EMBLIDE AND TROCHELIN

TWO NOVEL DITERPENOID CONSTITUENTS

OF PACIFIC SOFT CORALS,

SARCOPHYTON SPP.

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
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By

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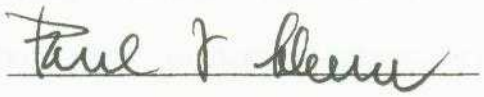

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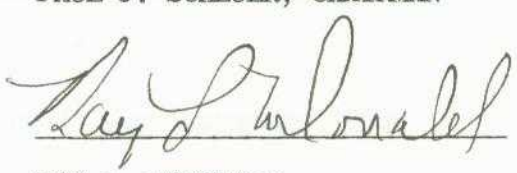

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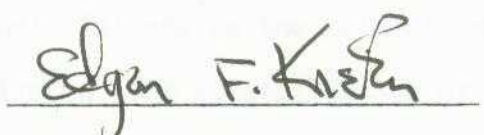

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The support of my parents, Andras and Theresia, has always been greatly appreciated. Without their love, understanding and encouragement, this work would not have been brought to completion.

The numerous friendships which have resulted from pursuing this degree have served to make this period most enjoyable.

ABSTRACT

The discovery of prostoglandins from the gorgonian, *Plexaura homomalla*, rendered recognition in the chemical community of a previously obscure subclass of coelenterates, the Alcyonaria or Octocorallia, to which the gorgonians belong (Chapter I). Since then a number of investigators have scrutinized the Gorgonacea and other alcyonarians. While numerous interesting compounds have been isolated, C_{20} diterpenoids with the 14-membered cembrane ring system are emerging as the characteristic constituents of the octocorals.

A number of cembrane diterpenoids have been isolated from various sources (Chapter II). Those isolated from terrestrial sources exist largely as olefins and alcohols. Highly functionalized cembrane derivatives are emerging as characteristic metabolites of some marine invertebrates.

In the course of the sytematic investigation into the chemistry of marine invertebrates within our research group, several Pacific soft corals were examined. Emblide, a novel marine cembranolide bearing acetoxy, dienoic ester and an unsaturated seven-membered lactone, was isolated from the soft coral, *Sarcophyton glaucum*. The soft coral, *Sarcophyton trocheliophorum*, yielded another diterpenoid, trochelin. The isolation and structure elucidation of emblide and trochelin is presented (Chapters III and IV).

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LIST OF ABBREVIATIONS AND SYMBOLS

boiling point	bp
broad	br
carbon-13 nuclear magnetic resonance	^{13}C nmr
complex signal	cs
doublet	d
doublet of doublets	dd
editor	ED.
melting point	mp
nuclear magnetic resonance	nmr
angle of optical rotation	α
proton magnetic resonance	^{1}H nmr
thin layer chromatography	tlc
ultraviolet	uv

PREFACE

Coral reef coelenterates are among the most successful marine organisms; because of their symbiotic zooxanthellae they produce organic matter in waters poor in essential nutrients. The primary productivity of coral reefs is far greater than that of any other marine environment except for certain specialized grass communities.[1,2] Recently, attention has been called to the coral reefs as a possible source of petroleum as there are several examples of reefs that are potential precursors of petroleum with the Leduc Field as an example of a commercial oil pool associated with an ancient reef.[3]

The biosynthetic versatility of reef coelenterates is indicated by a growing list of novel compounds recently isolated from horny corals (Gorgonacea) and from soft corals (Alcyonacea): prostoglandins, sterols, secosterols with unusual sidechains, butenolides, sesquiterpenes and diterpenes including cembranolides. The coral reef offers tantalizing prospects for the biochemist with its inexhaustible supply of novel compounds with potential value as drugs or as tools for pharmacological research.

Happy is he who has knowledge
from research and does not turn
to injury of his fellows or to
unjust deeds, but looks upon the
ageless order of eternal nature
to learn in what way and where
and how it came to be.

Anonymous

CHAPTER ONE

INTRODUCTION TO COELENTERATE BIOLOGY

The phylum Cnidaria, or Coelenterata, includes the hydras, jellyfish, sea anemones and corals. The coelenterates were known to the ancients as the writings of Aristotle show. The brilliant coloring of many species combined with the radial symmetry often creates a beauty surpassed by few other animals. Because of the plant-like form of many species, the coelenterates were thought to be marine plants by the scholars of the Renaissance period. The seeming combination of plant and animal charracteristics bedeviled scientists, as awareness of the living world grew with the development of sophisticated means of observation.

Although the coelenterates are of relatively little direct economic value, they are important to man in many other ways. Corals of the remote geological past formed reef structures that were highly favorable sites for the accumulation of petroleum deposits. A knowledge of the biology of modern reefs provides an insight into the circumstances that led to the production of oil in ages past. Recently, the chemical constituents of the coelenterates have come under observation yielding many interesting compounds of unique structural and biological characteristics.

GENERAL FEATURES

The coelenterates are among the lowest eumatazoan phyla - that is, animals constructed of well-defined tissues having distinct form and symmetry and with a digestive tube called a *coelenteron*. The digestive tract opens to the exterior by a mouth but lacks an anus. Unlike sponges, coelenterates have a continuous surface that is not perforated by numerous pores. Although in their high degree of individuality the coelenterates show a marked advance over the sponges; they lack cephalization and show no trace of a centralized nervous system. They have simple digestive, muscular, nervous and sensory systems constructed of the basic epithelial, muscular and connective tissues. Definite respiratory, circulatory and excretory systems are not present. The genital systems consist only of sex cells localized in gonads. Definite symmetry which appears first in the coelenterates is of a radial nature and is arranged around the main oral-aboral axis of the body. Coelenterate construction is of the tissue level of complexity with two fundamental layers, the ectoderm and the endoderm, and a layer of mesogloeal jelly in between. The mesogloea ranges from a thin noncellular membrane to a thick, fibrous jellylike, mucoid material. The cnidarians have remained rather primitive although anticipating some of the specializations found in higher metazoans with only a limited degree of organ development (Figure 1).

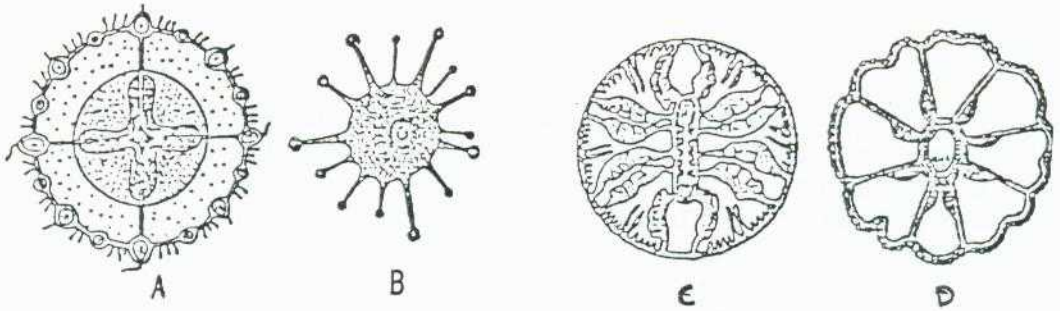


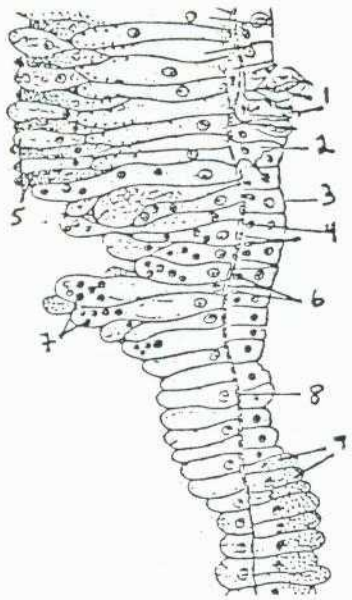
Figure 1. Types of coelenterate symmetry (by author). A. Hydrozoan medusa. B. Hydrozoan polyp. C. Actinian polyp, cross section of pharynx. D. Octocoral polyp, cross section of pharynx.

The epidermis may be ciliated or flagellated. Frequently, cell boundaries are ill-defined and the epidermis is a multinucleated mass of protoplasm. Two kinds of gland cells are found among the supporting cells of the epidermis, *mucous* and *granular*. *Mucous* cells are more abundant and produce secretions which serve adhesive or protective functions; *granular* cells are found only sparingly in the epidermis but are concentrated in the pharyngeal lining of the anthozoans, where they produce secretions with a digestive function.

The primitive nervous system consists of sensory cells arranged in an irregular nerve net located beneath the epidermis and concentrated around the mouth. They apparently serve as undifferentiated receptors. The presence of free sensory nerve endings have been alleged but not adequately demonstrated.

Situated here and there among the epidermal cells are the interstitial cells which are capable of development into various cell types, such as cnidoblasts and germ cells and thus are important in regeneration activities (Figure 2). The *nematocysts* are the most characteristic structure in the coelenterates. Aside from one known instance in the phylum Ctenophora, they are not found outside the Coelenterata. Though

Figure 2. Diagrammatic cross section of hydrozoan polyp wall[4]. 1. Nematocysts. 2. Sensory cells. 3. Granular border of epidermal cell. 4. Interstitial cell. 5. Gland cells. 6. Epidermal cells. 7. Food vacuolaes. 8. Muscle bases of epidermal cells. 9. Epidermal gland cells of pedal disc.



they are frequently known as "stinging cell," they are actually structures produced by special cells, the cnidoblasts or nematocysts. There are two main structural types: the true nematocysts with double walls and a tube usually armed with spines and often with a thicker basal and a thinner terminal part; and the spirocysts with single walls and an unarmed tube of uniform diameter. Nematocysts are found in all coelenterate groups but spirocysts are confined to the subclass Zoantharia (Figure 3) The capsules of the nematocysts contain a mixture

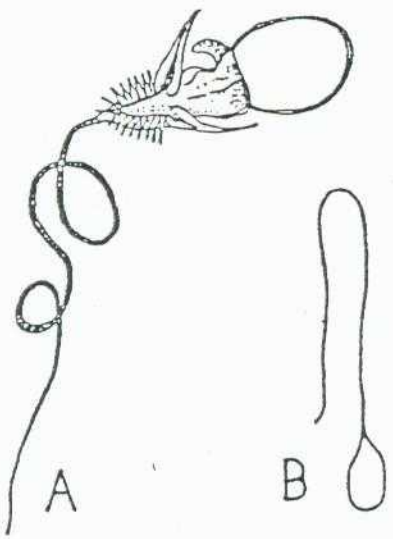


Figure 3. Nematocyst structural types (by author). A. The true nematocyst with a double wall and a tube usually armed with spines and often with a thicker basal and thinner terminal part. B. The spirocyst with single walls and an unarmed tube of uniform diameter.

proteins and phenols whereas those of the spirocysts contain mucoprotein or glycoprotein.[5] Although the chemistry of the nematocysts is still not completely known, their toxicity is appreciated by those who have been stung by sea nettles or Portuguese men-of-war. The toxin is sufficiently powerful to subdue active animals such as fishes and in some species of the Cubomedusae it is virulent enough to be fatal to man.

In spite of their small size, the nematocysts have very distinctive features showing modifications in various taxa. Systematists have made much use of them in classification and have evolved an elaborate terminology for the various types of nematocysts.

All coelenterates are basically tentaculate and radially symmetrical with two different structural types found within the phylum. The sessile form is known as the polyp while the free-swimming form is called the medusa. Typically, the body of a polyp is a tube or a cylinder in which the oral end, bearing the mouth and the tentacles, is directed upward and the opposite, or aboral end is attached. On the other hand, the medusa resembles a bell with the convex side upward and the mouth located in the center of the concave undersurface The tentacles hang down from margin of the bell. In contrast to the polypoid mesogloea (middle layer) which is more or less thin, the medusoid mesogloea is thick and makes up the bulk of the animal and because of this mass of jellylike material, these cnidarian forms are commonly known as jellyfish. Some cnidarians exhibit only the polypoid form, some only the medusoid while others pass through both in their life

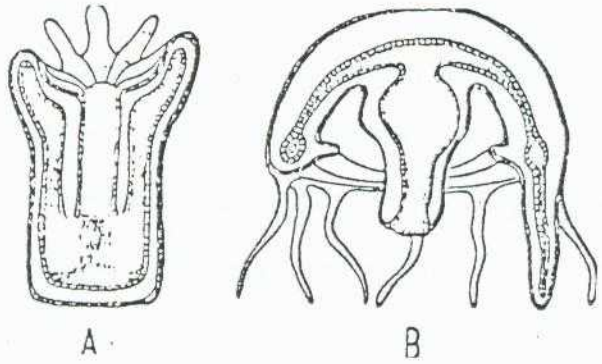


Figure 4.
General coelenterate body plan (by author). A. polyp. B. medusa.

cycles (Figure 4). Because of their simple construction and great adaptability, the fundamental medusoid and polypoid body plans of the coelenterates, by relatively minor modification, have given rise to the various distinctive orders of the three great orders, Hydrozoa, Scyphozoa and Anthozoa. The diversity of these animals is astounding, and it is difficult to believe that such different-looking organisms as a hydra, a jellyfish, a Portuguese man-of-war, a reef coral and a sea pen are really nothing more than a variation of the simple body plan.

In many shallow-water coelenterates, the gastrodermis is densely packed with symbiotic algal cells. These are green in the freshwater species and are called zoochlorellae but are typically brownish or yellowish in most marine forms, where they are called zooxanthellae. The former when outside their coelenterate hosts prove to be the algae of the genus *Chlorella*, and the latter, long resistant to identification, have been fairly well established as belonging to the class Dinoflagellata of the algal phylum Pyrrophyta.

A brief representation and discussion of the orders and classes of coelenterates with emphasis on the class Anthozoa, subclass Octocorallia, follows. An extensive treatement on the phylum Coelenterata is beyond the scope of this report.[6,7]

Class Hydrozoa

All members of the class Hydrozoa share certain distinctive characteristics that set them apart from all the other coelenterates. For the most part they have both polypoid and medusoid stages in their life history, although some exist only as polyps and others only as medusae. Their symmetry is radial and either tetramerous or polymerous; the mouth is situated on an elongated *manubrium* and lacks a pharynx; and there are neither septa nor nematocyst-bearing structures in the coelenteron. The hydroid form is the familiar one in this class, as the medusae are mostly small, transparent and rarely seen by the casual observer. The hydroid form is usually colonial and consists of the simplest of the polypoid type of individual (Figure 5) which is divided into the *base*, *stem* and *hydranth*. The *base* is an area of attachment and ordinarily consists of a glandular zone that secretes adhesive substances. In solitary forms, as in the hydra, the proximal end terminates in a pedal disc. In colonial hydroids (Figure 6) tubular branches called *stolons* grow out from the base and produce a complicated network, the *hydrorhiza*, which extends along the substrate anchoring the colony firmly. The *hydrorhiza* gives forth either simple stalks with terminal hydranths, or the main stem of a lony with numerous side branches bearing hydranths.

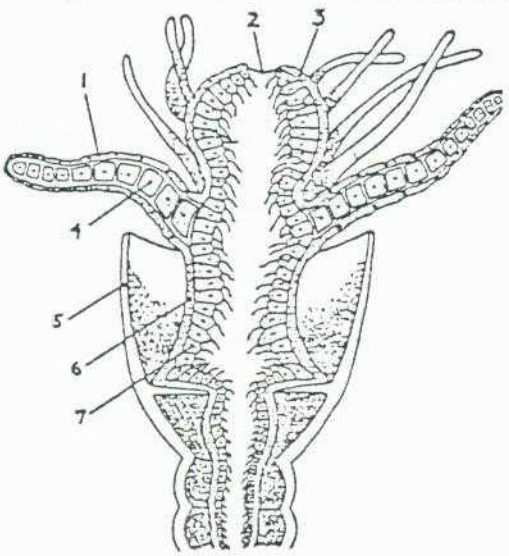


Figure 5. Longitudinal section through hydranth of hydroid (by author). 1. tentacle. 2. mouth. 3. hypostome. 4. cellular core. 5. hydrotheca. 6. ectoderm of hydranth. 7. gastrodermis.

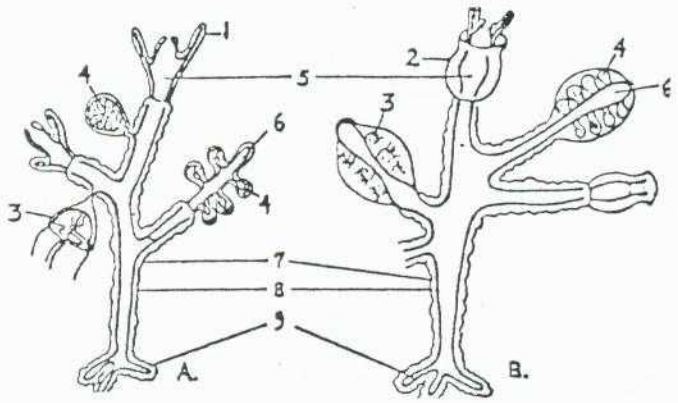


Figure 6. Colonial hydroids (by author). A. Athecate hydroid colony showing various reproductive structures. B. Thecate hydroid colony showing sporesacs and medusa. Code: 1, tentacles; 2, hydrotheca 3, medusa; 4, sporosac; 5, hydranth; 6, blastostyle; 7, perisarc; 8, hydrorhiza, 9, hydrocaulus.

Class Scyphozoa

The class Scyphozoa includes the most familiar of all the coelenterates, the common jellyfish and sea nettles, as well as the largest, the gigantic *Cyanea*, a medusa that may measure as much as two meters across the umbrella. Among the coelenterates, the *Cyanea* is exceeded in size only by the massive reef corals, but these are colonies, not individuals. The medusoid phase dominates the polypoid stage and is reduced to an inconspicuous organism often lacking in some species.

Scyphozoan medusae are much larger than the hydromedusae previously discussed, which are commonly 15 to 30 cm in diameter. The tetramerous symmetry is a conspicuous feature. The umbrella varies in form from cuboidal or a tall dome-shape to a shallow saucer. It is ordinarily of a firm cartiligeous consistency of the mesogloea containing many fibers and wandering amoeboid cells. The edge of the bell is notched and fringed with tentacles. Sense organs occur in the notches and alternate with the tentacles in a definite sequence arranged in some multiple of four (Figure 7).

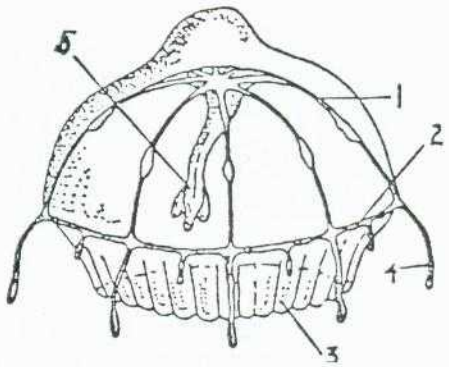


Figure 7. Narcomedusa, *Cunina prolifera*. Code: 1, marradial canal; 2, ring canal 3, velum; 4, tentacle; 5 marginal lappet

Class Anthozoa

The Anthozoa, third of the coelenterate classes, is characterized by complete suppression of the medusoid stage. Included here are the well known stony corals, soft corals, sea anemones and sea pens. The polyps differ from those of the hydrozoans in a number of ways noticeably by being larger, short and squat instead of tall and slender with a flattened oral end, the oral disc, which is usually surrounded by hollow tentacles. Unlike the situation in the hydrozoan polyp, the mesogloea is usually quite thick and richly cellular. In some anthozoans, the cells of the mesogloea secrete calcareous spicules and there may be a horny ectodermal secretion that forms a supporting structure for the colonial aggregates of polyps.

On the basis of their symmetry and other characteristics, the Anthozoa are divided into two subclasses, one homogeneous as to all essentials but showing diversified specialization, the other quite heterogeneous but showing much less diversity. The subclass Octocorallia, or Alcyonaria, is comprised of colonial forms whose polyps are basically of uniform construction. Their symmetry is invariably octomerous (Figure 8a) with eight septa and eight pinnate tentacles. The colonies most always produce a calcareous skeleton in the form of spicules in the mesogloea. The nematocysts are uniformly of a single

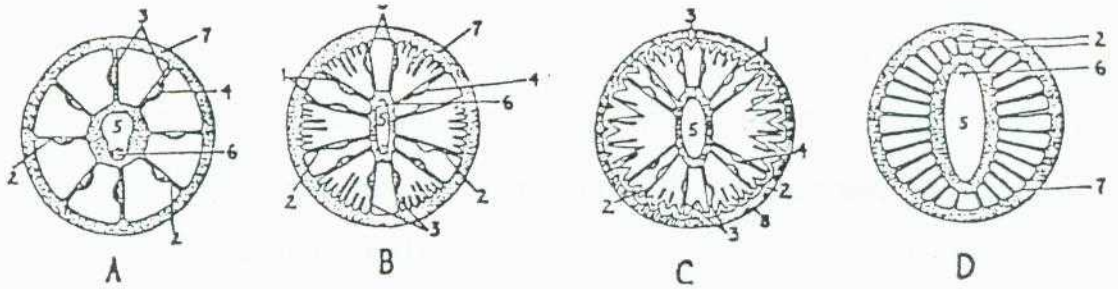


Figure 8. Anthozoan symmetry. Diagrammatci cross sections of polyps. A. Octocorallia. B. Actinaria. C. Scleractinia. D. Ceriantharia. Code: the septa marked 1 are pairs, those marked 2 are couples; 3, directives, 4, retractor muscles; 5, pharynx; 6, siphonoglyph; 7, body wall and 8, skeleton.

type. Diversity of the alcyonarians is expressed in the various modifications of the calcareous spicules and in the variations in structure of axis and organization of the colony.

The other subclass, the Hexacorallia or Zoantharia, is a heterogeneous assemblage of forms whose symmetry as the name implies, is based on multiples of six (Figure 8). Polyps may be either solitary or colonial with or without a skeleton of epidermal origin.

Although the size may vary, the basic plan of the alcyonarian subclass is universally cylindrical, the upper part encircled by a ring of eight tentacles which surround the flattened oral disc. The tentacles bear a series of fingerlike processes, the pinnules, a featherlike arrangement along both sides. The mouth leads from the center of the oral disc into the flattened tubular pharynx. Alternating with the tentacles are eight radial septa all of which extend from the body wall to the pharynx. As alcyonarian colonies are for the most part immovably fixed, their chief movements consist of the food capturing actions of the polyps and the withdrawal of the upper parts of the polyps into the *coenenchyme*, the common tissue connecting the polyps, in times of danger.

The subclass Octocorrallia is divided into six orders. Members of the order Stolonifera (Figure 9a) consist of simple cylindric polyps connected at the base by narrow ribbons of coenenchyme. Species of the order Telestacea are similar, but the polyps grow tall and bud off daughter polyps (Figure 9b). Colonies of the order Alcyonacea, the soft corals, are usually embedded in a thick mass of mesogloea filled with spicules (Figures 9c and 9d).

The order Gorgonacea contains the sea fans or gorgonians and related forms (Figure 10). These all have firm supporting skeletons, often branched, over which the polyps are spread in a layer of coenenchyme containing spicules. In some, the axis may be bright red or pink as in the familiar precious corals. These four orders show a regular progression of complexity and perhaps an evolutionary sequence.

The remaining two orders are the massive blue corals, order Coenothecallia, which resembles millipores or stony corals, and the sea pens, order Pennatulacea. The Pennatulacea are the most complex of the octocorals, being composed of several modified types of polyps. The highly organized colonies (Figure 11) are built around one primary polyp which buds off lateral polyps in a very regular way.

The members of the subclass Zoantharia are as diverse as the Alcyonarians are homogeneous, and it is seemingly impossible to describe the group in terms compatible with all the others. Included in this group are the Actinaria or sea anemones, the Madreporaria or stony corals, the Anthipatharia or black corals and the Ceriantharia or burrowing sea anemones.

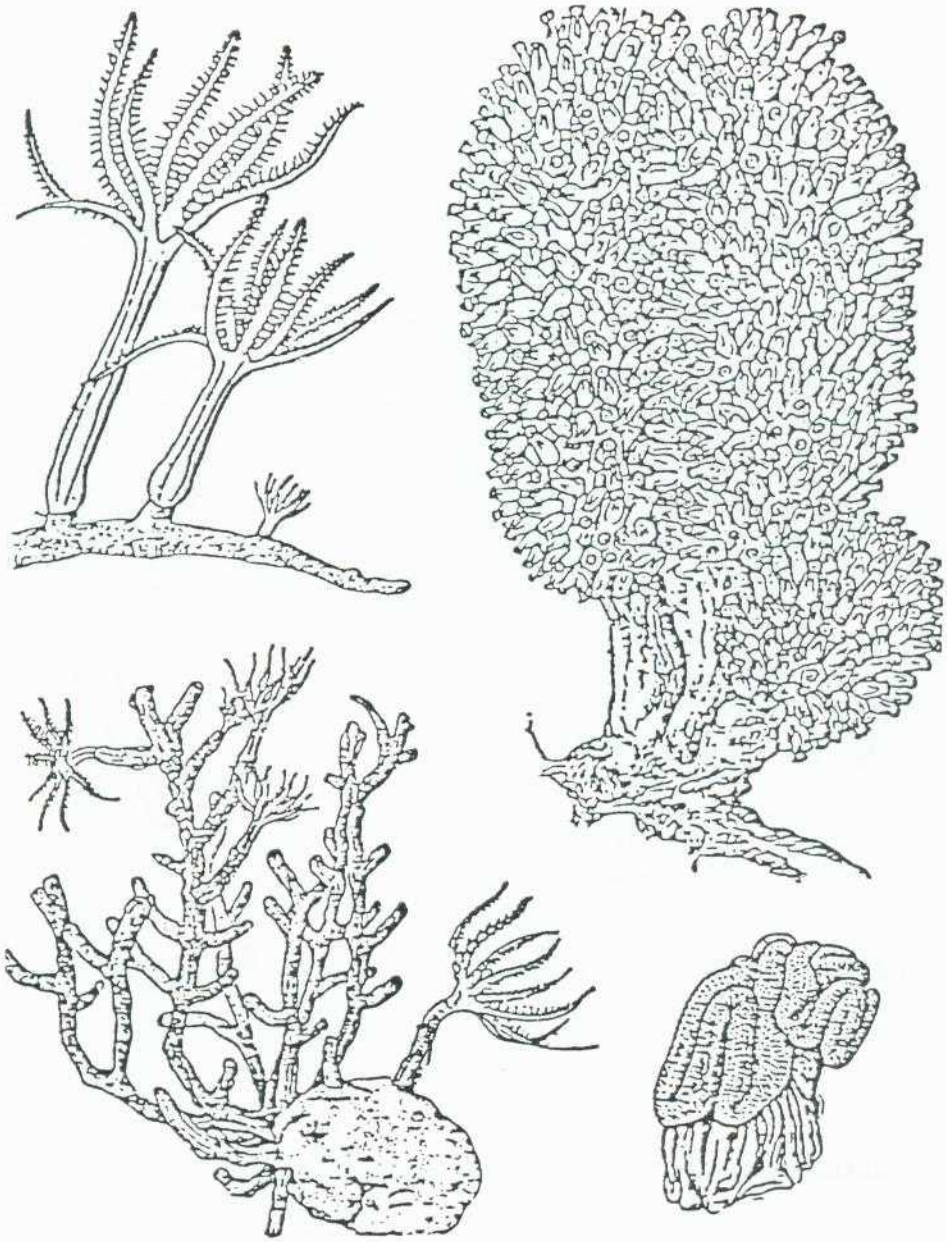


Figure 9. Colonial forms in octocorals. A. *Clavularia*[7] (order Stolonifera). B. Polyps of *Tolesta*[7] (order Telestacea). Colony of *Alcyonium*[8] (order Alcyonacea). D. *Sarcophyton* (order Alcyonacea, by author).

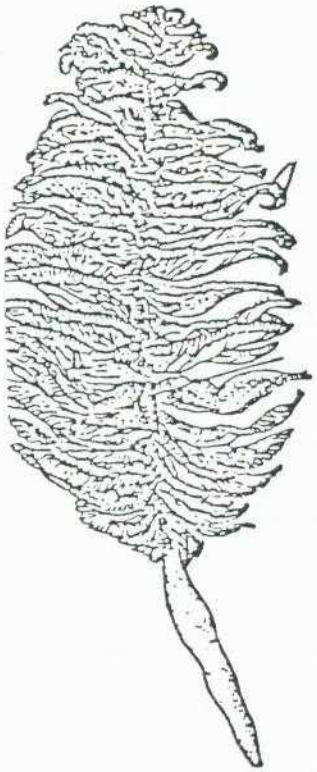


Figure 10. Sea pen.[8]

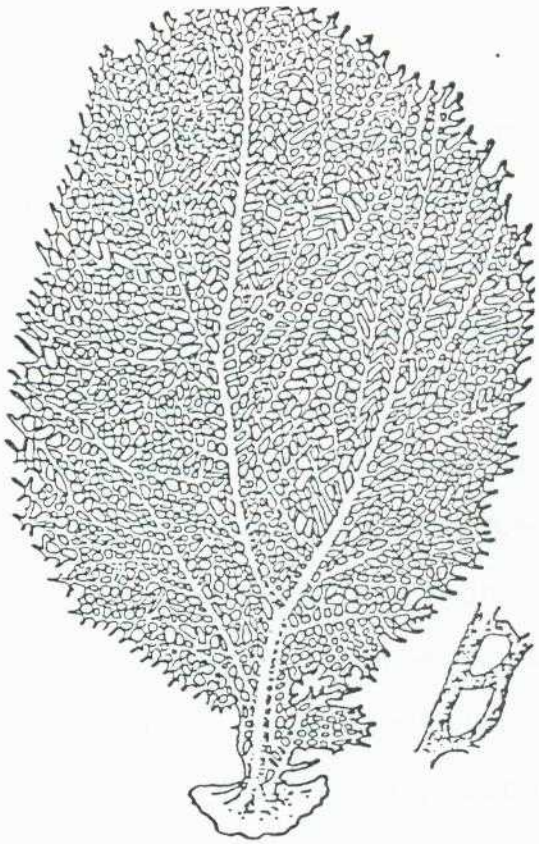


Figure 11. Gorgonian.[9]

organization of the alcyonarian polyp, differing in details. The principal difference between the Alcyonaria and the Zoantharia is the tendency toward hexamerous symmetry. Also, the nematocysts show a greater diversity than in the alcyonarians, which is further indication of the heterogeneous nature of the Zoantharia.

Summary

The coelenterates are a highly diverse and abundant marine form - anemones, jellyfish, corals and hydras are all members of the phylum. The common body form shows radial symmetry. Most have tentacles about the mouth and "stinging cells" for food gathering and defense. They have more complex organization than sponges - the body wall has two distinct germ layers and in most a third, the mesogloea, is evident.

The "tissue level of organization" has led to more specialization including a nerve plexus tthat gives the animal a certain degree of co-ordination. Coelenterates may have two different body forms in the same species. One form is the polyp, which is usually attached to the sub-strate with the free end containing the mouth usually surrounded with tentacles. The other form is the medusa, which is free-swimming and has tentacles and a mouth projecting from the underside of the bell-shaped body,

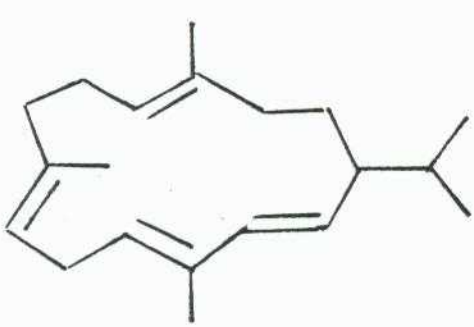
If we are alert with minds and eyes open we will see meaning in the commonplace; we will see very real purposes in situations which we might otherwise shrug off and call 'chance'.

CHAPTER TWO

NATURALLY OCCURRING DITERPENOIDS HAVING A CEMBRANE SKELETON

The chance discovery of prostaglandins in extracts of the gorgonian, *Plexaura homomalla*,[10] rendered recognition to a previously obscure subclass of coelenterates, the Alcyonaria, or Octocorallia, to which the gorgonians belong. A number of investigators have scrutinized the Gorgonacea and other coelenterates. While numerous interesting compounds have been isolated, C_{20} diterpenoids with the 14-membered cembrane ring system are emerging as the characteristic constituents of the octocorals.

Naturally occurring diterpenoids containing a 14-membered carbocyclic ring were characterized only relatively recently. Cembrene (1) also known as thunbergene, d-tumbelene, and the Weinhaus hydrocarbon was the first 14-carbon ring compound to be identified.[11-13] Cembrane (2) is the generic name for octahydrocembrene.



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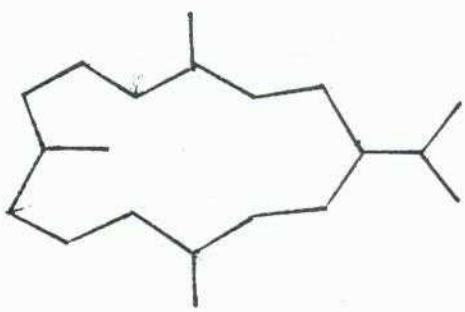
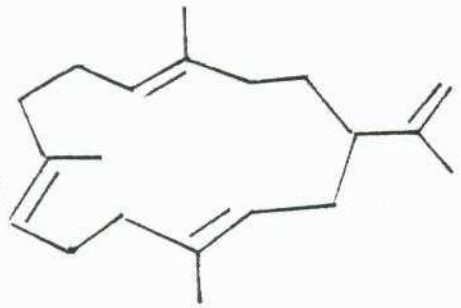


2

The crystal structure of cembrene, determined by x-ray diffraction confirmed the molecular structure and showed that three double bonds are

trans-oriented and only the C-4 olefin is cis-oriented.[13]

Three cembrene isomers, (3) (4), and (5) have been isolated from various sources. Cembrene-A(3) was isolated from a tree in India[14,15] and possesses the same gross structure as compounds isolated from a Siberian spruce,[16,17] from termites in Australia,[18] and from a Pacific soft coral.[19]



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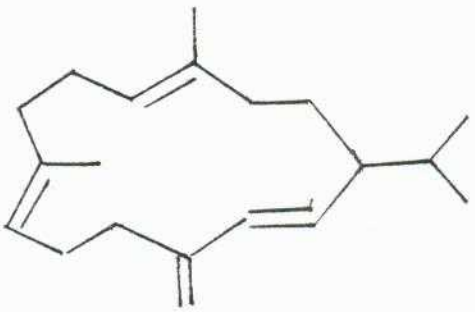
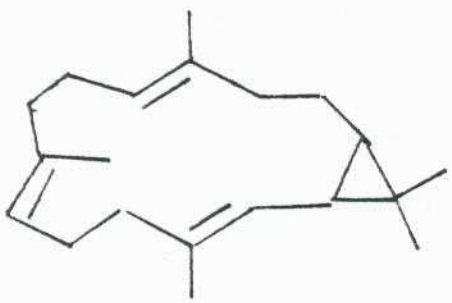
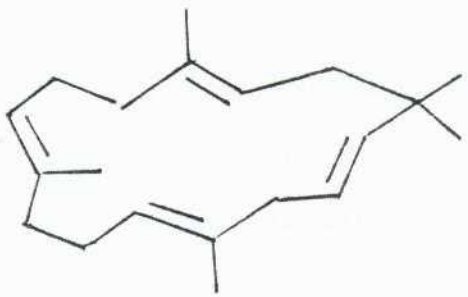


4

The two hydrocarbons, isocembrene (4) and casbene (5), were isolated from a Russian pine tree [20] and from castor beans,[21,22] respectively. (Table I.)



5



6

A soft coral recently yielded the hydrocarbon flexibilene (6).[23] Although flexibilene (6) has a 15-membered carbocyclic ring, it is interesting to note the similarity with casbene (5).

TABLE I. Physical Data for Cembrene and Isomers

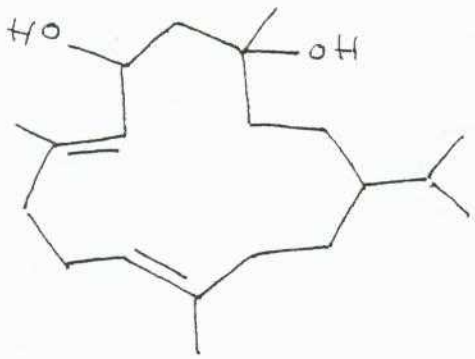
Name(number)	Formula	MP (^{o}C)	$[\alpha]$
(+)-Cembrene(1)	$C_{20}H_{32}$	59-60	+23.8
(-)-Cembrene-A(3)	$C_{20}H_{32}$	oil	
(+)-Isocembrene(4)	$C_{20}H_{32}$	---	
Casbene(5)	$C_{20}H_{32}$	---	
Flexibilene (6)	$C_{20}H_{32}$	oil	

Concurrent with the establishment of the structure of cembrene, hydroxylated derivatives were shown to be present in tobacco leaves and cigarette smoke;[24-26] they are related to 1-isopropyl-4,6,12-trimethyl-1,7,8-cyclotetradecatriene-4,6-diol (7). Cembrene[27] appears to be the trivial name of choice in the literature, yet the authors assigned the name duvane to structure 2 and named the macrocylic diterpenoids from tobacco as derivatives of duvane (Chart I).

Literature reports include six monohydric cembrane alcohols, thunbergol (15),[28,29] isocembrol (16),[19] mukulol (17),[15] cembrol (18)[30] nepthenol (19), 2-hydroxynepthenol (20),[31] and an alcohol isolated from the soft coral, *Sarcophyton glaucum*, which has been tentatively assigned structure 21 (Chart II).[32] The macrocyclic diterpenes incensole (22) and incensole oxide (23) were isolated from frankincense.[33,34]

A diepoxy cembrane compound with the epoxides occurring at the 3,4 and 11, 12 positions was isolated from a soft coral, 3,4,11,12-diepoxy-cembrane (24).[35]

CHART I



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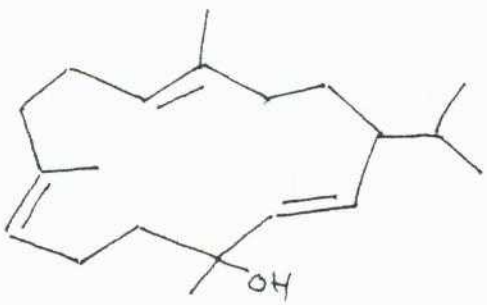
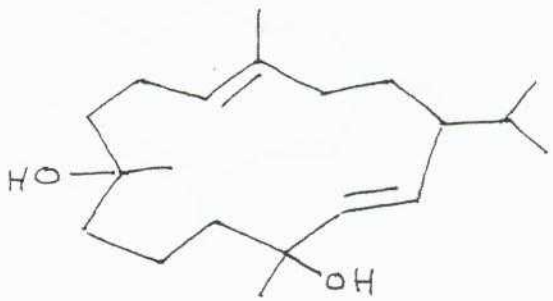
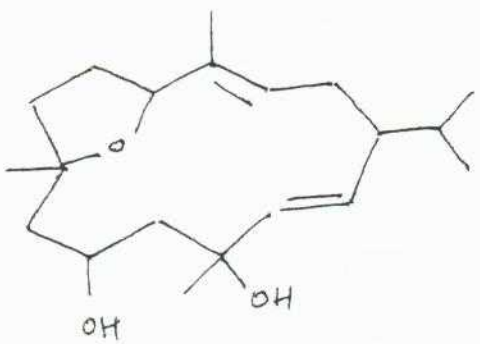


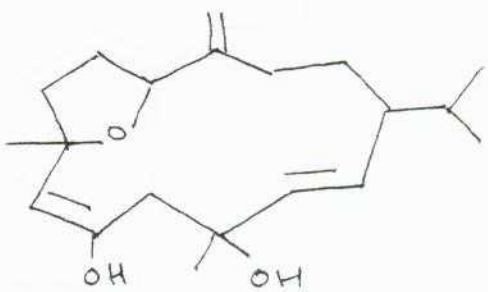
8 and 9



10 and 11



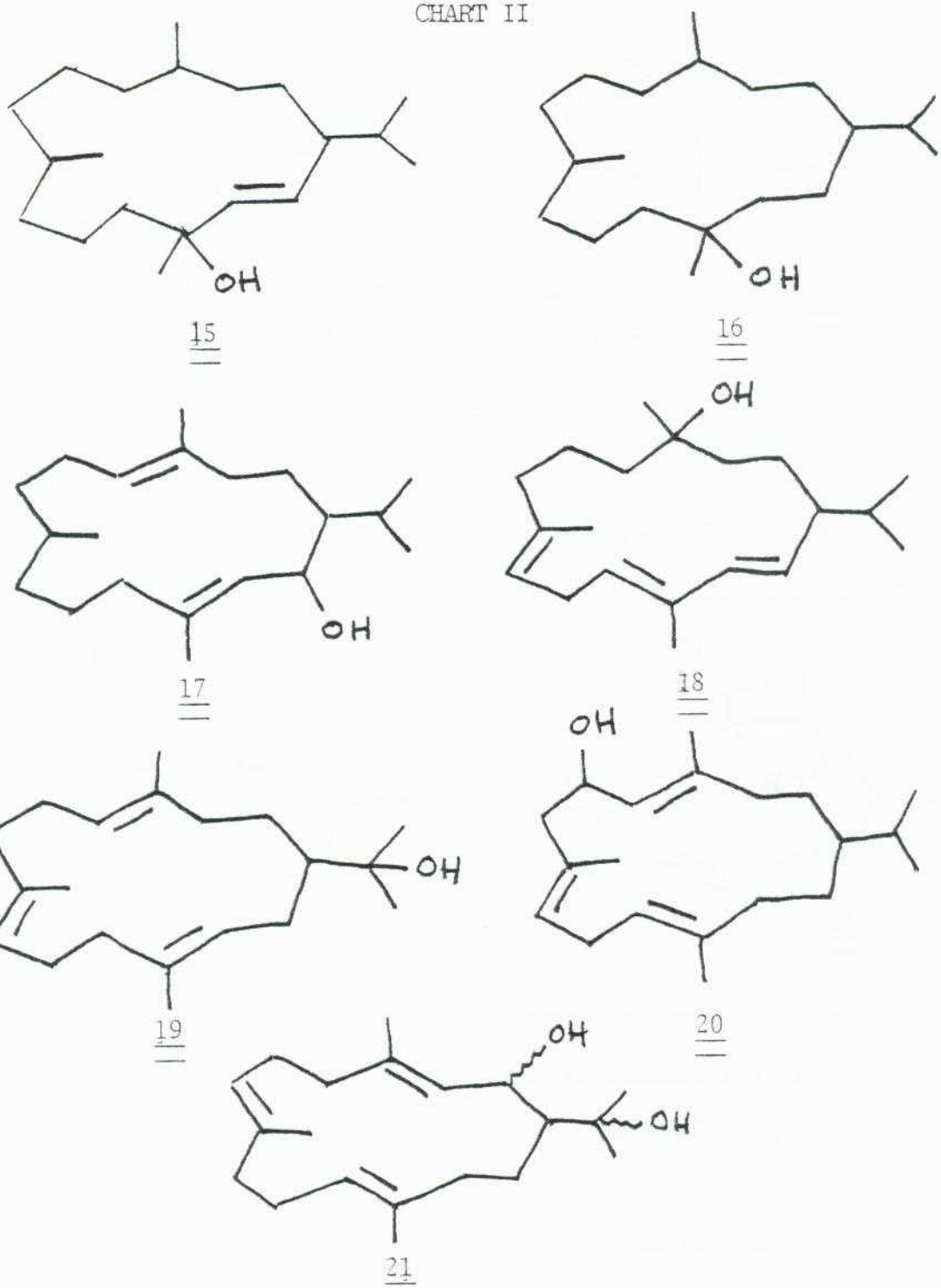
12



13 and 14

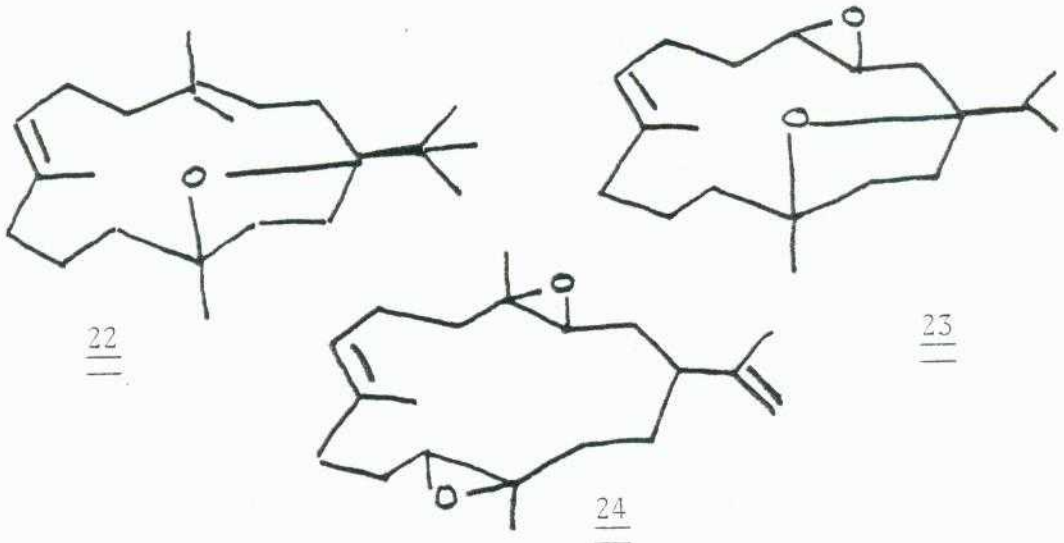
1-isopropyl-4,6,12-trimethyl-1,7,8-cyclotetradecatriene-4,6-diol (7) and related duvane derivatives

CHART II



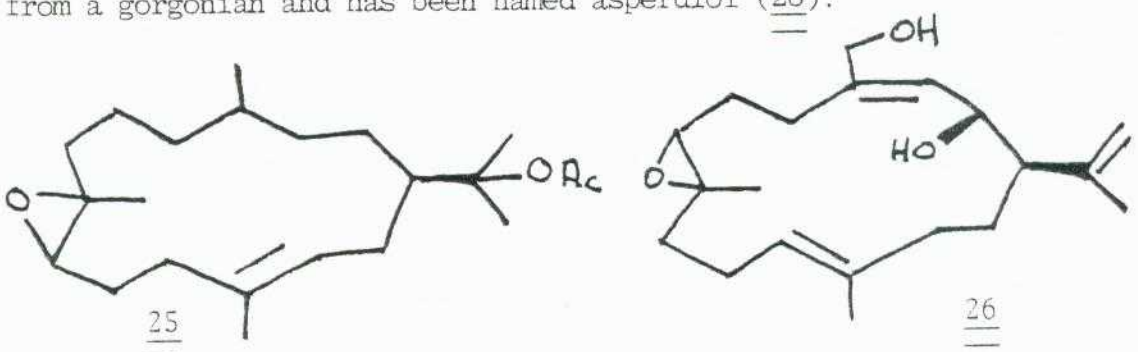
15 16 17 18 19 20 21

Cembrane alcohols: thunbergol (15), isocembrol (16), mukulol (17), cembrol (18), nepthenol (19), 2-hydroxynephthenol (20), and the alcohol isolated from the soft coral with tentative structure 21



22 23 24

An acetylated cembrane derivative, epoxynephthenol acetate (25), was isolated from a Pacific soft coral.[36] A related diol was isolated from a gorgonian and has been named asperdiol (26).[37]



25 26

Several naturally occurring diterpene lactones with the cembrane skeleton have been reported. The first representative of these macrocyclic diterpenes, ovatodiolide (27), was isolated from the Vietnamese herb, *Anisomeles ovata*.[38]

A large group of these diterpenoids possessing various levels of antibacterial activity have been encountered in gorgonians (Coelenterata, Octocorallia, Gorgonacea).[18,39-42] Eunicin (28) and jeunicin (29), crassin acetate (30) and eupalmerin acetate (31) were isolated

from Caribbean gorgonians (Chart III). Eupalmerin acetate (31) can be looked at as the possible biogenetic precursor of both eunicin (28) and jeunicin (29), yielding one or the other by reaction of the epoxide group with the hydroxyl radical released by the hydrolysis of the acetate group. Peunicin (32)[43] and Cueunicin (33)[19] were also recently isolated from gorgonians.

Recently, a number of novel cembranolides have been isolated from another group of coelenterates - the soft corals (Coelenterata, Octocorallia, Alcyonacea). Lobophytolide (34) has been isolated from the soft coral, *Lobophytum cristigalii*, and is closely related to the cembranolides, eunicin (28) and jeunicin (29).[44]

An epoxycembtranolide with interesting pharmacological properties is sarcophine (35) isolated from the soft coral *Sarcophyton glaucum*. Four additional cembranolides (36, 37, 38 and 39) have been isolated from *Sarcophyton glaucum* and as can be expected are related to sarcophine.[45,46]

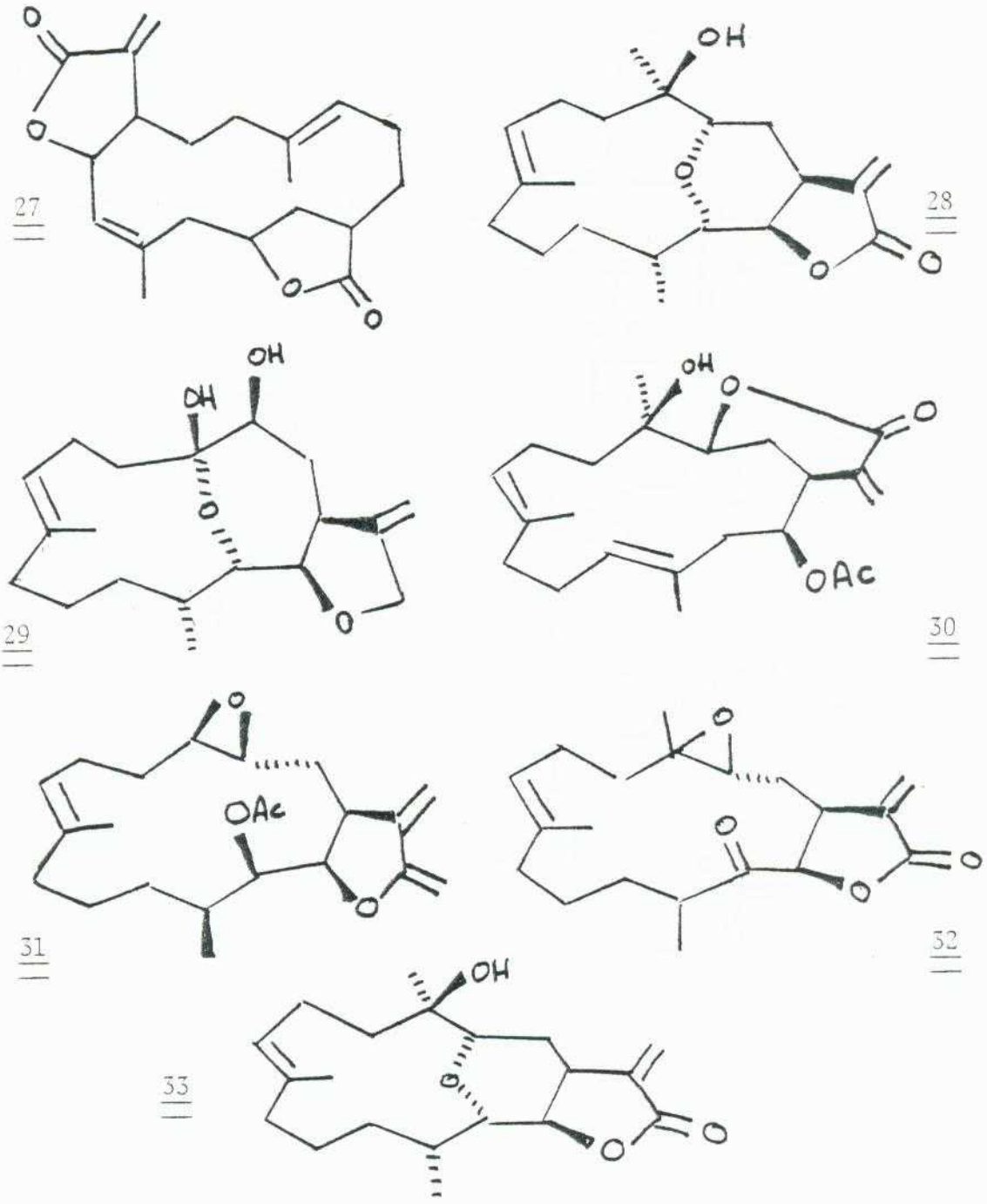
The soft coral *Sinularia flexibilis* has also yielded a series of cembranolides - sinulariolide (41),[47] 11-dehydrosinulariolide (42), 6-hydroxysinulariolide (43),[48] sinularin (44) and dihydrosinularin (45).[49]

In the course of the sytematic investigation into the chemistry of marine invertebrates within our research group, a Pacific soft coral, *Sinularia abrupta* was examined. The octocoral was collected off the Blowhole on the island of Oahu, Hawaii, and yielded pukalide (46) a novel epoxycembranolide containing a furan moiety.[50] Table III illustrates the available data for cembranolides.

TABLE II Summary of Oxygenated Cembrane Derivatives (excluding lactones)

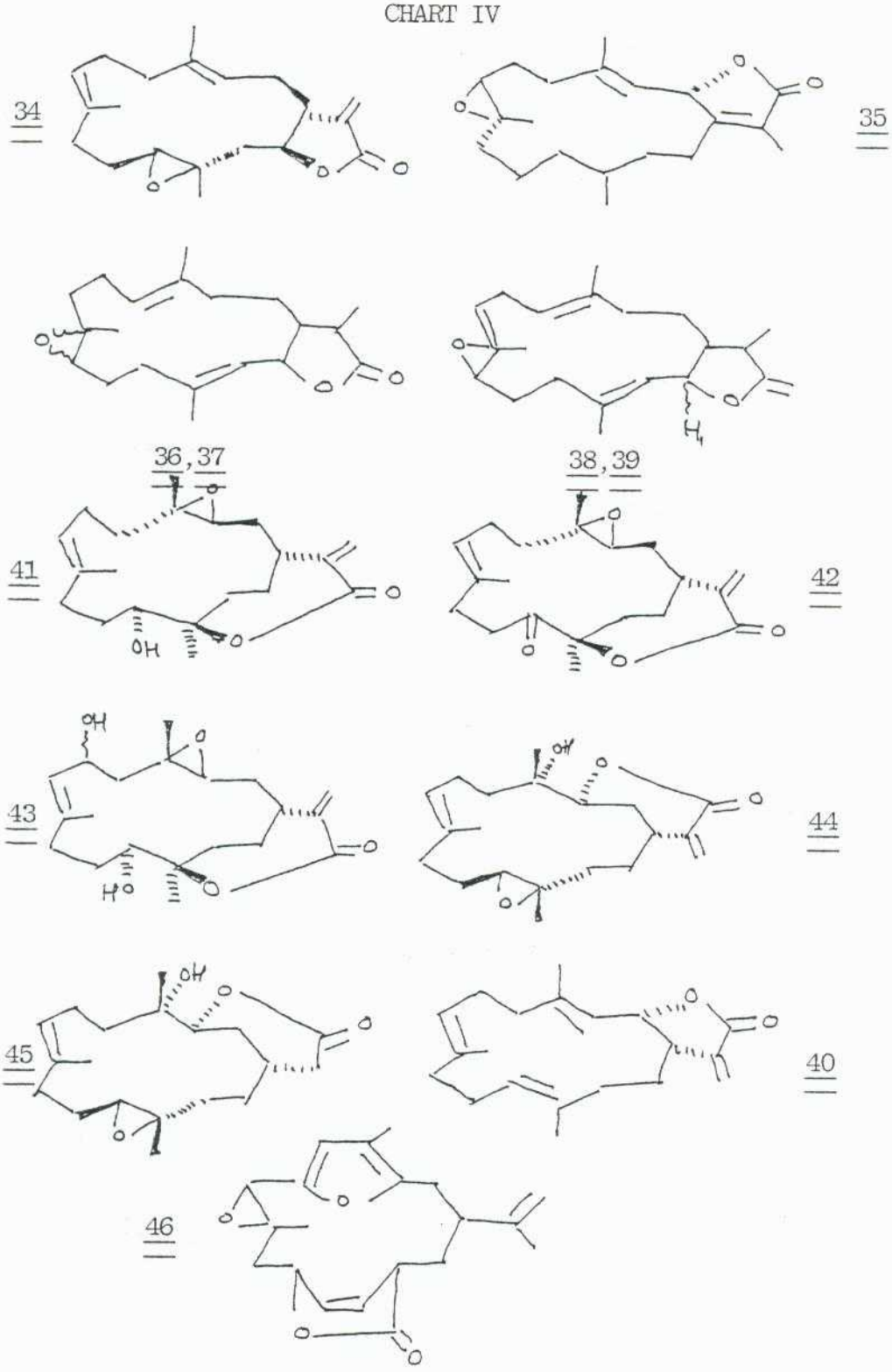
Name (Structure)	Composition	MP °C	[α]
α-2,7,11-Duvatriene-4,6-diol (8)	$C_{20}H_{34}O_2$	65-66	+281.6
β-2,7,11-Duvatriene-4,6-diol (9)	$C_{20}H_{34}O_2$	127-28	+162
α-2,6,11-Duvatriene-4,8-diol(10)	$C_{20}H_{34}O_2$	118-20	+100
β-2,6,11-Duvatriene-4,8-diol(11)	$C_{20}H_{34}O_2$	150-52	+ 40
α-8,11-Oxido-2,6,12-duvatriene-4-ol(12)	$C_{20}H_{34}O_2$	95-96	+ 86
α-8,11-Oxido-2,6,12-duvatriene-4-ol(13)	$C_{20}H_{34}O_2$	109-10	+ 77.4
β-8,11-Oxido-2,6,12-duvatriene-4-ol(14)	$C_{20}H_{34}O_2$	108-09	+ 72.5
Thunbergol (15)	$C_{20}H_{34}O$	oil	+ 74.4
Isocembrol (16)	$C_{20}H_{34}O$	------	+ 80.1
Mukulol (17)	$C_{20}H_{34}O$	37-38	+ 53
Cembrol (18)	$C_{20}H_{34}O$	------	+ 59.6
Nepthenol (19)	$C_{20}H_{34}O$	oil	------
2-Hydroxynepthenol (20)	$C_{20}H_{34}O_2$	98-99	-104
Alcohol 21	$C_{20}H_{32}O$	143-45	------
Incensole (22)	$C_{20}H_{34}O_2$	oil	- 77.5
Incensole oxide (23)	$C_{20}H_{34}O_3$	164-5	- 48
3,4,11,12-diepoxycembrane (24)	$C_{20}H_{32}O_2$	66-68	+ 63
Epoxynephthenol acetate (25)	$C_{22}H_{36}O_3$	oil	- 20.7
Asperdiol (26)	$C_{20}H_{32}O_3$	109-110	- 87

CHART III



Ovatodiolide (27) and naturally occurring cembranolides from gorgonians: eunicin (28), jeunicin (29), crassin acetate (30), eupalmerin acetate (31), peunicin (32) and cueunicin (33)

CHART IV



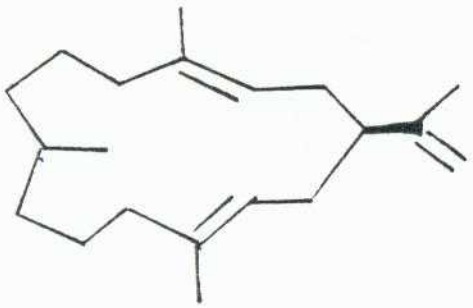
Naturally occurring cembranolides from soft corals

Table III. Summary of Naturally Occurring Cembranolides

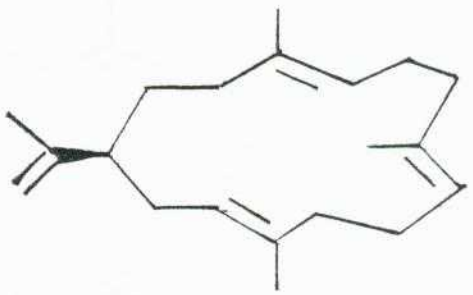
Name	Formula	MP °C	[α]
Ovatodiolide (27)	$C_{20}H_{24}O_4$	150	
Eunicin (28)	$C_{20}H_{30}O_4$	155	-89.4
Jeunicin (29)	$C_{20}H_{30}O_4$	141	+2.8
Crassin Acetate (30)	$C_{22}H_{32}O_5$	140	+70.4
Eupalmerin acetate (31)	$C_{22}H_{32}O_5$	159	+ 8
Peunicin (32)	$C_{20}H_{26}O_4$	175-176	-----
Cuenicin (33)	$C_{20}H_{30}O_4$	oil	-147
Lobophytolide (35)	$C_{20}H_{28}O_3$	137-138	
Sarcophine (36)	$C_{20}H_{28}O_3$	---	
36	$C_{20}H_{30}O_2$	---	
37	$C_{20}H_{30}O_2$	---	
38	$C_{20}H_{28}O_3$	70	
39	$C_{20}H_{28}O_3$	---	
40			
Sinulariolide (41)	$C_{20}H_{24}O_6$	170-173	+76
11-Dehydrosinulariolide (42)	$C_{20}H_{28}O_4$	120	+ 87
6-Hydroxysinulariolide (43)	$C_{20}H_{30}O_5$	192-119	+54.5
Sinularin (44)	$C_{20}H_{30}O_4$	150-152	-127
Dihydrosinularin (45)	$C_{20}H_{32}O_4$	110-112	- 45
Pukalide (46)	$C_{21}H_{24}O_6$	210-212	+310

Proposed Biogenetic Scheme for Cembrane-type Compounds

The biosynthesis of these compounds has not been established, but some speculation can be presented. A hydrocarbon (47) can be drawn to represent the carbon skeleton for crassin acetate (30).

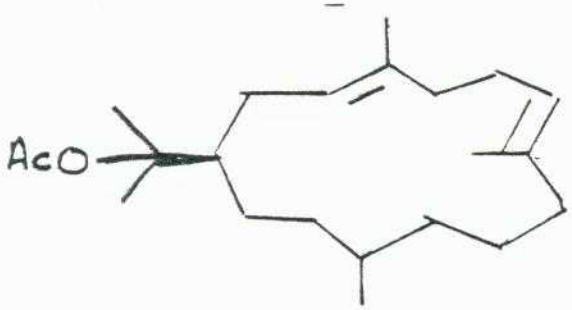


47



3

As can be seen from above, 47 is enantiomeric with (-)-cembrene-A (3), a well-known natural product isolated from a tree in India as previously described. Sesquiterpene hydrocarbons isolated from Caribbean gorgonians have also been shown to possess an enantiomeric relationship with the common forms of the corresponding hydrocarbons found in terrestrial plants.[51,52] It should be noted, however, that a cembrane derivative isolated from a soft coral, epoxynephthenol acetate (25) has the carbon skeleton 3.

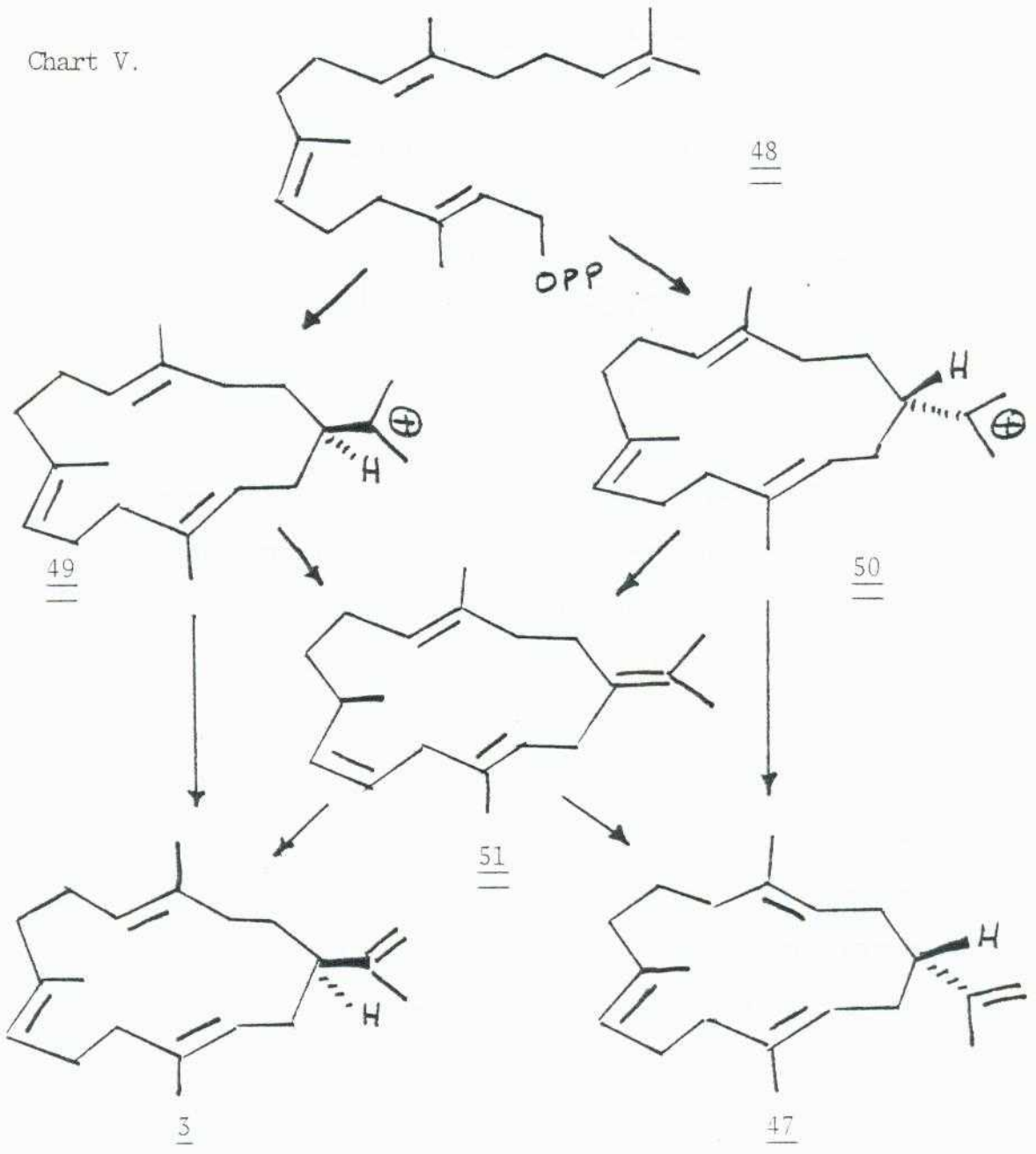


25

It seems reasonable to assume,[14,21,22] that biogenesis of these

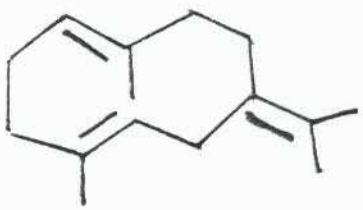
compounds involves the single cyclization of the normal diterpenoid precursor, geranylgeranyl pyrophosphate (48) which can be envisaged to produce 47, 3 and 51 via deprotonation of the cations 49 and 50 (Chart V).

Chart V.



(+)-cembrene-A (47), which has the carbon skeleton of crassin acetate (30) emerges as the likely precursor for eunicin (28), jeunicin (29), crassin acetate (30), eupalmerin acetate (31), lobophytolide (34), sarcophine (35), and the four related compounds, (36), (37), (38), (39), sinulariolide (41) and pukalide (46). Cembrene-B (51) also represents a possible intermediate since it is analogous to germacrene-B (52) long postulated as an intermediate in sesquiterpene biogenesis.[53,54]

52



If (-)-cembrene-A (3) is envisaged as the precursor of the corresponding cembranoids, then an additional step must be incorporated into the biogenetic scheme involving an inversion of configuration at C-1 or an isomerization of the double bond at C-7, C-8 to C-8, C-9.

The same reasoning can be employed to envisage (-)-cembrene-A (3) or cembrene-B (49) as the precursor for epoxynephthenol acetate (25). Once again if (+)-cembrene-A (47) is envisaged as the precursor for 25, then the additional step of inversion at C-1 or the isomerization at C-8, C-9 to C-7, C-8 must be incorporated into the biogenetic scheme.

As illustrated, diterpenoids possessing the 14-membered cembrane ring system have been found to occur in diverse natural sources. They have been found relatively seldom in terrestrial sources and exist largely as olefins and alcohols. Conversely, highly functionalized cembrane derivatives are emerging as common metabolites of marine invertebrates of the phylum Coelenterata.

During the course of the investigation into the chemistry of invertebrates within our research group, several coft corals collected from Enewetak Atoll were scrutinized. The isolation and structure elucidation of emblide and trochelin, two novel diterpenoids, is discussed in the following chapter.

There lies the port; the vessel puffs her sail;
There gloom the dark, broad seas. My mariners...
That ever with a frolic welcome took
The thunder and the sunshine... Come my friends...
Push off, and sitting well in order smite
The sounding furrows; for my purpose holds
To sail beyond the sunsets, and baths
Of all the western stars...
Some work of noble note, may yet be done...

Chapter Three

Experimental Section

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Solvents were distilled prior to use. Chromatographic supports were Bio-Sil A(Bio-Rad, 200-235 mesh) for column chromatography and silica gel for thin-layer chromatography (EM Reagents, HF 254+ 366).

Preparative thin layer chromatography plates were prepared by coating glass plates with EM silica gel HF to a thickness of approximately 1 mm. The developed chromatograms were visualized with short-long wavelength ultraviolet, iodine vapor or sulfuric acid spray.

Rotations were run on a Bendix-Ericsson Automatic Polarimeter type 143A. Infrared spectra were obtained on a Perkin-Elmer 467 or a Beckman IR-10 instruments. ^{1}H nmr spectra were obtained on a Varian HA-100 or a Varian XL-100 using TMS as an internal standard. ^{1}H nmr chemical shifts are reported in J-values (ppm from tetramethylsilane as an internal standard) and are followed by the multiplicity of the signal, the number of protons absorbing at that frequency, the coupling constants or line separations in Hertz (Hz). The multiplicities are reported as follows: s=singlet; d=doublet; dd=doublet of doublets; t=triplet; cs=complex signal; q=quartet; b=broad and m=multiplet.

The high resolution mass spectrum of emblide was provided by the Massachusetts Institute of Technology, Cambridge, Massachusetts. The low resolution mass spectra were obtained on a Varian MAT-311 Spectrometer.

Elemental analysis was performed by Micro-Analytical Laboratories, U.C. Berkeley, Berkeley, California.

The octocorals, *Sarcophyton glaucum* and *Sarcophyton trocheliophorum*, were collected from the coral pinnacle near Bogen island, Enewetak atoll, Marshall Islands. The samples were kept in seawater containers until frozen a short time after collection. Samples were then packed in dry ice containers for shipment to Hawaii.

Preparation of Jones reagent: Dissolved 8.7g of CrO_3 in 10ml of conc H_2SO_4. Solution was then diluted to 50ml with distilled H_2O.

Preparation of Diazomethane (3g total): EtOH (95%, 30ml) is added to 6.0g of KOH in 10ml of H_2O in a 250ml rb flask. A solution of Diazald (Aldrich Chem Co.) was then added to the alkaline solution at a rate equal to the rate of distillation. Mixture was heated to approx. 60-65°C with a hot water bath.

Isolation of emblide: A wet sample (650g) of *Sarcophyton glaucum*, collected in February, 1974 at a depth of 10 m was steeped in several portions of fresh EtOH. The EtOH extract was then partitioned between H_2O and Et_2O. The ether extract yielded 17g of an oily residue. The residue was then subjected to preparative thin layer chromatography (10% ether - 90% CH_2Cl_2, silica gel HF) in 250mg portions. Recrystallization of the component with R_f value 0.6 gave emblide (270mg); $C_{23}H_{32}O_6$; M^+404; mp 119-120°C; $[\alpha]_D^{25.5}$=+92° (c 1.3, $CHCl_3$); ir (Figure 12, in

$CHCl_3$) $\nu_{C=O}$ 1740 cm^{-1}, $\nu_{C=O}$ 1720 cm^{-1}, $\nu_{C=O}$ 1695 cm^{-1}, $\nu_{C=C}$ 1660 cm^{-1}, $\nu_{C=C}$ 1475cm^{-1}, 1380cm^{-1} and 1370cm^{-1} (gem-dimethyl), ν_{C-O} 1240cm^{-1} (acetate); uv(EtOH) 284 nm (ε 15,6000), 220 nm (ε 8,800).

^1H nmr in $CDCl_3$ (Figure 13): 7.11 ppm (d, 1H, J=12Hz); 6.15 ppm (d, 1H, J=12Hz); 6.02 ppm (br t, 1 H, J=4Hz), 5.25 ppm (dd, 1H), 3.70 ppm (s, 3H), 3.10 ppm (br m, 1H), 2.35 ppm (br m, 8H), 2.0 ppm (br s, 5H), 1.80 ppm (br s, 1H), 1.77 ppm (br m, 1H), 1.43 ppm (s, 3H, methyl) and 1.15 ppm (dd. 6H, isopropyl).

25.2 MHz proton decoupled ^{13}C nmr in $CDCl_3$ (Figure 14): 169.4 ppm (-C-O), 168.0 (-C-O-), 166.1 ppm (-C-O-), 154.9 ppm (C=C), 141.9 (H-C=C), 135.4 (H-C=C), 131.9 (C=C), 124.4 (C=C), 120.8 (H-C=C), 82.3 (-C-O-), 68.2 (H-C-O-), 51.2 (CH_3-O-), with the remaining eleven sp^3 carbons at 37.1, 35.9, 34.3, 27.1, 26.3, 25.3, 23.7, 22.7, 21.9, 20.9 ppm.

Emblide showed a molecular ion at 404 corresponding to the formula $C_{23}H_{32}O_6$ (Figure 12). The mass spectrum (70 eV) m/e (relative intensity): M^+404(4) 373(3), 372(6), 362(2), 361(5), 344(2), 330(6), 319(6), 312(12), 284(8), 269(10), 259(6), 241 (9), 239(6), 227(6), 213(5), 201(6), 200(7), 199(7), 197(6), 195(5). 189(9), 187(6), 185(6), 179(6), 177(7), 175(6), 173(9), 171(7), 169(5), 165(9), 164(31), 163(8), 162(7), 161(10), 159(8), 157(6), 155(5), 153(5), 152(25), 151(6), 150(6), 149 (22), 148(13), 147(15), 146(11), 144(10), 143(7), 141(5), 137(10), 136(5), 135(10), 134(5), 133(17), 131(12), 129(7), 128(5), 123(6), 122(5), 121(15), 120(7), 119(31), 118(7), 117(11), 115(5), 109(6), 108(5), 107(16), 106(5), 105(29), 103(4), 95(11), 94(6), 93(23), 92(7), 91 (33), 81 (15), 79(24), 77(14), 71(6), 69(11), 67(19), 65(7), 59(12),

57(5), 55(19), 53(16), 45(5), 44(8), base peak 43(100) and 41(33).

Analyses: Calculated for $C_{23}H_{32}O_6$, C, 68.3%, H, 8.0%, O 23.6%. Found (elemental analysis by Micro-Analytical Lab) C, 65.7%, H 8.1%, O, 26.2%. Molecular weight calculated for $C_{23}H_{32}O_6$, 404.21957. Found molecular weight (high resolution mass spectrum) 404.21689.

Hydrogenation of emblide

A solution of emblide (51.5mg) in 20ml of isopropyl alcohol was hydrogenated for 20 hr. over 10% Pd/charcoal. The reaction residue showed three spots on tlc. The hydrogenated product was isolated as an oil; $C_{23}H_{38}O_6$; ir in $CHCl_3$ (Figure 15) $\nu_{C=O}$ 1735cm^{-1}, ν_{C-C} 1450cm^{-1}, 1380cm^{-1}, 1370cm^{-1}(gem dimethyl); ν_{C-O} 1240cm^{-1}(acetate); uv(EtOH) 275nm (ε 1150).

The ^{1}H nmr in $CDCl_3$ (Figure 16) showed two sharp spikes at 3.615 ppm and 3.605 ppm (3H), two sharp spikes at 2.005 and 2.010 ppm (3H), 1.8-1.4 ppm (br m, 23H), 1.43 ppm (s, 3H), and 0.9 ppm (dd, 6H, gem dimethyl).

The product showed a molecular ion at 410 corresponding to the formula $C_{23}H_{38}O_6$ (Figure 16). The mass spectrum (70 ev) m/e (relative intensity): 410(1), 383(2), 382(9), 368(4), 367(4), 350(3), 337(2), 336(4), 335(2), 332(4), 326(2), 322(3), 321(3), 319(3), 318(4), 308(6), 307(7), 306(2), 304(3), 303(3), 300(3), 295(3), 294(2), 293(4), 292(2), 291(4), 290(5), 289(7), 279(2), 275(5), 273(2), 272(2). 271(2). 265(2),

263(5), 261(4), 257(4), 255(2), 254(2), 253(5), 249(3), 248(2), 247(6), 245(2), 239(2), 236(2), 235(5), 233(3), 231(2), 229(4), 221(4), 219(2), 217(2), 215(2), 213(2), 211(4), 210(2), 209(3), 208(3), 207(4), 206(2), 205(3), 203(3), 201(4), 196(3), 195(3), 194(2), 193(3), 192(2), 191(4), 189(3), 187(2), 185(3), 183(3), 182(2), 181(6), 180(3), 179(4), 178(2), 177(4), 175(4), 173(3), 172(2), 171(3), 170(4), 169(3), 168(2), 167(4), 166(3), 165(6), 164(2), 163(7). 162(3), 161(6), 159(4), 157(3), 156(2), 155(3), 154(3), 153(5), 152(4), 151(6), 150(5), 149(10), 148(4), 147(9), 146(2), 145(5), 144(2), 143(3), 142(10), 141(3), 140(3), 139(5), 138(5), 137(9), 136(7), 135(13), 134(4), 133(8), 132(2), 131(3), 130(3), 129(3), 128(3), 127(8), 126(5), 125(7), 124(7), 123(13), 122(7), 121(15), 120(4), 119(7), 118(2), 117(2), 115(2), 114(4), 113(6), 112(6). 111(10), 110(8), 109(23), 108(14), 107(20), 106(5), 105(9), 102(3), 101(2), 100(4), 99(5), 98(5), 97(14), 96(10), 95(32), 94(11), 93(19), 90(8), 87(12), 85(45), 84(17), 83(65), 82(14), 81(33), 80(5), 79(16), 77(4), 74(5), 73(3), 72(2), 71(15), 70(7), 69(31), 68(7), 67(26), 60(7), 59(5), 58(4), 57(13), 56(6), 55(44), 54(4), 53(5), 50(2), 49(5), 48(8), 47(18), 46(7), 44(9), base peak 43(100) and 41(35).

Deacetylation of emblide

Emblide (40.0mg) was dissolved in 15ml of MeOH and a catalytic amount (1mg) of Na was added. The solution was gently refluxed 1 hr after it was found that emblide would not deacetylate by stirring at room temperature. After the reflux period, the reaction mixture was stirred at room temperature for 4 hr as the mixture showed no starting materials present. Extraction with chloroform and evaporation gave 28mg of deacetylated emblide; $C_{21}H_{30}O_5$; ir in $CHCl_3$ (Figure 17) ν_{OH} 3450cm^{-1}, $\nu_{C=O}$ 1720cm^{-1}, $\nu_{C=C}$ 1680cm^{-1}, ν_{C-C} 1450cm^{-1}, 1380cm^{-1} and 1370cm^{-1} (gem dimethyl).

The ^{1}H nmr in $CDCl_3$ (Figure 18): 6.94 ppm (d, 1H, J=12Hz), 6.25 ppm (d, 1H, J=12Hz), 6.08 (t, 1H, J=4Hz), 4.12 (dd, 1H, J=8Hz), 3.80 ppm (s, 3H), 1.43 ppm (s, 3H, CH_3), 1.28 ppm (br s, 15H) and 1.10 ppm (dd, 6H, gem dimethyl).

The product showed a molecular ion at 362 corresponding to the formula $C_{21}H_{30}O_5$ (Figure 17). The mass spectrum (70ev) m/e (relative intensity), M^+ 362(7), 344(6), 331(10), 330(33), 319(17), 312(21), 287(11), 285(12), 284(19), 279(18), 269(18), 259(17), 241(20), 201(17), 189(34), 163(20), 162(20), 161(22), 152 (33), base peak 149(100), 148(39), 147(32), 121(38), 119(39), 107(34), 105(45), 97(32), 95(36), 93(42), 91(48), 85(41), 83(39), 81(36), 79(41), 77(25), 71(70), 69(57), 67(39), base peak 57(100), 55(61), 43(95), and 41(67).

Attempted Lemieux-Jones oxidation and derivatization of the resulting carboxylic acids with diazomethane

Emblide (53 mg) was dissolved in 25 ml t-BuOH and added dropwise to 4.0 g $NaIO_4$ and 0.2 g $KMnO_4$ in 60 ml of H_2O. $NaCO_3$ was then added to bring the pH of the mixture to 8. Dil H_2SO_4 was then added until the mixture was acidic. Na_2SO_3 was then added to reduce IO_4^-, I_2, and IO_3^- to I^-. Enough dil NaOH was added to make the solution basic. The t-BuOH was removed under reduced pressure. The solution was then acidified with dil H_2SO_4. The solution was then washed with Et_2O. The Et_2O was then removed C_3H_6O (acetone) used as the solvent. Approximately 3ml of Jones reagent was then added and evidence (frothing, change of color) of oxidation was immediate. The acetone layer was then extracted with Et_2O and $CHCl_3$. The extracts were then combined and added to a prepared diazomethane solution in Et_2O. The reaction mixture showed four spots on tlc:

Reaction product 1, R_f= 0.7; ir in CH_2Cl_2 (Figure 20) 2960cm^{-1}, 2930cm^{-1}, 1710cm^{-1} (weak), 1260cm^{-1}, 1100cm^{-1}, 1020cm^{-1}; ^{1}H nmr in $CDCl_3$ (Figure 19) 1.6 ppm (br s), 1.4 ppm (br s) and 0.9 ppm (br s);

Reaction product 2, R_f= 0.5; ir in CH_2Cl_2 (Figure 22) 2980cm^{-1}, 2940cm^{-1}, 1700 (weak), 1260cm^{-1}, 1100cm^{-1}, 1020cm^{-1}; ^{1}H nmr in $CDCl_3$ (Figure 21) 5.2 ppm (br t) and 1.3 ppm (br m);

Reaction product three, R_f= 0.4; ir in CH_2Cl_2 (Figure 24), 2960cm^{-1}, 2940cm^{-1}, 2880cm^{-1}, 1700cm^{-1}(strong), 1450cm^{-1}, 1380cm^{-1}, 1370cm^{-1}, 735cm^{-1}; ^{1}H nmr in $CDCl_3$ (Figure 23) 7.7 ppm (m), 7.5 ppm (m), 6.35 ppm (d), 1.5 ppm (m) and 0.9 ppm (dd, 6H);

Reaction product four, R_f= 0.1; ir in CH_2Cl_2 (Figure 26) 2970cm^{-1}, 2940cm^{-1}, 2870cm^{-1}, 1735cm^{-1}, 1700cm^{-1}, 1260cm^{-1}, 1100cm^{-1}, and 1020 cm^{-1}; ^{1}H nmr in $CDCl_3$ (Figure 25)

Attempted ozonolysis and derivatization with diazomethane

Emblide (50 mg), dissolved in 20ml CH_2Cl_2, was ozonized at 0°C. When ozonolysis was complete, 3ml of 30% H_2O_2 was added to the solution. The reaction mixture was allowed to come to room temperature. Pt was added to the excess H_2O_2. A prepared CH_2N_2 in Et_2O solution was then added to the reaction mixture. The solution was then allowed to come to room temperature and the excess CH_2N_2 was allowed to evaporate. The mixture showed four spots by tlc corresponding to the mixture resulting from the oxidation previously described.

Attempted hydrolysis of hexahydroemblide

Hexahydroemblide (10mg) was dissolved in 9.6 ml of 1N alc KOH (57mg/ml) in 20ml of distilled H_2O. The solution was refluxed for 1 hr. The mixture was then allowed to come to room temperature. Then the reaction mixture was neutralized with HCl and extracted with $CHCl_3$. The solvent was then removed at reduced pressure and 9.8 mg of crude product remained and appeared to be homogeneous: 1 spot on several solvent systems with tlc; R_f=0.8; M^+284.

Isolation of trochelin

A wet sample (370 g) of *Sarcophyton trocheliophorum* was extracted with several portions of EtOH. The EtOH extract was then partitioned between H_2O and Et_2O. The Et_2O extract yielded 11.5 g of a brown oily residue. The residue was subjected to preparative tlc (10% ether-90% CH_2Cl_2; silica gel HF). Recrystallization of the component with R_f value of 0.54 gave 250 mg of trochelin; $C_{22}H_{34}O_4$; mp 140-141°C; ir in $CHCl_3$ (Figure 27) $\nu_{C=C}$ 1610 cm^{-1}, $\nu_{C=C}$ 1450 cm^{-1}, 1380 cm^{-1} and 1370 cm^{-1} (isopropyl methyls), ν_{C-O} 1240 cm^{-1} (acetate); uV (EtOH) 246 nm (ε 16,600) and 258 nm (ε 8500).

The ^1H nmr in $CDCl_3$ (Figure 28): 5.95 ppm (s, 2H), 4.50 ppm (d, 1H, J=12 Hz), 4.12 ppm (d, 1H, J=12 Hz), 3.10 ppm (m, 2H), 2.60-2.45 ppm (br m, 1H), 2.45-2.20 (cs, 4H), 2.20-1.80 ppm (br s, 5H), 1.78 ppm (s, 3H), 1.68 ppm (br s, 2H), 1.26 ppm (s, 3H) and 1.06 ppm (d, 6H, J=4 Hz).

The 25.2 MHz decoupled ^{13}C nmr spectrum in d_6-benzene(Figure 30): 170.4 (s, 1C), 147.9 (s, 1C), 134.7 (s, 1C), 120.0 (d, 1C), 117.9 (d, 1C), 64.2 (s, 1C), 62.3 (d, 1C), 60.8 (t, 1C), 60.0 (d, 1C), 59.3 (s, 1C) with the remaining twelve carbons in the sp^3 region, 37.9, 35.5, 34.6, 31.4, 29.6, 25.0 (two carbons), 23.3, 21.9 (two carbons), 20.8 and 17.6.

Trochelin showed a molecular ion at 362 corresponding to the formula $C_{22}H_{34}O_4$ (Figure 27). The mass spectrum (70 ev) m/e (relative intensity): M^+ 362(10), 344(2), 319(5), 303(3), 302(6), 289(2), 287(2), 284(3), 259(10), 187(11), 183(10), 175(21), 163(14), 162(14), 161(22), 159(19), 157(13), 153(11), 151(12), 150(13), 149(35), 148(48), 147(32),

143(11), 137(21), 136(40), 135(63), 134(28), 133(61), 122(27), 121(85), 120(21), 119(67), 112(13), 109(40), 108(19), 107(94), 106(19), 105(65), 97(14), 95(40), 94(19), 93(98), 92(16), 91(57), 83(16), 81(58), 80(14), 79(45), 77(29), 71(17), 69(46), 67(33), 57(13), 55(59), 53(17), base peak 43(100) and 41(63).

Analyses for trochelin

Calculated molecular weight for $C_{22}H_{34}O_4$ is 362. 245717. Observed molecular weight via high resolution mass spectrum is 362.245911.

Deacetylation of trochelin

Trochelin (40 mg) was dissolved in 10 ml MeOH. A catalytic amount of Na was added. The reaction mixture was stirred at room temperature for 2 hr until tlc showed no presence of starting material. Upon extraction with $CHCl_3$ and removal of solvent, 35 mg of a white oily substance remained; $C_{20}H_{32}O_3$; ir in $CHCl_3$ (Figure 32) ν_{OH} 3600 cm^{-1} and ν_{OH} 3450 cm^{-1} and $\nu_{C=C}$ 1600 cm^{-1}.

^1H nmr in d_6-benzene (Figure 31): 6.0 ppm (dd, 2H), 4.50 ppm (d, 1H, J=6 Hz), 4.12 ppm (d, 1H, J=6 Hz), 3.10 ppm (m, 2H), 2.60-2.45 ppm (m, 1H), 2.45-2.20 ppm (cs, 4H), 2.20 (s, 1H), 2.18 ppm (s, 3H), 2.10-1.80 ppm (cs, 5H), 1.78 ppm (s, 3H), 1.68 ppm (br s, 2H), 1.26 ppm (s, 3H) and 1.06 ppm (d, 6H, J=4 Hz, isopropyl group).

The deacetylated trochelin showed a molecular ion at 320 (Figure 32) with the mass spectrum (70 ev) m/e (real intensity): M^+ 320(7), 305(2), 302(5), 291(5), 275(2), 273(2), 259(3), 251(5), 167(10), 150(13),

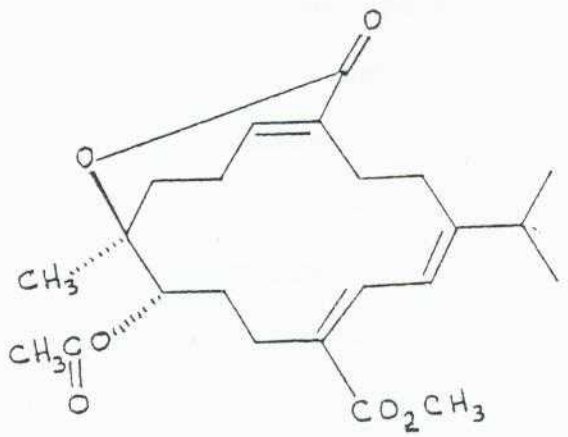
149(12), 148(12), 147(11), 141(10), 139(10), 137(25), 136(16), 135(26), 134(12), 133 (21), 131(11), 127(12), 126(11), 125(20), 123(23), 121(25), 119(20), 109(16), 108(10), 107(28), 106(7), 97(5), 94(7), 93(33), 92(6), 91(30), 83(10), 81(25), 80(6), 79(30), 77(14), 71(15), 69(25), 67(17), 65(5), 57(25), 55(60), 53(20), 44(45) and base peak 43(100).

CHAPTER IV

RESULTS AND DISCUSSION

A Pacific soft coral, *Sarcophyton glaucum*, was collected from the coral pinnacle near Bogen Island, Enewetak Atoll, Marshall Islands. Ethanol extraction of the marine invertebrate followed by partitioning between ether and water yielded a brown oily residue. The residue was then subjected to chromatography and resulted in the isolation of a highly functionalized cembranolide which subsequently was found to have structure 53. Recrystallization from ethanol afforded emblide[a] (53), mp 119-120°, $\alpha_D^{25.5}$ + 92. The following evidence supports the structure.

53



High resolution mass spectrum showed a molecular ion at 404 which corresponds to the formula $C_{23}H_{32}O_6$.

[a] We have chosen the name in recognition of the Enewetak Marine Biological Laboratory, recently redesignated the Mid-Pacific Marine Laboratory, which has been maintained for the benefit of the marine community by the U.S. Atomic Energy Commission (now the Department of Energy).

The functional groups and olefinic sites are apparent from the spectral characteristics of emblide and its derivatives (Figures 12-18).

The infrared spectrum (Figure 12) showed a number of strong absorptions in the carbonyl region at $\nu_{C=O}$ 1740 cm^{-1} and 1720 cm^{-1}. The strongest absorption was at 1695 cm^{-1} indicating a conjugated olefin. Characteristic bands for an isopropyl (gem dimethyl) group occurred at 1475 cm^{-1}, 1380 cm^{-1} and 1370 cm^{-1}. A ν_{C-O} at 1240 cm^{-1} indicated the possibility of an acetate group in emblide (53).

The proton decoupled ^{13}C nmr spectrum (Figure 14) of emblide confirmed the presence of twenty-three carbons including three ester carbons (169.4 ppm, 168.0 ppm and 166.1 ppm); six olefinic carbons (154.9 ppm, 141.9 ppm, 135.4 ppm, 131.9 ppm, 124.4 ppm and 120.8 ppm); three carbons singly bound to oxygen (82.3 ppm, 68.2 ppm and 51.2 ppm), with the remaining eleven carbons appearing in the sp^3 region. The ^{13}C nmr data and assignments are summarized in Table IV.

The 100 MHz ^1H nmr spectrum of emblide (53) in deuterated chloroform (Figure 13) showed five signals for methyls: an overlapping doublet of doublets at 1.14 ppm (6H) for an isopropyl group; a singlet at 1.43 ppm (3H) corresponding to a methyl attached to a deshielded carbon atom; a broad singlet at 2.0 ppm (5H) which includes the three protons of a methyl of an acetate; and a singlet methyl at 3.70 ppm (3H) corresponding to the methyl of a carbomethoxy group. The mass spectrum (Figure 12) shows a parent peak at 404 and attested to the presence of the methyl ester and acetoxy groups previously described by fragments at 372 for a loss of methanol and at 344 for the loss of acetic acid.

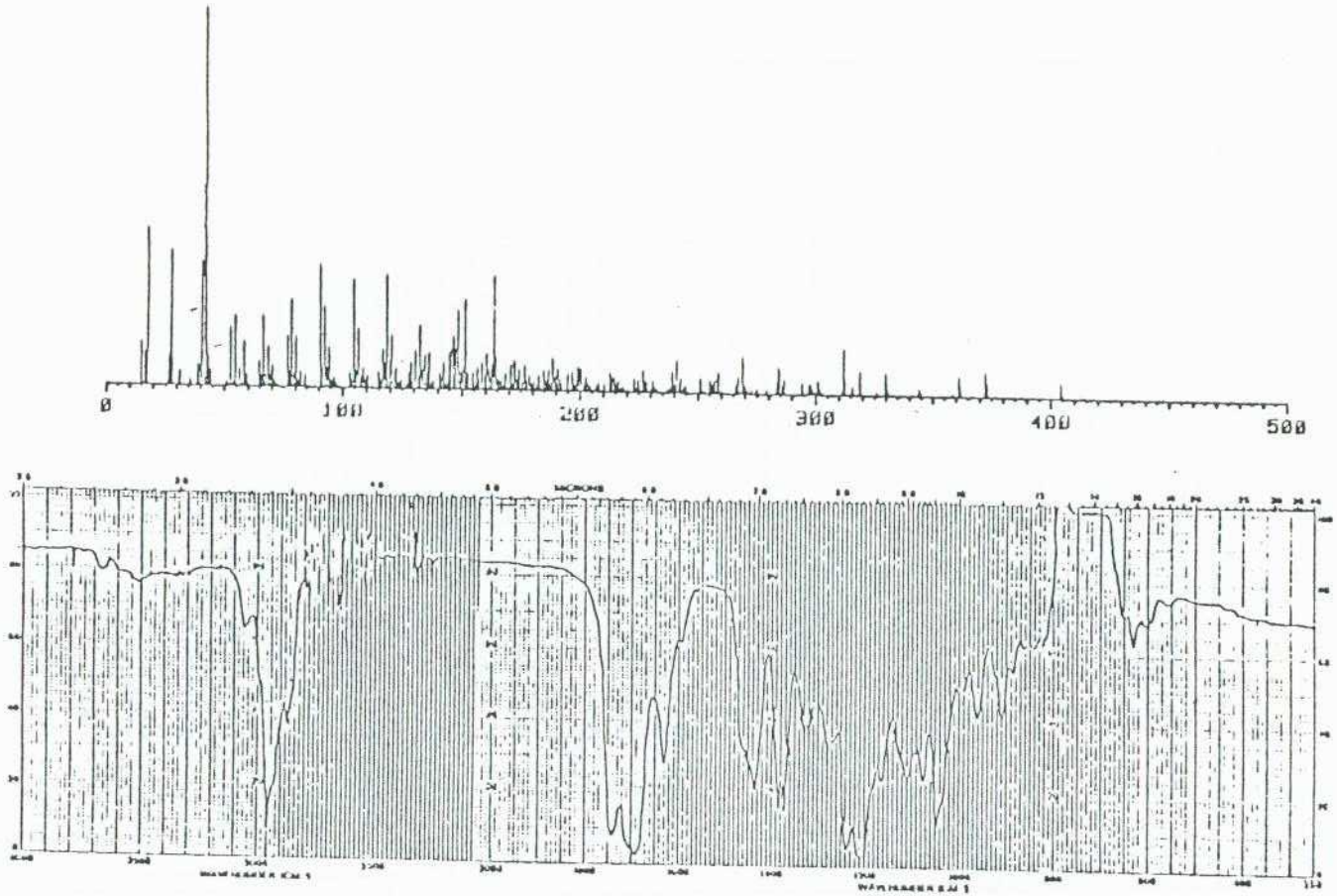


Figure 12. Mass spectrum of emblide (top). Infrared spectrum of emblide in CH_2Cl_2.

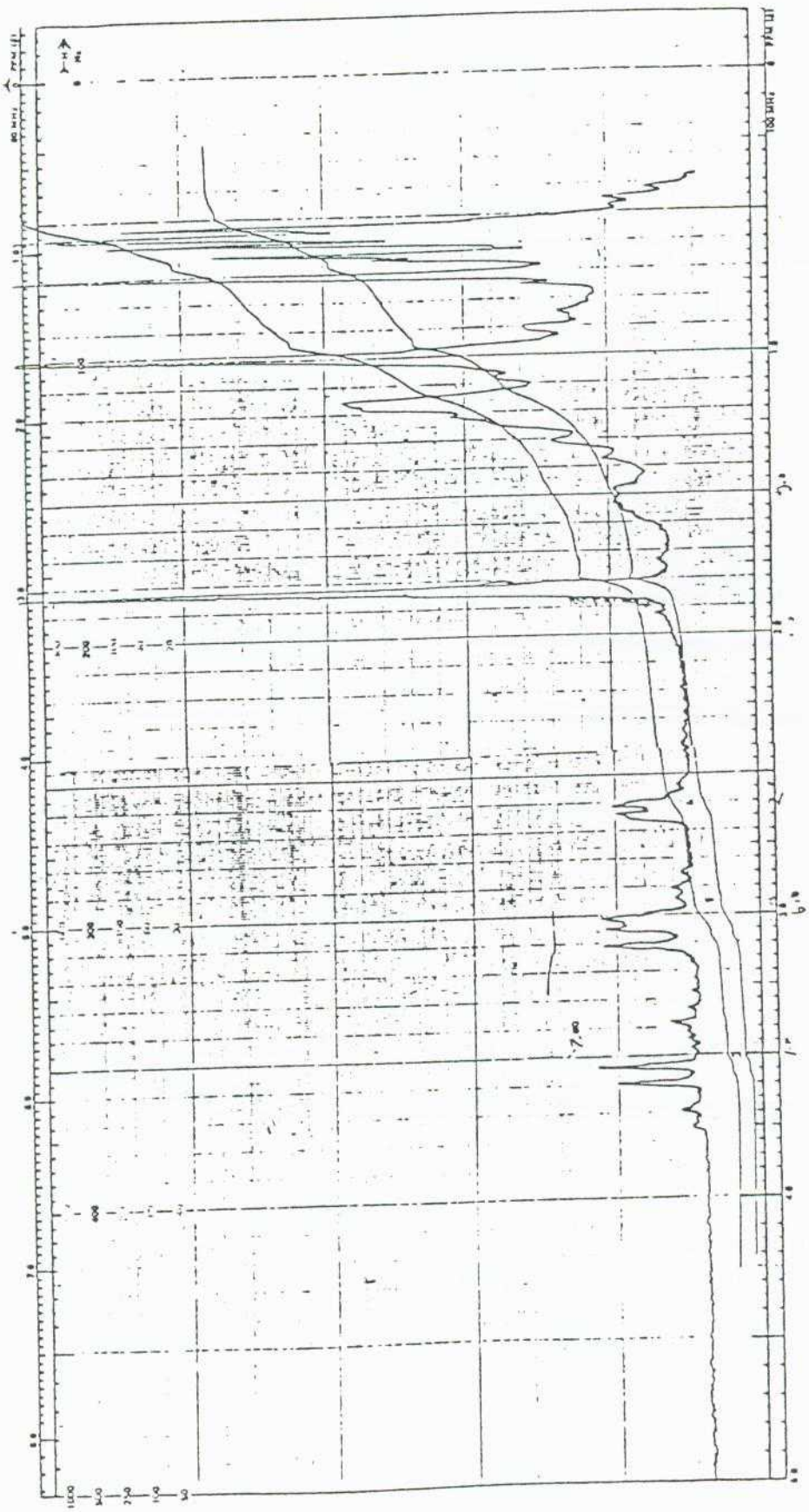


Figure 13. ^{1}H nmr spectrum of enblide in $CuCl_3$

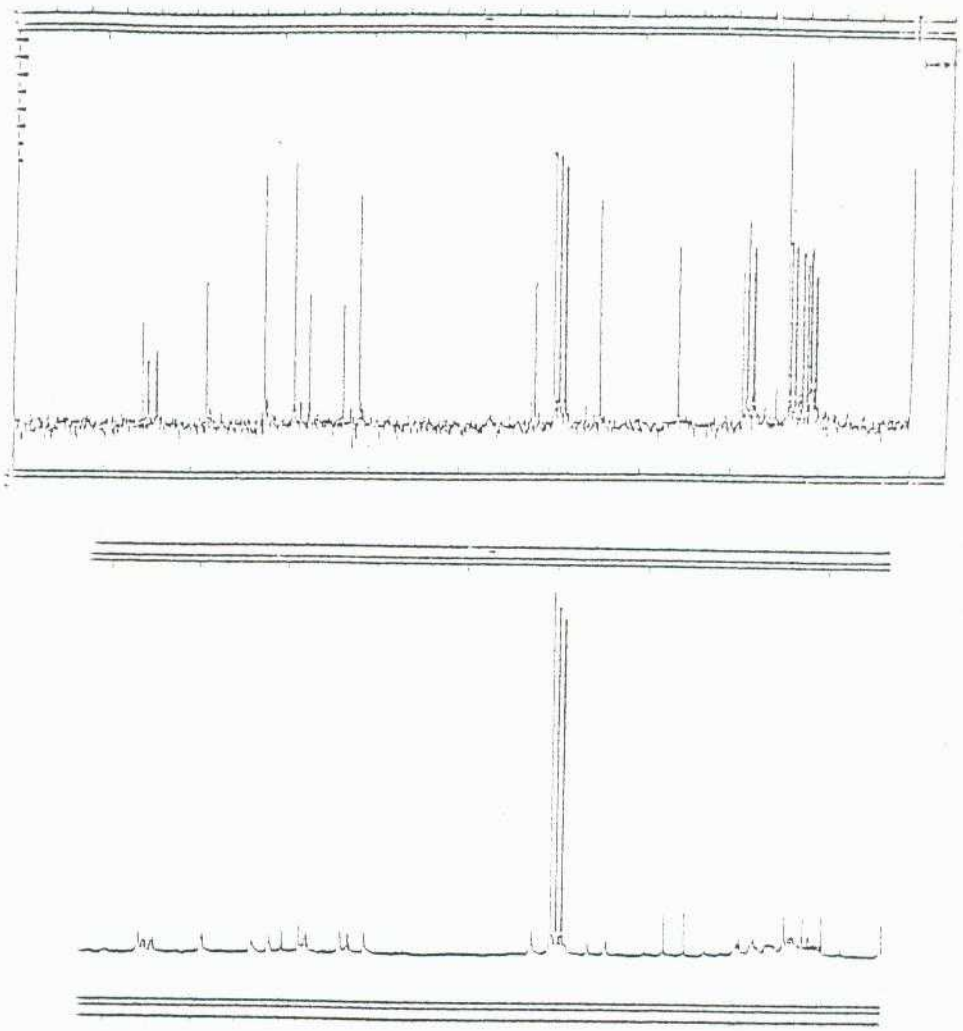
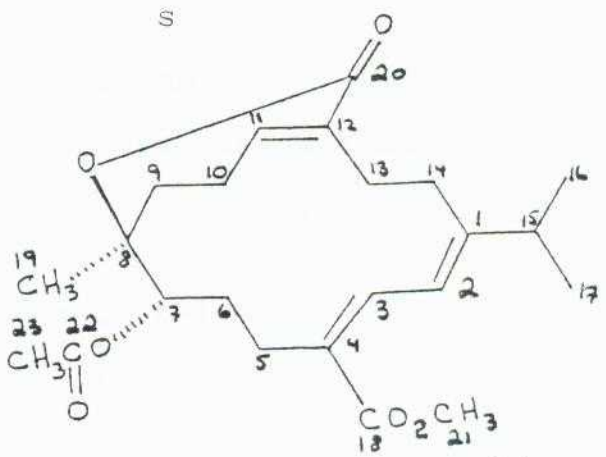


Figure 14. Proton decoupled ^{13}C nmr spectrum of emblide; resonance decoupled spectrum of emblide.

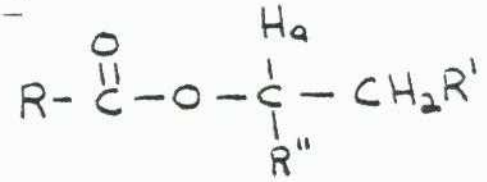
Table IV. Assignments of ^{13}C Nmr Signals in the Spectrum of Emblide (52).

Chemical Shift (δ)	Carbons	Multiplicity	Assignment
169.4	1	s	C-18
168.0	1	s	C-20
166.1	1	s	C-22
154.9	1	s	C-4
141.9	1	d	C-3
135.4	1	d	C-2
131.9	1	s	C-12
124.4	1	s	C-1
120.8	1	d	C-11
82.3	1	s	C-8
68.2	1	d	C-7
51.2	1	q	C-21
37.1	1	-	-
35.9	1	q	C-23
34.3	1	s	
27.1	2		
26.3	1		
25.3	1		
23.7	1		
22.7	1		
21.9	1		
20.9	q	-	C-1



The ^{1}H nmr spectrum also exhibited a signal at 5.26 ppm (1H, dd, J= 6,2 Hz) which may be assigned to an ether methine (OCH_a) as in partial structure A.

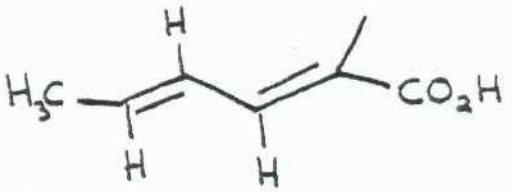
A



Signals are also recorded at 7.22 ppm (1H, d, J=12 Hz), 6.15 ppm (1H, d, J=12 Hz) and at 6.02 ppm (1H, br t, J=4 Hz). ^{1}H nmr data and assignments are summarized in Table V.

Emblide (53) exhibited a strong absorption with λ_{max} at 284 nm (ε 15,600) with a shoulder at 220 nm (ε 8,800). The chromophore responsible for this ultraviolet spectral charactersitic is unlike any of the chromophores encountered in other cembranoids. The ultraviolet spectral data did suggest the existence of a diene system. Acyclic 2,4-dienoic acids and the corresponding methyl or ethyl esters such as sorbic acid (B) absorb at 259 $\pm$ 5 nm (ε 20,000-25,000).[66,67]

B



Alkyl substituents produce a bathochromic shift of 5-9 nm. By using sorbic acid as a model, the ultraviolet absorption and additional spectroscopic evidence suggested the presence of a methyl sorbate (C).

C

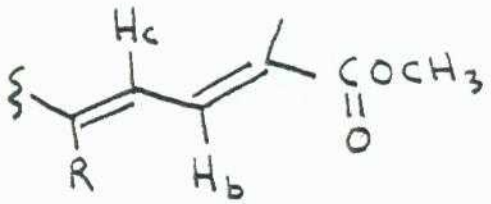
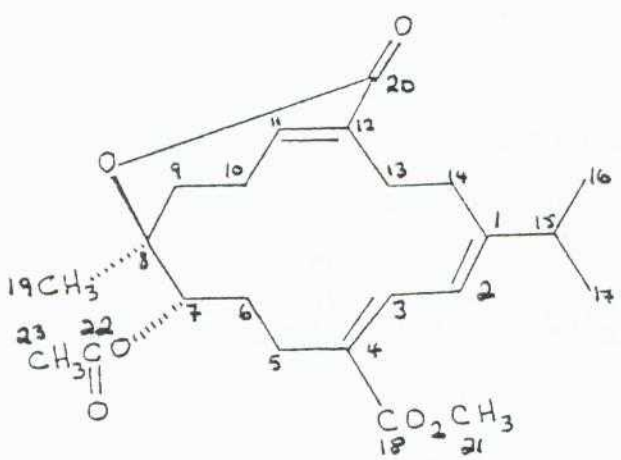


Table V. Assignments of ^{1}H Nmr Signals in the Spectrum of Emblide (53).

Chemical shift (δ)	Protons	Multiplicity	J (Hz)	Assignment
7.11	1	d	12	H_b
6.15	1	d	12	H_c
6.02	1	br t	4	H_d
5.25	1	dd		H_a
3.70	3	s	-	C-21 protons
3.10	1	br m	-	-
2.35	8	cs	-	-
2.0	5	br s	-	C-23 protons and others
1.80	1	br s	-	
1.77	1	br m	-	C-15 proton
1.43	3	s	-	C-19 protons
1.15	6	dd	-	C-16 and C-17 protons

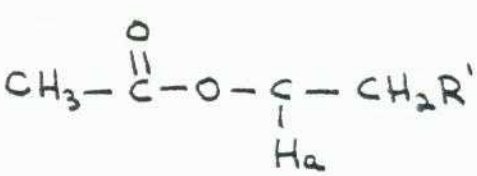


The proton signals at 7.11 ppm and 6.15 ppm may be assigned to the beta (H_b) and gamma (H_c) protons of the dienoate system. ^{13}C nmr frequencies (doublets at 141.9 and 135.4) are also in agreement. The structure is probably responsible for the strong infrared absorbance at 1695 cm^{-1}.

Catalytic hydrogenation readily afforded a hexahydroemblide derivative 54 (Figures 15 and 16) with a molecular ion at 410 corresponding to the formula $C_{23}H_{38}O_6$. The infrared spectrum showed that the carbonyl frequency at 1740 cm^{-1} was indeed due to an aliphatic ester. It also showed the absence of all infrared absorbances associated with the olefinic portion of emblide. The 100 MHz ^1H nmr spectrum also exhibited the absence of olefinic protons with proton signals at 3.6 ppm (s, 3H), 2.0 ppm (s, 3H), 1.8-1.4 ppm (23 proton envelope), 1.4 ppm (s, 3H) and 0.9 ppm (dd, 6H) for an isopropyl group. The interesting phenomenon is the formation of a mixture of isomers as the signals (spikes) indicate the diene and monoene systems were sterically hindered (Figure 16). Otherwise, cis-addition of hydrogen to the unsaturated sites would have resulted in only one product.

Valuable information was obtained from the deacetylation product (55) of emblide (Figures 17 and 18). The doublet of doublets at 5.25 ppm in the 100 MHz ^1H nmr remained after acetylation implying structure D.

D



The methyl resonance at 1.43 ppm remained unchanged in the deacetylated product and can be assigned to partial structure E in emblide (53).

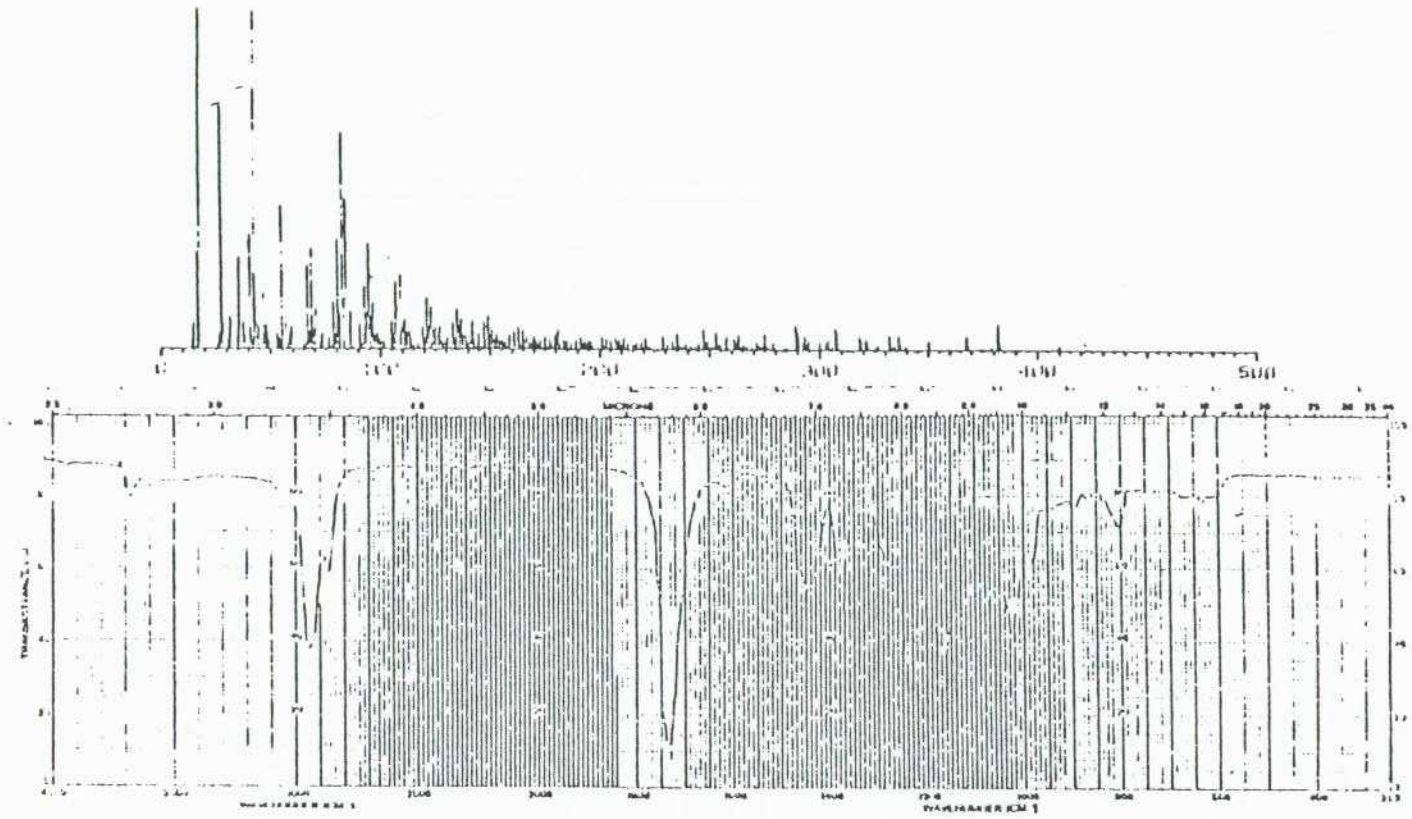


Figure 15. Mass spectrum ofhexahydroemblide (top). Infrared spectrum of hexahydroemblide (bottom).

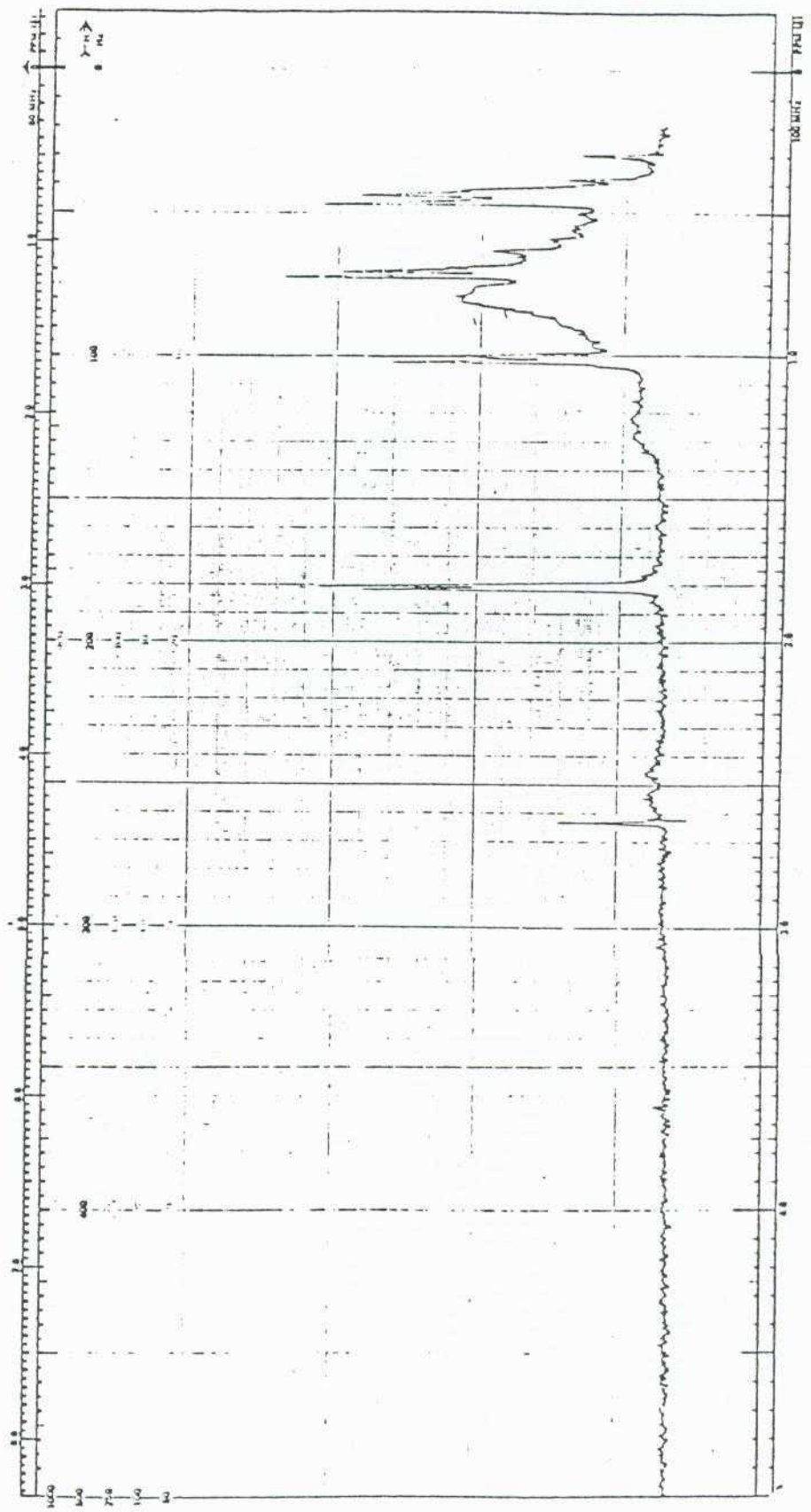


Figure 16. ^{1}H nmr spectrum of hexahydrocembl ide in $CDCl_3$.

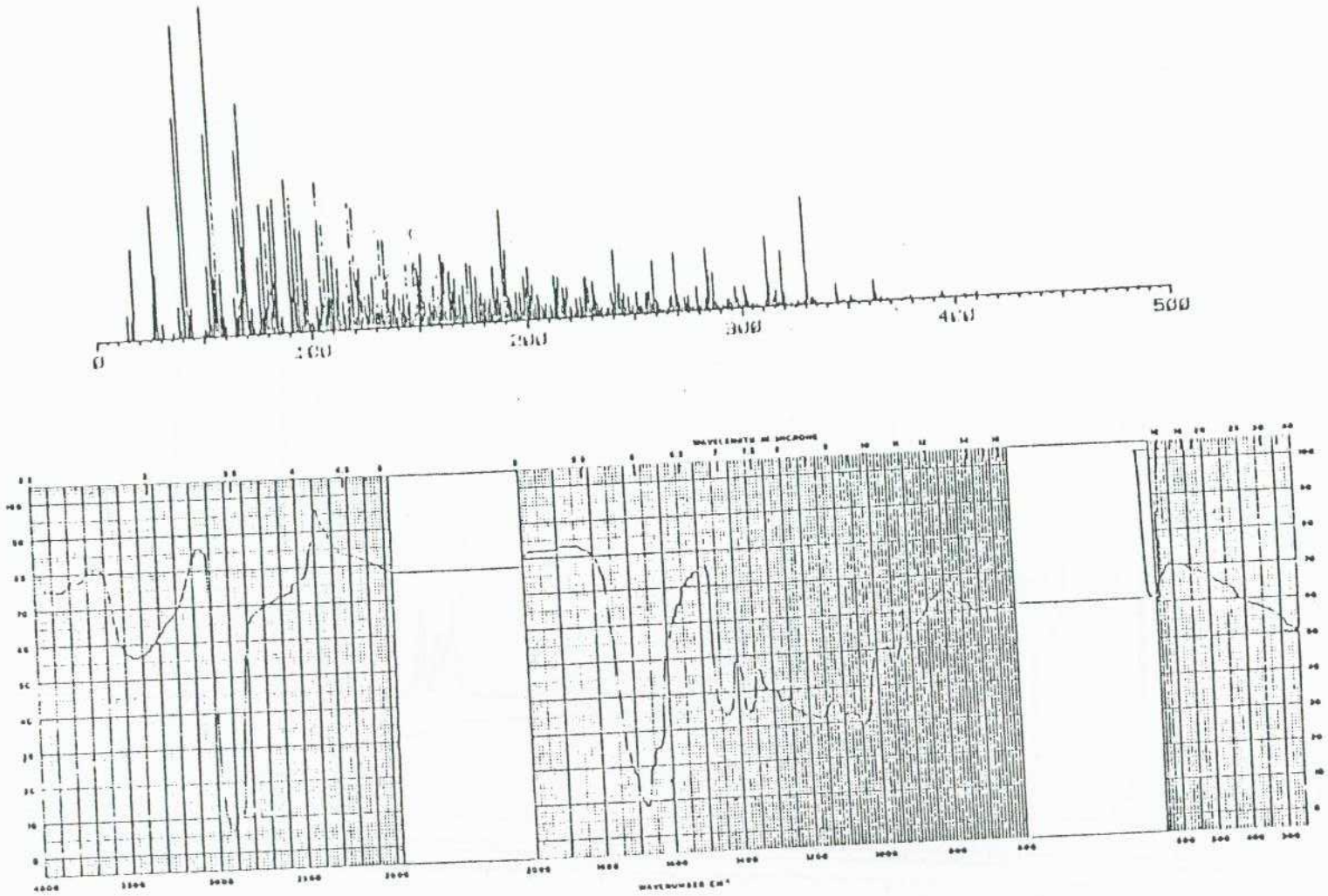


Figure 17. Mass spectrum of deacetylated emblide (top). Infrared spectrum of deacetylated emblide (bottom).

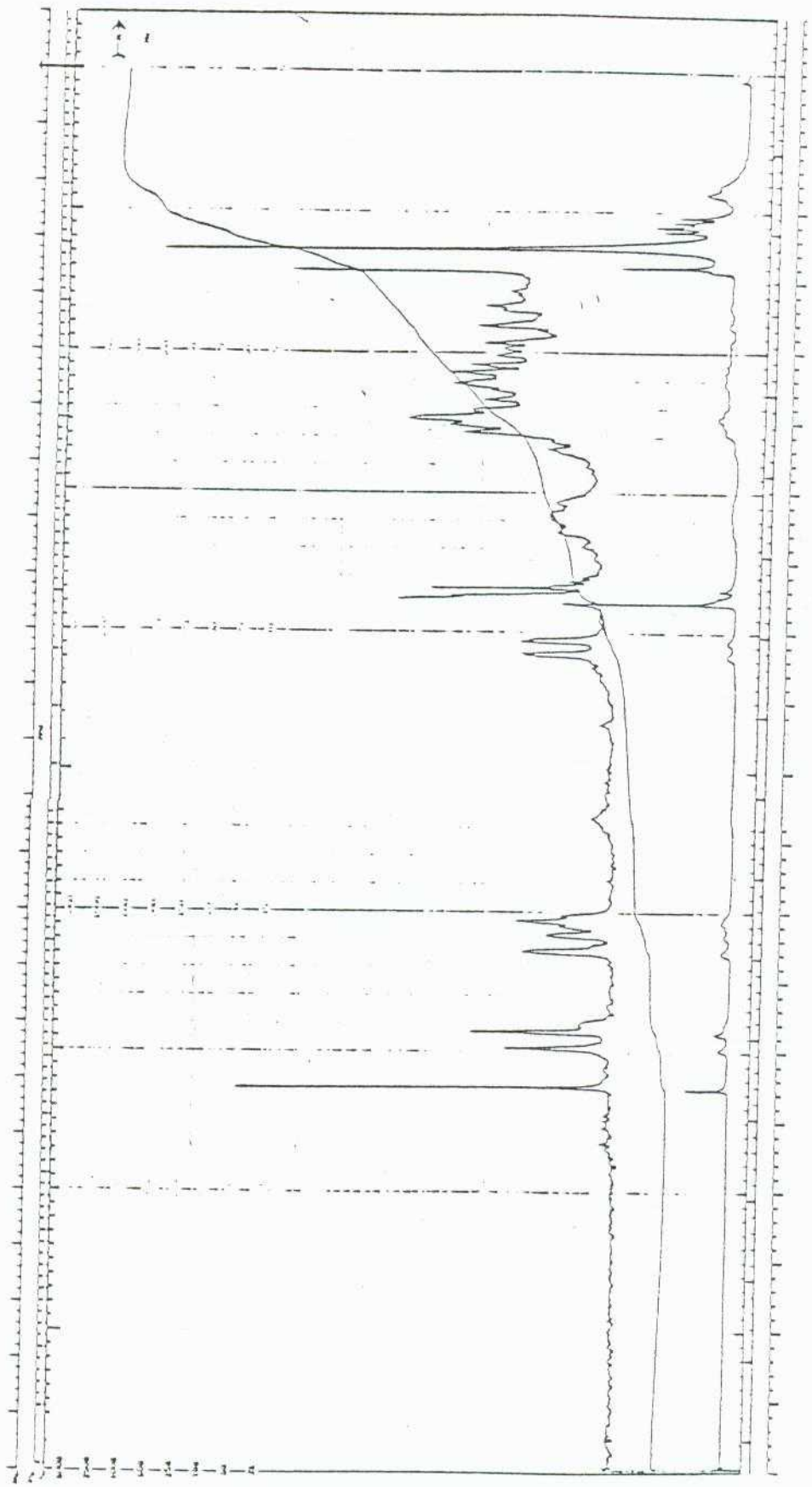
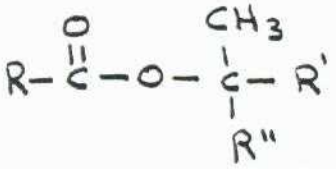


Figure 18. ^{1}H nmr spectrum of deacetylated emblide.

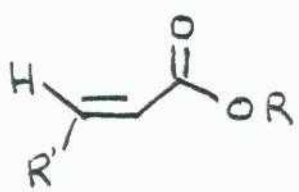
E



The deacetylation resolved that the methyl was attached to the quaternary carbon of an ester; otherwise, the chemical shift would have been altered.

Thus the spectral features of emblide (53), its hexahydro derivative (54) and the C-7 alcohol (55) secured the nature of all functional groups. Ready loss of methanol (m/e 372) and of acetic acid (m/e 344) from the molecular ion attested to the presence of the methyl ester and acetoxy groups which were also confirmed by appropriate ^{1}H nmr singlets at 3.7 ppm and 2.0 ppm. The ultraviolet maximum at 284mm (ε 15,600) suggested a dienoic ester, which was also supported by an infrared band at 1695 in agreement with absorption expected of a trialkylated methyl sorbate. The two ^{1}H nmr doublets can be assigned to the beta and gamma protons of the dienoate system. The third carbon-carbon double bond is indicated by the presence of six olefinic ^{13}C nmr signals (155.0 to 120.8 ppm)-three singlets and three doublets. The remaining olefinic proton, a broadened triplet at 6.02 ppm, must be attached to the remaining unassigned olefinic carbon doublet (from ^{13}C nmr) and appears to be the beta proton of a monoenoate as in structure F.

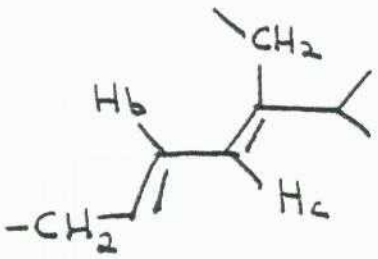
F



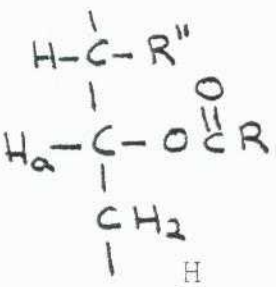
The signal at 5.26 ppm (dd,J=6,2Hz) in the ^{1}H nmr spectrum of emblide, assigned to an ester methine, cannot be associated with the α,β-unsaturated ester. It must be the methine proton of the acetate since in the deacetylated derivative, the resonance is seen at 4.12 ppm (dd,J=8,2Hz). On the other hand, the methyl singlet at 1.42 ppm in 53 remains in 54 and 55, thereby placing it at the C-8 carbon bearing the oxygen of the monoenoate. These assignments are further attested to by the corresponding ^{13}C nmr doublet at 682 ppm and a singlet at 82.3 ppm The three ester carbonyls at 169.3, 168 and 166.1, matched by the ir frequency at 1740cm^{-1} require that the remaining function be an α,β-unsaturated lactone of six or more members.

All functional groups of emblide have thus been established. In 53, three esters and three olefinic linkages accounted for all but two sites of unsaturation. Making the reasonable assumption that emblide arose from geranylgeranyl pyrophosphate (48), the normal diterpenoid precursor, the cembrane skeleton emerges as one of the rings in emblide. While 53 represented a reasonable structure for a marine cembranolide on biogenetic grounds, we had little direct evidence for the arrangement of the functional groups within the fourteen membered ring. A series of decoupling experiments further characterized the structural nature of emblide.

Emblide was not very amenable to decoupling experiments but did provide valuable information. The doublets at 7.11 ppm and 6.15 ppm (H_b, H_c) were found to be allylically coupled to the proton signal at 2.3 ppm which substantiates partial structure G. Additionally, the doublet of doublets at 5.25ppm (H_a) was found to be coupled to the proton signal at 1.85 ppm thereby showing that the methine of the ester is not allylic and substantiating partial structure H.



G



H

As has been the case with most cembranoids, further structural information on emblide was not accessible through chemical degradation. Several attempts at oxidation with the intention of cleavage at the olefinic sites resulted in mixtures of products. Several attempts with Lemieux-Jones oxidations (Figures 19-26) as well as ozonolysis succeeded only to deplete my supply of emblide. An attempt at hydrolysis of hexahydroemblide resulted in a yield of 1.8mg (19%) of product which appeared to be a mixture of several components, by tlc and glc analysis.

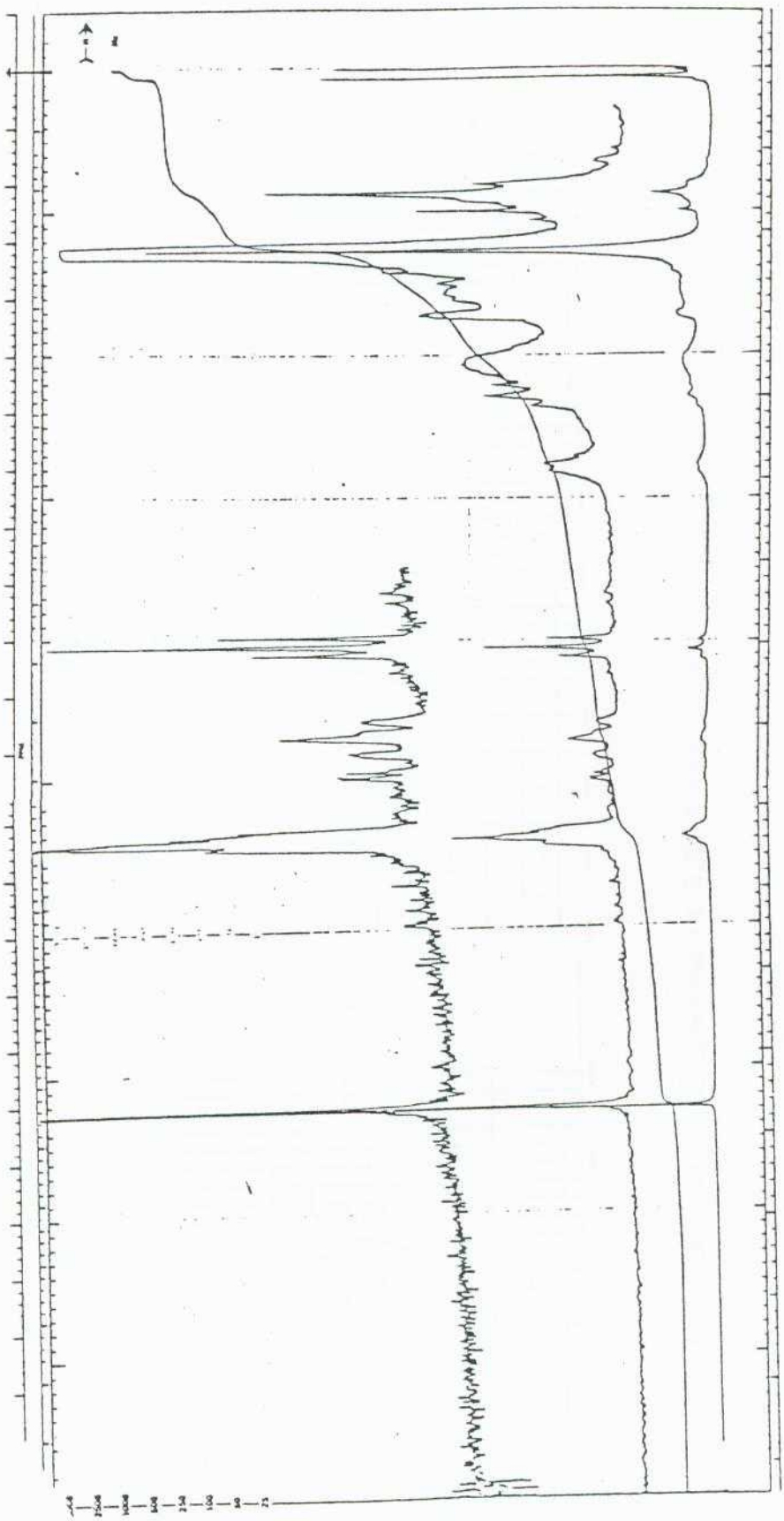


Figure 19. ^{1}H nmr spectrum of R.P. 1 from oxidation of emblide.

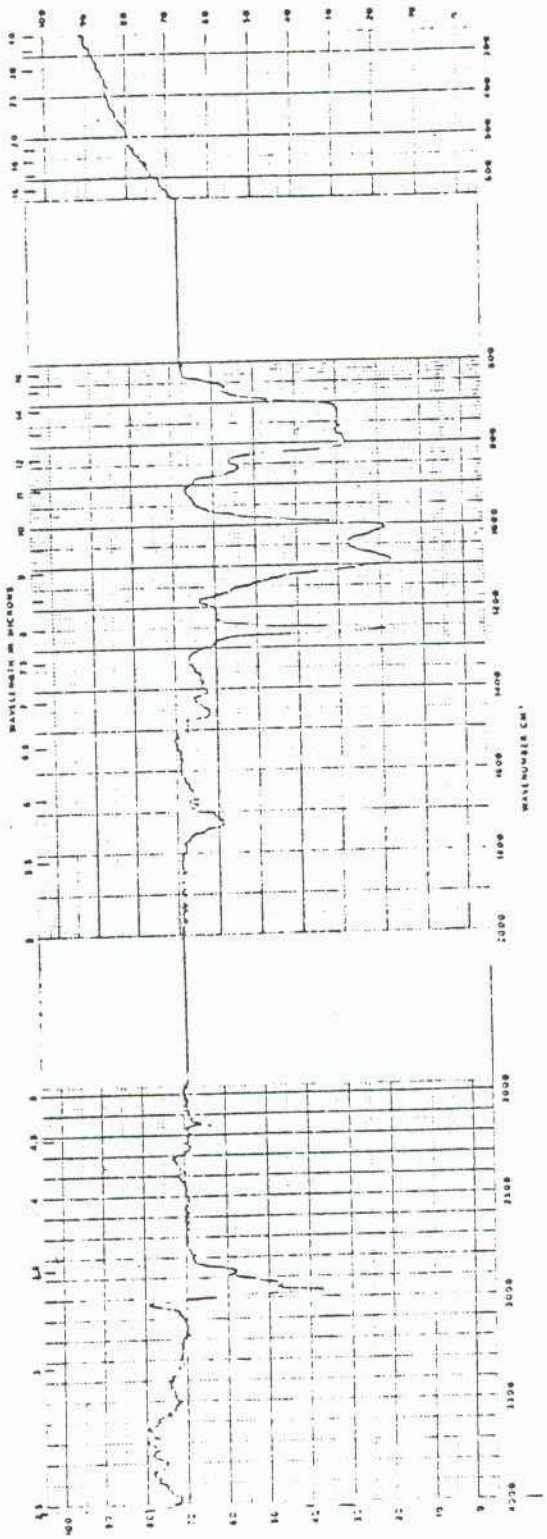


Figure 20. Infrared spectrum of R.P. 1 from oxidation of emblide.

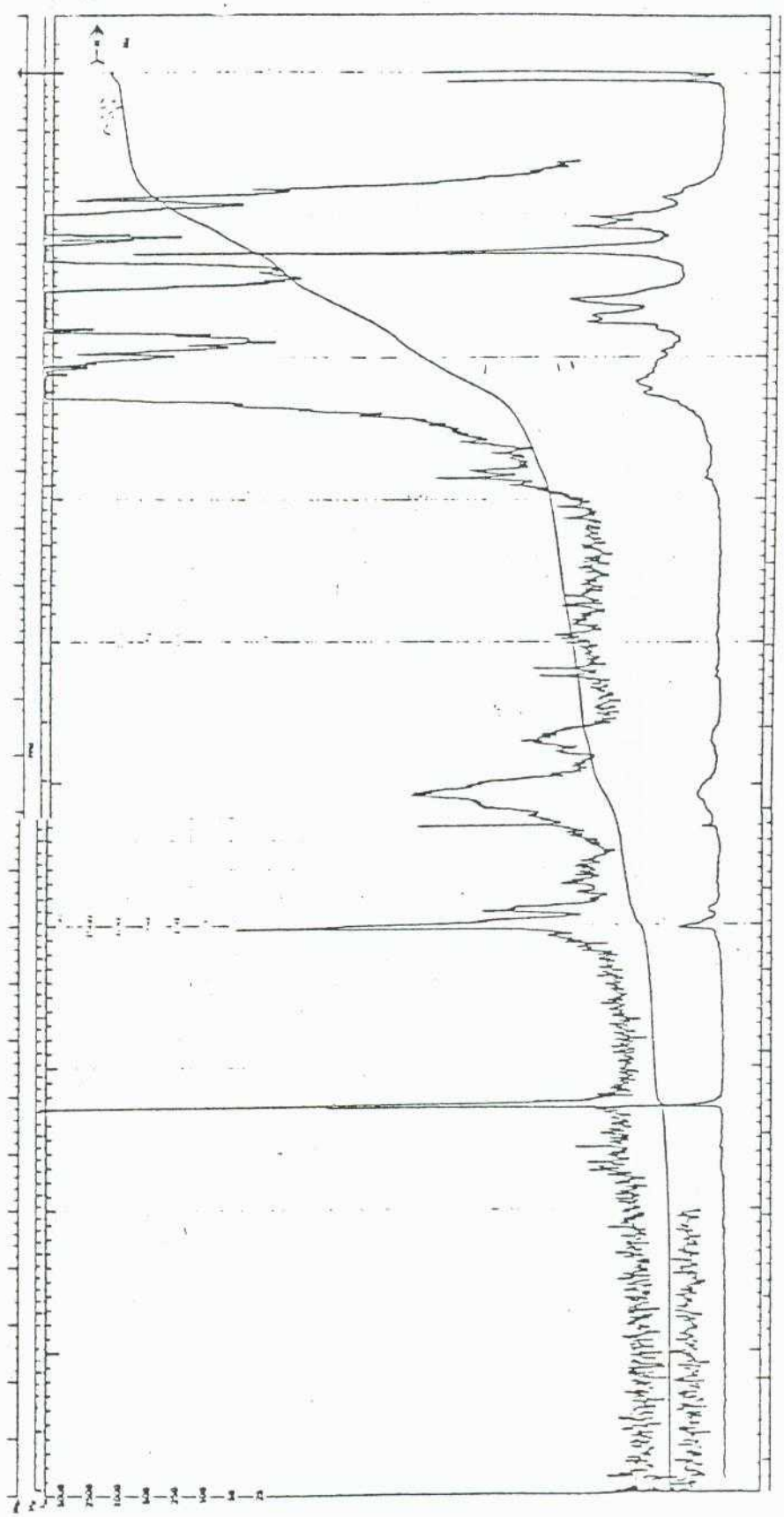


Figure 21. ^{1}H nmr spectrum of R.P. 2 from oxidation of emblide.

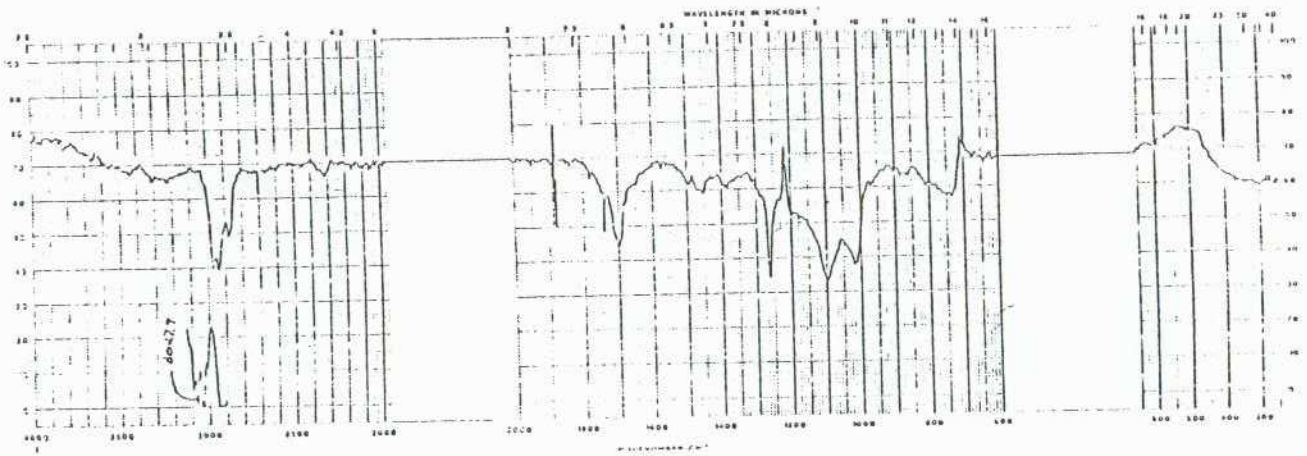


Figure 22. Infrared spectrum of R.P. 2 from oxidation of emblide.

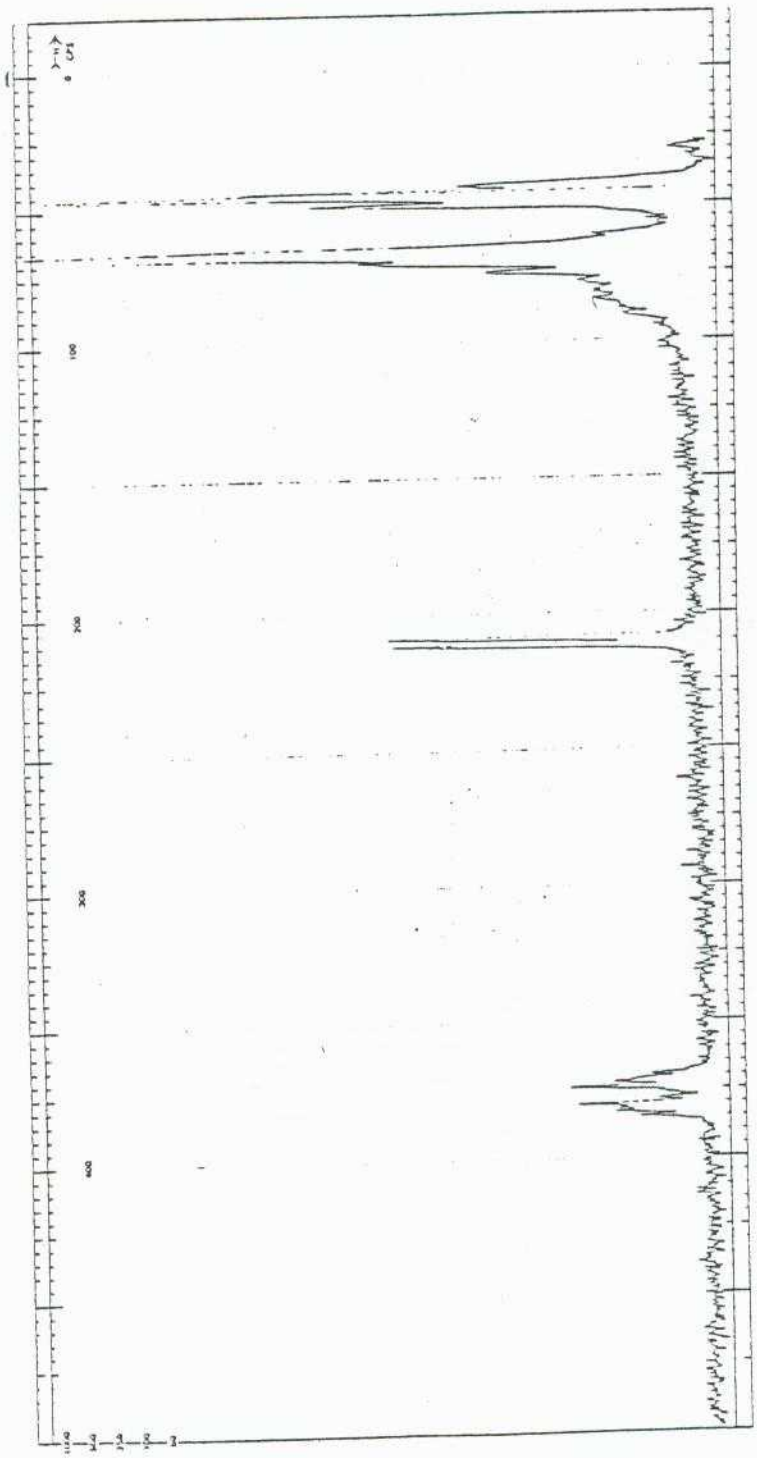


Figure 23. ^{1}H nmr spectrum of R.P. 3 from oxidation of emblide.

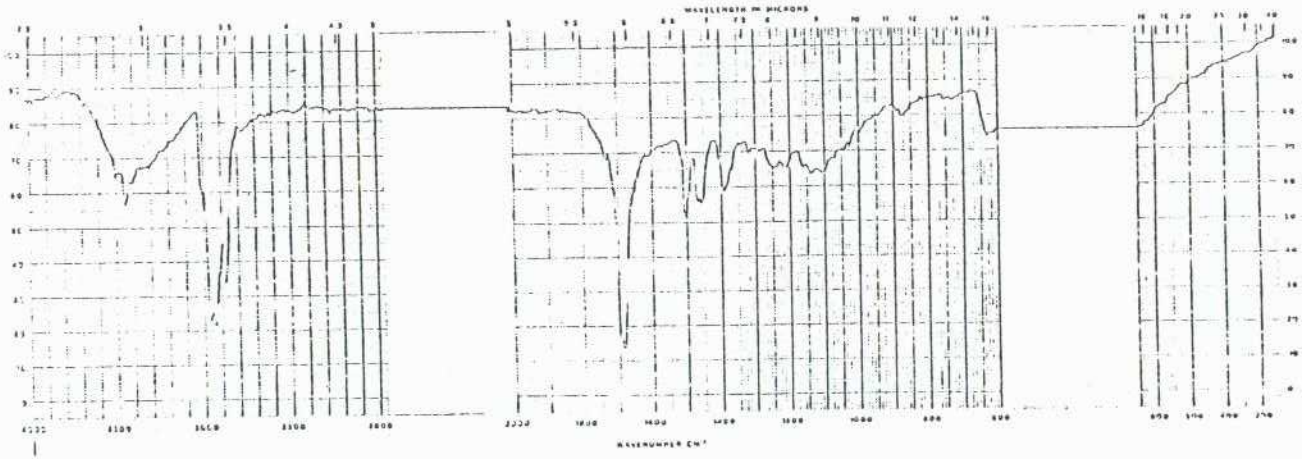


Figure 24. Infrared spectrum of R.P. 3 from oxidation of emblide.

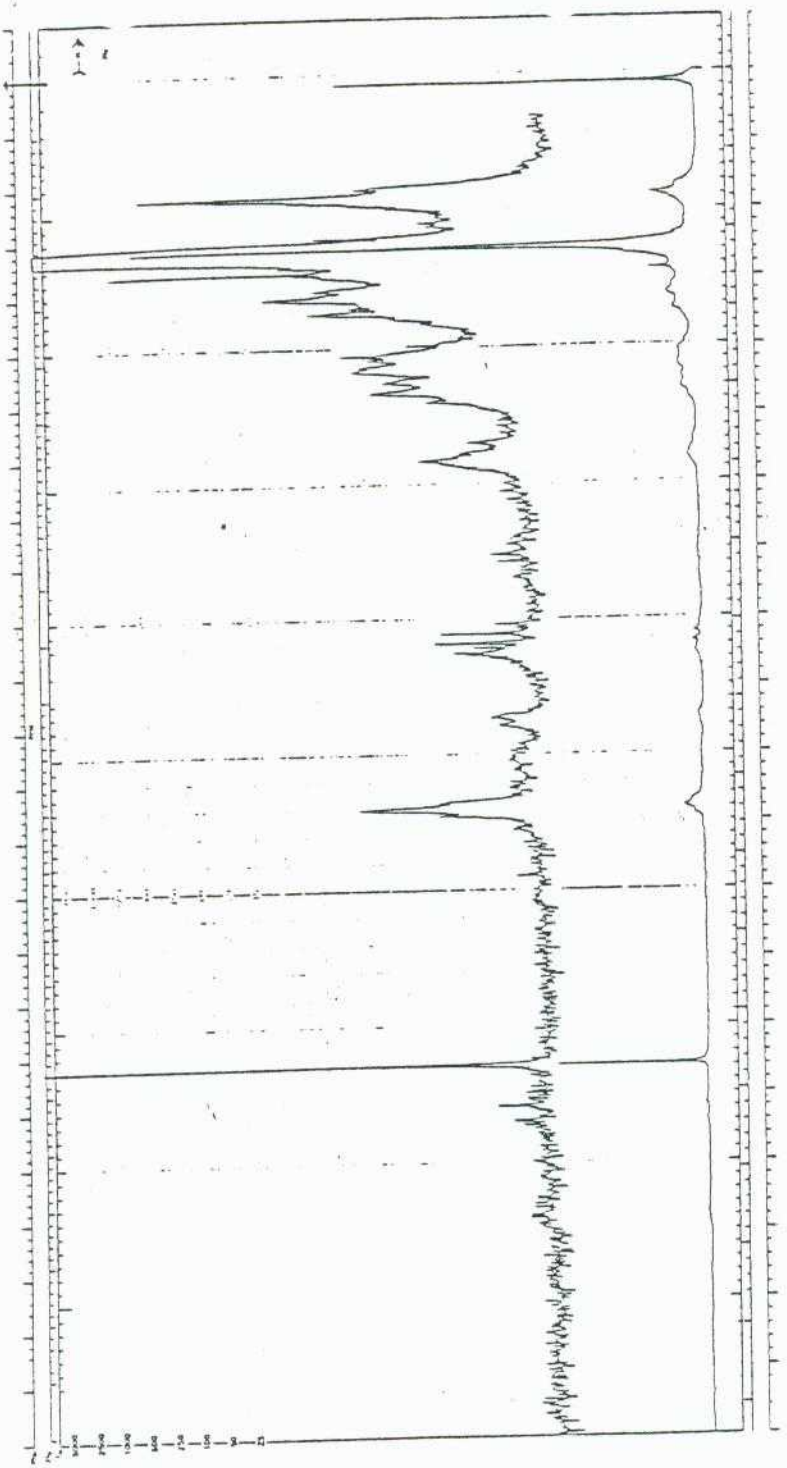


Figure 25. ^{1}H nmr spectrum of R.P. 4 from oxidation of emblide.

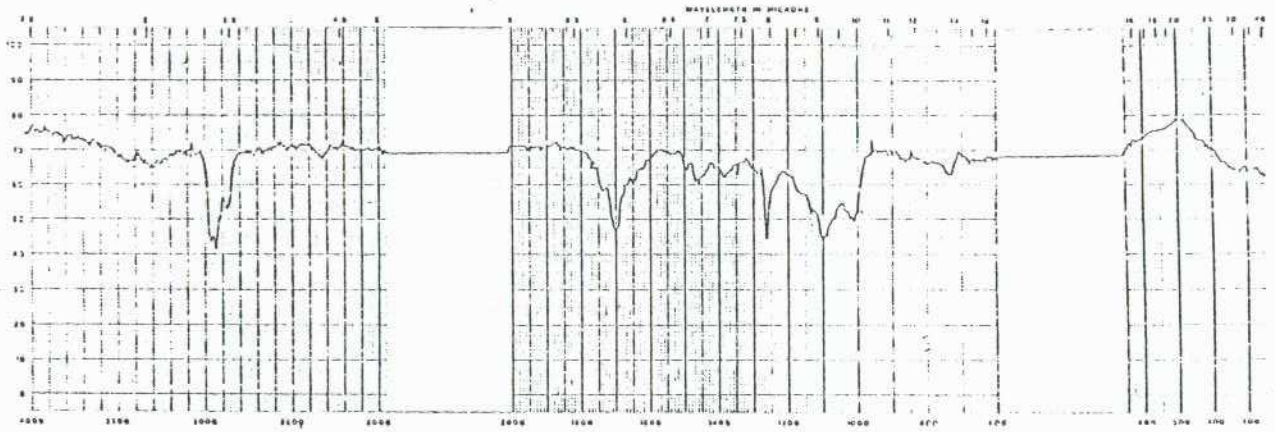
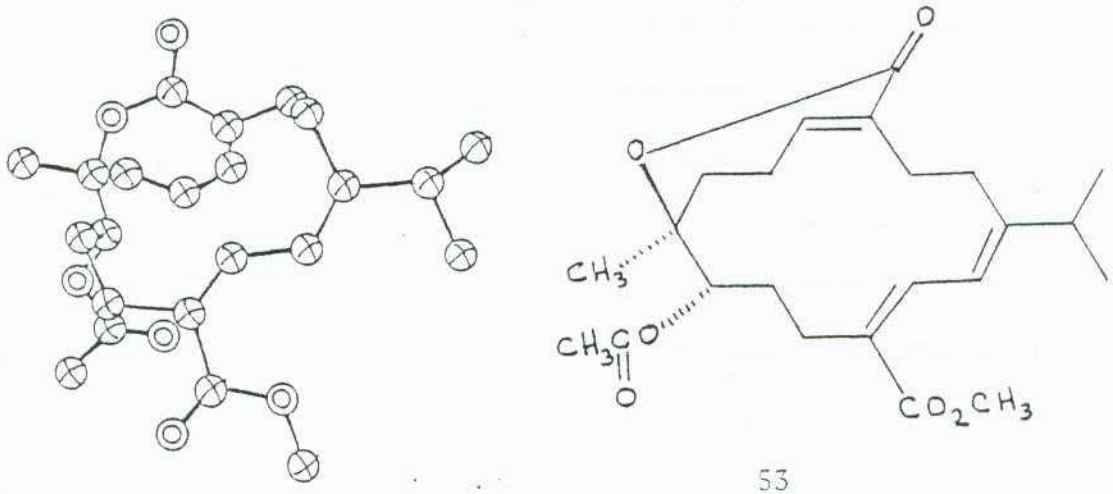


Figure 26. Infrared spectrum of R.P. 4 from oxidation of emblide.

With approximately 50mg of emblide remaining, a sample was subjected to x-ray diffraction studies [68] leading to the complete structure for emblide (53). A computer-generated drawing of emblide (53) is shown below.



53

In retrospect, the complete structure of emblide shows the complexity of this cembranolide. The position of the functional groups illustrates the potential for intramolecular rearrangements during oxidative degradation. The exact mechanism responsible for the unusual results obtained in the Lemieux-Jones oxidation and ozonolysis (Figures 19-26) is unknown but is assumed to have been catalyzed by the reagents.[69,70]

A search for additional quantities of emblide continued concurrently with the effort at structure elucidation. Many additional specimens of *S. glaucum* were scrutinized. Due to the relative inaccessibility to the area we had very little control over the collection of samples from the original site where the soft coral containing emblide had been collected. Additional samples of *S. glaucum* were subjected to the same tedious extraction without isolation of emblide.

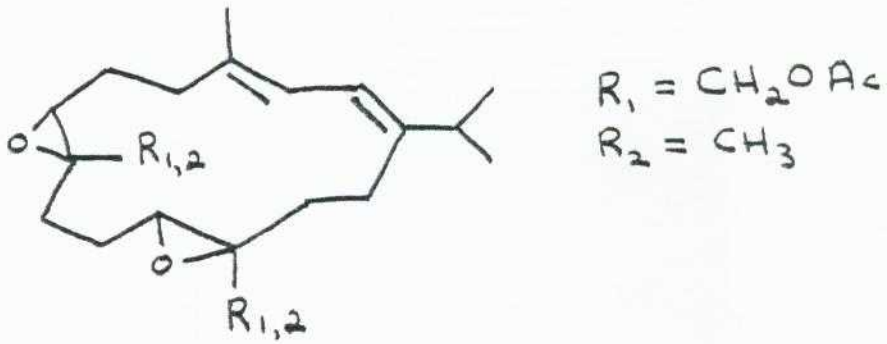
The initial samples of *S. glaucum* were harvested a few weeks after a typhoon had swept across Enewetak atoll. Since the animals were collected at a shallow depth (10 m) and storm damage was evident in the area, the formation of emblide may actually have been brought about by a stress condition.

Subsequent specimens of *S. glaucum* were collected at depths ranging from 15 to 25 m due to nonexistence of populations at the original site. The change in depth could affect the symbiotic zooxanthellae. Variation in the diterpene lactone content of soft corals and gorgonians of the same species suggests the possibility that the biosynthesis of the diterpenes may involve these zooxanthellae. Since the zooxanthellae may differ in identity, the metabolic capability may be affected in different specimens.

Continued search for emblide did result in the isolation of a diterpene constituent of another soft coral of genus Sarcophyton, *S. trocheliophorum*, which originally appeared to be the alcohol corresponding to emblide. A proposed structure for trochelin is presented in the following discussion.

A white crystalline material was isolated from the soft coral *S. trocheliophorum* by extraction with ethanol, partitioning between water and ether and chromatographic separation of the constituents. The new diterpene was designated trochelin and assigned tentative structure 56 pending complete structure elucidation.

56



The spectral data for trochelin are presented in Figures 28-32. The high resolution mass spectrum of trochelin showed a molecular ion at 362 corresponding to the formula of $C_{22}H_{34}O_4$. The infrared spectrum showed one carbonyl absorption at 1710 cm^{-1}; bands corresponding to an isopropyl (gem dimethyl) group at 1450 cm^{-1}, 1380 cm^{-1} and 1370 cm^{-1}; a band at 1240 cm^{-1} suggesting the presence of an acetate; and a band at 1610 cm^{-1} (C=C).

The 25.2 MHz proton decoupled ^{13}C nmr spectrum confirmed the presence of twenty two carbons, including the one ester carbonyl (170.4 ppm); four olefinic carbons (147.9ppm, 134.7 ppm, 120.0 ppm and 117.9); five carbons singly bound to oxygen (64.2 ppm, 62.3 ppm, 60.8 ppm, 60.0 ppm and 59.3 ppm); with the remaining twelve carbons appearing in the aliphatic region. Table VI summarizes the ^{13}C nmr data and assignments.

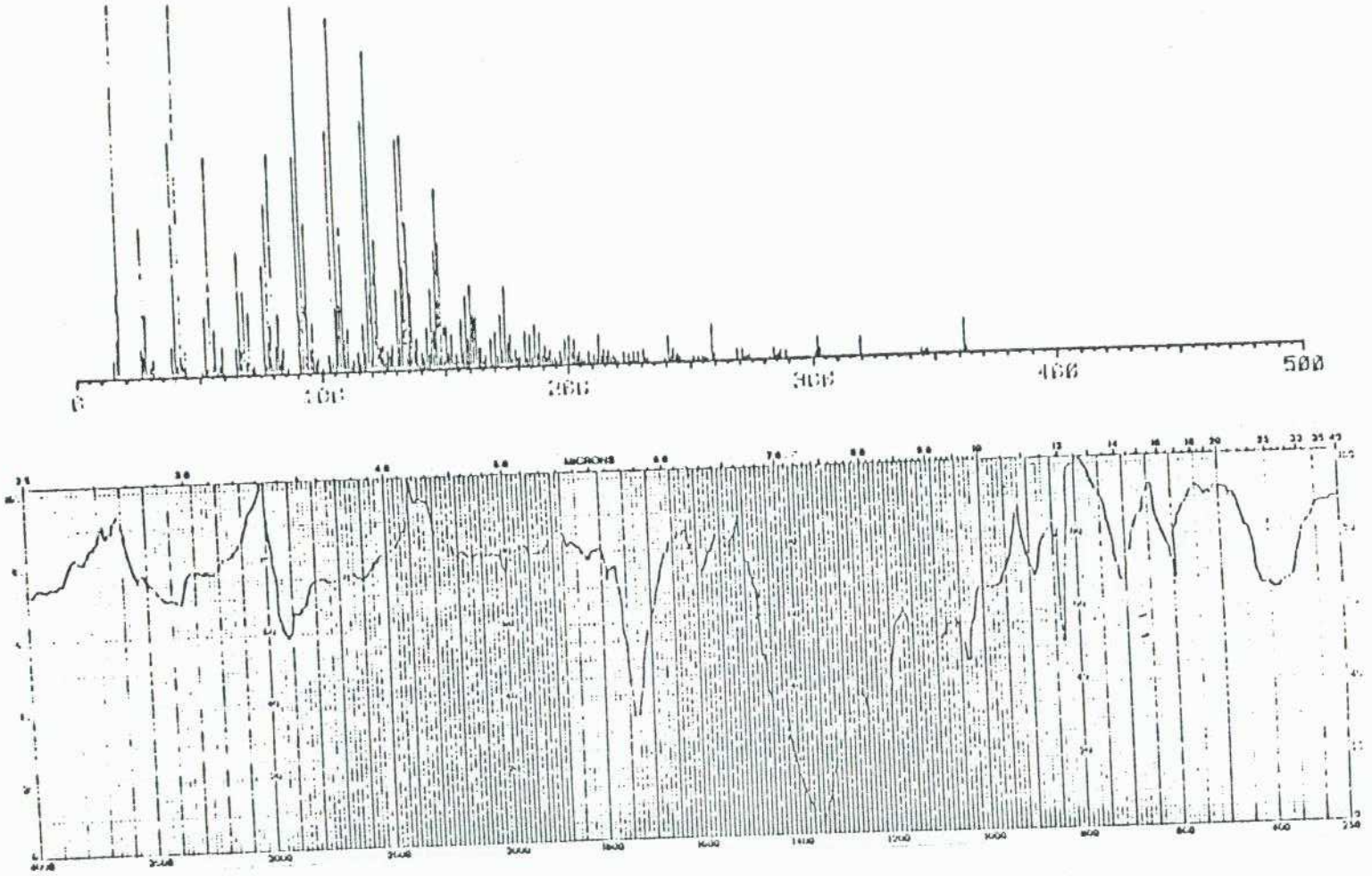


Figure 27. Mass spectrum of trochelin (top). Infrared spectrum of trochelin (bottom).

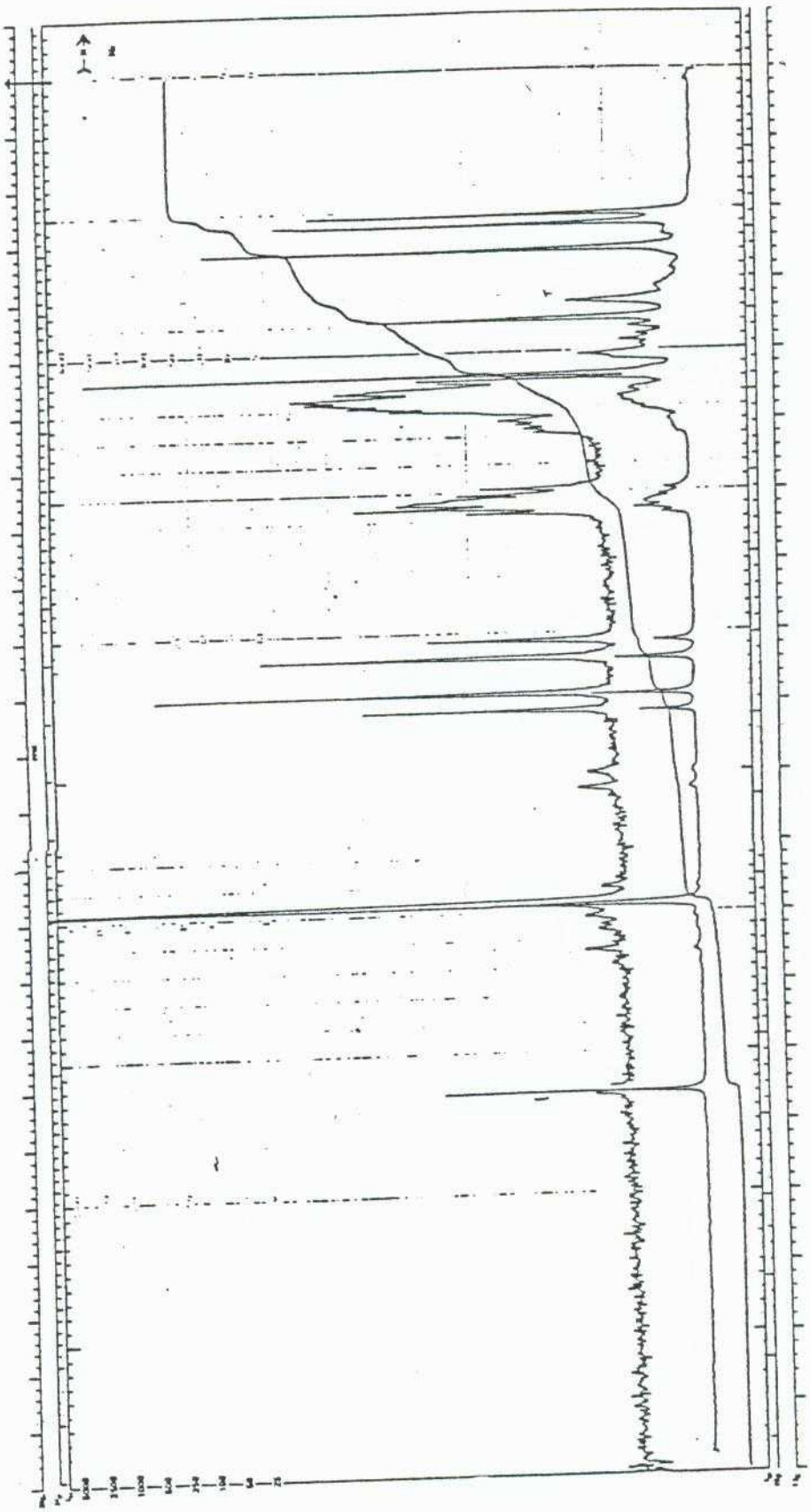


Figure 28. ^{1}H nmr spectrum of trochelin.

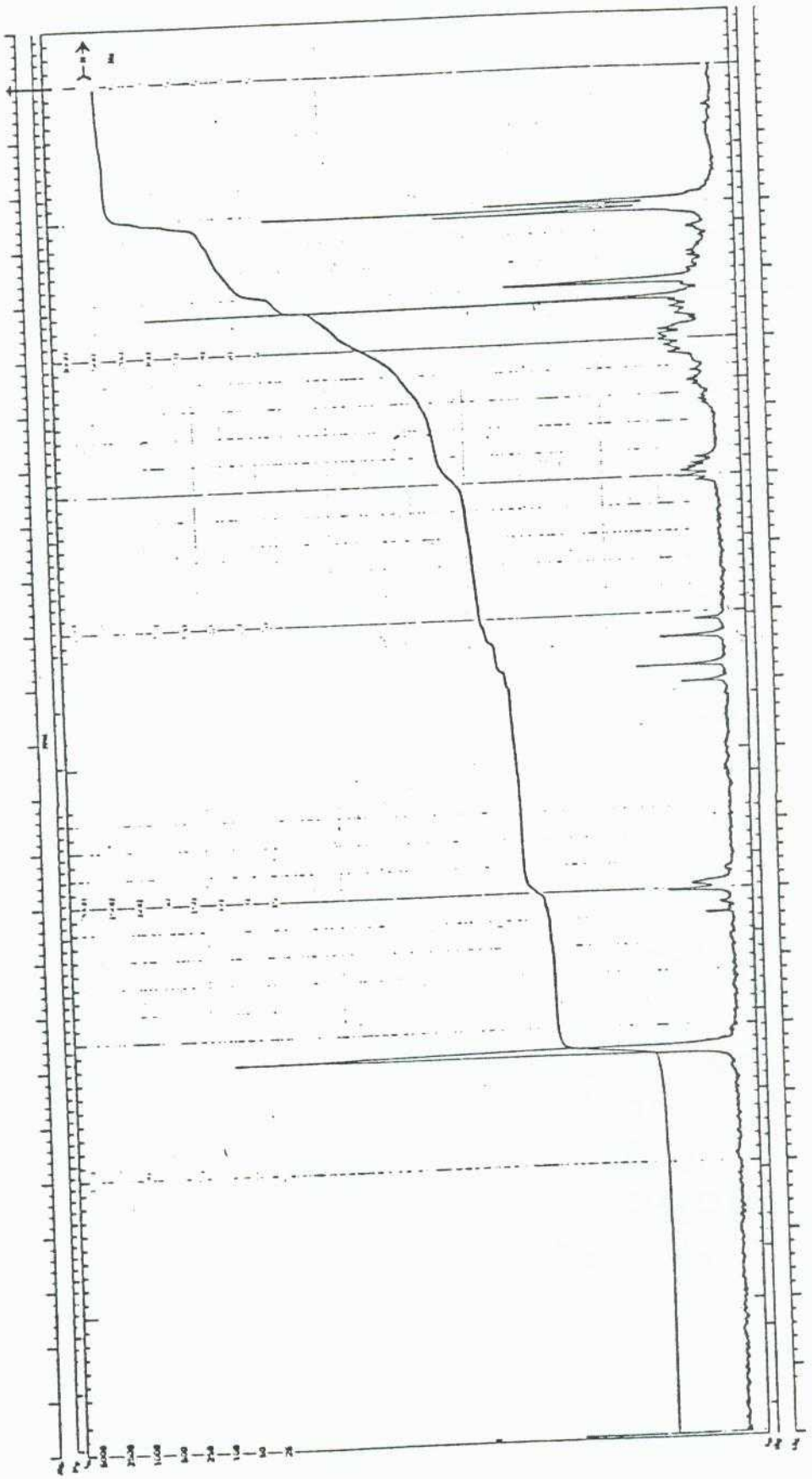
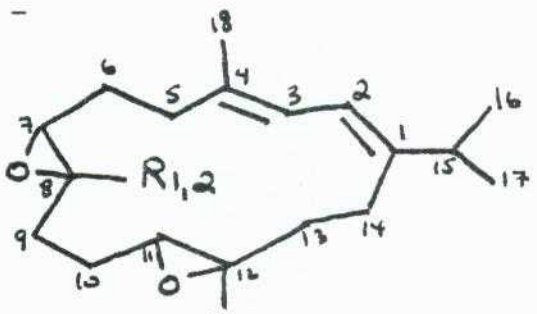


Figure 29: ^{1}H nmr spectrum of trochelin in d_6-benzene.

Table VI. Assignments of ^{13}C Nmr Signals in the Spectrum of Trochelin (56)

Chemical shift (δ)	Carbons	Multiplicity	Assignments
170.4	1	s	Acetate carbonyl
147.9	1	s	C-1
134.7	1	s	C-4
120.0	1	d	C-3
117.9	1	d	C-2
64.2	1	s	C-12 or C-8
62.3	1	d	C-7 or C-11
60.8	1	t	$-CH_2-O-$
60.0	1	d	-CH-O
59.3	1	s	-C-O-
37.9	1	-	
35.5	1		
34.6	1		
31.4	1		
29.6	1		
25.0	2		
23.3	1		
21.9	1		
20.8	1		
17.6	1		



$R_{1,2}$

$R_1 = CH_2OAc$

$R_2 = CH_3$

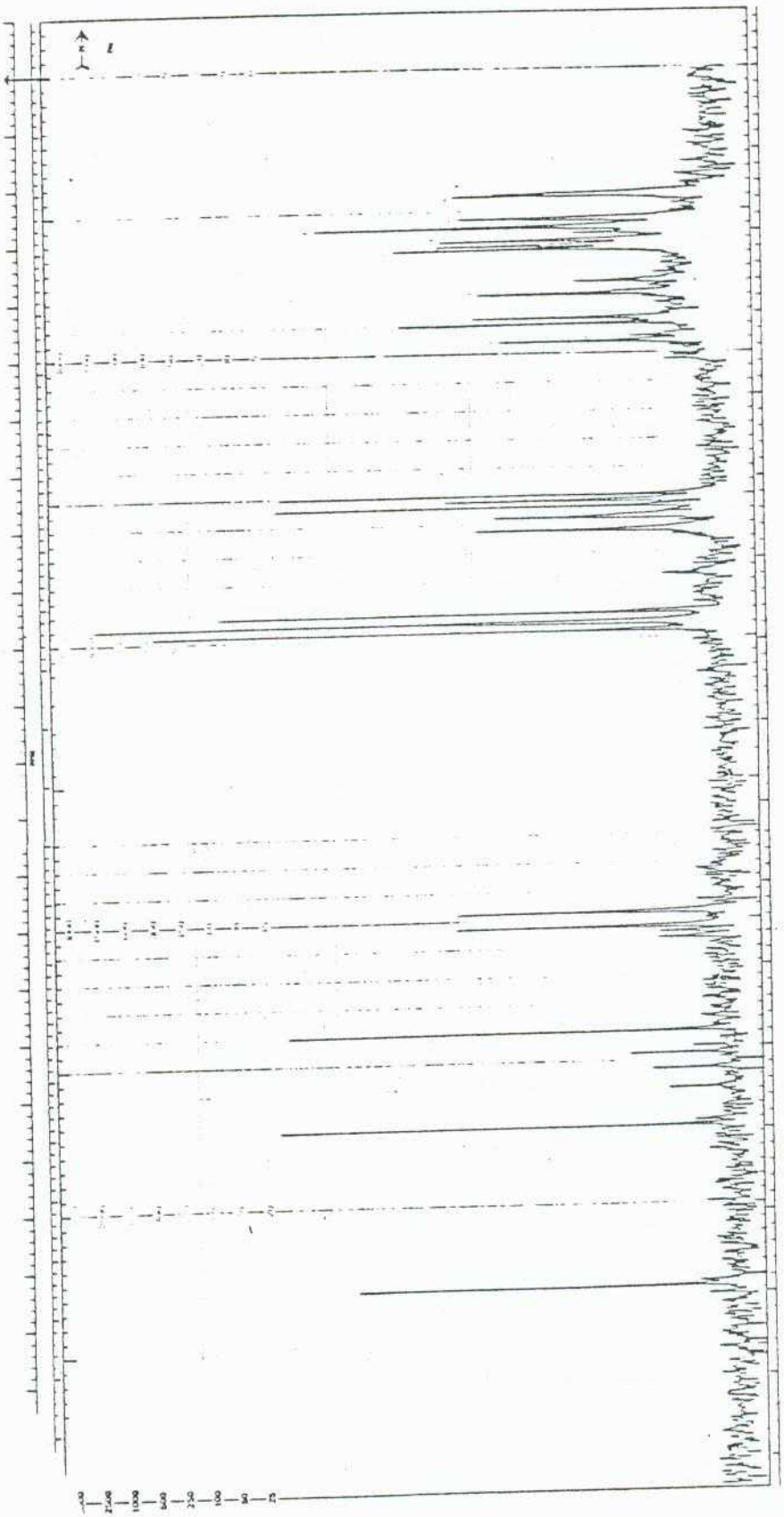


Figure 30a. Off-resonance ^{13}C nmr spectrum of trochelin.

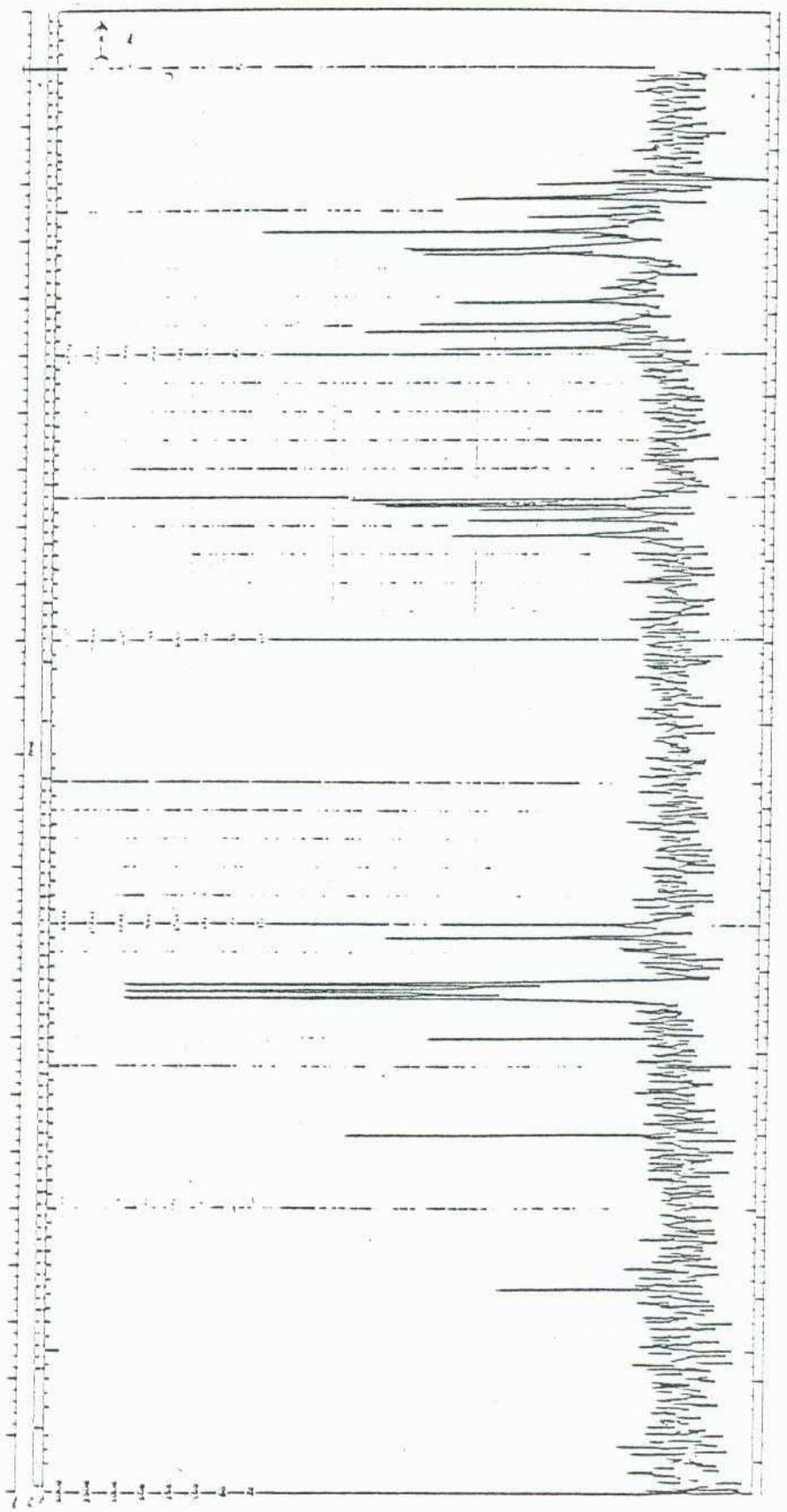
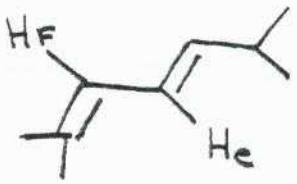


Figure 30b. Proton decoupled ^{13}C nmr spectrum of trochelin.

The 100 MHz ^1H nmr spectrum in $CDCl_3$ showed an interesting absorbance at 5.95 ppm (s, 2H). The absorption did not exchange with D_2O and therefore cannot be attributed to an exchangeable proton due to a hydroxyl. The spectrum of trochelin was then taken in benzene-d_6 (Figure 30) where the resonance at 6.0 ppm appears as an overlapping doublet of doublets akin to the conjugated diene system previously described for emblide (53) as in partial structure G.

G



Trochelin has a strong ultraviolet absorption at 246 nm (ε 16,600) which is very similar to the conjugated diene system in cembrene (1). Table VII summarizes the limited available information on reported conjugated diene systems in cembrane derivatives.

Table VII. Summary of Ultraviolet Maxima of the Conjugated Dienes in Some Cembrene Derivatives

Compound	uV Maximum Absorption (extinction coefficient)
Trochelin	246 nm (ε 16,600)
Cembrene (1)	246 nm (ε 16,600)
Cembrol (17)	246 nm (ε 17,100)

Treatment of trochelin with a trace of sodium in methanol afforded the deacetylated derivative of trochelin (Figure 32). Partial structure H was established in trochelin when the AB quartet (4.3 ppm) shifted up-

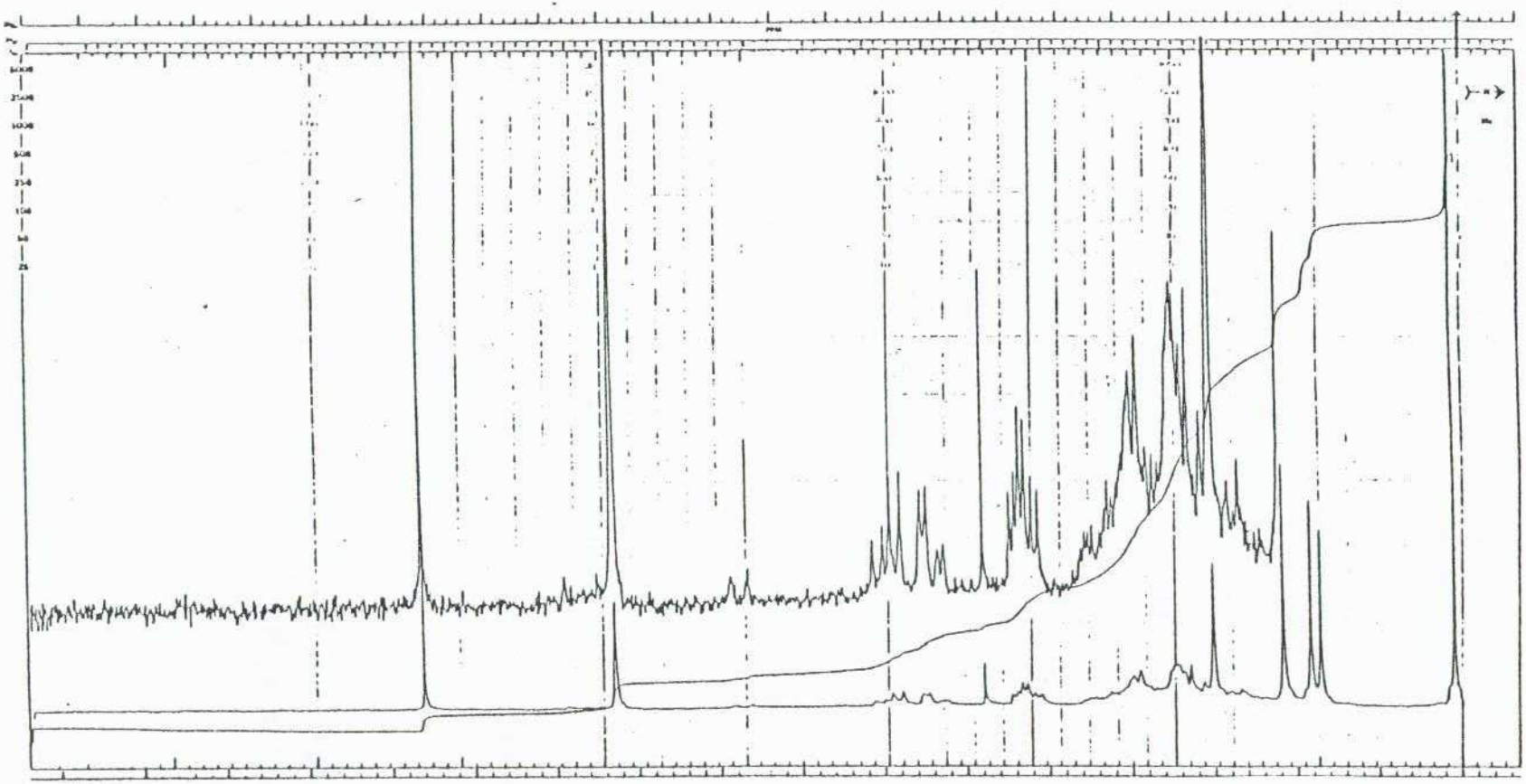
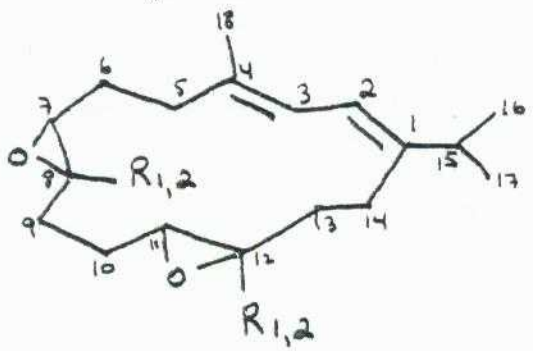


Figure 31. ^{1}H nmr spectrum of deacetylated trochelin.

Table VIII. Assignments of ^1H Nmr Data in the $CDCl_3$ Spectrum of Trochelin (56)

Chemical shift (δ)	Protons	Multiplicity	J (Hz)	Assignment
5.95*	2	s*		H_e, H_f
4.50	1	d	12	$-CH_2-O-$
4.12	1	d	12	
3.10	2	m		two epoxide hydrogens
2.50-2.40	1	br m		
2.40-2.20	4	cs		
2.20	1	s		
2.10-1.80	7	cs		
1.78	3	s		C-18 protons
1.68	2	s		
1.56	3	s		
1.40	1	cs		
1.20	1	cs		
1.06	6	d	4	isopropyl group

* resolved doublet in d_6-benzene



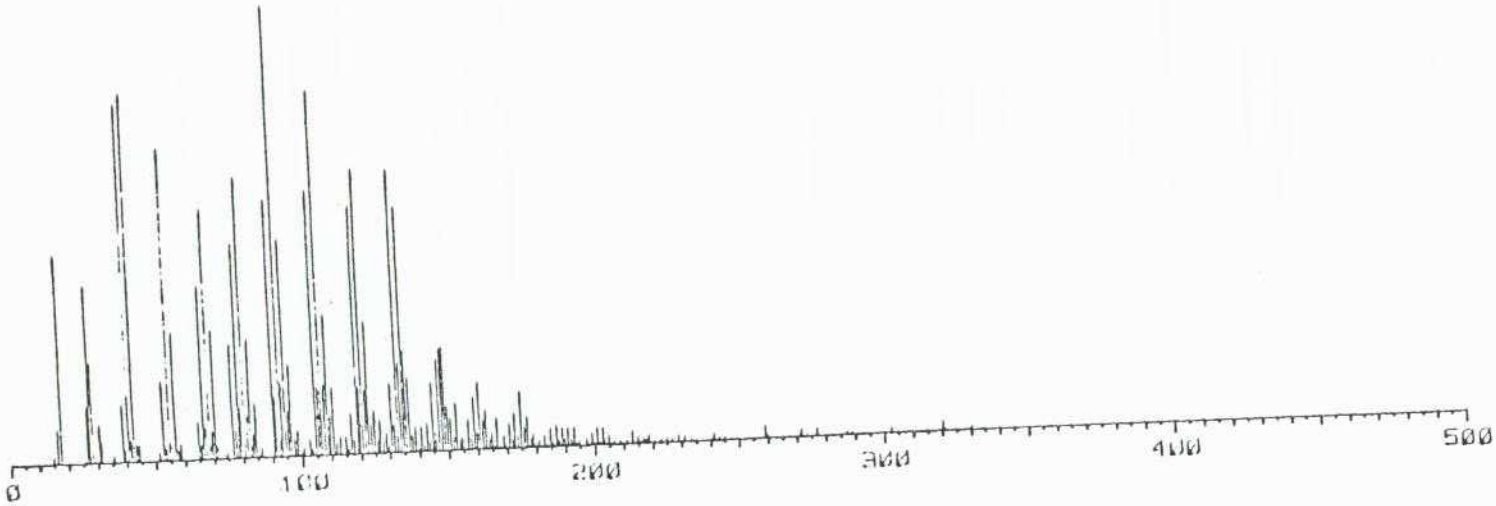


Figure 32. Mass spectrum of deacetylated trochelin.

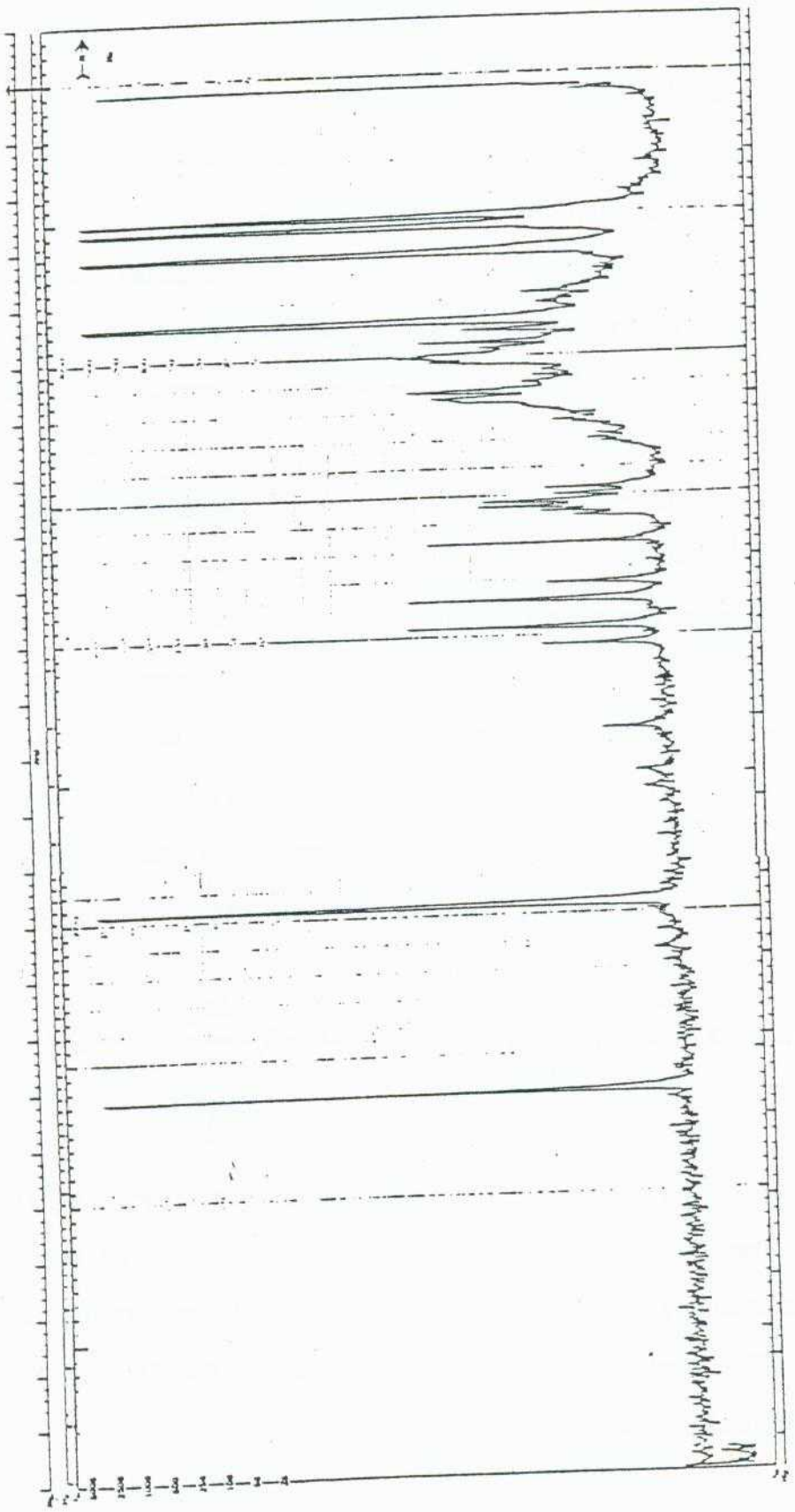
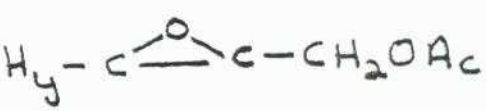


Figure 33. ^{1}H nmr spectrum of deacetylated trochelin after addition of D_2O.

field to a pair of quartets at 3.8 ppm. The multiplicity increase was reduced with the addition of D_2O and the AB quartet reappeared at 4.3 ppm (Figure 34). The observed geminal coupling was in general agreement with typical values.

Two of the oxygen atoms in trochelin are present in an acetate function. The formula for trochelin, $C_{22}H_{34}O_4$, allows for two more oxygen atoms. Trochelin did not exhibit hydroxyl absorptions nor did the deacetylated product show any carbonyl absorptions. The two remaining oxygen atoms, as suggested from the 25.2 MHz ^{13}C nmr spectrum, must be ether functions. The 100 MHz ^1H nmr spectrum additionally suggests the presence of two epoxide hydrogens as displayed by absorptions at 3.10 ppm (m,2H). The ^1H nmr spectrum signals also remained in the deacetylated derivative. One of the epoxide hydrogens (H_x) may be part of structure I, the methyl signal of which occurs at 1.26 ppm; the other epoxide hydrogen (H_y) can be in structure J.

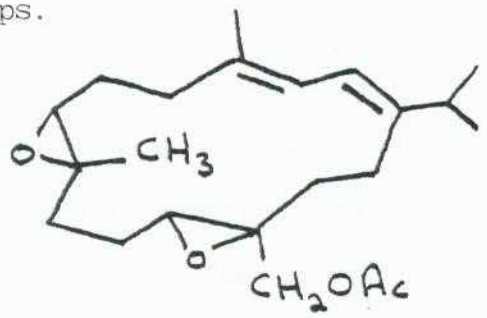
I



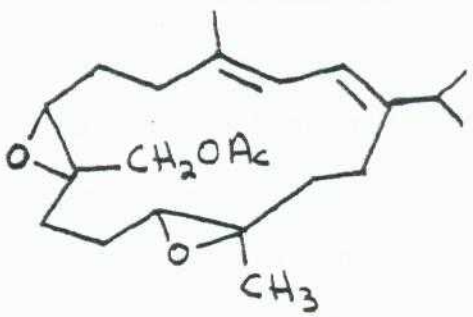
J

It was previously shown that a $-CH_2OAC$ group was present and that the carbon to which it was attached was fully substituted. The second epoxide can reasonably be associated with the fully substituted center alpha to the $-CH_2OAC$ group, as in partial structure J. The chemical shifts of the methylene protons at 4.50 and 4.12 ppm are in accord with the assignment in J.

The partial structures accounted for all but one site of unsaturation allowed by $C_{22}H_{34}O_4$. Making a reasonable assumption that trochelin arose from geranylgeranyl pyrophosphate (48),[21,22] the cembrane ring system emerges as the probable macrocycle in trochelin. In the absence of additional chemical evidence, the partial structures established for trochelin were combined to form possible total structures. Two of the probable structures are 56 and 57. The difference between the two structures is the interchange of the methyl and methylene acetate groups.



56



57

A sample was submitted for x-ray crystallography, but the crystals were unsuitable for analysis.

CHAPTER FIVE

SUMMARY AND CONCLUSION

Numerous compounds with the cembrane ring system have been isolated from diverse sources. The terrestrially derived cembranes have been largely olefins and alcohols. The marine cembranes have tended to be more highly functionalized and are recognized as fairly common metabolites of some marine invertebrates.

The coelenterates are a highly diverse and abundant marine form. A variety of free-swimming, solitary and colonial forms are known. The gorgonians are especially abundant in the tropical Western Atlantic while the alcyonarians are dominant in the Indo-Pacific reefs.[70] These two orders are often represented by large colonies within easy reach of the chemist because of their occurrence in shallow waters.

The cembranoids isolated from marine sources have included a wide and interesting spectrum of functional groups. Many of the structures were determined by X-ray diffraction analysis. Others were determined by classical spectroscopic methods and chemical degradations and correlations.

In any event, progress toward structure elucidation is dependent upon availability of suitable material. Although organic extracts from the alcyonarians and gorgonians often exceed one to five per cent of the dry weight of the animal, the extracts may differ in constituents from different samples of the same species. No systematic research has been

reported on this topic, but there are a number of scattered observations in addition to our experience with the *Sarcophyton spp.*

Neeman and coworkers[31] found that relative amounts of the cembranolide sarcophine (35) isolated from *Sarcophyton glaucum* changed from 3% of the dry weight to trace amounts depending upon time and place of collection. Comparison of samples of *Sarcophyton trocheliophorum* also showed variation in terpenoid content.[71]

Other factors, such as age and sex of the colonies or changes in the symbiotic association between the polyps and zooxanthellae may also account for these variations. The possibility of identification errors also exists. The systematics of the Octocorallia is sometimes delicate and identification of the symbiotic zooxanthellae is still a matter of controversy.

A number of cembrane derivatives do exhibit interesting pharmacological properties. Sinulariolide (41), toxic to mice and fish, is thought to be part of the defense mechanism of the soft coral to ward off predators. Sinulariolide(41), sinularin(43) and its dihydro derivative(44),[49] crassin acetate(30)[72] eupalmerin acetate(31),[72] jeunicin (29) and eunicin(28)[72] and apserdiol (26)[37] have shown antitumor activity. Sinulariolide (41) and lobophytolide(34) markedly inhibit the growth of marine unicellular algae when present in trace amounts in culture media.

This is not to infer that all coelenterate diterpenoids are endowed with pharmacological charactersitics. The amount of emblide (53) remaining after structure elucidation precluded determination of biological activity although its unusual spectrum of functional groups

indicates possible activity. The potential biological properties of trochelin (56) also remain untested.

The cembrane derivatives are dominant in the alcyonarians and gorgonians. The structure elucidation and biological activities of this recently discovered class of compounds have opened new avenues of research for the scientist. It is interesting to note that the remaining orders of the coelenterates, Pennatulacea, Stolonifera and Telestacea, have not received much attention. Several diterpenoids isolated from Pennatulacea await final structure determination but do appear to deviate from the cembrane derivatives.

The coral reefs with their apparent biosynthetic versatility offer a supply of novel compounds with potential value as drugs or tools for pharmacological research. Once the remaining coelenterates come under scrutiny, many new compounds will be added to the growing list of those already isolated from the gorgonians and alcyonarians.

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