

## INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.
2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.
3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.
4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.
5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

**Xerox University Microfilms**

300 North Zeeb Road  
Ann Arbor, Michigan 48106

75-21,835

VANDERAH, David John, 1946-

I. DACTYLYNE, A REVISED STRUCTURE, AND ISODACTYLYNE  
A NOVEL HALOGENATED ACETYLENIC COMPOUND. II. THREE  
NEW SESQUITERPENES FROM THE SEA HARE APLYSIA  
DACTYLOMELA (RANG): DACTYLENOL, DACTYLENOL ACETATE,  
AND DACTYLOL. III. ISOLATION OF DENDROLASIN FROM  
THE SPONGE OLIGOCERAS HEMORRHAGES. IV. ISOLATION  
OF TWO KNOWN SAPOGENINS FROM THE SEA CUCUMBER  
HOLOTHURIA ATRA. V. A NOVEL POLACETYLATED COMPOUND  
FROM A SOFT CORAL. VI. CEMBRENE-A AND TWO NEW

**Xerox University Microfilms**, Ann Arbor, Michigan 48106

75-21,835

VANDERAH, David John, 1946-

CEMBRENE DERIVATIVES FROM A PACIFIC SOFT CORAL,  
NEPHTHEA SP.

The University of Oklahoma, Ph.D., 1975  
Chemistry, organic

**Xerox University Microfilms**, Ann Arbor, Michigan 48106

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

- I. DACTYLYNE, A REVISED STRUCTURE, AND ISODACTYLYNE A NOVEL HALOGENATED ACETYLENIC COMPOUND.
- II. THREE NEW SESQUITERPENES FROM THE SEA HARE *APLYSIA DACTYLOMELA* (RANG): DACTYLENOL, DACTYLENOL ACETATE, AND DACTYLOL.
- III. ISOLATION OF DENDROLASIN FROM THE SPONGE *OLIGOCERAS HEMORRHAGES*.
- IV. ISOLATION OF TWO KNOWN SAPOGENINS FROM THE SEA CUCUMBER *HOLOTHURIA ATRA*.
- V. A NOVEL POLACETYLATED COMPOUND FROM A SOFT CORAL.
- VI. CEMBRENE-A AND TWO NEW CEMBRENE DERIVATIVES FROM A PACIFIC SOFT CORAL, *NEPHTHEA SP.*

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

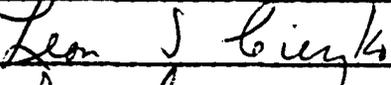
DAVID JOHN VANDERAH

Norman, Oklahoma

1975

- I. DACTYLYNE, A REVISED STRUCTURE, AND ISODACTYLYNE, A NOVEL HALOGENATED ACETYLENIC COMPOUND.
- II. THREE NEW SESQUITERPENES FROM THE SEA HARE *APLYSIA DACTYLOMELA* (RANG): DACTYLENOL, DACTYLENOL ACETATE, AND DACTYLOL.
- III. ISOLATION OF DENDROLASIN FROM THE SPONGE *OLIGOCERAS HEMORRHAGES*.
- IV. ISOLATION OF TWO KNOWN SAPOGENINS FROM THE SEA CUCUMBER *HOLOTHURIA ATRA*.
- V. A NOVEL POLYACETYLATED COMPOUND FROM A SOFT CORAL.
- VI. CEMBRENE-A AND TWO NEW CEMBRENE DERIVATIVES FROM A PACIFIC SOFT CORAL, *NEPHTHEA SP.*

APPROVED BY

  
\_\_\_\_\_  
  
\_\_\_\_\_  
  
\_\_\_\_\_  
  
\_\_\_\_\_  
  
\_\_\_\_\_

DISSERTATION COMMITTEE

## Acknowledgements

My deepest appreciation is expressed to Professor F. J. Schmitz for his direction and guidance during the course of these studies. His patience and understanding during periods of slow results is appreciated.

A special note of gratitude is extended to Professor Leon S. Ciereszko who was most generous in providing several of the marine organisms mentioned in this dissertation. His enthusiasm was a great morale booster.

Appreciation is extended to the National Heart Institute (HE-056075) for support through a predoctoral traineeship, to the University of Oklahoma for financial assistance through teaching assistantships and to the Graduate College for support in the summer of 1974.

The author wishes to thank Stan Siegel, Oklahoma State University, for certain 100 MHz spectra and spin decoupling experiments. Dr. D. John Faulkner, Scripps Institute of Oceanography, was most gracious in providing 220 MHz spectra of dactylol and X-518. A special thanks goes to Dr. C. Hignite, Massachusetts Institute of Technology, for high resolution mass spectra.

The cmr and 100 MHz spectra displayed herein were provided by Mr. Larry W. Wilson and Mr. Marvin Taylor; low resolution mass spectra were kindly provided by Mr. Herman Curtis, Mr. Robert W. Allen and Mr. J. Michael Wilson.

A special debt of appreciation goes to Dr. Tommy K. B. Karns for his friendship and aid over the years.

A note of gratitude is extended to Dr. Dev, National Chemical Laboratory, Poona, India and Dr. L. Minale, Laboratorio per la Chimica e Fisica di Molecole di Interesse biologico del C.N.R., Arco Felice, Napoli, Italy for providing samples of cembrene-A and perhydrodendrolasin, respectively.

To my fellow graduate students goes my fondest thanks and best wishes. The friendships made here will be lasting for a long time. The pleasant comradery made difficult times easier to bear.

To Mr. Neal Rutledge goes my sincere appreciation for his faithful and excellent assistance down the home stretch. As the months passed not only did Neal provide the hands in the lab, but his friendship outside of the lab became equally valuable.

The author wishes to thank Mrs. Lucille Stellner, who typed job-seeking letters; Betty Rupp, dissertation typist; and Jeannie Kelley, who faithfully plotted the mass spectra.

Finally, to my parents, whose support never waned, goes my wholehearted thanks.

TABLE OF CONTENTS

	Page
LIST OF TABLES . . . . .	
LIST OF ILLUSTRATIONS . . . . .	
 Chapter	
I. DACTYLYNE, A REVISED STRUCTURE, AND ISODACTYLYNE, A NOVEL HALOGENATED ACETYLENIC COMPOUND	
INTRODUCTION . . . . .	1
RESULTS AND DISCUSSION . . . . .	3
SUMMARY . . . . .	23
EXPERIMENTAL . . . . .	24
BIBLIOGRAPHY . . . . .	30
 II. THREE NEW SESQUITERPENES FROM THE SEA HARE <i>APLYSIA DACTYLOMELA</i> (RANG): DACTYLENOL, DACTYLENOL ACETATE, AND DACTYLOL	
INTRODUCTION . . . . .	33
RESULTS AND DISCUSSION . . . . .	34
SUMMARY . . . . .	47
EXPERIMENTAL . . . . .	48
BIBLIOGRAPHY . . . . .	53
 III. ISOLATION OF DENDROLASIN FROM THE SPONGE <i>OLIGOCERAS HEMORRHAGES</i>	
INTRODUCTION . . . . .	54
RESULTS AND DISCUSSION . . . . .	55
EXPERIMENTAL . . . . .	59
BIOLOGIGRAPHY . . . . .	62
 IV. ISOLATION OF TWO KNOWN SAPOGENINS FROM THE SEA CUCUMBER <i>HOLOTHURIA ATRA</i>	
INTRODUCTION . . . . .	63
RESULTS AND DISCUSSION . . . . .	64
SUMMARY . . . . .	68
EXPERIMENTAL . . . . .	69
BIBLIOGRAPHY . . . . .	73

	Page
V. A NOVEL POLYACETYLATED COMPOUND FROM A SOFT CORAL	
INTRODUCTION . . . . .	74
RESULTS AND DISCUSSION . . . . .	75
SUMMARY . . . . .	86
EXPERIMENTAL . . . . .	87
BIBLIOGRAPHY . . . . .	90
VI. CEMBRENE-A AND TWO NEW CEMBRENE DERIVATIVES FROM A PACIFIC SOFT CORAL, <i>NEPHTHEA SP.</i>	
INTRODUCTION . . . . .	91
RESULTS AND DISCUSSION . . . . .	93
SUMMARY . . . . .	115
EXPERIMENTAL . . . . .	116
BIBLIOGRAPHY . . . . .	124

LIST OF TABLES

Section I

Table	Page
1. Pmr data of Laureatin and Laurencin . . . . .	12
2. Pmr data of Several Natural Products Containing a Conjugated Enyne System . . . . .	16
3. Structures of the Compounds Listed in Table 2 . . . . .	17
4. UV Data of Compounds Listed in Table 2 . . . . .	19

Section II

1. Double Irradiation of Dactylenol . . . . .	40
---	----

Section V

1. Double Irradiation of X-518 in $\text{CDCl}_3$ . . . . .	81
2. Double Irradiation of X-518 in Benzene- $\text{d}_6$ . . . . .	82

Section VI

1. Double Irradiation of Epoxynephthenol Acetate (I) . . . . .	96
--	----

## LIST OF ILLUSTRATIONS

### Section I

1. Spectra of Dactylyne (II) . . . . . 6
2. Spectra of Octahydromonodebromodactylyne (X) . . . . . 9
3. Spectra of Isodactylyne (XVIII) . . . . . 14
4. Spectra of Octahydromonodebromodactylyne (X) from  
Isodactylyne (XVIII) . . . . . 20

### Section II

1. Spectra of Dactylenol Acetate . . . . . 35
2. Spectra of Dactylenol . . . . . 38
3. Chemical Ionization Mass Spectra of Dactylenol and Dactylenol  
Derived from Dactylenol Acetate . . . . . 39
4. Gas Chromatogram of Reaction Mixture Obtained from Reaction of  
Dactylenol and p-TsOH . . . . . 42
5. Spectra of Dactylol . . . . . 43

### Section III

1. Spectra of Dendrolasin (I) . . . . . 56
2. Spectra of Perhydrodendrolasin (II) . . . . . 57

#### Section IV

1. Spectra of 22,25-oxidoholothurinogenin Acetate (III) . . . . .	65
2. Spectra of 17-desoxy-22,25-oxidoholothurinogenin Acetate (IV) .	66

#### Section V

1. Spectra of X-518 . . . . .	76
2. More Spectra of X-518 . . . . .	77
3. Possible Combinations of Carbons Singly Bonded to Oxygen In X-518 . . . . .	79

#### Section VI

1. Spectra of Epoxynephthenol Acetate (I) . . . . .	94
2. Spectra of Epoxynephthenol (II) . . . . .	98
3. Spectra of (-) Homoterpenyl Methyl Ketone (III) . . . . .	100
4. Spectra of (±) III Derived from (±) $\alpha$ -terpineol . . . . .	102
5. Spectra of Diketo Lactone (IV) . . . . .	104
6. Mechanism of Formation of III and IV from II . . . . .	106
7. Mechanisms for m/e 98 Fragment Ion in the Mass Spectrum of III . . . . .	107
8. Spectra of Nephthenol (V) . . . . .	109
9. Spectra of NEP-3 (VI) . . . . .	112
10. Spectra of Cembrene-A . . . . .	114

DACTYLYNE, A REVISED STRUCTURE, AND ISODACTYLYNE,  
A NOVEL HALOGEN CONTAINING ACETYLENIC COMPOUND

Introduction

The sea hare *Aplysia dactylomela* (Rang) is a large (approximately 10 inches in length) shell-less opisthobranch commonly found in the West Indian region. Aplysiids or sea hares have a world-wide distribution and number about thirty-five species.<sup>1</sup> Sea hares are found in all sizes, ranging from small ones not much larger than a human thumb nail to species that are about 12-18 inches in length. The term "sea hare" is a Roman appellation stemming from the fact that aplysiids resemble a sitting rabbit.<sup>2</sup> The major staples of their diet are algae<sup>2</sup> and seaweed<sup>3</sup>, but they have been known to devour animal substances also.<sup>2</sup>

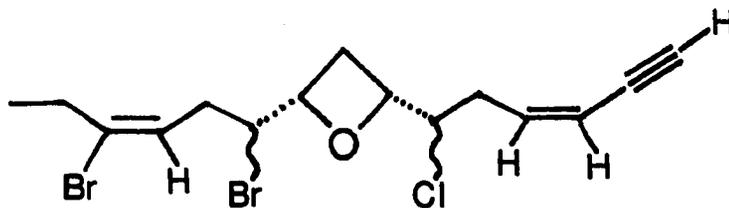
Sea hares and the algae upon which they feed have been rich sources of halogenated compounds.<sup>4-27,40,41</sup> Some of the halogenated compounds found in the sea hares are the result of ingestion<sup>27</sup> while others may be the result of further metabolism by the animal.

Interest in the sea hare *Aplysia dactylomela* (Rang) was initially generated in this lab because the lipid portion of the extracts of this animal showed tumor inhibitory activity. In the course of attempting to isolate the antitumor agent(s) from the extracts, a non anticancer active halogenated compound named dactylyne was isolated and a structure was proposed for it.<sup>28</sup> The objective of the work described

in this section was to isolate more dactylyne and investigate certain aspects of its structure in more detail. Dactylyne had been shown to contain three halogen atoms (two bromines and one chlorine), a cyclic ether and a conjugated enyne group. In the course of reisolating dactylyne, a second halogen containing compound called isodactylyne was isolated and its structure was determined. *Aplysia dactylomela* (Rang) is also the source of three new non-halogenated sesquiterpene ethers.<sup>29,30,31</sup>

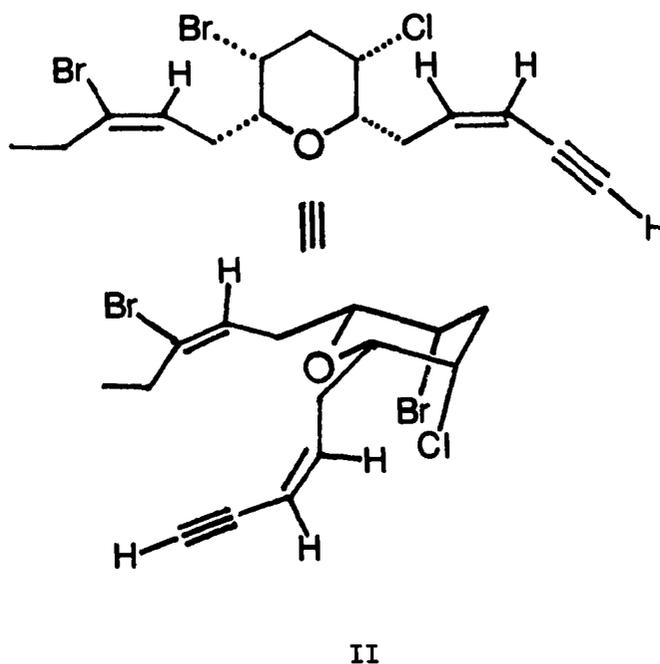
## Results and Discussion

The sea hares [*Aplysia dactylomela* (Rang)] used in the study presented here were collected in the environs of Bimini, Bahamas, in the latter part of May of 1972. The whole animals were sacrificed shortly after collection and immersed immediately in 2-propanol for shipment to Oklahoma. Soon after the specimens arrived sufficient water was added to bring the preservative liquid to a 40/60 (v/v) water/alcohol mixture and the animals were allowed to soak in this mixture for 1-2 days. The aqueous alcoholic solution was removed from the specimens by decantation and filtration. Further processing of this extract will not be discussed in this dissertation. The recovered sea hare bodies were air dried and then extracted in a Soxhlet apparatus with hexane for 2-4 days. From the hexane extracts a novel halogenated acetylenic compound called dactylyne,  $C_{15}H_{19}Br_2ClO$ , had been isolated by earlier workers and assigned the structure I based on its spectral properties and extensive spin decoupling experiments.<sup>28</sup>



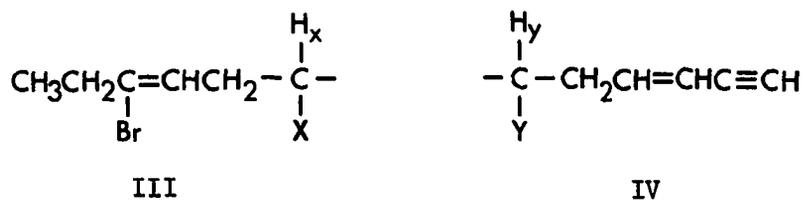
I

Confirmation of the relative positions of the halogens attached to saturated carbons and the stereochemistry of C-7 and C-9 of the oxetane ring in I warranted reisolation and further experiments. Dactylyne had previously<sup>28</sup> been an oil which decomposed rapidly at room temperature in the absence of solvent. Surprisingly, upon silica gel chromatography using a different solvent system (5% ether/hexane, see experimental section), crystalline dactylyne was obtained. Combustion analysis of of crystalline dactylyne confirmed the molecular formula  $C_{15}H_{19}Br_2ClO$ . Recrystallization of dactylyne from an ether/hexane mixture yielded crystals, mp 62.5-63.3°C (stable at room temperature indefinitely), suitable for X-ray analysis. A single crystal X-ray analysis was promptly carried out<sup>32</sup> and showed that dactylyne had the relative and absolute configuration shown in structure II.

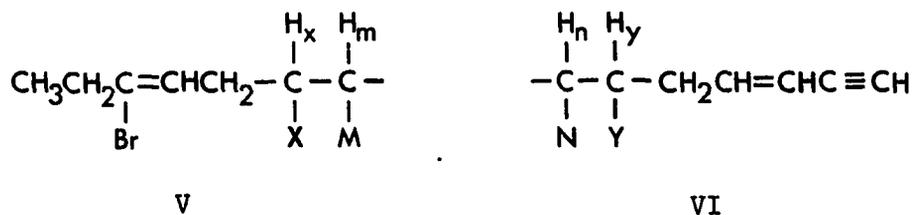


The initial postulation of structure I for dactylyne rather than structure II was due to incorrect proton assignments in the proton magnetic resonance (pmr) spectrum of dactylyne. A brief discussion relating how the original structure, I, for dactylyne was arrived at is presented below.

Analysis of the infrared (ir), pmr, ultraviolet and mass spectra led to the conclusion that the structural units III and IV were present in dactylyne. Various spectra of dactylyne are shown in Figure I. Proton decoupling experiments further established that  $H_x$  was vicinally coupled



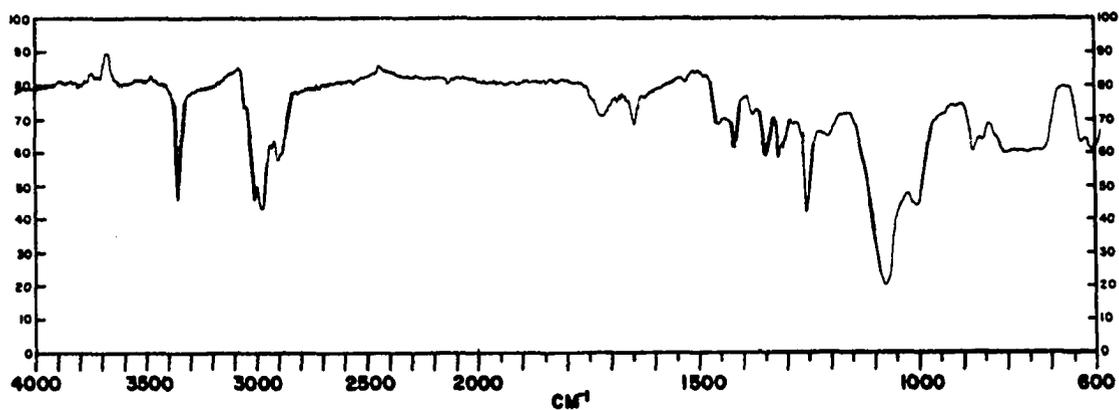
to a proton on a carbon bearing a heteroatom and that  $H_y$  was vicinally coupled to a proton on a carbon bearing a heteroatom, but  $H_x$  was not coupled to  $H_y$ . The partial structures III and IV were then expanded to V and VI.



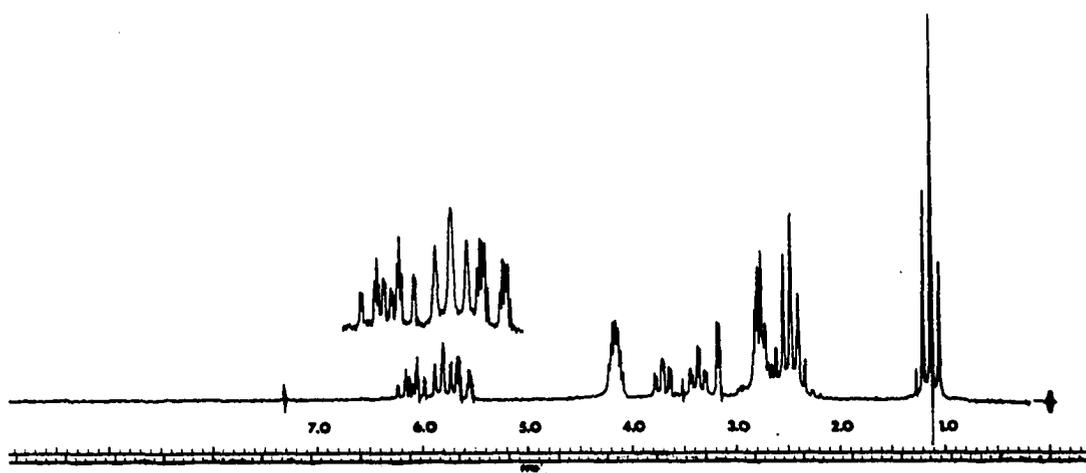
Partial structures V and VI defined fourteen of the fifteen carbon atoms and all but two of the required number of hydrogen atoms of dactylyne. It was concluded that V and VI must be joined by a methylene group as is shown in VII, to complete the carbon skeleton of dactylyne.

Figure I

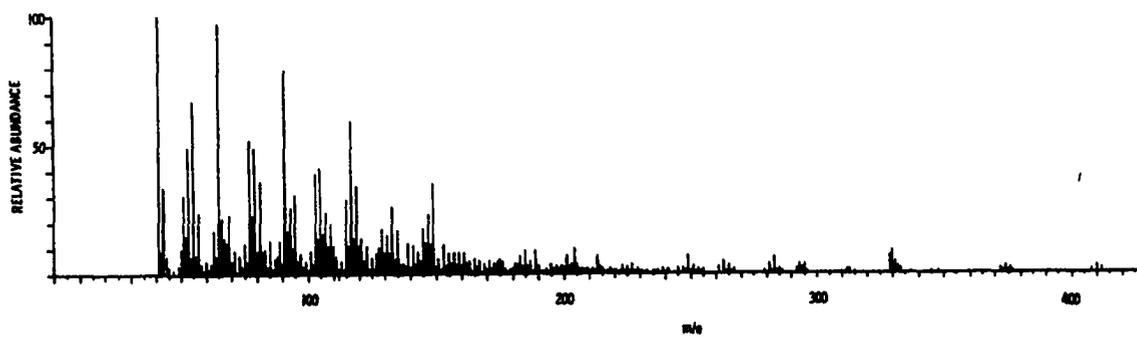
## Spectra of Dactylyne (II)



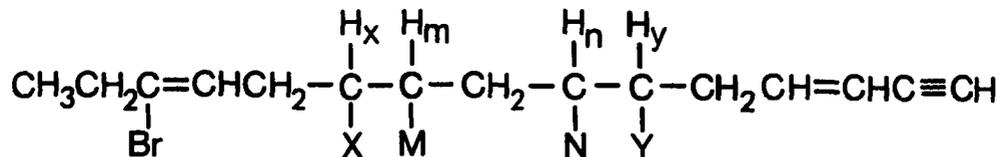
IR Spectrum of II



100 MHz PMR Spectrum of II



Mass Spectrum of II

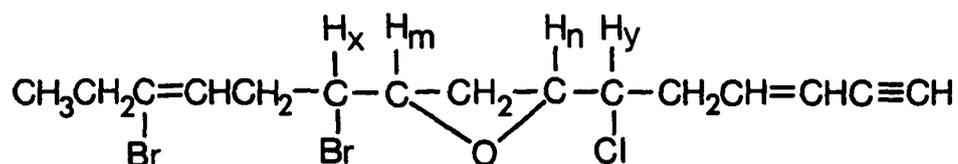


## VII

Structure VII accounted for all of dactylyne's atoms except three: one chlorine, one bromine and one oxygen - but indicated four carbon atoms attached to heteroatoms. Structure VII could only be in accord with the molecular formula of dactylyne (with regard to number of atoms and number of degrees of unsaturation) if two of the four hypothetical heteroatoms (X, Y, M and N) were one and the same, i.e., an ether oxygen atom. Four protons in the pmr spectrum of dactylyne, absorbing at a chemical shift position consistent for protons on carbons bearing heteroatoms, appear in the region 4.5 to 3.2 ppm (see Figure I). The farthest downfield signal in this region,  $\delta$  4.16, which integrated for two protons, was assigned to the protons on the carbons bearing the ether oxygen. This was based on the assumption that the oxygen atom would have a greater deshielding effect than the bromine or chlorine. The protons on the carbons bearing the halogens were assigned to the absorptions centered at  $\delta$  3.71 and  $\delta$  3.37.

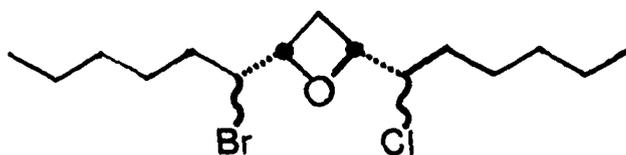
Upon irradiation at  $\delta$  2.7 [the chemical shift position which is very close to that of the central methylene protons in oxetane itself ( $\delta$  2.7)<sup>33</sup> and the methylene protons in a naturally occurring oxetane ( $\delta$  2.9) isolated by Irie and coworkers]<sup>13,15</sup> the signal at  $\delta$  4.16 collapsed to a doublet. Thus, it was concluded that an oxetane was present

in dactylyne. An oxetane ring is produced by substituting the ether oxygen atom for M and N in structure VII. Substituting bromine for X and chlorine for Y then completed the gross structure VIII originally proposed.



VIII

This assignment was reinforced by a similar analysis of the product of hydrogenation of dactylyne, octahydromonodebromodactylyne (X) [See summary page; since the structure of dactylyne has been revised the structural assignment of octahydromonodebromodactylyne must also be revised. The structure shown below (X') is the one proposed in Campbell's dissertation.<sup>28</sup>]

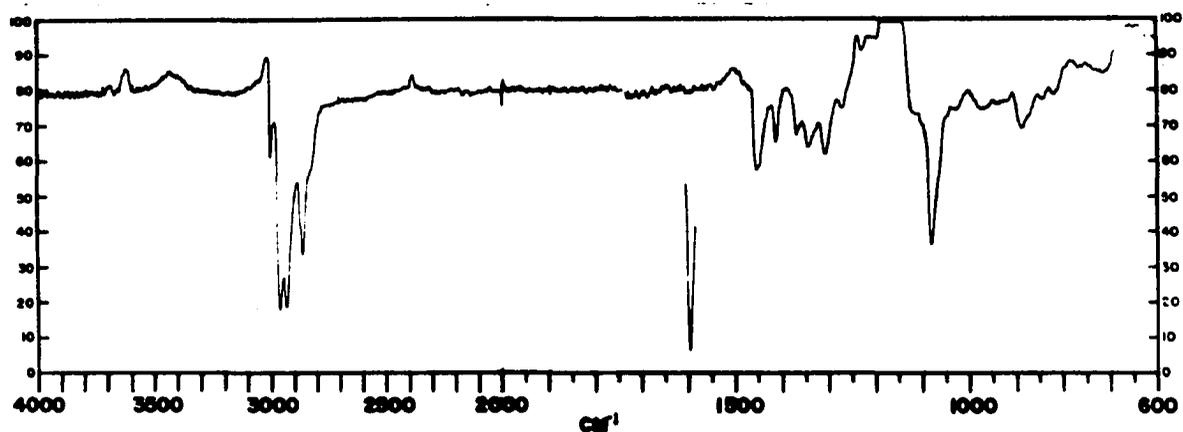


X'

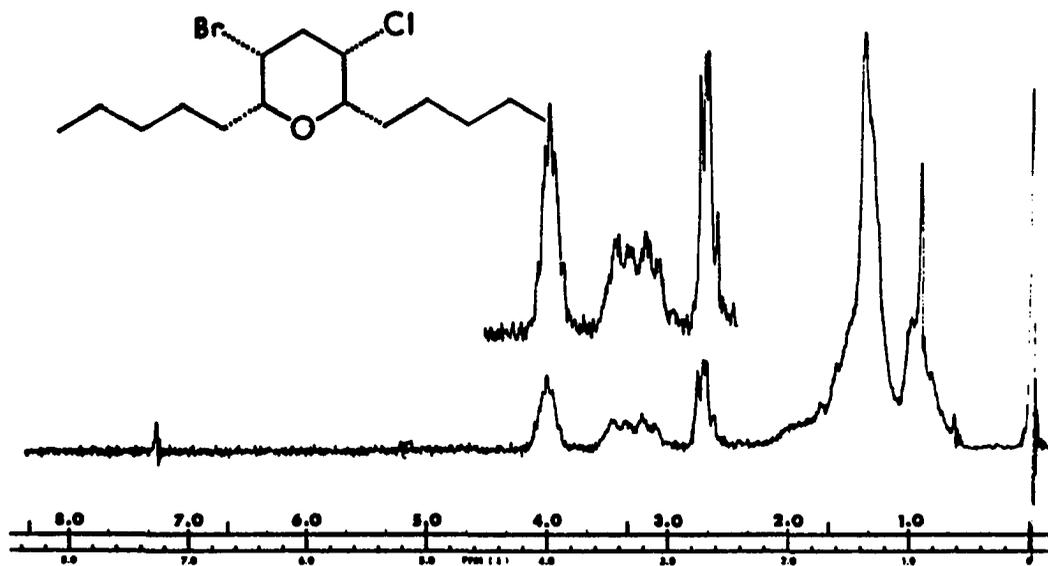
The pmr spectrum of X, see Figure II, displays a two proton absorption at  $\delta$  4.15 and two signals (one proton each) centered at  $\delta$  3.58 and  $\delta$  3.32. Clearly visible in the spectrum of X is a two proton multiplet centered at  $\delta$  2.78. Irradiation at  $\delta$  2.78 caused the  $\delta$  4.15 signal to collapse to a broad doublet. The presence of an oxetane ring as proposed in X' was concluded from this data.

Figure II

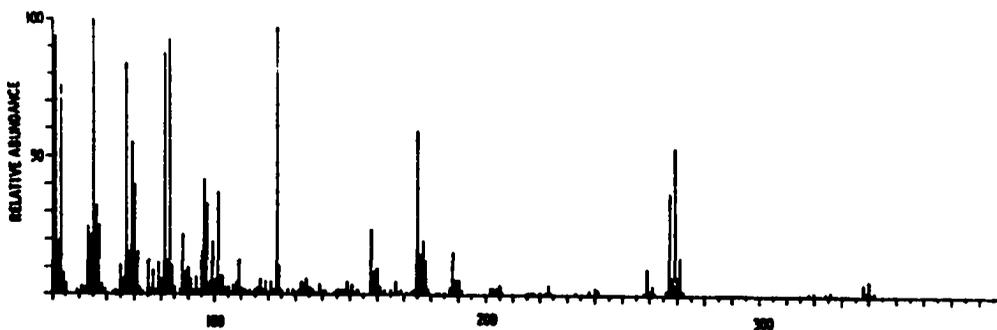
Spectra of Octahydromonodebromodactylyne (X)



IR Spectrum of X



60 MHz PMR Spectrum of X

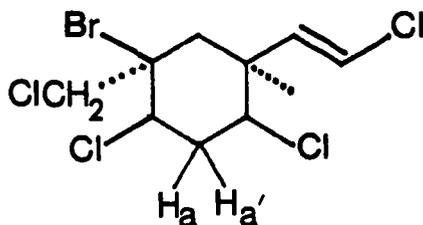


Mass Spectrum of X

In light of the X-ray analysis the pmr assignments are now correctly assigned as follows: the  $\delta$  4.16 absorption is due to the two protons on the carbons bearing the halogens and the signals at  $\delta$  3.71 and  $\delta$  3.37 are due to the protons on the carbons bearing the ether oxygen. The signals at  $\delta$  2.7 in dactylene and  $\delta$  2.78 in octahydromonodebromodactylene are produced by the protons of the methylene group situated between the carbons bearing the halogens. The chemical shift for the protons of a methylene of this nature is not unreasonable as can be seen from the nmr spectra of 1-bromo-3-chloropropane, IX, and 1,3-dichloropropane, XI.<sup>34</sup>

(a)	(b)	(c)	(a)	(b)	(c)
Cl-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -Br			Cl-CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -Cl		
IX			XI		
(a)	$\delta$ 3.70		(a)	$\delta$ 3.70	
(b)	$\delta$ 2.28		(b)	$\delta$ 2.20	
(c)	$\delta$ 3.55				

Violacene (XII), a natural product recently isolated by Faulkner and Mynderse,<sup>27</sup> has a methylene group situated between two carbons



XII

bearing halogen atoms in a six-membered ring. The two protons of this methylene group, H<sub>a</sub> and H<sub>a</sub>', absorb at  $\delta$  2.64 and  $\delta$  2.44. All three examples show a chemical shift of below 2 ppm for the protons of a

methylene group of this nature.

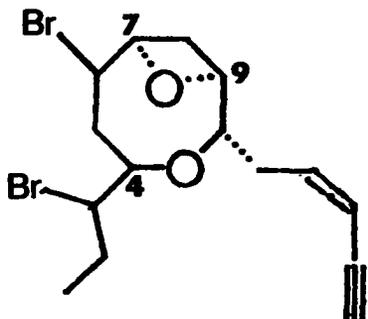
The assumption that the oxygen would produce a greater deshielding effect is in error as the pmr spectra of laureatin and laurencin illustrate in Table I.<sup>15,11</sup> Of the protons on carbons bearing the ether oxygens (large rings) only the proton on carbon 4 in laureatin absorbs at lower field than the protons on carbons bearing a halogen (the unusual chemical shift for this proton was attributed to its being situated close to the oxetane oxygen). Interestingly though, the protons on carbons 7 and 9 in laureatin which bear the oxetane oxygen absorb at lower field than the protons on the carbons bearing halogens. The chemical shift for the protons on the carbons bearing the oxygen atom in oxetane itself absorb at  $\delta$  4.73.<sup>33</sup> All this merely points to the necessity of using extreme caution when making pmr assignments in molecules which contain an ether oxygen and halogen atoms.

Since dactylyne was obtained in crystalline form, all its physical constants were retaken and, in the course of this, two changes were made. The rotation of dactylyne itself was changed from  $[\alpha]_D^{25} + 33^\circ$  ( $c$  6.5,  $\text{CHCl}_3$ ) to  $[\alpha]_D^{23} - 36^\circ$  ( $c$  15.2,  $\text{CHCl}_3$ ). This change was the result of an inadvertent error in the sign of the rotation in the earlier work.<sup>28</sup> The rotation of octahydromonobromodactylyne prepared by this investigator (see experimental section) was  $[\alpha]_D^{24} - 0.90^\circ$  ( $c$ , 5.45,  $\text{CHCl}_3$ ). The optical rotation of octahydromonobromodactylyne was earlier reported<sup>28</sup> to be  $[\alpha]_D^{25} - 7^\circ$  ( $c$  1.4,  $\text{CHCl}_3$ ).

Further investigations into the fractions from which dactylyne was obtained yielded a new polyhalogenated acetylenic compound which was named isodactylyne. Isodactylyne,  $\text{C}_{15}\text{H}_{19}\text{Br}_2\text{ClO}$  (low resolution mass

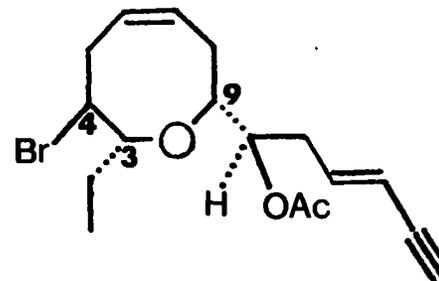
Table I

Pmr data of Laureatin and Laurencin



Laureatin

Proton on Carbon	Chemical Shift ( $\delta$ )
3	3.85
4	4.59
6	4.26
7	5.03
9	4.77
10	3.61



Laurencin

Proton on Carbon	Chemical Shift ( $\delta$ )
3 & 9	3.4
4	4.07
10	4.98

spectrum) was isolated as a clear, colorless oil,  $[\alpha]_D^{23} -8.06$  ( $c$  7.9,  $\text{CHCl}_3$ ), which decomposed rapidly at room temperature in the absence of solvent. To date, all efforts to induce isodactylyne to crystallize have failed. The mass spectrum of isodactylyne, which was virtually identical with that of dactylyne, indicated a molecular weight of 410 and showed the pattern expected for a compound containing two bromine atoms and one chlorine atom (408, 1.5%; 410, 3%, 412, 2%). The molecular formula  $\text{C}_{15}\text{H}_{19}\text{Br}_2\text{ClO}$  indicated that the molecule has five degrees of unsaturation.

The presence of a conjugated enyne functionality, similar to that in dactylyne, was indicated by the sharp strong absorption at  $3300\text{ cm}^{-1}$  and the weak absorption at  $2095\text{ cm}^{-1}$  in the infrared spectrum corresponding to the carbon hydrogen stretch and the carbon-carbon stretch respectively, of a terminal acetylenic group.<sup>35</sup> The ultraviolet spectrum of isodactylyne ( $\lambda_{\text{max}} 224\text{ nm}$ ,  $\epsilon_{\text{max}} = 15,500$ , with an inflection at  $233\text{ nm}$ ) is consistent with that of a molecule having a double bond in conjugation with a terminal acetylene.<sup>36</sup> Thus the partial structure XIII was indicated.

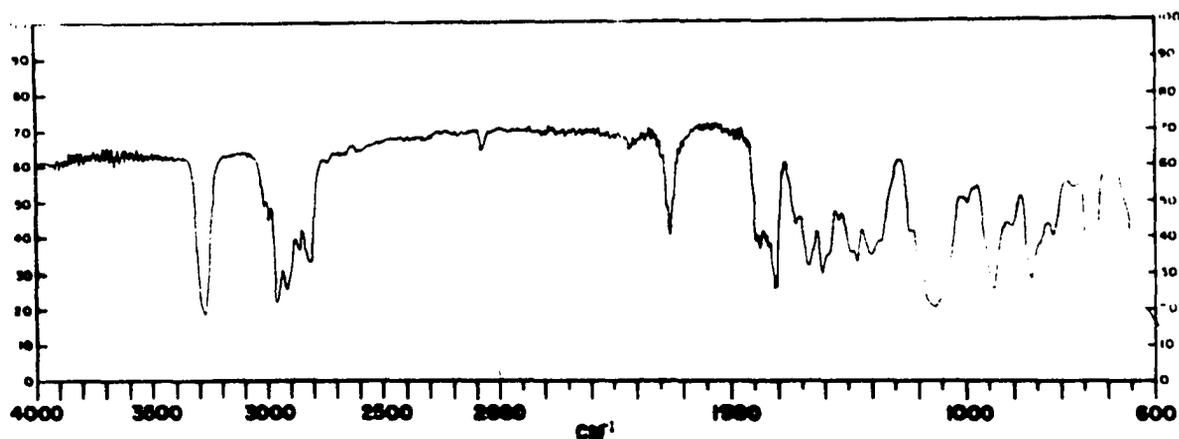


## XIII

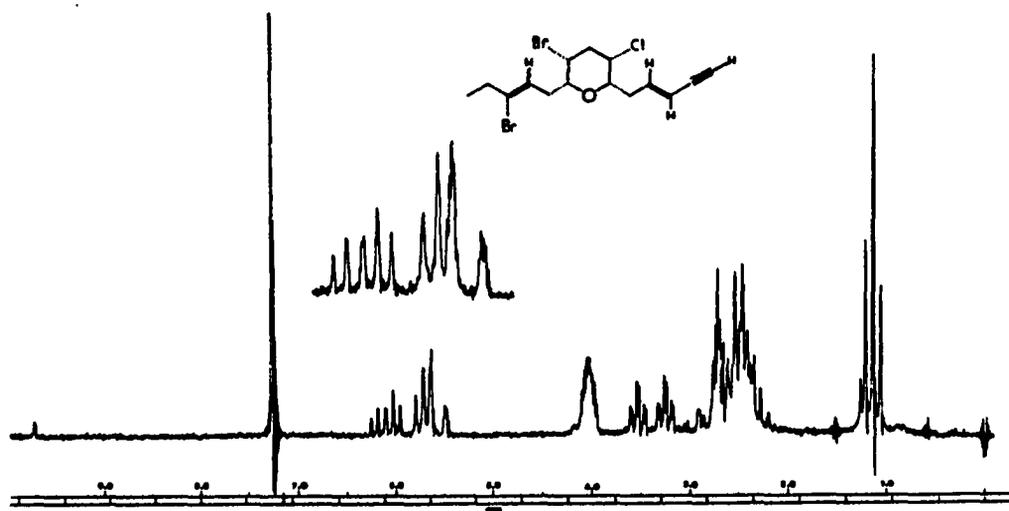
The similarity between the pmr and mass spectra of isodactylyne and dactylyne suggested considerable similarity in functionality between the two compounds (see Figures I and III). In the pmr spectrum of isodactylyne three sets of absorptions are observed in the olefinic region, the lowest field of these is a one proton

Figure III

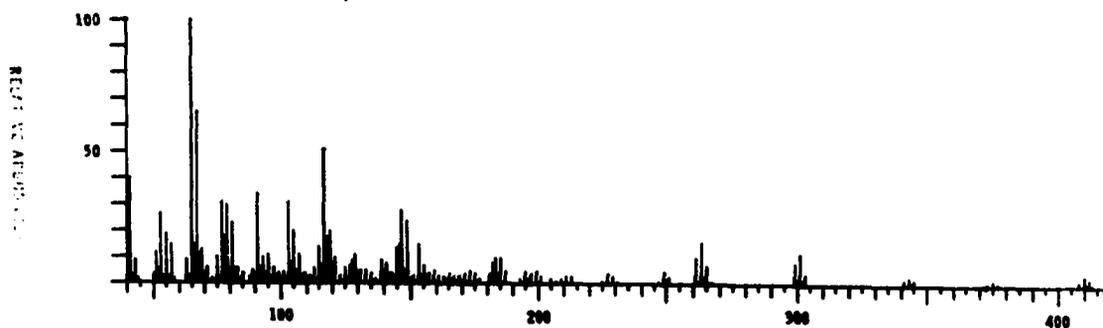
Spectra of Isodactylyne (XVIII)



IR Spectrum of XVIII

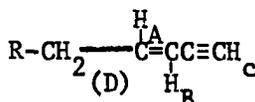


100 MHz PMR Spectrum of XVIII



Mass Spectrum of XVIII

doubled triplet ( $J = 14.8$  and  $6.5$  Hz) centered at  $\delta$  6.10. The second absorption is a slightly broadened one proton triplet centered at  $\delta$  5.70, characteristic of a normal, non conjugated olefinic proton. The third olefinic proton signal is a one proton doublet ( $J = 14.8$  Hz) centered at  $\delta$  5.56 which is also long range coupled ( $J \approx 1-2$  Hz) to at least two additional protons. The coupling constants, chemical shifts and splitting patterns for the absorptions at  $\delta$  6.10 and  $\delta$  5.57 are consistent for two olefinic protons, *trans* oriented ( $J = 14.8$  Hz), on a conjugated enyne system flanked by a methylene carbon as is shown in partial structure XIV.



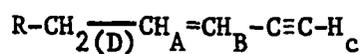
## XIV

The absorption at  $\delta$  6.10 is assigned to the olefinic proton,  $\text{H}_A$ , at the terminus of the enyne system, since it is vicinally coupled to three protons ( $J = 14.8, 6.5$  and  $6.5$  Hz). The absorption at  $\delta$  5.57 is assigned to  $\text{H}_B$  of partial structure XIV since it is vicinally coupled to one olefinic proton ( $\text{H}_A$ ) and long range coupled to three additional protons ( $\text{H}_C$ ,  $J = 2$  Hz, and the two methylene protons,  $\text{H}_D$ ,  $J = 1-2$  Hz).

The *trans* nature of the double bond in conjugation with the terminal acetylene is indicated by the large coupling constant between  $\text{H}_A$  and  $\text{H}_B$  of 14.8 Hz, typical for *trans* olefinic protons.<sup>37</sup> Irie and coworkers<sup>12-15</sup> have isolated a series of compounds containing the conjugated enyne functionality. Table II summarizes the chemical shift data and coupling constants for several of these compounds. In all cases a coupling constant of 11 Hz between the olefinic protons

Table II

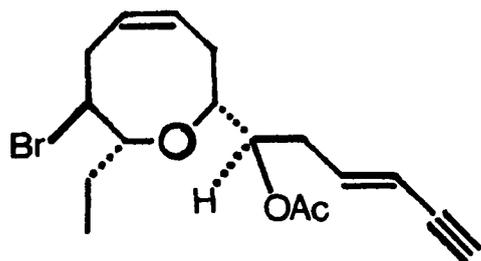
Pmr data of several natural products containing an enyne system



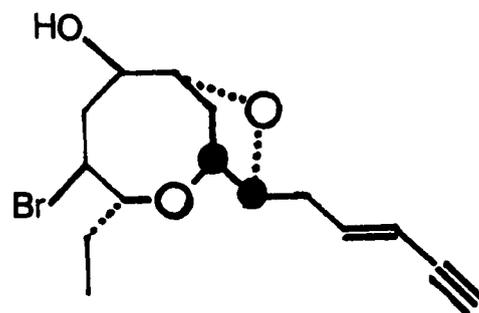
Compound	Chemical Shift ( $\delta$ )			Coupling Constants (Hz)		
	H <sub>A</sub>	H <sub>B</sub>	H <sub>C</sub>	J <sub>AB</sub>	J <sub>AD</sub>	J <sub>BC</sub>
laurencin <sup>12</sup> (trans)	6.25	5.52	2.83	15	6	2
laurefucin <sup>9</sup> (trans)	6.24	5.55	2.82	16	6	2
isoprelaurefucin <sup>19</sup> (trans)	6.15	5.52	2.66	16	7	2
isodactylyne (trans)	6.10	5.57	2.78	14.8	6.5	2
laureatin <sup>15</sup> (cis)	6.03	5.53	3.06	11	7	2
isolaureatin <sup>15</sup> (cis)	6.04	5.55	3.06	12	7	2
dactylyne <sup>28</sup> (cis)	6.12	5.6	3.18	11	8 & 6.5	2

Table III

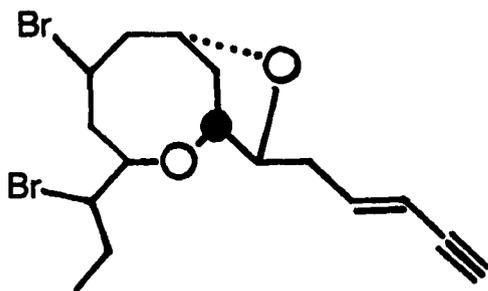
Structures of Compounds Containing Conjugated  
Enyne System Listed in Table II



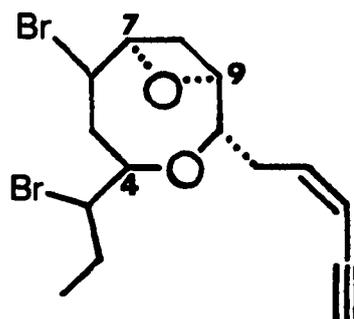
Laurencin



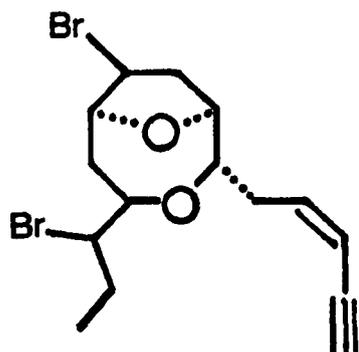
Laurefucin



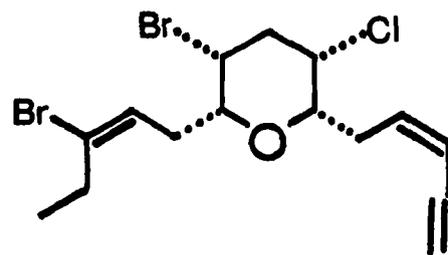
Isoprelaurefucin



Laureatin



Isolaureatin



Dactylyne

indicates *cis* double bond geometry while a coupling constant between olefinic protons of 15 Hz corresponds to a *trans* configuration.

One of the striking differences in the pmr spectra of dactylyne and isodactylyne is the position of absorption of the acetylenic proton,  $H_c$ .<sup>39</sup> As can be seen from Table II,  $H_c$  absorbs at a position of higher field (toward TMS) from 3.0 ppm for a *trans* enyne system and downfield from 3.0 ppm for a *cis* enyne system. By analogy this is further evidence for the *trans* nature of the double bond in isodactylyne.

Table IV summarizes the ultraviolet spectral data for the compounds listed in Table II. The molar extinction coefficient ( $\epsilon_{max}$ ) is larger in the compounds possessing the *trans* enyne system than those having the *cis* enyne system.<sup>38</sup> The 3,000 increase in the molar extinction coefficient of isodactylyne as compared to dactylyne is further evidence for the *trans* double bond geometry.

Hydrogenation of isodactylyne over  $PtO_2$  catalyst in ethyl acetate resulted in the formation of a white crystalline compound (mp 51.0 - 52.2°C) whose elemental formula,  $C_{15}H_{28}BrClO$ , was ascertained from its low resolution mass spectrum which displayed a molecular ion of 338 with an isotopic ratio expected for a compound that contains one bromine and one chlorine. The octahydromonodebromo product was found to be identical to octahydromonodebromodactylyne (X) in all respects -- ir, pmr, mass spectra (see Figures II and IV),  $R_f$  value, melting point, mixed melting point and optical rotation (see experimental section). The chemical identity of these hydrogenation products, especially as evidenced by the optical rotation data, indicated that isodactylyne had the same stereochemistry at the four chiral centers as dactylyne. Combining all the foregoing data the

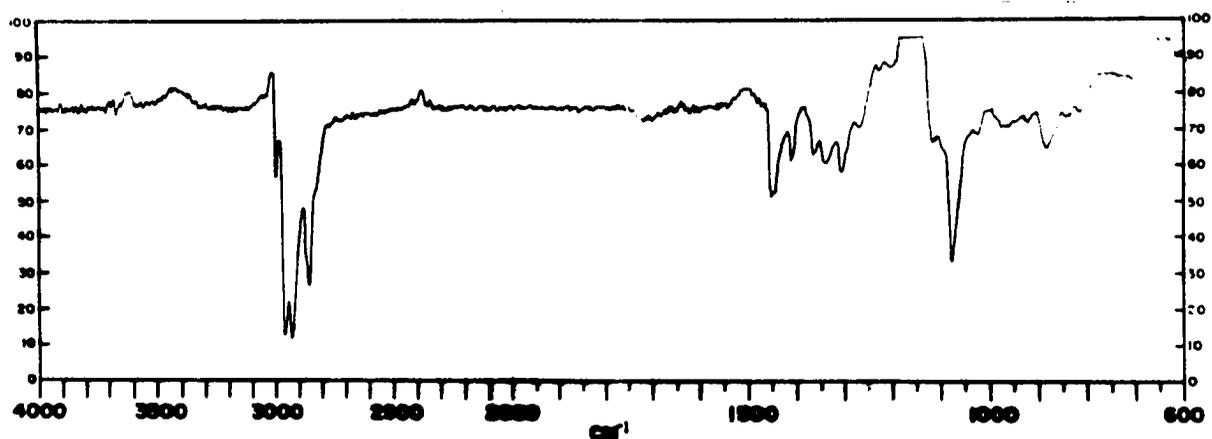
Table IV

UV spectral data of several natural products  
containing an enyne system.

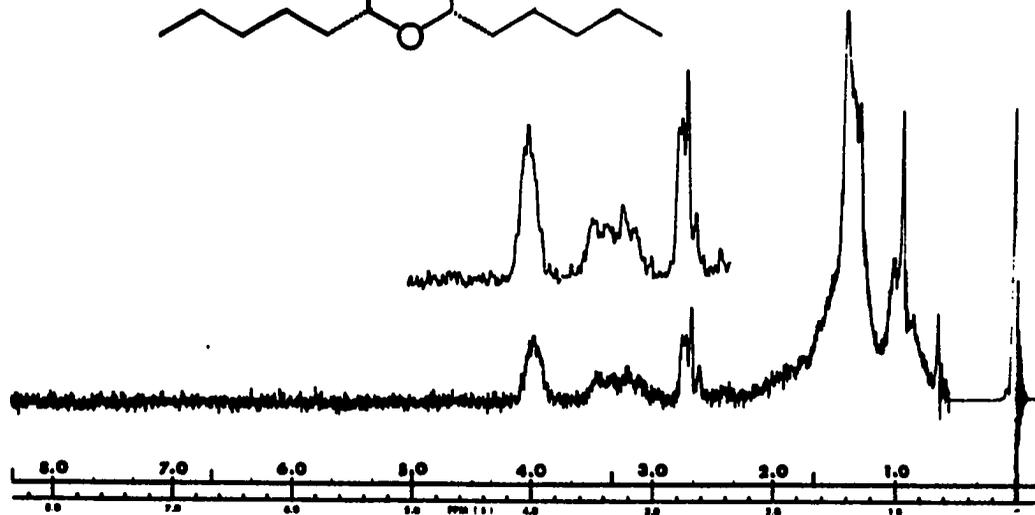
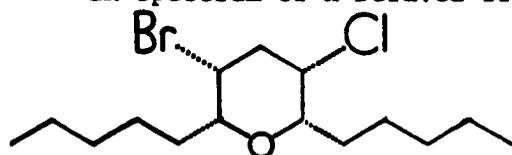
Compound	$\lambda_{\max}$ (nm)	$\epsilon_{\max}$
laurencin <sup>12</sup> (trans)	224, inf at 232	16,400
laurefucin <sup>9</sup> (trans)	224.5, inf at 232	17,900
isoprelaurefucin <sup>19</sup> (trans)	224, inf 219 and 232	13,200
isodactylyne <sup>39</sup> (trans)	224, inf at 233	15,500
laureatin <sup>15</sup> (cis)	223, inf at 229	12,800
isolaureatin <sup>15</sup> (cis)	223, inf at 229	12,400
dactylyne <sup>28</sup> (cis)	222.5, inf at 230	12,300

Figure IV

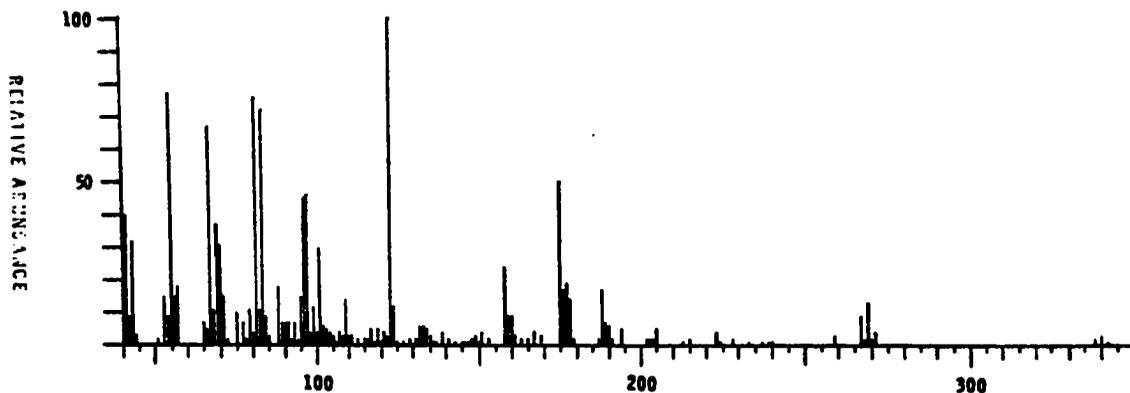
Spectra of Octahydromonodebromodactylyne (X) from Isodactylyne (XVIII)



IR Spectrum of X Derived from XVIII

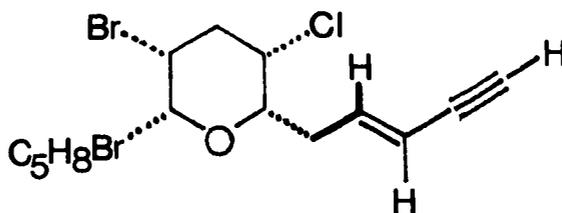


60 MHz Spectrum of X Derived from XVIII



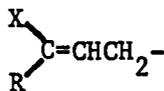
Mass Spectrum of X Derived from XVIII

structure XV may be drawn for isodactylyne.



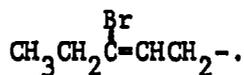
XV

To complete the detailed structure of isodactylyne the nature of the  $C_5H_8Br$  group needed to be ascertained. From the multiplicity (triplet) of the olefinic proton absorbing at  $\delta$  5.70 in the pmr spectrum it was concluded that the olefinic proton was vicinal to a methylene group while the other end of the double bond was fully substituted as shown in XVI.



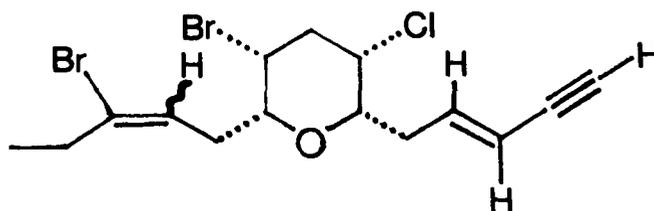
XVI

From the chemical shift ( $\delta$  1.12) and the multiplicity (triplet,  $J = 7$  Hz) of the methyl group in the pmr spectrum of isodactylyne, R cannot be a methyl group. By analogy with dactylyne and the pmr data just discussed it was concluded that R must be an ethyl group and X the bromine atom. Partial structure XVI was therefore expanded to XVII.



XVII

The geometry of the double bond in XVII is still to be rigorously determined. Campbell<sup>28</sup> presented an argument, based on the carbon-13 chemical shifts of dactylyne, for assigning the *cis* configuration between the bromine and hydrogen atoms, which was corroborated by the X-ray analysis. This argument is considered tenuous at best by the present author, and since the cmr spectrum of isodactylyne was not obtained due to an equipment breakdown, the stereochemistry of the trisubstituted double bond in isodactylyne remains unsettled. On the basis of all the foregoing data the structure XVIII is proposed for isodactylyne.

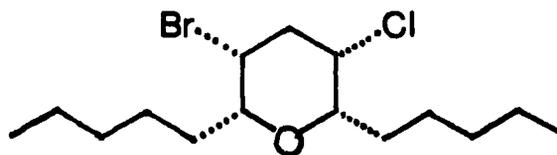
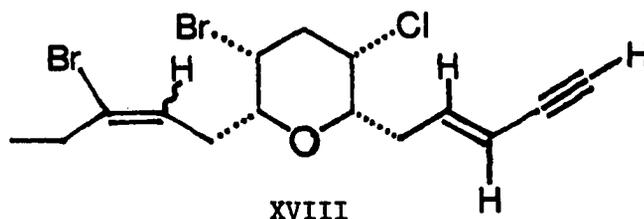
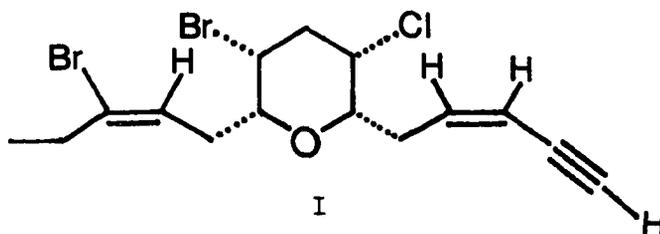


XVIII

It was interesting to note that in the pmr spectrum of octahydro-monodebromodactylyne(X) taken in benzene- $d_6$  the ring methylene proton signals appeared as a well resolved pair of doubled triplets ( $J = 16, 4$  Hz) and ( $J = 16, 2$  Hz) centered  $\delta$  1.78 and  $\delta$  2.45 respectively. This is probably the result of a preferential solvent cage or complexation by the benzene, presumably from the least hindered side of X, rendering the methylene protons magnetically non equivalent. The geminal and vicinal coupling constants are in accord with the halogen atoms being axially oriented in a six-membered ring.<sup>37</sup>

### Summary

Dactylyne, a polyhalogenated acetylenic straight chain  $C_{15}$  compound ( $C_{15}H_{19}Br_2ClO$ ) isolated by earlier workers as an oil from the sea hare *Aplysia dactylomela* (Rang), was induced to crystallize and a subsequent X-ray crystallographic analysis established II as its structure. A second polyhalogenated acetylenic straight chain  $C_{15}$  compound ( $C_{15}H_{19}Br_2ClO$ ), which was named isodactylyne, was isolated from the same animal and it was assigned the structure XVIII based on its spectral properties (ir, nmr, uv) and conversion to an octahydro derivative (X) identical to that obtained from dactylyne.



X

## Experimental

Melting points were taken on an A. H. Thomas Unimelt apparatus and are uncorrected. Solvents were distilled prior to use. Chromatographic supports were Florisil (Fisher, 100-200 mesh), silicic acid (Mallinckrodt, silicAR CC-7).

Thin layer chromatography plates were prepared by coating 5 x 20 cm glass plates with Merck (Darmstadt) silica gel H to a thickness of approximately 0.30 mm. The developed chromatograms were visualized with iodine vapor or 10% sulfuric acid spray.

Rotations were run on a Gaertner polarimeter or a Perkin-Elmer 141 polarimeter, ultraviolet spectra in isoctane on a Cary Model 118 (Varian) spectrometer, and infrared spectra on a Beckman IR-8 spectrophotometer. Low resolution mass spectra were obtained on a Hitachi Perkin-Elmer RMU-7 double focusing spectrometer. Proton magnetic resonance spectra were obtained on a Varian T-60 or XL-100 spectrometer; carbon-13 spectra were obtained on the Varian XL-100 spectrometer. Pmr chemical shifts are reported as  $\delta$ -values (ppm from tetramethylsilane as internal standard) and are followed by the multiplicity of the signal, the number of protons absorbing at that frequency, the coupling constants in Hertz (Hz), and, when assignable, the specific protons which give rise to the reported signal. The multiplicities are reported as follows: s, singlet; d, doublet; dd, double doublet; dt double triplet; t, triplet; q, quartet;

br s, broad singlet and m, multiplet.

High resolution mass spectra were obtained through the courtesy of Dr. C. Hignite, Massachusetts Institute of Technology. Certain 100 MHz spectra and decoupling experiments were kindly provided by Stan Siegel at Oklahoma State University. Combustion analyses were performed by Mr. E. Meier, Stanford University.

Isolation of Dactylyne and Isodactylyne: The sea hare bodies (3.5 kg) recovered after an extraction with 2-propanol/water (1/1) were air dried and then extracted in a Soxhlet apparatus with distilled hexane for 2-4 days. The hexane extract was filtered and the solvent was evaporated to yield 204 g of a dark green oil.

A portion of the crude hexane extract (100 g) was chromatographed on Florisil (1500 g). One liter fractions were collected employing the following elution scheme: hexane, fractions 1-7; benzene/hexane (1/3), fractions 8-11; benzene/hexane (1/1), fractions 12-18; benzene (two liter fractions), fraction 19-22; ethyl acetate (one eight liter fraction). The fractions were checked by tlc and on the basis of this analysis the following combinations were made: 9-11 (16.4550 g); 12-14 (9.0092 g); 15-18 (6.3791 g). A portion (2.5 g) of the material in fractions 9-11 (see above) was chromatographed on 40 g of thin layer mesh silica gel H using ether/hexane (5/95) as solvent and collecting 20 ml fractions. Dactylyne (175 mg) crystallized from the material obtained in fractions 18-20 (290 mg). Recrystallization from an ether/hexane mixture yielded large, colorless crystals roughly trapezoidal in shape which were utilized for X-ray crystallographic analysis. After the collection of 29 fractions (20 mls each) one 250 ml fraction was collected which yielded 170 mg of material, homogeneous by tlc. A follow-up chromatography of this material on thin-layer mesh silica gel yielded 160 mg of a pure compound, isodactylyne. Numerous attempts to crystallize isodactylyne were unsuccessful.

Pure isodactylyne had  $[\alpha]_D^{24} = -8.06^\circ$  ( $c$  7.97,  $\text{CHCl}_3$ );  $R_f = 0.46$  (1:1 benzene/hexane, silica gel H),  $R_f = 0.057$  (3% ether/hexane, silica

gel H); ir (neat 3300, 3030, 3010, 2970, 2970, 2930, 2830, 2100 (weak), 1640, 1415, 1345, 1315, 1080, 955, 880, 750 and 600  $\text{cm}^{-1}$ ; uv (isooctane)  $\lambda_{\text{max}}$  224 nm,  $\epsilon_{\text{max}}$  = 15,500, with an inflection at 233 nm; 100 MHz pmr ( $\text{CCl}_4$ )  $\delta$  6.10 (dt, 1 H,  $J = 14.8$  Hz,  $J = 6.5$  Hz,  $\text{CH}=\text{CHC}\equiv\text{CH}$ ), 5.70 (br t, 1 H,  $J = 7$  Hz,  $\text{RXC}=\text{CHCH}_2-$ ), 5.56 (br d, 1 H,  $J = 14.8$  Hz,  $J = 1-2$  Hz,  $-\text{CH}=\text{CHC}\equiv\text{CH}$ ), 4.04 (m, 2 H, protons on carbons bearing halogens), 3.53 (dt, 1 H,  $J = 2$  Hz,  $J = 6.5$  Hz, one of protons on carbon bearing oxygen), 3.26 (dt, 1 H,  $J = 2$  Hz,  $J = 6.5$  Hz, one of protons on carbon bearing oxygen), 2.95-2.20 (m, 9 H, protons on the four methylene carbons and the acetylenic proton), 1.12 ppm (t, 3 H,  $J = 7$  Hz,  $-\text{CH}_2\text{CH}_3$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$ , 412 (3), 410 (4), 408 (2), 377 (1), 375 (2), 373 (1), 345 (2), 343 (3), 341 (2), 265 (7), 263 (16), 261 (10), 251 (3), 249 (5), 247 (1), 229 (3), 227 (4), 225 (1), 187 (5), 185 (10), 183 (10), 182 (8), 153 (15), 149 (24), 147 (28), 146 (15), 145 (14), 129 (11), 119 (20), 118 (18), 117 (51), 115 (14), 107 (11), 105 (20), 103 (31), 95 (11), 93 (10), 91 (34), 81 (23), 79 (30), 78 (18), 75 (10), 69 (13), 67 (65), 65 (100) base peak, 57 (15), 55 (18), 53 (27), 51 (12), 41 (40).

Due to the highly unstable nature of isodactylyne no combustion analysis was obtained.

Pure dactylyne had mp 62.5 - 63.5°C;  $[\alpha]_D^{23}$  - 36.2° (c 15.16,  $\text{CHCl}_3$ );  $R_f = 0.57$  (1:1 benzene:hexane, silica gel H);  $R_f = 0.071$  (3%  $\text{Et}_2\text{O}$ /hexane, silica gel H).

The ir, pmr and mass spectral data for dactylyne are presented in Figure I; details are recorded in Campbell's dissertation<sup>28</sup> and will not be reproduced here.

Anal. Calcd. for  $C_{15}H_{19}Br_2ClO$ : C, 43.88; H, 4.66; Cl, 8.63; Br, 38.92.

Found: C, 44.20; H, 4.73; Cl, 8.59; Br, 37.61.

Octahydromonodebromodactylyne (X). To a solution of 30 ml of ethyl acetate containing a few milligrams of prerduced  $PtO_2$  in a hydrogen atmosphere was added 105 mg of dactylyne dissolved in 10 ml of ethyl acetate. The uptake of hydrogen was immediate. The flask's contents were maintained in the hydrogen atmosphere overnight. The catalyst was filtered from the solution, and the solvent was removed under reduced pressure on a rotary evaporator to yield 77.8 mg of a clear, colorless oil which solidified after the removal of all solvent. Recrystallization from 95% ethanol yielded pure octahydromonodebromodactylyne, X, homogenous by tlc, mp  $51.4^\circ - 52.5^\circ$ . Pure X had  $[\alpha]_D -0.90^\circ$  ( $c$  5.45,  $CHCl_3$ ). The ir, pmr and mass spectral data are presented in Figure II; details are recorded in Campbell's dissertation<sup>28</sup> and will not be reproduced here.

Octahydromonodebromodactylyne (X) from Isodactylyne: To a solution of 10 ml of ethyl acetate and a few mg of prerduced  $PtO_2$  in a hydrogen atmosphere was added 57.5 mg of isodactylyne dissolved in 2 ml of ethyl acetate. Uptake of hydrogen began immediately. The hydrogen atmosphere was maintained overnight. The catalyst was then filtered from the solution and the solvent was removed under reduced pressure on a rotary

evaporator to yield 36.7 mg of an oil which crystallized after the removal of all solvent. Recrystallization from 95% ethanol yielded a pure octahydromonodebromo product (homogeneous by tlc,  $R_f$  was identical to that of octahydromonodebromodactylyne derived from dactylyne), mp 51.0° - 52.2°C. Ir ( $\text{CHCl}_3$ ) 3000, 2960, 2930, 2860, 1450, 1415, 1370, 1345, 1310 and 1080  $\text{cm}^{-1}$ ; 60 MHz pmr ( $\text{CCl}_4$ )  $\delta$  3.97 (m, 2 H, protons on the carbons bearing the halogens), 3.40 (m, 1 H, one of the protons on the carbon bearing oxygen), 3.20 (m, 1 H, one of the protons on the carbon bearing oxygen), 2.70 (t, 1 H,  $J = 2$  Hz, one of the ring methylene protons), 2.65 (t, 1 H,  $J = 4$  Hz, one of the ring methylene protons), 2.1 - 1.08 (m, 14 H, methylene protons of side chain), 0.95 (m 6 H, terminal methyl group protons); mass spectrum (70 eV)  $m/e$  (relative intensity)  $M^+$  342 (1), 340 (3), 338 (2), 271 (4), 269 (13), 267 (9), 191 (2), 190 (6), 188 (17), 178 (14), 177 (19), 176 (17), 175 (50), 160 (9), 158 (24), 123 (100) base peak, 109 (14), 101 (30), 99 (12), 97 (36), 96 (35), 95 (15), 88 (18), 83 (72), 81 (76), 79 (11), 71 (15), 70 (31), 69 (37), 67 (37), 67 (67), 57 (18), 56 (15), 55 (77), 53 (15), 43 (32), 41 (40).

An admixture of samples of X obtained from dactylyne and iso-dactylyne had an undepressed melting point, mp 51.5-53.3°C.

## Bibliography

1. Lerner Marine Laboratory Newsletter, Vol. 4, #3, October 1973, Bimini, Bahamas.
2. B. W. Halstead, *Poisonous and Venomous Marine Animals of the World*, Vol. 1, United States Government Printing Office, Washington, D.C., 1965.
3. L. A. Borradile, F. A. Potts, L. E. S. Eastham and J. T. Saunders, *The Invertebrates*, Fourth Edition, Spottiswoode Ballantyne and Co. Ltd., London, 1963. Chap. XV.
4. S. Yamamura and Y. Hirata, *Tetrahedron*, 19, 1485 (1963).
5. T. Irie, M. Suzuki and Y. Hayakawa, *Bull. Chem. Soc. Japan*, 42, 843 (1969).
6. T. Irie, M. Suzuki, E. Kurosawa and Y. Masamune, *Tetrahedron Lett.*, 1837 (1966).
7. T. Irie, M. Suzuki, E. Kurosawa and T. Masamune, *Tetrahedron*, 26, 3271 (1970).
8. T. Irie, A. Fukuzawa, M. Izawa and E. Kurosawa, *Tetrahedron Lett.*, 1343 (1969).
9. A. Fukuzawa, E. Kurosawa and T. Irie, *ibid*, 3 (1972).
10. M. Suzuki, E. Kurosawa and T. Irie, *ibid*, 4495 (1970).
11. T. Irie, M. Suzuki and T. Masamune, *ibid*, 1091 (1965).
12. T. Irie, M. Suzuki and T. Masamune, *Tetrahedron*, 24, 4193 (1968).
13. T. Irie, M. Izawa and E. Kurosawa, *Tetrahedron Lett.*, 2091 (1968).
14. T. Irie, M. Izawa and E. Kurosawa, *ibid*, 2735 (1968).
15. T. Irie, M. Izawa and E. Kurosawa, *Tetrahedron*, 26, 851 (1970).
16. E. Kurosawa, A. Kukuzawa and T. Irie, *Tetrahedron Lett.*, 212 (1972).

17. A. Furusaki, E. Kurosawa, A. Fukuzawa, M. Izawa and T. Irie, *ibid*, 3857 (1973).
18. A. Kurosawa, A. Fukuzawa and T. Irie, *ibid*, 4135 (1973).
19. A. Furusaki, I. Kurosawa, A. Fukuzawa and T. Irie, *ibid*, 4570 (1973).
20. W. Fenical, J. J. Sims and P. Radlick, *ibid*, 313 (1973).
21. D. J. Faulkner, M. O. Stallard, J. Fayos and J. Clardy, *J. Amer. Chem. Soc.*, *95*, 3413 (1973).
22. J. J. Sims, W. Fenical, R. M. Wing and P. Radlick, *J. Amer. Chem. Soc.*, *93*, 3774 (1971).
23. J. J. Sims, W. Fenical, R. M. Wing and P. Radlick, *Tetrahedron Lett.*, 195 (1972).
24. J. J. Sims, W. Fenical, R. M. Wing and P. Radlick, *J. Amer. Chem. Soc.*, *95*, 972 (1973).
25. D. J. Faulkner, M. O. Stallard, *Tetrahedron Lett.*, 1171 (1973).
26. M. R. Willcott, R. E. Davis, D. J. Faulkner and M. O. Stallard, *ibid*, 3967 (1973).
27. J. S. Mynderse and D. J. Faulkner, *J. Amer. Chem. Soc.*, 96, 6771 (1974).
28. D. C. Campbell, Ph.D. Dissertation, The University of Oklahoma, Norman, Oklahoma, 1974.
29. F. J. Schmitz and F. J. McDonald, *Tetrahedron Lett.*, 2541 (1974).
30. F. J. McDonald, D. J. Vanderah and F. J. Schmitz, Abstracts, 30th Southwest Regional A.C.S. Meeting, Houston, Texas, 1974, private communication.
31. F. J. McDonald and F. J. Schmitz, unpublished results.
32. F. J. McDonald, D. C. Campbell, D. J. Vanderah, F. J. Schmitz, D. M. Washecheck, J. E. Burks, D. van der Helm, *J. Org. Chem.*, in press.
33. N. S. Bhacca, L. F. Johnson and J. N. Schoolery, *NMR Spectra Catalog*, Vol. 1, Spectrum #33, Varian Associates, Palo Alto, California, 1962.
34. N. S. Bhacca, L. F. Johnson, and J. N. Schoolery, *ibid*, Spectrum #29 and #31.

35. R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley & Sons, New York, N. Y., 1963, p 81 and 82.
36. E. A. Braud and E. R. H. Jones, *J. Chem. Soc.*, 122 (1946) and previous papers.
37. J. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice-Hall, Inc., London, 1965, p 99.
38. R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley & Sons, New York, N.Y., 1963, p 157.
39. Although the acetylenic proton is not clearly visible in the pmr spectrum in  $\text{CCl}_4$ , the spectrum in  $\text{CDCl}_3$  clearly shows the acetylenic proton absorbing at  $\delta$  2.81, a chemical shift position still at higher field from 3.00 ppm.
40. S. M. Waraszkiewicz, K. L. Erickson, *Tetrahedron Lett.*, 2003 (1974).
41. J. A. McMillan, I. C. Paul, R. H. White, L. P. Hager, *ibid*, 2039 (1974).

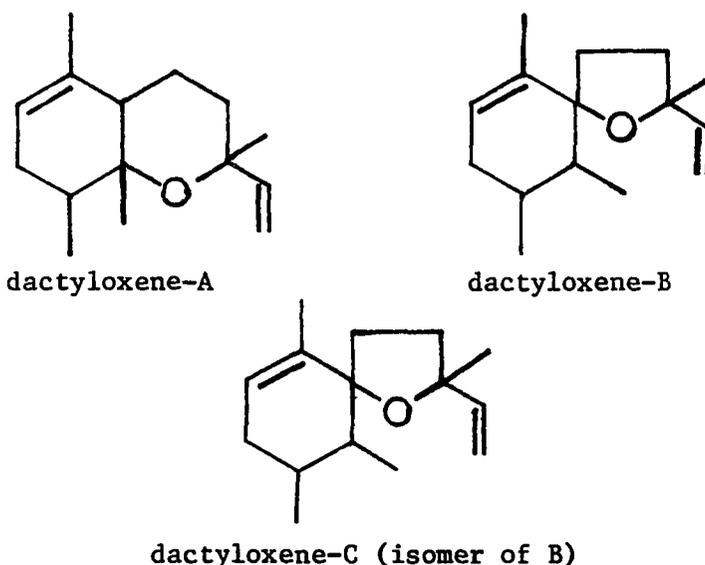
## INTRODUCTION

### ISOLATION OF THREE NEW SESQUITERPENES FROM THE SEA HARE

*APLYSIA DACTYLOMELA* (RANG): DACTYLENOL,

DACTYLENOL ACETATE AND DACTYLOL.

Three new sesquiterpene ethers - dactyloxene - A, dactyloxene-B, and dactyloxene-C<sup>1,2,3</sup> were isolated by earlier workers from the hexane extracts of the sea hare *Aplysia dactylomela* (Rang) and were assigned the structures shown below. The stereochemistry of these compounds is as yet undetermined.

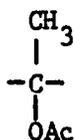


In the course of isolating dactylyne and isodactylyne (Part I of this dissertation) three new non-halogenated sesquiterpene compounds were isolated; structures or possible structures for these are discussed herein.

## RESULTS AND DISCUSSION

In the process of reisolating dactylyne from the extracts of the sea hare *Aplysia dactylomela* three new oxygenated sesquiterpenes were isolated by silica gel chromatography.

Dactylenol acetate,  $C_{17}H_{26}O_2$  (combustion analysis), was isolated as a clear colorless oil. The presence of an acetate was suggested in the infrared spectrum by the absorptions at 1740 and 1245  $cm^{-1}$ , and in the proton magnetic resonance (pmr) spectrum by the three hydrogen singlet at  $\delta$  1.95 (see Figure I). The presence of a methyl group attached to the carbon bearing the acetate as in partial structure A, was indicated by the three proton singlet at  $\delta$  1.48 in the pmr spectrum of dactylenol acetate.

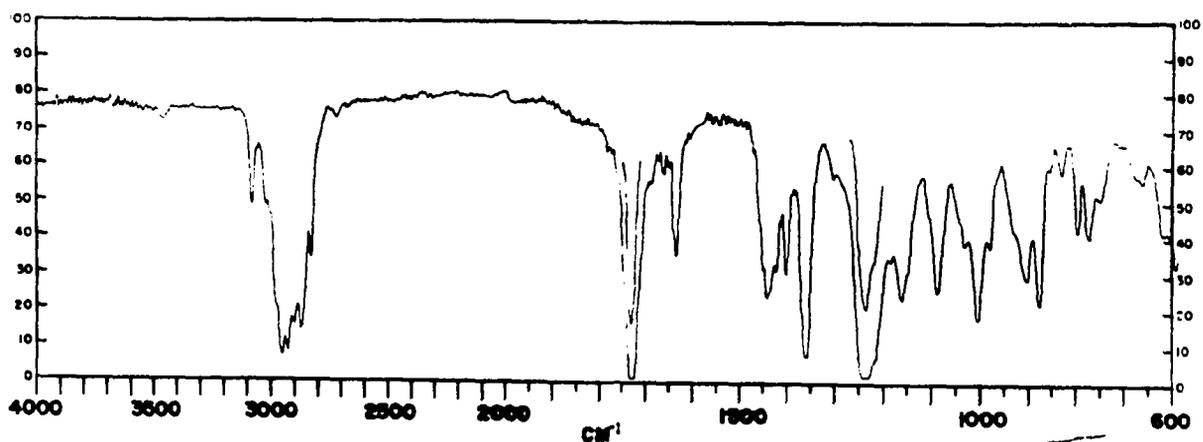


A

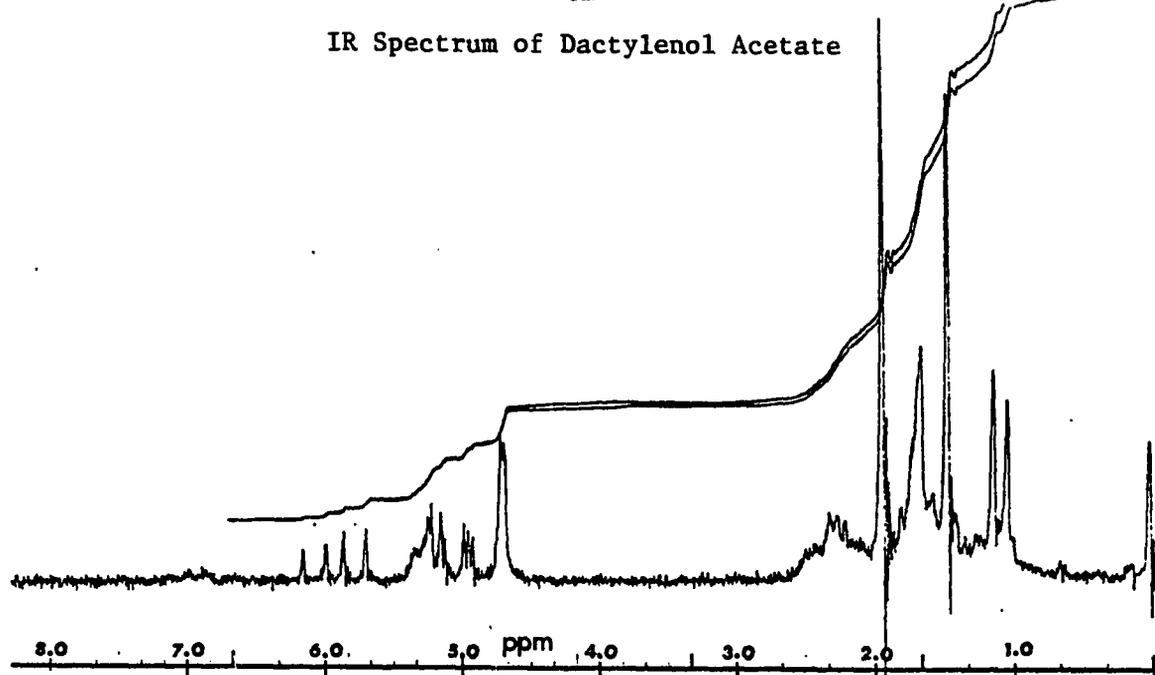
Olefinic functionality was suggested in the ir spectrum by the absorptions at 3090, 1645 and 885  $cm^{-1}$ . The presence of three different olefinic groups was indicated in the pmr spectrum of dactylenol acetate. The first olefinic functionality was denoted by the multiplets centered at  $\delta$  5.93 and  $\delta$  5.04 which constituted an AMX pattern and suggested the presence of a vinyl group attached to a fully substituted carbon, as shown

Figure I

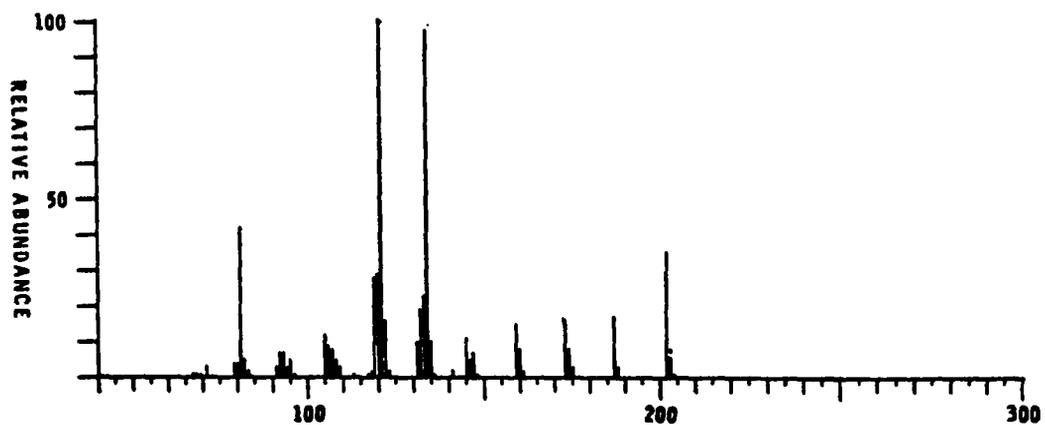
Spectra of Dactylenol Acetate



IR Spectrum of Dactylenol Acetate

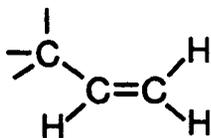


60 MHz PMR Spectrum of Dactylenol Acetate



Mass Spectrum of Dactylenol Acetate

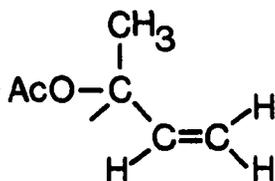
in B.<sup>1</sup> The second olefinic unit, an exomethylene group, was indicated by the two proton signal centered at  $\delta$  4.72 (d,  $J = 2$  Hz).<sup>4</sup> The third olefinic



B

group, a trisubstituted double bond on which one of the substituents was a methyl group, was implied by the one proton signal at  $\delta$  5.25 and the vinyl methyl group signal at  $\delta$  1.67. The presence of three double bonds and one acetate account for four of the five degrees of unsaturation implied by the molecular formula  $C_{17}H_{26}O_2$ , indicating a monocyclic structure.

By analogy with the dactyloxenes (A, B and C) partial structure B was expanded to C.



C

Functionality similar to that depicted in C is commonly found in natural products.<sup>5</sup>

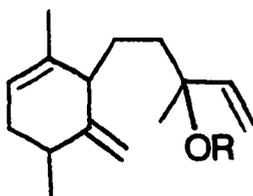
Dactylenol,  $C_{15}H_{24}O$  (combustion analysis), was also obtained as a clear, colorless liquid. The presence of hydroxyl functionality was clearly indicated in the ir spectrum by the broad absorption at  $3400\text{ cm}^{-1}$ .

The pmr spectrum of dactylenol (see Figure II) is virtually identical with that of dactylenol acetate except that: (1) the former lacks the acetate methyl group signal and (2) a methyl group signal appears at  $\delta$  1.21, as compared to  $\delta$  1.48 in dactylenol acetate, which indicates a methyl group is attached to the carbon bearing the hydroxyl group.

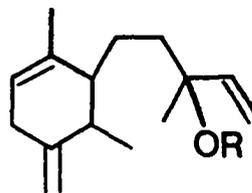
Dactylenol acetate was converted into dactylenol quantitatively by treatment with  $\text{LiAlH}_4$ . The product was identical in all respects with the naturally occurring dactylenol (see figure III). Dactylenol, in turn, was converted into dactylenol acetate in about 10-15% yield using acetic anhydride in pyridine at  $50^\circ$ . The acetate product was identified by gas chromatographic analysis only.

Double irradiation experiments were carried out at 100 MHz on dactylenol and are listed in Table I. These experiments confirmed the presence of the trisubstituted double bond, exomethylene and vinyl group functionalities. It further showed that the methine proton ( $\delta$  2.24) coupled to the secondary methyl group ( $\delta$  1.01) is allylic since irradiation at  $\delta$  2.24 collapsed the  $\delta$  1.01 doublet to a broad singlet.

Based on the data presented, and by analogy to dactyloxene-A, B and C isolated earlier from the same extracts, two possible structures D and E were formulated.



D

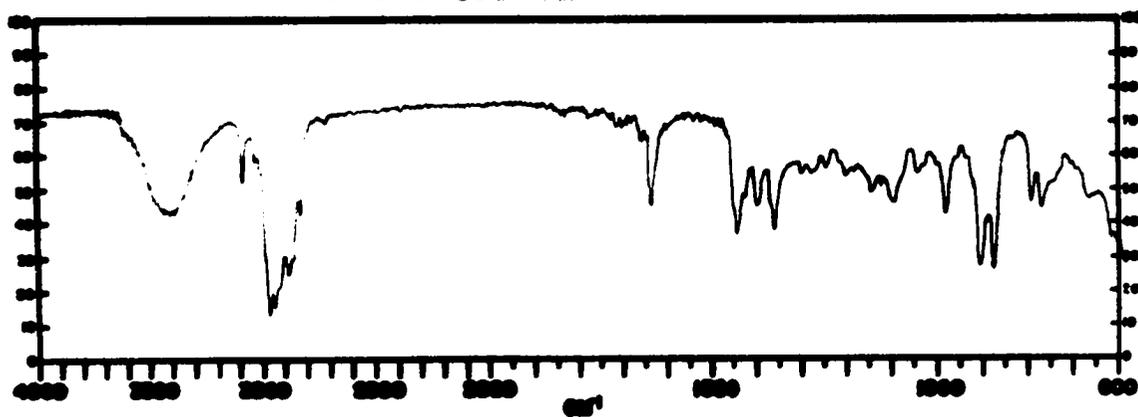


E

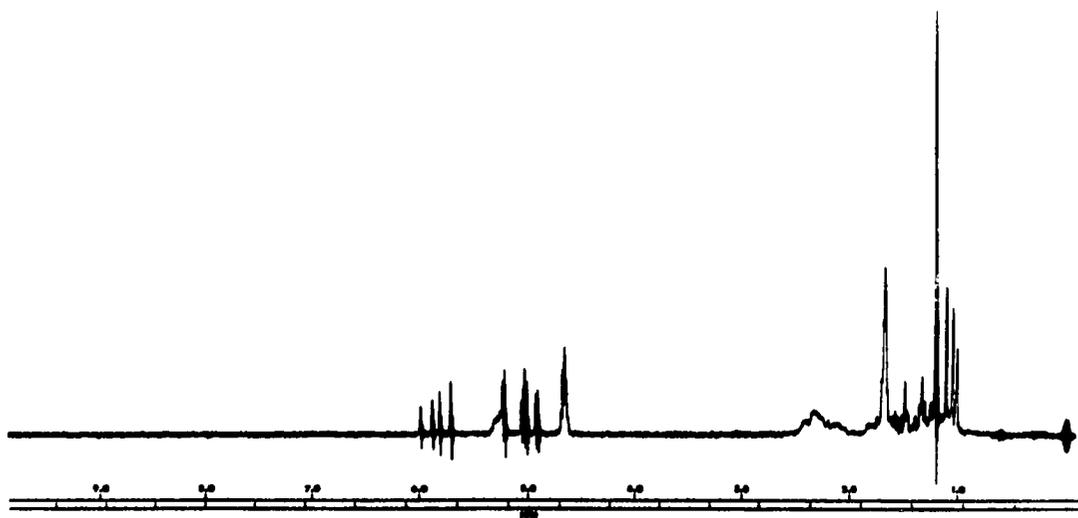
R = H      dactylenol

R = Ac      dactylenol acetate

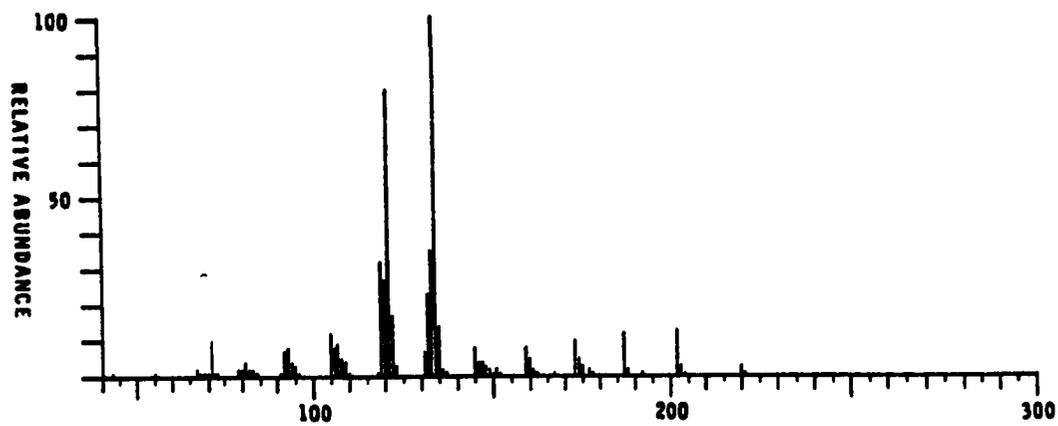
Figure II  
Spectra of Dactylenol



IR Spectrum of Dactylenol

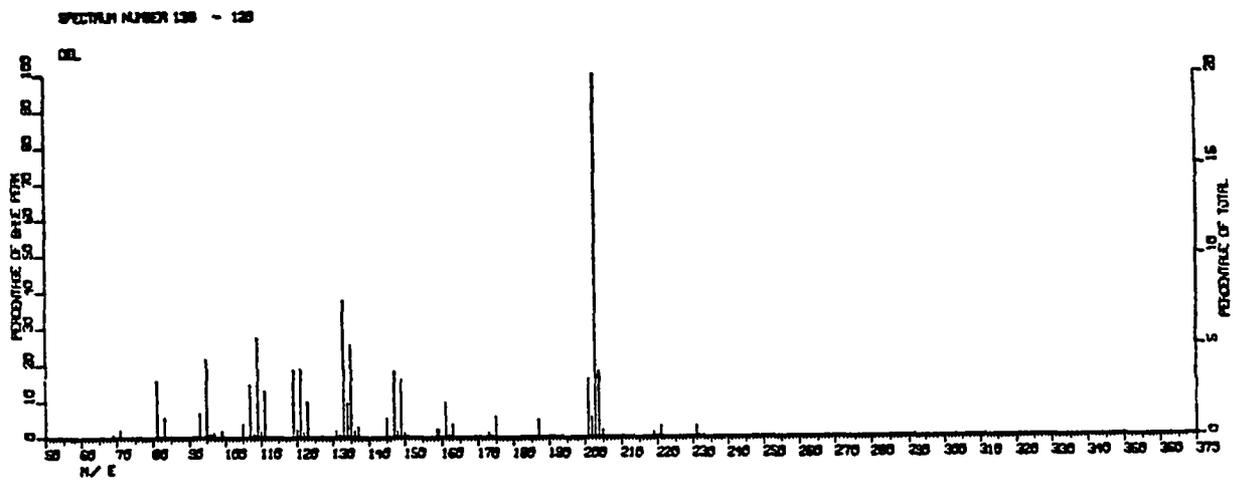
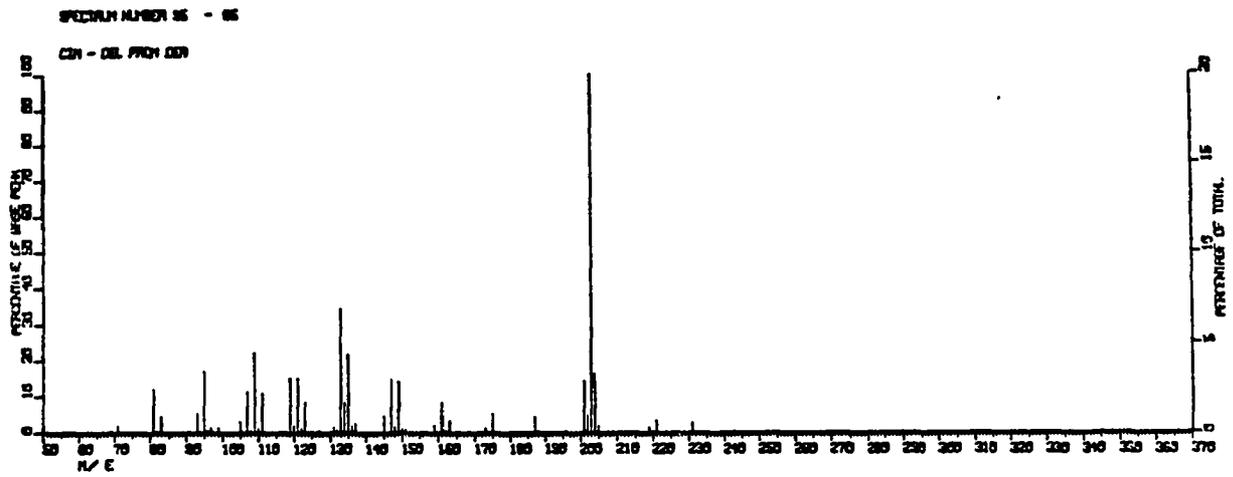


100 MHz PMR Spectrum of Dactylenol



Mass Spectrum of Dactylenol

Figure III



Chemical ionization mass spectra of dactylenol derived from dactylenol acetate (upper spectrum) and naturally occurring dactylenol (lower spectrum).

Table I

Double Irradiation<sup>a</sup> of dactylenol

Signal Irrad'd	Signal Obs'd	Change Obs'd
5.16	1.60	sharpened
4.96	5.76	dd (J = 10,17 Hz)→ dd (J = 10,2 Hz)
2.32	4.60	sharpened
2.24	1.01	d (J = 6 Hz)→ br s

<sup>a</sup>Experiments were carried out on a Varian XL 100 nmr spectrometer using a CCl<sub>4</sub> solution with tetramethylsilane as internal standard and using a proton lock. These experiments were kindly provided by Stan Siegel at Oklahoma State University.

Treatment of dactylenol with a catalytic amount of p-TsOH in ether at room temperature, or  $\text{BF}_3 \cdot 2\text{Et}_2\text{O}$  at  $0^\circ\text{C}$ , yielded a complex product mixture (see Figure IV) that was analyzed by gas chromatographic analysis on a 100' support coated open tubular (S. C. O. T.) FFAP column. All of the products formed had shorter retention times than dactylenol suggesting less polar functionality. Two components of the mixture had retention times identical to those of dactyloxene-A and dactyloxene-B. These two peaks were enhanced when the reaction mixture and authentic samples of dactyloxene-A and B were coinjected. The other peaks (at least 11) are most likely isomers of dactyloxene-A and B and other possible ethers. Although it is not definitive evidence, the formation of the ethers was considered to provide support for the structures postulated in D and E. The conversion of dactylenol to dactyloxene-A and B also raises the possibility that the alcohol is the biogenetic precursor of the ethers. Further work to distinguish between structures D and E for dactylenol and dactylenol acetate is still in progress.

Dactylol,  $\text{C}_{15}\text{H}_{26}\text{O}$  (combustion analysis), was isolated as an oil that crystallized after distillation (Kugelrohr), mp  $50.3\text{--}51.5^\circ\text{C}$ . The presence of a hydroxyl group in dactylol was indicated in the ir spectrum by the broad absorption at  $3500\text{ cm}^{-1}$  (see Figure V). A geminal dimethyl group was denoted by the doublet absorption at  $1370$  and  $1360\text{ cm}^{-1}$  in the ir spectrum and by a six proton singlet at  $\delta 0.90$  in the pmr spectrum. A trisubstituted double bond on which one of the substituents is a methyl group was indicated by the one proton absorption at  $\delta 5.31$  and by the vinyl methyl group signal at  $\delta 1.82$ . Proton decoupling experiments at 220 MHz confirmed the presence of the trisubstituted double bond, since irradiation of the  $\delta 5.31$  signal caused a sharpening of the  $\delta 1.82$  signal. The

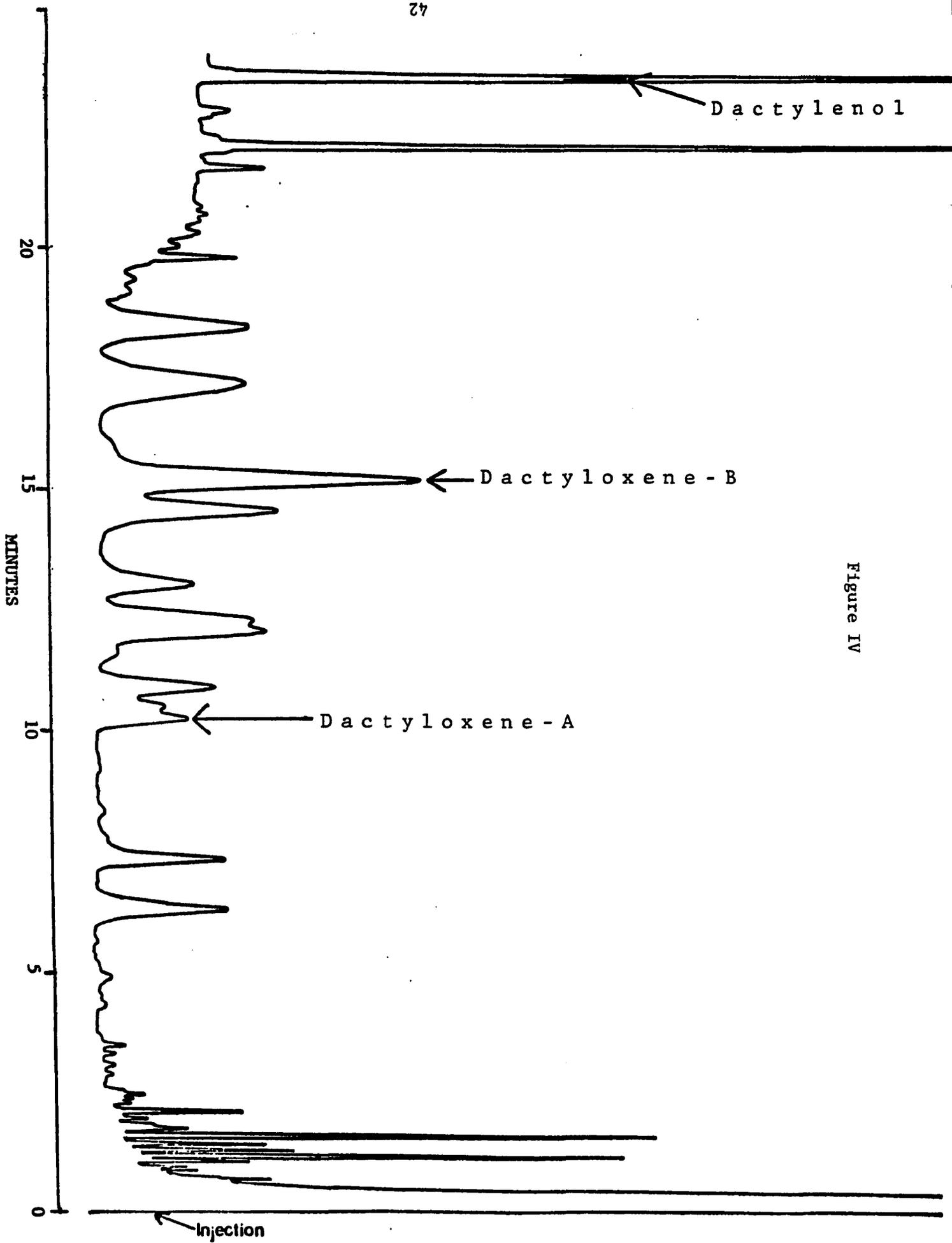
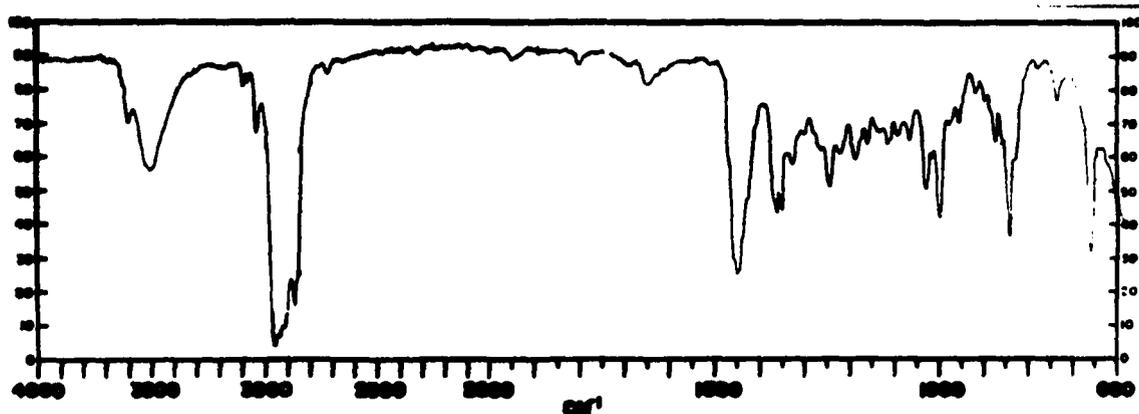
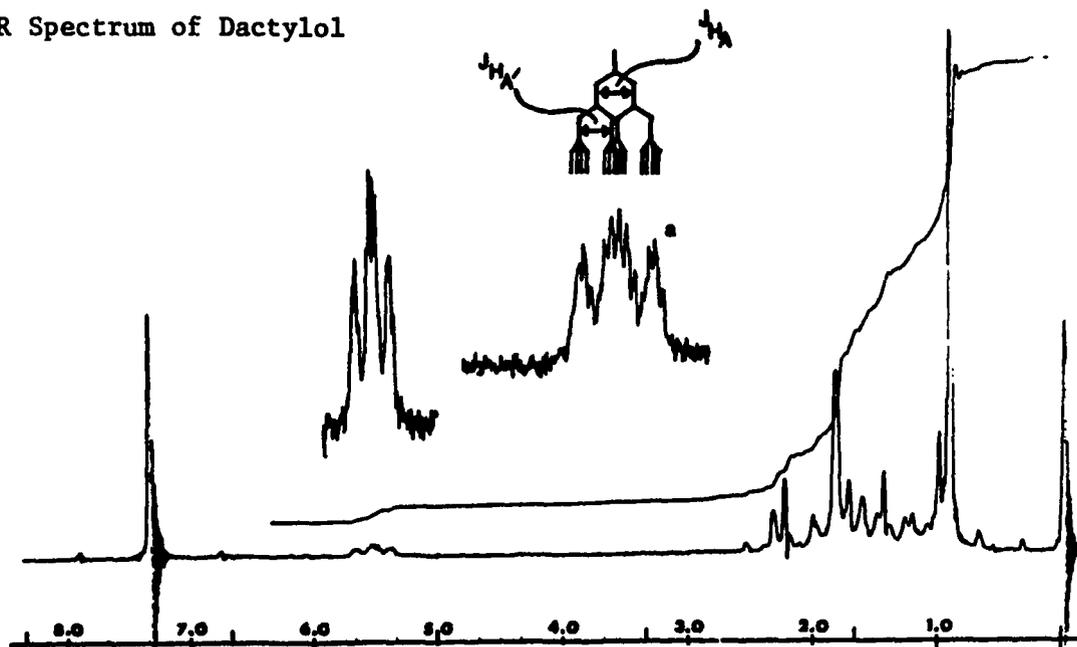


Figure IV

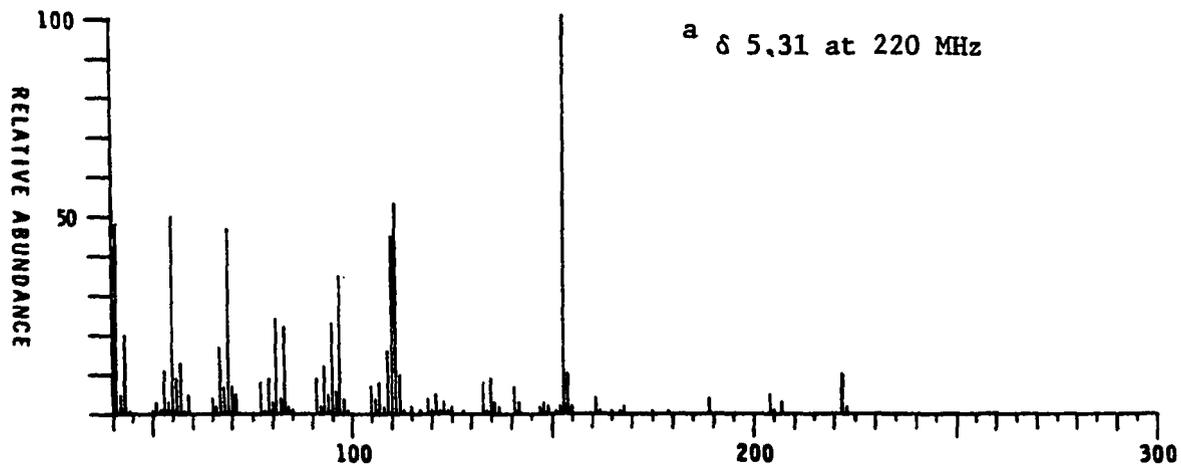
Figure V  
Spectra of Dactyolol



IR Spectrum of Dactyolol



60 MHz Spectrum of Dactyolol



Mass Spectrum of Dactyolol

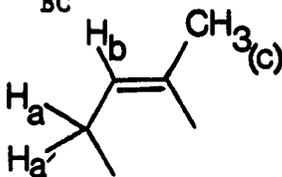
presence of only one double bond in dactylol was indicated by the proton decoupled  $^{13}\text{C}$ -magnetic resonance spectrum (cmr) which displayed only two absorptions in the olefinic carbon region ( $\delta$  135.4 and 125.5).

An absorption in the cmr spectrum consistent for a carbon bearing an oxygen atom was observed at  $\delta$  83.3; this signal remained a singlet in the off resonance decoupled cmr spectrum indicating that the carbinol carbon was tertiary. Since the pmr spectrum does not display any methyl group signal which would be consistent for a methyl group attached to a carbon bearing the hydroxyl oxygen, it was concluded that the carbon bearing the hydroxyl group was a ring junction carbon.

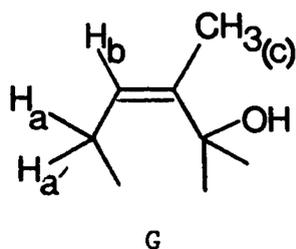
A secondary methyl group in dactylol was indicated in the pmr spectrum by the doublet absorbing at  $\delta$  0.92 ( $J = 5$  Hz). Although the doublet is partially obscured in the spectrum taken in  $\text{CDCl}_3$ , the spectrum taken in benzene- $d_6$  clearly displays the methyl doublet at  $\delta$  1.04 (see experimental section).

The formula  $\text{C}_{15}\text{H}_{26}\text{O}$  for dactylol indicates three degrees of unsaturation and since only one double bond functionality is present a bicyclic structure was indicated.

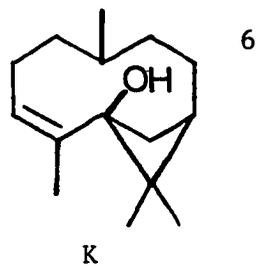
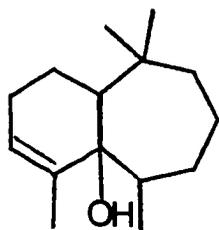
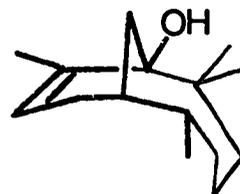
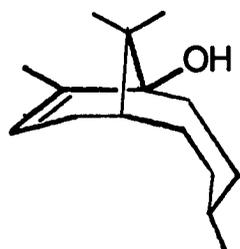
The olefinic absorption,  $\delta$  5.31, is a remarkably complex but sharply defined signal. Partial structure F was deduced from the multiplicity of the  $\delta$  5.31 signal at 220 MHz, where  $J_{\text{H}_A}$  and  $J_{\text{H}_{A'}}$  were  $\approx 7$  Hz but not exactly equal and  $J_{\text{BC}} \approx 1$  Hz. The sharp character of the signal



suggested the lack of further allylic coupling. Thus partial structure F was expanded to G.



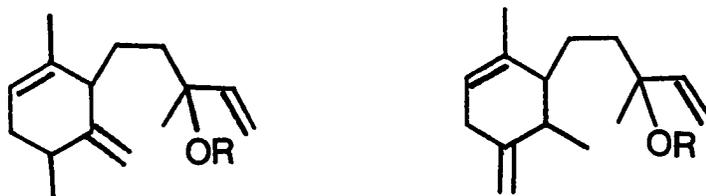
If one assumes an isoprenoid carbon skeleton for dactyolol, four possible ring systems are shown below.



Doubtless more ring systems could be constructed which fit the present data for dactylol. The systems depicted in I, J and K are derived from known systems in the sesquiterpene literature. Should H be shown to be the ring system for dactylol it would establish a new secondary<sup>7</sup> class of compounds for sesquiterpenes. The ring system of H was derived basically by analogy to the dactyloxenes (A, B and C), dactylenol and dactylenol acetate previously discussed.

### Summary

Three new sesquiterpene compounds - dactylenol, dactylenol acetate, and dactylol - were isolated from the hexane extracts of the sea hare *Aplysia dactylomela*. Two possible structures for dactylenol are proposed based on spectroscopic data and conversion of dactylenol into two known sesquiterpene ethers.



R = H      dactylenol

R = Ac      dactylenol acetate

Dactylol,  $C_{15}H_{26}O$ , was shown to be a bicyclic compound containing a tertiary carbinol group, a geminal dimethyl group, a trisubstituted double bond and a secondary methyl group.

## Experimental

Experimental conditions specified in section I apply with the following addition. Preparative layer chromatography was performed on 20 x 20 cm glass plates with a 2 mm layer of silica gel PF<sub>254+366</sub> (E. Merck AG, Darmstadt) impregnated with 9% silver nitrate by weight. Preparative gas chromatography was carried out on a Varian 1200 instrument using a 20% FFAP column (6' x 1/4") at 160°C.

Isolation of dactylenol and dactylenol acetate. The initial processing of *Aplysia* is described in the dactylyne/isodactylyne section. From the florisil chromatography described earlier, 16.4550 g was obtained in fractions 9-11, 9.01 g was obtained in fractions 12-14, and 6.3 g was obtained in fractions 15-18. A portion (2.62 g) of the material collected in fractions 9-11 was chromatographed on 40 g of thin-layer mesh silica gel employing benzene/hexane (1:1) as solvent and collecting 15 ml fractions. After collection of 31 fractions, three 100 ml fractions were collected. The first of these 100 ml fractions contained fairly pure (80-90%) dactylenol acetate. Further chromatographies on silica gel and 9% AgNO<sub>3</sub> impregnated silica gel did not enhance the purity of dactylenol acetate. Pure dactylenol acetate was obtained by preparative gas chromatography on a 6' x 1/4" 20% FFAP column.

Pure dactylenol acetate has  $[\alpha]_D + 168.0^\circ$  ( $c$  2.46, CHCl<sub>3</sub>), bp (Kugelrohr)  $\approx 75^\circ/1$  Torr;  $R_f = 0.36$  (5% ether/hexane, silica gel H); ir (neat) 3090, 2970, 2945, 2885, 2835, 1740 (acetate), 1645 (double bond),

1455, 1410, 1370, 1245, 1170, 1090, 1030, 915, 885, 805, and 780  $\text{cm}^{-1}$ ;  
 60 MHz pmr ( $\text{CCl}_4$ )  $\delta$  5.91 (dd, 1,  $J = 17$  Hz,  $J = 10$  Hz,  $-\underline{\text{CH}}=\text{CH}_2$ ), 5.23  
 (br s, 1,  $J = 6$  Hz,  $J < 1$  Hz,  $-\underline{\text{CH}}=\text{CCH}_3-$ ), 5.08 (dd, 1,  $J = 17$  Hz,  $J = 2$   
 Hz,  $\begin{array}{c} \text{H} \\ | \\ \text{C} = \text{C} \\ | \quad | \\ \text{H} \quad \text{H} \end{array}$ ), 5.05 (dd, 1,  $J = 10$  Hz,  $J = 2$  Hz,  $\begin{array}{c} \text{H} \\ | \\ \text{C} = \text{C} \\ | \quad | \\ \text{H} \quad \text{H} \end{array}$ ), 4.68 (br d,  
 2,  $J = 2$  Hz,  $\text{C}=\underline{\text{CH}}_2$ ), 2.6 - 0.8 (m, 8), 1.95 (s, 3,  $\text{CH}_3-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-$ ), 1.65 (br s,  
 3,  $-\text{CH}=\text{CCH}_3-$ ), 1.48 (s, 3,  $\text{CH}_3\text{CO}_2-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3-$ ), and 1.08 ppm (d, 3,  $J = 6$  Hz,  
 $\text{CH}-\underline{\text{CH}}_3$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity) no molecular ion  
 was observed, 202 [ $\text{M}^+ - 60$  (35)], 187 (17), 173 (16), 159 (15), 145 (11),  
 134 (97), 121 (100) base peak, 105 (12), and 81 (42).

Anal. Calcd. for  $\text{C}_{17}\text{H}_{26}\text{O}_2$ : C, 77.82; H, 9.99;

Found: C, 77.72; H, 9.95.

Chromatography of the material (6.3 g) obtained from the combined  
 fractions 15, 16, 17 and 18 (see above) on 60 g of thin-layer mesh silica  
 gel using benzene/hexane (7/3) as solvent and collecting 50 ml fractions  
 yielded, in fractions 15-18 and the 100% benzene flush,  $\approx 1.5$  g of  
 dactylenol, homogeneous by tlc analysis. Gas chromatographic analysis  
 of this material on a 5' x 1/8" 10% FFAP column indicated that the material,  
 homogeneous by tlc, was a mixture of at least five components. Pure  
 dactylenol was obtained by chromatography on 9%  $\text{AgNO}_3$  impregnated silica  
 gel thick-layer plates (20 x 20 cm, 2 mm thick) employing ethyl acetate/cyclo-  
 hexane (4/6) as the irrigating solvent. From  $\approx 100$  mg samples applied onto  
 the thick layer plates, about 40-50 mg of pure dactylenol was obtained.

Pure dactylenol has  $[\alpha]_D + 203.8$ ,  $R_f$  0.21 (70% benzene/hexane,  
 silica gel H); ir (neat) 3400 (broad), 3080, 2970, 2940, 2880, 2830, 1640,  
 1450, 1365, 1150, 1100, 985, 910, 880, 800 and 775  $\text{cm}^{-1}$ ; 100 MHz pmr ( $\text{CCl}_4$ )  
 $\delta$  5.84 (dd, 1,  $J = 17$  Hz,  $J = 10$  Hz,  $-\underline{\text{CH}}=\text{CH}_2$ ), 5.24 (m, 1,  $-\underline{\text{CH}}=\text{CCH}_3-$ ),  
 5.13 (dd, 1,  $J = 17$  Hz,  $J = 2$  Hz,  $\begin{array}{c} \text{H} \\ | \\ \text{C} = \text{C} \\ | \quad | \\ \text{H} \quad \text{H} \end{array}$ ), 4.96 (dd, 1 H,  $J = 10$  Hz,

$J = 2 \text{ Hz}$ ,  $\begin{array}{c} \diagup \\ \text{C} \\ \diagdown \end{array} = \begin{array}{c} \text{H} \\ | \\ \text{C} \\ | \\ \text{H} \end{array}$ , 4.66 (br d, 2,  $J = 2 \text{ Hz}$ ), 2.45 - 0.8 (m, 8), 1.66 (br s, 3,  $-\text{CH}=\text{CCH}_2-$ ), 1.19 (s, 3,  $\text{HO}-\text{CCH}_2$ ), 1.07 (d, 3,  $J = 6 \text{ Hz}$ ), and 1.01 ppm (s, 1,  $\text{H}-\text{O}-\text{R}$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$ , 220 (3), 202 (13), 187 (12), 173 (10), 159 (8), 145 (8), 134 (100), base peak, 132 (23), 121 (80), 119 (32), 105 (12), and 71 (10).

Anal. Calcd. for  $\text{C}_{15}\text{H}_{24}\text{O}$ : C, 81.76; H, 10.98;

Found: C, 81.94; H, 11.14.

Dactylenol from dactylenol acetate. To a 25 ml round bottomed flask containing 57.3 mg of dactylenol acetate and a magnetic stirring bar was added  $\approx 10$  ml of dry ether (distilled directly from lithium aluminum hydride) and then 20 mg of lithium aluminum hydride (LAH). The reaction mixture was stirred at room temperature for 30 minutes after the addition of the LAH. The excess LAH was destroyed by the addition of ethyl acetate followed by water. The water layer was extracted sequentially with two equal volumes of ethyl acetate and two equal volumes of ether. The organic layers were combined and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . solvent was removed under reduced pressure to yield 49.5 mg of a clear liquid. The reaction product was placed atop 10 g of thin-layer mesh silica gel and chromatographed employing 10% ether/hexane as solvent and collecting 7 ml fractions. Fractions 14-17 contained a single compound as judged by tlc and gas chromatographic analysis. The pure reaction product was identical to the naturally occurring dactylenol in all the following respects: ir, pmr (60 MHz), and mass spectra (chemical ionization using methane and isobutane as gas),  $R_f$  value, and gas chromatographic retention time (on the 5' x 1/8" 10% FFAP column).

Pure dactylenol derived from dactylenol acetate has  $[\alpha]_D + 204^\circ$  ( $c$  0.25,  $\text{CHCl}_3$ ); ir (neat) 3400, 3080, 3030, 2970, 2940, 2920, 2880, 2830, 1665, 1645, 1450, 1405, 1365, 1255, 1150, 1100, 980, 910, 880, 800 and  $780 \text{ cm}^{-1}$ ; 60 MHz pmr ( $\text{CCl}_4$ )  $\delta$  5.70 (dd, 1,  $J = 17 \text{ Hz}$ ,  $J = 10 \text{ Hz}$ ,  $\begin{array}{c} \diagup \text{C} = \text{C} \diagup \\ | \quad | \\ \text{H} \quad \text{H} \end{array}$ ), 5.27 (m, 1,  $-\text{CH}=\text{CCH}_3-$ ), 5.10 (dd, 1,  $J = 17 \text{ Hz}$ ,  $J = 2 \text{ Hz}$ ,  $\begin{array}{c} \diagup \text{C} = \text{C} \diagup \\ | \quad | \\ \text{H} \quad \text{H} \end{array}$ ), 4.95 (dd, 1,  $J = 10 \text{ Hz}$ ,  $J = 2 \text{ Hz}$ ,  $\begin{array}{c} \diagup \text{C} = \text{C} \diagup \\ | \quad | \\ \text{H} \quad \text{H} \end{array}$ ), 4.65 (d, 2,  $J = 2 \text{ Hz}$ ,  $\diagup \text{C}=\text{CH}_2$ ), 2.50 - 0.8 (m, 9), 1.65 (br s, 3,  $-\text{CH}=\text{CCH}_3-$ ), 1.20 (s, 3,  $-\text{COHCH}_3$ ), and 1.07 ppm (d, 3,  $J = 6 \text{ Hz}$ ,  $-\text{CHCH}_3$ ); mass spectrum (chemical ionization using methane as gas)  $m/e$  (rel. intensity) 243 (1), 231 (3), 221 ( $M^+ + 1$ ) (4), 220 ( $M^+$ ) (1), 219 (2), 204 (16), 203 (100) base peak, 201 (14), 187 (5), 175 (6), 161 (8), 149 (13), 147 (14), 135 (22), 133 (34), 123 (8), 121 (13), 119 (13), 111 (11), 109 (22), 107 (12), 95 (17), and 81 (12).

Anal. Calcd. for  $\text{C}_{15}\text{H}_{24}\text{O}$ : C, 81.76; H, 10.98

Found: C, 81.93, H, 10.91.

Acetylation of Dactylenol. To 25 mg of dactylenol was added 1.0 ml pyridine (dried over molecular sieves) and 1.0 ml acetic anhydride. The flask's contents were then placed under a nitrogen atmosphere and the temperature raised to  $50^\circ\text{C}$  for eight hours. The solvent was removed in vacuo yielding a semi-solid residue that was only partially soluble in hexane. Gas chromatographic analysis of the hexane solubles on a 5' x 1/8" 10% FFAP column showed a peak of retention time identical to that of dactylenol acetate. The gas chromatographic analysis also showed that only a small amount ( $\sim 10\%$ ) of the alcohol had been converted to the acetate.

Isolation of dactylol. During the course of isolating a dactylenol acetate and dactylenol another sesquiterpene alcohol., dactylol, was

obtained from silica chromatographies described earlier. Dactylol was eluted from the chromatographic support between dactylenol acetate and dactylenol. Impure dactylol (238.8 mg) was rechromatographed on 10 g of thin-layer mesh silica gel employing benzene/hexane as solvent and collecting 10 ml fractions. Fractions 12-16 yielded 109 mg of material that was homogeneous by tlc and that crystallized upon removal of all solvents. Recrystallization from hexane afforded pure dactylol.

Pure dactylol has mp 50.3 - 51.5°C,  $[\alpha]_D + 22.5^\circ$  ( $c$  1.76,  $\text{CHCl}_3$ );  $R_f$  0.22 (50% benzene/hexane, silica gel H); ir (neat) 3600, 3500, 3090, 3070, 3040, 2960, 2870, 1460, 1370, 1360, 1265, 1040, 1010, 855 and 670  $\text{cm}^{-1}$ ; 60 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  5.31 (complex t, 1 H,  $J = 7$  Hz,  $-\text{CH}_2-\text{CH}=\text{CCH}_3-$ ); 2.4-0.8 (m, 13 H), 1.82 (br s, 3 H,  $-\text{CH}=\text{CCH}_3-$ ), 0.90 (d, 3 H,  $J = 5$  Hz,  $-\text{CHCH}_3-$ ), and 0.90 ppm (s, 6 H,  $-\text{C}(\text{CH}_3)_2-$ ); 60 MHz pmr ( $d_6$ -benzene)  $\delta$  5.31 (complex t, 1 H,  $J = 7$  Hz,  $-\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)-$ ), 2.2 - 0.6 (m, 13 H), 1.87 (br s, 3 H,  $-\text{CH}=\text{CCH}_3-$ ), 1.04 (d, 3 H,  $J = 5$  Hz,  $-\text{CHCH}_3-$ ), 0.93 (s, 3 H, one of  $-\text{C}(\text{CH}_3)_2-$ ), and 0.90 ppm (s, 3 H, one of  $-\text{C}(\text{CH}_3)_2-$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$  222 (10), 207 (3), 204 (5), 189 (4), 161 (4), 153 (100) base peak, 111 (59), 110 (45), 97 (35), 81 (24), 69 (47), 55 (50), 43 (20), and 41 (48).

Anal. Calcd. for  $\text{C}_{15}\text{H}_{26}\text{O}$ : C, 81.02; H, 11.78.

Found: C, 81.05; H, 11.90.

## BIBLIOGRAPHY

1. F. J. Schmitz and F. J. McDonald, *Tetrahedron Lett.*, 2541 (1974).
2. F. J. McDonald, D. J. Vanderah and F. J. Schmitz, Abstracts, 30th Southwest Regional A.C.S. Meeting, Houston, Texas, 1974, private communication.
3. F. J. McDonald and F. J. Schmitz, unpublished results.
4. R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley and Sons, New York, N.Y., 1963, p 130.
5. T. K. Devon and A. I. Scott, "Handbook of Naturally Occurring Compounds," Vol. II, Terpenes, Academic Press, New York, N.Y., 1972.
6. Suggested by Dr. R. A. Gross, private communication.
7. See reference 5 p 2.

## ISOLATION OF DENDROLASIN FROM THE SPONGE

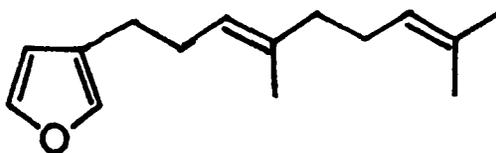
*OLIGOCERAS HEMORRHAGES* (de Laubenfels)

### Introduction

In the course of collecting sponges for evaluation as sources of drug materials, it was noted that freshly detached samples of *Oligoceras hemorrhages*, commonly called the bleeding sponge,<sup>1</sup> have an aroma reminiscent of terpene mixtures. This observation prompted us to search for sesquiterpenes in the extracts of *O. hemorrhages*. Minale and collaborators have isolated a number of furanosesquiterpenes, poly-prenylated benzoquinones, and polyprenylated benzoquinols from Mediterranean sponges.<sup>2</sup> One of the furanosesquiterpenes isolated from the sponge *Pleraplysilla spinifera* was 9,10-dehydrodendrolasin.<sup>2b</sup> Dendrolasin itself was first isolated<sup>3</sup> from the ant *Dendrolasius fuliginosus* and subsequently also from sweet potato fusel oil<sup>4</sup> and the wood oil of *Torreya nucifera*.<sup>5</sup> It has been suggested that dendrolasin may function as a juvenile hormone,<sup>6</sup> an alarm substance<sup>7</sup> and a defensive substance.<sup>8</sup> Two different syntheses of dendrolasin have been reported.<sup>9</sup> The purpose of this section is to report the isolation of dendrolasin (I) from the sponge *O. hemorrhages* collected in the lagoon at Bimini, Bahamas.

## RESULTS AND DISCUSSION

Dendrolasin (I) was isolated by chromatography over Florisil of the chloroform extracts of sponge specimens which had been immersed in 2-propanol immediately after collection and subsequently recovered by filtration and air dried. Dendrolasin isolated in this manner exhibited the same refractive index, ir, uv, nmr and mass spectra previously reported.<sup>3,5</sup> Catalytic hydrogenation of sponge-derived dendrolasin gave a perhydro product that was determined by infrared, nuclear magnetic resonance, mass spectral and gas chromatographic analyses to be identical to an authentic sample of perhydrodendrolasin.

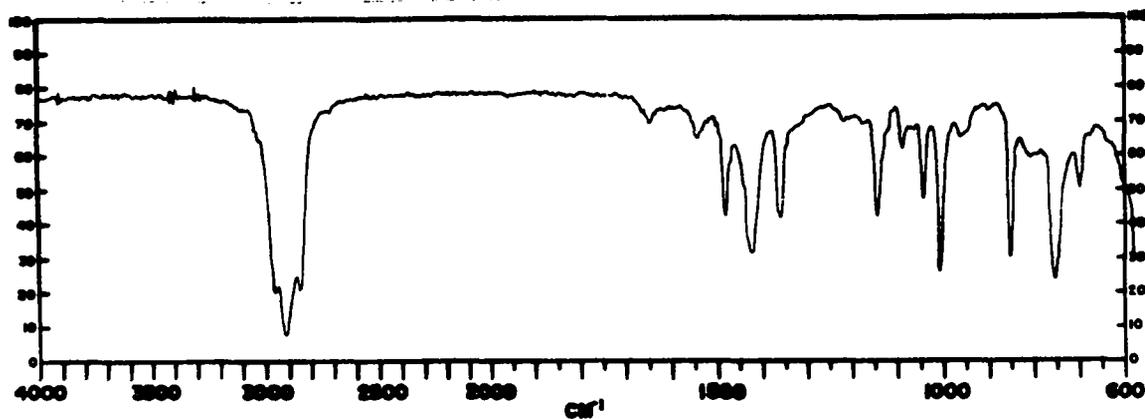


I

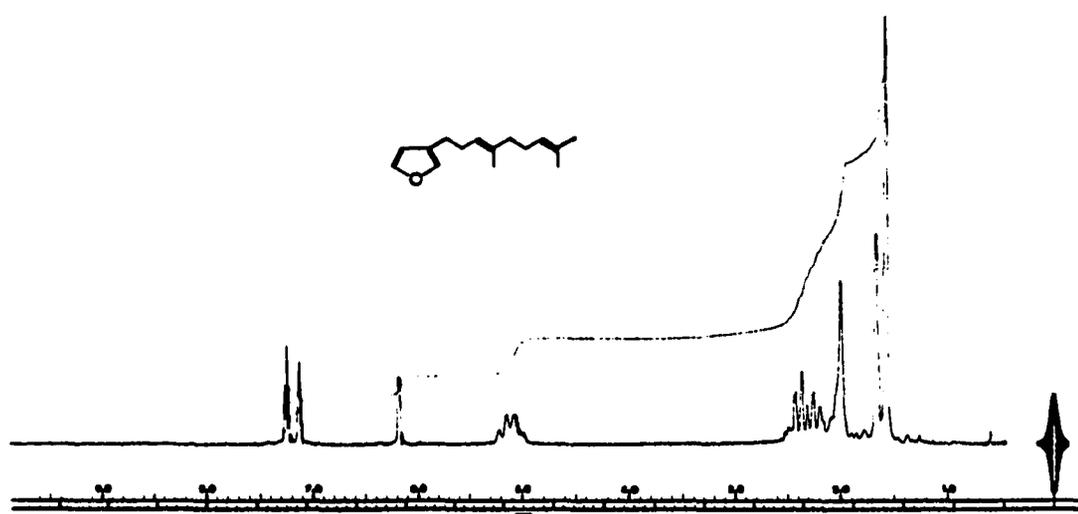
The original aqueous alcoholic extract of *O. hemorrhages* was concentrated on a rotary evaporator at reduced pressure. Simple extraction of this concentrate with hexane and then chloroform gave 99.2 g and 1.4 g batches of material, respectively. The hexane extract was shown by chromatography to contain dendrolasin also.

After extraction with hexane and chloroform, the aqueous concentrate was lyophilized and the lyophilizate was soaked in methanol. The methanol soluble fraction exhibited high levels of positive inotropic activity in a modified Langendorff test.<sup>11</sup> Further investigations<sup>12</sup> have

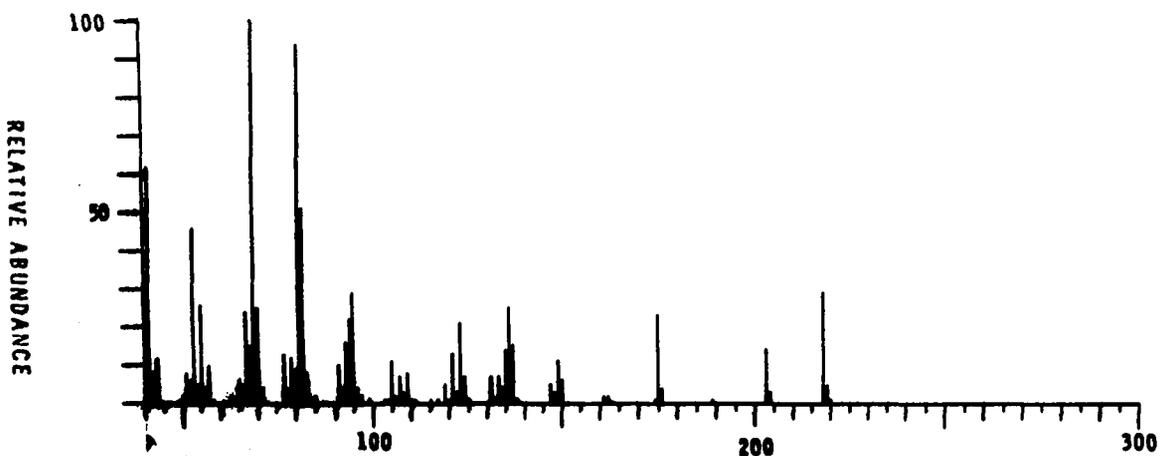
Figure I  
Spectra of Dendrolasin (I)



IR Spectrum of I



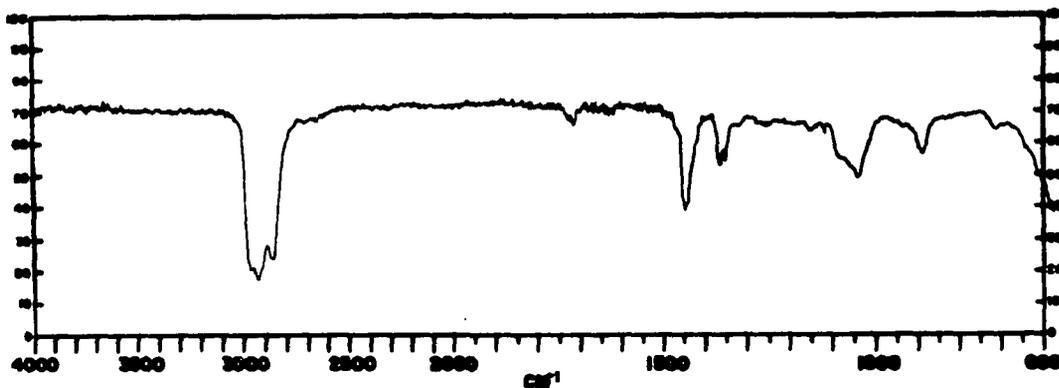
100 MHz PMR Spectrum of I



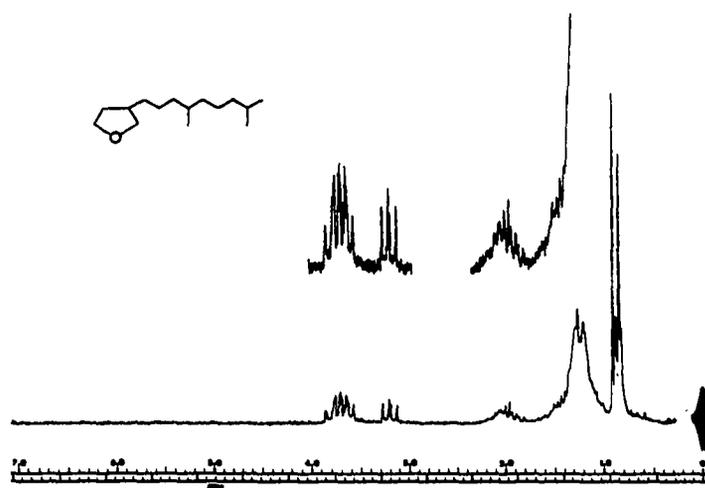
Mass Spectrum of I

Figure II

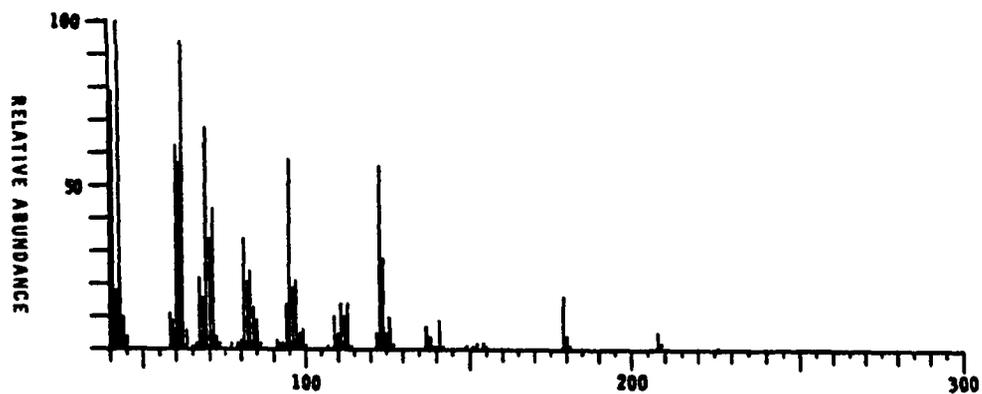
Spectra of Perhydrodendrolasin (II)



IR Spectrum of II



100 MHz PMR Spectrum of II



Mass Spectrum of II

now established that histamine was the principal inotropic agent of the methanol fraction.

## EXPERIMENTAL

Experimental conditions discussed in Section I apply.

Isolation of Dendrolasin. Specimens of the sponge *O. hemorrhages* were collected in late May/early June (1973, 1974) in the lagoon of Bimini, Bahamas. The freshly collected sponge material was shaken free of silt, cut into small pieces, and immediately immersed in 2-propanol for preservation during shipment. The alcohol was later removed by filtration, and after the sponge material was air dried, it was extracted by soaking 3 times at room temperature for 24 hr periods with 1.5 l portions of chloroform. The combined chloroform extracts weighed 33.9 g; the re-dried sponge residue weighed 2.33 kg.

The total chloroform extract was chromatographed on Florisil (450 g) and yielded the following fractions (solvent, volume used, eluate): a) hexane, 3 l, 1.8 g; b) benzene, 3 l, 5 g; c) chloroform, 3 l, 5.3 g and d) methanol, 3 l, 15 g. Rechromatography of the hexane eluate over 40 g of Florisil using hexane as solvent yielded 480 mg of dendrolasin which was homogeneous by tlc analysis on silica gel and gas chromatographic analysis on two different supports (5' X 1/8" 10% FFAP and 10' X 1/8" 1% OV-1).

Pure dendrolasin has bp (Kugelrohr), 65°/1 Torr;  $n_D^{23}$  1.4873 (Lit.  $n_D^{20}$  1.486); ir, pmr and mass spectra were the same as those reported;<sup>3,5</sup> ir (neat) 2960, 2910, 2858, 1500, 1440, 1375, 1160, 1103,

1060, 1022, 870 and 770  $\text{cm}^{-1}$ ; 100 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  7.24 (m, 1 H, one of the  $\alpha$  protons of furan ring), 7.12 (m, 1 H, one of the  $\alpha$  protons of the furan ring), 6.18 (m, 1 H, the  $\beta$  proton of the furan ring), 5.10 (m, 2 H,  $-\text{CH}=\text{C}-$ ), 2.56 - 1.95 (m, 6 H, methylene protons), 1.66 (br s, 3 H,  $-\text{CH}=\text{CCH}_3-$ ), 1.58 ppm (br s, 6 H,  $-\text{CH}=\text{CCH}_3-$ ); 25.2 MHz proton decoupled cmr ( $\text{CDCl}_3$ )  $\delta$  142.3, 138.6, 135.5, 131.1, 124.8, 124.2, 123.7, 39.7, 28.5, 26.7, 25.7, 25.1, 17.7, and 16.0 ppm; mass spectrum (145°C, 70 eV)  $m/e$  (rel. intensity)  $M^+$ , 218 (29), 203 (14), 175 (23), 136 (25), 123 (21), 95 (29), 81 (94), 69 (100) base peak, 41 (62).

Anal. Calcd. for  $\text{C}_{15}\text{H}_{22}\text{O}$ : C, 82.51; H, 10.16. Found: C, 82.75; H, 10.39.

Perhydrodendrolasin A 70 mg sample of dendrolasin dissolved in 10 ml of 95% ethanol was added to 15 ml 95% ethanol containing a few mg of pre-reduced  $\text{PtO}_2$ . Hydrogenation was conducted at atmospheric pressure for 12 hrs. Removal of the catalyst by filtration and evaporation of the solvent at reduced pressure gave 72.8 mg of a clear, colorless oil. Chromatography of this oil on silica gel gave pure perhydrodendrolasin: ir (neat) 2960, 2930, 2860, 1460, 1375, 1360, 1080, 1070, 1050, 903 and 750  $\text{cm}^{-1}$  (cf. ref. 3); 100 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  3.71 (br pentet, 3 H, protons on the  $\alpha$ -carbons of the tetrahydrofuran ring), 3.21 (dd, 1 H,  $J = 8$  Hz,  $J = 6$  Hz, proton on  $\alpha$ -carbon of the tetrahydrofuran ring *cis* to the alkyl group), 2.26 - 1.75 (m, 2 H), 1.70 - 0.97 (m, 14 H, methylene and methine protons of alkyl side chain), 0.88 (d, 6 H,  $J = 7$  Hz,  $-\text{CHCH}_3-$ ), and 0.86 ppm (d, 3 H,  $J = 6$  Hz,  $-\text{CHCH}_3-$ ); mass spectrum (70 eV)  $m/e$  (rel.

intensity), no molecular ion observed, 208 ( $M^+ - 18$ ) (5), 179 (16), 123 (56), 113 (14), 111 (14), 109 (10), 95 (58), 81 (34), 69 (68), 57 (94), 43 (100) base peak, 41 (79).

The hydrogenation product and an authentic sample of perhydro-dendrolasin exhibited identical gas chromatographic retention times on two different supports (see above for dendrolasin). A single symmetrical peak was obtained when the two samples were coinjected on either support.

## BIBLIOGRAPHY

1. M. W. deLaubenfels, *American Museum Novitates*, No. 1431, 1 (1949).
2. (a) G. Cimino, S. DeStefano and L. Minale, *Experientia*, 39, 846 (1974); (b) G. Cimino, S. DeStefano, L. Minale and E. Trivellone, *Tetrahedron*, 28, 4761 (1972); (c) G. Cimino, S. DeStefano and L. Minale, *ibid*, 28, 1315-1324 (1972).
3. A. Quillico, F. Piozzi and M. Pavan, *ibid*, 1, 177 (1957).
4. Y. Hirose, M. Abu and Y. Sekiya, *J. Chem. Soc. Japan, Pure Chem. Sec.*, 82, 725 (1961).
5. T. Sakai, K. Nishimura and Y. Hirose, *Bull. Chem. Soc. Japan*, 38, 381 (1965); T. K. Nishimura and Y. Hirose, *Tetrahedron Lett.*, 1171 (1963).
6. V. B. Wigglesworth, *J. Ins. Physiol.*, 9, 105 (1963); see however R. Bernardi, R. C. Cardani, D. Ghiringhelli, A. Selva and M. Pavan, *Tetrahedron Lett.*, 3893 (1967) for suggestion that observed activity may be due to farnesal impurity.
7. M. Pavan, *Atti. Accad. Naz. Ital. Entomol. Rend.*, 8, 228 (1961) as cited by G. W. K. Cavill, P. J. Williams and F. B. Whitfield, *Tetrahedron Lett.*, 2201 (1967).
8. M. Pavan, *Ric. Sci.*, 26, 144 (1956).
9. A. F. Thomas, *Chem. Comm.*, 1657 (1968); K. A. Parker and W. S. Johnson, *Tetrahedron Lett.*, 1329 (1968).
10. Sample and 100 MHz nmr kindly provided by Prof. L. Minale, Laboratorio per la Chimica e Fisica di Molecole di Interesse biologico del C. N. R., Arco Felice, Napoli, Italy.
11. R. Cherlier, "Coronary Vasodilators." Pergamon Press, Oxford, England, 1961.
12. K. H. Hollenbeak, F. J. Schmitz and Pushkar N. Kaul, Proceedings of the Fourth Food and Drugs from the Sea Conference, The Marine Technology Society, Washington, D. C., 1974, in press.

ISOLATION OF TWO KNOWN SAPOGENINS FROM THE  
SEA CUCUMBER *Holothuria Atra*.

INTRODUCTION

Sea cucumbers or Holothuridae (phylum Echinodermata, subphylum Eleutherozoa, order Aspidochirota) have been actively investigated in recent years.<sup>1,2,5</sup> Much of this research has concentrated on highly toxic compounds, called saponins (water soluble glycosides), which have been isolated from both the Cuvierian glands and body walls of several species of sea cucumbers.<sup>2,5,6</sup> Saponins isolated from both terrestrial and marine sources possess a broad range of biological activity.<sup>1,3</sup>

Acidic hydrolysis of the saponin mixtures yields aglycones, called sapogenins. The structure proof of many of the sapogenins has been carried out.<sup>2,5</sup> All of the sapogenins isolated from sea cucumbers have been found to be triterpenoids with a lanostane skeleton.

It is the purpose of this section is to report the isolation of two sapogenins, 22,25-oxidoholothurinogenin (I) and 17-desoxy-22,25-oxidoholothurinogenin (II), from the body walls of the sea cucumber *Holothuria atra* collected from the atoll at Enewetak, Marshall Islands in August 1969. The sapogenins I and II were first isolated from the sea cucumber *Actinopyga agassizi* and their structures were established by Chanley et al.<sup>7</sup> Subsequent to the start of our work on *Holothuria atra* a report of the isolation of I and II from the same sea cucumber collected at various places in the Western Pacific has appeared.<sup>6</sup>

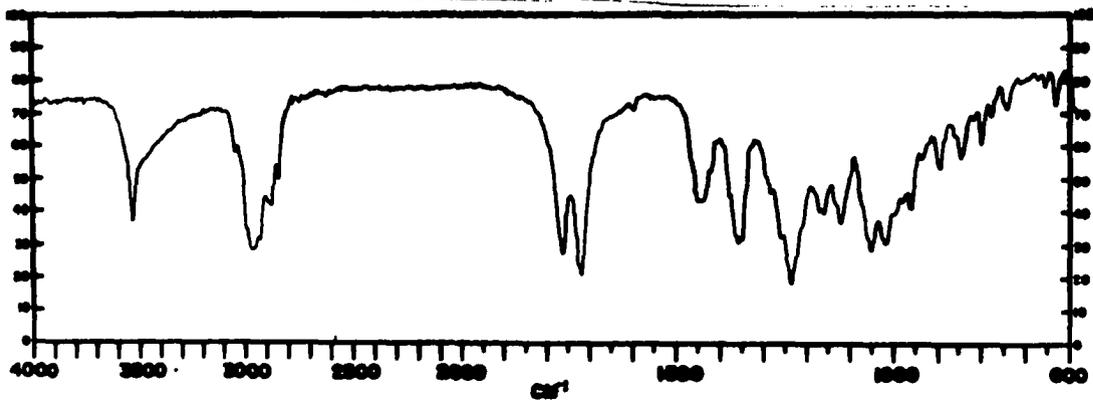
## RESULTS AND DISCUSSION

The body walls of the sea cucumber *Holothuria atra* (1.2 kg) were defatted (benzene) and then twice extracted with refluxing methanol/conc. hydrochloric acid (7/3) solution. This resulted in extraction of saponin and immediate hydrolysis to the sapogenin. The combined methanolic hydrochloric acid extracts were then diluted with water and extracted with chloroform in a separatory funnel to yield 12 g of chloroform soluble material. Chromatography of the chloroform soluble material on silica gel afforded 2 g of a white solid which thin layer chromatographic (tlc) analysis showed to be two components.

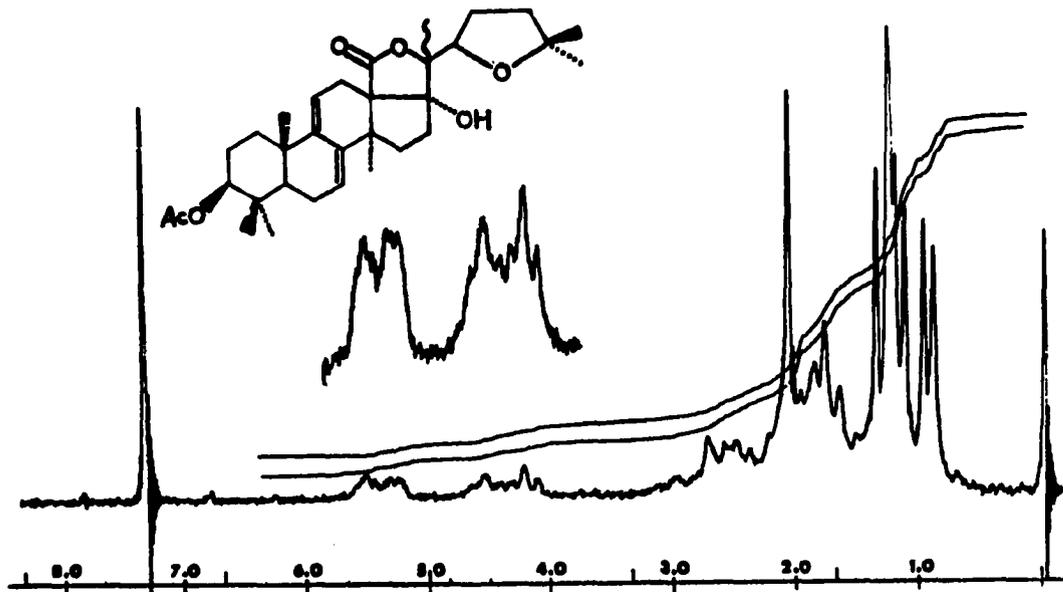
Acetylation of the white solid with acetic anhydride in pyridine resulted in the complete conversion of the original two components into two new products separable by tlc. Preparative thick layer chromatography of the acetylated material yielded two pure compounds which were shown to be monoacetate derivatives by analysis of their infrared (ir), proton magnetic resonance (pmr), and mass spectral data. The pure monoacetate derivatives III and IV (see Figures I and II) were identical in all respects (see experimental section) with the monoacetate derivatives of 22,25-oxidoholothurinogenin and 17-desoxy-22,25-oxidoholothurinogenin.<sup>7</sup>

Figure I

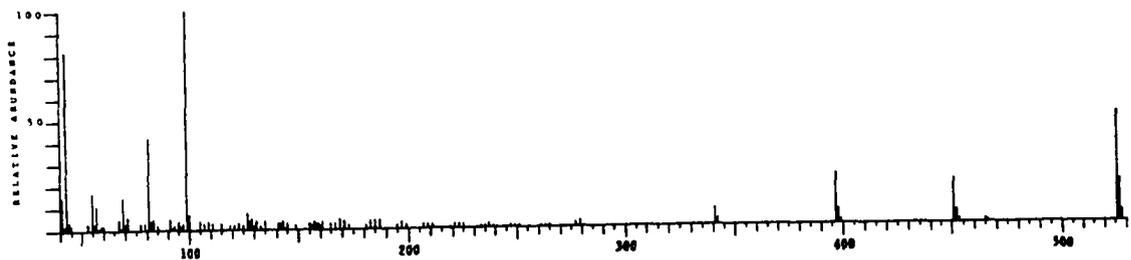
Spectra of 22,25-oxidoholothurinigenin Acetate (III)



IR Spectrum of III



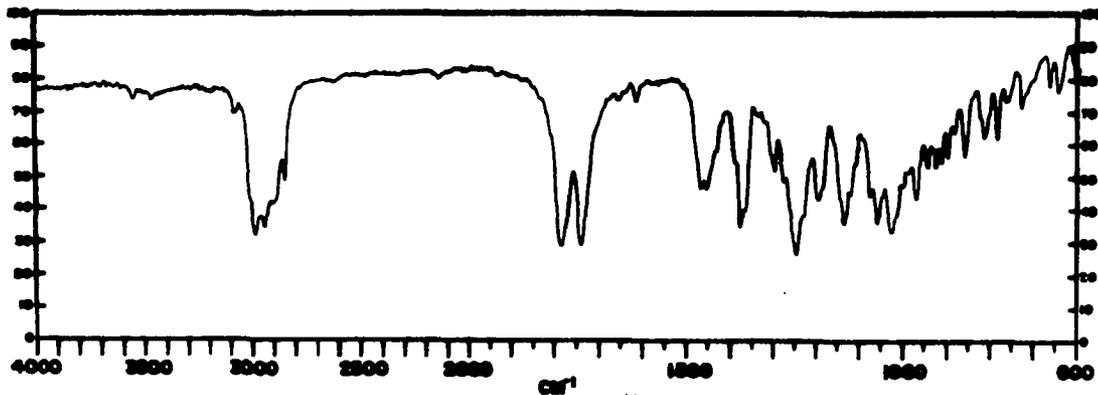
60 MHz Spectrum of III



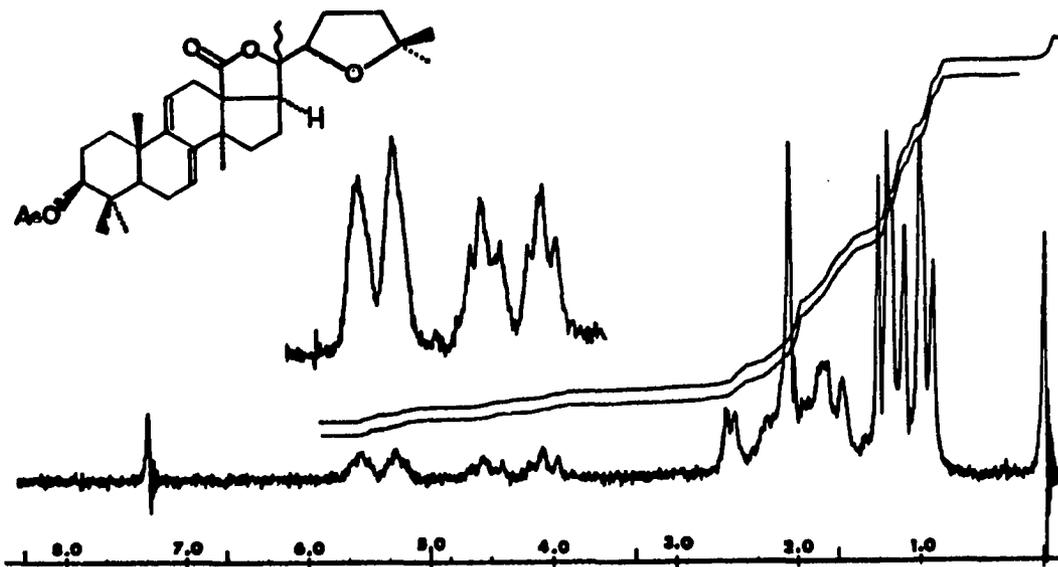
Mass Spectrum of III

Figure II

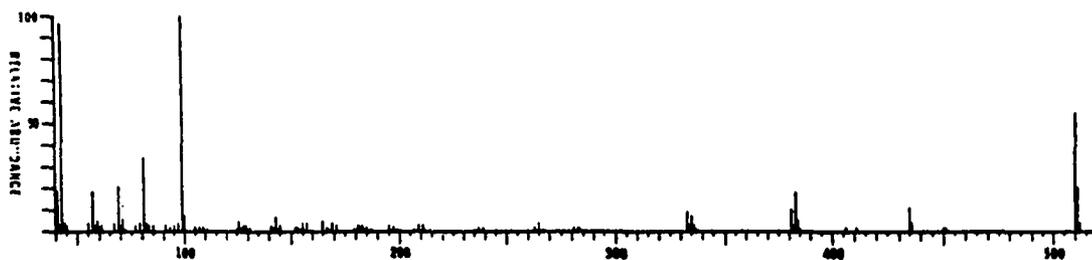
Spectra of 17-desoxy-22,25-oxidoholothurinigenin Acetate (IV)



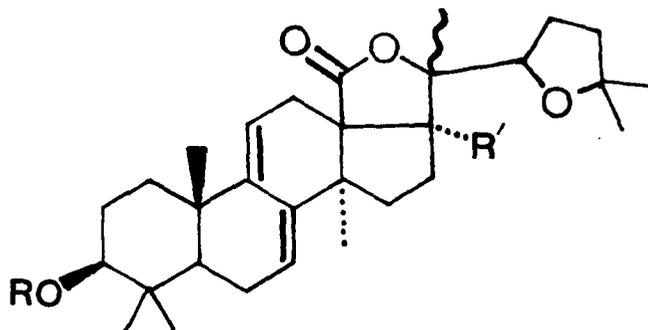
IR Spectrum of IV



60 MHz Spectrum of IV



Mass Spectrum of IV



I R = H, R' = OH

II R = H, R' = H

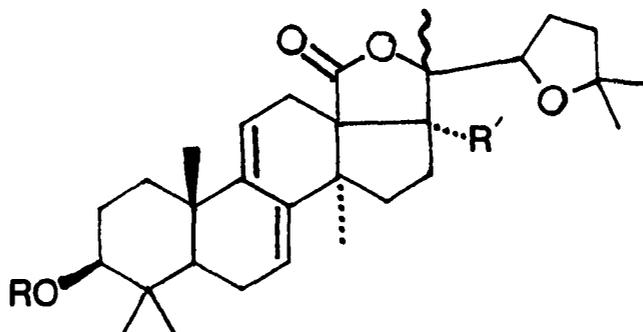
III R = Ac, R' = OH

IV R = Ac, R' = H

Hydrolysis of the acetates yielded the aglycones I and II. The latter were identified by comparison of their physical and spectral properties with those reported in the literature.<sup>7</sup>

## SUMMARY

Two known sapogenins, I and II, were isolated from the body walls of the sea cucumber *Holothuria atra*. The structures were determined by comparison of the physical and spectral properties of the monoacetate derivatives, as well as of the original glycones, with those found in the literature. The sapogenins were found in a relative ratio of 75/25 (I/II).



- I R = H, R' = OH
- II R = H, R' = H
- III R = Ac, R' = OH
- IV R = Ac, R' = H

## Experimental

Experimental conditions in section I apply.

Initial isolation of the aglycones I and II. The freshly collected (1.2 kg) sea cucumbers were immediately cut open and the inner organs were removed from the body walls. The body walls were oven dried (65°C) and cut into small pieces. The inner organs were air dried and kept, but no further work on this material was carried out. The body walls were thoroughly defatted by six room-temperature extractions with benzene. The defatted body walls were extracted twice with refluxing methanolic hydrochloric acid solution [methanol/conc. hydrochloric acid (7/3)]. The contents of the two methanolic extractions were combined, diluted with water (1 l) and extracted three times with chloroform (1 l each) in a separatory funnel. The chloroform was removed under reduced pressure to yield 12 g of a brown solid. The brown solid (12 g) was chromatographed on silica gel (75 g) employing ethyl acetate/benzene (15/85) and collecting 500 ml fractions. The second fraction yielded a yellow-orange solid upon removal of the solvent. Addition of diethyl ether to the yellow-orange solid resulted in the precipitation of a white solid (2 g) which was filtered from the solution. Thin layer chromatographic analysis of the white solid showed it to be a two component mixture [ $R_f$  0.27 and 0.24, ethyl acetate/benzene (15/85), silica gel H].

Acetylation of mixture of I and II. To a flask containing 6 ml of pyridine was added 217.5 mg of the white solid (I and II) dissolved in

6 ml of acetic anhydride. After 48 hours at room temperature the excess acetic anhydride was destroyed by the slow addition of water while the flask was being cooled in an ice bath. Extraction into chloroform and removal of solvent afforded 195.4 mg of a white solid product [ $R_f$  0.52 and 0.39, ethyl acetate/benzene (15/85), silica gel H].

Final separation of the acetylated derivatives was achieved using preparative thin layer chromatography on silica gel using ethyl acetate/benzene (15/85) as solvent. Recrystallization from ether yielded pure compounds.

Pure 22,25-oxidoholothurinogenin acetate (III), identical in all respects to that described in the lit.,<sup>7</sup> has mp 289.0-289.4°C;  $[\alpha]_D + 5.27^\circ$  ( $c$  7.8,  $\text{CHCl}_3$ ); uv (95% ethanol)  $\lambda_{\text{max}}$  243 m $\mu$  ( $\epsilon$  14,678),  $\lambda_{\text{sh}}$  236 m $\mu$  ( $\epsilon$  13,658),  $\lambda_{\text{sh}}$  252 ( $\epsilon$  10,091); ir (KBr) 3030, 315, 2980, 2940, 2880, 1770, 1725, 1460, 1370, 1255, 1135, 1060 1030, 990, 975, 920, 860 and 810  $\text{cm}^{-1}$ ; 60 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  5.51 (m, 1 H,  $-\underline{\text{CH}}=\text{C}-\text{C}=\text{CH}-$ ), 5.31 (m, 1 H,  $-\text{CH}=\text{C}-\text{C}=\underline{\text{CH}}-$ ), 4.53 (t, 1 H,  $J = 5$  Hz,  $-\underline{\text{CH}}-\text{OAc}$ ), 4.20 (t, 1 H,  $J = 6$  Hz, proton on carbon bearing ether oxygen), 2.07 (s, 3 H,  $-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\underline{\text{CH}}_3$ ), 1.37 (s, 3 H), 1.27 (s, 3 H), 1.25 (s, 3 H), 1.20 (s, 3 H), 1.13 (s, 3 H), 0.98 (s, 3 H), and 0.90 ppm (s, 3 H); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$  526 (50), 450 (20), 397 (23), 99 (100) base peak, 81 (42), 69 (15), 55 (17), and 43 (82).

Pure 17-desoxy-22,25-oxidoholothurinogenin acetate (IV) has mp 265.0-265.7°C;  $[\alpha]_D + 21.5^\circ$  ( $c$  0.58,  $\text{CHCl}_3$ ); (95% ethanol)  $\lambda_{\text{max}}$  243 ( $\epsilon$  15,077), 251 ( $\epsilon$  10,685),  $\lambda_{\text{sh}}$  236 ( $\epsilon$  13,827); ir (KBr) 3080, 3010, 2980, 2940, 2900, 2850, 1775, 1732, 1465, 1380, 1250, 1195, 1135, 1060, 1030, 970, 945, 925, 915, 900, 860, 815 and 780  $\text{cm}^{-1}$ ; 60 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  5.57 (m, 1 H,  $-\underline{\text{CH}}=\text{C}-\text{C}=\text{CH}-$ ), 5.29 (m, 1 H,  $-\text{CH}=\text{C}-\text{C}=\underline{\text{CH}}-$ ), 4.57 (t, 1 H,  $J = 5$  Hz,

-CHOAc), 4.10 (t, 1 H,  $J = 6$  Hz, proton on carbon bearing the ether oxygen), 2.08 (s, 3 H,  $-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$ ), 1.35 (s, 3 H), 1.27 (s, 3 H), 1.23 (s, 3 H), 1.13 (s, 3 H), 1.02 (s, 3 H), 0.98 (s, 3 H) and 0.90 ppm (s, 3 H); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$  510 (54), 450 (2), 435 (11), 383 (18), 381 (10), 335 (7), 333 (9), 99 (100) base peak, 81 (35), 69 (21), 57 (19), 43 (97) and 41 (19).

Deacetylation of 22,25-oxidoholothurinogenin acetate. To a flask containing 90 mg of 22,25-oxidoholothurinogenin acetate was added 25 ml of 5% methanolic KOH solution and the solution was refluxed for 7 hours. The base was neutralized with dilute HCl, and the precipitated product was recovered by extraction into chloroform. The chloroform was dried ( $\text{Na}_2\text{SO}_4$ ) and then removed under reduced pressure to yield 56.5 mg of I. Recrystallization from ethyl acetate afforded pure I.

Pure I has mp 314.9-315.4;  $[\alpha]_D -20.8$  ( $c$  2.82,  $\text{CHCl}_3$ ); uv (95% ethanol)  $\lambda_{\text{max}}$  243 ( $\epsilon$  15,500),  $\lambda_{\text{sh}}$  252 ( $\epsilon$  11,200),  $\lambda_{\text{sh}}$  237 ( $\epsilon$  14,800); ir (KBr) 3540, 3510, 3040, 2970, 2940, 2875, 2855, 1750, 1605, 1450, 1375, 1365, 1305, 1283, 1255, 1225, 1190, 1140, 1105, 1060, 1040, 1105, 985, 960, 860, 845, 805, 785 and  $620 \text{ cm}^{-1}$ ; 60 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  5.52 (m, 1 H,  $-\underline{\text{CH}}=\text{C}-\text{C}=\text{CH}-$ ), 5.24 (m, 1 H,  $-\text{CH}=\text{C}-\text{C}=\underline{\text{CH}}-$ ), 41.8 (t, 1 H,  $J = 6$  Hz, proton on the carbon bearing the ether oxygen), 3.24 (t, 1 H,  $J = 6$  Hz,  $-\underline{\text{CH}}-\text{OH}$ ), 1.33 (s, 3 H), 1.25 (s, 3 H), 1.23 (s, 3 H), 1.18 (s, 3 H), 1.08 (s, 3 H), 1.00 (s, 3 H), and 0.88 ppm (s, 3 H); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$  484 (57), 451 (21), 397 (21), 299 (8), 99 (100) base peak, 81 (36), 69 (15), 57 (13), 55 (20), 43 (59), and 41 (22).

Deacetylation of 17-desoxy-22,25-oxidoholothurinogenin acetate.

To a flask containing 14.8 mg of 17-desoxy-22,25-oxidoholothurinogenin acetate was added 50 ml of methanolic KOH solution, the solution was then refluxed for 6 hours. The base was neutralized with dilute HCl and the precipitated product was recovered by extraction into chloroform. The chloroform was dried ( $\text{Na}_2\text{SO}_4$ ) and the solvent was removed under reduced pressure to yield 10 mg of II. Despite repeated recrystallizations, a melting point for II corresponding to that recorded in the literature<sup>7</sup> was not obtained. The ir, pmr and mass spectra of II obtained from the above hydrolysis agree with that reported for 17-desoxy-22,25-oxidoholothurinogenin.

II has ir (KBr), 3340, 3040, 2970, 2930, 2860, 1765, 1450, 1380, 1365, 1275, 1195, 1135, 1050, 1025, 990, 940, 920, 875, 850, 800, 745, and 655  $\text{cm}^{-1}$ , 60 MHz pmr ( $\text{CDCl}_3$ ), 5.58 (m, 1 H,  $-\underline{\text{CH}}=\text{C}-\text{C}=\text{CH}-$ ), 5.28 (m, 1 H,  $-\text{CH}=\text{C}-\text{C}=\underline{\text{CH}}-$ ), 4.08 (t, 1 H,  $J = 6$  Hz, proton on the carbon bearing the ether oxygen), 3.27 (t, 1 H,  $J = 6$  Hz,  $-\underline{\text{CHOH}}$ ), 1.33 (s, 3 H), 1.25 (s, 3 H), 1.22 (s, 3 H), 1.10 (s, 3 H), 1.02 (s, 3 H), 1.00 (s, 3 H), and 0.90 (s, 3 H); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $\text{M}^+$  468 (43), 408 (2), 381 (13), 341 (26), 283 (14), 99 (100) base peak, 81 (31), 69 (14), 55 (12), 43 (36), and 41 (11).

## BIBLIOGRAPHY

1. P. J. Scheuer, *Fortschr. Chem. Org. Naturstoffe*, **27**, 332 (1969).
2. J. S. Grossert, *Chem. Soc. Rev.*, **1**, 1 (1973).
3. N. Basu, R. P. Rustogi, *Phytochemistry*, **6**, 1249 (1970).
4. T. Matsumo, A. Sakushima, T. Ishida, *Nippon Suisan Gakkaishi*, **39**, 307 (1973).
5. I. Rothberg, B. M. Tursch, D. Djerassi, *J. Org. Chem.*, **38**, 209 (1973).
6. G. B. Elyakov, V. A. Stonik, E. V. Levina, V. P. Slanke, T. A. Kuznetsova, V. S. Levin, *Comp. Biochem. Physiol.*, **44B**, 325 (1973).
7. J. D. Chanley, T. Mazzetti, H. Sobotka, *Tetrahedron*, **22**, 1857 (1966).

## A NOVEL POLYACETYLATED COMPOUND FROM A SOFT CORAL

### INTRODUCTION

The previous efforts of Steudler<sup>1</sup> resulted in the isolation of a novel polyacetylated compound from the hexane extracts of the soft coral *Xenia elongata* collected at Heron Island, Australia by Dr. L. S. Ciereszko, University of Oklahoma. The cooled hexane extract of *Xenia* yielded a brown precipitate from which was obtained a small quantity of a white crystalline compound by chromatography on silica gel. The white solid was shown to have a molecular weight of 518 (low resolution mass spectrum) and preliminary infrared and proton magnetic resonance spectra indicated a compound of unique structural features. This new compound was referred to as X-518.

It is the purpose of this section to report the reisolatoin of a greater quantity of X-518 and results of further investigation into the nature of its structure.

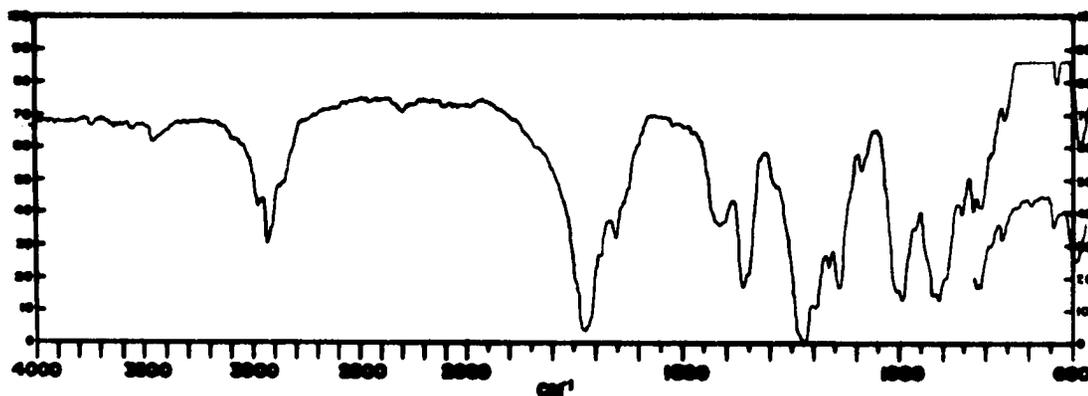
## RESULTS AND DISCUSSION

From the lyophilized aq. alcoholic extracts of the soft coral *Xenia elongata* was isolated a new compound, called X-518, by silica gel chromatography. X-518,  $C_{28}H_{38}O_9$  (combustion analysis and high resolution mass spectrum), is a white crystalline solid, mp 141.5-142.3°C. Its infrared spectrum lacked hydroxyl absorption, but displayed a strong broad absorption in the carbonyl region at  $1735\text{ cm}^{-1}$  (acetate) with a shoulder at  $1700\text{ cm}^{-1}$  (ketone or conjugated ester). The presence of olefinic functionality was indicated in the infrared spectrum by absorptions at 3100, 1665 and  $1635\text{ cm}^{-1}$ .

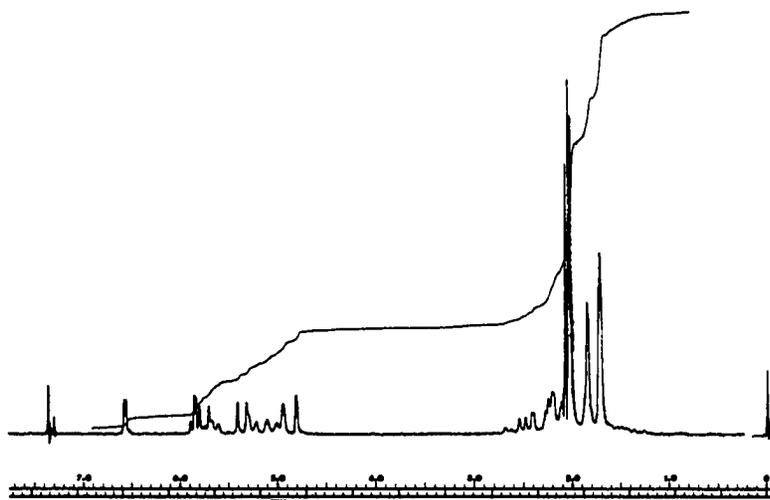
The presence of three acetate groups was indicated by the pmr spectrum ( $CDCl_3$  and benzene- $d_6$ ), see Figures I and II, and by the high resolution mass spectrum. In the pmr spectrum taken in  $CDCl_3$  three methyl group signals appear at  $\delta$  2.07, 2.04 and 2.02. The three acetate signals are more clearly resolved in the spectrum taken in benzene- $d_6$  in which these absorptions are displayed at  $\delta$  1.74, 1.73 and 1.60. The high resolution mass spectrum showed the sequential loss of three m/e 60 units (loss of acetic acid); 458.22831,  $C_{26}H_{34}O_7$ ; 398.21006,  $C_{24}H_{30}O_5$ ; 338.18856,  $C_{22}H_{26}O_3$ .

The presence of four double bonds was indicated by the proton decoupled  $^{13}C$  magnetic resonance spectrum (see Figure II) which displays eight signals in the olefinic region at  $\delta$  146.1, 142.1, 140.2, 133.9,

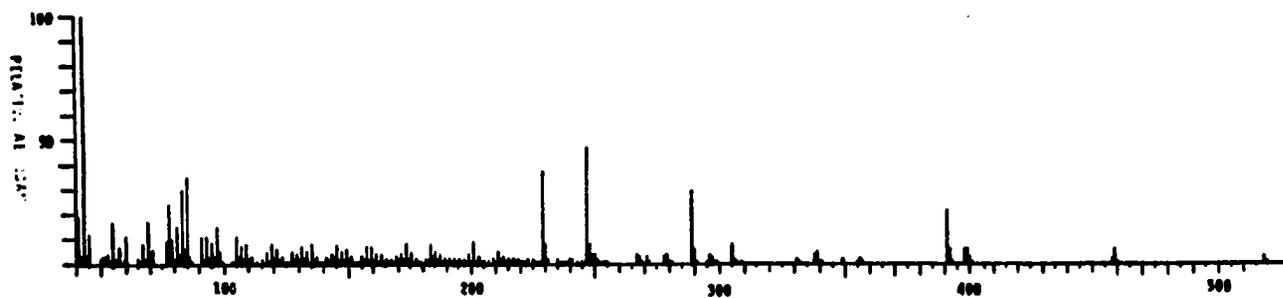
Figure I  
Spectra of X-518



IR Spectrum of X-518

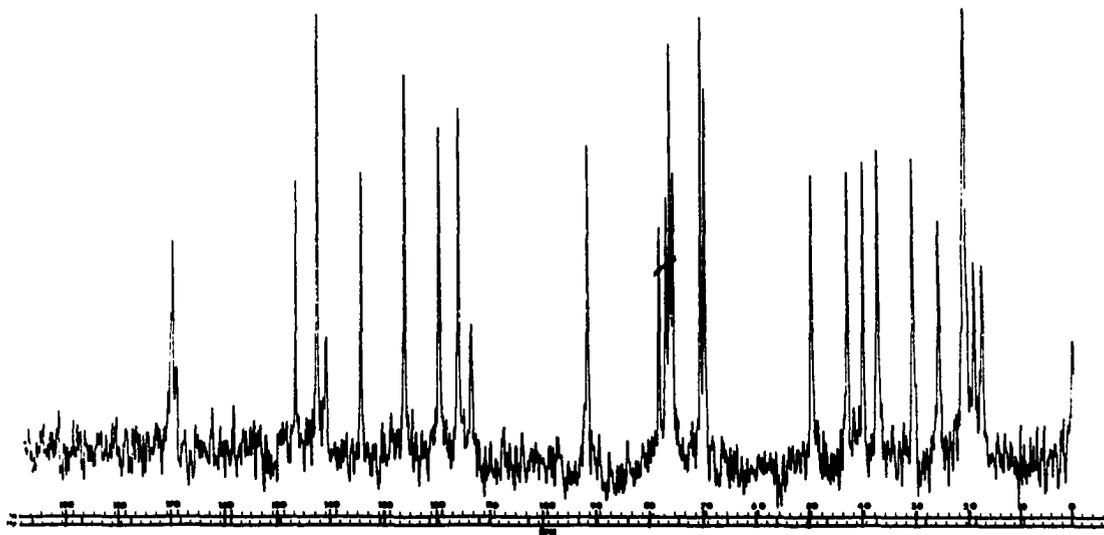


100 MHz (CDCl<sub>3</sub>) Spectrum X-518

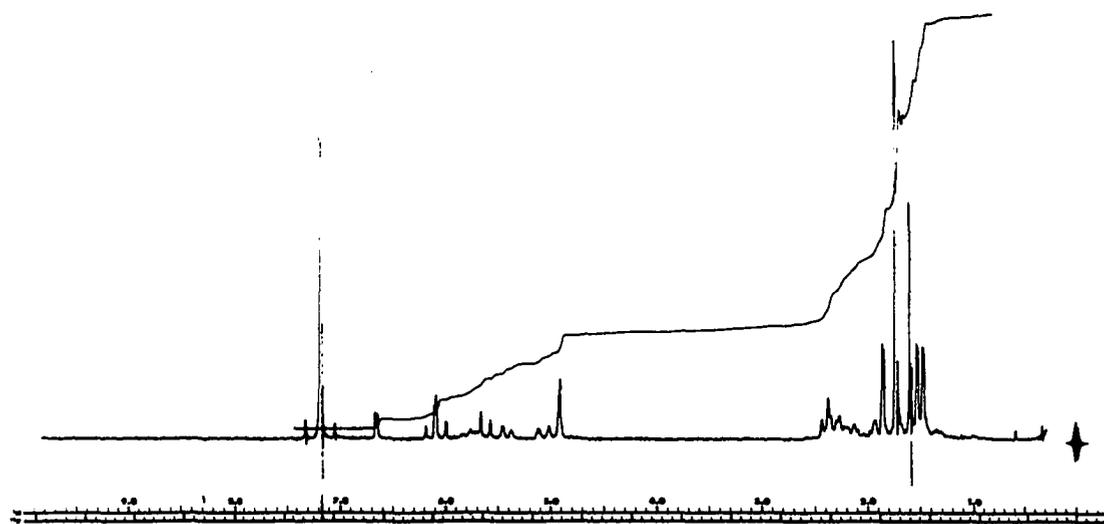


Mass Spectrum of X-518

Figure II



25.2 MHz CMR Spectrum of X-518



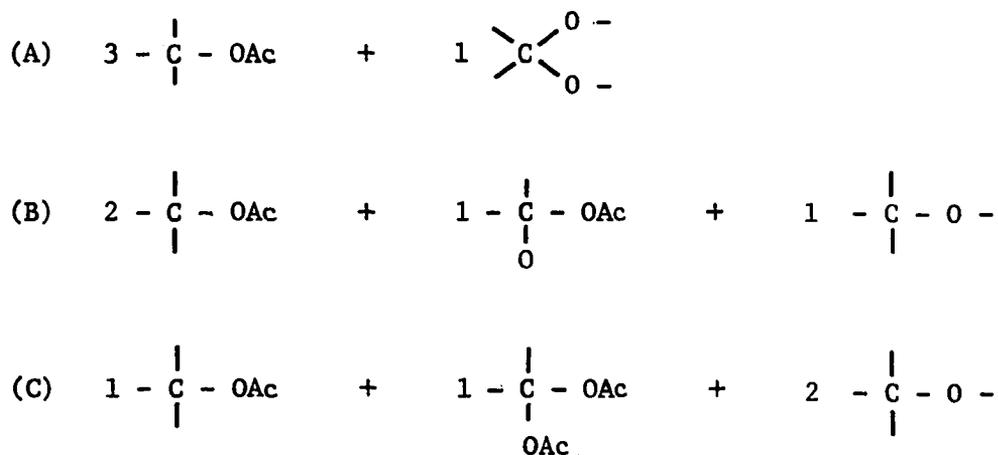
100 MHz (Benzene-d<sub>6</sub>) Spectrum of X-518

125.8, 119.2, 115.5 and 113.1 ppm. It was further concluded that three of the four double bonds bear methyl groups since in the pmr spectrum (benzene- $d_6$ ) there are three methyl group signals at  $\delta$  1.85 (d, 3,  $J = 1$  Hz), 1.53 (d, 3,  $J = 1$  Hz) and 1.47 (d, 3,  $J = 0.5$  Hz).

Four double bonds and three acetates account for seven of the ten degrees of unsaturation indicated by the molecular formula,  $C_{28}H_{38}O_9$ , of X-518. The three acetates account for six of the nine oxygen atoms. The nature of the remaining three oxygens is still unclear. Since the ir is devoid of hydroxyl absorption, the remaining oxygen atoms must be present as some combination of the following groups: ester, ketone, or ether. The cmr spectrum was void in the region where ketone carbonyl carbons absorb;<sup>2,4</sup> thus a ketone functionality was tentatively ruled out. Since some ketone carbonyl carbons absorb below 200 ppm, however, a ketone functionality cannot be rigorously excluded. Field sweep for the  $^{13}C$  nmr analysis terminated at 200 ppm. The cmr spectrum displays four absorptions consistent for  $sp^3$  carbons bearing oxygen atoms ( $\delta$  91.3, 76.0, 70.2 and 69.5).<sup>5</sup> The signal at  $\delta$  91.3 is sufficiently far downfield to be attributed to a carbon bearing two oxygen atoms.<sup>2</sup> The four absorptions in this region thus indicated 5 carbon to oxygen single bonds. Figure III lists three possible combinations of carbons singly bonded to oxygen that would be in accord with the cmr data.

Five oxygens singly bonded to carbon and three acetate carbonyl oxygens establishes eight of the nine oxygen atoms.

Figure III



The possibility of a fourth ester group in X-518 is likely. Owing to the very similar chemical shifts of the absorptions in the carbonyl carbon region of the cmr spectrum (see Figure II), the number of carbons giving rise to the signals in this region is unclear. The presence of three acetates requires at least three carbonyl carbon absorptions. The possibility of the presence of an  $\alpha,\beta$ -unsaturated ester group is indicated by the  $1700 \text{ cm}^{-1}$  shoulder of the carbonyl absorption and  $1665 \text{ cm}^{-1}$  absorption (consistent for a double bond in conjugation with a ketone or ester) in the ir spectrum, and by the strong end absorption in the ultraviolet spectrum (see experimental section). The high and low resolution mass spectra show a major fragment ion at  $m/e$  391 ( $M^+ - 127$ ),  $\text{C}_{21}\text{H}_{27}\text{O}_7$ , (at 10 eV  $m/e$  391 is the base peak) which corresponds to a loss of  $\text{C}_7\text{H}_{11}\text{O}_2$ . This

fragment ion contains two degrees of unsaturation and is consistent with the possibility of a fourth ester group. If indeed one of the three remaining oxygen atoms is a carbonyl oxygen, then eight degrees of unsaturation are accounted for. The last two degrees of unsaturation are presumed to be due to rings, thus a bicyclic system is indicated for X-518.

X-518 does not possess an integral multiple of  $C_5$  units (i.e. isoprene units); hence one can only speculate regarding its classification. Even if the carbons due to the acetates are not considered, the formula still contains 22 carbons. Thus X-518 could be a truncated sesterterpene ( $C_{25}$ ) or substituted diterpene ( $C_{20}$ ) or sesquiterpene ( $C_{15}$ ). Which of these is the case is not known at this time. The high resolution mass spectrum displayed a  $C_{15}H_{15}O$  fragment ion corresponding to the loss of the three molecules of acetic acid (see earlier discussion) and the 127 fragment ion ( $C_7H_{11}O_2$ ) [see earlier discussion]. Adding the elements of water to  $C_{15}H_{15}O$  for each loss of acetic acid, plus one -OH for the loss of the 127 fragment ion results in a formula of  $C_{15}H_{22}O_5$ . This formula indicates five degrees of unsaturation which is consistent with the loss of five degrees of unsaturation ( $3 \text{ CH}_3\overset{\text{O}}{\parallel}\text{C}-\text{OH}$  and  $C_7H_{11}O_2$ ) from X-518. This could indicate X-518 is a bicyclic sesquiterpene. This is the only evidence for such a conclusion.

Decoupling experiments were carried out at 100 MHz on the pmr spectrum of X-518 in both  $\text{CDCl}_3$  and benzene- $d_6$ . These results are listed in Table I and II. Since several of the absorption signals were at similar chemical shift positions, the possibility of the decoupling radiofrequency affecting more than one signal at a time (especially in the downfield region) makes any interpretation of the apparent decoupling results tentative. However, the decoupling experiments were informative

Table I

Double irradiation <sup>a</sup> of X-518

Signal Irrad'd	CDCl <sub>3</sub> Signal Obs'd	Change Observed
6.56	2.17	Affected
5.85	5.36	Collapsing to Singlet
	5.05	Collapsing to Singlet
5.80	5.36	Collapsed to Singlet
	5.25	Collapsing to Singlet
	5.05	Collapsed to Broad Singlet
5.36	5.80	Collapsed to Singlet
	5.05	Collapsed to Singlet
2.17	6.56	d → s
1.84	5.85	d → s
1.73	5.68	br t to a doublet t (J = 2 Hz, J = 7 Hz)
	5.25	br. d to sharp d
	5.05	q. d to sharpened d.

<sup>a</sup>Run on a Varian XL-100 spectrometer at 100 MHz in chloroform-d, solution using tetramethylsilane as internal standard. The spectrometer was locked on the deuterium signal. Abbreviations specified in the experimental section apply.

Table II

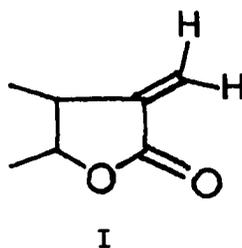
Double irradiation <sup>a</sup> of X-518

Signal Irrad'd	benzene-d <sub>6</sub> Signal Obs'd	Change Observed
6.65	2.26	Affected
6.09	5.62	d to br. s.
	5.07	q. d to br s.
	1.94	Affected
5.62	6.09	Affected
	1.47	d → s
5.42	1.47	d → s
5.07	6.09	t → d
	1.85	d → s
	1.55	d → s
2.26	6.65	d → s
1.85	6.09	d → s
1.55	5.76	br t → d t (J = 2 Hz, J = 7 Hz)
	5.42	Slightly Affected
	5.07	Slightly Affected
1.47	5.42	br d → sharp d
	5.76	Slightly Affected

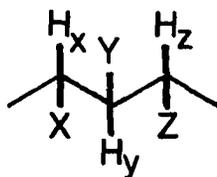
<sup>a</sup>Run on a Varian XL-100 spectrometer at 100 MHz in benzene-d<sub>6</sub> solution using tetramethylsilane as internal standard. The spectrometer was locked on the deuteron signal. Abbreviations specified in the experimental section apply.

and indeed confirmed some relationships. All of the following discussion will relate to the spectrum taken in benzene- $d_6$ .

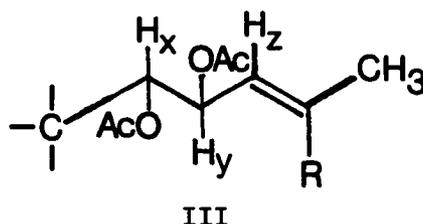
The signal at  $\delta$  6.65 is not coupled to the signal at  $\delta$  6.09, but is coupled to an allylic proton at  $\delta$  2.26. This rules out the partial structure I since, although the chemical shift positions of the protons absorbing at  $\delta$  6.65 and  $\delta$  6.09 are consistent for the protons of such an exomethylene group, these protons would be mutually coupled.



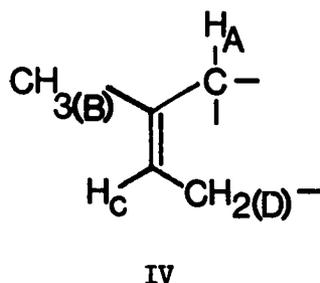
The proton resonating at  $\delta$  6.09 (that proton giving rise the triplet) (see Figure I,  $CDCl_3$  spectrum, and experimental section) is coupled to the proton absorbing at  $\delta$  5.62 and to the proton absorbing at  $\delta$  5.07. However, the signal at  $\delta$  5.07 is not coupled to the proton absorbing at  $\delta$  5.62. This suggests the proton absorbing at  $\delta$  6.09 (triplet) is vicinally coupled to two protons ( $J \approx 9.5$  Hz) on two different carbons as is shown in partial structure II where  $H_y$  is assigned to the  $\delta$  6.09 signal ( $J_{xy} = J_{yz} \approx 9.5$  Hz).



From the chemical shift and multiplicities of the signals at  $\delta$  6.09, 5.62 and 5.07 partial structure II was expanded to III where  $H_x$  is assigned to the  $\delta$  5.56 signal and  $H_z$  is assigned to the  $\delta$  5.07 signal.



The signal at  $\delta$  5.76 sharpens to a distinct double triplet when one of the vinyl methyl signals ( $\delta$  1.55) is irradiated, indicating the possibility of partial structure IV in X-518 where  $J_{CD} \approx 7$  Hz,  $J_{AC} = 2$  Hz and  $J_{CB} = 1$  Hz.



The nature of the two protons resonating at  $\delta$  6.65 and  $\delta$  6.09 (doublet) cannot be conclusively ascertained at this time. The chemical shift position of the  $\delta$  6.65 signal would be consistent with one of the following: (a) a proton on the  $\alpha$ -carbon of an  $\alpha,\beta$ -unsaturated ketone or ester group, (b) a proton on the  $\alpha$ -carbon of an  $\alpha$ -acetoxy ketone or ester group, or (c) a proton on the  $\alpha$ -carbon of an enol ether.<sup>3</sup> If X-518 has four ester groups, then the ninth oxygen is most likely an ether (a ketone functionality is tentatively ruled out from the cmr data). The pmr spectra of X-518 ( $CDCl_3$  and benzene- $d_6$ ) are void in the region one would

expect a proton to absorb if it were attached to a carbon under the deshielding effect of an ether oxygen only. Any ether functionality thus either has no protons on the carbons to which the oxygen is attached, or additional factors are present to effect a further deshielding for these protons (i.e., the ether carbons are also allylic, vinylic or attached to a carbon bearing a second oxygen atom).

Extensive chemical work is required to answer the many unsolved structural aspects of X-518. An attempted deacetylation using a small amount of sodium methoxide in methanol failed for some unknown reason. Attempted hydrogenation yielded a mixture which indicated (pmr and mass spectra) only partial hydrogenation. Due to a limited supply of X-518 further chemistry was delayed and crystals hopefully suitable for single crystal X-ray analysis were obtained from a hexane/benzene mixture.

X-518 was not found in the extracts of *Xenia elongata* collected at Picnic Bay, Magnetic Island, Australia or at the Fiji Islands.

### Summary

A novel polyacetylated compound, called X-518, first isolated by Steudler was reisolated by a different procedure. The molecular formula of X-518 was established as  $C_{28}H_{38}O_9$ . The presence of three acetates and four double bonds was deduced from the spectral data of X-518. Despite the accumulation of a great deal of spectral information, including extensive spin decoupling data, no ring system could be formulated that was in accord with all the data collected. Due to a limited supply of X-518 a sample has been provided Dr. D. van der Helm for low temperature X-ray crystallography.

X-518 was obtained only from the *Xenia elongata* collected at Heron Island, Australia. *Xenia elongata* collected at Picnic Bay, Magnetic Island, Australia or in the Fiji Islands did not contain any X-518. Thus the soft coral *Xenia elongata* is a species in which the chemical constituents are related in some fashion to specific geographical location.

## EXPERIMENTAL

Experimental Conditions Specified in Section I Apply.

Isolation of X-518. *Xenia elongata* ( ),  $\approx$  2 lbs, was collected by Dr. R. Schroeder in the environs of Heron Island, Australia. The freshly collected specimens were preserved in 2-propanol for shipment to Oklahoma. Water was then added to the preservative solution to obtain a water/2-propanol (1/1) composition. Removal of the 2-propanol under reduced pressure followed by the removal of the water by lyophilization, yielded 58.6 g of a light porous-looking brown solid. This material was placed in a soxhlet extractor and extracted with distilled hexane. The hexane was removed under reduced pressure to yield a brown residue. The residue was dissolved in chloroform and chromatographed on Florisil, 100 g, using chloroform as the solvent. The chloroform was then removed to yield 1.208 g of a brown solid. The 1.208 g of material was rechromatographed on 40 g of thin-layer mesh silica gel employing ethyl acetate/benzene (1/9) as solvent and collecting 20 ml fractions. Combination of fractions L-0 yielded 357.1 mg of a white solid, homogeneous by tlc. Recrystallization from a benzene/hexane mixture afforded 295.5 mg of pure X-518.

Pure X-518 has mp 141.5 - 142.3°C;  $[\alpha]_D^{23.5} - 36.7^\circ$  ( $c$  0.61,  $\text{CHCl}_3$ ) and  $[\alpha]_D^{23.5} - 36.0^\circ$  ( $c$  1.81,  $\text{CHCl}_3$ ); uv (95% ethanol) strong end absorption starting at 235 nm with no maxima above 215 nm; ir (KBr) 2980,

2940, 2860, 1735, 1700, 1665, 1635, 1440, 1375, 1235, 1205, 1180, 1155, 1105, 1020, 1005, 930, 870, 845, 825  $\text{cm}^{-1}$ ; 100 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  6.56 (d, 1,  $J = 2$  Hz), 5.85 (d, 1,  $J = 2$  Hz), 5.80 (t, 1,  $J = 9.5$  Hz), 5.68 (br t, 1,  $J = 6.5$  Hz), 5.36 (d, 1,  $J = 9.0$  Hz), 5.25 (br d, 1,  $J = 7$  Hz), 5.05 (quadrupled d, 1,  $J = 9.5$  Hz,  $J = 1$  Hz), 4.94 (br s, 1), 4.80 ppm (br s, 1), 2.7 - 1.2 (m, 11), 2.07 (s, 3), 2.04 (s, 3), 2.02 (s, 3), 1.84 (br s, 3,  $J = 1$  Hz), 1.73 ppm (br s, 6,  $J = 1$  Hz); 100 MHz pmr (benzene- $d_6$ )  $\delta$ , 6.65 (d, 1,  $J = 2$ , Hz), 6.09 (d, 1,  $J = 2$  Hz), 6.09 (t, 1,  $J = 9.5$  Hz), 5.75 (br t, 1,  $J = 7$  Hz), 5.62 (d, 1,  $J = 9.0$  Hz), 5.42 (br d, 1,  $J = 6$  Hz), 5.07 (quadrupled d, 1,  $J = 1$  Hz,  $J = 9.5$  Hz), 4.91 (br s, 2), 2.5 - 1.2 (m, 11), 1.85 (d, 3,  $J = 1$  Hz), 1.74 (s, 3), 1.73 (s, 3), 1.60 (s, 3), 1.55 (d, 3,  $J = 1$  Hz), 1.47 ppm (d, 3,  $J = 0.5$  Hz); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$ , 518 (3), 459 (6), 458 (2), 400 (3), 399 (5), 398 (5), 392 (52), 391 (21), 339 (5), 338 (4), 305 (8), 297 (3), 296 (4), 290 (5), 289 (29), 248 (8), 229 (37), 211 (5), 201 (9), 185 (5), 183 (7), 173 (8), 159 (7), 145 (7), 135 (8), 131 (7), 119 (8), 105 (11), 97 (15), 93 (11), 91 (11), 85 (35), 83 (20), 78 (23), 69 (17), 60 (11), 55 (17), 45 (12), base peak 43 (100) and 41 (19).

Anal. Calcd for  $\text{C}_{28}\text{H}_{38}\text{O}_9$ : C, 64.86; H, 7.38; mol. wt. 518.25158

Found: C, 64.90, 64.91; H, 7.37, 7.32; mol. wt (mass spectrum) 518.24888.

Attempted deacetylations of X-518. A small sample (131.9 mg) of X-518 was dissolved in 125 ml of methanol in a 250 ml round-bottomed flask. To this solution was added  $\approx 1$  mg of sodium metal. After the sodium disappeared the solution was allowed to stand for 48 hrs. The methanol was removed under reduced pressure on the rotary evaporator and the resultant

white solid was partitioned between 100 ml of water and two 150 ml portions of chloroform. The chloroform was dried ( $\text{Na}_2\text{SO}_4$ ) and removed under reduced pressure. Tlc analysis of the product showed mostly unreacted X-518. The reaction was repeated using same procedure as above and allowed to react for three days at room temperature. Work-up yielded no identifiable products.

Attempted hydrogenation of X-518. To a solution of 10 ml of ethyl acetate containing a few milligrams of prereduced  $\text{PtO}_2$  in a hydrogen atmosphere was added 45.9 mg of X-518 dissolved in 15 ml of ethyl acetate. After 15 hours the catalyst was filtered and the solvent was removed under reduced pressure to yield 56.6 mg of a glassy solid. Tlc analysis showed complete absence of X-518 and the presence of a mixture of four new compounds. Chromatography of the mixture from the hydrogenation on silica gel yielded 24.0 mg of a poorly crystalline, glassy solid, homogeneous by tlc; 100 MHz Fourier Transform pmr (512 scans) (benzene- $d_6$ )  $\delta$  6.57 (br s, 1), 6.06 (q or dd), 5.8 - 4.6 (m), 2.62 (br m), 1.67 (s, 3), 1.65 (s, 3), 1.52 (br s, 3), 1.42 (br s, 3), 1.25 (m), 0.87 (d, 3 J = 6.5 Hz), 0.75 ppm (d, 3, J = 6.5 Hz); mass spectrum (70 eV) m/e (rel. intensity) 402 (5), 393 (20), 351 (11), 291 (18), 249 (27), 231 (31), 43 (100) base peak.

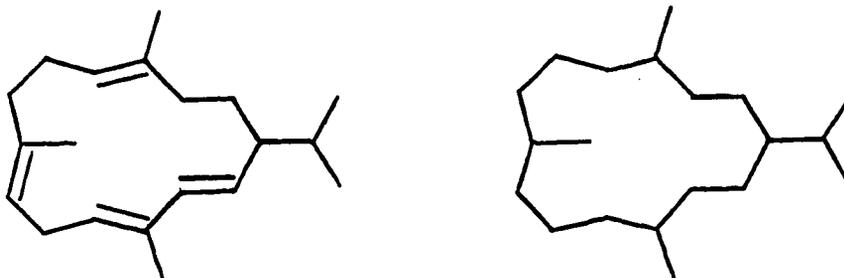
### Bibliography

1. P. A. Steudler, unpublished results.
2. J. B. Stothers, "Carbon-13 NMR spectroscopy," Academic Press, New York, N. Y., 1972.
3. L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N.Y., 1959.
4. R. A. Gross, Ph.D. Dissertation, The University of Oklahoma, 1974, Section III.
5. The signals at  $\delta$  77.9, 76.6 and 75.4 are due to the solvent,  $\text{CDCl}_3$ .

CEMBRENE-A AND TWO NEW CEMBRENE DERIVATIVES, NEPHTHENOL  
AND EPOXYNEPHTHENOL ACETATE, FROM A PACIFIC  
SOFT CORAL *NEPHTHEA SP.*

INTRODUCTION

The isolation of cembrene barely over a decade ago<sup>1-5</sup> established a new secondary class of diterpene (C<sub>20</sub>) compounds. Since that time a



growing number of compounds possessing the cyclotetradecane ring system have been found in nature from a variety of sources.<sup>6-10</sup> Speculations concerning the biosynthetic formation of these compounds from acyclic precursors have been lucidly described.<sup>6</sup> The synthesis of cembrene itself has just recently been accomplished.<sup>7</sup>

Work in this department<sup>6</sup> has shown that gorgonians (subclass Octocorallia, order Gorgonacea) contain a number of diterpene lactones

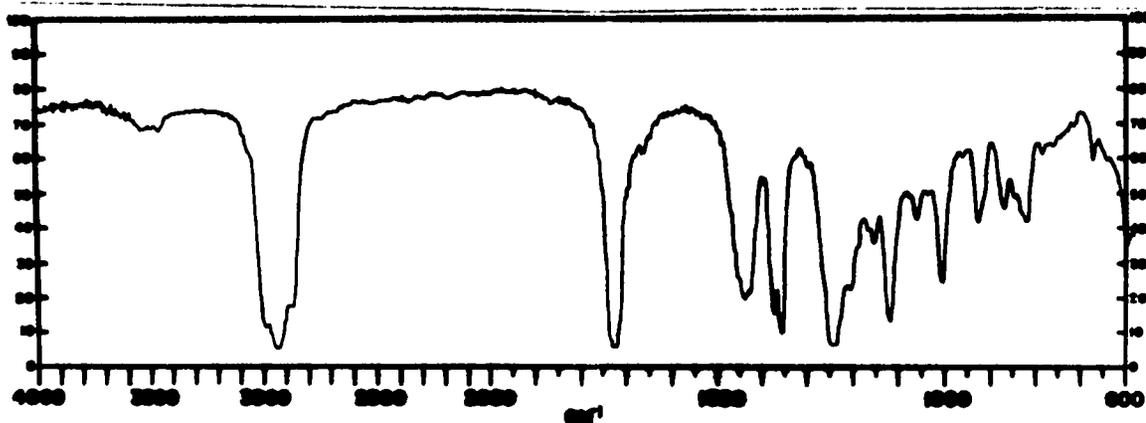
having the carbon skeleton of cembrene. In contrast, little information was available at the time this work was initiated regarding the chemical constituents of soft corals (subclass Octocorallia, order Alcyonacea) commonly found in the Indo-Pacific. In order to acquire some information regarding the chemistry of this order of octocorals, a study of the extracts of a soft coral, *Nephthea sp.*, was undertaken. From a collection of a soft coral (*Nephthea Sp.*) from the Enewetak atoll, Marshall Islands, two new cembrene derivatives, nephthenol and epoxynephthenol acetate, were isolated and their structures determined. Isolation of cembrene-A, a compound previously isolated from other sources,<sup>11-15</sup> is also described.

## RESULTS AND DISCUSSION

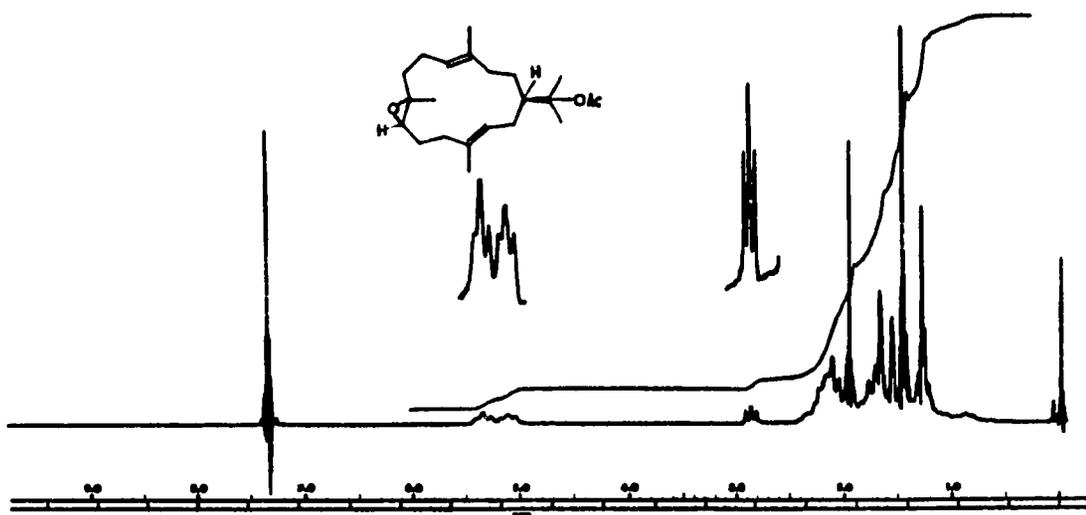
Two new oxygenated cembrene compounds and a known cembrene hydrocarbon, called cembrene-A,<sup>11-15</sup> were isolated from the hexane extracts of a soft coral, *Nephthea sp.* (species unidentified), collected at Enewetak, Marshall Islands.

The more abundant compound (\*0.75% of dry animal weight), called epoxynephthenol acetate (I), was shown to have a molecular formula of  $C_{22}H_{36}O_3$  (combustion analysis and high resolution mass spectrum). The presence of an acetate functionality in I was indicated by the absorptions at 1730 and 1255  $cm^{-1}$ , in the infrared (ir) spectrum, and by the signal at  $\delta$  1.97 (s, 3 H) in the proton magnetic resonance (pmr) spectrum (see Figure I). The ir spectrum indicated a lack of hydroxyl functionality, but showed the presence of olefinic functionality by the absorptions at 3100 and 1658  $cm^{-1}$ . The acetate group accounts for two of the three oxygens in I, and since neither hydroxyl nor any other carbonyl functionalities are present, the third oxygen must be an ether oxygen. The signal at  $\delta$  2.84 (t, 1 H,  $J = 5$  Hz) in the pmr spectrum of I suggested an oxirane ring.<sup>16</sup> A positive reaction of I with acidic silver periodate confirmed the presence of an epoxide group. The sharp triplet absorption pattern of the  $\delta$  2.84 signal and a methyl group signal at  $\delta$  1.30 suggested the epoxide was substituted as shown in partial structure A.

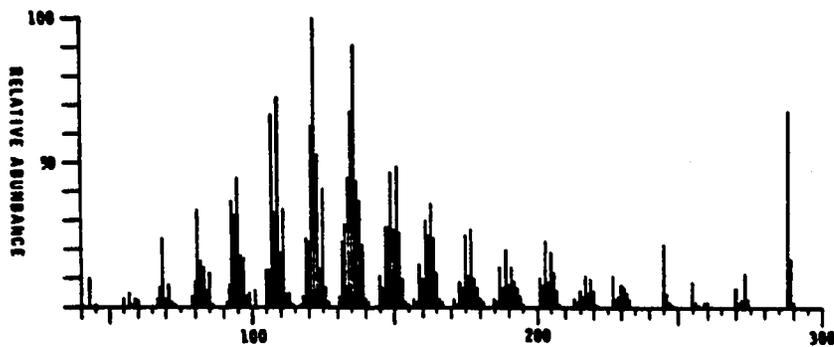
Figure I  
Spectra of Epoxynephenol Acetate (I)



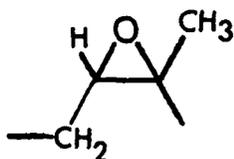
IR Spectrum of I



100 MHz Spectrum of I



Mass Spectrum of I



A

The presence of two trisubstituted double bonds was indicated by two absorptions (one proton each) in the olefinic region which appear as broad triplets centered at  $\delta$  5.36 and  $\delta$  5.13, and two vinyl methyl group signals which appear at  $\delta$  1.67 and  $\delta$  1.55. Two double bonds were indicated also by the proton decoupled  $^{13}\text{C}$ -magnetic resonance spectrum obtained on epoxynephtenol (vide infra) which displayed four olefinic carbon absorptions at  $\delta$  134.7, 131.5, 126.8 and 124.7. The acetate, oxirane and two double bonds in I account for four of the five degrees of unsaturation implied by the molecular formula  $\text{C}_{22}\text{H}_{36}\text{O}_3$ ; thus a monocyclic structure was indicated.

Double irradiation experiments on I were carried out on a Varian T-60 nmr spectrometer and are listed in Table I. Irradiation at  $\delta$  2.84 caused a barely discernible broad doublet absorption at approximately  $\delta$  1.90 to collapse to a broad singlet. Irradiation at  $\delta$  1.90 caused the triplet at  $\delta$  2.84 to collapse to a singlet. The chemical shift position of  $\delta$  1.90 is consistent for protons on a carbon(s) adjacent to an epoxide group. The four protons on the methylene carbons flanking the epoxide group of cyclohexene oxide appear as a broad multiplet at  $\delta$  1.82.

Irradiation in the allylic region at  $\delta$  2.13 caused the olefinic protons to collapse to nearly broad singlets, but did not affect the triplet at  $\delta$  2.84; thus at least one methylene unit must be interposed

TABLE I

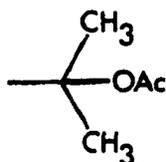
Double Irradiation<sup>a</sup> of Epoxynephthenol Acetate

Signal Irrad'd	Signal obs'd	Change obs'd
2.84	1.90	br d → br s
1.90	2.84	t → s
2.13	5.36	br t → br s
	5.13	br t → br s
	2.84	unaffected
5.36	1.67	br s → sharp s
5.13	1.55	br s → sharp s

<sup>a</sup>Run on a Varian T-60 nmr spectrometer in CCl<sub>4</sub> solution using tetramethylsilane as internal standard. Abbreviations specified in the experimental section apply.

between any allylic carbon atoms and the monosubstituted side of the oxirane unit. The trisubstituted nature of the two double bonds was confirmed when irradiation at the olefinic absorptions,  $\delta$  5.36 and  $\delta$  5.13, caused a sharpening of the vinyl methyl absorption at  $\delta$  1.67 and  $\delta$  1.55, respectively.

The pmr spectrum of I displays a six proton singlet at  $\delta$  1.48, consistent for two methyl groups attached to a carbon bearing an acetate; thus the partial structure B was concluded.



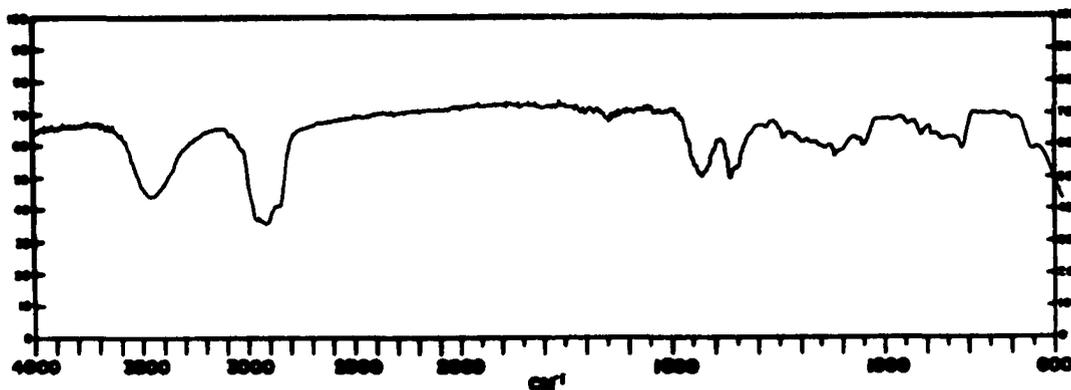
B

All of the foregoing data suggested that I was an acetylated diterpene. Since I was indicated to be monocyclic, the cembrane ring system became the working hypothesis.

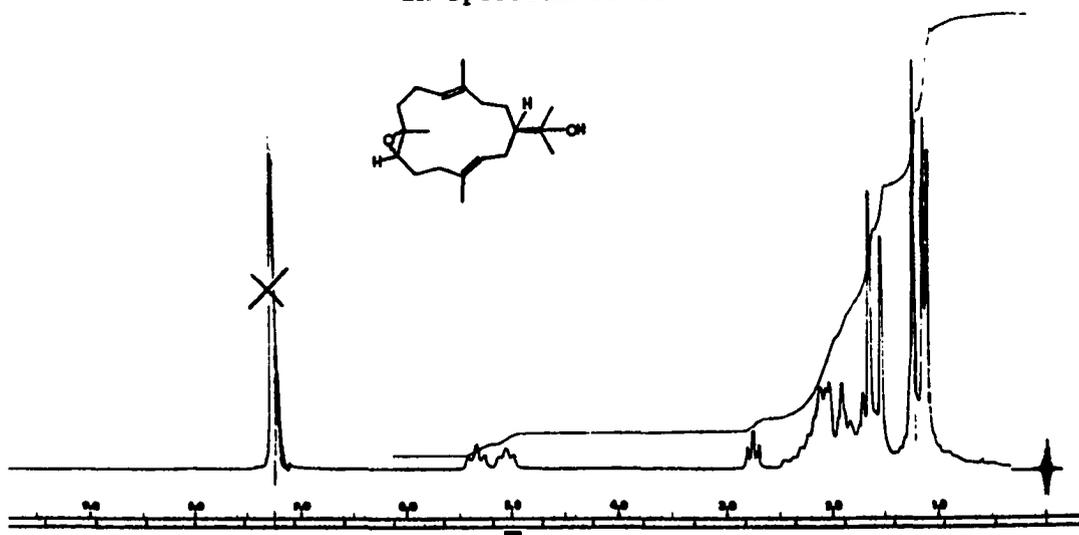
Mild reduction of I with  $\text{LiAlH}_4$  yielded epoxynephthenol. The spectral data for epoxynephthenol (II) is shown in Figure II. The pmr spectrum of II is similar to that of I. The differences are: (1) the pmr spectrum of II lacks the acetate methyl signal in I and (2) a six proton singlet (60 MHz) occurs at  $\delta$  1.18 in the pmr spectrum of II as compared to  $\delta$  1.48 in I. The 0.30 ppm upfield shift in the resonance of the geminal dimethyl group (see partial structure B) is expected as a result of the conversion of the acetoxyisopropyl group to the dimethyl-carbinol unit C.

Figure II

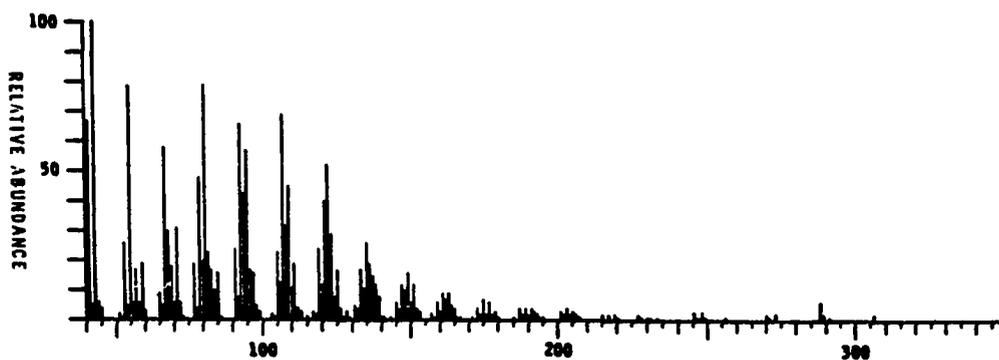
Spectra of Epoxynephthenol (II)



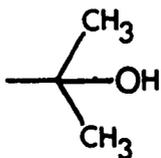
IR Spectrum of II



100 MHz Spectrum of II

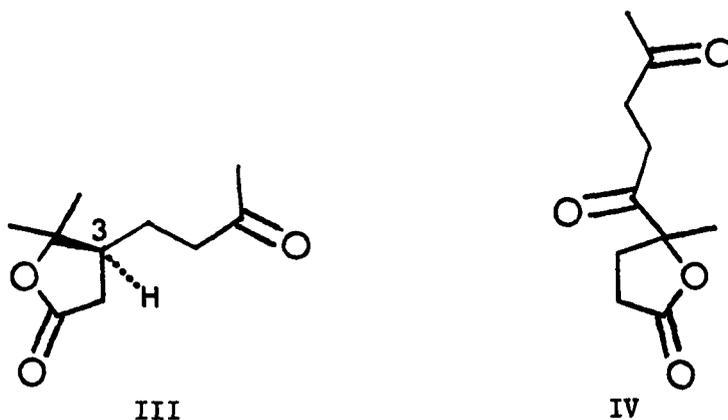


Mass Spectrum of II



c

Oxidative degradation of II using  $\text{OsO}_4/\text{NaIO}_4$  followed by Jones reagent ( $\text{CrO}_3$ ) yielded III and IV.

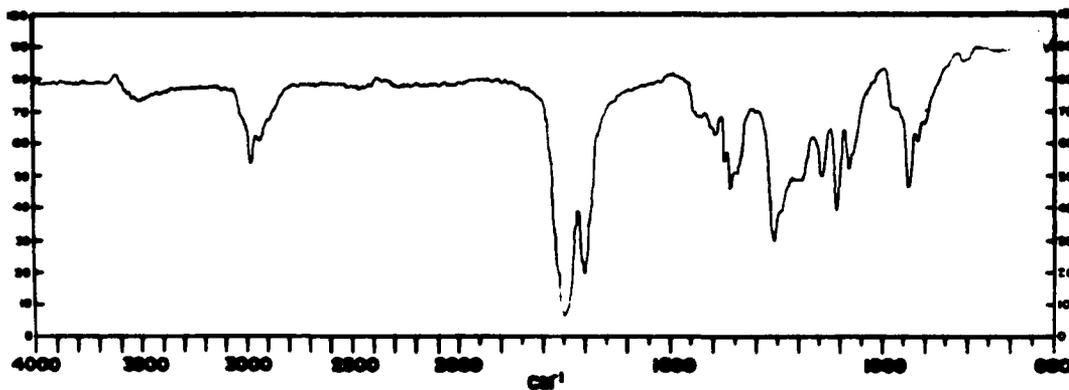


The molecular formula of  $\text{C}_{10}\text{H}_{16}\text{O}_3$  for III was established by high resolution mass spectroscopy and combustion analysis. The presence of a  $\gamma$ -lactone and a ketone in III was indicated in the ir spectrum (see Figure III) by the absorptions at  $1760$  and  $1715\text{ cm}^{-1}$ , respectively. The pmr spectrum of III shows three methyl group signals. The signal at  $\delta\ 2.18$  is assigned to the methyl of the acyl group. The signals at  $\delta\ 1.47$  and  $\delta\ 1.29$  are ascribed to the methyls on the carbon bearing the lactone ether oxygen.

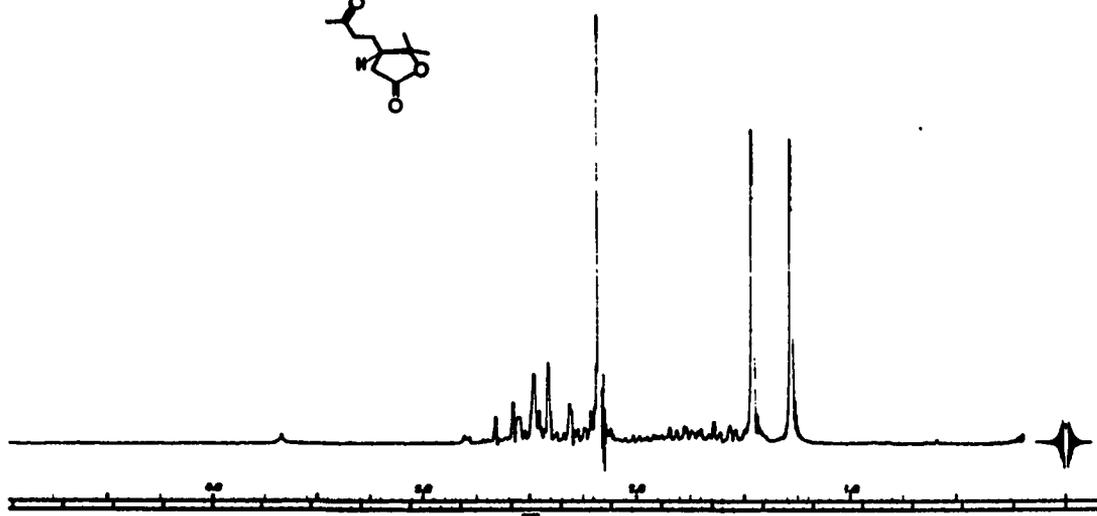
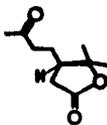
Preparation of an authentic sample of II was accomplished by oxidation of ( $\pm$ )  $\alpha$ -terpineol according to the procedure of Narang and Dutta<sup>17</sup> as shown below.

Figure III

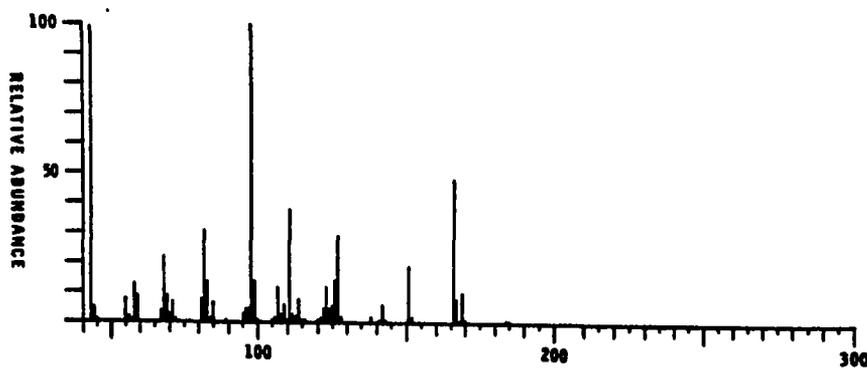
Spectra of (-) Homoterpenyl Methyl Ketone (III)



IR Spectrum of III



100 MHz Spectrum of III

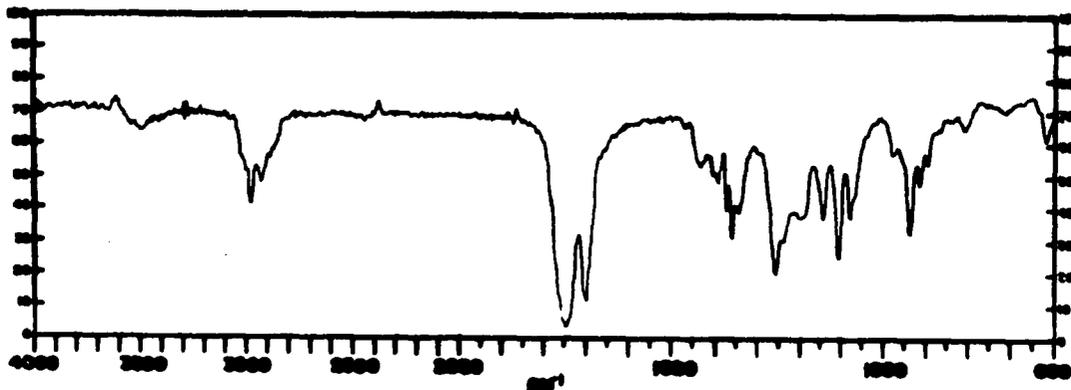


Mass Spectrum of III

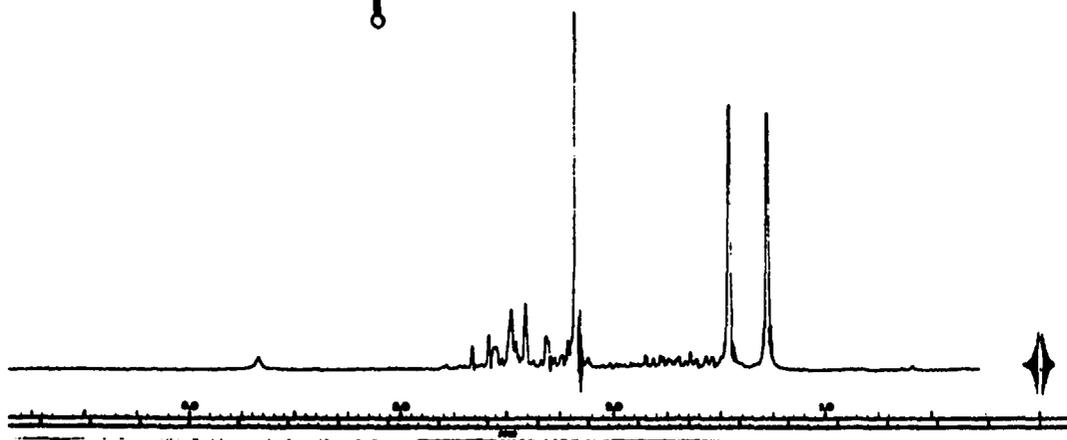
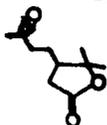


Figure IV

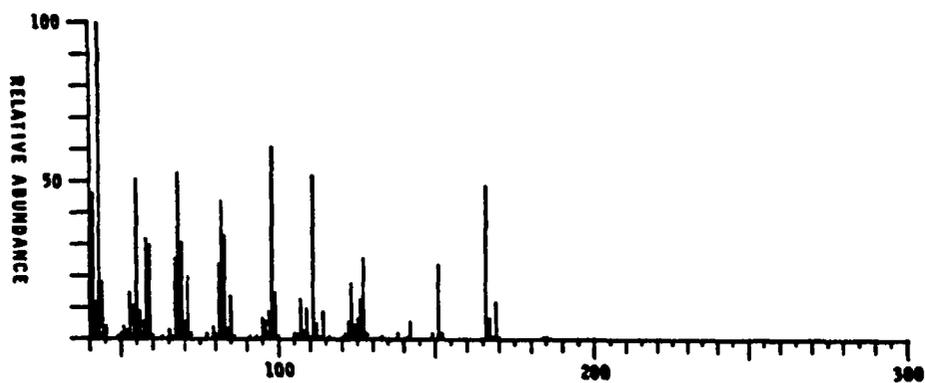
Spectra of ( $\pm$ ) III Derived from ( $\pm$ )  $\alpha$ -terpineol



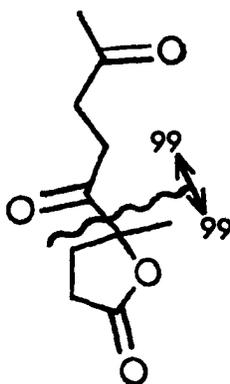
IR Spectrum of ( $\pm$ ) III



100 MHz Spectrum of ( $\pm$ ) III

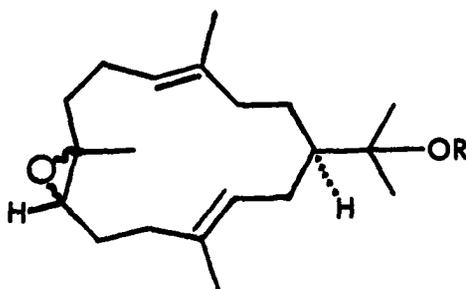


Mass Spectrum of ( $\pm$ ) III



The presence of a  $\gamma$ -lactone and aliphatic keto groups in IV was denoted by the absorptions at 1780 and 1720  $\text{cm}^{-1}$  in the infrared spectrum (see Figure V). The pmr spectrum of IV shows two methyl group signals. The methyl group signal at  $\delta$  2.12 was assigned to the methyl of the acyl group and the methyl group signal at  $\delta$  1.60 was ascribed to the methyl on the carbon bearing the lactone ether oxygen. A four proton broad singlet appears at  $\delta$  2.84 and was assigned to the four methylene protons between the two ketone carbonyl groups [in the pmr spectrum of 2,5-hexanedione,  $\text{CH}_3\overset{\text{O}}{\parallel}\text{C}\overset{\text{a}}{\text{C}}\overset{\text{b}}{\text{C}}\overset{\text{b}'}{\text{C}}\overset{\text{a}'}{\text{C}}\overset{\text{O}}{\parallel}\text{CH}_3$ <sup>24</sup>, the protons labelled b absorb at  $\delta$  2.60 (s)]. Structure IV is consistent with all of the foregoing data. Several attempts to synthesize IV were unsuccessful.

The formation of III and IV was interpreted as clear indication of the cyclotetradecane ring system for epoxynephthenol acetate (I).



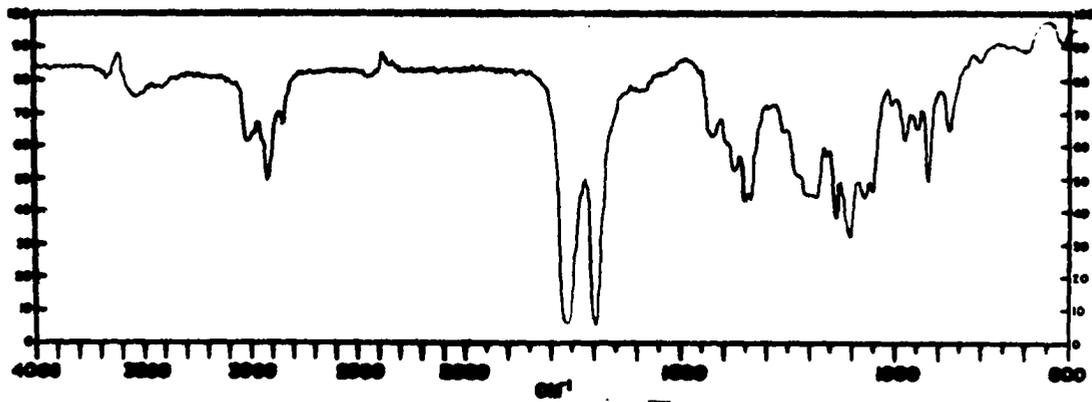
I: R = Ac

II: R = H

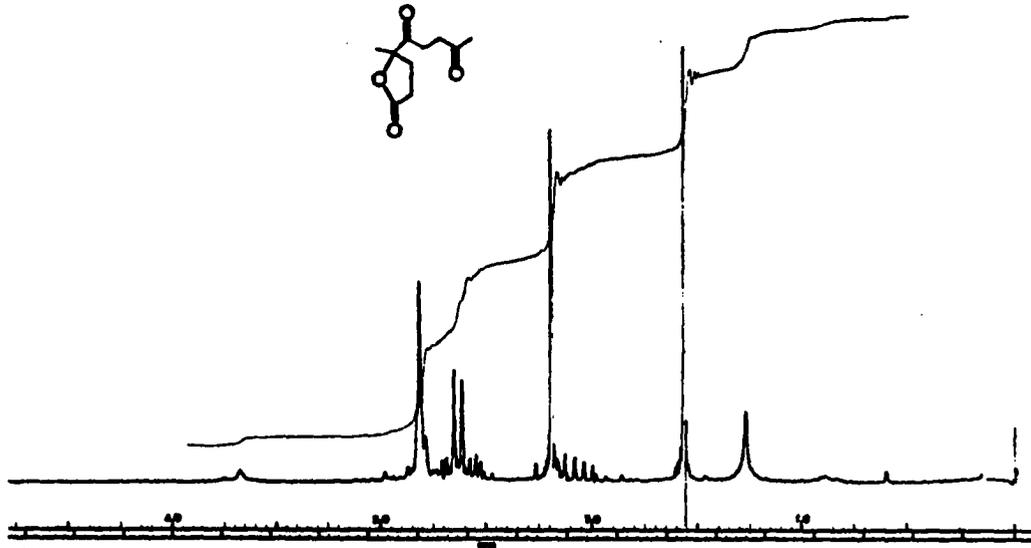
D

Figure V

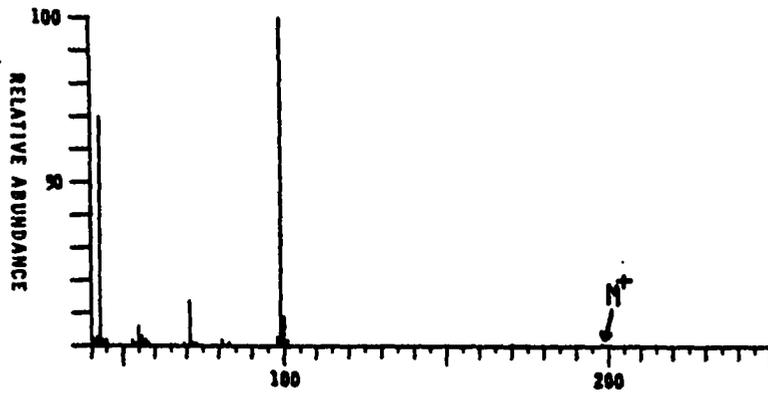
Spectra of Diketo Lactone (IV)



IR Spectrum of IV



100 MHz Spectrum of IV



Mass Spectrum of IV

The formation of (-) homoterpenyl methyl ketone (III) established the R configuration of the carbon bearing the acetoxyisopropyl side chain. The R stereochemical assignment requires the double bonds in the ring to be positioned as is depicted in D.<sup>6</sup> A proposed mechanism for the formation of III and IV from the oxidative degradation of epoxynephthenol (II) is shown in Figure VI.

The *trans* configuration for the two double bonds and the epoxide functionality was indicated by the failure to obtain any Nuclear Overhauser effect for the olefinic or epoxide methine proton signals upon irradiation at the position of the vinyl methyl and epoxide methyl group signals. Irradiation in the allylic region at  $\delta$  2.04 produced a 9%  $\pm$ 3% signal intensity enhancement for the olefinic protons.

On the basis of all the foregoing data, the structure I, as shown in D, is proposed for epoxynephthenol acetate, including the absolute configuration implied thereby; the only stereochemical feature unassigned was the chirality of the *transoid* epoxide.

An interesting feature was noted in the mass spectrum of (-) homoterpenyl methyl ketone (II). The spectrum displayed a large fragment ion at  $m/e$  98. Two mechanisms can be forwarded to account for this fragment ion. These are shown in Figure VII. The high resolution mass spectrum of II indicated that both mechanisms are operative since valid elemental compositions for both  $C_6H_{10}O^+$  (98.07520) and  $C_5H_6O_2^+$  (98.03678) [in approximately a 60:40 ratio] were observed.

The second new compound obtained from *Nephthea* was called nephthenol; it has a molecular formula of  $C_{20}H_{34}O$  (low resolution mass spectrum). Nephthenol, isolated as an unstable oil (bp (Kugelrohr)  $\approx$ 96°C/0.03 Torr), was determined to have a hydroxyl group on the basis

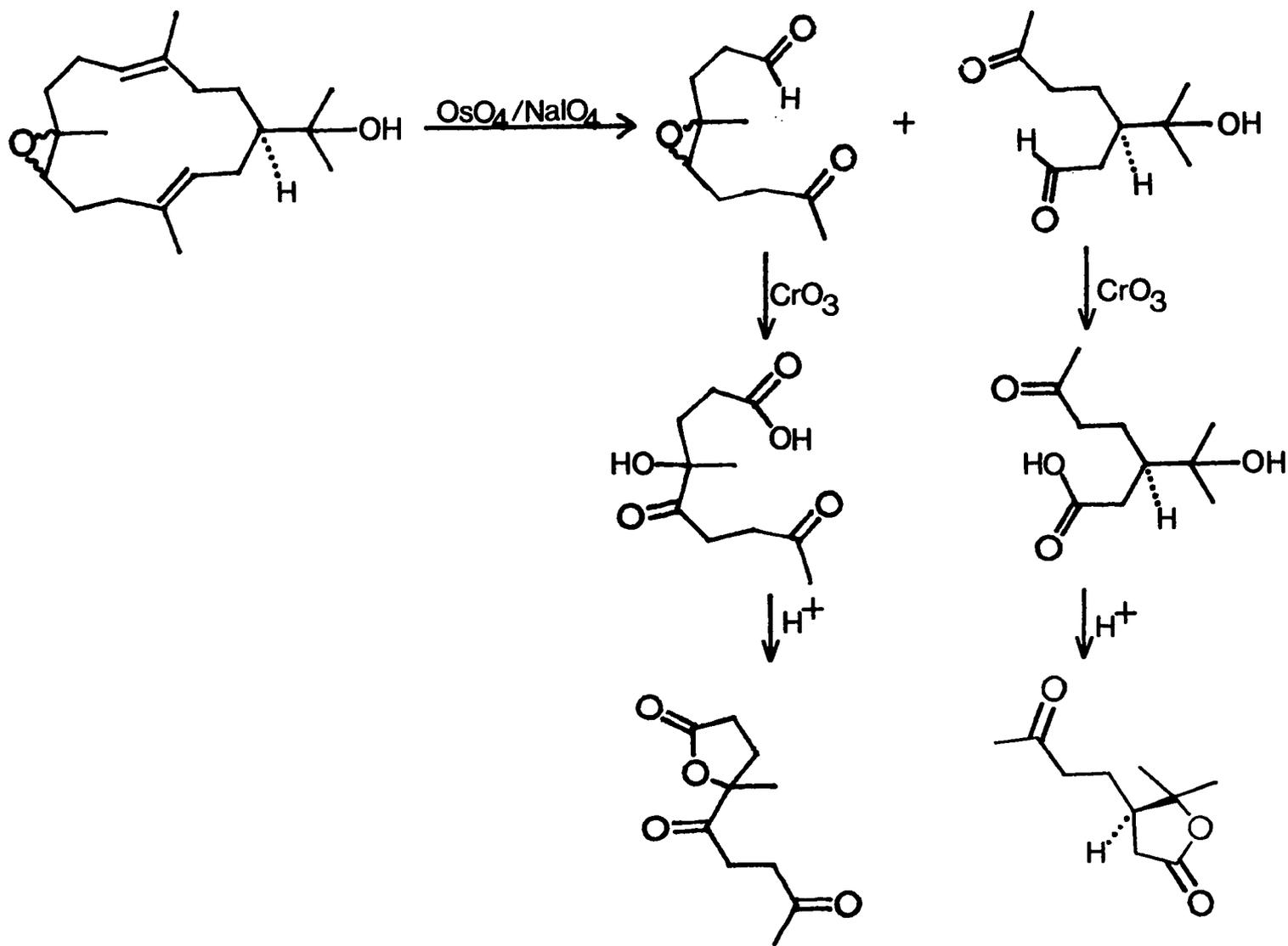
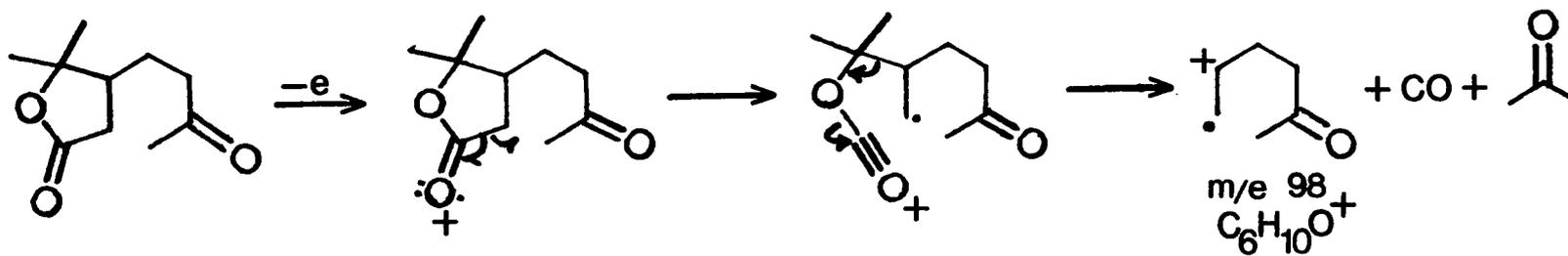


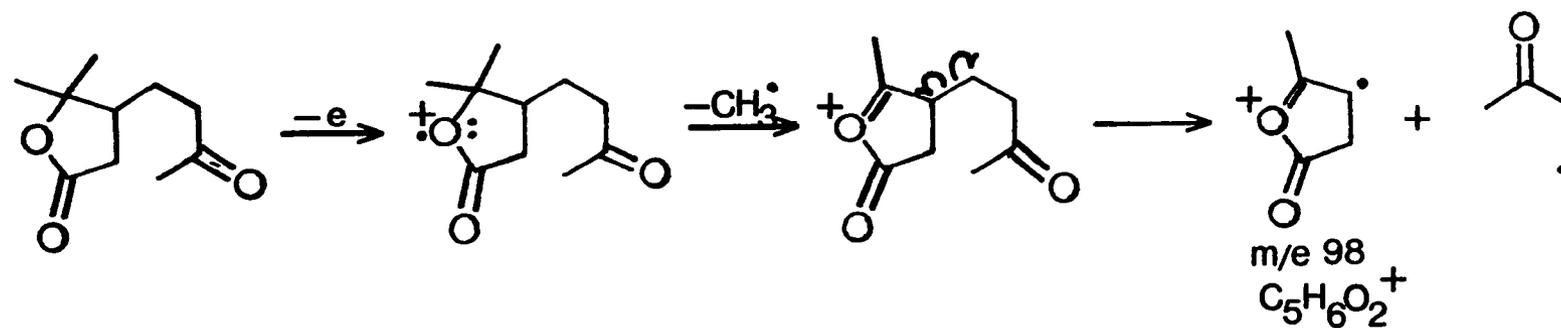
Figure VI

Figure VII

Mechanism 1:

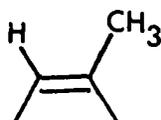


Mechanism 2:



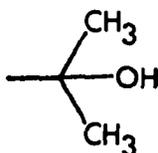
of the broad absorption at  $3460\text{ cm}^{-1}$  in its ir spectrum (Figure VIII).

Catalytic hydrogenation of nephthenol (molecular weight 290) yielded a saturated derivative whose molecular weight was determined to be 296 (low resolution mass spectrum). The increase in molecular weight of six mass units indicated three double bonds in nephthenol. The three double bonds were ascertained to be trisubstituted, as shown in E, from the pmr absorptions at  $\delta$  5.0 and  $\delta$  1.60 which integrated in a ratio of 1:3.



E

The presence of a dimethyl carbinol group, F, similar to that in epoxynephthenol (see Figure II), was denoted by the six proton absorption in the pmr spectrum at  $\delta$  1.20.

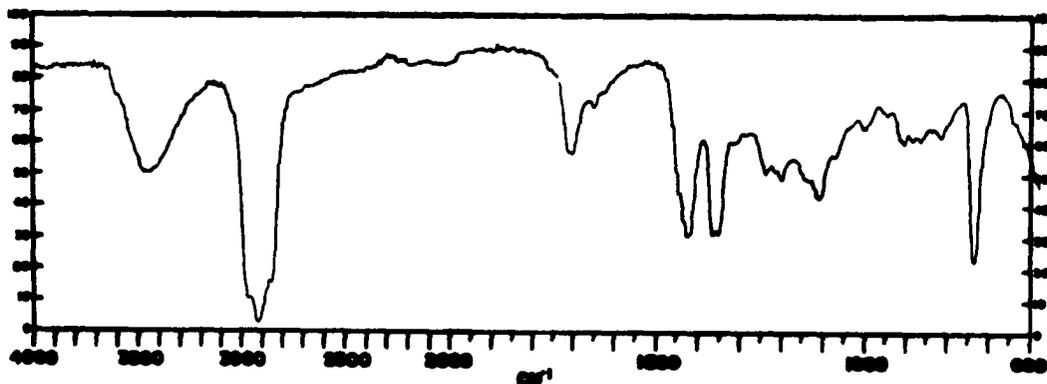


F

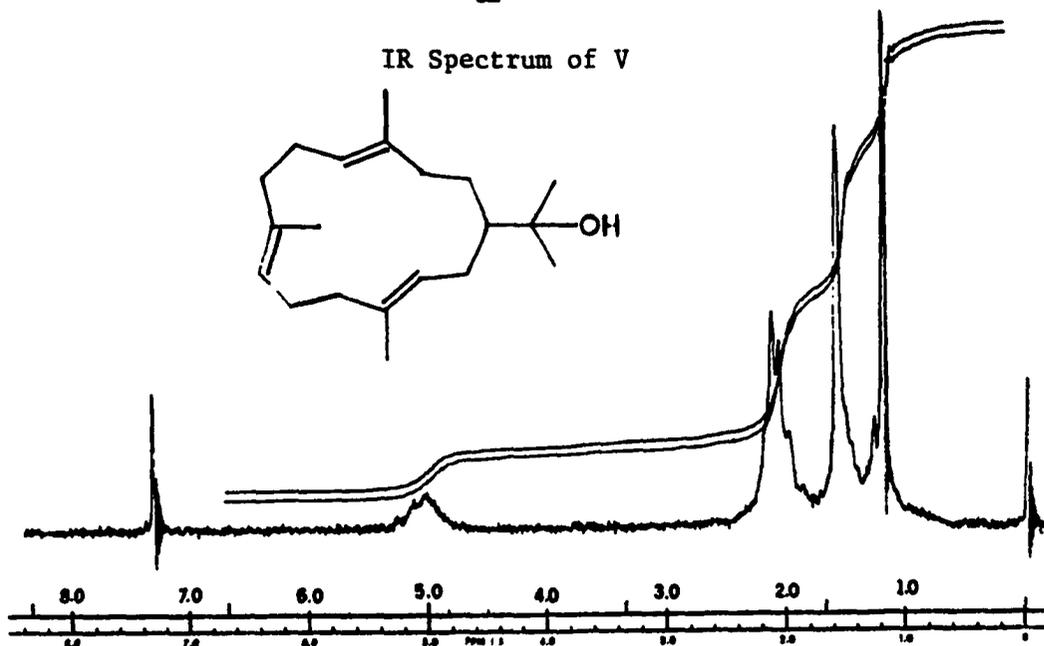
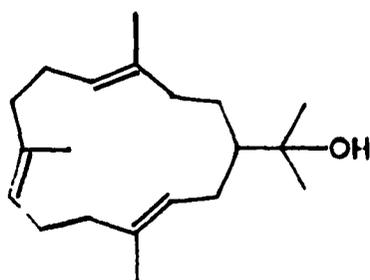
The molecular formula of  $\text{C}_{20}\text{H}_{34}\text{O}$  indicates four degrees of unsaturation. Three of the four are accounted for by the double bonds, thus nephthenol must be monocyclic. All of the foregoing data suggested that nephthenol was a diterpene and the cembrane ring system again became the working hypothesis.

Figure VIII

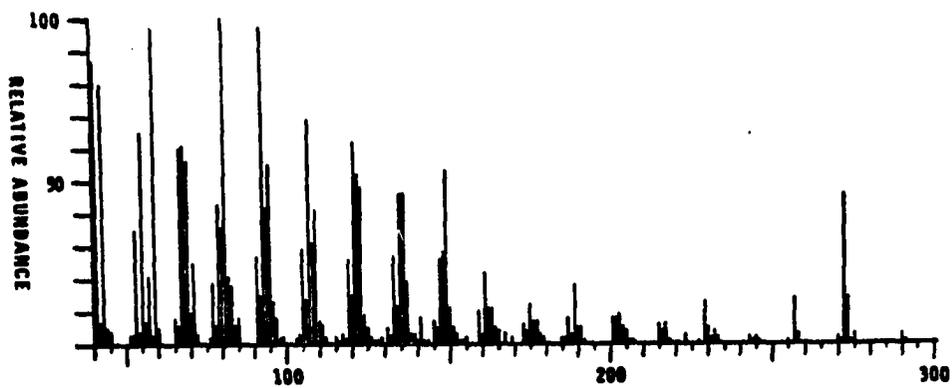
Spectra of Nephthenol (V)



IR Spectrum of V



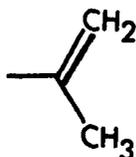
60 MHz Spectrum of V



Mass Spectrum of V

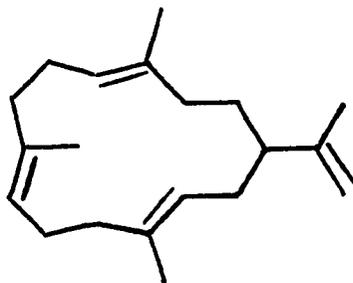


The third compound isolated from *Nephthea*,  $C_{20}H_{32}$  (combustion analysis and low resolution mass spectrum), was obtained as an oil and called NEP-3. The spectral data for NEP-3 is shown in Figure IX. The presence of three double bonds, similar to those in nephthenol, was indicated in the pmr spectrum by the absorptions at  $\delta$  5.0 and  $\delta$  1.56, which integrated in a ratio of 1:3. The presence of an isopropenyl group, G, was suggested by the absorption in the olefinic region at  $\delta$  4.80 (2 protons) and the vinyl methyl absorption at  $\delta$  1.67 (3 protons).<sup>6</sup>



G

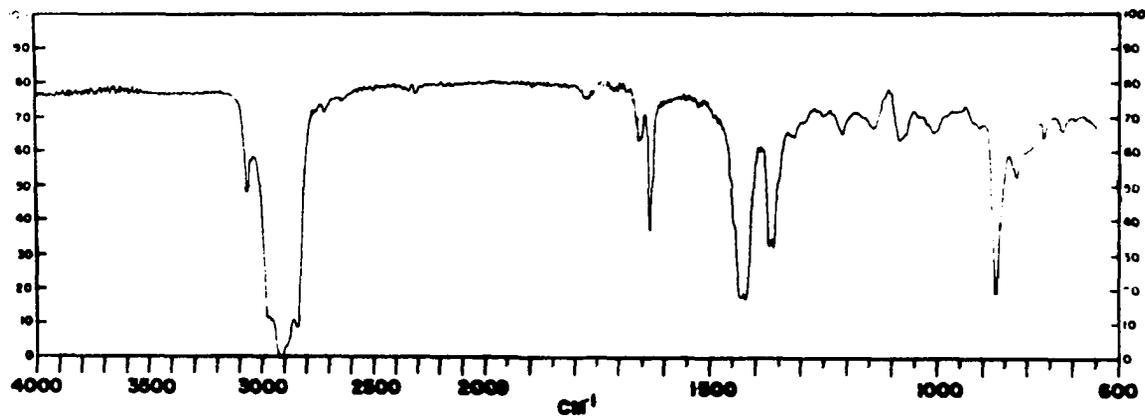
From the data discussed and by analogy with I and V the structure for NEP-3 was initially formulated as shown in VI.



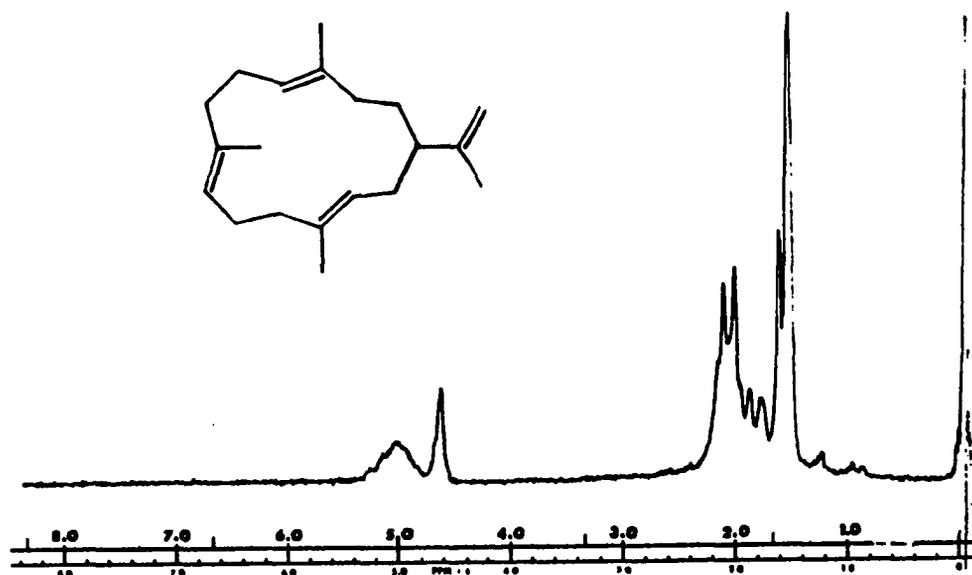
VI

A search of the literature revealed that a compound possessing the gross structural features of VI had previously been isolated from various natural sources: from a tree in India,<sup>11,12</sup> a Siberian spruce tree in Russia,<sup>13,14</sup> and termites in Australia.<sup>15</sup> The name neocembrene was used for this hydrocarbon by the Russian group,<sup>13,14</sup> neocembrene-A by the Australian group<sup>15</sup> and cembrene-A by the group in India.<sup>11,12</sup>

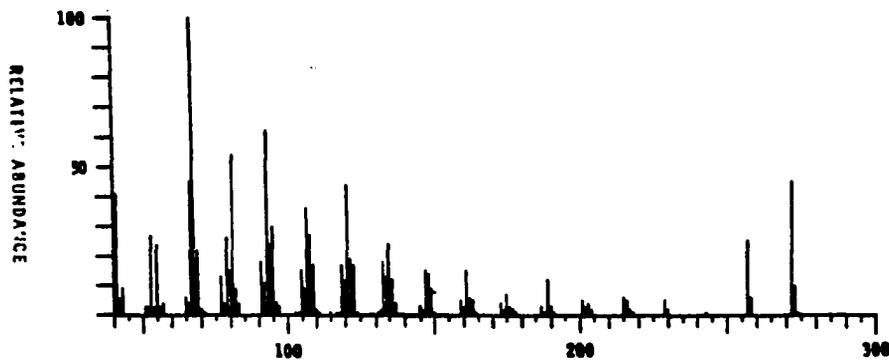
Figure IX  
Spectra of NEP-3 (VI)



IR Spectrum of VI



60 MHz Spectrum of VI

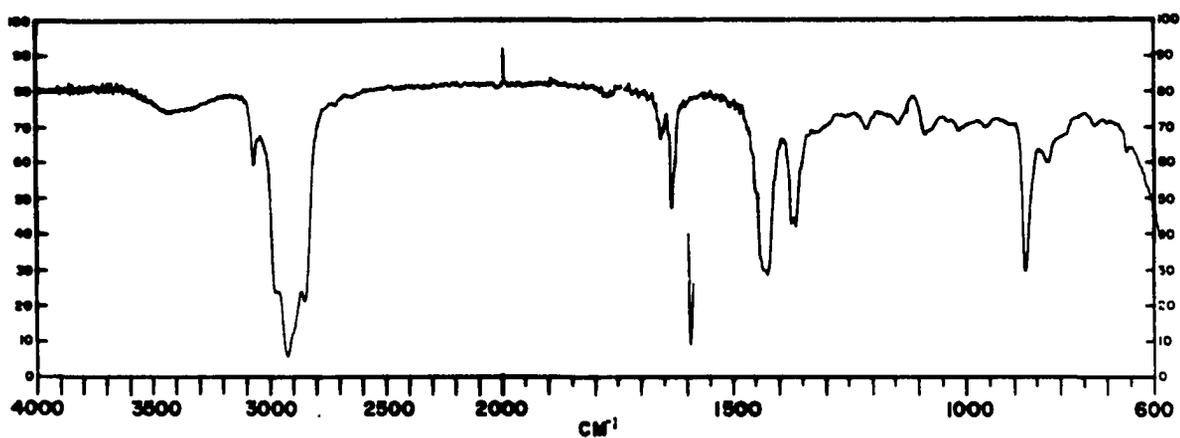


Mass Spectrum of VI

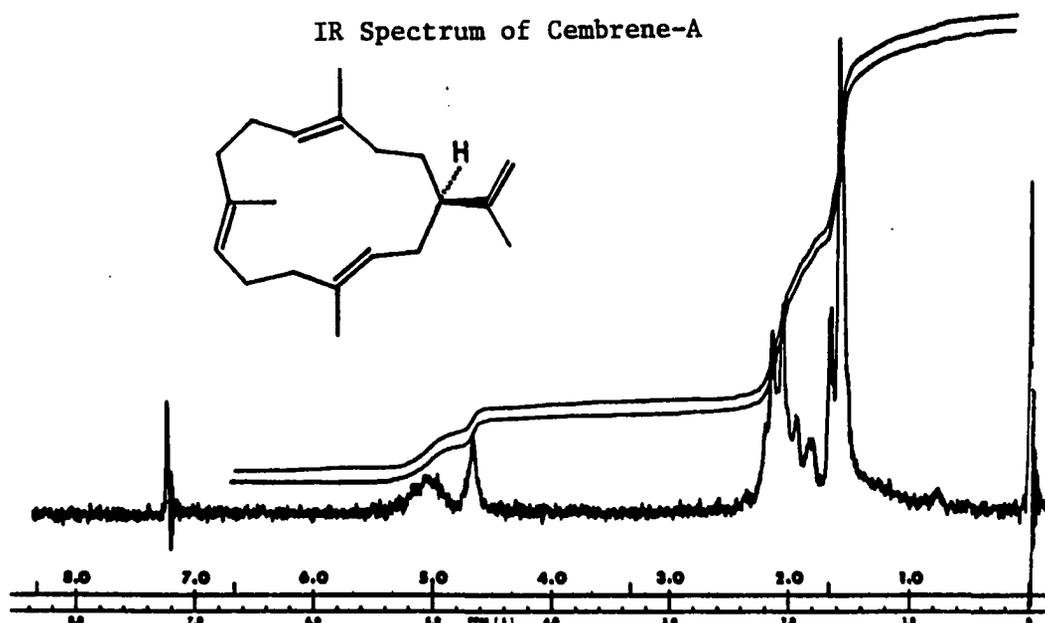
An authentic sample of cembrene-A was obtained from the research group in India. NEP-3 and cembrene-A showed identical physical and spectral properties (see experimental section and compare Figure IX and X) except for optical rotational data. The difference in optical rotations (cembrene-A  $[\alpha]_D -20^\circ$ , NEP-3  $[\alpha]_D - 0.37^\circ$ ) indicates that either the cembrene-A from *Nephthea* is a racemic mixture or that there is still an impurity present in our sample. While an impurity cannot be rigorously ruled out, our sample was homogeneous by tlc and gas chromatographic analysis and gave a satisfactory combustion analysis. The pmr, ir, and mass spectral comparison of NEP-3 with cembrene-A confirm the structural assignment formulated in VI.

Subsequent to the isolation of NEP-3, another novel hydrocarbon, termed NEP-4, has been isolated from *Nephthea sp.* Work on this hydrocarbon is presently in progress; structural details will not be discussed in this dissertation.

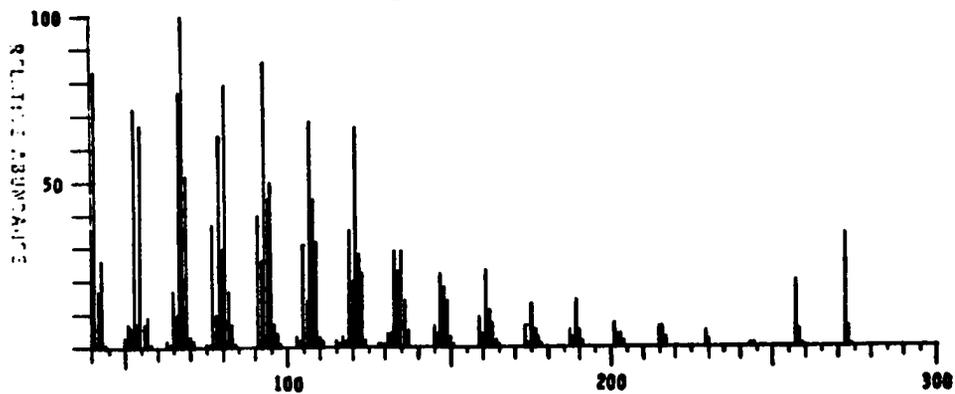
Figure X  
Spectra of Cembrene-A



IR Spectrum of Cembrene-A



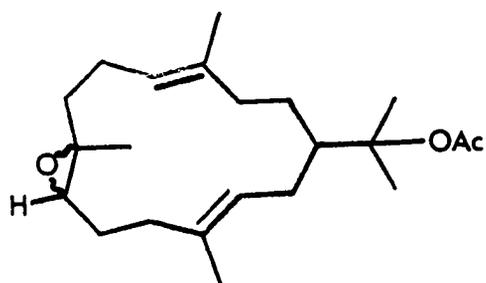
60 MHz Spectrum of Cembrene-A



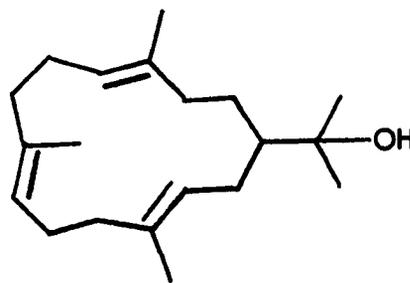
Mass Spectrum of Cembrene-A

### Summary

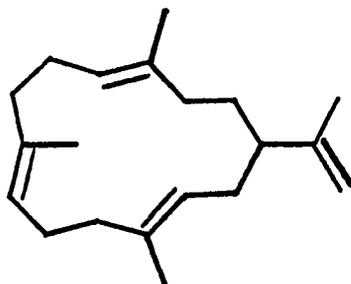
Two new cembrene derivatives, epoxynephthenol acetate (I) and nephthenol (V), were isolated from the hexane extracts of the soft coral *Nephthea* sp. and structures are proposed based on spectral and chemical degradation data. A third cembrene derivative, a hydrocarbon, was also isolated. Comparison of spectral data (ir, pmr, mass spectra) and of gas chromatographic retention times for this hydrocarbon with an authentic sample of cembrene-A established its structure as VI.



I



V



VI

## Experimental

Experimental conditions specified in section I apply

Isolation of Nephthenol (V) and Epoxynephthenol Acetate (I). The *Nephthea Sp.* was collected from the Enewetak atoll, Marshall Islands. The isolation procedure discussed directly below was carried out on specimens collected in August 1969 by the author. Subsequent collections were made by Dr. R. E. Middlebrook (1970 and 1972) and Dr. R. A. Gross (1973). These later collections were fractionated by the procedure discussed in the isolation of NEP-3.

The air dried specimens of *Nephthea sp.* (3.5 kg) were ground in a blender into small pieces and extracted with distilled hexane for 3 days. The hexane was removed under reduced pressure on a rotary evaporator to yield  $\approx$  150 g of a dark green viscous oil. The green oil was distilled through a falling film molecular still (Nester Faust) at 110°C/0.3 Torr to yield 13 g of a yellow liquid. A portion of the distillate ( $\approx$ 2.5 g) was chromatographed on 125 g of thin-layer mesh silica gel employing ethyl acetate/benzene (4/96) as solvent and collecting 60 ml fractions. Impure V was obtained in fractions 6 and 7 and impure I was collected in fractions 9 to 16. Preparative thin layer chromatography yielded pure I and V.

Pure I has  $[\alpha]_D -20.7$  ( $c$  2.3,  $\text{CHCl}_3$ );  $R_f = 0.31$  (10% ethyl acetate/benzene, silica gel H); ir (neat) 3060, 3000, 2940, 2870, 1735 (acetate), 1455, 1390, 1375, 1260, 1135, 1020, 940, 885, and 835  $\text{cm}^{-1}$ ;

100 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  5.35 (br t, 1 H,  $-\underline{\text{CH}}-\text{CCH}_3-$ ), 5.12 (br t, 1 H,  $-\underline{\text{CH}}-\text{CCH}_3-$ ), 2.86 (t,  $J = 5$  Hz,  $-\overset{\text{O}}{\text{C}}\underline{\text{H}}-\text{CCH}_3-$ ), 2.4 - 1.95 (m, 8 H, allylic protons), 1.98 (s, 3 H,  $-\text{C}(\text{CH}_3)_2\text{OCO}\underline{\text{C}}\text{H}_3$ ), 1.74 (m, 4 H, protons on carbons attached to carbons bearing the epoxide), 1.67 (br s, 3 H,  $-\text{CH}=\underline{\text{C}}\text{H}_3-$ ), 1.56 (br s, 3 H,  $-\text{CH}=\underline{\text{C}}\text{H}_3-$ ), 1.48 (s, 6 H,  $-\text{C}(\underline{\text{C}}\text{H}_3)_2-\text{OCO}\text{C}\underline{\text{H}}_3$ ), and 1.28 ppm (s, 3 H,  $-\overset{\text{O}}{\text{C}}\underline{\text{H}}-\text{C}\underline{\text{H}}_3$ ); mass spectrum (10 eV)  $m/e$  (rel. intensity) no molecular ion was obtained, 288 ( $\text{M}^+$  - acetic acid) (68), 273 (12), 270 (7), 255 (9), 245 (22), 189 (20), 175 (25), 163 (36), 161 (30), 151 (49), 149 (47), 136 (91), 122 (100) base peak, 109 (73), 93 (37), 81 (34), 69 (24) and 43 (10).

Anal. Calcd. for  $\text{C}_{22}\text{H}_{36}\text{O}_3$ : C, 75.82; H, 10.41, mol. wt. 348.26644.  
Found: C, 76.12; H 10.74; mol. wt. (mass spectrum) 348.26610.

Nephtenol has ir 3450, 3050, 2980, 2930, 2860, 1720 (due to impurity) 1660, 1440, 1380, 1365, 1250, 1210, 1125, and  $750\text{ cm}^{-1}$ ; 60 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  5.0 (m, 3 H,  $-\underline{\text{CH}}=\text{CCH}_3-$ ), 2.1 (br m, 12 H, allylic protons), 1.58 (br s, 9 H,  $-\text{CH}=\underline{\text{C}}\text{H}_3-$ ) and 1.18 ppm (s, 6 H,  $-\text{C}(\underline{\text{C}}\text{H}_3)_2\text{OH}$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $\text{M}^+$  290 (3), 272 (45), 257 (14), 189 (18), 175 (12), 161 (22), 149 (53), 135 (46), 121 (62), 107 (69), 93 (97), 81 (100) base peak, 68 (61), 59 (97), 43 (80) and 41 (87).

Deacetylation of Epoxynephthenol Acetate (I). To a flask containing 161.4 mg of I (0.46 mmoles) in 50 ml of diethyl ether (freshly distilled from  $\text{LiAlH}_4$ ) was added 35.0 mg of  $\text{LiAlH}_4$ . After 30 minutes at room temperature the excess  $\text{LiAlH}_4$  was destroyed with the addition of ethyl acetate ( $\approx 25$  mls) and water ( $\approx 15$  mls). The product was extracted into ether, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and the solvent was

removed under reduced pressure on a rotary evaporator to yield 139.7 mg of a white solid. Recrystallization from hexane yielded pure epoxy-nephtenol (II).

Pure II has mp 58.7 - 61.6°C; ir 3460, 3030, 2960, 2920, 2850, 1655 (weak), 1440, 1380, 1250, 1130, 1065, 930, 825 and 670  $\text{cm}^{-1}$ ; 100 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  5.34 (br t, 1 H  $J = 7$  Hz,  $-\text{CH}=\text{CCH}_3-$ ), 5.06 (br t, 1 H,  $J = 7$  Hz,  $-\text{CH}=\text{CCH}_3-$ ), 2.76 (t, 1 H,  $J = 5$  Hz,  $-\text{CH}-\overset{\text{O}}{\text{C}}\text{CH}_3-$ ), 2.5 - 1.9 (m, 8 H, allylic protons), 1.9 - 1.7 (m, 4 H, protons on the  $\alpha$  carbons to the epoxide), 1.66 (br s, 3 H,  $J < 1$  Hz,  $-\text{CH}=\text{CCH}_3-$ ), 1.44 (br s, 3 H,  $J < 1$  Hz,  $-\text{CH}=\text{CCH}_3-$ ), 1.24 (s, 3 H,  $-\text{CH}-\overset{\text{O}}{\text{C}}\text{CH}_3-$ ), 1.15 (s, 3 H, one of  $-\text{C}(\text{CH}_3)_2\text{OH}$ ), and 1.10 ppm (s, 3 H, one of  $-\text{C}(\text{CH}_3)_2\text{OH}$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$  306 (2), 288 (6), 273 (2), 270 (2), 248 (3), 177 (6), 163 (9), 151 (12), 149 (16), 135 (26), 122 (59), 107 (69), 93 (66), 81 (79), 67 (58), 55 (79), 43 (100) base peak, and 41 (67).

Anal. Calcd for  $\text{C}_{20}\text{H}_{34}\text{O}_2$ : C, 78.38; H, 11.18. Found: C, 78.04; H, 11.01.

The sample used in the Nuclear Overhauser experiment was dissolved in  $\text{CDCl}_3$  containing TMS, the solution was transferred to an nmr tube, frozen in a dry ice-acetone bath and a vacuum was applied. When full vacuum was attained the nmr tube was sealed with a microburner.

Hydrogenation of Epoxynephthenol Acetate. To a flask containing 25 ml of absolute alcohol and a few milligrams of prereduced  $\text{PtO}_2$  in a hydrogen atmosphere was added 100 mg of epoxynephthenol acetate dissolved in 5 ml of absolute alcohol. The uptake of hydrogen began immediately. The flask and contents were maintained in the hydrogen atmosphere overnight.

After the catalyst was filtered from the solution the solvent was removed under reduced pressure to yield an oily product.

The product of hydrogenation has ir ( $\text{CHCl}_3$ ) 2950, 2910, 2870, 1735, 1460, 1370, 1255, 1130, 1020, 930 and  $610 \text{ cm}^{-1}$ ; 60 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  2.80 (m, 1 H, epoxide methine proton), 1.97 (s, 3 H,  $-\text{OCOCH}_3$ ), 1.47 (s, 6 H,  $-\text{C}(\text{CH}_3)_2\text{OAC}$ ), 1.27 (s, 3 H,  $-\overset{\text{O}}{\text{C}}\text{H}-\text{CCH}_3$ ), and 0.91 (br t, 6 H,  $J = 7 \text{ Hz}$ ,  $-\text{CHCH}_3$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $M^+$  352 (1), 292 (11), 252 (13), 149 (11), 123 (27), 101 (30), 95 (35), 81 (31), 69 (37), 55 (45), 43 (100) base peak, and 41 (31).

Reaction of Epoxynephenol with  $\text{OsO}_4$ . To a solution of 25 mg of epoxynephenol (.084 mmoles) dissolved in 20 ml p-dioxane (freshly distilled from  $\text{LiAlH}_4$ ) and 5 ml water was added 19.2 mg  $\text{OsO}_4$  (.08 mmoles) at  $0^\circ\text{C}$ , followed by 157 mg of  $\text{NaIO}_4$  (0.65 mmoles). After the addition of  $\text{NaIO}_4$  the flask and contents were placed in a nitrogen atmosphere and stirred with a magnetic stirring bar for 48 hours. The resulting yellow solution was filtered from the insoluble  $\text{NaIO}_3$  and Jones reagent was added while the reaction flask was cooled in an ice-water bath. After the addition of the Jones reagent, water was added and the products were extracted into ether and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Gas chromatographic analysis on a 5' x 1/8" 10% FFAP column indicated there were two products; these were separated by preparative gas chromatography on a 12' x 1/4" 20% FFAP column. Overall 17.5 mg of (-) homoterpenyl methyl ketone (III) and 8.6 mg of IV were isolated.

Pure III has mp  $46.8 - 47.2$ ;  $[\alpha]_D -41.2^\circ$  ( $c$  0.5,  $\text{CHCl}_3$ ); ir ( $\text{CHCl}_3$ ) 3020, 2980, 2940, 2890, 1760, 1715, 1380, 1270, 1160, 1120, 1090,

950, 930, and 910  $\text{cm}^{-1}$ ; 100 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  2.8 - 2.1 (m, 4 H, protons on the carbons  $\alpha$  to the ketone and lactone carbonyl carbons), 2.18 (s, 3 H,  $-\overset{\text{O}}{\text{C}}\text{CH}_3$ ), 2.05 - 1.50 (m, 3 H), 1.47 (s, 3 H, one of the methyls attached to the carbon bearing the lactone ether oxygen), and 1.29 ppm (s, 3 H, one of the methyls attached to the carbon bearing the lactone ether oxygen); mass spectrum (10 eV) m/e (rel. intensity)  $M + 1$  185 (1),  $M^+$  184 (1), 169 (10), 166 (48), 151 (19), 127 (29), 123 (12), 111 (38), 107 (12), 99 (14), 98 (100) base peak, 82 (31), 68 (22), 58 (13), and 43 (99).

Anal: Calcd. for  $\text{C}_{10}\text{H}_{16}\text{O}_3$ ; C, 65.20; H, 8.74; mol. wt. 184.10994.  
Found: C, 65.22; H, 8.83; mol. wt. (mass spectrum) 184.11219.

Pure IV has ir ( $\text{CHCl}_3$ ) 2930, 2850, 1780, 1720, 1365, 1220, 1160, 1125, 1090, 1070, 1000, 970, 945, and 895  $\text{cm}^{-1}$ ; 100 MHz pmr ( $\text{CDCl}_3$ )  $\delta$  2.82 (br s, 4 H,  $-\overset{\text{O}}{\text{C}}\text{CH}_2\text{CH}_2-\overset{\text{O}}{\text{C}}\text{CH}_3$ ), 2.64 (m, 3 H), 2.20 (s, 3 H,  $-\overset{\text{O}}{\text{C}}\text{CH}_3$ ), 2.15 (m, 1 H), and 1.57 ppm (s, 3 H,  $-\text{CH}_3\text{C}-\text{O}$ ); mass spectrum (70 eV) m/e (rel. intensity)  $M^+$  198 (0.5), 99 (100) base peak, 71 (14), 55 (6), and 43 (70).

Oxidation of  $\alpha$ -terpineol. To 50 g of  $\alpha$ -terpineol was added 2 l of  $\text{H}_2\text{O}$  and 75 g of  $\text{KMnO}_4$ . After the solution was stirred for 12 hours the  $\text{MnO}_2$  was filtered from the solution. The water was then removed under reduced pressure on the rotary evaporator to yield a yellow-brown syrup. This was dissolved in 100 ml of  $\text{H}_2\text{O}$  and 90 ml of conc.  $\text{H}_2\text{SO}_4$  with cooling. Then 70 g of  $\text{CrO}_3$  was added slowly. The product was extracted into ether, dried ( $\text{Na}_2\text{SO}_4$ ) and distilled (Kugelrohr). The distillate solidified on cooling and was recrystallized from ether to yield large cube-like crystals, mp 60.1 - 61.8°C. The ( $\pm$ ) homoterpenyl methyl ketone had identical ir, pmr, mass spectra and gas chromatographic retention times as (-)

homoterpenyl methyl ketone derived from II.

Hydrogenation of Nephthenol. To a solution of 15 ml of absolute ethanol containing a few milligrams of prereduced  $\text{PtO}_2$  in a hydrogen atmosphere was added 15 mg nephthenol in 10 ml of absolute alcohol. The hydrogenation was allowed to proceed overnight. After the catalyst was filtered from the solution, the solvent was removed under reduced pressure to yield  $\approx$ 10 mg of an oily product. Spectral data for the hydrogenation product are as follows: 60 MHz pmr ( $\text{CDCl}_3$ ) 2.3 - 1.5 (m, ring methine protons), 1.27 (m, ring methylene protons), 1.21(br s,  $-\text{C}(\underline{\text{CH}}_3)_2\text{OH}$ ), 0.87 (d,  $J = 6$  Hz,  $-\text{CHCH}_3-$ ); mass spectrum (70 eV)  $m/e$  (rel. intensity)  $\text{M}^+$  296 (0.3), 281 (1), 278 (2), 276 (1), 236 (11), 149 (20), 97 (12), 83 (15), 71 (14), 69 (23), 59 (100) base peak, 55 (30), 43 (21), and 41 (22).

Lemieux-Von Rudloff Oxidation of Nephthenol. To a flask containing 10 ml of Lemieux-Von Rudloff reagent solution (0.74 mmoles of  $\text{KIO}_4$ ) was added 14.3 mg of nephthenol (0.06 mmoles) in 10 ml of tert-butyl alcohol. The reaction flask was maintained at room temperature for 48 hours with the pH adjusted to and maintained at 8-9 by  $\text{K}_2\text{CO}_3$ .

The pH was then adjusted to 6 using 10%  $\text{H}_2\text{SO}_4$  and the excess oxidants were destroyed by the addition of solid  $\text{K}_2\text{S}_2\text{O}_5$  until a color change was no longer observed; the pH drops to  $< 1$  in this process. Continuous ethereal extraction for two days resulted in the isolation of 3-8 mg of a product which was determined by gas chromatographic analysis on the 10% FFAP column to be essentially a two component mixture. The shorter

retention time component was identified as levulinic acid by a peak enhancement experiment on the FFAP column. Similarly the longer retention time product was identified as homoterpenyl methyl ketone (III) by peak enhancement using III obtained previously from the degradation of epoxynephthenol (II).

Isolation of NEP-3. Approximately 2.5 kg of *Nephthea sp.* (collected at Enewetak in the summer of 1973 by Dr. R. E. Middlebrook and Dr. R. A. Gross) was extracted with distilled hexane at room temperature for 7 days to yield 172 g of a dark green semi-viscous oil. The entire hexane extract was chromatographed on Florisil (2 lbs.) in the following manner:

A) Hexane (1 liter fractions)

1	↘	54.7613 g
2	↙	
3	-	1.9901 g
4	↘	1.8788 g
5	↙	
6	-	3.3991 g

B) Benzene (4 liters) 13.6729 g

C) Methanol (8 liters) 66.4908 g

The material eluted in fractions 1 and 2 was rechromatographed on Florisil (900 g) employing hexane as solvent and collecting 250 ml fractions. Fraction 9 contained 2.714 g of a clear colorless liquid which by gas chromatographic analysis on a 5' x 1/8" 10% FFAP column contained NEP-3 and NEP-4 (unknown hydrocarbon).

Chromatography of a portion ( $\approx 75$  mg) of fraction 9 on 10 g of thin-layer mesh silica gel yielded pure NEP-3 ( $\approx 35$  mg) and resulted in the complete destruction of NEP-4.

Pure NEP-3 has  $[\alpha]_D - 0.37^\circ$  ( $c$  2.72, hexane); ir 3070, 2980, 2930, 2850, 1665, 1640, 1435, 1380, 1370, 880, and 830  $\text{cm}^{-1}$ ; 60 MHz pmr ( $\text{CCl}_4$ )  $\delta$  5.00 (m, 3 H,  $-\underline{\text{CH}}=\text{CCH}_3-$ ), 4.60 (br s, 2 H,  $-\text{C}(\text{CH}_3)=\underline{\text{CH}}_2$ ), 2.4 - 1.60 (m, 13 H, allylic protons), 1.67 (br s, 3 H,  $-\text{C}(\underline{\text{CH}}_3)=\text{CH}_2$ ), and 1.50 ppm (br s, 9 H,  $-\text{CH}=\text{C}\underline{\text{CH}}_3-$ ); mass spectrum (70 eV) m/e (rel. intensity  $\text{M}^+$  272 (45), 357 (25), 189 (12), 161 (15), 148 (14), 147 (15), 135 (24), 121 (44), 107 (36), 93 (62), 81 (54), 68 (100) base peak, 55 (34), and 41 (41).

Anal. Calcd. for  $\text{C}_{20}\text{H}_{32}$ : C, 88.16; H, 11.84 Found: C, 87.85; H, 11.75.

## BIBLIOGRAPHY

1. W. G. Dauben, W. F. Thiessen, and P. R. Resnick, *J. Amer. Chem. Soc.*, **84**, 2015 (1962).
2. M. G. B. Drew, C. H. Templeton, and A. Salkin, *Acta. Crystallogr.*, **B25**, 261 (1969).
3. H. Kobayashi and S. Akiyoshi, *Bull. Chem. Soc. Japan*, **35**, 1044 (1962).
4. H. Kobayashi and S. Akiyoshi, *ibid*, **36**, 823 (1963).
5. W. G. Dauben, W. E. Thiessen, and P. R. Resnick, *J. Org. Chem.*, **30**, 1693 (1965).
6. R. A. Gross, Ph.D. Dissertation, The University of Oklahoma, Norman, Oklahoma, 1974, and all references cited therein.
7. W. G. Dauben, G. H. Beasley, M. D. Broadhurst, B. Muller, D. J. Peppard, P. Pesnelle, C. Suter, *J. Amer. Chem. Soc.*, **96**, 4724 (1974) and all references cited therein.
8. J. Bernstein, U. Shmeuli, E. Zadock, Y. Kashman, and I. Neeman, *Tetrahedron*, **30**, 2817 (1974).
9. Y. Kashman, E. Zadock and I. Neeman, *ibid*, **30**, 3615 (1974).
10. B. Tursch, J. C. Braekman, D. Dalozze, M. Herin, and R. Karlsson, *Tetrahedron Lett.*, 3769 (1974).
11. V. D. Patil, U. R. Nayak, and S. Dev, *Tetrahedron*, **28**, 2341 (1972).
12. V. D. Patil, U. R. Nayak, and S. Dev, *ibid*, **29**, 341 (1973).
13. E. N. Shmidt, N. K. Kashtanova, and V. A. Pentegova, *Khim. Prir. Soedin*, 694 (1970).
14. E. N. Shmidt and V. A. Pentagova, *ibid*, 769 (1970).
15. A. J. Birch, W. V. Brown, J. E. T. Corrie, and B. P. Moore, *J. Chem. Soc. (Perkin Trans)*, **1**, 2643 (1972).
16. L. M. Jackman, "Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, New York, N. Y., 1959, p 55-56.

17. S. A. Narang and P. C. Dutta, *J. Chem. Soc.*, 1119 (1964).
18. L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. 1, John Wiley & Sons, New York, N.Y., 1967, p 811.
19. R. U. Lemieux and E. Von Rudloff, *Can. J. Chem.*, 33, 1701 (1955).
20. R. U. Lemieux and E. Von Rudloff, *ibid*, 33, 1710 (1955).
21. E. Von Rudloff, *ibid*, 33, 1714 (1955).
22. R. Y. Naves, *Bull. Soc. Chim. France*, 1372 (1958).
23. M. Palmade, P. Pesnell, J. Streith and G. Ourisson, *ibid*, 1950 (1963),  $[\alpha]_D - 48^\circ$  ( $c$  0.6,  $\text{CHCl}_3$ ).
24. Sadtler Standard NMR Spectra No. 2709.