



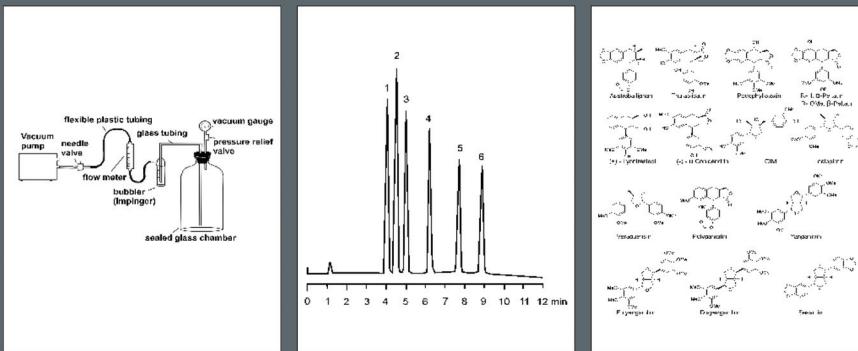
Isolation, Identification and Characterization of Allelochemicals/Natural Products

Editors

Diego A. Sampietro
Cesar A.N. Catalan
Marta A. Vattuone

Series Editor

S.S. Narwal



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Preface

Allelopathy is a newly emerging multidisciplinary field of agricultural research. Till now, a lot of allelopathy research work has been done in various fields of Agriculture and Plant Sciences. However, no standard methods are being used by various workers due to lack of compendium on the techniques, hence, the results obtained are not easily comparable with each other. This situation has caused a lot of problems to researchers working in underdeveloped/third world countries in small towns, where library and research facilities are not available. Therefore, to make available the standard methods for conducting allelopathy research work, this multi-volume book has been planned, with one volume each for each discipline. In all the conferences held worldwide since 1990's, a need has always been felt for a Manual of Allelopathy Research Methods. Hence, Prof. S.S. Narwal has planned this multivolume book **Research Methods in Plant Sciences : Allelopathy**. The objective of this book series is to provide research workers, this information about various methods, so that they can conduct research independently. The methods have been described in a simple manner.

Discovery of new bioactive compounds from natural products/secondary metabolites has played a major role in the development of organic chemistry. The ecological role of these allelochemicals/natural products as herbicides, insecticides and fungicides for ecological/organic sustainable agriculture has recently drawn great attention, due to increasing public concern against synthetic pesticides. Current researches on natural products emphasize not only the need of structural elucidation of the isolated compounds, but also of their natural biological function and possible utility for human purposes. These concerns require reliable protocols for such studies, which sometimes are not available worldwide. In allelopathy research, identification of allelochemicals is always required. Majority of researchers in under developed/third world countries, do not identify such allelochemicals, mainly because of non-compilation of such methods in book form.

This book is divided into three sections:

Section I. Sample Collection, Handling and Storage: It includes three chapters: Allelopathy and Allelochemicals, Soil, Air and Water Samples and Plant Sampling and Sample Preparation.

Section II. Isolation, Identification and Structural Elucidation : It has eight chapters: Colorimetric Reactions, TLC and PC, HPLC and LC- MS, Chromatography and Spectroscopy of Alkaloids, GC and GC-MS of Terpenoids, GC and GC-MS for Non-volatile Compounds, Spectrometry: Ultraviolet and Visible Spectra, Spectrometry: Infrared Spectra.

Section III. Biological Activity of Natural Products : It (has six Chapters: Bioassays with whole plants and plant organs, Bioassays on Plants: Plant cells and organelles, Bioassays on Microorganisms: Antifungal and antibacterial activities, Bioassays for Antioxidant, Genotoxic, Mutagenic and Cytotoxic activities, Bioassays: Inhibitors of insect chitin-degrading enzymes and Bioassays: insect behaviour, development and survival.

Section IV. Appendices: Abbreviations, Chemical Formulae and Molecular Weight of Solvents and Reagents, Molecular Weight of Organic Compounds.

This book will serve as a ready reference in the laboratory or class room and probably provide solutions to many problems of isolation, identification and characterization of allelochemicals/natural products. It will be particularly useful for UG and PG students pursuing this field, as well as for organic chemists.

We are indebted to all the contributors, who have actually used all these methods in their fields of specialization for the last 10 to 25 years and by accepting the challenging task of presenting the procedures of various methods in a very simple language, easily understood by beginners.

We would appreciate receiving valuable suggestions from students and researchers, to enable us to make further improvements in future editions of this book, so that it is more useful and meaningful.

May 28, 2008

Editors

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SECTION

I

Sample Collection, Handling and Storage

Allelopathy and Allelochemicals

S.S. Narwal^{1*} and D.A. Sampietro²

1. INTRODUCTION

Allelopathy refers to any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (Narwal, 1994). It has been established that allelopathy offers great potential to (a) increase agricultural production (food grains, vegetables, fruits and forestry), (b) decrease harmful effects of modern agricultural practices [multiple cropping, leaching losses from N fertilizers, indiscriminate use of pesticides (weedicides, fungicides, insecticides and nematicides), tolerant/resistant biotypes in pests] on soil health/productivity and on environment and (c) maintain soil productivity and a pollution-free environment for our future generations. It is likely that in the near future allelopathy will be used in crop production, crop protection, agroforestry and agrohorticultural practices in developed and developing countries. Allelopathy may become one of the strategic sciences to reduce the environmental pollution and to increase agricultural production in sustainable agriculture of the 21st century. Allelopathy provides the basis for sustainable agriculture, hence, currently allelopathy research is being done in most countries worldwide and is receiving more attention from agricultural and bioscientists.

Allelopathy is a new field of science and the term 'Allelopathy' was coined by Prof. Hans Molisch, a German plant physiologist in 1937. Therefore, till now

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there is no comprehensive book on Methodology of Allelopathy Research and is causing problems to researchers working in underdeveloped/third world countries, in small towns without library facilities. Therefore, to make available the standard methods for conducting allelopathy research independently, this multivolume book has been planned. Since allelopathy is a multi-disciplinary area of research, individual volumes have been planned for each discipline.

2. ALLELOCHEMICALS

Allelochemicals are secondary substances, biosynthesized from the metabolism of carbohydrates, fats and amino acids and arise from acetate or the shikimic acid pathway. These are biosynthesized and stored in the plant cells and do not affect the cell activities. However, after their release from the plant cells (through volatilization, leaching root exudates and decomposition of biomass), these allelochemicals start influencing the organisms (plants, pathogens, insect pests, etc.), when they come in contact. Rice (1984) indicated that allelopathic agents influence the plant growth through following physiological processes: (a) cell division and cell elongation, (b) phytohormone induced growth, (c) membrane permeability, (d) mineral uptake, (e) availability of soil phosphorus and potash, (f) stomatal opening and photosynthesis, (g) respiration, (h) protein synthesis and changes in lipid and organic acid metabolism, (i) inhibition of porphyrin synthesis, (j) inhibition or stimulation of specific enzymes, (k) corking and clogging of xylem elements, stem conductance of water and internal water relations and (l) miscellaneous mechanisms. Most of these physiological and biochemical processes are responses of cells to various allelochemicals.

2.1 Nature of Allelochemicals

Rice (1984) has divided these compounds into 14 chemical categories: (a) cinnamic acid derivatives, (b) coumarins, (c) simple phenols, benzoic acid derivatives, gallic acid and protocatechuic acid, (d) flavonoids, (e) condensed and hydrolysable tannins, (f) terpenoids and steroids, (g) water soluble organic acids, straight chain alcohols, aliphatic aldehydes and ketones, (h) simple unsaturated lactones, (i) long chain fatty acids, (j) naphthoquinones, anthraquinones and complex quinones, (k) amino acids and polypeptides, (l) alkaloids and cyanohydrins, (m) sulfides and mustard oil glycosides and (n) purines and nucleotides. However, Putnam and Tang (1986) grouped these chemicals into 11 classes: (a) toxic gases, (b) organic acids and aldehydes, (c) aromatic acids, (d) simple unsaturated lactones, (e) coumarins, (f) quinines, (g) flavonoids, (h) tannins, (i) alkaloids, (j) terpenoids and steroids, and (k) miscellaneous and unknown.

Rice (1984) outlined the following factors which affect the amount of allelochemicals produced: (a) radiation, (b) mineral deficiencies, (c) water stress, (d) temperature, (e) allelopathic agents, (f) age of plant organs, (g) genetics, and (h) pathogens and predators.

3. ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF ALLELOCHEMICALS

Allelochemicals are an integral part of allelopathy research. Hence, allelopathy research is not complete till the allelochemicals present in the experimental conditions are isolated, identified and characterized. Hence, the knowledge of chemistry methods is required. The preceding section 2.1 shows that allelochemicals belong to several distinct chemical classes and consequently the techniques for their isolation, identification and characterization are also different.

Discovery of new bioactive compounds from natural products/secondary metabolites has played a major role in the development of organic chemistry. The ecological role of these allelochemicals/natural products as herbicides, insecticides and fungicides for ecological/organic sustainable agriculture has recently drawn great attention, due to increasing public concern against the use of synthetic pesticides. Current researches on natural products emphasize not only the need for structural elucidation of the isolated compounds, but also for their natural biological function and possible utility for human purposes. These concerns require reliable protocols for such studies, which sometimes are not available worldwide. Hence, this book has been written to provide consolidated information about the isolation, identification and characterization of allelochemicals/natural products.

SUGGESTED READINGS

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