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NEW MARINE CEMBRANE DITERPENOIDS ISOLATED FROM THE CARIBBEAN GORGONIAN *EUNICEA MAMMOSA*[1]

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ABSTRACT.—Eleven cembrane-type diterpenes were isolated from the lipid extract of the Caribbean gorgonian *Eunicea mammosa*. Of these, four α-methylene-γ-lactone derivatives, **1–4**, and one α-methylene-δ-lactone, **5**, had been previously isolated from various gorgonians. The structures of six new cembrane derivatives, eupalmerone [**7**], pseudoplexauric acid methyl ester [**8**], (7E,11E)-(1S,3S,4R)-3,4;15,17-diepoxycembra7,11-diene [**11**], (−)-eunicenone [**12**], cembranoid chlorohydrin **14**, and diketone **15**, were determined by means of spectroscopic analyses, and compounds **7** and **8** were chemically correlated with known cembranoid diterpenes eupalmerin [**2**] and pseudoplexaurol [**9**], respectively. (−)-Eunicenone [**12**], a rare cembranoid diterpene having the uncommon 11-cis double bond, was found to be the (−)-antipode of cembrane derivative **13** isolated from a South Pacific soft coral. The structure and stereochemistry of chlorohydrin **14** were established in conjunction with a single crystal X-ray analysis.

With a few exceptions, all coelenterate diterpenoids reported thus far are cembrane or cyclized cembrane derivatives (1). In general, marine natural product chemists believe that the cembrane skeleton originates from the cyclization of geranylgeranyl pyrophosphate, a hypothesis supported in part by the observation that in almost all derivatives the geometry of the double bonds is *E*, as in geranylgeraniol. The initial cyclization can a priori yield two antipodes, **I** and **II** (Scheme 1). The *R* antipode **I** leads to the α series in which the isopropyl is α (pointing downward). All cembrane diterpenes of known absolute stereochemistry at C-1 that have so far been reported from the order Alcyonacea belong to the α series. In a similar fashion, the *S* antipode **II** leads to a series of compounds of corresponding configuration in which the isopropyl group is β (pointing upward). In contrast, all cembrane diterpenes isolated from marine animals within the order Gorgonacea belong to the β series (2).

The Caribbean gorgonian octocoral *Eunicea mammosa* (Lamouroux) (phylum Cnidaria) has been reported to yield the diterpenoids euniolide [**1**] (3), eupalmerin [**2**] (4), eupalmerin acetate [**3**] (5), jeunicin, eunicin, and cueunicin or cueunicin acetate depending on the location of collection (6). Analogous variations have also been observed in the case of *Eunicea palmeri* and *Eunicea succinea*. Moreover, *E. mammosa* also shows a geographic variation in its sesquiterpene hydrocarbon content (7). In contrast, Caribbean gorgonians belonging to the genus *Pseudoplexaura* collected over a wide geographical range exhibit a marked consistency in their content of the cembranoid diterpene crassin acetate [**6**] and sesquiterpenes (8). Thus, it appears likely that in a given species the variation in terpenoid content may arise only from the functionalization and not from the carbon skeleton. The identification and subsequent interpretation of these variations are not straightforward and to separate the various factors that might be involved (for
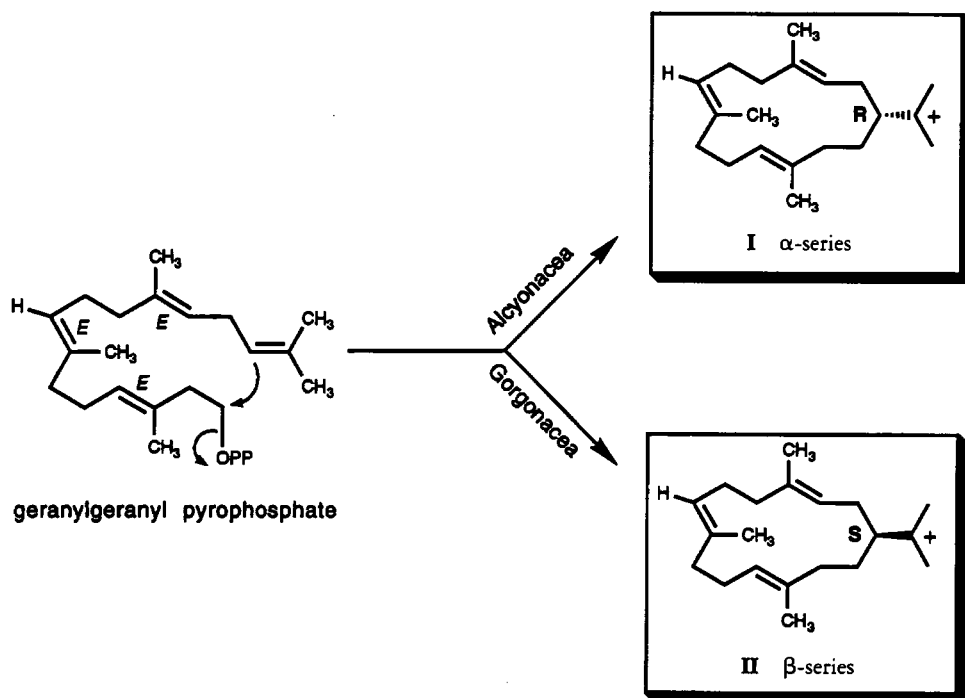
SCHEME 1. The enzyme-catalyzee cyclization of geranylgeranyl pyrophosphate could in principle lead to the two antipodal carbocations I and II, depending upon the the order being Alcyonaceae or Gorgonaceae, respectively.
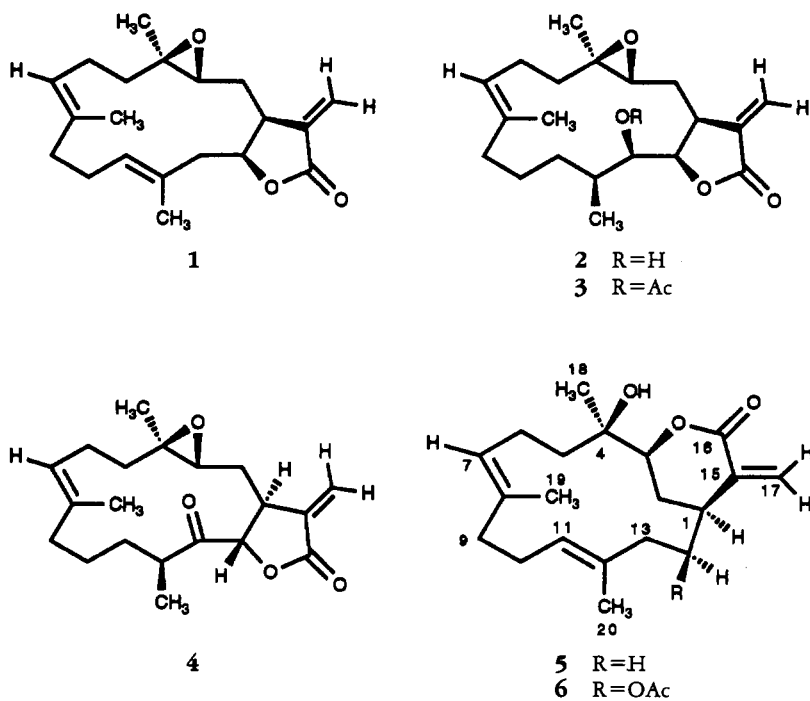
instance, geographic, individual, or seasonal variations, age and sex of the colonies, or changes in the symbiotic association between polyps and zooxanthellae) is not at this moment an easy task (2).

In our continuing investigations on the distribution of cembrane diterpenes across specimens of *E. mammosa* collected at various locations near Puerto Rico, we now report the structures of six new cembranoid diterpenes related to **II**, isolated from a specimen of *E. mammosa* collected off the northwestern coast (on the leeward side of Desecheo Island) of Puerto Rico.

A sample (324 g) of freeze-dried *E. mammosa* was blended with MeOH-CHCl$_3$ (1:1); the extract was concentrated, and the resulting crude extract was partitioned against hexane and H$_2$O. The hexane solubles were filtered and evaporated, giving an oil (12.90 g) which was subsequently fractionated by successive size exclusion (Bio-Beads SX-2; toluene) and adsorption cc (Si gel, 20% EtOAc in hexane). Repeated Si gel cc of some of the less polar fractions, followed in some instances by reversed-phase hplc, gave two major [**1** 0.52% and **3** 0.20% (based on the weight of freeze-dried coral)] and nine minor cembranoid diterpenes (**2, 4, 5, 7, 8, 11, 12, 14**, and **15**). Of these, compounds **1–5** are known and were identified by comparison of their ^{1}H- and ^{13}C-nmr, ms, ir, and uv spectral properties with values reported in the literature (see Experimental).

Compound **7** (eupalmerone, 0.049% dry wt), a uv-active (λ max 244 nm) white crystalline solid, mp 159–160°, had a molecular formula of C$_{20}$H$_{28}$O$_4$ which was established by hreims (*m/z* [M]$^+$ 332.19842) and ^{13}C-nmr data, thus indicating seven degrees of unsaturation in the molecule. Since the ^{13}C-nmr spectrum (Table 1) contained only four olefinic carbon resonances [131.25 (s) and 126.52 (d) assigned to a trisubstituted olefin and 137.18 (s) and 119.29 (t) assigned to an exocyclic olefin] and two carbon resonances at δ 208.60 and 169.15 for a ketone and ester carbonyl, respectively, the molecule was judged to be tricyclic. The presence of two additional oxygenated carbon atoms [62.05 (s) and 58.69 (d) ppm] suggested that the fourth oxygen in the molecule
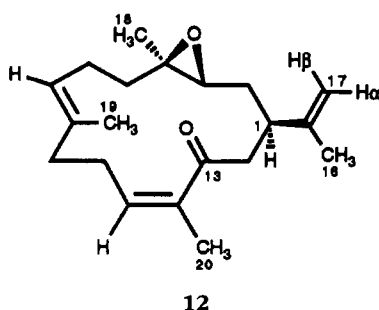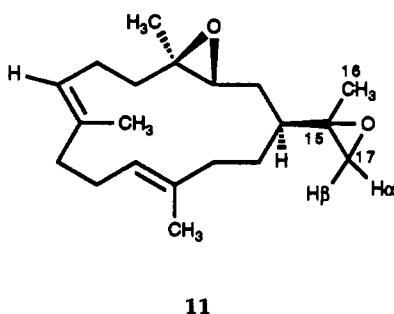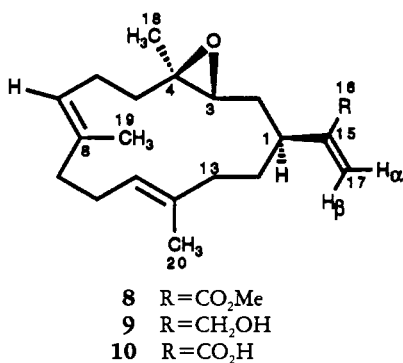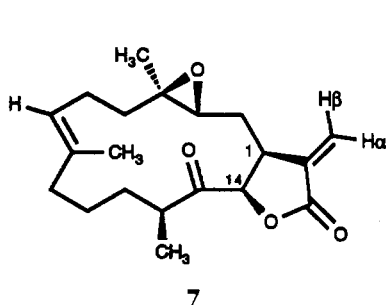

7

8   R=CO$_2$Me
9   R=CH$_2$OH
10   R=CO$_2$H

11

12

TABLE 1. ^{13}C-nmr (CDCl$_3$, 75 MHz) Data for Cembranoid Diterpenes **7, 8, 11, 12**, and **13**.[a]

Carbon	Compound				
	7	**8**	**11**	**12**	**13**[b]
C-1	40.89 (d)	34.75 (d)	38.94 (d)	38.34 (d)	38.4 (d)
C-2	26.42 (t)	34.25 (t)	28.95 (t)[c]	31.05 (t)	31.09 (t)
C-3	58.69 (d)	62.81 (d)	63.06 (d)	60.49 (d)	60.36 (d)
C-4	62.05 (s)	60.74 (s)	61.07 (s)	59.61 (s)	59.49 (s)
C-5	36.35 (t)	38.22 (t)	38.52 (t)	38.18 (t)	38.19 (t)
C-6	23.58 (t)	23.61 (t)	23.69 (t)	23.52 (t)	23.54 (t)
C-7	126.52 (d)	124.62 (d)[c]	124.48 (d)[d]	123.98 (d)	124.06 (d)
C-8	131.25 (s)	135.06 (s)[d]	134.72 (s)[e]	135.49 (s)	135.40 (s)
C-9	35.76 (t)	39.50 (t)	39.72 (t)	38.95 (t)	38.99 (t)
C-10	25.41 (t)	24.48 (t)	24.35 (t)	29.22 (t)	29.24 (t)
C-11	20.01 (t)	123.86 (d)[c]	124.16 (d)[d]	134.42 (d)	134.46 (d)
C-12	42.19 (d)	133.15 (s)[d]	132.25 (s)[e]	137.34 (s)	137.30 (s)
C-13	208.60 (s)	34.98 (t)	34.38 (t)	206.18 (s)	186.66 (s)
C-14	79.15 (d)	30.74 (t)	30.98 (t)[c]	44.89 (t)	44.89 (t)
C-15	137.18 (s)	144.64 (s)	59.36 (s)	147.28 (s)	147.25 (s)
C-16	169.15 (s)	167.39 (s)	16.62 (q)[f]	21.71 (q)	21.72 (q)
C-17	119.29 (t)	124.08 (t)	55.13 (t)	110.51 (t)	110.49 (t)
C-18	13.87 (q)	16.88 (q)	16.89 (q)[f]	16.67 (q)	16.71 (q)
C-19	13.87 (q)	16.88 (q)[e]	16.52 (q)[f]	16.82 (q)	16.71 (q)
C-20	13.87 (q)	15.63 (q)[e]	15.47 (q)[f]	20.51 (q)	20.54 (q)
C-21	—	51.69 (q)	—	—	—

[a] ^{13}C-nmr multiplicities for **7, 8, 11**, and **12** were obtained by Attached Proton Test (APT) sequences. Assignments were made on the basis of homo- and heteronuclear chemical shift correlation methods and comparison to known models. The δ values are in parts per million downfield from TMS. Values for **13** were recorded in CDCl$_3$ by Ravi and Faulkner (12).
[b] Prof. D.J. Faulkner has reevaluated the ^{13}C-nmr data for structure **13** and has agreed that the reported C=O chemical shift value (C-13) should be revised to that shown for (−)-eunicenone {12].
[c-f] Values with identical superscripts in each column may be interchanged.

was present as a trisubstituted epoxide. The ir spectrum indicated the absence of hydroxyl functions and the presence of an unsaturated lactone conjugated to an exomethylene (1773 cm^{-1}). Moreover, a strong band at 1716 cm^{-1} confirmed the presence of a ketone carbonyl function, and absorptions at 1264 and 811 cm^{-1} supported the presence of an epoxide ring. The ^{1}H-nmr spectrum of eupalmerone [two 1H sharp doublets at δ 6.20 (J=3.6 Hz) and 5.47 (J=3.3 Hz)] strongly suggested the presence of a γ-lactone conjugated to an exomethylene group (Table 2). The presence of two 3H singlets at δ 1.57 and 1.07 indicated a methyl substituted olefin and a methyl group in an E-trisubstituted epoxide, respectively, while a 3H doublet at δ 1.12 (J=7.5 Hz) indicated a methyl group bonded to a tertiary carbon atom. The ^{1}H-nmr spectrum also showed key signals at δ 5.18 (d, 1H, J=7.8 Hz, H-14), 5.16 (overlapped t, 1H, J=6.1 Hz, H-7), 3.40 (m, 1H, H-1), 3.05 (m, 1H, H-12), 2.67 (dd, 1H, J=3.1, 10.3 Hz, H-3), 2.48 (m, 1H, H-6α), and 1.89 (dt, 1H, J=4.0, 13.8 Hz, H-5α). The ^{1}H-^{1}H correlation COSY experiment showed that the complex multiplet at δ 3.40 (H-1) has responses to two nonequivalent methylene protons (δ 2.15, 1.66, H-2αβ), a methine proton (δ 5.18, H-14) and the two doublets centered at δ 5.47 (H-17β) and 6.20 (H-17α). The contour plot of the ^{1}H-^{1}H COSY spectrum of eupalmerone [**7**] also revealed that the doublet of doublets centered at δ 2.67 due to an epoxymethine proton has strong responses to each of the nonequivalent H-2 protons, thereby confirming the location of the epoxide ring. The methine proton resonating at δ 5.18 (H-14), which in turn showed

13   14

15   (tentative)
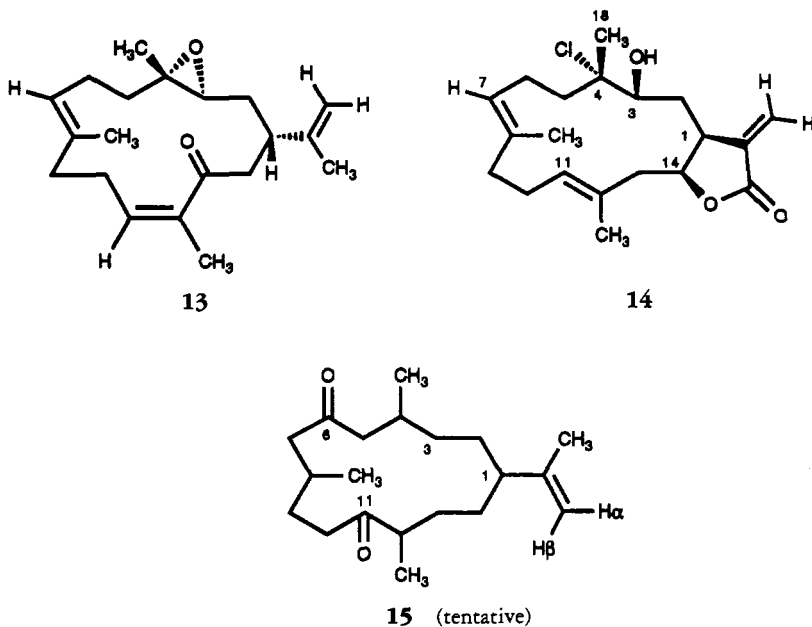
no further responses in the ^{1}H-^{1}H COSY spectrum, has a strong $^1J_{CH}$ coupling response to the carbon signal at δ 79.15 (d, C-14), indicating the location of the attachment of the oxygen of the γ-lactone and that of the ketone functionality. The coupling constant for the sharp doublet centered at δ 5.18 (J = 7.8 Hz, H-14) assigned to the hydrogen on the γ-carbon of the lactone, indicated that the two lactone protons (H-1 and H-14) are cis to each other. The remaining ^{1}H-^{1}H COSY responses were consistent with the proposed structure for eupalmerone [7]. The locus of the ketone functionality in **7** was also deduced from a long-range heteronuclear chemical shift correlation spectrum (Selective Pulse INEPT nmr experiment) (9). Thus, irradiation of the signals at δ 3.40 (H-1) and 3.05 (H-12) caused the enhancement of the ^{13}C-nmr signal at δ 208.60 (C-13). Based on all the spectroscopic data accumulated for this molecule, it appeared that compound **7** is the oxidation product of eupalmerin [2], which was found to occur as a minor component in the same specimen of *E. mammosa*, and that they must, therefore, possess identical relative stereochemistry (4). This contention was confirmed by oxidation of eupalmerin to eupalmerone using the Swern oxidation method (see Experimental). After purification, all the physical properties displayed by the semisynthetic oxidation product were identical with those recorded here for naturally occurring eupalmerone [7].

Compound **8** (0.031% dry wt), a uv-active (λ max 242 nm) colorless oil, was shown to have the molecular formula $C_{21}H_{32}O_3$ (m/z [M]$^+$ 332.23583, calcd 332.23513; Δ 0.7 mmu) by hreims. A strong ir band at 1720 cm^{-1} suggested the presence of an α,β-unsaturated ester group, and strong bands at 1260 and 801 cm^{-1} could be assigned to an epoxy group. The ^{13}C-nmr spectrum (Table 1) exhibited twenty-one signals divided by APT (10) into five quaternary carbons, four methine carbons, eight methylenes, and four methyl groups. The ^{1}H-nmr spectrum (Table 2) contained a proton signal at δ 2.82 (dd, 1H, H-3), which could be assigned to an α-epoxy proton, and showed additional signals at δ 6.18 (s, 1H, H-17α) and 5.50 (s, 1H, H-17β) due to a pair of terminal methylene protons; four methyl singlets at δ 3.73, 1.60, 1.54, and 1.20; and a signal at δ 2.74 (m, 1H, H-1). The chemical shift and multiplicity of the corresponding carbon [δ 34.75 (d), C-1] indicate that the latter signal is ascribable to H-1. Moreover, two overlapped triplets centered near δ 5.12, integrating as 2H, could be attributed to two

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TABLE 2.   Partial ^{1}H-NMR (300 MHz) Data for Compounds **7**, **8**, **11**, **12**, and **14** (CDCl$_3$, TMS).[a]

Proton	Compound				
	7	**8**	**11**	**12**	**14**
	δH (Hz)	δH (Hz)	δH (Hz)	δH (Hz)	δH (Hz)
H-1	3.40 (m, 1H)	2.74 (m, 1H)	—	2.89 (m, 1H))	3.41 (dt, 1H, 5.0, 12.3)
H-2α	2.15 (m, 1H)	1.82 (m, 1H)	—	1.70 (m, 1H)	2.02 (m, 1H)
H-2β	1.66 (m, 1H)	1.47 (m, 1H)	—	1.70 (m, 1H)	1.40 (m, 1H)
H-3	2.67 (dd, 1H, 3.1, 10.3)	2.82 (dd, 1H, 4.0, 9.0)	2.76 (br t, 1H, 6.9)	2.63 (t, 1H, 5.4)	3.60 (br d, 1H, 10.2)
H-7	5.16 (t, 1H, 6.1)	5.12 (t, 1H, 6.1)	5.03 (t, 1H, 6.3)	5.05 (t, 1H, 6.6)	5.53 (t, 1H, 6.9)
H-11	—	5.12 (t, 1H, 6.1)	5.03 (t, 1H, 6.3)	5.65 (t, 1H, 7.5)	5.13 (t, 1H, 6.5)
H-14α	5.18 (d, 1H, 7.8)	—	—	2.89 (m, 1H)	4.73 (m, 1H)
H-14β	—	—	—	2.74 (dd, 1H, 9.3, 19.5)	—
H-16	—	—	1.30 (s, 3H)	1.79 (s, 3H)	—
H-17α	6.20 (d, 1H, 3.6)	6.18 (br s, 1H)	2.58 (d, 1H, 5.0)	4.84 (s, 1H)	6.28 (br s, 1H)
H-17β	5.47 (d, 1H, 3.3)	5.50 (br s, 1H)	2.46 (d, 1H, 5.0)	4.72 (s, 1H)	5.73 (br s, 1H)
H-18	1.07 (s, 3H)	1.20 (s, 3H)	1.18 (s, 3H)	1.21 (s, 3H)	1.46 (s, 3H)
H-19	1.57 (s, 3H)	1.54 (s, 3H)[b]	1.55 (s, 3H)	1.61 (s, 3H)	1.60 (s, 3H)
H-20	1.12 (d, 3H, 7.5)	1.60 (s, 3H)[b]	1.55 (s, 3H)	1.85 (s, 3H)	1.66 (s, 3H)
H-21	—	3.73 (s, 3H)	—	—	—

[a]Assignments were aided by ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, spin splitting patterns and comparison of J values.
[b]Values with identical superscripts in each column may be interchanged.

olefinic protons (H-7 and H-11), and a broad envelope of allylic proton signals found between δ 1.80 and 2.30, integrating as 8H, could be ascribed to protons H-6, H-9, H-10, and H-13. The ^{1}H-^{1}H COSY spectrum revealed that the ^{1}H-nmr signal at δ 2.74, assigned to H-1, was coupled to a pair of diastereotopic protons resonating at δ 1.82 and 1.47 (H-2αβ) which were in turn coupled to the single proton doublet of doublets centered at δ 2.82 assigned to the α-epoxy proton H-3. This chain of coupling placed the epoxide ring between C-3 and C-4. This observation was confirmed by the Selective Pulse INEPT nmr spectrum; during irradiation of the ^{1}H-nmr signal at δ 2.82 (H-3), the ^{13}C-nmr signals at δ 34.25 (C-2) and 34.75 (C-1) experienced a strong enhancement thus confirming their ^{13}C-nmr chemical shift assignments. These combined data were consistent with the formula C$_{21}$H$_{32}$O$_3$ and the proposed cembrane skeleton for epoxy ester **8**.

In order to establish the relative configuration at chiral centers C-1, C-3, and C-4 by chemical correlation to a cembranoid diterpene of known structure, we carried out the reduction of cembranoid ester **8** at 0°–25° with LiAlH$_4$ in dry Et$_2$O, giving pseudoplexaurol [**9**], identical with regard to retention time by tlc and glc, specific rotation, ^{1}H nmr, and ms to authentic material isolated from the Caribbean gorgonian *Pseudoplexaura porosa* (**11**). Cembrane acid **10**, named pseudoplexauric acid (not isolated), having the proper stereochemistry to be a logical biosynthetic presursor to 14-deoxycrassin [**5**], is presumably the actual natural product present in *E. mammosa*, with ester **8** being produced as an artifact during the isolation process. An attempt to carry out the saponification of cembranoid ester **8** (Na$_2$CO$_3$ in refluxing MeOH) to give pseudoplexauric acid [**10**] which in principle could decompose by concomitant back-side attack of the carboxylic hydroxyl group at C-3 of the epoxide to afford 14-deoxycrassin [**5**], was not successful.

Characterization of cembrane diepoxide **11** (0.0057% dry wt), an optically active,

$[\alpha]^{25}$D$= -23.97°$, viscous oil, was carried out on a chromatographically homogeneous sample of formula $C_{20}H_{32}O_2$ as determined by hreims of its molecular ion. The molecular formula required five degrees of unsaturation: the ^{13}C-nmr spectrum (Table 1) contained only four sp^2 carbons [two singlets (134.72, 132.25) and two doublets (124.48 and 124.16)]; the molecule is, therefore, tricyclic. The ir and ^{1}H-nmr (Table 2) spectra indicate the presence of two epoxide rings: ir 1259, 1240, 816, and 808 cm^{-1}; ^{1}H nmr δ 2.76 (br t, 1H, $J=6.9$ Hz, H-3), 2.58 (d, 1H, $J=5.0$ Hz, H-17α), and 2.46 (d, 1H, $J=5.0$ Hz, H-17β). In addition, the ^{1}H-nmr spectrum showed two methyl-bearing trisubstituted double bonds (δ 1.55, two overlapped br s, 6H; 5.03, two overlapped br t, 2H, $J=6.3$ Hz), two methyl groups on a carbon bearing oxygen (δ 1.30, s, 3H and 1.18, s, 3H), and a broad envelope of allylic proton signals, integrating as 8H, between δ 1.94 and 2.30. The ^{1}H-^{1}H COSY spectrum confirmed the chain of coupling between H-3, the diastereotopic methylene protons H-2αβ, and the methine proton H-1. The presence of a 3,4-epoxide was, thus, established as shown in structure **11**. Since this cembrane derivative is a logical biosynthetic precursor to pseudoplexauric acid [**10**], their relative stereochemistry at carbons C-1, C-3, and C-4 must be identical. Therefore, we propose structure **11**, with unspecified stereochemistry at C-15, for the new cembranoid diepoxide.

Compound **12**, named (−)-eunicenone (0.0093% dry wt), was isolated as a uv-active (λ max 246 nm) oily substance that analyzed for $C_{20}H_{30}O_2$ by combined hreims and ^{13}C-nmr methods. The compound showed ir spectral characteristics consistent with the presence of an epoxide ring (1261 and 800 cm^{-1}) and a ketone carbonyl functionality (1709 cm^{-1}). Consideration of ^{13}C-nmr (Table 1) and ^{1}H-nmr data, and specifically the results of COSY analyses, allowed the complete structure of **12** to be assigned.

The ^{1}H-nmr spectrum of (−)-eunicenone (Table 2) showed two triplets at δ 5.65 (1H, $J=7.5$ Hz) and 5.05 (1H, $J=6.6$ Hz), due, respectively, to the β proton of an α,β-unsaturated Z-trisubstituted ketone (H-11) and the vinyl proton on a E-trisubstituted olefinic bond (H-7), and signals at δ 4.84 (s, 1H, H-17α), 4.72 (s, 1H, H-17β), 2.89 (complex m, 2H, H-1 and H-14α), 2.74 (dd, 1H, $J=9.3$, 19.5 Hz, H-14β), and 2.63 (t, 1H, $J=5.4$ Hz, H-3). The four 3H singlets at δ 1.85, 1.79, 1.61, and 1.21 have been attributed to the methyl groups placed, respectively, at C-12, C-15, C-8, and C-4, while the broad envelope found between δ 2.08 and 2.50, integrating as 6H, is ascribable to allylic protons H-6, H-9, and H-10. The ^{13}C-nmr spectrum of (−)-eunicenone [**12**] exhibited twenty signals divided by APT into five quaternary carbons (C-4, C-8, C-12, C-13, and C-15), four CH groups (C-1, C-3, C-7, and C-11), seven methylenes (C-2, C-5, C-6, C-9, C-10, C-14, and C-17), and four Me groups (C-16, C-18, C-19, and C-20), one ketone carbonyl carbon (δ 206.18), two oxygenated carbons (δ 60.49, 59.61), and six olefinic (δ 147.28, 137.34, 135.49, 134.42, 123.98, 110.51) carbons. The APT experiment indicated that one oxygenated carbon (δ 60.49) is tertiary, while the second (δ 59.61) belongs to a quaternary carbon. Three of the olefinic carbons (δ 147.28, 137.34, 135.49) are nonprotonated vinyls, two are singly protonated (δ 134.42, 123.98), and a sixth olefinic signal is of a terminal methylene carbon (δ 110.51).

By use of a ^{1}H-^{1}H correlation COSY and a Selective Pulse INEPT nmr experiments, the complete structure for **12** was assigned unequivocally, starting with the terminal methylene (δ 4.84 and 4.72, H-17α, -17β), which exhibits a COSY response (allylic coupling) corresponding to the 3H singlet resonating at δ 1.79 (Me-16). Irradiation of the ^{1}H-nmr signal at δ 4.84 (H-17α) caused the enhancement of the ^{13}C-nmr signals resonating at δ 38.34 (C-1) and 21.71 (C-16), thus establishing the attachment of the isopropenyl group at C-1. Irradiation of the ^{1}H-nmr signal at δ 2.89 (H-1 and H-14α), which caused enhancement of the ^{13}C-nmr signal at δ 147.28 (C-15), confirmed this contention. The single proton triplet centered at δ 2.63 (H-3) has responses in the ^{1}H-

¹H COSY spectrum to the nonequivalent methylene protons resonating near δ 1.70 (H-2), which in turn show strong coupling to H-1 resonating as a complex multiplet at δ 2.89. In turn, H-1 shows strong responses to the nonequivalent methylene protons resonating at δ 2.89 (H-14α) and 2.74 (H-14β), and since the connectivity of the C-14 protons ends there, C-13 (δ 206.18) must be a quaternary carbon. These results confirm the chain of coupling between protons H-3, H-2, H-1, and H-14, including the more remote terminal methylene protons H-17αβ. Moreover, the locus of the C-13 carbonyl group was also evident from the INEPT spectrum of (−)-eunicenone. Upon irradiating the ¹H-nmr signals at δ 5.65 (H-11) and 1.85 (Me-20), the ¹³C-nmr signal at δ 206.18 experienced significant enhancement, a fact consistent with the proposed structure for (−)-eunicenone [12].

A careful literature survey revealed that the nmr (Tables 1 and 2), ir, uv, and mass spectra of (−)-eunicenone, as well as the magnitude of its specific rotation, appeared to be almost identical with those reported by Ravi and Faulkner (12) for the known cembranoid diterpene 13 isolated from an unidentified *Sarcophytum* species collected in the South Pacific at Canton Island (Phoenix Island group). However, solutions of the two cembrane enones in CHCl₃ showed opposite signs of rotation. Therefore, 12 ([α]D −5.38°) and 13 ([α]D +8.8°) are clearly enantiomeric. Since the absolute stereochemistry of cembranoid diterpene 13 has been described, that of (−)-eunicenone must be as depicted in structure 12. These findings are consistent with the observation that cembranoids isolated from a number of soft corals have opposite absolute configurations at C-1 from those obtained from gorgonians. (−)-Eunicenone is of particular interest since it is the first example of a natural cembranoid diterpene isolated from a Caribbean gorgonian having the uncommon 11-cis double bond. The Z-trisubstituted C-11 olefinic bond in enones 12 and 13 is unique among cembranes from marine organisms.

The cembranoid chlorohydrin 14, C₂₀H₂₉O₃Cl by hreims, was isolated in 0.0063% yield (based on the weight of freeze-dried coral). The structure of the optically active compound, [α]²⁵D −22.36°, was deduced largely from nmr spectroscopy as follows. The molecular formula C₂₀H₂₉O₃Cl required six double-bond equivalents while the ¹³C-nmr spectrum revealed the presence of only four double bonds [one ester carbonyl (δ 170.00) and six olefinic carbons: three singlets (138.56, 134.18, 128.90), two doublets (127.84, 127.37), and one triplet (123.29)]. The compound was thus bicyclic. From the ir spectrum, two of the oxygen atoms in the molecule are ascribable to a lactone (1765 cm⁻¹); the third was suspected to be that of a hydroxyl group (3485 cm⁻¹). The ¹H-nmr spectrum (Table 2) and the low-field nature of the ¹³C-nmr signals at 80.50 (s), 78.24 (d), and 75.17 (d) ppm excluded the possibility of a trisubstituted epoxide ring. The downfield shift of these signals coupled with the absence of a corresponding epoxide methine signal near δ 2.90 in the ¹H-nmr spectrum indicated instead the presence of a lactone linkage and that of a secondary alcohol. The uv spectrum (λ max 216 nm) suggested the presence of conjugation; this information together with the strong ir band observed at 1765 cm⁻¹ is consistent with the presence of the α- methylene-γ- lactone ring shown in structure 14. Additional experimental evidence supporting this stems from the ¹H-nmr spectrum which shows two broad singlet peaks at δ 6.28 and 5.73 due, respectively, to H-17α and H-17β, and the complex multiplet character of the δ 4.73 absorption for H-14 which is vicinal to the C-13 methylene and the C-1 methine. In addition, the ¹H-nmr spectrum shows two vinyl protons on E-trisubstituted olefinic bonds (δ 5.53, t, 1H, J=6.9 Hz, H-7 and δ 5.13, t, 1H, J=6.5 Hz, H-11), a methine proton on a carbon bearing oxygen (δ 3.60, br d, 1H, J=10.2 Hz, H-3), an allylic sp³ methine proton (δ 3.41, dt, 1H, J=5.0, 12.3 Hz, H-1), two vinyl methyl groups (δ 1.66, s, 3H, Me-20 and 1.60, s, 3H, Me-19), a methyl group on a quaternary carbon bearing

a chlorine atom (δ 1.46, s, 3H, Me-18), and a broad envelope of allylic proton signals (δ 2.70–2.10, m, 8H, H-6, H-9, H-10, and H-13).

The proton-proton connectivity pathway around the bicyclic array constituted by the cembrane and lactone rings (from the carbinol methine proton H-3 to the C-13 methylene including the more remote exomethylene H-17αβ protons) was established from the ^{1}H-^{1}H COSY spectrum, which confirmed the allylic nature and ring size of the lactone ring system in **14**. A heteronuclear chemical shift correlation with broad band decoupling (CSCMBB) experiment (13,14) correlated the proton resonances at δ 4.73 and 3.60, respectively, with their corresponding carbon resonances at δ 78.24 (C-14) and 75.17 (C-3). The low-field nature of the quaternary carbon signal at δ 80.50 (C-4) must be attributed to a chlorine atom placed at that position. A chlorine atom bonded at C-4 is consistent with the observation that the Me-18 signal in the ^{13}C-nmr spectrum is at noticeable low field (δ 30.98) and that its associated protons give rise to a signal at δ 1.46 which is somewhat deshielded. The close proximity between the Me-18 group and the proton at chiral center C-3 was established by nOeds (15) involving the irradiation of the C-3 substituted proton (δ 3.60). This resulted in significant enhancement of the Me-18 protons at C-4 (δ 1.46) and the H-17β proton resonating at δ 5.73. With regard to the criteria for establishing the stereochemistry of the trisubstituted double bonds, the Me-19 and Me-20 signals in the ^{13}C-nmr spectrum (δ 16.04 and 15.02, respectively) are at high field, which indicates that these trisubstituted double bonds are *E* with respect to the continuous chain of carbons (12). The diterpenoid chlorohydrin **14** proved amenable to single-crystal X-ray analysis (Figure 1), establishing the relative stereochemistry at C-1, C-3, C-4, and C-14 as shown. The anti relationship of the vicinal chlorohydrin suggests that there is a strong possibility that **14** may be derived artificially from euniolide [**1**] during the extraction of the gorgonian through the acid-catalyzed cleavage of the *E*-trisubstituted epoxide.

A small amount (16.3 mg, 0.0050% dry wt) of diterpene **15** of molecular formula $C_{20}H_{34}O_2$ (as deduced on the basis of hreims, ^{1}H and ^{13}C nmr, and ir), was isolated. The compound contained a strong carbonyl group band at 1709 cm^{-1} in the ir but no hydroxyl functionality, and ^{13}C-nmr data revealed the presence of one terminal olefin [148.47 (s) and 109.95 (t)] and two ketone groups {214.66 (s) and 210.59 (s)}, but no epoxide moiety was detected. The two carbonyl functions in the molecule are unconjugated,
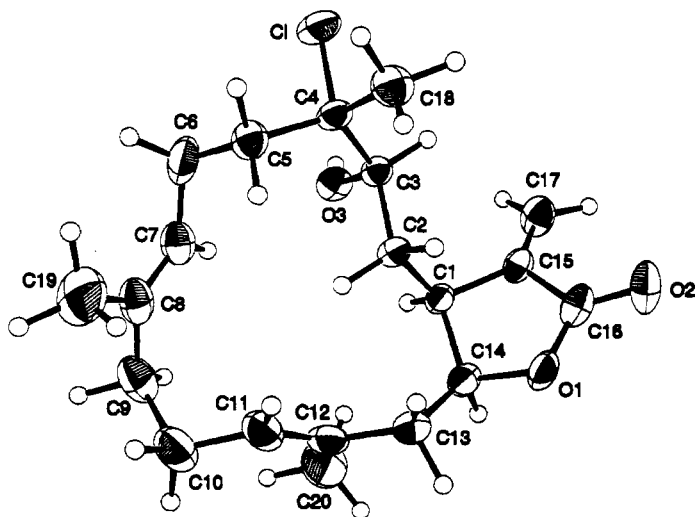

FIGURE 1.   Crystal structure (ORTEP representation) of chlorohydrin **14**.

as the uv showed only end absorption. These groups accounted for three of the four degrees of unsaturation implied by the molecular formula; hence **15** must be monocarbocyclic. The ^{1}H-nmr spectrum revealed the presence of an isopropenyl group (δ 4.69, br s, 1H, H-17α; 4.61, br s, 1H, H-17β; 1.62, s, 3H, Me-16) and three 3H doublets centered at δ 1.00 (J=6.9 Hz), 0.96 (J=6.6 Hz), and 0.91 (J=6.6 Hz) ascribable to three methyl groups bonded to tertiary carbons C-4, C-8, and C-12. A broad envelope, integrating as 8H, of allylic protons plus methylene and methine groups adjacent to ketone functions, was found between δ 1.70 and 2.50. Combination of these data suggested the substituted cembrane structure **15**, or an alternative cembrane with the C-11 carbonyl placed instead in the C-3 position. Structure **15**, without stereochemical specification, is reminiscent of the plexaurolone cembranoids isolated from the Caribbean gorgonian *Plexaura* A (16). Unfortunately, not enough sample was available to establish all details of the structure of this diterpenoid diketone, but the evidence for a cembranoid structure such as **15** is quite strong.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Details have been described previously (17). All hplc separations were performed on a Beckman Ultrasphere ODS semipreparative column (10 mm×25 cm). All separations were monitored simultaneously by refractive index and uv absorption.

COLLECTION AND EXTRACTION OF *E. MAMMOSA*.—The Caribbean gorgonian *E. mammosa* was collected by hand using SCUBA at depths of 15–20 m in July 1990 from Desecheo Island, Puerto Rico. The gorgonian was freeze-dried upon arrival and kept frozen until extraction. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The dried animal (324.0 g) was blended with MeOH-CHCl$_3$ (1:1)(3×1 liter), and after filtration the crude extract was evaporated under vacuum to yield a residue (27.20 g) that was partitioned between hexane and H$_2$O. The hexane extract was subsequently filtered, and the filtrate was concentrated in vacuo to yield 12.90 g of a dark green oily residue. This residue was dissolved in 10 ml of toluene and then was fractionated by size exclusion chromatography on a Bio-Beads SX-2 column using toluene as eluent. The combined terpenoid-rich fractions (tlc-guided) were concentrated to a yellow-orange oil (7.14 g) that was chromatographed over a Si gel column (280 g) with 20% EtOAc in hexane. The less polar portion of the lipids was fractionated roughly into fractions A through H on the basis of tlc analyses. Repeated cc of fraction B (0.68 g) on Si gel (40 g) with 5% EtOAc in hexane gave pseudoplexauric acid methyl ester [**8**] (ca. 100 mg) and a fraction that was subsequently purified by hplc [Ultrasphere-ODS Si gel with MeOH-H$_2$O (80:20)] to give pure (−)-eunicenone [**12**] (ca. 30.3 mg). Purification of fraction C (0.70 g) by cc on Si gel (52 g) using 10% EtOAc in hexane afforded a fast moving mixture (0.11 g), which was fractionated further by hplc [Ultrasphere-ODS Si gel with MeOH-H$_2$O (75:25)] giving pure compound **15** (ca. 16.3 mg) and cembranoid diepoxide **11** (ca. 18.7 mg), and a slow moving fraction (0.10 g) which was also purified by hplc [Ultrasphere-ODS Si gel with MeOH-H$_2$O (80:20)] to give pure cembranoid chlorohydrin **14** (ca. 20.4 mg). Fraction D (1.69 g) consisted of chromatographically pure euniolide [**1**], identified by 2D nmr techniques, uv, ir, ^{1}H and ^{13}C nmr, and ms (3). After purification by hplc [Ultrasphere-ODS Si gel with MeOH-H$_2$O (75:25)] and further spectral analyses (^{1}H and ^{13}C nmr, ir, uv, and ms), fraction E (0.16 g) was identified as succinolide [**4**] (49.6 mg, 0.015% dry wt) (17). Fraction F (0.65 g) was identified as the known cembranoid diterpene eupalmerin acetate [**3**] by comparison of the ir, ^{1}H nmr, ^{13}C nmr, and ms of the crystals with values reported elsewhere (5). Rechromatography of fraction G (0.65 g) on Si gel (30 g) with 20% EtOAc in hexane followed by hplc [Ultrasphere-ODS Si gel with MeOH-H$_2$O (90:10)] gave eupalmerone [**7**] (ca. 159 mg). The most polar fraction H (1.47 g) was passed through a column containing 80 g of Si gel and eluted with 20% EtOAc in hexane to afford ca. 50 mg (0.015% dry wt) of known cembranolide 14-deoxycrassin [**5**] (11) and ca. 150 mg (0.046% dry wt) of eupalmerin [**2**] (4). Both compounds were identified by comparison of their ir, ^{1}H-nmr, ^{13}C-nmr, and ms data with reported values.

Eupalmerone [**7**].—White crystalline solid: 159.0–160.0°; ir (neat) 2933, 1773, 1716, 1457, 1264, 1092, 986, 811, 755 cm^{-1}; uv λ max (CHCl$_3$) 244 nm (ε 3,300); [α]^{25}D −57.69° (c=0.52, CHCl$_3$); hreims m/z [M]$^+$ 332.19842 (8.0%) (C$_{20}$H$_{28}$O$_4$ requires 332.19874), 314 (3.0), 261 (2.3), 220 (5.2), 137 (16.0), 108 (61.5), 95 (94.1), 81 (100.0), 68 (78.3); ^{13}C nmr (75 MHz, CDCl$_3$) see Table 1; ^{1}H nmr (300 MHz, CDCl$_3$) see Table 2.

Pseudoplexauric acid methyl ester [**8**].—Colorless semisolid: ir (neat) 2980, 2931, 1720, 1625, 1439, 1384, 1260, 1148, 1096, 1020, 801 cm^{-1}; uv λ max (CHCl$_3$) 242 nm (ε 850); [α]^{25}D −15.18° (c=2.7, CHCl$_3$); hreims m/z [M]$^+$ 332.23583 (4.7%) (C$_{21}$H$_{32}$O$_3$ requires 332.23513), 314 (1.2), 300 (4.8), 285 (2.5),

272 (2.5), 255 (3.4), 219 (8.1), 178 (17.3), 147 (26.0), 119 (54.2), 107 (55.1), 93 (69.6), 81 (100.0), 68 (50.5); ^{13}C nmr (75 MHz, CDCl$_3$) see Table 1; ^{1}H nmr (300 MHz, CDCl$_3$) see Table 2.

(7E,11E)-(1S,3S,4R)-3,4;15,17-Diepoxycembra-7,11-diene [**11**].—Colorless oil: ir (neat) 2927, 2858, 1450, 1384, 1259, 1240, 1065, 1021, 897, 816, 808 cm^{-1}; [α]^{25}D −23.97° (*c*=1.46, CHCl$_3$); hreims *m/z* [M]$^+$ 304.23899 (2.4%) (C$_{20}$H$_{32}$O$_2$ requires 304.24022), 273 (3.7), 231 (1.2), 213 (1.5), 202 (4.4), 187 (4.9), 147 (16.2), 135 (24.6), 121 (39.8), 107 (58.0), 93 (68.2), 81 (100.0), 67 (48.9); ^{13}C nmr (75 MHz, CDCl$_3$) see Table 1; ^{1}H nmr (300 MHz, CDCl$_3$) see Table 2.

(−)-Eunicenone [**12**].—Colorless oil: ir (neat) 2963, 2931, 1709, 1674, 1666, 1645, 1450, 1382, 1261, 1092, 1022, 888, 800, 757 cm^{-1}; uv λ max (CHCl$_3$) 246 nm (ε 1,900); [α]^{25}D −5.38 ° (*c*=2.6, CHCl$_3$); hreims *m/z* [M]$^+$ 302.22414 (1.8%) (C$_{20}$H$_{30}$O$_2$ requires 302.22457), 205 (3.1), 177 (3.4), 163 (8.1), 151 (8.4), 137 (11.7), 121 (19.6), 107 (28.7), 93 (32.6), 81 (100.0), 67 (25.1); ^{13}C nmr (75 MHz, CDCl$_3$) see Table 1; ^{1}H nmr (300 MHz, CDCl$_3$) see Table 2.

Cembranoid chlorohydrin **14**.—White crystalline solid: ir (neat) 3485, 2963, 2932, 1765, 1449, 1261, 1161, 1092, 1059, 1021, 864, 800, 758 cm^{-1}; uv λ max (MeOH) 216 nm (ε 3,300); [α]^{25}D −22.36° (*c*=0.76, CHCl$_3$); hreims *m/z* [M]$^+$ 352.18205 (3.7%) (C$_{20}$H$_{29}$O$_3$Cl requires 352.18051), 334 (0.5), 316 (6.8), 283 (3.6), 217 (4.1), 181 (12.0), 135 (32.1), 121 (46.9), 107 (72.1), 93 (78.2), 81 (100.0), 68 (94.0), 55 (80.6); ^{13}C nmr (75 MHz, CDCl$_3$) δ 170.00 (s) 138.56 (s), 134.18 (s), 128.90 (s), 127.84 (d), 127.37 (d), 123.29 (t), 80.50 (s), 78.24 (d), 75.17 (d), 41.08 (t), 39.49 (t), 39.08 (t), 39.02 (d), 30.98 (q), 26.47 (t), 24.59 (t), 23.11 (t), 16.04 (q), 15.02 (q); ^{1}H nmr (300 MHz, CDCl$_3$) see Table 2.

Compound **15**.—Colorless semisolid: ir (neat) 2960, 2934, 2872, 1709, 1644, 1373 cm^{-1}; [α]^{25}D −9.09 ° (*c*=0.44, CHCl$_3$); hreims *m/z* [M]$^+$ 306.25719 (11.3%) (C$_{20}$H$_{34}$O$_2$ requires 306.25587), 288 (3.2), 263 (3.2), 220 (5.3), 177 (10.1), 159 (14.2), 136 (68.5), 121 (65.9), 109 (69.4), 93 (65.4), 81 (85.3), 69 (62.6), 55 (100.0); ^{13}C nmr (75 MHz, CDCl$_3$) δ 214.66 (s), 210.59 (s), 148.47 (s), 109.95 (t), 50.84 (t), 47.98 (t), 46.93 (d), 46.84 (d), 37.44 (t), 33.85 (t), 31.63 (t), 31.38 (d), 30.34 (t), 29.58 (t), 29.58 (t), 28.18 (d), 20.97 (q), 20.44 (q), 20.25 (q), 16.55 (q); ^{1}H nmr (300 MHz, CDCl$_3$) see text.

SWERN OXIDATION OF EUPALMERIN [**2**] TO EUPALMERONE [**7**] (18).—Oxalyl chloride (0.5 ml) dissolved in dry CH$_2$Cl$_2$ (3 ml) was taken in a 3-necked round-bottom flask fitted with a rubber septum under N$_2$ and placed over a magnetic stirrer. To the cooled flask (−60°) was added DMSO (1 ml) dissolved in CH$_2$Cl$_2$ (3 ml) over a period of 2–3 min, and stirring was continued for 6–8 min. A solution of eupalmerin [**2**] (ca. 50 mg) dissolved in CH$_2$Cl$_2$ (2 ml) was added slowly over a period of 2–3 min, and after stirring at −60° for another 15–20 min triethylamine (5 ml) was added dropwise over a 4–5 min period. After stirring the resulting mixture for another 15–20 min, the cooling bath was removed, and distilled H$_2$O (20 ml) was added. Stirring was continued for the next 15 min, the organic layer was separated, and the aqueous layer was extracted with CH$_2$Cl$_2$ (4×20 ml). The combined organic extract was washed with distilled H$_2$O (1×30 ml) and dried throughly over MgSO$_4$. After removal of the solvent, the oily residue was loaded on a Si gel column (8 g) and eluted with EtOAc-hexane mixture (8:92) to afford semisynthetic eupalmerone [**7**] (ca. 10 mg), identical with regard to its spectral properties to the natural product. After cc, most of the starting material was recovered unreacted.

LiAlH$_4$ REDUCTION OF CEMBRANE ESTER **8** TO PSEUDOPLEXAUROL [**9**].—To a solution of cembrane ester **8** (40.2 mg, 0.12 mmol) in dry Et$_2$O (5 ml) kept at 0° under an inert atmosphere of argon, was added powdered LiAlH$_4$ (15 mg). After stirring the resulting mixture for 1 h at 0°, the temperature was allowed to rise slowly to 25° over a period of 6–8 h after which a few drops of aqueous 0.1 N HCl were added. The oil crude product obtained after removal of the solvents under vacuum was taken up with 20% EtOAc in hexane. The concentrate was passed through a small column of Si gel (2.0 g, 20% EtOAc in hexane), and one of the homogeneous oily products obtained from the complex product mixture was identified as pseudoplexaurol [**9**] by comparison of its retention time (by tlc and glc analyses), its specific rotation in CHCl$_3$ ([α]^{27}D −21.1°), and its ^{1}H-nmr and mass spectra with those of authentic material (11).

X-RAY DIFFRACTION STUDY OF CHLOROHYDRIN **14**[3].—A prism of dimensions 0.25×0.25×0.30 mm was selected for data collection The density of the crystalline material was not measured. Data were collected on an Enraf-Nonius CAD-4 diffractometer. Cell constants were determined from the setting angles of 25 reflections with 30≤2θ≤40°, using CuKα radiation; a=8.5205 (6), b=12.4434 (6), c=10.0465 (8) Å, β=111.482 (3)°. Space group *P2$_1$* was assigned from systematic absences 0*k*0, *k*=2n+1, and successful

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[3]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

TABLE 3.   Atomic Parameters x, y, z, and B_{ISO} for Compound **14**.[a]

Carbon	x	y	z	B or B_{eq}
C-1	0.42517 (6)	0.44270	0.07342 (6)	4.388 (22)
C-1	0.04813 (21)	0.75588 (15)	−0.23265 (18)	2.72 (7)
C-2	0.23475 (22)	0.71607 (15)	−0.16833 (19)	2.75 (7)
C-3	0.25091 (22)	0.60994 (15)	−0.08793 (18)	2.70 (7)
C-4	0.43891 (22)	0.57262 (16)	−0.01000 (19)	3.01 (7)
C-5	0.54229 (24)	0.55510 (19)	−0.10234 (22)	3.56 (8)
C-6	0.4823 (3)	0.47309 (21)	−0.2269 (3)	4.63 (11)
C-7	0.3696 (3)	0.51857 (20)	−0.37289 (24)	4.11 (10)
C-8	0.4215 (3)	0.56482 (21)	−0.4677 (3)	4.27 (10)
C-9	0.2929 (4)	0.60604 (25)	−0.61018 (24)	4.94 (12)
C-10	0.3015 (4)	0.7275 (3)	−0.6316 (3)	5.08 (12)
C-11	0.2806 (3)	0.79378 (22)	−0.51386 (24)	4.40 (10)
C-12	0.1338 (3)	0.82297 (20)	−0.50711 (22)	3.98 (9)
C-13	0.1367 (3)	0.89652 (18)	−0.38707 (23)	3.81 (9)
C-14	0.02223 (24)	0.86486 (17)	−0.31051 (20)	3.21 (7)
C-15	−0.01179 (21)	0.78411 (16)	−0.11471 (20)	3.06 (7)
C-16	0.02058 (25)	0.90069 (18)	−0.08611 (23)	3.44 (8)
O-1	0.05204 (19)	0.94501 (13)	−0.19444 (16)	3.79 (6)
O-2	0.02592 (23)	0.95297 (18)	0.01741 (21)	5.05 (8)
C-17	−0.0847 (3)	0.72496 (21)	−0.0457 (3)	4.22 (11)
O-3	0.14731 (16)	0.53385 (12)	−0.18924 (14)	3.36 (6)
C-18	0.5372 (3)	0.64765 (22)	0.11451 (23)	4.23 (9)
C-19	0.6075 (4)	0.5816 (3)	−0.4409 (4)	6.61 (18)
C-20	−0.0390 (3)	0.7912 (3)	−0.6184 (3)	5.50 (13)

[a]ESDs refer to the last digit printed.

refinement. Data was collected to 2θ max = 140°, with $-10 \leq h \leq 9$, $0 \leq k \leq 15$, and $0 \leq l \leq 12$. Three standard reflections measured after every 3600 sec of X-ray exposure were used for scaling and showed no significant decay during the data collection period. A total of 2784 reflections were measured, of which 2505 were unique. The small subset of reflections which were averaged gave a merging R factor, $R_i = 0.023$. Of the unique reflections, 2450 were considered observed $[I > 2.0\sigma(I)]$. The structure was solved by the direct methods. Refinement was on $|F|$, and the function minimized during refinement was $\Sigma w(|F_o| - |F_c|)^2$, with $w = 1/[\sigma^2(F) + 0.001(F^2)]$. Hydrogen atoms were located in difference Fourier maps and refined with fixed isotropic thermal parameters. Non-hydrogen atoms were refined with anisotropic thermal parameters. The final refinement with 304 parameters and the observed data gave $R = 0.036$, $R_w = 0.044$, and $S = 1.25$, with (Δ/σ) max in the final cycle less than 5%. Atomic parameters are given in Table 3. The Eta parameter, an indicator of absolute configuration, refined to 1.14 (3). The final difference map had maximum and minimum densities of +0.16 and −0.31 eÅ^{-3}, respectively. Atomic scattering factors and anomalous-dispersion corrections were taken from the *International Tables for X-ray Crystallography* (19). All calculations except as noted were performed with NRCVAX (20).

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