelines or regulations regarding naturally occurring toxicants in food. Quite likely, it may ultimately be considered immaterial whether a new variety of a common food is bred traditionally or with genetic engineering techniques; if a new variety has a production or marketing advantage, it will likely be produced and marketed. Historically, we have largely been unaware of the toxic potential of food plants entering the marketplace. Two examples, celery and potatoes, are discussed in this review. We continue to place new varieties on the market with no toxicological testing. We have always, in effect, experimented with foods on ourselves. It seems reasonable that a more solid scientific base regarding the chemical components in foods be developed and used in making properly informed judgements and decisions concerning our food supply; we have the tools and scientific talent to obtain the knowledge for that base. A basis for establishing change in our food supply should be established now.

We propose here a new strategy for plant breeders, Integrated Breeding and Environmental Chemicals (IBEC), to work in essence as follows: During the plant breeding process, the breeder evaluates levels of toxic compound(s) in all prospective new varieties. The variety with the lowest level(s) is ultimately used in the marketplace. This methodology was used with success while looking for leaf miner resistance in *Apium* accessions. We recognize that such an approach will require a multidisciplinary effort, which will require considerable effort and expense; however, the potential societal benefits are tangible and significant. As an example, a large reduction in the flavonol content of vegetables could probably be achieved through breeding using the IBEC approach. Using IBEC could be a very important step

forward in breeding programs for crops like celery, sweet potatoes, nightshade family crops, and other crops known to contain natural toxicants.

Rodricks (1978) states that "we remain abysmally ignorant of the chemical and toxicological properties of most natural chemicals to which humans have a lifetime of exposure." In our opinion, we need to more fully identify health hazards presented to humans by naturally occurring pesticides in foods. Scientific progress in this new frontier will require scientific cooperation and coordination, and support from appropriate institutional and public interest groups.

## ACKNOWLEDGMENT

Special thanks to the following individuals for their stimulating discussions or helpful critiques: B. N. Ames, J. A. Attaway, A. A. Bell, J. R. DeLoach, J. A. Duke, J. B. Harborne, R. B. Harvey, C. Holtzapple, W. E. Huff, G. W. Ivie, L. F. Kubena, U. Matern, N. I. Mondy, B. P. Mundy, J. O. Norman, D. M. Norris, L. D. Rowe, S. L. Sinden, M. R. Southerland, C. H. VanEtten, M. H. Wheeler, and D. A. Witzel.

## REFERENCES

- Abramovici, A., 1977, Mycotoxins and abnormal fetal development, Contributions to Microbiology and Immunology, 3:81-94.
- Adlercreutz, H., Fotsis, T., Bannwart, C., Wähälä, K., Brunow, G., and Hase, T., 1991, Isotope dilution gas chromatographic-mass spectrometric method for the determination of lignans and isoflavonoids in human urine, including identification of genistein, *Clinica Chimica Acta*, 199:263-278.
- Adzet, T., and Camarasa, J., 1986, Pharmacokinetics of polyphenolic compounds, p. 25-47, in: "Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships," V. Cody, E. Middleton, Jr., and J. B. Harborne, eds., Proceedings of a Symposium Held in Buffalo, New York, July 22-26, 1985, Alan R. Liss, Inc., New York, New York.
- Ahmed, E. M., Dennison, R. A., Dougherty, R. H., and Shaw, P. E., 1978, Effect of nonvolatile orange juice components, acid, sugar, and pectin on the flavor threshold of *d*-limonene in water, *Journal of Agricultural and Food Chemistry*, 26:192-194.
- Ahmed, S. S., and Müller, K., 1978, Effect of wound-damages on the glyco-alkaloid content in potato tubers and chips, *Lebensmittel-Wissenschaft + Technologie*, 11:144-146.
- Akkmeteli, M. A., 1977, Epidemiological features of the mycotoxicoses, *Annales de la Nutrition et de l'Alimentation*, 31:957-976.
- Allcroft, R., Rogers, H., Lewis, G., Nabney, J., and Best, P. E., 1966, Metabolism of aflatoxin in sheep: Excretion of the 'milk toxin', *Nature*, 209:154-155.
- Aleksandrowicz, J., and Smyk, B., 1973, The association of neoplastic diseases and mycotoxins in the environment, *Texas Reports on Biology and Medicine*, 31:715-726.
- Alfa, J., and Heyl, E., 1923, Potatoes of the 1922 harvest with an unusually high solanine content, *Zeitschrift fuer Untersuchung der Nahrungs-und Genussmittel sowie der Gebrauchsgegenstaende*, 46:306-309.
- Allen, E. H., and Kuć, J., 1968,  $\alpha$ -Solanine and  $\alpha$ -chaconine as fungitoxic compounds in extracts of Irish potato tubers, *Phytopathology*, 58:776-781.
- Alozie, S. O., Sharma, R. P., and Salunkhe, D. K., 1979, Inhibition of rat cholinesterase isoenzymes *in vitro* and *in vivo* by the potato alkaloid,  $\alpha$ -chaconine, *Journal of Food Biochemistry*, 2:259-276.

- Amat-Guerri, F., Martinez-Utrilla, R., and Pascual, C., 1984, Condensation of 3-hydroxymethylindoles with 3-substituted indoles. Formation of 2,3'-methylene-diindole derivatives, *Journal of Chemical Research, Synopsis* 160-161.
- American Cancer Society, 1984, Nutrition and cancer: Cause and prevention, *Ca-A Cancer Journal for Clinicians*, 34:121-126.
- American Council on Science and Health, 1987, Mother nature and her chemicals join us for Thanksgiving dinner, New York, New York.
- Ames, B. N., 1983, Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases, *Science*, 221:1256-1263.
- Ames, B. N., and Gold, L. S., 1989, Pesticides, risk, and applesauce, *Science*, 244:755-757.
- Ames, B. N., and Gold, L. S., 1990a, Chemical carcinogenesis: Too many rodent carcinogens, *Proceedings of the National Academy of Science*, 87:7772-7776.
- Ames, B. N., and Gold, L. S., 1990b, Too many rodent carcinogens: Mitogenesis increases mutagenesis, *Science*, 249:970-971.
- Ames, B. N., Magaw, R., and Gold, L. S., 1987, Ranking possible carcinogenic hazards, *Science*, 236:271-280.
- Ames, B. N., Profet, M., and Gold, L. S., 1990a, Dietary pesticides (99.99% all natural), *Proceedings of the National Academy of Science*, 87:7777-7781.
- Ames, B. N., Profet, M., and Gold, L. S., 1990b, Nature's chemicals and synthetic chemicals: Comparative toxicology, *Proceedings of the National Academy of Science*, 87:7782-7786.
- Anonymous, 1988, 1987 pesticide residue annual reports, California Department of Food and Agriculture, p. 1-32.
- Anonymous, 1989, Woman dies after tanning, *Bryan-College Station Eagle*, May 28, Bryan, Texas.
- Archer, A. W., 1988, Determination of safrole and myristicin in nutmeg and mace by high-performance liquid chromatography, *Journal of Chromatography*, 438:117-121.
- Armstrong, B., and Doll, R., 1975, Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices, *International Journal of Cancer*, 15:617-631.
- Arsecularatne, S. N., Gunatilaka, A. A. L., and Panabokke, R. G., 1981, Studies on medicinal plants of Sri Lanka: Occurrence of pyrrolizidine alkaloids and hepatotoxic properties in some traditional medicinal herbs, *Journal of Ethnopharmacology*, 4:159-177.
- Arsecularatne, S. N., Gunatilaka, A. A. L., and Panabokke, R. G., 1985, Studies on medicinal plants of Sri Lanka, Part 14: Toxicity of some traditional medicinal herbs, *Journal of Ethnopharmacology*, 13:323-335.
- Ashwood-Smith, M. J., Ceska, O., and Chaudhary, S. K., 1985, Mechanism of photosensitivity reactions to diseased celery, *British Medical Journal*, 290:1249.
- Ashwood-Smith, M. J., Poulton, G. A., Ceska, O., Liu, M., and Furniss, E., 1983, An ultrasensitive bioassay for the detection of furocoumarins and other photosensitizing molecules, *Photochemistry and Photobiology*, 38:113-118.
- Atluru, S., and Atluru, D., 1991, Evidence that genistein, a protein-tyrosine kinase inhibitor, inhibits CD<sub>28</sub> monoclonal-antibody-stimulated human T cell proliferation, *Transplantation*, 51:448-450.
- Attaway, J. A., 1992, Medical benefits of juice flavonoids, *Proceedings of the XI International Congress of Fruit Juices*, Sao Paulo, Brazil, 17-21 November 1991.
- Augustin, J., McDole, R. E., and Painter, G. C., 1977, Influence of fertilizer, irrigation, and storage treatments on nitrate-N content of potato tubers, *American Potato Journal*, 54:125-136.
- Austad, J., and Kavli, G., 1983, Phototoxic dermatitis caused by celery infected by *Sclerotinia sclerotiorum*, *Contact Dermatitis*, 9:448-451.

- Axelson, M., Kirk, D. N., Farrant, R. D., Cooley, G., Lawson, A. M., and Setchell, K. D. R., 1982, The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine, *Biochemical Journal*, 201:353-357.
- Bababunmi, E. A., 1978, Toxins and carcinogens in the environment: An observation in the tropics, *Journal of Toxicology and Environmental Health*, 4:691-699.
- Babish, J. G., and Stoewsand, G. S., 1975, Hepatic microsomal enzyme induction in rats fed varietal cauliflower leaves, *Journal of Nutrition*, 105:1592-1599.
- Babish, J. G., and Stoewsand, G. S., 1978, Effect of dietary indole-3-carbinol on the induction of the mixed-function oxidases of rat tissue, *Food and Cosmetics Toxicology*, 16:151-155.
- Bailey, G., Goeger, D., Hendricks, J., Nixon, J., and Pawlowski, N., 1985, Indole-3-carbinol promotion and inhibition of aflatoxin B<sub>1</sub> carcinogenesis in rainbow trout, Abstract No. 456, Proceedings of the 67th Annual Meeting of the American Association of Cancer Research, 26:115.
- Bailey, G. S., Hendricks, J. D., Shelton, D. W., Nixon, J. E., and Pawlowski, N. E., 1987, Enhancement of carcinogenesis by the natural anticarcinogen indole-3-carbinol, *Journal of National Cancer Institute*, 78:931-934.
- Bailey, J. A., and Mansfield, J. W., eds., 1982, *Phytoalexins*, John Wiley and Sons, New York, New York, p. 1-334.
- Baker, D., Keeler, R., and Gaffield, W., 1987, Lesions of potato sprout and extracted potato sprout alkaloid toxicity in syrian hamsters, *Clinical Toxicology*, 25:199-208.
- Balducci, L., Wallace, C., Khansur, T., Vance, R. B., Thigpen, J. T., and Hardy, C., 1986, Nutrition, cancer, and aging: An annotated review. I. Diet, carcinogenesis, and aging, *Journal of the American Geriatrics Society*, 34:127-136.
- Barbush, R. J., and Coutant, J. E., Welsh, M. B., and Setchell, K. D. R., 1989, The use of thermospray liquid chromatography/tandem mass spectrometry for the class identification and structural verification of phytoestrogens in soy protein preparations, *Biomedical and Environmental Mass Spectrometry*, 18:973-977.
- Barrett-Connor, E., and Bush, T. L., 1989, Estrogen replacement and coronary heart disease, *Cardiovascular Clinician*, 19:159-172.
- Barz, W., Bless, W., Börger-Papendorf, G., Gunia, W., Mackenbrock, U., Meier, D., Otto, Ch., and Süper, E., 1990, Phytoalexins as part of induced defence reactions in plants: Their elicitation, function and metabolism. Bioactive compounds from plants, Wiley, Chichester (Ciba Foundation Symposium 154), p. 140-156.
- Barzaghi, N., Perucca, E., Pisseri, G., and Crema, A., 1990, p. 551-556, in: "Flavonoids in Biology and Medicine III. Current Issues in Flavonoid Research," N. P. Das, ed., National University of Singapore.
- Bean, G. A., and Southall, A., 1983, Effect of pyridazinone herbicides on growth and aflatoxin release by *Aspergillus flavus* and *Aspergillus parasiticus*, *Applied and Environmental Microbiology*, 46:503-505.
- Beier, R. C., 1985, A reverse phase technique for separating the linear furanocoumarins in celery, *Journal of Liquid Chromatography*, 8:1923-1932.
- Beier, R. C., and Ivie, G. W., 1985, Linear furanocoumarins in the common herb parsley (*Petroselinum sativum*): Biologically active compounds, Abstract No. 181, 190th American Chemical Society National Meeting, Chicago, Illinois.
- Beier, R. C., Ivie, G. W., and Oertli, E. H., 1983a, Psoralens as phytoalexins in food plants of the family Umbelliferae: Significance in relation to storage and processing, p. 295-310, in: "Xenobiotics in Foods and Feeds," J. W. Finley and D. E. Schwass, eds., American Chemical Society Symposium, Series 234.
- Beier, R. C., Ivie, G. W., Oertli, E. H., and Holt, D. L., 1983b, HPLC analysis of linear furanocoumarins (psoralens) in healthy celery (*Apium graveolens*), *Food and Chemical Toxicology*, 21:163-165.

- Beier, R. C., and Norman, J. O., 1990, The toxic factor in white snakeroot: Identity, analysis, and prevention, p. 81-88, in: "Proceedings of the Symposium on Public Health Significance of Natural Toxicants in Animal Feeds," W. C. Keller, V. R. Beasley, and J. F. Robens, eds., Veterinary and Human Toxicology, 32 (supplement).
- Beier, R. C., Norman, J. O., Irvin, T. R., and Witzel, D. A., 1987, Microsomal activation of constituents of white snakeroot (*Eupatorium rugosum* Houtt) to form toxic products, American Journal of Veterinary Research, 48:583-585.
- Beier, R. C., and Oertli, E. H., 1983, Psoralen and other linear furocoumarins as phytoalexins in celery, Phytochemistry, 22:2595-2597.
- Bell, A. A., 1974, Biochemical bases of resistance of plants to pathogens, p. 403-462, in: "Proceedings of the Summer Institute on Biological Control of Plant Insects and Diseases," F. G. Maxwell and F. A. Harris, eds., The University Press of Mississippi.
- Bell, A. A., 1981, Biochemical mechanisms of disease resistance, Annual Review of Plant Physiology, 32:21-81.
- Bell, A. A., and Wheeler, M. H., 1986, Biosynthesis and functions of fungal melanins, Annual Review of Phytopathology, 24:411-451.
- Belogurov, A. A., and Zavilgelsky, G. B., 1981, Mutagenic effect of furocoumarin monoadducts and cross-links on bacteriophage lambda, Mutation Research, 84:11-15.
- Bennett, J. W., 1982, Genetics of mycotoxin production with emphasis on aflatoxins, p. 549-461, in: "Overproduction of microbial products," V. Krumphanzl, B. Sikyta, and A. Vanek, eds., Academic Press, London, England.
- Bennett, J. W., 1985, Mutants and mycotoxins: Aflatoxins as a model system, p. 271-280, in: "Trichothecenes and other mycotoxins," J. Lacey, ed., John Wiley and Sons, New York, New York.
- Bennetts, H. W., Underwood, E. J., and Shier, F. L., 1946, A specific breeding problem of sheep on subterranean clover pastures in Western Australia, The Australian Veterinary Journal, 22:2-12.
- Berberich, S., 1988, Potato plants make their own insect repellent, Agricultural Research, 36(2):15.
- Bergman, J., 1970, Condensation of indole and formaldehyde in the presence of air and sensitizers: A facile synthesis of indolo[3.2-*b*] carbazole, Tetrahedron, 26:3353-3355.
- Berkley, S. F., Hightower, A. W., Beier, R. C., Fleming, D. W., Brokopp, C. D., Ivie, G. W., and Broome, C. V., 1986, Dermatitis in grocery workers associated with high natural concentrations of furanocoumarins in celery, Annals of Internal Medicine, 105:351-355.
- Bezuidenhout, S. C., Gelderblom, W. C. A., Gorst-Allman, C. P., Horak, R. M., Marasas, W. F. O., Spiteller, G., and Vleggaar, R., 1988, Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*, Journal of the Chemical Society, Chemical Communications, p. 743-745.
- Bilyk, A., and Sapers, G. M., 1985, Distribution of quercetin and kaempferol in lettuce, kale, chive, garlic chive, leek, horseradish, red radish, and red cabbage tissues, Journal of Agricultural and Food Chemistry, 33:226-228.
- Bilyk, A., and Sapers, G. M., 1986, Varietal differences in the quercetin, kaempferol, and myricetin contents of highbush blueberry, cranberry, and thornless blackberry fruits, Journal of Agricultural and Food Chemistry, 34:585-588.
- Birmingham, D. J., Key, M. M., Tubich, G. E., and Perone, V. B., 1961, Phototoxic bullae among celery harvester, Archives of Dermatology, 83:73-85.

- Birt, D. F., Pelling, J. C., Pour, P. M., Tibbels, M. G., Schweickert, L., and Bresnick, E., 1987, Enhanced pancreatic and skin tumorigenesis in cabbage-fed hamsters and mice, *Carcinogenesis*, 8:913-917.
- Bjeldanes, L. F., and Chang, G. W., 1977, Mutagenic activity of quercetin and related compounds, *Science*, 197:577-578.
- Bjeldanes, L. F., Kim, J.-Y., Grose, K. R., Bartholomew, J. C., and Bradfield, C. A., 1991, Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol *in vitro* and *in vivo*: Comparisons with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, *Proceedings of the National Academy of Science*, 88:9543-9547.
- Blackard, C. E., Doe, R. P., Mellinger, G. T., and Byar, D. P., 1970, Incidence of cardiovascular disease and death in patients receiving diethylstilbestrol for carcinoma of the prostate, *Cancer*, 26:249-256.
- Bonner, W. A., and DeGraw, J. I., Jr., 1962, Ketones from "white snakeroot" *Eupatorium urticaefolium*, *Tetrahedron* 18:1295-1309.
- Bonner, W. A., DeGraw, J. I., Jr., Bowen, D. M., and Shah, V. R., 1961, Toxic constituents of white snakeroot, *Tetrahedron Letters*, p. 417-420.
- Booth, C., 1971, "The Genus *Fusarium*," p. 237, Commonwealth Mycological Institute, Kew, Surrey, England.
- Booth, A. N., Jones, F. T., and DeEds, F., 1958, Metabolic fate of hesperidin, eriodictyol, homoeriodictyol, and diosmin, *Journal of Biological Chemistry*, 230:661-668.
- Booth, A. N., Murray, C. W., Jones, F. T., and DeEds, F., 1956, The metabolic fate of rubin and quercetin in the animal body, *Journal of Biological Chemistry*, 223:251-257.
- Bordia, A., Bansal, H. C., Arora, S. K., and Singh, S. V., 1975, Effect of the essential oils of garlic and onion on alimentary hyperlipemia, *Atherosclerosis*, 21:15-19.
- Bourgeois, C. H., Shank, R. C., Grossman, R. A., Johnsen, D. O., Wooding, W. L., and Chandavimol, P., 1971, Acute aflatoxin B<sub>1</sub> toxicity in the macaque and its similarities to Reye's syndrome, *Laboratory Investigation*, 24:206-216.
- Bowen, D. M., DeGraw, J. I., Jr., Shah, V. R., and Bonner, W. A., 1963, The synthesis and pharmacological action of tremetone, *Journal of Medicinal Chemistry*, 6:315-319.
- Boyd, J. N., Babish, J. G., and Stoewsand, G. S., 1982, Modification by beet and cabbage diets of aflatoxin B<sub>1</sub>-induced rat plasma  $\alpha$ -foetoprotein elevation, hepatic tumorigenesis, and mutagenicity of urine, *Food and Chemical Toxicology*, 20:47-52.
- Boyd, M. R., 1976, Role of metabolic activation in the pathogenesis of chemically induced pulmonary disease: Mechanism of action of the lung-toxic furan, 4-ipomeanol, *Environmental Health Perspectives*, 16:127-138.
- Boyd, M. R., 1977, Evidence for the clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in lung, *Nature*, 269:713-715.
- Boyd, M. R., and Burka, L. T., 1978, *In vivo* studies on the relationship between target organ alkylation and the pulmonary toxicity of a chemically reactive metabolite of 4-ipomeanol, *Journal of Pharmacology and Experimental Therapeutics*, 207:687-697.
- Boyd, M. R., Burka, L. T., Harris, T. M., and Wilson, B. J., 1973, Lung-toxic furanoterpenoids produced by sweet potatoes (*Ipomoea batatas*) following microbial infection, *Biochimica et Biophysica Acta*, 337:184-195.
- Boyd, M. R., and Wilson, B. J., 1971, Preparative and analytical gas chromatography of ipomeamarone, a toxic metabolite of sweet potatoes (*Ipomoea batatas*), *Journal of Agricultural and Food Chemistry*, 19:547-550.
- Braden, A. W. H., Hart, N. K., and Lamberton, J. A., 1967, The oestrogenic activity and metabolism of certain isoflavones in sheep, *Australian Journal of Agricultural Research*, 18:335-348.

- Bradfield, C. A., and Bjeldanes, L. F., 1984, Effect of dietary indole-3-carbinol on intestinal and hepatic monooxygenase, glutathione S-transferase and epoxide hydrolase activities in the rat, *Food and Chemical Toxicology*, 22:977-982.
- Bradfield, C. A., and Bjeldanes, L. F., 1987a, High-performance liquid chromatographic analysis of anticarcinogenic indoles in *Brassica oleracea*, *Journal of Agricultural and Food Chemistry*, 35:46-49.
- Bradfield, C. A., and Bjeldanes, L. F., 1987b, Structure-activity relationships of dietary indoles: A proposed mechanism of action as modifiers of xenobiotic metabolism, *Journal of Toxicology and Environmental Health*, 21:311-323.
- Bradfield, C. A., and Bjeldanes, L. F., 1991, Modification of carcinogen metabolism by indolic autolysis products of *Brassica oleracea*, p. 153-163, in: "Nutritional and Toxicological Consequences of Food Processing," M. Friedman, ed., Plenum Press, New York, New York.
- Braun, R., Greeff, U., and Netter, K. J., 1980, Indications for nitrosamide formation from the mushroom poison gyromitrin by rat liver microsomes, *Xenobiotica*, 10:557-564.
- Braun, U., and Kalbhen, D. A., 1973, Evidence for the biogenic formation of amphetamine derivatives from components of nutmeg, *Pharmacology* 9:312-316.
- Brown, J., 1981, Care of the mentally handicapped, *East African Medical Journal*, 58:469-471.
- Brown, J. P., and Dietrich, P. S., 1978, Promutagenic plant phenolics in man's diet: Do mutagenic flavonols and anthraquinones play a role in environmental mutagenesis/carcinogenesis, Abstract Cb-5, 9th Annual Meeting of Environmental Mutagen Society.
- Buchanan, R. L., Hoover, D. G., and Jones, S. B., 1983, Caffeine inhibition of aflatoxin production: Mode of action, *Applied and Environmental Microbiology*, 46:1193-1200.
- Buchanan, R. L., and Lewis, D. F., 1984, Caffeine inhibition of aflatoxin synthesis: Probable site of action, *Applied and Environmental Microbiology*, 47:1216-1220.
- Buck, W. B., and Cysewski, S. J., 1986a, Nervous form of ergotism, p. 369, in: "Current Veterinary Therapy: Food Animal Practice," Volume 2, J. L. Howard, ed., W. B. Saunders Co., Philadelphia, Pennsylvania.
- Buck, W. B., and Cysewski, S. J., 1986b, Tremorgenic toxins, p. 378-379, in: "Current Veterinary Therapy: Food Animal Practice," Volume 2, J. L. Howard, ed., W. B. Saunders Co., Philadelphia, Pennsylvania.
- Buck, W. B., Haliburton, J. C., Thilsted, J. P., Lock, T. F., and Vesonder, R. F., 1979, Equine leukoencephalomalacia: Comparative pathology of naturally occurring and experimental cases, *Proceedings of the Annual Meeting of the American Association of Veterinary Laboratory Diagnostician*, 22:239-258.
- Bullerman, L. B., 1974, Inhibition of aflatoxin production by cinnamon, *Journal of Food Science*, 39:1163-1165.
- Bullerman, L. B., 1979, Significance of mycotoxins to food safety and human health, *Journal of Food Protection*, 42:65-85.
- Burka, L. T., 1978, 1-(3'-Furyl)-6,7-dihydroxy-4,8-dimethylnonan-1-one, a stress metabolite from sweet potatoes, *Ipomoea batatas*, *Phytochemistry*, 17:317-318.
- Burka, L. T., Bowen, R. M., and Wilson, B. J., 1974, 7-Hydroxymyoporone, a new toxic furanosesquiterpene from mold-damaged sweet potatoes, *Journal of Organic Chemistry*, 39:3241-3244.
- Burka, L. T., Felice, L. J., and Jackson, S. W., 1981, 6-Oxodendrolasin, 6-hydroxdendrolasin, 9-oxofarnesol and 9-hydroxyfarnesol, stress metabolites of the sweet potato, *Phytochemistry*, 20:647-652.
- Burka, L. T., and Iles, J., 1979, Myoporone and related keto alcohols from stressed sweet potatoes, *Phytochemistry*, 18:873-874.

- Busby, W. F., and Wogan, G. N., 1981, Psorlens, p. 105-119, in: "Mycotoxins and N-nitroso Compounds: Environmental Risks," Volume II, R. C. Shank, ed., CRC Press, Boca Raton, Florida.
- Bushway, R. J., Bureau, J. L., and McGann, D. F., 1983, Alpha-chaconine and alpha-solanine content of potato peels and potato peel products. *Journal of Food Science*, 48:84-86.
- Bushway, R. J., and Ponnampalam, R., 1981,  $\alpha$ -Chaconine and  $\alpha$ -solanine content of potato products and their stability during several modes of cooking, *Journal of Agricultural and Food Chemistry*, 29:814-817.
- Canada, A. T., Watkins, W. D., and Nguyen, T. D., 1989, The toxicity of flavonoids to guinea pig enterocytes, *Toxicology and Applied Pharmacology*, 99:357-361.
- Canton, J. H., Kroes, R., van Logten, M. J., van Schothorst, M., Stavenuiter, J. F. C., and Verhulsdonk, C. A. H., 1975, The carcinogenicity of aflatoxin M<sub>1</sub> in rainbow trout, *Food and Cosmetics Toxicology*, 13:441-443.
- Carman, A. S., Jr., Kuan, S. S., Ware, G. M., Francis, O. J., Jr., and Kirschenheuter, G. P., 1986, Rapid high-performance liquid chromatographic determination of the potato glycoalkaloids  $\alpha$ -solanine and  $\alpha$ -chaconine, *Journal of Agricultural Food and Chemistry*, 34:279-282.
- Carter, J. N., and Bosma, S. M., 1974, Effect of fertilizer and irrigation on nitrate-nitrogen and total nitrogen in potato tubers, *Agronomy Journal*, 66:263-266.
- Cassier, C., and Moustacchi, E., 1981, Mutagenesis induced by mono- and bi-functional alkylating agents in yeast mutants sensitive to photo-addition of furocoumarins (psos), *Mutation Research*, 84:37-47.
- Caster, W. O., Burton, T. A., Irvin, T. R., and Tanner, M. A., 1986, Dietary aflatoxins, intelligence and school performance in Southern Georgia, *International Journal for Vitamin and Nutrition Research*, 56:291-295.
- Casterline, C. L., 1980, Allergy to Chamomile tea, *Journal of the American Medical Association*, 244:330-331.
- Catalano, E. A., Hasling, V. C., Pons, W. A., Jr., and Schuller, W. H., 1979, Analysis of sweet potato products for lung edema toxins, *HortScience*, 14:124-125.
- Cawood, M. E., Gelderblom, W. C. A., Vleggaar, R., Behrend, Y., Thiel, P. G., and Marasas, W. F. O., 1991, Isolation of the fumonisin mycotoxins: A quantitative approach, *Journal of Agricultural and Food Chemistry*, 39:1958-1962.
- Ceska, O., Chaudhary, S., Warrington, P., Poulton, G., and Ashwood-Smith, M., 1986, Naturally-occurring crystals of photocarcinogenic furocoumarins on the surface of parsnip roots sold as food, *Experientia*, 42:1302-1304.
- Chang, R. S., and Yeung, H. W., 1988, Inhibition of growth of human immunodeficiency virus *in vitro* by crude extracts of Chinese medicinal herbs, *Antiviral Research*, 9:163-176.
- Chaudhary, S. K., Ceska, O., Tétu, C., Warrington, P. J., Ashwood-Smith, M. J., and Poulton, G. A., 1986, Oxypeucedanin, a major furocoumarin in parsley, *Petroselinum crispum*, *Planta Medica*, 52:462-464.
- Chaudhary, S. K., Ceska, O., Warrington, P. J., and Ashwood-Smith, M. J., 1985, Increased furocoumarin content of celery during storage, *Journal of Agricultural and Food Chemistry*, 33:1153-1157.
- Chauhan, Y., Nagel, D., Gross, M., Cerny, R., and Toth, B., 1985, Isolation of  $N^2$ -[ $\gamma$ -L-(+)-glutamyl]-4-carboxyphenylhydrazine in the cultivated mushroom *Agaricus bisporus*, *Journal of Agricultural and Food Chemistry*, 33:817-820.
- Chauhan, Y., Nagel, D., Issenberg, P., and Toth, B., 1984, Identification of *p*-hydrazinobenzoic acid in the commercial mushroom *Agaricus bisporus*, *Journal of Agricultural and Food Chemistry*, 32:1067-1069.
- Chesney, A. M., Clawson, T. A., and Webster, B., 1928, Endemic goiter in rabbits. I. Incidence and characteristics, *Bulletin Johns Hopkins Hospital*, 43:261-277.

- Childers, N. F., 1981, A diet to stop arthritis, the nightshades and ill health, Somerset Press, Inc., Somerville, New Jersey, p. 1-191.
- Cho, M.-J., and Harper, J. E., 1991a, Effect of inoculation and nitrogen on isoflavonoid concentration in wild-type and nodulation-mutant soybean roots, *Plant Physiology*, 95:435-442.
- Cho, M.-J., and Harper, J. E., 1991b, Effect of localized nitrate application on isoflavonoid concentration and nodulation in split-root systems of wild-type and nodulation-mutant soybean plants, *Plant Physiology*, 95:1106-1112.
- Christensen, W. I., 1965, Milk sickness: A review of the literature, *Economic Botany*, 19:293-300.
- Chu, D.-T., Wong, W. L., and Maylight, G. M., 1988, Immunotherapy with Chinese medicinal herbs. I. Immune restoration of local xenogeneic graft-versus-host reaction in cancer patients by fractionated *Astragalus membranaceus* *in vitro*, *Journal of Clinical Laboratory Immunology*, 25:119-123.
- Claringbold, W. D. B., Few, J. D., and Renwick, J. H., 1982, Kinetics and retention of solanidine in man, *Xenobiotica*, 12:293-302.
- Clark, C. A., Lawrence, A., and Martin, F. A., 1981, Accumulation of furanoterpenoids in sweet potato tissue following inoculation with different pathogens, *Phytopathology*, 71:708-711.
- Clark, W. G., and MacKay, E. M., 1950, The absorption and excretion of rutin and related flavonoid substances, *Journal of the American Medical Association*, 143(16):1411-1415.
- Cody, V., Middleton, E., Jr., and Harborne, J. B., 1986, "Plant Flavonoids in Biology and Medicine, Biochemical, Pharmacological, and Structure-Activity Relationships," Proceedings of a Symposium Held in Buffalo, New York, July 22-26, 1985, Alan R. Liss, Inc., New York, New York, 592 p.
- Cody, V., Middleton, E., Jr., Harborne, J. B., and Beretz, A., 1988, "Plant Flavonoids in Biology and Medicine II, Biochemical, Cellular, and Medicinal Properties," Proceedings of a Meeting on Plant Flavonoids in Biology and Medicine Held in Strasbourg, France, August 31-September 3, 1987, Alan R. Liss, Inc., New York, New York, 461 p.
- Committee On Food Protection, National Research Council, 1966, Toxicants occurring naturally in foods, National Academy of Sciences, Washington, D.C., p. 1-301.
- Committee On Food Protection, National Research Council, 1973, Toxicants occurring naturally in foods, National Academy of Sciences, Washington, D.C., p. 1-624.
- Committee Report, 1981, Weeds of the North Central States, p. 208, North Central Regional Research Bulletin No. 772, Publication No. 281, College of Agriculture, University of Illinois at Urbana-Champaign, Urbana-Champaign, Illinois.
- Common, R. H., and Ainsworth, L., 1961, Identification of equol in the urine of the domestic fowl, *Biochimica et Biophysica Acta*, 53:403-404.
- Concon, J. M., 1988a, Food toxicology: Principles and concepts, Part A, Marcel Dekker, Inc., New York, New York, p. 1-675.
- Concon, J. M., 1988b, Food toxicology: Contaminants and additives, Part B, Marcel Dekker, Inc., New York, New York, p. 676-1371.
- Conway, G. A., and Slocumb, J. C., 1979, Plants used as abortifacients and emmenagogues by Spanish New Mexicans, *Journal of Ethnopharmacology*, 1:241-261.
- Coppock, R. W., and Swanson, S. P., 1986, Aflatoxins, p. 363-367, in: "Current Veterinary Therapy: Food Animal Practice," Volume 2, J. L. Howard, ed., W. B. Saunders Co., Philadelphia, Pennsylvania.
- Cordell, G. A., 1981, Introduction to alkaloids: A biogenetic approach, John Wiley and Sons, New York, New York, p. 118-137.

- Correa, P., 1981, Epidemiological correlations between diet and cancer frequency, *Cancer Research*, 41:3685-3690.
- Corrier, D. E., Holt, P. S., and Mollenhauer, H. H., 1987, Regulation of murine macrophage phagocytosis of sheep erythrocytes by T-2 toxin, *American Journal of Veterinary Research*, 48:1304-1307.
- Corrier, D. E., and Ziprin, R. L., 1986a, Enhanced resistance to listeriosis induced in mice by preinoculation treatment with T-2 mycotoxin, *American Journal of Veterinary Research*, 47:856-859.
- Corrier, D. E., and Ziprin, R. L., 1986b, Immunotoxic effects of T-2 toxin on cell-mediated immunity to listeriosis in mice: Comparison with cyclophosphamide, *American Journal of Veterinary Research*, 47:1956-1960.
- Couch, J. F., 1927, The toxic constituent of richweed or white snakeroot (*Eupatorium urticaefolium*), *Journal of Agricultural Research*, 35:547-576.
- Cox, R. I., 1984, Immunophysiological control of phyto-oestrogen toxicity, p. 98-108, in: "Plant Toxicology," Proceedings of the Australia-U.S.A. Poisonous Plants Symposium, Brisbane, Australia, May 14-18, 1984.
- Coxon, D. T., Curtis, R. F., and Howard, B., 1975, Ipomeamarone, a toxic furanoterpenoid in sweet potatoes (*Ipomoea batatas*) in the United Kingdom, *Food and Cosmetics Toxicology*, 13:87-90.
- Crawford, L., McDonald, G. M., and Friedman, M., 1990, Composition of sicklepod (*Cassia obtusifolia*) toxic weed seeds, *Journal of Agricultural and Food Chemistry*, 38:2169-2175.
- Crosby, D. G., and Aharonson, N., 1967, The structure of carotatoxin, a natural toxicant from carrot, *Tetrahedron Letters*, 23:465-472.
- Culvenor, C. C. J., Edgar, J. A., Smith, L. W., Kumana, C. R., and Lin, H. J., 1986, *Heliotropium lasiocarpum* Fisch. and Mey. identified as cause of veno-occlusive disease due to a herbal tea, *Lancet*, 1:978.
- Cyong, J.-C., Matsumoto, T., Arakawa, K., Kiyohara, H., Yamada, H., and Otsuka, Y., 1987, Anti-*Bacteroides fragilis* substance from rhubarb, *Journal of Ethnopharmacology*, 19:279-283.
- Daigle, D. J., Conkerton, E. J., Sanders, T. H., and Mixon, A. C., 1988, Peanut hull flavonoids: Their relationship with peanut maturity, *Journal of Agricultural and Food Chemistry*, 36:1179-1181.
- Das, N. P., ed., 1990, "Flavonoids in Biology and Medicine III, Current Issues in Flavonoids Research," Proceedings of 3rd International Symposium on Flavonoids in Biology & Medicine Held in Singapore, November 13-17, 1989, National University of Singapore, 602 p.
- Das, N. P., and Ratty, A. K., 1986, Effects of flavonoids on induced non-enzymic lipid peroxidation, p. 243-247, in: "Plant Flavonoids in Biology and Medicine: Biochemical Pharmacological and Structure-Activity Relationships," V. Cody, E. Middleton, and J. B. Harborne, eds., Alan R. Liss, Inc., New York, New York.
- da Silva, J. M. R., Darmon, N., Fernandez, Y., and Mitjavila, S., 1991, Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds, *Journal of Agricultural and Food Chemistry*, 39:1549-1552.
- Davidson, J. N., Babish, J. G., DeLaney, K. A., Taylor, D. R., and Phillips, T. D., 1987, Hydrated sodium calcium aluminosilicate decreases the bioavailability of aflatoxin in the chicken, *Poultry Science*, 66(Supplement 1):89.
- Dawson, K. P., and Mitchell, E. A., 1990, Asthma in New Zealand children, *Journal of Asthma*, 27:291-297.
- Deichmann, W. B., Herschler, D., Holmstedt, B., and Keil, G., 1986, What is there that is not poison: A study of the third defense by Paracelsus, *Archives of Toxicology*, 58:207-213.

- DeLoach, J. R., and Kachatourians, G. G., 1988, Interaction of T-2 toxin with cell membranes, No. 12, in: "Proceedings of the Symposium on Cellular and Molecular Mode of Action of Selected Microbial Toxins in Foods and Feeds, The National 4-H Center, Chevy Chase, Maryland.
- DeLoach, J. R., Gyongyossy-Issa, M. I. C., and Khachatourians, G. G., 1989, Species-specific hemolysis of erythrocytes by T-2 toxin, Toxicology and Applied Pharmacology, 97:107-112.
- Denning, D. W., 1987, Aflatoxin and human disease, Adverse Drug Reactions and Acute Poisoning Reviews, 4:175-209.
- Dercks, W., Trumble, J., and Winter, C., 1990, Impact of atmospheric pollution on linear furanocoumarin content in celery, Journal of Chemical Ecology, 16:443-454.
- de Riera, M. V. Q., Noriega, M. T., Gonzalez, J. A., and Seeligmann, P., 1988, Influencia de la luz solar sobre la sintesis de flavonoides en la germinacion de *Arachis hypogaea*, Lilloa, XXXVII(1):33-35.
- Dick, A. J., Redden, P. R., DeMarco, A. C., Lidster, P. D., and Grindley, T. B., 1987, Flavonoid glycosides of spartan apple peel, Journal of Agricultural and Food Chemistry, 35:529-531.
- Dickinson, J. M., Smith, G. R., Randel, R. D., and Pemberton, I. J., 1988, *In vitro* metabolism of formononetin and biochanin A in bovine rumen fluid, Journal of Animal Science, 66:1969-1973.
- Diener, J. L., and Davis, N. D., 1969, Aflatoxin formation by *Aspergillus flavus*, p. 13-54, in: "Aflatoxin. Scientific Background, Control and Implications," L. A. Goldblatt, ed., Academic Press, New York, New York.
- Doll, R., and Peto, R., 1981, The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today, Journal of the National Cancer Institute, 66:1191-1308.
- Dollahite, J. W., and Anthony, W. V., 1957, Poisoning of cattle with *Gutierrezia microcephala*, a perennial broomweed, Journal of the American Veterinary Medical Association, 130:525-530.
- Doster, A. R., Farrell, R. L., and Wilson, B. J., 1983, An ultrastructural study of bronchiolar lesions in rats induced by 4-ipomeanol, a product from mold-damaged sweet potatoes, American Journal of Pathology, 111:56-61.
- Downum, K. R., Swain, L. A., and Faleiro, L. J., 1991, Influence of light on plant allelochemicals: A synergistic defense in higher plants, Archives of Insect Biochemistry and Physiology, 17:201-211.
- Draughon, F. A., and Ayres, J. C., 1981, Inhibition of aflatoxin production by selected insecticides, Applied and Environmental Microbiology, 41:972-976.
- Dubick, M. A., 1986, Historical perspectives on the use of herbal preparations to promote health, Journal of Nutrition, 116:1348-1354.
- Duke, J. A., 1985, CRC handbook of medicinal herbs, CRC Press, Boca Raton, Florida, p. 1-677.
- Duke, J. A., 1987a, Father nature's pharmacy: Yarrow (*Achillea millefolium*), HerbalGram 12:6-7.
- Duke, J. A., 1987b, Father nature's pharmacy: The mint family, HerbalGram, 14:6.
- Duke, J. A., 1988a, These compounds have been compiled from a large number of references by J. A. Duke and are unpublished in the present form.
- Duke, J. A., 1988b, Bishop's weed (*Ammi majus* L., Apiaceae), Notes on economic plants, Economic Botany, 42:442-445.
- Duke, J. A., 1988c, Father nature's pharmacy: The carrot family, HerbalGram, 15:11.
- Durham, S. K., Babish, J. G., and Castleman, W. L., 1987, 4-Ipomeanol-induced effects on sendai viral pneumonia in mice, American Journal of Pathology, 126:364-375.

- Durham, S. K., Boyd, M. R., and Castleman, W. L., 1985, Pulmonary endothelial and bronchiolar epithelial lesions induced by 4-ipomeanol in mice, American Journal of Pathology, 118:66-75.
- Eisner, G., 1931, Ueber die lebensrettende Wirkung von Pflanzenteilen und daraus isolierten Säften bei der tödlich verlaufenden, subakuten Uranvergiftung, Biochemische Zeitschrift, 232:218-228.
- Elwood, J. H., and MacKenzie, G., 1973, Associations between the incidence of neurological malformations and potato blight outbreaks over 50 years in Ireland, Nature, 243:476-477.
- Emanuel, I., and Sever, L. E., 1973, Questions concerning the possible association of potatoes and neural-tube defects, and an alternative hypothesis relating to maternal growth and development, Teratology, 8:325-332.
- Engelhardt, J. A., Carlton, W. W., and Tuite, J. F., 1989, Toxicity of *Fusarium moniliforme* var *subglutinans* for chicks, ducklings and turkey poult, Avian Diseases, 33:357-360.
- Ernster, V. L., Bush, T. L., Huggins, G. R., Hulka, B. S., Kelsey, J. L., and Schottenfeld, D., 1988, Benefits and risks of menopausal estrogen and/or progestin hormone use, Preventive Medicine, 17:201-223.
- Farmakalidis, E., and Murphy, P. A., 1984, Oestrogenic response of the CD-1 mouse to the soya-bean isoflavones genistein, genistin and daidzin, Food and Chemical Toxicology, 22(3):237-239.
- Fenwick, G. R., Heaney, R. K., and Mullin, W. J., 1983, Glucosinolates and their breakdown products in food and food plants, Critical Reviews in Food Science and Nutrition, 18:123-201.
- Field, B., and Kerr, C., 1973, Potato blight and neural-tube defects, Lancet, 2:507-508.
- Fisher, G. S., Frank, A. W., and Cherry, J. P., 1988, Total gossypol content of glandless cottonseed, Journal of Agricultural and Food Chemistry, 36:42-44.
- Fisher, J. F., and Trama, L. A., 1979, High-performance liquid chromatographic determination of some coumarins and psoralens found in citrus peel oils, Journal of Agricultural and Food Chemistry, 27:1334-1337.
- Floss, H. G., Guenther, H., and Hadwiger, L. A., 1969, Biosynthesis of furanocoumarins in diseased celery, Phytochemistry, 8:585-588.
- Fleury, Y., and Maguolato, D., 1990, Changes in isoflavone content during maturation of soybean (*Glycine max* Mer.), p. 19-26, in: "Flavonoids in Biology and Medicine III. Current Issues in Flavonoid Research," P. Das, ed., National University of Singapore.
- Food and Drug Administration Pesticide Program, 1988, Residues in Foods - 1987, Journal of the Association of the Official Analytical Chemists, 71:156A-174A.
- Forlot, P., 1990, 5-Methoxysoralen and other furocoumarins as jet lag suppressants, European Patent Application 352198, 5 p.
- Forsyth, A. A., 1954, British Poisonous Plants, Bulletin No. 161, Her Majesty's Stationery Office, London, England, p. 1-116.
- Förster, H., 1975, Absorption and metabolism of flavonoids, p. 257-268, in: "Topics in Flavonoid Chemistry and Biochemistry," L. Farkas, M. Gábor, and E. Kallay, eds., Proceedings of the 4th Hungarian Bioflavonoid Symposium, Keszthely, 1973, Elsevier, New York, New York.
- Francis, A. R., Shetty, T. K., and Bhattacharya, R. K., 1989, Modifying role of dietary factors on the mutagenicity of aflatoxin B<sub>1</sub>: *In vitro* effect of plant flavonoids, Mutation Research, 222:393-401.
- Franke, S., Freimuth, U., and List, P. H., 1967, Ueber die Giftigkeit der Frühjahrslorchel, *Gyromitra* (*Helvella*) *esculenta* Fr., Archives of Toxicology, 22:293-332.

- Franzblau, S. G., and Cross, C., 1986, Comparative *in vitro* antimicrobial activity of Chinese medicinal herbs, *Journal of Ethnopharmacology*, 15:279-288.
- Friedman, M., and Henika, P. R., 1991, Mutagenicity of toxic weed seeds in the Ames test: Jimson weed (*Datura stramonium*), velvetleaf (*Abutilon theophrasti*), morning glory (*Ipomoea* spp.), and sicklepod (*Cassia obtusifolia*), *Journal of Agricultural and Food Chemistry*, 39:494-501.
- Friedman, M., and Smith, G. A., 1984, Factors which facilitate inactivation of quercetin mutagenicity, Volume 77, p. 527-544, in: "Advances in Experimental Medicine and Biology: Nutritional and Toxicological Aspects of Food Safety," M. Friedman, ed., Plenum Press, New York, New York.
- Fritz, J. C., Mislove, P. B., Pla, G. W., Harrison, B. N., Weeks, C. E., and Dantzman, J. G., 1973, Toxicogenicity of moldy feed for young chickens, *Poultry Science*, 52:1523-1530.
- Fu-Sun, Y., and Kong-Nien, S., 1986, Epidemiology and early diagnosis of primary liver cancer in China, *Advances in Cancer Research*, 47:297-329.
- Futami, H., Eader, L. A., Komschlies, K. L., Bull, R., Gruys, M. E., Ortaldo, J. R., Young, H. A., and Wiltrot, R. H., 1991, Flavone acetic acid directly induces expression of cytokine genes in mouse splenic leukocytes but not in human peripheral blood leukocytes, *Cancer Research*, 51:6596-6602.
- Garreau, B., Vallette, G., Adlercreutz, H., Wähälä, K., Mäkelä, T., Benassayag, C., and Nunez, E. A., 1991, Phytoestrogens: New ligands for rat and human  $\alpha$ -fetoprotein, *Biochimica et Biophysica Acta*, 1094:339-345.
- Geibel, M., Geiger, H., and Treutter, D., 1990, Tectochrysin 5- and genistein 5-glucosides from the bark of *Prunus cerasus*, *Phytochemistry*, 29(4):1351-1353.
- Gelderblom, W. C. A., Jaskiewicz, K., Marasas, W. F. O., Thiel, P. G., Horak, R. M., Vleggaar, R., and Kriek, N. P. J., 1988, Fumonisins—Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*, *Applied and Environmental Microbiology*, 54:1806-1811.
- Gelderblom, W. C. A., Marasas, W. F. O., Steyn, P. S., Thiel, P. G., van der Merwe, K. J. (in part), van Rooyen, P. H., Vleggaar, R., and Wessels, P. L., 1984, Structure elucidation of fusarin C, a mutagen produced by *Fusarium moniliforme*, *Journal of the Chemical Society, Chemical Communications*, p. 122-124.
- Gelderblom, W. C. A., Marasas, W. F. O., Vleggaar, R., Thiel, P. G., and Cawood, M. E., 1992, Isolation, chemical characterization and toxicological effects of fumonisins, *Mycopathologia* (in press).
- Gelderblom, W. C. A., Thiel, P. G., Jaskiewicz, K., and Marasas, W. F. O., 1986, Investigations on the carcinogenicity of fusarin C - a mutagenic metabolite of *Fusarium moniliforme*, *Carcinogenesis*, 7:1899-1901.
- Gelderblom, W. C. A., Thiel, P. G., van der Merwe, K. J., Marasas, W. F. O., and Spies, H. S. C., 1983, A mutagen produced by *Fusarium moniliforme*, *Toxicon*, 21:467-473.
- Géro, E., 1946, Étude de l'élimination urinaire de l'épicatéchine, *Archives Internationales de Physiologie*, LIV (Fasc.2):201-204.
- Gigliotti, H., and Levenberg, B., 1964, Studies on the  $\gamma$ -glutamyltransferase of *Agaricus bisporus*. *Journal of Biological Chemistry*, 239:2274-2284.
- Gillner, M., Bergman, J., Cambillau, C., Fernström, B., and Gustafsson, J., 1985, Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver, *Molecular Pharmacology*, 28:357-363.
- Gmelin, R., and Virtanen, A. I., 1961, Glucobrassicin, the precursor of 3-indolylacetonitrile, ascorbigen, and SCN<sup>-</sup> in *Brassica oleracea* species, *Suomen Kemistilehti B*, 34:15-18.
- Gmelin, R., and Virtanen, A. I., 1962, Neoglucobrassicin, ein zweiter SCN<sup>-</sup>-Precursor vom Indoltyp *Brassica*-Arten, *Acta Chemica Scandinavica*, 16:1378-1384.

- Gold, J., and Cates, W., Jr., 1980, Herbal abortifacients, Journal of the American Medical Association, 243:1365-1366.
- Goldblatt, L. A., 1972, Implications of mycotoxins, Clinical Toxicology, 5:453-464.
- Goldin, B. R., and Gorbach, S. L., 1988, Effect of diet on the plasma levels, metabolism, and excretion of estrogens, American Journal of Clinical Nutrition, 48:787-790.
- Goldin, B. R., Adlercreutz, H., Gorbach, S. L., Woods, M. N., Dwyer, J. T., Conlon, T., Bohn, E., and Gershoff, S. N., 1986, The relationship between estrogen levels and diets of Caucasian American and Oriental immigrant women, American Journal of Clinical Nutrition, 44:945-953.
- Goupy, P. M., Varoquaux, P. J. A., Nicolas, J. J., and Macheix, J. J., 1990, Identification and localization of hydroxycinnamoyl and flavonol derivatives from endive (*Cichorium endivia* L. cv. geante maraichere) leaves, Journal of Agricultural and Food Chemistry, 38:2116-2121.
- Graham, S., 1983, Results of case-control studies of diet and cancer in Buffalo, New York, Cancer Research, 43:2409s-2413s.
- Graham, S., Dayal, H., Swanson, M., Mittelman, A., and Wilkinson, G., 1978, Diet in the epidemiology of cancer of the colon and rectum, Journal of the National Cancer Institute, 61:709-714.
- Graham, S., Schotz, W., and Martino, P., 1972, Alimentary factors in the epidemiology of gastric cancer, Cancer, 30:927-938.
- Graham, T. L., 1991, Flavonoid and isoflavonoid distribution in developing soybean seedling tissues and in seed and root exudates, Plant Physiology, 95:594-603.
- Gray, A. I., and Waterman, P. G., 1978, Coumarins in the Rutaceae, Phytochemistry, 17:845-864.
- Greenhalgh, R., Blackwell, B. A., Pare, J. R. J., Miller, J. D., Levandier, D., Meier, R.-M., Taylor, A., and Apsimon, J. W., 1985, Isolation and characterization by mass spectrometry and NMR spectroscopy of secondary metabolites of some *Fusarium* species, p. 22-25, in: "Mycotoxins and Phycotoxins," P. S. Steyn and R. Vleggaar, eds., Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa.
- Grekin, D. A., and Epstein, J. H., 1981, Psoralens, UVA (PUVA) and photocarcinogenesis, Photochemistry Photobiology, 33:957-960.
- Griebel, C., 1924, Solanine content of potatoes of the 1922 crop, Zeitschrift fuer Untersuchung der Nahrungs- und Genussmittel sowie der Gebrauchsgegenstaende, 47:436-438.
- Griffiths, L. A., 1964, Studies on flavonoid metabolism: Identification of the metabolites of (+)-catechin in rat urine, Biochemical Journal, 92:173-179.
- Griffiths, L. A., 1975, The role of intestinal microflora in flavonoid metabolism, p. 201-213, in: "Topics in Flavonoid Chemistry and Biochemistry," L. Farkas, M. Gabor, and E. Kallay, eds., Proceedings of the 4th Hungarian Bioflavonoid Symposium, Keszthely, 1973, Elsevier, New York, New York.
- Grisebach, H., and Ebel, J., 1978, Phytoalexins, chemical defense substances of higher plants?, Angewandte Chemie International Edition in English, 17:635-647.
- Gross, T. P., Ratner, L., DeRodriguez, O., Farrell, K. P., and Israel, E., 1987, An outbreak of phototoxic dermatitis due to limes, American Journal of Epidemiology, 125:509-514.
- Gugler, R., Leschnik, M., and Dengler, H. J., 1975, Disposition of quercetin in man after a single intravenous dose. European Journal of Clinical Pharmacology 9:229-233.
- Gunderson, E. L., 1988, FDA total diet study, April 1982 - April 1984, dietary intakes of pesticides, selected elements, and other chemicals, Journal of the Association of Official Analytical Chemists, 71:1200-1209.

- Hackett, A. M., 1986, The metabolism of flavonoid compounds in mammals, p. 177-194, in: "Plant Flavonoids in Biology and Medicine, Biochemical, Pharmacological, and Structure—Activity Relationships," V. Cody, E. Middleton, Jr., and J. B. Harborne, eds., Proceedings of a Symposium Held in Buffalo, New York, July 22-26, 1985, Alan R. Liss, Inc., New York, New York.
- Hagerty, M. A., Howie, B. J., Tan, S., and Shultz, T. D., 1988, Effect of low- and high-fat intakes on the hormonal milieu of premenopausal women, *American Journal of Clinical Nutrition*, 47:653-659.
- Haliburton, J. C., and Buck, W. B., 1986, Equine leukoencephalomalacia, *Current Topics in Veterinary Medicine and Animal Science*, 33:75-79.
- Hamerski, D., Beier, R. C., Kneusel, R. E., Matern, U., and Himmelsbach, K., 1990, Accumulation of coumarins in elicitor-treated cell suspension cultures of *Ammi majus*, *Phytochemistry*, 29:1137-1142.
- Hamilton, P. B., 1982, Mycotoxins and farm animals, *Refuah Veterinarith*, 39:17-45.
- Hann, S. K., Cho, M. Y., and Park, Y.-K., 1990, UV treatment of generalized prurigo nodularis, *International Journal of Dermatology*, 19:436-437.
- Hannuksela, M., Stenback, F., and Lahti, A., 1986, The carcinogenic properties of topical PUVA, *Archives of Dermatological Research*, 278:347-351.
- Hansen, A. A., 1925, Two fatal cases of potato poisoning, *Science*, 61:340-341.
- Hanson, C. H., ed., 1974, The effect of FDA regulations (GRAS) on plant breeding and processing, *CSSA Special Publication Number 5*, Crop Science Society of America, Madison, Wisconsin, p. 1-63.
- Harborne, J. B., 1986a, Nature, distribution and function of plant flavonoids in plant flavonoids, p. 15-24, in: "Plant Flavonoids in Biology and Medicine, Biochemical, Pharmacological, and Structure-Activity Relationships," V. Cody, E. Middleton, Jr., and J. B. Harborne, eds., Proceedings of a Symposium Held in Buffalo, New York, July 22-26, 1985, Alan R. Liss, Inc., New York, New York.
- Harborne, J. B., 1986b, The role of phytoalexins in natural plant resistance, p. 22-35, in: "Natural Resistance of Plants to Pests. Roles of allelochemicals," M. B. Green and P. A. Hedin, eds., *American Chemical Society Symposium Series 296*.
- Harborne, J. B. ed., 1988, "The Flavonoids, Advances in Research Since 1980," Chapman and Hall, New York, New York, 621 p.
- Harborne, J. B., Mabry, T. J., and Mabry, H., eds., 1975a, "The Flavonoids, Part 1," Academic Press, New York, New York, 631 p.
- Harborne, J. B., Mabry, T. J., and Mabry, H., eds., 1975b, "The Flavonoids, Part 2," Academic Press, New York, New York, 1204 p.
- Hardigree, A. A., and Epler, J. L., 1978, Comparative mutagenesis of plant flavonoids in microbial systems, *Mutation Research*, 58:231-239.
- Hardin, J. W., 1973, Stock-poisoning plants of North Carolina, p. 121, North Carolina Agricultural Experiment Station, Technical Bulletin No. 414 (Revised).
- Harris, C. C., Tsung-Tang, S., 1986, Interactive effects of chemical carcinogens and hepatocellular carcinoma, *Cancer Surveys (Cold Spring Harbor)*, 5:765-780.
- Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E., and Cole, J. R., 1990, Pulmonary edema and hydrothorax in swine produced by fumonisins B<sub>1</sub>, a toxic metabolite of *Fusarium moniliforme*, *Journal of Veterinary Diagnostic Investigation*, 2:210-212.
- Harvey, R. B., Kubena, L. F., Phillips, T. D., and Huff, W. E., 1988a, Possible methods to combat the mycotoxin problem, *Proceedings of the Plains Nutrition Council Symposium Mycotoxins Livestock Feeds*, Texas A&M Research Center, Amarillo, Texas, p. 1E-4E.
- Harvey, R. B., Kubena, L. F., Phillips, T. D., Huff, W. E., and Corrier, D. E., 1988b, Approaches to the prevention of aflatoxicosis, *Proceedings of the Maryland Nutritional Conference*, Baltimore, Maryland, p. 102-107.

- Harvey, R. B., Phillips, T. D., Kubena, L. F., and Huff, W. E., 1988c, Dietary hydrated sodium calcium aluminosilicate and its impact on aflatoxin toxicity in pigs and milk residues in dairy cows, Proceedings of the 31st Annual Meeting of the American Association of Veterinary Laboratory Diagnosis, Little Rock, Arkansas, p. 67.
- Harvill, A. M., Jr., Bradley, T. R., Stevens, C. E., Wieboldt, T. F., Ware, D. M. E., and Ogle, D. W., 1986, Atlas of the Virginia Flora, p. 48, Second edition, Virginia Botanical Associates, Farmville, Virginia.
- Hayashi, M., Kishi, M., Sofuni, T., and Ishidate, M., Jr., 1988, Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals, *Food and Chemical Toxicology*, 26(6):487-500.
- Hayes, A. W., 1980, Mycotoxins: A review of biological effects and their role in human diseases, *Clinical Toxicology*, 17:45-83.
- Heaney, R. K., and Fenwick, G. R., 1980, Glucosinolates in *Brassica* vegetables: Analysis of 22 varieties of Brussels sprout (*Brassica oleracea* L. var. *gemmifera*), *Journal of the Science of Food and Agriculture*, 31:785-793.
- Hearst, J. E., 1989, Photochemistry of the psoralens, *Chemical Research in Toxicology*, 2:69-75.
- Henderson, B. E., Benton, B., Jing, J., Yu, M. C., and Pike, M. C., 1979, Risk factors for cancer in the testis in young men, *International Journal of Cancer*, 23:598-602.
- Henseler, T., Christophers, E., Höningmann, H., Wolff, K., and nineteen other investigators, 1987, Skin tumors in the European PUVA study, *Journal of the American Academy of Dermatology*, 16:108-116.
- Herrmann, K., 1976, Flavonols and flavones in food plants: A review, *Journal of Food Technology*, 11:433-448.
- Hesseltine, C. W., 1976, Conditions leading to mycotoxin contamination of foods and feeds, p. 1-22, in: "Mycotoxins and Other Fungal Related Food Problems," Volume 149, J. V. Rodericks, ed., Advances in Chemistry Services, American Chemical Society, Washington, D.C.
- Hesseltine, C. W., 1979, Introduction, definition, and history of mycotoxins of importance to animal production, p. 3-18, in: "Interactions of Mycotoxins in Animal Production," National Academy of Science (U.S.A.), Washington, D.C.
- Hesseltine, C. W., 1985, Global significance of mycotoxins, p. 1-18, in: "Mycotoxins and Phycotoxins," P. S. Steyn and R. Vleggaar, eds., Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa.
- Hirayama, T., 1977, Changing patterns of cancer in Japan with special reference to the decrease in stomach cancer mortality, p. 55-75, in: "Origins of Human Cancer. Book A. Incidence of Cancer in Humans," Volume 4, H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds., Cold Spring Harbor Laboratory Press, New York, New York.
- Hogan, R. P., III, 1983, Hemorrhagic diathesis caused by drinking an herbal tea, *Journal of the American Medical Association*, 249:2679-2680.
- Holt, P. S., Corrier, D. E., and DeLoach, J. R., 1988, Suppressive and enhancing effect of T-2 toxin on murine lymphocyte activation and interleukin 2 production, *Immunopharmacology Immunotoxicology*, 10:365-385.
- Holt, P. S., and DeLoach, J. R., 1988a, T-2 mycotoxin alters splenocyte activation and interleukin-2 production, *Proceedings of the 26th Symposium on Cellular and Molecular Mode of Action of Selected Microbial Toxins in Foods and Feeds*, Chevy Chase, Maryland.
- Holt, P. S., and DeLoach, J. R., 1988b, *In vitro* effect of T-2 mycotoxin on the immune response of mice, *American Journal of Veterinary Research*, 49:1480-1484.
- Holzapfel, C. W., Steyn, P. S., and Purchase, I. F. H., 1966, Isolation and structure of aflatoxins M<sub>1</sub> and M<sub>2</sub>, *Tetrahedron Letters*, 25:2799-2803.

- Howie, B. J., and Shultz, T. D., 1985, Dietary and hormonal interrelationships among vegetarian Seventh-Day Adventists and nonvegetarian men, *The American Journal of Clinical Nutrition*, 42:127-134.
- Huang, M.-T., Wood, A. W., Newmark, H. L., Sayer J. M., Yagi, H., Jerina, D. M., and Conney, A. H., 1983, Inhibition of the mutagenicity of bay-region diol-epoxides of polycyclic aromatic hydrocarbons by phenolic plant flavonoids, *Carcinogenesis*, 4(12):1631-1637.
- Hudson, J. B., Miki, N., and Towers, G. H. N., 1987, Isopimpinellin is not phototoxic to viruses and cells, *Planta Medica*, 53:306-307.
- Huet, R., 1982, Constituants des agrumes à effet pharmacodynamique: les citroflavonoïdes, *Fruits*, 37:267-271.
- Huisgen, R., and Reimlinger, H., 1956, Nitroso-acyl-amine und diazo-ester. X. Die Isomerisierung der Nitroso-acyl-alkylamine zu Diazo-estern und ihre Kinetik, *Justus Liebigs Annalen der Chemie*, 599:161-182.
- Hungria, M., Joseph, C. M., and Phillips, D. A., 1991a, Anthocyanidins and flavonols, major *nod* gene inducers from seeds of a black-seeded common bean (*Phaseolus vulgaris* L.), *Plant Physiology*, 97:751-758.
- Hungria, M., Joseph, C. M., and Phillips, D. A., 1991b, *Rhizobium nod* gene inducers exuded naturally from roots of common bean (*Phaseolus vulgaris* L.), *Plant Physiology*, 97:759-764.
- Inoue, H., and Uritani, I., 1979, Biosynthetic correlation of various phytoalexins in sweet potato root tissue infected by *Ceratostysis fimbriata*, *Plant and Cell Physiology*, 20:1307-1314.
- Irving, G. W., Fontaine, T. D., and Doolittle, S. P., 1946, Partial antibiotic spectrum of tomatin, an antibiotic agent from the tomato plant, *Journal of Bacteriology*, 52:601-607.
- Ivie, G. W., 1978, Linear furanocoumarins (psoralens) from the seed of Texas *Ammi majus* L. (Bishop's weed), *Journal of Agricultural and Food Chemistry*, 26:1394-1403.
- Ivie, G. W., 1987, The chemistry of plant furanocoumarins and their medical, toxicological, environmental, and coevolutionary significance, *Revista Latinoamericana de Química*, 18:1-6.
- Ivie, G. W., Holt, D. L., and Ivey, M. C., 1981, Natural toxicants in human foods: Psoralens in raw and cooked parsnip root, *Science*, 213:909-910.
- Ivie, G. W., MacGregor, J. T., and Hammock, B. D., 1980, Mutagenicity of psoralen epoxides, *Mutation Research*, 79:73-77.
- Jadhav, S. J., Sharma, R. P., and Salunkhe, D. K., 1981, Naturally occurring toxic alkaloids in foods, *Critical Review in Toxicology*, 9:21-104.
- Jaskiewicz, K., Marasas, W. F. O., and Taljaard, J. J. F., 1987a, Hepatitis in vervet monkeys caused by *Fusarium moniliforme*, *Journal of Comparative Pathology*, 97:281-291.
- Jaskiewicz, K., van Rensburg, S. J., Marasas, W. F. O., and Gelderblom, W. C. A., 1987b, Carcinogenicity of *Fusarium moniliforme* culture material in rats, *Journal of the National Cancer Institute*, 78:321-325.
- Jelinek, R., Kyzlink, V., and Blattny, C., Jr., 1976, An evaluation of the embryo-toxic effects of blighted potatoes on chicken embryos, *Teratology*, 14:335-342.
- Jeschke, N., Nelson, P. E., and Marasas, W. F. O., 1987, Toxicity to ducklings of *Fusarium moniliforme* isolated from corn intended for use in poultry feed, *Poultry Science*, 66:1619-1623.
- Johns, A. N., 1980, Beware the bay leaf, *British Medical Journal*, 281:1682.
- Johnson, C., Brannon, D. R., and Kuć, J., 1973, Xanthotoxin: A phytoalexin of *Pastinaca sativa* root, *Phytochemistry*, 12:2961-2962.

- Jones, E., and Hughes, R. E., 1982, Quercetin, flavonoids and the life-span of mice, *Experimental Gerontology*, 17:213-217.
- Jones, S. B., Jr., and Coile, N. C., 1988, Distribution of the vascular flora in Georgia, p. 97, Department of Botany, University of Georgia, Athens, Georgia.
- Kaplan, D. T., Keen, N. T., and Thomason, I. J., 1980, Association of glyceollin with the incompatible response of soybean roots to *Meloidogyne incognita*, *Physiological Plant Pathology*, 16:309-318.
- Karasawa, D., Shibata, H., Horiuchi, N., Andou, Y., and Simada, M., 1990, Photoactive furocoumarins in diseased celery (*Apium graveolence*), *Agricultural and Biological Chemistry*, 54:2141-2142.
- Keeler, R. F., 1973, Comparison of the teratogenicity in rats of certain potato-type alkaloids and the veratrum teratogen cyclopamine, *Lancet*, 1:1187-1188.
- Keeler, R. F., 1986, Teratology of steroid alkaloids, p. 389-425, in: "Alkaloids: Chemical and Biological Perspectives," S. W. Pelletier, ed., John Wiley and Sons, Inc., New York, New York.
- Keeler, R. F., Brown, D., Douglas, D. R., Stallknecht, G. F., and Young, S., 1976a, Teratogenicity of the solanum alkaloid solasodine and of "Kennebec" potato sprouts in hamsters, *Bulletin of Environmental Contamination and Toxicology*, 15:522-524.
- Keeler, R. F., Young, S., and Brown, D., 1976b, Spina bifida, exencephaly, and cranial bleb produced in hamsters by the solanum alkaloid solasodine, *Research Communication in Chemical Pathology and Pharmacology*, 13:723-730.
- Keeler, R. F., Young, S., Brown, D., Stallknecht, G. F., and Douglas, D., 1978, Congenital deformities produced in hamsters by potato sprouts, *Teratology*, 17:327-334.
- Keji, C., 1981, Certain progress in the treatment of coronary heart disease with traditional medicinal plants in China, *American Journal of Chinese Medicine*, 9:193-196.
- Kellerman, T. S., Marasas, W. F. O., Pienaar, J. G., and Naude, T. W., 1972, Mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. A preliminary communication, *Onderstepoort Journal of Veterinary Research*, 39:205-208.
- Kelly, R. B., Daniels, E. G., and Hinman, J. W., 1962, Agaritine: Isolation, degradation, and synthesis, *Journal of Organic Chemistry*, 27:3229-3231.
- Kew, M. C., 1986, The development of hepatocellular cancer in humans, *Cancer Surveys (Cold Spring Harbor)*, 5:719-739.
- Kingsbury, J. M., 1964, Poisonous plants of the United States and Canada. Prentice-Hall, Englewood Cliffs, New Jersey, p. 287.
- Kinlen, L., and Hewitt, A., 1973, Potato blight and anencephalus in Scotland, *British Journal of Preventive and Social Medicine*, 27:208-213.
- Kitts, D. D., 1987, Studies on the estrogenic activity of a coffee extract, *Journal of Toxicology and Environmental Health*, 20:37-49.
- Klyne, W., and Wright, A. A., 1957, Steroids and other lipids of pregnant goat's urine, *Biochemical Journal*, 66:92-101.
- Klyne, W., and Wright, A. A., 1959, Steroids and other lipids of pregnant cow's urine, *Journal of Endocrinology*, 18:32-45.
- Knogge, W., Kombrink, E., Schmelzer, E., and Hahlbrock, K., 1987, Occurrence of phytoalexins and other putative defense-related substances in uninfected parsley plants, *Planta* 171:279-287.
- Kömives, T., and Casida, J. E., 1983, Acifluorfen increases the leaf content of phytoalexins and stress metabolites in several crops, *Journal of Agricultural and Food Chemistry*, 31:751-755.
- König, H., 1953, Untersuchungen über Solaninwirkung bei Rind und Schaf im Zusammenhang mit Kartoffelkraut-Fütterung, *Schweiz Arch Tierheilkd*, 95:97-120.

- Korfmacher, W. A., Bloom, J., Chiarelli, M. P., Lay, J. O., Jr., Holcomb, M., and McManus, K. T., 1991, Comparison of thermospray, FAB, and electrospray for the MS characterization of the mycotoxin fumonisin B<sub>1</sub>, Abstracts of the 39th ASMS Conference on Mass Spectrometry and Allied Topics, p. 710-711.
- Kriek, N. P. J., Kellerman, T. S., and Marasas, W. F. O., 1981a, A comparative study of the toxicity of *Fusarium verticillioides* (= *F. moniliforme*) to horses, primates, pigs, sheep, and rats, Onderstepoort Journal of Veterinary Research, 48:129-131.
- Kriek, N. P. J., Marasas, W. F. O., and Thiel, P. G., 1981b, Hepato- and cardiotoxicity of *Fusarium verticillioides* (*F. moniliforme*) isolates from southern African maize, Food and Cosmetic Toxicology, 19:447-456.
- Krogh, P., 1976, Mycotoxic nephropathy, p. 147-170, in: "Advances in Veterinary Science and Comparative Medicine," Volume 20, C. A. Brandy, C. E. Cornelius, and W. I. B. Beveridge, eds., Academic Press, New York, New York.
- Kubena, L. F., Harvey, R. B., Phillips, T. D., and Heidelbaugh, N. D., 1987, Novel approach to the preventive management of aflatoxicosis in poultry, Proceedings of the 91st Annual Meeting of the United States Animal Health Association, Salt Lake City, Utah, p. 302-304.
- Kubena, L. F., Harvey, R. B., Phillips, T. D., and Huff, W. E., 1988, Modulation of aflatoxicosis in growing chickens by dietary addition of a hydrated sodium calcium aluminosilicate, Poultry Science, 67(Supplement 1):106.
- Kuć, J., 1972, Phytoalexins, Annual Review of Phytopathology, 10:207-232.
- Kuhn, R., and Löw, I., 1961, Zur Konstitution der Leptine, Chemische Berichte, 94:1088-1095.
- Kühnau, J., 1976, The flavonoids. A class of semi-essential food components: Their role in human nutrition, World Review of Nutrition and Dietetics, 24:117-191.
- Kumar, P., Dixit, V. P., and Khanna, P., 1989, Antifertility studies of kaempferol: Isolation and identification from tissue culture of some medicinally important plant species, Plantes médicinales et phytothérapie, Tome XXIII:193-201.
- Lai, D. Y., and Woo, Y.-t., 1987, Naturally occurring carcinogens: An overview, Journal of Environmental Science and Health, C5(2):121-173.
- Langer, P., 1966, Antithyroid action in rats of small doses of some naturally occurring compounds, Endocrinology, 79:1117-1122.
- Langer, P., and Greer, M. A., 1977, Antithyroid substances and naturally occurring goitrogens, Karger, New York, New York, p. 1-178.
- Langer, P., and Štolc, V., 1964, Studien über Beziehungen zwischen Rhodanbildung und kropfbildender Eigenschaft von Nahrungsmitteln, V. Vergleich der Wirkung von Weisskohl und Rhodanid auf die Rattenschilddrüse, Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie, 335:216-220.
- Langer, P., Štolc, V., and Kutka, M., 1964, Naturally occurring goitrogens and thyroid function, Publishing House of the Slovak Academy of Sciences, p. 201-208.
- Lee, H. K., Kim, Y. K., Kim, Y.-H., and Roh, J. K., 1987, Effect of bacterial growth-inhibiting ingredients on the Ames mutagenicity of medicinal herbs, Mutation Research, 192:99-104.
- Lee, L. S., Dunn, J. J., DeLucca, A. J., and Ciegler, A., 1981, Role of lactone ring of aflatoxin B<sub>1</sub> in toxicity and mutagenicity, Experientia, 37:16-17.
- Legrain, P. M., and Barthe, R., 1926, Dermite professionnelle des mains et des avant-bras chez un ramasseur de celeris, Bulletin de la Societe Francaise de Dermatologie et de Syphiligraphie, 33:662-664.
- Lepper, W., 1949, Solanine content of 58 varieties of potatoes, Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung, 89:264-273.
- Lerman, S., 1986, Photosensitizing drugs and their possible role in enhancing ocular toxicity, Ophthalmology, 93:304-318.

- Levenberg, B., 1961, Structure and enzymatic cleavage of agaritine, a phenylhydrazide of L-glutamic acid isolated from *Agaricaceae*, Journal of American Chemical Society, 83:503-504.
- Li, M., Lu, S., Ji, C., Wang, Y., Wang, M., Cheng, S., and Tian, G., 1980, Experimental studies on the carcinogenicity of fungus contaminated food from Linxian county, p. 139-148, in: "Genetic and Environmental Factors in Experimental and Human Cancer," H. V. Gelboin, ed., Japan Science Society Press, Tokyo, Japan.
- Libert, B., and Franceschi, V. R., 1987, Oxalate in crop plants, Journal of Agricultural and Food Chemistry, 35:926-938.
- Liewen, M. B., and Marth, E. H., 1985, Growth and inhibition of microorganisms in the presence of sorbic acid: A review, Journal of Food Protection, 48:364-375.
- Lightfoot, R. J., Croker, K. P., and Neil, H. G., 1967, Failure of sperm transport in relation to ewe infertility following prolonged grazing on oestrogenic pastures, Australian Journal of Agricultural Research, 18:755-765.
- Lin, P., and Tang, W., 1980, Zur Epidemiologie und Ätiologie des Oesophaguscancers in China, Journal of Cancer Research and Clinical Oncology, 96:121-130.
- Linsell, C. A., 1977, The mycotoxins and human health hazards, Annales de la Nutrition et de l'Alimentation, 31:997-1004.
- Lipsitz, D. J., 1984, Herbal teas and water intoxication in a young child, Journal of Family Practice, 18:933-937.
- List, P. H., and Luft, P., 1967, Gyromitrin, das Gift der Fruehjahrslorchel, *Gyromitra (Helvella) esculenta* Fr., Tetrahedron Letters, 20:1893-1894.
- Ljunggren, B., 1990, Severe phototoxic burn following celery ingestion, Archives of Dermatology, 126:1334-1336.
- Locci, R., and Kuć, J., 1967, Steroid alkaloids as compounds produced by potato tubers under stress, Phytopathology, 57:1272-1273.
- Lord, K. M., Epton, H. A. S., and Frost, R. R., 1988, Virus infection and furanocoumarins in celery, Plant Pathology, 37:385-389.
- Loub, W. D., Wattenberg, L. W., and Davis, D. W., 1975, Aryl hydrocarbon hydroxylase induction in rat tissues by naturally occurring indoles of cruciferous plants, Journal of the National Cancer Institute, 54:985-988.
- MacGregor, J. T., 1984, Genetic and carcinogenic effects of plant flavonoids: An overview, Advances in Experimental Medicine and Biology, 177:497-526.
- MacGregor, J. T., and Jurd, L., 1978, Mutagenicity of plant flavonoids: Structural requirements for mutagenic activity in *Salmonella typhimurium*, Mutation Research, 54:297-309.
- MacRae, H. F., Dale, D. G., and Common, R. H., 1960, Formation *in vivo* of 16-epiestriol and 16-ketoestradiol-17 $\beta$  from estriol by the laying hen and occurrence of equol in hen's urine and feces, Canadian Journal of Biochemistry and Physiology, 38(6):523-532.
- Mäkinen, S. M., Kreula, M., and Kauppi, M., 1977, Acute oral toxicity of ethylidene gyromitrin in rabbits, rats and chickens, Food and Cosmetics Toxicology, 15:575-578.
- Malinow, M. R., Bardana, E. J., Jr., Pirofsky, B., Craig, S., and McLaughlin, P., 1982, Systemic lupus erythematosus-like syndrome in monkeys fed alfalfa sprouts: Role of a nonprotein amino acid, Science, 216:415-417.
- Mannon, J., and Johnson, E., 1985, Fungi down on the farm, New Scientist, 105(1445):12-16.
- Marasas, W. F. O., 1982, Mycotoxicological investigations on corn produced in oesophageal cancer areas in Transkei, Volume 1, p. 29-40, in: "Cancer of the Oesophagus," C. J. Pfeifer, ed., CRC Press, Boca Raton, Florida.

- Marasas, W. F. O., Kellerman, T. S., Gelderblom, W. C. A., Coetzer, J. A. W., Thiel, P. G., and van der Lugt, J. J., 1988, Leucoencephalomalacia in a horse induced by fumonisins B<sub>1</sub> isolated from *Fusarium moniliforme*, Onderstepoort Journal of Veterinary Research, 55:197-203.
- Marasas, W. F. O., Kriek, N. P. J., Fincham, J. E., and van Rensburg, S. J., 1984a, Primary liver cancer and oesophageal basal cell hyperplasia in rats caused by *Fusarium moniliforme*, International Journal of Cancer, 34:383-387.
- Marasas, W. F. O., Nelson, P. E., and Toussoun, T. A., 1984b, Toxigenic *Fusarium* species. Identity and Mycotoxicology, p. 216, Pennsylvania State University Press, University Park, Pittsburgh, Pennsylvania.
- Marasas, W. F. O., van Rensburg, S. F., and Mirocha, S. J., 1979, Incidence of *Fusarium* species and the mycotoxins, deoxynivalenol and zearalenone, in corn produced in esophageal cancer areas in Transkei, Journal of Agricultural and Food Chemistry, 27(5):1108-1112.
- Marasas, W. F. O., Wehner, F. C., van Rensburg, S. J., and van Schalkwyk, D. J., 1981, Mycoflora of corn produced in human oesophageal cancer areas in Transkei, southern Africa, Phytopathology, 71:792-796.
- Markham, R. J. F., Erhardt, N. P., Dininno, V. L., Penman, D., and Bhatti, A. R., 1987, Flavonoids protect against T-2 mycotoxins both *in vitro* and *in vivo*, Journal of General Microbiology, 133:1589-1592.
- Marrian, G. F., and Haslewood, G. A. D., 1932, Equol, a new inactive phenol isolated from the ketohydroxyoestrin fraction of mares' urine, Biochemical Journal, 26:1227-1232.
- Maruanovic, D. R., Holt, P., Norred, W. P., Bacon, C. W., Voss, K. A., Stancel, P. C., and Ragland, W. L., 1991, Immunosuppressive effects of *Fusarium moniliforme* corn cultures in chickens, Poultry Science, 70:1895-1901.
- Maryland Department of Health and Mental Hygiene, 1984, Outbreak of phototoxic dermatitis from limes, Morbidity and Mortality Weekly Report, 34:462-464.
- Masri, S. M., 1984, Defenses against aflatoxin carcinogenesis in humans, Advances in Experimental Medicine and Biology, 177:115-146.
- Massaldi, H. A., and King, C. J., 1974, Retention of *d*-limonene during freeze drying of orange juice, Journal of Food Science, 39:445-448.
- Matovinovic, J., 1983, Endemic goiter and cretinism at the dawn of the third millennium, Annual Review of Nutrition, 3:341-412.
- Mathews, F. P., 1936, The toxicity of broomweed (*Gutierrezia microcephala*) for sheep, cattle and goats, Journal of the American Veterinary Medical Association, 88:55-61.
- Matsuura, M., Obata, A., and Fukushima, D., 1989, Objectionable flavor of soy milk developed during the soaking of soybeans and its control, Journal of Food Science, 54(3):602-605.
- Mattocks, A. R., 1980, Toxic pyrrolizidine alkaloids in Comfrey, Lancet, 2:1136-1137.
- McClure, J. W., 1975, Physiology and function of flavonoids, p. 970-1055, in: "The Flavonoids Part 2," J. B. Harborne, T. J. Mabry, and H. Mabry, eds., Academic Press, New York, New York.
- McClusky, G. A., Cooks, R. G., and Knevel, A. M., 1978, Direct analysis of mushroom constituents by mass spectrometry, Tetrahedron Letters, 46:4471-4474.
- McCue, P. M., 1989, Equine leukoencephalomalacia, The Compendium, 11:646-651.
- McDanell, R., McLean, A. E. M., Hanley, A. B., Heaney, R. K., and Fenwick, G. R., 1988, Chemical and biological properties of indole glucosinolates (glucobrassicins): A Review, Food and Chemical Toxicology, 26:59-70.
- McEvoy, M. T., and Stern, R. S., 1987, Psoralens and related compounds in the treatment of psoriasis, Pharmacology and Therapeutics, 34:75-97.
- McKee, R. K., 1955, Host-parasite relationships in the dry-rot disease of potatoes, Annals of Applied Biology, 43:147-148.

- McKinley, E. R., and Carlton, W. W., 1980a, Patulin mycotoxicosis in the Syrian hamster, *Food and Cosmetics Toxicology*, 18:173-179.
- McKinley, E. R., and Carlton, W. W., 1980b, Patulin mycotoxicosis in Swiss ICR mice, *Food Cosmetics Toxicology*, 18:181-187.
- McLean, E. K., 1970, The toxic actions of pyrrolizidine (*senecio*) alkaloids, *Pharmacology Reviews*, 22:429-483.
- McMahon, B., Yen, S., and Rothman, K. J., 1973, Potato blight and neural-tube defects, *Lancet*, 1:598-599.
- McMillan, M., and Thompson, J. C., 1979, An outbreak of suspected solanine poisoning in schoolboys: Examination of criteria of solanine poisoning, *Quarterly Journal of Medicine*, 48:227-243.
- Meltz, M. L., and MacGregor, J. T., 1981, Activity of the plant flavonol quercetin in the mouse lymphoma L5178Y TK<sup>+/−</sup> mutation, DNA single-strand break and Balb/c 3T3 chemical transformation assays, *Mutation Research*, 88:317-324.
- Michnovicz, J. J., and Bradlow, H. L., 1990, Induction of estradiol metabolism by dietary I3C in humans, *Journal of the National Cancer Institute*, 82:947-949.
- Middleton, E., 1988, Plant flavonoid effects on mammalian cell systems, Volume 3, p. 103-144, *in: "Herbs, Spices and Medicinal Plants,"* L. E. Craker and J. E. Simon, eds., Oryx Press, Phoenix, Arizona.
- Mirocha, C. J., Pathre, S. V., and Christensen, C. M., 1977, Zearalenone, p. 345-364, *in: "Mycotoxins in Human and Animal Health,"* J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, eds., Pathotox Publishers, Inc., Park Forest South, Illinois.
- Molyneux, R. J., and James, L. F., 1990, Pyrrolizidine alkaloids in milk: Thresholds of intoxication, p. 94-103, *in: "The Proceedings of the Symposium on Public Health Significance of Natural Toxicants in Animal Feeds,"* W. C. Keller, V. R. Beasley, and J. F. Robens, eds., Veterinary and Human Toxicology 32 (supplement).
- Mondy, N. I., and Gosselin, B., 1988, Effect of peeling on total phenols, total glycoalkaloids, discoloration and flavor of cooked potatoes, *Journal of Food Science*, 53:756-759.
- Mondy, N. I., Leja, M., and Gosselin, B., 1987, Changes in total phenolic, total glycoalkaloid, and ascorbic acid content of potatoes as a result of bruising, *Journal of Food Science*, 52:631-633.
- Morimoto, I., Watanabe, F., Osawa, T., and Okitsu, T., 1982, Mutagenicity screening of crude drugs with *Bacillus subtilis* rec-assay and *Salmonella*/microsome reversion assay, *Mutation Research*, 97:81-102.
- Morin, Y. L., and Daniel, P., 1967, Quebec beer-drinkers cardiomyopathy: Etiological considerations, *Canadian Medical Association Journal*, 97:926-928.
- Morley, F. H. W., Axelsen, A., and Bennett, D., 1964, Effects of grazing red clover (*Trifolium pratense* L.) during the joining season on ewe fertility, *Proceedings of the Australian Society of Animal Production*, 5:58-61.
- Morris, S. C., and Lee, T. H., 1984, The total toxicity and teratogenicity of *Solanaceae* glycoalkaloids, particularly those of the potato (*Solanum tuberosum*): A review, *Food Technology in Australia*, 36:118-124.
- Mosel, H.-D., and Herrmann, K., 1974, The phenolics of fruits. III. The contents of catechins and hydroxycinnamic acids in pome and stone fruits, *Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung*, 154:6-11.
- Moule, G. R., Braden, A. W. H., and Lamond, D. R., 1963, The significance of oestrogens in pasture plants in relation to animal production, *Animal Breeding Abstracts*, 31(2):139-157.
- Mullin, W. J., and Sahasrabudhe, M. R., 1977, Glucosinolate content of cruciferous vegetable crops, *Canadian Journal of Plant Science*, 57:1227-1230.

- Mun, A. M., Barden, E. S., Wilson, J. M., and Hogan, J. M., 1975, Teratogenic effects in early chick embryos of solanine and glycoalkaloids from potatoes infected with late-blight *Phytophthora infestans*, *Teratology*, 11:73-78.
- Murray, R. D. H., Méndez, J., and Brown, S. A., 1982, *The natural coumarins: Occurrence, chemistry and biochemistry*, John Wiley and Sons, New York, New York, p. 1-702.
- National Institute of Environmental Health Sciences, 1982, Psoralens, National Toxicology Program, Technical Bulletin No. 6, p. 8.
- National Research Council, 1982, *Diet, nutrition and cancer*, National Academy Press, Washington, D.C.
- National Toxicology Program, 1989, Fiscal Year 1989 Annual Plan, U.S. Department of Health and Human Services, NTP-89-167, June 1989, Public Health Service, Research Triangle Park, North Carolina, 265 p.
- National Toxicology Program, 1991, NTP Technical Report on the Toxicology and Carcinogenesis Studies of Quercetin (CAS No. 117-39-5) in F344/N rats (feed studies) NTP TR 409, NIH Publication No. 91-3140, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, March 11-12, 1991, Research Triangle Park, North Carolina, 276 p.
- Nevin, N. C., and Merrett, J. D., 1975, Potato avoidance during pregnancy in women with a previous infant with either anencephaly and/or spina bifida, *British Journal of Prevention and Social Medicine*, 29:111-115.
- Newsome, F. E., and Kitts, W. D., 1980, Action of phyto-estrogens coumestrol and genistein on cytosolic and nuclear oestradiol-17 $\beta$  receptors in immature rat uterus, *Animal Reproduction Science*, 3:233-245.
- Niederhofer, R. E., 1985, The milk sickness, *Journal of the American Medical Association*, 254:2123-2125.
- Nikaido, T., Ohmoto, T., Kinoshita, T., Sankawa, U., Monache, F. D., Botta, B., Tomimori, T., Miyaichi, Y., Shirataki, Y., Yokoe, I., and Komatsu, M., 1989, Inhibition of adenosine 3',5'-cyclic monophosphate phosphodiesterase by flavonoids, III, *Chemical and Pharmaceutical Bulletin*, 37(5):1392-1395.
- Nishie, K., and Daxenbichler, M. E., 1980, Toxicology of glucosinolates, related compounds (nitriles, *R*-goitrin, isothiocyanates) and vitamin U found in cruciferae, *Food Cosmetic Toxicology*, 18:159-172.
- Norred, W. P., Plattner, R. D., Voss, K. A., Bacon, C. W., and Porter, J. K., 1989, Natural occurrence of fumonisins in corn associated with equine leukoencephalomalacia (ELEM), *The Toxicologist*, 9:258, Abstract No. 1084.
- Ogawara, H., Akiyama, T., and Watanabe, S.-i., 1989, Inhibition of tyrosine protein kinase activity by synthetic isoflavones and flavones, *The Journal of Antibiotics*, XLII(2):340-343.
- Omidiji, O., and Ehimidu, J., 1990, Changes in the content of antibacterial isorhamnetin 3-glucoside and quercetin 3'-glucoside following inoculation of onion (*Allium cepa* L. cv. Red Creole) with *Pseudomonas cepacia*, *Physiological and Molecular Plant Pathology*, 37:281-292.
- O'Neill, M. J., Adesanya, S. A., and Roberts, M. F., 1983, Antifungal phytoalexins in *Phaseolus aureus* Roxb., *Zeitschrift fuer Naturforschung Teil C*, 38:693-697.
- Orgell, W. H., 1963, Inhibition of human plasma cholinesterase *in vitro* by alkaloids, glycosides, and other natural substances, *Lloydia*, 26:36-43.
- Orgell, W. H., and Hibbs, E. T., 1963, Cholinesterase inhibition *in vitro* by potato foliage extracts, *American Potato Journal*, 40:403-405.
- Orgell, W. H., Vaidya, K. A., and Dahm, P. A., 1958a, Cholinesterase inhibition *in vitro* by extracts of potato, *Proceedings of the Iowa Academy of Science*, 65:160-162.

- Orgell, W. H., Vaidya, K. A., and Dahm, P. A., 1958b, Inhibition of human plasma cholinesterase *in vitro* by extracts of solanaceous plants, *Science* 128:1136-1137.
- Osol, A., and Farrar, G. E., 1947, The dispensatory of the United States of America, Volume 24, J. B. Lippincott Company, Philadelphia, Pennsylvania, p. 334-335.
- Ownbey, G. C., and Morley, T., 1992, Checklist and atlas of the flora of Minnesota, University of Minnesota Press, Minneapolis, Minnesota (in press).
- Pantuck, E. J., Hsiao, K.-C., Loub, W. D., Wattenberg, L. W., Kuntzman, R., and Conney, A. H., 1976, Stimulatory effect of vegetables on intestinal drug metabolism in the rat, *Journal of Pharmacology and Experimental Therapeutics*, 198:278-283.
- Pantuck, E. J., Pantuck, C. B., Anderson, K. E., Wattenberg, L. W., Conney, A. H., and Kappas, A., 1984, Effect of Brussels sprouts and cabbage on drug conjugation, *Clinical Pharmacology and Therapeutics*, 2:161-169.
- Pantuck, E. J., Pantuck, C. B., Garland, W. A., Min, B. H., Wattenberg, L. W., Anderson, K. E., Kappas, A., and Conney, A. H., 1979, Stimulatory effect of Brussels sprouts and cabbage on human drug metabolism, *Clinical Pharmacology and Therapeutics*, 1:88-95.
- Panzer, P. E., 1983, The dangers of cooking with bay leaves, *Journal of the American Medical Association*, 250:164-165.
- Park, D. L., Lee, L. S., Price, R. L., and Pohland, A. E., 1988, Review of the decontamination of aflatoxin by ammoniation: Current status and regulation, *Journal of the Association of Official Analytical Chemists*, 71:685-703.
- Parsons, B. J., 1980, Psoralen photochemistry, *Photochemistry and Photobiology*, 32:813-821.
- Paszkowski, W. L., and Kremer, R. J., 1988, Biological activity and tentative identification of flavonoid components in velvetleaf (*Abutilon theophrasti* Medik.) seed coats, *Journal of Chemical Ecology*, 14(7):1573-1582.
- Pathak, M. A., Daniels, F., and Fitzpatrick, T. B., 1962, The presently known distribution of furocoumarins (psoralens) in plants, *Journal of Investigative Dermatology*, 39:225-240.
- Patil, B. C., Sharma, R. P., Salunkhe, D. K., and Salunkhe, K., 1972, Evaluation of solanine toxicity, *Food and Cosmetics Toxicology*, 10:395-398.
- Paxton, J., 1980, A new working definition of the term "phytoalexin," *Plant Disease*, 64:734.
- Peckham, J. C., Mitchell, F. E., Jones, O. H., and Doupnik, B., Jr., 1972, Atypical interstitial pneumonia in cattle fed moldy sweet potatoes, *Journal of American Veterinary Medical Association*, 160:169-172.
- Pence, B. C., Buddingh, F., and Yang, S. P., 1986, Multiple dietary factors in the enhancement of dimethylhydrazine carcinogenesis: Main effect of indole-3-carbinol, *Journal of the National Cancer Institute*, 77:269-276.
- Penrose, L. S., 1957, Genetics of anencephaly, *Journal of Mental Deficiency Research*, 1:4-15.
- Pestka, J. J., 1988, Enhanced surveillance of foodborne mycotoxins by immunochemical assay, *Journal of the Association of Official Analytical Chemists*, 71:1075-1081.
- Phillips, R. L., 1975, Role of life-style and dietary habits in risk of cancer among Seventh-Day Adventists, *Cancer Research*, 35:3513-3522.
- Phillips, T. D., Clement, B. A., Kubena, L. F., and Harvey, R. B., 1988a, Mycotoxins: Detection and detoxification, *Proceedings of the 23rd National Meeting of Poultry Health Condemnations*, Delmarva Poultry Industry Inc., Ocean City, Maryland, p. 94-106.
- Phillips, T. D., Kubena, L. F., Harvey, R. B., Taylor, D. R., and Heidelbaugh, N. D., 1988b, Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxin, *Poultry Science*, 67:243-247.

- Pienaar, J. G., Kellerman, T. S., and Marasas, W. F. O., 1981, Field outbreaks of leukoencephalomalacia in horses consuming maize infected by *Fusarium verticillioides* (*F. moniliforme*) in South Africa, Journal of South African Veterinary Association, 52:21-24.
- Pierpoint, W. S., 1985, Phenolics in food and feedstuffs: the pleasures and perils of vegetarianism, p. 427-451, in: "The Biochemistry of Plant Phenolics," C. F. Van Sumere and P. J. Lea, eds., Annual Proceedings of the Phytochemical Society of Europe, Volume 29, Clarendon Press, Oxford, England.
- Pierpoint, W. S., 1986, Flavonoids in the human diet, p. 125-140, in: "Plant Flavonoids in Biology and Medicine, Biochemical, Pharmacological, and Structure-Activity Relationships," V. Cody, E. Middleton, Jr., and J. B. Harborne, eds., Proceedings of a Symposium Held in Buffalo, New York, July 22-26, 1985, Alan R. Liss, Inc., New York, New York.
- Pierpoint, W. S., 1990, Flavonoids in human food and animal feedstuffs, p. 497-514, in: "Flavonoids in Biology and Medicine III. Current Issues in Flavonoid Research," N. P. Das, ed., National University of Singapore.
- Pierro, L. J., Haines, J. S., and Osman, S. F., 1977, Teratogenicity and toxicity of purified  $\alpha$ -chaconine and  $\alpha$ -solanine, Teratology, 15(2):31A.
- Plattner, R. D., Norred, W. P., Bacon, C. W., Voss, K. A., Peterson, R., Shackelford, D. D., and Weisleder, D., 1990, A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalacia, Mycologia, 82:698-702.
- Plattner, R. D., Ross, P. F., Reagor, J., Stedelin, J., and Rice, L. G., 1991, Analysis of corn and cultured corn for fumonisin B<sub>1</sub> by HPLC and GC/MS by four laboratories, Journal of Veterinary Diagnostic Investigation, 3:357-358.
- Plattner, R. D., Weisleder, D., Shackelford, D. D., Peterson, R., and Powell, R. G., 1992, A new fumonisin from solid cultures of *Fusarium moniliforme*, Mycopathologia (in press).
- Plott, R. T., and Wagner, R. F., 1990, Modern treatment approaches to vitiligo, CUTIS, 45:311-316.
- Polacco, J. C., and Sands, D. C., 1977, The mycotoxin patulin inhibits respiration of higher plant cells, Plant Science Letters, 9:121-128.
- Pollock, G. A., 1982, Mycotoxins in animal feeds, Modern Veterinary Practice, 4:285-287.
- Ponnampalam, R., and Mondy, N. I., 1986, Effect of foliar application of indoleacetic acid on the total glycoalkaloids and nitrate nitrogen content of potatoes, Journal of Agricultural and Food Chemistry, 34:686-688.
- Pratesi, G., Manzotti, C., Tortoreto, M., Audisio, R. A., and Zunino, F., 1991, Differential efficacy of flavone acetic acid against liver versus lung metastases in a human tumour xenograft, British Journal of Cancer, 63:71-74.
- Purchase, I. F. H., 1967, Acute toxicity of aflatoxins M<sub>1</sub> and M<sub>2</sub> in one-day-old ducklings, Food and Cosmetics Toxicology, 5:339-342.
- Pyysalo, H., 1976, Identification of volatile compounds in seven edible fresh mushrooms, Acta Chemica Scandinavica B, 30:235-244.
- Pyysalo, H., and Niskanen, A., 1977, On the occurrence of *N*-methyl-*N*-formylhydrazones in fresh and processed false morel, *Gyromitra esculenta*, Journal of Agricultural and Food Chemistry, 25:644-647.
- Pyysalo, H., Niskanen, A., and von Wright, A., 1979, Formation of toxic methylhydrazine during cooking of false morels, *Gyromitra esculenta*, Journal of Food Safety, 1:295-299.
- Ramarathnam, N., Osawa, T., Namiki, M., and Kawakishi, S., 1989, Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a C-glycosyl flavonoid, Journal of Agricultural and Food Chemistry, 37:316-319.

- Raynal, J., Moutounet, M., and Souquet, J.-M., 1989, Intervention of phenolic compounds in plum technology. 1. Changes during drying, *Journal of Agricultural and Food Chemistry*, 37:1046-1050.
- Reindel, J. F., Ganey, P. E., Wagner, J. G., Slocum, R. F., and Roth, R. A., 1990, Development of morphologic, hemodynamic, and biochemical changes in lungs of rats given monocrotaline pyrrole, *Toxicology and Applied Pharmacology*, 106:179-200.
- Renwick, J. H., 1972, Hypothesis: Anencephaly and spina bifida are usually preventable by avoidance of a specific but unidentified substance present in certain potato tubers, *British Journal of Preventive and Social Medicine*, 26:67-88.
- Renwick, J. H., 1982a, Vitamin supplementation and neural tube defects, *Lancet*, 1:748.
- Renwick, J. H., 1982b, Food and malformation, *Practitioner*, 226:1947-1953.
- Renwick, J. H., Claringbold, W. D. B., Earthy, M. E., Few, J. D., and McLean, A. C. S., 1984, Neural-tube defects produced in Syrian hamsters by potato glycoalkaloids, *Teratology*, 30:371-381.
- Riazuddin, S., Malik, M. M., and Nasim, A., 1987, Mutagenicity testing of some medicinal herbs, *Environmental and Molecular Mutagenesis*, 10:141-148.
- Rich, J. R., Keen, N. T., and Thomason, I. J., 1977, Association of coumestans with the hypersensitivity of lima bean roots to *Pratylenchus scribneri*, *Physiological Plant Pathology*, 10:105-116.
- Richards, D. E., 1972, The isolation and identification of toxic coumarins, p. 36-43, in: "Microbial Toxins: Fungal Toxins," Volume VIII, S. Kadis, A. Ciegler, and S. J. Ajl, eds., Academic Press, New York, New York.
- Ries, C. A., and Sahud, M. A., 1975, Agranulocytosis caused by Chinese herbal medicines: Dangers of medications containing aminopyrine and phenylbutazone, *Journal of the American Medical Association*, 231:352-355.
- Robbins, R. C., 1980, Medical and nutritional aspects of citrus bioflavonoids, p. 44-59, in: "Citrus Nutrition and Quality," S. Nagy and J. A. Attaway, eds., American Chemical Society Symposium Series 143, American Chemical Society, Washington, D.C.
- Robinson, T., 1991, The organic constituents of higher plants: Their chemistry and interrelationships, Sixth Edition, Cordus Press, North Amherst, Massachusetts, p. 120.
- Rodricks, J. V., 1978, Food hazard of natural origin, *Federation Proceedings*, 37:2587-2593.
- Roitman, J. N., 1981, Comfrey and liver damage, *Lancet*, 1:944.
- Roitman, J. N., and James, L. F., 1985, Chemistry of toxic range plants. Highly oxygenated flavonol methyl ethers from *Gutierrezia microcephala*, *Phytochemistry*, 24(4):835-848.
- Rose, E. F., and Fellingham, S. A., 1981, Cancer patterns in Transkei, *South African Journal of Science*, 77:555-561.
- Ross, A. E., Nagel, D. L., and Toth, B., 1982, Occurrence, stability and decomposition of  $\beta$ -N[ $\gamma$ -L(+)-glutamyl]-4-hydroxymethylphenylhydrazine (agaritine) from the mushroom *Agaricus bisporus*, *Food and Chemical Toxicology*, 20:903-907.
- Ross, P. F., Rice, L. G., Plattner, R. D., Osweiler, G. D., Wilson, T. M., Owens, D. L., Nelson, H. A., and Richard, J. L., 1991a, Concentrations of fumonisin B<sub>1</sub> in feeds associated with animal health problems, *Mycopathologia*, 114:129-135.
- Ross, P. F., Rice, L. G., Reagor, J. C., Osweiler, G. D., Wilson, T. M., Nelson, H. A., Owens, D. L., Plattner, R. D., Harlin, K. A., Richard, J. L., Colvin, B. M., and Banton, M. I., 1991b, Fumonisin B<sub>1</sub> concentrations in feeds from 45 confirmed equine leukoencephalomalacia cases, *Journal of Veterinary Diagnostic Investigation*, 3:238-241.

- Rubin, B., Penner, D., and Saettler, A. W., 1983, Induction of isoflavonoid production in *Phaseolus vulgaris* L. leaves by ozone, sulfur dioxide and herbicide stress, Environmental Toxicology and Chemistry, 2:295-306.
- Rusul, G., and Marth, E. H., 1988, Food additives and plant components control growth and aflatoxin production by toxic aspergilli: A review, Mycopathologia, 101:13-23.
- Rutledge, A. E., 1976, The environmental-public health significance of mycotoxins, Proceedings of the 1st International Symposium on Feed Composition, Animal Nutrient Requirements, and Computerization of Data, Utah State University, Logan, Utah, p. 345-350.
- Rutledge, A. E., 1977, The public health significance of mycotoxins, Annales de la Nutrition et de l'Alimentation, 31:1019-1030.
- Ryan, C. A., 1973, Proteolytic enzymes and their inhibitors in plants, Annual Review of Plant Physiology, 24:173-196.
- Ryan, C. A., 1988, Oligosaccharides as recognition signals for the expression of defensive genes in plants, Biochemistry, 27(25):8879-8883.
- Ryan, C. A., Bishop, P. D., Graham, J. S., Broadway, R. M., and Duffey, S. S., 1986, Plant and fungal cell wall fragments activate expression of proteinase inhibitor genes for plant defense, Journal of Chemical Ecology, 12(5):1025-1036.
- Salaman, R. N., 1985, The history and social influence of the potato, Cambridge University Press, Cambridge, England, p. 1-685.
- Sato, K., Ishiguri, Y., Doke, N., Tomiyama, K., Yagihashi, F., Murai, A., Katsui, N., and Masamune, T., 1978, Biosynthesis of the sesquiterpenoid phytoalexin rishitin from acetate via oxylubimin in potato, Phytochemistry, 17:1901-1902.
- Saxe, T. G., 1987, Toxicity of medicinal herbal preparations, American Family Physician (U.S.), 35:135-142.
- Scheel, L. D., Perone, V. B., Larkin, R. L., and Kupel, R. E., 1963, The isolation and characterization of two phototoxic furanocoumarins (psoralens) from diseased celery, Biochemistry, 2:1127-1133.
- Schlatter, C., 1988, The importance of mycotoxins in foods, Bibliotheca Nutrition Dieta, 41:55-65.
- Schmidlin-Mészáros, J., 1974, Gyromitrin in Trockenlorcheln (*Gyromitra esculenta* sicc.), Mitteilungen aus dem Gebiete Lebensmitteluntersuchung und Hygiene, 65:453-465.
- Schmidlin-Mészáros, J., 1975, Sind die getrockneten Lorcheln, *Gyromitra* (*Helvella*) *esculenta*, ungiftig?, Schweizerische Zeitschrift fuer Pilzkunde, 53:106-111.
- Schneider, J. A., Lee, J., Naya, Y., Nakanishi, K., Oba, K., and Uritani, I., 1984, The fate of the phytoalexin ipomeamarone: Furanoterpenes and butenolides from *Ceratocystis fimbriata*-infected sweet potatoes, Phytochemistry, 23:759-764.
- Schneider, J. A., and Nakanishi, K., 1983, A new class of sweet potato phytoalexins, Journal of the Chemical Society Chemical Communications, p. 353-355.
- Schoental, R., 1968, Toxicology and carcinogenic action of pyrrolizidine alkaloids, Cancer Research, 28:2237-2246.
- Schoental, R., 1976, Hazards of oral contraceptives to future generations, International Journal of Environmental Studies, 9:81.
- Schoental, R., 1977a, Health hazards due to oestrogenic mycotoxins in certain foodstuffs, International Journal of Environmental Studies, 11:149-150.
- Schoental, R., 1977b, The role of nicotinamide and of certain other modifying factors in diethylnitrosamine carcinogenesis, Cancer, 40:1833-1840.
- Schoental, R., 1980, Disorders and tumors associated with alcoholic drinks and mycotoxins, Nutrition and Cancer, 2:88-92.
- Schoental, R., 1981, Mycotoxins and fetal abnormalities, International Journal of Environmental Studies, 17:25-29.

- Schoental, R., 1984, Mycotoxins and the Bible, Perspectives in Biology and Medicine, 28:117-120.
- Schoental, R., and Joffe, A. Z., 1974, Lesions induced in rodents by extracts from cultures of *Fusarium poae* and *F. sporotrichioides*, Journal of Pathology, 112:37-42.
- Schoental, R., Joffe, A. Z., and Yagen, B., 1976, Chronic lesions in rats treated with crude extracts of *Fusarium poae* and *F. sporotrichioides*. The role of mouldy food in the incidence of wesophageal, mammary and certain other abnormalities and tumours in livestock and man, Abstracts of the Proceedings of the British Association for Cancer Research, 17th Annual Meeting, University College, Swansea, British Journal of Cancer, 34:310.
- Schoental, R., Joffe, A. Z., and Yagen, B., 1978, The induction of tumours of the digestive tract and of certain other organs in rats given T-2 toxin: A secondary metabolite of *Fusarium sporotrichioides*, Abstracts of the Proceedings of the British Association for Cancer Research, 19th Annual Meeting, University of Oxford, England, British Journal of Cancer, 38:171.
- Schriber, K., 1968, Steroid Alkaloids: The Solanum Group, Volume X, p. 1-192, in: "The Alkaloids," R. H. F. Manske, ed., Academic Press, New York, New York.
- Scott, B. R., Pathak, M. A., and Mohn, G. R., 1976, Molecular and genetic basis of furocoumarin reactions, Mutation Research, 39:29-74.
- Sears, M. R., 1991, Growing number of Asthma cases, deaths, baffles physicians, Canadian Medical Association Journal, 145:379-380.
- Segelman, A. B., Segelman, F. P., Karliner, J., and Sofia, D., 1976, Sassafras and herb tea, Journal of the American Medical Association, 236:477.
- Seifulla, K. I., and Ryzhova, K. E., 1972, Effect of hydrocortisone, solasodine, and solanine on the growth and development of fetuses of pregnant rats, Mater Vses Konf Issled Lek Rast Perspekt Ikh Ispol'z Proizvod Lek Prep 1970:160-162; Chemical Abstracts, 83:22383t (1975).
- Seligman, P. J., Mathias, C. G. T., O'Malley, M. A., Beier, R. C., Fehrs, L. J., Serrill, W. S., and Halperin, W. E., 1987, Phytophotodermatitis from celery among grocery store workers, Archives of Dermatology, 123:1478-1482.
- Setchell, K. D. R., Borriello, S. P., Hulme, P., Kirk, D. N., and Axelson, M., 1984, Nonsteroidal estrogens of dietary origin: Possible roles in hormone-dependent disease, The American Journal of Clinical Nutrition, 40:569-578.
- Shane, B. S., Littman, C. B., Essick, L. A., Gutenmann, W. H., Doss, G. J., and Lisk, D. J., 1988, Uptake of selenium and mutagens by vegetables grown in fly ash containing greenhouse media, Journal of Agricultural and Food Chemistry, 36:328-333.
- Shank, R. C., Bourgeois, C. H., Keschamras, N., and Chandavimol, P., 1971, Aflatoxins in autopsy specimens from Thai children with an acute disease of unknown aetiology, Food and Cosmetics Toxicology, 9:501-507.
- Sharby, T. F., Templeton, G. E., Beasley, J. N., and Stephenson, E. L., 1973, Toxicity resulting from feeding experimentally molded corn to broiler chicks, Poultry Science, 52:1007-1014.
- Shier, W. T., Abbas, H. K., and Mirocha, C. J., 1991, Toxicity of the mycotoxins fumonisins B<sub>1</sub> and B<sub>2</sub> and *Alternaria alternata* f. sp. *lycopersici* toxin (AAL) in cultured mammalian cells, Mycopathologia, 116:97-104.
- Shih, M. J., and Kuć, J., 1974,  $\alpha$ - and  $\beta$ -Solamarine in Kennebec *Solanum tuberosum* leaves and aged tuber slices, Phytochemistry, 13:997-1000.
- Shirahata, S., Murakami, H., Nishiyama, K., Yamada, K., Nonaka, G., Nishioka, I., and Omura, H., 1989, DNA breakage by flavan-3-ols and procyanidins in the presence of cupric ion, Journal of Agricultural and Food Chemistry, 37:299-303.
- Shore, L. S., and Lytle, C. R., 1986, Interactions of isoflavones, zearalenone and DES with rat uterine peroxidase enzyme, p. 253-256, in: "Plant Flavonoids in Biology

- and Medicine, Biochemical, Pharmacological, and Structure-Activity Relationships," V. Cody, E. Middleton, Jr., and J. B. Harborne, eds., Proceedings of a Symposium Held in Buffalo, New York, July 22-26, 1985, Alan R. Liss, Inc., New York, New York.
- Shultz, T. D., Wilcox, R. B., Spuehler, J. M., and Howie, B. J., 1987, Dietary and hormonal interrelationships in premenopausal women: Evidence for a relationship between dietary nutrients and plasma prolactin levels, *American Journal of Clinical Nutrition*, 46:905-911.
- Shutt, D. A., and Braden, A. W. H., 1968, The significance of equol in relation to the oestrogenic responses in sheep ingesting clover with a high formononetin content, *Australian Journal of Agricultural Research*, 19:545-553.
- Shutt, D. A., and Cox, R. I., 1972, Steroid and phyto-oestrogen binding to sheep uterine receptors *in vitro*, *Journal of Endocrinology*, 52:299-310.
- Siegel, R. K., 1976, Herbal intoxication: Psychoactive effects from herbal cigarettes, tea, and capsules, *Journal of the American Medical Association*, 236:473-476.
- Sinden, S. L., 1972, Effect of light and mechanical injury on the glycoalkaloid content of greening resistant potato tubers, *American Potato Journal*, 49:368.
- Sinden, S. L., Sanford, L. L., and Deahl, K. L., 1986, Segregation of leptine glycoalkaloids in *Solanum chacoense* Bitter, *Journal of Agricultural and Food Chemistry*, 34:372-377.
- Sinden, S. L., Sanford, L. L., and Osman, S. F., 1980, Glycoalkaloids and resistance to the Colorado potato beetle in *Solanum chacoense* Bitter, *American Potato Journal*, 57:331-343.
- Sinden, S. L., and Webb, R. E., 1972, Effect of variety and location on the glycoalkaloid content of potatoes, *American Potato Journal*, 49:334-338.
- Singleton, V. L., 1981, Naturally occurring food toxicants: Phenolic substances of plant origin common in foods, p. 149-242, *in: "Advances in Food Research," Volume 27*, C. O. Chichester, E. M. Mrak and G. F. Stewart, eds., Academic Press, New York, New York.
- Sinnhuber, R. O., Lee, D. J., Wales, J. M., Landers, M.K., and Keyl, A. C., 1970, Aflatoxin M<sub>1</sub>, a potent liver carcinogen in rainbow trout, *Federation Proceedings*, 29:568 (Abstract).
- Smalley, E. B., 1978, Salivary syndrome in cattle, p. 121-141, *in: "Mycotoxic fungi, mycotoxins, and mycotoxicoses,"* T. D. Wyllie and L. G. Morehouse, eds., Marcel Dekker, New York, New York.
- Smith, D. A., 1982, Toxicity of phytoalexins, p. 218, *in: "Phytoalexins,"* J. A. Bailey and J. W. Mansfield, eds., John Wiley and Sons, New York, New York.
- Smith, D. M., 1985, Occupational photodermatitis from parsley, *Practitioner*, 229:673-675.
- Smith, G. R., Randel, R. D., and Bradshaw, C., 1986, Influence of harvest date, cultivar, and sample storage method on concentration of isoflavones in subterranean clover, *Crop Science*, 26:1013-1016.
- Smith, O., 1977, Chemical composition of the potato, p. 59-109, *in: "Potatoes: Production, Storing, Processing,"* 2nd ed, AVI Publishing Co., Westport, Connecticut.
- Snively, W. D., Jr., and Furbee, L., 1966, Discoverer of the cause of milk sickness, *Journal of the American Medical Association*, 196:1055-1060.
- Sorenson, W. G., 1990, Mycotoxins as potential occupational hazards, *Development of Industrial Microbiology*, 31:205-211, reprinted in *Journal of Industrial Microbiology, Supplement No. 5.*
- Spanos, G. A., and Wrolstad, R. E., 1990, Influence of processing and storage on the phenolic composition of Thompson seedless grape juice, *Journal of Agricultural and Food Chemistry*, 38:1565-1571.

- Spencer, G. F., Tjarks, L. W., and Powell, R. G., 1987, Analysis of linear and angular furanocoumarins by dual-column high-performance liquid chromatography, *Journal of Agricultural and Food Chemistry*, 35:803-805.
- Sperry, O. E., Dollahite, J. W., Hoffman, G. O., and Camp, B. J., 1964, Texas plants poisonous to livestock, *Texas Agricultural Experiment Station Bulletin No. B-1028*, p. 59.
- Spiers, P. S., Pietrzyk, J. J., Piper, J. M., and Glebatis, D. M., 1974, Human potato consumption and neural-tube malformation, *Teratology*, 10:125-128.
- Stanley, W. L., and Jurd, L., 1971, Citrus coumarins, *Journal of Agricultural and Food Chemistry*, 19:1106-1110.
- Steinbauer, C. E., and Cushman, L. J., 1971, Sweet potato culture and diseases, *Agriculture Handbook No. 388*, Agricultural Research Service, United States Department of Agriculture, Washington, D.C.
- Steiner, R. P., 1986, Folk medicine: The art and the science, *American Chemical Society*, Washington, D.C., p. 1-223.
- Steiner, W. E., Rieker, R. H., and Battaglia, R., 1988, Aflatoxin contamination in dried figs: Distribution and association with fluorescence, *Journal of Agricultural and Food Chemistry*, 36:88-91.
- Steinmetz, M.-D., Monlin-Traffort, J., and Régli, P., 1987, Transmission and scanning electronmicroscopy study of the action of sage and rosemary essential oils and eucalyptol on *Candida albicans*, *Mycoses*, 31:40-51.
- Stephenson, M., 1978, The confusing world of health food, *FDA Consumer*, 12:18-22.
- Stern, R. S., Abel, E., Wintroub, B., Epstein, J. H., Tschen, J., Wolf, J., Nigra, T. P., Voorhees, J., Anderson, T. F., Armstrong, R., Harber, L., Muller, S., Taylor, J. R., Frost, P., Horwitz, S., Urbach, F., Arndt, K. A., Baughman, R. D., Braverman, I. M., Murray, J., Petrozzi, J., Gonzalez, E., Parrish, J. A., Fitzpatrick, T. B., Lange, R., and Bleich, H. I., 1990, Genital tumors among men with psoriasis exposed to psoralens and ultraviolet A radiation (PUVA) and ultraviolet B radiation, *The New England Journal of Medicine*, 322:1093-1097.
- Stern, R. S., Thibodeau, L. A., Kleinerman, R. A., Parrish, J. A., Fitzpatrick, T. B., and 22 participating investigators, 1979, Risk of cutaneous carcinoma in patients treated with oral methoxalen photochemotherapy for psoriasis, *New England Journal of Medicine*, 300:809-813.
- Stevens, D. K., and R. I. Krieger, 1991, Effect of route of exposure and repeated doses on the acute toxicity in mice of the cyanobacterial nicotinic alkaloid anatoxin-A, *Toxicon*, 29(1):134-138.
- Stich, H. F., and Rosin, M. P., 1984, Naturally occurring phenolics as antimutagenic and anticarcinogenic agents, *Advances in Experimental Medicine and Biology*, 177:1-29.
- Stoessl, A., Stothers, J. B., and Ward, E. W. B., 1974, Lubimen: A phytoalexin of several Solanaceae. Structure revision and biogenetic relationships, *Journal of the Chemical Society, Chemical Communications*, p. 709-710.
- Stoessl, A., Stothers, J. B., and Ward, E. W. B., 1976, Sesquiterpenoid stress compounds of the Solanaceae, *Phytochemistry*, 15:855-872.
- Stoessl, A., Unwin, C. H., and Ward, E. W. B., 1972, Postinfectional inhibitors from plants. I. Capsidiol, an antifungal compound from *Capsicum frutescens*, *Phytopathologische Zeitschrift*, 74:141-152.
- Stoewsand, G. S., Babish, J. B., and Wimberly, H. C., 1978, Inhibition of hepatic toxicities from polybrominated biphenyls and aflatoxin B<sub>1</sub> in rats fed cauliflower, *Journal of Environmental Pathology Toxicology*, 2:399-406.
- Stoloff, L., 1980, Aflatoxin M in perspective, *Journal of Food Protection*, 43:226-230.
- Stotts, R., 1984, White snakeroot toxicity in dairy cattle, *Veterinary Medicine & Small Animal Clinician*, 79:118-120.

- Stransky, L., and Tsankov, N., 1980, Contact dermatitis from parsley (*Petroselinum*), Contact Dermatitis, 6:233-234.
- Stürckow, B., and Löw, I., 1961, Die Wirkung einiger *Solanum*-Alkaloidglykoside auf den Kurtoffelkäfer, *Leptinotarsa decemlineata* Say, Entomologia Experimentalis et Applicata, 4:133-142.
- Sullivan, J. M., Zwaag, R. V., Hughes, J. P., Maddock, V., Kroetz, F. W., Ramanathan, K. B., and Mirvis, D. M., Estrogen replacement and coronary artery disease: Effect on survival in postmenopausal women, Archives of Internal Medicine, 150:2557-2562.
- Surak, J. G., 1978, Phytoalexins and human health—A review, Proceedings of the Florida State Horticultural Society, 91:256-258.
- Sydenham, E. W., Gelderblom, W. C. A., Thiel, P. G., and Marasas, W. F. O., 1990, Evidence for the natural occurrence of fumonisin B<sub>1</sub>, a mycotoxin produced by *Fusarium moniliforme*, in corn, Journal of Agricultural and Food Chemistry, 38:285-290.
- Szeto, S. Y., Vernon, R. S., and Brown, M. J., 1983, Degradation of disulfoton in soil and its translocation into asparagus, Journal of Agricultural and Food Chemistry, 31:217-220.
- Taira, T., and Fukagawa, Y., 1958, Bitter substance separated from alcohol distillation of sweet potato mash, Nippon Nôgei Kagaku Kaishi, 32:513-514; Chemical Abstracts, 53:4645c (1959).
- Takács, O., and Gábor, M., 1975, New data on the metabolism of flavonoids, p. 227-231, in: "Topics in Flavonoid Chemistry and Biochemistry," L. Farkas, M. Gábor, and E. Kallay, eds., Proceedings of the 4th Hungarian Bioflavonoid Symposium, Keszhely, 1973, Elsevier, New York, New York.
- Takeuchi, T., 1946, Bitter substance, produced in black-rotted sweet potatoes, Scientific Insect Control (Kyoto), 12:26-29; Chemical Abstracts, 43:8453 (1949).
- Talib, S., and Banerjee, A. K., 1982, Covalent attachment of psoralen to a single site on vesicular stomatitis virus genome RNA blocks expression of viral genes, Virology, 118:430-438.
- Tanew, A., Hönigsmann, H., Ortel, B., Zussner, C., and Wolff, K., 1986, Nonmelanoma skin tumors in long-term photochemotherapy treatment of psoriasis, Journal of the American Academy of Dermatology, 15:960-965.
- Tang, B. Y., and Adams, N. R., 1980, Effect of equol on oestrogen receptors and on synthesis of DNA and protein in the immature rat uterus, Journal of Endocrinology, 85:291-297.
- Tang, J., Zhang, Y., Hartman, T. G., Rosen, R. T., and Ho, C.-T., 1990, Free and glycosidically bound volatile compounds in fresh celery (*Apium graveolens* L.), Journal of Agricultural and Food Chemistry, 38:1937-1940.
- Tatum, J. H., and Berry, R. E., 1979, Coumarins and psoralens in grapefruit peel oil, Phytochemistry, 18:500-502.
- Temple, N. J., and El-Khatib, S. M., 1987, Cabbage and vitamin E: Their effect on colon tumor formation in mice, Cancer Letters, 35:71-77.
- Tietjen, K. G., Hunkler, D., and Matern, U., 1983, Differential response of cultured parsley cells to elicitors from two non-pathogenic strains of fungi. 1. Identification of induced products as coumarin derivatives, European Journal of Biochemistry, 131:401-407.
- Tietjen, K. G., and Matern, U., 1984, Induction and suppression of phytoalexin biosynthesis in cultured cells of safflower, *Carthamus tinctorius* L., by metabolites of *Alternaria carthami* Chowdhury, Archives of Biochemistry and Biophysics, 229:136-144.
- Timberlake, C. F., 1981, Anthocyanins in fruits and vegetables, Chapter 12, p. 221-247, in: "Recent Advances in the Biochemistry of Fruits and Vegetables."

- Tingey, W. M., Mackenzie, J. D., and Gregory, P., 1978, Total foliar glycoalkaloids and resistance of wild potato species to *Empoasca fabae* (Harris), American Potato Journal, 55:577-585.
- Tomás-Barberán, F. A., García-Grau, M. M., and Tomás-Lorente, F., 1991, Flavonoid concentration changes in maturing broad bean pods, Journal of Agricultural and Food Chemistry, 39:255-258.
- Toth, B., 1979, Hepatocarcinogenesis by hydrazine mycotoxins of edible mushrooms, Journal of Toxicology and Environmental Health, 5:193-202.
- Toth, B., 1983, Carcinogens in edible mushrooms, p. 99-108, in: "Carcinogens and Mutagens in the Environment: Naturally Occurring Compounds," Volume III, H. F. Stich, ed., CRC Press, Inc., Boca Raton, Florida.
- Toth, B., and Nagel, D., 1981, Studies of the tumorigenic potential of 4-substituted phenylhydrazines by the subcutaneous route, Journal of Toxicology and Environmental Health, 8:1-9.
- Toth, B., Nagel, D., and Ross, A., 1980, Occurrence and the carcinogenic action of 4-(hydroxymethyl)benzene diazonium ion (4-HMBD), Proceedings of the American Association for Cancer Research, 21:73.
- Toth, B., Raha, C. R., Wallcave, L., and Nagel, D., 1981, Attempted tumor induction with agaritine in mice, Anticancer Research, 1:255-258.
- Toth, B., and Sornson, H., 1984, Lack of carcinogenicity of agaritine by subcutaneous administration in mice, Mycopathologia, 85:75-79.
- Truitt, E. B., Jr., Callaway, E., Braude, M. C., and Krantz, J. C. Jr., 1961, The pharmacology of myristicin, a contribution to the psychopharmacology of nutmeg, Journal of Neuropsychiatry, 2:205-210.
- Trumble, J. T., Dercks, W., Quiros, C. F., and Beier, R. C., 1990, Host plant resistance and linear furanocoumarin content of *Apium* accessions, Journal of Economic Entomology, 83:519-525.
- Trumble, J. T., and Quiros, C. F., 1988, Antixenotic and antibiotic resistance in *Apium* species to *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), Journal of Economic Entomology, 81:602-607.
- Tuite, J., 1977, Mycology of mycotoxic fungi, The genus *Aspergillus*, p. 21-39, in: "Mycotoxic Fungi, Mycotoxins, and Mycotoxicoses," Volume 1, T. D. Wyllie and L. G. Morehouse, eds., Marcel Dekker, New York, New York.
- Ueno, Y., 1977, Trichothecenes: Overview address, p. 189-207, in: "Mycotoxins in Human and Animal Health," J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, eds., Pathotox Publishers, Inc., Forest Park South, Illinois.
- Umeda, M., Tsutsui, T., and Saito, M., 1977, Mutagenicity and inducibility of DNA-single strand breaks and chromosome aberrations by various mycotoxins, Gann, 68:619-625.
- Uritani, I., and Miyano, M., 1955, Derivatives of caffeic acid in sweet potato attacked by black rot, Nature, 175:812.
- van der Linde, J. A., Frens, A. M., de Jongh, H., and Vles, R. O., 1964, Inspection of milk from cows fed aflatoxin-containing groundnut meal, Tijdschrift voor Diergeneeskunde, 89:1082-1088.
- Van Egmond, H. P., 1989, Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis, Food Additives and Contaminants, 6:139-188.
- VanEtten, C. H., Daxenbichler, M. E., Tookey, H. L., Kwolek, W. F., Williams, P. H., and Yoder, O. C., 1980, Glucosinolates: Potential toxicants in cabbage cultivars, Journal of the American Society for Horticultural Science, 105:710-714.
- VanEtten, C. H., Daxenbichler, M. E., Williams, P. H., and Kwolek, W. F., 1976, Glucosinolates and derived products in cruciferous vegetables: Analysis of the

- edible part from twenty-two varieties of cabbage, *Journal of Agricultural and Food Chemistry*, 24:452-455.
- VanEtten, C. H., and Tookey, H. L., 1983, Glucosinolates, p. 15-30, *in: "CRC Handbook of Naturally Occurring Food Toxicants,"* M. Rechcigl, Jr., ed., CRC Press, Boca Raton, Florida.
- Vannier, S. H., and Stanley, W. L., 1958, Fluorometric determination of 7-geranoxycoumarin in lemon oil: Analysis of mixtures of grapefruit oil in lemon oil, *Journal of the Association of Official Agricultural Chemists*, 41:432-435.
- van Rensburg, S. J., Marasas, W. F. O., Gelderblom, W. C. A., Thiel, P. G., and Rabie, C. J., 1982, Mycotoxins and oesophageal cancer, *Proceedings of the Vth International IUPAC Symposium on Mycotoxins and Phycotoxins*, Vienna, Austria, p. 265-268.
- Van Scott, E. J., 1976, Therapy of psoriasis 1975, *Journal of the American Medical Association*, 235:197-198.
- Varma, S. D., Mikuni, I., and Kinoshita, J. H., 1975, Flavonoids as inhibitors of lens aldose reductase, *Science*, 188:1215-1216.
- Varma, S. D., Mizuno, A., and Kinoshita, J. H., 1977, Diabetic cataracts and flavonoids, *Science*, 195:205-206.
- Vederas, J. C., 1985, Biosynthetic studies on mycotoxins using multiple stable isotope labelling and NMR spectroscopy, p. 97-108, *in: "Mycotoxins and Phycotoxins,"* P. S. Steyn and R. Vleggaar, eds., Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa.
- Veech, J. A., 1979, Histochemical localization and nematoxicity of terpenoid aldehydes in cotton, *Journal of Nematology*, 11:240-246.
- Virtanen, A. I., 1965, Studies on organic sulphur compounds and other labile substances in plants, *Phytochemistry*, 4:207-228.
- Vo-Dinh, T., 1987, Evaluation of an improved fiberoptics luminescence skin monitor with background correction, *American Industrial Hygiene Association Journal*, 48:594-598.
- Vo-Dinh, T., and Gammage, R. B., 1981, The lightpipe luminoscope for monitoring occupational skin contamination, *American Industrial Hygiene Association Journal*, 42:112-120.
- Vo-Dinh, T., White, D. A., O'Malley, M. A., Seligman, P. J., and Beier, R. C., 1988, Fluorescence detection of phototoxic psoralens in vegetable products, *Journal of Agricultural and Food Chemistry*, 36:333-337.
- Von Der Hude, W., and Braun, R., 1983, On the mutagenicity of metabolites derived from the mushroom poison gyromitrin, *Toxicology*, 26:155-160.
- Voss, K. A., Norred, W. P., Plattner, R. D., and Bacon, C. W., 1989, Hepatotoxicity and renal toxicity in rats of corn samples associated with field cases of equine leukoencephalomalacia, *Food and Chemical Toxicology*, 27:89-96.
- Wall, M. E., Wani, M. C., Manikumar, G., Abraham, P., Taylor, H., Hughes, T. J., Warner, J., and McGivney, R., 1988, Plant antimutagenic agents, 2. Flavonoids, *Journal of Natural Products* 51(6):1084-1091.
- Wallace, R. B., Hoover, J., Sandler, D., Rifkind, B. M., and Tyroler, H. A., 1977, Altered plasma-lipids associated with oral contraceptive or oestrogen consumption; The lipid research clinic program, *Lancet*, 2:11-14.
- Walters, C. L., Carr, F. P. A., Dyke, C. S., Saxby, M. J., Smith, P. L. R., and Walker, R., 1979, Nitrite sources and nitrosamine formation *in vitro* and *in vivo*, *Food and Cosmetics Toxicology*, 17:473-479.
- Ward, E. W. B., Unwin, C. H., Hill, J., and Stoessl, A., 1975, Sesquiterpenoid phytoalexins from fruits of eggplants, *Phytopathology*, 65:859-863.
- Watanabe, T., Shiraishi, T., Sasaki, H., and Oishi, M., 1989, Inhibitors for protein-tyrosine kinases, ST638 and genistein, induce differentiation of mouse

- erythroleukemia cells in a synergistic manner, *Experimental Cell Research*, 183:335-342.
- Wattenberg, L. W., 1971, Studies of polycyclic hydrocarbon hydroxylases of the intestine possibly related to cancer: Effect of diet on benzpyrene hydroxylase activity, *Cancer*, 28:99-102.
- Wattenberg, L. W., 1983, Inhibition of neoplasia by minor dietary constituents, *Cancer Research*, 43:2448s-2453s.
- Webster, B., Marine, D., and Cipra, A., 1931, The occurrence of seasonal variations in the goiter of rabbits produced by feeding cabbage, *Journal of Experimental Medicine*, 53:81-91.
- Wehner, F. C., Thiel, P. G., and DuRand, M., 1979, Mutagenicity of the mycotoxin emodin in the *Salmonella*/microsome system, *Applied and Environmental Microbiology*, 37:658-660.
- Wells, J. M., Cole, R. J., and Kirksey, J. W., 1975, Emodin, a toxic metabolite of *Aspergillus wentii* isolated from weevil-damaged chestnuts, *Applied Microbiology*, 30:26-28.
- Wheeler, M. H., and Bell, A. A., 1988, Melanins and their importance in pathogenic fungi, p. 338-387, in: "Current Topics in Medical Mycology," Volume 2, M. R. McGinnis, ed., Springer-Verlag, New York, New York.
- Wheeler, M. H., and Bhatnagar, D., 1991, Inhibition of aflatoxin biosynthesis by the fungicides blastin, fthalide and pyroquilon, Abstracts of the Annual Meeting of the American Society of Microbiology, Dallas, Texas, p. 261.
- Wheeler, M. H., Bhatnagar, D., and Bennett, J. W., 1988, Inhibition of aflatoxin biosynthesis by *Aspergillus flavus* and *A. parasiticus* with chlobenthiazone, Abstracts of the Annual Meeting of the American Phytopathology Society, St. Paul, Minnesota, *Phytopathology*, 78:1617.
- Wheeler, M. H., Bhatnagar, D., and Klich, M. A., 1991, Effects of Chlobenthiazone on aflatoxin biosynthesis in *Aspergillus parasiticus* and *A. flavus*, *Pesticide Biochemistry and Physiology*, 41:190-197.
- Wheeler, M. H., Bhatnagar, D., and Rojas, M. G., 1989, Chlobenthiazone and tricyclazole inhibition of aflatoxin biosynthesis by *Aspergillus flavus*, *Pesticide Biochemistry and Physiology*, 35:315-323.
- White, J. W., Jr., 1975, Relative significance of dietary sources of nitrate and nitrite, *Journal of Agricultural and Food Chemistry*, 23:886-891.
- Willimott, S. G., 1933, An investigation of solanine poisoning, *Analyst*, 58:431-439.
- Wilson, B. J., 1973, Toxicity of mold-damaged sweet potatoes, *Nutrition Reviews*, 31:73-78.
- Wilson, B. J., 1978, Hazards of mycotoxins to public health, *Journal of Food Protection*, 41:375-384.
- Wilson, B. J., Boyd, M. R., Harris, T. M., and Yang, D. T. C., 1971, A lung oedema factor from mouldy sweet potatoes (*Ipomoea batatas*), *Nature*, 231:52-53.
- Wilson, B. J., and Burka, L. T., 1983, Sweet potato toxins and related toxic furans, p. 3-42, in: "Handbook of Natural Toxins: Plant and Fungal Toxins," Volume I, R. F. Keeler and A. T. Tu, eds., Marcel Dekker Inc., New York, New York.
- Wilson, B. J., and Maronpot, R. R., 1971, Causative fungal agent of leucoencephalomalacia in equine animals, *The Veterinary Record*, 88:484-485.
- Wilson, B. J., Maronpot, R. R., and Hildebrandt, P. K., 1973, Equine leukoencephalomalacia, *Journal of the American Veterinary Medical Association*, 163(11):1293-1295.
- Wilson, B. J., Yang, D. T. C., and Boyd, M. R., 1970, Toxicity of mould-damaged sweet potatoes (*Ipomoea batatas*), *Nature*, 227:521-522.
- Wilson, G. S., 1959, A small outbreak of solanine poisoning, *Monthly Bulletin of the Ministry of Health and Public Health Laboratory Services*, 18:207-210.

- Wilson, R. H., Poley, G. W., and DeEds, F., 1961, Some pharmacologic and toxicologic properties of tomatine and its derivatives, *Toxicology and Applied Pharmacology*, 3:39-48.
- Wilson, T. M., Nelson, P. E., and Knepp, C. R., 1985a, Hepatic neoplastic nodules, adenofibrosis, and cholangiocarcinomas in male Fisher 344 rats fed corn naturally contaminated with *Fusarium moniliforme*, *Carcinogenesis*, 6:1155-1160.
- Wilson, T. M., Nelson, P. E., Ryan, T. B., Rouse, C. D., Pittman, C. W., Neal, T. P., Porterfield, M. L., and Saunders, G. K., 1985b, Linking leukoencephalomalacia to commercial horse rations, *Equine Practice*, 80(11):63-69.
- Wilson, T. M., Ross, F. P., Rice, L. G., Osweiler, G. D., Nelson, H. A., Owens, D. L., Plattner, R. D., Reggiano, C., Noon, T. H., and Pickrell, J. W., 1990, Fumonisin B<sub>1</sub> levels associated with an epizootic of equine leukoencephalomalacia, *Journal of Veterinary Diagnostic Investigation*, 2:213-216.
- Witkin, L. B., 1956, Acute toxicity of hydrazine and some of its methylated derivatives, *American Medical Association Archives of Industrial Health*, 13:34-36.
- Wogan, G. N., and Paglialunga, S., 1974, Carcinogenicity of synthetic aflatoxin M<sub>1</sub> in rats, *Food and Cosmetics Toxicology*, 12:381-384.
- Wolf, M. J., and Duggar, B. M., 1946, Estimation and physiological role of solanine in the potato, *Journal of Agricultural Research*, 73:1-32.
- Wollenweber, E., and Dietz, V. H., 1981, Occurrence and distribution of free flavonoid aglycones in plants, *Phytochemistry*, 20(5):869-932.
- Woo, Y.-T., Lai, D. Y., Arcos, J. C., and Argus, M. F., 1988, Plant flavonoids, p. 384-396 in: "Chemical Induction of Cancer, Structural Bases and Biological Mechanisms," Academic Press, New York, New York.
- Wood, F. A., and Young, D. A., 1974, TGA in potatoes, Publication No. 1533, Canada Department of Agriculture.
- Woolcock, A. J., Blackburn, C. R. B., Freeman, M. H., Zylstra, W., and Spring, S. R., 1970, *American Review of Respiratory Disease*, 102:575-590.
- World Health Organization, 1987, Evaluation of certain food additives and contaminants, World Health Organization, Technical Report Series, 759:1-53.
- Wray, B. B., and O'Steen, K. G., 1975, Mycotoxin-producing fungi from house associated with leukemia, *Archives of Environmental Health*, 30:571-573.
- Wright, A. V., Niskanen, A., Pyysalo, H., and Korpeila, H., 1978, The toxicity of some N-methyl-N-formylhydrazones from *Gyromitra esculenta* and related compounds in mouse and microbial tests, *Toxicology and Applied Pharmacology*, 45:429-434.
- Wu, C. M., Koehler, P. E., and Ayres, J. C., 1972, Isolation and identification of xanthotoxin (8-methoxypsoralen) and bergapten (5-methoxypsoralen) from celery infected with *Sclerotinia sclerotiorum*, *Applied Microbiology*, 23:852-856.
- Wu, M. T., and Salunkhe, D. K., 1978, Difference between light- and wound-induced biosynthesis of  $\alpha$ -solanine and  $\alpha$ -chaconine in potato tubers, *Biologia Plantarum*, 20:149-151.
- Yamaguchi, T., 1980, Mutagenicity of isothiocyanates, isocyanates and thioureas on *Salmonella typhimurium*, *Agricultural and Biological Chemistry*, 44:3017-3018.
- Yang, C. S., 1980, Research on esophageal cancer in China: A review, *Cancer Research*, 40:2633-2644.
- Yates, S. G., and England, R. E., 1982, Isolation and analysis of carrot constituents: Myristicin, falcarinol, and falcarindiol, *Journal of Agricultural and Food Chemistry*, 30:317-320.
- Young, A. R., 1986, Aspects of psoralen phototumorigenesis with emphasis on the possible role of tumour promotion, *Biochimie*, 68:885-889.
- Yourtee, D. M., and Kirk-Yourtee, C. L., 1986, The mutagenicity of aflatoxin Q<sub>1</sub> to *Salmonella typhimurium* TA 100 with or without rat or human liver microsomal

- preparations, Research Communications in Chemical Pathology and Pharmacology, 54:101-113.
- Yusof, S., Ghazali, H. M., and King, G. S., 1990, Naringin content in local citrus fruits, Food Chemistry, 37:113-121.
- Zaika, L. L., and Buchanan, R. L., 1987, Review of compounds affecting the biosynthesis or bioregulation of aflatoxins, Journal of Food Protection, 50:691-708.
- Zaynoun, S. T., Aftimos, B. G., Ali, L. A., Tenekjian, K. K., Khalidi, U., and Kurban, A. K., 1984, *Ficus carica*; isolation and quantification of the photoactive components, Contact Dermatitis, 11:21-25.
- Ziprin, R. L., and McMurray, D. N., 1988, Differential effect of T-2 toxin on murine host resistance to three facultative intracellular bacterial pathogens: *Listeria monocytogenes*, *Salmonella typhimurium*, and *Mycobacterium bovis*, American Journal of Veterinary Research, 49:1188-1192.
- Zitnak, A., 1961, The occurrence and distribution of free alkaloid solanidine in netted gem potatoes, Canadian Journal of Biochemistry and Physiology, 39:1257-1265.

## FUTURE FOR NATURAL PRODUCTS

Herbert N. Nigg

Citrus Research and Education Center  
University of Florida  
700 Experiment Station Road  
Lake Alfred, FL 33850

David S. Seigler

Department of Plant Biology  
University of Illinois  
289 Morrill Hall  
505 S. Goodwin Street  
Urbana, IL 61801

### INTRODUCTION

A host of plant, animal, fungal, and bacterially derived chemicals, often known as natural products, are involved in many aspects of human existence. These natural products may be used as purified compounds or as components of complex mixtures which serve as medicines, pesticides, flavorings, herbicides, dyestuffs, tanning agents, rubber, food preservatives, detergents, perfumes, resins, gums, etc., as well as such esoteric uses as arrow poisons or piscicides. Some are the active components of masticatories such as tobacco, khat, and betel; others provide the stimulating effects of beverages; some provide sweetness, while yet others are basic components of the foods we eat. Natural products produced by fungi are frequent food contaminants. Man has even used some natural products to poison other human beings. What is the future of man's usage of these compounds?

To determine the future, one should examine the past. Why have so many chemical entities arisen? The answer to this may lie in ecological and biological interactions. In the course of evolution of all major kinds of organisms, new pathways arose which differed from the basic steps involved in respiration and photosynthesis. The products of these pathways were almost always deleterious but, in rare instances, gave some selective advantage to the producing organism. Some interfered with the proper functioning of other organisms, i.e., they served as defensive compounds for the organism which produced them. In some instances, there was also selection for the ability to recognize these chemical warfare agents; and they became cues by which a predatory organism could locate the producing organism. As evolution progressed, the skies, water, and ground were laced with a variety of these biologically active

"messages." Compounds with many types of activity are known; these may interact with most biochemical processes and affect many systems and receptors of organisms in the world around us. No living being has escaped the selective effects exerted on this chemical battleground. Man is no exception.

How did primitive man encounter natural products? First of all, he found them in the foods he ate. Most animals have developed search strategies to locate acceptable food items, none of which are devoid of chemical toxins (Beier and Nigg, this volume; Duke, this volume). Man "learned" (or was selected?) to prefer food items containing toxins which were tolerable in metabolic terms. No doubt, many of these experiments were empirical; but in nature, few wild primates are seriously poisoned by food items and the same was probably true for early man. The utilization of spices and herbs for flavoring and for food preservation predates written human history; poisoning by fungal and bacterial contaminants undoubtedly does as well. At some point man learned to use these compounds to poison arrows and to capture fish (piscicides). These advancements provided a more constant and secure food supply. Other organisms provided dyestuffs. Man learned to preserve hides as leather with others. And so the discovery of natural products was initiated, and continues to the present. Although modern man is, in some regards, more sophisticated than our remote ancestors, we interact with and use many natural products in similar ways.

No human society, however undeveloped, lacks a knowledge of medicinal products (Lewis and Elvin-Lewis, 1977), but even non-human primates appear to have learned to use certain plants in this manner (Rodriguez et al., 1985). Among primitive peoples, the practice of medicine was intimately intermixed with religion. For example, many medicinal properties were attributed to frankincense and myrrh (Miller and Goodell, 1968). Frankincense, cited in Celsus' *De Medicina*, was used to treat paralyzed limbs; stop hemorrhages; mature abscesses; clean wounds; heal bruises, anal fissures, piles, and canker; and was a poison antidote and emollient ointment. Myrrh was used for a 'broken head', inflammation, fissures, piles, canker, and to induce menstruation. It was also used for infections of the eye, ear, throat, and mouth, as well as an antidote for poison and as an additional ingredient in frankincense preparations. *De Medicina* was written between 25 and 35 AD, but Egyptian commerce in frankincense and myrrh was flourishing by at least 2900 BC (Miller and Goodell, 1968). However, both of these resins were also extensively used as an incense ingredient in religious practice. Although resins such as frankincense and myrrh have limited use, many other medicinal products were derived from Near Eastern cultures, such as morphine from opium poppies, ergot alkaloids from ergot, and atropine from *Atropa belladonna* and other solanaceous plants.

But what about current usage of these natural products? Biologically active compounds from a wide range of sources are still important in many aspects of modern civilization. Just as they were for primitive man, poisonous substances in the food we eat are still a significant problem. For example, quercetin, one of the most widespread natural products in fruits and vegetables, is positive in the Ames test, the chromosome breakage test, and is a carcinogen in male mice [these results call into question the practice of testing a single compound at the maximum tolerated dose and Ames has challenged the way these tests are conducted (Hay, 1991)]. But other types of compounds such as furanocoumarins in celery, parsley, and celery root are less easily dismissed (Beier and Nigg, this volume).

Many of the natural products responsible for medicinal activity from natural drugs have been isolated and a large number are now available through synthesis; others have served as templates for the synthesis of derivative drugs with greater effectiveness, or special properties (Balandrin et al., 1985).

The use of compounds from microorganisms has a long history in medicine

(Kinghorn, this volume). A recent example of the importance of these compounds is the development of avermectin and ivermectin by Merck & Co., Inc. (1991). In 1987, ivermectin was Merck's second largest selling drug; since 1984, it has been the largest selling animal health product in the world (Eckholm, 1989). In 1980, about \$8.1 billion was spent for pharmacy dispensed plant-derived drugs in the U.S. This was 25% of the total prescriptions (synthetic and plant-derived) filled in pharmacies, but did not include hospital dispensed drugs (Farnsworth and Loub, 1983; Kinghorn, this volume). If these ratios still hold (corrected for inflation), consumers will spend about \$16.2 billion for plant-derived drugs in 1992. About 75% of the world's population still relies on traditional medicine (Abelson, 1990).

In 1990, herbicides were applied to 75%, insecticides to 84%, and fungicides to 62% of the vegetable acres in Florida, Arizona, Michigan, and Texas (USDA, 1991a). In 47 corn producing states, herbicides were applied to 92.4% of the acreage and, for upland cotton (six states), 94.6% of the acreage (USDA, 1991b). In eleven potato states, herbicides were used on 79.2% and insecticides on 88.2% of the acreage (USDA, 1991b). Large amounts of pesticides are also marketed for home and garden use. In 1990, the end user market for pesticides was \$20 billion. Of this, the U.S. used 22%, Japan 17%, France 10%, the Soviet Union 7%, Brazil 5%, and Italy 4% (Menn, 1990). However, the public perception that synthetic pesticides are 'bad' makes the future for these compounds less certain than in the past.

How can we most effectively identify new biologically active and useful natural products? Modern man has used many approaches to find new biologically active compounds. These range from random synthesis and screening (Menn, 1983; Menn and Henrick, 1981, 1984), basic biochemical, physiological, and behavioral research (Masler et al., 1991; Menn, 1985; Menn and Pallos, 1975; Menn et al., 1981, 1989), to the study of folklore and ethnobotany (Balick, 1990; Fox, 1991; Kinghorn, 1987; Waterman, 1990). Other useful natural products have been discovered by bioassay-guided serendipity, straight serendipity, chemotaxonomy, or selecting plants with few or no pest problems (Waterman, 1990). Examination of both the biology and chemistry of the organisms involved in biological interactions (a field of study known as "chemical ecology") can yield information about the mechanisms of biological communication, but also can provide many new leads to useful natural products.

Most pesticide companies appear to use the random synthesis and screening approach (Menn, 1980; Menn and Henrick, 1984) though several important types of insecticides are directly derived from plants and others, such as the nitroketenedimethyl mercaptols, were based on a knowledge of the structure and activity of nicotine and insect antifeedants (Menn, 1980). One of the most successful types of insecticidal and helminthicidal compounds, the avermectins, was discovered by random screening (Menn and Henrick, 1984).

In an effort to discover new medicinal as well as other types of bioactive compounds, considerable attention has been given to ethnobotanical uses of plant drugs (Balick, 1990).

Bioactive compounds from a variety of natural sources have been proposed to be useful for treating a number of human maladies such as schistosomiasis (Hamburger and Hostettmann, 1991; Hostettmann, 1984), cancer (Kingston et al., 1990; Svoboda, 1983), viral diseases (Che, 1991; Vanden Berghe et al., 1986), and AIDS (Nonaka et al., 1990). Other medically important natural products include anti-inflammatory (Sertié et al., 1990), antihepatotoxic (Houghton and Hikino, 1989), antiphlogistic and antiallergic (Wagner, 1989), antimutagenic (Wall et al., 1988), antimicrobial (Metzner et al., 1979; Řeháček, 1990), and fungicidal drugs (Langcake, 1981; Lwande et al., 1986).

Bioactive compounds with other activities such as herbicides (Saito et al., 1989),

insecticides and insect repellents and attractants (Jacobson, 1983), and molluscicides (Lemma, 1983) have been reported.

Despite the fact that many important drugs are derived from plants, there has been a decline in new drugs from plants in the U.S. This is linked with a strong emphasis on synthetic chemistry and structure-activity relationships. According to Farnsworth, only three plant-derived drugs were developed and marketed by U.S. industry from 1950 to 1983. No new plant-derived pesticides became commercial products during this period.

The lack of discovery and development of new bioactive natural products, with some notable exceptions, appears to result from lack of investigation rather than failure of programs to detect active compounds. In contrast to the U.S., the Japanese and Germans have active programs for screening of plant-derived as well as other natural products (Abelson, 1990; Farnsworth and Loub, 1983) for a number of types of activity. Many corporate efforts in the U.S. include plant materials, but only as a small proportion of the materials to be tested. Although the National Cancer Institute has evaluated many biological extracts and natural products for anti-tumor and anti-AIDS activity, the materials used apparently are not evaluated for other types of biological activity (Hamburger and Hostettmann, 1991). Because of their importance, the National Toxicology Program has emphasized testing of synthetic compounds.

Yet the breeding community is rushing to produce 'synthetic' plants with biotechnology (Constabel, 1990). These plants, unique to Earth, hold the promise of higher yields and resistance to cold, drought, and pests. We should not hope that these new plants will provide safer food for the consumer, inasmuch as we know precious little about the relationship between food plant chemistry and human health.

Conflicting claims of carcinogenic and anti-tumor activity of natural products from common foods (American Cancer Society, 1984; Beier and Nigg, this volume; National Research Council, 1982) and the tremendous public interest in "safe" foods suggest that many natural products from foods should be studied for adverse health affects.

A vast body of literature on ethnobotanical and folkloric uses of natural products exists, but many of these reports and data are found in relatively obscure and poorly indexed publications. Others are in older works that are not widely available. Nonetheless, these sources are of major importance because either the peoples studied no longer exist, or their cultures have been sufficiently modified to result in loss of much valuable information. At present, there is great interest in ethnobotany, especially because of the rapid destruction of both peoples of rain forests and rain forests (Balick, 1990). Despite the paramount importance of access to published natural product information, the USDA's economic botany data base was taken off-line for lack of funding (Duke, 1983).

In order to take full advantage of the host of natural products that have been produced in the evolution of living organisms, several steps should be taken. We should undertake studies of chemical ecology while many of the organisms yet exist. This type of interdisciplinary research is, at present, poorly supported. Timing is crucial; a large fraction of the world's organisms, especially those of rain forests where some of the most pronounced and intricate biological interactions occur, are being destroyed at a rapid pace. Collection and testing of plants, animals, fungi, bacteria, etc. should be accelerated, but sensitivity to the problems of dwindling populations of many organisms is required. Funding of studies of ethnobotanical, folkloric, and traditional uses of natural products by various people groups should be established before the few remaining intact cultures disappear and the information is forever lost. New information, as well as extant literature, needs to be surveyed and stored in data bases that are accessible to a wide range of investigators. Probably this would best be done through the oversight of the National Science Foundation, the National

Institutes of Health, or the Department of Agriculture. New methods of biotechnology need to be developed to maintain organisms and to produce natural products in the laboratory. A recent example of the importance of this approach involves taxol. In order to evaluate the anti-cancer potential of this intriguing diterpene, the species which produces it most abundantly may be exterminated (Hamburger and Hostettmann, 1991). Many (if not most) previously reported data on biological activity of natural products are not valid because of the lack of specificity or sensitivity of the bioassay methods employed. New methods of bioassay must be developed and made more widely available. Many capable investigators have been discouraged from entering this field because of the lack of research funding, positions, and associated problems.

Finally, there is a widespread perception that natural products research is not good, solid science, but rather a "fishing expedition" or "merely screening" biological materials. Work in this area has been poorly funded by the National Science Foundation, at least in part, because it is too "practical" and lacks "theoretical" robustness. Projects are rarely sufficiently ecological, systematic, or chemical to warrant support by programs representing these disciplines. Similar criticisms apply to other funding agencies as well.

Changes are evident in the prospective utilization of natural products. It is predicted that, whereas biological methods of pest control accounted for 1% of the market in 1987 (\$200 million), they will account for 10% by the year 2000 (Menn, 1990). Attitudes toward interdisciplinary research and funding also seem to be changing. The realization that most natural products oriented problems require collaborative efforts of many scientific disciplines is being recognized. There has been renewed interest at the National Science Foundation, National Institutes of Health, and USAID in funding natural product oriented projects from a broad perspective. Corporations also have expressed renewed interest in this area of research. Heightened public interest in better quality food products will undoubtedly force new studies in this vital area. While problems remain, the future payoff is too great not to attract many entrepreneurs and visionaries. The future for natural products promises to be bright and exciting as we systematically tap this vast resource for improvement of the quality of life.

## REFERENCES

- Abelson, P. H., 1990, Medicine from plants, *Science*, Volume 247, Number 4942, p. 513.
- American Cancer Society, 1984, Nutrition and cancer: Cause and prevention, *California Cancer Journal of Clinicians*, 34:121-126.
- Balandrin, M. F., Klocke, J. A., Wurtele, E. S., and Bollinger, W. H., 1985, Natural plant chemicals: Sources of industrial and medicinal materials, *Science*, 228:1154-1160.
- Balick, M. J., 1990, Ethnobotany and the identification of therapeutic agents from the rainforest, p. 22-39, in: "Bioactive Compounds from Plants," D. J. Chadwick and J. Marsh, eds., Ciba Foundation Symposium No. 154, John Wiley and Sons, Chichester, United Kingdom.
- Che, C., 1991, Marine products as a source of antiviral drug leads, *Drug Development Research*, 23:201-218.
- Constabel, F., 1990, Medicinal plant biotechnology, *Planta Medica*, 56(5):421-492.
- Duke, J., 1983, The USDA economic botany laboratory's data base on minor economic plant species, p. 196-214, in: "Plants: The Potentials for Extracting Protein, Medicines, and Other Useful Chemicals—Workshop Proceedings"

- (Washington, D.C.: U.S. Congress, Office of Technology Assessment, OTA-BP-F-23, September 1983.
- Eckholm, E., 1989, River blindness: Conquering an ancient scourge, *The New York Times Magazine*, January 8, 1989, Section 6, p. 20-27, 58-59.
- Farnsworth, N. R. and Loub, W. D., 1983, Information gathering and data bases that are pertinent to the development of plant-derived drugs, p. 178-195, *in*: "Plants: The Potentials for Extracting Protein, Medicines, and Other Useful Chemicals—Workshop Proceedings" (Washington, D.C.: U.S. Congress, Office of Technology Assessment, OTA-BP-F-23, September 1983).
- Fox, B. W., 1991, Medicinal plants in tropical medicine. 2. Natural products in cancer treatment from bench to the clinic, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85:22-25.
- Hamburger, M., and Hostettmann, K., 1991, Bioactivity in plants: The link between phytochemistry and medicine, *Phytochemistry*, 30:3864-3874.
- Hay, A., 1991, Carcinogenesis: Testing times for the tests, *Nature*, 350:555-556.
- Hostettmann, K., 1984, On the use of plants and plant-derived compounds for the control of schistosomiasis, *Naturwissenschaften*, 71:247-251.
- Houghton, P. J., and Hikino, H., 1989, Anti-hepatotoxic activity of extracts and constituents of *Buddleja* species, *Planta Medica*, 55:123-126.
- Jacobson, M., 1983, Insecticides, insect repellants, and attractants from arid/semiarid-land plants, p. 138-146, *in*: "Plants: The Potentials for Extracting Protein, Medicines, and Other Useful Chemicals—Workshop Proceedings" (Washington, D.C.: U.S. Congress, Office of Technology Assessment, OTA-BP-F-23, September 1983), U.S. Government Printing Office, Washington, D.C.
- Kinghorn, A. D., 1987, Biologically active compounds from plants with reputed medicinal and sweetening properties, *Journal of Natural Products*, 50(6):1009-1024.
- Kingston, D. G. I., Samaranayake, G., and Ivey, C. A., 1990, The chemistry of taxol, a clinically useful anticancer agent, *Journal of Natural Products*, 53(1):1-12.
- Langcake, P., 1981, Alternative chemical agents for controlling plant disease, *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 295:83-101.
- Lemma, A., 1983, Molluscicidal and other economic potentials of Endod, p. 111-125, *in*: "Plants: The Potentials for Extracting Protein, Medicines, and Other Useful Chemicals—Workshop Proceedings" (Washington, D.C.: U.S. Congress, Office of Technology Assessment, OTA-BP-F-23, September 1983), U.S. Government Printing Office, Washington, D.C.
- Lewis, W. H., and Elvin-Lewis, M. P. F., 1977, *Medical Botany*, John Wiley and Sons, New York, New York.
- Lwande, W., Hassanali, A., Bentley, M. D., and Monache, F. D., 1986, 8-C-Prenylated flavones from the roots of *Tephrosia hildebrandtii*, *Journal of Natural Products*, 49:1157-1158.
- Masler, E. P., Kelly, T. J., and Menn, J. J., 1991, Biologically active insect peptides: Prospects for applied and fundamental knowledge, Chapter 2, p. 6-18, *in*: "Insect Neuropeptides: Chemistry, Biology, and Action," J. J. Menn, T. J. Kelly, and E. P. Masler, eds., American Chemical Society Series 453, Washington, D.C.
- Menn, J. J., 1980, Contemporary frontiers in chemical pesticide research, *Journal of Agricultural and Food Chemistry*, 28:2-8.
- Menn, J. J., 1983, Present insecticides and approaches to discovery of environmentally acceptable chemicals for pest management, Chapter 1, p. 5-31, *in*: "Natural Products for Innovative Pest Management," D. L. Whitehead, ed., Pergamon Press, Oxford and New York, New York.

- Menn, J. J., 1985, Prospects of exploitation of insect antijuvenile hormones for selective insect control, p. 37-46, in: "Approaches to New Leads for Insecticides," von Keyserlingk et al., eds., Springer-Verlag, Berlin and Heidelberg.
- Menn, J. J., 1990, Current trends and new directions in crop protection, American Journal of Industrial Medicine, 18:499-504.
- Menn, J. J., and Henrick, C. A., 1981, Rational and biorational design of pesticides, Philosophical Transactions of the Royal Society of London B Biological Sciences, 295:57-71.
- Menn, J. J., and Henrick, C. A., 1984, Newer chemicals for insect control, Chapter 21, p. 247-265, in: "Agricultural Chemicals of the Future," J. L. Hilton, ed., BARC Symposium 8, Rowman & Allanheld, Totowa.
- Menn, J. J., Henrick, C. A., and Staal, G. B., 1981, Juvenoids: Bioactivity and prospects for insect management, p. 735-748, in: "Regulation of Insect Development and Behaviour," Scientific Papers of the Institute of Organic and Physical Chemistry of Wroclaw Technical University, No. 22, Conferences 7, Karpacz, Poland.
- Menn, J. J., King, E. G., and Coleman, R. J., 1989, Future control strategies for *Heliothis* in cotton, Chapter 10, p. 101-121, in: "Pest Management in Cotton," published by Ellis Horwood Limited.
- Menn, J. J., and Pallos, F. M., 1975, Development of morphogenetic agents in insect control, Environmental Letters, 8(1):71-88.
- Merck & Co., Inc., 1991, News Release, 5 p.
- Metzner, J., Bekemeier, H., Paintz, M., und Schneidewind, E., 1979, Zur antimikrobiellen Wirksamkeit von Propolis und Propolisinhaltsstoffen, Pharmazie, 34:97-102.
- Miller, J. M. and Goodell, H. B., 1968, Frankincense and Myrrh, The Surgeon's Library, The Book Shelf, Surgery, Gynecology & Obstetrics, 127:360-365.
- National Research Council, 1982, Diet, nutrition and cancer, National Academy Press, Washington, D.C.
- Nonaka, G.-I., Nishioka, I., Nishizawa, M., Yamagishi, T., Kashiwada, Y., Dutschman, G. E., Bodner, A. J., Kilkuskie, R. E., Cheng, Y.-C., and Lee, K.-H., 1990, Anti-AIDS agents, 2: Inhibitory effects of tannins on HIV reverse transcriptase and HIV replication in H9 lymphocyte cells, Journal of Natural Products, 53(3):587-595.
- Reháček, Z., 1990, Research into biogenesis of biologically active microbial metabolites in Czechoslovakia: A review, Process Biochemistry International, 25(6):197-209.
- Rodriguez, E., Aregullin, M., Shida, T. N., Wrangham, R., Abramowski, Z., Finlayson, A., and Towers, G. H. N., 1985, Thiarubrin A—A bioactive constituent of *Aspilia* (Asteraceae) consumed by wild chimpanzees, Experientia, 41:419-420.
- Saito, K., Matsumoto, M., Sekine, T., Murakoshi, I., Morisaki, N., and Iwasaki, S., 1989, Inhibitory substances from *Myriophyllum brasiliense* on growth of blue-green algae, Journal of Natural Products, 52(6):1221-1226.
- Sertié, J. A. A., Basile, A. C., Panizza, S., Matida, A. K., and Zelnik, R., 1990, Anti-inflammatory activity and sub-acute toxicity of artemetin, Planta Medica, 56:36-40.
- Svoboda, G. H., 1983, The role of the alkaloids of *Catharanthus roseus* (L.) G. Don (*Vinca rosea*) and their derivatives in cancer chemotherapy, p. 154-169, in: "Plants: The Potentials for Extracting Protein, Medicines, and Other Useful Chemicals—Workshop Proceedings" (Washington, D.C.: U.S. Congress, Office

- of Technology Assessment, OTA-BP-F-23, September 1983), U.S. Printing Office, Washington, D.C.
- United States Department of Agriculture, 1991a, "Agricultural Chemical Usage, 1990 Vegetables Summary," Agricultural Statistics Board, NASS, USDA, Washington, D.C., 118 p.
- United States Department of Agriculture, 1991b, "Agricultural Chemical Usage, 1990 Field Crops Summary," Agricultural Statistics Board, NASS, USDA, Washington, D.C., 154 p.
- Vanden Berghe, D. A., Vlietinck, A. J., and Van Hoof, L., 1986, Plant products as potential antiviral agents, *Bulletin de l'Institut Pasteur*, 84:101-147.
- Wagner, H., 1989, Search for new plant constituents with potential antiphlogistic and antiallergic activity, *Planta Medica*, 55:235-241.
- Wall, M. E., Wani, M. C., Hughes, T. J., and Taylor, H., 1988, Plant antimutagenic agents. 1. General bioassay and isolation procedures, *Journal of Natural Products*, 51(5):866-873.
- Waterman, P. G., 1990, Searching for bioactive compounds: Various strategies, *Journal of Natural Products*, 53(1):13-22.

## TAXONOMIC INDEX

- Abies balsamea*, 44, 232  
*Abies cilicia*, 27  
*Abutilon avicennae*, 218  
*Abutilon theophrasti*  
    flavonoid antigermination, 222  
    flavonoids, 266  
    herbicide, 212  
    seed germination, 214  
*Acacia concinna*, 196  
*Acacia senegal*, 44  
*Acanthamoeba* spp., 119, 155  
*Acanthus mollis*  
    herbicidal activity, 211  
    herbicidal compounds, 212  
*Achillea millefolium*, 44, 277, 278  
*Acokanthera oppositifolia*, 44  
*Aconitum lycocotonum*  
    wolf killer, 8  
*Aconitum napellus*, 44  
*Acouanthera schimperi*, 44  
*Agaricus bisporus*, 308, 310, 330  
*Agathosma betulina*, 44  
*Agavaceae*, 49  
*Ageratum houstonianum*, 233  
*Agrimonia pilosa*, 145  
*Alangiaceae*, 120  
*Alangium lamarkii*, 120  
*Albizia chinensis*, 196  
*Aletris farinosa*, 44  
*Alliaceae*, 140  
*Allium* spp., 24, 44, 140, 154, 198, 203,  
    237, 238, 266  
*Allium cepa*  
    allelopathic response, 266  
    mummy packing, 24  
*Allium fistulosum*, 198  
*Allium grayi*, 198, 203  
*Allium sativum*  
    allicin, 140  
    antibiotic, 44  
    dog-tick repellent, 238  
*Aloe ferox*, 44  
*Aloe perryi*, 44  
*Aloe vera*, 44, 62  
*Althaea officinalis*, 44  
*Amanita* spp., 48  
*Amanita ceasarea*, 4  
*Amanita phalloides*, 4, 5  
*Amaranthus retroflexus*, 218  
*Ammi majus*, 44, 281, 282, 284  
*Ammi visnaga*, 43, 44, 73  
*Anacardiaceae*, 48, 60  
*Anamirta cocculus*, 44  
*Ananas comosus*, 44, 58  
*Anethum graveolens*, 44  
*Angelica archangelica*, 44  
*Angelica pubescens*, 189  
*Anguina tritici*, 186  
*Annona montana*, 123, 153  
*Annona reticulata*, 228  
*Annonaceae*, 123, 124, 133, 138  
*Anthyllis vulneraria*, 168  
*Aphelenchoides besseyi*  
    motility bioassay, 144  
    nematicidal activity, 188, 189, 192  
    odoracin activity, 198  
*Apiaceae*, 43, 44, 46, 47, 189  
*Apium* spp., 242, 299, 332  
*Apium graveolens*, 242  
*Apium nodiflorum*, 299  
*Apocynaceae*, 44, 48, 50, 57, 120, 123,  
    127, 129  
*Arachis hypogaea*  
    nematicidal activity, 192  
    herbicidal activity, 222

*Aralia racemosa*, 44  
*Araliaceae*, 44, 47  
*Arctium lappa*, 44  
*Arctostaphylos uva-ursi*, 44  
*Ardisia japonica*, 44  
*Areca catechu*, 44, 145  
*Arecaceae*, 44, 48, 145  
*Argemone mexicana*, 192, 203  
*Artabotrys uncinatus*, 133  
*Artemia salina*, 213  
*Artemisia absinthium*, 44  
*Artemisia annua*  
    antiparasite activity, 137, 138  
    artemisinin, 65, 84, 97, 130  
*Artemisia apiacea*, 65  
*Artemisia maritima*, 44, 143  
*Arundo donax*, 212  
*Asclepias capricornu*, 28  
*Asparagus adescendens*, 196  
*Asparagus officinalis*, 44, 195  
*Aspergillus* spp., 293, 315, 318, 320, 322, 326, 327  
*Aspergillus clavatus*, 320  
*Aspergillus flavus*  
    aflatoxin production, 315  
    biosynthesis inhibition, 327  
    liver cancer, 318  
    moisture and growth, 326  
*Aspergillus ochraceus*, 322  
*Aspergillus parasiticus*, 315, 327  
*Aspergillus terreus*, 318  
*Aspergillus wentii*, 322  
*Aspidosperma* spp., 123  
*Aspilia* spp., 145  
*Aspilia mossambicensis*, 145  
*Aspilia pluriseta*, 145  
*Aspilia rufis*, 145  
*Aspleniaceae*, 45  
*Asteraceae*, 44-46, 48, 69, 130, 134, 140, 143-146, 156, 186-188, 197-199, 375  
*Astragalus gummifer*, 44  
*Astragalus membranaceus*, 283  
*Atherospermataceae*, 125  
*Atropa acuminata*, 44  
*Atropa belladonna*  
    anticholinergic, 44  
    drugs, 248  
    future, 370  
    narcotic, 53

*Atropa belladonna* (continued)  
    pupil dilation, 64  
    symptoms, 9  
*Avena sativa*, 44  
*Azadirachta indica*  
    antiparasite activity, 137  
    nematicidal activity, 197  
    uses, 44  
  
*Bacillus cereus*, 210  
*Bacillus subtilis*, 210  
*Bacteroides fragilis*, 283  
*Baillonella toxisperma*, 217  
*Berberidaceae*, 44, 45, 47, 52, 123, 125  
*Berberis* spp., 44, 123, 125  
*Berberis vulgaris*, 44  
*Beta vulgaris*, 181, 255  
*Betula lenta*, 44  
*Betulaceae*, 44  
*Bidens pilosa*, 140, 154  
*Bignoniaceae*, 137  
*Bocconia cordata*, 187  
*Boletus edulis*, 308  
*Boraginaceae*, 48  
*Botrytis cinerea*  
    alkaloid activity, 187  
    EC<sub>50</sub> fungicides, 173  
    nematode culture, 188  
*Bradyrhizobium japonicum*, 269  
*Brassica* spp., 44, 195, 216, 253, 255-257, 260  
*Brassica canolorapa*, 255  
*Brassica hirta*, 255  
*Brassica juncea*, 216  
*Brassica napus*, 255  
*Brassica nigra*  
    allyl isothiocyanate, 44  
    goiter, 255  
    nematicidal activity, 195  
*Brassica oleracea*, 255, 257  
*Brassica pekinensis*, 255  
*Brassica rapa*, 255  
*Brassicaceae*, 44  
*Bromeliaceae*, 44, 58  
*Brucea javanica*, 136  
*Brugia pahangi*, 197, 200  
*Bursaphelenchus xylophilus*, 187, 188  
*Burseraceae*, 45  
*Butea frondos*, 145

- Caenorhabditis elegans*, 145, 156, 189  
*Calendula officinalis*, 44  
*Callistemon lanceolatus*, 193  
*Caloglossa leprieurii*, 144  
*Camellia sinensis*, 44, 55  
*Campanulaceae*, 47  
*Camptotheca acuminata*, 83  
*Canavalia ensiformis*, 198  
*Candida* spp., 242, 277  
*Candida albicans*, 277  
*Cannabaceae*, 44, 46  
*Cannabidaceae*, 138  
*Cannabis sativa*  
  amoebastatic, 138  
  drugs, 44  
  figure, 39  
  psychoactive, 38  
  production, 43  
*Cantharellus* spp., 308  
*Caprifoliaceae*, 49  
*Capsicum annuum*, 44  
*Capsicum baccatum*, 44  
*Capsicum chinense*, 44  
*Capsicum frutescens*, 44  
*Capsicum oleoresin*, 44  
*Carica papaya*, 44, 58, 80  
*Caricaceae*, 44, 58  
*Carthamus tinctorius*  
  nematicidal activity, 188  
  polyacetylenes, 144  
  useful products, 44  
*Carum carvi*, 44, 242  
*Cassia* spp., 18, 21, 24, 44, 62, 67, 218,  
  266, 285  
*Cassia angustifolia*, 44, 67  
*Cassia auriculata*, 285  
*Cassia italica*, 44  
*Cassia obtusifolia*, 266  
*Cassia senna*, 44  
*Cassia tora*, 218  
*Castanosperma australe*, 86, 92  
*Catha* spp., 98, 99, 111-116  
*Catha abbotii*, 98  
*Catha cassinooides*, 98  
*Catha edulis*  
  khat history, 98  
  polymorphism, 99  
*Catha spinosa*, 98  
*Catha transvaalensis*, 98, 112  
  
*Catharanthus roseus*, 44, 50, 78, 80, 94,  
  376  
*Caulophyllum thalictroides*, 45  
*Cedri succus*, 30  
*Celastraceae*, 98, 101, 112, 113, 115,  
  137  
*Celastrus paniculatus*, 137, 155  
*Cephaelis ipecacuanha*  
  amoebicide, 119  
  emetine, 80  
  ipecac extract, 55  
  useful products, 45  
*Cephalotaxaceae*, 45, 129  
*Cephalotaxus harringtonia*, 45, 129  
*Ceratocystis fimbriata*  
  6-methoxymellein, 173  
  phytoalexin elicitor, 306  
  xanthotoxin EC<sub>50</sub>, 175  
*Cercariae*, 147  
*Ceropithecus pygerythus*, 324  
*Chamaemelum nobile*, 45  
*Chenopodiaceae*, 45, 178  
*Chenopodium* spp., 45, 117, 281  
*Chenopodium ambrosioides*, 45, 281  
*Chondrodendron tomentosum*, 45, 49,  
  80  
*Chonemorpha fragrans*, 129  
*Chrysanthemum cinerariaefolium*, 205,  
  228  
*Chrysanthemum coccinum*, 205  
*Chrysanthemum coronarium*, 233  
*Chrysanthemum onethifolium*, 205  
*Chusquea cumingii*, 212  
*Cibarius* spp., 308  
*Cimicifuga racemosa*, 45  
*Cinchona* spp.  
  amoebicidal activity, 123  
  antimalarial, 84  
  bark extracts, 57  
  cinchophyllines, 120  
  plant source, 45  
  quinine, 64, 80, 118  
  world consumption, 67  
*Cinchona calisaya*, 45  
*Cinchona ledgeriana*  
  amoebicidal activity, 120  
  aricine, 123  
  drug source, 80  
  malaria, 84  
*Cinchona officinalis*, 45, 67  
*Cinchona pelletierana*, 123

- Cinnamomum camphora*, 45  
*Cinnamomum cassia*, 24  
*Cinnamomum verum*, 45  
*Cinnamomum zeylanicum*, 24  
*Cirsium japonicum*, 188  
*Citrobacter freundii*, 210  
*Citrullus colocynthis*, 45  
*Citrus aurantifolia*, 242  
*Citrus aurantium*, 45, 242  
*Citrus limon*, 45, 50, 242  
*Citrus reticulata*, 242  
*Citrus sinensis*, 45, 242  
*Cladosporium fulvum*, 322  
*Claviceps purpurea*, 45, 64  
*Clavicipitaceae*, 45, 64  
*Clibadium*, 69  
*Clusiaceae*, 46, 138  
*Cocculus trilobus*, 228  
*Coffea* spp., 45, 239  
*Coffea arabica*, 45  
*Coffea canephora*, 45  
*Coffea liberica*, 45  
*Coix lacryma-jobi*, 212  
*Colchicum autumnale*  
    figure, 37  
    for gout, 36, 59  
    microtubule function, 129  
    as poison, 3  
    useful products, 45  
*Coleus barbatus*, 45  
*Colletotrichum circinans*, 160  
*Colletotrichum graminicola*, 178  
*Combretaceae*, 144  
*Commiphora abyssinica*, 45  
*Commiphora opobalsamum*, 45  
*Commiphora pedunculata*, 24  
*Compositae*, 165, 178, 200, 201, 215,  
    233, 277  
*Conium maculatum*, 2  
*Convolvulaceae*, 46, 165, 175  
*Copaifera officinalis*, 45  
*Coreopsis lanceolata*, 188  
*Cornaceae*, 45  
*Cornus florida*, 45  
*Coronilla eumurus*, 168  
*Corydalis* spp., 125  
*Crataegus laevigata*, 45  
*Crataegus monogyna*, 45  
*Crithidia fasciculata*, 122, 123, 137  
*Crotalaria* spp., 187, 285  
*Crotalaria juncea*, 285  
*Crotalaria spectabilis*, 187  
*Crotalaria verrucosa*, 285  
*Croton* spp., 45, 69  
*Croton tiglium*, 45  
*Cruciferae*, 195, 253  
*Cucumis sativus*, 216  
*Cucurbita moschata*, 145  
*Cucurbitaceae*, 45, 49, 145  
*Cupressaceae*, 46, 49  
*Curcuma longa*, 45  
*Cutaneous leishmaniasis*, 123, 154  
*Cyamopsis tetragonolobus*, 45  
*Cymbopogon caesius*, 194  
*Cymbopogon nardus*, 45  
*Cynara scolymus*, 45  
*Cynoglossum* spp., 187  
*Cyperus* spp., 70, 73  
*Cyperaceae*, 73, 213  
*Cytisus scoparium*, 45  
*Cytisus scoparius*, 64  
*Cytospora persoonii*, 276  
*Daphnandra micrantha*, 125  
*Daphne genkwa*, 45  
*Daphne odora*, 198, 201  
*Daphnia magna*, 251  
*Datura metel*, 53, 80  
*Datura stramonium*, 170, 266, 344  
    useful products, 45  
*Derris* spp., 228  
*Dichroa febrifuga*, 129  
*Digitalis lanata*, 67  
    digoxin, 57  
    history, 78  
    useful products, 45  
*Digitalis purpurea*  
    digoxin, 57, 77  
    folkloric use, 78  
    useful products, 45  
*Dionaea muscipula*, 45  
*Dioscorea* spp., 60, 67, 80  
*Dioscorea floribunda*, 67  
*Diphyllolothrium mansoni*, 145  
*Diplodia macrospora*, 222  
*Ditylenchus destructor*, 194  
*Ditylenchus dipsaci*, 186, 187, 194  
*Droseraceae*, 45  
*Dryopteris filix-mas*, 45

*Duboisia myoporoides*, 45, 53  
*Dyera* spp., 120

*Ecballium elaterium*, 45  
*Echinacea angustifolia*, 46  
*Echinacea purpurea*, 46  
*Echinochloa crus-galli*, 211, 218  
*Echinochloa frumentacea*, 216  
*Eimeria* spp., 118  
*Elettaria cardamomum*, 46, 242  
*Elymus gayanus*, 212  
*Enantia chlorantha*, 124  
*Ensete ventricosum*, 99  
*Entamoeba histolytica*, 117, 119, 123, 124, 126, 129, 134, 140  
*Ephedra* spp., 34-37, 46, 55  
*Ephedra alata*, 35  
*Ephedra equisetina*, 36  
*Ephedra gerardiana*, 36, 46  
*Ephedra intermedia*, 36  
*Ephedra major*, 35  
    figure, 36  
*Ephedra sinesis*, 37  
*Ephedra sinica*, 36, 46  
*Ephedraceae*, 34, 46, 55  
*Eragrostis curvula*, 192, 203  
*Eremochloa ophiuroides*, 210  
*Ericaceae*, 44, 46, 48  
*Erigeron philadelphicus*, 188, 201  
*Eriodictyon californicum*, 46  
*Erysiphe graminis*, 216  
*Erythroxylaceae*, 46  
*Erythroxylon coca*  
    drug, 80  
    production, 43  
    psychoactive, 38  
    useful products, 46  
*Erythroxylon novogranatense*  
    figure, 42  
    production, 43  
    psychoactive, 38  
    useful products, 46  
*Escherichia coli*, 210, 311  
*Eucalyptus* spp., 46, 138, 218  
*Eucalyptus citriodora*, 218  
*Eucalyptus globulus*, 46  
*Eucalyptus robusta*, 138  
*Eugenia caryophyllata*, 137, 194  
*Eupatorium cannabinum*, 46  
*Eupatorium perfoliatum*, 46

*Eupatorium rugosum*, 248  
*Euphorbiaceae*, 45, 48, 178

*Fabaceae*, 44-47, 49, 60, 62-64, 142, 145, 187, 192, 196, 198  
*Fagopyrum esculentum*, 46  
*Falciparum* spp., 66, 84, 85, 118, 123-126, 129, 131-133, 136-138, 140  
*Ferula assa-foetida*, 46  
*Ferula galbaniflua*, 46  
*Ficus carica*, 302  
*Foeniculum vulgare*, 46, 242  
*Fraxinus rhynchophylla*, 46  
*Fumariaceae*, 125  
*Fusarium moniliforme*, 315, 319, 322-325  
*Fusarium nivale*, 212  
*Fusarium solani*  
    phytoalexin elicitor, 306, 309  
    phytoalexin response, 83  
    tomatine resistance, 162  
*Fusarium subglutinans*, 325

*Galipea officinalis*, 46  
*Garcinia hanburyi*, 46  
*Gaultheria procumbens*, 46  
*Gaura rusbyi*, 46  
*Gelidium amansii*, 46  
*Gelidium cartilagineum*, 46  
*Gelsemium sempervirens*, 46  
*Gentiana lutea*, 46  
*Gentianaceae*, 46  
*Gibbium psylloides*, 27  
*Ginkgo biloba*, 46  
*Ginkgoaceae*, 46  
*Globodera rostochiensis*  
    asparagusic toxicity, 196  
    mustard seedling toxicity, 195  
     $\alpha$ -terthienyl toxicity, 186  
*Globodera tabacum*, 192  
*Glycine max*, 60, 255  
*Glycosmis* spp., 127  
*Glycyrrhiza glabra*, 46, 67  
*Gossypium* spp., 46, 87, 133, 143, 173, 194, 281  
*Gossypium arboreum*, 46  
*Gossypium hirsutum*  
    nematode response, 143  
    phytoalexin response, 194  
    useful products, 46

- Gracilaria confervoides*, 46  
*Gramineae*, 171, 178, 192, 194  
*Gutierrezia* spp., 269, 275  
*Gutierrezia microcephala*, 269  
*Gyrocarpaceae*, 125  
*Gyrocarpus americanus*, 125  
*Gyromitra esculenta*  
  cooking, toxicity, 330  
  gyromitrin, 310  
  volatile compounds, 308
- Hamamelidaceae*, 46, 47  
*Hamamelis* spp., 46, 57  
*Hamamelis virginiana*, 46  
*Hannoia undulata*, 192  
*Hedeoma* spp., 46, 238, 244, 281  
*Hedeoma oblongifolia*, 281  
*Hedeoma pulegioides*, 46, 238  
*Helenium* spp., 188, 197  
*Heliopsis longipes*, 228  
*Heliotropium lasiocarpum*, 285  
*Helminthosporium maydis*, 212  
*Helvella esculenta*, 310  
*Heterodera glycines*, 196, 198  
*Heterodera schachtii*, 193  
*Holarrhena antidysenterica*  
  connessine, 127  
  pyrrolizidine alkaloids, 285  
*Homo sapiens*, 35  
*Huanghuahao*, 64, 65  
*Humulus lupulus*, 46  
*Hydrangea* spp., 129, 208, 209, 285  
*Hydrangea macrophylla*, 208, 209  
*Hydrangeaceae*, 129  
*Hydrastis canadensis*, 46  
*Hydrophyllaceae*, 46  
*Hyoscyamus* spp., 11, 46, 53, 55  
*Hyoscyamus albus*, 55  
*Hyoscyamus niger*, 11, 46, 53  
*Hypericum japonicum*, 138  
*Hypericum perforatum*, 46  
*Hypericum triquetrifolium*, 86  
*Hyssopus officinalis*, 46
- Ilex guayusa*, 69, 72  
*Illiciaceae*, 46  
*Illicium verum*, 46, 242  
*Inula helenium*, 198  
*Ipomoea* spp., 46, 266, 305  
*Ipomoea batatas*, 83, 305
- Ipomoea orizabensis*, 46  
*Ipomoea purga*, 46  
*Iris japonica*, 192  
*Isocoma wrightii*, 249
- Juniperus drupacea* L., 24, 26  
*Juniperus mexicana*, 46  
*Juniperus phoenicea* L., 24, 26  
*Juniperus sabina*, 46  
*Juniperus virginiana*, 24, 46
- Krameria triandra*, 46  
*Krameriaceae*, , 46
- Labiatae*, 193  
*Lactarius rufus*, 308  
*Lactarius torminosus*, 308  
*Lactarius trivialis*, 308  
*Lactuca sativa*, 214  
*Lamiaceae*, 45-49, 134  
*Laminaria* spp., 46  
*Lathyrus hirsutus*, 168  
*Lathyrus odoratus*, 168  
*Lauraceae*, 45, 47, 48, 123  
*Lavandula angustifolia*, 46  
*Lavandula stoechas*, 46  
*Lawsonia alba*, 24  
*Lawsonia inermis*, 24  
*Leguminosae*, 165, 167, 169-171  
*Leishmania* spp., 117, 119, 122, 123,  
  125, 137, 140, 242  
*Leishmania donovani*, 119, 137, 140  
*Leishmania mexicana amazonensis*,  
  122, 123  
*Leishmania tropica*, 140  
*Lemna minor*, 213  
*Lepidium sativum*, 211  
*Levisticum officinale*, 46  
*Liliaceae*, 36, 44, 45, 48, 49, 57, 59,  
  129, 195, 196, 198  
*Linum usitatissimum*, 47, 255  
*Liquidambar orientalis*, 47  
*Liquidambar styraciflua*, 47  
*Liriomyza trifolii*, 299, 363  
*Lobelia inflata*, 47  
*Locusta* spp., 4  
*Loganiaceae*, 46, 48  
*Lunularia* spp., 208  
*Lunularia cruciata*, 208  
*Lupus erythematosus*, 252

*Lycopersicon esculentum*, 170

*Lycopodiaceae*, 47

*Lycopodium clavatum*, 47

*Lyta vesicatoria*, 3

*Malpighia punicifolia*, 47

*Malpighiaceae*, 47

*Malus domestica*, 47

*Malvaceae*, 44, 46, 133, 143, 173, 194

*Mammea longifolia*, 228

*Mandragora autumnalis*, 55

*Mandragora officinarum*, 53, 55

*Manihot esculenta*, 68

*Marrubium vulgare*, 47

*Medicago sativa*, 166, 214

*Melaleuca cajuputi*, 47

*Melaleuca quinquenervia*, 47

*Melia azedarach*, 143

*Melia toosendan*, 143

*Meliaceae*, 44, 46, 137, 143, 197

*Melissa officinalis*, 47

*Meloidae*, 3

*Meloidogyne hapla*, 196

*Meloidogyne incognita*

*Allium* compounds, 198

    asparagus toxicity, 195

    butyric acid toxicity, 192

    glyceollin toxicity, 143, 194

    monocrotaline toxicity, 187

    neem toxicity, 197

    saponin toxicity, 196

*Meloidogyne javanica*, 143, 192-194, 197

*Menispermaceae*, 44, 45, 49, 125

*Mentha arvensis*, 47, 238

*Mentha piperatum*, 193

*Mentha spicata*, 242

*Mentha x gentilis*, 47

*Mentha x piperita*, 47

*Mentha x spicata*, 47

*Mesostenopa* spp., 28

*Minquartia guianensis*, 145

*Monimiaceae*, 47

*Moraceae*, 178

*Murraya paniculata*, 87

*Musa x paradisiaca*, 99

*Mycobacterium thermospectum*, 210

*Myrica cerifera*, 47

*Myricaceae*, 47

*Myristica fragrans*, 47, 242

*Myristicaceae*, 47

*Myroxylon balsamum*, 47

*Myroxylon balsamum* var. *pareirae*, 47

*Myrsinaceae*, 44

*Myrtaceae*, 46-48, 137, 138, 193

*Naegleria* spp., 117, 138

*Naegleria fowleri*, 138

*Nectandra megapotamica*, 123, 150

*Nicotiana debneyi*, 163, 180

*Nicotiana tabacum*, 170, 228

*Ochrosia* spp., 120, 123

*Ocimum basilicum*

    compounds in, 277, 280

    juvenile hormone, 232

    nematicide, 193

*Ocimum sanctum*, 193

*Octodon degus*, 273

*Oenothera* spp., 239

*Olacaceae*, 145

*Olea europaea*, 47

*Oleaceae*, 46, 47

*Orchidaceae*, 49, 178

*Origanum vulgare*, 277, 279

*Panagrellus redivivus*, 187, 192, 199

*Panagrolaimus* spp., 187

*Panax ginseng*, 47, 67

*Panax quinquefolium*, 47, 67

*Panicum crus-galli*, 218

*Papaver* spp., 38, 43, 47, 55, 57, 80

*Papaver bracteatum*, 47

*Papaver somniferum*

    codeine, 55

    drugs, 80

    figure, 38

    papaverine, 57

    production, 43

    useful products, 47

*Papaveraceae*, 47, 48, 57, 124, 187, 192

*Paratrichodorus christiei*, 195

*Paratylenchus curvitatus*, 196

*Parmelia furfuracea*, 24

*Parthenium hysterophorus*, 134

*Passiflora incarnata*, 47

*Passifloraceae*, 47

*Pastinaca sativa*, 228

*Pausinystalia johimbe*, 47

*Pedaliaceae*, 48

*Penicillium* spp., 293, 320, 322

*Penicillium patulum*, 320  
*Penicillium rugulosum*, 322  
*Peronospora tabacina*, 162, 163  
*Persea americana*, 47  
*Petroselinum crispum*, 47  
*Peumus boldus*, 47  
*Pharbitis purpurea*, 218  
*Phaseolus lunatus*, 142, 286  
*Phaseolus vulgaris*, 198, 210, 269  
*Phleum penetrans*, 192  
*Phleum pratense*, 192  
*Phoenix dactylifera* L., 24  
*Physostigma venenosum*  
drugs, 80  
lead compound, 228  
nematicide, 187  
physostigmine, 63  
stigmasterol, 60  
useful products, 47  
*Phytophthora infestans*, 162, 171, 216  
*Picrasma excelsa*, 47  
*Picrasma javanica*, 123, 155  
*Picrolemma pseudocoffea*, 136  
*Pilocarpus* spp., 47, 62, 63, 80  
*Pilocarpus jaborandi*, 47, 62, 80  
*Pilocarpus pinnatifolius*, 47, 62  
*Pimenta dioica*, 47  
*Pimpinella* spp., 47, 220  
*Pimpinella anisum*, 47, 220  
*Pimpinella diversifolia*, 220  
*Pimpinella major*, 220  
*Pimpinella peregrina*, 220  
*Pimpinella saxifraga*-1, -2 and -3, 220  
*Pimpinella tragium*, 220  
*Pinaceae*, 44, 47  
*Pinus halepensis*, 27  
*Pinus mugo*, 47  
*Pinus pinea*, 27  
*Pinus strobus*, 47  
*Pinus sylvestris*, 47  
*Piper cubeba*, 47  
*Piper methysticum*, 47  
*Piper nigrum*, 24, 228  
*Piperaceae*, 47  
*Pisum sativum*, 165  
*Plantaginaceae*, 47  
*Plantago afra*, 47, 62  
*Plantago indica*, 62  
*Plantago ovata*, 47, 62, 67  
*Plantago psyllium*, 47

*Plasmodium* spp., 66, 84, 117, 118, 123-127, 129, 131-133, 136-138, 140  
*Plasmodium berghei*, 124, 127, 136, 138, 140, 154  
*Plasmodium chabaudi*, 126  
*Plasmodium cynomolgi*, 131  
*Plasmodium falciparum*  
alkaloid activity, 124  
artemisinin derivatives, 132  
artemisinin treatment, 66  
bruceantin activity, 136  
gossypol activity, 133  
haemaline activity, 123  
homoharringtonine activity, 129  
malaria, 84  
pipoxide activity, 138  
polyacetylene activity, 140  
pristimerin, lapachol activity, 137  
pycnamine activity, 125  
qinghaosu, 85  
quinoline antimarialials, 118  
tetrandrine activity, 126  
*Plasmodium knowlesi*, 131  
*Plasmodium vinckeii*, 127  
*Plasmodium vivax*, 66, 85  
*Plasmodium yoelii*, 127, 129, 131  
*Plasmopora viticola*, 216  
*Poaceae*, 44, 45, 49  
*Podophyllum peltatum*, 47, 52, 82  
*Pogonopsis tubulosis*, 120  
*Poleo chino*, 281  
*Polygala senega*, 47  
*Polygalaceae*, 47  
*Polygonum lapathifolium*, 222  
*Polygonum multiflorum*, 47  
*Polygonum persicaria*, 222, 225  
*Populus balsamifera*, 47  
*Populus nigra*, 47  
*Pratylenchus brachyurus*, 197  
*Pratylenchus coffeae*, 188, 189, 192  
*Pratylenchus penetrans*, 186, 188, 196  
*Pratylenchus scribneri*, 142, 156, 194  
*Prunella* spp., 239  
*Prunus amygdalus*, 48  
*Prunus armeniaca*, 48  
*Prunus domestica*, 48  
*Prunus virginiana*, 48  
*Prurigo nodularis*, 296  
*Pseudomonas cepacia*, 266

- Pseudomonas fluorescens*, 266  
*Pseudomonas marginalis*, 266  
*Psyllium* spp., 47, 63  
*Pycnanthemum* spp., 242  
*Pycnanthemum muticum*, 238  
*Pyricularia oryzae*, 216, 327  
*Pythium aphanidermatum*, 216  
  
*Qinghao* spp., 64  
*Quassia amara*, 48  
*Quassia simarouba*, 48  
*Quercus infectoria*, 48  
*Quisqualis indica*, 144  
  
*Rabdosia eriocalyx*, 216  
*Ranunculaceae*, 44-46  
*Rauvolfia* spp., 77  
*Rauvolfia canescens*, 55, 80  
*Rauvolfia serpentina*, 48, 55, 67, 80  
*Rauvolfia tetraphylla*, 55  
*Rauvolfia vomitoria*, 48  
*Remijia* spp., 48, 57, 118, 123  
*Remijia pedunculata*, 48, 57  
*Remijia purdieana*, 48, 57, 123  
*Rhabditis* spp., 187, 192  
*Rhamnaceae*, 48, 62  
*Rhamnus frangula*, 48  
*Rhamnus purshiana*, 48, 62  
*Rheum emodi*, 62  
*Rheum officinale*, 48, 62, 283  
*Rhizoctonia leguminicola*, 293  
*Rhizoctonia solani*, 216  
*Rhodnius prolixus*, 137  
*Rhododendron molle*, 48  
*Rhus cortaria*, 48, 60  
*Ricinus communis*  
    drug, 80  
    laxative, 62  
    useful products, 48  
*Rosa gallica*, 48  
*Rosaceae*, 45, 47, 48, 145  
*Rosmarinus officinalis*  
    compounds in, 277, 281  
    useful products, 48  
*Rubiaceae*, 45, 47-49, 55, 57, 64, 118,  
    120  
*Rubus idaeus*, 48  
*Rumex crispus*, 48  
*Rumex hymenosepalus*, 48  
*Ruscus aculeatus*, 48  
  
*Ruta graveolens*, 48, 277  
*Rutaceae*, 44-48, 50, 62, 295, 302  
  
*Salicaceae*, 47, 48  
*Salix alba*, 48  
*Salmonella typhimurium*  
    aflatoxin mutagenesis, 275, 321  
    flavonoid mutagen bioassay, 273  
    isocyanate mutagen bioassay, 257  
*Salvia officinalis*, 48, 277, 283  
*Sanguinaria*, 48, 124  
*Sanguinaria canadensis*, 48  
*Santalaceae*, 48  
*Santalum album*, 48  
*Sassafras albidum*, 48  
*Saussurea lappa*, 284  
*Schistosoma japonicum*, 146, 147  
*Schistosoma mansoni*, 146  
*Scirpus maritimus*, 213  
*Sclerotinia sclerotiorum*, 293, 296, 322  
*Sclerotinia trifoliorum*, 212  
*Scrophulariaceae*, 45, 49, 57  
*Secale cereale*, 192, 211  
*Serenoa repens*, 48  
*Sesamum indicum*, 48, 229, 232  
*Setaria viridis*, 218  
*Severinia* spp., 127  
*Silybum marianum*, 48  
*Simaroubaceae*, 47, 48, 123, 134, 136,  
    192  
*Skimmia laureda*, 284  
*Smilax aristolochiifolia*, 48  
*Smilax febrifuga*, 48  
*Solanaceae*, 44-46, 50, 53, 55, 64, 165,  
    171, 248, 287, 294  
*Solanum* spp., 213, 248, 291  
*Solanum acaule*, 162  
*Solanum melanogena*, 170  
*Solanum nigrum*, 248  
*Solanum tuberosum*, 170, 213, 359  
*Solidago altissima*, 188  
*Solidago canadensis*, 188  
*Sonchus tuberifer*, 215  
*Sophora* spp., 187, 239  
*Sophora flavescens*, 187, 202  
*Spigelia marilandica*, 48  
*Spodoptera frugiperda*, 213  
*Stachys officinales*, 48  
*Stephania tetrandra*, 126  
*Sterculia urens*, 48

- Sterculiaceae*, 48, 49, 55  
*Stevia rebaudiana*, 88  
*Strophanthus gratus*, 48, 57, 80  
*Strophanthus kombe*, 48, 57  
*Strychnos nux-vomica*, 1, 48  
*Styracaceae*, 48  
*Styrax benzoin*, 48  
*Styrax officinalis*, 48  
*Swertia chiraita*, 284  
*Symphytum* spp., 48, 285, 286  
*Symphytum officinale*, 48, 286  
*Syphacia obvelata*, 146  
*Syzygium aromaticum*, 48  
  
*Tabernanthe iboga*, 48  
*Taenia* spp., 145, 146  
*Taenia hydatigena*, 145  
*Taenia pisiformis*, 145  
*Taenia solium*, 145  
*Tagetes* spp., 186, 199  
*Tagetes erecta plena*, 186  
*Tanacetum cinerariifolium*, 48  
*Taraxacum*, 239  
*Taxaceae*, 48, 52, 134  
*Taxus baccata*, 11  
*Taxus brevifolia*  
    antiparasitic activity, 134  
    taxol, 52, 83  
    useful products, 48  
*Tetradenia riparia*, 134  
*Tetragonolobus maritimus*, 168  
*Theaceae*, 44, 55  
*Theobroma cacao*, 49, 55  
*Thuja occidentalis*, 49  
*Thymelaeaceae*, 45, 198  
*Thymus vulgaris*, 49, 242  
*Tiliacora triandra*, 125  
*Tineacide*, 240  
*Trichomonas* spp., 117, 124, 134, 137, 140  
*Trichomonas vaginalis*, 124, 134, 137, 140  
*Trichosanthes kirilovii*, 49, 87  
*Triclisia patens*, 125, 155  
*Trifolium pratense*, 166  
*Trifolium repens*, 166  
*Trifolium subterraneum*, 269  
*Trigonella foenum-graecum*, 49  
*Triticum aestivum*, 49, 210, 222  
  
*Trypanosoma* spp., 117, 122-125, 129, 134, 137, 140  
*Trypanosoma brucei brucei*, 125  
*Trypanosoma cruzi*  
    colchicine activity, 129  
    fatty acid activity, 140  
    harmaline activity, 122  
    lapachol activity, 137  
    obabерine activity, 125  
    olivacine, ellipticine activity, 123  
    taxol activity, 134  
*Trypanosoma cruzi* epimastigotes, 140  
*Trypanosoma equiperdum*, 124  
*Trypanosoma lewisi*, 124  
*Trypanosoma rhodesiense*, 125  
*Tylophora asthmaticus*, 126  
  
*Ulmaceae*, 49  
*Ulmus rubra*, 49  
*Umbelliferae*, 2, 173, 295  
*Uncaria gambir*, 49  
*Urginea maritima*, 49  
*Urtica dioica*, 49  
*Urticaceae*, 49  
*Usnea* spp., 49, 206  
*Usnea barbata*, 206  
*Usnea longissima*, 206  
*Uvaria ferruginea*, 138  
  
*Valeriana officinalis*, 49  
*Valerianaceae*, 49  
*Valerianella* spp., 239  
*Vanilla planifolia*, 49  
*Venturia inaequalis*, 216  
*Veratrum album*, 49, 57  
*Veratrum viride*, 57  
*Verbascum thapsus*, 4, 49  
*Vernonia colorata*, 134, 146  
*Verticillium* spp., 173  
*Viburnum opulus*, 49  
*Viburnum prunifolium*, 49  
*Vicia faba*, 168  
*Vinca rosea*, 78  
*Viscaceae*, 49  
*Viscum album*, 49  
*Vitaceae*, 178  
  
*Wikstroemia sandwicensis*, 2  
*Wrightia tomentosa*, 127, 152

- Xanthium strumarium*, 5  
*Xanthomonas campestris*, 173  
*Yucca aloifolia*, 49  
*Zea mays*  
herbicide, 210  
3-hydroxyuridine, 218

- Zea mays* (continued)  
microplot experiments, 197  
mycotoxin, 322  
useful compounds, 49  
*Zingiber* spp., 49, 237  
*Zingiber officinale*, 49  
*Zingiberaceae*, 45, 46, 49

## CHEMICAL INDEX

- Acetaldehyde  
apple pie, 252  
in false morel, 310  
*N*-methyl-*N*-formylhydrazone, 308  
MFM, 308
- Acetaminophen, 257
- Acetic acid  
biological activity, 240  
flavone, 275  
indole-3, 212  
*meta*-hydroxyphenyl, 261  
in penny royal, 239  
reaction w/fumonisins, 324
- 3-Acetyl, 314
- Acetylcholine, 7  
pilocarpine, 62  
structure, 50  
tubocurarine, 49
- Acetylenes, 171, 187-189, 251, 254
- Acetylfalcarindiol, 251
- Acetylgitoxin, 45
- O(23)-Acetylleptinidine, 289
- Achilleine, 278
- Achilletin, 278
- Acifluorfen  
phytoalexin simulation, 250, 330
- Aconine  
poisoning, 9
- Aconite, 12  
lethal dose, human, 9  
poisoning symptoms, 8
- Aconitine  
heart treatment, liniment, 44  
poisoning symptoms, 8  
structure, 9
- Acridone alkaloids  
antimalarial, 127
- Adiphenine  
antispasmodic, 55  
structure, 53
- Aesculetin, 46
- Afinin  
structure, 228
- Aflatoxicol  
reduction of B<sub>1</sub>, 321  
structure, 322
- Aflatoxin B1  
carcinogen, 315  
fetal abnormalities, 320  
in figs, 303  
flavonoid mutagen inhibition, 275  
hepato cancer promotion, 258  
in human tissue, 317  
metabolism, 321  
mutagenicity, 322  
in peanuts, figs, 330  
removal from feeds, 325-327  
Reye's syndrome, 318  
structure, 321
- Aflatoxin B1 and B2  
structure, 321
- Aflatoxin D1, 325  
structure, 326
- Aflatoxin Q1  
structure, 321
- Agaritine  
hydrolysis, 309  
from mushroom, 308, 310
- Agrimony, 145
- Agrimophol  
histosome toxicity, 147  
structure, 146  
as taeniafuge, 145
- Ajmaline, 48

- Alantolactone  
     nematicide, 198  
     structure, 197  
 Alar, 244, 331  
 Allantoin, 48, 49  
 Allethrin  
     structure, 229  
 Allicin  
     structure, 142  
     trophozoite toxicity, 140  
     useful products, 44  
 Allodesacetylconfertiflorin, 243  
 Alloimperatorin, 284  
*cis*-Allo-ocimene, 280  
*trans*-Allo-ocimene, 280  
 Allopurinol, 38  
     structure, 41  
 Allygrin  
     structure, 198  
 Allyl isothiocyanate  
     in cabbage, 256  
     in crucifers, 329  
     goitrogenic activity, 254  
     mutagenicity, 257  
     nematicide, 195  
     structure, 196  
     Thanksgiving Day dinner, 252  
     useful products, 44  
 Aloin, 62, 63  
 Alstonine, 123  
     structure, 124  
 $\alpha$ -Amanitin  
     latent poisoning phase, 5  
     structure, 6  
 Aminocamptothecin, 83  
     structure, 84  
 S-(-)- $\alpha$ -Aminopropiophenone, 102  
 Aminopyrine, 284  
 Aminoquinoline, 67, 84  
 Ammirin, 281  
 Amodiaquine, 64  
     structure, 65  
 $\alpha$ -Amorphene, 280  
 Amphetamine  
     khat, 97, 102  
     lead compound, 36  
     mechanism of action, 109  
     structure, 41, 103  
     symptoms, 105, 108  
 S-(+)-Amphetamine, 102  
     structure, 103  
     Anagyrine, 187  
         structure, 189  
 Anatoxin A, 322  
 Anethole, 279  
 Angular pyranoacridones, 127  
 Anhydro- $\beta$ -rotunol, 288  
 Anisodamine, 79  
     structure, 81  
 Anisidine, 79  
     structure, 81  
 Annomontine, 123  
     structure, 122  
 Anodyne, 278  
 Anonane, 228  
 Anthraquinone, 266  
 Antipyrine, 257  
 Apigenin  
     in carrot, 270  
     lipid peroxidation, 271  
     metabolism, 261, 262  
     in oregano, 279  
     in yarrow, 278  
 Apigeninidin  
     in sorghum, 178  
     structure, 177  
 Apiol, 277  
 Apotrichothecene, 314  
 Arabinosyl-5-O-apigeninidin, 178  
     structure, 177  
 Arachidonic acid, 162, 171  
 Arecoline, 44, 145  
     structure, 146  
 Aricine, 123  
     structure, 124  
 Aromoline, 125  
     structure, 128  
 Arsenic, 252  
 Arteannuin, 130  
 Arteether, 147, 150  
     antimalarial activity, 131, 132  
     structure, 85, 133  
 Artelinic acid, 131  
     structure, 133  
 Artemether  
     antimalarial activity, 131, 132  
     semisynthetics, 146  
     structure, 66, 85, 133  
 Artemetin, 137, 140, 375  
 Artemisinin, 65, 67, 84, 85, 97, 126  
     activity w/cloroquine, 138  
     antimalarial activity, 130, 132

- Artemisinin (continued)**
- semisynthetics, 146
  - structure, 66, 133
- Artesunate, 130-132**
- structure, 66, 85, 133
- Ascorbic acid, 271, 280, 289**
- Asparagusic acid, 196**
- structure, 197
- Asparanin B, 196**
- structure, 197
- Asparanin I, 196**
- Aspartame, 88**
- Atalaphillinine**
- antimalarial activity, 127
  - structure, 131
- Atracurium, 50, 73, 82**
- structure, 51
- Atracurium besylate, 82**
- Atranorin, 206**
- Atropine**
- antitussive, 55
  - in henbane, 11
  - history, 75
  - mydriasis, 63
  - as poison, 9
  - in solanacene, 248, 370
  - structure, 10, 53
  - therapeutic agent, 77
  - useful products, 45
- Aubergenone, 171**
- structure, 170
- Avenacin, 164**
- Avermectin, 371**
- Azadirachtin**
- antiparasitic activity, 137
  - nematicide, 197
  - structure, 138
- 3'-Azido-2',3'-dideoxythymidine, 85**
- AZT, 85**
- Azulene, 278**
- Balanitin, 243**
- Barbatic acid, 206**
- structure, 207
- Bayogenin, 243**
- Benzimidazoles, 140**
- Benzofurans, 169, 178**
- Benzoin**
- dermatological use, 57
  - plant source, 47, 48
  - structure, 61
- Benzophenanthridine, 86, 125**
- Benzophenanthridine alkaloid, 125**
- Benz[a]pyrene, 252, 257**
- 2(3H)-Benzoxazolinone, 164, 211**
- structure, 212
- Benzyl acetate, 280**
- Benzyl alcohol, 280, 308, 330**
- Benzyl isothiocyanate, 256**
- Berberine**
- amoebicide, 124
  - antigiardia, 241
  - leishmaniasis treatment, 123
  - medicinal qualities, 242, 244
  - plant source, 44
  - structure, 125
- Berberine sulphate, 124, 148, 150, 153, 157**
- cis- $\alpha$ -Bergamotene, 280**
- trans- $\alpha$ -Bergamotene, 240, 280**
- Bergamottin, 301, 302**
- Bergapten**
- in Bishop's weed, 284
  - in celery, 297, 298
  - in citrus, figs, 302
  - in parsley, 301
  - in parsnip, 300
  - molluscicide, 243
  - photosensitization, 329
  - PUVA treatment, 296, 297
  - structure, 297
- Bergaptol, 301, 302**
- Bergemin, 44**
- Betagarin, 178**
- structure, 176
- Betaine, 278**
- Betavulgarin, 176, 178**
- Bibenzyl stilbene derivative, 210**
- Bicarbonate, 22**
- Bicycloelemene, 280**
- Bicyclogermacene, 280**
- Bio-allethrin, 229**
- Biochanin A, 269, 275, 276**
- Bioresmethrin**
- structure, 229
- Bisabolene, 280**
- Bisbenzyltetrahydroisoquinoline alkaloids, 125**
- Blastin, 327**
- Bocconine, 187**
- structure, 188
- Borneol, 240**

- Borneol (continued)
  - in basil, 280
  - in oregano, 279
  - in rosemary, 281
  - in sage, 283
  - in yarrow, 278,
- Bornyl acetate, 278, 280, 283
- Boron, 239
- $\beta$ -Bourbonene, 280
- Bruceantin, 151
  - structure, 136
- Bruceines A, B, and D, 136
- Brusatol, 136
- $\epsilon$ -Bulgarene, 280
- $\beta$ -Bungarotoxins, 7
- Butanol, 310
- 5-(3-Buten-1-ynyl)-2,2'-bithienyl, 186
  - structure, 187
- Butenolide, 314
- Butenyl isothiocyanate, 256
- Butylated hydroxyanisole, 271
- Butylphthalide, 298
- 3-*n*-Butyroyl- $\beta$ -ionone, 172, 173
- Butyric acid, 192, 193, 239
- Cadalenes, 251
- Cadinane sesquiterpene, 133
- $\alpha$ - $\gamma$ -Cadinene, 280
- $\delta$ -Cadinene, 278
- 10-Cadinol, 280
- Caffeic acid, 279, 287
  - apigeninidin ester, 177, 178
  - in pears, 239
  - in potato peel, 160
  - as phytoalexin, 165
  - structure, 161
  - in yarrow, 278
- Caffeine
  - aflatoxin inhibition, 327
  - antisage remedy, 277
  - Jívaro stimulant, 69
  - khat analysis, 100
  - plant source, 44, 45
  - structure, 70
  - synthetic manufacture, 76
  - Thanksgiving Day dinner, 252
- 1-Caffeoyl, 171
- Calamene, 280
- Calonectrin, 314
- Camphene
  - in mountain mint, 240
- Camphene (continued)
  - in oregano, 280
  - in yarrow, 278
- Camphor
  - in celery, 298
  - in mountain mint, 280
  - plant source, 45
  - in rosemary, 281
  - in sage, 283
  - in yarrow, 278
- Camptothezin, 83
  - structure, 84
- Cantalasaponin-2, 243
- Cantharidic acid, 3
- Cantharidin, 3
  - structure, 4
- Capnoidine, 124
  - structure, 126
- Capsidiol
  - in green pepper, 294
  - in jimson weed, 173
  - in pepper, 251
  - as phytoalexin, 171
  - structure, 170, 295
- Carbaryl
  - structure, 230
- Carbazole, 259
- Carbofuran
  - structure, 230
- Carboxyatractyloside, 5
- Cardol, 243
- Carene, 279, 280
- Carnosic acid, 283
- Carotatoxin, 251, 252
- Carvacrol, 279, 281
  - trans*-Carveol, 298
- Carvone, 281
- Caryophyllene, 239, 240, 278-279
  - in basil, 280
  - in celery, 298
- Casbene, 178, 251
  - structure, 176
- Castanospermine
  - structure, 86
- Casticin, 137
  - structure, 140
- Catechin
  - antioxidiand, 271
  - carcinogenicity, 274
  - DNA breaks, 275
  - in fruits, 270

- Catechin (continued)  
     in jimson weed, 266  
     metabolism, 261
- Catechol, 160, 182  
     structure, 161
- Cathedulin-E3  
     structure, 107
- Cathedulin-E5  
     structure, 107
- Cathedulin-K1  
     structure, 106
- Cathedulin-K12  
     structure, 106
- Cathedulins, 102, 109
- Cathidine, 100, 101
- Cathidine D, 101
- Cathine, 100, 101
- Cathinine, 100
- Cathinone  
     in khat, 102-105  
     pharmacology, 109  
     structure, 101  
     symptoms, 110
- $\beta$ -Cedrene, 280
- Celastrine, 100
- Cephaeline  
     decongestant, 55  
     structure, 56
- Cephalosporin, 45
- Chaconine  
     birth defects, 293  
     cholinesterase inhibition, 288  
     compliance level, 294  
     concentration in potato, 289, 291, 294  
     cooking effects, 290  
     in damaged potato, 289  
     ED<sub>50</sub> nematodes, 187  
     effect of cooking, 290  
     fungicide, 162  
     nematicidal, 187  
     as phytoalexin, 162, 251  
     phytoalexin response, 251, 329  
     post deterrent, 290  
     in potato, 288, 289, 329  
     in potato varieties, 291  
     structure, 163, 189, 287  
     teratogenicity, 293  
     Thanksgiving Day dinner, 252
- Chalepensin, 243
- Chamazulene, 278
- Chaparrinone, 192  
     structure, 193
- Chelerythrine, 187  
     structure, 188
- Chelidonium, 124
- Chlobenthiazone, 327  
     structure, 328
- Chlordane, 230
- m*-Chlorobenzoic acid, 219
- Chlorogenic acid  
     carrot phytoalexin, 251  
     in coffee bean, 239  
     constitutive antibiotic, 287  
     ineffective nematicide, 194  
     in morning glory seeds, 266  
     as phytoalexin, 171  
     resistance to scab, 160  
     structure, 161  
     Thanksgiving Day dinner, 252
- Chloroquine  
     antimalarial, 64, 84, 127  
     biochemical mechanism, 138  
     malarial resistance, 67, 126, 129  
     receptor, 118  
     structure, 66
- Chlorpromazine  
     schizophrenia, 55  
     structure, 56
- Chonemorphine, 129  
     structure, 132
- Chromones, 43, 169, 222
- Chuanliansu, 143
- Cinchonamine, 123  
     structure, 124
- Cinchonidine, 118
- Cinchonine, 118
- Cinchophylline, 120  
     structure, 121
- Cineole  
     in basil, 280  
     biological activity, 240  
     in mountain mint, 240  
     nematicide, 194  
     in oregano, 279  
     in pennyroyal, 239  
     in rosemary, 281  
     in sage, 283  
     structure, 195  
     in yarrow, 278
- Cinnamic acid, 262
- Cinnamoylethylamine, 103

Cinnamylphenols, 251  
Citral, 193  
    structure, 195  
Citric acid, 326  
Citrinin, 314, 320, 328  
Citronellol, 280  
Cobaltous chloride, 307  
Cocaine  
    drug, 98  
    history, 75  
    lead compound, 82  
    local anesthetic, 42  
    plant source, 46, 80  
    structure, 41  
    topical anesthetic, 49  
Cocsuline, 125, 128  
Codeine, 47  
    antitussive decongestant, 55  
    dermatology, 57  
    drug, 77  
    lead compound, 80  
    from poppy, historical, 39  
    production, 43  
    structure, 40  
Colchicine  
    antiparasitic, 129  
    cell division inhibition, 134  
    for gout, 38, 59  
    plant source, 45  
    as poison, 3  
    structure, 41, 132  
Columbine, 8  
Commersonine, 289  
Concanavalin A, 198  
Confertiflorin, 243  
Coniine, 2  
    structure, 3  
Connessine, 127  
    structure, 132  
Copaene, 278-280  
Coralyne, 125  
    structure, 126  
Cornine, 45  
Corpaine, 124  
    structure, 126  
*p*-Coumaric acid, 160  
    structure, 161  
Coumestans, 142, 203  
Coumesterol, 275  
Coumestrol  
    in lima bean, 286

Coumestrol (continued)  
    phytoalexin response, 194  
    phytoestrogen, 269  
    structure, 142, 165, 195, 272  
Cromolyn, 43  
    structure, 50  
Cryptopleurine, 127  
    structure, 130  
     $\alpha$ -Cubebene, 280  
     $\beta$ -Cubebene, 280  
Cucurbitine, 145-147  
Cuminaldehyde, 278  
Cumorin, 314  
Curare, 7, 69  
Curcumin, 45  
Cyanidin, 262, 266  
    structure, 264  
Cyanogenic glycosides, 252  
Cyclamate, 88  
Cyclo-2,3-dehydrokievitone hydrate,  
    286  
Cyclokievitone hydrate, 286  
Cyclopiazonic acid, 314  
Cycloprothrin, 229  
Cyclosativene, 280  
Cymene  
    in basil, 280  
    in celery, 298  
    in mountain mint, 240  
    in oregano, 279  
    in sage, 283  
    in yarrow, 278  
Cynarin, 45  
Cypermethrin, 229  
Cystine, 280  
Cytisine  
    molluscicide, 243  
    nematicide, 187  
    structure, 189  
Daidzein  
    infertility syndrome, 275  
    nematicide, 194  
    in soybean, 269  
    structure, 272  
Daidzin  
    antioxidant, 271  
    in soybean, 269  
Damnacanthin, 243  
Daphnandrine, 125  
    structure, 127

Deacetyl-sendanin, 143  
Debneyol, 163, 164  
    structure, 180  
2-*cis*,8-*cis*-Deca-2,8-diene-4,6-dynoate, 188  
Decyclic acid, 239  
1",2"-Dehydrocyclokievitone, 286  
2,3-Dehydroemetine, 119  
    structure, 120  
Dehydroipomeamarone, 178  
    structure, 175  
2,3-Dehydrokievitol, 286  
2,3-Dehydrokievitone, 286  
*cis*-Dehydromatricaria ester, 188  
    structure, 191  
Dehydromatricarianol, 188  
    structure, 191  
*trans*-Dehydromatricaria ester  
    structure, 191  
*trans*-Dehydromatricarianol  
    structure, 191  
Dehydromatricarianyl acetate, 188  
    structure, 191  
*trans*-Dehydromatricarianyl acetate  
    structure, 191  
Dehydrosafynol, 178  
    structure, 176  
Dehydrotremetone, 249  
Delphinidin, 266  
Demerol, 10  
9,10-Demethoxyemetine  
    amoebicide, 121  
    structure, 120  
9,10-Demethoxyisoemetine, 120  
Demethylbarbatic acid, 206  
    structure, 207  
Demethylepipodophyllotoxin- $\beta$ -D-ethidene glucoside, 82  
Demethylhomoterocarpin, 166  
Demethylpterocarpin, 166  
6-Demethylvignafuran, 168  
Demissidine, 288, 289  
Demissine, 162, 289  
Deoxyartemisinin  
    antimalarial, 65  
    structure, 66  
6-Deoxyhemigossypol  
    structure, 174  
5-Deoxykievitol, 251, 286  
5-Deoxykievitone, 286

Deoxynivalenol  
    analysis, 317  
    in corn, 323  
    food contamination, 318  
    regulatory level, 329  
    worldwide importance, 314  
Derivatives of dihydroartemisinin, 66  
Derivatized stilbene, 209, 210  
Desacetylphytuberin, 288  
Deserpidine, 80  
DHC  
    increase w/sunlight, 173  
DHN, 327  
    structure, 328  
Di- and tri-sulfides, 252  
DIBOA, 211, 212  
Di-*n*-butyl succinate, 192  
    structure, 193  
Di-*tert*-butyl thiosulfinate  
    structure, 196  
Diacetylmorphine, 39  
    structure, 40  
Diazonium compound, 308  
Dibenzo-1,4-dioxin alkaloids, 125  
Dibutyl thiosulfinate, 198  
Dicyclomine  
    antispasmodic, 55  
    structure, 53  
Dieldrin, 230  
Diethylpropione  
    structure, 104  
    synthetic from khat, 102  
Diethylstilbesterol  
    cardiovascular disease, 318  
    compared to coumesterol, 275  
    structure, 272  
Diffractive acid, 206  
    structure, 207  
Digitalis  
    consumption, 67  
    for heart edema, 57  
    ethnomedical use, 78  
    lead compound, 77  
    plant source, 45  
Digitoxin  
    atrial fibrillation, 77  
    as cardiovascular drug, 57  
    history, 78  
    plant derived drug, 75  
    plant source, 45  
    structure, 58, 79

- Digitoxose, 79  
 2,10-Di( $\gamma$ , $\gamma$ -dimethylallyl)glycinol, 286  
 Digoxin  
     cardiotonic, 77  
     as cardiovascular drug, 57  
     history, 78  
     plant-derived drug, 75  
     plant source, 45  
     structure, 58, 79  
 Dihydroartemisinin  
     antimalarial, 65, 130, 131  
     structure, 66, 133  
 Dihydrohydrangeic acid, 209  
 2,3-Dihydro-2-hydroxy-3-methylene-6-methylbenzofuran, 197  
 Dihydroisocoumarin  
     herbicide, 208  
     from plant, 209  
 Dihydroisocoumarin glucosides, 209  
 Dihydrophenanthrenes, 178  
 Dihydrostilbene derivative, 209  
 1,8-Dihydroxyanthraquinone, 283  
 2,4-Dihydroxy-1,4(2H)-benzoxazin-3-ne, 211  
     structure, 212  
 2,4-Dihydroxy-1,4-benzoxazin-3-one  
     glucoside, 212  
 2,7-Dihydroxycadalene, 173  
     structure, 174  
 5,7-Dihydroxychromone  
     herbicide, 222  
     in peanut, 270  
 8,2'-Dihydroxygenistein  
     in lima bean, 286  
     phytoalexin, 251  
 2,3-Dihydroxygermacrene, 172, 173  
 1,8-Dihydroxynaphthalene, 327  
 3,4-Dihydroxy or *meta*-hydroxyphenylacetic acid, 261  
 3,4-Dihydroxyphenylacetic acid, 260  
 3,3'-Diindolylmethane, 260  
     cancer inhibition, 257  
     MFO induction, 259  
     UV absorption, 260  
 2-(Indol-3-ylmethyl)-3,3'-diindolylmethane, 260  
 Dimeric pyranoacridone, 127  
 6,6'-Dimethoxygossypol, 173  
     structure, 174  
 3'-( $\gamma$ , $\gamma$ -Dimethylallyl)kievitone, 286  
 4-( $\gamma$ , $\gamma$ -Dimethylallyl)phaseollidin, 286  
 3,6-Dimethyl-2,5-diphenylpyrazine,  
     102  
     structure, 104  
 7,12-Dimethylbenz[a]anthracene, 259  
 1,2-Dimethylhydrazine, 258  
 Dimethylmorphine, 39  
 $\alpha$ -*p*-Dimethylstyrene, 280  
 Dimetilan, 230  
 Diosgenin, 60  
     structure, 62  
 Diosmetin  
     biological activity, 279  
     flavonoid metabolism, 261  
     lipid peroxidation, 271  
     in oregano, 279  
     structure, 263  
 Diosmin  
     flavonoid metabolism, 261  
     in pennyroyal, 239, 240  
     structure, 263  
 1,4-Dioxygenated-1-(3-furyl)pentanes, 305  
 Dipentene, 239  
 Dipentyl thiosulfinate, 196  
 Diplodia toxin, 314  
 Diplodiol  
     structure, in fungi, 222  
 Diplosporin  
     structure, in fungi, 222  
 Dipropyl thiosulfinate  
     structure, 198  
 Dodonoside, 243  
 Dopamine, 109  
 Duvane, 163  
 Duvatrien 1,3-diols,-1-ols  
     structure, 164  
 Duvatrieniols, 162  
     fungal resistance, 162  
     structure, 164  
 DVT, 162, 163  
 Eduline, 100  
 Eicosapentaenoic, 171  
 $\alpha$ -Elemene, 280  
 $\beta$ -Elemene, 280  
 Ellipticine  
     structure, 123  
 Emetine  
     for amoebic dysentery, 119  
     antiamoebic, 126  
     lead compound, 121

- Emetine** (continued)  
 mechanism of action, 127  
 pharmacological activity, 77  
 from plant, 80  
 structure, 120
- Emodin**, 321, 322
- Ent-kaurene diterpenoids**, 216
- Ent-kaurene glycosides**, 88
- Ephedrine**  
 antitussive, 55  
 in khat, 100, 102  
 neanderthal, 35  
 plant source, 36, 46  
 structure, 41, 54, 104
- R,S-(-)-Ephedrine**, 102  
 structure, 104
- Ephedron**, 35
- 1-Epibicyclosesquiphellandrene**, 280
- Epicatechin**, 271  
 DNA, 262, 275  
 in jimson weed, 266
- Epicatechin 3-O-gallate**, 271
- 2-Epi- $\alpha$ -cedrene**, 280
- (-)Epigallocatechin-3-O-gallate**, 275
- Epinephrine**, 35, 272  
 structure, 41
- 9-Epiquinine**, 118
- Epirosmanol**, 281
- 9,10-Epoxyheptadec-16-en-4,6-diyne-8-ol**  
 nematicide, 188  
 structure, 190
- Equol**, 275, 276  
 structure, 272
- Ergometrine**, 75
- Ergonovine**, 64, 65
- Ergosterol**, 60, 62
- Ergot alkaloids**, 45, 64, 70, 370
- Ergotamine**, 75
- Eriodictyol**, 261, 270, 272  
 structure, 263
- Esculetin**, 160, 262  
 structure, 161
- Esculin**, 288
- Estradiol-17 $\beta$**   
 structure, 272
- Estragole**, 280
- Estrogen**, 275, 318
- Estrone-glucuronide**, 275
- 7-Ethoxycoumarin**, 302  
 structure, 304
- Ethoxycoumarin O-deethylase**, 257
- Ethyl carbamate**, 252
- Ethylene**, 167, 173
- $\alpha$ -Ethyltryptamine**  
 structure, 122
- Etoposide**, 47, 50, 82  
 structure, 52, 83
- Etretinate**, 57  
 structure, 61
- Eucalyptol**, 46, 277
- Eucatropine**, 63
- Eudesmanes**, 305
- Eugenol**  
 in basil, 280  
 in cranberry sauce, 251  
 $IC_{50}$  for *T. vaginalis*, 137  
 nematicide, 194  
 plant source, 48  
 structure, 139, 195  
 Thanksgiving Day dinner, 252  
 in yarrow, 278
- 3,3-(Ethylenedioxy) eudesm-11(12)-eno-13,6 $\alpha$ -lactone**, 216
- Eupatorin**, 46
- Europine**, 285
- Evernic acid**, 206  
 structure, 207
- Evonine**, 101
- Falcarindiol**  
 constitutive resistance, 173  
 structure, 172  
 in tomato, 251  
 tomato phytoalexin, 171
- Falcarinol**  
 carrot phytoalexin, 173  
 in carrot, tomato, 251  
 $EC_{50}$  for *E. cinerea*, 175  
 structure, 172  
 tomato phytoalexin, 171
- Falcarinolone**, 251
- Farnesene**, 239, 279, 280
- trans- $\beta$ -Farnesene**, 239
- Farnesol**, 280
- Febrifugine**, 129  
 structure, 132
- Fenchone**, 103, 280
- Fenetyl acetate**, 280
- Fenetyl alcohol**, 280
- Fenoxycarb**, 231  
 structure, 232

- Fenvalerate  
    structure, 229
- Ferulic acid, 160  
    structure, 161
- Feruloyl, 171
- $\alpha$ -Fetoprotein  
    estrone binding and  
    phytoecdysones, 276
- Fisetin, 271
- Flavan-3-ols, 274
- Flavone acetic acid, 275
- Flavonoids, 86, 103, 138, 159, 178,  
    222, 260-264, 266, 269,  
    270-276, 301, 329
- Flavonoid content, 260, 264, 266, 270,  
    273
- Flavonoid metabolism, 260, 262
- Flavonoid mutagen, 273
- Flavonol, 137, 269, 273, 274, 332
- Formic acid, 239, 240, 312
- Formononetin, 269, 275  
    structure, 272
- (*-*)-*N*-Formylnorephedrine  
    structure, 103
- Forskolin, 45
- Fumarprotocetraric acid, 206
- Fumonisins B1, 324, 325, 330
- Fumonisins B2, 325
- Fumonisins B3, 325
- Fumonisins B4, 325
- Fumonisins mycotoxin, 316, 325
- Fumonisins, 314-316, 322, 324, 325,  
    328
- Fumonisins A1 and A2, 324
- Fumonisins B1 and B2, 324, 325  
    structures, 315
- Fumonisins B3 and B4, 325  
    structures, 316
- Furan derivatives, 252
- Furano- and pyranocoumarins, 302
- Furanoacetylenes, 169
- Furanocoumarins, 249, 251, 282, 291,  
    293, 295-303, 322, 329, 370
- Furanosesquiterpene, 175, 305
- Furanoterpene, 178, 305
- Furanoterpeneoids, 159, 305, 309
- Furfural, 280
- Fusarin C, 323  
    structure, 324
- G-strophanthin, 57
- Galacturonic acid, 168
- Galbanum, 28, 46
- (*+*)-Gallocatechin, 275
- Gedunin, 137  
    structure, 138
- Gelsemine, 46
- Genistein  
    cancer induction, 276  
    estrogenic activity, 275  
    in lima bean, 286  
    in soybean, clover, 269  
    structure, 272
- Genistin, 269, 271
- Geraniol, 193, 194, 279, 280  
    structure 195
- 7-Geranoxycoumarin, 301
- 8-Geranoxypсорален, 302
- Geranyl acetate, 280
- Germacrene-D, 239, 240
- Germacrenolides, 134
- GHPH, 308, 310
- Gitalin, 57
- Glaucarbinone  
    structure, 136
- Glaucarubin, 48  
    structure, 136
- Glaucarubol  
    structure, 136
- Glaucarubolone, 192  
    structure, 193
- $\beta$ -1,3-Glucanases  
    fungal cell wall, 169  
    fungicide, 159  
    phytoalexin, 171
- Glucobrassicin, 257, 259
- N2-[ $\gamma$ -L-(*+*)-Glutamyl]-4-  
    carboxyphelhydrazine, 310
- N2-[ $\gamma$ -L-(*+*)-Glutamyl]-4-(hydroxy-  
    methyl), 310
- Glutinosone, 251
- Glutamyl-4-hydroxymethylphenyl-  
    hydrazine, 308-310
- Glyceofuran  
    structure, 166
- Glyceollin  
    nematostatic effect, 194  
    in soybean, 251  
    soybean phytoalexin, 143  
    structure, 142, 166, 195

- Glyceollin I  
structure, 166
- Glyceollin II  
structure, 166
- Glyceollin III  
structure, 166
- Glyceollin IV  
structure, 166
- sn*-Glycerol-1-eicosa-9,12-dienoate-2-palmitoleate-3-linoleate  
structure, 193
- Glycinoeclepin A  
structure, 198
- Glycinol, 286  
structure, 166
- Glycobismine A, 127  
structure, 131
- Glycocitrine I, 127  
structure, 131
- Glycolic acid, 281
- Glycoside, 46, 57, 77, 78, 86, 97, 149, 195, 196, 243, 252, 295
- Glycyrrhizin, 46  
structure, 86
- Goitrin, 252, 254, 256, 329
- Gossypetin, 271
- Gossypol  
antifertility agent, 87, 253  
antiparasitic activity, 133  
in cotton, 251  
cotton phytoalexin, 143  
 $EC_{50}$  for fungal spores, 173  
nematode resistance, 194  
plant source, 46  
structure, 134, 174, 196, 255
- Gossyvertin  
structure, 174
- Graveolone, 300  
structure, 302
- Guaianolides, 134  
structure, 135
- $\alpha$ -Guaiene, 280
- $\delta$ -Guaiene, 280
- $\gamma$ -Gurjunene, 280
- Gyrocarpine, 125  
structure, 127
- Gyromitrin  
in false morel, 308, 310  
hydrolysis, 312  
structure, 311
- Harmaline, 123  
structure, 122
- Harmalol  
structure, 122
- Harman  
structure, 122
- Harmine  
structure, 122
- Harmol  
structure, 122
- Hederagenin, 243
- Heleurine  
structure, 285
- Hemerocallin  
structure, 146
- Hemigossypol, 194, 251  
structure, 174, 196
- Heptadeca-1,9-diene-4,6-diyne-3,8-diol, 188  
structure, 190
- Heraclenin, 284
- Herniarin  
antimicrobial activity, 302  
in Bishop's weed, 281  
structure, 304
- Heroin, 39, 47, 98
- Hesperetin  
antioxidant activity, 271  
metabolism, 261  
structure, 263
- Hesperidin  
antioxidant inactivity, 271  
metabolism, 261  
structure, 263  
vitamin P, 272
- 5,6,11,12,17,18-Hexahydrocyclonona[1.2-b:4.5-b':7.8-b"]triindole, 260
- Hexanol, 279, 280, 298, 310
- Hircinol, 178  
structure, 177
- Holacanthone  
structure, 136
- Homatropine, 63
- Homoeriodictyol, 261  
structure, 263
- Homoharringtonine, 45, 129  
structure, 132
- Homovanillic acid, 260, 261
- Honyumine, 127  
structure, 131

- Hordatine**, 164  
***m*-HPAA**, 260  
***m*-HPPA**, 261  
     structure, 263  
**Humulene**, 278  
     in basil, 280  
     in pennyroyal, 239  
**Hydrangeic acid**, 208  
**Hydrangenol**, 208, 209  
**Hydrastine**, 46  
**Hydrazine**, 330  
     cooking, 310  
     LD<sub>50</sub>s, 311  
     lethal dose, apes, 312  
     in mushrooms, 251, 308  
     Thanksgiving Day dinner, 252  
***p*-Hydrazinobenzoic acid**, 310  
**Hydrazones**, 312, 330  
**Hydrocortisone**, 57, 60  
     structure, 61  
**Hydrogen peroxide**, 137, 252  
**Hydromorphone**, 39  
     structure, 40  
**7-Hydroxy**, 314  
**3-Hydroxy-flavone**, 271, 273  
**3-Hydroxy-β-ionone**, 173  
**3-Hydroxy-4-methoxyphenyl-hydacrylic acid**, 261  
***p*-Hydroxybenzoic**, 262  
***p*-Hydroxybenzoic acid**, 173, 175  
**Hydroxychloroquine**, 64  
     structure, 66  
**Hydroxycinnamic acid**, 160, 288  
**2'-Hydroxydaidzein**, 286  
**11-Hydroxy-9,10-dehydroneolidol**, 173  
     structure, 172  
**Hydroxydiffractive acid**, 206  
     structure, 207  
**9-Hydroxyellipticine**  
     structure, 123  
**Hydroxyethyl rutosides**, 262  
**2'-Hydroxygenistein**, 286  
**8-Hydroxygenistein**, 286  
***m*-Hydroxyhippuric acid**, 261  
**5, 7, 3', 4'-Hydroxylated flavonoid**, 262  
**Hydroxylated neo-menthol**, 219  
**Hydroxylubimin**, 173, 251, 287  
**Hydroxymenthol**, 219  
**2-Hydroxy-7-methoxycadalene**, 173  
**2-Hydroxy-7-methoxycadalene**  
     (continued)  
     structure, 174  
**4-Hydroxymethylphenylhydrazine**, 308  
     structure, 309  
**7-Hydroxymyoporone**  
     structure, 305  
**4-Hydroxymyoporonal**, 306  
**9-Hydroxynerolidol**  
     jimson weed, 173  
     phytoalexin, 173  
     structure, 172  
***m*-Hydroxyphenyl propionic acid**, 261  
***m*-Hydroxyphenyl propionic acid**, 261  
***m*-Hydroxyphenylacetic acid**, 260  
     structure, 263  
**Hydroxytremetone**, 249  
     structure, 250  
**3-Hydroxyuracil**, 218  
**3-Hydroxyuridine**, 217  
     structure, 218  
**Hydroxyvernolide**, 134, 146  
     structure, 135  
**Hyoscine**, 10  
**Hyoscyamine**  
     in belladonna, 10  
     decongestant, 55  
     plant source, 46  
     in solanaceous plants, 248  
     structure, 54  
**Hypericin**, 46  
     structure, 86  
**I3C**, 257-260  
**IAA**, 292  
**Ibogaine**, 48  
**ICZ**, 259, 260  
**Imidazole alkaloid**, 77  
**Imperatorin**, 243, 301  
**Indirubin**, 79  
     structure, 81  
**Indole alkaloid**, 77-79, 86, 87, 93, 122, 129, 212, 254, 257-259, 329  
**Indole glucosinolates**, 254, 259  
**Indole-3-acetic acid**, 212  
**Indole-3-carbinol**, 257, 258, 329  
     structure, 259  
**Indoleacetic acid**, 292  
**3-Indoleacetonitrile**, 257  
**3-Indolemethanol**, 257  
**Indolo[3.2-b]carbazole**, 259

- 3-Indolylacetonitrile, 257, 259  
 3-Indolylmethyl, 256  
 Indolylmethylglucosinolate, 257  
     structure, 258  
 $\beta$ -Ionone  
     blue mold resistance, 173  
     structure, 172  
 1,4-Ipomeadiol  
     structure, 305  
     in sweet potato, 309  
 Ipomeamarone  
     concentration, 306  
     maximum concentration, 309  
     structure, 175, 304  
     in sweet potato, 251  
     sweet potato toxicity, 305  
 Ipomeamaronol, 178  
     structure, 175  
 Ipomeanine  
     concentration, 306  
     potato toxicity, 305  
     structure, 304  
 Ipomeanol  
     LD<sub>50</sub> in mice, 307  
     lung edema, 330  
     lung toxin, 83, 307, 330  
     maximum concentration, 309  
     metabolism and lung binding, 307  
     potato toxicity, 305  
     structure, 84, 305  
     in sweet potato, 309  
 3-Isobutyroyl- $\beta$ -ionone, 172  
 Iso-menthol, 219, 220  
 Isoartemisia, 278  
 Isocaryophyllene, 280  
 Isocorynantheol, 123  
     structure, 124  
 Isocoumarins, 159, 249  
 Isocyanates, 257  
 Isoeugenol, 137, 280  
     structure, 139  
 Isoferreirin, 286  
 Isoflavones, 252, 269, 275, 276  
 Isoflavonoid, 159, 165, 169-171, 178,  
     249, 273, 333  
 Isohemigossypol  
     structure, 174  
 Isoheptylic acid, 239  
 Isoimperatorin, 300  
     structure, 302  
 Isomenthone, 239, 240  
 Isomeric stilbene, 208  
 Isopimpinellin, 243, 298, 301  
     structure, 297  
*cis*-Isopulegone, 240  
*trans*-Isopulegone, 240  
 Isoquinoline  
     antiviral activity, 86  
     capnoidine, 124  
     drugs, 77  
     tetrahydropalmatine, 79  
 Isoquinoline alkaloid, 77, 79, 82, 123,  
     124  
 Isorhamnetin, 266  
 Isorosmanol, 281  
 Isosilychristin  
     structure, 271  
 Isotenulin, 243  
 Isothiocyanates, 257  
 Isotretinoin, 57  
     structure, 61  
 Isotrichodermin, 314  
 Isovitexin, 271  
 Ivermectin, 371  
 Japonicine A, 138  
     structure, 141  
 Jatrorrhizine, 124  
 Juvabione  
     structure, 125, 232  
 Juvenile Hormone, 186  
     analogs, 231-233  
     JH, 230, 231, 233  
     JH B<sub>3</sub>  
         structure, 231  
 JH biosynthesis, 233  
     JH I  
         structure, 231  
     JH II  
         structure, 231  
     JH III  
         structure, 231, 232  
     JH O  
         structure, 231  
 Juvocimene, 280  
 Juvocimene, II  
     structure, 232  
 Kaempferol  
     bowel disease, 275  
     in fruits, 268, 269  
     in khat, 103

- Kaempferol** (continued)  
 mutagenicity, 273  
 in oregano, 279  
 in vegetables, 267  
 structure, 274  
**Kainate**, 144  
**Kainic acid**  
 antihelmintic, 144  
 structure, 143  
**Katin**, 100  
**1-Keto**, 314  
**Ketone**, 119, 136, 189, 278  
**Khat phenylpropylamines**, 102  
**Khatamine**, 103-105  
**Khatamines**, 102, 104  
**Khellin**, 43  
 structure, 50  
**Kievitol**, 286  
**Kievitone**, 286  
 structure, 165  
**Kievitone hydrate**, 286  
**Kinetin**, 217  
**Klaineanone**, 192  
 structure, 193  
  
**Labiatic acid**, 283  
**trans-Lachnophyllic acid**  
 structure, 191  
**Lachnophyllol**, 188  
 structure, 191  
**trans-Lachnophyllol**  
 structure, 191  
**cis-Lachnophyllum ester**  
 structure, 191  
**trans-Lachnophyllum ester**  
 structure, 191  
**Lacinilene C**, 173  
 structure, 174  
**Lacinilene C** 7-methyl ether, 173  
 structure, 174  
**Lacinilenes**, 251  
**Laetrile**, 48  
**Lanatoside A-C**, 57  
**Lanatoside C**, 45  
**Lanatosides A, B, and C**  
 structure, 59  
**Lapachol**, 137  
 structure, 139  
**Lapachone**  
 antiparasitic, 137  
 structure, 139  
  
**Lasiocarpine**  
 structure, 285  
**Lathodoratin**  
 structure, 168  
**Lecanoric acid**, 206  
 structure, 207  
**Lecithin**, 239  
**Lectin**, 48, 198  
**Ledene**, 280  
**Lemmatoxin**, 243  
**Leptidine**, 289  
**Leptine I**, 289-290  
 structure, 288  
**Leptine II**, 289  
**Leptinine I**, 289  
**Leptinine II**, 289  
**Leptinines**, 291  
**Leurocristine**, 78, 94  
**Levophanol**, 82  
 structure, 81  
**Lignans**, 86  
 structure, 83  
**Lignin**, 159, 160, 169  
**cis-Ligustilide**, 298  
**Limonene**  
 biological effects, 241  
 in citrus juice, 301, 303  
 in pennyroyal, 240  
 structure, 195, 302  
**Limonoids**, 85  
**Linalool**  
 in basil, 281  
 in khat, 103  
 nematicide, 194  
 in oregano, 279  
 in sage, 283  
 structure, 195  
**Linalyl acetate**, 239, 280  
**Linear furanocoumarins**, 249, 291,  
 293, 295-303, 322  
**Linear pyranoacridone**, 127  
**Linoleic acid**, 298  
 structure, 193  
**Linoleic acids**, 192  
**Lobeline**, 47  
**Loroglossol**, 178  
 structure, 177  
**Lubimin**  
 in egg plant, 251  
 egg plant phytoalexin, 294  
 in jimson weed, 173

- Lubimin (continued)**
- potato phytoalexin, 162, 171, 287
  - structure, 170, 295
- Lunularic acid**
- liverwort, herbicide, 208
  - relationship to resveratrol, piceatannol, 213
  - structure, 209
- Lupanine**, 243
- Luteolin**
- in carrot, 270
  - in oregano, 279
  - in yarrow, 278
- Luteone**, 286
- Maackiain**
- structure, 166
- Macrophyllidosides A, B, and C**, 209
- Malathion**, 288
- Malonaldehyde**, 252
- Malonyl-daidzin**, 271
- Malonyl-genistin**, 271
- Mansonones**, 164
- Maoecrystal I**, 216
- structure, 217
- Maoecrystal I and J**, 216
- structure, 217
- Maoecrystal J**
- structure, 217
- Marmesin**, 284
- Marmesinin**, 284
- 2-cis-8-cis-Matricaria ester**
- structure, 190
- 2-trans-8-cis-Matricaria ester**
- structure, 190
- Maytoline**, 101
- Medicagenic acid**, 243
- Medicarpin**, 171, 214, 251
- structure, 166, 215
- Melanin**, 327
- structure, 328
- Menthane 3,8 diols**
- structure, 219
- Menthane-3,8-diols**, 218, 225
- Menthol**
- in basil, 280
  - biological activity, 240
  - herbicide, 219
  - nematicide, 194
  - in pennyroyal, 239
  - plant source, 47
- Menthol (continued)**
- structure, 195, 220
  - in yarrow, 278
- Menthone**
- in basil, 280
  - in mountain mint, 240
  - in pennyroyal, 239
- D-menthone**, 240
- 1-Menthyl acetate**, 219
- Mepenzolate**
- antispasmodic, 55
  - structure, 54
- Mercuric chloride**
- elicitor inactivity, 171
  - phytoalexin elicitor, 169, 173
- Mercuric or cupric chloride**, 167
- Merucathine**, 105
- structure, 103
- R,S-(+)-Merucathine**
- structure, 103
- Merucathinone**, 101, 103, 105
- S-(+)-Merucathinone**, 103
- structure, 101
- Mesaconitine**, 9
- p-Methane-3,8-diol**
- structure, 219
- Methionine**, 280
- Methoprene**
- structure, 232
- 3-Methoxy-4-hydroxyphenylacetic acid**, 260
- 4-Methoxy-14-hydroxytylophorine**
- structure, 130
- 4-Methoxy-6-hydroxy-1-vinyl- $\beta$ -carboline**
- structure, 122
- N-methoxy-3-indolylmethyl isothiocyanate**, 256
- 6-Methoxy-N-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline**, 123
- structure, 122
- 3-Methoxy-4,5-methylene-dioxyamphetamine**, 251
- Methoxyannomontine**, 123
- structure, 122
- 2'-Methoxychalcone**, 251
- 10-Methoxycinchonamine**, 123
- structure, 124
- 2'-Methoxygenistein**, 286
- 6-Methoxygossypol**, 173, 194
- structure, 174, 196

- 6-Methoxyharmalan  
structure, 122
- 6-Methoxyhemigossypol, 194  
structure, 174, 196
- 4-Methoxymedicarpin, 214  
structure, 215
- 6-Methoxymellein, 173, 251  
structure, 175
- 5-Methoxypsoralen  
in celery, 299  
jet lag, 296  
in parsley, 301  
in parsnip, 300  
photosensitivity, 296
- Methoxysativan, 214  
structure, 215
- 4-Methoxy-1-vinyl- $\beta$ -carboline, 123  
structure, 122
- Methyl 2-*trans,8-cis*-deca-2,8-diene-4,6-dioate, 188
- N*-methyl-*N*-formylhydrazine, 310, 311
- N*-methyl-*N*-formylhydrazone, 310
- Methyl 4'-hydroxycinnamate, 198  
structure, 197
- Methyl 4-hydroxybenzoate, 198  
structure, 197
- Methyl cation alkylating agent, 312, 330
- Methyl cinnamate, 280
- Methyl decanoate, 298
- Methyl isothiocyanate, 195
- Methyl salicylate, 44, 49, 239, 240  
structure, 50
- 2-Methylanthraquinone, 243
- 3-Methylbutanol, 310
- Methylcatichol, 280
- Methylcyclohexanone, 239
- N*-methylcytisine, 187, 189
- Methyldithiocarbamate, 195
- Methylergonovine, 64  
structure, 65
- Methyleugenol, 280
- N*-methylformylhydrazine, 312  
structure, 311
- Methylglyoxal, 252
- Methylhexahydrophthalic anhydride, 145
- N*-methylhydrazine, 312, 330  
structure, 311
- 7-Methyljuglone, 243
- Methylorsellinic acid, 206
- Methylorsellinic acid (continued)  
structure, 207
- Methylorsellinic acid ethyl ester, 206
- 4-Methylphenylhydrazine, 308
- 3-Methylsulfinylpropyl isothiocyanate, 256
- 4-Methylsulfinylbutyl isothiocyanate, 256
- 4-Methylsulfonylbutyl isothiocyanate, 256
- 4-Methylthiobutyl isothiocyanate, 256
- 3-Methylthiopropyl isothiocyanate, 256
- N*-methyltryptamine, 122
- Metronidazole, 134
- Mevinolin, 318  
structure, 319
- Milbemycins, 140
- Minquartynoic acid, 145  
structure, 144
- Miotine  
structure, 63
- Momilactone A, 178  
structure, 176
- Momilactone B, 178  
structure, 176
- Momilactones, 180, 251
- Monocrotaline, 187  
structure, 188, 285
- Monocrotaline pyrrole  
structure, 285
- Moracin A, 178  
structure, 176
- Moracin B, 178  
structure, 176
- Morin, 271, 320
- Morphine  
antitussive, 55  
drug, 57, 75, 77  
lead compound, 80, 82, 370  
opium poppy, history, 39  
plant source, 47  
production, 43  
seminarcosis, 10  
structure, 40, 81
- 4-MPH, 308
- MTI-800  
structure, 229
- Mukaadial, 243
- $\alpha$ -Murolene, 280
- Myoporone, 338

- Myrcene**  
 in basil, 280  
 biological activity, 240  
 in oregano, 279  
 in pennyroyal, 239  
 in yarrow, 278
- Myricetin**  
 biological activity, 275  
 biological effects, 271  
 in jimson weed, 266  
 in khat, 103  
 metabolism, 261  
 mutagenic activity, 273
- Myristic acid**, 192  
 structure, 193
- Myristicin**, 251-253  
 structure, 228
- Nabilone**, 40  
 structure, 41
- Naphthoquinone**, 243
- 1,2-Naphthoquinone**, 137, 153
- Naringenin**, 271, 279
- Naringin**, 261, 270
- Natron**, 16, 18, 21-24, 27
- Neoglucobrassicin**, 259
- Neoisomenthol**, 240
- Neomenthol**, 219, 220, 240
- Neosilyhermin A**  
 structure, 266
- Neosilyhermin B**  
 structure, 266
- Neostigmine**  
 structure, 63
- Nerol**, 103, 280
- Nerolidol**, 280
- Nerylacetate**, 279
- Nicotine**  
 insecticide, 227  
 lead compound, 371  
 nematicide, 187  
 structure, 228, 189
- Nimbolide**, 137  
 structure, 138
- Niridazole**, 147
- Nitrate**, 252, 291, 292
- Nitriles**, 257
- Nitroketenedimethyl mercaptols**, 371
- Nitrosamide**, 312
- N*-nitroso compounds**, 291
- N*-nitroso-bis-(2-oxopropyl) amine**, 258
- Nivalenol**, 314
- NMFA**, 312
- Noradrenaline**, 109, 114
- Norephedrine**  
 cardiovascular effects, 109  
 concentration in khat, 104-105  
 in khat, 102  
 structure, 103
- R,S-(-)-Norephedrine**, 102  
 structure, 103
- Norepinephrine**, 35, 272  
 structure, 41
- Norpseudoephedrine**  
 cardiovascular effects, 109  
 concentration in khat, 104, 105  
 in khat, 100, 102  
 structure, 101
- Norpseudoephedrine oxalate**, 100
- Nortiliacorinine A**, 125  
 structure, 129
- Nortriterpenoids**, 198
- Norwogonin**, 273
- Noscapine**  
 common cold, 55  
 from opium poppy, 80  
 structure, 54
- Notexins**, 7
- Obabерine**, 125  
 structure, 127
- Ochratoxin A**, 314, 320, 328, 330
- Ochratoxin residues**, 316
- Ochrolifuanine A**, 120  
 structure, 121
- Ochrolifuanine alkaloids**, 120
- Ochrolifuanines**, 120, 121
- Ocimene**  
 in basil, 280  
 in khat, 103  
 in mountain mint, 240
- cis-Ocimene**, 240, 280
- trans-Ocimene**, 280
- Octanal**, 310
- Octanol**  
 in basil, 280  
 in pennyroyal, 239  
 structure, 198
- 3-Octanone**, 280

- 1-Octen-3-ol  
     in basil, 280  
     biological activity, 240  
     in mushroom, 308  
     in pennyroyal, 239  
*cis*-2-Octenal, 310  
*trans*-2-Octenal, 310  
 Octylic acid, 239  
 Odoracin, 201  
     structure, 198  
 Odoratrin  
     structure, 198  
 Oleanolic acid, 243, 279  
*trans*-Olefinic cathinone, 103  
 Oleoresin filicin, 45  
 Olivacine  
     structure, 123  
 Ophiuroidins, 210, 211  
 Orchinol, 178  
     structure, 176  
 $\beta$ -Orcinol type depsides, 206  
 Orcinocarboxylic acid  
     herbicide, 207  
     lichen herbicide, 206  
     structure, 207  
 Orsellinic acid, 206  
     structure, 207  
 Oruwacin, 243  
 Oruwal, 243  
 Oryzalexins, 251  
 Ouabain  
     arrow poison, 57  
     plant source, 44, 48, 80  
     structure, 60  
 Oxalate, 100, 251, 351  
 Oxalic acid, 252  
 $(11S)$ -3-Oxoeudesm-11(12)-eno-13,6  $\alpha$ -lactone  
     as herbicide, 216  
     structure, 217  
 9-Oxonerolidol  
     jimson weed, 173  
     phytoalexin, 173  
     structure, 170  
 Oxymorphone, 39  
     structure, 40  
 Oxypeucedanin  
     in Bishop's weed, 284  
     mutagenicity, 300  
     in parsley, 301  
     structure, 302  
     Oxypeucedanin hydrate  
         in Bishop's weed, 284  
         in parsley, 300  
         structure, 302  
 Oxyphencyclimine  
     antispasmodic, 55  
     structure, 53  
 Oxytocin, 64  
 Pabulenol, 284  
 Palasonin  
     structure, 145  
 Palmatine, 124  
     structure, 125  
 Palmitic acid, 192  
     structure, 193  
 $(+)$ -Pandoxide  
     inactivity, 138  
     structure, 141  
 Papaverine  
     antitussive, 55  
     coronary muscle relaxant, 57  
     drug, 75  
     lead compound, 80  
     structure, 61  
     synthetic, 76  
 Parthenin, 134  
     structure, 135  
 Patulin  
     alar relationship, 331  
     human disease, 328  
     neurotoxin, lung toxin, 330  
     source, 320  
     structure, 315  
     toxicology, 321  
     worldwide importance, 314  
 Penicillic acid, 315  
 Pentanal, 310  
 Pentazocine, 82  
     structure, 81  
 Pentyl alcohol, 279  
 Pentylbenzene, 298  
 2-Pentylfuran, 298  
 Permethrin  
     structure, 229  
 Petaline, 82  
 Phaeanthine, 125, 126  
     structure, 128  
 Phaseolin, 175  
     structure, 165  
 Phaseollidin, 286

- Phaseollidin** (continued)  
 structure, 165  
**Phaseollininisoflavan**  
 structure, 165  
**Phebalosin**, 243  
**β-Phellandrene**, 103  
**Phellopterin**, 302  
**Phenacetin**, 257  
**Phenanthroindolizidine**, 126, 127  
**Phenanthroquinolizidine**, 126, 127  
**1-Phenyl-1,2-propandione**  
 structure, 104  
**1-Phenyl-1,3,5-heptatriyne**, 140  
 structure, 142  
**1-Phenylhepta-1,3,5-triyne**, 188  
 structure, 190  
**Phenols**, 86, 159, 160, 169, 266, 289, 290  
**Phenylacetic acid**, 262  
**Phenylalkylamine**, 100, 102-105, 108  
**Phenylbutazone**, 284  
**Phenylethyl alcohol**, 280  
**Phenylethyl isothiocyanate**, 256  
**Phenylhydrazine**, 310  
**Phenylpentenylamines**, 102, 105  
**Phenylpropanoid**, 76, 137, 171, 220, 221  
**Phenylpropionic acid**, 262  
**Phenylpropyl isothiocyanate**, 257  
**Phenylpropylamine**, 102, 104, 109  
**2-Phenyl-5-(1'-propynyl)-thiophene**, 188  
 structure, 190  
**Phenylvalerolactone**, 261  
**Phlorizin**, 252, 270  
**Phloroglucinol**, 138  
**Phocion**, 2  
**Phomopsin**, 314  
**Phthalide isoquinoline alkaloid**, 124  
**Phyllodulcin**, 209  
**Physostigmine**  
 drug, 77, 80  
 nematostatic, 187  
 plant source, 47  
 structure, 63, 188, 228, 230  
**Phytoestrogens**, 275, 276  
**Phytosterol**, 280  
**Phytuberin**  
 in potato, 287  
 potato phytoalexin, 162, 171, 251  
 structure, 170  
**Phytuberol**, 171, 251  
 structure, 170  
**Piceatannol**  
 structure, 213  
**Picrotoxin**, 44  
**Pilocarpine**  
 drug, 75, 77, 80  
 miotic, 62  
 plant source, 47  
 structure, 63  
**Pinene**, 279  
 in basil, 280  
 in celery, 298  
 in khat, 103  
 in mountain mint, 240  
 in pennyroyal, 239  
 in rosemary, 281  
 in sage, 283  
 in yarrow, 278  
**Pinosylvin**, 164  
**Pipericide**  
 structure, 228  
*trans*-**Piperitol**, 240  
**Piperitone**, 239, 240  
**Piperitonene**, 240  
**Piperonyl butoxide**, 228, 307  
 structure, 229  
**(-)-Pipoxide**  
 anti-helmintic, 141  
 antiprotozoal, 138  
**Pisatin**  
 in pea, 171  
 pea phytoalexin, 175, 251  
 structure, 165  
**Podophyllin**, 47  
**Podophyllotoxin**  
 lead compound, 82  
 plant source, 47  
 structure, 52, 83  
**Polyacetylenes**, 140, 144, 145, 165, 171, 173, 178, 187-189  
**Polyenes**, 159  
**Polygodial**, 243  
**Polymethoxyflavones**, 137  
**Polysaccharides**, 76, 168  
**Polythienyls**, 186  
**Precocene I**  
 structure, 233  
**Precocene II**  
 structure, 233  
**Prednisolone**, 57

- Prednisolone (continued)  
     structure, 61
- Prednisone  
     structure, 61
- Prenylated hydroxynaphthoquinone,  
     137
- Primaquine, 64, 84  
     structure, 66
- Primulic acid, 243
- Pristimerin, 137  
     structure, 139
- Procaine, 82
- Procyanidin B-1 and C-1, 275
- Procyanidin B2 3'-O-gallate, 271
- Procyanidins, 271
- Propanol, 310
- Propantheline  
     antispasmodic, 55  
     structure, 53
- Propionic acids, 326
- Propoxur  
     structure, 230
- Protoberberine alkaloids, 124
- Protocatechuic acid, 160  
     structure, 161
- Protocetraric acid, 206
- Protoveratrine A and B, 57  
     structure, 58
- Prunetin, 276
- Pseudoephedrine  
     decongestant, 55  
     structure, 54
- Pseudoguaianolide, 134
- Pseudohypericin  
     structure, 86
- Pseudomerucathine, 103, 105  
     structure, 101
- Pseudotubulosine, 120  
     structure, 121
- Psoralen, 296-302, 329  
     structure 297
- Psoralidin, 142, 194  
     structure, 196
- Pterocarpan, 215
- Pterostilbene, 178  
     structure, 177
- trans*-Pterostilbene, 178
- Pulegone, 238-240
- Pycnamine, 125  
     structure, 128
- Pyrazole, 307
- Pyrethrins, 48, 205, 227, 228, 229,  
     234, 235
- Pyrethrin I  
     structure, 228, 229
- Pyridocarbazole alkaloids, 123, 124
- Pyrocatechol, 192, 194
- Pyroligneous acid, 24
- Pyroquilon, 327
- Pyrrolizidine alkaloids, 286, 329  
     structure, 285
- Qinghaosu, 84, 97, 126, 130, 146  
     structure, 85
- Quassinooids, 85, 134, 136, 137
- Quercetagetin, 271
- Quercetin  
     Ames test, 370  
     antioxidant, 271  
     antiparasitic action, 137  
     carcinogenicity, 274  
     cataract prevention, 273  
     in fruits, 268, 269  
     in jimson weed, 266  
     in khat, 103  
     life span reduction, 275  
     metabolism, 260  
     nematicide, 192  
     structure, 140, 194, 262  
     tyrosine protein kinase inhibition,  
         276
- Thanksgiving Day dinner, 252
- in vegetables, 267
- in yarrow, 278
- Quercetin glycosides, 252
- Quiesone, 173  
     structure, 172
- Quinacrine, 64  
     structure, 65
- Quinidine, 57  
     malaria treatment, 118  
     plant source, 45, 48, 80  
     structure, 61, 119
- Quinidinone, 119
- Quinine, 48  
     antimalarial, 84, 118  
     compared to febrifugine, 129  
     drug, 75, 77, 80, 97  
     plant source, 45, 64  
     structure, 65, 85, 119
- Quinoline, 77, 83, 84, 86, 118
- Quinoline alkaloid, 77, 83, 84

- Quinones**, 137  
**Quinonoid triterpene**, 137, 151  
**Quinuclidinylindole alkaloids**, 123  
**Quisqualate**, 144  
**Quisqualic acid**, 144  
  structure, 143  
  
**Rebaudioside A**, 88  
  structure, 89  
  
**Rescinnamine**, 80  
  
**Reserpine**  
  antihypertensive, 77  
  as drug, 75  
  hypotension, 55  
  hypotensive, 97  
  plant source, 48, 80  
  structure, 56  
  
**Resveratrol**, 178, 182, 251  
  structure, 168, 213  
  
**Retinoic acid**  
  structure, 61  
  
**Rhein**, 62  
  structure, 63  
  
**Rhein anthrones**, 62  
  
**Rhisitin**, 287  
  
**Rhomitoxin**, 48  
  
**Ricin**, 48  
  
**Rishitin**, 162, 171, 194, 251  
  structure, 170, 195  
  
**Rishitinol**, 288  
  
**Robustadials A and B**, 138  
  structure, 141  
  
**Robustaol**, 138  
  structure, 141  
  
**Rosmanol**, 281  
  
**Rosmarinic acid**  
  in basil, 280  
  in oregano, 279  
  in *Prunella*, 239  
  in rosemary, 281  
  in sage, 283  
  
**Rotenone**, 194, 227  
  structure, 228  
  
**Rubratoxin B**, 319  
  
**Rutin**  
  metabolism, 261, 262  
  mutagenicity, 273  
  noncarcinogenicity, 274  
  in pagota tree, 239  
  plant source, 46  
  in yarrow, 278  
  
**Rutosides**, 262  
  
**Sabinene**  
  in basil, 280  
  in mountain mint, 240  
  in pennyroyal, 239  
  in yarrow, 278  
  
*cis*-**Sabinene hydrate**, 280  
  
*trans*-**Sabinene hydrate**, 280  
  
**Saccharin**, 88  
  
**Safrole**  
  in basil, 280  
  in liver, 277  
  in rosemary, 281  
  Thanksgiving Day dinner, 252  
  
**Safynol**, 178  
  structure, 176  
  
**Salazinic acid**, 206  
  
**Salicin**, 47, 48, 82  
  
**Salicylic acid**  
  biological activity, 240  
  in pennyroyal, 239  
  in yarrow, 278  
  
**Saligenin**, 48  
  
**Salvin**, 283  
  
**Sambucinol**, 314  
  
**Sambucoin**, 314  
  
**8(14),15-Sandaracopimaradiene-7 $\alpha$ , 18-diol**  
  antitrichome, 134  
  structure, 135  
  
**Sanguinarine**, 124, 187  
  structure, 125, 188  
  
**Santonin**  
  ancient anthelmintic, 117  
  antimalarial, 133  
  compared to quisqualate, 144  
  herbicide, 216  
  lead compound, 133  
  plant source, 44  
  structure, 143  
  
**Saponin**, 196, 290  
  
**Sativan**, 214, 251  
  structure, 215  
  
**Saxalin**, 284  
  
**Scirpusin A**, 213  
  structure, 214  
  
**Scirpusin B**, 213  
  structure, 214  
  
**Scopolamine**  
  antitussive, 55

- Scopolamine** (continued)  
     in belladonna roots, 10  
     drug, 80  
     mydriatic, 63  
     plant source, 45  
     in *Solanaceae*, 248  
         structure, 11, 53
- Scopoletin**, 160  
     structure, 161
- Scopolin**, 287
- Sedanenolide**, 298
- Sedanolide**, 298
- $\alpha$ -Selinene**  
     in basil, 280  
     in celery, 298
- $\beta$ -Selinene delta**, 280
- $\beta$ -Senepoxide**  
     inactivity, 140  
     *P. falciparum*, 140  
     structure, 141
- Septicine**, 126  
     structure, 130
- Sergeolide**, 136
- Sesamin**, 228  
     structure, 229
- Sesamolin**, 228  
     structure, 229, 232
- Sesquiterpene endoperoxide**, 130, 133
- Sesquiterpene lactones**, 134, 146
- Sesquiterpene phytoalexin capsidiol**, 294
- Sesquiterpenes**, 130, 294
- Sesquiterpenoid naphthols**, 173
- Sesquiterpenoid phytoalexins**, 162, 165, 171
- Sesquiterpenoids**, 159, 162, 165, 171, 230
- Sesquithujene**, 280
- Sexangularetin**, 273
- Shikimate**, 170, 171
- Silandrin**  
     structure, 265
- Silybin**, 264  
     structure, 265
- Silychristin**  
     structure, 271
- Silydianin**  
     structure, 265
- Silyhermin**  
     structure, 266
- Silymonin**  
     structure, 265
- Simalikalactone D**, 137  
     structure, 136
- Sinigrin**, 257
- $\beta$ -Sitosterol**  
     hypcholesteremics, 60  
     in medical preparations, 49  
     in sicklepod, 266  
     structure, 62  
     in yarrow, 278
- SKF-525A**, 307, 312
- Slaframine**, 293
- Sodium artesunate**  
     antimalarial, 66  
     i.v. injection, 131  
     mechanism, 132  
     structure, 66, 85
- Sodium carbonate**, 22
- Sodium sulfate**, 22
- Solamargine**, 243
- $\alpha$ -Solamarine**  
     fungal stress, 162  
     in potato, 289  
     structure, 163
- $\beta$ -Solamarine**  
     fungal stress, 162  
     in potato, 289  
     structure, 163
- 5  $\beta$ -Solanidan-3  $\alpha$ -ol**, 289
- Solanidine**, 287, 289, 293  
     structure, 294
- Solanine**  
     cholinesterase inhibition, 288-290, 329  
     concentration and illness, 289  
     dark content, 289  
     effect of cooking, 290  
     fungicide, 162  
     human birth defects, 293  
     illness, 289  
     inhibitor, teratogen, 329  
     phytoalexin, 251  
     plasma cholinesterase and illness, 288  
     in potato, 251  
     potato phytoalexin, 162  
     in potato varieties, 288, 289, 291  
     structure, 163, 287, 294  
     synthesis w/injury, 288  
     temperature, 289

- Solanine (continued)**
- teratogenic effects, 293
  - Thanksgiving Day dinner, 252
  - toxic testing, 294
  - USDA guidelines, 288
- Solasodine**, 294
- structure, 295
- Solasonine**, 243
- Solavetivone**
- potato phytoalexin, 162, 171, 251, 288
  - structure, 170
- Sonunin III**, 196
- Soranjidiol- $\alpha$ -acetate**, 243
- Sorbate**, 326
- Sorbic acid**, 326
- Sparteine**, 45, 64, 243
- structure, 65
- Spirobenzylisoquinoline alkaloid**, 124
- Spirosolane alkaloid**, 294
- Stachybotrys toxin**, 314
- Stachydrine**, 278
- Sterigmatocystin**, 314
- structure, 315
- Steroid**
- alkaloids, 127
  - glycoalkaloid, 162, 171, 187, 291, 295, 296
  - glycoside, 76, 77
  - sapogenins, 60
- Stevioside**, 88
- structure, 89
- Stigmasterol**, 60
- structure, 62
- Stilbene**
- fungicide, 178
  - herbicide, 208, 209
  - phytoalexin, 159, 169, 250
  - structure, 210
- Stilbene derivatives**, 208
- Stilbene metabolites**, 213
- Strophanthin**, 48, 57.
- Strychnine**, 1, 48
- Suberin**, 160
- Succinylcholine**
- structure, 50
- Sucrose**, 88, 209
- Sucrose substitutes**, 88
- Sulfinothioic acid**, 140
- Sumilarv**, 231
- structure, 232
- Surangin B**
- structure, 228
- T-2 toxin**, 317, 320, 321
- Tabernanthine**, 48
- Taipoxins**, 7
- Tannin**
- antiviral, 237
  - antiviral activity, 86
  - constipation, 110
  - hemorrhoidal preparation, 60
  - human effects, 108
  - in khat, 103
  - in oregano, 279
  - in pennyroyal, 239
  - plant source, 48
  - Thanksgiving Day dinner, 252
  - in yarrow, 278
- Taspine**, 69, 70
- Taxifolin**, 271
- Taxine**, 11
- Taxol**
- anticancer activity, 83
  - antiparasitic activity, 134
  - drug, 97
  - plant source, 48
  - structure, 52, 84, 135
- Temik**
- structure, 230
- Terpenoids**, 76, 86, 130, 143, 171, 181, 193, 194, 223, 250
- Terpinen-4-ol**, 278-281, 283
- $\alpha$ -Terpinene**, 278
- in basil, 280
  - in celery, 298
- Terpineol**, 279
- in basil, 280
  - in khat, 103
- Terpinolene**, 103, 278
- Terpinoline**, 280
- $\alpha$ -Terpinyl acetate**
- in basil, 280
- $\alpha$ -Terthienyl**
- nematicide, 186
  - photosynthesizing, 145
  - structure, 187
- 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin**
- receptor induction, 259
- Tetrachlorophthalide**, 327
- Tetracyclic**, 120, 187

- cis*-Tetradeca-6-ene-1,3-dyne-5,8-diol, 171  
 structure, 172
- Tetrahydro- $\beta$ -carboline, 120, 122
- Tetrahydrocannabinol, 40, 138, 141  
 amoebastatic, 138  
 structure, 41, 141
- Tetrahydroisoquinoline, 120
- Tetrahydropalmatine, 79  
 structure, 81
- Tetrandrine, 126
- Thebaine, 39  
 structure, 40
- Theobromine  
 bronchodilator, 55  
 in native holly, 69  
 plant source, 49  
 structure, 56, 255  
 testicular atrophy, 252
- Theophylline  
 bronchodilator, 55  
 in native holly, 69  
 plant source, 44  
 structure, 56  
 as synthetic, 76
- Thiarubrine A, 145  
 structure, 144
- Thiocyanate, 254, 257, 329
- Thionins, 159, 169
- Thiosulfinate, 196, 198
- Thiosulfonates, 198
- Thujaplicins, 164
- Thujone, 278-279  
 in basil, 280  
 in khat, 103
- Thymol  
 in oregano, 279  
 plant source, 49  
 in rosemary, 281  
 in sage, 283
- Tiliacorine, 125  
 structure, 129
- Tiliacorinine  
 activity towards *P. falciparum*, 125, 126  
 structure, 129
- Tiliroside, 274
- Tingenone, 137  
 structure, 139
- $\alpha$ -Tocopherol, 271
- Tomatidenol, 288, 289
- Tomatidine, 295
- Tomatine  
 biological activity, 243  
 distribution in tomato, 295  
 $ED_{50}$ , nematode, 187  
 illness, tomato content, 295  
 nematicide, 187  
 structure, 163, 189, 296  
 Thanksgiving Day dinner, 252  
 in tomato, 251  
 tomato phytoalexin, 162, 251
- Total glycoalkaloid, 288
- Totaquine, 118
- Tremetone, 248, 249  
 structure, 250
- Tretinoin, 57
- Trichosanthin, 49, 87
- Trichothevenes, 220, 314, 328
- Tricyclazole, 327  
 structure, 328
- Tricyclazole (t) inhibition, 327
- Tricyclene, 278
- Tridec-1-en-3,5,7,9,11-pentayne, 188  
 structure, 190
- 3-cis-11-trans-Trideca-1,3,11-triene-5,7,9-triyne  
 structure, 190
- Triganelline, 278
- Trigilletimine, 125  
 structure, 128
- Triglycerides, 276, 318, 330
- 2',4',7-Trihydroxyisoflavone, 194
- 4',5,7-Trihydroxyisoflavone, 194
- 4',5,7-Trihydroxyflavone, 271
- (3S)-3',4',5'-Trimethoxyphenyl-8- $\beta$ -D-glucopyranosyl dihydroisocoumarin, 209
- 4,5',8-Trimethylpsoralen, 297
- Triterpenes, 134
- Tryptophan, 239
- Tuberiferin, 215, 216
- Tubocurarine  
 anesthetic, blowgun, 49  
 drug, 75, 77, 80  
 lead compound, 50  
 plant source, 45  
 structure, 51
- Tubulosine, 120  
 structure, 121
- Tylophorine, 126  
 structure, 130

- Tyramine, 251, 252
- Umbelliferone**  
in Bishop's weed seed, 281  
fungicide, 160  
potato phytoalexin, 288  
structure, 161
- 2-Undecylenic acid**, 192
- Undulatone**, 136
- Ursolic acid**  
in oregano, 279  
in rosemary, 281  
in sage, 283
- Usnic acid**, 206
- 3-n-Valeroyl- $\beta$ -ionone**, 172, 173
- Vanillin**, 49
- Vecuronium**, 50  
structure, 51
- Verapamil**, 126  
structure, 129
- cis-Verbenone**, 298
- Vernolide**, 134, 146  
structure, 135
- Vesitol**, 251
- Vinblastine**  
anticancer activity, 50, 82, 97  
antiparasitic mechanism, 129  
discovery, 78  
drug, 75, 80  
plant source, 44  
structure, 51, 79
- Vincaleukoblastine**, 78
- Vincristine**  
anticancer activity, 50  
drug, 75, 77, 80, 97  
ethnopharmacological connection, 78  
lead compound, 82  
plant source, 44  
structure, 51, 79
- Viniferin**  
fungicide, 178  
grape phytoalexin, 251
- Viniferin (continued)**  
herbicide, 213  
phytoalexin, 178  
from *S. maritimus*, 213  
structure, 177
- Vomitoxin**, 318
- Warburganal**, 243
- Warfarin**, 1
- Wyerone**, 251  
structure, 168
- Wyerone acid**  
structure, 168
- Wyerone epoxide**  
structure, 168
- Xanthine alkaloids**, 69
- Xanthotoxin**  
biological activity, 243  
in Bishop's weed seed, 284  
celery, parsley, parsnips, 329  
celery phytoalexin, 298  
in parsley, 300  
plant source, 44  
structure, 175, 297
- Yingzhaosu A**, 133  
structure, 134
- Yohimbine**, 47
- Yuanhuacine**, 45
- Yuehchukene**  
structure, 87
- Zearalenol**, 275, 330  
structure, 318
- Zearalenone**  
cancer, 323  
ELISA, 317  
estrogenic agent, 330  
fetal abnormalities, 320  
from *Fusarium* spp., 314  
reproductive effects, 275  
structure, 318

## SUBJECT INDEX

- Aborigines, 53  
Abortifacient  
*Daphne genkwa*, 45  
herbal, 277  
plant, New Mexico, 281  
*Tricosantheo kirilovii*, 49  
yarrow, thujone, 278  
Abortion, 269,  
flavonoids, 275, 329  
herbal, 277  
plants, New Mexico, 281  
zearylone, 318  
Abscesses  
frankincense, 370  
marijuana, 40  
Absinthium oil, 44  
Abyssinian, Arabian, or Somali tea, 98  
Acaricidal, 244  
Acesulfame K, 88  
Acetone breath, 248  
AChE-inhibitor, 241  
Achuar tribe, 67  
Acidic air pollution, 330  
Acidic fog  
phytoalexin simulation, 250, 298, 330  
Acne  
amarillid treatment, 69  
vitamin A synthetics, 57  
Aconitum, 44  
as poison, 7-9  
Actium, 7  
Acute aflatoxicosis, 317  
Acute lymphocytic leukemia, 50  
Adrenergic antagonist, 55  
Adrenergic blocker, 47  
Adrenergic stimulants, 36  
Adrenocorticotropic hormone, 108  
Afghanistan  
khat, 98  
psychoactive drugs, 43  
yellow rain, 314  
Aflatoxicosis, 317, 326  
Aflatoxin  
B1 and G1, 303, 330  
B1 and M1, 317  
biosynthesis, 327  
cancer and vegetables, 258  
contaminated feed, 330  
dietary carcinogens, 328  
food guidelines, 332  
fumonisins structures, 315, 316  
G1, 322  
global importance, 314  
global perspective, 313  
inhibition of production, 326  
liver cancer, 316  
M, 321  
M1, 321, 322, 326, 330  
mutagenicity inhibition, 275  
removal from food, 325  
residues, 316  
structures, 312-322  
synthesis, 327, 338  
Thanksgiving day dinner, 252  
African sleeping sickness, 117  
African trypanosomes, 125, 137, 148, 152  
Agar bulk laxative, 46  
Agranulocytosis, 284  
Agrippina, 3-5  
AHH, 257-260  
AHH-receptor, 260  
AIDS  
castanospermine, 86

- AIDS (continued)  
 marijuana, 40  
 natural compounds, 371  
 plant extracts, 85  
 plant metabolites, 75  
 trichosanthin, 87
- Air pollution, 298, 330
- Akai, 2
- Alaska, 15
- Albichinin II, 196
- Alcoholic cirrhosis, 319
- Alcoholic poisoning, 5
- Aleppo pine, 27
- Aleutia, 15
- Alexander the Great, 99
- Alexandria, 6, 7
- Alfalfa  
 growth regulatory problems, 214  
 medicarpin, 171  
 phytoalexins, 251  
 sprouts, 252
- Alga, 76, 92, 206, 208, 322, 375
- Alginate, 46
- Alkaloids  
 from *Aconitum* sp., 9  
 amoebicidal, 123  
 ancient poisons, 12  
 antimalarial, 85, 127  
 antiparasitic, 118  
 antiprotozoal, 122  
 antispasmodic, 53  
 antitrypanosome, 129  
 antitussive, 55  
 antiviral, 86  
 birth defects, 293  
 curare, 69  
 drug, 76  
 hypotensive, 57  
 jimson weed, 266  
 khat, 100-102, 108  
 lead compound, 49  
 liver cancer, 286  
 liver disease, 285  
 nematicide, 187  
 opium, 43  
 opium poppy, 39  
 oxytocic, 64, 70  
 plant source, 45  
 poisoning, potato, 290  
 related to emetine, 121  
 tropane, 50
- Alkaloids (continued)  
 tubulosine, 120
- Allelochemic, 205, 211, 243, 278, 281, 283
- Allergens, 277
- Allergic, 36, 43, 277
- Allergic conjunctivitis, 43
- Allergic or anaphylactic reactions, 277
- Allergic rhinitis, 43
- Allergies, 43
- Allspice oil, 47
- Aloe gel, 44
- Althaea root syrup, 44
- Altitude sickness, 42
- Amaryllid bulb, 69
- Amastigotes, 119, 137, 156
- Amazonia, 35, 73
- Amazonian lowlands, 145
- Amenemope, 17
- American colonies, 40
- American juniper, 24
- American mandrake, 82
- American Pennyroyal, 239
- American Revolution, 248
- Amerindians, 40
- Ames assay, 273
- Ames test, 284, 300, 322, 325, 370
- Amino acids, 76, 86, 114
- Ammoniation processes, 325
- Amoeba, 242
- Amoebiasis, 118, 119, 127, 129, 134, 138, 157
- Amoebic dysentery, 119
- Amoebicide, 48, 64, 119-124, 127, 149-152, 156, 244
- Amset, 22
- Amylase inhibitors, 252
- Anacardic, 243
- Analgesic, 2, 10, 39, 40, 43-49, 77, 80-82, 92, 240, 244, 278, 279, 281, 283
- Analgesic and tonic prep, 45
- Ancient medicines, 117
- Ancient poisons, 1
- Androgen dependent sialic acid, 275
- Anesthesia, 49, 50
- Anesthetic, 42, 46, 49, 80, 82, 240, 278, 279, 281, 283
- Angina pectoris, 43
- Angostura bark, 46
- Animal-parasitic nematode, 197

- Animal toxicoses**, 315  
**Anise oil**, 46, 47  
**Anointing agent**, 26  
**Anointing fluid**, 23  
**Anopheles**, 84  
**Anorectal drugs**, 102  
**Anorexia**, 110  
**Anthelminthic**, 44-48, 58, 64, 117, 140, 142-145, 185, 279, 281  
**Anthocyanins**, 264, 362  
**Anti-acne drugs**, 57  
**Anti-AIDS**, 75, 85-87, 91, 372  
**Anti-asthmatic**, 45  
**Anti-implantation**, 87, 93  
**Anti-infective**, 48  
**Anti-inflammatory**  
 analgesic, 44  
 berberine, 242, 244, 273  
 corticosteroids, 57  
 molluscicide, 243  
 in oregano, 279  
 in pennyroyal, 240  
 plant source, 44, 48  
 in yarrow, 278  
**Anti-schistosomal**, 147  
**Anti-seasick medicines**, 10  
**Anti-tumor**, 372  
**Anti-ulcer**, 46  
**Antiallergenic**, 278, 279, 281, 283, 371, 376  
**Antiamoebic**, 77, 80, 119, 120, 126, 127, 134, 136, 137, 148-150, 155, 156, 158  
**Antiapertif**, 243  
**Antiappetant**, 243  
**Antiarrhythmias**, 57  
**Antiarrhythmic**, 45, 80  
**Antiasthmatic**, 240, 278, 279, 281, 283  
**Antiatherogenic**, 278  
**Antibacterial**, 45, 49, 124, 173, 180, 198, 206  
**Antibiotic**, 43, 44, 70, 69, 97, 151, 157, 167, 169, 227, 250, 261, 287, 295, 315  
**Antibronchitic**, 240, 278, 279, 281, 283  
**Anticancer**, 50, 82, 83, 91, 92, 94, 97, 240-242, 273, 275, 276, 373, 374  
**Anticancer drugs**, 82, 83, 275  
**Anticancer nutrients**, 276  
**Anticapillary-fragility**, 240  
**Anticariogenic**, 279  
**Anticestodal agents**, 145  
**Anticholeric**, 244  
**Anticholinergic**, 44-46, 63, 77, 79  
**Anticoagulant**, 277, 278  
**Anticonjunctivitis**, 45  
**Anticonvulsant**, 243, 244, 281, 283  
**Antidepressant**, 48  
**Antidermatotic**, 240  
**Antidiabetic**, 52  
**Antidiarrheic**, 45, 47, 50, 244, 278, 279  
**Antidysenteric**, 244  
**Antieczemic**, 240  
**Antiedemic**, 278  
**Antiestrogen**, 276  
**Antifeedant**, 197, 213, 227, 233, 243, 371  
**Antifertility**  
 gossypol, 87, 133  
 Jívaro, sedge, 70  
 molluscicide, 243  
 in oregano, 279  
**Antiflatulent**, 44  
**Antiflu**, 279  
**Antifungal activity**, 159, 160, 162, 164, 173, 178, 179, 251  
**Antigenic stimulation**, 320  
**Antigenically sensitized mice**, 320  
**Antigermination activity**, 222, 225  
**Antigiardial**, 244  
**Antiglaucoma**, 47  
**Antihemorrhoidal**, 46, 240  
**Antihepatotoxic**, 264, 279, 371  
**Antiherapeutic rosmarinic acid**, 239  
**Antihistamine**, 55, 243, 278, 279  
**Antihypercholesterolemic**, 278  
**Antihyperlipoproteinemic**, 49  
**Antihypertensive**, 48, 77, 80  
**Antijuvenile hormones**, 231, 233, 375  
**Antilaryngitic**, 240, 278, 279, 281, 283  
**Antileishmanial activity**, 119, 137, 150, 244  
**Antileukemic**, 44, 78, 83, 95, 129, 134, 136  
**Antileukodermic**, 243  
**Antilithic**, 241  
**Antimalarial**, 45, 64-67, 73, 75, 77, 80, 84, 85, 91-94, 97, 118, 119, 124, 126, 127, 129-134, 136, 138, 146-155, 157, 158, 244

- Antimalarial drugs, 67, 73, 84, 85, 91, 92, 94, 147-149, 151, 152, 154, 155  
Antimetabolites, 233  
Antimetrorrhagic, 240  
Antimicrobial, 94, 150-152, 154, 155, 157, 158, 160, 164, 182, 203, 249, 371  
Antimitotic, 129, 216  
Antimitotic alkaloid, 129  
Antimutagenic, 273, 275, 371, 376  
Antimyoatrophic, 278  
Antineoplastic, 3, 33, 48, 50, 75, 77, 78, 80, 82-84, 92, 129, 216  
Antineuritic, 240  
Antineuritic, 279  
Antinutrients, 244  
Antioch, 7  
Antioxidant, 244, 260, 271, 272, 279, 281, 283  
Antiparasitic activity, 125  
Antiperiodic, 240  
Antipharyngitic, 240, 278, 279, 281, 283  
Antiphlogistic, 371, 376  
Antipodagric, 240  
Antiprostatadenomic, 278  
Antiprostatitic, 278  
Antiprotozoal activity, 117, 118, 122  
Antipruritic, 240, 278, 281, 283  
Antipsoriac, 240, 243  
Antipyretic, 240, 278, 279  
Antirheumatic, 240, 278  
Antirhinitic, 240, 278, 279, 281, 283  
Antisarcomic, 279  
Antiseborrheic, 240  
Antiseptic, 44, 46-49, 237, 238, 240, 241, 243, 278, 279, 281, 283  
Antihistosomal, 216  
Antispasmodics, 10, 43, 45, 46, 48, 49, 53, 55, 243, 273, 278, 279  
Antisyncopic, 240  
Antithrombogenic, 278  
Antithyroid activity, 251  
Antitoxins, 244  
Antitrachomic, 244  
Antitrematodal agents, 146  
Antitypanosomals, 64  
Antituberculic, 244  
Antitumor, 34, 73, 94, 95, 123, 125, 134, 152, 157, 206, 241, 243, 244, 278, 279, 281, 283  
Antitumor-promoter, 241  
Antitussives, 39, 47, 55, 77, 80, 240, 278, 279, 281, 283  
Antiulcer, 242, 244  
Antivaginitic, 240  
Antiviral activity, 33, 46, 75, 85, 86, 91, 92, 94, 273, 339, 373, 376  
Antivitiligo, 243  
Antony, Mark, 7, 9  
Anubis, 5, 20  
Anxiety, 108  
Apes, 312  
Aphicide, 243  
Aphrodisiac, 3, 68, 278  
Apple  
    alar, 331  
    flavonoid content, 270  
    Jívaro diet, 68  
    patulin, 320, 330  
    pectin, 50  
    psychoactive thorn apple, 285  
    quercetin and kaempferol, 268  
    Thanksgiving Day dinner, 252  
Apricot, 268, 270, 320  
Arab, 23, 111, 113-115  
Arabian Peninsula, 98  
Arabian physicians, 36  
Arabic, 15, 44, 98  
Arnica, 44  
Aromatic, 44, 46, 86, 90, 120, 132, 133, 153, 158, 231, 234, 235, 238, 301  
    mummification, 18, 21, 24, 28  
Arrhythmia, 8, 108, 110  
Arrow-poison, 57  
Arrhythmia, 108  
Arthritis, 68, 253, 284, 292  
    hemlock, 2  
Asafetida, 46  
ASB, 292, 293  
Ascariasis, 144  
Ascaricidal activity, 144  
Ascaricidal agents, 143  
Ascarids, 143, 144  
Ascomycete, 206  
Asia, 2, 26, 39, 67, 78, 285, 314  
Asia Minor, 26  
Asparagus, 44, 195, 196, 292  
Asphyxiation, 7, 35  
Aspirin, 82  
Asthma, 35, 42-44, 308, 330

- Asthma conditions, 330  
 Astringent, 23, 24, 39, 45, 46, 48, 49, 60, 213, 240, 242, 244  
 Ataxia, 257, 323  
 Atherosclerosis, 62, 276  
 Atrial fibrillation, 77  
 Atrial flutter, 57  
 Atropos, 9  
 Augustus, 5  
 Augustus Caesar, 5  
 Auricular fibrillation, 57  
 Australia, 53, 218  
 Autoclaved corn, 323  
 Autopsy reports, 317  
 Autotoxic effect, 214  
 Autumn crocus, 36  
 Avermectins, 140, 371  
 Avifuge, 240  
 Avocado oil, 47  
 Awedai, 110  
 Aztec, 283
- Baboons, 319  
 Back pain, 58, 284  
 Bacteria, 69, 142, 167, 169, 173, 210, 242, 269, 302, 304, 305, 372  
 Bacterial and fungal skin problems, 68  
 Bactericide, 173, 239, 240, 241, 243, 244, 278, 279, 281, 283, 312  
 Bacteriostatic, 238, 273  
 Baked fish, 68  
 Baked goods, 301, 326  
 Baked potato, 252  
 Baking, boiling, or microwaving, 290  
 Balm, 47  
 Balm of Gilead, 47  
 Balsam of Peru, 47  
 Balsam of Tolu, 47  
 Bamboo, 212  
 Banana, 68, 99, 320  
 Banewort, 9  
 BAP database, 237-239, 242, 243  
 Barbiturate antidote, 44  
 Barbiturates, 10  
 Bark and needle oil, 47  
 Bark canker, 276  
 Bark mucilage, 49  
 Barley, 36, 293, 316, 318, 320, 322, 330  
 Barnyardgrass, 211  
 Basidiomycete, 206  
 Basil, 193, 234, 277, 280
- Bay leaf, 282, 348  
 Bean, 60, 142, 156, 169-171, 175, 178, 183, 187, 194, 198, 203, 210, 228, 239, 250-252, 266, 267, 269, 286, 292, 327, 330, 331  
 Bean seedlings, 210  
 Bear's breech, 212  
 Beer  
 alcoholic cirrhosis, 319  
 estrogenic agents, 330  
 estrogenic substance, 318  
 flavonoids, 264  
 Jívaro Indian, 68  
 T-2 toxin, 320  
 toxin food chain, 248
- Beer drinkers, 319, 320  
 Beeswax, 23, 28  
 Beetle-attractant, 278, 281, 283  
 Beetle resistance to DDT, 291  
 Beets, 178, 291, 292  
 Belgium, 318, 320  
 Bell pepper, 267  
 Belladonna, 9, 10, 44, 53, 60, 64, 77, 248, 370  
 Belladonna extract, 44, 60  
 Benzoin gum in friar's balsam, 48  
 Berries, 9, 10, 26, 48, 326  
 Betel nut, 145  
 BHA, 271  
 Bilberry, 268  
 Bile duct, 274, 312  
 Bile duct tumors, 274  
 Biocidal, 244  
 Bioflavonoid, 272  
 Biological methods, 373  
 Biosynthesis pathway of DHN melanin, 328  
 Biotechnology, 90, 233, 372  
 Birch oil, 44  
 Birth control, 60, 87  
 Birth weight, 108  
 Bishop's weed seed, 281, 282, 284, 329  
 Bitter orange oil, 301  
 Bitter tonic, 46  
 Bitterness, 290  
 Bitters, 46-48  
 Bitumen, 15, 27, 28  
 Black currant, 268  
 Black masses, 11  
 Black mustard seed, 195, 255  
 Black pepper, 252

- Black peppercorns, 24  
 Blackberry, 268, 269  
 Bladder tumors, 274  
 Blemished, damaged, or infected sweet potatoes, 306  
 Blindness, 323  
 Blistering, 297  
 Blood pressure, 8, 35, 108, 251  
 Blood vessel tumors, 308  
 Blow-gun, 49, 69  
 Blue mold, 163, 173  
 Blume, 44, 83  
 BOA, 211, 212  
 Body aches, 68  
 Bogs, 15, 16  
 Boiling, 290, 306, 310  
 Boils, 39, 69  
 Boldo leaves, 47  
 Bolivia, 42, 43  
 BOP, 258  
 Botanical insecticides, 227, 228  
 Bottle brush, 193  
 Brain  
     *Aconitum* poisoning, 8  
     aflatoxins, 317  
     edema, fumonisins, 324  
     edema, patulin, 320  
     mummification, 18, 21  
     quisqualate, kainate binding, 144  
     tetrahydrocannabinol accumulation, 138  
 Brazilian rain forest, 63  
 Bread, 9, 36, 64, 252, 330  
 Breast cancer, 82, 241, 257, 276  
 Brine shrimp, 145, 213  
 Britannicus, 5  
 Broad bean, 168, 251, 267  
 Broccoli, 251-253, 255, 256, 258, 267, 292, 329  
 Broken bones, 68  
 Bromelain, 44, 58  
 Bronchial asthma, 43, 44  
 Bronchial congestion, 43  
 Bronchitis, 42, 44  
 Bronchodilator, 46, 55  
 Bronchogenic cancers, 307  
 Bronze Age, 38  
 Broom, 8, 269, 285  
 Broom snakeweed, 269  
 Broomweed, 269  
 Brown mustard, 216  
 Bruce Ames, 237  
 Bruising, 289  
 Brussels sprouts, 253-260, 329  
 Bryophytes, 76  
 Buchu oil, 44  
 Bug bites, 241  
 Bulb nematode, 186  
 Bulgaria, 317  
 Bulk laxative, 46, 47  
 Burkitt's lymphoma, 50  
 C-mitosis, 3  
 Cabbage, 117, 238, 253-259, 267, 292, 329  
 Caesar, 3, 5-7, 12  
 Cajeput oil, 47  
 Calabar bean, 62, 187, 228, 230  
 Calcium channel blocking agent, 126  
 Calculated lethal doses, 312  
 California Department of Food and Agriculture, 331  
 California poppy, 285  
 Callose, 159, 160, 169  
 Cameroon, 217  
 Canada, 44, 58, 67, 73, 275, 318  
 Canada balsam oil, 44  
 Cancer  
     aflatoxin, 321  
     aflatoxin w/alcohol, 319  
     bioassays, 331  
     cholinesterase inhibition, 288  
     colon, 82, 83, 257, 258  
     cruciferous vegetables, 256  
     from diet, 253  
     fumonisins, 323, 324  
     genital, 245, 297  
     I3C and TCDD, 260  
     khat tannins, 108  
     marijuana, 40  
     mushrooms, 308  
     mycotoxins, 315-317  
     mycotoxins/hepatitis, 318  
     nitrates, 291  
     N-methylhydrazine, 312  
     potential, 319  
     preventive, 238-241  
     prostate, dietary fat, 276  
     PUVA, 296  
     quercetin, NTP, 274  
     safrole, 277  
     T-cell deficiency, 284

- Cancer (continued)
- testicular and lung, 52
  - Thanksgiving Day dinner, 251
  - tomatidine, 295
  - tuberiferine, 215
  - vincristine, 97
  - vincristine bioassay, 78
  - Candidicide, 241, 243, 244, 279
  - Candoshi tribe, 67
  - Cannabis resin, 44
  - Canopic jars, 6, 22
  - Cantaloupes, 331
  - Capillary-fortificant, 279
  - Capillary permeability, 273
  - Caraway, 2, 239, 242
  - Carbamate, 10, 228, 230, 252
  - Carbohydrates, 86, 88
  - Carcinogenic potency, 321
  - Carcinogenic risk, 296
  - Cardamom, 46, 242
  - Cardanol, 243
  - Cardiac arrhythmias, 8
  - Cardiac depressants, 57
  - Cardiac glycoside, 77, 78, 97
  - Cardiac output, 57
  - Cardiodepressant, 242, 244
  - Cardiomyopathy, 320, 353
  - Cardiotonic, 44, 45, 48, 49, 77, 80, 86, 243, 278, 279
  - Cardiotonic glycosides, 86
  - Cardiovascular collapse, 251
  - Cardiovascular drugs, 55
  - Caries, 43, 68, 88
  - Carminative, 2, 44-49, 242, 244, 278, 279, 281, 283
  - Carrot
    - acetylenes, figure, 254
    - caffeic acid, 165
    - cancer preventative, 238
    - falcarindiol, 173
    - falcarinol, 175
    - flavonoid content, 270
    - nitrate content, 292
    - phenylpropenoid herbicide, 221
    - phytoalexin, 251
    - Thanksgiving Day dinner, 252  - Cascara, 48, 62
  - Cassava, 68
  - Cassius, 4, 7
  - Castor, 48, 62, 80, 117, 178, 251
  - Castor bean, 178, 251
  - Castor oil, 48, 62, 80, 117
  - Cat, 98, 110
  - Cataract formation, 282, 296
  - Cataracts, 273, 364
  - Cathartic, 39, 62, 63, 80, 143, 243
  - Cathartic agent, 143
  - Catnip, 285
  - Cattle, 269, 275, 293, 305, 318
  - Cauliflower, 173, 183, 253, 256-259, 292, 329, 335, 361
  - Cauliflower mosaic virus, 173, 183
  - CDC, 296, 298, 299, 330
  - CDFA, 331
  - Cedar, 18, 22-26, 28, 49
  - Cedar juice, 28
  - Cedar leaf oil, 49
  - Cedar-oil, 18, 22-24, 26
  - Cedarwood oil, 46
  - Celery
    - anthelmintic, 117
    - celery production, 322
    - cultivar, 298, 299, 329
    - dermatitis, 293, 296
    - handlers, field workers, and processors, 293, 295, 296, 329
    - human toxicity, 291
    - juice, 297
    - limonene, 242
    - nitrate content, 292
    - phytoalexin response, 250
    - phytoalexins, 251, 330
    - plant breeding, 332
    - root, 297, 370
    - Thanksgiving Day dinner, 252  - Cell wall polymers, 169
  - Cell wall components of fungi, bacteria and plants, 167
  - Centers for Disease Control, 296
  - Centipede grass, 210, 224
  - Central America, 78
  - Central nervous system depressant, 10
  - Central stimulant, 46, 48
  - Cephalobus, 192
  - Cephalosporium, 45
  - Cerberus, 7
  - Cereal crops, 314
  - Cerebral excitations, 251
  - Cerebral malaria, 131, 154, 156
  - Cerebrodepressant, 279
  - Cerebrovascular accidents, 318

- Cestodes, 117, 145, 146  
Cestofugal, 145  
Ceylon, 24  
Chagas disease, 117, 137  
Chamomile, 57, 277  
Chard, 255  
Charon's ferry, 9  
Chat, 98  
Cheese, 316  
Chelate, 272  
Chemical ecology, 203, 223-225, 228, 371, 372  
Chemopreventive, 238  
Chemotaxonomy, 201, 371  
Chenopodium oil, 45  
Cheops, 16  
Cherry tomatoes, 252  
Chick embryos, 320, 325, 293, 320  
Chickens, 68, 319, 321, 323  
Childhood leukemias, 82  
Chilling injury, 295  
Chills, 35, 47  
Chinese hamster ovary cells, 274  
Chinese medicinal plant, 133  
Chinese medicinal herbs, 283  
Chinese traditional medicine, 79, 81, 89  
Chitinases, 159, 169, 171  
Chive, 237, 267  
Chloroquine resistance, 10, 126, 129  
Cholera, 242  
Choleretic, 45, 240, 242, 244, 278, 279  
Cholesterol, 60, 241, 275, 276, 318, 329, 330  
Choline, 278  
Cholinergic-ophthalmic, 47  
Cholinesterase inhibitor, 77, 80, 288, 290, 329  
Cholinesterase isoenzymes, 288  
Choriocarcinoma, 50  
Christ, 2, 268  
Christian cemeteries, 26  
Chromosomal aberration tests, 274  
Chromosomal DNA, 5  
Chromosome breakage test, 370  
Chronic bronchitis, 44  
Chronic kidney disease, 317  
Chronic liver disease, 285, 316  
Chronic lung disease, 308  
Chronic mitogenesis, 331  
Chronic myelocytic leukemia, 79  
Chronic toxic hepatitis, 324  
Chrysanthemums, 277  
Chymopapain, 44, 58  
Cilician fir, 27  
Cinnamon, 22, 24, 45, 252, 285, 327  
Circulation, 35, 48, 57, 68  
Circulation of legs, 48  
Cirrhosis, 285, 319, 323, 329  
Cirrhosis of the liver, 285, 319, 329  
Citrus  
    antimalarial, 127  
    bioflavanoids, 60  
    cancer preventative, 238  
    dermatitis, 295  
    drugs, 45  
    flavonoids, 271  
    furanocoumarins, 302  
    juice, 301, 303  
    limonene, 242  
    nematicide, 193  
    oil, 193, 301, 303  
    pectin, 50  
    peel oils, 301, 303  
Civil War, 40  
Clara bronchiolar cells, 83  
Clara cell necrosis, 307, 330  
Claudius, 3-5  
Cleopatra, 6, 7  
Clitoral glands, 312  
Clove oil, 48, 137, 156, 194  
Clover sickness, 215, 224, 225  
CNS depressant, 240, 279  
CNS stimulant, 36, 40, 240, 279, 281  
Cobra venom, 6  
Cobras, 7  
Coca, 38, 40, 42, 43, 46, 80, 99  
Coccidiosis, 118  
Coccidiostats, 129  
Cocculolidine, 228  
Cocilliana extract with rusbyine, 46  
Cocklebur, 5  
Cocoa, 252, 255, 264  
Coffee  
    chlorogenic acid, 239  
    in Ethiopia, 110  
    flavonoids, 264  
    Thanksgiving Day dinner, 252  
Cohosh, 277  
Cola, 264  
Cold prep, 47  
Colds, 42, 46, 47, 55

Colic, 35, 43  
Collyrial, 242  
Collyrium, 244  
Colocynth, 45  
Colombia, 40, 42, 43  
Colon cancer, 82, 83, 257, 258  
Colorado, 277, 290  
Colorado potato beetle, 290  
Colorectal cancer, 82  
Coma, 5, 248, 317  
Comfrey, 285, 286  
Comfrey root tea, 286  
Commercial potatoes, 288  
Common cold, 55  
Community pharmacies, 77  
Compound C, 233  
Compound syrup of spikenard, 44  
Compound tincture benzoin, 47  
Conception, 73, 318  
Cone, 46  
Congenital spina bifida, 292, 293  
Congestion of visceral organs, 320  
Congestive heart failure, 57  
Conjunctivitis, 43, 68  
Constipation, 82, 108, 110, 248  
Contact dermatitis, 295, 299  
Contaminated bread w/ergot, 64  
Contaminated figs w/aflatoxin, 303, 330  
Contaminated food w/mycotoxins, 313-316  
Contaminated food w/fungi, 319  
Contaminated fruit w/patulin, 320  
Contaminated grain w/weed seeds, 266, 274  
Contaminated water w/anatoxin, 322-324  
Contraceptive steroids, 80  
Convulsant, 279  
Convulsions, 5, 317  
Copaiba oil, 45  
Corn  
    for bioassay, herbicide, 210  
    BOA, 212  
    boron, 239  
    corn remover, 46  
    drugs, 49  
    genetic engineering, 332  
    hepatitis, 319  
    intercropping w/khat, 99, 110  
    kernels, 323

Corn (continued)  
    mint, 238  
    mycotoxins, 315, 316, 330  
    mycotoxins, cancer, 323-325  
    nitrate content, 292  
    oil, 49, 62  
    salad, 239  
    silk, 49  
     $\beta$ -Sitosterol, 60  
Coronary heart disease, 283  
Corticosteroid, 80  
Cotton  
    abortifacients, 281  
    antifertility, 87  
    EC<sub>50</sub>, 173  
    gossypol, 253  
    gossypols, nematicide, 194  
    Jívaro Indians, 68  
    phytoalexin, 251, 330  
    phytoalexin elicitor, 250  
    phytoalexin response, 143  
    root bark, 281  
Cottonseed, 255, 316, 330  
    oil, 46, 62, 252, 253  
Cough, 35, 39, 49, 55  
    and colds, 46, 47  
    mixtures and lozenges, 47  
    preparation, 44, 45, 47, 48  
    remedies, 48  
    syrups, 47  
Coumarin content, 301  
Coumarins, 159, 249, 277, 278, 282, 301, 302, 304  
Counterirritant, 44, 45, 47, 49, 240, 278  
Cow, 275, 322  
Cranberry, 251, 252, 268, 269  
Cranial bleb, 293, 294  
Crécy, 11  
Cress, 117, 211  
Crito, 2  
Croton oil, 45  
Crotoloxins, 7  
Crown gall tumors, 213  
Cruciferous plants, 254  
Cruciferous vegetables, 253, 254, 256-258  
Crucifers, 253, 329  
Crustacicide, 243  
CT, 260  
Cubeb oil, 47  
Cucumbers, 216-218, 221, 292

Cuprea bark, 48  
Cupric, 167, 169, 171, 173, 275  
Cupric chloride, 167, 169, 171  
Curarimimetic neurotoxins, 7  
Cured meats, 291  
Custard apple, 68  
Cuts, 39, 68, 69, 98  
Cyst nematode, 186, 192, 193, 196  
Cytochalasins, 220  
Cytochrome P-448, 259  
Cytochrome P-450, 257, 273, 307, 312  
Cytokinins, 217  
Cytotoxic, 40, 119-121, 123, 134, 136, 145, 216, 238, 243, 275, 284  
Cytotoxic activity, 136  
Cytotoxic drugs, 40  
Cytotoxic effects, 275  
Cytotoxicity, 82, 119-123

Dairy products, 326  
Dalmatian insect powder, 205  
Dandelion flowers, 239  
Danish pork, 316  
Darts, 49, 69  
Data bases, 372, 374  
Date-palm, 24  
Day lily, 147  
De Medicina, 370  
Dead Sea, 27, 28  
Deadly nightshade, 9  
Death  
    from aflatoxin, 326  
    anatoxin A, 322  
    beetles in mummy, 28  
    embalmer's workshop, 20  
    from green tomato plants, 295  
    isothiocyanate, 257  
    milk sickness, 248  
    mummification, 21  
    from mycotoxins, 317  
    nematicides, 185  
    of Pliny, 35  
    from poisons, 2-10  
    St. Anthony's Fire, 64  
    from sweet potato, 308  
    trees by lichens, 206  
Decongestants, 35, 55  
Deer-tick, 238  
Defensive chemicals, 227, 233  
Degu, 273

Delayed pulmonary microvascular leak, 285  
Deliriant  
    in oregano, 279  
    in rosemary, 281  
    in sage, 283  
    in yarrow, 278  
Delirium, 9  
Delos, 2  
Delphic pythoness, 99  
Demulcent, 46  
Dental caries, 43, 88  
Department of Agriculture, 247, 372, 376  
Depressants, 57, 68  
Derivative drugs, 370  
Dermatitigenic, 279  
Dermatological aid, 44-46, 49  
Dermatological diseases, 57  
Dermatological preparations, 57  
Desiccation, 15, 18, 22, 23  
Desprouted potatoes, 293  
Diagnostic products, 76  
Diaphoresis, 44, 46, 63  
Diarrhea  
    amanitin, 5  
    antidiarrheal preparations, 50  
    from chamomile, 277  
    Jívaro, 68  
    opium juice treatment, 39  
    plants for treatment, 48  
    from potato glycoalkaloids, 290  
Diarrheagenic toxicant, 322  
Diet  
    arthritis prevention, 292  
    cancer incidence, 253  
    cancer prevention, 257  
    carcinogens, 245, 328  
    cultural differences, 266  
    d-limonene, 241  
    dietary I3C, 260  
    estrogenic compounds, 275  
    fat, 276  
    flavonoid content, 264  
    flavonoid metabolism, 261  
    lack of guidelines, 332  
    NRC recommendation, 258  
    onions and garlic, 276  
    pesticides, 331  
    potato glycoalkaloids, 288  
    quercetin intake, 273

- Diet (continued)  
 supplement in hyper-cholesterolemia,  
 44
- Digestion, 40  
 Digestive aid, 44, 47  
 Dill oil, 44  
 Dio Cassius, 4  
 Diodorus Siculus, 18, 19, 21-24, 27  
 Dioscorides, 23, 25, 35, 36, 38, 39  
 Directed screening, 80  
 Disease resistance  
   carrot, cotton, 173  
   to fungi, 160, 162  
   phytoalexins, 164  
   phytoalexin response, 167  
   plant family specificity, 165  
   resistance complex, 169  
   Solanaceae, 171  
   systemic resistance, 178, 179
- Diseased celery, 296-299, 322  
 Disorientation, 69  
 Diuresis, 78  
 Diuretic  
   from *Digitalis* spp., 57  
   from oregano, 279  
   plant compounds, 243  
   plant sources, 44-49  
   from rosemary, 281  
   from *S. maritimus*, 213  
   from sage, 283  
 Diuretic and laxative prep, 44  
 Dizziness, 290  
 Djibouti, 98, 110  
 DMBA, 259  
 DNA and RNA adducts, 296  
 DNA topoisomerase, 83  
 Dog  
   dog-tick repellent, 238  
   *Ephedra* extracts, 35  
   hemolysis by T-2 toxin, 320  
   kidney cells and fumonosin, 324  
   LD<sub>50</sub> hydrazine, 311  
   LD<sub>10</sub> salicylic acid, 240  
 Donkeys, 323  
 Double curled parsley, 300  
 Double vision, 10  
 Dream life, 8  
 Dried figs, 303, 330  
 Dropsy, 40, 74, 77, 287  
 Drug design, 80, 90  
 Drug resistance, 118
- Drusus, 5  
 Duamutef, 22  
 Ducklings, 319  
 Duckweed bioassay, 213  
 Ductular carcinoma of the liver, 323  
 Dusting powders, 76  
 Dwale, 9  
 Dyes, 69, 157, 206, 207  
 Dysentery, 46, 79, 117, 119, 127, 242
- Ear rot pathogens, 322  
 Earache, 39, 68  
 East Africa, 98, 109, 112, 113, 114, 317  
 Ecbolic, 279  
 ECD, 257  
 Ectoparasites, 117  
 Ecuador, 42, 145  
 Ecuador's Amazonian lowlands, 145  
 Edema  
   brain, 324  
   from coumarins, 297  
   digitalis, 57, 78  
   *Ephedra*, 35  
   lungs and brain, 320, 330  
   from MRC 826, 323  
   from sweet potatoes, 305-307
- Edible fresh mushrooms, 308  
 Edward I, 11  
 Eggplant  
   birth defects, 294  
   nitrate content, 292  
   phytoalexins, 171, 251, 287, 329  
   solanaceous plants, 248  
   structure, 170, 172
- Egyptian cobra, 7  
 Egyptian hoodless cobra, 6  
 Egyptian plants, 23  
 EH, 257  
 Elaterin resin, 45  
 Elderberry, 268  
 Elicitor, 162, 167-169, 171, 178, 298  
 ELISAs, 317  
 Embalmer's workshop, 20  
 Embalmers, 21, 22, 24  
 Embryonal death, 257  
 Embryotoxic, 294, 317  
 Emetic, 49, 68, 69, 77, 80, 82, 278, 279,  
 283  
 Emmenagogue, 45, 46, 278, 281  
 Emollient, 44, 46-48, 76, 370  
 Emperor Claudius, 3

- Encephalocele, 293  
Endemic nephropathy, 317  
Endive, 267, 330, 345  
Endogenous elicitors, 169  
English Yew, 11  
Enhancers, 171, 182  
Enterocytes, 275  
Environmental chemicals, 299, 319, 332  
EPA, 331  
Epididymides, 275  
Epileptogenic, 279  
Epimastigotes, 122, 125, 140  
Episiotomy, 58  
Epithelial cancers, 3  
Equine leukoencephalomalacia, 323, 330  
Ergot, 45, 57, 64, 70, 313, 314, 370  
Ergotism, 293, 314  
Ericaceous, 137  
Erisipelas, 40  
EROD, 259  
Erythema, 297  
Erythrocytes, 132, 320  
Erythrocytic stages, 130, 137, 149  
Esophageal and liver cancer, 323, 324, 330  
Esophageal cancer, 319, 323  
Essential oil, 26, 100, 102, 103, 193, 194, 238-240, 245, 278-281, 283  
Estrogen, 87, 275, 276, 278, 318, 320, 330  
Estrogen replacement, 318  
Estrone binding sites, 276  
Ethiopia, 98, 99, 103, 105, 110  
Ethnobotany, 33, 34, 38, 68, 371, 372  
Ethnomedicine, 34, 70, 78, 87, 89  
Ethnopharmacological approach, 78  
Eucalyptus oil, 46  
Eucharis, 69  
Euphoria, 39, 40, 98, 105, 110  
Eurasia, 213  
European, 34, 36, 64, 308, 323  
Evening-primrose seed, 239  
Evisceration, 6, 18, 21-23  
Excessive salivation, 63, 293  
Execution, 2, 3  
Exencephaly, 293, 294, 349  
Expectorant, 45-47, 49, 240, 278, 279, 281, 283  
External healing, 48  
Extracting teeth, 68  
Eye drops, 43, 44  
Eyewash, 44, 46  
Falciparum malaria, 84, 118, 131  
Fall armyworm, 213  
Fallopian tube sperm, 275  
False banana, 99  
False morel, 308, 310, 312  
FAO, 316  
Far East, 316  
Farm workers, 297  
Father Nature's Farmacy, 237  
Fats, 76  
FDA, 49, 238, 299, 331  
Febrifuge, 240, 244  
Female disorders, 44  
Fennel, 46, 242  
Fennel oil, 46  
Fens, 15  
Fertility-regulating activity, 75, 87, 89, 90  
Fetal abnormalities, 320  
Fever, 35, 64, 67, 84, 238, 242  
Fever-reducing, 242  
Fibrillation, 8, 57, 77  
Fig  
    aflatoxin contamination, 303, 330  
    dermatitis, 302  
    leaf sap, 302  
Fire ant, 230  
Fish, 23, 68, 69, 133, 248, 317, 326, 330, 370  
Fixed oils, 76  
Flakes, 300, 301  
Flatulence, 40  
Flax, 255  
Fleas, 230  
Flies, 230  
FNF, 237-239  
FNF database, 238, 239  
Focal necrosis, medulla oblongata, 324  
Folk medicine, 34, 78, 145, 361  
Folklore  
    indexing, 372  
    monkshood, 8  
    podophyllotoxin, 82  
    preselection, 34  
    product discovery, 371  
    remedies, 78

Food and Drug Administration, 238, 331  
Food contaminants, 319, 369  
Food products, 292  
Forestomach, 312  
Foxglove, 57, 77  
Frame shift mutagens, 322  
France, 64, 99, 371  
Frangula bark, 48  
Frankincense, 370  
Free-living nematode, 145, 185, 187, 189, 192  
Fresh and dried parsley, 300  
Fresh frozen mushrooms, 308  
Fresh fruits, 68  
Fresh potatoes, 294  
Freshwater blue-green algae, 322  
Fructose syrups, 88  
Fruit juices, 264  
Fungal glucan, 167  
Fungal-infected sweet potatoes, 305  
Fungal invasion, 293  
Fungi on grains, 313  
Fungicidal, 169, 207, 209, 212, 213, 240, 241, 243, 244, 250, 277-279, 281, 283, 298, 327, 371  
Fungitoxic, 160, 162, 163, 278, 286, 287  
Fungus, 4, 62, 64, 70, 76, 159, 160, 162, 165, 167, 169, 171, 173, 178, 187, 206, 209, 216, 222, 242, 293, 294, 296, 305, 313, 315, 318, 319, 322, 323, 327, 328, 330  
Fusarium, 83, 162, 212, 306, 309, 314, 318, 322, 323  
  
Gaius and Lucius Caesar, 5  
Galen, 39  
Gallbladder, 312  
adenocarcinoma, 258  
Gallstones, 241  
Gangrene, 64  
Garden pea, 251  
Garden peppers, 248, 287  
Garlic  
allicin, 140  
anthelmintic, 117  
biological activity, 276  
cancer preventative, 238  
drugs, 44  
phytochemicals, 237

Garlic (continued)  
quercetin, kaempferol content, 267  
serum cholesterol, 329  
symptoms, 277  
Garlic chive, 267  
Garlic oil, 44  
Gastric cancer, 257  
Gastric disturbances, 108  
Gastroenteritis, 277, 290  
Gastrostimulant, 279  
GCPH, 310  
Gels, 46  
Gemmae, 208  
Gemmalings, 208  
Generally Recognized as Food, 237  
Generally Recognized as Safe, 237, 326  
Genetic engineering, 291, 332  
Genital tumors, 297  
Giant reed, 212  
Giardia, 117, 241, 242  
Ginger, 237  
oleoresin and oil, 49  
Glandless cotton, 253  
Glaucoma, 40, 62  
Glioma cancer, 82  
Global birth rate, 87  
Glucan, 167, 169, 171  
Glucosidase inhibitor, 86  
Glucosinolate, 251, 252, 254, 256, 257, 259  
Glucuronide conjugation, 257  
Glutathione S-transferase, 257  
Glycoalkaloid, 162, 249, 287-292, 294  
content, 289, 293  
extracts, 293  
poisoning, 289  
Glycoside bitters, 46  
Goat, 275, 326  
Goiter, 254  
Goitrogen, 253-255, 329  
Golden hamsters, 129  
Goldenrod, 188, 249  
Gooseberry, 268  
Gout, 2, 36, 38, 45, 60  
GRAF, 237, 241, 245  
Grain, 5, 241, 248, 266, 274, 313, 316, 320, 325, 326, 330  
Grain sorghums, 330  
Grape juice, 269  
Grapefruit, 303

- Grapefruit (continued)  
     juice, 301  
 Grapes, 251, 320  
 GRAS, 237, 241, 242, 245, 326  
 Great Britain, 99  
 Greece, 38  
 Greek, 2, 7-9, 18, 22, 23, 25, 142, 313  
 Greek Herbal, 23  
 Green and cull potatoes, 287  
 Green bean  
     chemical translocation, 331  
     phytoalexins, 169-171, 251  
     pisatin, 175  
 Green or damaged potato tubers, 294  
 Green potatoes, 248, 287  
 Green tomato, 295  
 Greened or sprouted potatoes, 287  
 Grocery store workers, 298, 299, 330  
 Growth hormone, 108  
 Grubs, 68  
 GST, 257  
 Guar gum, 45  
 Guillotine, 2  
 Guinea pig, 87, 110, 119, 260-262, 275,  
     320  
 Gum arabic, 44  
 Gum galbanum, 46  
 Gum resins, 24, 27  
 Gum sarcocolla, 44  
 Gum tragacanth, 44  
 Gums, 76, 369  
 Gymnosperms, 76  
  
 Hades, 7, 9  
 Haemin, 118  
 Hair, 25, 40  
 Hallucination, 8-10, 40, 68, 69, 251,  
     253  
 Halotus, 4  
 Hamsters, 129, 134, 258, 274, 290, 293,  
     294, 307, 312, 319-321  
 Hapy, 22  
 Harris tweeds, 206  
 Hatching stimulants, 196, 198  
 Hawaii, 2  
 HB, 310  
 HDL, 277  
 Head cancer, 82  
 Head pressing, 323  
 Headache, 5, 39, 68, 79, 277, 290  
 Healthy celery, 296, 299  
  
 Heart, 6, 21, 35, 57  
     disease, 78, 283  
     treatment, 44  
 Hebona, 12  
 Helicicide, 243  
 Helminth diseases, 140  
 Helminthiasis, 117, 157  
 Helminthicidal, 371  
 Hemlock, 2, 8  
 Hemocoagulant, 278  
 Hemolyzed, 320  
 Hemorrhage, 35, 269, 323  
 Hemorrhagic cystitis, 83  
 Hemorrhoidal preparations, 60  
 Hemorrhoids, 60, 65, 68  
 Hemostat, 39, 45, 244, 278  
 Hemostatic, 46, 60, 242, 277  
 Hemp, 39  
 Hen, 275  
 Henbane, 11  
 Henna, 24  
 Hepatic amoebiasis, 129, 134  
 Hepatic and renal necrosis, 320, 321  
 Hepatic elimination, 320  
 Hepatic ethoxyresorufin O-deethylase  
     activity, 259  
 Hepatic veno-occlusive disease, 285  
 Hepatitis  
     cancer w/mycotoxins, 318  
     insidious and progressive, 324  
     Jívaro Indians, 68, 69  
     w/mycotoxins, 319  
 Hepatitis B, 69, 318  
 Hepatocarcinogenic, 258, 277, 286,  
     308, 312, 317  
 Hepatocellular cancer, 318, 319, 323  
 Hepatocellular cancer risk populations,  
     319  
 Hepatomas, 274, 318  
 Hepatoprotective, 279  
 Hepatosis, 323  
 Hepatotoxic, 240, 279  
 Hepatotoxic, 243, 305, 312, 316, 317,  
     323, 324, 330, 374  
 Hepatotoxic and carcinogenic MFH,  
     312  
 Hepatotropic, 278  
 Herb Research Foundation, 238  
 Herbal abortifacients, 277  
 Herbal medicines, 276, 284, 285, 316  
 Herbal plants, 331

- Herbal preparations, 276, 277, 283-285  
Herbal remedies, 78, 89  
Herbal teas, 277, 285, 319  
Herbal Vineyard, 241  
Herbicide, 205, 212, 213, 215, 216, 221, 223, 224, 241, 243, 250, 269, 298, 327, 330, 369, 371  
Herbs, 57, 237, 238, 241, 242, 248, 276, 277, 283-285, 316, 327, 329, 370  
Hermonthis, 7  
Herodotus, 18, 19, 21-24, 39  
Heterocyclic amines, 252  
High density lipid, 277  
High tannin, 48, 108  
Highblush Blueberry, 269  
Hippocrates, 3, 39  
Historia Naturalis, 11  
HIV  
    cell binding, 86  
    natural product testing, 85  
    trichosanthin, 87  
Hodgkin's disease, 78, 82  
Hokkaido, 206  
Holy men, 55  
Homegrown corn, 330, 324  
Homegrown maize, 323  
Homocidal, 242  
Hops, 285  
Horace, 1, 7, 205  
Hormesis, 242  
Horned viper "fu," 6  
Horse rations, 323  
Horseradish, 267  
Horses, 319, 320, 323, 324, 325  
HRF, 238  
Huambisa tribe, 67  
Human  
    abnormalities, 319  
    anencephaly, 292, 293  
    cancer, 78, 82, 331  
    colon cancer, 83, 92  
    esophageal cancer, 323  
    immunodeficiency virus, 85, 283  
    liver disease, 319  
    plasma cholinesterase, 288, 290, 291, 329  
    plasma cholinesterase inhibitors, 329  
    urine, 275  
Hump spider beetle, 27  
Hyaluronidases, 272  
Hydrazine carcinogens, 312  
Hydrocolloids, 76  
Hydroxyproline-rich glycoproteins, 159, 169  
Hyoscyamus oil, 46  
Hyperactivity, 105, 110  
Hypercholesterolemia, 241  
Hypermotility, 110  
Hyperplasia of the basal cells, 323  
Hyperstimulation, 69  
Hypertension, 110, 285  
Hyperthermia, 110  
Hypnotics, 43  
Hypocholesteremics, 62  
Hypocholesterolemic agent, 241, 318, 319  
Hypoglycemia, 5, 78, 243, 278, 317  
Hypokalemia, 87  
Hypomania, 105  
Hyponatremic seizures, 277  
Hypotension, 55, 57, 97, 240, 242-244, 278  
Hypothermia, 8  
IBEC, 299, 332  
IGR, 231  
Immune functions, 273  
Immune system, 160, 164, 242, 244, 317, 330  
Immunochemical assays, 317  
Immunomodulatory activity, 283  
Immunostimulant, 242, 244  
Immunosuppressant, 276, 320, 321, 324  
Impotence, 108  
Incan Empire, 42  
Increased oxygen consumption respiration, 110  
Indian, 39, 42, 48, 63, 69, 126, 127, 145, 285  
Indian herbal teas, 285  
Indian medicinal plant, 126, 127  
Indian tragacanth, 48  
Indians of Asia, 39  
Indigenous peoples, 33, 35, 38, 50, 52, 68, 70  
Indolent ulcers, 47  
Infected sweet potatoes, 305, 306  
Infections, 67, 68, 117, 125, 136-138, 143, 145, 146, 283, 370  
Infertility syndrome, 275  
Inflammation, 68, 285, 370

- Inflammatory bowel disease, 275  
 Inhalation expectorant, 47  
 Immortal, 281  
 Insect  
     antifeedants, 371  
     attractant, 279  
     bites, 68  
     growth regulators, 228, 230, 234  
     hormones, 230  
     insecticidal compounds, 227  
     juvenile hormones, 231  
     lichen resistance, 207  
     neem, 197  
     and nematode damage, 167  
     precocenes, 233  
     repellent, 45, 46, 238, 240, 241  
     tobacco resistance, 162  
 Insecticide, 44, 48, 84, 137, 185, 189, 197, 205, 227, 228, 229, 230, 233, 234, 235, 239, 240, 241, 243, 279, 281, 288, 327, 371, 374, 375  
 Insectifuge, 240, 241, 278, 279, 281, 283  
 Insomnia, 110, 241  
 Integrated Breeding and Environmental Chemicals, 299, 332  
 Interstitial edema, 307  
 Interstitial inflammation, 285  
 Intestinal amoebae, 117  
 Intestinal amoebiasis, 129  
 Intestinal aryl hydrocarbon hydroxylase, 257  
 Intestinal damage, 5  
 Intestinal mucosal necrosis, 290  
 Intestinal parasitic infections, 145  
 Intestinal tumors, 274  
 Intraocular pressure, 63  
 Intratubular debris, 305  
 Intraventricular cardiac thrombosis, 323  
 Invasive squamous-cell carcinoma, 297  
 Iodine uptake, 277  
 Ipecac, 45, 49, 55, 77  
 Iraq, 35  
 Ireland, 9, 293  
 Irish, 287, 289  
 Irrigation, 292  
 Irritability, 69  
 Irritant, 137, 241  
 Irritation, 108, 290  
 Israel, 208  
 Italy, 10, 99, 371  
 Itching, 68  
 Itip, 68  
 Ivory Coast, 146  
 Izmir, 8  
 Jackbean, 198  
 Jacques Louis David, 2  
 Jalap, 46  
 Jamestown, 40  
 Japan, 88, 206, 286, 320, 323  
 Japanese millet, 216  
 Jimmyweed, 249  
 Jimson weed, 170, 173, 266  
 Jívaro, 67-70  
 Job's tears, 212  
 Joseph, 5, 159  
 Julia Agrippina, 3  
 Julius Caesar, 6, 7  
 Juniper, 24, 26, 285  
 Juniper berries, 26  
 Juniper wood, 24, 26  
 Kachi grass, 194  
 Kala azar, 117  
 Kale, 255, 267  
 Karaya gum, 48  
 Kat, 98, 114, 115  
 Kawain, 47  
 Kenya, 98, 99, 103-105  
 Keratinocytes, 119  
 Keratoacanthomas, 296  
 Khat, 97-105, 108-111  
 Khios, 8  
 Khu-fu, 6  
 Kidney bean, 198  
 Kidney cancer, 82  
 Killing jaundice, 69  
 Kohlrabi  
     diet recommendation, 258  
     goiter, 255  
     quercetin, kaempferol content, 267  
 Kola, 285  
 Kraits, 7  
 Kraut, 256  
 Kuwait, 111  
 LA40221, 118  
 Labor, 64, 70

- Lack of fatigue, 110  
 Lactagogue, 279  
 Lactating rats, 321  
 Lactation, 108  
 Larvicide, 278, 279, 281, 283  
 Lassitude, 87  
 Late-blight  
     pathogen, 171  
     spina bifida incidence, 293  
 Lavender oil, 46  
 Laxative, 44-48, 108  
 Laxative tonic, 48  
 LDL, 276  
 Leaf juice, 44  
 Leaf miner, 299, 332  
 Leaves antibiotic, 44  
 Leek, 18, 117, 237, 267  
 Legume forages, 293  
 Legume phytoalexins, 214  
 Leishmaniasis, 68, 118, 123, 148  
 Lemon, 50, 242, 301-303  
 Lemon oil, 301  
 Lemon peel, 50  
 Lenape, 289, 291, 329  
 Lens aldose reductase, 273  
 Lepidius, 7  
 Lesion nematode, 186, 188, 192, 194, 197  
 Lethargy, 108  
  
 Lettuce  
     herbicide bioassay, 206, 214, 217-219, 221  
     mutagen absorption, 330  
     nitrate content, 292  
     psychoactive, wild lettuce, 285  
     quercetin, kaempferol content, 267  
 Leukemia, 50, 78, 79, 82, 83, 213, 216, 319, 330  
 Leukocytosis, 277  
 Leukoderma, 296  
 Leukoencephalomalacia, 323, 330  
 Leukopenia, 78, 83  
 Leydig cells, 275  
 Li Shih-Chen, 35, 64  
 Lice, 68  
 Lichen, 24, 76, 207, 208  
 Licorice extract, 46  
 Light-headedness, 10  
 Lignification, 171  
  
 Lima bean  
     coumesterol, nematode infection, 194  
     phytoalexins, 142, 251, 286  
     Thanksgiving Day dinner, 252  
 Lime  
     coca chewing containers, 42  
     contact dermatitis, 295  
     flavonoid content, 270  
     lime in fens, 15  
     limonene content, 242  
     in mummification, 22  
     oil, 301  
     solids in oil, 303  
 Lincoln, Nancy Hanks, 248  
 Linear furanocoumarins into DNA, 296  
 Liniment, 44, 76  
 Linseed oil, 47  
 Lipid peroxidation, 132, 271  
 Lipid synthesis, 276  
 Lipotropic, 278  
 Liquifaction necrosis of the white matter, 323  
 Litholytic, 241  
 Litmus test, 206  
 Liver, 39  
     *Amanita* poisoning, 5  
     Amset, mummification, 22  
     cancer, 258, 316, 317, 318, 319, 323, 324, 330  
     cell tumors, 312  
     cirrhosis, 319, 329  
     cytochrome P-450, 312  
     disease, 316, 319  
     flavonoid metabolism, 262  
     hepatotoxic compounds, 305  
     ipomeanol binding, 307  
     microsomal enzymes, 284  
     microsomal monooxygenase, 312  
     microsomes, 321  
     parasites, 146  
     preneoplastic foci, 274  
     solanidine, post mortum samples, 294  
         veno-occlusive disease, 285  
 Liverwort, 208  
 Livestock, 248, 282, 287, 295, 318, 322  
 Livia Drusilla, 5, 13  
 Livy, 12  
 Local analgesic, 48

- Local anesthesia, 49, 42, 80, 82  
 Local antiseptic, 46  
 Logorrhoea, 110  
 Lonchocarpus, 228  
 London, 16, 36, 99  
 Long-wave UV light, 296  
 Longbow, 11  
 Loquacity, 105  
 Loss of appetite, 109, 248  
 Loss of righting reflex, 257  
 Low density lipids, 276  
 LT, 260  
 Lucas, 16, 21-24, 26-28, 270  
 Lung  
     *Amanita* poisoning, 5  
     cancer, 82, 83, 91  
     clara cell necrosis, 330  
     clara cells, 307  
     edema, 305, 320, 330  
     edema toxin, 306, 307  
 Hapy, mummification, 22  
 mummification, 6  
*N*-methylhydrazine tumors, 312  
 toxin, 304, 305, 307, 330  
 tumor incidence, 308  
 tumors, 308, 312  
 Lyme Disease, 238  
 Lymphoid cell depletion, 320  
 Lymphoid cells, 320  
  
 Macbeth, 9  
 Macrocytis, 46  
 Macrophage phagocytic activity, 320  
 Madagascar, 50, 98, 103, 104  
 Madagascar periwinkle, 50, 78  
 Mahatma Gandhi, 55  
 Mahonia, 123, 125  
 Mahuang, 36, 37  
 Malaria, 35, 64, 66, 68, 84, 117-119,  
     131, 242  
 Male  
     antifertility agent, 133  
     contraceptive, 87  
     hormones, 276  
     sexual function, 108  
 Malignant histiocytomas, 312  
 Malnutrition, 109  
 Malonate, 170, 171  
 Malonate pathway, 171  
 Mambas, 7  
 Mammals, 69, 242, 317, 330  
  
 Mandarin orange, 270  
 Marcellus, 5  
 Marcus Salvius Otho, 3  
 Marigolds, 186, 199  
 Marijuana, 38-40, 98  
 Mark Antony, 7, 9  
 Maximum tolerated dose, 331, 370  
 Mayna tribe, 67  
 MCPBA, 219  
 Meadow saffron, 3, 36  
 Meat products, 314, 326  
 Mechanical wounding, 169  
 Meckel's diverticulum, 282  
 Medea, 8  
 Medical ethnobotany, 68  
 Medicinal activities, 239, 241, 242, 370  
 Medicinal plants, 34, 35, 67, 75-77, 79,  
     87, 88, 283, 285  
 Medicinal properties, 7, 15, 35, 281,  
     283, 370  
 Melanin biosynthesis, 327  
 Melanoma, 82  
 Melissa oil, 47  
 Melons, 292  
 Meningoencephalitis, 117  
 Menstrual irregularities, 42  
 Mental confusion, 5  
 Mental patients, 55  
 Mental retardation, 317, 330  
 Merck & Co., Inc., 371, 375  
 Mesolithic, 39  
 Messenger RNA, 5  
 Mestizo tribe, 67  
 Metastatic testicular cancer, 82  
 Mevalonate pathway, 170, 171  
 Mexican folk medicines, 283  
 Mexican lime, 270  
 Mexican yams, 80  
 MFH, 310, 312  
 MFHO, 310  
 MFO, 257, 259, 307  
 Microbial metabolites, 167, 375  
 Microphthalmics, 293  
 Microsomal epoxide hydrolase, 257  
 Microtubule function, 129  
 Middle East, 39, 282  
 Midwives, 64  
 Migraine headaches, 40, 79  
 Milk  
     aflatoxin B<sub>1</sub> content, 322  
     and eggs, 314

- Milk (continued)
- mycotoxins in, 315, 316
  - novasil aflatoxin reduction, 326
  - poisoning, 248
  - products, 248, 318, 330
  - sickness, 248, 249, 360
  - soybean, cancer preventive, 241
  - soybean flavonoids, 269
  - toxin, 321
- Minerals, 244
- Minotaur, 8
- Mint, 117, 238-240, 242
- Miotic, 47, 63
- Miraa, 98
- Mirra, 98
- Mirungi, 98
- Mites, 68
- Mitogenesis, 273, 331
- Mitomycin C, 276
- Mixed-function oxidases, 257
- MMDA, 251
- Maori and Pacific Island children, 308
  - Mold metabolites, 313
  - Mold-damaged sweet potatoes, 305
  - Moldy brewers' grains, 320
  - Molluscicide, 238, 241, 243, 371, 374
  - Monkeys, 131, 252, 319, 321, 324, 330
  - Monkshood, 7, 8
  - Morbidity, 109, 258, 282, 352
  - Morning glory, 266
  - Morocco, 111
  - Mosquitoes, 230
  - Motion sickness, 45, 49
  - Mount Kenya, 111
  - Mountain mint, 239-240, 242
  - Mouthwash, 45, 49
  - MRC 826
    - cancer enhancement, 323
    - F. moniliforme*, 319
    - mycotoxin production, 325
    - structure, fusarin C, 324  - MTD, 331
  - Mucilages, 76
  - Mucoirritant, 279
  - Muhulo, 98
  - Muirungi, 98
  - Mulberry, 178
  - Multidrug resistant *P. falciparum*, 123, 137
  - Multifunction oxidase inducers, 273
  - Multiple drug-resistant strain, 131
  - Multiple hepatic nodules, 323
  - Multiple sclerosis, 40
  - Mumia, 15
  - Mummification, 6, 15-28
  - Mummy, 15, 16, 17, 18, 19-29
  - Mummy Mountain, 15
  - Murder, 1
  - Muscle cramps, 251
  - Muscle relaxant, 45, 46, 69, 77, 80
  - Mushroom
    - Amanita* poisoning, 4, 5
    - false morel toxins, 310
    - poisoning, 36
    - Thanksgiving Day dinner, 251, 252
    - toxic compounds, 308
    - toxic steam, 330  - Musitate, 98
  - Musk lime, 270
  - Muslim drug, 98
  - Mustard seedlings, 195
  - Mutagenesis
    - flavonoid antimutagens, 275
    - furanocoumarins, 300
    - gyromitrin, *N*-methylhydrazine, 312
    - herbs, 284
    - isopimpinellin, 243
    - isothiocyanates, 257
    - mycotoxins, 317, 322-325, 330
    - nitrates, 291
    - maximum tolerated dose, 331
    - quercetin, myricetin, kaempferol, 273  - Mutagenic flavonols, 274
  - Mutagens from fly ash, 330
  - Mutations in a mammalian cell, 321
  - Mycotoxicosis, 313, 314, 318
  - Mycotoxicosis of man, 313
  - Mycotoxin, 223, 258, 271, 275, 293, 303, 313-328, 330-333
  - Mydriasis, 10, 45, 64, 108, 110
  - Myocardial infarction, 318
  - Myocardiodepressant, 244
  - Myoneural synapses, 7
  - Myrosinase, 257, 259
  - Myrrh, 2, 18, 21, 22, 24, 27, 45, 370
  - NAPRALERT, 93, 147, 238
  - Narcissus, 70
  - Narcotics, 8, 10, 11, 39, 53, 55, 80, 101, 102, 251, 279
  - Narcotic effect, 8

- Narcotic or hallucinogenic effect, 251  
Nasal, 35, 43-47  
Nasal decongestant, 35, 47  
Nasal sprays, 43, 46  
National Academy of Sciences, 271  
National Cancer Institute, 52, 82, 83, 86, 245, 372  
National diets, 264  
National Institutes of Health, 82, 372, 373  
National Research Council, 247, 258, 271, 332, 372  
National Science Foundation, 33, 372, 373  
National Toxicology Program, 274, 372  
Native holly, 69  
Natriuretic, 279  
Natural dyes, 207  
Natural goitrogens, 253  
Natural pesticides, 237, 242, 244, 285, 287, 305, 317, 330, 331  
Natural plant products, 34  
Natural product prototype, 76  
Natural product pyrimidine, 217  
Natural Products Alert Database, 238  
Natural toxicants, 247, 248, 253, 293, 299, 331, 332  
Nausea, 2, 10, 53, 277, 290  
NCI, 82, 238, 241, 245  
Neanderthal, 35  
Neck cancer, 82  
Neem, 137, 152, 197  
Nematocidal Agents, 142-145, 185-189, 192, 194-204, 243  
Nematode, 117, 142-144, 145, 146, 157, 167, 185-189, 192-198, 250  
Nematotoxic polyacetylenes, 187  
Neolithic, 38, 39  
Neoplasms, 52, 78, 82  
Nephrosis, 305, 323  
Nephrotoxin, 320  
Nero, 3-5  
Nero Claudius Caesar, 3  
Nervous ergotism syndrome, 293  
Nervousness, 105  
Neural tube defects, 293  
Neurologic diseases, 293  
Neuromuscular blocker, 49  
Neuromuscular blocking agent, 82  
Neuronophagia, 323  
Neurotoxic, 251, 320, 323, 330  
Neurotoxicity, 127  
Neurotoxin, 7, 69, 322  
New Guinea, 308  
New Kingdom, 18, 19  
New Mexico, 281  
New Zealand, 308  
Niaouli oil, 47  
Nicotinic acetylcholine receptors, 7  
Nightmares, 105  
Nightshade family, 287, 292, 299, 332  
Non-human primates, 370  
Norman Conquest, 9  
North America, 2, 42, 72, 88, 213  
North Yemen, 103, 104, 109  
Northeast Thailand, 317  
Northern Belgium, 318  
Nut, 145, 241, 252, 285, 316, 330  
Nutmeg  
    drug, 47  
    limonene, 242  
    narcotic effects, 251  
    oil, 47  
    psychoactive substances, 285  
    Thanksgiving Day dinner, 252  
Nutrients, 244, 248, 276, 287
- Oak, 60  
Oatmeal, 44, 57  
Oats, 322  
Obstetrics, 64, 70, 375  
Occupational exposure, 315  
Octavia, 3, 4, 7  
Oil of juniper, 24, 26  
Oil of sassafras, 277  
Oil of turpentine, 24  
Ointment, 22, 24, 76, 370  
Old World, 35, 38  
Oleoresin, 44, 45, 49  
Oligogalacturonates, 169  
Oligosaccharides and polysaccharides, 168  
Olive oil, 47, 117, 308  
Onion  
    antibiotic, 70  
    health effects, 276, 277, 329  
    mummification, 24  
    phytoalexin fungi, 160  
    phytochemicals, 237  
    quercetin, kaempferol content, 252, 266, 267, 292

- Onion and garlic juices, 276, 329  
 Onion scales, 160  
 Ophthalmology, 42, 49, 350  
 Opium, 38, 39, 43, 47, 55, 57, 60, 72,  
     80, 98, 370  
 Oral hypoglycemic effects, 78  
 Orange, 45, 242, 270, 301-303  
 Orange juices, 301  
 Orange oil, 45, 301  
 Oregano  
     LD<sub>50</sub>s compounds in, 279  
     natural pesticides, 244  
     tannins, oils, 237  
 Organogenesis, 319  
 Orient, 55  
 Oriental sore, 117, 123  
 Orythroleukemia cells, 276  
 Osiris, 21  
 Osteolytic, 240  
 Otolaryngology, 42  
 Ovarian and breast cancers, 52  
 Ovarian cancer, 82, 83, 97  
 Ovid, 8  
 Oxytocic, 45, 64, 70, 243  
  
 Paleolithic, 39  
 Pagoda tree, 239  
 Pain  
     *Aconitum* poisoning, 8  
     *Amanita* poisoning, 5  
     antispasmodics, 53  
     bay leaf, rectal, 282  
     chymopapain, 58  
     gout treatment, 36  
     herbal adulteration, 284  
     historical plant selection, 33  
     Jívaro Indians, 68  
     killing, 242  
     lead compound, 80  
     opium, 39  
 Pakistani, 284  
 Palm wine, 18, 21, 24  
 Pancreatic ductular carcinoma, 258  
 Pansy, 239  
 Papain, 44, 58, 80  
 Papaya, 44, 58, 80  
 Papillomas, 296  
 Paracelsus, 329  
 Paraguay, 88  
 Paralysis, 2, 6, 7, 49, 87  
 Parasites, 64, 66, 117, 133, 146, 185  
  
 Parasiticidal, 47, 64, 117, 134  
 Parasympathomimetic, 77, 80  
 Parched and sore throat, 10  
 Parkinson's disease, 10  
 Parmelia, 24, 206  
 Parsley  
     anthelmintic, 117  
     furanocoumarin content, 300-302  
     phytoalexins, 251  
     phytoalexins, disease, 329  
     pickers and cutters, 295  
     poisonous hemlock, 2  
 Parsnip  
     furanocoumarin content, 300  
     furanocoumarins, 295  
     hemlock, 2  
     phytoalexins, 251  
     phytoalexins, disease, 329  
     xanthotoxin, 175  
 Pathogens, 142, 160, 162, 167, 169, 173,  
     216, 227, 247, 249, 260, 283,  
     306, 309, 322  
 Pea  
     acifluorfen elicitor, 250, 251  
     nitrate content, 292  
     phytoalexins, 165, 169-171, 251  
     pisatin, 175  
 Peach, 268, 270, 320  
 Peanut  
     butter, 330  
     di-n-butyl succinate, nematicide, 192  
     flavonoid content, 270  
     genetic engineering, 332  
     goiter, 255  
     hulls, 222  
     mycotoxins, 314, 330  
     mycotoxins, cancer, 315  
     mycotoxins, consumption, 316  
     phytoalexins, 168, 251  
 Pear  
     flavonoid content, 270  
     patulin contamination  
     quercetin, kaempferol content, 268  
 Pectin, 45, 47, 50  
 Peels, 287, 290, 291, 293  
 Peking Union Medical College, 35  
 Pennyroyal, 46, 238-240, 277  
 Pennyroyal oil, 46, 277  
 People's Republic of China, 78, 84, 85,  
     87

- Pepper**  
 capsidial, 171  
 chemical translocation, 331  
 nightshade family, 248, 287  
 nitrate content, 292  
 phenolic accumulation, 295  
 phytoalexin, 170, 251  
 phytoalexin, capsidiol, 294  
 phytoalexin response, 329  
 quercetin, kaempferol content, 267  
 Thanksgiving Day dinner, 252
- Peppermint**, 193  
 oil, 47
- Pepsin**, 58
- Perennial snakeweed**, 269
- Perfumed oils**, 30
- Perfumery**, 279
- Peripheral edema**, 57
- Perivascular hemorrhage**, 323
- Periwinkle**, 50, 52, 78, 285
- Peroxidase**, 159, 169, 171, 186, 275
- Persian insect powder**, 205
- Peru**, 15, 40, 42, 43, 47, 287
- Pesticidal activity**, 237-244, 281, 283
- Pesticidal and medicinal properties**,  
 281, 283
- Pets**, 322
- Pharaoh**, 5, 6
- Pharmaceutical aid**, 44, 46, 49
- Pharmaceutical prep**, 44, 48
- Pharmaceutical solvent**, 48
- Pheromone**, 233, 239, 240, 279
- Phillipi**, 7
- Phosphate and carbamate poisoning**,  
 10
- Photoactive compounds**, 296, 302, 303
- Photoactive furanocoumarins**, 298, 303,  
 299
- Photocarcinogenic**, 329
- Photodermatitis**, 293, 295, 300
- Photomutagenic**, 329
- Photophytodermatitis**, 298, 299, 330
- Photosensitization**, 296, 329
- Photosensitization in humans**, 296
- Photosensitized skin reactions**, 297
- Photosensitizing linear**  
 furanocoumarins, 296
- Photosensitizing toxins**, 295
- Phototoxic linear furanocoumarins**,  
 293, 300, 303
- Phytoalexin**, 142, 143, 159, 162, 164,  
 165, 167-171, 173, 175, 178,  
 192, 194, 244, 249-251, 253,  
 260, 286, 287, 288, 293, 294,  
 295, 298, 299, 304, 305, 307,  
 322, 329, 330, 332
- Phytobiocides**, 244
- Phytochrome**, 208
- Phytogeography**, 38
- Phytomolluscicides**, 243
- Phytoparasitic nematodes**, 185-187,  
 194, 195, 197
- Phytophotodermatitis**, 296, 302
- Pickles**, 292
- Pig**, 87, 110, 119, 254, 260-262, 275,  
 295, 318, 319, 320, 323
- Pimples**, 69
- Pin nematode**, 196
- Pineapples**, 58, 68, 320
- Pinewood nematode**, 187
- Pipe & Siam gamboge**, 46
- Piscicide**, 243
- Pitch**, 15, 27, 28
- Plant**  
 derived drugs, 75-77, 79, 81, 88-89,  
 371, 372, 374
- derived sweetening agents, 89
- parasitic nematodes, 185, 186
- Plant breeding**, 299, 329, 332
- Plant defensive chemicals**, 227
- Plant diuretic and laxative prep**, 44
- Plant lectins**, 198
- Plant sterols**, 80
- Plant tissue culture**, 217
- Plantain**, 68
- Plaque**, 68
- Plasma cholinesterase**, 288, 291, 329
- Plasma levels of cholesterol and**  
 triglycerides, 318, 330
- Platelet aggregation**, 273
- Plato**, 2
- Plectus**, 192
- Pliny**, 11, 18, 35
- Plum**  
 flavonoid content, 270  
 patulin contamination, 320  
 quercetin, kaempferol content, 268,  
 269
- Plutarch**, 9
- Pneumocystis**, 118
- Pneumonia**, 42, 118, 307, 330

Pneumotoxicities, 285  
Pods, 44, 170  
Poison hemlock, 2, 8  
Poisonous parsley, 2  
Poland, 319, 375  
Polyacetylene nematocidal agents, 144  
Polymerase, 123  
Pome fruits, 271  
Pomegranate pips, 117  
Pompeii, 4, 11  
Poplar buds, 47  
Poppea Sabina, 3, 13  
Poppy juice, 39  
Porcine pulmonary edema, 324, 330  
Positive inotropic and chronotropic effect, 110  
Posset, 12  
Postmortem liver samples, 294  
Postpartum, 64, 70  
Potassium quisqualate, 144  
Potato, 287-294, 299, 304-309, 329, 330, 332, 371  
anticancer ipomeanol, 83  
avoidance by pregnant women, 292, 293  
birth defects, 293  
breeders, 288  
cancer preventive, 238  
chaconine, solanine fungicide, 162  
chips, 289  
cholinesterase inhibition, 288  
chronic lung disease, 308  
cooking; poisoning symptoms, 290  
cultivar, 291, 293  
cyst nematode, 186  
fungus resistance, 160, 162, 165  
genetic engineering, 332  
glycoalkaloids, 288-290, 294  
glycoalkaloids, concentrations, 289  
ice cream, 292  
illness, 287  
intercropping w/khat, 99  
ipomeamarone phytoalexin, 175  
ipomeanol, lung toxin, 307  
isothiocyanate yield improvement, 195  
late-blight, 293  
lung edema, 330  
lung toxin concentrations, 309  
new variety toxicants, 299  
nightshade family, 248

Potato (continued)  
nitrate content, 292  
peels, 290  
pesticide use on, 371  
phytoalexin elicitors, 171  
phytoalexin response, 329  
phytoalexins, 165, 178, 251  
phytoalexins, sweet potato, 306, 307  
postmortem concentration, 294  
quercetin, kaempferol content, 267  
rishitin phytoalexin, 194  
rot nematode, 194  
scab, 160  
solanine, chaconine concentrations, 291  
sweet potato, 305  
Thanksgiving Day dinner, 252  
tissues, 289  
toxins, sweet potato, 304  
tuber discs, 194, 213  
tuber tissues, 290  
varieties, 288, 290-294  
vines, 248, 287  
Poultry, 282, 317, 330  
PPE, 324, 325  
Precious ointments, 22  
Preemergence herbicides, 215, 221  
Pregnancies, 68  
Pregnant mares, 275  
Premeditated crime, 1  
Preplant soil fumigants, 195  
Preputial glands, 312  
Primary hepatocellular carcinoma, 318  
Primitive man, 370  
Produce workers, 297  
Promastigotes, 125, 140  
Proprietary medicine, 46  
Proprietary prep, 44  
Prostate cancer, 82, 276, 257, 318  
Proteases, 169  
Protein digestants, 58  
Proteolytic, 80  
Protisticide, 244  
Protozoa, 117, 242  
Protozoans, 117, 119  
Prune prep, 48  
*Prurigo nodularis* lesions, 296  
Psoralen treatments, 297  
Psoralens, 252, 284, 296, 297, 303, 330  
Psoriasis, 57, 296, 297  
Psychic dependence, 108

- Psychoactive substances, 38-40, 43, 68, 98, 103, 109, 110, 243, 284, 285  
 Psychopharmacology, 55  
 Psychotic patients, 55  
 Psychotropic, 77, 99, 103, 110  
 Pteridophytes, 76  
 Ptolemaic Period, 17, 28  
 Ptolemy, 6, 7  
 Ptolemy Caesar, 7  
 Ptolemy XIII, 6  
 Ptolemy XIV, 6, 7  
 Ptolemy XV Philopator Philometor, 7  
 Pulifuge, 240  
 Pulmonary congestion, 57  
 Pulmonary edema, 323, 324, 330  
 Pulmonary hypertension, 285  
 Pulse rate, 108  
 Pummelo, 270  
 Pumpkin, 145, 252, 292  
 Pumpkin pie, 252  
 Punt, 24  
 Purine, 86, 217, 218  
 Purple foxglove, 77  
 PUVA, 296, 297  
 Pyrethrin insecticide, 48  
 Pyrethrum, 205, 227, 228, 235  
 Pyridazinone herbicides, 327  
 Pyrus, 239  
  
 Qat, 98, 111, 114-116  
 Qebehsenuef, 22  
 Quassia bitters, 47  
 Queen Hetepheres, 16  
 Quijos Quichua, 145  
 Quince, 268  
  
 R-gene resistance, 171, 182  
 Rabbit, 3, 110, 260-262, 320  
 Radioimmunoassay, 317  
 Radish  
     chemical translocation, 331  
     herbicide bioasssay, 210, 217, 218, 221  
     quercetin, kaempferol content, 267  
     Thanksgiving Day dinner, 252  
 Ragweed, 277  
 Rain forests, 34, 63, 68, 217, 372  
 Rainbow trout, 321  
 Rameses II, 24  
 Rameses IV, 24  
  
 Random screening, 80, 371  
 Random synthesis, 371  
 Rangoon creeper, 144  
 Rape seed or meal, 255  
 Rash, 8, 277  
 Raspberry, 203, 268  
 Raw and processed milk, 322  
 Rayless goldenrod, 249  
 Reactive depression, 108  
 Rectal pain, 282  
 Recurrent bad dreams, 108  
 Red cabbage, 256, 267  
 Red clover  
     medicarpin, 171  
     phytoalexins, 171  
 Red Currant, 268  
 Red wine, 252  
 Renal colic, 43  
 Renal failure, 5  
 Renal insufficiency, 251  
 Renal lesions, 285  
 Renal toxicity, 323  
 Renal tubular cell adenomas, 274  
 Repellents, 227, 233, 371  
 Reproduction, 188, 192, 193, 197, 230, 233, 275  
 Republic of Transkei, Africa, 323  
 Research funding, 373  
 Resins, 16, 18, 21-24, 27, 28, 30, 44, 45, 47, 217, 369, 370  
 Resistance  
     asparagus, 195  
     beetles to leptines, 291  
     and birth defects, 293  
     blue mold, 173  
     chloroquine, 67, 126  
     and contact dermatitis, 299  
     defense mechanism, 169  
     genetic engineering, 332  
     induction, 178  
     insect, 233  
     lima bean, 142  
     malaria, 118  
     nematicidal alkaloids, 187  
     nematicidal fatty acids, 192  
     nematicide polythienyls, 186  
     phytoalexin accumulation, 167  
     due to phytoalexins, 165, 249  
     post infectional compounds, 194  
     potato, 160  
     potato varieties, 291

- Resistance (continued)**
- in Solanaceae, 171
  - soybean, 143
  - tobacco, 163
  - tobacco, potato, 162
  - and toxicity, 329
- Respiratory stimulant**, 47
- Respirodepressant**, 244
- Respiroinhibitor**, 279
- Respirostimulant**, 242
- Restlessness**, 110, 248
- Retrovirus**, 85
  - carcinogenicity, 276
- Reye's syndrome**, 317, 318, 330
- Rhatany root**, 46
- Rheumatism**, 36, 68
- Rhizome**, 45, 48
- Rhododendron**, 48, 137
- Rhubarb**, 62, 331
  - root, 283
- RIA**, 317
- Rice**
  - aflatoxin inhibitor, 327
  - aflatoxin reduction w/novasil, 326
  - blast disease, 327
  - fumonisins, 322
  - herbicide bioassay, 209, 217-219
  - hull, 271
  - isovitexin, 271
  - momilactone B, 178
  - mycotoxin contamination, 316
  - phytoalexins, 251
    - white tip nematode, 188
- River Styx**, 9
- RNA**, 5, 87, 124, 218, 244, 296
- RNA-depressant**, 244
- Roasted birds**, 68
- Rocky Mountain Spotted Fever**, 238
- Rolls with butter**, 252
- Roman Empire**, 6, 13
- Romania**, 317
- Rome**, 3, 6-8
- Root**, 2, 3, 10, 36, 52, 55, 57, 60, 65, 69, 87, 104, 117, 129, 142, 145, 152, 157, 170, 173, 175, 180, 181, 193, 195, 197, 198, 204, 209, 211, 213, 215, 216, 217, 218, 219, 255, 260, 283, 269, 286, 297, 300, 370, 374
  - anthelmintic, 48
  - bark, 47, 49, 68, 277, 281
- Root (continued)**
- lesion nematode, 186, 188, 192
  - nematodes, 143, 187, 192, 194, 196
- Root diuretic**, 44
- Rose petal infusion**, 48
- Rosemary**, 48, 277, 281
- Rosemary oil**, 48
- Rough lime**, 270
- Rowan or mountain ash trees**, 326
- Rubefacient**, 44, 45, 47, 240, 243, 278, 279, 281, 283
- Rue**, 117, 277
- Rufus Crispinus**, 3
- Rutabaga**, 255
- Rye**
  - BOA, DIBOA, 212
  - ergot, 64
  - germ oil, 62
  - herbicides, 211
  - nematicide, 192
  - nervous ergotism syndrome, 293
  - $\beta$ -sitosterol, 62
- Ryegrass**, 221
- Sacred Ship**, 2
- Safflower**, 178
- Sage**, 277, 283
  - oil, 48
- Salivation**, 63, 293
- Salmonella assays**, 274
- Salt**, 22, 26, 43, 76, 83, 131, 173
- Sandalwood oil**, 48
- Sanguinaria root**, 48
- Sap**, 69, 302
- Sarcoma**, 82, 321
- Sassafras**
  - oil, 48
  - root bark, 277
- Satellitosis**, 323
- Saudi Arabia**, 110
- Saudi Arabian khat**, 103
- Sauerkraut**, 292
- Savin oil**, 46
- Sawdust**, 18, 24, 26
- Saw palmetto berries**, 48
- Scabicide**, 47
- Scarlatina**, 10
- Scented oils**, 30
- Schistosome**, 146
- Schistosomiasis**, 241, 371
- Schistosomicide**, 243

Schistosomula, 147  
Schizophrenia, 55, 105  
Sclerosis, 40  
Scotland, 9, 11, 206, 287, 320  
Screening, 52, 76, 80, 82, 85, 103, 118, 127, 178, 371-373  
Scythians, 39  
Sea-snakes, 7  
Secondary chemistry, 227, 233  
Secondary metabolite, 75, 76, 78, 82, 85, 87, 88  
Sedative, 2, 10, 46, 47, 49, 53, 79, 80, 240, 244, 257  
Sedges, 70  
Seed oil, 195, 228  
Seed oil toothpaste, 44  
Seeds, 2, 39, 43, 55, 60, 62, 63, 68, 99, 117, 144, 145, 192, 197, 206, 211, 266, 271, 274  
Selenium, 133, 330  
Self-heal, 239  
Seminarcosis, 10  
Seneca, 5  
Senega fluid extract, 47  
Senna, 44, 62  
Senna leaves, 44  
Septic shock, 79  
Serendipity, 80, 371  
Serotonin antagonist, 48  
Serum cholesterol, 276, 329  
Sesame oil, 48, 85, 228  
Sesquiterpenoid cyclase, 171  
Seutonius, 4  
Severe acidosis, 248  
Severe generalized phototoxic reaction, 297  
Severe headache, 277  
Sex accessory glands, 275  
Shakespeare, 7-9, 11  
Sheep, 275, 319, 323, 333  
Shepses-ka-f, 6  
Shikimate pathway, 171  
Shuar tribe, 67  
Sick Stomach, 248  
Sicklepod, 266  
Simonsen hamsters, 293  
Sir Edward Howe, 36  
Skeletal muscle relaxant, 49, 77, 80  
Skin  
    aid, 44  
    blemishes, 69

Skin (continued)  
    depigmentation, 282, 296  
    healing, 47  
    papilloma, 258  
    patches, 303  
    protectant, 48  
    surfaces, 303  
    ulcer therapy, 47  
    vesicant, 3  
Slaves, 281  
Sleep, 4, 10, 39, 53, 257  
Sleeplessness, 108  
Small cell lung cancer, 52, 82  
Smoked game, 68  
Smooth muscle relaxant, 46, 80  
Smudge disease, 160  
Snake, 5-7, 68  
Snakebite death, 6  
Snuff, 273  
Soap, 44  
Socrates, 2, 12  
Soft-tissue infections, 283  
Soft-tissue tumors, 308  
Solanaceae glycoalkaloids, 294  
Somalia, 98  
Sorbent technology, 326  
Sore muscles, 42  
Sore throats and coughs, 49  
Sorghum, 99, 110, 178, 316  
Sour cherry, 268, 270  
Sour orange, 242  
South Africa, 98, 321  
South America, 2, 38, 49, 67  
South Yemen, 109  
Southeast Asia, 78, 314  
Southern Georgia, 317  
Soy products, 275  
Soy protein, 275, 335  
Soya meal, 275  
Soybean  
    acifluorfen elicitor, 330  
    cyst nematode, 196  
    fumonisins, 322  
    glyceollin phytoalexin, 143  
    glyceollin nematode response, 194  
    goiter, 255  
    milk, 241  
    oil, 60  
    phytoalexin, 142, 166, 167, 171, 250, 251  
    phytoestrogens, 269

Spanish fly, 3  
Sparta, 314  
Spasmolytic, 240, 242, 243, 278, 279  
Spearmint, 47, 242  
Spearmint oil, 47  
Sperm, 40, 87, 275  
Spermatids, 275  
Spermatogenic cell abnormalities, 252  
Spermatorrhea, 108  
Spermicide, 243, 279  
Sphagnum, 15  
Spices, 18, 21, 22, 24, 28, 237, 244, 264, 327, 370  
Spina bifida, 292-294  
Spinach  
    acifluorfen elicitor, 330  
    chemical translocation, 331  
    nitrate content, 291, 292  
    oxylate, 251  
    phytoalexins, 250  
Spores  
    absorbent, 47  
Sprengel, 44  
Sprouts, 162, 252-260, 287, 290, 293, 329  
Spurge trees, 69  
Squalene synthetase, 171  
Squamous cell carcinomas, 296  
Squill extract, 49  
Sri Lanka, 67, 285, 316  
St. Anthony's Fire, 64  
Staph, 69  
Star anise, 46, 242  
Stem nematode, 194  
Stereochemistry, 102, 120, 121, 138  
Stereotyped oral activity, 110  
Sterility, 253, 318  
Stimulants, 35, 36, 40, 44-49, 62, 68, 69, 73, 99, 100, 102, 196, 198, 240, 278, 279, 281, 283  
Stinkweed, 269  
Stomach cramps, 290  
Stomachache, 48  
Stomachic, 244  
Stone or Umbrella Pine, 27  
Strabo, 18, 27  
Stramonium, 45, 266  
Stress, 83, 159, 162, 167-169, 171, 178, 214, 250, 292, 294, 305, 330  
Structure-activity, 121, 134, 206, 210, 372

Suberization, 171  
SubSahara Africa, 64  
Subterranean clover  
    isoflavones, 269  
    phytoestrogens, 275  
Sudan, 67  
Sugar beets, 178  
Sumac, 60  
Suntan parlor, 297  
Suppository, 76  
Suppressers, 171  
Suppressing or preventing jet lag, 296  
Surgeon General, 241, 271  
Sweet, 9  
    wine w/ephedron, 35  
Sweet and bitter almond oil, 48  
Sweet cherries, 320, 268, 270  
Sweet peppers, 292  
Sweet potatoes, 83, 99, 165, 175, 178, 251, 252, 292, 304-309, 329, 330, 332  
Sweetening, 75, 88, 89  
Sweetening Activity, 88  
Swelling, 8, 36, 68, 216  
Swine, 324  
Swiss mice, 308, 312  
Switzerland, 97, 99, 110  
Synergists, 228, 229  
Syria, 38  
Syrian hamsters, 290, 293  
Syrup, 44, 46  
Systemic resistance, 173, 178  
T-cell function deficiency, 284  
Tachycardia, 108  
Tacitus, 4  
Taeniafuge, 145  
Tamarind extract, 28  
Tangerine, 242  
Tannic acid galls, 48  
Tanning booths, 297  
Tanning parlor, 297  
Tanzania, 98  
Tapeworms, 145  
Taproot, 2  
Taro, 68  
Tarsus, 7  
Taster Halotus, 4  
TCDD, 259, 260, 329  
TCDD receptor, 329

- Tea**
- cancer herbal teas, 319
  - chamomile allergens, 277
  - comfrey root, 286
  - flavonoid intake, 266
  - hepatic disease, 285
  - stimulant, 55
  - testicular atrophy, 252
  - theobromine, 255
- Tecomaria**, 137
- Tenderizing meats**, 58
- Teratogenic effects**, 257, 277, 293-295, 317, 319, 320
- Teratogens**, 292, 319, 329-331
- Termites**, 230
- Termiticide**, 243
- Testicular atrophy**, 252
- Testicular cancer**, 82
- Testicular cholesterol**, 275
- Testicular tumors**, 50
- Textured soya**, 275
- TGA**, 288-292
- TGA contents**, 289, 290
- TGA poisoning**, 290
- Thailand**, 67, 317
- Thang Shen-Wei**, 35, 37
- Thanksgiving Day dinner**, 250, 252
- Thebes**, 17
- Theobroma oil**, 57
- Theramenes**, 2
- Theseus**, 8
- Thirst**, 10
- Thorn**, 285
- Thoy**, 17
- Thracians**, 39
- Thromboembolic disease**, 318
- Thujone abortifacient**, 278
- Thyme**, 242
- Tiberius**, 5
- Tiberius Claudius Nero**, 5
- Tick repellent**, 238
- Timothy**, 192
- Tineacide**, 240
- Titus Ollius**, 3
- TMP**, 297, 298
- Tobacco**
- capsidiol, phytuberin, 171
  - cyst nematode, 192
  - induced resistance, 173
  - insect resistance, 162
  - masticatories, 369
- Tobacco (continued)**
- nightshade family, 248
  - phytoalexins, 170, 251
  - resistance w/age, 163
  - rutin in snuff, 273
- Tomato**
- herbicide bioassay, 221
  - neem nematode bioassay, 197
  - nematode bioassay, 195
  - nightshade family, 287
  - nitrate content, 292
  - phytoalexin response, 329
  - phytoalexins, 171, 172, 251
  - terpenoid nematicides, 193
  - Thanksgiving Day dinner, 252
  - tomatine, 162
  - tomatine biological properties, 295
  - vines, 248
- Tonic**, 44-49, 68
- Tonic and diuretic prep**, 47
- Tonic prep**, 45
- Tonics**, 44, 49, 68
- Toothache**, 68
- Toothpaste**, 44
- Topical**, 44, 46-49, 57, 60, 69, 296
- Topical analgesic**, 46
- Topical anesthetic**, 46, 49
- Topical antibiotics**, 69
- Topical parasiticide**, 47
- Topical protectant**, 48
- Topical PUVA**, 296, 346
- Total psoralens**, 284
- Toxic bacillary dysentery**, 79
- Toxic furanoterpenoids**, 305
- Toxic glycoalkaloids**, 290
- Toxic psychosis**, 105
- Toxic sweet potatoes**, 306
- Toxic weed seeds**, 266
- Toxicosis**, 326
- Toxins**, 7, 244, 248, 253, 295, 304-306, 330, 331, 370
- Toxoplasma**, 117
- Toxoplasmosis**, 118
- TPA**, 259
- Tracheorelaxant**, 279, 281, 283
- Traditional medicine**, 36, 38, 43, 49, 52, 57-60, 62, 76-79, 81, 89, 117, 371
- Tranquilizer**, 48, 55, 79, 80
- Transitional bogs**, 16
- Transkei**, 323, 324

- Tree nuts, 316  
Trematodes, 117  
Trembles, 248, 249  
Trembling, 108, 248  
Tremorgenic mycotoxins, 293  
Trichomes, 162  
Trichomonads, 242  
Trichomonicide, 244  
Trimmed celery, 299  
Trojan, 8  
Tropane alkaloid, 49, 50, 53, 55, 77, 79, 266  
Tropane types, 86  
Trophozoite, 140  
Tropical rain forests, 34, 78, 217  
True resins, 24, 27  
Truth serum, 10  
Trypanads, 242  
Trypanocidal activity, 123, 124, 129, 132, 148, 244  
Trypanosome, 122, 137  
Trypanosomiasis, 118  
Trypomastigote, 129, 140  
Tubular nephrosis, 305  
Tulsi, 193  
Tumors  
    antitumor, 213  
    cecum, 312  
    digestive tract, 323  
    esophogeal, 323  
    flavonoids, 274  
    gallbladder and bile ducts, 312  
    genital, 297  
    Jívaro Indians, 68  
    limonene, antitumor, 241  
    liver, 312  
    liver, lungs, gallbladder, 312  
    lung, blood vessel, 308  
    lungs, 312  
    PUVA therapy, 296  
    treatment, testing, 82  
    vincristine, 50  
Turkestan, 98  
Turkey, 8, 252, 302, 313, 319  
Turkey poult, 319  
Turkey X, 313  
Turnip root, 255  
Turpentine oil, 47  
Turpentine weed, 269  
Tyrosine protein kinase, 276  
Uganda, 98  
Ulcerative amoebiasis, 119  
Ulcerogenic, 240  
Ulcers, 2, 47  
Ultraviolet radiation, 296, 297, 327  
Uncomplicated falciparum malaria, 131  
UN Food and Agriculture Organization, 316  
Unguents, 18, 30  
U.K., 266, 306  
U.S. Environmental Protection Agency, 260, 331  
United States Food and Drug Administration, 331  
United States Surgeon General, 271  
Urinary anti-infective, 48  
Urinary antiseptic, 47  
Urinary estrogen, 275  
Urogenital infections, 117  
USAID, 373  
USDA, 1, 185, 205, 237, 238, 241, 245, 288, 371  
Uterine, 45  
Uterine antispasmodic, 45, 49  
Uterine bleeding, 64  
Uterine contractions, 64  
Uterine sedative, 49  
Uterotonic, 242, 244, 279  
UV-exposed skin, 297  
UV light, 250, 296, 298  
UV radiation, 167  
UVA, 296  
UVB irradiation, 296  
Vaginal douches, 137  
Valdivia Culture, 42  
Valerian oil, 49  
Vasoconstriction, 35  
Vasoconstrictor, 45, 48, 242, 244, 281  
Vasoconstrictor action, 281  
Vasopressor, 35, 278  
Vegetables, 68, 241, 248, 253, 254, 256-258, 260, 264, 267, 270, 271, 274, 291, 292, 326, 332, 370  
Velvet bean, 266  
Velvetleaf  
    food contamination, 266  
    herbicide bioassay, 214, 221, 222  
    seed, 212

- Vermicide, 281, 283  
 Vermifuge, 279  
 Verrucolytic, 240  
 Vervet monkeys, 324  
 Very low density lipids, 276  
 Veterinary medicine, 145  
 Vetii, 4  
 Vetispiranes, 288  
 Vibrio, 242  
 Vibriocide, 240  
 Village medicine men, 55  
 Vinegar, 2  
 Viola, 239  
 Viper venom, 6  
 Viral diseases, 69, 371  
 Viral pneumonia, 307  
 Virgil, 12  
 Viricide, 237, 240, 244, 278, 279  
 Viruses, 85, 142, 167, 169, 171, 242  
 Vitamin  
     A, 57  
     C, 47, 272  
     E, 133  
     flavonoids, 329  
     K, 60, 62  
     P, 272  
     in plants, 244  
 Vitiligo, 296  
 VLDL, 276  
 Vomiting, 2, 4, 5, 248, 277, 290, 317,  
     321  
 Vulvovaginitis, 318  
 Walking ability, 68  
 Walnuts, 117, 255  
 Water intoxication, 277  
 Wavelength, 296  
 Waxes, 76  
 Wayus, 69  
 W'bt, 20  
 Weak pulse, 10  
 Welsh, 11, 335  
 Western yew, 83  
 West Indies, 78  
 Wheat  
     BOA, DIBOA, 212  
     germ agglutinin, 199  
     germ oil, 49, 62  
     herbicide bioassays, 210, 222  
     hypcholesteremics, 60  
     mycotoxin contamination, 316  
 Wheat (continued)  
     nervous ergotism syndrome, 293  
     seed gall nematode, 186  
     useful products, 49  
 White cabbage, 256  
 White clover, 166, 171  
 White Currant, 268  
 White mustard seed, 255  
 White potato, 248, 287  
 White snakeroot, 248-250  
 Whole leaf, 45, 57  
 Wild cherry bark, 48, 55  
 Wild chimpanzees, 145  
 Wild false morel, 308  
 Wild garlic, 277  
 Wild lettuce, 285  
 Wild onion, 277  
 Wild rye, 212  
 Wildlife, 322, 329  
 Wilm's tumor, 50  
 Wine  
     *Belladonna*, 9  
     ephedron in, 35  
     flavonoid intake, 264  
     henbane, 11  
     monkshood, 8  
     natural toxicant exposure, 248  
     palm, 18, 24  
     palm w/spices, 21  
     Thanksgiving Day dinner, 252  
     tyramine, blood pressure, 251  
 Winter rye, 211  
 Wintergreen oil, 46  
 Witch hazel extract, 46  
 Withdrawal symptoms, 108  
 Wolf killer, 8  
 Wolfsbane, 8  
 Wood pitch, 28  
 Wood tar, 24, 27, 28  
 Worms, 68, 143, 146  
 Wormseed, 45, 281  
 Wormseed oil, 45  
 Wormwood, 117, 143  
 Wounding, 169, 214, 266, 289  
 Wounds, 40, 68, 69, 370  
 Xenophon, 4  
 X-radiation, 273  
 Yams, 68, 80  
 Yarrow, 44, 277, 278

Yarrow oil, 44  
Yellow jaundice, 69  
Yellow rain, 314  
Yemen, 98, 99, 104, 105, 109, 110

Yerba santa syrup, 46  
Yew, 11, 83  
Zebra mussel, 241