THE UNIVERSITY OF OKLAHOMA

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I. EUPALMERIN ACETATE: A NEW MARINE EPOXY CEMBRANOLIDE

II. *EUNICEA PALMERI* BAYER: AN AMBIVALENT SPECIES

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APPROVED BY

DISSERTATION COMMITTEE

DEDICATION

To my family

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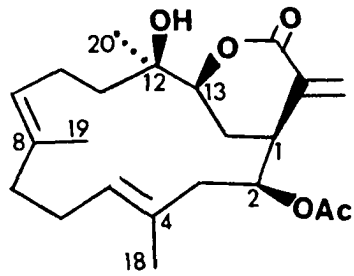
I. EUPALMERIN ACETATE: A NEW MARINE EPOXY CEMBRANOLIDE

INTRODUCTION

Diterpene lactones have been isolated from marine sources on several[1] occasions. It has been found by this laboratory that two genera of gorgonians have produced diterpene lactones, Pseudoplexaura and Eunicea. It was thought that these diterpenes were interrelated with the animal's defense mechanism and were produced, at least in part, by the symbiotic zooxanthellae of the parent gorgonian.[1,2]

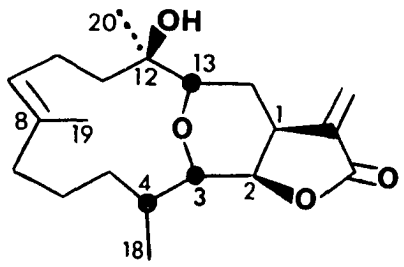
The diterpene lactone crassin acetate was found in the gorgonian Pseudoplexaura porosa, as well as in Ps. wagenaari and Ps. flagellosa. This particular diterpene lactone was shown by x-ray crystallography[3] to have the following structure.

Crassin acetate
(1S,2S,12R,13S)

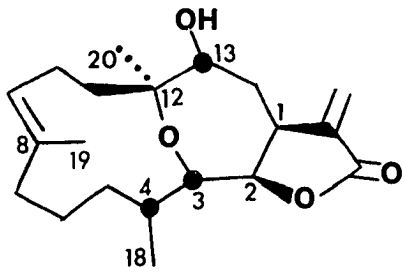


Two other diterpene lactones, eunicin[4,5,6,7] and jeunicin[8], were found in the gorgonian, Eunicea mammosa Lamouroux. Eunicin was isolated from samples collected from Bimini and the Florida Keys, whereas jeunicin was found only in those samples collected from Jamaica. Both of these

compounds have a common structural feature, that is, both are cembrane-type γ-lactones. They do, however, have additional features which emphasize their structural similarities. Both compounds contain an α,β-unsaturated-γ-lactone, a trisubstituted olefinic bond, a secondary methyl group, and oxygenation at the 3, 12, and 13 positions of the fourteen-membered ring. The structures of eunicin[9] and jeunicin[10], as determined by x-ray crystallography, are shown below and illustrate these similarities.



Eunicin
(1S,2R,3S,4R,12R,13S)



Jeunicin
(1S,2R,3S,4R,12S,13R)

It is worthwhile to note that all of the centers of asymmetry in these molecules have the same configuration except for inverted centers at the 12 and 13 positions of the fourteen-membered ring.

The purpose of this study was to elucidate the structure of a new diterpene lactone, eupalmerin acetate, isolated from the gorgonian, Eunicea palmeri Bayer, initially collected by Professor Leon S. Ciereszko in shallow water (4-8 ft) on the seaward side of the Ragged Keys, Florida.

RESULTS AND DISCUSSION

Eupalmerin acetate (I), a white crystalline solid, m.p. 157-159° C, $[\alpha]_D^{25}$ +8.00 (c 2.0, $CHCl_3$), was isolated from the hot hexane extract of the dried gorgonian Eunicea palmeri. Elemental analysis indicated that the compound had an empirical formula, $C_{22}H_{32}O_5$, which was substantiated (figure 1) by low resolution mass spectrometry (M^+, m/e 376). Preliminary infrared (1738 and 1232 cm^{-1}), nmr (δ 1.90, 3H, s), and mass spectral data (M^+-42, ketene and M^+-60, acetic acid) indicated that the compound contained an acetate moiety. In addition, the infrared (1420 and 1380 cm^{-1}) and nmr (δ 0.85, 3H; δ 1.33, 3H; and δ 1.63, 3H) spectra implied methyl branching of an isoprenoid structure. Thus, the compound can be classified as a diterpenoid acetate.

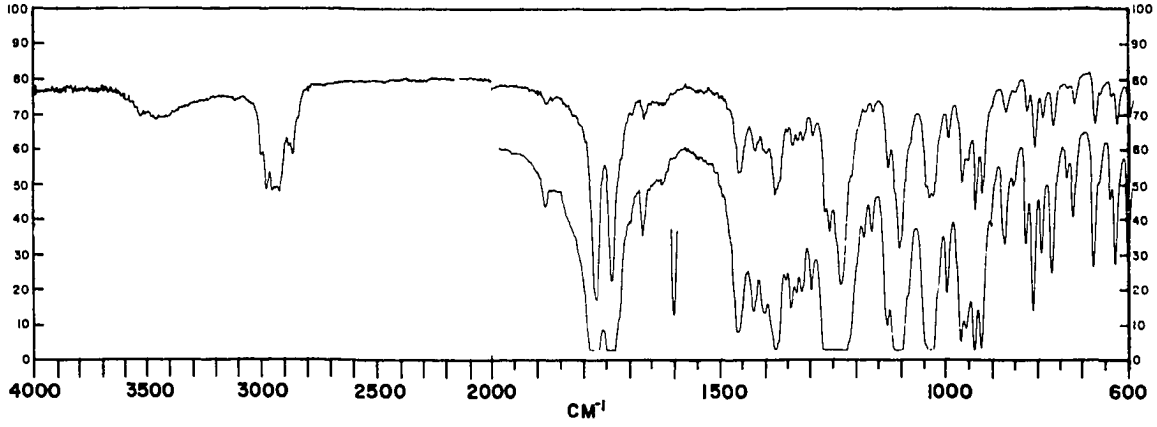
Part One

The infrared spectrum (KBr) of I (figure 1) indicated the presence of a γ-lactone (1775 cm^{-1}, slightly split at 1770 cm^{-1}), an acetate (1738, 1232 cm^{-1}), an exocyclic olefinic bond (3110, 1628, and 870 cm^{-1}), and a trisubstituted olefinic bond (1670, 828, and 810 cm^{-1}). The spectrum contained no strong hydroxyl absorption ($\sim$3500 cm^{-1}).

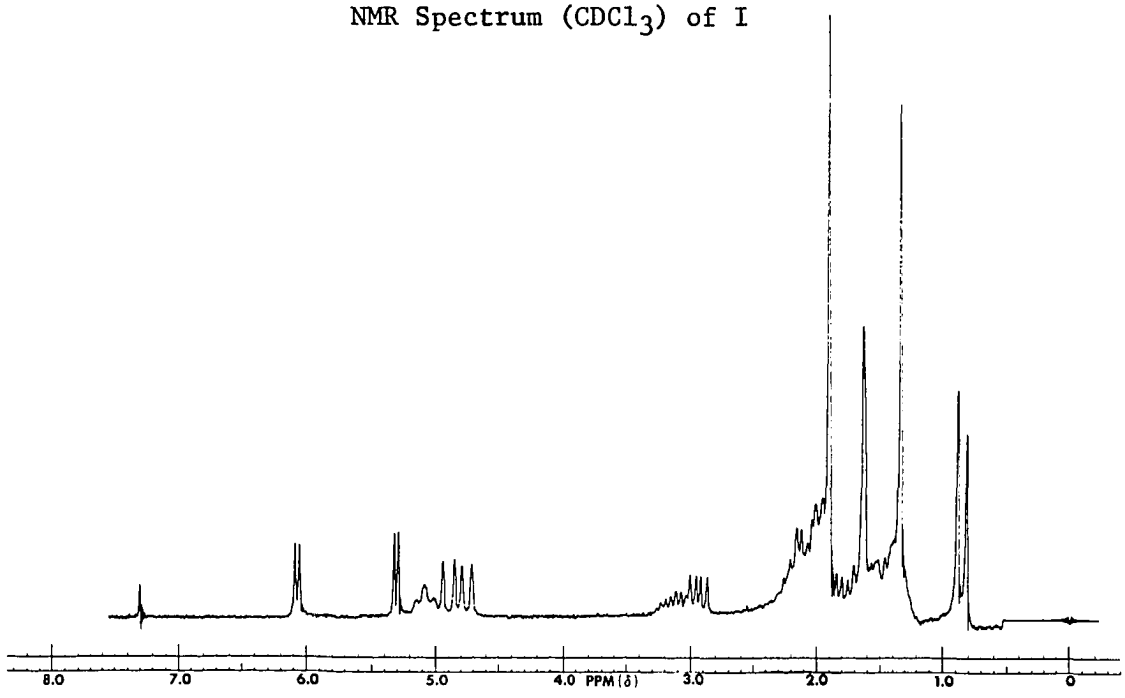
The 100 MHz nmr spectrum ($CDCl_3$) of I (figure 1) showed four methyl absorptions: δ 0.85 (3H, d, J=7 Hz, secondary methyl), δ 1.33 (3H, s, quaternary methyl under oxygen), δ 1.63 (3H, brd s, vinylic methyl),

Figure 1
Spectra of Eupalmerin Acetate (I)

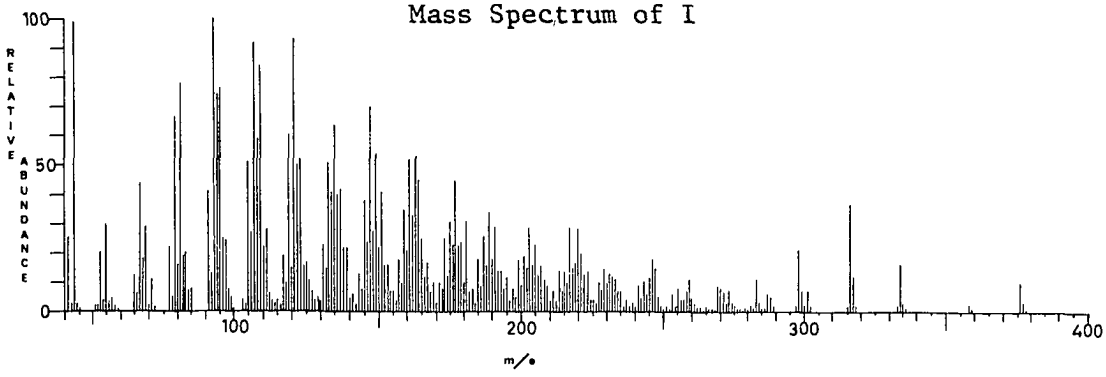
IR Spectrum (KBr) of I



NMR Spectrum ($CDCl_3$) of I



Mass Spectrum of I



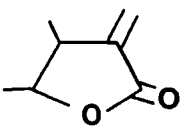
and δ 1.90 (3H, s, methyl adjacent to a carbonyl, i.e., in an acetate). In addition to these absorptions, seven lowfield proton signals could be distinguished. A pair of doublets were found at δ 5.31 (1H, d, J=3.5 Hz) and δ 6.07 (1H, d, J=3.5 Hz) characteristic of exomethylenic protons conjugated with the carbonyl of a γ-lactone. Absorption signals were also found at δ 5.10 (1H, complex triplet, J=6.5 Hz vinylic proton adjacent to a methylene group), δ 4.91 (1H, d, J=9.5 Hz, proton under the acetate oxygen), δ 4.76 (1H, d, J=8 Hz, proton under the lactone oxygen), δ 2.94 (1H, dd, J=5.5 and 9 Hz, compatible with an ethereal proton), and δ 3.15 (1H, 7-line multiplet, which can be assigned to the proton beta to the lactone carbonyl, typical of the appearance and chemical shift of the proton beta to the lactone carbonyl in eunicin). A summary of these results from the 100 MHz spectrum are found in Table I.

TABLE I

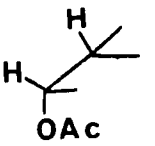
Proton Assignments Made from the
100 MHz NMR Spectrum of Eupalmerin Acetate (I)

Signal (δ)	Multiplicity	Protons	J (Hz)	Assignment
0.85	d	3	7	Secondary methyl
1.33	s	3		Methyl under oxygen
1.63	brd, s	3		Vinylic methyl
1.90	s	3		Acetate methyl
2.94	dd	1	5.5, 9	Ethereal proton
3.15	m	1		Proton beta to the lactone carbonyl
4.76	d	1	8.0	Proton under the lactone
4.91	d	1	9.5	Proton under the acetate
5.10	ct	1	≈6.5	Vinylic proton
5.31	d	1	3.5	Exomethylenic proton
6.07	d	1	3.5	Exomethylenic proton

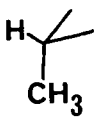
As indicated by the molecular formula, $C_{22}H_{32}O_5$, eupalmerin acetate has seven degrees of unsaturation, i.e., rings plus double bonds. From the above spectral data, five degrees of unsaturation can be counted: one for the acetate, two for the γ-lactone, and one each for the exo-methylenic and trisubstituted olefinic bonds. Moreover, four of the oxygen atoms in the molecule were specified, i.e., both the acetate and lactone functions each contained two oxygen atoms. The nature of the remaining oxygen atom and two degrees of unsaturation still need to be elucidated. It seems probable that the oxygen atom and the remaining unsaturations can be accounted for in terms of an oxygen containing ring and a carbocyclic ring. Indeed, eupalmerin acetate exhibited a positive 1,2-glycol test with acidic silver periodate solution, even though it was apparent from the infrared spectrum that no hydroxyl groups were present in the molecule. Since neither the acetate nor the lactone would be expected to react under these mild conditions, the positive test must be attributed to an oxirane function in the molecule, which under the test conditions would be expected to be rapidly hydrolyzed, and hence give a positive test. Due to the fact that only one proton in the nmr spectrum was compatible with an ethereal proton, it was concluded that the epoxide was trisubstituted. A methyl group was indicated as one of the epoxide substituents from the singlet methyl absorption in the nmr spectrum at δ 1.33 and a methylene group as another of the substituents, from the double doublet nature of the epoxide proton at δ 2.94. Illustrated below are all of the structural features in the molecule which were suggested by these interpretations.



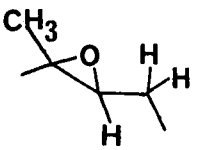
an α methylenic γ-lactone



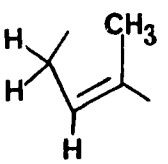
a secondary acetate



a secondary methyl



a trisubstituted epoxide with methyl and methylene substituents



a trisubstituted olefin with methyl and methylene substituents

A monocarbocyclic ring was indicated as the remaining unsaturation, evidenced spectrally by the lack of a suitable alkyl chain terminus. The ring size was determined from simple bookkeeping of the molecular elements, as shown below.

Molecular Elements

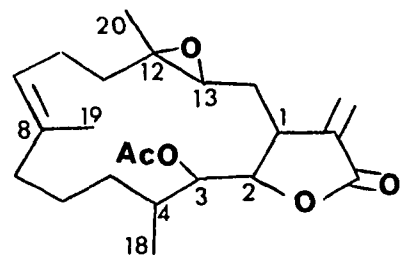
	C	O	
Eupalmerin acetate	22	5	
	-3	-2	Exomethylenic γ-lactone
	-2	-2	Acetate
		-1	Epoxide
	-3		Methyls
Ring size	14		

Indeed, a fourteen-membered ring, characteristic of other oxygenated

diterpenes isolated from marine sources, was compatible with the observed spectral data.

A working model was made which incorporated those structural features which were determined spectrally. In addition, the model was constructed from a regular isoprenoid skeleton, which had the same oxygenation pattern as observed in the diterpenoid lactones, eunicin and jeunicin. That structure is shown below as Model I.

Model I



Model I sufficed for all the spectral data so far mentioned except for the fact that the protons under the lactone and acetate oxygens appeared as doublets in the nmr spectrum. In this particular model, however, it would be expected that each of these protons would produce a double doublet signal, unless through fortuitous circumstances the dihedral angle between these protons was a 90° angle. Further evidence is needed to clarify this point. In the meantime, it must be kept in mind that Model I is merely one of many possible structures.

Part Two

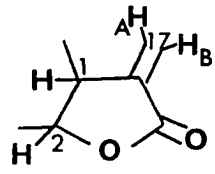
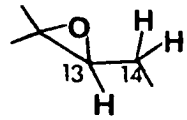
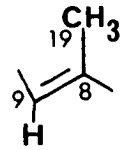
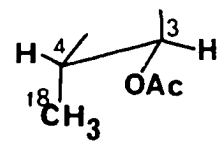
Double resonance (100 MHz) confirmed some of the assignments made for Model I. Irradiation at δ 3.09 (H-1 signal) caused the collapse of the H-2 (δ 4.76, d), H-17A (δ 5.31, d), and H-17B (δ 6.07, d) signals into singlets as expected for a γ-lactone of this type. It is noteworthy that this irradiation frequency, δ 3.09, corresponded to the sixth

member of the seven-line, H-1, multiplet. Separate irradiations at δ 5.31 and δ 6.07 each resulted in the collapse of the H-1 absorption forming a new six-line pattern which was shifted ∿2 Hz toward the H-13 (δ 2.94, dd) signal. In addition, there appeared in the decoupled spectrum several new peaks which were sandwiched in among the H-13 signal. This suggested that the observed seven-line multiplet of H-1 represented only a portion of the H-1 absorption and that the remaining lines of the multiplet were obscured by the overlapping of the H-13 absorption. H-1 was also affected when the δ 4.76, H-2, signal was irradiated. However, the decoupled pattern of the H-1 signal was still too complex to permit evaluation of the other coupling constants of H-1.

Three other regions in the spectrum gave useful double resonance results. The proton H-13 (δ 2.94, dd) collapsed to a doublet when irradiated at δ 2.14 or δ 1.78. This indicated that the epoxide proton was coupled to two protons, i.e., a methylene group. Also, irradiation at δ 5.10, H-9, caused the vinylic methyl, C-19, (δ 1.63) to narrow to a sharp singlet, indicating that this methyl was coupled to the vinylic proton by small allylic coupling. Finally, irradiation at δ 1.62 caused both the secondary methyl, C-18, at δ 0.85 and the proton under the acetate, H-3, to collapse to singlets. Inability to distinguish the proton at this irradiation frequency, however, made this decoupling assignment tentative. The decoupling results are summarized in Table II, together with the partial structures.

TABLE II

Results from the 100 MHz NMR
Double Resonance Experiment on Eupalmerin Acetate

Irradiation Frequency	Proton Assignment	Observed Frequency	Multiplicity Change
H-1, δ 3.09		H-2, δ 4.76	d to s
		H-17B, δ 6.07	d to s
		H-17A, δ 5.31	d to s
H-14A, δ 1.78		H-13, δ 2.94	dd to d
H-14B, δ 2.14		H-13, δ 2.94	dd to d
H-9, δ 5.10		C-19, δ 1.63	brd s to sharp s
H-4, δ 1.62		C-18, δ 0.85	d to s
		H-3, δ 4.91	d to s

Part Three

The initial reactions of eupalmerin acetate (I), hydrogenation and hydrolysis, led either to non-crystalline products or inseparable mixtures. Selective catalytic hydrogenation (5% Pd/C) of the conjugated exomethylenic double bond (nmr showed complete reduction of the exomethylenic olefinic bond) produced three products, none of which could be induced to crystallize. Simple alkaline hydrolysis produced a multicomponent, inseparable mixture (tlc). The nmr of the crude hydrolysate nevertheless showed the quantitative absence of the acetoxy methyl signal at δ 1.90. Likewise, attempted hydrolysis under acidic conditions resulted in many products. Moreover, the product distribution from either acid or base hydrolysis could not be effectively controlled by alteration of the reaction conditions. Thus, the inherent lability of the molecule toward even the mildest of reaction conditions was attributed to the notoriously reactive epoxide functionality. Since most degradative schemes eventually would involve either acidic or basic conditions, the removal of the epoxide moiety seemed the first goal toward the structure elucidation.

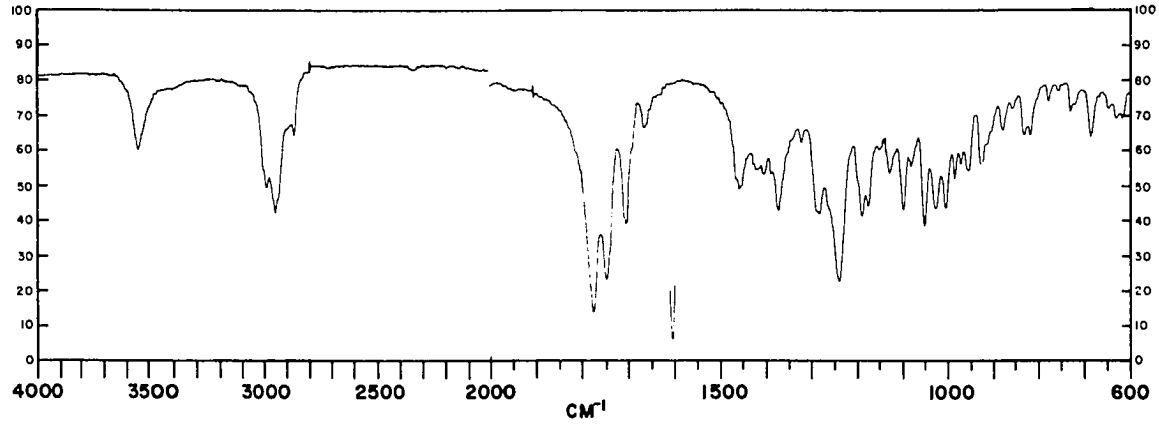
Jones oxidation[11] succeeded in removing the reactive epoxide functionality. After chromatography of the Jones product, a crystalline derivative (II) was isolated (30%) which was apparently derived from the oxidation of the secondary hydroxyl of the 1,2-diol formed initially by the acid hydrolysis of the oxirane. It is well documented that trisubstituted epoxides form α-ketols[12] with the Jones reagent, and indeed the infrared spectrum of the product satisfied the spectral requirements

of an α-ketol. The ir spectrum (KBr) of II (figure 2) showed three carbonyl absorptions, at 1775 cm^{-1} (γ-lactone), 1748 cm^{-1} (acetate), and 1703 cm^{-1} (ketone) and a strong hydroxyl absorption at 3540 cm^{-1}.

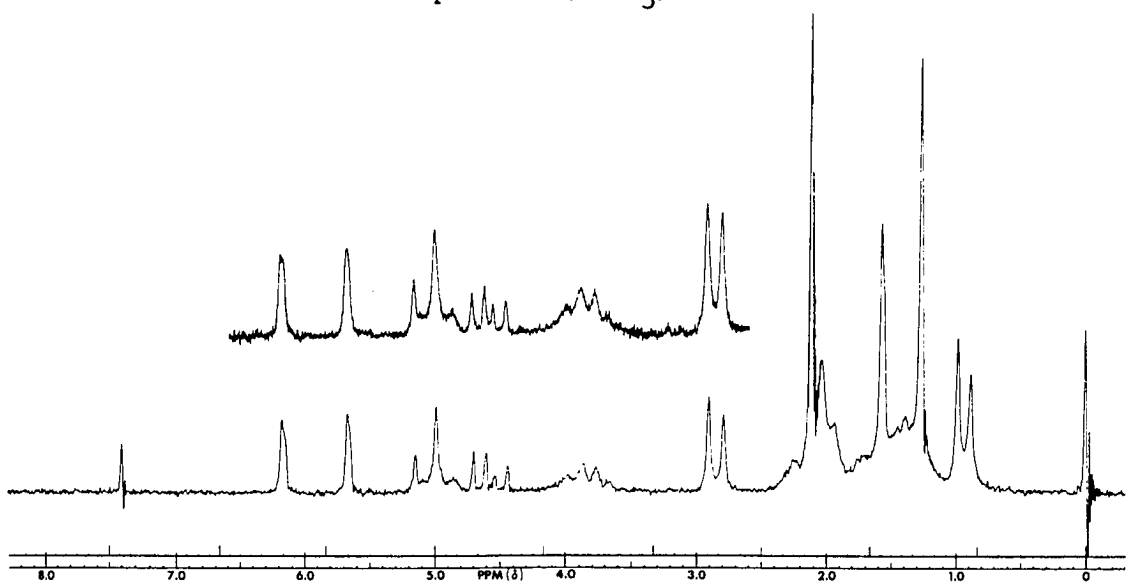
The 70 ev mass spectrum (figure 2) of the α-ketol (II) displayed a molecular ion, M^+, m/e 392, as anticipated for a $C_{22}H_{32}O_6$ compound, as well as the ions m/e 374, 349, 332, and 314 which were related to the loss of either or both the hydroxyl and acetate moieties from the parent ion. A metastable ion at m/e 358 indicated the decomposition of the parent ion (m/e 392) via dehydration to form the m/e 374 ion.

The 100 MHz nmr spectrum ($CDCl_3$) (figure 2, 60 MHz spectrum) showed four methyl absorptions: δ 0.93 (3H, d, J=6.5 Hz secondary methyl), δ 1.25 (3H, s, quaternary methyl under hydroxyl oxygen), δ 1.57 (3H, d, J<1 Hz, vinylic methyl), and δ 2.12 (3H, s, acetate methyl). In addition to these absorptions, eight lowfield signals could be distinguished: a doublet at δ 2.83 (2H, d, J=6.5 Hz, methylene protons alpha to a carbonyl), a quartet at δ 3.79 (1H, q, J≈6 Hz, probably the proton beta to the lactone carbonyl which is additionally deshielded), a broad singlet at δ 4.05 (1H, s, hydroxyl proton, exchangeable in D_2O), a double doublet at δ 4.54 (1H, dd, J=6, 9.5 Hz, proton under the lactone oxygen), a doublet at δ 5.06 (1H, d, J=9.5 Hz, proton under the acetate oxygen), a multiplet at δ 4.96 (1H, m, vinylic proton adjacent to a methylene group), and two hyperfine doublets at δ 5.66 (1H, d, J<1 Hz) and δ 6.18 (1H, d, J<1 Hz, conjugated exomethylene protons split by small allylic coupling). At first inspection, it appeared that several absorptions in the spectrum exhibited unexpected multiplicities. First, the lactone proton at δ 4.54 appeared as a

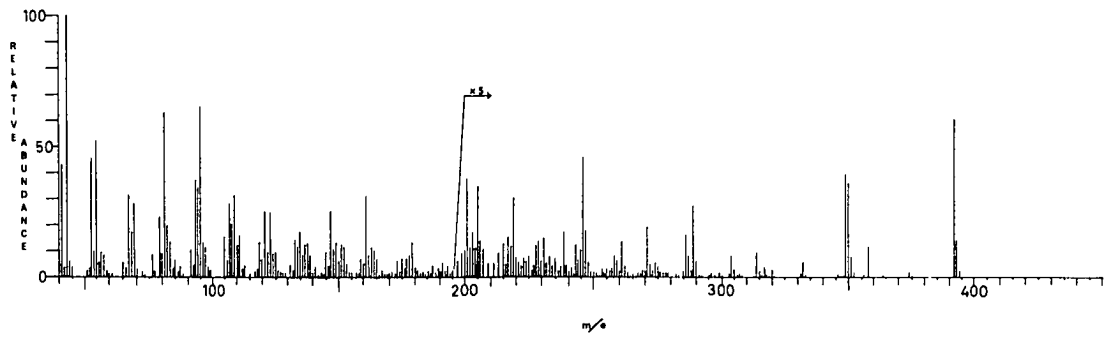
Figure 2
Spectra of Eupalmerin Acetate α-Ketol (II)
IR Spectrum (KBr) of II



NMR Spectrum ($CDCl_3$) of II

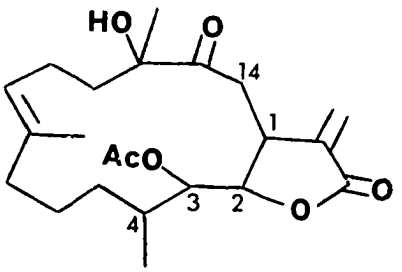


Mass Spectrum of II



doublet in I. Secondly, the absorption at δ 2.83, supposedly the methylene protons alpha to the ketone, appeared as a mere doublet, and finally, the proton beta to the lactone carbonyl at δ 3.79 appeared as a simple quarter (1:3:3:1 ratio) of peaks. However, all of these apparent multiplicity anomalies can be rationalized upon re-examination of the proposed structure.

(II) α-Ketol



Apparently, in the α-ketol (II), the lactone proton is coupled to two proton neighbors. Indeed, the coupling constants exhibited in the double doublet signal at δ 4.54 (J=9.5 and 6 Hz) were reflected in the doublet at δ 5.06 (J=9.5 Hz, proton under the acetate) and the quartet at δ 3.79 (J=6 Hz, proton β to the lactone carbonyl). Thus, the lactone proton (H-2) of II appeared to be coupled to both the proton under the acetate and the proton beta to the lactone carbonyl. The non-coupling of the proton under the acetate (H-3) and the lactone (H-2) in the parent molecule (I), thus, can be rationalized to result from a 90° dihedral angle between the two protons for a coupling constant of zero; however, in the α-ketol (II) dúe to a change in molecular conformation, these protons are coupled. Furthermore, the proton under the acetate in II no longer appeared to be coupled to the H-4 methine proton as a consequence of the new conformation.

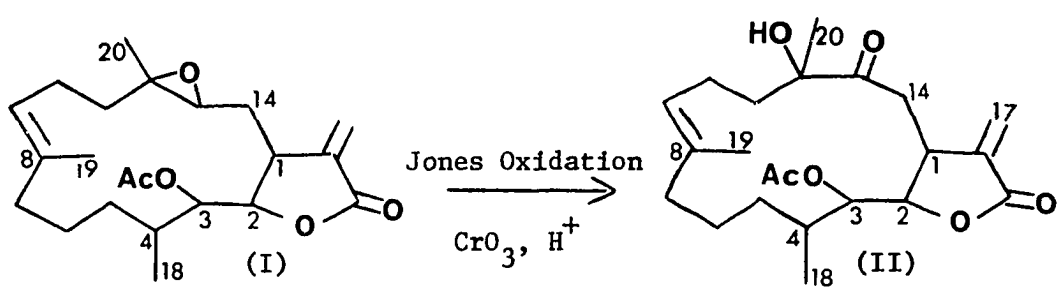
The doublet at δ 2.83 (2H, d, J=6.5 Hz) was interpreted as magnet-

ically equivalent methylene protons coupled only to a vicinal proton and not to each other. Again the coupling constant of this methylene signal was reflected in the quartet at δ 3.79. Since there were two sets of absorptions (three protons) in the spectrum which were apparently coupled to the proton β to the lactone carbonyl, each with approximately the same coupling constant (J=6 Hz versus J=6.5 Hz), the simple combination of the coupling constants led to the basic 1:3:3:1 quartet. Thus, the methylene protons alpha to the ketone appeared to be coupled to the proton β to the lactone. In regard to this interpretation, it appeared that in II that all of the oxygen functionality, the α-ketol, the lactone, and the acetate, could be assigned. Therefore, the epoxide in I, separated by a methylene ($-CH_2-$) from the beta position of the lactone, was designated to occupy the C-12 and C-13 positions of the monocarbocyclic fourteen-membered ring. Found in Table III are the nmr assignments that were made for II.

TABLE III

Proton Assignments Made from the
100 MHz NMR Spectrum of the α-Ketol (II)

Assignment	Signal (δ)	Protons	Multiplicity	J (Hz)
C-18	0.93	3	d	6.5
C-20	1.25	3	s	
C-19	1.57	3	d	<1
C-22	2.12	3	s	
H-14	2.83	2	d	6.5
H-1	3.79	1	dt (q)	6, 6.5
H-2	4.54	1	dd	6, 9.5
H-9	4.96	1	m	
H-3	5.06	1	d	9.5
H-17A	5.66	1	d	<1
H-17B	6.18	1	d	≤1
HO	4.05	1	brd s	Exchange-able in D_2O



H-3 is coupled to one neighbor H-4
H-2 is coupled to H-1 but not to H-3
H-3 is coupled to H-4 but not to H-2

H-2 is coupled to H-1 and H-3
H-14 is coupled to H-1
H-3 is not coupled to H-4

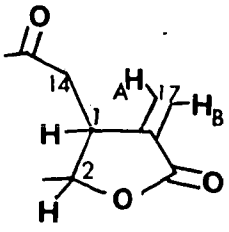
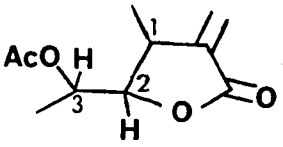
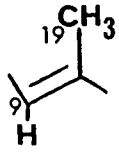
Part Four

The structural interpretations which were made for the α-ketol (II) were verified by a double resonance experiment (100 MHz). Irradiation of the H-1 signal at δ 3.79 (q, the proton beta to the lactone carbonyl) caused decoupling of H-14, δ 2.83 (doublet collapsed to a singlet), H-2, δ 4.54 (double doublet collapsed to a doublet, J=9.5 Hz), H-17A, δ 5.66 (hyperfine doublet to a sharp singlet), and H-17B, δ 6.18 (hyperfine doublet to a sharp singlet), signals. This indicated that the proton β to the lactone carbonyl was coupled to five other protons: the two exomethylene protons, the lactone proton, and two methylene protons alpha to a ketone. Similarily, irradiation of H-14 at δ 2.83 (d, methylene alpha to a ketone) caused decoupling of H-1, δ 3.79 (quartet collapsed to a doublet, J=6 Hz); and irradiation of H-2 at δ 4.54 (dd, lactone proton) caused decoupling of H-1, δ 3.79 (quartet collapsed to a triplet, J=6.5 Hz), and H-3, δ 5.06 (doublet collapsed to a singlet), signals. Likewise, irradiation of H-3 at δ 5.06 (d, proton under acetate), showed decoupling of H-2 (double doublet collapsed to doublet, J=6 Hz). Thus, it was shown that the acetate function was adjacent to the lactone oxygen and that the methylene protons adjacent to the ketol (formerly, the methylene protons vicinal to the epoxide proton) were adjacent to the proton β to the lactone carbonyl. Again, irradiation of H-9, δ 4.96 (multiplet, vinylic proton) caused decoupling of the vinylic methyl, C-19, δ 1.57 (signal narrowed to a sharp singlet).

The results of this experiment are summarized in Table IV along with the partial structures.

TABLE IV

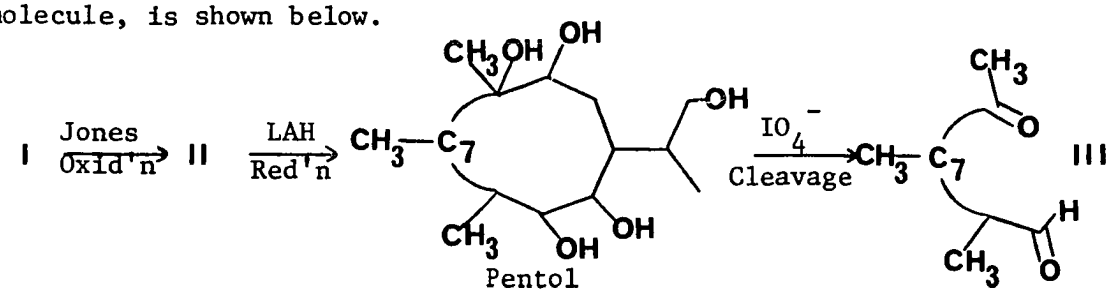
100 MHz NMR Decoupling Experiment on the α-Ketol (II)

Irradiation Frequency	Assignments	Observed Frequency	Multiplicity Change
H-1, δ 3.79		H-14, δ 2.83	d to s
		H-2, δ 4.54	dd to d
		H-17A, δ 5.66	d to s
		H-17B, δ 6.18	d to s
H-14, δ 2.83		H-1, δ 3.79	q to d
H-2, δ 4.54		H-1, δ 3.79	q to t
		H-3, δ 5.06	d to s
H-3, δ 5.06		H-2, δ 4.54	dd to d
H-9, δ 4.96		C-19, δ 1.57	d to s

Thus, the relative positions of the oxygen functionalities in the molecule were correlated and, as in the case of eunicin and jeunicin, the 3, 12, and 13 positions of the fourteen-membered ring were oxygenated. Yet to be determined is the position of the tri-substituted olefinic bond relative to these oxygen functionalities and the assignment of stereochemistry to the molecule.

Part Five

An oxidative cleavage was sought which would afford an isolable product that could be used to determine the position of the trisubstituted olefinic bond. The reaction sequence, designed to utilize the reaction of periodic acid with the vicinal oxygen functionalities within the molecule, is shown below.

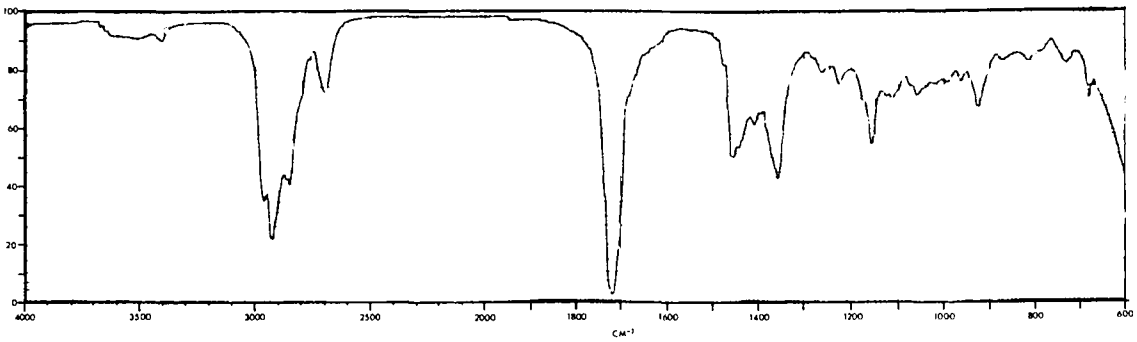


The cleavage product from this sequence moreover would contain the nmr labels of a methyl ketone from the epoxide end of the molecule (C-12) and an α-methyl aldehyde from the carbon bearing the acetate (C-3), which would enable the determination of the olefinic bond.

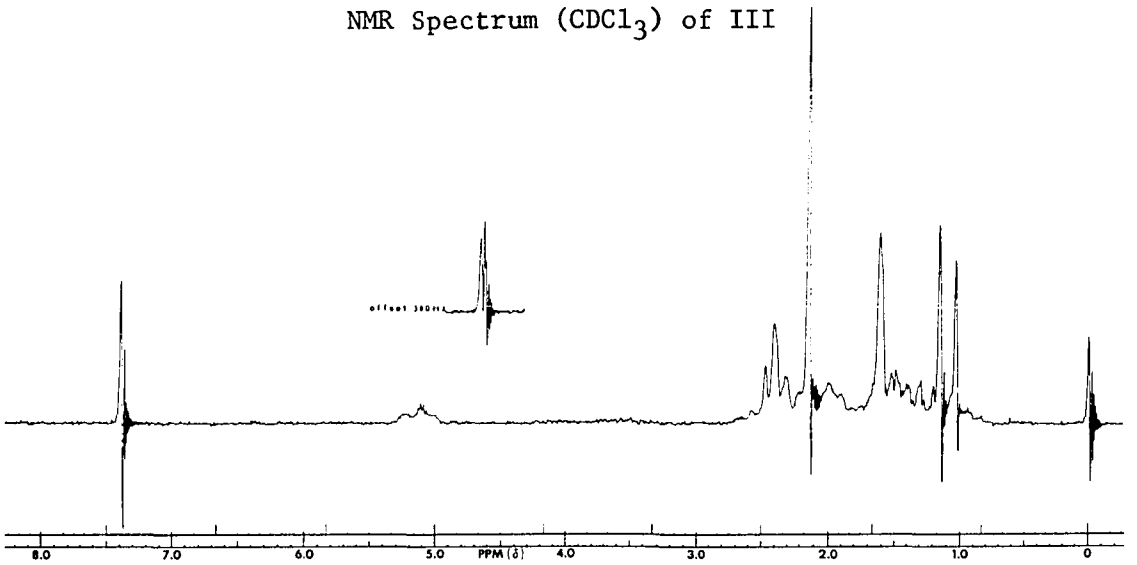
The α-ketol (II) was reduced by lithium aluminum hydride (LAH) to a mixture of pentols which were then subjected to periodate cleavage. A neutral fragment was isolated which after silica gel chromatography gave a colorless, pleasant-smelling oil (III). The ir spectrum (film, figure III) showed only one carbonyl absorption at 1717 cm^{-1}. Other prominent features in the spectrum were absorptions at 2705 and 2855 cm^{-1} (aldehyde proton) and at 1358 and 1457 cm^{-1} (methyl ketone), which were compatible with those expected for a ketoaldehyde.

The 60 MHz nmr spectrum of III ($CDCl_3$, figure 3) showed three methyl absorptions: δ 1.10 (3H, d, J=7 Hz), δ 1.62 (3H, brd s), and δ 2.13 (3H, s) which corresponded to a deshielded secondary methyl (C-18), a vinylic methyl (C-19), and a methyl adjacent to a carbonyl

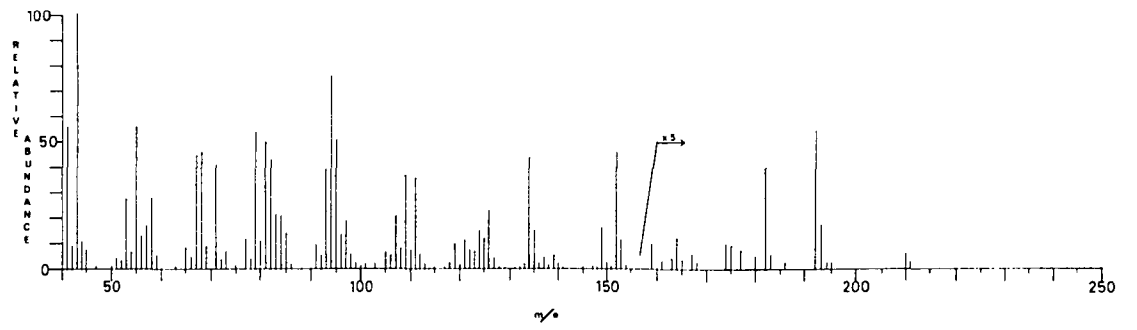
Figure 3
Spectra of the Ketoaldehyde Cleavage Product (III)
IR Spectrum (film) of III



NMR Spectrum ($CDCl_3$) of III



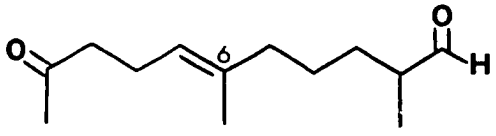
Mass Spectrum of III



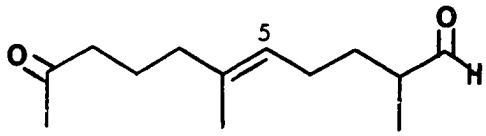
(C-20), respectively. In addition, two lowfield absorptions were distinguishable. These absorptions occurred at δ 5.13 (1H, ct, J≈7 Hz, vinylic proton adjacent to a methylene) and δ 9.63 (1H, d, J=2 Hz, aldehyde proton).

Although the cleavage product contained one asymmetric center, III was optically inactive. Apparently, the very susceptible alpha position of the aldehyde was racemized during the reaction. III therefore was not useful in the determination of the stereochemistry at C-4.

The 70 ev mass spectrum of III (figure 3) gave a molecular ion, m/e 210, expected for a molecular formula of $C_{13}H_{22}O_2$. Assuming that III was produced from the cleavage of an isoprenoid compound, the double bond can occur at only two positions and satisfy the spectral data so far presented.



2,6-Dimethyl-10-Oxo-Undec-6-Ene-Al
(A)



2,6-Dimethyl-10-Oxo-Undec-5-Ene-Al
(B)

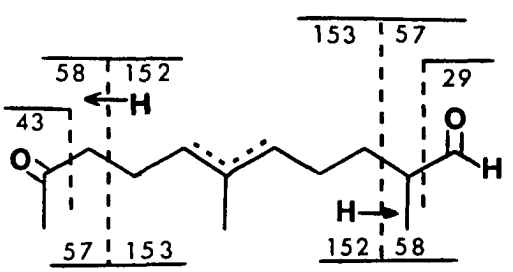
A close inspection of the mass spectrum of III provided the information necessary to characterize the position of the olefinic bond. The ions of relative intensity representing approximately 10% of the base peak are shown below in Table V.

TABLE V

Mass Spectrum Fragmentations of III
Ten Percent of the Relative Intensity of the Base Peak

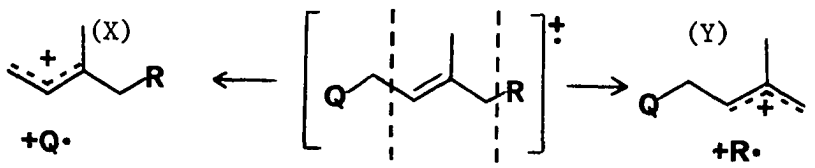
m/e	Rel. Int.	m/e	Rel. Int.	m/e	Rel. Int.
41	56	82	43	124	15
*43	100	83	21.5	*125	12
53	27	84	21	126	23
55	56	93	39	134	44
*57	17	94	76	135	15
*58	28	95	51	149	16
67	45	107	21	*152	46
68	46	108	11	*153	12
79	54	109	37	*182	8
81	50	111	36	*192	11

The starred ions (*) can be attributed to typical cleavages of ketones and aldehydes. For example, the ions m/e 192 and 182 indicated the loss of water (M^+-18) and loss of ethylene (M^+-28) from the molecular ion (typical of aldehydes).[13a,14] The ion m/e 43 was typical of the α-cleavage of methyl ketones (i.e., CH_3CO^+); m/e 57 and 153 indicated β-cleavage of the ketone and aldehyde; and m/e 58 and 152 indicated rearrangement with hydrogen transfer (McLafferty) from either the ketone or aldehyde end of the molecule.



These cleavages occurred with the cationic charge localized on the oxygen functionality. Since the olefin also competed for the charge,

albeit not as successfully as the carbonyl groups,[13b, 15] there were also some cleavages typical of olefins, as well as the production of alkyl fragments at lower mass units. Although the mass spectra of di-substituted mono-olefins usually result in the migration of the radical sites along the chain,[13c] the competition of the trisubstituted double bond with the carbonyl groups for the cationic charge resulted in the preferential cleavage of allylically activated carbon-carbon bonds with limited migration of the radical site.[16] This type of cleavage is shown below.



A, (X)=m/e 153
(Y)=m/e 125

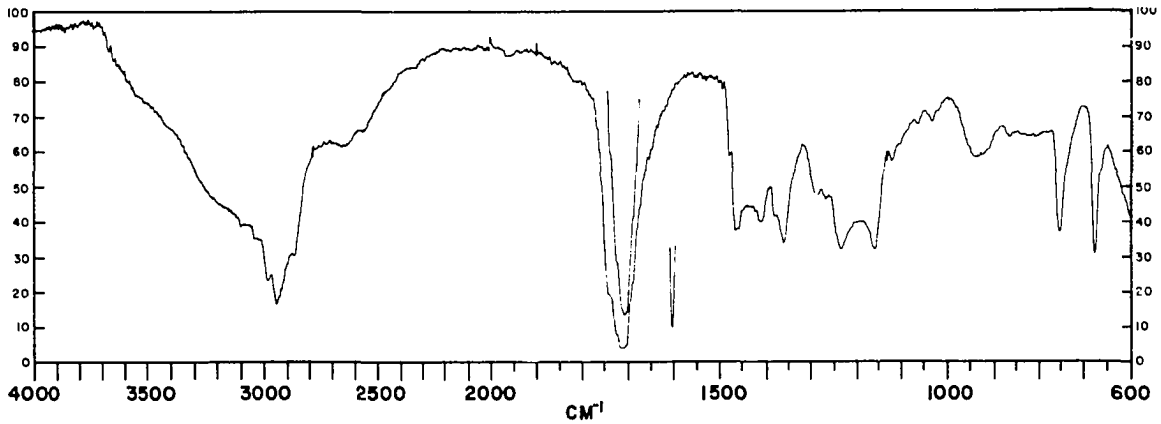
B, (X)=m/e 139
(Y)=m/e 139

These expected allylic cleavages (m/e 125--12%, 139--5.5%, and 153--12%), although not completely unambiguous, indicated that III was the 6-ene-ketoaldehyde (A), since the 5-ene structure (B), symmetrical with respect to this allylic type cleavage, was expected to produce a large m/e 139 ion due to the statistically increased abundance of that ion. The odd electron ion pair, m/e 126 and 84, possibly resulting from a McLafferty-type rearrangement of the olefin, likewise supported this assignment.

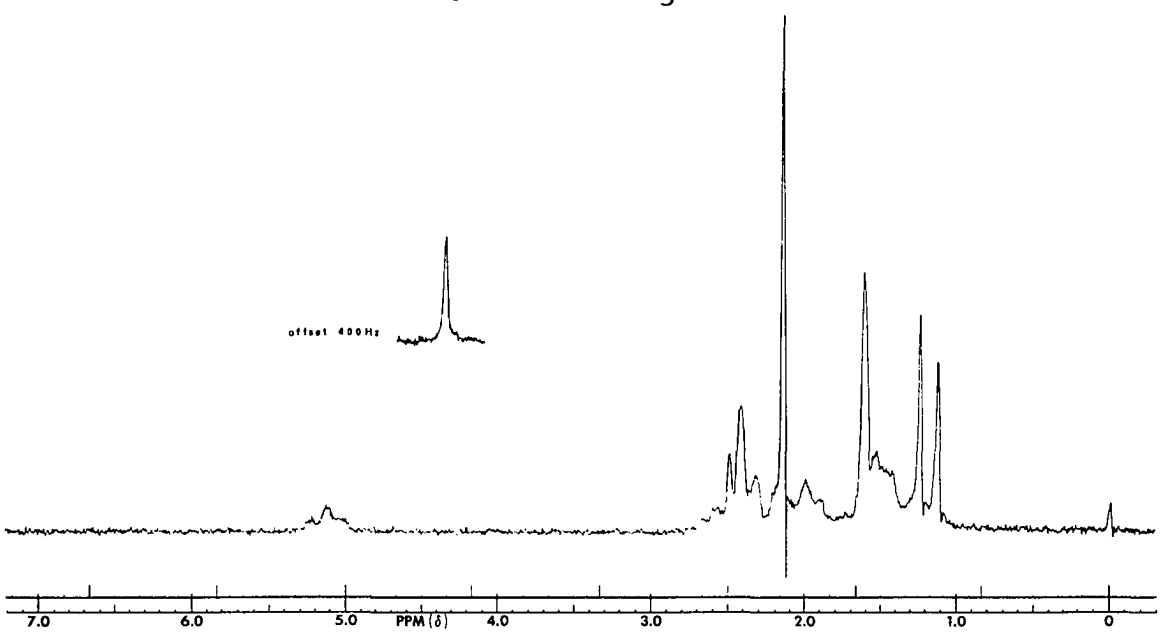
Oxidation of the ketoaldehyde (III) with Jones reagent gave an acid (IV) which was readily soluble in alkaline solution. The ir spectrum of IV (film, figure 4) exhibited a broad band at 3500∿2500 cm^{-1}, characteristic of the absorption of an acid, as well as a carbonyl absorption at 1703 cm^{-1} (shoulder at 1721 cm^{-1}).

Figure 4
Spectra of the Ketoacid (IV)

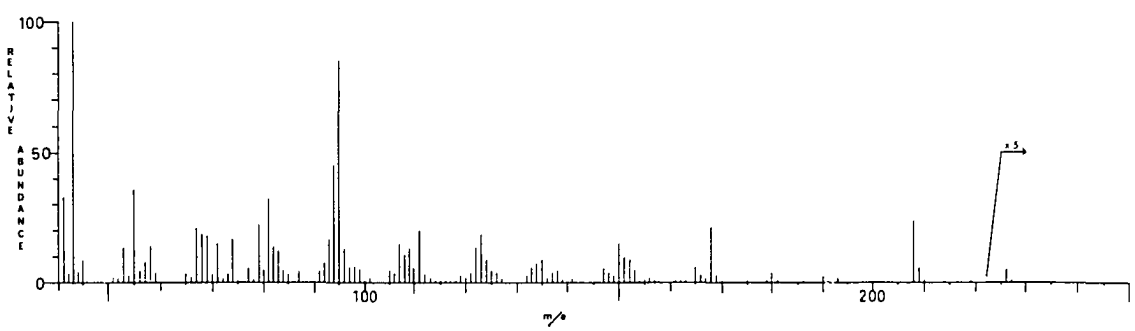
IR Spectrum (film) of IV



NMR Spectrum ($CDCl_3$) of IV



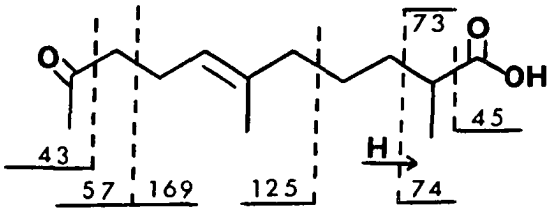
Mass Spectrum of IV



The 60 MHz nmr spectrum ($CDCl_3$, figure 4) of IV showed three methyl absorptions: δ 1.18 (3H, d, J=7 Hz, deshielded secondary methyl), δ 1.61 (3H, brd s, vinylic methyl), and δ 2.14 (3H, s, methyl adjacent to a carbonyl). Only two other absorptions were distinguishable: δ 5.12 (1H, ct, J≈6 Hz, vinylic proton adjacent to a methylene) and δ 11.0 (1H, s, acid proton). This spectrum, aside from a few changes, mirrored that of the ketoaldehyde cleavage product (III). The principal change in the spectrum involved that of the secondary methyl group. As expected from substituent effects,[17a] the secondary methyl showed a small paramagnetic shift, 0.09 ppm, as the electronegativity of the alpha substituent decreased from -CHO to -COOH.

The 70 ev mass spectrum of IV (figure 4) displayed a molecular ion at m/e 226, as expected. The loss of water, m/e 208, from the molecular ion was an intense fragmentation (33.5%). Odd electron ion fragments were found for both the acid and ketone ends of the molecule giving the pairs of ions, m/e 152--8.8%, 74--16.5% and m/e 168--21%, 58--14%, respectively. Carbon-carbon cleavages typical of ketones and acids were also found. The ketone end of the molecule produced ions at m/e 43--100% (α-cleavage), 57--7.8% (β-cleavage), and 169--2.9% (β-cleavage). The acid end of the molecule produced ions at m/e 45--8.7% (α-cleavage), 73--3.2% (β-cleavage), and 153--4.6% (β-cleavage). By utilizing the favorable allylic carbon-carbon bond cleavages about the olefin, the ions m/e 125--4.5% and 169--2.9%, compared to m/e 155--1% and 139--1% for the 5-ene structure, indicated the 6-ene-ketoacid structure was most compatible for IV. The appearance of odd electron ion fragments (even mass) from the methyl ketone end of the molecule

was attributed to the nature of alkyl methyl ketones in their ability at high ionization potentials to produce the m/e 58 ion.[13d]



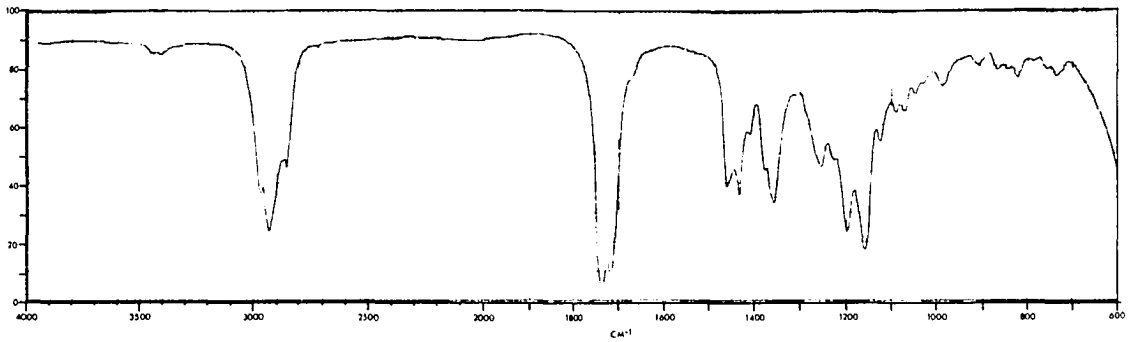
Esterification of the ketoacid (IV) with diazomethane produced the ester (V) which exhibited two carbonyl absorptions in the infrared (film, figure 5), at 1735 and 1717 cm^{-1} (ester and ketone). No hydroxyl absorption was found in the 3500 cm^{-1} region.

The 60 MHz nmr spectrum ($CDCl_3$, figure 5) of the ketoester (V) mirrored that of III and IV. It exhibited four methyl absorptions: δ 1.14 (3H, d, J=7 Hz, secondary methyl beta to a carbonyl), δ 1.60 (3H, brd s, vinylic methyl), δ 2.13 (3H, s, methyl adjacent to a carbonyl), and δ 3.68 (3H, s, methyl on oxygen--ester). The only other interpretable absorption occurred at δ 5.11 (1H, ct, J≈6 Hz, vinylic proton adjacent to a methylene).

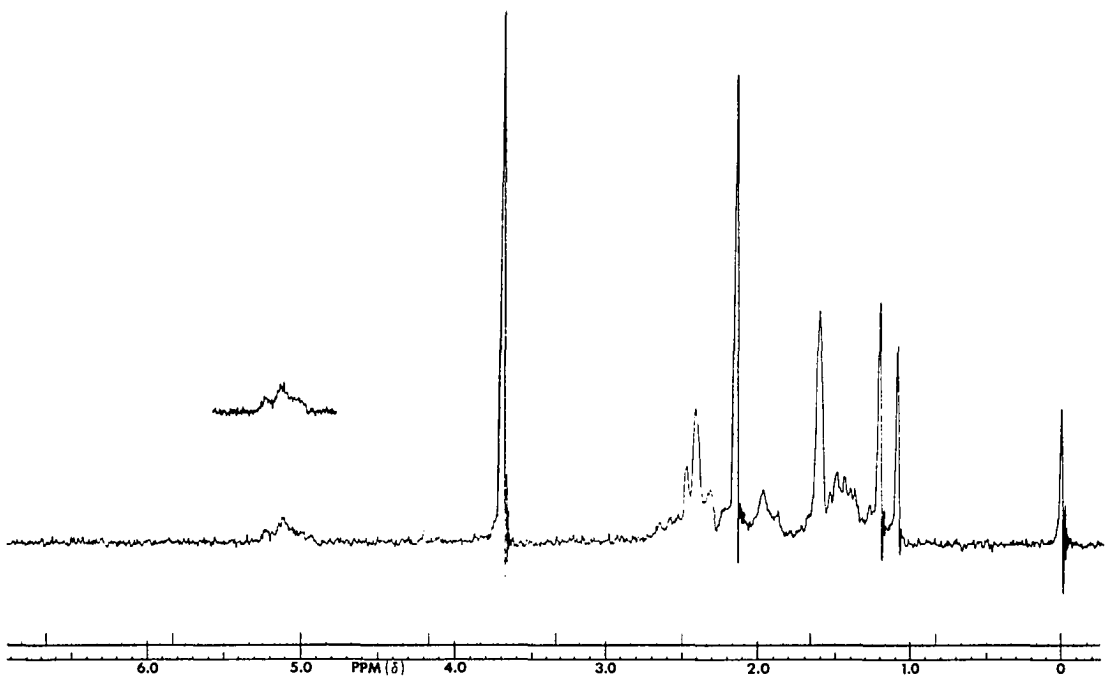
Elemental analysis of the ketoester (V) verified the expected molecular formula, $C_{14}H_{24}O_3$. The 20 ev mass spectrum of V (figure 5) also attested this molecular formula (M^+, m/e 240). The loss of water (m/e 222) and methanol (m/e 208) from the molecular ion were also observed. The ions m/e 43--40% (α-cleavage), 57--4.7% (β-cleavage), and 183--5.6% (β-cleavage) associated with typical cleavages of methyl ketones were again observed. The base peak in the spectrum of V was m/e 88. The odd electron ion pair m/e 58--5.8% and 182--43% was interpreted as a rearrangement with hydrogen transfer but not a McLafferty-

Figure 5
Spectra of the Ketoester (V)

IR Spectrum (film) of V



NMR Spectrum ($CDCl_3$) of V



Mass Spectrum of V

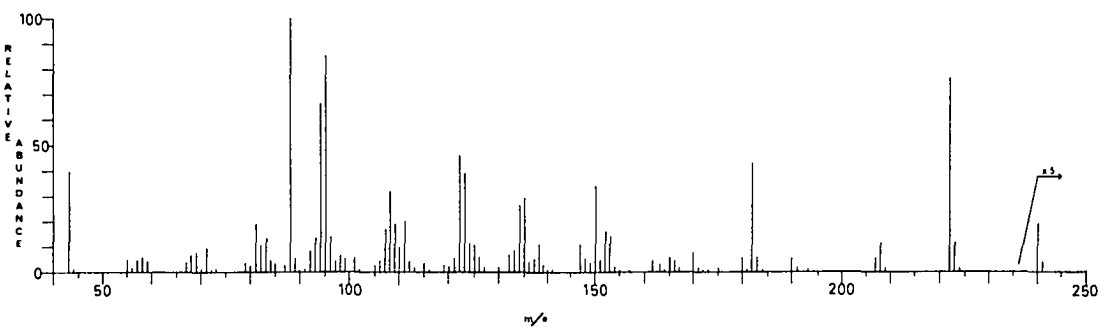
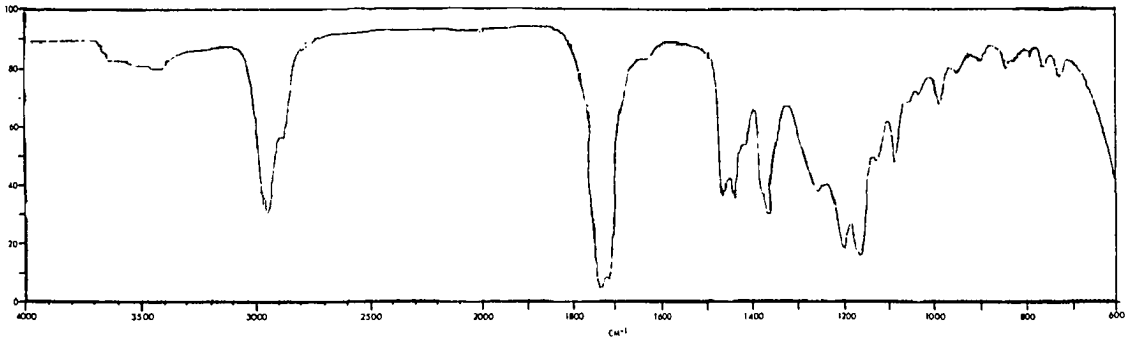
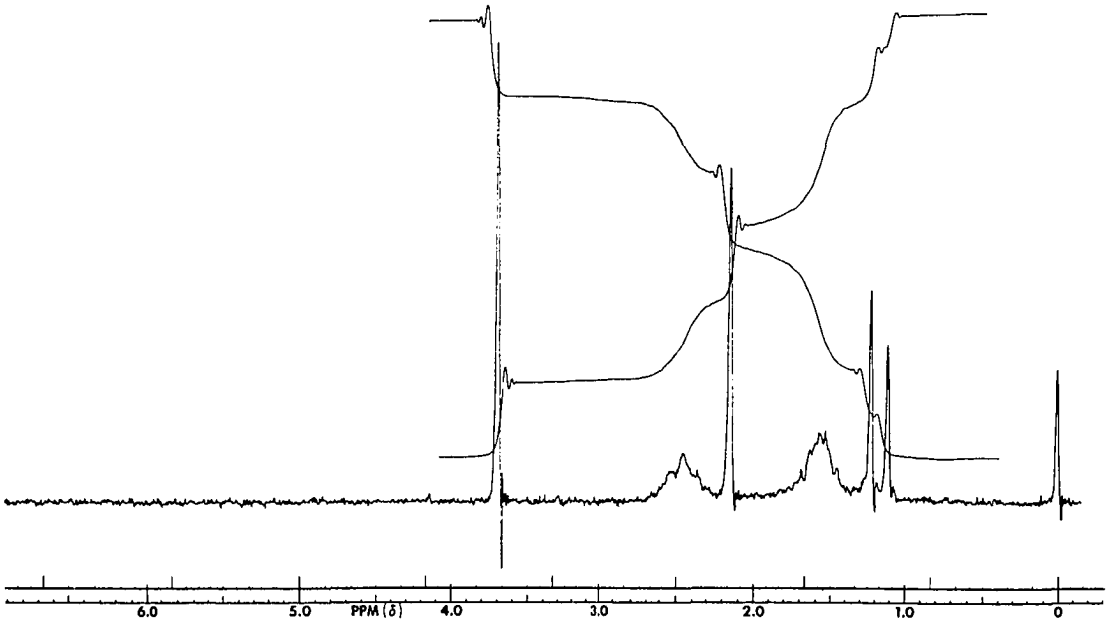


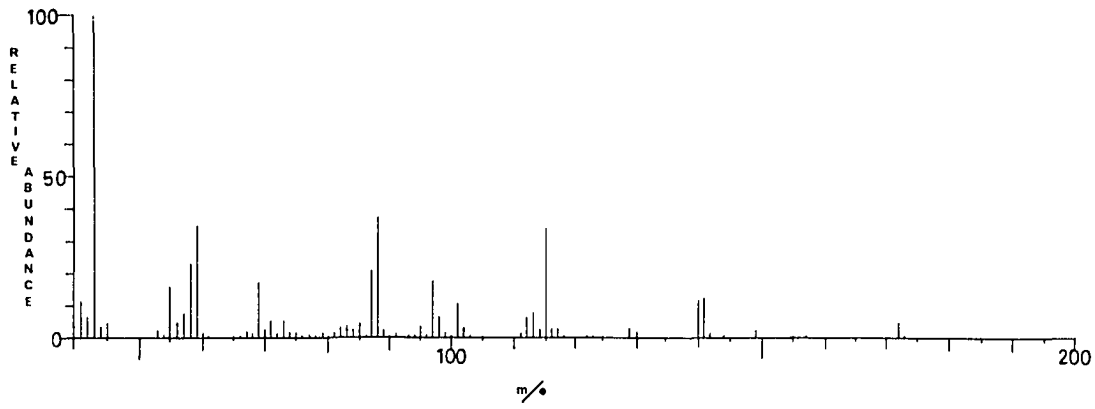
Figure 6
Spectra of Methyl 2-Methyl-6-Oxo-Heptanoate (VI)
IR Spectrum (film) of VI



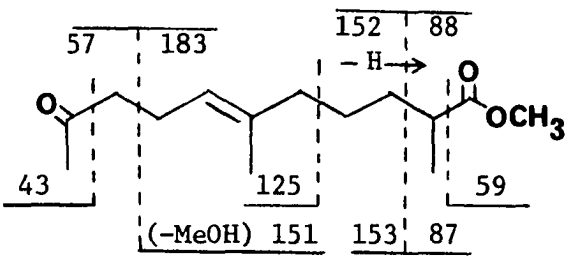
NMR Spectrum ($CDCl_3$) of VI



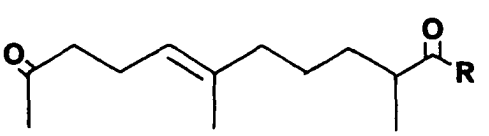
Mass Spectrum of VI



type[18] since the intensity of the m/e 58 ion was predicted to be much larger from such a fragmentation. The ester portion of the molecule also produced typical cleavages. These ions were found at m/e 59--4.4% (α-cleavage), 87--2.2% (β-cleavage), and 153--14% (β-cleavage). The odd electron ion pair m/e 88--100% and 152--16% indicated a McLafferty rearrangement from the ester end of the molecule.



The ions predicted for allylic carbon-carbon bond cleavages in V were m/e 125--9.5% and m/e 183--5.6% for the 6-ene-structure, compared to m/e 139--2.4% and 169--0% for the 5-ene structure. Thus, the mass spectral analysis of compounds III, IV, and V, indicated that the olefinic bond occurred at C-6 (C-8 in eupalmerin acetate) giving the structure shown below.



III, R=H
IV, R=OH
V, R=OCH_3

Part Six

Ozonolysis of V produced two isolable products. VI, methyl 2-methyl-6-oxo-heptanoate, was partitioned into dichloromethane, while VII, levulinic acid, was partitioned into water. Methyl 2-methyl-6-oxo-heptanoate (VI) was obtained pure from preparative thin layer chromatography, and

the elemental analysis indicated the expected molecular formula $C_9H_{16}O_3$, which was verified by mass spectrometry (M^+, m/e 172). The ir spectrum (film, figure 6) of VI exhibited two carbonyl absorptions, at 1732 (ester) and 1716 cm^{-1} (ketone), and the 60 MHz nmr spectrum ($CDCl_3$, figure 6) showed three methyl absorptions: δ 1.16 (3H, d, J=7 Hz, secondary methyl beta to the ester carbonyl), δ 2.13 (3H, s, methyl adjacent to a ketone carbonyl), and δ 3.68 (3H, s, methyl on oxygen, ester). In addition, there were two other broad absorptions in the spectrum centered at δ 2.45 (3H, m, protons adjacent to carbonyls) and at δ 1.55 (4H, m, methylene protons). The above spectra as well as the retention time of VI in the gas chromatogram proved identical to the corresponding data for 2-methyl-6-oxo heptanoic acid methyl ester prepared independently.

The water soluble component VII exhibited an ir spectrum (film) identical with that of levulinic acid. When VII reacted with diazomethane, VIII was obtained which gave an elemental analysis expected for a molecular formula, $C_6H_{10}O_3$, and produced spectra identical with those of an authentic sample of methyl levulinate. The gas chromatographic retention time of VIII, likewise, was identical to that of the authentic sample. Therefore, as predicted from mass spectral analyses of the derivatives III, IV, and V, the trisubstituted double bond in eupalmerin acetate was demonstrated to occur at C-8 in the monocarbocyclic fourteen-membered ring. Thus, the relative position of all the functionality within the molecule was established. Derivatives of eupalmerin acetate (I) were then made to determine the stereochemistry within the molecule.

Part Seven

Eupalmerin acetate (I) was derivatized by hydrogenation (IX), methanolysis of the dihydroproduct (X), bromination (XI), and the addition of diazomethane (XII). From these derivatives the assignment of stereochemistry to the molecule was made.

Atmospheric catalytic hydrogenation (5% Pd/C) of eupalmerin acetate afforded a three component mixture in which the ratio of the products was 10:16:74 by gas chromatography. A sample of the major component (IX) was obtained by chromatography of the hydrogenation mixture on silicic acid; however, IX failed to crystallize even though it appeared as a single component in tlc and gc.

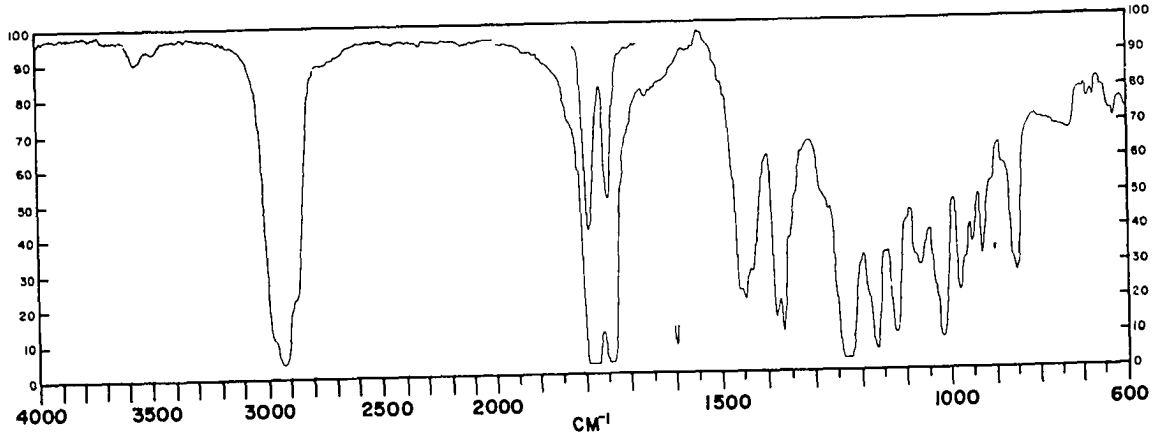
The ir spectrum (figure 7, CCl_4) of IX exhibited two carbonyl absorptions at 1792 (γ-lactone) and 1749 cm^{-1} (ester). There was also the strong absorption at 1235 cm^{-1} (acetate).

The 60 MHz nmr spectrum (CCl_4, figure 7) showed complete reduction of the exomethylenic double bond. In addition, five methyl absorptions could be distinguished: δ 0.93 (3H, d, J=6.5 Hz, secondary methyl, C-18), δ 1.15 (3H, d, J≈7 Hz, secondary methyl adjacent to the lactone carbonyl, C-17), δ 1.21 (3H, s, epoxide methyl, C-20), δ 1.58 (3H, brd s, vinylic methyl, C-19), and δ 2.07 (3H, s, acetate methyl). Additionally, three lowfield absorptions were found at δ 4.42 (1H, dd, J=4, 9.5 Hz, proton under the lactone oxygen, H-2), δ 5.20 (1H, m, vinylic proton, H-9), and δ 5.27 (1H, d, J=9.5 Hz, proton under the acetate, H-3).

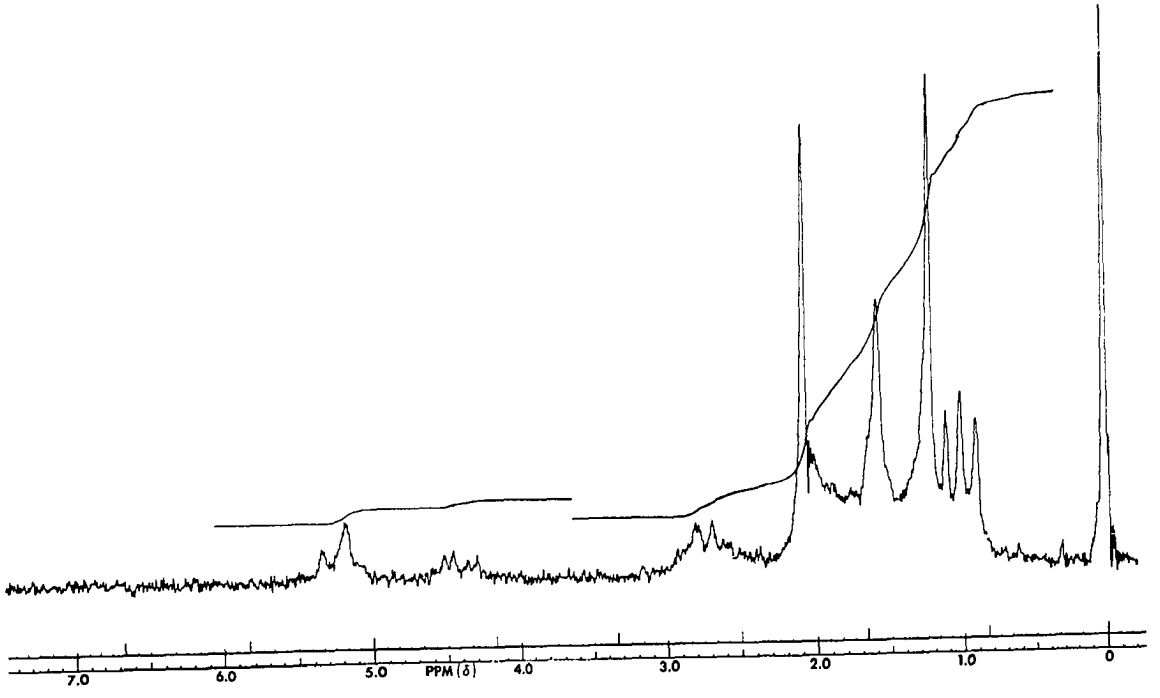
When dihydroeupalmerin acetate (IX) was allowed to reflux with anhydrous methanol and a catalytic amount of p-toluenesulfonic acid,

Figure 7
Spectra of Dihydroeupalmerin Acetate (IX)

IR Spectrum (CCl_4) of IX



NMR Spectrum (CCl_4) of IX

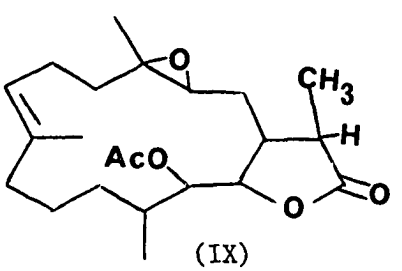


a mixture of products was obtained. The principal component (X) of this mixture after chromatography (Florisil) crystallized.

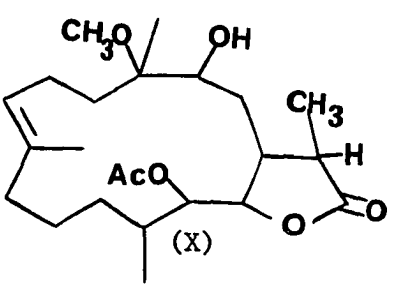
Elemental analysis indicated that X had a molecular formula of $C_{23}H_{38}O_6$, indicative of the addition of methanol to dihydroeupalmerin acetate. The 70 ev mass spectrum (figure 8) of this derivative produced as expected a molecular ion at m/e 410. Ions at m/e 392, 378, and 350 indicated the loss of water, methanol, and acetic acid from the molecular ion.

The ir spectrum (KBr, figure 8) showed absorptions at 3500 (hydroxyl), 1780 (γ-lactone), 1730 (ester), and 1240 cm^{-1} (acetate).

The 60 MHz nmr ($CDCl_3$, figure 8) showed six methyl absorptions: δ 0.91 (3H, d, J=7 Hz, secondary methyl, C-18), δ 1.02 (3H, s, methyl on the quaternary carbon bearing the methoxyl, C-20), δ 1.30 (3H, d, J=7 Hz, secondary methyl adjacent to the lactone carbonyl, C-17), δ 1.55 (3H, d, J=1 Hz, vinylic methyl, C-19), δ 2.08 (3H, s, acetoxyl methyl), and δ 3.25 (3H, s, methyl on oxygen, methyl ether). In addition, four distinguishable lowfield proton signals were found in the spectrum: δ 3.73 (1H, dd, J=5, 9.5 Hz, methine proton under the hydroxyl, H-13), δ 4.43 (1H, dd, J=4, 10 Hz, proton under the lactone oxygen, H-2), δ 5.01 (1H, dd, J=1, 10 Hz, proton under the acetate, H-3), and δ 5.80 (1H, ct, J≈7 Hz, vinylic proton, H-9).

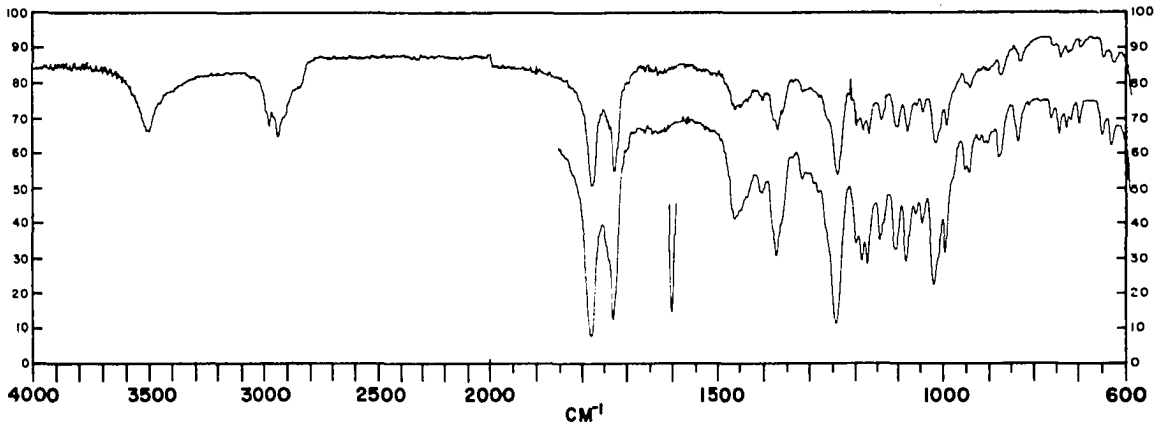


(IX)

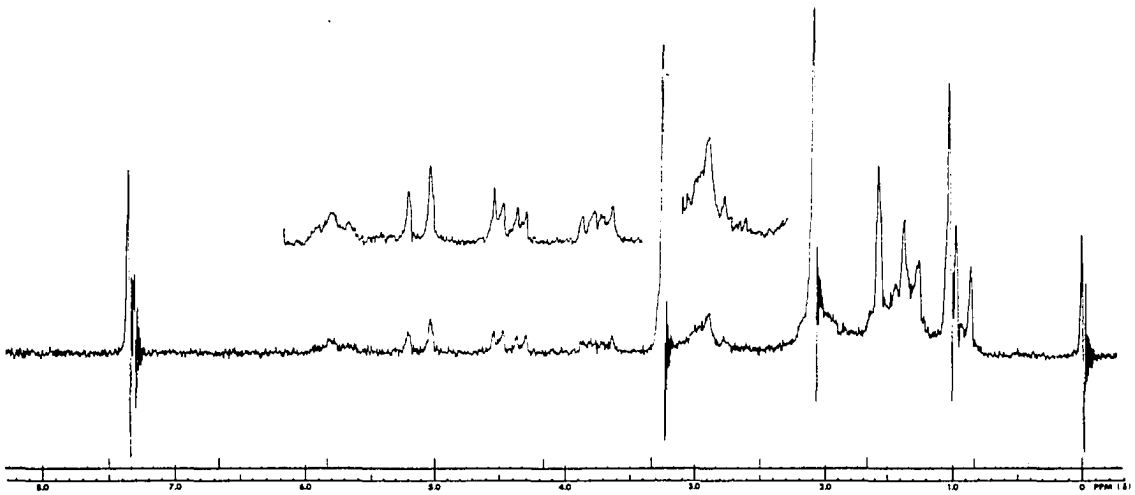


(X)

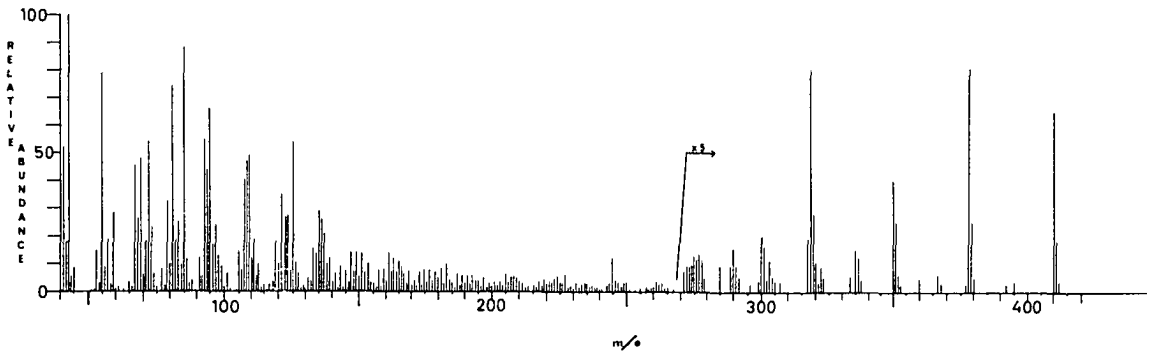
Figure 8
Spectra of Dihydroeupalmerin Acetate Methanol Adduct (X)
IR Spectrum (KBr) of X



NMR Spectrum ($CDCl_3$) of X



Mass Spectrum of X



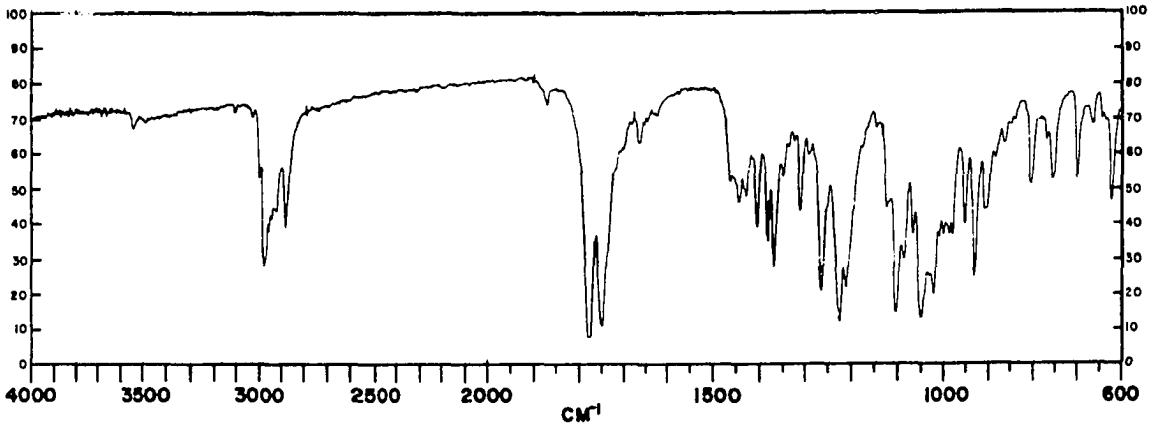
It was noticed that in each of the derivatives IX and X, the vicinal coupling constants of H-2 and H-3 ($J_{2,3}$) had become quite large, compatible for trans-anti coupling. Likewise the vicinal coupling constants between H-3 and H-4 ($J_{3,4}$) had approached zero, indicative of a 90° dihedral angle between H-3 and H-4. This meant that upon hydrogenation (and also subsequent reaction of that product with methanol), the conformations at C-3 and C-4 in these derivatives apparently had each rotated 90° from the conformations found in eupalmerin acetate at these positions.

When eupalmerin acetate reacted with one equivalent of bromine (a 10% solution in CCl_4), a crystalline compound (XI) was isolated and the elemental analysis, $C_{22}H_{32}O_5Br_2$, indicated that XI was the product of the simple addition of two bromine atoms to I. The 70 ev mass spectrum (figure 9) displayed molecular ions in the proper isotopic ratio (1:3:1) expected for a compound containing two bromine atoms. Ion fragments were found in the spectrum which contained one and two bromine atoms.

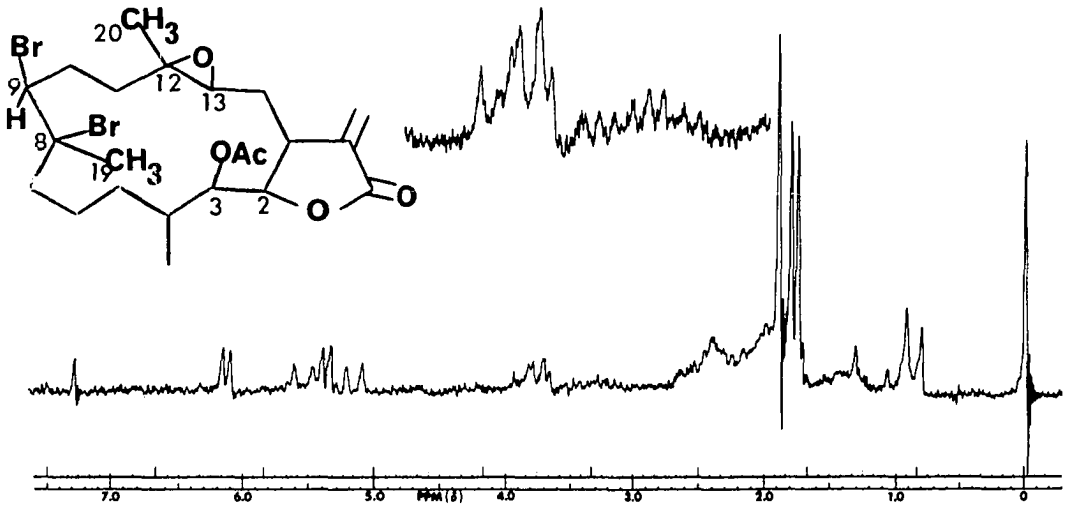
The ir spectrum (KBr, figure 9) showed absorptions at 1777 (γ-lactone), 1750 (ester), 1225 (acetate), and 3030 cm^{-1} (strained epoxide).

The 60 MHz nmr spectrum ($CDCl_3$, figure 9) of XI showed four methyl absorptions: δ 0.88 (3H, d, J=7 Hz, secondary methyl, C-18), δ 1.75 (3H, s), δ 1.80 (3H, s), and δ 1.89 (3H, s, acetate methyl). Protons were also observed at δ 3.30 (1H, m, proton β to the lactone carbonyl, H-1), δ 3.76 [2H, m, coincident absorption of the epoxide proton and the proton under the bromine--resolved in benzene, as two absorptions: δ 3.85 (1H, dd, J=3.5, 10 Hz) and δ 3.76 (1H, dd, J=2, 7 Hz)], δ 5.18

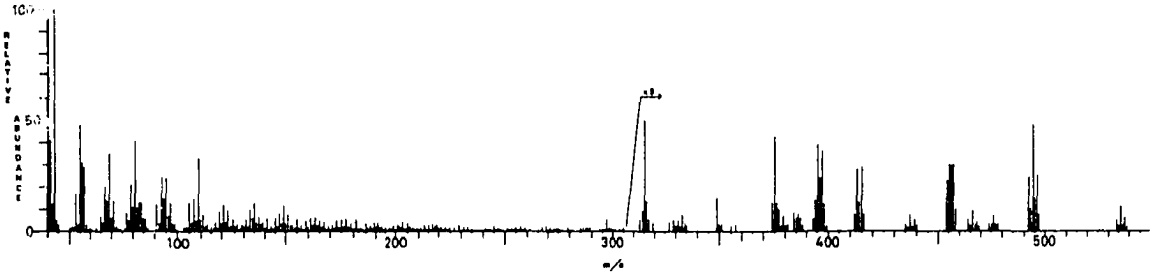
Figure 9
Spectra of Dibromoeupalmerin Acetate (XI)
IR Spectrum (KBr) of XI



NMR Spectrum ($CDCl_3$) of XI



Mass Spectrum of XI



(1H, d, J=8 Hz, proton under the lactone oxygen, H-2), δ 5.39 (1H, d, J=3.5 Hz, conjugated olefinic proton, H-17A), δ 5.57 (1H, d, J=8.5 Hz, proton under the acetate, H-3), and δ 6.14 (1H, d, J=3.5 Hz, conjugated olefinic proton, H-17B).

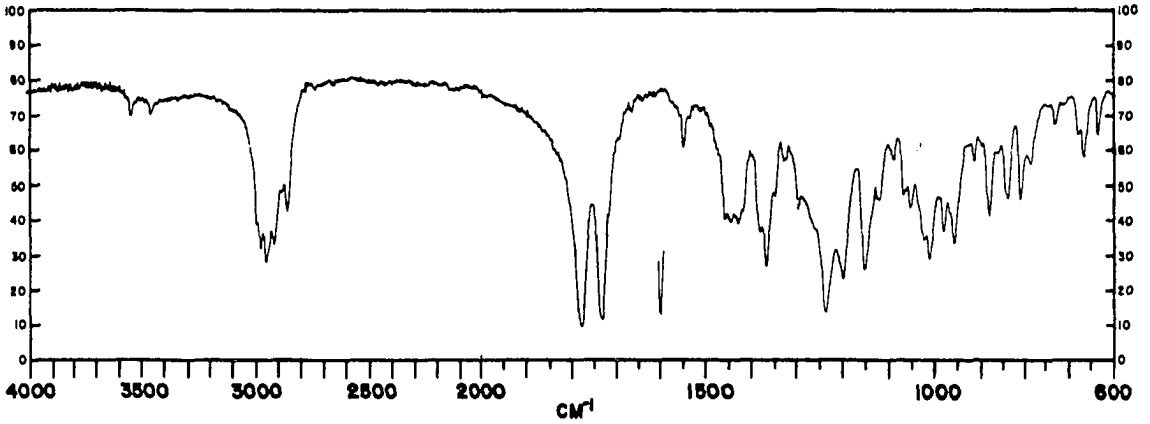
When eupalmerin acetate reacted with diazomethane, a crystalline produce (XII) was obtained. The elemental analysis indicated that eupalmerin acetate had added one molecule of diazomethane to give the molecular formula, $C_{23}H_{34}O_5N_2$. The 70 ev mass spectrum (figure 10) in addition to the molecular ion, m/e 418, displayed ions expected for the pyrazoline of eupalmerin acetate.

The ir spectrum of XII (KBr, figure 10) exhibited absorptions at 1780 (γ-lactone, 1736 (ester), 1551 (νN=N), and 1238 cm^{-1} (acetate).

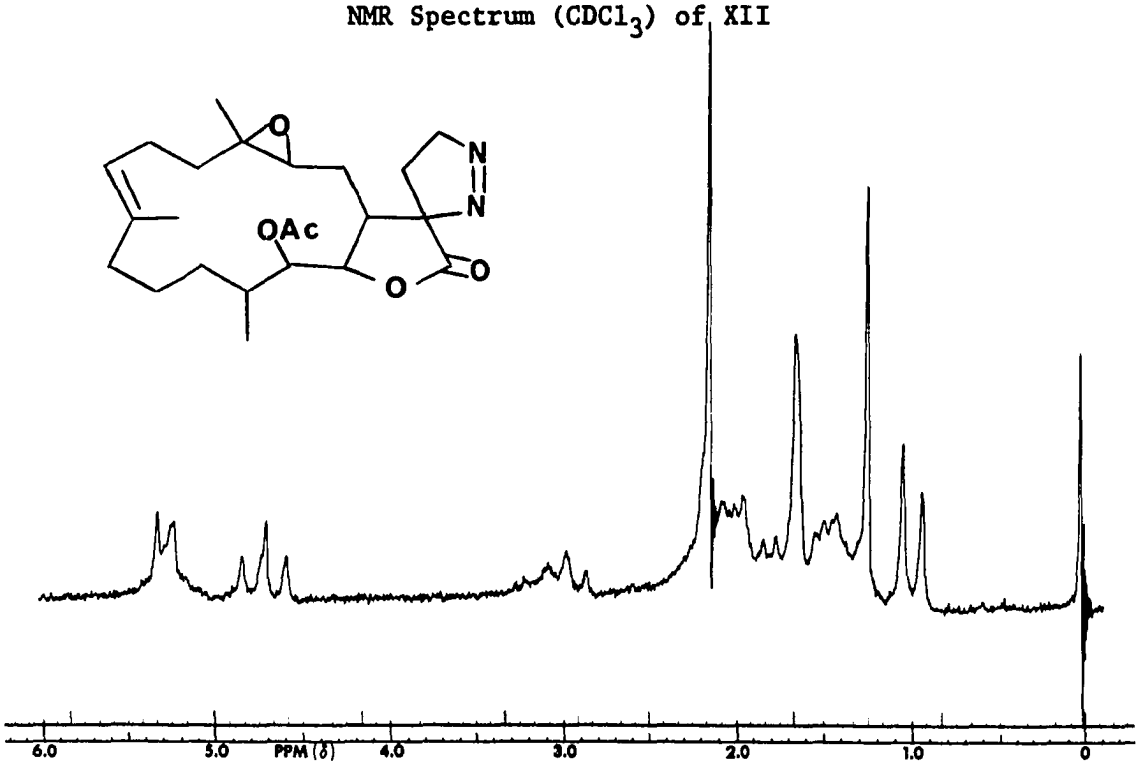
The 60 MHz nmr ($CDCl_3$, figure 10) exhibited four methyl signals: δ 0.98 (3H, d, J=7 Hz, secondary methyl, C-18), δ 1.22 (3H, s, epoxide methyl, C-20), δ 1.63 (3H, brd s, vinylic methyl, C-19), and δ 2.12 (3H, s, acetate methyl). Other absorptions in the spectrum occurred at δ 2.97 (4H, m), δ 4.63 (1H, d, J=8 Hz, proton under the lactone, H-2), δ 4.77 (1H, d, J=8.5 Hz, proton under the acetate, H-3), δ 5.18 1H, m, vinylic proton, H-9), and δ 5.23 (2H, m, methylene adjacent to the N=N). These features were all consistent with the expected structure for the pyrazoline XII.

Pascual and co-workers[19] have correlated the configuration of the trisubstituted olefinic bond by the chemical shift of the olefinic proton. The calculated value of the various olefinic bonds of this type are shown in Table VI.

Figure 10
Spectra of Eupalmerin Acetate Diazomethane Adduct (XII)
IR Spectrum (KBr) of XII



NMR Spectrum ($CDCl_3$) of XII



Mass Spectrum of XII

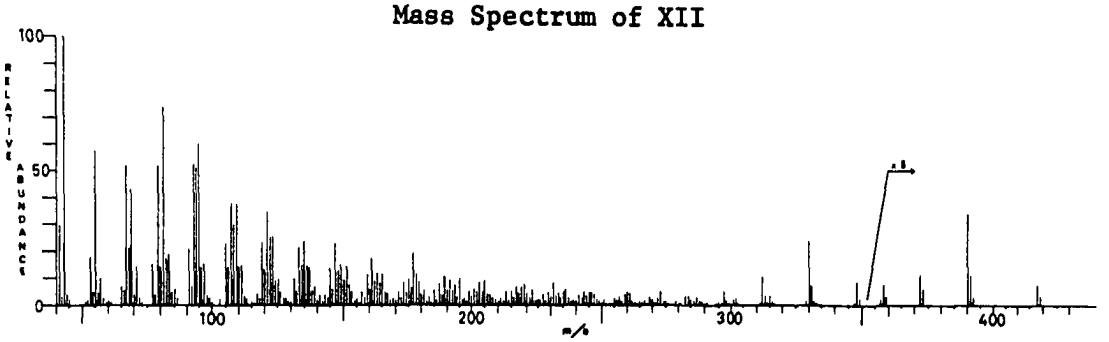
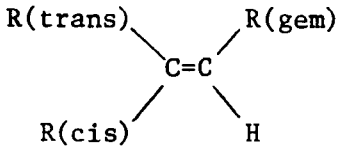


TABLE VI

Calculated Values of the Chemical Shift of the Trisubstituted Olefinic Bonds



$\delta_{C=CH} = 5.28 + \sum_i Z_i(R's)$

Transoid Cyclic	Cisoid Cyclic	Acyclic	
5.28	5.28	5.28	
+0.71	+0.71	+0.44	R(gem)
-0.33	-0.26	-0.26	R(cis)
-0.29	-0.30	-0.29	R(trans)
5.37	5.43	5.17	$\delta_{C=CH}$

The calculated values were notably higher than those found in the derivatives of eupalmerin acetate. The chemical shift of the H-9 proton for various diterpenoid derivatives is found in Table VII.

TABLE VII

Observed Chemical Shift of the Trisubstituted Olefinic Bonds in Eupalmerin Acetate and its Derivatives

δ, H-9	Cyclic Diterpenoid Lactones	δ, H-7 (H-9)	Acyclic
5.10	I	5.13	III
4.96	II	5.12	IV
5.20	IX	5.11	V
5.18	XII		
5.80	X		
5.08	Eunicin*		
5.17	Dihydroeunicin*		
5.57	Jeunicin*		
5.58	Dihydrojeunicin*		

(*) Transoid Olefinic Bond

The chemical shift of the H-9 proton in I, II, IX, and XII compared favorably with that found in the eunicin-series. It also appeared that the α,β-unsaturated lactone was responsible for some of the shielding

of the H-9 proton, since upon hydrogenation, the H-9 proton was shifted 0.10 ppm to lower field, nearly the same value observed in the eunicin-series (0.09 ppm). The fact that the chemical shift of the H-9 proton was considerably lower than the calculated value, implied that the deviation from that value was due to additional shielding effects within the molecule. Since the chemical shift of the H-9 proton corresponded so well with the olefinic proton in the eunicin series in which the olefin was transoid, and because these results were also compatible with the calculated values, the trisubstituted olefin in eupalmerin acetate was interpreted as transoid.

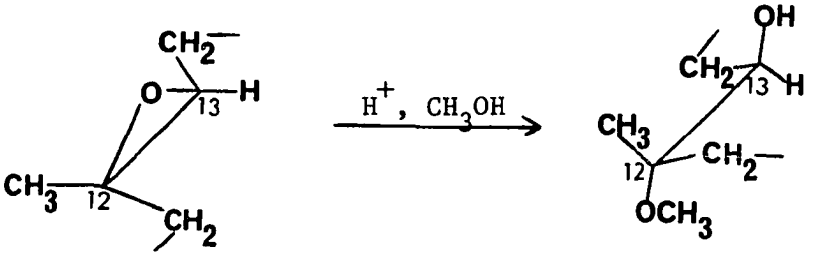
The only values of H-9 which exceeded the calculated chemical shifts for transoid olefinic bonds were X and the jeunicin series. Although jeunicin contained a transoid olefinic bond, x-ray crystallographic studies[10] showed that the vinylic proton, H-9, was very close to the ethereal oxygen (nearly within a van der Waals radius), which is the probable cause of the deshielding of that proton. The conformational change of the oxygen functionalities at C-12 and C-13 from a eunicin-like molecule, dihydroeupalmerin acetate (IX), to a jeunicin-like molecule, dihydroeupalmerin acetate methanol adduct (X), indicated that the newly introduced oxygen functionality at C-12 was the cause of the increased deshielding of the H-9 proton. The apparent similarities between X and jeunicin were likewise observed in the comparison of the analogous protons in each molecule (Table VIII).

TABLE VIII

Comparison of the Chemical Shift of Protons in Jeunicin and the Methanol Adduct of Dihydroeupalmerin Acetate (X)

Proton	X, δ	Jeunicin, δ
H-2	4.43	4.45
C-18	0.91	0.94
H-13	3.73	3.73
H-9	5.80	5.57

In the construction of a molecular model, it was found that the β-epoxide of eupalmerin acetate upon methanolysis would produce a jeunicin-like product, presuming inversion at C-12 and a transoid skeleton,



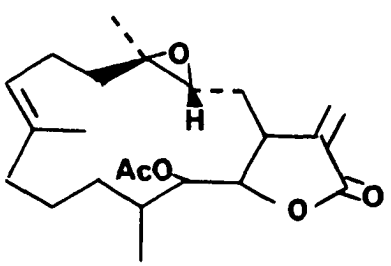
Eunicin-like (IX) Jeunicin-like (X)

in which the H-9 proton was very close to the methoxyl. Although this evidence seems compelling, it is not proof of the transoid skeleton of the β-epoxide, nor does it preclude the possibility that other isomers of I might also compare favorably. However, the fact that X compared very favorably with jeunicin made that structure most appealing.

In the bromination of eupalmerin acetate, it was observed that the C-20 methyl on the epoxide and the H-13 proton of the epoxide were considerably deshielded. After having observed a strong anisotropic effect in X produced by the methoxyl at C-12 upon the olefinic proton H-9, it was not surprising that the introduction of bromine atoms at

C-8 and C-9 might have a similarly strong anisotropic effect upon the C-20 methyl and the H-13 epoxide proton. Dreiding models were made of the dibromo adduct of the β-epoxide of eupalmerin acetate to check this supposition. Assuming that normal trans addition of bromine had occurred to the transoid olefinic bond from the less hindered face of the molecule, the Dreiding model of XI showed that both the C-20 methyl and the H-13 proton were each very near different bromine atoms. The β-epoxide in this model was derived from a trans olefinic bond at C-12. Models in which the epoxide was derived from a cis olefinic bond at C-12 did not compare as favorably with the nmr data. In these models the cisoid skeleton allowed for a larger, more flexible ring in which the ring substituents were less crowded, and therefore a small anisotropic effect was expected of the C-20 methyl and the H-13 proton signals.

In addition, it was also noticed that in dibromoeupalmerin acetate (XI) the non-coupled protons, H-2 and H-3, were also deshielded. This meant that the dihedral angle between H-2 and H-3 was 90°. Since the acetoxy methyl was not deshielded in the nmr of dibromoeupalmerin acetate (XI), only the α-acetoxy isomer of I was compatible with that fact. Thus, the structure most compatible with the data derived from derivatives of eupalmerin acetate possessed a transoid olefinic bond and a β-epoxide on a transoid backbone. That structure is shown below.



Part Eight

The fusion of the γ-lactone to the fourteen-membered ring was not easily determined. It had been shown previously[20] that in rigid systems, such as six-membered rings, cis- and trans-fused γ-lactones can be differentiated on the basis of the magnitude of the coupling constant between the proton under the lactone oxygen (H-2) and the proton β to the lactone carbonyl (H-1). However, in situations in which the γ-lactone was attached to a ring containing more than six carbon atoms, the increased flexibility of the molecule made differentiation of the ring fusion by these coupling constants unreliable. Thus, the criterion for distinguishing between cis- and trans-fused γ-lactones did not appear to be particularly applicable to the fourteen-membered ring system in eupalmerin acetate. However, Herz and Samek[21] have shown by analyzing a large group of analogous γ-lactones (fused to seven-membered rings) with well-established stereochemistry that for first-order analysis of the values of $J_{1,2}$, the general rule

$$0 < J_{1,2}(\text{cis}) < 8\text{-}9 \text{ Hz} < J_{1,2}(\text{trans})$$

appeared applicable in determining the fusion of the γ-lactone. The logical extension of this rule to larger ring systems may be a useful tool in the designation of the fusion of the γ-lactone. Although the fourteen-membered ring system has the potential for greater flexibility (compared to the seven-membered ring), the rigidity imposed within this ring system by the carbon-carbon double bond and the epoxide severely limited the inherent flexibility of the ring. By cautious application of the above rule, it was possible to correlate the ring fusion of the

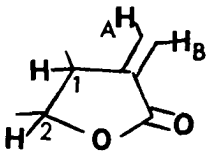
γ-lactone in the fourteen-membered ring system.

TABLE IX

Vicinal Coupling Constants of the Protons at the γ-Lactone Ring Fusion ($J_{1,2}$) and the Allylic Coupling Constants of the Exomethylene Protons

Compound	$J_{1,2}$ (Hz)	$^4J_{1,A}$	$^4J_{1,B}$
I	8.5	3.5	3.5
II	6.0	1.0	<1.0
IX	4.0		
X	4.0		
XI	8.0	3.5	3.5
XII	8.0		
	ave 6.4 ± 1.7 Hz	ave 2.7 ±	1.1 Hz

Table IX shows that the $_{ave}J_{1,2}$ for derivatives of eupalmerin acetate is compatible with the above rule for cis-fused γ-lactones, and is comparable to the cis-fused fourteen-membered γ-lactones eunicin and jeunicin ($_{ave}J_{1,2}$ = 8.7 ± 0.9 Hz; and $_{ave}J_{1,2}$ = 6.8 ± 1.1 Hz).



Recently, it was shown that the stereochemistry of α,β-unsaturated γ-lactones (type 1) could be correlated by the long-range allylic coupling constants of the exomethylene protons.[22] By analyzing a large number of naturally occurring sesquiterpene lactones of well established stereochemistry containing six-, seven-, and ten-membered rings, the following rule was formulated:

$$^4J(\text{cis}) \leq 3 \text{ Hz}; \text{ and } ^4J(\text{trans}) \geq 3 \text{ Hz}.$$

Results from this study also showed that as the size of the accompanying fused ring increased, so did the average value for the respective ^4J(cis) or ^4J(trans) allylic coupling constant.

Thus, the designation of the ring fusion according to the magnitude of the allylic coupling constant (^4J) was made more tenuous as the size of the fused ring increased. In particular, coupling constants of greater than 3 Hz were no longer applicable in the assignment of a trans-ring fusion.

$^4J > 3$ Hz: No Assignment of the Lactone Fusion

However, a coupling constant of less than 3 Hz was generally an indication of a cis-fused ring, even in the larger ring systems.

$^4J < 3$ Hz: cis-fused γ-lactone

The low value of $^4J_{1,A}$ and $^4J_{1,B}$ (Table IX) in II clearly indicated that the γ-lactone was cis-fused. Also, the values of $^4J_{1,A}$ found in I and XI although of no diagnostic consequence were compatible with the respective values obtained from the derivatives of the cis-fused fourteen-membered γ-lactones, jeunicin and eunicin. Thus, the long range allylic coupling constants of eupalmerin acetate and its derivatives also indicated that the fusion of the γ-lactone was cis.

Circular dichroism (CD) measurements of XII likewise supported the assignment of a cis-fused γ-lactone in I. It had been shown that diazomethane adducts of α,β-unsaturated lactones (type 1) in the terpene series were good derivatives for the assignment of stereochemistry to the lactone ring.[23] Molecular models of various fourteen-membered cis- and trans-fused γ-lactones were made for comparison. It was ob-

Figure 11
CD Curves of Eupalmerin Acetate I(a) and its Pyrazoline Derivative XII(b).

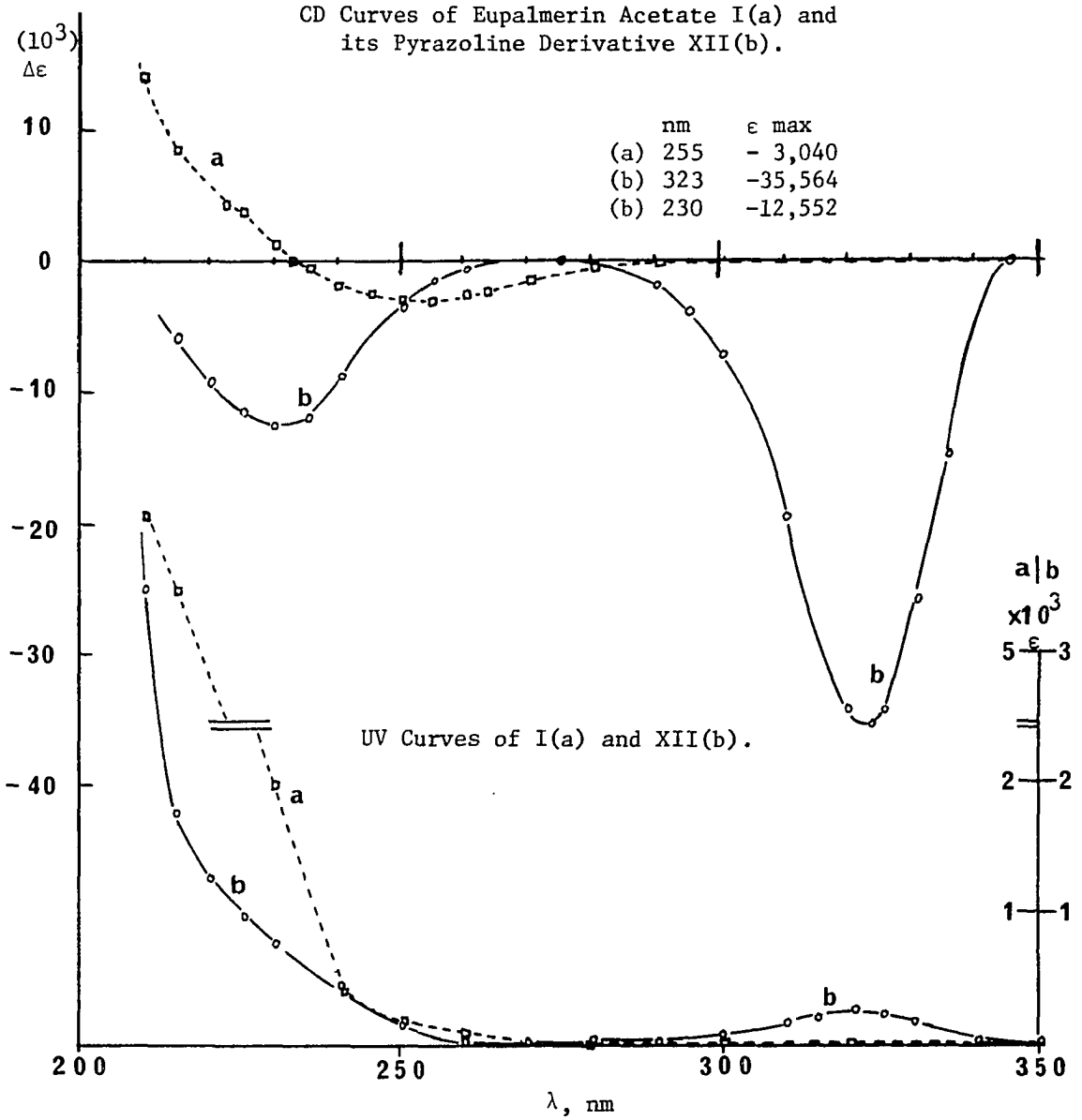


Figure 12
CD Curves of Eunicin (a) and
its Pyrazoline Derivative (b).

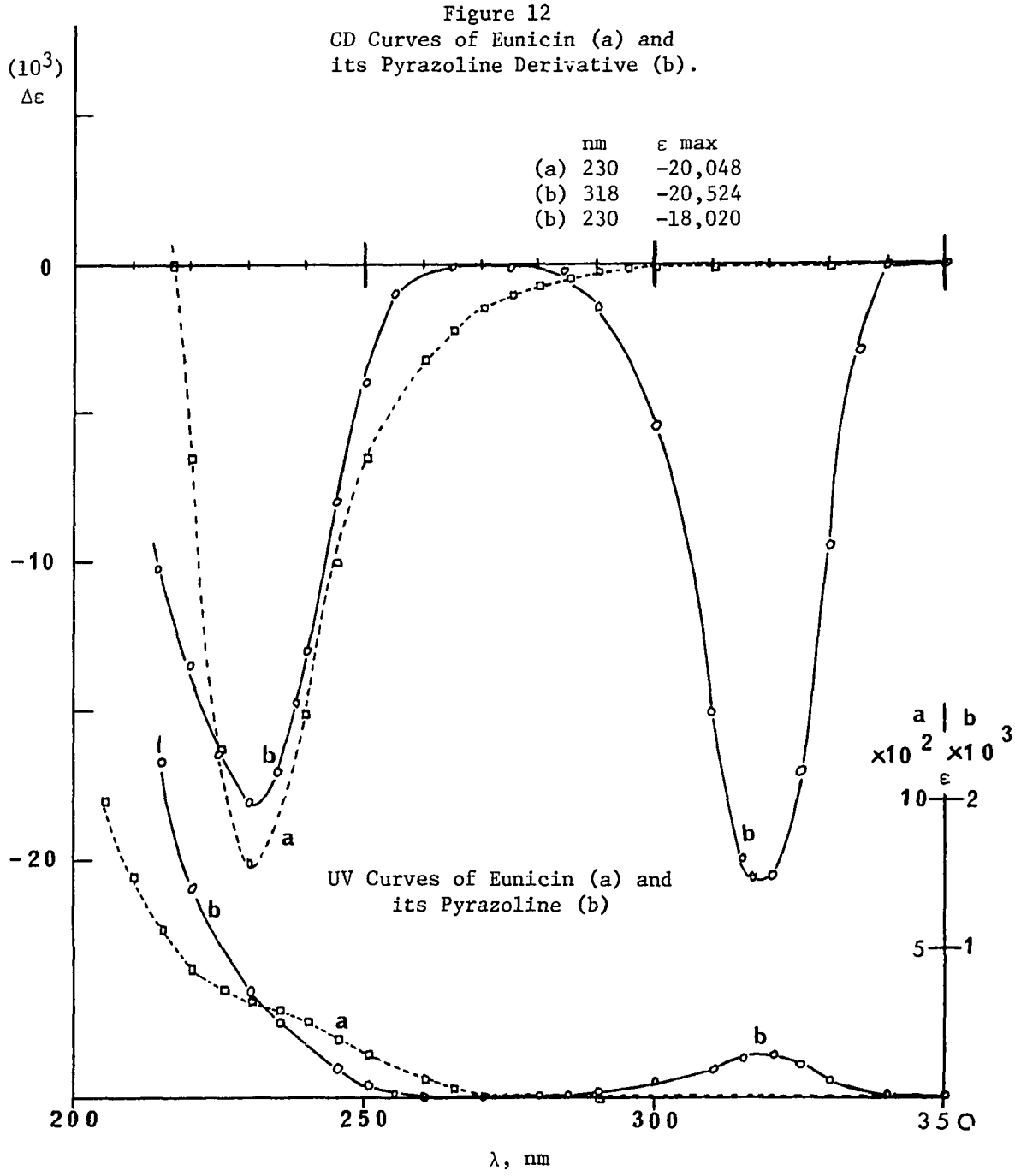
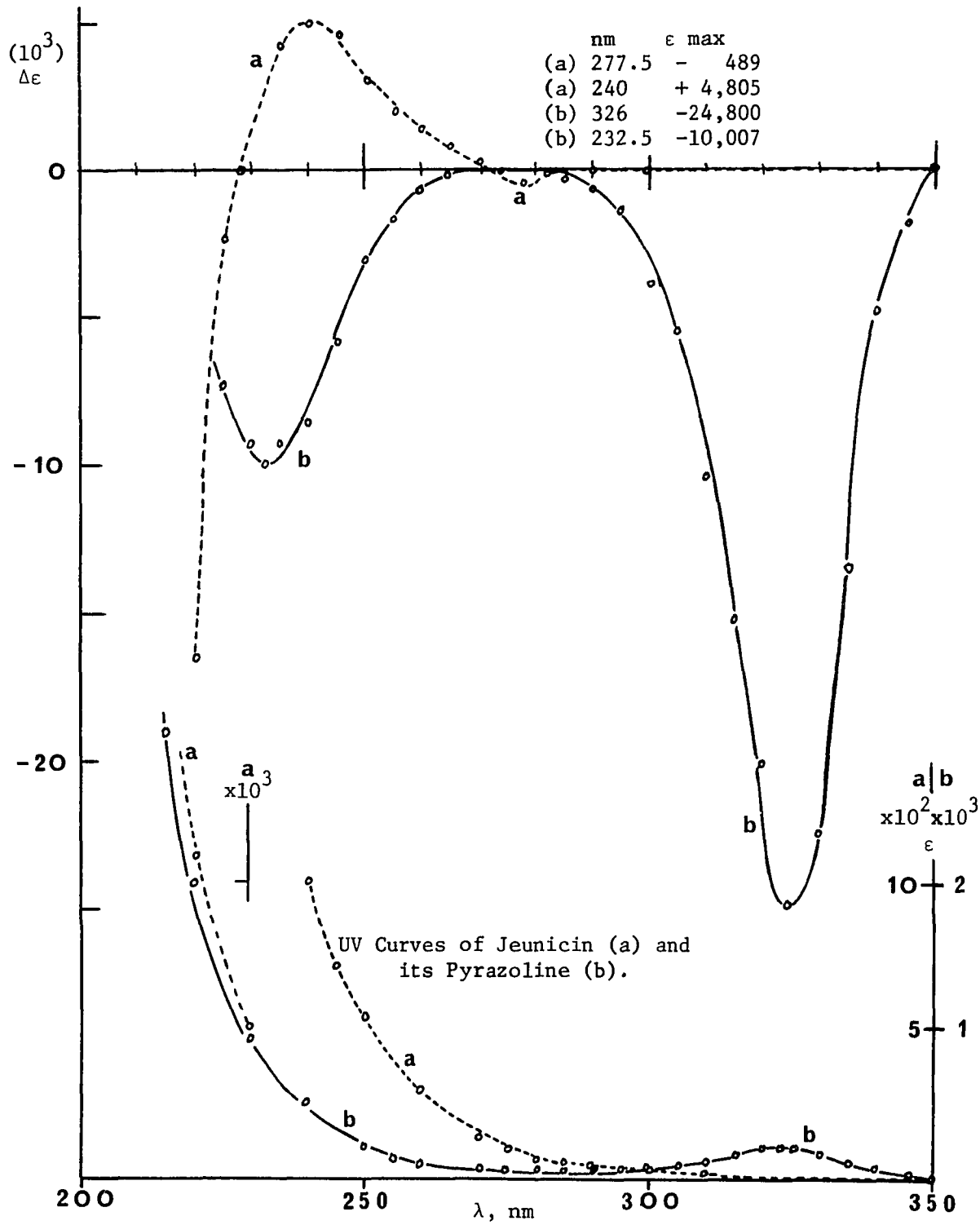
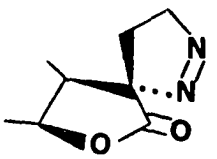


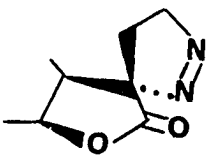
Figure 13
CD Curves of Jeunicin (a) and
its Pyrazoline Derivative (b).



served that for trans-fused γ-lactones, as a result of the trans fusion, the plane of the lactone effectively bisected the molecule, and therefore a small Cotton effect was predicted. However, cis-fused γ-lactones in this system were expected to exhibit large Cotton effects, by reason of the fact that the fourteen-membered ring was found almost exclusively beneath the plane of the lactone. As predicted, the CD curves of the pyrazolines of eunicin (figure 12) and jeunicin (figure 13) exhibited strongly negative Cotton effects in the 320 nm region of the spectrum. Since the ring fusion of these compounds was known by x-ray crystallography,[9,10] the CD curves were directly related to the absolute stereochemistry. Because the stereoselective reaction of γ-lactones (type 1) with diazomethane has been well documented, the CD curves merely confirmed that the attack of diazomethane had occurred from the less hindered plane of the lactone.

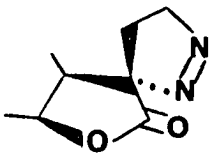


Pyrazoline of Eunicin
$\Delta\varepsilon = -20,524$
$\lambda_{max} = 318$ nm



Pyrazoline of Jeunicin
$\Delta\varepsilon = -24,800$
$\lambda_{max} = 326$ nm

The CD spectrum of XII (figure 11) also exhibited a strongly negative Cotton effect $\Delta\varepsilon = -35,564$. The magnitude and direction of the Cotton effect was comparable to that found for the pyrazolines of eunicin and jeunicin, and therefore, the stereochemistry about the γ-lactone of eupalmerin acetate was judged to be the same as that of eunicin and jeunicin, namely cis-fused.



Pyrazoline of Eupalmerin Acetate
$\Delta\varepsilon = -35,564$
$\lambda_{max} = 323$ nm

Knowledge of the absolute stereochemistry of the γ-lactone in eupalmerin acetate enabled the configuration of the two adjoining asymmetric centers, C-3 and C-4, to be established. This was accomplished by the use of Dreiding models and the data compiled in Table X from the derivatives of eupalmerin acetate.

TABLE X

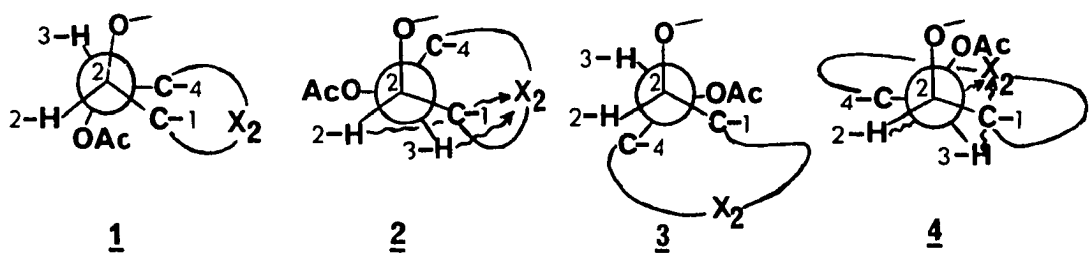
Chemical Shifts of the H-2, H-3, C-18, and Acetate Methyl Protons and the Coupling Constants $J_{2,3}$ and $J_{3,4}$ in the Derivatives of Eupalmerin Acetate

Compound	$J_{2,3}$(hz)	$J_{3,4}$(Hz)	H-2 (δ)	H-3 (δ)	C-18 (δ)	Acetate Methyl (δ)
I	0.0	9.5	4.76	4.91	0.85	1.90
II	9.5	0.0	4.54	5.06	0.93	2.12
IX	9.5	0.0	4.42	5.27	0.93	2.07
X	10.0	1.0	4.43	5.01	0.91	2.08
XI	0.0	8.5	5.18	5.57	0.88	1.89
XII	0.0	8.5	4.63	4.77	0.98	2.12

Two very pertinent statements concerning the data in Table X can be made. First, the protons H-2 and H-3 both showed unusual variation in their chemical shift (δ) compared to I; and second, the vicinal coupling constants, $J_{2,3}$ and $J_{3,4}$, showed an inverse relationship, being either very large or very small. This latter observation should permit the assignment of preferred conformations from an application of

the Karplus rule.

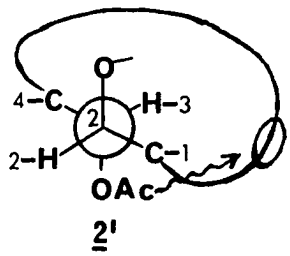
In the jeunicin-eunicin series, the $J_{2,3}$≈10 Hz indicated trans-anti vicinal coupling. Using this criterion for trans-anti conformations, it was noted that in compounds II, IX, and X the large $J_{2,3}$ was compatible with a trans-anti vicinal coupling. However, in compounds I, XI, and XII, the $J_{2,3}$=0.0 Hz indicated that the dihedral angle between H-2 and H-3 in this conformation was 90°. Dreiding molecular models were made of the various conformations to determine the stereochemistry of the molecule by application of the Karplus rule. Although molecular models may not necessarily reflect the actual geometry of the molecule, they do provide defined limits with which the molecule can be compared. Newman projections were made for molecular models of I (which were also assumed applicable to XI and XII) with the restriction of a 90° angle between the H-2 and H-3 protons.



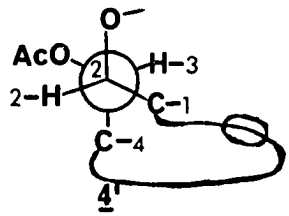
1 2 3 4

The fact that both the H-2 and H-3 protons were strongly deshielded in dibromoeupalmerin acetate (XI) indicated that they were situated so as to be similarly influenced by the ring substituents. Since H-3 was considerably more deshielded, compared to H-2, it necessarily must be nearer the sphere of influence of the bromine atoms. Two conformations, 2 and 4, could satisfy this requirement. However, upon consideration of the conformations 2' and 4' (180° H-2, H-3 dihedral angle),

knowing that the acetate methyl did not exhibit an appreciably different chemical shift upon bromination, but was slightly deshielded in the other derivatives, it was concluded that only 2, 2' conformations were compatible with all the observed data and therefore were most representative of the stereochemistry at C-3.

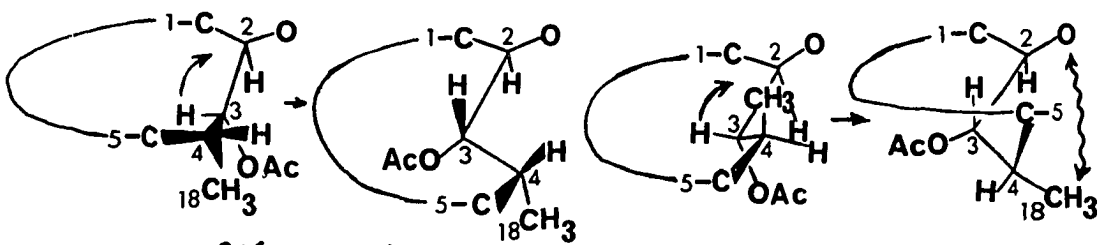


2'

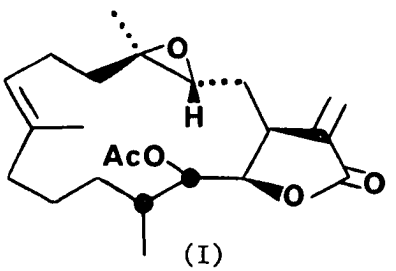


4'

Similarly, the stereochemistry at C-4 was determined. Knowing that in compounds II, IX, and X, the dihedral angle between H-3 and H-4 protons was approximately 90° (as observed by the 0.0-1.0 Hz coupling constants),[17b] again model conformations (2'/1' and 2'/2') were constructed. Since it was not expected during any of these reactions (for example, hydrogenation) that drastic molecular conformational change should occur, the additional restriction that any change of conformation would involve only minor alterations of the gross structure was invoked. Moreover, if it were assumed that the large $J_{3,4}$ indicated a trans-anti conformation, then only two sterically feasible models (2/1 and 2/2) were possible for their precursors.



Of these model pairs, only 2/1-2'/1' could meet all of the features found in Table X (in particular, the chemical shifts of the C-18 and acetate methyls). Thus, the structure of eupalmerin acetate (I) which was most compatible with the spectral data and integrated all the subtleties found in and expected for conformational changes upon derivatization is shown below.



(I)

The stereochemistry of eupalmerin acetate at C-1 through C-4 using the Cahn-Ingold-Prelog convention for designation of absolute configuration was the same as that found in eunicin and jeunicin, namely, 1(S), 2(R), 3(S), 4(R). In addition, the C-12 and C-13 positions whose configurations were designated as 12(R) and 13(R) were the chemical complement to the 12(R), 13(S) configuration in eunicin and the 12(S), 13(R) configuration in jeunicin. Since these diterpenoid lactones, eunicin, jeunicin, and eupalmerin acetate, were all isolated from gorgonians in the genus Eunicea, it was not unexpected that the stereochemistry of each molecule was interrelated with the others. In fact, the compatibility of the stereochemistry in eupalmerin acetate determined spectrally with that anticipated biosynthetically provided additional support for this structure. Moreover, the biosynthesis of eunicin, jeunicin, and even crassin acetate can be envisioned to occur through the intermediacy of eupalmerin acetate. Therefore, the marine

epoxy cembranolide, eupalmerin acetate, may be the "missing link" which interrelates the other marine diterpenoid lactones.

EXPERIMENTAL

All melting points are uncorrected. All solvents, except diethyl ether, were redistilled before use. Hexane and tetrahydrofuran were distilled from calcium hydride. Acetone was distilled from potassium permanganate and pyridine from barium oxide. Diazomethane was prepared from EXR-101 (N,N'-dinitroso-N,N'-dimethyl terephthalamid, from DuPont). Chromatographic supports used were Florisil (Floridin Co., 100-200 mesh), SilicAR CC-7 (Mallinckrodt, 100-200 mesh), and silica gel (Fisher-Grace, 60-200 mesh).

Gas chromatographic analyses were conducted on a Hewlett-Packard (F&M), model 402, using 4 ft x 8 mm glass columns containing either 1% OV-17, 1½% JXR, or 2% OV-1 on gas chromQ (100-120 mesh). Thin layer chromatography was performed either on 5 x 20cm glass plates or on microscope slides coated with Merck (Darmstadt silica gel H or silica gel $PF_{254+366}$. The plates were placed in an iodine vapor tank, sprayed with ceric acid, or placed under a uv lamp to effect visualization of the chromatogram.

Infrared absorption spectrum (ir) were recorded on a Beckman IR-8 or IR-18A spectrophotometer. Samples for ir spectra were run as thin films between salt discs, as a potassium bromide (KBr) pellets, or as solutions in chloroform or carbon tetrachloride. Nuclear magnetic resonance (nmr) spectra were taken on a Varian A-60 spectrophotometer

using tetramethylsilane (TMS) as an internal reference. Samples were run in varying concentrations in deuterochloroform, carbon tetrachloride, benzene or as the neat liquid. Chemical shifts were reported in δ-values (ppm from TMS), and are followed by the proton integration, the multiplicity of the signal, and the corresponding coupling constants. The multiplicities are noted by the symbols: s, singlet; d, doublet; dd, double doublet; t, triplet; ct, complex triplet; q, quartet; brd s, broad singlet; and m, multiplet. Coupling constants are reported in Hz. 100 MHz nmr and double resonance spectra were kindly provided by Dr. Norman S. Bhacca at Louisiana State University and Stanford University laboratories.

Mass spectra were obtained on a Hitachi-Perkin Elmer RMU-7, double focusing spectrometer. Elemental analyses were carried out by the Alfred Bernhardt Laboratories, Mulheim, Germany, and by Erick Meier of Stanford University Laboratories.

Isolation of Eupalmerin Acetate (I). Eupalmerin acetate was isolated from the hot hexane extract of Eunicea palmeri collected near the Ragged Keys, Florida. From 5.24 kilograms of the dried gorgonian, 259 g (4.94%) of lipid extract was obtained after forty-eight hours of continuous hot hexane extraction. After trituration of the lipid extractibles with hexane (2 liters) and storage in the cold (-20°C), a reddish crystalline solid was isolated. The remainder of the extract was then placed on dual 65 x 650 mm Florisil columns (750 g) and eluted at first with hexane (5 liters) then with benzene. The eluents from one column were used as the solvent for the second column. Five hundred milliliter fractions were taken; the solvent was removed on a rotary

evaporator. The material which was eluted in benzene (5 liters) was concentrated, then triturated with hexane, placed in the cold(-20°C), and whitish crystals of eupalmerin acetate formed. These crystals were collected by filtration on a sintered glass funnel and combined with those initially filtered from the total extract. The combined crude crystalline material was then placed on a 32 x 500 mm silicAR CC-7 column and chromatographed using benzene as the solvent. Two hundred and fifty milliliter fractions were taken, the solvent was removed on a rotary evaporator, and the eluted material crystallized upon trituration with hexane. Recrystallization several times from hexane-chloroform (4:1) gave 11.03 g of very pure eupalmerin acetate. mp 158.5-159.5°C. $[\alpha]_D^{25}$ + 8.00(c=2.0, chloroform).

Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57; O, 21.25. Found: C, 70.12; H, 8.47; O, 21.74.

The 70 ev mass spectrum displayed peaks at m/e 376 (molecular ion), 368, 334, 316, 298, 183 and 43 (base peak).

The ir spectrum (KBr) showed absorptions at 1775 cm^{-1}, split at 1770 cm^{-1} (γ-lactone), 1738 and 1232 cm^{-1} (acetate), and 1670 cm^{-1} (double bond).

In the 100 MHz nmr spectrum ($CDCl_3$), methyl signals were observed at δ 0.85, 3H, d, J=7 Hz; δ 1.33, 3H, s; δ 1.63, 3H, brd s; and δ 1.90, 3H, s. Olefinic protons appeared at δ 5.10, 1H, ct, J≈6.5 Hz; δ 5.31, 1H, d, J=3.5 Hz; and δ 6.07, 1H, d, J=3.5 Hz. Protons under oxygen appeared at δ 4.91, 1H, d, J=9.5 Hz (acetate); δ 4.76, 1H, d, J=8 Hz (lactone); and δ 2.94, 1H, dd, J=5.5, 9 Hz (epoxide proton). An allylic proton appeared at δ 3.15, 1H, m (typical in appearance to the

proton beta to a lactone oxygen and allylic to an exomethylenic group).

Preparation of the α-Ketol (II) from Eupalmerin Acetate (I). A sample of 3.8579 g (10.26 mmol) of eupalmerin acetate was added to 50 ml of acetone, cooled to -22.4°C (freezing point of CCl_4), and 2.5 ml of Jones reagent added over a 30 min period to the stirred solution. The reaction flask was allowed to warm to room temperature and 2 ml of Jones reagent added to compensate for oxidation of solvent. After a short time, 5 ml of isopropyl alcohol was added to the reaction mixture with cooling. Then, 100 ml of distilled water was added to the solution which was extracted three times with equal volumes of chloroform. The chloroform layer was washed first with saturated sodium chloride solution, then with distilled water, and dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator. However, not all of the starting material had reacted, and the mixture was resubmitted to the same reaction conditions stated above, whereupon, 3.78 g of a greenish material was isolated. This material was filtered through a 10 g Florisil column, and then chromatographed on a 50 g silicAR CC-7 column. The material eluted in the first liter of benzene was concentrated on a rotary evaporator and triturated with hexane. White needles crystallized from solution and were collected by filtration through a sintered glass funnel. Upon recrystallization from hexane-chloroform (40:1), 1.176 g (29.3%) of the α-ketol (II) was isolated. mp 147-148°C.

Anal. Calcd for $C_{22}H_{32}O_5$: C, 67.32; H, 8.22; O, 24.46. Found: C, 67.32; H, 8.05; O, 24.65 (by difference).

The 70 ev mass spectrum displayed peaks at m/e 392 (molecular ion),

374, 358 (metastable), 350, 349, 332, 320, 314, 304, 289, 286, 271, 261, 246, 238, 219, and 43 (base peak).

The ir spectrum (KBr) exhibited absorptions at 3540 (hydroxyl), 1775 (γ-lactone), 1748 (acetate), 1703 (ketone), 1662 (olefin), and 1238 cm^{-1} (acetate).

The 100 MHz nmr spectrum exhibited methyl absorptions at δ 0.93, 3H, d, J=6.5 Hz; δ 1.25, 3H, s; δ 1.57, 3H, brd s; and δ 2.12, 3H, s. Olefinic protons were found at δ 6.18, 1H, 3, J<1Hz; δ 5.66, 1H, d, J=1 Hz; and δ 4.96, 1H, m. Protons under oxygen appeared at δ 5.06, 1H, d, J=9.5 Hz (acetate); and δ 4.54, 1H, dd, J=6, 9.5 Hz (lactone). The protons adjacent to the ketone appeared at δ 2.83, 2H, d, J=6.5 Hz. A proton allylic to the exomethylene and beta to the lactone appeared at δ 3.79, 1H, q, J≈6 Hz. A proton exchangeable in heavy water was found at δ 4.05, 1H, brd s.

Preparation of the Cleavage Product (III) from the α-Ketol (II). A 1.111 g sample of the α-ketol (II) was dissolved in 50 ml of anhydrous tetrahydrofuran (THF). Thirty milliliters of approximately 1 M lithium aluminum hydride (LAH) in ether was added to this solution and then refluxed for two hours. The reaction was worked up by the addition of 10 ml of 10% sodium hydroxide, followed by 10 ml of distilled water. Such a voluminous precipitate formed that filtration was deemed impractical. The gel was finally dissolved in 10% hydrochloric acid and extracted three times with 100 ml portions of ether. The ether extract was back extracted with water, dried over anhydrous sodium sulfate, and concentrated on a rotary evaporator. It was determined later that the pentol which was formed was somewhat soluble in

water, especially in the volume required to solublize the aluminate gel. A very poor yield of the pentol was isolated, 383 mg. An alternate workup procedure, which was employed in subsequent reactions called for the addition of a 100-fold molar excess of water to hydrolyze the aluminate salts. Hi-flo celite was added to form a slurry which was filtered through a sintered glass funnel. In this alternate sequence, the pentol could be quantitatively recovered. The pentol was then added to 20 ml of an ethanol-water (1:1) solution and an aqueous solution of 505 mg of $NaIO_4$ was added. The reagents were stirred at room temperature for 6 hours. The $NaIO_3$ which precipitated during the reaction was filtered off. After the addition of water, the solution was extracted three times with ether. The ethereal solution was washed with barium hydroxide, water, and then dried over anhydrous sodium sulfate. A three component mixture, 314 mg, was chromatographed on a silicAR CC-7 (10 g) column using hexane-benzene (1:1) as the solvent. The first component eluted, 96 mg, was the cleavage product (III). No elemental analysis was obtained due to the apparent lability of the molecule toward oxidation.

The 70 ev mass spectrum displayed peaks at m/e 210 (molecular ion), 192, 182, 152, 134, 126, 124, 111, 109, 94, and 43 base peak).

The ir spectrum (film) showed absorptions at 2855, 2705, 1717 (νC=O), 1457, and 1358 cm^{-1}.

In the 60 MHz nmr spectrum ($CDCl_3$), methyl absorptions occurred at δ 1.10, 3H, d, J=7 Hz; 1.62, 3H, brd s; and δ 2.13, 3H, s. An olefinic proton appeared at δ 5.13, 1H, ct, J≈7 Hz; and an aldehyde proton occurred at δ 9.63, 1H, d, J=2 Hz.

Preparation of the Cleavage Product III from Eupalmerin Acetate (I). A 3.7761 g sample of eupalmerin acetate (10 mmol) was added to 125 ml of THF. Then, 50 ml of a 5.6% $HClO_4$ solution was added and allowed to react for 40 hr at room temperature. The mixture was concentrated on a rotary evaporator, removing as much THF as possible; then, 75 ml of water was added and extracted twice with equal volumes of chloroform and twice with equal volumes of dichloromethane. The solution was dried over anhydrous sodium sulfate, and the solvent removed on a rotary evaporator. When most of the solvent was removed, the residue frothed and bubbled.

The glassy diol (tlc showed that no starting material remained) was then added to 120 ml of dry THF, cooled to -78°C, and 10 ml of a 2.45 M lithium aluminum hydride solution (in ether) was added. A vigorous reaction took place. Another 10 ml of LAH solution was added and the reaction mixture was allowed to warm to room temperature. After 20 hr, 8 ml of water was added along with several scoops of celite. The slurry was then filtered through a sintered glass funnel and washed with 300 ml of dichloromethane and 50 ml of dry THF. The solution was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The ir spectrum showed incomplete reduction of the carbonyl groups, and the reaction was repeated. The material was dissolved in 50 ml of dry THF, cooled to -78°C, and 15 ml of 2.45 M LAH added. The reagents were then warmed to room temperature, and refluxed on a steam bath for 2 hr, whereupon, 5 ml of water was added along with celite to the reaction flask. The solution was filtered and washed with 25 ml of THF and 200 ml of dichloromethane. The solu-

tion was then concentrated on a rotary evaporator, and 3.0414 g of a viscous residue remained. The pentol showed no C=O absorption in the ir spectrum.

The pentol was added to 250 ml of THF, 25 ml of water, and 3.9951 g of sodium periodate, and allowed to react at 19-22°C for 67 hr. Sodium iodate precipitated during the course of the reaction; the reaction mixture was filtered, and washed with ether. Then, 75 ml of water was added to the filtrate and the aqueous phase was extracted four times with ether. The ethereal solution was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator, giving 2.58 g of an oily multicomponent mixture. The crude cleavage product was chromatographed on a 100 g, 23 x 360 mm silica gel column using benzene as the eluent. Two hundred milliliter fractions were taken. After 1200 ml of solvent had been eluted, the solvent was changed to 2% ethyl acetate in benzene. The fractions 9 through 13 contained the cleavage product (III). The solvent was removed and 367 mg of a clear pleasant smelling oil was obtained. The cleavage product isolated by this procedure gave spectra identical with that obtained from the α-ketol (II). The overall yield in this reaction sequence was 17.5%.

Oxidation of the Ketoaldehyde (III) to the Ketoacid (IV). A 198 mg sample of the ketoaldehyde (III) was dissolved in 25 ml of acetone and cooled to -22°C. Then, 0.26 ml of Jones reagent[11] was added dropwise while the solution was vigorously stirred. After the addition was complete, the reagents were allowed to stir for one minute. Then, the reaction was quenched with 1 ml of isopropyl alcohol, and 100 ml of water was added, and the resulting solution was extracted three

times with 50 ml portions of dichloromethane. The organic phase was backwashed with distilled water. Then, the organic phase was extracted twice with saturated sodium bicarbonate solution and once with water. The combined basic extracts were then back-extracted once with 50 ml of dichloromethane and then neutralized with 20% hydrochloric acid. The aqueous phase was extracted three times with 75 ml of chloroform, and the chloroform extract was dried over anhydrous sodium sulfate. Upon concentration on a rotary evaporator 168.3 mg of the oily ketoacid (IV) was isolated. No elemental analysis was performed.

The 70 ev mass spectrum displayed peaks at m/e 226 (molecular ion), 208, 193, 190, 168, 150, 135, 123, 111, 95, 81, 74, 68, 58, 55, and 43 (base peak).

The ir spectrum exhibited absorptions at 3600∿2400, 1721 (shoulder), 1702 (C=O), 1479, 1464, 1458, 1411, 1360, 1286, 1269, 1235, 1159, 940, 756, and 680 cm^{-1}.

The 60 MHz nmr ($CDCl_3$) showed three methyl absorptions: δ 1.18, 3H, d, J=7 Hz; δ 1.61, 3H, brd s; and δ 2.14, 3H, s. An olefinic proton was observed at δ 5.12, 1H, ct, J≈6 Hz. The acid proton appeared at δ 11.0, 1H, s.

Esterification of the Ketoacid (IV) to the Ketoester (V). A 168.3 mg sample of the ketoacid (IV) was dissolved in 10 ml of ether and 30 ml of an ethereal diazomethane solution (0.3 M) was added and allowed to stand for twenty minutes. Then the excess diazomethane and ether were removed on a rotary evaporator without heating. IV was quantitatively converted to the ketoester (V), a pleasant smelling colorless oil.

Anal. Calcd for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07; O, 19.97. Found:

C, 69.89; H, 10.04; O, 20.07 (by difference).

The 20 ev mass spectrum displayed peaks at m/e 240 (molecular ion), 222, 208, 190, 182, 165, 153, 152, 150, and 88 (base peak).

The ir spectrum exhibited absorptions at 1735, 1717, 1460, 1432, 1358, 1258, 1200, 1159, 1125, 1090, 1070, 1046, 987, 910, 870, 821, and 786 cm^{-1}.

The 60 MHz nmr ($CDCl_3$) showed four methyl absorptions: δ 1.14, 3H, d, J=7 Hz; δ 1.60, 3H, brd s; δ 2.13, 3H, s; and δ 3.68, 3H, s; and one olefinic proton at δ 5.11, 1H, ct, J=6 Hz.

Ozone Cleavage of the Ketoester (V). A: A sample of 296.4 mg of V was dissolved in 10 ml of dichloromethane and then 60 ml (excess) of a saturated ozone solution (approximately 0.04 M) was added while the solution was maintained at -78°C. The solution was then concentrated on a rotary evaporator without heating. After the solvent was removed, 25 ml of acetone was added, and 2.5 ml of Jones reagent was added while the stirred solution was maintained at 0°C. After five minutes, 1 ml of isopropyl alcohol was added to destroy any excess Jones reagent. The solution was again concentrated on a rotary evaporator removing all the acetone (and isopropyl alcohol). Then, 30 ml of water was added, and the mixture was extracted five times with equal volumes of dichloromethane. The aqueous phase from this extraction was submitted for continuous ether extraction (Part B). The dichloromethane phase was washed once with saturated sodium bicarbonate solution and once with distilled water. Then, the dichloromethane solution was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. A 210.5 mg sample of the neutral ozonolysis product was obtained. A tlc

of this product showed two components which were DNP-positive, indicative of a ketone or aldehyde. Upon chromatography (preparative tlc), 64.5 mg of the major component was isolated.

Anal. Calcd for $C_9H_{16}O_3$: C, 62.77; H, 9.36; O, 27.87. Found: C, 62.51; H, 9.38; O, 28.11 (by difference).

The gas chromatogram of VI at 90°C (on 3% JXR on Gas Chrom Z, 80-100 mesh, 4 ft x 8 mm glass column) produced a single peak at 5.55 min. An authentic sample of 2-methyl-6-oxo-heptanoic acid methyl ester had the same retention time.

The 70 ev mass spectrum displayed abundant ions at m/e 172, 141, 140, 115, 113, 107, 88, 87, 68, 59, 58, and 43 (base peak).

The ir spectrum (film) exhibited absorptions at 1732, 1716, 1463, 1435, 1362, 1255, 1199, 1160, 1126, 1082, 985, 845, 760, and 726 cm^{-1}.

The 60 MHz nmr spectrum ($CDCl_3$) showed three methyl absorptions: δ 1.16, 3H, d, J=7 Hz; δ 2.13, 3H, s; and δ 3.68, 3H, s. Two other absorptions were observed at δ 1.55, 4H, m; and δ 2.45, 3H, m.

The above spectra were identical to those produced by an authentic sample of 2-methyl-6-oxo-heptanoic acid methyl ester produced by the ozonolysis of 1,3-dimethyl cyclohexene (ChemSampCo), followed by oxidative (Jones) workup, and esterification.

B: After 40 hr of continuous ether extraction, the ether was dried over sodium sulfate and concentrated on a rotary evaporator. The sample apparently contained small amounts of water and after azeotropic distillation with anhydrous benzene and absolute alcohol, 71.8 mg of VII was obtained.

The ir spectrum exhibited broad absorptions at 3700∿2300 cm^{-1} and

1712 cm^{-1}. The spectrum was identical with that of an authentic sample of levulinic acid (Quaker Oats). Esterification with excess ethereal diazomethane (30 ml of 0.3 M), followed by filtration through a Florisil pipet column gave 69.8 mg of VIII. DNP mp = 141° Lit.[24] mp = 142° Mixture mp = 140°.

Anal. Calcd for $C_6H_{10}O_3$: C, 55.37; H, 7.74; O, 36.88. Found: C, 55.39; H, 7.76; O, 36.84 (by difference).

Gas chromatography showed that VIII was a single component having a retention time identical with an authentic sample of methyl levulinate.

The 70 ev mass spectrum displayed peaks at m/e 130 (molecular ion), 115, 99, 98, 88, 87, 71, 69, 59, 57, 55, and 43 (base peak).

The 60 MHz nmr spectrum ($CDCl_3$) showed two methyl absorptions: δ 2.18, 3H, s; and δ 3.67, 3H, s. Only one other absorption occurred in the spectrum; centered at δ 2.66, 4H, symmetrical eight-line signal (A_2B_2, of the type $X-CH_2-CH_2-Y$).

The above spectra were identical with that produced by an authentic sample of methyl levulinate.

Hydrogenation of Eupalmerin Acetate. A sample of 7.543 g of eupalmerin acetate (20.6 mmol) was dissolved in 200 ml of ethyl acetate and a small amount of benzene. To this solution 250.9 mg of 5% Pd/C catalyst was added, and the mixture was hydrogenated at atmospheric pressure for one hour. After hydrogen absorption had ceased, the solution was filtered through celite and a glass filter. Concentration on a rotary evaporator gave 7.58 g of hydrogenated product. The nmr of the crude residue showed that the exomethylene alpha to the lactone carbonyl had been quantitatively reduced. The trisubstituted double bond did not

appear to be reduced since the vinylic multiplet and the vinylic methyl signals were not diminished. TLC and gc showed that the hydrogenated product consisted principally of three components. The relative area of these components (determined by the width at half peak height times the height divided by the sum total of the areas of the three components) was 9.7, 16.1, and 74.2%, as determined in their order of volatility at 200°C on 2% OV-1 Gas Chrom Q, 100-120 mesh. However, none of these products could be made crystalline although after chromatography on silicAR CC-7, the major component by tlc and gc was apparently pure.

The ir spectrum of the major dihydroderivative exhibited absorptions at 1792 (γ-lactone), 1749, 1662, 1449, 1384, 1368, 1235, 1168, 1133, 1070, 1020, 980, 954, 931, and 855 cm^{-1}.

The 60 MHz nmr (CCl_4) showed five methyl absorptions: δ 0.93, 3H, d, J=6.5 Hz; δ 1.15, 3H, d, J=7 Hz; δ 1.21, 3H, s; δ 1.58, 3H, brd s; and δ 2.07, 3H, s. One olefinic proton appeared at δ 5.20, 1H, m; and two protons under oxygen appeared at δ 4.42, 1H, dd, J=4, 9.5 Hz; and δ 5.27, 1H, d, J=9.5 Hz.

Electrophilic Addition of Methanol to the Epoxide of Dihydroeupalmerin Acetate. A sample of 177 mg of the major dihydroderivative (IX) of eupalmerin acetate was added to 25 ml of dry methanol and a trace of p-toluenesulfonic acid. The material was refluxed on a steam bath for several hours and left four days at room temperature. The course of the reaction was monitored by tlc. When the reaction appeared complete, the solution was concentrated on a rotary evaporator and then filtered through Florisil. Upon elution in benzene and concentration, the first component eluted crystallized upon trituration with hexane.

Recrystallization from benzene-hexane (1:4) produced 50 mg of the pure derivative. mp 166-167°C.

Anal. Calcd for $C_{23}H_{38}O_6$: C, 67.29; H, 9.33; O, 23,38. Found: C, 67.19; H, 9.24; O, 23,57 (by difference).

The 70 ev mass spectrum displayed peaks at m/e 410 (molecular ion), 378, 350, 335, 318, 300, 290, and 43 (base peak).

The ir spectrum (KBr) showed absorptions: 3500, 1780, 1730, 1460, 1405, 1372, 1240, 1182, 1170, 1142, 1102, 1080, 1018, 992, 942, 867, and 838 cm^{-1}.

The 60 MHz nmr ($CDCl_3$) showed six methyl absorptions: δ 0.91, 3H, d, J=7 Hz; δ 1.02, 3H, s; δ 1.30, 3H, d, J=7 Hz; δ 1.55, 3H, d, J=1 Hz; δ 2.08, 3H, s; and δ 3.25, 3H, s. Protons under oxygen appeared at δ 3.73, 1H, dd, J=5, 9.5 Hz; δ 4.43, 1H, dd, J=4, 10 Hz; and δ 5.01, 1H, dd, J=1, 10 Hz. An olefinic proton appeared at δ 5.80, 1H, ct, J≈7 Hz.

Bromination of Eupalmerin Acetate. A 376.8 mg sample (1 mmol) of eupalmerin acetate was dissolved in 50 ml of CCl_4 and vigorously stirred at 0°C while 1 meq of bromine (1.6 ml of a 10% solution in CCl_4) was added dropwise to the solution. After the addition was complete (five minutes), a slightly orangish color persisted. The material was concentrated on a rotary evaporator and filtered through a 5 g (12 mm x 130 mm) Florisil column using benzene as the solvent. The first several fractions were combined, concentrated, and then triturated with hexane. A white crystalline compound was isolated. mp = 184-185.5°C.

Anal. Calcd for $C_{22}H_{32}Br_2O_5$: C, 49.27; H, 6.01; Br, 29.80; O, 14.92. Found: C, 49.51; H, 6.00; Br, 29.76; O, 14.73 (by difference).

The 70 ev mass spectrum displayed peaks containing two bromine

atoms (identified according to their isotopic ratios) with the center peak at m/e 536 (molecular ion), 494, 476, 466, 414, and 396. Peaks containing one bromine atom were displayed at m/e 454, 456; 455, 457; 413, 415; and 395, 397. Peaks which were apparently composed of only CHO were displayed at m/e 376, 375, 374, 315, and 43 (base peak).

The ir spectrum (KBr) exhibited absorptions at 3030, 1777, 1750, 1462, 1428, 1404, 1381, 1369, 1310, 1263, 1225, 1210, 1105, 1088, 1069, 1050, 1023, 952, 932, 909, 804, 756, and 700 cm^{-1}.

The 60 MHz nmr spectrum ($CDCl_3$) showed four methyl absorptions: δ 0.88, 3H, d, J=7 Hz; δ 1.75, 3H, s; δ 1.80, 3H, s; and δ 1.89, 3H, s. In addition, there were two olefinic protons: δ 5.39, 1H, d, J=3.5 Hz; and δ 6.14, 1H, d, J=3,5 Hz; and two protons under oxygen: δ 5.18, 1H, d, J=8 Hz; and δ 5.57, 1H, d, J=8.5 Hz. Lowfield proton absorptions were also observed at δ 3.30, 1H, m (proton beta to the lactone and allylic to the exomethylene); and at δ 3.76, 2H, m (resolved in benzene as a pair of double doublets).

The 60 MHz spectrum (benzene) showed four methyl absorptions: δ 0.86, 3H, d, J=7 Hz; δ 1.50, 3H, s; δ 1.525, 3H, s; and δ 1.53, 3H, s Olefinic protons occurred at δ 4.91, 1H, d, J=3.5 Hz; and δ 6.03, 3H, d, J=3.5 Hz. Protons under oxygen occurred at δ 4.58, 1H, J=8 Hz; and δ 5.47, 1H, d, J=8.5 Hz. Three other discernable lowfield absorptions occurred at δ 2.87, 1H, m (proton beta to the lactone carbonyl and allylic to the exomethylene); δ 3.85, 1H, dd, J=3.5, 10 Hz; and δ 3.76, 1H, dd, J=2, 7 Hz.

Diazomethane Adduct, Pyrazoline (XII), of Eupalmerin Acetate. A sample of 192.2 mg of eupalmerin acetate was dissolved in a small amount

of ether and added to 40 ml of an ethereal diazomethane solution (approximately 0.3 M). After seven days at -20°C, the solution was concentrated on a rotary evaporator without heating. After the solvent was removed, 5 ml of ether and 1 ml of dichloromethane were added to dissolve the residue. Then the solution was triturated with hot hexane. The pyrazoline crystallized as fine needles upon standing. A 167.9 mg sample was recrystallized by the above procedure (dissolution in ether followed by trituration with hot hexane). mp = 125.5-126°C (decomposition, with evolution of a gas).

Anal. Calcd for $C_{23}H_{34}N_2O_5$: C, 66.01; H, 8.19; N, 6.69; O, 19.11. Found: C, 66.16; H, 8.18; N, 6.86; O, 18.80 (by difference).

The 70 ev mass spectrum displayed peaks at m/e 418 (molecular ion), 390, 372, 358, 348, 330, 315, 312, and 43 (base peak).

The uv spectrum (95% ethanol) of the pyrazoline, XII, exhibited an absorption, λ_{max} 321 nm (ε 242).

The 60 MHz nmr spectrum showed four methyl absorptions: δ 0.98, 3H, d, J=7 Hz; δ 1.22, 3H, s; δ 1.63, 3H, brd s; and δ 2.12, 3H, s. One olefinic proton appeared at δ 5.18, 1H, m. Two protons under oxygen appeared at δ 4.63, 1H, d, J=8 Hz; and δ 4.77, 1H, d, J=8.5 Hz. In addition, there were absorptions centered at δ 5.23, 2H, m; and 2.97, 4H, m.

The ir spectrum exhibited absorptions: 1780, 1736, 1551 (N=N), 1462, 1450, 1432, 1381, 1370, 1238, 1200, 1156, 1014, 960, 885, 843, 813, 790, 734, and 670 cm^{-1}.

The Ketoester (VIII) from Eupalmerin Acetate. A sample of 382.9 mg (1.0125 mmol) of eupalmerin acetate was dissolved in 35 ml of dichloro-

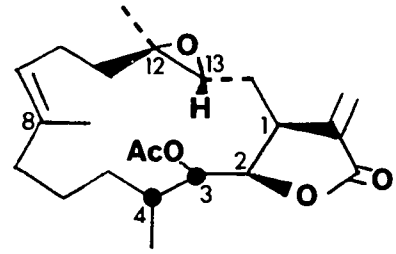
methane, stirred at 0°C while 240.1 mg of m-chloroperbenzoic acid (85%, 1.185 mmol) dissolved in 25 ml of dichloromethane was added dropwise to the solution of I. After the addition was complete, the reagents were allowed to warm to room temperature and stirred for an additional hour. The mixture was transferred to a separatory funnel and extracted with 50 ml of a 6 mM solution of sodium sulfite (0.3 mmole) to destroy excess peroxide. The organic phase was extracted once with 5% sodium bicarbonate and then washed with water. After drying over anhydrous sodium sulfate and concentration on a rotary evaporator, a two component (tlc) epoxidation product was isolated. Without further purification, the epoxidation product was dissolved in 25 ml of THF; 5 ml of a 5.6% perchloric acid solution was added and allowed to stir overnight. Then, the solution was concentrated on a rotary evaporator to remove the THF; 50 ml of water was added; and the material extracted four times with equal volumes of chloroform. A yellowish glass was isolated. The glass was dissolved in THF, 478.5 mg of metaperiodic acid was added, and allowed to stand for several days at room temperature. The precipitated iodic acid from the reaction mixture was filtered, and the THF was removed on a rotary evaporator. The residue was then dissolved in 10 ml of acetone and 1.5 ml of Jones reagent was added. After five minutes, the acetone was removed, and 20 ml of water was added. The solution was neutralized with sodium carbonate ($\sim$1.1 g) and extracted five times with equal volumes of chloroform. The water portion was then continuously ether extracted for 24 hr. After the ethereal extract was dried over sodium sulfate, a small amount of an oily substance was isolated. The ir spectrum compared favorably with

that of an authentic sample of levulinic acid. Esterification with 10 ml of ethereal diazomethane gave 4.5 mg of VIII upon concentration. VIII at 110°C (1.6% OV-17, on Gas Chrom Q, 100-120 mesh; 5 ft x 1/8 in stainless steel column) gave an identical retention time identical with that of an authentic sample of methyl levulinate (26 sec relative to the solvent ether). The small amount of sample did not permit further characterization.

Attempted Acetate Pyrolysis of Eupalmerin Acetate. A sample of 376 mg (1 mmol) of eupalmerin acetate was dissolved in a small amount of benzene and placed in a separatory funnel. The top of a 25 in x 18 mm glass column, filled with glass helices and maintained at 480°C, was fitted with an adaptor which allowed nitrogen (2 psi) to flow through the column while the solution of eupalmerin acetate was slowly added at the top of the column. Collection of the sample from the bottom of the column was made difficult due to aerosoling of the product. However, the product was identical to the starting material, aside from slight discoloration.

SUMMARY

The structure of eupalmerin acetate ($C_{22}H_{32}O_5$), a new epoxy cembranolide, isolated from the Caribbean gorgonian Eunicea palmeri Bayer has been determined from its spectral characteristics and those of its transformation products.



The absolute stereochemistry postulated for eupalmerin acetate in (I) is based upon normal trans openings of the epoxide ring, and is also consistent with the relative configurations of positions 1, 2, 3, and 4 deduced from nmr and CD data. The trans arrangement of the isolated carbon-carbon double bond is based upon analogy with the other cembranolides.

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II. EUNICEA PALMERI BAYER: AN AMBIVALENT SPECIES

INTRODUCTION

The study of octocorals, their biology and chemistry, has been undertaken by several laboratories. The organic marine chemistry laboratory at the University of Oklahoma, headed by Professor A. J. Weinheimer, has concentrated its investigations mainly within the order GORGONACEA (Holaxonia) and the family PLEXAURIDAE. Within this family, all of the genera (Plexaura, Pseudoplexaura, Eunicea, Muricea, Muriceopsis, and Plexaurella) endemic to the West Indies have been studied chemically to various extents. It has been this author's privilege to have worked with the species, Eunicea palmeri Bayer. Professor Frederick Bayer described this species as "colonies with exceptionally soft and flexible, long, and slender branches 3-4 mm in diameter and up to 35 cm in length. Small colonies may be branched in one plane, in an irregular candelabrum form with rather few branches; larger colonies tend to become quite bushy. Calyces (wart-like projecting, lower part of the body of the polyp) are developed in some specimens, chiefly near the branch tips, as a low, shelf-like lip beneath each aperture, but in other specimens they are not present at all. The anthocodiae (upper, tentacular part of the polyp) are very weakly spiculated (calcareous skeletal elements of the mesogloea), having only a few tiny, flat rods

crosswise in the tentacles. The axial sheath contains deep reddish purple spindles, some of them slender and acute, sculptured with simple processes, others stout, not so sharp, and covered with complex tubercles; toward the base of the colonies, they may increase in size, and some coarse, oval bodies may occasionally be found. The middle layer of rind has slender, purple spindles usually about 0.8 mm long but reaching 1.0 mm in some specimens. The outer layer of rind contains purple spindles with strong thorns on one side, and colorless torches with sharply laciniate heads; toward the base of the colonies many of the clubs may be very coarse and stout, with short, tuberculate handles and elaborately foliate and laciniate heads. In some colonies, these exceptionally ornate clubs are very abundant and may be found even in the terminal branches. The color of fresh and alcoholic specimens is purplish gray, purplish brown, or grayish brown; the deep purple spindles may be seen at the surface among the colorless torches. Dry colonies are brown; the coenenchyme becomes so friable that the specimens are easily damaged. Ecologically, E. palmeri is abundant in 3-6 ft of water on the seaward shore of Soldier and Ragged Keys, where it grows together with Pseudopterogorgia acerosa, P. americana, Plexaurella dichotoma, and others."[1] It may be added that dry colonies of this species can also be light brown or purplish brown.

The primary emphasis while working with this species was the elucidation of the structure of a new diterpenoid lactone, eupalmerin acetate. The numerous collection trips for additional specimens, moreover, provided an excellent opportunity to study the reef fauna and, to some extent, reef ecology. It was discovered in the summer of 1968

after a collection trip to the Florida Keys that a collected specimen of E. palmeri did not afford the expected diterpenoid lactone upon chemical analysis. Instead, the diterpenoid lactone, eunicin (initially isolated from E. mammosa Lamouroux) was isolated. This unexpected phenomenon was at first confusing and frustrating. Therefore, it was decided that a chemical map of this species** might in some way relate to the zoogeography or possibly to the biochemistry of this species. In addition this laboratory has produced a tremendous amount of data on the genus Eunicea. It should be pointed out (1) that the species within this genus have shown remarkable chemical variations in other than the diterpenoid lactones; and (2) that the diterpenoid lactones which were isolated from Euniceas have an interrelated stereochemistry, (3) have shown activities against cancer cells, and (4) have proven to be toxic to most organisms.

** The author is indebted to the assistance received from Professor Leon S. Ciereszko who collected specimens in the West Indies while on sabbatical leave.

RESULTS AND DISCUSSION

Part One

Thin layer chromatography (tlc) seemed best suited to demonstrate chemical taxonomical relationships within the species. Since the diterpenoid lactones, eupalmerin acetate (I) and eunicin (II), are readily identified by their R_{Dye} values and characteristic stain with ceric acid, these compounds would be excellent labels in mapping the gorgonian. Specimens identified by this laboratory and by Professor Bayer (now with the Smithsonian Institution), as E. palmeri were collected from the Ragged Keys, Florida, Molasses Key, Florida, and Spanish Harbor, Florida. The chromatographic pattern of the specimens collected near the Ragged Keys, including Soldier's Key, all demonstrated the presence of eupalmerin acetate as the only diterpenoid lactone. Specimens collected further into the Florida Keys (Molasses Key and Spanish Harbor) exhibited only eunicin. At first, it was thought that inadvertently E. mammosa Lamouroux was collected instead of E. palmeri. However, Professor Bayer, at that time, confirmed out initial designation of the gorgonian, as E. palmeri. This led us to the conclusion that possibly chemical taxonomy of this species may be invalid. Therefore, it was necessary to collect other specimens and determine if the production of these diterpenoid lactones was merely a function of

geography and not indigenous to the species. Although the subgenus Eunicea is well represented (six species) in the West Indies, the species E. palmeri, had not been collected except from the Florida Keys. Several small Euniceas were collected from various marine laboratories in the West Indies, with the hope that several of the specimens would be the gorgonian, E. palmeri.

Specimens from different locations in the West Indies--Jamaica, Cornelissbaai, Curacao, and Puerto Rico--were characterized for diterpenoid lactone content. Only one collection site provided a specimen which could be positively identified as E. palmeri. That specimen was from Puerto Rico. A view of the thin layer chromatogram (tlc) in 25% ethyl acetate--benzene (1:3) demonstrated that the upper Florida Keys specimen and the Puerto Rican specimen contained the diterpenoid lactone, eupalmerin acetate, R_{Dye}=0.67, while the lower Florida Keys specimen contained only eunicin, R_{Dye}=0.37. The tlc's of these species are illustrated below.

TABLE XI

Thin Layer Chromatograms of Eunicea Palmeri
From Puerto Rico, Lower Florida Keys, and Upper Florida Keys

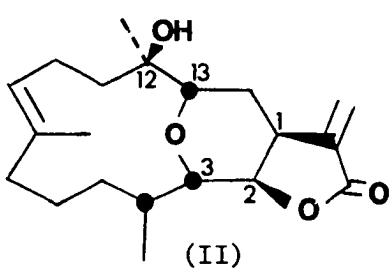
Puerto Rico		Lower Florida Keys		Upper Florida Keys	
III	IV	I	II	V	VI
118	119	122	120	117	117
103	103				
97	94	94	97	97	97
87	87	86	86	88	88
76	80	77	77	78	77
67	67			67	67
60	60			59	60
	50				
44		45	44	44	44
		37	36		
	33			32	32
14	14	14	14	14	14

R_{Dye}=100 Dye, Sudan Yellow Solvent front=17 cm

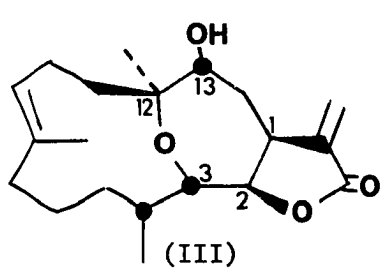
From Table I, one can definitely point to the fact (1) that the species E. palmeri is known outside the immediate Florida coastal region, and (2) that the diterpenoid lactone found in this species can be either eupalmerin acetate or eunicin, and (3) that specimens collected from any one geographic location produced only one of these diterpenoid lactones, and (4) that simple chemical taxonomical identification cannot be made with certainty with other members of the subgenus Eunicea. Professor Bayer on one occasion mentioned the possibility that E. palmeri was a morphological variant of E. mammosa. Indeed, the apparent similarities of these two species would tend to support this contention.

Part Two

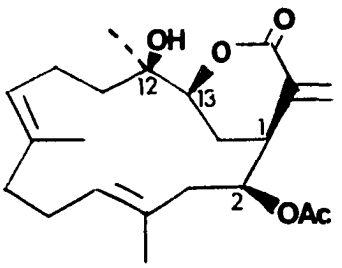
It was pointed out previously that the Oklahoma University laboratories have so far isolated four diterpenoid lactones,[2,3,4,5,6,7] and that x-ray crystallographic studies[8,9,10] have shown that the absolute stereochemistry of three of these molecules can be interrelated. The absolute stereochemistry of eunicin (II) and crassin acetate (IV) are identical at all common centers of asymmetry, while jeunicin (III) differs only by a simple inversion about two of these centers.



(II)

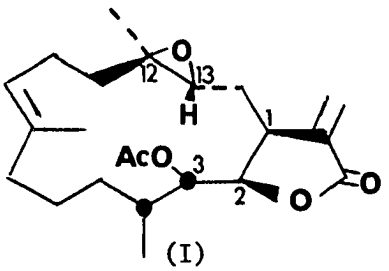


(III)



(IV)

It can readily be seen from an inspection of the proposed structure of eupalmerin acetate, shown below,



(I)

that through simple enzymatic reactions, both jeunicin and eunicin could be formed with the appropriate, assigned stereochemistry. These two oxa-bridged cembranolides, II and III, may be considered to be derived from (I) via transannular displacement by the C-3 oxygen function of I upon either C-12 (jeunicin) or C-13 (eunicin) of the epoxide function. Thus, the fourth member of this sequence might act as a substrate or intermediate in the formation of the other diterpenoid lactones. This possibility cannot be discounted although obviously much more work must be done before such a claim can be given credence. However, in support of this hypothesis, it can be pointed out that a specimen of E. mammosa collected from Jamaica contained both diterpenoid lactones, eunicin and jeunicin.[11] The reason why a single specimen

produces both diterpenoid lactones is unknown, but that both were found in the same colony made reasonable the availability of a common intermediate, i.e., possibly eupalmerin acetate. The chemical reactivity of this compound likewise would suffice for its potentiality as an intermediate in the formation of the other diterpenoid lactones.

Another point which can be emphasized is the apparent relationship between E. palmeri and E. mammosa or for that matter, any species in the subgenus Eunicea, as well as the relationship between the genera Eunicea and Pseudoplexaura.[2] The University of Oklahoma laboratories have studied the sesquiterpene, sterol, and diterpenoid lactone content of E. palmeri and E. mammosa, as well as the Pseudoplexauras (Ps. porosa, Ps. wagenaari, and Ps. flagellosa). The similarities between these genera are outstanding. For example. E. palmeri (Ragged Keys) and Ps. porosa (Bache Shoal) have as common sesquiterpenes, α-muurolene and β-copaene. While E. palmeri (lower Keys) contained several sesquiterpenes common to E. mammosa (upper Keys or Bimini), β-elemene and α-muurolene and germacrene-A, although the relative percentages of each component differed in each location.

Professor David Anderson at the University of Miami marine biological laboratory has found that the diterpenoid lactone of Ps. porosa seemed to be made exclusively by the symbiotic zooxanthellae, and that the sesquiterpenes are produced primarily by the host gorgonian.[12] The logical extension of his work would be (1) that the gorgonian produced sesquiterpenes (for what reason, it is not yet clear) and that the sesquiterpenes for any given species should very nearly establish a chemical fingerprint for that species; (2) that the sesquiterpene

content of any species is dependent to a large degree on its environment (i.e., temperature, salinity, illumination, depth of water, current, and symbiotic zooxanthellae); (3) that the diterpenoid lactones which are produced must come from the zooxanthellae, which are different to the extent that each is capable of producing a highly toxic compound which differ from one another by apparent recombinations of the chemical functionality within the molecule; and (4) that the diterpenoid lactones so far isolated arise from only two genera, Eunicea and Pseudoplexaura, and thereby, the zooxanthellae from these genera may have in the biosynthesis of the diterpenoid lactones a common substrate. Whether the production of the diterpenoid lactones by the zooxanthellae is the result of different species of zooxanthellae which are capable of producing the diterpenoid lactones, of environmental differences due to variations in geography which are indirectly causing the zooxanthellae to produce the diterpenoid lactones, or of some other salient feature is unknown to date. However, the fact that within the genus, Eunicea, three different diterpenoid lactones are produced, suggests the possibility that the zooxanthellae themselves are distinct, having synthesized the diterpenoid lactones from a common intermediate in which the algae have produced by independent enzymatic routes the different diterpenoid lactones. Again much more work must be done before such propositions are verified.

Part Three

This laboratory as a routine matter has sent samples to be assayed for biological activity. As a result, a wealth of data has been accum-

ulated. These diterpene lactones gave active Walker KB tests.[13] The KB tests received from Dr. Sigel are found in Table XII.

TABLE XII

Diterpenoid Lactones: Walker KB Test

Compound	ED_{50} (micrograms/ml) in DMF
Eupalmerin Acetate	5.0
Crassin Acetate	4.6-4.7
Jeunicin	4.8
Eunicin	4.8

These compounds also have a high degree of toxicity. Dr. P. Kaul and Mr. K. Likes[14] at the University of Oklahoma (Pharmacy School) have studied the effects of crassin acetate on the respiratory system of the rat and have shown that this compound is highly toxic. Crassin acetate produced a LD_{50} of 160 mg/kg for the rat. Their work suggested that the death of the animal was due to a respiratory failure with the locus of action occurring within the central nervous system. Likewise, Dr. Perkins (Zoology) has shown that eunicin, jeunicin, and crassin acetate are also toxic, killing all aquatic life (snails, fish) to which it was exposed. In particular, Dr. Perkins found that these compounds possessed remarkable antiprotozoal activity.[15] Professor Ciereszko had previously shown that these compounds were also antimicrobials.[16,17]

Thus, the relative toxicity and biological activity of these diterpenoid lactones has been well documented. The toxic compounds and the quantities in which they are produced (0.05-1.5% of the dry weight of the animal) lead one to conclude that these secondary metabolites may be part of the animal's (i.e., zooxanthellae) defense mechanism and

ensure its existence in a most inhospitable sea.

Thus, the gorgonian Eunicea palmeri, a simple colonial animal, is in reality an ambivalent species. On one hand, it is a morphologically uniform species with a well established taxonomy; yet on the other hand, it has an unpredictable biochemistry, producing qualitatively the same sesquiterpenes and sterols but different diterpenoid lactones. This ambivalence in morphology and biochemistry only point out how little we know of this simple reef-dweller.

EXPERIMENTAL

All solvents were redistilled before use. Column chromatography support was Florisil (Floridin Co., 100-200 mesh). Thin layer chromatography was performed on 5 x 20 cm or 20 x 20 cm glass plates coated with 0.2 mm of Merck (Darmstadt) silica gel H. The plates were placed in an iodine tank for initial visualization and a permanent chromatogram was developed with ceric acid spray (1% ceric sulfate in 35% sulfuric acid) heated to 100-105°C for twenty minutes.

Gas chromatographic analyses were performed on a Hewlett-Packard (F & M) model 402, using 4 ft x 8 mm glass columns containing 20% carbowax 20M on Gas Chrom Z support (80-100 mesh). Carrier gas (helium) was maintained at 100 ml per minute. Flash and detector heaters were maintained at 233 and 251°C respectively, while the oven was maintained at 131°C.

Comparison of the TLC's of E. palmeri Collected from Different Locations in the West Indies. A small amount of the dried gorgonian (several tips, approximately 6 cm or 300 mg) was placed in a 1 dram vial and 1 ml of ethyl acetate was added. After five minutes, the specimen furnished sufficient quantities of extractibles to give representable chromatograms. Samples from Puerto Rico, collected in 1969 and 1970 gave chromatograms identical with samples collected from

the Ragged Keys, Florida, in 1966, 1969, and 1970. Samples from the lower Florida Keys showed significant color variations among the specimens collected; however, the chromatograms were identical regardless of the color of the specimen. Standards were prepared of eunicin, jeunicin, and eupalmerin acetate by dissolution in chloroform. The reference dye used was Sudan Yellow (1-phenyl-azo-2-naphthol). The spots in the chromatogram indicative of the diterpenoid lactones turned yellowish-tan after spraying with ceric acid and warming in an oven at 105°C for a few minutes. Eventually the entire chromatogram charred, displaying even minor components. Sterols likewise were easily visualized by the color change to a reddish purple on spraying with ceric acid. The co-addition of a small quantity of standard to alternate tlc applications ensured competent evaluation of the chromatogram in terms of diterpenoid lactone content and correlation of the remaining portions of the chromatogram. The observed R_{Dye}'s of two samples collected at Spanish Harbor, Florida are shown below. The first chromatogram was obtained from a brown specimen, and the second from a purple specimen.

The most intense spots of the chromatogram are underlined.

I) 1.22, 0.94, 0.86, 0.77, 0.63, 0.45, 0.37, 0.14.

II) 1.22, 0.97, 0.86, 0.77, 0.62, 0.44, 0.36, 0.14.

Two samples were collected at Puerto Rico (both brown).

III) 1.18, 1.03, 0.97, 0.87, 0.76, 0.67, 0.60, 0.44, 0.14.

IV) 1.19, 1.03, 0.94, 0.87, 0.80, 0.67, 0.60, 0.50, 0.33, 0.14.

Two samples were collected from the Ragged Keys, Florida (brown).

V) 1.17, 0.97, 0.88, 0.78, 0.67, 0.59, 0.44, 0.32, 0.14.

VI) 1.17, 0.97, 0.88, 0.77, 0.67, 0.60, 0.44, 0.32, 0.14.

The spot R_D=0.67, compared to the standard, was identified as eupalmerin acetate. The spot R_D=0.37, compared to the standard, was identified as eunicin.

Comparison of the Sesquiterpene Hydrocarbons of Eunicea palmeri (Spanish Harbor). A cold hexane wash (three volumes) of the dried gorgonian gave a lipid fraction (4.04% by weight of the dry animal). A portion of this extract was filtered three times through Florisil columns, eluted with hexane each time. A gas chromatogram (gc) of hexane elutents on a 20% carbowax column (131°) showed nine components. The relative retention time (minutes) and the relative area of each component is shown below.

Component	Retention Time	Percentage of the Total SQHC	After Heating
1	4.2	0.4%	0.6%
2	4.75	0.8%	0.7%
3	6.2	13.3%	22.3%
4	8.35	2.7%	2.1%
5	8.7	11.6%	6.6%
6	11.2	55.5%	62.7%
7	13.3	6.5%	2.4%
8	15.4	9.0%	3.1%
9	17.6	0.7%	-

The column of percentages at the far right represents the hydrocarbon fraction after it was heated at 128°C for three hours and allowed to stand for 15 hr at 25°C. By using a reference sample supplied by Dr. William Youngblood and Dr. Tommy Karns, the components 3, 6, and 7 were tentatively identified as (+)-β-elemene, (+)-α-muurolene, and germacrene-A.

SUMMARY

Studies of Eunicea palmeri Bayer indicate that the chemistry of this species is more complex than initially thought. Chemically this species is ambivalent with regard to the production of diterpenoid lactones; as a result, diterpenoid lactones alone are no longer a valid method for species differentiation. There is however a remarkable similarity between this species and Eunicea mammosa Lamouroux, observed in the production of common sesquiterpene hydrocarbons and a common diterpenoid lactone, eunicin. It is therefore quite possible that E. palmeri may be a morphological variant of E. mammosa.

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