REARRANGED SPONGIAN DITERPENOIDS FROM THE NUDIBRANCH *CHROMODORIS CAVAE* THAT MAY SERVE AS A CHEMICAL DEFENSE.

By

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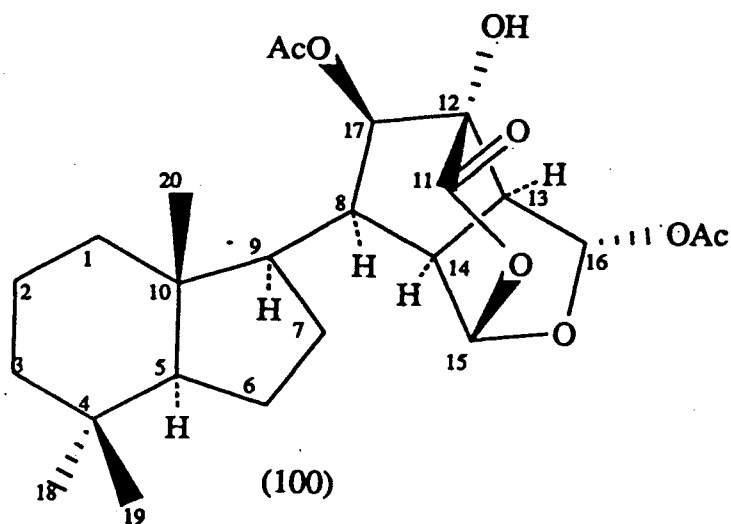
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Date 5ᵗʰ January 1989

Abstract

Nudibranchs of the genus *Chromodoris* are known to selectively sequester sponge metabolites which have novel structures and often serve as deterrents to predation. Spectroscopic and X-ray diffraction studies on a biologically active metabolite from *Chromodoris cavae* have led to the discovery of a diterpenoid, chromodorolide A (100), with a new carbon skeleton.

(100)

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ABBREVIATIONS

AcOH	acetic acid
AcO	acetate
APT	Attached Proton Test
BB	BroadBand ^{1}H decoupled
C_6D_6	benzene-d$_6$
calcd.	calculated
CaSO$_4$	calcium sulphate
CDCl$_3$	chloroform-d
CHCl$_3$	chloroform
CH$_2$Cl$_2$	dichloromethane
CH$_2$N$_2$	diazomethane
CI	Chemical Ionization
COSY	COrrelated SpectroscopY
COSYPHDQ	PHase sensitive Double Quantum COrrelated SpectroscopY
CrO$_3$	chromium (VI) trioxide
1D	one-dimensional
2D	two-dimensional
2DJ	J-Resolved spectroscopy

D_2O water-d_2

EI   electron impact

Et_2NH diethylamine

Et_2O diethyl ether

fig.   figure

HETCOR   HETeronuclear CORrelation

HCO_2H formic acid

hplc   high performance liquid chromatography

hrms   high resolution mass spectrum (electron impact)

ir   infrared

K_2CO_3 potassium carbonate

LAH   lithium aluminum hydride

Li   lithium

MeOH   methanol

mp   melting point range

ms   mass spectrum (low resolution)

NH_3 ammonia

^1H nmr   proton nuclear magnetic resonance

^{13}C nmr   carbon-13 nuclear magnetic resonance

nOe   nuclear Overhauser enhancement

Pd/C	palladium on activated carbon
POCl$_3$	phosphoryl chloride
ppm	parts per million
Py	pyridine
ref.	reference
rel. int.	relative intensity
s	solvent signal
tlc	thin layer chromatography
TMS	tetramethylsilane
uv	ultraviolet
w	water signal

Acknowledgements

I wish to sincerely thank my research supervisor, Dr. Raymond Andersen, for his encouragement and guidance during the course of this work. It has been my pleasure to work with him.

Introduction

A. Chemical defense in nudibranchs.

Shell-loss in marine opisthobranchs has been compensated for by the evolution of secondary defensive mechanisms [1]. Nowhere is this more clearly demonstrated than in the completely shell-less nudibranchs (Fig. 1), whose secondary defensive mechanisms can be classified into three categories; behavioral (hiding), morphological and chemical.[2]

Morphological defenses include camouflage, spicule incorporation and partial automization. Camouflaging can be achieved through cryptic colorization (homochromy) which enables the molluscs to resemble their food source; countershading which is thought to make their outline indistinct; or disruptive colorization which makes it hard to spot the organism as a whole. Some nudibranchs reinforce their mantles with spiny spicules from sponges, making them difficult to chew. Many nudibranchs employ autonomy; the ability to shed portions of their anatomy while under attack. Aeolid nudibranchs readily discard their cerata when harassed and some discodorids have been observed to shed their mantle edges [3]. The most astounding morphological defense, however, can be observed in certain coelenterate-feeding aeolids which manage to salvage intact nematocysts from their diet and transport them to specialized areas at the tips of their cerata [4]. When disturbed the nudibranchs will release and fire these stinging cells. A few aeolids of this type are able to selectively retain only the most potent nematocysts of their

[1] Faulkner D.J.; *Marine Ecology Progress Series* 1983, *13*, 295.
[2] Karuso, P. "Chemical Ecology of the Nudibrachs", in *Bioorganic Marine Chemistry*, P.J. Scheuer, Ed., Springer Verlag, Berlin, 1987: Vol. 1, pp. 31-60.
[3] Ibid.
[4] a) Grosvenor, G.H. *J. Roy. Soc. London*, 1903, *72*, 462. b) Thompson, T.E. and Bennett, I. *Science*, 1969, *166*, 1532.

TYPICAL CRYPTOBRANCH DORID NUDIBRANCH

Rhinophore

Dorsum

Branchial Plume

Foot
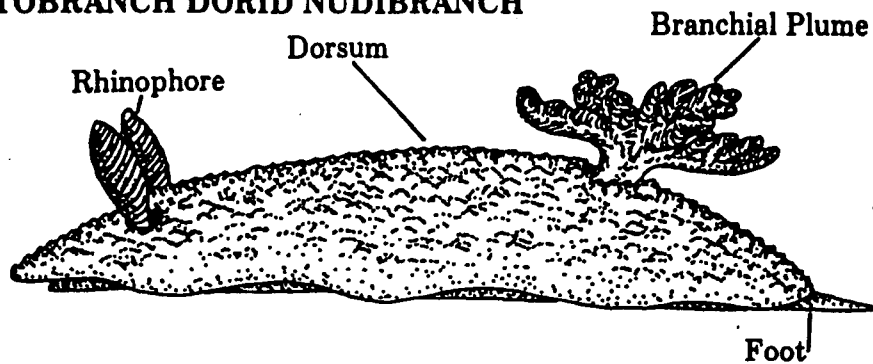
TYPICAL AEOLID NUDIBRANCH

Rhinophores

Ceratal Group

Ceras

Foot Corner

Oral Tentacle

Branchial Plume

Rhinophores

Oral Tentacles

Extra-branchial Appendage

Extra-rhinophoral Appendage

Foot Corner
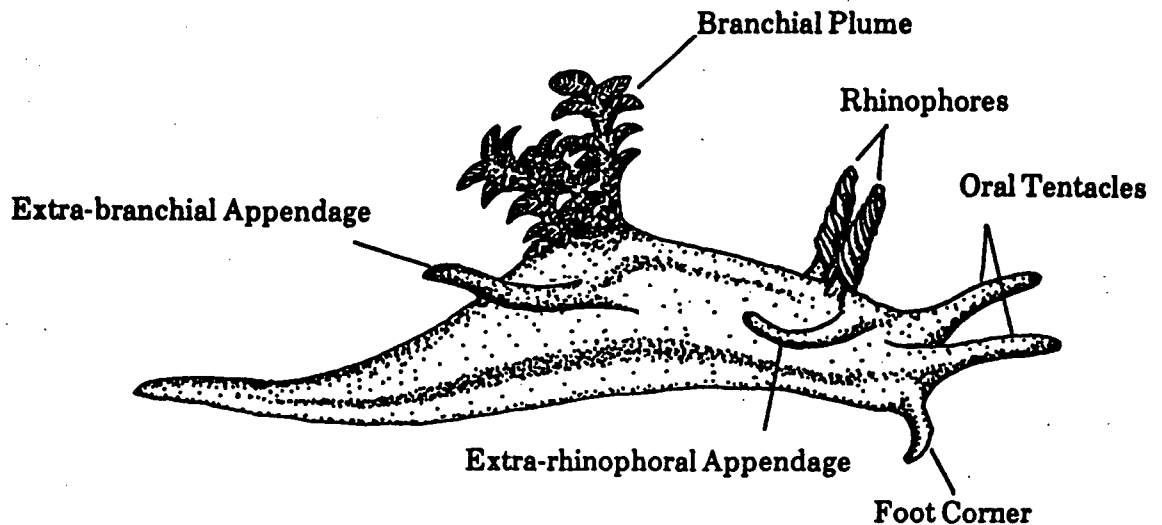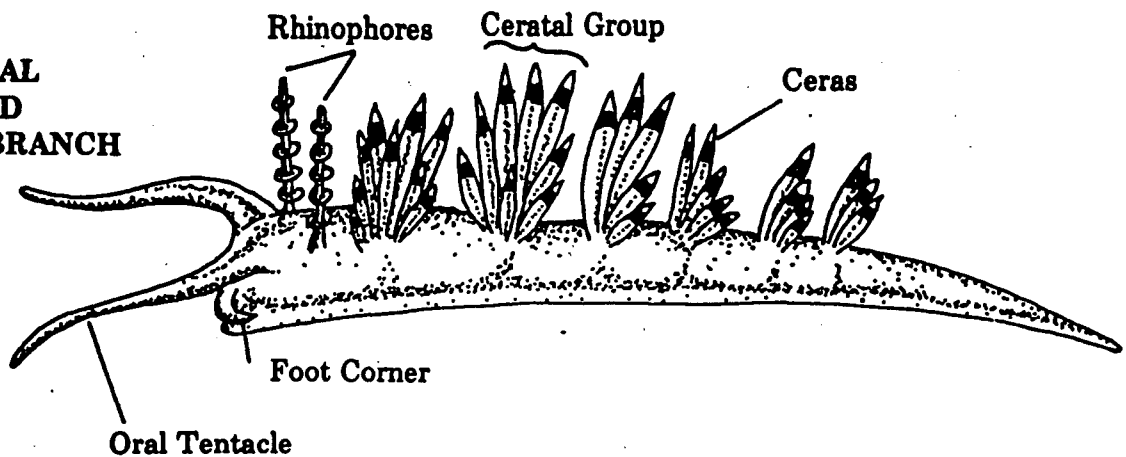
TYPICAL PHANEROBRANCH DORID NUDIBRANCH

Fig. 1   Typical Nudibranchs

coelenterate food, although how they distinguish among the various nematocysts or transport these alien cells intact through their bodies to their cerata tips is still a mystery.

Perhaps the most interesting defense mechanism developed in nudibranchs is the selective aggregation of defensive metabolites from their food sources, using these in turn as their own chemical deterrents. Garstang observed this amazing ability in 1890 when he noted that many nudibranchs secreted acid as a defense, especially those observed to feed on acidic tunicates [5]. Others subsequently noted that live nudibranchs would not be accepted as food by aquarium fishes and certain nudibranch species even killed fish that shared the aquarium. In 1960 Thompson found specialized skin glands in non-acidic nudibranchs containing bitter or tasteless fluids which he postulated contained compounds employed as chemical defenses by the shell-less molluscs [6].

Since then, researchers have amassed considerable evidence supporting the role of unique secondary metabolites as chemical defenses in nudibranchs. Species from five families of nudibranchs contain organic compounds in their skin extracts which serve as either antifeedants or icthyotoxins [7]. Such chemical defenses are particularly noticeable in dorids that feed on sponges. Sponges have long been recognized as having evolved elaborate chemical mechanisms to solve such problems as predation, fouling, and establishing a "home" within the marine environment. Screening and identifying any of these substances which might benefit humans has become a growing research concern [8].

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[5] see note 2 above.
[6] a) Thompson, T.E. *J. Mar. Biol. Ass. U.K.*, 1960, *39*, 115. b) Thompson, T.E. *J. Mar. Biol. Ass. U.K.*, 1960, *39*. 123. c) Thompson, T.E. *Aust. J. Zool.* 1969, *17*, 755.
[7] Gunthorpe, L. and Cameron, A.M. *Mar. Biol.*, 1987, *94*, 39.
[8] Some current general references include: a) Thompson, J.E.; Walker, R.P.; Faulkner, J.D. *Mar. Biol.*, 1985, *88*, 11. b) McCaffrey, E.J. and Endean, R. *Mar. Biol.*, 1985, *89*, 1. c) Munro, M.H.G.; Luibrand, R.T.; Blunt, J.W. "The Search for Antiviral and Anticancer Compounds from Marine Organisms", in *Bioorganic Marine Chemistry, Vol. I*, 1987, Springer-Verlag.

Nudibranchs in the family *Chromodoridae* are spongiverous, brightly colored and rarely, if ever, eaten by reef fishes making them prime candidates for studies of chemical defenses. Roughly twenty species of this family have been examined to date and all have been found to contain interesting metabolites that may be defensive allomones.

The primitive chromodorid *Cadlina luteomarginata* has proven to be a rich source of interesting secondary metabolites [9]. This common, fragrant nudibranch has been found to contain terpenes with isonitrile, isothiocyanate and furan functionalities. The isonitriles and associated isothiocyanates, such as **1** and **2**, from this nudibranch are icthyotoxic and inhibit feeding by goldfish and sculpin in lab assays. Furodysinin (**3**), idadione (**4**) and pallescensin-A (**5**) also serve as defensive allomones for *C. luteomarginata*, while the terpenoid albicanylacetate (**6**), isolated from specimens collected in British Columbian coastal waters, is the most potent antifeedant found in this nudibranch. Seasonal and regional variations indicate that *C. luteomarginata* obtains these metabolites from dietary sources.

(1)   X = NC

(2)   X = NCS

(3)
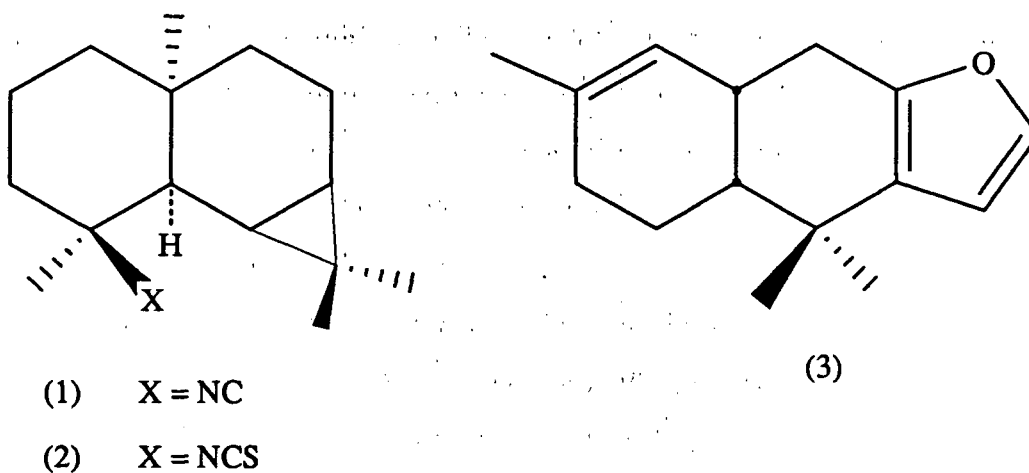
[9] a) Thompson, J.E.; Walker, R.P.; Wratten, S.J.; Faulkner, D.J. *Tetrahedron*, **1982**, *38*, 1865. b) Hellou, J.; Andersen, R.J.; Thompson, J.E. *Tetrahedron*, **1982**, *38*, 1875. c) Hellou, J. and Andersen, R.J. *Tetrahedron Lett.*, **1981**, *22*, 4173.

(4)

(5)

(6)
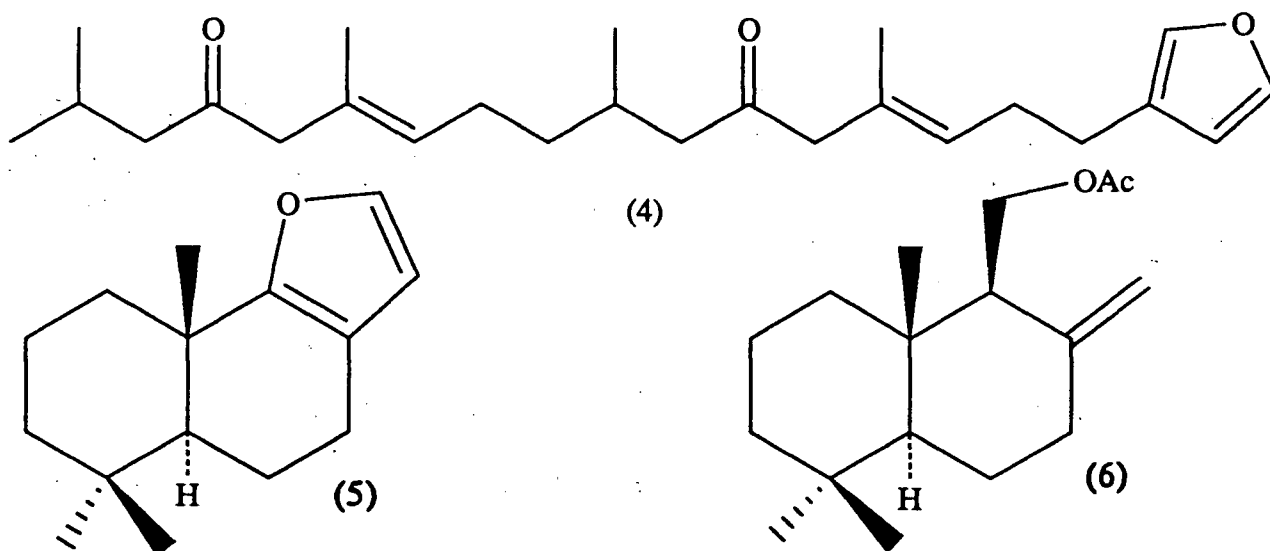
Chromodoris elisabethina employs latrunculin-A (7), an icthyotoxin, for its defense [10], as does *C. lochi* which obtains the metabolite from the sponge *Spongia mycofijiensis*[11]. The isomer, latrunculin-B (8), is the major defensive allomone for *Glossodoris quadricolor* and its prey, the red sponge *Latrunculia magnifica* [12].

(7)

(8)
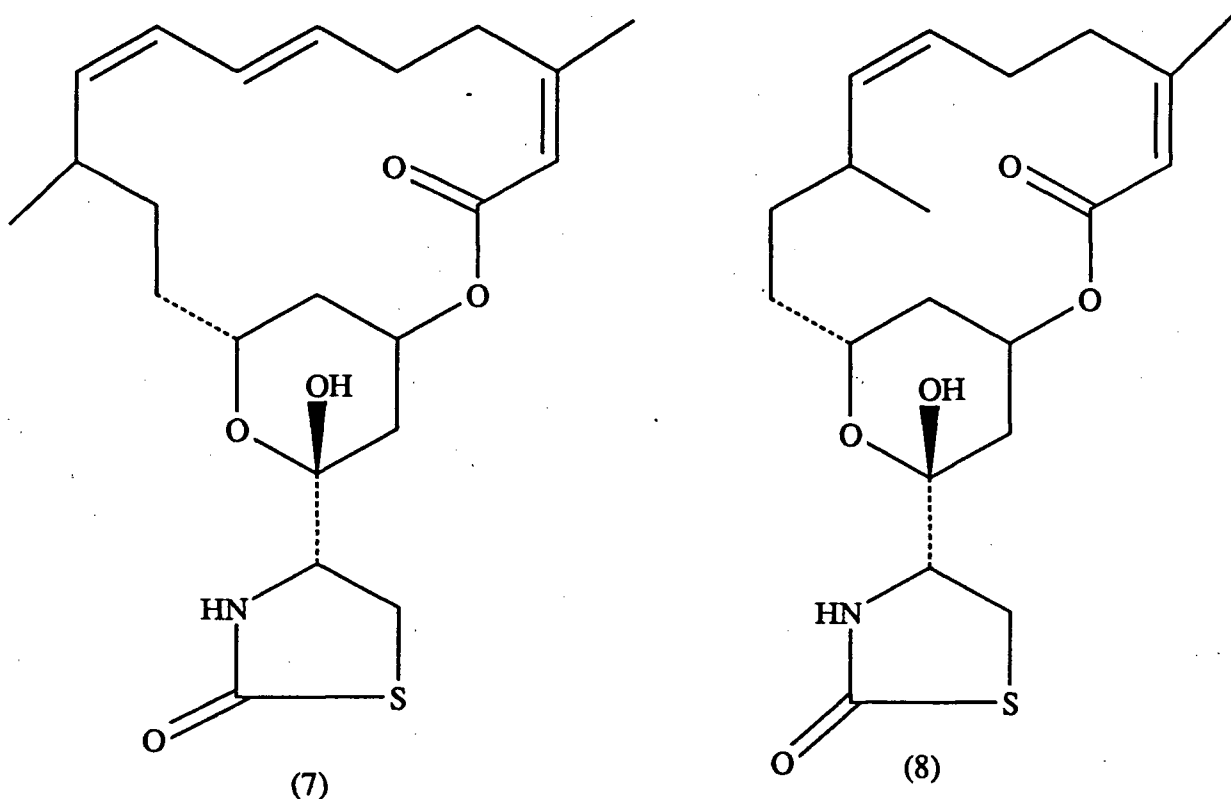
[10] Okuda, R.K. and Scheur, P.J. *Experentia*, 1985, *41*, 1355.
[11] Kakou, Y.; Crews, P.; Bakus, G.J. *J. Nat. Prod.*, 1987, *50*, 482.
[12] Mebs, D. *J. Chem. Ecol.*, 1985, *11*, 713.

Dysidea fragilis, a Pacific marine sponge, and two nudibranchs observed feeding on it, *Chromodoris maridadilus* and *Hypselodoris godeffroyana*, were all found to contain the furanosesquiterpenoids nakafuran-8 (9) and -9 (10) [13]. These two compounds proved to be effective antifeedants against common reef fishes. Other metabolites, found in *D. fragilis* but not common to all three organisms, failed to show any antifeedant activity suggesting that the nudibranchs selectively acquire only the effective defensive compounds in their diet.

(9)   (10)
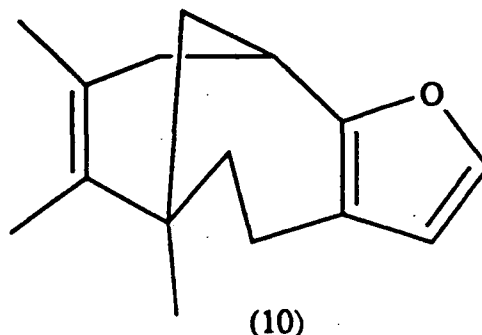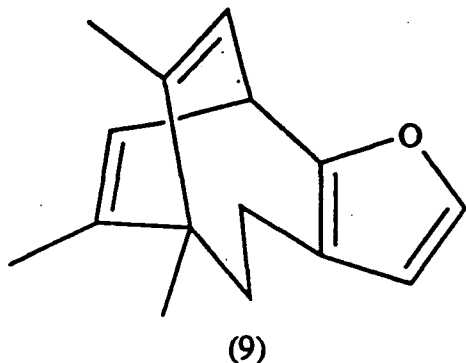
Although only compounds **1 - 10** above are proven antifeedants, many other compounds of similar structure have been isolated from chromodorid nudibranchs. Potential defensive metabolites called scalarins, similar to **11** , have been isolated from three species of *Chromodoris; C. funerea, C. sedna* and *C. youngbleuthi*[14]. A variety of linear and cyclic furans such as marislin (12) and furodysinin (13) have been isolated from a number of chromodorid nudibranchs[15]. Spiniferin-2 (14), a compound similar to albicanylacetate, has been isolated from Hawaiian specimens of *C. albonotata* [15d]. The rearranged diterpenoids norrisolide (15) and the macfarlandins (16-20), from *C. norrisi* and *C. macfarlandi* respectively[16], are degradation products of a class of sponge metabolites called "spongians" which may serve as defensive allomones in both phyla.

[13] Shulte, G.; Scheuer, P.J. and McConnell, O.J. *Helv. Chim. Acta*, 1980, *63*, 2159.
[14] a) Kernan, M.R.; Barrabee, E.B. and Faulkner, D.J. *Comp. Biochem. Physiol.*, 1988, *89B*, 275. b) Hochlowski, J.E.; Faulkner, D.J.; Bass, L.S.; Clardy, J. *J. Org. Chem.*, 1983, *48*, 1738. c) Terem, B. and Scheuer, P.J. *Tetrahedron*, 1986, *42*, 4409.
[15] a) Hochlowski, J.E. and Faulkner, D.J. *Tetrahedron Lett.*, 1981, *22*, 271. b) Cimino, G.; De Stefano, S.; De Rosa, S.; Sodano, G.; Villani, G. *Bull. Chem. Soc. Belg.*, 1980, *89*, 1069. c) Hochlowski, J.E.; Walker, R.P.; Ireland, C.; Faulkner, D.J. *J. Org. Chem.*, 1982, *47*, 88. d) Shulte, G.R. and Scheuer, P.J. *Tetrahedron*, 1982, *38*, 1857.
[16] see notes [37], [39] and [42]

(11)

(12)

(13)

(14)

(15)

(16) $R_1 = H$ $R_2 = OAc$

(17) $R_1 = OAc$ $R_2 = H$

(18)

(19)

(20) $R_1 = H$ $R_2 = OAc$
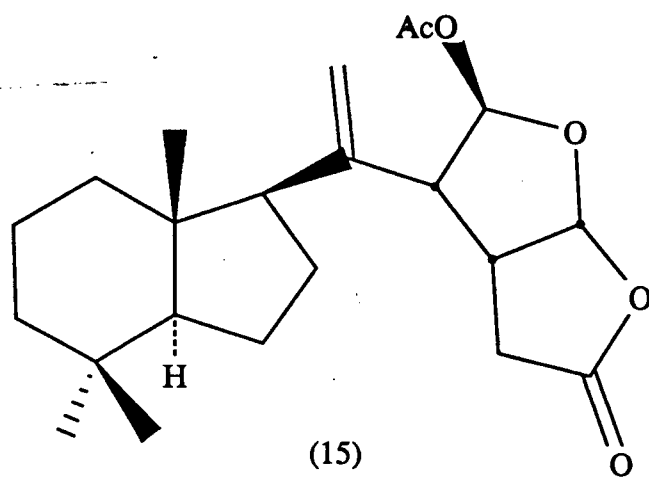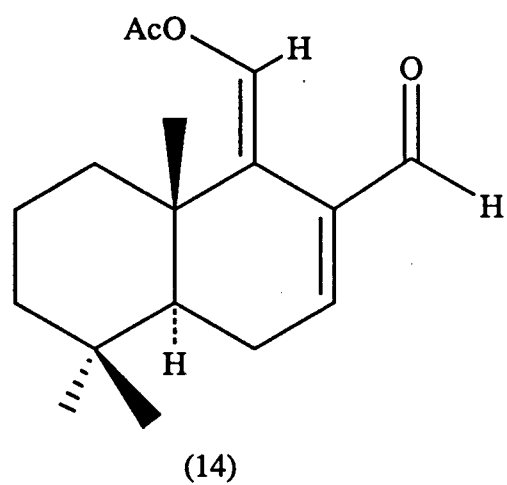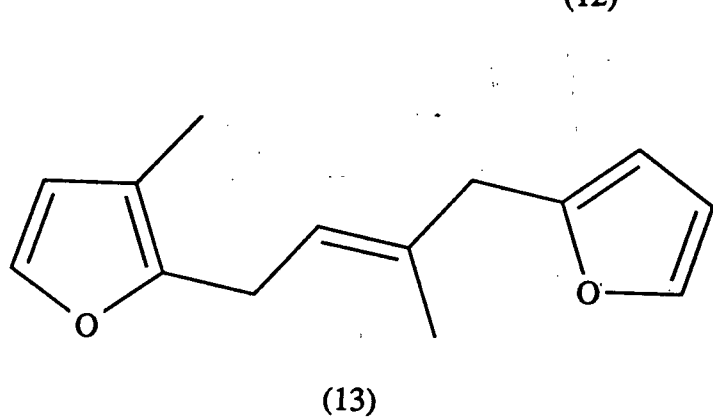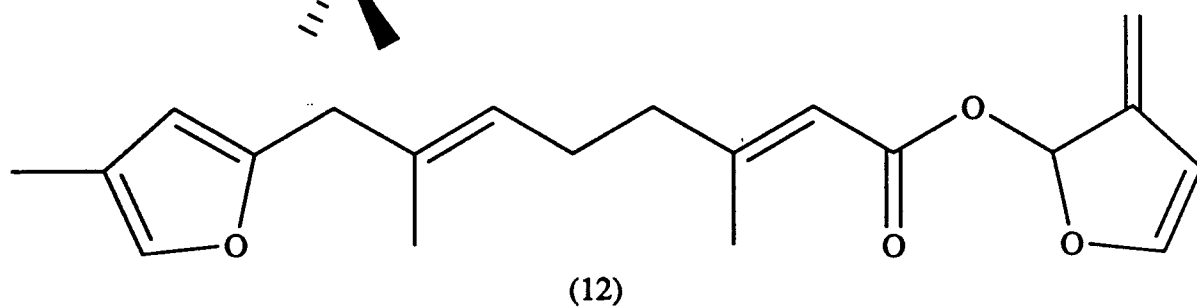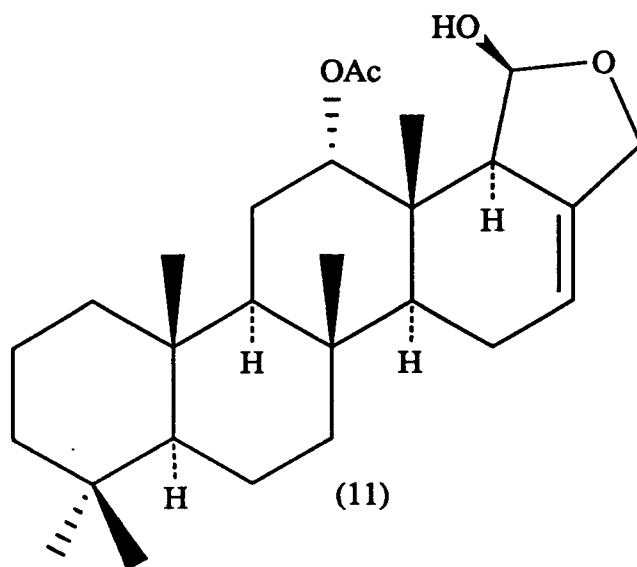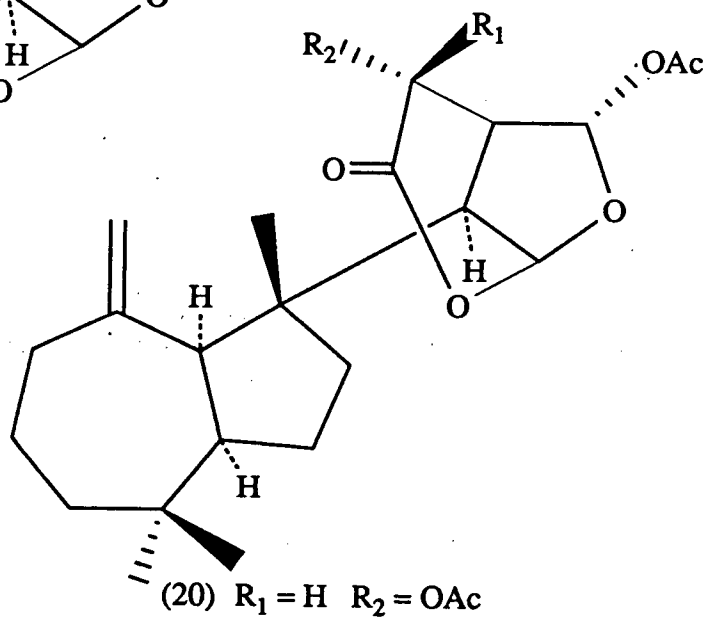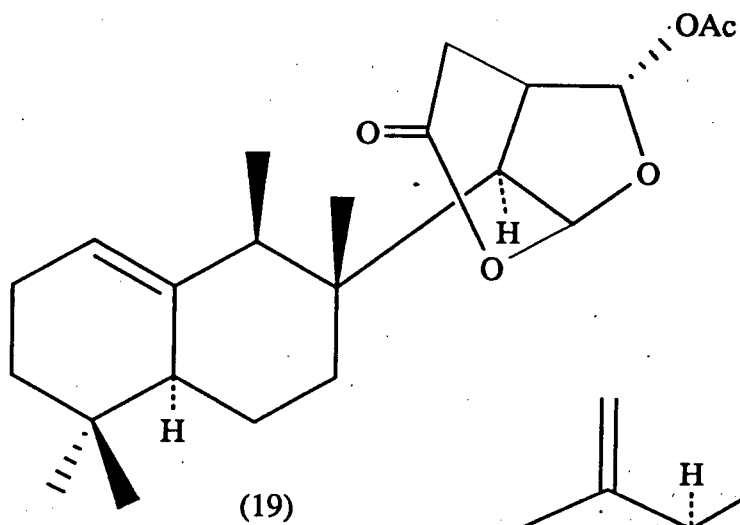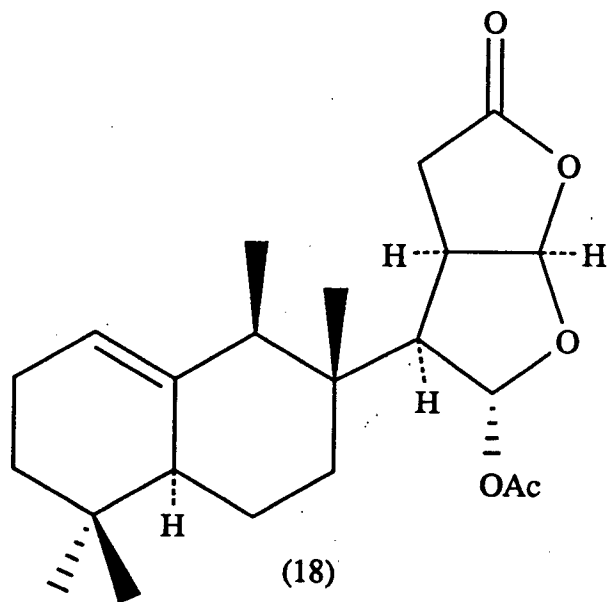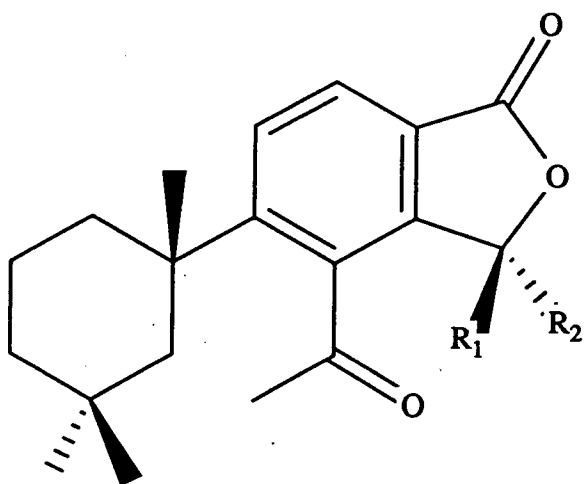
B. Spongian Diterpenes.

Among the most interesting of the secondary metabolites found in skin extracts from various Chromodoridae nudibranchs are those classified as containing or deriving from the novel hypothetical "spongian" diterpene skeleton (21). The large number of reports detailing the isolation and structures of related compounds published in recent years as well as discoveries in our laboratory have sparked our interest in this novel class of marine diterpenes. In light of the current interest in these compounds, a detailed review of previous work on their isolation and synthesis is warranted.

(21)
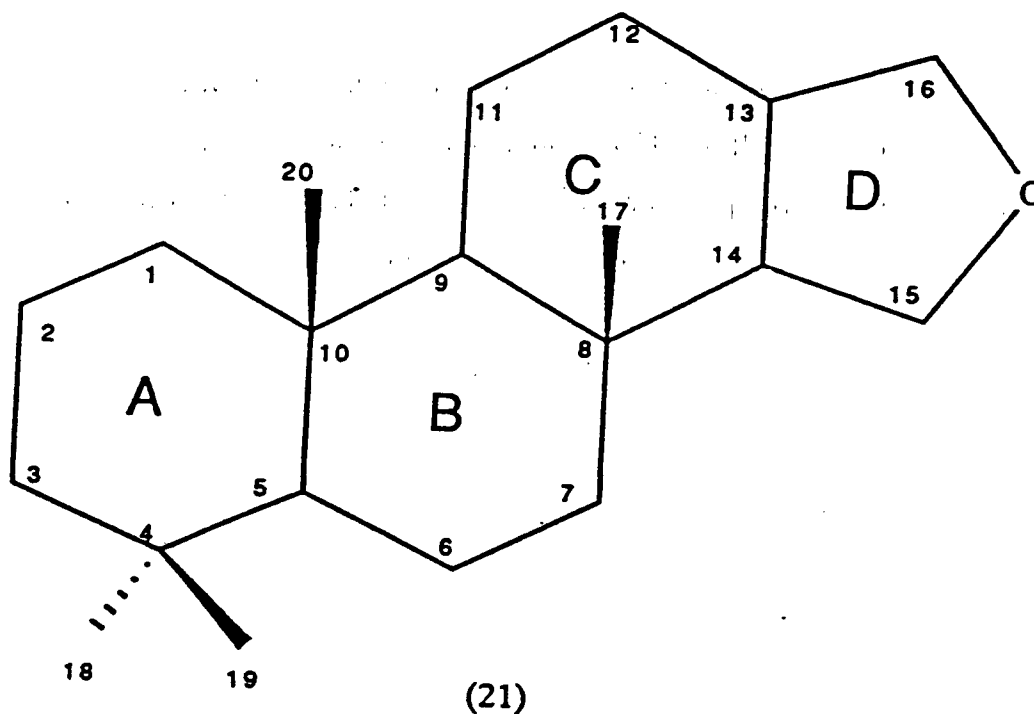
The first diterpene of this novel class was reported by Cimino et. al. [17] who isolated it from the marine sponge *Spongia officinalis*. Because of its relation to isoagathic acid (23) the compound was named isoagatholactone (22). The mass spectral fragmentation pattern led to conclusion that the new compound is related to methyl isoagathate (23, R = Me). A detailed series of chemical reactions outlined in scheme I was used to confirm this hypothesis. Identical optical rotations and spectra were observed for isoagathic alcohol (25, scheme I) obtained from both grindellic acid (24, scheme I) and isoagatholactone ($[\alpha]_D = -9^0, -10^0$ respectively) which also proved the absolute stereochemistry of the new terpenoid.

(22)

(23) R = H, Me
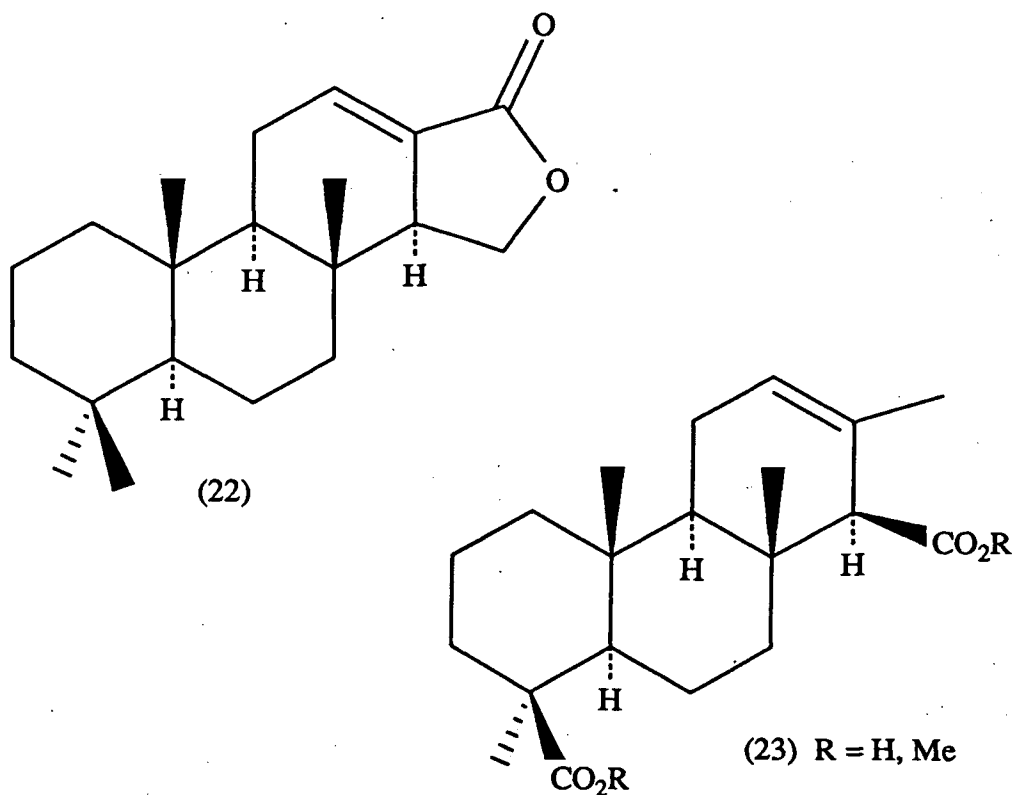
[17] Cimino, G.; de Rosa, D.; de Stefano, S.; Minale, L. *Tetrahedron*, 1974, *30*, 645.

Scheme I - 11 -

(24)

LAH

Li, EtNH₂

CrO₃, Py
CH₂N₂

(reagents labeled: 1 CrO₃, Py; 2 CH₂N₂)

POCl₃, Py

+

HCO₂H

LAH

(22)

LAH

H₂, Pd/C

(25)
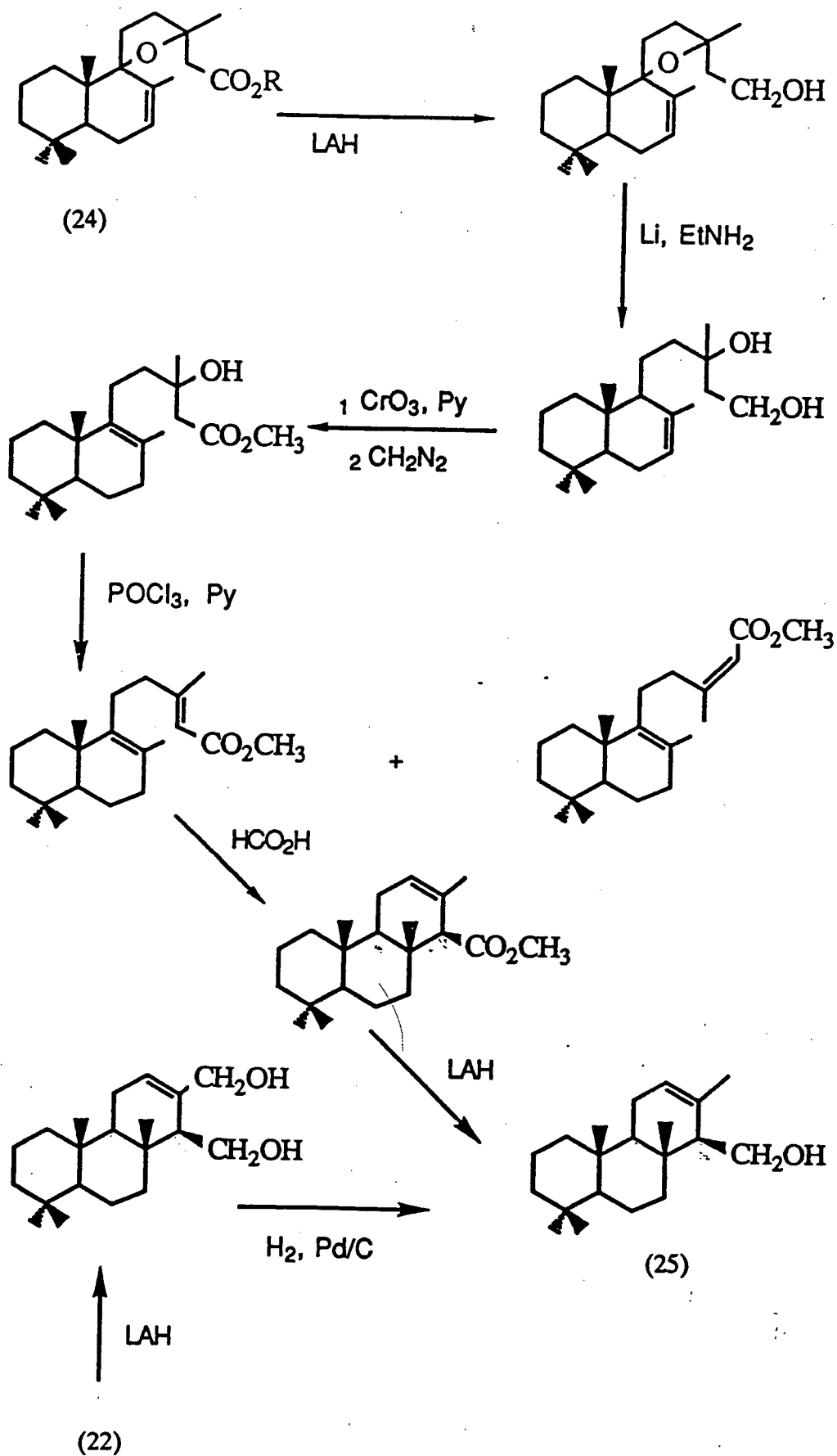
Five years later Kazlaukas and coworkers reported the isolation and structure of a related spongian diterpene isolated from the sponge *Aplysilla rosea*[18]. The compound, aplysillin (26, 12α,15α,16α-triacetoxy-spongian), had its structure confirmed by a single crystal X-ray experiment. However, this work is not without controversy. A later effort to establish chemotaxonomic evidence for the classification of the so-called advanced sponges (those with reduced or absent spicules) has reclassified the sponge as a member of the genus *Darwinella*[19] and failed to detect any trace of aplysillin in fresh extracts of the sponge. A result which is consistent with chemotaxonomic evidence from closely related species.

(26)
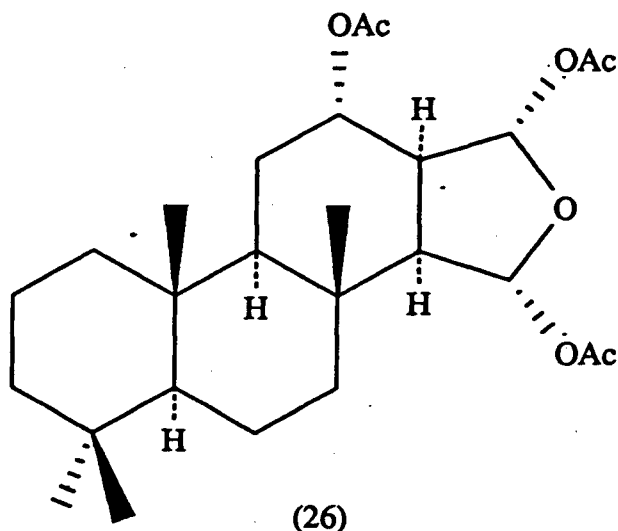
[18] Kazlauskas, R.; Murphy, P.T.; Wells, R.J. Tetrahedron Lett. 1979, 10, 903.

[19] Karuso, P.; Bergquist, P.R.; Cambie, R.C.; Buckleton, J.S.; Clark G.R.; Rickard, C.E.F Aust. J. Chem., 1986, 39, 1643.

The unique skeleton shared by the spongians has been of some interest to synthetic chemists. Two South American groups have reported synthetic studies on the spongian diterpenes in the last seven years [20]. Although to date no stereospecific synthesis has been carried out on the spongian skeleton, enantiomeric isoagatholactone (28) as well as the parent hydrocarbon isocopalane (29) have been synthesized from methyl isocopalate (27) and racemic isoagatholactone has been synthesized from racemic labda-8(20),13-dien-15-oic acid (30).
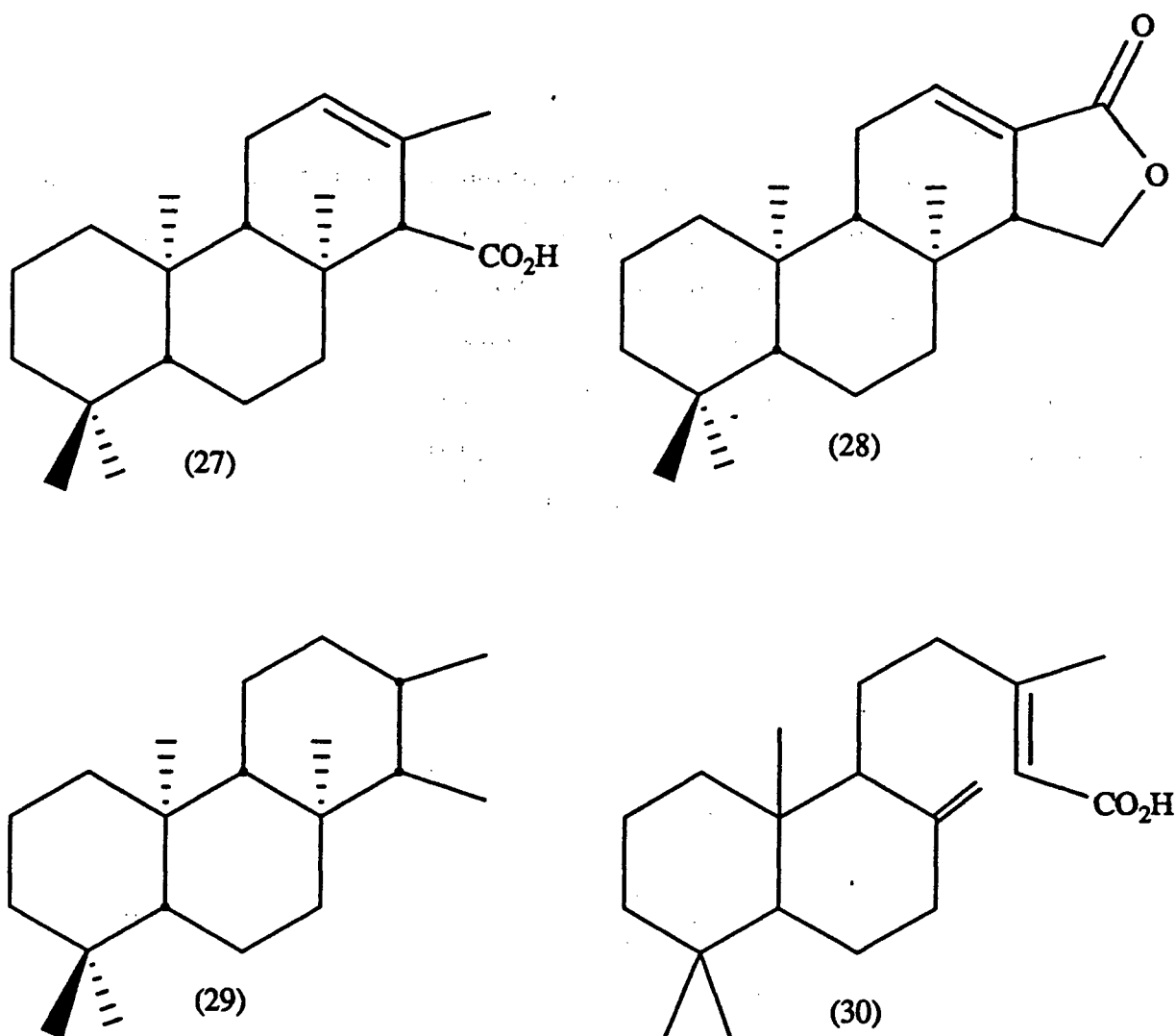
[20] a) de Miranda, D.S.; Brendolan, G.; Imamura, P.M.; Sierra, M.G.; Marsaioli, A.J.; Rùveda, E.A. *J. Org. Chem.*, 1981, *46*, 4851. b) Nakano, T. and Hernàndez, M.I. *Tetrahedron Lett.*, 1982, *14*, 1423. c) Nakano, T. and Hernàndez, M.I. *J. Chem. Soc. Perkin. Trans. I.*, 1983, 135. d) Mischne, M.P.; Sierra, M.G.; Rùveda, E.A. *J. Org. Chem.*, 1984, *49*, 2035.

Following up earlier work on *S. officinalis*[17], Cimino and coworkers examined a fresh specimen of the sponge and reported the isolation of four new metabolites 31-34[21]. In addition to isoagatholactone (22), *S. officinalis* was found to contain 15α,16α-diacetoxyspongian (31), a compound similar to aplysillin (26) but lacking the C-12 acetoxy function, and three tricyclic diterpenes lacking the D ring system 32-34. These opened structures, *ent*-isocopal-12-en-15,16-dials, are considered to be possible precursors to the spongian system and are named from the base hydrocarbon isocopalane (29) previously synthesized from methyl isocopalate [20a].
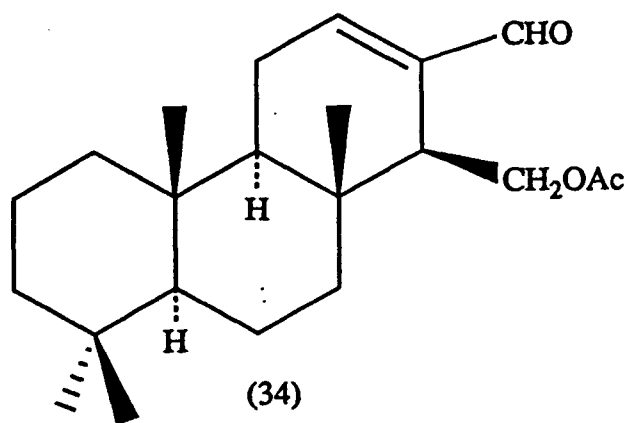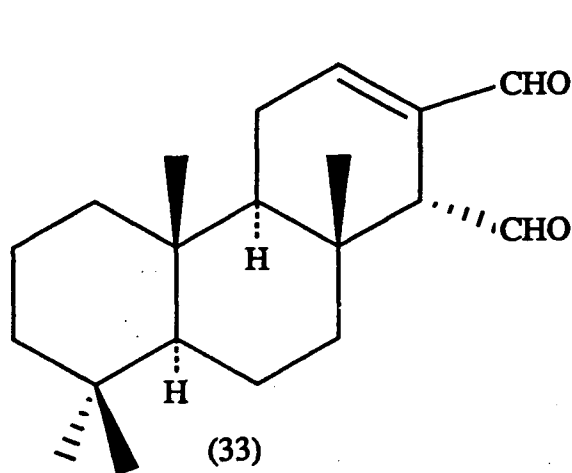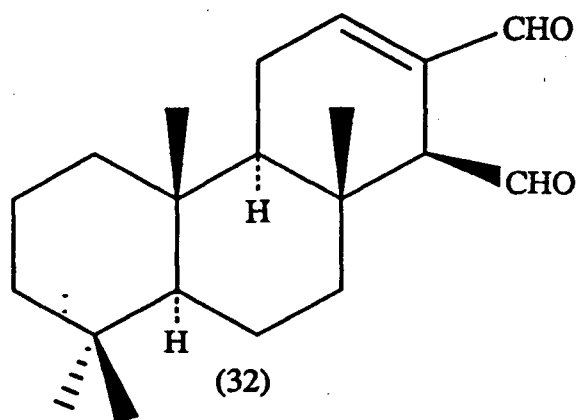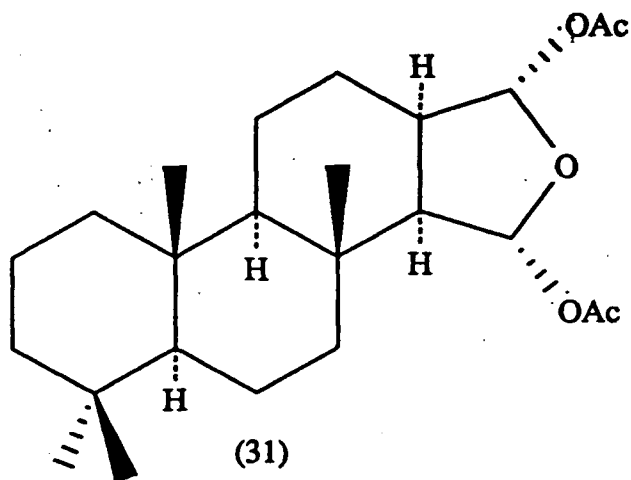
[21] Cimino, G.; Marrone, R.; Sodano, G. *Tetrahedron Lett.*, 1982, *23*, 4139.

Another group examining *Spongia officinalis* from the Canary Islands [22] isolated aplysillin (26) as well as four new spongian diterpenes closely related to isoagatholactone (22) indicating that this sponge is a rich source of compounds with the spongian carbon skeleton. Of the hydroxyspongia-12-en-16-ones (35-38) isolated, the 11β-hydroxy- and 11β-acetoxy- compounds are biologically active against broad spectrum bacteria and inhibit HeLa cell growth.
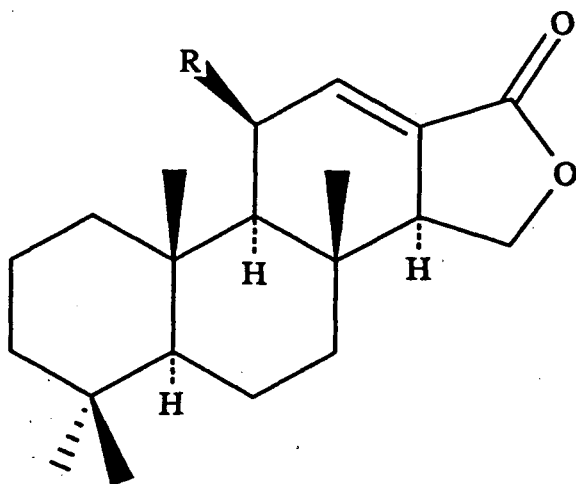
(35)   R = OH

(36)   R = OAc
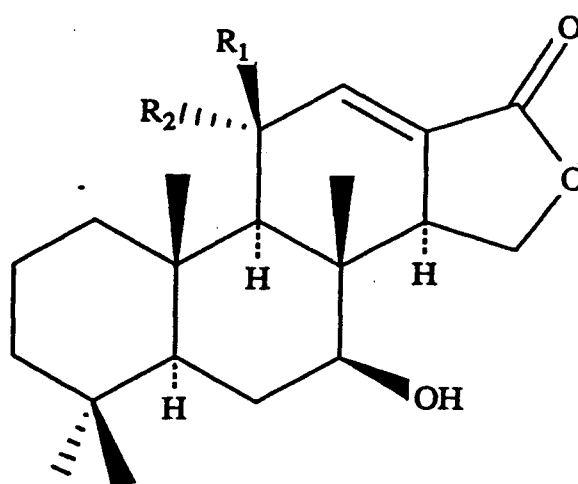
(37) R_1 = OH R_2 = H

(38) R_1 = H R_2 = OH

[22] Gonzalez, A.G.; Estrada, D.M.; Martin, J.D.; Martin, V.S.; Perez, C.; Perez, R. *Tetrahedron*, 1984, *40*, 4109.

Karuso and Taylor have also isolated spongian diterpenes closely related to isoagatholactone[23]. These two compounds, 7α-Acetoxyspongia-16-one (39) and 7α,17-diacetoxyspongia-16-one (40), were isolated from specimens of *Aplysilla rosea* collected in the Great Barrier Reef.

(39)   (40)
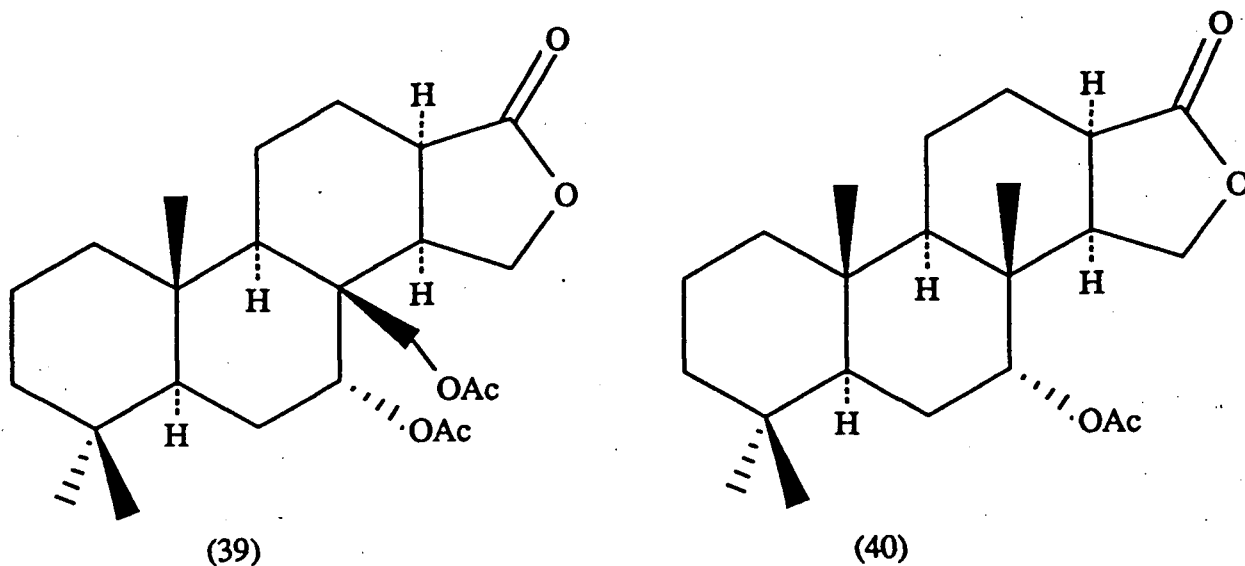
The same year they reported the structure of aplysillin, Kazlaukas *et. al.*[24] reported the isolation of another variety of diterpenes related to spongian. The spongiols, 41-48, isolated from various species of *Spongia* sponges collected in the Great Barrier Reef, were the first diterpenoids related to isoagatholactone (22) to be reported. The term "spongian" was used first in this paper and, following IUPAC guidelines, they proposed a semisystematic nomenclature for naming diterpenes with the same carbon skeleton. Any functionality on the tetracyclic hydrocarbon is listed by the numbering scheme of the hypothetical spongian skeleton (21). Hence, isoagatholactone (22) is named under the new system as spongia-12-en-16-one and the compounds isolated by Kazlaukas and coworkers are named 3α,19-dihydroxyspongia-13(16),14-dien-2-one (41, spongiadiol); 3α,19-diacetoxyspongia-13(16),14-dien-2-one (42, spongiadiol diacetate); 3α,17,19-trihydroxyspongia-13(16),14-dien-2-one (43, spongiatriol); 3α,17,19-triacetoxyspongia-13(16),14-dien-2-

[23] Karuso, P. and Taylor, W.C. *Aust. J. Chem.*, 1986, *39*, 1629.
[24] Kaslaukas, R.; Murphy, P.T.; Wells, R.J.; Noack, K.; Oberhänsli, W.E.; Schönholzer, P. *Aust. J. Chem*, 1979, *32*, 867.

one (**44**, spongiatriol triacetate) as well as the 3β epimers of these four compounds (**45-48**, epispongiadiol etc.). While trivial names still persist in the literature, especially for highly functionalized derivatives, most authors follow the naming scheme outlined in this paper. Some years later another Australian group reexamined the same sponge Kazlaukas had worked on and classified it as belonging to a new genus, *Rhopaloeides* [25]. *R. odorablie*, as the species is now named, was found consistently to contain the four 3α epimers **41-44**, though samples genetically identical to each other produced varying amounts of the spongiols depending on environmental factors. No evidence was found for the natural presence of any 3β-spongiol and it was suggested that these compounds may have resulted by silica catalyzed epimerization in Kazlaukas' purification .
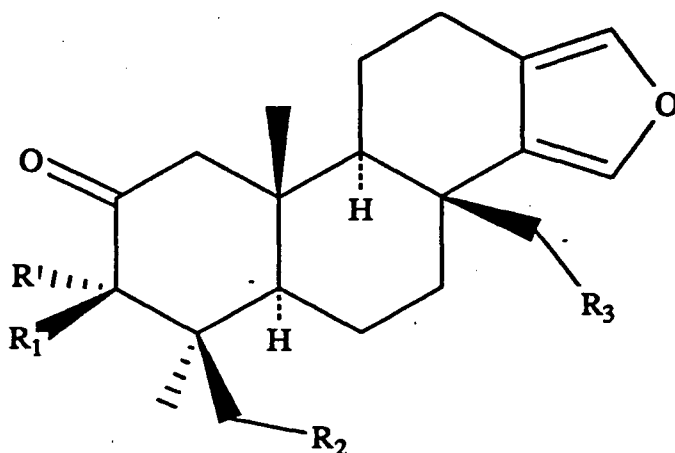
	R	R₁	R₂	R₃
(41)	OH	H	OH	H
(42)	OAc	H	OAc	H
(43)	OH	H	OH	OH
(44)	OAc	H	OAc	OAc
(45)	H	OH	OH	H
(46)	H	OAc	OAc	H
(47)	H	OH	OH	OH
(48)	H	OAc	OAc	OAc

[25] Thompson, J.E.; Murphy, P.T.; Bergquist, P.R.; Evans, E.A. *Biochem. System. Ecol.* 1987, *15*, 595.

The marine sponge *Spongia officinalis* continued to provide diterpenes related to isoagatholactone in subsequent research efforts. A Belgian group working on specimens of this sponge collected in the waters near Papua-New Guinea isolated three new spongians 49-51 in 1980[26]. These three compounds all contain the furan moiety of the spongiadiols but lack any oxidation of the A ring system. In fact, spongia-13(16),14-dien-19-oic acid (49) is remarkably similar to the isoagathic acid and may represent an early stage in biosynthesis.

(49)

(50)

(51)
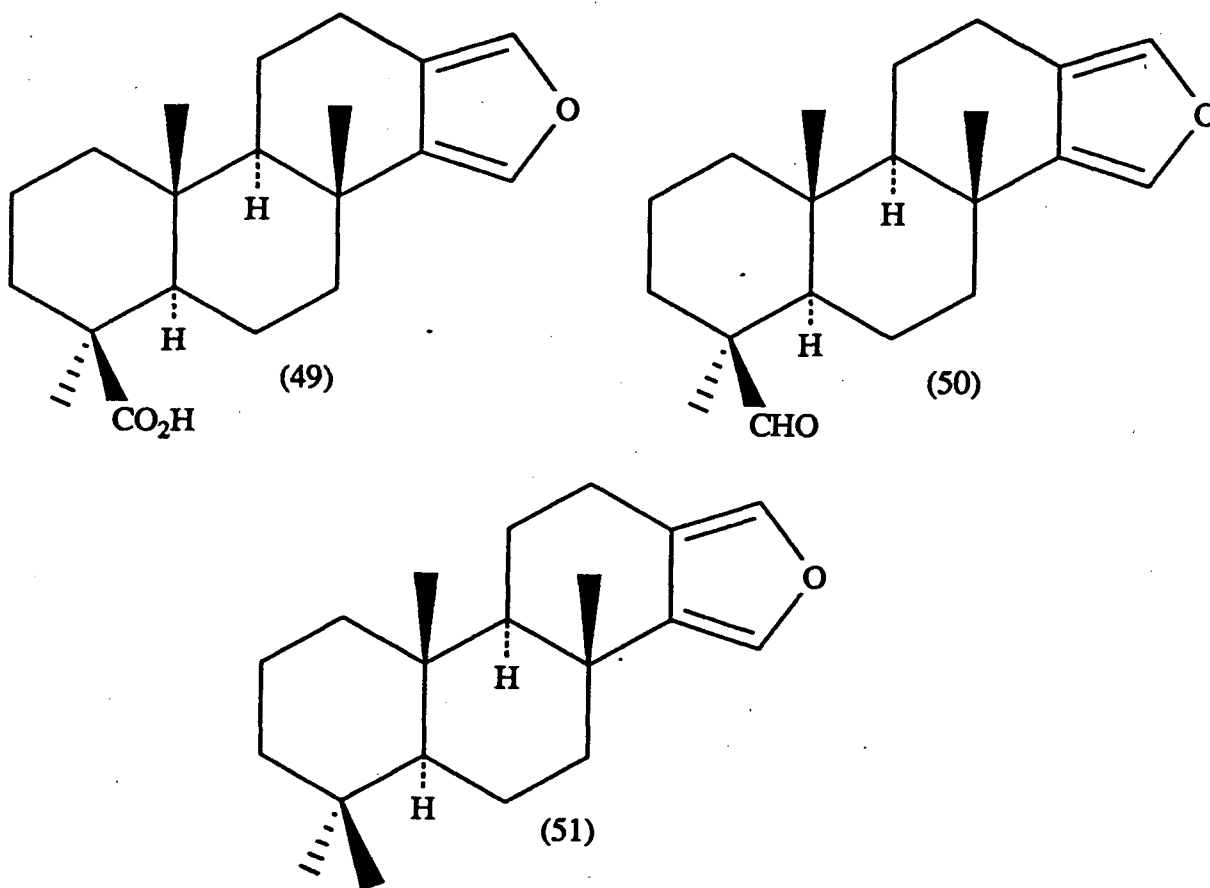
[26] Capelle, N.; Braekman, J.C.; Daloze, D.; Tursch, B. *Bull. Soc. Chim. Belg.*, 1980, *89*, 399.

Six spongian diterpenes **42, 44, 52-55** were isolated from a dorid nudibranch, *Casella atromarginata*, by de Silva and Scheuer in 1982 [27]. Two of these were the previously reported peracetates of spongiadiol and spongiatriol (**42** and **44**); two were the 3α hydroxyl derivatives of the same structures **52-53**; and two were enolized diones **54-55**. These last two, 2,17,19-trihydroxyspongia-13(16),14-dien-3-one (**54**) and its 17,19-diacetoxy ester are related closely enough to poriferan spongian diterpenes that the authors suggest that *C. atromarginata* has oxidized the A ring of a dietary precursor.
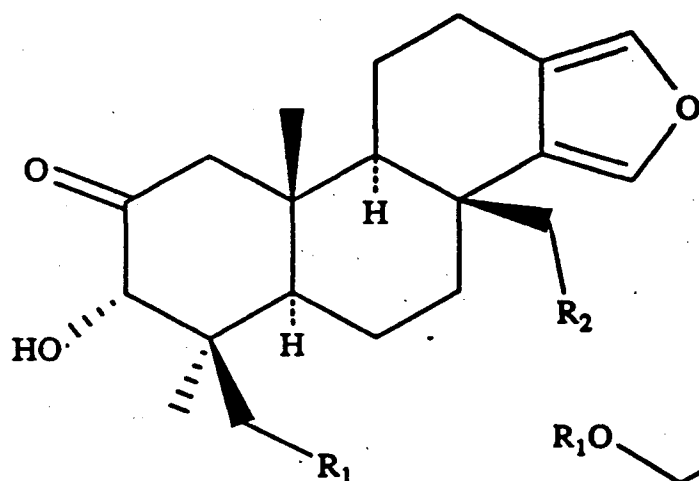
(52) $R_1 = OAc$ $R_2 = OAc$

(53) $R_1 = OAc$ $R_2 = H$
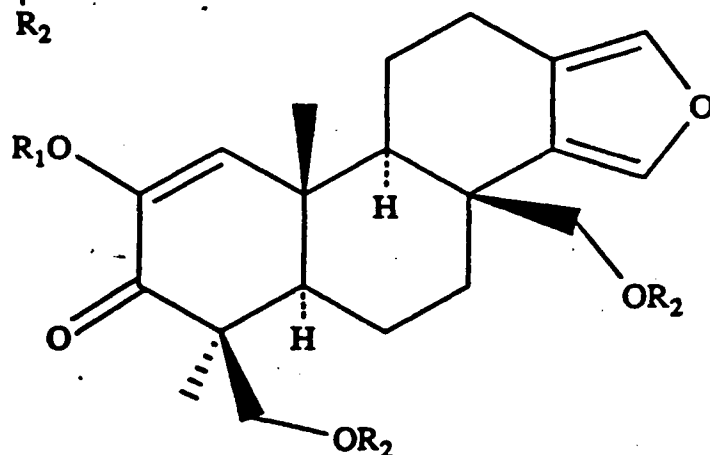
(54) $R_1 = R_2 = H$

(55) $R_1 = H$ $R_2 = Ac$

[27] de Silva, E.D. and Scheuer, P.J. *Heterocycles*, 1982, *17*, 167.

While reports of isolation and structural elucidation of spongian-related terpenoids have been fairly frequent, details concerning biological activity of these interesting compounds have been relatively rare. One such report came from the SeaPharm Project Laboratories [28]. A deep water Caribbean species of *Spongia* was found to contain previously reported spongiadiol and epispongiadiol (41 and 45) as well as the new compound isospongiadiol (56, 2α,19-dihydroxyspongia-13(16),14-dien-3-one). Cytotoxicity assays against P388 cells of 41, 45 and 56 yielded IC_{50} values of 0.5,8,and 5 μg/ml, respectively. Antiviral activity against Herpes Simplex Virus I gave the values of 0.25, 12.5, and 2 μg/ml, respectively. Comparison with standards indicates that while only mildly cytotoxic, spongiadiol and isospongiadiol are fairly effective as antiviral agents in *in vitro* assays.
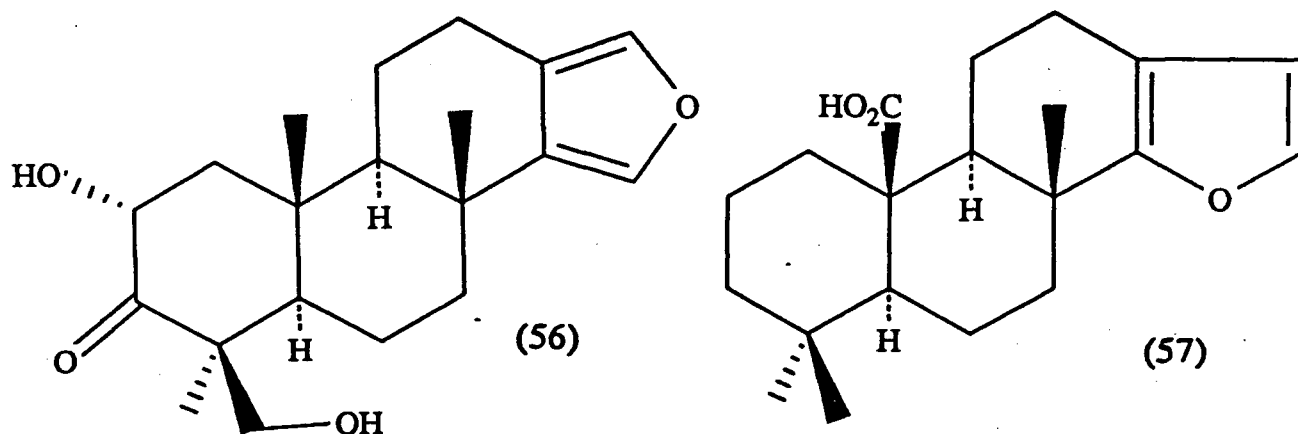
While technically not a spongian diterpene, marginatafuran (57), isolated from the nudibranch *Cadlina luteomarginata* by Gustafson and Andersen [29], has a remarkably similar skeleton. The nudibranch was collected in the waters off the Queen Charlotte Islands of British Columbia, a region thought to have remained ice-free during the last glaciation period. Perhaps the resulting evolutionary isolation from the Indo-Pacific marine basin has allowed unique development in the marine invertebrates near the islands. The amazingly slight difference in structure between marginatafuran and diterpenoids from other parts of the world certainly may be evidence for such a claim.

[28] Kohmoto, S.; McConnell, O.J.; Wright, A.J.; Cross, S. *Chem. Lett.*, 1987, 1687.

[29] Gustafson, K. and Andersen, R.J. *Tetrahedron Lett.*, 1985, *26*, 2521.

A Caribbean sponge, *Igernella notabilis*, has also proven to contain spongian terpenoids [30]. The 7α,17β-dihydroxy-15,17-oxidospongian-16-ones isolated from this sponge **58-60** have lactone-ketal functionalities and an interesting cyclization pattern from Me-17. Whether this functionality serves any biological function may be pondered but no evidence has been offered in either case.
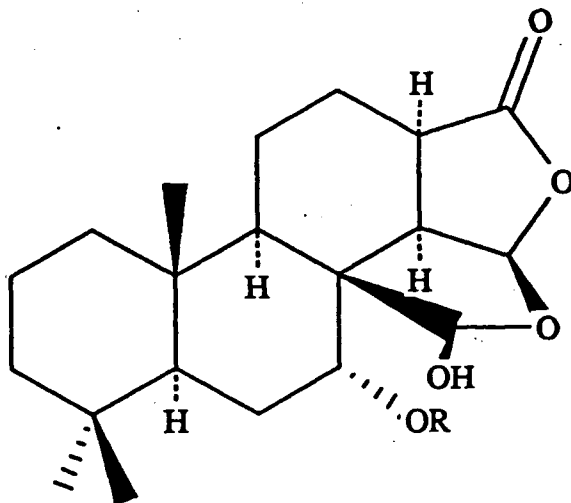
(58)   R = COPr

(59)   R = Ac

(60)   R = H

Several reports of compounds related to those from *Igernella notabilis* came out the following year [31]. Molinski and Faulkner reported the isolation of 6α,7α,17β-trihydroxy-15β,17-oxidospongian-16-one 7-butyrate **(61)** from an Australian *Aplysilla* species. Karuso and Taylor reported the isolation of the above compounds **(58, 59, and 61)** from *Aplysilla rosea* as well as three related compounds which he called the aplyroseols **(62-64)**. The aplyroseols and four related compounds **65-68**, were also isolated from the sponge *Dendrilla rosea*.

[30] Schmitz, F.J.; Chang, J.S.; Hossain, M.B.; van der Helm, D. *J. Org. Chem.*, **1985**, *50*, 2862.
[31] a) Molinski, T.F. and Faulkner, D.J. *J. Org. Chem.*, **1986**, *51*, 1144. b) see note [23]. c) see note [19].
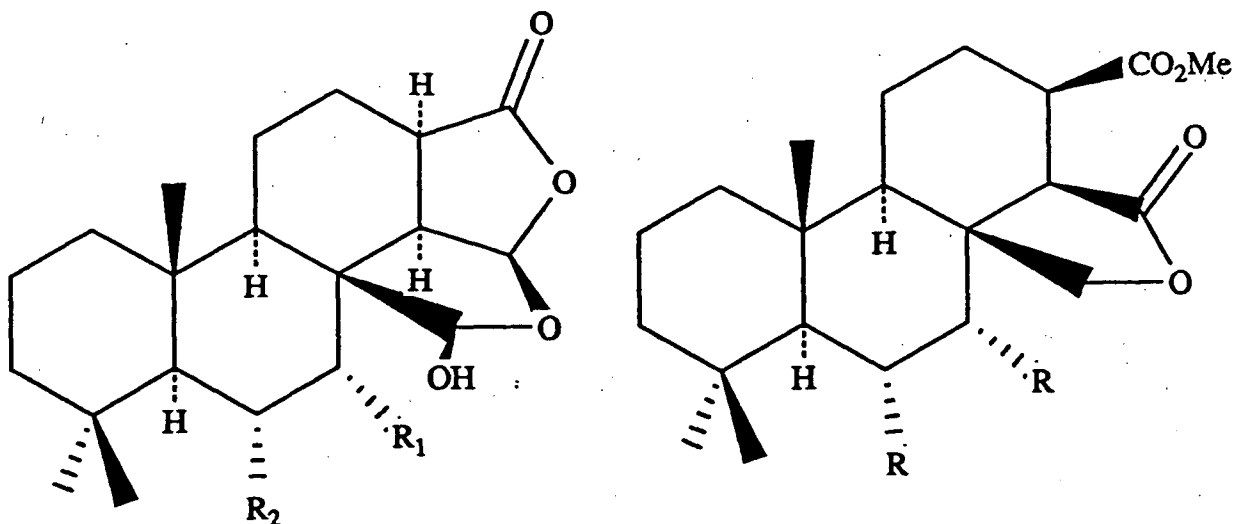
	R_1	R_2
(61)	OCOPr	OH
(62)	OCOPr	OAc
(63)	OH	OCOPr
(64)	OAc	OCOPr
(65)	H	H
(66)	OAc	OAc

(67) R = H

(68) R = OH

Eight compounds **69-76** related to the aplyroseols **61-66** were reported isolated from an Australian nudibranch classified as *Ceratosoma brevicaudatum*[32]. The wide variety in oxidation and cyclization patterns of these nine compounds poses some interesting questions about the role of functionality in the spongians.

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[32] Ksebati, M.B. and Schmitz, F.J. *J. Org. Chem.*, 1987, *52*, 3766.
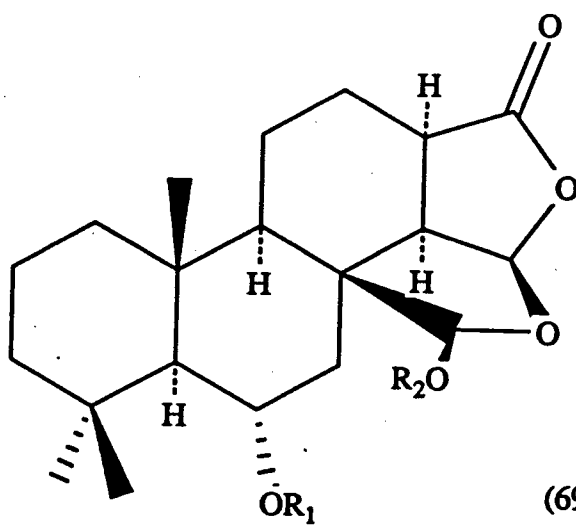
	R_1	R_2
(69)	COPr	H
(70)	Ac	H
(71)	Ac	Ac
(72)	COPr	Ac
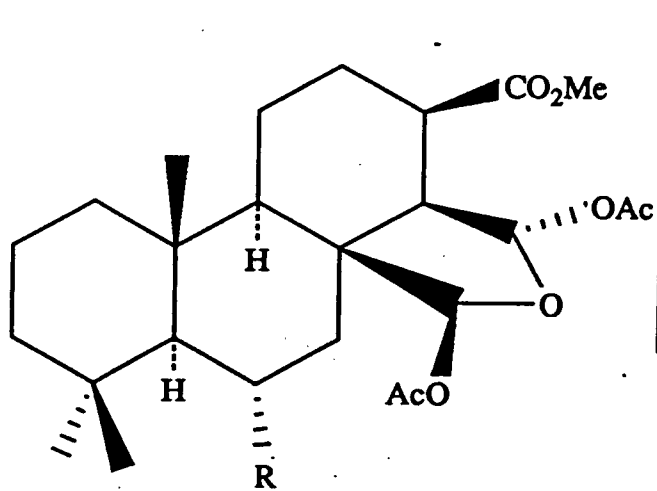
(73) R = OAc

(74) R = OCOPr

(75) R = H
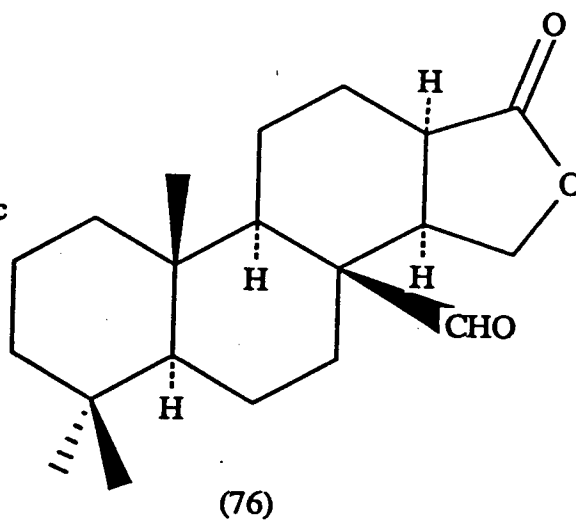
(76)

An interesting degraded spongian diterpene was isolated from eastern Australian samples of *Aplysilla sulphurea*[33]. Aplysulphurin (77), is related to the spongian skeleton through a single methyl shift (C17) and an opening of ring B. Karuso and coworkers later reexamined the same sponge[34], now reclassified as *Darwinella oxeata*, and again found aplysulphurin as well as the tetrahydroaplysulphurins 1 (78), 2 (79) and 3 (80).

(77)

(78)

(79)

(80)
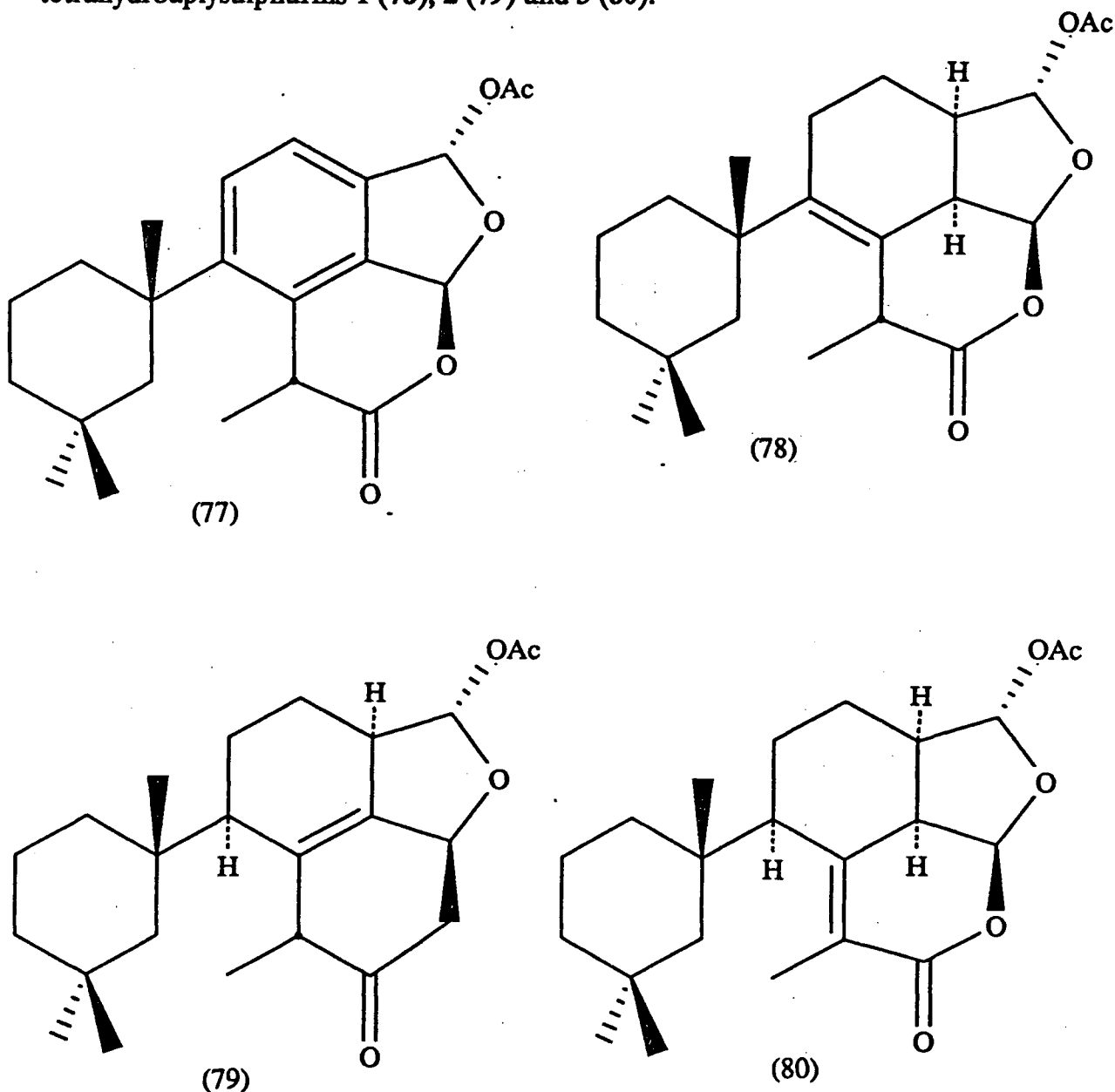
[33] Karuso, P.; Skeleton, B.W.; Taylor, W.C.; White, A.H. *Aust. J. Chem.*, 1984, *37*, 1081.
[34] see note [19] above.

The sponge *Spongionella gracilis* provided a compound related to the aplysulphurins but completely lacking the C17 methyl[35]. Gracilin A (81), a norditerpene which is similar to 15α,16α-diacetoxyspongian (31) could possibly be derived from a precursor similar to this undegraded terpenoid isolated earlier by Cimino *et al* from *Spongia officinalis*. A dinorditerpene, gracilin B (82), found in conjunction with gracilin A in *S. gracilis*[36], has a highly unusual carbon skeleton that appears to derive from a spongian-type diterpene, but if so rearrangement involves a more complex mechanism than the simple cationic shifts and oxidations which account for other rearrangements.

(81)

(82)
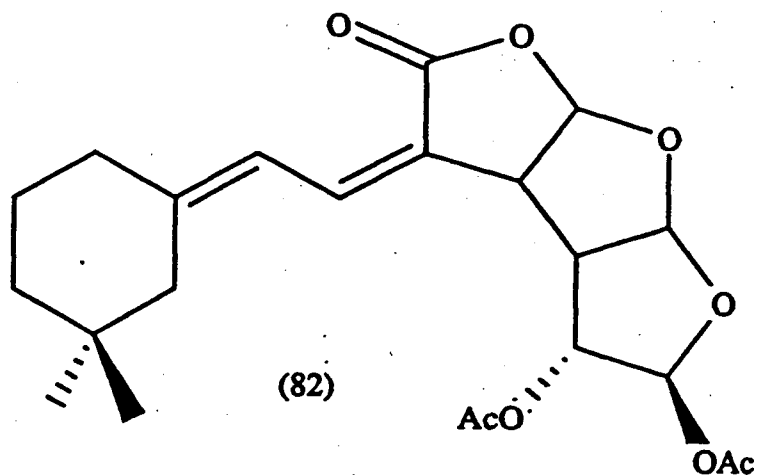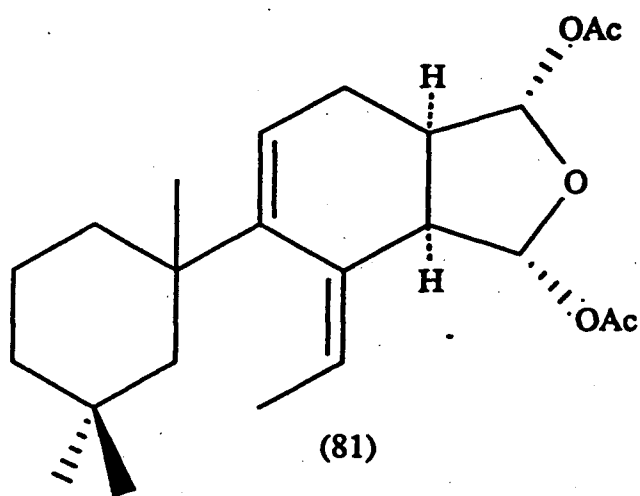
[35] Mayol, L.; Piccialli, V.; Sica, D. *Tetrahedron Lett.*, 1985, *26*, 1357.
[36] Mayol, L.; Piccialli, V.; Sica, D. *Tetrahedron Lett.*, 1985, *26*, 1253.

From the nudibranch *Chromodoris macfarlandi* two terpenoids similar to aplysulphurin have been isolated [37]. Macfarlandins A and B (16 and 17) are epimeric aromatic norditerpenes, familial to aplysulphurin (77) but without the C-17 methyl and the δ lactone, and appear to represent more highly oxidized relatives of gracilin A (81).
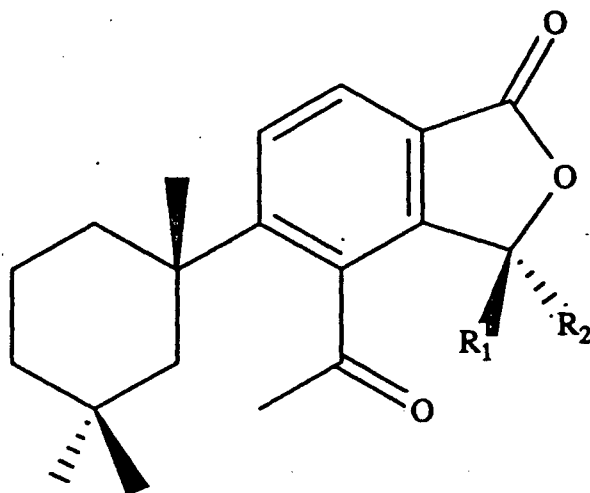
(16) $R_1 = H$ $R_2 = OAc$

(17) $R_1 = OAc$ $R_2 = H$

Molinski and Faulkner reported the isolation of two more terpenoids in this family, 9,11-dihydrogracilin A (83) and membranolide (84), found in the Antarctic sponge *Dendrilla membranosa*[38]. Since *D. membranosa* grows slowly, contains no spicules and has never been observed to be eaten, it is likely that some form of chemical defense exists. These two compounds are likely candidates.
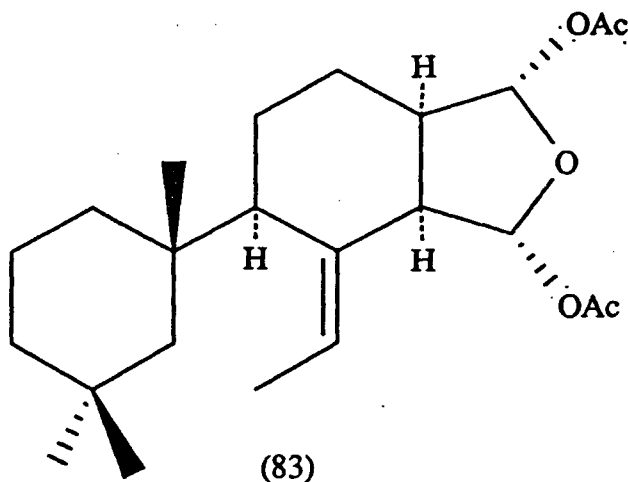
(83)
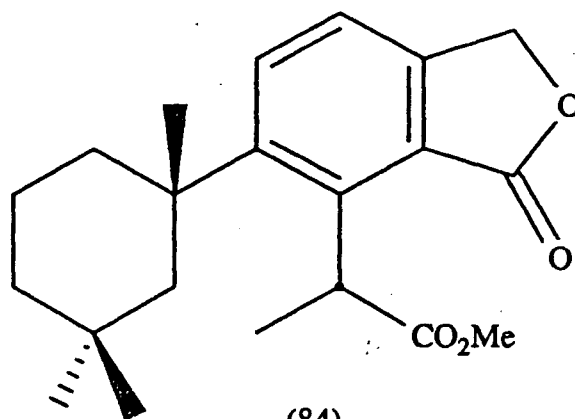
(84)

[37] Molinski, T.F. and Faulkner, D.J. *J. Org. Chem.*, 1986, *51*, 2601.
[38] Molinski, T.F. and Faulkner, D.J. *J. Org. Chem.*, 1987, *52*, 296.

Faulkner's research group at Scripps Institution of Oceanography was the first to report the elucidation of a "rearranged spongian diterpene" containing a novel carbon skeleton related to a spongian precursor. Norrisolide (15), which they isolated from the nudibranch *Chromodoris norrisi*[39], has also been found as a trace component in extracts of the sponge *Dendrilla* sp. collected at Palau, though this sponge is not native to the Gulf of California where *C. norrisi* was collected. A possible biogenesis of norrisolide was proposed starting from a spongian skeleton and following the series of bond transformations outlined in scheme II.

(15)
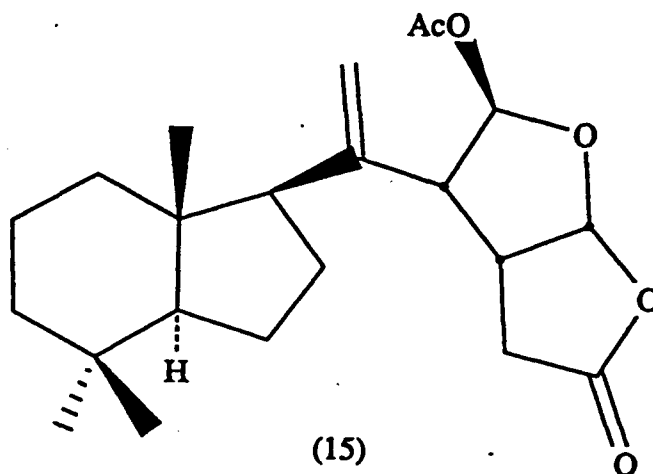
Scheme II
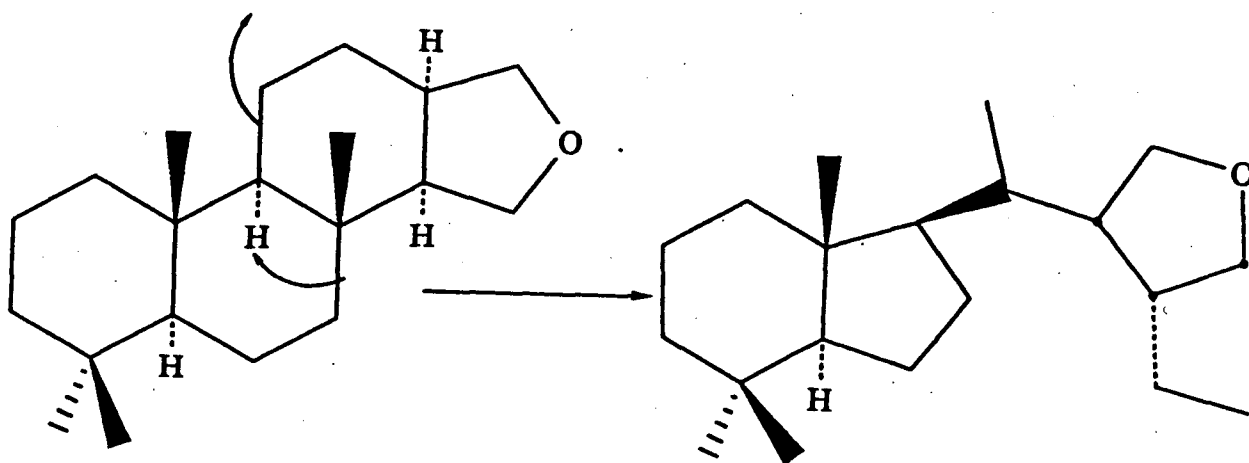
[39] Hochlowski, J.E.; Faulkner, D.J.; Matsumoto G.K.; Clardy, J. J. Org. Chem., 1983, 48, 1141.

Besides norrisolide, *Dendrilla* sp. contains three other diterpenes which can be envisioned as being derived through rearrangement of the spongian skeleton. The dendrillolides A, B and C (85-87), isolated from Palauan marine lake specimens of the sponge by Sullivan and Faulkner[40], can be thought of as arising from spongian diterpenes via the mechanism proposed in scheme III.

(85)

(86)

(87)

Scheme III
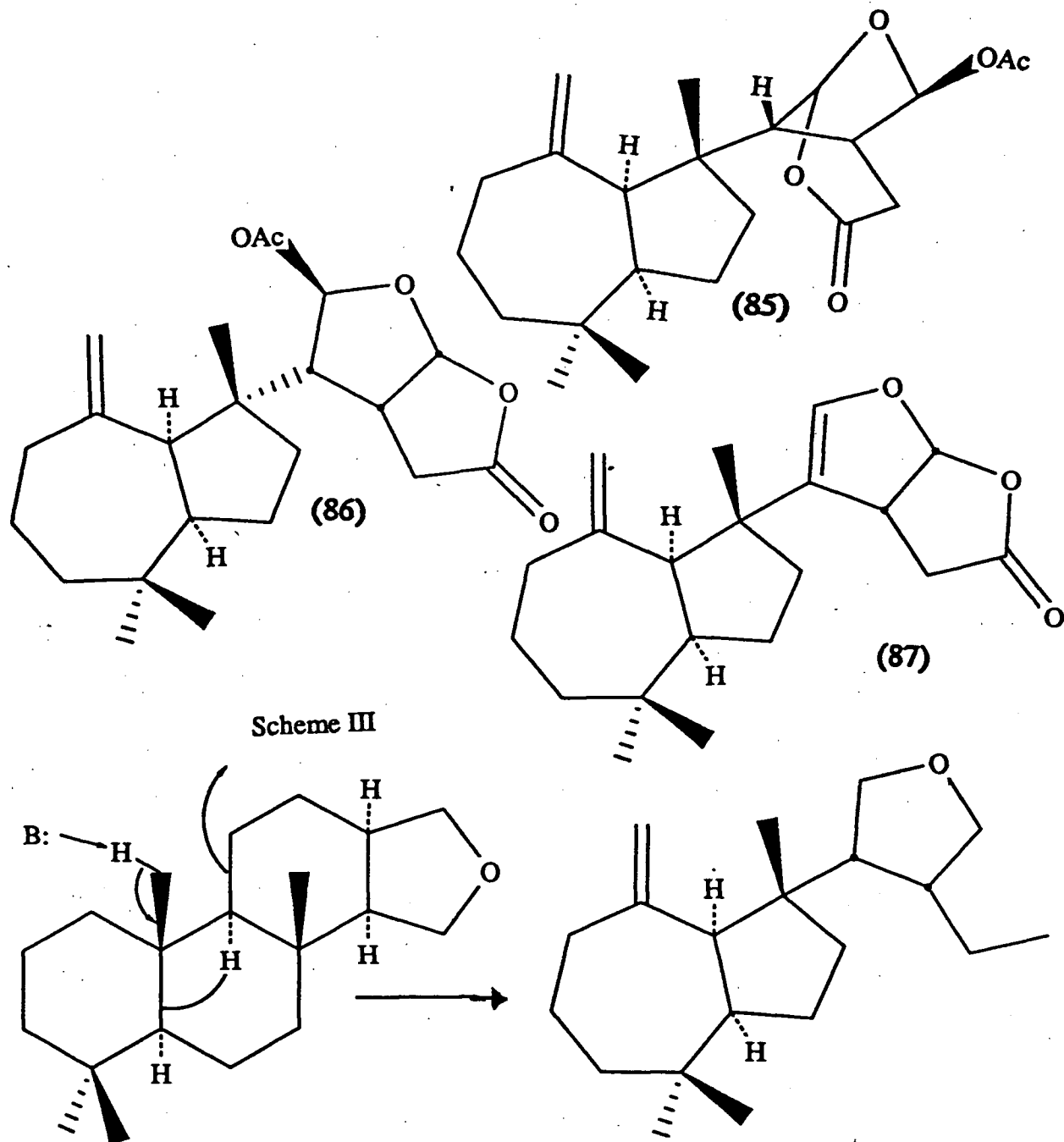
[40] Sullivan, B and Faulkner, D.J. *J. Org. Chem.*, 1984, *49*, 3204.

Rearranged spongians continued to be found in a variety of organisms. The marine sponge *Chelonaplysilla violacea* has been found to contain the two terpenoids aplyviolene (**88**) and aplyviolacene (**89**)[41]. Aplyviolene has the same structure proposed by Sullivan and Faulkner as dendrillolide A (**50**), but differences in spectral data have brought into question the actual structure of dendrillolide A. Aplyviolene was solved by an X-ray diffraction study.

(88)
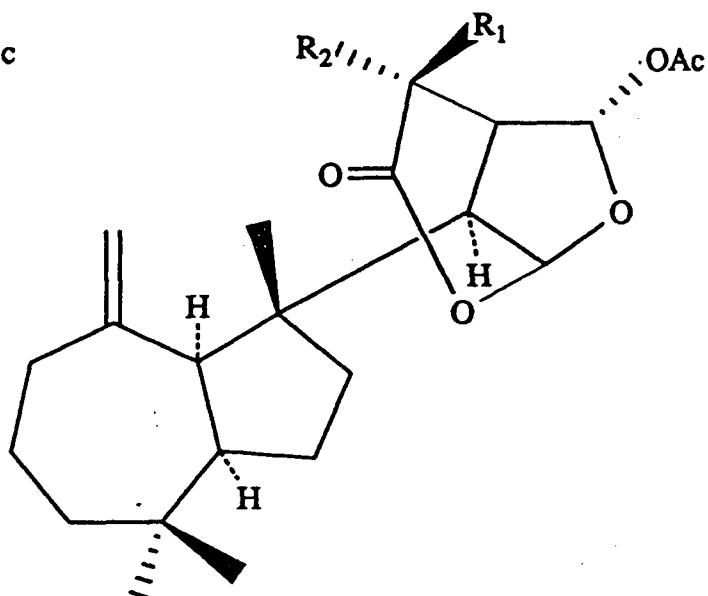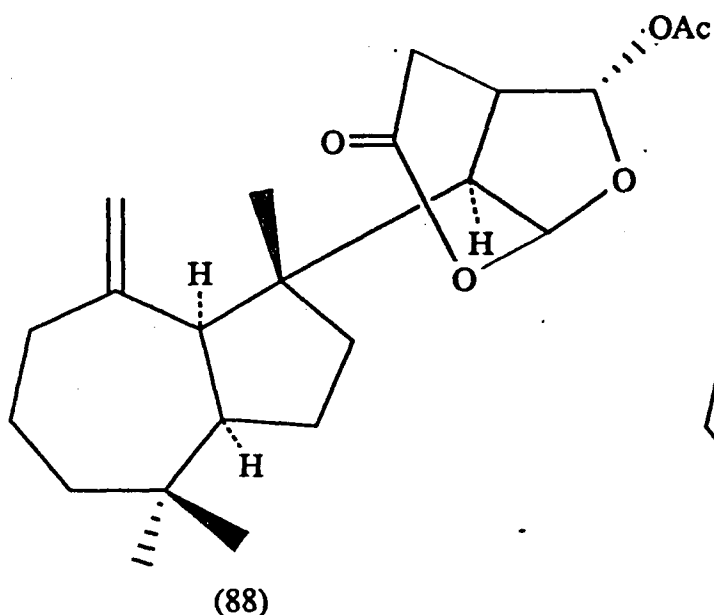
(89) R_1 = OAc R_2 = H

(20) R_1 = H R_2 = OAc

From the nudibranch *Chromodoris macfarlandi* three more rearranged spongian terpenoids have been isolated[42]. Macfarlandins C (**18**) and D (**19**) are lactone cyclization isomers of each other, bearing a carbon skeleton that is only slightly modified through shift of Me-20 and cleavage of the C9-C11 bond of a spongian precursor. Macfarlandin E (**20**) on the other hand bears the carbon skeleton of the dendrillolides and appears to be an epimer of aplyviolacene (**89**).

[41] Hambley, T.W.; Poiner, A.; Taylor, W.C. *Tetrahedron Lett.*, 1986, *28*, 3281.
[42] Molinski, T.F.; Faulkner, D.J.; Cun-heng, H.; Van Duyne, G.D.; Clardy, J. *J. Org. Chem.*, 1986, *51*, 4564.

(18)

(19)
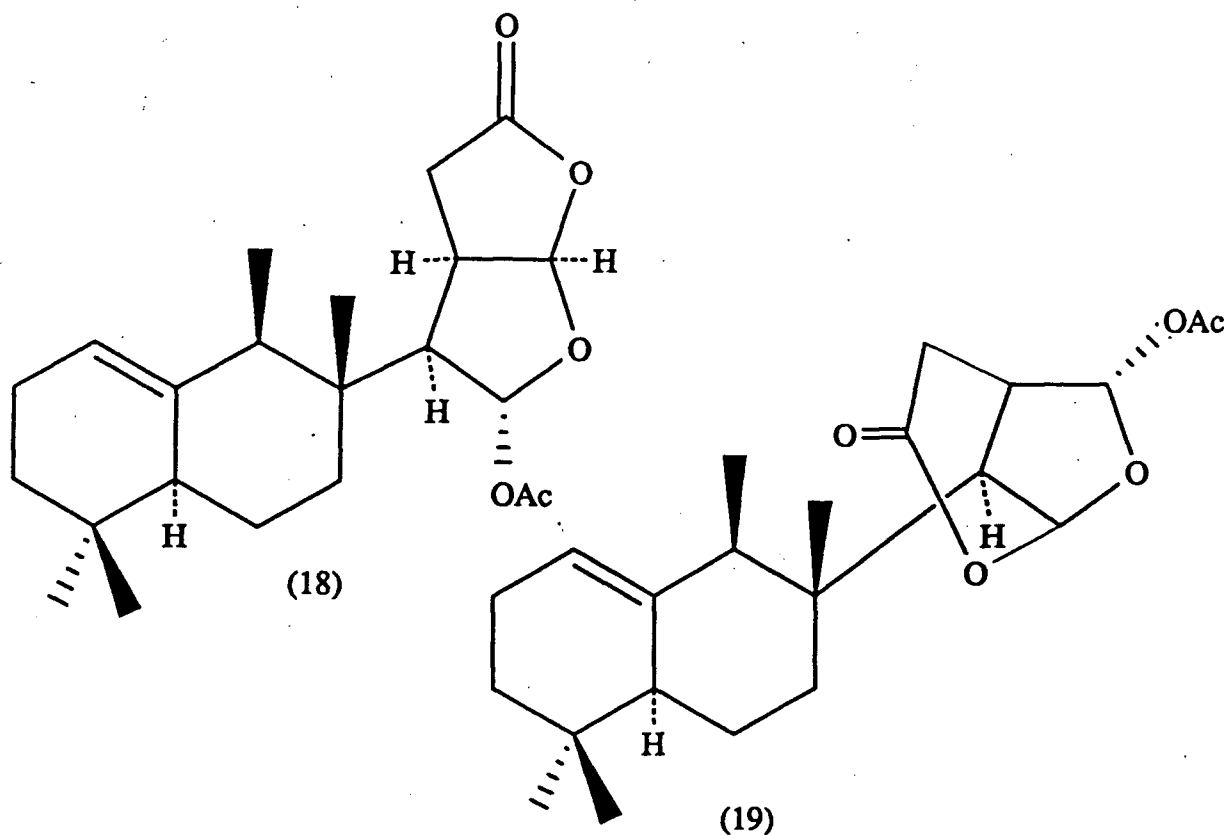
The latest report of spongian type diterpenoids reports the structure of ten compounds related to the macfarlandins[43]. Five compounds (**90-94**, shahamins A-E) related to macfarlandin E have been isolated from one *Dysidea* species of sponge and five others (**95-99**, shahamins F-J), related to macfarlandins C and D, but containing a unique C5-C10 double bond, have been isolated from another. Shahamins A-E display a wide variety of cyclization in the heterocyclic portion of the molecule and again calls into question the biological role the various cyclization isomers may play. Shahamins F-J are unique in that these five compounds contain a tetrasubstituted double bond across the decalin system. Such compounds are rare in marine systems and this once again indicates the spongians are a novel and fascinating class of compounds.

[43] Carmely, S.; Cojocaru, M.; Loya, Y.; Kashman, Y. *J. Org. Chem.*, **1988**, *53*, 4801.

(90)

(91)

(92)

(93) R = Ac

(94) R = H
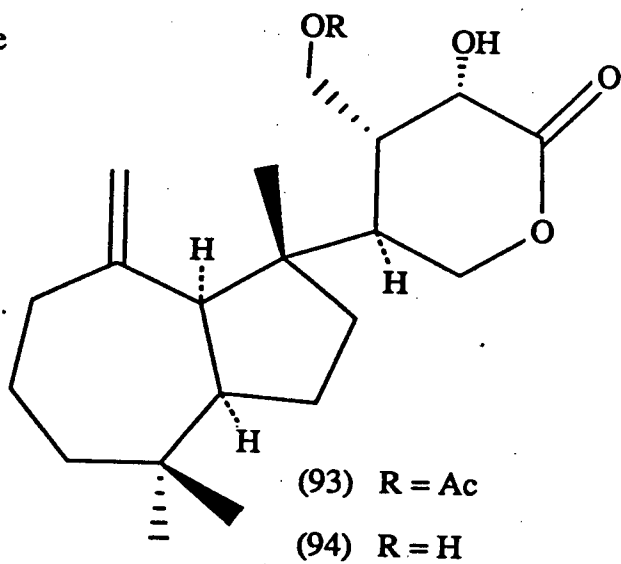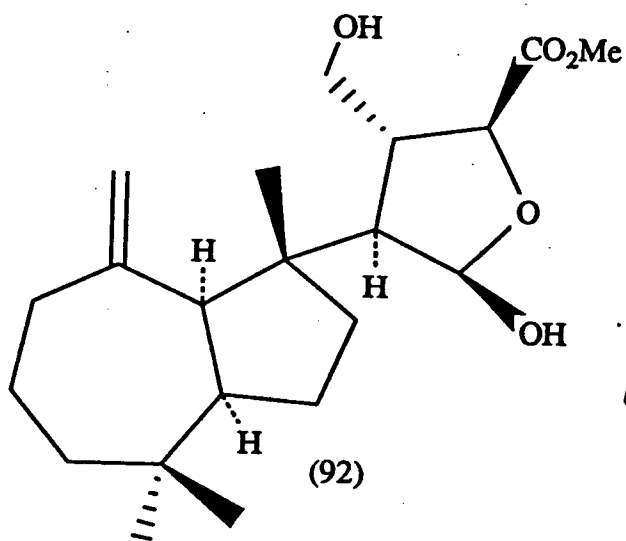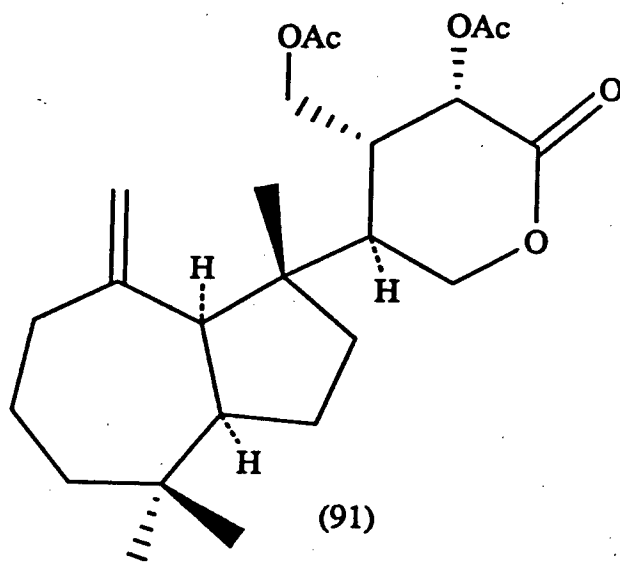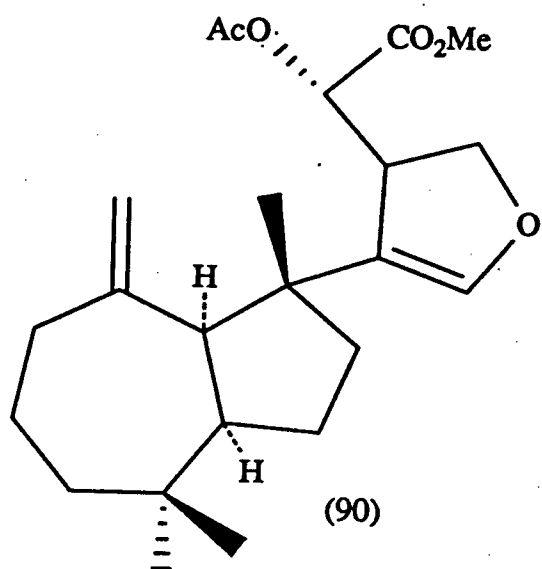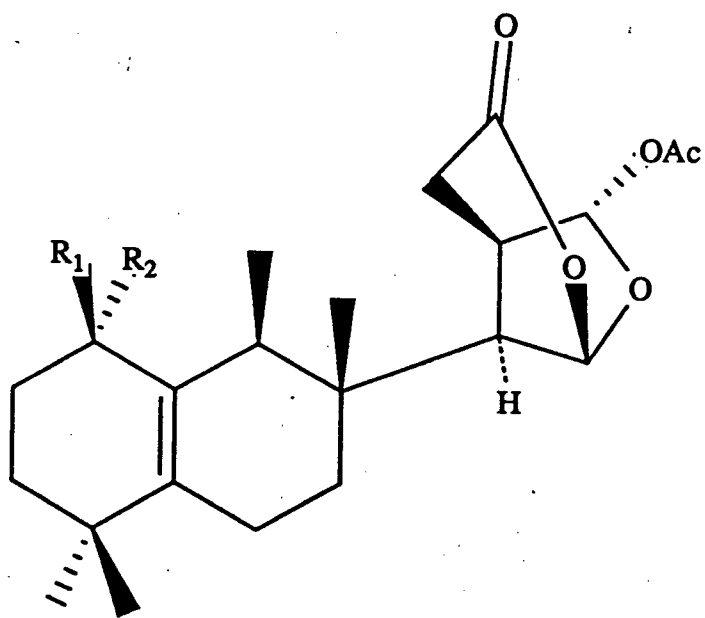
(95) R$_1$ = H   R$_2$ = H

(96) R$_1$ = H   R$_2$ = OH

(97) R$_1$ = OH   R$_2$ = H
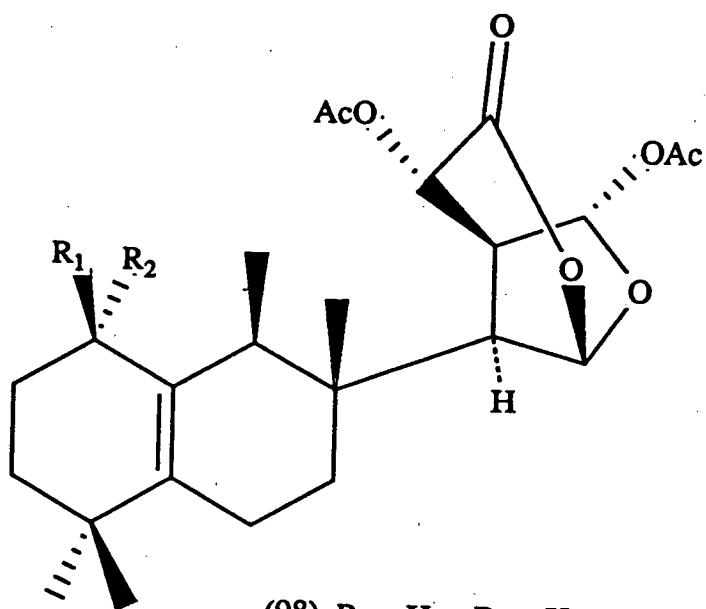
(98) R$_1$ = H   R$_2$ = H

(99) R$_1$ = OH   R$_2$ = H

II.   Our Current Work

The opportunity arose to study the organic skin extract of a previously uninvestigated chromodorid nudibranch, *Chromodoris cavae*, collected in the Indian Ocean. In light of the interesting metabolites isolated to date from molluscs in this genus, we were very interested in investigating the metabolites a new species collected in a different geographical region.

A series of silica flash and thin-layer chromatography procedures led to the purification of three diterpenoid metabolites **100-102** from the crude skin extract of specimens of *Chromodoris cavae*. The metabolite isolated in greatest quantity, chromodorolide A **(100)** proved to be a rearranged spongian diacetate with a new carbon skeleton. The two minor metabolites each proved to be rearranged spongian monoacetates, one of these, **101**, being the previously isolated dendrillolide A.
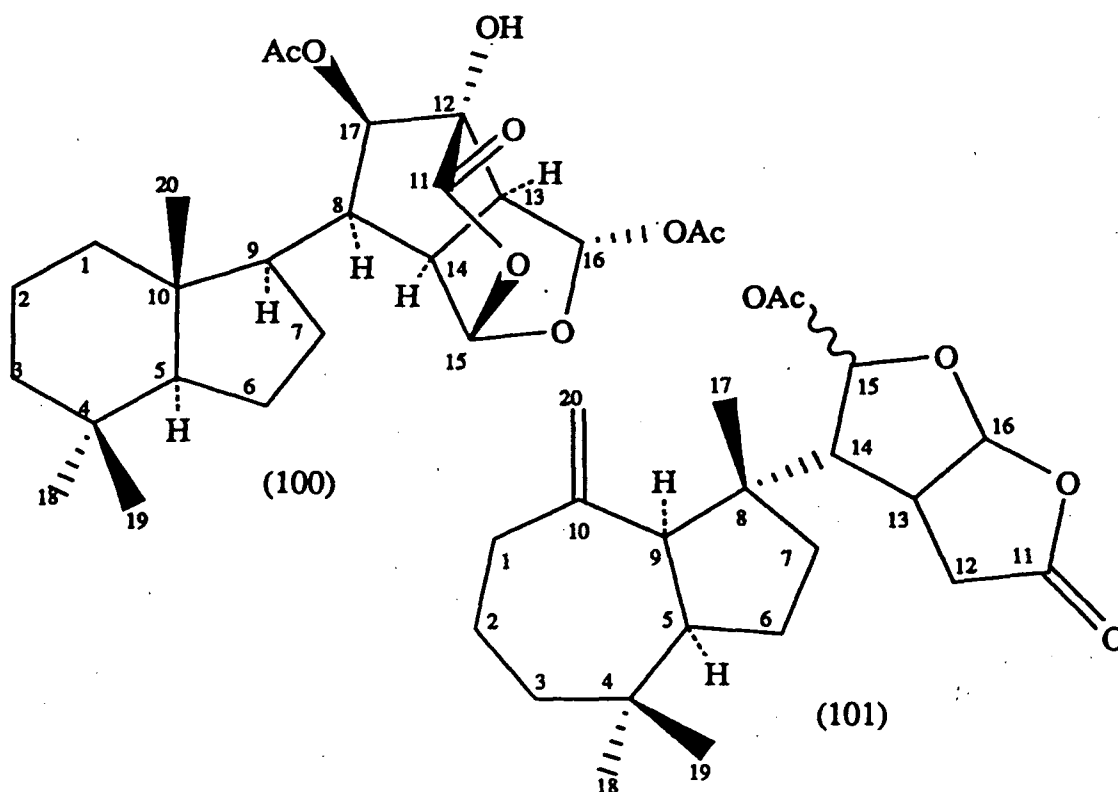
Discussion

Part I---Chromodorolide A

Electron impact mass spectrometry (Fig. 2) failed to show a parent ion for the compound. A peak at 390.2043 amu corresponding to a formula of $C_{22}H_{30}O_6$ (calcd. 390.2044) was the highest mass observed, due to the facile loss of acetic acid from the molecular ion. Losses of a methyl (375.1807 $C_{21}H_{27}O_6$, calcd. 375.1807), CO_2 (346.2144 $C_{21}H_{30}O_4$, calcd. 346.2144) and another acetic acid molecule (330.1825 $C_{20}H_{26}O_4$, calcd. 330.1831) from the M-60 mass at 390 gave further indication of a parent formula of $C_{24}H_{34}O_8$. A CI mass spectrum (Fig. 3) did show this parent ion as an M+1 peak at 451 amu while the largest peak observed in the CI was at 468 indicating incorporation of the ionizing agent NH_3.

^{1}H nmr spectroscopy of chromodorolide A in $CDCl_3$ (Fig. 4) revealed many interesting features. Two acetate methyl resonances were present at δ 2.12 and 2.08 ppm. Three aliphatic methyl resonances at δ 0.88, 0.86, and 0.85 ppm, an exchangeable proton signal at δ 3.31 ppm, and two ketal protons resonating at δ 6.36(s) and 5.79 (dd, J=2.9,1.8 Hz) ppm gave some indication that the molecule might be a rearranged spongian diterpenoid. One dimensional decoupling experiments helped to work out the spin system for the heterocyclic portion of the molecule (see structure A below). Irradiation of the multiplet at δ 5.79 ppm simplified the signals at 3.06 ppm. Irradiation of the doublet at δ 4.83 ppm simplified the 2.45 ppm resonance to a doublet of doublets. Irradiation at δ 2.45 ppm simplified 3.06 ppm, collapsed 4.83 ppm into a singlet and increased the intensity of an upfield signal near 1.3 ppm. When complications arose due to two coincidental multiplet signals at δ 3.06 ppm, careful examination of the decoupled spectra suggested that these two protons were coupled to each other giving the spin system outlined below.
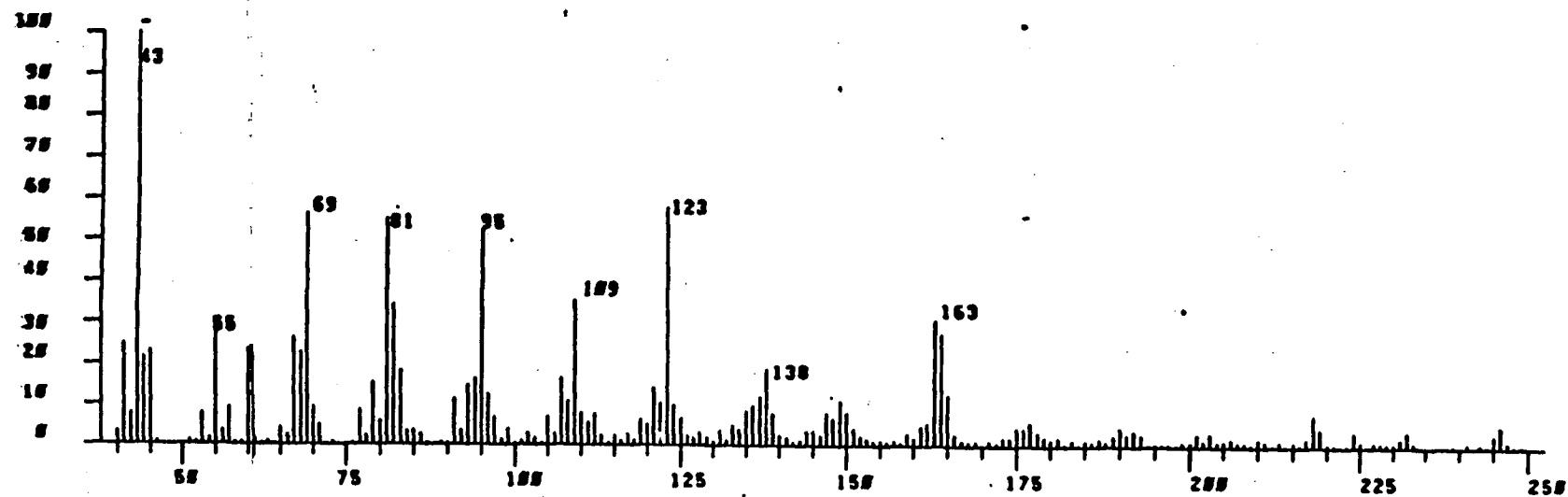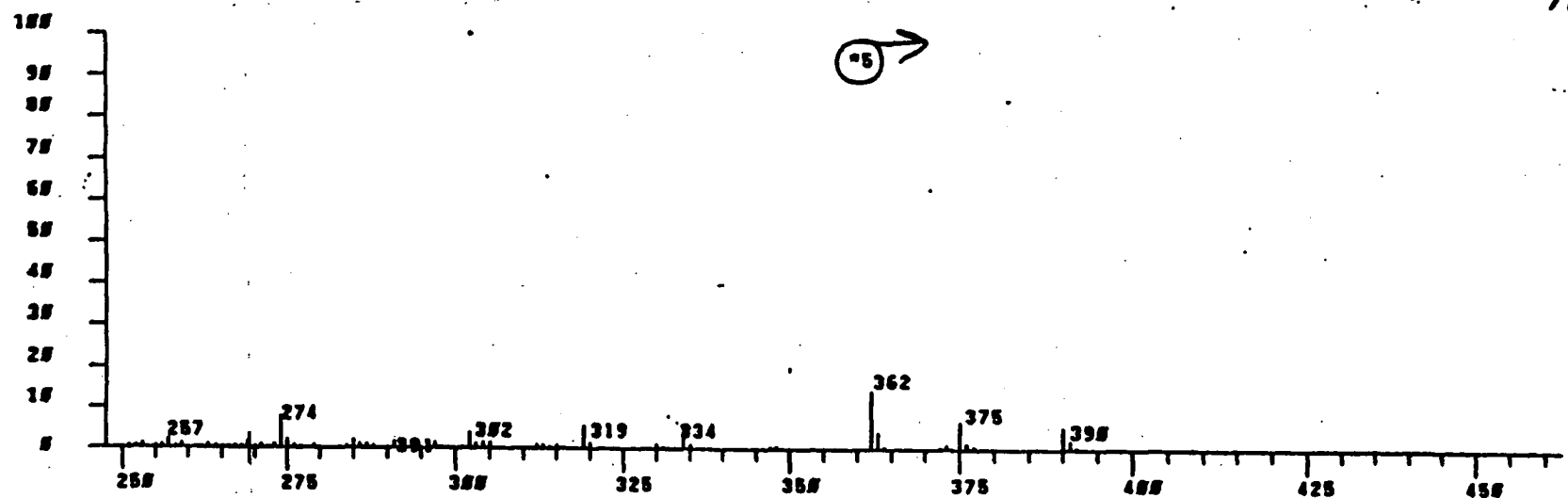
Figure 2. Electron Impact mass spectrum of (100)
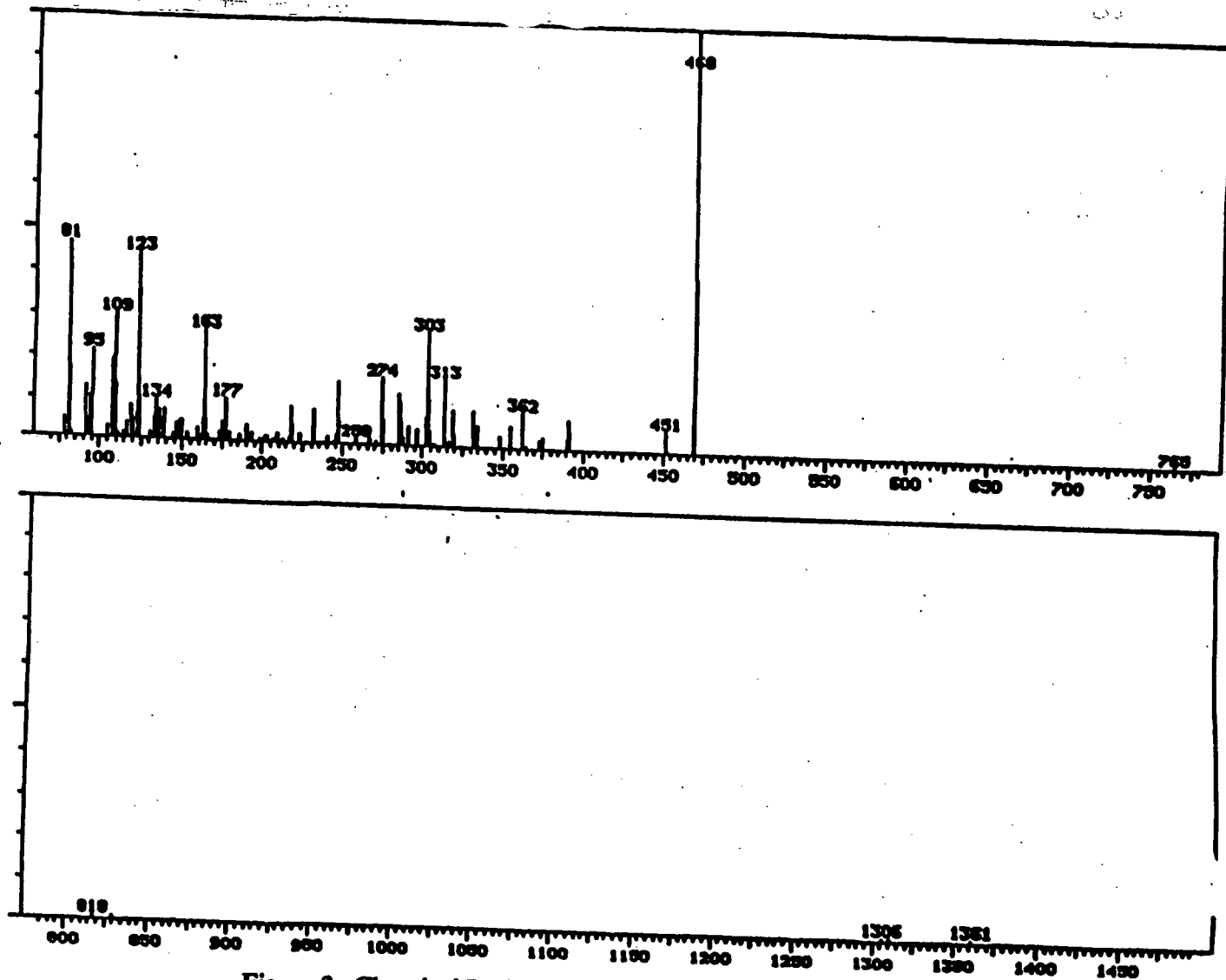
Figure 3. Chemical Ionization (NH$_3$) mass spectrum of (100)

CHROMODOROLIDE A (CDCL3)
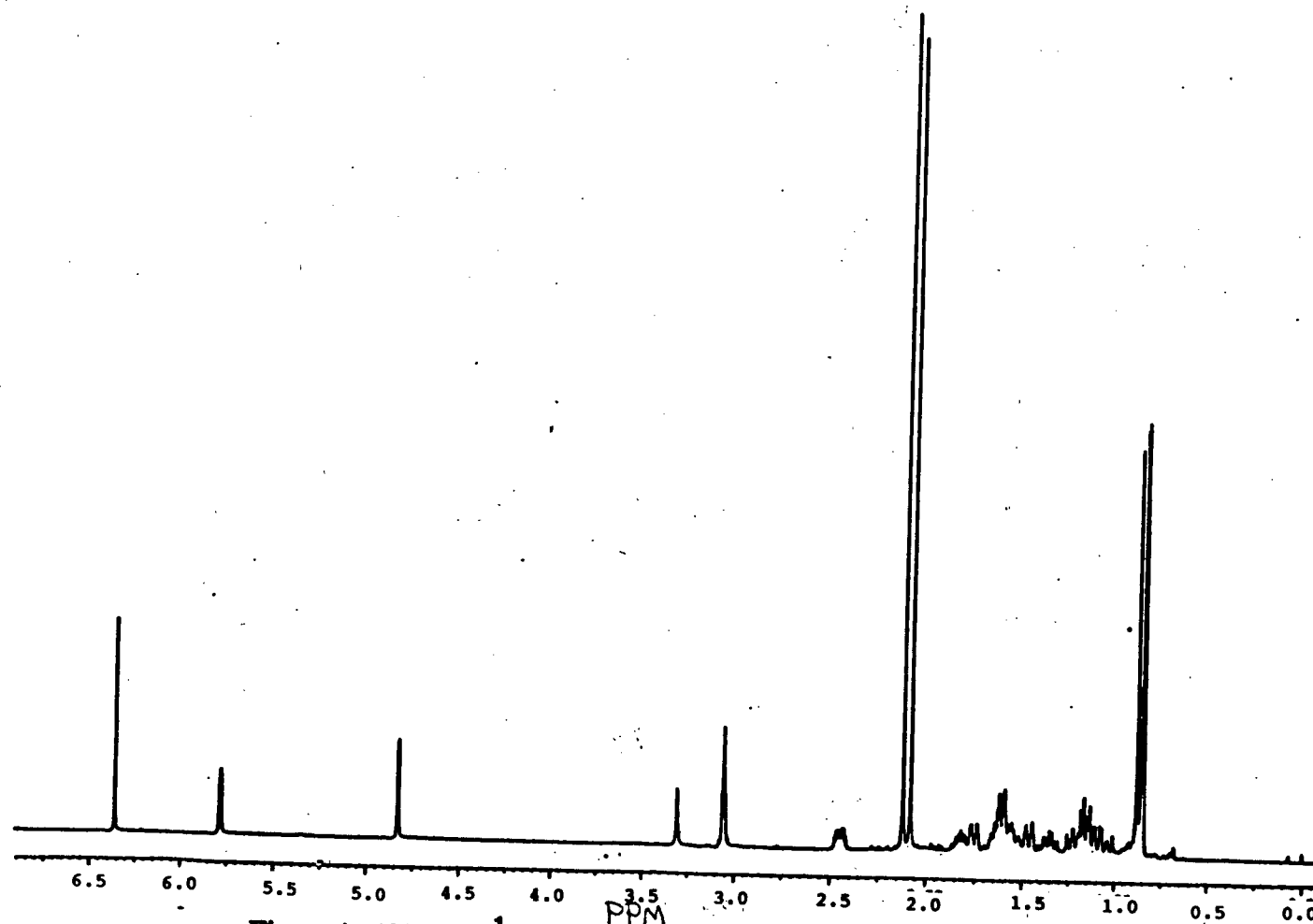
Figure 4   400 MHz ^{1}H nmr spectrum of Chromodorilide A (100) in CDCl$_3$.

CHROMODOROLIDE A (CDCL3)
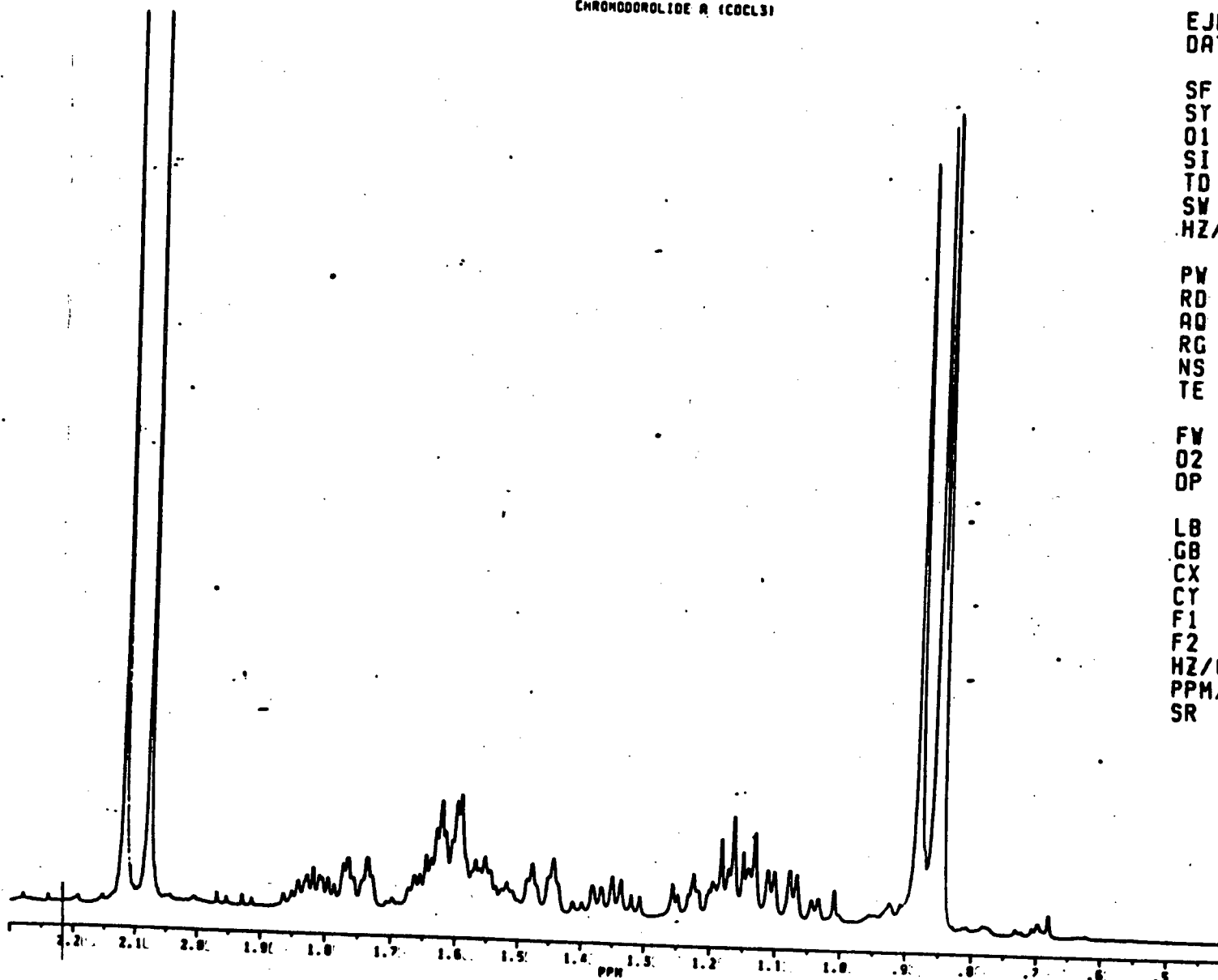
Figure 4a 400 MHz ^{1}H nmr CDCl$_3$ upfield region
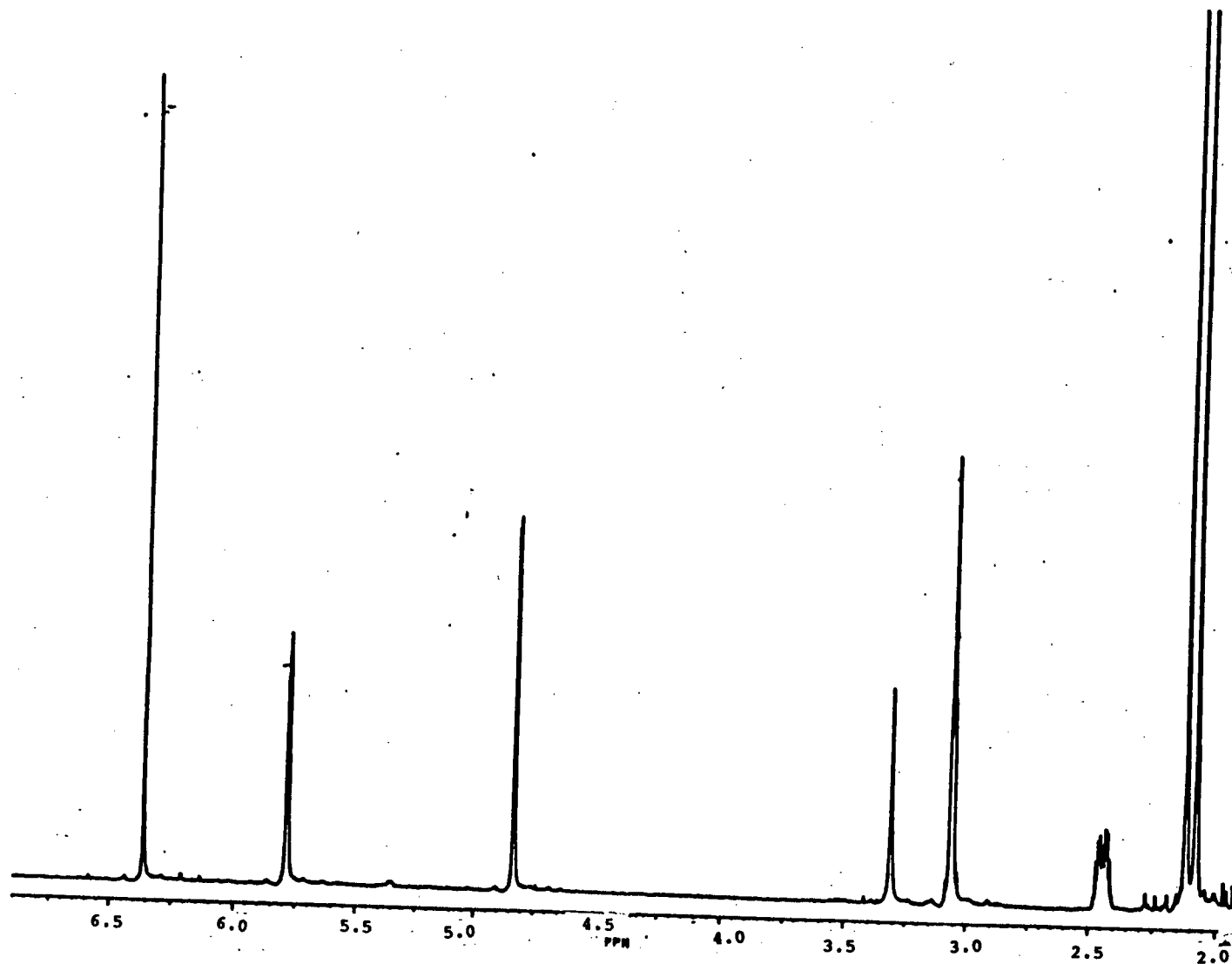
Figure 4b. 400 MHz ^{1}H nmr CDCl$_3$ downfield region

A
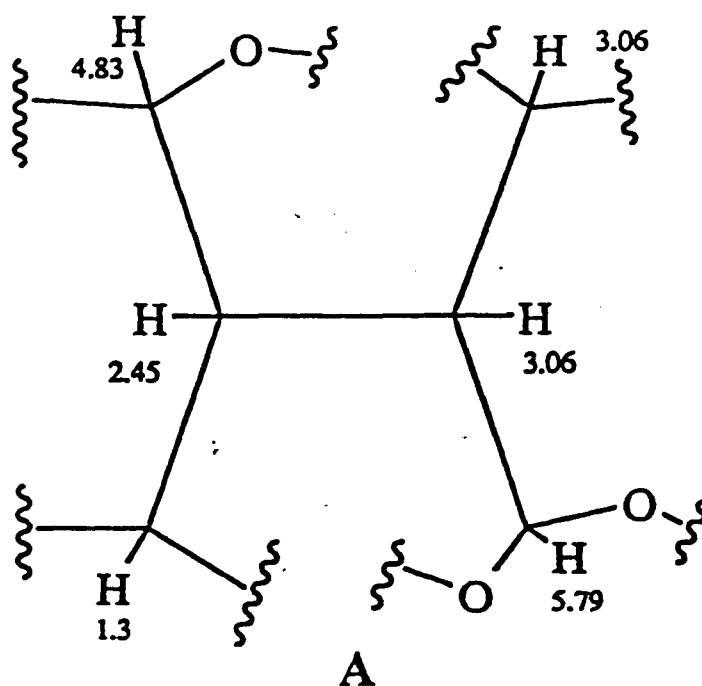
^{13}C nmr spectroscopy in CDCl$_3$ (Fig. 5) revealed twenty-two of the twenty-four signals expected for a diterpenoid diacetate. Three carbonyl signals at δ 172, 170 and 169 ppm accounted for the diacetate and indicated the possible presence of a lactone in the molecule. Two ketal methines at δ 104 and 95.5 ppm as well as highly deshielded quaternary and methine resonances at 80 and 79 ppm respectively accounted for the remaining oxygen containing sites. Only three methyl resonances were observed clearly, at δ 33.4, 20.8 and 13.6 ppm, however, the peak at 20.8 is the most intense in the entire spectrum. The relatively low intensity of this peak in the APT spectrum[46] indicates that the signal may be due to more than one carbon.

In order to clear up some of the ambiguities created by coincidental signals in the CDCl$_3$ nmr spectra of chromodorolide A (100), C$_6$D$_6$ was tried as a solvent. Some fairly dramatic shifts were observed in both the ^{1}H and ^{13}C nmr spectra (Figs. 6 and 7, respectively) as a result.

[46] Patt, S.L.; Schoolery, J.N. J. Mag. Reson. 1982, 46, 535.

CHROMODOROLIDE A
APT
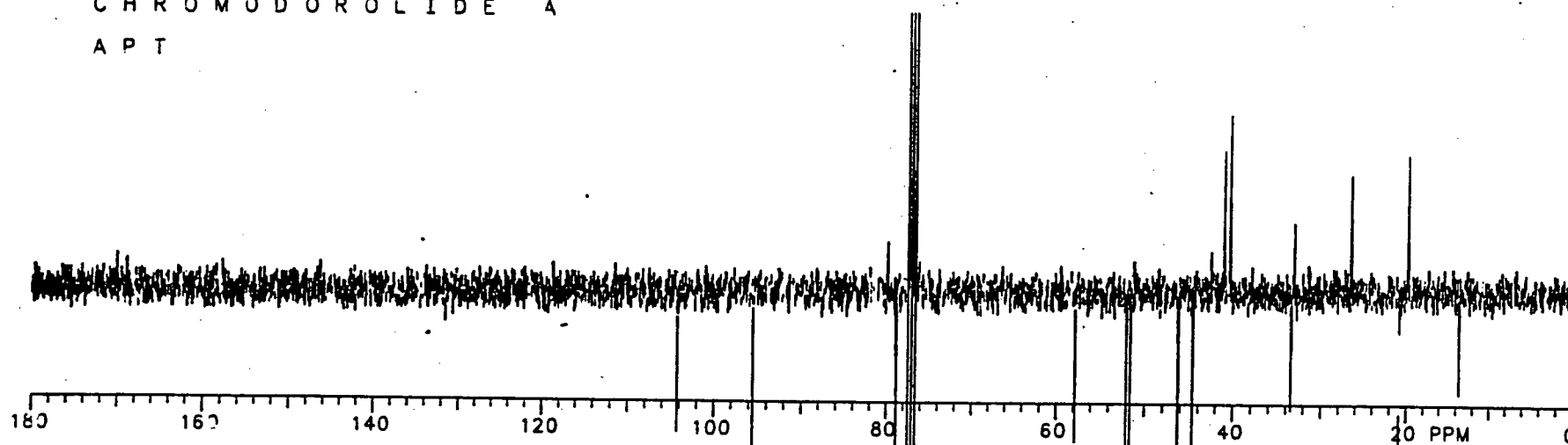
1 3 C
BB DECOUPLED
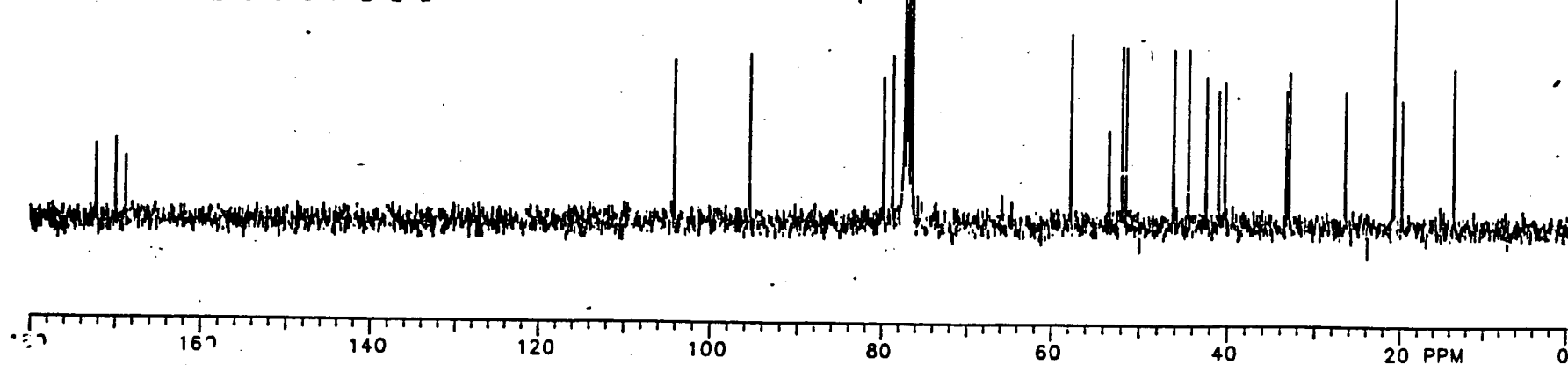
Figure 5.   75.4 MHz ^{13}C nmr spectrum and APT of (100) in $CDCl_3$

CHRONODOROLIDE A

- 42 -

6.5   6.0   5.5   5.0   4.5   4.0   3.5   3.0   2.5   2.0   1.5   1.0   0.5   0.0
PPM   PPM

Figure 6. 400 MHz ^{1}H nmr spectrum of (100) in C_6D_6
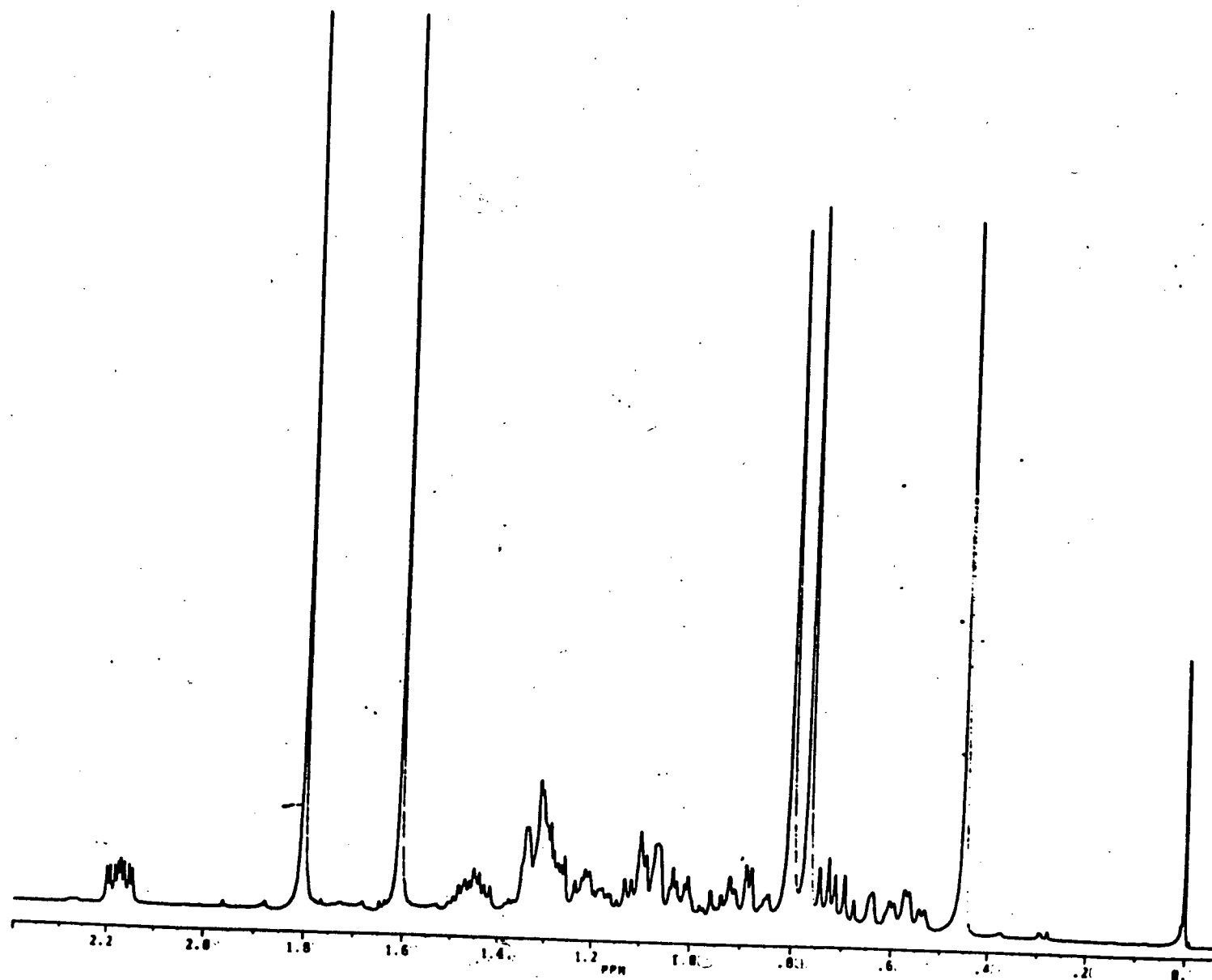
Figure 6a. Expanded upfield region of fig. 6

6.80 6.60 6.40 6.20 6.00 5.80 5.60 5.40 5.20 5.00 4.80 4.60 4.40 4.20 4.00 3.80 3.60 3.40 3.20 3.00 2.80 2.60
PPM
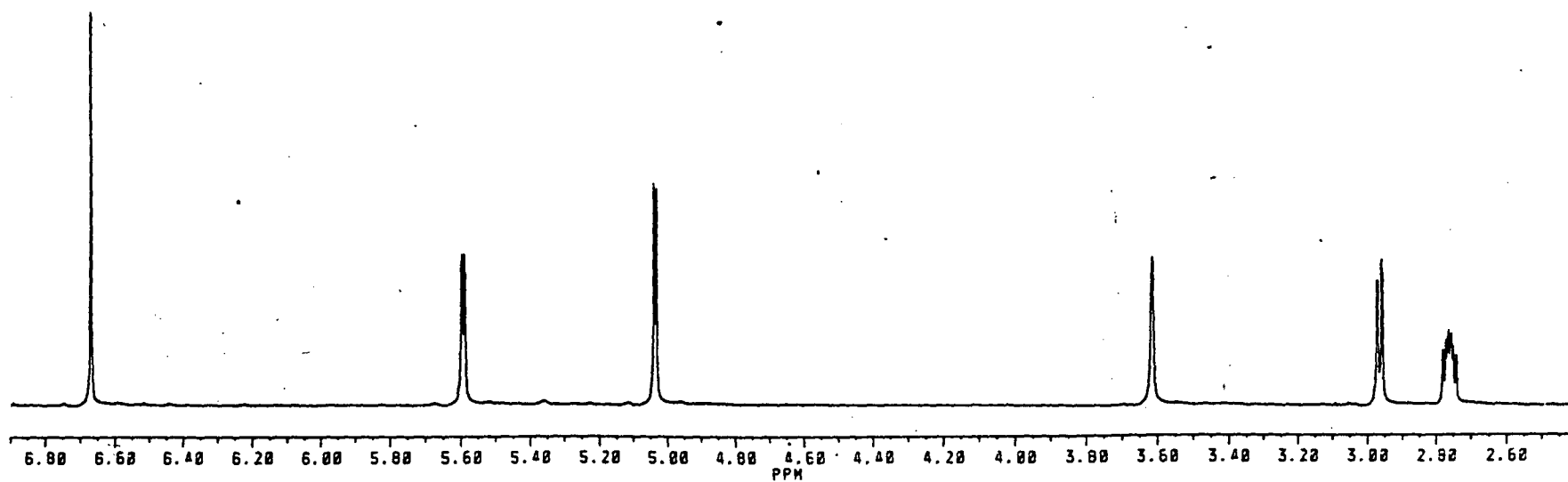
Figure 6b. Expanded downfield region of fig. 6

CHROMODOROLIDE A
APT

13 C
BB DECOUPLED
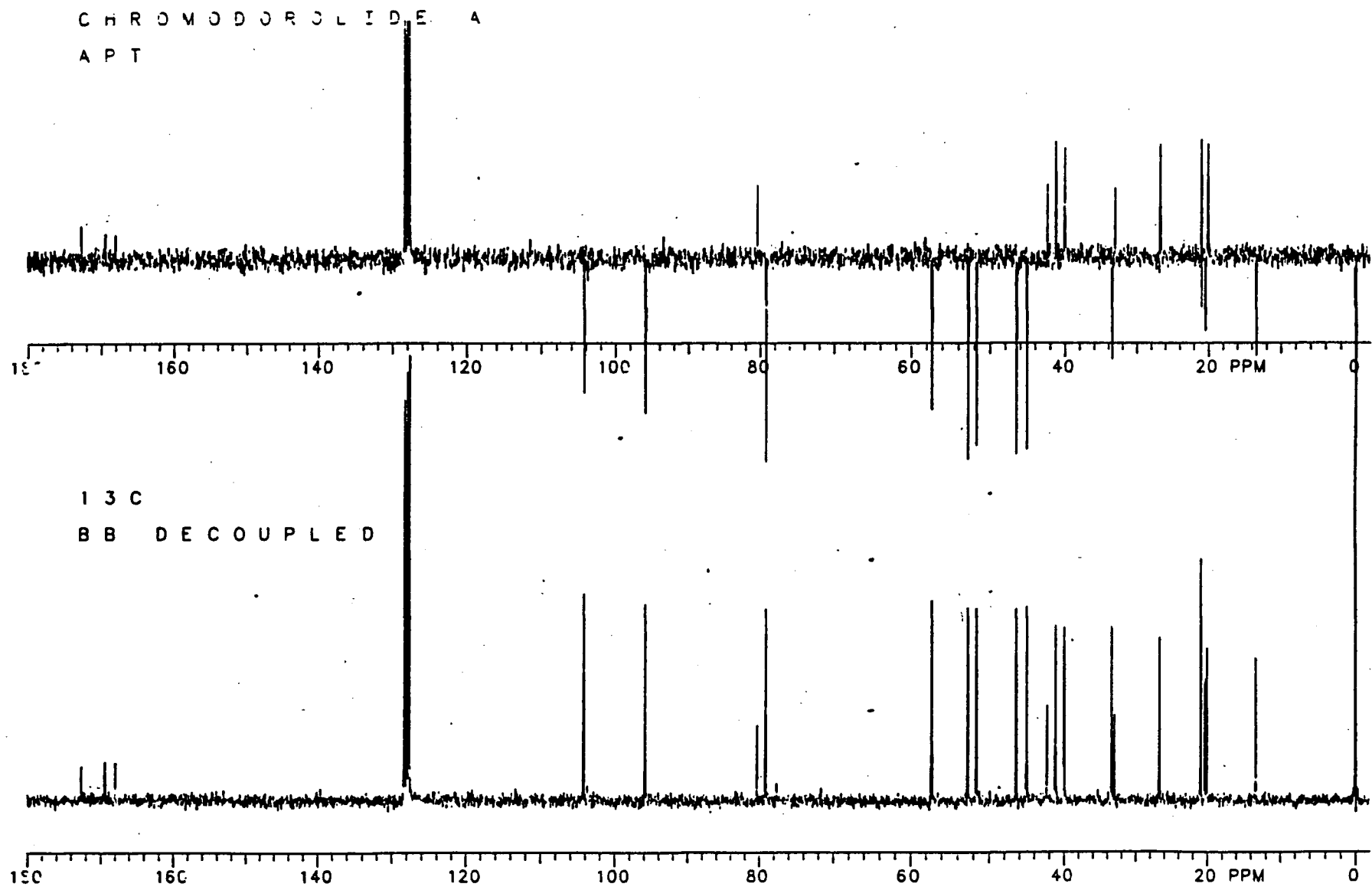
Figure 7. 75.4 MHz ^{13}C nmr spectrum and APT of (100) in C_6D_6

The proton ketal singlet at δ 6.36 ppm was shifted downfield to 6.67 ppm in deuterobenzene. The apparent triplet at δ 5.79 in CDCl₃ clearly became a doublet of doublets at 5.59 ppm in deuterobenzene and the doublet at 4.83 ppm moved downfield to 5.03 ppm. The exchangeable proton shifted downfield slightly and the coincidental signals forming a multiplet at δ 3.06 in CDCl₃ resolved into a doublet of doublets at 2.96 ppm and a double doublet of doublets at 2.76 ppm in C₆D₆. The acetates moved upfield to δ 1.80 and 1.60 ppm and the aliphatic methyls shifted to 0.80, 0.77 and 0.45 ppm. Two highly shielded multiplets at δ 0.87 and 0.57 ppm which each integrated to a single proton were also now clearly resolved.

The ^{13}C nmr spectrum recorded in C_6D_6 again showed three carbonyl signals, two ketal methines, as well as down field quaternary and methine carbons. Five methines, two quaternary and five methylene resonances accounted for all but the five methyls. Four of the methyls were easily observed at δ 33.4, 20.44, 20.36 and 13.4 ppm. The remaining methyl resonance appears to be nearly coincident with the methylene at δ 20.9 ppm. The intensity of the peak in the BB decoupled spectrum, as well as a small negative peak in the APT spectrum, indicates that this is indeed the case.

The molecular formula of $C_{24}H_{34}O_8$, with three sites of unsaturation accounted for by the carbonyls, indicated that the molecule was pentacyclic. Although 1D decoupling, COSY[47] (Fig. 8) and COSYPHDQ[48] (Fig. 9) experiments helped to work out the spin system in structure A, this system could not be made to fit any previously reported pentacyclic spongian diterpenoid carbon skeleton. An aliphatic [4,3,0] bicyclic system as in norrisolide could be deduced from the five methylene resonances in the ^{13}C nmr spectrum as well as the mass spectral fragment at m/z 165 corresponding to the ionic form of structure B. Therefore the heterocyclic portion of the molecule must be a tricyclic system. Since no such

[47] Bax, A "Two-Dimensional Nuclear Magnetic Resonance in Liquids", Reidel, Boston, 1982. Chapter 2.
[48] Sanders, J.K.M. and Hunter, B.K. "Modern NMR Spectroscopy, A Guide for Chemists", Oxford Univ. Press, New York, 1987. Chapter 4.
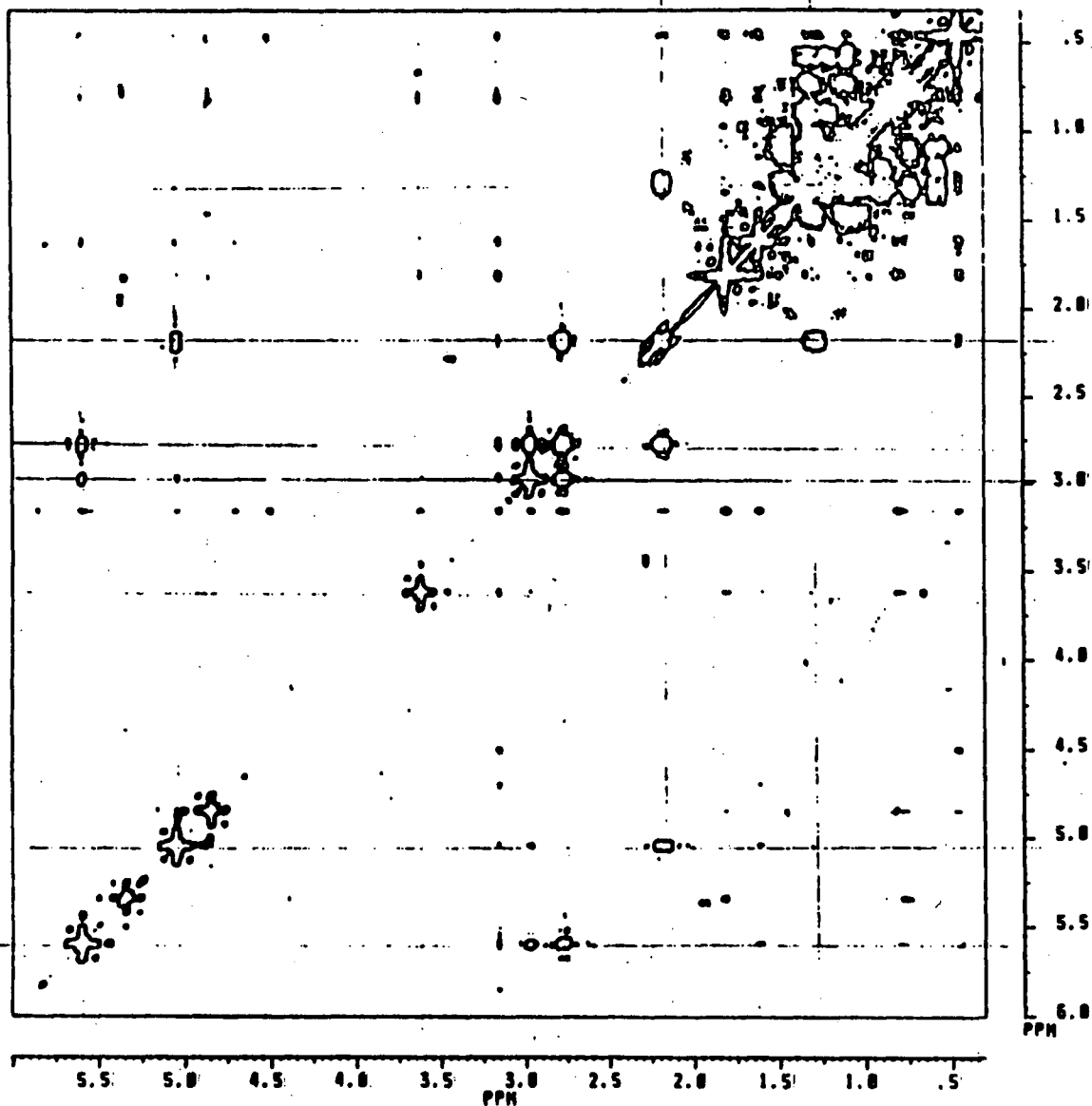
Figure 8. ^1H-^1H COSY of (100) in C_6D_6

BRUKER

EJD14CSY.SMX
AU PROG:
COSY.AU
DATE 5-11-88

SI2   1024
SI1   512
SW2   2283.105
SW1   1141.553
NO0   1

VDW2   S
VDW1   S
SSB2   0
SSB1   0
MC2   M
PLIM ROW:
F1   3.227P
F2   .386P
AND COLUMN:
F1   3.294P
F2   .386P

D1   1.0000000
P1   16.50
D0   .0000030
P2   10.70
RO   0.0
PW   0.0
DE   273.80
NS   16
DS   2
NE   1
IN   .0004300
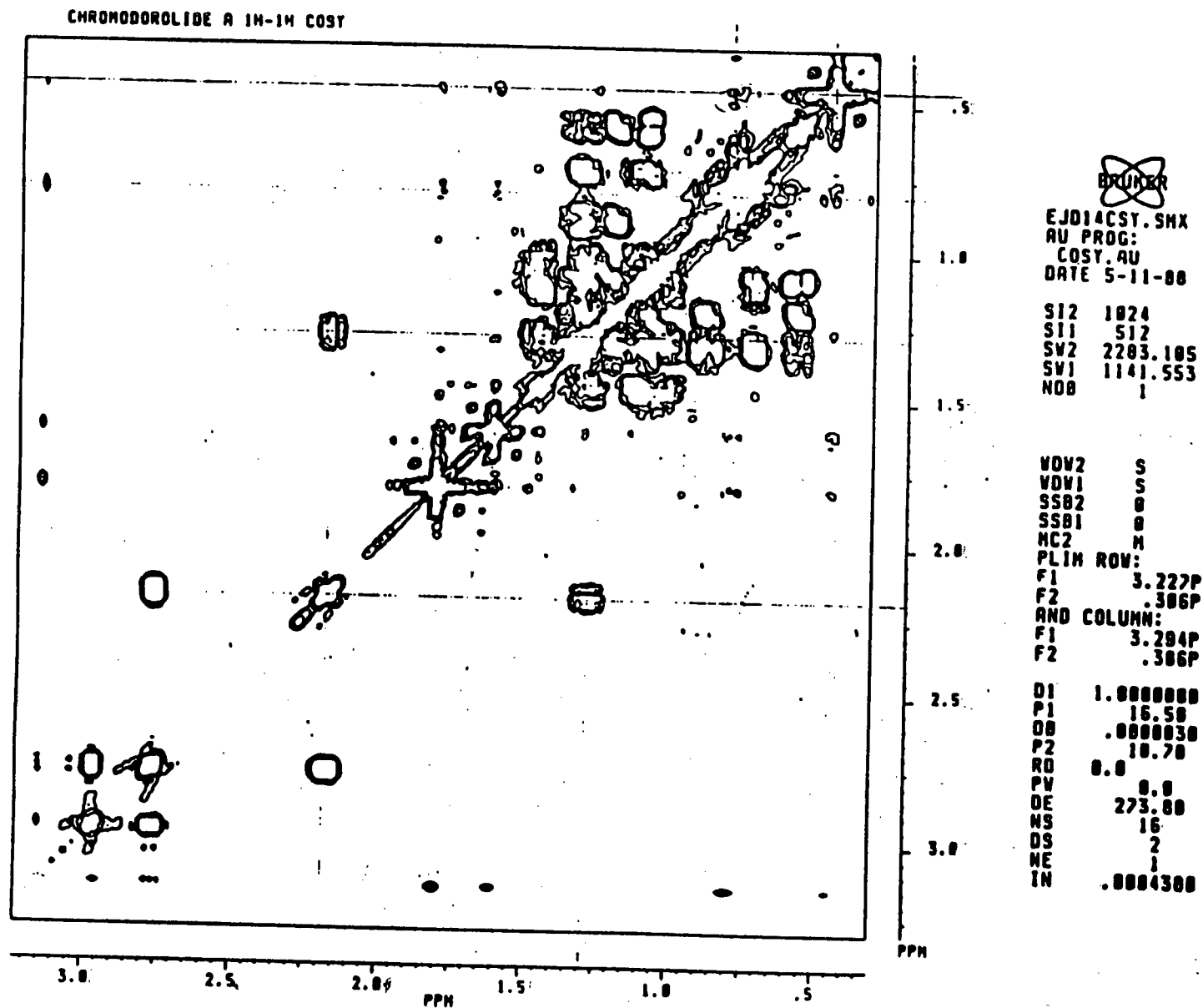
Figure 8a. Expanded upper right quadrant of fig. 8
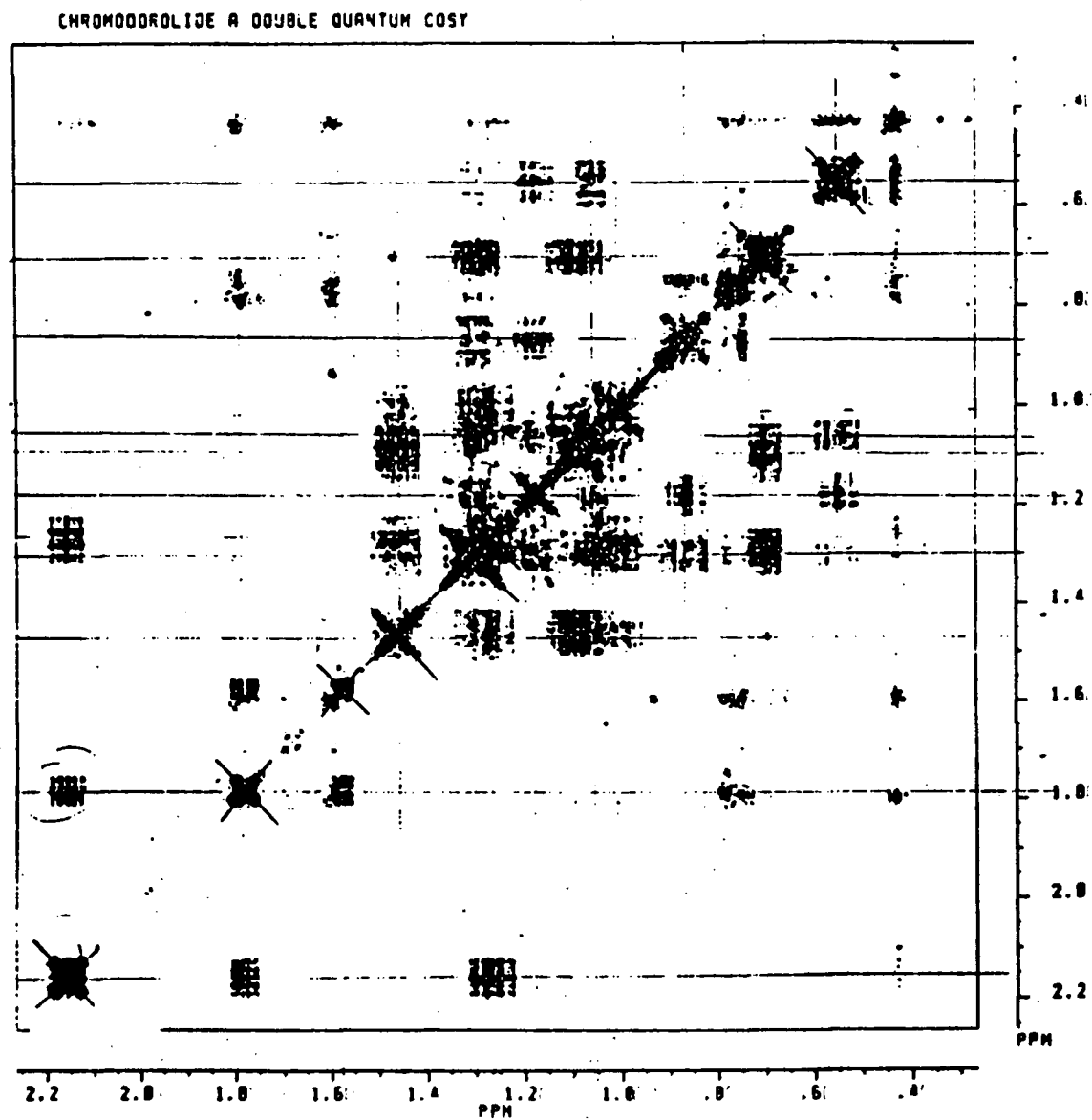
BRUKER

EJD14PDQ.SMX
AU PROG:
  COSYPHDQ.AU
DATE 18-9-88

SI2   1024
SI1   1024
SW2   800.000
SW1   400.000
ND0   2

WDW2   S
WDW1   S
SSB2   0
SSB1   0
MC2   W
PLIM ROW:
F1   2.261P
F2   .265P
AND COLUMN:
F1   2.26

Figure 9. ^{1}H-^{1}H PHDQCOSy of (100, aliphatic resonances) in C_6D_6

structure had been reported to date and nmr experiments could not unequivocally determine the pattern of cyclization, an X-ray diffraction experiment was performed, by Jon Clardy and coworkers at Cornell University, on a chromodorolide A crystal grown from hot methanol.[49]
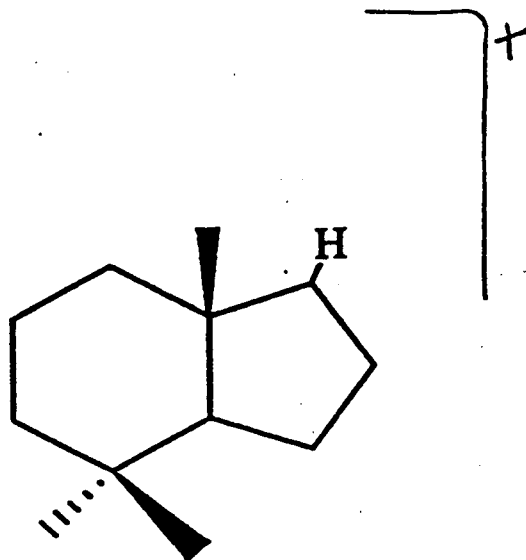
B

The computer-generated drawing of the X-ray crystal structure of chromodorolide A (**100**) (Fig. 10) indicated that the molecule generally followed the norrisane (scheme II) skeleton but a new ring was formed through an unprecedented C-17-C-12 carbon-carbon bond. We suggest the new carbon skeleton resulting from this bond be named "chromodorane" (structure C).
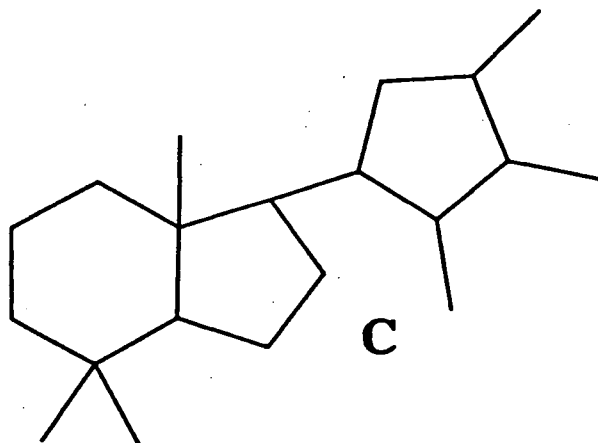
C

[49] Crystals of chromodorolide A belonged to the space group $P2_12_12_1$ with a=8.653(2), b=9.662(3), c=30.743(9) Å and one molecule of composition $C_{24}H_{34}O_8$ CH_3OH forming the asymmetric unit. Additional data can be found in the appendix.
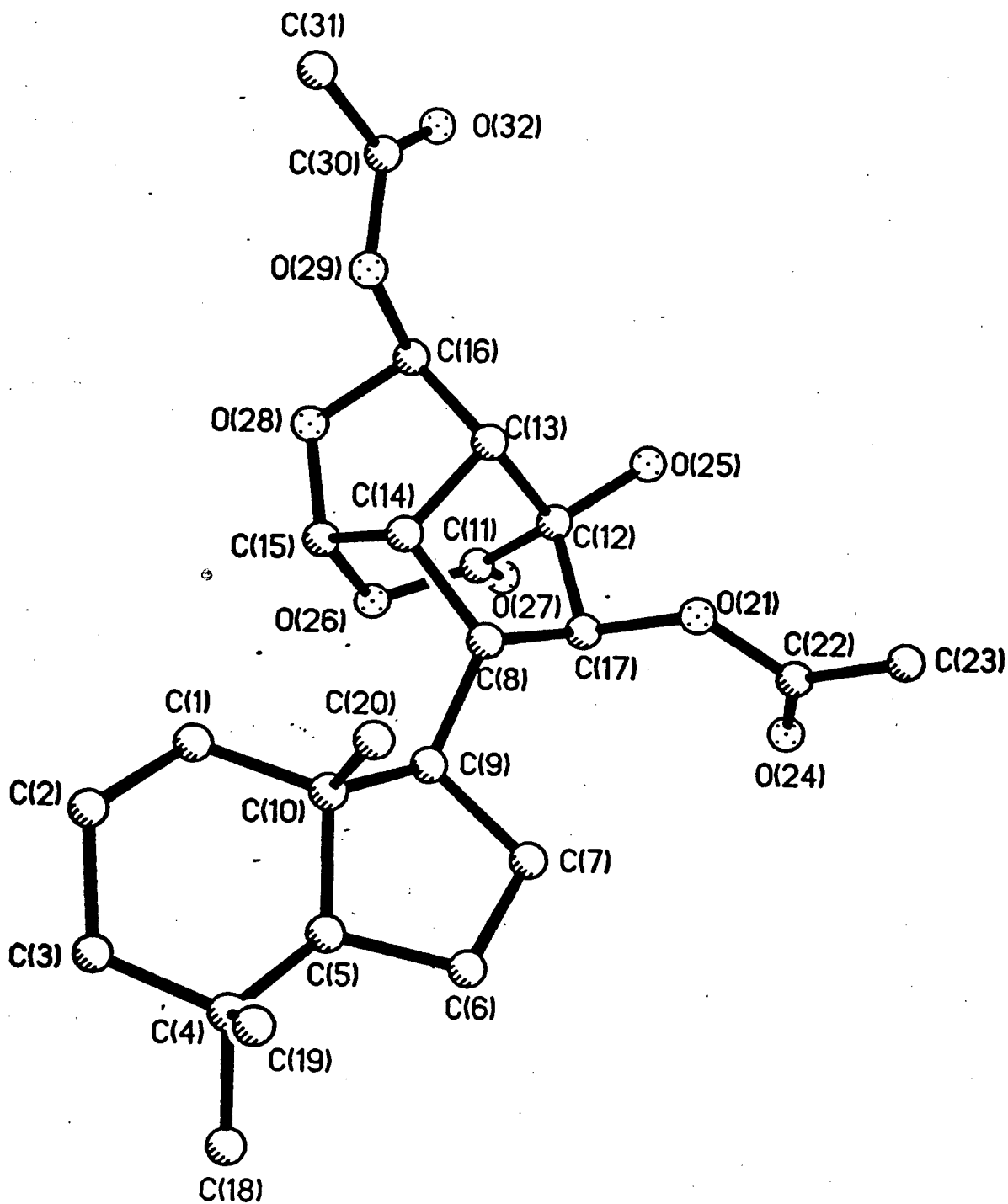
Figure 10. Computer generated X-ray structure of (100)

With the structure of chromodorolide A (100)[50] in hand, assignment of most of the ^{1}H and ^{13}C nmr resonances was fairly straightforward. It is interesting to note that the downfield ketal is a singlet due to a dihedral angle of nearly 90 degrees between H-16 and H-13. All other resonances in the down field ^{1}H nmr spectrum (Fig. 6b) could be assigned to appropriate protons by coupling patterns and chemical shifts (see Table I).

In order to assign the carbons and decipher the couplings observed in the PHDQCOSY plot of the aliphatic system, a single bond [1]H-^{13}C heteronuclear correlation (HETCOR) experiment[51] was performed (Fig. 11). Since the ^{1}H nmr spectrum for the heterocyclic portion of the molecule had already been assigned, assignment of this portion of the ^{13}C was easily accomplished by examination of the HETCOR contour plot (Table I). Assignment of the aliphatic portion was more difficult owing to the fact that two isolated spin systems with many overlapping multiplets were involved. In an attempt to sort out the chemical shifts of all the aliphatic protons, a 2-D J-resolved[52] experiment was performed (Fig. 12). By careful correlation between the HETCOR, PHDQCOSY and 2D J contour plots it could be seen that the H-8 methine (δ 2.16 ppm) was coupled into a methine at δ 1.26 ppm (H-9). This methine was further coupled into one methylene proton at δ 1.47 ppm (H-7α). This resonance was coupled into its upfield geminal partner at δ 1.03 ppm (H-7β) as well as another methylene proton at 1.10 ppm (H-6α). Coupling can be seen from H-6α into its geminal partner at δ 1.28 ppm (H-6β) and an upfield methine at 0.67 ppm (H-5). Correlations from δ 1.28 ppm (H-6β) to 0.67 (H-5) and 1.03 (H-7β) ppm complete the spin system.

Assignment of the remaining six protons was more difficult since they form a completely isolated spin system. It was possible to get a handle on the system

[50] The absolute stereochemistry shown in (100) as well as the numbering system used is based on the assumption that the chromodorane skeleton is generated from a spongian precursor.
[51] Bax, Chapter 2.
[52] Bax, Chapter 3.

Figure 11. ^1H-^{13}C HETCOR of (100) in C_6D_6

VARIAN XL-300
13C OBSERVE

EXP9   PULSE SEQUENCE: HETCOR
DATE   04-10-88
SOLVENT C6D6
FILE   HETCOR

ACQUISITION		DEC. & VT	
TN	13.750	DN	1.750
SW	12500.0	DO	-800.0
AT	0.082	DM	NNY
NP	2048	DMM	CCS
PW	18.0	DMF	8000
P1	0	DHP	N
D1	1.000	DLP	0
D2	0	PP	81.9
TO	-400		
NT	128	PROCESSING	
CT	0	RE	0.005
PW90	18.0	FN	2048
SW2	700.0	AF	0.020
NI	32	MATH	F
BS	64	FN2	128
SS	0	RE2	0.003
IL	Y	AF2	0.011
IN	Y		
DP	Y	DISPLAY	
HS	NN	SP	672.0
J1XH	80.0	WP	12500.0
JNXH	8.0	VS	180
PRESAT	N	SP2	55.6
HMULT	Y	WP2	700.0
GAIN	0	SC	0
		WC	200
		IS	500
		RFL	8981.0
		RFP	9654.9
		TH	9
		SC2	290
		WC2	200
		INS	1.000
		RFL2	-51.6

F2 (PPM)

Figure 11a. Expanded lower right quadrant of fig. 11

^{1}H PPM.

VARIAN XL-300
13C OBSERVE

EXP9   PULSE SEQUENCE: HETCOR
DATE   23-09-88
SOLVENT C6D6
FILE   HETCOR

ACQUISITION		DEC. & VT	
TN	13.750	DN	1.750
SW	8703.2	DO	-100.0
AT	0.118	DM	NNY
NP	2048	DMM	CCS
PW	18.0	DMF	6700
P1	0	DHP	N
D1	0.400	DLP	0
D2	0	PP	81.9
TO	-3000		
NI	512	PROCESSING	
CT	512	RE	0.007
PW90	18.0	FN	2048
SW2	2100.0	AF	0.029
NI	64	MATH	F
BS	32	FN2	256
SS	0	RE2	0.002
IL	Y	AF2	0.008
IN	Y		
DP	Y	DISPLAY	
HS	NN	SP	8.5
J1XH	140.0	WP	5019.5
JNXH	0	VS	3028
PRESAT	N	SP2	-16.5
HMULT	N	WP2	1256.7
		SC	0
		WC	200
		IS	500
		RFL	9681.5
		RFP	9692.6
		TH	9
		SC2	290
		WC2	200
		INS	1.000
		RFL2	2161.9
		RFP2	2144.6
		AI	DC AV

- 54 -

CHROMODOROLIDE A 2DJRES
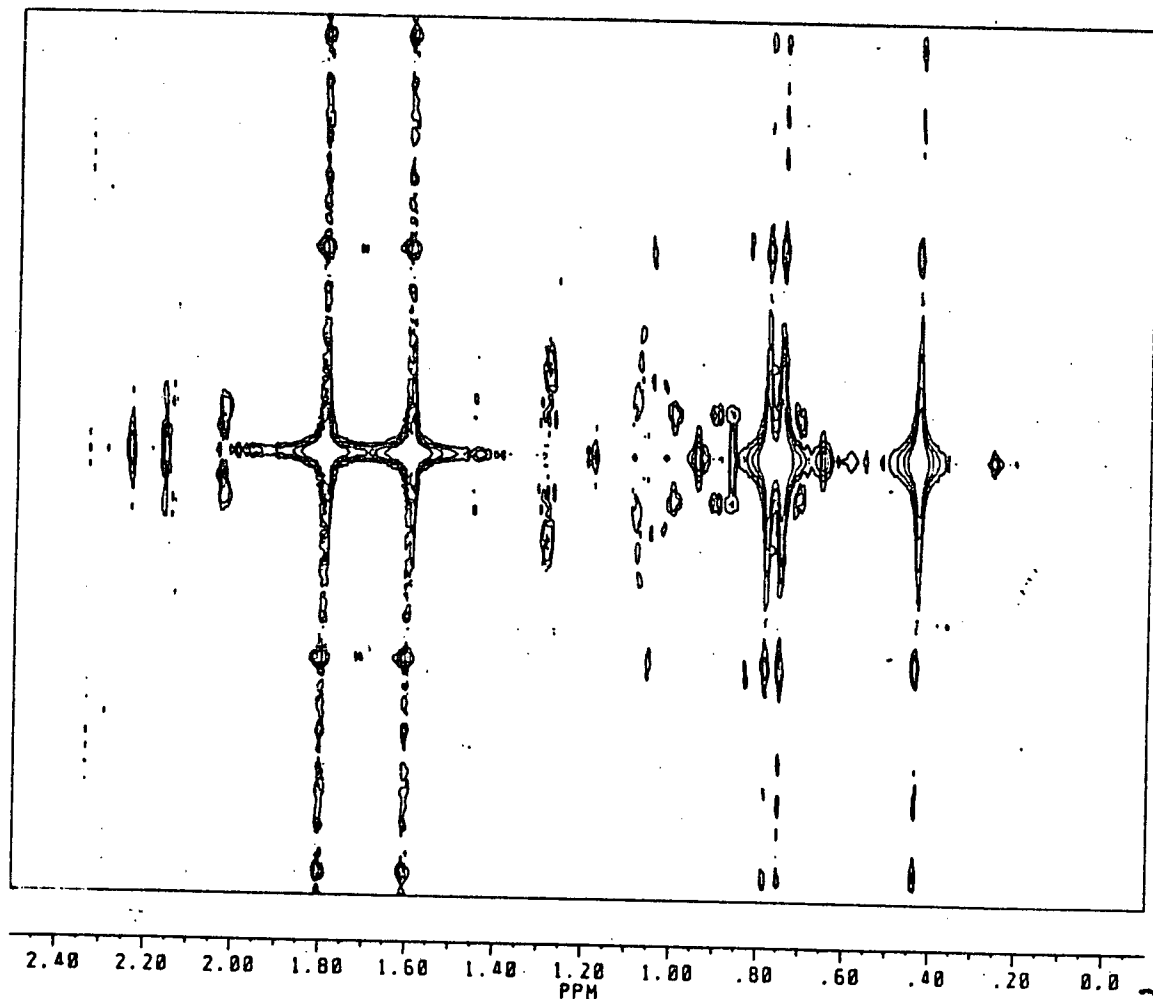
Figure 12. 2D-JRES plot of (100) in C_6D_6
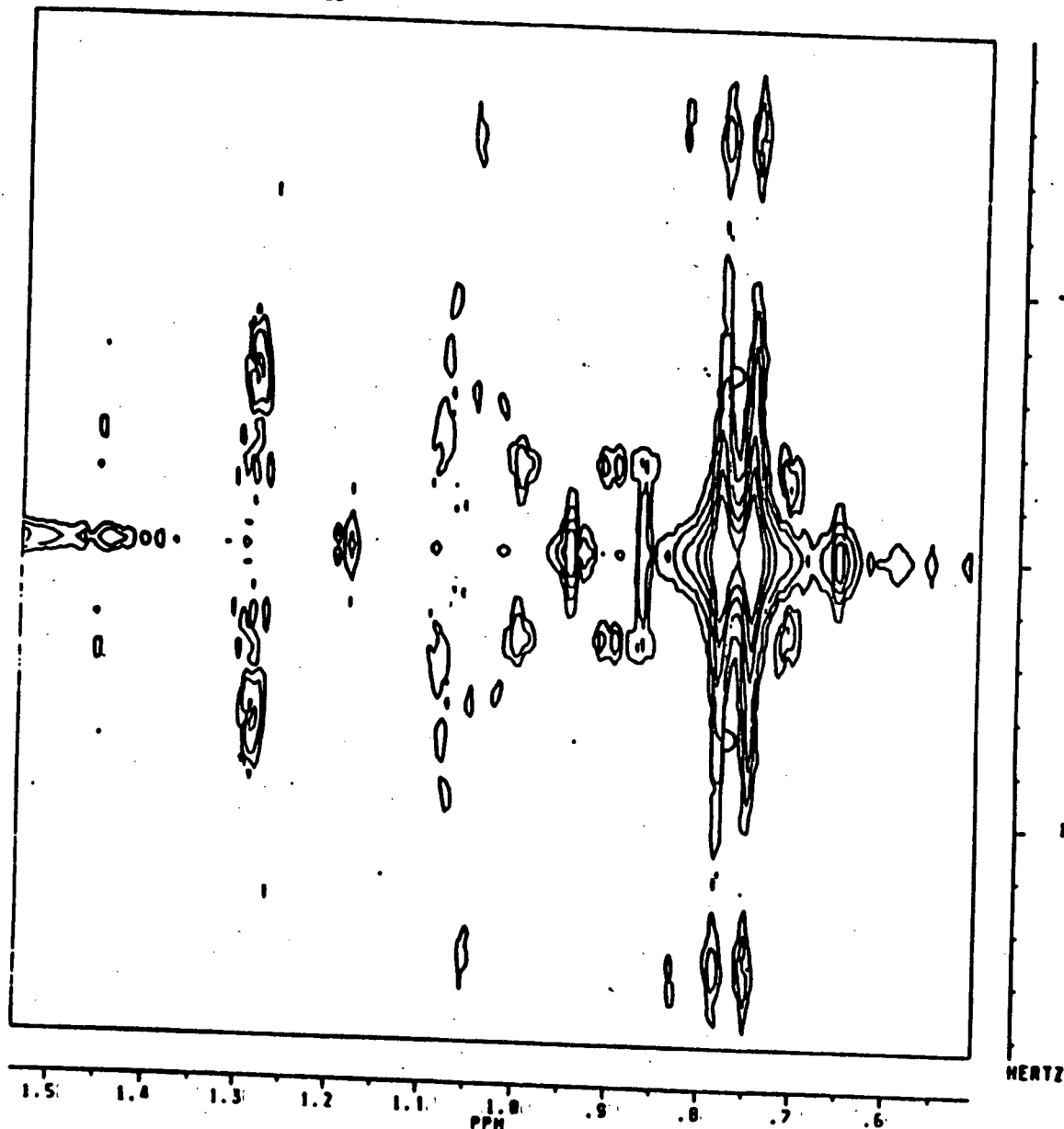
Figure 12a. Expansion of figure 12

through the use of nOe difference spectroscopy.[53] Irradiation of the methine at δ 5.59 ppm (H-15) resulted in a 6% positive enhancement of the multiplet at 0.56 ppm (H-1α), and a 6.2% enhancement of H-14. Irradiation of the upfield multiplet at δ 0.56 ppm (H-1α) resulted in a 12.5% enhancement of the ketal proton at 5.59 ppm (H-15) as well as a 27.5% enhancement to a proton signal at 1.05 ppm (H-1β). Irradiation of the methine at δ 2.75 ppm (H-14) also displayed a 3.9% positive nOe to the same 1.05 ppm (H-1β) proton signal. Such nOes can be explained by examining a molecular model of chromodorolide A (see structure D, below). If, as the X-ray structure shows, ring A exists in a chair conformation with both Me-20 and Me-19 axial, then H-15 can be brought close enough to H-1α and H-1β to account for the observed enhancements. The H-14 methine on the other hand is only close enough to the H-1β proton to exhibit a significant nOe.
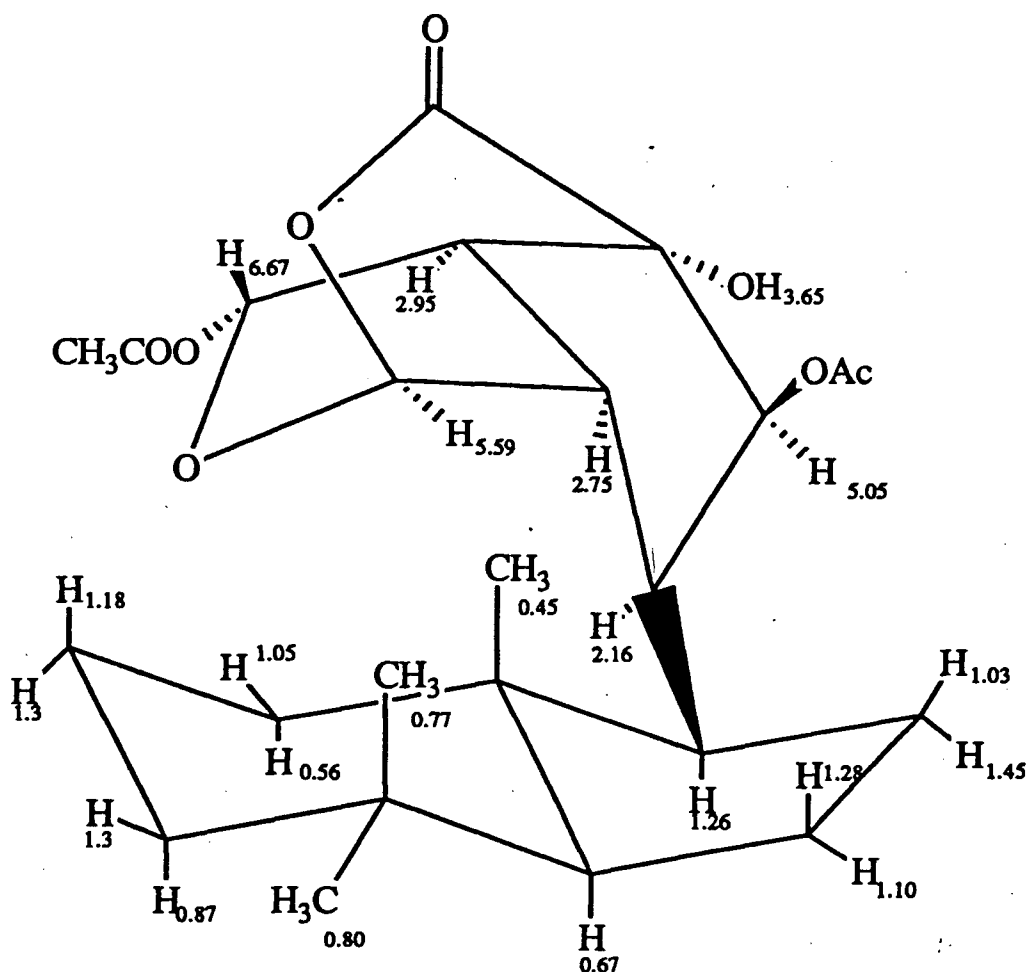
[53] Sanders and Hunter, Chapter 6.

Examination of the COSYPHDQ contour plot (Fig. 9) showed the H-1α proton at δ 0.56 ppm coupling into its geminal partner at 1.05 (H-1β) as well as another methylene signal at 1.18 ppm (H-2β). This latter proton signal showed coupling into protons at δ 1.05 (H-1β), 1.3 (H-2α or H-3β) and 0.87 ppm (H-3α). Examination of the HETCOR plot indicated that a δ 1.3 ppm proton was geminal to the proton at 1.18 ppm (H-2α and H-2β respectively). However, the proton at δ 0.87 ppm (H-3α) also has a geminal partner at 1.3 ppm (H-3β). H-3α couples into one of the protons at δ 1.3 ppm as well as the H-2β signal. Examination of the PHDQCOSY and 2D-J contour plots did not permit the exact determination of the chemical shifts or coupling patterns of the two equatorial protons at δ 1.3 ppm (H-2α and H-3β).

All that remained to be assigned were the five methyl peaks and the quaternary carbon signals. Irradiation of the methyl at δ 0.45 ppm (Me-20) resulted in a 6.5% positive nOe to the methine at 2.16 ppm (H-8) as well as a 7.5% enhancement of the methyl at 0.77 ppm (Me-19). Me-20 is thus assigned as the upfield resonance while δ 0.77 must be Me-19. The ^{1}H nmr methyl resonance at δ 0.80 ppm (Me-18) has a highly deshielded methyl carbon (33.4 ppm C-18) which is characteristic of Me-18 in other spongian diterpenoids, thus supporting the other aliphatic methyl assignments.

Acetate methyls were assigned to their respective carbonyls by a long range heteronuclear correlation experiment (Fig. 13) optimized for a 2 or 3 bond coupling constant of 8 Hz. A clear correlation can be seen between the methyl ^{1}H resonance at 1.80 and the carbonyl at 169.5 ppm as well as acetate methyl signal at 1.60 ppm to the carbonyl at 168.0 ppm. Tentative assignment of the acetates to their respective methinyl protons (H-16, H-17) was based on the observation of a weak nOe (3.8%) from the methyl resonance at 1.60 ppm to the methine proton at 6.67 ppm, however this assignment may be erroneous, since no other nOe's were observed to either acetate. Aliphatic quaternary carbons are assigned by comparison to other spongians. Complete assignments of both ^{1}H and ^{13}C spectra are listed in Table I.
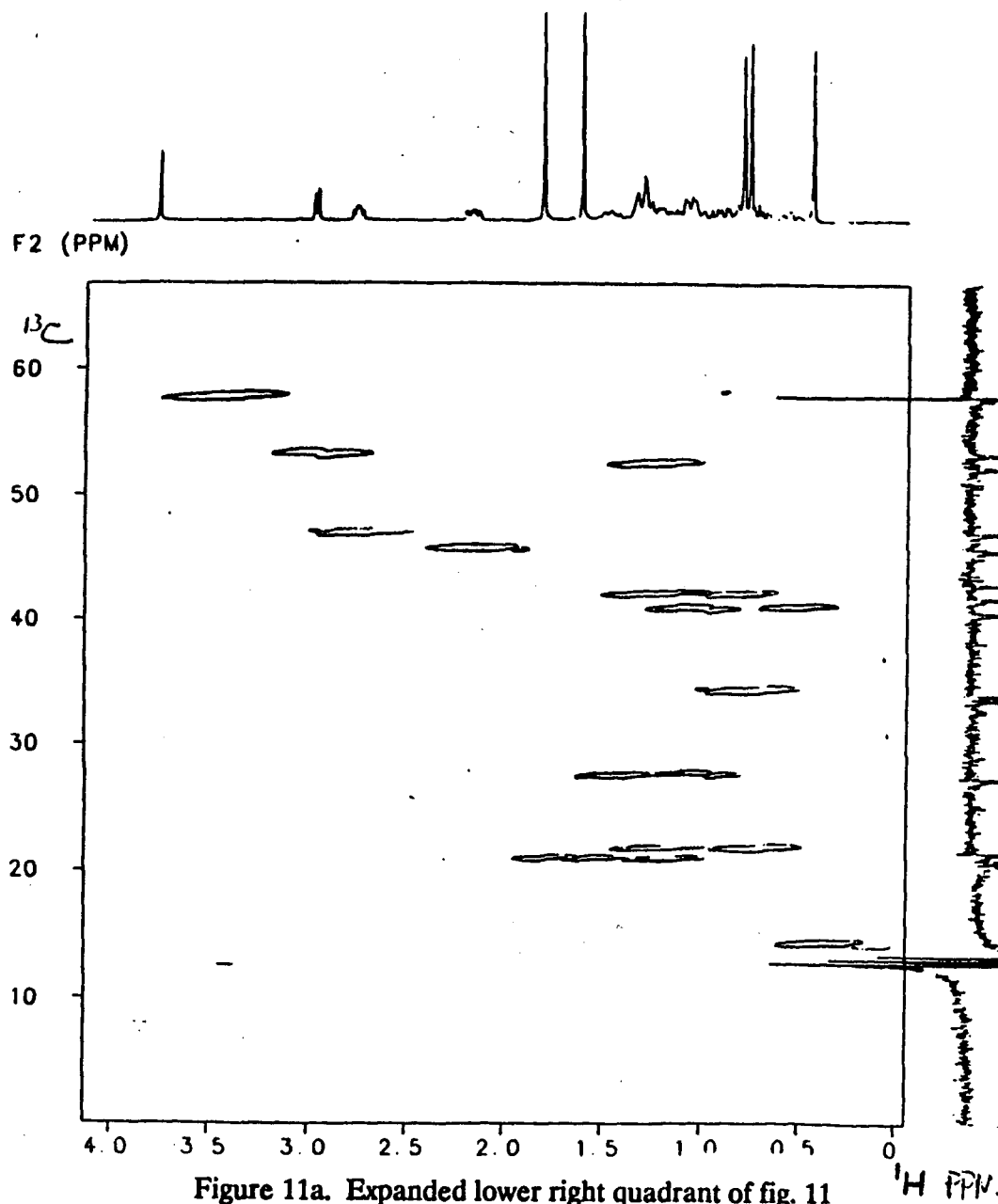
EXP9   PULSE SEQUENCE: HETCOR
DATE   04-10-88
SOLVENT C6D6
FILE   LRHTCR

ACQUISITION		DEC. & VT	
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SW	12500.0	DO	-800.0
AT	0.082	DM	NNY
NP	2048	DMM	CCS
PW	18.0	DMF	8000
P1	0	DHP	N
D1	1.000	DLP	0
D2	0	PP	81.9
TO	-400		
NT	128	PROCESSING	
CT	0	RE	0.005
PW90	18.0	FN	2048
SW2	700.0	AF	0.020
NI	32	MATH	F
BS	64	FN2	128
SS	0	RE2	0.003
IL	Y	AF2	0.011
IN	Y		
DP	Y	DISPLAY	
HS	NN	SP	806.5
J1XH	80.0	WP	6109.5
JNXH	8.0	VS	175
PRESAT	N	SP2	111.1
HMULT	Y	WP2	255.6
		SC	0
		WC	200
		IS	500
		RFL	8981.0
		RFP	9654.9
		TH	9
		SC2	290
		WC2	200
		INS	1.000

F2 (PPM)

¹³C

160 140 120 100 80 60 40 20

¹H 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 PPM

Figure 13. Long range ^{1}H-^{13}C HETCOR of (100) in C$_6$D$_6$

110.78
%T

22.65

88/11/10 10:54
sln14: 16 scans, 4.0cm-1
chromodorolide A

Figure 14. Infrared spectrum of (100). thin film

TABLE I

Complete Assignment of ^{1}H and ^{13}C nmr Spectra ofChromodorolide A (100).

CARBON NUMBER	[1]H(C$_6$D$_6$)	[13]C (C$_6$D$_6$)
1α	0.57	
1β	1.05	40.0
2α	1.3	
2β	1.18	20.1*
3α	0.87	
3β	1.3	41.2
4		33.0
5	0.72	56.8
6α	1.10	
6β	1.28	21.0*
7α	1.45	
7β	1.03	26.7
8	2.17	44.5
9	1.26	51.2
10		42.3
11		172.6
12	3.65 (O-H)	80.4
13	2.96	52.7
14	2.76	46.5
15	5.59	104.2
16	6.67	95.9
17	5.05	79.2
18	0.80	33.4
19	0.77	20.9
20	0.45	13.4
16-M$_E$CO$_2$		168.0
16-H$_3$C-CO$_2$	1.60	20.4
17-M$_E$CO$_2$		169.5
17-H$_3$C-CO$_2$	1.80	20.4

*   assignments may be exchanged

Part II—Dendrillolide (101).

One of the monoacetates isolated has been identified as the previously reported dendrillolide A. Only 6.1 mg of this terpenoid was isolated and it degraded in a subsequent purification step. However, comparison of the ^{1}H nmr spectrum (Fig. 15) with that of a monoacetate isolated in good quantity by E. Dilip de Silva from *Chromodoris gleniei* proved that the two metabolites were identical. Dr de Silva has identified the compound as dendrillolide A based on a comparison of ^{13}C nmr chemical shifts. Table II lists the ^{13}C nmr shift assignments of the monoacetate compared to reported values for dendrillolide A. Dr de Silva is currently trying to establish the stereochemistry of the heterocyclic portion of the molecule in order to resolve the conflicting evidence on the structure of dendrillolide A. The structure reported previously, 85, has been shown to be incorrect through comparison to spectral data of aplyviolene (88). Such discrepancies are thought to have arisen by differences in the stereochemistry at position 15.

(101)
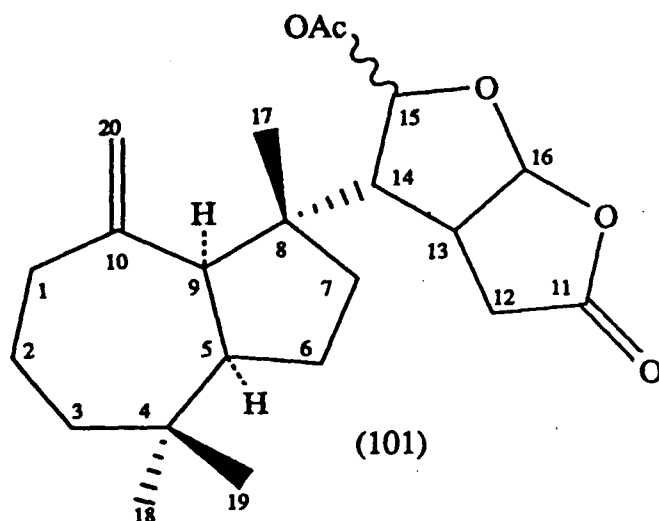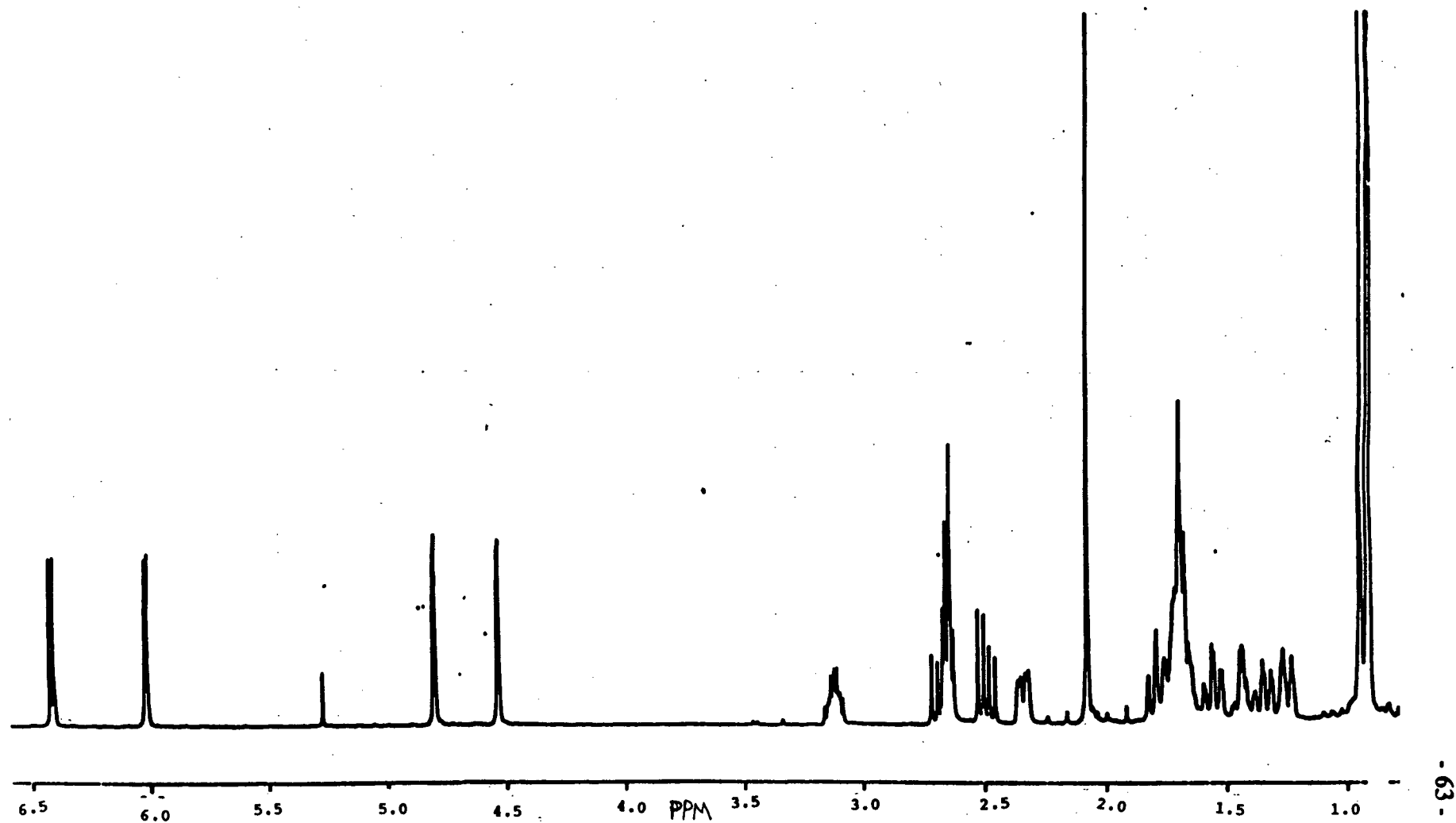
Fig. 15 ^{1}H nmr spectrum of dendrillolide (101) in CDCl$_3$

Table II

Comparison of ^{13}C nmr Chemical shifts (C_6D_6) for Dendrillolide A isolated from *C. gleniei* to reported values.

Carbon #	Found	Reported[54]
1	28.7	28.7
2	38.1	38.7
3	37.7	37.7
4	46.7	46.7
5	54.9	54.9
6	27.1	27.1
7	37.7	37.7
8	36.0	36.0
9	55.7	55.7
10	153.8	153.7
11	175.1	175.2
12	28.8	28.8
13	41.9	41.9
14	54.6	54.5
15	105.0	105.0
16	97.1	97.1
17	24.1	24.1
18	25.7	25.8
19	34.4	34.5
20	114.3	113.3
OAc	20.7	20.7
	169.0	169.1

[54] see note [40] above.

Part III---Chromodorolide B (102)

The second monoacetate (chromodorolide B, 102) seems to be related to chromodorolide A (100). The ^{1}H nmr of (102) (Fig. 16) is similar to that of (100), (Fig. 4), but has some very interesting differences. Three aliphatic methyls at δ 0.70, 0.77 and 0.80 ppm and a single acetate resonance at 2.13 ppm are present in the $CDCl_3$ [1]H nmr spectrum of (102). Interestingly, however, a methyl ether resonance at δ 3.33 ppm is present instead of a second acetate. The heterocyclic portion of the chromodorolide B also displays a different coupling pattern than that observed in (100). One-dimensional decoupling and ^{1}H-^{1}H COSY (Fig. 19) experiments have worked out the spin system outlined in partial structure E below. The downfield doublet at δ 6.04 ppm (J = 7 Hz) is coupled into a doublet of doublets coincidental with the methyl ether at 3.33 ppm (dd, J=7,10 Hz). The proton at δ 3.33 ppm is further coupled into a resonance at 2.85 ppm (ddd, J=5.5,7,10 Hz) which is coupled into the two protons resonating at 5.07 ppm (d, J=5.5 Hz) and 2.35 (ddd, J=7,9,11 Hz). The methine proton at δ 2.35 ppm also couples into the ketal doublet at 5.48 (J=9 Hz) and into a multiplet at 1.80 ppm.
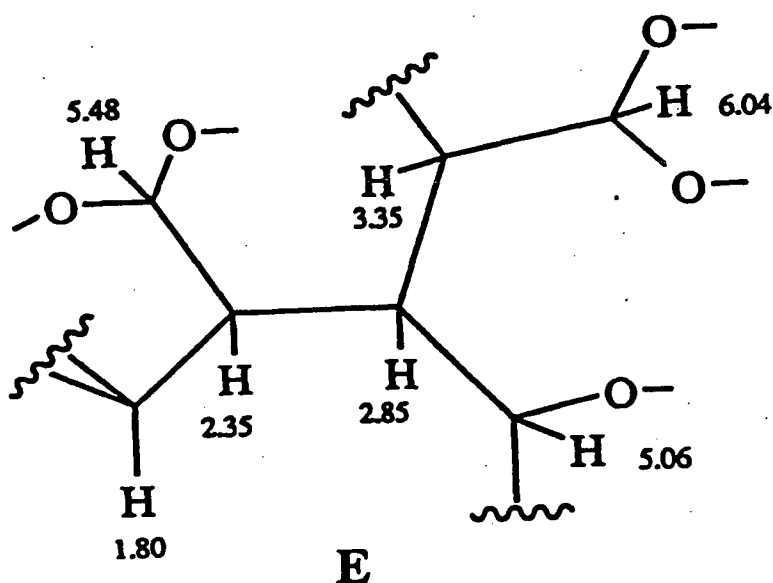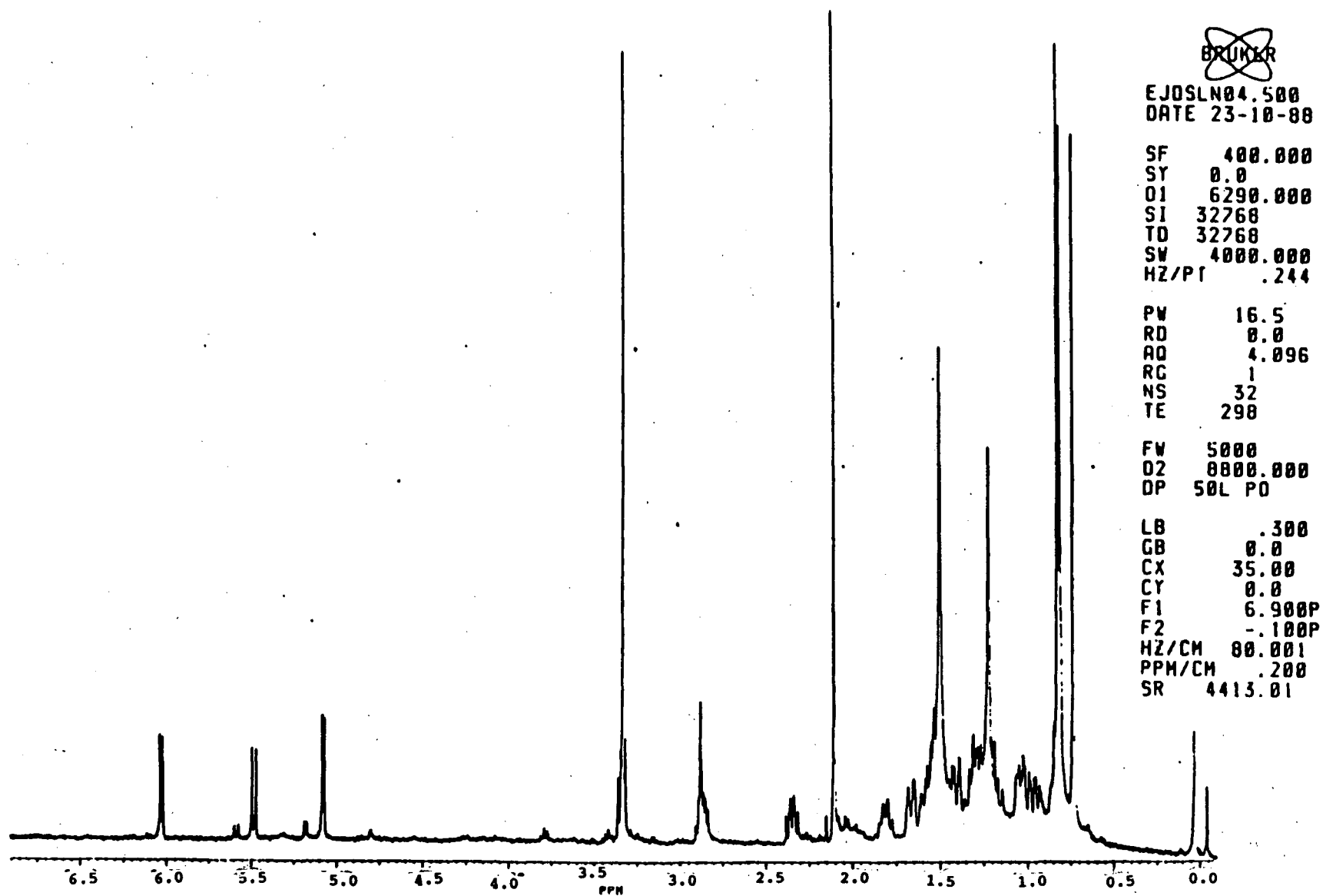
Fig. 16 ^{1}H nmr spectrum of chromodorolide B (102) in CDCl$_3$
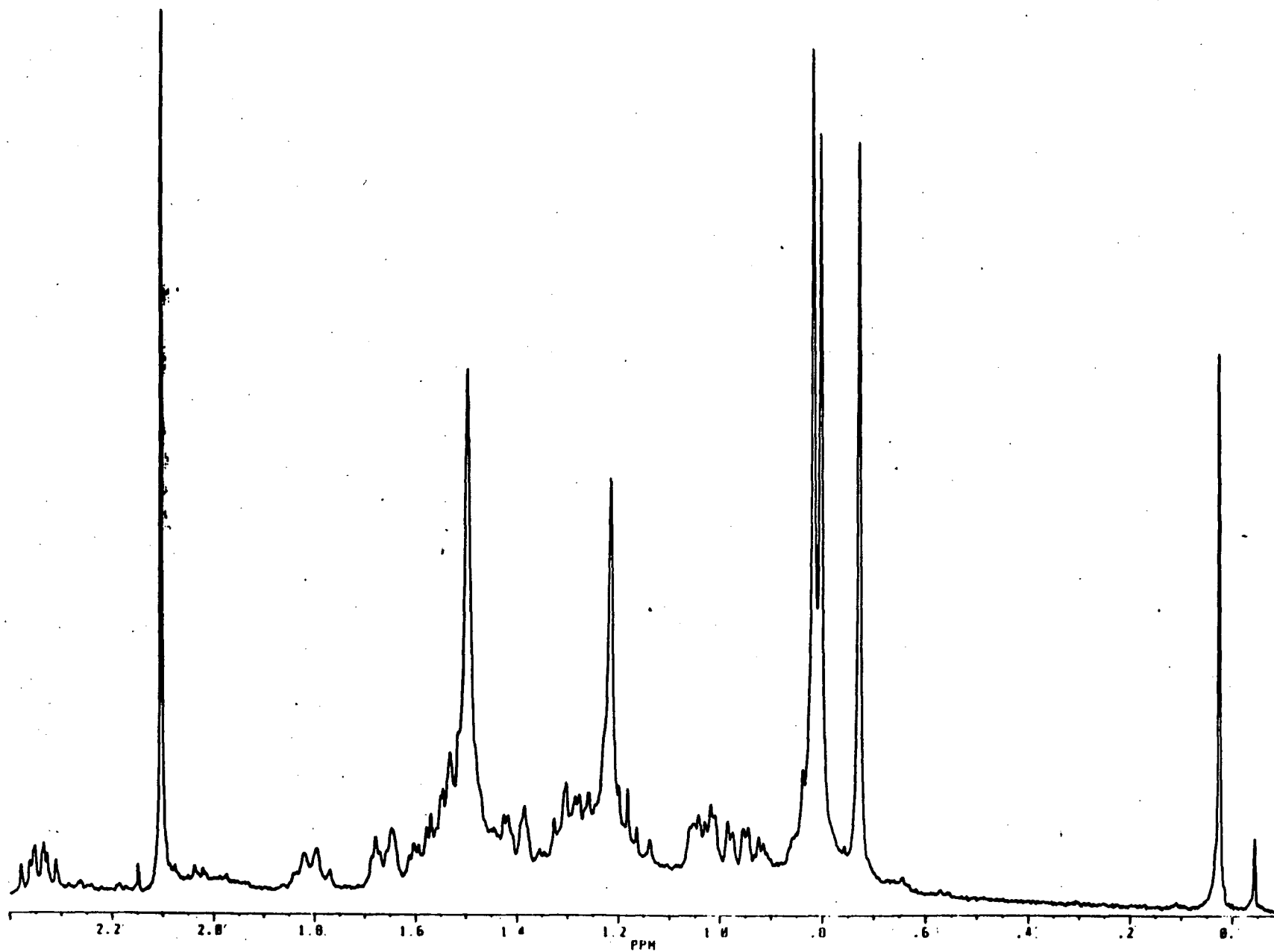
Fig. 16a   Expansion of upfield region of fig. 16
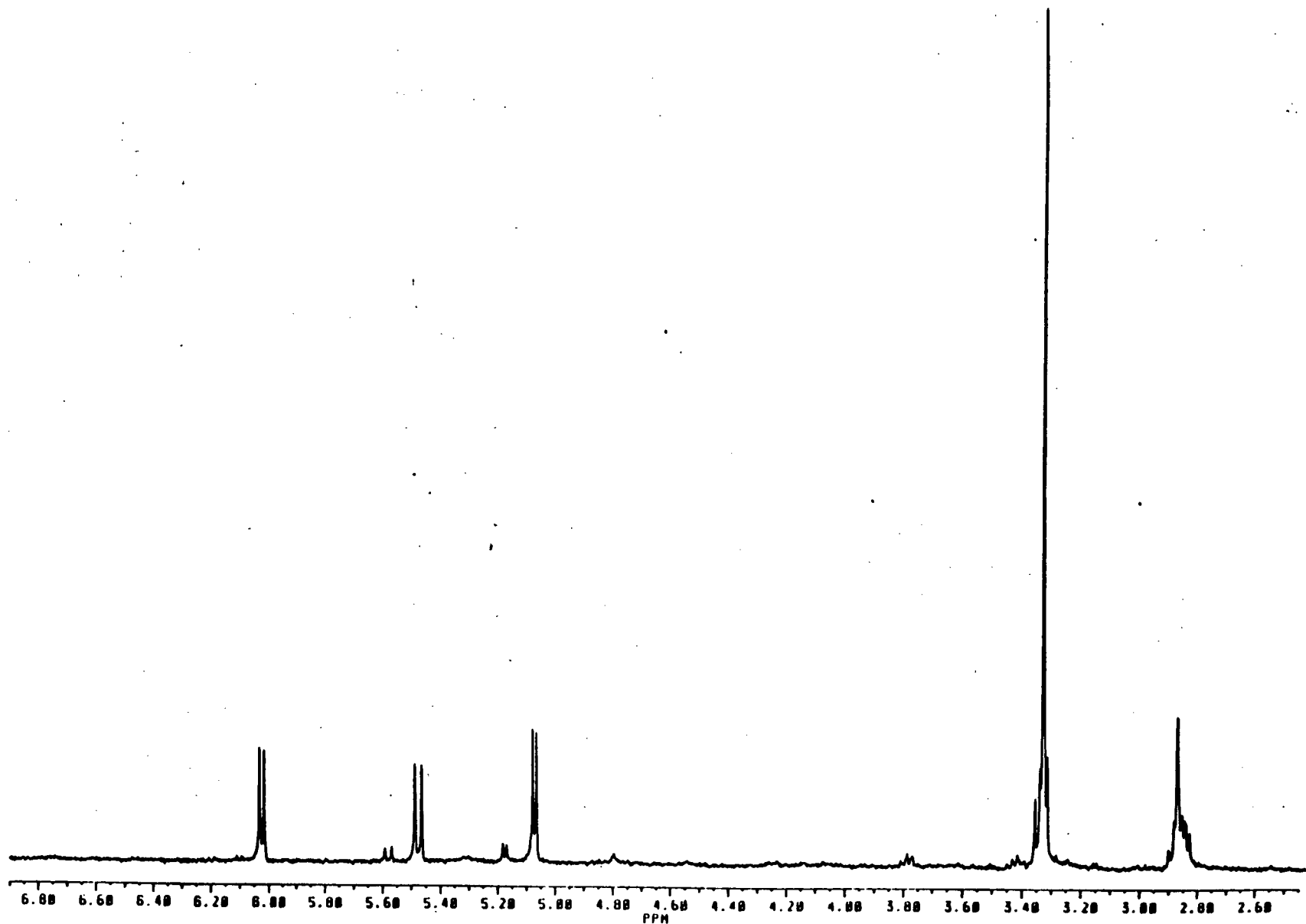
Fig. 16b   Expansion of downfield region of fig. 16

The strongest evidence supporting a relationship between (100) and (102) is the mass spectral fragmentation patterns of the two compounds (Figs. 2 and 17 respectively). Chromodorolide B has an almost identical fragmentation pattern in the low mass (<200 amu) region of the spectra. The peak at m/z 163 seems to indicate that the chromodorolides both contain the [4,3,0] bicyclic system of partial structure **B**.

Comparison of the ^{13}C nmr spectra of (100) and (102) (Figs. 5 and 18 respectively) also implies a familial relationship between the two compounds. Two ketal methines at δ 106.7 and 104.1 ppm in the chromodorolide B spectrum as well as another oxymethine at 77.7 ppm are very reminiscent of chromodorolide A. Five aliphatic methines and five methylenes indicate a skeleton very similar to (100) in chromodorolide B. All five methyl resonances can be observed. A downfield methyl at δ 33.4 ppm is indicative of Me-18 in spongian diterpenoids. Two resonances at δ 21.3 and 20.7 ppm account for the acetate and Me-19 while the peak at 13.3 is likely Me-20. The fifth methyl is a methyl ether resonance δ 54.7 ppm. Because of small sample size and its relative impurity, quaternary carbons and carbonyls could not be observed. Table III lists a comparison of ^{13}C nmr resonances for chromodorolide A (100) and B (102).

Although undeniably similar to (100), the spin system of chromodorolide B outlined in partial structure **E** cannot be reconciled with the chromodorane skeleton. Work is currently underway to elucidate the structure of chromodorolide B which should prove to be another highly unusual rearranged spongian diterpenoid.
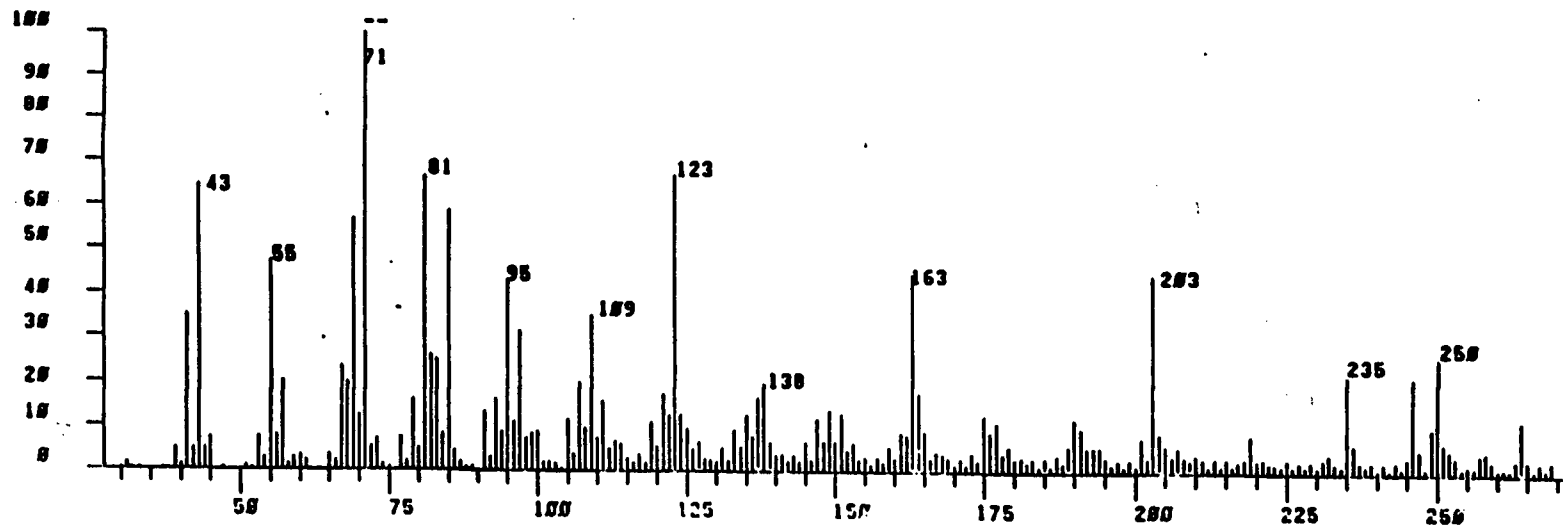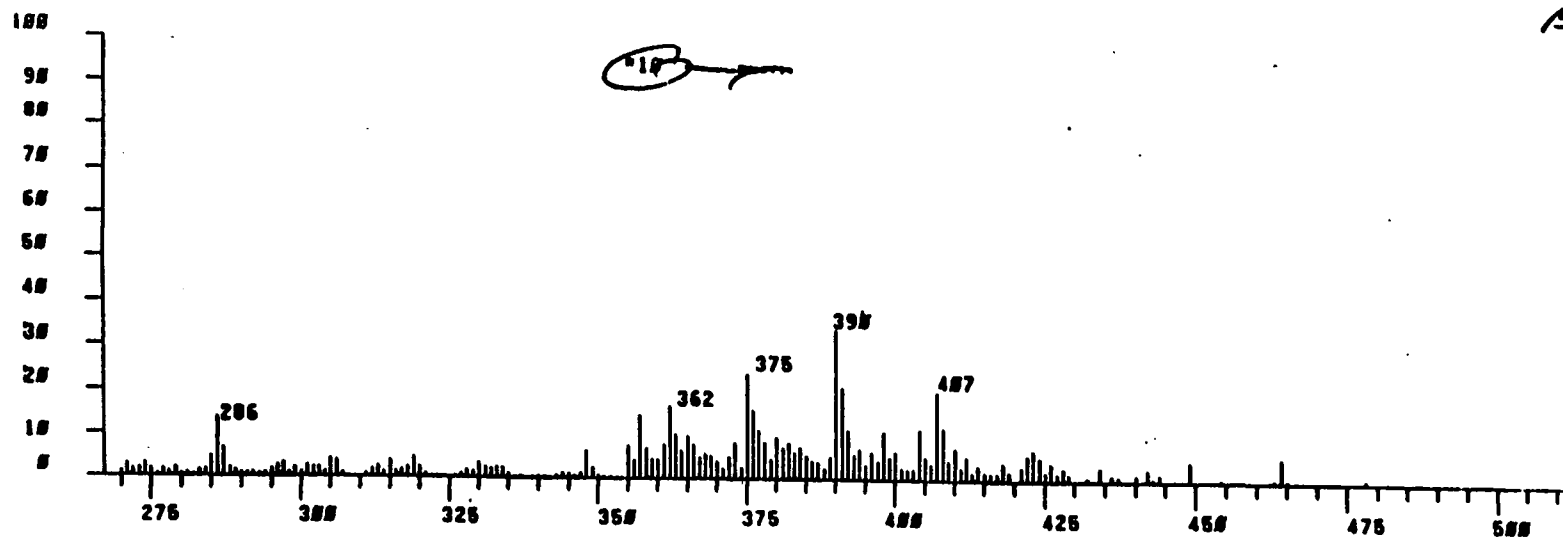
Fig. 17   EI mass spectrum of chromodorolide B (102)

CHROMODOROLIDE B
APT

13 C
BB DECCUPLED
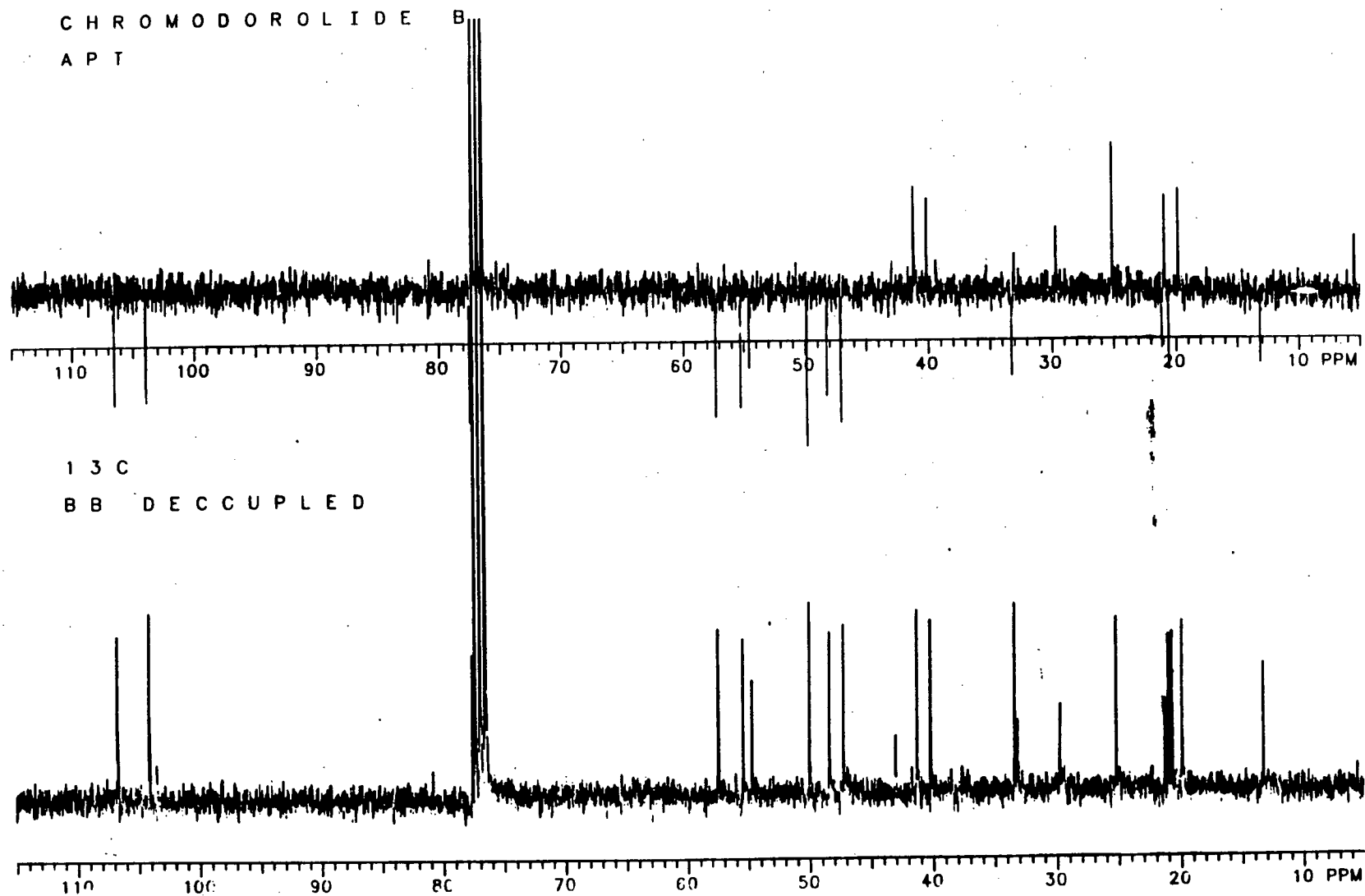
Fig. 18 ^{13}C nmr spectrum of (102) in CDCl$_3$

BRUKER

EJDSLN04.SMX
AU PROG:
 COSY.AU
DATE 23-10-88

SI2   1024
SI1   512
SW2 2283.105
SW1 1141.553
ND0   1

WDW2   S
WDW1   S
SSB2   0
SSB1   0
MC2   M
PLIM ROW:
F1   6.228P
F2   .531P
AND COLUMN:
F1   6.228P
F2   .531P

D1   1.0000000
P1   16.50
D0   .0000030
P2   16.50
RD   0.0
PW   0.0
DE   273.80
NS   80
DS   2
NE   256
IN   .0004380

Fig. 19 ^1H-^1H COSY of (102) in $CDCl_3$

Fig. 19a   Expansion of upper right quadrant of fig. 19
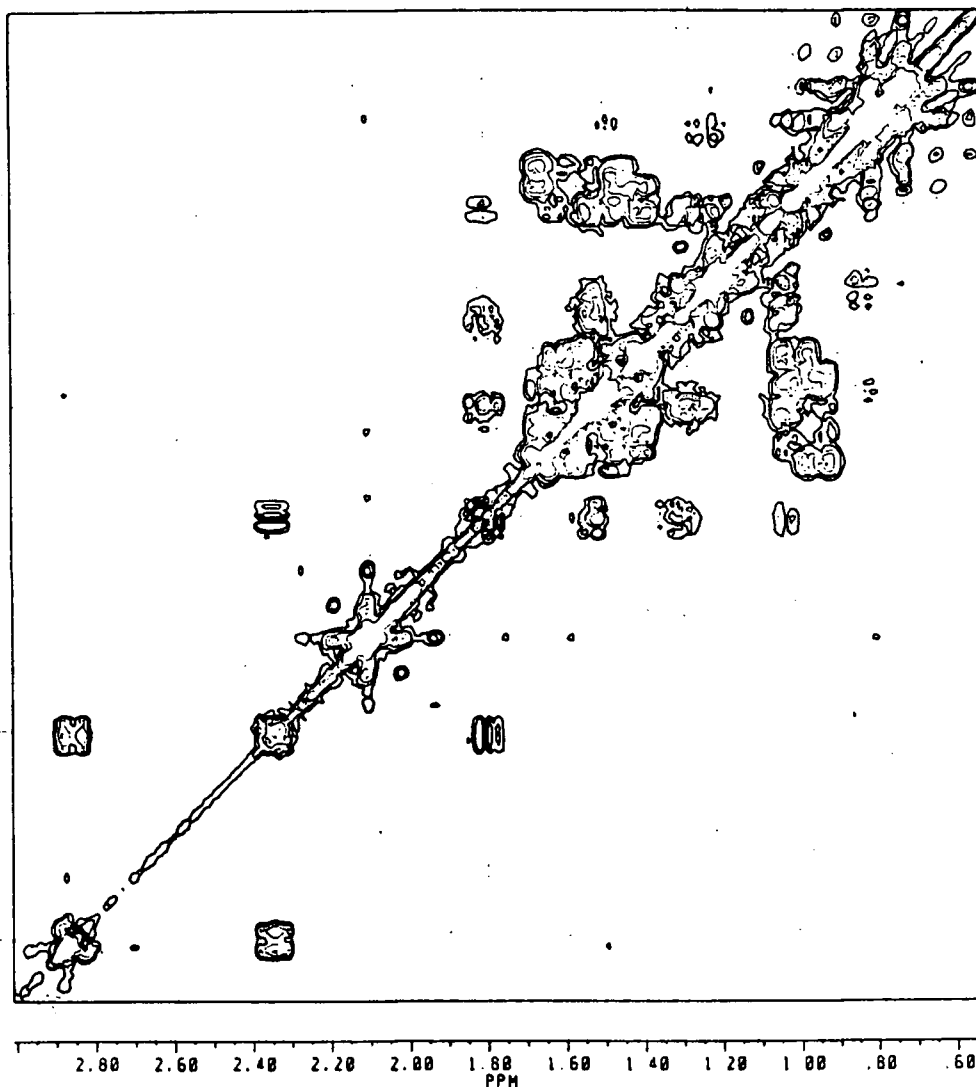
BRUKER

EJDSLN04.SMX
AU PROC:
 COSY.AU
DATE 23-10-88

SI2   1024
SI1   512
SW2   2283.105
SW1   1141.553
ND0   1

WDW2   S
WDW1   S
SSB2   0
SSB1   0
MC2   M
PLIM ROW:
F1   3.006P
F2   .531P
AND COLUMN:
F1   3.006P
F2   .531P

D1   1.0000000
P1   16.50
D0   .0000030
P2   16.50
RD   0.0
PW   0.0
DE   273.80
NS   80
DS   2
NE   256
IN   .0004380

P-E

100.63
XT

92.34

4000   3500   3000   2500   2000   1500   1000   cm⁻¹

Fig. 20   Infrared spectrum of (102), thin film.
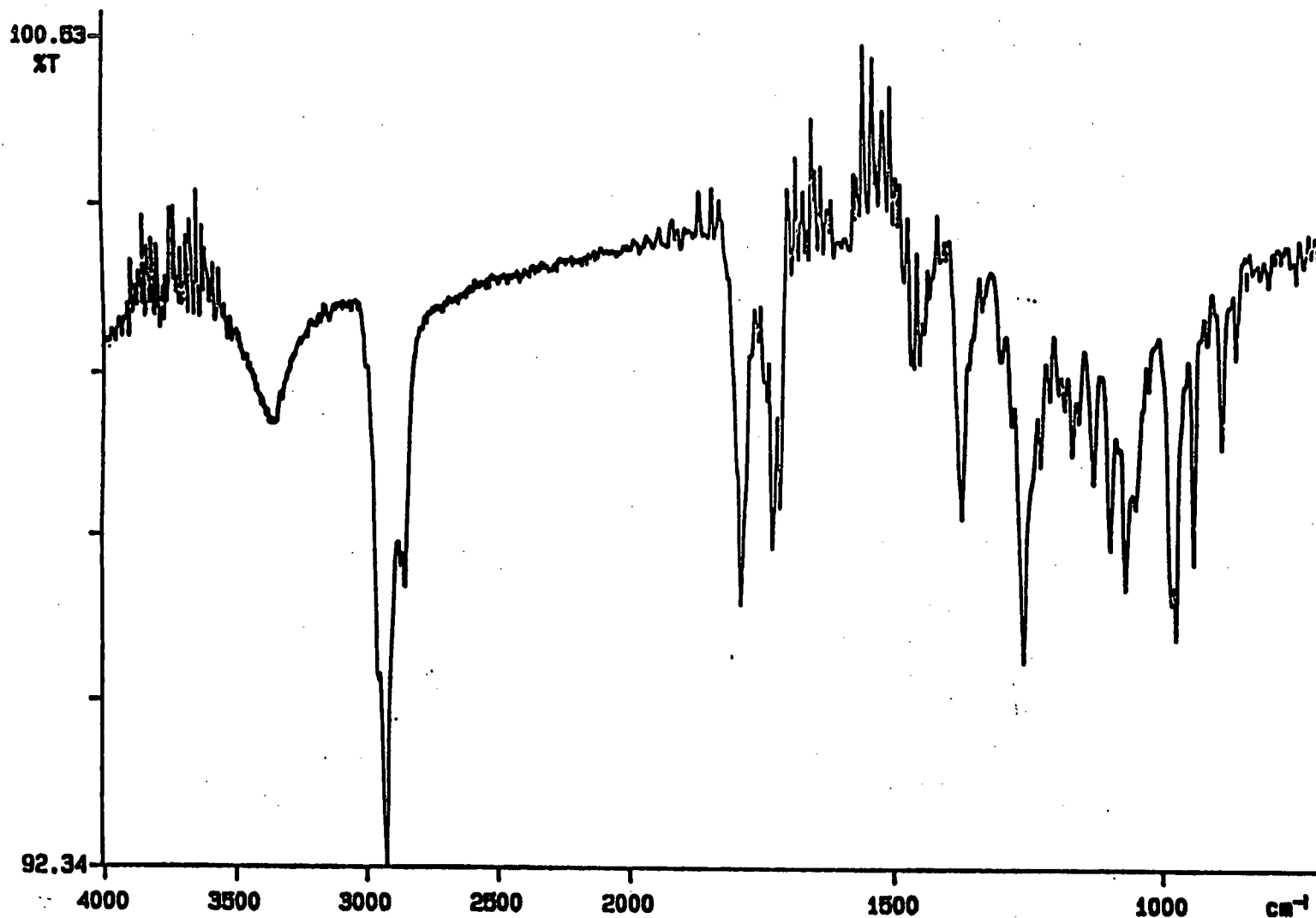
88/09/13 16: 01
SCAN: 64 scans, 4.0cm-1
sln04 monoacetate #2

TABLE III

Partial Comparison of ^{13}C nmr resonances for Chromodorolides A (100) and B (102).

CARBON NUMBER	(100)	(102)
1	40.4	40.2
2	19.8	19.9
3	41.1	41.3
5	57.8	57.4
6	20.8	21.0
7	26.6	25.2
8	44.6	47.2
9	51.6	50.0
13	52.1	55.4
14	46.3	48.4
15	104.3	106.7
16	95.6	104.1
17	78.8	77.7
18	33.4	33.4
19	20.8	20.7 (21.3)
20	13.6	13.3

CONCLUSION

Three rearranged spongian metabolites **100-102** have been isolated from the Indian Ocean nudibranch *Chromodoris cavae*. At least one of these, chromodorolide A, (**100**), displays a wide range of biological activity and may serve as a chemical antifeedant. Work is currently underway to elucidate the structures of the other two minor metabolites, and plans to examine their possible role in inhibiting fish predation are being made.

Experimental

The ^{1}H and ^{13}C nmr spectra were recorded on Bruker WH-400 and Varian XL-300 spectrometers. Most spectra were run using either $CDCl_3$ or C_6D_6 as an internal standard, though the peaks were referenced to internal tetramethylsilane ($\delta = 0$ ppm) in order to correct for solvent variance. Low-resolution electron impact mass spectra were recorded on an A.E.I. MS-902 spectrometer and high resolution mass spectra were recorded on an A.E.I. MS-50 spectrometer. Chemical ionization mass spectra were recorded on a Nermag R 10-10 C spectrometer employing NH_3 as the ionizing agent. Infrared spectra were recorded on a Perkin-Elmer series 1600 FTIR. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter using a 1.000 dm microcell, and a Fisher-Johns apparatus was used to determine melting points which are uncorrected.

A Perkin-Elmer Series 2 instrument was used for hplc wherein a Perkin-Elmer LC-55 uv detector linked to a Hewlett-Packard 18850A recorder was employed for peak detection. A Whatman Magnum-9 Partisil 10 column was used for preparative hplc. All hplc, chromatotron and crystallization solvents were BDH Omni-solv grade or Fisher hplc grade. All other chromatography solvents were reagent grade. Merck Silica Gel 230-400 mesh was used for flash chromatography employing N_2 as the propulsive gas. Preparative tlc was carried out with Merck Silica Gel 60 PF-254 on glass plates. A Harrison Research model 7924 Chromatotron was used with Merck Silica Gel 60 PF-254 / $CaSO_4 \cdot \frac{1}{2}H_2O$ for radial tlc.

Collection Data

Ninety specimens of *Chromodoris cavae* [44] were collected from the waters near Jaffna on the northern coast of Sri Lanka during the months of January to March 1988.

Extraction and Purification

The fresh animals were preserved in dichloromethane/methanol (1:1) and stored in the freezer. The organic layer was separated from thawed samples, the aqueous layer was concentrated at reduced pressure and extracted with more methylene chloride. The combined organic layers were evaporated at reduced pressure to yield 2.74 g of crude extract as a red-orange oil.

One half of the residue was dissolved in dichloromethane (10 mL) and fractionated by silica flash chromatography using a step gradient elution of increasing amounts of diethyl ether or methanol in dichloromethane. The less polar fractions (0-10% diethyl ether / methylene chloride) were found to contain mainly long chain hydrocarbons and steroids, while those eluting at high polarity (60% diethyl ether and 10-50% methanol in dichloromethane) contained mainly triacyl glycerides and fatty acids. The fractions eluting between twenty and thirty percent diethyl ether in dichloromethane were shown by tlc and nmr to be of the greatest interest.

These fractions were further purified by preparative scale silica thin layer chromatography (prep tlc) using a solvent system of 12% diethyl ether in methylene chloride. The major component, (100), (Rf 0.42) was shown to be pure by tlc and nmr. Minor components failed to purify completely on a series of silica high

[44] Identified by Sandra Millen, Zoology Dept. UBC. A voucher sample is deposited at UBC.

performance chromatography (hplc) which followed. The very weak chromophores of the minor components made it difficult to interpret the ultraviolet detector traces. Better response was often seen for impurities than the compounds of interest.

Further purification was achieved using the chromatotron with a solvent system of 15% Et_2O/CH_2Cl_2. Analysis of the eluting fractions by nmr spectroscopy indicated a high degree of purity for both a diacetate (chromodorolide A, **100**) and two monoacetates. Chromodorolide A was easily separated from trace colored impurities by crystalization from hot methanol to give 31 mg of colorless needles (melting point 133-134°C), $[\alpha]_D$ -0.4 (c 3, $CDCl_3$). Further purification of the monoacetates was not judged necessary. Evaporation under reduced pressure yielded 6.2 mg of dendrillolide A (**101**) and 4 mg of a previously unreported diterpene (chromodorolide B, **102**).

Chromodorolide A (**100**) is biologically active, inhibiting growth of *Bacillus subtilis* and *Rhizoctonia solani* at a minimum inhibitory concentration of 60 μg/disc. Cytotoxic activity against L1210 gave an ED_{50} value of 20μg/ml, and antineoplastic activity in the P388 mouse leukemia assay resulted in a T/C value of 125% at 4mg/kg with no toxicity observed at this level.[45]

Chromodorolide A (**100**):Electron Impact Mass Spectrometry m/z (rel. int.): Low Resolution: 390 (1), 375 (1), 362 (3), 334 (5), 319 (6), 274 (8) 164 (27), 163 (31), 123 (58), 109 (36), 95 (53), 81 (55), 69 (57), 55 (30), 43 (100); High Resolution: 390.2043 ($C_{22}H_{30}O_6$, calcd. 390.2042), 375.1807 ($C_{21}H_{27}O_6$ calcd. 375.1807), 334.2152 ($C_{20}H_{30}O_4$ calcd. 334.2144), 330.1825 ($C_{20}H_{26}O_4$ calcd. 330.1831), 274.1939

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[45] ED_{50} refers to an "effective dose" causing 50% inhibition of cell growth. L1210 is a human lymphocytic leukemia cell line. T/C is a ratio of survivors in test/control groups of leukemiatic mice. For particulars on the assay procedures employed contact Dr. T. Allen, Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alta, Canada.

(C$_{18}$H$_{26}$O$_2$ calcd. 274.1933). Chemical Ionization Mass Spectroscopy m/z: 469, 468, 451, 408, 392, 391, 362, 332, 319, 287, 267, 224, 218, 177, 163, 138, 123, 109, 95, 81. Fourier Transform Infrared Spectroscopy, thin film, cm^{-1}: 3852, 3464, 2948, 2869, 2846, 1770, 1654, 1220. ^{1}H nmr in CDCl$_3$ (400 MHz, ppm from TMS):δ 6.36 (s,1H), 5.79 (dd,1H, J=1.9,1.7 Hz), 4.83 (d,1H, J=2.9 Hz), 3.31 (s,1H,exch.), 3.06 (m,2H), 2.45 (ddd,1H, J=2.9,5.6,12.2 Hz), 2.12 (s,3H), 2.08 (s,3H), 1.82 (m,1H), 1.34 (m,1H), 1.07 (m,1H), 0.88 (s,3H), 0.86 (s,3H,), 0.85 (s,3H). ^{13}C nmr in CDCl$_3$ (75.4 MHz, ppm from TMS):δ 172.3 (C), 170.0 (C), 168.9 (C), 104.3 (CH), 95.55 (CH), 79.9 (C), 78.8 (CH), 57.8 (CH), 53.4 (C), 52.1 (CH), 51.6 (CH), 46.3 (CH), 44.6 (CH), 42.5 (C), 41.1 (CH$_2$), 40.4 (CH$_2$), 33.4 (CH$_3$), 26.6 (CH$_2$), 20.8 (CH$_2$,CH$_3$), 19.8 (CH$_2$), 13.6 (CH$_3$). ^{1}H nmr in C$_6$D$_6$ (400 MHz, ppm from TMS):δ 6.67 (s,1H), 5.59 (dd,1H,J=3.5,1.0 Hz), 5.03 (d,1H,J=2.6Hz), 3.61 (s,1H,exc.), 2.96 (dd,1H,J=5.4,1.0Hz), 2.76 (ddd,1H,J=3.5,6.8,5.5 Hz), 2.17 (ddd,1H,J=6.8,2.9,12.0Hz), 1.80 (s,3H), 1.60 (s,3H), 1.45(m,1H), 0.87(m,1H), 0.80 (s,3H), 0.77 (s,3H), 0.74 (dd,1H,J=5.3,11.6 Hz), 0.57 (m,1H), 0.45 (s,3H). ^{13}C nmr in C$_6$D$_6$ (75.4 MHz, ppm from TMS):δ 172.6 (C), 169.4 (C), 168.0 (C), 104.2 (CH), 95.9 (CH), 80.4 (C), 79.2 (CH), 57.3 (CH), 52.7 (CH), 51.7 (CH), 46.5 (CH), 45.1 (CH), 42.3 (C), 41.2 (CH$_2$), 40.0 (CH$_2$), 33.4 (CH$_3$), 33.0 (C), 26.7 (CH$_2$), 20.9 (CH$_2$,CH$_3$), 20.44 (CH$_3$), 20.36 (CH$_3$), 20.1 (CH$_2$), 13.4 (CH$_3$).

Dendrillolide (101). Electron impact mass spectrometry, low resolution, m/z (rel. int.); 374 (1), 316 (5), 301 (6), 255 (5), 237 (5), 166 (25), 137 (51), 121 (28), 93 (28), 69 (53), 55 (33), 43 (100). Fourier transform infrared spectrometry, thin film, cm^{-1}; 2950, 1799, 1752, 1451, 1366, 1225, 986. ^{1}H nmr (400 MHz, ppm from TMS in CDCl$_3$):δ 6.49 (d,1H, J=7.5Hz), 6.10 (d,1H, J=4.4Hz), 4.87 (d,1H, J=2.2Hz), 4.60 (d,1H, J=2.2Hz), 3.20 (m,1H), 2.74 (m,2x1H), 2.56 (dd,1H, J=9.4,17Hz), 2.16 (s,3H), 1.01 (s,3H), 0.98 (s,3H), 0.97 (s,3H). ^{13}C nmr (75.4 MHz, ppm from TMS in CDCl$_3$):δ 175.48 (C), 169.63 (C), 153.25 (C), 114.41 (CH$_2$), 104.86 (CH), 96.95 (CH), 55.89 (CH), 54.44 (CH), 54.34 (CH), 46.61 (C), 41.85 (CH), 37.95 (CH$_2$), 37.84 (CH$_2$), 37.41 (CH$_2$), 35.88 (C), 34.23 (CH$_3$), 28.92 (CH$_2$), 28.28 (CH$_2$), 26.75

(CH$_2$), 25.56 (CH$_3$), 23.97 (CH$_3$), 21.07 (CH$_3$). See Table II for comparison to reported ^{13}C nmr values of dendrillolide A.

Chromodorolide B (102): Electron impact mass spectrometry, low resolution, m/z (rel. int.); 407 (2), 391 (2), 390 (3), 375 (2), 362 (2), 286 (14), 250 (26), 235 (22), 203 (45), 163 (45), 123 (67), 109 (35), 95 (44), 85 (59), 71 (100). Fourier transform infrared spectrometry, thin film, cm^{-1}; 2924, 1787, 1727, 1691, 1462, 1451, 1258, 974. ^{1}H nmr spectroscopy (CDCl$_3$, 400 MHz, ppm from TMS); δ 6.04 (d,1H, J=7 Hz), 5.48 (d,1H, J=9 Hz), 5.07 (d,1H, J=5.5 Hz), 3.33 (dd,1H, J=7,10 Hz, s,3H), 2.85 (ddd,1H, J=5.5,7,10 Hz), 2.35 (ddd,1H, J=7,9,11 Hz), 2.13 (s,3H), 0.80 (s,3H), 0.77 (s,3H), 0.70 (s,3H). ^{13}C nmr spectroscopy (CDCl$_3$, 75.4 MHz, ppm from TMS); δ 106.7 (CH), 104.1 (CH), 77.7 (CH), 57.5 (CH), 55.4 (CH), 54.7 (CH), 50.0 (CH), 48.4 (CH), 47.2 (CH), 43.0 (C), 41.3 (CH$_2$), 40.2 (CH$_2$), 33.4 (CH$_3$), 33.1 (C), 29.8 (CH$_2$), 25,2 (CH$_2$), 21.3 (CH$_3$), 21,0 (CH$_2$), 20.8 (CH$_3$), 19.9 (CH$_2$), 13.2 (CH$_3$).

^{1}H and ^{13}C nmr experiments: Parameters used in the individual experiments are listed to the left of the spectra. Abbreviations used are as follows. SF, standard proton frequency; O1, deuterium lock reference; SI, size (in bits) of FID acquired; SW, sweep width in Hz; PW, pulse length in μsec; RD, relaxation delay; AQ (or AT), acquisition time in sec; NS (or NT), number of scans; NE, number of experiments.

2D nmr Experiments: All parameters used in the acquisition and processing of two-dimensional nmr experiments (i.e. FID size, sweepwidths, offsets, etc.) can be found in the parameter lists contained with the contour plots within the discussion. Variations on the parameters' abbreviations above are as follows: P1 P2, pulse lengths in μsec; SI1 SI2, relative FID sizes in two dimensions; ND0, number of phase switches; D1, relaxation delay. General guidelines and pulse sequences are listed below.

^{1}H-^{1}H COSY: All COSY experiments were performed on a Bruker WH-400 nmr instrument using the following pulse sequence:

D1 - P1 - D0 - P2 - FID

where D1 is a relaxation delay of 1.0 second, P1 is a 90 degree pulse, P2 is either a 60 or 90 degree pulse, and D0 is a small delay of 3 μsec to allow the shift and coupling patterns to evolve. Generally, 256 1K FIDs completed the matrix which allowed zero-filling in the F1 domain but not in F2.

COSYPHDQ: A PHase sensitive Double Quantum COSY experiment was performed on a Bruker WH-400 nmr spectrometer employing the following pulse sequence;

D1 - P1 - D0 - P1 - D3 - P1 - FID

where D1 is a relaxation delay of 1.2 seconds, P1 is a 90 degree pulse, D0 is a short (3 μsec) delay for evolution and D3 is a fixed delay (3 μsec) for phase switching. Phase correction on the 2D matrix was accomplished by obtaining phasing parameters from a 1D spectrum acquired using a 90 degree pulse and a relaxation delay of 1.2 seconds. A 512K matrix was employed for this experiment.

Homonuclear J-Resolved 2-D nmr: 2D-J spectroscopy was performed on the Bruker WH-400 nmr spectrometer using the Hahn spin-echo pulse sequence below;

D1 - P1 - D0 - P2 - D0 - FID

where D1 is a relaxation delay of 1.0 seconds, D0 is an evolution delay of 3 μsec, and P1, P2 are 90 and 180 degree pulses respectively. Sweep width in the F1 domain was set equal to or larger than one half the width of the largest multiplet, chosen such that the ratio I2D (Hz/pt/Hz/pt) is a multiple of two, in order to allow symmetrization and tilting of the transformed 128K 2D matrix.

^{1}H-^{13}C HETCOR: Both single bond and long range HETCOR experiments were performed on the Varian XL-300 nmr spectrometer with the following pulse sequence;

^{1}H   D1 - 90^o_x - t1 - Δ_1 - 90^o_y - Δ_2 - ^{1}H-BB

^{13}C   180^o_x 90^o_x FID

where D1 is a set relaxation delay and all other delays (t1, Δ_1 and Δ_2) are set by choice of single and multiple bond coupling constants. The single bond experiment was performed using a D1 of 0.4 sec and a coupling constant of 140.0 Hz. The long range HETCOR was run using a D1 of 1.0 sec, a single bond J value of 80.0 Hz and a multiple bond J value of 8.0 Hz.

Appendix

Material in this appendix includes interatomic distances and angles determined in the X-ray crystallography experiment performed at Cornell University. The numbering system employed is one arbitrarily assigned by the computer and bears no relation to the spongian numbering system employed throughout the text of this thesis. A copy of the X-ray structure numbered as per the data listed in this appendix precedes the tables of interatomic distances and angles.
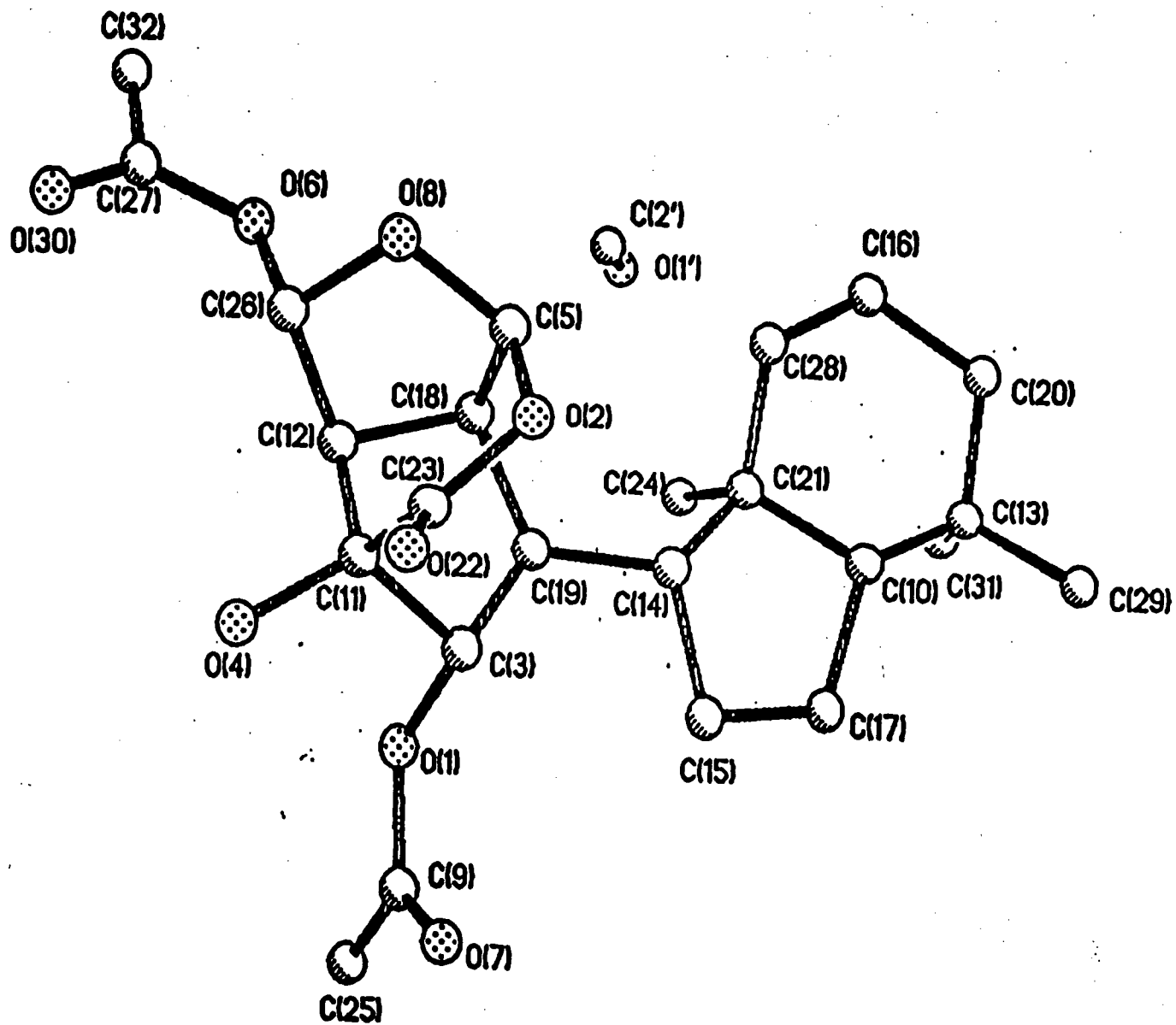
Table 1. Atomic coordinates ($\times 10^4$) and equivalent isotropic
displacement parameters ($\mathring{A}^2 \times 10^3$)

	x	y	z	U(eq)
C(1)	4325(9)	6654(10)	593(3)	62(3)
C(2)	3340(12)	5612(12)	334(3)	78(4)
C(3)	4134(11)	5108(11)	-89(3)	78(4)
C(4)	5749(10)	4455(10)	-17(3)	56(3)
C(5)	6635(10)	5486(9)	262(2)	50(3)
C(6)	8331(10)	5139(9)	392(3)	60(3)
C(7)	8724(10)	6241(9)	737(3)	56(3)
C(8)	7205(10)	7551(9)	1306(3)	51(3)
C(9)	7198(10)	7025(9)	834(2)	50(3)
C(10)	5918(10)	6008(9)	692(3)	49(3)
C(11)	7620(12)	10775(11)	1300(3)	66(4)
C(12)	7933(10)	9741(10)	1663(3)	57(3)
C(13)	6435(10)	9169(9)	1867(3)	56(3)
C(14)	5795(10)	8366(10)	1475(3)	54(3)
C(15)	5209(11)	9619(10)	1225(3)	64(3)
C(16)	5227(10)	10236(12)	1956(3)	73(4)
C(17)	8608(10)	8461(9)	1423(3)	50(3)
C(18)	6536(13)	4343(11)	-466(3)	88(4)
C(19)	5624(13)	2981(9)	167(3)	82(4)
C(20)	5721(11)	4840(9)	1047(3)	67(3)
O(21)	9541(7)	7662(6)	1720(2)	56(2)
C(22)	11134(11)	7657(10)	1658(3)	59(4)
C(23)	11866(12)	6640(9)	1963(3)	77(4)
O(24)	11745(8)	8359(8)	1399(2)	80(3)
O(25)	8871(7)	10330(7)	1987(2)	74(2)
O(26)	6450(7)	10399(6)	1023(2)	64(2)
O(27)	8350(9)	11778(7)	1227(2)	83(3)
O(28)	4540(7)	10522(7)	1540(2)	73(3)
O(29)	4008(7)	9577(8)	2217(2)	83(3)
C(30)	3590(13)	10179(13)	2586(3)	83(5)
C(31)	2208(11)	9485(13)	2775(3)	102(5)
O(32)	4259(11)	11133(11)	2729(3)	153(5)
C(33)	1746(17)	2883(13)	1652(6)	200(11)
O(34)	1565(9)	1576(9)	1738(3)	126(4)

* Equivalent isotropic U defined as one third of the
trace of the orthogonalized U_{ij} tensor

Table 2. INTERATOMIC DISTANCES (Å)

C(1)-C(2)	1.542 (14)		C(1)-C(10)	1.544 (12)
C(2)-C(3)	1.548 (13)		C(3)-C(4)	1.549 (13)
C(4)-C(5)	1.522 (12)		C(4)-C(18)	1.543 (12)
C(4)-C(19)	1.536 (13)		C(5)-C(6)	1.557 (12)
C(5)-C(10)	1.545 (11)		C(6)-C(7)	1.540 (12)
C(7)-C(9)	1.552 (12)		C(8)-C(9)	1.538 (11)
C(8)-C(14)	1.543 (12)		C(8)-C(17)	1.541 (12)
C(9)-C(10)	1.544 (12)		C(10)-C(20)	1.580 (12)
C(11)-C(12)	1.523 (13)		C(11)-O(26)	1.373 (12)
C(11)-O(27)	1.178 (12)		C(12)-C(13)	1.542 (12)
C(12)-C(17)	1.555 (12)		C(12)-O(25)	1.403 (10)
C(13)-C(14)	1.538 (12)		C(13)-C(16)	1.493 (14)
C(14)-C(15)	1.520 (13)		C(15)-O(26)	1.453 (11)
C(15)-O(28)	1.425 (11)		C(16)-O(28)	1.437 (11)
C(16)-O(29)	1.470 (12)		C(17)-O(21)	1.442 (10)
O(21)-C(22)	1.391 (11)		C(22)-C(23)	1.498 (13)
C(22)-O(24)	1.172 (12)		O(29)-C(30)	1.325 (13)
C(30)-C(31)	1.489 (15)		C(30)-O(32)	1.175 (16)
C(33)-O(34)	1.299 (15)			

Table 3. INTERATOMIC ANGLES (°)

C(2)-C(1)-C(10)	109.3(8)	C(1)-C(2)-C(3)	113.2(8)
C(2)-C(3)-C(4)	114.2(7)	C(3)-C(4)-C(5)	105.5(7)
C(3)-C(4)-C(18)	107.4(7)	C(5)-C(4)-C(18)	109.1(7)
C(3)-C(4)-C(19)	111.5(8)	C(5)-C(4)-C(19)	115.8(7)
C(18)-C(4)-C(19)	107.2(8)	C(4)-C(5)-C(6)	118.6(7)
C(4)-C(5)-C(10)	119.5(7)	C(6)-C(5)-C(10)	103.2(6)
C(5)-C(6)-C(7)	103.7(7)	C(6)-C(7)-C(9)	106.3(7)
C(9)-C(8)-C(14)	118.9(7)	C(9)-C(8)-C(17)	114.3(7)
C(14)-C(8)-C(17)	104.7(7)	C(7)-C(9)-C(8)	109.8(7)
C(7)-C(9)-C(10)	104.2(7)	C(8)-C(9)-C(10)	118.7(7)
C(1)-C(10)-C(5)	108.8(6)	C(1)-C(10)-C(9)	116.0(7)
C(5)-C(10)-C(9)	99.3(6)	C(1)-C(10)-C(20)	109.2(7)
C(5)-C(10)-C(20)	113.8(7)	C(9)-C(10)-C(20)	109.6(6)
C(12)-C(11)-O(26)	114.4(8)	C(12)-C(11)-O(27)	125.7(9)
O(26)-C(11)-O(27)	119.7(9)	C(11)-C(12)-C(13)	112.6(7)
C(11)-C(12)-C(17)	103.9(7)	C(13)-C(12)-C(17)	102.9(7)
C(11)-C(12)-O(25)	110.9(8)	C(13)-C(12)-O(25)	110.1(6)
C(17)-C(12)-O(25)	116.2(7)	C(12)-C(13)-C(14)	99.5(6)
C(12)-C(13)-C(16)	114.5(8)	C(14)-C(13)-C(16)	103.8(7)
C(8)-C(14)-C(13)	103.7(7)	C(8)-C(14)-C(15)	120.1(7)
C(13)-C(14)-C(15)	96.5(7)	C(14)-C(15)-O(26)	112.5(7)
C(14)-C(15)-O(28)	106.3(7)	O(26)-C(15)-O(28)	105.9(7)
C(13)-C(16)-O(28)	105.1(7)	C(13)-C(16)-O(29)	107.7(8)
O(28)-C(16)-O(29)	105.8(7)	C(8)-C(17)-C(12)	105.6(7)
C(8)-C(17)-O(21)	106.4(7)	C(12)-C(17)-O(21)	109.6(6)
C(17)-O(21)-C(22)	118.1(6)	O(21)-C(22)-C(23)	109.6(8)
O(21)-C(22)-O(24)	122.5(9)	C(23)-C(22)-O(24)	127.9(9)
C(11)-O(26)-C(15)	114.6(7)	C(15)-O(28)-C(16)	108.5(7)
C(16)-O(29)-C(30)	118.3(8)	O(29)-C(30)-C(31)	110.9(10)
O(29)-C(30)-O(32)	122.1(11)	C(31)-C(30)-O(32)	127.0(10)

Table 4. Anisotropic displacement parameters ($\text{Å}^2 \times 10^3$)

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(1)	53(6)	73(6)	59(6)	-5(6)	-6(5)	10(6)
C(2)	73(7)	82(7)	79(7)	0(7)	-11(6)	-2(7)
C(3)	79(7)	81(7)	73(6)	-5(6)	-18(6)	-13(7)
C(4)	62(6)	58(6)	49(5)	-5(5)	-13(5)	0(5)
C(5)	62(6)	46(5)	43(5)	-1(4)	5(5)	-1(5)
C(6)	63(6)	60(6)	57(5)	-7(5)	0(5)	6(5)
C(7)	55(6)	59(6)	53(5)	-10(5)	-1(5)	5(5)
C(8)	65(6)	41(5)	47(5)	-1(4)	2(5)	7(5)
C(9)	62(6)	48(5)	39(4)	-1(4)	5(4)	-8(5)
C(10)	56(5)	49(5)	42(5)	-4(4)	-2(4)	-2(5)
C(11)	74(7)	60(7)	63(6)	-10(6)	5(6)	2(6)
C(12)	60(6)	64(6)	47(5)	-11(5)	-5(5)	-9(5)
C(13)	60(6)	65(6)	44(5)	-3(5)	9(5)	-3(6)
C(14)	53(5)	57(6)	52(5)	-7(5)	10(5)	-3(5)
C(15)	66(6)	70(6)	57(5)	-14(5)	-2(5)	7(6)
C(16)	59(6)	92(8)	69(7)	-35(7)	20(6)	-7(6)
C(17)	51(5)	59(6)	40(5)	3(5)	-1(5)	-1(5)
C(18)	102(8)	101(8)	60(6)	-27(6)	-14(6)	0(8)
C(19)	105(9)	59(6)	82(7)	-11(5)	-18(7)	-4(7)
C(20)	89(7)	59(6)	54(5)	7(5)	-6(5)	-10(6)
O(21)	55(4)	62(4)	50(3)	7(3)	0(3)	-1(3)
C(22)	58(7)	55(6)	64(6)	-5(5)	-3(6)	-9(6)
C(23)	72(7)	69(6)	90(7)	6(6)	-17(7)	10(6)
O(24)	57(4)	100(6)	83(5)	26(5)	3(4)	-5(4)
O(25)	65(4)	98(5)	61(4)	-16(4)	1(3)	-16(4)
O(26)	72(4)	62(4)	59(4)	7(3)	2(4)	4(4)
O(27)	87(5)	56(4)	105(6)	9(4)	2(5)	-11(4)
O(28)	69(4)	79(5)	72(4)	-21(4)	8(4)	14(4)
O(29)	78(5)	97(5)	75(4)	-42(4)	28(4)	-14(5)
C(30)	82(8)	93(8)	74(7)	-35(7)	11(7)	-4(8)
C(31)	84(8)	145(11)	79(7)	-41(8)	31(7)	-21(9)
O(32)	152(9)	192(10)	116(7)	-102(7)	58(6)	-77(8)
C(33)	94(11)	80(10)	425(31)	-68(15)	46(17)	-17(9)
O(34)	65(5)	95(6)	219(10)	-14(7)	25(6)	-20(5)

The anisotropic displacement exponent takes the form:

$$-2\pi^2(h^2a*^2U_{11} + \ldots + 2hka*b*U_{12})$$

Table 5. H-Atom coordinates $(x10^4)$ and isotropic
displacement parameters $(\text{Å}^2 x10^3)$

	x	y	z	U
H(1A)	3804	6850	862	80
H(1B)	4441	7506	436	80
H(2A)	2374	6037	258	80
H(2B)	3114	4817	510	80
H(3B)	4242	5888	-280	80
H(3C)	3479	4447	-231	80
H(5B)	6718	6304	86	80
H(6A)	9011	5219	147	80
H(6B)	8410	4219	508	80
H(7A)	9505	6862	631	80
H(7B)	9104	5797	995	80
H(8A)	7270	6747	1489	80
H(9B)	7163	7815	645	80
H(13A)	6625	8573	2111	80
H(14B)	4965	7764	1560	80
H(15B)	4451	9343	1014	80
H(16B)	5638	11053	2091	80
H(17B)	9170	8726	1167	80
H(18B)	7542	3935	-437	80
H(18C)	5913	3781	-655	80
H(18D)	6633	5254	-587	80
H(19B)	6636	2593	205	80
H(19C)	5107	3020	443	80
H(19D)	5040	2413	-30	80
H(20C)	4929	4201	960	80
H(20D)	6680	4354	1082	80
H(20E)	5437	5265	1318	80
H(23A)	12963	6675	1919	80
H(23B)	11632	6871	2259	80
H(23C)	11497	5724	1901	80
H(25B)	9675	10679	1872	80
H(31D)	1951	9951	3041	80
H(31E)	1349	9538	2578	80
H(31F)	2438	8532	2835	80
H(33A)	937	3416	1785	80
H(33B)	1704	2998	1342	80
H(33C)	2730	3194	1758	80
H(34A)	1009	793	1849	80

TORSION ANGLES FOR CHROMODOROLIDE A

C10	C1	C2	C3	-55.7(0.0)	C2	C1	C10	C5	52.8(0.0)	C2	C1	C10	C9	163.7(0.0)
C2	C1	C10	C20	-71.9(0.0)	C1	C2	C3	C4	56.1(0.0)	C2	C3	C4	C5	-50.1(0.0)
C2	C3	C4	C18	-166.4(0.0)	C2	C3	C4	C19	76.5(0.0)	C3	C4	C5	C6	-179.7(0.0)
C3	C4	C5	C10	52.9(0.0)	C18	C4	C5	C6	-64.5(0.0)	C18	C4	C5	C10	168.1(0.0)
C19	C4	C5	C6	56.5(0.0)	C19	C4	C5	C10	-70.9(0.0)	C4	C5	C6	C7	-169.3(0.0)
C10	C5	C6	C7	-34.6(0.0)	C4	C5	C10	C1	-56.5(0.0)	C4	C5	C10	C9	-178.2(0.0)
C4	C5	C10	C20	65.5(0.0)	C6	C5	C10	C1	169.3(0.0)	C6	C5	C10	C9	47.6(0.0)
C6	C5	C10	C20	-68.8(0.0)	C5	C6	C7	C9	7.6(0.0)	C6	C7	C9	C8	150.2(0.0)
C6	C7	C9	C10	22.0(0.0)	C14	C8	C9	C7	179.9(0.0)	C14	C8	C9	C10	-60.4(0.0)
C17	C8	C9	C7	55.4(0.0)	C17	C8	C9	C10	175.1(0.0)	C9	C8	C14	C13	-159.0(0.0)
C9	C8	C14	C15	-53.0(0.0)	C17	C8	C14	C13	-29.9(0.0)	C17	C8	C14	C15	76.1(0.0)
C9	C8	C17	C12	133.1(0.0)	C9	C8	C17	O21	-110.5(0.0)	C14	C8	C17	C12	1.3(0.0)
C14	C8	C17	O21	117.8(0.0)	C7	C9	C10	C1	-158.8(0.0)	C7	C9	C10	C5	-42.5(0.0)
C7	C9	C10	C20	76.9(0.0)	C8	C9	C10	C1	78.7(0.0)	C8	C9	C10	C5	-165.0(0.0)
C8	C9	C10	C20	-45.6(0.0)	O26	C11	C12	C13	-42.4(0.0)	O26	C11	C12	C17	68.2(0.0)
O26	C11	C12	O25	-166.2(0.0)	O27	C11	C12	C13	143.0(0.0)	O27	C11	C12	C17	-106.4(0.0)
O27	C11	C12	O25	19.1(0.0)	C12	C11	O26	C15	32.9(0.0)	O27	C11	O26	C15	-152.2(0.0)
C11	C12	C13	C14	65.9(0.0)	C11	C12	C13	C16	-44.2(0.0)	C17	C12	C13	C14	-45.3(0.0)
C17	C12	C13	C16	-155.4(0.0)	O25	C12	C13	C14	-169.8(0.0)	O25	C12	C13	C16	80.1(0.0)
C11	C12	C17	C8	-89.9(0.0)	C11	C12	C17	O21	155.8(0.0)	C13	C12	C17	C8	27.6(0.0)
C13	C12	C17	O21	-86.7(0.0)	O25	C12	C17	C8	148.0(0.0)	O25	C12	C17	O21	33.7(0.0)
C12	C13	C14	C8	46.7(0.0)	C12	C13	C14	C15	-76.5(0.0)	C16	C13	C14	C8	165.0(0.0)
C16	C13	C14	C15	41.9(0.0)	C12	C13	C16	O28	77.4(0.0)	C12	C13	C16	O29	-170.1(0.0)
C14	C13	C16	O28	-30.0(0.0)	C14	C13	C16	O29	82.4(0.0)	C8	C14	C15	O26	-34.8(0.0)
C8	C14	C15	O28	-150.3(0.0)	C13	C14	C15	O26	75.1(0.0)	C13	C14	C15	O28	-40.3(0.0)
C14	C15	O26	C11	-52.8(0.0)	O28	C15	O26	C11	62.9(0.0)	C14	C15	O28	C16	24.3(0.0)
O26	C15	O28	C16	-95.6(0.0)	C13	C16	O28	C15	3.9(0.0)	O29	C16	O28	C15	-109.9(0.0)
C13	C16	O29	C30	127.1(0.0)	O28	C16	O29	C30	-120.9(0.0)	C8	C17	O21	C22	136.5(0.0)
C12	C17	O21	C22	-109.8(0.0)	C17	O21	C22	C23	-173.2(0.0)	C17	O21	C22	O24	6.2(0.0)
C16	O29	C30	C31	172.3(0.0)	C16	O29	C30	O32	-7.5(0.0)					

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