UNIVERSITY OF CALIFORNIA

San Diego

Natural Products Chemistry of Burrowing Sponges of the Genus Siphonodictyon

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Oceanography

by

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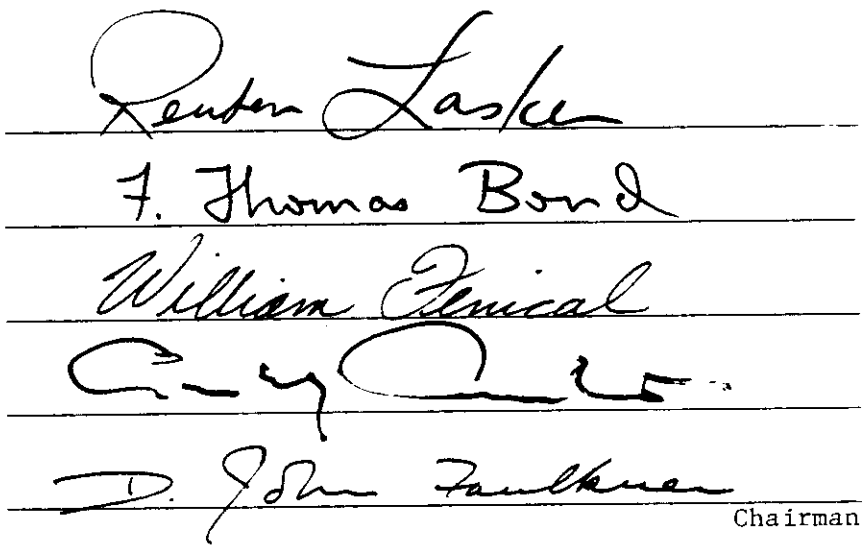

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FIELDS OF STUDY

Major Field: Marine Natural Products Chemistry

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ABSTRACT OF THE DISSERTATION

Natural Products Chemistry of Burrowing Sponges

of the Genus Siphonodictyon

by

Brian William Sullivan

Doctor of Philosophy in Oceanography

University of California, San Diego, 1985

Professor D. John Faulkner, Chairman

Sponges of the genus Siphonodictyon belong to a small group of sponges which burrow into limestone substrata. Siphonodictyon sponges have the unique ability to burrow deep inside living coral heads, leaving only the oscular chimneys exposed. This thesis reports the isolation and structural elucidation of seven novel secondary metabolites from eighteen samples of Siphonodictyon collected from five different locations.

Fifteen samples of two forms of Siphonodictyon coralliphagum were collected from Belize and the Bahamas. Four sesquiterpene quinol metabolites were isolated from S. coralliphagum forma typica while only two of these metabolites were present in S. coralliphagum forma

tubulosa. Two additional samples of S. coralliphagum from Ponape and Kwajalein were found to contain three closely related compounds. Also, one metabolite was found to be common to both the Caribbean and Pacific samples.

An undescribed Siphonodictyon species, collected from Palau, produced a guanidine-containing compound that is unrelated to the sesquiterpene quinol metabolites of S. coralliphagum.

Results from coral toxicity assays conducted by Leith Webb and calcium chelation assays were used to propose ecological roles for the Siphonodictyon metabolites.

Chemotaxonomy is often suggested as a useful tool in classifying sponges. This technique is evaluated for Siphonodictyon based on the results of this chemical study.

Chapter 1

Introduction

A variety of marine plants and invertebrate animals are known to excavate hard, calcareous substrata by chemical or mechanical means, or by a combination of both. Much has been written concerning the systematics, distribution, ecology and physiology of these organisms, as well as the geological, chemical and biological effects they cause.[1] These organisms contribute to coastal erosion and sedimentation, influence the calcium balance of the sea, and often control the structure of marine communities where calcium carbonate-producing organisms are dominant.[2]

The mechanisms by which organisms other than sponges penetrate calcareous substrata have received much attention, but investigators disagree on the details of the mechanisms. Most of the work has been observational and has defined whether the mechanisms are chemical or mechanical, but little has been done to resolve the specific details of the mechanisms.[3,4] Many investigators have stated that chemicals are involved in the mechanisms, but few studies have been aimed at identifying the specific chemicals. Organic and inorganic acids, chelators, and enzymes have all been suggested as possible candidates.[5,6]

A lack of agreement exists concerning the use of the terms "boring" and "burrowing" to define penetration of hard calcium carbonate material by organisms. In this thesis, "boring" refers to the penetration into the calcareous exoskeleton of a prey organism by a

predator for the purpose of obtaining food. "Burrowing" implies excavation of a space by an organism for the purpose of living part or all of its life cycle within the substratum.[7]

Burrowing algae produce an etching pattern that resembles the pattern remaining when calcium carbonate crystals (Iceland spar) have been treated with inorganic acids.[8] The secretion from the pallial glands of the rock burrowing bivalve Lithophaga lithophaga was found to have a pH of 6.5 indicating that it did not contain a free acid.[9] Histochemical tests demonstrated that the secretion was a neutral mucoprotein and indicated the presence of soluble calcium in the pallial gland. This was interpreted as evidence that the neutral mucoprotein was capable of chelating calcium and was involved in the burrowing mechanism.[9] The hypothesis of the chelating ability of the neutral mucoprotein in L. lithophaga has been perpetuated in the literature even though the mucoprotein was not tested specifically for calcium chelating ability.

Studies of the boring mechanism of the gastropod Urosalpinx cinerea are the most detailed and complete. The boring action consists of alternating periods of chemical dissolution and mechanical rasping. The chemical process involves the accessory boring organ (ABO) which secretes a viscid acid substance with a pH of 3.8-4.1.[10] The patterns of dissolution etched by the ABO secretion and those produced artificially by HCl and EDTA are very similar.[6] Carbonic anhydrase activity has been demonstrated on the tip of the ABO[10,11] of U. cinerea. Carbonic anhydrase activity has also been found in the boring gastropod Purpura (Thais) lapillus.[12] From this work, the chemical mechanism of shell dissolution by boring gastropods is believed to involve a combination of an

inorganic acid, enzymes, and chelating agents.[13]

Burrowing sponges play an important role in the tropical reef environment as well as in temperate coastal waters.[14] In temperate waters clionid sponges burrow into clam and oyster shells causing extensive damage, and sometimes death. This represents an important economic problem to molluscan fisheries.[15]

In the tropics, burrowing sponges can account for up to 90% of the total burrowing in reef corals.[16,17] Most burrowing sponges attack the dead basal portion of reef corals, severely weakening the coral and making it susceptible to physical damage.[18]

Since sponges remove 90-97% of the calcium carbonate as chips,[2,19] sponge bioerosion produces large amounts of sediment, with estimates ranging from 0.25 to 25.0 $kg/m^2/yr$.[20,21] The fine grained sediment produced by sponges of the genus Cliona, accounts for 2-3% (Persian Gulf and North Adriatic Sea) to 30% (Fanning Island) of the total sediment.[22]

The burrowing mechanism employed by sponges was debated for many years but is now accepted to be a combination of chemical and mechanical processes.[2,15] The mechanical removal of the substratum is carried out by etching cells. The etching cells are characterized by a prominent rough endoplasmic reticulum that extends to the distal portion of the cells, a golgi apparatus, abundant mitochondria, lysosomes, phagosomes, small vacuoles, and many cytoplasmic bodies.[2,23] This indicates the cells have the capability for synthesis of proteins, secretion of substances into extracellular spaces, absorption of substances from

extracellular spaces, and digestion of absorbed substances intracellularly.[15] A unique feature of the etching cells is the presence of apical filopodia which interconnect to form basket-like structures. A secretory product, possibly synthesized by the etching cells, is concentrated on the filopodia causing a localized dissolution of the substratum. The filopodial basket cores out characteristicaly shaped chips from calcium carbonate, as well as from conchiolin layers of mollusc shells. Dislodged chips are displaced by new etching cells or by other tissue components, forced into the exhalent canal system, and discarded through the oscula. Exhausted etching cells are not regenerated, but are either phagocytized or removed in the same manner as the chips. Calculations indicate these chips account for 97-98% of the total substratum removed.[2,19,24] A detailed review of the cytological mechanism of excavation by burrowing sponges has recently been published.[15]

Most of the detailed ultrastructural examinations of burrowing sponges have been performed on sponges of the genus Cliona. However, the cytological mechanism of burrowing has been found to be similar for eleven species of sponges, representing the four genera Cliona, Spheciospongia, Anthosigmella and Siphonodictyon.[23] The first detailed examination of the fine structure of the burrowing mechanism of C. celata reported a different mode of penetration by the etching cells, but it is believed that this study was hampered by the limits of resolution of light microscopy.[24,25]

The nature of the chemical or chemicals involved in the dissolution of the substratum is not known. The mechanism is thought to be similar to that of boring gastropods, possibly involving acids,

chelators, enzymes, or any combination of these.

Acids have been implicated as the primary reagent in the removal of calcium carbonate by burrowing sponges.[19] Few researchers have investigated whether acids play a role in the excavation process, and no increase in hydrogen ion concentration has been measured in burrowing sponges.[19] Since only 2-3% of the excavated calcium carbonate is dissolved and dissolution occurs in a localized area, sensitive detection techniques must be utilized to determine if an acid is responsible for the dissolution of substratum.

The implication of the involvement of carbonic anhydrase in the excavation of substratum by boring gastropods[11,12] led to the speculation that the enzyme was also active in burrowing sponges.[26] Carbonic anhydrase catalyzes the reaction $H_2O + CO_2 \rightarrow H^+ + HCO_3^-$. Solubilization of calcium carbonate could occur through regulation of the hydrogen ion concentration by this enzyme.[15] Carbonic anhydrase in seawater was found to be incapable of causing shell dissolution.[27]

Carbonic anhydrase activity was demonstrated in Cliona celata by electrometric assay.[28] The enzyme was found to be membrane- or particle-bound. Evidence for the enzyme's participation in the burrowing process was the correlation of the highest carbonic anhydrase activity with the most actively excavating sponges and inhibition of the excavation rate by a specific enzyme inhibitor. However, the gamma growth form of C. celata, which has completely excavated the calcium carbonate substratum and is no longer actively burrowing, contained 1.5 to 2.0 times more carbonic anhydrase than the actively burrowing

forms.[28] Similar results were obtained for C. delitrix and C. caribbaea.[15] It was suggested that the high enzyme concentration in the gamma growth form represented the potential for burrowing when new calcium carbonate substratum was contacted.[28] This explanation is considered unsatisfactory,[15] since it is unlikely that the sponge would produce an enzyme for future use.

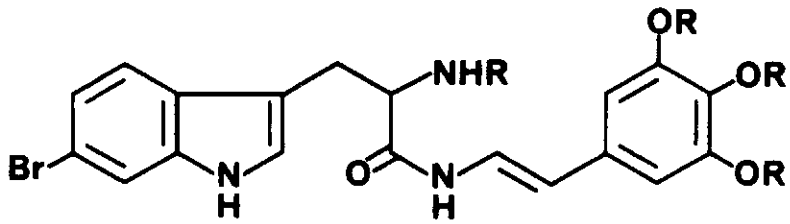
Cytochemical assays for acid phosphatase, a lysosomal marker, have shown enzyme activity on the rough endoplasmic reticulum, golgi apparatus, lysosmes, and phagosomes.[29] This suggests the enzyme is being synthesized by the endoplasmic reticulum and golgi apparatus, lysosmes are being formed, and intracellular digestion in phagosomes is occurring.[29] Acid phosphatase activity in the extracellular spaces between cell processes and on the outer membrane of cell processes was interpreted as evidence that the chemical etching agent is localized on the membranes of etching cell processes.[15] Based on these facts, a hypothesis was proposed for the chemical dissolution of calcium carbonate substratum by two mechanisms: "1) enzymatic digestion of organic components of skeleton carbonates, both intracellularly and extracellularly, via the lysosomal system and the membranes of etching cell processes; and 2) solubilization of mineral components via carbonic anhydrase regulation of hydrogen ion concentration at the etching cell membrane."[15]

Though many investigators have suggested that chelators are involved in the chemical dissolution of calcium carbonate by burrowing organisms, no evidence has been found to support this hypothesis. However, natural products produced by burrowing sponges contain functional

groups that may be capable of chelating calcium. Only two species of burrowing sponges, Cliona celata and Siphonodictyon coralliphagum have been investigated for the presence of secondary metabolites. Five compounds were isolated from C. celata as their acetylated derivatives, tetra-acetylclionamide (1),[30] hexa-acetylcelenamides A (2) and B (3),[31] penta-acetylcelenamide C (4),[32] and nona-acetylcelenamide D (5).[32] A small amount of clionamide (6)[33] was isolated and Anderson suggested that the triphenolic portion of this molecule might be a good calcium chelator, but could not show the compound's chleating ability.[34] Two other phenolic metabolites, the siphonodictyals A (7) and B (8) were isolated from S. coralliphagum.[35] No tests were performed to determine whether these compounds could chelate calcium.

In summary, the mechanical portion of the burrowing mechanism by sponges is well defined, but very little is known about the chemical mechanism. The enzymes, carbonic anhydrase and acid phosphatase, have been shown to be active in burrowing sponges, but their role in the burrowing process has not been confirmed. A flocculent secretory product is visible on etching cell filopodia in SEM micrographs, but the chemical nature of this product is unknown.

Sponges of the genus Siphonodictyon belong to a small group of sponges that burrow into limestone substrata. Most burrowing sponges, like the relatively common Cliona species, burrow into shells, rocks or dead coral. Siphonodictyon sponges have the unique ability to burrow deep inside living coral heads, leaving only the oscular chimneys exposed (Figure 1).[36] In order to survive, these sponges must be able to prevent overgrowth by coral polyps and there is indeed a 1-2 cm zone of



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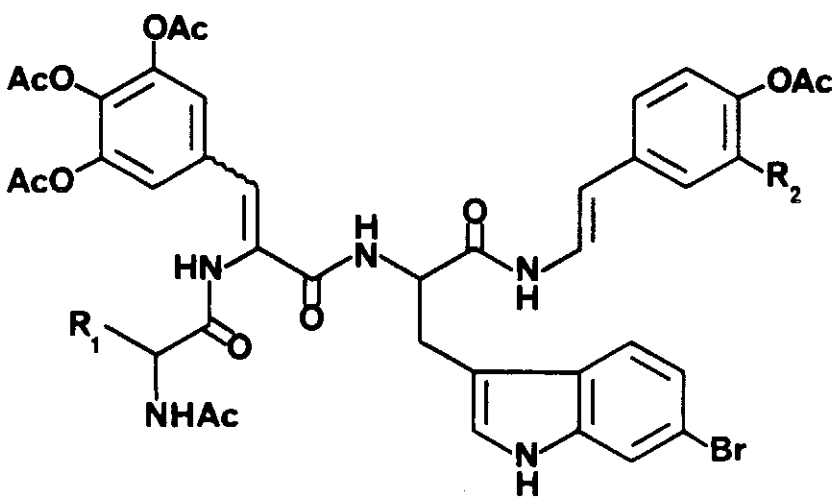
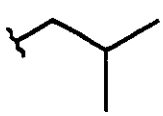


2 R_1 =  , R_2 = OAc

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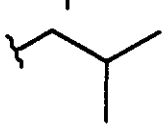
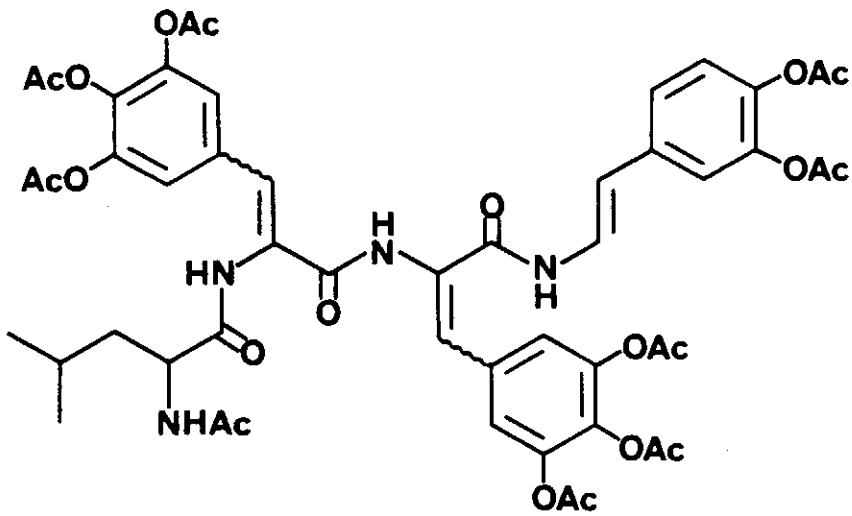
4 R_1 =  , R_2 = H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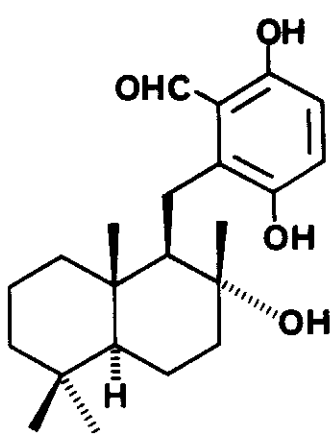
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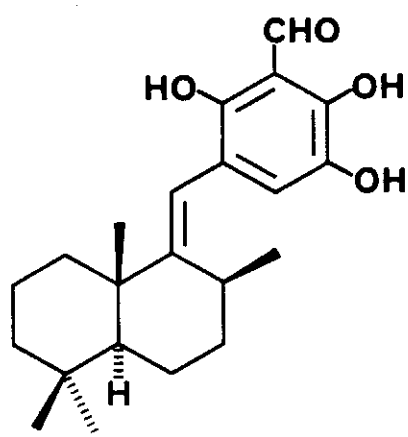
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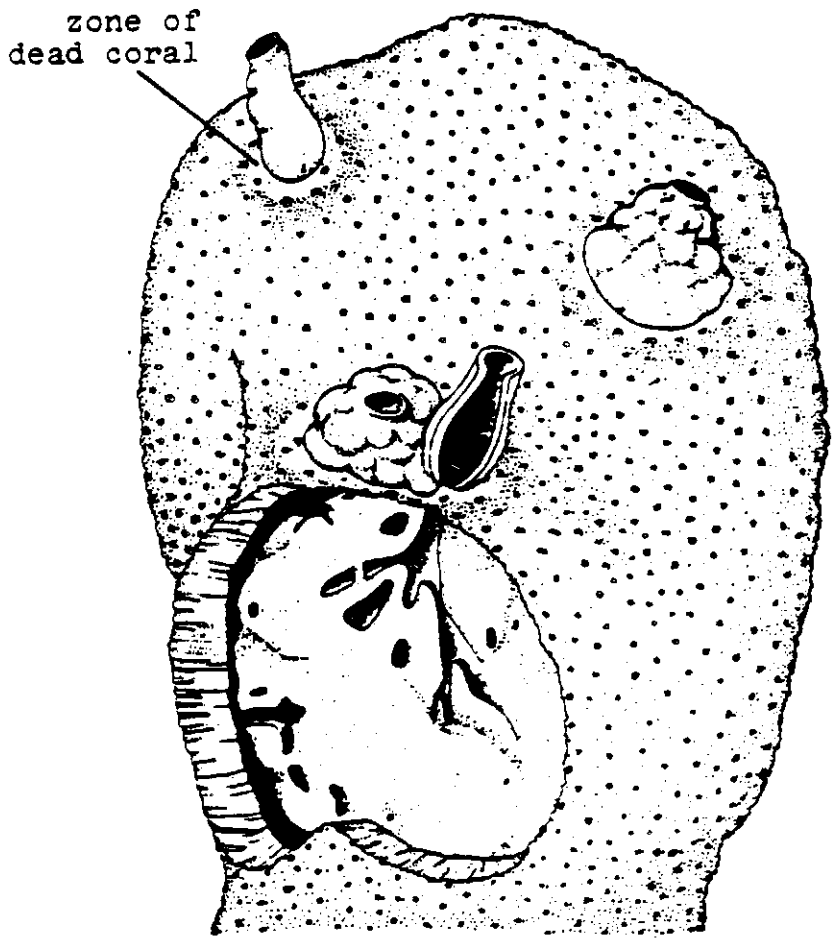


Figure 1. Cutaway view of Siphonodictyon coralliphagum growing out of Stephanocoenia michelinii (from Rützler)[36]

dead coral polyps around the base of most oscular chimneys. It has been suggested that this dead zone is maintained by the production of mucus that flows down the oscular chimney and spreads around the base.[36] I propose that the mucus may act as an efficient carrier of toxic secondary metabolites. These metabolites may also play a role in the burrowing process by chelating calcium.

The main goal of this research project was to isolate novel, biologically active, marine natural products from burrowing sponges of the genus Siphonodictyon, and to determine possible ecological roles for these compounds. Sponges of the genus Siphonodictyon were collected from five different locations: Belize, the Bahamas, Palau, Ponape, and Kwajalein. The secondary metabolites in these sponges were isolated and characterized. Only one sponge, an undescribed Siphonidictyon sp. from Palau, did not contain metabolites of the sesquiterpene quinol class. This sponge contained a guanidine metabolite.

Marine natural products chemists have begun to stress the importance of the ecological roles of the compounds produced by the organisms they study. Most secondary metabolites are considered to be involved in defensive roles such as detering predation or inhibiting the settlement of potential competitors. This field has recently been reviewed.[37] The Siphonodictyon metabolites were tested for antimicrobial activity, toxicity to coral polyps, and their ability to chelate calcium. Ecological roles for these compounds are discussed on the basis of these test results.

Chemotaxonomy is often mentioned as a useful criterion in sponge classification. The present state of the field is discussed and evaluated based both on past studies and the present work on Siphonodictyon.

Chapter 2

Sesquiterpene Quinols from *Siphonodictyon coralliphagum*

Sesquiterpene quinols and quinones, have been found in both brown algae and sponges. Sesquiterpene quinols have the general structure shown in Figure 2. Six metabolites, zonarol (9),[38] isozonarol (10),[38] zonaroic acid (11),[39] chromazonarol (12),[40] isochromazonarol (13),[40] and the chromenol 14,[41] have been isolated from the brown alga *Dictyopteris undulata*, collected from California,[39,40] Mexico[39,40] and Japan.[41]

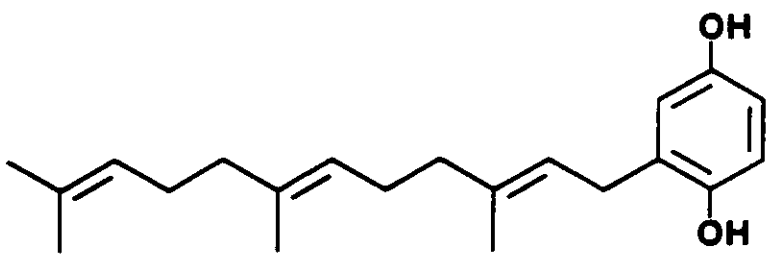
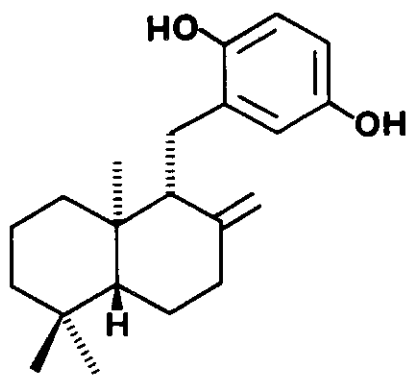


Figure 2. Farnesol hydroquinone, the hypothetical precursor of all sesquiterpene quinols.

The majority of the sesquiterpene quinols and quinones from sponges are found in three families, the Spongiidae, the Thorectidae, and the Dysideidae, all in the order Dictyoceratida. Three species of *Dysidea* (family Dysideidae) contain this class of metabolites. *Dysidea avara* was found to contain avarol (15)[42] as the major metabolite, together with avarone (16)[42] and two other minor compounds 17[43] and



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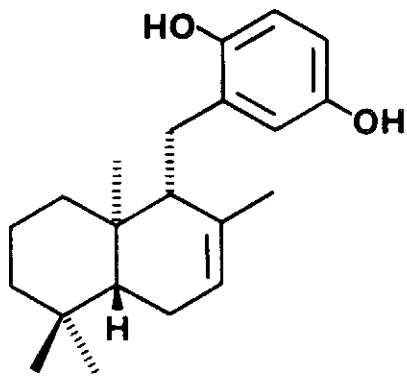

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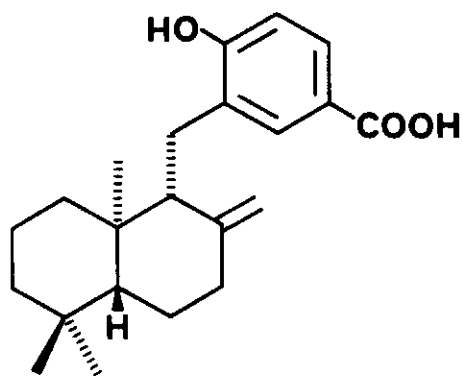

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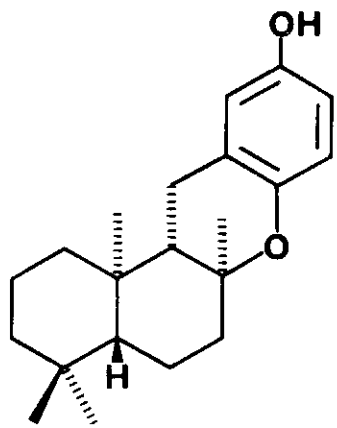

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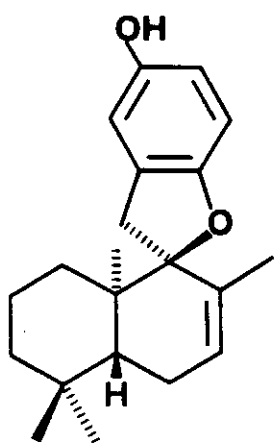

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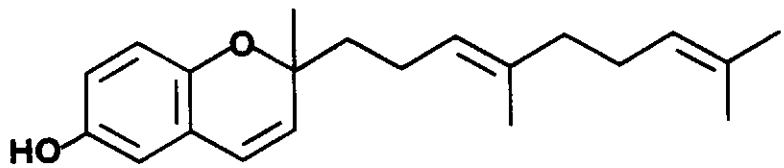

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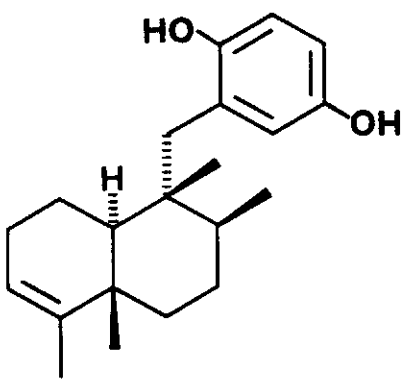

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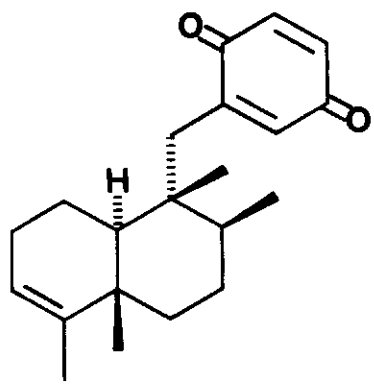

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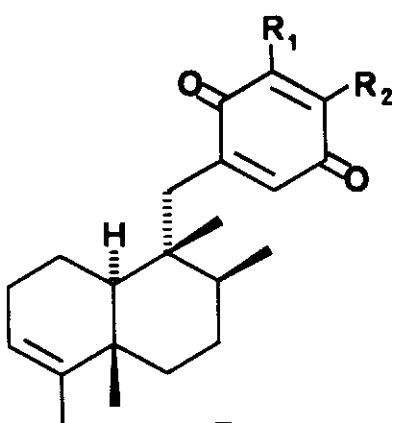

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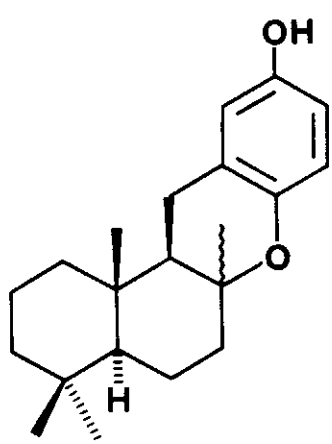


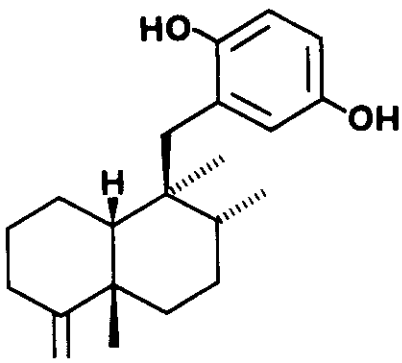
19

18,[43] all of which contain a trans-clerodane sesquiterpene skeleton. Ent-chromazonarol (19)[44] was isolated from Dysidea pallescens, while a sesquiterpene quinol containing the cis-clerodane skeleton, arenarol (20),[45] and the corresponding quinone, arenarone (21)[45] were isolated from Dysidea arenaria.

Three genera of sponges, Fasciospongia, Smenospongia and Hyrtios, in the family Thorectidae produce sesquiterpene quinols and quinones. The Australian Great Barrier Reef sponge Fasciospongia rimosa contained the five related quinones 22-26.[46] One sample of Smenospongia aurea produced aureol (27), while a second sample gave 8-epichromazonarol (28).[47] The monocyclic sesquiterpene quinol 29 was isolated from Smenospongia echina.[47] Two compounds, puupehenone (30) and puupehenone dimer (31) were obtained from Hyrtios eubamma.[48] A Hyrtios sp. from Hawaii also contained puupehenone (30) while another Hyrtios sp. from Enewetak Atoll, in the Marshall Islands, contained chloro-puupehenone (32) and bromo-puupehenone (33).[49]

In the family Spongiidae, only the sponge Hippospongia metachromia is known to produce sesquiterpene quinols or quinones. The major metabolite of this sponge has been identified as illimaquinone (34).[50]

Only two genera of sponges outside the order Dictyoceratida, Halichondria (order Halichondrida, family Halichondriidae) and Siphonodictyon (order Haplosclerida, family Adociidae), are known to produce sesquiterpene quinols or quinones. H. panicea was found to contain five unusual aromatic metabolites, the paniceins A (35), B (36), B_2 (37), B_3



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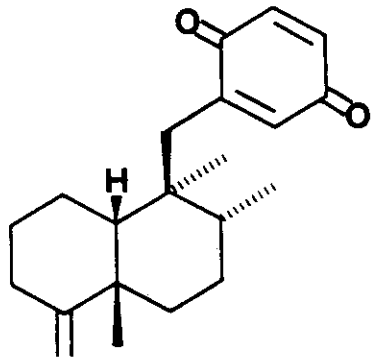

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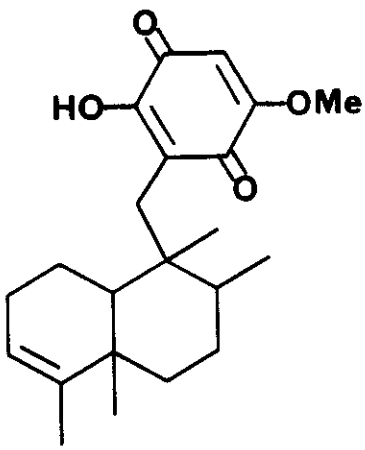

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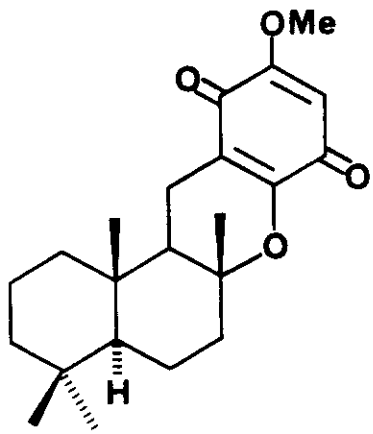

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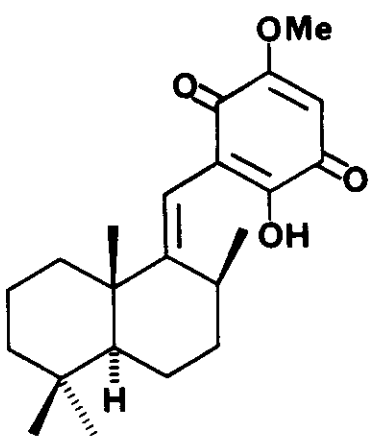

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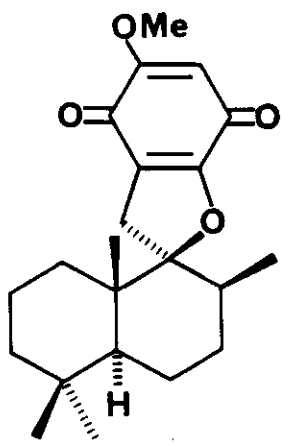

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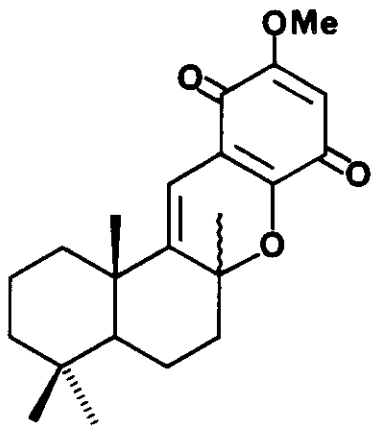

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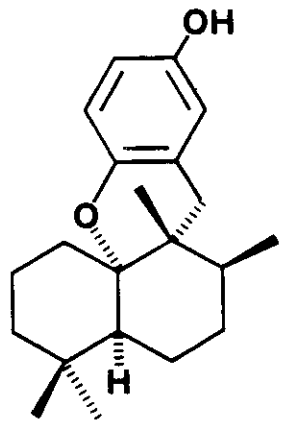

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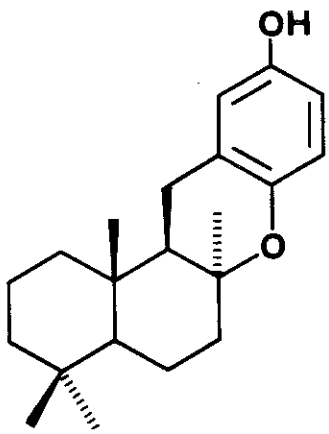

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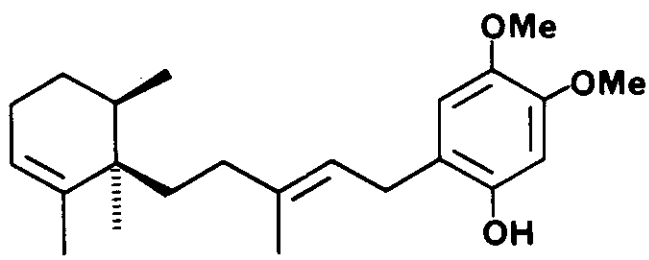

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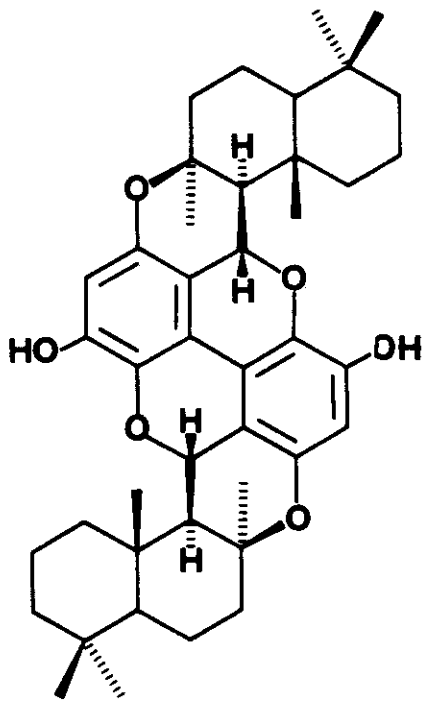

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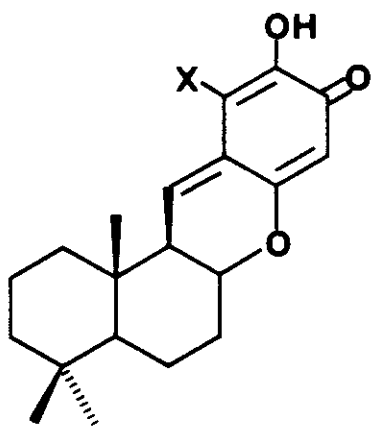
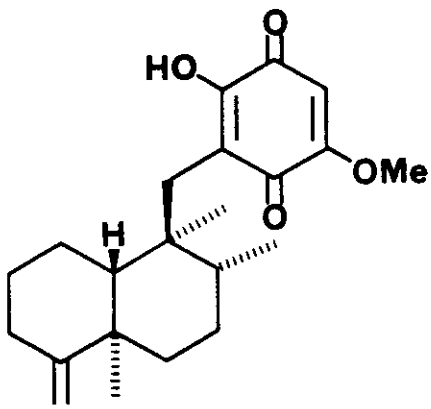


30 X = H
32 X = Cl
33 X = Br



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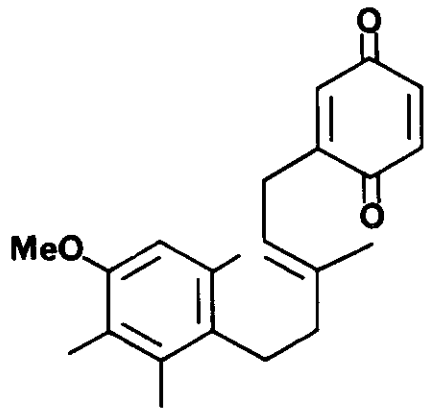

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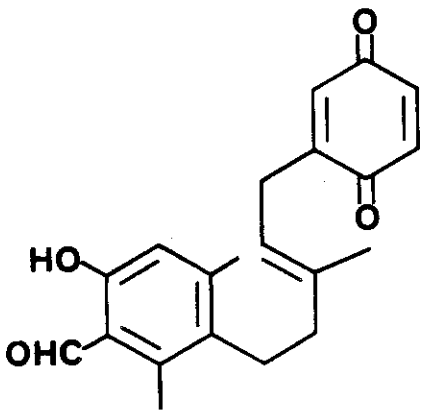

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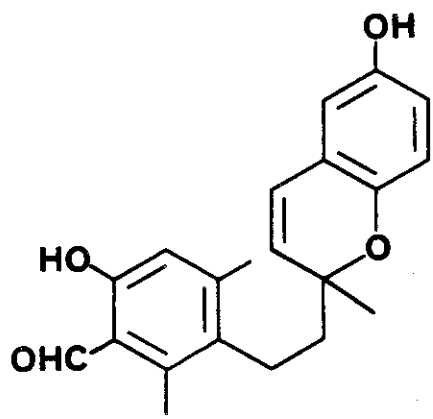

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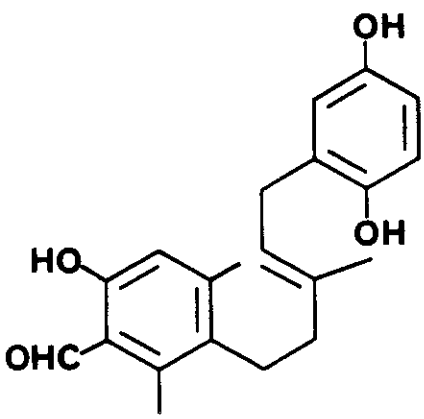

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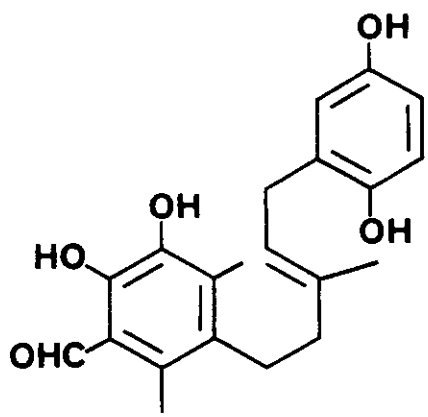


39

(38), and C (39).[51] As previously mentioned, the siphonodictyals A (7) and B (8) were isolated from S. coralliphagum. The isolation and structural elucidation of six additional Siphonodictyon metabolites is presented in this Chapter.

The structural elucidation of each metabolite is divided into two parts. The substitution pattern of the aromatic ring and the sesquiterpene portion of the molecule are elucidated independently. The phenolic nature of these compounds is characterized by the downfield shift of the aromatic carbons in the ^{13}C NMR spectrum to 140-150 ppm and a broad adsorption in the infrared spectrum centered at ~3400 cm^{-1}. An ortho-hydroxy aldehyde functionality is indicated by the ^{13}C NMR chemical shift of the aldehyde carbon at δ 198 and an infrared adsorption at 1650-1685 cm^{-1}. A benzoic acid group is indicated by a signal in the ^{13}C NMR spectrum at δ ~170 and peaks in the infrared spectrum at ~3450 and 1710 cm^{-1}. A benzylic alcohol functionality is characterized by signals in the ^{13}C NMR spectrum at δ 65 (t) and in the ^{1}H NMR spectrum at δ 4.5 (s, 2 H). The substitution pattern of the aromatic ring is determined by interpretation of the ^{1}H NMR spectrum, calculation of the expected ^{13}C NMR chemical shifts of the aromatic carbons for all possible structures, and nuclear Overhauser effect experiments.

Nuclear Overhauser effect (nOe)[52] experiments are based on proton spin lattice relaxation phenomena. An nOe is measured by the change in integrated intensity of a proton after saturation of a proximal proton. The measurement is accomplished by nOe difference spectroscopy (NOEDS)[53] which involves subtracting a control spectrum from the enhanced spectrum, leaving only signals showing an nOe in the final

spectrum. This allows enhancements of as little as 1.0% to be observed. An nOe experiment is useful in determining the proximity of protons less than 3.0 Å apart in space. This provides important information about the spatial relationship of protons that are not directly coupled. Approximate distances are determined using Dreiding molecular models.

The sesquiterpene portion of the molecules are assumed to be derived from a farnesol precursor. The number of rings in each molecule is deduced from the molecular formula and unsaturation data. Structures are assigned based on interpretation of ^{1}H and ^{13}C NMR and mass spectral data. The structures of the bicyclic ring systems can be predicted from the cyclization of a farnesol precursor to produce an intermediate cation that undergoes loss of a proton or rearrangement by 1,2-hydride and/or 1,2-methyl shifts, followed by proton loss (Figure 3). Confirmation of the structures is accomplished by ^{1}H NMR decoupling studies and comparison of the spectral data with those of known compounds.

Siphonodictyon coralliphagum[36] from the Caribbean was described as having four distinct morphological forms, forma typica, forma obruta, forma tubulosa, and forma incrustans, all belonging to one population. These sponges excavate in both living and dead coral. This chapter reports the secondary metabolites of two of the forms, forma typica, excavating in living coral, and forma tubulosa, excavating in dead coral, as well as one sample of Siphonodictyon coralliphagum from Ponape and one sample from Kwajalein.

The original work on S. coralliphagum collected from Lighthouse Reef and Glover Reef, Belize, resulted in the identification of two

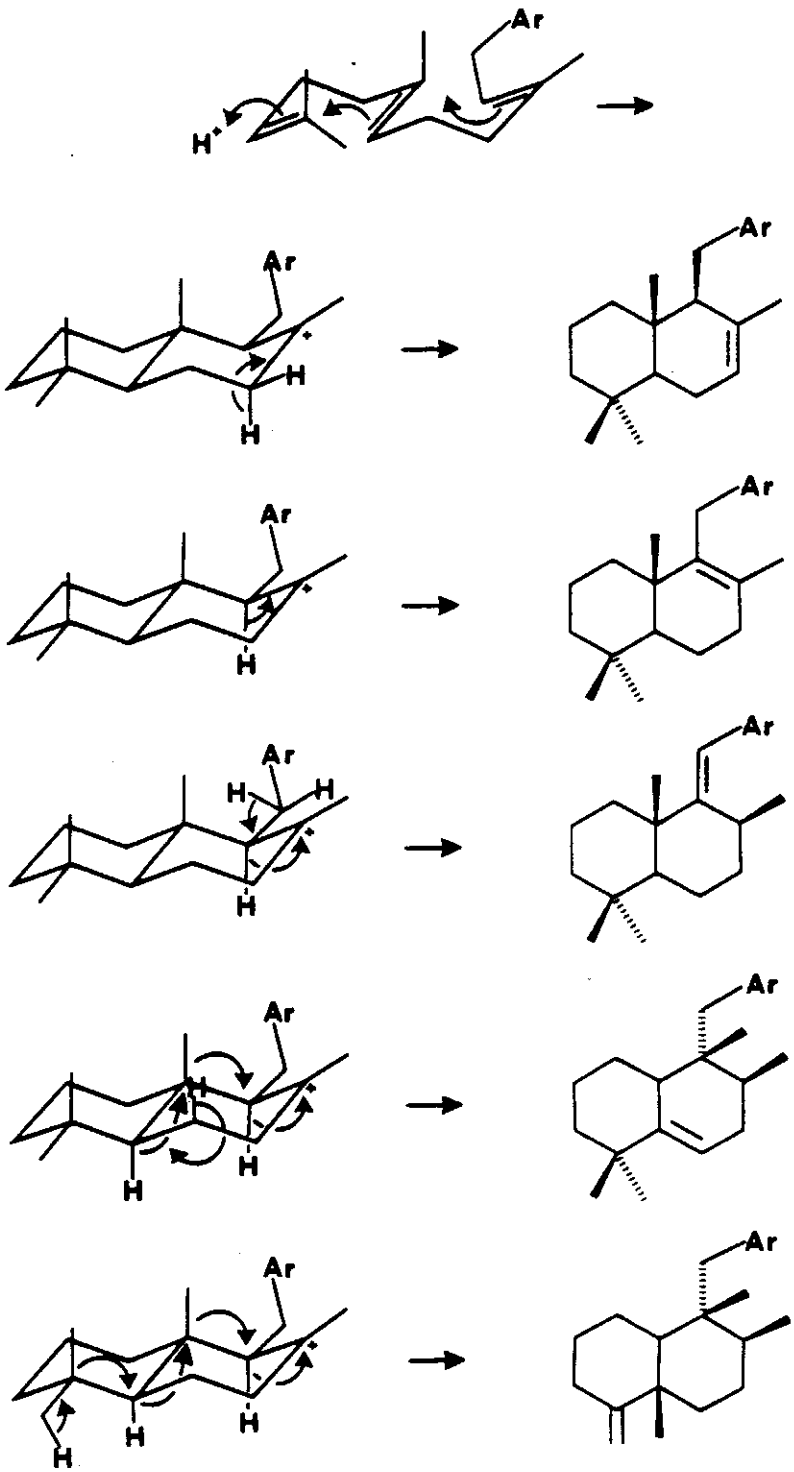
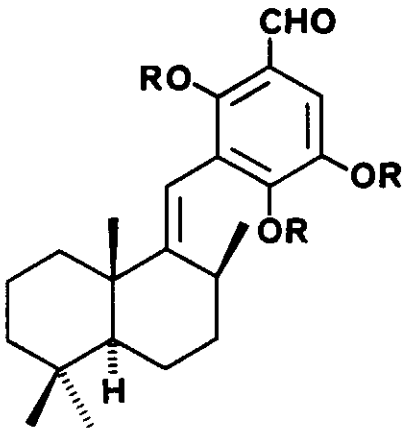


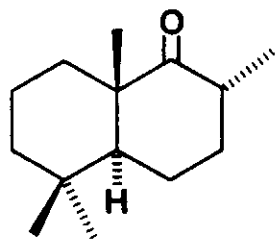
Figure 3. Cyclization and rearrangements of a farnesol precursor to produce the siphonodictyon metabolites ring systems.

metabolites, the siphonodictyals A (7) and B (40). The structure of siphonodictyal A (7) was determined by interpretation of spectral data and synthesis of a derivative. The structure of siphonodictyal B (40) is one of two possible structures based on the identification of two degradation products. Ozonolysis of trimethoxy siphonodictyal B (41) produced two compounds, the ketone 42, and the dialdehyde 43. The spectral data of ketone 42 was identical to that reported for the ozonolysis product of spongiaquinone (24).[46] The structure of the dialdehyde (43) was confirmed by synthesis from 4,5-dimethoxysalicaldehyde (44). The ketone 42 and the dialdehyde 43 can be formed from either the trimethoxy derivative of 40 or the alternative structure 8. Structure (40) was assigned to siphonodictyal B based on calculations of the expected chemical shift of the aromatic proton signal of 9,11-dihydrosiphonodictyal-B trimethyl ether (45).[35]

The structure of siphonodicytal B (8) was reassigned as a result of the following experiments. Siphonodictyal B (8) was treated with methyl iodide and potassium carbonate in acetone at 80°C for 8 hours to obtain the dimethoxy derivative 46. A series of nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiments were conducted to determine the substitution pattern on the benzene ring. An nOe was considered positive only if it could be observed in both directions. An nOe was observed between the aldehyde proton signal at δ 10.9 and both the hydroxyl proton signal at 11.42 and the methoxyl signal at 3.99. This indicated that the aldehyde was ortho to the hydroxyl group as well as to one methoxyl group. The aromatic proton signal at δ 7.01 showed an nOe to both the olefinic proton signal at 6.13 and the methoxyl



40 R = H
41 R = Me



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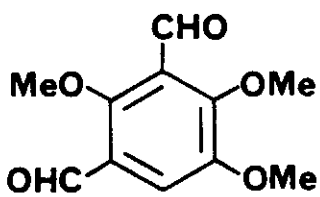

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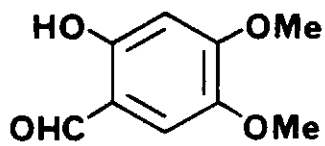

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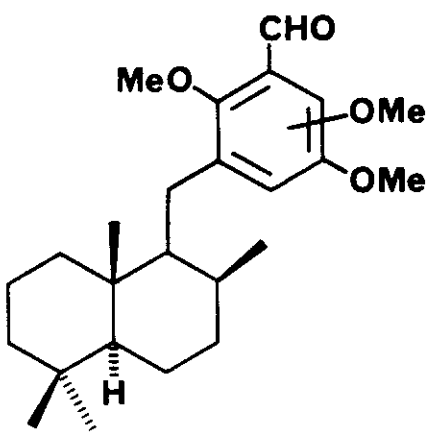

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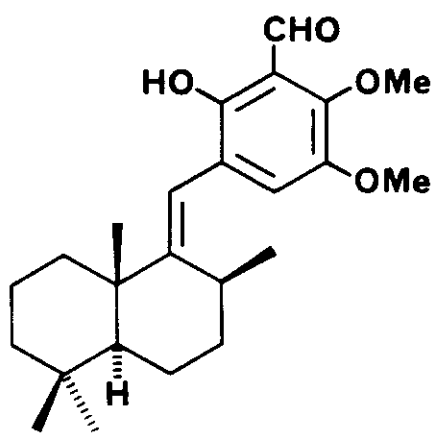


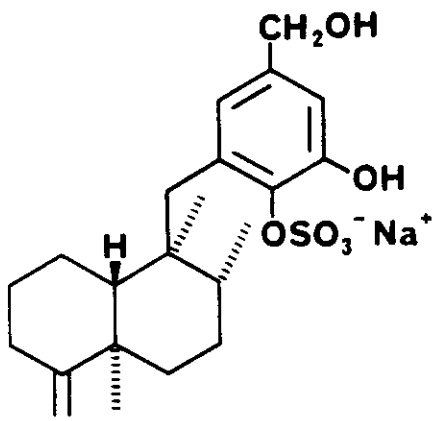
46

signal at 3.83 indicating that it was ortho to the sesquiterpene side chain and to the second methoxyl group. Only structure (46) is consistent with all of the experimental data: the structure of siphonodictyal B (8) must therefore be reassigned.

Siphonodictyon coralliphagum was recollected at Carrie Bow Cay, Belize, in November, 1983. Two forms were obtained, forma typica, which was growing in living coral with a zone of dead coral polyps around the base of the oscular chimneys, and forma tubulosa, which was growing in dead coral and was heavily fouled by coralline algae, other sponges, bryozoans, tunicates and other algae. Eight samples of forma typica (83-258 A-H) and six samples of forma tubulosa (83-256 A-E) were collected for chemical comparsions. Each sample was extracted separately and the crude extracts compared by ^{1}H NMR. Samples with identical ^{1}H NMR spectra were combined. The six samples of forma tubulosa were identical and all samples were combined. The eight samples of forma typica showed more variability. Samples A, G, and H were combined as were samples B, C, and D. Sample 83-258E was too small to analyze and 83-258F was different from the other samples. Two additional specimens of S. coralliphagum were collected from the Bahamas, one sample of forma tubulosa (84-090) and one sample of forma typica (83-185).

The ethyl acetate soluble material of methanol-water extracts of S. coralliphagum forma tubulosa (83-256 A-F and 84-090) were chromatographed on a Sephadex LH-20 column using 1:1 methanol:dichloromethane as eluant. The Belize collection yielded one major compound, siphonodictyol G (47) (0.089% dry weight). The Bahama sponge (84-090) contained siphonodictyal E (48) (0.048% dry weight) and siphonodictyol G (47)

(0.069% dry weight).



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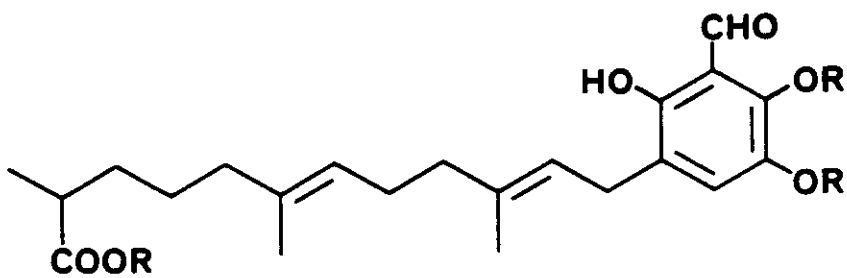


48 R = H

49 R = Me

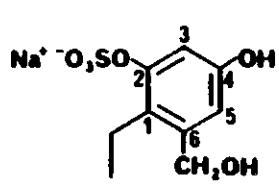
The molecular formula of siphonodictyol G (47), as determined by fast atom bombardment (FAB) mass spectrometry, is $C_{22}H_{31}O_6SNa$. The presence of a $-SO_3^- Na^+$ group was suggested by the molecular formula and bands in the infrared (IR) spectrum at 1250 and 1050 cm^{-1}. Two signals in the ^1H NMR spectrum at δ 6.83 (d, 1 H, J = 1.7 Hz) and 6.63 (d, 1 H, J = 1.7 Hz) and six signals in the ^{13}C NMR spectrum at δ 150.7 (s), 140.5 (s), 139.2 (s), 135.2 (s), 123.4 (d) and 115.3 (d) indicated that the compound contained a tetrasubstituted aromatic ring with two meta protons. A primary benzylic alcohol was indicated by signals in the ^{13}C

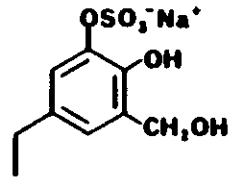
NMR spectrum at δ 64.9 (t) and in the ^{1}H NMR spectrum at δ 4.50 (s, 2 H). A broad peak in the infrared spectrum centered at 3450 cm^{-1} confirmed the presence of hydroxyl groups.

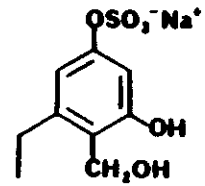
Ten non-symmetrical structures (A-J) could be proposed for the aromatic portion of siphonodictyol G (47) given the four substituents, primary alcohol, hydroxyl, sulfate and sesquiterpene sidechain and two meta coupled aromatic protons. The expected ^{13}C NMR chemical shifts of the aromatic carbons of each possible structure were calculated from empirical values[54] utilizing correction terms for replacement of -OH with $-OSO_3K$[55] (Table 1). The correction terms were derived from a ^{13}C NMR investigation of nine different phenol sulfate esters. The calculated values were compared with the observed values and the absolute value of the difference in chemical shifts, summed over all aromatic ring carbons, was used as an indicator of agreement. Structure J showed the best agreement with observed values and the substitution pattern was confirmed by NOEDS experiments. An nOe was observed between the benzylic alcohol protons at δ 4.50 and both aromatic signals at 6.63 and 6.83. Only structure J is consistent with all of the data.

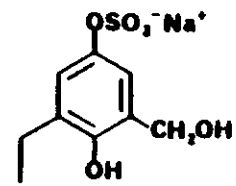
The ^{1}H NMR spectrum of siphonodictyol G (47) showed the presence of one secondary and two tertiary methyl groups, an exocyclic methylene and two benzylic protons. This indicated that the sesquiterpene portion of the molecule had a rearranged drimane skeleton as shown. Comparison of the ^{1}H and ^{13}C NMR data of siphonodictyol G (47), illimaquinone (34),[50] palauolide (50),[56] and siphonodictyoic acid (51) (whose structural elucidation appears later in this chapter, Table 2) confirmed the assignment and suggests they all have the same relative stereochemistry.

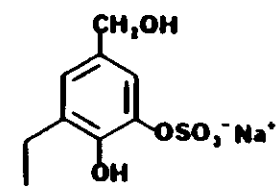
Table 1. Comparison of Calculated ^{13}C NMR Chemical Shifts of Possible Aromatic Structures with Observed Values for Siphonodictyol G



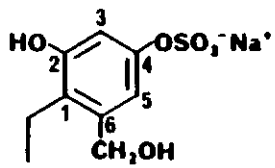


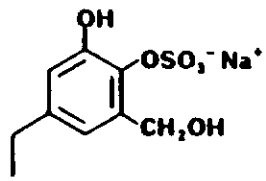


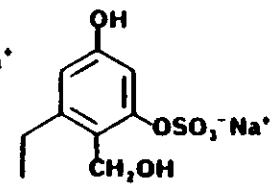


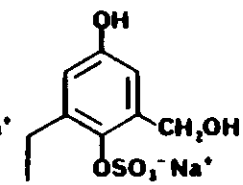


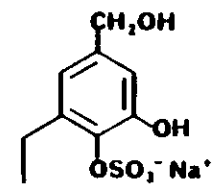
	A	B	C	D	E	Observed Values
C_1	129.0	138.4	146.1	133.0	134.4	139.2 s
C_2	152.2	121.7	114.0	121.7	126.6	123.4 d
C_3	108.0	135.5	151.6	143.9	136.2	140.5 s
C_4	154.2	145.0	106.4	119.5	121.4	115.3 d
C_5	112.9	129.4	156.0	129.4	139.9	150.7 s
C_6	143.1	126.6	125.5	152.1	149.1	135.2 s
$\Sigma(\lvert obsd-expd \rvert)$	43.1	36.7	51.3	22.7	15.3	



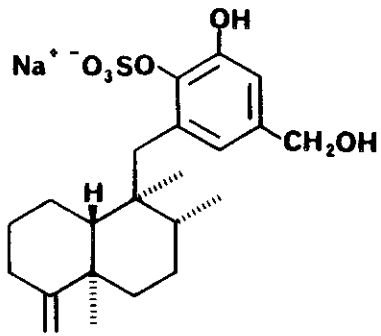


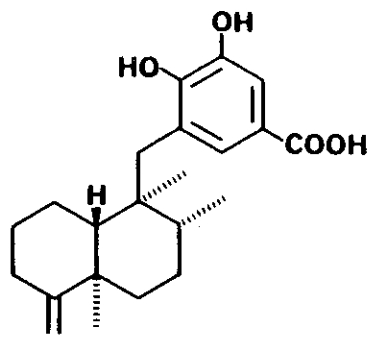


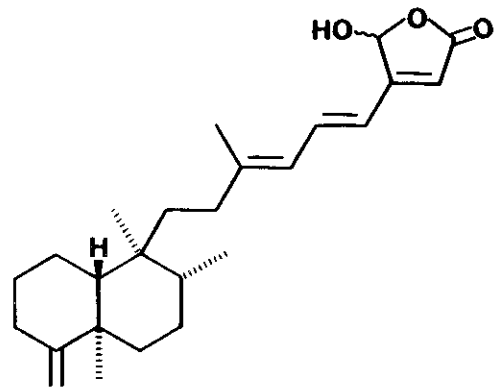


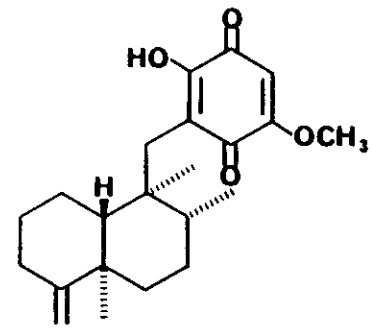


	F	G	H	I	J
C_1	128.5	143.6	146.1	138.7	140.6
C_2	156.6	116.0	113.5	116.0	122.8
C_3	108.0	148.6	156.0	153.5	141.8
C_4	150.0	134.9	106.4	113.8	115.2
C_5	113.4	135.1	151.6	135.1	150.5
C_6	143.1	121.4	126.0	142.5	138.5
$\Sigma(\lvert obsd-expd \rvert)$	43.3	13.3	50.3	14.3	6.9







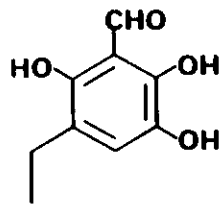


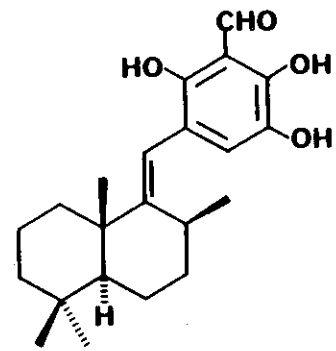
47	51	50	34
161.29 s	161.29 s	160.2 s	160.46 s
103.09 t	103.24 t	102.4 t	102.06 t
49.35 d	49.28 d	48.5 d	50.22 d
43.20 s	43.31 s	39.8 s	43.34 s
41.30 s	41.34 s	39.2 s	40.47 s
38.76 t	38.59 t	37.1 d	38.16 d
37.88 t	37.95 t	36.5 t	36.69 t
37.42 d	37.52 d	36.2 t	33.00 t
34.10 t	34.15 t	33.6 t	32.44 t
29.33 t	29.15 t	28.5 t	28.65 t
28.88 t	28.89 t	27.3 t	27.99 t
24.24 t	24.36 t	21.5 t	23.21 t
21.09 q	21.07 q	20.6 q	20.56 q
18.43 q	18.51 q	17.9 q	17.87 q
18.13 q	18.13 q	17.6 q	17.32 q

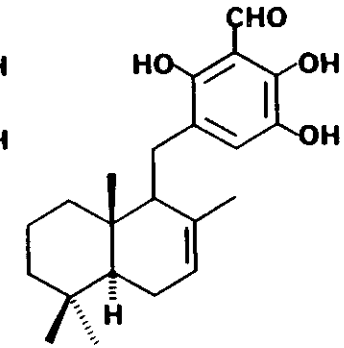
Table 2. Comparison of ^{13}C NMR data of sesquiterpene portion of siphonodictyol G 47, siphonodictyoic acid 51, palauolide 50, and ilimaquinone 34.

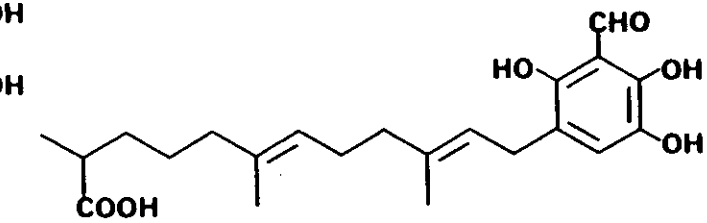
Siphonodictyal E (48) had the molecular formula $C_{22}H_{30}O_6$. Initial inspection of the spectral data indicated that this compound was a sesquiterpene quinol. A penta-substituted aromatic ring was indicated by a signal in the ^1H NMR spectrum at δ 7.06 (s, 1 H) and six signals in the ^{13}C NMR spectrum at δ 149.4 (s), 145.0 (s), 143.9 (s), 128.0 (s), 125.5 (d) an 116.6 (s). An aromatic aldehyde was suggested by a signal in the ^1H NMR at δ 10.27 (s, 1 H) and a signal in the ^{13}C NMR spectrum at δ 198.0 (d). Comparison of the chemical shifts of these signals with the corresponding signals for siphonodictyal B (8) and siphonodictyal C (51) (whose structural elucidation appears later in this chapter, Table 3) implied that all three compounds had the same aromatic substitution pattern. Treatment of siphonodictyal E (48) with methyl iodide and potassium carbonate in acetone at 80°C for 8 hours produced the dimethoxy-methyl ester derivative (49). The ortho-hydroxy aldehyde functionality was indicated by a sharp singlet in the ^1H NMR spectrum at δ 11.47 (s, 1 H). A series of NOEDS experiments confirmed the substitution pattern. An nOe was observed between the aldehyde proton signal at δ 10.29 and both the methoxyl signal at δ 3.97 and the phenolic proton signal at δ 11.47. Also, an nOe was observed between the aromatic proton signal at δ 7.06 and both the methoxyl signal at δ 3.82 and the benzylic proton signal at δ 3.29.

The sesquiterpene portion ($C_{15}H_{25}O_2$) of the molecule contained three degrees of unsaturation. An absorption in the IR spectrum at 1710 cm^{-1} and a signal in the ^{13}C NMR spectrum at 180.8 (s) indicated the presence of a carboxylic acid functionality. The two remaining degrees of unsaturation were assigned to two tri-substituted olefins which









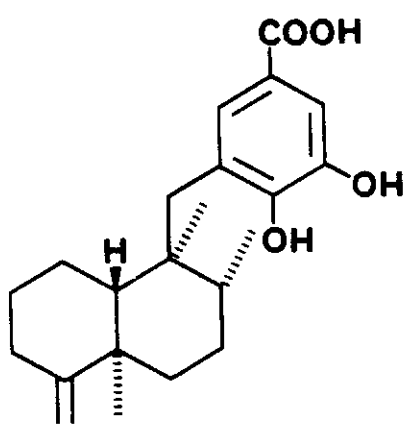
Calculated	7	51	47
126.1 (s)	127.7 (s)	130.5 (s)	128.0 (s)
150.4 (s)	149.6 (s)	149.1 (s)	149.4 (s)
113.1 (s)	116.6 (s)	116.6 9s)	116.6 (s)
142.8 (s)	144.8 (s)	144.8 (s)	145.0 (s)
136.0 (s)	142.9 (s)	143.7 (s)	143.9 (s)
123.7 (d)	125.2 (d)	123.4 (d)	125.5 (d)

Table 3. Comparison of ^{13}C NMR chemical shifts of the aromatic carbons of siphonodictyal B, C and E and calculated values.

accounted for the four remaining low field signals in the ^{13}C NMR spectrum at δ 137.5 (s), 135.8 (s) and two at 123.9 (d). The ^{1}H NMR spectrum indicated the presence of one secondary methyl and two olefinic methyl groups; the compound is therefore a linear sesquiterpene with one of the terminal methyl groups oxidized to an acid. Proton decoupling experiments confirmed the assigned structure. The benzylic proton signal at δ 3.47 (d, 2 H, $\underline{J}$ = 7 Hz) was coupled to an olefinic proton signal at 5.27 (t, 1 H, $\underline{J}$ = 7 Hz). The second olefinic proton signal at δ 5.09 (t, 1 H, $\underline{J}$ = 7 Hz) was coupled to a two proton signal at 2.10 (q, 2 H, $\underline{J}$ = 7 Hz) that was further coupled to a two proton signal at 2.04 (t, 2 H, $\underline{J}$ = 7 Hz). The two double bonds were assigned the E stereochemistry based on the ^{13}C NMR chemical shifts of the olefinic methyl groups at δ 15.9 (q) and 16.3 (q). The signal at δ 1.14 (d, 1 H, $\underline{J}$ = 7 Hz), due to a secondary methayl group was coupled to a multiplet at 2.41 (m, 1 H) that was further coupled to a one proton signal at 1.60 (m, 1 H) and another proton that was part of a three proton multiplet at δ 1.38 (m, 3 H). Also, a signal at δ 1.96 (t, 2 H, $\underline{J}$ = 7 Hz) was coupled to the multiplet at 1.38 (m, 3 H) indicating the partial structure $CH_3CH(COOH)CH_2CH_2\overset{CH_3}{\underset{|}{C}}=$. Only the assigned structure fits all of the spectral data.

The combined ethyl acetate soluble material of an aqueous methanolic extract of each sample of Siphonodictyon coralliphagum forma typica (83-258 A, G and H, 83-258 B, C and D, 83-258 F and 83-185) was chromatographed on a Sephadex LH-20 column using 1:1 methanol:dichloromethane as eluant. Sponges 83-258 F and 83-258 A, G and H contained the same metabolites but in different quantities.

Sample 83-258 A, G and H yielded siphonodictyal B (8) (0.21% dry weight), siphonodicytal E (48) (0.32% dry weight) and siphonodictyol G (47) (0.04% dry weight). Sample 83-258 F produced siphonodictyal B (8) (0.20% dry weight), siphonodictyal E (48) 0.04% dry weight) and siphonodictyol G (47) (0.09% dry weight). The major component of sample 83-258B, C and D was a unidentified mixture of two compounds. This sample also contained siphonodictyal B (8) (0.11% dry weight), siphonodictyal E (47) (0.03% dry weight), siphonodictyol G (47) (0.12% dry weight), and siphonodictyoic acid (51) (0.01% dry weight). All of the Belize samples (83-258A-H) include sponges from both the interior and exterior of coral heads. The Bahamas' sample (83-185) consisted only of sponges from the exterior of the coral head. This sample yielded siphonodictyal B (8) (3.41% dry weight), siphonodictyal E (48) (0.25% dry weight) and siphonodictyol G (47) (0.507% dry weight).



51

Siphonodictyoic acid (51) had the molecular formula $C_{22}H_{28}O_4$. The spectral data indicated that the compound was a sesquiterpene quinol. A tetrasubstituted aromatic ring with two meta protons was

suggested by signals in the ^{1}H NMR spectrum at δ 7.46 (d, 1 H, J = 1.9 Hz) and 7.42 (d, 1 H, J = 1.9 Hz) and by signals in the ^{13}C NMR spectrum at δ 151.2 (s), 145.3 (s), 135.6 (s), 128.2 (s), 126.77 (d) and 118.0 (d). The benzoic acid functionality gives rise to a signal in the ^{13}C NMR spectrum at δ 169.7 (s) and a band in the IR spectrum at 1710 cm^{-1}. The two remaining substituents on the aromatic ring were assigned as hydroxyls due to the large, broad absorption in the IR spectrum centered at 3450 cm^{-1} and the absence of any other carbonyl signals in the ^{13}C NMR spectrum. Five non-symmetrical structures (A-E) could be proposed for the aromatic portion of siphonodictyoic acid (51) (Table 4). Comparison of the observed ^{13}C and ^{1}H NMR chemical shifts with the calculated values[54,57] indicated E was the correct substitution pattern.

The ^{1}H NMR spectrum indicated the presence of a secondary and two tertiary methyl groups, an exocyclic olefin and two benzylic protons. This indicated that the sesquiterpene portion of the molecule had a rearranged drimane skeleton as shown. Comparison of the ^{13}C and ^{1}H NMR data of siphonodictyoic acid (51), siphonodictyol G (47) illimaquinone (34),[50] and palauolide (50)[56] confirmed the assignment and suggests that they all have the same relative stereochemistry (Table 2).

The ethyl acetate soluble material from a methanol-water extract of Siphonodictyon coralliphagum[54] from Kwajelein (84-030) was chromatographed on a Sephadex LH-20 column using 1:1 methanol:dichloromethane. Two compounds were obtained from this, siphonodictyal C (52) (0.23% dry weight) and siphonodictyal E (48) (0.05% dry weight).

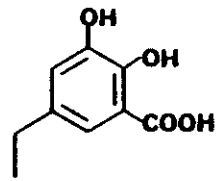
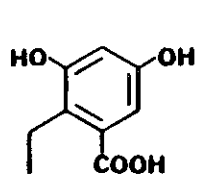
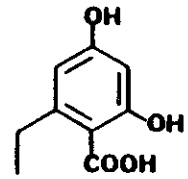
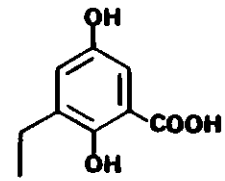
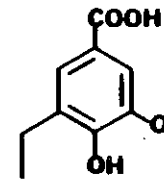
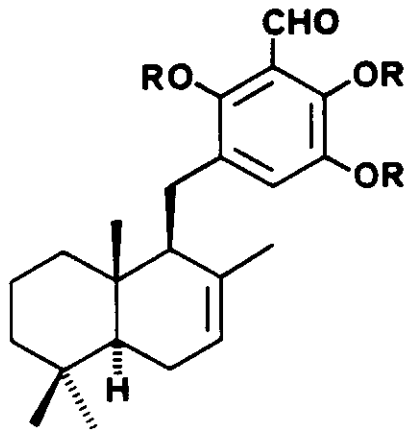
						Observed Values
^{1}H NMR						
H_2	6.68		6.36	6.68	7.38	7.46
H_3		6.41				
H_4			6.23	7.25	7.25	7.42
H_5		7.11				
H_6	7.38					
^{13}C NMR						
C_1	138.2	125.6	148.4	132.8	132.8	135.5
C_2	121.2	156.4	108.1	146.7	123.7	126.7
C_3	129.2	108.2	161.9	129.2	124.7	128.2
C_4	139.1	154.2	100.5	113.6	116.1	118.0
C_5	142.7	110.0	158.3	148.1	142.7	145.3
C_6	115.8	133.0	110.2	115.8	147.4	151.1

Table 4. Possible structures of aromatic portion of siphonodictyoic acid, 51, with calculated ^{1}H and ^{13}C NMR chemical shifts.



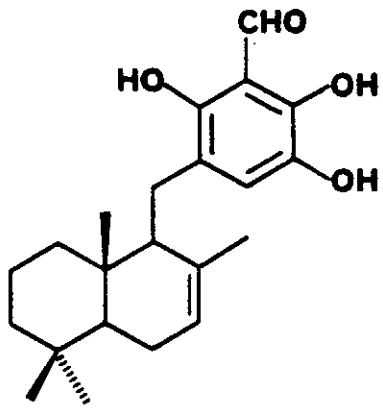
52 R = H
53 R = Me

The molecular formula of siphonodictyal C (52) $C_{22}H_{30}O_4$, was established by high resolution mass spectrometry and requires eight degrees of unsaturation. This compound is isomeric with siphonodictyal B (8) and the ^1H NMR spectrum indicated they were simply double bond isomers. The aromatic portion of the molecule was a pentasubstituted benzaldehyde as indicated by signals in the ^1H NMR spectrum at δ 10.17 (s, 1 H) and 7.03 (s, 1 H), seven signals in the ^{13}C NMR spectrum at δ 198.1 (d), 149.1 (s), 144.8 (s), 143.7 (s), 130.5 (s), 123.4 (d), and 116.6 (s) and an absorption in the infrared spectrum at 1645 cm^{-1}. A very large and broad peak in the IR spectrum centered at 3500 cm^{-1} supported the poly-hydroxyl functionality. The aromatic substitution pattern was deduced by comparison of the ^{13}C NMR chemical shifts of the aromatic carbons of siphonodictyal C (52) with those of siphonodictyal B (8) and E (48) (Table 3). The substitution pattern was confirmed by conducting NOEDS experiments on the trimethoxy derivative of siphonodictyal C (53). This compound was obtained by treating siphonodictyal C

(52) with methyl iodide and potassium carbonate in relfuxing acetone for 18 hours. An nOe was observed between the aldehyde signal at δ 10.40 and the methoxyl signals at 3.95 and 3.78 and between the aromatic signal at δ 7.05 and the methoxyl signal at 3.90. Only the assigned structure is consistent with these results.

The sesquiterpene portion of siphonodictyal C (51), $C_{15}H_{25}$, was elucidated as follows. The ^{13}C NMR spectrum contained two downfield signals at δ 136.5 (s) and 122.9 (d) and signals in the ^{1}H NMR at δ 5.32 (bs, 1 H) and 1.48 (bs, 3 H) indicated the existence of a trisubstituted olefin with a methyl substituent. A base peak in the mass spectrum at m/z = 191 ($C_{14}H_{23}$) and the presence of three tertiary methyl groups in the ^{1}H NMR spectrum at δ 0.93 (s, 3 H), 0.89 (s, 3 H) and 0.88 (s, 3 H) suggested the sesquiterpene portion of the molecule had a $\Delta^{7,8}$ drimene ring system. Decoupling experiments supported this structure and comparison of the ^{1}H and ^{13}C NMR data of siphonodictyal C (52) with that of isozonarol (10)[38] (Table 5) confirmed that they had the same bicyclic ring system and relative stereochemistry.

The oscular chimneys of sponge 83-076 were collected from living coral heads on the barrier reef of Ponape. This sponge has been identified as Siphonodictyon coralliphagum.[61] The ethyl acetate soluble material of a methanol-water extract was chromatographed on a Sephadex LH-20 column using 1:1 methanol:dichloromethane as eluant. Four compounds were obtained, two major metabolites, siphonodictyal C (52) (0.21% dry weight) and siphonodictyal D (54) (0.51% dry weight) and two minor metabolites, siphonodictyal E (48) (0.10% dry weight) and siphono-



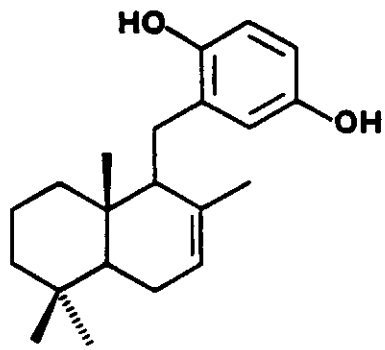
siphonodictyal C, 51

^{1}H NMR ($CDCl_3$ + CD_3OD)

0.88 s, 3 H
0.89 s, 3 H
0.93 s, 3 H
1.48 bs, 3 H
1.94 m, 2 H
2.28 m, 1 H
2.48 dd, 1 h, J = 3, 15 Hz
2.60 dd, 1 H, J = 9, 15 Hz
5.32 bs, 1 H

^{13}C NMR (CD_3OD)

136.49 s
122.93 d
56.39 d
51.63 d
43.40 t
40.80 t
37.95 s
33.92 s
33.81 q
27.20 t
24.73 t
22.65 q
22.40 q
19.92 t
14.40 q



isozonarol, 9

^{1}H NMR ($CDCl_3$)

0.88 s, 3 H
0.89 s, 3 H
0.91 s, 3 H
1.47 bs, 3 H
1.92 m, 2 H
2.34 m, 1 H
2.59 m, 2 H
4.66 s, 1 H
4.70 s, 1 H
5.38 bs, 1 H

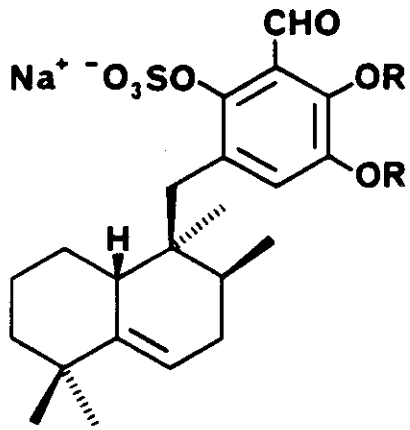
D_2O exchangeable

^{13}C NMR (CD_3OD)

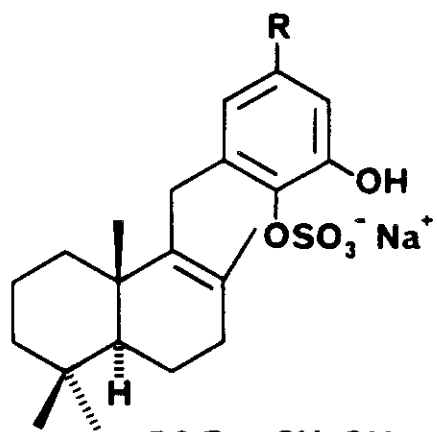
137.06 s
122.61 d
55.74 d
51.72 d
43.49 t
40.72 t
37.96 s
33.97 s
33.89 q
27.17 t
24.81 t
22.63 q
22.47 q
20.02 t
14.42 q

Table 5. Comparison of ^{1}H and ^{13}C NMR data for sesquiterpene portion of isozonarol and siphonodictyal C.

dictyol H (56) (0.08% dry weight).



54 R = H
55 R = Me



56 R = CH_2OH
59 R = CHO

Siphonodictyal D (54) had the molecular formula $C_{22}H_{29}O_7SNa$. The presence of a $-SO_3^- Na^+$ group was indicated by bands in the IR spectrum at 1230 and 1050 cm^{-1} and the molecular formula. The ^1H and ^{13}C NMR spectra were extremely difficult to interpret due to the low intensity and broadness of some of the signals. However, it was apparent that this compound was related to the other sesquiterpene quinol

metabolites from Siphonodictyon. Signals in the ^{1}H NMR at δ 10.28 (s, 1 H), 6.92 (bs, 1 H) and 5.41 (bs, 1 H) and in the ^{13}C NMR at δ 194.6 (s), 155.7 (s), 150.0 (s), 148.8 (s), 137.7 (s), 130.8 (d), 118.7 (s), 117.2 (d), and 112.4 (s), indicating the compound contained a pentasubstituted benzaldehyde moiety as well as a trisubstituted olefin. Methylation of siphonodictyal D (54) with methyl iodide and potassium carbonate in refluxing acetone for 18 hours yielded a dimethoxy derivative (55). This suggested that there were two hydroxyls on the aromatic ring in addition to an aldehyde, a sulfate group, and the sesquiterpene side chain.

The spectral data for the sesquiterpene portion of the molecule was very difficult to interpret (see Figures 4 and 5). The ^{1}H NMR spectrum contained signals for four methyl groups at δ 1.08 (s, 6 H), 1.01 (d, 3 H, J = 7 Hz) and 0.76 (bs, 3 H). The high field signal appeared as a broad, low intensity peak as did the aromatic proton at δ 6.92 (bs, 1 H). Very little structural information could be obtained from the remaining signals in the ^{1}H NMR spectrum due to their broad peak shape. Many of the signals in the high field region of the ^{13}C NMR spectrum were also broad and of reduced intensity. The ^{13}C NMR was run in both methanol-d_4 and acetonitrile-d_3 as well as at elevated temperatures, 40°C and 60°C. None of these changes affected the appearance of the spectrum. Due to the complexity of the spectral data, a crystal of siphonodictyal D (54) was submitted to Professor Jon Clardy of Cornell University for single cyrstal X-ray diffraction analysis. The structure obtained from this study is show in Figure 6.

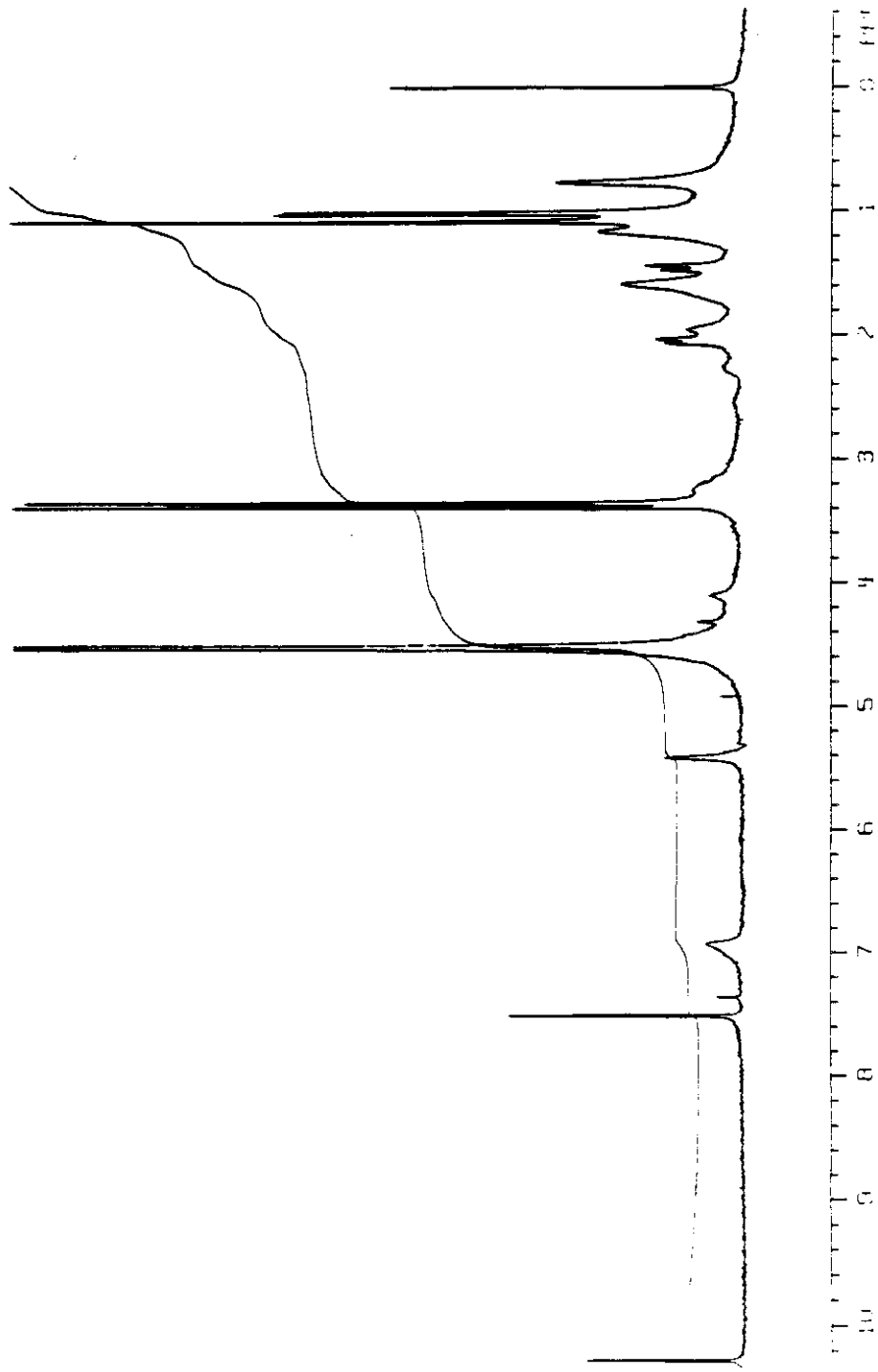


Figure 4. ^{1}H NMR spectrum of siphonodictyal D (54).

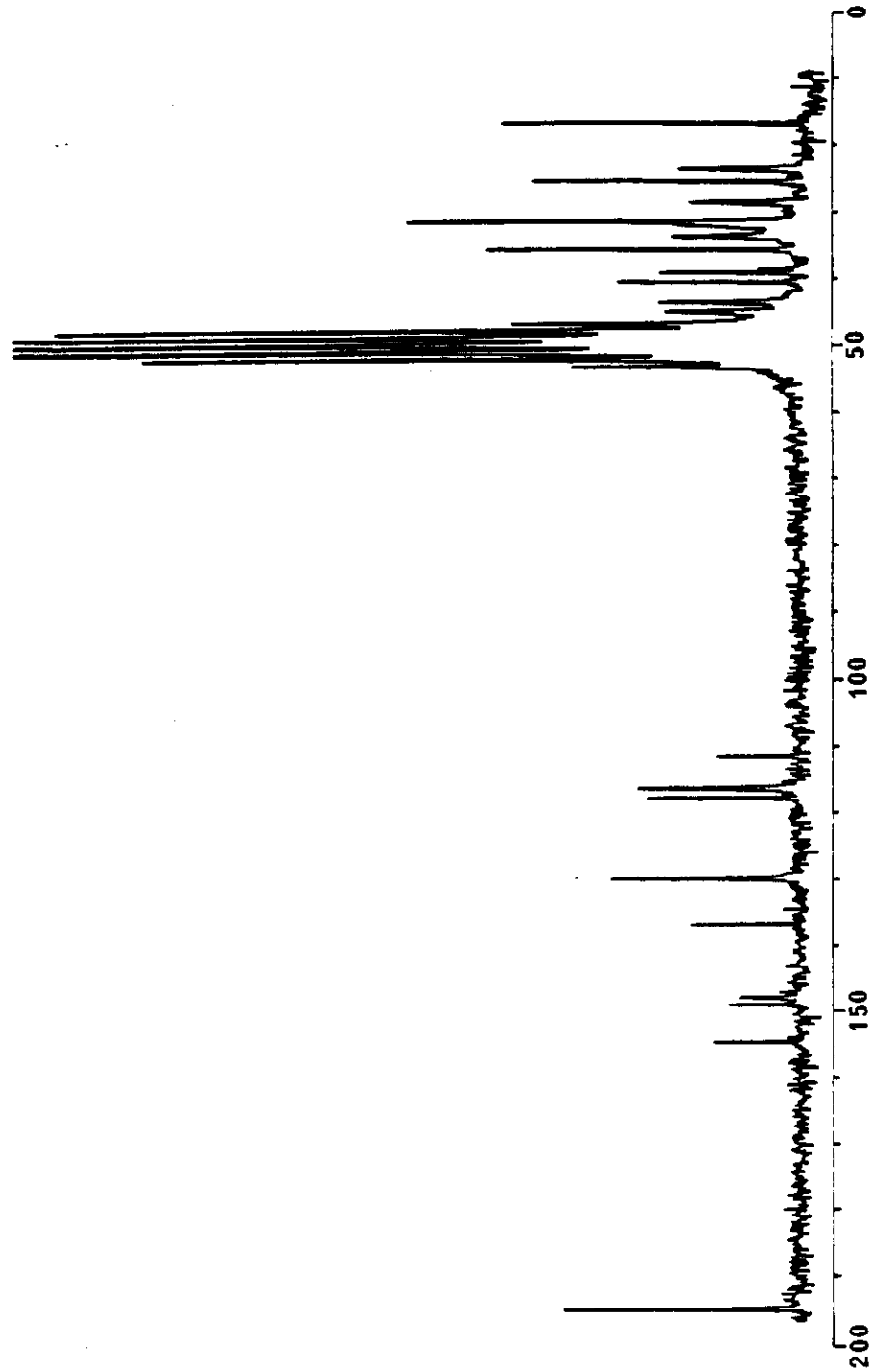


Figure 5. ^{13}C NMR spectrum of siphonodictyal D (54).

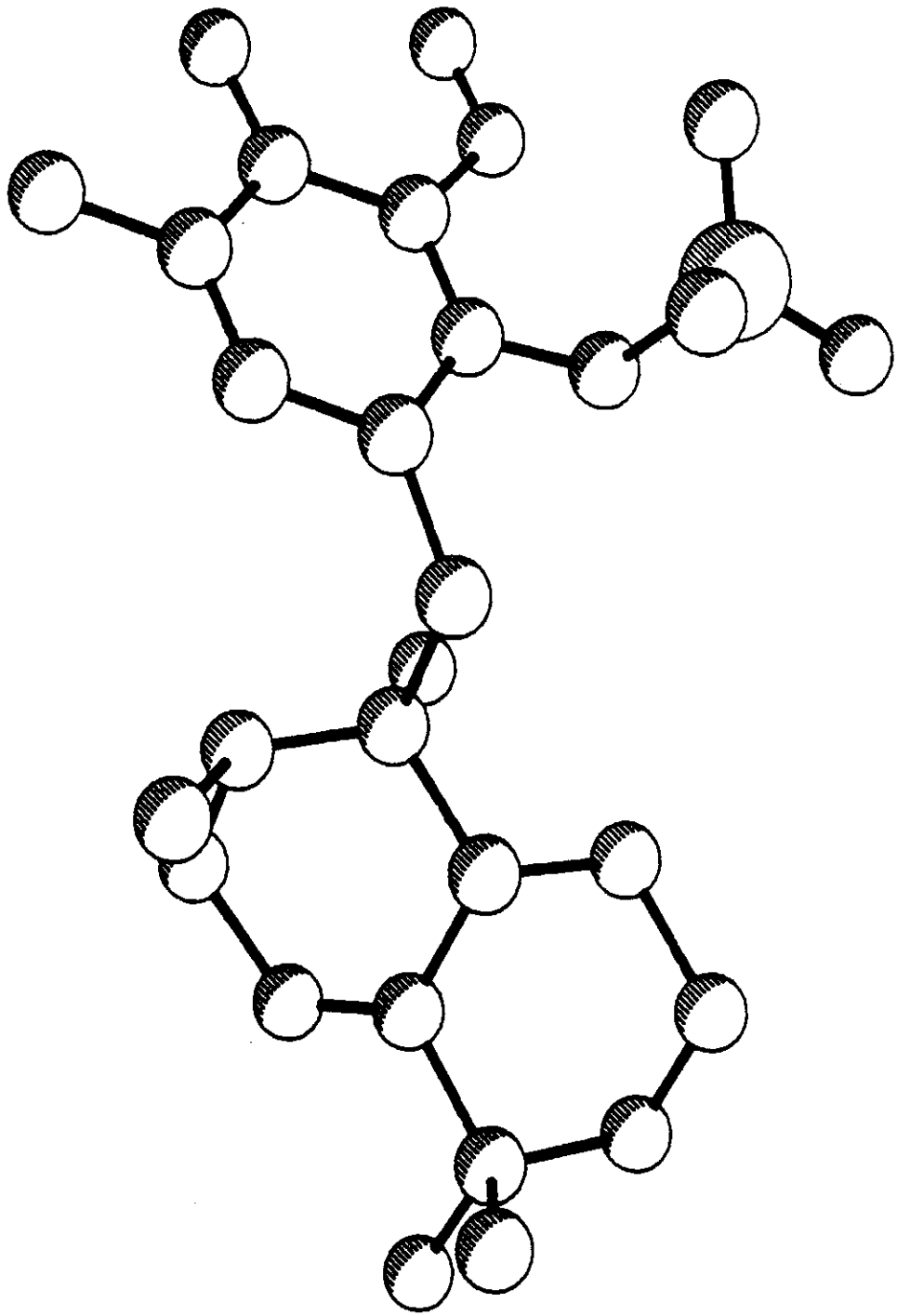


Figure 6. Computer generated drawing of siphonodictyal D (54).

An interesting feature of this structure is the relative stereochemistry at carbons 8 and 9. The two methyl groups are predicted to be cis to one another if the compound arises from a trans-antiparallel cyclization of a farnesol precursor (Figure 3). The reason for the observed stereochemistry is not known.

The molecular formula for siphonodictyol H (56) was determined by FAB mass spectrometry to be $C_{22}H_{31}O_6SNa$, indicating that it was isomeric with siphonodictyol G (47). Two mutually coupled signals in the ^1H NMR spectrum at δ 6.69 (d, 1 H, J = 1.9 Hz) and 6.56 (d, 1 H, J = 1.9 Hz) suggested the presence of an aromatic ring with two meta protons. Signals in the ^{13}C NMR spectrum at δ 151.3 (s) 139.9 (s), 139.0 (s), 138.9 (s), 137.6 (s), 129.8 (s), 119.5 (d) and 114.9 (d) confirmed the tetrasubstituted aromatic ring and indicated the presence of a tetrasubstituted double bond. A primary benzylic alcohol was indicated by signals at δ 65.2 (t), in the ^{13}C NMR spectrum, and δ 4.32 (s, 2 H) in the ^1H NMR spectrum. The presence of a $-SO_3^- Na^+$ group was evident from the molecular formula and absorptions in the infrared spectrum at 1250 and 1045 cm^{-1}. The final aromatic substituent was assigned as a hydroxyl group due to a large band in the infrared spectrum at 3450 cm^{-1} and the lack of carbonyl signals in the IR and ^{13}C NMR spectra.

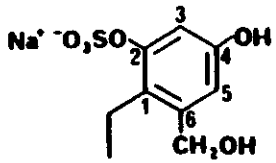
Given the four substituents, primary alcohol, hydroxyl, sulfate and sesquiterpene sidechain and two meta coupled aromatic protons, ten nonsymmetrical structures (A-J) could be proposed for the aromatic portion of siphonodictyol H (56). For each structure, the expected ^{13}C NMR chemical shifts of the aromatic carbons were calculated from empirical values[54] utilizing correction terms for replacement of -OH with $-OSO_3K$[55]

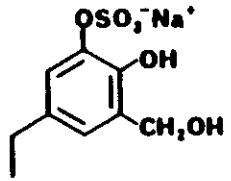
(Table 6). The calculated values were compared with the observed values and the absolute value of the difference in chemical shifts, summed over all aromatic ring carbons, was used as an indicator of agreement. Structure J showed the best correlation with observed values. This assignment was confirmed by NOEDS experiments. An nOe was observed between the benzylic alcohol proton signal at δ 4.32 and both aromatic proton signals at 6.69 and 6.56. Only the assigned substitution pattern is consistent with all of the spectral data.

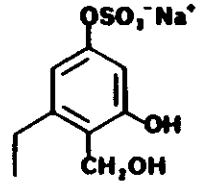
The appearance of one olefinic methyl and three tertiary methyl groups, suggested that the sesquiterpene portion of the molecule had a drimane ring system. The double bond must be between C-8 and C-9 to account for the chemical shift of the olefinic methyl group and the downfield shift of the benzylic protons to δ 3.50 (d, 1 H, J = 17.7 Hz) and 3.41 (d, 1 H, J = 17.7 Hz). Comparison of the ^{13}C NMR data of siphonodictyol H (56) with compounds 57[59] and 58[60] (Table 7) confirmed the structure and suggested that they have the same relative stereochemistry.

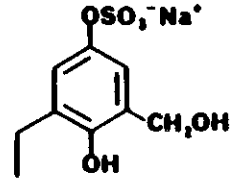
The corresponding aldehyde (59) of siphonodictyol H (56) was present in some fractions, but could not be separated from siphonodictyol H (56). Treatment of the mixture with sodium borohydride in methanol produced pure siphonodictyol H (56) in quantitative yield.

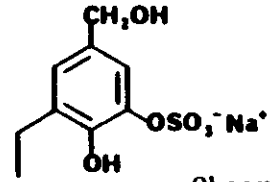
Table 6. Comparison of calculated ^{13}C NMR chemical shifts of possible aromatic structures with observed values for siphonodictyol H.



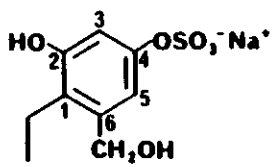


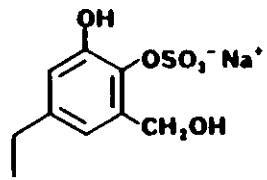


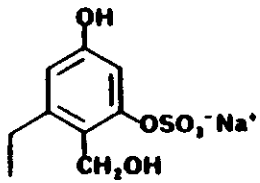


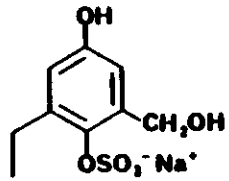


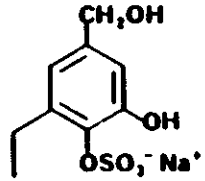
	A	B	C	C	E	Observed Values
C_1	129.0	138.4	146.1	133.0	134.4	138.8 s
C_2	152.2	121.7	114.0	121.7	126.6	119.9 d
C_3	108.0	135.5	151.6	143.9	136.2	140.0 s
C_4	154.2	145.0	106.4	119.5	121.4	114.7 d
C_5	112.9	129.4	156.0	129.4	139.9	150.6 s
C_6	143.1	126.6	125.5	152.1	149.1	137.7 s
Σ(|obsd-expd|)	42.5	31.5	50.7	26.1	20.7	



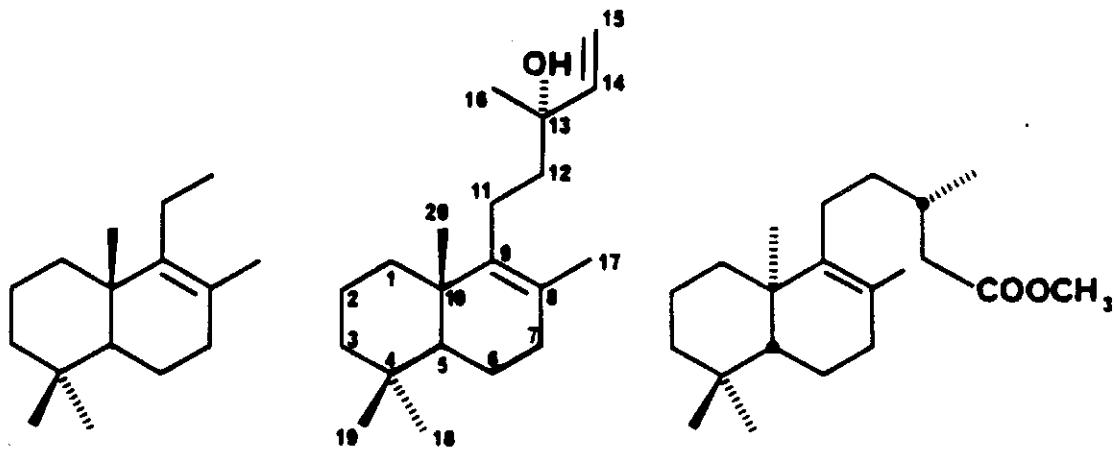








C#	F	G	H	I	J
1	122.5	143.6	146.1	138.7	140.6
2	156.6	116.0	113.5	116.0	122.8
3	108.0	148.6	156.0	153.5	141.8
4	150.0	134.9	106.4	113.8	115.2
5	113.4	135.1	151.6	135.1	150.5
6	143.1	121.4	126.0	142.5	138.5
Σ(|obsd-expd|)	48.7	18.9	50.7	12.9	7.9



56 57 58

^{13}C NMR (CD_3OD)	Carbon #	^{13}C NMR ($CDCl_3$)	Carbon #	^{13}C NMR ($CDCl_3$)
37.2 t	1	37.1	1	36.9
20.3 t	2	19.0	2	19.1
43.0 t	3	41.8	3	41.8
34.3 s	4	33.3	4	33.3
53.3 d	5	51.8	5	51.8
19.9 t	6	19.0	6	19.4
34.9 t	7	33.6	7	33.3
129.8 s	8	129.6	8	125.4
139.9 s	9	139.8	9	140.4
40.2 s	10	39.1	10	37.3
28.6 t	11	22.0	11	21.8
	12	42.4	12	41.5
	13	72.4	13	31.4
	14	144.7	14	38.9
	15	111.8	15	173.5
	16	27.6	16	20.1
20.5 q	17	19.4	17	25.5
33.8 q	18	33.3	18	33.6
22.2 q	19	21.7	19	21.6
20.7 q	20	20.1	20	19.1

Table 7. Comparison of ^{13}C NMR data of sesquiterpene portion of siphonodictyol H, 56, with compounds 57 and 58.

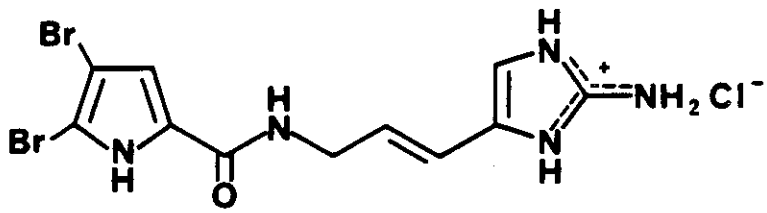
Chapter 3

Siphonodictidine, a Guanidine-Containing Metabolite from the Burrowing Sponge *Siphonodictyon* Sp.

Metabolites that incorporate a guanidine group are found in several sponges. The major metabolite of many species of the genus *Agelas* is oroidin (**60**), originally isolated from *Agelas oroides*.[61] The dimeric molecule, sceptrin (**61**), was the major metabolite of *Agelas sceptrum*.[62]. An unidentified species of *Agelas* contained keramadine (**62**),[63] an antagonist of serotonergic receptors. *Phakellia flabellata* contained dibromophakellin (**63**)[64] and monobromophakellin (**64**)[64] as well as compound **65**.[66] A brominated derivative, **66**,[66] of compound **65** was isolated from *Axinella verrucosa* and *Acanthella aurantiaca*. Both compounds **65** and **66**, together with the free base of **65**, were reported from *Hymeniacidon aldis*.[67]

Aplysinopsin (**67**) was isolated from *Fascaplysonpsis reticulata*[68] from Australia and from *Smenospongia echina*[69] from the Caribbean. Methylaplysinopsin (**68**), a serotonergic agent and potential antidepressant, was also obtained from *F. reticulata*.[70] A *Dercitus* sp. produced the two aplysinopsin derivatives **69** and **70**.[71]

The cyclic guanididnes, ptilocaulin (**71**) and isoptilocaulin (**72**), from *Ptilocaulis* aff. *P. spiculifera*[72] were found to have antimicrobial activity and to be cytotoxic. Only four acyclic guanidine metabolites, the acarnidines (**73**-**75**) from *Acarnus erithacus*,[73] and agelasidine-A (**76**) from an *Agelas* sp. from Japan,[74] have been isolated



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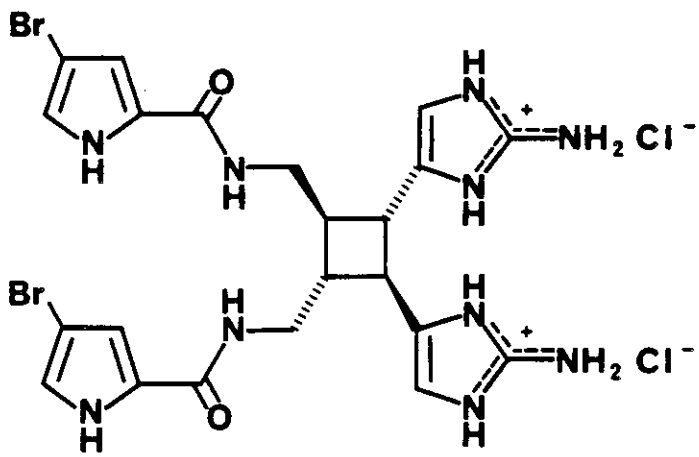

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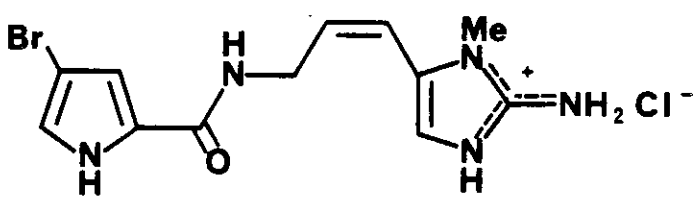

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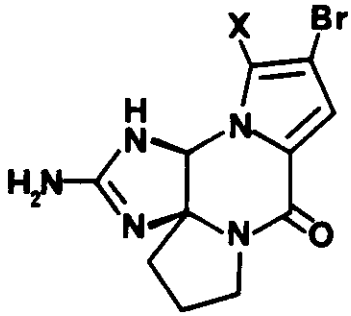
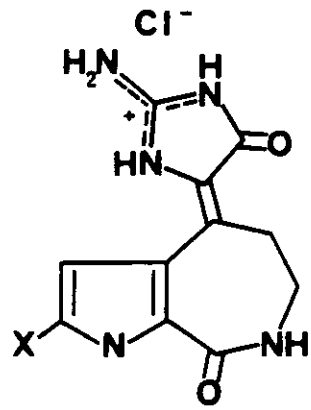
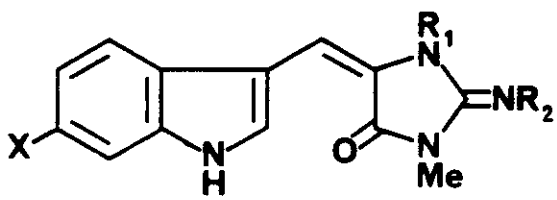


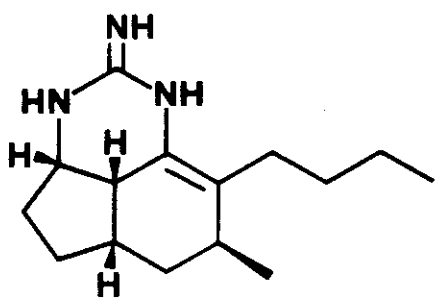
63 X = Br
64 X = H



65 X = H
66 X = Br



67 R_1 = Me, R_2 = X = H
68 R_1 = R_2 = Me, X = H
69 R_1 = R_2 = X = H
70 R_1 = R_2 = H, X = Br



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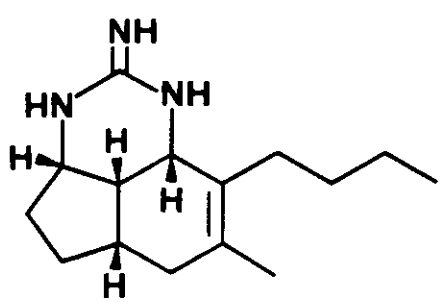

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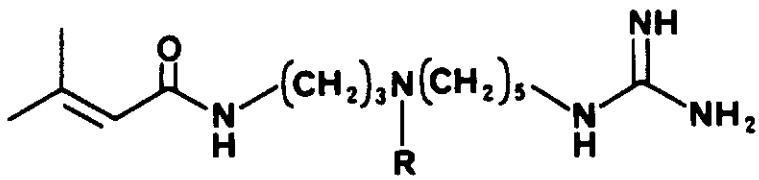
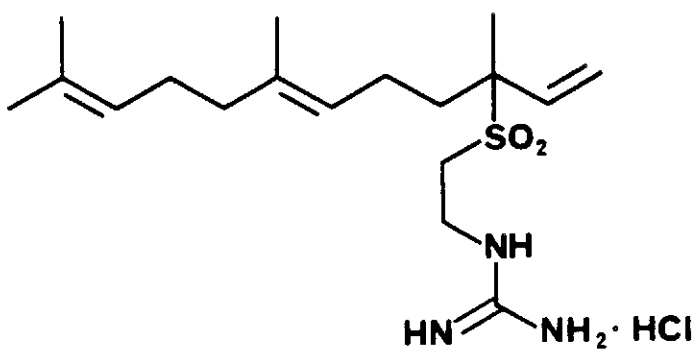


73 R = $COC_{11}H_{23}$
74 R = $COC_{13}H_{21}$
75 R = $CO(CH_2)_3CH{=}CHC_6H_{13}$



76

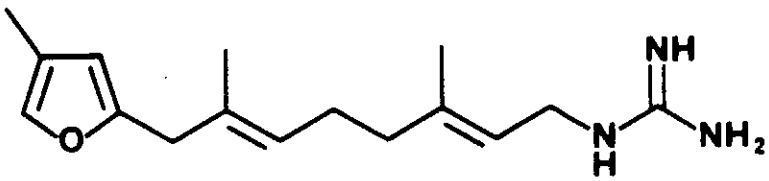
from sponges. The acarnidines (73-75) all showed antiviral and antimicrobial activity and agelasidine-A (76) has antispasmodic activity.

This chapter reports on the isolation and structural elucidation of siphonodictidine (77), the major metabolite of an undescribed Siphonodictyon species.

Sponge 81-063 was collected at Palau, Western Caroline Islands and identified as an undescribed Siphonodictyon species.[58] The sponge was growing in living coral heads with a zone of dead coral polyps around the base of the oscular chimneys. The burrowed habitat was similar to that of the other Siphonodictyons studied, but the oscular chimneys were off-white in contrast to the bright yellow color of the others. Only the protruding oscular chimneys, which exuded large quantities of mucus, could be collected. The ethyl acetate soluble material from an aqueous methanol extract of the sponge was chromatographed on Sephadex LH-20 using 1:1 methanol:dichloromethane as eluant, to obtain siphonodictidine (77) (1.06% dry weight) as the major metabolite.

Siphonodictidine (77) had the molecular formula $C_{16}H_{25}N_3O$, requiring six degrees of unsaturation. A signal in the ^{13}C NMR spectrum at δ 157.4 (s) and a positive Sakaguchi test[75] indicated the presence of a guanidine group.[76] Condensation of siphonodictidine (77) with 2,4-pentadione yielded the corresponding 4,6-dimethylpyrimidine derivative (78), confirming the guanidine functionality.

The structure of the sesquiterpene portion of siphonodictidine was deduced from spectral data and by comparison with model compounds. The ^{13}C NMR spectrum contained signals at δ 153.9 (s), 137.4 (d), 120.3



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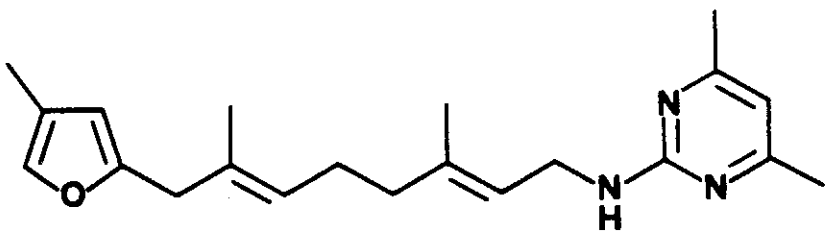

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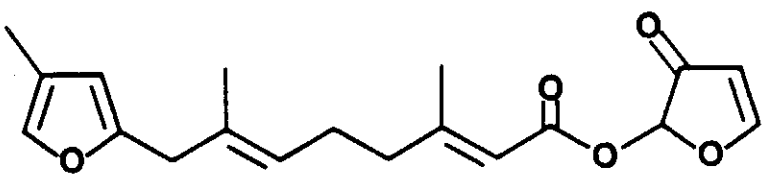

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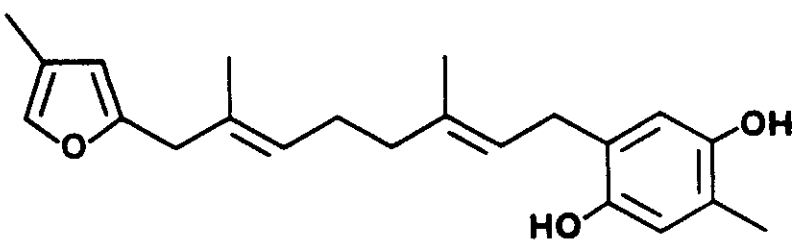


80

(s), 108.6 (d), and 9.6 (q), for an 2,4-disubstituted methyl furan, and 141.2 (s), 132.2 (s), 125.5 (d), and 125.5 (d) due to two trisubstituted olefins. The ^{1}H NMR spectrum contained signals at δ 7.06 (s, 1 H) and 5.86 (s, 1 H) due to an 2,4-disubstituted furan, 1.97 (s, 3 H) assigned to a 4-methyl substituent on a furan, 5.21 (t, 1 H, $\underline{J}$ = 7 Hz) and 5.18 (t, 1 H, $\underline{J}$ = 7 Hz) due to two olefinic protons, 3.78 (t, 2 H, J = 7 Hz) due to an allylic methylene adjacent to a guanidine group, 3.22 (s, 2 H) assigned to an allylic methylene adjacent to a furan and 1.58 (s, 3 H) and 1.68 (s, 3 H) due to two olefinic methyl groups. Comparison of the ^{1}H NMR data (Table 8) and ^{13}C NMR data (Table 9) of siphonodictidine (77) with those of the model compounds, marislin (79)[77] from Chromodoris marislae and the hydroquinone 80[78] from Sinularia lochmodes, indicated that the molecules were identical in the C-4 to C-14 region. The signal at δ 3.78 (t, 2 H, $\underline{J}$ = 7 Hz) in the ^{1}H NMR spectrum was coupled to a -NH proton signal at 7.80 (br t, 1 H) and an olefinic proton signal at 5.21 (t, 1 H, $\underline{J}$ = 7 Hz). Addition of methanol-d_4 to the sample resulted in exchange of the -NH protons, causing the signal at δ 3.78 to appear as a sharp doublet. The signal at δ 3.78 was assigned to the C-1 methylene group attached to the guanidine group. The final evidence supporting this structure were the major mass spectral fragmentation peaks at m/z of 126 (100%, $C_6H_{12}N_3$), 148 (7.6%, $C_{10}H_{12}O$), and 149 (5%, $C_{10}H_{13}O$) which result from cleavage of the bond between C-4 and C-5.

Table 8. ^{1}H nuclear magnetic resonance data for siphonidictidine (77), marislin (79) and hydroquinone 80; recorded in $CDCl_3$ solution at 360 MHz (100 MHz for 80) with internal tetramethylsilane as standard ($\delta = 0$). Abbreviations: s, singlet; t, triplet; m, multiplet; and J, coupling constant.

H at C#	77	79	80
1	3.78 (t, 2 H, J = 7 Hz)	--	3.35
2	5.21 (t, 1 H, J = 7 Hz)	5.73	5.3
4	2.05 (m, 2 H)	∿2.2	∿2.15
5	2.12 (m, 2 H)	∿2.2	∿2.15
6	5.18 (t, 1 H, J = 7 Hz)	5.18	5.3
8	3.22 (s, 2 H)	3.22	3.3
10	5.86 (s, 1 H)	5.86	5.95
12	7.06 (s, 1 H)	7.06	7.1
13	1.97 (s, 3 H)	1.98	2.0
14	1.58 (s, 3 H)	1.60	1.65
15	1.68 (s, 3 H)	2.20	1.75

Table 9. ^{13}C nuclear magnetic resonance data for siphonodictidine (77), marislin (79) and hydroquinone 80; recorded in $CDCl_3$ solution (C_6D_6 for 79) at 20 MHz with internal tetramethylsilane as standard ($\delta = 0$).

Carbon #	77	79	80
1	39.6[a]	188.8	28.8
2	117.8	115.4	122.9*
3	141.2	162.3	137.6*
4	39.0[a]	40.7	39.6
5	26.2	26.0	26.5
6	125.5	125.4	126.2*
7	132.2	133.0	132.3*
8	38.2[a]	38.7	38.4
9	153.9	154.5	154.3
10	108.6	109.2	108.8
11	120.3	120.7	120.5
12	137.4	138.2	137.6
13	9.6	9.8	9.7
14	15.9[b]	15.9	16.0
15	16.3[b]	19.0	16.0

[a,b]Signals may be interchanged.

*We have reassigned these signals.

Chapter 4

Biological Activity of the *Siphonodictyon* Metabolites and Possible Ecological Roles

A large number of secondary metabolites have been isolated from marine sponges,[79-82] but the ecological role of most of these compounds is unknown. Until recently, the biological activity of these metabolites was considered only in terms of pharmacological utility, such as antimicrobial activity and cytotoxicity. Although knowledge of this activity is interesting, it provides no information regarding the compound's role in nature.

Several hypotheses have been proposed for the roles of sponge metabolites. These compounds may be important in spatial competition, prevention of overgrowth by other organisms, detering predation, aiding in bacterial clumping for sponge feeding, or as antifouling agents.

Jackson and Buss[83] proposed that sponge metabolites serve as a specific mechanism in interference competition on coral reefs. This is accomplished by killing competitors or rendering the substrate around the sponge, or the sponge itself, unsuitable for other organisms. Their hypothesis is based on assays using dilute sponge homogenates rather than pure compounds.

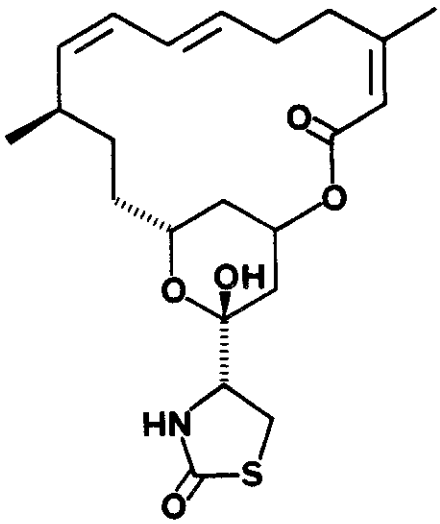
Bakus and Green[84,85] postulated that toxic sponge metabolites evolved in response to predation by fish. They showed that toxicity in sponges increased with decreasing latitude, paralleling the increasing diversity in fishes.

Bergquist and Bedford[86] suggested that antimicrobial sponge metabolites have a role in enhancing the efficiency with which sponges retain food by causing bacterial clumping.

Bergquist[87] has also suggested that sponge metabolites may make the sponge surface unsuitable for the settlement of larvae of other organisms. The role of sponge mtabolites in preventing surface fouling of sponges has recently been investigated.[88]

Sponge metabolites are now routinely tested for fish toxicity, feeding inhibition, and toxicity to other organisms to determine possible ecological roles for the compounds. The active fraction from the sponge Latrunculia magnifica was toxic to the fish Gambusia affinis[89] and contained a mixture of latrunculin A (81) and B (82).[90] Two furanosesquiterpenes, the nakafurans-8 (83), and -9 (84), isolated from Dysidea fragilis, showed feeding inhibition against two common reef fishes (Chaetodon spp).[91] Three sesterterpenes isolated from Spongia idia have been tested against marine organisms other than fish. Idiadione (85), and 12-deacetyl-12,18-diepi-scalaradial (86), are toxic to the sea star Pisaster giganteus at 5 mg/L and to the brine shrimp Artemia sp. at 10 mg/L. Both compounds immobilize the larvae of the red abalone Haliotis rufescens at 1 mg/L, while heteronemin (87) is toxic to the gametes of the giant kelp Macrocystis pyrifera.[92]

These assays are designed to show the metabolite's role in detering predation or inhibiting the settlement and growth of other organisms. A number of sponge extracts inhibit the growth of marine bacteria,[86,88] but their ability to cause bacterial clumping has not



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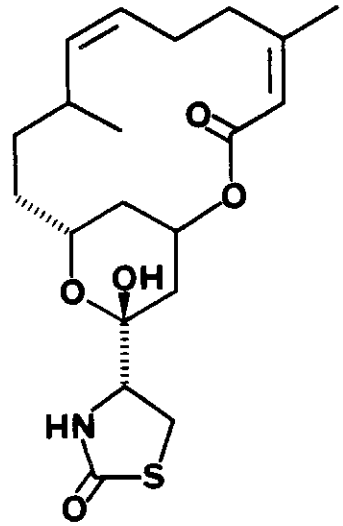

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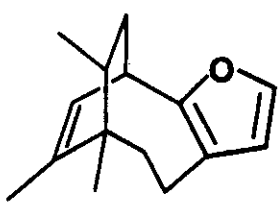

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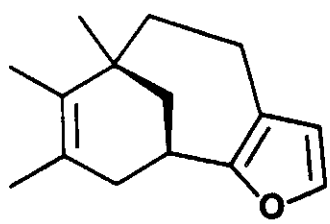

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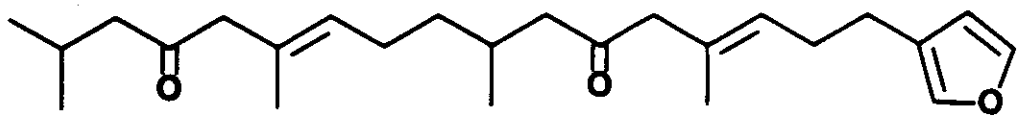

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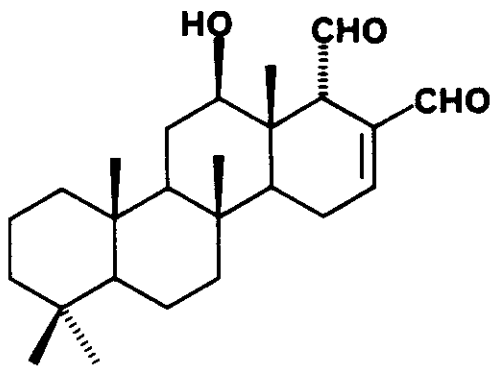

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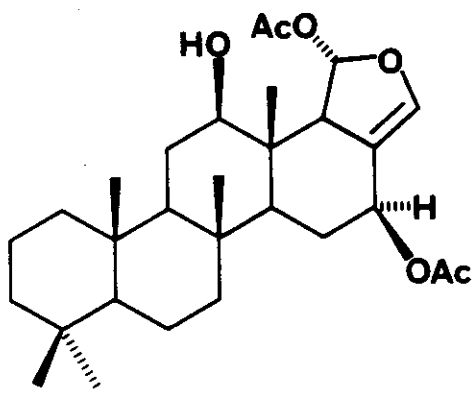

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been shown. No pure sponge metabolites have been tested for their involvement in the competition for space on coral reefs.

The metabolites described in this thesis were tested for antimicrobial activity, coral polyp toxicity, and calcium chelating ability. The antimicrobial assay was used to simply indicate biologically active compounds. The coral polyp toxicity assay was used to determine if the secondary metabolites produced by the sponges were responsible for the dead zone of coral polyps around the base of the oscular chimneys. The calcium chelation assay was used to indicate if the compounds could be involved in the removal of calcium carbonate by the sponge.

Antimicrobial assays were performed using the standard paper disc assay method, against five human pathogenic bacteria, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes and Pseudomonas aeruginosa, two marine bacteria, Beneckea harveyi-392 and Vibrio anguillarum and a yeast, Candida albicans. Results are shown in Table 10.

Coral toxicity tests were performed by Leith Webb of James Cook University of North Queensland, Australia. Due to lack of material only four metabolites, the siphonodictyals B (8), and C (52), siphonodictyol G (47) and siphonodictidine (77), could be tested. Acropora formosa was exposed to various concentrations of the compounds in seawater. Acute toxicity of the compounds was studied by monitoring the rates of respiration and photosynthesis of the coral.

Siphonodictidine (77) increased respiration at 10^{-2} to 10^{-1} ppm, decreased photosynthesis at 10 ppm, and caused acute toxicity at 100 ppm

Table 10. Antimicrobial Activity of Siphonodictyon Metabolites

Sample	µg/disc	S. aur.	B. sub.	E. coli	E. aero.	P. aeru.	C. alb.	V. ang.	B-392
Siphonodictyal B	100	11	8	--	--	--	--	--	--
	50	9	S1	--	--	--	--	--	--
	25	9	S1	--	--	--	--	--	--
Siphonodictyal C	100	14	12	--	--	--	S1	11	--
	50	12	10	--	--	--	--	10	--
	25	11	8	--	--	--	--	9	--
Siphonodictyal D	100	9	12	--	--	--	--	13	--
	50	8	11	--	--	--	--	11	--
	25	S1	9	--	--	--	--	9	--
Siphonodictyal E	100	--	S1	--	--	--	--	--	--
	50	--	--	--	--	--	--	--	--
	25	--	--	--	--	--	--	--	--
Siphonodictyol G	100	8	8	--	--	--	--	--	--
	50	S1	S1	--	--	--	--	--	--
	25	--	--	--	--	--	--	--	--
Siphonodictyol H	100	10	9	--	--	--	--	--	--
	50	8	S1	--	--	--	--	--	--
	25	--	--	--	--	--	--	--	--
Siphonodictidine	100	20	20	10	11	--	17	--	--
	50	19	17	9	9	--	14	--	--
	10	S1	10	--	--	--	S1	--	--

Numbers equal diameter of zone of inhibition including disc.

S1 - slight, inhibition visible but less than 8 mm.

with cell lysis occurring as the tissue was stripped from the skeleton. At higher concentrations, siphonodictidine (77), was a quick acting toxin, showing an effect in 5 to 30 minutes. "At high doses, siphonodictyal B (8) and siphonodictyol G (47) were toxic. At moderate doses, only siphonodictyal B was toxic. Siphonodictyal C (52), was not toxic although it gave a response which was significantly different from the controls (Student's test at the 0.05% level). Acropora formosa recovered from the acute shock of siphonodictyal C (52)."[93]

The sponges containing siphonodictyal B (8) and siphonodictidine (77) had zones of dead coral polyps around the base of the oscular chimneys. The toxicity of these compounds to coral polyps of Acropora formosa indicates that they are responsible for these dead zones. It is reasonable to propose that the mucus produced by the sponge could hold a toxic concentration of the metabolites in contact with the coral tissue to maintain the zones of dead coral polyps. The mucus from the above sponges was not analyzed separately from the sponges. However, the mucus from Siphonodictyon coralliphagum from Ponape contained both siphonodictyal C (52) and D (54), but in lower concentrations than the sponge itself. It is remarkable that two sponges of the same genus can produce such different compounds to potentially perform the same ecological function.

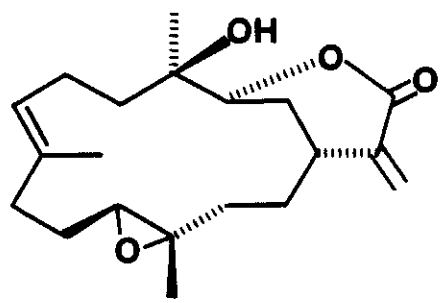
Siphonodictyon coralliphagum from Kwajalein was found growing in dead coral and the major metabolite of this sponge, siphonodictyal C (52), was not toxic to Acropora formosa. Since siphonodictyal B (8) and siphonodictyal C (52) are simple double bond isomers, one would expect them to have similar biological activity. The reason for the difference

in coral polyp toxicity for these two compounds is unknown.

Siphonodictyon coralliphagum forma tubulosa grows mainly in dead coral. Occasionally the sponge is found in living coral, but no zone of dead coral appears around the base of the sponge. The major metabolite of this sponge, siphonodictyol G (47) is toxic to coral polyps. This compound may function to prevent coral overgrowth but may not be toxic enough to maintain a dead zone of coral polyps around the base of the oscular chimneys.

These results indicate that the metabolites produced by sponges of the genus Siphonodictyon can prevent overgrowth by hard corals. Some of these metabolites can also kill coral polyps that are in contact with, or in close proximity to, the base of the sponge. Soft coral metabolites have been implicated as the cause of stunted growth and local mortality in hard corals.[94] Three soft coral metabolites, furanoquinol (80), flexibilide (88), and sarcophytoxide (89), were shown to be toxic to the hard corals Acropora formosa and Porities andrewsi.[95] This evidence suggests that some marine natural products are important in the competition for space on coral reefs.

The four major Siphonodictyon metabolites, the siphonodictyals B (8), C (52) and D (54) and siphonodictyol G (47) were tested for calcium chelating ability. The chelating ability of these compounds was difficult to determine due to their low solubility in most organic solvents and high solubility in water. Butanolic solutions of the metabolites were used to extract aqueous calcium solutions (5 ppm). The concentration of Ca^{2+} was measured by atomic absorption before and after



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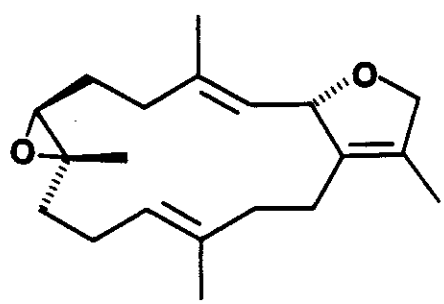

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extraction (Table 11). There was a significant reduction in the Ca^{2+} concentration after extraction for each metabolite. The exact amount of Ca^{2+} removed could not be determined due to the residual butanol in the aqueous solution. To confirm that the metabolites were responsible for the decreased Ca^{2+} concentration, isozonarol (10) was used to extract a 5 ppm Ca^{2+} solution. Isozonarol's structure is similar to that of the Siphonodictyon metabolites, but does not contain a functional group capable of chelating calcium. The results showed that isozonarol (10) extracted less than half of the calcium that the Siphonodictyon metabolites did, confirming the calcium chelating ability of these metabolites. X-ray analysis of siphonodictyal D (54) before and after extraction of a 200 ppm Ca^{2+} solution showed the presence of calcium after extraction but not before. These experiments show that the Siphonodictyon metabolites tested can sequester calcium from an aqueous solution. This suggests that they may participate in the burrowing process by chelating calcium as the $CaCO_3$ is dissolved.

Table 11. Results of calcium chelation experiment

Compound	AA value of Ca^{2+} solution after extraction with butanol	AA value of Ca^{2+} solution after extraction with butanol + compound
Siphonodictyal B*	0.103	0.027
Siphonodictyal C	0.510	0.330
Siphonodictyal D	0.510	0.334
Siphonodictyol G	0.510	0.360
Isozonarol	0.520	0.445

*A 2 ppm Ca^{2+} solution was used in this experiment.

Chapter 5

Chemotaxonomy

Classification of Demospongia (phylum Porifera) has traditionally been based on characteristics of the mineral skeleton. The inadequacy of this method became obvious and new parameters have been incorporated into the classification scheme. Reproductive characteristics were first used to redefine subclass and ordinal categories.[96] Since then, information such as biochemical, histological, reproductive, and ultrastructural characteristics along with skeletal features have been used in redefining the entire Demospongia.[97,98]

This difficulty in sponge classification has generated an interest in chemotaxonomy, the integration of chemical and biological studies.[99,100] Bergquist and Wells[100] have defined three criteria that must be met before a chemotaxonomic study can provide useful taxonomic information. "First, the chemical characterization of the compounds must be precise. Second, the taxonomic assignment of each specimen from which the compounds were extracted must be accurate, and reference specimens of an acceptable standard must be retained. Third, the spectrum of species samples in any given investigation must be sufficiently broad to permit the limits of natural variation within the taxa concerned to be established."[100]

The original sponge chemotaxonomic work dealt mainly with the sterol pattern in various organisms, but also covered a number of other classes of compounds.[101,102] Since that work, a number of chemotaxonomic

studies have been conducted using a variety of compounds such as sterols and pigments,[103,104] free amino acids,[105,106] sterols,[107,108,109] acid mucopolysaccharides,[110,111] fatty acids,[112] and dibromotyrosine-derived compounds, furanoterpenes, and bromopyrrole derivatives.[113] The results of these studies have been reviewed.[98,100]

The most convincing argument supporting chemotaxonomy involves the order Verongida. This group of sponges was recently separated from the rest of the Dictyoceratida based on "the different and complex histology of the Vergonida, their distinct pigmentation and fiber construction, and their apparent oviparous reproduction as opposed to the viviparity in the Dictyoceratida and Dendroceratida."[98] This new classification is supported by the unique biochemistry of the Verongida. All members contain brominated metabolites derived from tyrosine, found in no other marine organism; many possess sterols with novel aplystane skeletons; all have a moderately high percentage of sterol in the lipid fraction; and all lack terpenes. No other group of sponges is as well defined biochemically as the Verongida.

Taxonomic interpretations have been made of the chemical patterns in Australian sponges of the orders Dictyoceratida and Dendroceratida.[100] This was accomplished by utilizing the large number of terpenoid sponge metabolites reported in the chemical literature. A few examples of metabolites from sponges collected outside of Australia were included, but no basis for their selectivity was given. Six major classes of terpenoid compounds, A-F (Figure 7), were utilized to analyze the metabolites of these sponges. Class E members are the only metabolites that are limited in distribution to one family, the Aplysillidae.

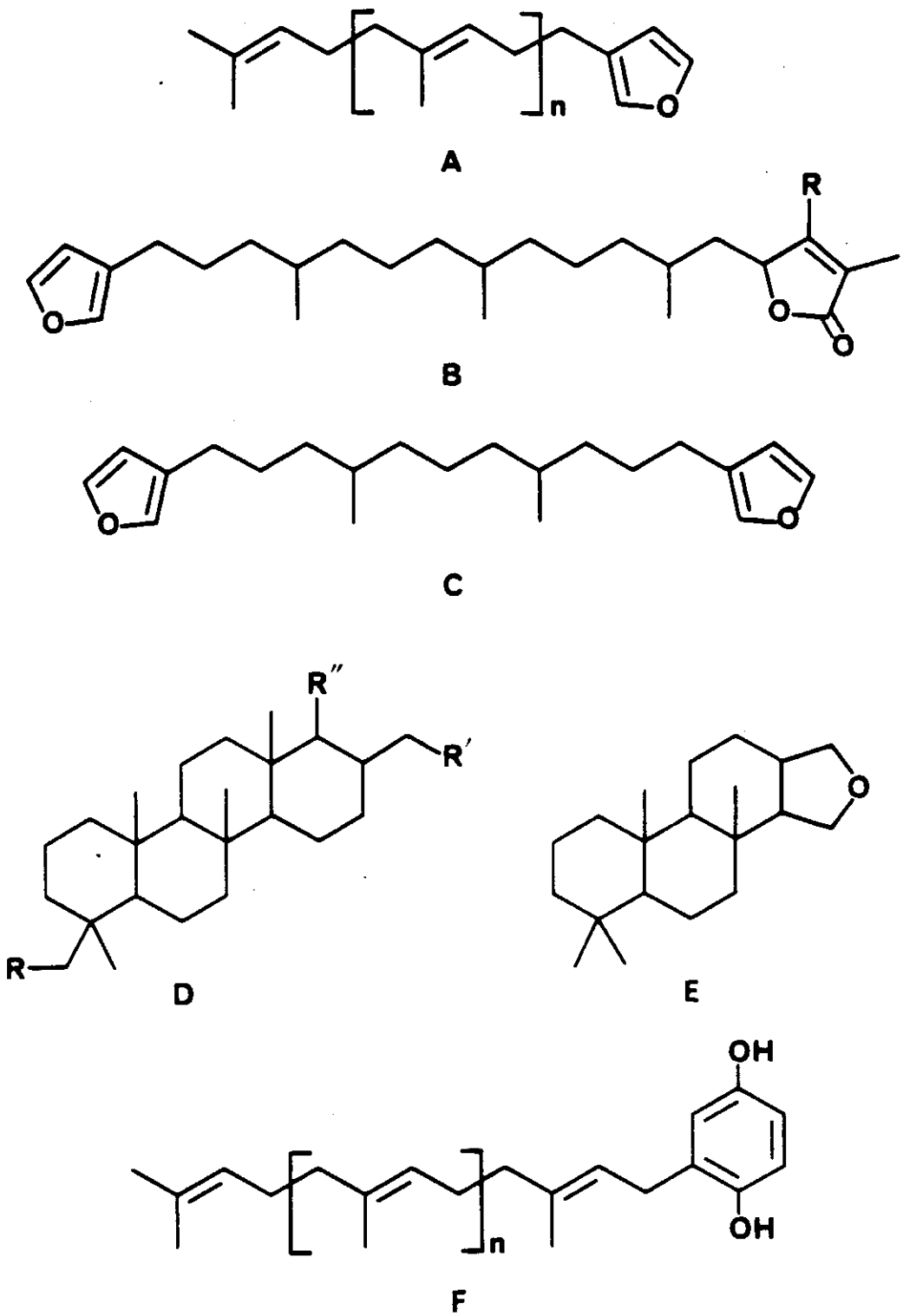

Figure 7. Terpenoid classes used in chemotaxonomy.[100]

All other classes occur in at least two families, while class A terpenes occur in all four families investigated. Also, the metabolite composition was found to vary within a species, depending on where the sponge was collected. Two examples where this occurs are Dysidea herbacea and Spongia officinalis. Overall, the authors felt there was a good correlation between major terpene classes and ordinal- and family-level groupings.[100] Some groups were considered distinctive at the generic level such as Dysidea, Sarcotragus, Ircinia, Carteriospongia and Lendenfeldia, while other genera were found to contain compounds belonging to a number of the defined terpenoid classes.[100] These conclusions are based mainly on metabolites isolated from Australian sponges and may not be valid for sponges from other locations.

The present study on sponges of the genus Siphonodictyon fulfills the three requirements[100] necessary for a useful chemotaxonomic study. Eighteen samples were investigated to compare the secondary metabolites present in a single species collected from different geographic locations and in different species (Table 12). Seventeen samples of S. coralliphagum were examined. These samples represent two different forms of S. coralliphagum collected from Belize and the Bahamas and two additional samples of S. coralliphagum from Ponape and Kwajalein. The remaining sample was of an undescribed Siphonodictyon species from Palau.

All samples of S. coralliphagum forma typica investigated contained siphonodictyal B (8) and siphonodictyal E (48). Siphonodictyol G (47) was present in all six of the Belize samples, but was not found in the Bahamas sample. Three of the Belize samples (83-258B, C, and D)

Table 12. Distribution of Siphonodictyon metabolites (% dry weight)

Compound \ Sample	81-063	77-065[35]	83-256 A-E	84-090	83-185	83-258 B,C&D	83-258F	83-258 A,G&H	83-076	84-030
Siphonodictyal A		0.12%								
Siphonodictyal B		0.90%			3.41%	0.11%	0.20%	0.21%		
Siphonodictyal C									0.21%	0.23%
Siphonodictyal D									0.51%	
Siphonodictyal E				0.05%	0.25%	0.03%	0.04%	0.03%	0.10%	0.05%
Siphonodictyol G			0.09%	0.07%	0.51%	0.12%	0.09%	0.04%		
Siphonodictyol H									0.08%	
Siphonodictyoic Acid						0.01%				
Siphonodictidine	1.06%									

contained siphonodictyoic acid (51) while one sample (83-258F) had as its major component a mixture of two compounds which could not be separated.

The seven samples of Siphonodictyon coralliphagum forma tubulosa all possessed siphonodictyol G (47) as the major component. This was the only compound identified from the six Belize samples and comprised ~20% of the organic extract. The Bahamas' sample also contained siphonodictyal E (48) as a minor component.

The most important difference in the secondary metabolites from the two forms of S. coralliphagum is the presence or absence of siphonodictyal B (8). Whether the presence of siphonodictyal B (8) in S. coralliphagum forma typica is a cause or an effect of the sponge's ability to burrow into living coral heads is unknown. There are also small variations in the minor constituents from forms collected in one location as well as in those from different locations. However, there is at least one common metabolite in all samples of each form individually, and two compounds, siphonodictyol G (47) and siphonodictyal E (48) are found in both forms.

The metabolites of Siphonodictyon coralliphagum collected from two Pacific islands were very similar to those of S. coralliphagum from the Caribbean. The sample from Ponape contained the siphonodictyals C (52), D (54), and E (48) and siphonodictyol H (56), while both siphonodictyal C (52) and E (48) were isolated from the Kwajalein sample. A number of interesting points can be made from a comparison of the metabolites present in the various samples of S. coralliphagum. All of the

metabolites isolated from this species are sesquiterpene quinols and contain 22 carbons. Only one metabolite, sphonodictyal E (48), is present in samples from both the Caribbean and the Pacific. Siphonodictyal B (8) the major metabolite of S. coralliphagum forma typica from Belize and the Bahamas, and siphonodictyal C (52), a major metabolite of S. coralliphagum from both Ponape and Kwajalein, are double bond isomers. All of the metabolites isolated from S. coralliphagum are closely related, but there are distinct differences between the Pacific and Caribbean samples.

The undescribed Siphonodictyon species collected in Palau was similar to S. coralliphagum in that it burrowed into living coral heads and maintained a zone of dead coral polyps around the base of the oscular chimneys. However, the physical appearance was very different. The oscular chimneys were thin walled fragile tubes, in contrast to the tough, massive oscular chimneys of S. coralliphagum. Also, the sponge was off-white in color compared to the bright yellow color of S. coralliphagum. The natural products of these sponges were also very different. The undescribed Siphonodictyon species contained siphonodictidine (77) as the major metabolite. This metabolite is a furanosesquiterpene and is completely unrelated to the sesquiterpene quinols found in S. coralliphagum.

These results show that sponges of the genus Siphonodictyon produce an array of metabolites. The metabolites present in S. coralliphagum vary with the form of the sponge and with geographic location. The major metabolite of the undescribed Siphonodicton species belongs to a different terpenoid class than the metabolite of S. coralliphagum.

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Due to this variability in metabolites produced by these sponges, chemotaxonomy is not useful for sponges of the genus Siphonodictyon.

In a limited survey of secondary metabolites from a group of sponges, specific chemical patterns may emerge. However, when the survey is expanded to include a large number of samples, these patterns often break down. It is obvious from the above results that any use of chemotaxonomy in a taxonomic study must be done judiciously. Chemotaxonomy may be useful in the classification of some sponges where classical techniques fail, but it should only be used as a secondary criterion.

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Experimental Section

Infrared spectra were recorded on either a Perkin-Elmer 621 Grating Infrared Spectrophotometer or a Perkin-Elmer 783 Spectrophotometer. Ultraviolet spectra were recorded on either a Gary 219 or a Uvikon 820 spectrophotometer. ^{1}H NMR spectra were recorded on either a Varian HR-220 NMR spectrometer or a 360-MHz spectrometer constructed from an Oxford narrow-bore magnet and a Nicolet 1180E FT data system by Dr. John M. Wright for the UCSD NMR Facility. ^{13}C NMR spectra were recorded on either a Varian CFT-20 spectrometer or a Nicolet 200-MHz wide-bore multinuclear spectrometer operating at 50 MHz in the ^{13}C mode. All chemical shifts in both ^{1}H and ^{13}C NMR spectra are reported with respect to tetramethylsilane ($\delta = 0$). Low resolution mass spectra were recorded on a Hewlett-Packard 5930A mass spectrometer. High resolution mass specctra were obtained from the Space Sciences Laboratory, UC Berkeley, under the direction of Professor A.L. Burlingame (sponsored by NIH Grant RR00719) and University of Minnesota Mass Spectrometery Service Laboratory. High resolution fast atom bombardment mass spectra were obtained from the Mass Spectrometry Lab, UC Riverside. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter usng a 10-cm microcell. Melting points were determined on a Fisher-Johns apparatus and are reported uncorrected. All solvents were either spectral grade or distilled from glass prior to use.

Experimental Section, Chapter 2

Siphonodictyon coralliphagum forma *tubulosa* was collected by hand using SCUBA at Carrie Bow Cay, Belize (-20 m) in November, 1983 and Bimini, the Bahamas (-20 m) in August, 1984. The portion of the sponge protruding from the coral head was collected.

Five samples were collected in Belize (83-256 A-E). Each sample was stored in methanol for 4 months and then worked up in an identical manner. The solvent was decanted and the sponge was soaked in fresh methanol for 1 day. The combined methanol extracts were evaporated to dryness and then dissolved in water (400 mL). The aqueous material was extracted with hexane (2 x 300 mL), dichloromethane (2 x 300 mL) and ethyl acetate (3 x 300 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated. Each extract was examined by ^{1}H NMR. The extracts from all five sponges were identical by ^{1}H NMR and were combined.

The ethyl acetate extract (83-256 A-E, 494 mg, 0.44% dry weight), which was found to contain the majority of the material, was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyol G (47, 100 mg, 0.09% dry wt.).

The Bahamas sample (84-090, 145.7 g dry wt.) was frozen immediately after collection and stored frozen for two weeks prior to extraction. The sponge was then steeped in methanol at 4°C for one week. The solvent was decanted and the sponge was soaked in fresh methanol for 1 day. The combined methanol extracts were evaporated to dryness and then

dissolved in water (400 mL). The aqueous material was extracted with hexane (2 x 100 mL), dichloromethane (2 x 400 mL), and ethyl acetate (3 x 400 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated.

The ethyl acetate extract (643 mg, 0.44% dry wt.), which contained the compounds of interest, was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyol G (47, 100 mg, 0.07% dry wt.) and siphonodictyal E (48, 70 mg, 0.05% dry wt.).

Siphonodictyol G (47): $[\alpha]_{578}$ -24.92 (c 0.65), $[\alpha]_{546}$ -28.02 (c 0.65, MeOH); UV (MeOH) 282 nm (ε 1440), 276 nm (ε 1390), 220 nm, sh (ε 6875), 212 nm (ε 8340); UV (MeOH+KOH) 299 nm (ε 1700), 284 nm (ε 1550), 244 nm (ε 2950), 218 (ε 10,275); IR (KBr) 3450 br, 1250, 1050 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD) δ 0.86 (s, 3 H), 1.04 (d, 3 H, J = 7 Hz), 1.05 (s, 3 H), 2.79 (d, 1 H, J = 14 Hz), 2.83 (d, 1 H, J = 14 Hz), 4.34 (bs, 1 H), 4.38 (bs, 1 H), 4.50 (s, 2 H), 6.63 (d, 1 H, J = 1.7 Hz), 6.83 (d, 1 H, J = 1.7 Hz); ^{13}C NMR (CD_3OD) δ 161.3 (s), 150.7 (s), 140.5 (s), 139.2 (s), 135.2 (s), 123.4 (d), 115.3 (d), 103.1 (t), 64.9 (t), 49.4 (d), 43.2 (s), 41.3 (s), 38.8 (t), 37.9 (t), 37.4 (d), 34.1 (t), 29.3 (t), 28.9 (t), 24.2 (t), 21.1 (q), 18.4 (q), 18.1 (q); high resolution fast atom bombardment mass measurement, observed m/z 469.1633, $C_{22}H_{31}O_6SNa_2$ (M^++1+Na) requires 469.1637.

Siphonodictyal E (48): UV (MeOH) 366 nm (ε 1600), 276 nm (ε 5500); IR (KBr) 3400 br, 1710, 1650 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD) δ 1.14 (d, 3 H, J = 7 Hz) 1.38 (m, 3 H), 1.57 (bs, 3 H), 1.60 (m, 1 H), 1.70

(bs, 3 H), 1.96 (t, 2 H, J = 7 Hz), 2.04 (t, 2 H, J = 7 Hz), 2.10 (q, 2 H, J = 7 Hz), 2.41 (m, 1 H), 3.47 (d, 2 H, J = 7 Hz), 5.09 (t, 1 H, J = 7 Hz), 5.27 (t, 1 H, J = 7 Hz), 6.96 (s, 1 H), 10.27 (s, 1 H); ^{13}C NMR (CD_3OD) δ 198.0 (d), 180.8 (s), 149.4 (s), 145.0 (s), 143.9 (s), 137.5 (s), 135.8 (s), 128.0 (s), 125.5 (d), 123.9 (d), 123.9 (d), 116.6 (s), 40.8 (t), 40.5 (d), 40.5 (t), 34.4 (t), 23.8 (t), 27.5 (t), 26.5 (t), 17.6 (q), 16.3 (q), 15.9 (q); high-resolution mass measurement, observed m/z 390.2076 $C_{22}H_{30}O_6$ requires 390.2042.

Methylation of Siphonodictyal E (48): Siphonodictyal E (8 mg) was dissolved in dry acetone (10 mL) and treated with methyl iodide (1 mL) and potassium carbonate. The reaction was stirred at 80°C for 8 hours. The mixture was poured into acidified ice water (20 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were dried over sodium sulfate and the solvent evaporated to leave a yellow oil. The product was purified by HPLC on μ-Partisil using 30% ethyl acetate in hexane as eluant to obtain 49 (2.5 mg, 28% theoretical yield). IR (CCl_4) 1745, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (d, 3 H, J = 7 Hz), 1.36 (m, 4 H), 1.57 (bs, 3 H), 1.71 (bs, 3 H), 1.95 (t, 2 H, J = 7 Hz), 2.07 (m, 4 H), 2.44 (m, 1 H), 3.29 (d, 2 H, J = 7 Hz), 3.66 (s, 3 H), 3.82 (s, 3 H), 3.97 (s, 3 H), 5.11 (t, 1 H, J = 7 Hz), 5.29 (t, 1 H, J = 7 Hz), 7.06 (s, 1 H), 10.29 (s, 1 H), 11.47 (s, 1 H); mass spectrum, m/z 432 (M^+).

Siphonodictyon coralliphagum forma typica was collected by hand using SCUBA at Chub Cay, the Bahamas (-20 m) in August, 1983 and Carrie Bow Cay, Belize (-20 m) in November, 1983. The Bahamas sample consisted of only the oscular chimneys, while the Belize samples included both

oscular chimneys and sponge from the interior of the coral heads.

The Bahamas sample (83-185, 15.0 g dry wt.) was frozen immediately after collection and stored frozen for 4 months. The sponge was then steeped in methanol for two weeks at 4°C. The solvent was decanted and the sponge was washed with fresh methanol. The combined methanol extracts were evaporated to dryness and then dissolved in water (400 mL). The aqueous material was extracted with hexane (2 x 400 mL), dichloromethane (2 x 400 mL), and ethyl acetate (3 x 400 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated to obtain 40 mg (0.26% dry wt.) of the hexane extract, 60 mg (0.4 % dry wt.) of the dichloromethane extract and 1.145 g (7.63% dry wt.) of the ethyl acetate extract.

The ethyl acetate extract was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyal B (8, 512 mg, 3.41% dry wt.), siphonodictyol G (47, 76 mg, 0.51% dry wt.), and siphonodictyal E (48, 37 mg, 0.25% dry wt.).

Eight samples were collected in Belize (83-258 A-H). Each sample was stored in methanol for 1-2 months and then worked up in an identical manner. The solvent was decanted and the sponge was washed with fresh methanol. The combined methanol extracts were evaporated to dryness and then dissolved in water (200-400 mL). The aqueous material was extracted with hexane (2 x 300 mL), dichloromethane (2 x 300 mL), and ethyl acetate (3 x 400 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated. Each extract was examined by ^{1}H NMR and those extracts which were identical were

combined. The hexane and dichloromethane extracts contained very little material and were not further investigated. The following ethyl acetate extracts were combined: 83-258 A, G and H, and 83-258 B, C, and D. Sample 83-258 E was too small to work on.

The ethyl acetate extract (83-258, A, G and H, 366 mg, 1.16% dry wt.) was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyal B (8, 67 mg, 0.21% dry wt.). Siphonodictyol G (47, 12 mg, 0.04% dry wt.) and siphonodictyal E (48, 10 mg, 0.03% dry wt.) were present in two earlier fractions and were purified by thin layer chromatography on C_8 reversed phase plates using methanol/water (7/3) as eluant.

The ethyl acetate extract (83-258 B, C and D, 1.107 g, 1.49% dry wt.) was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant. One pure compound, siphonodictyoic acid (51, 7 mg, 0.01% dry wt.) was obtained from this column. A fraction containing siphonodictyal E (48, 25 mg, 0.03% dry wt.) was further purified by thin layer chromatography on C_8 reversed phase plates. Siphonodictyal B (8, 80 mg, 0.11% dry wt.) could be crystalized from a fraction that also contained siphonodictyol G (47, 90 mg, 0.12% dry wt.) which was purified by thin layer chromatography on C_8 reversed phase plates using methanol/water (7/3) as eluant.

The ethyl acetate extract (83-258 F, 531 mg, 1.06% dry wt.) was chromatographed as above to obtain siphonodictyl B (8, 100 mg, 0.20% dry wt.), siphonodictyol G (47, 80 mg, 0.16% dry wt.), and siphonodictyal E (48, 18 mg, 0.04% dry wt.).

Siphonodictyoic Acid (51): $[\alpha]_{578}$ -5.69 (c 0.25, MeOH), $[\alpha]_{546}$ -12.60 (c 0.25, MeOH); UV (MeOH) 294 nm (ε 1650), 245 nm (ε 3340), 216 nm (ε 8400); UV (MeOH+KOH) 295 nm (ε 1640), 244 nm, sh (ε 3710), 216 nm (ε 8800); IR (KBr) 3450 br, 1710, 1250, 1050 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD) δ 0.87 (s, 3 H), 1.06 (s, 3 H), 1.07 (d, 3 H, J = 7 Hz), 2.19 (m, 2 H), 2.33 (m, 1 H), 2.82 (d, 1 H, J = 14 Hz), 2.89 (d, 1 H, J = 14 Hz), 4.36 (bs, 1 H), 4.39 (bs, 1 H), 7.42 (d, 1 H, J = 1.9 Hz), 7.46 (d, 1 H, J = 1.9 Hz); ^{13}C NMR (CD_3OD) δ 169.7 (s), 161.3 (s), 151.2 (s), 145.3 (s), 135.6 (s), 128.2 (s), 126.7 (d), 118.0 (d), 103.2 (t), 49.3 (d), 43.3 (s), 41.3 (s), 38.6 (t), 38.0 (t), 37.5 (d), 34.2 (t), 29.2 (t), 28.9 (t), 24.4 (t) 21.1 (q), 18.5 (q), 18.1 (q); high resolution mass measurement, observed m/z 358.2145, $C_{22}H_{30}O_4$ requires 358.2144.

Siphonodictyon coralliphagum from the Pacific was collected from two locations, Ponape (-15 m) in January, 1983 and Nini Island, Kwajalein (-11 m) in December, 1983. The Ponape sample (83-076) consisted of only the portion of the sponge that was protruding from the coral heads, while the Kwajalein sample was mainly the sponge from inside the coral heads.

The Kwajalein sample (84-030, 55.1 g dry wt.) was frozen immediately after collection. The sample was then placed in methanol and stored at 4°C for one month. The solvent was decanted and the sponge was washed with fresh methanol. The combined methanol extracts were evaporated to an aqueous suspension (500 mL). The aqueous material was extracted with hexane (2 x 300 mL), dichloromethane (2 x 300 mL) and ethyl acetate (3 x 300 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated. Each extract was

examined by ^1H NMR.

The ethyl acetate extract (485 mg, 0.88% dry wt.) was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyal C (52, 127 mg, 0.23% dry wt.) and siphonodictyal E (48, 30 mg, 0.05% dry wt.).

The Ponape sample (83-076, 245.9 g dry wt.) contained large quantities of mucus (12.3 g dry wt.) which was collected separately. Both the sponge and the mucus were frozen immediately after collection. The frozen sponge was lyophilized and the dried sponge extracted successively with dichloromethane (2 x 1 L), ethyl acetate (2 x 1 L) and methanol (3 x 1 L). The methanol extract (44.7 g, 18.19% dry wt.) contained the compounds of interest, and was dissolved in water (400 mL). The aqueous material was extracted with ethyl acetate (5 x 300 mL) to obtain a yellow gum (6.784 g, 2.76% dry wt.).

The ethyl acetate extract (3.392 g) was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyal C (52, 260 mg, 0.11% dry wt.) and siphonodictyal D (54, 650 mg, 0.26% dry wt.). A fraction containing siphonodictyal E (48, 120 mg, 0.05% dry wt.) was purified by thin layer chromatography on C_8 reversed phase plates. Siphonodictyol H (56, 105 mg, 0.04% dry wt.) was present with the corresponding aldehyde 57 as a 3:1 mixture. Purification by thin layer chromatography on C_8 reversed phase plates resulted in the loss of the aldehyde 57.

The frozen mucus was lyophilized and the dried material dissolved in water (100 mL). The aqueous material was extracted with ethyl

acetate (3 x 100 mL) to obtain a brown gum (60 mg, 0.49% dry wt.). Analysis of the ^{1}H NMR of this extract indicated that it contained both siphonodictyal C (52) and D (54).

Siphonodictyal C (54): $[\alpha]_{578}$ -23.60 (c 0.47, MeOH), $[\alpha]_{546}$ -30.47 (c 0.47, MeOH), UV (MeOH) 368 nm (ε 1600), 276 nm (ε 5800), 228 nm, sh (ε 7900), 209 nm (ε 11,880); UV (MeOH+KOH) 406 nm (ε 1800), 291 nm (ε 4470), 244 nm (ε 7840), 212 nm (ε 16,360); IR (KBr) 3500 br, 1645, 1240, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.88 (s, 3 H), 0.89 (s, 3 H), 0.93 (s, 3 H), 1.48 (bs, 3 H), 1.94 (m, 2 H), 2.28 (m, 1 H), 2.48 (dd, 1 H, J = 3, 15 Hz), 2.60 (dd, 1 H, J = 9, 15 Hz), 5.32 (bs, 1 H), 7.01 (s, 1 H), 10.17 (s, 1 H); ^{13}C NMR (CD_3OD) δ 198.1 (d) 149.1 (s), 144.8 (s), 143.7 (s), 136.5 (s), 130.5 (s), 123.4 (d), 122.9 (d), 116.6 (s), 56.4 (d), 51.6 (d), 43.4 (t), 40.8 (t), 37.9 (s), 33.9 (s), 33.8 (q), 27.2 (q), 24.7 (t), 22.6 (q), 22.4 (q), 19.9 (t), 14.4 (q); high resolution mass measurement, observed m/z 358.2122 $C_{22}H_{30}O_4$ requires 358.2144.

Methylation of Siphonodictyal C (52): Siphonodictyal C (54) (8 mg) was dissolved in dry acetone (6 mL) and treated with methyl iodide (1 mL) and potassium carbonate (~100 mg). The reaction was stirred at 80°C for 20 hours, cooled and poured into acidified ice water (10 mL). The aqueous material was extracted with ethyl acetate (3 x 15 mL) and the combined organic extracts dried over anhydrous sodium sulfate. The solvent was evaporated to leave a yellow oil which was purified by HPLC on μ-Partisil with 20% ethyl acetate in hexane as eluant to obtain the trimethoxy derivative 53 (2.0 mg, 22.4% theoretical yield). ^{1}H NMR ($CDCl_3$) δ 0.89 (s, 3 H), 0.90 (s, 3 H), 0.92 (s, 3 H), 1.43 (bs, 3 H), 1.92 (m, 2 H), 2.31 (m, 1 H), 2.66 (m, 2 H), 3.75 (s, 3 H), 3.88 (s, 3

H), 3.92 (s, 3 H), 5.40 (bs, 1 H), 7.06 (s, 1 H), 10.41 (s, 1 H); mass spectrum, m/z 400 (M^+).

Siphonodictyal D (54): m.p. 131-132°C; $[\alpha]_{478}$ -56.54 (c 1.12, MeOH), $[\alpha]_{546}$ -67.65 (c 1.12, MeOH); UV (MeOH) 367 nm (ε 2700), 276 nm (ε 9350), 228 nm, sh (ε 12,360), 214 nm (ε 14,110); UV (MeOH+KOH) 401 nm (ε 2965), 288 nm (ε 6620), 244 nm (ε 13,165), 212 nm (ε 17,530); IR (KBr) 3450 br, 1650, 1230, 1050 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD) δ 0.76 (bs, 3 H), 1.01 (d, 3 H, J = 7 Hz), 1.08 (s, 6 H), 5.41 (bs, 1 H), 6.92 (bs, 1 H), 10.28 (s, 1 H); ^{13}C NMR (CD_3OD) 194.6 (d), 155.7 (s), 150.0 (s), 148.8 (s), 137.7 (s), 130.8 (d), 118.7 (s), 117.2 (d), 112.4 (s), 44.6 (d), 43.2 (d), 40.1 (s), 38.6 (s), 35.2, 33.2 (t), 31.5, 31.3, 31.0 (q), 28.0, 24.8 (t), 23.0 (q), 16.8 (q); high resolution fast atom bombardment mass measurement, observed 483.1427 $(M+1+Na)^+$, $C_{22}H_{29}O_7SNa_2$ requires 483.1429.

Methylation of Siphonodictyal D (54): Siphonodictyal D (54) (20 mg) was dissolved in dry acetone (10 mL) and treated with methyl iodide (0.5 mL) and potassium carbonate. The reaction was stirred at 80°C for 3 hours. The mixture was poured into acidified ice water (15 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were dried over anhydrous sodium sulfate and the solvent evaporated to leave a yellow oil. The product was purified by HPLC on μ-Partisil using 25% ethyl acetate in hexane as eluant to obtain 55 (6.0 mg, 27% theoretical yield); ^1H NMR ($CDCl_3$) δ 0.76 (bs, 3 H), 0.97 (d, 3 H, J = 7 Hz), 1.09 (s, 6 H), 3.81 (s, 3 H), 3.99 (s, 3 H), 5.44 (bs, 1 H), 6.97 (bs, 1 H), 10.30 (s, 1 H).

Siphonodictyol H (56): no rotation observed; UV (MeOH) 280 nm (ε 1550), 274 nm (ε 1500), 213 nm (ε 9100); UV (MeOH+KOH) 292 nm (ε 1575), 282 nm (ε 1650), 244 nm (ε 3100), 218 nm (ε 10,900); IR (KBr) 3450 br, 1250, 1045 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD) δ 0.85 (s, 3 H), 0.91 (s, 3 H), 1.00 (s, 3 H), 1.45 (bs, 3 H), 2.07 (dd, 1 H, J = 6, 17 Hz), 2.17 (m, 1 H), 3.41 (d, 1 H, J = 18 Hz), 3.50 (d, 1 H, J = 18 Hz), 4.50 (s, 2 H), 6.56 (d, 1 H, J = 1.8 Hz), 6.69 (d, 1 H, J = 1.8 Hz); ^{13}C NMR (CD_3OD) δ 151.3 (s), 139.9 (s), 138.9 (s), 137.6 (s), 129.8 (s), 119.5 (d), 114.9 (d), 65.2 (t), 53.3 (d), 43.0 (t), 40.2 (s), 37.2 (t), 34.9 (t), 34.3 (s), 33.8 (q), 28.6 (t), 22.2 (q), 20.7 (q), 20.5 (q), 20.3 (t), 19.9 (t); high resolution fast atom bombardment mass measurement, observed m/z 469.1633 $(M+1+Na)^+$, $C_{22}H_{31}O_6SNa_2$ requires 469.1637.

Experimental Section, Chapter 3

An undescribed Siphonodictyon species was collected by hand using SCUBA from the fringing reef west of Koror, Palau (-20 m) in March, 1983. Only the oscular chimneys could be collected. The sponge was placed in methanol immediately after collection and stored at 4°C for two months. The solvent was decanted, the sponge washed with fresh methanol and the combined extracts were evaporated to dryness. The resulting material was dissolved in water (250 mL) and extracted with ethyl acetate (3 x 250 mL). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated to obtain a clear oil (1.393 g, 4.25% dry wt.).

The ethyl acetate extract was chromatographed on Sephadex LH-20 using methanol as eluant. Fractions showing antimicrobial activity against Staphylococcus aureus and Bacillus subtilis were combined and rechromatographed on Sephadex LH-20 with a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictidine (77, 350 mg, 1.06% dry wt.).

Siphonodictidine (77): UV (MeOH) 270 nm (ε 1350); IR ($CHCl_3$) 3350 br, 1580, 1210 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.58 (bs,3 H), 1.68 (bs, 3 H), 1.97 (s, 3 H), 2.05 (m, 2 H), 2.12 (m, 2 H), 3.22 (s, 2 H), 3.78 (t, 2 H, J = 7 Hz), 5.18 (t, 1 H, J = 7 Hz), 5.21 (t, 1 H, J = 7 Hz) 5.86 (s, 1 H), 7.06 (s, 1 H), 7.80 (br t, 1 H); ^{13}C NMR ($CDCl_3$) δ 157.4 (s), 153.9 (s), 141.2 (s), 137.4 (d), 132.2 (s), 125.5 (d), 120.3 (s), 117.8 (d), 108.6 (d), 39.6 (t), 38.2 (t), 26.2 (t),16.3 (q), 15.9 (q), 9.6 (q); high resolution mass measurement, observed m/z 275.2001, $C_{16}H_{25}N_3O$

requires 275.1998.

Condensation of Siphonodictidine (77): Siphonodictidine (45 mg, 0.16 mMoles) was refluxed (100°C) with 2,4-pentadione (1.5 mL) and sodium bicarbonate (50 mg) in 95% ethanol (10 mL) for 4 hours. The solution was cooled, poured into acidified ice water (20 mL) and extracted with ethyl ether (3 x 15 mL). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated to obtain a yellow oil. The 4,6-dimethylpyrimidine derivative 78 (5.0 mg, 8.9% theoretical) was obtained by thin layer chromatography of the crude product on a silica gel prep plate. ^{1}H NMR ($CDCl_3$) δ 1.59 (bs, 3 H), 1.70 (bs, 3 H), 1.98 (s, 3 H), 2.02 (t, 2 H, J = 7 Hz), 2.13 (q, 2 H, J = 7 Hz) 2.28 (s, 6 H), 3.22 (s, 2 H), 3.99 (t, 2 H, J = 7 Hz), 4.88 (bs, 1 H), 5.22 (t, 1 H, J = 7 Hz), 5.30 (t, 1 H, J = 7 Hz), 5.87 (s, 1 H), 6.30 (s, 1 H), 7.09 (s, 1 H); high resolution mass measurement, observed m/z 339.2311 $C_{21}H_{29}N_3O$ requires 339.2308.

Experimental Section, Chapter 4

Antimicrobial Assays. Sterile paper discs (6.5 mm) were saturated with methanolic solutions of the compounds to give specific concentrations (100 µg/disc, 50 µg/disc, 25 µg/disc). The discs were placed on agar plates that had been inoculated with one of eight microorganisms (the five human pathogenic bacteria Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, and Pseudomonas aeruginosa, the two marine bacteria Beneckea harveyi and Vibrio anguillarum and the yeast Candida albicans). The plates were incubated overnight at 37°C (30°C for the marine bacteria) and the diameters of the zones of inhibition measured.

Calcium Chelation Assay: The compounds were dissolved in butanol (15 mL) and the resulting solutions were used to extract 5 ppm Ca^{2+} solutions (20 mL). The mixtures were centrifuged and the two layers were separated. The aqueous phases were washed with fresh butanol (15 mL) and then analyzed by atomic absorption. A 5 ppm Ca^{2+} standard solution (20 mL) was extracted with butanol (2 x 15 mL) and analyzed for use as a control.