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MICHAUD, DENNIS PAUL

# AN INVESTIGATION INTO THE NATURAL PRODUCTS CHEMISTRY OF THE SEA HARE APLYSIA DACTYLOMELA

*The University of Oklahoma* PH.D. 1980

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

# AN INVESTIGATION INTO THE NATURAL PRODUCTS CHEMISTRY OF THE SEA HARE *APLYSIA DACTYLOMELA*

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

By

DENNIS PAUL MICHAUD

Norman, Oklahoma

1980

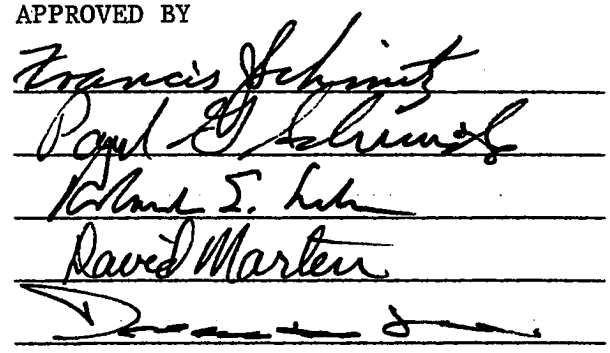
AN INVESTIGATION INTO THE

NATURAL PRODUCTS CHEMISTRY

OF THE

SEA HARE *APLYSIA DACTYLOMELA*

APPROVED BY



DISSERTATION COMMITTEE

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## INTRODUCTION

The sea hare, *Aplysia dactylomela* Rang, is a marine mollusc in the family Aplysiidae of the subclass Opisthobranchia. It is a common species found in the West Indies and its large yellow body, which is approximately ten inches in length, is randomly covered with brown spots and rings.

Sea hares, which lack the external shell that is characteristic of other molluscs, number about thirty-five species.[1] They have a world-wide distribution and range in size from small ones not much larger than a human thumbnail to species that are up to 18 inches in length. The trivial name "sea hare", of Roman origin, refers to their resemblance to a sitting rabbit.[2] Sea hares are found from tidal zones to depths of 200 feet wherever large populations of algae occur. Sea hares graze on algae and seaweed, but they have been known to devour animal substances also.[2]

Sea hares, especially *Aplysia*, are well known to cellular neurophysiologists for their large neurons.[3] The nerve cells within the central ganglia of these animals are readily visible. Because of their size, the neurons can be probed with microelectrodes and synaptic activities can be readily recorded.

The chemistry of sea hares has only recently been actively

investigated, however. The toxicity of sea hares has been known since antiquity and early investigators attempted to determine the origin and structure of the toxins. Toxins have been reported[4] from various glands in *Aplysia*, but attention was focused on the digestive gland of this genus when Yamamura and Hirata (1963)[5] reported the isolation of aplysin (16), debromoaplysin (17), and aplysinol (18) from *Aplysia kurodai*, see Figure 3. Since this discovery, the chemistry of the digestive gland, which occupies one-third of the body cavity, of *Aplysia* species has been extensively investigated. The fact that extracts of the gland are toxic[4] suggests that the sea hare uses these toxins as defensive agents[6] to compensate for lack of a protective shell.

The structures of the nearly 100 secondary metabolites isolated to date from various opisthobranch molluscs (excluding sterols, common carotenoids, and compounds discussed later in this thesis) are illustrated in Figures 1-5. The compounds are grouped into five classes: 1) monoterpenoids (Figure 1), 2) acetylenic nonterpenoids (Figure 2), 3) sesquiterpenoids (Figure 3), 4) diterpenoids (Figure 4), and 5) miscellaneous compounds (Figure 5).

The majority of the compounds isolated from sea hares are halogenated compounds. The subsequent discovery of many of the same compounds in algae has led to the conclusion that the sea hare concentrates algal metabolites in its digestive gland and that the algae on which *Aplysia* feeds are the ultimate sources of these compounds.[7]

Although the chemical research has been concentrated on species of *Aplysia*, other opisthobranch molluscs have also been investigated. Two unusual isocyanide sesquiterpenes (43, 44)[8,9] that are lethal to

fish have been isolated from *Phyllidia varicosa* and a number of interesting diterpenes (56-71)[10,11,12] have been found in the sea hare genus, *Dolabella*. The dietary sources of the compounds from these genera have also been determined.

A number of nonisoprenoid substances have also been isolated from various opisthobranch molluscs. The antileukemic substance, aplysiatoxin (81),[13] and two nontoxic amides (77 and 78),[14] were isolated from *Stylocheilus longicauda*. Four propionate-derived compounds (72-75)[15,16,17] were obtained from *Tridachia crispata* and *Tridachiella diomedea*, but it is not known at present whether these metabolites originate from the mollusc or from the functional chloroplasts within the mollusc. A dietary source is unlikely.[17]

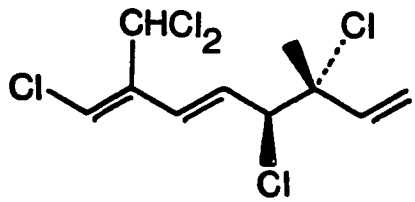
A number of trail-breaking pheromones have been isolated from *Navanax inermis*. This species is a carnivorous opisthobranch mollusc which moves along slime trails left by other *Navanax* individuals. When molested, *Navanax* secretes a yellow substance which, when encountered by other individuals, halts tail-following behavior. Seven substances (92-98) that are responsible for this alarm pheromone response have so far been isolated from the yellow slime.[18,19,20]

Some opisthobranch molluscs (order Nudibranchia) are exquisitely colored. Studies have shown[21,22] that the pigments are mixtures of carotenoids which are obtained from the diet and deposited in the integument of the animal. A change in diet will often result in a change in the color of these nudibranchs.

A number of the compounds in Figures 1-5 display interesting pharmacological activity. Several compounds such as brasilenyne (11),[23]

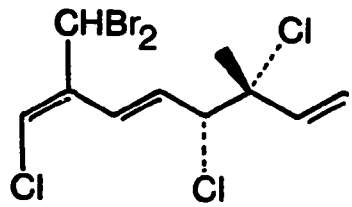
panacene (19),[24] and the isocyanopupukeananes (43[8] and 44[9]) protect the mollusc from predators by acting as potent feeding deterrents to fish. Routine pharmacological screening of the sea hare extracts by some investigators has led to the isolation of the cytotoxic compounds aplysiastatin (41)[25] and deodactol (31),[26] the antileukemic compounds dolatriol (70)[12] and aplysiatoxin (81),[13] and the hypotensive agent doridosine (76).[27] Dactylyne (7)[28] and isodactylyne (8),[29] isolated from *Aplysia dactylomela*, have been shown to be potent inhibitors of pentobarbital metabolism.[30]

Because of the tumor inhibitory activity displayed by lipophilic extracts of the sea hare, *Aplysia dactylomela*, a number of investigations have been carried out by previous workers in this laboratory to determine the source of the activity. The result was the isolation of sixteen new compounds.[31] Some had interesting activities while others were biologically inactive. The objective of this work is to continue the investigations into the chemistry of this sea hare in the search for antitumor compounds and to describe the chemistry of the animal obtained from a location different from that of previous collections. This research resulted in the isolation of a brominated indole and several sesquiterpenoid compounds from *Aplysia dactylomela*. In addition, five diterpenoid compounds possessing a novel skeleton were described. Some of them exhibited significant anticancer activity.



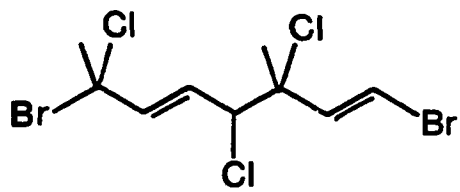

1

*Aplysia limacina*[32]



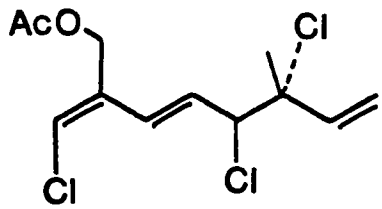

2

*Aplysia limacina*[32]



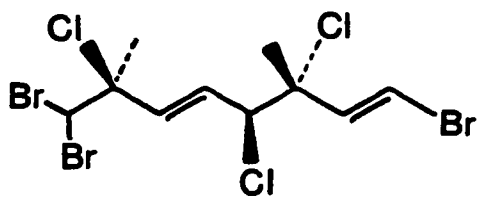

3

*Aplysia californica*[33]



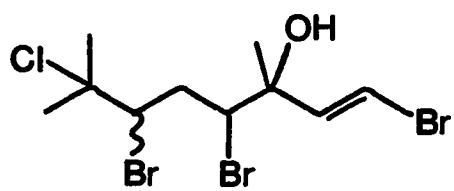

4

*Aplysia californica*[33]




5

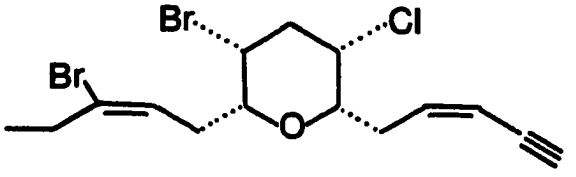
*Aplysia californica*[34]




6

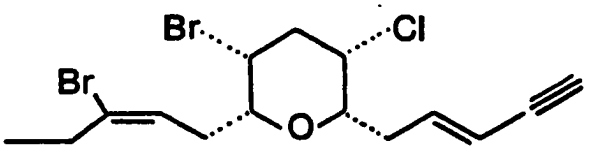
*Aplysia californica*[35]

**Figure 1. Monoterpenoid Derivatives from Opisthobranchs.**



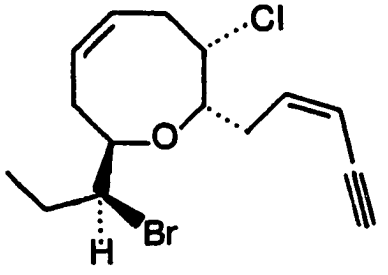

7 dactylyne

*Aplysia daotylomela*[28]



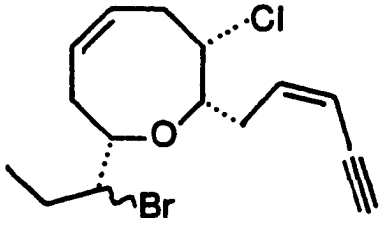

8 isodactylyne

*Aplysia dactylomela*[29]



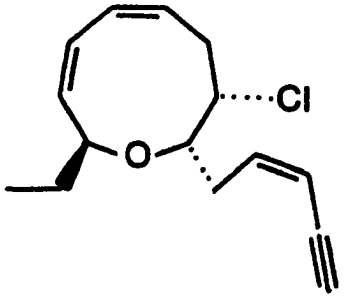

9 cis-dihydrorhodophytin

*Aplysia brasiliana*[23]




10 cis-isodihydrorhodophytin

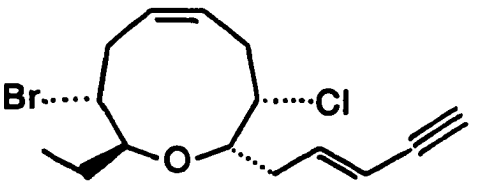
*Aplysia brasiliana*[23]




11 brasilenyne

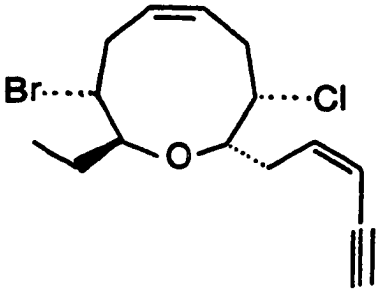
*Aplysia brasiliana*[23]

**Figure 2. Acetylenic Nonterpenoid Derivatives from Opisthobranchs.**



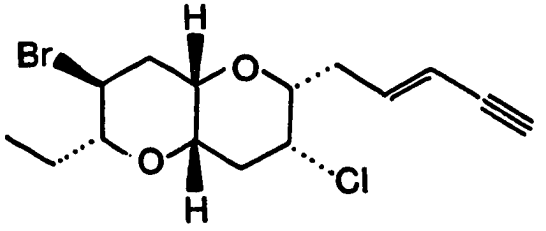

12

*Aplysia dactylomela*[31]



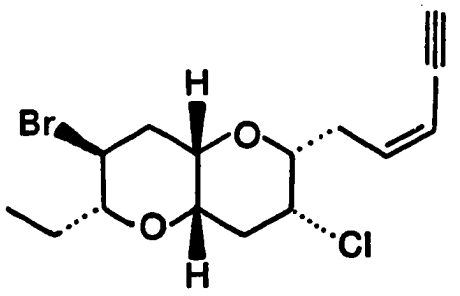

13

*Aplysia dactylomela*[31]




14

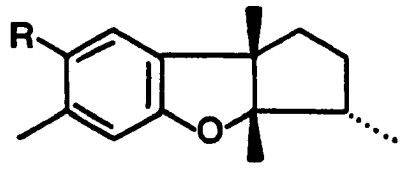
*Aplysia dactylomela*[31]




15

*Aplysia dactylomela*[31]

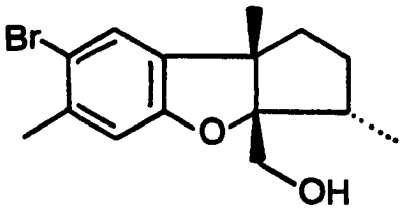
**Figure 2 (continued).**




16 R = Br, aplysin

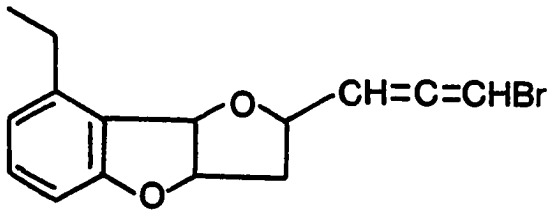
17 R = H, debromoaplysin

*Aplysia kurodai*[36]



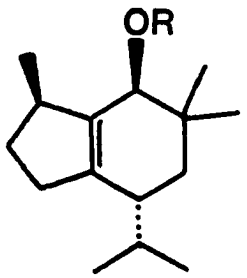

18 aplysinol

*Aplysia kurodai*[36]




19 panacene

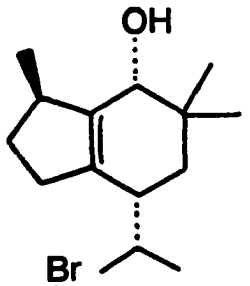
*Aplysia brasiliana*[24]




20 R = H, brasilenol

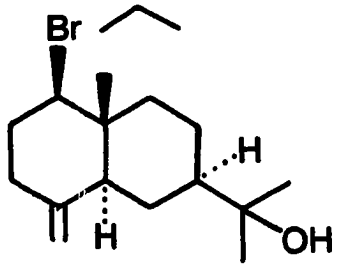
21 R = Ac, brasilenol acetate

*Aplysia brasiliana*[37]




22 epibrasilenol

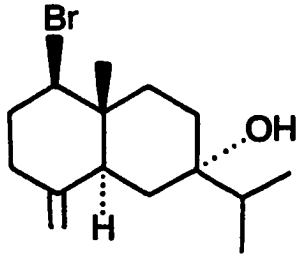
*Aplysia brasiliana*[37]




23 brasudol

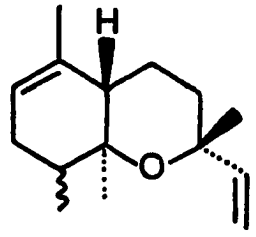
*Aplysia brasiliana*[38]

**Figure 3. Sesquiterpenoid Derivatives from Opisthobranchs.**



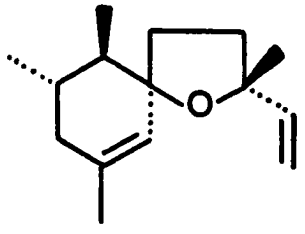

24 isobrasudol

*Aplysia brasiliana*[38]



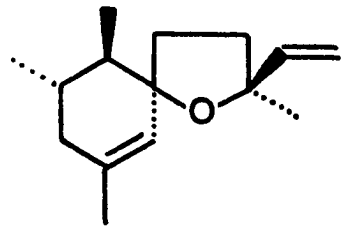

25 dactyloxene-A

*Aplysia dactylomela*[40]



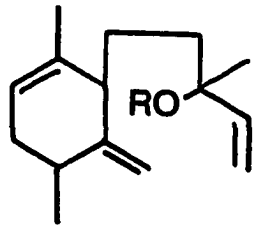

26 dactyloxene-B

*Aplysia dactylomela*[31,39,41]




27 dactyloxene-C

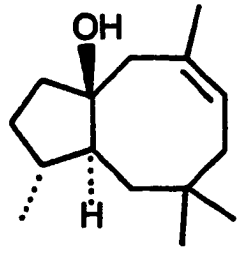
*Aplysia dactylomela*[31,40]




28 R = H, dactylenol

29 R = Ac, dactylenol acetate

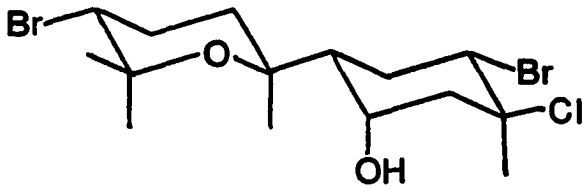
*Aplysia dactylomela*[40]




30 dactylol

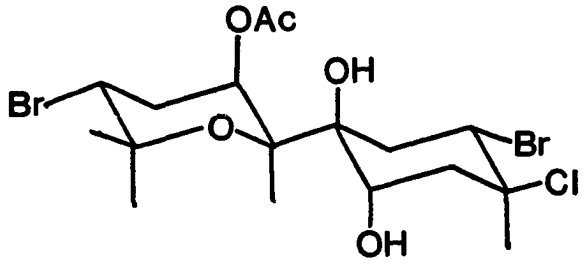
*Aplysia dactylomela*[42]

Figure 3 (continued).



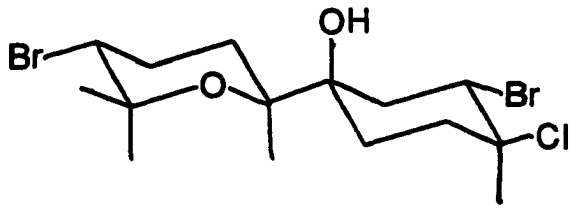

31 deodactol

*Aplysia dactylomela*[26]



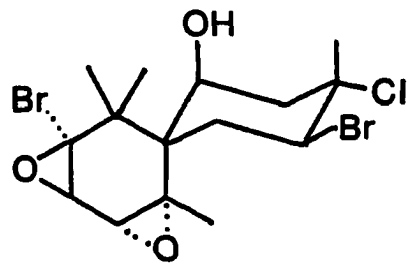

32 dihydroxydeodactol monoacetate

*Aplysia dactylomela*[43]



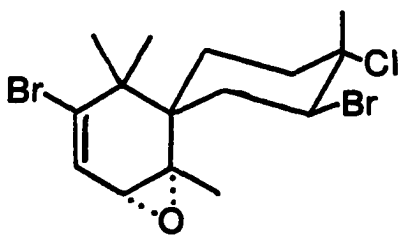

33

*Aplysia dactylomela*[31]




34 prepacifenol epoxide

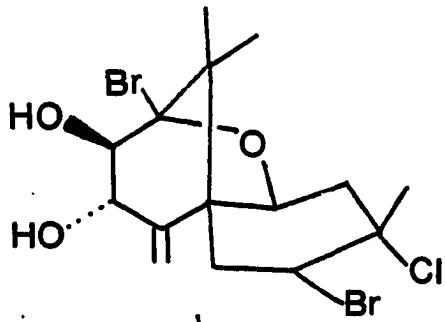
*Aplysia californica*[33,44]




35

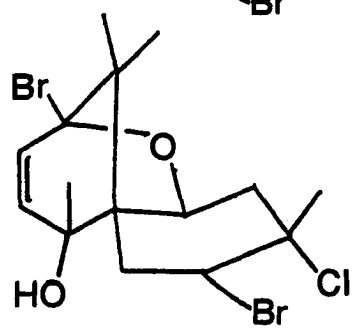
*Aplysia californica*[33]

Figure 3 (continued).



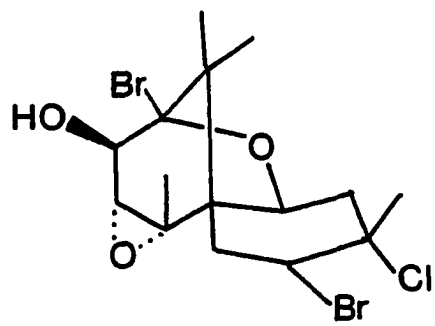

36

*Aplysia californica*[33]



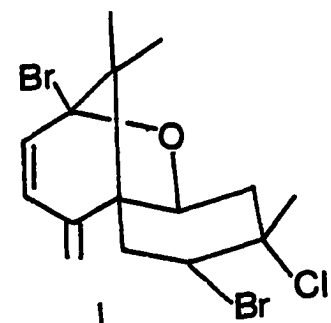

37 pacifenol

*Aplysia californica*[7,45]



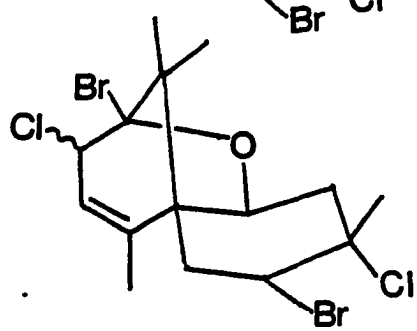

38 johnstonol

*Aplysia californica*[7]




39 pacifidiene

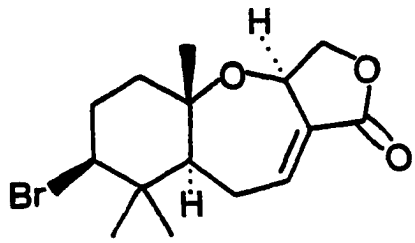
*Aplysia californica*[45,7]




40

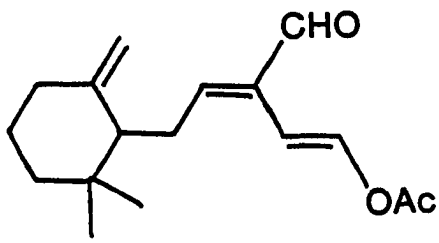
*Aplysia californica*[45]

Figure 3 (continued).



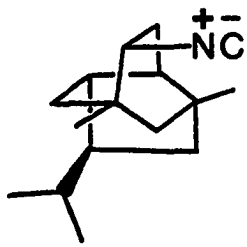

41 aplysiastatin

*Aplysia angasi* [25]



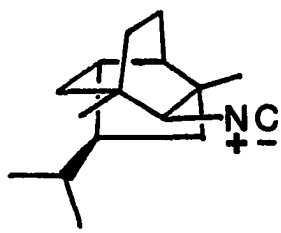

42 onchidal

*Onchidella binneyi* [46]




43 9-isocyanopupukeanane

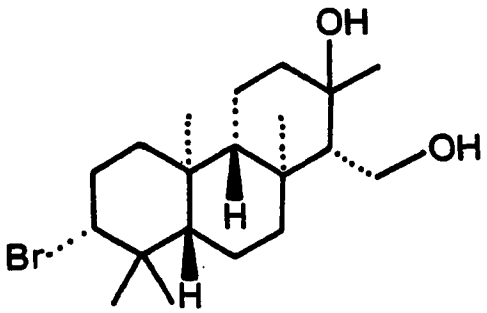
*Phyllidia varicosa* [8]




44 2-isocyanopupukeanane

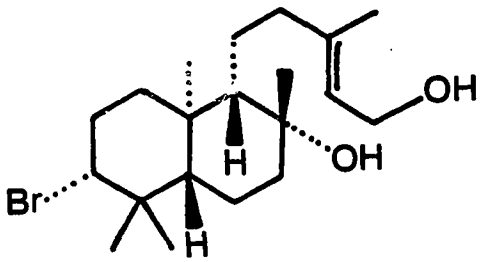
*Phyllidia varicosa* [9]

**Figure 3 (continued).**



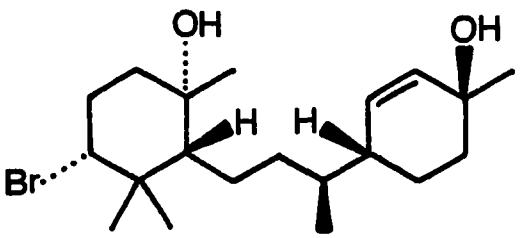

45 isoaplysin-20

*Aplysia kurodai*[47]



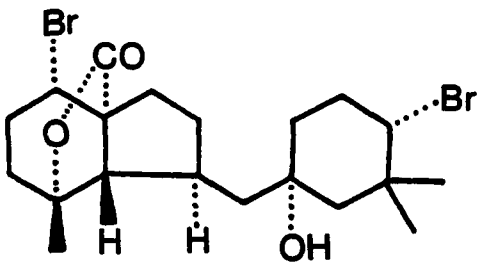

46 aplysin-20

*Aplysia kurodai*[48]



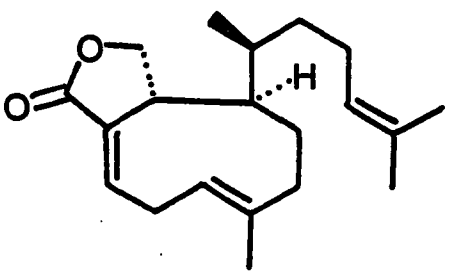

47 14-bromoobtus-1-ene-3,11-diol

*Aplysia dactylomela*[49]




48 angasiol

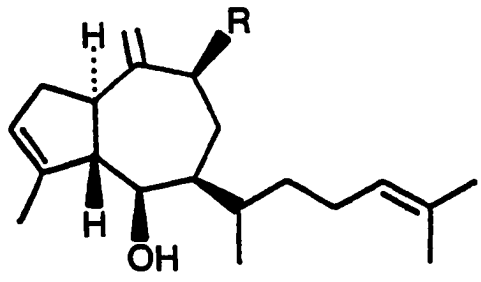
*Aplysia angasi*[50]




49 dictyolactone .

*Aplysia depilans*[51]

**Figure 4. Diterpenoid Derivatives from Opisthobranchs.**

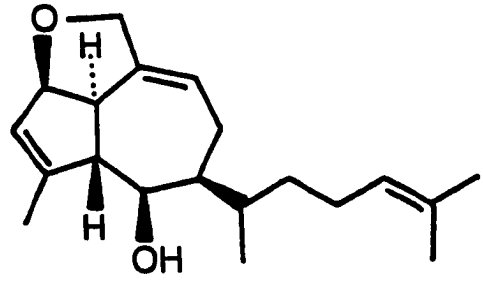



50 R = H, pachydictyol A

51 R = OH, dictyol B

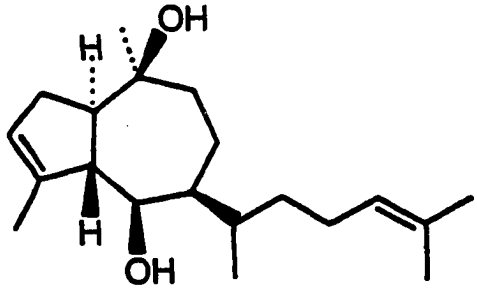
*Aplysia depilans*[52]

*Aplysia vaccaria*[20]



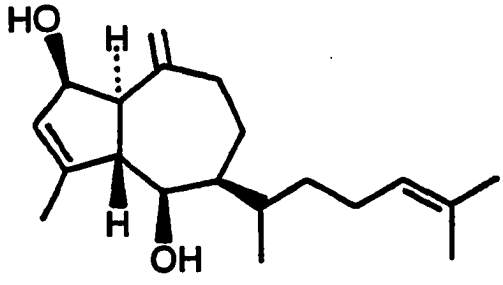

52 dictyol A

*Aplysia depilans*[52]



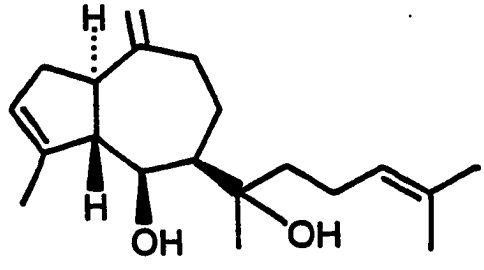

53 dictyol C

*Aplysia depilans*[53]




54 dictyol D

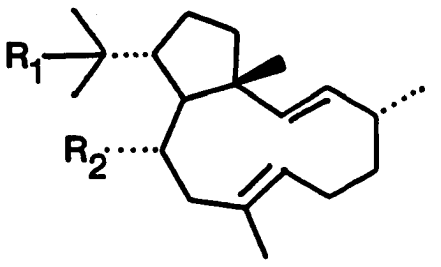
*Aplysia depilans*[53]




55 dictyol E

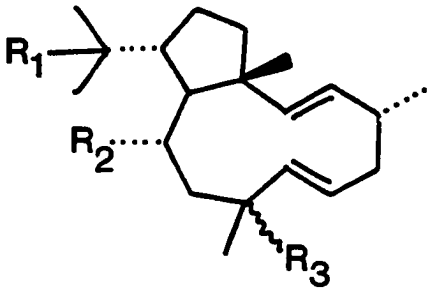
*Aplysia depilans*[53]

**Figure 4 (continued).**

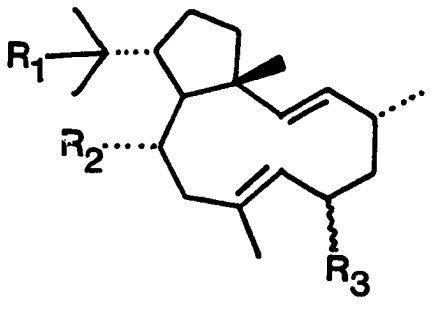


| | $R_1$ | $R_2$ |
|---|---|---|
| 56 | OAc | OAc |
| 57 | OH | OAc |
| 58 | OH | OH |

dolabelladienes

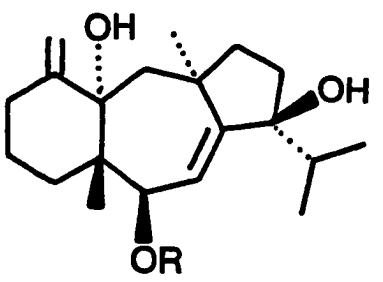


| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 59 | OAc | OAc | OAc |
| 60 | OAc | OH | OAc |
| 61 | OAc | OH | OH |
| 62 | OAc | OAc | OH |
| 63 | OH | OAc | OAc |
| 64 | OH | OH | OH |



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 65 | OH | OAc | OAc |
| 66 | OH | OAc | OH |
| 67 | OAc | OH | OH |
| 68 | OH | OH | OH |
| 69 | OH | H | H |

*Dolabella californica*[10,11]

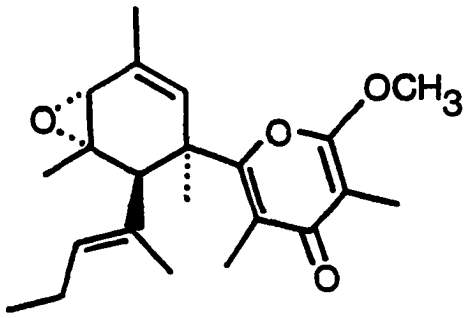


70 R = H, dolatriol

71 R = Ac, dolatriol 6-acetate

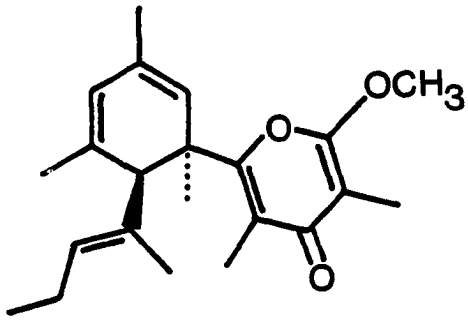
*Dolabella auricularia*[12]

**Figure 4 (continued).**




72 tridachione

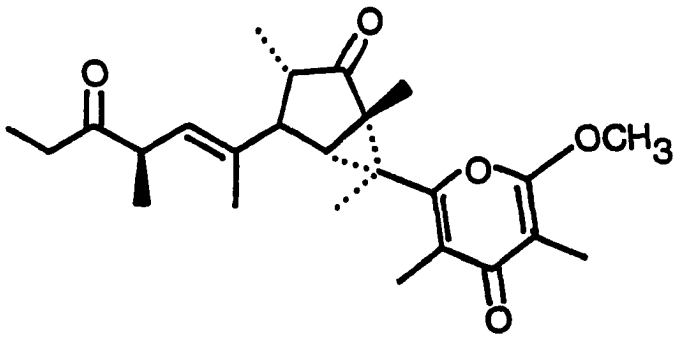
*Tridachiella diomedea*[15]




73 9,10-deoxytridachione

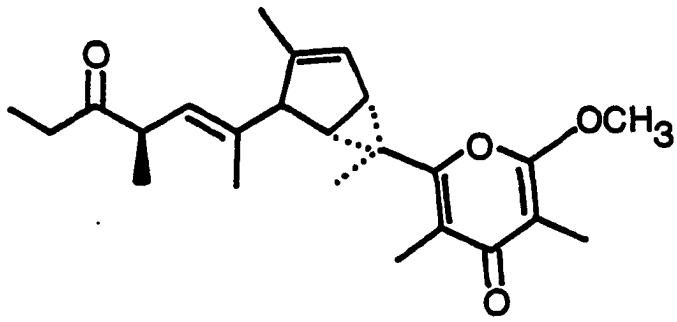
*Tridachiella diomedea*[15]

*Placobranchus ocellatus*[16]




74 crispatone

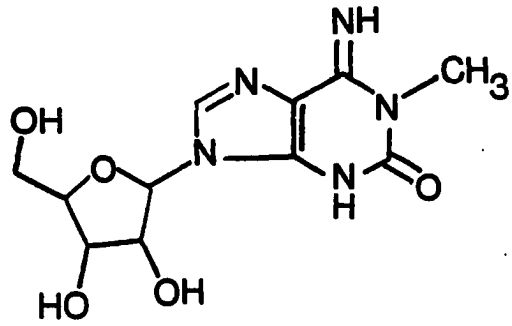
*Tridachia crispata*[17]




75 crispatene

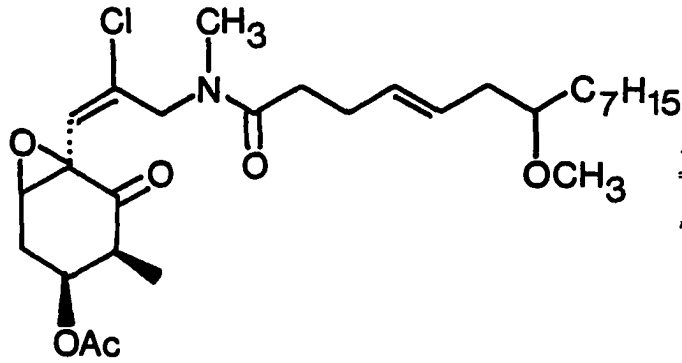
*Tridachia crispata*[17]

Figure 5. Miscellaneous Derivatives from Opisthobranchs.



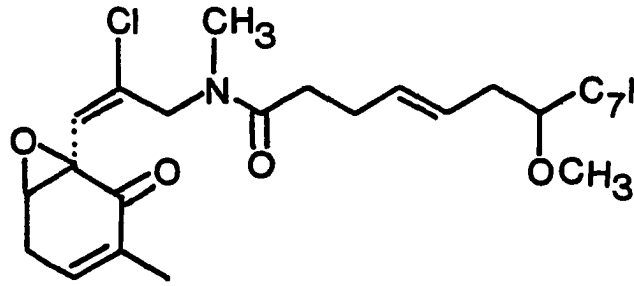
76 doridosine

*Anisodoris nobilis*[27]



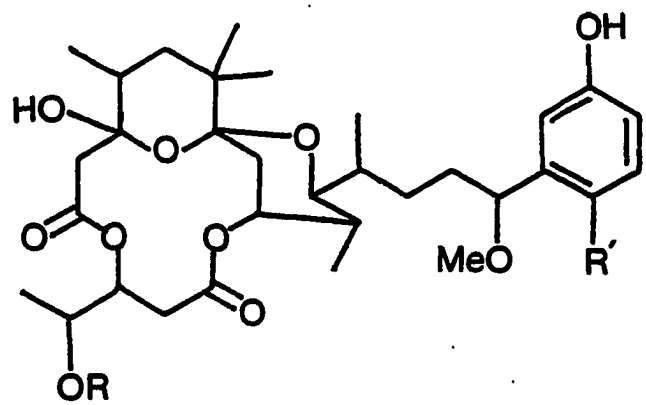
77 stylocheilamide

*Stylocheilus longicauda*[14]



78 deacetoxystylocheilamide

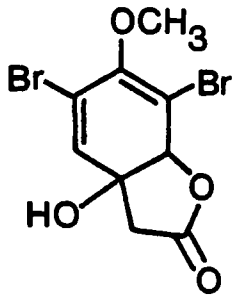
*Stylocheilus longicauda*[14]



| | R | R' | |
|---|---|---|---|
| 79 | H | H | debromoaplysiatoxin |
| 80 | Ac | H | |
| 81 | H | Br | aplysiatoxin |
| 82 | Ac | Br | |

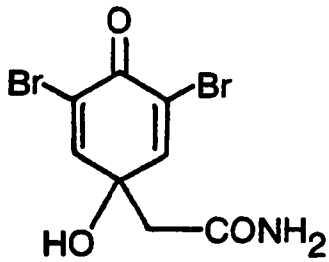
*Stylocheilus longicauda*[13,54]

Figure 5 (continued).



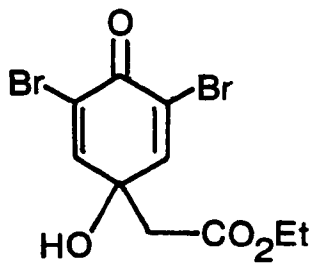

83 aeroplysinin-2

*Tylodina fungina*[55]




84

*Tylodina fungina*[55]



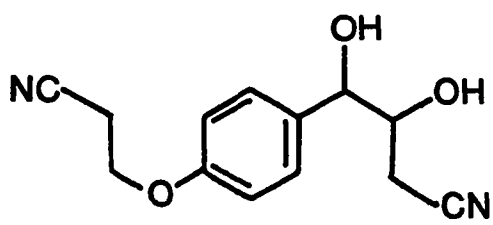

85

*Tylodina fungina*[55]




86

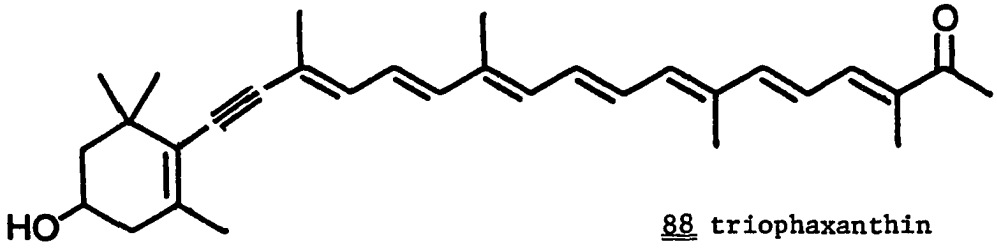
*Bursatella leachii*[20]




87 bursatellin

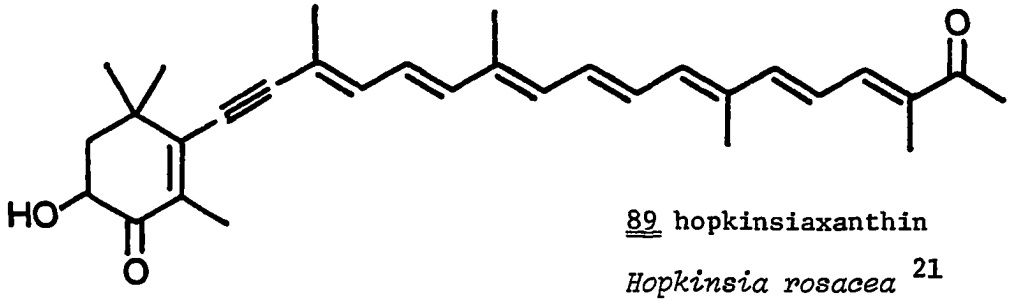
*Bursatella leachii pleii*[31]

Figure 5 (continued).



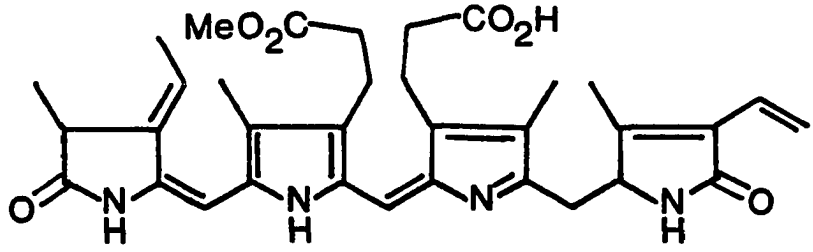

88 triophaxanthin

*Triopha carpenteri*[22]



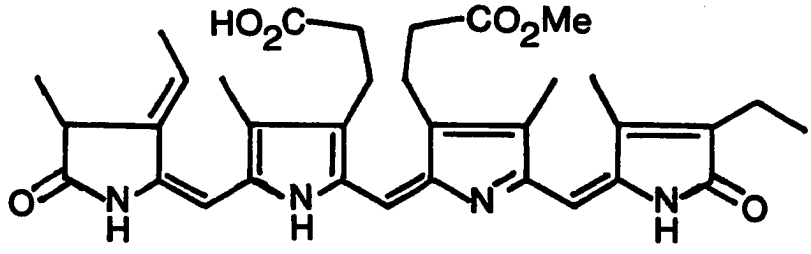

89 hopkinsiaxanthin

*Hopkinsia rosacea* [21]




90 aplysiaviolin

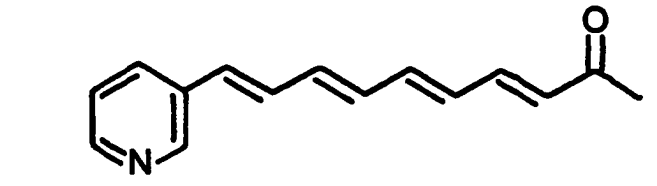
*Aplysia limacina*[56]




91 aplysiaverdin

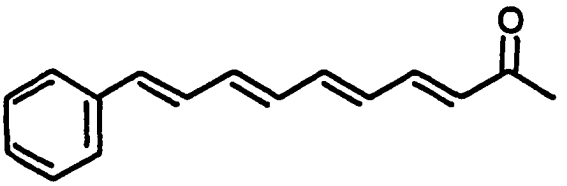
*Aplysia* [57]

Figure 5 (continued).



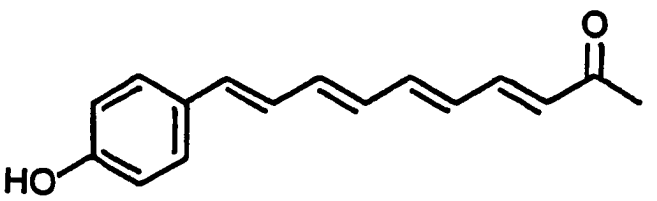
92 navenone-A

*Navanax inermis*[19,18]



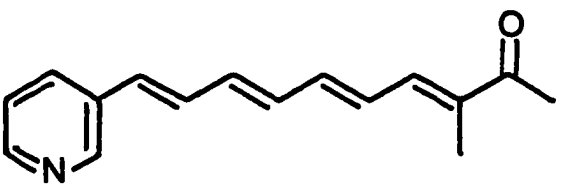
93 navenone-B

*Navanax inermis*[19,18]



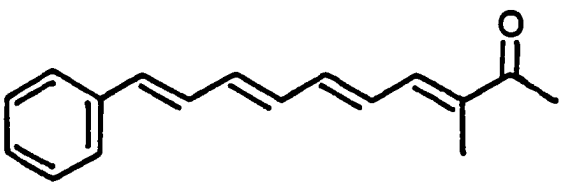
94 navenone-C

*Navanax inermis*[19,18]



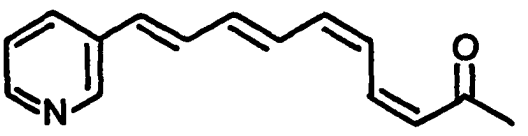
95

*Navanax inermis*[20]



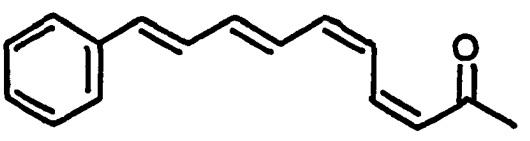
96

*Navanax inermis*[20]



97

*Navanax inermis*[20]



98

*Navanax inermis*[20]

Figure 5 (continued).

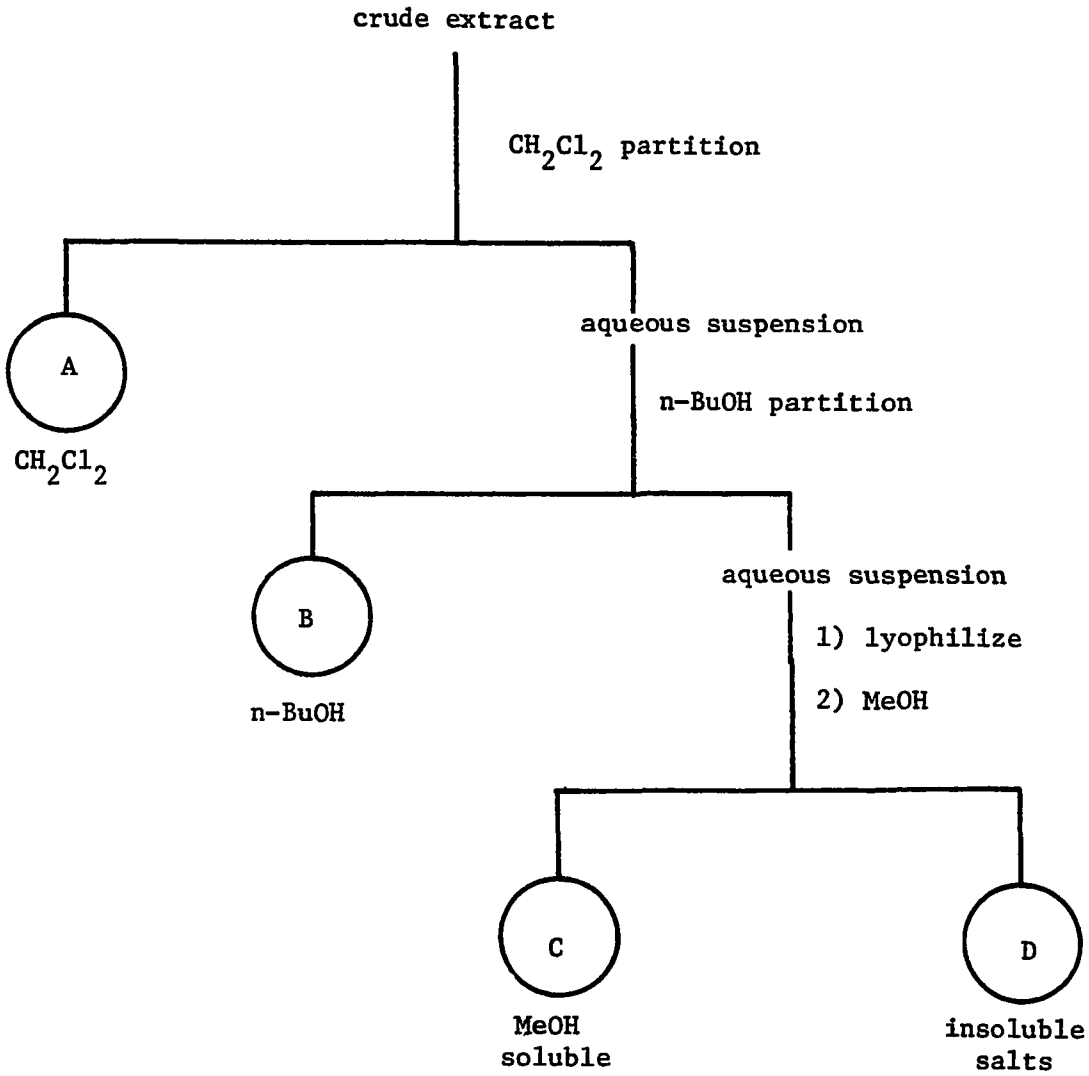
## RESULTS AND DISCUSSION

The sea hares, *Aplysia dactylomela* Rang, used in this study were collected near La Parguera, Puerto Rico in December 1977. The digestive glands of the three hundred specimens collected were excised and preserved in 70% isopropanol for shipment to Oklahoma.

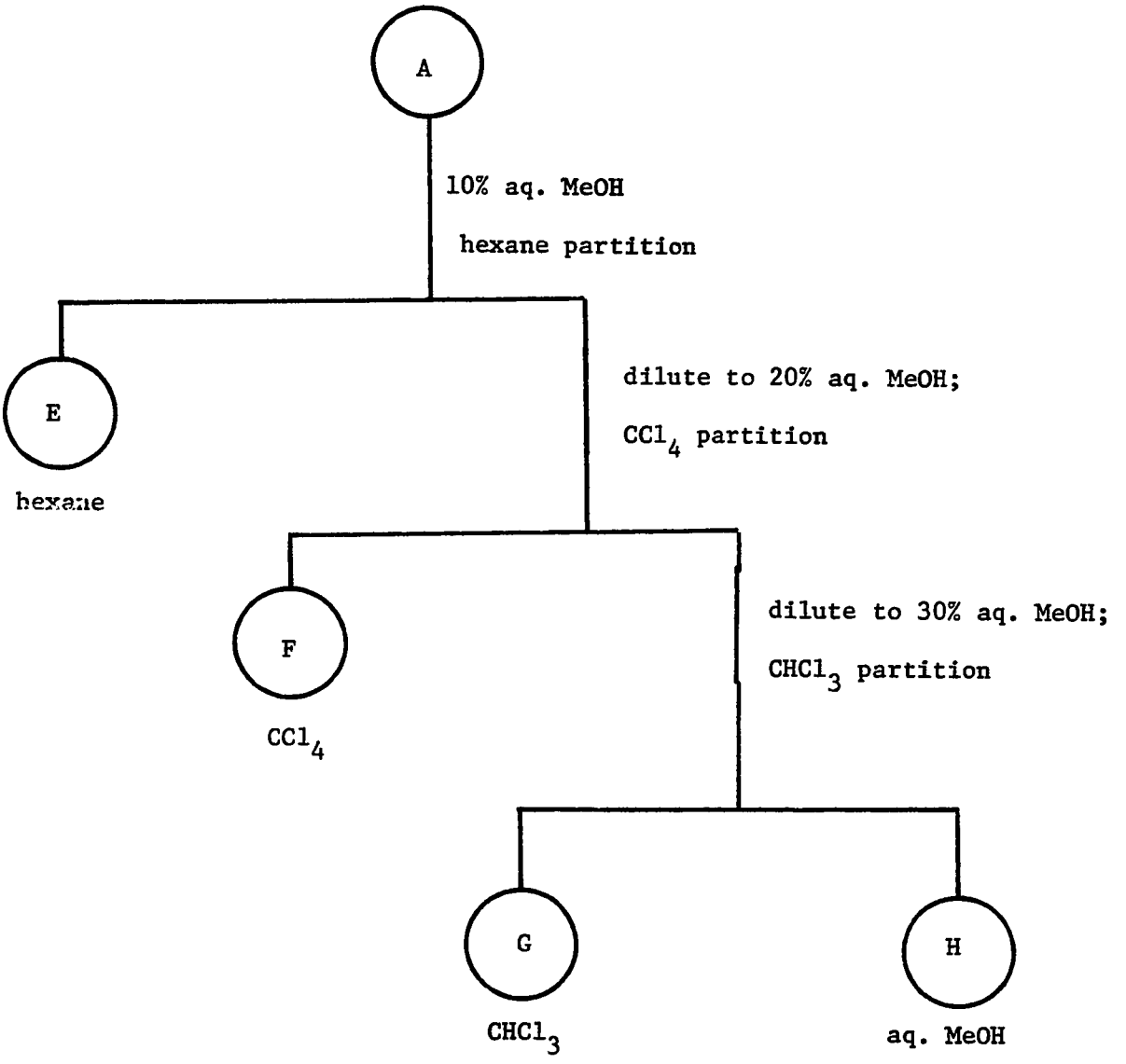
The alcoholic solution, which was removed from the glands by decantation and filtration, was concentrated and fractionated according to Scheme I. Samples of each fraction were sent for in vitro KB (nasopharynx carcinoma), PS-388 (lymphocytic leukemia), and L1210 (lymphoid leukemia) anticancer testing.[58,59] Fractions which exhibited significant activity were further fractionated.

The digestive glands were homogenized in a blender with chloroform-methanol (2:1) and filtered through cheesecloth. The resulting solution was also concentrated and fractionated according to Scheme I. The fraction which displayed the greatest biological activity (KB 23, PS 15, L1210 30) was fraction A of Scheme I. Fraction A was concentrated and further partitioned between aqueous methanol and hexane, carbon tetrachloride, and chloroform according to Scheme II. The greatest activity was found in fraction F (KB 2.5, PS 2.4, L1210 1.3) and fraction G (KB 2.1, PS 1.1, L1210 0.96). These two fractions and others were chromatographed extensively to yield a number of pure compounds which are described individually below.

SCHEME I


crude extract
$CH_2Cl_2$ partition
A
$CH_2Cl_2$
aqueous suspension
n-BuOH partition
B
n-BuOH
aqueous suspension
1) lyophilize
2) MeOH
C
MeOH
soluble
D
insoluble
salts

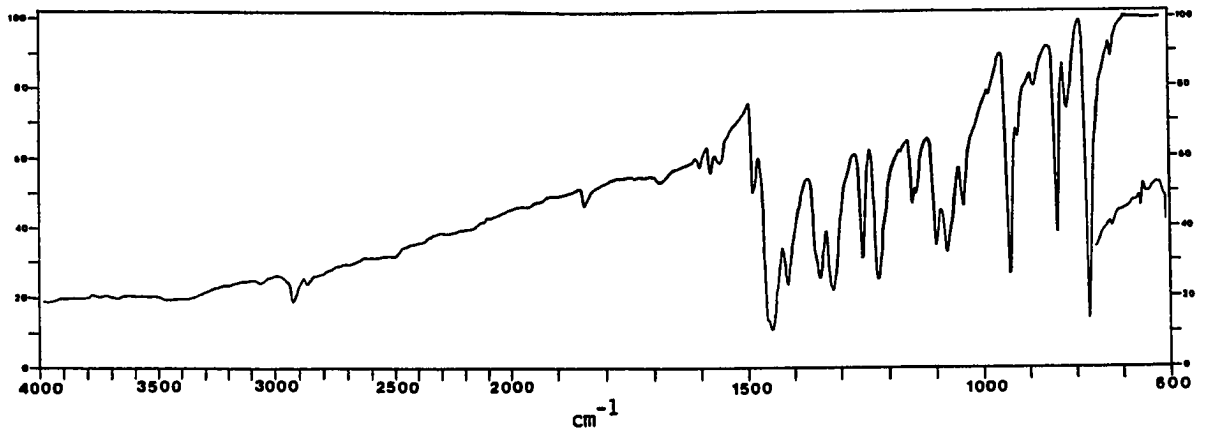
SCHEME II


A
10% aq. MeOH
hexane partition
E
hexane
dilute to 20% aq. MeOH;
$CCl_4$ partition
F
$CCl_4$
dilute to 30% aq. MeOH;
$CHCl_3$ partition
G
$CHCl_3$
H
aq. MeOH

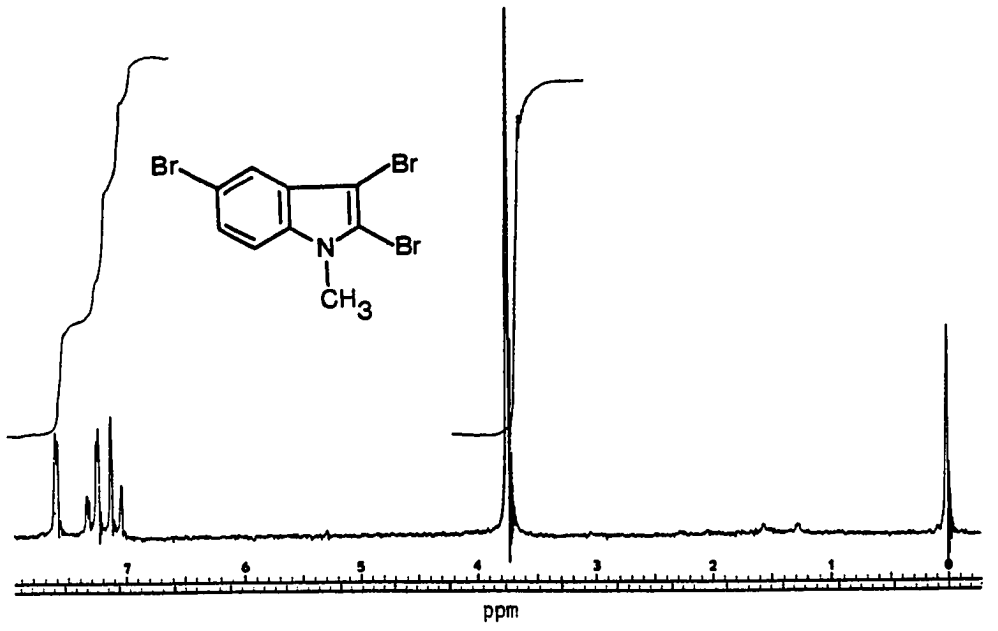
2,3,5-Tribromo-N-methylindole (99)

Silica gel chromatography of fraction F yielded, in the initial fractions, a white crystalline material, mp. 124-124.8°, $C_9H_6NBr_3$ (high resolution mass spectral analysis). The 100 MHz nmr spectrum contained a three proton singlet at 3.73 ppm, indicative of a methyl group attached to nitrogen. The remainder of the nmr spectrum contained three one-proton multiplets in the region 7.10-7.60 ppm. The formula and the nmr spectrum thus suggested the presence of a substituted indole. Further evidence in favor of an N-methyl indole nucleus was provided by uv absorptions at 292, 300, and 284 nm. The locations of the three bromine atoms on the indole nucleus were determined by comparison of the observed coupling constants to that of known substituted[60] indole systems and by Nuclear Overhauser Enhancement (NOE) experiments. Irradiation of the methyl group caused a 15% increase in the height of the doublet at 7.10 ppm indicating that this was the proton at C-7. The coupling constant (J=8 Hz) of this proton was consistent with an ortho-coupling to the C-6 proton which was in turn meta-coupled to the C-4 proton. Hence, the location of the bromine atoms were C-2, C-3, and C-5. The structure elucidation of 99 was accomplished independently of a report[60] of this indole and several other indoles isolated from the red alga, *Laurencia brongniartii*. The bromoindole isolated from *Aplysia* was identical in all respects to that isolated from *Laurencia*.

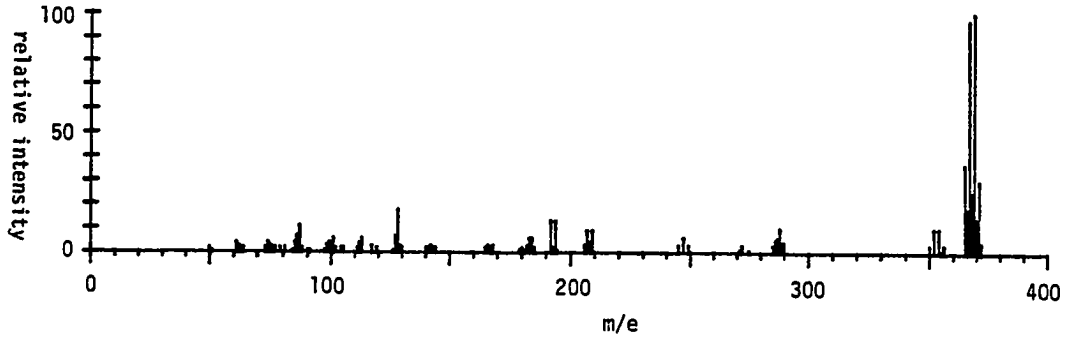
Figure 6. Spectra of 2,3,5-tribromo-N-methylindole (99).



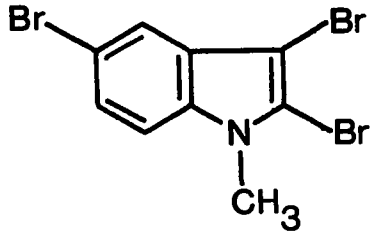

IR Spectrum of 99




100 MHz PMR Spectrum of 99 in $CDCl_3$




Mass Spectrum of 99




99

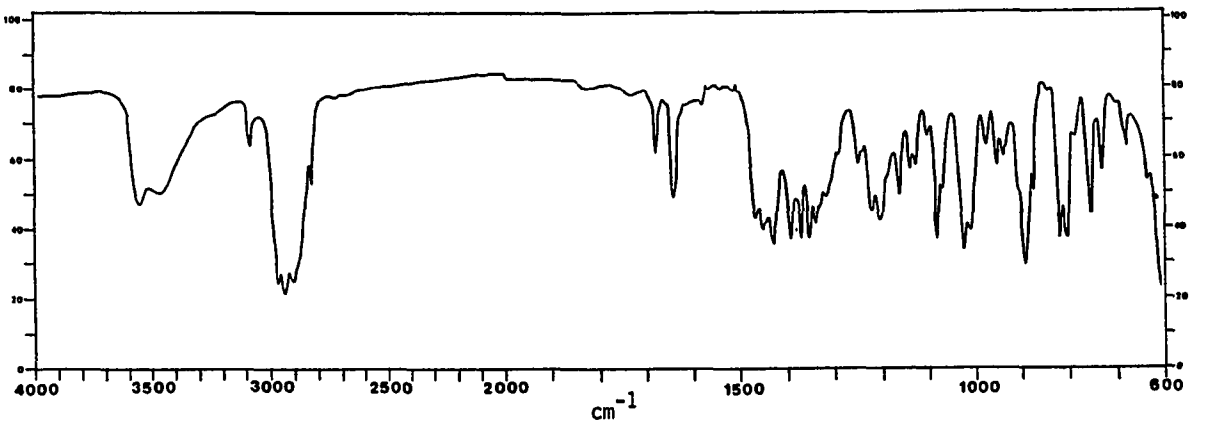
Elatol (100)

Another constituent isolated from the carbon tetrachloride fraction, fraction F, Scheme II, was elatol (100). Elatol was obtained as a yellowish oil which slowly solidified. Recrystallization from methanol yielded elatol as a sticky mass of crystals, mp. 62-66°. Elatol obtained[61] from the alga *Laurencia elata*, however, was reported as a colorless oil.

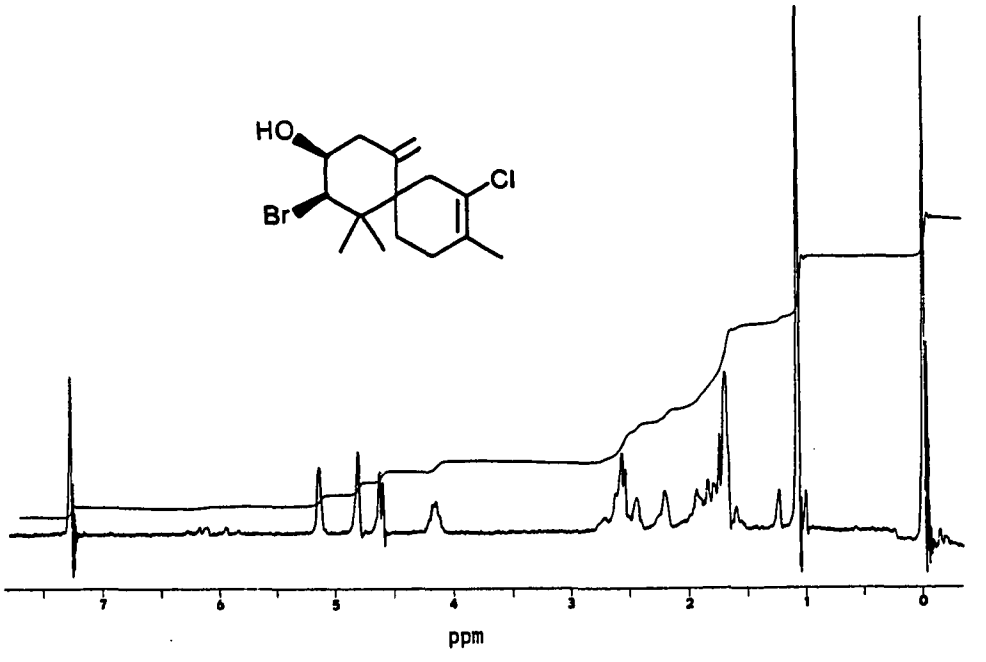
Acetylation of elatol yielded a crystalline acetate, mp. 155-156°. The melting point of the acetate, the proton magnetic resonance, and the carbon-13 magnetic resonance spectra of elatol were identical to those of elatol obtained from other sources.[61]

Elatol exhibits strong antifungal activity as determined by tests conducted in this laboratory according to the procedure of Klarman and Sanford.[62] A suspension of centrifuged V-8 juice with spores from the mold, *Cladosporium cucumerinum*, were sprayed on developed TLC plates containing samples of pure compounds and crude fractions. After a four day incubation period, the presence of antifungal active

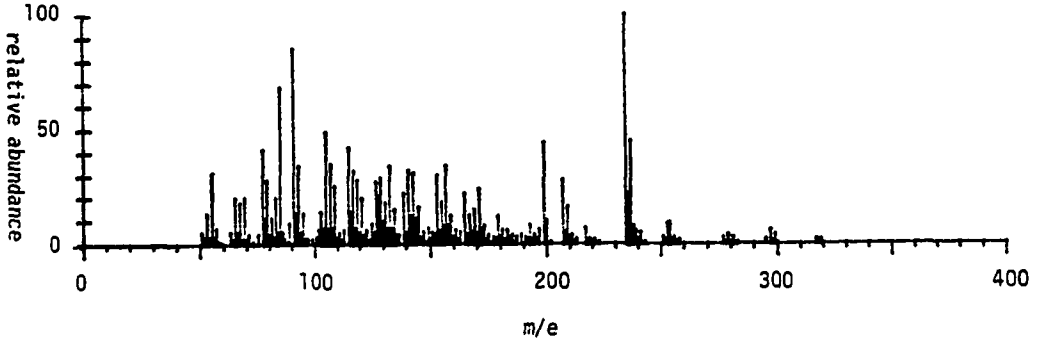
Figure 7. Spectra of Elatol (100)




IR Spectrum of 100

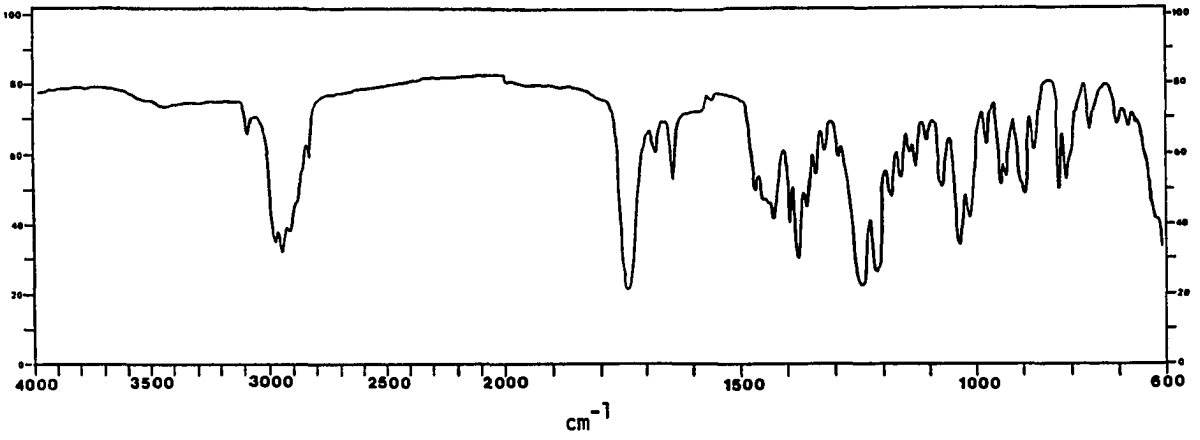



100 MHz PMR Spectrum of 100 in $CDCl_3$

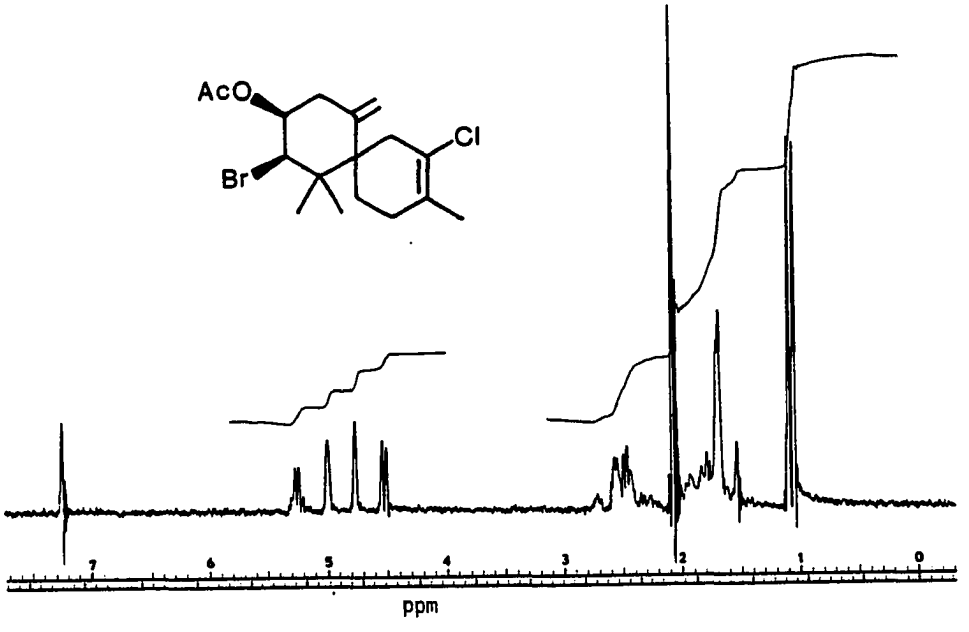



Mass Spectrum of 100

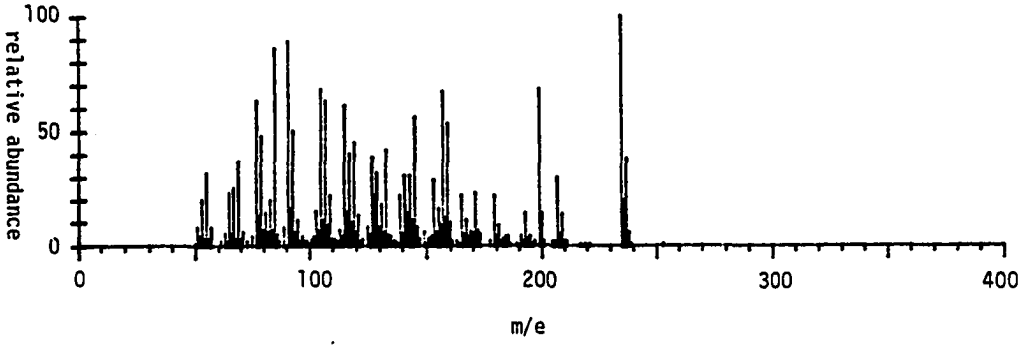
Figure 8. Spectra of Elatol Acetate




IR Spectrum of Elatol Acetate

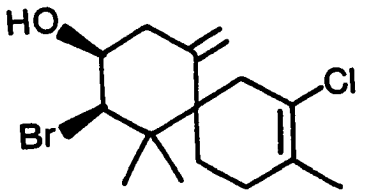



100 MHz PMR Spectrum of Elatol Acetate in $CDCl_3$




Mass Spectrum of Elatol Acetate

compounds was indicated by white zones of inhibition on a fuzzy green background of thriving organism.



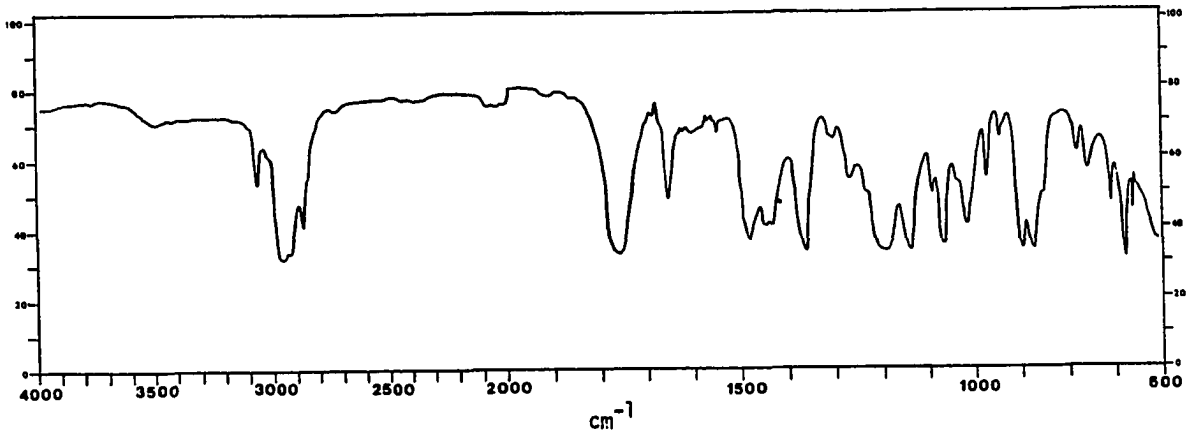

100

Characterization of allolaurinterol acetate (101)

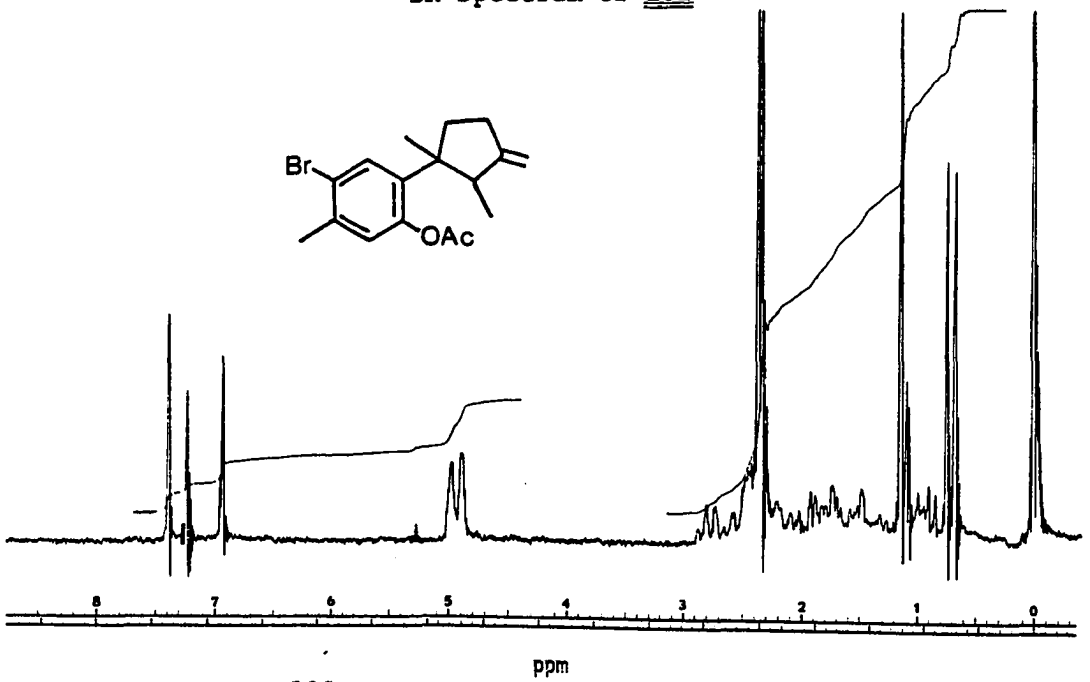
The mother liquor from recrystallizations of elatol was examined by HPLC. One of the fractions contained a crystalline compound (101), mp. 80-82°. The highest mass ions observed in the low resolution mass spectrum of 101 appeared at m/e 336, 338, consistent with a formula of $C_{17}H_{21}BrO_2$. Acetate functionality was indicated in the infrared spectrum by an absorption at 1763 $cm^{-1}$ and in the proton nmr spectrum by a methyl singlet at 2.33 ppm. The remainder of the pmr spectrum very closely resembled the spectra of isolaurinterol (102)[63] and allolaurinterol (103).[64]

The only difference in the structures of isolaurinterol and allolaurinterol is the substitution pattern on the methylenecyclopentane ring. In the pmr spectrum of allolaurinterol, the methine proton on the cyclopentane ring is vicinally coupled only to a methyl group and,

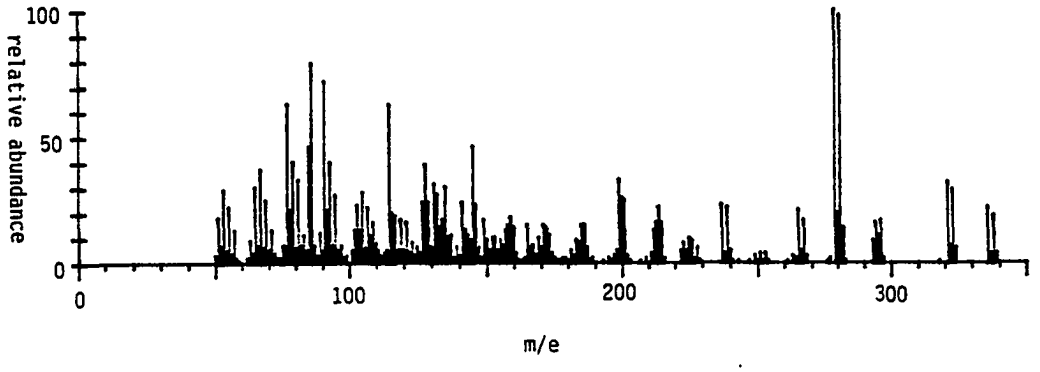
Figure 9. Spectra of allolaurinterol acetate (101)




IR Spectrum of 101



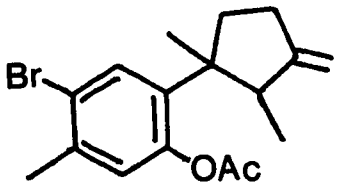

100 MHz PMR Spectrum of 101 in $CDCl_3$



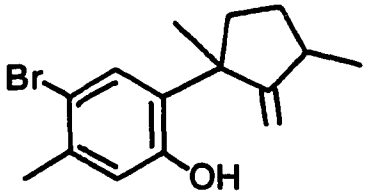

Mass Spectrum of 101

therefore, appears as a quartet. In the spectrum of isolaurinterol, the methine proton appears more complex since it is coupled to the methyl group and to adjacent ring protons. In order to establish whether the acetate of mp. 80-82° possessed the isolaurinterol or allolaurinterol type structure, a proton decoupling experiment was conducted. Irradiation of the methyl doublet at 0.70 ppm collapsed a quartet at 2.77 ppm to a singlet, a result which is consistent with the methyl group arrangement of allolaurinterol, and hence the acetate, mp. 80-82°, was confirmed to be allolaurinterol acetate (101).

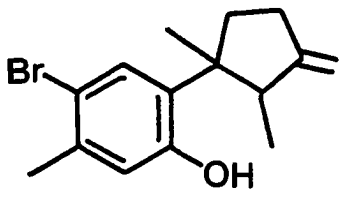
Although allolaurinterol acetate was not previously reported as a natural product, it was a known[64] synthetic derivative of allolaurinterol. The physical constants and spectral data for the naturally obtained allolaurinterol acetate were identical to those reported[64] for synthetic allolaurinterol acetate.




101



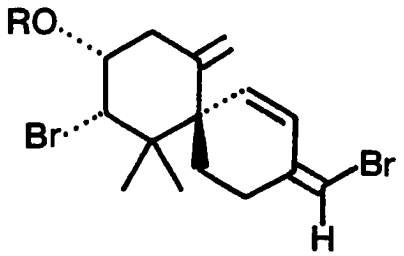

102




103

Bromochamigrene (104a) and Characterization of Bromochamigrene (105a)

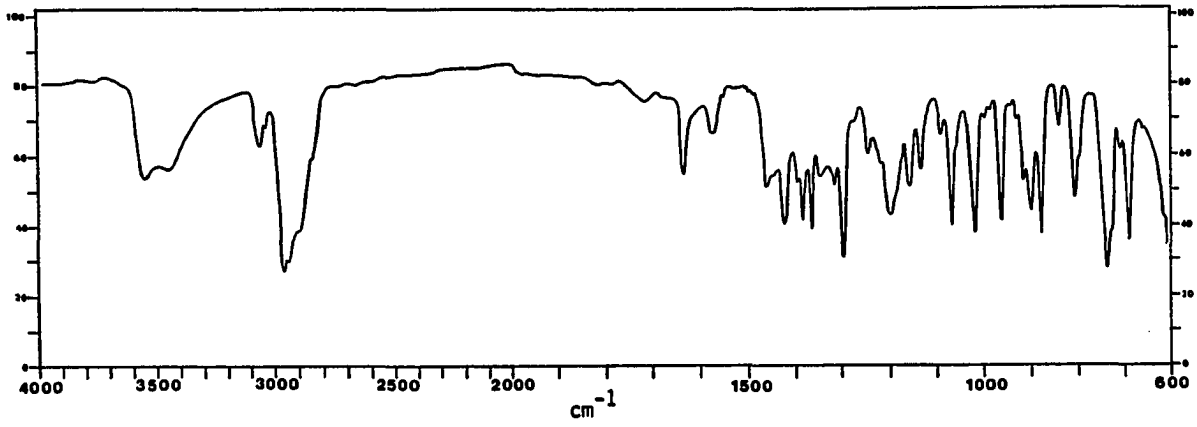
Accompanying elatol in the general fractionation of fraction F were two closely related compounds, 104a and 105a, which were separated by HPLC. Compound 104a was obtained as a colorless oil, $C_{15}H_{20}Br_2O$ (low resolution mass spectrum). Its physical and spectral properties were identical in all respects to those of a halogenated chamigrene alcohol (104a) reported[65,66] from *Laurencia majuscula*. Acetylation of compound 104a with acetic anhydride and pyridine yielded an acetate, 104b, mp. 115-117°, with the same physical and spectral properties as the reported[65] acetate.



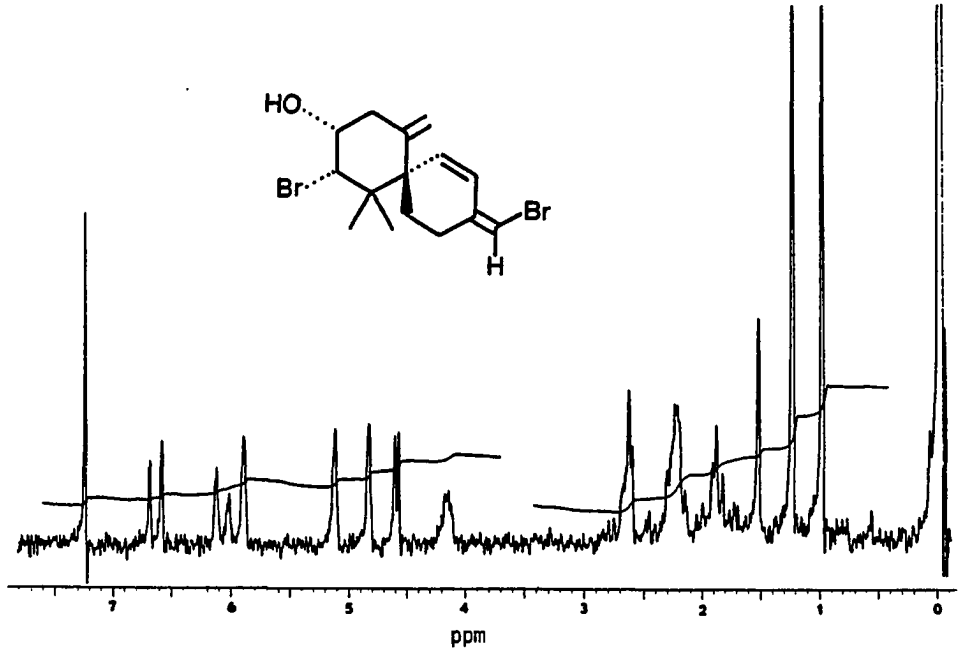

104a R=H

104b R=Ac

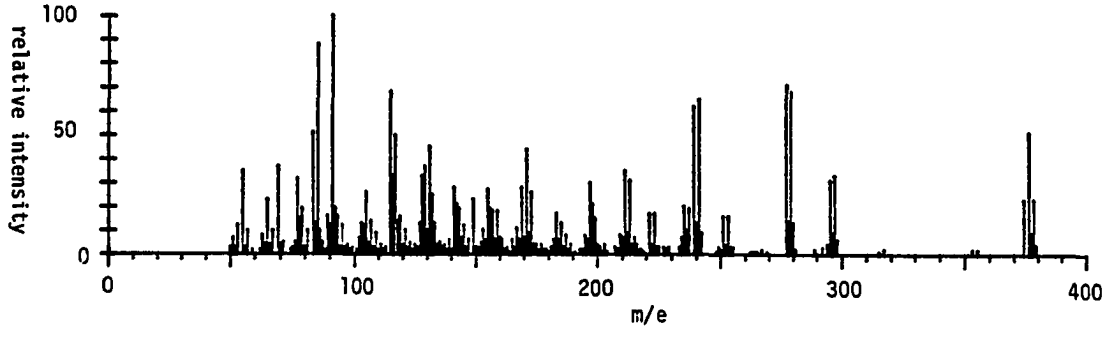
Figure 10. Spectra of Chamigrene 104a.




IR Spectrum of 104a

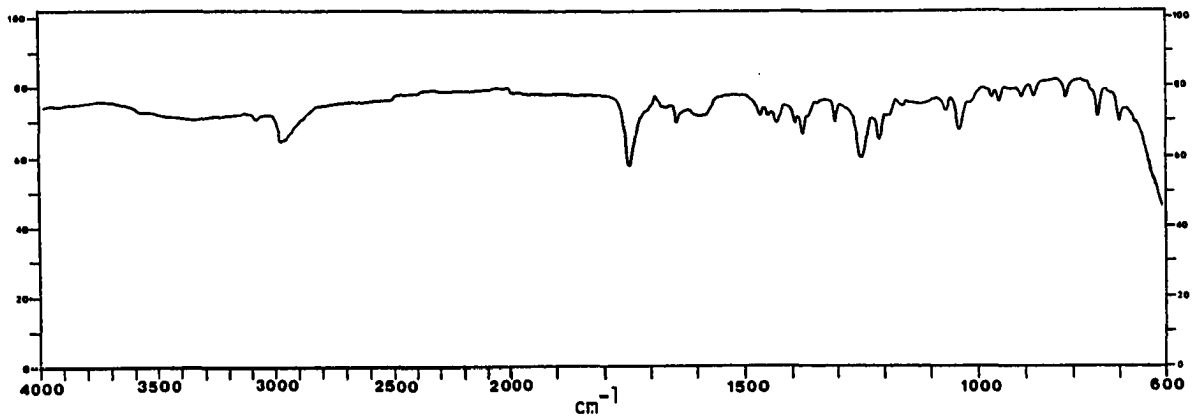



100 MHz PMR Spectrum of 104a in $CDCl_3$

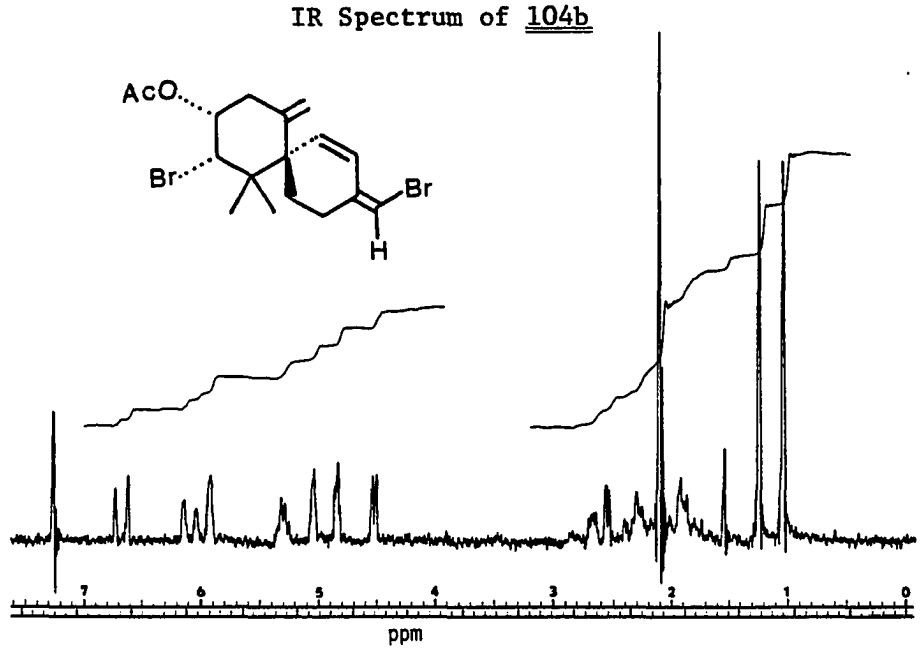



Mass Spectrum of 104a

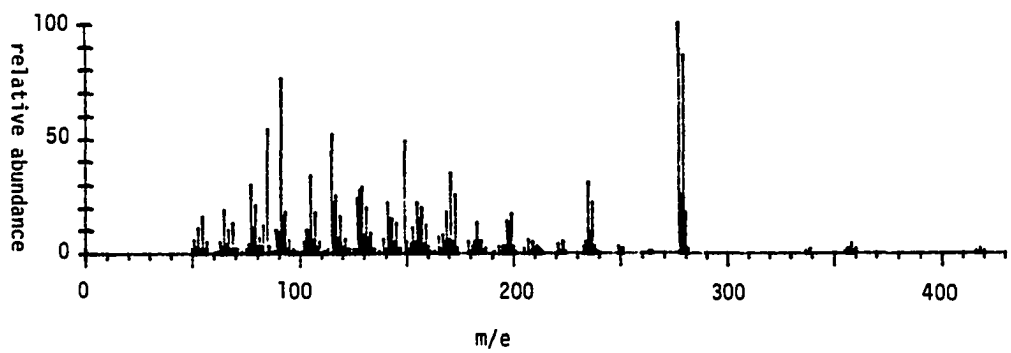
Figure 11. Spectra of Chamigrene 104b




IR Spectrum of 104b




100 MHz PMR Spectrum of 104b in $CDCl_3$

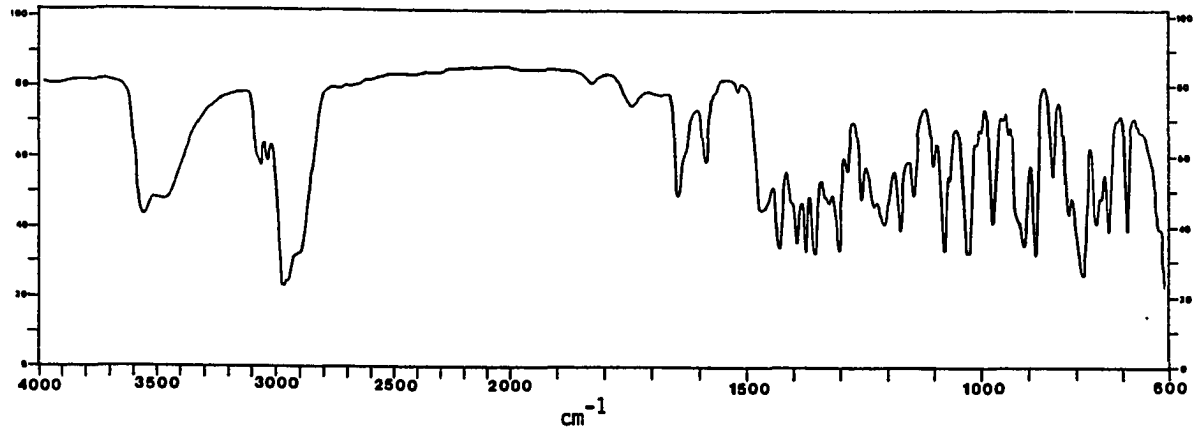



Mass Spectrum of 104b

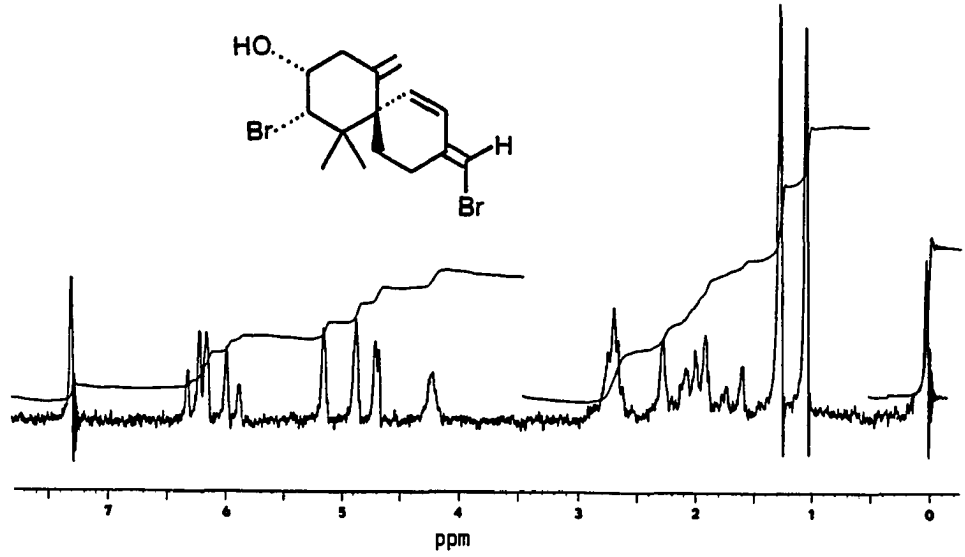
Compound 105a was also isolated as a colorless oil, $C_{15}H_{20}Br_2O$ (low resolution mass spectrum). The close similarity in the proton nmr and mass spectra of compounds 104a and 105a suggested that they were isomers. Indeed, after a few days standing, fractions originally containing one of the pure compounds were found to be contaminated with varying amounts of the other isomer.

The infrared spectrum of compound 105a indicated the presence of hydroxyl (3460 $cm^{-1}$) and a variety of olefinic groups (3060, 3030, 1638, 905, and 882 $cm^{-1}$). Ultraviolet absorption at 247 nm (16,000) was consistent for a conjugated diene. The proton nmr spectrum of 105a contained two methyl singlets at 1.01 and 1.24 ppm. Additionally, several signals appeared downfield which were assigned by reference to the known compound 104a. A sharp doublet (J=3 Hz) at 4.66 ppm was assigned to an axial proton deshielded by bromine. A multiplet at 4.20 ppm was indicative of a hydroxyl deshielded methine proton. An exocyclic methylene group appeared as two singlets at 4.84 and 5.12 ppm. The three remaining olefinic signals for compound 105a, however, were found at substantially different chemical shifts from the signals of compound 104a, see Table 1. The singlet at 6.13 ppm was assigned to the trisubstituted bromo olefinic proton. The coupling constants (J=10 Hz) for the absorptions at 5.95 and 6.24 ppm were consistent for two cis-oriented olefinic protons. The downfield shift of the cis olefinic protons in compound 104a relative to 105a is due to the deshielding effect of bromine. In compound 105a, the trisubstituted bromoolefinic bond has the E configuration with the bromine atom as far away as possible from the cis olefinic bond. In

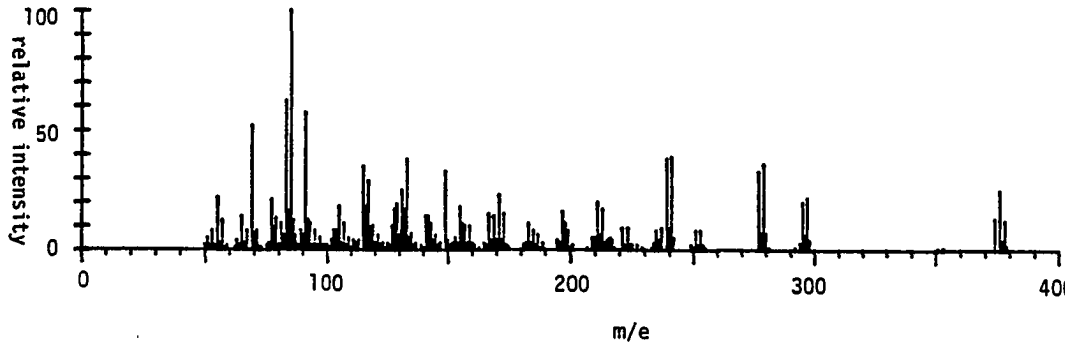
Figure 12. Spectra of Chamigrene 105a.




IR Spectrum of 105a

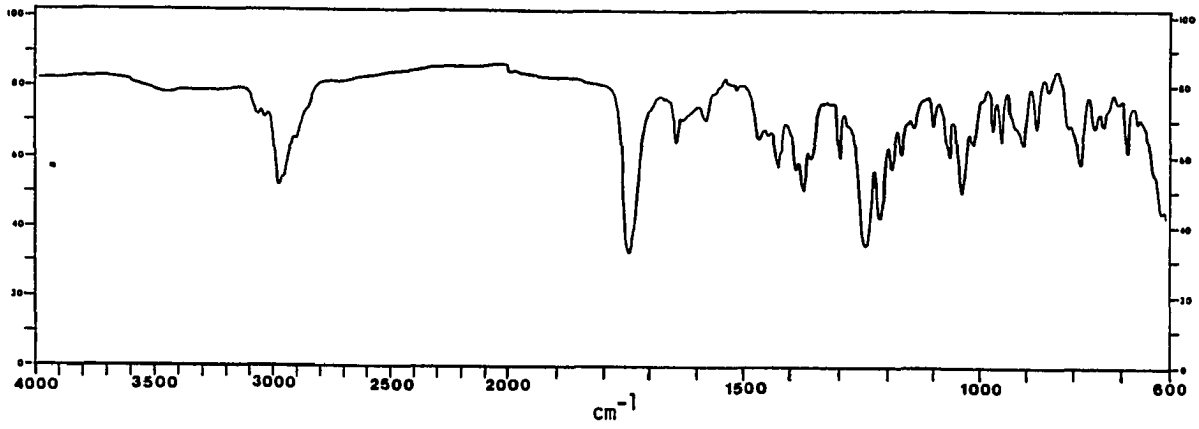



100 MHz PMR Spectrum of 105a in $CDCl_3$

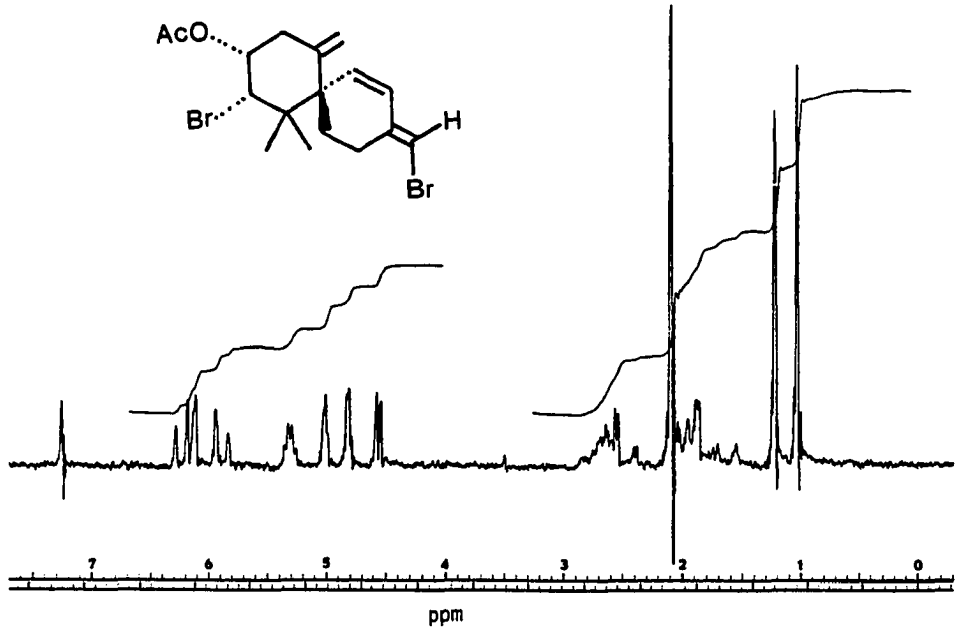



Mass Spectrum of 105a

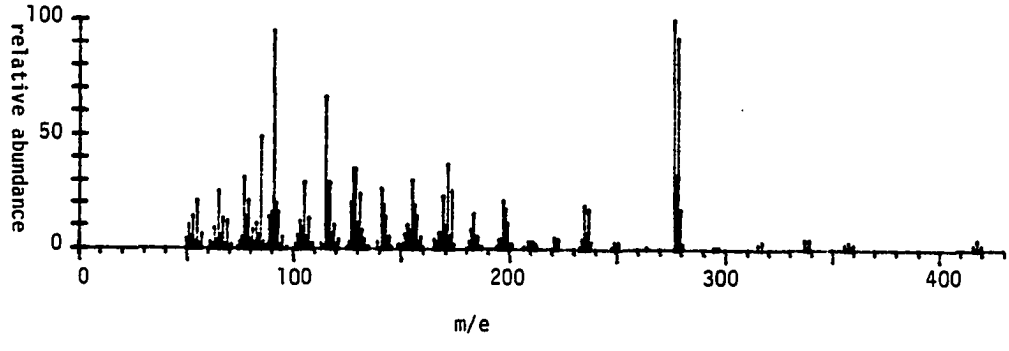
Figure 13. Spectra of Chamigrene 105b




IR Spectrum of 105b

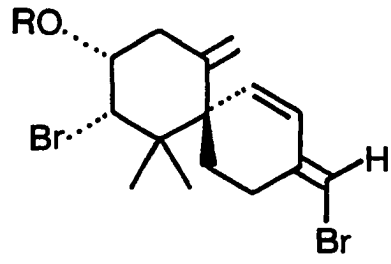



100 MHz PMR Spectrum of 105b in $CDCl_3$




Mass Spectrum of 105b

compound 104a, the trisubstituted bromoolefinic bond has the Z configuration bringing the bromine atom in close proximity to the cis olefinic protons and deshielding them. Additionally, the proton geminal to the bromine atom is shielded in the Z isomer relative to the E isomer because of its increased distance from the deshielding effects of the cis olefinic bond.




105a R=H

105b R=Ac

These differences in the chemical shifts are well illustrated in the oxidation product of compound 104a which was reported[65] by Suzuki and Kurosawa to spontaneously isomerize about the bromoolefinic bond into the E (106) and Z (107) isomers upon exposure to light. Table 1 summarizes the chemical shifts of the cis olefinic and bromo olefinic protons of the compounds discussed.

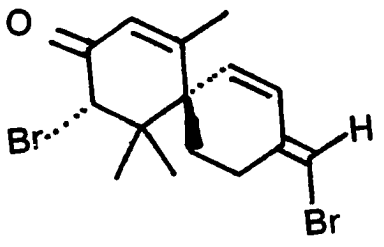
Compound 105a was acetylated with acetic anhydride and pyridine to yield an acetate 105b, mp. 121-123°C. The proton nmr spectrum of the acetate 105b exhibited the same general chemical shifts as the parent alcohol 105a and the values are also summarized in Table 1.

Table 1

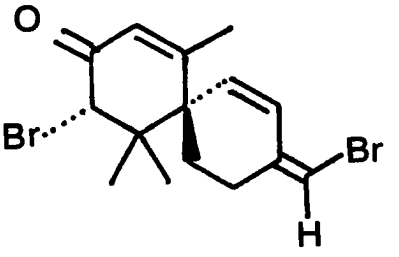
Chemical Shifts of Selected Olefinic Protons of 104-107

| Compound | -CH=CH- | -CH=CH- | C=CHBr (E or Z) |
|---|---|---|---|
| | br d | d | s |
| | J=10 Hz | J=10 Hz | |
| 105a[1] | 5.95 | 6.24 | 6.13 (E) |
| 105b[1] | 5.88 | 6.23 | 6.10 (E) |
| 106[2] | 5.75 | 6.39 | 6.26 (E) |
| 104a[1] | 6.10 | 6.65 | 5.92 (Z) |
| 104b[1] | 6.09 | 6.66 | 5.92 (Z) |
| 104a[2] | 6.06 | 6.61 | 5.87 (Z) |
| 104b[2] | 6.06 | 6.63 | 5.88 (Z) |
| 107[2] | 5.95 | 6.80 | 6.00 (Z) |

[1]This work.
[2]See Ref. 65.




106



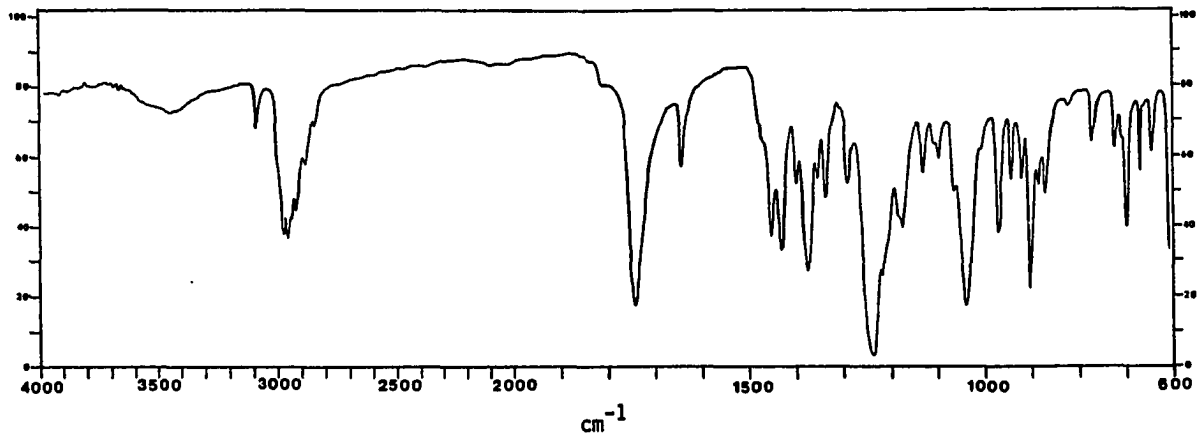

107

Characterization of isoobtusol acetate (108)

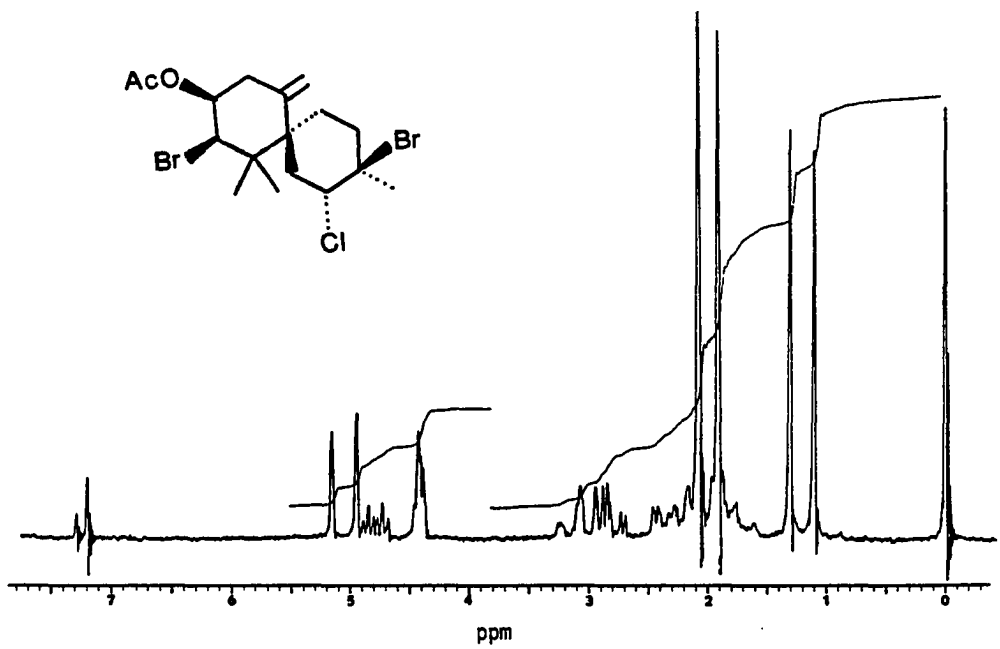
The hexane fraction, fraction E of scheme II, was chromatographed over coarse silica gel using the elution sequence hexane, chloroform, ethyl acetate, and methanol. Sephadex LH-20 chromatography of the chloroform eluate yielded isoobtusol acetate (108), $C_{17}H_{25}Br_2ClO_2$ (high resolution mass spectrum), as colorless crystals, mp. 170-173°C, $[\alpha]_D$ +57.0 ($CHCl_3$). The presence of an acetate group was determined by infrared absorptions at 1745 and 1235 $cm^{-1}$ and in the proton nmr spectrum by a methyl singlet at 2.11 ppm.

The remainder of the pmr spectrum resembled that of isoobtusol.[67] Three quaternary methyl singlets were present at 1.12, 1.32, and 1.92 ppm. The latter signal was in a range typical for a methyl group deshielded by bromine and in good agreement with one of the methyl signals in isoobtusol.[67] A two proton multiplet at 4.46 ppm was consistent with two methine protons, each deshielded by halogen. The small width of the multiplet indicated that both methine protons lack a large

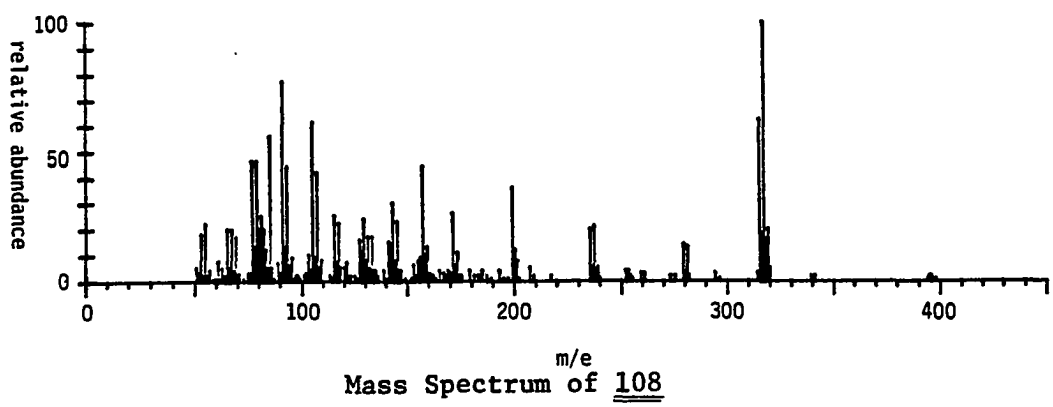
Figure 14. Spectra of Isoobtusol Acetate (108)




IR Spectrum of 108




100 MHz PMR Spectrum of 108 in $CDCl_3$




Mass Spectrum of 108

coupling. Hence, they were equatorial and the halogen atoms were axial. A distinct double triplet at 4.83 ppm was assigned to a methine deshielded by acetate. The presence of a large diaxial coupling established the methine as axially oriented. Two one proton singlets at 4.99 and 5.21 ppm were ascertained to be exocyclic methylene protons.

The proton nmr spectrum of isoobtusol acetate in $CDCl_3$ at 270 MHz (see experimental) was greatly resolved in the high field region, and proton decoupling experiments established the identity of all proton signals. Specifically, irradiation of the acetoxy deshielded methine proton at 4.83 ppm resulted in a change in the multiplet at 4.46 ppm, removed a small coupling from the double doublet at 2.40 ppm, and collapsed the triplet at 2.96 ppm to a doublet. The remaining coupling of the 2.96 ppm triplet was found to be due to geminal coupling to the 2.40 ppm signal. Hence the protons resonating at 2.96 and 2.40 ppm are due to a methylene group attached to a fully substituted carbon as shown in partial structure A. The low field at which these protons resonate indicate that this methylene group is allylic.

$$-CHBr-CHOAc-CH_2-\overset{|}{C}=CH_2$$

A

In order to determine whether or not the bromine deshielded methine in structure A was also attached to a fully substituted carbon, the spectrum was reobtained in benzene-$d_6$. Table 2 summarizes the decoupled spectrum in benzene-$d_6$. The two proton multiplet observed at 4.46 ppm in $CDCl_3$ was resolved in benzene-$d_6$ into two narrow multiplets at 4.43 and 4.17 ppm. Irradiation of the bromine deshielded

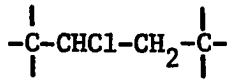
Table 2

Double Irradiation of Isoobtusol Acetate[a] (108)

| Signal Irrad'd | Signal Obs'd | Change Observed |
|---|---|---|
| 4.81 | 4.43 | sharpened |
| | 3.12 | br t→d |
| | 2.30 | dd→d |
| 4.17 | 2.77 | sharpened |
| | 1.47 | dq→dt |
| 2.77 | 4.17 | sharpened |
| | 3.12 | affected |
| 2.30 | 4.81 | dt→dd |
| | 3.12 | br t→br d |
| 1.83 | 1.47 | dq→br s |
| | 1.34 | dq→br s |
| 1.34 | 3.12 | sharpened |
| | 1.95 | sharpened |
| | 1.83 | affected |

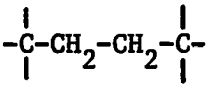
[a]Experiments were carried out at 270 MHz using benzene-$d_6$ as solvent.

methine signal at 4.43 ppm resulted only in a change of the acetoxy deshielded methine indicating that the bromine bearing carbon is also attached to a quaternary carbon. The chlorine deshielded methine signal at 4.17 ppm was found by decoupling to be coupled only with an adjacent methylene group to form partial structure B. Assignment of the specific locations for each halogen atom in 108 was ascertained by analogy with the chemical shifts observed in isoobtusol.



B

The remaining four protons were accounted for in two adjacent methylene groups, as determined by decoupling to form structure C.



C

A measurable W-coupling was observed between the equatorial protons of structure B, resonating at 4.17 and 3.12 ppm, with the equatorial protons of structure C, resonating at 1.47 and 1.34 ppm. Assuming an overlap of the quaternary carbons in both partial structures, structures B and C were combined to form a cyclohexane ring. Since the presence of four quaternary carbons was established from the $^{13}C$-nmr, partial structure A and the geminal dimethyl group were incorporated into a cyclohexane ring which was spiro fused to the first cyclohexane ring. The bromine and methyl group were attached to the remaining quaternary carbon to define the structure for isoobtusol acetate. Although isoobtusol acetate was not heretofore reported as a natural product, it

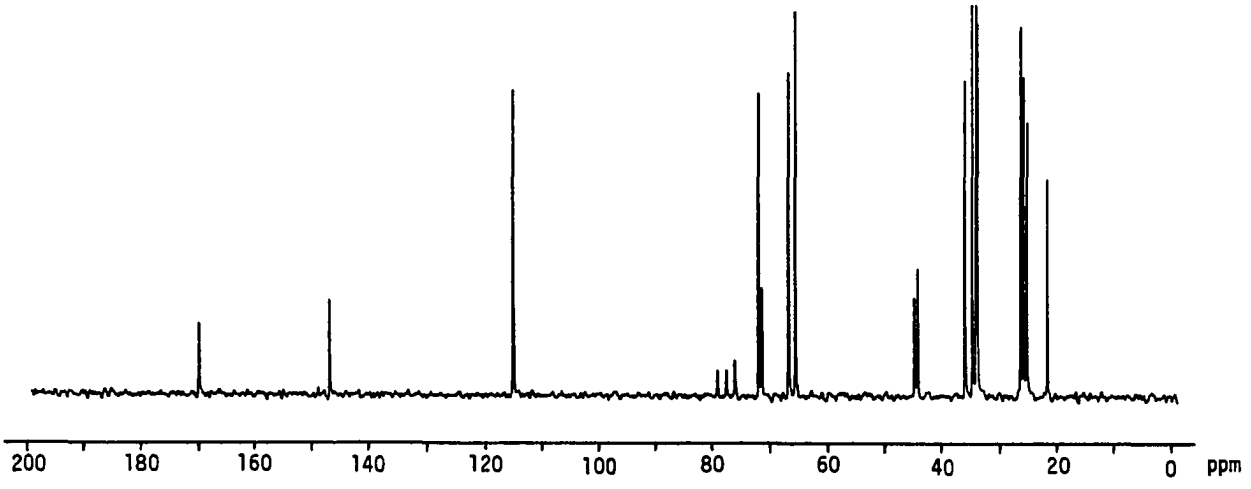
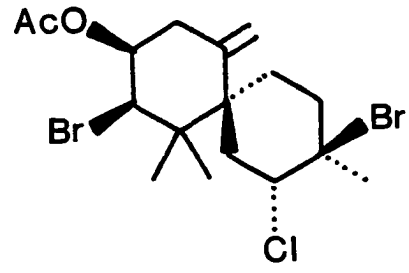



Figure 15. 22.50 MHz $^{13}C$-NMR ($CDCl_3$) Spectrum of Isoobtusol Acetate (108).

was synthetically available.[68] Natural isoobtusol acetate was identical in spectral, physical properties, and mixture melting point to synthetic isoobtusol acetate.



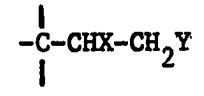

108

Structure elucidation of Parguerol (109)

Although fractions E and F yielded several interesting compounds, attention was also focused on the chloroform extract fraction G (PS 1.1). Sephadex LH-20 and silica gel chromatographies of fraction G led to the isolation of a pale yellow glass, parguerol (109), 185 mg, [α] -40.0 (c 0.03, $CHCl_3$). All attempts to crystallize parguerol failed. The molecular ion was not observed in the high resolution mass spectrum. A peak corresponding to $M^+$-18, however, was observed at 440.13898 which is correct for the formula $C_{22}H_{31}Br^{81}O_4$. Combustion analysis established the formula of parguerol to be $C_{22}H_{33}BrO_5$, indicating six degrees of unsaturation. The infrared spectrum displayed a strong, broad hydroxyl absorption at 3380 $cm^{-1}$ and a carbonyl absorption at 1725 $cm^{-1}$ indicative of acetate. The 270 MHz NMR spectrum in $CDCl_3$ (Figure 16) revealed

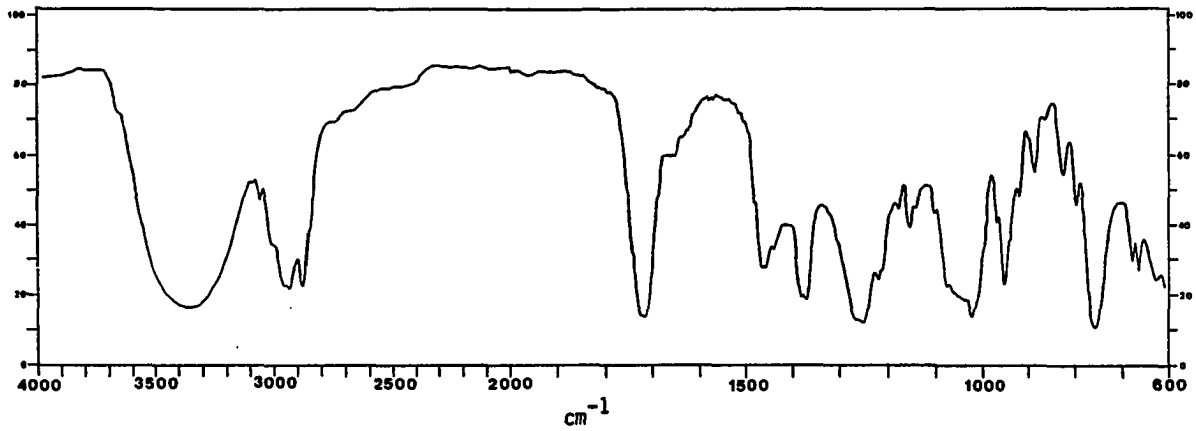
the presence of two quaternary methyl groups at 1.06 and 1.13 ppm and an acetate methyl group at 2.07 ppm. Additionally, two high field multiplets at 0.87 and 0.09 ppm indicated the presence of a cyclopropane ring. The proton decoupled $^{13}C$ magnetic resonance spectrum revealed the presence of one trisubstituted double bond (117.4(d), 142.9(s) ppm). The acetate group was also confirmed by a carbonyl signal at 170.5 ppm. The carbonyl group, double bond, and cyclopropane ring account for three of the six degrees of unsaturation in parguerol. Three additional rings are therefore present in parguerol.

In the 270 MHz nmr spectrum, a number of signals due to protons deshielded by heteroatoms was observed. A one proton multiplet at 3.12 ppm, was assigned to a methine proton of a secondary hydroxyl group. This signal is present at a higher field position than normally expected for a methine proton deshielded by a hydroxyl group. This suggests that the proton likely has an axial orientation in a ring. An isolated AB pattern, confirmed by decoupling, was observed at 3.38 and 3.52 ppm, J=12 Hz, and was assigned to a primary alcohol attached to a quaternary carbon. Three one-proton signals at 3.84, 3.95, and 4.26 ppm were found by decoupling experiments to comprise an isolated ABX spin system, indicative of an ethyl side chain with vicinal substituents. The limiting coupling requires that this substituted ethyl side chain be bonded to a quaternary carbon.

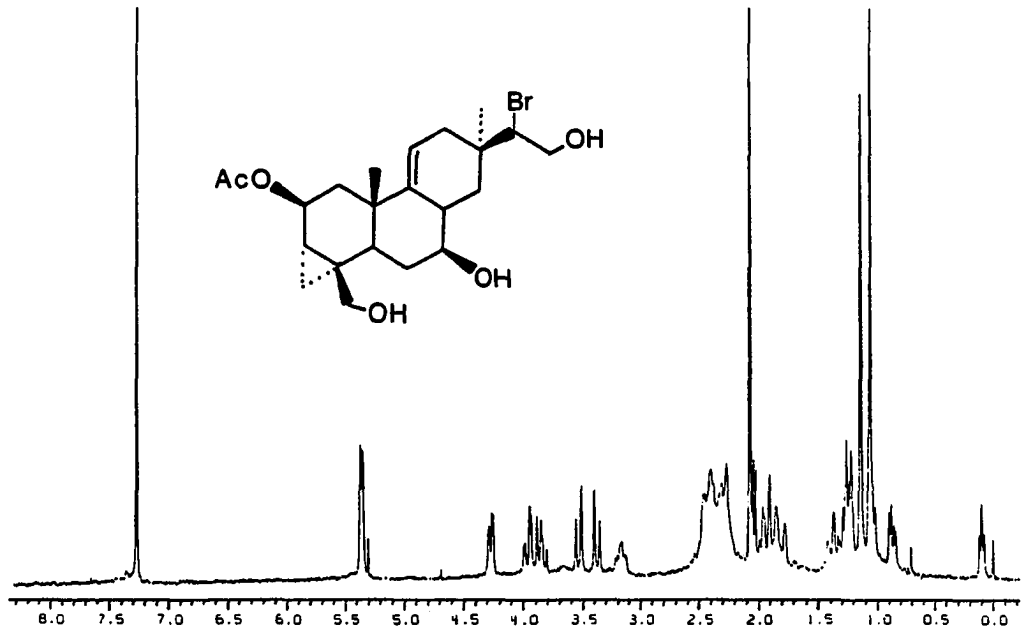


The remaining downfield signal, a two-proton doublet at 5.35 ppm, was due to the overlap of the olefinic hydrogen and a methine proton deshielded by the acetate group. Since this acetate methine appeared

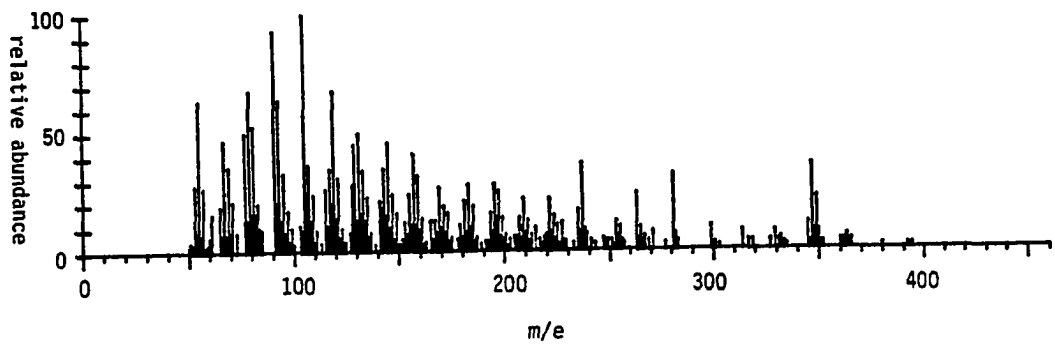
Figure 16. Spectra of Parguerol (109).




IR Spectrum of 109




270 MHz PMR Spectrum of 109 in $CDCl_3$




Mass Spectrum of 109

at lower field than normal, it was initially assumed that the proton was also probably allylic.

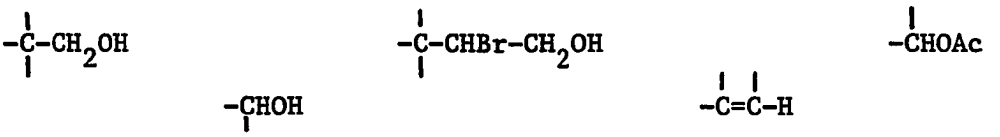
Upon acetylation, parguerol yielded a tetraacetate (110a) indicated by the presence of four acetoxy methyl groups at 2.08, 2.08, 2.10, and 2.14 ppm in the $^1H$ nmr spectrum. The addition of three new acetate groups to parguerol indicates that all five oxygens of the formula have been accounted for, and no ether oxygen or tertiary hydroxyl group is present in parguerol. Acetylation of parguerol (109) caused a downfield shift of signals in the region of 3 to 6 ppm. The signal at 3.12 ppm in parguerol shifted downfield by 1.29 ppm, a change typical of acetylation of a secondary alcohol. The AB pattern originally at 3.38 and 3.52 ppm was shifted to 3.71 and 4.04 ppm suggesting the presence of a primary alcohol attached to a chiral quaternary carbon, see Table 3. The remaining hydroxyl group and the bromine atom must therefore be assigned to X and Y on the ethyl side chain. Two possible orientations can exist, one with the hydroxyl group on the primary carbon and bromine on the secondary. The other orientation has the bromine on the primary carbon and the hydroxyl group on the secondary. In the acetylated product, two of the protons, geminally coupled (J=12 Hz), of the ethyl side chain are shifted to a greater extent than the third suggesting the presence of a primary hydroxyl group and a secondary bromine.

$$-\overset{|}{\underset{|}{C}}-CHOH-CH_2Br \qquad\qquad -\overset{|}{\underset{|}{C}}-CHBr-CH_2OH$$

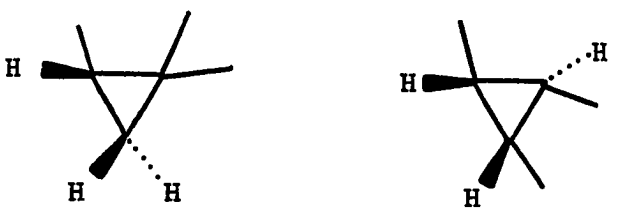
The $-CHBr-CH_2OH$ arrangement was confirmed by a series of reactions originally conceived to test for the presence of an allylic

acetate. Reduction of parguerol with lithium aluminum hydride to the tetraol followed by manganese dioxide oxidation yielded an aldehyde, indicated in the NMR by a doublet at 9.56 ppm. Irradiation of the aldehydic proton doublet collapsed a methine doublet deshielded by bromine at 4.18 ppm. No other changes were observed in the spectrum. The other hydroxyl groups were unaffected by the oxidation which meant that the acetate in parguerol was not allylic.

On the basis of the foregoing evidence, the protons resonating in the region of 3 to 6 ppm of parguerol could be incorporated in the following moieties involving olefinic or heteroatom deshielding.



A key distinctive feature in the NMR spectrum of parguerol is the presence of cyclopropane proton signals. Irradiation of the triplet at 0.17 ppm in parguerol acetate removed the small coupling from the double doublet at 0.93 ppm and caused a sharpening in the region of 1.13 ppm. No changes were observed in the spectrum below 1.13 ppm upon irradiation of any of the cyclopropane proton signals suggesting that the cyclopropane ring was an isolated AMX spin system. The magnitudes of the coupling constants suggested either of the two following arrangements.



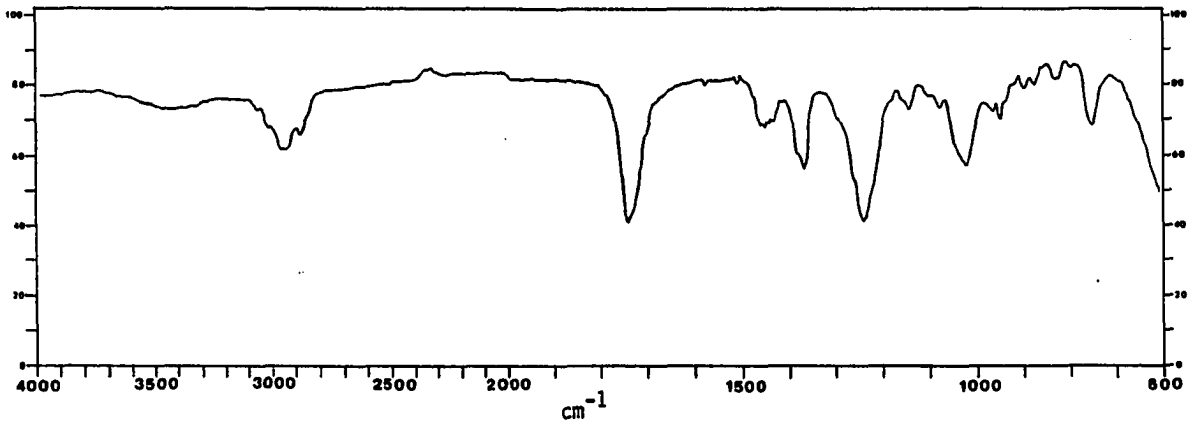
The former structure is considered most likely since it would require only one non-coupling arrangement, such as quaternary carbon attachment or angle-dependent zero coupling. The latter structure is unlikely because it would require three such non-coupling arrangements in order to fit the observations.

Most of the double irradiation experiments were carried out on the spectrum of parguerol acetate, see Figure 17, since the signals were more clearly resolved. Irradiation, in the spectrum of the tetraacetate, of the doublet at 5.36 ppm, which was due to the methine proton deshielded by the original acetate group of parguerol, was carried out to investigate the extent of this spin system. The small coupling constant (J=4 Hz) of this doublet indicated that the neighboring proton was equatorial. Irradiation of the doublet signal produced a change in the region at 1.25 ppm and the latter signal was further coupled to a doublet at 1.94 ppm. No other changes were observed indicating an isolated spin system. This spin system could arise from the following two possible arrangements.

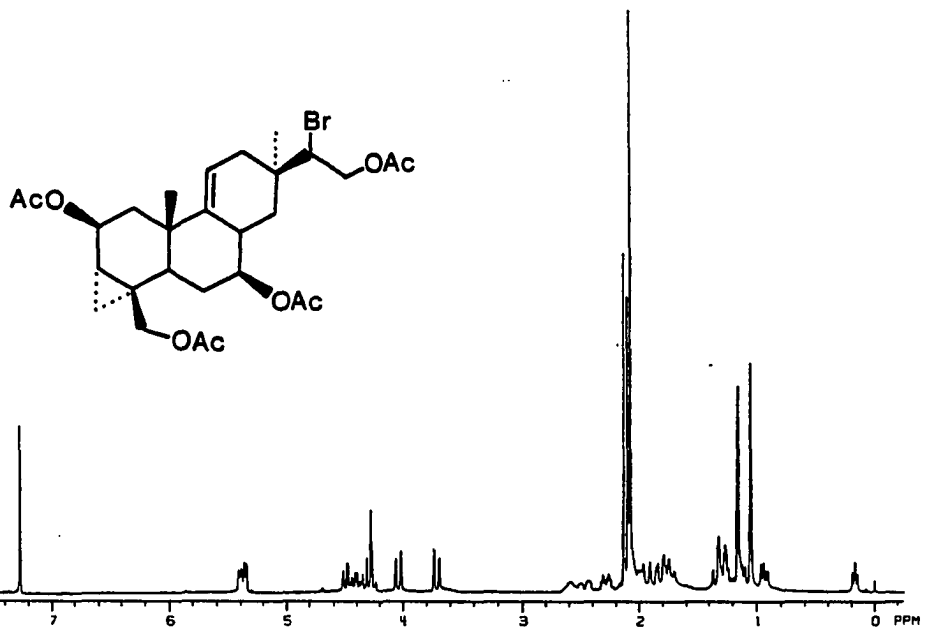
$$-CHOAc-CH_2- \qquad\qquad -CHOAc-\underset{|}{\overset{|}{C}}H-\underset{|}{\overset{|}{C}}H-$$

The former is possible if the assumption is made that one of the vicinal coupling constants is zero. This is possible in certain conformations of a rigid ring structure. The latter structure is unlikely since it would require bonding to four quaternary carbons. The methine proton of the secondary alcohol appears as a double triplet at 4.41 ppm in the tetraacetate spectrum. Irradiation of the double triplet substantially reduces the width at 1/2 height of a broad triplet at 2.59 ppm indicating

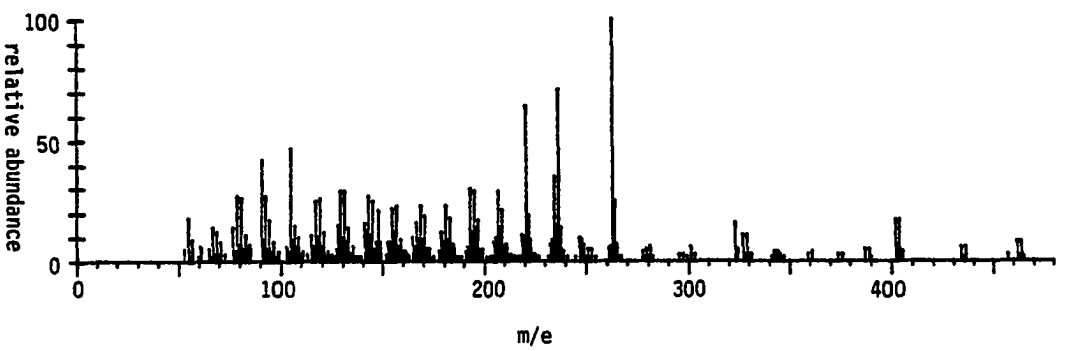
Figure 17. Spectra of Parguerol Acetate (110a).




IR Spectrum of 110a

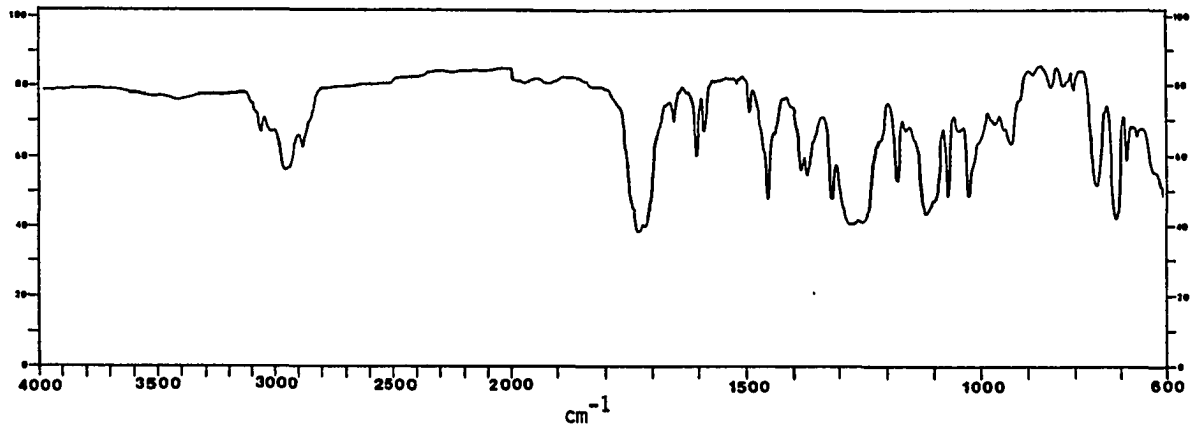



270 MHz PMR Spectrum of 110a in $CDCl_3$

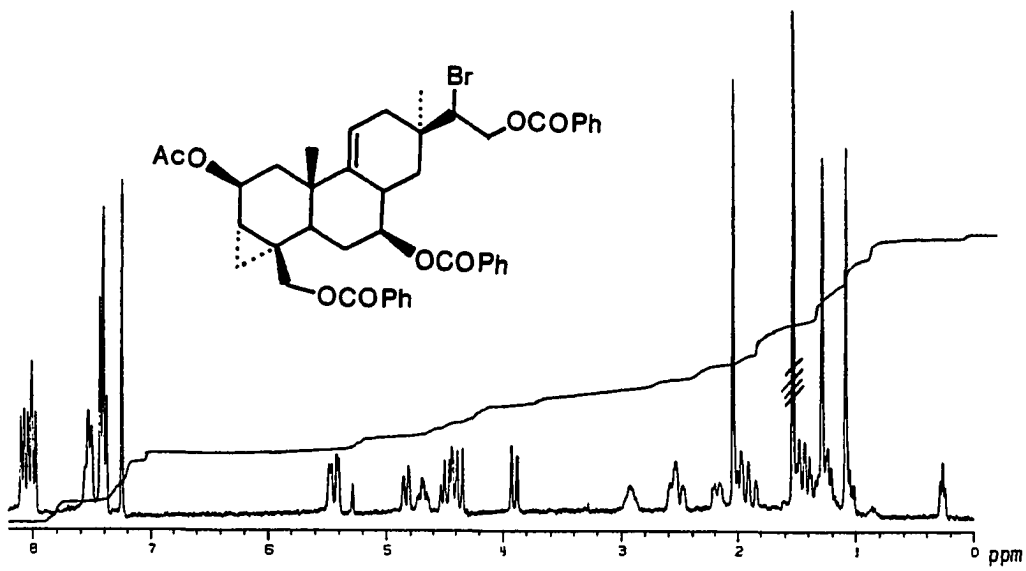



Mass Spectrum of 110a

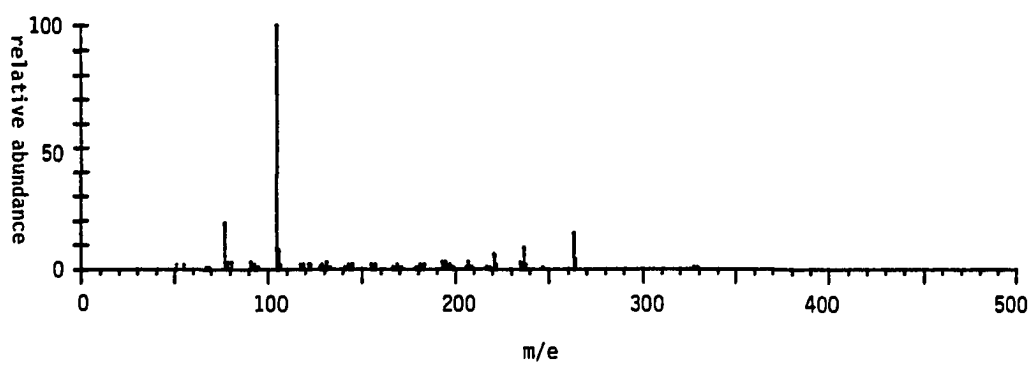
Figure 18. Spectra of Parguerol Tribenzoate (110b)




IR Spectrum of 110b

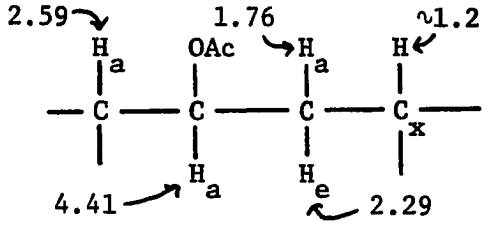



270 MHz PMR Spectrum of 110b in $CDCl_3$




Mass Spectrum of 110b

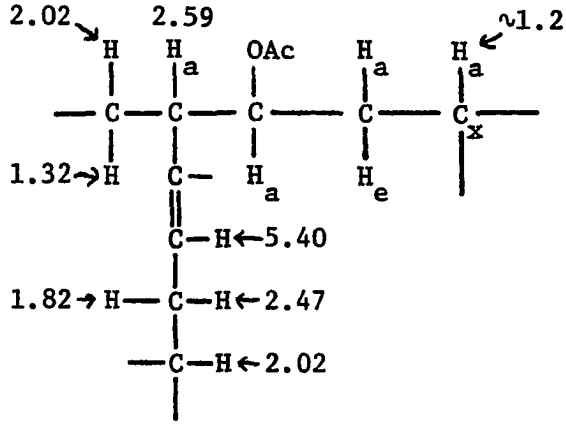
that a large diaxial coupling was removed. Two other changes also occurred. A doubled triplet at 2.29 ppm was reduced to a doubled doublet by removal of a small axial-equatorial coupling and a change occurred at 1.76 ppm. This latter change would have to involve removal of a large diaxial coupling to account for the doubled triplet appearance of the signal at 4.41 ppm. This resulted in the [illegible] spin system:



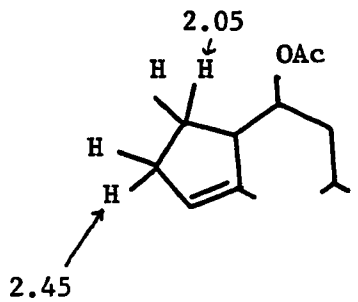

The protons at 2.29 and 1.76 ppm were also geminally coupled to each other (J=12 Hz) and both protons were vicinally coupled to another proton in the region at ∿1.2 ppm. This region contained several overlapping signals, so it was difficult to determine how many protons were attached to $C_x$ in the above structure. The clear doubled triplet multiplicity of the 2.29 ppm signal suggested that there was only one proton on $C_x$.

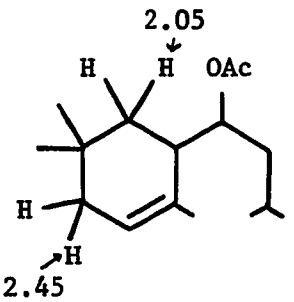
The broad triplet at 2.59 ppm was assumed to be due to an allylic proton from its chemical shift. Irradiation of this signal, in addition to affecting the doubled triplet at 4.41 ppm, produced some changes in the regions of 2.02 and 1.32 ppm indicating that it was coupled to a methylene group. A broad doublet at 2.47 ppm, also assumed to be an allylic proton from its chemical shift, was vicinally coupled to the olefinic proton signal at 5.40 ppm, geminally coupled to a signal at 1.82 ppm, and further coupled to a signal at 2.02 ppm (J=4 Hz). The partial structure above can now be expanded to the

following:




The number and multiplicity of the proton(s) in the region near 2 ppm had to be ascertained. Since this region was obscured by acetate methyl signals, the tribenzoate ester (110b) of parguerol (109) was made, but its nmr spectrum was not particularly helpful. However, the nmr spectrum of parguerol acetate in benzene-$d_6$ proved to be more useful. Solvent-induced shifts had more clearly resolved the signals in the region near 2 ppm. A double double doublet unobscured by any other signals appeared at 2.05 ppm. From decoupling, this proton was shown to be equatorially coupled to a broad triplet at 2.78 ppm (corresponding to the 2.59 ppm signal in $CDCl_3$), geminally coupled to a signal at 1.26 ppm and further coupled to a broad doublet at 2.45 ppm. This last coupling is significant in that it allows the ends of the partial structure above to be connected to form a ring. Two connections are possible, one to form a five-membered ring and the other to form a six-membered ring.



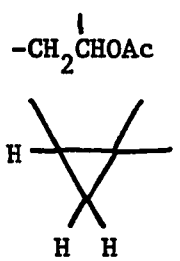

The substructure with the five membered ring can be ruled out because it would require coupling of the signal at 2.05 ppm to four proton signals rather than the three observed. In a six membered ring, a logical mechanism to explain the coupling between the signals at 2.45 and 2.05 ppm is W-coupling between 1,3-diequatorial hydrogens. The W-coupling in this partial structure is abnormally large (J=4 Hz) for a cyclohexane ring, the normal range being about 1 Hz. Large couplings are characteristic of the more highly strained rings.[69]

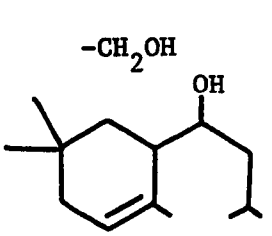
The partial structures inferred thus far from the nmr data can now be summarized as follows.

$-CHBrCH_2OH$

$-CH_3$

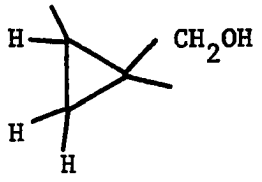
$-CH_3$



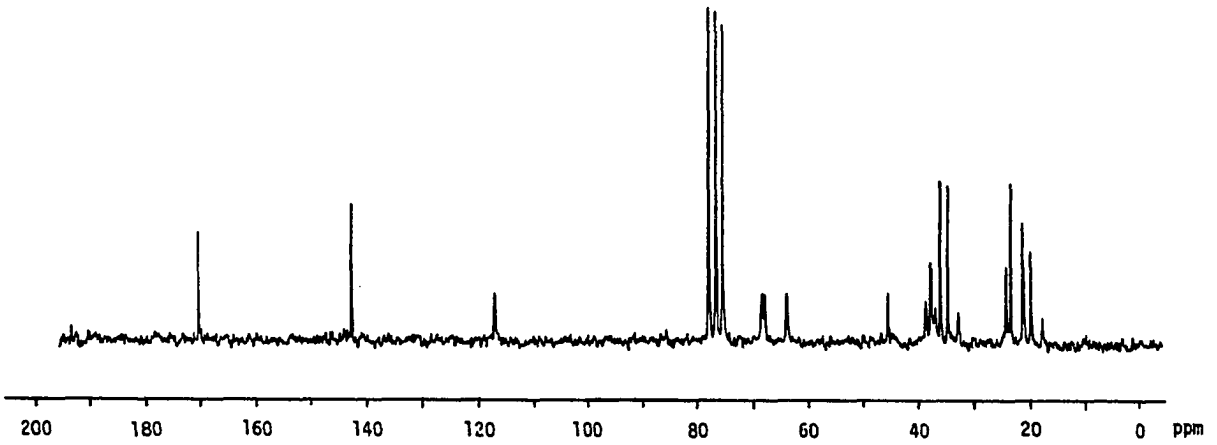
These partial formulas account for all atoms of the molecular formula of parguerol except for one carbon atom which must be quaternary. Further information was necessary to combine these fragments.

Comparison of the chemical shifts of parguerol and parguerol acetate reveal that, in addition to the substantial downfield shifts experienced by the α-protons of the alcohols upon acetylation, all three cyclopropane protons show significant downfield shifts, see Table 3. The only alcohol containing moiety which, when directly bound to the cyclopropane ring, would induce such shifts is the hydroxymethylene group, $-CH_2OH$. On these grounds, the cyclopropane segment could be extended to form,

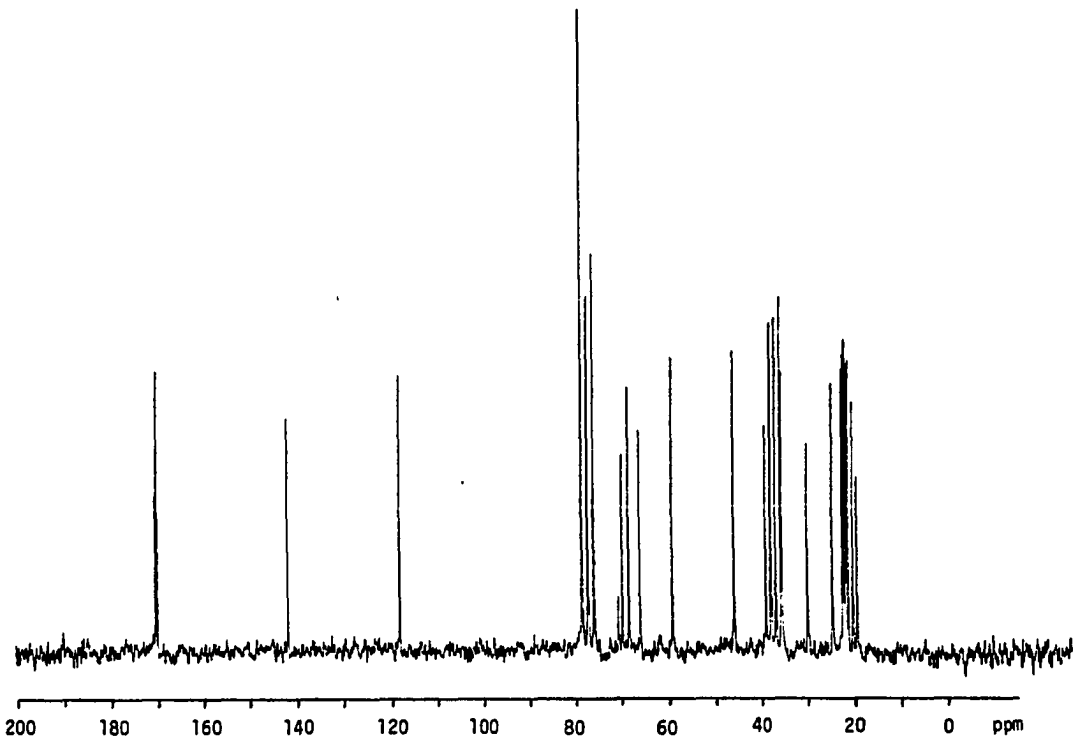



In order to simplify the high field region in the nmr spectrum of parguerol and its acetate, an ozonolysis was carried out. This was accomplished in the hope that it would remove the long range coupling observed in the cyclohexene ring and hopefully shift protons to less congested areas of the nmr spectrum. Ozonolysis of parguerol acetate in methanol-methylene chloride followed by dimethyl sulfide reduction yielded a dimethyl acetal (111). The 270 MHz spectrum of the acetal in benzene-$d_6$ revealed for the first time, all three cyclopropane protons unobscured by other signals. Irradiation of the triplet at -0.36 ppm removed a small coupling from each of the double doublets at 0.49 and 0.98 ppm. Additionally, the singal at 0.98 ppm was slightly

Figure 19. $^{13}C$-NMR ($CDCl_3$) Spectra of 109 and 110a

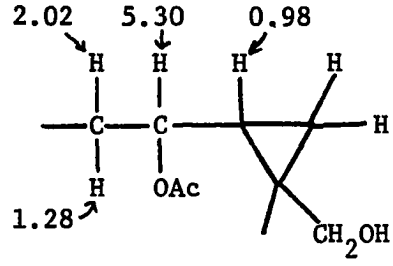



25.05 MHz $^{13}C$-NMR ($CDCl_3$) Spectrum of Parguerol (109).




25.05 MHz $^{13}C$-NMR ($CDCl_3$) Spectrum of Parguerol (110a).

more broadened than the signal at 0.49 ppm indicating a long range coupling. Irradiation of the signal at 2.02 ppm produced a perceptible sharpening of the double doublet at 0.98 ppm. The signal at 2.02 ppm is also geminally coupled (J=16 Hz) to a proton at 1.28 ppm which is in turn coupled (J=5 Hz) to the methine deshielded by acetate at 5.30 ppm. The only arrangement possible to explain these couplings also invokes the use of two zero couplings.




This clearly indicates that this moiety is held in a rigid ring system with the cyclopropane oriented axially and the protons at 2.02 and 0.98 ppm held equatorially. Anisotropy of the cyclopropane ring provides an explanation for the abnormally low field position of the methine deshielded by acetate.

The NMR spectrum of the acetal also displayed a double doublet at 0.88 ppm. This was shown to be coupled (J=3.4 Hz) to the double triplet at 2.40 ppm and diaxially coupled (J=13.9 Hz) to a signal at 1.98 ppm, both of which were further coupled to a methine proton deshielded by an acetate group, indicating the following partial structure, $-\overset{|}{C}H-CH_2-\overset{|}{C}H(OAc)$. This reinforces the assumption made earlier in the discussion of the cyclohexene-containing substructure that the secondary alcohol of parguerol was coupled to a methylene group which

Table 3

270 MHz Chemical Shifts for Parguerol and Related Compounds

| | 109 a | 110a a | 110a c | 110a b | 110b a | 111 a | 111 b | 118 a | 119 a | 120 a | 121 a | 122 a | 122 b | 123 a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1ax | | | | 0.88 | 1.35 | 1.42 | 1.28 | | 1.21 | 1.26 | 1.65 | 1.88 | 1.29 | |
| 1eq | | 1.94 | 1.89 | 1.69 | 2.00 | 1.96 | 2.02 | 1.94 | 1.89 | 1.91 | | 1.68 | 1.82 | |
| 2 | 5.35 | 5.36 | 5.27 | 5.34 | 5.40 | 5.32 | 5.30 | 5.36 | 5.29 | 5.28 | 4.96 | 4.95 | 4.88 | 4.97 |
| 3 | 1.04 | 1.13 | 1.08 | 1.01 | | 1.16 | 0.98 | 1.04 | 0.79 | 0.78 | | 2.48 | 2.11 | 2.54 |
| 5 | | | | 0.97 | | | 0.88 | | 1.06 | 1.14 | | | | |
| 6ax | | 1.76 | 1.74 | 1.86 | 1.97 | | 1.98 | | 1.68 | 1.70 | | 1.85 | 1.88 | 1.78 |
| 6eq | | 2.29 | 2.25 | 2.36 | 2.57 | 2.48 | 2.40 | | 2.12 | 2.16 | | | | 2.38 |
| 7 | 3.12 | 4.41 | 4.31 | 4.58 | 4.69 | 4.42 | 4.56 | 3.15 | 3.16 | 4.38 | 3.29 | 4.50 | 4.58 | 3.27 |
| 8 | | 2.59 | 2.62 | 2.78 | 2.92 | 3.07 | 3.12 | | 2.22 | 2.56 | | 2.60 | 2.75 | 2.45 |
| 11 | 5.35 | 5.40 | 5.38 | 5.12 | 5.46 | 4.54 | 4.57 | 5.35 | 5.34 | 5.36 | 5.44 | 5.48 | 5.15 | 5.44 |
| 12ax | | 1.82 | 1.78 | 1.60 | 1.86 | 1.75 | 1.92 | | 1.79 | 1.82 | | 1.82 | 1.62 | |
| 12eq | | 2.47 | 2.45 | 2.45 | 2.51 | 1.75 | 1.92 | 2.45 | 2.40 | 2.43 | 2.45 | 2.48 | 2.45 | |
| 14ax | | 1.32 | | 1.26 | 1.43 | 1.21 | 1.45 | 1.38 | 1.35 | 1.33 | | 1.35 | 1.25 | |
| 14eq | | 2.02 | 1.97 | 2.05 | 2.18 | 2.52 | 2.74 | 2.34 | 2.25 | 2.02 | | | 2.05 | |
| 15 | 4.26 | 4.49 | 4.47 | 4.21 | 4.42 | 4.24 | 4.42 | 4.60 | 4.27 | 4.53 | 4.29 | 4.49 | 4.24 | 4.28 |
| 16α | 3.84 | 4.27 | 4.13 | 4.52 | 4.49 | 4.42 | 4.67 | 4.23 | 3.83 | 4.28 | 3.84 | 4.25 | 4.50 | 4.60 |
| 16β | 3.95 | 4.31 | 4.19 | 4.52 | 4.83 | 4.57 | 4.77 | 4.26 | 3.90 | 4.28 | 3.94 | 4.30 | 4.50 | 4.28 |
| 17 | 1.06 | 1.05 | 1.06 | 0.91 | 1.08 | 0.96 | 1.09 | 1.06 | 1.04 | 1.02 | 1.08 | 1.05 | 0.88 | 1.09 |
| 18endo | 0.09 | 0.17 | 0.17 | -0.25 | 0.27 | 0.20 | -0.36 | 0.08 | 0.01 | 0.01 | | | 0.72 | |
| 18exo | 0.87 | 0.93 | 0.94 | 0.56 | 1.04 | 0.98 | 0.49 | 0.87 | 0.66 | 0.65 | | | 1.35 | |
| 19α | 3.38 | 3.71 | 3.74 | 3.56 | 3.90 | 3.72 | 3.56 | 3.42 | 1.02 | 1.00 | | | 1.10 | |
| 19β | 3.52 | 4.04 | 3.96 | 3.85 | 4.36 | 4.10 | 3.76 | 3.48 | 1.02 | 1.00 | | | 1.41 | |
| 20 | 1.13 | 1.16 | 1.12 | 1.20 | 1.28 | 1.38 | 1.26 | 1.13 | 1.12 | 1.12 | 1.21 | 1.21 | 1.26 | 1.22 |
| OAc | 2.07 | 2.08 | 2.02 | 1.64 | 2.04 | 2.06 | 1.61 | 2.07 | 2.05 | 2.08 | 2.03 | 2.02 | 1.61 | 2.03 |
| OAc | | 2.08 | 2.03 | 1.71 | | 2.09 | 1.65 | 2.11 | | 2.10 | | 2.05 | 1.66 | 2.11 |
| OAc | | 2.10 | 2.06 | 1.73 | | 2.11 | 1.74 | | | 2.14 | | 2.11 | 1.69 | |
| OAc | | 2.14 | 2.08 | 1.77 | | 2.13 | 1.84 | | | | | | | |

[a] $CDCl_3$; [b] $C_6D_6$; [c] $CCl_4$

Table 4

Induced Shifts of Proton NMR Signals for 110a

| Proton | $CCl_4$ | $Eu(fod)_3$ (.32:1) | $Eu(fod)_3$ (.75:1) |
|---|---|---|---|
| 1ax | | 1.95 | |
| 1eq | 1.89 | 3.12 | 4.79 |
| 2 | 5.27 | 7.33 | 10.14 |
| 3 | 1.08 | 2.50 | 4.32 |
| 5 | | 1.95 | 2.96 |
| 6ax | 1.74 | 2.95 | 4.62 |
| 6eq | 2.25 | 3.62 | 5.55 |
| 7 | 4.31 | 5.74 | 7.88 |
| 8 | 2.62 | | |
| 11 | 5.38 | 5.74 | 6.22 |
| 12ax | 1.78 | | 2.13 |
| 12eq | 2.45 | | 2.81 |
| 14ax | | | 3.93 |
| 14eq | 1.97 | | 2.90 |
| 15 | 4.47 | 5.17 | 6.29 |
| 16α | 4.13 | 4.67 | 5.40 |
| 16β | 4.19 | 4.78 | 5.55 |
| 17 | 1.06 | 1.21 | 1.40 |
| 18endo | 0.17 | 0.62 | 1.20 |
| 18exo | 0.94 | 1.50 | 2.14 |
| 19α | 3.74 | 5.17 | 7.04 |
| 19β | 3.96 | 5.40 | 7.32 |
| 20 | 1.12 | 2.09 | 3.39 |
| OAc | 2.02 | 2.69 | 3.62 |
| OAc | 2.03 | 3.21 | 4.90 |
| OAc | 2.06 | 3.36 | 5.05 |
| OAc | 2.08 | 3.56 | 5.62 |

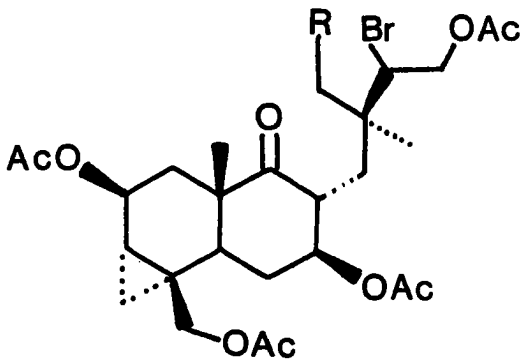
Table 5

270 MHz Coupling Constants for Parguerol and Related Compounds

| | 109 | 110a | 110a[1] | 110b | 119 | 120 | 118 | 121 | 111[1] |
|---|---|---|---|---|---|---|---|---|---|
| 1ax | | | | | 6,15 | | | | |
| 1eq | | | | | 15 | 15 | | | |
| 2 | | 5 | 5 | 5 | 6 | 5 | 6 | 5 | 5 |
| 3 | | | | | 6,11 | 6,10 | | | 7,11 |
| 5 | | | | | | | | | 3,14 |
| 6ax | | 10,11,13 | 12,12,12 | | | 12,12,12 | | | |
| 6eq | | 4,4,12 | 4,4,13 | | | 4,4,13 | | | 3,5,13 |
| 7 | 5,10,10 | 5,11,11 | | 4,11,11 | 5,10,10 | 5,11,11 | 5,10,10 | 6,12,12 | |
| 8 | | 10,10 | | | | 11,11 | | | |
| 11 | 5 | 2,2.5,6 | 6.6 | 6 | 6 | 6.7 | 6.5 | 6 | |
| 12ax | | 3,4,18 | | | | 2,4,18 | | | |
| 12eq | | 18 | | | 18 | 7,18 | | | |
| 14ax | | | 11,14 | | | | | | 14 |
| 14eq | | | 4,6,14 | 15 | | 4,6,15 | | | 8,14 |
| 15 | 3,9 | 9.5 | | | 4,9 | | | 4,10 | 4,8 |
| 16α | 3,12 | | | 3,12 | | | | 12 | 4,12 |
| 16β | 9,12 | | | | | | | 9,11 | 8,12 |
| 17 | | | | | | | | | |
| 18endo | 5.4 | 5.5 | 6 | 6 | 5.6 | 5 | 6 | | 6 |
| 18exo | 5,10 | 5,10 | 5,11 | 5,11 | 5,11 | 4,10 | 5,11 | | 5,11 |
| 19α | 12 | 12 | 12 | 12 | | | 13 | | 12 |
| 19β | 12 | 12 | 12 | 12 | | | 13 | | 12 |
| 20 | | | | | | | | | |

[1]Determined in benzene-$d_6$

was in turn coupled to only one other proton. The acetal (111) was hydrolyzed and oxidized with Jones reagent to the acid (112) in order to remove the methoxy groups whose signals obscured the signal at 3.12 ppm (corresponding to the signal at 2.59 ppm in $CDCl_3$ for 110a).

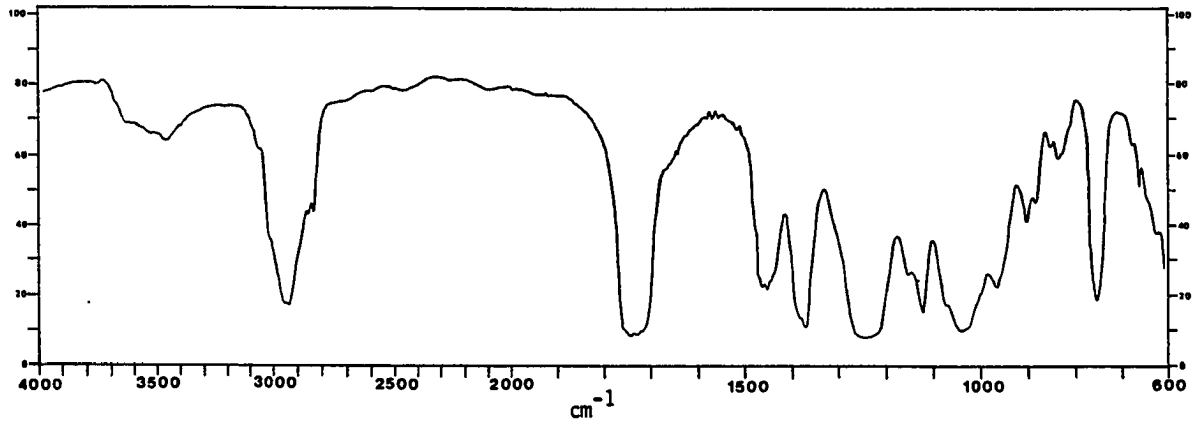



111 R = $-CH(OMe)_2$

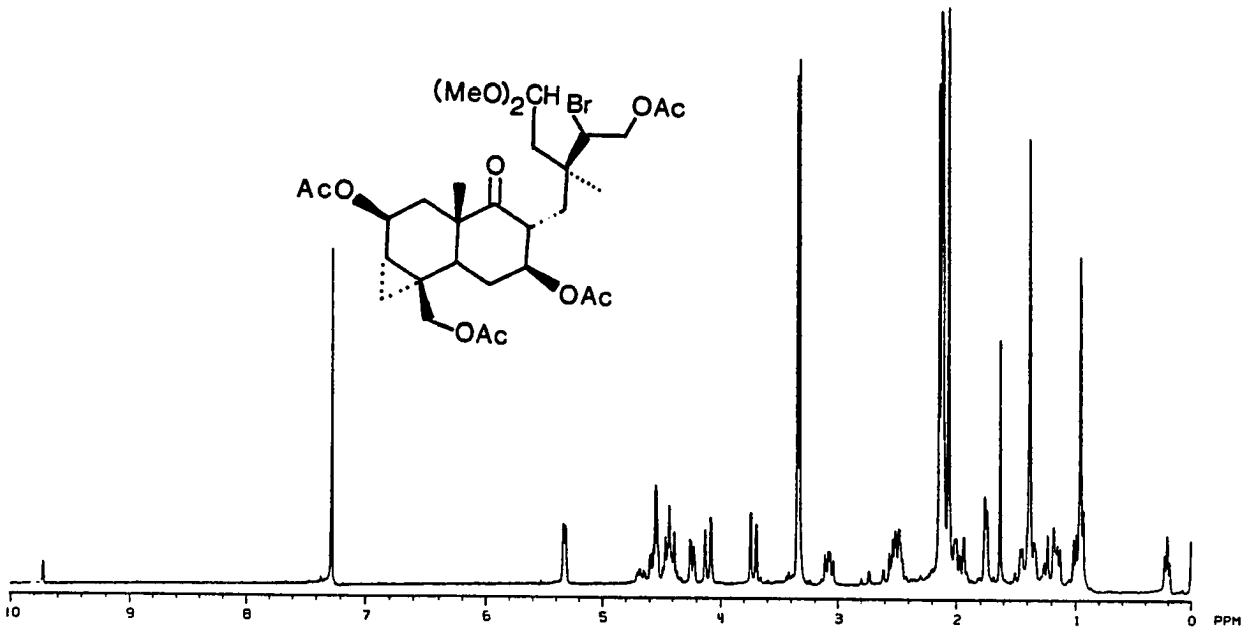
112 R = $-CO_2H$

Reduction of parguerol with zinc-acetic acid yielded a mixture of products, one of which contained an isolated vinyl group. This is conceivably formed from the 1,2-elimination of hydroxyl and bromine from the substituted ethyl side chain. The NMR spectrum of the reduction product contained, among other signals, a one proton double doublet at 5.73 ppm and two one proton overlapping doublets centered at 4.89 ppm which form an ABX system. Irradiation of the signal at 5.73 ppm, in addition to collapsing the doublets at 4.89 ppm, also sharpened slightly a methyl group signal at 1.05 ppm. This sharpening, due to W-coupling or a NOE effect, established the

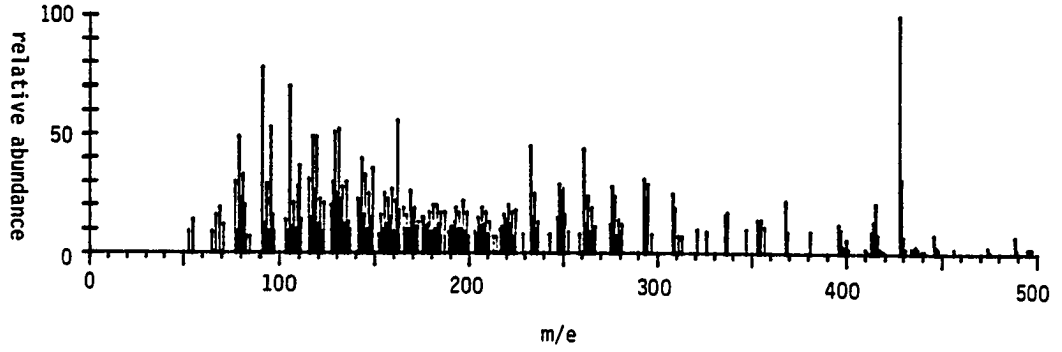
Figure 20. Spectra of Acetal 111.




IR Spectrum of 111




270 MHz PMR Spectrum of 111 in $CDCl_3$




Mass Spectrum of 111

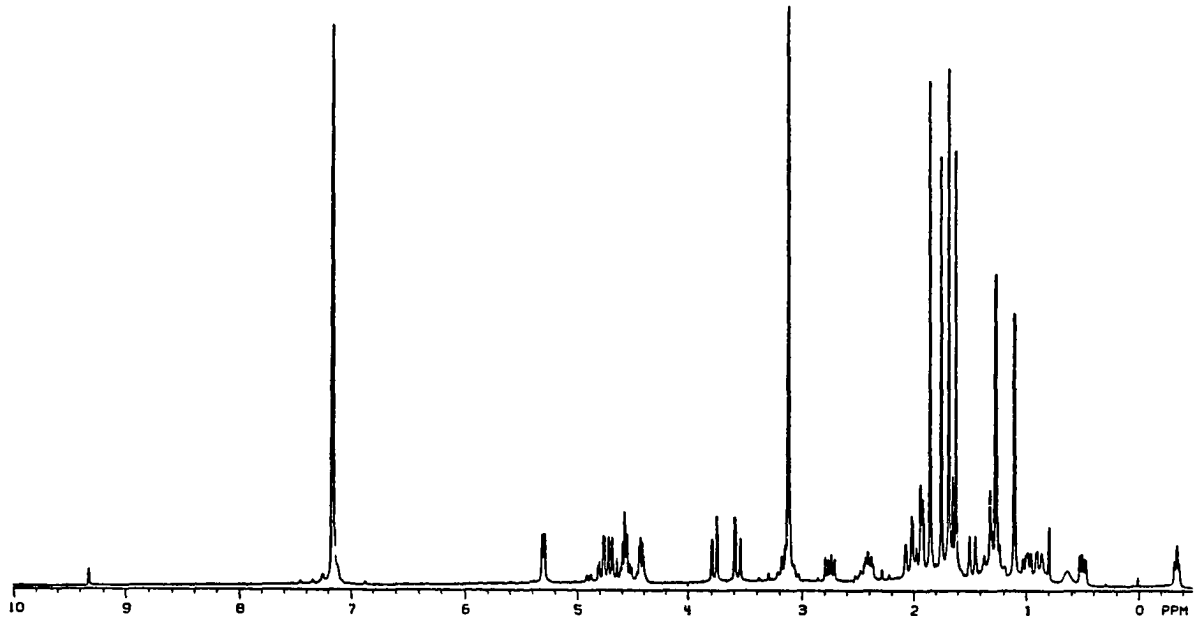
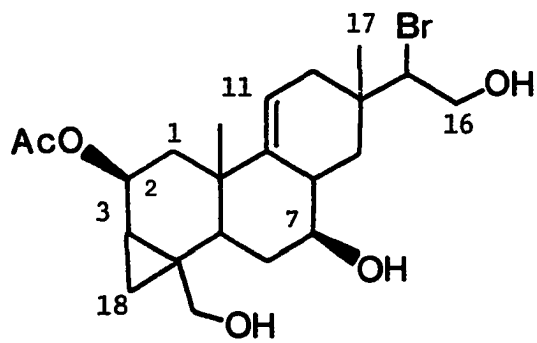



Figure 21. 270 MHz NMR Spectrum of 111 in Benzene-$d_6$

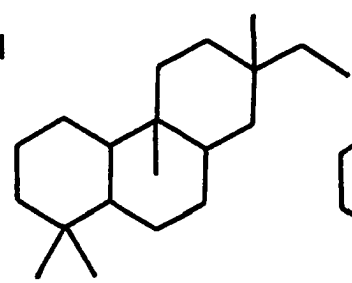
proximity of a methyl group to the ethyl side chain, forming the expanded partial structure:



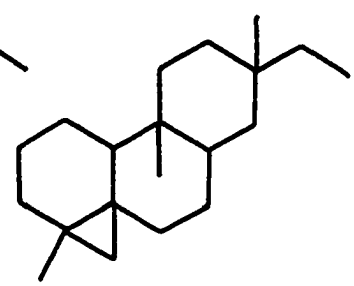
Assembly of the partial structures to form the completed diterpene structure was aided by reference to known[70] diterpene skeletons. The presence of a geminal methyl ethyl moiety in parguerol quickly narrowed the possible choices to the pimarane skeleton. The pimarane skeleton is consistent with all of the data if it is assumed that one of the gem-dimethyls is joined to C-3 to form a cyclopropane ring. Several features are at once noticeable in the structure of parguerol with respect to other pimaranes. First, the cyclopropane ring is a novel feature in pimaranes. Only a few examples of gem-dimethyl involvement in ring closure exist in diterpenes, and they are in the rosane skeleton (113)[71] to form the devadarenes (114).[72] Another feature is the uncommon substitution at C-2 and C-7. Oxygen functionality is most often seen at C-3. The double bond at position 9(11),[73] is also rare, the usual positions being at 7, 8(14), or 15.




109



113



114

Molecular models of parguerol demonstrate very well that if the cyclopropane is α-oriented, there is a 90° relationship between $H_{1eq}$ and $H_2$ and also between $H_2$ and $H_3$. This accounts for the lack of coupling between these respective protons. Also $H_{1eq}$ and $H_3$ are ideally suited for the W-coupling interaction which would explain the broadened appearance of $H_3$.

Recently, several new techniques in data manipulation have emerged in Fourier transform (FT) NMR. Many of these techniques involve computer alteration of the free induction decay (FID). One such manipulation that has been used frequently in the NMR studies of proteins and other macromolecules is sine bell resolution enhancement (SBRE).[74] To effect SBRE, a normal FID is acquired which is then filtered with a sinusoidal window function available in standard FT-NMR software. This procedure zeroes out the beginning of the FID and alters the lineshape of the signals. The broad components of the spectrum are removed by this technique while the sharper lines are emphasized.

The SBRE technique has been applied to the 360 MHz spectrum of parguerol acetate. Even at this high field strength, the olefinic proton consistently appeared in the normal FT spectrum only as a broadened doublet, although it is coupled vicinally to two protons and allylically to one proton. SBRE at 360 MHz revealed that the olefinic signal at 5.40 ppm was indeed a double triplet with one large coupling constant and two small coupling constants (J=6,2,2 Hz). Irradiation of the broad triplet at C-8 (2.59 ppm) collapsed the double triplet to a double doublet by removing a small allylic coupling. Likewise, irradiation of the signal at 1.82 ppm ($H_{12ax}$) removed a

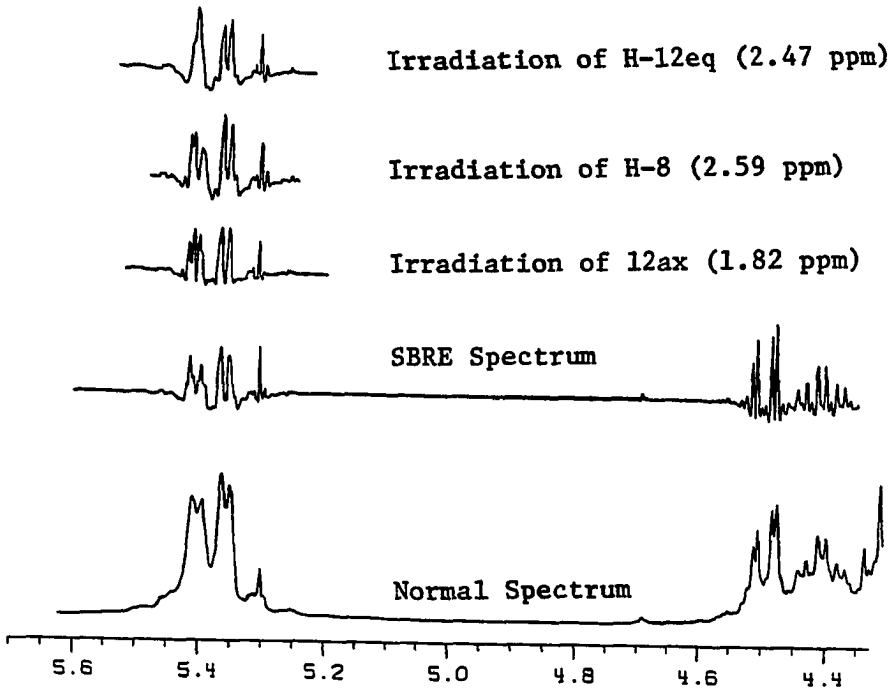



Figure 22. SBRE of parguerol acetate (110a) at 360 MHz.

small coupling. The same irradiations without SBRE only very slightly sharpened the olefinic doublet and neither of the small coupling constants could be measured. Using SBRE, the olefinic signal is reduced to a broad triplet by irradiation of the $H_{12eq}$ proton (2.47 ppm). See figure 22. In addition, a homoallylic coupling (J=2 Hz) was detected between $H_8$ and $H_{12ax}$.

Another recently introduced technique which has proven useful is difference spectroscopy. This has been applied to spin decoupling and nuclear Overhauser enhancement (NOE) experiments. Difference double resonance spectroscopy (DDR)[75,76] is accomplished by the acquisition of two spectra, one with the decoupling frequency set at the proton signal to be decoupled and another with the decoupling frequency set in a blank region of the spectrum (e.g., 8 ppm) as a control. The spectra are then digitally subtracted. Those signals which experience no change by irradiation of the proton in question are nulled while those experiencing a change remain. The pattern of the changed signal is generally centrosymmetric with the unperturbed signal appearing on one side of the baseline and the perturbed signal appearing on the other side. See Figure 23. This technique is extremely useful in resolving signals hidden in a complex absorption envelope containing several proton resonances.

An example of DDR is shown in figure 23. Upon irradiation of the double triplet arising from H-7 (4.58 ppm), the resonances of the allylic axial proton at C-8 and of the methylene group at C-6 are the only signals affected. All other signals have been removed. The signals before decoupling appear above the baseline while the

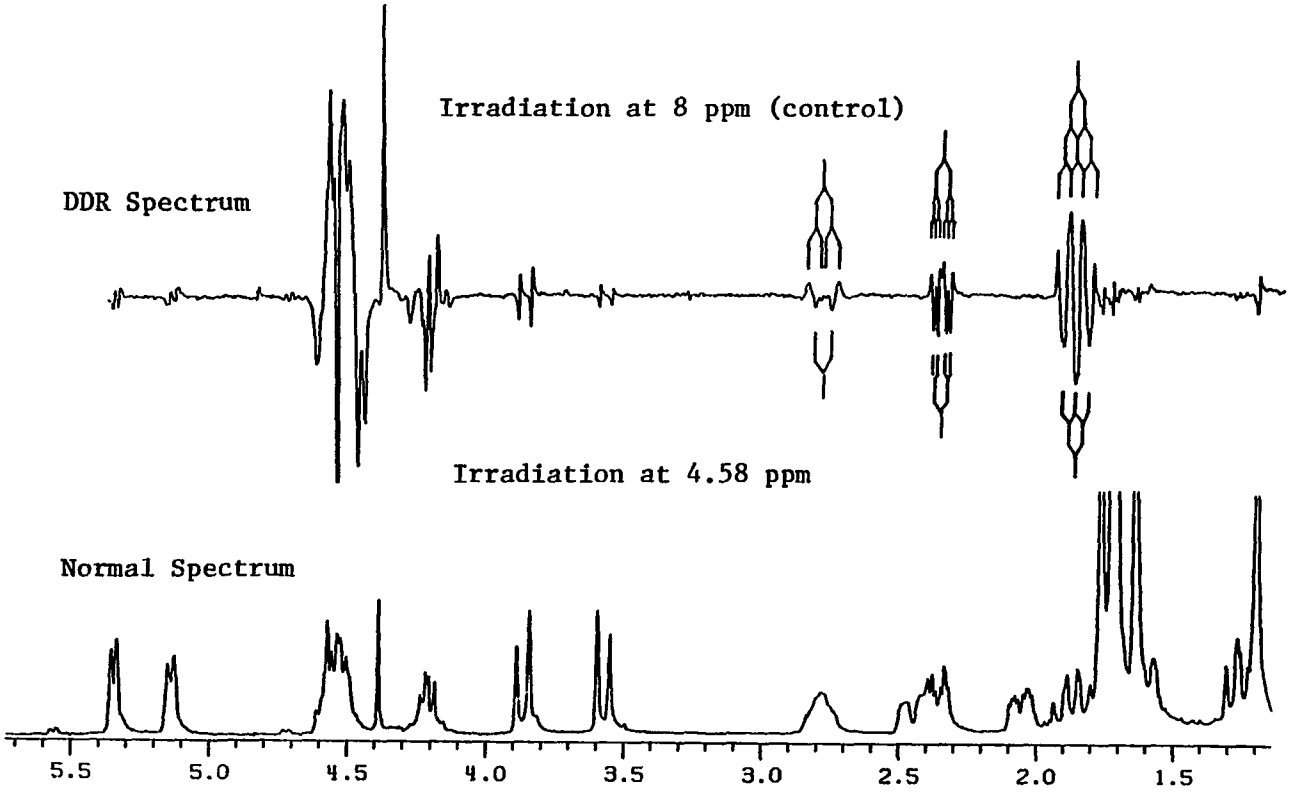



Figure 23. DDR Spectrum of Parguerol acetate (110a) in benzene-$d_6$.

signals after decoupling appear below. Note that signals of protons having chemical shifts close to that of the proton being irradiated appear even though no coupling exists between them. This is due to the Bloch-Siegert shift.[77] The signals on either side of the irradiation point will be shifted away from that point and, when DDR is employed, the peaks do not match and are not cancelled. This effect drops off with increasing chemical shift difference and is dependent on the power of the irradiation frequency.

Difference spectroscopy may also be used to determine NOE effects.[75,78] In a typical Nuclear Overhauser Enhancement Difference Spectroscopy (NOEDS) experiment, two spectra are acquired alternately with and without gated decoupling to reduce effects due to small drifts in the magnetic field. Gated decoupling eliminates problems associated with spin decoupling or Bloch-Siegert shifts.

As in DDR, all signals are nulled except those experiencing a NOE effect. In the structure elucidation of parguerol, NOEDS was of great value in determining the stereochemistry about C-13 since both pimaranes and isopimaranes, which differ in their configuration about C-13, are well known.[70] Irradiation of the axial proton at C-8 would resolve the question, since it can enter into a 1,3-diaxial relationship with one or both of the methyl groups in parguerol depending on the C-13 stereochemistry. When the C-8 proton was irradiated, only one of the methyl groups experienced a NOE effect. The proton at C-15 also experienced an effect since it too is 1,3-diaxial with the proton at C-8. Thus, the configuration at C-13 in paraguerol is like that for the pimarane skeleton, and parguerol can

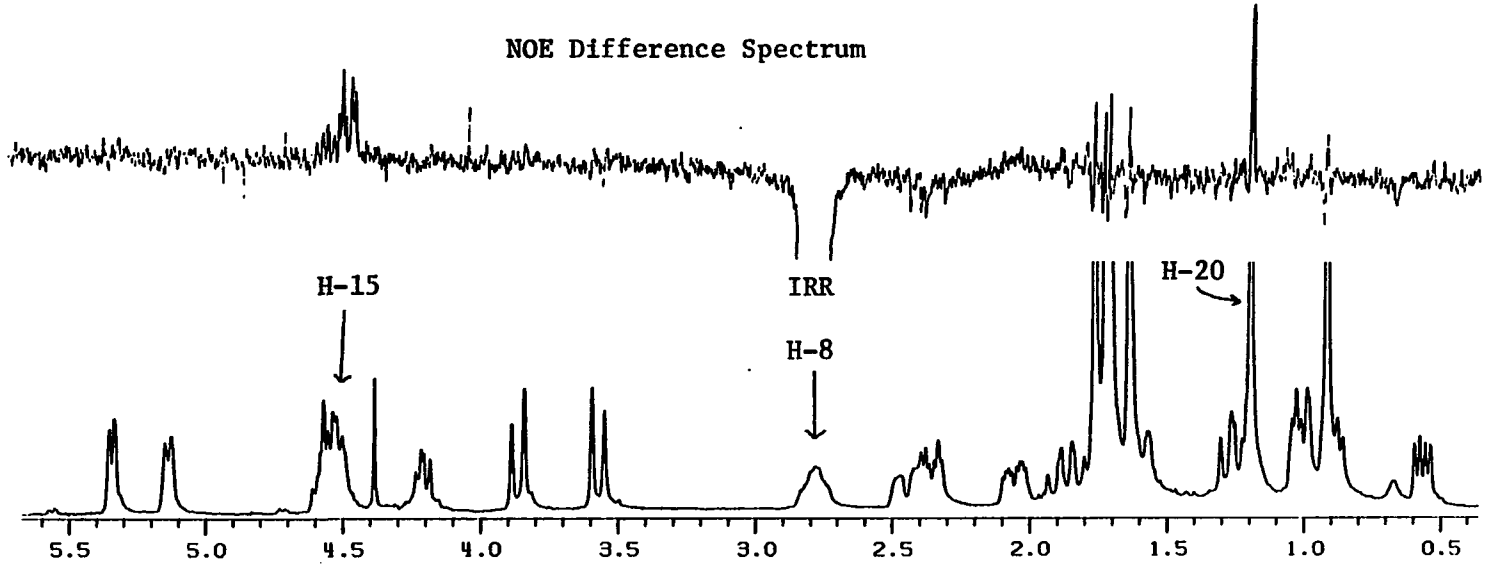
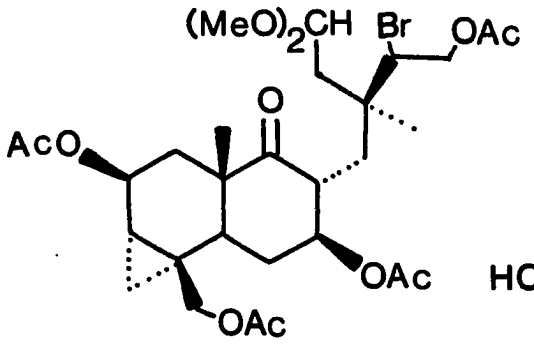


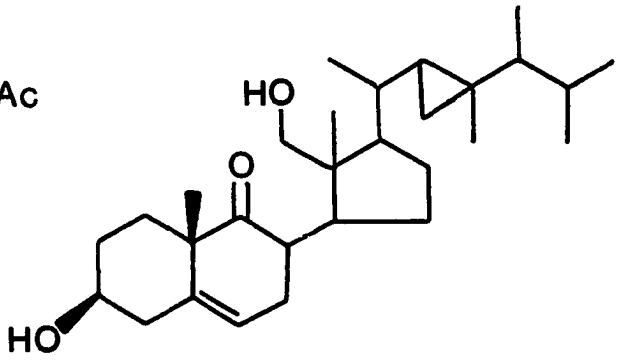

Figure 24. NOE Difference Spectrum of Parguerol Acetate (110a).

be classified as a 3α,18-cyclo-2β-acetoxy-7β,16,19-trihydroxy-15-bromopimar-9(11)-ene.

In order to determine the absolute configuration of parguerol, the circular dichroism (CD) curve of the acetal (111) was obtained. Secogorgosterol (115)[79] was selected as a model for comparison. Since the acetal displayed a positive Cotton effect (+6374°) while the diacetate of secogorgosterol displayed a negative Cotton effect (-10,000°), it was tentatively concluded that parguerol had the absolute configuration opposite to that illustrated in this work. It should be noted that this is a very tentative assignment because there are significant structural differences in the decalin portions of the two compounds.



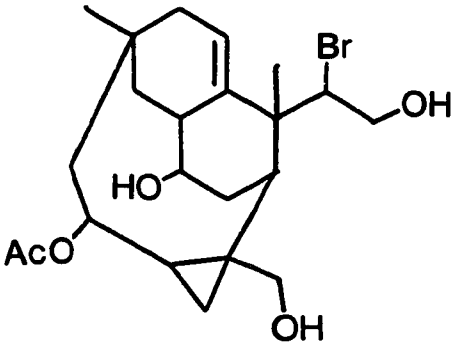

111



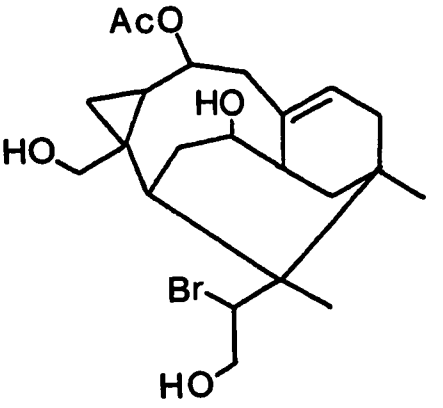

115

The structural fragments deduced from the chemical and spectral analysis of parguerol and its derivatives were processed in the CONGEN computer program. CONGEN is a program for computer-assisted structure elucidation developed by the DENDRAL group at Stanford University.[80] In addition to 109, two other structures 116, 117 (stereoisomers not considered) were generated by the program. Structure 109, however,

is the only which is chemically sensible.




116

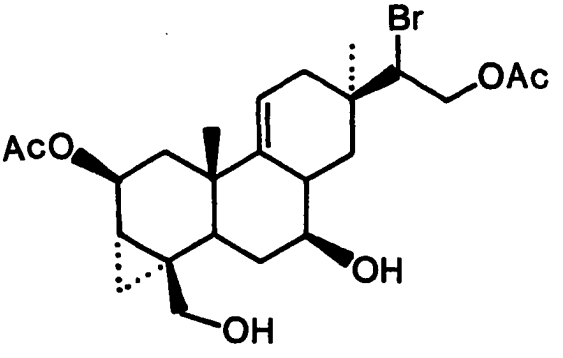



117

Molecular models of 116 and 117 were made to determine if their geometries were consistent with the spectral analysis. In structure 116, severe steric interactions were encountered between the double bond and the methine deshielded by an acetate group. In addition, this proton forms a 120° angle with each proton on the adjacent methylene group. Since the multiplicity of the methine proton would be more complex than actually observed, structure 116 can be ruled out. Structure 117 also encounters a similar constraint. The acetoxy-deshielded methine proton in 117 forms a 180° angle with a cyclopropane proton. Clearly, a significant coupling between these two protons would have been observed. From the model, the proton in 117, corresponding to H-8 in structure 109, must be equatorial. Irradiation of this proton could not produce the observed NOE effect on both the methyl group and the -CHBr- group if structure 117 were considered.

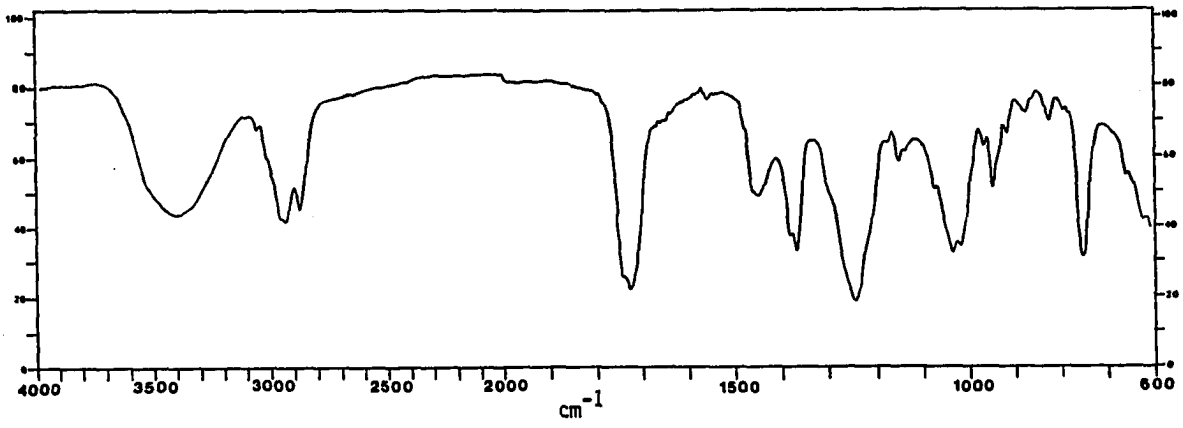
Characterization of Parguerol 16-acetate (118)

Further Sephadex LH-20 and silica gel chromatographies of fraction G followed by purification by HPLC led to the isolation, in trace quantities, of an oil which crystallized as white needles, mp. 121-123°, $[\alpha]_D$ -40.5 (c. 0.40, $CHCl_3$). A doublet in the low resolution mass spectrum at m/e 480, 482 was consistent with the formula $C_{24}H_{33}BrO_5$. Hydroxyl and acetate functionalities were clearly indicated in the infrared spectrum by absorptions at 3400, 1742, and 1726 $cm^{-1}$. The proton nmr spectrum was nearly identical to that of parguerol (109) except for the presence of an additional acetate methyl group at 2.11 ppm and a downfield shift of the protons on C-16 to 4.23 and 4.26 ppm. It was concluded that this compound was parguerol 16-acetate, $C_{24}H_{35}BrO_6$, and that the mass ions observed at m/e 480, 482 represent $M^+-H_2O$. Acetylation of parguerol 16-acetate produced a compound whose nmr was superimposable with that of parguerol peracetate (110) thus confirming the structure and stereochemistry of parguerol 16-acetate.

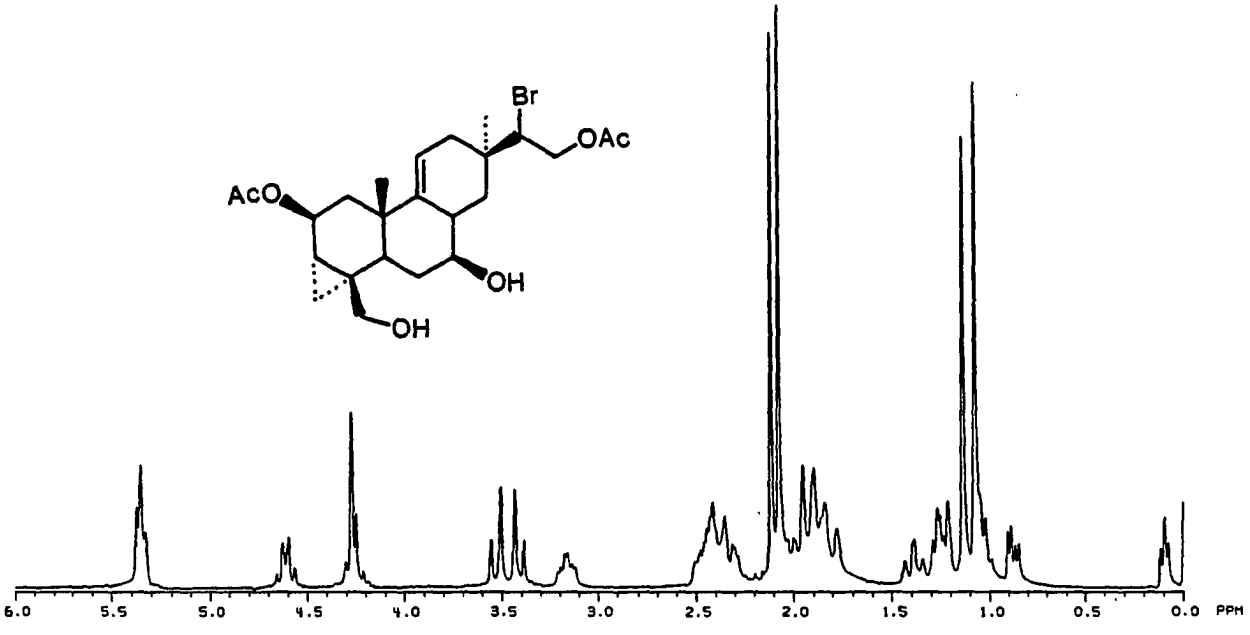



118

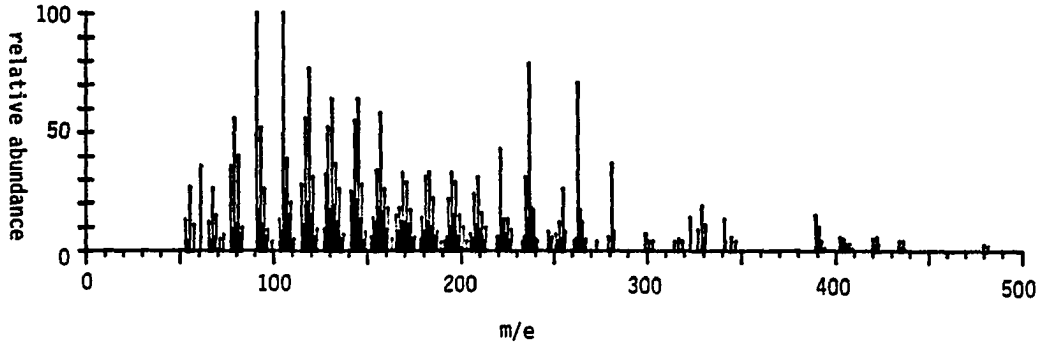
Figure 25. Spectra of Parguerol 16-Acetate (118)




IR Spectrum of 118



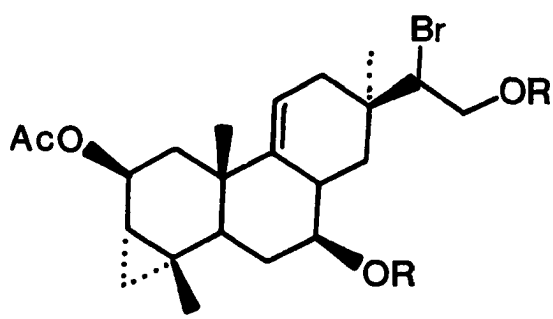

270 MHz PMR Spectrum of 118 in $CDCl_3$




Mass Spectrum of 118

Characterization of Deoxyparguerol (119)

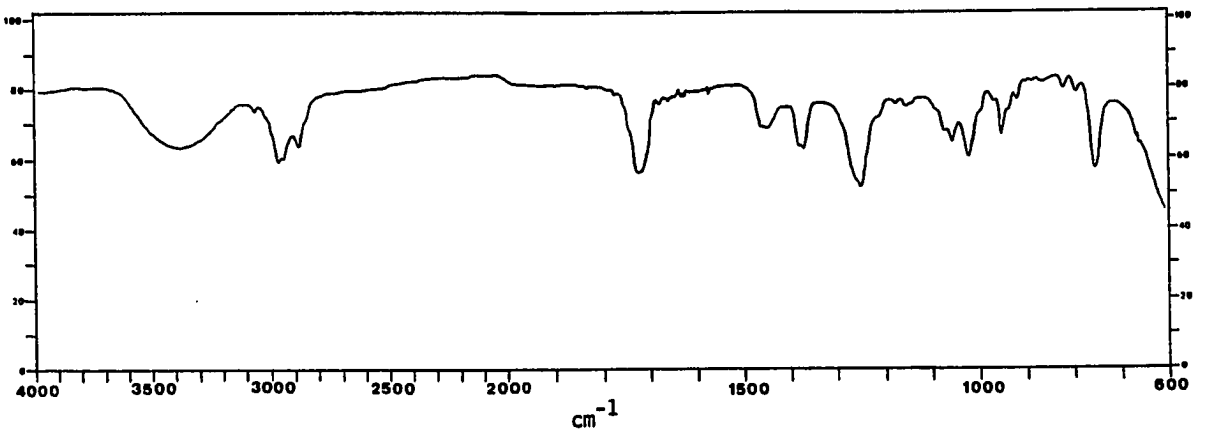
Further silica gel chromatography of the Sephadex LH-20 fraction which had yielded parguerol yielded deoxyparguerol (119), obtained as a clear colorless glass, $[\alpha]_D$ -35.8 (c. 0.62, $CHCl_3$). The highest mass ions observed in the low resolution mass spectrum of deoxyparguerol appeared at m/e 422, 424, consistent with a formula of $C_{22}H_{31}BrO_3$ (however, see the mass spectrum derived formula for deoxyparguerol acetate below). The presence of hydroxyl was indicated by an infrared absorption of 3360 $cm^{-1}$. Acetate functionality was confirmed in the infrared by a carbonyl absorption at 1725 $cm^{-1}$ and in the proton nmr spectrum by a methyl singlet at 2.05 ppm. The nmr spectrum of deoxyparguerol contained all the downfield proton signals observed in parguerol except that the AB pattern due to the hydroxymethylene group at C-19 in parguerol was missing in deoxyparguerol. In addition, a new quaternary methyl group (1.02 ppm) appeared and all three cyclopropane protons were shifted upfield relative to parguerol.



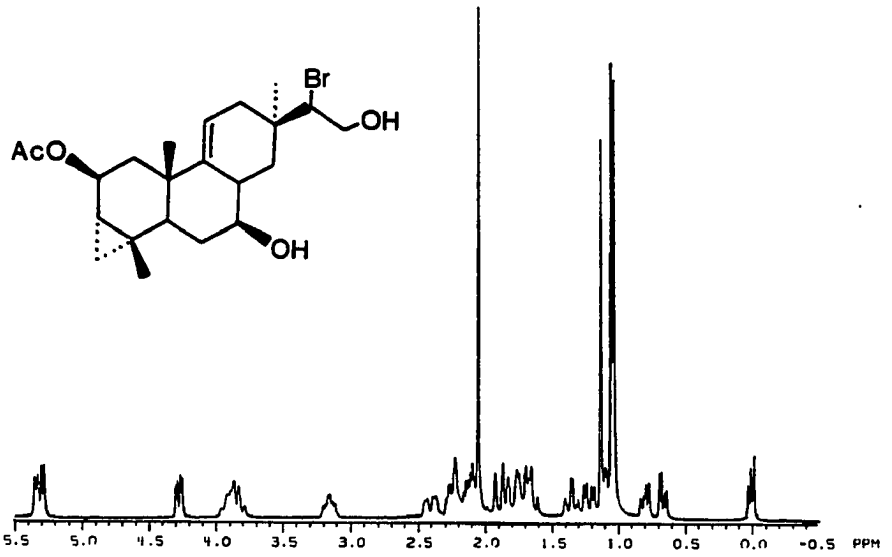

119 R=H

120 R=Ac

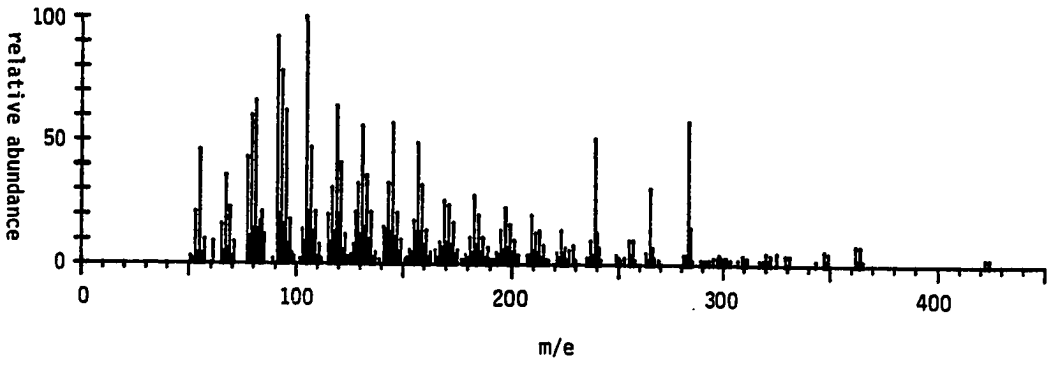
Figure 26. Spectra of Deoxyparguerol (119)




IR Spectrum of 119

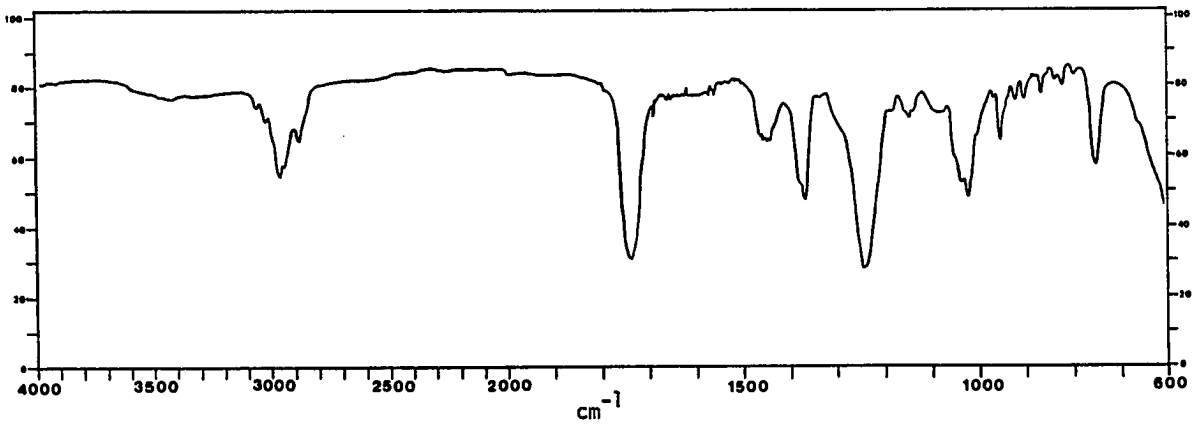



270 MHz PMR Spectrum of 119 in $CDCl_3$

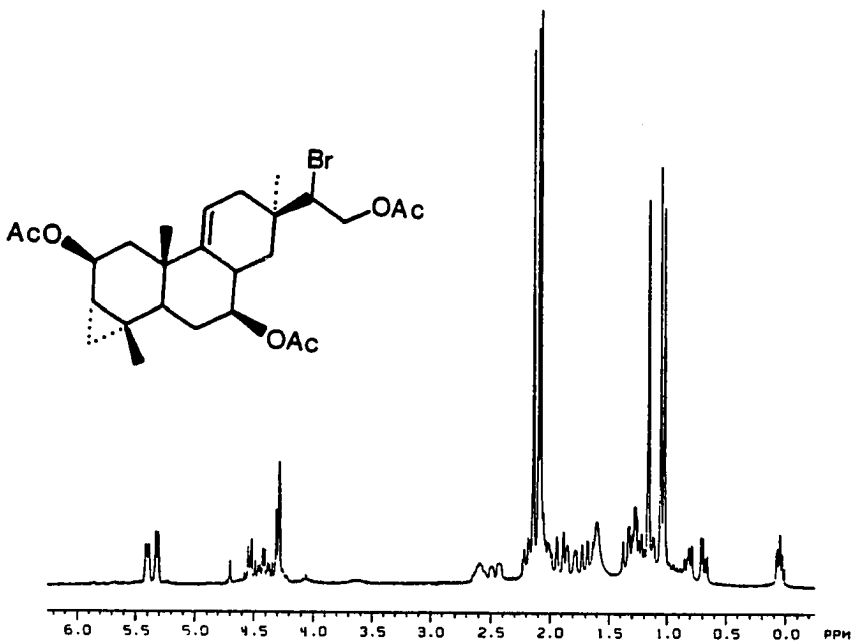



Mass Spectrum of 119

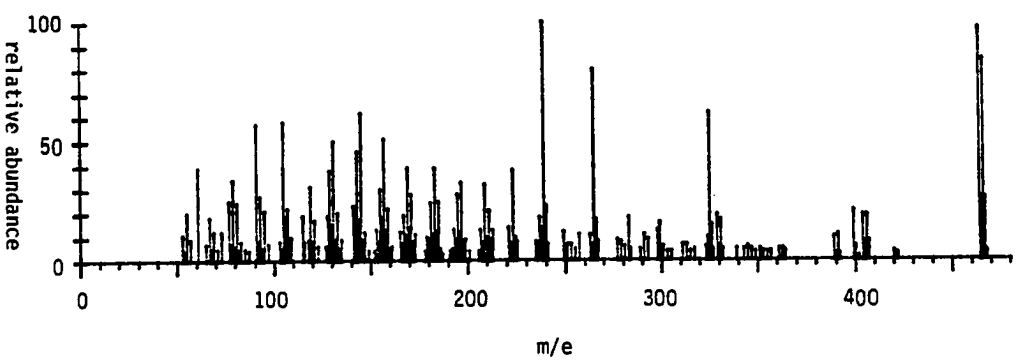
Figure 27. Spectra of Deoxyparguerol Acetate (120)




IR Spectrum of 120




270 MHz PMR Spectrum of 120 in $CDCl_3$




Mass Spectrum of 120

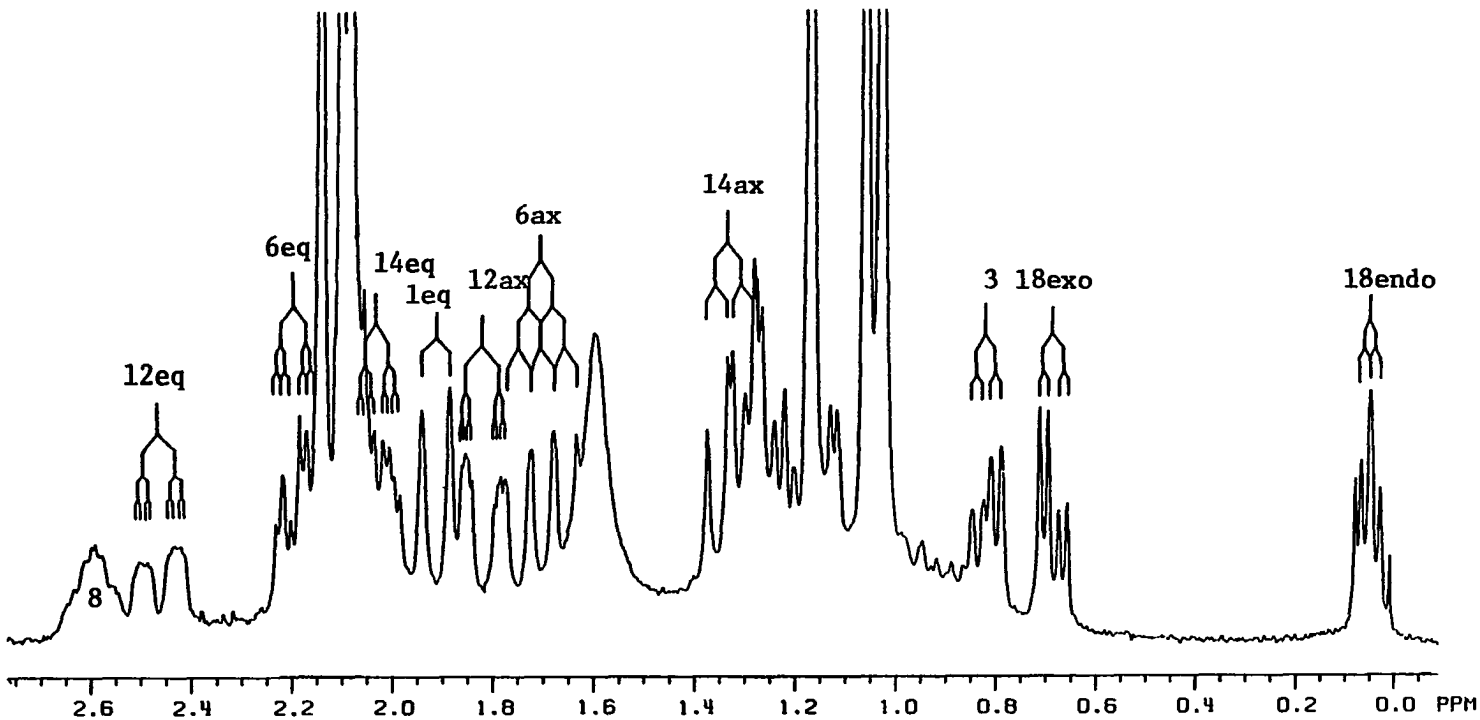



Figure 28. High Field Portion of the 270 MHz PMR Spectrum of 120 in $CDCl_3$.

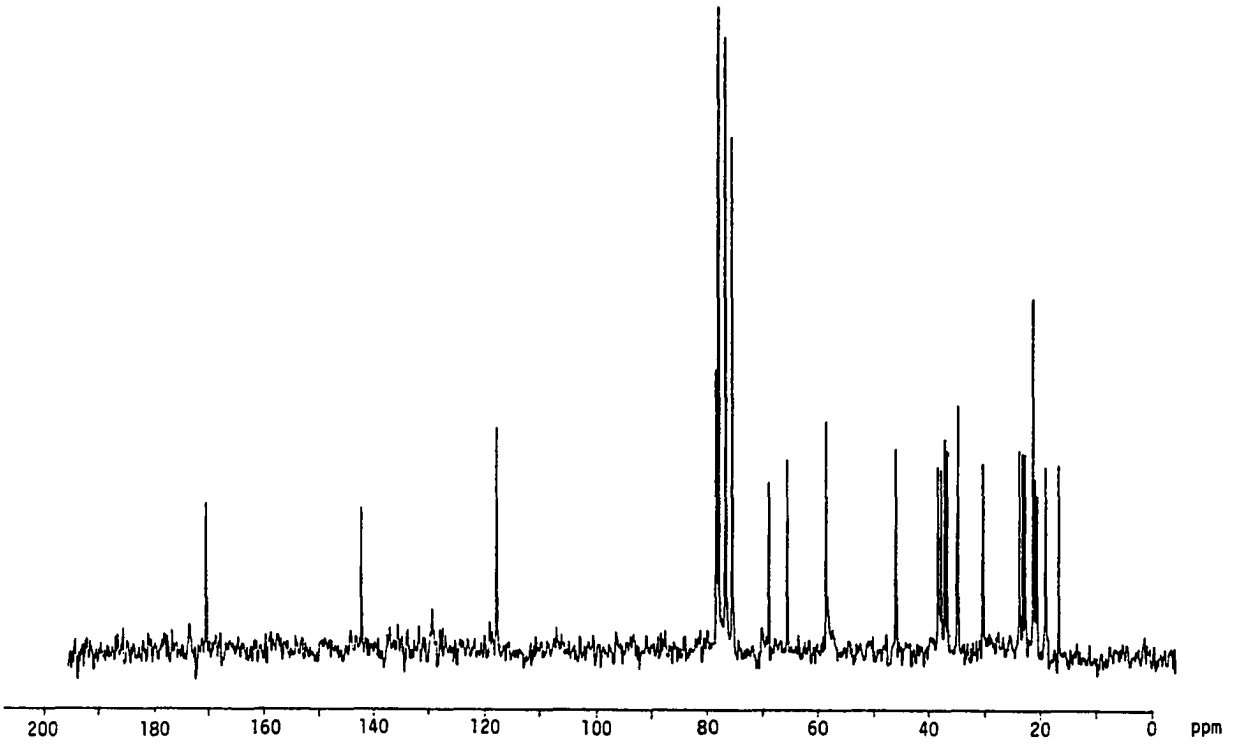



Figure 29. $^{13}C$-NMR ($CDCl_3$) Spectrum of Deoxyparguerol Acetate (120).

Upon acetylation, deoxyparguerol yielded a triacetate (120) with the formula $C_{26}H_{37}BrO_6$ (high resolution mass spectrum) and indicating that the highest mass ion observed for deoxyparguerol itself corresponded to $M^+$-18. The presence of only three acetate groups was confirmed by acetate methyl signals at 2.08, 2.10, and 2.14 ppm in the nmr spectrum. The signals corresponding to the protons at C-7 and C-16 in deoxyparguerol, 3.16, 3.83, and 3.90 ppm, were shifted downfield to 4.38, and 4.28 ppm in deoxyparguerol peracetate, thus supporting the assignment of hydroxyl groups to these positions as in parguerol.

It can therefore be concluded that the absence in the nmr spectrum of deoxyparguerol of an AB pattern at ∿3.4 ppm, coupled with the presence of a third quaternary methyl group signal, and upfield shift of the cyclopropane protons, is consistent with replacement of the hydroxyl group at C-19 by hydrogen. Decoupling established that the remainder of the structure was identical with parguerol. See Table 3. Also, since the chemical shifts of the C-17 and the C-20 protons of deoxyparguerol were nearly identical to those of parguerol, the same stereochemistry was inferred. Hence structure 119 was assigned to deoxyparguerol, $C_{22}H_{33}BrO_4$.

A derivative of deoxyparguerol, deoxyparguerol 16-acetate, was isolated from the red alga, *Laurencia obtusa*.[81] This compound has not yet been found in *Aplysia dactylomela*, but its presence in algae suggest the diet of *Aplysia* as the ultimate source of these diterpenes.

Characterization of Isoparguerol (121)

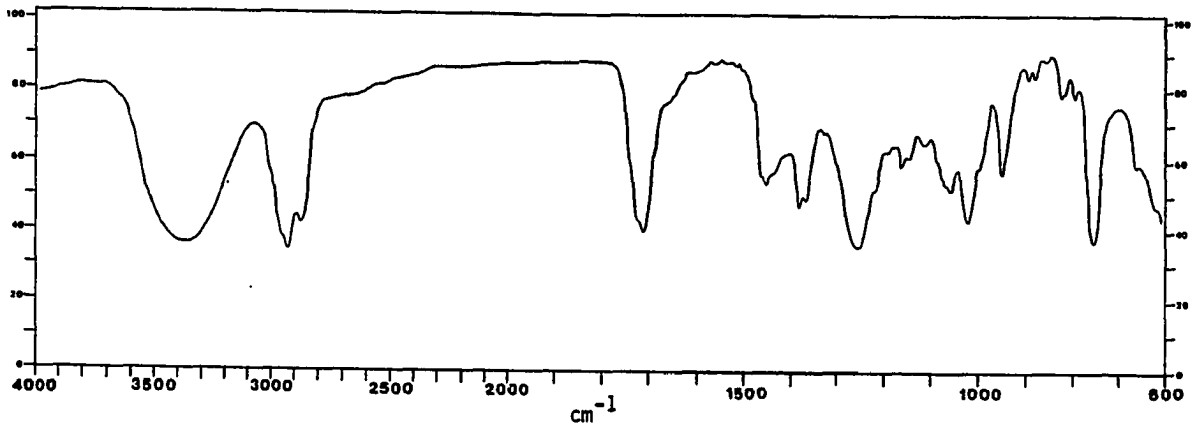
Isoparguerol was isolated as fine needles mp. 139-141°, $[\alpha]_D$

+3.6 (c. 0.14, $CHCl_3$) by HPLC from fractions of crude parguerol (109). The highest mass ions observed in the field desorption mass spectrum of isoparguerol appeared at m/e 438, 440, consistent with a formula of $C_{22}H_{31}BrO_4$. The infrared spectrum of isoparguerol indicated hydroxyl absorption at 3360 $cm^{-1}$ and acetate carbonyl absorption at 1710 $cm^{-1}$. The nmr spectrum confirmed the presence of acetate by a three proton singlet at 2.03 ppm. Two quaternary methyl signals were also present at 1.21 and 1.08 ppm.

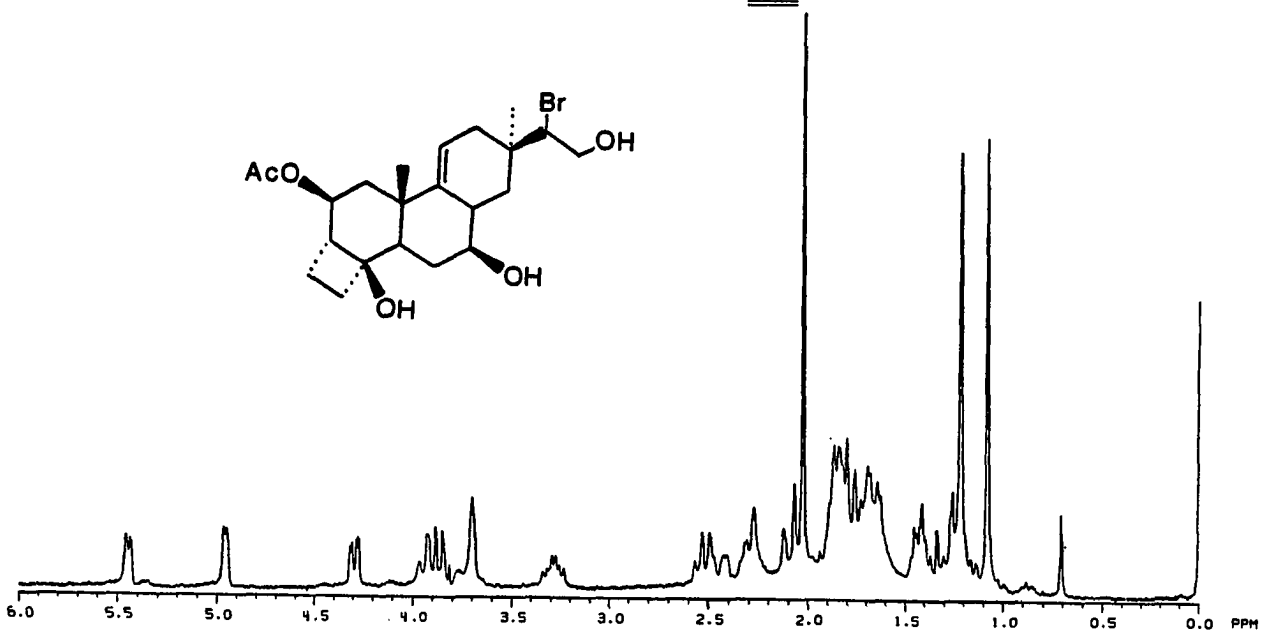
Although the nmr spectrum of isoparguerol resembles that of parguerol, there are some striking differences. A notable difference is the absence of cyclopropane proton absorptions and also of absorptions due to the isolated AB pattern at ∿3.4 ppm as in parguerol. In addition, the proton signal at 5.35 ppm in paraguerol corresponding to the -CH-OAc proton has shifted upfield to 4.96 ppm in isoparguerol, a position more characteristic of a normal -CH-OAc proton. This shift was consistent with the absence of a cyclopropane at C-3,4 and the attendant loss of the deshielding effect caused by the cyclopropane ring. The pmr spectrum of isoparguerol also contained a methine signal at 3.29 ppm, due to a proton deshielded by hydroxyl, an olefinic proton signal at 5.44 ppm, and an ABC pattern at 3.84, 3.94, and 4.29 ppm. These signals were nearly identical to those found in parguerol. Hence, the major portion of the carbon skeleton of isoparguerol was inferred to be like that of parguerol.

Upon acetylation of isoparguerol, two new acetate groups were incorporated. However, the infrared spectrum still showed hydroxyl absorption at 3500 $cm^{-1}$, which was therefore attributed to a tertiary

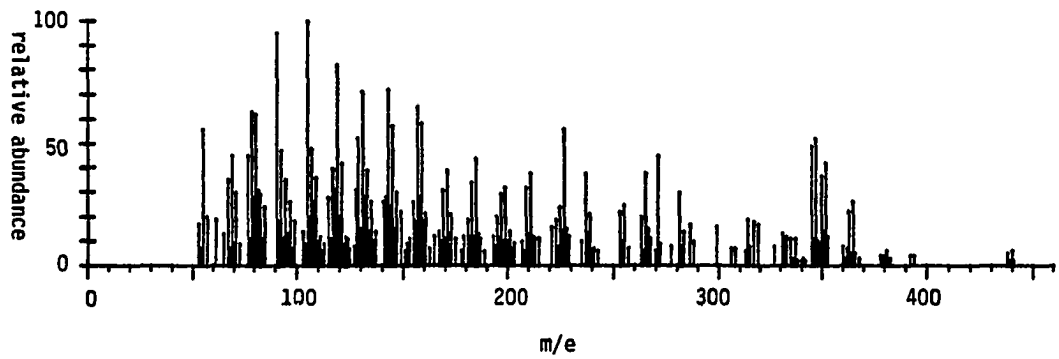
Figure 30. Spectra of Isoparguerol (121)




IR Spectrum of 121

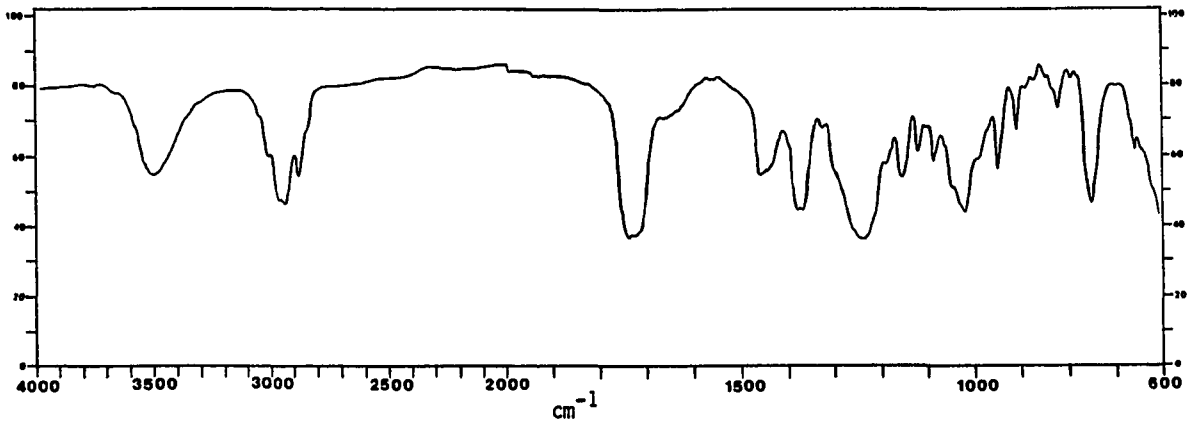



270 MHz PMR Spectrum of 121 in $CDCl_3$

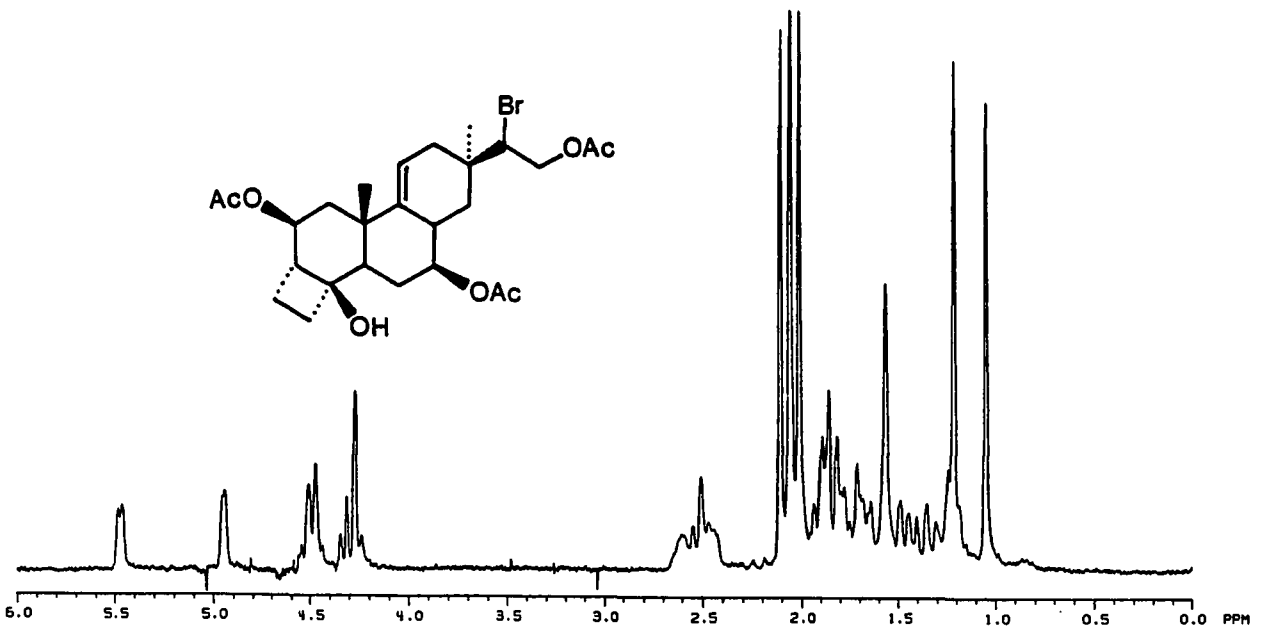



Mass Spectrum of 121

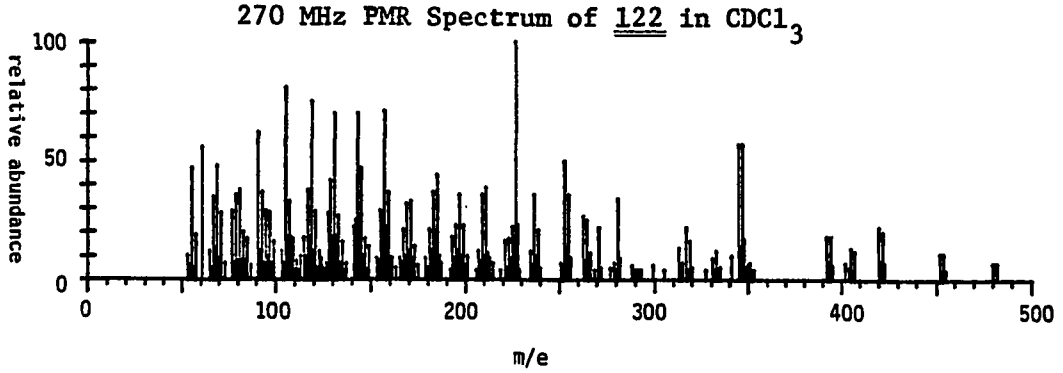
Figure 31. Spectra of Isoparguerol Acetate (122)




IR Spectrum of 122

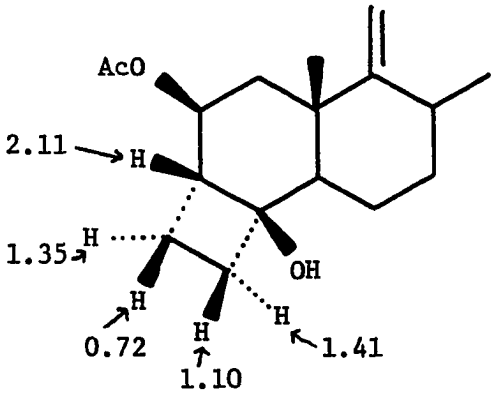



270 MHz PMR Spectrum of 122 in $CDCl_3$




Mass Spectrum of 122

hydroxyl group. The presence of this tertiary hydroxyl group suggested that the formula for isoparguerol (121) was $C_{22}H_{33}BrO_5$, to account for all oxygenated functionalities. The observed ions at m/e 438, 440 were due to $M^+-H_2O$. Loss of the cyclopropane absorptions with formation of a tertiary hydroxyl without generation of a new methyl group can best be explained by an opening of the cyclopropane ring to form a cyclobutane ring. Indeed, decoupling studies involving the DDR technique performed on isoparguerol acetate (122) in benzene-$d_6$ did identify all protons of the expected substituted cyclobutane ring. Thus, irradiation of the triplet at 2.11 ppm collapsed two quintets at 0.72



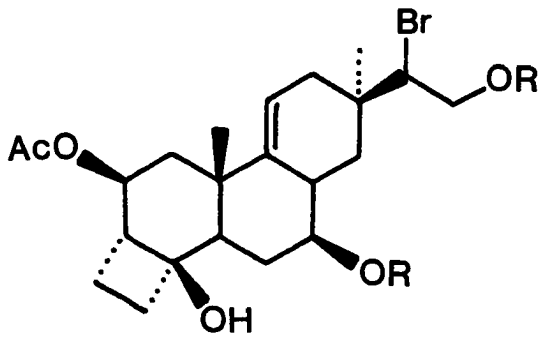

and 1.35 ppm to quartets. Irradiation of the quintet at 0.72 ppm collapsed two quartets at 1.10 and 1.41 ppm to triplets, reduced the quintet at 1.35 ppm to a quartet, and converted the triplet at 2.11

Table 6

Induced Shifts of Proton nmr for 122

| Proton | $C_6D_6$ | $Eu(fod)_3$(.1:1) | $Eu(fod)_3$(.34:1) |
|---|---|---|---|
| 1ax | 1.29 | 1.40 | |
| 1eq | 1.82 | 2.08 | |
| 2 | 4.88 | 5.34 | 6.34 |
| 3 | 2.11 | 2.34 | 2.93 |
| 5 | | | |
| 6ax | 1.88 | 2.02 | |
| 6eq | | 2.07 | |
| 7 | 4.58 | 4.88 | 5.60 |
| 8 | 2.75 | 2.99 | 3.61 |
| 11 | 5.15 | 5.21 | 5.45 |
| 12ax | 1.62 | 1.60 | |
| 12eq | 2.45 | 2.46 | |
| 14ax | 1.25 | | 1.75 |
| 14eq | 2.05 | 2.20 | |
| 15 | 4.24 | 4.32 | 4.63 |
| 16α | 4.50 | 4.58 | 4.92 |
| 16β | 4.50 | 4.64 | 5.05 |
| 17 | 0.88 | 0.88 | 1.04 |
| 18endo | 0.72 | 0.75 | 1.00 |
| 18exo | 1.35 | 1.40 | 1.53 |
| 19α | 1.10 | 1.15 | |
| 19β | 1.41 | 1.47 | |
| 20 | 1.26 | 1.44 | 1.91 |

ppm to a doublet. No other protons were affected and it was concluded that the cyclobutane ring was an isolated spin system. The presence of a downfield shift of the C-20 protons in isoparguerol (1.21 ppm) relative to parguerol (1.13 ppm) indicated that the tertiary hydroxyl group was on the same side as the C-20 protons. Europium shift reagent studies on isoparguerol acetate confirmed the β-nature of the tertiary hydroxyl group since the C-20 protons were shifted much farther downfield than the C-17 protons. See Table 6.




121 R=H

122 R=Ac

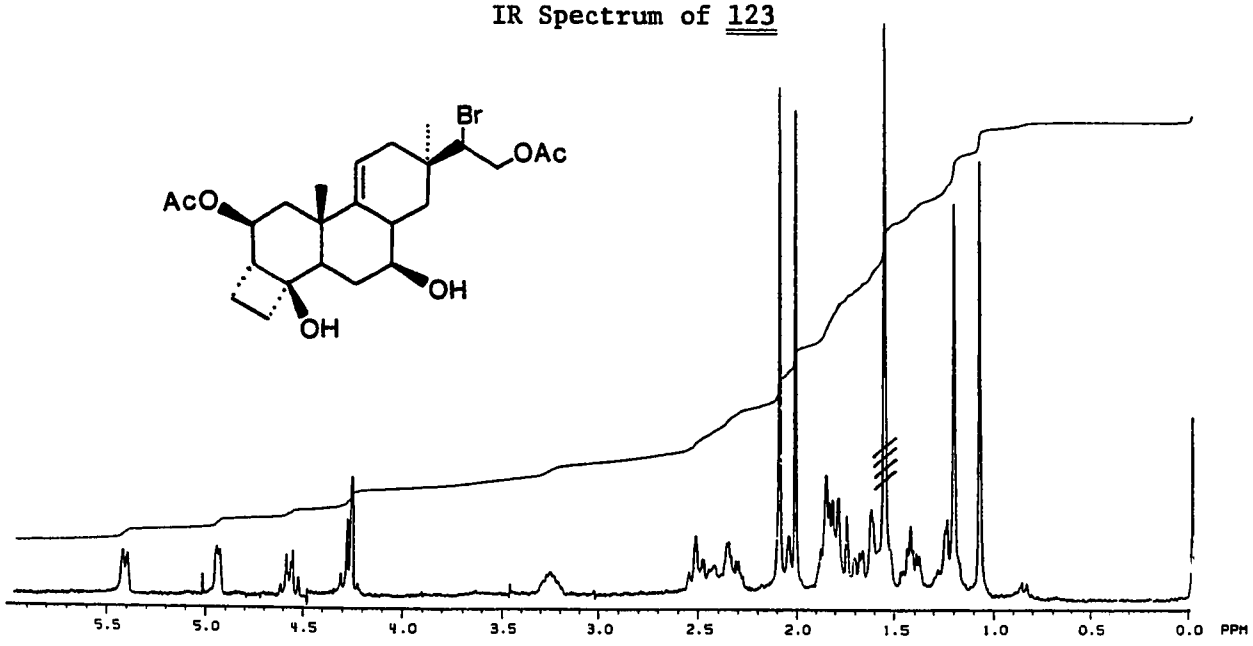
Characterization of Isoparguerol 16-acetate (123)

Further Sephadex LH-20 and silica gel chromatographies of fraction G followed by purification by HPLC led to the isolation of isoparguerol 16-acetate as fine needles, mp. 180-182°, $[\alpha]_D$ -18.8 (c. 0.09, $CHCl_3$). The highest mass ions observed in the low resolution mass spectrum of 123 appeared at m/e 480, 482, consistent with a formula of $C_{24}H_{33}BrO_5$, (see final formula below, however). The

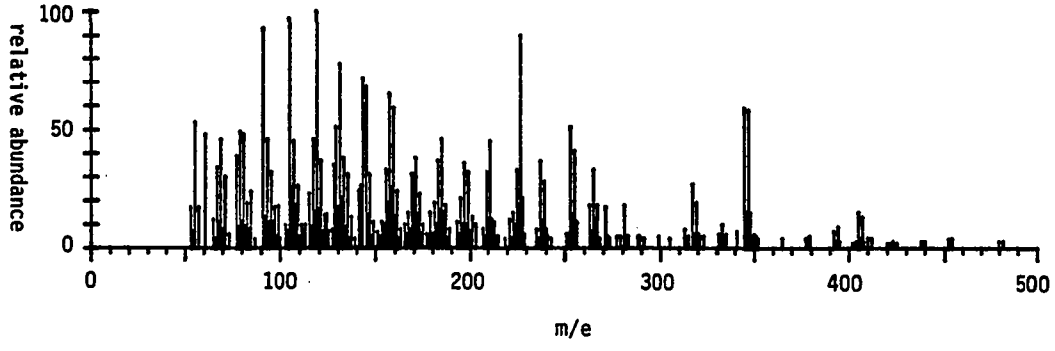
Figure 32. Spectra of Isopargueroi 16-acetate (123)




IR Spectrum of 123

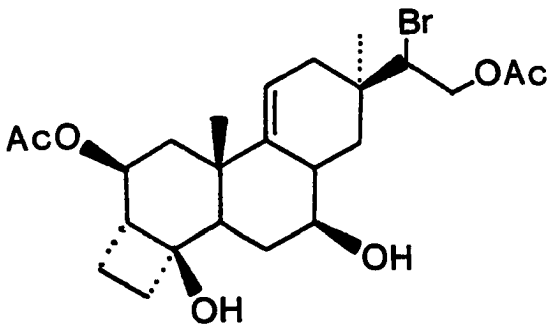



270 MHz PMR Spectrum of 123 in $CDCl_3$




Mass Spectrum of 123

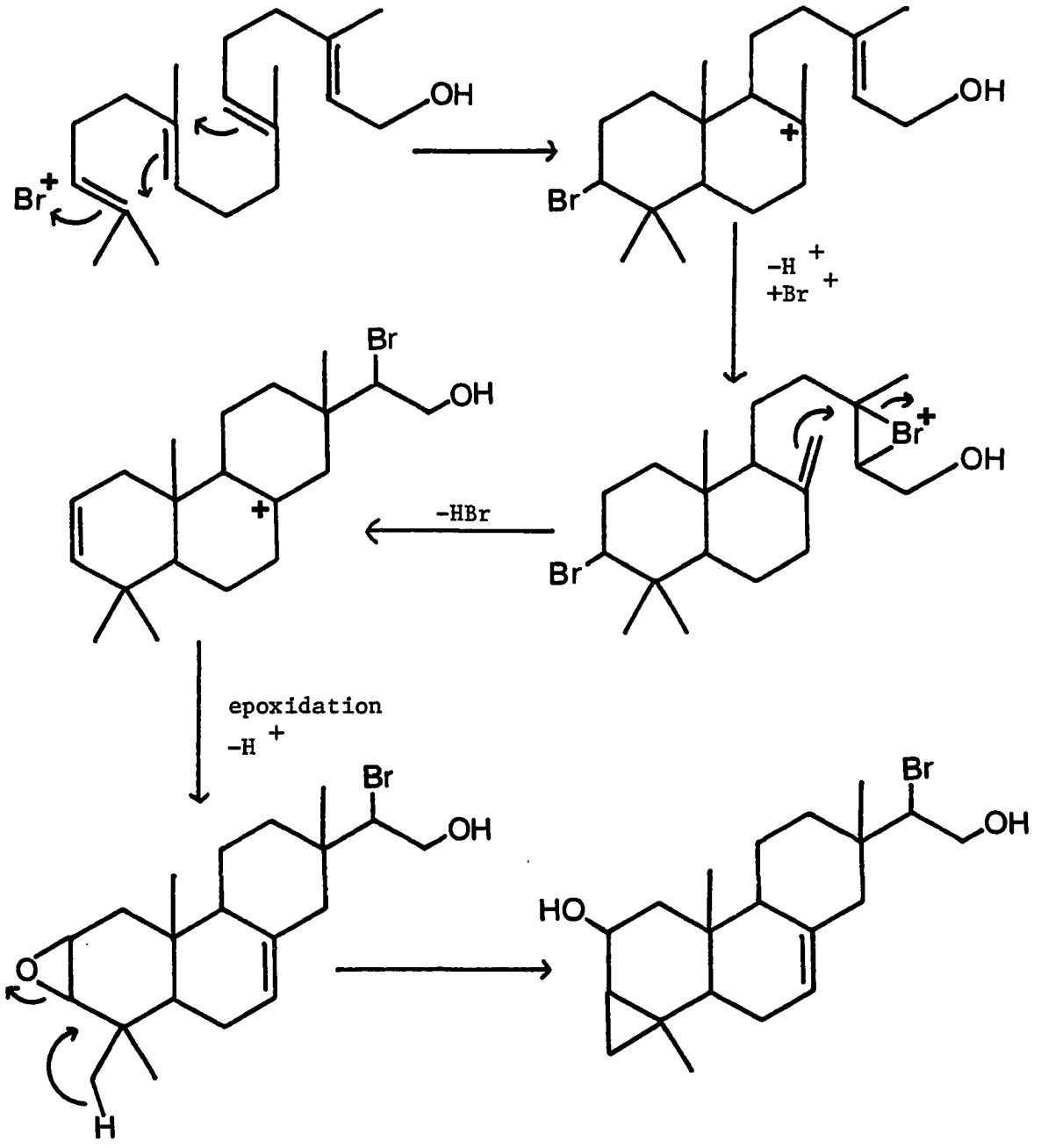
infrared spectrum indicated hydroxyl (3600 $cm^{-1}$) and acetate (1725 $cm^{-1}$). Two acetate methyl signals were observed at 2.03 and 2.11 ppm in addition to two quaternary methyl signals at 1.09 and 1.22 ppm. Comparison of the nmr of isoparguerol 16-acetate to that of isoparguerol indicated that the protons at C-16 in 123 had been shifted downfield by about 0.5 ppm. Hence, it was concluded that the second acetate was located at C-16. Acetylation of isoparguerol 16-acetate produced a peracetate whose nmr spectrum was identical to that of isoparguerol peracetate (122). Therefore, the structure and stereochemistry of isoparguerol 16-acetate was confirmed to be that shown by formula 123, $C_{24}H_{35}BrO_6$. The highest mass ions in the mass spectrum of 123 must thus be due to $M^+$-18.




123

The mass spectral fragmentation patterns of parguerol, deoxyparguerol and their acetates are all dominated by losses of neutral molecules and radicals such as $H_2O$, HOAc, $Br^{\cdot}$, and $CH_3^{\cdot}$. In fact, loss of four neutral molecules of acetic acid and loss of a bromine radical is responsible for the base peak in parguerol acetate (110a). No retro Diels-Alder fragmentation typical of other pimarenes was

SCHEME III



observed.[82,83] The mass spectra of isoparguerol and isoparguerol 16-acetate were distinguished from those of parguerol and deoxyparguerol by prominent losses of ethylene from the molecular ion or fragment ions. This loss is attributed to cleavage of the cyclobutane ring. In addition, losses of $H_2O$, HOAc, $Br^{\cdot}$, and $CH_3^{\cdot}$ were also observed for isoparguerol and its acetates.

A suggested biosynthetic pathway to parguerol and related compounds is outlined in Scheme III. The first step involves a bromonium ion initiated cyclization of geranylgeraniol to form a bicyclic intermediate. Cyclizations of this type have been proposed for a number of halogenated compounds.[84] Loss of a proton, addition of a bromonium ion and subsequent cyclization would lead to a pimarane diterpene with a bromohydrin located on the ethyl side chain. Loss of hydrogen bromide to form a double bond at C-2 followed by epoxidation would produce an intermediate which is ideally set up for cyclopropanation. Loss of a proton from one of the geminal methyls and cyclization can lead to formation of a cyclopropane ring and functionalization of C-2 as in parguerol and related compounds. This last reaction step is akin to that proposed for the biosynthesis of cycloartenol.[85]

Characterization of 4,9,14-trihydroxydolasta-1(15),7-diene (124)

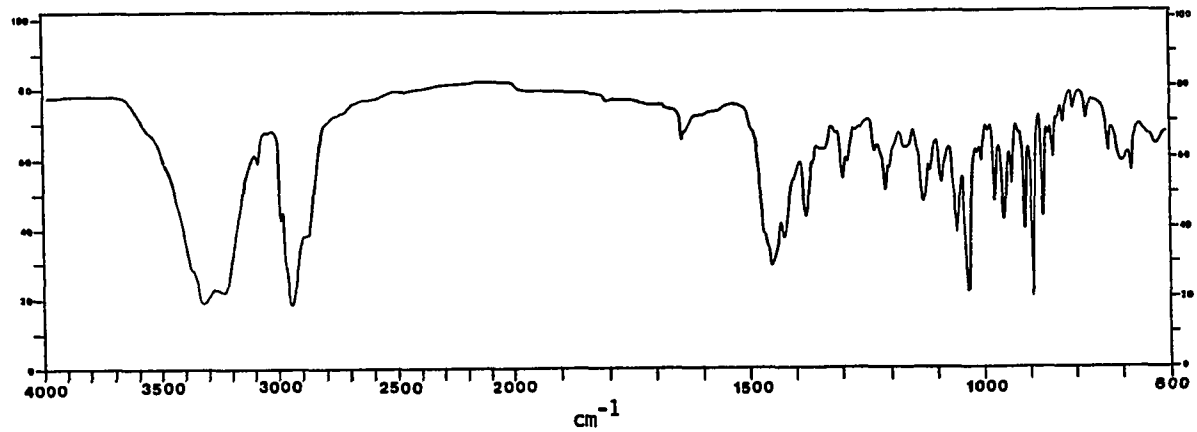
In the process of isolating deoxyparguerol (119), a small amount of a crystalline material precipitated from solution. The compound, mp. 220-222°, $[\alpha]_D$ -211° (c. 0.05, $CHCl_3$), was shown to have a molecular formula of $C_{20}H_{32}O_3$ (high resolution mass analysis). The presence of hydroxyl was established by a strong infrared absorption at 3325 $cm^{-1}$. The proton nmr contained two quaternary methyl group signals at 0.78 and 1.23 ppm, in addition to two doublet methyl signals at 0.84 and 1.03 ppm. An exocyclic methylene group was indicated by infrared absorptions at 3090,

1642, and 896 $cm^{-1}$. A one proton double doublet signal was present at 5.63 ppm indicating the presence of a trisubstituted olefinic unit coupled to two other protons. The exocyclic methylene group was confirmed by two signals at 4.79 and 4.93 ppm. The double bonds account for two of the five degrees of unsaturation. Since there was no carbonyl absorption in the infrared, the remaining three degrees of unsaturation were assumed to be accounted for by rings. Only one signal, a multiplet at 3.45 ppm, was observed which was indicative of a methine proton deshielded by a hydroxyl group. It was inferred that any other hydroxyl groups present must be tertiary. The singlets at 2.66, 3.71, and 3.74 ppm disappeared upon addition of $D_2O$ and hence were due to hydroxyl protons.

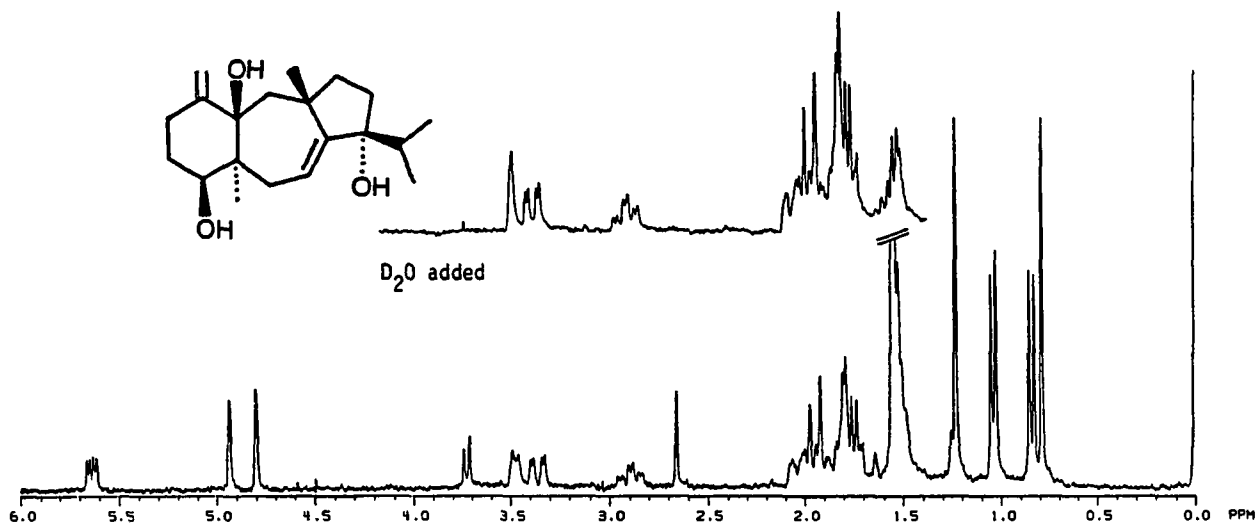
A literature search of the formula $C_{20}H_{32}O_3$ identified dolatriol[12] (70) as having the same formula and very similar nmr data. Decoupling experiments were carried out on 124 to confirm the presence of a dolastane carbon skeleton.

Irradiation of the olefinic double doublet at 5.63 ppm resulted in the removal of a small coupling from the double doublet at 3.35 ppm and produced changes in the region of 1.74 ppm. Application of the difference double resonance technique, described earlier, confirmed that the signal at 1.74 ppm was a double doublet which was geminally coupled (J=15 Hz) to the signal at 3.35 ppm and vicinally coupled (J=4 Hz) to the olefinic proton at 5.63 ppm. Irradiation of the signal at 3.35 ppm, in addition to affecting the 1.74 and 5.63 ppm signals, sharpened a quaternary methyl signal to 0.78 ppm. The observed long range coupling thus established the presence of the methyl group (0.78 ppm) as being next to the allylic methylene group, shown in the following partial structure.

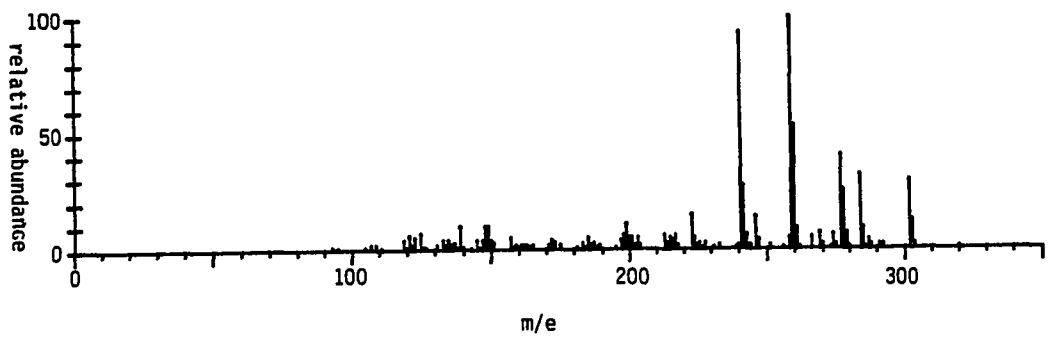
Figure 33. Spectra of 4,9,14-Trihydroxydolas-1(15),7-diene (124)



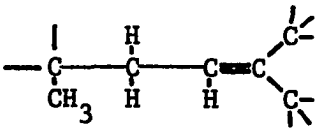

IR Spectrum of 124



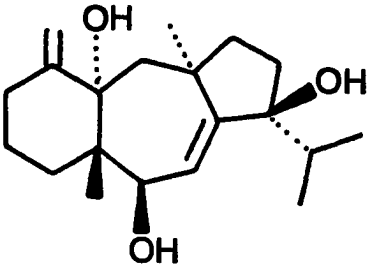

270 MHz PMR Spectrum of 124 in $CDCl_3$




Mass Spectrum of 124



The fact that no allylic coupling was observed for the olefinic proton indicates that the double bond is also attached to two quaternary centers.



70

In order to establish if an isopropyl group in compound 124 was attached to a quaternary carbon bearing a hydroxyl group as in dolatriol (70), further decoupling experiments were carried out. Irradiation of one of the isopropyl methyl signals produced a barely perceptible change in the complex region at 1.95 ppm. With the DDR technique, the methine at 1.95 ppm appeared as an isolated septet appearing above the base line and a quartet appearing below, See Figure 34. Hence, the isopropyl group was attached to a quaternary carbon. The chemical shift of the isopropyl methine proton, which was considered unlikely for an allylic proton, suggested that the isopropyl group was attached to a quaternary carbon bearing a hydroxyl group as in dolatriol.

Pettit and coworkers, however, reported[12] that the isopropyl methine proton in dolatriol absorbed at 2.6 ppm. Compound 124 contained a double triplet with a similar chemical shift, 2.88 ppm, which was assumed to be due to a proton allylic to the exocyclic double bond by analogy with other dolastane compounds.[86] Irradiation of one of the exomethylene protons at 4.79 ppm sharpened the double triplet.

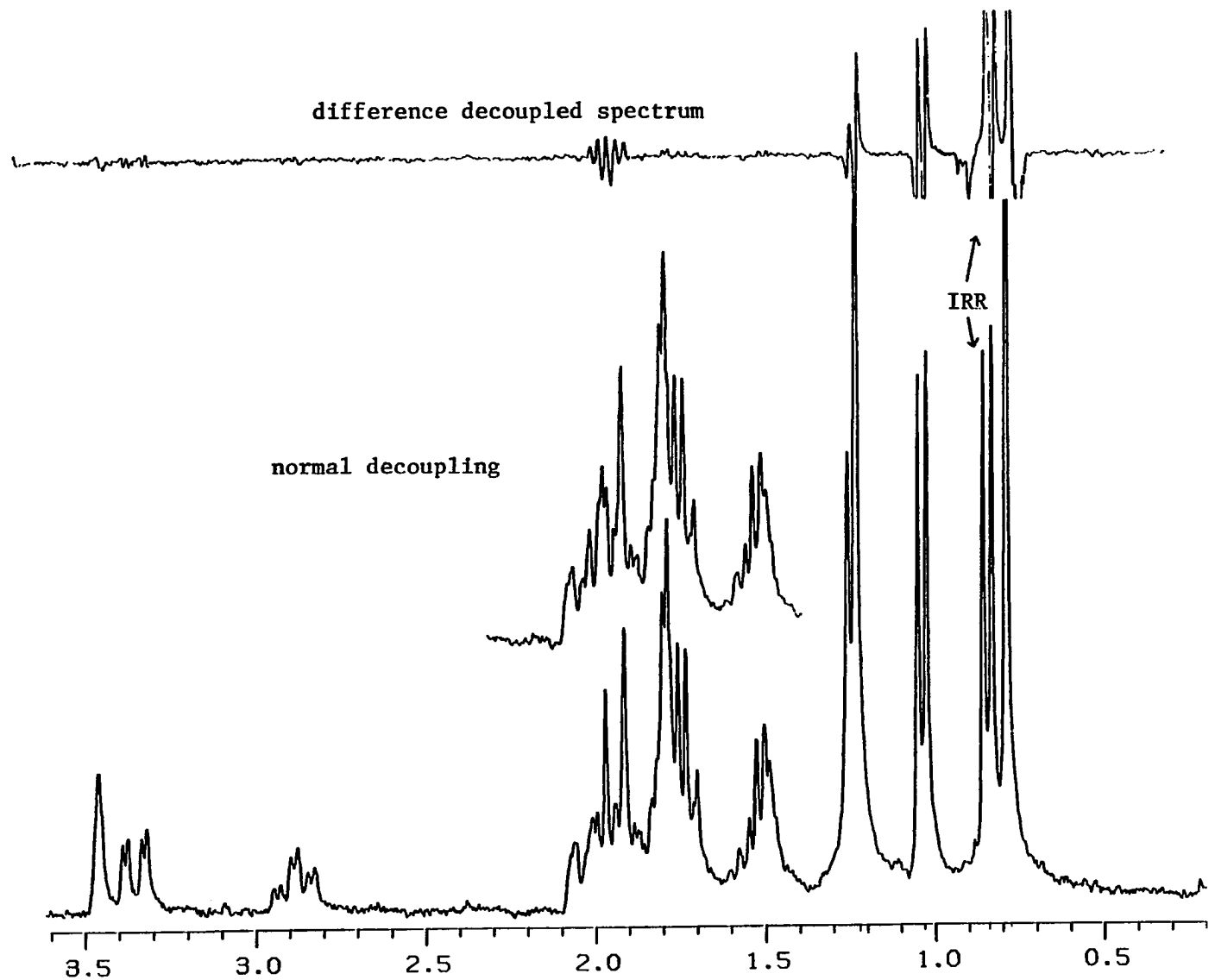
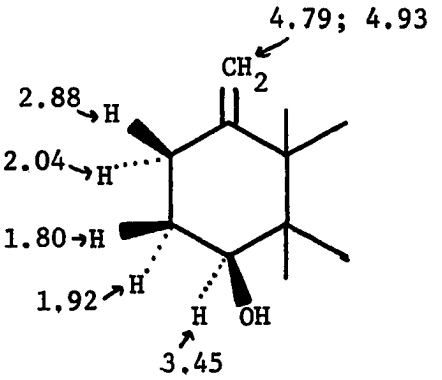



Figure 34. Difference Decoupled Spectrum of 124

Hence, the allylic nature of the signal at 2.88 ppm was confirmed and the reported assignment of the 2.6 ppm signal in dolatriol is likely in error.

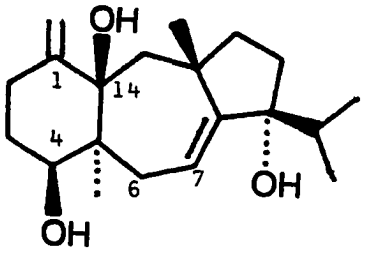
The location of the secondary hydroxyl group in compound 124 was inferred from further proton decoupling experiments. Irradiation of the double triplet at 2.88 ppm removed a geminal coupling from a broad doublet at 2.04 ppm and produced changes in the signals at 1.80 and 1.92 ppm. These two signals were further coupled to the methine proton at 3.45 ppm which was not further coupled to other protons. The methine proton displays only small couplings which indicate an equatorial orientation. The hydroxyl is therefore axially oriented on C-4 of the dolastane skeleton.




The third hydroxyl group of compound 124, which must be tertiary, is placed at C-14. The unusually low field positions of the methyl group signal at 1.23 ppm and the allylic proton signal at 2.88 ppm can best be explained by 1,3-diaxial interactions with the β-oriented

hydroxyl group.

From the above spectral evidence and by analogy with other dolastane compounds, compound 124 was assigned as 4,9,14-trihydroxy-dolas-1(15),7-diene.




124

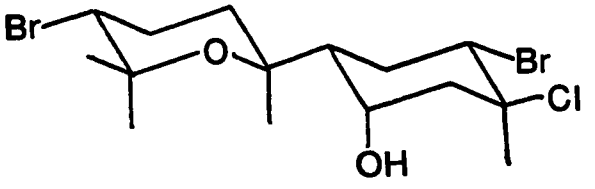
Several of the peak assignments in the nmr spectrum reported for dolatriol are in error. Pettit and coworkers reported[12] that the exocyclic methylene protons appear as doublets at 4.97 and 5.45 ppm (J=6 Hz). However, protons of olefins exocyclic to a six-membered ring, such as those found in elatol[61] and other chamigrenes,[65] are known to have insignificant geminal couplings.[69] Also, the large shifts these protons undergo upon acetylation suggests that these signals are indeed due to the protons at C-6 and C-7. The exocyclic methylene protons of dolatriol should be assigned to the singlets at 4.73 and 4.89 ppm.

Structure Elucidation of Dihydroxydeodactol Monoacetate (32)

Sephadex LH-20 and silica gel chromatography, carried out by an earlier worker in this laboratory on extracts of *Aplysia*

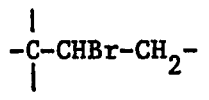
*dactylomela* from Bimini, led to the isolation of a new sesquiterpenoid (32). Compound 32, $C_{17}H_{27}O_5Br_2Cl$ (high resolution mass analysis), was isolated as colorless crystals, mp. 168-169°C, $[\alpha]_D$+40.5 ($CHCl_3$). The presence of hydroxyl functionality was determined by an infrared absorption at 3440 $cm^{-1}$. The presence of an acetate group was ascertained from a carbonyl absorption at 1730 $cm^{-1}$ and from a methyl singlet absorption in the nmr at 2.14 ppm. No olefinic proton signals were present. Since 32 had an unsaturation number of three, it was concluded that 32 was probably bicyclic.

The proton nmr spectrum of 32 was found to very closely resemble that of deodactol[26] (31). Three methyl groups deshielded by




31

an ether oxygen were observed at 1.42, 1.44 and 1.47 ppm. One methyl group deshielded by a halogen and a 1,3-diaxial interaction with a hydroxyl group was observed at 1.87 ppm. As in deodactol, the presence of two units of partial structure A was inferred from the bromine deshielded axial protons (4.24, 4.78 ppm, dd, J=14, 4 Hz each) in six membered rings.




A

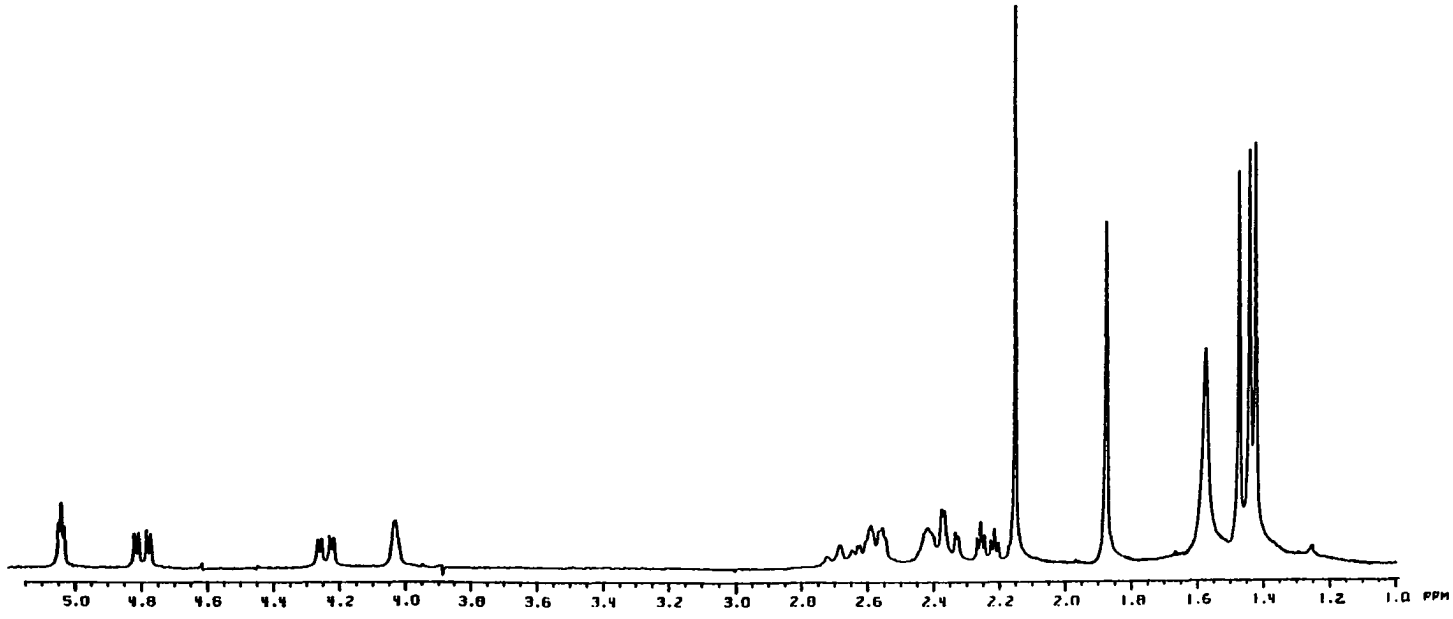
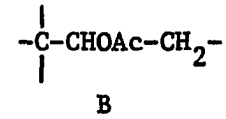



Figure 35. 360 MHz PMR Spectrum of Dihydroxydeodactol Monoacetate (32)

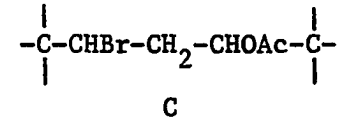
A one-proton triplet at 5.04 ppm (J=4 Hz) was assigned as an equatorial proton deshielded by an acetoxy group as in partial structure B.



B

A signal observed at 4.03 ppm was assigned to an equatorial methine proton deshielded by a hydroxyl group.

Decoupling experiments at 360 MHz fully elucidated the three isolated spin systems of 32. The acetoxy methine proton absorbing at 5.04 ppm and the bromine deshielded methine signal at 4.24 ppm were both coupled to the same methylene group and to no other protons. Partial structure B and one of the two partial structures A could thus be combined to give partial structure C.

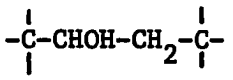


C

By analogy with deodactol, partial structure C was expanded into a tetrahydropyran ring. The prominent fragment ions at m/e 263 and 265 in the low resolution mass spectrum which correspond to such a substituted tetrahydropyran ring, and the coupling constants which were typical of a six-membered ring, all support this formulation.

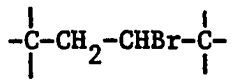
Irradiation of the methine proton signal at 4.04 ppm disturbed the proton absorbing in the region of 2.57 ppm and removed the small coupling from a double doublet at 2.35 ppm. Since the 2.57 and 2.35 ppm signals were also geminally coupled (J=14 Hz) to each other and to no further signals, these signals must be due to a methylene group

flanked on one side by a quaternary carbon. Thus partial structure D can be formulated.



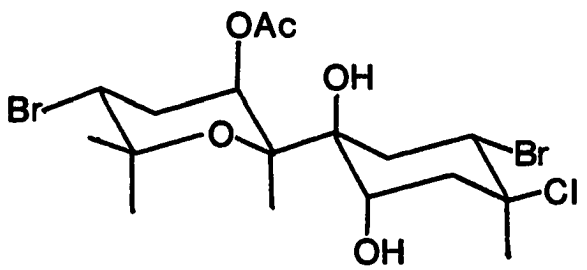
D

Irradiation of the signal at 4.78 ppm resulted in the removal of a diaxial coupling from the triplet at 2.68 ppm (J=14 Hz) and sharpened the signal at 2.43 ppm. The signals at 2.68 and 2.43 ppm were also geminally coupled (J=14 Hz) to each other but to no further proton indicating that these protons are due to a methylene group flanked on one side by a quaternary carbon to give rise to the partial structure E.



E

Joining partial structures D and E with overlap of the quaternary carbons leads to a cyclohexane ring. The addition of the chlorine, the tertiary hydroxyl group, the methyl groups, and joining of the tetrahydropyran and cyclohexane rings as in deodactol yielded the structure for 32.




32

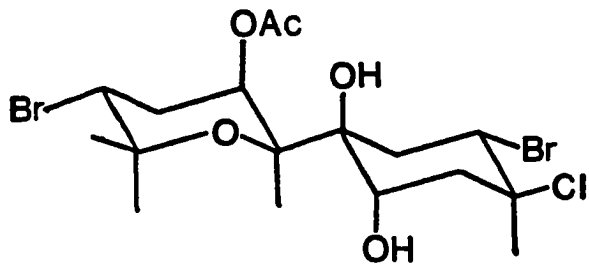
To confirm structure 32, chemical conversions were performed as outlined in Scheme IV. Oxidation of 32 gave the ketone 125 having hydroxyl (3475 $cm^{-1}$) and carbonyl (1740, 1725 $cm^{-1}$) absorptions in the infrared. The absorption at 1725 $cm^{-1}$ is consistent with an α-hydroxy ketone in a six-membered ring. Dehydrochlorination of ketone 125 with diazabicyclononene yielded the α,β-unsaturated ketone 126. The ketone absorption in 126 had shifted to 1685 $cm^{-1}$ and its proton nmr spectrum contained a vinyl methyl signal at 2.09 ppm and an olefinic proton singlet at 6.11 ppm in agreement with the proposed structure 126. The isotopic ratio of 1:2:1 at m/e 466, 468, 470 for the molecular ion in the low resolution mass spectrum confirmed the presence of two bromines, and hence chlorine had been lost.

Shift reagent studies were performed on 126 to rule out a possible alternate structure in which the acetoxy and bromine groups on the tetrahydropyran ring are interchanged.

From Table 7, it can be seen that the proton at C-8 is shifted to a greater extent than the C-10 proton. Since the opposite order would have been observed for the alternate structure, the final structures for 126 and 32 are as proposed.

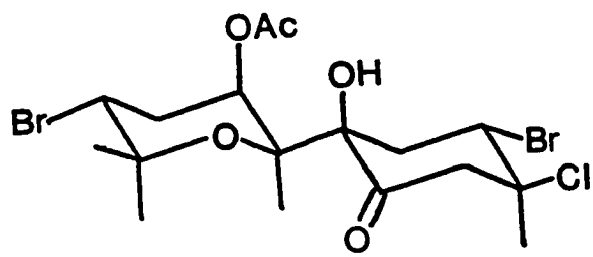
The relative stereochemistry of 32 was based on the proton nmr data as discussed above, chemical evidence, and conformational analysis. A trans-diaxial relationship between the secondary and tertiary hydroxyl groups of 32 was inferred from the failure of 32 to form a boronate ester when heated with phenylboronic acid.[87] Also, the six-membered rings are held equatorial relative to each other in order to avoid a severe 1,3-diaxial interaction. Observation of a W-coupling between $H_a$ and $H_b$ in 32 confirmed the conformation of the

SCHEME IV

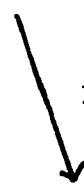


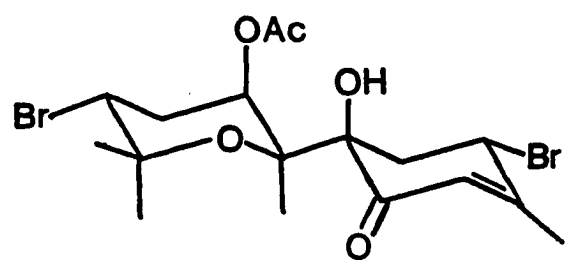

32




125




126

Table 7

Induced Shifts of Proton nmr Signals for 126

| Proton | $CDCl_3$, no shift reagent | $CCl_4$, $Eu(fod)_3$[a] |
|---|---|---|
| 4 | 6.11 (br s) | 7.10 |
| 2ax | 4.98 (dd, 5, 10) | 5.54 (dd, 5, 11) |
| 1ax | | 2.82 (dd, 11, 15) |
| 1eq | 3.08 (dd, 5, 14) | 3.76 (dd, 5, 15) |
| 8 | 4.95 (t, 2) | 9.40 (t, 2.5) |
| 9ax | ∿2.48 (dt, 3, 13) | 3.28 (dt, 2, 14) |
| 9eq | 2-2.5 (m) | 4.16 (dt, 4, 15) |
| 10ax | 4.08 (dd, 5, 12) | 5.32 (dd, 4, 12) |
| 15 | 2.08 (s) | 2.46 (s) |
| 14 | 1.57 (s) | 2.33 (s) |
| 12,13 | 1.28 (s) | 1.85 (s) |
| | 1.48 (s) | 1.88 (s) |
| OAc | 2.0 (s) | 4.97 (s) |
| OH | 4.10 (s) | |

[a]The $Eu(fod)_3$/126 molar ratio was ∿1:4.

cyclohexane ring. Thus, the most probable conformation is that shown in 32.

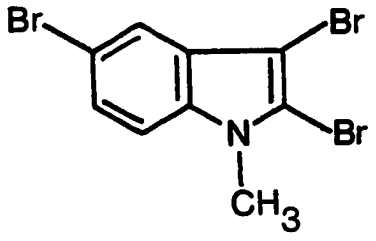
## SUMMARY

Five known compounds and eight new compounds have been isolated from *Aplysia dactylomela*. The known natural products, tribromoindole 99, elatol (100), and the chamigrene 104a, have been previously isolated from algae. Isoobtusol acetate (108) and allolaurinterol acetate (101) have not been reported as natural products, but are known synthetic derivatives of natural products.

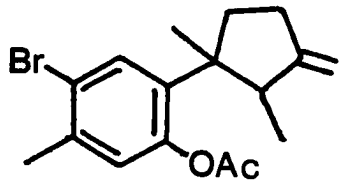
Of the eight novel compounds described, the chamigrene 105a and the dolastadiene 124 are isomers of known compounds. Dihydroxydeodactol monoacetate (32) is a more functionalized molecule, but similar to deodactol (31).

The five remaining compounds are related in having a novel carbon skeleton based on the known pimarane skeleton. The most distinctive feature in parguerol (109), parguerol 16-acetate (118), and deoxyparguerol (119) is the presence of a cyclopropane ring, while that of isoparguerol (121) and its 16-acetate (123) is a cyclobutane ring. The location of the double bond, in the otherwise rare position at C-9 (11), in all of these diterpenoids is another novel feature.

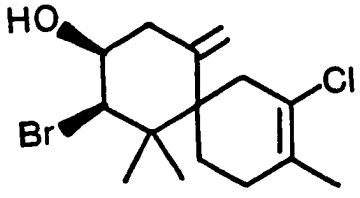
Many of the compounds isolated gave poor to marginal results in PS-388 *in vitro* testing, for example 99, $ED_{50}$=47 μg/ml; 100, $ED_{50}$=26 μg/ml; 108, $ED_{50}$=40 μg/ml; and 124, $ED_{50}$=22 μg/ml. Elatol (100) did however show a strong antifungal activity. The diterpenes of the



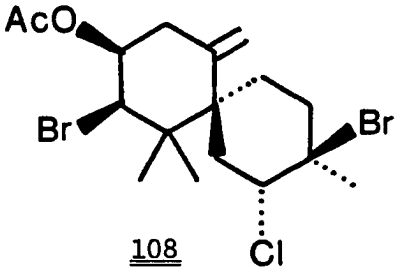

99



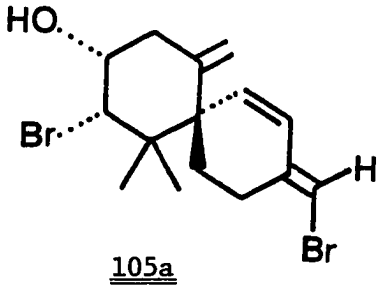

101



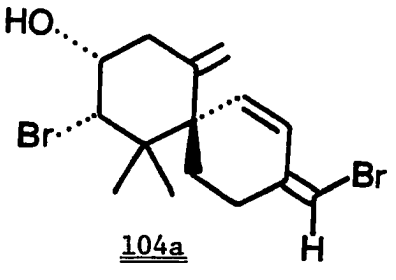

100



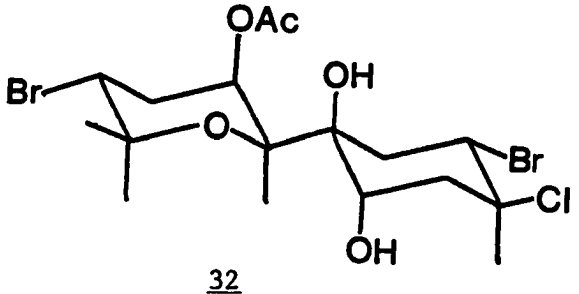

108



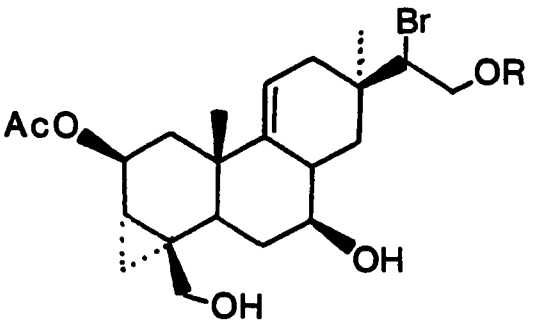

105a




104a

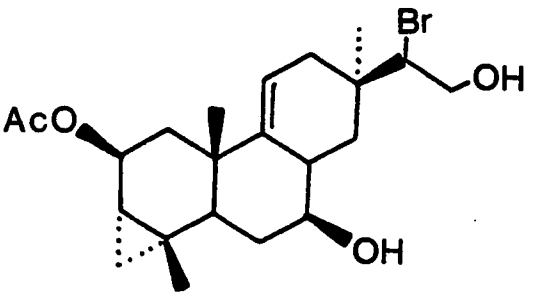



32

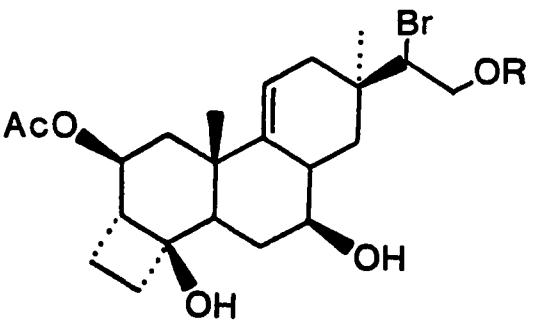



109 R=H

118 R=Ac

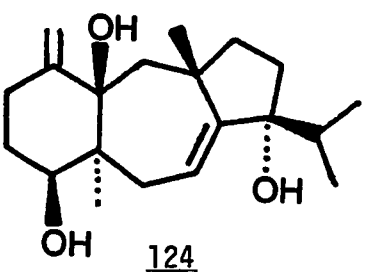



119




121 R=H

123 R=Ac




124

pimarane-type skeleton such as parguerol ($ED_{50}$=3.8 μg/ml) and deoxy-parguerol ($ED_{50}$=3.0 μg/ml) did display significant cytotoxicity.

Of the 12 compounds isolated from *Aplysia dactylomela* from Puerto Rico, none are in common with the 16 compounds isolated from *Aplysia dactylomela* from Bimini, Bahamas. Since the isolation procedures used were generally the same for all *Aplysia* specimens regardless of origin, this difference is probably a reflection of the diet of the sea hares at different locations.

## EXPERIMENTAL

Melting points were taken on an A. H. Thomas Unimelt apparatus and are uncorrected. Solvents were distilled prior to use. Chromatographic supports were Mallinckrodt silicAR CC-7, and Merck Silica Gel 60 (230-400 mesh).

Some liquid chromatographies were carried out on a Hitachi CLC-5 centrifugal preparative liquid chromatograph. High performance liquid chromatography was carried out on a Waters M-6000A pump equipped with a UV Model 440 absorbance detector and a R401 differential refractometer.

Thin layer chromatography was run on precoated Macherey-Nagel Polygram sil G/$UV_{254}$ (0.25 mm) plates. The developed chromatograms were visualized with sulfuric acid-vanillin spray.

Rotations were run on a Perkin-Elmer 141 polarimeter, ultraviolet spectra in ethanol on a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer, and infrared spectra on a Perkin-Elmer 298 infrared spectrophotometer. Low resolution mass spectra were obtained on a HP 5985 GC/MS system. 100 MHz proton magnetic resonance spectra were obtained on a Varian XL-100 spectrometer with Nicolet TT-100 Fourier Transform computer. 270 MHz proton magnetic resonance spectra were run on a Brucker magnet and probe with Nicolet 1180 computer interfaced with in-house radio frequency electronics. Carbon-13 spectra

were obtained on a Jeol FX-100 at the Colorado State Regional NMR Center. Pmr chemical shifts are reported as δ-values (ppm from tetramethylsilane as internal standard) and are followed by the signal multiplicity, the number of protons absorbing at that frequency, the coupling constants in Hertz, and the assignment if known.

High resolution mass spectra were obtained on a CEC (Dupont, Monrovia, CA) 110 mass spectrometer through the courtesy of Professor K. Biemann, Massachusetts Institute of Technology. Combustion analyses were performed by Mr. Eric Meier, Stanford University.

Initial partitioning procedure: Specimens of the sea hare, *Aplysia dactylomela*, were collected in December of 1977, off the coast of La Paguera, Puerto Rico. The excised digestive glands (8 kg wet wt.) from three hundred specimens were removed from the 70% isopropanol preservative and homogenized in a Waring blender with chloroform-methanol (2:1) and filtered through cheesecloth. The resulting aqueous suspension was partitioned against methylene chloride (2000 ml). The methylene chloride solution was evaporated to give 314.0 g of extract, designated as fraction A. Further partitioning of the aqueous suspension with 1-butanol (1000 ml) produced 21.49 g of butanol solubles, fraction B. The remaining aqueous suspension was then lyophilized and the solids obtained were washed with methanol. The methanol soluble portion, fraction C, amounted to 63.32 g, while the insoluble salts remaining, fraction D, accounted for 71.70 g. The isopropanol solution which was used as a preservative for the glands was first concentrated and then partitioned in the same manner to give parallel fractions A, 15.9 g; B, 11.9 g; C, 107.2 g; and D, 120.0 g.

A portion of fraction A (308 g) was dissolved in one liter of 10% aqueous methanol and partitioned against two liters of hexane. The hexane extract, 232.52 g, was designated fraction E. Water (125 ml) was added to the aqueous methanol solution which was then partitioned versus 1.5 liters of carbon tetrachloride to give 16.44 g of $CCl_4$ solubles, fraction F. The aqueous methanol solution was further diluted with water (260 ml) and partitioned with 1.5 liters of chloroform to give 6.83 g of chloroform solubles, fraction G. The remaining aqueous methanol solution was concentrated and lyophilized affording fraction H, 3.61 g.

Isolation of 2,3,5-tribromo-N-methylindole (99): A portion (1.1373 g) of fraction F was chromatographed over Silica Gel 60 PF-254 using chloroform as the eluant. The first 50 ml fraction collected contained an off-white solid. Recrystallization from hexane yielded pure 2,3,5-trioromo-N-methylindole as white crystalline needles, 32.4 mg (.0004%); mp. 124-124.8°; lit. mp. 120-122°[61]; ir (KBr) 2930, 1488, 1448, 1414, 1345, 1318, 1254, 1220, 1100, 1075, 945, 842, 772 $cm^{-1}$; uv (EtOH) $\lambda_{max}$ 292 nm (log $\varepsilon_{max}$ 4.01), with inflections at 300 and 284 nm; 100 MHz pmr ($CDCl_3$) $\delta$ 3.73 (s, 3H, $-NCH_3$), 7.10 (d, 1H, J=8 Hz, H-7), 7.29 (dd, 1H, J=2, 8 Hz, H-6), 7.60 (d, 1H, J=2 Hz, H-4); mass spectrum (70 eV) m/e (relative intensity) 371(30), 369(100) base peak, 367(97), 365(37), 356(3), 354(10), 352(10), 350(3), 290(4), 289(4), 288(10), 287(6), 286(5), 285(3), 275(1), 273(3), 271(1), 249(3), 247(6), 245(3), 209(9), 208(4), 207(9), 206(3), 194(13), 192(13), 128(18), and 87 (11) high resolution mass measurement $M^+$ 364.80523, $C_9H_6NBr_3^{79}$ requires 364.80502.

Isolation of elatol (100): A portion (2.13 g) of fraction F was chromatographed over silica gel eluting with chloroform and collecting 50 ml fractions. Fractions 3-8 (883.4 mg) contained a yellow oil. This oil was further chromatographed on silica gel using 5% ethyl acetate in hexane to give another oil (688.5 mg). The chromatography was repeated using 1% ethyl acetate-hexane (335 ml) as eluant followed by 2% ethyl acetate-hexane. After a total of 535 ml of solvent had been collected, crude elatol was isolated as a pale yellow oil (284.1 mg) which slowly solidified. The crystals were filtered, washed, and recrystallized in methanol to give elatol as an off-white sticky mass of crystals, 123.7 mg (.0015%).

Pure elatol has mp. 62-66°; $[\alpha]_D$ +55.2° (c. 3.68, $CHCl_3$); ir(neat) 3550, 3470, 3080, 2970, 2940, 2900, 1678, 1640, 1425, 1204, 1082, 1025, 892, 820, 805, 755 $cm^{-1}$; 100 MHz pmr ($CDCl_3$) δ 1.08 (s, 6H, $-CH_3$), 1.70 (s, 3H, $-\underset{|}{C}ClCH_3$), 1.98 (m, 5H), 2.42-2.56 (m, 4H), 4.15 (dd, 1H, J=3, 7 Hz, $-\underline{C}HOH$), 4.62 (d, 1H, J=3 Hz, $-\underline{C}HBr$), 4.80 (s, 1H, $-\underset{|}{C}=CH_2$), 5.13 (s, 1H, $-\underset{|}{C}=CH_2$); 25.05 MHz $^{13}$C-nmr ($CDCl_3$) 19.4(q), 20.7(q), 24.2(q), 25.6(t), 29.3(t), 38.0(t), 38.6(t), 43.0(s), 49.1(s), 70.6(d), 72.0(d), 115.6(t), 123.9(s), 127.8(s), 140.6(s); mass spectrum (70 eV) m/e (relative intensity) 334(1), 319(2), 317(2), 299(4), 297(6), 237(45), 236(22), 235(100) base peak, 209(16), 207(28), 200(10), 199(43), 179(12), 171(24), 157(34), 133(34), 115(42), 105(49), 93(34), 91(85), 85(68), 77(41).

Isolation of allolaurinterol acetate (101), chamigrene (104a), and chamigrene (105a): The mother liquor (1.5 g) from a recrystallization of elatol was chromatographed on TLC mesh silica gel in a CLC-5 centrifugal liquid chromatograph using 100 ml each of 2.5%, 5%, and 10% ethyl acetate-hexane followed by 200 ml of 20% ethyl acetate-hexane. After an initial collection of 150 ml, 8 ml fractions were collected. Fractions 2-5 (105 mg) were concentrated and rechromatographed on an Altex LiChrosorb Si60 5μ HPLC column using 5% ethyl acetate-hexane. Allolaurinterol acetate was thus isolated as colorless needles (17.7 mg, 0.0002%, $R_v$=30 ml).

Fractions 9-11 (1115.3 mg) of the preliminary chromatography were combined to give a pale yellow oil. A portion (85.0 mg) of this oil was chromatographed by HPLC using the same column as described above with a solvent system of 10% ethyl acetate-hexane. The mixture yielded chamigrene (105a) (21.3 mg, 0.0003%, $R_v$=40.2 ml), elatol (100) (47.2 mg, $R_v$=45.0 ml), and chamigrene (104a) (10.6 mg, .0001%, $R_v$=60.6 ml).

Pure allolaurinterol acetate (101) has mp. 80-82°; $[\alpha]_D$ +41° (c. 0.2, $CHCl_3$); lit. mp. 86.6-89.1°, $[\alpha]_D$ +48.2°; ir(neat) 3065, 2960, 2930, 2870, 1763, 1656, 1480, 1364, 1200, 1140, 1070, 900, 875, and 682 $cm^{-1}$; uv(EtOH) $\lambda_{max}$ 208 nm (7900), 268 nm (600), and 276 nm (600); 100 MHz pmr ($CDCl_3$) δ 0.70 (d, 3H, J=7.5 Hz, $-CHC\underline{H}_3$), 1.13 (s, 3H, $-CH_3$), 2.33 (s, 3H, $-OCOCH_3$), 2.36 (s, 3H, $ArC\underline{H}_3$), 2.77 (q, 1H, J=7.5 Hz, $-C\underline{H}CH_3$), 4.90 (br s, 1H, $C=CH_2$), 4.98 (br s, 1H, $C=CH_2$), 6.95 (s, 1H, ArH), 7.39 (s, 1H, ArH); mass spectrum (70 eV) m/e (relative intensity) 338(19), 336(22), 323(29), 321(32), 296(17), 295(11), 294(16), 293(9),

282(14), 281(98), 280(20), 279(100) base peak, 267(17), 265(21), 239(22), 237(23), 215(16), 214(22), 213(16), 212(13), 201(25), 200(26), 199(33), 145(46), 115(63), 91(72), 86(79), and 77(63).

Pure chamigrene (104a) has $[\alpha]_D$ -4.8° (c. 0.77, $CHCl_3$); lit.[66] $[\alpha]_D$ -4°; ir(neat) 3550, 3460, 3065, 3040, 2965, 1635, 1575, 1465, 1425, 1300, 1200, 1072, 1025, 882, 813, 742, 695 $cm^{-1}$; uv(EtOH) $\lambda_{max}$ 234 nm (16,000), 242 nm (16,500), and 250 nm (14,000); 100 MHz pmr ($CDCl_3$) δ 1.00 (s, 3H, $-CH_3$), 1.24 (s, 3H, $-CH_3$), 1.5-3.0 (m, 7H), 4.18 (m, 1H, -CHOH), 4.62 (d, 1H, J=3 Hz, -CHBr), 4.86 (s, 1H, $C=CH_2$), 5.14 (s, 1H, $C=CH_2$), 5.92 (s, 1H, C=CHBr), 6.10 (br d, 1H, J=11 Hz, -CH=CH-), 6.65 (d, 1H, J=10 Hz, -CH=CH-); mass spectrum (70 eV) m/e (relative intensity) 379(4), 378(23), 377(9), 376(51), 374(23), 297(33), 295(31), 279(68), 277(71), 253(16), 251(16), 241(65), 239(62), 237(19), 235(20), 223(17), 221(17), 213(31), 211(35), 198(21), 197(30), 183(17), 173(26), 171(44), 169(28), 167(11), 155(27), 149(23), 141(28), 131(45), 117(50), 115(68), 107(14), 105(26), 91(100) base peak, 85(88), and 83(51).

Pure chamigrene (105a) has $[\alpha]_D$ -33.3° (c. 1.47, $CHCl_3$); uv(EtOH) $\lambda_{max}$ 247 nm (16,000); ir(neat) 3550, 3460, 3060, 3030, 2960, 1638, 1578, 1460, 1425, 1348, 1295, 1200, 1072, 1022, 905, 882, 780, 685 $cm^{-1}$; 100 MHz pmr ($CDCl_3$) δ 1.01 (s, 3H, $-CH_3$), 1.24 (s, 3H, $-CH_3$), 1.5-3.0 (m, 7H), 4.20 (m, 1H, -CHOH), 4.66 (d, 1H, J=3 Hz, -CHBr), 4.84 (s, 1H, $C=CH_2$), 5.12 (s, 1H, $C=CH_2$), 5.95 (br d, 1H, J=11 Hz, CH=CH-), 6.13 (s, 1H, C=CHBr), 6.24 (d, 1H, J=10 Hz, -CH=CH-); mass spectrum (70 eV) m/e (relative intensity); 379(2), 378(12), 377(4), 376(25), 374(13), 297(22), 295(20), 279(36), 277(33), 253(8), 251(8), 241(39), 239(38), 237(9), 235(8), 213(17), 211(20), 198(11), 197(16), 183(11),

173(15), 171(24), 169(14), 167(15), 149(33), 133(38), 117(29), 115(35), 107(11), 105(18), 91(57), 85(100) base peak, 83(63), and 69(52).

Acetylation of elatol (100), 104a, and 105a: A mixture of 100, 104a, and 105a, (32 mg) was allowed to react with pyridine (1 ml) and acetic anhydride (2 ml) overnight at room temperature. The mixture was then poured over crushed ice, washed with 1N sulfuric acid, extracted into chloroform, and dried over sodium sulfate. The acetates were separated by HPLC on an Altex LiChrosorb Si60 column using 15% ethyl acetate in hexane as the solvent to give 105b (13.9 mg, $R_v$=17.6 ml), elatol acetate (15.2 mg, $R_v$=19.2 ml), and 104b (2.7 mg, $R_v$=21.6 ml).

Pure elatol acetate has: mp. 155-156°; $[\alpha]_D$ +83.5° (c. 1.46, $CHCl_3$); ir(neat) 3090, 2975, 2950, 1743, 1680, 1643, 1375, 1245, 1215, 1038, 1015, 900, 825 $cm^{-1}$; 100 MHz pmr ($CDCl_3$) δ 1.03 (s, 3H, $-CH_3$), 1.08 (s, 3H, $-CH_3$), 1.68 (s, 3H, $C=C-CH_3$), 1.5-2.8 (m, 8H), 2.06 (s, 3H, $-OCOCH_3$), 4.52 (d, 1H, J=3 Hz, -CHBr), 4.76 (s, 1H, $C=CH_2$), 5.00 (s, 1H, $C=CH_2$), 5.26 (dd, 1H, J=3, 7 Hz, -CHOAc); mass spectrum (70 eV) m/e (relative intensity) 238(6), 237(38), 236(20), 235(100) base peak, 209(14), 207(30), 199(68), 193(14), 179(22), 159(53), 157(67), 155(16), 153(29), 145(56), 143(31), 141(31), 133(42), 119(45), 117(40), 115(61), 107(63), 105(68), 93(50), 91(89), 85(86), 77(63).

Pure chamigrene acetate 104b has: mp. 115-117° (lit.[66] mp. 113-114°); $[\alpha]_D$ -25° (c. 0.18, $CHCl_3$), lit.[66] $[\alpha]_D$ -25°; ir(neat) 3080, 2970, 1743, 1642, 1375, 1245, 1205, 1038, 745, 695 $cm^{-1}$; 100 MHz pmr ($CDCl_3$) δ 1.01 (s, 3H, $-CH_3$), 1.21 (s, 3H, $-CH_3$), 2.08 (s, 3H, $-OCOCH_3$), 1.5-3.0 (m, 6H), 4.51 (d, 1H, J=3 Hz, -CHBr), 4.83 (br s, 1H, $C=CH_2$),

5.03 (br s, 1H, C=$CH_2$), 5.30 (dd, 1H, J=3, 6 Hz, -CHOAc), 5.92 (br s, 1H, C=CHBr), 6.09 (br d, 1H, J=10 Hz, -C<u>H</u>=CH-), 6.66 (d, 1H, J=10 Hz, -CH=C<u>H</u>-); mass spectrum (70 eV) m/e (relative intensity) 420(1), 418(2), 416(1), 360(2), 358(4), 356(2), 339(1), 337(1), 280(17), 279(86), 278(25), 277(100) base peak, 251(2), 249(2), 237(22), 235(31), 223(5), 221(4), 119(17), 198(13), 197(14), 183(13), 173(25), 171(35), 169(18), 155(22), 149(49), 141(22), 129(29), 128(28), 127(24), 115(52), 91(76), 85(54).

Pure chamigrene acetate <u>105b</u> has: mp. 121-123°; $[\alpha]_D$ -39.3° (c. 1.37, $CHCl_3$); ir(neat) 3060, 3040, 2970, 1745, 1642, 1375, 1245, 1215, 1038, 788, and 690 $cm^{-1}$; 100 MHz pmr ($CDCl_3$) δ 1.01 (s, 3H, $-CH_3$), 1.20 (s, 3H, $-CH_3$), 2.07 (s, 3H, $-OCOCH_3$), 1.5-3.0 (m, 6H), 4.55 (d, 1H, J=3 Hz, -CHBr), 4.80 (br s, 1H, C=$CH_2$), 5.00 (br s, 1H, C=$CH_2$), 5.30 (dd, 1H, J=4, 7 Hz, -CHOAc), 5.88 (br d, 1H, J=11 Hz, -C<u>H</u>=CH-), 6.10 (br s, 1H, C=CHBr), 6.23 (d, 1H, J=10 Hz, -CH=C<u>H</u>-); mass spectrum (70 eV) m/e (relative intensity) 420(2), 418(4), 416(2), 360(2), 358(3), 356(2), 339(4), 337(4), 280(17), 279(92), 278(32), 277(100) base peak, 251(3), 249(3), 237(17), 235(19), 223(4), 221(5), 199(11), 198(17), 197(21), 183(15), 173(25), 171(37), 169(23), 155(30), 141(26), 129(35), 128(35), 115(66), 105(29), 91(95), 85(45).

<u>Isolation of isoobtusol acetate (108)</u>: A portion (130.44 g) of fraction E was chromatographed over Silcar CC-7 in a total of eleven runs. For each run, approximately one liter each of hexane, chloroform, ethyl acetate, and methanol were used in that sequence. After removal of solvent, the weight distributions were: the hexane fraction, 1.93 g; the chloroform fraction, 57.66 g; the ethyl acetate fraction, 35.69 g;

and the methanol fraction, 22.38 g. The chloroform fraction (44.25 g) was chromatographed on Sephadex LH-20 collecting 60 ml fractions. Fractions 15-17 (1.7313 g) were combined and filtered to give isoobtusol acetate as colorless crystals from benzene-hexane, 86 mg (0.001%).

Pure isoobtusol acetate has mp. 170-173°; $[\alpha]_D$ + 57.0 (c. 0.405, $CHCl_3$); authentic sample mp. 167-170°, $[\alpha]_D$ + 51.0 (c. 5.14, $CHCl_3$); ir(KBr) 3085, 2970, 2950, 1738, 1640, 1450, 1428, 1370, 1234, 1035, 968, 900, 698 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 1.12 (s, 3H, $-CH_3$), 1.32 (s, 3H, $-CH_3$), 1.82 (dq, 1H, J=14, 3, 3, 3 Hz, $-C\underline{H}_2CH_2-$), 1.86 (dq, 1H, J=14, 3, 3, 3Hz, $-CH_2C\underline{H}_2-$), 1.92 (s, 3H, $-\overset{|}{C}BrC\underline{H}_3$), 2.11 (s, 3H, $-OCOCH_3$), 2.08 (m, 1H, $-C\underline{H}_2CH_2-$), 2.26 (m, 1H, $-CH_2C\underline{H}_2-$), 2.40 (dd, 1H, J=12.4, 4.6 Hz, $-C\underline{H}_2CHOAc$), 2.83 (dd, 1H, J=16, 4.2 Hz, $-CH_2CHCl$), 2.96 (t, 1H, J=12.2 Hz, $-C\underline{H}_2CHOAc$), 3.18 (br d, 1H, J=15.6 Hz, $-CH_2CHCl$), 4.46 (m, 2H, $-\overset{|}{C}HBr$ and $-\overset{|}{C}HCl$), 4.83 (dt, 1H, J=12.2, 4.4, 4.4 Hz, $-C\underline{H}OAc$), 4.99 (s, 1H, $C=CH_2$), 5.21 (s, 1H, $C=CH_2$); 270 MHz pmr (benzene-$d_6$) δ 0.77 (s, 3H, $-CH_3$), 1.12 (s, 3H, $-CH_3$), 1.34 (dq, 1H, J=14, 3, 3, 3 Hz, $-C\underline{H}_2CH_2-$), 1.47 (dq, 1H, J=14, 3, 3, 3 Hz, $-CH_2C\underline{H}_2-$), 1.72 (s, 3H, $-\overset{|}{C}BrCH_3$), 1.75 (s, 3H, $-OCOCH_3$), 1.83 (dt, 1H, J=14, 3, 3 Hz, $-C\underline{H}_2CH_2-$), 1.95 (br t, 1H, J=15 Hz, $-C\underline{H}_2CH_2-$), 2.30 (dd, 1H, J=13, 4.5 Hz, $-C\underline{H}_2CHOAc$), 2.77 (dd, 1H, J=16, 3 Hz, $-C\underline{H}_2\overset{|}{C}HCl$), 3.12 (m, 2H, $-C\underline{H}_2\overset{|}{C}HCl$ and $-C\underline{H}_2\overset{|}{C}HOAc$), 4.17 (m, 1H, $-CH_2C\underline{H}Cl$), 4.43 (m, 1H, $-C\underline{H}BrCHOAc$), 4.65 (s, 1H, $C=CH_2$), 4.81 (dt, 1H, J=12, 4, 4 Hz, $C\underline{H}OAc$), 4.94 (s, 1H, $C=CH_2$); 25.05 MHz $^{13}$C-nmr ($CDCl_3$) 21.0(q), 24.5(q), 25.0(q), 25.5(t), 33.0(q), 33.2(t), 33.9(t), 35.1(t), 43.6(s), 44.2(s), 65.0(d), 66.2(d), 70.9(s), 71.4(d), 114.5(t), 146.7(s), 169.8(s); mass spectrum (70 eV) m/e (relative intensity) no molecular ion was observed, 398(1), 396(2),

394(.8), 341(2), 339(2), 320(5), 319(20), 318(16), 317(100) base peak, 316(18), 315(62), 314(3), 281(13), 279(14), 237(21), 235(20), 200(12), 199(36), 173(11), 171(26), 159(13), 157(44), 145(23), 143(30), 133(17), 131(17), 129(24), 117(22), 115(25), 107(42), 105(61), 93(44), 91(77), 85(56), 79(46), and 77(46).

Isolation of parguerol (109), deoxyparguerol (119), and 4,9,14-trihydroxydolasti-1(15),7-diene (124): A portion (5.5 g) of the chloroform extract, fraction G, was chromatographed on Sephadex LH-20 using chloroform-methanol (1:1) and collecting 60 ml fractions. A brown band (420 ml) of polymeric material was eluted followed by a green band (300 ml). The latter part of this green band (fractions 13-14) weighing 0.9392 g was collected. Chromatography of this fraction on silica gel TLC mesh type H (62 g) using 4% methanol in chloroform and collecting 25 ml fractions yielded parguerol (109) (184.6 mg, 0.0023%) as a pale yellow glass in fractions 16-18. Fraction 5-7 from the above chromatography were combined (275 mg) and further chromatographed on thin layer mesh silica gel using 3% methanol in chloroform. Fractions 11-16 of the last chromatography each contained a single compound as determined by TLC and were combined to give deoxyparguerol (119) (76.5 mg, 0.001%) as a glassy solid. All attempts to crystallize either parguerol or deoxyparguerol failed. Fractions 2-10 of the last silica gel chromatography were combined and white crystals which precipitated out were collected and washed to give 6.7 mg (.0001%) of the trihydroxydolastadiene (124).

Pure 4,9,14-trihydroxydolast-1(15),7-diene (124) has mp. 220-

222°; $[\alpha]_D$ -211° (c. 0.05, $CHCl_3$); ir(KBr) 3325, 3240, 3090, 2990, 2950, 1642, 1455, 1380, 1034, 896 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 0.78 (s, 3H, C-$CH_3$), 0.84 (d, 3H, J=7 Hz, -CHC$H_3$), 1.03 (d, 3H, J=7 Hz, -CHC$H_3$), 1.23 (s, 3H, $CCH_3$), 1.2-2.1 (m, 11H), 2.66 (s, 1H, -OH), 2.88 (dt, 1H, J=14, 14, 6 Hz, -CH-C=$CH_2$), 3.35 (dd, 1H, J=15, 4 Hz, -CH-CH=C-), 3.45 (m, 1H, -CHOH), 3.71 (s, 1H, -OH), 3.74 (s, 1H, -OH), 4.79 (s, 1H, -C=$CH_2$), 4.93 (s, 1H, -C=$CH_2$), 5.63 (dd, 1H, J=9.6, 4.5 Hz, -CH-CH=C-); mass spectrum (70 eV) m/e (relative intensity) 320(1), 302(29), 287(4), 284(32), 278(25), 277(40), 274(6), 269(7), 266(5), 260(53), 259(100) base peak, 251(2), 246(14), 242(27), 241(93), 223(15), 199(11), 149(10), and 139(10); high resolution mass measurement $M^+$ 320.23635, $C_{20}H_{32}O_3$ requires 320.23514.

Pure parguerol (109) has $[\alpha]_D$ -40.0° (c. .03, $CHCl_3$); ir(neat) 3380(broad), 3060, 2935, 2880, 1725, 1380, 1368, 1250, 1020, 952, 755 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 0.09 (t, 1H, J=5 Hz, H-18 endo), 0.87 (dd, 1H, J=5, 10.5 Hz, H-18 exo), 1.00-1.50 (m, 4H), 1.06 (s, 3H, H-17), 1.13 (s, 3H, H-20), 1.70-2.10 (m, 4H), 2.07 (s, 3H, -OCO$CH_3$), 2.15-2.60 (m, 6H), 3.12 (dt, 1H, J=5, 10, 10 Hz, H-7), 3.38 (d, 1H, J=11.5 Hz, H-19), 3.52 (d, 1H, J=11.5 Hz, H-19), 3.84 (dd, 1H, J=9, 12.5 Hz, H-16), 3.95 (dd, 1H, J=3, 12.5 Hz, H-16), 4.26 (dd, 1H, J=3, 9 Hz, H-15), 5.35 (d, 2H, J=5 Hz, H-11 and H-2); 25 MHz $^{13}$C-NMR ($CDCl_3$) 18.3, 20.3, 21.6, 21.8, 23.9, 24.6, 33.4, 35.3, 36.6, 37.4. 38.2, 39.0, 45.8, 64.4, 68.2, 68.6, 68.8, 76.8, 117.4, 142.9, 170.5 ppm; mass spectrum (70 eV) m/e (relative intensity) no molecular ion was observed, 394(2), 392(2), 365(4), 363(5), 362(4), 360(4), 350(8), 349(22), 348(8), 347(35), 345(11), 319(4), 317(4), 303(3), 301(3), 299(10), 281(32), 263(24),

255(10), 253(11), 237(37), 157(41), 145(46), 131(50), 129(45), 119(68), 105(100) base peak, 93(64), 91(93), 81(53), 79(68), and 55(64).

Anal. Calcd for $C_{22}H_{33}BrO_5$: C, 57.77; H, 7.27; Br, 17.47; Found: C, 58.04, H, 7.01; Br, 16.72; high resolution mass spectrum for $C_{22}H_{31}Br^{81}O_4$ ($M^+-H_2O$), calc: 440.13864; detm: 440.13898.

Pure deoxyparguerol (119) has $[\alpha]_D$ -35.8° (c. 0.62, $CHCl_3$); ir(neat) 3360, 3055, 2960, 2870, 1725, 1455, 1380, 1365, 1250, 1055, 1020, 955, 755 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 0.01 (t, 1H, J=5.6 Hz, H-18 endo), 0.66 (dd, 1H, J=5.3, 10.5 Hz, H-18 exo), 0.79 (dd, 1H, J=6.1, 10.5 Hz, H-3), 1.02 (s, 3H, H-19), 1.04 (s, 3H, H-17), 1.12 (s, 3H, H-20), 1.21 (dd, 1H, J=6, 15 Hz, H-1 ax), 1.89 (d, 1H, J=15 Hz, H-1 eq), 2.05 (s, 3H, $-OCOCH_3$), 2.40 (br d, 1H, J=18 Hz, H-12 eq), 3.16 (dt, 1H, J=5, 10, 10 Hz, H-7), 3.83 (m, 1H, H-16), 3.90 (m, 1H, H-16), 4.27 (dd, 1H, J=3.6, 9 Hz, H-15), 5.29 (d, 1H, J-5.7 Hz, H-2), 5.34 (d, 1H, J=6 Hz, H-11); mass spectrum (70 eV) m/e (relative intensity) 424(3), 422(3), 364(8), 362(8), 349(5), 347(6), 331(4), 329(4), 325(5), 322(4), 321(2), 320(5), 284(15), 283(58), 265(31), 255(10), 240(13), 239(51), 197(23), 185(20), 183(28), 171(24), 169(26), 157(49), 145(57), 131(56), 121(41), 119(64), 105(100) base peak, 93(78), 91(92), 81(66), 79(60), 55(46).

Acetylation of Parguerol (109): Parguerol (109, 19.2 mg) was allowed to react with pyridine (1 ml) and acetic anhydride (2 ml) for 24 hours. The mixture was poured into cold water, extracted into chloroform, washed with 1N sulfuric acid, and dried over sodium sulfate to give parguerol acetate (110a, 24 mg).

Pure parguerol acetate (110a) has ir(neat) 3020, 2960, 2880, 1745, 1370, 1240, 1025, 755 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 0.17 (t, 1H, J=5.5 Hz, H-18 endo), 0.93 (dd, 1H, J=5, 10.5 Hz, H-18 exo), 1.05 (s, 3H, H-17), 1.16 (s, 3H, H-20), 1.10-1.40 (m, 4H), 1.65-2.05 (m, 4H), 2.08 (s, 6H, $-OCOCH_3$), 2.10 (s, 3H, $-OCOCH_3$), 2.14 (s, 3H, $-OCOCH_3$), 2.29 (dt, 1H, J=4, 4, 12 Hz, H-6 eq), 2.47 (br d, 1H, J=18 Hz, H-12eq), 2.59 (br t, 1H, J=10, 10 Hz, H-8), 3.71 (d, 1H, J=12 Hz, H-19), 4.04 (d, 1H, J=12 Hz, H-19), 4.20-4.35 (m, 2H, H-16), 4.41 (dt, 1H, J=5, 11, 11 Hz, H-7), 4.49 (d, 1H, J=9.5 Hz, H-15), 5.36 (d, 1H, J=5 Hz, H-2), 5.40 (d, 1H, J=6.2 Hz, H-11); 270 MHz pmr ($CCl_4$) δ 0.17 (t, 1H, J=6 Hz, H-18 endo), 0.94 (dd, 1H, J=5, 10.7 Hz, H-18 exo), 1.06 (s, 3H, H-17), 1.00-1.40 (m, 4H), 1.12 (s, 3H, H-20), 1.76 (m, 2H, H-6ax and H-12ax), 1.89 (d, 1H, J=15.4 Hz, H-1eq), 1.97 (m, 1H, H-14eq), 2.02 (s, 3H, $-OCOCH_3$), 2.03 (s, 3H, $-OCOCH_3$), 2.06 (s, 3H, $-OCOCH_3$), 2.08 (s, 3H, $-OCOCH_3$), 2.25 (dt, 1H, J=4, 4, 12 Hz, H-6eq), 2.45 (br d, 1H, J=17.4 Hz, H-12eq), 2.62 (br t, 1H, J=11, 12 Hz, H-8), 3.74 (d, 1H, J=12 Hz, H-19), 3.96 (d, 1H, J=12 Hz, H-19), 4.16 (m, 2H, H-16), 4.31 (dt, 1H, J=5, 11, 11 Hz, H-7), 4.47 (dd, 1H, J=3, 11 Hz, H-15), 5.27 (d, 1H, J=5 Hz, H-2), 5.38 (d, 1H, J=6 Hz, H-11); 270 MHz pmr (benzene-$d_6$) δ -0.25 (t, 1H, J=6 Hz, H-18 endo), 0.56 (dd, 1H, J=5.6, 10.6 Hz, H-18 exo), 0.91 (s, 3H, H-17), 0.80-1.10 (m, 3H), 1.20 (s, 3H, H-20), 1.26 (dd, 1H, J=11.4, 13.8 Hz, H-14ax), 1.50-1.75 (m, 2H), 1.64 (s, 3H, $-OCOCH_3$), 1.71 (s, 3H, $-OCOCH_3$), 1.73 (s, 3H, $-OCOCH_3$), 1.77 (s, 3H, $-OCOCH_3$), 1.86 (ddd, 1H, J=12, 12, 12 Hz, H-6ax), 2.05 (ddd, 1H, J=3.6, 6, 13.8 Hz, H-14eq), 2.36 (dt, 1H, J=4, 4, 13 Hz, H-6eq), 2.45 (br d, 1H, J=18 Hz, H-12eq), 2.78 (br t, 1H, J=11 Hz,

H-8), 3.56 (d, 1H, J=12.4 Hz, H-19), 3.85 (d, 1H, J=12.4 Hz, H-19), 4.21 (m, 1H, H-15), 4.55 (m, 3H, H-7 and H-16), 5.12 (d, 1H, J=6.6 Hz, H-11), 5.34 (d, 1H, J=5.4 Hz, H-2); 25.05 MHz $^{13}$C-nmr ($CDCl_3$) 18.9, 19.7, 20.7, 21.0, 21.2, 21.3, 21.7, 22.0, 24.2, 29.6, 35.1, 35.4, 36.4, 37.4, 38.6, 45.6(d), 58.9(d), 65.8(t), 68.2(d), 69.5(t), 78.3(d), 118.1(d), 142.0(s), 170.2(s), 170.4(s), 170.4(s), 170.7(s); mass spectrum (70 eV) m/e (relative intensity) 464(10), 462(10), 436(8), 434(6), 405(6), 404(22), 403(6), 402(21), 389(6), 387(6), 376(3), 374(3), 361(4), 359(3), 329(12), 327(12), 323(22), 264(26), 263(100) base peak, 237(66), 235(36), 221(64), 193(33), 169(22), 157(21), 131(26), 105(37), 93(21), 91(33).

Parguerol tribenzoate (110b): Parguerol (109, 5 mg) was added to pyridine (1 ml) and freshly distilled benzoyl chloride (2 drops) and allowed to react at room temperature overnight. The mixture was chromatographed by preparative TLC using 20% ethyl acetate in hexane to give parguerol tribenzoate, 2.8 mg, $R_f$ .33; ir(neat) 3065, 2960, 2880, 1725, 1604, 1585, 1450, 1385, 1370, 1315, 1275, 1245, 1175, 1115, 1068, 1025, 755, 710 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) $\delta$ 0.27 (t, 1H, J=5.7 Hz, H-18 endo), 1.04 (dd, 1H, J=5.4, 10.8 Hz, H-18 exo), 1.08 (s, 3H, H-17), 1.28 (s, 3H, H-20), 1.15-1.55 (m, 4H), 1.80-2.05 (m, 3H), 2.04 (s, 3H, $-OCOCH_3$), 2.18 (br d, 1H, J=15 Hz, H-14eq), 2.54 (m, 2H, H-6eq and H-12eq), 2.92 (br t, 1H, J=11 Hz, H-8), 3.90 (d, 1H, J=12 Hz, H-19), 4.36 (d, 1H, J=12 Hz, H-19), 4.46 (m, 2H, H-15 and H-16), 4.69 (dt, 1H, J=4, 11, 11 Hz, H-7), 4.83 (dd, 1H, J=3, 12 Hz, H-16), 5.40 (d, 1H, J=5 Hz, H-2), 5.46 (d, 1H, J=6 Hz, H-11), 7.35-8.10 (m, 15H, ArH); mass spectrum (70 eV) m/e (relative intensity) 329(1), 327(1),

264(4), 263(15), 237(9), 221(6), 106(8), 105(100) base peak, 77(19).

Deoxyparguerol acetate (120): Deoxyparguerol (119, 20 mg) was allowed to react with acetic anhydride (2 ml) and pyridine (1 ml) overnight at room temperature. The mixture was poured into cold water and the product was extracted into chloroform, washed with water, and dried over sodium sulfate to give 120 in quantitative yield.

Pure deoxyparguerol acetate (120) has ir(neat) 3055, 3020, 2955, 2870, 1737, 1365, 1240, 1020, 750 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) $\delta$ 0.01 (t, 1H, J=5.4 Hz, H-18 endo), 0.65 (dd, 1H, J=4.2, 10.4 Hz, H-18 exo), 0.78 (dd, 1H, J=5.8, 10 Hz, H-3), 1.00 (s, 3H, H-19), 1.02 (s, 3H, H-17), 1.12 (s, 3H, H-20), 1.10-1.40 (m, 3H), 1.70 (ddd, 1H, J=12, 12, 12 Hz, H-6ax), 1.82 (br d, 1H, J=2, 4, 18.3 Hz, H-12ax), 1.91 (d, 1H, J=15 Hz, H-1eq), 2.02 (m, 1H, J=3.7, 6.4, 14.7 Hz, H-14eq), 2.08 (s, 3H, $-OCOCH_3$), 2.10 (s, 3H, $-OCOCH_3$), 2.14 (s, 3H, $-OCOCH_3$), 2.16 (dt, 1H, J=4, 4, 13 Hz, H-6eq), 2.43 (br d, 1H, J=6.8, 18.3 Hz, H-12eq), 2.56 (br t, 1H, J=11, 11 Hz, H-8), 4.28 (m, 2H, H-16), 4.38 (dt, 1H, J=5, 11, 11 Hz, H-7), 4.53 (d, 1H, H-15), 5.28 (d, 1H, J-5 Hz, H-2), 5.36 (d, 1H, J=6.7 Hz, H-11); 25 MHz $^{13}C$-nmr ($CDCl_3$) 17.2(s), 19.5(d), 21.1,21.4, 21.7, 23.2, 23.6, 24.2, 30.7, 35.2, 35.4, 37.0, 37.5, 38.1, 38.7, 46.4(d), 59.0(d), 66.0(t), 69.2(d), 78.7(d), 117.9(d), 142.4(s), 170.5(s); mass spectrum (70 eV) m/e (relative intensity) 467(26), 466(84), 465(26), 464(97), 422(3), 420(4), 407(8), 406(19), 405(8), 404(19), 399(20), 391(11), 389(10), 331(17), 329(19), 325(62), 299(16), 283(18), 266(17), 265(80), 240(23), 239(100) base peak, 223(38), 209(32), 197(33), 183(39), 169(39), 157(51), 145(62), 131(50),

119(31), 105(58), 91(57), 81(24), 79(34), 55(20); high resolution mass measurement $M^+$-HOAc 464.1544, $C_{24}H_{33}O_4Br^{79}$ requires 464.1562.

Ozonolysis of parguerol acetate: Parguerol acetate (110a) (24 mg) was dissolved in 8 ml of methylene chloride-methanol (1:1). The solution was cooled in a dry ice-acetone bath and ozone was bubbled in at a rate of 4 ml/sec. When a persistent pale blue color appeared, the gas flow was discontinued and the reaction vessel was kept cold for 1/2 hour. Dimethyl sulfide (4 drops) was added and the flask was slowly allowed to come to room temperature. The keto acetal product (111), 21 mg, was washed with water and dried over sodium sulfate. Chromatography of the product gave pure 111: ir(neat) 2930, 2850, 2830, 1735, 1450, 1365, 1240, 1150, 1120, 1040, 965, 900, 752 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 0.20 (t, 1H, H=6 Hz, H-18 endo), 0.96 (s, 3H, H-17), 0.90-1.60 (m, 5H), 1.37 (s, 3H, H-20), 1.85-2.05 (m, 2H), 1.75 (m, 2H, H-12), 2.06 (s, 3H, $-OCOCH_3$), 2.09 (s, 3H, $-OCOCH_3$), 2.11 (s, 3H, $-OCOCH_3$), 2.13 (s, 3H, $-OCOCH_3$), 2.50 (m, 2H, H-6eq and H-14 eq), 3.07 (dd, 1H, J=8, 11 Hz, H-8), 3.32 (s, 3H, $-OCH_3$), 3.34 (s, 3H, $-OCH_3$), 3.72 (d, 1H, J=12 Hz, H-19), 4.10 (d, 1H, J=12 Hz, H-19), 4.24 (dd, 1H, J=3, 9 Hz, H-15), 4.50 (m, 4H, H-7, H-11, H-16), 5.32 (d, 1H, J=5 Hz, H-2); 270 MHz pmr (benzene-$d_6$) δ -0.36 (t, 1H, J=6 Hz, H-18 endo), 0.49 (dd, 1H, J=5.5, 10.8 Hz, H-18 exo), 0.88 (dd, 1H, J=3.4, 14 Hz, H-5), 0.98 (dd, 1H, J=7.4, 10.8 Hz, H-3), 1.09 (s, 3H, H-17), 1.26 (s, 3H, H-20), 1.15-1.40 (m, 1H), 1.45 (d, 1H, J=14.3 Hz, H-14a), 1.61 (s, 3H, $-OCOCH_3$), 1.65 (s, 3H, $-OCOCH_3$), 1.74 (s, 3H, $-OCOCH_3$), 1.84 (s, 3H, $-OCOCH_3$), 1.80-2.10 (m, 4H), 2.40 (ddd, 1H, J=3.4, 5,

12.8 Hz, H-6eq), 2.74 (dd, 1H, J=7.7, 14.3 Hz, H-14eq), 3.11 (s, 6H, $-OCH_3$), 3.12 (m, 1H, H-8), 3.56 (d, 1H, J=12 Hz, H-19), 3.76 (d, 1H, J=12 Hz, H-19), 4.42 (dd, 1H, J=4, 8 Hz, H-15), 4.56 (m, 2H, H-7 and H-11), 4.67 (dd, 1H, J=8, 12.3 Hz, H-16), 4.77 (dd, 1H, J=4, 12.3 Hz, H-16), 5.30 (d, 1H, J=5 Hz, H-2); mass spectrum (70 eV) (relative intensity) 497(2), 495(2), 428(100) base peak, 415(21), 368(22), 353(14), 337(17), 308(25), 295(29), 293(31), 261(44), 233(45), 162(56), 105(70), 91(78), 95(53), 79(49).

Oxidation of the keto-acetal (111): Jones reagent (5 drops) was added dropwise to a solution of the keto-acetal (111) (21 mg) in acetone (1 ml) until an orange color persisted. The resulting acid was dissolved in chloroform, washed with water and dried to give an oil, 6 mg. Keto-acid (112) has the following spectral properties: ir(neat) 3400 (broad), 3020, 2960, 2930, 2850, 1740, 1450, 1370, 1240, 1035, 755 $cm^{-1}$; 270 MHz pmr (benzene-$d_6$) $\delta$ -0.36 (t, 1H, J=6 Hz, H-18 endo), 0.52 (dd, 1H, J=6, 11.3 Hz, H-18 exo), 0.88 (s, 3H, H-17), 0.80-2.10 (m, 8H), 1.26 (s, 3H, H-20), 1.65 (s, 3H, $-OCOCH_3$), 1.67 (s, 3H, $-OCOCH_3$), 1.70 (s, 3H, $-OCOCH_3$), 1.89 (s, 3H, $-OCOCH_3$), 2.35-2.65 (m, 2H), 3.04 (dd, 1H, J=8, 11 Hz, H-8), 3.56 (d, 1H, J=12 Hz, H-19), 3.73 (d, 1H, J=12 Hz, H-19), 4.45 (m, 2H, H-7 and H-16), 4.84 (dd, 1H, J=3, 12.5 Hz, H-16), 5.09 (br d, 1H, J=9 Hz, H-15), 5.29 (d, 1H, J=5.7 Hz, H-2); mass spectrum (70 eV) m/e (relative intensity) 491(19), 448(10), 431(63). 417(13), 389(30), 388(31), 375(56), 370(32), 266(42), 199(48), 119(49), 105(100) base peak, 91(68), 79(52).

Manganese dioxide oxidation of Parguerol: Parguerol (10.5 mg) was

dissolved in tetrahydrofuran (1 ml) and reacted for five minutes at room temperature with a saturated solution of lithium aluminum hydride in tetrahydrofuran (3 ml). The reaction was quenched with water and the mixture was extracted into chloroform. The product was purified by preparative TLC (10% methanol in chloroform solvent) to yield a tetraol (2.7 mg, $R_f$ = .13-.29). The tetraol was then stirred at room temperature with 25.6 mg of activated manganese dioxide for one hour. The mixture was filtered and the product was purified by HPLC on an Altex LiChrosorb Si60 column using 15% isopropanol in chloroform as solvent. The oxidation product (2.3 mg, $R_v$=22 ml) had the following spectral properties: ir(neat) 3360, 2925, 2855, 1722, 1600, 1455, 1378, 1015, 750 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) $\delta$ 0.08 (t, 1H, J=6 Hz, H-18endo), 0.90 (dd, 1H, J=5, 10 Hz, H-18 exo), 1.21 (s, 3H, $-CH_3$), 1.28 (s, 3H, $-CH_3$), 1.1-2.7 (m, 14H), 3.20 (m, 1H, H-7), 3.38 (d, 1H, J=12 Hz, H-19), 3.64 (d, 1H, J=12 Hz, H-19), 4.18 (d, 1H, J=4 Hz, H-15), 4.44 (d, 1H, J=4 Hz, H-2), 5.43 (d, 1H, J=5 Hz, H-11), 9.56 (d, 1H, J=4 Hz, H-16).

Reduction with zinc-acetic acid: Parguerol (5 mg) was dissolved in tetrahydrofuran (10 ml) with zinc (87.4 mg) and glacial acetic acid (2 drops) added. After one hour of reflux, mostly starting material was recovered. The procedure was repeated with 5 drops of acetic acid and, after an additional hour of reflux, the mixture was stirred at

room temperature for 5 hours. Preparative TLC of the mixture with 10% isopropanol in chloroform as solvent separated three compounds (1.7 mg total, $R_f$ = .36-.59) from the starting material. Separation by HPLC of the mixture of compounds on Partisil PXS (10/25 mm) using 10% isopropanol in chloroform as the solvent system yielded a compound (0.2 mg) which had the following spectral properties: 270 MHz pmr ($CDCl_3$) δ 0.08 (m, 1H, H-18 endo), 0.86 (m, 1H, H-18 exo), 1.02 (s, 3H, H-20), 1.05 (s, 3H, H-17), 2.08 (s, 3H, $-OCOC\underline{H}_3$), 4.89 (d, 1H, J=17 Hz, $-C=C\underline{H}_2$), 4.89 (d, 1H, J=12 Hz, $-CH=C\underline{H}_2$), 5.73 (dd, 1H, J=11,18 Hz, $-C\underline{H}=CH_2$). Lack of sufficient material prevented complete spectral characterization.

Isolation of isoparguerol (121), parguerol 16-acetate (118), and isoparguerol 16-acetate (123): The green band (1.0 g) obtained from a Sephadex LH-20 column (see isolation of parguerol above) was chromatographed on silica gel 60 (100 g, 230-400 mesh) using 10% methanol in chloroform. The first fraction (150 ml) contained 661 mg of material and the second fraction (50 ml) contained 309 mg. This second fraction was quickly passed through 1.6 g of neutral alumina (activity II) to remove the green pigmentation which accompanies parguerol. A portion of crude parguerol (160 mg) was chromatographed on an Altex LiChrosorb Si60 HPLC column 5μ (10 x 250 mm) using 10% isopropanol in chloroform as eluant. Parguerol (109) (150.6 mg, 0.002%, $R_v$=34.8 ml) was isolated along with a minor component, isoparguerol (6.8 mg, 0.0001%, $R_v$=33.2 ml). The first fraction (660 mg) from the above silica gel chromatography was rechromatographed using 3-5% methanol in chloroform

collecting 100 ml fractions. Fractions 3 and 4 (244.9 mg) were combined, passed through neutral alumina, and separated by HPLC on a 10 micron μBondapak $C_{18}$ reverse phase column (7.8 x 300 mm) using 20% aqueous methanol. Two fractions of interest were collected: fraction IV (67.5 mg, $R_v$=19.2 ml) and fraction V (34.8 mg, $R_v$=20.8 ml). Fraction IV was separated by preparative TLC using 4% methanol in chloroform to give parguerol 16-acetate (21.6 mg, 0.0003%, $R_f$=.13-.33). Fraction V (31.5 mg) was rechromatographed by adsorption HPLC using 5% isopropanol in chloroform to give isoparguerol 16-acetate (4.9 mg, 0.00006%, $R_v$=32 ml).

Pure isoparguerol (121) has $[\alpha]_D$ +3.6° (c. .14, $CHCl_3$); mp. 139-141°; ir(neat) 3360, 2925, 2870, 1710, 1375, 1365, 1250, 1020, 945, and 750 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 1.08 (s, 3H, H-17), 1.21 (s, 3H, H-20), 1.00-2.60 (m, 18H), 2.03 (s, 3H, $-OCOCH_3$), 3.29 (dt, 1H, J=6.5, 11.6, 11.6 Hz, H-7), 3.84 (dd, 1H, J=9, 11 Hz, H-16), 3.94 (br d, 1H, J=12 Hz, H-16), 4.29 (dd, 1H, J=4, 10 Hz, H-15), 4.96 (d, 1H, J=4.8 Hz, H-2), 5.44 (d, 1H, J=6 Hz, H-11); mass spectrum (70 eV) m/e (relative intensity) 440(4), 438(4), 422(1), 420(1), 394(2), 392(2), 383(3), 381(3), 380(2), 378(2), 365(15), 363(16), 362(6), 360(5), 352(30), 350(28), 347(36), 345(35), 337(8), 335(8), 319(12), 317(14), 299(14), 281(30), 271(42), 237(36), 227(50), 197(29), 185(36), 183(32), 159(59), 157(55), 145(53), 143(62), 133(42), 131(66), 119(79), 105(100) base peak, 91(81), 79(55), 55(49).

Pure parguerol 16-acetate (118) has mp. 121-123°; $[\alpha]_D$ -40.5 (c. 0.40, $CHCl_3$); ir(neat) 3400, 3060, 2940, 2880, 1742, 1726, 1450, 1380, 1368, 1245, 1035, 1020, 950, 775 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 0.08

(t, 1H, J=5.8 Hz, H-18 endo), 0.87 (dd, 1H, J=5, 11 Hz, H-18 exo), 1.06 (s, 3H, H-17), 1.13 (s, 3H, H-20), 0.95-1.50 (m, 4H), 1.70-2.10 (m, 5H), 2.07 (s, 3H, $-OCOCH_3$), 2.11 (s, 3H, $-OCOCH_3$), 2.20-2.55 (m, 4H), 3.15 (dt, 1H, J=5, 10, 10 Hz, H-7), 3.42 (d, 1H, J=13 Hz, H-19), 3.48 (d, 1H, J=13 Hz, H-19), 4.25 (m, 2H, H-16), 4.60 (m, 1H, H-15), 5.35 (d, 1H, J=6.5 Hz, H-11), 5.36 (d, 1H, J=5.8 Hz, H-2); mass spectrum (70 eV) m/e (relative intensity) 482(2), 480(3), 436(4), 434(4), 422(6), 420(5), 407(3), 405(3), 404(5), 402(6), 391(10), 389(15), 341(13), 331(11), 329(19), 327(9), 323(14), 281(37), 263(71), 255(26), 237(79), 221(43), 157(58), 145(64), 131(64), 119(77), 105(100) base peak, 91(100) base peak, 79(58).

Pure isoparguerol 16-acetate (123) has mp. 180-182°; $[\alpha]_D$ -18.8 (c. 0.09, $CHCl_3$); ir($CHCl_3$) 3600, 2920, 2850, 1725, 1380, 1365, 1250, 1015 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) $\delta$ 1.09 (s, 3H, H-17), 1.22 (s, 3H, H-20), 1.10-2.10 (m, 13H), 2.03 (s, 3H, $-OCOCH_3$), 2.11 (s, 3H, $-OCOCH_3$), 2.20-2.60 (m, 4H), 3.27 (dt, 1H, $w_{1/2}$ = 22.7 Hz, H-7), 4.28 (m, 2H, H-15 and H-16), 4.60 (m, 1H, H-16), 4.97 (d, 1H, J=4 Hz, H-2), 5.44 (d, 1H, J=6.3 Hz, H-11); mass spectrum (70 eV) m/e (relative intensity) 482(3), 480(3), 454(4), 452(4), 440(3), 438(3), 425(2), 423(3), 422(2), 420(2), 412(4), 410(4), 407(13), 405(15), 394(8), 392(7), 379(5), 377(4), 365(4), 363(3), 347(57), 345(58), 319(19), 317(26), 281(18), 271(17), 265(33), 255(41), 253(51), 239(28), 237(37), 227(90), 157(65), 145(68), 143(72), 131(78), 119(100) base peak, 105(97), 91(93), 79(49), 55(53).

Isoparguerol triacetate (122): Isoparguerol (121, 1.5 mg) was allowed to react with acetic anhydride (.2 ml) and pyridine (.1 ml) overnight at room temperature. The mixture was poured into cold water and the product was extracted into chloroform, washed with water, and dried over sodium sulfate. Chromatography of the product gave pure isoparguerol triacetate: ir(neat) 3500, 2930, 2875, 1735, 1460, 1380, 1365, 1240, 1150, 1020, 950, 755 $cm^{-1}$; 270 MHz pmr ($CDCl_3$) δ 1.05 (s, 3H, H-17), 1.21 (s, 3H, H-20), 1.30-1.90 (m, 5H), 2.02 (s, 3H, $-OCOCH_3$), 2.05 (s, 3H, $-OCOCH_3$), 2.11 (s, 3H, $-OCOCH_3$), 2.40-2.70 (m, 3H, H-3, H-8, and H-12eq), 4.27 (m, 2H, H-16), 4.50 (m, 2H, H-7 and H-15), 4.95 (d, 1H, J=3.6 Hz, H-2), 5.48 (d, 1H, J=6 Hz, H-11); 270 MHz pmr (benzene-$d_6$) δ 0.72 (quintet, 1H, J=10.4 Hz, H-18 endo), 0.88 (s, 3H, H-17), 1.00-1.70 (m, 8H), 1.26 (s, 3H, H-20), 1.61 (s, 3H, $-OCOCH_3$), 1.66 (s, 3H, $-OCOCH_3$), 1.69 (s, 3H, $-OCOCH_3$), 1.75-1.95 (m, 3H), 2.00-2.20 (m, 2H, H-14eq and H-3), 2.45 (br d, 1H, J=17 Hz, H-12eq), 2.75 (br t, 1H, H-8), 4.24 (dd, 1H, J=10, 13 Hz, H-15), 4.45-4.65 (m, 3H, H-16 and H-7), 4.88 (d, 1H, J=4 Hz, H-2), 5.15 (d, 1H, J=5.8 Hz, H-11); mass spectrum (70 eV) m/e (relative intensity) 482(7), 480(7), 454(11), 452(11), 423(7), 422(20), 421(7), 420(21), 407(12), 405(13), 404(5), 402(7), 394(18), 392(18), 347(56), 345(57), 333(11), 331(9), 319(16), 317(21), 281(34), 271(22), 265(25), 263(27), 255(36), 253(50), 237(35), 227(100) base peak, 211(39), 209(36), 185(44), 157(71), 143(70), 131(70), 119(75), 105(81), 91(62), 69(48), 61(56).

Isolation of Dihydroxydeodactol Monoacetate (32): The 13th fraction of the Sephadex LH-20 chromatography of the chloroform extract (fraction G) was chromatographed over TLC mesh silica gel with chloroform to give 61 mg of 32.

Pure dihydroxydeodactol monoacetate (32) has: mp. 168-169°; $[\alpha]_D$+40.5° ($CHCl_3$); ir(KBr) 3440, 3000, 2945, 1730, 1395, 1270, and 1055 $cm^{-1}$; 360 MHz NMR ($CDCl_3$) δ 1.42, 1.44 (s, 3H ea, C-12-13), 1.47 (s, 3H, C-14), 1.58 (br s, 2H, -OH), 1.87 (s, 3H, C-15), 2.14 (s, 3H, -OAc), 2.24 (dt, 1H, J=3.9, 14.8 Hz, C-9 $H_{eq}$), 2.35 (dd, 1H, J=2.7, 14.3 Hz, C-4 H), 2.42-2.74 (m, 4H), 4.03 (br s, 1H, C-5 H), 4.24 (dd, 1H, J=4.2, 12.8 Hz, C-10 H), 4.78 (dd, 1H, J=4.2, 12.8 Hz, C-2 H), 5.04 (t, 1H, J=3.6 Hz, C-8 H); mass spectrum (70 eV) m/e (relative intensity) 510(.34), 508(1.2), 506(1.7), 504(.85), 490(.88), 488(1.2), 486(0.6), 471(.85), 469(1.3), 467(.71), 265(29), 263(30), 223(6), 221(6), 206(11), 205(9), 204(12), 203(8), 186(28), 141(11), 125(17), 124(14), 123(27), 109(32), 81(22), 43(100).

Anal. Calcd for $C_{17}H_{27}O_5Br_2Cl$: C, 40.30; H, 5.37; Br, 31.54; Cl, 7.00. Found: C, 41.90; H, 5.48; Br, 32.21; Cl, 6.86.

Oxidation of dihydroxydeodactol monoacetate (32): Jones reagent (.1 ml) was added dropwise to a solution of dihydroxydeodactol monoacetate (32) (35.4 mg) in acetone (5 ml) at room temperature until a permanent yellow color was observed. The ketone (126) was extracted with ether, washed with water, and recrystallized from hexane to give white crystals in quantitative yield, mp. 154.5-155°; ir(KBr) 3475, 2990, 2975, 1740, 1725, 1385, 1380, 1250, 1110, 977, and 808 $cm^{-1}$;

100 MHz NMR ($CDCl_3$) δ 1.47, 1.48, 1.53 (s, 3H ea, C-12-13-14), 1.83 (s, 3H, C-15), 2.00 (s, 3H, -OAc), 2.10-2.70 (m, 3H), 2.94 (dd, 1H, J=12, 14 Hz, C-1 $H_{ax}$), 3.20 (d, 1H, J=13 Hz, C-4 $H_{ax}$), 3.73 (d, 1H, J=13 Hz, C-4 $H_{eq}$), 3.83 (s, 1H, -OH), 4.12 (dd, 1H, J=4, 12 Hz, C-10 H), 4.36 (dd, 1H, J=4, 12 Hz, C-2 H), 5.02 (dd, 1H, J=3, 4, Hz, C-8 H); mass spectrum (70 eV) m/e (relative intensity) 427(1.3), 425(5), 423(4), 398(.7), 396(2), 394(2), 329(.5), 327(1), 325(.5), 287(.9), 285(2), 283(2), 265(13), 263(13), 223(4), 221(4), 205(6), 203(5), 186(11), 141(8), 125(12), 123(16), 69(16), 43(100).

Dehydrochlorination of Ketone (126): The ketone (126) (13.4 mg) was dissolved in benzene (.1 ml) in a nitrogen atmosphere. DBN (3.4 mg) was added and the mixture was heated to reflux for 15 minutes. The mixture was then poured on ice, washed with 1N sulfuric acid and extracted with hexane to yield a clear oil (127), ir(KBr) 3450, 2990, 1750, 1685, 1382, 1245, 1120, 1055, 860, and 760 $cm^{-1}$; 100 MHz NMR ($CDCl_3$) δ 1.29, 1.48, 1.57 (s, 3H ea, C-12-13-14), 2.01 (s, 3H, -OAc), 2.09 (br s, 3H, C-15), 2.10-2.70 (m, 3H), 3.08 (dd, 1H, J=5, 14 Hz, C-1 $H_{ax}$), 4.08 (dd, 1H, J=5, 12 Hz, C-10 H), 4.10 (s, 1H, -OH), 4.96 (m, 2H, C-2 H and C-8 H), 6.11 (br s, 1H, C-4 H); mass spectrum (heating elements off) m/e (relative intensity) 470(.6), 468(1), 466(.6), 265(88), 263(85), 223(22), 221(21), 206(22), 205(32), 204(22), 203(32), 141(21), 125(58), 124(32), 123(69), 109(32), 95(30), 43(100).

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