NOVEL METABOLITES FROM BRITISH COLUMBIA NUDIBRANCHS

BY

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ABSTRACT

Skin extracts from a number of British Columbia nudibranchs have proven to be a rich source of novel metabolites. This thesis describes the structural investigation of several of these compounds and includes some preliminary studies of their biosynthetic origin and physiological role.

The unique diacylguanidine triophamine (75) has been isolated from Triopha catalinae and Polycera tricolor. Its structure was deduced by classical chemical methods and confirmed by an unambiguous synthesis. Anisodoris nobilis provided the odoriferous degraded sesquiterpene dihydroapofarnesal (15). The structure of aldehyde 15 was assigned by interpretation of spectral data. Extracts of Archidoris montereyensis contain the diterpenoic acid glyceride 17, its two monoacetates 18 and 19, the drimane sesquiterpenoic acid glyceride 20 and its monoacetate 21, the monocyclofarnesic acid glyceride 22 and the glyceryl ether 23. These compounds were established by a combination of spectral analysis, chemical interconversion and/or single crystal X-ray diffraction analysis. The structure of a free diterpenoic acid found in only one collection of A. montereyensis, was also tentatively identified. The isomeric farnesic acid glyceride 27 in addition to

glycerides 17, 18, 20, 22 and ether 23 have also been isolated from Archidoris odhneri. The drimenoic acid glyceride 20 and the glyceryl ether 23 exhibit antifeedant activity against fish. Biosynthetic studies showed that ^{14}C labeled mevalonic acid is incorporated into the terpenoid portion of glycerides 17 and 20 by A. montereyensis and into the farnesic acid portion of glyceride 24 by Archidoris odhneri. Preliminary results suggest that triophamine (75) is synthesized in situ by T. catalinae and the drimane skeleton of albicanyl acetate (36) is biosynthesized by Cadlina luteomarginata.

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The reliable and friendly support of Mike LeBlanc and Steven Ayer is greatly appreciated. Warm thanks are extended to all of the divers who aided in the collection of the marine organisms that were essential to this research. I am grateful to Rosemary Armstrong, who kindly supplied a sample of authentic drimenol. The patient and understanding staff of the departmental NMR and MS laboratories are also acknowledged.

LIST OF ABBREVIATIONS

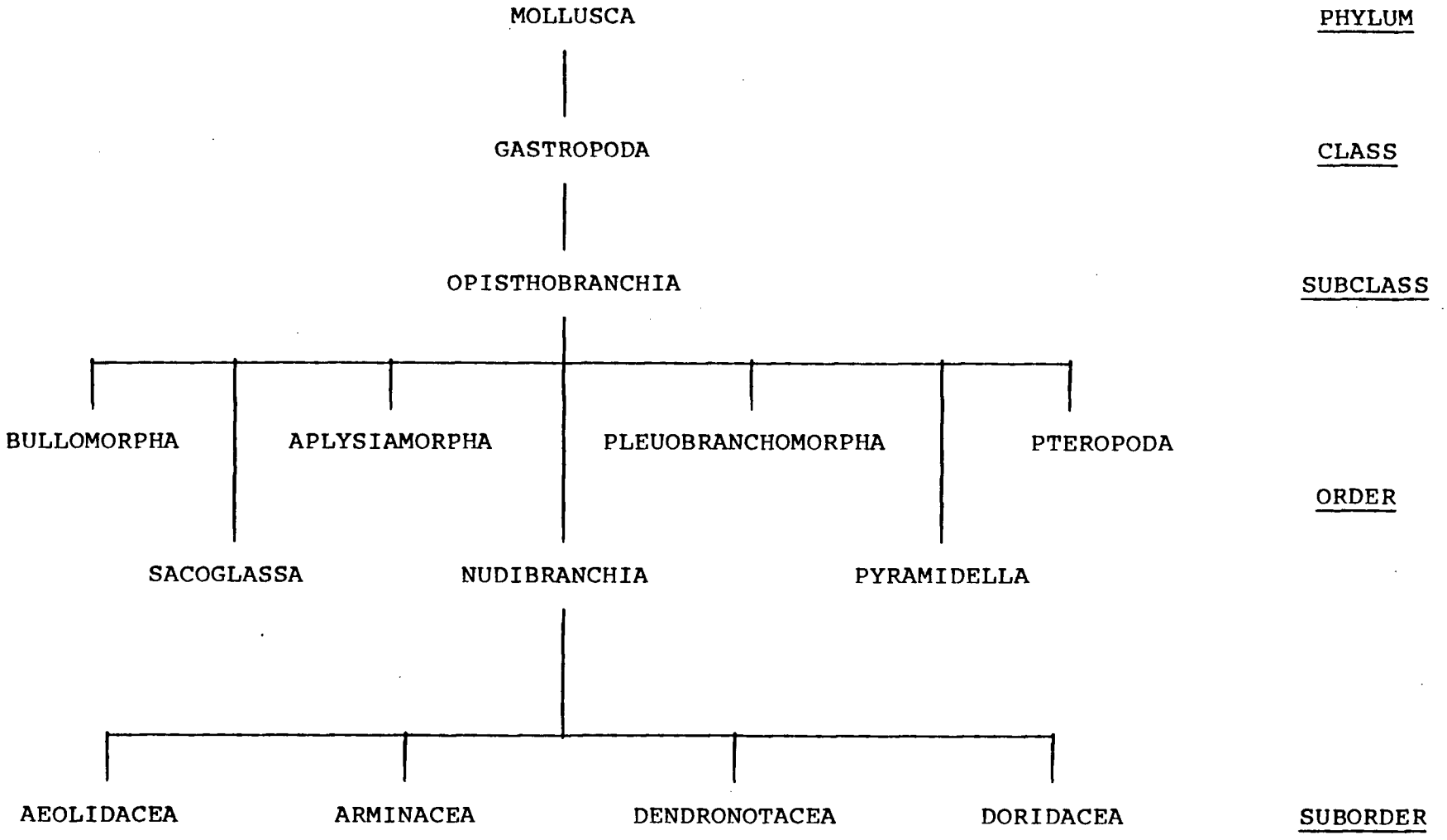
DCC	=	Dicyclohexylcarbodiimide
DIBAL	=	Diisobutyaluminum hydride
DMSO	=	Dimethyl sulfoxide
DNPH	=	2,4-Dinitrophenylhydrazine
E	=	Electronic noise
EtOH	=	Ethanol
GC	=	Gas chromatography
HPLC	=	High pressure liquid chromatography
HRMS	=	High resolution mass spectroscopy
IR	=	Infrared
LC	=	Liquid chromatography
MeOH	=	Methanol
NEN	=	New England Nuclear
^{1}H NMR	=	Proton nuclear magnetic resonance
^{13}C NMR	=	Carbon-13 nuclear magnetic resonance
NOE	=	Nuclear Overhauser enhancement
RT	=	Room temperature
S	=	Solvent signal
SFORD	=	Single frequency off-resonance decoupled
THF	=	Tetrahydrofuran
TLC	=	Thin-layer chromatography
UV	=	Ultraviolet
W	=	Water signal

INTRODUCTION

I. NUDIBRANCH DEFENSES

Molluscs of the class Gastropoda, subclass Opisthobranchia have been the subject of intense scrutiny by natural product chemists in recent years. Adult opisthobranchs are slow moving animals whose synonyms include sea slug and naked snail. While most opisthobranchs are shell-less, some forms have internal or greatly reduced shells that provide very little protection. Even though they have exposed soft tissue and are often brightly colored, these animals have few known predators[1]. This relative impunity from predation has led to speculation that opisthobranchs utilize some form of chemical defense.

Nudibranchs comprise the largest of seven orders in the subclass Opisthobranchia (Figure 1). They are characterized by the complete absence of a shell and, as their name implies, they possess external respiratory appendages. Adult nudibranchs range in size from 3-300 mm and their diets include sponges, bryozoans and coelenterates[2]. Coloration ranges from camouflaged color schemes that mimic the preferred substrate of a species to very bright, conspicuous colors that seem to blatantly advertise the presence of the organism. Selected members of four groups of animals, opisthobranchs, crustaceans, asteroids and fishes, are known to prey on nudibranchs[3], but the level of



N.B. Organisms classified according to Behrens[2].

Figure 1. Phylogenetic classification of nudibranchs

predation is very low. Therefore, while their soft body form and limited mobility might suggest that nudibranchs are particularly vulnerable to attack, this is clearly not the case. It is believed that they employ a variety of sophisticated defensive strategies to deter potential predators.

Cryptic coloration in concert with complementary habitat selection reduces the conspicuousness of certain nudibranchs. Several species directly utilize pigments acquired from their prey[4], while others use pigments which they produce themselves to match the color scheme of their surroundings. Such coloration may reduce harassment by potential predators, but Thompson[1] found that nudibranchs were still not acceptable to fish when placed in a setting where color offered no concealment. Once discovered, cryptic nudibranchs apparently rely on a second level of defense.

The outer covering of a nudibranch is termed the mantle or dorsum and it comprises the bulk of the organism's exposed tissue (Figure 2). Other external features include the rhinophores, branchial plume and foot. Rhinophores are paired sensory apparatus found around a nudibranch's head, while the branchial plume is a respiratory structure (gill) usually located near the anus. The foot anchors the animal to its substrate and is involved in locomotion. When nudibranchs are disturbed, most species retract these

external structures leaving only the dorsum exposed to would be attackers. The presence of spicules in the mantle of dorid (suborder Doridacea) nudibranchs has been postulated as a possible deterent to predation[5]. Spicules are calcareous spines acquired from dietary sponges. However, the relative importance of spicules in thwarting predators is believed to be quite low[3].

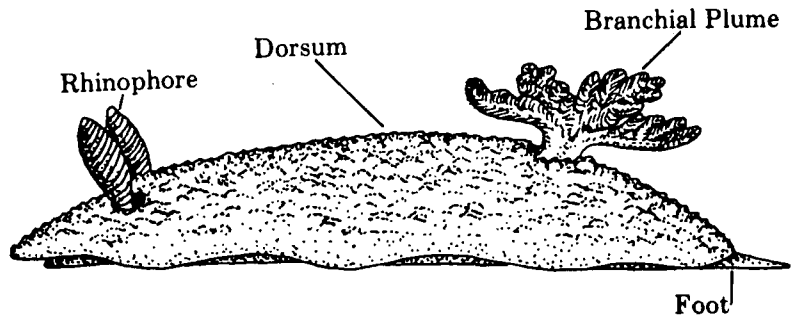


Figure 2. Typical dorid nudibranch

Nematocysts, which are stinging organelles produced by coelenterates, provide a deterent to potential predators for certain aeolid, (suborder Aeolidacea), nudibranchs[1]. Unfired nematocysts obtained from dietary organisms travel through the digestive gland of the nudibranch and are stored in a functional state in specialized cynidocyst cells[3]. These storage cells are localized in projections from the nudibranchs skin surface. Thus situated, they are readily

encountered during the initial inspection by an inquisitive predator. When an aeolid is disturbed, the cynidocyst cells release clouds of stinging nematocysts as a defensive response. Nematocyst utilization by nudibranchs illustrates the remarkable ability of an animal to selectively sequester and utilize for its own defense an intact cell organelle from a dietary animal of a different phylum.

It has become increasingly apparent that a large number of nudibranchs employ chemical secretions as part of their defensive arsenal. Most species are known to possess an abundance of non-mucous skin glands whose functions are speculated to be defensive[1]. Several nudibranchs can secrete highly acidic (H_2SO_4, pH $\cong$ 1) solutions along their skin surface when molested[6]. While the release of strong acid provides protection for some species, the skin secretion of most nudibranchs is non-acidic[7]. The chemical nature of these secretions and their possible defensive role has prompted much of the interest by natural product chemists.

II. CHEMISTRY OF OTHER OPISTHOBRANCHS

Prior to discussing the chemical defense of nudibranchs, an overview of the chemistry of other opisthobranchs is in order. An exhaustive review of opisthobranch chemistry is beyond the scope of this thesis. Rather, the highlights and general trends are presented

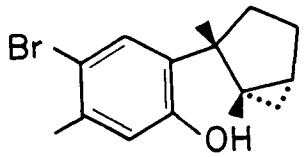
along with findings that have been mirrored in nudibranch work.

Initial opisthobranch studies focused on Aplysia species because they are large (average length several hundred mm) and often contain high concentrations of novel compounds. Products isolated from Aplysia spp. include monoterpenes[8], sesquiterpenes[9], diterpenes[10] and C-15 acetate derivatives[11]. Many of these constituents contain one or more halogen atoms.

Strong circumstantial evidence indicates that Aplysia accumulates compounds from dietary sources, primarily algae. Invariably the highest metabolite concentration is found in the digestive gland of these herbivors. The composition of a species' extracts can vary with the location and time of year that the animals were collected, due to geographical and seasonal differences in the availability of dietary algae. Numerous products isolated from Aplysia have also been found in one or more species of algae[10a,10c,12]. Stallard et al.[8b] clearly demonstrated that A. californica concentrates and stores in its digestive gland halogenated monoterpenes obtained from the alga Plocamium coccineum var. pacificum.

In some instances, metabolites concentrated from the diet are chemically modified following ingestion. The algal products laurinterol (1) and pacifenol (2) are converted to aplysin (3) and pacifidiene (4) respectively, within the

digestive gland of A. californica[13].



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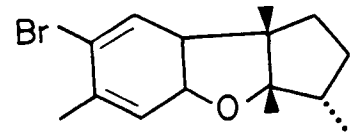

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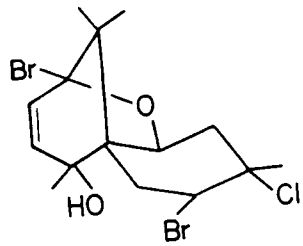

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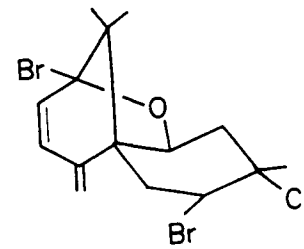
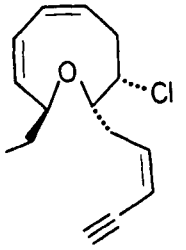


4

Many of the known Aplysia compounds possess some type of biological activity[9a,9b,14] and apparently are active principles of the animals' defensive secretions. Brasilenyne (5) and cis-dihydrorhodophytin (6)[11], which are found both in the body wall and digestive gland of A. brasiliana, have demonstrated antifeedant activity toward fish.

Soft-bodied marine molluscs other than Aplysia also employ chemical defensive substances to thwart predators. The opisthobranch Onchidella binneyi produces a repugnant exudate containing the sesquiterpene onchidal (7)[15] and a polypropionate derivative, pectinatone (8)[16], has been implicated as the deterrent in a mucous secretion from the siphonarid Siphonaria pectinata.



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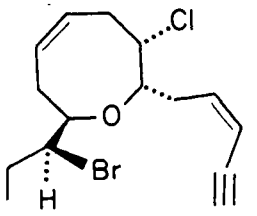

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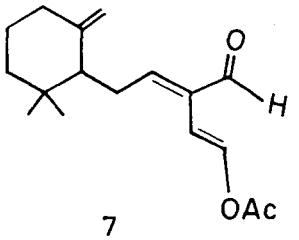

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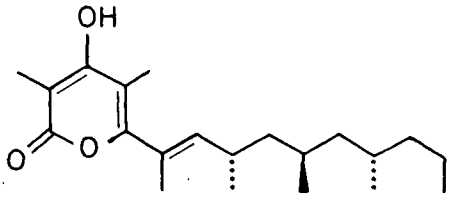

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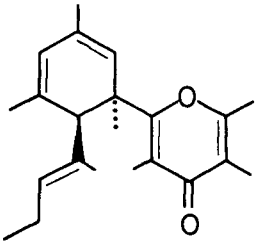


9

The ability to acquire functional organelles from dietary organisms is not unique to aeolid nudibranchs. Several opisthobranchs of the order Sacoglossa are known to assimilate photosynthetically active chloroplasts from their algal diet. Ireland et al.[17] showed that polypropionate metabolites such as 9,10-deoxytridachione (9), from Placobranchus ocellatus, are biosynthesized in situ by the mollusc-chloroplast pair. This was the first demonstration that an opisthobranch actively participates in the biosynthesis of secondary metabolites.

It is clear that opisthobranch molluscs have developed many sophisticated systems of chemical defense. Using a variety of potentially noxious chemicals they successfully resist predation. The source of these compounds is often directly from the diet, although some dietary constituents are chemically transformed following ingestion. In addition, several species are capable of *in situ* production of unique metabolites via a symbiotic association with algal chloroplasts.

III. NUDIBRANCH CHEMISTRY

Nudibranchs have been the focus of numerous chemical investigations in recent years. Reviews by Thompson *et al.*[25] and Schulte *et al.*[29] nicely summarize the work up to 1982. To date, natural products have been reported from over forty species of nudibranchs. These compounds encompass an amazing diversity of molecular size, functionality and structural type. For a compilation of all known nudibranch metabolites with comments about their source and biological activity see Table I.

It can be seen that the majority of nudibranch skin chemicals are terpenoid in nature. Sesquiterpenes comprise the largest structural class and exhibit a number of recurring structural features. In addition, they have provided much of our insight into the chemical ecology of nudibranchs.

TABLE I: NUDIBRANCH METABOLITES

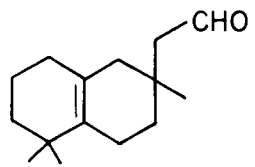
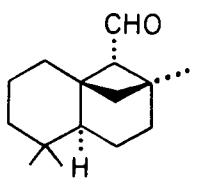
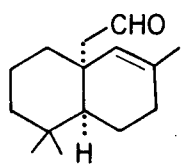
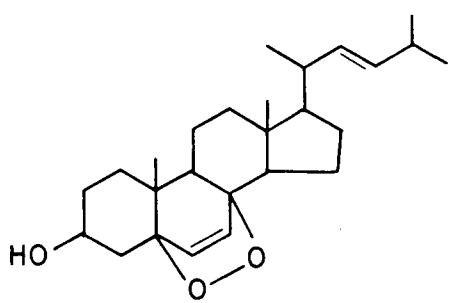
SPECIES	METABOLITES	COMMENTS
Acanthodoris nanaimoensis (Ref. 18)	 nanaimoal (10)  acanthodoral (11)  isoacanthodoral (12)	These isomeric aldehydes represent three new carbon skeletons. A biogenetic proposal suggests that acanthodoral (11) is the precursor of nanaimoal (10) and isoacanthodoral (12).
Adalaria sp. (Ref. 19)	 13	Compound (13) is the major component of a mixture of steroidal peroxides.

TABLE I: continued

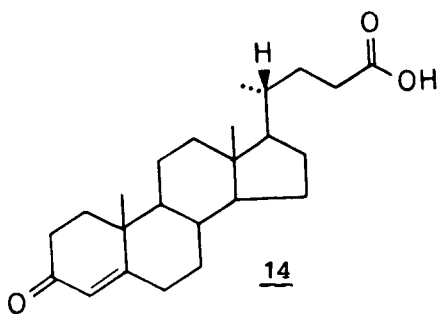
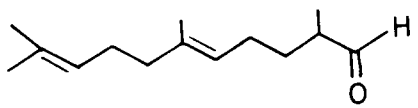
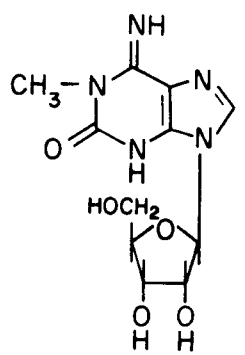
SPECIES	METABOLITES	COMMENTS
Aldisa sanguinea cooperi (Ref. 20, 21)	 14	In addition to steroidal ketone 14, its analog with an αβ-unsaturated acid was also present. Acid 14, the major steroid, has antifeedant properties toward goldfish. It is suggested that cholestenone obtained from the sponge Anthroarcuata graceae may be converted by the nudibranch to fish-antifeedant 14. Glyceryl ether 23, reported for Archidoris montereyensis is also found in A. sanguinea cooperi.
Anisodoris nobilis (Ref. 21, 22)	 dihydroapofarnesal (15)  doridosine (16)	The degraded sesquiterpene aldehyde dihydroapofarnesal (15) is the principle odoriferous constituent of the nudibranch. Doridosine (16) which was isolated from the nudibranch's digestive gland, is water soluble and exhibits a variety of pharmacological activities. It has also been isolated from the Australian sponge Tedania digitata.

TABLE I: continued

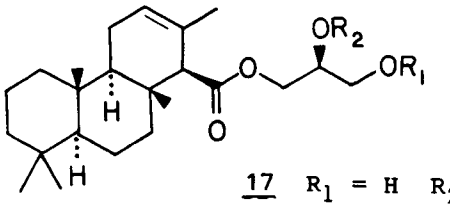
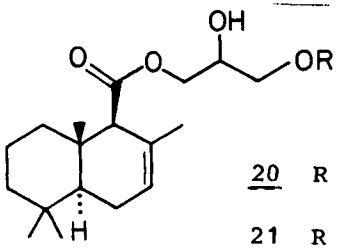
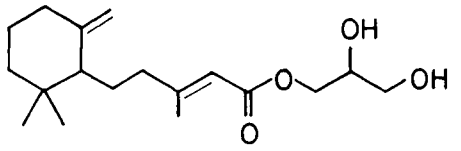
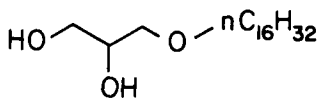
SPECIES	METABOLITES	COMMENTS
Archidoris montereyensis (Ref. 21, 23)	 17 R_1 = H R_2 = H 18 R_1 = Ac R_2 = H 19 R_1 = H R_2 = Ac  20 R = H 21 R = Ac  22  23	Compound 17 and its 1° and 2° monoacetates are the major metabolites. Glyceride 20 and glyceryl ether 23 act as feeding deterents to the tide pool sculpin Oligocottus maculosus while 17 is inactive. Compound 23 has strong antibiotic activity and is also found in the dietary sponge Halichondria panicea. ^{14}C-labeled mevalonic acid fed to A. montereyensis is incorporated into the terpenoid portions of compounds 17 and 20. Thus the nudibranch seems to acquire glycerol derivatives from both dietary sources and de novo biosynthesis. Compounds 17, 18, 20, 22 and 23 have also been isolated from the nudibranch Archidoris odhneri while compound 23 has been found in Aldisa sanguinea cooperi.

TABLE I: continued

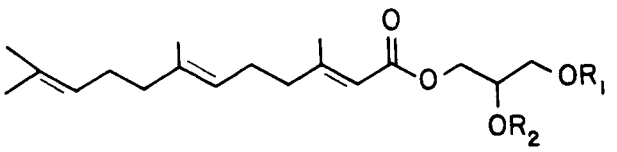
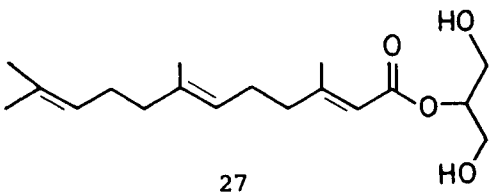
SPECIES	METABOLITES	COMMENTS
Archidoris odhneri (Ref. 24)	 24 R_1 = H R_2 = H 25 R_1 = Ac R_2 = H 26 R_1 = H R_2 = Ac  27	Farnesic acid glyceride 24 and its 1° and 2° monoacetates are the principle metabolites. The isomeric glyceride 27 is found at a much lower abundance. Compounds 17, 18, 20, 22 and 23, reported for A. montereyensis, are also present in trace amounts. ^{14}C labeled mevalonic acid fed to the nudibranch is incorporated into the farnesic acid portion of 24. Glyceride 24 shows antibiotic activity against gram positive bacteria but no antifeedant activity towards fish.

TABLE I: continued

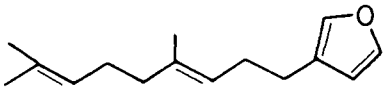
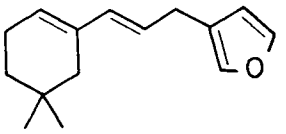
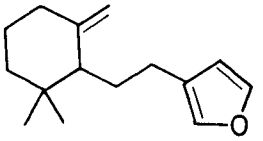
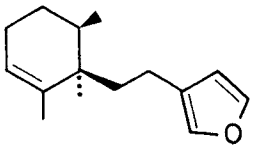
SPECIES	METABOLITES	COMMENTS
Cadlina luteomarginata (Ref. 25, 26, 27)	 dendrolasin (28)  pleraplysillin-1 (29)  30  microcionin-2 (31)	The compounds present in C. luteomarginata extracts show wide variability depending on the location and time of year of collection. Many of these metabolites can be traced to dietary sponge sources. Furodysinin (33), pallescensin-A (34) and possibly the other furanosesquiterpenes are obtained from Dysidea amblia, idiadione (37) comes from Leiosella idia and compounds 39 and 40 have been traced to Axinella sp. Albicanol (35) is known from the liverwort Diplophyllum ablicans and is present in the nudibranch primarily as albicanyl acetate (36). The source of the degraded terpenoid luteone (38) is not clear. While several of these metabolites have been detected in the gut contents of C. luteomarginata, they are all stored in the dorsum of the nudibranch. Furodysinin, pallescensin-A, idiadione and compounds 39 and 40 are toxic to goldfish. They also show varying degrees of antifeedant activity toward goldfish and the sculpin Clinocottus analis. Albicanyl acetate is also an antifeedant toward goldfish. The nudibranch Hypselodoris ghiselini is known to contain dendrolasin while H. porterae contains furodysinin and H. californiensis contains dendrolasin, furodysinin and pallescensin-A.

TABLE I: continued

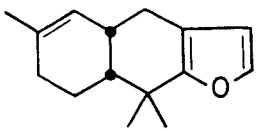
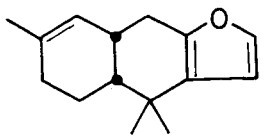
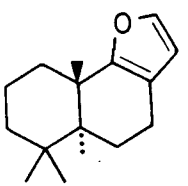
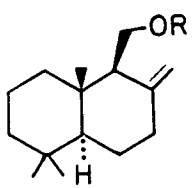
SPECIES	METABOLITES	COMMENTS
Cadlina luteomarginata (continued)	 furodysin (32)  furodysinin (33)  pallescensin-A (34)  35 R = H 36 R = Ac	

TABLE I: continued

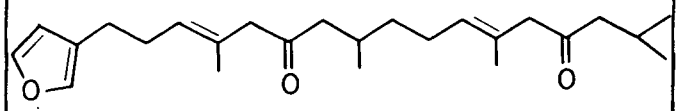
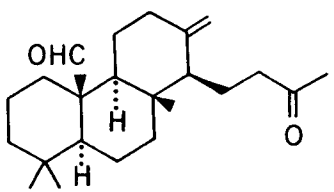
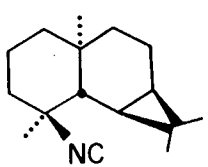
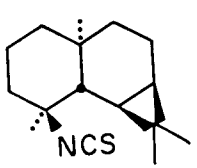
SPECIES	METABOLITES	COMMENTS
Cadlina luteomarginata (continued)	 idiadione (37)  luteone (38)  39  40	

TABLE I: continued

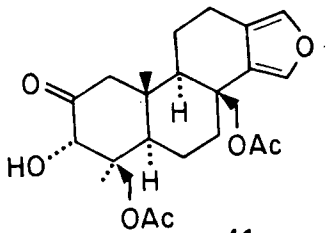
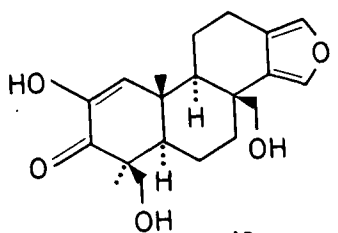
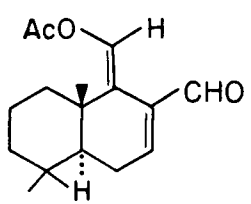
SPECIES	METABOLITES	COMMENTS
Casella atromarginata (Ref. 28)	 41  42	A total of four acetylated derivatives of 41 and 42 were also reported. These furanoditerpenes are identical to, or are structurally related to, compounds previously isolated from an Australian Spongia sp.
Chromodoris albonotata (Ref. 29)	 pu'ulenal (43)	Pu'ulenal (43) is readily hydrolyzed to polygodial (55) which is known to deter predation by fish.

TABLE I: continued

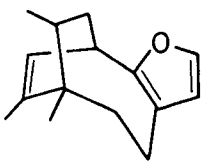
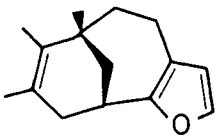
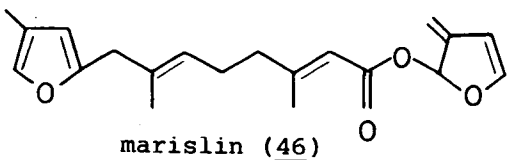
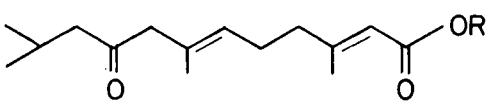
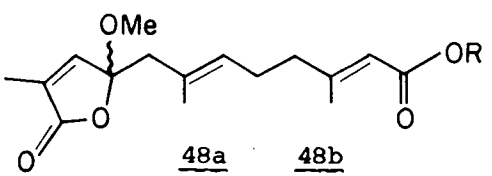
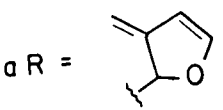
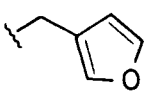
SPECIES	METABOLITES	COMMENTS
Chromodoris maridadilus (Ref. 30)	 nakafuran-8 (44)  nakafuran-9 (45)	Nakafuran-8 (44) and nakafuran-9 (45) were isolated from the nudibranchs C. maridadilus and Hypselodoris godeffroyana and traced to the dietary sponge Dysidea fragilis. Both compounds possess antifeedant properties against the common reef fishes Chaetodon spp. The nudibranch Hypselodoris ghiselini also contains nakafuran-9 and a related methoxy butenolide 67 while Hypselodoris californiensis contains nakafuran-8.
Chromodoris marislae (Ref. 31)	 marislin (46)  47a 47b  48a 48b  a R =  b R =	Marislin (46) is the principal constituent and it readily rearranges to the sponge metabolite pleraplysillin-2 (formally a [3,3] sigmatrophic rearrangement). This close structural relationship indicates that there may be chemical alteration by the nudibranch of a metabolite that it obtains from its diet.

TABLE I: continued

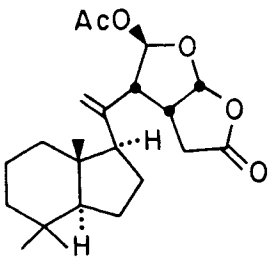
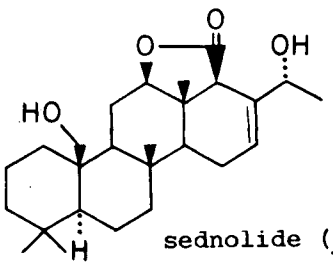
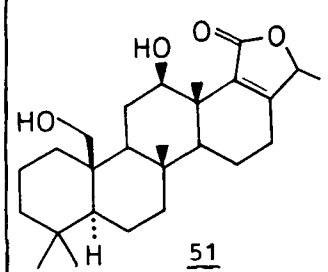
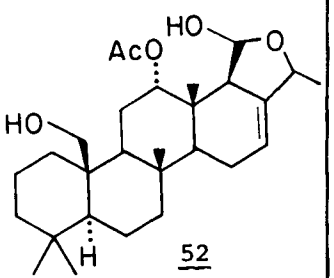
SPECIES	METABOLITES	COMMENTS
Chromodoris norrisi (Ref.32)	 norrisolide (49)	The rearranged diterpene norrisolide (49) was also found in the south Pacific sponge Dendrilla sp. A biosynthetic proposal for the formation of the norrisane skeleton from the spongian skeleton was presented.
Chromodoris sedna (Ref. 33)	 sednolide (50)	A total of five scalarin type C_{26} tetracyclic terpenes were isolated. Sednolide (50) inhibits the growth of the marine bacterium Vibrio anguillarum. An Australian dorid nudibranch, Chromodoris splendida was also found to contain a mixture of 24-methylscalarins. Similar C_{26} scalarin derivatives have been isolated from sponges of the genus Phyllospongia.
	 51  52	

TABLE I: continued

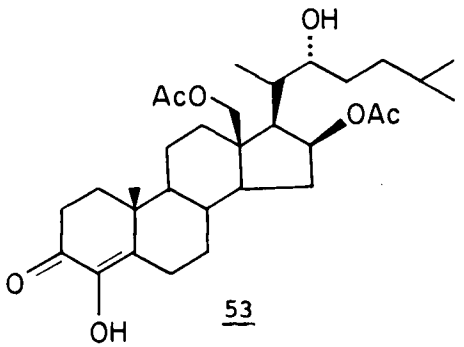
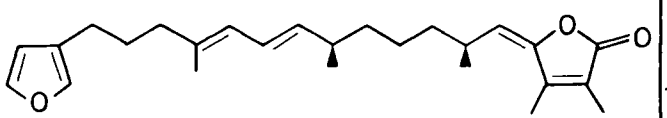
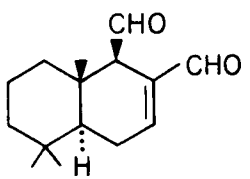
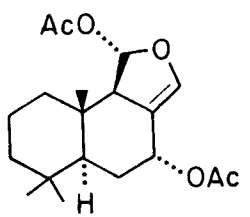
SPECIES	METABOLITES	COMMENTS
Chromodoris splendida (Ref. 33)	see Chromodoris sedna	
Coryphella lineata (Ref. 34, 35)	 53	A number of polyhydroxylated steriods were isolated from C. lineata and from the hydroid Eudendrium sp. upon which they prey. The major steroid is compound 53. A similar mixture of polyhydroxylated steroids was also found in the nudibranchs Flabellina affinis and Hervia peregrina.
Dendrodoris grandiflora (Ref. 35)	 fasciculatin (54)	Fasciculatin (54) is a furanosesterterpene isolated from the digestive gland of D. grandiflora. It is known to be a metabolite of the sponge Ircinia fasciculata.
Dendrodoris krebsii (Ref. 36)	 polygodial (55)  olepupuane (56)	The nudibranchs D. krebsii, D. nigra and D. tuberculosa were found to contain polygodial (55) and olepupuane (56). Polygodial is a known fish and insect antifeedant while olepupuane was found to inhibit feeding of the Pacific damsel fish Dascyllus aruanus. Polygodial has also been isolated from the nudibranch Dendrodoris limbata while olepupuane has been found in Doriopsilla albopunctata and Doriopsilla janaina.

TABLE I: continued

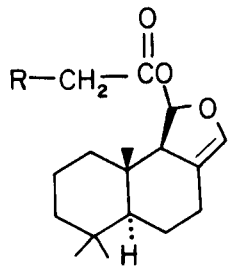
SPECIES	METABOLITES	COMMENTS
Dendrodoris limbata (Ref. 37, 38)	 57	A sesquiterpene with the drimane skeleton was found esterified to a series of fatty acids with varying degrees of unsaturation. These esters were localized in the digestive gland while polygodial (55) was found in extracts of the mantle. Polygodial is a potent antifeedant against fish but the sesquiterpene esters are inactive. ^{14}C-labeled mevalonic acid fed to D. limbata is incorporated into polygodial and the sesquiterpene portion of the esters. It is suggested that the sesquiterpene esters arise from the detoxification of polygodial. Similar sesquiterpene esters have also been isolated from the nudibranchs Doriopsilla albopunctata and Doriopsilla janaina.
Dendrodoris nigra (Ref. 36)	see Dendrodoris krebsii	
Dendrodoris tuberculosa (Ref. 36)	see Dendrodoris krebsii	

TABLE I: continued

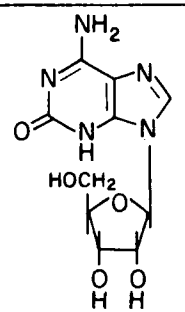
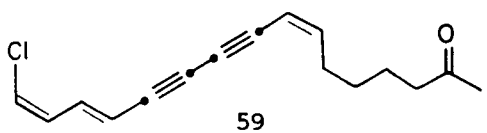
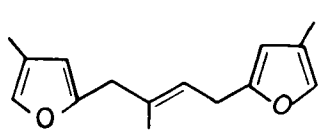
SPECIES	METABOLITES	COMMENTS
Diaulula sandiegensis (Ref. 39, 40)	 isoguanosine (58)	Isoguanosine (58) was isolated from a water extract of the nudibranch's digestive gland. It is a purine riboside and exhibits a variety of pharmacological activities. A series of nine straight chain chlorinated acetylenes of general structure 59 were also isolated from skin extracts. Compound 59 shows antimicrobial activity against several bacteria and a yeast.
	 59	
Doriopsilla albopunctata (Ref. 36)	see Dendrodoris limbata and Dendrodoris krebsii	
Doriopsilla janaina (Ref. 36)	see Dendrodoris limbata and Dendrodoris krebsii	
Flabellina affinia (Ref. 34, 35)	see Coryphella lineata	
Glossodoris gracilis (Ref. 35)	 longifolin (60)	Longifolin (60) is a known metabolite from the sponge Pleraplysilla spinifera. It is a deterent to feeding by the marine fish Chromis chromis and has also been isolated from the mantle and digestive gland of the nudibranch Glossodoris valenciennesi.

TABLE I: continued

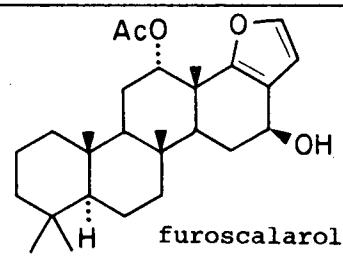
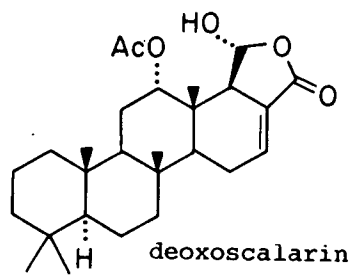
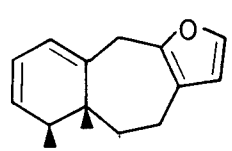
SPECIES	METABOLITES	COMMENTS
Glossodoris tricolor (Ref. 41)	 furoscalarol (61)	Furoscalarol (61) and deoxoscalarin (62) were also isolated from the dietary sponge Cacospongia mollior. Both compounds show antifeedant properties toward the marine fish Chromis chromis.
	 deoxoscalarin (62)	
Glossodoris valenciennesi (Ref. 41)	see Glossodoris gracilis	
Hervia peregrina (Ref. 34, 35)	see Coryphella lineata	
Hypselodoris agassizi (Ref. 42)	 agassizin (63)	Agassizin (63) is structurally related to the sponge metabolite pallescensin-G.

TABLE I: continued

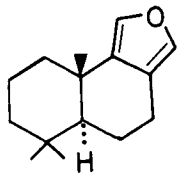
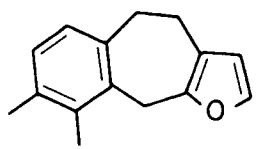
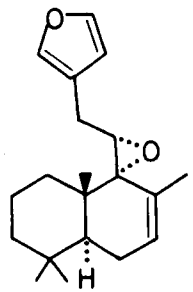
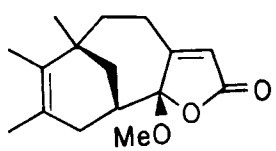
SPECIES	METABOLITES	COMMENTS
Hypselodoris californiensis (Ref. 42)	 euryfuran (64)	Euryfuran (64) is a metabolite of the sponge Euryspongia sp. Four other sponge metabolites, dendrolasin (28), nakafuran-8 (44), furodysinin (33) and pallescensin-A (34) were also isolated from H. californiensis. Hypselodoris porterae is also known to contain euryfuran.
Hypselodoris danielae (Ref. 29)	 spiniferin-2 (65)	Spiniferin-2 (65) is a known metabolite of the sponge Pleraplysilla spinifera.
Hypselodoris ghiselini (Ref. 42)	 ghiselinin (66)  67	In addition to ghiselinin (66), H. ghiselini contains dendrolasin (28), nakafuran-9 (45) and a methoxy butenolide 67 related to nakafuran-9.

TABLE I: continued

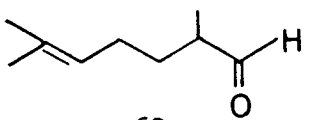
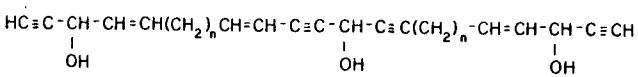
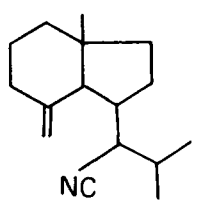
SPECIES	METABOLITES	COMMENTS
Hypselodoris godeffroyana (Ref. 42)	see Chromodoris maridadilus	
Hypselodoris porterae (Ref. 42)	see Hypselodoris californiensis and Cadlina luteomarginata	
Melibe leonina (Ref. 43)	 68	Compound 68 is the principal odoriferous constituent of M. leonina. This degraded monoterpene is also present as a carboxylic acid.
Petodoris atromaculata (Ref. 44)	 69	A series of high molecular weight straight chain polyacetylenes (C_{46}, C_{49}, C_{52} and C_{59}) of general structure 69 were isolated from the nudibranch's digestive gland and from the dietary sponge Petrosia ficiformis.
Phyllidia pulitzeri (Ref. 41)	 axisonitrile-1 (70)	Axisonitrile-1 (70) originates in the dietary sponge Axinella cannabina. It is inactive as a fish antifeedant but is toxic to fish at concentrations as low as 8 ppm.

TABLE I: continued

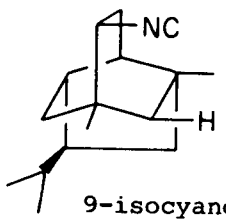
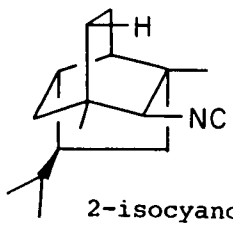
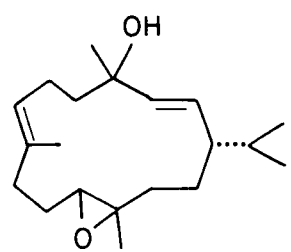
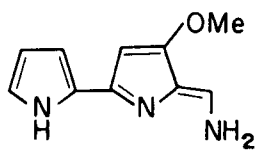
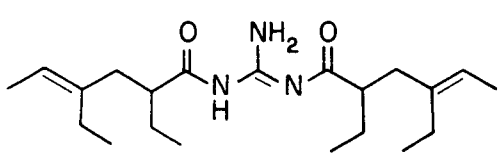
SPECIES	METABOLITES	COMMENTS
Phyllidia varicosai (Ref. 45, 46)	 9-isocyanopupukeanane (71)  2-isocyanopupukeanane (72)	The isocyanosesquiterpenes 71 and 72 are sequestered from the dietary sponge Hymeniacidon sp. They are the active constituents of the nudibranch's mucous skin secretion that is lethal to fish and crustaceans.
Phyllodesmium longicirra (Ref. 47)	 trocheliophorol (73)	Trocheliophorol (73) is obtained from the dietary soft coral Sarcophyton trocheliophorium and is concentrated in the cerata of the nudibranch. Two other cembranoid diterpenes related to trocheliophoral were also found in both the nudibranch and the soft coral.

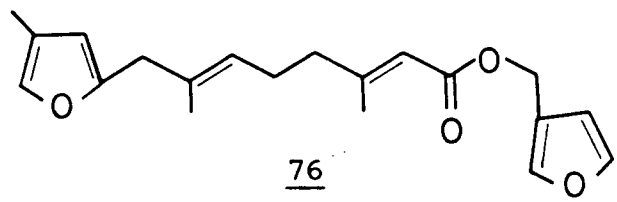
TABLE I: continued

SPECIES	METABOLITES	COMMENTS
Polycera tricolor (Ref. 21)	see Triopha catalinae	
Roboastra tigris (Ref. 48)	see Tambje abdere	
Tambje abdere (Ref. 48)	 tambjamine-A (74)	Tambjamine-A (74) and three related bipyrroles were traced to the dietary bryozoan Sessibugula translucens. Tambjamines B, C and D differ from Tambjamine A by the presence of bromine and/or an isobutylamine functionality. These compounds shown antibiotic activity and inhibit cell division of fertilized sea urchin eggs. They have also been found in the nudibranchs Tambje eliora and Roboastra tigris.
Tambje eliora (Ref. 48)	see Tambje abdere	
Triopha catalinae (Ref. 49)	 triophamine (75)	Triophamine (75) is present primarily in the mucous secretion of T. catalinae but does not deter feeding by goldfish. It has also been isolated from the nudibranch Polycera tricolor.

The first natural products reported from a nudibranch were the rearranged sesquiterpene 9-isocyanopupukeanane (71)[45] and its 2-isocyano isomer 72[46]. These two isonitriles are the active constituents of a toxic mucous secretion produced by Phyllidia varicosa and were both found in the dietary sponge Hymeniacidon sp. This clearly established that nudibranchs are capable of sequestering and storing sponge metabolites for use as defensive allomones. Biologically active isocyanosesquiterpenes of sponge origin are also known from Phyllidia pulitzeri[41] and Cadlina luteomarginata[25]. These compounds have regular isoprenoid skeletons and are toxic to fish. In marine sponges, isocyano compounds often occur along with their isothiocyanate and formamide derivatives. Except for the isothiocyanate analog 40 from C. luteomarginata, such derivaties have not been reported from nudibranchs.

Another common functionality in nudibranch sequiterpenes is the furan ring. A series of seven related furanosesquiterpenes has been isolated from C. luteomarginata[25]. These constituents are localized in the nudibranch's mantle and several of them have demonstrated toxicity or antifeedant activity toward fish. Sesquiterpenes, or their derivatives, which possess a furan residue are also known from Chromodoris marislae[31], Glossodoris gracilis[35] and a number of Hypselodoris species[29,42]. All of these furan-containing compounds can

be traced to a sponge source or are structurally related to known sponge products. Circumstantial evidence suggests that C. marislae can chemically transform the sponge metabolite pleraplysillin-2 (76)[50] into marislin (46). These two furan compounds are formally related by a [3,3] sigmatropic rearrangement and the transformation of 46 to 76 occurs spontaneously during routine handling[31].



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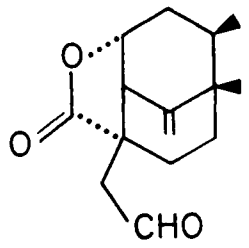
When a conclusive predator-prey relationship has been established, the extracts of a nudibranch and its sponge prey can still show substantial variance. Some metabolites are retained by the nudibranch while others are absent or in reduced concentration. Chromodoris maridadilus and Hypselodoris godeffrayana are known to obtain nakafuran-8 (44) and nakafuran-9 (45) from the sponge Dysidea fragilis[32]. These two furanosesquiterpenes show antifeedant activity against fish. Another Dysidea sesquiterpene, upial (77) has no repellent properties and is not present in the nudibranchs. The nudibranchs apparently retain sponge metabolites from which they derive benefit and reject those that are inactive.



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Carbon skeletons observed in the majority of nudibranch sesquiterpenes are well known from other sources. Compounds previously discussed from P. varicosa, H. godeffroyana and C. maridadilus, in addition to the isomeric aldehydes nanaimoal (10), acanthodoral (11) and isoacanthodoral (12) from Acanthodoris nanaimoensis[18], represent notable exceptions. The drimane carbon skeleton occurs in terrestrial and marine natural products, including nudibranch constituents such as euryfuran (64)[42], olepupuane (56)[36], pu'ulenal (43)[29] and albicanol (35)[26]. While the significance of the carbon skeleton is not clear, it is interesting to note that many drimane derivatives exhibit antifeedant or other biological activities.

The acquisition of secondary metabolites from dietary sources is firmly established for nudibranchs, however Cimino et al.[38] recently demonstrated that Dendrodoris limbata is capable of de novo synthesis of a drimane sesquiterpene. ^{14}C-labeled mevalonic acid fed to the

nudibranch was incorporated into the dialdehyde polygodial (55). Polygodial, localized in the nudibranch's mantle, is a potent antifeedant against fish. It has previously been isolated from a number of higher plants where it serves as an insect antifeedant[51]. The mixed esters 57, in which a drimane sesquiterpene is esterified to a series of fatty acids, were also found to contain the ^{14}C label. It was suggested they arise from the detoxification of polygodial[28], but this relationship remains ambiguous. Polygodial has been isolated from three other species of Dendrodoris which did not contain the mixed esters and two species of Doriopsilla contain the esters but no polygodial[36]. While they are not directly related to the esters 57, farnesic acid glycerides from Archidoris odhneri[24] are another example of nudibranch sesquiterpenes of mixed biogenesis.

Diterpenes found in nudibranchs include furanoditerpenes of sponge origin from Casella atromarginata[28], Cadlina luteomarginata[25] and Hypselodoris ghiselini[42]. Norrisolide (49), a rearranged diterpene from Chromodoris norrisi[32], is also obtained from a sponge while a number of cembranoids from Phyllodesmium longicirra[47] were traced to a dietary soft coral.

Sesterterpenes from nudibranchs fall into two groups; scalarin derivatives and straight-chain furano compounds. These metabolites apparently originate in dietary sponges or

result from transformation of sponge products. A series of C_{26} methylated scalarins, including the novel derivative sednolide (50), was found in Chromodoris sedna[33]. Biological activity of sesterterpenes is evidenced by the ichthyotoxicity of idiadione (37)[25] and the feeding deterrence of two scalarins from Glossodoris tricolor[41].

Degraded terpenes are represented by the C_9 aldehyde 68 from Melibe leonina[43] and C_{23} luteone (38) from Cadlina luteomarginata[27]. Nudibranchs are also the source of interesting steroidal peroxides[19] and polyhydroxylated steroids[34]. Bile acid derivatives from Aldisa sanguinea cooperi[20] possibly arise from chemical modification of a dietary compound. It is suggested that Δ4-cholesta-3-one, obtained from the sponge Anthroarcuata graceae, may be converted by the nudibranch to the observed bile acids.

A growing number of non-terpenoid constituents have recently been reported. A series of chlorinated polyacetylenes were obtained from Diaulula sandiegensis[40], and Peltodoris atromaculata[44] provided another series of acetylenic alcohols. Tambjamine-A (74)[48] and three related bipyrroles occur in several nudibranch species and originate in a dietary bryozoan. Other nitrogenous metabolites include novel purine ribosides from Anisodoris nobilis[22] and Dialula sandiegensis[39].

The array of natural products identified from nudibranchs is truely remarkable. Like the other

opisthobranchs, nudibranchs have developed the ability to obtain complex molecules from their diet and may be able to chemically modify them following ingestion. These organisms are selective in the dietary compounds they concentrate and store. In some cases nudibranchs are directly responsible for the biosynthesis of secondary metabolites. Antifeedant activity associated with nudibranch constituents supports their ascribed function as defense allomones. Adaptation of a chemical defense has apparenty deterred predation and allowed nudibranchs to eliminate the metabolic load required to produce and maintain a protective shell.

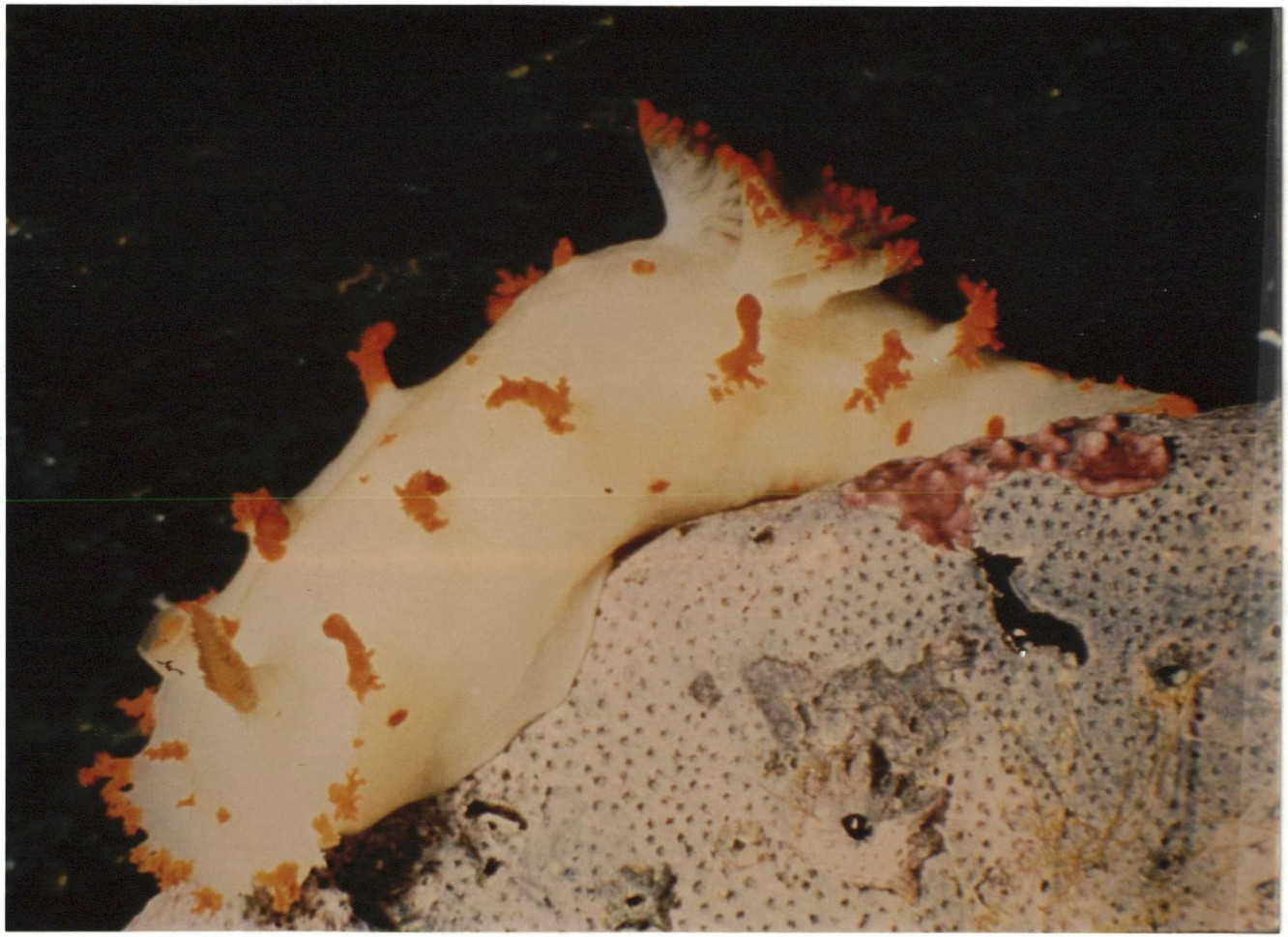


Plate 1. *Triopha catalinae*. Photographer: Ron Long, Simon Fraser University.

CHEMICAL STUDIES OF TRIOPHA CATALINAE

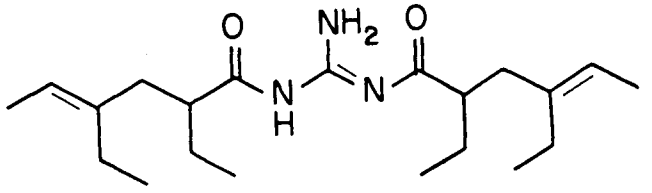
I. ISOLATION AND STRUCTURAL ELUCIDATION OF TRIOPHAMINE (75)

Triopha catalinae (recently Triopha carpenteri)[2] is a nudibranch of the suborder doridacea. It is beautifully decorated with orange tipped rhinophores, gills and papillae (finger like processes extending from the dorsum) projecting from a white body surface. With an average length of approximately 70 mm, T. catalinae's white, orange-spotted body is quite conspicuous in the marine environment. This striking nudibranch feeds on bryozoans[4] and is found along the Pacific coast of North America from Vancouver Island, British Columbia to San Diego, California[2].

Due to its relative abundance, conspicuous coloration and apparent lack of predation, an investigation of the natural products from Triopha catalinae was initiated in 1980. The specimens used in this study were collected in Barkley Sound, along the west coast of Vancouver Island. Although found in an assortment of habitats, T. catalinae were most abundant on exposed rocky outcroppings which support large bryozoan communities. This dorid nudibranch was collected throughout the year from depths of 1-13 meters.

Freshly collected specimens of Triopha catalinae were rapidly immersed in methanol and stored at room temperature.

The solvent was decanted and the nudibranchs washed with additional methanol. Evaporation *in vacuo* of the combined extracts produced an aqueous suspension that was partitioned between chloroform and water. The chloroform soluble material was concentrated to a brownish oil composed primarily of fats, pigments, steroids and the compound of interest, triophamine (75)[49].



75

The oil was fractionated by silica gel flash chromatography using step gradients of ethyl acetate in hexane. Non-polar lipids were eluted with 5% ethyl acetate/hexane. Triophamine (75) and several bright orange pigments were eluted with 15% ethyl acetate/hexane while the steroid fraction was eluted with more polar solvent mixtures. Attention was drawn to triophamine by its strong UV absorbance and the presence of interesting signals in the ^{1}H NMR of the crude fraction from the flash column. Triophamine was further purified to a light yellow oil by preparative thin-layer chromatography (1:1 hexane/diethyl ether, $R_f \cong 0.5$).

Triophamine (75) is optically active ($[\alpha]_D$ -7.0° (C

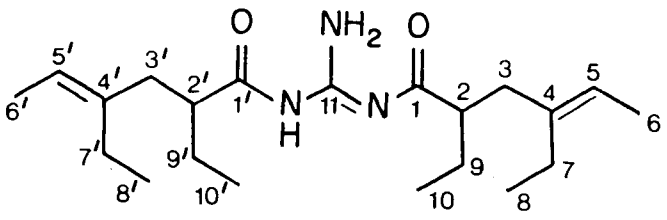
1.7, MeOH)) and it contains a UV chromophore with a λ_{max} = 250 nm (ε 12000, MeOH). Treatment with acid causes a hypsochromic shift in the λ_{max} to 214 nm (ε 14500, MeOH/HCl), while treatment with base (MeOH/NaOH) causes triophamine to decompose to a new substance with a λ_{max} = 232 nm ($T_{1/2} \cong$ 30 min, ε 10000).

The molecular formula of triophamine (75) was established as $C_{21}H_{37}N_3O_2$ by electron-impact high resolution mass spectrometry (M^+, m/z 363.2885, calc'd 363.2885). Major fragment ions resulting from loss of CH_3 (m/z 348), C_2H_5 (m/z 334), C_6H_{10} (m/z 281) and C_9H_{17} (m/z 238) residues were also observed.

Examination of the ^1H and ^{13}C NMR spectra of triophamine (75) led to the conclusion that in the NMR time scale the molecule must exhibit an element of symmetry. Only 11 signals were observed in the ^{13}C NMR spectrum and the ^1H NMR spectrum showed well resolved resonances for only 17 nonexchangeable protons (Table II). When the ^1H NMR spectra were obtained with DMSO-d_6 as solvent one additional exchangeable proton at δ9.4 and a very broad exchangeable signal that integrated for less than one proton at 11 ppm was observed.

It was apparent that each ^1H resonance must account for double the number of protons indicated by integration. Thus, triophamine (75) contains 3 exchangeable and 34

TABLE II. ^{1}H and ^{13}C NMR data for Triophamine (75)



Carbon	^{1}H NMR,[a] δ	^{13}C NMR,[b] δ
1,1'		185.6 (s)
2,2'	2.30-2.48 (m, 1H)	50.3 (d)
3,3'	2.12 (dd, J = 6, 13 Hz, 1H)	40.1 (t)
	2.30-2.42 (m, 1H)	
4,4'		140.4 (s)
5,5'	5.21 (q, J = 7 Hz, 1H)	120.5 (d)
6,6'	1.56 (d, J = 7 Hz, 3H)	12.1 (q)[d]
7,7'	2.01 (q, J = 7 Hz, 2H)	23.2 (t)[e]
8,8'	0.95 (t, J = 7 Hz, 3H)	12.9 (q)[d]
9,9'	1.46-1.67 (m, 2H)	26.1 (t)[e]
10,10'	0.91 (t, J = 7 Hz, 3H)	13.1 (q)[d]
11		158.9 (s)
NH_2[c]	9.4 (br, 1H)	
NH[c]	11.0 (br, 0.5H)	

[a] 400 MHz, $CDCl_3$
[b] 100 MHz, acetone-d_6
[c] 100 MHz, DMSO-d_6
[d] May be reversed.
[e] May be reversed.

nonexchangeable protons. In addition, 10 of the 11 resonances observed in the ^{13}C NMR spectrum must each account for 2 carbons. A ^{13}C SFORD NMR experiment (Figure 7) clearly showed signals for 3 methyl groups, 3 methylene carbons, a methine carbon and a trisubstituted olefin. These observations and a facile loss of C_9H_{17} in the mass spectrum demonstrated that triophamine contains two identical 9-carbon segments, each with 17 nonexchangeable protons.

The structure of the C_9H_{17} residue was assigned by analysis of 'H NMR spectra and a series of proton decoupling experiments.

TABLE III. 'H NMR[a] Decoupling Studies of Triophamine (75)

Resonances Irradiated	Observed Spectral Changes		
δ 1.46-1.67	δ0.91 (t)	→	singlet
	5.21 (q)	→	singlet
2.01	0.95 (t)	→	singlet
2.12	2.30-2.48	→	sharpen up
2.30-2.48	2.12 (d,d)	→	singlet
2.43	2.12 (d,d)	→	(d, J = 13 Hz)

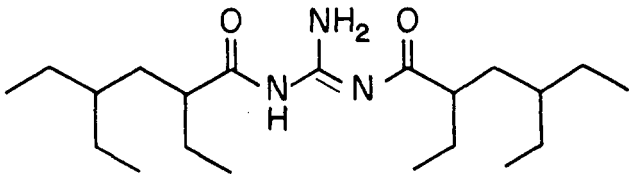
[a]400 MHz, $CDCl_3$

The ^{13}C SFORD NMR experiment had already established the presence of a trisubstituted double bond. An olefinic methyl signal at δ1.56 (3H, d, J = 7Hz) coupled to a

one-proton quartet at 5.21 (J = 7Hz) ppm indicated a methyl group and a proton were attached to the same carbon of the olefin. A methylene resonance at δ2.01 (2H, q, J = 7Hz) coupled to a three-proton triplet at 0.95 (J = 7Hz) ppm revealed that the third olefinic substituent was an ethyl group. An aliphatic ethyl residue on the hydrocarbon chain was evidenced by a methyl triplet at δ0.91 (J = 7Hz) coupled to a two-proton multiplet at 1.46-1.67 ppm.

Catalytic hydrogenation (Pd/C, EtOH) of triophamine (75) gave tetrahydrotriophamine (78). The 'H NMR spectrum of 78 (Figure 10) revealed that resonances for a pair of geminal methylene protons observed at δ2.12 (dd, J = 6, 13Hz, 1H) and 2.30-2.42 (m, 1H) ppm in triophamine had undergone an upfield shift. This established that a methylene group was attached to the ethyl substitued carbon of the olefin. A prominant peak in the mass spectrum of 78 at m/z 283, resulting from a McLafferty rearrangement, supported this assignment. A one-proton multiplet deshielded to δ2.37 ppm in tetrahydrotriophamine indicated that the hydrocarbon chain was attached to the remainder of the molecule via a methine moiety. Decoupling experiments with natural triophamine showed that the methine proton was coupled to both the allylic methylene protons at δ2.12 and 2.30-2.42 ppm and the methylene protons of the aliphatic ethyl group. This led to the conclusion that triophamine contains two 9-carbon residues of general structure 79,

which was consistent with the observed mass spectral fragmentation pattern (see Figure 4).



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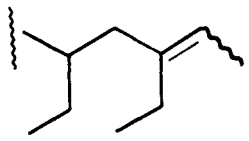


79

The geometry about the double bond was elucidated by means of a difference Nuclear Overhauser Enhancement (NOE)[52] experiment (Figure 9). Irradiation of the olefin proton at δ5.21 caused a positive enhancement of the allylic methylene proton at 2.12 and a negative enhancement of the proton at 2.30-2.42 ppm. Negative homonuclear NOE's of proton signals are not uncommon in small molecules. Certain geometrical arrangements of spins can lead to direct positive NOE's and indirect negative NOE's in the same molecule[52b]. No enhancement was observed in the olefinic ethyl resonances. This demonstrated that the olefin proton contributes significantly to the relaxation of the methylene in the main hydrocarbon substituent. Since the magnitude of this effect is inversely proportional to the sixth power of the internuclear distance[53], the olefin proton and the main chain methylene group must be cis. The olefin therefore has

an (E) configuration.

The remaining portion of triophamine (75) consisted of $C_3H_3N_3O_2$ and it had to incorporate three units of unsaturation. The ^{13}C NMR resonance observed at δ158.9 ppm was highly suggestive of a guanidine substituent. An infrared absorption at 1700 cm^{-1} (Figure 8) and the remaining ^{13}C resonance at δ185.6 ppm suggested the presence of two carbonyl carbons. Low temperature 'H NMR studies showed substantial signal broadening at -40°C which increased at -60°C. The element of symmetry observed on the NMR time scale at room temperature was lost as the temperature was lowered. From these observations it was clear that triophamine consisted of a diacylated guanidine moiety. Monoacylguanidines are known to exist almost exclusively as their acyl imine tautomer[54]. The symmetry observed in the NMR spectra of triophamine results from rapid equilibrium between its two acyl imine tautomers.

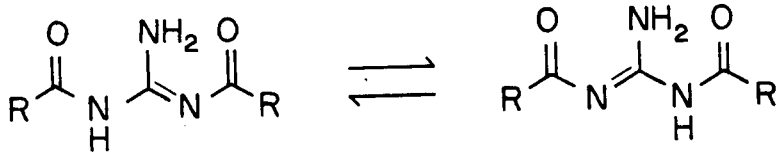
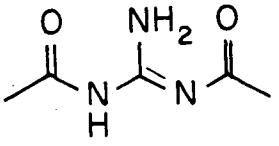


Figure 3. Acylimine tautomers of triophamine (75).

To test this conclusion diacetylguanidine 80 was prepared by reacting guanidine carbonate with acetic anhydride. The UV characteristics of compound 80 [λ_{max} = 248 nm (ε 16800, MeOH) and λ_{max} = 212 nm (ε 19900, MeOH/HCl)] were comparable to those observed for triophamine (75). It underwent rapid base-catalyzed hydrolysis (MeOH/NaOH, room temperature) to give monoacetylguanidine with a λ_{max} = 230 nm (ε 14300, MeOH). Under similar reaction conditions triophamine was converted to its monoacyl derivative 81 whose UV absorbance [λ_{max} = 232 nm (ε 10000, MeOH)] correlates well with monoacetylguanidine. The ^{13}C NMR resonances of diacetylguanidine at δ159.0 and 180.1 ppm are in good agreement with those of triophamine. The deshielding of the carbonyl carbons in triophamine relative to diacetylguanidine is almost equal in magnitude to the difference between propionamide (δ177.2) and acetamide (δ172.7) reported by Levy et al.[55].



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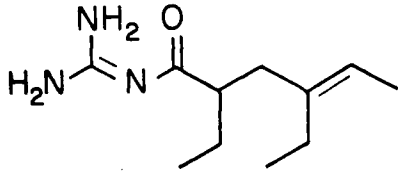

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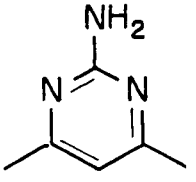
Final proof of the structure was obtained by base-catalyzed hydrolysis (MeOH/NaOH, 48h) of triophamine (75). Using the FCNP spray reagent[56] (ferricyanate - sodium nitroprusside), guanidine could be detected in the reaction mixture by TLC [$CHCl_3$/MeOH/acetic acid (50:35:5), $R_f \cong 0.3$]. Treatment with acetyl acetone allowed the isolation of guanidine as its 4,6-dimethylpyrimidine derivative 82. After repeated attempts, the 10-carbon carboxylic acid 83 was also isolated in low yield as a product of hydrolysis.



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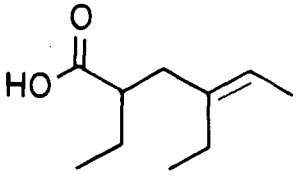

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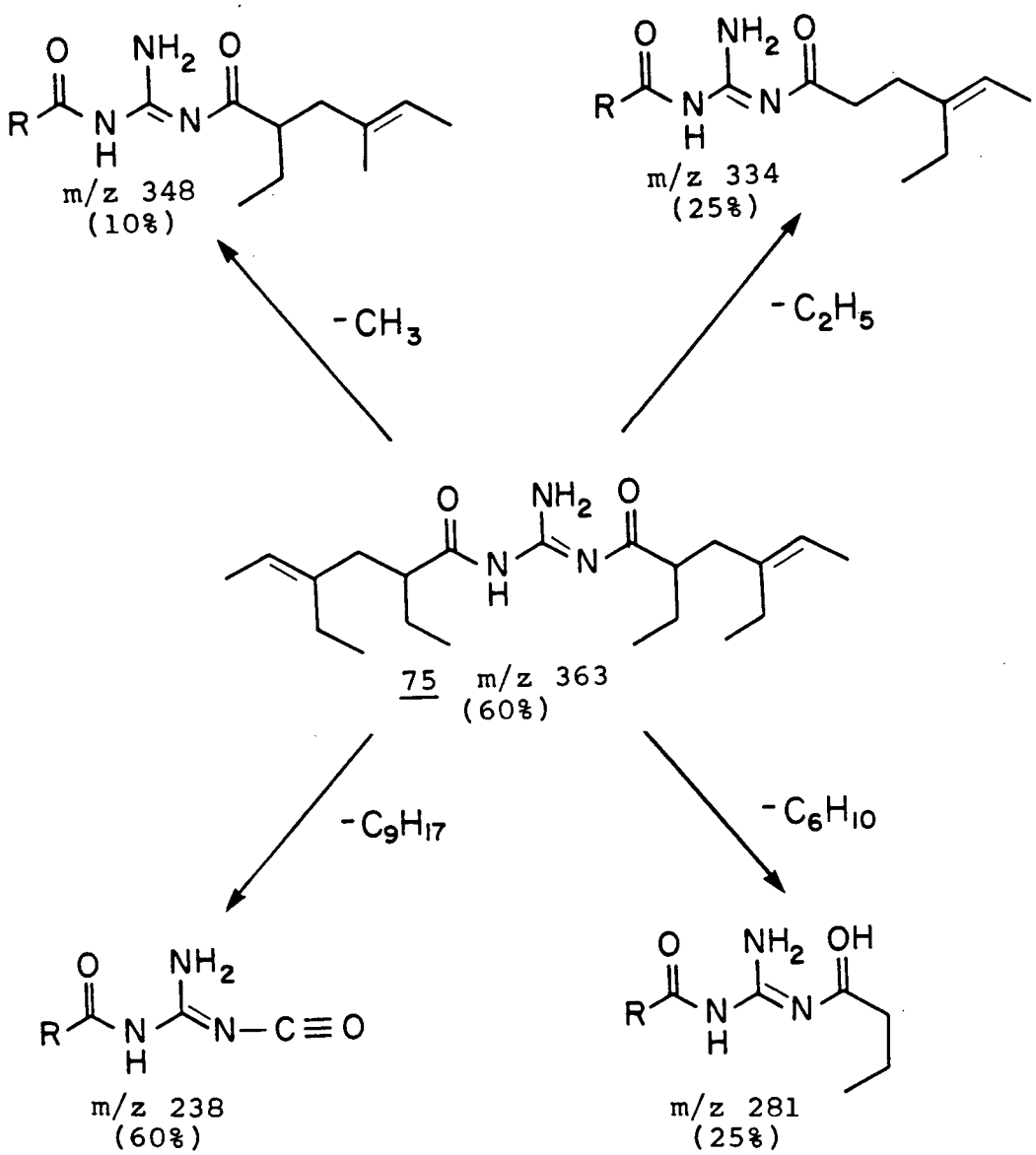


Figure 4. Interpretation of the HRMS of 75.

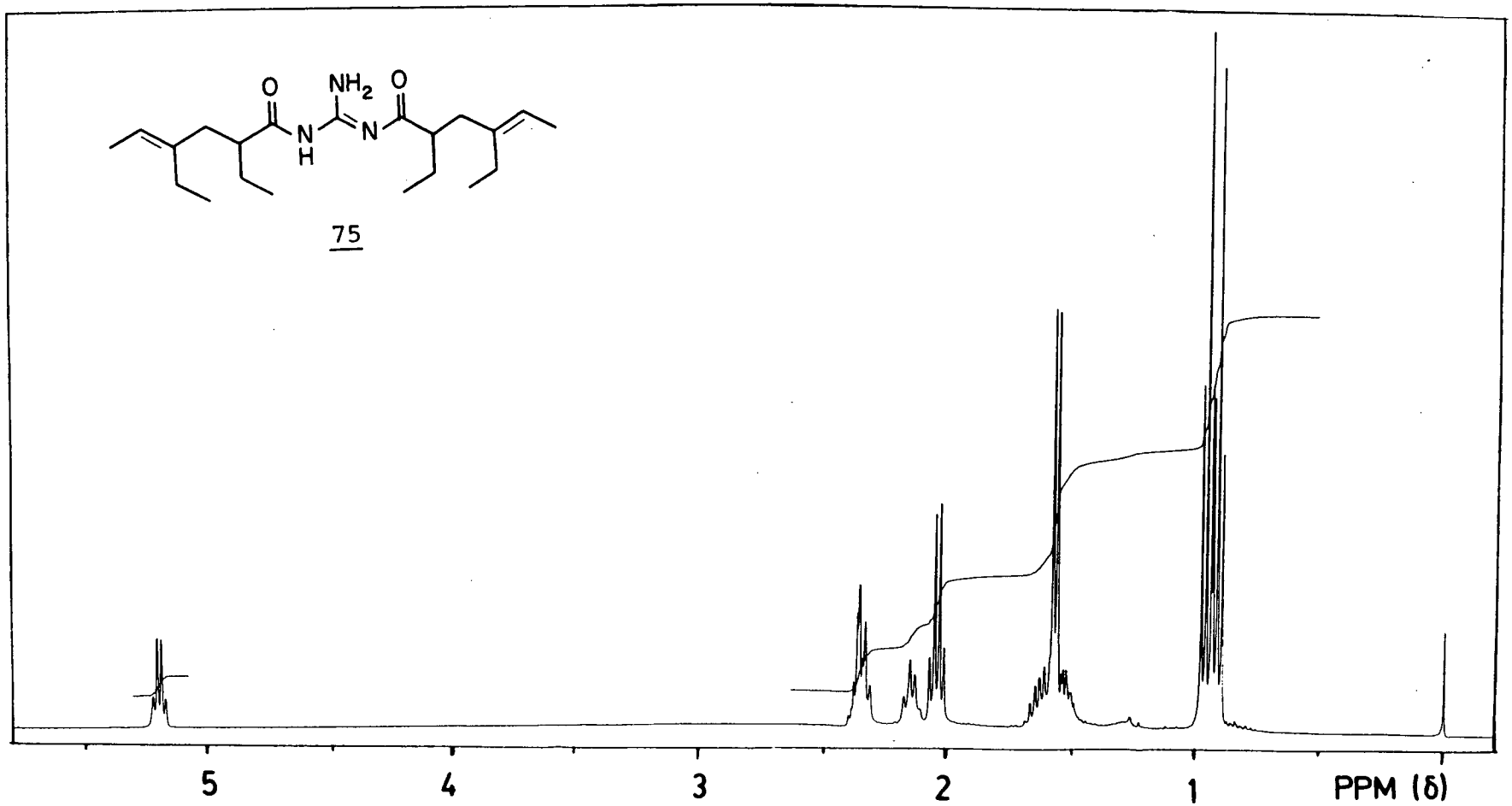


Figure 5. 400 MHz 'H NMR spectrum of 75 in $CDCl_3$.

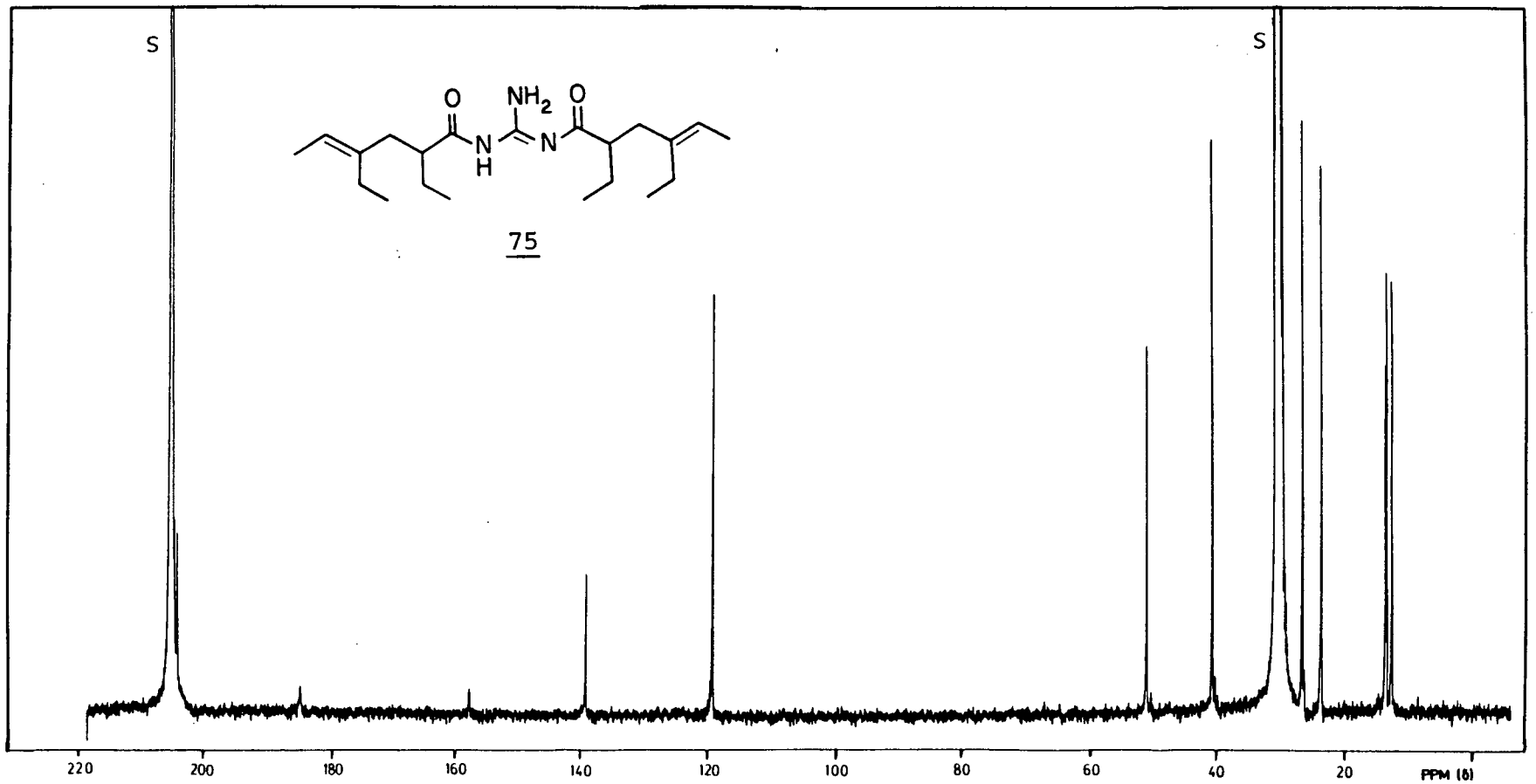


Figure 6. 100 MHz broad band decoupled ^{13}C NMR spectrum of 75 in acetone-d_6.

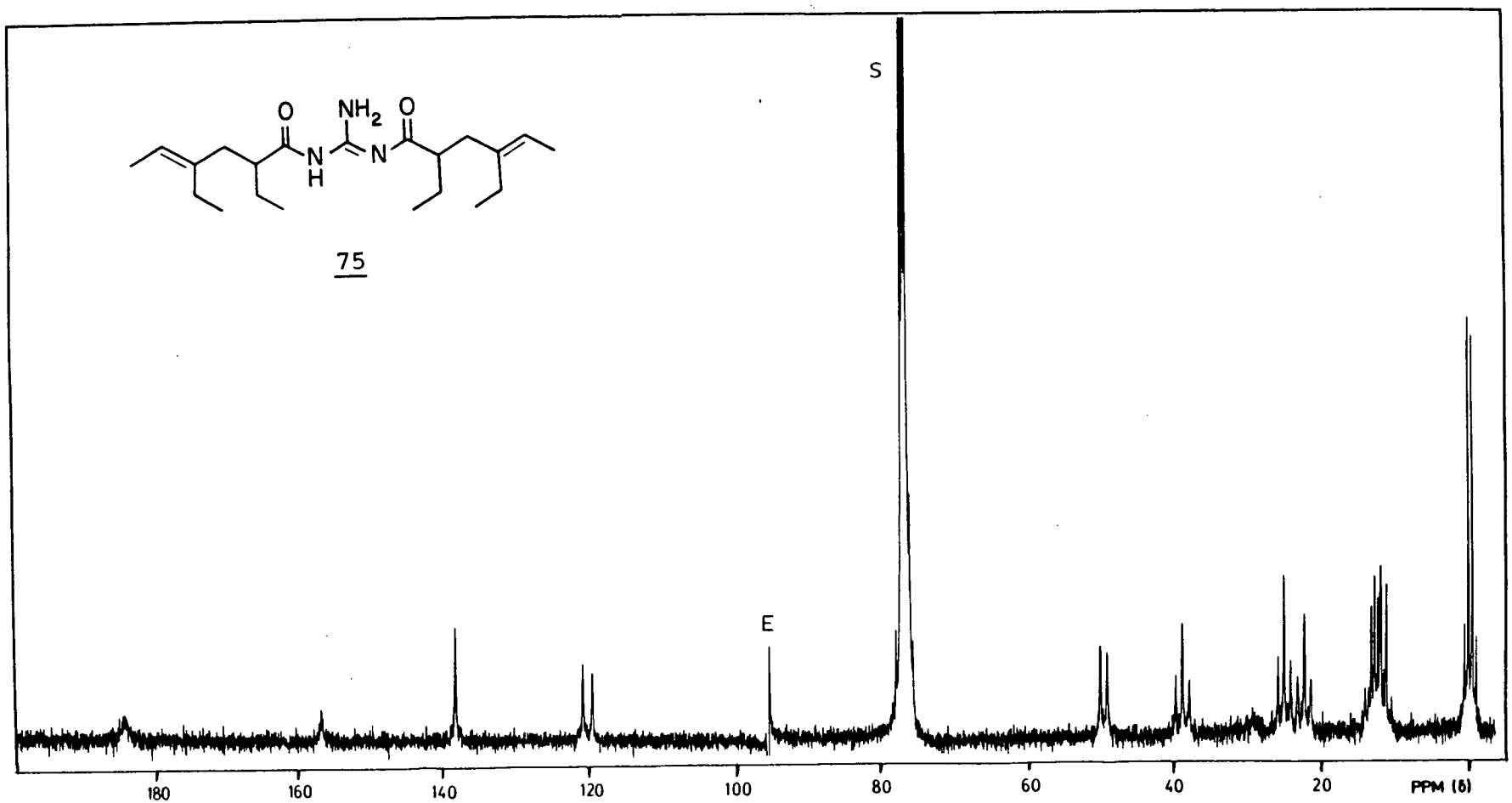


Figure 7. 100 MHz SFORD ^{13}C NMR spectrum of 75 in $CDCl_3$.

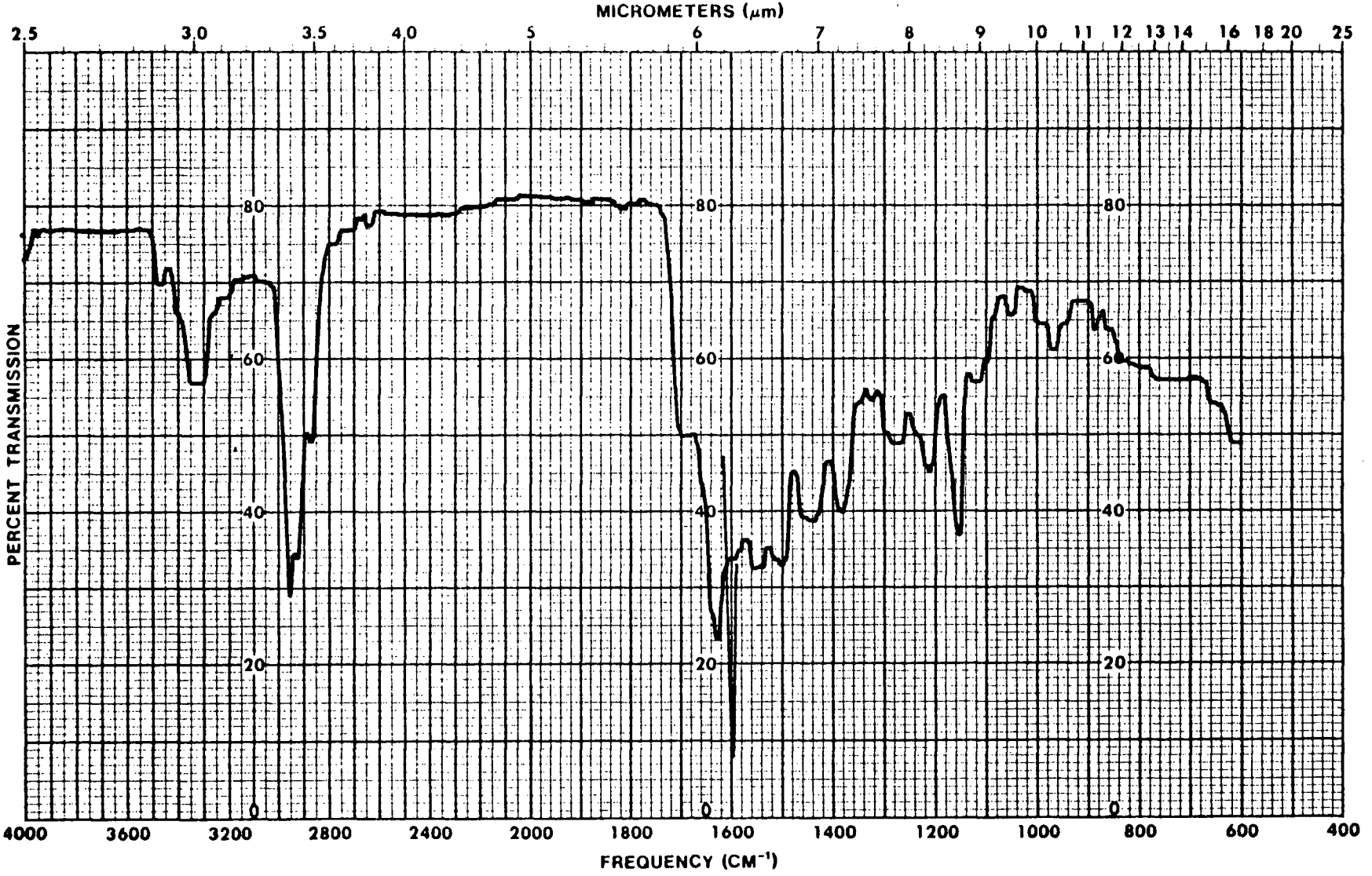


Figure 8. IR spectrum of 75 in $CHCl_3$.

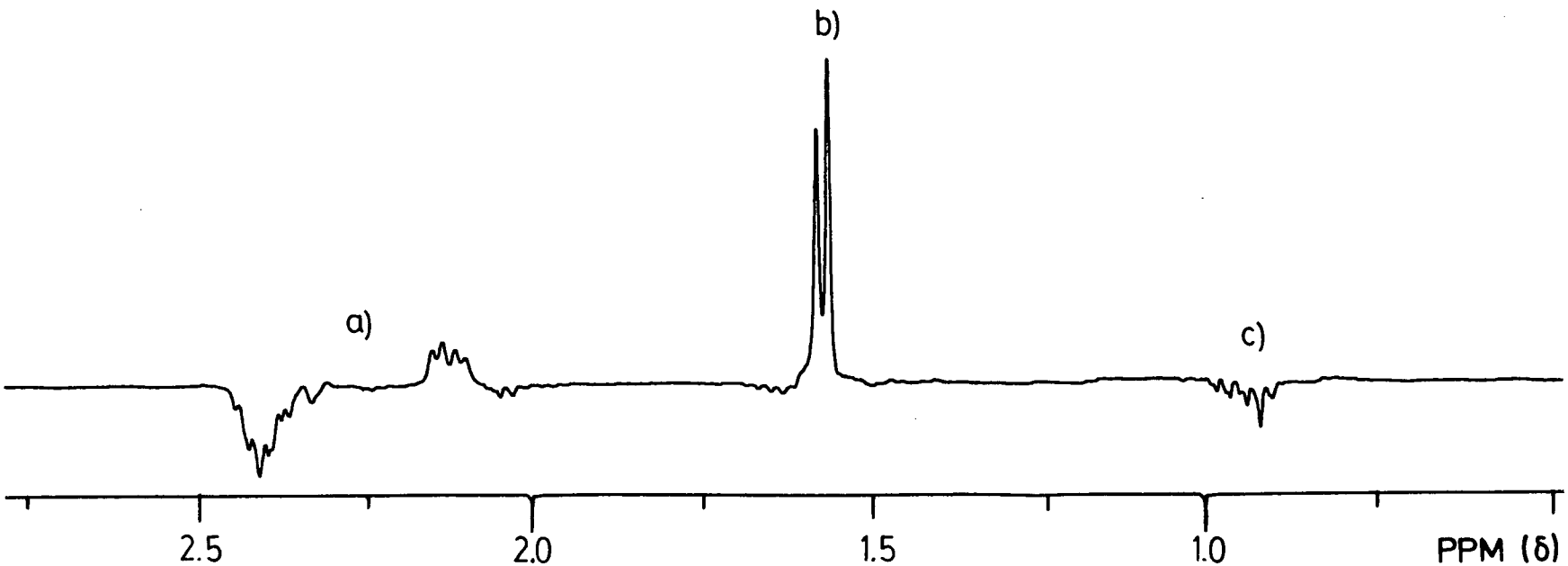


Figure 9. 400 MHz 'H NMR difference NOE spectrum of 75 when the olefinic proton at δ5.21 ppm was irradiated: a) enhanced signals of the main chain methylene protons; b) enhanced signal of the vinyl methyl group c) aliphatic methyls.

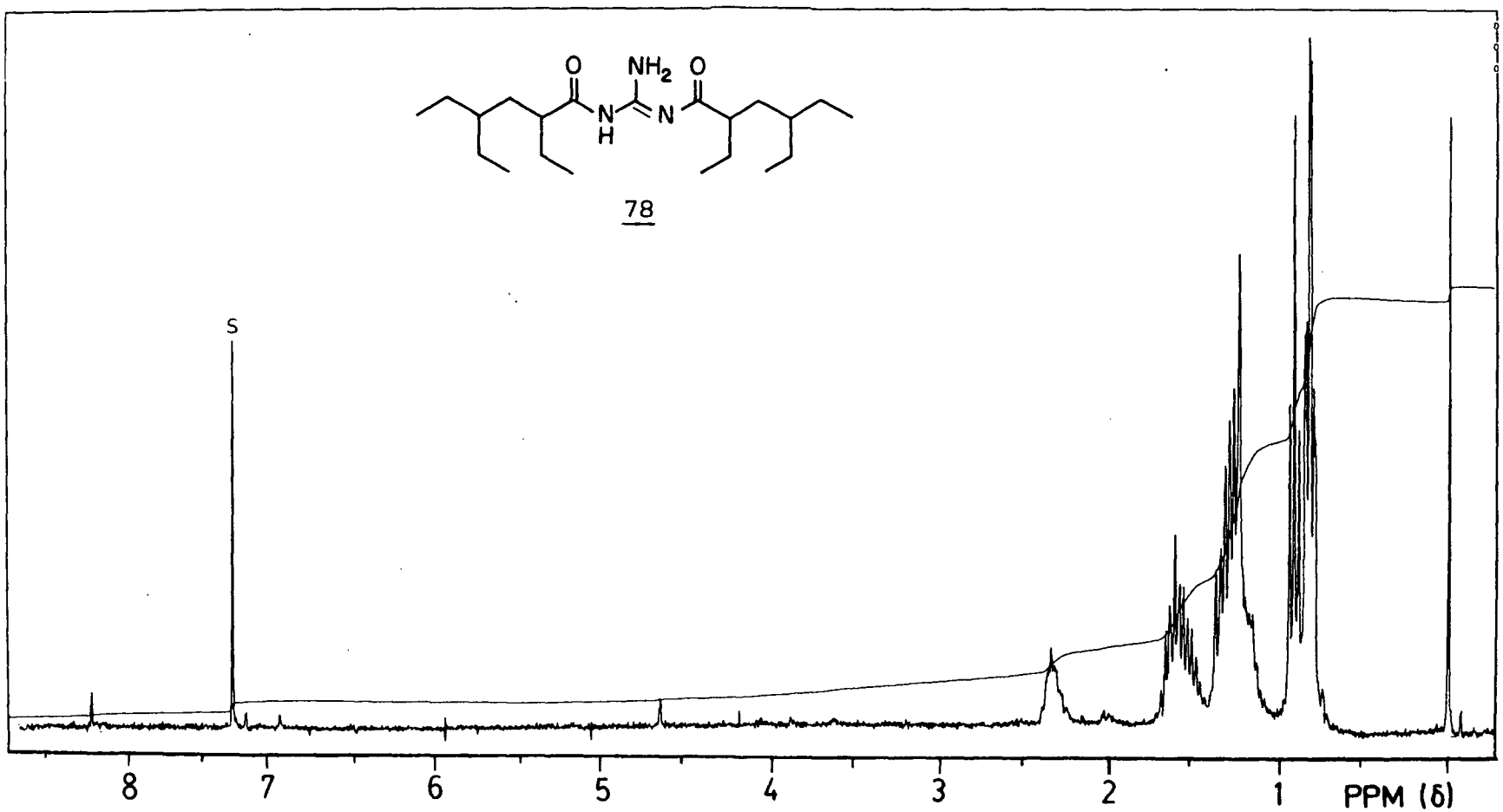


Figure 10. 270 MHz 'H NMR spectrum of 78 in $CDCl_3$.

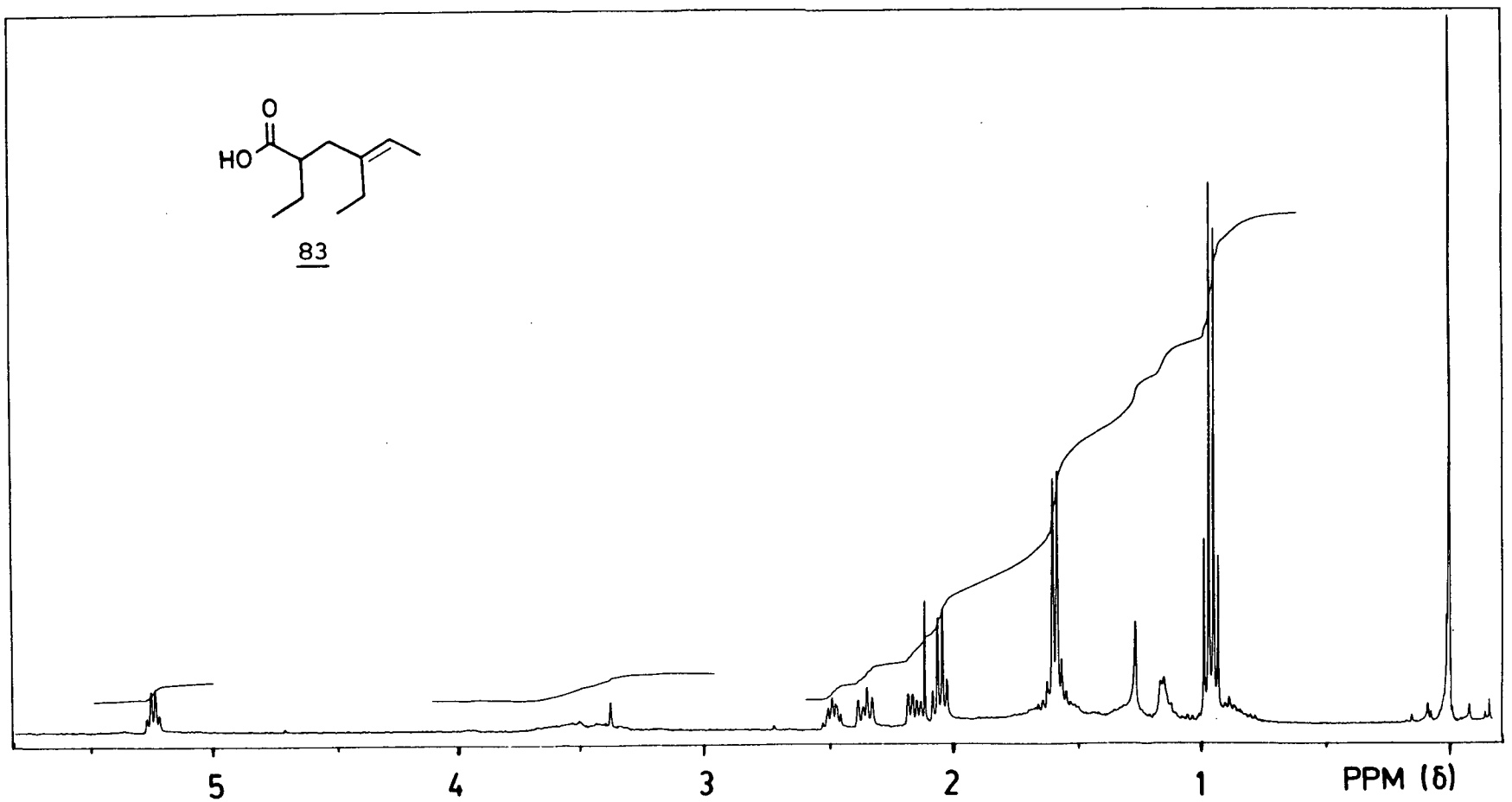


Figure 11. 400 MHz 'H NMR spectrum of 83 in $CDCl_3$.

II. BIOLOGICAL CONSIDERATIONS

A number of interesting and unique guanidine containing compounds have been reported from marine organisms. A thorough review of these compounds was provided by Chevolot[57] in 1981. Since then several other guanidine derivatives of marine origin have also been described[58,59]. However, to the best of our knowledge, triophamine (75) is the first naturally occurring diacylaguanidine.

The impetus for studying Triopha catalinae was the implicit assumption that the nudibranch employed a chemical defense system. Dissecting the organism prior to extraction showed that triophamine (75) was localized exclusively in the mantle. When prodded repeatedly, live specimens produced a mucous exudate rich in 75. Triophamine seems to be strategically stored near the outer surface of the nudibranch where it can be rapidly mobilized in a mucous secretion. Such logistics are ideally suited for a defensive substance, but in a fish antifeedant bioassay using goldfish, triophamine did not deter feeding. In addition, in a standard assay against gram positive bacteria triophamine failed to inhibit growth. While a large number of compounds containg the guanidine unit possess powerful biological activity, in these two tests triophamine proved to be inactive. On the basis of these negative results, it should not be concluded that triophamine has no value as a chemical deterent. Freshwater goldfish might respond

differently than their marine counterparts or it may be that triophamine deters predators other than fish.

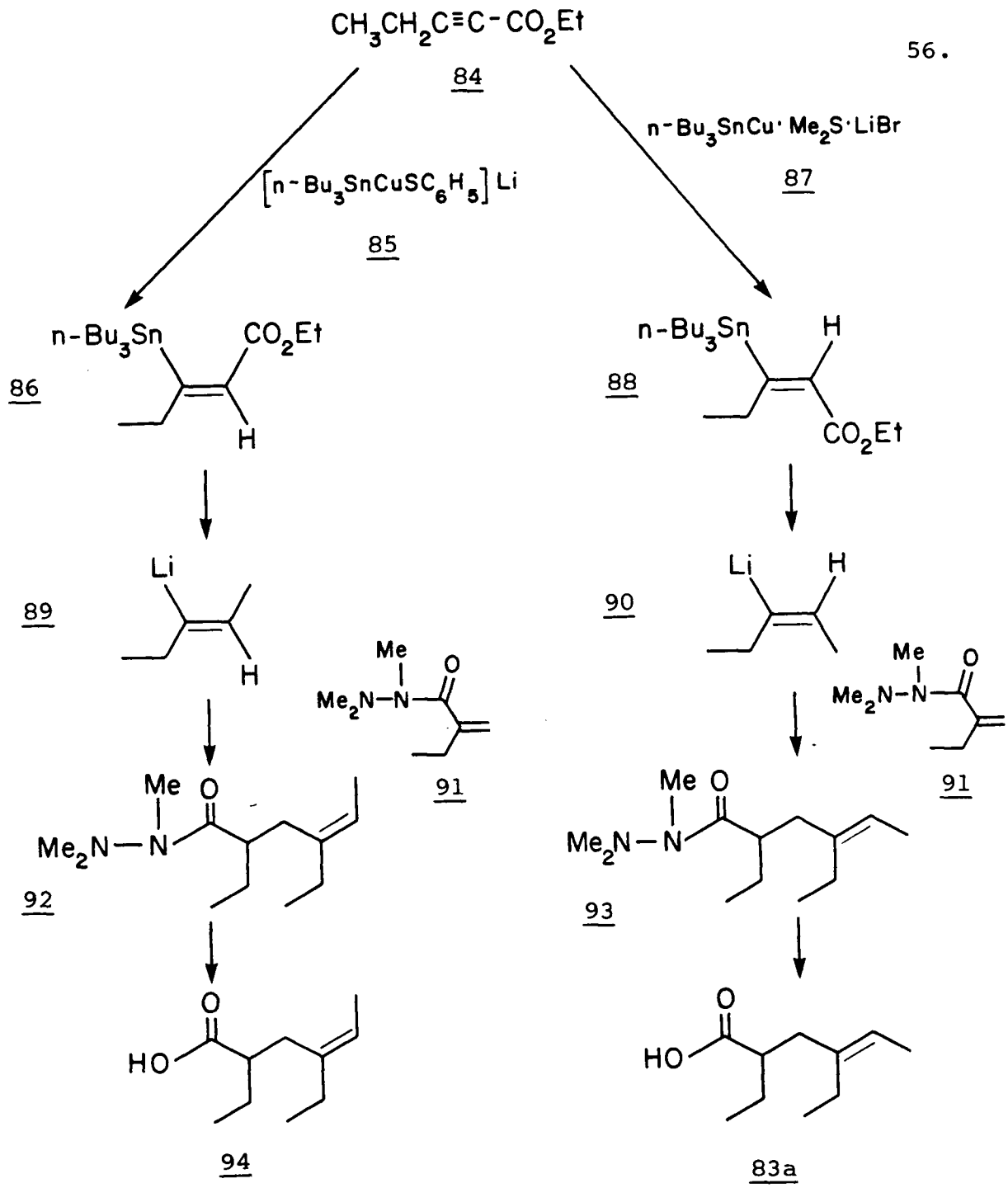
The egg masses of numerous nudibranch species consist of concentric rings of soft, spongy material. These structures are often brightly colored and deposited on unobstructed substrates. Nudibranch eggs would appear to be a suitable food source for many animals but predation is rarely observed. The conspicuous nature of T. catalinae egg masses prompted a search for chemical antifeedants. Methanol extracts of Triopha egg masses produced lipids and several steroids but no triophamine (75) or other unusual metabolites. The apparent immunity to predation enjoyed by these egg masses remains an unanswered puzzle.

Triophamine (75) was subsequently found in extracts of the vividly colored dorid nudibranch Polycera tricolor. P. tricolor also feeds exclusively on bryozoans, a group of sessile marine animals from which nitrogenous compounds have previously been isolated[60]. Since many nudibranchs have the ability to sequester metabolites obtained through their diet, a search was undertaken to find a dietary source for triophamine. Chemical analysis of bryozoan species encountered at the collection sites failed to reveal the presence of triophamine. While it is possible that triophamine originates in an undetected dietary source, a preliminary ^{14}C incorporation study suggests it is produced within the nudibranch (see Chapter 4, V).

III. SYNTHESIS OF (±)-TRIOPHAMINE (75a)

A synthesis of triophamine (75) was undertaken to provide definative proof of the proposed structure. The synthetic scheme involved production of the C_{10}-acid 83 and coupling two of these moieties to guanidine. This project was performed in conjunction with Piers and Chong[61] who synthesized the hydrocarbon portion of the molecule. At the time the synthesis was initiated the geometry about the olefinic bonds in triophamine had not been established. Piers and Chong applied a recently developed method[62] employing organocopper reagents to sterospecifically produce C_{10}-acids with (E) and (Z) olefins. Their syntheses of the C_{10}-acids are summarized below (see Scheme 1).

Reaction of the α, β-acetylenic ester 84 with lithium (phenylthio) (tri-n-butylstannyl) cuprate 85 in THF at -48°C afforded upon workup, ethyl (Z)-3-(tri-n-butylstannyl)-2-pentenoate 86. Alternately, when 84 was reacted in THF at -78°C with the (tri-n-butylstannyl) copper reagent 87 the corresponding geometric isomer 88 was produced. Complete reduction of the ester moieties to methyl groups in 86 and 88 followed by transmetalation yielded 89 and 90 respectively. Conjugate addition of the vinylithium species 89 to the N,N,N'-trimethylhydrazide derivative of 2-ethylpropenoic acid 91 provided the trimethyl hydrazide 92. In a similar fashion 93 was produced via 1,4-addition of 90 to 91. The trimethylhydrazide residues were removed



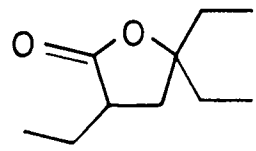
Scheme 1. Synthetic sequence for production of the isomeric acids 94 and 83a. Note: the "a" designates a racemic synthetic product that structurally corresponds with material derived from a natural source.

via reductive cleavage. Oxidation of the crude reduction products of 92 and 93 produced 94 and 83a respectively.

Synthetic product 83a provided IR and 'H NMR spectra identical in all respects to those of the olefinic acid 83 isolated from natural triophamine (75). Spectra (IR and 'H NMR) of the isomeric acid 94 were very similar to, but distinctly different from, the spectra of the natural acid. This confirmed the structure of the hydrocarbon portion of triophamine which had previously been deduced by interpretation of spectral data. The (E) configuration of the double bond in triophamine, predicted from the difference NOE experiment, was now definitively established by the unambiguous synthesis of 83a.

It should be noted that acid-catalyzed hydrolysis of 94 using HCl, acetic acid, p-toluenesulfonic acid or oxalic acid produced 2,4,4-triethylbutyrolactone 95 as the major product. This indicated that the carbon-carbon double bond participated in the hydrolysis process. With this observation in mind, the hydrolysis of triophamine (75) was re-examined. While hydrolysis of 75 in the presence of base is a facile process, the persistent low yield of acid 83 from the reaction mixture was difficult to explain. It is now apparent that the hydrocarbon portion of 75 is prone to lactonization in the presence of acid. Acidification of the hydrolysis mixture leads to Markovnikov protonation of the olefin. The 3° carbonium ion which is generated can

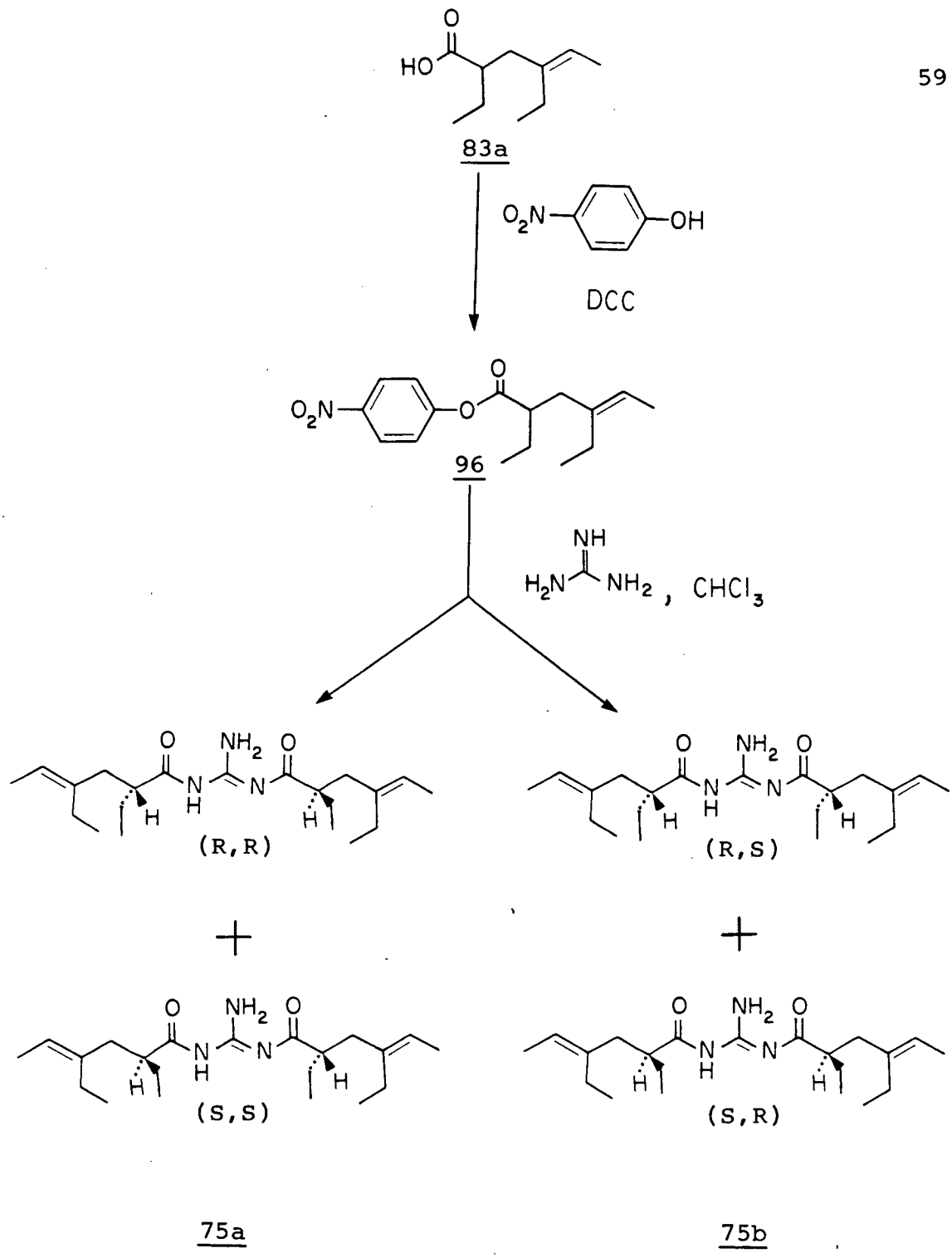
then be attacked by the carbonyl oxygen of the carboxylic acid. The γ-lactone 95 was subsequently isolated from the acidified hydrolysate of 75.



95

The strategy of the remainder of the synthesis involved converting acid 83a into a suitable derivative and then coupling it to guanidine. Since the coupling reaction requires nucleophilic attack of guanidine on the acid carbonyl, a derivative of 83a was made which replaced the hydroxyl residue with a more favorable leaving group. Treatment of the carboxylic acid 83a with p-nitrophenol in the presence of N,N'-dicyclohexylcarbodiimide afforded the p-nitrophenyl ester 96 in excellent yield. The ability of the p-nitrophenyl unit to stabilize a negative change increased the reactivity of 96 toward nucleophiles. Coupling the activated ester 96 to guanidine in a 2:1 stoichiometric ratio would complete the synthesis (see Scheme 2).

Previous studies have shown that while the products obtained on acylation of guanidine are dependent on the conditions used, N, N'-diacyl derivatives can be produced in



75a 75b

Scheme 2 Final steps in the synthesis of the (R,R) and (S,S) enantiomers of (±)-triophamine (75a) and the (R,S) and (S,R) diastereomers 75b.

good yield[63]. From our own studies, it was known that treatment of guanidine carbonate with acetic anhydide produced a mixture of the diacetyl and triacetyl derivatives. Triacetylguanidine could be hydrolyzed to diacetylguanidine in refluxing ethanol. We set out to find suitable conditions for diacylating guanidine with the p-nitrophenyl ester 96 or if the triacyl derivative predominated, achieve selective hydrolysis of a single acyl group.

Initial attempts to acylate the hydrochloride or carbonate salts of guanidine were unsuccessful as 96 failed to react with the guanidine salts. Employing a procedure described by Banker et al.[64], the free base of guanidine was treated with acylating agent 96 in the presence of N,N,N',N'-tetramethyl guanidine. Under these conditions ester 96 reacted with the tetramethylguanidine to yield its monoacyl derivative, but no reaction of 96 with free guanidine was observed. By systematically varying the reaction conditions, it was found that the desired diacyl product 75a could be formed by adding small amounts of free guanidine to an excess of 96 in chloroform. Guanidine free base is extremely hygroscopic so an accurate weight of the total guanidine added was not determined. Rather, small portions were added at 1h intervals and the course of the reaction monitored by TLC (the appearance of 75a and the disappearance of 96). If the reaction was allowed to go to

completion, as determined by the disappearance of 96, appreciable quantities of side product(s) formed and the yield of 75a was very low. Therefore, when approximately 50-75% of the starting material 96 had reacted, the reaction was stopped by filtering off the chloroform soluble material. Chromatography on silica provided a 48% yield (based on unrecovered 96) of a mixture of (±)-triophamine (75a) and its diastereomers 75b.

The identical hydrocarbon moieties of triophamine (75) each contain a single chiral center [the C-2(2') methine carbon]. As natural triophamine is optically active, the configuration about the two chiral centers must be (R,R) or (S,S). In the synthesis of (±)-triophamine, the olefinic acid 83a is produced as a racemic mixture. When two acid residues are coupled to guanidine four combinations of configurations are formed, (R,R), (S,S), (R,S) and (S,R). The mixture of the (R,R) and (S,S) products constitute (±)-triophamine (75a) while the (R,S) and (S,R) products represent a mixture of unwanted diastereomers 75b.

Separation of (±)-triophamine (75a) from its diastereomers 75b was achieved by repeated preparative thin-layer chromatography. The 'H NMR and IR spectra of (±)-triophamine were identical in all respects to those of the natural product. The spectra ('H NMR and IR) of the diastereomers showed subtle but distinct differences from authentic triophamine. Resonances between δ1.5 and 2.5 ppm

in the 'H NMR spectra were most useful in distinguishing the desired product (see figure 12). The diastereomers displayed a slightly altered coupling pattern in the two-proton multiplet at δ1.49-1.67 and a sharpened signal at 2.12 (dd, J = 7, 14Hz, 1H) ppm. The clearest spectral difference was the two one-proton multiplets at δ2.28-2.37 and 2.37-2.47 in the diastereomers that were coalesced to a two-proton multiplet at 2.30-2.48 ppm in authentic triophamine.

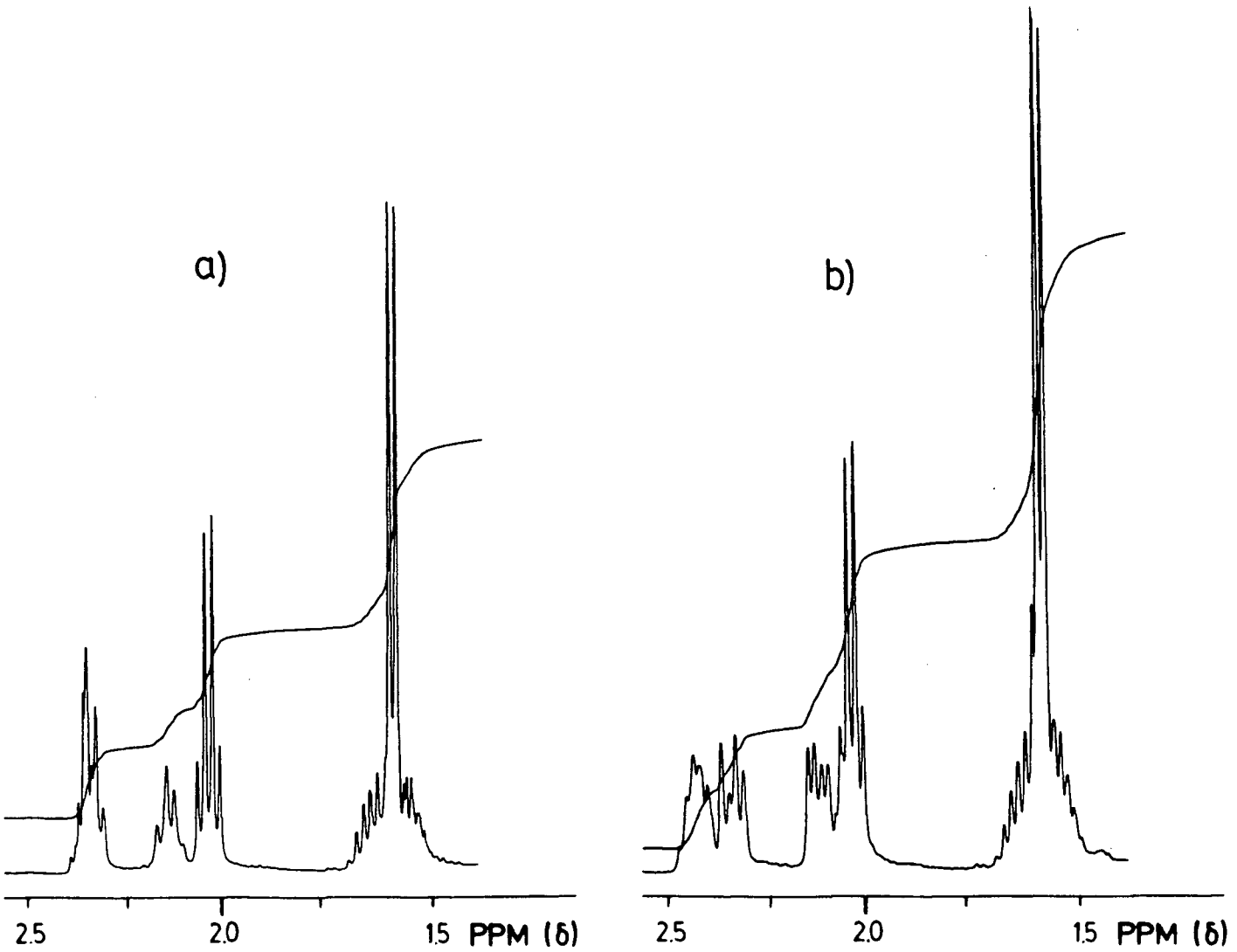


Figure 12. Comparison of ^{1}H NMR (400 MHz, $CDCl_3$) resonances between δ1.5-2.5 ppm of: a) natural triophamine (75) and b) mixture of synthetic diastereomers 75b.

Plate 2. Anisodoris nobilis. Photographer: Ron Long, Simon Fraser University.

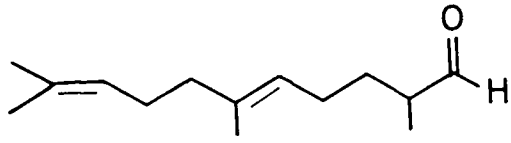
CHEMICAL STUDIES OF ANISODORIS NOBILIS

I. DIHYDROAPOFARNESAL (15)

The dorid nudibranch Anisodoris nobilis is large (average size 80-100mm) and fairly abundant along the coast of British Columbia. It feeds on sponges and is found in a variety of habitats from the intertidal zone to depths of greater than 100 m. When removed from the water, the nudibranch emits a pleasant, fruity aroma. Because of its pleasant smell and bright yellow coloration, A. nobilis is commonly referred to as the speckled sea lemon. A. nobilis was the subject of a previous chemical investigation. In 1980, Fuhrman et al.[22] isolated the unique N-methylpurine riboside doridosine (16) from aqueous extracts of the nudibranch's digestive gland. It is pharmacologically active and is probably responsible for the toxicity to mice and crabs originally observed in these extracts[65]. Doridosine is also a constituent of the Australian sponge Tedonia digitata[66].

The present investigation of Anisodoris nobilis was prompted by the nudibranch's pleasant odor. When perturbed, A. nobilis produces a voluminous mucous secretion which retains the smell of the organism. Freshly collected specimens were extracted with chloroform, as methanol formed an emulsion with the mucous. Successive chloroform extracts were combined and concentrated to a fragrant brown oil.

Fractionation of the crude extract on silica gel provided lipids, steroids and a single odoriferous compound that was DNPH positive. Final purification was achieved by preparative gas chromatography to yield the olefinic aldehyde 15. The volatile nature and low natural abundance of 15 resulted in a total yield of only 2.8 mg (approximately 0.1 mg/animal).

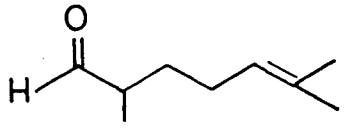


15

The molecular formula $C_{14}H_{24}O$ was established by electron impact HRMS and revealed that 15 possessed three units of unsaturation. The 400 MHz ^{1}H NMR spectrum (Figure 14) displayed a one-proton doublet at δ9.58 (J = 1 Hz) ppm, indicative of an aldehyde residue. Three olefinic methyl groups were evidenced by slightly broadened singlets at δ 1.60, 1.61 and 1.69 ppm that integrated for three protons each. Two olefinic protons were observed as a multiplet at δ5.12 ppm. It was evident the compound was acyclic, as the three units of unsaturation were accounted for by one aldehyde and two olefin functionalities. The ^{1}H NMR spectrum also revealed a methyl doublet at δ1.10 (J = 7 Hz)

and six allylic methylene protons as a broad multiplet at 1.96-2.13 ppm. One-proton multiplets at δ1.42 and 1.78 ppm were appropriate for a methylene that is β to both an olefin and aldehyde substituent.

The 'H NMR spectrum of 15 showed great similarity to that of compound 68, recently isolated as the odoriferous principle of the nudibranch Melibe leonina[43].



68

The fragrant compound from Anisodoris nobilis was evidently the C_{14}-analog, dihydroapofarnesal (2,6,10-trimethyl-5,9-undecadien-1-al). The proposed structure is supported by a prominent mass spectral fragment ion at m/z 150 (M^+-CH_3CHCHO + H) resulting from a McLafferty rearrangement and the base peak at m/z 69 resulting from a doubly allylic cleavage which expels the terminal isoprene unit (Figure 13). The geometry of the central olefin has arbitrarily been assigned as trans. Aldehyde 15 is clearly a degraded sesquiterpene.

The mucous secretion of Anisodoris nobilis which contains dihydroapofarnesal 15 is known to be repugnant to the predatory sea star Pycnopodia helianhoides[4]. It is interesting to note that the exudate from Melibe leonina

elicits a similar escape response from P. helianthoides[67] and contains 68 as its principal component. The low yield and volatile nature of 15 prevented its testing in a fish antifeedant bioassay. Dihydroapofarnesal has not been reported previously as a natural product but appears to have found use in the perfume industry[68].

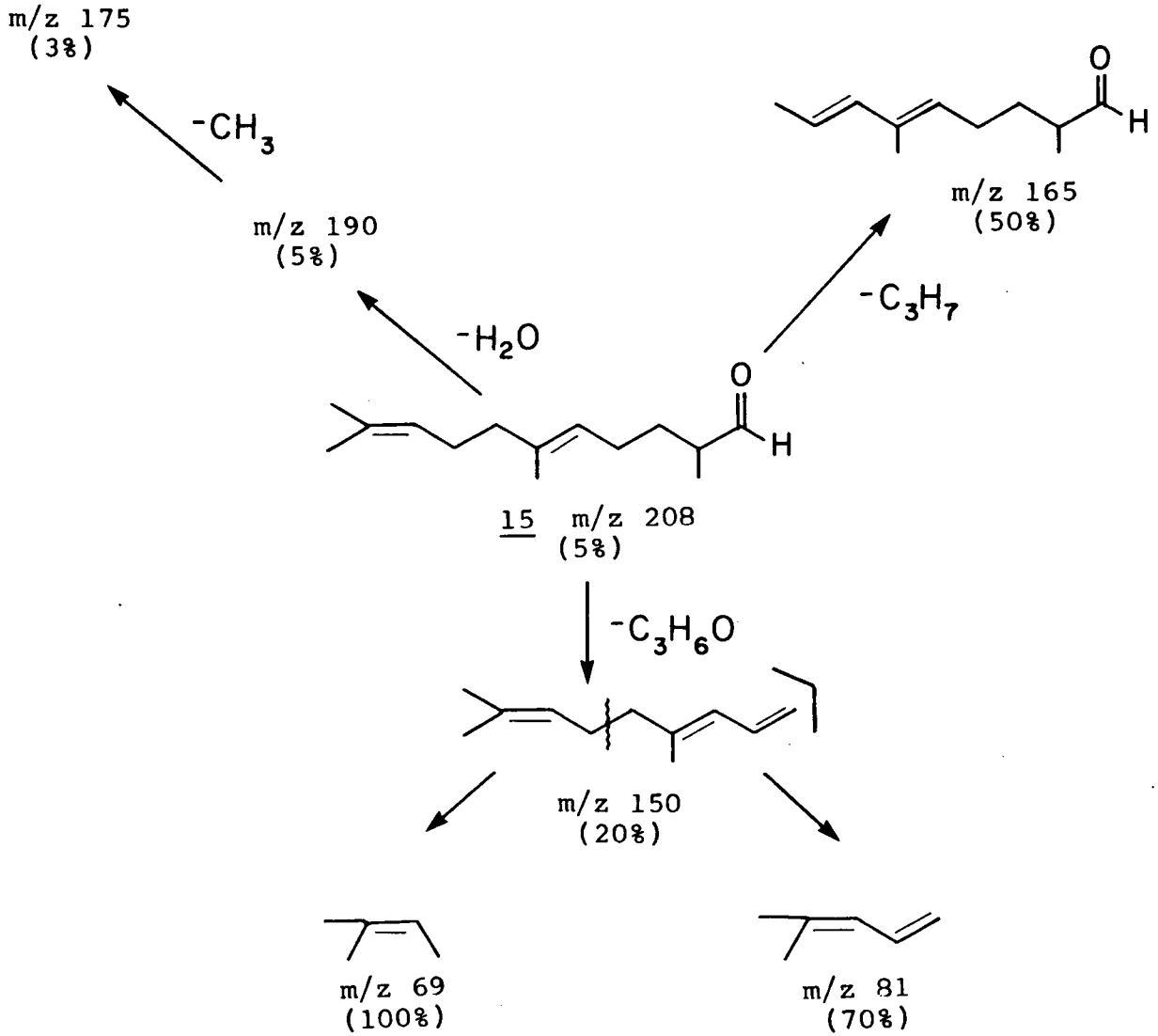


Figure 13. Interpretation of the HRMS of 15.

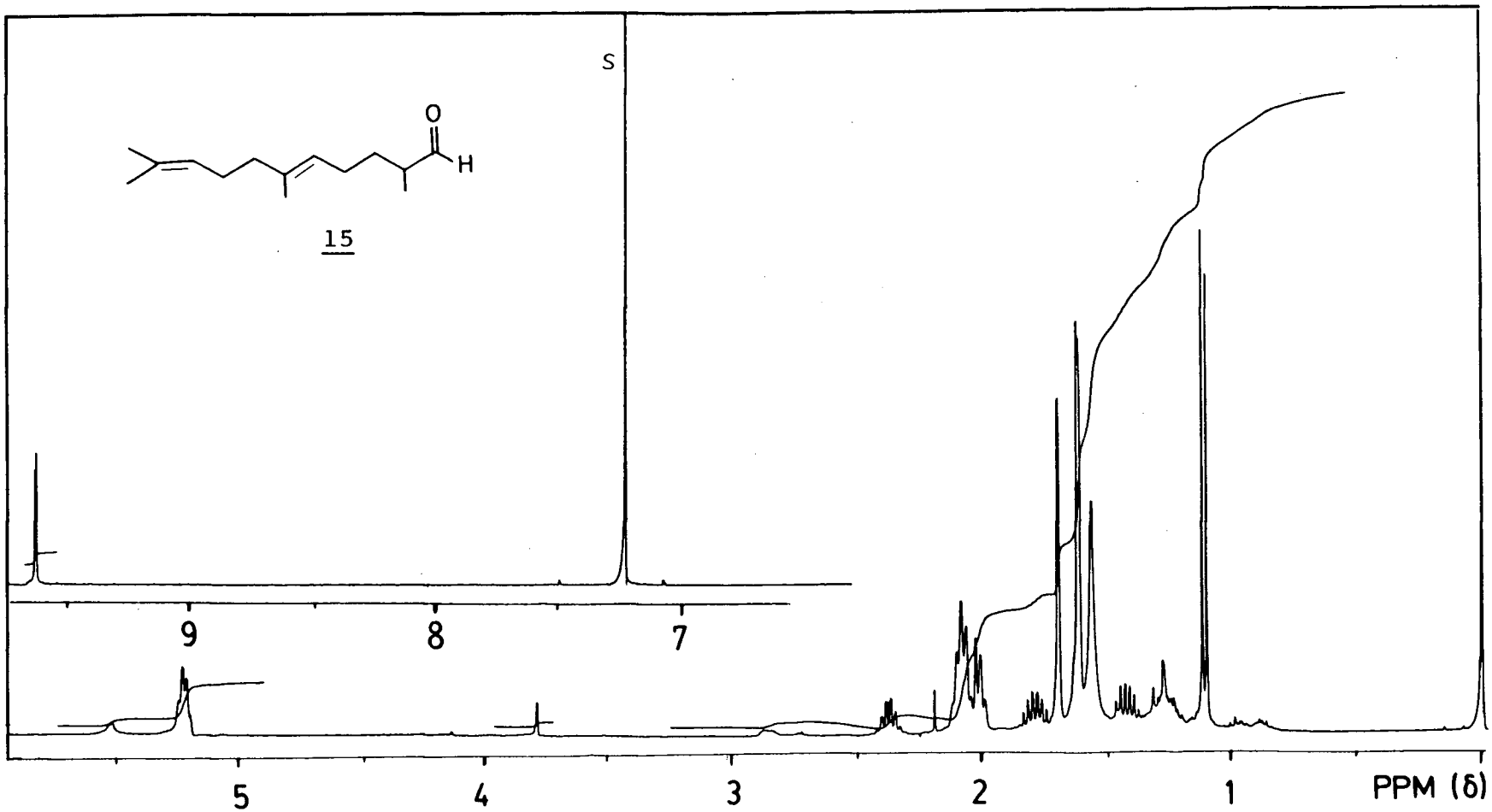


Figure 14. 400 MHz 'H NMR spectrum of 15 in $CDCl_3$.

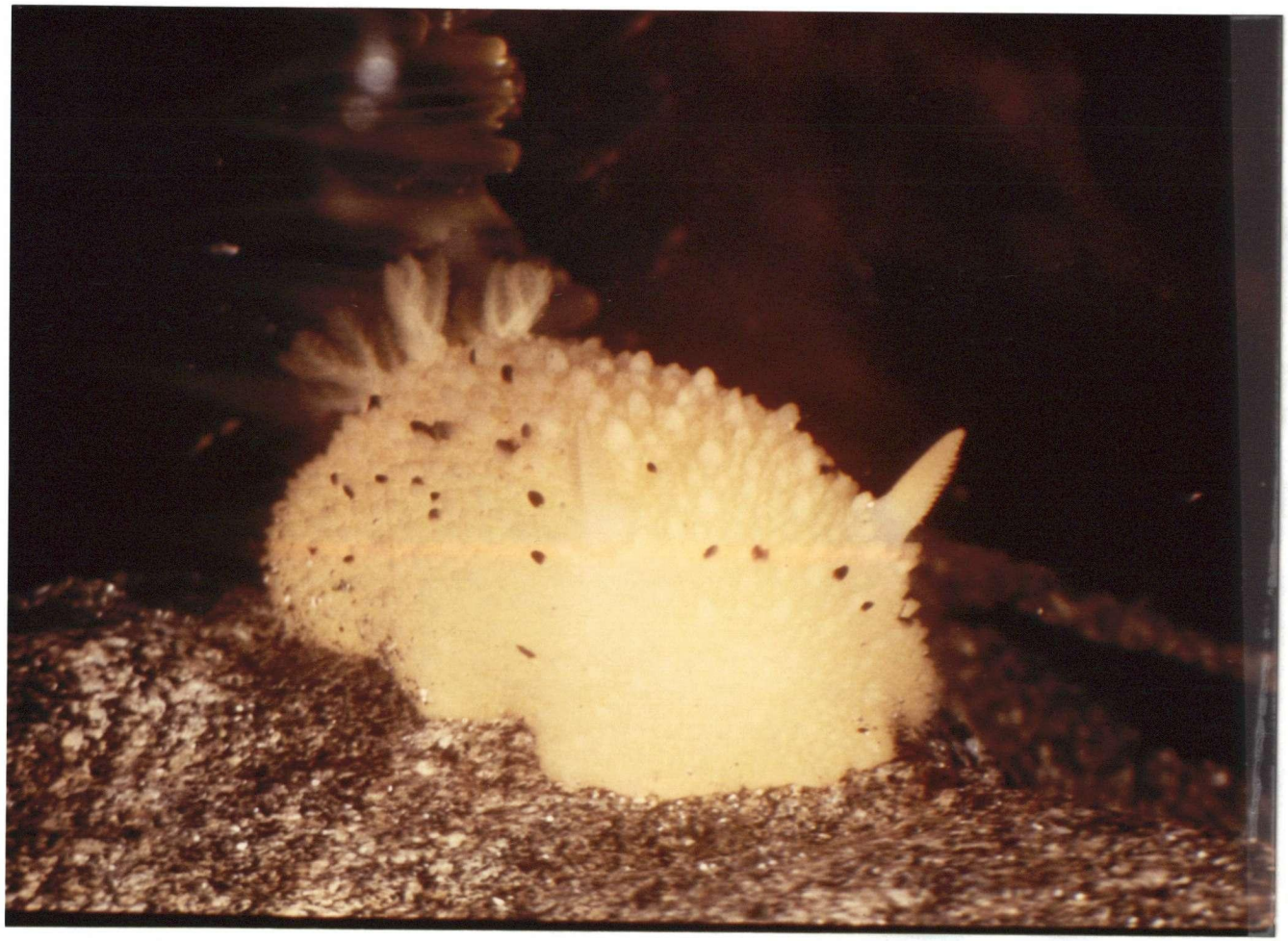


Plate 3. Archidoris montereyensis. Photographer: Ron Long, Simon Fraser University.

CHEMICAL STUDIES OF ARCHIDORIS MONTEREYENSIS AND A. ODHNERI

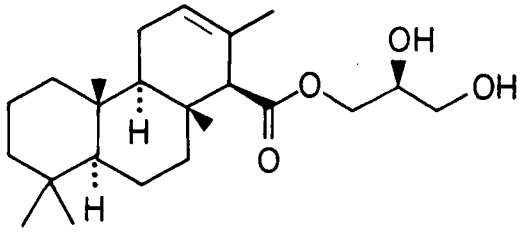
I. Archidoris montereyensis

The dorid nudibranch Archidoris montereyensis superficially resembles the speckled sea lemon Anisodoris nobilis. A. montereyensis ranges from subtidal to 25 m depth, but is most common in relatively shallow, sponge dominated communities. The observation that its crude methanol extracts exhibited antibiotic activity against the gram positive bacteria Staphylococcus aureus and Bacillus subtilis prompted the present chemical investigation[21].

Specimens of Archidoris montereyensis were collected in Barkley Sound, B.C. and immediately immersed in methanol. The methanol was decanted and concentrated under reduced pressure to an aqueous suspension. Partitioning between chloroform and water provided an organic extract which retained the antibiotic activity. Bioassay guided fractionation was performed by flash chromatography on silica gel using step gradients of hexane-ethyl acetate mixtures. The material eluted with 100% ethyl acetate was subjected to radial thin-layer chromatography eluting with chloroform-methanol (95:5). Final separation by HPLC with a hexane-ethyl acetate-methanol (50:40:2) eluant provided pure samples of compounds 17, 20, 22 and 23.

The major metabolite 17, was an optically active ($[\alpha]_D$ = -12.5°, $CHCl_3$), crystaline solid. Electron impact

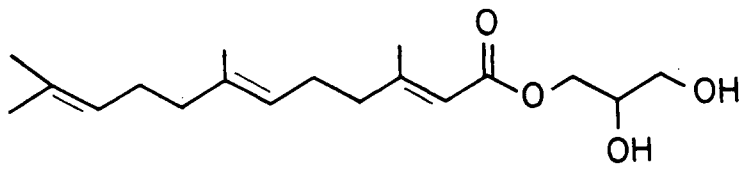
HRMS established its molecular formula as $C_{23}H_{38}O_4$. Absorbances in the IR spectrum revealed the presence of hydroxyl (3600-3300 cm^{-1}) and ester (1730 cm^{-1}) functionalities. A resonance at δ173.4 ppm in the ^{13}C NMR (100 MHz, $CDCl_3$) spectrum supported the presence of an ester.



17

The 'H NMR (400 MHz, $CDCl_3$) contained resonances highly suggestive of a 1-acyloxy-2,3-dihydroxypropane moiety. The carbinol protons of a primary alcohol were seen as two doubled doublets at δ3.63 (J = 6, 12 Hz) and 3.70 (J = 4, 12 Hz) ppm that each integrated for one proton. A one-proton multiplet at δ3.95 ppm was indicative of a secondary alcohol. A broad, two-proton singlet at δ2.44 ppm that exchanged with D_2O supported the presence of two hydroxyl groups. Two doubled doublets deshielded to δ4.15 (J = 7, 12 Hz, 1H) and 4.22 (J = 5, 12 Hz, 1H) ppm were appropriate for an esterified primary alcohol. These distinctive resonances were virtually identical to those of the glycerol unit in the farnesic acid derivative 24, previously isolated from

Archidoris odhneri[24]. Prominent peaks in the mass spectrum at m/z 286 ($M^+-C_3H_8O_3$) and 258 ($M^+-C_4H_8O_4$) confirmed that the alcohol component of the ester was a glycerol residue.



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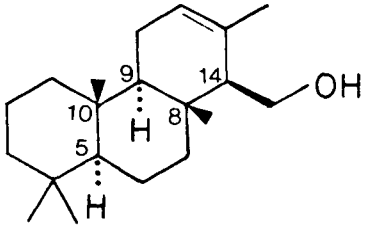
The molecular formula ($C_{20}H_{31}O$) of the acyl portion of the ester suggested that compound 17 was a diterpenoic acid esterified to glycerol. Two of the five total units of unsaturation in the parent glyceride could be accounted for by the ester carbonyl and a trisubstituted olefin that was evidenced by ^{13}C NMR resonances at δ124.3 (d) and 128.6 (s) ppm and a single olefinic proton signal at δ5.54 (bs) ppm. The terpenoid portion of 17 clearly contained three carbocyclic rings. The 'H NMR spectrum revealed four methyl singlets at δ0.82, 0.87, 0.92 and 0.96, a vinyl methyl at 1.61 and a broad, one-proton singlet at 2.93 ppm appropriate for an allylic methine that is also α to an ester carbonyl. The olefin, ester and methyl substituents accounted for eight of the twenty carbons in the diterpenoid acyl residue. A ^{13}C SFORD NMR experiment established that the remaining twelve consisted of six methylene, three methine and three quarternary carbons. This spectral data suggested that compound 17 had the regular isoprene structure shown. The

base peak in the mass spectrum at m/z 192, resulting from a retro Diels-Alder cleavage of the C ring, supported this hypothesis. However, trace contamination by compound 20 resulted in a persistent peak at m/z 310 which was presumed to be a loss of C_5H_8 from the parent glyceride. A rational mechanism for such a loss from 17 could not be envisioned. Due to this spectral ambiguity and a limited quantity of the natural product, the structure was verified by single crystal X-ray diffraction analysis conducted by Clardy and Chen[23].

The X-ray experiment confirmed the structure but defined only the relative stereochemistry of the molecule. The absolute configuration was determined by reducing 17 with DIBAL to the known alcohol 97. Comparison of the specific rotation ($[\alpha]_D$ = -9°) of the reduction product with the literature value[69] ($[\alpha]_D$, = -9°) confirmed the absolute configuration as shown (5S, 8R, 9R 10S, 14R).



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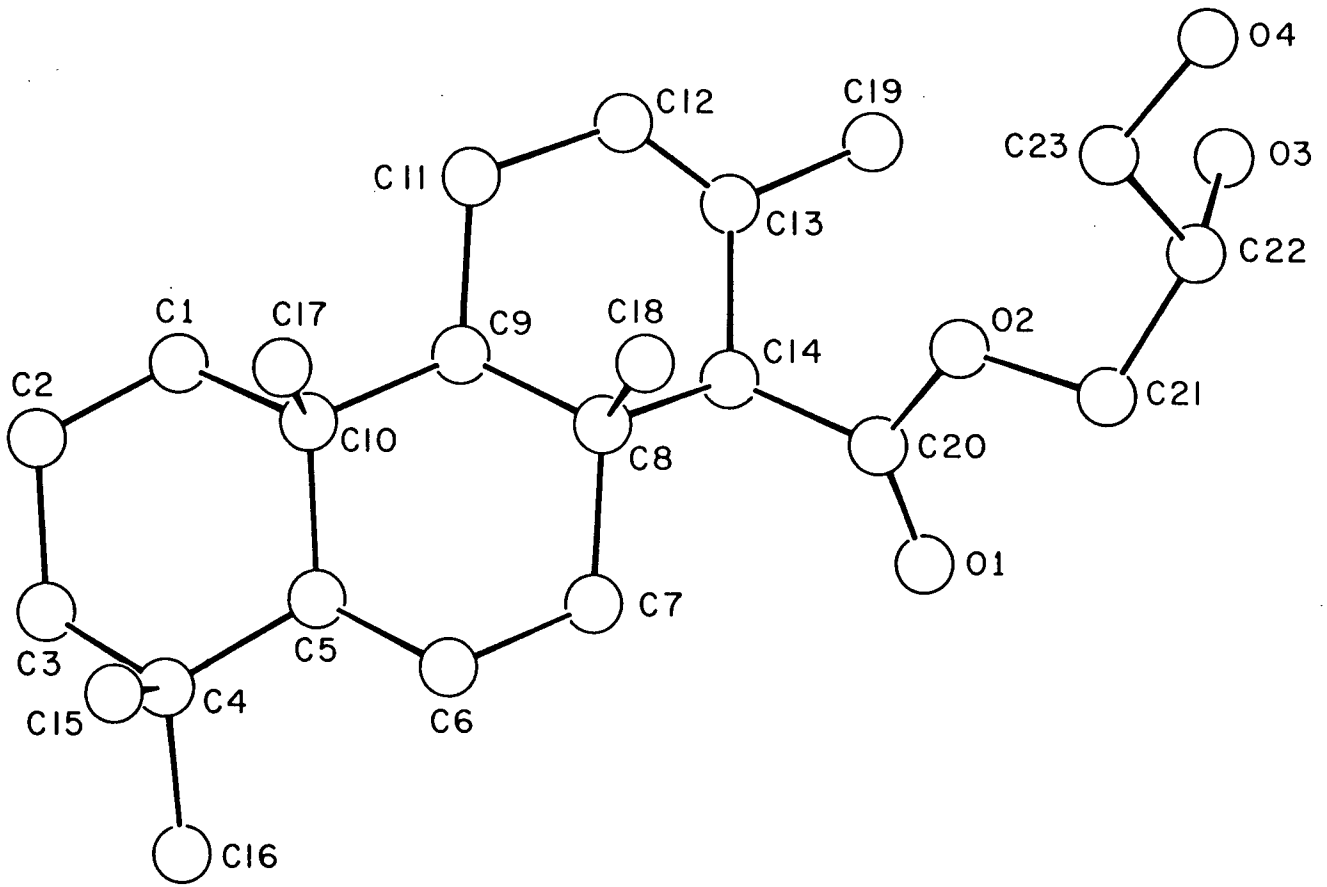


Figure 15. Computer generated stereochemical representation of 17 obtained by single crystal X-ray diffraction analysis.

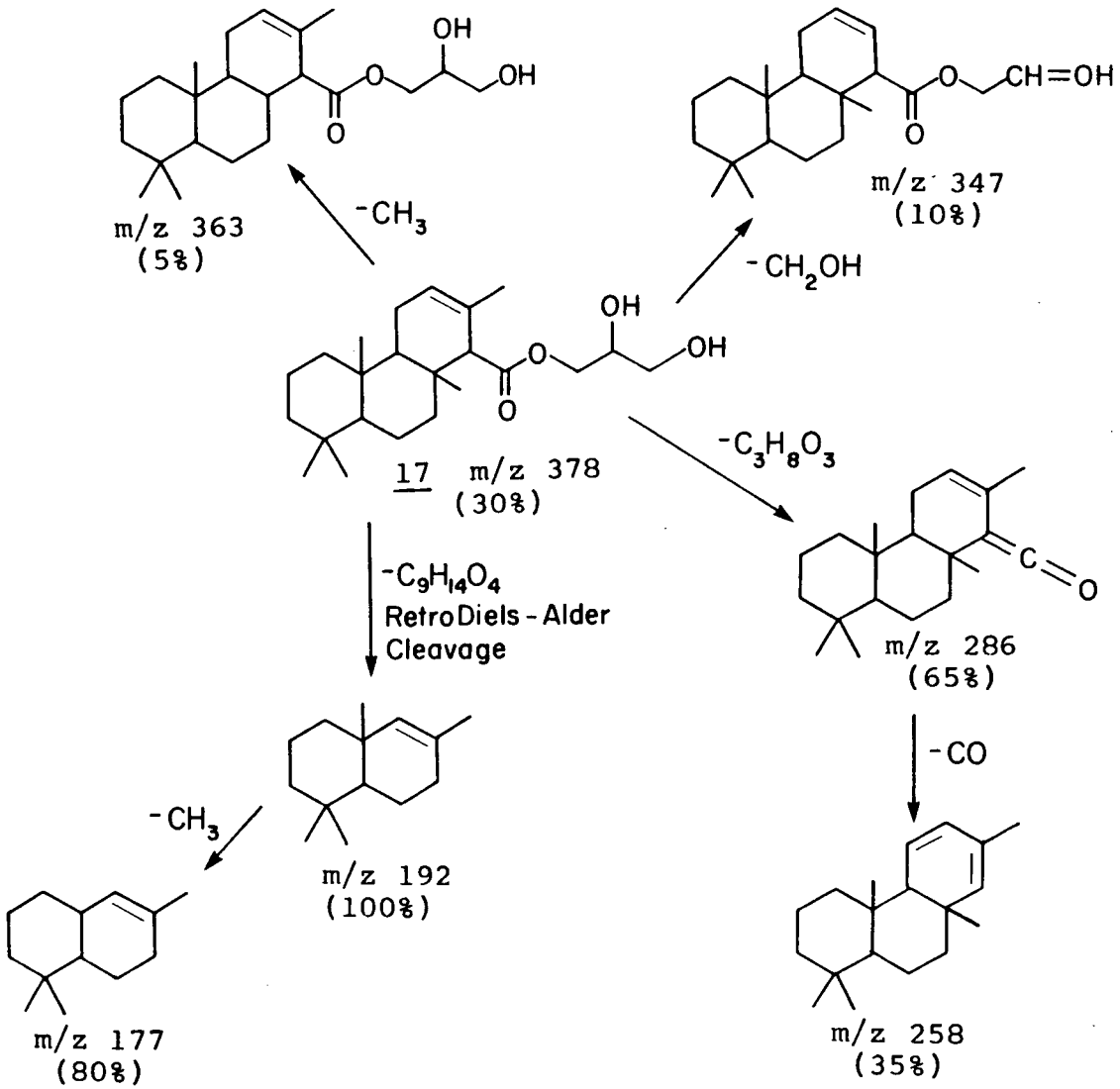


Figure 16. Interpretation of the HRMS of 17.

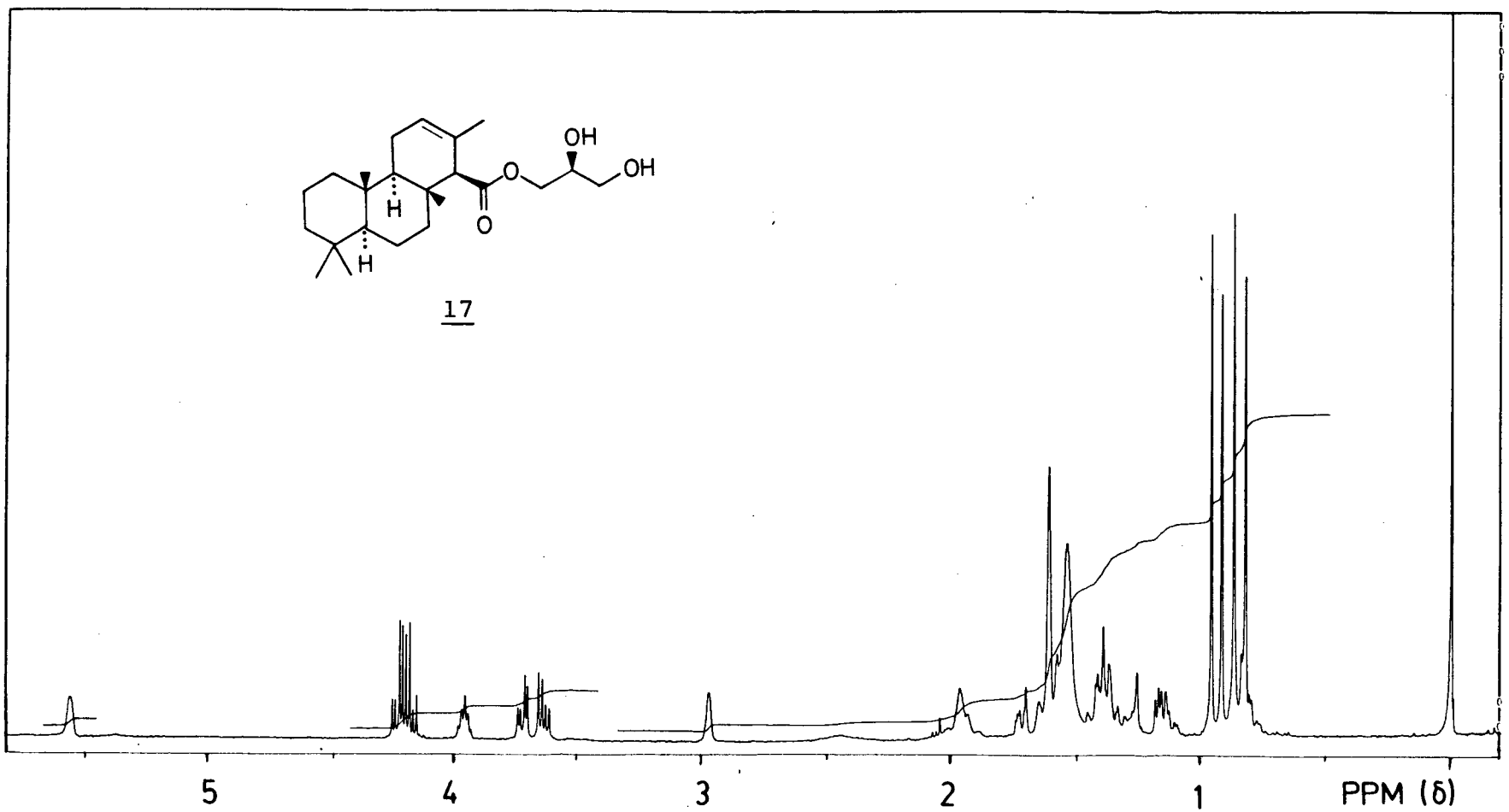


Figure 17. 400 MHz ^{1}H NMR spectrum of 17 in $CDCl_3$.

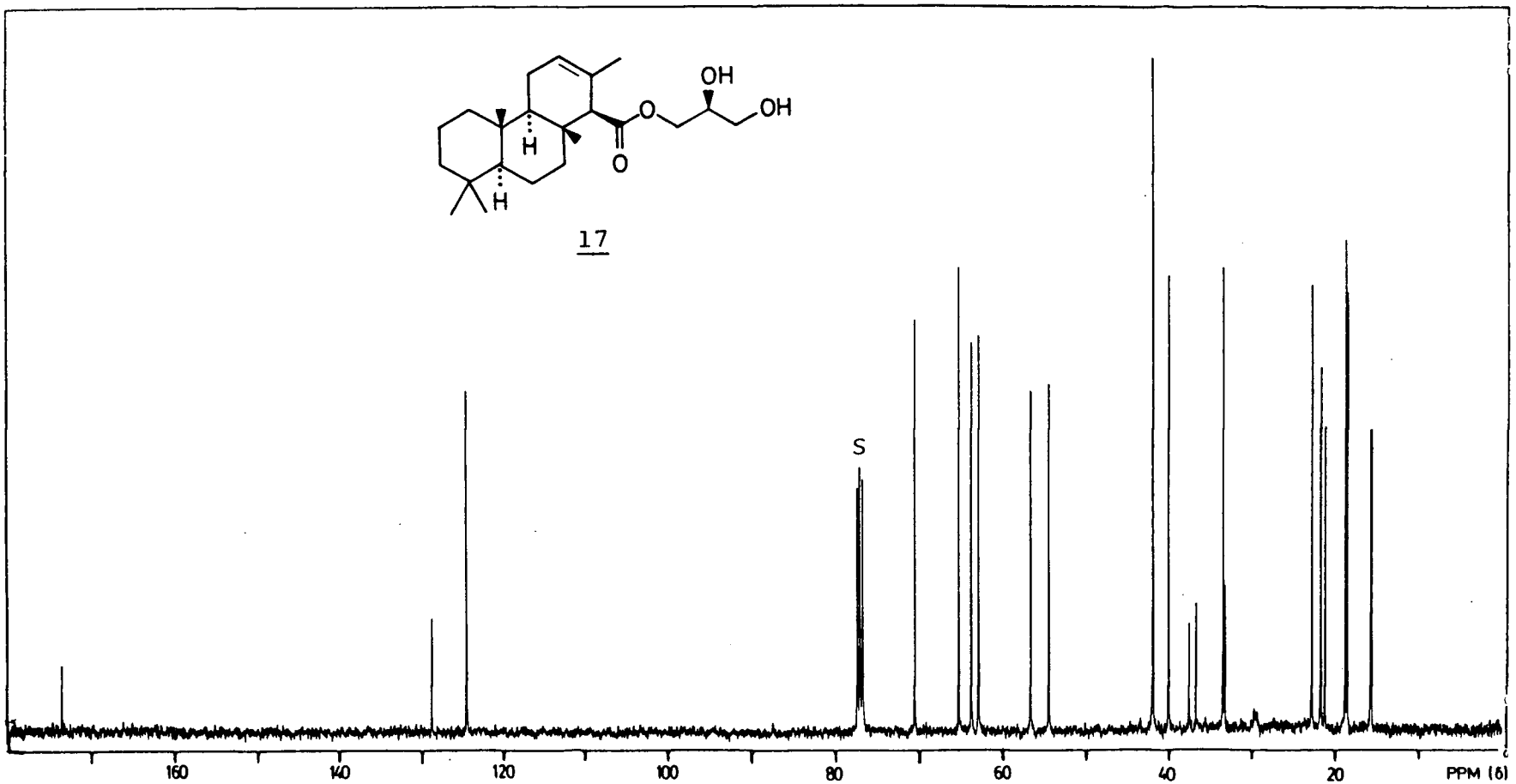


Figure 18. 100 MHz broad band decoupled ^{13}C NMR spectrum of 17 in $CDCl_3$.

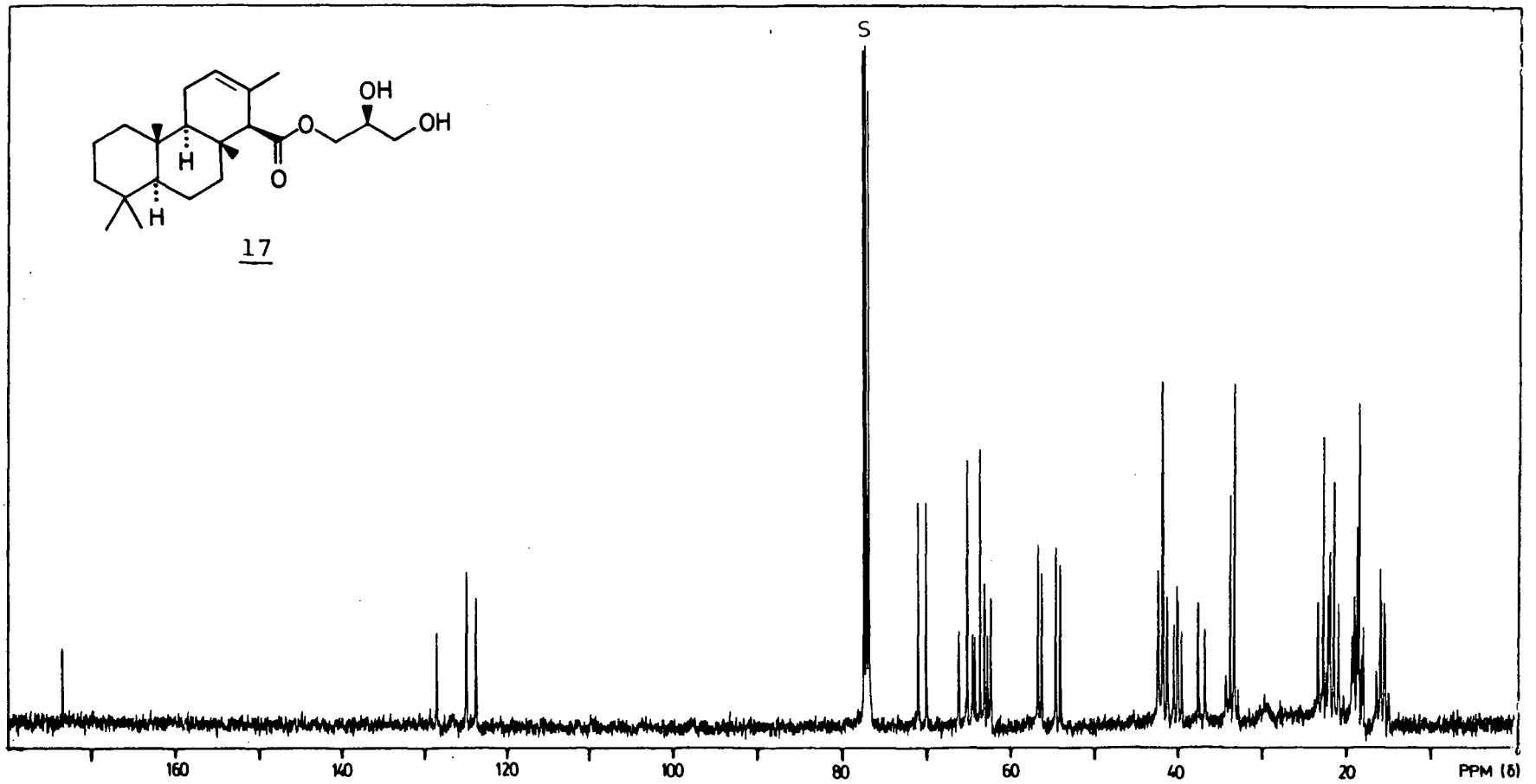


Figure 19. 100 MHz SFORD ^{13}C NMR spectrum of 17 in $CDCl_3$.

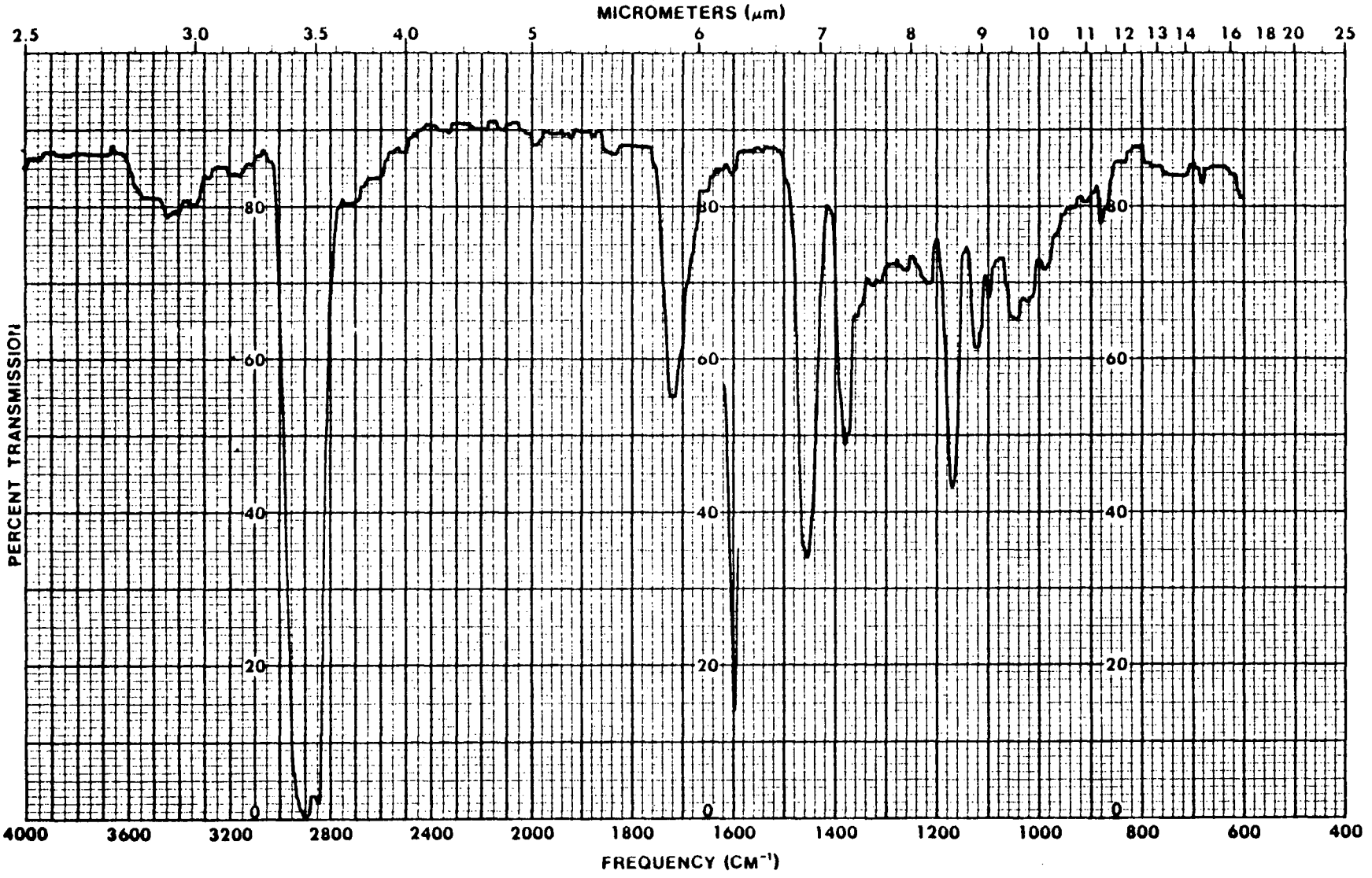


Figure 20. IR spectrum of 17 in $CHCl_3$.

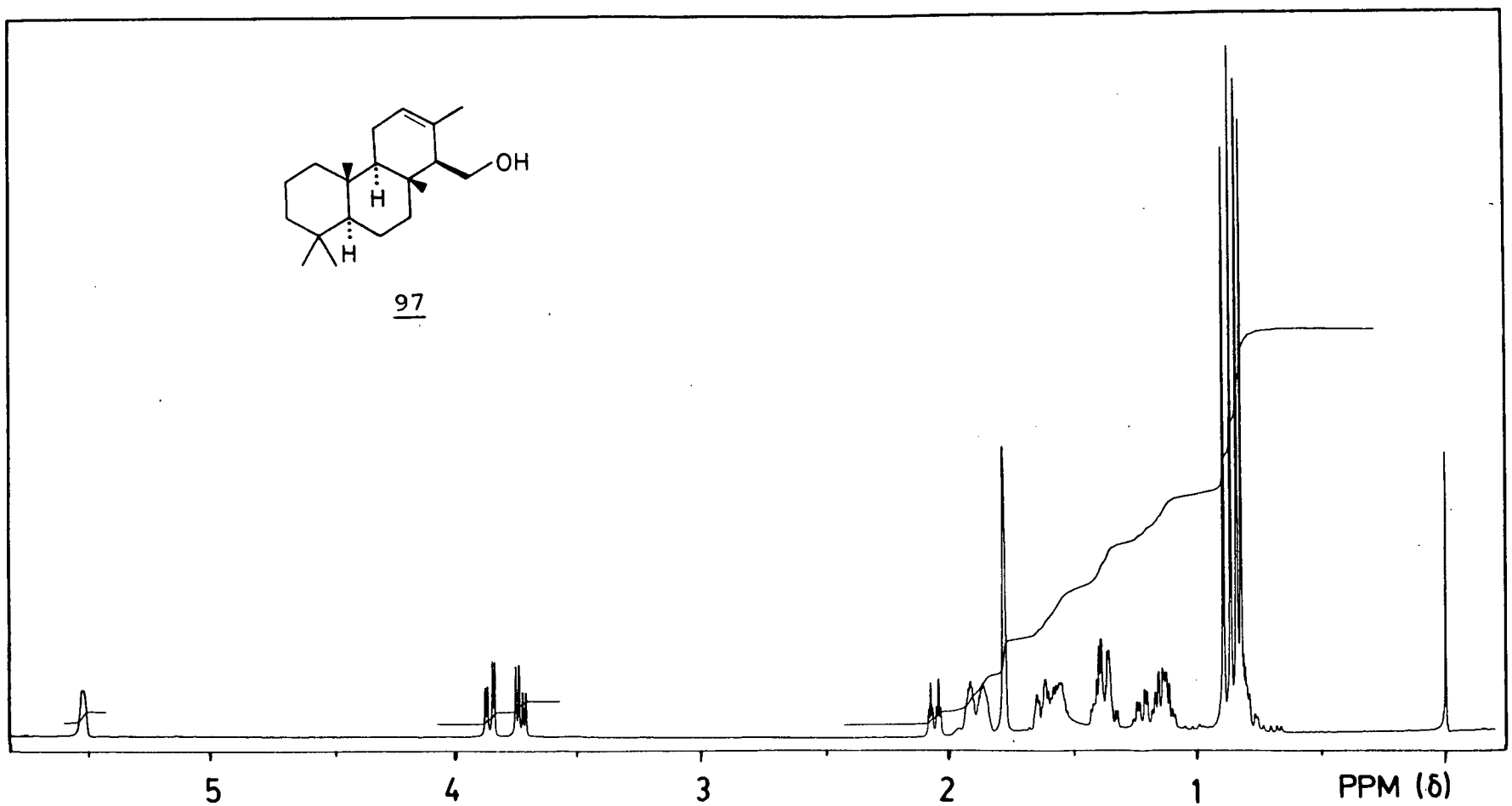


Figure 21. 400 MHz ^{1}H NMR spectrum of 97 in $CDCl_3$.

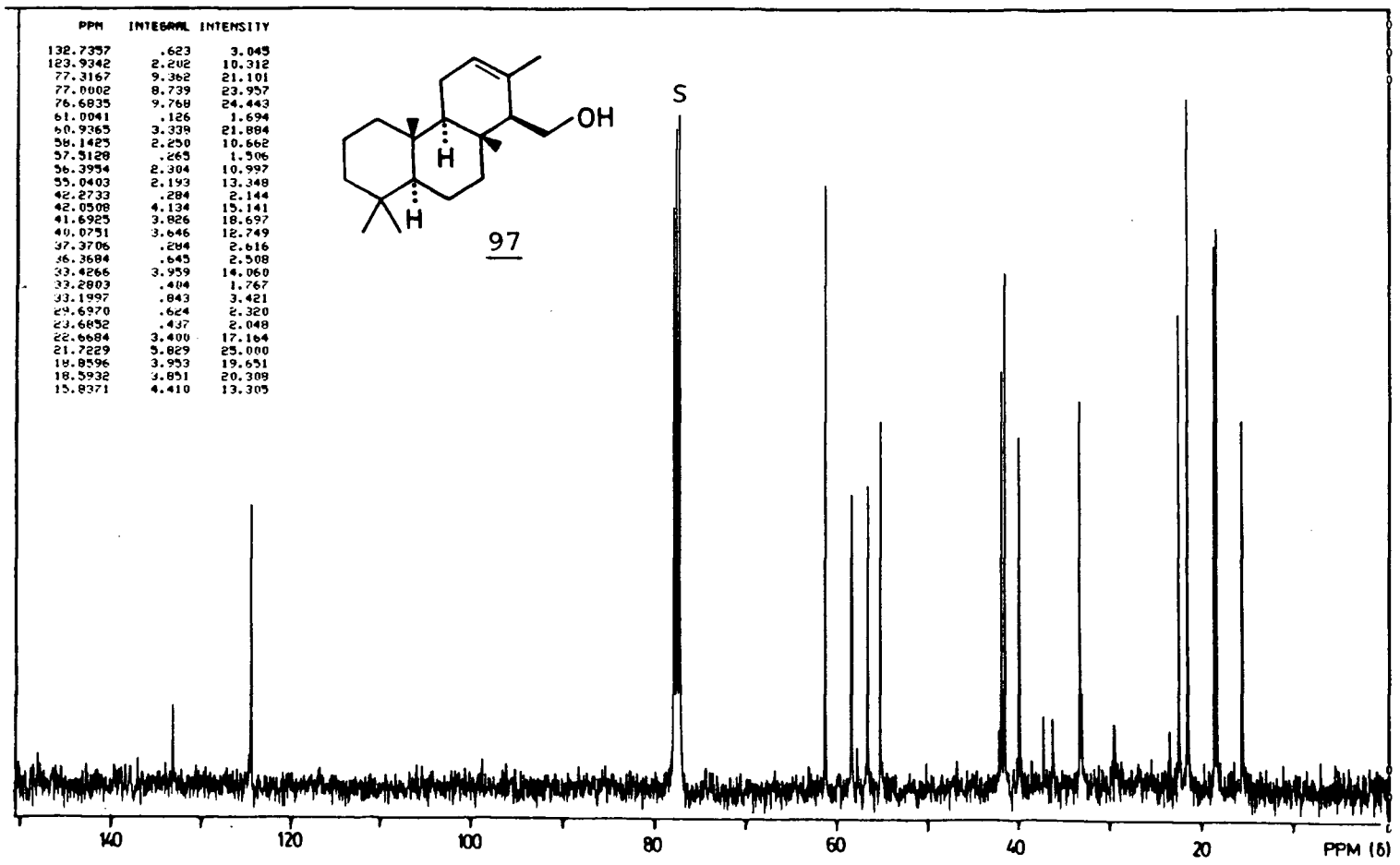


Figure 22. 100 MHz broad band decoupled ^{13}C NMR spectrum of 97 in $CDCl_3$.

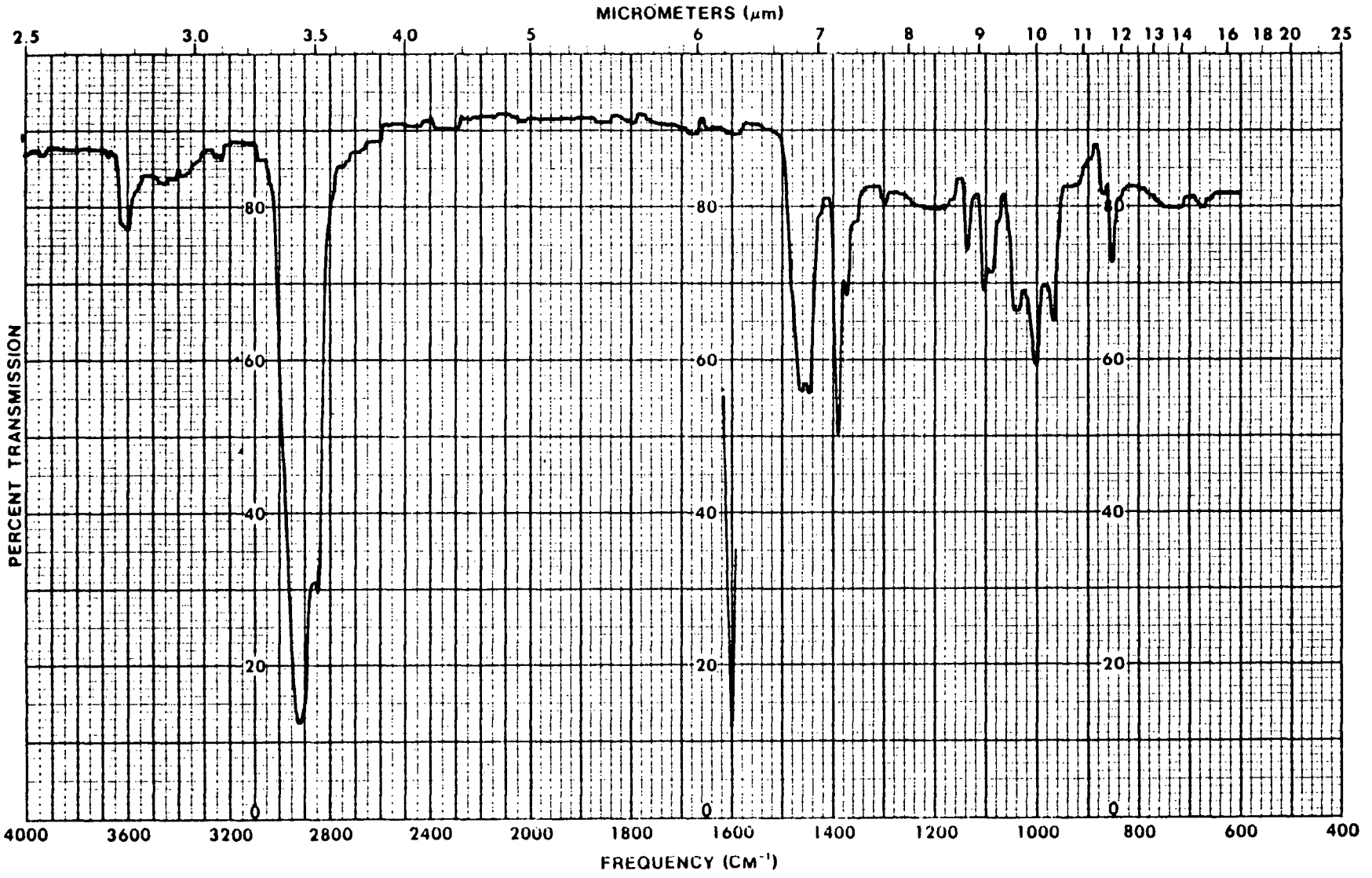
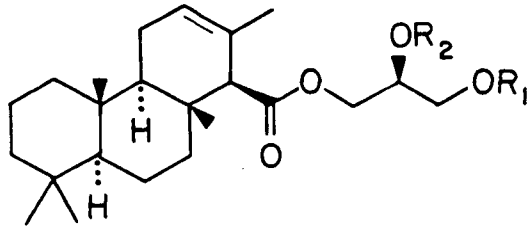


Figure 23. IR spectrum of 97 in $CHCl_3$.

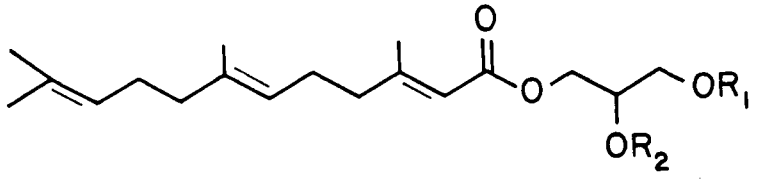
Material eluted with hexane-ethyl acetate (1:1) in the initial fractionation of A. montereyensis extracts contained two minor metabolites related to 17. The molecular formula of 18, established by HRMS as $C_{25}H_{40}O_5$, and intense mass spectral peaks at m/z 286 ($M^+-C_5H_{10}O_4$) and 258 ($M^+-C_6H_{10}O_5$) suggested it differed from 17 by addition of acetate to the glycerol fragment. The 'H NMR spectrum of 18 contained resonances virtually identical to those of the diterpenoid portion of 17, in addition to a sharp methyl singlet at δ2.11 and a single exchangeable proton at 2.48 ppm. A deshielded five-proton multiplet at δ4.08-4.25 ppm, which corresponded well with the chemical shifts and coupling patterns previously reported for the glyceryl acetate protons in 25, established the 1-acyloxy-2-hydroxy-3-acetoxypropane residue in 18. Reduction of acetate 18 with DIBAL gave alcohol 97 which was identical in all respects to the reduction product of 17.



18 R_1 = Ac R_2 = H

19 R_1 = H R_2 = Ac

A second monoacetyl glyceride 19, appeared to be a positional isomer of 18 as it also had a molecular formula of $C_{25}H_{40}O_5$ and showed mass spectral losses of $M^+-C_5H_{10}O_4$ and $M^+-C_6H_{10}O_5$. The ^1H NMR spectrum contained resonances characteristic of the terpenoid portion of 17 and a methyl singlet at δ2.09 ppm. Resonances of the glyceryl acetate residue at δ3.76 (d, J = 6 Hz, 2H), 4.26 (dd, J = 7, 12 Hz, 1H), 4.32 (dd, J = 6, 14 Hz, 1H) and 5.06 (m, 1H) ppm were identical to those previously observed for 26 and confirmed the glycerol moiety of 19 was acetylated at the 2-position. Reduction of 19 with DIBAL also gave alcohol 97.



25 R_1 = Ac R_2 = H

26 R_1 = H R_2 = Ac

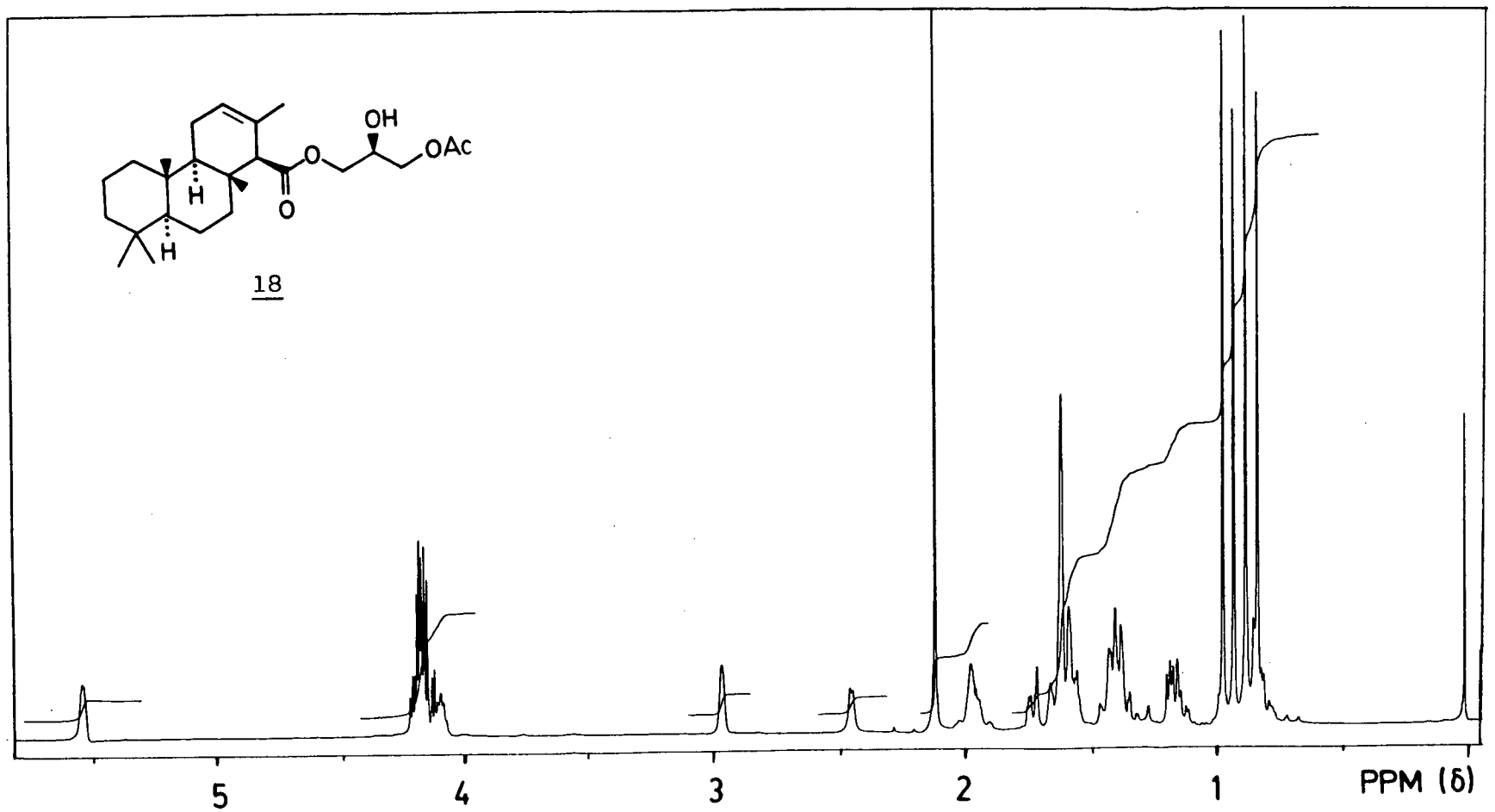


Figure 24. 400 MHz 'H NMR spectrum of 18 in $CDCl_3$.

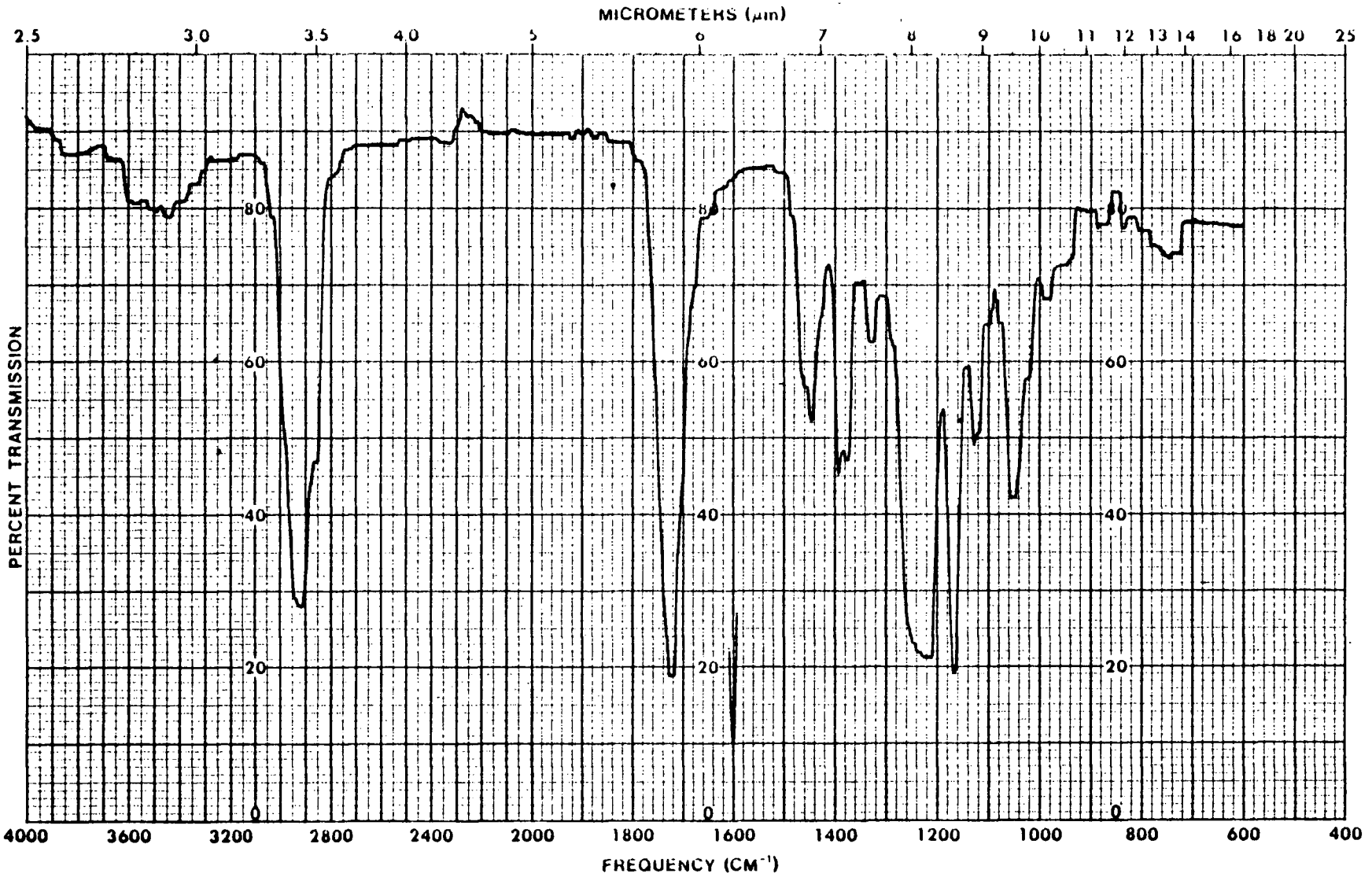


Figure 25. IR spectrum of 18 in $CHCl_3$.

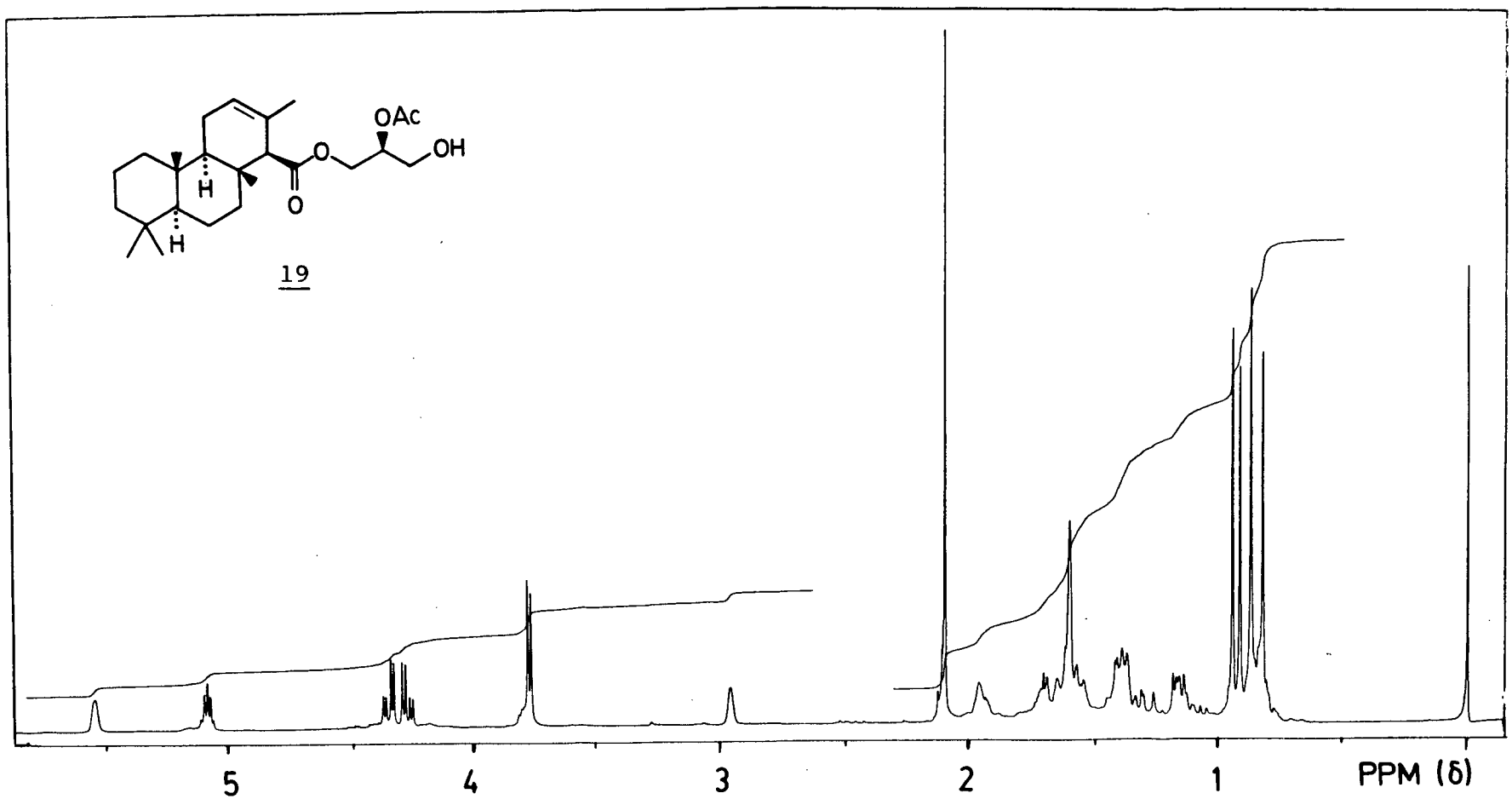


Figure 26. 400 MHz 'H NMR spectrum of 19 in $CDCl_3$.

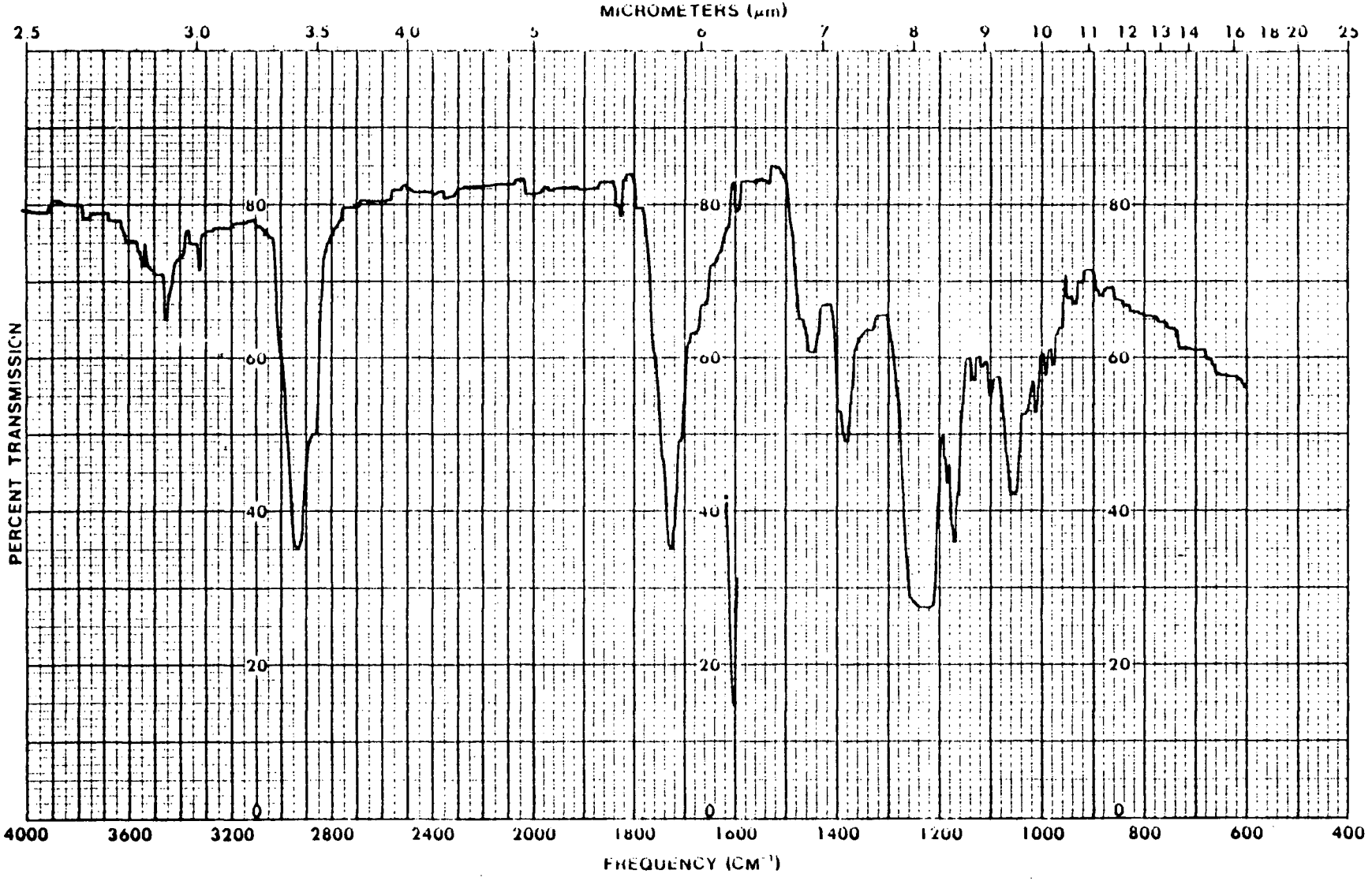
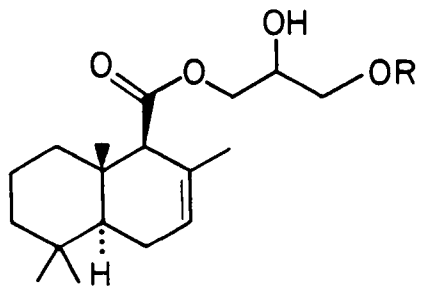


Figure 27. IR spectrum of 19 in $CHCl_3$.

A third minor metabolite was isolated during HPLC purification of glyceride 17. Compound 20 was an optically active solid ($[\alpha]_D$ = +23.1°) with a molecular formula of $C_{18}H_{30}O_4$. Its infrared spectrum showed hydroxyl (3600-3300 cm^{-1}) and ester carbonyl (1730 cm^{-1}) absorbance bands. Fragments in the mass spectrum at m/z 218 ($M^+-C_3H_8O_3$) and 190 ($M^+-C_4H_8O_4$) revealed that 20 was a glyceride ester.

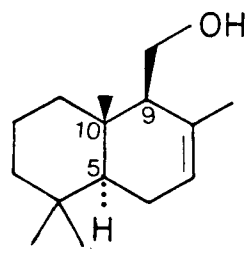


20 R = H

21 R = Ac

Resonances in the ^1H NMR (400 MHz, $CDCl_3$) spectrum at δ2.48 (2H, exchangeable), 3.63 (dd, J = 6, 12 Hz, 1H), 3.70 (dd, J = 4, 12 Hz, 1H), 3.95 (m, 1H), 4.15 (dd, J = 7, 12 Hz, 1H) and 4.22 (dd, J = 5, 12 Hz, 1H) ppm, established that the glycerol fragment was acylated on a primary alcohol. Methyl singlets at δ0.89, 0.92 and 0.98, a vinyl methyl at 1.62, and a single olefinic proton at 5.57 ppm suggested that the C_{15} acyl portion of the ester was a

bicyclic sesquiterpenoic acid. A broadened one-proton singlet deshielded to δ2.96 ppm was characteristic of an allylic methine α to an ester carbonyl. A NOE difference experiment showed that this proton participates significantly in the relaxation of two alkyl protons (see Figure 32). The observed NOE indicated a 1-3 diaxial orientation between the methine proton and two other protons in the bicyclic ring system. The drimane derivative 20 was an obvious structural proposal that satisfied all of the spectral data. Further support for this hypothesis could be found in prominent mass spectral fragments at m/z 124, retro Diels-Alder cleavage of the B ring, and 109, loss of an additional methyl. To confirm the structure, 20 was reduced with DIBAL to the known sesquiterpene drimenol 98. The spectral data (NMR, HRMS) and GC retention time of alcohol 98 were identical in all respects to those obtained from authentic (±)-drimenol (kindly supplied by R. Armstrong)[70]. Comparison of the optical rotation ($[\alpha]_D$ = -20°) of 98 with the literature value of drimenol ($[\alpha]_D$ = -20°)[71] proved that the absolute configuration of the sesquiterpenoid portion of 20 is as shown (5S, 9S, 10S).



98

A related monoacetate 21, was also recovered from A. montereyensis extracts. The molecular formula $C_{20}H_{32}O_5$, diagnostic mass spectral fragments at $M^+-C_5H_{10}O_4$ and $M^+-C_6H_{10}O_5$ and a resonance at δ2.11 (s, 3H) ppm in the ^1H NMR spectrum revealed it to be a monoacetylated glyceryl ester. A five-proton multiplet at δ4.05-4.24 ppm demonstrated that the glycerol fragment was 1,3-diacylated. Resonances characteristic of the sesquiterpenoid portion of 20 were also observed in 21. Reduction of 21 with DIBAL gave drimenol 98.

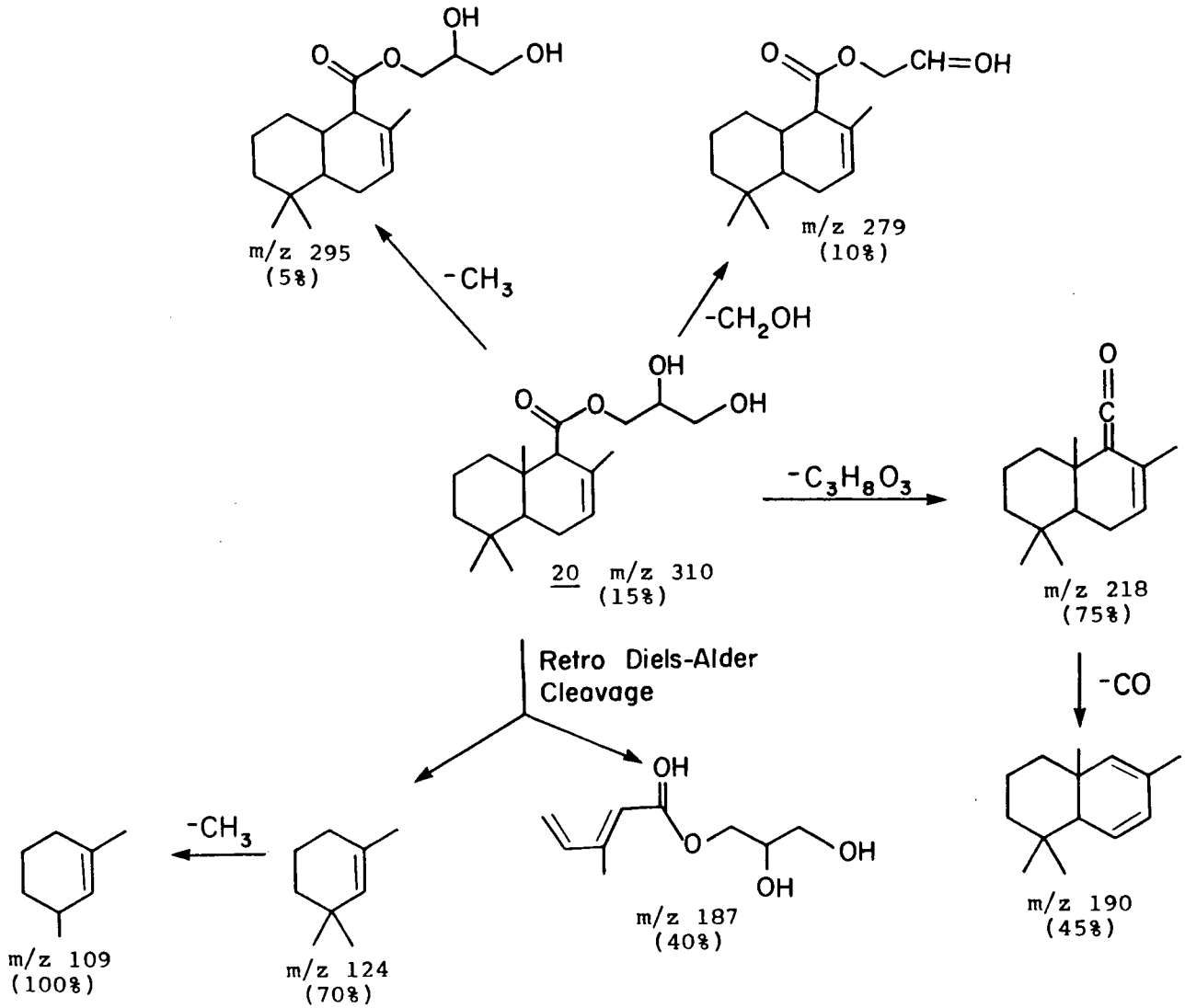


Figure 28. Interpretation of the HRMS of 20.

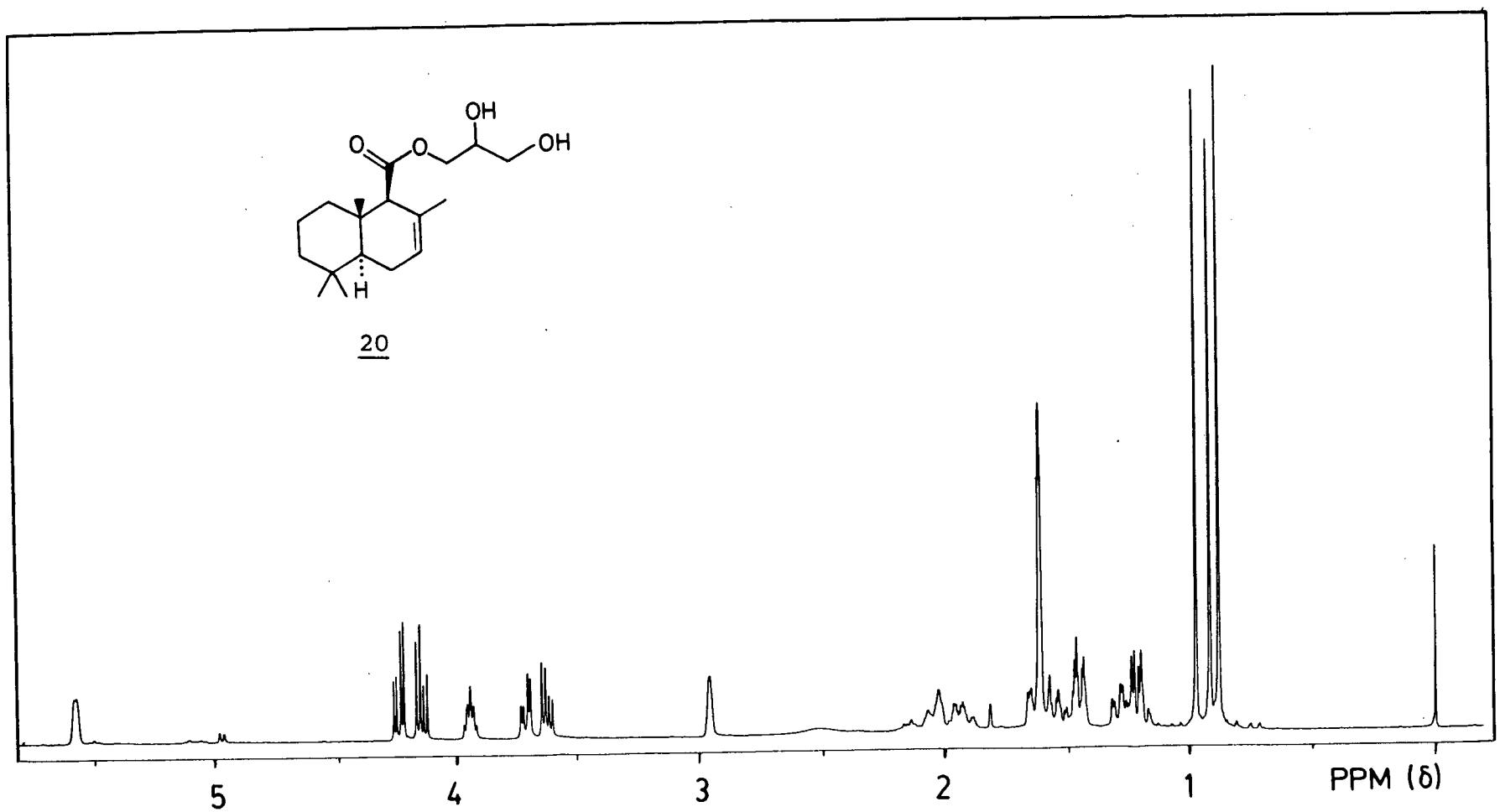


Figure 29. 400 MHz 'H NMR spectrum of 20 in $CDCl_3$.

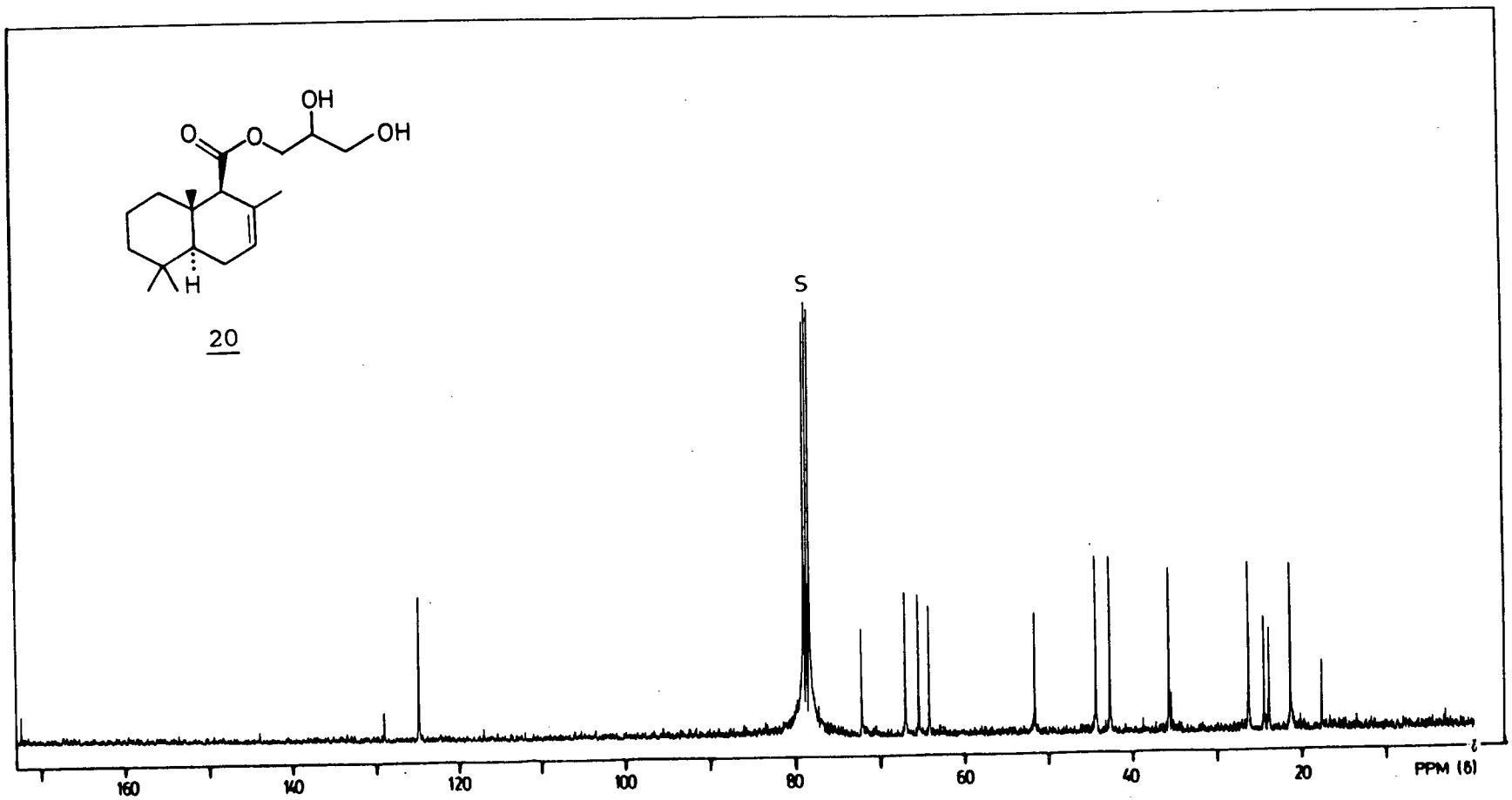


Figure 30. 100 MHz broad band decoupled ^{13}C NMR spectrum of 20 in $CDCl_3$.

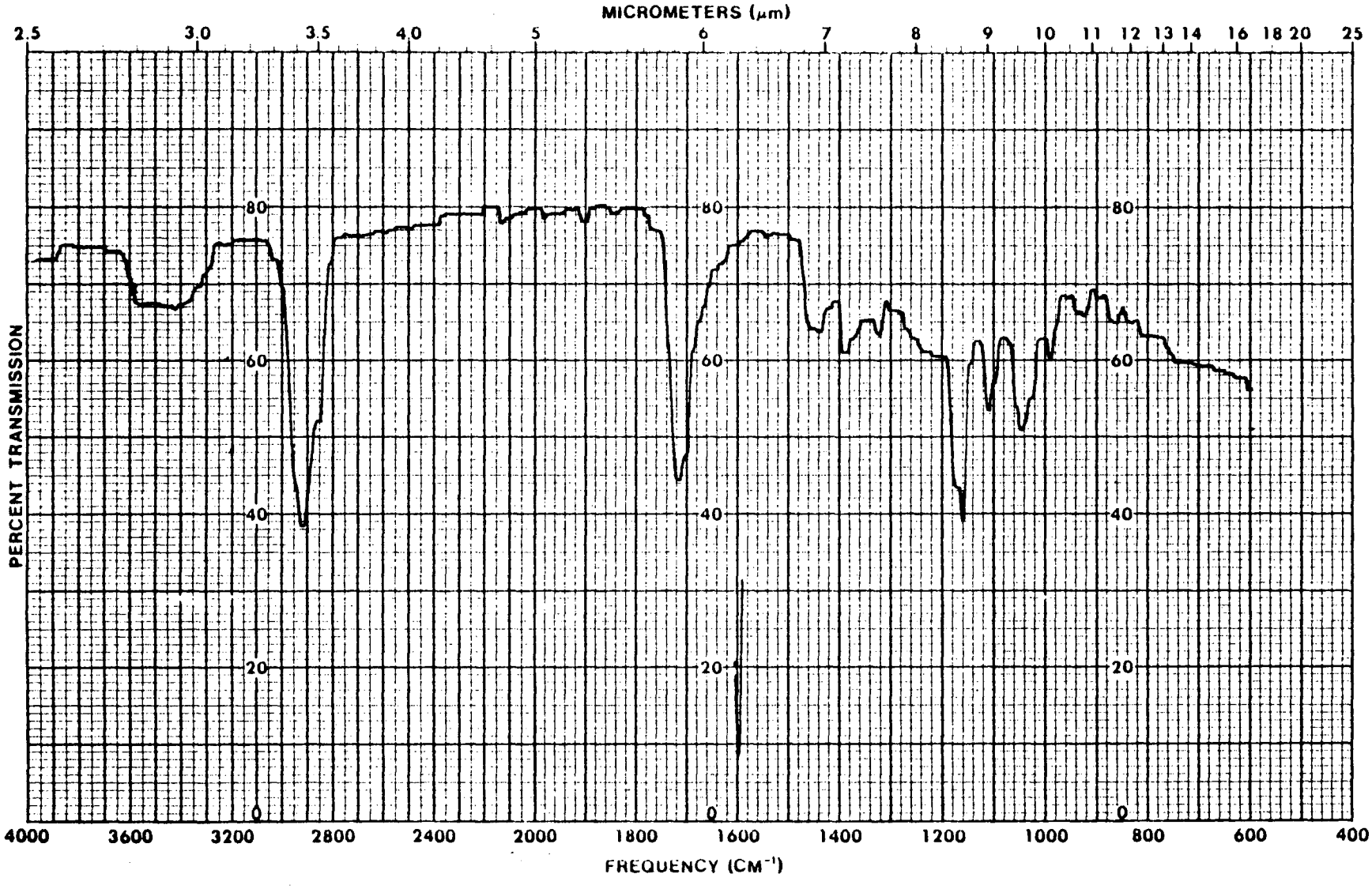


Figure 31. IR spectrum of 20 in $CHCl_3$.

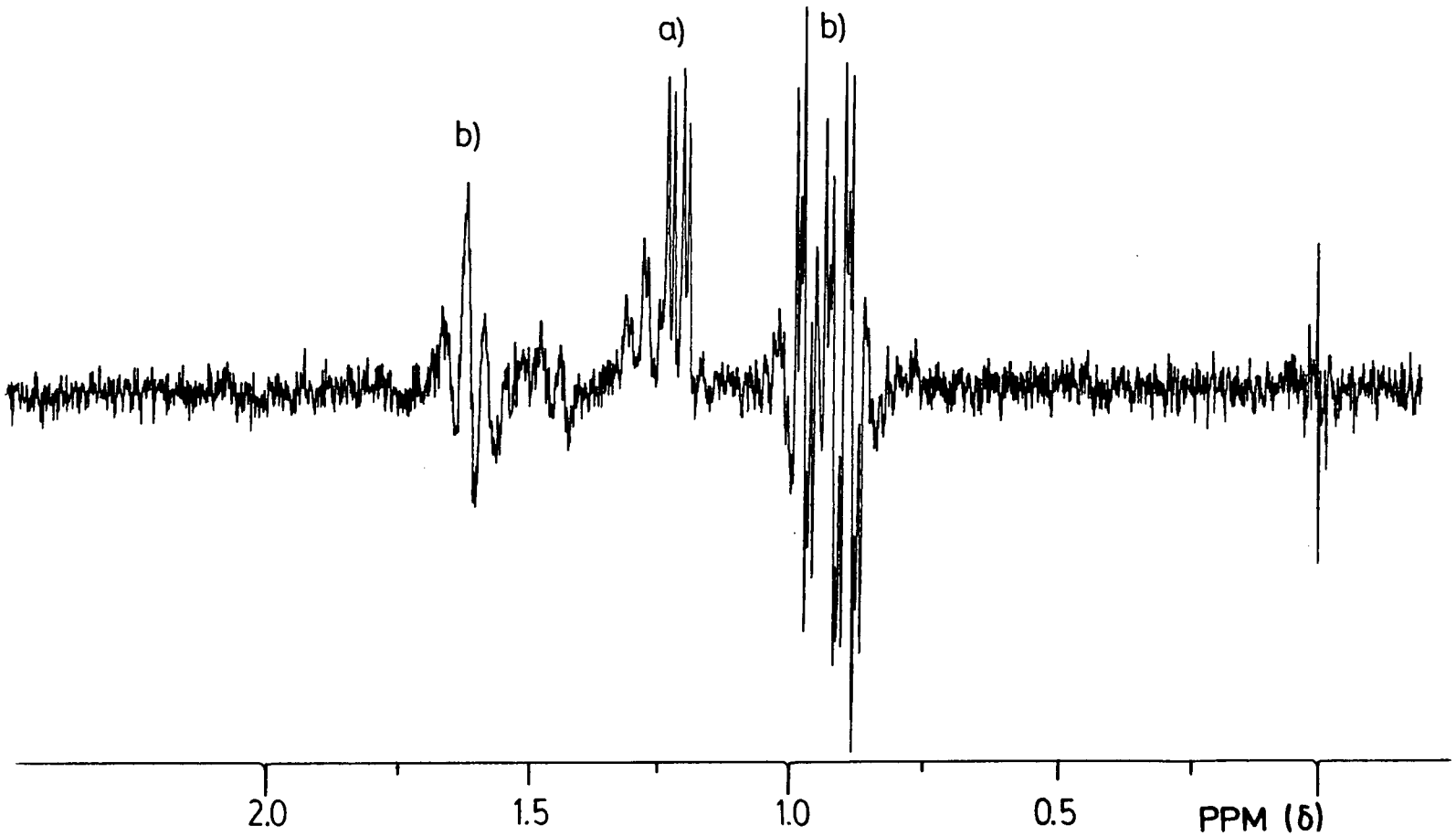


Figure 32. 400 MHz 'H NMR difference NOE spectrum of 20 when the allylic methine proton at δ2.96 ppm was irradiated: a) enhanced signals of two alkyl protons; b) incompletely subtracted methyl signals.

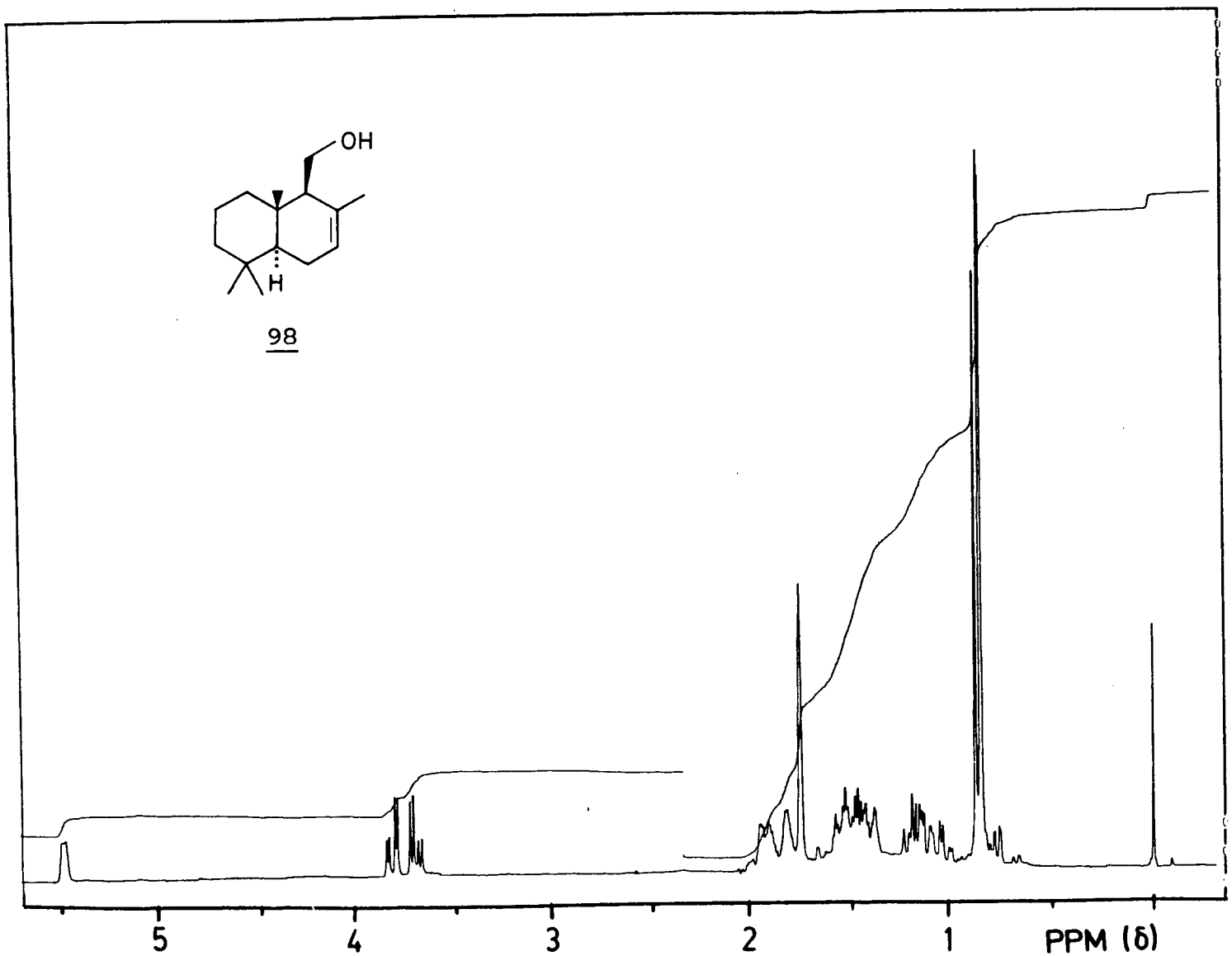


Figure 33. 270 MHz 'H NMR spectrum of 98 in $CDCl_3$.

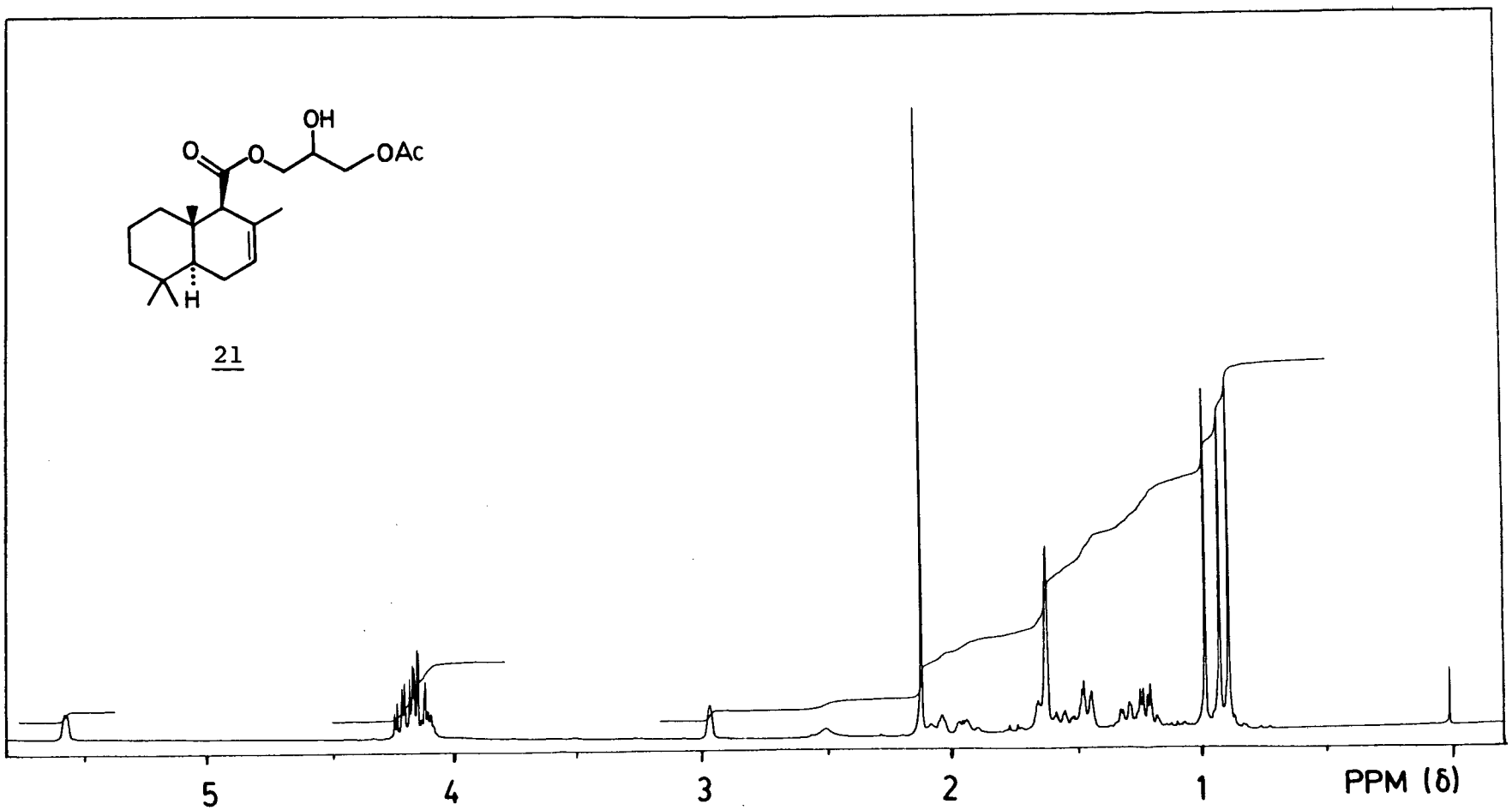


Figure 34. 400 MHz 'H NMR spectrum of 21 in $CDCl_3$.

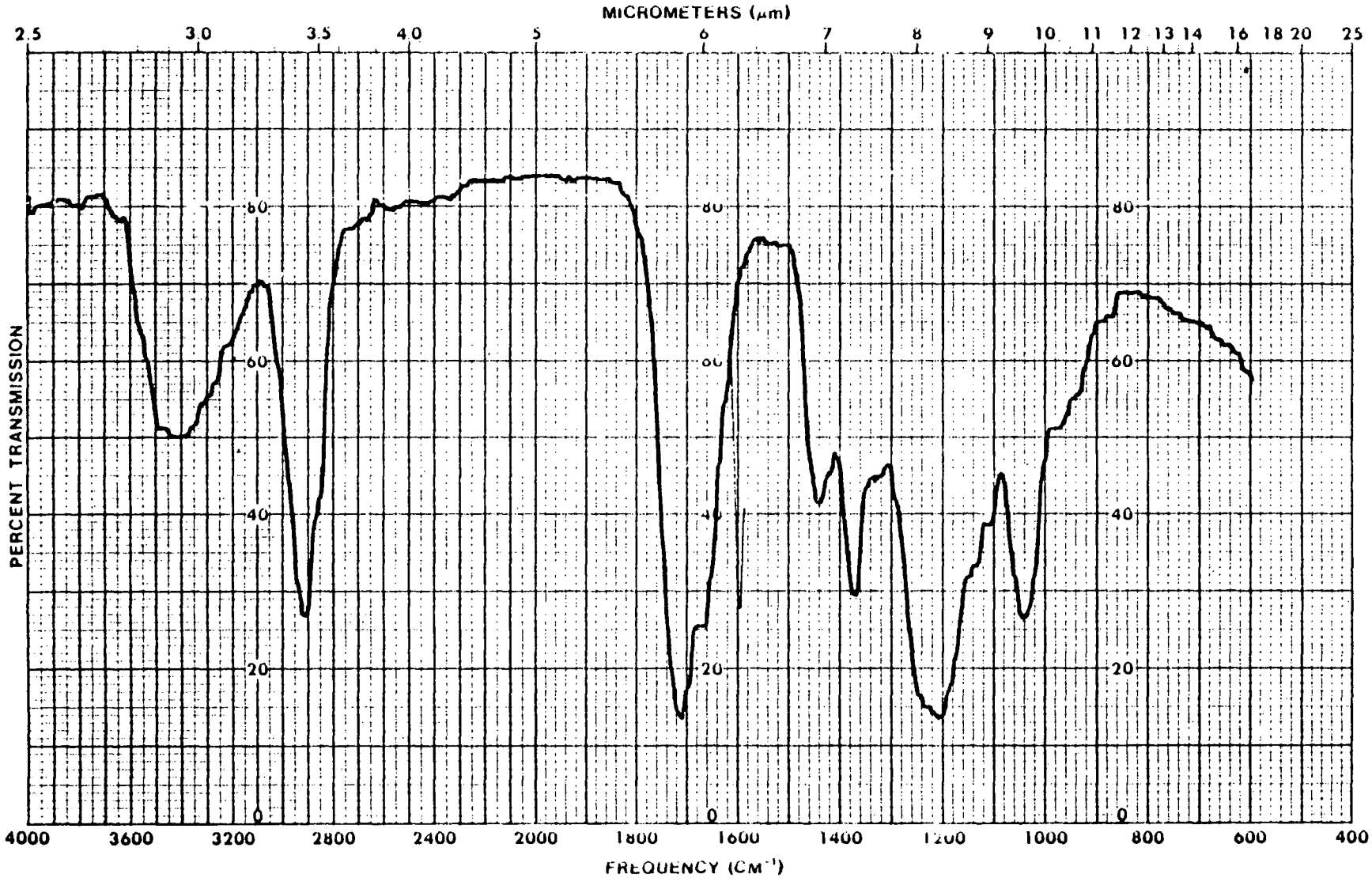
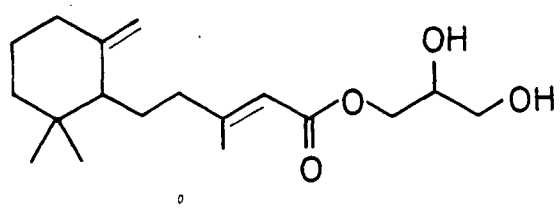


Figure 35. IR spectrum of 21 in $CHCl_3$.

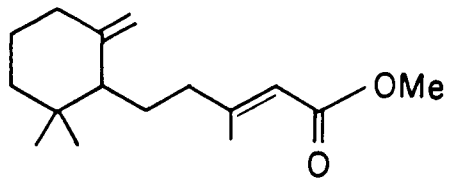
Another trace metabolite could only be isolated in low yield after extensive HPLC purification. It was an optically active oil ($[\alpha]_D$ = +9.7°) with a molecular formula of $C_{18}H_{30}O_4$.



22

Compound 22 showed infrared absorbances (3425, 1700 cm^{-1}), a mass spectral fragment ion ($M^+-C_3H_8O_3$) and ^1H NMR resonances [δ3.62 (dd, J = 7, 12 Hz, 1H), 3.70 (dd, J = 5, 12 Hz, 1H), 3.95 (m, 1H), 4.18 (dd, J = 7, 12 Hz, 1H), 4.24 (dd, J = 5, 12 Hz, 1H) ppm] diagnostic of a 1-acyloxy-2,3-dihydroxypropane residue. The presence of an αβ unsaturated ester moiety could be readily deduced from the UV absorbance (λ_{max} = 228 nm, MeOH), IR spectrum (1700 cm^{-1}) and resonances in the ^1H NMR for a deshielded vinyl methyl (δ2.16 ppm) and an olefinic proton (δ5.70 ppm). Methyl singlets were observed at δ0.85 and 0.93 and an exocyclic methylene was evidenced by singlets at 4.55 and 4.79 ppm. The αβ unsaturated ester and exocyclic olefin accounted for three of the four total units of unsaturation in 22, indicating the C_{15} acyl portion was a monocyclic

sesquiterpenoid. Resonances of the acyl residue were identical to those reported for the methyl ester of trans-γ-monocyclofarnesic acid (99)[72], thus the structure of the minor metabolite 22 was assigned as shown. Its mass spectral fragmentation pattern, which included prominent peaks at m/z 176 ($C_{13}H_{20}$) and 109 (C_8H_{13}), was consistent with this proposal (see Figure 36).



99

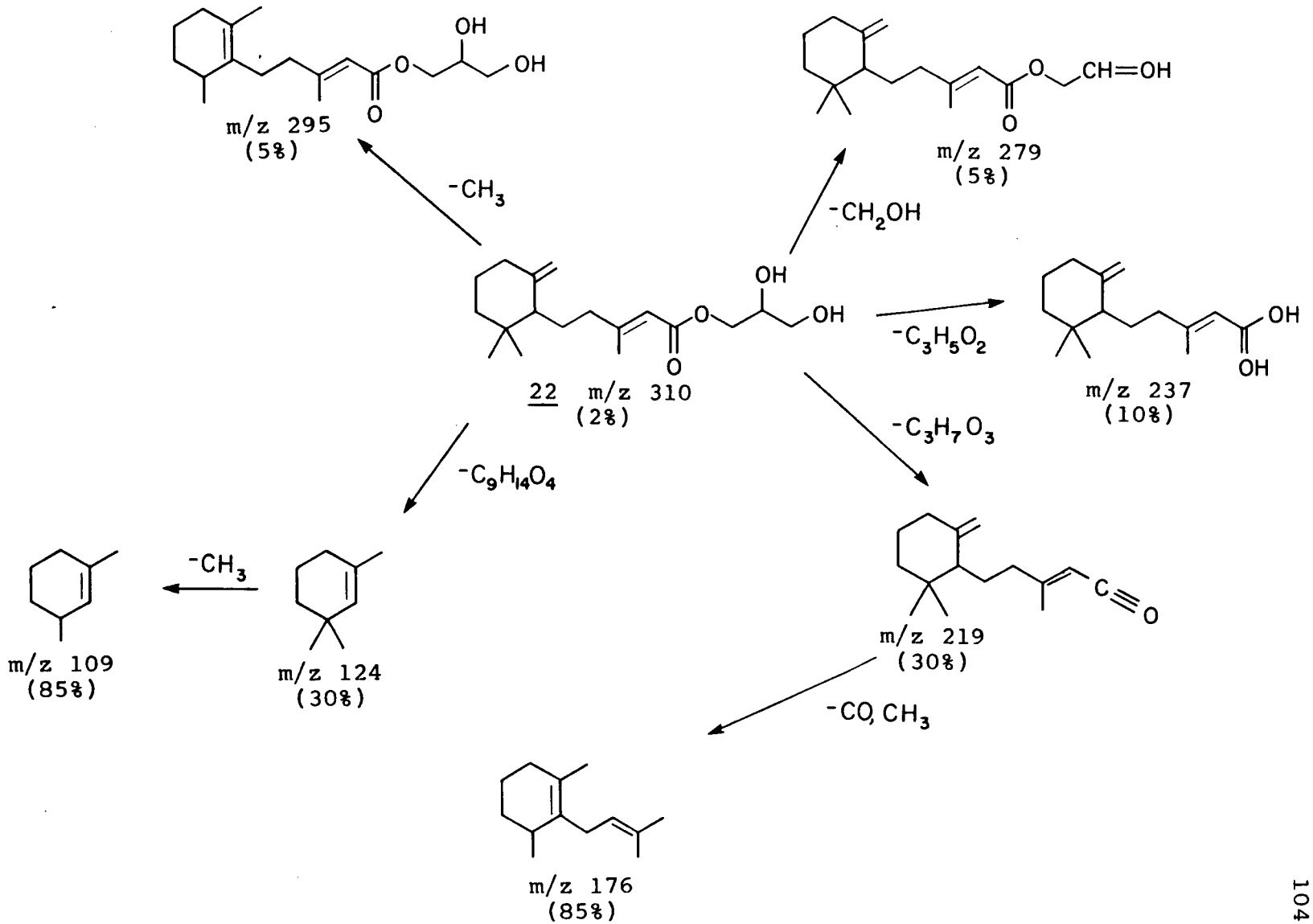


Figure 36. Interpretation of the HRMS of 22.

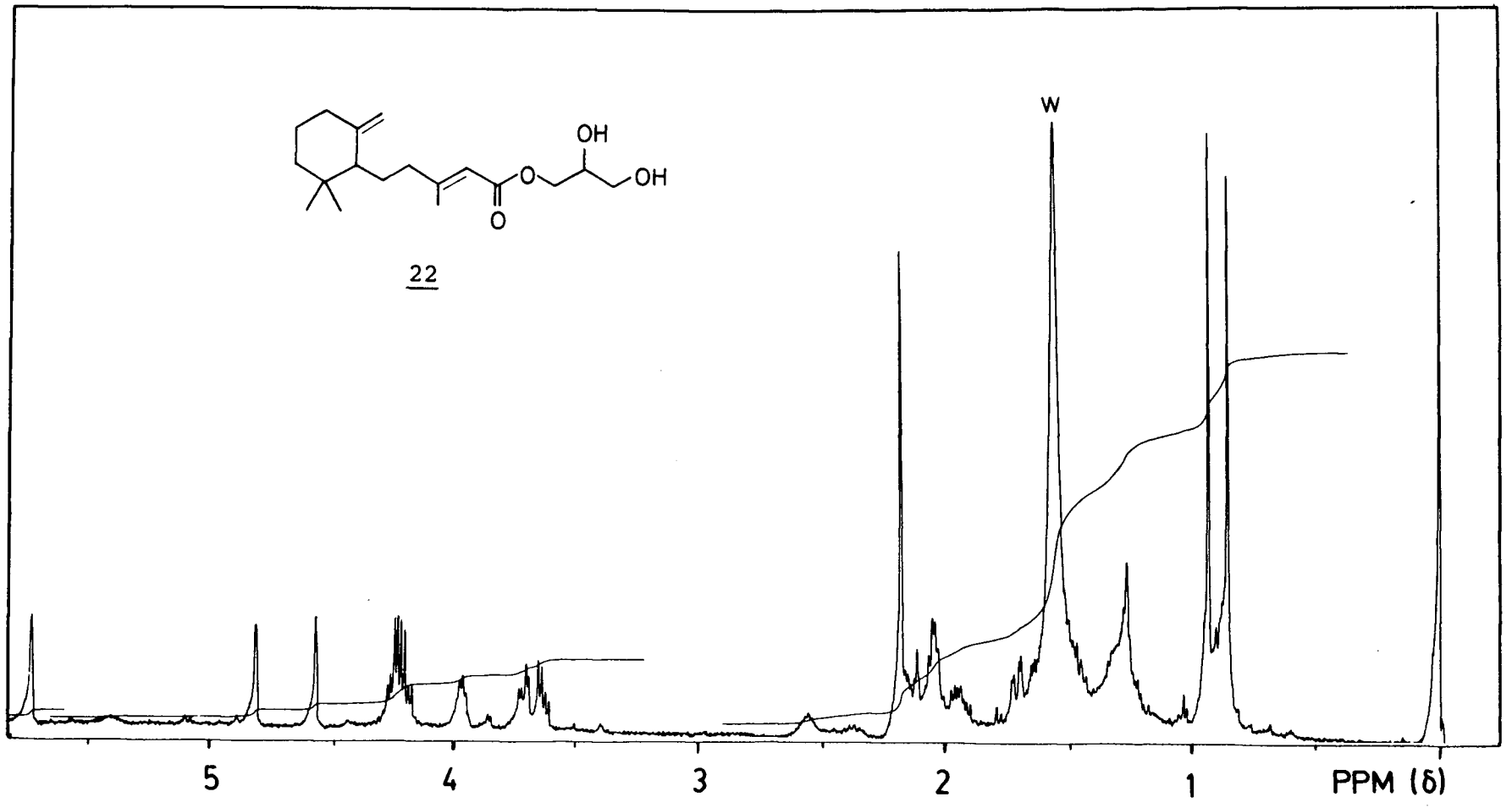


Figure 37. 400 MHz 'H NMR spectrum of 22 in $CDCl_3$.

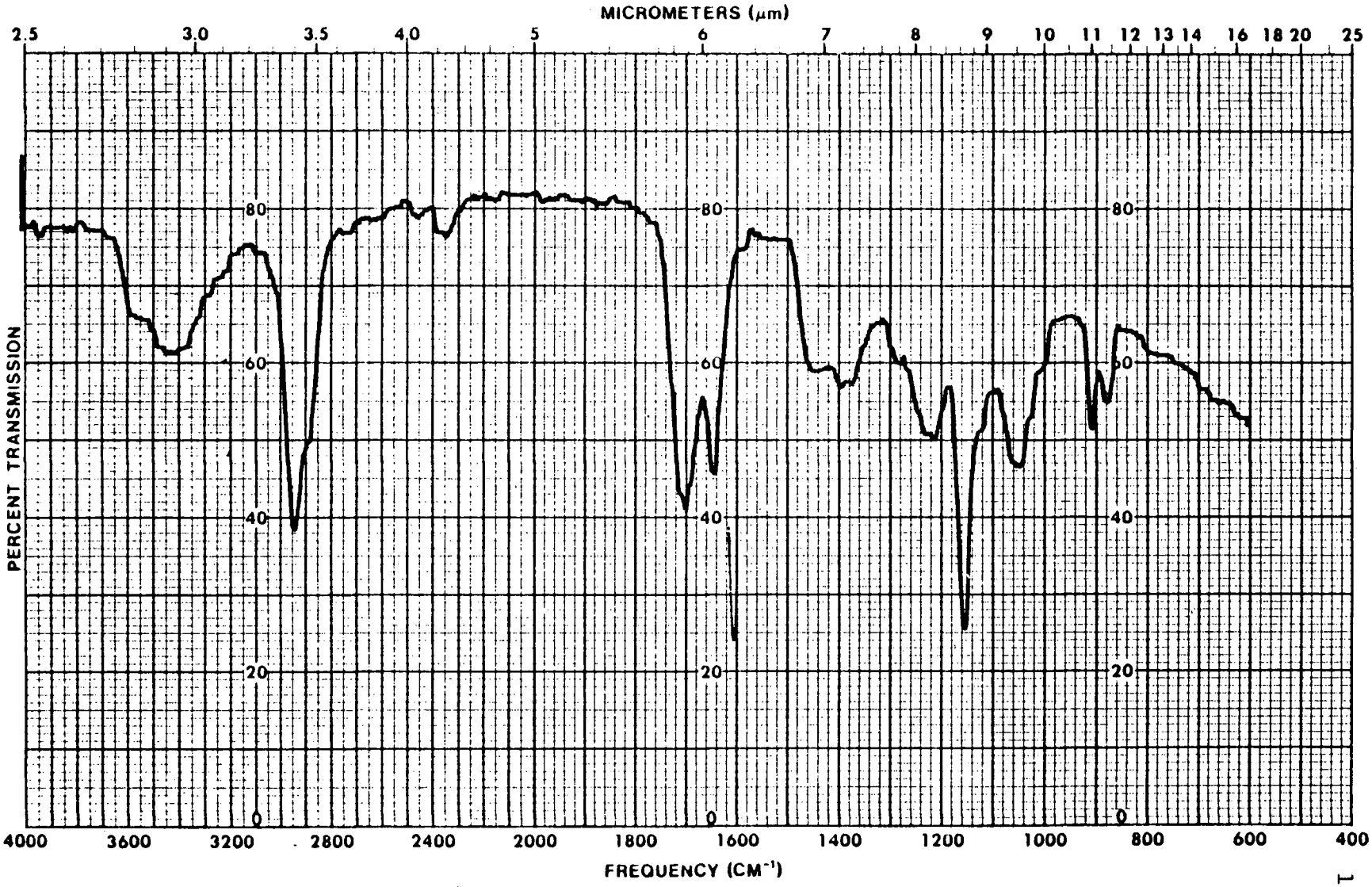


Figure 38. IR spectrum of 22 in $CHCl_3$.

A very minor constituent was found in only a single collection of A. montereyensis. It had a molecular formula of $C_{20}H_{32}O_2$ indicating a total of 5 units of unsaturation. Infrared absorbances at 3225-2500 and 1695 cm^{-1} revealed the presence of an acid functionality. Resonances in the 'H NMR spectrum of an olefinic proton δ5.70 (bs, 1H) and a vinyl methyl 2.20 (bs, 3H) ppm were appropriate for an αβ unsaturated acid with the methyl group attached to the β carbon. A second trisubstituted olefin was evidenced by a one-proton multiplet at δ5.07 and a vinyl methyl singlet at 1.60 ppm. Two aliphatic methyl singlets at δ0.83 and 0.91 and resonances of an exocyclic methylene at 4.53 (bs, 1H) and 4.76 (bs, 1H) ppm suggested the metabolite was a monocyclic diterpenoic acid.

An obvious structural proposal that is reasonable on biosynthetic grounds and consistent with the NMR data is compound 100. The mass spectrum showed intense signals at m/z 289 (M^+-CH_3), 124 (C_9H_{16}), 109 (C_8H_{13}) and a base peak at 81 (C_6H_9) which support this hypothesis (Figure 39). However, a prominent peak observed at m/z 177 ($C_{13}H_{21}$) is difficult to rationalize from fragmentation of 100. While the isomeric compound 101 could be expected to produce the m/z 177 fragment ion (Figure 40), this structure is not entirely consistent with the mass spectrum either. A doubly allylic cleavage of the bond between C-9 and C-10 of 101 should result in a strong signal at m/z 123 (C_9H_{15}). While

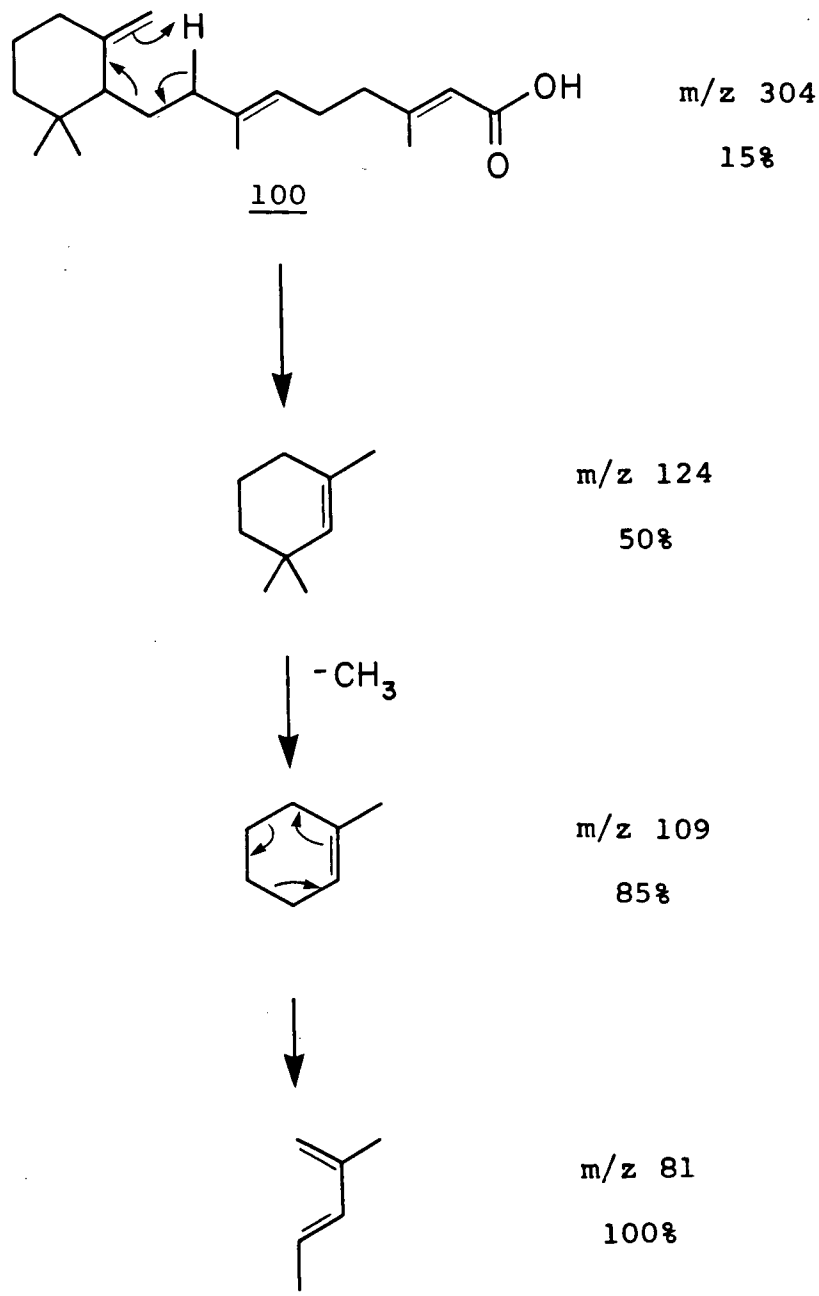


Figure 39. Possible mass spectral fragmentation of 100.

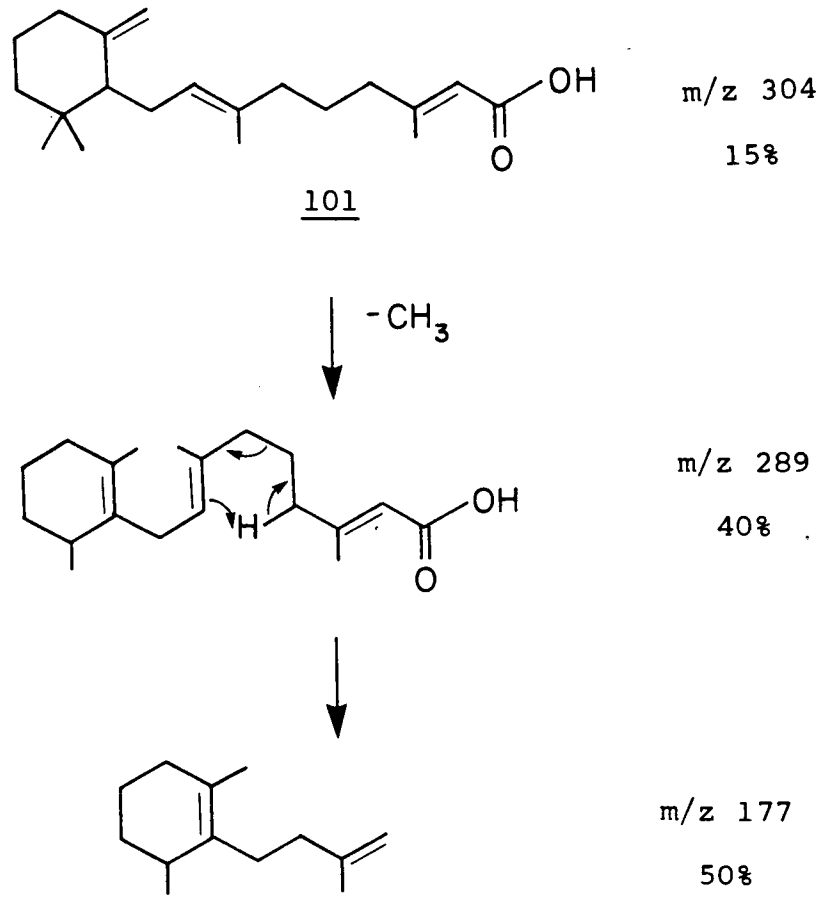


Figure 40. Possible mass spectral fragmentation of 101 to produce a peak at m/z 177.

a peak at m/z 123 is observed, the peak at 124 is considerably more intense. An allylic McLafferty rearrangement in compound 100 could readily provide the m/z 124 ion, but an analogous proton transfer and cleavage in compound 101 seems less plausible.

It is possible that a mixture of the two olefinic isomers was isolated, and the mass spectrum shows fragment ions from both 100 and 101. Resonances in the ^{1}H NMR are generally quite sharp, but the asymmetric nature of the olefin proton at δ5.07 ppm may support this argument. Definative proof of the structure(s) was prevented by a low yield of the natural product and its absence from extracts of all but one collection of A. montereyensis.

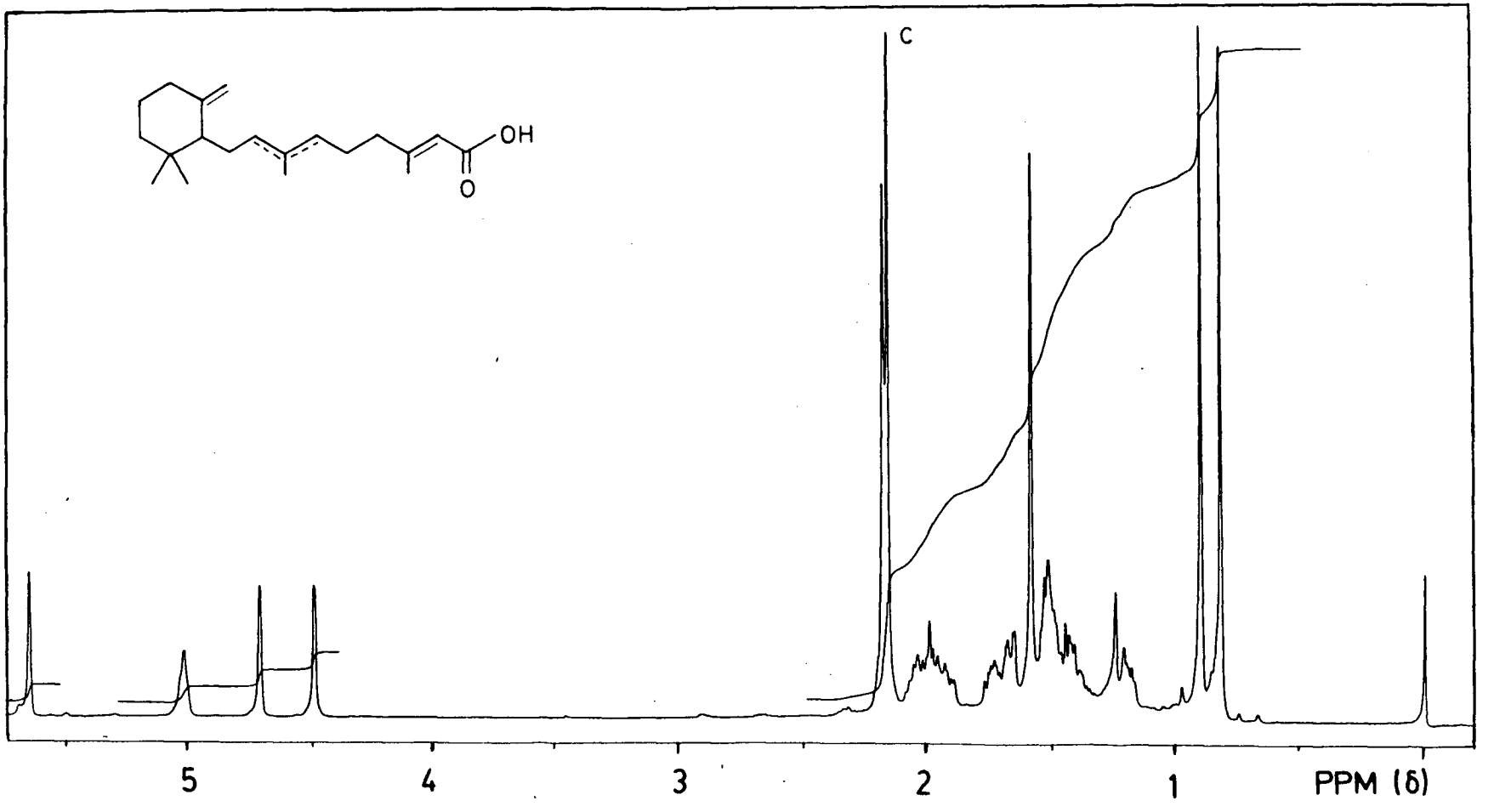


Figure 41. 400 MHz 'H NMR spectrum of the diterpenoic acid(s) tentatively identified as 100 and/or 101. Peak C is an unidentified contaminant.

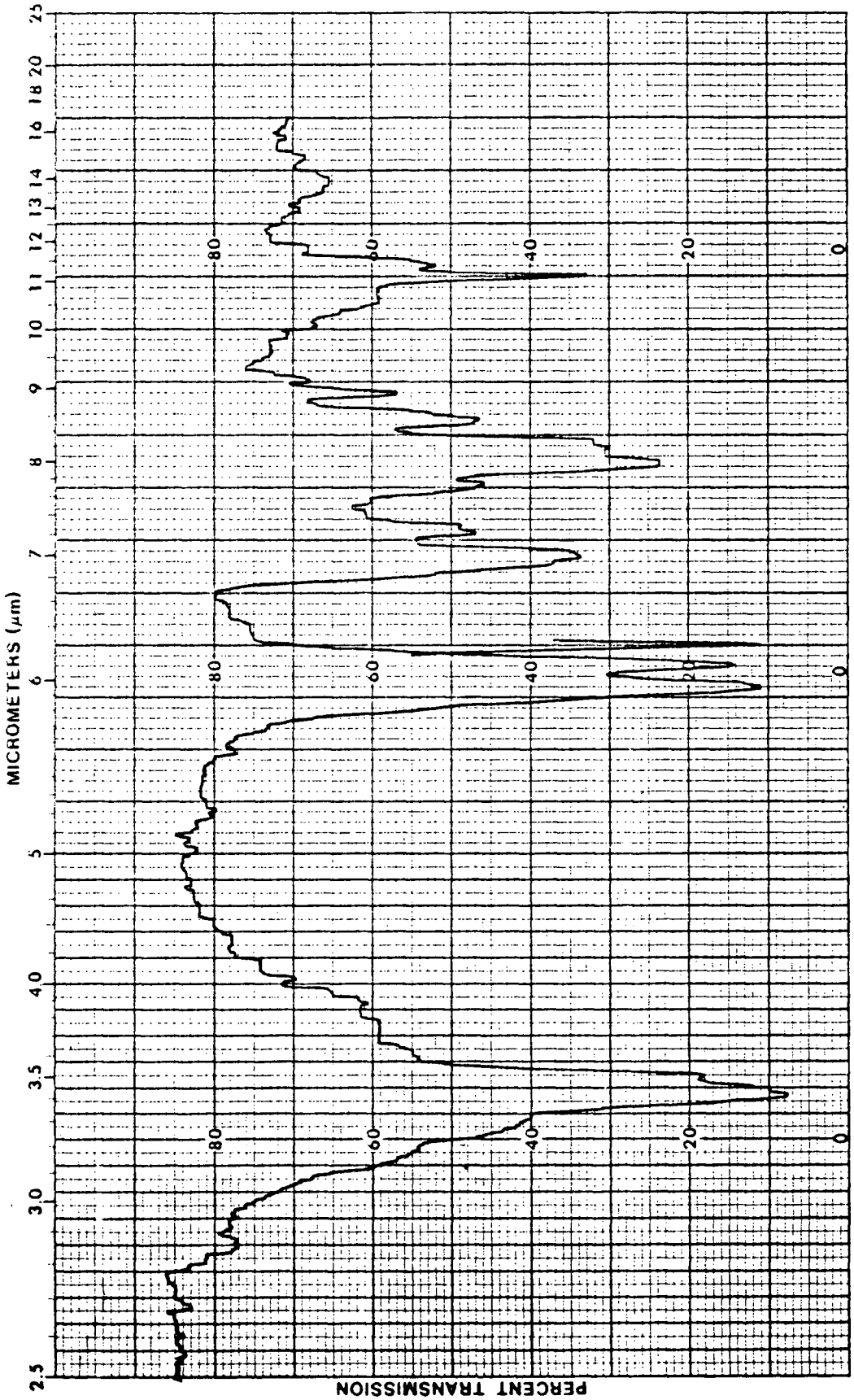
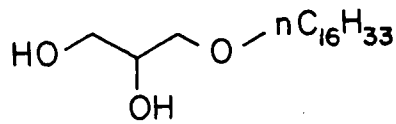


Figure 42. IR spectrum of 100 and/or 101 in $CHCl_3$.

The final trace constituent isolated from A. montereyensis had a molecular formula $C_{19}H_{40}O_3$ and mass spectral fragment ions, m/z 255 ($M^+-C_2H_5O_2$) and 225 ($M^+-C_3H_7O_3$) suggestive of a glyceryl ether moiety. The ^1H NMR resonances at δ3.41-3.53 (m, 4H), 3.62 (dd, J = 6, 12 Hz, 1H), 3.68 (dd, J = 5, 12 Hz, 1H) and 3.82 (m, 1H) ppm were in good agreement with reported literature values for glycerol ethers[73]. A C_{16} reduced fatty acid alkyl residue was clearly discernable in the ^1H NMR spectrum establishing the metabolite as the known compound 1-O-hexadecyl glycerol (23)[74]. Ether 23 shows potent in-vitro antibiotic activity against Staphylcoccus aureus and Bacillus subtilis, and it alone appears to be responsible for the antibacterial activity observed for crude A. montereyensis extracts.



23

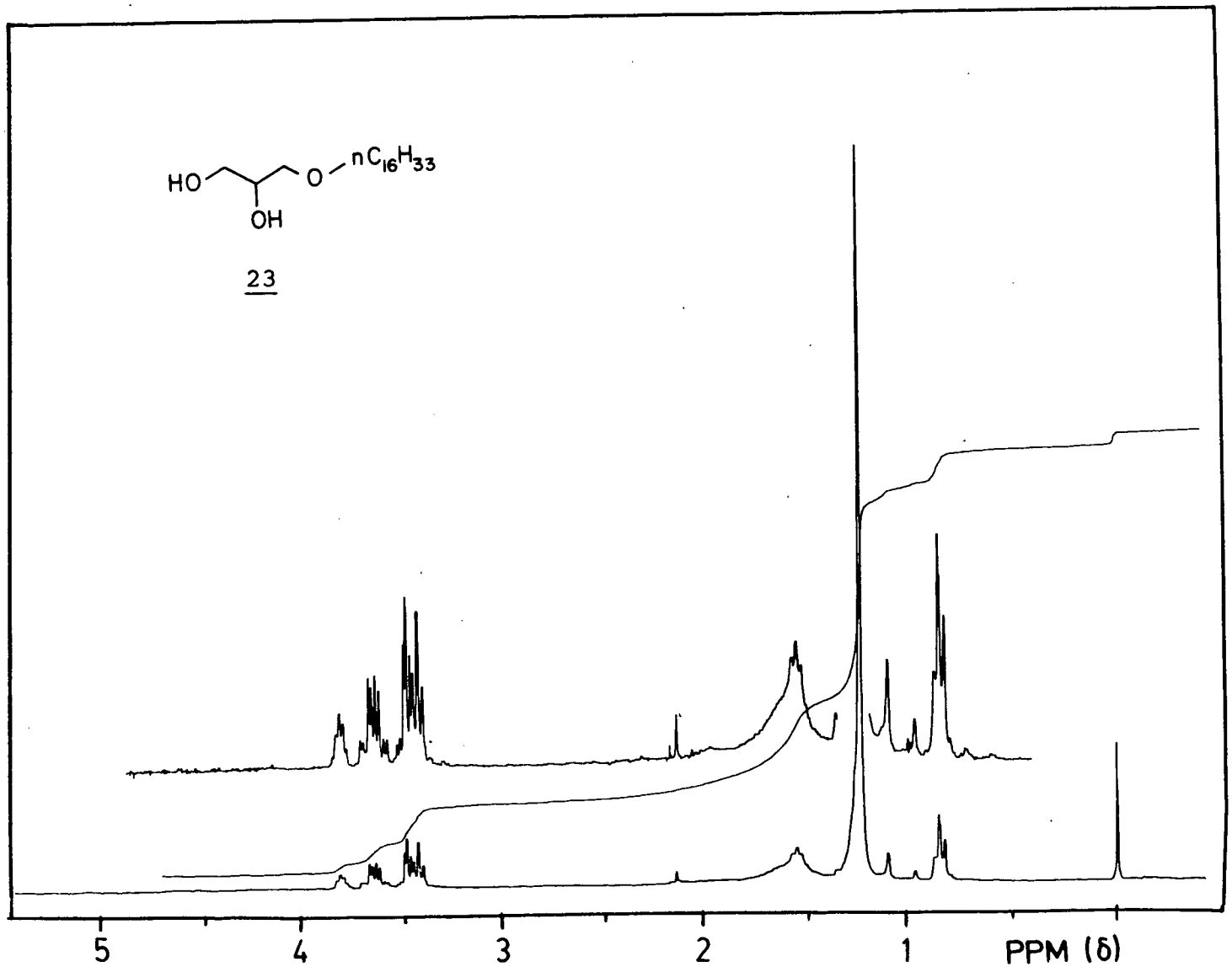


Figure 43. 270 MHz 'H NMR spectrum with vertical expansion of 23 in $CDCl_3$.

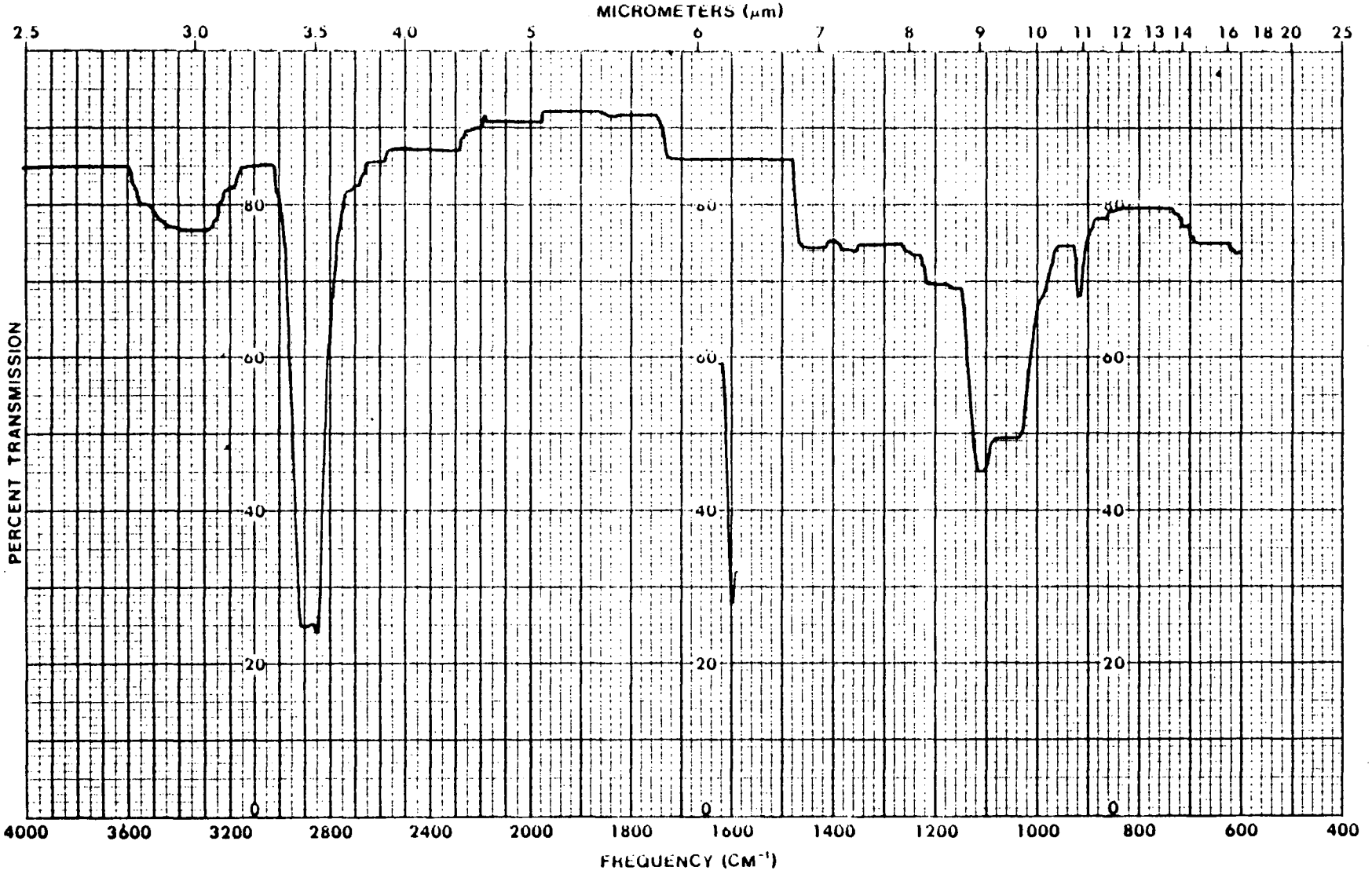
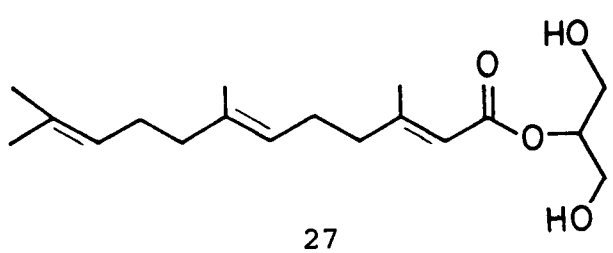


Figure 44. IR spectrum of 23 in $CHCl_3$.

II. Archidoris odhneri

The principal metabolites in extracts of Archidoris odhneri were previously reported to be the three farnesic acid glycerides 24, 25 and 26[24]. Methanol extracts of A. odhneri were re-examined for trace constituents. After rigorous HPLC purification a positional isomer of glyceride 24 was isolated in low yield. Compound 27 had a molecular formula of $C_{18}H_{30}O_4$ and 'H NMR signals characteristic of a farnesic acid acyl residue. Resonances of the glycerol fragment at δ3.81 (t, J = 6 Hz, 4H) and 4.90 (pentet, J = 6 Hz, 1H) demonstrated it was esterified at the secondary hydroxyl. The observed fragmentation pattern in the mass spectrum was consistent with structure 27. The diterpenoic acid glycerides 17 and 18 were subsequently isolated from A. odhneri extracts in low yield. They were identical in all respects to the corresponding metabolites of A. montereyensis. Glycerides 20 and 22 and ether 23 were also present in extremely trace amounts. They were evidenced by characteristic retention times in the HPLC trace and diagnostic signals in the 'H NMR spectrum of a partially purified mixture of the terpenoic glycerides.



27

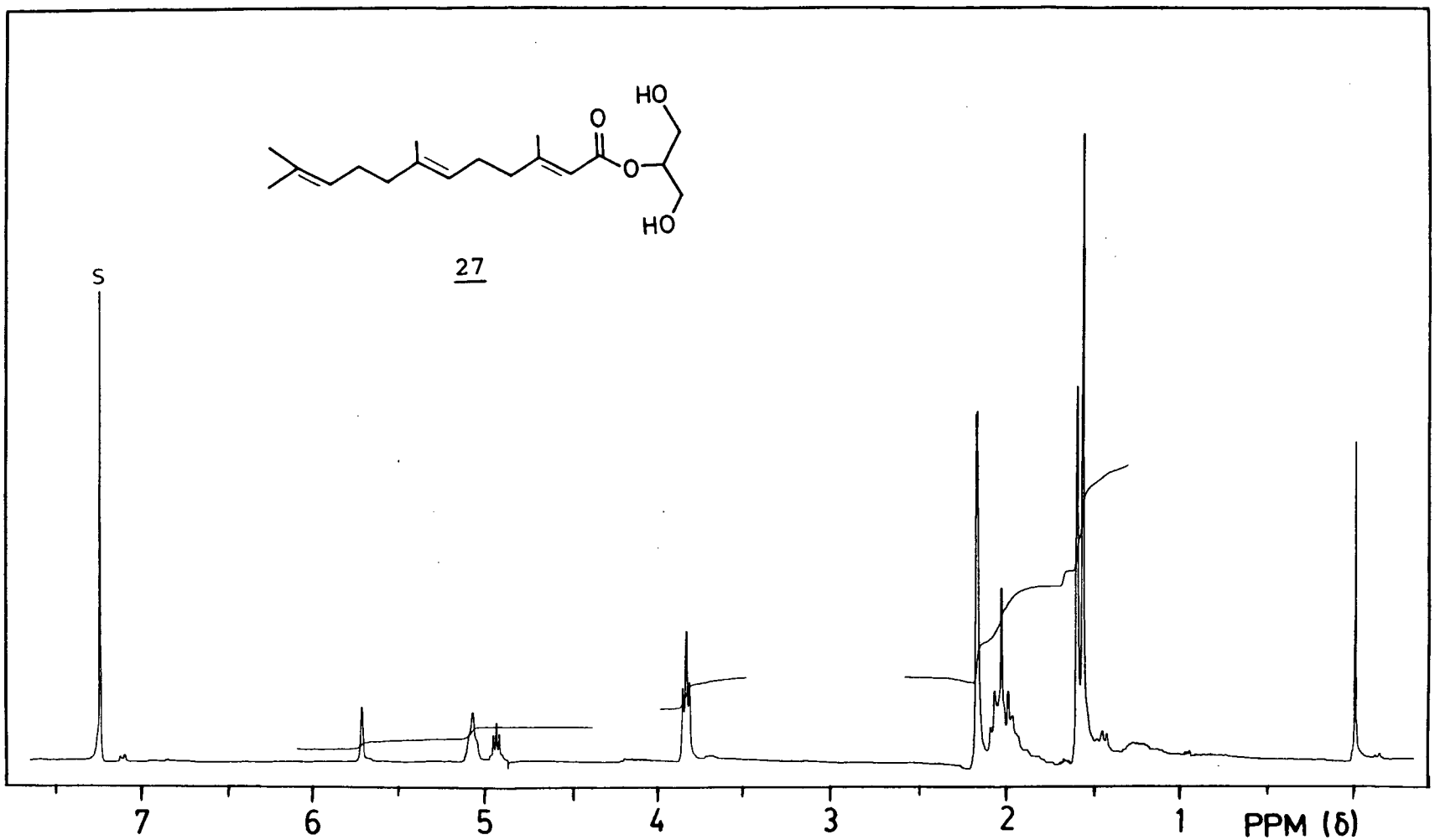


Figure 45. 270 MHz 'H NMR spectrum of 27 in $CDCl_3$.

III. Antifeedant Bioassay

The function of nudibranch skin chemicals is generally presumed to involve chemical defense of the organism. To test this theory, glycerol derivatives from A. montereyensis and A. odhneri were evaluated for fish antifeedant activity. Since the assays often required more compound than was available from the nudibranchs, only those constituents isolated in sufficient quantity were screened. The tide pool sculpin Oligocottus maculosus was chosen as a test fish. These fish are regularly encountered in areas where the nudibranchs were collected and thus represent a legitimate potential predator. The sculpins were collected intertidally and starved for 24 h prior to testing. Commercial shrimp pellets were coated with varying concentrations of nudibranch metabolites in acetone. Control pellets were treated only with acetone. The assay involved alternately offering sculpins control and treated pellets and monitoring their feeding response during a fixed period of time. The feeding ability of the fish was verified by offering untreated pellets both before and after the testing of the treated pellets. Feeding inhibition was assessed as a marked cessation of feeding behavior followed by indifference to, or avoidance of, the treated pellet. Although the active pellets were mouthed by the fish, they were quickly rejected. In all cases where feeding inhibition was observed, subsequently offered control

pellets were rapidly consumed. The results of the bioassay are shown in Table IV. The major glycerides from A. montereyensis and A. odhneri proved inactive as fish antifeedants. Two of the trace constituents, drimane derivative 20 and glyceryl ether 23 exhibited potent antifeedant activity. While the role of the more abundant skin chemicals has yet to be defined, this preliminary study showed that at least two nudibranch metabolites do inhibit the feeding behavior of fish.

Table IV. Fish Antifeedant Bioassay Results

Compound	Concentration µg/mg	Activity[a]	Concentration µg/mg	Activity
Diterpenoic acid glyceride 17	5	-[a]	160	-
1°-acetate 18	5	-	110	-
Sesquiterpenoic acid glyceride 20	18	+	NT	NT
Farnesic acid glyceride 24	26	-	111	-
Glyceryl ether 23	18	+	NT	NT

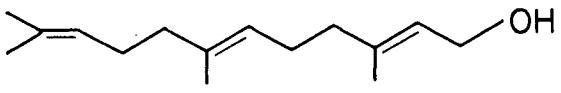
a) + pellet not eaten
- pellet eaten
NT) no test

IV. Biosynthesis of Archidoris Metabolites

We were interested in determining the source of metabolites in A. montereyensis and A. odhneri. Compounds found in nudibranchs can often be traced to an organism in their diet. The sponge Halichondria panicea is a dietary component of both Archidoris species, and A. montereyensis was frequently found eating the sponge at collection sites. Sponge extracts provided glyceryl ether 23, but none of the terpenoic acid glycerides. As mentioned in Chapter 1, de novo biosynthesis of the sesquiterpene polygodial (55), by the dorid nudibranch Dendrodoris limbata has previously been demonstrated[38]. Since a predator-prey relationship was established for only one of the glycerol derivatives, the possibility that the others were synthesized by the nudibranchs was investigated.

^{14}Carbon labeled mevalonic acid-dibenzylethylenediamine salt was injected into the digestive gland of live A. montereyensis and A. odhneri specimens. The nudibranchs were incubated for 24 h in an aquarium and then extracted with methanol. The extracts were fractionated in the usual fashion and compounds 17, 20 and 24 were taken to purity by HPLC. To remove any radioactivity associated with the glycerol moiety or with trace impurities, the glycerides were reduced with DIBAL to alcohols. Purification by HPLC provided alcohols 97 and 98 which crystalized from solution and farnesol (102), which was an oil. Reaction of farnesol

with dinitrobenzoyl chloride in the presence of 4-dimethylaminopyridine gave the crystaline 3,5-dinitrobenzoate derivative 103.



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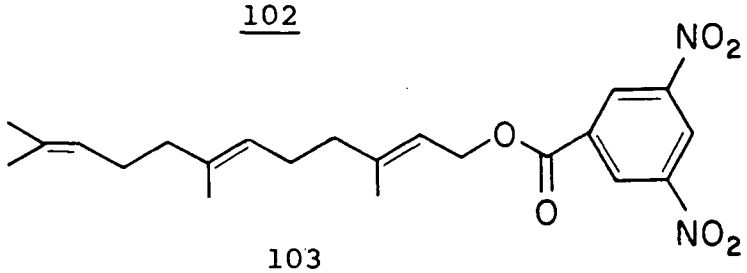
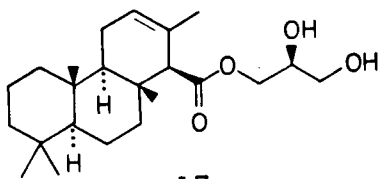
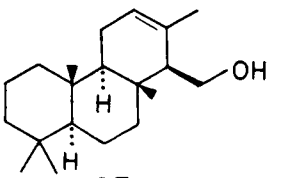
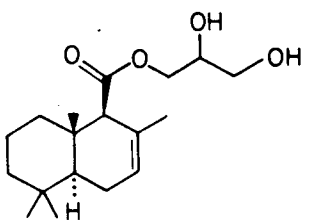
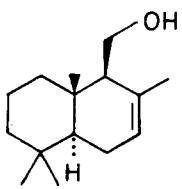
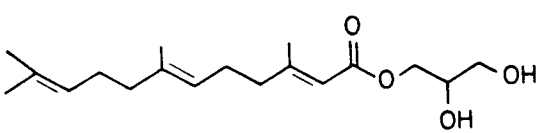
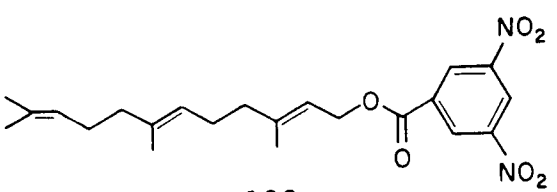


103

All derivatives were crystalized prior to counting, the results of which are shown in Table V. Significant levels of ^{14}C mevalonic acid incorporation were observed for the terpene portion of terpenoic acid glycerides 17, 20 and 24.

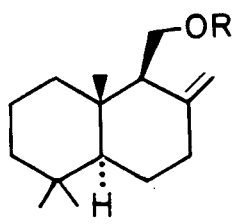
Table V. Results of ^{14}C Mevalonic Acid Incorporation Experiments

Nudibranch	Metabolite	Derivative used in counting	Activity[a] DPM/mg
Archidoris montereyensis	 17	 97	550
	 20	 98	3260
Archidoris odhneri	 24	 103	1810

a) disintegrations per minute/milligram

V. Additional Biosynthetic Studies

Preliminary biosynthetic investigations were also carried out with Triopha catalinae and Cadlina luteomarginata. Base hydrolysis of triophamine (75), isolated from T. catalinae that had been fed ^{14}C labeled acetate, gave the monoacyl derivative 81 which showed significant (1201 dpm/mg) radioactivity. In a similar experiment, albicanyl acetate (36) was recovered from C. luteomarginata that were injected with ^{14}C labeled mevalonic acid. The acetate moiety was cleaved with base to give albicanol (35) with an activity of 3815 dpm/mg. While these results indicate in situ biosynthesis by the nudibranchs, they should be interpreted with caution. The natural products were taken to HPLC purity and converted to derivatives prior to counting, but crystals of the derivatives were not obtained. The possibility that the observed radioactivity was actually associated with trace impurities can not be ruled out. It was not possible to repeat the experiments but questions raised by these preliminary results certainly warrent further study.



35 R = H

36 R = Ac

SUMMARY AND DISCUSSION

Chemical investigation of nudibranchs requires the isolation and structural identification of constituents that are often present in trace quantities. Recent success of these studies is due in large part to advances in spectroscopic and separation techniques. Natural product chemists are also interested in the source, biosynthesis and physiological function of novel compounds. Addressing these latter questions remains a formidable challenge. It is difficult to design experiments that can clearly define the origin or precise role of secondary metabolites. With natural products from marine organisms, such studies are often made more difficult by logistical limitations imposed by the marine environment. The work presented in this thesis concentrates on structural assignments of nudibranch products. Several investigations beyond the intial structural studies were also attempted.

The structure of triophamine (75), isolated from Triopha catalinae, was solved by classical methods of spectral analysis and degradation. This assignment was then confirmed by an unambiguous synthesis of the natural product. The presence of guanidine is not uncommon in compounds from marine sources, however the diacyl guanidine functionality represents a new class of natural compound. The acyl residue of triophamine has an unusual carbon

skeleton and its biosynthetic origin is not obvious. It is difficult to rationalize the biogenesis of the C_{10}-unit from an isoprenoid precursor, although this possibliity can not be ruled out. Alternatively, the hydrocarbon moiety could result from condensation of acetyl-CoA and two butyric acid equivalents (Figure 46). Such a pathway is purely speculative as appropriate biosynthetic studies of triophamine were not attempted.

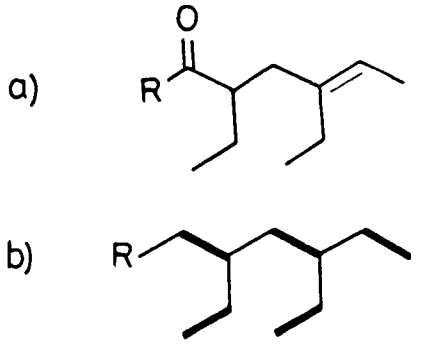


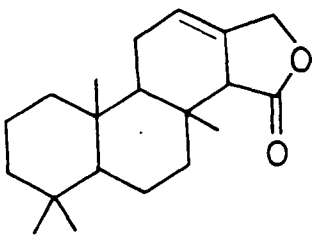
Figure 46 a) C_{10}-hydrocarbon portion of triophamine (75)
b) possible position of acetate units

The presence of triophamine in the mucous secretion of the nudibranch suggests it plays a defensive role, but this was not substantiated in a fish antifeedant bioassay. A more thorough investigation of the natural product's effect on potential predators is in order. Triophamine was also found in the dorid Polycera tricolor. Since a dietary

source for triophamine could not be located, the possibility of *de novo* biosynthesis was investigatged. Tentative results indicate that triophamine is indeed produced by the nudibranch.

The odoriferous constituent of *Anisodoris nobilis*, dihydroapofarnesal (15), was isolated in only trace amounts. Its structure was proposed on the basis of spectral interpretation and correlation with related aldehyde 68. Dihydroapofarnesal contains only 14 carbons, yet is clearly derived from a regular isoprenoid precursor. As such, it represents the first nor-sesquiterpene isolated from a nudibranch.

Chemical studies of *Archidoris montereyensis* revealed a series of terpenoic acid glycerides. The structure of the principal metabolite 17 was deduced from spectral data but finally proven by X-ray analysis. Its absolute configuration was established *via* reduction to a known compound. The diterpene carbon skeleton of glyceride 17 has previously been observed in furanoditerpenes from the nudibranch *Casella atromarginata*[28] and a series of sponge metabolites related to isoagathalactone (104)[75] and aplysillin (105)[76]. While this skeleton is rather rare in nature, it can arise from a straightforward cyclization of the acyclic precursor all-*trans*-geranylgeraniol pyrophosphate.



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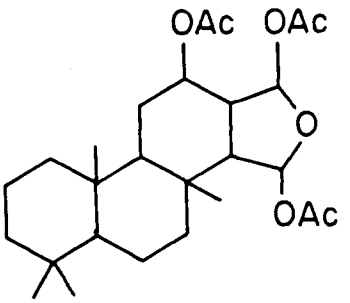

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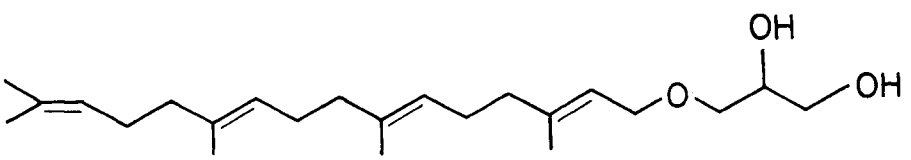
Diterpenes of mixed biogenesis are known from a variety of marine organisms[77]. In most cases, the diterpenoid component is acyclic and bound to an aromatic or carbohydrate moiety. Compound 17 represents a rare polycyclic diterpene of mixed biogenesis. The only other diterpene glycerol derivative previously reported from a marine source is the ether O-geranylgeranylglycerol 106, isolated from the brown seaweed Dilaphus fasciola[78].



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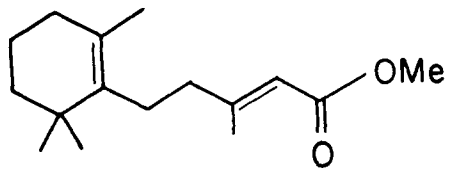
The structure and absolute stereochemistry of the sesquiterpene portion of glyceride 20 was established by chemical interconversion to drimenol 98. The drimane carbon skeleton, found in sesquiterpenes from terrestrial and

marine organisms, is a common structural feature of several nudibranch metabolites. Compound 20, like many drimane sesquiterpenes, exhibits antifeedant activity. The diverse array of chemical functionalities observed in these sesquiterpenes suggests that the biochemical basis for their activity is intimately associated with the drimane carbon skeleton.

The sesquiterpenoic acid glyceride 22 was isolated in extremely low yield and its structure deduced from spectral interpretation. Relatively few sesquiterpenes possessing the simple monocyclofarnesyl skeleton have been found in nature, however such compounds have been reported from algae[79] and sponges[80]. The methyl ester of trans-monocyclofarnesic acid 107 has been isolated from the dietary sponge Halichondria panicea[81]. This sponge has also been the source of mixed sesquiterpene-hydroquinone derivatives[82]. H. panicea was the suspected source of A. montereyensis metabolites, but analysis of sponge samples showed no sign of any terpenoid compounds. A single constituent, glyceryl ether 23, was found in both the nudibranch and sponge. This suggests that ether 23 is obtained from the sponge diet.



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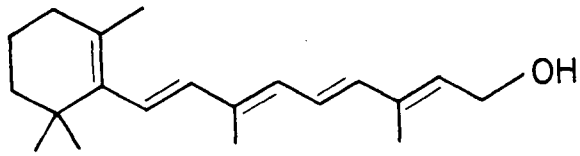
The nudibranch A. odhneri was known to contain a family of farnesic acid glycerides[24]. Close examination revealed that it also contained a number of terpenoic acid glycerides identified from A. montereyensis. We were unable to find a dietary source for any of these substances.

Combining spectral analysis and biogenetic considerations, the structure of a free diterpenoic acid isolated from A. montereyensis could be tentatively assigned as 100 or 101. There is some evidence that the isolated product was actually a mixture of the two olefinic isomers. The proposed structures are clearly related to other natural compounds such as retinol (108) and the algal metabolite caulerpol (109)[83]. While the free acid only occured in extracts from a single collection of A. montereyensis, the composition of the terpenoic acid glycerides was quite consistent in all collections. The cause of this apparent discrepancy is not known.



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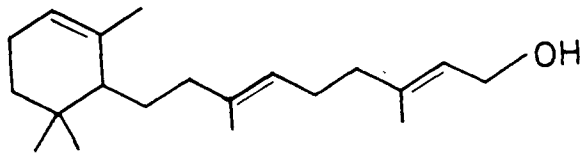

109

An incorporation study with ^{14}C labeled mevalonic acid indicated that the two Archidoris species are capable of terpene biosynthesis. Care was taken throughout this experiment to eliminate trace contaminants and their associated radioactivity. We are confident that the measured radioactivity resulted from de novo incorporation of the labeled precursor into the terpene residues. This is only the second instance where direct biosynthesis of secondary metabolites has been demonstrated in nudibranchs.

The physiological function of nudibranch skin chemicals is often presumed to be defensive, but evaluating a substance's defensive potential remains a problem. Antifeedant bioassays require a negative response from the assay organism, making it difficult to avoid subjective interpretation of the test subjects behavior. The bioassay of Archidoris constituents utilized fish that were active and voracious feeders. Rejection of food pellets treated

with the sesquiterpenoic acid glyceride 20 and glyceryl ether 23 was in such dramatic contrast to the rapid consumption of the other treated pellets that we feel these metabolites can truely inhibit feeding by fish.

EXPERIMENTAL

General

The 'H and ^{13}C NMR spectra were recorded on Bruker WH-400, Bruker WP-80, Nicolet-Oxford 270, Varian XL-100 and Varian CFT-20 spectrometers. Tetramethylsilane was employed as an internal standard and $CDCl_3$ as solvent unless otherwise indicated. Low-resolution mass spectra were recorded on an A.E.I. MS-902 spectrometer and high-resolution mass spectra were recorded on an MS-50 spectrometer. Infrared specta were recorded on a Perkin-Elmer model 710B spectrometer with $CHCl_3$ as solvent and ultraviolet absorbances were measured with a Cary-14 or Bausch and Lomb Spectronic 2000 spectrophotometer. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter using a 10 cm cell and $CHCl_3$ or MeOH as solvent. A Fisher-Johns apparatus was used to determine melting points and these values are uncorrected.

Merck Silica Gel 60 PF-254 was used for preparative TLC, Merck Silica Gel 230-400 Mesh was used for flash chromatography and Merck Silica Gel 60 PF-254 with $CaSO_4 \cdot 1/2\ H_2O$ was employed in radial TLC. High pressure liquid chromatography was performed on a Perkin-Elmer Series 2 instrument with a Perkin-Elmer LC-25 refractive index detector. A Whatman Magnum-9 Partisil 10 column was used for preparative HPLC. All chromatography solvents were HPLC

or reagent grade. Gas chromatography was performed on a Hewlett-Packard 5830 A instrument utilizing a thermal conductivity or flame ionization detector.

Collection Data

All specimens were collected by hand using SCUBA. Collections were made at a depth of 1-20 m in Barkley Sound, British Columbia. Samples were immediately immersed in methanol or chloroform and stored at low temperature.

Extraction and Chromatography of Triophamine (75)

A variety of procedures were employed to extract and purify triophamine. The following sequence was found to be the most efficient. Seventy-five freshly collected specimens of Triopha catalinae were immediately immersed in methanol. The solvent was decanted and the nudibranchs washed 3 times with 500 mℓ methanol. Evaporation of the combined extracts gave an aqueous suspension that was partitioned between water and chloroform. The chloroform fraction was dried over sodium sulfate and evaporated to yield a dark viscous oil (3.1 g, 7.8% dry weight). The oil was fractionated by flash chromatography on a column of silica gel (160 g) using 15% ethyl acetate in hexane as eluant. Triophamine was visualized as a strong UV (254 nm) absorbing spot on TLC. Fractions from the column which contained triophamine were further purified by preparative

thin-layer chromatography (1:1 hexane/diethyl ether, $R_f \cong$ 0.5) to yield 75 (51 mg) as a light yellow oil. In some instances, final purification by HPLC on Partisil-10 with chloroform was required to remove a persistent lipid impurity. 75 oil; $[\alpha]_D$ = -7° (C = 1.7, MeOH); UV λmax = 251nm (ε12000, MeOH); HRMS obs. 363.2885, $C_{21}H_{37}N_3O_2$ cal. 363.2885; IR ($CHCl_3$) 3330, 1700, 1635, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ0.91 (t, J = 7Hz, 3H), 0.95 (t, J = 7 Hz, 3H), 1.56 (d, J = 7Hz, 3H), 1.46-1.67 (m, 2H), 2.01 (q, J = 7Hz, 2H), 2.12 (dd, J = 6, 13Hz, 1H), 2.30-2.48 (m, 2H), 5.21 (q, J = 7Hz, 1H) ppm; ^{13}C NMR (100 MHz, acetone-d_6) δ12.1 (q), 12.9 (q), 13.1 (q), 23.2 (t), 26.1(t), 40.1 (t), 50.3 (d), 120.5 (d), 140.4 (s), 158.9 (s), 185.6 (s) ppm; MS, m/z 363 (60), 348 (10), 334 (25), 281 (25), 238 (60), 223 (50), 205 (45), 86 (100). The methanol extracts of 30 specimens of Polycera tricolor were fractionated in an identical manner to provide 5 mg of triophamine (75).

Hydrogenation of Triophamine (75)

A solution of 75 (8 mg) in ethanol with 5% Pd on charcoal as catalyst was stirred under H_2 for 48h. The charcoal was filtered off and the solution evaporated to yield a yellow oil. Preparative thin-layer chromatography (1:1 hexane/ ether, $R_f \cong$ 0.4) provided 4 mg (49% yield) of tetrahydrotriophamine (78). HRMS, obs 367.3198, $C_{21}H_{41}N_3O_2$ cal. 367.3198; ^1H NMR (270 MHz, $CDCl_3$) δ0.83 (t, J = 7Hz,

3H), 0.84 (t, J = 7Hz, 3H), 0.93 (t, J = 7Hz, 3H), 1.15-1.40 (m, 6H), 1.55-1.65 (m, 3H), 2.79 (m, 1H) ppm; MS, m/z 367 (10), 338 (15), 296 (30), 283 (75) 268 (30), 240 (70).

Monoacyltriophamine 81

A methanolic solution of triophamine (10 mg) was treated with several drops of 1 N NaOH and stirred at room temperature for 2h. The reaction mixture was evaporated in vacuo and partitioned between water and ethyl acetate. The ethyl acetate soluble material was purified by preparative thin-layer chromatography (9:1 $CHCl_3$/MeOH $R_f \cong 0.1$) to yield 2 mg (34% yield) of 81. UV λ_{max} = 232 (ε 10000, MeOH); MS, m/z 211 (M^+), 196 (10), 182 (15), 129 (15), 86 (100).

Dimethylpyrimidine Derivative of Guanidine 82

To 15 mg of triophamine (75) in 1.0 mℓ MeOH was added 5 drops of 5 N NaOH. The reaction was stirred at room temperature for 24h at which time guanidine could be detected by TLC (60:35:5 $CHCl_3$/MeOH/acetic acid, $R_f \cong 0.3$) using the FCNP spray reagent[56]. The solution was acidified with HCl and partitioned between H_2O and ether. The aqueous layer was evaporated to dryness yielding 5 mg of FCNP positive material. This was stirred with 12 mg $NaHCO_3$ and 1.0 mℓ 2,4-pentanedione at 130° for 30 min. The reaction mixture was evaporated in vacuo and triturated with $CHCl_3$.

Preparative thin-layer chromatography (1:4 acetonitrile/ethyl acetate, $R_f \cong 0.15$) of the $CHCl_3$ soluble material produced 1.0 mg (20% yield overall) of the 4,6-dimethylpyrimidine derivative 82. UV λ_{max} = 228 and 289 nm (MeOH); MS, m/z 123 (M^+); ^1H NMR (80 MHz, $CDCl_3$) δ2.31 (s, 6H), 5.05 (bs, 2H), 6.38 (s, 1H) ppm.

C_{10}-Carboxylic Acid 83

After repeated attempts, the C_{10}-carboxylic acid 83 was isolated in low yield from base-catalyzed hydrolysis of triophamine (75). To 15 mg of 75 in 3 mℓ MeOH was added 1 mℓ of 1 N NaOH. After refluxing for 1h, 5 mℓ H_2O was added and the mixture extracted with ethyl acetate. The aqueous layer was acidified with HCl and rapidly extracted with ethyl acetate (3 x 15 mℓ). The ethyl acetate fractions were dried over Na_2SO_4 and purified by preparative thin-layer chromatography (100:1:1 $CHCl_3$/MeOH/acetic acid, $R_f \cong 0.3$) to provide 1.5 mg (11% yield) of 83. HRMS, obs 170.1298, $C_{10}H_{18}O_2$ cal. 170.1307; IR ($CHCl_3$) 3400-2400, 1700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ0.95 (t, J = 7Hz, 3H), 0.97 (t, J = 7Hz, 3H), 1.57 (d, J = 7Hz, 3H), 1.52-1.62 (m, 2H), 2.04 (q, J = 7Hz, 2H), 2.14 (dd, J = 7, 15Hz, 1H), 2.33 (dd, J = 9, 15Hz, 1H), 2.47 (m, 1H), 5.23 (q, J = 7Hz, 1H) ppm; MS, m/z 170 (20), 141 (15), 83 (85), 73 (70), 55 (100). If extraction of the acidified hydrolysate is not carried out promptly, the C_{10}-acid 83 lactonizes to the γ-lactone 95.

95, IR 1750 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ0.93 (t, J = 7Hz, 6H), 0.99 (t, J = 7Hz, 3H), 1.55-1.80 (m, 8H), 2.62 (m, 1H) ppm.

Diacetylguanidine 80

To 390 mg of guanidine carbonate was added an excess of freshly distilled acetic anhydride. The mixture was kept under N_2 and heated to 100°C for 90 min. Excess acetic anhydride was evaporated in vacuo and the resulting solid triturated in acetone. The acetone soluble material was purified by preparative thin-layer chromatography (1:4 acetone/$CHCl_3$, $R_f \cong 0.3$) to give 87 mg (28% yield) of 80. UV λ_{max} = 248nm (ε 16800, MeOH), λ_{max} = 212nm (ε 19900, MeOH/HCl); IR 3350, 1710, 1645 cm^{-1}; MS m/z 143 (M^+); ^{13}C NMR (20 MHz, acetone-d_6) δ26.0, 159.0, 180.1 ppm. Base-catalyzed hydrolysis of 80 with methanolic NaOH at room temperature rapidly produced monoacetylguanidine [UV λ_{max} = 230 nm (ε 14300, MeOH)].

p-Nitrophenyl (E)-2,4-Diethyl-4-Hexenoate (96)

To a stirred solution of the carboxylic acid 83a (120 mg, 0.71 mmol) and p-nitrophenol (150 mg, 1.08 mmol) in 3 mℓ of dry acetonitrile was added 300 mg (1.47 mmol) of N, N'-dicyclohexylcarbodiimide, and the resultant mixture was stirred at room temperature for 12h. Removal of the solvent under reduced pressure provided a solid which was triturated

with chloroform. Filtration of the mixture, followed by removal of the solvent from the filtrate, gave a solid which was purified by flash chromatography on 28 g of silica gel. Elution of the column with 1:3 hexane-chloroform afforded 180 mg (87% yield) of the desired ester 96. mp 45-46°; HRMS, obs. 291.1496, $C_{16}H_{21}NO_4$ cal. 291.1471; IR ($CHCl_3$), 1755 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ1.01 (t, J = 7 Hz, 3H), 1.04 (t, J = 7 Hz, 3H), 1.42-1.86 (m, 2H), 1.62 (d, J = 7Hz, 3H), 2.13 (q, J = 7Hz, 2H), 2.28-2.85 (m, 3H), 5.33 (q, J = 7Hz, 1H), 7.23 (d, J = 9Hz, 2H), 8.31 (d, J = 9Hz, 2H) ppm.

(±)-Triophamine (75a)

Subjection of 14.3 g of guanidine hydrochloride to ion-exchange chromatography on a column of 64.5 g of Dowex 1-X8 (OH^- form) provided free guanidine as a hygroscopic solid. Small amounts of the latter material were added at 1h intervals to a stirred solution of the p-nitrophenyl ester 96 (34 mg, 0.12 mmol) in 2 mℓ of chloroform. The progress of the reaction was monitored by TLC (silica gel, 1:1 hexane/diethyl ether). It was found that if the reaction was allowed to proceed to completion (disappearance of 96), appreciable quantities of side product(s) were formed and the yield of desired material was low. Therefore, after approximately 50-75% of the starting material 96 had reacted, the reaction mixture was filtered and the collected solid was washed with 4 x 5 mℓ of

chloroform. Removal of the solvent from the combined filtrate gave a yellow oil which was subjected to preparative thin-layer chromatography. There was thus obtained 9.1 mg of the ester 96 and 7.4 mg (48% yield based on unrecovered 96) of a mixture of (±)-triophamine 75a and its diastereomers 75b. This mixture was separated by repeated (three developments) preparative thin-layer chromotography on silica gel 60 PF-254 (19:1 hexane/isopropyl alcohol) to give 4.0 mg (26% yield) of (±)-triophamine which exhibited infrared, mass and 'H NMR spectra identical with those of the natural product. The diastereomers (2.5 mg; 16% yield) provided spectra which were similar to, but clearly different from natural triophamine. The spectral features of the diastereomers are as follows: HRMS obs. 363.2883, $C_{21}H_{37}N_3O_2$ cal. 363.2885; IR ($CHCl_3$) 3323, 1700, 1630, 1160 cm^{-1}; 'H NMR (400 MHz, $CDCl_3$) δ0.91 (t, J = 7Hz, 3H), 0.95 (t, J = 7Hz, 3H), 1.46-1.67 (m, 2H), 1.56 (d, J = 7Hz, 2H), 2.03 (q, J = 7Hz, 2H), 2.11 (dd, J = 6, 13Hz, 1H), 2.28-2.37 (m, 1H), 2.37-2.47 (m, 1H), 5.20 (q, J = Hz, 1H) ppm.

Extraction and Chromatography of Dihydroapofarnesal (15)

A total of 22 specimens of Anisodoris nobilis were extracted with chloroform (2 x 800 mℓ). The chloroform was dried over Na_2SO_4 and evaporated to obtain a pleasant smelling light brown oil (820 mg). The oil was

separated by flash chromotography on silica gel (hexane-chloroform step gradient). Material eluting with 15% chloroform in hexane was further purified by radial thin-layer chromatography using 100% hexane as the eluant. Final purification was achieved by preparative GC (3% OV-17 on Chromosorb, initial temperature 100°, rate 4°/min, RT 14 min.) providing 2.8 mg of 15 as a sweet smelling, colorless oil. HRMS, obs. 208.1825, $C_{14}H_{24}O$ cal. 208.1823; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (d, J = 7 Hz, 3H), 1.42 (m, 1H), 1.60 (s, 3H), 1.61 (s, 3H) 1.69 (s, 3H), 1.78 (m, 1H), 1.96-2.13 (m, 6H), 2.36 (m, 1H), 5.12 (m, 2H), 9.58 (d, J = 1 Hz, 1H) ppm; MS, m/z 208 (5), 190 (5), 175 (3), 165 (50), 150 (20), 95 (25), 81 (70), 69 (100).

Extraction and Chromatography of Archidoris Metabolites

A total of 80 specimens of Archidoris montereyensis were extracted with methanol (3 x 1 ℓ) for 3 days. The combined methanol extracts were evaporated to an aqueous suspension and extracted with chloroform (3 x 500 mℓ). The combined extracts were dried over sodium sulfate and evaporated to yield a brown gum (2.7 g, 8.3% dry weight, 33.7 mg/animal).

The gum (1.5 g) was subjected to flash chromatography on a column of silica gel (130 g) with a step gradient of hexane-ethyl acetate mixtures. Elution with 20% ethyl acetate in hexane removed non-polar fats and pigments. The

acetylated glycerides were eluted with a 1:1 solution of hexane-ethyl acetate while the non-acetylated glycerides were eluted with 100% ethyl acetate.

Material eluted with (1:1) hexane-ethyl acetate was fractionated by radial thin-layer chromatography (silica gel), eluting solvent chloroform-methanol (99:1). Final separation was acheived by LC on Partisil-10 with hexane-ethyl acetate-methanol (85:15:1) eluant to yield in the order of their elution, compounds 18 (24 mg; 1.6% of crude extract), 21 (2 mg; 0.13% of crude extract) and 19 (3 mg; 0.2% of crude extract).

18 mp 117-119° (Hexane/Et_2O); $[\alpha]_D$ = -53.7° (C = 0.13, $CHCl_3$); HRMS obs. 420.2884, $C_{25}H_{40}O_5$ cal. 420.2876; IR ($CHCl_3$) 3600-3350, 2930, 1730, 1230 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.87 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.61 (s, 3H), 2.11 (s, 3H), 2.41 (bs, 1H, exchangable), 2.97 (bs, 1H), 4.08-4.25 (m, 5H), 5.53 (bs, 1H); MS, m/z 420 (2), 402 (5), 347 (5), 286 (100), 192 (65), 177 (70), 117 (60).

21 oil; $[\alpha]_D$ = +15.3° (C = 1.2, $CHCl_3$); HRMS obs. 352.2221, $C_{20}H_{32}O_5$ cal. 352.2250, IR ($CHCl_3$) 3400, 2930, 1720, 1230 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.92 (s, 3H), 0.98 (s, 3H), 1.61 (s, 3H), 2.11 (s, 3H), 2.48 (bs, 1H, exchangable), 2.95 (bs, 1H), 4.05-4.24 (m, 5H), 5.55 (bs, 1H). MS, m/z 352 (3), 334 (4) 279 (5), 218 (55), 190 (45), 124 (80), 117 (80), 109 (100), 95 (80).

19 oil; $[\alpha]_D$ = -33.0° (C = 0.83, $CHCl_3$); HRMS obs. 420.2876, $C_{25}H_{40}O_5$ cal. 420.2876; IR ($CHCl_3$) 3600-3210, 2925, 1725, 1230, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.87 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 1.60 (s, 3H), 2.09 (s, 3H), 2.94 (bs, 1H), 3.76 (d, J = 6 Hz, 2H), 4.26 (dd, J = 7 and 12 Hz, 1H), 4.32 (dd, J = 6 and 13 Hz, 1H), 5.06 (m, 1H), 5.52 (bs, 1H); MS, m/z 420 (5), 402 (3), 286 (90), 258 (20), 192 (55), 191 (50), 177 (75), 117 (100), 95 (80), 81 (80).

The 100% ethyl acetate fraction from the flash column was subjected to radial thin-layer chromatography eluting with chloroform-methanol (95:5). Final separation was achieved by LC on Partisil-10 with hexane- ethyl acetate-methanol (50:40:2) eluant to yield in the order of their elution, compounds 17 (38 mg; 2.5% of crude extract), 23 (2 mg; 0.13% of crude extract), 20 (3 mg; 0.2% of crude extract) and 22 (2 mg; 0.13% of crude extract).

17, mp = 125-126° (Hexane/Et_2O), $[\alpha]_D$ = -12.5° (c = 0.4, $CHCl_3$); HRMS, obs. 378.2772, $C_{23}H_{38}O_4$ cal. 378.2770; IR ($CHCl_3$) 3600-3300, 2900, 2850, 1730, 1460, 1170 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.87 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.61 (bs, 3H), 1.92-1.99 (m, 2H), 2.44 (bs, 2H, exchangable), 2.96 (bs, 1H), 3.63 (dd, J = 6 and 12 Hz, 1H), 3.70 (dd, J = 4 and 12 Hz, 1H), 3.95 (m, 1H), 4.15 (dd, J = 7 and 12 Hz, 1H), 4.22 (dd, J = 5 and 12 Hz, 1H), 5.54 (bs, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) 15.63 (q), 15.73 (q),

18.52 (q), 18.73 (q), 21.15 (t), 21.68 (t), 22.74 (t), 33.20 (s), 33.43 (q), 36.70 (s), 37.53 (s), 40.01 (t), 41.95 (t), 54.45 (d), 56.58 (d), 62.76 (d), 63.64 (t), 65.17 (t), 70.47 (d), 124.32 (d), 128.60 (s), 173.35 (s) ppm; MS, m/z 378 (30), 363 (5), 347 (10), 286 (65), 258 (35), 192 (100), 177 (80), 95 (75).

23 HRMS, obs. 316.2973, $C_{19}H_{40}O_3$ cal. 316.2977; IR ($CHCl_3$) 3600-3250, 2900, 1110 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J = 7 Hz, 3H), 1.26 (bs, 28H), 1.59 (m, 2H), 3.44 (t, J = 6 Hz, 2H), 3.50 (m, 2H), 3.64 (dd, J = 6 and 12 Hz, 1H), 3.67 (dd, J = 4 and 12 Hz, 1H), 3.82 (m, 1H) ppm; MS, m/z 316 (2), 285 (5), 255 (15), 225 (15), 71 (80), 57 (100).

20 mp = 94-95°; $[\alpha]_D$ = +23.1 (c = 0.93, $CHCl_3$); HRMS, obs. 310.2142, $C_{18}H_{30}O_4$ cal. 310.2144; IR ($CHCl_3$) 3600-3300, 2900, 1730, 1165 cm^{-1}; ^1H NMR (400 MHz,$CDCl_3$) δ 0.89 (s, 3H), 0.92 (s, 3H), 0.98 (s, 3H), 1.62 (bs, 3H), 2.48 (bs, 2H, exchangable), 2.96 (bs, 1H), 3.63 (dd, J = 6 and 12 Hz, 1H), 3.70 (dd, J = 4 and 12 Hz, 1H), 3.95 (m, 1H), 4.15 (dd, J = 7 and 12 Hz, 1H), 4.22 (dd, J = 5 and 12 Hz, 1H), 5.57 (bs, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ14.9, 18.7, 21.3, 21.9, 23.7, 33.1, 33.3, 40.5, 42.2, 42.3, 49.5, 62.3, 63.6, 65.2, 70.5, 124.6, 128.8, 173.4 ppm; MS, m/z 310 (15), 295 (5), 279 (10), 218 (75), 190 (45), 187 (40), 124 (70), 109 (100), 95 (75).

22 oil; $[\alpha]_D$ = +9.7° (C = 0.3, $CHCl_3$); UV λ_{max} = 228 nm (ε 1800, MeOH); HRMS obs. 310.2146, $C_{18}H_{30}O_4$ cal.

310.2144; IR ($CHCl_3$) 3430, 2945, 1700, 1645, 1155 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 3H), 0.93 (s, 3H), 2.16 (s, 3H), 3.62 (dd, J = 12 and 7 Hz, 1H), 3.70 (dd, J = 12 and 5 Hz, 1H), 3.95 (m, 1H), 4.18 (dd, J = 12 and 7 Hz, 1H), 4.24 (dd, J = 12 and 5 Hz, 1H), 4.55 (s, 1H), 4.79 (s, 1H), 5.70 (s, 1H); MS: m/z 310 (2), 295 (5), 279 (5), 237 (10), 219 (30), 203 (35), 176 (85), 124 (30), 109 (85), 95 (90), 82 (100), 69 (90).

The diterpenoic acid(s) tentatively identified as 100 and/or 101 was isolated from only one of the many collections of A. montereyensis made during the course of this study. Fractionation of the chloroform soluble material by flash chromatography using 30% ethyl acetate in hexane as eluant provided a crude mixture which did not contain any of the terpenoic glycerides. Final purification by preparative thin-layer chromatography (1% MeOH in $CHCl_3$, $R_f \cong 0.4$) gave 2.3 mg of the acid(s) as a light yellow oil. HRMS 304.2356 obs. $C_{20}H_{32}O_2$ cal. 304.2402; IR ($CHCl_3$) 3225-2500, 1695, 1650 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ0.83 (s, 3H), 0.91 (s, 3H), 1.60 (s, 3H), 2.20 (bs, 3H), 4.53 (bs, 1H), 4.76 (bs, 1H), 5.07 (bs, 1H), 5.70 (s, 1H) ppm; MS m/z 304 (15), 289 (40), 177 (50), 124 (50), 109 (85), 81 (100).

Ten specimens of Archidoris odhneri were extracted with methanol and subjected to a separation and purification scheme identical to that used with A. montereyensis. In

addition to 43 mg of farnesic acid glyceride 24, 1.5 mg of the positional isomer 27 were recovered.

27 HRMS 310.2143 obs. $C_{18}H_{30}O_4$ cal. 310.2144; 'H NMR (270 MHz, $CDCl_3$) δ1.56 (s, 6H), 1.60 (s, 3H), 2.16 (s, 3H), 3.81 (t, J = 6Hz, 4H), 4.90 (pentet, J = 7Hz, 1H), 5.03 (bs, 2H), 5.68 (s, 1H) ppm; MS, m/z 310 (15), 219 (20), 218 (20), 82 (90), 69 (100). Repetative HPLC separation of A. odhneri extracts provided pure samples of glycerides 17 and 18. Trace quantities of 20, 22 and 23 were also evidenced by characteristic retention times in the HPLC trace and diagnostic signals in the 'H NMR spectrum of a partially purified mixture.

Reduction of 17

A solution of 17 (20 mg) in 0.5 mℓ of toluene was stirred at RT for 8 h in the presence of excess DIBAL. Addition of 5 mℓ of EtOH, followed by filtration of the mixture and evaporation of the solvents, resulted in one major product. Preparative thin-layer chromatography (1:4, ethyl acetate/hexane $R_f \cong 0.3$) provided 13 mg (85% yield) of white crystalline 97. mp 126-127° (hexane/Et_2O) (lit. 125-126°)[69]; $[\alpha]_D$ = -9° (C = 0.1, $CHCl_3$), (lit. -9°)[69]; HRMS, obs. 290.2623, $C_{20}H_{34}O$ cal. 290.2609; IR ($CHCl_3$) 3600-3300, 2920, 2850, 1450, 1390 cm^{-1}; 'H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.84 (s, 3H), 0.87 (s, 3H), 0.90 (s, 3H), 1.78 (s, 3H), 2.06 (dt,J = 4 and 13 Hz, 1H), 3.72 (dd,

J = 6 and 12 Hz, 1H), 3.85 (dd, J = 4 and 12 Hz, 1H), 5.50 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ15.8, 18.6, 18.9, 21.7, 22.7, 23.7, 29.7, 33.2, 33.4, 36.4, 37.4, 40.1, 41.7, 42.1, 55.0, 56.4, 58.1 60.9, 123.9, 132.7; MS, m/z 290 (40), 275 (5), 260 (5), 245 (5), 192 (100), 177 (95), 95 (40), 81 (60), 69 (85).

Reduction of 18 and 19

Separate solutions of 18 (15 mg) and 19 (6 mg) were treated with DIBAL and subsequently purified in a manner identical to the reduction of 17. This produced 8 mg (77% yield) and 3 mg (72% yield) respectively of white crystalline 97, which was identical in all respects to the reduction product of 17.

Reduction of 20

A solution of 20 (6 mg) was treated with DIBAL and subsequently purified in a manner identical to the reduction of 17. This produced 2.6 mg (50% yield) of white crystaline 98. mp 94-95° (lit. 95-96°)[71]; $[\alpha]_D$ = -20° (c = 0.08, $CHCl_3$) (lit. - 20°)[71]; HRMS, obs. 222.1984, $C_{15}H_{26}O$ cal. 222.1984; 'H NMR (400 MHz, $CDCl_3$) δ 0.837 (s, 3H), 0.844 (s, 3H), 0.867 (s, 3H), 1.76 (s, 3H), 3.73 (dd, J = 13 and 7.2 Hz, 1H), 3.84 (dd, J = 13 and 5 Hz, 1H) ppm; MS, m/z 222 (10), 191 (10), 124 (45), 109 (75), 85 (75), 83 (100), 69 (60). Reduction product 98 was identical to authentic

drimenol by 'H NMR, MS, TLC (ethyl acetate/hexane (1:4), $R_f \cong 0.3$ on Polygram 0.25 mm silica gel plates) and GC (co-injection of 98 and authentic drimenol gave a single peak: initial temperature 140°, rate 10°/min, RT = 6.75 min on 3% OV-17).

^{14}C Incorporation Studies of Archidoris Metabolites

A total of 22 specimens of A. montereyensis and 13 specimens of A. odhneri were employed in the incorporation studies. Two uCi of [2-^{14}C] RS-mevalonic acid-dibenzylethylenediamine salt (New England Nuclear; 45 mCi/mmole) in (1:1) ethanol/sterile seawater (0.05 mℓ) was directly injected into the digestive gland of each animal by means of a syringe. After injection, the nudibranchs were placed in a running seawater aquarium for 24 h and then extracted with methanol in the normal manner. The extracts were fractionated and the glyceride metabolities purified by HPLC as previously described for both species. Compounds 17 and 20 obtained from A. montereyensis, were reduced to alcohols 97 and 98 respectively by DIBAL in toluene. The alcohols were purified by HPLC (Partisil-10, 8% ethyl acetate/hexane) and crystalized from ether/hexane prior to ^{14}C counting. Compound 24, obtained from A. odhneri was reduced to farnesol (102) and then converted to its 3,5-dinitrobenzoate derivative 103. Compound 103 was crystalized from ether/hexane prior to counting. The

samples (0.5-4 mg) were dissolved in 10 mℓ Aquasol (NEN) scintillation counting fluid. Radioactive counts were determined in a Unilux III liquid scintillation system. Quenching was corrected by external standardization.

Reduction of 24

A solution of 24 (52 mg) was treated with DIBAL and subsequently purified in a manner identical to the reduction of 17. Final purification by HPLC (Partisil-10, 8% ethyl acetate in hexane) provided 26 mg (65% yield) of farnesol (102) as a colorless oil.

3,5-dinitrobenzoate Derivative of Farnesol (102)

To a solution of 102 (26 mg; 0.117 mmole) in 1.0 mℓ pyridine was added 4-dimethylaminopyridine (20 mg, 0.164 mmole) and 3,5-dinitrobenzoyl chloride (31 mg, 0.135 mmole). After stirring at RT for 12 h, 5 mℓ methanol was added and the mixture was stirred for an additional 2 h. The reaction mixture was filtered through a plug (1 x 1 cm dia.) of silica gel using chloroform as eluant. Removal of the solvent under reduced pressure followed by preparative thin-layer chromatography (25% ethyl acetate in hexane) provided 39 mg (86% yield) of 103 mp 77-79°; ^{1}H NMR (80 MHz, $CDCl_3$); 1.58 (s, 6H), 1.64 (s, 3H), 1.79 (s, 3H), 1.90-2.19 (m, 8H), 4.93 (d, J = 8Hz, 2H), 5.04 (m, 2H), 5.45 (t, J = 8Hz, 1H), 9.21 (m, 3H); MS m/z 416 (20), 401 (5), 195 (50),

136 (60), 93 (50), 81 (170), 69 (100).

Additional ^{14}C Incorporation Studies

A total of 10 specimens of Cadlina luteomarginata were each injected with five uCi of [2-^{14}C] RS-mevalonic acid-dibenzylethylenediamine salt (45 mCi/mmol) in 0.05 mℓ sterile seawater. After incubating for 24h the nudibranchs were extracted with methanol. The chloroform soluble portion of the extract was fractionated by flash chromatography on a column of silica with chloroform as eluant. Fractions containing albicanyl acetate (36) were combined and further purified by HPLC (Partisil-10, 5% ethyl acetate/hexane) to give 3.2 mg of pure 36. This material was added to 6.8 mg of "cold" albicanyl acetate and then hydrolyzed by stirring at RT in a methanolic potassium carbonate solution. After 12h the reaction mixture was filtered and partitioned between water and ether. The ether soluble material was purified by preparative thin-layer chromatography to yield 3.5 mg of albicanol (35) with an activity of 1221 dpm/mg. After correcting for the 68% dilution with "cold" material, the albicanol had a ^{14}C activity of 3815 dpm/mg. 35 'H NMR δ0.73 (s, 3H), 0.81 (s, 3H), 0.88 (s, 3H), 3.76 (dd, J = 11, 11Hz, 1H), 3.86 (dd, J = 11, 4Hz, 1H), 4.65 (bs, 1H), 4.93 (bs, 1H) ppm.

A total of 10 specimens of Triopha catalinae were each injected with 5 uCi of [2-^{14}C] acetic acid sodium salt (1-3

mCi/mmol) in 0.05 mℓ sterile seawater. After incubating for 24 h the nudibranchs were extracted and worked up as previously described to yield 3.5 mg of triophamine (75). This material was combined with 6.5 mg of "cold" triophamine, dissolved in 1 mℓ methanol and stirred at RT in the presence of 0.5 mℓ 1 N sodium hydroxide. The reaction mixture was extracted with ethyl acetate and purified by preparative thin-layer chromatography to give 2.2 mg of the monoacyl derivative 81 with an activity of 420.4 dpm/mg. When the 65% dilution with "cold" material was accounted for, compound 81 showed an activity of 1201 dpm/mg.

Fish Antifeedant Bioassay

Antifeedant activity was assessed by observing the feeding response of the tide pool sculpin Oligocottus maculosus toward food pellets (Wardely Shrimp Pellets) treated with varying concentrations of dorid metabolities. The sculpins were collected intertidally at Barkley Sound, British Columbia and were starved for 24 h prior to testing. Test compounds were applied to food pellets with acetone which was evaporated at room temperature. Control pellets were treated with solvent only. Single pellets were randomly added to a group of 40 fish (5-8 cm in length) in a 10 gallon aquarium and the feeding response was observed for up to 1 h. The fish exhibitied voracious feeding behaviour toward control pellets and pellets treated with inactive

compounds. These pellets were aggressively attacked by groups of fish and consumed within 5-10 min. Indigestible hard parts in the pellets were ignored. Test compounds that were active caused a marked cessation of feeding. After initial mouthing and rejection, the fish avoided the active pellets which remained uneaten after 1 h. The feeding ability of the fish was verified by feeding them untreated pellets both before and after each test. In all cases the untreated pellets were rapidly consumed.

BIBLIOGRAPHY

1. Thompson, T.E. J. Mar. Biol. Assoc. U.K. 1960, 39, 123.
2. Behrens, D.W. "Pacific Coast Nudibranchs"; Sea Challengers: Los Osos, California, 1980.
3. Harris, L.G. Current Topics Comp. Pathobiol. 1973, 2, 213.
4. McDonald, G.; Nybakken, J.W. "Guide to Nudibranchs of California"; American Malacologists Inc.: Melbourne, Florida, 1980.
5. Paine, R.J. Veliger 1963, 6, 1.
6. Thompson, T.E. J. Mar. Biol. Assoc. U.K. 1960, 39, 115.
7. Edmunds, M. Proc. Malac. Soc. Lond. 1968, 38, 121.
8. For example see: a) Imperato, F.; Minale, L. and Riccio, R. Experientia 1977, 33, 1273. b) Stallard, M.O. and Faulkner, D.J. Comp. Biochem. Physiol. 1974, 49B, 25.
9. For example see: a) Pettit, G.R.; Herald, C.L.; Allen, M.S.; Von Dreele, R.B.; Vanell, L.D.; Kao, J.P.Y. and Blake, W. J. Am. Chem. Soc. 1977, 99, 262. b) Hollenbeak, K.H.; Schmitz, F.J.; Hossain, M.B. and Van Der Helm, D. Tetrahedron 1979, 35, 541. c) Yamamura, S. and Hirata, Y. Tetrahedron 1963, 19, 1485.

10. For example see: a) Minale, L. and Riccio, R. Tetrahedron Lett. 1976, 2711. b) Matsuda, H.; Tomiie, Y.; Yamamura, S. and Hirata, Y. Chem. Comm. 1967, 898. c) Pettit, G.R.; Herald, C.L.; Einck, J.J.; Vanell, L.D.; Brown, P. and Gust, D. J. Org. Chem. 1978, 43, 4685.

11. Kinnel, R.B.; Dieter, R.K.; Meinwald, J.; Van Engen, D.; Clardy, J.; Eisner, T.; Stallard, M.O. and Fenical, W. Proc. Natl. Acad. Sci. U.S.A. 1979, 76, 3576.

12. Imperato, F.; Minale, L. and Riccio, R. Experientia 1977, 33, 1273.

13. Stallard, M.O. and Faulkner, D.J. Comp. Biochem. Physiol. 1974, 49B, 37.

14. See for example: Schmitz, F.J.; Michaud, D.P. and Schmidt, P.G. J. Am. Chem. Soc. 1982, 104, 6415.

15. Ireland, C. and Faulkner, D.J. Biorg. Chem. 1978, 7, 125.

16. Biskupiak, J.E. and Ireland, C.M. Tetrahedron Lett. 1983, 3055.

17. Ireland, C. and Scheuer, P.J. Science 1979, 205, 922.

18. a) Ayer, S.W.; Hellou, J.; Tischler, M. and Andersen, R.J. Tetrahedron Lett. 1984, 141. b) Ayer, S.W.; Andersen, R.J.; Cun-heng, H. and Clardy, J. J. Org. Chem. 1984, in press.

19. Stonard, R.J.; Petrovich, J.C. and Andersen, R.J. Steroids 1980, 36, 81.

20. Ayer, S.W. and Andersen, R.J. Tetrahedron Lett. 1982, 1039.

21. Gustafson, K. and Andersen, R.J. Tetrahedron 1984, submitted for publication.

22. Fuhrman, F.A.; Fuhrman, G.J.; Kim, Y.H.; Pavelka, L.A. and Mosher, H.S. Science 1980, 207, 193.

23. Gustafson, K.; Andersen, R.J.; Chen, M.H.M.; Clardy, J. and Hochlowski, J.E. Tetrahedron Lett. 1984, 25, 11.

24. Andersen, R.J. and Sum, F.W. Tetrahedron Lett. 1980, 797.

25. Thompson, J.E.; Walker, R.P.; Wratten, S.J. and Faulkner, D.J. Tetrahedron 1982, 38, 1865.

26. Hellou, J.; Andersen, R.J. and Thompson, J.E. Tetrahedron 1982, 38, 1875.

27. Hellou, J.; Andersen, R.J.; Rafii, S.; Arnold, E. and Clardy, J. Tetrahedron Lett. 1981, 4173.

28. de Silva, E.D. and Scheuer, P.J. Heterocycles 1982, 17, 167.

29. Schulte, G.R. and Scheuer, P.J. Tetrahedron 1982, 38, 1857.

30. Schulte, F.; Scheuer, P.J. and McConnell, O.J. Helv. Chim. Acta 1980, 63, 2159.

31. Hochlowski, J.E. and Faulkner, D.J. Tetrahedron Lett. 1981, 271.

32. Hochlowski, J.E.; Faulkner, D.J.; Matsumoto, G.K. and Clardy, J. J. Org. Chem. 1983, 48, 1142.

33. Hochlowski, J.E.; Faulkner, D.J.; Bass, L.S. and Clardy, J. J. Org. Chem. 1983, 48, 1738.

34. Cimino, G.; De Rosa, S.; De Stefano, S. and Sodano, G. Tetrahedron Lett. 1980, 3303.

35. Cimino, G.; De Stefano, S.; De Rosa, S.; Sodano, G. and Villani, G. Bull. Soc. Chim. Belg. 1980, 89, 1069.

36. Okuda, R.K.; Scheuer, P.J.; Hochlowski, J.E.; Walker, R.P. and Faulkner, D.J. J. Org. Chem. 1983, 48, 1866.

37. Cimino, G.; De Rosa, S.; De Stefano, S. and Sodano, G. Tetrahedron Lett. 1981, 1271.

38. Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. and Villani, G. Science 1983, 219, 1237.

39. Fuhrman, F.A.; Fuhrman, G.J.; Nachman, R.J. and Mosher, H.S. Science 1981, 212, 557.

40. Walker, R.P. and Faulkner, D.J. J. Org. Chem. 1981, 46, 1475.

41. Cimino, G.; De Rosa, S.; De Stefano, S. and Sodano, G. Comp. Biochem. Physiol. B 1982, 73, 471.

42. Hochlowski, J.E.; Walker, R.P.; Ireland, C. and Faulkner, D.J. J. Org. Chem. 1982, 47, 88.

43. Ayer, S.W. and Andersen, R.J. Experientia 1983, 39, 255.

44. Castiello, D.; Cimino, G.; De Rosa, S.; De Stefano, S. and Sodano, G. Tetrahedron Lett. 1980, 5047.

45. Burreson, B.J.; Scheuer, P.J.; Finer, J. and Clardy, J. J. Am. Chem. Soc. 1975, 97, 4763.

46. Hagadone, M.R.; Burreson, B.J.; Scheuer, P.J.; Finer, J. and Clardy, J. Helv. Chim. Acta 1979, 62, 2484.

47. Coll, J.C.; Bowden, B.F.; Tapiolas, D.M.; Willis, R.H.; Djura, P.; Streamer, M.; Trott, L. and Webb, L. Tetrahedron 1984, submitted for publication.

48. Carté, B. and Faulkner, D.J. J. Org. Chem. 1983, 48, 2314.

49. Gustafson, K. and Andersen, R.J. J. Org. Chem. 1982, 47, 2167.

50. Cimino, G.; De Stefano, S. and Minale, L. Experientia 1974, 30, 846.

51. Nakanishi, K. and Kubo, I. Isr. J. Chem. 1977, 16, 28.

52. a) Hall, L.D. and Sanders, J.K.M. J. Am. Chem. Soc. 1980, 102, 570. b) Sanders, J.K.M. and Mersh, J.D. Org. Mag. Res. 1982, 18, 122.

53. Noggle, J.H. and Schirmer, R.E. "The Nuclear Overhauser Effect: Chemical Applications"; Academic Press: New York, 1971.

54. Masumoto, K. and Rapoport, H. J. Org. Chem. 1968, 33, 552.

55. Levy G.C. and Nelson, G.L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists"; Wiley-Interscience: New York, 1972.

56. Fishbein, L. and Cavanaugh, M.A. J. Chromatog. 1965, 20, 283.

57. Chevolot, L. in "Marine Natural Products, Chemical and Biological Perspectives Vol. IV", P.J. Scheuer Ed.; Academic Press, 1981.

58. Nakamura, H.; Wu, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y.; Higashijima, T. and Miyazawa, T. Tetrahedron Lett. 1983, 4105.

59. Cimino, G.; De Rosa, S.; De Stefano, S.; Self, R. and Sodano, G. Tetrahedron Lett. 1983, 3029.

60. For example see: Carlé J.S. and Christophersen, C. J. Org. Chem. 1981, 46, 3440.

61. Piers, E.; Chong, J.M.; Gustafson, K. and Andersen, R.J. Can. J. Chem. 1984, 62, 1.

62. Piers, E.; Chong, J.M.; Morton, H.E. Tetrahedron Lett. 1981, 4905.

63. Greenhalgh, R. and Bannard, R.A.B. Can. J. Chem. 1961, 39, 1017.

64. Barker, P.L.; Bendler, P.L. and Rapoport, H. J. Org. Chem. 1981, 46, 2455.

65. Fuhrman, F.A.; Fuhrman, G.J. and De Riemer, K. Biol. Bull. 1979, 156, 289.

66. Quinn, R.J.; Gregson, R.P.; Cook, A.F. and Bartlett, R.T. Tetrahedron Lett. 1980, 567.

67. Nybakken, J. and Ajeska, R.A. Veliger 1967, 19, 19.

68. Gora, J. and Antczak, U. Perfumer Flavorist 1980, 5, 31.

69. Cimino, G.; De Rosa, S.; De Stefano, S. and Minale, L. Tetrahedron 1974, 30, 645.

70. Armstrong, R.J. Ph.D. thesis 1983, Chemistry Dept., Univ. of British Columbia.

71. Pelletier, S.W.; Lajsia, S.; Ohtsuka, Y. and Djarmati, Z. J. Org. Chem. 1975, 40, 1607.

72. Schulte-Elte, K.H.; Muller, B.L. and Ohloff, G. Nouveau J. Chim. 1978, 2, 427.

73. Do, M.N. and Erickson, K.L. Tetrahedron Lett. 1983, 5699.

74. Pettit, G.R. and Fujii, Y. J. Natural Prods. 1982, 45, 640.

75. Cimino, G.; De Rosa, S.; De Stefano, S. and Minale, L. Tetrahedron 1974, 30, 645.

76. Kazlauskas, R.; Murphy, P.T. and Wells, R.J. Tetrahedron Lett. 1979, 903.

77. Fenical, W. in "Marine Natural Products, Chemical and Biological Perspectives, Vol. II", P.J. Scheuer Ed.; Academic Press: New York, 1978, p. 174.

78. Amico, V.; Oriente, G.; Piattelli, M.; Tringali, C.; Fattorusso, E.; Magno, S. and Mayol, L. Experientia 1977, 33, 1274.

79. Howard, B.M. and Fenical, W. Tetrahedron Lett. 1976, 41.

80. a) Cimino, G.; De Stefano, S.; Guerriero, A. and Minale, L. Tetrahedron Lett. 1975, 1417. b) Cimino, G.; De Stefano, S.; Guerriero, A. and Minale, L. Tetrahedron Lett. 1975, 3723.

81. Cimino, G.; De Stefano, S. and Minale, L. Experientia 1973, 29, 1063.

82. Cimino, G. in "Marine Natural Products Chemistry", D.J. Faulkner and W.H. Fenical, Eds.; Plenum Press: New York 1977, p. 61.

83. Blackman, A.J. and Wells, R.J. Tetrahedron Lett. 1976, 2729.