THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

I. CUEUNICIN AND CUEUNICIN ACETATE-- TWO NEW MARINE CEMBRANOLIDES

II. STRUCTURE AND PMR SPECTRA OF NATURAL PRODUCTS. THE ASSOCIATION OF HIGH FIELD VINYL METHYL SIGNALS WITH TRANS DOUBLE BOND GEOMETRY IN GERMACRENE DERIVATIVES

III. NEW MARINE DITERPENOIDS

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RAY AUTRY GROSS, JR.

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III. NEW MARINE DITERPENOIDS

APPROVED BY

A. J. Weinheimer

Aaron P. Hayes

Leon S. Ciereszko

J. R. Gruender

F. J. Schmitz

DISSERTATION COMMITTEE

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TABLE OF CONTENTS

LIST OF TABLES

Chapter 1

LIST OF ILLUSTRATIONS

Chapter I

..."The history of organic chemistry provides in abundance instances of the major role played by the study of natural products in revealing, extending, and shaping the fundamental bases of the science. Time and again the penetration of a new sector of the vast, often surprising and always beautiful panorama of natural products has led to new insights which could hardly have been achieved by more self-conscious fundamental investigations. This role of natural product studies is in no way diminished in our day, and it will certainly continue in the future.".....

R. B. Woodward, Pure and Applied Chemistry, 17, 519 (1968).

I. CUEUNICIN AND CUEUNICIN ACETATE -- TWO NEW MARINE CEMBRANOLIDES

INTRODUCTION

Several naturally occurring diterpene lactones possessing the 20-carbon cembrane skeleton (1) have been reported (Table I). One of them, ovatodiolide (2), was isolated from a Southeast Asian herb.[1,2] The others, eunicin (3), jeunicin (4), crassin acetate (5), and eupalmerin acetate (6) were isolated from Caribbean gorgonians.[3-15] The structures for 1-6 are shown in Chart I. The stereochemical features of 2 have not been elucidated, while 3-6 possess the absolute configurations shown. The structural similarities of the five cembranolides are obvious; each cyclotetradecane ring possesses a normal isoprenoid substitution pattern.

The biosynthetic formation of these compounds has not been established, nevertheless, some preliminary speculations can be made. The hydrocarbon 7 (Chart II) can be drawn to represent the skeleton of crassin acetate. The double bond at C-12, C-13 follows from C-12, C-13 oxygenation in crassin acetate. As seen in Chart II, 7 is enantiomeric with (-)-Cembrene-A (8) a known natural product isolated from a tree in India.[16] (See Section III for a discussion of other cembrane derivatives.) Sesquiterpene hydrocarbons isolated from

Table I

Naturally Occurring Cembranolides[a]

Compound	Formula	Mol wt	Mp[b]	α_D[b]
Ovatodiolide (2)	$C_{20}H_{24}O_4$	328	150°	+228°
Eunicin (3)	$C_{20}H_{30}O_4$	334	155°	- 89°
Jeunicin (4)	$C_{20}H_{30}O_4$	334	141°	+ 13°
Crassin Acetate (5)	$C_{22}H_{32}O_5$	376	140°	+ 70°
Eupalmerin Acetate (6)	$C_{22}H_{32}O_5$	376	159°	+ 8°

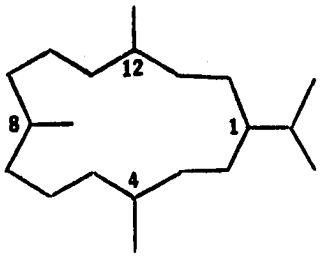
[a]See reference 13 for a discussion of the sources of the marine cembranolides. [b]Values rounded to nearest whole number.

Caribbean gorgonians have also been shown[17-19] to possess an enantiomeric relationship with the common forms of the corresponding hydrocarbons found in terrestrial plants. It should be noted, however, that a cembrane derivative isolated from a Pacific soft coral has recently been reported[20] to have the structure 9, which clearly has a skeleton identical to 8 (Chart II). Thus, an ubiquitous land-sea enantiomeric relationship does not exist.

It seems reasonable to assume that the biosynthesis of the gorgonian cembranolides involves a single cyclization of the normal diterpenoid precursor, geranylgeranyl pyrophosphate (10),[16,21-23] which can be envisaged to produce 7, 8 or 13 via deprotonation of the cations 11 or 12 as shown in Chart III.

(+) Cembrene-A (7), which is the skeleton of crassin acetate, emerges as the likely precursor for 3-6. Cembrene-B (13) also represents a possible intermediate; 13 is analogous to germacrene-B (14),

Chart I



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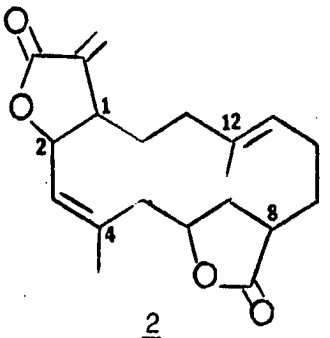

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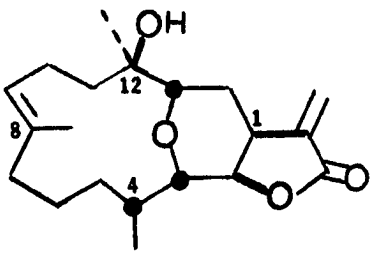

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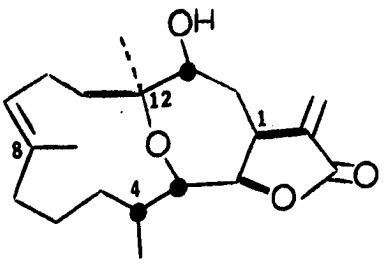

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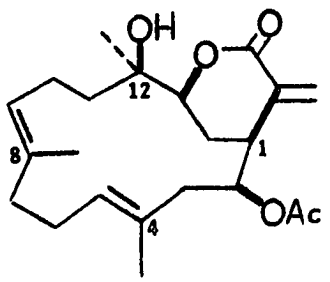

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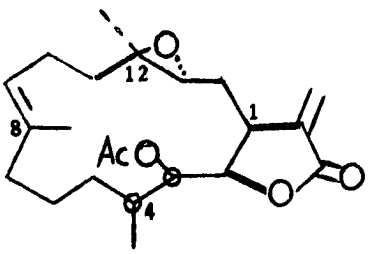
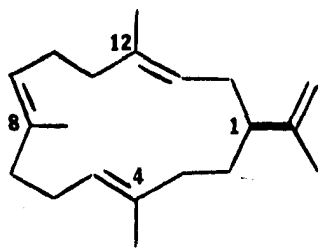


6

Cembrane (1) and Naturally Occurring Cembranolides: ovatodiolide (2), eunicin (3), jeunicin (4), crassin acetate (5), and eupalmerin acetate (6).

Chart II



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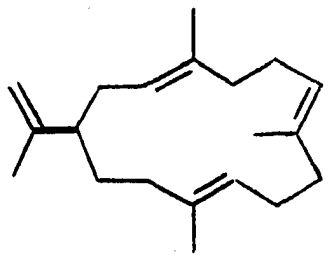

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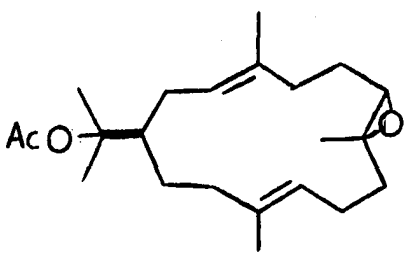

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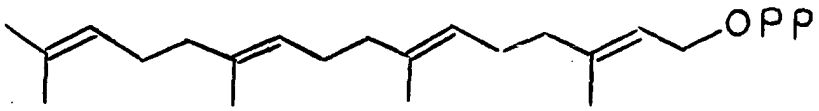
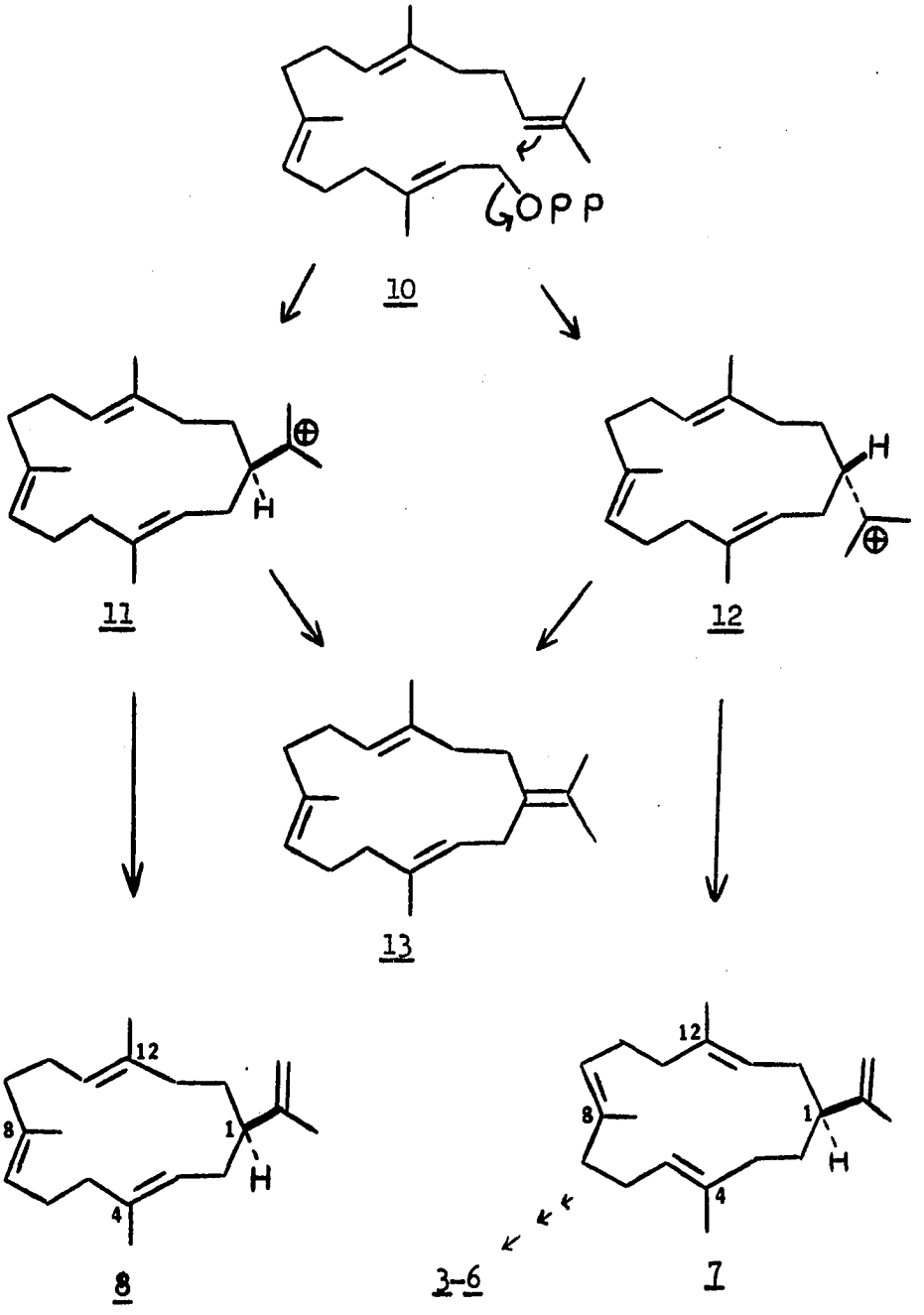


10

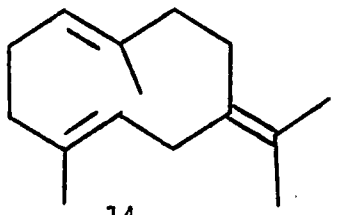
(+)-Cembrene-A (7), (-)-cembrene-A (8), Epoxynepthenol acetate (9), and geranylgeranyl pyrophosphate (10).

Chart III



Cyclization of geranylgeranyl pyrophosphate (10) to (+)-cembrene-A (7), (-)-oembrene-A (8) and cembrene-B (13).

long postulated as an intermediate in sesquiterpene biosynthesis.[24-25] Interestingly, neither 7 nor 13 has yet been found in nature.



14

If (-)-cembrene-A (8) is envisaged as a precursor for 3-6, then an additional step must be incorporated into the biosynthetic scheme in order to arrive at the crassin acetate structure (5), i.e., either a subsequent inversion of configuration at C-1 or an isomerization of the double bond at C-7--C-8 to C-8--C-9. At this time there is no compelling reason to incorporate an additional step in the sequence.

By the same reasoning either 8 or 13 is a logical precursor for 9. Thus, 5 and 9 might have arisen via a common intermediate 13, or independently via the enantiomeric pair 7 or 8.

It is also noteworthy that the orientation of the double bonds in 7 and 8 governs the stereochemistry of the C-1 side chain, owing to the symmetry of the cembrane skeleton about the C-1, C-8 axis; i.e., 7 and 8 may not be superimposed because their double bonds would be misaligned. Therefore, until a convention is established (e.g., one similar to that proposed[26] for germacranolides), it is quite ambiguous to refer to an alpha or beta orientation of the C-1 side chain in general terms; i.e., without reference to the endocyclic double bonds; such descriptions must be confined to individual mole-

cules. In other words, the three carbon side chains in either 7 or 8 may now be drawn to possess beta stereochemistry, although 7 and 8 are enantiomers. This also applies equally to enantiomeric pairs (e.g., cembranolides) derived from 7 and 8.

In the present work, two new cembranolides have been isolated from the gorgonian Eunicea mammosa Lamouroux[27-29] collected at Curacao, Netherlands, Antilles. The isolation and structure elucidation of these new cembranolides is reported in the following section.

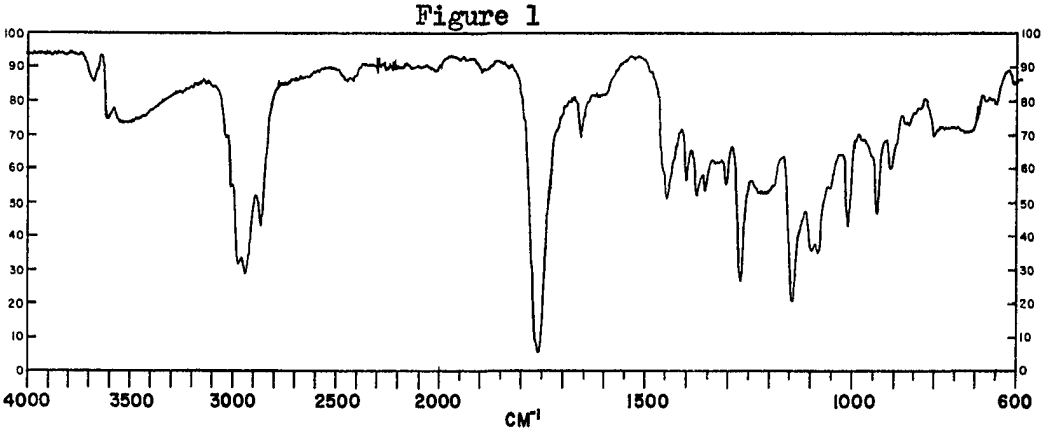
RESULTS AND DISCUSSION

A. Structure of Cueunicin and Cueunicin Acetate

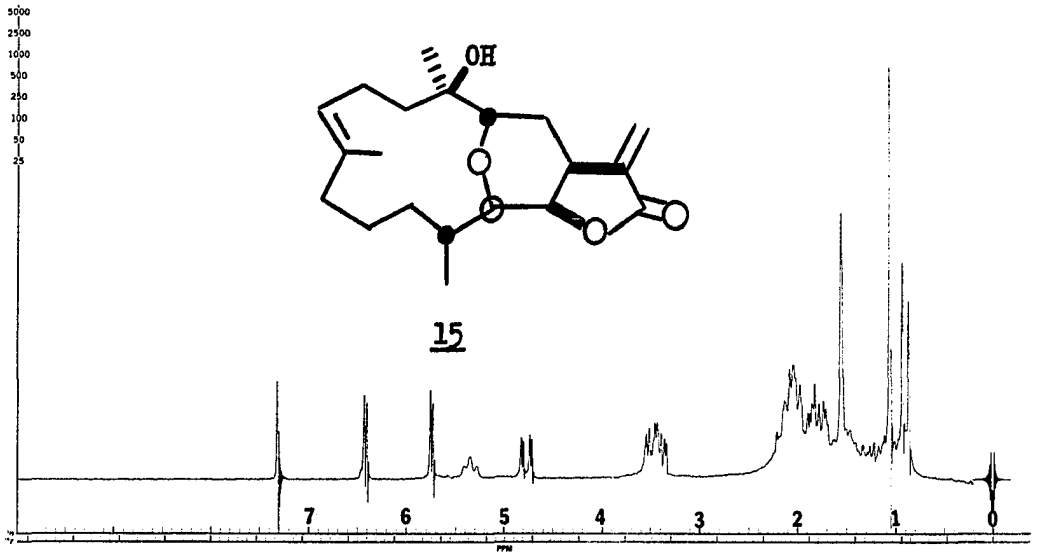
Two new diterpenoids, cueunicin (15) and cueunicin acetate (16)[15,30] were isolated from the hexane extract of the gorgonian Eunicea mammosa Lamouroux. Cueunicin was readily converted to cueunicin acetate, when treated with acetic anhydride and a trace of boron trifluoride etherate at -20°. Cueunicin, $C_{20}H_{30}O_4$ (high-resolution mass spectrum), was isolated as a white gum which could not be induced to crystallize. The ir, pmr and mass spectral data for cueunicin is displayed in Figure 1. Cueunicin contained a conjugated γ-lactone[31] (ir bands at 1762 and 1660 cm^{-1} and very strong uv end absorption), and also possessed a hydroxyl function (ir 3605 and 3500 cm^{-1}).

The pmr spectrum of 15 showed three methyl signals: a broad singlet at δ1.56, a sharp singlet at δ1.09 and a doublet at δ0.92 corresponding to a vinyl methyl, a methyl attached to a carbon bearing oxygen and a secondary methyl group, respectively.[31-32] The pmr spectrum exhibited the characteristic doublets[31] of H_a and H_b in partial structure A at δ6.44 and δ5.74. Pmr signals were also recorded at δ5.36, a one proton triplet characteristic of an isolated olefinic proton; at δ4.78, a double doublet ascribable to the absorption of a proton (H_d, structure A) attached to a carbon bearing the lactone ether oxygen; and at δ3.3-3.5, overlapping signals due to three protons, which

Figure 1



IR Spectrum of Cueunicin (15) in $CHCl_3$



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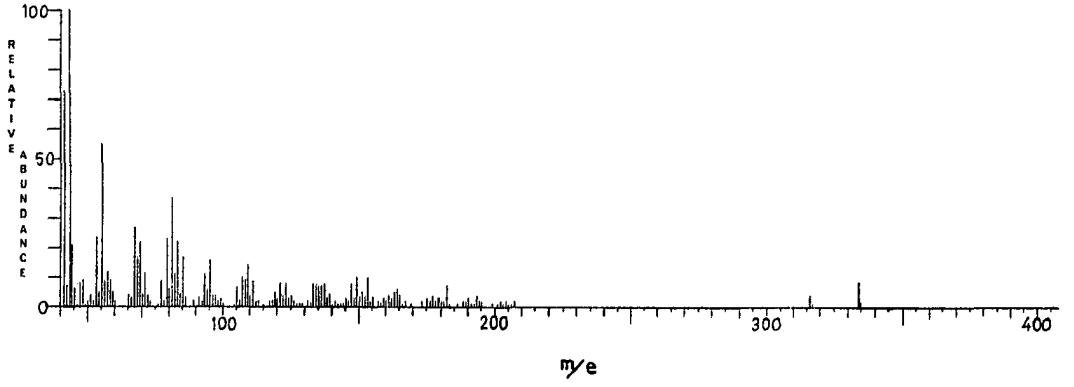
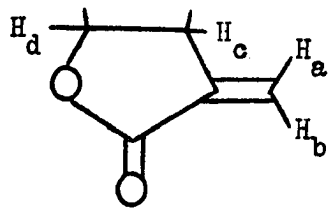
100 MHz PMR Spectrum ($CDCl_3$) of 15



Mass Spectrum of 15



A

partially obscured the H_c signal. Therefore, cueunicin acetate proved to be more amenable to spin decoupling experiments, as its pmr spectrum (Figure 2) was nicely resolved in the 3-4 ppm region.

Cueunicin acetate (Spectral data in Figure 2) was a white crystalline solid, $C_{22}H_{32}O_5$ (high-resolution mass spectrum) mp 141-142°, and it contained a conjugated γ-lactone (ir bands at 1759 and 1660 cm^{-1}; uv 215 nm, ε=6,000)[34-38] and an acetate moiety (ir 1728 and 1255 cm^{-1}; pmr δ1.95, 3 proton singlet). Table II shows the proton assignments as determined from the pmr spectrum of cueunicin acetate.

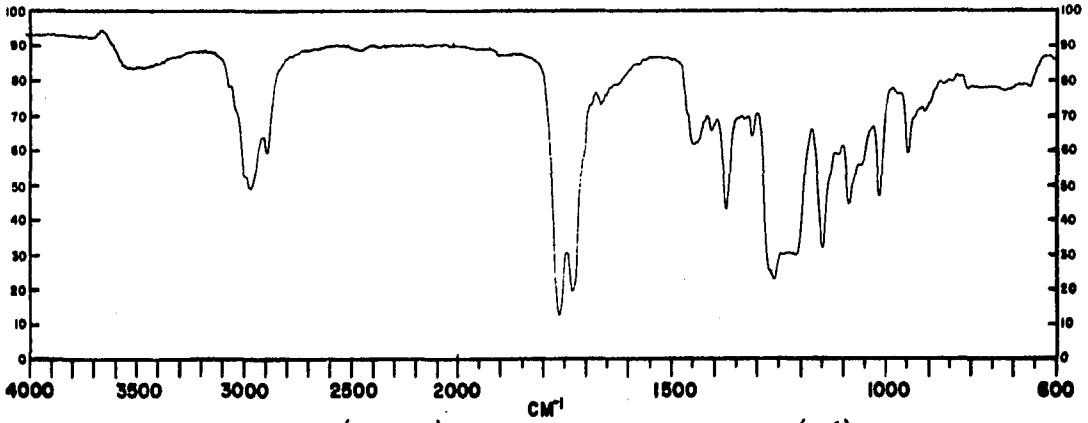
Four of the five oxygen atoms in 16 were accounted for by the presence of the γ-lactone and acetate functions. The remaining oxygen atom was attributed to a disecondary ether shown in partial structure B, which accounted for the two midrange proton absorptions at δ3.34 and

$$H_e\text{-C-O-C-}H_f$$

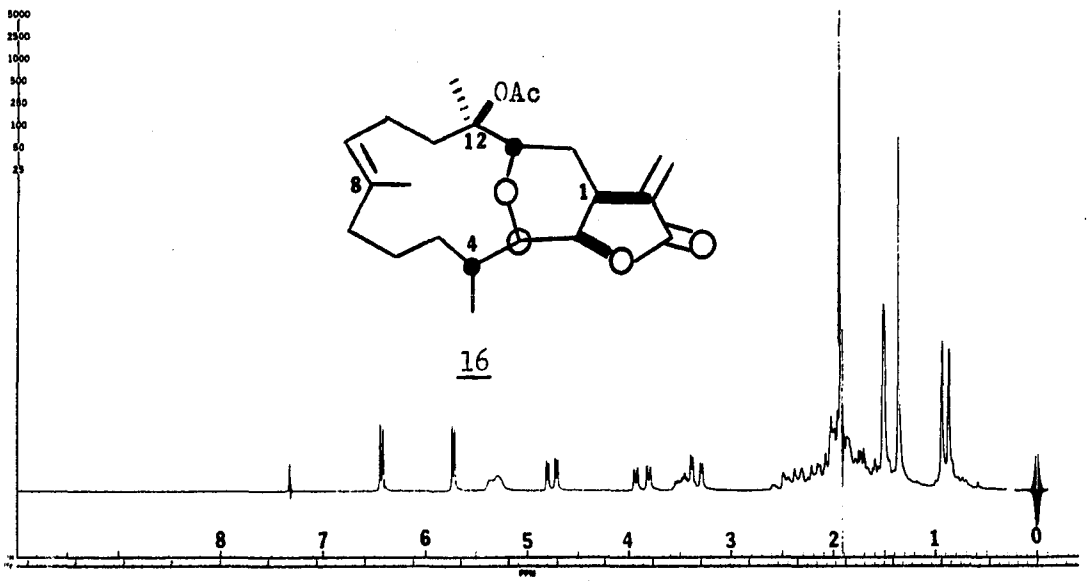
B

δ3.87 in 16 assigned to H_e and H_f, respectively. The possibility of an oxirane was eliminated when 16 failed to give a positive test when

Figure 2

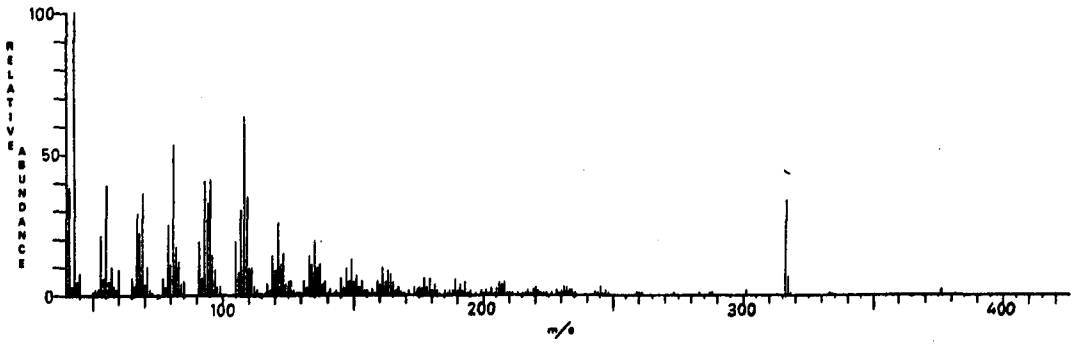


IR Spectrum ($CHCl_3$) of Cueunicin Acetate (16)



16

100 MHz PMR Spectrum ($CDCl_3$) of 16

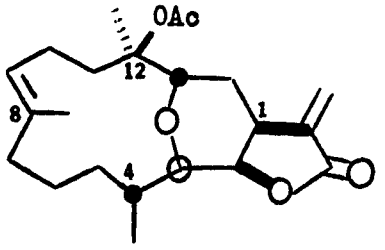


Mass Spectrum of 16

Table II

Proton Assignments from the 100 MHz PMR Spectrum of Cueunicin Acetate (16)

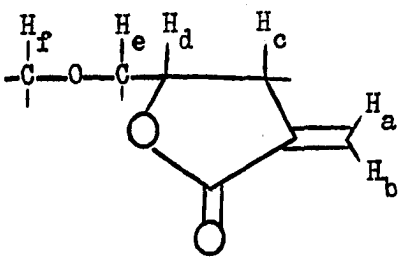
Signal (δ)	Multiplicity	Protons	J (Hz)	Assignment
6.43	d	1	2.4	Conjugated exomethylene proton (H_a)
5.72	d	1	2.4	Conjugated exomethylene proton (H_b)
5.30	br	1		Olefinic proton (H_i)
4.76	dd	1	2,8	Proton on carbon bearing a lactone ether oxygen (H_d)
3.87	dd	1	3.5,12	Ether proton (-CHOR) (H_f)
3.46	m	1		Proton beta to the lactone carbonyl (H_c)
3.34	dd	1	2,9	Ether proton (-CHOR) (H_e)
1.95	s	3		Acetate Methyl
1.52	s	3		Vinyl methyl (H_h)
1.38	s	3		Methyl on carbon bearing oxygen (H_g)
0.91	d	3	7	Secondary methyl (H_j)



16

treated with acidic silver periodate solution, or indeed when the ether function failed to react on treatment with any acidic or basic reagent (vide infra).

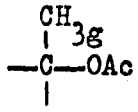
Irradiation at the frequencies of H_a and H_b in 16 established the chemical shift of H_c at 3.46 ppm in the usual fashion.[39] Irradiation at the frequency of H_c collapsed the doublet signals of H_a and H_b to singlets and also collapsed a double doublet at 4.76 ppm to a doublet thus establishing the latter signal as that of H_d. Structure A could be expanded to C when irradiation at the frequency of H_d not only simplified the signal due to H_c but also collapsed a double doublet at 3.34 ppm (H_e) to a doublet. The acetate function in 16



C

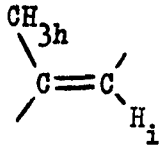
was shown to be part of partial structure D when the three proton signal (H_g) at δ1.09 in cueunicin shifted downfield to δ1.38 in cueunicin acetate.[3,6] The pmr spectrum of cueunicin displayed a singlet at δ4.41 for the hydroxyl proton in dimethyl sulfoxide (DMSO), confirming the tertiary nature of the carbinol system.[40-41] The proton-decoupled carbon magnetic resonance (cmr) spectrum of cueunicin acetate firmly established structure D by the observation that the

signal at δ85.6 due to the carbon bearing the acetate function remained a singlet in the off-resonance decoupled cmr spectrum.[42-44] Figure 3 displays the cmr spectra of 16.



D

The presence of partial structure E in 16



E

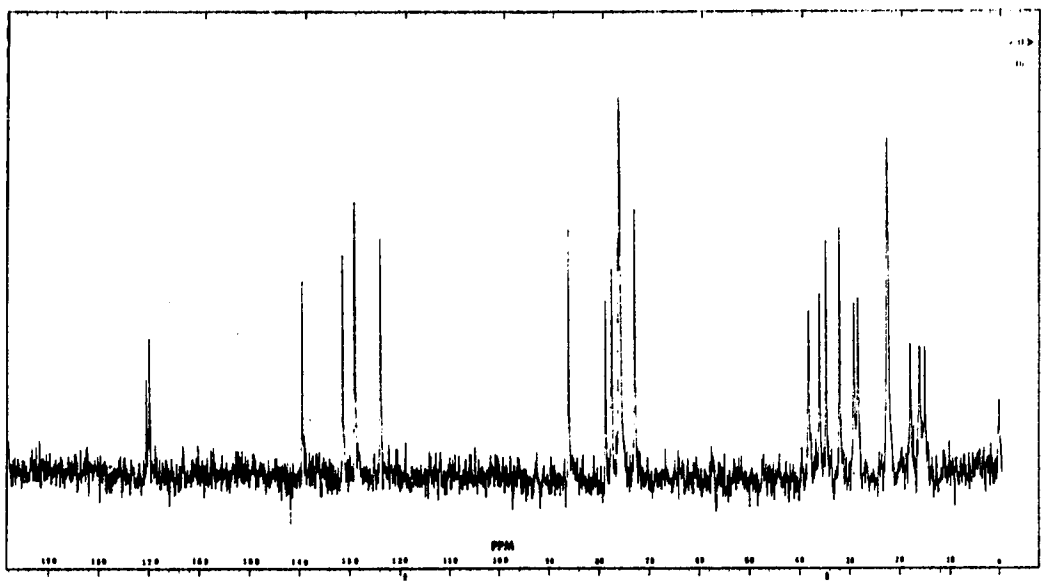
was inferred from the broadened three-proton singlet (H_h) at δ1.52 which sharpened on irradiation of the one proton signal, H_i, at δ5.30. Likewise, irradiation of the olefinic proton H_i at δ5.36 in 15 caused the vinyl methyl signal at δ1.56 to sharpen.[26]

A secondary methyl group, partial structure F,

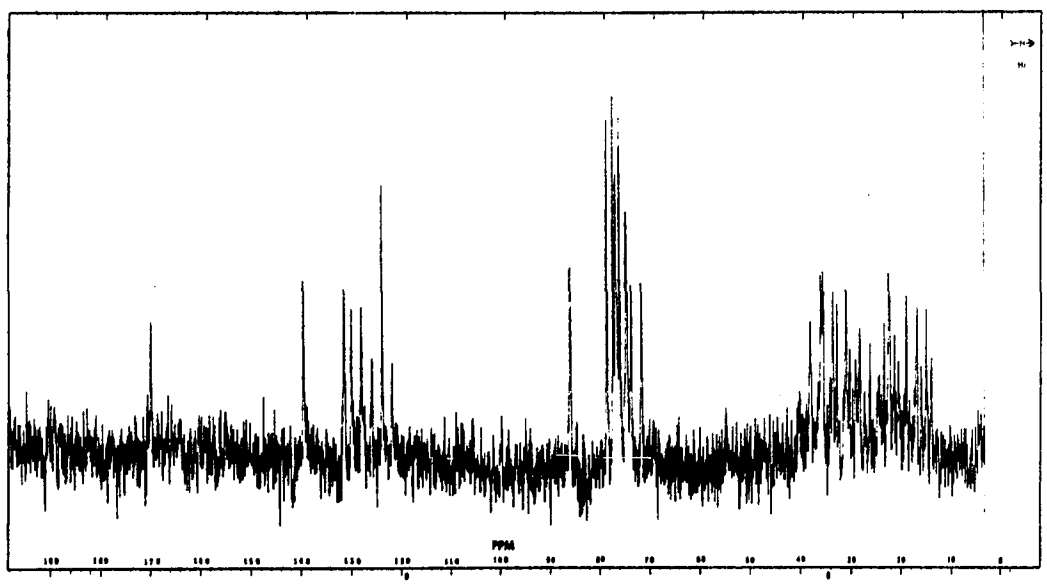
F

gave rise to a three-proton doublet (H_j) at 0.92 ppm in 15 and 0.91 ppm

Figure 3



25.2 MHz Proton-Decoupled CMR Spectrum ($CDCl_3$)
of Cueunicin Acetate (16)



25.2 MHz Off-Resonance Decoupled CMR Spectrum ($CDCl_3$)
of Cueunicin Acetate (16)

in 16.

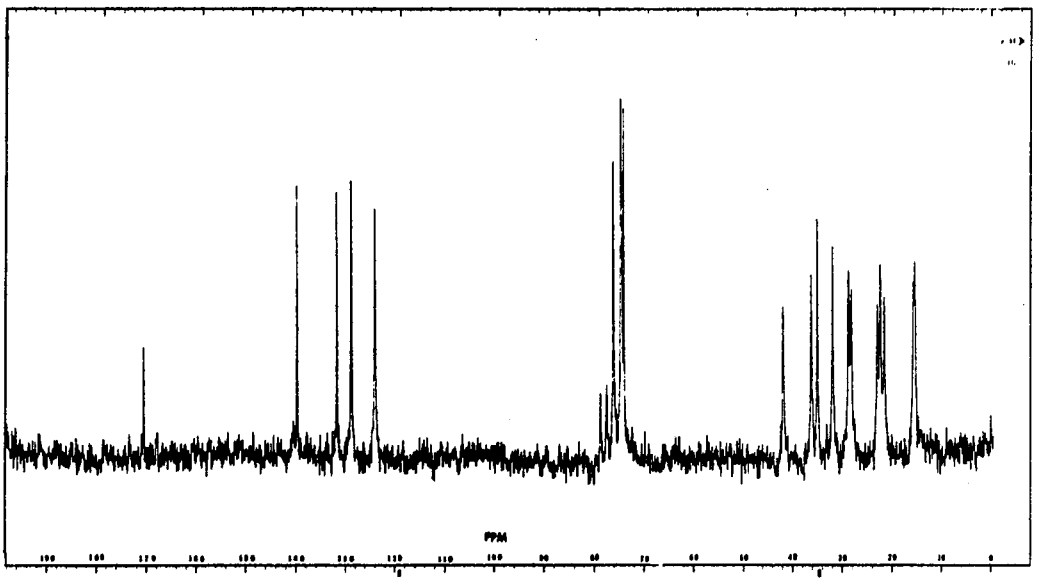
The proton-decoupled cmr spectrum of 15 (Figure 4) confirmed the presence of twenty carbon atoms, including one carbonyl carbon (δ170.1), four olefinic carbons (δ139.4, 131.3, 128.3 and 123.6) and four carbons bound to oxygen by single bonds (δ75.8, 74.3, 74.3 and 73.7). The signal at δ170.1 remained a singlet in the off-resonance decoupled spectrum (Figure 4), while the signals noted above due to olefinic carbons were respectively a singlet, singlet, doublet and triplet. The triplet and one of the singlet signals could be attributed to the olefinic carbons in structure A, while the doublet and remaining singlet were ascribable to the olefinic carbons in structure E.

As expected two additional signals due to the carbonyl and methyl carbons of the acetate group were observed in the proton decoupled cmr spectrum of cueunicin acetate (Figure 3).

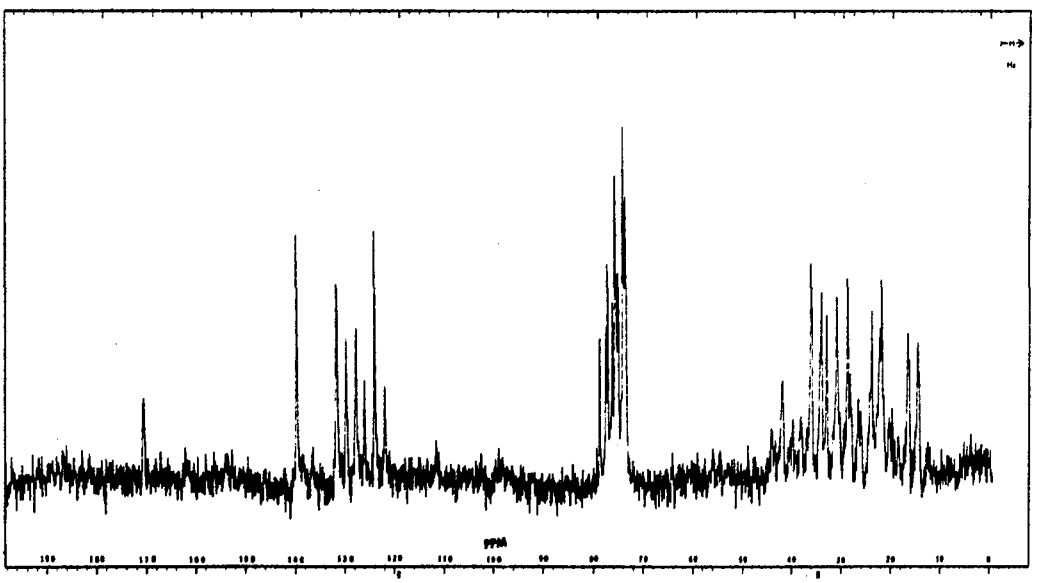
The presence of the α-methylene-γ-butyrolactone (A) and trisubstituted double bond (E) moieties in cueunicin accounted for four degrees of unsaturation (rings plus double bonds); therefore, two rings remained to be identified.

When cueunicin acetate was allowed to react with sodium in refluxing n-butanol,[45] two products were obtained by column chromatography, which proved to be the epimers, dihydrocueunicin-I (17) and dihydrocueunicin-II (18) (Spectral data in Figures 5 and 6, respectively). Not only was the conjugated double bond reduced, but the acetate function was removed as well. This was inferred spectrally by the absence of the conjugated exomethylene doublets and the appearance of a new methyl doublet in the pmr spectra of 17 and 18. Partial

Figure 4

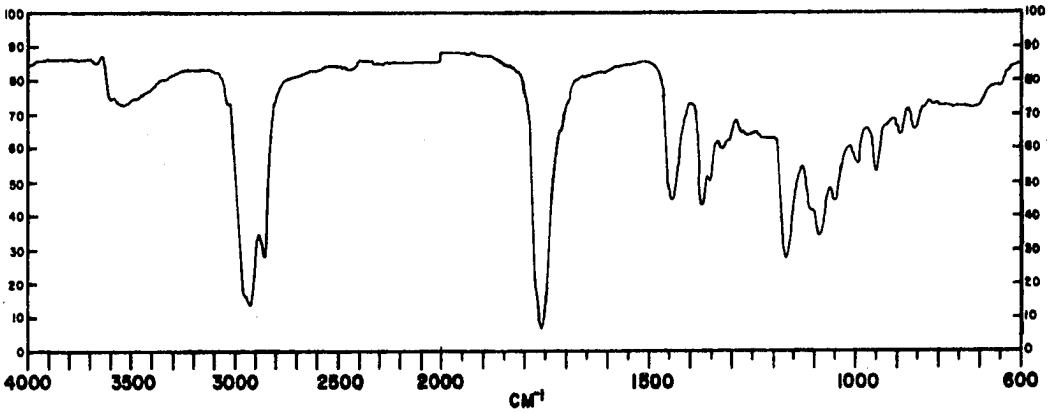


25.2 MHz Proton-Decoupled CMR Speotrum ($CDCl_3$)
of Cueunicin (15)

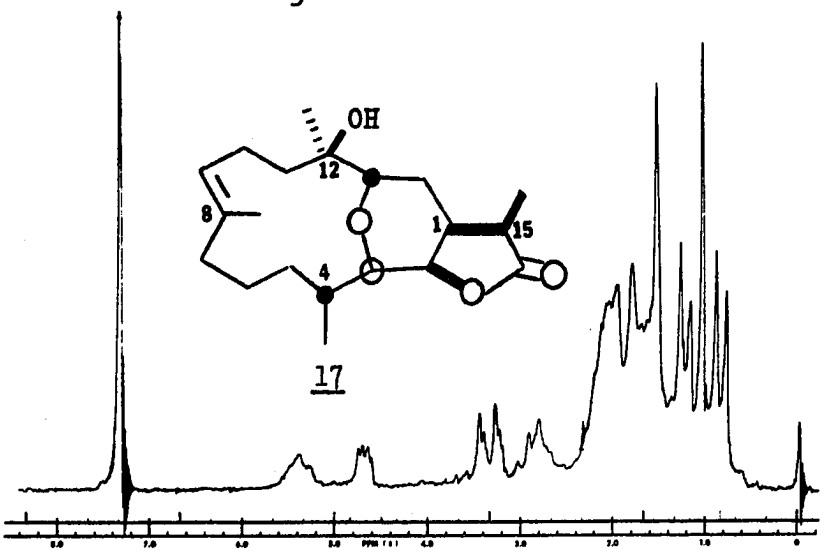


25.2 MHz Off-Resonance Decoupled CMR Spectrum ($CDCl_3$)
of Cueunicin (15)

Figure 5

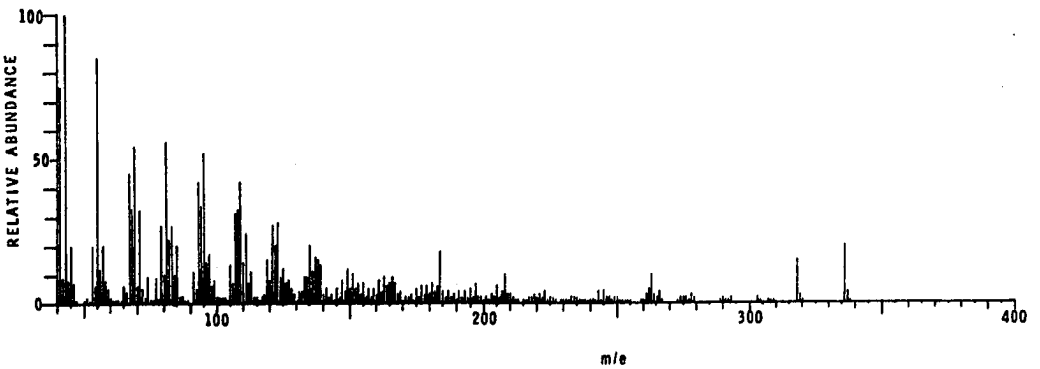


IR Spectrum ($CHCl_3$) of Dihydrocoeunicin-I (17)



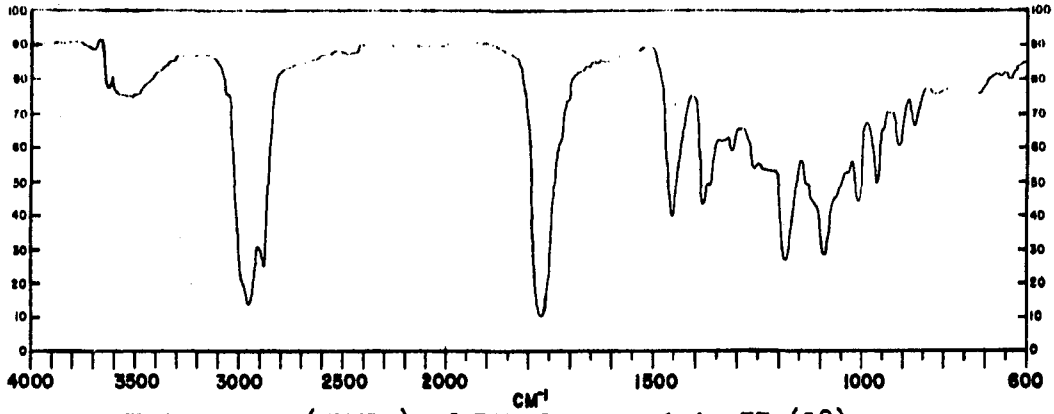
17

60 MHz PMR Spectrum ($CDCl_3$) of 17

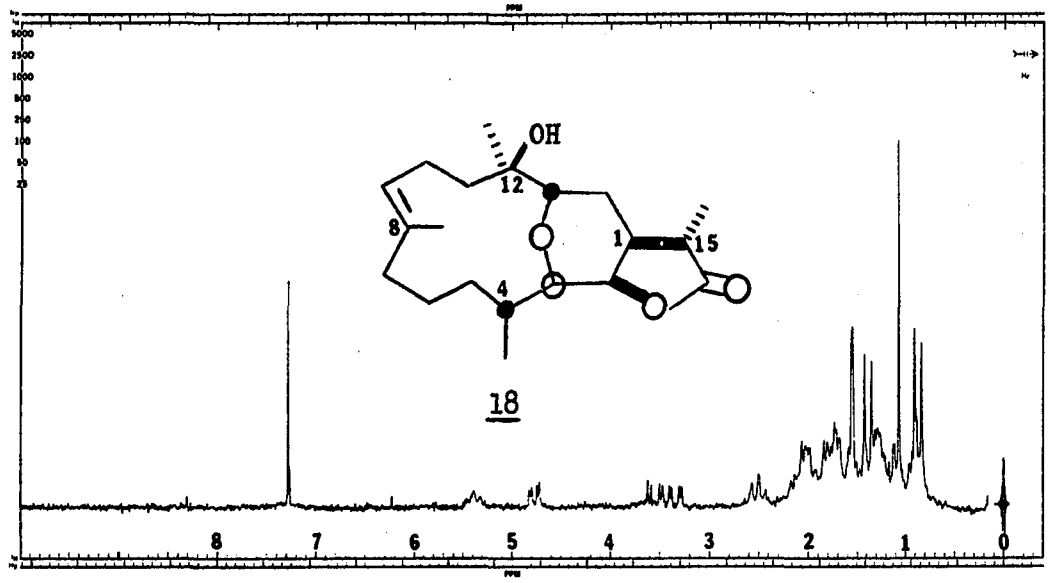


Mass Spectrum of 17

Figure 6

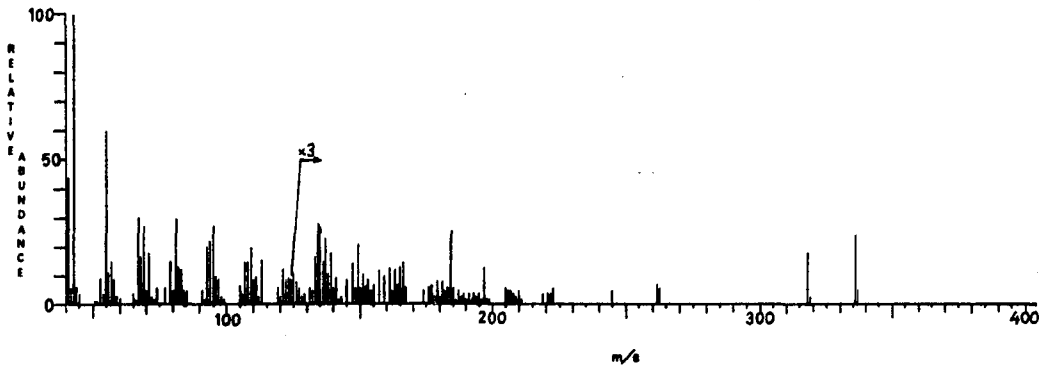


IR Spectrum ($CHCl_3$) of Dihydrocueunicin-II (18)



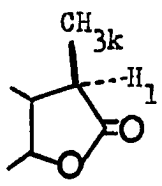
18

100 MHz PMR Spectrum ($CDCl_3$) of 18

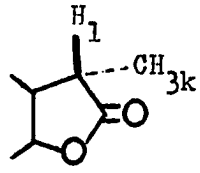


Mass Spectrum of 18

structures G and H show the relationship of the epimeric methyl groups in the dihydrocueunicin derivatives. Stereochemical assignments could not be made for the two compounds at this point; however, the two compounds could be readily differentiated on the basis of the respective chemical shifts of the new methyl doublet (H_k) and proton (H_1) signals. High field methyl (δ1.23) and low field proton (δ2.80)



G



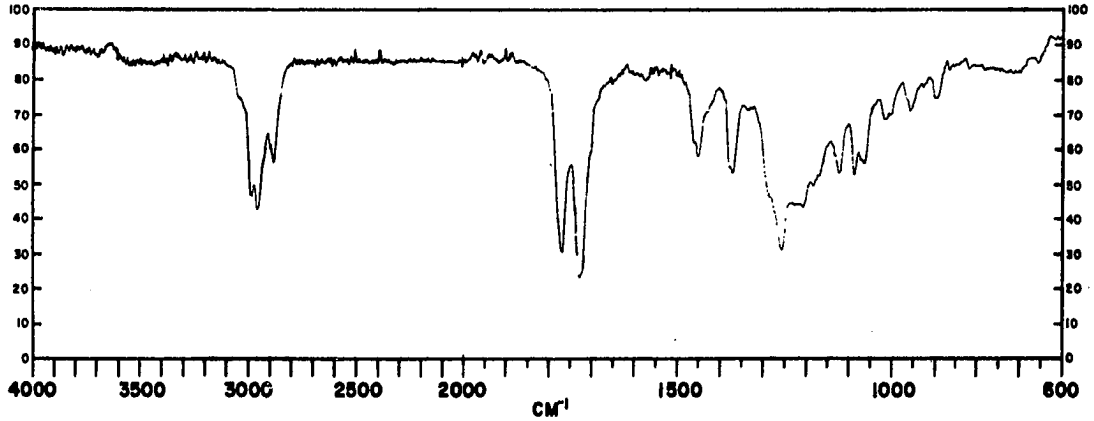
H

signals characterized 17, while low field methyl (δ1.39) and high field proton (δ2.54) signals were displayed by 18.

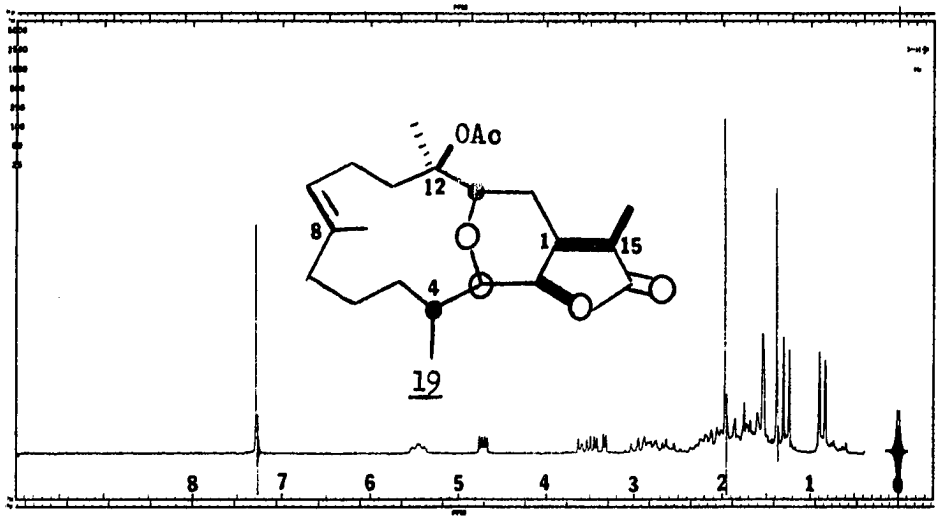
Sodium borohydride reduction[46-50] of cueunicin in ethanol produced 17 exclusively. Likewise, sodium borohydride reduction of cueunicin acetate afforded a single dihydrocueunicin acetate (19) in high yield. Spectral data for 19 is shown in Figure 7. Again, the downfield doublets of H_a and H_b were replaced by a new methyl doublet (H_k). Although numerous authors have used methanol (possibly because of solubility requirements) as solvent for the sodium borohydride reduction of partial structure A, three noteworthy references[51-53] have advised against the use of methanol and recommended ethanol. The latter solvent is said to react much less rapidly with the reagent to evolve hydrogen.

Catalytic hydrogenation[54] of 16 smoothly produced tetrahydro-

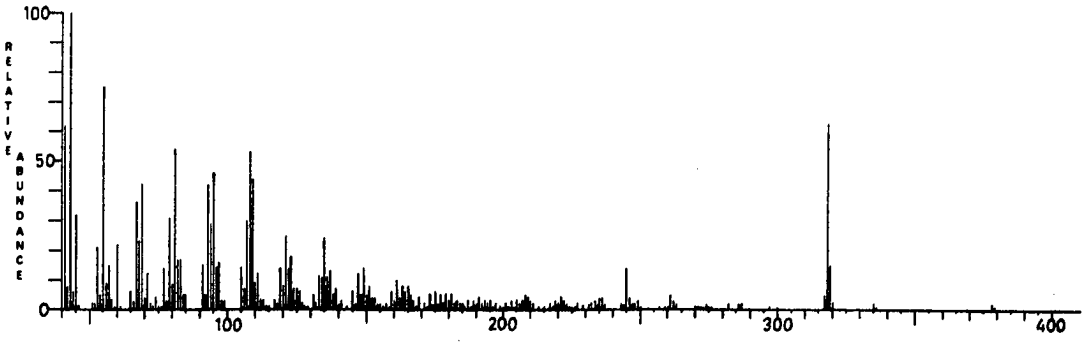
Figure 7



IR Spectrum ($CHCl_3$) of Dihydrocueunicin Acetate (19)



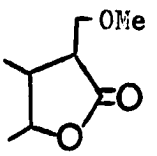
100 MHz PMR Spectrum ($CDCl_3$) of 19



Mass Spectrum of 19

cueunicin acetate, 20. The spectral data for 20 (Figure 8) indicated a single compound. The absorption due to the three olefinic protons in 16 had disappeared and two new methyl doublets appeared at 1.30 and 0.87 ppm, as expected for the reduction of partial structures A and E, respectively. The formula for 20, $C_{22}H_{36}O_5$, allowed five degrees of unsaturation. Three degrees of unsaturation were accounted for by the lactone and acetate carbonyl groups and the γ-lactone ring, again demonstrating that 2 rings exist in the residue of the compound.

Treatment of 16 with methanolic potassium hydroxide[55-58] did not deacetylate it, but rather produced a Michael adduct (21) of methanol to the conjugated γ-lactone (partial structure I). One of the two possible epimers was isolated pure (spectral data in Figure 9),

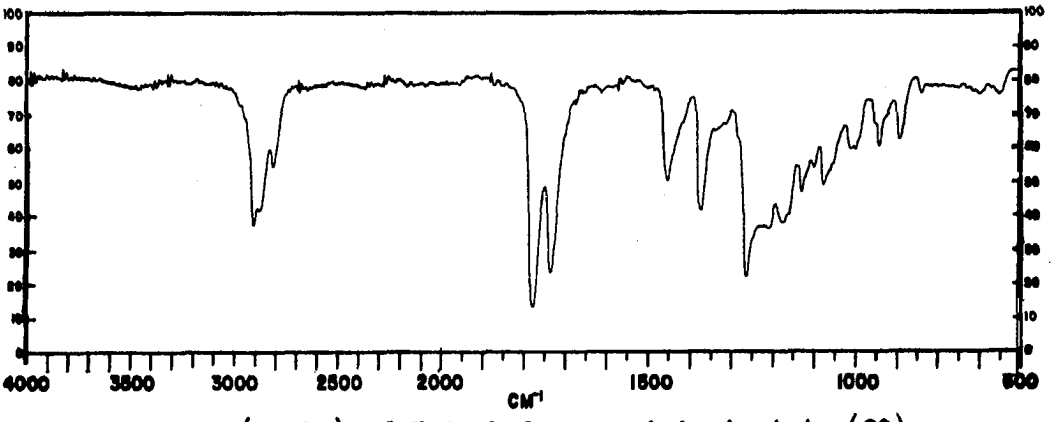


I

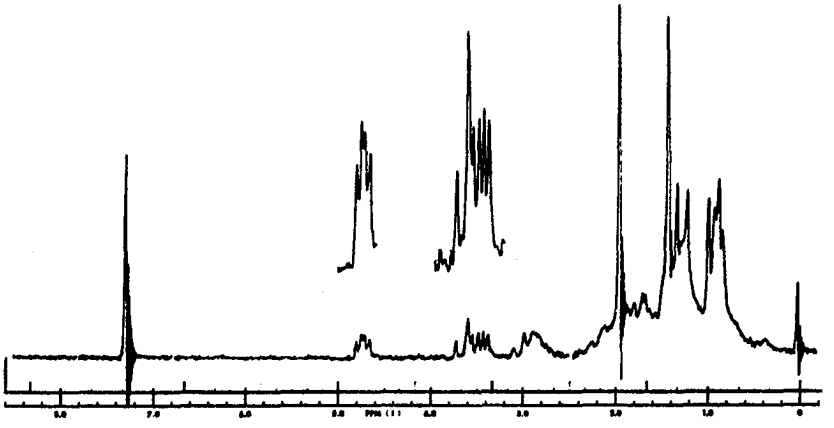
although both epimers were produced in the reaction. The tertiary nature of the acetate accounted for its resistance to deacetylation. However, deacetylation of 16 was accomplished[59] under more severe conditions (KOH/ethylene glycol) at 110° affording 15.

Both 15 and 16 formed crystalline epoxides, 22 and 23, respectively, when allowed to react with meta-chloroperbenzoic acid.[60] The spectral data for the epoxides 22 and 23 shown in Figures 10 and 11 indicated that both were pure compounds. In 22 the methyl signal

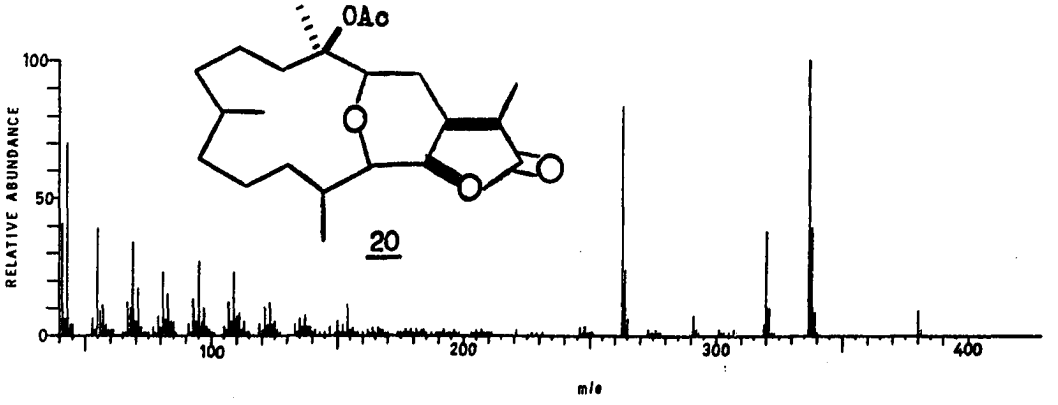
Figure 8



IR Spectrum ($CHCl_3$) of Tetrahydrocueunicin Acetate (20)

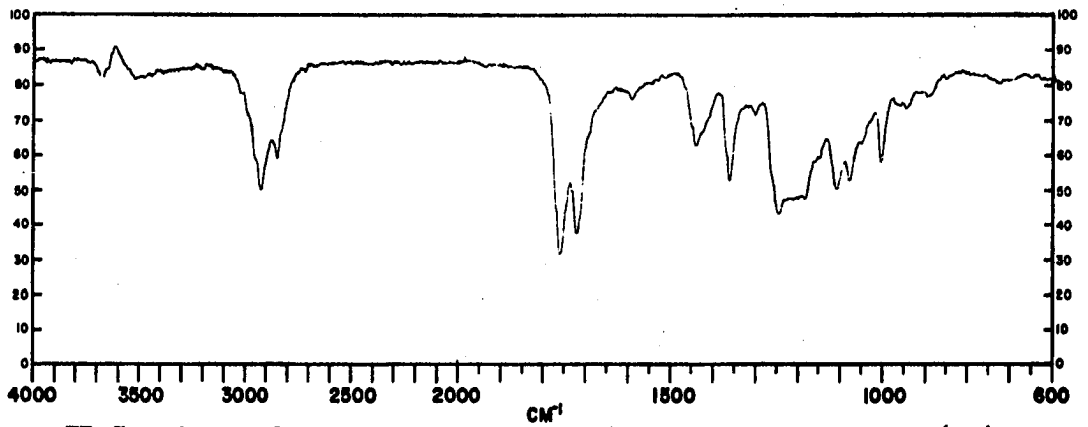


60 MHz PMR Spectrum ($CDCl_3$) of 20

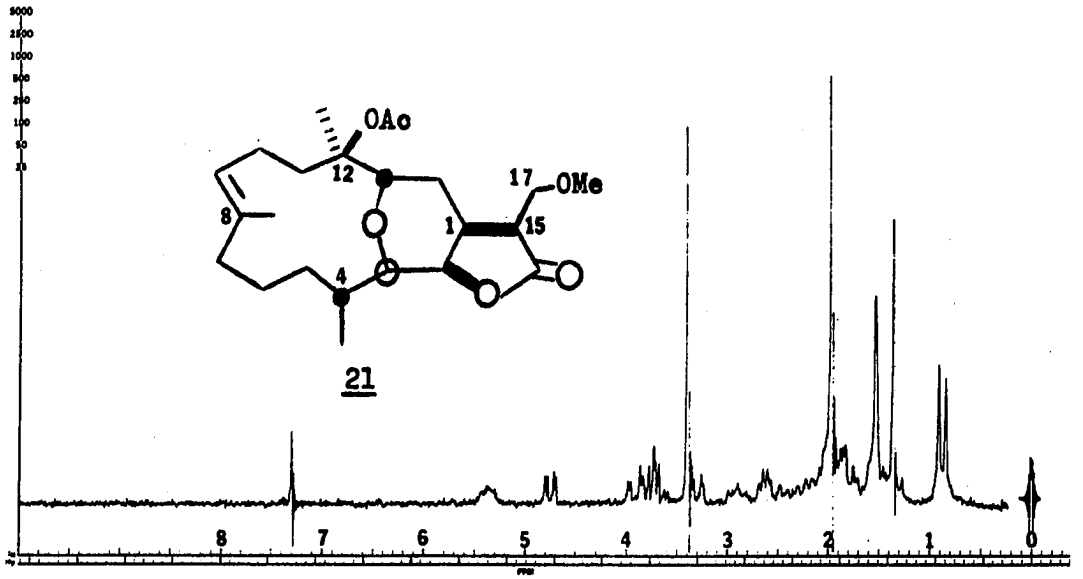


Mass Spectrum of 20

Figure 9

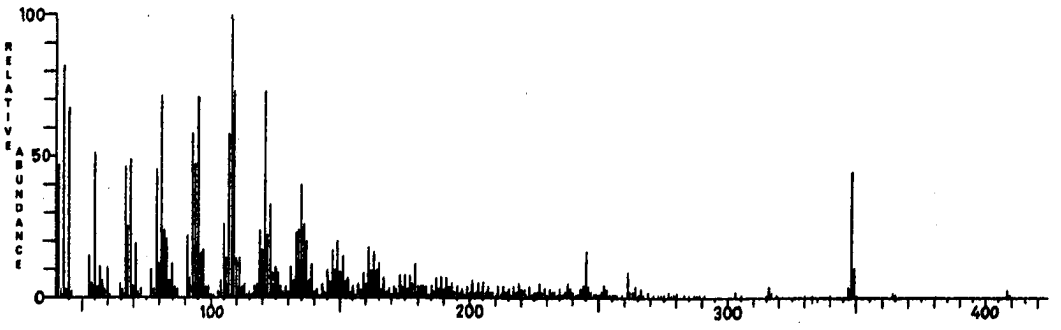


IR Spectrum of the Methanol Adduct of Cueunicin Acetate (21)



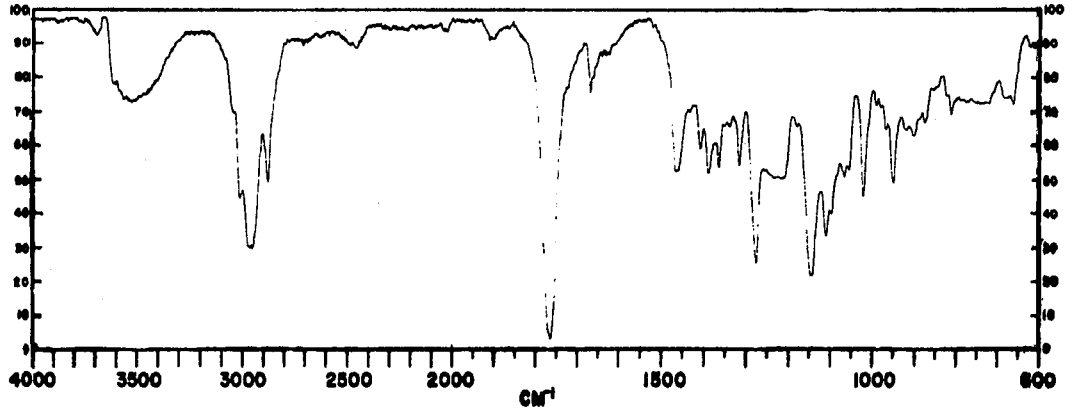
21

100 MHz PMR Spectrum ($CDCl_3$) of 21

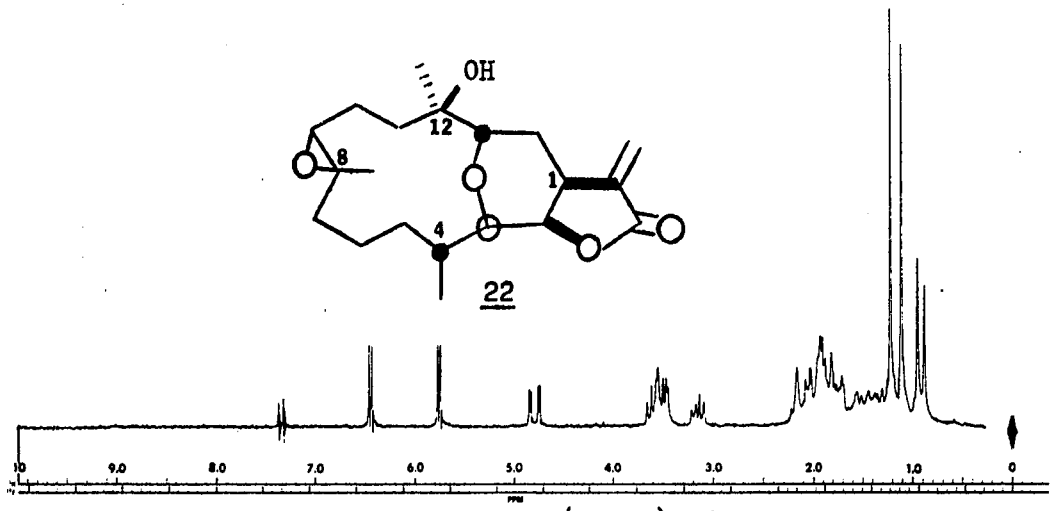


Mass Spectrum of 21

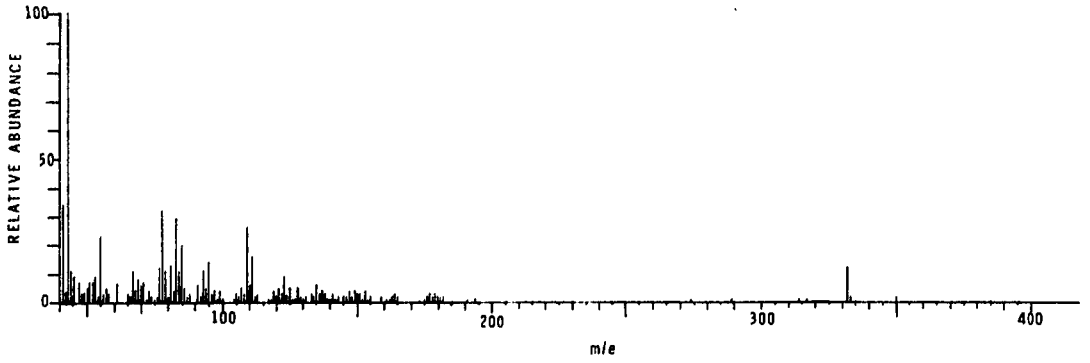
Figure 10



IR Spectrum of Epoxycueunicin (22)

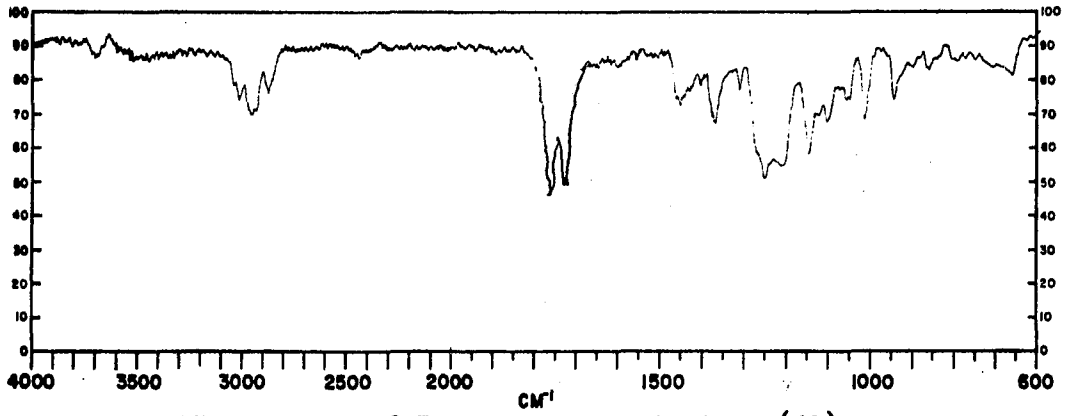


100 MHz PMR Spectrum ($CDCl_3$) of 22

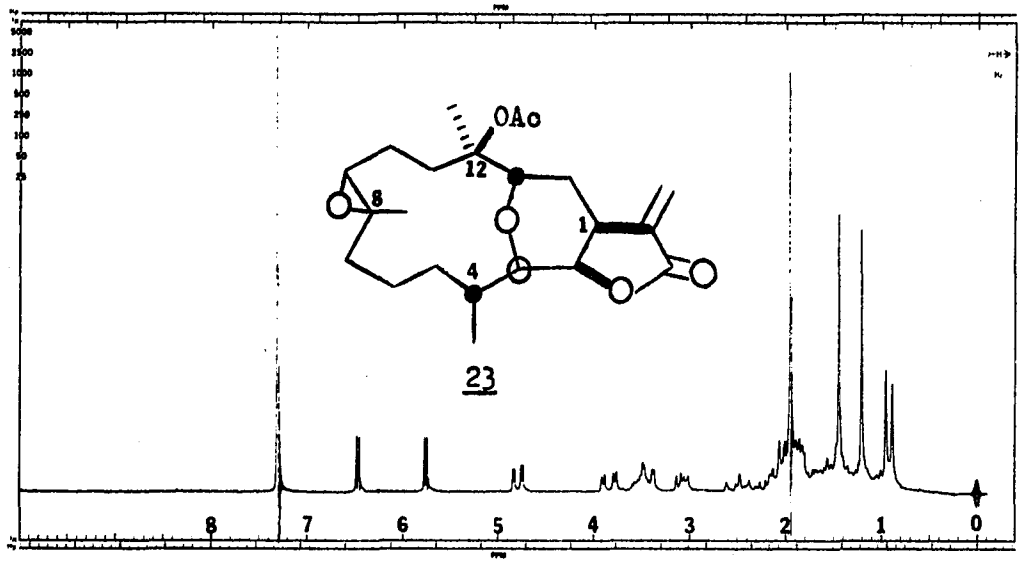


Mass Spectrum of 22

Figure 11

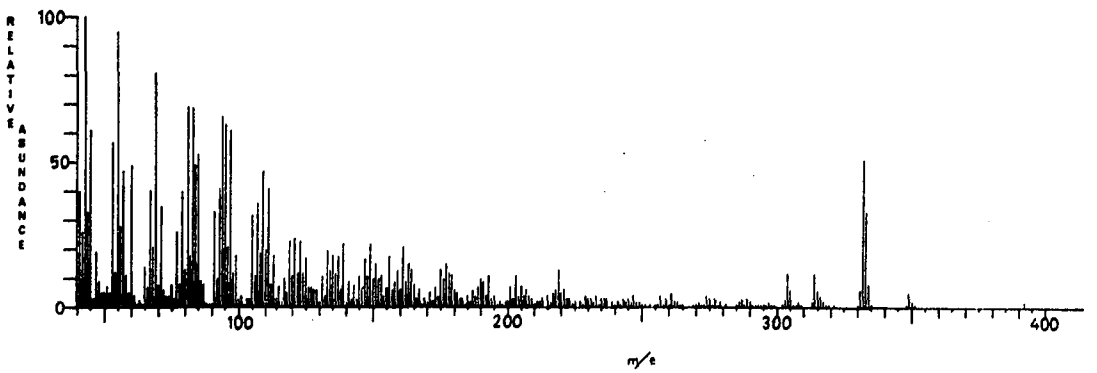


IR Spectrum of Epoxycueunicin Acetate (23)



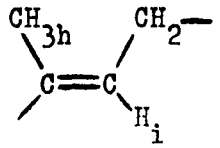
23

100 MHz PMR Spectrum ($CDCl_3$) of 23



Mass Spectrum of 23

due to H_h had sharpened and moved upfield[61] (δ1.56 to δ1.23), while the one proton signal (H_i) now appeared as a double doublet (δ3.17). Likewise, 23 displayed a sharp methyl singlet at δ1.20 as opposed to the broad singlet at δ1.52 in 16. The multiplicity of H_i in 23 was also a double doublet (δ3.03). These results demonstrated the presence of a methylene group adjacent to H_i and allowed E to be expanded to J. The multiplicity of H_i in 15 was a broadened triplet which rein-



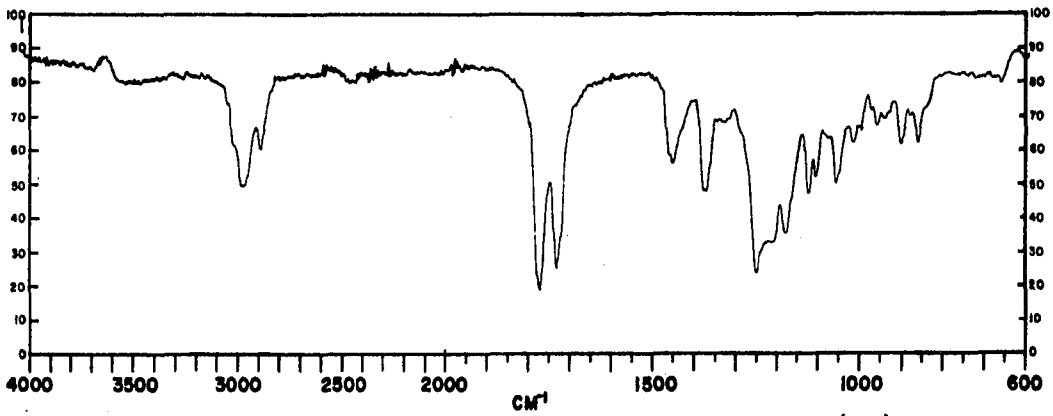
J

forced the assignment of the methylene group in J.

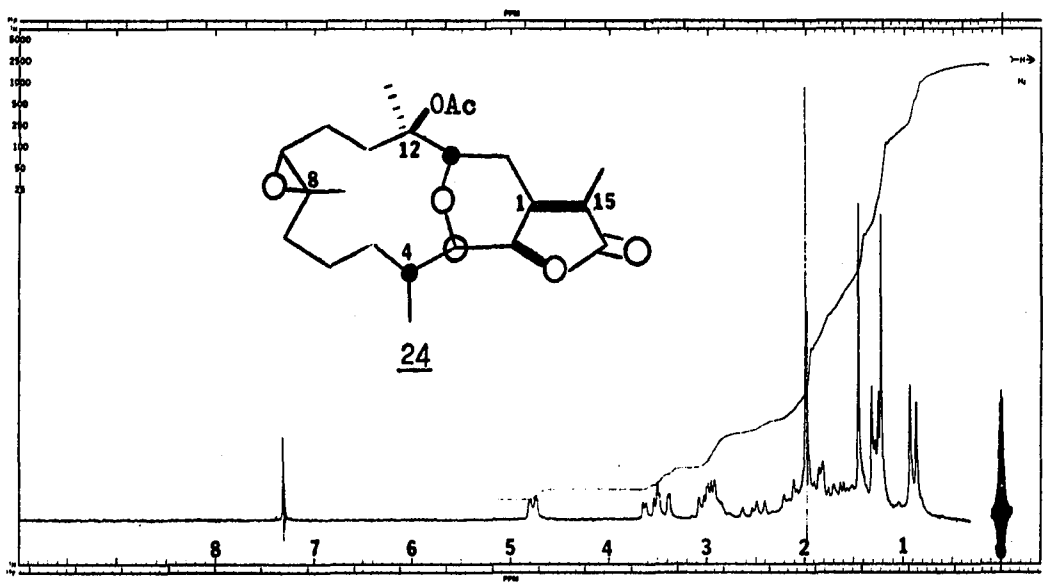
Catalytic hydrogenation[54] of 23 smoothly afforded a single dihydro derivative (24) of epoxycueunicin acetate. The spectral data for 24 (Figure 12) substantiated this assignment, in that a normal γ-lactone absorption was observed in the infrared spectrum at 1770 cm^{-1} and a new secondary methyl doublet at δ1.26 replaced the doublets of H_a (δ6.42) and H_b (5.74) in 23.

Catalytic hydrogenation[54] of 22, on the other hand, produced two new compounds 25 ($C_{20}H_{32}O_5$) and 26 ($C_{20}H_{30}O_5$), which were separated by column chromatography. 25 was the expected dihydroepoxycueunicin as evidenced spectrally (Figure 13) by infrared absorption at 3595, 3450 (hydroxyl) and 1769 cm^{-1} (γ-lactone) as well as the new methyl doublet at δ1.22 in the pmr spectrum.

Figure 12

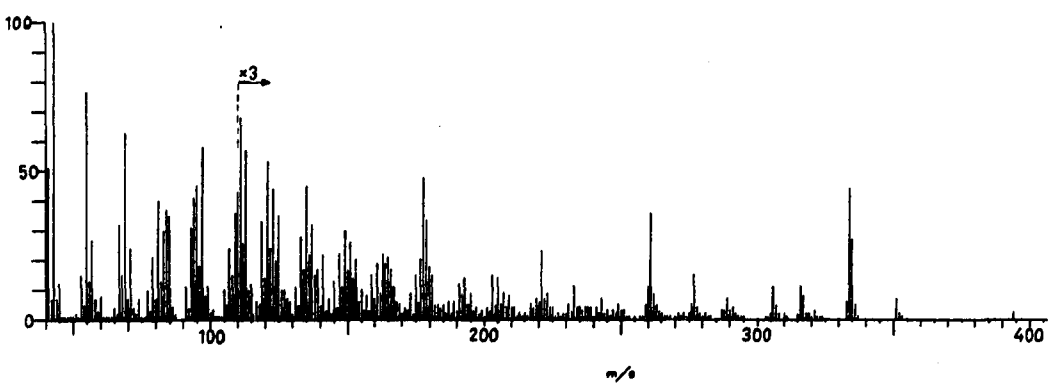


IR Spectrum of Dihydroepoxycoueunicin Acetate (24)



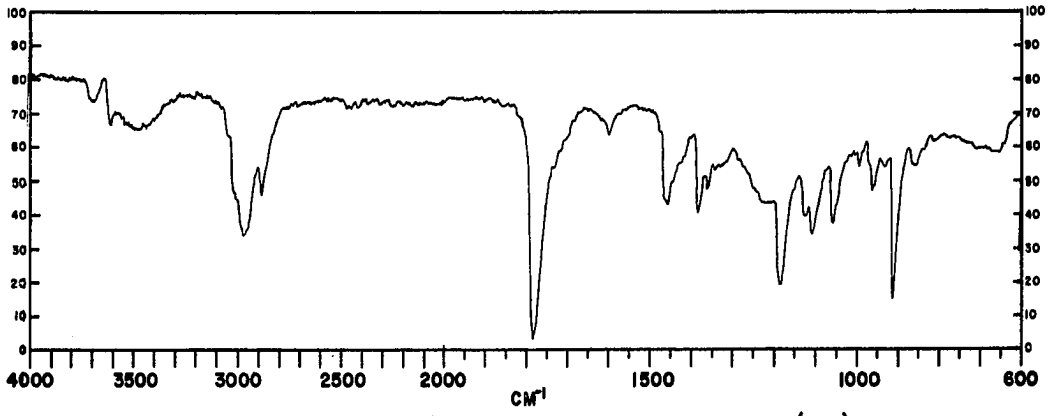
24

100 MHz PMR Spectrum ($CDCl_3$) of 24

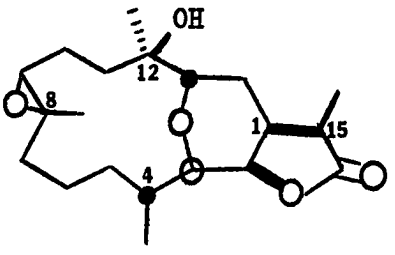


Mass Spectrum of 24

Figure 13

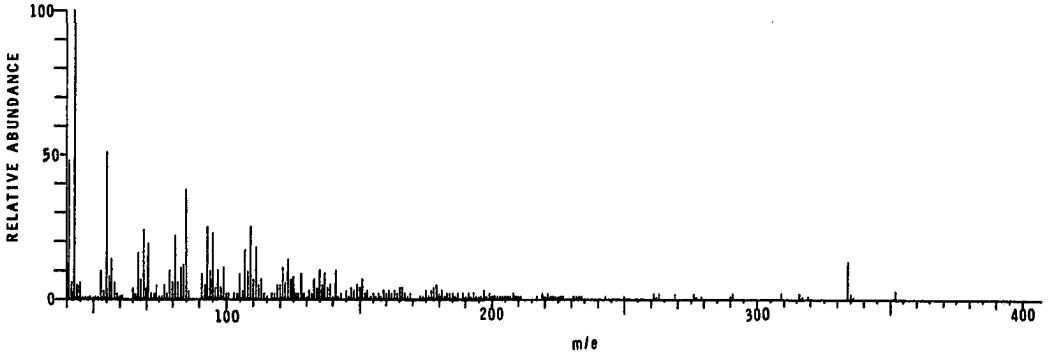


IR Spectrum of Dihydroepoxyoueunicin (25)



25

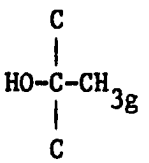
100 MHz PMR Spectrum ($CDCl_3$) of 25



Mass Spectrum of 25

At this point it was possible to derive significant data by comparing the pmr spectra of cueunicin and its derivatives with those of the corresponding compounds containing the acetate group; therefore, consideration of 26 will be deferred until these pmr analyses are completed.

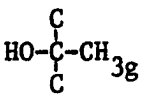
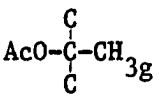
As mentioned earlier, a tertiary carbinol system, K, is present in cueunicin. Table III shows the paramagnetic shift (ave. value 0.35 ppm) of the tertiary methyl (H_g) signal arising from acetylation of the hydroxyl function in cueunicin and its derivatives.



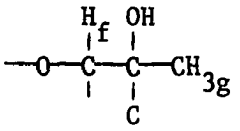
K

Table III

Paramagnetic Shift of the Tertiary Methyl Signal (H_g) in Cueunicin Derivatives upon Acetylation

			
	H_g (δ)	H_g (δ)	Δν (ppm)
Cueunicin ($CDCl_3$)	1.09	1.38	0.29
Cueunicin (PhH-d_6)	1.01	1.43	0.42
Epoxide	1.08	1.42	0.34
Dihydro-I	1.02	1.38	0.36
Dihydro-I-epoxide	1.06	1.46	0.36

It was further noted (Table IV) that acetylation of cueunicin and its derivatives produced a similar paramagnetic shift (ave. value 0.31 ppm) in the signal of H_f, one of the protons on a carbon attached to the disecondary ether oxygen. This suggested that H_f and the methyl protons (H_g) were in similar magnetic environments with respect to the hydroxyl group, namely situated on carbon atoms alpha to the carbinol group. This inference would allow K to be expanded to L. The presence of partial structure L in cueunicin will be firmly established presently

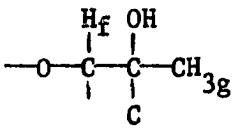


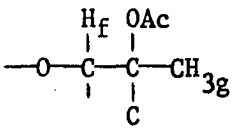
L

(vide infra).

Table IV

Paramagnetic Shift in the pmr Signal of the Secondary Ether Proton (H_f) in Cueunicin Derivatives Upon Acetylation

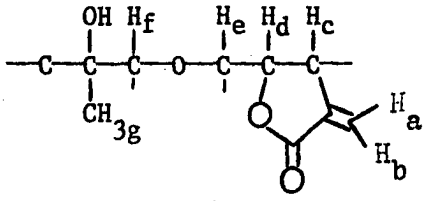




	H_f	H_f	$\Delta\nu$ (ppm)
Cueunicin ($CDCl_3$)	3.48	3.87	0.39
Cueunicin ($PhH-d_6$)	3.44	4.01	0.57
Epoxide	3.6*	3.80	0.2
Dihydro-I	3.38*	3.56	0.18
Dihydro-I-epoxide	3.38	3.58*	0.20

*Partially obscured signal due to overlapping absorption at this frequency.

Partial structures C and L, which both contain H_f were combined to produce partial structure M.

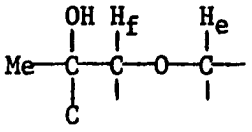


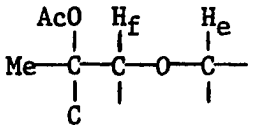
M

Acetylation of cueunicin and its derivatives had no pronounced effect (Table V) on the position of absorption of the signal due to H_e, as it is remote from the deshielding influence of the acetate function.

Table V

Invariability of the pmr Signal of the Secondary Ether Proton H_e on Acetylation of Cueunicin and its Derivatives

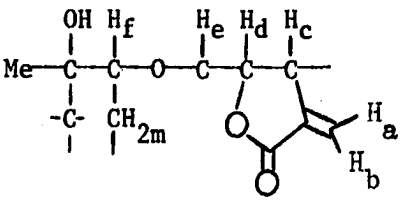




	H_e (δ)	H_e (δ)	Δν (ppm)
Cueunicin ($CDCl_3$)	3.40	3.34	-0.06
Cueunicin ($PhH-d_6$)	2.95	2.97	0.02
Epoxide	3.44	3.40	-0.04
Dihydro-I	3.35	3.39	0.04
Dihydro-I-epoxide	3.38	3.44	0.06

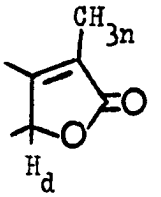
The multiplicity of H_f was either a double doublet or a triplet in all of the compounds reported herein. Since there were no protons on one of its neighboring carbon atoms, the other adjacent carbon necessarily possessed two protons, i.e., was a methylene group,

and M was elaborated to N.



N

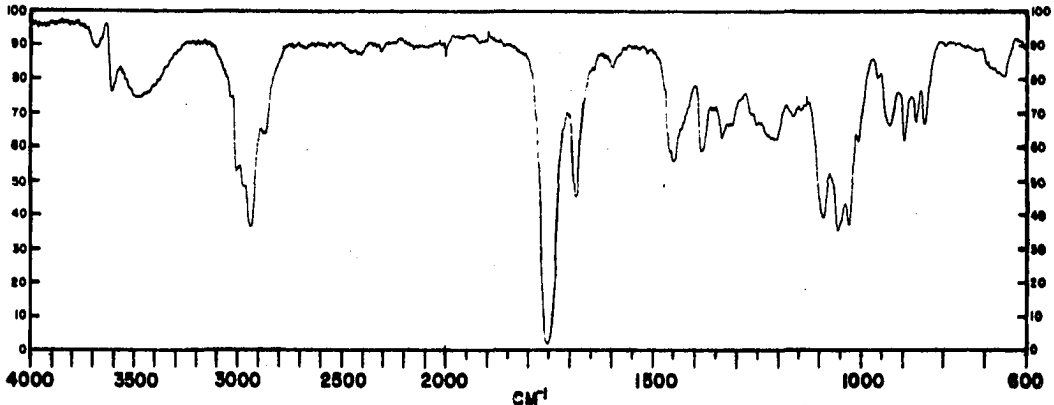
The unexpected product 26, obtained on catalytic hydrogenation of epoxycueunicin (22), displayed spectral characteristics (Figure 14), which were quite different from those of 25. Combustion analysis and low resolution mass spectrometry showed that the molecular formula of 26 was identical to that of 22 ($C_{20}H_{30}O_5$); therefore, 26 was named isocueunicin oxide. The infrared spectrum of 26 showed hydroxyl absorption (3450 and 3600 cm^{-1}) and absorptions (1750 and 1681 cm^{-1}) characteristic of an α,β-unsaturated γ-lactone of the type shown in partial structure O.[61] (See reference 6, p. 48.) An isomerization of this type on a hydrogenation catalyst is not unusual,[62] but was



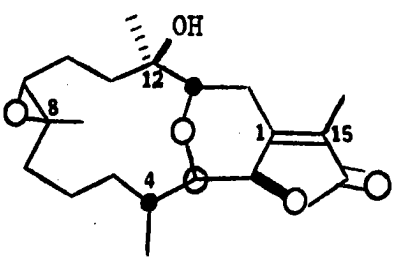
O

unexpected here because both 16 and 23 had undergone only normal hydrogenation. The presence of O was substantiated by the pmr spectrum of 26. The doublets due to H_a and H_b in 22 were replaced by a new methyl signal (H_n) at δ1.88, characteristic of a methyl group situated

Figure 14

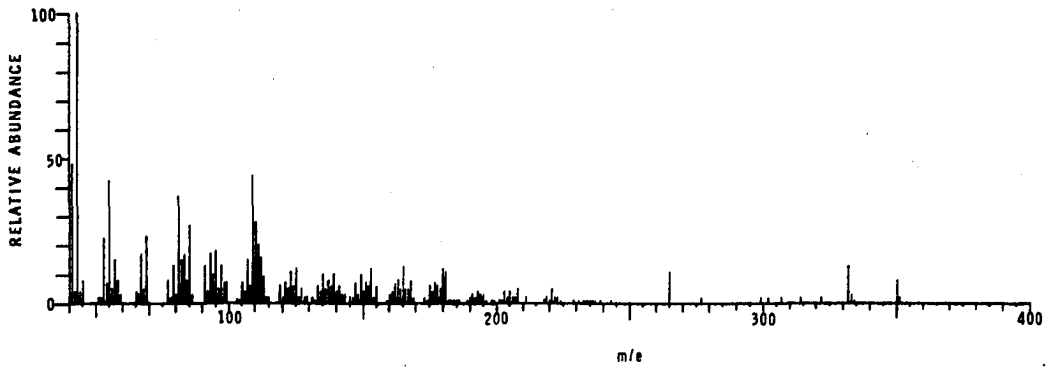


IR Spectrum ($CHCl_3$) of Isocueunicin Oxide (26)



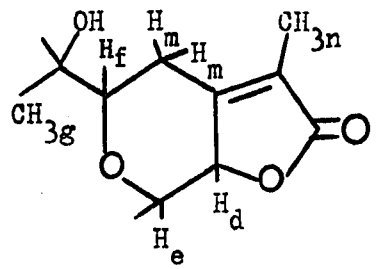
26

100 MHz PMR Spectrum ($CDCl_3$) of 26



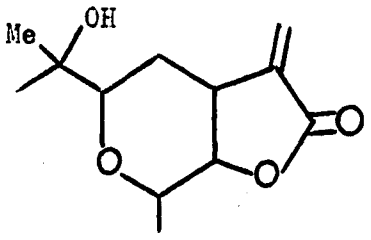
Mass Spectrum of 26

on such a conjugated π-system.[31-32] Proton H_d appeared as a doublet at 5.01 ppm. Irradiation at the frequency of H_d collapsed a double doublet at δ4.00 to a doublet, establishing the latter signal as that due to H_e. Structure O was expanded to P when irradiation of the one proton signal (δ2.40) in the allylic region (H_m) collapsed the double doublet due to H_f (δ3.62) to a doublet and sharpened the vinyl methyl signal (H_n). Conversely, irradiation at the frequencies of H_f or H_n affected the allylic proton signals (H_m). These experiments established that the methylene group CH_{2m} was <u>alpha</u> to H_f and homoallylic to the vinyl methyl (CH_{3n}) group. Homoallylic couplings of the type observed here are well documented.[63]

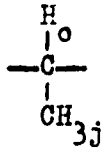


P

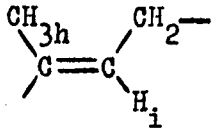
Thus, cueunicin possesses the partial structure Q, which together with the previously adduced structures F and J account for all of the molecule except for 4 methylene groups.



Q

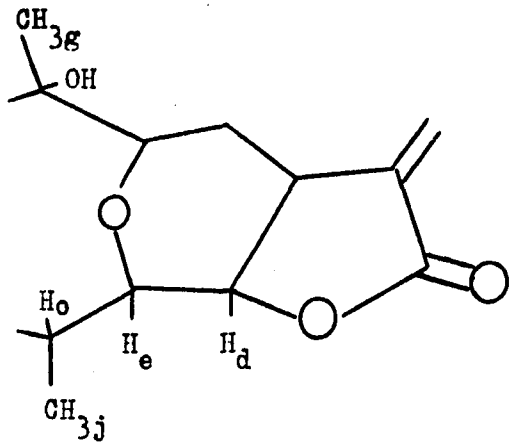


F



J

The multiplicity of the pmr signal due to H_e appeared as a double doublet in every compound described herein and H_e has already been shown to be adjacent to the lone proton H_d; therefore, H_e required one other neighboring proton. Since H_i has been shown to be flanked by a methylene group (partial structure J) and clearly was not coupled to H_e, the methinyl proton (H_o) in partial structure F was the only proton which could be adjacent to H_e. This allowed Q to be expanded to R.



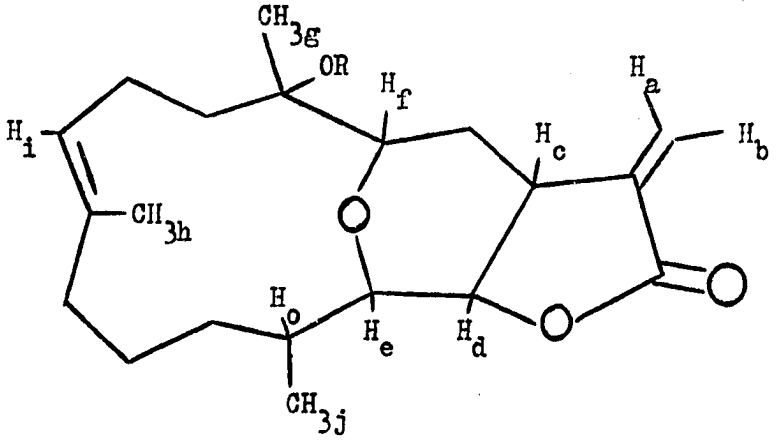
R

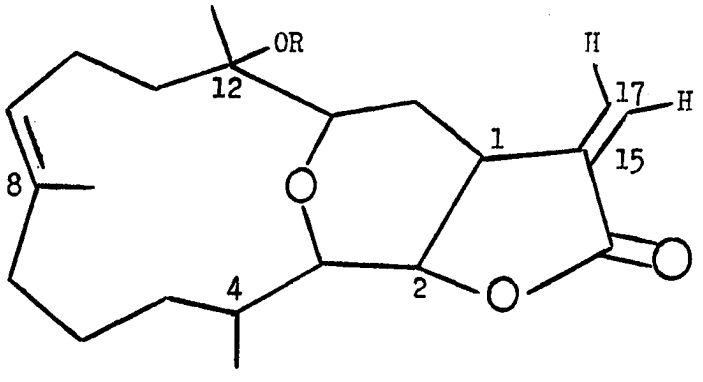
Completion of the structure assignments for cueunicin (15) and cueunicin acetate (16) (Chart IV) was accomplished with evidence from the dihydro-keto-dilactone (27) obtained from dihydrocueunicin-I (17) on treatment with the Lemieux-von Rudloff[64-68] ($KMnO_4/KIO_4$) reagent (Chart V). The spectral data for 27 (Figure 15) showed two γ-lactones (ir 1770 cm^{-1}) and a methyl ketone (ir 1710 cm^{-1} and pmr δ2.15, 3H, singlet). The tertiary methyl (12-Me) signal in 17 underwent a paramagnetic shift of 0.41 ppm (δ1.02 to δ1.43), when 17 was converted to 27, a fact which was compatible with new lactone formation at that site.[6] The spontaneous lactonization observed in the above reaction demonstrated that the carbon atom bearing the olefinic proton (H-9, formerly H_1) of the trisubstituted double bond bore a 1,4 relationship with the carbon bearing the tertiary hydroxyl group. This accounted for one of the four remaining methylene moieties, and, by difference, left three methylene groups separating the carbons bearing the secondary methyl (4-Me) and vinyl methyl (8-Me) groups. The structure thus adduced for cueunicin contains a regular isoprenoid skeleton.

Chart IV

Cueunicin 15 and Cueunicin Acetate 16

(Stereochemistry is not implied.)





15 R = H

16 R = Ac

Chart V

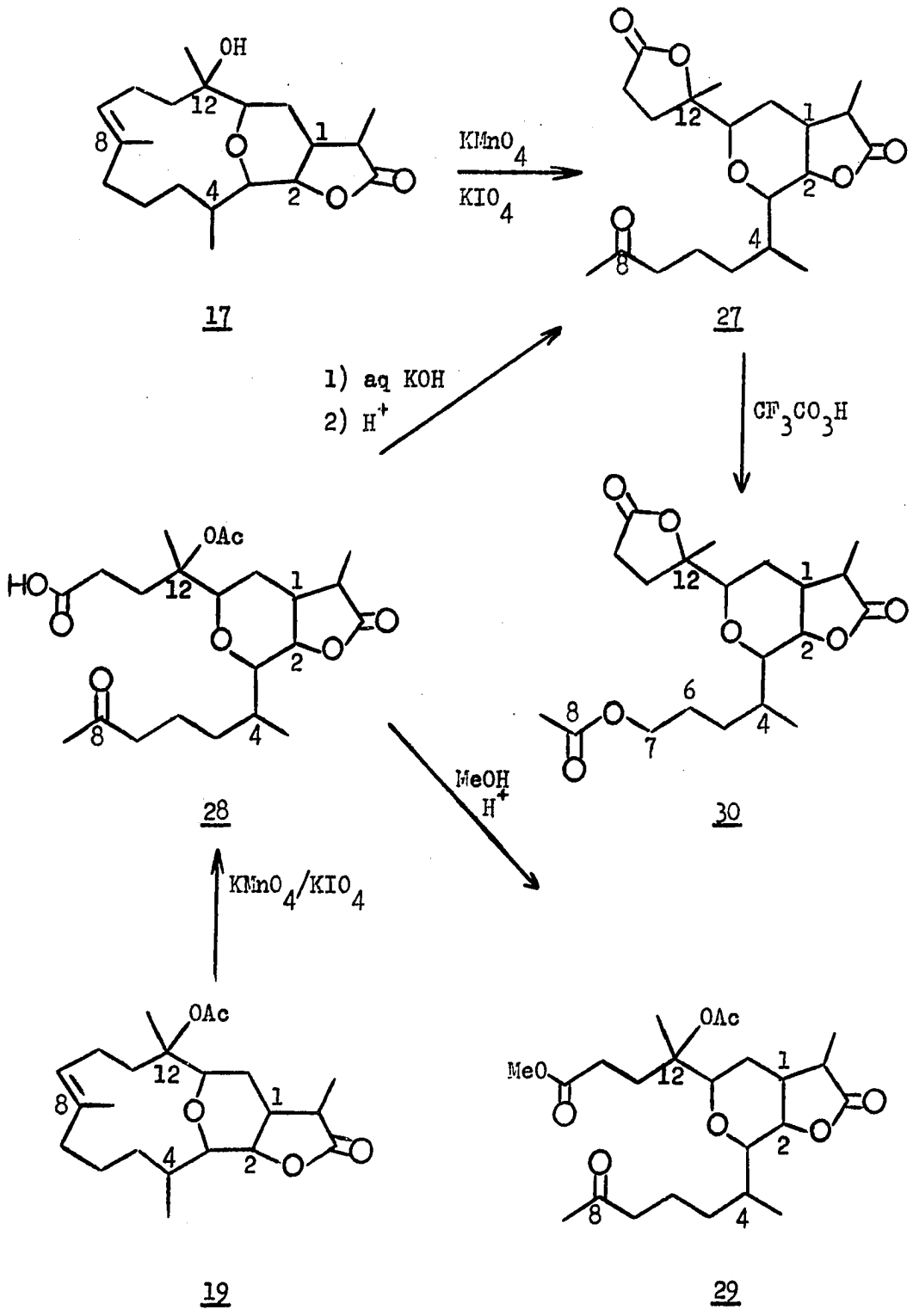
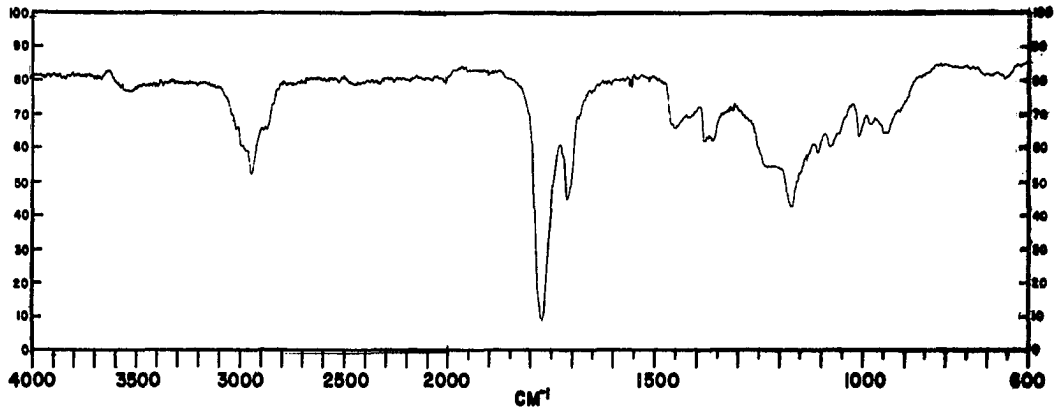
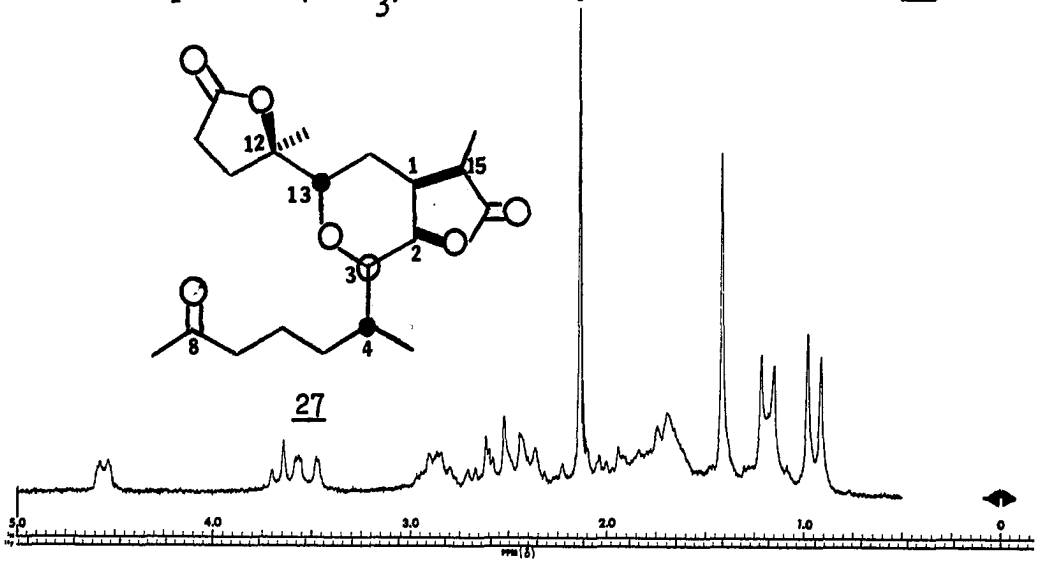

OH
12
8
1
4
2
O
KMnO4
KIO4
17
27
1) aq KOH
2) H+
CF3CO3H
OAc
HO
28
30
MeOH
H+
KMnO4/KIO4
MeO
19
29
6
7

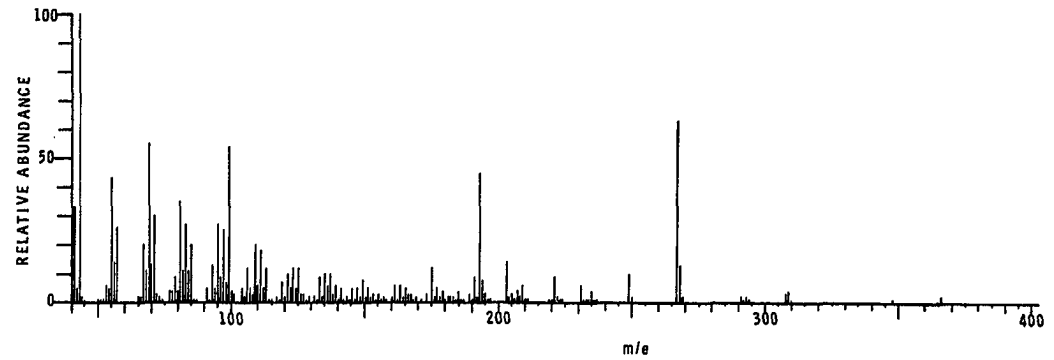
Figure 15



IR Spectrum ($CHCl_3$) of the Dihydro-keto-dilactone (27)



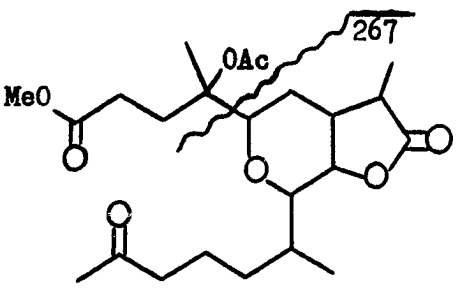
100 MHz PMR Spectrum ($CDCl_3$) of 27



Mass Spectrum of 27

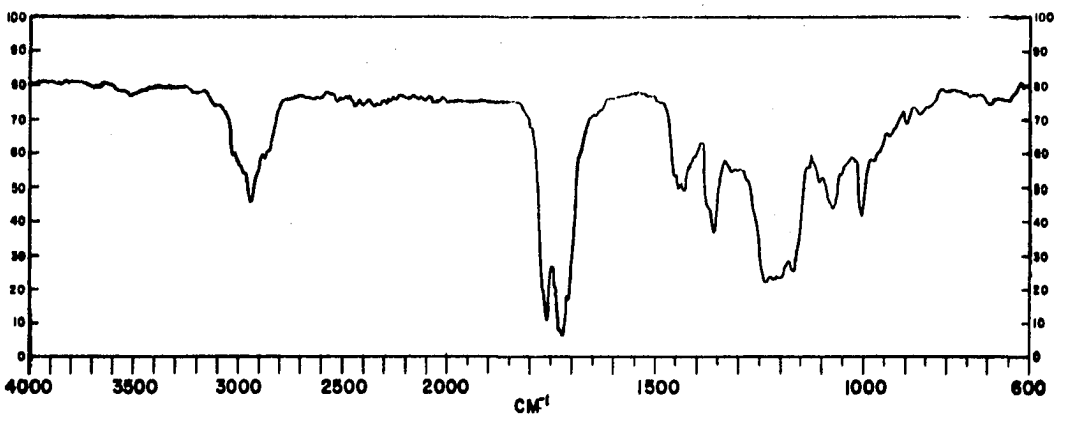
27 was also prepared from cueunicin acetate via dihydrocueunicin acetate (19) (Chart V). When 19 was allowed to react with the Lemieux-von Rudloff reagent, the tertiary acetate remained intact producing 28. This compound was not fully characterized but was converted to 27 on saponification (KOH/water) of the acetate followed by acidification.

In another experiment an attempt was made to convert 28 to 27 by hydrolyzing 28 with methanolic KOH followed by acidification to effect lactonization. As noted earlier, the acetate function was quite resistant to deacetylation and on refluxing 28 in methanolic KOH the acetate was only partially removed, and on acidification of the resulting solution with HCl, Fischer esterification[69] was effected producing 29 (Chart V). 29 was a mixture of epimers at C-15, owing to the prior treatment with methanolic KOH, which effectively epimerized the center alpha to the lactone carbonyl. Spectral data for 29 are shown in Figure 16. The low resolution mass spectrum of 29 did not show a molecular ion, but showed facile loss of acetic acid (m/e 380) and the side chain bearing the acetate and carbomethoxy groups (m/e 267) from the parent.

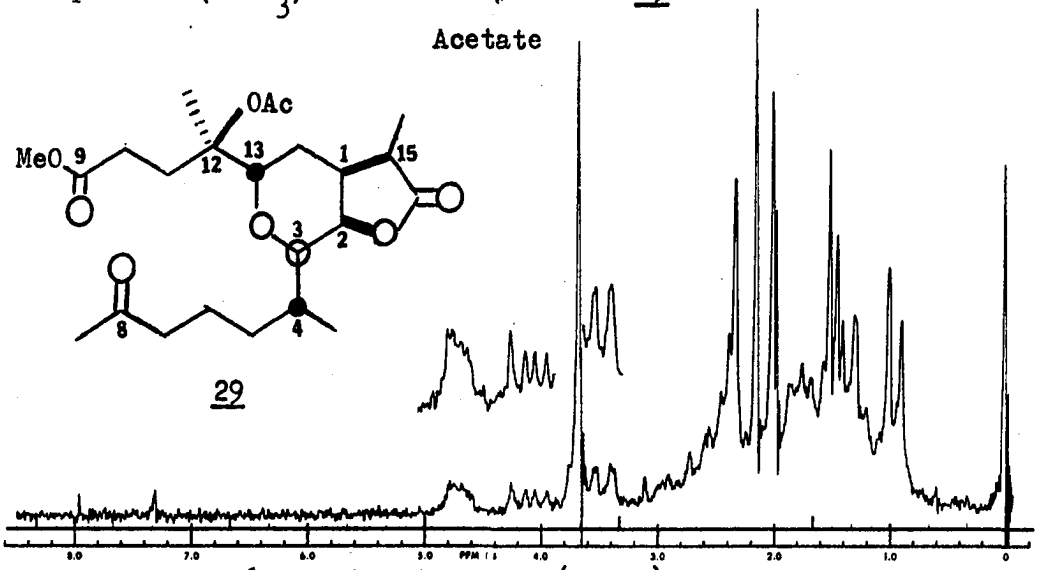


29

Figure 16

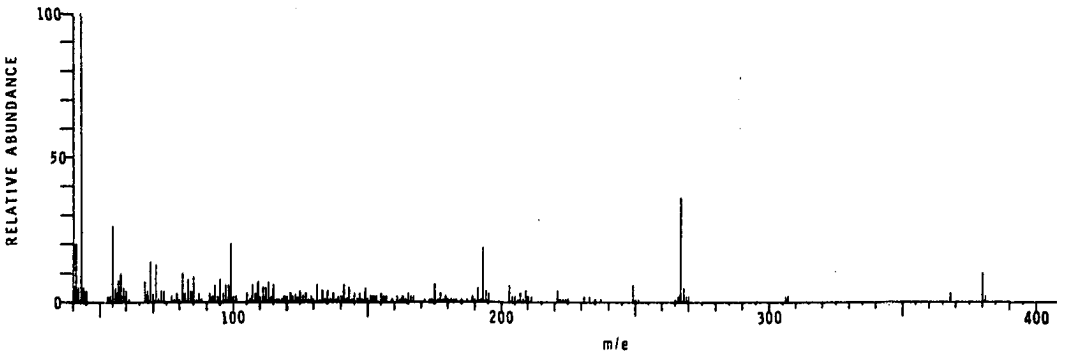


IR Spectrum ($CHCl_3$) of the Methyl Ester 29 Derived From Cueunicin Acetate



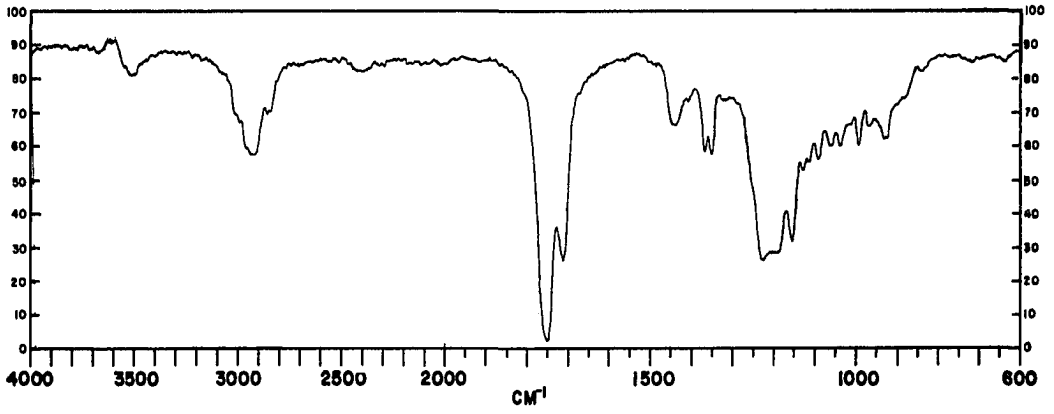
29

60 MHz PMR Spectrum ($CDCl_3$) of 29

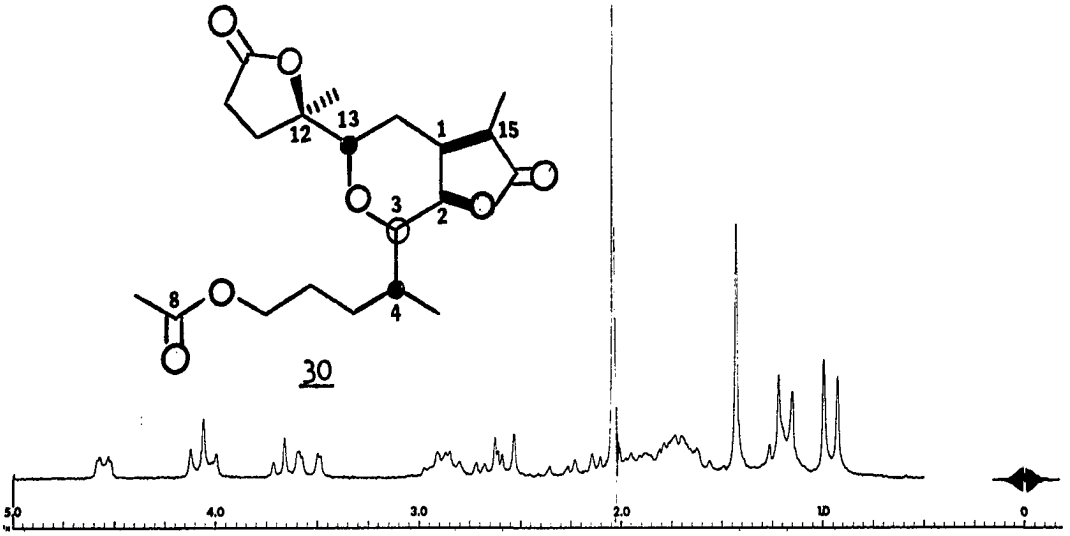


Mass Spectrum of 29

Figure 17

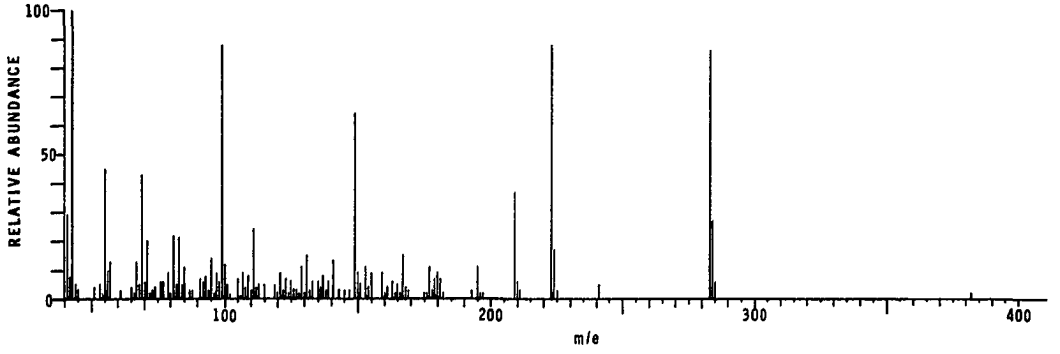


IR Spectrum ($CHCl_3$) of the Dihydroacetoxy-dilactone 30



30

100 MHz PMR Spectrum ($CDCl_3$) of 30

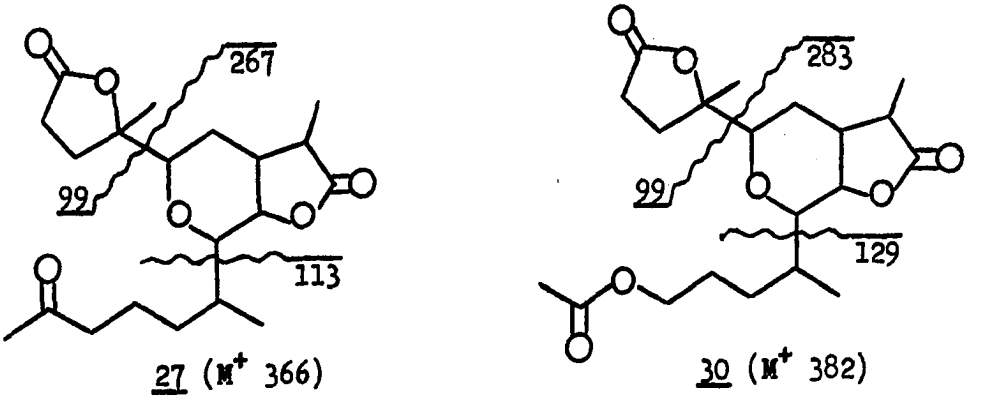


Mass Spectrum of 30

Baeyer-Villiger oxidation of 27 (Chart V) with pertrifluoroacetic acid[70-71] produced the dihydroacetoxy-dilactone 30 (spectral data in Figure 17). The appearance of a new two proton triplet (H-7) at δ4.06 demonstrated the simple methylene character at C-6 and C-7, and verified that the quaternary carbon at C-12 in cueunicin bore the tertiary methyl rather than the vinyl methyl group, i.e., that the presence of partial structure L inferred earlier from pmr spectral data was in fact correct.

The mass spectra of compounds 27 and 30 strongly supported the assigned structures. Significant fragments were observed with m/e 99 ($C_5H_7O_2$), 267 ($C_{15}H_{23}O_4$) and 113 ($C_7H_{13}O$) for 27 and m/e 99 ($C_5H_7O_2$), 283 ($C_{15}H_{23}O_5$) and 129 ($C_7H_{13}O_2$) for 30 (see Figure 18). Fragmentations due to a Mc Clafferty rearrangement of the methyl ketone in 27 and elimination of the acetate group in 30 were also observed; 27 showed a large m/e at 55 (113-58) and 30 a large m/e at 69 (129-60). The compositions indicated above for the ions were confirmed by high resolution mass spectrometry.

Figure 18

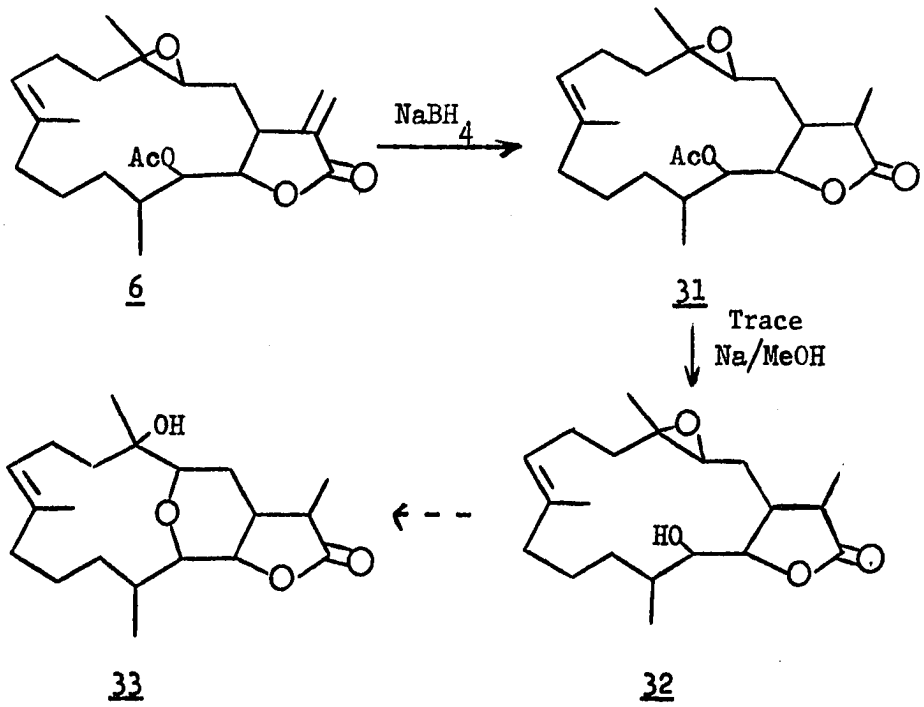


27 (M^+ 366) 30 (M^+ 382)

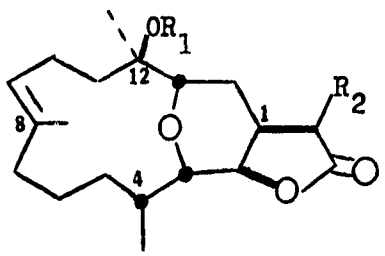
This mass spectral data clearly defines the structural detail of the non-oxygenated portions of cueunicin and cueunicin acetate, and leads to the assignment of structures 15 and 16 (Chart IV) to these compounds.

During the course of these studies an attempt was made to prepare a new oxybridged cembranolide (33) possessing the gross structure of dihydroeunicin. The approach followed is outlined in Chart VI. Eupalmerin acetate (6) was isolated and compounds 31 and 32 prepared as indicated, but the formation of 33 was not realized, owing in part to a paucity of 32.

Chart VI

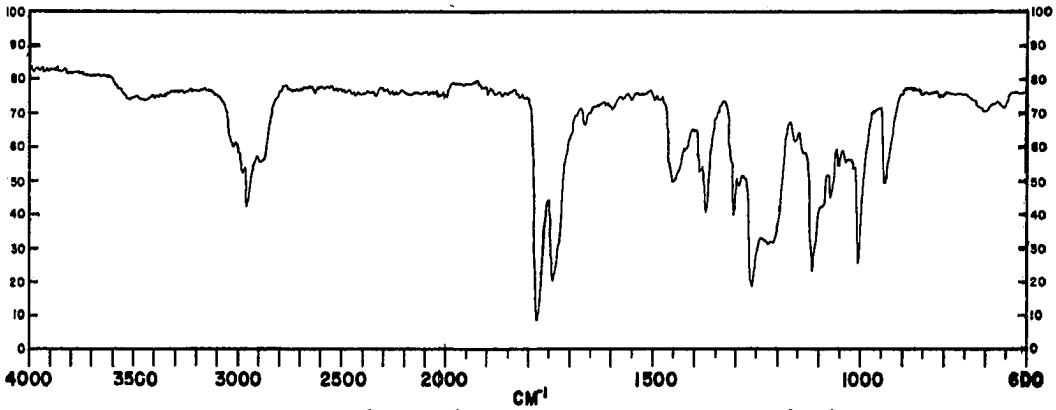


The structure elucidated for cueunicin is stereoisomeric with that of eunicin (3). Since stereoisomers are not expected to show appreciable differences in their mass spectra,[72] eunicin was isolated and four derivatives were prepared in order to provide analogs for comparison with the corresponding compounds in the cueunicin series. Dihydroeunicin (34) was prepared from eunicin by sodium/n-butanol reduction. (See reference 6, p. 62 for spectral data of 3 and 34). Eunicin acetate, 35 (spectral data in Figure 19) was prepared by acetylation of eunicin with acetic anhydride and boron trifluoride etherate. Treatment of 34 with the Lemieux-von Rudloff reagent,[64-68] afforded 36, the compound analogous to 27; 36 (Spectral data in Figure 20) was identical to the compound obtained from 34 by Bledsoe[6] using the procedure of Narang and Dutta.[73] Pertrifluoracetic acid converted 36 to 37 (Spectral data in Figure 21).

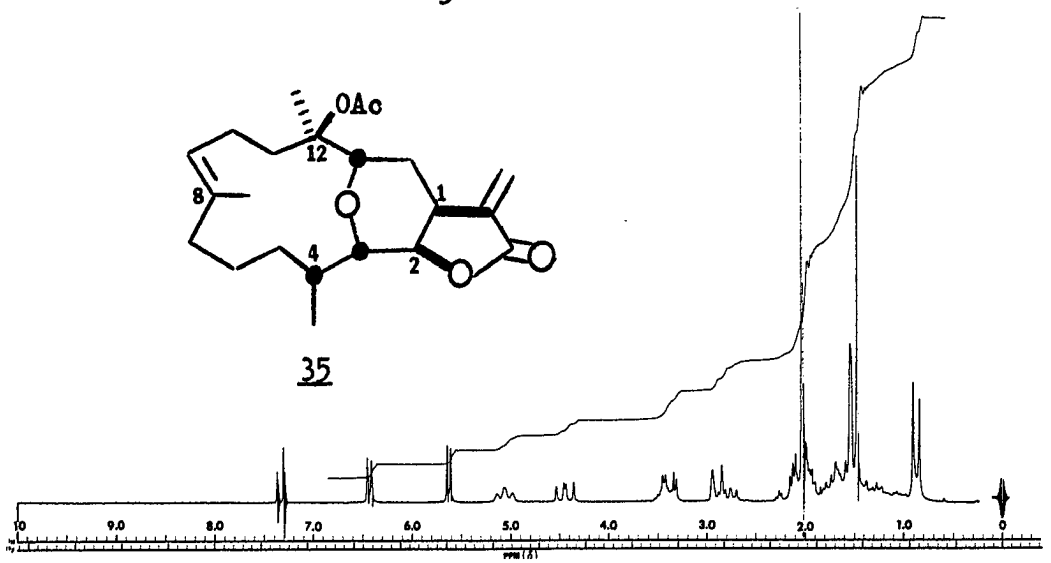


3 R_1 = H, R_2 = CH_2
34 R_1 = H, R_2 = CH_3
35 R_1 = Ac, R_2 = CH_2

Figure 19

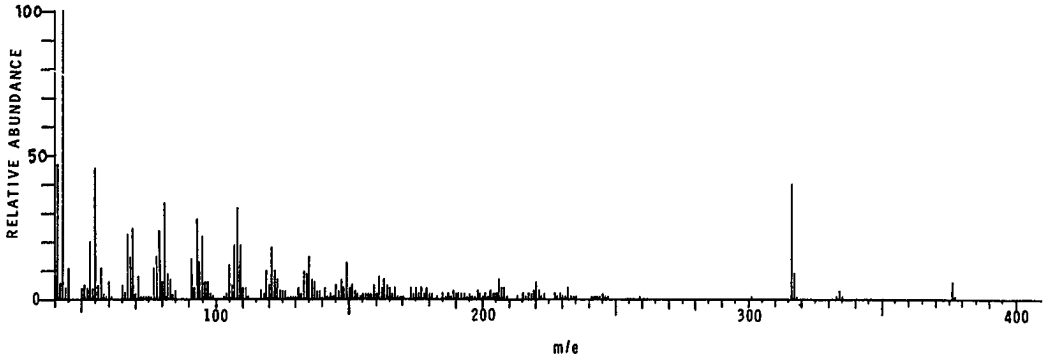


IR Spectrum ($CHCl_3$) of Eunicin Acetate (35)



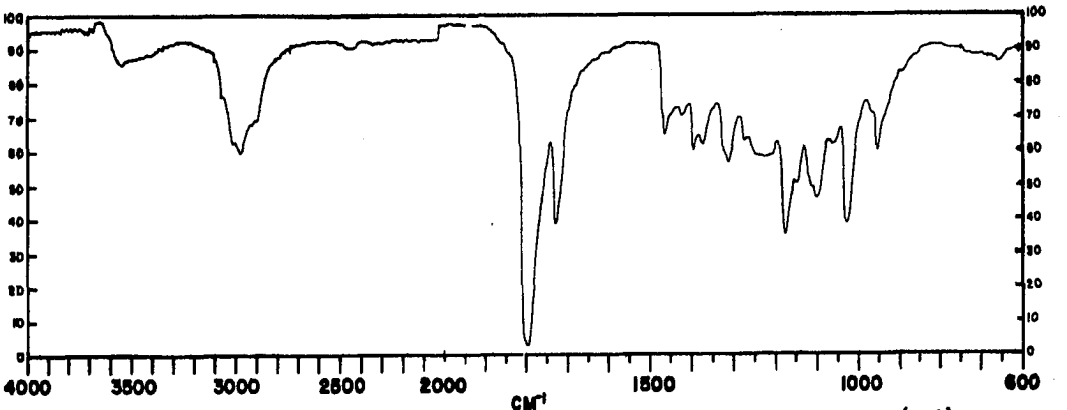
35

100 MHz PMR Spectrum ($CDCl_3$) of 35

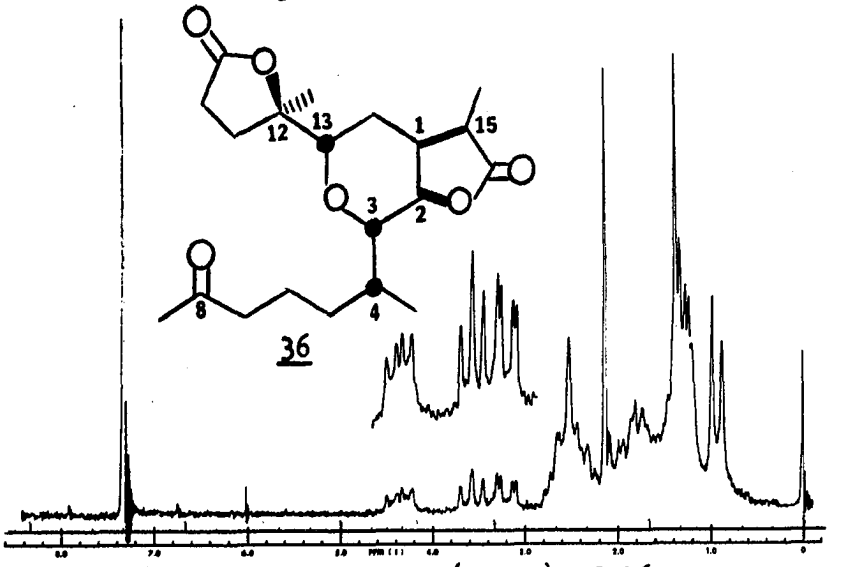


Mass Spectrum of 35

Figure 20

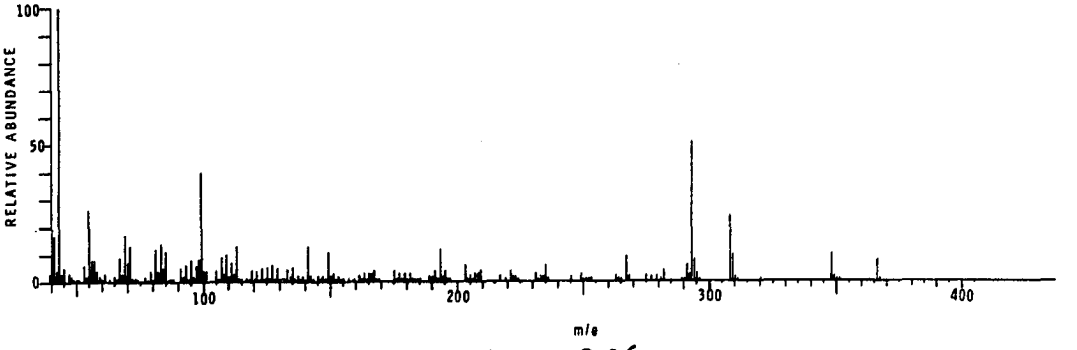


IR Spectrum ($CHCl_3$) of the Dihydro-keto-dilactone (36)



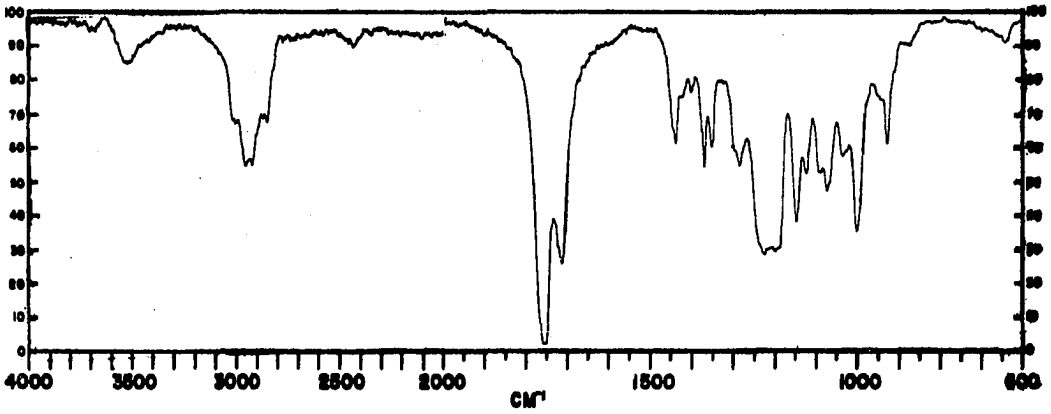
36

60 MHz PMR Spectrum ($CDCl_3$) of 36

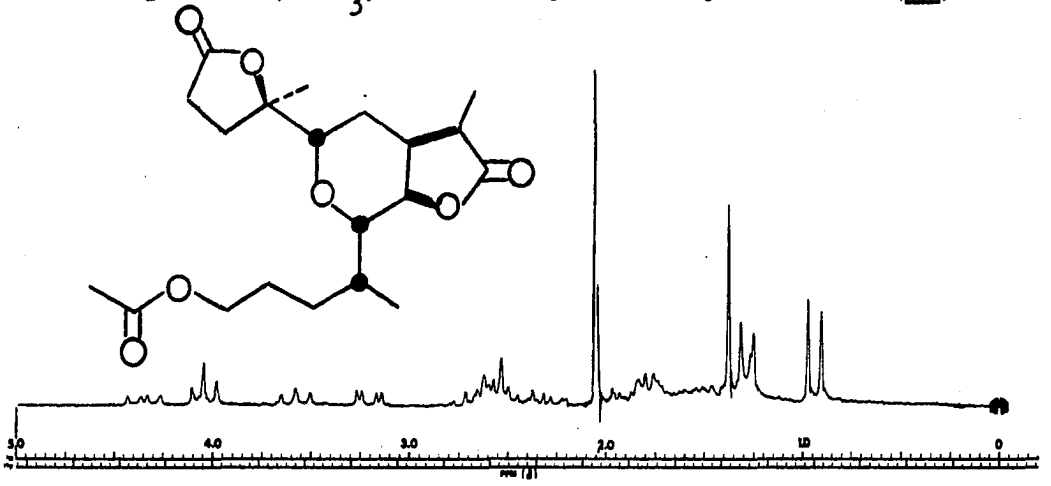


Mass Spectrum of 36

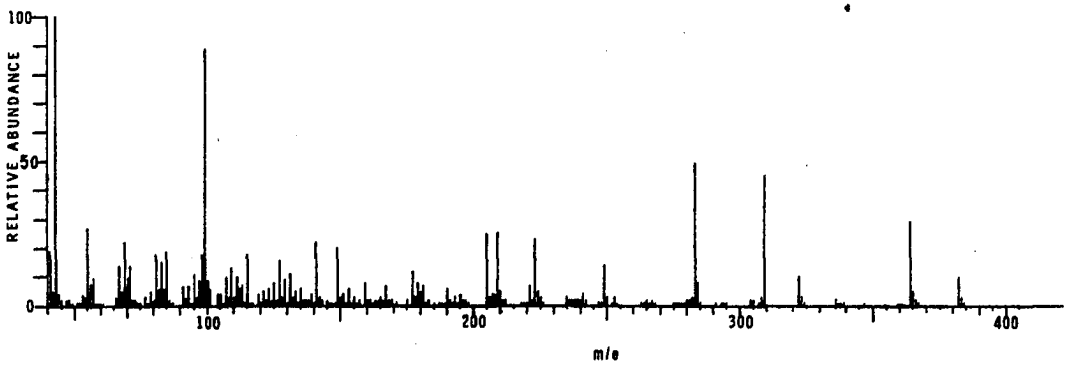
Figure 21



IR Spectrum ($CHCl_3$) of the Dihydroacetoxy-dilactone (37)

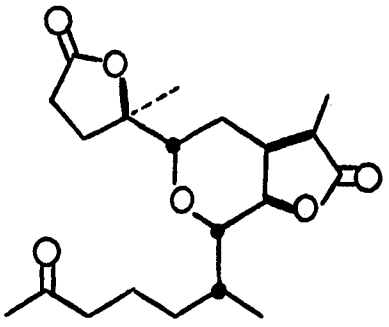


100 MHz PMR Spectrum ($CDCl_3$) of 37

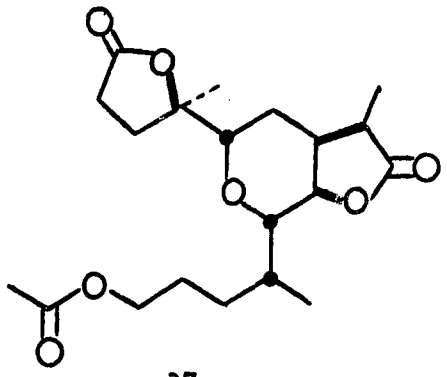


Mass Spectrum of 37

Significantly, the five compounds in the eunicin series (3, 34, 35, 36, and 37) displayed 70 eV mass spectra, which were quite similar to those of the corresponding six compounds (15, 17 and 18, 16, 27, and 30) in the cueunicin series. The high-resolution mass spectra of 36 and 37 contained major fragmentations identical to those of 27 and 30, respectively, shown in Figure 18 and specifically, those important to the characterization of the non-oxygenated portion of the carbocyclic ring.



36

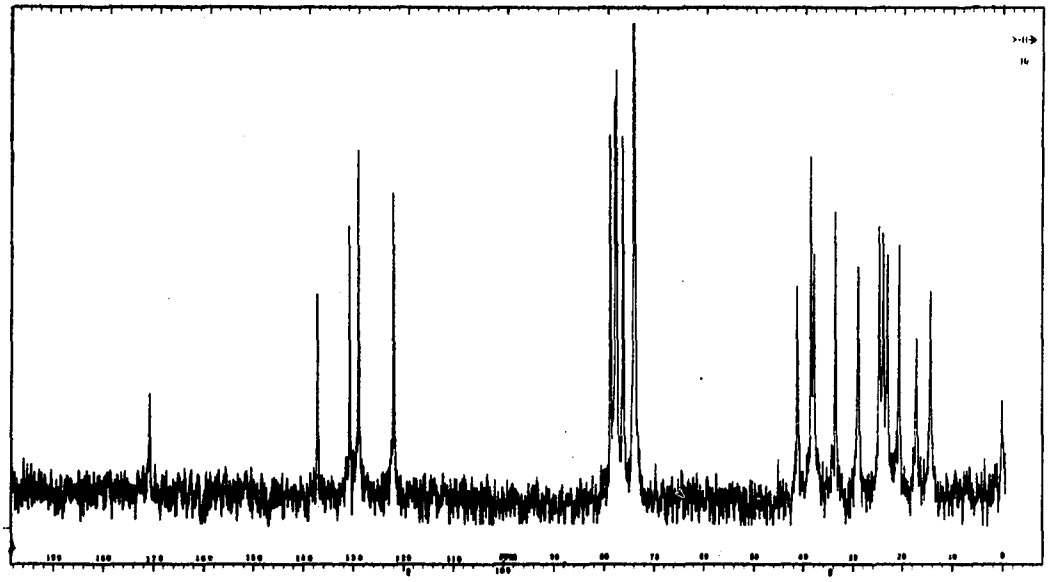


37

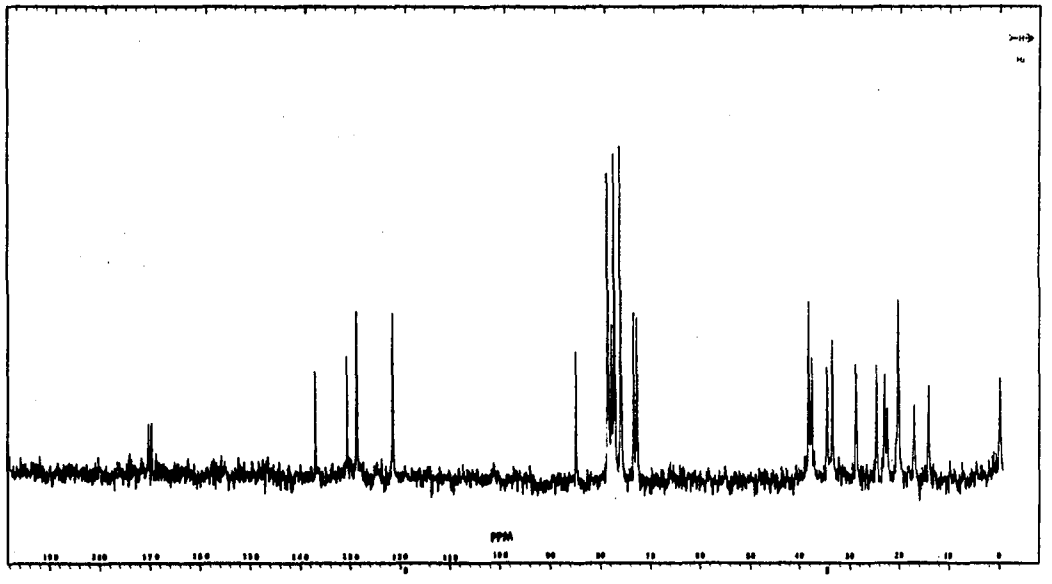
As expected, the proton-decoupled cmr spectra of eunicin and eunicin acetate (Figure 22) were quite similar to their cueunicin analogs (Figures 3 and 4).

In conclusion, the data presented above provided powerful evidence for the structures 15 and 16 for cueunicin and cueunicin acetate. It is noteworthy that cueunicin possesses six asymmetric centers and a trisubstituted double bond, which allows 128 possible stereostructures.

Figure 22



25.2 MHz Proton-Decoupled CMR Spectrum ($CDCl_3$) of Eunicin (3)



25.2 MHz Proton-Decoupled CMR Spectrum ($CDCl_3$)
of Eunicin Acetate (35)

B. Stereochemistry of Cueunicin and Cueunicin Acetate

The α-methylene-γ-butyrolactone moiety is a rather common group found in over 400 naturally occurring sesquiterpenes[74-75] alone. Accordingly, the cis or trans nature of the lactone ring fusion to carbocyclic rings has been the subject of several investigations.

One study[76] has shown circular dichroism (CD) curves of pyrazoline derivatives of the α-methylene-γ-lactone group to be well suited for assigning the bridgehead geometry of the lactone in sesquiterpenes based on the sign of the Cotton effect arising from the pyrazoline chromophore.

Rehm[13] correlated the absolute configuration of the cis-fused γ-lactones in the cembranolides 3, 4 and 6 with a strongly negative Cotton effect in the 320-nm region of the CD curves of their respective pyrazoline derivatives (Table VI). The cis nature of the lactone in eupalmerin acetate (6) has recently been verified by an x-ray diffraction study.[14] (See Chart I, page 3 for structures of 3,4 and 6.)

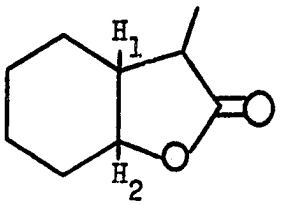
Table VI

Cotton Effects in the CD Curves of Cembranolide Pyrazoline Derivatives

Pyrazoline	[θ]	λmax (nm)	Lactone Geometry
Eunicin (3)	-20,524	318	cis (beta)
Jeunicin (4)	-24,800	326	cis (beta)
Eupalmerin Acetate (6)	-36,564	324	cis (beta)

Treatment of cueunicin acetate with diazomethane[77-78] afforded the pyrazoline derivative 38 in quantitative yield. The spectral data of 38 (Figure 23) indicated, as expected, that only one stereoisomer had been formed, i.e., attack of diazomethane had occurred stereospecifically presumably from the less sterically hindered face of the lactone. 38 exhibited a strong negative Cotton effect [θ] = -13,700) at 324 nm, showing that the lactone fusion in cueunicin acetate was the same as that in 3, 4, and 6 (Chart I, page 3).

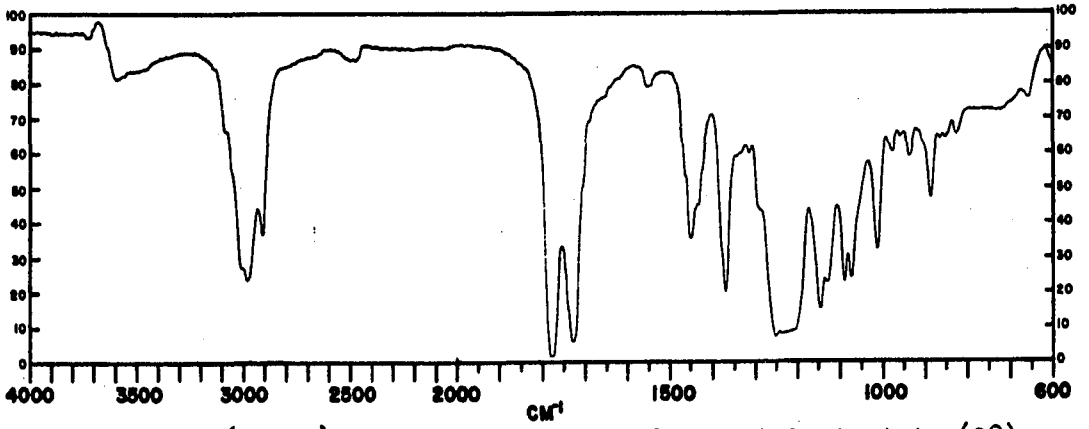
In another study, Pinhey and Sternhell[79] observed that the vicinal coupling constant between H-1 and H-2 in partial structure S was particularly useful in assigning stereochemistry to the lactone ring fusion when the lactone was fused to a six-membered ring (Table VII).



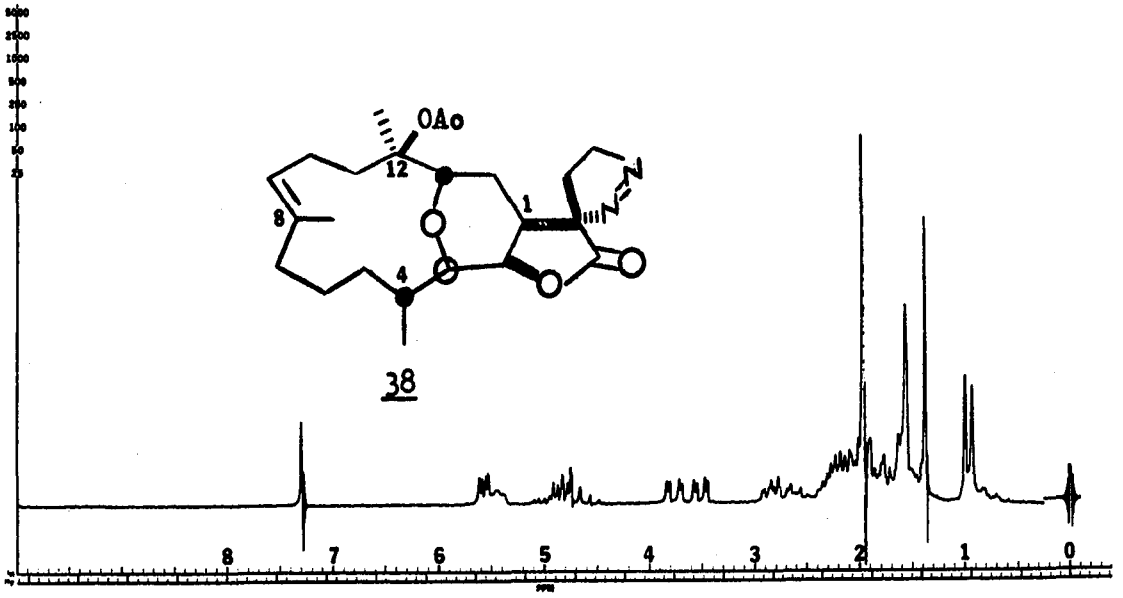
S

Both eunicin and cueunicin possess γ-lactones annealed to six-membered rings; therefore, the coupling constants ($J_{1,2}$) of the available dihydro derivatives of eunicin and cueunicin were compared (Table VIII). The vicinal coupling constants $J_{1,2}$ for the cueunicin derivatives corresponded nicely with those of the eunicin derivatives, which suggested that the lactone fusion in cueunicin was like that of eunicin, i.e., cis. The data for both eunicin and cueunicin were

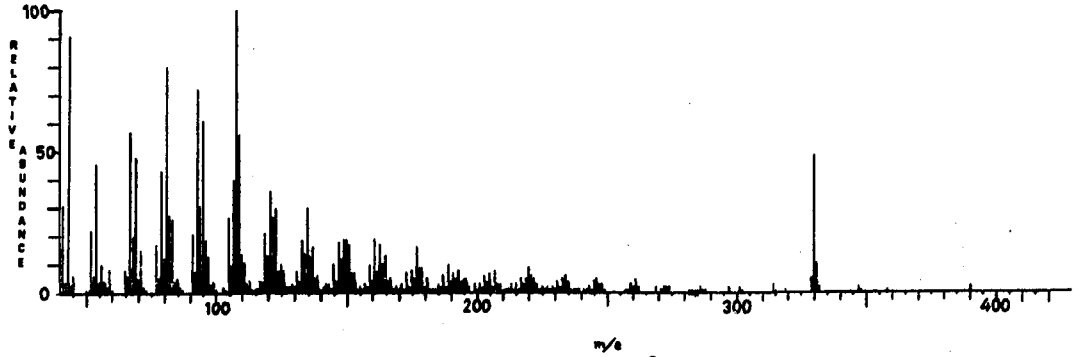
Figure 23



IR Spectrum ($CHCl_3$) of the Pyrazoline of Cueunicin Acetate (38)



100 MHz PMR Spectrum ($CDCl_3$) of 38



Mass Spectrum of 38

Table VII

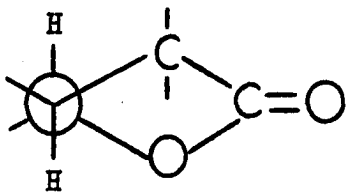
Vicinal Coupling Constants[a] between H-1 and H-2 in α-Methyl-γ-lactones Fused to Six Membered Rings (S)

Trans Lactone		Cis Lactone	
Compound	$J_{1,2}$ (Hz)	Compound	$J_{1,2}$ (Hz)
α-Santonin	9	6-Epi-α-santonin	5
β-Santonin	11	6-Epi-β-santonin	4
Artemisin	12	6-Epi-artemisin	5
Artemisin Acetate	12	6-Epi-artemisin acetate	6
α-Hydroxysantonin	10	ψ-Santonin	6
Lumisantonin	10	1-Desmotroposantonin Acetate	6

[a]Values taken from Reference 79 and rounded to nearest whole number.

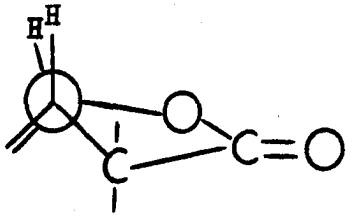
comparable with the data in Table VII for a cis rather than trans-fused lactone.

Herz, Aota, Holub, and Samek,[80] on the other hand, formulated the general rule for the vicinal coupling constant ($J_{1,2}$) of partial structure S as shown below. By this rule eunicin and cueunicin were again determined to have cis-fused lactones. (See also reference 81.)



Trans

$^3J_{H,H} > 10$ Hz



Cis

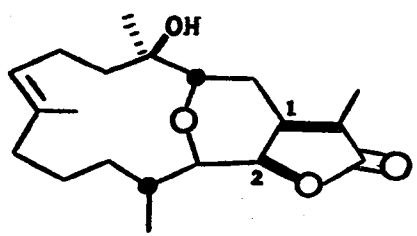
$^3J_{H,H} \leqslant 8$–9 Hz

(^3J = vicinal coupling constant)

Table VIII

Vicinal Coupling Constants* ($J_{1,2}$) in Dihydroeunicin and Dihydrocuenicin Derivatives

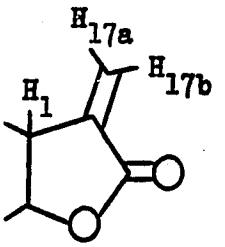
Dihydroeunicin Derivatives	$J_{1,2}$ (Hz)	Dihydrocueunicin Derivatives	$J_{1,2}$ (Hz)
Keto-dilactone (36)	7	Keto-dilactone (27)	7
Acetoxy-dilactone (37)	6	Acetoxy-dilactone (30)	6
Dihydroeunicin (34)	7	Dihydrocueunicin-I (17)	7
		Dihydrocueunicin-II (18)	8
		Dihydrocueunicin Acetate (19)	6
		Tetrahydrocueunicin Acetate (20)	6
		Dihydroepoxycueunicin (25)	6
		Dihydroepoxycueunicin Acetate (24)	7



Dihydroeunicin or
Dihydrocueunicin

*Values rounded to nearest whole number

The cis lactone assignment for 15 was reinforced by considering the magnitude of the allylic couplings of H-17_a and H-17_b with H-1. It has been found (Samek's rule) by analyzing a large number of naturally occurring sesquiterpene γ-lactones fused to 6-membered rings of well-defined stereochemistry that $^4J_{cis} \leq 3$ and $^4J_{trans} \geq 3$ Hz.[82] The application of this rule to the case at hand (Table IX) again suggested that cueunicin contained a cis-fused lactone. (^4J = allylic coupling constant)



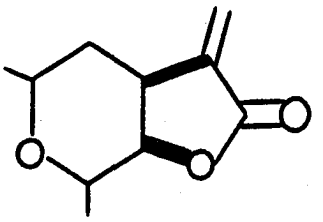
Lactone Ring in Cueunicin

Table IX

Allylic Coupling Constants $J_{1,17a}$ and $J_{1,17b}$ for Cueunicin and Derivatives

Compound	$J_{1,17a}$ (Hz)	$J_{1,17b}$ (Hz)
Cueunicin	2.5	2.5
Cueunicin (PhH-d_6)	2.4	2.4
Cueunicin Acetate	2.4	2.4
Cueunicin Acetate (PhH-d_6)	2.4	2.4
Epoxycueunicin	2.4	2.4
Epoxycueunicin Acetate	2.4	2.4

The observations described above allowed the partial stereoformula 15A to be drawn for cueunicin.



15A

Having determined the lactone fusion to the 14-membered ring, attention was turned to the remaining stereochemical features of cueunicin. Toward this end numerous pmr spin decoupling experiments were performed.[83] It was previously noted that H-1 and H-2 were coupled, as were H-2 and H-3. Since the signal due to H-2 occurred considerably downfield from those of H-1 and H-3, the favorable $\Delta\nu/J$ established first order conditions for these couplings.[33] First order conditions were assumed for all other spin systems.

Detailed spin decoupling data are presented in Tables X and XI for cueunicin (15) and isocueunicin oxide (26), and in Tables XII through XV for cueunicin acetate (16) and its pyrazoline, methanol adduct (21), and epoxide (23) derivatives, respectively.

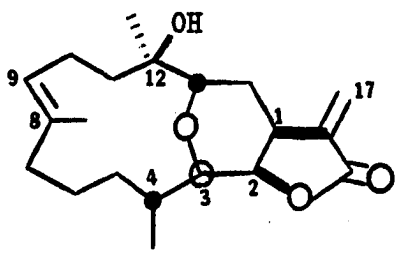
The coupling constants $J_{1,2}$ and $J_{2,3}$ obtained by double irradiation (Tables X-XV) were identical to the corresponding line separations of the H-2 signal in the pmr spectra of the compounds examined; therefore, these coupling constants for the remaining derivatives of cueunicin were obtained directly (line separations) from the pmr spectra rather than by decoupling experiments. The H-3

Table X

Double Irradiation[a] of Cueunicin (15)

Signal irrad'd	Signal obs'd	Change obs'd	Inference
4.78 (H-2)	3.45 (m, H-1)	Affected	
	3.34 (dd, H-3)	Collapsed to d	$J_{3,4}$=10
6.44 (H-17a)	3.45 (m, H-1)	Affected	
5.74 (H-17b)	3.45 (m, H-1)	Affected	
3.34 (H-3)	4.78 (dd, H-2)	Collapsed to d	$J_{1,2}$=8
5.36 (H-9)	1.56 (br, 8-Me)	Sharpened	
2.08 (H-10)	5.34 (t, H-9)	Affected	

[a]Run on a Varian XL-100 nmr spectrometer at 100 MHz in chloroform-d_1 solution using tetramethylsilane as internal standard. The spectrometer was locked on the deuteron signal. Experiments were kindly performed by Mr. L. W. Wilson. Abbreviations specified in the experimental section apply.



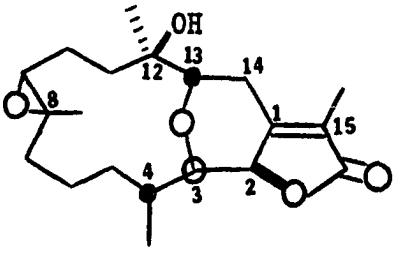
15

Table XI

Double Irradiation[a] of Isocueunicin Oxide (26)

Signal irrad'd	Signal obs'd	Change obs'd	Inference
5.02 (H-2)	4.00 (dd, H-3)	Collapsed to d	$J_{3,4}$=11
4.00 (H-3)	5.02 (d, H-2)	Collapsed to s	$J_{2,3}$=7
2.40 (H-14a)	3.62 (dd, H-13)	Collapsed to d	$J_{13,14b}$=11
			$J_{13,14a}$=4
	1.88 (m, 15-Me)	Sharpened	
	0.90 (d, 4-Me)	None	
1.88 (15-Me)	2.40 (H-14a)	Affected	
3.62 (H-13)	2.40 (H-14a)	Affected	
	2.92 (H-14b)[b]	Affected	

[a]Conditions specified in Table X apply. [b]Partially obscured signal.



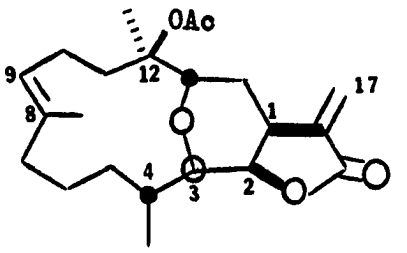
26

Table XII

Double Irradiation[a] of Cueunicin Acetate (16)

Signal irrad'd	Signal obs'd	Change obs'd	Inference
6.43 (H-17a)	3.46 (m, H-1)	Affected	
5.72 (H-17b)	3.46 (m, H-1)	Affected	
3.46 (H-1)	6.43 (d, H-17a)	Collapsed to s	$J_{1,17a}$=2.4
	5.72 (d, H-17b)	Collapsed to s	$J_{1,17a}$=2.4
	4.76 (dd, H-2)	Collapsed to d	$J_{2,3}$=2
4.76 (H-2)	3.34 (dd, H-3)	Collapsed to d	$J_{3,4}$=9
	3.46 (m, H-1)	Affected	
3.34 (H-3)	4.76 (dd, H-2)	Collapsed to d	$J_{1,2}$=8
1.52 (8-Me)	5.30 (C, H-9)	Sharpened	
5.30 (H-9)	1.52 (br, 8-Me)	Sharpened	

[a]Conditions specified in Table X apply



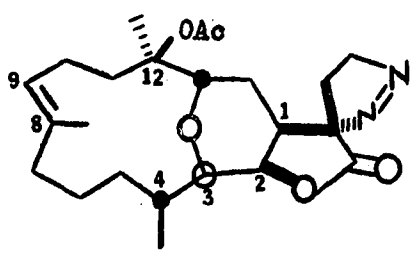
16

Table XIII

Double Irradiation[a] of the Pyrazoline of Cueunicin Acetate (38)

Signal irrad'd	Signal obs'd	Change obs'd	Inference
5.56 (H-2)	3.54 (dd, H-3)	Collapsed to d	$J_{3,4}$=10
	2.85 (m, H-1)	Affected	
2.85 (H-1)	5.56 (dd, H-2)	Collapsed to d	$J_{2,3}$=3
3.54 (H-3)	5.56 (dd, H-2)	Collapsed to d	$J_{1,2}$=6
1.56 (8-Me)	5.42 (c, H-9)	Sharpened	
5.42 (H-9)	1.56 (br, 8-Me)	Sharpened	

[a]Conditions specified in Table X apply



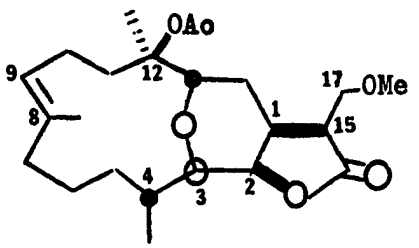
38

Table XIV

Double Irradiation[a] of the Methanol Adduct of Cueunicin Acetate (21)

Signal irrad'd	Signal obs'd	Change obs'd	Inference
4.76 (H-2)	3.30 (dd, H-3)	Collapsed to d	$J_{3,4}$=10
	2.90 (m, H-1)	Affected	
2.90 (H-1)	4.76 (dd, H-2)	Simplified to a distorted d	$J_{2,3}$=2
3.30 (H-3)	4.76 (dd, H-2)	Collapsed to d	$J_{1,2}$=8
1.54 (8-Me)	5.38 (c, H-9)	Affected	
5.38 (H-9)	1.54 (br, 8-Me)	Sharpened	

[a]Conditions specified in Table X apply



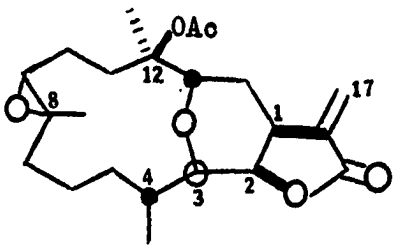
21

Table XV

Double Irradiation[a] of the Epoxide of Cueunicin Acetate (23)

Signal irrad'd	Signal obs'd	Change obs'd	Inference
6.42 (H-17a)	3.45 (m, H-1)	Affected	
5.74 (H-17b)	3.45 (m, H-1)	Affected	
3.45 (H-1)	6.42 (d, H-17a)	Collapsed to s	$J_{1,17a}$=2.4
	5.74 (d, H-17b)	Collapsed to s	$J_{1,17b}$=2.4
	4.76 (dd, H-2)	Collapsed to d	$J_{2,3}$=2.5
4.76 (H-2)	3.40 (dd, H-3)	Collapsed to d	$J_{3,4}$=10
	3.45 (m, H-1)	Simplified	
3.40 (H-3)	4.76 (dd, H-2)	Collapsed to d	$J_{1,2}$=7

[a]Conditions specified in Table X apply



23

signal was invariably a double doublet; i.e., H-3 was coupled to H-2 and H-4. The line separations of the H-2 and H-3 signals taken together established $J_{2,3}$ and hence $J_{1,2}$ unequivocally for all compounds discussed herein.

The vicinal coupling constant, $J_{2,3}$ in every cueunicin derivative was small (∿2 Hz), whereas the corresponding value in the eunicin series was invariably large (∿10 Hz),[6,7] which suggested that cueunicin and eunicin possess opposite configurations at C-3. The data are shown in Table XVI.

H-2 and H-3 are known to be anti in eunicin; therefore, the large values observed for $J_{2,3}$ were expected. On the other hand, the consistent occurrence of small values of $J_{2,3}$ suggested that H-2 and H-3 were syn in the cueunicin derivatives. This assignment was not unequivocal, however, since the tetrahydropyran ring could well exist in a different conformation in 15 than in 3 resulting in a narrowing of the dihedral angle between H-2 and H-3 even though H-2 and H-3 were transoid. Such a possibility is illustrated in the Newman projection shown in Figure 24 in which H-2 and H-3 are trans, but possess a small $J_{2,3}$ owing to the small dihedral angle separating them. Final assignment of the configuration at C-3 in cueunicin will be deferred until the chirality at C-13 is considered.

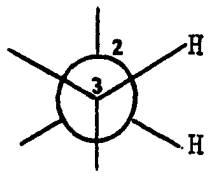
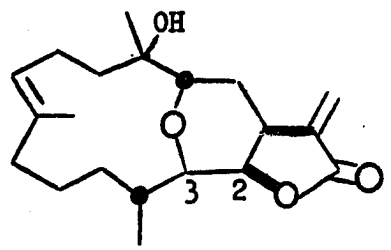


Figure 24

Table XVI

Vicinal Coupling Constants ($J_{2,3}$) for Eunicin and Cueunicin Derivatives

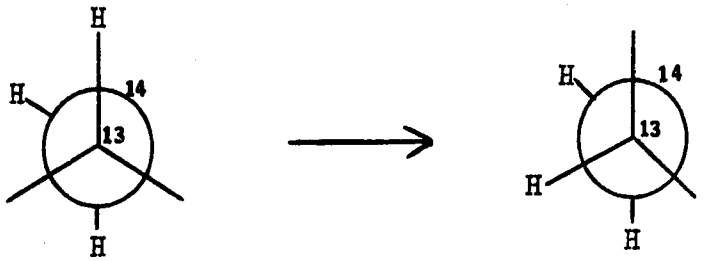
Cueunicin Series		Eunicin Series	
Compound	$J_{2,3}$	Compound	$J_{2,3}$
Cueunicin (15)	2	Eunicin (3)	10
Cueunicin Acetate (16)	2	Dihydroisoeunicin*	9
Dihydrocueunicin-I (17)	3	Isoeunicin*	10
Dihydrocueunicin-II (18)	2	Dihydroeunicin (34)	10
Dihydrocueunicin Acetate (19)	3	Eunicin Acetate (35)	10
Epoxycueunicin (22)	2	Keto-dilactone (36)	10
Epoxycueunicin Acetate (23)	2	Acetoxy-dilactone (37)	10
Keto-dilactone (27)	1.5		
Acetoxy-dilactone (30)	1.5		
Tetrahydrocueunicin Acetate (20)	3		
Methanol Adduct of 16 (21)	2		
Pyrazoline of 16 (38)	3		
Dihydroepoxycueunicin Acetate (24)	2		



Eunicin or Cueunicin

*Data taken from reference 6.

The first indication of the orientation of H-13 was gleaned from the multiplicity change incurred by its pmr signal in going from 17 to 27 (Chart V). The multiplicity of the signal in 17 was a double doublet (J=4 Hz and J=11 Hz), while in 27 it was a triplet (J=5.5 Hz). This suggested that the conformation along the C-13, C-14 bond underwent a change, i.e., H-13 was anti and gauche to the C-14 protons in 17 and gauche to both C-14 protons in 27 (See Figure 25[84]). A priori there were two possible structures from which the multiplicity change could be rationalized, one in which H-13 was beta oriented in 17, or one in which H-13 was alpha oriented in 17. These possibilities are considered in Figures 26 and 27. The two possibilities arise from the



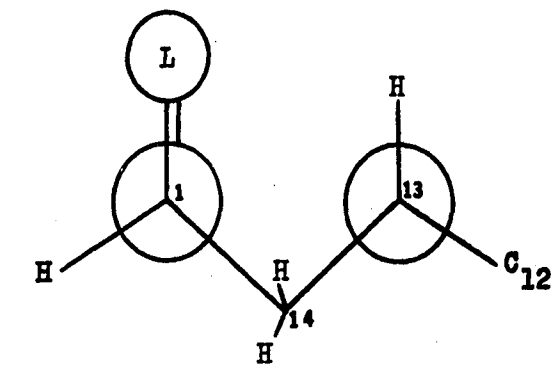
17A 27A

Figure 25

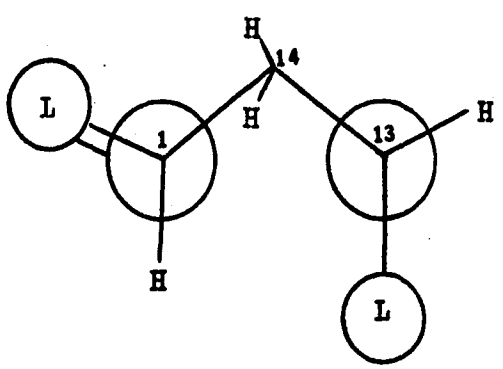
fact that H-13 in 17 is situated on a 6-membered ring and must have a 180° dihedral angle (J=11 Hz) with one of its H-14 neighbors as shown in the two conformations depicted for 17 in Figures 26 and 27. It is noteworthy that this is true regardless of the configuration at C-3 or the conformation of the tetrahydropyran ring, i.e., whether the residue of the ring generates a boat or chair conformation. The actual conformation of the ring will become clear later.

Figure 26

Conformational analysis of dihydrocoueunicin-I (17) going to the dihydro-keto-dilactone (27) with the C-13 proton beta oriented. (See Chart V for structures of 17 and 27)

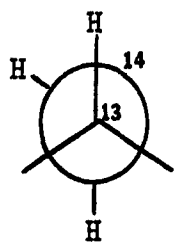


17B

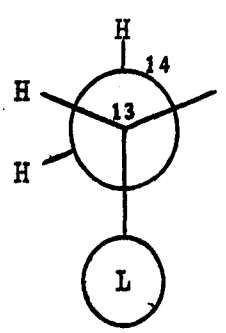


27B

Newman projection of the tetrahydropyran ring as viewed from C-1—C-14—C-13



17C



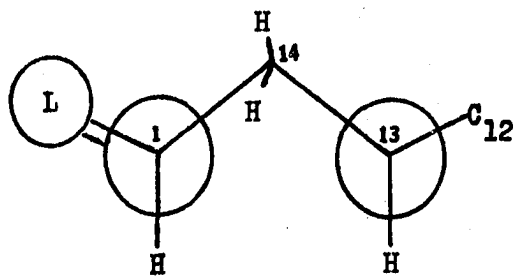
27C

Newman projection of the C-13—C-14 bond

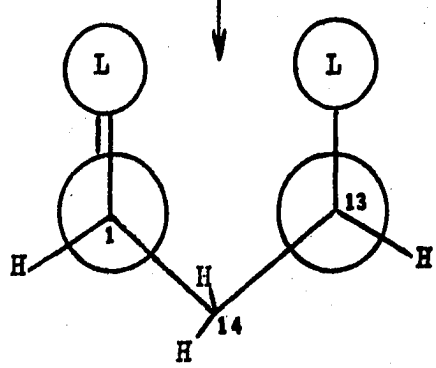
L = Lactone

Figure 27

Conformational analysis of dihydrocueunicin-I (17) going to the dihydro-keto-dilactone (27) with the C-13 proton alpha oriented. (See Chart V for structures of 17 and 27)

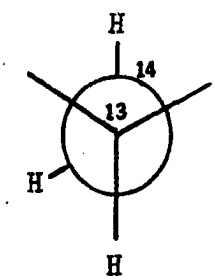


17D

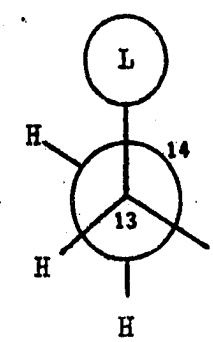


27D

Newman projection of the tetrahydropyran ring as viewed from C-1—C-14—C-13



17E



27E

Newman projection of the C-13—C-14 bond

L = Lactone

From these analyses it was clear that the situation depicted in Figure 26 was much more favorable than the one in Figure 27. In the former case the new lactone generated by the reaction would be sterically relatively unopposed while in the latter case the new lactone would encounter an extremely unfavorable interaction with the original γ-lactone. This latter situation would be analogous to the severe 1,3-diaxial interactions encountered by bulky substituents on a cyclohexane ring. Thus, H-13 was inferred to be beta. It should be pointed out, that if the triplet signal for H-13 in 27 arose from the rapid interconversion of one conformer to the other,[85] the above analysis would be invalid; therefore, additional evidence for the C-13 configuration was sought.

It was noted that the chemical shift of the H-13 pmr signal moved progressively upfield in the series 16, 38 and 19 (Table XVII).

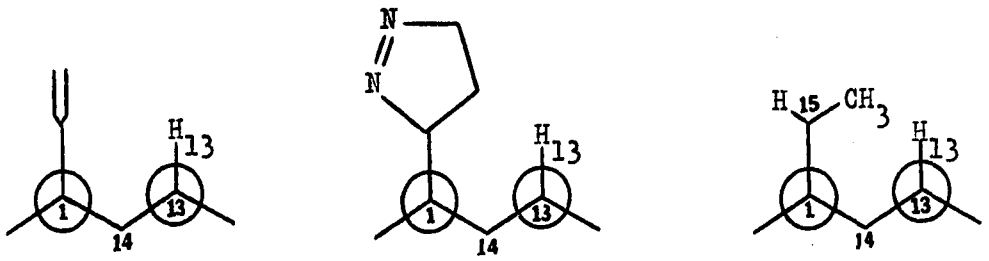
Table XVII

Shielding of the H-13 Proton in Cueunicin Acetate Derivatives

Compound	δ H-13 (ppm)	Multiplicity ($J_{13,14}$)
Cueunicin Acetate (16)	3.87	dd (3.5, 12)
Pyrazoline of 16 (38)	3.74	dd (3, 11)
Dihydro-16 (19)	3.56	dd (4, 11)

This differential shielding of the H-13 proton was interpreted to mean that H-13 and the γ-lactone were residing on the same side of the 6-membered ring. This interpretation would allow H-13 to be

successively shielded in the manner tabulated above by a methylene group in 38 and a methyl group in 19. Figure 28 shows the relative spatial orientations suggested by the data in Table XVII.

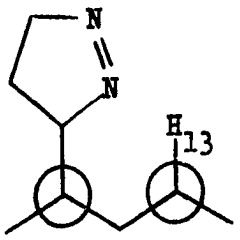


16A 38A 19A

Figure 28

Experimentally, the existence of a strong (17%) nuclear Overhauser effect (NOE)[83,86-90] between the 15-Me and H-13 in 19 confirmed the fact that these two groups were spatially very near one another, and firmly established H-13 as beta.

The firm beta assignment of H-13 reinforced the conclusion that the pyrazoline of cueunicin acetate had resulted from alpha approach of the diazomethane reagent producing the adduct 38 (Figure 28). Since H-13 in the alternative adduct 38B (beta approach of CH_2N_2) would lie in the deshielding cone of the diazo group, it would be expected to give rise to a deshielded pmr signal relative to that of H-13 in cueunicin acetate, rather than the observed shielded signal (Table XVII). In addition, models clearly demonstrated that the proton (H-2) attached to the carbon bearing the lactone ether oxygen

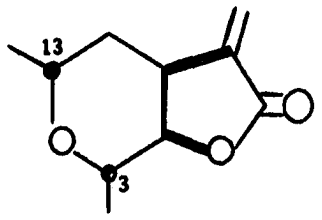


38B

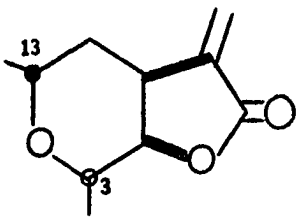
would lie in the deshielding cone of the diazo group in 38, but not in 38B; therefore, the significant paramagnetic shift ($\Delta\nu$ = 0.80 ppm) incurred by the pmr signal of H-2 on formation of the pyrazoline derivative also lent support to the assignment of the pyrazoline adduct 38A rather than 38B.

The assignment of H-13 as beta allowed H-3 to be unequivocally assigned alpha. This derived from the fact that, with the cis lactone ring fusion established, there were four possible substitution patterns for the tetrahydropyran ring (Figure 29). Only two of these structures (T and U) possessed H-13 beta, and one of them (T) corresponded to the eunicin system; therefore, the other (U) represented cueunicin. The latter structure was compatible with the aforementioned small values of $J_{2,3}$ in the cueunicin series (Table XVI). Thus, the partial stereoformula of cueunicin was further elaborated as 15B.

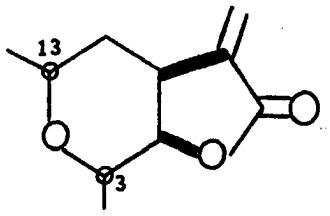
The partial structure 15B was found to be fully in accord with the results deduced from the conformational analysis in Figure 26 if the residue of the tetrahydropyran ring were assumed to generate



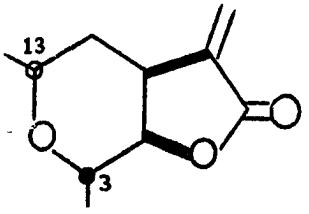
T



U

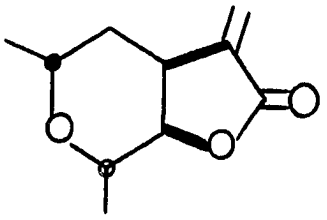


V



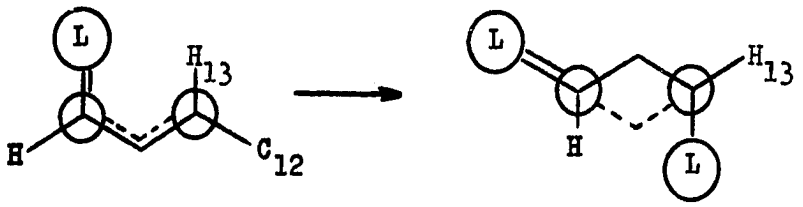
W

Figure 29



15B

a boat conformation in 17 and a chair in 27 (Figure 30). These conformations were not only compatible with the observed coupling constants of H-2, H-3, and H-13 in both compounds, but also allowed the bulky alkyl substituents attached to the 6-membered ring at C-3 and C-13

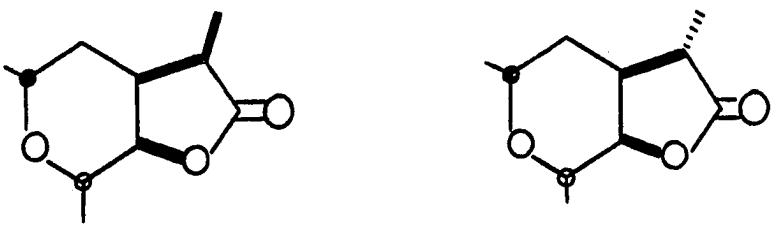


17F 27F

Figure 30

to assume pseudoequitorial orientations with respect to that ring in 17. Severing the 14-membered ring in 17 to produce 27 presumably allowed the tetrahydropyran ring to adopt the normally energetically more favorable chair conformation despite the pseudoaxial orientation of the new lactone moiety attached at C-13 necessitated by the chair conformer.

The NOE determined in 19 provided additional evidence for the gross structure of cueunicin as 15, and allowed assignments to be made to the stereochemistry of the epimeric methyl groups in dihydrocueunicin-I and dihydrocueunicin-II. A single product (19) was obtained on sodium borohydride reduction of cueunicin acetate. The

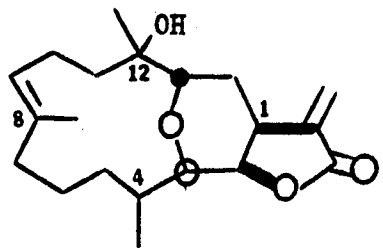


17A 18A

solvent would be expected to approach from the less hindered (alpha) side of the lactone ring to produce a new beta 15-Me. (See 19 in Figure 28.) The NOE confirmed the presence of a beta 15-Me in 19 and hence alpha attack by the proton from solvent (ethanol). Since cueunicin afforded dihydrocueunicin-I (17) exclusively on sodium borohydride reduction, then by analogy with 19,17 must have been the 15-Me beta isomer and 18 the 15-Me alpha isomer. The opposite configurations at C-15 accounted for the different chemical shifts of the new methyl and proton signals discussed earlier, as well as the different mobility of 17 and 18 on tlc.

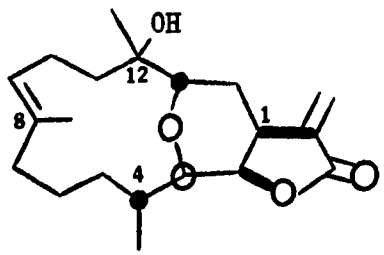
The success of the NOE experiment in 19 in establishing the configuration at C-13 prompted an NOE investigation of the trisubstituted double bond in the same molecule. Irradiation of the 8-Me signal produced no noticable effect on the integral of the H-9 signal and Δ^{8-9} was thus established trans.[91-92] The trans nature of the trisubstituted double bond in cueunicin accounted for the relatively high field at which the 8-Me group was found to absorb in the pmr spectrum, e.g., δ1.56 in cueunicin and 1.52 in cueunicin acetate.[93-95] Cueunicin could then be represented by 15C below, in which the double bond at C-8, C-9 is trans.

The configurations at C-4 and C-12 remained to be established. The configuration at C-4 was deduced by analyzing the magnitude of the H-3, H-4 couplings in cueunicin and its derivatives (Table XVIII). The large values of $J_{3,4}$ indicated that H-3 and H-4 were transoid[96] and presuming that the 4-Me would prefer a spatial orientation outward from the 14-membered ring rather than an inward orientation, the



15C

H-4 proton was inferred to be beta as shown below (15D). It is noteworthy that the corresponding $J_{3,4}$ values in the eunicin series are small (Table XVIII) owing to the cisoid nature of H-3 and H-4.



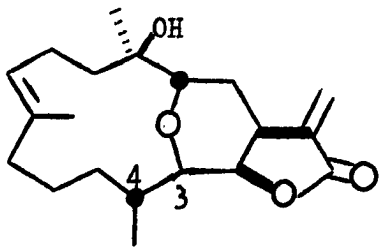
15D

Data which would rigorously demonstrate the configuration at C-12 was not obtained; however, the data presented earlier in Table III suggested that the hydroxyl at C-12 and the proton at C-13 were syn,[97] due to the large paramagnetic shift induced in the H-13 pmr signal on acetylation of the 12 hydroxyl group in the various cueunicin derivatives. The downfield shift of the H-13 signal in eunicin was only 0.11 ppm when eunicin was converted to eunicin acetate compared to a shift of 0.39 ppm upon conversion of cueunicin

Table XVIII

Vicinal Coupling Constants ($J_{3,4}$) for Cueunicin and Eunicin Derivatives

Cueunicin Series		Eunicin Series	
Compound	$J_{3,4}$	Compound	$J_{3,4}$
Cueunicin (15)	10	Eunicin (3)	0
Cueunicin Acetate (16)	9	Dihydroeunicin (34)	0
Dihydrocueunicin-II (18)	10	Eunicin Acetate (35)	0
Dihydrocueunicin Acetate (19)	10	Keto-dilactone (36)	2.5
Epoxycueunicin Acetate (23)	10	Acetoxy-dilactone (37)	2.5
Keto-dilactone (27)	9		
Acetoxy-dilactone (30)	9		
Tetrahydrocueunicin Acetate (20)	10		
Methanol Adduct of 16 (21)	10		
Pyrazoline of 16 (38)	10		
Dihydroepoxycueunicin Acetate (24)	10		
Isocueunicin oxide	11		



Eunicin or Cueunicin

to cueunicin acetate (Table XIX). This indicated that H-13 in cueunicin was in even closer proximity to its neighboring hydroxyl function than was the corresponding proton in eunicin. The substituents are known to be syn (beta) in eunicin which suggests that the dihedral angle between the 12-OH and H-13 groups in cueunicin is less than that in eunicin although both are syn and beta.

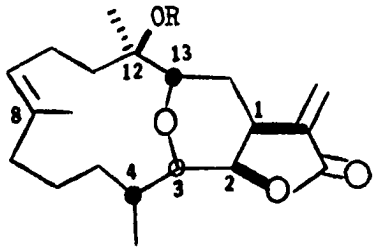
Similarly, a comparison of the chemical shift of the H-13 proton in cueunicin with that of eunicin revealed that the signal occurred 0.20 ppm further downfield in cueunicin. Likewise, a comparison of the chemical shifts of H-13 in cueunicin acetate and eunicin acetate showed that the signal occurred 0.48 ppm further downfield in cueunicin acetate (Table XIX). Assuming that H-13 is similarly situated (pseudoaxially) on the tetrahydropyran ring in all four compounds, then the downfield shift of the signal in the cueunicin derivatives is attributable to an increased deshielding influence by its neighboring hydroxy or acetoxyl functions. The double doublet nature of the H-13 signal in all four compounds supported the inference that H-13 bore a pseudoaxial relation to the tetrahydropyran ring, i.e., it was anti and gauche to the C-14 protons in all four compounds. These comparative analyses pointed to a 12-beta-hydroxyl function in cueunicin; a beta-hydroxyl would be in a more favorable disposition to produce the noted effects on the beta proton at C-13. Therefore, structure 15 below is proposed to represent the absolute configuration of cueunicin and 16 its acetate.

In conclusion, the structures of cueunicin and its acetate elucidated above represent novel diterpene lactones, and mark the first

Table XIX

Comparison of the Chemical Shift of the H-13 PMR Signal in Cueunicin, Eunicin, and Their Acetates

		δ H-13 (ppm)		δ H-13 (ppm)	Δν
Cueunicin Acetate		3.87	Cueunicin	3.48	0.39
Eunicin Acetate		3.39	Eunicin	3.28	0.11
	Δν	0.48		0.20	



15 R = H

16 R = Ac

reported instance in which the free hydroxyl and acetylated forms of a cembranolide coexist in a Caribbean gorgonian.

The discovery of cueunicin and cueunicin acetate in Curacao E. mammosa also establishes another case in which the compounds produced by a given gorgonian are tied to a specific geographical location. This chemical geospecificity has been noted previously for E. mammosa, E. palmeri, and P. homomalla. E. mammosa produces eunicin (Florida),[3] and jeunicin (Jamaica);[8] E. palmeri[13] yields eupalmerin acetate (Miami) and eunicin (Florida Keys); and P. homomalla contains

(15R) PGA_2 (Florida)[100] and (15S)-PGA_2 (other Caribbean sites).[101] This variation of chemical constituents appears to be a valid measure of some of the dynamic evolutionary processes at work in these sessile marine organisms, and underscores the importance of widespread geographical studies for a given marine invertebrate.

The 14-membered ring in cueunicin can be considered to be formed biogenetically in a manner analogous to that discussed earlier for crassin acetate and the other gorgonian derived cembranolides.

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-4 and CC-7 special for column chromatography and silica gel (Grace, 60-200 mesh). The Lemieux-von Rudloff reagent was prepared by diluting 0.20 g of potassium permanganate and 11.50 g of potassium periodate to 500 ml with water and heating on a hot plate (a steam bath proved insufficient) until all solid material had dissolved.[66]

Thin layer chromatography plates were prepared by coating 5 x 20 cm glass plates with Merck (Darmstadt) silica gel H or silica gel G to a thickness of approximately 1 mm. The developed chromatograms were visualized with iodine vapor, sulfuric acid spray or 1% ceric sulfate in 35% sulfuric acid.

Rotations were run on a Gaertner polorimeter, ultraviolet spectra in 95% ethanol on a Cary model 14 recording spectrophotometer or a Hitachi Perkin-Elmer model 124 spectrophotometer, and infrared spectra on a Beckman IR-8 spectrophotometer. Low resolution mass spectra were obtained by Mr. H. Curtis on a Hitachi Perkin-Elmer RMU-7 double focusing spectrometer. 100 MHz and 25.2 MHz nuclear magnetic resonance spectra were run by Mr. L. Wilson on a Varian XL-100 spectro-

meter. 60 MHz proton magnetic resonance (pmr) spectra were obtained on a Varian T-60 spectrometer. Pmr chemical shifts are reported in δ-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 or line separations in Hertz (Hz), and, when assignable, the specific protons which give rise to the reported signal. The numbering system used herein for natural product derivatives is derived from the corresponding carbon atoms of the parent natural product. The multiplicities are reported as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; dt, doubled triplet; c, complex signal; q, quartet; br, broad singlet; and m, multiplet.

High resolution mass spectra are courtesy of Dr. C. Hignite, Massachusetts Institute of Technology. Circular dichroism (CD) curves were kindly provided by Dr. E. Bunnenberg, Stanford University. Certain decoupling experiments were performed by Dr. N. S. Bhacca, Louisiana State University, and carbon-13 magnetic resonance spectra of the gorgonian diterpenoids of established structure were kindly provided by Dr. E. Wenkert, Rice University.

Analyses were performed by Mr. E. Meier, Stanford University.

Isolation of Cueunicin (15) and Cueunicin Acetate (16) -- Eunicea mammosa (Lamouroux), wt 1.01 kg, collected by Dr. Leon S. Ciereszko on December 29, 1972 off the coral reef (Pest Baai) neighboring Curacao, Netherlands Antilles, was extracted with hexane.[102]

The concentrated hexane extract was placed in a refrigerator at 7°C overnight. The insoluble portion was filtered and afforded 11.29 g of green pigmented solid material on drying. The solid was halved and each portion chromatographed on a 300 g silica gel (Grace 60-200 mesh) column. Fractions (120 ml) were collected employing 4:1 benzene-ethyl acetate as solvent. Fractions 9-13 from both chromatographies showed a major spot on tlc and were combined and rechromatographed as before to yield, after several recrystallizations from benzene, 1.82 g of pure cueunicin acetate (16), a white crystalline solid. Fractions 23-35 likewise showed a major spot on tlc and the combined fractions from both chromatographies were rechromatographed yielding 542 mg of cueunicin (15) as a white gum. Various attempts to induce crystallization failed.

Pure cueunicin (15) had $[\alpha]_D^{23.5}$ - 147° (c 1.1, $CHCl_3$); R_f = 0.23 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 3605, 3500 (hydroxyl), 1762, 1660 (α,β'-unsaturated γ-lactone), 1448, 1274, 1148, 1012, 944 cm^{-1}; uv absorption at 218 nm (ε 4300); 100 MHz pmr ($CDCl_3$) δ 6.44 (d, 1H, J = 2.5 Hz, H-17a), 5.74 (d, 1H, J = 2.5 Hz, H-17b), 5.36 (t, 1H, J = 8 Hz, H-9), 4.78 (dd, 1H, J = 2 Hz, J = 8 Hz, H-2), 3.48 (dd, 1H, J = 3 Hz, J = 11.5 Hz, H-13), 3.45 (m, 1H, H-1), 3.40 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 1.56 (s, 3H, 8-Me), 1.09 (s, 3H, 12-Me), and 0.92 ppm (d, 3H, J = 7 Hz, 4-Me); 100 MHz pmr ($PhH-d_6$) δ 6.28 (d, 1H, J = 2.4 Hz, H-17a), 5.44 (t, 1H, J = 7 Hz, H-9), 5.25

(d, 1H, J = 2.4 Hz, H-17b), 4.15 (dd, 1H, J = 2 Hz, J = 8 Hz, H-2), 3.44 (dd, 1H, J = 6.5 Hz, J = 8 Hz, H-13), 2.95 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 2.78 (m, 1H, H-1), 1.51 (s, 3H, 8-Me), 1.01 (s, 3H, 12-Me) and 0.78 ppm (d, 3H, J = 7 Hz, 4-Me); 25.2 MHz proton decoupled cmr ($CDCl_3$) δ 170.1 (C=O), 1.39.4 (C=C), 131.3 (C=C), 128.3 (-C=CH), 123.6 (C=CH_2), 75.8 (C-O), 74.3 (C-O-), 74.3 (C-O-), 73.7 (C-O-), 41.7, 36.0, 34.8, 31.7, 28.5, 28.0, 22.7, 22.1, 21.3, 15.4 and 15.4 ppm; Mass spectrum (70 eV) m/e (rel intensity) M^+, 334 (18), 316 (8), 193 (4), 164 (6), 163 (5), 161 (4), 153 (10), 151 (4), 149 (10), 147 (8), 137 (8), 136 (7), 135 (7), 134 (8), 133 (8), 123 (8), 121 (8), 119 (5), 111 (9), 109 (14), 108 (9), 107 (10), 105 (7), 95 (16), 94 (6), 93 (11), 91 (3), 85 (17), 83 (22), 82 (11), 81 (37), 79 (23), 77 (9), 71 (12), 69 (22), 67 (27), 57 (12), 55 (55), 53 (24), base peak 43 (100) and 41 (73).

Anal. Calcd for $C_{20}H_{30}O_4$: mol wt, 334.21441. Found: mol wt (mass spectrum) 334.21470.

Cueunicin acetate (16) had mp 141-142°; $[\alpha]_D^{23}$ -152° (c 1.2, $CHCl_3$); R_f = 0.55 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 1759, 1660 (α,β'-unsaturated γ-lactone), 1728, 1255 (acetate), 1368, 1145, 1085, 1012, 942 cm^{-1}; ir (CCl_4) 1772, 1660 (α,β'-unsaturated γ-lactone), 1732, 1240 cm^{-1} (acetate); uv absorption at 215 nm (ε 6000); CD curve 263 nm ([θ] + 1730, c 1.01); 100 MHz pmr ($CDCl_3$) δ 6.43 (d, 1H, J = 2.4 Hz, H-17a), 5.72 (d, 1H, J = 2.4 Hz, H-17b), 5.30 (br, 1H, H-9), 4.76 (dd, 1H, J = 2 Hz, J = 8 Hz, H-2), 3.87 (dd, 1H, J = 3.5 Hz, J = 12 Hz, H-13), 3.46 (m, 1H, H-1), 3.34 (dd, 1H, J = 2 Hz, J = 9 Hz, H-3), 1.95 (s, 3H, OAc), 1.52 (s, 3H, 8-Me), 1.38 (s, 3H, 12-Me), and

0.91 ppm (d, 3H, J = 7 Hz, 4-Me), 60 MHz pmr (PhH-d_6) δ 6.38 (d, 1H, J = 2.4 Hz, H-17a), 5.21 (m, 1H, H-9), 5.35 (d, 1H, J = 2.4 Hz, H-17b), 4.25 (dd, 1H, J = 2 Hz, J = 9 Hz, H-2), 4.01 (dd, 1H, J = 5 Hz, J = 11 Hz, H-13), 2.97 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 1.76 (s, 3H, OAc), 1.52 s, 3H, 8-Me), 1.43 (s, 3H, 12-Me), and 0.92 ppm (d, 3H, J = 7 Hz, 4-Me); 25.2 MHz proton decoupled cmr ($CDCl_3$) δ 169.9 (C=O), 169.3 (C=O), 139.0 (C=C), 130.9 (C=C), 128.5 (C=C-H), 123.5 ($C=CH_2$), 85.6 (C-OAc), 75.4 (HC-O), 75.4 (HC-O), 72.4 (HC-O), 37.9 35.8, 34.4, 31.7, 28.9, 28.1, 22.2, 22.2, 22.2, 17.7, 15,8 and 14.8 ppm; mass spectrum (70 eV) m/e (rel intensity) M^+, 376 (2), 334 (1), 333 (1), 316 (34), 301 (2), 259 (1), 245 (3), 232 (3), 220 (3), 208 (5), 207 (4), 206 (5), 177 (6), 163 (9), 161 (10), 149 (13), 147 (10), 135 (20), 133 (14), 121 (26), 119 (14), 109 (35), 108 (63), 107 (30), 105 (19), 95 (41), 93 (40), 91 (19), 81 (53), 79 (25), 69 (37), 67 (29), 55 (39), 53 (21), base peak 43 (100), and 41 (38). The high resolution mass spectrum exhibited a significant M^+ peak (35%).

Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57; mol wt 376.22497. Found: C, 70.24; H, 8.41; mol wt (mass spectrum) 376.22240.

Cueunicin Acetate (16) from Cueunicin (15) -- To a solution of 83.9 mg of cueunicin in 3 ml of acetic anhydride and 3 ml of dry ether at -30° C was added 3 drops of boron trifluoride etherate. The boron trifluoride etherate was distilled immediately before use and cooled to -20°C prior to its addition to the reaction mixture. The reaction was monitored by tlc, and after 20 min a spot corresponding to cueunicin acetate was observed. The reaction mixture was quenched after 25 min. by pouring it into a mixture of pyridine (0.6 ml) and water (25 ml). A white precipitate formed immediately. The mixture was extracted with

chloroform (3 x 50 ml) and the chloroform extract washed with water (5 x 25 ml), 2% HCl (2 x 25 ml), 5% sodium bicarbonate (2 x 25 ml), and water (2 x 25 ml) and then dried ($MgSO_4$). Tlc indicated that a trace of cueunicin remained together with the desired cueunicin acetate. The mixture was chromatographed on 16 g (1.5 x 47 cm) of silicAR CC-7 (Mallinckrodt) at a flow rate of 10 ml/5 min using 4:1 benzene-ethyl acetate as solvent. Fractions (10 ml) were collected and fractions 5 and 6 were combined and recrystallized three times from benzene-hexane to yield 24.2 mg of cueunicin acetate, identical in all respects (ir, pmr, rotation, tlc, mass spectrum, mp and mmp undepressed) with naturally occurring cueunicin acetate (16).

Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57. Found: C, 70.04; H, 8.34.

Fractions 7-9 afforded 8.8 mg of unreacted cueunicin (15).

Deacetylation of Cueunicin Acetate -- A solution of cueunicin acetate (86.1 mg) and 20 ml of 1N potassium hydroxide in ethylene glycol (20 ml) was stirred at 110°C for 5 hr. The reaction mixture was cooled, acidified (conc. HCl), and extracted with ether (3 x 50 ml). The ether extract was concentrated to 40 ml and washed with 5% sodium bicarbonate (2 x 25 ml) and water (2 x 25 ml), dried ($MgSO_4$) and evaporated under reduced pressure giving a yellow gum. The yellow color was removed with decolorizing charcoal affording 48.7 mg of a white gum, which resisted crystallization from a variety of solvents. The gum was identical (tlc, ir, pmr, and ms) to authentic cueunicin isolated from Curacao E. mammosa.

Sodium-n-Butanol Reduction of Cueunicin Acetate -- A solution of cueunicin acetate (88.0 mg) in n-butanol (12 ml) was refluxed under

a nitrogen atmosphere. Finely divided sodium (281 mg, freshly cut) was added as rapidly as possible and refluxing continued for 40 min. The mixture was acidified (10% HCl) and most of the n-butanol evaporated under reduced pressure. The mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with 5% sodium bicarbonate and water, then dried ($MgSO_4$). Tlc revealed two spots R_f = 0.24 and 0.19, (3:1 benzene-ethyl acetate, silica gel H). The mixture was chromatographed on 16 g of silicic acid (silicAR CC-7, 1.2 x 52 cm) with 4:1 benzene-ethyl acetate (10 ml fractions). Fractions 12-15 afforded 21.8 mg of a white gum, which resisted crystallization. Dihydrocueunicin-I (17) had: ir ($CHCl_3$) 3605, 3500 (hydroxyl), and 1767 cm^{-1} (γ-lactone); R_f = 0.24; 60 MHz pmr ($CDCl_3$) δ 5.43 (m, 1H, H-9), 4.68 (dd, 1H, J = 3 Hz, J = 6.5 Hz, H-2), 3.35 (m, 2H, H-3, H-13), 2.80 (m, 1H, H-15), 1.53 (s, 3H, 8-Me), 1.23 (d, 3H, J = 7 Hz, 15-Me), 1.02 (s, 3H, 12-Me), and 0.93 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 336 (20), 318 (15), 263 (10), 262 (5), 245 (4), 223 (4), 219 (3), 197 (7), 193 (4), 184 (18), 181 (7), 177 (6), 166 (9), 165 (7), 163 (9), 161 (8), 159 (5), 157 (5), 151 (10), 149 (12), 147 (8), 139 (13), 137 (15), 135 (20), 123 (28), 121 (27), 111 (24), 109 (42), 108 (33), 107 (31), 95 (52), 94 (34), 93 (42), 81 (56), 79 (27), 71 (32), 69 (54), 57 (20), 55 (85), base peak 43 (100), 41 (75).

An analysis was not obtainable for 17 because it decomposed.

Fractions 17-21 afforded a solid, which was recrystallized from benzene-hexane to yield 21.0 mg of a white crystalline solid, dihydrocueunicin-II (18): mp 142-143°; R_f = 0.19; ir ($CHCl_3$) 3605, 3500

(hydroxyl), and 1767 cm^{-1} (γ-lactone); 100 MHz pmr ($CDCl_3$) δ 5.36 (t, 1H, J = 7 Hz, H-9), 4.74 (dd, 1H, J = 2 Hz, J = 8 Hz, H-2), 3.56 (dd, 1H, J = 3 Hz, J = 12 Hz, H-13), 3.33 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 2.54 (q, 1H, J = 7 Hz, H-15), 1.56 (s, 3H, 8-Me), 1.39 (d, 3H, J = 7 Hz, 15-Me), 1.06 (s, 3H, 12-Me), and 0.92 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 336 (24), 318 (18), 263 (5), 262 (6), 245 (5), 223 (6), 219 (4), 197 (13), 193 (4), 184 (26), 181 (8), 177 (7), 166 (15), 165 (13), 163 (12), 161 (13), 159 (10), 157 (12), 151 (11), 149 (21), 147 (13), 139 (18), 137 (23), 135 (27), 123 (11), 121 (13), 111 (10), 109 (20), 108 (15), 107 (15), 95 (27), 94 (22), 93 (20), 81 (30), 79 (15), 71 (18), 69 (28), 57 (15), 55 (60), base peak 43 (100), 41 (43).

Anal. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.29; H, 9.75.

Dihydrocueunicin-I (17) -- Cueunicin (3.1144 g) in absolute ethanol (50 ml) was cooled in an ice bath. To the stirred solution was added 662.3 mg of sodium borohydride. The ice bath was removed and the reaction mixture allowed to warm to room temp. Stirring was continued for 2 hr. The reaction mixture was acidified with 10% HCl and most of the ethanol removed under reduced pressure leaving a white residue. The residue was taken up in chloroform, and washed successively with water (30 ml), 5% sodium bicarbonate (20 ml) and water (20 ml) then dried ($MgSO_4$). Removal of the solvent gave 2.6866 g of dihydrocueunicin-I which was identical (tlc, ir, pmr, mass spectrum) with dihydrocueunicin-I (17) obtained on treatment of cueunicin acetate with sodium/n-butanol. No dihydrocueunicin-II (18) was detected.

Dihydrocueumicin Acetate (19) -- A solution of 95.6 mg of cueumicin acetate in 6 ml of absolute ethanol was stirred with 18.4 mg of sodium borohydride at room temp for 2 hr. The mixture was acidified and the solvents evaporated under reduced pressure. The mixture was diluted with water and extracted with chloroform. The chloroform layer was washed (H_2O) and dried ($MgSO_4$). Evaporation of the chloroform afforded a white solid which was recrystallized from ethanol to yield 87.5 mg of white needles: mp 159.5-161°; R_f = 0.67 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 1769 (γ-lactone), 1728 and 1250 cm^{-1} (acetate); 100 MHz pmr ($CDCl_3$) δ 5.46 (t, 1H, J = 7 Hz, H-9), 4.74 (dd, 1H, J = 3 Hz, J = 6 Hz, H-2), 3.56 (dd, 1H, J = 4 Hz, J = 11 Hz, H-13), 3.39 (dd, 1H, J = 3 Hz, J = 10 Hz, H-3), 2.93 (m, 1H, H-15), 1.98 (s, 3H, OAc), 1.54 (s, 3H, 8-Me), 1.38 (s, 3H, 12-Me), 1.28 (d, 3H, J = 7 Hz, 15-Me), and 0.88 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 378 (2), 318 (63), 261 (5), 245 (14), 149 (14), 147 (12), 137 (13), 136 (11), 135 (24), 134 (11), 133 (12), 123 (18), 122 (14), 121 (25), 119 (14), 111 (12), 109 (44), 108 (53), 107 (30), 105 (14), 97 (16), 96 (14), 95 (46), 94 (29), 93 (42), 91 (15), 83 (17), 82 (17), 81 (54), 79 (31), 77 (14), 71 (12), 69 (42), 68 (23), 67 (36), 60 (22), 57 (15), 55 (75), 53 (21), 45 (32), base peak 43 (100) and 41 (62).

Anal. Calcd for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.84; H, 9.29.

Tetrahydrocueumicin Acetate (20) -- A solution of 125.4 mg of cueumicin acetate in 20 ml of absolute ethanol was hydrogenated at atmospheric pressure and room temp over prereduced platinum oxide (7.3 mg)

for 10 hr. The filtered solution was evaporated in vacuo, and the residue recrystallized from benzene-hexane affording 119.2 mg of white prisms: mp 133-134°; R_f = 0.59 (1:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 1770 (γ-lactone), 1728 and 1250 cm^{-1} (acetate); 60 MHz pmr ($CDCl_3$) δ 4.73 (dd, 1H, J = 3 Hz, J = 6 Hz, H-2), 3.58 (t, 1H, J = 7.5 Hz, H-13), 3.50 (dd, 1H, J = 3 Hz, J = 10 Hz, H-3), 2.84 (m, 1H, H-15), 1.95 (s, 3H, OAc), 1.43 (s, 3H, 12-Me), 1.30 (d, 3H, J = 7 Hz, 15-Me), 0.95 (d, 3H, J = 7 Hz, 4-Me) and 0.87 ppm (d, 3H, J = 7 Hz, 8-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 380 (9), base peak 337 (100), 320 (38), 291 (7), 263 (83), 205 (2, meta stable), 123 (12), 121 (10), 109 (23), 107 (12), 97 (10), 95 (27), 83 (15), 81 (23), 71 (17), 69 (34), 67 (12), 55 (39), 43 (70), and 41 (41).

Anal. Calcd for $C_{22}H_{36}O_5$: C, 69.43; H, 9.54. Found: C, 69.60; H, 9.44.

Methanol Adduct of Cueunicin Acetate (21) -- A solution of 324 mg of cueunicin acetate in 35 ml of 60% aqueous methanol containing 279 mg of KOH was stirred at room temperature for 21 hrs. Most of the methanol was removed by evaporation at reduced pressure and the aqueous solution acidified with 10% H_2SO_4. A white precipitate formed which was taken up in chloroform, washed with 5% sodium bicarbonate and dried ($MgSO_4$). Evaporation of the solvent left an oily residue which crystallized on trituration with hexane. Recrystallization of the solid from chloroform-hexane afforded 166 mg of a white solid which proved to be a mixture (two distinct - OMe signals at δ 3.33 and 3.40) of the epimeric Michael adducts of methanol to the exomethylene group of cueunicin acetate. An attempt to increase the yield by vigorous extraction of

the acidified aqueous solution with chloroform resulted in the recovery of an additional 80 mg of solid which proved to consist entirely of only one of the two possible Michael adducts: mp 114-114.5°; R_f = 0.79 (1:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 1766 (γ-lactone), 1728 and 1250 cm^{-1} (acetate); 100 MHz pmr ($CDCl_3$) δ 5.38 (c, 1H, H-9), 4.76 (dd, 1H, J = 2 Hz, J = 8 Hz, H-2), 3.91 (dd, 1H, J = 3 Hz, J = 12 Hz, H-13), 3.72 (m, 2H, H-17), 3.40 (s, 3H, OMe), 3.30 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 2.91 (m, 1H, H-15), 1.98 (s, 3H, OAc), 1.54 (s, 3H, 8-Me), 1.36 (s, 3H, 12-Me), and 0.88 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 408 (3), 365 (2), 348 (45), 316 (4), 261 (9), 245 (16), 179 (12), 165 (12), 164 (10), 163 (16), 162 (10), 161 (18), 151 (15), 149 (20), 148 (10), 147 (17), 145 (10), 137 (20), 136 (26), 135 (40), 134 (24), 133 (23), 126 (10), 125 (11), 123 (33), 122 (22), 121 (73), 120 (17), 119 (24), 111 (13), base peak 108 (100), 107 (58), 105 (26), 97 (17), 96 (16), 95 (71), 94 (47), 93 (58), 91 (22), 85 (12), 83 (21), 82 (24), 81 (71), 80 (12), 79 (45), 77 (10), 71 (19), 69 (49), 68 (25), 67 (46), 60 (11), 57 (11), 55 (51), 53 (15), 45 (67), 43 (82), and 41 (37).

Anal. Calcd for $C_{22}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.78; H, 8.93.

Epoxidation of Cueunicin -- To a stirred solution of 1.6346 g of cueunicin in 70 ml of chloroform cooled in an ice bath was added 1.0 g of m-chloroperbenzoic acid. The ice bath was removed and the solution was allowed to warm to room temp. The solution was stirred for 16 hr. At this time tlc showed the reaction to be complete. The chloroform solution was washed successively with 30 ml of 10% sodium

sulfite, 40 ml of 1M sodium bicarbonate and 50 ml of water, dried ($MgSO_4$) and the solvent removed leaving a clear residue which crystallized on trituration with hexane. The solid was recrystallized from chloroform-benzene-hexane affording 746.8 mg of white crystals (22): mp 170-172°; R_f = 0.21 (1:1 benzene-ethyl acetate; silica gel G), ir ($CHCl_3$) 3500 (hydroxyl), 1765 and 1658 cm^{-1} (α,β'-unsaturated γ-lactone); 100 MHz pmr ($CDCl_3$) δ 6.45 (d, 1H, J = 2.4 Hz, H-17a), 5.72 (d, 1H, J = 2.4 Hz, H-17b), 4.80 (dd, 1H, J = 2 Hz, J = 9 Hz, H-2), 3.56 (m, 3H, H-1, H-3, and H-13), 3.17 (dd, 1H, J = 5 Hz, J = 8 Hz, H-9), 1.23 (s, 3H, 8-Me), 1.12 (s, 3H, 12-Me), and 0.93 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 350 (2), 332 (12), 111 (16), 109 (25), 95 (14), 93 (11), 85 (20), 84 (11), 83 (29), 81 (13), 79 (11), 78 (32), 77 (12), 67 (11), 55 (23), 44 (11), base peak 43 (100), and 41 (34).

Anal. Calcd for $C_{20}H_{30}O_5$: C, 68.55; H, 8.63. Found: C, 68.62; H, 8.61.

An additional 652.1 mg of epoxycueunicin was recovered from the mother liquor.

Epoxide of Cueunicin Acetate (23) -- A solution of cueunicin acetate (100 mg) and m-chloroperbenzoic acid (190 mg) was stirred at room temp in 15 ml of chloroform. After 20 hr tlc showed the reaction to be complete. The solution was washed successively with 10% sodium sulfite, 1M sodium bicarbonate and water, then dried ($MgSO_4$). Most of the chloroform was removed under reduced pressure. Trituration with hexane afforded a solid which was recrystallized (chloroform-hexane) to yield 97.9 mg of a white crystalline compound: mp 151-152°; R_f

= 0.29 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 1765 (γ-lactone), 1729 and 1250 cm^{-1} (acetate); 100 MHz pmr ($CDCl_3$) δ 6.42 (d, 1H, J = 2.4 Hz, H-17a), 5.74 (d, 1H, J = 2.4 Hz, H-17b), 4.76 (dd, 1H, J = 2 Hz, J = 8 Hz, H-2), 3.80 (dd, 1H, J = 3.5 Hz, J = 12 Hz, H-13), 3.45 (m, 1H, H-1), 3.40 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 3.03 (dd, 1H, J = 4 Hz, J = 8 Hz, H-9), 1.97 (s, 3H, OAc), 1.42 (s, 3H, 12-Me), 1.20 (s, 3H, 8-Me), and 0.90 ppm (d, 3H, J = 6 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 392 (2), 349 (5), 332 (51), 304 (12), 219 (13), 203 (11), 193 (11), 190 (10), 177 (15), 163 (15), 161 (21), 156 (18), 151 (15), 149 (22), 147 (17), 139 (22), 137 (18), 135 (18), 133 (20), 125 (17), 123 (23), 121 (24), 119 (23), 113 (18), 111 (41), 110 (20), 109 (47), 108 (19), 107 (36), 105 (32), 97 (61), 95 (63), 94 (66), 93 (41), 91 (33), 85 (53), 83 (69), 81 (69), 79 (40), 71 (35), 69 (81), 67 (40), 60 (49), 57 (47), 55 (95), 53 (57), base peak 43 (100), 41 (40).

Anal. Calcd for $C_{22}H_{32}O_6$: C, 67.32; H, 8.22. Found: C, 67.10; H, 8.16.

Hydrogenation of the Monoepoxide of Cueunicin Acetate -- A solution of 80 mg of the epoxide of cueunicin acetate in 20 ml of absolute ethanol was hydrogenated at atmospheric pressure and room temp over platinum oxide (17 mg). The filtered solution was evaporated in vacuo to yield 71 mg of an oily residue. Chromatography on 20 g of silicic acid (silicAR CC-4, 4:1 benzene-ethyl acetate) afforded in fractions 14-20, after removal of the solvents, 33.2 mg of a white crystalline compound (24): mp 172-173.5°; R_f = 0.24 (3:1 benzene-ethyl acetate, silica gel H), ir ($CHCl_3$) 1770 (γ-lactone), 1730 and 1250 cm^{-1} (acetate);

100 MHz pmr ($CDCl_3$) δ 4.77 (dd, 1H, J = 2 Hz, J = 7 Hz, H-2), 3.58 (dd, 1H, J = 3 Hz, J = 12 Hz, H-13), 3.44 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 3.0 (c, 2H, H-9, H-15), 2.01 (s, 3H, OAc), 1.46 (s, 3H, 12-Me), 1.26 (d, 3H, J = 7 Hz, 15-Me), 1.23 (s, 3H, 8-Me), and 0.91 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity x 3) M^+, 394 (2), 351 (7), 334 (44), 316 (11), 306 (11), 289 (7), 277 (15), 261 (36), 260 (11), 233 (11), 221 (23), 205 (14), 203 (15), 193 (14), 191 (12), 181 (15), 180 (18), 179 (34), 178 (48), 177 (20), 175 (15), 166 (17), 165 (21), 164 (19), 163 (22), 161 (19), 159 (15), 155 (10), 153 (20), 152 (14), 151 (26), 150 (17), 149 (30), 148 (11), 147 (22), 145 (13), 141 (22), 139 (17), 138 (15), 137 (32), 136 (22), 135 (45), 134 (17), 133 (28), 131 (11), 125 (35), 124 (20), 123 (44), 122 (24), 121 (53), 120 (14), 119 (33), 113 (57), 112 (26), 111 (68), 110 (43), (rel intensity x 1) 109 (36), 108 (15), 107 (24), 97 (58), 96 (18), 95 (45), 94 (41), 93 (31), 91 (11), 85 (35), 84 (37), 83 (30), 81 (40), 79 (21), 71 (24), 57 (27), 55 (77), base peak 43 (100), 41 (51).

Anal. Calcd for $C_{22}H_{34}O_6$: C, 66.98; H, 8.69. Found: C, 66.87; H, 8.59.

Hydrogenation of Epoxycueunicin -- A solution of 261.5 mg of cueunicin epoxide in 30 ml of absolute ethanol was hydrogenated for 16 hr at atmospheric pressure in the presence of 25.1 mg of prereduced platinum oxide. The solution was filtered and the solvent removed leaving an oily residue that showed two overlapping spots on tlc. The residue was chromatographed (16 g silicAR CC-7, 1:1 benzene-ethyl acetate, 7 ml/5 min). Fractions 12-14 showed a single spot on tlc, and were combined and the solvents removed to give, after recrystallization

from benzene-hexane, 48.6 mg of dihydroepoxycueunicin (25): mp 151-153°; R_f = 0.23 (1:1 benzene-ethyl acetate, silica gel G), ir ($CHCl_3$) 3595, 3450 (hydroxyl) and 1769 cm^{-1} (γ-lactone); 60 MHz pmr ($CDCl_3$) δ 4.70 (dd, 1H, J = 1 Hz, J = 5.5 Hz, H-2), 3.38 (m, 2H, H-3 and H-13), 2.85 (m, 2H, H-9 and H-15), 1.22 (d, 3H, 15-Me), 1.17 (s, 3H, 8-Me), 1.08 (s, 3H, 12-Me), and 0.86 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 352 (3), 334 (13), 316 (2), 141 (10), 135 (10), 123 (14), 121 (11), 111 (18), 109 (25), 107 (17), 99 (11), 97 (10), 95 (23), 93 (25), 85 (38), 81 (22), 79 (10), 71 (19), 69 (24), 67 (16), 57 (14), 55 (51), base peak 43 (100), and 41 (48).

Anal. Calcd for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15. Found: C, 68.44; H, 9.08.

Fractions 15-21 showed a single spot on tlc and were combined. Removal of the solvents afforded a white solid which was recrystallized from benzene-hexane to give 118.2 mg of isocueunicin oxide (26): mp 171-172.5°; R_f = 0.17 (1:1 benzene-ethyl acetate, silica gel G), ir ($CHCl_3$) 3450, 3600 (hydroxyl), 1750 and 1681 cm^{-1} (α,β-unsaturated γ-lactone); uv absorption at 236 nm (ε 15,000); 100 MHz pmr ($CDCl_3$) δ 5.01 (d, 1H, J = 7 Hz, H-2), 4.00 (dd, 1H, J = 7 Hz, J = 11 Hz, H-3), 3.62 (dd, 1H, J = 3 Hz, J = 11 Hz, H-13), 2.92 (m, 2H, H-9 and H-14b), 2.40 (m, 1H, H-14a), 1.88 (t, 3H, J ∿ 1 Hz, 15-Me), 1.28 (s, 6H, 8-Me and 12-Me), and 0.90 ppm (d, 3H, J = 6.5 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 350 (8), 332 (13), 265 (11), 221 (5), 181 (11), 180 (12), 165 (13), 153 (12), 149 (10), 139 (10), 135 (10), 125 (12), 123 (11), 112 (16), 111 (20), 110 (28), 109 (44), 107 (15), 97 (13), 95 (18), 94 (10), 93 (17), 85 (27), 83 (17), 82 (15), 81 (37),

79 (13), 69 (23), 67 (17), 57 (15), 55 (42), 53 (22), base peak 43 (100), and 41 (48).

Anal. Calcd for $C_{20}H_{30}O_5$: C, 68.55, H, 8.63. Found: C, 68.66; H, 8.64.

Dihydro-Keto-dilactone (27) from Cueunicin Acetate -- A solution of 103.8 mg of cueunicin acetate in 7 ml ethanol was stirred with 20 mg of sodium borohydride at room temp for 2 hr. The ethanol was evaporated under reduced pressure and a white solid formed. The solid was taken up in chloroform and washed (H_2O).

The crystalline residue (106 mg) was dissolved in 20 ml of 40% aqueous t-butyl alcohol and the pH adjusted to 8-10 by the addition of potassium carbonate. The alkaline solution was stirred for 3 days with 22 ml of the Lemieux-von Rudloff reagent. The reaction mixture was then acidified (10% H_2SO_4) and potassium metabisulfite ($K_2S_2O_5$) added until the solution was clear. The mixture was extracted with chloroform, washed (H_2O) and dried ($MgSO_4$). Evaporation of the solvents left a solid (28) which liberated CO_2 when treated with sodium bicarbonate. The solid had: ir ($CHCl_3$) 3500, 1768 1730 -- 1710, and 1240 cm^{-1}. 28 was not further characterized but treated directly with 20 mg of potasium hydroxide in 10 ml of water and gently refluxed overnight. The mixture was acidified (10% HCl), stirred at room temp for 8 hr, then extracted with chloroform, washed with water, dried ($MgSO_4$), and filtered through a short silicic acid (SilicAR CC-7, 1.5 x 15 cm) column. Evaporation of the chloroform and trituration with hexane afforded 53.1 mg of white feathers (27): mp 143-143.5°; R_f = 0.23 (1:1 benzene-ethyl acetate, silica gel G); ir ($CHCl_3$) 1770 (2 γ-lactones) and 1710 cm^{-1} (ketone);

100 MHz pmr ($CDCl_3$) δ 4.56 (dd, 1H, J = 1.5 Hz, J = 6 Hz, H-2), 3.65 (t, 1H, J = 5.5 Hz, H-13), 3.52 (dd, 1H, J = 1.5 Hz, J = 9 Hz, H-3), 2.87 (m, 1H, H-15), 2.15 (s, 3H, 8-Me), 1.43 (s, 3H, 12-Me), 1.19 (d, 3H, J = 6.5 Hz), and 0.96 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 366 (2), 348 (1), 308 (3), 293 (2), 267 (63), 193 (45), 149 (8), 141 (5), 113 (12), 109 (20), 107 (5), 99 (54), 83 (27), 81 (35), 69 (55), 55 (43), base peak 43 (100), 41 (33).

Anal. Calcd for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25; mol wt, 366.20424. Found: C, 65.58; H, 8.38; mol wt (mass spectrum) 366.20310.

Lemieux-von Rudloff Reaction on Dihydrocueunicin-I -- A solution of dihydrocueunicin-I (532.9 mg) in 35 ml of 40% aqueous t-butyl alcohol was adjusted to pH 8-10 by the addition of powdered potassium carbonate. To the stirred solution was added 100 ml of the Lemieux-von Rudloff reagent. Stirring was continued for 3 days. The solution was then acidified (5% sulfuric acid) and powdered potassium metabisulfite ($K_2S_2O_5$, Mallinckrodt) was added until the solution turned clear. Most of the t-butyl alcohol was removed on the rotary evaporator at reduced pressure. The aqueous solution was extracted with chloroform (3 x 50 ml). The chloroform layer was washed with 5% sodium bicarbonate (20 ml) and water (20 ml), then dried ($MgSO_4$). The chloroform was removed leaving a colorless oil which solidified on trituration with hexane. The product was chromatographed on 100 g of silicAR CC-7 (1:1 benzene-ethyl acetate; 100 ml/20 min). Fractions 8 and 9 contained a single spot by tlc (R_f = 0.23, 1:1 benzene-ethyl acetate, silica gel G) and were combined to yield, after recrystallization from benzene-hexane, 134.0 mg of the dihydro-keto-dilactone (27): mp 143-144°. The compound was identical

[tlc, pmr, ir, mp and mmp (undepressed)] with the dihydro-keto-dilactone obtained from cueunicin acetate via the Lemieux-von Rudloff reaction.

Anal. Calcd for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25. Found: C, 65.19; H, 8.22.

Methyl Ester of the Keto-acid derived from Dihydrocueunicin Acetate via the Lemieux-von Rudloff Reaction -- A solution of 587.7 mg of dihydrocueunicin acetate in 35 ml of 40% aqueous t-butyl alcohol was adjusted to pH 9 by the addition of powdered potassium carbonate. To the stirred solution was added 122 ml of the Lemieux-von Rudloff reagent. Additional potassium carbonate was added to maintain the pH at 8-10. The magenta solution was stirred at room temp for 3 days. The solution was then acidified with 10% sulfuric acid followed by the addition of powdered potassium metabisulfite ($K_2S_2O_5$) until the solution turned clear. Most of the t-butyl alcohol was removed on the rotary evaporator under reduced pressure. The colorless solution was then extracted with chloroform (3 x 200 ml) and dried ($MgSO_4$). The chloroform was evaporated leaving an oily residue. The residual oil was gently refluxed in methanolic potassium hydroxide (0.4 g KOH in 40 ml methanol) overnight, acidified with HCl and stirred for 3 hr, followed by removal of most of the methanol on the rotary evaporator. The mixture was extracted with chloroform (3 x 20 ml), dried ($MgSO_4$) and the chloroform evaporated to yield an oily residue. Tlc indicated the presence of a mixture; one spot (R_f = 0.23, 1:1 benzene-ethyl acetate, silica gel G) corresponded to the dihydro-keto-dilactone (27) derived from cueunicin, while the other spot (R_f = 0.56) indicated a new compound.

The oily residue was chromatographed (120 g silicAR CC-7, 1:1 benzene-ethyl acetate, 50 ml/20 min). Fractions 12-14 contained one

spot by tlc, and were combined to yield, after removal of the solvents, 106.1 mg of a colorless oil. The oil could not be induced to crystallize; it apparently consisted of a mixture of epimers at C-15. The oil had: ir ($CHCl_3$) 1767 (γ-lactone), 1738 (ester), 1730 (ester), 1712 (ketone), and 1240 cm^{-1} (acetate); 60 MHz pmr ($CDCl_3$) δ 4.70 (1H, H-2), 4.12 (1H, H-13), 3.64 (s, 3H, OMe), 3.44 (1H, H-3), 2.31 (2H, $\underline{CH_2}$-C=O), 2.13 (s, 3H, $\underline{Me}$-C=O), 1.97 (s, 3H, OAc), 1.47 (s, 3H, 12-Me), 1.23 (d, 3H, J = 7 Hz, 15-Me), and 0.92 ppm (d, 3H, j + 7 Hz, 4-Me); mass spectrum (70 eV) $\underline{m/e}$ (rel intensity) M^+-HOAc, 380 (10), 367 (3), 267 (36), 249 (6), 221 (4), 193 (19), 175 (6), 143 (5), 141 (6), 131 (6), 99 (20), 81 (10), 71 (13), 69 (14), 58 (10), 55 (26), base peak 43 (100), and 41 (20).

Fractions 21-25 showed a single spot on tlc and were combined. Removal of the solvents afforded 57.5 mg of the dihydro-keto-dilactone (27). The balance of the product was assumed to be 28, and was presumably lost at the origin on chromatography.

Baeyer-Villiger Oxidation of the Dihydro-Keto-dilactone (27) -- A solution of pertrifluoroacetic acid was prepared by dropwise addition of 1.0 ml of trifluoroacetic anhydride (Columbia Organic Chemicals, Inc.) to a suspension of 0.2 ml of 98% hydrogen peroxide (FMC Corp.) in 3 ml of cold methylene chloride. This solution was then added dropwise to a stirred suspension containing 0.2 g of finely powdered anhydrous disodium hydrogen phosphate and 131.4 mg of the dihydro-keto-dilactone (27) derived from cueunicin. The reaction vessel was warmed until the mixture began to reflux. At this point heating was discontinued and the exothermic nature of the reaction caused the mixture to continue to reflux without heating. After 1 hr the salts were filtered and washed

with 20 ml of methylene chloride. The filtrate was washed with 10 ml of 5% sodium bisulfite solution and 10 ml of 5% sodium carbonate solution. The methylene chloride layer was then dried ($MgSO_4$). Removal of the solvents afforded a colorless oil that crystallized on trituration with hexane. Recrystallization from benzene-hexane gave 108.2 mg of a white crystalline solid. Tlc showed a trace impurity. The solid was chromatographed (16 g silicAR CC-7; 2:1 benzene-ethyl acetate; 7 ml/5 min).

Fractions 13-20 showed a single spot by tlc and were combined. The solvents were removed to yield, after recrystallization from benzene-hexane, 58.1 mg of white crystals (30): mp 109-109.5°; R_f = 0.22 (1:1 benzene-ethyl acetate, silica gel G); ir ($CHCl_3$) 1770 (two γ-lactones), 1728 and 1240 cm^{-1} (acetate); 100 MHz pmr ($CDCl_3$), δ 4.56 (dd, 1H, J = 1.5 Hz, J = 6 Hz, H-2), 4.06 (t, 2H, J = 6.5 Hz, H-7), 3.66 (t, 1H, J = 5.5 Hz, H-13), 3.53 (dd, 1H, J = 1.5 Hz, J = 9 Hz, H-3), 2.86 (m, 1H, H-15), 2.05 (s, 3H, OAc), 1.43 (s, 3H, 12-Me), 1.18 (d, 3H, J = 7 Hz, 15 Me), and 0.96 ppm (d, 3H, J = 7 Hz, 4 Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 382 (2), 283 (86), 223 (88), 209 (37), 149 (64), 141 (13), 131 (15), 129 (11), 99 (88), 83 (20), 81 (21), 69 (43), 55 (45), base peak 43 (100), and 41 (29).

Anal. Calcd for $C_{20}H_{30}O_7$: C, 62.81; H, 7.91; mol wt 382.19915. Found: C, 63.10; H, 7.81; mol wt 382.20049.

Pyrazoline of Cueunicin Acetate (38) -- An ethereal solution of diazomethane (40 ml of approximately 0.3 M) was added to a solution of cueunicin acetate (163.2 mg) in ether. After standing seven days at -20°, the solvent was evaporated leaving a solid residue which was

recrystallized from chloroform-hexane to yield 180 mg of white prisms: mp 136-136.5° (gas evolved); ir ($CHCl_3$) 1777 (γ-lactone), 1732 and 1240 cm^{-1}; uv absorption at 226 nm (ε 744), and 324 nm (ε 232); CD curve λ_{max} 324 nm ([θ]-13,700; c 0.101); 100 MHz pmr ($CDCl_3$) δ 5.56 (dd, 1H, J = 3 Hz, J = 6 Hz, H-2), 5.42 (c, 1H, H-9), 4.80 (c, 2H, H-17), 3.74 (dd, 1H, J = 3 Hz, J = 11 Hz, H-13), 3.54 (dd, 1H, J = 3 Hz, J = 10 Hz, H-3), 2.85 (m, 1H, H-1), 1.97 (s, 3H, OAc), 1.56 (s, 3H, 8-Me), 1.38 (s, 3H, 12-Me), and 0.96 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity M^+-N_2, 390 (2), 358 (1), 330 (49), 261 (5), 233 (5), 220 (9), 207 (8), 189 (10), 177 (17), 163 (17), 161 (19), 150 (10), 149 (19), 148 (12), 147 (18), 137 (16), 135 (30), 133 (19), 125 (10), 123 (30), 122 (27), 121 (36), 119 (21), 110 (14), 109 (56), base peak 108 (100), 107 (40), 105 (27), 97 (13), 96 (19), 95 (61), 94 (31), 93 (72), 91 (21), 83 (26), 82 (27), 81 (80), 79 (43), 77 (17), 71 (15), 69 (48), 68 (20), 67 (57), 56 (10), 54 (46), 52 (22), 43 (91), and 41 (31).

Anal. Calcd for $C_{23}H_{34}N_2O_5$: C, 66.01; H, 8.19; N, 6.69. Found: C, 66.18; H, 8.11; N, 6.80.

Eupalmerin Acetate (6) -- The isolation of eupalmerin acetate has been described.[13] A crude hexane extract (106 g) of E. palmeri (Miami area) was placed on 400 g of silicAR CC-4 and eluted with 2:1 benzene-ethyl acetate at 100 ml/20 min. Fractions 10-12 were combined and rechromatographed on 300 g of silicAR CC-4 with 9:1 benzene-ethyl acetate at 100 ml/20 min. Fractions 6-17 afforded 2.92 g of eupalmerin acetate: mp 153-154.5° [lit[13] mp 158.5-159.5°]; R_f = 0.49 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 1770, 1665 (α,β'-unsaturated

γ-lactone), 1730, and 1235 cm^{-1} (acetate); 100 MHz pmr ($CDCl_3$) δ 6.07 (d, 1H, J = 3.5 Hz, H-17a), 5.31 (d, 1H, J = 3.5 Hz, H-17b), 5.1 (m, 1H, H-9), 4.76 (d, 1H, J = 8 Hz, H-2), 3.15 (m, 1H, H-1), 2.94 (dd, 1H, J = 5.5 Hz, J = 9 Hz, H-13), 1.90 (s, 3H, OAc), 1.63 (s, 3H, 8-Me), 1.33 (s, 3H, 12-Me), and 0.85 ppm (d, 3H, J = 7 Hz, 4-Me). A sample of eupalmerin acetate was given to Dr. E. Wenkert for cmr studies.

Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57. Found: C, 70.31; H, 8.52.

Dihydroeupalmerin Acetate (31) -- A suspension of eupalmerin acetate (1.1162 g) in absolute ethanol (70 ml) was stirred at ice bath temp. Sodium borohydride (214 mg) was added and the ice bath removed. Stirring was continued at room temp for 2.5 hr. The mixture was acidified (10% HCl), evaporated at reduced pressure to remove most of the ethanol, diluted with water, and extracted with chloroform. The chloroform extract was dired ($MgSO_4$) and the chloroform evaporated, yielding an oily residue. Tlc showed one major spot and 4 minor components. The residue was chromatographed (150 g silicAR CC-7, 9:1 benzene-ethyl acetate, 70 ml/20 min). Fractions 22-26 showed a single spot on tlc and were combined. Removal of the solvents afforded a white solid which was recrystallized three times from hexane to yield 390.0 mg of white prisms (31): mp 111-112°; R_f = 0.38 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 1770 (γ-lactone), 1740, and 1240 cm^{-1} (acetate); 100 MHz ($CDCl_3$) δ 5.35 (dd, 1H, J = 2 Hz, J = 10 Hz, H-3), 5.16 (m, 1H, H-9), 4.47 (dd, 1H, J = 4.5 Hz, J = 10 Hz, H-2), 2.90 (m, 1H), 2.88 (dd, 1H, J = 4.5 Hz, J = 7.5 Hz, H-9), 2.50 (m, 1H), 2.41 (s, 3H, OAc), 1.58 (d, 3H, J = 1 Hz, 8-Me), 1.28 (s, 3H, 12-Me), 1.24 (d,

3H, J = 7 Hz, 15-Me), and 0.99 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 378 (2), 360 (1), 336 (2), 318 (6), 299 (2), 245 (5), 219 (3), 205 (3), 187 (3), 183 (3), 165 (7), 135 (13), 133 (10), 123 (13), 122 (15), 121 (21), 119 (12), 111 (10), 109 (26), 108 (28), 107 (24), 97 (10), 96 (11), 95 (41), 94 (44), 93 (35), 83 (14), 82 (11), 81 (41), 79 (24), 71 (11), 69 (28), 67 (25), 55 (39), base peak 43 (100), and 41 (41).

Anal. Calcd for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.74; H, 9.01.

Dihydroeupalmerin (32) -- Sodium (∿ 1 mg) was added to a solution of 58.9 mg of dihydroeupalmerin acetate in 20 ml ethanol. After 8 hr, tlc indicated the complete absence of starting material (R_f = 0.56, 1:1 benzene-ethyl acetate, silica gel H) and the appearance of a new spot (R_f = 0.34). Most of the methanol was removed in vacuo on a rotary evaporator. Water (10 ml) was added and the mixture extracted with chloroform (3 x 15 ml). The chloroform layer was dried ($MgSO_4$) and the solvent evaporated leaving 41.3 mg of a white crystalline solid. The solid was filtered through a short (1.5 x 18.5 cm) silicAR CC-7 column affording white prisms: mp 164-165°; R_f = 0.34 (1:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 3595, 3450 (hydroxyl), and 1765 cm^{-1} (γ-lactone); 60 MHz pmr ($CDCl_3$) δ 5.04 (m, 1H, H-9), 4.50 (d, 1H, J = 6 Hz, H-2), 3.53 (m, 1H, H-3), 2.90 (m, 2H, H-13 and H-15), 1.60 (s, 3H, 8-Me), 1.30 (s, 3H, 12-Me), 1.18 (d, 3H, J = 7 Hz, 15-Me) and 1.03 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 336 (9), 318 (8), 290 (3), 263 (5), 245 (4), 219 (5), 209 (6), 205 (5), 183 (11), 169 (15), 165 (14), 153 (10), 151 (12),

149 (12), 139 (13), 138 (10), 137 (14), 136 (13), 135 (20), 133 (10), 125 (11), 124 (11), 123 (22), 122 (14), 121 (30), 119 (15), 111 (22), 110 (17), 109 (43), 108 (39), 107 (30), 105 (10), 99 (11), 97 (26), 96 (21), 95 (61), 94 (65), 93 (63), 91 (11), 85 (16), 84 (16), 83 (26), 82 (21), 81 (55), 80 (10), 79 (18), 78 (29), 77 (10), 72 (12), 71 (26), 70 (10), 69 (60), 68 (45), 67 (54), 57 (26), 56 (11), 55 (77), 53 (21), base peak 43 (100), 42 (12), and 41 (76).

Anal. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.59; H, 9.64.

Eunicin (3) -- The isolation of eunicin has been described.[5-7] An insoluble portion (6.3438 g) of a hexane extract of E. mammosa (Lamouroux) collected off the Florida Keys was placed on 300 g of silicAR CC-4 and eluted with 4:1 benzene-ethyl acetate at 100 ml/20 min. Fractions 16-26 showed a single spot on tlc. From fraction 17 was isolated 457.3 mg of eunicin, which was given to Dr. E. Wenkert for cmr studies. Fractions 16 and 18-26 afforded after several recrystallizations 5.0221 g of pure eunicin (3): mp 152-153° [lit[6] mp 155°]; R_f = 0.30 (3:1 benzene-ethyl acetate, silica gel H); ir 3625, 3500 (hydroxyl), 1765, and 1664 cm^{-1} (α,β'-unsaturated γ-lactone); 100 MHz pmr ($CDCl_3$) δ 6.42 (dd, 1H, J = 0.5 Hz, J = 3.5 Hz, H-17a), 5.68 (d, 1H, J = 3.5 Hz, H-17b), 5.06 (t, 1H, J = 7.5 Hz, H-9), 4.44 (dd, 1H, J = 8 Hz, J = 10 Hz, H-2), 3.42 (m, 1H, H-1), 3.28 (dd, 1H, J = 2.5 Hz, J = 12 Hz, H-13), 2.88 (d, 1H, J = 10 Hz, H-3), 1.56 (s, 3H, 8-Me), 1.17 (s, 3H, 12-Me), and 0.87 (d, 3H, J = 6.5 Hz, 4-Me); 25.2 MHz proton decoupled cmr ($CDCl_3$) δ 170.2 (C=O), 136.6 (C=C), 128.3 (C=C), 121.3 (C=C), 77.2 (C-O), 73.4 (C-O), 40.8, 38.2, 37.6, 33.3, 28.8, 24.4, 23.6, 22.8, 20.4, 17.0, and

14.1 ppm; mass spectrum (70 eV) m/e (rel intensity) M^+, 334 (54), 316 (31), 193 (20), 164 (22), 163 (18), 161 (19), 153 (20), 151 (19), 149 (19), 147 (20), 137 (20), 136 (15), 135 (34), 134 (19), 133 (20), 123 (22), 119 (26), 111 (20), 109 (53), 108 (57), 107 (47), 105 (26), 95 (70), 94 (41), 93 (57), 91 (26), 85 (10), 83 (22), 82 (24), 81 (81), 79 (55), 77 (21), 71 (41), 69 (56), 67 (63), 57 (22), 55 (84), 53 (52), base peak 43 (100), and 41 (83).

Anal. Calcd for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04; mol wt, 334.21440. Found: C, 72.01; H, 8.89; mol wt, 334.21319.

Dihydroeunicin (34) -- A solution of eunicin (0.9700 g) in 60 ml of n-butanol was heated to reflux under nitrogen. Freshly cut and finely divided sodium (2 g) was added to the refluxing solution as rapidly as possible. After 1 hr the solution was cooled; at this point all of the sodium had dissolved. Water (150 ml) was added and most of the n-butanol removed in vacuo on the rotary evaporator. The resulting aqueous solution was extracted with ether (100 ml) and acidified (10% HCl). A white precipitate formed and was extracted with ether (2 x 100 ml) and chloroform (2 x 100 ml). The ether and chloroform extracts were dried ($MgSO_4$) separately and, after removal of the solvents, were combined into a single chloroform extract which afforded 879.1 mg of white crystalline solid on removal of the solvent. Tlc showed the presence of two spots R_f = 0.25 and R_f = 0.42 (3:1 benzene-ethyl acetate, silica gel H); therefore, 438.2 mg of the solid was chromatographed (400 g silicAR CC-7, 3:1 benzene-ethyl acetate, 50 ml/20 min). Fractions 24-30 showed a single spot on tlc (R_f = 0.42) and were combined. Removal of the solvents afforded a white solid which was recrystallized from

benzene-hexane to give 355.1 mg of white crystalline dihydroeunicin (34): mp 160-163° [lit[6] mp 157°]; ir ($CHCl_3$) 3605, 3500 (hydroxyl), and 1770 cm^{-1} (γ-lactone); 60 MHz pmr ($CDCl_3$) δ 5.10 (t, 1H, J = 7 Hz, H-9), 4.30 (dd, 1H, J = 7 Hz, J = 10 Hz, H-2), 3.35 (dd, 1H, J = 4.5 Hz, J = 9 Hz, H-13), 3.07 (d, 1H, J = 10 Hz, H-3), 2.58 (m, 1H, H-15), 1.63 (s, 3H, 8-Me), 1.27 (d, 3H, J = 6 Hz, 15-Me), 1.18 (s, 3H, 12-Me), and 0.87 ppm (d, 3H, J = 6.5 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 336 (36), 318 (16), 263 (12), 262 (10), 245 (7), 223 (3), 219 (3), 197 (3), 193 (4), 184 (4), 181 (6), 177 (4), 166 (6), 165 (6), 163 (7), 161 (7), 159 (6), 157 (2), 151 (5), 149 (10), 147 (6), 139 (10), 137 (10), 135 (18), 123 (16), 121 (24), 111 (12), 109 (36), 108 (34), 107 (24), 95 (36), 94 (21), 93 (33), 81 (39), 79 (22), 78 (62), 71 (28), 69 (34), 57 (75), 55 (68), base peak 43 (100), and 41 (85).

Anal. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.36; H, 9.64.

Reaction of Eunicin with Acetic Anhydride and Boron Trifluoride Etherate -- A solution of eunicin (983 mg) in 30 ml of acetic anhydride and 10 ml of dry ether was cooled to -20°C. To the cold solution was added 5 ml of boron trifluoride etherate. This solution was allowed to stand at -20° for 1.5 hr, then poured into a cold mixture of 10:1 water-pyridine (110 ml). The mixture was shaken for several minutes then extracted with chloroform (3 x 100 ml). The combined chloroform extracts were washed with water (5 x 100 ml), 2% hydrochloric acid (2 x 100 ml), 5% sodium bicarbonate (100) ml) and water (2 x 100 ml). The extract was dried ($MgSO_4$) and the chloroform removed on the rotary evaporator. Hexane was added and a white solid formed. Tlc showed two spots. The

solid was then chromatographed (150 g silicAR CC-7, 9:1 benzene-ethyl acetate, 5 ml/5 min). Fractions 54-70 showed a single spot by tlc and were combined. Removal of the solvents afforded 380.4 mg of eunicin acetate. Recrystallization from benzene-hexane afforded 182.4 mg of pure eunicin acetate (35); mp 159-162° [lit[6] mp 155-157°]; R_f = 0.60 (3:1 benzene-ethyl acetate, silica gel H), ir ($CHCl_3$) 1767, 1662 (α,β'-unsaturated γ-lactone), 1730 and 1250 cm^{-1} (acetate); 100 MHz pmr ($CDCl_3$) δ 6.44 (d, 1H, J = 3.5 Hz, H-17a), 5.63 (d, 1H, J = 3.5 Hz, H-17b), 5.08 (t, 1H, J = 8 Hz, H-9), 4.45 (dd, 1H, J = 8 Hz, H-2), 3.41 (m, 1H, H-1), 3.39 (dd, 1H, J = 3 Hz, J = 11 Hz, H-13), 2.90 (d, 1H, J = 10 Hz, H-3), 2.78 (m, 1H), 2.01 (s, 3H, OAc), 1.53 (br, 3H, 8-Me), 1.46 (s, 3H, 12-Me), and 0.88 ppm (d, J = 6.5 Hz, 4-Me); 25.2 MHz proton decoupled cmr ($CDCl_3$) δ 169.9 (C=O), 169.2 (C=O), 136.5 (C=C), 130.2 (C=C), 128.1 (C=C-H), 121.0 ($C=CH_2$), 84.5 (C-OAc), 77.5 (H-C-O), 73.0 (H-C-O), 72.4 (H-C-O), 38.1, 37.5, 34.5, 33.4, 28.7, 24.5, 22.8, 22.3, 20.1, 20.1, 16.9, and 14.1 ppm; mass spectrum (70 eV) m/e (rel intensity) M^+, 376 (6), 334 (3), 333 (1), 316 (40), 301 (1), 259 (1), 245 (2), 232 (4), 220 (6), 208 (4), 207 (4), 206 (7), 177 (4), 163 (7), 161 (8), 149 (13), 147 (7), 135 (15), 133 (10), 121 (18), 119 (10), 109 (19), 108 (32), 107 (19), 105 (12), 95 (22), 93 (28), 91 (14), 81 (34), 79 (24), 69 (25), 67 (23), 55 (46), 53 (20), base peak 43 (100), and 41 (47).

Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57; mol wt, 376.22496. Found: C, 70.17; H, 8.35; mol wt, 376.22489.

Fractions 81-105 appeared to contain a single compound by tlc and were combined. Removal of the solvents and trituration with hexane afforded 636.3 mg of a mixture consisting of the endo and exocyclic

olefins expected upon acylation of the trisubstituted double bond in eunicin,[103,6] and concomitant acetylation of the tertiary alcohol function.

Lemieux-von Rudloff Reaction on Dihydroeunicin -- A solution of 141 mg of dihydroeunicin in 20 ml of 40% aqueous t-butyl alcohol was adjusted to pH 8-10 by the addition of powdered potassium carbonate. To the basic solution was added 24 ml of the Lemieux-von Rudloff reagent. The reaction mixture was stirred overnight, acidified with 10% sulfuric acid and treated with potassium metabisulfite until the solution turned clear. The aqueous solution was extracted with chloroform (2 x 30 ml) and dried ($MgSO_4$). The solvents were removed under reduced pressure leaving a white amorphous solid. The solid was filtered through a short Florisil column (1.5 x 6 cm, 1:1 benzene-ethyl acetate). After removal of the solvents, a white crystalline solid formed. Recrystallization from benzene-hexane afforded 60.5 mg of white crystals (36): mp 84-85° [lit[6] mp 85-86°]; R_f = 0.30 (1:1 benzene-ethyl acetate, silica gel H), ir ($CHCl_3$) 1774 (two γ-lactones) and 1712 cm^{-1} (ketone); 60 MHz pmr ($CDCl_3$) δ 4.37 (dd, 1H, J = 7 Hz, J = 10 Hz, H-2), 3.58 (t, 1H, J = 7.5 Hz, H-13), 3.21 (dd, 1H, J = 2.5 Hz, J = 10 Hz, H-3), 2.17 (s, 3H, Me-C=O, 8-Me), 1.41 (s, 3H, 12-Me), 1.30 (d, 3H, J = 6.5 Hz, 15-Me), and 0.94 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 366 (8), 348 (24), 308 (24), 293 (51), 267 (9), 193 (12), 149 (11), 141 (13), 113 (13), 109 (10), 107 (9), 99 (40), 83 (14), 81 (12), 69 (17), 55 (26), base peak 43 (100), and 41 (17).

Anal. Calcd for $C_{20}H_{30}O_6$: C, 65.55, H, 8.25; mol wt, 366.20424. Found: C, 65.30, H, 8.22; mol wt (mass spectrum), 366.20495.

Baeyer-Villiger Oxidation of the Dihydro-keto-dilactone (36) derived from Eunicin -- A solution of pertrifluoroacetic acid was

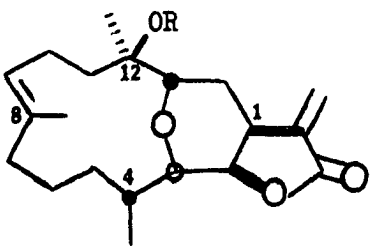
prepared by dropwise addition of 1.0 ml (7.2 mmol) of trifluoroacetic anhydride to a suspension of 0.2 ml of 98% hydrogen peroxide in 3 ml of cold methylene chloride. This solution was added dropwise to a stirred suspension containing 0.2 g of finely powdered anhydrous disodium hydrogen phosphate and 173.4 mg of the dihydro-keto-dilactone (36) derived from eunicin in 3 ml of methylene chloride. The exothermic reaction caused the solution to boil gently. The reaction mixture was stirred for 40 min. The salts were filtered and washed with 20 ml of methylene chloride. The filtrate was washed with 10 ml of 5% sodium bisulfite solution and 10 ml of 5% sodium carbonate solution and dried ($MgSO_4$). The solvent was removed in vacuo to yield an oil, which crystallized on trituration with warm hexane. The solid was brownish and was chromatographed (16 g silicAR CC-7, 2:1 benzene-ethyl acetate, 2ml/5 min). Fractions 11-16 each showed a single spot by tlc and were combined and the solvents removed on the rotary evaporator leaving a colorless oil which crystallized on trituration with warm hexane. The solid was recrystallized from benzene-hexane to yield 47.4 mg of the dihydro-acetoxy-dilactone (37): mp 135-136° [lit.[6] mp 132-133°]; R_f = 0.27 (1:1 benzene-ethyl acetate, silica gel G), ir ($CHCl_3$) 1775 (two γ-lactones), 1730 and 1230 cm^{-1} (acetate); 100 MHz pmr ($CDCl_3$) δ 4.35 (dd, 1H, J = 7 Hz, J = 10 Hz, H-2), 4.05 (t, 2H, J = 6.5 Hz, H-7), 3.58 (t, 1H, J = 7.5 Hz, J = 10 Hz, H-2), 3.21 (dd, 1H, J = 2.5 Hz, J = 10 Hz, H-3), 2.62 (m, 1H, H-15), 2.06 (s, 3H, OAc), 1.38 (s, 3H, 12-Me), 1.28 (d, 3H, J = 6.5 Hz, 15-Me), and 0.94 ppm (d, 3H, J = 7 Hz, 4-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 382 (10), 364 (29), 336 (3), 322 (10), 309 (45), 283 (49), 249 (14), 223 (23), 221 (7), 209 (25), 205 (18), 181 (7), 177 (12), 167 (7), 159 (8), 149 (20), 141 (22), 131 (11), 129 (9), 127 (16), 115 (18), 111 (10),

109 (13), 107 (10), 99 (89), 95 (11), 85 (19), 83 (15), 81 (18), 71 (14), 69 (22), 67 (14), 57 (10), 55 (27), base peak 43 (100), and 41 (19).

Anal. Calcd for $C_{20}H_{30}O_7$: C, 62.81; H, 7.91; mol wt, 382.19915. Found: C, 62.58; H, 7.81; mol wt, 382.20162.

SUMMARY

The gorgonian E. mammosa Lamouroux, taken at Curacao, Netherlands Antilles, was found to contain two novel diterpene lactones, cueunicin ($C_{20}H_{30}O_4$) and its acetate ($C_{22}H_{32}O_5$). The presence of an α-methylene-γ-lactone, a di-secondary ether function, a secondary methyl group, a vinyl methyl group and a tertiary methyl carbinol system established cueunicin as being very similar to the oxa-bridged cembranolides, eunicin and jeunicin, previously isolated from E. mammosa collected at other Carribbean locations. The absolute configurations 15 and 16 are proposed for these new cembranolides based on chemical and spectroscopic evidence. 15 is 3-epi-eunicin.



15 R = H

16 R = Ac

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..."The Reviewer has endeavored to delineate the usefulness of the better established correlations between the magnitudes of interproton spin-spin coupling constants and structure in an essentially empirical manner. This approach although clearly more closely related to 'stamp collecting' than to 'physics'* is considered justified because it is obvious that the underlying cause of the boom in n.m.r. spectroscopy is its usefulness as an analytical tool in organic chemistry rather than its (very considerable) inherent interest. It is clear that at the present state of the art, the empirical approach, which amounts to argument by analogy, is less likely to lead to errors of interpretation than any attempt to apply the necessarily simplified, theoretical treatments."

*With apologies to Lord Rutherford.

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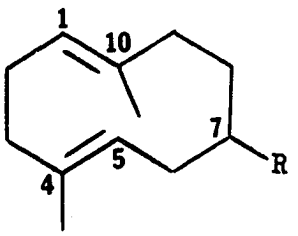
II. STRUCTURE AND PMR SPECTRA OF NATURAL PRODUCTS. THE ASSOCIATION OF HIGH FIELD VINYL METHYL SIGNALS WITH TRANS DOUBLE BOND GEOMETRY IN GERMACRENE DERIVATIVES

In their early report on the small but characteristic differences in the chemical shifts of vinyl methyl groups on trisubstituted double bonds of terpenoid compounds, Bates and Gale[1] observed that compounds of the germacrane system often displayed two distinct vinyl methyl absorptions, one of which typically fell at higher field than the normal range of δ 1.74-1.59 for such signals. The vinyl methyl groups in this system normally occur at positions 4 and 10, with the double bonds disposed between positions 4 and 5, and 1 and 10 (see below). Bates and Gale attributed the high field vinyl methyl signals to a shielding effect produced by the transannular double bond on the absorbing methyl protons. Their observations were made without knowledge of the configurations of the double bonds in question.



A Typical Germacrene Ring

Subsequently, Sathe, Kulkarni, and Kelkar[2] demonstrated that, in most instances, the C-10 methyl was responsible for the abnormally high field absorption. They also attributed the shielding to the transannular π-system, but pointed out that the presence of oxygen substituents caused considerable variability in the chemical shifts of the vinyl methyl signals because of deshielding effects. These observations were also made without taking into account the geometry of the endocyclic double bonds, or the conformation of the 10-membered ring.

In the interim, the absolute configurations of a vast number of germacrane-derived natural products have been determined and the endocyclic double bonds are now known to occur in either the cis or trans forms at either site.[3,4]

A useful rule[5] which correlates the high field chemical shift noted above with trans double bond geometry is presented herein.

RESULTS AND DISCUSSION

Table I shows the chemical shifts of the vinyl methyl pmr signals of 81 natural products (Structures in Table II) which possess the germacrane ring system. The geometry of the associated double bond is also listed where known. As shown by the first 34 entries in Table I, a vinyl methyl absorption occurring at δ 1.55[6] or higher field is invariably associated with trans geometry for the double bond to which the methyl group is attached. The illustration below shows the correlation graphically. If the vinyl methyl signal occurs between δ 1.12 and δ 1.55 then the methyl group resides on a trans double bond. If the signal is between δ 1.55 and δ 2.26 then the double bond may

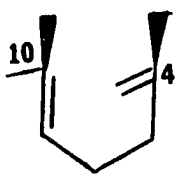
be cis or trans.

Double Bond Geometry	Cis or Trans	Trans
Chemical Shift (ppm)	2.26	1.55	1.12

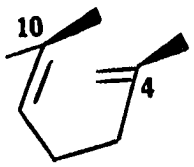
This relationship holds not only for hydrocarbons in the series, but also for compounds possessing most of the commonly encountered types and patterns of oxygen substitution. Thus, it appears that the dominant parameter resulting in strong shielding must be close approach of the shielded methyl to the transannular π-system,[8] and that the effects of oxygen substituents are of secondary importance.

Of the first 34 entries, the high field absorption is invariably associated with the C-10 methyl[9] in compounds possessing (with the exceptions 15, 20, and 34) trans, trans configurations. The signals of methyl groups situated on cis double bonds (17 examples) all lie downfield from the δ 1.55 limit, as do those of a large number of methyl groups on trans double bonds.

Although it is impossible to make conformational arguments embracing all of these observations, certain conclusions may reasonably be drawn concerning those compounds displaying highly shielded methyl signals. The common preferred conformation for the trans, trans system (to the extent known) is the crossed conformation[10-15] in which the two methyl groups are syn, and disposed either alpha or beta. In the idealized form of this conformation, the planes of the two π-systems are perpendicular to the plane of the ten-membered ring, and are



A



B

roughly parallel to each other. In such a conformation (A), the two methyl groups would be equidistant from the shielding zones of the opposed π-systems, and would be equally shielded. The identity of the chemical shifts of the germacrene hydrocarbons 24 and 25 suggest that these compounds prefer this conformation in solution; this conclusion is reinforced by the extreme ease with which they undergo the Cope rearrangement,[16,17] the transition state for which requires precisely this geometry.

The predominance of examples of highly shielded C-10 methyls appear to be explainable in terms of a variation on this idealized conformation in which the planes of the two π-systems remain roughly parallel, but tilted with respect to the plane of the ten-membered ring, as in B. In this conformation, the C-10 methyl preferentially confronts the opposing π-system and is shielded, whereas the C-4 methyl is not shielded. Models suggest that this conformation (B) is readily accessible, but that the converse one, resulting from tilt of both planes in the opposite direction, is not, thus explaining the infrequent observation of a highly shielded C-4 methyl group.

Models further suggest that the planes of the two π-systems can be separately tilted from the perpendicular in opposite directions,

resulting in conformations in which neither of the methyls confronts the shielding zone of the transannular π-system, thus accounting for lower field signals. The influence of substituents on conformational preferences is extremely obscure in this system, and it is impossible at this time to correlate substituent types and positions with conformational preferences.

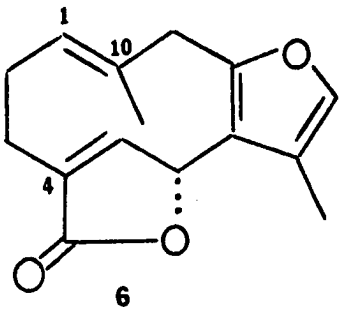
Incorporation of the transannular double bond into a conjugated system increases the likelihood of shielding of the opposed methyl group (see examples 1, 2, and 3). This probably results both from an increase in the area of the shielding zone of the opposed π-system[18] as well as increased rigidity of the ring which would restrict outward tilt of the methyl group.

In systems in which one of the double bonds has the cis configuration, published drawings[13,14] suggest a superficial similarity to to the crossed conformation with respect to the disposition of the two vinyl methyl groups toward the opposed double bonds. Models suggest, however, that in cis, trans systems, the center of the cis double bond is farther from the average plane of the ten-membered ring than that of the trans double bond, thus increasing the distance of the cis methyl group from the face of the opposed double bond (even though the planes of the two double bonds can adopt roughly parallel orientations). This conformational effect is consistent with the failure of any cis methyl group to be strongly shielded.

Using the rule presented above, the methyl group at C-10 in laserolide (13), a germacranolide of unestablished stereochemistry,[19] can be predicted with a high degree of certainty to reside on a carbon

atom which forms part of a trans double bond system.

The rule is further brought into focus when two compounds, which differ only in configuration of one trisubstituted double bond, are compared directly. Linderalactone (6)[20] and neolinderalactone (44)[21] differ only in the configuration of the double bond at C-1, C-10.



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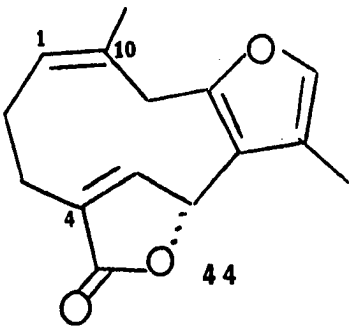
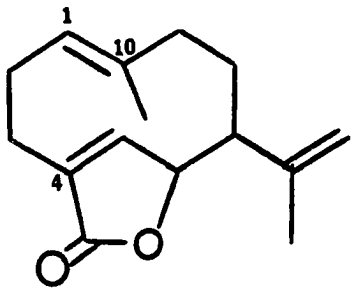


44

The C-10 methyl signal of 6 ($\Delta^{1(10)}$ trans) occurs at δ 1.27, while that of 44 ($\Delta^{1(10)}$ cis) is at δ 1.60. The latter signal is at remarkably low field, considering that the methyl group is across the ring from two conjugated π-systems. Aristolactone (27)[22] and isoaristolactone (38)[22] also differ in geometry at $\Delta^{1(10)}$. The signals in question occur at 1.48 and 1.59 ppm in the trans and cis cases, respectively.



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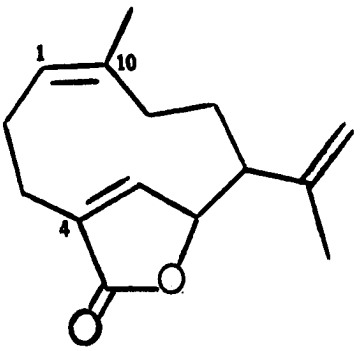
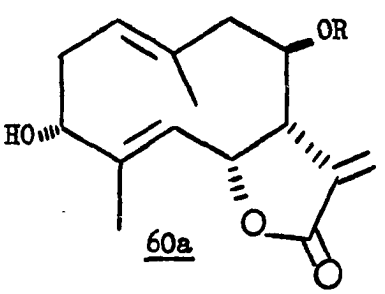
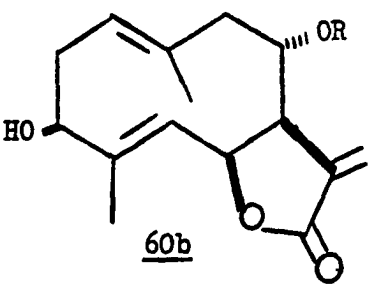


38

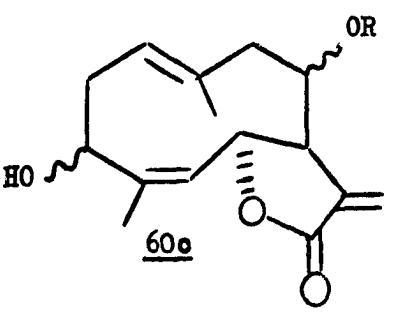
The only germacranolide reported to possess trans, trans geometry and methyl groups on both endocyclic double bonds, and which does not possess one vinyl methyl signal at relatively high field (δ1.68 or higher), is nobilin (60a or its enantiomer 60b).[23] The simplest and most probable explanation for this apparent anomaly is that the geometrical arrangement of the 10-membered ring at present assigned to nobilin is incorrect. We suggest that nobilin, which was isolated by Sorm, et al., from a plant of the compositae family is most probably represented by 60c. This view not only accords with the low field vinyl methyl signals (1.83 and 1.86 ppm), but is supported by the recent work of Hertz and Wahlberg who demonstrated that Samek's rule, which was used in assigning cis-fusion to the lactone ring in nobilin, is inoperable in germacranolides possessing Δ^4 cis.[24-25] The pmr spectrum of nobilin (60)[23] bears a striking resemblance to that reported for provincialin (55) and eucannobinolide (57),[25] germacranolides containing Δ^4 cis, $\Delta^{1(10)}$ trans and a trans fused γ-lactone as in 60c, and both of which are seen to be situated very near nobilin (60) in Table I.



60a



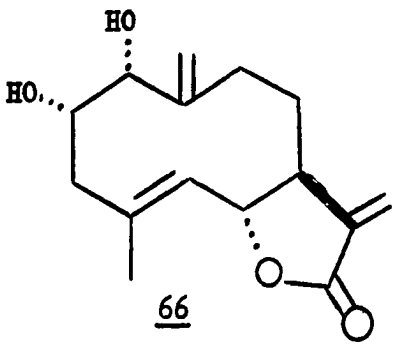
60b



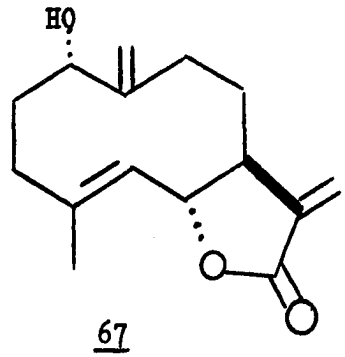
60c

R = -C-C=CHMe
O Me

The compounds verlotorin (66),[26] artemorin (67),[26] artevasin (76),[27] and ridentin (77),[28] comprise an interesting array of structures, all of which possess a single endocyclic double bond of unknown configuration at Δ^4. It is interesting to speculate on the geometry of these unknown configurations based on the data revealed by Table I. Compounds 66 and 67 possess C-4 methyl signals at δ1.69 and δ1.71, values which are quite in accord with a large number of compounds in Table I with Δ^4 trans. The corresponding methyl signals in 76 and 77 are at δ1.85 and δ1.93, respectively. The rather low field at which these latter signals occur appears to be inexplicable by deshielding of their adjacent hydroxyl groups alone,[29] suggesting that the methyl groups in 76 and 77 reside on cis double bonds. These observations await corroboration by other techniques.



66



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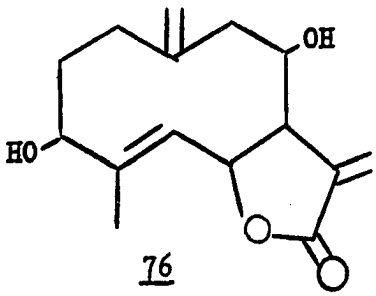

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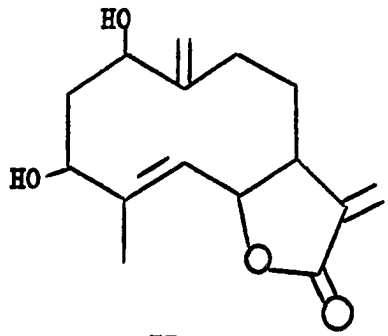

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Table I

Chemical Shifts of the Vinyl Methyl PMR Signals in Some Naturally Occurring Germacrane-Type Rings[a]

Compound	Solvent[b]	C_{10}-Me[c] (δ)	Geometry[d] $\Delta^{1(10)}$	C_4-Me[c] (δ)	Geometry[d] Δ^4	Ref.
1 Acoragermacrone	A	1.12	*E*	1.97	*E*	1
2 Germacrene-C	B	1.15	*E*	1.56	*E*	2, 3
3 Pregeijerene	B	1.17	*E*	1.55	*E*	4, 5
4 Neosericenine	C	1.20	*E*	----	*E*	6
5 Neosericenyl acetate	C	1.25	*E*	----	*E*	6, 7
6 Linderalactone	C	1.27	*E*	----	*E*	8
7 Furanodiene	C	1.28	*E*	1.59	*E*	9
8 Furanodienone	C	1.30	*E*	1.99	*E*	10
9 Salonitenolide	E	1.3	*E*	----	*E*	11, 12
10 Litsealactone	C	1.35	*E*	----	*E*	8
11 Salonitolide[e]	D	1.37	*E*	----	*E*	45, 81
12 Preisocalamendiol	B	1.37	*E*	----	-	13, 14
13 Laserolide	C	1.40		1.73		15
14 Dihydrocostunolide	B	1.41	*E*	1.67	*E*	16, 17
15 Sericenine	C	1.41	*E*	----	*Z*	18, 19

Table I (continued)

Compound	Solvent[b]	C_{10}-Me[c] (δ)	Geometry[d] $\Delta^{1(10)}$	C_4-Me[c] (δ)	Geometry[d] Δ^4	Ref.
16 Onopordopicrin[e]	E	1.41	*E*	----	*E*	12,20,21
17 Costunolide	C	1.42	*E*	1.70	*E*	22, 23
18 Balchanolide	I	1.43	*E*	1.66	*E*	24, 25
19 Tamaulipin-B	C	1.44	*E*	1.63	*E*	26
20 Sericenic acid	C	1.44	*E*	----	*Z*	18, 19
21 Bicyclogermacrene	F	1.46	*E*	1.63	*E*	27
22 Cnicin	D	1.46	*E*	----	*E*	12, 28
23 Agerol	C	1.47	*E*	[f]	*E*	29
24 Germacrene-A	B	1.48	*E*	1.48	*E*	30
25 Germacrene-B	I	1.48	*E*	1.48	*E*	31, 32
26 Hedycaryol	B	1.48	*E*	1.57	*E*	33, 34
27 Aristolactone	B	1.48	*E*	----	*E*	35
28 Arctiopicrin[e]	C	1.48	*E*	----	*E*	20, 36
29 Tamaulipin-A	C	1.49	*E*	1.74	*E*	37, 38
30 Eupatoriopicrin	C	1.50	*E*	1.78	*E*	22, 39
31 Scabiolide[e]	C	1.50	*E*	----	*E*	12, 21
32 Epitulipinolide	C	1.52	*E*	1.76	*E*	39
33 Germacrene-D	I	1.52	*E*	----	-	40
34 Isofuranodienone	C	1.55	*E*	1.89	*Z*	10
35 Tulipinolide	C	1.58	*E*	1.71	*E*	39
36 Linderane	C	1.58	*E*	----	-	41, 42
37 Chihuahuin	C	1.59	*E*	1.63	*E*	43

Table I (continued)

Compound	Solvent[b]	C_{10}-Me[c] (δ)	Geometry[d] $\Delta^{1(10)}$	C_4-Me[c] (δ)	Geometry[d] Δ^4	Ref.
38 Isoaristolactone	I	1.59	*Z*	----	*E*	35
39 Germacrone	B	1.60	*E*	1.42	*E*	32, 44
40 Artemisiifolin	C	1.60	*E*	----	*E*	12, 45
41 Zederone	C	1.60	*E*	----	-	46
42 Dehydrocurdione	B	1.60	*E*	----	-	47
43 Curdione	B	1.60		----	-	48
44 Neolinderalactone[g]	I	1.60	*Z*	----	*E*	49
45 Isabelin[g]	C	1.60	*E*	----	*E*	12
46 Eupatolide	C	1.63	*E*	1.73	*E*	22, 40
47 Ketopelenolide-B	C	1.63	*E*	----	-	50
48 Shiromool	C	1.64		----	-	56
49 Ketopelenolide-A	C	1.65	*E*	----	-	50
50 Chamissonin	G	1.67	*E*	2.26	*E*	51, 52
51 Laurenobiolide	C	1.68	*E*	1.68	*E*	53
52 Hydroxypelenolide	C	1.71	*E*	----	-	50
53 Dihydroparthenolide	C	1.71		----	-	54, 58
54 Parthenolide	C	1.72		----	-	54
55 Provincialin	C	1.77	*E*	1.83	*Z*	55
56 Shiromodiol diacetate	C	1.78	*E*	----	-	56, 57
57 Eucannabinolide	C	1.79	*E*	1.82	*Z*	22, 55
58 Lanuginolide	C	1.80	*E*[h]	----	-	58

Table I (continued)

Compound	Solvent[b]	C_{10}-Me[c] (δ)	Geometry[d] $\Delta^{1(10)}$	C_4-Me[c] (δ)	Geometry[d] Δ^4	Ref.
59 Baileyin	I	1.80		----	-	59
60 Nobilin	D	1.83		1.86		60
61 Zeylanine	C	1.89	*Z*	----	*E*	61
62 Zeylanane	C	1.91	*Z*	----	-	61
63 Eupacunin	F	1.97	*Z*	1.80	*Z*	62
64 Eupatocunin	C	1.97	*E*	1.89	*Z*	62
65 Frutescin	C	----	*Z*	1.69	*E*	65
66 Verlotorin	I	----	-	1.69		66
67 Artemorin	I	----	-	1.71		66
68 Chrysanolide	C	----	-	1.76	*E*	67
69 Woodhousin	C	----	-	1.77	*Z*	73
70 Anhydroverlotorin	I	----	-	1.77		66
71 Heliangin	C	----	-	1.8	*Z*	64,68,69
72 Deoxyelephantopin	C	----	*E*	1.83	*E*	80
73 Erioflorin	C	----	-	1.84	*Z*	55, 70
74 Pyrethrosin	I	----	-	1.86	*E*	44, 71
75 Calaxin[i]	C	----	-	1.87	*Z*	72, 73
76 Artevasin	H	----	-	1.88		74
77 Ridentin	I	----	-	1.93		75
78 Liatrin	C	----	-	1.94	*Z*	76
79 Uvedalin	C	----	*Z*	1.96	*E*	77, 78
80 Polydalin	C	----	*Z*	2.00	*E*	77, 78
81 Melampodin	C	----	*Z*	2.10	*E*	63, 64

Footnotes for Table I

[a]The data compiled in Table I were obtained from the references cited. Chemical shifts are in ppm (δ) from tetramethylsilane. Various authors have assigned configurations to the double bonds based on the results of acid catalyzed cyclizations, Cope rearrangements, x-ray analyses, chemical correlations, circular dichroism, and nuclear Overhauser effect studies. [b]A = benzene-d_6, B = carbon tetrachloride, C = chloroform-d_1, D = dimethylsulfoxide-d_6, E = dimethylsulfoxide-d_6 plus chloroform-d_1, F = acetone-d_6, G = pyridine, H = pyridine-d_5, I = unspecified solvent. [c]A dash indicates the absence of a vinyl methyl group. [d]Cis double bonds are denoted by *Z*, and trans double bonds by *E*. A dash indicates the absence of a double bond, and unknown configurations are left blank. [e]Ref. 12 established trans configurations for the double bonds in 9 and 22. The 10-membered rings in 9 and 22 had previously been correlated with 16, 28 and 31 via 11.[20,21] Therefore, all of these compounds possess trans, trans double bonds. [f]The C-4 methyl signal in 23 is uncertain: it is either δ 1.56 or 1.77. [g]Only the major conformer is listed for 44 and 45. The minor (20%) conformer of 44 (C-10 Me δ 1.46 at -40°C) constitutes the single exception to the generalization presented herein; however, the abnormally high field chemical shift could be anticipated owing to the strained geometry in this conformer, and the anisotropic effect of the transannular α,β-unsaturated lactone.[79] [h]The trans assignment was based on a conformational analysis. [i]75 has been correlated with ciliarin.[72] Ciliarin possesses Δ^4 cis.[73]

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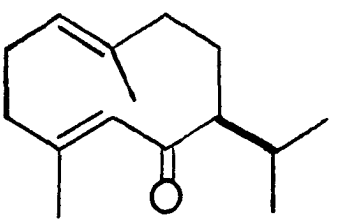
Table II

Structures For The Compounds Listed In Table I



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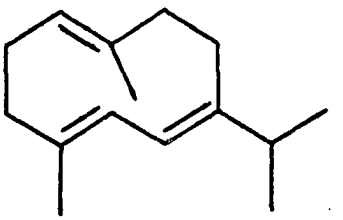

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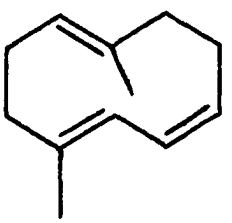

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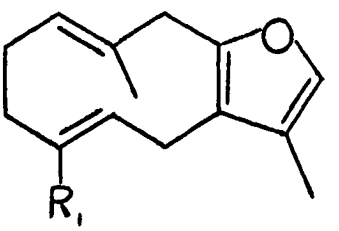

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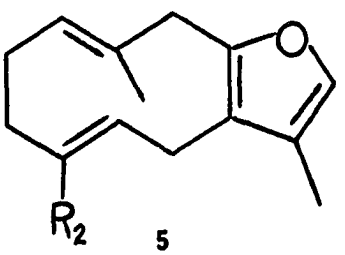

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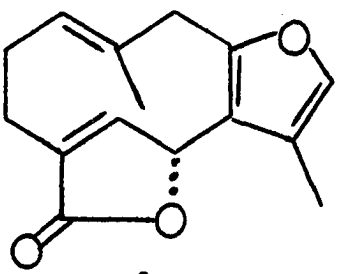

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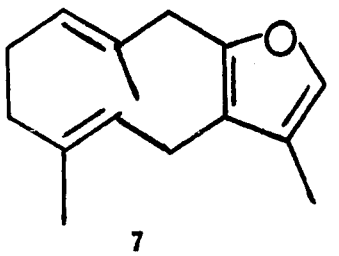
$R_1 = CO_2CH_3$, $R_2 = CH_2OAc$

Table II



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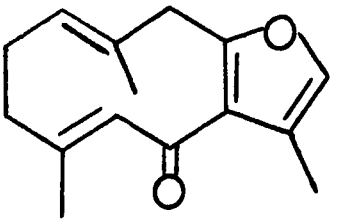

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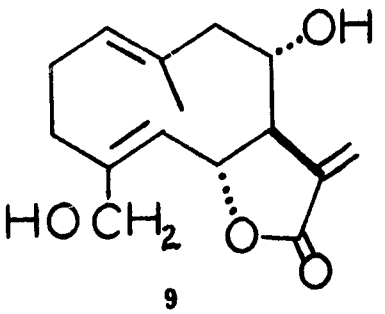

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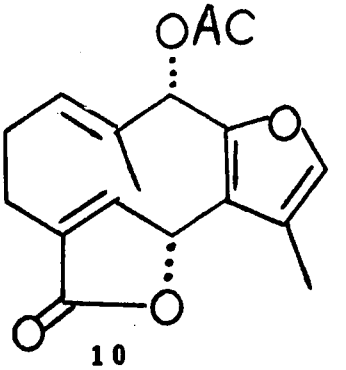

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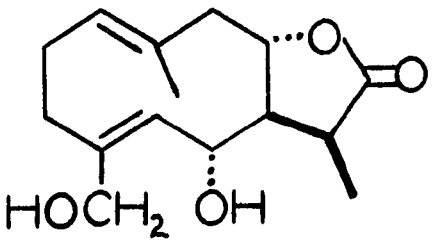

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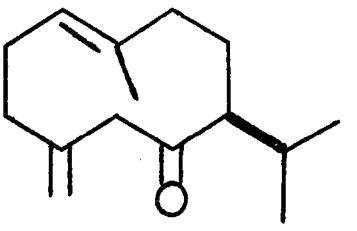

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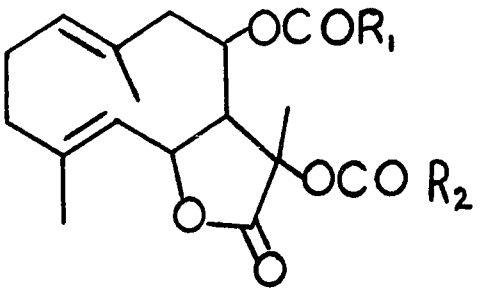

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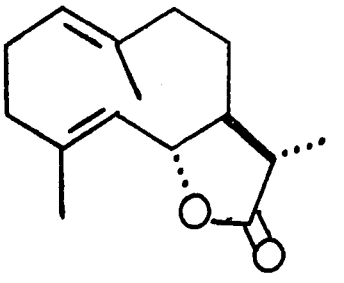
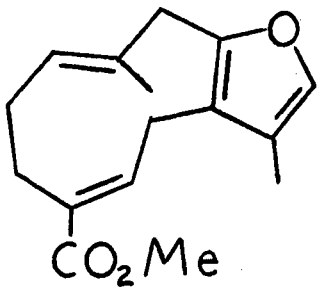


14

R_1 = $C(CH_3)=CHCH_3$, R_2 = CH_3

Table II



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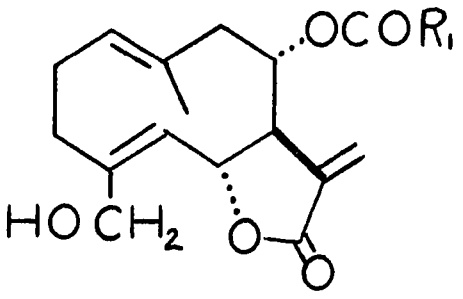

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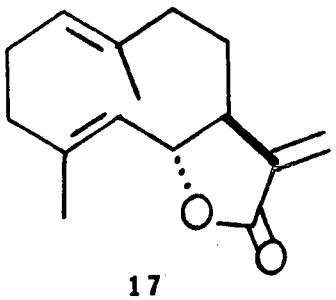

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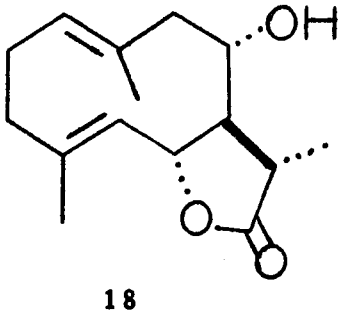

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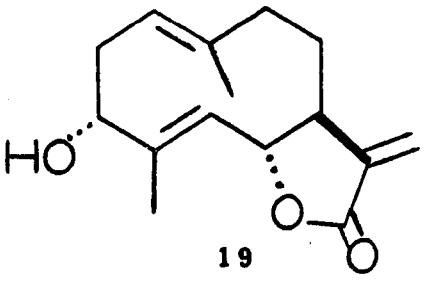

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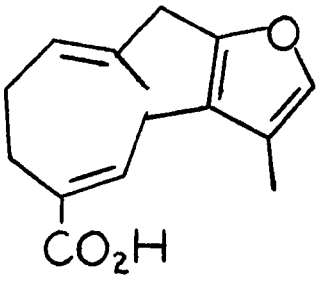

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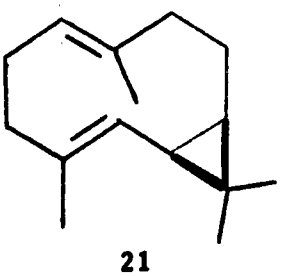

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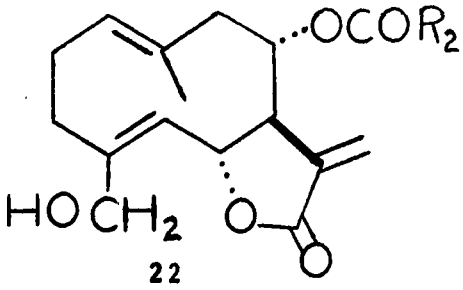
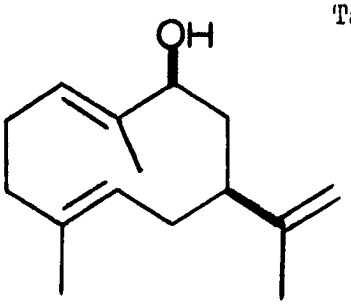


22

R_1 = $C(=CH_2)CH_2OH$, R_2 = $C(=CH_2)CHOHCH_2OH$

Table II



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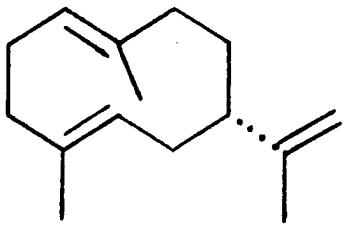

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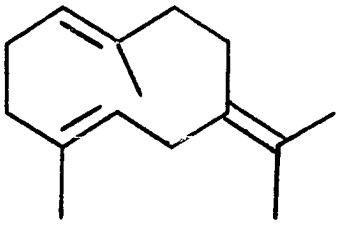

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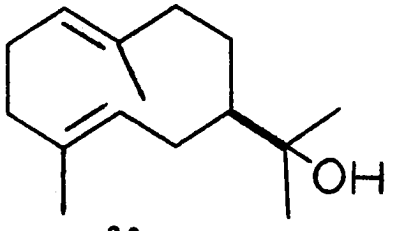

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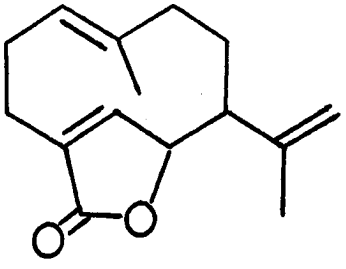

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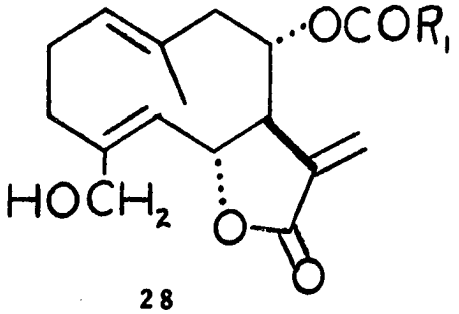

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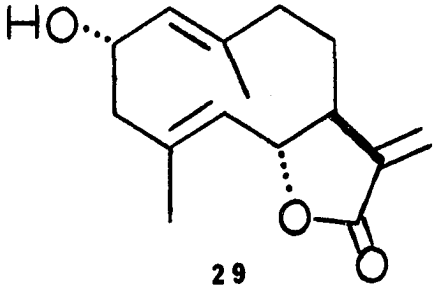

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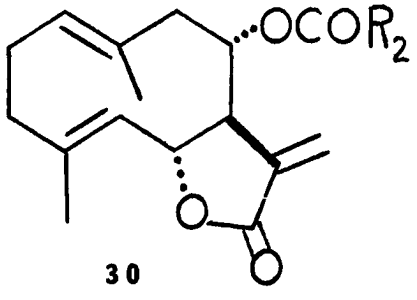
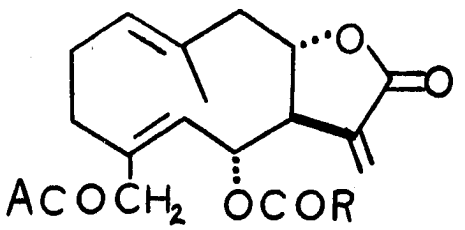


30

R_1 = $CH(CH_3)CH_2OH$, R_2 = $\cdot C(CH_2OH)=CHCH_2OH$

Table II



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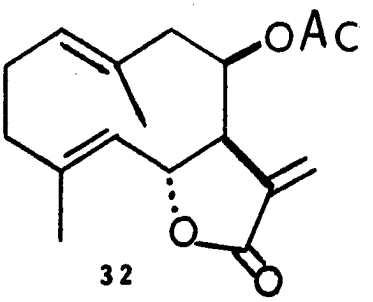

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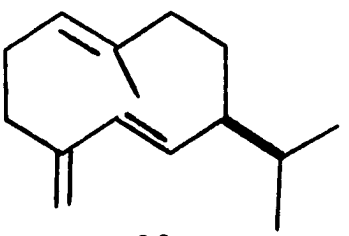

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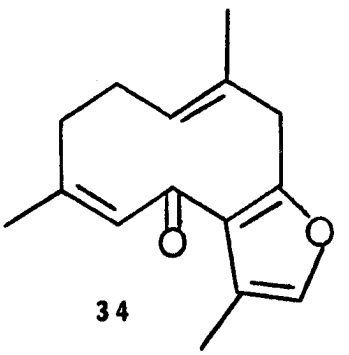

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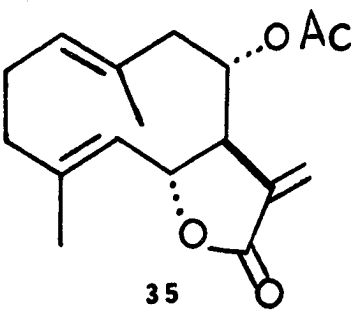

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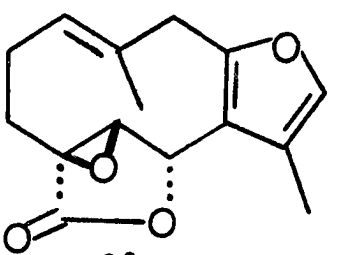

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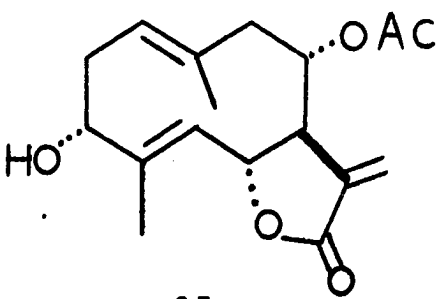

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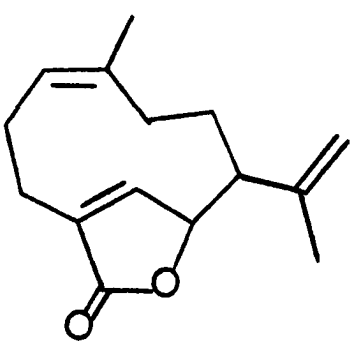
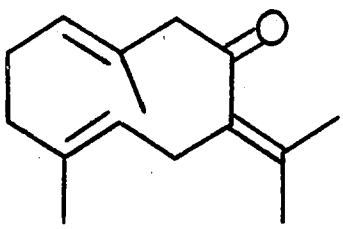


38

R = $C(CH_3)OHCH_2OH$

Table II



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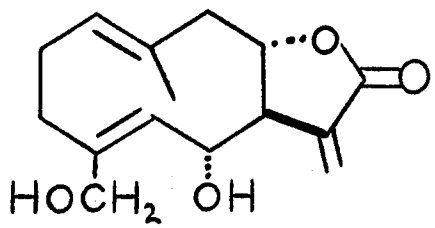

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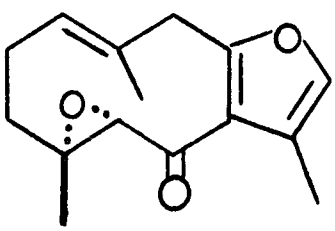

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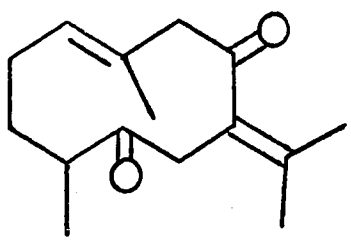

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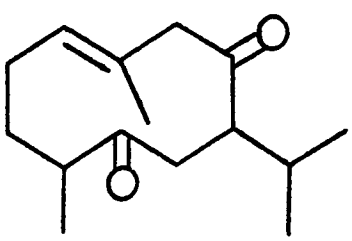

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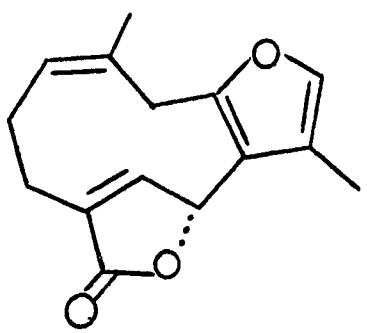

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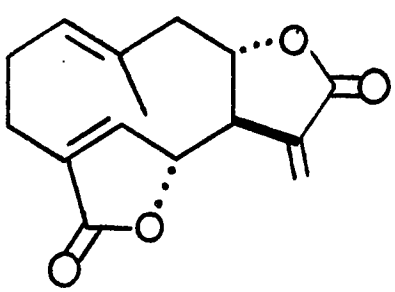

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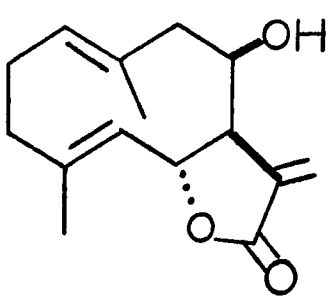

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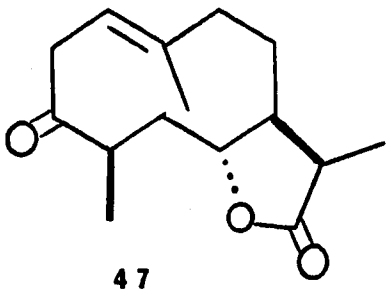

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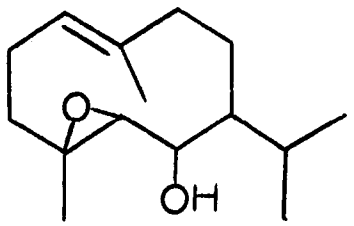

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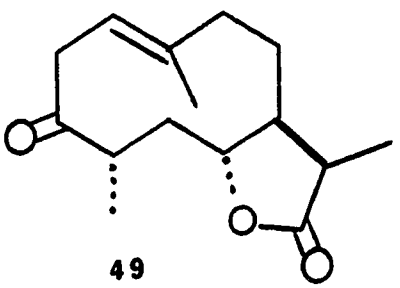

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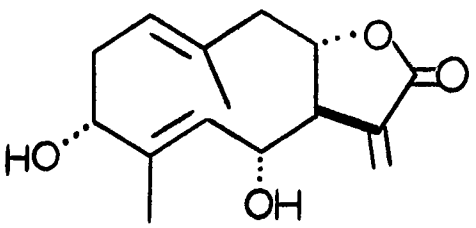

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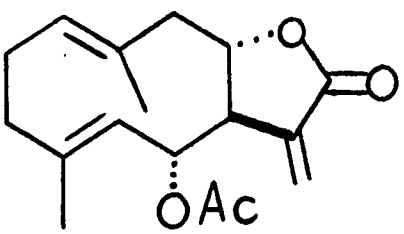

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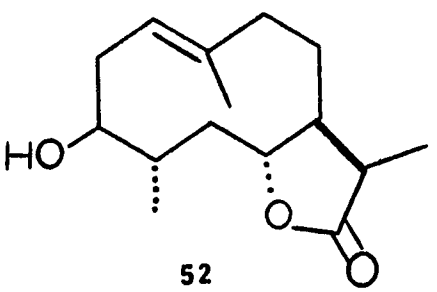

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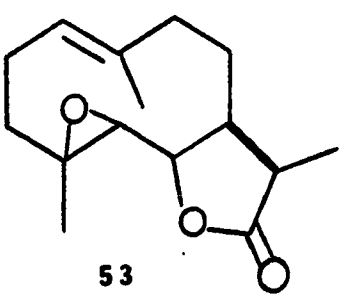

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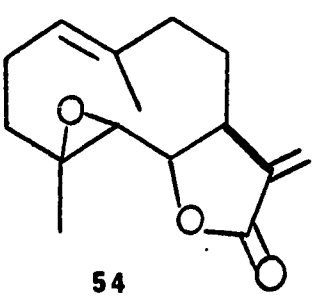

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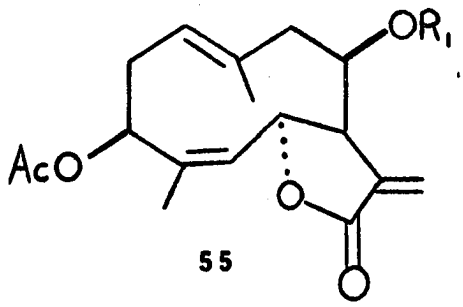
Table II



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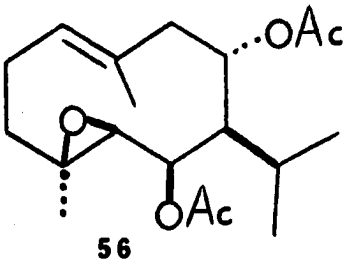

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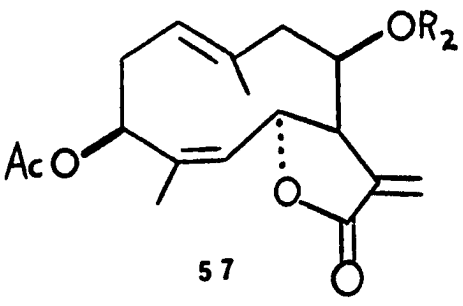

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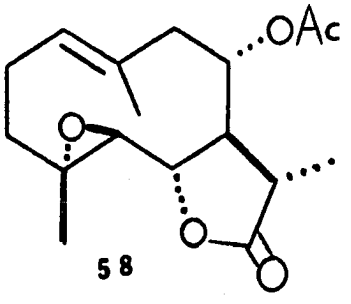

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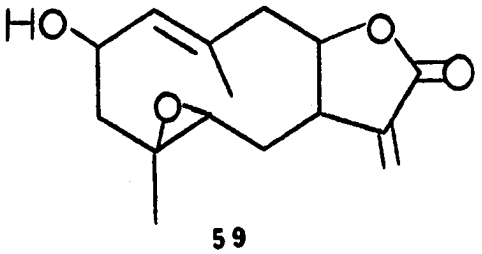

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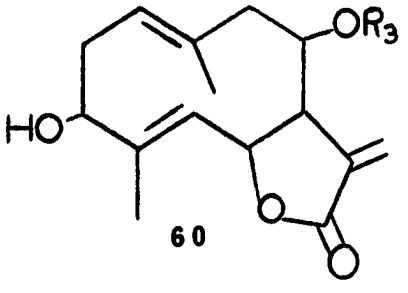

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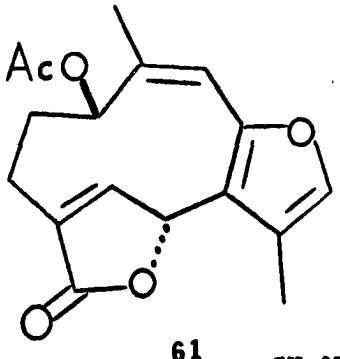

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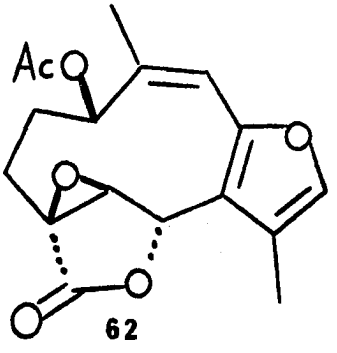


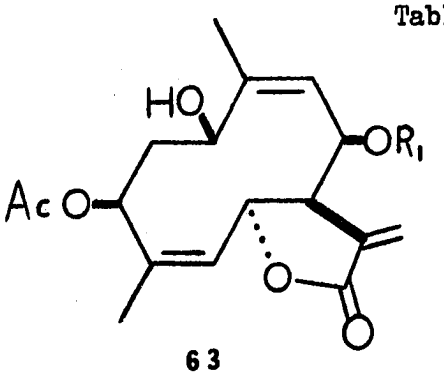
62

$R_1 = -C(=O)-C(CH_2-O-C(=O)-C(CH_2OH)=CHCH_3)=CHCH_2OH$

$R_2 = -C(=O)-C(CH_2OH)=CHCH_2OH$

$R_3 = -C(=O)-C(CH_3)=CHCH_3$

Table II



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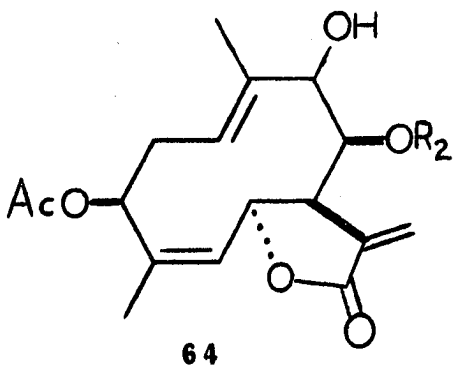

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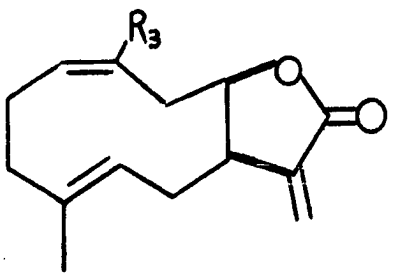

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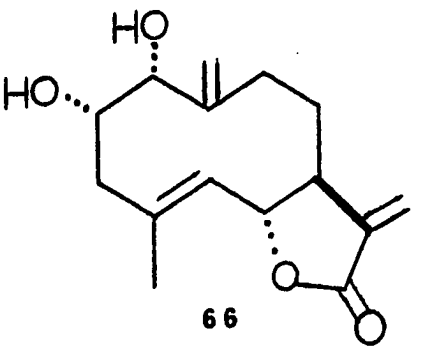

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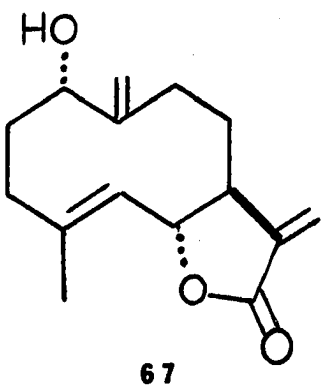

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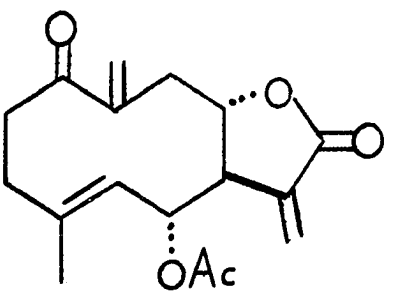

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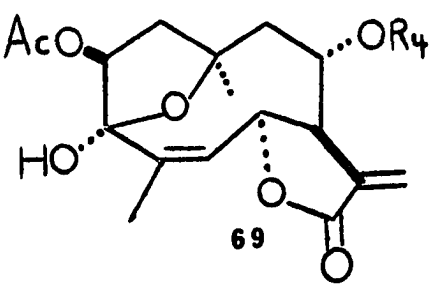

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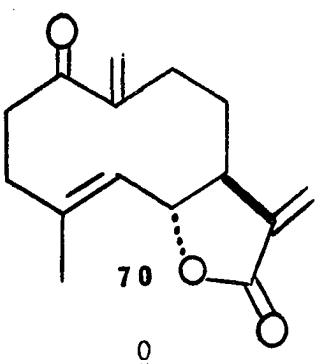
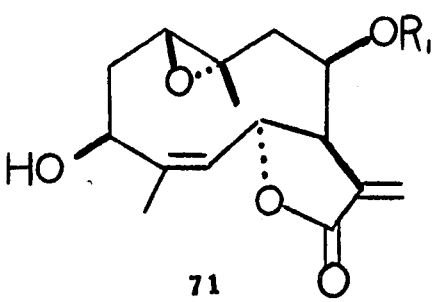


70

$R_1 = R_2 = -\overset{O}{\overset{\|}{C}}-\underset{CH_3}{\overset{|}{C}}=CHCH_3$ $\quad R_3 = -\overset{O}{\overset{\|}{C}}-H$ $\quad R_4 = -\overset{O}{\overset{\|}{C}}-CH(CH_3)_2$

Table II



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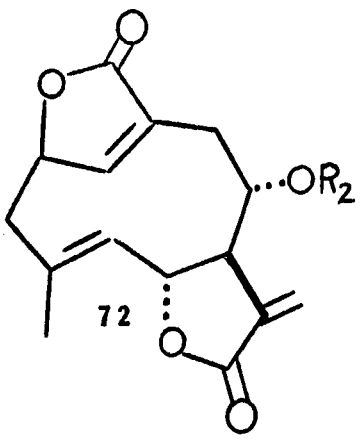

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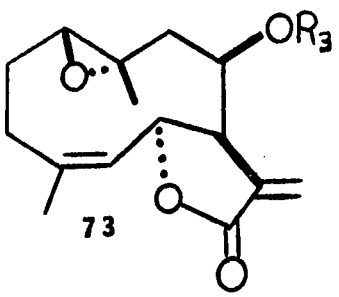

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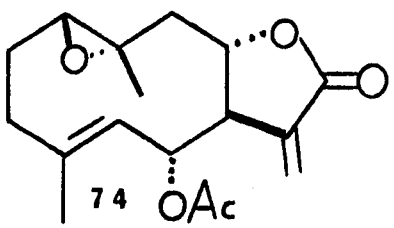

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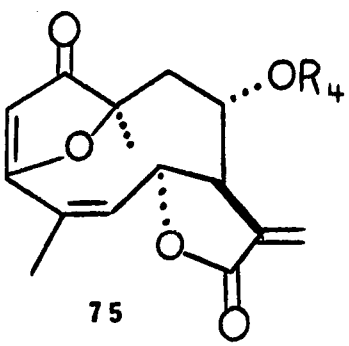

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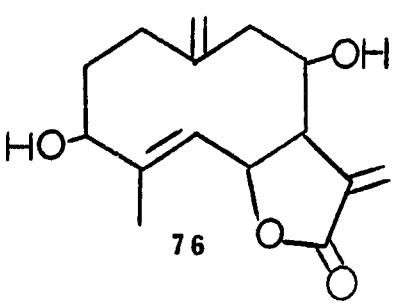

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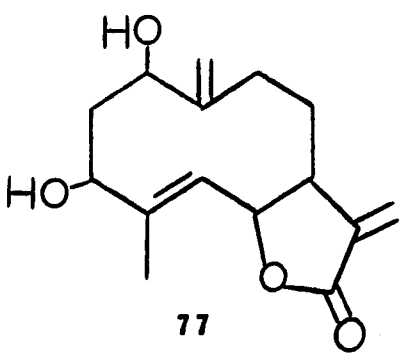

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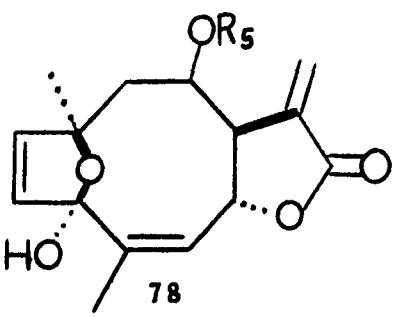
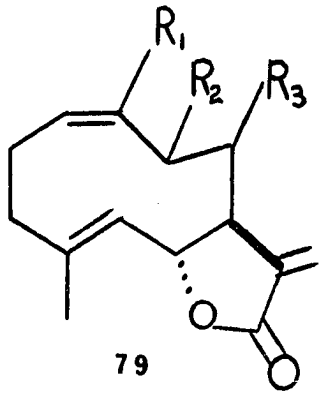


78

R_1 = $-\overset{\overset{O}{\|}}{C}-\overset{\overset{CH_3}{|}}{C}=CHCH_3$ $R_2 = R_3 = R_4$ = $-\overset{\overset{O}{\|}}{C}-\overset{\overset{CH_2}{\|}}{C}-CH_3$ R_5 = $-\overset{\overset{O}{\|}}{C}-\overset{\overset{CH_2OAc}{|}}{C}=CHCH_3$

Table II



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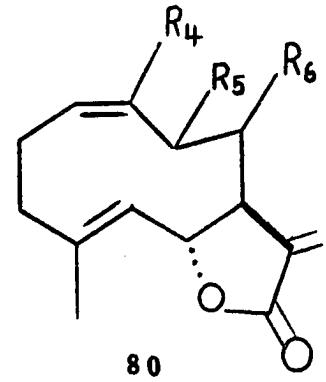

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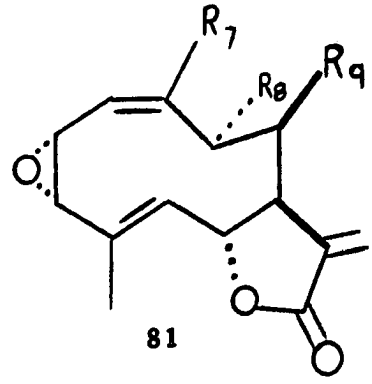

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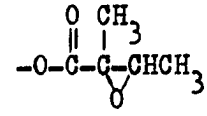
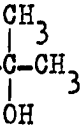
$R_1 = R_4 = R_7 = CO_2CH_3$ $\quad R_2 = R_5 = OAc$

$R_3 = R_9 =$  $\quad R_6 =$ 

$R_8 = -OH$

SUMMARY

Consideration of the vinyl methyl pmr signals of some 80 natural products of the germacrene system reveals that a vinyl methyl absorption occurring at δ 1.55 or higher field (over 30 examples) is invariably associated with trans geometry for the double bond to which the methyl group is attached. This observation was discussed in terms of variations from the idealized crossed conformation commonly adopted by the trans, trans-1(10), 4-diene system. The utility of the correlation as a tool in structure determinations is indicated and exemplified.

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6. The value of δ 1.55 is selected even though δ 1.59 represents the highest field for a cis example in Table I. This allows for new compounds which might lower the value; e.g., cis, cis-germacrone, a synthetic compound has been reported[7] to have vinyl methyl absorptions at δ 1.74 and δ 1.57.

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9. That the C-10 methyl is responsible for the high field signal follows from the thirteen samples (compounds 4, 5, 6, 9, 10, 11, 15, 16, 20, 22, 27, 28, and 31) which possess two endocyclic double bonds, but have a methyl group only at the C-10 carbon. Sathe, Kulkarni, and Kelkar's original observation was made from three examples of this type.

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29. A vinylic methyl signal is shifted to lower field by approximately 0.1 ppm on the introduction of a neighboring hydroxyl group (Cf. 23 and 24, and 35 and 37).

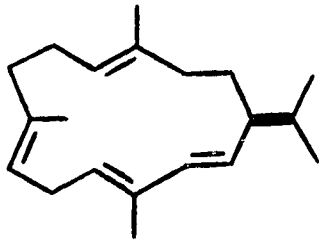
..."The majority of germacranolides contain a double bond conjugated with the bound carboxyl which makes them rather sensitive and unstable. When determining their structures by chemical methods we committed errors in some cases but we corrected them gradually by applying modern physical methods to their study."...

F. Sorm, Pure and Applied Chemistry, 21, 263 (1970).

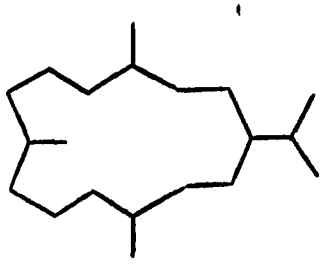
III. NEW MARINE DITERPENOIDS

INTRODUCTION

Naturally occurring diterpenoids containing a fourteen-membered carbocyclic ring have been recognized as such only relatively recently. Cembrene (1) also known as thunbergene, d-tumbelene and the Wienhaus hydrocarbon, was the first 14-carbon ring compound to be identified.[1-5] 1 occurs in the oleoresin of many pine trees of the subgenus Haploxylon of which Cembrae is a group. Thus, cembrane is the trivial name for the saturated cembrene derivative 2. The absolute configuration of the



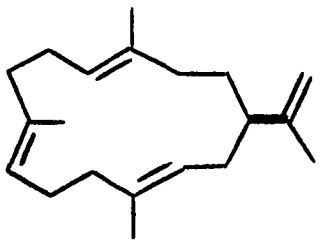
1



2

asymmetric carbon in cembrene is S, which follows from a degradation to the known (-)-S-2-isopropyl-5-oxohexanoic acid.[5,6] The all trans stereochemistry of the trisubstituted double bonds is known from an x-ray study of cembrene.[2] (Reference 2 shows R chirality for cembrene; i.e., the enantiomer of the natural form is depicted.)

Three hydrocarbons (3, 4 and 5) have been isolated which are isomeric with cembrene (Table I). Cembrene-A (3) isolated from a tree in India[7,8] possesses the identical gross structure found for compounds isolated about the same time from a Siberian spruce[9,10] in Russia, from termites in Australia,[11] and from a Pacific soft coral.[12] The compound isolated in Russia was called neocembrene and the Australian compound was named neocembrene-A.



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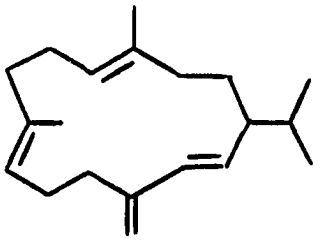
The other two hydrocarbons, isocembrene (4) and casbene (5), were isolated from a Russian pine tree[13] and castor beans,[14,15] respectively.



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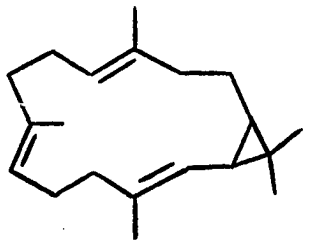


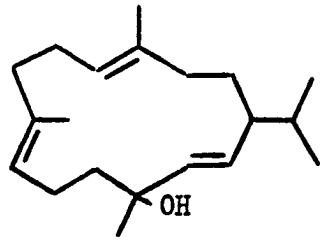
5

The stereochemistry has not been vigorously demonstrated for the trisubstituted double bonds in 3, 4 or 5.

Table II summarizes the available data on oxygenated cembrane derivatives. (Cembranolides were discussed in Section I.) Five monohydric alcohols, thunbergol (6),[16,17] isocembrol (7),[13] mukulol (8),[8] cembrol (9),[18] and nepthenol (10)[12,19] have been reported (Chart I).

Chart I

Naturally Occurring Cembrols



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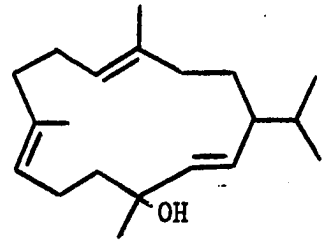
Thunbergol



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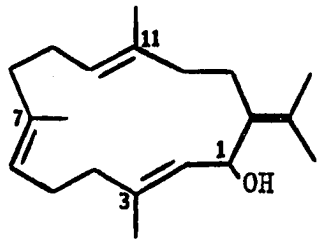
Isocembrol



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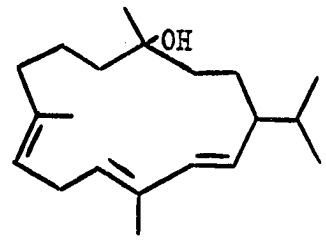
Mukulol



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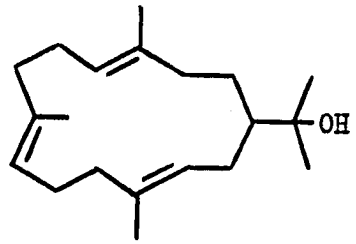
Cembrol



10

Nepthenol

Table I

Cembrane Derived Hydrocarbons

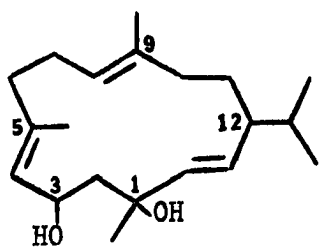
	Name	Formula	Mol Wt	Mp	α_D
(+)	Cembrene (1)	$C_{20}H_{32}$	272	59-60°	+238°
(-)	Cembrene-A (3)	$C_{20}H_{32}$	272	oil	-19.7°
(+)	Isocembrene (4)	$C_{20}H_{32}$	272	--	+60.3°
	Casbene (5)	$C_{20}H_{32}$	272	--	--

Compounds 6 and 7 differ only in configuration at the carbinol carbon atom. The configuration of the alcohol carbon has not been established for compounds 6, 7, 8 or 9. The chirality of the asymmetric carbon bearing the 3-carbon side chain has been firmly established only in 8, which was converted to (+)-cembrene. (The configuration was incorrectly assigned, however, due to a misinterpretation of the stereochemistry of cembrene.) The geometry of the double bonds in 6, 7 and 9 are all trans: assignment of the geometry of the double bonds in 10 has not been made. The 6,7 and 10,11 double bonds of 8 possess trans geometry, while Δ^2 remains unassigned.

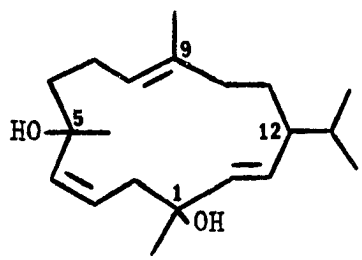
Seven oxygenated diterpenoids (Chart II) possessing the cembrane skeleton have been isolated from tobacco (Nicotiana tabacum) and characterized by the R. J. Reynolds group.[20-22] These authors assigned the name duvane (from duvan, Serbian for tobacco) to the structure 2, and named the macrocyclic diterpenoids from tobacco as derivatives of duvane, e.g., α-4,8,13-duvatriene-1,3-diol (11). Cembrane[23] appears to be the trivial name of choice in the literature, although the duvane nomenclature is commonly used when reference is made to the tobacco derived compounds.[24]

Chart II

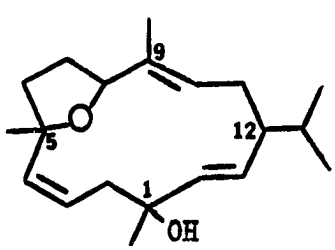
Oxygenated Diterpenoids From Tobacco[a]



11 and 12



13 and 14



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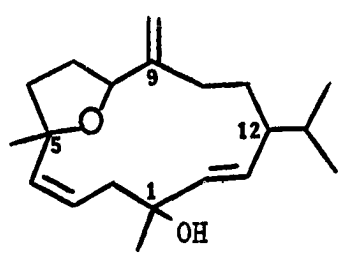


16 and 17

[a]See Table II for the names of the compounds shown above.

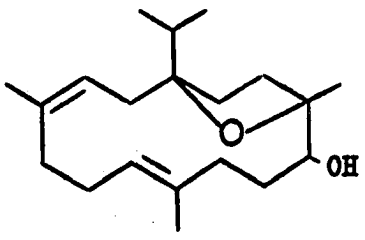
Table II

Oxygenated Cembrane Derivatives

Name	Formula	Mol Wt	Mp	α_D
Thumbergol (6)	$C_{20}H_{34}O$	290	oil	+74.4°
Isocembrol (7)	$C_{20}H_{34}O$	290	--	+80.1°
Mukulol (8)	$C_{20}H_{34}O$	290	37-38°	+53°
Cembrol (9)	$C_{20}H_{34}O$	290	--	+59.6°
Nepthenol (10)	$C_{20}H_{34}O$	290	oil	--
α-4, 8, 13-Duvatriene-1, 3-diol (11)	$C_{20}H_{34}O_2$	306	65-66°	+281.6°
β-4, 8, 13-Duvatriene-1, 3-diol (12)	$C_{20}H_{34}O_2$	306	127-127.5°	+162°
α-3, 8, 13-Duvatriene-1, 5-diol (13)	$C_{20}H_{34}O_2$	306	118-120°	+100°
β-3, 8, 13-Duvatriene-1, 5-diol (14)	$C_{20}H_{34}O_2$	306	150-152°	+40°
α-5, 8-Oxido-3, 9, 13-duvatrien-1-ol (15)	$C_{20}H_{32}O_2$	304	95-96°	+86°
α-5, 8-Oxido-3, 9(17), 13-duvatrien-1-ol (16)	$C_{20}H_{32}O_2$	304	109-110°	+77.4°
β-5, 8-Oxido-3, 9(17), 13-duvatrien-1-ol (17)	$C_{20}H_{32}O_2$	304	108-109°	+72.5°
Incensole (18)	$C_{20}H_{34}O_2$	306	oil	-77.5°
Incensole oxide (19)	$C_{20}H_{34}O_3$	322	164-5°	-48°
Epoxynepthenol Acetate (20)	$C_{22}H_{36}O_3$	348	oil	-20.7°

The alpha and beta designations for the tobacco diterpenes (Table II) have no absolute stereochemical significance, as the chirality has not been firmly established for any of the asymmetric centers in the seven compounds. The alpha designation was given to the diol 11 isolated from tobacco in largest yield. The compounds 11, 13, 15 and 16 in the alpha series possess identical chirality at C-1, and the compounds 12, 14 and 17 of the beta series possess precisely the opposite configuration at this site. The configuration at C-12 is identical in all seven of the tobacco diterpenes (probably S, as in (+)-cembrene), but as yet not established as R or S. The geometry of the disubstituted double bond at C-13 is trans in all seven compounds. The trisubstituted double bonds in 11, 12 and 13 have been assigned all trans geometry.[20-22,24] Although the absolute configurations are unknown, the respective C-5 and C-8 configurations are identical in 15, 16 and 17. No other stereochemical assignments have been made for these interesting compounds.

The macrocyclic diterpenes incensole (18) and incensole oxide (19), which contain the cembrane skeleton were isolated by Italian chemists from frankincense.[25,26] Neither the geometry of the double bonds, nor the chirality of any of the asymmetric centers in 18 or 19 has been established.



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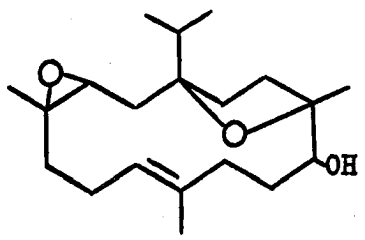
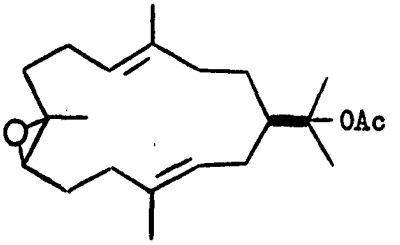


19

An acetylated cembrene derivative, epoxynepthenol acetate, 20, was isolated from a Pacific soft coral. The absolute stereochemistry shown below was established for 20.[12,19] The only stereochemical feature unassigned was the chirality of the transoid epoxide.



20

Thus, diterpenoids possessing the cembrane ring system have been found to occur around the globe in diverse natural sources. The isolation from a Caribbean gorgonian and preliminary characterization of three diterpenoids, which appear to be cembrane derivatives, is reported herein. Certain structural features of a fourth, non-cembrane derived diterpenoid isolated from a Pacific soft coral are also discussed.

RESULTS AND DISCUSSION

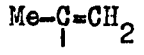
A. New Diterpenoids From A Caribbean Gorgonian

In the course of systematic investigations into the chemistry of marine invertebrates an unidentified gorgonian[27,28] taken at Bonaire, Netherlands Antilles, was examined. The previously unknown gorgonian was classified by Dr. L. S. Ciereszko of this department as a member of the Plexaura or Pseudoplexaura species. Three new diterpenoids were isolated from the hexane extract of the gorgonian. One of the compounds (B-1) proved to be identical to a compound already under investigation in this laboratory as part of a collaborative effort with Dr. R. E. Middlebrook, University of Puerto Rico, Mayaguez, Puerto Rico. Dr. Middlebrook had isolated the compound from the same unidentified gorgonian taken at Puerto Rico. Dr. Middlebrook has initiated efforts to have the gorgonian properly classified in the zoological sense. The thin layer chromatograms of the extracts of the Puerto Rican and Bonaire gorgonians were identical. The gorgonian was also identical by tlc to a specimen collected by Dr. Ciereszko at St. Thomas, Virgin Islands. The new compounds obtained from the gorgonian were prefixed with a B (Bonaire), and labeled B-1, B-2 and B-3 in the order in which they were rendered pure.

B-1 (21), $C_{20}H_{34}O_3$ (high resolution mass spectrum), was a non-conjugated, unsaturated ketone (ir bands at 1700 and 1640 cm^{-1}, no significant uv absorption). The ir spectrum also showed hydroxyl absorption

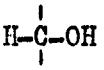
(3500 cm^{-1}). The pmr spectrum indicated the presence of the following structural features: three secondary methyl groups (doublets at 1.04, 1.00 and 0.97 ppm), a vinylic methyl (δ 1.69), a secondary alcohol (a one proton doublet of triplets at δ 3.36) and an exomethylene group (broad two proton signal at δ 4.71). The spectral data are shown in Figure 1.

By double irradiation[8] it was shown that the exomethylene signal (4.71 ppm) and the vinyl methyl signal (1.69 ppm) were mutually coupled ($J \sim 1$ Hz), thus partial structure A was present.



A

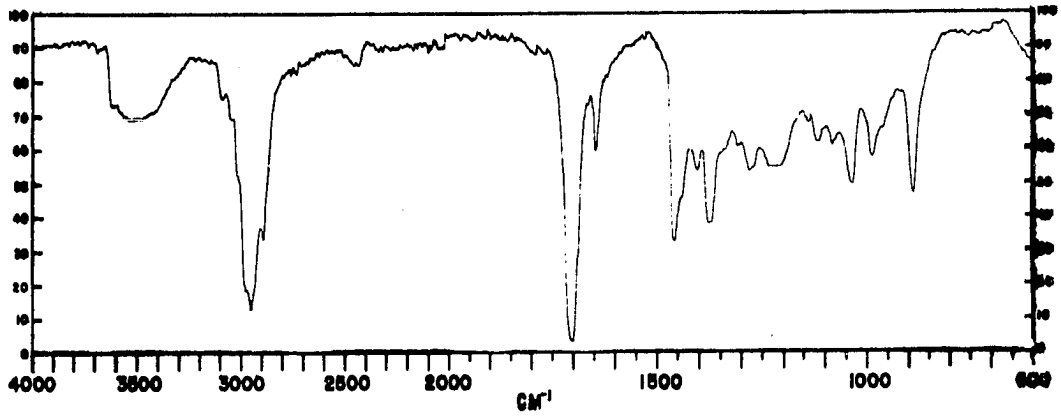
The secondary nature of the alcohol (partial structure B) was confirmed when the one proton signal at δ 3.36 in B-1 moved downfield (δ 4.6) in B-1 acetate (22). Spectral data for 22 are shown in Figure 2.



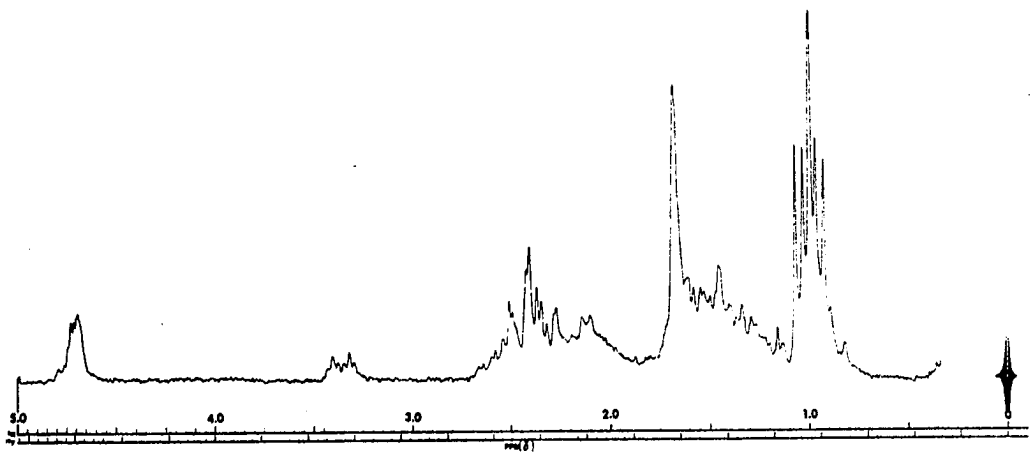
B

Carbon-13 magnetic resonance (cmr) spectroscopy[29] (Figure 3) proved very informative. The presence of A and B in B-1 were firmly established. Two olefinic carbon absorptions occurred at 148.2 and 109.6 ppm in the proton decoupled cmr spectrum. The former signal remained a singlet, while the latter was triplet in the off resonance decoupled cmr spectrum, showing that the single double bond consisted of a carbon atom bearing no hydrogens and one bearing two hydrogens, respectively. The singlet at 70.1 ppm in the proton decoupled cmr spectrum, attributed to the carbinol carbon atom, became a doublet in the off resonance decoupled spectrum, confirming the presence of a secondary

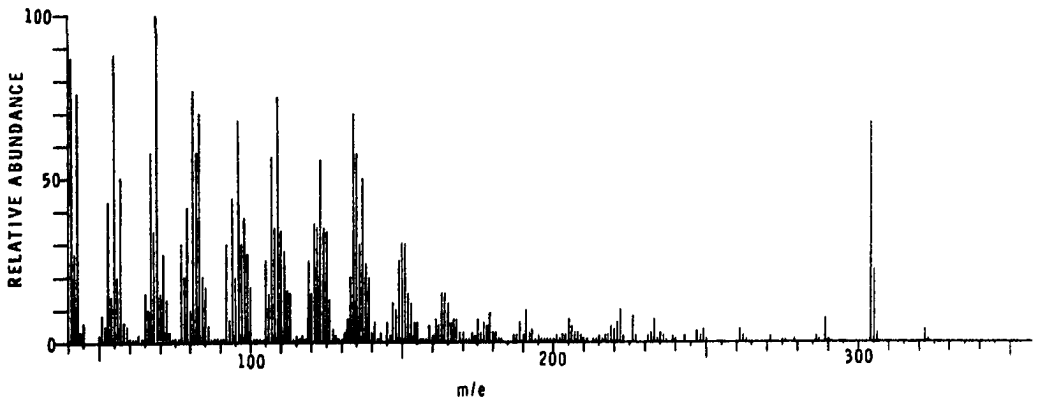
Figure 1



IR Spectrum ($CHCl_3$) of B-1 (21)

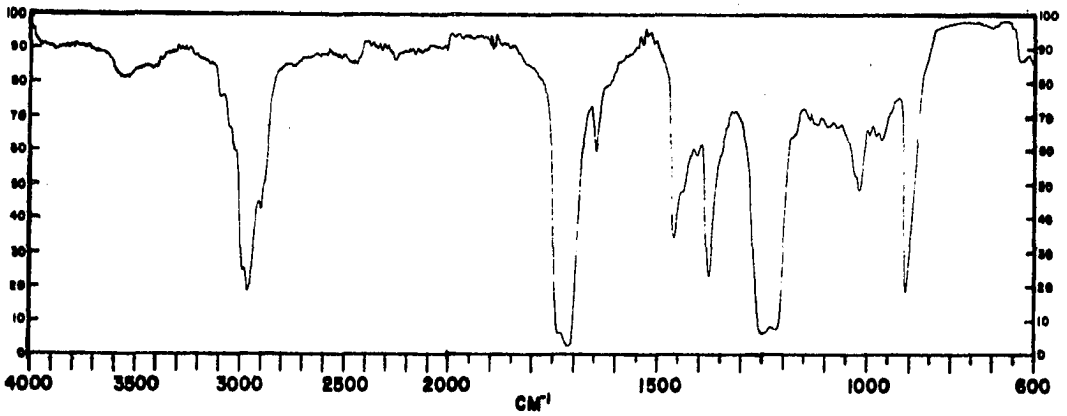


100 MHz PMR Spectrum ($CDCl_3$) of 21

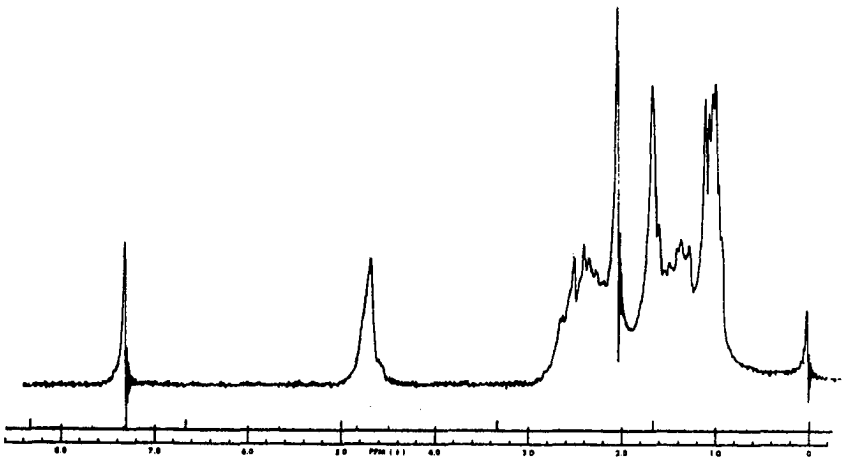


Mass Spectrum of 21

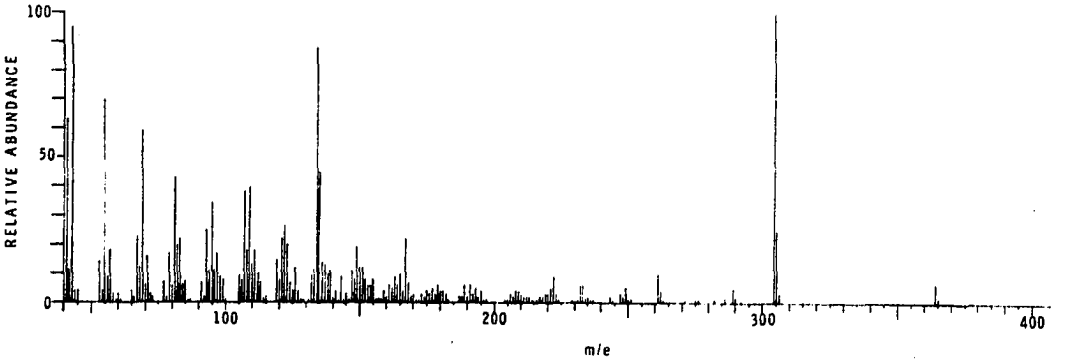
Figure 2



IR Spectrum ($CHCl_3$) of B-1 Acetate (22)

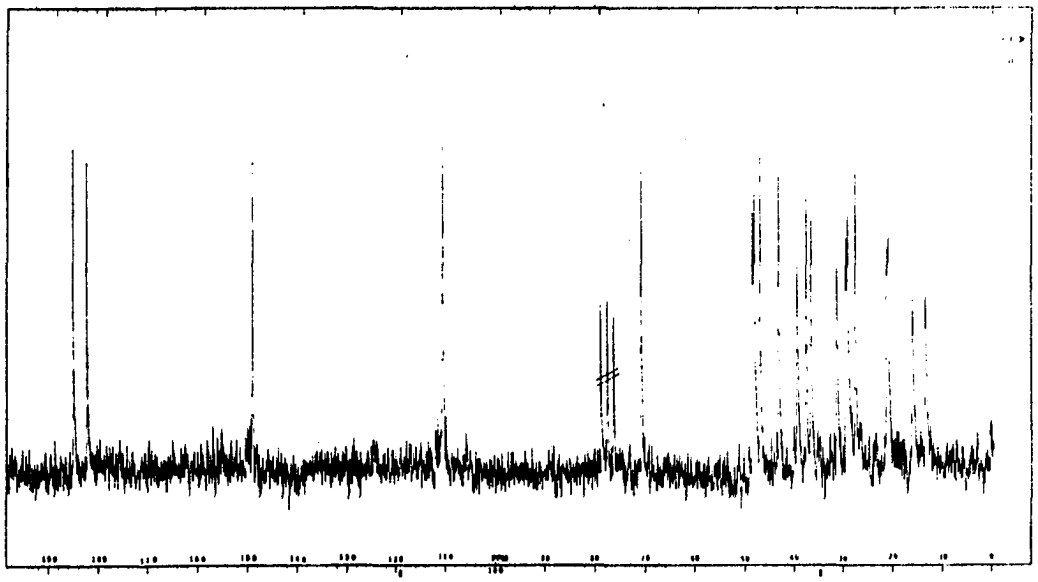


60 MHz PMR Spectrum ($CDCl_3$) of 22

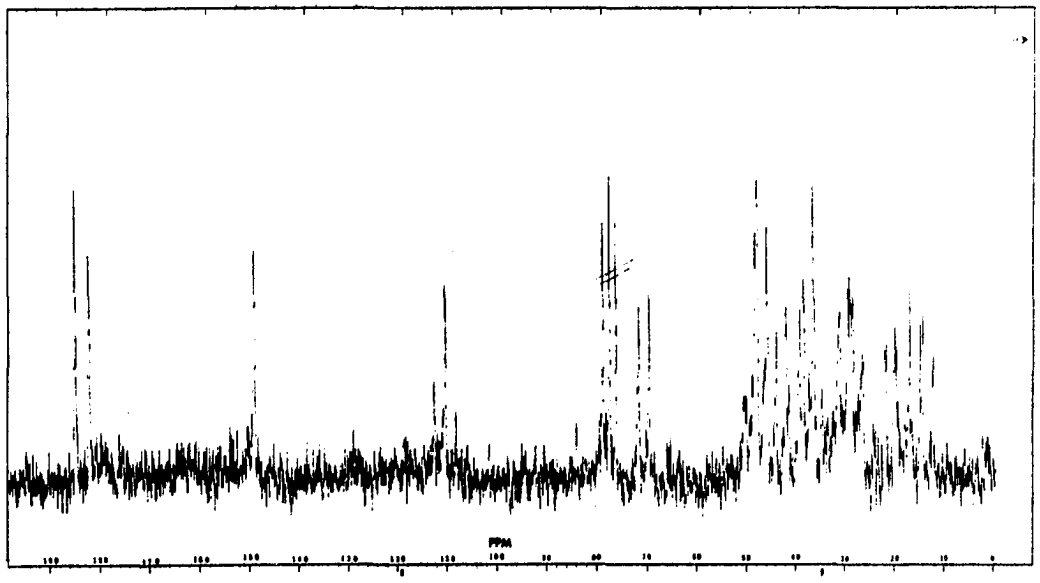


Mass Spectrum of 22

Figure 3



25.2 MHz Proton-Decoupled CMR Spectrum ($CDCl_3$) of B-1



25.2 MHz Off-Resonance Decoupled CMR Spectrum ($CDCl_3$) of B-1

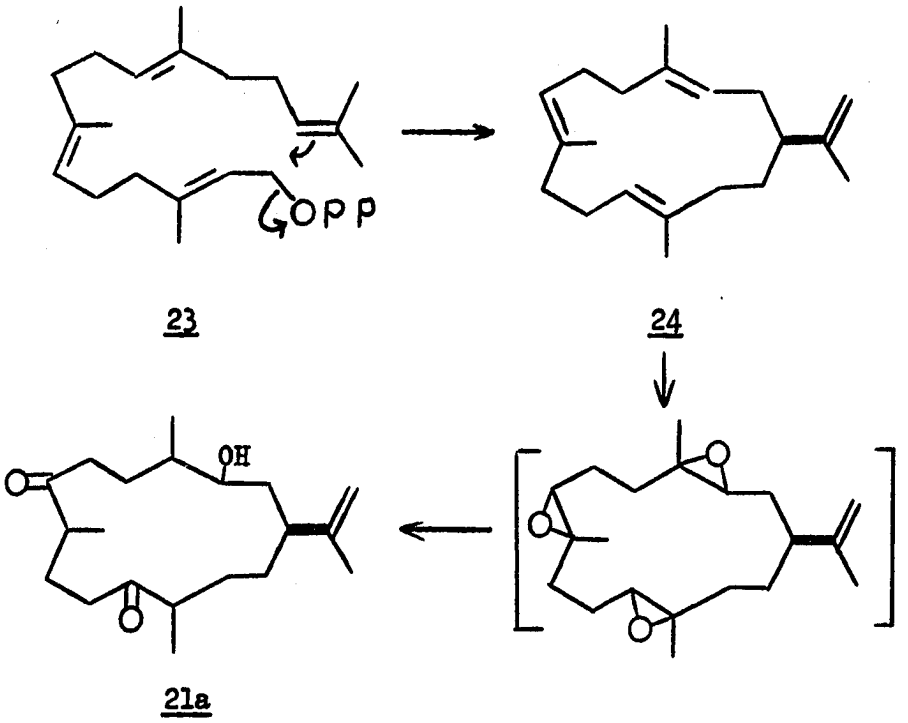
alcohol. The compound possessed two carbonyl carbon atoms as evidenced by the pair of singlets at δ 184.3 and δ 181.5 in both cmr spectra in Figure 3. The absence of absorption attributable to an aldehyde proton in the pmr spectrum of B-1 established the two carbonyl groups as ketones. It is noteworthy that only one ketone group was obvious from the other forms of spectroscopy available. Thus, the new compound, B-1, had a total of 3 unsaturations (2 ketones and one olefin) and being $C_{20}H_{34}O_3$, must be monocyclic and, from its structural features, was clearly a diterpene.

Making the reasonable assumption[8] that the compound arose in nature from geranylgeranyl pyrophosphate (23), the normal diterpenoid precursor,[14,15,30] by a single cyclization, then the cembrane skeleton (2), emerged as a ring system for B-1. If it be assumed that B-1 arose via (+)-cembrene-A (24) (Chart III), postulated in Section I as a precursor for other gorgonian derived cembrane derivatives, then the biosynthesis of B-1 required that the three endocyclic double bonds in 24 be saturated during the course of the biosynthesis. In addition, three oxygen atoms must be incorporated into the 14-membered ring. One possible sequence is proposed in Chart III, in which 21a represents one of the many possible structures for B-1.

Consideration of alternative ring systems led to either a 6 or 10-membered ring as the most logical ring size.[8] If the ring contained 6 carbon atoms, then the 1700 cm^{-1} absorption in the ir spectrum would be inexplicable. The standard value of 1715 cm^{-1} for a 6-membered ring ketone could be shifted lower by 15 cm^{-1} by intramolecular hydrogen bonding[31] between one of the ketone groups and the hydroxyl function,

Chart III

A Proposed Biosynthetic Sequence for B-1



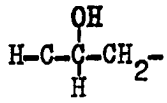
23 24

21a

but the second ketone function should then possess a normal 1715 cm^{-1} absorption, which was not observed. A 10-membered ring cannot be ruled out, but the hitherto absence of 10-membered ring monocyclic diterpenoids in nature[32] and the fact that an isopropylidene rather than an isopropenyl group would be expected for this 20-carbon system on biogenetic grounds,[8] again favored the cembrane ring system for B-1.

The ir absorption at 1700 cm^{-1} was in accord with the assignment of a 14-membered carbocyclic ring to B-1. Table III shows three natural product derivatives (25, 26, and 27) all of which possess a 14-membered ring ketone and all of which have been reported to have carbonyl absorption at 1700 cm^{-1}. In addition, the doubled triplet pmr signal of

the proton attached to the carbon atom bearing the hydroxyl group, indicated that the carbinol system was flanked by methylene and methinyl protons, as in partial structure C, and obviated the possibility of an α-ketol group.



C

The p-iodobenzoate derivative (28) was prepared from B-1 in order to obtain the structure by x-ray crystallography. Spectral data for 28 are in Figure 4. 28 was crystalline, but crystallized in dendritic needles from a variety of solvents. The crystals were quite

Table III

Cembrone derivatives

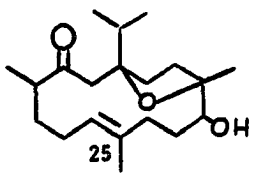
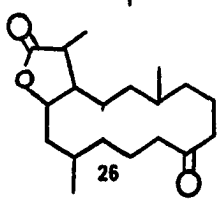
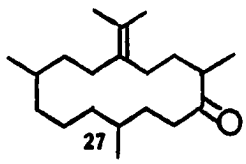
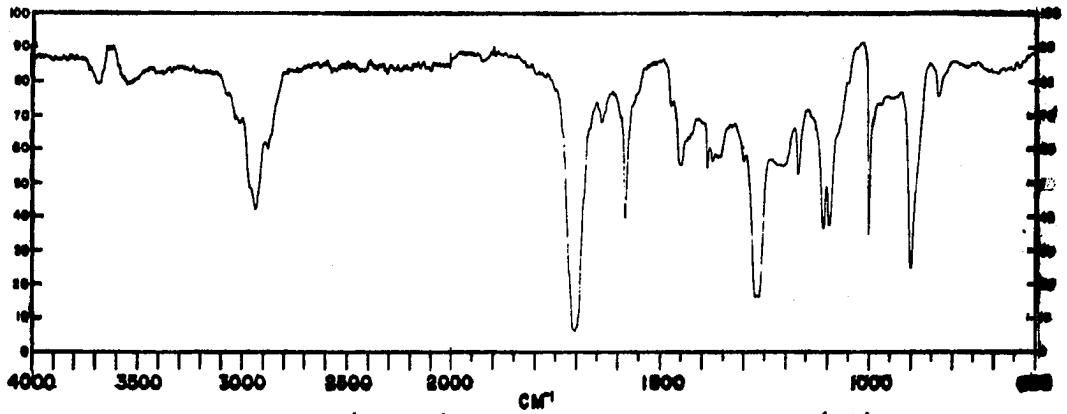
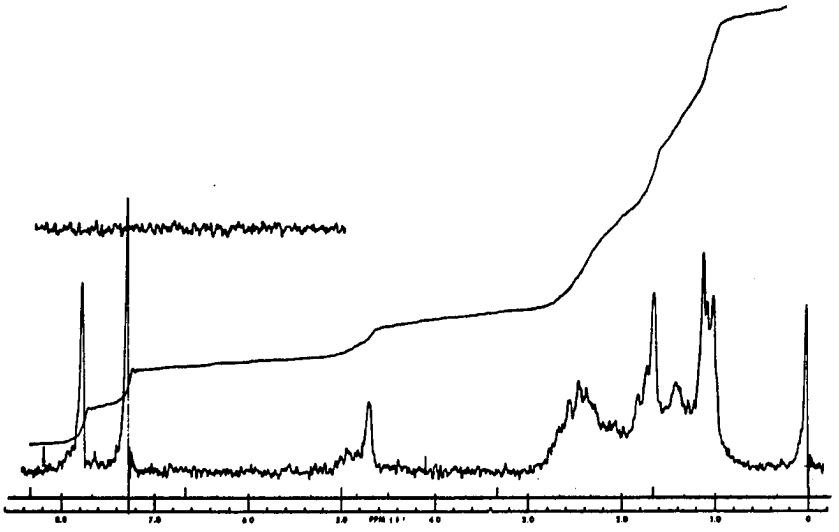
Compound	IR (C=O) ν cm^{-1}	Ref.
 25	1700 (CCl_4)	26
 26	1700 ($CHCl_3$)	40
 27	1700 ($CHCl_3$)	25

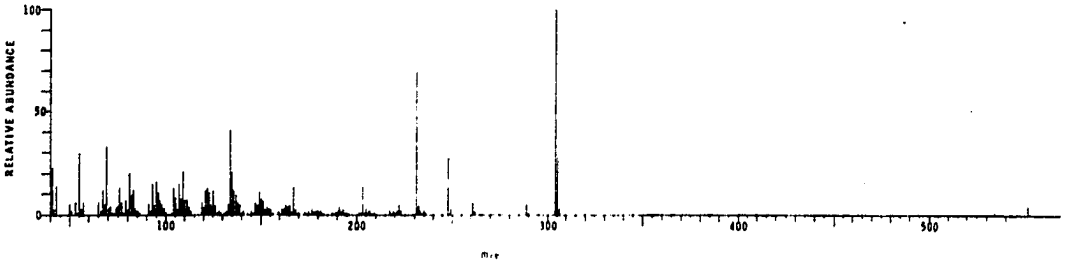
Figure 4



IR Spectrum ($CHCl_3$) of B-1 p-Iodobenzoate (28)



60 MHz PMR Spectrum ($CDCl_3$) of 28



Mass Spectrum of 28

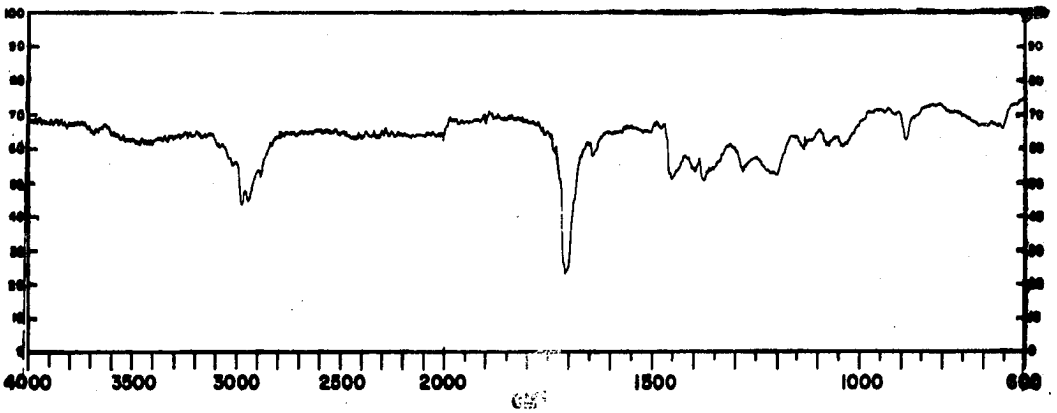
unsatisfactory for an x-ray study. The possibility of determining the structure of B-1 acetate (22) by direct method x-ray crystallographic techniques is currently being explored by Dr. D. van der Helm and Mr. S. Ealick of this department.

The second diterpene B-2 (29), $C_{20}H_{32}O_3$ (high resolution mass spectrum) was obtained only in very small yield. B-2 had no hydroxyl absoprtion in the ir spectrum, but did have an intense carbonyl absorption at 1700 cm^{-1}, indicating a cembrone (Table III). The absence of an alcohol group in B-2 was further indicated by its considerably greater mobility on tlc (R_f = 0.76, 3:1 benzene-ethyl acetate, silica gel H) than the monohydric B-1 (R_f = 0.51). Like B-1, B-2 possessed an isopropenyl group as shown by the following double irradiation experiment. Irradiation of the broad 2 proton singlet at 4.73 ppm sharpened the broad vinyl methyl signal at 1.70 ppm, and conversely irradiation at δ 1.70 caused the signal at δ 4.73 to sharpen. Figure 5 depicts the spectral data for B-2.

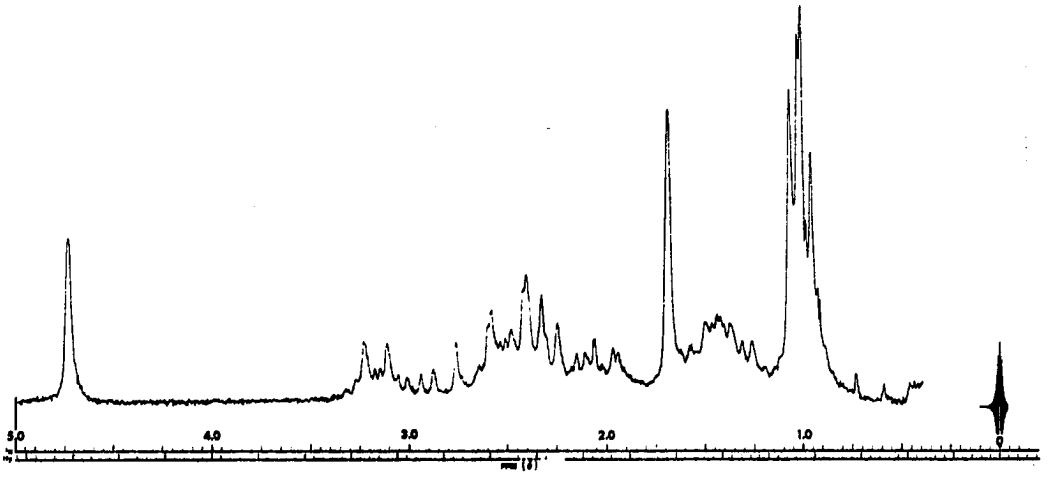
B-2 also contained three secondary methyl groups (δ 1.02, 9H, three overlapping doublets). The presence of an alpha proton of a secondary ether was suggested by the doublet of triplets at δ 3.17. There was no positive indicator for the third oxygen atom; however, the integrated intensity of the absorption in the pmr spectrum of B-2 between 2 and 3 ppm, suggested that B-2, like B-1, was a diketone, possessing a similar number of hydrogen atoms alpha to keto groups.

B-3 (30) $C_{20}H_{36}O_3$ (combustion analysis) had ir absorption at 3600 and 3450 (hydroxyl), 1700 (ketone), and 1640 cm^{-1} (double bond). Spectral data for B-3 is shown in Figure 6. The large OH band in the

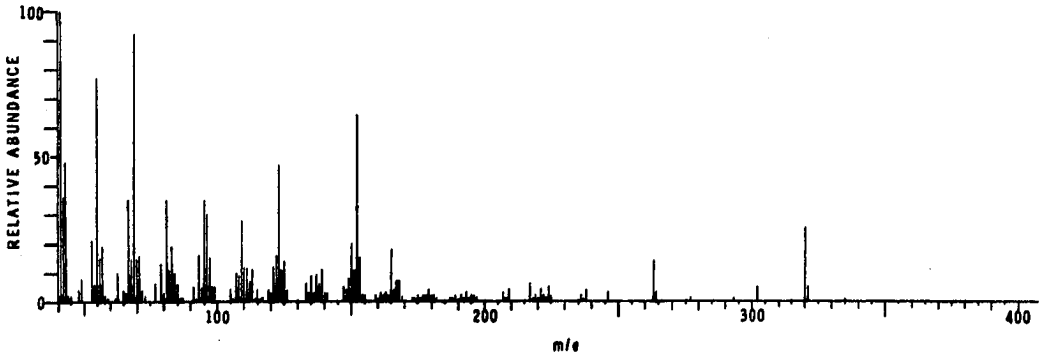
Figure 5



IR Spectrum ($CHCl_3$) of B-2 (29)

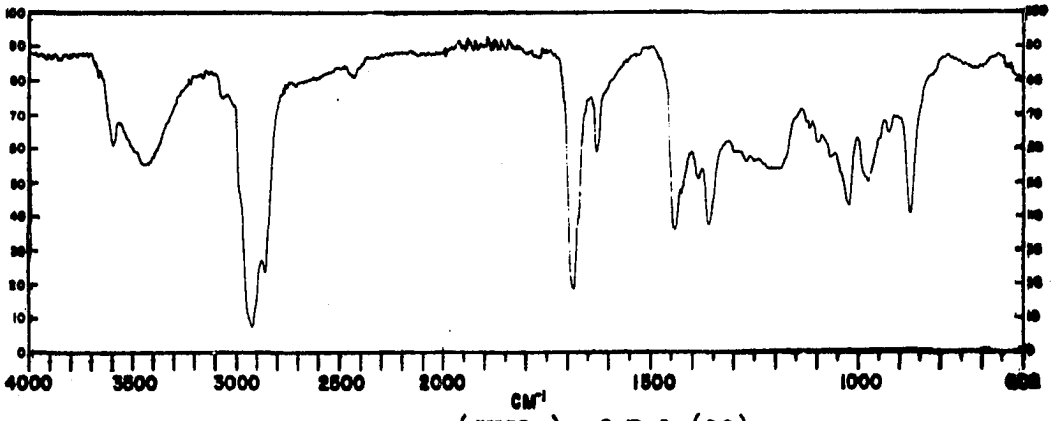


100 MHz PMR Spectrum of 29

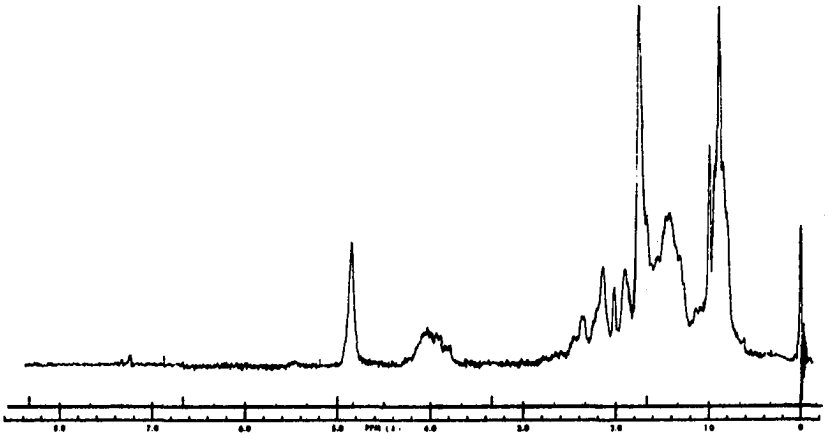


Mass Spectrum of 29

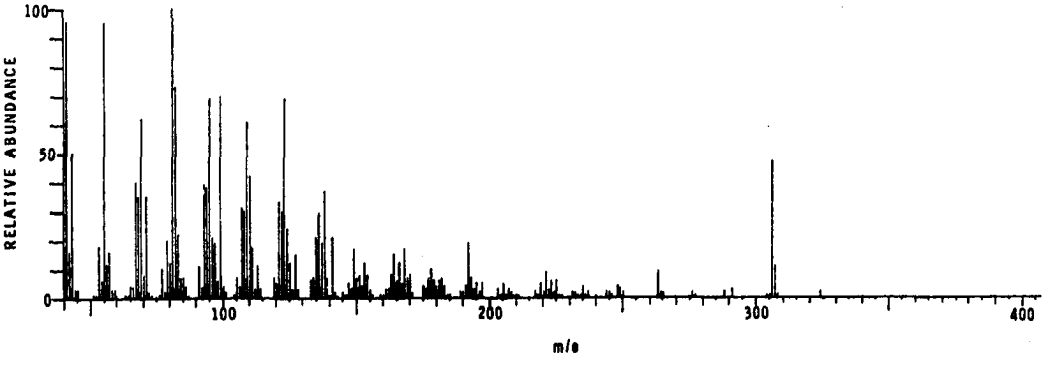
Figure 6



IR Spectrum ($CHCl_3$) of B-3 (30)



60 MHz PMR Spectrum ($CDCl_3$) of 30



Mass Spectrum of 30

ir spectrum coupled with its relatively slow mobility on tlc (R_f = 0.26, 3:1 benzene-ethyl acetate, silica gel H) and the 2 proton multiplet at 4.00 ppm, suggested that B-3 was a disecondary diol. This was confirmed when B-3 formed a diacetate (31); the pmr absorption arising from both protons attached to the secondary carbinol carbons moved downfield approximately 1 ppm. The spectral data for 31 is presented in Figure 7.

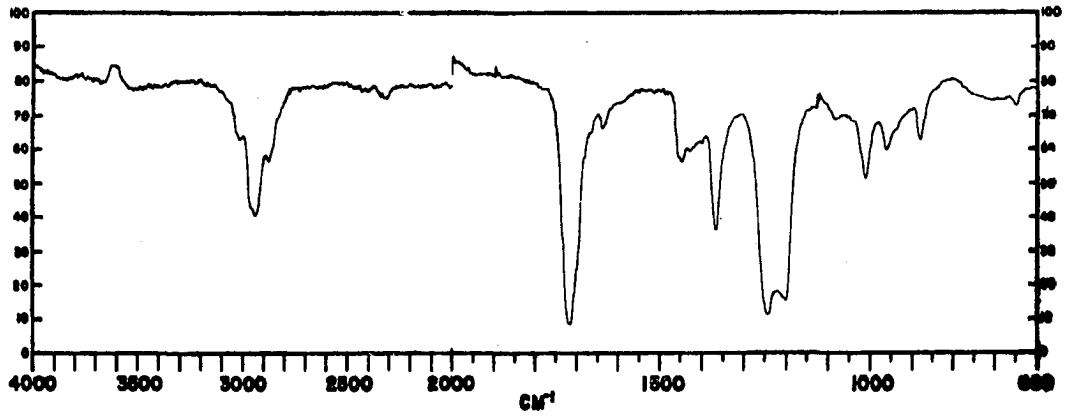
The pmr spectrum of 31 clearly revealed the presence of three secondary methyl doublets (δ 1.02, 0.92 and 0.82). An isopropenyl group was also shown to be present by double irradiation as before for B-1 and B-2.

B-1, B-2, and B-3 all possessed 20 carbon and 3 oxygen atoms; further they possessed the following common structural features: three secondary methyl groups, an isopropenyl group and at least one ketone. Thus, they were closely related. Specifically, B-1 was a monohydroxy-diketone and B-3 was a dihydroxy-ketone, suggesting that oxidation of one of the hydroxyl groups in B-3 would produce B-1.

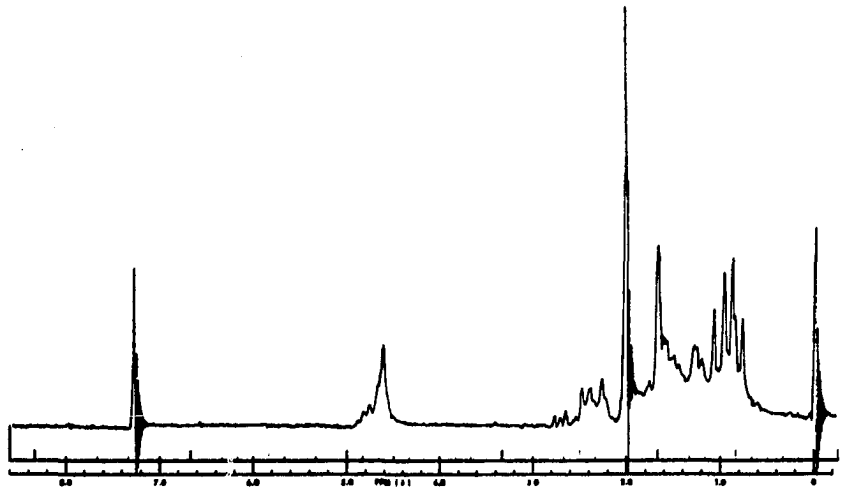
The observations made above represent an attempt to correlate the chemical evidence at hand with a logical biosynthetic sequence which would evolve the B-compounds. The large number of compounds possessing the 14-membered ring (cembrane) skeleton and the established monocyclic nature of B-1 and B-3 make the cembrane skeleton very attractive; however, it should be reiterated that many other structures satisfy the raw data presented above. Chemical studies to elucidate the unknown structures are continuing.

This marks the first report of naturally occurring monocyclic diterpenoids which possess ketone functionality. The number of nuclear

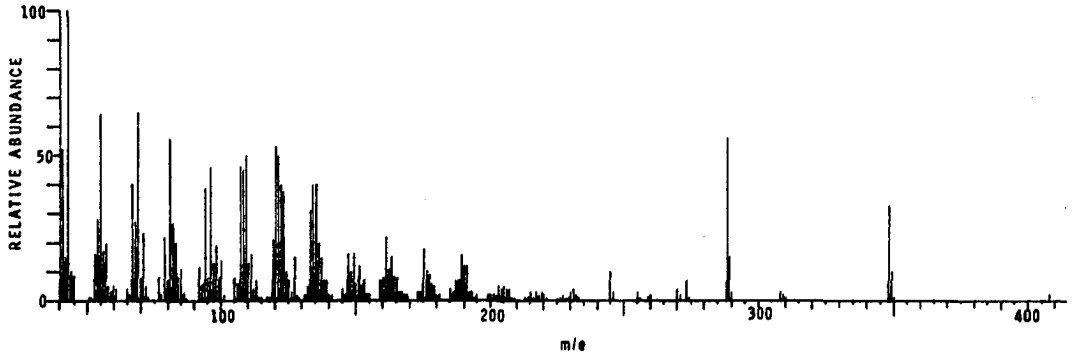
Figure 7



IR Spectrum ($CHCl_3$) of B-3 Diacetate (31)



60 MHz PMR Spectrum ($CDCl_3$) of 31



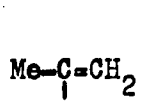
Mass Spectrum of 31

oxygen atoms in the three diterpenoids places them between nepthenol (soft coral) and the cembranolides (gorgonians) and may indicate the level of chemical sophistication of the new gorgonian in the overall classification scheme.

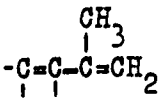
B. A Novel Diterpenoid From A Pacific Soft Coral

A preliminary report[33] has appeared which described the isolation of a diterpene acetate from Sclerophytum capitalis (formerly called Sclerophytum capitale). The new diterpenoid was designated SCC pending structural elucidation. The present effort constituted an extension of the previous work.

SCC (32), $C_{22}H_{34}O_4$ (high resolution mass spectrum), had ir absorption at 1730, 1240 (acetate) and 1610 cm^{-1} (double bond). The spectral data for SCC is displayed in Figure 8. The presence in SCC of partial structure A (isopropenyl group) was established when irradiation of the two proton signal at 5.94 ppm caused the vinylic methyl signal at 1.76 ppm to sharpen, conversely irradiation at 1.76 ppm caused the signal at 5.94 ppm to sharpen. The exomethylene absorption at δ 5.94 occurred at approximately 1 ppm lower field than a non-conjugated exomethylene signal normally occurs, indicating that the exomethylene was at the terminus of a conjugated system (D).[34] The strong uv absorption at 247 nm (ε = 17,000) supported the assignment of the diene D.

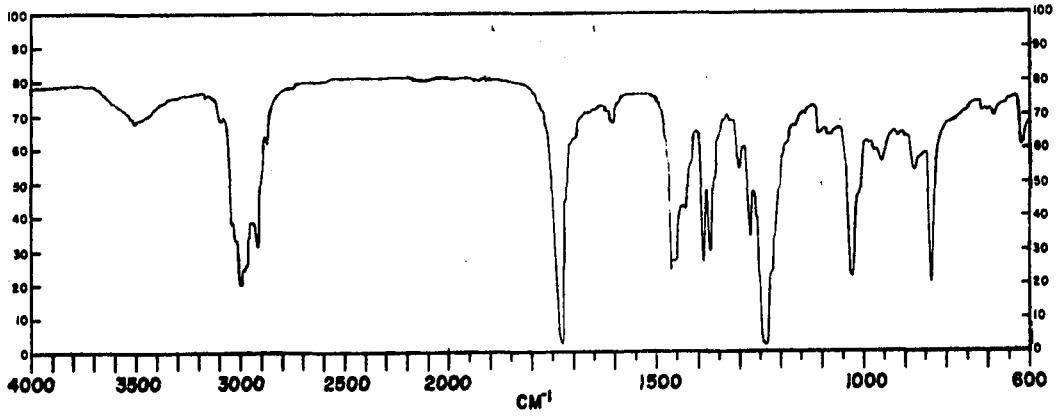


A

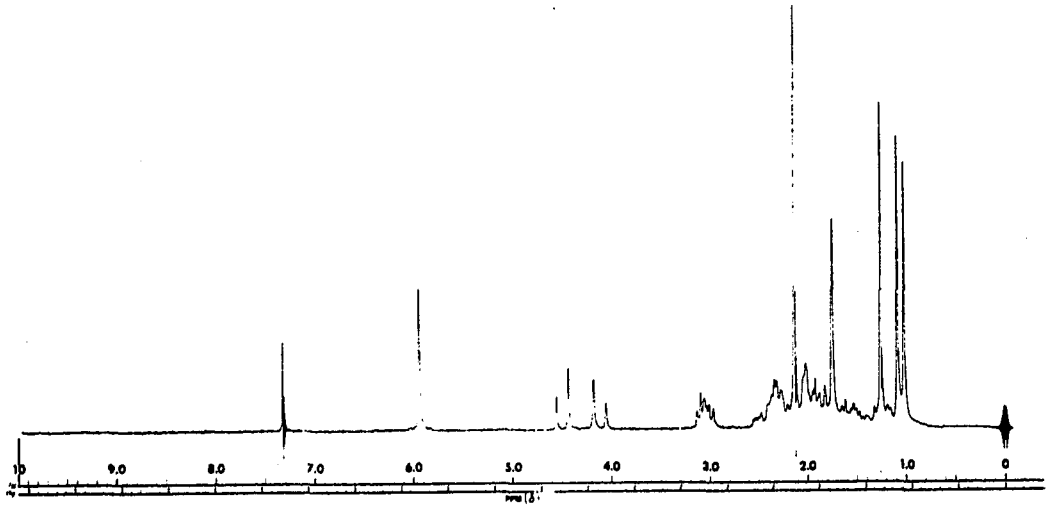


D

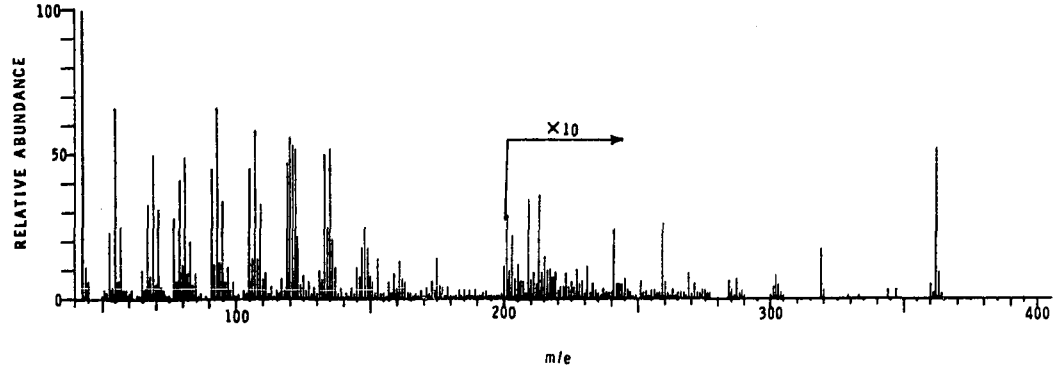
Figure 8



IR Spectrum (KBr) of SCC (32)



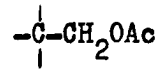
100 MHz PMR Spectrum ($CDCl_3$) of 32



Mass Spectrum of 32

The absence of further olefinic proton absorption demanded that the second double bond be tetrasubstituted.

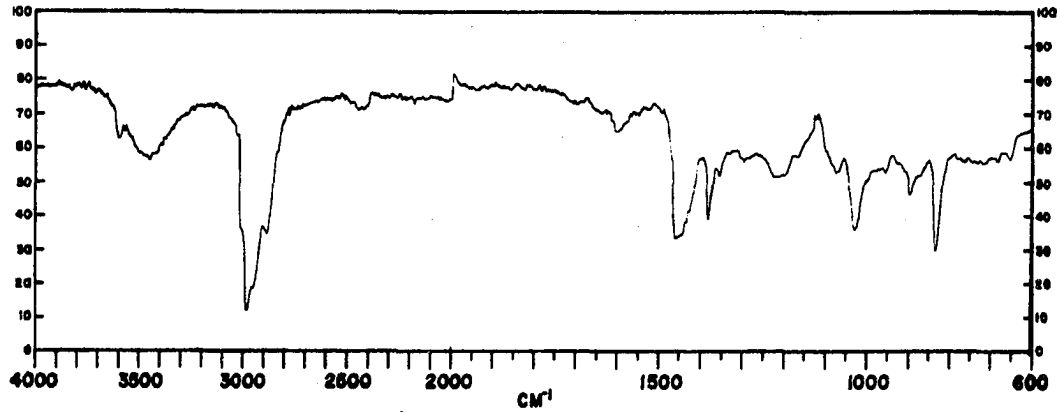
Treatment of SCC with a trace of sodium in methanol afforded deacetyl SCC (33); spectral data for 33 is presented in Figure 9. Partial structure E, previously postulated[33] to be present in SCC was established when the AB quartet (4.3 ppm) due to the methylene protons in E of the parent compound 32 changed to a pair of quartets at higher field in 33. That the multiplicity increase was due to coupling between the methylene protons in E and the hydroxyl proton of the newly formed primary alcohol was demonstrated by rerunning the pmr spectrum of 33 with a drop of D_2O added. Figure 10a shows the result; the AB quartet reappeared. The geminal coupling between the methylene protons of E was attributed to the presence of a fully substituted carbon atom alpha to the $-CH_2OAc$ group.[36] The observed geminal coupling constant of 11 Hz was in agreement with typical values.[36]



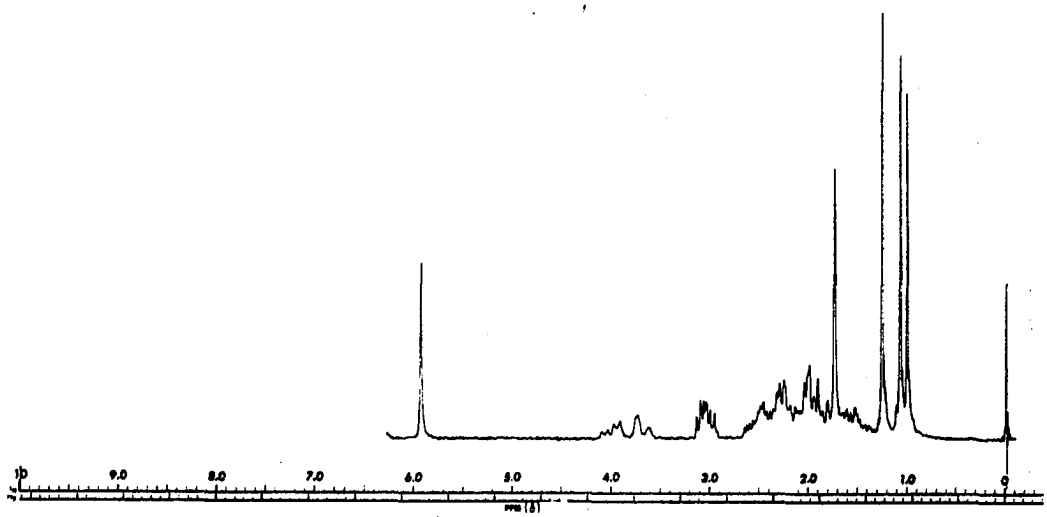
E

An isopropyl group was also present in SCC (ir doublet 1380 cm^{-1}, pmr 6H doublet at 1.06 ppm). Irradiation in the allylic region (δ 2.33) in 32 and (δ 2.33) in 33 caused the six proton doublet at δ 1.06 in 32 and δ 1.05 in 33 to collapse to a singlet, showing that the methinyl proton of the isopropyl group was allylic, and, therefore, that the isopropyl group was one of the substituents of the tetrasubstituted double bond as shown in partial formula F. (A non-allylic methinyl proton of an isopropyl group normally absorbs at ca. 1.60 ppm. See Sadtler Standard NMR Spectra Nos. 3416, 3432 and 3956.)

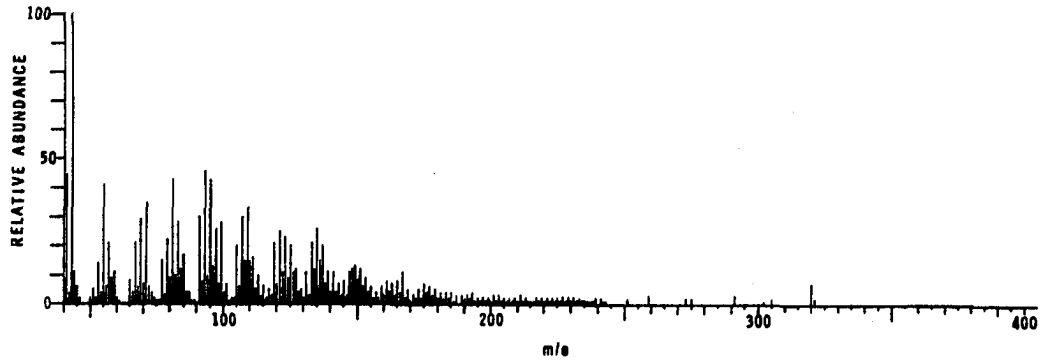
Figure 9



IR Spectrum ($CHCl_3$) of Deacetyl SCC (33)

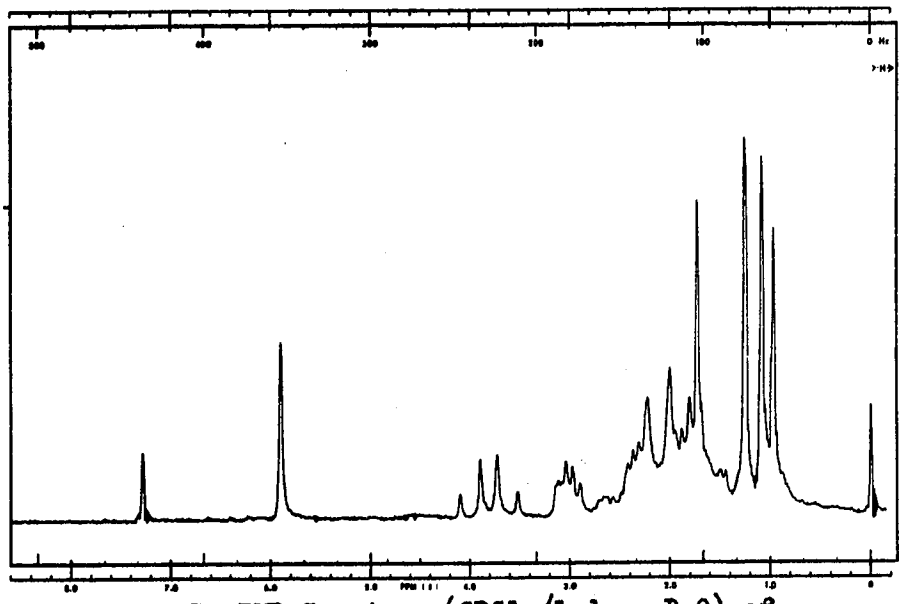


100 MHz PMR Spectrum ($CDCl_3$) of 33



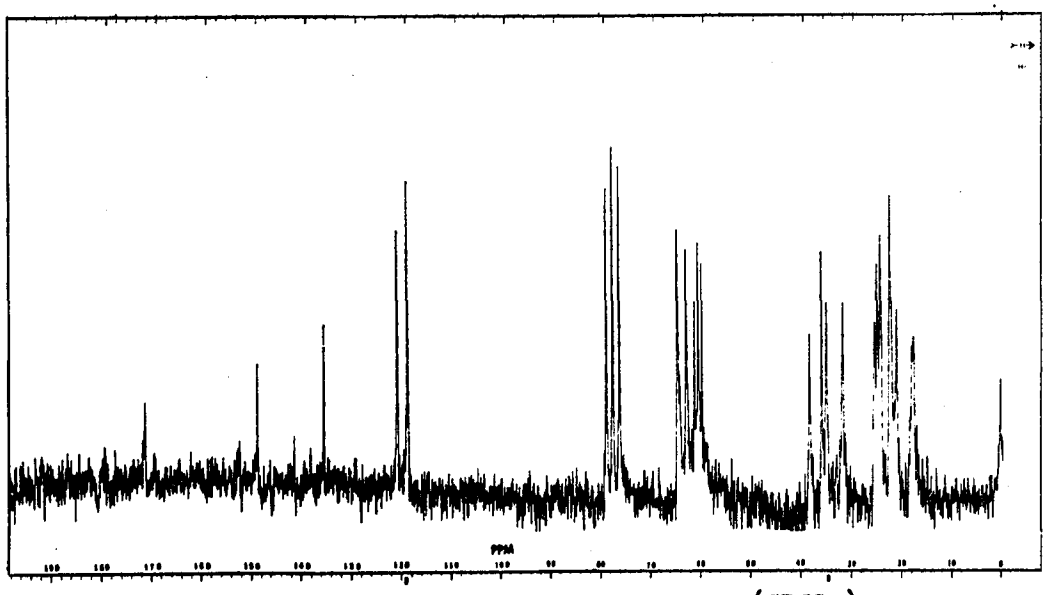
Mass Spectrum of 33

Figure 10a

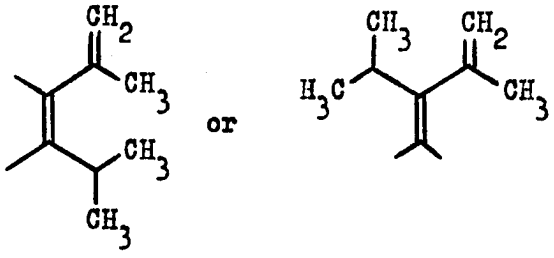


100 MHz PMR Spectrum ($CDCl_3$/1 drop D_2O) of Deacetyl SCC (33)

Figure 10b

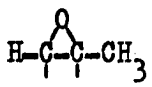


25.2 MHz Proton-Decoupled CMR Spectrum ($CDCl_3$) of SCC (32)



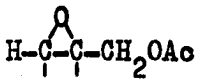
F

Two oxygen atoms in SCC were shown above to be present in an acetate function. The formula for SCC allowed for two more oxygen atoms. SCC did not exhibit hydroxyl absorption and deacetyl SCC did not show carbonyl absorption, therefore, the remaining two oxygen atoms were ethereal. Definitive evidence for the nature of the ether oxygens was not obtained; however, the pmr spectra of 32 and 33 displayed absorption at 3.0 ppm which integrated for 2 protons, indicating the presence of two epoxide hydrogens. One of the epoxide protons was judged to be part of partial structure G, the methyl signal of which occurred at 1.27 ppm in 32.



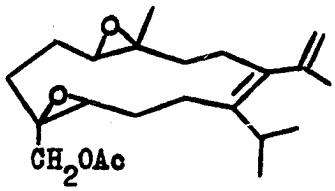
G

It was shown above that a $-CH_2OAc$ group was present and that the carbon to which the group was attached was fully substituted. The second epoxide would appear to be reasonably associated with the fully substituted center alpha to the $-CH_2OAc$ group, as in partial structure H. It is noteworthy that the chemical shift and geminal coupling of the methylene protons in H are quite in accord with this assignment.[38,39]

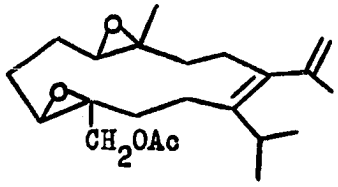


H

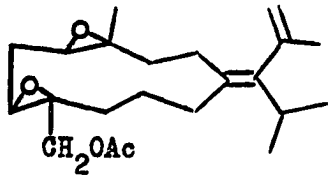
The partial structures F, G, and H together with one more ring accounted for all the rings and double bonds allowed by $C_{22}H_{34}O_4$. In the absence of further chemical evidence, the partial structures established above for SCC together with six methylene groups, required by the formula for SCC, were combined to form possible total structures. Three of the vast number of possible structures which incorporate the functionality deduced for SCC are represented by 32a, 32b, and 32c.



32a



32b



32c

The proton decoupled cmr spectrum of SCC (Figure 10b) confirmed the presence of one carbonyl (acetate) carbon (δ 170.4), four olefinic

carbons (δ 148.0, 134.8, 120.0, and 118.0), five carbons bearing singly bound oxygen (δ 64.2, 62.4, 60.8, 60.0, and 59.3) and twelve other carbon atoms.[29]

The presence of partial structure F alone makes SCC unique,[32] but even more intriguing is the likely presence of an eleven or twelve membered ring. It is interesting that the 12-membered ring in 32a and 32b is isoprenoid while the 11-membered ring in 32c is nonisoprenoid.

Chemical studies leading to the structural elucidation of SCC have been slowed due to its relative inaccessability; Sclerophytum capitalis is collected in the Marshall Islands. Therefore, a sample of SCC has been provided to Dr. D. van der Helm for low temperature x-ray crystallography.

EXPERIMENTAL

Experimental conditions specified in Section I apply.

New Diterpenoids B-1, B-2 and B-3 - An unidentified gorgonian which exhibits taxonomic characteristics of both plexaura and pseudoplexaura species was collected by Dr. Leon S. Ciereszko on June 1-2, 1973 off the island of Bonaire in the Carribbean Sea. The unknown species, air dried, (432 g) was extracted with hexane.

The extract was concentrated and allowed to stand overnight in 100 ml of hexane. A solid crystallized and was collected. Tlc showed two major spots. The solid was chromatographed (400 g silic AR CC-7, 4:1 benzene-ethyl acetate, 50 ml/20 min). After 4 l of 4:1 benzene-ethyl acetate had been placed on the column, the polarity of the solvent was increased to 1:1 benzene-ethyl acetate; 1 l of this solvent was eluted. Fractions 23-29 showed a single spot by tlc and were combined and the solvents removed to yield a white crystalline solid. The solid was recrystallized several times from benzene-hexane affording 1.3868 g of pure B-1. Fractions 43-60 likewise displayed a single spot on tlc, and after combining the fractions, evaporating the solvents and recrystallizing several times from benzene-hexane, 1.5855 g of pure B-3 was obtained. B-1 and a third diterpenoid, B-2 had previously been isolated from this same unidentified gorgonian in small yield by Dr. Robert E. Middlebrook from a specimen collected at Puerto Rico. The mother liquor from which B-1 and B-3 coprecipitated was shown by tlc to contain B-2.

B-1 had: mp 111.5-112°; $[\alpha]_D^{24}$ - 7.8° (c, 0.902, $CHCl_3$); R_f = 0.51 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 3500 (broad, hydroxyl), 1700 (C=O), and 1640 cm^{-1} (C=C); 100 MHz pmr ($CDCl_3$) δ 4.71 (m, 2H, C=CH_2), 3.36 (dt, 1H, J = 8.5 Hz, J = 2.5 Hz, -CHOH), 1.69 (d, 3H, J = 1 Hz, C=C-Me), 1.04 (d, 3H, J = 6.5 Hz, H-C-Me), 1.00 (d, 3H, J = 6.5 Hz, H-C-Me), and 0.97 ppm (d, 3H, J = 6.5 Hz, H-C-Me); 25.2 MHz proton decoupled cmr ($CDCl_3$) δ 184.3 (C=O), 181.5 (C=O), 148.2 (C=C), 109.6 (C=CH_2), 70.1 (-CHOH), 48.0, 47.6, 46.5, 42.6, 38.9, 37.1, 36.1, 30.9, 29.0, 28.7, 27.1, 20.5, 20.3, 15.6 and 13.0 ppm; mass spectrum (70 eV) m/e (rel intensity) M^+, 322 (4), 304 (67), 289 (7), 286 (2), 261 (4), 249 (4), 233 (7), 226 (8), 222 (10), 219 (5), 191 (10), 189 (6), 179 (9), 165 (12), 164 (15), 163 (15), 151 (30), 150 (30), 137 (50), 134 (70), 123 (56), 109 (75), 107 (57), 98 (38), 96 (68), 94 (44), 83 (70), 81 (67), 79 (41), 71 (27), base peak 69 (100), 67 (58), 57 (50), 55 (88), 53 (43), 43 (76), and 41 (87).

Anal. Calcd. for $C_{20}H_{34}O_3$: C, 74.48; H, 10.63; mol wt 322.25079. Found: C, 74.66; H, 10.39; mol wt (mass spectrum) 322.24770.

B-2 had: mp 86-87°; $[\alpha]_D^{24}$ +61° (c 0.115, $CHCl_3$); R_f = 0.76 (3:1 benzene-ethyl acetate, silica gel H), ir ($CHCl_3$) 1700 (C=O), and 1640 cm^{-1} (C=C); δ 4.73 (br, 2H, C=CH_2), 3.17 (dt, 1H, J = 11.5 Hz, J = 6 Hz, -OC-H), 1.70 (br, 3H, C=C-Me), 1.02 (m, 9H, three H-C-Me); mass spectrum (70 eV) m/e (rel intensity) M^+, 320 (25), 302 (5), 293 (1), 277 (1), 263 (14), 165 (18), 153 (15), 152 (64), 151 (11), 150 (20), 139 (11), 125 (14), 123 (47), 122 (16), 121 (12), 113 (11), 111 (12), 110 (12), 109 (28), 107 (10), 97 (15), 96 (30), 95 (35), 93 (16), 84 (10), 83 (19), 82 (11), 81 (35), 79 (13), 71 (15), 70 (14), 69 (92),

68 (15), 67 (35), 63 (10), 57 (19), 56 (15), 55 (77), 53 (21), 43 (48), 42 (36), and base peak 41 (100).

Anal. Calcd for $C_{20}H_{34}O_3$: mol wt 320.23514. Found: mol wt (mass spectrum) 320.23330.

B-3 had mp 119-121°; R_f = 0.26 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 3600, 3450 (hydroxyl), 1700 (C=O) and 1640 cm^{-1} (C=C); 60 MHz pmr ($CDCl_3$) δ 4.83 (s, 2H, C=C$\underline{H}_2$), 4.00 (m, 2H, two C$\underline{H}$OH), 1.78 (s, 3H, C=C-$\underline{Me}$), and 0.96 ppm (m, 9H, three H-C-$\underline{Me}$); mass spectrum (70eV) m/e (rel intensity) M^+, 324 (2), 306 (47), 291 (3), 288 (2), 276 (2), 263 (9), 248 (4), 235 (4), 221 (9), 205 (5), 192 (19), 168 (17), 164 (15), 153 (12), 149 (17), 141 (21), 138 (37), 136 (29), 127 (15), 123 (69), 121 (33), 109 (61), 99 (70), 95 (69), base peak 81 (100), 71 (35), 69 (62), 67 (40), 55 (95), 43 (50), and 41 (96).

Anal. Calcd for $C_{20}H_{34}O_3$: C, 74.02; H, 11.19. Found: C, 74.24; H, 11.05.

B-1 Acetate - A solution of B-1 (98.5 mg) in 2 ml of acetic anhydride and 2 ml of pyridine was stirred overnight at room temp. Tlc of the reaction mixture indicated complete conversion of B-1 to its acetate. The reaction mixture was diluted with chloroform (10 ml) and washed successively with 5% HCl (2x10 ml) and water 10 ml). The chloroform layer was dried ($MgSO_4$) and the solvent removed under reduced pressure on the rotary evaporator affording a white solid. Recrystallization from benzene-hexane gave 109.1 mg of B-1 acetate as white feathers: mp 95°; R_f = 0.70 (1:1 benzene-ethyl acetate, silica gel H), ir ($CHCl_3$) 1730, 1245 (acetate), 1700 (C=O), and 1640 cm^{-1} (C=C); 60 MHz pmr ($CHCl_3$) δ 4.70 (s, 2H, C=C$\underline{H}_2$), 2.04 (s, 3H, OAc), 1.70 (s, 3H, C=C-$\underline{Me}$), and

1.04 ppm (m, 9H, three H-C-Me); mass spectrum (70eV) m/e (rel intensity) M^+, 364 (7), base peak 304 (100), 289 (5), 286 (2), 261 (10), 222 (9), 191 (6), 189 (6), 179 (6), 167 (22), 165 (10), 151 (12), 149 (19), 135 (45), 134 (88), 126 (12), 123 (20), 122 (27), 121 (22), 119 (15), 112 (10), 111 (18), 110 (13), 109 (40), 108 (18), 107 (28), 105 (10), 97 (17), 95 (35), 93 (25), 83 (22), 82 (20), 81 (43), 79 (17), 71 (16), 69 (59), 68 (12), 67 (23), 57 (18), 55 (70), 53 (14), 43 (95), and 41 (63).

Anal. Calcd for $C_{22}H_{36}O_4$: C, 72.49; H, 9.96. Found: C, 72.85; H, 10.04.

B-1 - p-iodobenzoate - A solution of 0.9243 g of p-iodobenzoic acid in 10 ml of thionyl chloride was refluxed for 3.5 hr. After removing the excess thionyl chloride on the rotary evaporator, the last traces of gaseous byproducts were removed under vacuum, affording a white solid (p-iodobenzoyl chloride) which was not further purified. B-1 (136.5 mg) was dissolved in 7 ml benzene and 3 ml pyridine containing 0.483 g of the p-iodobenzoyl chloride prepared previously. After stirring for 2 days at room temp, tlc indicated essentially no reaction. The mixture was then refluxed for 2 days. Tlc showed the absence of starting material. The residue obtained after removing the solvents was chromatographed (100 g silic AR CC-7, 6:1 benzene-ethyl acetate, 50 ml/20 min). Fractions 7-9 showed a single spot by tlc, and proved to be the p-iodobenzoate derivative of B-1 (26 mg). A variety of solvents was employed in recrystallizing the solid, but suitable crystals for an x-ray study were not obtained; the crystals, although well formed, were either too small or dendritic. B-1 p-iodobenzoate had: mp 129-131°;

R_f = 0.76 (3:1 benzene-ethyl acetate); ir ($CHCl_3$) 1705 (broad, aromatic ester and ketone) and 1640 cm^{-1} (C=C); 60 MHz pmr ($CDCl_3$) δ 7.78 (s, 4H, aromatic), 4.90 (m, 1H, ArCOOC<u>H</u>), 4.70 (s, 2H, C=C<u>H</u>$_2$), 1.68 (s, 3H, C=C-<u>Me</u>), and 1.08 ppm (m, 9H, three H-C-<u>Me</u>); mass spectrum (70 eV) <u>m/e</u> (rel intensity) M^+, 552 (4), 534 (1), base peak 304 (100), 289 (5), 261 (6), 248 (28), 231 (71), 203 (14), 167 (14), 149 (12), 137 (10), 136 (12), 135 (21), 134 (41), 125 (12), 123 (11), 122 (13), 121 (12), 109 (21), 107 (15), 104 (13), 96 (11), 95 (16), 93 (15), 83 (12), 82 (10), 81 (20), 76 (13), 69 (33), 67 (12), 55 (30), 43 (14), and 41 (23).

<u>Anal</u>. Calcd for $C_{27}H_{37}O_4I$: C, 58.68; H, 6.75. Found: C, 58.90; H, 6.79.

<u>B-3 diacetate</u> - A solution of 106 mg of B-3 in 2 ml of pyridine and 2 ml of acetic anhydride was refluxed for 30 min, after which time tlc showed the absence of B-3 and the appearance of a new spot. The mixture was diluted with chloroform (10 ml), washed with 5% HCl (3x10 ml) and water (10 ml), dried ($MgSO_4$) and the chloroform evaporated to give a yellowish solid, tlc of which showed a single spot. The solid was filtered through a short Florisil column (0.5x8 cm, 1:1 benzene-ethyl acetate) and recrystallized from benzene-hexane, affording 119 mg of white needles: mp 82-83°; R_f = 0.51 (3:1 benzene-ethyl acetate, silica gel G); ir ($CHCl_3$) 1720 (broad) and 1245 (acetate), 1700 (ketone), and 1240 cm^{-1} (C=C); 60 MHz pmr ($CDCl_3$) δ 4.75 (m, 2H, two <u>H</u>-C-OAc), 4.64 (s, 2H, C=C<u>H</u>$_2$), 2.01 (s, 6H, two OAc), 1.68 (br, 3H, C=C-<u>Me</u>), 1.02 (d, 3H, J = 6.5 Hz, H-C-<u>Me</u>), 0.92 (d, 3H, H-C-<u>Me</u>), and 0.84 ppm (d, 3H, J = 6.5 Hz, H-C-<u>Me</u>); mass spectrum (70 eV) <u>m/e</u> (rel intensity) M^+, 408 (2), 348 (33), 308 (3), 288 (56), 237 (7), 270 (4), 255 (3), 245 (10),

231 (4), 217 (3), 203 (5), 189 (16), 175 (18), 161 (22), 149 (16), 147 (16), 135 (40), 134 (40), 121 (50), 120 (50), 109 (50), 95 (46), 81 (56), 69 (65), 67 (40), 55 (64), base peak 43 (100), and 41 (52).

Anal. Calcd for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.73; H, 9.67.

Isolation of SCC - Sclerophytum capitalis, air dried, wt 3.88 kg, collected by Dr. Robert E. Middlebrook and Ray Gross off the islet of Japtan in the Marshall Islands on August 7, 1973, was extracted with hexane. The concentrated hexane extract was placed in a refrigerator overnight. The insoluble portion was filtered and recrystallized several times from benzene-hexane to afford 1.5 g of SCC as white prisms: mp 111.5-112°; R_f = 0.71 (3:1 benzene-ethyl acetate, silica gel H); ir (KBr) 1730, 1240 (acetate), and 1610 (C=C); uv (95% ethanol) 247 nm, ε = 17,000; 100 MHz pmr ($CDCl_3$) δ 5.94 (s, 2H, C=C-C=C$\underline{H}_2$), 4.50 (d, 1H, J = 11 Hz, $\underline{H}$-CHOAc), 4.12 (d, 1H, J = 11 Hz, $\underline{H}$-CHOAc), 3.06 (m, 2H, two $\underline{H}$-C-O), 2.15 (s, 3H, OAc), 1.76 (s, 3H, C=C-$\underline{Me}$), 1.27 (s, 3H, $\underline{Me}$-C-O), and 1.06 ppm (d, 6H, J = 7 Hz, H-C-$\underline{Me}_2$); 25.2 MHz proton decoupled cmr ($CDCl_3$) δ 170.4 ($\underline{C}$=O), 148.0 ($\underline{C}$=C), 134.8 ($\underline{C}$=C), 120.0 ($\underline{C}$=C), 118.0 $\underline{C}$=C), 64.2 ($\underline{C}$-O), 62.4 ($\underline{C}$-O), 60.8 ($\underline{C}$-O), 60.0 ($\underline{C}$-O), 59.3 ($\underline{C}$-O), 37.9, 35.5, 34.6, 31.3, 25.0, 24.5, 23.8, 21.9, 21.9, 20.8, 17.4 and 17.4 ppm; mass spectrum (70 eV) m/e (rel intensity) M^+, 362 (5), 319 (2), 302 (1), 259 (3), 241 (2), 231 (1), 227 (1), 215 (2), 213 (4), 209 (4), 205 (1), 203 (2), 201 (3), 200 (1), 175 (14), 161 (13), 153 (14), 149 (18), 148 (25), 147 (18), 145 (11), 137 (11), 136 (21), 135 (52), 134 (25), 133 (50), 131 (10), 123 (22), 122 (52), 121 (53), 120 (56), 119 (47), 109 (33), 108 (13), 107 (58), 106 (14), 105 (45), 97 (11), 95 (34),

94 (13), 93 (66), 92 (12), 91 (45), 83 (20), 81 (49), 80 (12), 79 (41), 77 (28), 71 (31), 69 (50), 67 (33), 65 (10), 57 (25), 55 (66), 53 (23), 44 (11), and base peak 43 (100).

Anal. Calcd for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45; mol wt 362.24571. Found: C, 72.84; H, 9.46, mol wt (mass spectrum) 362.24654.

Deacetyl SCC - SCC (152.2 mg) was dissolved in 20 ml of methanol and a catalytic amount (1 mg) of sodium was added. The mixture was allowed to stand overnight at which time tlc indicated the absence of SCC. Most of the methanol was removed on the rotary evaporator under reduced pressure without heating. Water (10 ml) was added and a white solid formed which was taken up in chloroform. The chloroform layer was washed (water) and dried ($MgSO_4$). After removal of the solvent, a white solid formed, which was recrystallized from hexane, affording 124.7 mg of white feathers: mp 119-121°; R_f = 0.11 (3:1 benzene-ethyl acetate, silica gel H); ir ($CHCl_3$) 3600, 3450 (hydroxyl) and 1600 cm^{-1} (C=C); 100 MHz pmr ($CDCl_3$) δ 5.92 (s, 2H, C=C$\underline{H}_2$), 4.01 (dd, 1H, J = 6.5 Hz, J = 12 Hz, $\underline{H}$-CHOH), 3.67 (dd, 1H, J = 2 Hz, J = 12 Hz, $\underline{H}$-CHOH), 3.04 (m, 2H, two $\underline{H}$-C-O), 1.75 (s, 3H, C=C-$\underline{Me}$), 1.28 (s, 3H, $\underline{Me}$-C-O) and 1.05 ppm (d, 6H, J = 7 Hz, H-C-$\underline{Me}_2$); 60 MHz pmr ($CDCl_3$ + 1 drop D_2O) δ 5.92 (s, 2H, C=C$\underline{H}_2$), 4.00 (d, 1H, J = 12 Hz, $\underline{H}$-CHOD), 3.63 (d, 1H, J = 12 Hz, $\underline{H}$-CHOD), 3.04 (m, 2H, two $\underline{H}$-C-O), 1.75 (s, 3H, C=C-$\underline{Me}$), 1.28 (s, 3H, $\underline{Me}$-C-O), and 1.05 ppm (d, 6H, J = 7 Hz, HC$\underline{Me}_2$); mass spectrum (70 eV) $\underline{m/e}$ (rel intensity) M^+, 320 (7), 305 (2), 302 (1), 291 (3), 275 (2), 273 (2), 259 (3), 251 (2), 167 (11), 151 (12), 149 (13), 148 (12), 147 (11), 141 (11), 139 (11), 137 (20), 136 (15), 135 (26), 134 (12), 133 (21), 131 (11), 127 (12), 126 (11), 125 (20), 123 (23), 121 (25), 119 (21),

113 (10), 111 (16), 110 (15), 109 (33), 108 (15), 107 (30), 105 (20), 99 (28), 97 (24), 95 (43), 93 (46), 91 (30), 85 (17), 83 (28), 81 (43), 79 (22), 77 (15), 71 (35), 69 (29), 67 (21), 57 (21), 55 (41), 53 (14), 44 (11), base peak 43 (100), and 41 (45).

SUMMARY

Four novel diterpenoids were isolated from marine invertebrates and partially characterized. Three of the new compounds ($C_{20}H_{32}O_3$, $C_{20}H_{34}O_3$, $C_{20}H_{36}O_3$) appeared to possess the cembrane skeleton and ketone functionality; they were found in an unidentified Caribbean gorgonian. The fourth new marine diterpenoid ($C_{22}H_{34}O_4$) isolated from a pacific soft coral (S. capitalis) appeared to contain an unusual terminal conjugated diene system substituted by methyl and isopropyl groups.

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