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Marine natural products: Chemistry and Chemosystematics of the Gorgonian Genus Eunicea and Exploratory Studies of the Secondary Metabolites of Marine Fungi

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University of California, San Diego, 1989

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SAN DIEGO

Marine Natural Products: Chemistry and Chemosystematics of the Gorgonian Genus *Eunicea* and Exploratory Studies of the Secondary Metabolites of Marine Fungi

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

by

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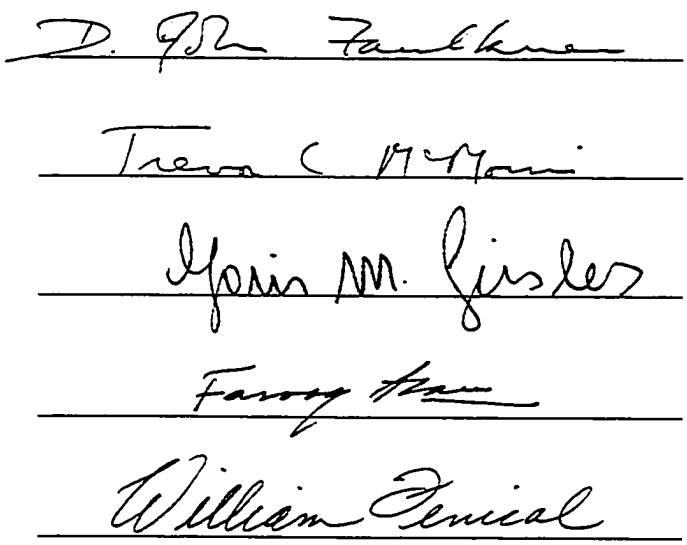
1989

The dissertation of Jongheon Shin is approved, and it is acceptable in quality and form for publication on microfilm:



Chairman

University of California, San Diego

1989

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PUBLICATIONS

Shin, J., Paul, P.J. and Fenical, W., "New macrocyclic α- and γ- pyrones from the marine red alga *Phacelocarpus labillardieri*," *Tetrahedron Lett.*, **1986**, 27, 5189.

Shin, J. and Fenical, W., "Isolation of gliovictin from the marine deuteromycete *Asteromyces cruciatus*," *Phytochemistry*, **1987**, 26, 3347.

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Shin, J., Park, M. and Fenical, W., "The Junceellolides, new anti-inflammatory diterpenoids of the briarane class from the Chinese gorgonian *Junceella fragilis*," *Tetrahedron*, **1989**, 45, 1633.

FIELDS OF STUDY

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ABSTRACT OF THE DISSERTATION

Marine Natural Products: Chemistry and Chemosystematics
of the Gorgonian Genus *Eunicea* and Exploratory
Studies of the Secondary Metabolites from Marine Fungi

by

Jongheon Shin
Doctor of Philosophy in Oceanography
Scripps Institution of Oceanography
University of California, San Diego, 1989
Professor William H. Fenical, Chairman

Secondary metabolites of the Caribbean gorgonian genus *Eunicea* were extensively investigated through the systematic collection and assortment based on thin layer chromatographic analysis. In addition, the chemical characters of *Eunicea* species were compared to the morphological classifications. In total, 792 individual colonies were collected from various locations. Based upon the TLC characters, 780 colonies were divided into 11 chemotypes which cover 8 of 12 taxonomically defined species and 4 new species.

Detailed chemical investigation resulted in the isolation of 39 new metabolites, along with 9 previously described compounds. Diterpenoids were the major group of

metabolites, and cembranes were the most commonly encountered class. Other diterpenoids were dolabellanes, cubitanes, asperketals, fuscol and fuscol glycosides. Metabolites of three unprecedented classes were also isolated: C_{28} reduced quinones, trisnorditerpenoids and a diterpene glycoside of the "extended eremophilane" class. The irregular diterpenoid cubitanes were determined to be formed by a photochemically induced 1,3-acyl migration of a cembrane precursor.

Several chemotypes were collected from more than one location. Each chemotype contained only one or two classes of very distinct metabolites. In the case where metabolites of a single class were isolated from more than one chemotype, there were great structural similarities among metabolites from the same chemotype, while metabolites from different chemotypes often showed very distinct patterns of functionalization.

Chemical characteristics of each chemotype were compared to the morphological classification. The chemical contents were clearly different between the *Eunicea* subgenera, *Eunicea sensu strictu* and *Euniceopsis*. *Eunicea s.s.* was a chemically homogeneous group, all containing cembrane lactones. In contrast, *Euniceopsis* showed species-specific distribution of metabolites. Comparison of chemical contents revealed that for the chemosystematics of the *Eunicea*, types and distributions of functional groups were as important characters as the carbon skeletons of metabolites.

One hundred twenty one marine fungal strains were either isolated from various habitats or obtained from mycologists. The fungi were cultivated in liquid media. Thirty eight extracts showed significant anti-microbial activities and/or cytotoxicity. Based upon the results of bioactivity tests, TLC analysis, and proton NMR spectroscopic analysis of the extracts, several strains were chemically investigatigated.

From *Asteromyces cruciatus*, gliovictin, a metabolite of the gliotoxin class was

isolated. Also, several trichothecenes of the verrucarin and roridin classes were isolated from an unknown fungus. In addition, a few small-sized metabolites were isolated. The future of marine fungi for chemical investigation is discussed.

Part I.

Chemistry and Chemosystematics of the Gorgonian Genus *Eunicea*

Chapter I

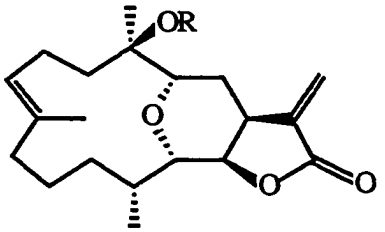
Introduction

Gorgonians (Octocorallia; Gorgonacea) are one of the most prolific and conspicuous elements of the shallow water benthic invertebrate communities of the West Indian region, representing 38% of the total known fauna.[1] West Indian gorgonians are dominated by the two families Plexauridae (42% of total species) and Gorgonidae (40%). Of the seven known plexaurid genera, *Eunicea* is taxonomically divided into twelve species, the largest in terms of number of recognized species.[1] Moreover, this genus includes some of the most important reef-dwelling gorgonians of the west Atlantic. Including its type organism, *Eunicea tourneforti*, several species range over most of the tropical northwestern Atlantic Ocean. In addition, *Eunicea* species are frequently dominant in the number of individual colonies within a given area.[2,3]

Caribbean octocorals are very rich with secondary metabolites.[4-7] Several chemical investigations led to the isolation of numerous secondary metabolites, some of which appear to possess defensive properties.[8-10] The predominant metabolites are terpenoids and mixed biosynthesis products containing isoprene units. The great number and structural diversity of these compounds are beyond the scope of review in this dissertation.

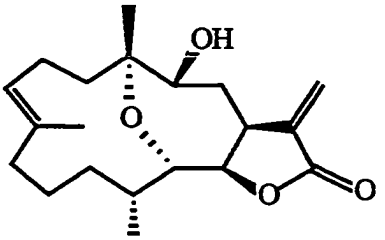
In the genus *Eunicea*, all of the reported metabolites are sesqui- and diterpenoids (Figure 1). Since Ciereszko and coworkers isolated eunicin (**1**) from *E. mammosa* collected at Bimini,[11] several cembrane lactones (**2** - **9**) have been isolated from various

Figure 1. Known metabolites from gorgonians of the genus *Eunicea*.

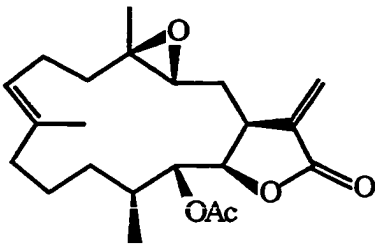


1 eunicin (R = H) — *E. mammosa*[11], *E. succinea*[17]

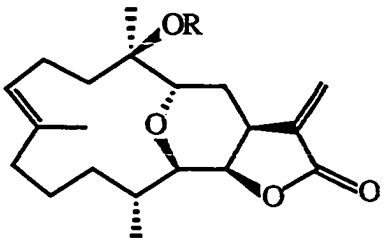
2 eunicin acetate (R = Ac) — *E. succinea*[17]



3 jeunicin — *E. mammosa*[14]



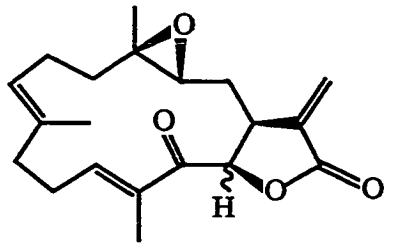
4 eupalmerin acetate — *E. mammosa*[13,17], *E. succinea*[13,17], *E. palmeri*[13,17]



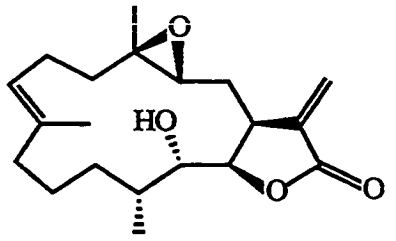
5 cueunicin (R = H) — *E. mammosa*[12]

6 cueunicin acetate (R = Ac) — *E. mammosa*[12]

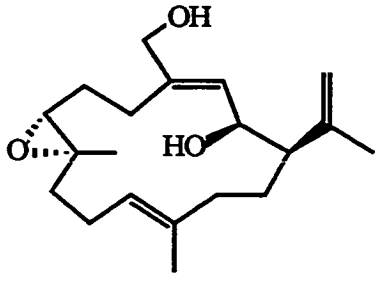
Figure 1. (continued)



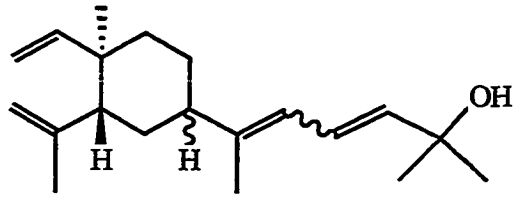
7 peunicin (14α-H) *E. succinea*[15,16]
8 epipeunicin (14β-H) *E. succinea*[16]



9 12,13-bisepieupalmerin *E. succinea*[17]

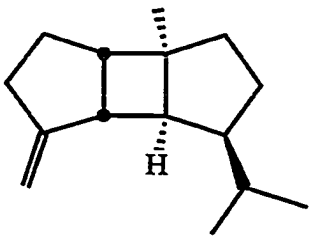


10 asperdiol *E. asperula*[19]
E. tourneforti[19]

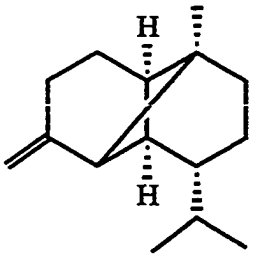


11 fuscol *E. fusca*[21]

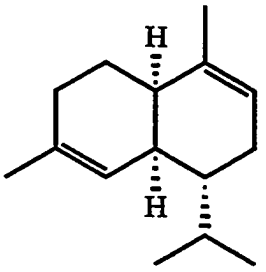
Figure 1. (continued)



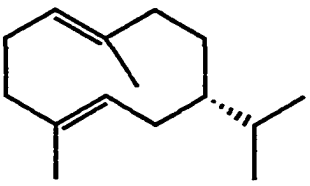
12 (+) - β - epibourbonene — *E. mammosa*[17]



13 (+) - β - copaene — *E. mammosa*[17], *E. palmeri*[24]

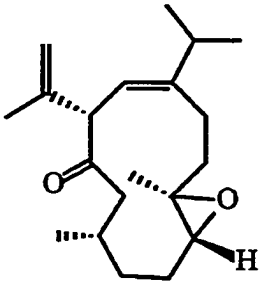


14 (+) - α - muurolene — *E. palmeri*[24], *E. mammosa*[17,22,24]

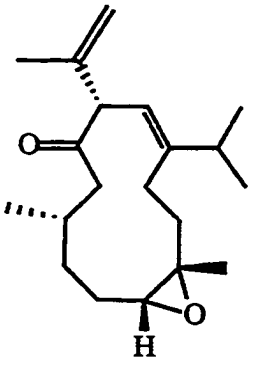


15 (-) - germacrene A — *E. mammosa*[22,23]

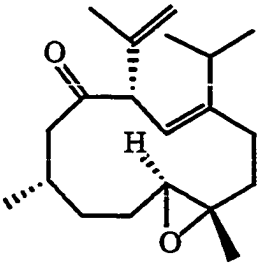
Figure 1. (continued)



16 calyculone A — *E. calyculata*[26]

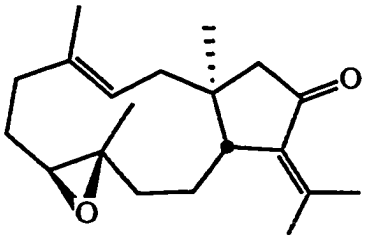


17 calyculone B — *E. calyculata*[26]

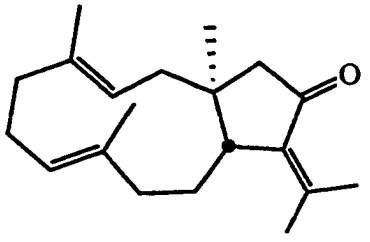


18 calyculone C — *E. calyculata*[26]

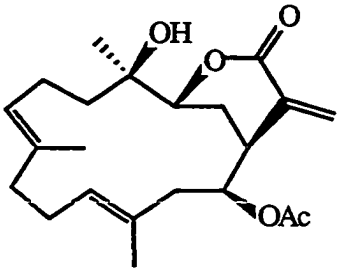
Figure 1. (continued)



19 *E. calyculata*[25]



20 *E. calyculata*[25]



21 crassin acetate *E. calyculata*[25]

locations.[12-18] Asperdiol (**10**) is the only cembranoid which does not possess the lactone ring.[19,20] Fuscol (**11**) has a carbon skeleton related to the sesquiterpene, elemane, apparently derived from a different biosynthetic pathway.[21] Also, volatile sesquiterpene hydrocarbons (**12** - **15**) have been isolated.[17,22-24] Recently, Look and coworkers isolated diterpenoids (**16** - **21**) of three different carbon skeletons, cubitane, dolabellane and cembrane from *E. calyculata* collected in the Belize and Bahamas.[25,26] Their interesting work revealed the possibility that the secondary metabolites from *Eunicea* might be much more diverse than generally believed.

The major problem which hindered the chemical investigation of *Eunicea* octocorals is the difficulty of insuring homogeneous collection. Comparison of chemical characters of individual colonies is very helpful for obtaining chemically homogeneous collections. There are few techniques available for this purpose. Kashman and coworkers used a Gas-Liquid chromatographic (GLC) technique to "finger print" Mediterranean soft corals.[27] Caccamese and coworkers used Gas chromatography-mass spectrometry (GC-MS) for the separation of three different collections of the red alga *Laurencia* which were morphologically very similar but chemically distinct.[28] Fenical and Norris used a thin layer chromatographic (TLC) tenique for the separation of three chemically different groups in the *Laurencia "pacifica"* complex.[29] The use of TLC is particulary useful for distinguishing between chemically different organisms and ensuing a particular organism. Since most of the Caribbean gorgonians are very rich with secondary metabolites, TLC is an ideal tool for this purpose. Burch exclusively used TLC to distinguish between ten species of the gorgonian genus *Pseudopterogorgia*.[30]

Gorgonians are among the dominant members of benthic invertebrate fauna in the West Indies.[1] Despite the obvious importance of gorgonians in general, and of *Eunicea* in particular, to Caribbean reef ecosystems, information on their ecology is scarce. The paucity of ecological studies of Caribbean gorgonians have been attributed to the difficulty

of identifying specimens in the field.[31] In particular, substantial numbers of *Eunicea*, one of taxonomically most dubious gorgonians, have been reported without speciation.[10,32-34] The success of ecological studies can only be expected if based upon reliable taxonomic classification. Despite successive revisions[35-38] however, taxonomy of the octocorals is still large by subjective, and "it is not an exaggeration to state that we still do not know what an alcyonarian species is".[1] This taxonomic uncertainty is due to the shortage of specimens for defining a taxon and the lack of reliable systematic characters. The definition of a taxon without sufficient number of specimens will, regardless to say, led to confusion on identifying taxonomic variants. The systematic characters which are regarded as being of practical value by octocoral systematists fall into two categories; colonial morphological features and microscopic features.[1] The features of colonial morphology used for classification are colony size, shape and color, the pattern of branching, and the axis structure. Also, microscopic features such as the color, shape, and distribution of coenenchymal sclerities are frequently used for classical taxonomy. However, the degree to which variation in these characters reflects genetic difference between colonies is unknown.[1] Moreover, growth forms of colonies are frequently affected by depth, current and other physical factors.[1,8,39-42] Microscopic features can be also affected by environmental factors. In *Eunicea clavigera* and *Gorgonia mariae*, spicular morphology in the growing tips changed upon transplantation to a shallower depth.[41] Although their susceptivity to environmental factors does not rule out their importance for classification, the range of variation and extent of environmental influence on morphological characters must be well known before they can be used successfully.[1,42]

When classical taxonomy can not define a taxon successfully, chemosystematics can be a very useful tool. Since it emerged as a subdiscipline of organic chemistry a few decades ago, chemical systematics has been developed to a point that even allows addressing evolutionary studies.[43] Chemosystematics have contributed greatly to the

taxonomy of terrestrial plants, lichens, and colonial insects.[43-49] In some taxa (bacteria and lichens), the use of chemical characters is the entire basis for classification. Although marine natural products chemistry has progressed to the point where it is often easier to identify compounds from colonial marine organisms than it is to identify the animal from which the compounds were isolated, chemosystematics has been applied only to the taxonomy of limited numbers of marine organisms; algae and sponges (Porifera), dinoflagellets, octocorals, and holothurians.[27-29,42,50-60]

Chemosystematics are in the most part used to clarify taxonomic assignments where a dispute exists. Chemical clues can assist in indicating taxonomic anomalies and provide impetus for closer morphological scrutiny in order to reaffirm or alter existing classifications. Rarely is a taxonomic assignment based solely on chemical data, because the greatest acceptance of chemical data as taxonomic characters can be achieved only when they have been used in conjunction with other characters as part of a comprehensive re-evaluation. As in the case of some angiosperms, taxonomy based solely on either morphological or chemical characters often leads to the unexplainable anomalies.[61]

Due to the their great diversity, the secondary metabolites have been the most useful chemical compounds in chemosystematic analysis.[61] They consists of indeed wide variety of chemicals, including flavonoids, alkaloids, terpenoids, anthraquinones, polyacetylenes, saponins, phyerythlins, phenolics, etc. In the Caribbean octocorals, and the genus *Eunicea* in particular, the predominant secondary metabolites are terpenoids.

Analysis of terpenoid compounds has been used with some success in the taxonomy of terrestrial plants and colonial insects, such as bees, ants, and termites.[49] Terpenoids are also successfully used for the chemosystematics of the marine organisms. Fenical and Norris were able to distinguish between three morphologically similar types of the red alga *Laurencia "pacifica"* based on the type of halogenated terpenoids and other halogenated metabolites.[29] Bergquist and Wells applied terpenoids to the global scale

taxonomy of sponges and succeeded in relocating some disputed species.[53] Kashman and coworkers used the volatile sesquiterpene contents as "finger prints" to aid the identification of Mediterranean soft corals *Sinularia* and *Sarcophyton* species.[27] Gerhart approached the phylogeny of gorgonians by terpenoid characters.[42] His cladistic analysis on published chemical data agreed for the most part with classical taxonomy. Although his approach was based on only a limited amount of data, the results showed great potential of chemotaxonomy for the study of the systematics of gorgonians and other colonial animals. More recently, Burch successfully applied terpenoid characters for chemosystematics of the Caribbean gorgonian genus *Pseudopterogorgia*.[30] The result of his work showed remarkable species-specific variations on the terpenoid contents.

Taxonomic study of colonial marine animals (e.g., sponges and octocorals) is notoriously difficult. Related to the taxonomic difficulty, the major obstracle which hinders the progress on the natural products chemistry of these organisms is difficulty of assuring chemically homogeneous collection. The separation of organisms based on their chemical characters, followed by chemical investigation of each group can contribute significantly to both natural products chemistry and taxonomy of the organisms. Taking into account the established techniques of chemical systematics and realizing the problems of classical taxonomy and the difficulty of chemically homogeneous collection, the following techniques were used for chemical and chemosystematic studies of *Eunicea*:

1) Reproducible analysis of gorgonian tissue extracts using thin layer chromatography (TLC) which allowed the unambiguous recognition of all the metabolites used as chemical characters.
2) TLC analysis of individual colonies from collections which differ in both time and location.
3) Classification of individual colonies into chemically consistant groups called chemotypes.

4) Identification of all the secondary metabolites in each chemotypes.

5) Examination of the morphological characters of colonies within chemically consistent groups.

For this work, major collections were made at the Tobago Cays, in July 1986 and in the Florida Keys, in July 1987. Also, minor collections were made at Martinique in July 1986 and at St. Croix Island, in Nobember 1987. The work for part 1), 2), and 3) is described in the general experimental section. Chemical investigations (part 4) of each chemotype resulted in the isolation of 6 known compounds and 39 new compounds. In addition, 3 compounds previously isolated from different octocorals were also isolated from *Eunicea*. The detail of this work is described in chapters 2 - 8. Part 5) was performed by Dr. Frederick M. Bayer, an octocoral taxonomist and curator of marine invertebrates at the Smithonian Institution. The results of this work and discussions are in chapter 9.

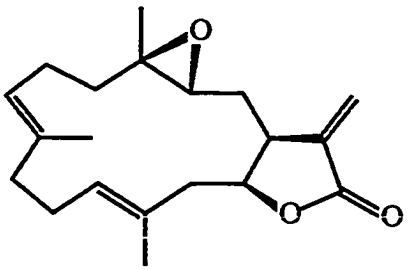
Chapter II

Cembrane lactones from *Eunicea* species (subgenus *Eunicea*)

Gorgonians of the geuns Eunicea were divided taxonomically into 12 distinct species by F. M. Bayer.[1] Upon the basis of morphological affinities, he assigned them into two subgenera; *Eunicea sensu strictu* and *Euniceopsis*. Subgenus *Eunicea* consists of five species, *mammosa* (type), *succinea*, *palmeri*, *laxispica*, and *pinta*. The chemistry of the former three species has been extensively studied by the Oklahoma group who reported several metabolites of the cembrane class from these gorgonians.[11-20] All of the metabolites are structurally similar to each other, and as a common feature possess a γ-lactone with a conjugated exocyclic double bond. Therefore, it seemed likely that the species in the subgenus *Eunicea* s.s. are chemically, if not identical, very closely related with one another. For this work, several collections of species in the subgenus *Eunicea* were made and their constituents were again similar to each other.

The major collection was made at the Tobago Cays in the eastern Caribbean Sea, in July 1986. Despite some variation in their gross morphological features, the results of TLC analyses showed that these gorgonians were chemically homogeneous. Five vouchers were sent to Dr. Bayer, and all were identified as *Eunicea mammosa*. Extraction of the dried gorgonians with dichloromethane gave 35 g of the crude organic extract (from 2 kg, dry gorgonians). Six metabolites were isolated by silica vacuum flash chromatography followed by silica HPLC (Figure 2). Four were the previously reported metabolites eunicin (**1**), eunicin acetate (**2**), 12,13-bisepieupalmerin (**9**) and crassin acetate (**21**). The structures of two new metabolites, **22** and **23**, were elucidated by spectral methods, especially two-dimensional NMR techniques. Eunicin (**1**) was the major metabolite (ca 10% of the crude extract) and the other compounds were isolated in nearly equal amounts (0.8 - 1%).

Figure 2. Cembrane lactones from *E. mammosa*.



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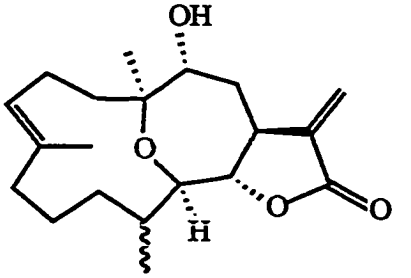

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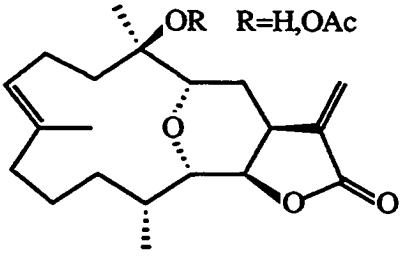
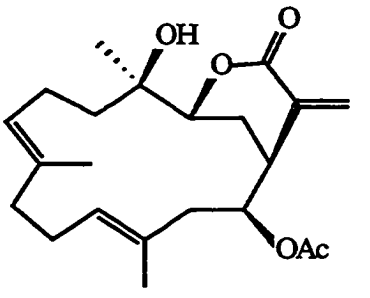
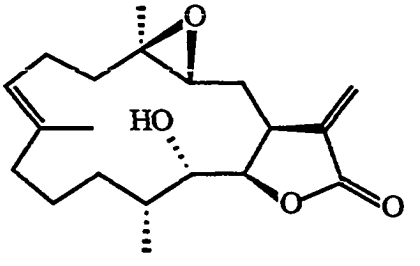


R = H, eunicin (1)

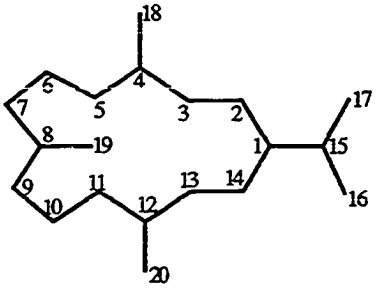
R = Ac, eunicin acetate (2)



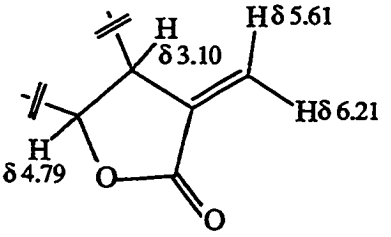
crassin acetate (21)



12,13-bisepieupalmerin (9)



Compound **22** was isolated as an oil by HPLC (20% EtOAc in isooctane) and analyzed for $C_{20}H_{28}O_3$ by high resolution mass and ^{13}C NMR spectrometry. Compound **22** showed a carbonyl peak at δ 169.2 (s) in the ^{13}C NMR spectrum (Table 1). A strong absorption band at 1770 cm^{-1} in the IR spectrum indicated that the carbonyl was part of a γ-lactone. ^1H NMR spectrum showed two low field proton signals at δ 6.21 (1 H, dd, *J*=1.8, 1.4) and 5.61 (1 H, dd, 1.7, 1.4). The deshielded nature of these protons was characteristic of the protons of an exocyclic methylene in conjugation with the lactone carbonyl. A ^1H NMR correlation spectroscopy (COSY) experiment showed that the methylene protons were coupled to a proton at δ 3.10 (brddd, 11.0, 5.8, 1.3) which was also coupled to a low field proton at δ 4.79 (ddd, 10.7, 5.8, 2.7). Thus, all the protons of the γ-lactone were assigned as followes:

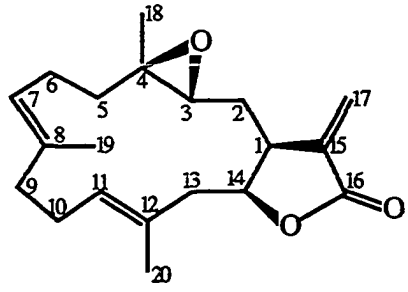


Four carbon signals at δ 134.7 (s), 129.9 (s), 125.7 (d), and 124.5 (d) in the ^{13}C NMR spectrum indicated **22** to possess two additional double bonds. The presence of only end absorption in the UV spectrum revealed that they were not conjugated olefins. The olefinic protons at δ 5.02 (brdd, 7.0, 6.2) and 4.97 (m) in the ^1H NMR spectrum were coupled to methyl protons at δ 1.58 (brs) and 1.68 (brs), respectively. Therefore, the double bonds were both trisubstituted. The carbon NMR spectrum showed two signals at δ 60.2 (s) and 59.2 (d) which were characteristic of an epoxide. The chemical shift of a methyl group at δ 1.31 (d, 1.4) indicated that it was connected to the epoxide. Thus, all of the key functionalities of **22** were confidently identified.

Table 1. ^{1}H and ^{13}C NMR assignments and result of a COLOC experiment with compound **22**.

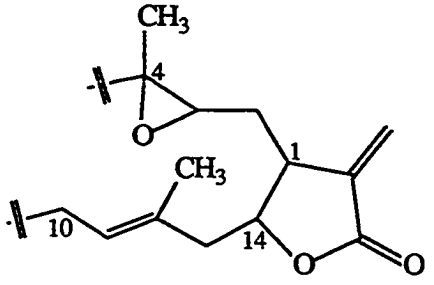
#	proton[a]	carbon[b,c]		COLOC[d]
1	3.10 brddd(11.0,5.8,1.3)	41.0	d	2(1.38), 17(6.21), 17(5.61)
2	2.25 - 2.15[e] 1.38[e]	30.6	t	3
3	2.82 ddd(9.6,3.8,1.2)	59.2	d	2(1.38), 18
4	----	60.2	s	18
5	2.10 - 1.95[e] 1.34[e]	37.5	t	18
6	2.25 - 2.15[e] 2.10 - 1.95[e]	22.5	t	5(1.34)
7	5.02 brdd(7.0,6.2)	124.5	d	5(1.34), 19
8	----	134.7	s	19
9	2.25 - 2.15[e] 2.10 - 1.95[e]	38.7	t	19
10	2.33 m 2.10 - 2.06[e]	24.8	t	—
11	4.97 m	125.7	d	13(2.61), 13(2.42), 20
12	----	129.9	s	13(2.61), 13(2.42), 20
13	2.61 brd(16.8) 2.42 dd(17.0,10.7)	37.2	t	11, 20
14	4.79 ddd(10.7,5.8,2.7)	78.9	d	2(1.38), 13(2.61), 13(2.42)
15	----	140.9	s	2(1.38), 17(6.21), 17(5.61)
16	----	169.2	s	1, 17(6.21), 17(5.61)
17	6.21 dd(1.8,1.4) 5.61 dd(1.7,1.4)	120.8	t	1
18	1.31 d(1.4)	16.8	q	—
19	1.58 brs	15.6	q	—
20	1.68 brs	17.5	q	11, 13(2.61)

Experiments were performed in $CDCl_3$ solutions. [a] Assignments were made by COSY and one-dimensional decoupling experiments. [b] Numbers of attached protons were determined by DEPT experiments. [c] Assignments were made by XHCORR and COLOC experiments. [d] Parameters were optimized for couplings of 6 Hz. [e] Coupling constants were not determined.



22

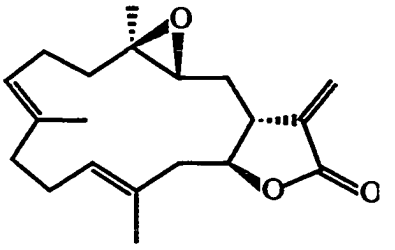
The connectivity of these groups were made by ^{1}H NMR COSY[67] and direct carbon-proton correlation (XHCORR)[69,70] experiments. Both of the protons at δ 3.10 (C-1 of the lactone) and 2.82 (epoxide) were coupled to the same methylene protons at δ 2.25 - 2.15 and 1.38 (C-2). Therefore, the position of the epoxide was confidently assigned at C-3 and C-4. The other connectivity of the lactone was also determined by the combination of ^{1}H NMR COSY and XHCORR experiments. The C-14 proton at δ 4.79 was coupled to the methylene protons at δ 2.61 and 2.42, which were also coupled to a vinyl methyl at δ 1.68. Thus, the position of a double bond was assigned as C-11 and C-12. The C-11 olefinic proton at δ 4.97 was coupled to protons at δ 2.33 and 2.10 - 2.06. Due to the overlapping of the proton signals however, further assignments could not be made.



A long range carbon-proton correlation (COLOC) experiment helped to determine the remaining part of the structure (Table 1). A long range coupling between the C-18 methyl protons and the methylene carbon at δ 37.5 positioned this carbon to C-5. One of the C-5 protons at δ 1.34 was coupled to the carbons at δ 22.5 (t) and 124.5 (d). Therefore, a double bond was assigned at C-7 and C-8. The carbon at δ 38.7 (t) was coupled to the C-19 methyl protons and was assigned as C-9. Also, several carbon-proton long range couplings confirmed the partial structure determined by ^{1}H NMR COSY and XHCORR experiments. Thus, the structure of compound **22** was defined.

The two-dimensional structure of **22** was identical with a known metabolite,

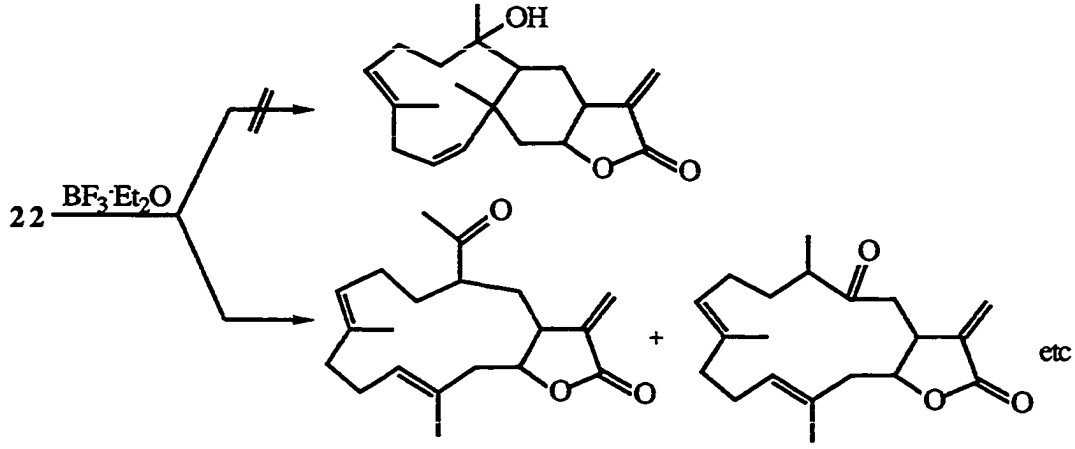
isolobophytolide (**24**), isolated earlier from the Australian soft coral *Lobophytum crassum*.[62]



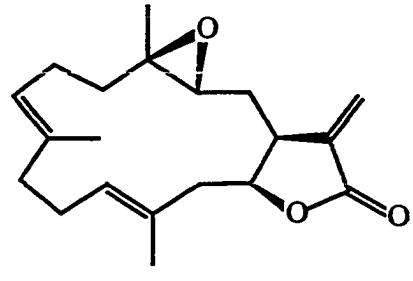
isolobophytolide (**24**)

However, the ^{1}H and ^{13}C NMR data for **22** were significantly different from the reported data for **24**. In particular, the chemical shift of the C-14 proton (δ 4.10) of **24** was at higher field than the same proton (δ 4.79) of **22** by 0.69 ppm. Therefore, it seemed very likely that **22** possessed a different stereochemistry at the asymmetric centers. Compound **22** has two double bonds (C-7,8 and C-11,12) and four asymmetric carbon centers (C-1, 3, 4, and 14). High field chemical shifts of the C-19 (δ 15,6) and 20 (δ 17.5) methyl carbons revealed that both of the double bonds possess the ***E*** configurations, identical with **24**.

The stereochemistry of the four asymmetric carbon centers was first approached by a chemical conversion. Due to the size of the 14-membered ring, and the flexibility of the cembrane ring, the configurations of the asymmetric centers are very difficult to determine. Formation of an additional bond by transannular cyclization would make the conformation of the molecule more rigid, thus readily allowing stereochemical assignment of the asymmetric centers. The nature of the functional groups (an epoxide and two double bonds) on the 14-membered ring of **22** suggested that the transannular reaction could be achieved under acidic condition. However, **22** readily decomposed in acidic media, and treatment of **22** with $BF_3{\cdot}Et_2O$ resulted in the formation of several rearranged products, which were incompletely characterized.



The configurations of the asymmetric centers in **22** were determined by ^1H NMR nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiments.[72] Irradiation of the C-1 proton enhanced the C-3 (16.9% enhancement), C-14 (11.7%), and C-17 (δ 5.61, 4.1%) protons. Also, irradiation of the C-14 proton enhanced the C-1 (5.0%), C-3 (1.3%), and C-11 (3.9%) protons. In addition, the C-1 proton was consistently, but weakly, enhanced by irradiation of the C-3 proton (1.2%) . The C-18 methyl protons were not enhanced by irradiation of any key protons. Since most cembranoid double bonds possess the ***E*** configuration, consideration of the epoxidation mechanism revealed that the C-18 methyl and C-3 proton were *trans*-oriented to the epoxide ring. Thus, the overall relative configurations of the asymmetric centers of **22** was defined as 7(***E***), 11(***E***), 1**S***, 3**S***, 4**S***, and 14**S***.



22

Compound **23** was isolated as an oil by HPLC (40% EtOAc in isooctane) and

analyzed for $C_{20}H_{30}O_4$ by high resolution mass and ^{13}C NMR spectrometry. Spectral analyses showed **23** to possess the γ-lactone identical with **22**; δ 170.4 (s) in ^{13}C NMR spectrum, δ 6.13 and 5.41 in ^{1}H NMR spectrum, and 1760 cm^{-1} in the IR spectrum. However, **23** was unstable at room temperature, and most of spectral analyses were performed on its acetylation product, **25** (Table 2). Spectral data for compound **25** were very similar with **23**. Beside the ^{1}H and ^{13}C NMR resonances corresponding to an acetyl group, the only significant difference in the NMR spectra was the down field shift of a proton signal from δ 3.90 to 5.07 in compound **25**. Therefore, **23** must possess a secondary hydroxyl group. The carbon NMR spectrum of **23** showed four oxygen bearing carbon signals at δ 85.7 (d), 80.5 (s), 79.6 (d), and 77.9 (d). Consideration of the molecular formula revealed that besides signals corresponding to the lactone and hydroxy-bearing carbons two other resonances of oxygen-bearing carbons originated from an ether functionality.

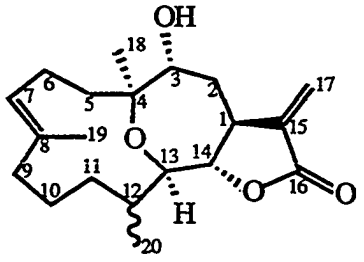
A ^{1}H NMR COSY experiment illustrated proton signals at δ 6.13 (C-17), 5.39 (C-17), 3.69 (C-14), and 2.97 (C-1) in the ^{1}H NMR spectrum assigned as the lactone protons. The C-1 proton at δ 2.97 was coupled to two protons at δ 2.12 - 2.02 and 1.90 which were also coupled to the proton at δ 5.07 attached to the acetoxy-bearing carbon. Therefore, attachment of the acetyl group was confidently assigned at C-3. Direct (XHCORR) and long range (COLOC) carbon-proton correlation experiments showed that the C-3 carbon at δ 77.9, as well as carbons at δ 80.5 (s) and 41.0 (t), were coupled to the methyl protons at δ 1.14 (s). Thus, the partial structure of C-1 ~ C-5 was determined.

Further connectivity of the lactone was also determined by the combination of two-dimensional experiments. ^{1}H NMR COSY experiments revealed that the C-14 proton at δ 3.69 was coupled to a proton at δ 3.25. An XHCORR experiment showed that this proton was attached to a carbon resonance at δ 79.6 (C-13) in the ^{13}C NMR spectrum. Since the molecular formular of **23** indicated the presence of an ether, it was concluded

Table 2. ^{1}H and ^{13}C NMR assignments for compounds **23** and **25**.

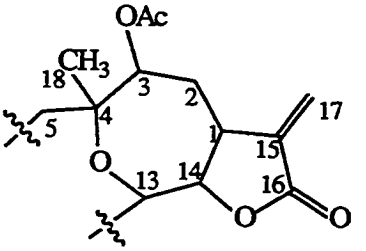
	proton		carbon		COLOC of 25[a]	
#	23	25	23	25	carbon	proton[b]
1	3.20 brdd(10.4,10.4)	2.97 brddd(10.4,10.4, 1.9)	41.7	42.9	1	17 (6.13)
2	2.10 - 2.03[c] 1.89 ddd(14.0,10.5, 2.8)	2.12 - 2.02[c] 1.90 ddd(14.4,10.6, 2.4)	27.0	27.0	2	—
3	3.90 ddd(4.9,4.8,2.8)	5.07 dd(5.2,2.4)	77.9	77.9	3	18
4	—	—	80.5	80.5	4	18
5	1.82 m 1.62 - 1.41[c]	1.87 m 1.59 m	41.0	41.0	5	18
6	2.44 dddd(15.2,11.3, 11.3,3.9) 2.10 - 2.03[c]	2.42 dddd(15.1,11.3, 11.3,3.8) 2.12 - 2.02[c]	23.5	23.5	6	—
7	5.23 brdd(10.9,1.5)	5.20 brd(10.4)	128.6	128.5	7	—
8	—	—	128.2	128.3	8	19
9	2.10 - 2.03[c] 1.62 - 1.41[c]	2.12 - 2.02[c] 1.46 - 1.40[c]	36.2	36.0	9	—
10	2.10 - 2.03[c] 1.62 - 1.41[c]	2.12 - 2.02[c] 1.46 - 1.40[c]	21.1	20.9	10	—
11	1.62 - 1.41[c] 0.92 m	1.55 - 1.46[c] 0.90 m	30.7	30.7	11	—
12	1.62 - 1.41[c]	1.55 - 1.46[c]	36.1	36.0	12	—
13	3.26 dd(8.9,8.8)	3.25 dd(9.0,8.8)	79.6	79.6	13	—
14	3.68 dd(10.3,9.3)	3.69 dd(10.4,9.3)	85.7	85.4	14	—
15	—	—	139.8	139.1	15	1
16	—	—	170.4	170.0	16	17 (6.13, 5.39)
17	6.13 d(3.5) 5.41 d(3.2)	6.13 d(3.4) 5.39 d(3.2)	117.0	117.3	17	—
18	1.23 s	1.14 s	17.0	16.9	18	—
19	1.56 brs	1.54 d(1.1)	14.8	14.7	19	7
20	1.01 d(7.0)	1.02 d(7.0)	16.5	16.3	20	—
OAc	—	2.09 s	—	170.1 21.1		

Spectra were recorded in $CDCl_3$ solution. [a] Parameters were optimized for couplings of 6 Hz. [b] The numbers in parentheses are chemical shifts of the protons which correlate. [c] Coupling constants were not determined.



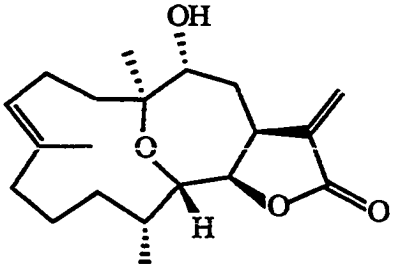
23, R = H
25, R = Ac

that the C-13 carbon was connected to the C-4 carbon by an oxygen bridge and formed a 7-membered cyclic ether.



The remaining part was also determined by two-dimensional NMR experiments. The olefinic proton at δ 5.20 was coupled to protons at δ 2.42 (1 H), 2.12 - 2.02 (1 H), and 1.54 (3 H). The protons at δ 2.42 and 2.12 - 2.02 were also coupled to the C-5 methylene protons at δ 1.87 and 1.59. Thus, the double bond was confidently assigned at C-7 and C-8. The C-13 proton at δ 3.25 and two high field proton bands at δ 1.02 (3 H) and 0.90 (1 H) coupled to a common methine resonance at δ 1.55 - 1.46. Therefore, the corresponding carbon signal at δ 36.0 in the ^{13}C NMR spectrum was assigned at C-12. Due to the overlapping of proton resonances, the region of C-9 ~ C-11 was determined by their carbon chemical shifts and an XHCORR experiment.

The two-dimensional structure of **23** was identical with a previously known metabolite, jeunicin (**3**), from *Eunicea mammosa* collected in Jamaica.[14] Recently, jeunicin was also isolated from a mollusc, *Planaxis sulcatus*.[63]



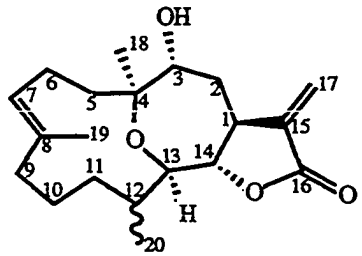
jeunicin (**3**)

However, ^{1}H and ^{13}C NMR data of **23** were significantly different from jeunicin (Table 3). Differences were more distinct in the chemical shifts of some proton and

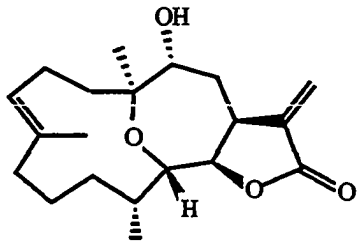
Table 3. Comparison of NMR data between **23** and jeunicin (**3**).[a]

	proton		carbon	
#	**23**[b]	**3**[c]	**23**	**3**
1	3.20 brdd (10.4,10.4)	3.36	41.7	40.0
2	2.10 - 2.03[d]	2.36	27.0	30.3
	1.89 ddd(14.0,10.5,2.8)	1.89		
3	3.90 ddd(4.9,4.8,2.8)	3.67	77.9	71.9
4	—	—	80.5	80.0
5	1.82 m	1.71	41.0	32.3
	1.62 - 1.41[d]	1.50		
6	2.44 dddd(15.2,11.3, 11.3,3.9)	2.27	23.5	22.8
	2.10 - 2.03[d]	2.08		
7	5.23 brdd(10.9,1.5)	5.53	128.6	125.4
8	—	—	128.2	133.5
9	2.10 - 2.03[d]	2.04	36.2	40.4
	1.62 - 1.41[d]	1.93		
10	2.10 - 2.03[d]	1.63	21.1	22.8
	1.62 - 1.41[d]	1.27		
11	1.61 - 1.41[d]	1.72	30.7	29.6
	0.92 m	1.14		
12	1.62 - 1.41[d]	1.86	36.1	36.5
13	3.26 dd(8.9,8.8)	3.18	79.6	72.1
14	3.68 dd(10.3,9.3)	4.42	85.7	80.0
15	—	—	139.8	137.6
16	—	—	170.4	169.4
17	6.13 d(3.5)	6.31	117.0	120.0
	5.41 d(3.2)	5.61		
18	1.23 s	1.25	17.0	19.1
19	1.56 brs	1.58	14.8	15.5
20	1.01 d(7.0)	0.92	16.5	15.8

[a] Reference 63. [b] Assigned by COSY experiment. [c] Coupling constants were not reported. [d] Coupling constants were not determined.



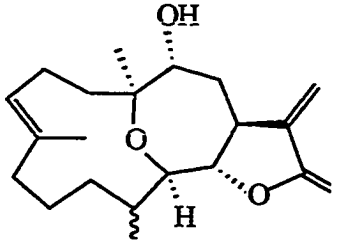
23



3

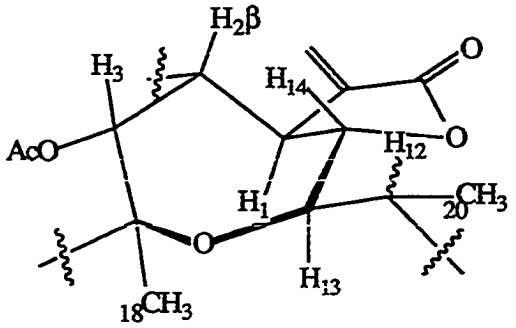
carbon signals corresponding to the asymmetric centers. Therefore, compound **23** was concluded to be a diastereomer of jeunicin. Compound **23** has asymmetric carbon centers at C-1, -3, -4, -12, -13, and -14. ^{1}H NMR NOEDS experiments helped to assign the configurations of these centers (Figure 3). Irradiation of the C-1 proton enhanced the C-13 proton signal by 7.8%. Also, irradiation of the C-13 proton enhanced the C-1 and C-18 protons by 9.8 and 2.6%, respectively. The C-14 proton was not enhanced by irradiation of either the C-1 or C-13 protons. Therefore, it was apparent that **23** was at least epimeric to **3** at C-13 and C-14.

The stereochemistries of other asymmetric centers were also pursued by ^{1}H NMR NOEDS experiments. Irradiation of the C-14 proton enhanced the C-2β proton signal by 7.4%. Also, both the C-2β and C-14 protons were enhanced by irradiation of the C-3 proton (7.1 and 1.0%, respectively). Therefore, all of these protons are *syn*-oriented to the plane of the molecule. However, the stereochemistry of C-12 was not determined by the ^{1}H NMR NOEDS experiment. Irradiation of the C-20 methyl protons enhanced both the C-13 and C-14 protons by 2.3 and 1.3%, respectively. Inspection of the molecular model revealed that when the conformation of the C-20 methyl was equatorial to the plane of molecule, both the C-13 and 14 protons were proximal to the C-20 methyl protons. Since the C-20 methyl could be in an equatorial conformation in both configurations however, the stereochemistry of C-12 remained unknown. The overall relative configurations of other centers are 1**S***, 3**R***, 4**S***, 13**R***, and 14**S***.



23

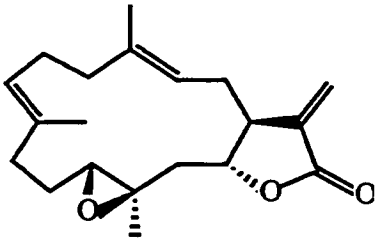
Figure 3. Results of a ^1H NMR NOEDS experiment with compound **23**.



proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
1	13	7.8
3	2β, 14	7.1, 1.0
13	1, 18	9.8, 2.6
14	2β	7.4
20	13, 14	2.3, 1.3

In addition to compounds **22** and **23**, silica HPLC gave four known metabolites: eunicin (**1**), eunicin acetate (**2**), 12,13-bisepieupalmerin (**9**), and crassin acetate (**21**).[11,17,18,26] ^{1}H NMR data of these compounds were in full accord with reported data.

All of the compounds except crassin acetate (**21**) have the γ-lactone as a common feature. Cembranoids with γ-lactone functionalities have been isolated from various gorgonians (Gorgonacea) and soft corals (Alcyonacea). One of the frequently encountering difficulties in the structural elucidations of these compounds is the determination of stereochemistry of the lactone ring fusion (C-1 and C-14). It is well known that the determination of stereochemistry of asymmetric centers in 5-membered rings are very difficult and often require painstaking labor.[64] To solve this problem, Coll and coworkers used the intensity of coupling between the protons at the ring junction.[62] They compared the vicinal coupling constant of isolobophytolide (**24**, $J_{1,14}$ = 7 Hz) with two previously known compounds, eunicin (**1**, *cis*, $J_{1,14}$ = 8 Hz)[11] and lobophytolide (**26**, *trans*, 5 Hz)[65], and proposed a *cis* ring fusion. However, the structure of **24** determined by X-ray crystallographic methods showed a *trans* ring fusion and revealed that coupling constant analysis was an unreliable indicator.[5,66]



lobophytolide (**26**)

The results of ^{1}H NMR NOEDS experiments with compounds **22** and **23** showed that nOe measurement between protons at the ring junction could be an effective predictor for its stereochemistry. Since the lactone is fixed to the 14-membered ring by two adjacent positions (C-1 and C-14), conformational variations on the ring junction would

be minimal. Therefore, a *cis* ring junction would result in eclipse between the C-1 and C-14 protons, and very large nOe. When *trans*, the protons will be *anti*-oriented to the plane of molecule and be outside nOe range.

Eunicea succinea forma *plantaginea* (specimen number CI 86 - 192) was also collected from the Tobago Cays. Silica vacuum flash chromatography of the CH_2Cl_2 extract, followed by silica HPLC yielded compound **22** as the dominant metabolite (ca 35% of the crude extract). The ^1H NMR spectra of other HPLC fractions showed the presence of various cembranoid lactones (characteristic chemical shifts of the C-17 protons) as minor metabolites. However, there was a minor metabolite (**27**) which showed a very distinct ^1H NMR spectrum. Both of the exocyclic methylene (C-17) protons were replaced by two broad singlet peaks at δ 5.16 and 4.99 in the ^1H NMR spectrum. The lack of the carbonyl peaks in the ^{13}C NMR spectrum confirmed this change. It was not clear whether this metabolite is a compound of the same structural class or a different class of diterpene. Due to the difficulty of purification and also due to the low yield (less than 0.1% of the extract) of **27**, the structure was not pursued.

Two groups of gorgonians of the subgenus *Eunicea* were also collected from the Florida Keys, in July 1987. Although their morphological features were different, the results of TLC analyses revealed that their constituents were similar with *Eunicea mammosa* (specimen number CI 86-192) from Tobago Cays. These were identified by F. M. Bayer as *E. mammosa* (F 87-24) and *E. palmeri* (F 87-26).

General Experimental

Collection. The gorgonians were collected by hand using SCUBA at 15 to 25 m depth. The major collections were made at the Martinique - Tobago Cays area, eastern Caribbean Sea (July, 1986) and the Florida Keys (July, 1987). Also, few specimens were collected at the Island of St. Croix, U. S. Virgin Islands (November, 1987).

TLC Analysis, Chemotype, and Identification. The collections were surface air-dried in the shade for 1 to 2 hours. Each of the specimens were labelled with serial numbers. A small piece (3 - 5 mm) of tissue was taken from the tip of each colony, and the remaining part was immediately frozen. All the pieces of tissue were extracted with dichloromethane (0.5 - 1 ml). The extracts were analyzed by thin layer chromatography (TLC) by using ethyl ether and a mixture of ethyl ether and n-hexane (1:1, v:v) as developing solvents. The results of the TLC analyses were used to separate individual gorgonian colonies. The TLC characters which the separation was based on were: 1) the Rf, which is the ratio of the distance traveled by the metabolites to the distance traveled by the solvent front, 2) UV activity, i. e., fluoresence observed under an ultraviolet light, and 3) the primary color produced by acid charring and heating. When a colony (or colonies) showed distinct TLC characters, that (or those) organism(s) were defined as a chemotype. The homogenity of each chemotype was checked by repeated and more precisely performed TLC analysis in the laboratory. Depending on the size of collections and gross morphological variations (shape of colony and calyx), one to five vouchers were selected from each chemotype. The vouchers were sent to Dr F. M. Bayer, at the Smithsonian Institution for identification.

Extraction and Isolation. Samples were freeze-dried, and exhaustively extracted with dichloromethane. The solvent was evaporated under vacuum, and the crude organic extract was eluted from a vacuum flash silica chromatography column using

mixtures of ethyl acetate and isooctane. The metabolites were isolated by silica and C-18 reverse phase high performance liquid chromatography (HPLC) on the flash chromatographic fractions. The solvents used for HPLC were mixtures of ethyl acetate and isooctane (silica), and water and methanol (C-18 reverse phase). Also, lipophilic Sephadex-LH-20 (chloroform and isooctane) column chromatography was used for some metabolites. HPLC separations were carried out on Waters Model 6000 or SSI Model 300 solvent delivery system monitored by a Waters differential refractometer R-401 or 403. Solvents used were distilled in glass prior to use.

Spectral Analysis. Infrared spectra were recorded on a Perkin-Elmer 783 spectrophotometer. Ultraviolet spectra were obtained in methanol solution using Perkin-Elmer Lamda 3B spectrophotometer. Proton NMR, homonuclear correlation spectroscopy (COSY)[67] and relay coherence transfer (RCT)[68] spectra were recorded in solutions on a 360 MHz spectrometer constructed from an Oxford narrow-bore magnet and a Nicolet Fourier transform data system by Dr. John M. Wright of the UCSD NMR Facility: all chemical shifts are reported with respect to Me_4Si. Nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiments were performed in general as outlined by Hall and Sanders.[72] Carbon-13 NMR spectra were recorded on Nicolet wide-bore (50 MHz) and IBM WP-200 SY (50 MHz) spectrometers. Direct (XHCORR)[69,70] and long range (COLOC)[71] carbon-proton correlation spectra were obtained on an IBM WP-200 SY (50 MHz) spectrometer: all chemical shifts are reported with respect to Me_4Si. High resolution mass measurements were supplied from mass spectral laboratories at either the University of California, Riverside, the University of Iowa, or the University of Minnesota. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter with a 10-cm microcell. Circular dichroic measurement was performed on a Cary 61 spectropolarimeter with a 0.02 cm cell by signal-averaging 20 scans and were run under the direction of Joseph Taulane. Melting points were determined on a

Fisher-Johns apparatus and are reported uncorrected. X-ray crystallographic spectra were provided by Professor Jon Clardy, Cornell University.

Experimental Section

A. *Eunicea mammosa* from the Tobago Cays

Collection and Extraction. Gorgonians (specimen number CI 86-192) were collected by hand using SCUBA at -20 to 25 m in July 1986, along the offshore islands of the Tobago Cays, eastern Caribbean Sea. The freeze-dried samples were repeatly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 35 g of the crude organic materials (from 2.5 kg, dry weight of the gorgonian). Compounds **1**, **2**, **9**, **21**, **22**, and **23** were eluted from a vacuum flash silica column with sequential mixtures of EtOAc and isooctane and further purified by silica and C-18 reverse phase HPLC.

Compound 22. The cembranoid **22** was obtained as an oil by HPLC (25% of EtOAc in isooctane). Final purification by reverse phase HPLC (95% MeOH in water) gave 190 mg (0.5% of the extract) of **22**. Compound **22** exhibited $[\alpha]_D$+25.3° (c 0.9, $CHCl_3$) and displayed the following spectral features: IR (film) 2920, 1770, 1665, 1440, 1385, 1270, 1160, 1120, 980 cm^{-1}; HRMS: M^+, m/z obsd 316.2048, $C_{20}H_{28}O_3$ required 316.2038; low resolution MS: m/z (relative intensity) 316 (3), 283 (2), 163 (9), 147 (10), 133 (15), 119 (25), 107 (41), 93 (46), 81 (51), 68 (84), 55 (59), 43 (100); UV (MeOH) no λmax.

Reaction of 22 with BF_3·Et_2O. To a stirred solution of **22** (145 mg, 0.46 mmol) in 3 ml of anhydrous ethyl ether at 0°, 200 µl of BF_3·Et_2O was added. After 30 min, distilled water (20 ml) and ethyl ether (30 ml) were added. The ether layer was separated and washed with saturated $NaHCO_3$ (2 x 20 ml), water (2 x 20 ml) and dried under vacuum. Separtation by HPLC (25% EtOAc in isooctane) gave six products. ^1H NMR spectra showed that all of these compounds were simple rearranged products of **22** (chemical shifts and coupling patterns of signals corresponding the C-3, -4, -5, and -18

protons). The structures of these products were not pursued.

Compound 23. The cembranoid **23** was isolated as an oil by HPLC (50% EtOAc in isooctane). Compound **23** showed $[\alpha]_D$-7° (c 0.5, $CHCl_3$) and displayed the following spectral features: IR (film) 3500, 2940, 1760, 1670, 1440, 1260, 1160, 1040, 980 cm^{-1}; HRMS: M^+, m/z obsd 334.2149, $C_{20}H_{30}O_4$ required 334.2144; low resolution MS: m/z (relative intensity) 334 (28), 164 (18), 151 (13), 147 (13), 135 (13), 121 (21), 107 (24), 95 (34), 81 (46), 69 (23), 43 (100); UV (MeOH) no λmax,

Acetylation of 23. To a stirred solution of **23** (41 mg, 0.12 mmol) in 2 ml of dry pyridine, 1.5 ml of acetic anhydride was added. After 3 hrs, pyridine and excess acetic anhydride were removed under vacuum. The acetylated product **25** was quantitatively obtained.

Isolation of eunicin acetate (2), crassin acetate (21), eunicin (1), 12,13-bisepieupalmerin (9). Compounds **2**, **21**, **1**, and **9** (polarity order) were isolated as white solids by HPLC (25, 70, 70, and 70% EtOAc in isooctane, respectively). The amounts of the isolated materials were 0.3, 0.3, 2.1, and 0.2 g, respectively. The ^1H NMR spectrum of **2** ($CDCl_3$) showed bands at δ 6.42 (1 H, d), 5.60 (1 H, d), 5.03 (1 H,brt), 4.42 (1 H, dd), 3.40 (1 H, dd), 2.86 (1 H, d), 2.03 (3 H, s), 1.54 (3 H, brs), 1.47 (3 H, s), 0.87 (3 H, d). The ^1H NMR spectrum of **21** ($CDCl_3$) showed peaks at δ 6.47 (1 H, d), 5.71 (1 H, d), 5.40 (1 H, m), 5.25 (1 H, m), 5.05 (1 H, brt), 3.95 (1 H, d), 1.96 (3 H, s), 1.72 (3 H, s), 1.61 (3 H, s), 1.43 (3 H, s). The ^1H NMR spectrum of **1** ($CDCl_3$) showed peaks at δ 6.40 (1 H, d), 6.85 (1 H, d), 5.03 (1 H, brt), 4.42 (1 H, dd), 3.40 (1 H, m), 3.25 (1 H, d), 2.85 (1 H, d), 1.53 (3 H, brs), 1.16 (3 H, s), 0.86 (3 H, d). The ^1H NMR spectrum of **9** ($CDCl_3$) showed peaks at δ 6.42 (1 H, d), 5.80 (1 H, d), 5.02 (1 H, brt), 4.30 (1 H, m), 3.77 (1 H, brd), 3.42 (1 H, m), 1.57 (3 H, brs), 1.24 (3 H, s), 0.92 (3 H, d). All of the ^1H NMR data were in full accord with reported data.

B. *Eunicea succinea* forma *plantaginea* from the Tobago Cays

Collection and Extraction. Gorgonians (specimen number CI 86-191) were collected by hand using SCUBA at -20 to -25 m along the offshore islands of the Tobago Cays, eastern Caribbean Sea, in July 1986. Extraction with CH_2Cl_2 of the dried sample (2 kg, dry weight) gave 36 g of the crude extract. The extract was separated by silica vacuum flash chromatography eluting with sequential mixtures of isooctane and EtOAc. All of the flash chromatographic fractions were separated by silica HPLC (the same solvents). ^1H NMR spectra were obtained for all of the HPLC fractions.

Isolation of compound 22. The cembranoid **22** was isolated as an oil from HPLC (20% EtOAc in isooctane). The extract gave ca 12 g (35% of the extract) of **22**. The ^1H and ^{13}C NMR data for **22** was identical with the metabolite isolated from *Eunicea mammosa* (CI 86-192).

Isolation of compound 27. Compound **27** was isolated as an oil by HPLC (60% EtOAc in isooctane). The extract gave 30 mg (0.08% of the extract) of **27**. The ^1H NMR spectrum of **27** ($CDCl_3$) showed peaks at δ 5.23 (1 H, dd), 5.16 (1 H, brs), 5.02 (1 H, dd), 4.99 (1 H, brs), 4.12 (2 H, brs), 3.84 (2 H, m), 1.62 (3 H, s), 1.53 (3 H, s), 1.12 (3 H, s). The ^{13}C NMR spectrum ($CDCl_3$) of **27** showed peaks at δ 148.9 (s), 132.6 (s), 130.6 (s), 128.4 (d), 128.1 (d), 110.0 (t), 83.1 (d), 80.6 (d), 73.9 (s), 65.4 (t), 46.6 (d), 42.5 (t), 39.8 (t), 39.1 (t), 33.5 (t), 25.2 (t), 23.3 (q), 22.1 (t), 17.2 (q), 15.2 (q).

C. *Eunicea mammosa* and *E. palmeri* from the Florida Keys

Collection. Two groups of gorgonians (specimen numbers F 87-24 and F 87-26) were collected at 15 to 20 m depth at the Florida Keys, in July 1987. TLC analyses of these gave same results with *E. mammosa* (CI 86-192) from The Tobago Cays. Vouchers were sent to Dr. F. M. Bayer who identified them as *E. mammosa* (F 87-24) and *E.*

palmeri (F 87-26), respectively.

Chapter III

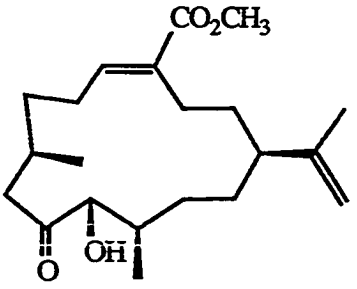
Cembranes and reduced quinones from *Eunicea* sp (CI 86 - 193, *Euniceopsis*)

Gorgonians (specimen number CI 86 - 193) were collected at the Tobago Cays, in July 1986. They were defined by Bayer as animals which belong to the genus *Eunicea* (subgenus *Euniceopsis*), but morphologically distinct from known species. Extraction of the dried gorgonians with dichloromethane (1.5 kg, dry weight) gave 25 g of the crude organic materials. Silica vacuum flash chromatography, followed by silica and C-18 reverse phase HPLC, gave five metabolites. Compounds **28** - **30** (Figure 4) were diterpenoids of the cembrane class. Compounds **35** and **36** (Figure 6) were determined subsequently to be products of mixed biogenesis possessing a rare skeleton of 28 carbons.

The major metabolite, **28**, was isolated as a white solid, and analyzed for $C_{21}H_{34}O_4$ by high resolution mass and ^{13}C NMR spectrometry. The infrared spectrum showed strong absorption bands at 3500 and 1710 cm^{-1}. Corresponding carbon signals at δ 210.4 (s), 167.6 (s), and 81.0 (d) in the ^{13}C NMR spectrum (Table 4) illustrated **28** to possess a ketone, an unsaturated ester and a secondary alcohol. Thus, functionalities of all of the four oxygens in the molecular formula were confidently assigned. A carbon signal at δ 51.1 (q) and corresponding proton signal at δ 3.71 (3 H, s) in the ^1H NMR spectrum (Table 5) revealed that the ester was a methyl ester.

Most of spectral analyses were performed in C_6D_6 solutions in which proton signals were well dispersed. A ^1H NMR COSY experiment was very informative for the determination of proton spin systems. With the help of a XHCORR experiment (all of the 17 proton-bearing carbons and their protons were precisely matched), four structural units were confidently identified as a - d.

Figure 4. Structures of compounds **28 - 31**.



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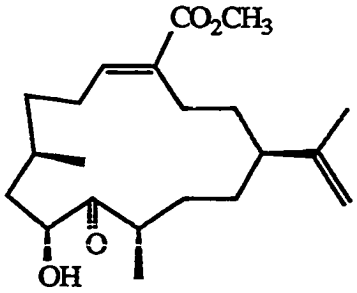

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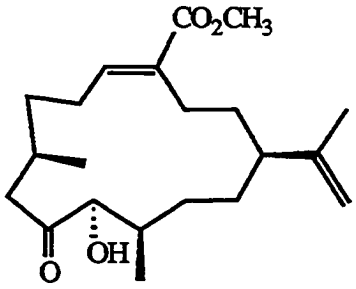

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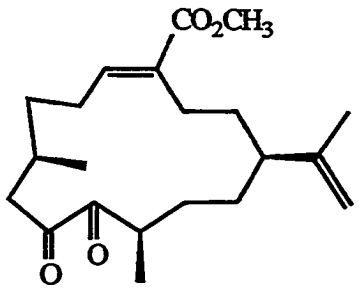

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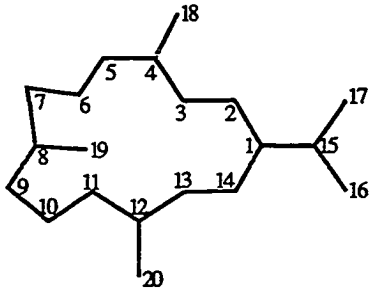


Table 4. ^{13}C NMR assignments for compounds **28 - 31**.

#	28		29		30		31	
1	43.9	d	44.4	d	45.2	d	44.3	d
2	29.9	t	30.0	t	31.6	t	30.8	t[a]
3	24.9	t	25.6	t	25.5	t	26.1	t
4	133.0	s	132.4	s	133.7	s	132.5	s
5	141.6	d	142.6	d	142.2	d	141.3	d
6	24.2	t	22.2	t	25.4	t	23.6	t
7	34.4	t	31.2	t	35.8	t	38.5	t
8	25.4	d	28.2	d	29.1	d	27.6	d
9	42.3	t	38.7	t	43.4	t	44.0	t
10	210.4	s	72.5	d	212.2	s	199.9	s[b]
11	81.0	d	215.2	s	78.9	d	202.5	s[b]
12	34.0	d	37.7	d	33.0	d	34.8	d
13	23.0	t	29.7	t	28.9	t	31.8	t[a]
14	28.2	t	28.8	t	29.9	t	29.7	t[a]
15	147.7	s	147.5	s	147.8	s	147.6	s
16	110.3	t	111.2	t	110.9	t	110.7	t
17	19.1	q	19.1	q	18.2	q	19.8	q
18	167.6	s	168.1	s	168.0	s	167.7	s
19	20.7	q	20.0	q	21.1	q	20.5	q
20	16.5	q	12.4	q	13.0	q	13.8	q
OMe	51.1	q	51.6	q	51.5	q	51.1	q

Compounds **28 - 30** were measured in $CDCl_3$ solutions, and **31** was measured in C_6D_6 solution. Multiplicities were measured by DEPT experiments. Assignments for **28** were made by XHCORR and COLOC experiments. Assignments for others were made by XHCORR and comparison with **28**. [a,b] might be reversed.

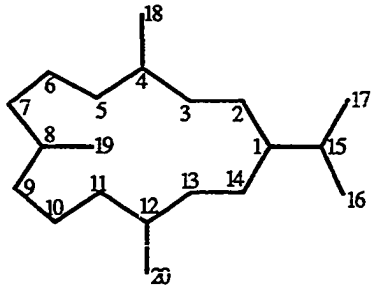


Table 5. ^{1}H NMR assignments for compounds **28 - 31**.

#	28[a]	28[b]	29[a]	30[b]	31[a]
1	c	1.95[d]	2.18-2.10[d]	2.00[d]	c
2	c	1.47 dddd(13.2,13.2, 9.7,5.3)	1.56-1.50[d]	1.58-1.53[d]	c
	c	0.88[d]	1.28-1.20[d]	1.08-1.06[d]	c
3	2.3-2.2[d]	2.47 ddd(12.9,12.9, 5.3)	2.40 ddd(13.1,13.1, 5.2)	2.49-2.45[d]	c
	2.1-2.0[d]	1.89 ddd(13.2,12.9, 3.3)	2.03 ddd(13.1,13.1, 3.5)	1.96-1.94[d]	c
5	6.65 dd(10.9,4.8)	6.72 dd(11.1,4.8)	6.75 ddd(11.5,4.1, 0.5)	6.67 dd(10.8,4.3)	6.72 dd(9.3, 7.3)
6	2.3-2.2[d]	1.84[d]	2.30-2.20[d]	1.93-1.92[d]	c
	2.1-2.0[d]	1.65[d]	2.15-2.05[d]	1.67-1.66[d]	c
7	c	1.41[d]	1.70-1.60 (2H)[d]	1.29-1.20[d]	c
	c	1.15[d]		1.05-1.00[d]	c
8	2.3-2.2[d]	2.11[d]	2.24-2.16[d]	1.60-1.50[d]	c
9	2.85 dd(19.3,5.9)	2.27 dd(19.4,6.2)	1.82 ddd(14.0,10.9, 1.2)	2.47 dd(15.2,4.5)	c
	2.1-2.0[d]	1.30[d]	1.16 ddd(14.0,11.5, 3.7)	1.82 dd(15.2,9.2)	c
10	----	-----	4.36 ddd(11.5,5.6, 1.2)	----	-----
11	4.12 brd(3.8)	3.80 brdd(4.4,0.8)	-----	3.82 brd(5.0)	-----
12	2.0-1.9[d]	1.60[d]	2.83 ddq(11.1,2.5, 6.5)	1.7-1.6[d]	3.40 m
13	c	1.10[d]	1.76-1.66[d]	1.65-1.53[d]	c
		0.90[d]	1.32-1.25[d]	1.25-1.17[d]	c
14	c	1.4-1.2 (2H)[d]	1.64-1.56[d] 1.54-1.44[d]	1.46-1.31 (2H)[d]	c
16	4.75 brs	4.81 brs (2H)	4.79 brs	4.86 brs	4.71 brs
	4.70 brs		4.78 brs	4.83 brs	4.60 brs
17	1.68 brs	1.65 brs	1.73 brs	1.68 brs	1.62 brs
19	1.01 d(6.8)	0.78 d(6.8)	1.03 d(7.0)	0.76 d(6.7)	1.07 d(6.8)
20	1.14 d(6.7)	1.12 d(6.7)	1.10 d(6.5)	0.77 d(6.6)	1.10 d(5.3)
OMe	3.71 s	3.44 s	3.73 s	3.45 s	3.70 s
OH	3.31 d(5.4)	3.41 d(5.7)	3.42 d(5.6)	2.80 brs	-----

[a,b] were obtained in $CDCl_3$ and C_6D_6 solutions, respectively. Assignments were made by one-dimensional decoupling and COSY experiments. [c] Nonassignable resonances. [d] Coupling constants were not measured.

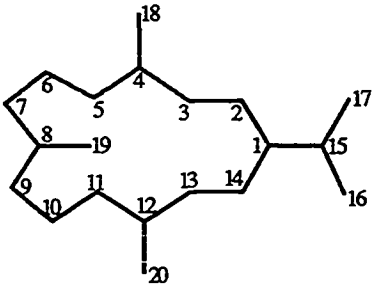
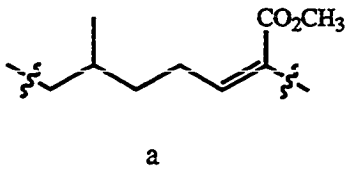


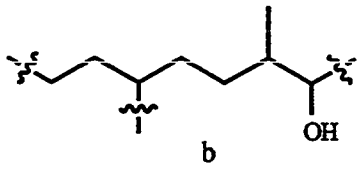
Table 6. Results of a COLOC experiment with compound **28**.

carbon	proton
1	16, 17
2	3 (1.89)
4	3 (2.47), 3 (1.89)
7	19
8	19
9	19
10	9 (2.27), 11
11	20
12	11
13	11
15	17
16	17
17	16
18	3 (1.89), 5, OMe
20	11, 12

Experiment was performed in C_6D_6 solution. Parameters were optimized for couplings of 6 Hz. Numbers in parentheses are chemical shifts of the correlated protons.



a



b

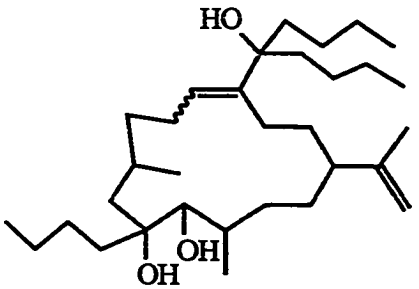
c

d

Combination of the partial structures was made by a COLOC experiment (Table 6). The methine carbon at δ 43.9 was observed to be coupled to the isopropylene protons at δ 4.81 and 1.65. Therefore, the partial structure c must be attached to the methine carbon of b. The carbon signals at δ 167.6 and 133.0 were found to be coupled to the proton at δ 1.89. At the same time, the olefinic carbon at δ 133.0 was also coupled to the proton at δ 2.47. Thus, the connection between the olefinic terminus of a and methylenic terminus of b was determined. The carbonyl carbon at δ 210.4 (d) was coupled to the protons at δ 3.80 and 2.27, indicating the connection of d to the partial structures a and b. Additional data supporting this assignment came from COSY NMR experiment which showed a long range couplings between protons at δ 3.80 and 1.30. Thus, the structure of **28** was unambiguously determined as a diterpenoid of the cembrane class possessing an unusual α-hydroxy ketone functionality.

Compound **28** has an asymmetric double bond (Δ^4) and four asymmetric carbon centers (C-1, -7, -11, -12). Due to the size of the 14-membered ring however, the conformation of **28** was very flexible in the solution and the stereochemistry was not determined by NOEDS experiment. Attempts to impart rigidity to the molecule by forming a transannular bond via a Michael-type reaction failed. Reaction of **28** with lithium diisopropylamide (LDA) resulted in the formation of no isolable products. Treatment of **28** with n-butyllithium (nBuLi) gave the n-butyl adducts **32** and **33**, and

two other products. The structures of these latter compounds were later determined to be the other metabolites isolated, **29** and **30**.



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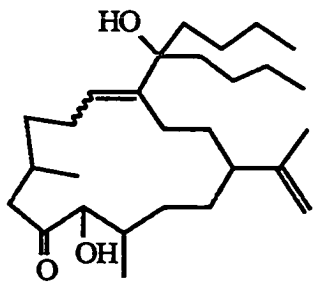
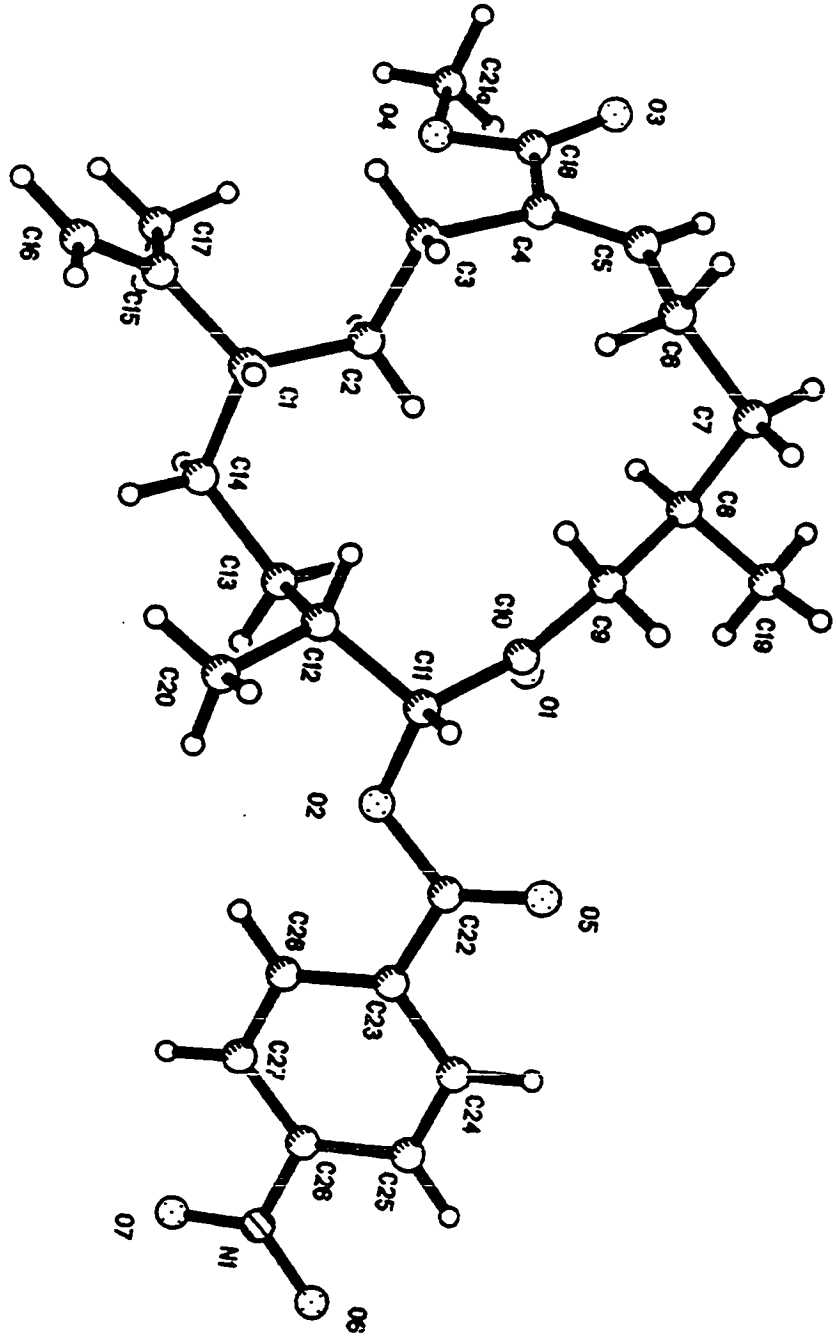


33

The stereochemistry of the asymmetric centers were finally determined by X-ray crystallographic methods. Treatment of **28** with p-nitrobenzoyl chloride gave **34**, the 11-(p-nitrobenzoyl) derivative of **28** as the major product. The X-ray drawing of **34** is shown in Figure 5. Thus, the stereochemistry of **28** was unambiguously determined. Following the general rule that the gorgonian cembranoids possess the C-1 proton down configuration,[4] the absolute configuration was defined as 4(*E*), 1**S**, 8**R**, 11**R**, and 12**R**.

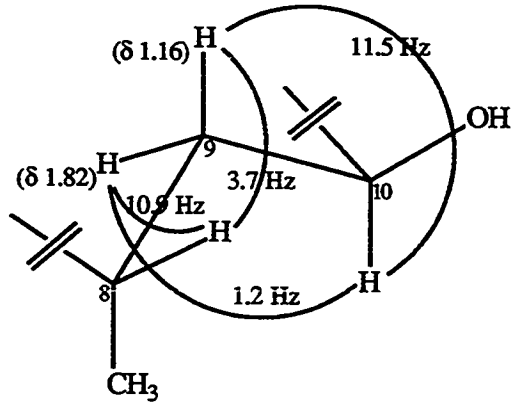
Compound **29** was isolated as an oil and analyzed for $C_{21}H_{34}O_4$ by high resolution mass and ^{13}C NMR spectrometry. Spectral data of **29** were very similar to those of **28**. However, there were several significant differences in the ^1H and ^{13}C NMR spectra. Carbon signals at δ 210.4 (s) and 81.0 (d) in compound **28** were shifted to δ 215.2 (s) and 72.5 (d), respectively (Table 4). In the ^1H NMR spectrum of **29**, both the chemical shift and splitting pattern of the α-hydroxy proton (δ 4.36) were significantly different from the same proton in **28** (Table 5). COSY NMR data showed that in contrast to **28**, the α-hydroxy proton of **29** was directly coupled to the methylene protons at δ 1.82 and 1.16 (J = 1.2 and 11.5 Hz, respectively). All of the differences could be accommodated by the exchange of functionalities between the ketone (C-10) and hydroxy-bearing carbon (C-11). Simple chemical conversion confirmed this interpretation. Oxidation of **28** by pyridinium chlorochromate (PCC) gave an α-diketone **31** as the major product (Figure 4).

Figure 5. A computer-generated perspective drawing of compound **34**.



Compound **31** was fully characterized by spectral methods. Treatment of **29** with PCC gave the same product. Thus, the structure of **29** was unambiguously determined as a diterpenoid of the cembrane class.

Chemical conversions proved not only the structure of **29**, but also the stereochemistry of most of its asymmetric centers. However, compound **29** has a new asymmetric carbon center at C-10. The stereochemistry of this center was approached by the consideration of vicinal proton coupling constants. Of the C-9 methylene protons at δ 1.82 and 1.16 (all the protons of **29** were confidently assigned by COSY NMR and XHCORR experiments), the proton at δ 1.82 showed large coupling (J = 10.9 Hz) with the C-8 methine proton and very small coupling (J = 1.2) with the C-10 proton. In contrast, the other proton at δ 1.16 showed large (J = 11.5) and small (J = 3.7) couplings with the C-10 and C-8 protons, respectively.

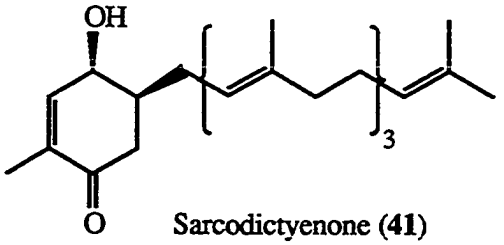


Therefore, the orientation of the C-8 and C-10 protons is *anti* to the plane of the molecule. Since **29** has the same R configuration as **28** for the C-8 center, the configuration at C-10 was assigned as S. The overall configurations of the asymmetric centers are thus 4(***E***), 1**S**, 8**R**, 10**S** and 12**R**.

An apparently related metabolite, **30**, was isolated as a white solid. Spectral data for **30** were very similar to that from **28**. However, signals of protons attached to the

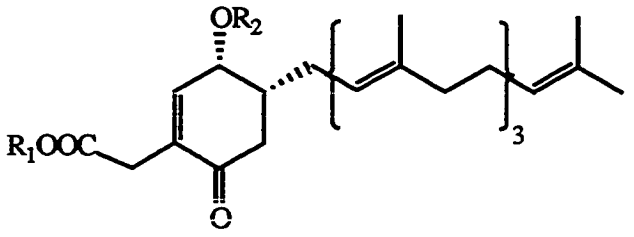
carbons at C-8, -9, -13, and -20 were considerably different from **28** (Table 5). In addition, the hydroxyl proton in **28** was significantly changed in both chemical shift (from δ 3.41 to 2.80) and coupling constant (from d, J = 5.7 Hz to brs) in **30**. Therefore, compound **30** was concluded to be a stereoisomer of **28**. Oxidation of **30** by PCC gave the same product, **31**, which was obtained from the oxidation of **28** and **29**. Thus, the structure of **30** was confidently determined as the C-11 epimer of **28**. The overall configurations are 4(***E***), 1**S**, 8**R**, 11**S**, and 12**R**.

In addition to the compounds **28** - **30**, ^{1}H NMR spectra of more polar flash chromatographic fractions (60 - 100% EtOAc in isooctane) showed the presence of structurally very different metabolites. Separation by C-18 reverse phase HPLC (95% MeOH in water) gave compounds **35** and **36** (Figure 6). These metabolites possess an unique C-28 carbon skeleton related to the polyprenylquinones and hydroquinones. In the marine environment, the polyprenylquinones and hydroquinones are widely distributed in brown algae of the order Fucales and sponges of the order Dictyoceratida.[5-7] Related metabolites also have been isolated from the ascidian *Aplidium californicum*,[73] the soft coral *Nepthea* sp.[74] and the gorgonian *Plexaura flava*.[75] Recently, Pietra and coworkers reported the isolation of sarcodictyenone (**41**), a ring-reduced tetraprenylquinone from the Mediterranean stolonifer *Sarcodictyon roseum*.[76] The structures of compounds **35** and **36** were similar to sarcodictyenone in possessing the reduced quinone ring. Instead of methyl substituents in sarcodictyenone and other quinones (hydroquinones) however, these metabolites possessed oxidized ethyl substituents on the reduced quinone ring.

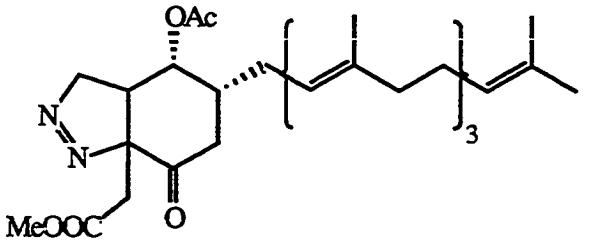


Sarcodictyenone (**41**)

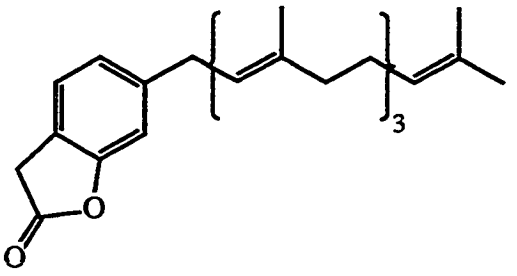
Figure 6. Structures and reactions of compounds 35 - 40.



	R_1	R_2
35	Me	H
36	H	Ac
37	Me	Ac
40	Me	COPh



38



39

35 $\xrightarrow[\text{room temp.}]{Ac_2O/Pyr.}$ 37

36 $\xrightarrow[0°]{CH_2N_2/THF}$ 37 and 38

36 $\xrightarrow[\text{room temp.}]{CHCl_3,SiO_2}$ 39

35 $\xrightarrow[\text{Pyr. 80°}]{\text{PhCOCl, 4-Dimethylaminopyr.}}$ 40

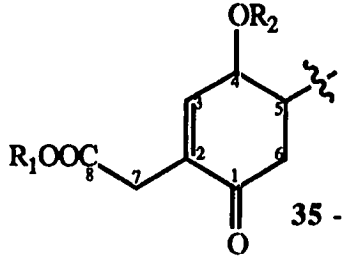
Compound **35** was isolated as an oil and analyzed for $C_{29}H_{44}O_4$ by high resolution mass and ^{13}C NMR spectrometry. The presence of a tetraprenyl unit was easily recognized by ^1H and ^{13}C NMR spectra (experimental section) and through a COSY NMR experiment. This assignment was supported by mass spectral fragments at m/z 387 (M^+-69, relative intensity 8), 251 (M^+-69 -68 -68, 6), and 183 (M^+-69 -68 -68 -68, 8). In particular, the fragment at m/z 183 was analyzed for $C_9H_{11}O_4$ by high resolution mass measurement, indicating the loss of a $C_{20}H_{33}$ (tetraprenyl) unit. High field chemical shifts for four vinyl methyl carbons at δ 17.7, 16.2, 16.1, and 16.1 in the ^{13}C NMR spectrum revealed that all of the four double bonds possessed the *E* configurations.

The remaining part of **35** was also identified by spectral analysis. ^{13}C NMR signals at δ 197.4 (s) and 171.9 (s) illustrated **35** to possess two carbonyl groups (Table 7). Absorption bands at 1680 and 1740 cm^{-1} in the IR spectrum indicated that the carbonyls were a conjugated enone and an ester. A proton signal at δ 3.68 (3 H, s) in the ^1H NMR spectrum and a corresponding carbon signal at δ 51.7 (q) in the ^{13}C NMR spectrum were assigned to a methyl ester methoxy group. Another ^{13}C NMR signal at δ 66.3 (d), and absorption band at 3450 cm^{-1} in the IR spectrum, revealed the presence of a secondary alcohol. Thus, all of the four oxygens in the molecular formula of **35** were confidently assigned. One-dimensional proton decoupling and COSY NMR experiments revealed that the remaining eight protons form a chain of sequential couplings (Table 7). That is, the olefinic proton at δ 6.82 was coupled (J = 0.6 Hz) to two proton resonances at δ 3.22. The olefinic proton was also coupled to a proton at δ 4.45, which was in turn, coupled to a proton at δ 2.22. Finally, two mutually coupled (J = 16.7 Hz) protons at δ 2.56 and 2.40 coupled to the proton at δ 2.22. With the help of XHCORR data, all of the protons were precisely matched to the corresponding carbons.

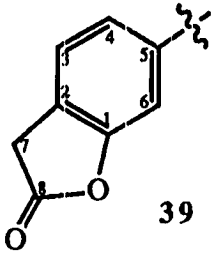
Table 7. ^{1}H and ^{13}C NMR assignments for C-1 ~ C-8 parts of compounds **35**, **36**, **37**, and **39**.

	proton[a,c]				carbon[b,d]			
#	**35**	**36**	**37**	**39**	**35**[e]	**36**[f]	**37**[f]	**39**[f]
1	—	—	—	—	197.4 s	196.5 s	197.4 s	154.9 s
2	—	—	—	—	133.9 s	137.8 s	138.2 s	120.2 s
3	6.82 dd(4.6,0.6)	6.87 brd(4.8)	6.84 dd(4.8,0.9)	7.16 brd(7.7)	147.0 d	142.5 d	142.5 d	124.2 d
4	4.45 dd(4.6,3.4)	5.43 dd(4.7,3.4)	5.43 dd(4.8,3.5)	6.95 brd(7.6)	66.3 d	68.5 d	68.4 d	123.9 d
5	2.22 m	2.34 m	2.35 m	—	40.8 d	38.6 d	38.6 d	143.4 s
6	2.56 dd(16.7, 10.0) 2.40 dd(16.7, 3.9)	2.58 dd(16.9, 10.1) 2.49 dd(16.9, 4.4)	2.58 dd(16.8, 10.2) 2.48 dd(16.8, 4.3)	6.93 brs	39.0 t	39.3 t	39.2 t	110.7 d
7	3.22 AB	3.24 AB	3.22 AB	3.69 s	35.0 t	35.1 t	34.8 t	34.2 t
8	—	—	—	—	171.9 s	176.4 s	171.0 s	174.5 s
OAc	—	2.10 s	2.10 s	—	—	169.7 s	170.4 s	—
					—	20.2 q	20.9 q	—
OMe	3.68 s	—	3.68 s	—	51.7 q	—	52.1 q	—

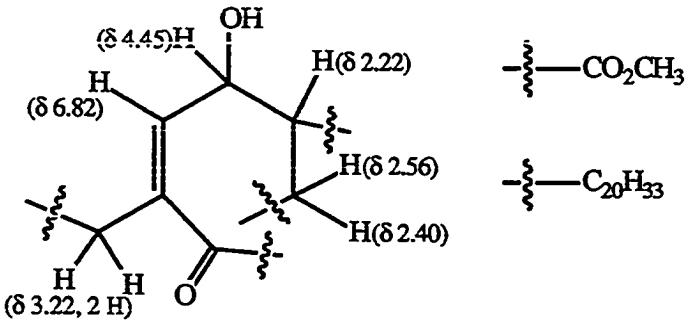
[a] measured in $CDCl_3$ solution. [b] Spectra for **35** and **36** were measured in C_6D_6 solution. Data for **37** and **39** were obtained in $CDCl_3$ solution. [c] Assignments were made by proton decoupling and COSY experiments. [d] Multiplicities were determined by DEPT experiments. [e] Assignments were made by XHCORR and COLOC (6 Hz) experiments. [f] Assignments were made by comparison with compound **35**.



35 - 37



39



The connectivity of the methyl ester was determined by a COLOC experiment. The ester carbon at δ 171.9 was coupled to the protons at δ 3.68 and 3.22. Also, the olefinic carbon at δ 133.9 was coupled to the protons at δ 3.22. Therefore, the methyl ester was connected to the allylic carbon at δ 35.0 in the ^{13}C NMR spectrum. The lowfield chemical shifts of both protons (δ 2.56 and 2.44) and carbon (δ 39.0) signals confidently allowed assignment of the methylene to the α-carbonyl position. Thus, compound **35** was unambiguously defined as a product of mixed biogenesis possessing an unique C_{28} skeleton. Compound **35** has two asymmetric carbon centers at C-4 and C-5. The stereochemistry of these centers were determined by a chemical reaction and CD measurement, and these experiments are discussed in the final section.

Compound **36** was isolated as an oil and analyzed for $C_{30}H_{44}O_5$ by high resolution mass and ^{13}C NMR spectrometry. Spectral data of **36** were very similar to those of **35**. However, there were several significant differences in the ^1H NMR, ^{13}C NMR and IR spectra. The methoxy proton at δ 3.68 in the ^1H NMR spectrum of **35** was replaced by an upfield proton resonance at δ 2.10 (3 H, s) in compound **36** (Table 7). Also, the C-4 proton at δ 4.45 was significantly shifted to lower field (δ 5.43) in the ^1H NMR spectrum of **36**. Corresponding differences were found in the ^{13}C NMR spectrum, in which the methoxy carbon at δ 51.7 of **35** was replaced by acetyl carbons at δ 169.7 (s) and 20.2 (q). In addition, instead of the hydroxyl absorption at 3450 cm^{-1} in **35**, the IR spectrum

of **36** showed absorption bands at 3500 - 2500 (broad) and 1715 cm^{-1}. These changes were accommodated in compound **36** by replacement of the methyl ester and hydroxyl group in **35** with a free carboxylic acid and an acetoxyl group, respectively.

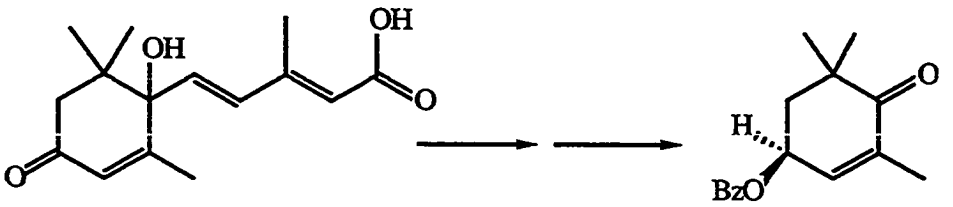
The structure of compound **36** was confirmed by simple chemical conversions (Figure 6). Treatment of **35** with acetic anhydride quantitatively yielded compound **37** as the sole product. The acetate **37** was fully characterized by spectral analysis (Table 7). Treatment of **36** with diazomethane gave compounds **37** and **38** as the major products. The spectral data of compound **37** derived from two different precursors (**35** and **36**) were identical with each other. Also, the structure of **38** was defined by spectral analysis as a product formed by a 2 + 3 cycloaddition of diazomethane to the conjugated enone of **37** (experimental section). Thus, the structure of compound **36** was unambiguously determined.

Compounds **35** and **36** possess asymmetric carbon centers at C-4 and -5. Consideration of vicinal coupling constants between the C-5 proton at δ 2.22 and C-6 protons at δ 2.56 ($J_{5,6}$=10.0 Hz for **35**) and 2.44 ($J_{5,6}$=3.9 Hz for **35**) revealed that the orientation of the C-5 proton is axial to the cyclohexenone ring. Subsequently, small couplings (J=3.4 Hz) between the C-4 (δ 4.45) and C-5 (δ 2.22) proton showed the equatorial orientation for the C-4 proton. The equatorial orientation of the C-4 proton was further confirmed by a chemical conversion. Compound **36** was very unstable in both basic and acidic media. Treatment of **36** with acetic anhydride in pyridine gave compound **39** as a major product (Figure 6). Compound **39** was also produced by treatment of a chloroform solution of **36** with silica.

Compound **39** was isolated as an oil and analyzed for $C_{28}H_{38}O_2$ by high resolution mass and ^{13}C NMR spectrometry. In contrast to **35** and **36**, compound **39** was optically inactive ($[\alpha]_D = 0^o$). ^1H and ^{13}C NMR spectra of **39** showed that the tetraprenyl chain of **36** was intact. However, signals of protons and carbons corresponding to the acetyl

group of **36** had disappeared. In addition, there were numerous changes on both ^{1}H and ^{13}C NMR signals corresponding to the cyclohexenone ring of **36** (Table 7). Consideration of ^{1}H and ^{13}C NMR spectra and a COSY NMR experiment revealed that the cyclohexenone ring was transformed to a phenol group. Displacement of absorption maxima (from 274 and 280 nm to 240 and 296 nm in 1 N KOH solution) in the UV spectrum of **39** confirmed this interpretation. Another transformation also occurred on the carboxyl group of **36**. Since the molecular formula showed the presence of only two oxygens in **39**, the carboxylic group must have been converted to a phenyl ester. A characteristic absorption band at 1815 cm^{-1} in the IR spectrum confirmed this. Thus, compound **39** was defined as a 2-coumaranone derivative. It is well known that the deacetylation of the acetate readily occurs when the orientation of the acetyl group is axial to the ring. Therefore, the C-4 acetyl must be axial to the cyclohexenone ring. The relative configurations are thus **4R*** and **5R***.

The absolute stereochemistry of the asymmetric centers were determined by circular dichroic (CD) measurement on a derivative. The 4-hydroxyl cyclohexenone moiety of **35** is identical with (+)-abscisic acid (**42**). Nakanishi and coworkers determined the absolute stereochemistry of **42** by performing CD measurements on a derivative, **43**.[77]

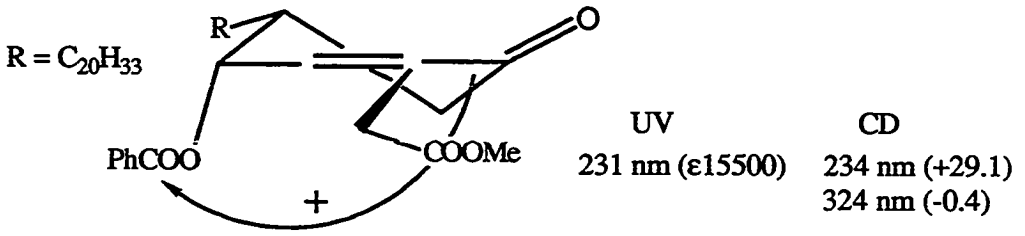


42 **43**

Treatment of **35** with benzoyl chloride gave **40** as a major product (Figure 6). The ^{1}H NMR spectrum and a COSY NMR experiment of **40** revealed that besides the

replacement of the C-4 hydroxyl by a benzoyl group, the structure of **40** was identical to **35**. The UV spectrum (MeOH) of **40** showed an absorption maximum at 231 nm (ε15500). The CD spectrum (MeOH) of **40** showed a maximum at 234 nm (MD + 29.1) corresponding to the first Cotton effect (Figure 7). However, the second Cotton effect which was usually the weaker, was buried in a strong positive background ellipticity. It is not unusual that the CD spectrum showed very disproportionate maxima or only one maximum.[78] Since the position (234 nm) of the first Cotton effect was very near to the expected one (235 nm), the CD of **40** was confidently assigned as a positive split Cotton effect. Thus, the absolute stereochemistry of the asymmetric centers at C-4 and 5 were unambiguously determined. The overall configurations are 4R and 5R for both compounds **35** and **36**.

Figure 7. Absolute stereochemistry of compound **40**.



40

Experimental Section

Collection, Extraction and Isolation. *Eunicea* sp.(specimen number CI 86-193) was collected by hand using SCUBA at 20 to 25 m depth in July, 1986, along the offshore of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonian was next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 25 g of crude organic materials (from 1.5 kg, dry weight of the gorgonian). Compounds **28-30** were eluted from a vacuum flash silica gel column with 20-40% EtOAc in isooctane and further purified with the same solvents by HPLC using preparative silica gel columns. Compounds **35** and **36** were eluted with 60-100% EtOAc in isooctane and further purified with 95% MeOH in water by reverse phase HPLC using Dynamax C-18 columns.

Compound 28. The ester **28** was isolated as a white solid by HPLC (30% EtOAc in isooctane). Recrystallization from MeOH gave 5 g (20% of the crude extract) of **28**; mp 124-125°. Compound **28** exhibited $[\alpha]_D$ +104° (c 0.7, $CHCl_3$) and displayed the following spectral features; HRMS: M^+, m/z obsd 350.2465, $C_{21}H_{34}O_4$ required 350.2457; low-resolution MS: m/z (relative intensity) 350 (6), 318 (23), 290 (11), 197 (16), 178 (17), 165 (68), 136 (28), 107 (41), 81 (86), 69 (64), 41 (100); IR ($CHCl_3$) 3500, 2940, 1710, 1640, 1460, 1440, 1380, 1270 cm^{-1}; UV (MeOH) 220 nm (ε24000).

Compound 29. The ester **29** was isolated as an oil by HPLC (25% EtOAc in isooctane). The extract yielded 1.3 g (5% of the crude extract) of **29**. Compound **29** showed $[\alpha]_D$ +106° (c 2.4, $CHCl_3$) and displayed the following spectral features; HRMS: M^+, m/z obsd 350.2462, $C_{21}H_{34}O_4$ required 350.2457; low-resolution MS : m/z (relative intensity) 350 (5), 290 (27), 207 (15), 165 (31), 121 (33), 109 (46), 93 (50), 81 (76), 69 (77), 55 (92), 41 (100); IR ($CHCl_3$) 3500, 2940, 1710, 1640, 1460, 1440, 1380, 1200

cm^{-1}; UV (MeOH) 220 nm (ε18000).

Compound 30. The ester **30** was isolated as a white solid by HPLC (20% EtOAc in isooctane). Recrystallization from MeOH yielded 240 mg (1% of the crude extract) of **30**; mp 72-74°. Compound **30** showed $[\alpha]_D$ -70° (c 2.2, $CHCl_3$) and displayed the following spectral features; HRMS: M^+, m/z obsd 350.2455, $C_{21}H_{34}O_4$ required 350.2457; low-resolution MS : m/z (relative intensity) 350 (5), 318 (15), 290 (14), 207 (10), 169 (28), 165 (64), 136 (28), 121 (35), 109 (43), 93 (55), 81 (87), 69 (67), 55 (90), 41 (100); IR ($CHCl_3$) 3480, 2940, 1706, 1640, 1460, 1440, 1270, 1170 cm^{-1}; UV (MeOH) 220 nm (ε16800).

Oxidation of 28, 29, and 30. To a stirred suspension of 86 mg (0.4 mmol) of pyridium chlorochromate (PCC) and 20 mg of sodium acetate (0.2 mmol) in 1.2 ml of dry CH_2Cl_2, a solution of 73 mg of **28** (0.21 mmol) in 0.7 ml of dried CH_2Cl_2 was added. After stirring the mixture for 3 hrs, a new spot began to appear on analysis by TLC of the reaction mixture. The reaction was complete after 48 hrs, and the solvent was removed under vacuum. Organic materials were extracted with ethyl ether (2x20 ml). A small scale silica vacuum flash chromatography of the extract removed the decomposed and inorganic materials. Final purification by HPLC (30% EtOAc in isooctane) gave 46 mg (0.14 mmol, 67% yield) of **31** as the sole product; mp 52.5-54°. The diketone **31** exhibited $[\alpha]_D$ +48° (c 0.8, $CHCl_3$) and displayed the following spectral features; HRMS: M^+, m/z obsd 348.2299, $C_{21}H_{32}O_4$ required 348.2301; low-resolution MS: m/z (relative intensity) 348 (3), 316 (8), 197 (25), 177 (13), 169 (20), 165 (56), 137 (25), 109 (48), 91 (42), 81 (77), 69 (48), 67 (81), 55 (100); IR ($CHCl_3$) 2940, 1770, 1710, 1645, 1460, 1430, 1270, 1190 cm^{-1}; UV (MeOH) 220 nm (ε17800). Oxidations of **29** and **30** were performed by the same procedure. In each case, a diketone was obtained as the sole product. HRMS, ^1H and ^{13}C NMR spectra of these were identical with **31** obtained from **28**.

Reaction of 28 with LDA. To a stirred solution of 105 mg (0.3 mmol) of **28** in 5 ml of dry THF at -78° under N_2, 450 µl of 10% LDA in hexane solution was added. The mixture was stirred for 2 hrs and followed for 3 hrs at room temperature. To quench the reaction, 3 ml of distilled water were added and the mixture was extracted with 20 ml of ethyl ether. After removal of solvents under vacuum, more than 90% of the reactant was recovered by HPLC (30% EtOAc in isooctane) and no sizable amount of product was found.

Reaction of 28 with nBuLi. To a stirred solution of 180 mg (0.5 mmol) of **28** in 2 ml of dry THF at 0° under N_2, 250 µl (0.7 mmol) of 2.6 M nBuLi in n-hexane was added. After 2 hrs, stirring was continued overnight at room temperature. To remove excess nBuLi, 100 µl of distilled water was added. Organic materials were separated by 20 ml of ethyl ether and 20 ml of water. The ether layer was washed with 10% $NaHCO_3$ (2x15 ml), water (2x10 ml), and solvent removed under vacuum. Separation by HPLC (15% EtOAc in isooctane) gave 45 mg of **32**, 19 mg of **33**, 16 mg of **29**, 11 mg of **28**, and 9 mg of **30**. Yields were 18, 7, 9, 6, and 5%, respectively. The major product **32** was isolated as a white solid; mp 113-114°, and showed the following spectral data; HRMS: $(M-H_2O)^+$, m/z obsd 474.4439, $C_{32}H_{60}O_3$ required 474.4441; low-resolution MS: m/z (relative intensity) 474 (9), 417 ($M^+-H_2O-C_4H_9$, 65), 399 (18), 334 (11), 234 (11), 205 (13), 191 (23), 123 (23), 109 (31), 95 (40), 85 (100), 57 (75); IR (NaCl) 3400, 2960, 2940, 1645, 1470, 1455, 1380, 1210, 880, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.46 (1 H, dd, 8.0, 8.0), 4.74 (1 H, brs), 4.68 (1 H, brs), 3.40 (1 H, d, 6.7), 1.69 (3 H, brs), 1.00 (3 H, d, 6.9), 0.99 (3 H, d, 6.9), 0.88 (9 H, m); ^{13}C NMR ($CDCl_3$) δ 149.0 (s), 142.4 (s), 123.0 (d), 109.8 (t), 78.1 (s), 78.1 (d), 77.4 (d), 44.2 (d), 39.8 (t), 39.3 (t)x2, 38.6 (t), 37.9 (t), 32.0 (d), 30.0 (t), 29.3 (t), 27.5 (t), 26.8 (d), 26.1 (t), 25.7 (t), 25.6 (t), 24.0 (t), 23.3 (t), 23.1 (t)x3, 22.5 (q), 20.3 (q), 17.3 (q), 14.1 (q)x3; $[\alpha]_D$-8.5° (c 0.8, $CHCl_3$). Compound **33** was isolated as an oil and

displayed the following spectral features; HRMS: M^+, m/z obsd 434.3743, $C_{28}H_{50}O_3$ required 434.3762; low-resolution MS: m/z (relative intensity) 434 (0.3), 416 (M^+-H_2O, 3), 377 (M^+-C_4H_9, 100), 359 (6), 109 (14), 95 (17), 85 (63), 69 (27), 57 (39); IR (film) 3500, 2960, 2940, 1710, 1640, 1460, 1380, 890 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.28 (1 H, dd, 8.6, 5.7), 4.73 (1 H, brs), 4.67 (1 H, brs), 4.13 (1 H, d, 5.9), 3.33 (1 H, d, 5.8), 2.90 (1 H, dd, 19.6, 8.3), 2.43 (1 H, m), 1.65 (3 H, brs), 1.14 (3 H, d, 6.7), 1.00 (3 H, d, 7.0), 0.88 (6 H, m); ^{13}C NMR ($CDCl_3$) δ 210.9 (s), 148.3 (s), 143.1 (s), 124.8 (d), 110.4 (t), 81.3 (d), 78.0 (s), 44.5 (d), 43.3 (t), 40.1 (t), 39.5 (t), 35.6 (t), 34.5 (d), 30.8 (t), 28.8 (t), 26.0 (t), 25.7 (t), 25.6 (t), 25.4 (d), 23.4 (t), 23.3 (t), 23.1 (t)x2, 21.0 (q), 19.7 (q), 16.8 (q), 14.1 (q)x2; $[\alpha]_D$+3.4° (c 1.3, $CHCl_3$).

Reaction of 28 with p-nitrobenzoyl chloride. To a stirred solution of 126 mg (0.36 mmol) of **28** in 2 ml of pyridine, 204 mg (1.10 mmol) of p-nitrobenzoyl chloride was added. After stirring the mixture overnight at room temperature, pyridine was removed under vacuum. To remove excess p-nitrobenzoyl chloride, 40 ml of benzene was added, and the mixture was filtered. Separation of benzene-soluble materials by HPLC (35% EtOAc in isooctane) gave 81 mg (0.16 mmol, 45% yield) of **34**, the p-nitrobenzoyl ester, and 46 mg (0.13 mmol, 37%) of unreacted **28**. Compound **34** showed the following ^1H NMR ($CDCl_3$) data; δ 8.30 (2 H, d, 9.0), 8.20 (2 H, d, 9.0), 6.66 (1 H, dd, 10.9, 4.8), 5.23 (1 H, d, 1.6),4.79 (1 H, brs), 4.76 (1 H, brs), 3.72 (3 H, s), 2.82 (1 H, dd, 18.9, 5.8), 2.35-1.95 (9 H, m), 1.73 (3 H, brs), 1.65-1.40 (7 H, m), 1.18 (3 H, d, 6.8), 1.02 (3 H, d, 6.7).

Compound 35. The ester **35** was isolated as a viscous oil by reverse phase HPLC (95% MeOH in water). The extract yielded 240 mg (1% of the crude extract) of **35**. Compound **35** exhibited $[\alpha]_D$ +39.8° (c 1.3, $CHCl_3$) and displayed the following spectral features; HRMS: M^+, m/z obsd 456.3227, $C_{29}H_{44}O_4$ required 456.3239: {M-$C_{20}H_{33}$ (tetraprenyl)}$^+$, m/z obsd 183.0662, $C_9H_{11}O_4$ required 183.0657; low-resolution

MS: m/z (relative intensity) 456 (35), 319 (8), 205 (11), 204 (17), 183 (6), 179 (14), 167 (27), 149 (24), 137 (31), 136 (49), 95 (21), 93 (25), 81 (57), 69 (100); IR (film) 3420, 2920, 1740, 1680, 1440, 1380, 1170, 1110 cm^{-1}; UV (MeOH) 225 nm (sh, ε10900); ^1H NMR ($CDCl_3$) δ 6.82 (1 H, dd, 4.6, 0.6), 5.18-5.05 (4 H, m), 4.45 (1 H, dd, 4.6, 3.4), 3.68 (3 H, s), 3.22 (2 H, AB), 2.56 (1 H, dd, 16.7, 10.0), 2.40 (1 H, dd, 16.7, 3.9), 2.29 (1 H, m), 2.22 (1 H, m), 2.15-1.90 (13 H, m), 1.68 (3 H, brs), 1.63 (3 H, brs), 1.60 (9 H, brs); ^{13}C NMR (C_6D_6) δ 197.4 (s), 171.9 (s), 147.0 (d), 137.4 (s), 135.2 (s), 135.0 (s), 133.9 (s), 131.1 (s), 124.9 (d), 124.8 (d), 124.7 (d), 122.1 (d), 66.3 (d), 51.7 (q), 40.8 (d), 40.3 (t), 40.2 (t) x 2, 39.0 (t), 35.0 (t), 28.7 (t), 27.2 (t), 27.1 (t), 27.0 (t), 25.8 (q), 17.7 (q), 16.2 (q), 16.1 (q) x 2.

Compound 36. The acid **36** was isolated as a viscous oil. Reverse phase HPLC (95% MeOH in water) gave 350 mg (1.5% of the crude extract) of **36**. Compound **36** showed $[\alpha]_D$ +101° (c 0.8, $CHCl_3$) and displayed the following spectral features; HRMS: M^+, m/z obsd 484.3177, $C_{30}H_{44}O_5$ required 484.3189; low-resolution MS: m/z (relative intensity) 484 (21), 204 (13), 137 (22), 136 (47), 135 (28), 123 (23), 93 (23), 81 (49), 69 (100), 49 (21); IR (film) 3400-2700 (broad), 2920, 1740, 1715, 1685, 1440, 1370, 1230, 1030 cm^{-1}; UV (MeOH) 225 nm (sh, ε9900); ^1H NMR ($CDCl_3$) δ 6.87 (1 H, brd, 4.8), 5.43 (1 H, dd, 4.7, 3.4), 5.15-5.00 (4 H, m), 3.24 (2 H, AB), 2.58 (1 H, dd, 16.9, 10.1), 2.49 (1 H, dd, 16.9, 4.4), 2.34 (1 H, m), 2.22 (1 H, m), 2.10 (3 H, s), 2.05-1.90 (13 H, m), 1.68 (3 H, brs), 1.60 (9 H, brs), 1.57 (3 H, brs); ^{13}C NMR (C_6D_6) δ 196.5 (s), 176.4 (s), 169.7 (s), 142.5 (d), 137.8 (s), 135.7 (s), 135.3 (s), 135.0 (s), 131.1 (s), 124.9 (d), 124.8 (d), 124.5 (d), 121.2 (d), 68.5 (d), 40.2 (t) x 3, 39.3 (t), 38.6 (d), 35.1 (t), 28.5 (t), 27.2 (t), 27.1 (t), 27.0 (t), 25.8 (q), 20.2 (q), 17.7 (q), 16.1 (q) x 3.

Acetylation of compound 35. To a stirred solution of 14 mg (0.03 mmol) of **35** in 1 ml of dry pyridine, 0.5 ml of acetic anhydride was added. After stirring for 2 hrs,

pyridine and excess anhydride were removed under vacuum. The acetate **37** was quantitatively obtained as a viscous oil. Compound **37** showed the following spectral data; HRMS: M^+, m/z obsd 498.3351, $C_{31}H_{46}O_5$ required 498.3345; low-resolution MS: m/z (relative intensity) 498 (32), 204 (14), 167 (47), 137 (22), 136 (39), 107 (26), 93 (26), 81 (50), 69 (100), 43 (25); IR (film) 2920, 1740, 1730, 1685, 1440, 1375, 1240, 1170, 1020 cm^{-1}; UV (MeOH) 224 nm (sh, ε12500); ^1H NMR ($CDCl_3$) δ 6.84 (1 H, dd, 4.8, 0.9), 5.43 (1 H, dd, 4.8, 3.5), 5.13-5.05 (4 H, m), 3.68 (3 H, s), 3.22 (2 H, AB), 2.58 (1 H, dd, 16.8, 10.2), 2.48 (1 H, dd, 16.8, 4.3), 2.35 (1 H, m), 2.21 (1 H, m), 2.10 (3 H, s), 2.10-1.95 (13 H, m), 1.68 (3 H, brs), 1.60 (9 H, brs), 1.58 (3 H, brs); ^{13}C NMR ($CDCl_3$) δ 197.4 (s), 171.0 (s), 170.4 (s), 142.5 (d), 138.2 (s), 135.8 (s), 135.3 (s), 134.9 (s), 131.2 (s), 124.4 (d), 124.2 (d), 123.9 (d), 120.3 (d), 68.4 (d), 52.1 (q), 39.7 (t) x 3, 39.2 (t), 38.6 (d), 34.8 (t), 28.4 (t), 26.8 (t), 26.6 (t) x 2, 25.7 (q), 20.9 (q), 17.7 (q), 16.1 (q), 16.0 (q) x 2.

Reaction of compound 36 with diazomethane. The reaction was performed by using a micro-scale diazomethane-generation apparatus. A solution of 26 mg (0.05 mmol) of **36** in 2 ml of THF was put into the outer tube of the apparatus, and 12.4 mg (0.08 mmol) of 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) was into the inner tube. To prevent vigorous reaction, the bottom half of the apparatus was cooled with ice. Diazomethane was formed by dropwise addition of 1 ml of 5 N NaOH solution into the inner tube. After 20 min, the solvent was removed under vacuum and the products were separated by HPLC (30% EtOAc in isooctane). The reaction yielded 11.2 mg (0.02 mmol, 45% yield) of **37** and 10.6 mg (0.02 mmol, 39%) of **38**. Proton and carbon NMR spectra of **37** were identical to those from the compound obtained from the acetylation of **35**. Compound **38** was obtaind as an oil and exhibited the following spectral features; low resolution MS : m/z (relative intensity) 540 ($C_{32}H_{48}N_2O_5$, 9) 217 (14), 193 (14), 181 (100), 155 (12), 154 (42), 149 (51), 147 (12); IR ($CHCl_3$) 2970,

2920, 2400, 1730, 1520, 1435, 1040, 845 cm^{-1}; UV (MeOH) no λmax; ^1H NMR ($CDCl_3$) δ 5.15-5.05 (3 H, m), 4.97 (1 H, brt, 6.9), 4.87 (1 H, dd, 19.1, 9.0), 4.75 (1 H, dd, 19.2, 2.8), 4.60 (1 H, brs), 3.65 (3 H, s), 3.30 (1 H, d, 16.8), 2.97 (1 H, brd, 9.0), 2.45 (1 H, dd, 17.4, 7.9), 2.33 (1 H, dd, 17.5, 10.8), 2.09 (3 H, s), 2.10-1.94 (16 H, m), 1.68 (3 H, s), 1.60 (9 H, s), 1.53 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 201.6 (s), 170.2 (s), 170.1 (s), 138.2 (s), 135.4 (s), 135.1 (s), 131.1 (s), 124.9 (d), 124.7 (d), 124.4 (d), 121.0 (d), 99.1 (s), 83.0 (t), 71.9 (d), 51.2 (q), 40.8 (d), 40.2 (t) x 2, 40.0 (t), 39.6 (t), 35.7 (t), 33.3 (d), 30.6 (t), 27.2 (t), 27.1 (t), 26.9 (t), 25.8 (q), 20.7 (q), 17.7 (q), 16.1 (q) x 2, 15.9 (q).

Deacetylation of 36. The acid **36** was very unstable in both acidic and basic media. This phenomenon was accidently found during exploratory attempts at acetylation. Standard work-up procedure resulted in the decomposition of most of **36** and formation of (ca 40% yield) of **39** as the major product. Also, **36** was converted to compound **39**, when a chloroform solution of **36** was stirred with TLC grade silica gel at room temperature overnight. Compound **39** was optically inactive {$[\alpha]_D$ 0° (c 0.5, $CHCl_3$)}, and exhibited the following spectral features; HRMS: M^+, m/z obsd 406.2861, $C_{28}H_{38}O_2$ required 406.2872; low-resolution MS: m/z (relative intensity) 406 (31), 337 (6), 173 (24), 147 (22), 136 (58), 123 (28), 81 (50), 69 (100), 41 (24); IR (film) 2920, 1810, 1630, 1595, 1500, 1435, 1385, 1060, 960 cm^{-1}; UV (MeOH) 280 (ε6000), 274 (ε5900), 217 nm (ε34000); UV (MeOH + 1 N KOH) 296 (ε4700), 240 (ε8500), 215 nm (ε34000); ^1H NMR ($CDCl_3$) δ 7.16 (1 H, brd, 7.7), 6.95 (1 H, brd, 7.6), 6.93 (1 H, brs), 5.30 (1 H, brt, 7.9), 5.15-5.08 (3 H, m), 3.69 (2 H, s), 3.36 (2 H, d, 7.2), 2.15-1.95 (12 H, m), 1.71 (3 H, brs), 1.68 (3 H, brs), 1.60 (6 H, brs), 1.56 (3 H, brs); ^{13}C NMR ($CDCl_3$) δ 174.5 (s), 154.9 (s), 143.4 (s), 137.1 (s), 135.2 (s), 134.9 (s), 131.2 (s), 124.4 (d), 124.2 (d) x 2, 123.9 (d) x 2, 122.2 (d), 120.2 (s), 110.7 (d), 39.7 (t) x 3, 34.2 (t), 32.9 (t), 26.8 (t), 26.6 (t), 26.5 (t), 25.7 (q), 17.7 (q), 16.2 (q), 16.0 (q) x 2.

Benzoyl esterification of 35. To a solution of 32 mg (0.07 mmol) of **35** in 1 ml of pyridine with catalytic amount of 4-dimethylamino pyridine, an excess amount of benzoyl chloride was added, and stirred at 40° overnight. Pyridine was removed under vacuum. The residue was dissolved with 2 ml of THF. To convert excess benzoyl chloride to benzoic acid, a few drops of distilled water were added and the mixture was stirred for 12 hrs. After removing THF under vacuum, 20 ml of CH_2Cl_2 and 20 ml of water were added, and separated. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ (15 ml x 2), water (15ml x 2) and dried under vacuum. Purification by HPLC (30% EtOAc in isooctane) gave 12.9 mg (0.02 mmol, 33% yield) of **40**. The benzoate **40** showed the following spectral features; ^1H NMR (C_6D_6): δ 8.10 (2 H, d, 7.3), 7.11 (1 H, m), 7.05 (2 H, m), 6.56 (1 H, d, 4.3), 5.59 (1 H, t, 3.5), 5.35-5.20 (3 H, m), 4.98 (1 H, m), 3.30 (3 H, s), 3.06 (2 H, AB), 2.58 (1 H, dd, 16.6, 9.2), 2.28 (1 H, dd, 16.5, 3.3), 2.25-1.99 (15 H, m), 1.68 (3 H, s), 1.62 (6 H, s), 1.57 (3 H, s), 1.44 (3 H, s); UV (MeOH) 231 nm (ε15500); CD (MeOH) 217 (+1.56 MD), 234 (+29.1 MD), 324 nm (-0.41 MD).

Chapter IV

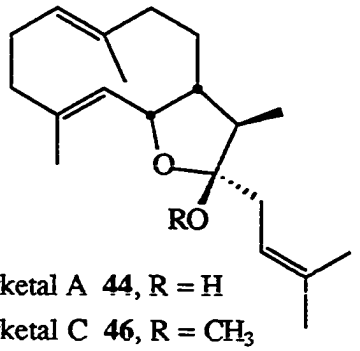
Asperketals, norasperenals, and a cembranoid from *Eunicea asperula* and *Eunicea* sp (F 87 - 32, *Euniceopsis*)

Eunicea asperula (specimen number CI 86 - 194) was collected offshore of the Tobago Cays, eastern Caribbean Sea, in July 1986. Extraction with dichloromethane gave 36 g of the crude organic materials (from 1 kg, dry weight of the gorgonian). Seven metabolites (**44 - 50**) were purified by vacuum flash silica gel chromatography of the crude extract followed by HPLC of the relatively nonpolar fractions (Figure 8). The structures of these compounds were elucidated by spectral methods. Asperketals A - E (**44 - 48**) are ketals and hemi-ketals related to the dilophol class of 10-membered ring diterpenoids, while another, asperketal F (**49**), belongs to the fuscol class. In addition, a known diterpenoid, obscuronatin (**50**) was isolated as a minor metabolite. Obscuronatin was originally isolated from the Red Sea soft coral *Xenia obscuronata*.[79,80] Diterpenoids of the dilophol class were first observed from brown marine algae of the family Dictyotacea.[81-84] Although the asperketals were not isolated in large amounts, it is believed that the actual concentrations of these compounds in the living organisms are much higher. Compounds **44** and **45**, in particular, were highly unstable and decomposed under the methods of isolation.

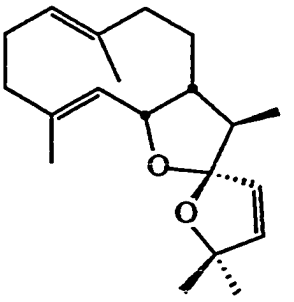
In addition to the asperketals and obscuronatin, several diterpenoids of the cembrane class were isolated as minor metabolites. The structure of a cembranoid (**51**) was determined by spectral methods and is discussed after asperketals.

Asperketals A and B were also isolated from an unknown *Eunicea* sp. (F 87 - 32, *Euniceopsis*) collected from the Florida Keys, in July 1987. In addition to the asperketals however, the organic extract contained trisnorditerpenoids, the norasperenals A - D (**52 - 55**, Figure 10), which are structurally related to the asperketals.

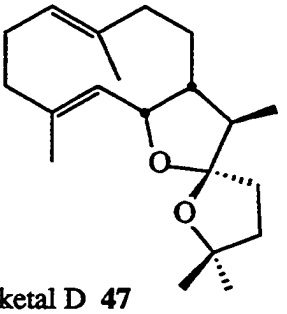
Figure 8. Asperketals and obscuronatin from *E. asperula*.



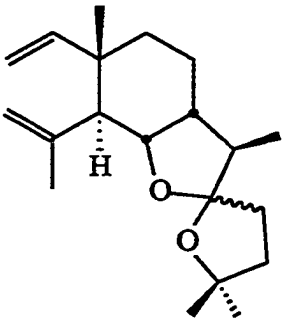
asperketal A **44**, R = H
asperketal C **46**, R = CH_3



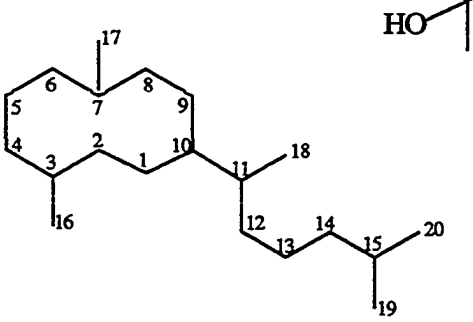
asperketal B **45**

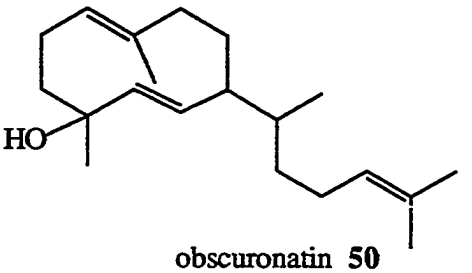


asperketal D **47**
asperketal E **48** (12-epi)



asperketal F **49**





obscuronatin **50**

Asperketal A (**44**) crystallized from CH_2Cl_2 after HPLC purification, and was found to be highly unstable in $CDCl_3$ solution. More than 90% of the compound decomposed within a few hours during NMR experiments. The compound analyzed for $C_{20}H_{32}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Six ^{13}C NMR bands (Table 8) at δ 140.2 (s), 135.2 (s), 133.5 (s), 128.2 (d), 125.2 (d), and 119.7(d) illustrated asperketal A to possess three double bonds and, thus, two rings. The proton NMR spectrum of **44** (Table 9) showed four low field resonances at δ 5.53 (brdd, 8.7, 6.6), 5.08 (dd, 10.9, 8.3), 4.93 (dd, 10.9, 0.3) and 4.82 (brt, 8.1, 8.1). Since there were only three olefinic protons, one of these four resonances was concluded to have originated from a proton attached to an oxygen-bearing carbon. A direct carbon-proton NMR correlation experiment (XHCORR) confirmed this assumption by illustrating that the proton at δ 5.08 corresponded to a carbon at δ 73.7 (d). This proton was coupled (J=10.9 Hz) to an adjacent olefinic proton (δ 4.93), which showed asperketal A to bear an allylic oxygen bond (C-1). Proton decoupling and COSY NMR experiments showed that protons at δ 5.53 (1 H, brdd, 8.7, 6.6), 2.53 (1 H, brdd, 14.0, 8.7), 2.35 (1 H, brdd, 14.0, 6.6), 1.59 (one of two 3 H, brs) and 1.53 (3 H, brs) were part of a terminal isoprene unit in **44**. This assignment was supported by mass spectral fragments at m/z 235 (M^+-69, relative intensity 39), 217 (M^+-H_2O-69, 9), and 69 (18) which illustrate a characteristic loss of the C_5H_9 fragment

Further consideration of ^1H NMR single frequency decoupling data, and COSY data showed that all of the protons in **44** belong to four isolated spin systems suggesting four partial structures. With the help of XHCORR data (all of the 16 proton-bearing carbons and their protons were precisely matched), three of these four units were confidently identified as a-c.

Table 8. ^{13}C NMR assignments for asperketals A-F (**44-49**).[a]

C	**44**[b]	**45**[c]	**46**[c]	**47**[c]	**48**[c]	**49**[c]
1	73.7 d	74.4 d	73.7 d	72.9 d	75.7 d	81.2 d
2	128.2 d	128.2 d	128.0 d	127.8 d	130.8 d	41.2 d[d]
3	133.5 s	133.4 s	133.1 s[e]	132.8 s	131.2 s	144.8 s
4	40.5 t	40.4 t	40.3 t	40.4 t	40.4 t	113.6 t
5	26.2 t	26.0 t	26.0 t	26.0 t	25.9 t	109.8 t
6	125.2 d	125.4 d	125.3 d	125.3 d	126.3 d	149.8 d
7	140.2 s	140.2 s	140.2 s	140.3 s	139.2 s	40.8 s
8	38.7 t	38.4 t	38.7 t	38.8 t	38.4 t	35.8 t
9	33.3 t	33.1 t	33.1 t	33.3 t[f]	32.9 t[f]	20.7 t
10	50.1 d	49.6 d[g]	50.6 d[g]	50.1 d[g]	51.5 d[g]	42.7 d[d]
11	50.7 d	49.3 d[g]	51.0 d[g]	49.3 d[g]	51.0 d[g]	55.2 d
12	104.4 s	118.2 s	107.0 s	114.1 s	116.8 s	114.9 s
13	37.7 t	140.8 d	31.8 t	37.7 t	37.3 t	37.7 t
14	119.7 d	126.8 d	120.4 d	33.7 t[f]	34.5 t[f]	34.4 t
15	135.2 s	86.8 s	132.8 s[e]	81.5 s	80.8 s	77.2 s
16	17.1 q	16.9 q	16.9 q	16.9 q	17.1 q[h]	25.4 q
17	20.7 q	20.5 q	20.6 q	20.6 q	21.1 q	18.1 q
18	13.8 q	13.8 q	13.5 q	13.3 q	16.5 q[h]	11.9 q
19	18.0 q	29.3 q[i]	17.9 q	30.3 q[i]	30.7 q[i]	30.3 q[i]
20	26.0 q	27.7 q[i]	25.9 q	28.9 q[i]	29.1 q[i]	28.5 q[i]
OCH3			47.6 q			

[a] ^{13}C NMR spectra were recorded at 50 MHz in C_6D_6 solutions. Multiplicities were determined from DEPT experiments. The δ values are in ppm downfield from Me_4Si. [b] Assignments were made from XHCORR and COLOC experiments. [c] Assignments were made by comparison with **44**. [d-i] Signals within a column may be reversed.

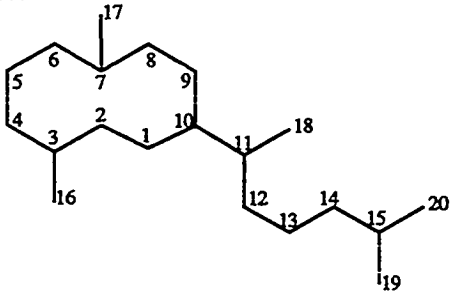
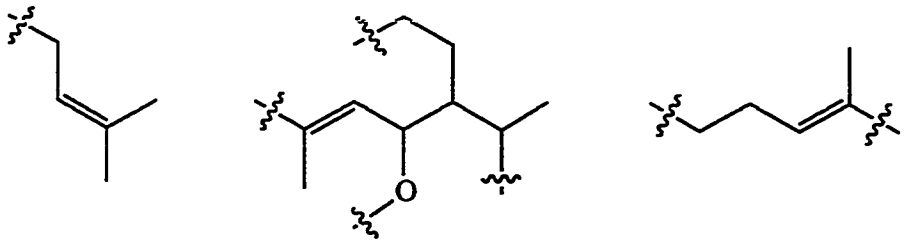


Table 9. ^{1}H NMR assignments for asperketals A-F (44-49).[a]

#	44	45	46	47	48	49
1	5.08 (1H,dd,10.9,8.3)	5.11 (1H,dd,10.8,8.3)	4.81 (1H,dd,10.9,8.3)	4.99 (1H,dd,10.9,7.8)	4.87 (1H,dd,10.4,6.9)	4.31 (1H,dd,10.8,7.8)
2	4.93 (1H,dd,10.9,0.3)	4.92 (1H,dd,10.9,0.8)	4.93 (1H,brd,10.9)	4.93 (1H,brd,10.4)	5.21 (1H,brd,10.4)	1.90 (1H,m)[c]
4	2.05 (1H,m)[c]	2.01 (1H,m)[c]	1.99 (1H,m)[c]	2.05 (1H,m)[c]	b	5.08 (1H,dd,1.6,1.6)
	1.74 (1H,m)[c]	1.70 (1H,m)[c]	1.75 (1H,m)[c]	1.96 (1H,m)[c]	b	4.80 (1H,brd,0.8)
5	2.00 (1H,m)[c]	1.95 (1H,m)[c]	2.00 (1H,m)[c]	2.00 (1H,m)[c]	b	4.90 (1H,dd,10.8,1.4)
	1.95 (1H,m)[c]	1.92 (1H,m)[c]	1.94 (1H,m)[c]	1.73 (1H,m)[c]	b	4.87 (1H,dd,17.5,1.4)
6	4.82 (1H,brdd,8.1,8.1)	4.81 (1H,brdd,8.2,7.7)	4.80 (1H,ddq,8.1,8.1, 1.5)	4.82 (1H,ddq,8.1,8.1, 1.5)	4.86 (1H,m)[c]	5.78 (1H,dd,17.5, 10.8)
8	2.44 (1H,brddd,13.4, 3.4,3.4)	2.44 (1H,brddd,13.7, 3.2,3.2)	2.42 (1H,brddd,13.3, 3.8,3.8)	2.43 (1H,brddd,13.3, 3.9,3.9)	2.05 (1H,m)[c]	1.40 (1H,m)[c]
	1.60 (1H,m)[c]	1.55 (1H,m)[c]	1.59 (1H,m)[c]	1.61 (1H,m)[c]	1.61 (1H,m)[c]	1.06 (1H,ddd,13.1,4.3, 4.3)
9	1.80 (1H,m)[c]	1.80 (1H,m)[c]	1.78 (1H,m)[c]	1.80 (1H,m)[c]	1.82 (1H,m)[c]	1.58 (1H,m)[c]
	1.41 (1H,ddd,14.0,4.1, 4.1)	1.45 (1H,m)[c]	1.40 (1H,dddd,14.1, 3.8,3.8,1.0)	1.42 (1H,dddd,13.7, 4.1,4.1,1.1)	1.45 (1H,m)[c]	1.47 (1H,m)[c]
10	2.09 (1H,m)[c]	2.10 (1H,m)[c]	2.05 (1H,m)[c]	2.00 (1H,m)[c]	1.85 (1H,m)[c]	2.27 (1H,m)[c]
11	1.54 (1H,m)[c]	1.60 (1H,m)[c]	1.70 (1H,m)[c]	1.55 (1H,m)[c]	2.08 (1H,m)[c]	1.82 (1H,m)[c]
13	2.53 (1H,brdd,14.0,8.7)	5.71 (1H,d,5.7)	2.62 (1H,brdd,15.2,6.9)	2.05 - 1.95 (2H,m)[c]	b	2.00
	2.35 (1H,brdd,14.0,6.6)		2.56 (1H,brdd,15.2,7.9)			
14	5.53 (1H,brdd,8.7,6.6)	5.46 (1H,d,5.7)	5.44 (1H,brdd,7.9,6.9)	1.85 (1H,m)[c]	b	- 1.78 (3H,m)[c]
				1.50 (1H,m)[c]		1.47 (1H,m)[c]
16	1.59 (3H,brs)	1.54 (3H,d,1.3)	1.59 (3H,d,1.4)	1.59 (3H,d,1.4)	1.50 (3H,d,1.4)	1.85 (3H,brs)
17	1.25 (3H,brs)	1.21 (3H,brs)	1.23 (3H,d,1.5)	1.21 (3H,d,1.5)	1.33 (3H,brs)	0.83 (3H,s)
18	1.19 (3H,d,6.8)	1.06 (3H,d,6.8)	1.23 (3H,d,6.8)	1.07 (3H,d,6.8)	0.99 (3H,d,7.2)	0.97 (3H,d,6.6)
19	1.53 (3H,brs)	1.44 (3H,s)[d]	1.57 (3H,brs)	1.46 (3H,s)[d]	1.47 (3H,s)[d]	1.41 (3H,s)[d]
20	1.59 (3H,brs)	1.25 (3H,s)[d]	1.68 (3H,d,1.1)	1.16 (3H,s)[d]	1.15 (3H,s)[d]	1.09 (3H,s)[d]
others	2.29 (1H,brs,-OH)		3.30 (3H,s,-OCH_3)			

[a] Assignments were aided by spin coupling, COSY and NOEDS experiments. *J* values are reported in Hz and chemical shifts are given in δ units.
[b] Nonassignable resonances. [c] Coupling constants were not determined. [d] Signals within a column may be reversed.



a b c

The infrared spectrum of asperketal A showed strong hydroxyl absorption at 3450 cm^{-1}. This observation, taken with the presence of a D_2O-exchangeable broad singlet at δ 2.22 in the ^1H NMR spectrum, showed **44** to possess a hydroxyl group. Since the only unassigned carbon in the ^{13}C NMR spectrum of **44** was a quaternary carbon resonance at δ 104.4, the oxygenation in asperketal A was concluded to be a cyclic hemiketal.

A long range carbon-proton correlation experiment, COLOC (Table 10), allowed partial structures a-c and the hemiketal to be combined. The hemiketal carbon at δ 104.4 (C-12) showed couplings with two protons at δ 2.53 and 2.35 (C-13), and with a methyl group at δ 1.19 (C-18). Also, couplings between the carbon at δ 125.2 (C-6) and the proton at δ 1.60 (C-8), and between the carbon at δ 38.7 (C-8) and the methyl protons at δ 1.25 (C-17) were observed. Thus, the structure of asperketal A could be unambiguously assigned as a cyclic hemiketal of the dilophol class. Another datum supporting this conclusion was a ^1H NMR resonance for a vinyl methyl (C-17) which was unusually high field shifted (δ 1.25 in C_6D_6, 1.37 in $CDCl_3$ solution).[85] The phenomenon is due to transannular shielding by the 2,3 double bond in germacrene rings, which requires an ***E*** configuration for both sites of unsaturation. The high field ^{13}C NMR resonance assigned to C-16 (δ 17.1) is also characteristic of the 10-membered ring system. Determination of the relative stereochemistry at four asymmetric carbons (C-1, -10, -11, and -12) was accomplished by NOE studies and these experiments will be discussed together with the other asperketals.

Table 10. Results of a COLOC NMR experiment with asperketal A (44).[a]

carbon #	protons
1	9 (1.80)[b]
2	16
3	16
6	8 (1.60)[b], 17
7	17
8	9 (1.80)[b], 17
9	8 (1.60)[b]
10	8 (2.44)[b], 18
11	18
12	13 (2.53)[b], 13 (2.35)[b], 18
14	19, 20
15	19, 20
16	2
17	8 (2.44)[b]
19	20
20	19

[a] Experiments were performed at 50 MHz in C_6D_6 solution. Parameters were optimized for couplings of 6 Hz.

[b] The numbers in parentheses are the ^1H NMR chemical shifts of the protons which correlate.

Asperketal B (**45**) analyzed for $C_{20}H_{30}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Comparison of ^1H NMR, ^{13}C NMR and COSY spectra showed similarities between **45** and compound **44**, and showed that partial structures (b) and (c) were also present. However, there were several significant differences in the spectral data. The high resolution mass spectrum, and ^{13}C NMR data, for asperketal B indicated the presence of one additional ring. The IR spectrum of **45** lacked hydroxyl absorption, and instead of one low field proton at δ 5.53, the ^1H NMR spectrum of **45** showed two protons at δ 5.71 (d, *J*=5.7) and 5.46 (d, 5.7). Also, the methyl resonances at δ 1.59 and 1.53 in **44** were shifted to 1.44 and 1.25 in **45** and were much sharper. Finally, ^{13}C NMR data revealed significant changes in the chemical shifts and multiplicities of the four carbons (C-12 ~ C-15) associated with the terminal isoprene unit in asperketal A. These changes were accommodated in asperketal B by the construction of a bicyclic ketal constellation between C-12 and C-15. A small coupling constant (5.7 Hz) between two adjacent olefinic protons (δ 5.71 and 5.46) showed that the double bond had migrated to the C-13 ~ C-14 position. The high field resonances of the C-17 methyl (δ 1.21) in the ^1H NMR spectrum and the C-16 methyl (δ 16.9) in ^{13}C NMR spectrum of **45** indicated that asperketal B also possesses two ***E*** double bonds in identical transannular positions.

Asperketal C (**46**) analyzed for $C_{21}H_{34}O_2$ by high resolution mass and ^{13}C NMR spectrometry. The spectral data for **46** were very similar to **44**, with the only apparent difference a change of hydroxyl group in **44** to methoxy in **46**. The presence of methoxy was indicated by new resonances in the ^{13}C NMR spectrum (δ 47.6, q), and in the ^1H NMR spectrum at δ 3.30 (s). A change of fragmentation pattern in the mass spectrum from M^+-H_2O, M^+-69-H_2O in **44** to M^+-OCH_3, M^+-69-$HOCH_3$ further supported this assignment. Therefore, asperketal C was identified as the methyl ketal analog of **44**. Transannular shielding effects and ^{13}C NMR data again led to the conclusion that both the Δ^2 and Δ^6 double bonds have ***E*** configurations.

Asperketal D (**47**) analyzed for $C_{20}H_{32}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Examination of ^{1}H, ^{13}C and COSY NMR data revealed that the 10-membered ring is also present in **47**. The absence of the two low field olefinic resonances (δ 140.8 and 126.8) in the ^{13}C NMR spectrum of **47**, and the presence of two new methylene carbons at δ 37.7 and 33.7, led to the conclusion that the asperketal D is the 13,14-dihydro derivative of **45**. Complete ^{1}H NMR analysis fully supported this assignment.

An obviously related diterpenoid, asperketal E (**48**), also analyzed for $C_{20}H_{32}O_2$ by combined spectral methods. This compound was highly comparable with **47**, in that there were similar NMR bands and coupling constants observed. There were, however, several significant differences in the chemical shifts of protons at C-1, -2, -10, -18, and -19. In particular, the C-11 methine proton was shifted 0.53 ppm to low field in relation to its chemical shift in asperketal D (**47**). Therefore, **48** was assigned as the ketal epimer (C-12) of **47**. Asperketal E (**48**), like the other metabolites in this series, showed spectral features consistent with the assignment as a 10-membered ring-containing diterpenoid.

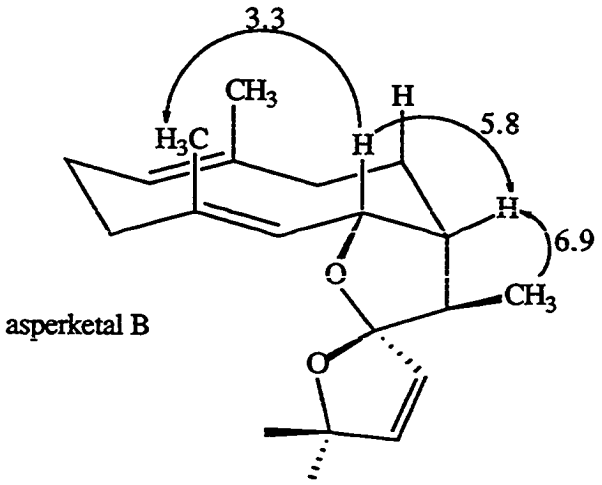
Asperketal F (**49**) analyzed for $C_{20}H_{32}O_2$ by high resolution mass and ^{13}C NMR spectrometry. The presence of two double bonds [δ 149.8 (d), 144.9 (s), 113.6 (t), and 109.8 (t)] in ^{13}C NMR spectrum showed that **49** was also tricyclic. ^{1}H, ^{13}C and COSY NMR data revealed that **49** also possessed the bicyclic ketal system formed with 5-membered rings. Analysis of ^{1}H and ^{13}C NMR data showed, however, that asperketal F possessed an entirely different carbon skeleton. Proton resonances at δ 5.08 (1 H, dd, *J*=1.6, 1.6), 4.80 (1 H, brd, 0.8) and 1.85 (3 H, brs), which were mutually coupled, along with carbon resonances at δ 149.8 (s), 113.6 (t) and 25.4 (q), were interpreted to indicate an isopropenyl group. Another cluster of olefinic protons at δ 5.78 (1H, dd, 17.5, 10.8), 4.90 (1 H, dd, 10.8, 1.4) and 4.87 (1 H, dd, 17.5, 1.4), which correlated with carbons at δ 149.8 (d) and 109.8 (t), were readily assigned to a terminal vinyl group.

A proton at δ 4.87 showed long range coupling with a high field singlet methyl at δ 0.83 (C-17). Since these protons do not couple with other protons, both the vinyl and methyl groups must be connected to a quaternary carbon (δ 40.8) assigned as C-7. The remaining component of asperketal F was assigned as a 6-membered ring on the basis of typical proton NMR features. Thus, ketal **49** was identified as a cyclic ketal possessing an "elemane" type divinylcyclohexane ring. This skeleton might arise via a Cope rearrangement of either of the ketals **47** or **48**. Thermolysis of **47** and **48**, in separate experiments at 180 °C under N_2, failed to generate asperketal F, and resulted instead in rapid decomposition. No data could be obtained to conclusively define the stereochemistry of asperketal F at C-12.

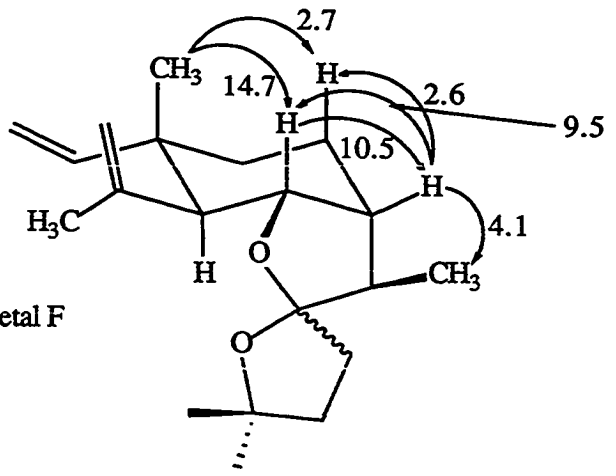
Obscuronatin (**50**) was isolated as an oil in very small amounts. ^1H, ^{13}C and COSY NMR data showed a very close correlation with published data for this compound. Obscuronatin, previously isolated from *Xenia obscuronata*,[79,80] is the only diterpene of the dilophol class to be isolated from marine animals. Further studies to determine the stereochemistry of this compound have not been pursued.

Asperketals A - E (**44** - **48**) possess four asymmetric centers at C-1, C-10, C-11, and C-12. Compound **49** has two additional centers at C-2 and C-7. Application of the nuclear Overhauser enhancement difference spectroscopy method (NOEDS) in the analysis of ^1H NMR data for these compounds allowed the configurations at these centers to be determined. However, because of the overlapping of many proton resonances, this method was only partially successful. Irradiation of the C-1 proton of asperketal B (Figure 9) enhanced the C-16 methyl proton by 3.3%, and confirmed the ***E*** configuration of the Δ^2 olefin. At the same time, the C-10 proton was enhanced by 5.8%, which implied that the C-1 and C-10 protons were oriented *cis* on the 10-membered ring. The C-10 proton was also enhanced (6.9%) by irradiation of the C-18 methyl protons. Thus, the relative configurations at three contiguous asymmetric centers were found to be 1S*,

Figure 9. Results of ^{1}H NMR NOEDS experiments with asperketals B (**45**) and F (**49**).*



asperketal B



asperketal F

* NOE enhancement in percent

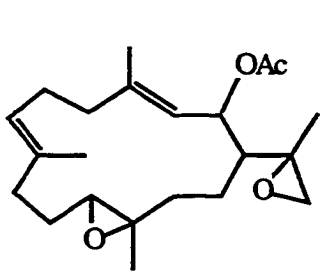
10R* and 11R*. Irradiation of the C-18 methyl protons failed to produce significant enhancements to either the C-13 or C-14 protons.

The stereochemistry of asperketal F, (**49**), was pursued using the same methods (Figure 9). Irradiation of the C-17 methyl enhanced the C-1 (14.7%) and C-9β (2.7%) protons (1,3-diaxial substituents), while the C-2 proton showed no enhancement. Thus, the ring juncture of **49** was assigned as the expected *trans* diaxial arrangement at C-2 and C-7. NOEDS methods were also used to probe the stereochemistry of the bicyclic ketal constellation in asperketal F. Irradiation of the C-1 proton enhanced the C-10 proton by 10.5%, while irradiation of the C-10 proton enhanced the proton resonances for the C-1, C-9β and C-18 methyl protons by 9.6, 2.6 and 4.1%, respectively. Therefore, the relative configurations of the three adjacent asymmetric centers are 1S*, 10R* and 11R*, identical with those of **45**.

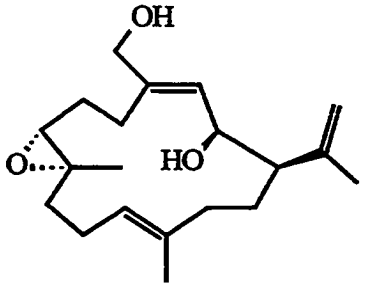
The configurations at C-12 in metabolites **44** - **48** were not confidently assignable by NOEDS methods (irradiation of C-18 methyl protons showed no enhancement of either the C-13 or -14 protons). This problem could be approached, however, by analysis of the chemical shifts of the C-11 protons. Consideration of a three-dimensional model of the ketal containing tetrahydrofuran rings in the asperketals, showed that the C-11 proton chemical shift could be an effective predictor of the relative stereochemistry at C-12. When oriented *cis* on the five-membered ring, the ketal oxygen eclipses the C-11 proton deshielding it. When *trans*, the C-11 proton is distant from oxygen and would not be deshielded. Using this approach, and comparing the chemical shifts of the C-11 protons in various asperketals, the oxygen and C-11 protons are proposed to be *trans* oriented in diterpenoids **44** - **47** and *cis* in the epimeric asperketal E (**48**). Using the R*S* notation, compounds **44**, **46**, and **48** possess the C-12 = S* configurations, while **45** and **47** possess the C-12 = R* configurations. This translates to overall relative stereochemistries of 1S*, 10R*, 11R*, and 12S* for asperketals A, C and E (**44**, **46** and **48**), and relative

configurations of 1S*, 10R*, 11R*, 12R* for asperketals B and D (**45** and **47**).

Careful re-examination of the nonpolar silica 'vacuum flash' chromatographic fractions revealed the presence of structurally very different diterpenoids. Several structurally related minor metabolites were isolated by silica HPLC. Spectral analyses revealed that these compounds possessed the well known cembrane skeleton. Due to the conformational variations in solution however, most signals in ^{1}H and ^{13}C NMR spectra were very broad and unable to be characterized. As a result, only the structure of a cembranoid (**51**) was elucidated. Previous investigations of gorgonians identified as *Eunicea asperula* resulted in the isolation of asperdiol (**10**).[20] However, asperdiol was not found from this collection.



51



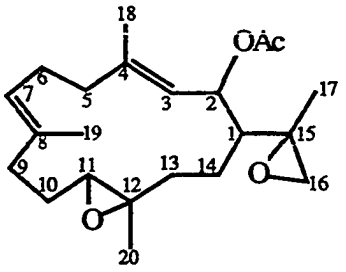
asperdiol (**10**)

Compound **51** crystallized from CH_2Cl_2 after HPLC purification. The compound analyzed for $C_{22}H_{34}O_4$ by high resolution mass and carbon NMR spectrometry. Strong absorption band at 1730 cm^{-1} in the IR spectrum and a corresponding carbon peak at δ 169.2 (s) in ^{13}C NMR spectrum (Table 11) revealed the presence of an ester. A singlet methyl resonance at δ 1.73 (2.06 in $CDCl_3$) in the ^{1}H NMR spectrum indicated that the ester is an acetate group. Four lowfield resonances at δ 138.6 (s), 133.5 (s), 126.0 (d), and 124.5 (d) in the ^{13}C NMR spectrum showed **51** to possess two double bonds, thus three rings. The absence of absorption maximum in the UV spectrum indicated that the double bonds are not conjugated with each other. Since

Table 11. ^{1}H and ^{13}C NMR assignments and results of a COLOC experiment with compound **5i**.

	proton[a]		carbon[b,c]		COLOC[d,e]
#	$CDCl_3$	C_6D_6	C_6D_6		C_6D_6
1	f	1.70 m	48.3	d	17
2	5.70 brd	6.16 dd (8.3,2.4)	72.1	d	—
3	5.21 brs	5.29 dd (8.3,1.2)	124.5	d	18
4	----	—	138.6	s	18
5	f	1.97 m (2H)	39.5	t	3, 18
6	f	1.97 m (2H)	24.7	t	—
7	5.05 brs	5.02 m	126.0	d	19
8	----	—	133.5	s	19
9	f	1.92 m 2.10 m	36.7	t	7, 19
10	f	1.30 m 1.65 m	24.7	t	—
11	2.60 dt	2.71 dd (6.4,6.4)	59.4	d	20
12	----	—	61.6	s	20
13	f	1.35 m 1.81 m	34.9	t	20
14	f	1.55 m 1.60 m	22.5	t	—
15	----	—	56.7	s	17
16	2.56 brd 2.68 brd	2.29 brd (5.3) 2.66 brd (5.3)	54.6	t	17
17	1.33 s[g]	1.25 s	19.7	q	—
18	1.63 s	1.56 d (1.2)	15.5	q	3
19	1.61 s	1.41 brs	14.8	q	7
20	1.26 s[g]	1.11 s	18.1	q	—
OAc	2.06 s	1.73 s	169.2 20.9	s q	2, CH_3

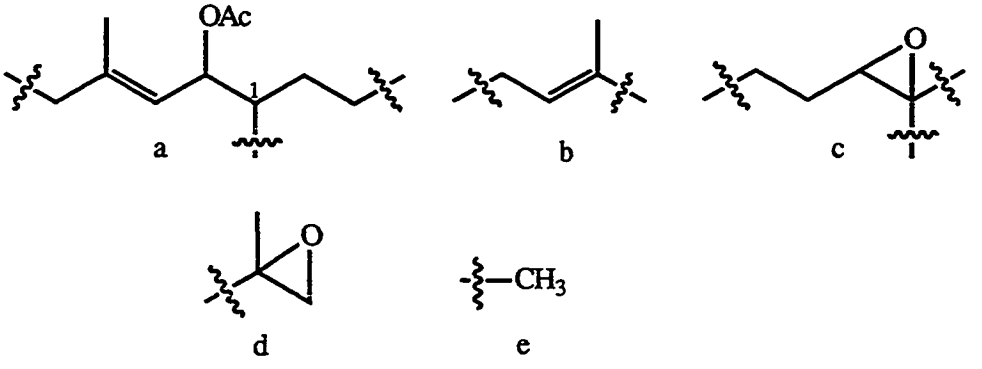
[a] Assignments were made by COSY and one-dimensional decoupling experiments. [b] Numbers of attached protons were determined by DEPT experiments. [c] Assignments were made by XHCORR and COLOC experiments. [d] Parameters were optimized for couplings of 6 and 8 Hz. [e] Numbers in the column are protons correlated. [f] Unassignable resonances. [g] may be reversed.



there are three low field proton resonances at δ 6.16 (dd, J = 8.3, 2.4), 5.29 (dd, 8.3, 1.2) and 5.02 (m), one of these was concluded to have originated from a proton attached to the acetoxy-bearing carbon. A direct carbon-proton correlation experiment (XHCORR) confirmed this assumption by illustrating that the proton at δ 6.16 corresponded with a carbon at δ 72.1 (d). One-dimensional decoupling and COSY experiments showed that this proton was coupled directly to an olefinic proton at δ 5.29 (J = 8.3) which was further coupled to a vinyl methyl at δ 1.56 and a complicated high field resonance at δ 1.97.

Compound **51** is a tricyclic diterpene. Four carbon resonances at δ 61.6 (s), 59.4 (d), 56.7 (s), and 54.6 (t) revealed that two of three rings are epoxides. A proton at δ 2.66 was coupled to another proton at δ 2.29 (J = 5.3 Hz) and a methyl at δ 1.25 (J = vs), illustrating the presence of an epoxy isopropyl group. The other epoxide proton at δ 2.71 (t, 6.4, 6.4) was coupled to protons at δ 1.65 and 1.30 which were further coupled to protons at δ 2.10 and 1.92.

Further consideration of the ^{1}H NMR single frequency decoupling data, and COSY data showed that all of the protons in **51** belong to five isolated spin systems. With the help of XHCORR data, these five units were confidently identified as a-e.



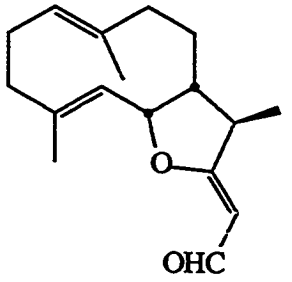
a b c d e

Confirmation of these partial structures and connectivities were made by long

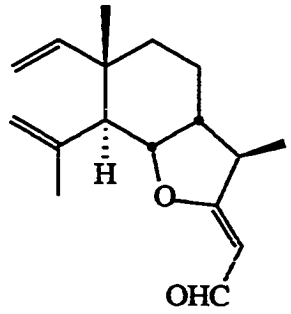
range carbon-proton correlation experiment (COLOC, Table 11). Carbons at δ 56.7, 54.6 and 48.3 (C-15, -16, and -1, respectively) were coupled to a common methyl resonance at δ 1.25 (C-17). Thus, the epoxy isopropyl group (d) was assigned to be attached to the C-1 of partial structure a. Several long range correlations to the methyl protons at δ 1.41 (C-19) and 1.11 (C-20) revealed connectivities around quaternary carbons at C-8 and -12, and revealed the connections of a, b, and e to the partial structure c. Due to the similar chemical shifts of the C-5 and C-6 protons, the connection between a and b was not determined by COLOC experiment. However, consideration of molecular formula and carbon multiplicities revealed the connection. Thus, the structure of compound **51** was unambiguously defined as (3*E*, 7*E*)-2-acetoxy-11,15-diepoxycembra-3,7-diene. The stereochemistries of the asymmetric carbon centers were not determined.

Asperketals A (**44**) and B (**45**) were also isolated from an unknown *Eunicea* sp. collected at the Florida Keys. F. M. Bayer concluded that these animals taxonomically belong to the subgenus *Euniceopsis*, but that they are morphologically distinct from the known species. In addition to the asperketals however, the dichloromethane extract of the dried gorgonian contained a series of other metabolites. Silica vacuum flash chromatography followed by silica and C-18 reverse phase HPLC gave four metabolites. Norasperenals A - D (**52** - **55**) were trisnorditerpene aldehydes in which the terminal isopropyl units (C-15, -19 and -20) of the asperketals were removed (Figure 10). Thus, norasperenals A and C were related to the asperketals A - E in possessing the 10-membered ring, while norasperenals B and D were related to the asperketal F. To the best of my knowledge, these are the first examples of trisnorditerpenoids from marine organisms. However, norasperenals were highly unstable and slowly decomposed even at -20°. In addition, norasperenals C and D were rapidly converted to their isomers, norasperenals A and B, respectively. As a result, norasperenals C and D were only characterized by ^{1}H and ^{13}C NMR (norasperenal C) spectra.

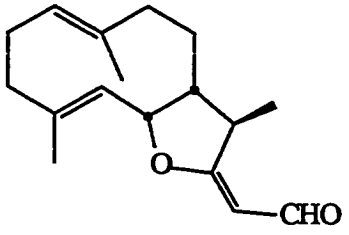
Figure 10. Structures of norasperenals A-D (**52-55**).



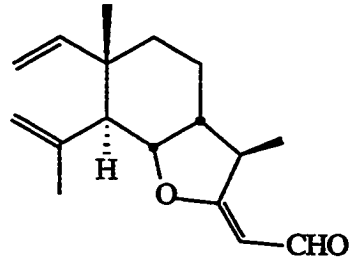
52, norasperenal A



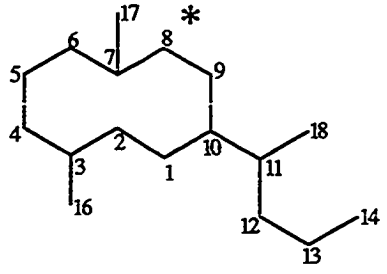
53, norasperenal B



54, norasperenal C



55, norasperenal D



* followed the numberings for asperketals

Table 12. Comparison of ^{13}C NMR data for asperketals A (**44**), F (**49**) and norasperenals A - C (**52** - **54**).

C	44	52	54	49	53
1	73.7 d	82.3 d	80.6 d	81.2 d	83.9 d
2	128.2 d	125.6 d	126.0 d	41.2 d[a]	41.8 d[a]
3	133.5 s	136.0 s	135.3 s	144.8 s	143.2 s
4	40.5 t	39.9 t	39.7 t	113.6 t	113.6 t
5	26.2 t	25.7 t	25.5 t	109.8 t	110.9 t
6	125.2 d	127.5 d	127.4 d	149.8 d	147.5 d
7	140.2 s	137.9 s	137.7 s	40.8 s	40.7 s
8	38.7 t	36.6 t	37.0 t	35.8 t	33.0 t
9	33.3 t	31.5 t	32.6 t	20.7 t	19.5 t
10	50.1 d	48.3 d	49.6 d	42.7 d[a]	38.7 d[a]
11	50.7 d	47.7 d	47.3 d	55.2 d	53.3 d
12	104.4 s	180.2 s	183.3 s	114.9 s	179.6 s
13	37.7 t	101.3 d	102.2 d	37.7 t	102.6 d
14	119.7 d	187.8 d	188.4 d	34.4 t	188.0 d
15	135.2 s			77.2 s	
16	17.1 q	16.7 q	16.5 q	25.4 q	25.4 q
17	20.7 q	20.7 q	21.4 q	18.1 q	16.8 q
18	13.8 q	18.4 q	19.9 q	11.9 q	14.9 q
19	18.0 q			30.3 q[b]	
20	26.0 q			28.5 q[b]	

Data were obtained in C_6D_6 solutions. Multiplicities were determined from DEPT experiments. Assignments for **44** and **52** were made from XHCORR and COLOC experiments. Assignments for others were made by comparison with **44** and **52**. [a,b] Signals within a column may be reversed.

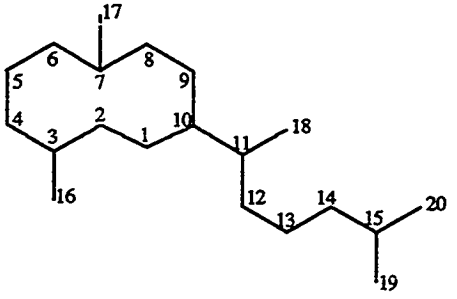
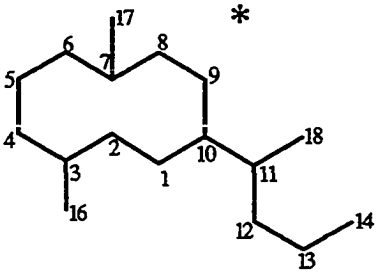


Table 13. ^{1}H NMR assignments for norasperenals A-D (**52-55**).

#	52	53	54	55
1	4.89 dd (9.4,6.1)	4.25 dd (10.9,7.4)	4.94 dd (9.8,4.8)	4.23 dd (7.8,6.6)
2	4.66 brd (9.4)	1.56 d (11.0)	4.75 brd (9.7)	1.88 d (8.0)
4	1.95 - 1.87[b] 1.72 m	5.01 brs 4.64 brs	1.98 - 1.85[b] 1.83 - 1.68[b]	4.64 brs 4.61 brs
5	1.95 - 1.87 (2H)[b]	4.87 dd (10.8,1.1) 4.80 dd (17.5,1.1)	1.98 - 1.85 (2H)[b]	4.89 brd (10.3) 4.82 brd (17.6)
6	4.63 m	5.57 dd (17.5,10.8)	4.65 brd (7.6)	5.66 dd (17.5,10.9)
8	2.16[b]	1.02 ddd (13.7,13.7, 4.3)	2.23[b]	a
	1.45[b]	0.90 ddd (13.6,4.7, 2.8)	1.46[b]	a
9	1.45[b]	1.30 dddd (14.1,14.1, 5.9,4.9)	1.74[b]	a
	0.97 m	1.13 m	0.85 m	a
10	1.50 m	1.42 m	1.60 m	a
11	2.10 m	2.15 ddq (12.1,1.3, 6.6)	2.92 m	2.62 m
13	5.22 d (8.3)	5.22 dd (8.3,1.5)	5.87 d (7.3)	5.89 d (7.5)
14	10.56 d (8.3)	10.47 d (8.3)	9.77 d (7.3)	9.88 d (7.7)
16	1.32 brs	1.64 d (0.6)	1.29 brs	1.60 brs
17	1.18 brs	0.67 s	1.21 brs	0.73 s
18	0.77 d (7.1)	0.66 d (6.7)	0.91 d (6.9)	0.92 d (6.8)

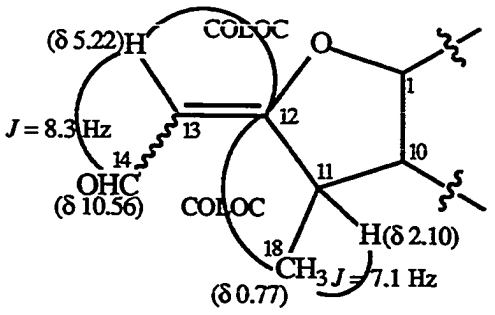
^{1}H NMR spectra were obtained in C_6D_6 solutions. Assignments were made by spin decoupling and COSY experiments. [a] Nonassignable proton resonances. [b] Coupling constants were not determined.



* followed the numberings for asperketals

Norasperenal A (**52**) was isolated as a white solid, and analyzed for $C_{17}H_{24}O_2$ by high resolution mass and ^{13}C NMR spectrometry. A low field carbon signal at δ 187.8 (d) in the ^{13}C NMR spectrum, and a corresponding proton signal at δ 10.56 (d, J = 8.3 Hz) showed the presence of an aldehyde functionality (Table 12 and 13). An absorption band at 1655 cm^{-1} in the IR spectrum, and UV absorption at 271 nm, indicated that the aldehyde was in conjugation with a double bond. A large coupling constant (J = 8.3 Hz) of the aldehyde proton to a proton at δ 5.22 in the ^1H NMR spectrum confirmed this conclusion. The chemical shifts of several signals in the ^{13}C NMR spectrum of **52** were similar to those from compound **44** (Table 13). Careful examination revealed that compound **52** possessed the same 10-membered ring as **44**. A combination of ^1H NMR COSY and XHCORR experiments confirmed this interpretation.

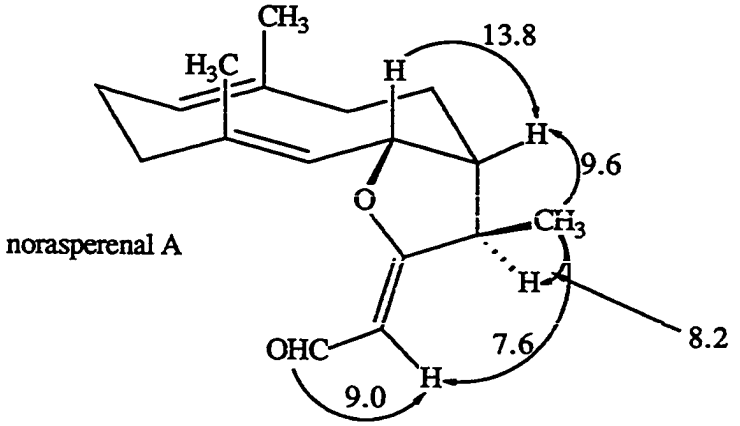
The remaining components of structure were also determined by spectral analyses. An XHCORR experiment assigned the carbon signal at δ 101.2 (d) to the α carbon of the double bond in conjugation with the aldehyde. The unusually large difference between the chemical shifts of the α and β {δ 180.2 (s)} carbons indicated the attachment of an oxygen to the β carbon. Therefore, the β carbon must be connected to the C-1 carbon by an ether linkage. COSY NMR data showed coupling between a methine proton at δ 2.10 and methyl protons at δ 0.77. The low field shift of the methine proton indicated the attachment of the methine carbon to the β carbon of the unsaturated aldehyde. Thus, the connectivity of the α,β-unsaturated aldehyde was fully determined. Additional data supporting this connectivity came from a COLOC experiment (optimized for 6 Hz) which showed long range couplings between the β carbon and the olefinic proton at δ 5.22 and methyl protons at δ 0.77. Thus, norasperenal A was unambiguously assigned as a trisnorditerpene aldehyde in which a terminal three carbon unit (C-15, -19, and -20) had been removed.



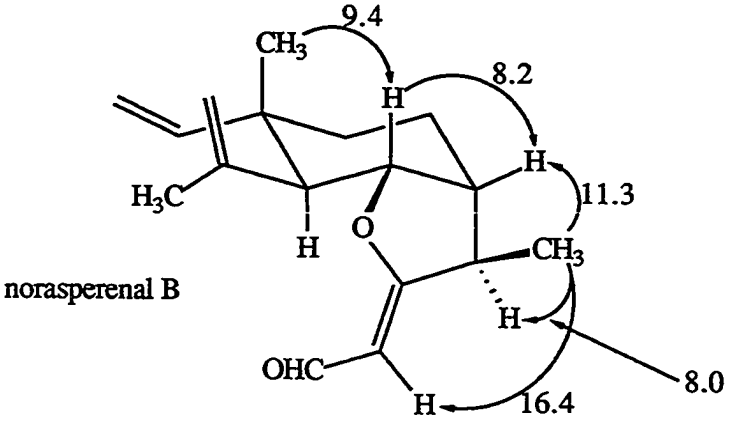
Norasperenal A has three asymmetric double bonds (Δ^2, Δ^6 and Δ^{12}) and three asymmetric methine centers (C-1, -10, and -11). The high field shifts of the C-16 carbon (δ 16.7) and the C-17 protons (δ 1.18) revealed **52** to possess the same ***E*** configurations for both the Δ^2 and Δ^6 double bonds as in asperketal A. The stereochemistries of the other centers were determined by ^{1}H NMR NOEDS experiments (Figure 11). Irradiation of the C-1 proton enhanced the C-10 proton by 13.8%. The C-10 proton was also enhanced by the irradiation of the C-18 methyl protons (9.6%). Therefore, compound **52** has the same **1S***, **10R***, and **11R*** configurations as found in asperketal A. Irradiation of the C-18 protonas also enhanced the C-11 and -13 protons (8.2 and 7.6%, respectively), indicating the ***Z*** configuration for the Δ^{12} double bond. This assignment was supported by the irradiation of the aldehyde proton which enhanced only the C-13 proton by 9.0%. Thus, the stereochemistry of norasperenal A was unambiguously assigned. The overall relative configurations were thus 2(***E***), 6(***E***), 12(***E***), **1S***, **10R***, and **11R***.

Norasperenal B (**53**) was isolated as a white solid which analyzed for $C_{17}H_{24}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Spectral analyses readily indicated **53** to possess the same α,β-unsaturated aldehyde as **52**; signals at δ 188.0 (d) and 102.6 (d) in the ^{13}C NMR spectrum, δ 10.47 (1 H, d, 8.3) in the ^{1}H NMR spectrum, and 1655 cm^{-1} in the IR spectrum. The remaining structure was also determined by spectral analyses. ^{13}C NMR data from **53** were very similar to that derived from asperketal F

Figure 11. Results of ^{1}H NMR NOEDS experiments with norasperenals A (52) and B (53).*



norasperenal A



norasperenal B

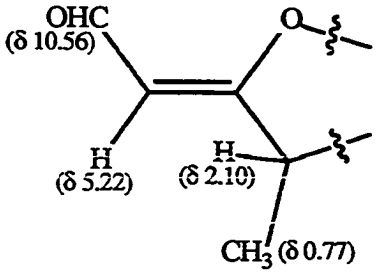
* NOE enhancement in percent

(**49**, Table12). Careful examination of NMR data revealed that **53** had the same divinylcyclohexane ring as in **49**. This interpretation was confirmed by a ^{1}H NMR COSY experiment, in which the same coupling patterns were found. Thus, compound **53** was identified as a trisnorditerpene aldehyde possessing diterpene equivalent of the "elemane" type carbon skeleton.

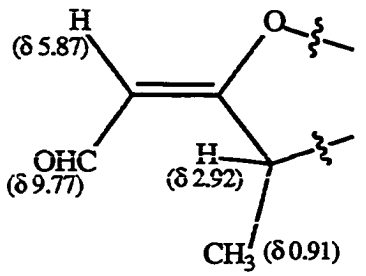
Norasperenal B has an asymmetric double bond (Δ^{12}) and five asymmetric carbon centers (C-1, -2, -7, -10, and -11). ^{1}H NMR NOEDS measurements were again used to determine the relative stereochemistry at these centers (Figure 11). Irradiation of the C-17 methyl protons enhanced the C-1 proton by 9.4%, while the C-2 proton showed no enhancement. Thus, the ring juncture of **53** was assigned as the expected *trans*-diaxial arrangement at C-2 and C-7. Another datum supporting this assignment was large vicinal coupling constant (J = 11.0 Hz) between the C-1 and -2 protons. From these observations, the cyclohexane ring of **53** was concluded to possess the chair conformation. Irradiation of the C-1 proton enhanced the C-10 proton by 8.2%, indicating the *cis* orientation at C-1 and C-10. The C-10 proton was also enhanced by irradiation of the C-18 methyl protons (11.3%). At the same time, the C-11 and -13 protons were enhanced by 8.0 and 16.4%, respectively. The aldehyde proton was not enhanced by the irradiation of the C-18 protons. Therefore, the Δ^{12} double bond of **53** has the same *Z* configuration as **52**. Thus, the relative stereochemistries of the asymmetric centers of norasperenal B were unambiguously determined by ^{1}H NMR NOEDS experiments as 12(*Z*), 1**S***, 2**S***, 7**S***, 10**R***, and 11**R***.

A highly unstable metabolite, norasperenal C (**54**) was isolated as an oil. ^{1}H, ^{13}C NMR and COSY NMR data for **54** were highly compatible with **52**, suggesting **54** as an isomer of compound **52**. The only significant differences were downfield shifts of the C-11, -13, and -18 protons, and upfield shift of the C-14 proton in the ^{1}H NMR spectrum of **54** (Table 13). This could be readily explained by a change of the geometry at the Δ^{12}

double bond. That is, the resonance of the olefinic proton is less upfield shifted when the β-oxygen is *cis* to the olefinic proton. In contrast, the aldehyde proton is less deshielded by the ether oxygen. The downfield shifts of the C-11 and -18 protons are the results of deshielding by the aldehyde carbonyl group.



52

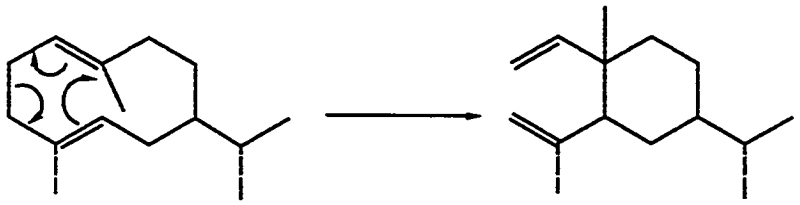


54

Thus, the Δ^{12} double bond in norasperenal C was assigned the ***E*** configuration. Additional evidence supporting the structural determination of **54** as a isomer of **52** was the rapid conversion of **54** to **52** at room temperature. Even in very mild conditions (acetone or benzene, -20°), **54** was slowly converted to **52**. Due to the highly unstable nature of this compound, norasperenal C could not be fully characterized. However, the interconversion confirmed the structure of **54** as an isomer of **52** and suggested the same stereochemistry for the asymmetric centers (C-1, -10, and -11) between them.

The last metabolite, norasperenal D (**55**) was isolated as an oil. Due to the highly unstable nature, compound **55** was only characterized by its ^{1}H NMR features (Table 13). The ^{1}H NMR data of **55** were very similar to **53**, indicating the presence of the same elemane type skeleton. Shifts of the proton resonances at C-11, -13, -14, and -18 suggested the ***E*** configuration for the Δ^{12} double bond. Rapid conversion of **55** to **53** at room temperature supported the assignment for **55** as a isomer of **53**. As in the case of norasperenal C (**54**), the stereochemistry of asymmetric centers in norasperenal D was not pursued by independent measurements.

Asperketals and norasperenals possess carbon skeletons related to the sesquiterpene germacrane and elemane. Interconversion between these sesquiterpene skeletons is well known.[4] That is the formation of elemane by a Cope rearrangement of a corresponding germacrane precursor.



germacrane elemane

Isolation of diterpenoids (and trisnorditerpenoids) of these skeletons from the same *Eunicea* sample suggests that similar type of rearrangement might also occur between diterpenoids. However, it is unknown whether this interconversion occurs via *in vivo* process or *in vitro*.

Experimental Section

A. *Eunicea asperula*

Collection and Extraction. *E. asperula* (specimen number CI 86-194) was collected by hand using SCUBA at 20 to 25 m depth in July 1986, along the offshore islands of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonian was next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 36 g of crude organic materials (from 1 kg dry weight of the gorgonian). Asperketals A-F (**44-49**) and obscuronatin (**50**) were eluted from a vacuum flash silica gel column with 10-20% EtOAc in isooctane. Compound **51** was eluted with 30% EtOAc in isooctane. All of the compounds were further purified by HPLC using semi preparative silica columns.

Asperketal A (44). The hemiketal **44** was isolated as a white solid after final purification by HPLC (Partisil 10 silica with 10% EtOAc in isooctane). Recrystallization gave 190 mg (0.5% of the crude extract) of **44**, mp 75-77°. Asperketal A showed $[\alpha]_D$+75° (c 0.97, C_6H_6) and the following spectral features: IR (film) 3450, 2920, 1660, 1465, 1380, 1160, 1100, 1050, 970, 960 cm^{-1}; HRMS: M^+, m/z obsd 304.2396. $C_{20}H_{32}O_2$ required 304.2402; low resolution MS: m/z (relative intensity) 304 (6), 286 (47), 235 (39), 217 (9), 161 (100), 125 (40), 81 (44), 69 (18).

Asperketal B (45). The ketal **45** was obtained as a white solid after final purification by HPLC (5% EtOAc in isooctane). The extract yielded 47 mg (0.14% of the crude extract) of **45**, mp 62-63°. Asperketal B showed $[\alpha]_D$+88° (c 0.65, C_6H_6) and the following features: IR (film) 2940, 1660, 1450, 1360, 1100, 1040, 990, 965 cm^{-1}; HRMS: M^+, m/z obsd 302.2237. $C_{20}H_{32}O_2$ required 302.2245; low resolution MS: m/z (relative intensity) 302 (55), 273 (100), 163 (53), 161 (18), 151 (44), 109 (64), 81 (23).

Asperketal C (46). The ketal **46** was isolated as a white solid after final

purification by HPLC (5% EtOAc in isooctane). The extract yielded 23 mg (0.06% of the crude extract) of **46**, mp 72-73°, Asperketal C showed $[\alpha]_D$+190° (c 1.88, C_6H_6) and the following spectral features: IR (film) 2920, 1660, 1460, 1370, 1080, 1050, 940, 920, 890, 840 cm^{-1}; HRMS: M^+, m/z obsd 318.2562. $C_{21}H_{34}O_2$ required 318.2558; low resolution MS: m/z (relative intensity) 318 (1), 287 (6), 249 (50), 217 (14), 161 (100), 81 (89), 69 (28).

Asperketal D (47). The ketal **47** was obtained as a white solid after final purification by HPLC (5% EtOAc in isooctane). The extract yielded 10 mg (0.03% of the extract) of **47**, mp 75-76°. Asperketal D exhibited $[\alpha]_D$+126° (c 0.60, C_6H_6) and the following spectral features: IR (film) 2940, 1660, 1450, 1360, 1140, 1010, 970, 880 cm^{-1}; HRMS: M^+, m/z obsd 304.2388. $C_{20}H_{32}O_2$ required 304.2404; low resolution MS: m/z (relative intensity) 304 (44), 275 (45), 165 (46), 161 (69), 153 (59), 126 (76), 81 (100), 69 (87).

Asperketal E (48). The ketal **48** was obtained as an oil after final purification by HPLC (5% EtOAc in isooctane). The extract yielded 9 mg (0.03% of the extract) of **48**, which showed $[\alpha]_D$+54° (c 0.57, C_6H_6) and the following features: IR (film) 2940, 1665, 1450, 1360, 1030, 960, 880 cm^{-1}; HRMS: M^+, m/z obsd 304.2406, $C_{20}H_{32}O_2$ required 304.2402; low resolution MS: m/z (relative intensity) 304 (100), 275 (63), 165 (27), 161 (67), 152 (23), 126 (43), 81 (47), 69 (37).

Asperketal F (49). The ketal **49** was isolated as a white solid after final purification by HPLC (5% of EtOAc in isooctane). The extract yield 14 mg (0.04% of the extract) of **49**, mp 54-55°. Asperketal F showed $[\alpha]_D$+71° (c 0.77, C_6H_6) and the following spectral features: IR (film) 2940, 1640, 1455, 1360, 1010, 980, 905, 885 cm^{-1}; HRMS: M^+, m/z obsd 304.2394, $C_{20}H_{32}O_2$ required 304.2402; low resolution MS: m/z (relative intensity) 304 (34), 275 (100), 165 (24), 161 (63), 153 (1), 134 (40), 126 (51), 81 (84), 69 (99).

Obscuronatin (50). Obscuronatin (**50**), was obtained as an oil after purification by HPLC (5% EtOAc in isooctane). The extract yielded 9 mg (0.03% of the extract) of **50**. The proton NMR spectrum of **7** (in C_6D_6) showed peaks at 5.31 (1 H, dd, 15.7, 10.1 Hz), 5.25 (1 H, brt, 7.0), 5.04 (1 H, brd, 15.7), 4.92 (1 H, brd, 11.0), 2.62 (1 H, m), 2.27 - 1.85 (7 H, m), 1.69 (3 H, brs), 1.55 - 1.10 (6 H,m), 1.54 (3 H, brs), 1.06 (3 H, s), 0.92 (3 H, d, 6.7) ppm, in full accord with published data. ^{13}C NMR resonances were also within $\pm$ 0.2 ppm of the reported data for this compound.[79]

Compound 51. The acetate **51** was isolated as a white solid after final purification by HPLC (30% EtOAc in isooctane). Recrystallization from CH_2Cl_2 gave 35 mg (0.1% of the extract) of **51**, mp 133.5-134.5°. Compound **51** showed $[\alpha]_D$ -9.1° (c 0.9, $CHCl_3$) and the following spectral features: IR (film) 3020, 1730, 1665, 1245, 1020 cm^{-1}; HRMS: M^+-OAc m/z obsd 303.2315, $C_{22}H_{34}O_4$ required 303.2324; low resolution MS: m/z (relative intensity) 362 (1, M^+), 303 (10), 285 (8), 269 (8), 201 (16), 154 (56), 133 (91), 107 (98), 93 (100), 81 (78), 69 (30), 43 (67).

B. *Eunicea* sp (F 87 - 32)

Collection and Extraction. A collection of *Eunicea*, specimen number F 87 - 32, was made at the Florida Keys in July 1987. After the same process as used for *E. asperula*, extraction of dried gorgonians with CH_2Cl_2 (3 Kg, dry weight) gave 17 g of crude extract. Asperketals A (**44**) and B (**45**) were eluted from silica vacuum flash chromatography using 20% EtOAc in isooctane as an elutant. Purifications were made by silica HPLC (10% EtOAc in isooctane). Norasperenals A - D (**52** - **55**) were eluted by 30% EtOAc in isooctane. Purifications were made by silica HPLC (30% EtOAc in isooctane), followed by C-18 reverse phase (RP) HPLC (100% methanol).

Norasperenal A (52). The aldehyde **52** was isolated as a white solid after final purification by C-18 RP-HPLC (100% methanol). The extract gave 165 mg (1.0%

of the extract) of **52**, mp 101.5-102.5°. Compound **52** showed $[\alpha]_D$+131° (c 1.2, MeOH) and the following spectral features: IR (NaCl) 2920, 1655, 1625, 1400, 1170, 1125, 1030, 995, 890, 800 cm^{-1}; HRMS: M^+, m/z 260.1769, $C_{17}H_{24}O_2$ required 260.1776; low-resolution MS: m/z (relative intensity) 260 (19), 189 (22), 161 (44), 145 (23), 133 (14), 119 (24), 105 (36), 91 (35), 79 (39), 58 (100); UV (MeOH) 271 nm (ε18500).

Norasperenal B (53). The aldehyde **53** was obtained as a white solid from final purification by C-18 RP-HPLC (100% methanol). The extract yielded 28 mg (0.2% of the extract) of 54, mp 123-124.5°. Norasperenal B showed $[\alpha]_D$-29° (c 0.5, MeOH) and the following spectral features: IR (NaCl) 2930, 2860, 1655, 1620, 1460, 1405, 1390, 1240, 1185, 960, 810 cm^{-1}; HRMS: M^+, m/z obsd 260.1776, $C_{17}H_{24}O_2$ required 260.1776; low-resolution MS: m/z (relative intensity) 260 (8), 189 (14), 161 (60), 145 (29), 133 (23), 121 (30), 109 (41), 81 (69), 69 (35), 43 (100); UV (MeOH) 271 nm (ε17000).

Norasperenal C (54). The aldehyde **54** was isolated as an oil from final purification by C-18 RP-HPLC (100% methanol). The extract yielded 60 mg (0.4% of the extract) of **54**. Due to the unstable nature of **54**, spectral data other than ^1H and ^{13}C NMR data for **54** were not obtained.

Norasperenal D (55). The aldehyde **55** was obtained as an oil from final purification by C-18 RP-HPLC (100% methanol). The extract gave 3 mg (0.02% of the extract). Due to its instability, only ^1H NMR data were obtained for **55**.

Isolations of asperketals A (44) and B (45). Asperketals A and B were purified by HPLC (10% EtOAc in isooctane). The extract gave ca 400 mg of **44** and 25 mg of **45** (2.3% and 0.15% of the extract, respectively). ^1H NMR spectra for **44** and **45** were identical with those from authentic samples.

Chapter V

Cembranoids from *Eunicea* sp (CI 86 - 222, *Euniceopsis*)

Gorgonian samples (specimen number CI 86 - 222) were collected at the Tobago Cays, eastern Caribbean Sea in July 1988. They were defined by F. M. Bayer as organisms which belong to the genus *Eunicea* (subgenus *Euniceopsis*) but possessed morphological features different from all of the known species. Extraction of the dried gorgonians with dichloromethane gave 15 g of crude organic materials (from 1 kg, dry weight). Silica vacuum flash chromatography, followed by silica and reverse phase HPLC of the nonpolar and moderately polar fractions, yielded seven compounds (**56** - **62**, Figure 12). The structures of these compounds were elucidated by spectral methods, chemical reactions and X-ray crystallographic techniques. All of these were diterpenoids of the well known cembrane class.

Compound **56** analyzed for $C_{20}H_{32}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Four low field ^{13}C NMR (Table 14) bands at δ 147.8 (s), 135.2 (s), 122.1 (d), and 118.0 (d) revealed **56** to possess two double bonds, thus, three rings. Absorption maxima at 249 and 242 nm in the UV spectrum indicated that the double bonds were conjugated to each other. A ^1H NMR COSY experiment showed that one of the olefinic protons at δ 6.15 (brd, J =10.9) was coupled to proton signals at δ 1.56 (3 H, brs) and 2.2 - 2.0 (1 H, multiplicity unknown) as well the other olefinic proton at δ 6.07 (brd, 11.0, Table 15). Therefore, the diene consisted of two tri-sbstituted double bonds.

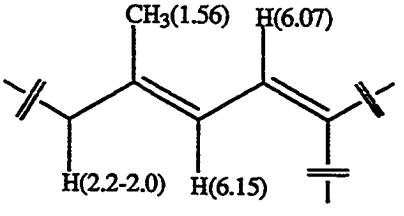
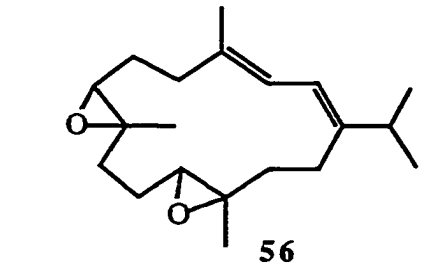
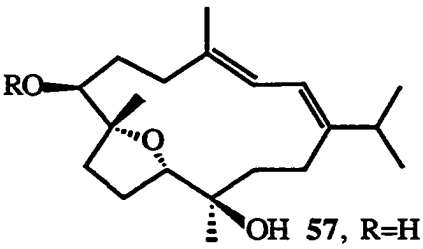


Figure 12. Structures of compounds **56 - 63**.

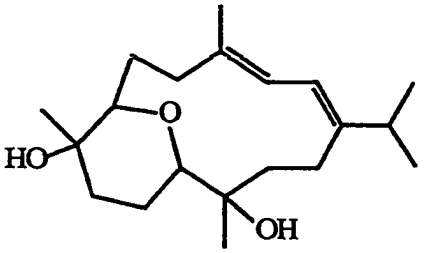


56



57, R=H

58, R=Ac



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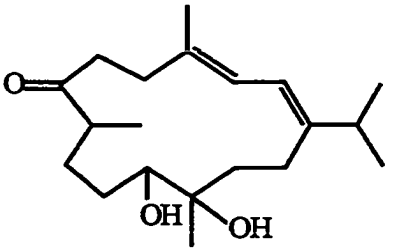
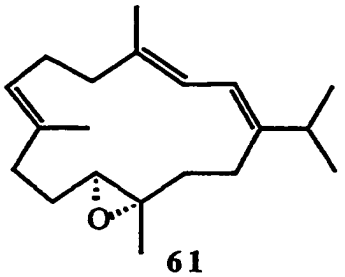


60



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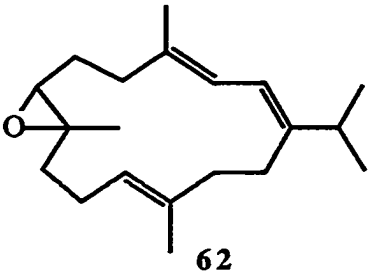

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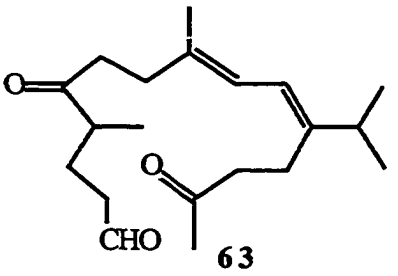

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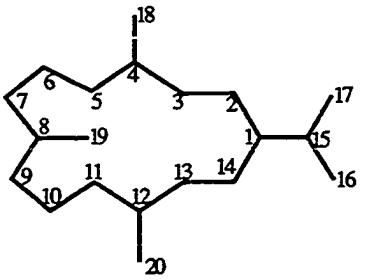


Table 14. ^{13}C NMR assignments for compounds **56 - 60** and **63**.

#	56	57	58	59	60	63
1	147.8 s	147.4 s	147.2 s	150.9 s	146.2 s	145.8 s
2	118.0 d	117.7 d	118.7 d	119.0 d	117.5 d	118.8 d
3	122.1 d	120.2 d	121.4 d	122.4 d	118.3 d	121.0 d
4	135.2 s	133.1 s	132.6 s	134.6 s	135.8 s	135.7 s
5	37.0 t	32.2 t	32.4 t	40.5 t	37.1 t^a	34.0 t
6	26.5 t	27.7 t	26.0 t	22.8 t	39.6 t^a	43.7 t^a
7	63.8 d	73.9 d	74.8 d	87.9 d	212.9 s	213.1 s
8	60.7 s	84.9 s	83.6 s	71.0 s	45.6 d	45.2 d
9	35.2 t	36.7 t	37.0 t	33.0 t	27.8 t^b	24.6 t^b
10	23.7 t	27.0 t	26.9 t	21.1 t	29.1 t^b	39.7 t^a
11	59.9 d	88.1 d	89.1 d	70.9 d	75.2 d	201.6 d
12	61.2 s	72.4 s	72.1 s	75.6 s	74.6 s	208.5 s
13	34.9 t	35.5 t	35.7 t	41.4 t	30.9 t^b	41.4 t^a
14	23.7 t	24.7 t	25.1 t	24.1 t	24.9 t	23.5 t^b
15	33.0 d	36.8 d	37.2 d	37.8 d	34.4 d	35.3 d
16	22.6 q	21.7 q	21.9 q	22.2 q	22.8 q	22.0 q
17	22.1 q	21.6 q	21.7 q	22.1 q	22.0 q	22.0 q
18	16.1 q	18.9 q	19.2 q	18.1 q	18.5 q	16.5 q
19	16.8 q	20.2 q	21.5 q	27.3 q	17.2 q	16.5 q
20	18.9 q	25.8 q	26.1 q	23.8 q	25.0 q	29.9 q
OAc			170.2 s			
			20.6 q			

Carbon NMR data for **56, 57, 60** and **63** were obtained in $CDCl_3$ solution. Data for **58** and **59** were obtained in C_6D_6 and CD_3OD solutions, respectively. Multiplicities were measured by DEPT experiments. Assignments for **56, 58** and **59** were made by XHCORR and COLOC experiments. Assignments for others were made by comparison with compounds **56, 58** and **59**. [a,b] Signals within a column may be reversed.

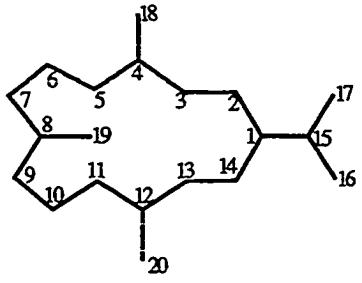


Table 15. ^{1}H NMR assignments for compounds **56 - 60.**

#	56[a]	57[b]	58[a]	59[c]	60[b]
2	6.07 brd(11.0)	6.02 brd(11.3)	6.27 brs	5.82 brd(8.9)	6.02 brd(11.0)
3	6.15 brd(10.9)	6.13 brd(11.2)	6.27 brs	6.05 brd(8.6)	5.55 brd(10.8)
5	2.2 - 2.0 (2H)[e]	2.1 - 1.9 (2H)[e]	2.28[e]	2.49 brdd(17.0,7.9)	2.66 - 2.56[e]
			1.86[e]	2.05 brdd(17.0,10.9)	2.20[e]
6	d	2.4 - 2.3[e]	2.30[e]	2.21 ddd(15.4,10.9, 9.3)	2.93 ddd(19.0, 11.2, 1.5)
	1.5 - 1.4[e]	1.4 - 1.3[e]	1.49[e]	1.50 - 1.46[e]	2.66 - 2.56[e]
7	2.86 dd(8.8,3.4)	3.49 d(9.8)	5.15 d(10.2)	3.45 brd(9.3)	-----
8	----	----	----	----	2.81[e]
9	d	2.28[e]	1.81[e]	1.95 - 1.82[e]	2.06[e]
		1.74[e]	1.30[e]	1.64[e]	1.75 - 1.67[e]
10	d	1.69[e]	1.44[e]	1.95 - 1.82[e]	1.75 - 1.67[e]
	1.4 - 1.3[e]	1.56[e]	1.26[e]	1.56[e]	1.44[e]
11	2.96 dd(8.0,4.2)	3.94 dd(11.3,4.3)	3.81 dd(11.3,4.5)	3.90 dd(11.6,2.8)	3.48[e]
13	2.0 - 1.9[e]	2.4 - 2.3[e]	2.23[e]	1.82 - 1.70[e]	1.96[e]
	1.60[e]	2.1[e]	1.34[e]	1.52[e]	1.64[e]
14	2.42[e]	2.4 - 2.3[e]	2.60 ddd(13.2,9.2, 2.8)	2.35 brdd(12.6,12.6)	2.11[e]
	2.0 - 1.9[e]	1.4 - 1.3[e]	2.13[e]	1.95 - 1.82[e]	1.48[e]
15	2.19 h(6.8)	2.4 - 2.3[e]	2.36 h(6.8)	2.30 h(6.8)	2.29 m
16	1.01 d(6.8)	1.04 d(6.7)	1.09 d(6.8)	1.03 d(6.8)	1.04 d(7.0)
17	1.00 d(6.8)	1.03 d(6.7)	1.09 d(6.8)	1.03 d(6.8)	1.02 d(7.8)
18	1.56 brs	1.77 brs	1.82 brs	1.68 brs	1.81 brs
19	1.07 s	1.22 s	1.23 s	1.13 s	1.14 d(7.1)
20	1.09 s	1.15 s	1.07 s	1.15 s	1.27 s
OAc			1.66 s		

[a,b,c] measured in C_6D_6, $CDCl_3$, and CD_3OD solutions, respectively. Assignments were made by one-dimensional decoupling and COSY experiments. [d] Nonassignable proton resonances. [e] Coupling constants were not determined.

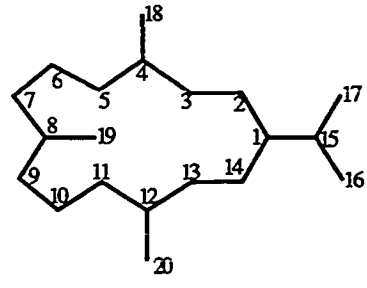
Other four carbon bands at δ 63.8 (d), 61.2 (s), 60.7 (s), and 59.9 (d) in the ^{13}C NMR spectrum and corresponding proton resonances at δ 2.96 (dd, J = 8.0, 4.2) and 2.86 (dd, 8.8, 3.4) in the ^{1}H NMR spectrum revealed the presence of two epoxides. A ^{1}H NMR COSY experiment showed that the epoxide protons were coupled to protons at higher field. The proton signal at δ 2.86 was coupled to a proton at δ 1.5 - 1.4 which further coupled to two proton resonances at δ 2.2 - 2.0. Due to the overlapping of proton signals however, the coupling pattern of the other epoxide proton at δ 2.96 was not clear. The remaining carbons in **56** were all protonated. Therefore, the two singlet methyls at δ 1.09 and 1.07 in the ^{1}H NMR spectrum must be attached to the epoxides. However, at this stage, their precise connections to the epoxides were unknown. Another spin system revealed by COSY NMR data consisted of two methyl protons at δ 1.01 (d, 6.8) and 1.00 (d, 6.8) which were coupled to an isolated methine proton signal at δ 2.19 (hep, 6.8), hence indicating an isopropyl group. Finally, a spin system consist of four protons at δ 2.42 (1 H), 2.0 - 1.9 (2 H) and 1.60 (1 H) was found by COSY NMR data. Since there was only one high field methine resonance in the ^{13}C NMR spectrum, this was confidently assigned as an ethylene group.

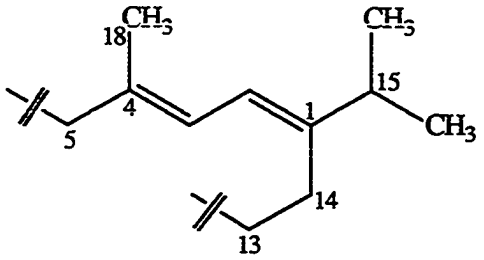
The confirmation and connection of the proton spin systems were made by direct (XHCORR) and long range (COLOC) carbon-proton correlation experiments (Table 16). A vinyl methyl (δ 16.1, C-18) and a methylene (δ 37.0, C-5) carbon were coupled to the olefinic proton at δ 6.15 (C-3), confirming their connectivities to the diene. Several long range carbon-proton couplings between the diene and isopropyl group illustrated the connection of the isopropyl to C-1 of the diene. The coupling between the carbon at δ 33.0 (C-15) and the olefinic proton at δ 6.07 (C-2) confirmed this arrangement. In addition, the coupling between this proton and the methylene carbon at δ 23.7 (C-14) showed the connectivity between the diene and ethylene groups (C-13 and C-14).

Table 16. Results of COLOC experiments with **56**, **58** and **59**.

carbon	**56**	**58**	**59**
1	14 (2.42), 16, 17	14 (2.60), 16, 17	3, 14 (2.35), 16, 17
2	—	14 (2.60), 14 (2.13)	14 (2.35)
3	18	18	5 (2.05), 18
4	18	18	18
5	3, 18	3, 7, 18	3, 7, 18
6	—	—	5 (2.05), 7
7	19	19	5 (2.49), 6 (2.21), 19
8	19	7, 19	7, 19
9	19	—	19
10	—	—	—
11	20	20	7, 20
12	20	11, 14 (2.13), 20	11, 14 (2.35), 20
13	14(2.42), 20	14 (2.60), 14 (2.13), 20	20
14	2	2	—
15	2, 14(2.42), 17	2, 14 (2.60), 14 (2.13), 16, 17	2, 14 (2.35), 16, 17
16	17	17	17
17	16	16	16
18	3	3	3
19	—	7	—
20	—	—	11
carbonyl		7, CH_3	

COLOC experiments with **56** and **58** were performed in C_6D_6 solution. The experiment with **59** was performed in CD_3OD solution. Parameters were optimized for couplings of 8 Hz for **56**, and 6 Hz for **58** and **59**. The numbers in parentheses are ^1H NMR chemical shifts of the protons which correlate.



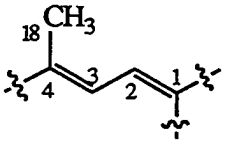


The COLOC experiment also revealed the presence of two very similar carbon systems. Each of the methyl proton resonances at δ 1.09 (C-20) and 1.07 (C-19) were coupled to three carbons (Table 16). As a result, two partial structures were established consisting of four carbons each; two epoxides (one d, another s), one methyl and one methylene. Therefore, compound **56** was concluded to possess two tri-substituted epoxides. Since the correlation between protons at the epoxide (δ 2.86) and C-5 was illustrated by ^{1}H NMR COSY and XHCORR experiments, the position of the epoxide was confidently assigned at C-7 and C-8. The position of the other epoxide was also assigned by the long range coupling between the epoxy methyl protons at δ 1.09 (C-20) and the methylene carbon at δ 34.9 (C-13). Although the connectivity between the epoxides was not determined by spectral methods, consideration of the molecular formula revealed that they were connected to each other via two methylene carbons (C-9 and -10). Thus, the structure of compound **56** was identified as 7,8;11,12-diepoxycembra-1,3-diene. The structure of **56** was confirmed by its acid-catalyzed hydrolysis which is discussed later.

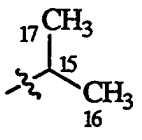
A highly unstable compound, **57**, was isolated as a white solid; m.p 131-132°, which analyzed for $C_{20}H_{34}O_3$ by high resolution mass and ^{13}C NMR spectrometry. Comparison of ^{1}H, ^{13}C NMR and UV spectra showed that **57** had the same diene system as compound **56**. Instead of carbon signals corresponding the epoxides however, the ^{13}C NMR (Table 14) spectrum of **57** showed four lower field carbon bands at δ 88.1 (d), 84.9 (s), 73.9 (d), and 72.4 (s). In addition, the IR spectrum showed a strong hydroxyl

absorption at 3400 cm^{-1}. Comparison of the molecular formula and carbon multiplicities revealed that the four low field carbons were two hydroxyl-bearing and two ether-bearing carbons. Although chemical shifts of many signals in the ^1H NMR spectra were different, ^1H COSY NMR data revealed **57** to possess very similar coupling patterns with compound **56**. For example, two methyl protons at δ 1.04 (d, 6.7) and 1.03 (d, 6.7) were coupled with a proton at δ 2.4 - 2.3, indicating an isopropyl group (Table 15). The low field proton at δ 3.94 (dd, 11.3, 4.3) was coupled to two protons at δ 1.69 and 1.56 which were further coupled to another two protons at δ 2.28 and 1.74. Therefore, it seemed likely that two epoxides in **56** were converted to an ether and two hydroxyl groups in compound **57**. Due to the unstable nature of **57** at room temperature however, the structure was determined by the acetylation of **57** to another natural product, the acetate **58**.

Compound **58** crystallized from a mixture of acetone and hexane after HPLC purification; m.p 96-98°. The compound analyzed for $C_{22}H_{34}O_3$ by high resolution mass and ^{13}C NMR spectrometry. The IR spectrum indicated the presence of hydroxyl (3450 cm^{-1}) and ester (1740 cm^{-1}) functionalities. The ^{13}C NMR data for **58** were very similar to those derived from compound **57**. Two additional carbon signals at δ 170.2 (s) and 20.6 (q) illustrated **58** to possess an acetoxyl group. Accordingly, the only significant difference in the ^1H NMR spectra was the downfield shift of a proton signal from δ 3.49 (d, 9.8) to 5.15 (d, 10.2) in **58**, which was interpreted as the α-acetoxyl proton (Table 15). ^1H NMR COSY and XHCORR experiments allowed the partial structures to be determined. As a result, seven partial structures (a - g) were defined.



a



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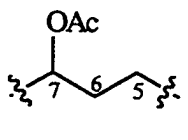

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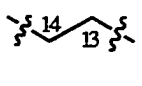

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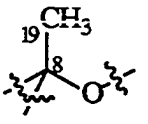

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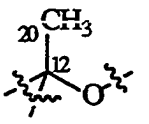

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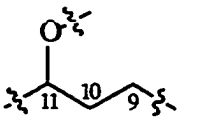


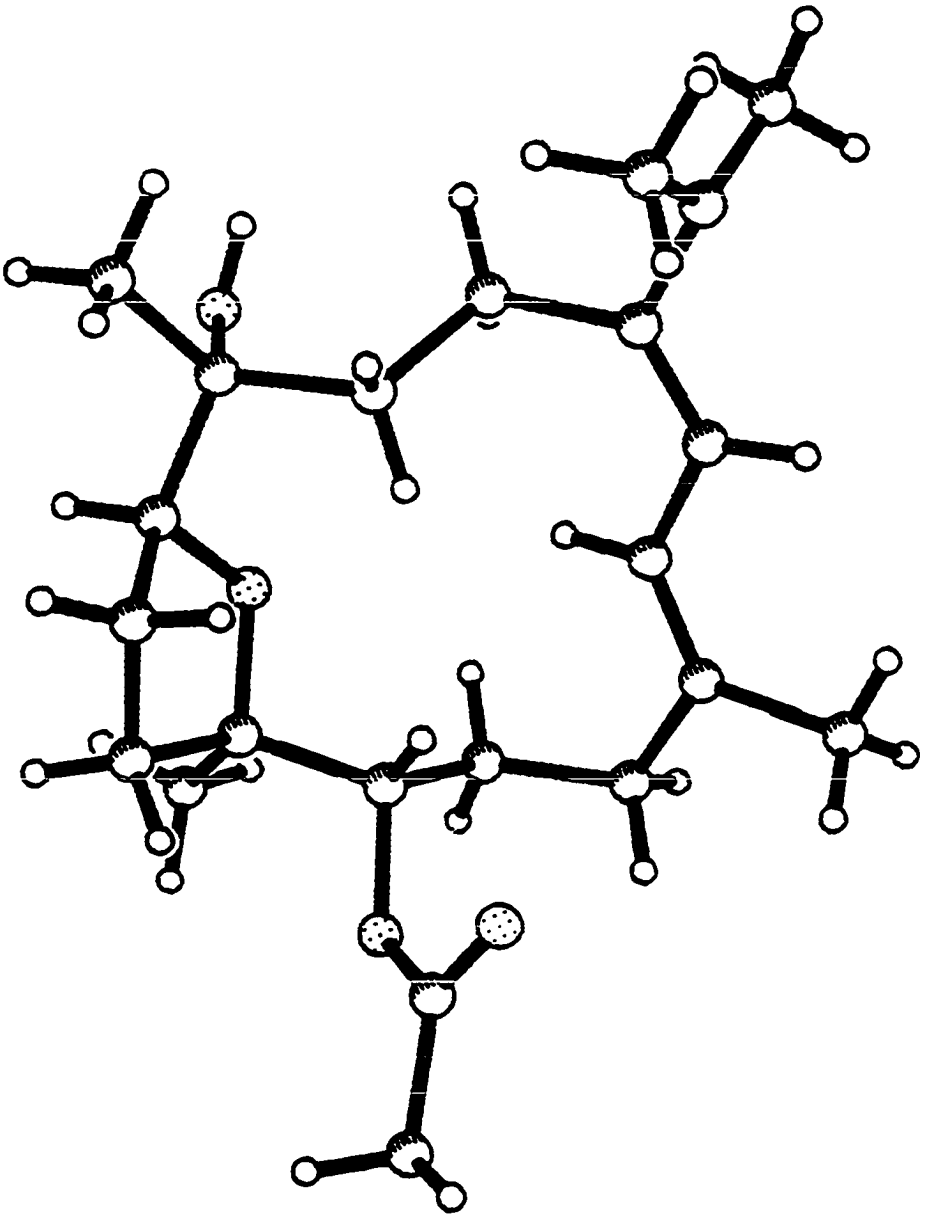
g

The connectivities of the partial structures were made possible by a COLOC experiment (Table 16). Long range couplings of the C-5 carbon with the C-3, -7 and -18 protons (δ 6.27, 5.15 and 1.82, respectively) revealed the connection between partial structures a and c . Several carbon-proton couplings showed the isopropyl group (b) to be connected to C-1 of a. In the same way, couplings between the carbons at C-1, -2, -13, and -15 with the C-14 protons (δ 2.60 and 2.13) determined the connection of d to a. The remaining part was also determined by long range couplings of several carbons with two methyls at C-19 (e), and C-20 (f) . Thus, compound **58** was unambiguously defined as a diterpene acetate of the cembrane class.

The remaining problem was the assignment of the location of the ether and hydroxyl groups. That is, either the C-11 or C-12 carbon was connected to the C-8 carbon by an oxygen bridge. Since a diacetate was not produced by acetylation of **57** (acetic anhydride in pyridine), it seemed likely that compound **58** possessed a 5-membered cyclic ether (C-11) and a tertiary hydroxyl (C-12) group. Other data supporting this conclusion were the ^{13}C NMR shifts of the C-11 (δ 89.1) and C-12 (δ 72.1) carbons. In general, the signal of an ether-bearing carbon is shifted further downfield than a hydroxyl-bearing carbon.

The structure of **58** was confirmed by X-ray crystallographic methods. The X-ray drawing of compound **58** is shown in Figure 13. Thus, the structure of **58** as well that of **57** were fully defined. The stereochemistry of the asymmetric centers were also determined by the X-ray techniques and the overall configurations are 1(*E*), 3(*E*), 7S*, 8R*, 11S*, and 12R*. The absolute stereochemistry of **58** was not determined in the X-

Figure 13. A computer-generated perspective drawing of compound **58**.



ray experiment.

Compound **59** was isolated as an oil which analyzed for $C_{20}H_{34}O_3$ by high resolution mass and ^{13}C NMR spectrometry. Comparison of spectral data showed similarities between compounds **59** and **57**. However, there were significant differences in the resonances between δ 90 and 70 in the ^{13}C NMR spectra, in which one of the carbon resonances in **59** was shifted to upfield by ca. 15 ppm (Table 14). XHCORR experiments revealed another significant difference. In compound **57**, carbons at δ 88.1 and 73.9 were directly coupled to the protons at δ 3.94 and 3.49, respectedly. In contrast, carbons at δ 87.9 and 70.9, in compound **59**, were coupled to protons at δ 3.45 and 3.90, respectively (Table 14 and 15).

Careful examination of ^1H NMR COSY, XHCORR, and COLOC (Table 16) data revealed that **59** and **57** possessed not only the same carbon skeleton but also the same substitution patterns. Therefore, the structural differences between these compounds should be the exchange of oxygen functionalities. To determine the differences, several attempts failed to acetylate compound **59**. This suggested that **59** possesses two tertiary hydroxyl groups. Thus, there must be an ether linkage between C-7 and C-11, and hydroxyl groups at C-8 and C-12. The high field shift of the C-11 carbon resonance might result from the difference of the ether ring size (5-membered in **57**, 6-membered in **59**). It is not uncommon among compounds possessing several oxygens that a change in ether ring size results in significant differences in the carbon chemical shifts.[86] Other data supporting this assignment were the reactions of compounds **57** - **59** with trichloroacetyl isocyanate (Figure 14), and the comparison of chemical shifts of relevant protons (Table 17). Proton resonances for the C-7 and C-11 protons in **59** were shifted downfield much less in **57** and **58**. Therefore, the C-7 and C-11 protons of **59** must be attached to ether not to hydroxy-bearing carbons. The possibility that **59** was a diastereomer of **57** was dismissed because there were no significant differences in either the chemical shifts or the

Figure 14. Reactions of compounds **57** - **59** with $Cl_3CCONCO$.

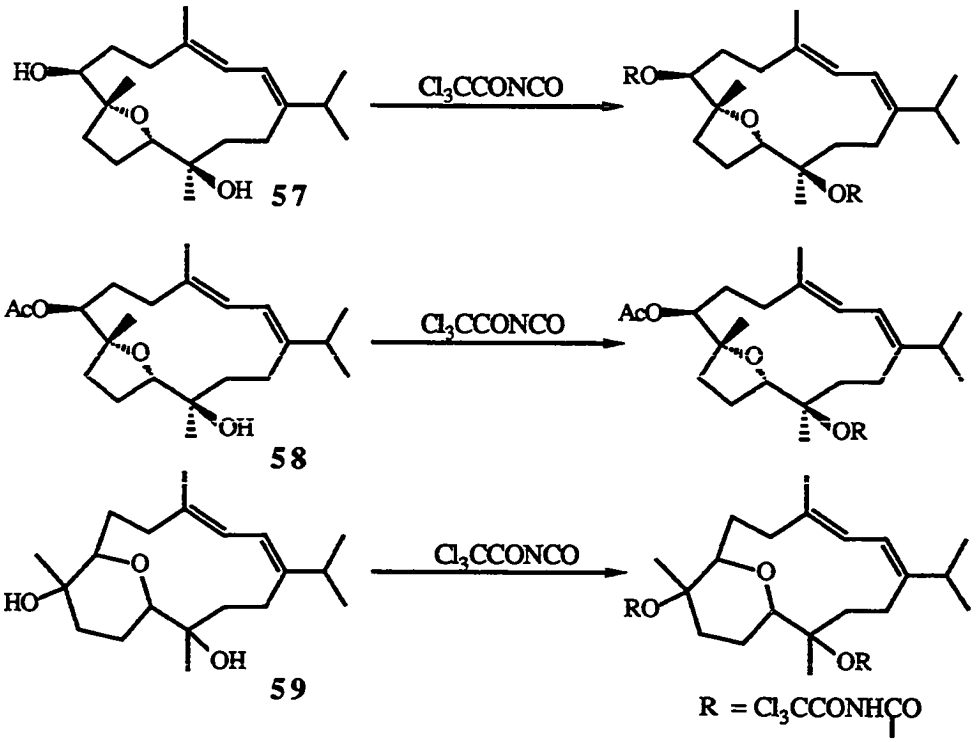


Table 17. Differences of proton chemical shifts for compounds **57** - **59** upon reaction with trichloroacetyl isocyanate.[a]

	H-7	H-11	H-19	H-20
57	3.30	3.78	1.14	1.09
57+2R[b,c]	5.28	4.52	1.29	1.43
58	5.15	3.81	1.25	1.04
58+R[b,c]	5.25	4.55	1.21	1.42
59	3.42	3.76	1.05	1.04
59+2R[b,c]	4.48	4.14	1.29	1.65
Δ**57**[d]	-1.98	-0.74	-0.15	-0.34
Δ**58**[d]	-0.10	-0.74	+0.04	-0.35
Δ**59**[d]	-1.06	-0.38	-0.24	-0.61

[a] Chemical shifts were measured in C_6D_6 solutions. [b] The number of reactive hydroxyl groups was determined by the number of amide protons produced. [c] R is -$Cl_3CCONHCO$. [d] Differences on proton chemical shifts

coupling constants of the C-7 and C-11 protons between these compounds.

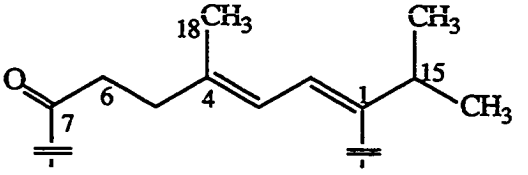
The structure of **59**, as well as **56**, were confirmed by a chemical reaction. Acid hydrolysis of **56** in aqueous acetone gave **57** and **59** as the major products. This result clearly indicated that the positions of the epoxides in **56** were C-7, -8 and C-11, -12. Also, the configuration of the diene must be the same as in **57**; 1(***E***), 3(***E***). Thus, the structure of **56** was confirmed. In addition, the assignment of the oxygen functionalities in **59** was confirmed by the hydrolysis reaction. Theoretically, acidic hydrolysis of **56** could afford four different ethers; linkage between C-8 and -11 (compound **57**), C-7 and -11, C-7 and -12, C-8 and -12. Since the possibility of the latter two linkages were eliminated by the acetylation and esterification (isocyanate), **59** possessed an ether linkage between C-7 and -11. Thus, the structure of **59** was unambigously defined.

Compounds **56** and **59** have asymmetric carbon centers in the same positions as **57**. ^{1}H NMR NOEDS experiments could not determine their overall relative stereochemistries. Among the protons at C-7, 11, 19 and 20, irradiations of any protons failed to enhance other protons, and only implied that they were distant to each other. The results of the acidic hydrolysis of **56** was also not very informative. Since there were several possible mechanisms for the acid hydrolysis of **56**, the stereochemistry of the asymmetric centers were not determined. The formation of both **57** and **59** by the same reaction only implied that, in compound **59**, the relative configurations in the vicinal centers (C-7 and -8, also C-11 and -12) were opposite to each other, and confirmed the negative results of the NOEDS experiment.

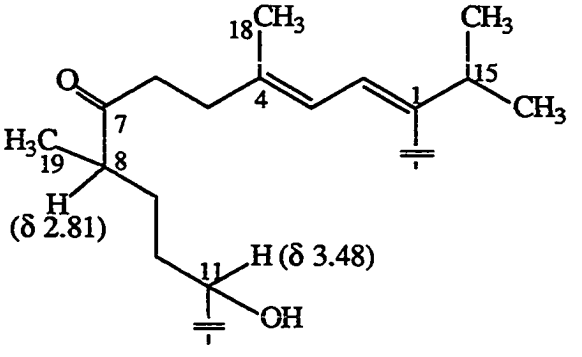
Compound **60** analyzed for $C_{20}H_{34}O_3$ by high resolution mass and ^{13}C NMR spectrometry. The infrared spectrum showed absorption bands at 3630 (hydroxyl) and 1715 (carbonyl) cm^{-1}. Corresponding carbon signals at δ 212.9 (s), 75.2 (d), and 74.6 (s) in the ^{13}C NMR spectrum (Table 14) indicated **60** possessed a ketone and two hydroxyl groups. ^{1}H NMR spectra (Table 15) and COSY NMR experiments revealed that

60 has the same diene and isopropyl functionalities as compounds **56** - **59**. Four mutually coupled protons were found at δ 2.93 (1 H, ddd, 19.0, 11.2, 1.5), 2.66 - 2.56 (2 H, m), and 2.20 (1 H, m) in the ^{1}H NMR spectrum of **60**. The proton at δ 2.20 showed a long range coupling to the methyl resonance at δ 1.81 (brs) which was also coupled to the olefinic proton at δ 5.55 (brd, 10.8, C-3). Thus, the connectivities around C-4 of the diene were determined.

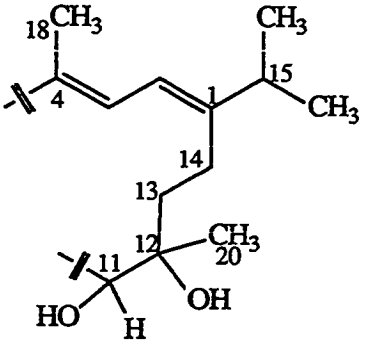
The low field chemical shift (δ 2.93) and large germinal coupling constant (19.0 Hz) of one of the C-6 methylene protons indicated the presence of the carbonyl group at the adjacent position (C-7).



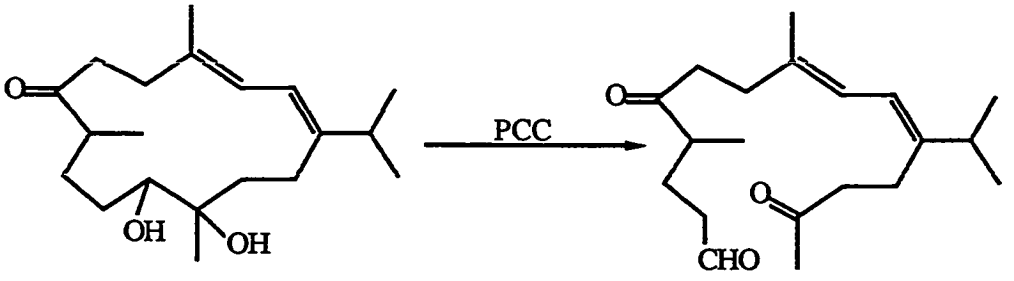
^{1}H NMR COSY experiments revealed the presence of a spin system that consisted of seven proton resonances, including the hydroxyl methine proton at δ 3.48 (Table 15). Consideration of the ^{13}C NMR spectrum showed that the protons were attached to five carbons; one methyl, two methylene, and two methine. The low field chemical shifts of both a methine proton (δ 2.81) and its corresponding carbon (δ 45.2) signal indicated the presence of an adjacent carbonyl group. Thus, the partial structure from C-8 to C-11 was determined.



Another spin system of four mutually coupled protons (δ 2.11, 1.96, 1.64, and 1.48) was also found by the ^{1}H NMR COSY experiment. Consideration of the ^{13}C NMR data from **60** confidently allowed assignment of this as an ethylene group. Finally, an isolated methyl signal was found at δ 1.27 in the ^{1}H NMR spectrum and the methyl group was connected to the quaternary carbon at δ 74.6 in the ^{13}C NMR spectrum. Since there was no coupling between the C-11 proton (δ 3.48) and the ethylene protons, the quaternary carbon must be between them (C-12). So, the ethylene was assigned as C-13 and C-14. Thus, the structure of **60** was defined.



Due to the poor solubility of **60** in NMR solvents, its structure could not be confirmed by two-dimensional carbon-proton correlation (XHCORR and COLOC) experiments. The confirmation was made, however, by a chemical reaction of **60**. Treatment of **60** with pyridinium chlorochromate (PCC) gave **63** as the major product.



60 **63**

Compound **63** was characterized by high resolution mass, ^{1}H and ^{13}C NMR spectral data (Table 14). Comparison of these data revealed that the two hydroxyl groups of **60** were oxidized to a ketone and an aldehyde. Also, the proton signal for the α-hydroxy methyl (C-20) at δ 1.27 in the ^{1}H NMR spectrum of **60** was shifted downfield to δ 2.16 (α-carbonyl). Therefore, compound **63** must be produced by an oxidative cleavage of the vicinal hydroxyl groups of **60**. Apparently, the only positions for vicinal hydroxyl groups (one secondary, the other tertiary) in **60** are at C-11 and C-12. Thus, the structure of **60** was unambigously defined. The stereochemistry of the asymmetric carbon centers were not determined. Several attempts to produce, either directly or via derivatization, a suitable crystal for an X-ray crystallographic study was failed.

Two previously reported diterpenoids, **61** and **62** were also isolated in small quantities. ^{1}H and ^{13}C NMR data of these were in full accord with reported data. Compound **61** was previously isolated from the Australian soft corals, *Sinularia grayi*,[87] *Nephthea* sp.,[88] and *Lobophytum* sp.[89] The absolute configurations of the asymmetric carbon centers (C-11 and -12) were assigned by application of the Horeau method.[90] Compound **62** was isolated from the soft coral *Sarcophyton* sp.[91] collected from the same area.

Experimental Section

Collection and Extraction. *Eunicea* sp.(specimen number CI 86-222) was collected by hand using SCUBA at -20 to 25 m in July 1986, along the offshore islands of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonians were next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 15 g of crude organic materials (from 1 kg, dry weight of the gorgonian). Compounds **56** - **62** were eluted from a 'vacuum flash' silica column with sequential mixtures of EtOAc and isooctane and further purified by HPLC using preparative silica gel columns.

Compound 56. The diepoxide **56** was isolated as a white solid after final purification by HPLC (30% EtOAc in isooctane). Recrystallization from CH_2Cl_2 gave 300 mg (2.0% of the crude extract) of **56**, mp 99-101°. Compound **56** showed $[\alpha]_D$ -76.4° (c 0.4, methanol) and exhibited the following spectral features; UV (methanol) 249 nm (ε 27000), 242 nm (ε 25000); IR (NaCl) 2960, 2940, 1670, 1460, 1380, 1070 cm^{-1}; HRMS: M^+, m/z obsd 304.2426, $C_{20}H_{32}O_2$ required 304.2402; low resolution MS: m/z (relative intensity) 304 (55), 261 (15), 243 (12), 193 (10), 175 (33), 161 (35), 148 (64), 135 (100), 125 (49), 121 (81), 107 (69), 93 (59).

Compound 57. The diol **57** was isolated as a white solid after final purification by HPLC (50% EtOAc in isooctane). Recrystallization from acetone gave 60 mg (0.4% of the crude extract) of **57**, mp 131-132°. Compound **57** showed $[\alpha]_D$ -85.8° (c 0.8, methanol) and displayed the following spectral features; UV (methanol) 249 nm (ε 27000), 243nm (ε 25000); IR (NaCl) 3400, 2980, 1620, 1460, 1380, 1070 cm^{-1}; HRMS: M^+, m/z obsd 322.2500, $C_{20}H_{34}O_3$ required 322.2508; low resolution MS: m/z (relative intinsity) 322 (81), 261 (11), 217 (21), 193 (16), 183 (18), 175 (22), 161 (25), 148 (32), 135 (70), 125 (59), 109 (53), 85 (96), 43 (100).

Compound 58. The acetate **58** was isolated as a white solid after final purification by HPLC (40% EtOAc in isooctane). Recrystallization from a mixture of hexane and acetone gave 80 mg (0.7% of the crude extract) of **58**, mp 96-98°. Compound **58** showed $[\alpha]_D$ -35.3° (c 0.5, methanol) and exhibited the following spectral features; UV (methanol) 249 nm (ε 33000), 241 nm (ε 25000); IR (KBr) 3450, 2960, 1740, 1660, 1610, 1440, 1370, 1030 cm^{-1}; HRMS: M^+, m/z obsd 364.2619, $C_{22}H_{36}O_4$ required 364.2614; low resolution MS: m/z (relative intensity) 364 (100), 346 (16), 175 (16), 150 (12), 135 (26), 127 (30), 107 (10), 85 (22).

Compound 59. The diol **59** was isolated as an oil after final purication by HPLC (70% EtOAc in isooctane); 50 mg (0.3% of the crude extract). Compound **59** showed $[\alpha]_D$ -85.8° (c 0.9, methanol) and displayed the following spectral features; UV (methanol) 250 nm (ε 23000), 243 nm (ε 22000); IR (NaCl) 3440, 2960, 1660, 1460, 1370, 1070 cm^{-1}; HRMS: M^+, m/z obsd 322.2534, $C_{20}H_{34}O_3$ required 322.2508; low resolution MS: m/z (relative intensity) 322 (42), 304 (10), 193 (21), 175 (21), 161 (12), 148 (18), 135 (64), 121 (59), 107 (50), 93 (82), 69 (30), 58 (100).

Compound 60. The ketone **60** was isolated as a white solid after final purification by HPLC (70% EtOAc in isooctane). Recrystallization from methanol gave 100 mg (0.7% of the crude extract) of **60**; mp 156-157°. Compound **60** showed $[\alpha]_D$ +11.3° (c 0.7, $CHCl_3$) and displayed the following spectral features; UV (methanol) 246 nm (ε 38000), 241 nm (ε 37000); IR ($CHCl_3$) 3630, 3030, 2980, 1715, 1620, 1420, 1050 cm^{-1}; HRMS: M^+, m/z obsd 322.2507, $C_{20}H_{34}O_3$ required 322.2508; low resolution MS: m/z (relative intensity) 322 (74), 304 (36), 261 (15), 176 (40), 161 (20), 148 (100), 136 (76), 133 (71), 121 (71), 107 (51), 93 (38).

Hydrolysis of 56. To a stirred emulsion of 35 mg of **56** (0.12 mmol) in 1.5 ml of a mixture of acetone and distilled water (2:1, v/v), 0.1 ml of conc. H_2SO_4 was added. After 15 min, the mixture was extracted with 20 ml of ethyl ether. The ether layer

was washed with saturated $NaHCO_3$ (2 x 15 ml), water (2 x 15 ml) and dried under vacuum. Separation by HPLC (50% EtOAc in isooctane) gave 15.4 mg (0.05 mmol, 42% yield) of **57** and 13.0 mg (0.04 mmol, 35%) of **59**. ^1H and ^{13}C NMR data of the products were identical with the natural products.

Acetylation of 57. To a stirred solution of 10 mg (0.03 mmol) of **57** in 1.0 ml of dry pyridine, 0.5 ml of acetic anhydride was added. After stirring for 2 hrs, pyridine and excess acetic anhydride were removed under vacuum. The monoacetate was obtained in quantitative yield. The ^1H and ^{13}C NMR spectra of the acetate were identical with those from compound **58**.

Reaction of 57 - 59 with trichloroacetyl isocyanate. Reactions were performed in NMR tubes. After measuring the chemical shifts of proton resonances in C_6D_6 solutions, one drop of neat isocyanate was added. Products were formed instantly, and NMR spectra showed that none of the natural product remained. The numbers of hydroxyl groups in compounds **57 - 59** were found by the numbers of urethane NH protons formed by the reaction.

Oxidation of 60. To a stirred solution of 21 mg (0.06 mmol) of **60** in 2 ml of dry CH_2Cl_2, 30 mg (0.13 mmol) of PCC and 6 mg (0.1 mmol) of sodium acetate were added. After stirring for 3 hrs at room temperature, organic materials were obtained by a small scale silica column chromatography (elution with ether) of the mixture. Final purification by HPLC (40% EtOAc in isooctane) gave 9 mg (0.03 mmol, 43% yield) of **63** as an oil; HRMS: M^+, m/z obsd 320.2357, $C_{20}H_{32}O_3$ required 320.2353; low resolution MS: m/z (relative intensity) 320 (19), 302 (12), 192 (100), 177 (26), 167 (15), 161 (17), 159 (30), 151 (13); ^1H NMR ($CDCl_3$): δ 9.75 (1 H, brs), 6.00 (1 H, d, 11.3), 5.94 (1 H, brd, 11.5), 2.6-2.2 (11 H, m), 2.16 (3 H, brs), 1.99 (1 H, m), 1.75 (3 H, brs), 1.65 (1 H, m), 1.11 (3 H, d, 7.0), 1.03 (6 H, d, 6.8). ^{13}C NMR data of **63** are shown in Table 14.

Isolation of compounds 61 and 62. The epoxides **61** and **62** were isolated as oils after final purification by HPLC (10% EtOAc in isooctane); 25 mg (0.15% of the extract) each. The ^{1}H NMR data of these compounds were in full accord with published data.[87-89, 91] The ^{13}C NMR resonances were also within $\pm$ 0.1 ppm of the reported data for these compounds.

Chapter VI

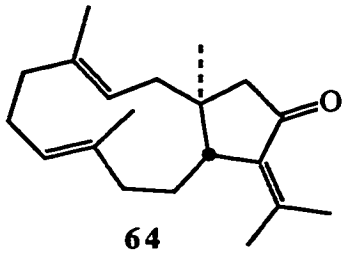
Dolabellanes from *Eunicea laciniata* and *E. cf calyculata*

Twelve specimens of stout gorgonians (height 1.5 ft) were collected at -20 to 25 m in July 1986, along the offshore of the Tobago Cays, eastern Caribbean Sea. Although they looked morphologically very similar, careful examination of the results of TLC analysis in various media revealed that they were indeed a mixture of two chemically distinct groups. As a result, the gorgonians were separated into two groups (one group of 4 specimens and another of 8), and identified by Bayer as *E. laciniata* (the former, specimen number CI 86 - 228) and *E. calyculata* (the latter, CI 86 - 227, Chapter VII), respectively.

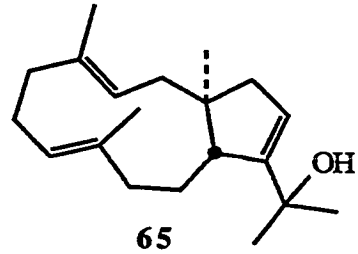
Extraction of the dried *E. laciniata* (0.6 kg) with dichloromethane yielded 13 g of crude extract. Silica flash chromatography, followed by HPLC (100 - 80% isooctane in EtOAc) of the crude extract gave six compounds (**64** - **69**) possessing the well known dolabellane skeleton (Figure 15). The dolabellanes were originally isolated from the sea hare *Dolabella californica*.[92,93] Subsequently, related metabolites were found from its dietary source, the brown alga *Dictyota* and taxonomically related organisms.[94-101] Among the gorgonians, Look and Fenical reported two metabolites from a collection of *Eunicea calyculata* from Belize.[25]

The major compound, **64**, was isolated as an oil (1.5 g, 12% of the extract). High resolution mass, proton NMR and carbon NMR spectra of **64** were in full accord with one of the previously known dolabellanes from *Eunicea calyculata*. Careful examination of spectral data ended in full agreement with the proposed structure. Compound **64** has two asymmetric carbon centers at C-1 and -11. The absolute stereochemistry of these were originally identified as 1S and 11R by combination of NOEDS, chemical reactions and the application of the Mosher method[102] to a derivative.

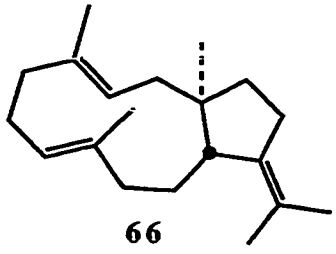
Figure 15. Dolabellane metabolites from *E. laciniata*.



64



65



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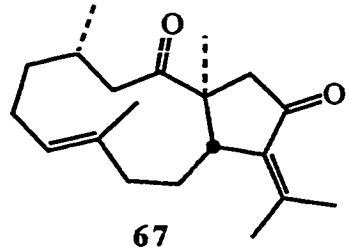

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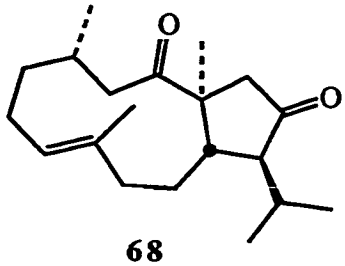

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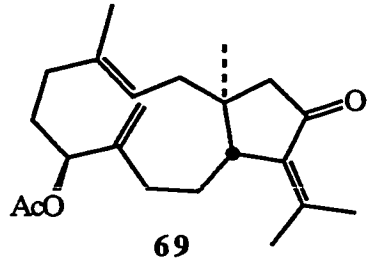

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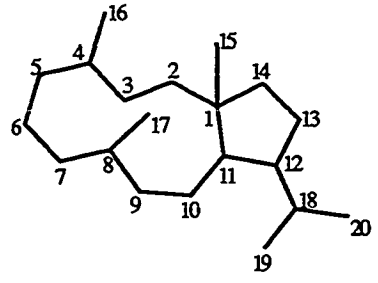


Since the optical rotation of **64** was very similar to the reported value (+32°, lit. value +31°), the stereochemistry of **64** must be identical to the known metabolite.

Compound **65** was isolated as a white solid (160 mg, 1.2% of the extract), and analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. Comparison of the NMR data showed similarities between **64** and **65**. However, there were several significant differences in spectral data. Carbon signals at δ 206.7 (s), 147.8 (s) and 137.9 (s) which corresponded to an eneone functionality in **64** were shifted to δ 153.9 (s), 122.6 (d) and 71.6 (s) (Table 18). In the proton NMR spectrum, two methyl signals at δ 2.22 (brs) and 1.83 (brs), whose chemical shifts were of vinyl methyls attached to a conjugated enone, were shifted to δ 1.43 (s) and 1.38 (s) in **65**. In addition, a new lowfield signal appeared at δ 5.48 (brs). This proton showed couplings to upfield resonances at δ 2.25 and 1.93. Finally, the carbonyl absorption at 1700 cm^{-1} in the IR spectrum of **64** was replaced by a hydroxyl absorption at 3620 cm^{-1} in **65**. All of these changes could be accommodated by the migration of the C-12,18 double bond to C-12,13 and formation of a tertiary alcohol at C-18.

A two-step chemical reaction confirmed this interpretation (Figure 16). Reduction of compound **64** using diisobutylaluminium hydride (DIBAL-H) in THF gave two major products, **70** and **71**. Spectral analyses showed that these were the C-13 hydroxyl derivatives of **64**, epimeric to each other at the same center. Due to the overlapping of the upfield proton signals however, the configurations of the new asymmetric centers were not determined. Attempts to convert these allylic alcohols to compound **65** in acidic media failed, resulting in very rapid decomposition of both alcohols. Inspection of the proton NMR spectrum of the decomposed mixture indicated that two tertiary double bonds at C-3 and -7 of the allylic alcohols had disappeared. This might be related to the conformation of the 11-membered ring (B ring). It is well known that in germacranes and related compounds π-bond interaction between two double bonds determine the 'crown'

Table 18. ^{1}H and ^{13}C NMR assignments for compounds **64 - 66**.[a,b]

#	64 ^{1}H	64 ^{13}C		65 ^{1}H	65 ^{13}C		66 ^{1}H	66 ^{13}C	
1	—	40.9	s	—	47.4	s	—	48.4	s
2	2.13 (1H,dd,11.7, 11.2) 1.60 (1H,dd,11.7, 5.0)	40.1	t	2.23-2.19 (1H)[d] 1.56 (1H,dd,13.4, 5.0)	40.7	t	c	40.2	t
3	5.24 (1H,dd,11.2, 5.0)	124.8	d	5.22 (1H,ddd,11.7, 5.0,1.2)	125.3	d	5.24 (1H,brdd, 11.0,4.6)	125.9	d
4	—	135.4	s	—	134.5	s	—	134.7	s
5	c	38.1	t[f]	c	38.1	t[f]	c	38.2	t[f]
6	c	24.2	t	2.34-2.29 (1H)[d] 2.10-2.05 (1H)[d]	24.4	t	c	24.3	t
7	4.93 (1H,brd,10.6)	130.3	d	4.85 (1H,brdd,10.4, 1.0)	128.6	d	4.89 (1H,brd, 10.0)	129.3	d
8	—	131.4	s	—	133.3	s	—	132.4	s
9	c	39.8	t[f]	c	40.0	t[f]	c	39.9	t[f]
10	c	27.9	t	c 1.45-1.35 (1H)[d]	26.1	t	c	27.9	t
11	2.83 (1H,brd,12.2)	41.4	d	2.37 (1H,brd,10.4)	46.0	d	c	42.0	d
12	—	137.9	s	—	153.9	s	—	142.5	s
13	—	206.7	s	5.48 (1H,brs)	122.6	d	c	28.3	t
14	2.36 (1H,d,18.4) 2.10 (1H,d,18.3)	54.7	t	2.25 (1H)[d] 1.93 (1H,dd,16.5, 3.2)	47.8	t	c c	38.7	t
15	1.23 (3H,brs)	23.1	q	1.17 (3H,brs)	22.7	q	1.19 (3H,brs)	23.6	q
16	1.44 (3H,brs)	16.1	q	1.51 (3H,brs)	16.2	q	1.44 (3H,brs)	16.3	q
17	1.64 (3H,brs)	15.5	q	1.63 (3H,brs)	15.5	q	1.64 (3H,brs)	15.3	q
18	—	147.8	s	—	71.6	s	—	121.9	s
19	1.83 (3H,brs)	24.4	q	1.43 (3H,s)[e]	31.8	q	1.61 (3H,brs)[e]	21.7	q[g]
20	2.22 (3H,brs)	21.3	q	1.38 (3H,s)[e]	31.8	q	1.59 (3H,brs)[e]	21.3	q[g]

[a] ^{1}H NMR spectra were obtained in $CDCl_3$ solution using Me_4Si as internal references. Assignments were made by COSY experiments. [b] ^{13}C NMR spectra were obtained in $CDCl_3$ solution using Me_4Si as internal references. Multiplicities were determined by DEPT experiments. Assignments were aided by comparison with compound **67** (Table 19). [c] Nonassignable resonances. [d] Coupling constants were not determined. [e-g] Signals within a column may be reversed.

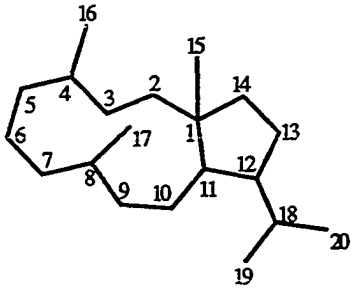


Figure 16. Interconversion of dolabellane **64** to the alcohol **65**.

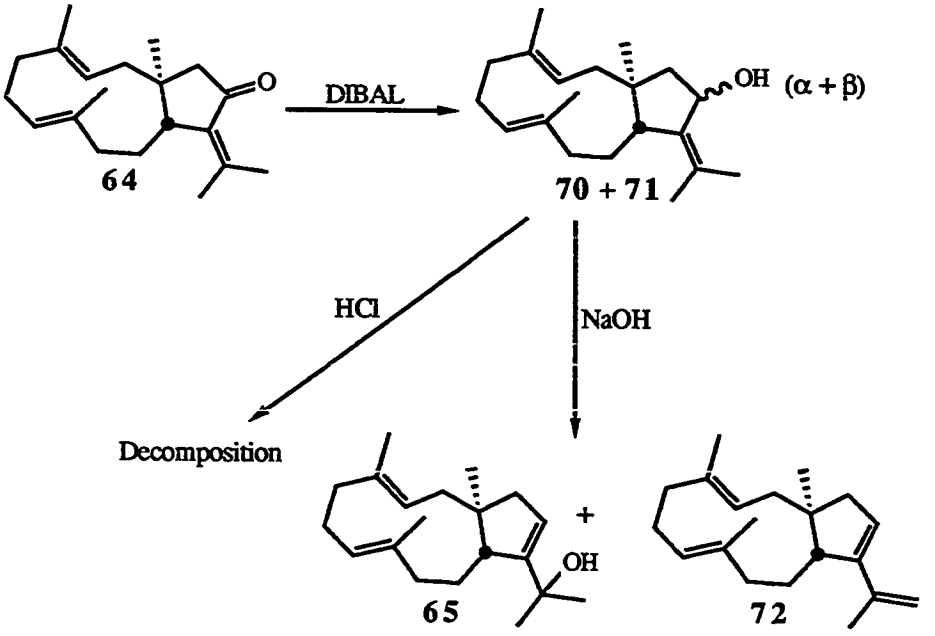
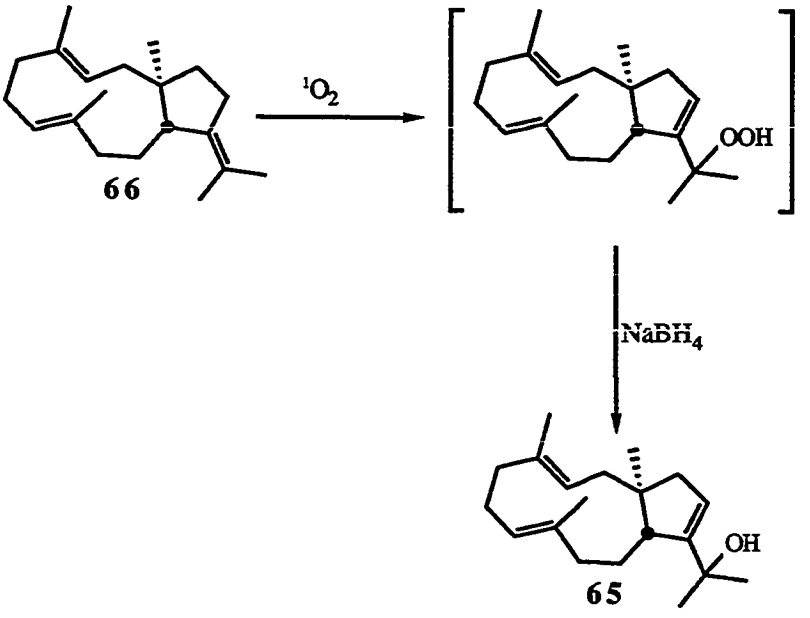


Figure 17. Photooxidation of compound **66.**



shape conformation of the 10-membered ring. Nuclear Overhauser enhancement difference spectroscopy (NOEDS) showed **64** to possess the same type of conformation. This interaction needs not only that two double bonds be proximal but also parallel to each other. As a result, the π-bond electrons of one double bond readily move to a carbonium ion formed on the other. In basic media however, the B ring was inert, and treatment of **70** with NaOH in aqueous acetone gave **65** and the tetraene, **72** (Figure 16). Thus, the structure of **65** was unambiguously defined.

A volatile hydrocarbon, **66**, was isolated as an oil (100 mg, 0.8% of extract) which analyzed for $C_{20}H_{32}$ by high resolution mass and ^{13}C NMR spectrometry. Analyses of NMR spectra revealed **66** to possess a B ring identical with **64** and **65** (Table 18). Differences were found however, in part of the 5-membered ring (A ring). In the ^{13}C NMR spectrum, the carbonyl resonance at δ 206.7 in **64** was replaced by a methylene signal at δ 28.3 or 27.9. Corresponding changes were also found in the ^{1}H NMR spectrum in which the vinyl methyl signals at δ 2.22 and 1.83 in **64** were shifted to δ 1.61 and 1.59. Therefore, compound **66** must be a derivative of **64** in which the carbonyl group was reduced to a methylene.

A simple photooxidation[103] followed by reduction confirmed this interpretation (Figure 17). Compound **66** was readily oxidized with singlet oxygen, using visible light and methylene blue as a sensitizer. Treatment of the reaction mixture with $NaBH_4$ gave **65** as the only product. Thus, the structure of **66** was defined as a diterpene of the dolabellane class.

Compound **67** was isolated as an oil (35 mg, 0.3% yield), and analyzed for $C_{20}H_{30}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Spectral analyses revealed **67** to possess an A ring identical with compound **64**. However, the ^{13}C NMR spectrum showed the appearence of a new carbonyl signal at δ 214.8 (Table 19). In addition, signals corresponding to one of the double bonds in the B ring of **64** disappeared. In the

Table 19. ^{13}C NMR assignments for compounds **67 - 69** and **73 - 76**.

#	67	68	69	73	74	75	76
1	50.4 s	52.3 s	40.4 s	52.3 s	52.4 s	48.5 s	48.5 s
2	214.8 s	213.2 s	40.8 t	212.5 s	216.0 s	73.1 s	75.2 s
3	44.4 t	44.6 t	124.2 d	44.5 t	55.9 d	41.4 t	42.3 t
4	27.3 d	27.8 d	136.5 s	27.6 d	40.8 d	27.5 d[a]	28.6 d[a]
5	31.2 t	32.0 t	37.6 t	28.7 t	34.7 t	34.5 t	34.5 t
6	23.2 t	24.2 t	32.3 t	23.1 t	28.9 t	28.1 t	26.4 t
7	130.6 d	130.2 d	76.1 d	64.7 d	55.2 d	48.3 d	53.6 d
8	131.7 s	132.1 s	149.7 s	61.5 s	75.7 s	150.3 s	76.2 s[d]
9	40.0 t	39.3 t	34.3 t	41.3 t	47.4 t	36.1 t	44.5 t
10	27.8 t	28.7 t	30.6 t	24.8 t	23.2 t	27.1 t	23.3 t
11	48.8 d	46.7 d	43.5 d	51.3 d	51.9 d	38.3 d	36.3 d
12	135.8 s	59.7 d	137.6 s	135.0 s	134.1 s	58.6 d	60.8 d
13	204.8 s	218.1 s	206.3 s	204.4 s	204.2 s	220.2 s	220.1 s
14	54.7 t	55.3 t	55.8 t	51.3 t	49.5 t	51.0 t	52.4 t
15	20.9 q	20.9 q	22.0 q	22.5 q	22.4 q[b]	20.7 q[b]	20.3 q[b]
16	21.8 q	22.4 q	15.8 q	19.8 q	19.1 q	22.3 q[b]	22.2 q[b]
17	16.8 q	17.0 q	114.0 t	16.7 q	19.1 q	113.4 t	22.9 q
18	147.9 s	28.9 d	147.8 s	150.0 s	149.6 s	29.1 d	28.8 d[a]
19	24.3 q	18.5 q[c]	24.1 q	24.6 q	24.5 q	19.2 q[c]	19.6 q[c]
20	21.5 q	18.1 q[c]	21.5 q	21.0 q	21.9 q[b]	18.3 q[c]	17.8 q[c]
OAc			170.8 s				
			21.4 q				

Carbon NMR spectra were recorded at 50 MHz in $CDCl_3$ solution. Multiplicities were determined from SFORD (compounds **67 - 69**) and DEPT (**73 - 76**) experiments. Assignments of **67** and **69** were made from XHCORR and COLOC experiments. Assignments of others were made by comparison with compounds **67** and **69**. [a-c] Signals within a column may be reversed. [d] Chemical hift was obtained in C_6D_6 solution.

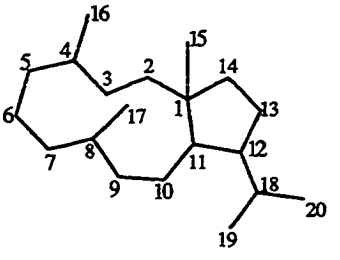


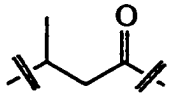
Table 20. ^{1}H NMR assignments for compounds **67 - 69** and **73 - 76**.

#	67	68	73	74	75	76	69
2	----	----	----	----	----	----	2.21 - 2.14[b] 1.84 brdd(13.1,4.5)
3	2.57 dd(19.7,10.9) 2.24 - 2.17[b]	2.54 dd(19.4,9.1) 2.35 dd(19.4,3.5)	2.65 dd(19.3,3.0) 2.42 dd(19.3,10.0)	2.78 dd(7.9,7.7)	1.58 m 1.12 ddd(12.7,12.7, 2.1)	1.42 - 1.33[b] 1.16 dd(13.3,12.8)	5.52 ddd(11.6,4.4, 0.8)
4	2.20 - 2.12[b]	2.17 - 2.10[b]	2.23 m	2.51 m	1.78 - 1.70[b]	1.63 - 1.56[b]	----
5	2.18 - 2.03[b] 1.43 m	1.98 - 1.90[b] 1.55 - 1.44[b]	1.88 m 1.69 m	1.98 - 1.85[b] 1.24 m	1.78 - 1.70[b] 0.85 m	1.85 - 1.80[b] 0.88 m	[a]
6	2.18 - 2.03[b]	2.12 - 2.02[b]	2.12 dddd(14.8,10.3, 4.1,1.5)	1.98 - 1.85(2H)[b]	1.78 - 1.70[b]	2.24 dddd(13.4,3.2, 3.2,3.1)	2.03 - 1.91(2H)[b]
	1.89 m	1.98 - 1.90[b]	1.37 m		1.50 m	1.47 dddd(13.3,13.3, 13.1,3.3)	
7	5.02 m	5.03 brdd(7.5,7.5)	2.69 dd(9.8,2.1)	2.27 m	2.28 dd(12.7,3.6)	1.63 m	5.21 brdd(4.8,4.2)
9	2.18 - 2.03 (2H)[b]	2.22 - 2.15[b]	2.35 brdd(13.9,8.1)	2.16 m	2.54 ddd(13.6,10.9, 2.7)	2.08 - 1.95[b]	2.35 - 2.26 (2H)[b]
		1.89 ddd(12.7,12.7, 2.9)	1.23 m	1.68 brdd(13.2,9.1)	2.19 m	1.88 - 1.80[b]	
10	1.82 m	1.76 brddd(11.7, 7.2,2.6)	1.91 m	2.05 - 1.96[b]	1.88 - 1.79[b]	2.08 - 1.95[b]	1.75 m
	1.61 m	1.55 - 1.44[b]	1.72 dddd(16.0,8.3, 4.5,1.3)	1.98 - 1.85[b]	1.46 m	1.39 m	1.53 m
11	3.09 brd(6.0)	2.30 ddd(10.9,7.5, 3.6)	2.72 m	2.74 m	2.14 m	2.87 ddd(10.9,10.9, 6.8)	2.48 brd(10.6)
12	----	1.83 ddd(10.9,3.4, 2.1)	----	----	1.83 - 1.78[b]	2.08 - 1.95[b]	----
14	2.84 brd(15.9) 2.00 d(15.7)	2.70 brd(17.0) 2.05 dd(17.1,2.0)	3.12 brd(16.2) 2.01 d(16.2)	3.06 brd(16.1) 2.00 d(16.1)	2.77 dd(17.1,1.0) 1.98 dd(17.1,1.8)	2.72 brd(17.0) 2.01 - 1.95[b]	[a]
15	1.22 s	1.15 s	1.25 s	1.27 s	1.03 d(0.9)	1.11 brs	1.24 s
16	0.98 d(7.0)	0.99 d(7.0)	1.00 d(7.1)	0.94 d(6.7)	0.88 d(6.0)	0.90 d(6.4)	1.54 brs
17	1.67 brs	1.64 brs	1.39 s	1.26 s	5.00 brs 4.94 brs	1.32 s	5.31 brs 5.13 brs
18	----	2.05 - 1.97[b]	----	----	1.90 m	2.08 - 1.94[b]	----
19	1.85 d(1.6)	0.99 d(7.1)[c]	1.81 d(2.0)	1.85 d(1.5)	1.00 d(6.9)[c]	1.01 d(6.9)[c]	1.80 d(0.8)
20	2.15 d(2.2)	1.11 d(6.9)[c]	2.22 d(2.3)	2.19 d(2.1)	0.90 d(6.7)[c]	0.94 d(7.0)[c]	2.18 d(1.5)
Others					2.01 d(1H,2.4,-OH)	1.61 brs(2H,-OH)	2.06 s(3H,-OAc)

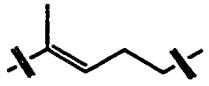
Proton NMR spectra were recorded at 360 MHz. Chemical shifts were measured in $CDCl_3$ solutions using TMS as internal references. Assignments were made by COSY and RCT (67 and 68) experiments. [a]Nonassignable resonances. [b]Coupling constants were not determined. [c]Signals within a column may be reversed.

^{1}H NMR spectrum (Table 20), a vinyl methyl resonance shifted upfield to δ 0.98 (d, 7.0). A COSY NMR experiment helped to interpret these changes. A proton peak at δ 2.57 (dd, 19.7, 10.9) was found to be coupled to two protons at δ 2.24 - 2.17 and 2.20 - 2.12. The new methyl resonance at δ 0.98 was also coupled to a proton signal in the same region (δ 2.20 - 2.12). Therefore, it seemed very likely that both were connected to the identical proton. However, this proton signal was obscured by others, and its assignment was not clear. This problem was solved by a proton-proton relay coherence transfer (RCT) experiment, in which a correlation was observed between protons at δ 2.57 and 0.98. The low field chemical shift (δ 2.57) and the very large coupling constant (19.7 Hz) indicated that this proton belonged to a methylene adjacent to a carbonyl.

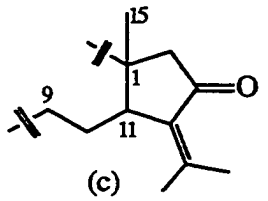
Combination of COSY, RCT and direct carbon-proton correlation (XHCORR) experiments revealed the presence of three partial structures (a-c).



(a)



(b)



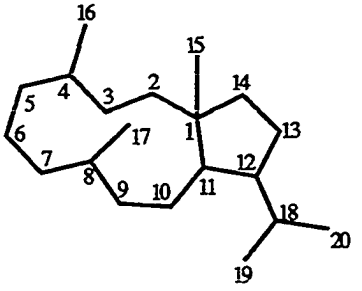
(c)

The chemical shift of the carbon at C-1 in (c) was significantly lower (9.5 ppm) than in **64**, indicating the carbonyl of partial structure (a) was attached to C-1 of (c). This was supported by the long range carbon proton correlation spectroscopy (COLOC) experiment (Table 21), in which a coupling between the carbonyl carbon (δ 214.8) and the C-15 methyl protons (δ 1.22) was found. The remaining problem was the attachment of (b) to (a) and (c). Since both of the proton and carbon chemical shifts of C-9 were substantially lower than C-10, C-9 must be attached to the olefinic end of (b). Thus, the structure of **67** was defined as a diterpene of the dolabellane class.

Table 21. Results of COLOC experiments with compounds **67** and **69**.[a]

carbon	proton	
	67[b]	**69**[b]
1	14 (2.84), 15	2 (2.21-2.14)[c], 2 (1.84), 11, 15
2	15	15
3	—	2 (2.21-2,14)[c], 2 (1.84), 16
4	—	2 (2.21-2.14)[c], 16
5	—	16
6	—	—
7	17	17 (5.31), 17 (5.13)
8	17	9 (2.35-2.26)
9	—	17 (5.31), 17 (5.13)
10	—	9 (2.35-2.26), 11
11	—	15
12	14 (2.00), 19, 20	11, 19, 20
13	14 (2.84), 14 (2.00)	11
14	15	15
15	—	2 (2.21-2.14)[c]
16	—	3
17	—	—
18	19, 20	19, 20
19	20	20
20	19	19

[a] Experiments were performed at 50 MHz in $CDCl_3$ solutions. Parameters were optimized for couplings of 6 Hz. [b] The numbers in parentheses are the ^1H NMR chemical shifts of the protons that correlate. [c] Due to the overlapping with other protons, correlation was not clear.

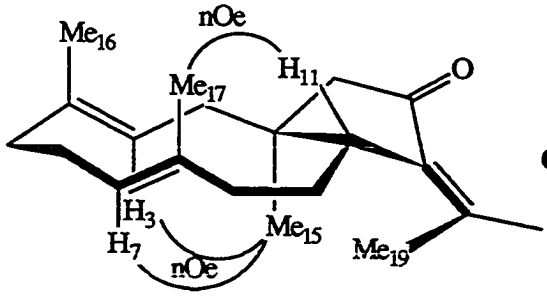


Compound **67** has three asymmetric carbon centers at C-1, -4 and -11. ^{1}H NMR NOEDS was used to determine the stereochemistry at these centers (Figure 18). The proton signals at C-3 (δ 2.57) and 15 (1.22) were enhanced when each was irradiated. Irradiation of the methine proton at C-11 enhanced the C-19 methyl. Since there was no enhancement between the protons at C-11 and -15, the configurations are 1S and 11R, identical with compound **64**.

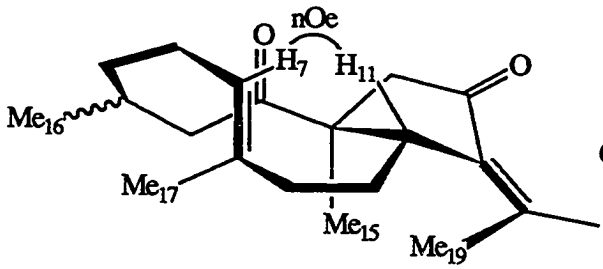
An interesting feature of the structure of **64** was found in these NOEDS experiments (Figure 18). Irradiation of the C-11 methine signal enhanced the olefinic proton at C-7 and vice versa. If the B ring of **67** is similar in conformation to **64**, enhancement would have been expected between protons at C-11 and -17. However, there was no nOe observed between these protons. These results suggested a change in conformation of the B ring in which, as compared to compound **64**, the C-7 double bond of **67** had rotated by 180°. Inspection of a molecular model revealed that with this new conformation, the C-7 double bond is not only proximal but coplanar with the C-2 carbonyl group. Thus, as the π bond interaction between two double bonds determined the 'crown' shape conformation in **64**, the same interaction between a double bond and a carbonyl resulted in the 'L' shape conformation for the B ring of compound **67**.

The stereochemistry of the asymmetric center at C-4 could not be determined by NOEDS, since irradiation of the C-16 methyl protons failed to enhance any key proton signals. The configuration of this center was thus determined by chemical reactions (Figure 19). Epoxidation of **67** with m-chloroperbenzoic acid (mCPBA) gave **73** as the major product. The structure of **73** was determined by HRMS, ^{1}H and ^{13}C NMR and COSY experiments (Table 19 and 20). Treatment of **73** with lithium diisopropylamide (LDA) gave **74** as the major product. The structure of **74** was elucidated by spectral methods, and found to possess a carbon skeleton of the fusicoccane class. Diterpenes of this class have been encountered from some fungi and liverworts.[104] To the best of our

Figure 18. Ring conformations of compounds **64** and **67**.



64 : B ring is 'crown' shaped.



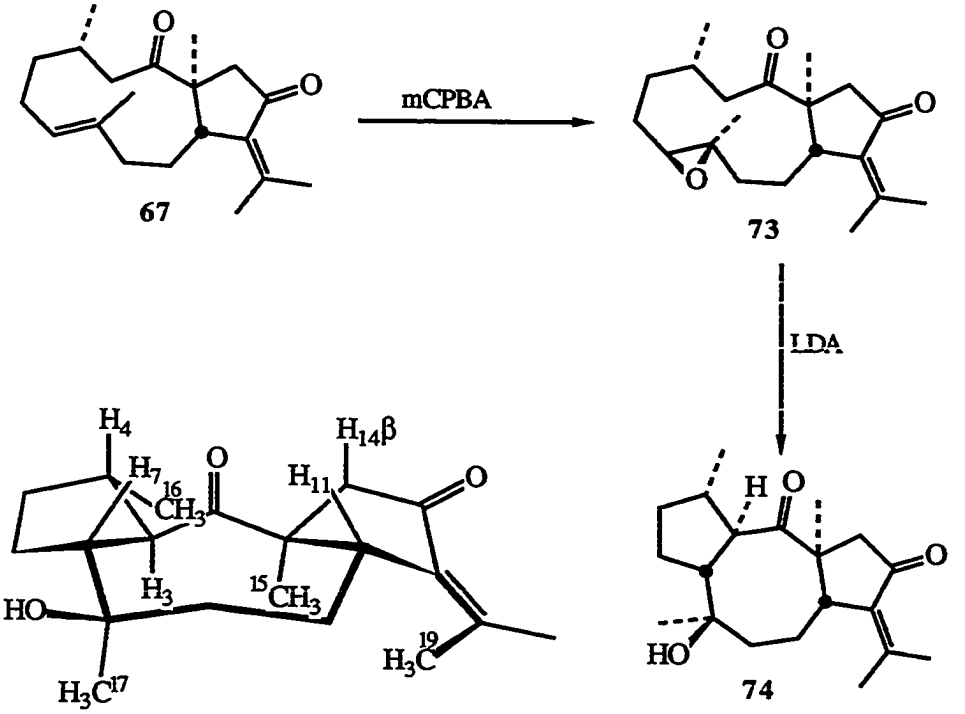
67 : B ring is 'L' shaped.

Results of a ^{1}H NMR NOEDS experiment with compound **67**.

proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
3α(2.57)[a]	15	3.0
7	11	4.3
11	7, 19	3.3, 4.4
15	3α(2.57)[a], 14α(2.00)[a]	8.5, 2.6

[a] Chemical shifts of protons

Figure 19. Formation and stereochemistry of compound **74.**



Results of a ^1H NMR NOEDS experiment with compound **74.**

proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
3	15, 16, 17	7.9, 5.5, 5.0
4	16	9.8
7	9β(2.05–1.96)[a], 11	1.8, 17.9
11	7, 14β(3.06)[a]	7.0, 1.1
16	3, 4	4.4, 7.6
17	3	5.0

[a] Chemical shifts of protons.

knowledge however, in the marine environment, epoxidictymene isolated from the brown alga *Dictyota dichotoma* is the only metabolite possessing this skeleton.[105]

All of the key protons of **74** were unambiguously assigned by a ^{1}H NMR COSY experiment (Table 20). The configurations of the asymmetric centers (C-1, -3, -4, -7, -8, and -11) were determined by NOEDS (Figure 19). Irradiation of the proton at C-3 enhanced both methyls signals at C-15 and -16. Also, irradiation of the C-16 methyl signals enhanced the C-3 proton. Therefore, these protons must be *syn* oriented to the plane of the molecule. Another datum supporting this interpretation was the lack of enhancement between protons at C-3 and -4. Mutual enhancements between the C-7 and -11 protons determined the configuration at C-7. The stereochemistry of a newly formed C-8 asymmetric center was determined by the nOe between the C-3 and C-17 protons. Thus, the stereochemistries of **74** and **67** were unambigously defined. Based upon the results of NOEDS, the overall configuration of **67** was defined as 1S, 4S and 11R. The configuration of **74** is 1S, 3R, 4S, 7R, 8R and 11R.

Compound **68** was isolated as an oil (35 mg, 0.3% of the extract) which analyzed for $C_{20}H_{32}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Spectral data for **68** were very similar to those from compound **67**. However, in the ^{13}C NMR spectrum of **67** (Table 19), one pair of olefinic carbon signals was replaced by two high field carbons at δ 59.7 (d) and 28.9 (d). Also, the carbonyl at δ 204.8 was shifted downfield to 218.1. Corresponding differences were also found in the ^{1}H NMR spectrum (Table 20). Vinyl methyls at δ 2.15 (d, 2.2) and 1.85 (d, 1.6) in **67**, were shifted to δ 1.11 (d, 6.9) and 0.99 (d, 7.1). Finally, the carbonyl absorption at 1710 cm^{-1} in the IR spectrum shifted to 1735 cm^{-1} for **68** indicating the presence of a cyclopentanone system. Based on these data, the structure of **68** was defined as the 12,18-dihydro derivative of **67**.

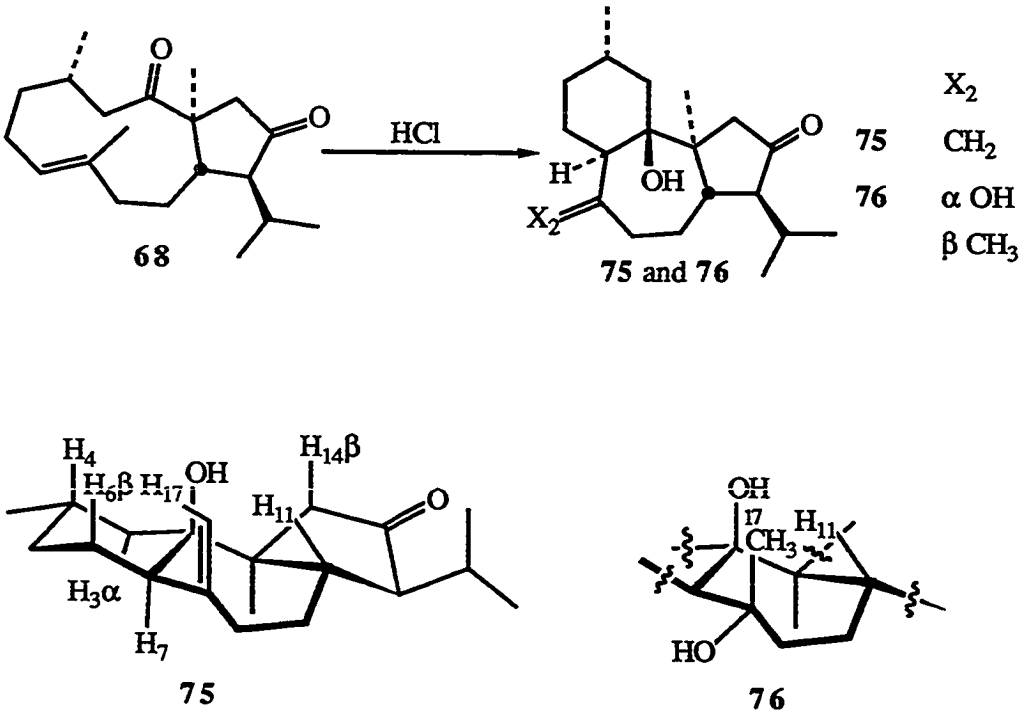
Compound **68** has an additional asymmetric carbon center at C-12. The methine proton at this center (δ 1.83) showed a long range coupling with one of the methylene

protons at C-14 (δ 2.05). Since the other proton (δ 2.70) of the same carbon displayed W-coupling to the C-15 methyl (δ 1.15) and was β-oriented to the A ring, it seemed very likely that the orientation of the C-12 proton is α. This conclusion was supported by the result of NOEDS experiments. Irradiation of the methyl at δ 1.11 (C-19 or -20) enhanced the proton at δ 2.30 (C-11) by 10.0%. Therefore, the configuration of C-12 was assigned as **S**.

To confirm the structure of **68**, a transannular cyclization was performed (Figure 20). Treatment of **68** with HCl in aqueous acetone afforded two major products, **75** and **76**. The structures of these tricyclic derivatives were elucidated by the combination of high resolution mass, ^{1}H and ^{13}C NMR, proton decoupling and COSY experiments (Table 19 and 20). Both **75** and **76** possess an unusual carbon skeleton. The only known metabolite possessing this skeleton was recently isolated from a Chinese stolonifer *Clavularia* sp.[106]

Compounds **75** and **76** possess several asymmetric carbon centers, the stereochemistries of which were determined by ^{1}H NMR NOEDS measurements (Figure 20). In compound **75**, the hydroxyl proton signal at δ 2.01 (d, 2.3, C-2) was enhanced by irradiation of both protons at δ 2.77 (dd, 17.1, 1.0, C-14β) and 4.94 (brs, C-17). Also, the proton at δ 2.28 (dd, 12.7, 3.6, C-7) was enhanced by irradiation of the proton at δ 1.12 (ddd, 12.7, 12.7, 2.1, C-3). Since there was no nOe enhancement found between the C-3 proton and the hydroxyl proton at C-2, but there was a W-coupling between these protons, their orientations to the ring must be α and β, respectively. The large coupling (12.7 Hz) between the C-3α and C-4 protons suggested that the methyl at C-16 is α-oriented to the cyclohexane ring. Thus, the stereochemistry of **75** was fully defined. Because of the overlapping of many proton resonances, ^{1}H NMR NOEDS experiments with **76** were less successful. However, irradiation of the proton at δ 2.87 (ddd, 10.9, 10.9, 6.8, C-11) enhanced the methyl at δ 1.32 (s, C-17), indicating that

Figure 20. Formation and stereochemistry of compounds **75** and **76**.



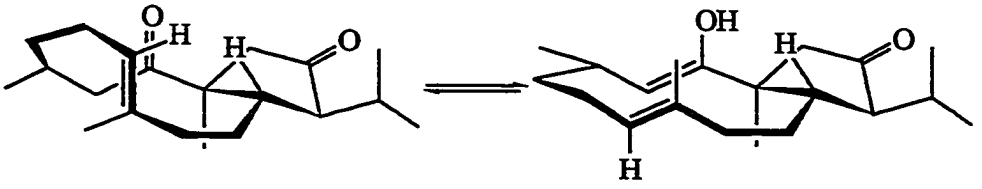
Results of ^{1}H NMR NOEDS experiments with compounds **75** and **76**.

	proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
75	3α(1.12)[a]	7	5.3
	14β(2.77)[a]	OH	5.6
	17(4.94)[a]	6β(1.78–1.70)[a], OH	4.2, 3.4
76	11	17	3.9
	19	11	1.7

[a] Chemical shifts of protons.

the orientation of the methyl group is β to the ring. Although, the orientation of the C-7 proton was not determined, the lack of enhancement of the methyl at C-17 suggested that they were *anti* to each other. Thus, the overall configuration of compounds **75** and **76** were defined as 1**S**, 2**R**, 4**S**, 7**R**, 8**S** (**76**), 11**S**, and 12**S**.

The results of ^{1}H NMR NOEDS studies showed another interesting feature (Figure 20). The stereochemistries of C-7 and C-8 of compounds **75** and **76** were opposite those of **74**. That is, during the condensation, the conformation of the C-7 double bond of **68** had inverted. This could be explained by either a) a shift of equilibrium in the keto-enol tautomerism or b) a difference in the reaction rates between the keto and enol forms.

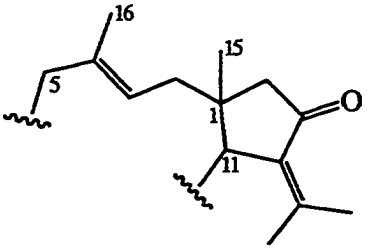


keto form enol form

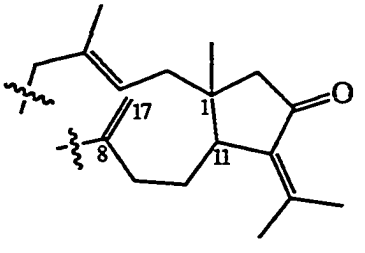
As shown in compound **67**, the conformation of the C-7 double bond is influenced by the C-2 carbonyl. If the enol form is dominant in the reaction medium (aqueous acetone), a shift of the π bond electrons from C-2 and oxygen to C-2 and C-3 will invert the conformation of the C-7 double bond. Also, the total conformation of the B-ring of **67** would change from 'L' to 'crown' shape. This would result in the inverse configurations at C-7 and 8 of the products, **75** and **76**. If the reactivity of the enol form is much higher than the keto form, the result might be also inversion.

Compound **69** was isolated as a white solid (33 mg, 0.3% of the extract), and analyzed for $C_{22}H_{32}O_3$ by high resolution mass and ^{13}C NMR spectrometry. Compound **69** had two more carbons than the other compounds. A sharp methyl singlet at δ 2.06 in

^{1}H NMR spectrum (Table 20) and two carbon signals at δ 170.8 (s) and 21.4 (q) in the ^{13}C NMR spectrum (Table 19) indicated the presence of an acetoxyl group. Therefore, compound **69** is a diterpene acetate. Comparison of the NMR spectra revealed that **69** has the same A ring as compounds **64** and **67**. The structure of the remaining part was determined by a combination of ^{1}H NMR COSY, XHCORR and COLOC experiments (Table 21). An olefinic proton signal at δ 5.52 (ddd, 11.6, 4.4, 0.8) was coupled with protons at δ 1.54 (3 H, brs), 2.21-2.14 (1 H, multiplicities unknown) and 1.84 (1 H, brdd, 13.1, 4.5). An XHCORR experiment showed that the latter two protons were attached to same carbon at δ 40.8 (C-2). Several carbon-proton long range correlations linked this methylene to adjacent centers, thus determined the region of C-1 ~ C-5 (Table 21).

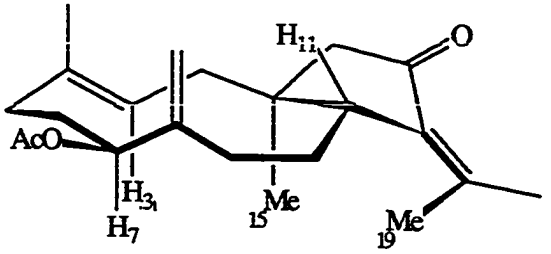


Long range proton couplings with methyl protons at δ 1.80 (C-19) and 2.18 (C-20) readily assigned a proton at δ 2.48 (brd, 10.6) to C-11. In addition, this proton was coupled to protons at δ 1.75 and 1.53, which were further coupled to two proton resonances at δ 2.35 - 2.26. A COLOC experiment involving correlations of these protons and corresponding carbons confirmed the partial structure C-8 ~ C-11 (Table 21).



The acetoxyl-bearing carbon at δ 76.1 (d) was coupled to the exocyclic methylene protons (C-17), indicating the position of the acetoxyl group at C-7. Although the connection between C-5 and C-6 was not determined by two dimensional NMR experiments, consideration of spectral data indicated they were adjacent to each other. Thus, the structure of **69** was defined as a diterpene acetate of the dolabellane class.

Compound **69** has an additional asymmetric carbon center at C-7. The ^{1}H NMR NOEDS experiment again helped to determine the stereochemistry of this center.



Irradiation of the olefinic proton at C-3 enhanced the C-7 proton by 4.1%. Signals of both protons were subsequently enhanced by the irradiation of the C-15 methyl protons (4.5% for the C-3 proton, and 4.7% for the C-7 proton). Therefore, the configuration of C-7 must be S. Irradiation of the C-11 proton enhanced the methyl protons at C-19 by 5.6% and confirmed the stereochemistry of the ring junction to be identical with the other compounds.

Another group of gorgonians (specimen number F 87 - 35) was collected at the Florida Keys, in July 1987. The results of TLC analysis showed that these were very similar chemically to *Eunicea laciniata* from the Tobago Cays. However, these animals were defined by Bayer as having morphological features different from *E. laciniata*, and somewhat similar to *E. calyculata* .[32] The same extraction process used for *E. laciniata* gave 25 g of crude extract (from 1.5 kg of dry weight of the gorgonian). Silica vacuum flash chromatography followed by silica HPLC gave compounds **64**, **65**, **66**, and **69**

from *E. laciniata* (Figure 15). Compounds **67** and **68** were not isolated from this extract. Compound **64** was the predominant metabolite. Quantification of the products was not pursued.

Experimental Section

Collection and Extraction. *Eunicea laciniata* (specimen number CI 86-228) was collected by hand using SCUBA at 20 to 25 m depth in July 1986, offshore of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonian was next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 13 g of crude organic materials (from 0.6 kg, dry weight of the gorgonian). Compounds **64** - **69** were eluted from a "vacuum flash" silica gel column with 0-20% EtOAc in isooctane and further purified with the same solvents by HPLC using preparative silica gel columns.

Eunicea cf. calyculata (specimen number F 87-35) was collected by hand using SCUBA at 15 to 20 m depth in August, 1987, offshore of the Florida Keys. After using the same process as for *E. laciniata*, 25 g of crude extract was obtained from 1.5 kg of the gorgonian (dry weight). Compounds **64**, **65**, **66**, and **69** were isolated by the same chromatographic method.

Compound 64. The triene **64** was isolated as an oil after final purification by HPLC (10% EtOAc in isooctane). The extract yielded 1.5 g (12% of the extract) of **64**, which displayed HRMS and NMR spectra in full accord with published data. Also the rotation, $[\alpha]_D$+32° (c 1.9, $CHCl_3$) was very similar to the reported value (+31°, c 0.9, $CHCl_3$).

Compound 65. The alcohol **65** was isolated as a white solid by HPLC (5% of EtOAc in isooctane). The extract yielded 160 mg (1.2% of the crude extract) of **65**, mp 52-53°. Compound **65** exhibited $[\alpha]_D$ -28° (c 0.7, $CHCl_3$) and displayed the following spectral features: HRMS; M+, m/z obsd 288.2465, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (10), 273 (14), 270 (80), 229 (56), 189 (38), 187 (59), 177 (53), 173 (49), 164 (37), 161 (40), 159 (100), 152 (84); IR ($CHCl_3$) 3620,

2980, 2920, 1690, 1450, 1390, 1160, 1120 cm^{-1}; UV (MeOH) no λmax.

Reduction of 64. To a stirred solution of **64** (60 mg, 0.21 mmol) in 3 ml of dry THF under N_2, 350 µl of 1 mol DIBAL solution in CH_2Cl_2 was added. After stirring for 1 hr, 5 ml of 1:1 (v/v) mixture of THF and water, and 150 µl of 1 N NaOH was added. After stirring for 5 min, 20 ml of ethyl ether and 20 ml of water were added. The ether layer was washed with saturated $NaHCO_3$ (15 ml), water (2 x 15 ml), and dried under vacuum. Purification by HPLC (10% EtOAc in isooctane) gave **70** (33 mg, 0.11 mmol, 55% yield) and **71** (20 mg, 0.06 mmol, 31%). Compound **70** was isolated as an oil and exhibited $[\alpha]_D$ -80° (c 0.7, $CHCl_3$). Compound **70** displayed the following spectral features: HRMS; M^+-H_2O, m/z obsd. 270.2350, $C_{20}H_{32}O$ required 270.2349; low- resolution MS: m/z (relative intensity) 270 (18), 255 (10), 187 (23), 173 (17), 162 (20), 159 (43), 147 (36), 134 (100), 119 (90), 105 (59); IR (film) 3400, 2920, 1670, 1620, 1450, 1385, 1370, 1040 cm^{-1}; UV (MeOH) no λmax; ^1H NMR ($CDCl_3$) δ 5.24 (1 H, dd, 11.0, 5.0, H-3), 4.89 (1 H, brd, 10.0, H-7), 4.65 (1 H, brd, 5.7, H-13), 1.82 (3 H, brs, H-20), 1.69 (3 H, brs, H-19), 1.63 (3 H, brs, H-17), 1.47 (3 H, brs, H-16), 1.10 (3 H, brs, H-15); ^{13}C NMR ($CDCl_3$) δ 147.7 (s, C-12), 134.8 (s, C-4), 132.1 (s, C-8), 129.9 (s, C-18), 129.5 (d, C-7), 125.9 (d, C-3), 71.7 (d, C-13), 49.8 (t, C-14), 47.8 (s, C-1), 42.9 (d, C-11), 40.0 (t, C-2), 39.8 (t, C-5 or -9), 38.1 (t, C-5 or -9), 27.9 (t, C-10), 24.3 (t, C-6), 23.4 (q, C-15), 22.1 (q, C-19 or -20), 21.7 (q, C-19 or -20), 16.1 (q, C-16), 15.4 (q, C-17). Compound **71** was isolated as a white solid, mp 124-125°. Compound **71** showed $[\alpha]_D$ -181° (c 0.7, $CHCl_3$) and displayed the following spectral features: HRMS: $M^{+\cdot}$ m/z obsd. 288.2480, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (6), 270 (100), 255 (36), 227 (21), 187 (68), 173 (42), 159 (83); IR ($CHCl_3$) 3600, 2980, 2920, 1670, 1445, 1390, 1375, 1050 cm^{-1}; UV (MeOH) no λmax; ^1H NMR ($CDCl_3$) δ 5.19 (1 H, dd, 11.4, 4.6, H-3), 4.87 (1 H, brd, 10.3, H-7), 4.65 (1 H, dd, 5.7, 5.1, H-13), 1.80 (3 H, brs, H-20), 1.67 (3 H, brs,

H-19), 1.63 (3 H, brs, H-17), 1.43 (3 H, brs, H-16), 1.18 (3 H, brs, H-15); ^{13}C NMR ($CDCl_3$) δ 145.4 (s, C-12), 135.0 (s, C-4), 132.2 (s, C-8), 129.5 (d, C-7), 129.2 (s, C-18), 125.6 (d, C-3), 71.8 (d, C-13), 51.5 (t, C-14), 46.4 (s, C-1), 42.0 (d, C-11), 40.7 (t, C-2), 39.9 (t, C-5 or -9), 38.2 (t, C-5 or -9), 29.0 (t, C-10), 24.3 (t, C-6), 23.9 (q, C-15), 21.5 (q, C-19 or -20), 20.9 (q, C-19 or -20), 16.0 (q, C-16), 15.4 (q, C-17).

Reaction of 70 with NaOH. To a stirred solution of **70** (13 mg, 0.05 mmol) in 1 ml of acetone, 0.5 ml of 1 N NaOH solution was added. The color of the solution turned yellow instantly. After 30 min, the solution was extracted with 20 ml of ethyl ether and dried under vacuum. Purification by HPLC (5% EtOAc in isooctane) gave **72** (2.9 mg, 0.01 mmol, 24% yield), **65** (3.6 mg, 0.01 mmol, 28%) and the reactant, **70** (4.9 mg, 0.02 mmol, 38%). ^1H NMR ($CDCl_3$) data of **72** are δ 5.60 (1 H, brs), 5.27 (1 H, dd, 11.6, 5.1), 4.88 (1 H, brd, 10.0), 4.86 (1 H, brs), 4.83 (1 H, brs), 1.88 (3 H, brs), 1.60 (3 H, brs), 1.47 (3 H, brs), 1.20 (3 H, brs). Further characterization was not pursued. Spectral data of **65** were identical with those of the natural compound.

Compound 66. The triene **66** was isolated as an oil by HPLC (100% isooctane). The extract yielded 100 mg (0.8% of the crude extract) of **66**. Compound **66** showed $[\alpha]_D$ -149° (c 0.8, $CHCl_3$) and displayed the following spectral features: HRMS; M^+, m/z obsd. 272.2500, $C_{20}H_{32}$ required 272.2504; low-resolution MS: m/z (relative intensity) 272 (11), 229 (20), 216 (22), 189 (26), 161 (36), 136 (84), 121 (100), 107 (37), 93 (31); IR (film) 2940, 2860, 1670, 1450, 1390, 1370, 920 cm^{-1}; UV (MeOH) no λmax.

Photooxidation of 66. Photooxidation of 66 was performed following the methods of Foote *et al.*.[101] To a stirred solution of **66** (19 mg, 0.07 mmol) in 10 ml of dried CH_2Cl_2, 2.5 mg of methylene blue was added. To keep the temperature low, the flask was immersed in ice water. A 100W tungsten lamp was used as the light source. After irradiating and air-bubbling (80 ml per min) for 15 min, all of the reactant

disappeared by TLC. The reaction mixture was filtered through a small silica column under vacuum and the CH_2Cl_2 was removed under vacuum. The residue was dissolved in 5 ml of ethanol, and 10 mg of $NaBH_4$ was added. After stirring the mixture for 30 min, the solution was extracted with ethyl ether. After evaporating the ether, and drying under vacuum, the residue was separated by silica HPLC (5% EtOAc in isooctane) which gave a single compound (4.5 mg) as the only product. NMR data of the product were identical with compound **65** (23% yield).

Compound 67. The ketone **67** was isolated as an oil by HPLC (10% EtOAc in isooctane). The extract yielded 35 mg (0.3% of the crude extract). Compound **67** exhibited $[\alpha]_D$ -27° (c 0.7, $CHCl_3$) and displayed the following spectral features: HRMS; M^+, m/z obsd. 302.2246, $C_{20}H_{30}O_2$ required 302.2246; low-resolution MS: m/z (relative intensity) 302 (42), 163 (11), 153 (15), 150 (100), 135 (40), 109 (29), 93 (27), 81 (33), 69 (55), 55 (37); IR (film) 2960, 1710, 1700, 1640, 1450, 1375, 1265, 1020 cm^{-1}; UV (MeOH) 254 nm (ε 3300).

Epoxidation of 67. To a stirred solution of **67** (27 mg, 0.09 mmol) in 5 ml of CH_2Cl_2 buffered with anhydrous Na_2HPO_4, 1.3 equiv of mCPBA was added. After stirring for 2 hr at room temperature, the CH_2Cl_2 layer was washed with 10% Na_2SO_3 (2 x 15 ml), 5% $NaHCO_3$ (2 x 15 ml), and finally with brine. After evaporating CH_2Cl_2 and drying under vacuum, HPLC (25% EtOAc in isooctane) separation gave **73** as a major product (13 mg, 0.04 mmol, 46% yield). The epoxide **73** was a white solid; mp 137-138° and exhibited the following spectral features: HRMS; M^+, m/z obsd. 318.2199, $C_{20}H_{30}O_3$ required 318.2196; low-resolution MS: m/z (relative intensity) 318 (4), 300 (5), 285 (3), 203 (6), 175 (9), 161 (20), 150 (100), 136 (46), 121 (29); IR (NaCl) 2960, 1710, 1695, 1625, 1470, 1390, 1260, 1195, 1030 cm^{-1}; UV (MeOH) 255 nm (ε 4000).

Transannular reaction of 73. To a stirred solution of **73** (6 mg, 0.02 mmol) in 1.5 ml of dry THF at -76° under N_2, 150 µl of LDA (10% wt. suspension in hexane)

was added. After stirring for 1 hr, excess LDA was removed and THF was evaporated. Purification by HPLC (50% EtOAc in isooctane) gave **74** (3.5 mg, 0.01 mmol, 58% yield) as a major product. The alcohol **74** was an oil and showed the following spectral features: HRMS; M^+, m/z obsd. 318.2209, $C_{20}H_{30}O_3$ required 318.2196; low-resolution MS: m/z (relative intensity) 318 (3), 300 (100), 285 (23), 257 (12), 207 (22), 193 (21), 177 (39), 165 (62), 161 (12); IR (film) 3480, 2960, 2930, 1705, 1700, 1620, 1460, 1380, 1370, 1205, 755 cm^{-1}; UV (MeOH) 250 nm (ε6000).

Compound 68. The ketone **68** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract yielded 35 mg (0.3% of the crude extract). Compound **68** exhibited [α] -44° (c 0.6, $CHCl_3$) and displayed the following spectral features: FABMS; $(M+H)^+$, m/z obsd. 305.2483, $C_{20}H_{32}O_2$ required 305.2481; low-resolution MS: m/z (relative intensity) 305 (100), 303 (47), 287 (86), 259 (16), 154 (85), 136 (80), 123 (39), 109 (40); IR (film) 2960, 1735, 1700, 1460, 1390, 1170 cm^{-1}; UV (MeOH) no λmax.

Transannular reaction of 68. To a stirred solution of **68** (17 mg, 0.06 mmol) in a mixture of 3 ml of acetone and water (2:1, v/v), 100 μl of conc. HCl was added. After stirring for 2 hr at room temperature, the mixture was extracted with ethyl ether (30 ml). The ether layer was washed with satd. $NaHCO_3$ (20 ml), water (20 ml), and then dried under vacuum. Purification by HPLC (30% EtOAc in isooctane) gave **75** (9 mg, 0.03 mmol, 53% yield) and **76** (7 mg, 0.02 mmol, 39%). Compound **75** was isolated as an oil which showed $[\alpha]_D$ -29° (c 0.7, $CHCl_3$). The alcohol **75** displayed the following spectral features; HRMS: M^+, m/z obsd. 304.2402, $C_{20}H_{32}O_2$ required 304.2403; low-resolution MS: m/z (relative intensity) 304 (38), 289 (18), 243 (12), 205 (15), 192 (31), 177 (22), 165 (100), 159 (11); IR (film) 3550, 2950, 2930, 1735, 1635, 1460, 1385, 1270, 1195, 950, 900 cm^{-1}; UV (MeOH) no λmax. Compound **76** was isolated as an oil which exhibited $[\alpha]_D$-26° (c 0.6, $CHCl_3$). The diol **76** displayed the following spectral features; HRMS: $(M-H_2O)^+$ m/z obsd 304.2393, $C_{20}H_{34}O_3$ required

304.2403; low-resolution MS: m/z (relative intensity) 304 (23), 289 (7), 209 (11), 205 (14), 192 (20), 166 (100), 165 (54), 163 (12); IR (film) 3480, 2960, 2930, 1725, 1460, 1390, 1370, 1275, 1205, 1090, 895, 760 cm^{-1}; UV (MeOH) no λmax.

Compound 69. The acetate **69** was isolated as a white solid by HPLC (15% EtOAc in isooctane). Recrystallization from acetone gave 33 mg (0.3% of the crude extract) of **69**, mp 103-104°. Compound **69** showed $[\alpha]_D$ +170° (c 0.7, $CHCl_3$) and displayed the following spectral features: FABMS; $(M+H)^+$, m/z obsd 345.2446, $C_{22}H_{32}O_3$ required 345.2430; low-resolution MS: m/z (relative intensity) 345 (15), 307 (64), 289 (37), 154 (100), 136 (100), 120 (26), 107 (36); IR (film) 2940, 1730, 1705, 1640, 1440, 1370, 1240, 1040 cm^{-1}; UV (MeOH) 254 nm (ε5200).

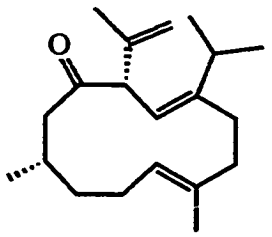
Chapter VII

Cubitanes and cembranes from *Eunicea calyculata*

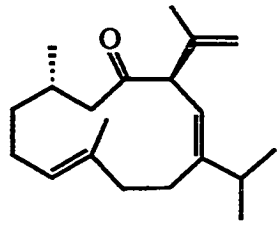
Gorgonians identified as *Eunicea calyculata* were collected at the Tobago Cays, eastern Caribbean Sea, in July 1986. Although they were morphologically very similar to *E. laciniata* (Chapter 6) collected at the same time, careful examination of the results of TLC analysis served to confidently distinguished them. Extraction of the dried gorgonians with dichloromethane (1 kg, dry weight) gave 6.7 g of crude organic extract. Proton NMR analysis of the crude extract revealed the presence of very large amounts of fats (> 90% of the extract). Silica vacuum flash chromatography, followed by Sephadex lipophilic LH-20 column chromatography of the nonpolar fractions gave 250 mg of fat-free materials. Four diterpenoids (**77**, **81**, **82**, and **83**, Figure 21) were isolated by HPLC (5% EtOAc in isooctane) of the fat-free materials. Three more diterpenoids (**78** - **80**, Figure 21) were isolated by preparative centrifugal chromatography (Chromatron, 4-6% EtOAc in isooctane). Although compounds **77** - **83** were not isolated in large amounts (less than 0.1% of the crude extract), it is believed that the actual concentrations of these compounds in the living organism are higher. Due to the highly volatile nature of the compounds, continuous loss made the structural elucidations very difficult.

Compounds **77** - **80** are diterpenoids of the unusual cubitane class. Cubitene, the first metabolite of this class was isolated from the East African termite, *Cubitermes umbratus*.[107] Since then, calyculones from *Eunicea calyculata* collected at Belize were the only known metabolites of the cubitane class.[25] The structurally related compounds **81** - **83** are diterpenoids of the well known cembrane class. The cembrane carbon skeleton has been proposed as a logical biosynthetic precursor to the irregularly isoprenoid cubitane ring system.[107] The structural relationship between the carbon skeletons is discussed later in detail.

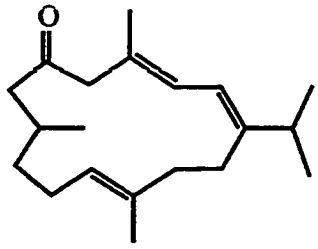
Figure 21. Cubitanes and cembranes from *E. calyculata*.



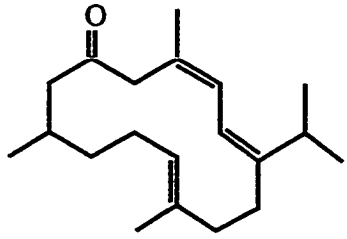
77, 78 (epimer)



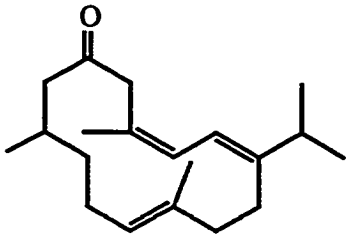
79, 80 (epimer)



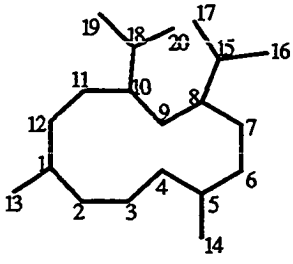
81



82



83



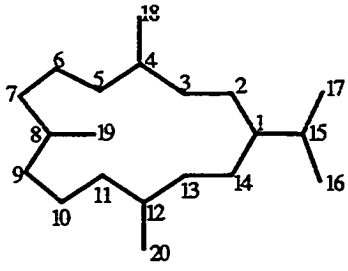
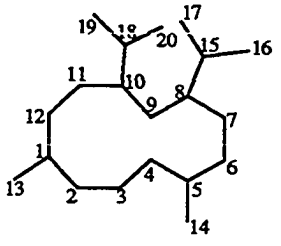


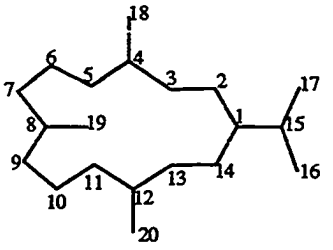
Table 22. ^{13}C NMR assignments for compounds **77 - 83.**

#	77	78	79	80	81	82	83
1	27.5 d	28.4 d	27.8 d	28.7 d	148.6 s	145.3 s	147.9 s
2	34.8 t	33.5 t	32.8 t	35.9 t	118.7 d	119.4 d	119.1 d
3	23.9 t	24.9 t	23.5 t	25.7 t	126.8 d	123.6 d^a	127.3 d^a
4	127.6 d	129.6 d	127.9 d	127.6 d	128.5 s	128.8 s	128.3 s
5	133.2 s	132.8 s	132.5 s	134.6 s	54.4 t	50.5 t^b	48.0 t^b
6	39.2 t	39.3 t	38.0 t	37.0 t	211.2 s	211.0 s	212.4 s
7	28.8 t	28.4 t	27.4 t	28.3 t	48.8 t	46.1 t^b	46.1 t^b
8	145.5 s	146.2 s	147.3 s	147.3 s	28.4 d	27.6 d	27.2 d
9	122.2 d	122.0 d	120.4 d	119.9 d	37.3 t	36.9 t^c	37.3 t^c
10	57.7 d	59.3 d	59.3 d	56.5 d	24.7 t	24.6 t	24.4 t
11	211.2 s	209.5 s	210.2 s	209.9 s	125.9 d	126.2 d^a	125.5 d^a
12	49.1 t	47.1 t	47.6 t	48.4 t	136.0 s	134.2 s	132.8 s
13	20.8 q	21.1 q^d	20.7 q^d	21.1 q^d	37.9 t	35.8 t^c	38.5 t^c
14	15.3 q	15.2 q	16.8 q	17.3 q	29.8 t	27.2 t	27.8 t
15	29.4 d	29.5 d	33.2 d	33.0 d	33.5 d	29.4 d	32.7 d
16	21.1 q	21.4 q^d	21.6 q^d	21.7 q^d	21.8 q	21.2 q^d	21.1 q^d
17	21.6 q	21.9 q^d	23.4 q^d	23.4 q^d	22.9 q	21.6 q^d	23.6 q^d
18	143.7 s	143.2 s	143.9 s	144.1 s	16.4 q	24.1 q	23.5 q^d
19	21.1 q	21.9 q^d	21.2 q^d	22.0 q^d	20.0 q	19.4 q	20.4 q
20	112.8 t	112.9 t	112.3 t	112.2 t	18.1 q	15.4 q	16.1 q

Carbon NMR spectra were recorded at 50 MHz in $CDCl_3$ solution. Multiplicities were determined by DEPT experiments. Assignments for **77** and **81** were made from XHCORR and COLOC experiments. Assignments of others were made by comparison with compounds **77** and **81**. $^{a-d}$ Signals within a column may be reversed.

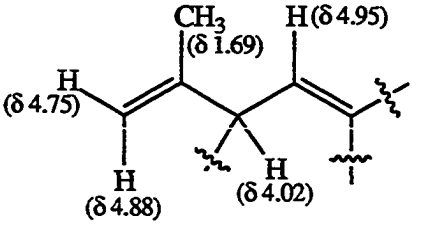


77 - 80



81 - 83

Compound **77** was isolated as an oil (5% EtOAc in isooctane) which analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. The carbonyl signal at δ 211.2 (s) in the ^{13}C NMR spectrum (Table 22) and the corresponding absorption band at 1715 cm^{-1} in the IR spectrum established the presence of a ketone which accounted for the oxygen atom in the molecular formula. Six low field carbon bands at δ 145.5 (s), 143.7 (s), 133.2 (s), 127.6 (d), 122.2 (d), and 112.8 (t) in the ^{13}C NMR spectrum indicated **77** to possess three double bonds, thus, one ring. The lack of absorption in the UV spectrum showed that the chromophores (ketone and double bonds) in **77** were not conjugated to each other. The ^1H NMR spectrum (Table 23) and a ^1H NMR COSY experiment identified several proton spin systems. Two methyl signals at δ 1.08 (d, *J*=7.0) and 0.95 (d, 6.9) in the ^1H NMR spectrum were coupled to an isolated proton resonance at δ 2.76 (heptet, 6.9), and together assigned to an isopropyl group. The presence of an isopropenyl group was established by couplings between proton signal of a methyl group at δ 1.69 (brs) and two olefinic protons at δ 4.88 (brt, 1.3, 1.3) and 4.75 (brs), in conjunction with bands at δ 112.8 (t) and 145.5 (s) or 143.7 (s) in the ^{13}C NMR spectrum. The olefinic proton at δ 4.88 and another olefinic proton at δ 4.95 (d, 9.6) were coupled to a highly deshielded proton at δ 4.02 (brd, 9.6) that was apparently bounded by a quaternary center since it was not further coupled.

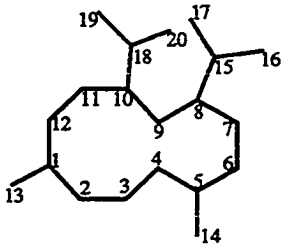


A proton peak at δ 2.48 (dd, 12.7, 6.5) in the ^1H NMR spectrum was coupled to another signal at δ 1.99 (dd, 12.7, 7.7). A direct carbon-proton NMR correlation

Table 23. ^{1}H NMR assignments for compounds **77** - **80**.

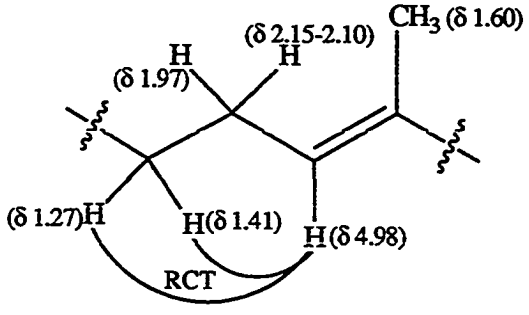
#	77	78	79	80
1	2.10 - 2.04 (1H)[a]	2.13 - 2.01 (1H)[a]	2.24 (1H)[a]	2.09 - 2.01 (1H)[a]
2	1.41 (1H,m)	1.48 (1H,m)	1.54 (1H,dddd,14.1, 10.3,3.8,3.8)	1.45 (1H,m)
	1.27 (1H,dddd,13.8, 7.0,7.0,4.5)	1.35 (1H,m)	1.28 (1H,dddd,14.0, 7.0,6.5,3.2)	1.08 (1H,m)
3	2.15 - 2.10 (1H)[a] 1.97 (1H)[a]	2.13 - 2.01 (2H)[a]	2.20 - 2.11 (1H)[a] 1.98 (1H,m)	2.05 - 1.93 (2H)[a]
4	4.98 (1H,dd,9.5,4.8)	5.03 (1H,dd,7.6,7.6)	4.87 (1H,dd,10.2,4.3)	5.02 (1H,brdd,7.3,7.3)
6	2.22 - 2.15 (2H)[a]	2.27 (1H,m)[b] 2.25 - 2.18 (1H)[a,b]	2.20 - 2.11 (1H)[a,b] 2.06 (1H,m)[b]	2.14 - 1.93 (1H,m)[b] 2.05 - 1.93 (1H)[a,b]
7	2.29 (1H,m)	2.25 - 2.18 (1H)[a,b]	2.32 (1H,ddd,12.3, 12.3,2.1)[b]	2.36 (1H,m)
	2.15 - 2.08 (1H)[a]	2.16 - 2.13 (1H)[a,b]	1.93 (1H,ddd,13.4,6.6, 2.1)[b]	2.05 - 1.93 (1H)[a,b]
9	4.95 (1H,d,9.6)	4.92 (1H,d,10.7)	5.32 (1H,d,10.1)	5.50 (1H,d,10.5)
10	4.02 (1H,d,9.6)	3.99 (1H,d,10.7)	3.82 (1H,d,10.1)	4.05 (1H,d,10.5)
12	2.48 (1H,dd,12.7,6.5) 1.99 (1H,dd,12.7,7.7)	2.98 (1H,dd,15.9,7.7) 2.13 - 2.01 (1H)[a]	2.46 (1H,dd,14.2,7.2) 2.04 (1H,dd,14.3,6.6)	2.43 (1H,dd,14.5,9.3) 2.24 (1H,dd,14.5,4.3)
13	0.95 (3H,d,6.6)	0.97 (3H,d,6.8)	0.91 (3H,d,6.9)	0.98 (3H,d,6.8)
14	1.60 (3H,brs)	1.57 (3H,brs)	1.73 (3H,brs)	1.62 (3H,brs)
15	2.76 (1H,hep,6.9)	2.83 (1H,hep,7.0)	2.27 (1H)[a]	2.31 (1H,hep,6.8)
16	1.08 (3H,d,7.0)	1.12 (3H,d,7.0)[c]	1.07 (3H,d,6.9)[c]	1.13 (3H,d,6.9)[c]
17	0.95 (3H,d,6.9)	1.00 (3H,d,6.9)[c]	1.03 (3H,d,6.8)[c]	1.03 (3H,d,6.8)[c]
19	1.69 (3H,brs)	1.72 (3H,brs)	1.69 (3H,brs)	1.67 (3H,brs)
20	4.88 (1H,brdd,1.5,1.5) 4.75 (1H,brs)	4.91 (1H,brs) 4.77 (1H,brs)	4.81 (1H,brdd,1.5,1.5) 4.73 (1H,brd,0.6)	4.82 (1H,brdd,1.5,1.5) 4.74 (1H,brs)

Proton NMR data were obtained in $CDCl_3$ solution. Assignments for **77** were made by COSY, RCT and XHCORR experiments. Assignments for others were made by COSY experiments. [a] Coupling constants were not measured. [b,c] Signals within a column may be reversed.



(XHCORR) experiment indicated that they were attached to a common methylene carbon at δ 49.1 in the ^{13}C NMR spectrum. COSY NMR data showed that the signals of the methylene protons and a methyl at δ 0.95 (d, 6.6) were both coupled to a common proton resonance in the region of δ 2.10-2.04. It seemed likely that the methylene and methyl groups were connected to a common methine carbon, although the assignment was not clear due to the partial overlapping of the proton signals in the region of the methine proton (δ 2.10-2.04). This problem was solved by a proton-proton relay coherence transfer (RCT) experiment, in which the correlations between protons of the methylene and methyl were found.

The combination of ^{1}H NMR COSY and RCT experiments helped to describe another spin system. COSY NMR data showed that an olefinic proton signal at δ 4.98 (brdd, 9.5, 4.8) was coupled to a vinyl methyl at δ 1.60 (brs) and was assigned as a tri-substituted double bond. The olefinic proton was also coupled to two proton resonances in the region of δ 2.15-2.10 and 1.97 both of which were hopelessly obscured by other proton signals. Two mutually coupled proton resonances at δ 1.41 (m) and 1.27 (dddd, 13.8, 7.0, 7.0, 4.5) were also coupled to protons at δ 2.15-2.10 and 1.97. A ^{1}H RCT experiment showed the correlation between the olefinic proton (δ 4.98) and two high field protons (δ 1.41 and 1.27) and established proton connectivities which might not have been recognized otherwise.

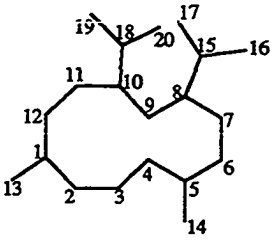


Finally, a spin system of four proton resonances at δ 2.29 (1 H), 2.22-2.15 (2 H) and 2.15-2.08 (1 H) in the ^{1}H NMR spectrum was described on the basis of a COSY

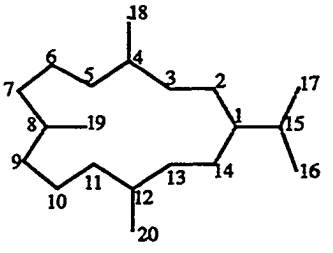
Table 24. Results of COLOC experiments with compounds **77** and **81**.[a]

carbon	proton	
	77[b]	**81**[b]
1	12 (2.48), 13	—
2	12 (2.00)	14 (2.55)
3	—	5 (3.20), 5 (2.88), 18
4	14	5 (3.20), 5 (2.88), 18
5	14	—
6	14	5 (3.20), 5 (2.88), 7 (2.65), 7 (1.66)
7	9	—
8	10, 16, 17	—
9	10	—
10	20 (4.88), 20 (4.75), 19	—
11	9, 10, 12 (2.48), 12 (2.00)	—
12	13	20
13	12 (2.48), 12 (2.00)	20
14	4	2
15	9, 16	2
16	17	—
17	16	16
18	10, 19, 20 (4.75)	3, 5 (3.20)
19	20 (4.88), 20 (4.75)	—
20	—	—

[a] Experiments were performed at 50 MHz in $CDCl_3$ solution. Parameters were optimized for couplings of 6 Hz. [b] The numbers in parentheses are the ^1H NMR chemical shifts of the protons that correlate.

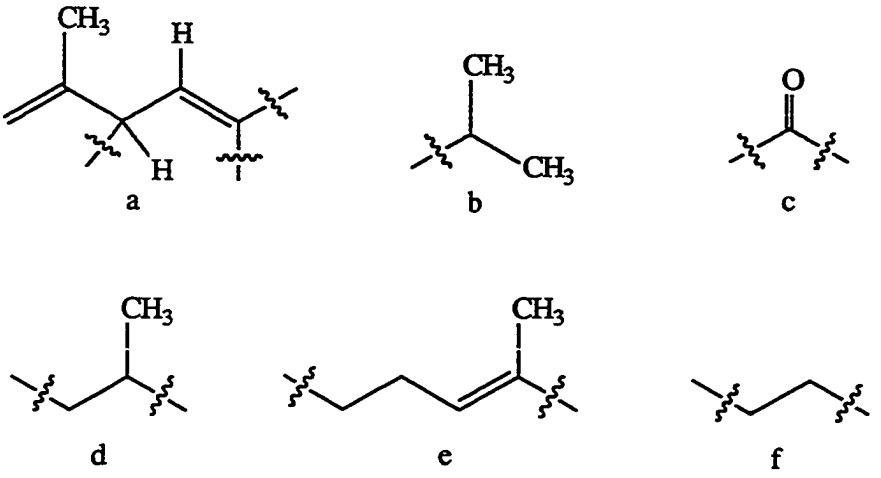


77



81

NMR experiment. An XHCORR experiment showed that these protons were attached to two methylene carbons at δ 39.2 (t) and 28.8 (t) in the ^{13}C NMR spectrum. Thus, all of the partial structures of compound **77** were confidently identified as a - f.



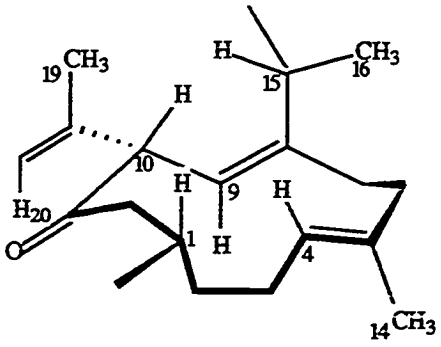
The combination of the partial structures were made by a long range carbon-proton correlation (COLOC) experiment (Table 24). The carbonyl carbon (c) was coupled to the protons at δ 4.95, 4.02, 2.48, and 1.99 (C-9, -10, -12, and -12, respectively). Therefore, the connections of c to the partial structures a and d were determined. The isopropyl carbon at δ 29.4 (d) of the partial structure b was found to be coupled to the C-9 proton. At the same time, the C-8 olefinic carbon at δ 145.5 (s) was coupled to the methyl protons at δ 1.08 and 0.95. Thus, the connection between a and b was defined. The methylene carbons at δ 39.2 and 28.8 of f were both coupled to the protons at δ 4.95 (C-9) and 1.60 (C-14), respectively. These data determined the connection of f to the partial structures a and e. Finally, the connection between d and e was found by a long range coupling between the C-2 carbon at δ 34.8 and the C-12 proton at δ 1.99. This assignment was supported by high field shifts of the C-2 proton resonances (δ 1.41 and 1.27) in the ^{1}H NMR spectrum. Thus, the structure of compound **77** was unambiguously defined as a diterpenoid of the cubitane class.

Compound **77** possesses two asymmetric double bonds (Δ^4 and Δ^8) and two asymmetric carbon centers (C-1 and C-10). The high field chemical shift of the C-14 methyl carbon (δ 15.3) in the ^{13}C NMR spectrum indicated the ***E*** configuration for the Δ^4 double bond. The stereochemistry of other asymmetric centers was determined by ^1H NMR NOEDS experiments (Figure 22). Irradiation of the C-15 proton (δ 2.76) enhanced the C-10 proton by 13.4%. Also, the irradiation of the C-10 proton enhanced the protons at C-1, C-15 and C-20 by 2.5, 12.5 and 3.8%, respectively. Finally, the C-9 proton was enhanced by irradiation of the C-14 methyl protons (4.9%). Thus, the stereochemistry of all the asymmetric centers of compound **77** was confidently defined. The overall relative configurations are 4(***E***), 8(***Z***), 1S*, and 10S*.

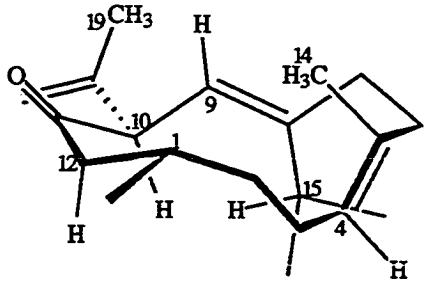
An apparently related compound, **78**, was isolated as an oil which analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. Spectral data for **78** were very similar to **77**. Moreover, COSY NMR data showed the identical coupling patterns as in **77**. The only significant difference was the downfield shift of one of the C-12 protons by 0.50 ppm in the ^1H NMR spectrum of **78** (Table 23). Therefore, compound **78** must be an isomer of **77**. A ^1H NMR NOEDS experiment helped to assign the stereochemistry of the asymmetric centers (Figure 22). Each of the protons at C-10 and C-15 were enhanced by irradiation of the other proton (8.2 % enhancement on C-10 and 8.6 % on C-15), while the C-9 proton was not enhanced. The C-10 proton was also enhanced by the irradiation of the C-12 proton (δ 2.99, 3.3 % enhancement). Irradiation of the C-14 methyl proton enhanced the C-9 proton by 1.1 %. However, the C-4 proton was not enhanced by this irradiation. Thus, **78** was assigned the same 4(***E***) and 8(***Z***) configurations as **77**. Since both compounds have only two asymmetric carbon centers (C-1 and -10), **78** must be an epimer of **77**. The overall relative configurations are 4(***E***), 8(***Z***), 1R* and 10S*.

Two other related metabolites, **79** and **80**, were isolated as oils. Spectral data of

Figure 22. Stereochemistry of compounds **77** and **78**.



77



78

Results of ^{1}H NMR NOEDS experiments with **77** and **78**.

	proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
77	10	1, 15, 20 (4.75)[a]	2.5, 12.5, 3.8
	14	9	4.9
	15	10, 16	13.4, 2.2
	16	15	5.6
78	12α(2.98)[a]	10	3.3
	10	15	8.6
	14	9	1.1
	15	10	8.2
	19	9, 10	3.0, 2.3

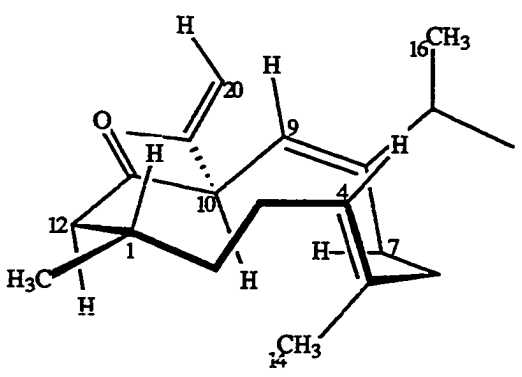
[a] Chemical shifts of the corresponding protons

these compounds were highly compatible with **77** and **78**. In addition, ^{1}H NMR COSY experiments with **79** and **80** showed the same coupling patterns as **77** and **78**. Therefore, these compounds must be also isomers of **77**. ^{1}H NMR NOEDS experiments again helped to determine the stereochemistry of the asymmetric centers (Figure 23). In compound **79**, irradiation of the C-9 proton enhanced the C-1, -16, and -20 protons, while the C-10 proton was not enhanced. The C-9 proton was enhanced by the irradiation of the C-16 methyl protons. Irradiation of the C-10 proton enhanced the C-7α, -12α, and -20 protons. The presence of an nOe between the C-9 and -16, also between the C-7α and -10 confidently assigned the ***E*** configuration for the Δ^8 double bond. Irradiation of the C-14 methyl protons enhanced the C-10 proton, while the C-4 olefinic proton was not enhanced, indicating the ***E*** configuration for the Δ^4 double bond. Another datum supporting this assignment was the high field shift of the C-14 carbon resonance (δ 16.8). The stereochemistries of two asymmetric carbon centers (C-1 and -10) were also determined by the ^{1}H NMR NOEDS experiment, in which enhancements between the C-1 and -9 and also between the C-10 and -12α were found. Thus, the stereochemistry of **79** was confidently assigned to possess the relative configurations of 4(***E***), 8(***E***), 1**S***, and 10**R***.

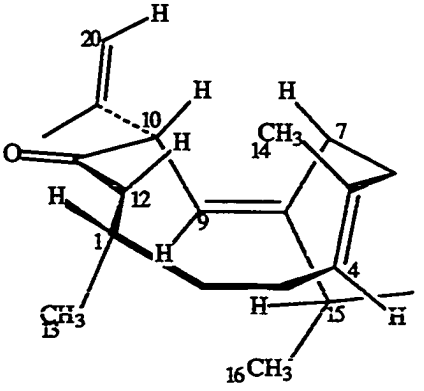
^{1}H NMR NOEDS again helped to determine the stereochemistry of **80**. NOe involving the C-9 and -10 protons indicated the ***E*** configuration for the Δ^8 double bond. The lack of nOe between the C-4 and -14 protons, together with the high field shift of the C-14 methyl carbon (δ 17.3) indicated the ***E*** configuration for the Δ^4 double bond. Thus, compound **80** was identified as an epimer of **79**. The overall relative configurations were thus assigned as 4(***E***), 8(***E***), 1**S***, and 10**S***.

In addition to the cubitanes, the crude extract contained metabolites of a different carbon skeleton. The major metabolite, **81**, was isolated as an oil by HPLC (5% EtOAc in isooctane), and analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR

Figure 23. Stereochemistry of compounds **79** and **80**.



79



80

Results of ^{1}H NMR NOEDS experiments with **79** and **80**.[a]

	proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
79	9	1, 16, 20(4.73)	b, 2.3, 1.1
	10	7α(2.32), 12α(2.46), 20(4.73)	b, 3.1, 2.3
	12α(2.46)	10	5.4
	14	10	2.3
	16	9	4.4
80	9	16, 20(4.74)	4.8, 2.3
	10	12β(2.43), 20(4.74)	b, 2.9
	16	9, 15	4.6, b

[a] Numbers in parentheses are chemical shifts of the corresponding protons. [b] Due to the overlapping of the proton signals, enhancements were not measured.

spectrometry. Comparison of the ^{1}H and ^{13}C NMR spectra showed similarities between **81** and compound **77**. However, there were several significant differences in the spectral data. Although the ^{13}C NMR spectrum of **81** showed the presence of three double bonds as **77**, the signal from the terminal methylene carbon {δ 112.8 (t)} in the ^{13}C NMR spectrum of **77** was replaced by a signal of different multiplicity {one of three peaks at δ 126.8 (d), 125.9 (d) and 118.7 (d)} in compound **81** (Table 22). Also, the α-carbonyl carbon at δ 57.7 (d) of **77** was replaced by a new signal at δ 54.4 (t) in the ^{13}C NMR spectrum of **81**. Corresponding differences were found in the ^{1}H NMR spectrum of **81** (Table 25). Proton signals from the terminal methylene (δ 4.88 and 4.75) and α-carbonyl (δ 4.02) carbons of **77** were replaced by three new signals at δ 6.23 (brd, 11.2) or 6.07 (d, 11.2), 3.20 (d, 12.7) and 2.88 (brd, 12.7) in compound **81**. In addition, one of the olefinic protons at δ 4.95 (C-9) in **77** was downfield shifted to δ 6.23 (brd, 11.2) or 6.07 (d, 11.2) in **81**. Finally, in contrast to the lack of absorption maximum in **77**, the UV spectrum of **81** showed maxima at 254 and 248 nm.

Several proton spin systems were determined by combination of ^{1}H NMR COSY and RCT experiments. Two low-field olefinic protons at δ 6.23 and 6.07 in the ^{1}H NMR spectrum of **81** were coupled (J=11.2 Hz) to one another. The olefinic proton signal at δ 6.23 was also coupled by signals of two mutually coupled protons at δ 3.20 (d, 12.7) and 2.88 (brd, 12.7), and a methyl at δ 1.71 (brs). The relation among these protons was further clarified by the result of the ^{1}H RCT experiment, in which correlations between the olefinic proton at δ 6.07 and protons at δ 3.20, 2.88, and 1.71 were revealed.

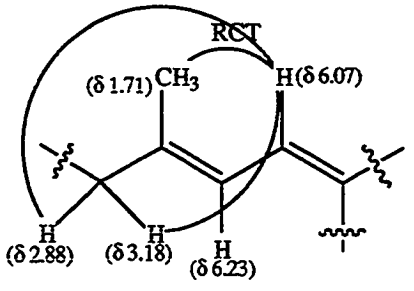
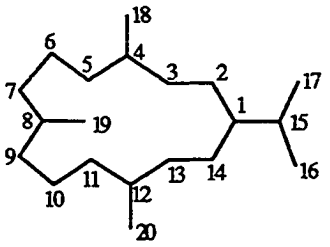


Table 25. ^{1}H NMR assignments for compounds **81** - **83**.

#	81	82	83
2	6.07 (1H,d,11.2)	5.91 (1H,brd,11.5)	6.14 (1H,d,10.5)
3	6.23 (1H,brd,11.2)	6.29 (1H,brd,11.5)	6.08 (1H,brd,10.9)
5	3.20 (1H,d,12.7) 2.88 (1H,brd,12.7)	3.22 (1H,brd,13.7) 3.17 (1H,brd,13.9)	3.72 (1H,d,13.7) 2.64 (1H,brd,13.7)
7	2.65 (1H,dd,12.6,4.1) 1.66 (1H)[a]	2.32 - 1.98 (2H)[a]	2.57 (1H,dd,12.6,4.5) 1.62 (1H)[a]
8	2.15 - 1.95 (1H)[a]	2.32 - 1.98 (1H)[a]	2.22 - 1.89 (1H)[a]
9	1.49 (1H,m) 1.21 (1H,dddd,13.7, 8.5,6.8,3.5)	1.27 - 1.20 (2H)[a]	1.40 (1H,dddd,13.7, 12.0,3.7,3.7) 1.15 (1H,m)
10	2.15 - 1.95 (2H)[a]	2.32 - 1.98 (2H)[a]	2.22 - 1.89 (2H)[a]
11	5.10 (1H,brdd,7.3,6.8)	5.07 (1H,brddd,7.7, 7.6,1.1)	4.73 (1H,ddd,10.4,2.0, 1.1)
13	2.23 (1H)[a] 2.15 - 1.95 (1H)[a]	2.32 - 1.98 (2H)[a]	2.22 - 1.89 (2H)[a]
14	2.55 (1H,m) 2.23 (1H)[a]	2.32 - 1.98 (2H)[a]	2.72 (1H,ddd,13.1,12.8, 3.2) 2.22 - 1.89 (1H)[a]
15	2.39 (1H,hep,6.9)	3.04 (1H,hep,6.9)	2.31 (1H,hep,6.9)
16	1.12 (3H,d,6.8)	1.06 (3H,d,7.1)[b]	1.09 (3H,d,6.8)[b]
17	1.07 (3H,d,6.8)	1.04 (3H,d,7.1)[b]	1.04 (3H,d,6.9)[b]
18	1.71 (3H,brs)	1.76 (3H,brs)	1.71 (3H,brs)
19	0.87 (3H,d,6.8)	0.84 (3H,d,6.3)	0.83 (3H,d,6.7)
20	1.58 (3H,brs)	1.60 (3H,brs)	1.62 (3H,brs)

Chemical shifts were measured in $CDCl_3$ solution. Assignments of **81** were made by combination of COSY NMR and RCT experiments. Assignments of others were made by COSY NMR experiments. [a] Coupling constants were not measured. [b] Signals within a column may be reversed.



Two mutually coupled protons at δ 2.65 (dd, 12.6, 4.1) and δ 1.66 (multiplicity unknown) were coupled to a proton resonance in the region of δ 2.15-1.95. The methyl proton signal at δ 0.87 (d, 6.8) was also coupled to a proton in the same region (δ 2.15-1.95). Since signals corresponding to four protons were overlapping in this region, however, the relation between the methyl and two protons (δ 2.65 and 1.66) was not clear. A ^{1}H RCT experiment showed correlations between them and indicated the presence of the same partial structure d as in **77**.

The olefinic proton at δ 5.10 (brdd, 7.3, 6.8) was coupled to two protons in the region of δ 2.15-1.95. Two high field protons at δ 1.49 (m) and 1.21 (dddd, 13.7, 8.5, 6.8, 3.5) were also coupled to the protons in the same region. ^{1}H RCT data again showed correlations between the olefinic proton and high field protons, and indicated **81** to possess the same partial structure e as **77**. Further consideration of the ^{1}H NMR spectrum, and a COSY NMR experiment, revealed the presence of several identical spin systems between **81** and compound **77**. With the help of XHCORR data (all of the 16 proton-bearing carbons and their protons were precisely matched), compound **81** was found to possess the identical partial structures b - f as in **77**.

The combination of the partial structures was made possible by a COLOC experiment (Table 24). The carbonyl carbon (c) at δ 211.2 in the ^{13}C NMR spectrum was coupled to the protons at δ 3.18, 2.88, 2.65, and 1.66 (C-5, -5, -7, and -7, respectively). Therefore, the connection of c to the diene, and to d was determined. The carbons at δ 33.5 and 29.8 (C-15 and -14) were coupled to the olefinic proton at δ 6.07 (C-2) and showed the connection of partial structures b and f to the diene. In addition, the connection of f to e was found by a long range coupling between the carbon at δ 37.9 (C-13) and the proton at δ 1.58 (C-20). Although the connection between d and e was not determined by the COLOC experiment, comparison of ^{1}H and ^{13}C NMR spectra of **81** with **77** revealed their connection to each other. Thus, the structure of **5** was

unambigously defined as a diterpenoid of the cembrane class.

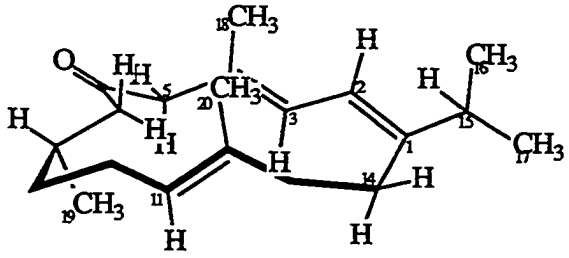
Compound **81** has three asymmetric double bonds (Δ^1, Δ^3, and Δ^{11}) and an asymmetric carbon center (C-8). The high field chemical shift of the C-20 carbon signal (δ 18.1) in the ^{13}C NMR spectrum indicated the ***E*** configuration for the Δ^{11} double bond. The stereochemistry of the other centers was determined by a ^1H NMR NOEDS experiment (Figure 24). Irradiation of the C-2 proton enhanced the proton signals at C-15, -16 and -17 by 3.7, 2.5 and 3.4%, respectively. At the same time, the signal for the C-18 methyl protons was enhanced by 4.9%. The signal of the C-2 proton was enhanced by irradiations of both the C-16 and -18 methyl protons (5.2 and 8.3%, respectively). On the other hand, irradiation of the C-3 proton enhanced the signals of the C-5α and C-14α protons by 2.5 and 9.8%, respectively. In addition, the signal of the C-3 proton was enhanced by the irradiation of the C-5α proton (5.1%). Therefore, the configurations of the diene double bonds were assigned as 1(***E***) and 3(***E***). Thus, the configurations of all the asymmetric double bonds of **81** were defined. The overall relative configurations are 1(***E***), 3(***E***), 11(***E***). Although the amount of the enhancement was not large (1.1%), irradiation of the C-19 methyl protons consistently enhanced the signal of the C-3 proton. Therefore, the C-19 methyl is assigned as axial to the plane of molecule. Since compound **81** has only one asymmetric carbon center at C-8, the stereochemistry of this center remains unknown.

An obiously related compound, **82**, was isolated as an oil by HPLC (5% EtOAc in isooctane) and analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. Spectral data for **82** were very similar to those from **81**. There were, however, several significant differences in the chemical shifts of protons at C-5β, -7, -9, and -14 (all of the key protons were confidently assigned by a ^1H NMR COSY experiment) in the ^1H NMR spectrum of **82** (Table 25). In particular, the C-15 methine proton was shifted 0.65 ppm to low field in relation to its chemical shift in compound **81**.

Therefore, **82** was assigned as a stereoisomer of **81**. ^{1}H NMR NOEDS experiment again helped to determine the stereochemistry of the asymmetric centers (Δ^1, Δ^3, Δ^{11} double bonds and a methine center at C-8, Figure 24). Irradiation of the C-3 proton enhanced the signal of the C-15 proton by 6.2%. In return, the C-3 proton was enhanced by irradiation of both the C-15 and C-18 protons by 21.8 and 8.4%, respectively. On the other hand, the signals of the C-2 and C-5 protons were enhanced by irradiation of each other (7.5% for the C-2, 4.3% for the C-5). Therefore, in contrast to the 1(*E*) and 3(*E*) configurations for **81**, compound **82** was determined to possess the 1(*Z*) and 3(*Z*) configurations. Irradiation of the C-20 methyl protons did not enhance the signal of the C-11 olefinic proton, suggesting the *E* configuration for the C-11 double bond. The chemical shifts of the protons around the Δ^{11} double bond of **82** were very similar to those of **81**, and supported the lack of nOe between the C-11 and C-20 protons. The orientation of the C-19 methyl group could not be determined by ^{1}H NMR NOEDS measurements.

Another related compound, **83**, was isolated as an oil by HPLC (5% EtOAc in isooctane), and analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. Spectral data for this compound were highly compatible with those for **81** and **82**. However, there were nonnegligible differences in the chemical shifts of protons at C-3, -11 and -14 in the ^{1}H NMR spectrum (all of the key protons were confidently assigned by a ^{1}H NMR COSY experiment, Table 25). In particular, the signal of one of the C-5 protons was shifted downfield to δ 3.72 in the ^{1}H NMR spectrum of **83**. Therefore, compound **83** was assigned as an additional isomer of **81**. Compound **83** possessed asymmetric centers at the same positions as the other cembranes. ^{1}H NMR NOEDS was again utilized to determine the stereochemistry of these centers (Figure 24). Irradiation of the C-18 methyl protons enhanced the signal of the C-3 proton by 5.2%. On the other hand, the C-2 proton was enhanced by irradiation of both the C-5 and C-16 protons by 13.2 and 2.5%, respectively. Therefore, the configurations of the diene

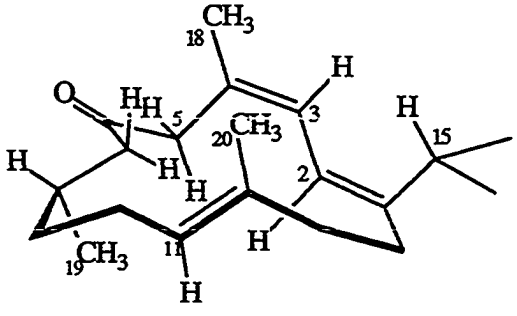
Figure 24. Stereochemistry of compounds **81** - **83**.



81

Results of a ^{1}H NMR NOEDS experiment with **81**.

proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
2	15, 16, 17, 18	3.7, 2.5, 3.4, 4.9
3	5α(3.20)[a], 14α(2.55)[a]	2.5, 9.8
5α	3	5.1
7β	18	5.7
16	2, 15	5.2, 3.7
18	2	8.3
19	3	1.1

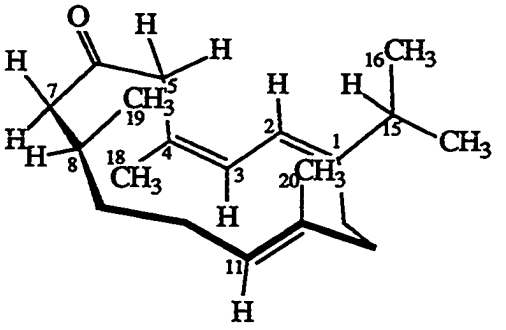


82

Figure 24. (continued)

Results of a ^{1}H NMR NOEDS experiment with **82**

proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
2	5[b]	4.3
3	15	6.2
5[b]	2	7.5
15	3	21.8
18	3	8.4



83

Results of a ^{1}H NMR NOEDS experiment with **83**

proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
5β(3.72)[a]	2	13.2
16	2	2.5
18	3, 5α(2.64)	5.2, 4.5
19	5β(3.72)[a]	4.0

[a] Chemical shift of the corresponding protons. [b] Both of the methylene protons were enhanced (irradiated).

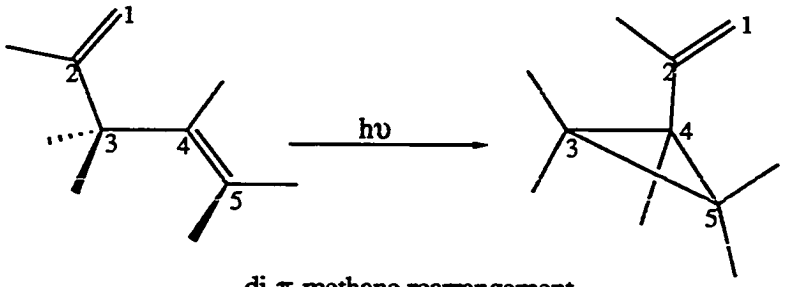
olefin were assigned as 1(***E***) and 3(***Z***). Irradiation of the C-11 olefinic proton did not enhance the signal of the C-20 methyl protons, suggesting the ***E*** configuration for the C-11 double bond. Irradiation of the C-19 methyl protons enhanced the C-5 proton by 4.0%, suggesting an axial orientation of the methyl to the plane of molecule.

Chemical investigations of *Eunicea calyculata* resulted in the isolation of diterpenoids of two different carbon skeletons; cubitanes (**77** - **80**) and cembranes (**81** - **83**). The origin of the irregular isoprenoid, cubitane has been unknown. Prestwich and coworkers suggested the co-occurring cembrenes as logical precursors of cubitene isolated from the termite *Cubitermes umbratus*, and proposed an oxidative chain-opening mechanism.[107] Comparison of the structures of compounds **77** - **83** revealed astounding similarities between the cubitanes and cembranes. The break-down of the bond between the C-2 and C-3 of the cembranes, and recyclization by joining C-2 and C-5 may form the cubitanes. Alternatively, bond breaking between C-5 and C-6, and recyclization by the C-3 and C-6 can also form the cubitanes.

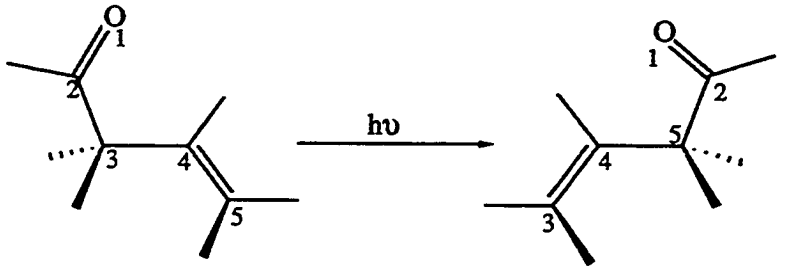
A possible mechanism, a photochemically induced 1,3-acyl migration was proposed for the conversion of the cembranes to the cubitanes. There are few known examples of photochemical rearrangements among marine natural products. Ireland and coworkers showed *in vivo* photochemical rearrangement of the polypropionate metabolites from the sacoglossan *Placobranchus ocellatus*.[108-110] Look and coworkers succeeded in the photochemical *in vitro* conversion of the erythrolides from the octocoral *Erythropodium caribaeorum*.[111] In both cases, the proposed mechanism was the photochemical di-π-methane rearrangement of a 1,3-diene (Figure 25).[110] The presence of the 1,3-diene in all of the cembranes (**81** - **83**) suggested the possibility of similar photochemical processes.

Careful examination of the structures of the cembranes revealed another important aspect; the ketone at C-6. In general, the C-6 carbon of the cembrane ring is usually inert,

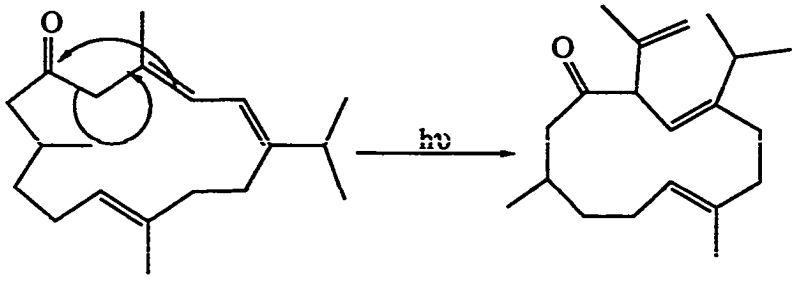
Figure 25. Di-π-methane rearrangement and 1,3-acyl migration.



di-π-methane rearrangement



1,3-acyl migration

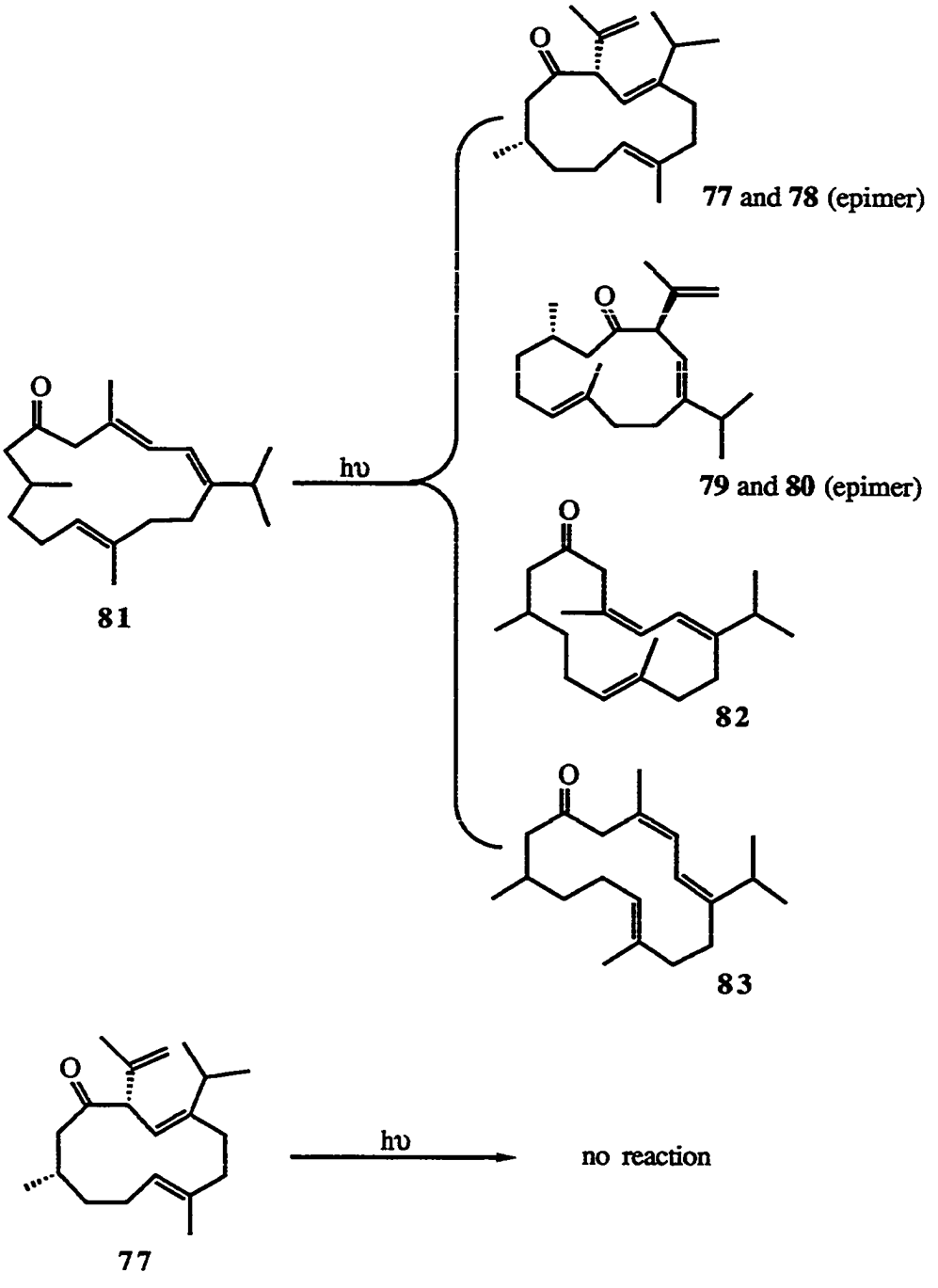


cembranes cubitanes

and rarely functionalized. It is well known that when a ketone is at the β-position of a double bond, photochemical rearrangement can readily occur.[112,113] Thus, a photochemically induced 1,3-acyl migration was proposed through combination of the susceptibility of the 1,3-diene and the β-position of the ketone (Figure 25).

To prove the proposed mechanism for the formation of the cubitanes, a small scale photochemical reaction was performed (Figure 26). Irradiation of compound **81** by Hg-emission UV light (3 hrs) produced several products. Purification by HPLC gave compounds **77**, **82** and **83** (8.8, 10.6 and 11.5% yields, respectively). Compounds **78** - **80** were also obtained as a mixture (6.2, 10.6 and 15.9% yields, respectively). This result clearly demonstrated that the irregular isoprenoid cubitanes are readily formed by a photochemically induced 1,3-acyl migration of the corresponding cembranes. In addition, this result showed that the geometric isomers of the cembranes might be also photochemically produced. The cubitane isomers might be produced either directly from a common cembrane precursor, or via rearranged cembranes. An alternative possibility is the conversion of the cubitanes to the cembranes. However, the cubitane **77** was inert under the same condition, and even after a prolonged irradiation (8 hrs), no sizable amounts of products were obtained. Although these results cannot rule out the possibility of an enzyme-mediated process, the shallow-water habitat of *Eunicea calyculata* and presence of the associating zooxanthellae may indicate that the cubitanes in the *E. calyculata* might be artifacts. On the contrary, considering the large numbers of the 1,3-cembradienes from the octocorals, isolation of the photochemically rearranged products only from *E. calyculata* suggested that these are produced *in vivo*. It is not known whether, as in the case of the sacoglossan *Placobranchus ocellatus*,[110] *E. calyculata* can convert metabolites by a photochemically induced *in vivo* process.

Figure 26. Photochemical reactions of compounds **77** and **81**.



Experimental Section

Collection, Extraction and Isolation. *Eunicea calyculata* (specimen number CI 86-227) was collected by hand using SCUBA at 20 to 25 m depth in July, 1986, along the offshore islands of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonian was next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 6.7 g of crude organic material (from 1 kg, dry weight of the gorgonian). The extract was separated by silica vacuum flash chromatography using sequential mixtures of EtOAc and isooctane as elutants. ^1H NMR spectra of the flash chromatographic fractions showed the presence of secondary metabolites in the fraction eluted with 10% EtOAc in isooctane. Separation by Sephadex lipophilic LH-20 column chromatography of the fraction gave 250 mg of the fat-free materials. Compounds **77**, **81**, **82**, and **83** were isolated by HPLC (5% EtOAc in isooctane) of the fat-free materials. Due to similar polarities, compounds **78** - **80** were not separable by HPLC. These compounds were isolated by a preparative centrifugal chromatography (Chromatron, 4-6% EtOAc in isooctane) using silica-gypsum-$AgNO_3$ (5% by weight) as the adsorbant.

Compound 77. The cubitane **77** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract yielded 49 mg (0.8% of the extract) of **77**. Compound **77** showed $[\alpha]_D$ +198° (c 1.0, $CHCl_3$) and the following spectral features; HRMS: M^+, m/z obsd 288.2447, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (100), 273 (29), 255 (12), 245 (83), 227 (35), 219 (21), 203 (29), 189 (57), 177 (61), 165 (43), 163 (47), 161 (51), 151 (33); IR (film) 2960, 2940, 1710, 1650, 1455, 1385, 890 cm^{-1}; UV (MeOH) no λmax.

Compound 78. The cubitane **78** was isolated as an oil by preparative centrifugal chromatography (6% EtOAc in isooctane). The extract gave 12 mg (0.2% of

the extract) of **78**. Compound **78** exhibited $[\alpha]_D$ -359° (c 0.5, $CHCl_3$) and the following spectral features; HRMS: M^+, m/z obsd 288.2463, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (10), 245 (6), 189 (11), 176 (12), 149 (11), 136 (100), 133 (13); IR (film) 2960, 2930, 2870, 1715, 1645, 1450, 1370, 1285, 890 cm^{-1}.

Compound 79. The cubitane **79** was isolated by preparative centrifugal chromatography (4% EtOAc in isooctane). The extract gave 11 mg (0.2% of the extract) of **79**. Compound **79** showed $[\alpha]_D$ +143° (c 0.4, $CHCl_3$) and the following spectral features; HRMS: M^+, m/z obsd 288.2474, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (54), 273 (25), 245 (68), 227 (29), 217 (10), 203 (15), 189 (84), 176 (100), 165 (71), 163 (34), 161 (58); IR (film) 2960, 2930, 2870, 1715, 1640, 1460, 1380, 890 cm^{-1}.

Compound 80. The cubitane **80** was isolated as an oil by preparative centrifugal chromatography (4% EtOAc in isooctane). The extract gave 15 mg (0.3% of the extract) of **80**. Compound **80** showed $[\alpha]_D$ -107° (c 0.5, $CHCl_3$) and the following spectral features; HRMS: M^+, m/z obsd 288.2460, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (24), 245 (27), 227 (11), 189 (100), 187 (17), 177 (18), 168 (25), 165 (25), 163 (11), 161 (21); IR (film) 2960, 2930, 2870, 1710, 1680, 1640, 1460, 1380, 1285, 890 cm^{-1}.

Compound 81. The cembrane **81** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract gave 51 mg (0.8% of the extract) of **81**. Compound **81** showed $[\alpha]_D$ +353° (c 0.6, $CHCl_3$) and the following spectral features; HRMS: M^+, m/z obsd 288.2443, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (24), 286 (46), 271 (11), 259 (12), 243 (15), 217 (18), 203 (100), 189 (20), 177 (45), 175 (35), 161 (40), 151 (59); IR (film) 2960, 2920, 1710, 1660, 1460, 1385 cm^{-1}; UV (MeOH) 254 nm (ε28500), 248 nm (ε28000).

Compound 82. The cembrane **82** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract gave 10 mg (0.2% of the extract) of **82**. Compound **82** showed $[\alpha]_D$ +23° (c 0.1, $CHCl_3$) and the following spectral features; HRMS: M^+, m/z obsd 288.2440, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (25), 245 (4), 227 (3), 177 (3), 165 (3), 151 (4), 136 (97), 121 (100), 108 (22), 93 (77); IR (film) 2960, 2920, 1710, 1660, 1465, 1380 cm^{-1}; UV (MeOH) 248 nm (ε21000), 245 nm (ε21000).

Compound 83. The cembrane **83** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract yielded 11 mg (0.2% of the extract) of **83**. Compound **83** showed $[\alpha]_D$ +283° (c 0.8, $CHCl_3$) and the following spectral features; HRMS: M^+, m/z obsd 288.2467, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (100), 273 (13), 270 (19), 245 (50), 242 (24), 227 (31), 203 (12), 190 (12), 177 (40), 175 (26), 165 (40), 161 (46), 151 (30); IR (film) 2960, 2930, 1705, 1665, 1460, 1440, 1380, 865 cm^{-1}; UV (MeOH) 248 nm (ε20500), 243 nm (ε21000).

Photochemical reaction of compound 81. A solution of compound **81** (11.3 mg, 0.04 mmol) in benzene (15 ml) was placed in a covered quartz test tube within 10 cm of a water-cooled photolysis apparatus and irradiated for 3 hr with light from a 450 watt Hanovia lamp. After evaporating the solvent, separation by HPLC (5% EtOAc in isooctane) gave four compounds; **77** (1.0 mg, 8.8% yield), **81** (2.1 mg, 18.6%), **82** (1.2 mg, 10.6%), and **83** (1.3 mg, 15.9%). The ^1H NMR spectra of these were identical with the natural products. Compounds **78** - **80** were obtained as a mixture (3.7 mg). Chemical shifts of the key protons (signals in the region of δ 2.8 - 5.8, and signals of high field methyl protons) in the ^1H NMR spectrum were within ± 0.002 ppm of the natural products. Quantification was made by integration of signals corresponding to the C-10 proton (δ 3.99 for **78**, 3.82 for **79** and 4.05 for **80**) in the ^1H NMR spectrum; **78** (0.7 mg, 6.2%), **79** (1.2 mg, 10.6%) and **80** (1.8 mg, 15.9%).

Photochemical reaction of compound 77. A solution of **77** (16.0 mg, 0.06 mmol) was dissolved in benzene (15 ml) and irradiated using the same apparatus and procedure as **81**. After 8 hr, the solvent was evaporated under vacuum. The ^{1}H NMR spectrum of the residue was identical with compound **77**.

Chapter VIII

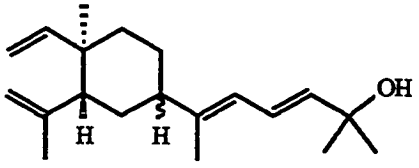
Fuscol and related diterpene glycosides from *Eunicea fusca*

Eunicea fusca (specimen number CI 86-195) was collected in Martinique and the Tobago Cays, in July 1986. Extraction of the dried gorgonians with dichloromethane (1.5 kg dry weight) gave 40 g of crude organic materials. Silica vacuum flash chromatography, followed by HPLC of the non-polar fractions gave fuscol (**84**) as the major metabolite. Fuscol was originally isolated from the *Eunicea fusca* collected at the South Caicos Island.[21] It was also isolated from an Australian soft coral *Lobophytum* sp.[114] ^{1}H NMR analysis of polar fractions showed the presence of several diterpene glycosides. Subsequently, C-18 reverse phase flash chromatography, followed by C-18 RP-HPLC of the polar fractions gave four additional metabolites (**85**, **87**, **88**, and **91**, Figure 27).

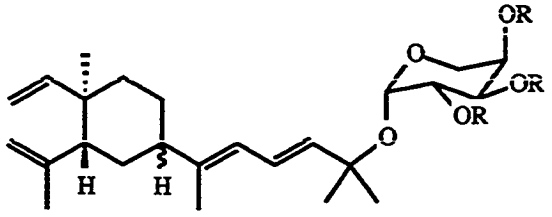
Compounds **85**, **87**, and **88** were diterpene arabinosides of the fuscol class, while **91** possessed a carbon skeleton related to the rearranged sesquiterpene eremophilane class. In contrast to the vast numbers of known terpenoids from the octocorals, only a few terpene glycosides have been reported. Look and coworkers isolated pseudopterosins and seco-pseudopterosins from various species of the Caribbean gorgonian *Pseudopterogorgia*.[115-117] The potent biological activities of these metabolites stirred new interest in marine natural products. Bandurraga and Fenical reported the muricins from the Pacific gorgonian *Muricea fructicosa*.[118] Another interesting metabolite, moritoside was isolated by Fusetani and coworkers from a Japanese gorgonian of the genus *Euplexaura*.[119] The structure of moritoside is unique in possessing a hydroquinone which connects a sesquiterpene and glycoside moiety.

The major compound, **84**, was isolated as an oil. The ^{1}H NMR spectrum of **84** was in full accord with the known metabolite, fuscol. Moreover, the optical rotation of **84** was very similar {$[\alpha]_D$+17.6°, fuscol +16.3°} to the reported value. Therefore, **84**

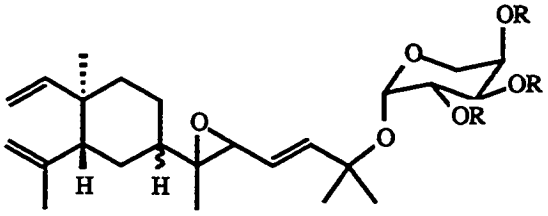
Figure 27. Structures of compounds **84** - **92**.



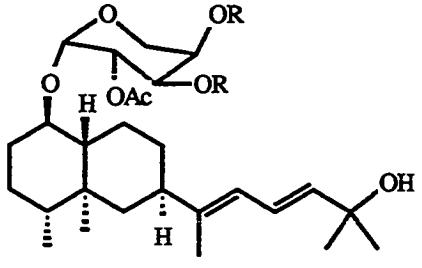
84 fuscol



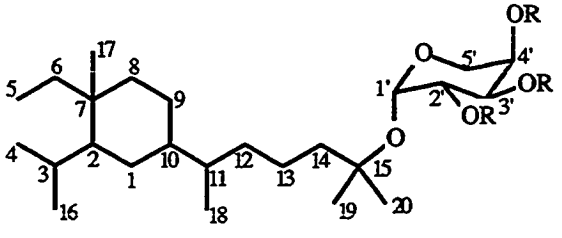
85 (R = H)
86 (R = Ac)

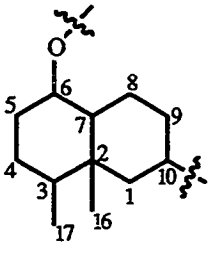


87 (RR or SS at the epoxide, R = H)
89 (same configurations as **87**, R = Ac)
88 (epimer at the epoxide, R = H)
90 (same configurations as **88**, R = Ac)

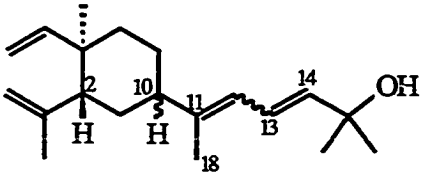


91 (R = H)
92 (R = Ac)





and fuscol must be identical. The originally reported structure of fuscol lacked the stereochemistry of the C-10 and diene functionalities.



the original structure of fuscol

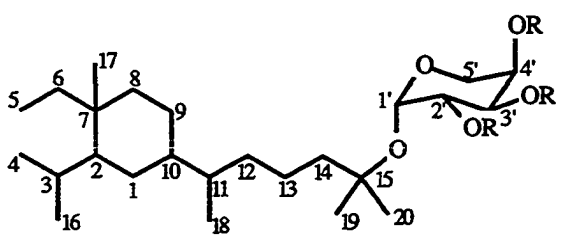
The large coupling constant between the C-13 and C-14 protons (J = 15.3 Hz) in the ^{1}H NMR spectrum allowed the assignment of the ***E*** configuration to the Δ^{13} double bond. Also, the high field shift of the C-18 carbon (δ 15.3) in the ^{13}C NMR spectrum indicated the ***E*** configuration for the Δ^{11} double bond (Table 26). However, the stereochemistry at C-10 was not clear. Due to the overlapping of the proton signals (the C-10 proton was partially overlapped with the C-2 proton), the coupling constants of the C-10 proton were unable to be measured. Based upon the comparison of ^{1}H NMR data with the known metabolites isolated from the same work, previous workers suggested that the C-10 and C-2 protons were *syn* oriented to the cyclohexane ring. The stereochemistry of this center is further discussed later.

Another major metabolite, **85**, was isolated as an oil which analyzed for $C_{25}H_{40}O_5$ by high resolution mass and ^{13}C NMR spectrometry. Chemical shifts of several signals in the ^{1}H and ^{13}C NMR spectra of **85** were very similar to fuscol (Table 26 and 27). However, five additional carbon signals at δ 93.9 (d), 70.9 (d), 69.6 (d), 69.0 (d), and 62.1 (t) indicated the presence of a sugar moiety. A corresponding difference was found in the ^{1}H NMR spectrum; δ 5.09 (1 H, d, J = 2.2) and 4.0 - 3.6 (5 H). Due to the overlapping of the proton signals however, the structure of the sugar part was determined by spectral analysis of a suitable derivative, **86**.

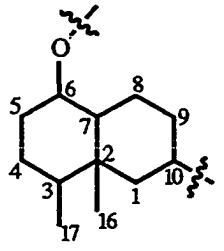
Table 26. ^{13}C NMR assignments for compounds **84** - **86** and **89** - **92**.

#	84	85	86	89	90	91	92
1	32.5 t	32.6 t	32.5 t	28.9 t	29.8 t	44.1 t	44.5 t
2	52.6 d	52.7 d	52.6 d	52.1 d	52.3 d	37.7 s	37.7 s
3	147.5 s	147.5 s	147.6 s	147.4 s	147.4 s	42.5 d	42.6 d
4	112.0 t	112.1 t	112.1 t	112.2 t	112.3 t	29.3 t	29.5 t
5	109.6 t	109.9 t	109.9 t	110.0 t	110.1 t	34.3 t	34.6 t
6	150.1 d	150.2 d	150.2 d	149.9 d	149.9 d	80.5 d	80.9 d
7	39.6 s	39.7 s	39.8 s	39.8 s	39.9 s	51.7 d	51.6 d
8	39.7 t	39.8 t	39.8 t	39.3 t	39.2 t	22.7 t	22.9 t
9	26.4 t	26.5 t	26.5 t	23.7 t	23.0 t	31.1 t	31.3 t
10	47.5 d	47.7 d	47.6 d	46.3 d	46.5 d	41.9 d	42.4 d
11	143.2 s	144.3 s	144.3 s	65.4 s	65.4 s	143.7 s	142.5 s
12	122.9 d[a]	122.3 d	122.2 d	61.9 d	62.2 d	122.5 d	123.2 d
13	123.0 d[a]	126.5 d	126.2 d	125.6 d	124.9 d	123.1 d	123.7 d
14	139.1 d	135.5 d	135.5 d	139.9 d	140.4 d	139.2 d	140.1 d
15	70.9 s	77.5 s	77.5 s	76.8 s	77.0 s	70.9 s	70.4 s
16	24.7 q	24.7 q	24.8 q	24.8 q	24.8 q	12.3 q	12.2 q
17	16.4 q	16.6 q	16.6 q	16.5 q	16.5 q	14.7 q	14.8 q
18	15.3 q	15.4 q	15.4 q	14.0 q	13.7 q	14.9 q	14.8 q
19	29.8 q	28.5 q	27.5 q	27.7 q	27.1 q	29.9 q	30.3 q
20	29.8 q	26.1 q	26.4 q	25.8 q	26.3 q	29.9 q	30.3 q
1'		93.9 d	91.5 d	91.5 d	91.7 d	98.4 d	98.9 d
2'		69.6 d	69.4 d[b]	69.3 d[b]	69.3 d[b]	72.5 d	69.7 d
3'		70.9 d[b]	68.5 d[b]	68.5 d[b]	68.4 d[b]	69.6 d	69.5 d
4'		69.0 d[b]	67.4 d[b]	67.3 d[b]	67.4 d[b]	67.7 d	67.9 d
5'		62.1 t	60.2 t	60.3 t	60.3 t	62.3 t	60.8 t
OAc			170.5 s	170.4 s	170.4 s	171.4 s	169.9 s
			170.3 s	170.3 s	170.3 s		169.9 s
			170.2 s	170.2 s	170.2 s		169.7 s
			21.0 q	20.9 q	21.0 q	20.9 q	20.4 q
			20.9 q	20.9 q	20.9 q		20.4 q
			20.8 q	20.8 q	20.8 q		20.4 q

Data for compounds **84, 85, 86, 89, 90** and **91** were obtained in $CDCl_3$ solution. Data for **92** was obtained in C_6D_6 solution. Multiplicities were determined by DEPT experiments. Assignments for **85, 91** and **92** were aided by XHCORR and COLOC experiments. Assignments for others were aided by comparison with **85**. [a,b] Carbons in the same column may be reversed.



84, 85, 86, 89 and **90**



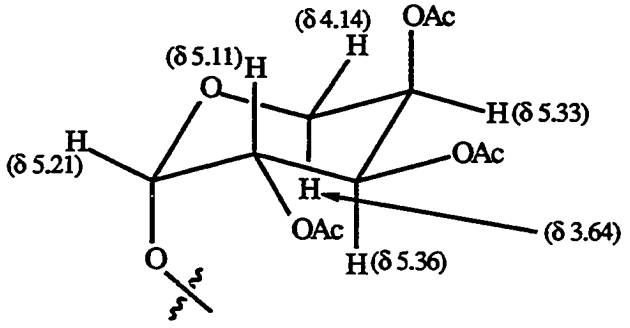
91 and **92**

Table 27. ^{1}H NMR assignments for compounds **85, 86** and **89 - 92.**

#	85[a]	86[a]	89[a]	90[a]	91[a]	92[a]	92[b]
1	1.61[d]	1.65 - 1.45 (2H)[d]	1.65 - 1.45 (2H)[d]	1.65 - 1.45 (2H)[d]	1.64 brd (12.7)	c	c
	1.46[d]				0.94 dd (12.6,12.6)	0.96[d]	
2	2.01 dd (12.1,3.8)	1.99[d]	2.05 - 1.95[d]	2.05 - 1.95[d]	---	---	---
3	---	---	---	---	1.24 - 1.19[d]	c	c
4	4.82 brs	4.81 brs	4.85 brs	4.83 brs	1.49 - 1.39 (2H)[d]	c	c
	4.59 brs	4.58 brs	4.61 brs	4.59 brs		2.05[d]	c
5	4.91 brd (15.6)	4.91 brd (16.2)	4.91 brd (16.5)	4.90 brd (16.4)	2.08[d]	c	c
	4.90 brd (12.0)	4.90 brd (12.0)	4.91 brd (11.8)	4.89 brd (11.7)	1.45[d]	c	c
6	5.82 dd (17.8,10.5)	5.82 dd (17.9,10.5)	5.81 dd (17.7, 10.7)	5.79 dd (17.8,10.5)	3.20[d]	3.18 ddd (10.1, 10.1,4.5)	3.04 m
7	---	---	---	---	1.15[d]	1.15[d]	c
8	1.58 - 1.44 (2H)[d]	1.65 - 1.45 (2H)[d]	1.65 - 1.45 (2H)[d]	1.65 - 1.45 (2H)[d]	2.01 m	1.97[d]	c
					1.15 - 1.09[d]	c	c
9	1.58 - 1.44 (2H)[d]	1.65 - 1.45 (2H)[d]	1.65 - 1.45 (2H)[d]	1.65 - 1.45 (2H)[d]	1.72 m	c	c
					1.24 - 1.19[d]		
10	1.98 m	1.99[d]	2.05 - 1.95[d]	2.05 - 1.95[d]	2.13 m	2.15[d]	c
12	5.88 brd (10.6)	5.83 brd (10.5)	3.22 d (6.6)	3.20 d (6.6)	5.83 brd (10.8)	5.84 brd (10.8)	6.02 brd (10.4)
13	6.44 dd (15.5,10.7)	6.39 dd (15.5,10.7)	5.57 dd (15.9,6.5)	5.53 dd (15.9,6.6)	6.47 dd (15.3,10.8)	6.47 dd (15.3,10.8)	6.66 dd (15.2,10.8)
14	5.60 d (15.4)	5.54 d (15.5)	5.79 d (15.8)	5.84 d (15.9)	5.75 d (15.3)	5.75 d (15.3)	5.73[d]
16	1.71 brs	1.70 brs	1.71 brs	1.71 brs	0.73 s	0.73 s	0.51 s
17	1.01 s	1.00 s	1.00 s	1.00 s	0.76 d (6.6)	0.76 d (6.4)	0.69 d (6.2)
18	1.78 brs	1.77 brs	1.22 s	1.21 s	1.74 d (0.9)	1.74 brs	1.70 brs
19	1.35 s[e]	1.31 s[e]	1.31 s[e]	1.30 s[e]	1.35 s	1.35 s	1.22 s
20	1.42 s[e]	1.35 s[e]	1.33 s[e]	1.33 s[e]	1.35 s	1.35 s	1.22 s
1'	5.09 d (2.2)	5.21 d (3.6)	5.23 d (3.5)	5.21 d (3.5)	5.00 d (3.7)	5.08 d (3.6)	5.30 d (3.1)
2'	3.76[d]	5.11 dd (10.1,3.7)	5.10 dd(10.3,3.5)	5.13 dd (10.3,3.5)	4.95 dd (9.6,3.7)	5.12 ddd (10.4,3.5, 1.4)	5.63 dd (11.2,3.2)
3'	3.76[d]	5.36 dd (10.4,3.6)	5.37[d]	5.35[d]	4.02 dd (9.9,3.6)	5.34[d]	5.74[d]
4'	3.98 brs	5.33 brs	5.33 brs	5.33 brs	4.00 brs	5.33 brs	5.56 brs
5'	3.95[d]	4.14 dd (12.9,1.0)	4.14 brd (12.6)	4.12 brd (13.0)	3.98[d]	4.06 brd (12.8)	3.85 brd (13.2)
	3.68 dd (12.6,1.8)	3.64 dd (13.0,1.9)	3.65 dd (12.9,1.3)	3.63 brd (12.8)	3.70 dd (10.9,1.8)	3.63 dd (12.9,1.3)	3.46 dd (13.0,1.2)
OAc		2.12 s	2.13 s	2.13 s	2.10 s	2.14 s	1.78 s
		2.07 s	2.08 s	2.08 s		2.04 s	1.77 s
		2.01 s	2.00 s	2.01 s		2.01 s	1.65 s

[a,b] measured in $CDCl_3$ and C_6D_6 solution, respectively. Assignments were made by ^{1}H NMR COSY experiments. [c] Unassignable proton resonances.
[d] Coupling constants were not measured. [e] Protons in the same column may be reversed.

Acetylation of **85** by acetic anhydride in pyridine quantitatively yielded the triacetate **86**. A ^{1}H NMR COSY experiment confidently assigned all of the sugar protons (Table 27). Comparison of the ^{1}H NMR spectra revealed that protons at C-2', -3', and -4' of **85** were significantly shifted to lower field (ca 1.5 ppm) in **86**, while the chemical shifts of the C-1' and -5' protons of **86** were similar to **85**, indicating the presence of a pyranose ring. The structure of the sugar was determined by measurment of proton coupling constants. A large vicinal coupling constant (10.3 Hz) between the C-2' and C-3' protons indicated the axial orientations for both protons. A small coupling (3.7 Hz) between the C-1' and C-2' protons indicated an equatorial orientation for the C-1' proton. Finally, the orientation of the C-4' was determined as equatorial by its small vicinal coupling constants (3.6, 1.0, and 1.9 Hz with the C-3', -4α' and -4β' protons, respectively). Thus, the sugar moiety was concluded to be an arabinose.



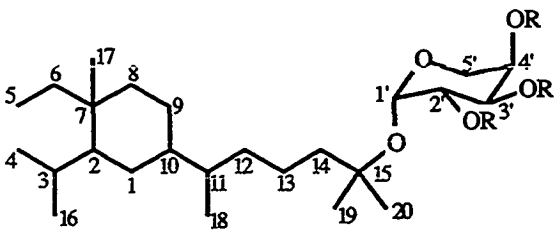
(δ 2.12, 2.07, and 2.01 for the acetyl protons)

The structure of **85** was confirmed by XHCORR and COLOC experiments (Table 28). All of the proton-bearing carbons and their protons were precisely matched. The long range coupling between the C-15 carbon and the C-1' proton confirmed the connection between the diterpene and arabinose components. Also, several carbon-proton couplings confirmed the structure of **85**. Thus, the structure of **85** was unambiguously determined as fuscol C-15 arabinoside.

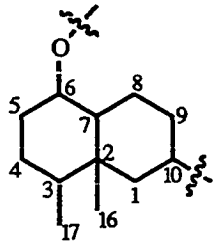
Table 28. Results of COLOC experiments with compounds **85**, **91** and **92**.

#	85[a]	91[a,c]	92[a,b,c,d]
1	—	10, 16 (17)[e]	16
2	—	16 (17)[e]	16, 17
3	16	16 (17)[e]	16, 17
4	—	—	17
5	—	—	—
6	17	—	—
7	5 (4.91)[f], 17	16	16
8	—	—	—
9	—	—	—
10	12, 18	18	18
11	18	18	18
12	13, 18	18	14, 18
13	14	—	12, 14
14	12, 13, 19 (20)[e]	12, 19 (20)[e]	12, 19 (20)[e]
15	13, 19 (20)[e], 1'	19 (20)[e]	13, 14, 19 (20)[e]
16	—	—	—
17	—	—	—
18	12	—	10, 12
19	—	—	—
20	—	—	—
1'	—	5' (3.70)[f]	5' (3.63)[f]
2'	—	—	—
3'	—	—	1', 5' (3.63)[f]
4'	—	5' (3.70)[f]	5' (4.06)[f], 5' (3.63)[f]
5'	1'	—	1'

[a-d] Parameters were optimized for 6, 8, 10, and 12 Hz of coupling constants, respectively. [e] Due to the proton overlapping, correlations were not clear. [f] Chemical shifts of protons which correlate.



85



91 and 92

At this point, the absolute stereochemistry of the sugar moiety was still in question. The arabinose could be either a D- or L-sugar. Several attempts to isolate the free sugar by the acid hydrolysis resulted in decomposition of **85**. Finally, the absolute stereochemistry of the sugar moiety was approached by comparison of the optical rotation between compounds **84** and **85**. This method, called Hudson's Rules of Isorotation,[120] assumes that the molecular rotation of a glycoside is made up of the sum of rotatory contribution of all the chiral centers in the molecule. The molecular rotation is defined as the molecular weight of the compound times the optical rotation divided by 100; $[M]_D = MW[\alpha]_D/100$. Therefore, in the case of **85**, the molecular rotation [M] was defined as sum of the $[M]_{fuscoi} + [M]_{sugar}$. From the measured optical rotations of fuscol and **85**, their molecular rotations were calculated as + 51° and -378°, respectively. Therefore, the molecular rotation of the sugar was expected to be -327°. The molecular rotations of D- and L-arabinose were -156° and +155°, respectively (after 22.5 hr).[121] Although there was substantial difference between the calculated and actual values of the molecular rotations of the sugar, expectation based on Hudson's rule revealed that the sugar was D-arabinose.

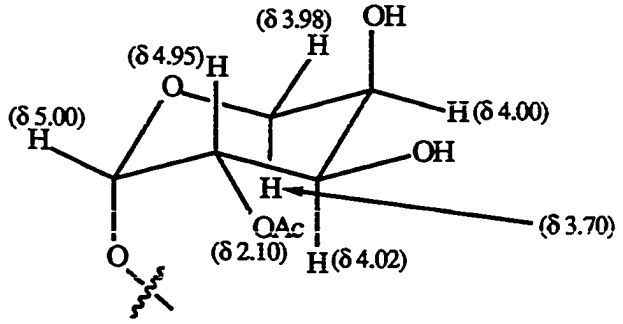
Two closely related metabolites **87** and **88** were isolated as a mixture. The ^1H NMR spectrum of the mixture looked like a pure diterpene glycoside. However, the ^{13}C NMR spectrum showed 34 signals instead of the expected 25 signals. Also, the relative intensity of carbon signals were very different from each other. Thus, it was found that this was a mixture of two structurally-related compounds. However, isolation of the pure metabolites was unsuccessful using both silica and C-18 reverse phase HPLC. Finally, the separation was achieved for the mixture of peracetyl derivatives which was produced by the reaction of the natural mixture with acetic anhydride in pyridine. Separation of the peracetate mixture by HPLC (30% EtOAc in isooctane) gave about equal amounts of two compounds **89** and **90** (12.1 and 12.9 mg, respectively).

Both compounds analyzed for $C_{31}H_{46}O_9$ by high resolution mass and ^{13}C NMR spectrometry. Spectral data of these compounds were highly compatible with that from the peracetate **86**. The only difference in the ^{13}C NMR spectra was the replacement of two olefinic carbons in **86** by epoxide signals (δ 66 - 60) in compounds **89** and **90** (Table 26).

Corresponding differences were also found in the ^1H NMR spectra (Table 27). The C-12 olefinic proton (δ 5.83) of **86** was significantly shifted upfield in **89** (δ 3.22) and **90** (δ 3.20). In addition, the C-18 vinyl methyl protons were shifted upfield (from δ 1.77 to 1.22 in **89** and 1.21 in **90**). Therefore, **89** and **90** must be the 11,12-epoxide derivatives of triacetate **86**. Other information supporting this assignment was the UV data, in which, in contrast to the strong absorption (241 nm, ε44000) of **85**, both epoxides were inactive, indicating that the diene of **86** was changed to another functionality in **89** and **90**. The structures of the epoxides were confirmed by a chemical conversion. Epoxidation of **85** with mCPBA gave **87** and **88** as the major products. Thus, the structures of **87** and **88** were determined as the 11,12-epoxide derivatives of **85**. Compounds **87** and **88** were epimeric to each other at the new asymmetric carbon centers. From the mechanism of the mCPBA epoxidation, their configurations must be 11R, 12R and 11S, 12S for one another. However, the absolute assignments were not made and the configurations of the epoxides remained unknown.

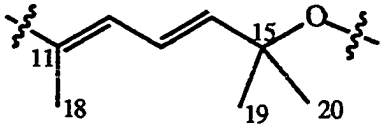
Compound **91** was isolated as an oil which analyzed for $C_{27}H_{44}O_7$ by high resolution mass and ^{13}C NMR spectrometry. A carbon signal at δ 171.4 (s) in the ^{13}C NMR spectrum (Table 26) and a strong absorption band at 1740 cm^{-1} in the IR spectrum indicated the presence of an ester. A sharp methyl singlet at δ 2.10 in the ^1H NMR spectrum revealed that the ester was an acetate (Table 27). A carbon signal at δ 98.4 and several carbons in the region between δ 75 and 60 illustrated **91** to possess a sugar moiety. A ^1H NMR COSY experiment revealed an isolated spin system of six protons in

the region of δ 5.0 - 3.7. With the help of XHCORR experiments, the sugar was identified as an arabinose pyranoside. The low field shift of the C-2' proton (δ 4.95) indicated that the acetoxyl group was attached to the C-2' carbon.



The presence of an arbinose pyranoside in compound **91** was confirmed by acetylation. Treatment of **91** with acetic anhydride in pyridine gave a triacetate, **92**, as the major product. The ^{1}H NMR spectrum of **92** was very similar to **91**, with the downfield shifts of the sugar methine protons as the only differences. A ^{1}H NMR COSY experiment confidently assigned all of the sugar protons (Table 27). Both the chemical shifts and coupling constants of the sugar protons of **92** were very similar to those from the triactate **86**. Thus, compound **91** was determined as a 2'-acetyl arabino pyranoside of a diterpene molecule.

Comparison of NMR data revealed that the chemical shifts of several protons and carbons of **91** were similar to **85**. Combination of ^{1}H NMR COSY and XHCORR data revealed **91** to possess the same acyclic part as found in **85**.

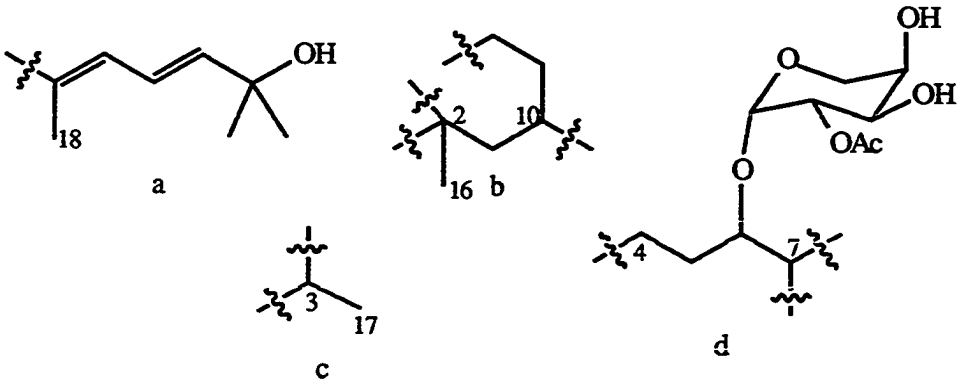


However, the chemical shift of the C-15 carbon (δ 70.9) was significantly higher

than the corresponding carbon shift (δ 77.5) in **85**, and rather similar to the same carbon shift (δ 70.9) from fuscol. Moreover, the chemical shift of the C-1' carbon (δ 98.9) of the triacetate **92** was much lower than the same carbon (δ 91.5) of another triacetate **86**. Therefore, unlike the other diterpene glycosides, compound **91** must possess a tertiary alcohol at C-15. This assignment was supported by reaction of **92** with trichloroacetyl isocyanate, and measurement of the chemical shifts of relevant protons. The chemical shifts of most protons were unchanged by the esterification, while the C-19 and -20 methyl protons were shifted from δ 1.22 (6 H, s) to 1.66 (6 H, s). Also, the C-14 olefinic proton was shifted from δ 5.73 to 6.42. Thus, compound **91** was determined to possess a free hydroxyl group at C-15.

The remaining part was also identified by interpretation of combined spectral data. Consideration of the molecular formula revealed that the remaining part was a component, $C_{12}H_{20}$, which was connected by the arabinose and the acyclic part. Since there were no olefinic carbons except the diene in the ^{13}C NMR spectrum, the remaining part must be a saturated bicyclic component. Combination of the ^{13}C NMR, COSY NMR and XHCORR data revealed several partial structures. A methyl singlet at δ 0.73 was readily assigned as attached to the carbon at δ 37.7, which was the only quarternary carbon of the bicyclic component. COSY NMR data revealed a proton spin system of seven protons. Consideration of the coupling pattern revealed that one end of the spin system {methylene protons at δ 1.64 (1 H, brd, 12.7) and 0.94 (1 H, dd, 12.6, 12.6)} was connected to the quarternary carbon. A proton at δ 3.20 was readily assigned as the α-arabinosyl proton. This proton was coupled by three protons at δ 2.08, 1.45 and 1.15. XHCORR data showed that these protons were attached to the carbons at δ 34.3 (t, the former two protons) and 51.7 (d, proton at δ 1.15). The proton at δ 2.08 was coupled with two proton resonances at δ 1.49 - 1.39, which were attached to a common methylene carbon at δ 29.3. A doublet methyl group at δ 0.76 (J = 6.6 Hz) was attached to a methine carbon.

Due to the overlapping of the high field proton signals, the methine proton was not able to be assigned precisely (δ 1.24 - 1.19). In total, four partial structures were determined by spectral analysis.



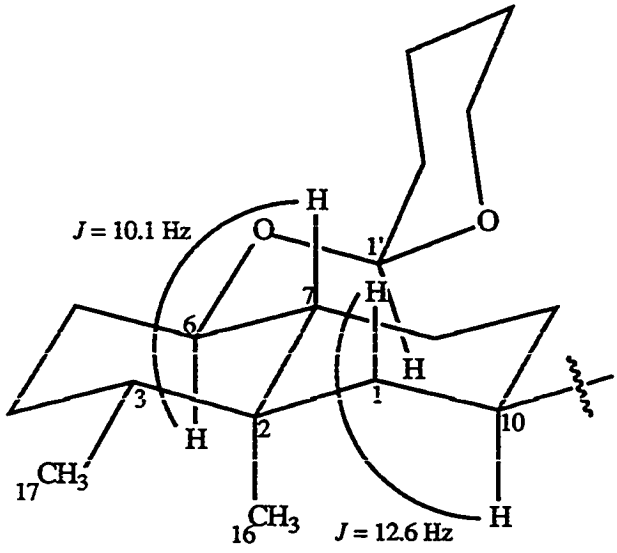
Combination of these assignments was made by COLOC experiments (Table 28). The long range coupling between the C-10 carbon and the C-18 protons readily connected the partial structures a and b. COLOC data showed several carbon-proton correlations associating the upfield methyl protons. Due to the similar chemical shifts of the C-16 and -17 methyl protons (δ 0.73 and 0.76, respectively) however, further interpretation was impossible. Finally, COLOC experiments were performed with the triacetate **92** in benzene-d_6 solution (in benzene-d_6, the C-16 and -17 protons were observed at δ 0.51 and 0.69, respectively, Table 27). Long range correlations of the C-1 and -2 carbons to the C-17 and -16 protons connected the partial structures b and c. Also, a coupling between the carbon at δ 29.5 (t, structure d) and the C-17 protons connected d to c. In addition, the connection between b and d was found by a coupling between the C-7 carbon (δ 51.6) and the C-16 methyl protons. Thus, the structure of compound **91** was determined as a diterpene arabino pyranoside. The carbon skeleton of the diterpene part was related to the rearranged sesquiterpene, eremophilane, which may be formed by a migration of the C-16 methyl group from C-7 to the C-2. To the best of my knowledge, this is a new

diterpenoid skeleton only distantly related to the eremophilane sesquiterpene system.

Compound **91** has five asymmetric carbon centers (C-2, -3, -6, -7, and -10). The stereochemistries of these centers were determined by combination of ^{1}H NMR, ^{13}C NMR and ^{1}H NMR NOEDS data (Figure 28). The high field chemical shift of the C-16 methyl carbon (δ 12.3) indicated a *trans* ring junction. Large vicinal coupling (J = 12.6 Hz) between the C-1 (δ 0.94) and C-10 protons revealed that the orientation of the C-10 proton was axial to the ring. By the same method, the large coupling (J = 10.1 Hz for compound **92**) between the C-6 and C-7 protons illustrated the *trans* diaxial orientation for the protons. These assignments were confirmed by the ^{1}H NMR NOEDS experiments. Irradiation of the C-16 methyl protons of **92** enhanced the C-6 and C-10 protons by 5.8 and 3.2 %, respectively, supporting the axial orientations of these protons. At the same time, the C-17 methyl protons were enhanced by 8.8 %, indicating the *syn* orientation between the C-16 and C-17 methyl groups. Irradiation of the C-6 proton enhanced both the C-16 and C-1' protons by 4.1 and 8.5 %, respectively. Finally, the C-16 protons were enhanced by the irradiation of the C-17 protons (4.2 %). Thus, the stereochemistry of compound **91** was unambiguously determined as 2**S***, 3**R***, 6**R***, 7**R***, and 10**R***.

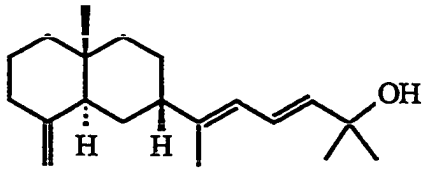
The stereochemistry at C-10 of compounds **84** - **90** is unknown. Based upon the comparison of spectral data, previous workers suggested that the C-2 and C-10 protons were *syn* oriented to the cyclohexane ring. On the other hand, coupling constants and ^{1}H NMR NOEDS experiments revealed that the stereochemistry of the same proton of compound **91** was opposite to fuscol. Assuming that fuscol and **91** derived from a common precursor, the stereochemistry of the C-10 must be identical between these metabolites. Reference study showed that a structurally related compound **93** was isolated from an Australian soft coral *Lobophytum hedleyi*.[122] The stereochemistry of the C-10 was found to be opposite to the proposed stereochemistry of fuscol.

Figure 28. Stereochemistry of compounds **91** and **92**.



Results of a ^1H NMR NOEDS experiment with **92**.

proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
6	16, 1'	4.1, 8.5
16	6, 10, 17	5.8, 3.2, 8.8
17	16	4.2



93

The methods used for the previous studies were degradation of compounds isolated with fuscol, followed by comparison of the optical rotation of the degraded products with authentic samples. Since the optical rotation of both compounds were very small (< 8°), the reliability of their results were questionable. As a result, the stereochemistry at C-10 in fuscol (**84**) and **85** - **90** remained unknown.

Samples of *Eunicea fusca* were also collected at the Florida Keys (July, 1987) and St. Croix Island (November, 1987). The results of TLC analysis showed that the chemical composition of these animals were identical with the Martinique and Tobago Cays collections.

Experimental Section

Collection and Extraction. *Eunicea fusca* (specimen number CI 86-195) was collected by hand using SCUBA at -20 to 25 m in July 1986, at the Martinique and Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonians were next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 40 g of crude organic materials (from 1.5 kg, dry weight of the gorgonian). Fuscol (**84**), the major metabolite was isolated from silica vacuum flash chromatography, followed by silica HPLC (15% EtOAc in isooctane) of the fraction eluted with 20% EtOAc in isooctane. Polar flash chromatographic fractions (70-100% EtOAc in isooctane) were combined and re-separated from C-18 reverse phase (RP) flash vacuum chromatography, in which the adsorbant was made by following the known method.[123] Final purifications by C-18 RP-HPLC (100-90% MeOH in water) gave four compounds (**85**, **87**, **88**, and **91**). *E. fusca* (specimen number F 87-23) was also collected at the Florida Keys, in July 1987. TLC analysis showed that the chemical components of the animals were identical with those collected from the eastern Caribbean Sea. Several specimens were also collected from St. Croix, U. S. Virgin Islands, in November 1987. The results of TLC analysis were again identical with the other collections.

Fuscol (84). Fuscol was isolated as an oil by HPLC (15% EtOAc in isooctane). The extract gave ca 2.5 g (6% of the extract) of **84**. Fuscol showed $[\alpha]_D$+17.6° (c 0.9, $CHCl_3$). ^1H NMR spectrum ($CDCl_3$) of **84** showed peaks at δ 6.49 (1 H, dd, 15.3, 10.8, H-13), 5.87 (1 H, brd, 10.7, H-12), 5.82 (1 H, dd, 17.8, 10.4, H-6), 5.77 (1 H, d, 15.2, H-14), 4.90 (1 H, d, 16.5, H-5), 4.90 (1 H, d, 10.6, H-5), 4.81 (1 H, d, 1.4, H-4), 4.58 (1 H, brs, H-4), 2.03 (1 H, dd, 12.1, 3.9, H-2), 1.99 (1 H, m, H-10), 1.79 (3 H, s, H-18), 1.70 (3 H, s, H-16), 1.36 (6 H, s, H-19 and H-20), and 1.01 (3 H, s,

H-17). ^{13}C NMR data of **84** is shown in Table 26.

Compound 85. The arabinoside **85** was isolated as an oil by C-18 RP-HPLC (90% MeOH in water). The extract yielded ca 1 g (2.5% of the extract) of **85**. Compound **85** showed $[\alpha]_D$-90° (c 1.0, $CHCl_3$) and displayed the following spectral features; HRFABMS: $(M+Na)^+$, m/z obsd 443.2773, $C_{25}H_{40}O_5Na$ required 443.2775; HREIMS: $(M-C_5H_8O_4)^+$, m/z obsd 288.2452, $C_{20}H_{32}O$ required 288.2455; low-resolution MS: m/z (relative intensity) 288 (2), 271 (35), 189 (14), 161 (18), 147 (21), 135 (22), 119 (51), 109 (63), 93 (50), 81 (49), 73 (100), 69 (43), 43 (50); IR (film) 3400, 2980, 2930, 1635, 1440, 1260, 1135, 1080, 990 cm^{-1}; UV (MeOH) 241 nm (ε44000).

Acetylation of 85. To a stirred solution of 24 mg (0.05 mmol) of **85** in 2 ml of dry pyridine, 1.5 ml of dry acetic anhydride was added. After stirring the mixture for 3 hr at 80°, pyridine and excess anhydride were removed under vacuum. The triacetate **86** was obtained quantitatively. ^1H and ^{13}C NMR data for **86** are shown in Tables 26 and 27.

Hydrolysis of 85. To a stirred solution of 16 mg (0.04 mmol) of **85** in 2 ml of MeOH, 2 ml of 1 N HCl was added. After stirring for 2 hr at room temperature and for 3 hr at 40°C, the mixture was separated by water (20 ml) and CH_2Cl_2 (30 ml). After evaporating the solvents under vacuum, the ^1H NMR spectra of both fractions showed that the reactant was decomposed to a mixture of several unrecognizable compounds.

Isolation of compounds 87 and 88. A mixture (~1:1 by integration of peaks in the ^1H NMR spectrum) of the epoxides **87** and **88** were isolated as an oil by C-18 RP-HPLC (90% MeOH in water). The extract gave 25 mg (0.06% of the extract) of the mixture of **87** and **88**. Several attempts to separate the mixture by C-18 RP-HPLC failed.

Acetylation of 87 and 88. To a stirred solution of 25 mg of the 1:1 mixture

of **87** and **88** in 2 ml of dry pyridine, 1 ml of dry acetic anhydride was added. After stirring the mixture for 2 hr, pyridine and excess anhydride were removed under vacuum. Separation by HPLC (30% EtOAc in isooctane) gave the triacetates **89** (12.1 mg) and **90** (12.9 mg) as the major products. Compound **89**, an oil, showed $[\alpha]_D$-62° (c 0.7, $CHCl_3$) and displayed the following spectral features; HRFABMS: $(M+H)^+$, m/z obsd 563.3199, $C_{31}H_{47}O_9$ required 563.3220; IR (film) 2980, 2940, 1750, 1640, 1440, 1375, 1250, 1230, 1135, 1070, 1015, 895, 750 cm^{-1}; UV (MeOH) no λmax. Compound **90**, an oil, exhibited $[\alpha]_D$-87° (c 0.5, $CHCl_3$) and displayed the following spectral features; HRFABMS: $(M+H)^+$, m/z obsd 563.3168, $C_{31}H_{47}O_9$ required 563.3220; IR (film) 2980, 2940, 1750, 1640, 1440, 1375, 1250, 1230, 1135, 1075, 1020, 895, 760 cm^{-1}; UV (MeOH) no λmax.

Epoxidation of 85. To a stirred solution of 30 mg (0.07 mmol) of **85** in 4 ml of CH_2Cl_2 buffered with anhydrous Na_2HPO_4, 1.3 equiv of mCPBA was added. After stirring the mixture for 2 hr at room temperature, the CH_2Cl_2 layer was washed with 10% Na_2SO_3 (2 x 20 ml), 5% $NaHCO_3$ (2 x 10 ml), and finally with brine. After evaporating the solvent and drying under vacuum, C-18 RP-HPLC separation (95% MeOH in water) gave 13.4 mg (0.03 mmol, 43% combined yield) of 1:1 mixture of **87** and **88**, and 14.6 mg (49%) of **85**. The ^1H NMR spectrum of the mixture was identical with the natural mixture.

Compound 91. The arabinoside **91** was isolated as an oil by C-18 RP-HPLC (90% MeOH in water). The extract yielded 95 mg (0.2% of the extract) of **91**. Compound **91** showed $[\alpha]_D$-64° (c 0.6, $CHCl_3$) and exhibited the following spectral features; HRMS: M^+, m/z obsd 480.3113, $C_{27}H_{44}O_7$ required 480.3088: $(M-C_7H_9O_6-H_2O)^+$ obsd 271.2429, $C_{20}H_{31}$ required 271.2427; low-resolution MS: m/z (relative intensity) 480 (4), 462 (7), 288 (7), 271 (14), 217 (9), 189 (14), 175 (53), 161 (17), 157 (40), 135 (13), 125 (22), 119 (16), 107 (71), 95 (31), 81 (39), 43 (100); IR (film) 3420,

2975, 2930, 1740, 1650, 1450, 1375, 1240, 1145, 1055, 1010, 760 cm^{-1}; UV (MeOH) 239 nm (ε28000).

Acetylation of 91. To a stirred solution of 55 mg (0.12 mmol) of **91** in 3 ml of dry pyridine, 2 ml of dry acetic anhydride was added. After stirring the mixture for 3 hr, pyridine and excess anhydride were removed under vacuum. Separation by HPLC (45% EtOAc in isooctane) gave 42 mg (0.07 mmol, 65% yield) of **92** as the major product. The acetate **92** was characterized by NMR and COLOC data (Table 26 - 28).

Chapter IX

Results (Chemistry) and Discussion (Chemosystematics)

Gorgonians of the genus *Eunicea* are among the most abundant octocorals in the shallow waters of the West Indies.[1] Despite their importance on the Caribbean reef ecosystem however, information on their ecology is scarse. This is due to the lack of reliable taxonomic classification, which is in turn derived from a lack of reliable systematic characters.[1,31] On the other hand, the major problem which hinders progress on the natural products chemistry of the octocorals, and *Eunicea* in particular, is the difficulty in the collection of chemically homogeneous specimens.

The goal of this work is first, to broaden our knowledge on the natural products chemistry of the *Eunicea* through systematic collection and extensive chemical investigation, and secondly, to provide reliable chemical characters which would be helpful for the systematics of these animals. For the first purpose, thin layer chromatography (TLC) of the tissue extracts was extensively used. Chemical investigation was performed using spectroscopic methods, especially by using modern two-dimensional NMR techniques. In addition, chemical reactions and X-ray crystallographic methods were used for the structural determination of some metabolites. For the second purpose, the chemistry of *Eunicea* species which were collected at both different times and locations were compared. Also, chemical characteristics of chemically consistent groups (chemotypes) were compared with morphological characters. Examination of morphological characters was performed by Dr. F. M. Bayer. In this chapter, the results of this work on the chemistry of *Eunicea* is summarized. In addition, morphological classification is discussed with the chemical characters of each chemotype.

The major collections were made at the Tobago Cays, eastern Caribbean Sea (July, 1986), and The Florida Keys (July, 1987). A few vouchers were collected at St. Croix

Island, U.S. Virgin Islands (November, 1987). In addition, specimens of *Eunicea fusca* were collected in Martinique (July, 1986). In total, 792 individual colonies were collected. Based upon TLC characters (Rf values, UV activity, colors produced by acid charring and heating), 780 (98.5 %) colonies were confidently divided into 11 chemotypes (Table 29).

Nine chemotypes were chemically investigated. In total, 39 new metabolites, along with 6 known metabolites were isolated. In addition, 3 compounds previously isolated from other octocorals were also isolated. Diterpenoids were the major group of metabolites. Cembranes were the most commonly encountered class. In most cases (except chemotypes H and Y) however, cembranes from different chemotypes significantly differed in both types and distributions of functionalities. Other metabolites were dolabellanes, cubitanes, asperketals, fuscol, and diterpene arabinosides of fuscol class. In addition, metabolites of three unprecedented classes were isolated; C-28 reduced quinones, trisnorditerpenoids and a diterpene arabinoside of "extended eremophilane" class.

Octocorals and other benthic organisms in the Caribbean Sea have been the focus of studies for their secondary metabolites for more than three decades. Considering the long history and numerous efforts on the chemistry of *Eunicea* and other octocorals, isolation of a large number of novel metabolites in this work emphasizes the importance of making chemically homogeneous collection. Due to immense morphological variations among individual colonies, homogeneous collection of colonial marine animals (corals and sponges) is notoriously difficult. On the other hand, most of these organisms are very rich in novel secondary metabolites. Among the available methods to detect the presence of secondary metabolites, TLC analysis is the most convenient for use in the field.[10,30] Collections based on TLC analysis will greatly contribute to the natural products chemistry and other work on these organisms.

Table 29. Separation of *Eunicea* specimens based on the results of TLC analysis.

chemotypes	collection site (s)[a]	numbers of the collected animals
H	T	150
	F	60
Y	T	16
E	T	20
A	T	14
L	F	56
	S	1
K	T, M	281
	F	65
	S	4
O	T	7
U	T	8
N	T	4
	F	12
I	F	11
G	T	43
	F	26
	S	2
11 chemotypes		780
unclassified[b]	T, F, S	12 (1, 8, 3)[c]
total		792

[a] Collection sites are shown by the abbrebiations of the locations (T = Tobago Cays, F = Florida Keys, M = Martinique, S = St. Croix). [b] Unclassified specimens lacked some of the distinct TLC characters of each chemotype. [c] Numbers in parenthesis are numbers of the unclassified specimens from each collection sites.

Chemosystematics have contributed greatly to the taxonomy of numerous organisms whose morphological classification is difficult. To be a suitable target for chemosystematical utility, chemical contents of the organism should be, if not consistent, minimal in the spatial and temporal variations.[42] Also, the chemical contents among organisms in the same hierarchy should be distinct from each other. The specimens of several *Eunicea* chemotypes were collected from more than one location, and the results of TLC analysis showed homogeniety (Table 29). Comparison of chemical contents revealed that most of chemotypes contained very distinct secondary metabolites. All of the studied chemotypes contained only one or two classes of metabolites. When compounds of a class were isolated from more than one chemotypes, compounds from the same chemotype showed great structural similarities. In contrast, compounds of the same class from different chemotypes often show very different pattern of functionalization. Cembranes were isolated from several chemotypes. However, all of the cembranes from the same chemotype showed similarities in both types and positions of functional groups, while cembranes from different chemotypes except H and Y were very distinct from each other (Figure 29). Although the asperketals were isolated from both chemotypes A and L, isolations of coexisting metabolites (cembranes from A and norasperenals from L) readily distinguished one from the other. Thus, the collection of chemically identical specimens from various locations, and the distinct chemical contents of most chemotypes showed the possibility that *Eunicea* might be a suitable organism for the chemosystematic work.

F. M. Bayer described 12 *Eunicea* species; *mammosa, succinea, palmeri, calyculata, asperula, laciniata, fusca, tourneforti, laxispica, pinta, clavigera, knighti.*[1] The first seven species were collected and chemically analyzed in this work. The *E. tourneforti* "complex" was collected but it has not been studied. In addition, four groups (chemotypes E, L, O, I) of *Eunicea* which possessed morphological characters different from known species were also found. Each of these undescribed collections has the

Figure 29. Major metabolites from the *Eunicea* chemotypes.

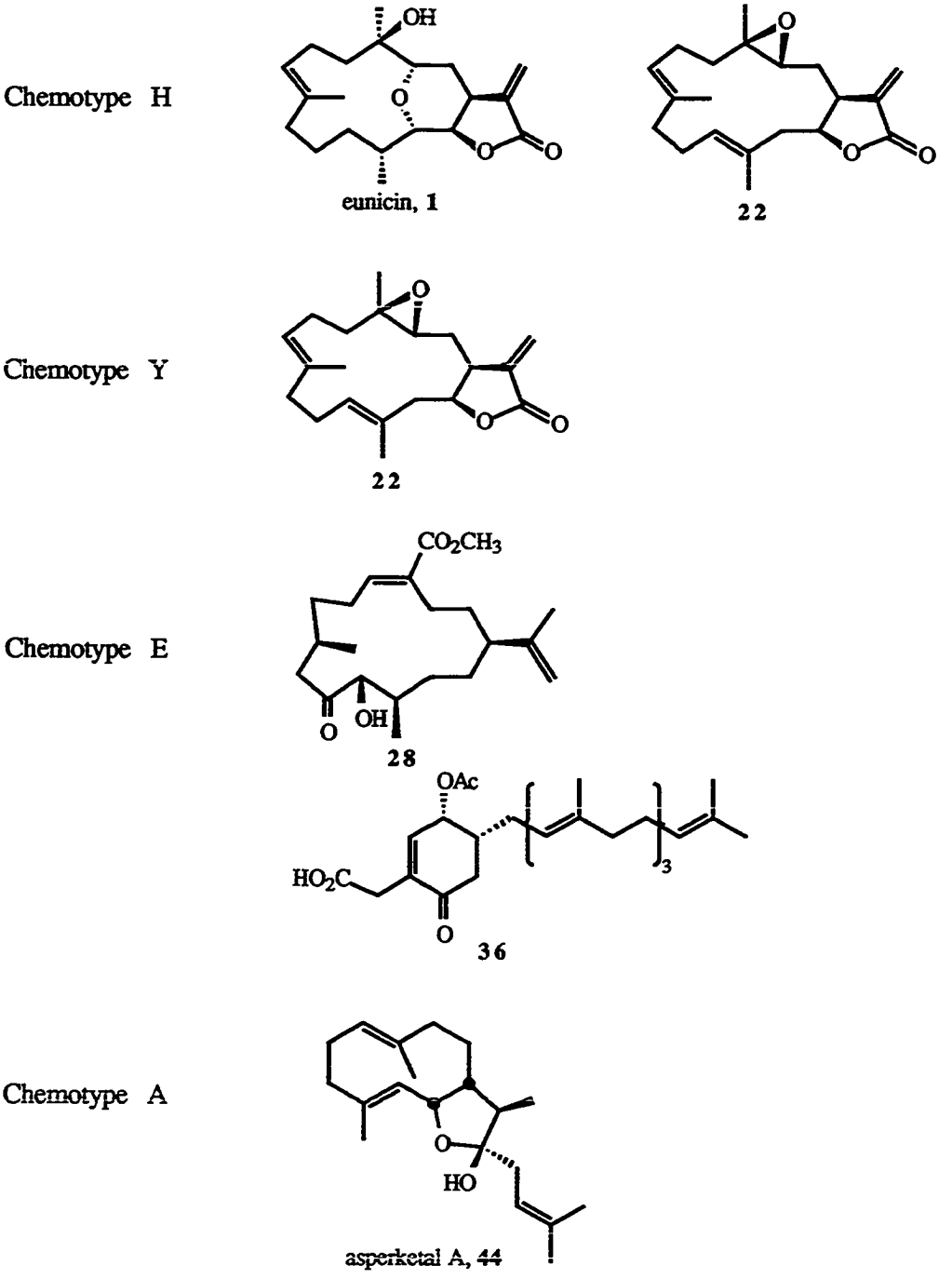
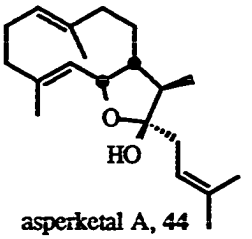
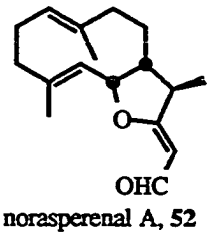


Figure 29. (continued)

Chemotype L

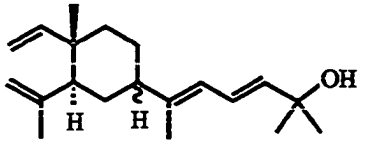


asperketal A, 44

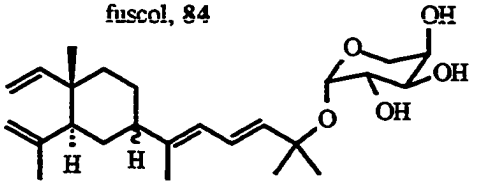


norasperenal A, 52

Chemotype K

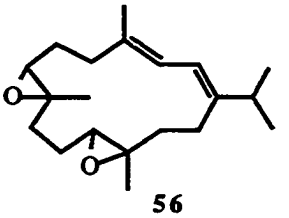


fuscol, 84

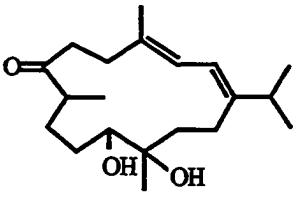


fuscol arabinoside, 85

Chemotype O

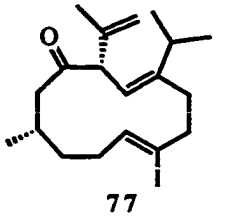


56

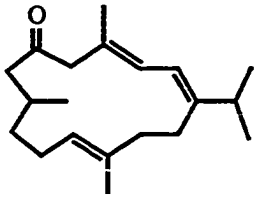


60

Chemotype U

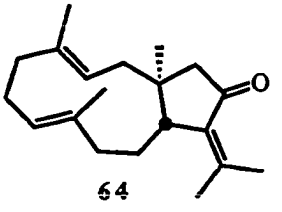


77



81

Chemotype N



64

potential to be classified as a new species. Although the chemical investigation of *Eunicea* is far from being complete, the chemical characteristics of each chemotype were compared with their morphological classifications. The TLC characters, major metabolites (Figure 29), and morphological classification of each chemotype are shown as below;

Chemotype: H

Definition: Purple spots at RF 0.6 (eunicin acetate and compound **22**) and 0.4 (eunicin and related cembrane lactones) in ethyl ether.

Major metabolite: eunicin (**1**)

Identification: *E. mammosa* (Tobago Cays and Florida Keys) and *E. palmeri* (Florida Keys).

Comments: Bayer found that some vouchers of *E. mammosa* and *E. palmeri* possessed morphological features approaching *E. laxispica* and *E. succinea*, respectively.

Chemotype: Y

Definition: Purple spot at RF 0.6 (compound **22**) in ethyl ether.

Major metabolite: compound **22**.

Identification: *E. succinea* forma *plantaginea*.

Chemotype : E

Definition: Yellow spot at RF 0.6 (cembrane esters **28** - **30**), purple spot at RF 0.3 (quinone **35**) and purple tail at 0.2 - 0.0 (quinone acid **36**) in ethyl ether.

Major metabolites: compounds **28** and **36**.

Identification: unknown (subgenus *Euniceopsis*).

Comments: Bayer defined that the sclerities of chemotype E were never seen before.

However, the TLC characters were among the most distinct of the chemotypes.

Chemotype: A

Definition: Brownish green spots at RF 0.7 - 0.6 (asperketals and cembranes) in ethyl ether.

Major metabolite: compound **44** (asperketal A).

Identification: *E. asperula.*

Chemotype: L

Definition: Brownish green spot at RF 0.6 (asperketals A and B) and yellow spot at RF 0.5 (norasperenals A - D) in ethyl ether.

Major metabolites: compounds **44** (asperketal A) and **52** (norasperenal A).

Identification: unknown (subgenus *Euniceopsis*).

Comments: Morphological characters of the specimens were highly variable.

Chemotype: K

Definition: Purple spots at RF 0.7 (fuscol, **84**), 0.2 (diterpene glycoside **91**), and 0.1 (diterpene glycosides **85**, **87** and **88**) in ethyl ether.

Major metabolites: compounds **84** (fuscol) and **85** (fuscol arabinoside)

Identification: *E. fusca.*

Chemotype: O

Definition: Purple spot at RF 0.8 (cembranes **61** and **62**) and brown spots at RF 0.6 and 0.5 (cembranes **56** - **60**) in ethyl ether.

Major metabolite: compounds **56** and **60**.

Identification: unknown (*Euniceopsis*).

Comments: Bayer found that the morphological features were well known, but never described in the literature.

Chemotype: U

Definition: brown spot at RF 0.8 (cubitanes **77 - 80** and cembranes **81 - 83**) in ethyl ether.

Major metabolites: compounds **77** and **81.**

Identification: *E. calyculata.*

Chemotype: N

Definition: Brownish yellow spots at RF 0.7 - 0.6 (dolabellanes **64 - 69**).

Major metabolite: compound **64.**

Identification: *E. laciniata* (Tobago Cays) and *E. cf calyculata* (Florida Keys).

Comments: The specimens of *E. cf calyculata* were similar to *E.calyculata*, but the shape of apertures were different from the typical *E. calyculata.*

Chemotype: I

Definition: Pink spots at RF 0.6 and 0.5, and a Red spot at 0.45 in the ethyl ether.

Major metabolite: Chemistry has not been studied.

Identification: unknown (*Euniceopsis*).

Comments: The morphological features were somewhat related to *E. asperula*, but highly variable among the vouchers.

Chemotype: G

Definition: 3 -4 purple spots at RF 0.7 - 0.5 in the ethyl ether.

Major metabolite: Chemistry has not been studied.

Identification: *E. tourneforti* "Complex"

Comments: Bayer found that there were limitless morphological variations in the *E. tourneforti* "complex". TLC characters were also variable by colonies. It seemed likely that this is due to the variations on the relative concentrations of metabolites by each colonies.

F. M. Bayer described 12 species of *Eunicea*. Based upon the morphological characters, he combined them into two subgenera; *Eunicea* s.s. (*sensu strictu*) and *Euniceopsis*.[1] He stated that "future work may demonstrate that the subgenera are actually of full generic importance, and the group of species treated under *Euniceopsis* might be split into two subgenera".[1] The subgenus *Eunicea* s.s. consists of five species; *mammosa, succinea, palmeri, laxispica,* and *pinta*. Several chemical investigations were made for the first three species.[11-18] There was no clear difference in the chemistry of these species, and all of the known metabolites were highly functionalized cembrane lactones. The results of this work were very similar to the previous results, and only cembranes with γ- and δ-lactones were isolated. *E. mammosa* and *E. palmeri* possessed the same chemical characters (chemotype H), while the chemistry of *E. succinea* forma *plantaginea* was slightly different (chemotype Y), possessing compound **22** as the only metabolite of significant quantity. Bayer reported two forms of *E. succinea*; forma *succinea* and forma *plantaginea*. Forma *plantaginea* was frequently confused with *E.calyculata* (chemotype U).[1] The result of this work showed that although the distinction between chemotypes Y and H was difficult, the chemical characteristics of Y were very different from chemotype U, supporting the classification of forma *plantaginea* into the subgenus *Eunicea* s. s..

Unlike the similar chemistry within the subgenus *Eunicea s.s.*, the chemical characteristics of the *Euniceopsis* were very different. Although cembranes were isolated

from both subgenera, oxidation patterns and positions of the functionalities on the carbon skeletons were very distinct between two subgenera. In his cladistic analysis of the gorgonians, Gerhart found that species in two subgenera were no more closely related to each other than they are to the species of other genera, supporting Bayer's idea that the *Eunicea* subgenera should be elevated to the level of genera.[42] The results of this work again support that idea. In addition to the chemically well studied species, Bayer reported two other species; *laxispica* and *pinta*. He stated that *E. laxispica* might just be a morphological extreme of *E. mammosa*, and *E. pinta* might be a deep water variant of *E. mammosa*. Neither were collected for this work. Further study of the chemistry of these species will more clearly demonstrate the biochemical relationships of the intra- and inter-subgenera.

While gorgonians of the subgenus *Eunicea* s.s. showed similar chemical patterns, animals of the other subgenus *Euniceopsis* showed great diversity in their chemical contents. Several chemotypes were defined by this work. Animals of four chemotypes (E, L, O, and I) possessed morphological characters different from the known species. Further study will demonstrate that some of these might be "new" species. *E. fusca* (chemotype K) was collected from each of the four collection locations. Regardless of the collection sites, the TLC characteristics of specimens were identical with each other. The major metabolites, fuscol (**84**) and fuscol arabinoside (**85**), were easily recognized by TLC analysis. Further work may show that these are the idiosyncratic chemicals which are useful as taxonomic markers.

F. M. Bayer found that there were limitless morphological variations in the *E. tourneforti* "complex".[32] TLC analysis also showed corresponding variations. While their TLC characters were very different from the other chemotypes, it seemed that there were significant variations of the relative concentrations of secondary metabolites among individual colonies. However, this would be clarified only after larger scale collections

and extensive chemical investigations.

Other confusing groups were chemotypes U and N. Chemotype U collected at the Tobago Cays was identified as *E. calyculata*, while chemotype N from the Tobago Cays and Florida Keys was identified as *E. laciniata* and *E. cf calyculata*, respectively. Regardless of their morphological classification, chemotype N from the different collection locations were chemically very similar to each other. Gorgonians from the Tobago Cays contained six diterpenoids of the dolabellane class. Specimens from the Florida Keys possessed four of the same six metabolites, but they did not possess the small amounts of two minor metabolites found in samples from the Tobago Cays (chapter 6). In addition, both of the extracts contained the same compound (**64**) as the dominant metabolite. Therefore, they are, from the point of chemical characters, the same organism. From *E. calyculata* collected at Carrie Bow Cay, Belize, and the Bahamas, Look and coworkers isolated metabolites of three different classes; dolabellanes, cubitanes, and cembranes.[25,26] In this work however, dolabellanes and cubitanes were not isolated from the same specimen. The sympatricity of *E. calyculata* and *E. laciniata* at the Tobago Cays suggests that the homogeniety of previous collections is questionable. Moreover, isolation of the cembrane, crassin acetate (**21**) from the Belize samples further supports the possibility of mixed collection. In this work, crassin acetate was isolated from *E. mammosa* (chemotype H). Crassin acetate, a highly functionalized cembrane lactone, does not structurally belong to the subgenus *Euniceopsis* but to the subgenus *Eunicea* s.s.. Specimens of *E. calyculata* and *E. laciniata* looked very similar to each other. However, they were readily distinguishable by TLC analysis. Although the real source of the each class of metabolites from the Belize samples is unknown, separation of *E. calyculata* and *E. laciniata* by TLC analysis emphasizes the importance of chemical characters for the separation of morphologically similar organisms.

The lack of reliable characters for morphological systematics with the gorgonians

is mainly due to the lack of information on the normal range of variations of important characters and the effects of the environment upon them.[1,42] Unfortunately, at present we also know little about the variations of chemical contents of gorgonians.[42] Systematic studies of spatial and temporal variations have not been undertaken, nor the effect of the environment been clarified. Information from a few scattered data showed that there are both spatial and temporal variations of the terpenoid contents of some gorgonians.[4] Among the *Eunicea*, *E. mammosa* yielded eunicin (**1**), jeunicin (**3**), cueunicin (**5**), cueunicin acetate (**6**), or eupalmerin acetate (**4**, chapter 1) depending on the location of the collection.[4] An analogous variation has been also observed in *E. palmeri* and *E. succinea*. Even in the same location, the terpenoid content showed a long term variation. *E. mammosa* collected at Jamaica, in 1960 yielded pure jeunicin, while successive annual collections at the same location showed a trend of jeunicin being replaced by eunicin.[4] Although these results do not rule out the use of chemical characters for the systematics, the interpretation of chemical characters should be carefully carried out.

All of the reported data on the variations of the terpenoid contents are limited to the change of functionalization on the carbon skeleton, not to the changes of the skeleton itself. This observation led Gerhart to the suggestion that emphasis on the carbon skeleton of a terpenoid rather than the presence of certain functional groups should reduce the problems posed by variation in chemical content.[42] Although chemical analysis of *Eunicea* is far from completion, chemical contents found by this work revealed that his suggestion may not comply with the systematics of *Eunicea*. Some chemotypes seemed to be better defined by the presence of the specific funtionality and/or partial structure than by the presence of the carbon skeleton of metabolites. Cembranes were isolated from several chemotypes. However, there were almost chemotype - specific patterns on types and sites of the functionalization on the carbon skeleton. The chemical criteria differentiating the subgenera *Eunicea* s.s. from the *Euniceopsis* is the presence of lactone moieties in only,

and all, the cembranes from the *Eunicea* s.s. Cembranes from *E. calyculata* (chemotype U) possessed the ketone at the unusual position (C-6) that might cause the rearrangement of cembranes to the coexisting cubitanes. Therefore, for the definition of chemotype U, the presence of cembranes with the C-6 ketone functionality might be as important as the presence of the cubitanes.

Similar trends were found in other carbon skeletons. Asperketals in chemotype A possess a common feature not of the carbon skeletons but of their rare ketalic and hemiketalic functionalities. The coexistence of diterpenoids of two "extended sesquiterpene" classes - germacrane and elemane - in the same chemotype suggested that in some cases, carbon skeletons themselves are subject to a change before functional groups. *E. fusca* (chemotype K) might be more distinct from the other *Eunicea* species by its ability of glycosidation rather than ability to form "extended elemane" carbon skeleton. Therefore, the comparison of chemical characters revealed that the classification of *Eunicea* by carbon skeleton of their metabolites only might be misleading. The presence of certain functional groups formed by species specific biosynthetic processes rather than the presence of carbon skeleton suits better for the chemical classification of some species. Thus, for the reliable classification of *Eunicea*, not only chemical data but also information on their biosynthetic process is required. One of the bases of chemosystematics is that the chemical characters reflect genetic differences.[42,48] The chemical characters are the result of certain biosynthetic process which in turn reflects the presence of particular genes in the organism. Therefore, correct information on the biosynthetic process of chemical characters will help to understand the genetic differences of species.

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86. For example, compare the 13C NMR data for eunicin (**1**)and jeunicin (**3**, Chapter 2).

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Part II.

Exploratory Studies of the Secondary Metabolites of Marine Fungi

Chapter I

Introduction

Fungi are widely distributed in the earth, and are active members of most ecosystems. Their great diversity in anatomy and life cycles makes even their definition controversial.[1] Due to the saprophytic and parasitic modes of their nutrition, fungi cause severe damage on wild life and economically valuable crops. Also, some fungi cause serious diseases of man and other domestic animals. This led to numerous investigations of the biology and ecology of these organisms. Consequently, more than a hundred thousand fungal species have been described from the terrestrial environment, and it is estimated that far more species may be yet discovered.[1]

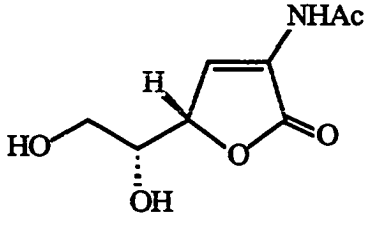
Fungi are also widely distributed in the marine environment, and they are particulary abundant in many coastal and shallow water habitats such as mangrove reefs, sand beaches, and salt marshes.[2-9] Despite the ecological importance of fungi in marine habitats however, marine micology is a relatively young field of science.[2,6] Even the definition of marine fungi is still under controversy among mycologists.[4-6] Several attempts to determine the physiological factors which would effectively distinguish the marine fungi from the terrestrial ones, have failed.[5] However, over 500 obligate marine fungi which grow and sporulate exclusively in a marine or estuarine habitats, are defined and their number will increase with time.[6]

Knowledge of the ecology and physiology of these organisms is very limited. First, ecological research has emphasized mainly taxonomy and morphology.[3-9]

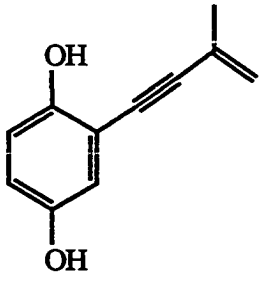
However, in drawing attention to the potential importance of these organisms in the food webs of marine and estuarine environment, few authors cited evidence suggesting specific nutritional relationships between marine fungi and detritus feeders.[10,11] Competition and antagonism among lignicolous higher marine fungi have been studied,[12,13] and a suggestion, although inconclusive, was made that competition is an important component of their interaction.

Secondly, information on the physiological aspects of marine fungi is restricted to environmental demands such as light, pH, salinity, and nutrients.[5,7,14] Diseases of marine plants and animals caused by pathogenic fungi are known.[15-17] However, these studies have focused only upon the symptoms in infected hosts, and direct mechanisms for the production of the diseases are unknown.

Consistent with the lack of ecological and physiological information, studies of the metabolites produced by marine fungi are also very scarse. In contrast to the numerous novel metabolites from the terrestrial fungi,[18] our knowledge of the metabolites of marine fungi is limited to a few common amino acids,[19] fatty acids,[20,21] steroids,[22-25] and two secondary metabolites: leptosphaerin and siccayne. Leptosphaerin, a 2-aminohexose derivative was isolated from the culture broth of the ascomycete *Leptosphaeria oraemaris*.[26-28] A monoprenyl hydroquinone, siccayne, originally isolated from the terrestrial deuteromycete *Helminthosporium siccans*, was isolated from the basidiomycete *Halocyphina villosa*.[18,29]



leptosphaerin



siccayne

From an ecological point of view, studies of the metabolites of marine fungi are meaningful from two aspects. Those are: 1) the adaptation of these organisms to their environment, and 2) competition with other organisms. First, despite the lack of knowledge on the biology of marine fungi, from the distribution of habitats and abundance it is apparent that these organisms successfully adapt to the marine environment.[5,6,8,9] As a result, metabolites of marine fungi, like other marine organisms, could be predicted to be different from those of terrestrial fungi.

Second, there is no doubt that marine fungi must interact with other organisms in the same habitat. Based upon the saprophitic and parasitic modes of their nutrition, a significant interaction might be antagonistic to regulate their competitors and hosts.[30-34] This antagonism has been observed in the case of mixed cultures of higher marine fungi within the same habitat.[13] It is well known that certain metabolites play important roles in antagonism among various microorganisms, plants, and some animals.[32,34] Studies focused on the antagonistic interaction of terrestrial fungi with other microorganisms led to the isolation of over 1000 metabolites which inhibit or destroy microorganisms.[34] Also, a vast number of biologically active metabolites have been isolated from plant and animal pathogenic terrestrial fungi. Some of these metabolites are known to play a role in the interaction between the host and parasitic fungi.[32]

From the point of successful adaptation of fungi to the marine environment, and the antagonistic interaction with other organisms, studies of metabolites from marine fungi can be focused on three different but somewhat overlapping groups of fungi. Those are: a) lignicolous marine fungi, b) fungi isolated from habitats other than cellulosic substances (mostly facultative fungi), and c) pathogenic marine fungi.

a. Lignicolous marine fungi

Wood and other cellulosic substances are dominant habitats of higher marine fungi, especially ascomycetes and deuteromycetes. Consequently, the lignicolous fungi are the largest group of known obligate marine fungi.[6] Moreover, their ecological aspects, such as host specificity, successions in habitat, and degradation rates of wood, have been studied more than any other type of marine fungi.[5,6,35] These organisms possess interesting features as follows:

1) There is no argument among mycologists regarding the identity of these fungi as being obligate marine. Also, it is known that these organisms play a very important role in coastal and estuarine ecosystems.[5,6]
2) In the terrestrial environment, most lignicolous fungi are basidiomycetes.[33] In contrast, all but two species of marine lignicolous fungi are ascomycetes and deuteromycetes.
3) These organisms severely compete with each other when they are grown in the same space. It was thought that the nature of competition is the production of antifungal metabolites.[12,13]

Lignicolous fungi are apparently a representative group of marine fungi. Studies of their metabolites will broaden our knowledge on both adaptation of marine fungi to the environment and their ecological roles. Also, the isolation of antifungal metabolites will enable us to underatand mechanisms of antagonism among these organisms.

b. Fungi isolated from habitats other than cellulosic substances (mostly facultative fungi)

In addition to the obligate marine fungi that are mostly lignicolous, numerous terrestrial fungi survive in the marine environment. Although many are in a dormant form, substantial numbers are active participants of the ecosystems (facultative marine

fungi), and apparently well adapted to the environment.[36] Therefore, their biochemical processes might be different from their relatives in the terrestrial environment, and they might produce different metabolites. It is well known that, among microorganisms, the production of bioactive agents through secondary metabolism is strain specific but not species-specific.[37] From cultures of several actinomycetes which are considered terrestrial but isolated from marine sources, Okami and coworkers have isolated several novel metabolites which were produced only in media of high salt concentration.[37] Thus, study of secondary metabolites from facultative marine fungi might be biochemically and ecologically as important as the obligate marine forms.

c. Pathogenic marine fungi

A vast number of biologically active metabolites have been isolated from plant and animal pathogenic terrestrial fungi and many of their structures have been determined. Some of these metabolites are proved to play a role in the interaction between the host and the parasitic fungus. Although the number of these metabolites are few, this is thought to be mainly because the amounts produced are too small to be detected by chemical methods.[32] In the marine environment, many fungi are known as pathogens of plants and animals. Taxonomic distributions of both hosts and pathogenic fungi are very wide.[15,17,38-41] In general, marine plants are infected by both higher and lower fungi and animals are infected by lower ones. *Pythium porphyrae*, a parasite on red algae of the genus *Porphyra*, *Labyrinthula* sp. on the marine angiosperm *Zostera marina*, and several fungi of the order Lagenidiales on various invertebrates, are a few of well known examples. Some of these pathogens are known to cause severe damage in aquaculture of valuable marine organisms.[39] As shown in the case of their relatives in terrestrial habitats, it would be quite reasonable to predict the presence of bioactive metabolites which are

responsible for the disease symptoms.

Metabolites of marine fungi are virtually unknown. The aim of this work is, through the study on their metabolites, not only to create better understanding in natural products chemistry itself, but also to contribute to the broadening of our knowledge on the ecology of marine microorganisms , especially their adaptations in the environment, and the mechanisms of antagonistic interactions with other marine organisms and themselves.

Due to the difficulty of obtaining bioactive strains, only one pathogenic fungus, *Lagenidium callinectus*, was studied, and most efforts were concentrated on the former two groups, lignicolous and facultative fungi. In total, 121 fungal strains were either isolated by field collections or obtained from our collaborating mycologists. All were successfully cultivated in liquid media. Based upon biological activity, thin layer chromatographic (TLC) analysis and proton NMR spectroscopic analysis of the organic extracts, a few strains were selected for further investigations. Several metabolites were isolated by the large scale cultivation and extraction, followed by separation using traditional chromatographic techniques. The structures of these metabolites were investigated using spectroscopic methods, especially proton and carbon NMR spectroscopic techniques. Unfortunately, all of the metabolites isolated were either those previously isolated from terrestrial fungi, common steroids, or simple aromatic compounds. Moreover, the amount of time and effort spent for the biological experiments made chemical progress very slow. As a result, this work still remains at an early stage. For progress in the future however, it would be worthwhile to summarize the results obtained so far, and to discuss the problems which hinder progress in this field. In the following sections, the biological methods used in this work and chemical investigations performed are briefly summarized. Also, the problems which this work faces are discussed in the later section.

Chapter II

Isolation and Cultivation

a. Isolation of fungal strains

Fungal strains were isolated from various marine algae and invertebrates such as sponges, bryozoans, tunicates, and gorgonians. Also, some strains were isolated from sediments, mangrove reefs and sea foams. The media used for the isolation were agar petri plates made with glucose (10 gm), yeast extract (3 gm), $MgSO_4$ (0.1 gm), and agar (18 gm) per liter of filtered sea water. To prevent bacterial contamination, each 1 l of media contained 0.1 gm of streptomycin sulfate and 0.1 gm of penicillin-G.[6] To remove terrestrial fungi which remained as dormant forms and germinated during the isolation process, isolated strains were grown on agar plates prepared with three different concentrations of sea water: 100 % distilled water, 1:1 mixture of distilled and sea water, and 100 % sea water. Growth of each strain on the agar plates was checked daily. Any strains which grew significantly faster in either distilled water or mixed water media were discarded. In total, 80 fungal strains were isolated by this process. Also, 41 strains of 35 obligate marine ascomycete and deuteromycete species were obtained from Dr. M. Speedie, University of Maryland (Dr. P.W. Kirk Jr.'s collections) and Dr. J. Kohlmeyer, University of North Carolina. These strains were also successfully transferred to the agar plates.

b. Cultivation

The media initially used for the cultivation of fungal strain was the one developed by Sguros and Myers: glucose (5 gm), $MgSO_4 \cdot 7H_2O$ (2.4 gm), NH_4NO_3 (2.4 gm), yeast extract (1 gm), Tris buffer (1.21 gm) and sea water (1 l).[42,43] However, growth of most

strains in this media was very slow, and even after prolonged cultivation (3 weeks) the amount of organic extract (extracted with ethyl acetate) were less than 10 mg per liter of media. Several different sources of carbon (glucose, arabinose, fructose, and glycerol) and nitrogen (peptone and NH_4NO_3) were tried. Based upon the amounts of the extracts and the results of TLC analysis, a final nutrient media was selected for the cultivation of fungi. The composition of the media was: glucose (20 gm), glycerol (3 ml), peptone (3 g), yeast extract (6 g), NH_4NO_3 (2.4 gm), Tris buffer (1 N solution, 10 ml) sea water (1 l). The initial pH was between 7.5 and 8.0.

Inoculations were performed by direct transfer of fungal strains from the agar plates to the liquid media (0.5 ~ 1 l) in 2.8 l Fernbach flasks. Inoculated flasked were stirred with an agitation speed of 100 rpm. Most of the strains grew very fast in this medium, and the broth could be harvested in 15-20 days. Harvest was performed by separation of mycelia from the media by filteration (due to the high density of seawater, separation by centrifugation was not successful), followed by extraction of the media by ethyl acetate. Filtered mycelia were freeze-dried and extracted with ethyl acetate and acetone. The extracts from the media and mycelia were, in most cases, not combined and each was tested separately.

c. Selection of strains for chemical investigation

Selection of a suitable strain for chemical investigation was made by combination of observed bioactivity, TLC analysis, and ^1H NMR analysis of the organic extracts. Bioactivity was tested against the pathogenic fungus *Candida albicans* and the bacteria *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus* sp. Also, several extracts were tested for cytotoxicity (provided by the Bristol-Myers Pharmaceutical Company and the University of Miami). From the extracts of 75 facultative fungal

strains, 29 (39 %) were significantly active against 2 or more test microorganisms (Table). Also, 7 extracts (of 48 extracts, 15 %) showed minimal levels of cytotoxicity. From the extracts of 38 obligate strains, 9 (24 %) were significantly active against microorganisms. Also, 7 extracts (of 36 extracts, 19 %), showed substantial cytotoxicity. TLC analyses were performed on preparative silica plates by with 75 % ethyl acetate in isooctane as developing solvent. The ^{1}H NMR spectra were measured in deuteriochloroform solutions. Based upon the results of bioactivity tests, TLC analyses and ^{1}H NMR analyses of the extracts, several strains were selected for chemical investigation.

d. Large scale cultivation

The medium used for large scale cultivation (10 ~ 100 l) was the same as that used for the initial cultivation. First, fungal strains were grown in 50 ml of media in test tubes for 7 ~ 10 days. Inoculation was performed by using a Waring blender. The homogenized suspensions were transferred to 4 l flasks (containing 2.5 l of media) or 20 l carboys (16 l of media). Flasks were shakn with an agitation speed of 100 rpm and carboys were stirred by air bubbling for 3 weeks. Harvest was performed by filtering and extraction of media was performed with ethyl acetate (2 x volume of media). Mycelia were freeze-dried and exhaustively extracted with ethyl acetate and acetone. The solvents were removed under vacuum.

e. Separation and identification of metabolites

The crude extracts were separated by either silica vacuum flash chromatography (solvents were mixtures of ethyl acetate in isooctane) or Sephadex LH-20 column chromatography (n-hexane-dichloromethane-methanol: 1:4:1). Purification of metabolites was performed by using silica high performance liquid chromatography (HPLC) using

Table. Results of bioactivity tests for fungal extracts.

extracts			antimicrobial activity[a,b]					cytotoxicity[c,d]
			1	2	3	4	5	
facultative fungi	A[e]	--	65	30	71	63	37	41
		+	8	28	2	4	17	
		++	2	11	2	6	10	6
		+++	--	6	--	2	11	1
		total	75	75	75	75	75	48
	B[e]	--	33	25	37	34	30	NT
		+	3	10	--	3	4	
		++	1	2	--	--	2	
		+++	--	--	--	--	1	
		total	37	37	37	37	37	NT
lignicolous fungi	A[e]	--	33	12	30	23	13	29
		+	4	26	8	15	22	
		++	1	--	--	--	3	7
		+++	--	--	--	--	--	
		total	38	38	38	38	38	36
	B[e]	--	38	34	38	35	37	NT
		+	--	3	--	3	1	
		++	--	--	--	--	--	
		+++	--	1	--	--	--	
		total	38	38	38	38	38	NT

[a] 1 ~5 are test microorganisms (1 = *Candida albicans*, 2 = *Bacillus subtilis*, 3 = *Escherichia coli*, 4 = *Streptococcus* sp., 5 = *Staphylococcus aureus*). [b] -- ~ +++ are activities against 20 µg of the extracts (-- inactive, + < 5 mm, ++ 5 ~ 10 mm, +++ >10 mm of inhibition zone, respectively). [c] Cytotoxicity was measured by ED_{50}. ED_{50} < 5 µg are active extracts (++ 0.1 ~ 5 µg, +++ < 0.1 µg). [d] NT are not tested. [e] A and B are extracts from the media and mycelia, respectively.

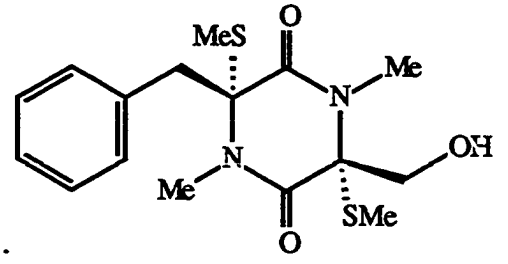
mixtures of ethyl acetate and isooctane). The structures of metabolites were determined by spectroscopic methods, especially ^{1}H and ^{13}C NMR techniques. Results are shown in next chapter.

Chapter III

Results and Discussion

a. Gliovictin from the deuteromycete *Asteromyces cruciatus*

The deuteromycete *Asteromyces cruciatus* F. et Moreau ex Hennebert is an obligate marine fungus, and is frequently isolated from drift wood and from both living and decaying marine algae. A strain (strain number F 156) of this fungus was originally obtained by Dr. P. W. Kirk Jr. in the lower Chesapeake Bay, and was provided to me by Dr. M. Speedie. After fermenting the fungus at 25 °C for 21 days, separation and extraction gave 0.8 gm of organic materials from the media and 2.6 gm of extract from the mycelia (from 5 x 2.5 l of media). Mycelia yielded only triglycerides and sterols, including common 5,8-peroxyergosterol, while TLC and ^{1}H NMR analyses of the extracts from the media showed the presence of single major metabolite, identified as the known fungal metabolite gliovictin.



gliovictin

The isolation of gliovictin was accomplished by combined Sephadex column chromatography (n-hexane-dichloromethane-methanol: 1:4:1) and silica HPLC (30 % ethyl acetate in isooctane). Gliovictin composed 27 % (219 mg) of the media extract, and was identified by comparison of its physical and spectral properties with those reported. The gliovictin isolated was confirmed as the originally isolated (-) isomer by comparison

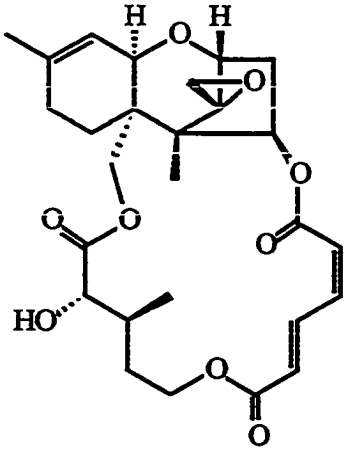
of its rotation {$[\alpha]_D$-62°} with the reported data {Its antipode has $[\alpha]_D$+64°}. Gliovictin is a highly functionalized diketopiperazine of the gliotoxin class. Compounds of this class have been isolated from various terrestrial deuteromycetes.[18] Gliovictin was originally isolated from the fungus *Helminthosporium victoriae*.[44] Its enantiomer (S for the both asymmetric centers) were isolated from the fungus *Hyalodendron* sp.[45]

Gliovictin showed following physical and spectral features: mp 134 - 135°; $[\alpha]_D$-62° (c 1.88, $CHCl_3$); HREIMS: M^+-SCH_3 obsd. 307.1110, $C_{16}H_{22}N_2O_3S_2$ required 307.1118; IR ($CHCl_3$) 3550, 1655, 1425 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.26 (3 H, m), 7.11 (2 H, m), 3.85 (1 H, dd, J = 12.1, 6.0 Hz), 3.72 (1 H, d, 13.8), 3.30 (3 H, s), 3.14 (1 H, d, 13.8), 3.13 (1 H, dd, 12.1, 6.0), 3.02 (3 H, s), 2.30 (3 H, s), 2.13 (3 H, s), 1.40 (1 H, brs); ^{13}C NMR ($CDCl_3$) δ 165.2 (s), 165.1 (s), 133.9 (s), 129.7 (d) x 2, 128.3 (d) x 2, 127.4 (d), 73.2 (s), 71.5 (s), 64.1 (t), 42.1 (t), 30.6 (q), 29.1 (q), 14.0 (q), 13.1 (q).

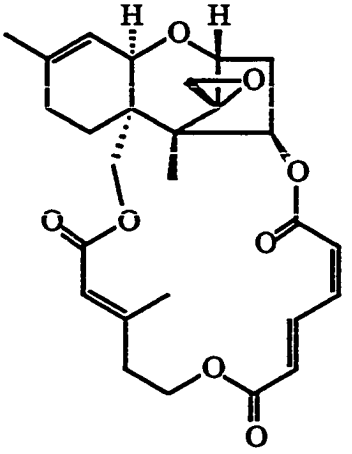
b. Trichothecenes from an unknown fungus

A white fungus (strain number P 58-1) was collected from sediment at Bahia del Los Angeles, Baja California. The extract of this fungus showed strong antimicrobial activity against *C. albicans, B. subtilis* and *S. aureus*. Also, extracts of the fungus showed very potent cytotoxicity (LD_{50} 0.01 μg). After the large scale culture (17.5 l), extraction with organic solvent gave 20 gm of the crude organic materials (Since TLC analysis of the media and mycelia extracts showed the same contents, they were combined). Analysis of the ^1H NMR spectra of the silica vacuum flash chromatographic fractions showed that the moderately polar and polar fractions (40 - 100 % ethyl acetate in isooctane, ca 12 gm) contained very large amounts of several related metabolites (The fractions contained neither fats nor other types of metabolites). Separation by silica HPLC (40 - 70 % ethyl acetate in isooctane) gave four trichothecenes of the verrucarin and

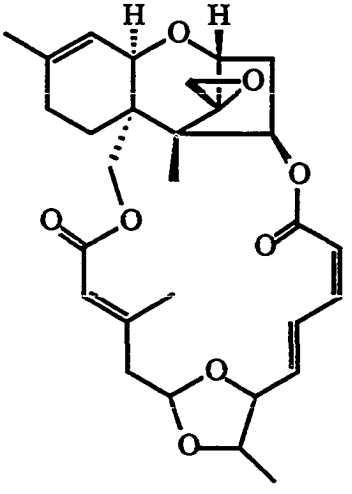
roridin classes. The compounds were verrucarins A, J, and roridins H, K acetate. The structure of verrucarin A was determined by ^{1}H NMR COSY NMR experiments and comparison of recorded ^{1}H NMR data with those of an authentic sample (purchased from the Sigma Chemical Company). The structures of the other metabolites were also determined by comparison of their ^{1}H NMR spectra with reported data.[46-50]



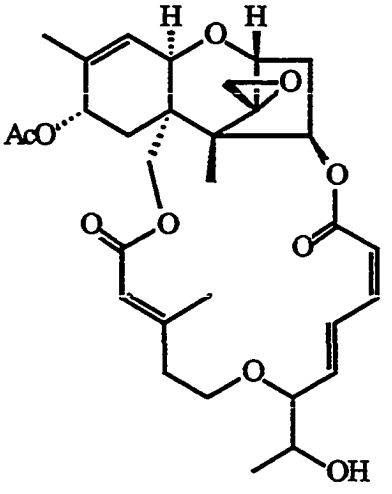
verrucarin A



verrucarin J



roridin H



roridin K acetate

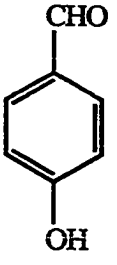
Verucarrins and roridins are among the most potent of the mycotoxins. Due to the wide spectrum of their bioactivities and their complicate structures, numerous investigations of these compounds have been reported. Since the isolation of verrucarin A from the fungus *Myrothecium verrucaria*,[46] successive efforts have resulted in the isolation of about 55 related metabolites.[18] Interestingly, similar metabolites have been isolated from the Brazilian shrub *Baccharis* sp.[51] Although it has not been confirmed, a suggestion that this might be the first known example of the incorporation of fungal gene to the plant gene was proposed.[52] In addition to these four metabolites, the ^{1}H NMR spectra of other fractions showed the presence of several related metabolites. Since metabolites of these classes are well known however, further studies were not pursued.

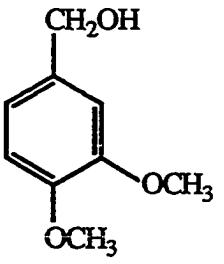
The ^{1}H NMR spectrum ($CDCl_3$) of verrucarin A had peaks at δ 8.04 (1 H, dd, 11.7, 15.6), 6.67 (1 H, dd, 11.3, 11.3), 6.15 (1 H, d, 11.1), 6.05 (1 H, d, 15.6), 5.81 (1 H, dd, 8.1, 3.9), 5.43 (1 H, brd, 4.9), 4.80 (1 H, d, 12.0), 4.51 (1 H, brd, 10.5), 4.21 (1 H, d, 12.1), 4.14 (1 H, brd, 5.0), 3.98 (1 H, dt, 3.0, 11.8), 3.86 (1 H, d, 5.0), 3.57 (1 H, brd, 5.1), 3.12 (1 H, d, 3.8), 2.80 (1 H, d, 3.8), 2.68 (1 H, brd, 7.3), 2.50 (1 H, d, 8.2), 2.46 (1 H, d, 8.4), 2.35 (1 H, m), 2.23 (2 H, m), 1.93 (4 H, m), 1.75 (3 H, s), 1.70 (2 H, m), 1.62 (2 H, brs), 0.88 (3 H, d, 6.5), 0.85 (3 H, s). The ^{1}H NMR spectrum ($CDCl_3$) of verrucarin J had peaks at δ 8.06 (1 H, dd, 15.7, 11.6), 6.62 (1 H, dd, 11.5, 11.1), 6.10 (1 H, d, 11.0), 6.00 (1 H, d, 15.8), 5.99 (1 H, dd, 7.3, 4.2), 5.83 (1 H, s), 5.46 (1 H, d, 5.1), 4.46 (1 H, m), 4.43 (1 H, d, 12.2), 4.15 (1 H, m), 3.96 (1 H, d, 12.6), 3.86 (1 H, d, 5.0), 3.75 (1 H, d, 5.1), 3.15 (1 H, d, 3.7), 2.82 (1 H, d, 3.8), 2.56 (2 H, m), 2.28 (3 H, s), 1.71 (3 H, s), 0.83 (3 H, s). The ^{1}H NMR spectrum ($CDCl_3$) of roridin H had peaks at δ 7.69 (1 H, brdd, 13.5, 13.1), 6.56 (1 H, dd, 11.4, 11.4), 5.95 (1 H, dd, 9.3, 2.5), 5.92 (1 H, m), 5.80 (1 H, d, 11.0), 5.68 (1 H, s), 5.54 (1 H, dd, 8.5, 3.2), 5.41 (1 H, brd, 4.6), 4.33 (1 H, d, 12.4), 4.07 (1 H, brd, 8.2), 4.04 (1 H, d, 12.3), 4.84 (1 H, d, 5.0), 3.67 (2 H, m), 3.13 (1 H, d, 10.0), 2.83 (1 H,

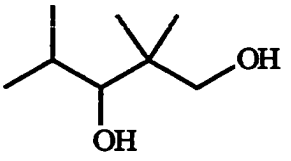
d, 4.1), 2.65 (1 H, dd, 12.4, 2.5), 2.48 (1 H, dd, 15.3, 8.3), 2.28 (3 H, s), 2.26 (1 H, dd, 12.8, 8.6), 2.17 (1 H, dt, 15.3, 4.8), 2.00 (2 H, m), 1.89 (2 H, m), 1.71 (3 H, s), 1.34 (3 H, d, 6.0), 0.86 (3 H, s). The ^{1}H NMR spectrum ($CDCl_3$) of roridin K acetate had peaks at δ 7.45 (1 H, dd, 15.4, 12.5), 6.57 (1 H, dd, 11.4, 11.3), 6.09 (1 H, dd, 8.0, 4.0), 5.92 (1 H, dd, 15.7, 3.0), 5.85 (1 H, brs), 5.74 (1 H, d, 11.2), 5.71 (1 H, brd, 5.4), 5.21 (1 H, d, 4.8), 4.52 (1 H, d, 12.6), 4.11 (2 H, m), 3.92 (1 H, d, 5.4), 3.84 (1 H, d, 5.1), 3.62 (3 H, m), 3.11 (1 H, d, 3.9), 2.84 (1 H, d, 4.0), 2.50 (2 H, m), 2.30 (3 H, s), 2.00 (3 H, s), 1.19 (3 H, d, 6.1), 0.78 (3 H, s).

c. Other metabolites

In addition to gliovictin and the trichothecenes, three small compounds were also isolated from cultures of marine fungi. First, p-hydroxybenzaldehyde was isolated from the lower fungus *Lagenidium callinectes*. The structure of this compound was determined by comparison of the ^{1}H NMR spectrum with the authentic sample. Also, 3,4-dimethoxy benzyl alcohol was isolated from an unknown fungus (strain number P15-1) collected from a blue-green algal (*Lyngbya majuscula*) mat at Puerto Rico. The structure was determined by interpretation of ^{1}H and ^{13}C NMR data. In addition, a small diol, 2,2,4-trimethyl-1,3-pentanediol was isolated from the ascomycete *Halosphaeria quadriremis*. The structure of the diol was determined by interpretation of ^{1}H and ^{13}C NMR data with that reported for similar compounds.







The NMR data of these metabolites are: p-hydroxybenzaldehyde; ^{1}H NMR ($CDCl_3$) δ 9.87 (1 H, s), 7.81 (2 H, d, 7.8), 6.96 (2 H, d, 7.9): 3,4-dimethoxy benzyl alcohol; ^{1}H NMR ($CDCl_3$) δ 6.9 - 6.8 (3 H, m), 4.63 (2 H, s), 3.89 (3 H, s), 3.88 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 149.2 (s), 148.7 (s), 133.7 (s), 119.4 (d), 111.3 (d), 110.7 (d), 65.2 (t), 55.9 (q), 55.8 (q): 2,2,4-trimethyl-1,3-pentanediol; ^{1}H NMR ($CDCl_3$) δ 3.56 (1 H, d, 10.5), 3.44 (1 H, d, 10.6), 3.40 (1 H, d, 2.4), 1.92 (1 H, m), 1.01 (3 H, d, 6.5), 0.97 (3 H, d, 6.6), 0.94 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 83.0 (d), 73.2 (t), 39.1 (s), 29.1 (d), 23.2 (q) x 2, 19.6 (q), 16.6 (q).

Discussion

Chemical investigation of marine fungi has logical reasonings because they antagonistically interact with other organisms and they live in different area from the terrestrial fungi. However, progress on this work has been very slow. It is believed that this is mainly due to the difficulty in finding appropriate culture media. It is well known that the production of secondary metabolites in microorganisms depends very much on the composition of the nutrient media. Okami reported that the production of aplasmomycin in marine *Streptomyces* depends on the concentrations of both salt and nutrients.[37] Also, it is found that certain marine bacterial metabolites are produced only in the presence of water-soluble algal extracts.[37]

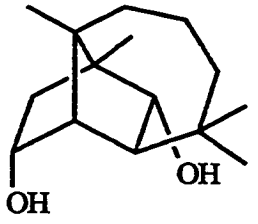
Unfortunately, no appropriate nutrient medium has been developed for the mass culture of marine fungi. Although there are several known media for the culture of these organisms,[5,53] the slow growth rate (4 - 6 weeks) and low yield of organic extract (less than 10 mg per litter of media) revealed that these are not appropriate for chemical investigations. Moreover, from the nature of this work, a medium suitable for several

fungi was needed. After several experiments, a nutrient medium with high concentrations of two carbon sources were chosen: 20 gm of glucose and 3 ml of glycerol per liter of media. Also, peptone (3 gm per liter) and NH_4NO_3 (2.4 gm) were chosen as nitrogen sources. The reason for using not one but two nutrients for carbon and nitrogen sources was to satisfy the different nutritional demands of various fungi. Another reason was the differentiation between the media for rapid growth of microorganisms and the one for the production of secondary metabolites. It is well known that many microorganisms do not produce secondary metabolites in the rapidly growing media (catabolic regulation).[54] Since marine fungi did not grow well in most media however, there was no choice but to use rapidly assimilated nutrients such as glucose and NH_4NO_3. It was expected that at the initial stage, fungi selectively uptake nutrients and rapidly increase their biomass. After the depletion of preferred nutrients, the large biomass of cells could utilize the other nutrients and begin to produce secondary metabolites. This medium seemed to work very well for the production of trichothecenes, and large amounts of the extract (1.1 g per liter of media) and high concentration of metabolites (ca 60 % of the extract) were obtained. This media also worked well for the obligate fungus *Asteromyces cruciatus* and yielded relatively large amounts of extract (64 mg per liter) and high concentration (27 % of the extract) of gliovictin. However, the possibility of the catabolic inhibition for the production of secondary metabolites in other fungi can not be ignored.[54]

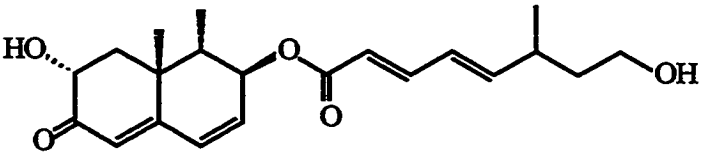
In addition to the problem of developing suitable media, another possible reason for slow progress might be the selection of unsuitable fungi for chemical investigation. It is well known that the distribution of biologically active metabolites among terrestrial microorganisms is generally restricted to a few groups of organisms such as soil actinomycetes and plant pathogenic fungi.[18] In this work major efforts were invested on the lignicolous fungi. Although their extracts showed substantial biological activities (Table), TLC and ^1H NMR analyses showed that the chemical contents of many extracts

were very similar. The biologically active metabolites might be simple phenolic compounds, triglycerides and/or some steroids, possibly 5,8-peroxy ergostrol derivatives which were isolated from a few lignicolous fungi. Thus, although it has not been conclusively shown, lignicolous fungi are suspected as organisms from which the possibility of isolation of secondary metabolites is relatively lower than other fungi.

Despite the slow progress on this work, the potential of marine fungi as a source of novel secondary metabolites has not been diminished overall. Findlay and coworkers recently isolated a known antifungal sesquiterpene, culmorin from the culture of the ascomycete *Leptosphaeria oraemaris*.[55,56] Also, Pietra and coworkers isolated dendryphiellin A, a novel trinoreremophilane from the marine deuteromycete *Dendryphiella salina*.[57]



culmorin



dendryphiellin A

Both the norterpenyl part and side chain of dendryphiellin A are unprecedented in terrestrial fungi. This is the first metabolite which could be claimed as true marine fungal metabolite. With more information on their ecology and physiolgy, development of optimal media and isolation of suitable strain will lead to the isolation of numerous biologically active and structurally unique metabolites from marine fungi.

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