

FURTHER STUDIES OF THE TERPENOID CONTENT IN THE GORGONIAN *EUNICEA SUCCINEA*: 12, 13-BISEPIEUPALMERIN, A NEW CEMBRANOLIDE

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ABSTRACT.—In a continuing comparative study of the sesquiterpenoid and diterpenoid content of the gorgonian *Eunicea succinea* from different locations, a new cembranolide, 12, 13-bisepieupalmerin (**6**), was isolated (structure by X-ray analysis) from specimens from St. Croix, U.S.V.I. 12, 13-Bisepieupalmerin possesses the correct stereochemistry to be a biosynthetic precursor for the related cembranolides eunicin (**1a**) and jeunicin (**2**). The latter compounds were found to occur with **6** in *E. succinea* from St. Croix. Eunicin and its acetate (**1b**) were obtained from a South Caicos collection. *E. succinea* from St. Croix, U.S.V.I. and South Caicos contain complex mixtures of hydrocarbons in which (–)-germacrene-A, (+)- β -elemene, (+)- β -epibourbonene, (+)- β -copaene, and (+)- α -muurolene were identified.

The occurrence of cembranolides in octocorals was discovered by Ciereszko (1) in 1955, when he crystallized eunicin (**1a**) from extracts of the gorgonian *Eunicea mammosa* Lamouroux collected at Bimini in the Bahamas. Since then, a deliberate search for compounds related to eunicin in gorgonians of the *Eunicea-mammosa-succinea* complex (2) collected in different locations in the Caribbean Sea has led to the discovery of jeunicin (**2**) in *E. mammosa* from Jamaica (3); of eupalmerin acetate (**3**) in *E. mammosa*, *Eunicea succinea* (Pallas), and *Eunicea palmeri* from the Florida Keys, and *E. succinea* from Puerto Rico (4-7); cueunicin (**4a**) and its acetate (**4b**) from *E. mammosa* collected in Curacao (8); and peunicin (**5a**) (9, 10) and epipeunicin (**5b**) (10) from *E. succinea* collected off the Atlantic coast of Panama.

Ciereszko (1) was initially attracted to *E. mammosa* in Bimini because of its odor, reminiscent of some of the essential oils of plants. Examination of the volatile components of extracts of *E. mammosa* indicated the presence of sesquiterpene hydrocarbons, and subsequent work resulted in the isolation of (+)- β -elemene and (–)-germacrene-A (11-13).

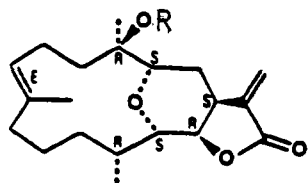
Preliminary examination of *E. succinea* collected at St. Croix, U.S. Virgin Islands, in 1976 yielded a lactone much less soluble in C_6H_6 than the cembranolides previously obtained from *Eunicea*. From a recollection of *E. succinea* at St. Croix in 1978, we have reisolated this lactone, obtained spectral data for it, and established by single crystal X-ray diffraction that it is a new cembranolide, 12, 13-bisepieupalmerin (**6**). Cembranolide **6** has the appropriate stereochemistry to make it a logical biosynthetic precursor to eunicin (**1a**) and jeunicin (**2**), both of which were found to occur in specimens of *E. succinea* from St. Croix.

In further comparative analysis, it was found that the major cembranolides in *E. succinea* from South Caicos are the previously known eunicin (**1a**) and its acetate (**1b**).

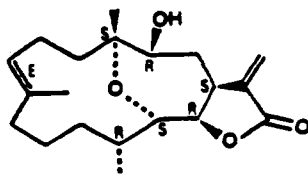
The sesquiterpene hydrocarbon content of *E. succinea* from both St. Croix and South Caicos was also examined for comparison with results from previous analyses of other gorgonians (11-13).

MATERIALS AND METHODS

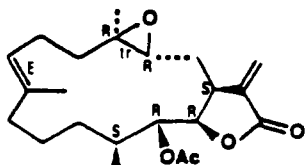
GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. IR spectra were taken on a Perkin-Elmer 298 spectrophotometer; nmr spectra were taken on a Varian XL-100 spectrometer in the solvent specified; signals are reported in parts per million (δ) downfield from internal TMS. Mass spectra were taken on a Hewlett-Packard 5985B spectrometer. A Perkin-Elmer 141 polarimeter was used for ob-



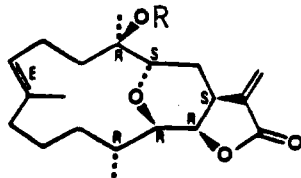
1a R=H
1b R=Ac



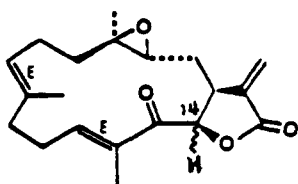
2



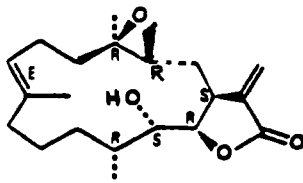
3



4a R=H
4b R=Ac



5a 14α-H
5b 14α-H



6

taining optical rotations. The chromatographic adsorbent used was Brinkmann silica gel 60 (230-400 mesh).

SPECIMEN SOURCES.—*E. succinea* was collected at a depth of 5 to 10 M at St. Croix, U.S. Virgin Islands and South Caicos, Turks, and Caicos Islands, during June, 1978. Specimens were air-dried immediately and stored until work-up.

ISOLATION OF EUNICIN (1a), JEUNICIN (2), 12,13-BISEPIEUPALMERIN (6) (–)-GERMACRENE-A, AND (+)-β-ELEMENE FROM *E. SUCCINEA* FROM ST. CROIX, U.S. VIRGIN ISLANDS.—Air-dried specimens (800 g) were cut into small pieces (~2.4 cm) and soaked first in hexane (3 liters) at room temperature for one day (Fraction A), and then in CHCl₃ MeOH (1:1), (3 liters) for 3 days (Fraction B). Fraction A was concentrated to a small volume (~100 ml) and then cooled at –10° overnight whereupon a white solid precipitated. The solid was recrystallized twice from Me₂CO-hexane and then twice from C₆H₆ to give 200 mg of pure 12,13-bisepieupalmerin (6): mp 144-145°, [α]_D –158.8° (c 1.57, CHCl₃); ir (KBr) 3560, 3110, 1760, 1660, 1465, 1430, 1280, 1175, 1112, 1100, 980, 850 cm⁻¹; 100 MHz pmr (CDCl₃) 0.91 (3H, d, 7 Hz, H-20), 1.24 (3H, s, H-18), 1.57 (3H, s, H-19), 3.42 (1H, brd t, H-3), 3.75 (1H, dd, 10, 4 Hz, H-13), 4.30 (1H, dd, 8, 10 Hz, H-14), 5.01 (1H, brd t, H-7), 5.78 (1H, d, 2 Hz, H-17), 6.40 (1H, d, 2 Hz, H-17); cmr (75 MHz, CDCl₃/CD₃OD) δ 12.05(q), 15.51(q), 16.21(q), 23.14(t), 23.43(t), 30.67(t), 31.36(t), 31.54(d), 37.02(t), 38.14(t), 39.19(d), 60.42(q), 60.49(d), 70.76(d), 79.02(d), 123.99(t), 124.18(d), 136.32(s), 139.20(s), 171.04(s); ms *m/z* (%) 334 (M⁺, 8), 319 (1), 317 (3), 316 (9), 306 (3), 273 (7), 207 (11), 195 (13), 189 (14), 177 (21), 163 (25), 151 (25), 149 (32), 135 (35), 123 (34), 105 (36), 95 (71), 91 (49), 81 (98), 79 (85), 67 (100). Analysis: calcd. for C₂₀H₃₀O₄: C, 71.85; H, 8.98. Found: C, 72.09; H, 9.2%.

Evaporation of the mother liquors from Fraction A yielded 18 g of an oil that was chromatographed on silica gel (600 g) using the step gradient (2.5 liter fractions) hexane, CHCl₃, EtOAc and MeOH. A portion (1.2 g) of the hexane eluates (7 g) was rechromatographed on AgNO₃ (10%) impregnated silica gel, eluting initially with hexane and then a hexane-EtOAc step gradient. Combination of like fractions yielded (–)-gemmaene-A (60 mg), [α]_D –4.8° (c 3.88, CHCl₃) and (+)-β-elemene (90 mg), [α]_D +14.16° (c 2.81, CHCl₃). Both were identified by comparison of their ir, pmr, and mass spectral data with reported values (10). The remaining sesquiterpenes present were not purified and identified.

The contents of a fraction B (~15 g) were chromatographed on Sephadex LH-20 (2" × 3¼') using CHCl₃-MeOH (1:1) and the fractions containing diterpenes (tlc analysis) were combined (~6 g) and chromatographed on silica gel (230-400 mesh) using C₆H₆-EtOAc (85:15). Some of the later fractions were combined (tlc analysis) and crystallized to give 1.25 g of **6**. The mother liquors from this crystallization combined with an earlier chromatographic fraction (total 0.95 g) were chromatographed again over silica gel with C₆H₆-EtOAc (85:15) to give in one of the fractions 250 mg of eunicin (**1a**), mp 154-156° [lit. (1,14) 154-155°]; pmr, (14). Cmr (75 MHz, CDCl₃) 14.14(q), 16.93(q), 20.47(t), 22.83(t), 23.63(q), 24.42(t), 28.75(t), 33.38(d), 37.70(t), 38.28(d), 40.84(t), 73.27(s), 73.41(d), 73.55(d), 77.28(d), 121.57(t), 128.59(d), 130.13(s), 136.73(s), 170.51(s). The chromatographic fraction (0.2 g) immediately following that containing eunicin contained a mixture of compounds and was further resolved by chromatography over AgNO₃ (10%) impregnated silica gel (40 g) using CH₂Cl₂-EtOAc-MeOH mixture (79:20:10 v/v). The later fractions yielded jeunicin (**2**, 20 mg), mp 137-140° [lit. (4) 139-140°]. Two of the earlier fractions were combined and chromatographed on reversed phase (C-18) hplc using 20% aqueous MeOH to give more **1a** (10 mg).

ISOLATION OF EUNICIN (1a), EUNICIN ACETATE (1b), (-)-GERMACRENE-A, (+)-β-EPIBOURBONENE, (+)-β-COPAENE, AND (+)-α-MUUROLENE FROM E. SUCCINEA FROM SOUTH CAICOS.—Air-dried specimens (1 kg) were cut into small pieces and soaked with hexane (4 liters) and the extracts were concentrated and cooled in a freezer to give (~4 g) of crystalline **1a**, Fraction A. The mother liquors (18.34 g), Fraction B, were chromatographed over silica gel using the step gradient hexane, CHCl₃, EtOAc, and MeOH (2.5 liters each). A portion (0.5 g) of the oil eluted with hexane (5 g) was rechromatographed over silica gel (50 g) using hexane. Pmr analysis indicated that one of these fractions was ~85% germacrene-A. On the basis of tlc analysis, various other fractions were combined and chromatographed on 10% AgNO₃ impregnated silica gel using hexane with increasing amounts of C₆H₆ to give (+)-α-muurolene (identification via its dihydrochloride, see below), (+)-β-copaene, and (+)-β-epibourbonene.

(+)-α-Muurolene: [α]_D +85.3° (c 3.1, CHCl₃) [lit. (12,15), ir (liquid film) 1445, 1380, 1360, 875, 840, 820, 792 cm⁻¹; 100 MHz pmr (CDCl₃) 0.82 (3H, d, 7 Hz), 0.88 (3H, d, 7 Hz), 1.67 (6H, s), 6.46 (2H, m); low resolution gc/ms *m/z* (%) 204 (M⁺, 21), 189 (7), 161 (35), 147 (7), 145 (10), 133 (17), 131 (15), 129 (12), 128 (10), 119 (29), 117 (21), 115 (21), 105 (100), 94 (21), 93 (38), 91 (77), 81 (24), 79 (44), 77 (46), 67 (14), 65 (16).

(+)-α-Muurolene dihydrochloride: Dry HCl gas was passed through a solution of (+)-α-muurolene (35 mg) in dry Et₂O (15 ml) and the solution was left at 0° overnight. The solvent was evaporated and the residue crystallized from EtOH to obtain 40 mg of dihydrochloride, mp 84-85°, [α]_D +18.4 (c 0.62, CH₃OH), [lit. (16) values for (-)-α-muurolene dihydrochloride: mp 83-84°; [α]_D -21.2 (c 0.44, CH₃OH)]; 100 MHz pmr (CDCl₃) δ 0.77 (3H, d, 7 Hz), 0.93 (3H, d, 7 Hz), 1.57 (3H, s), 1.64 (3H, s); ms *m/z* (%) 242 (M⁺-HCl, 3), 240 (10), 205 (16), 204 (14), 199 (16), 198 (10), 197 (55), 184 (13), 161 (100), 133 (13), 121 (15), 119 (23), 107 (22), 106 (24), 105 (77), 93 (48), 91 (51), 82 (25), 81 (52), 79 (54), 77 (45).

(+)-β-Epibourbonene: [α]_D +94° (c 1.02, CHCl₃), [lit. (12,15) [α]_D +68.7°] ir (liquid film) 3080, 1660, 1465, 1450, 1388, 1370, 1158, and 822 cm⁻¹, 100 MHz pmr (CDCl₃) δ 0.85 (6H, d, 6 Hz), 0.97 (3H, s), 4.72 (2H, bs); low resolution gc/ms *m/z* (%) 204 (M⁺, 2), 162 (4), 161 (30), 147 (2), 133 (6), 124 (9), 123 (72), 122 (7), 121 (8), 120 (5), 119 (10), 105 (19), 93 (9), 91 (24), 81 (100), 80 (61), 79 (47), 77 (25).

(+)-β-Copaene: [α]_D +1.4° (c 1.1, CHCl₃), [lit. (11,15), [α]_D +9.7°] ir (liquid film) 3075, 1640, 1465, 1385, 1368, 1168, and 870 cm⁻¹, 100 MHz pmr (CDCl₃) δ 0.69 (3H, s), 0.86 (6H, d, 6 Hz), 4.58 (1H, bs), 4.66 (bs); low resolution gc/ms *m/z* (%) 204 (M⁺, 28), 189 (22), 161 (100), 147 (24), 133 (36), 122 (22), 121 (41), 120 (22), 119 (45), 117 (10), 109 (15), 108 (17), 107 (52), 105 (79), 93 (46), 91 (69), 81 (60), 79 (42), 77 (33), 69 (16).

Some of the later fractions (2.9 g) from chromatography of Fraction B that were rich in eunicin (tlc analysis) were rechromatographed over silica gel using CHCl₃, and fractions rich in a component slightly different from **1a** indicated by tlc analysis were chromatographed again over silica gel using CH₂Cl₂ with increasing amounts (5 to 30%) of EtOAc to give 50 mg of eunicin acetate (**1b**).

Eunicin acetate: mp 156-158° (from hexane-C₆H₆); [α]_D -79.6° (c 0.55, CHCl₃); ir (KBr) 1775, 1738, 1460, 1310, 1260, 1205, 1110, 1005, 955 cm⁻¹; 100 MHz pmr (CDCl₃) δ 0.88 (3H, d, 6 Hz), 1.47 (3H, s), 1.54 (3H, s), 2.05 (3H, s), 2.93 (1H, d, 10 Hz), 3.36 (1H, dd, 11, 4 Hz), 4.47 (1H, dd, 8, 10 Hz), 5.08 (1H, t, 8 Hz), 5.65 (1H, d, 3 Hz), 6.46 (1H, d, 3 Hz); ms *m/z* (%) 376 (M⁺, 7), 334 (9), 317 (12), 306 (54), 245 (6), 232 (7), 220 (15), 206 (16), 161 (17), 135 (28), 133 (25), 121 (38), 119 (30), 109 (53), 105 (38), 93 (100), 81 (98), 67 (75).

Acetylation of Eunicin: To a solution of 0.20 of eunicin in 6 ml of Ac₂O and 2 ml of dry Et₂O at -20° was added 1 ml of boron trifluoride etherate. The reaction mixture was allowed to stand at -20° for 1.5 h and then poured into a cold solution of 2 ml of pyridine and 40 ml of H₂O. The white crystalline material

which separated immediately was filtered, washed with H₂O, and dried under vacuum. Recrystallization from hexane-C₆H₆ gave 120 mg of pure eunicin acetate, mp 164-166° [lit. (17) mp 157-163°, [α]_D -86.5° (c 0.48, CHCl₃)].

X-RAY CRYSTALLOGRAPHIC ANALYSIS OF 6.—Colorless single crystals of the compound were obtained by crystallization from C₆H₆ solution. A suitable crystal of size 0.51 × 0.43 × 0.17 mm³ was chosen for data collection. The crystal system is monoclinic, space group P2₁ with two molecules in the unit-cell. Unit-cell data (Table 1) and intensity data were measured at -135° on a CAD-4 automatic diffractometer equipped with a Enraf-Nonius cold-stream cooling device. The low temperature prevents decay of the natural product in the X-ray beam. Intensities of all 2010 unique reflections with 2 θ < 150° for CuK α radiation (λ = 1.5418 Å) were measured, using θ 2 θ scan technique. A variable scan width of (1.2 + 0.14 tan θ)° and a variable horizontal aperture width of (4 + 0.86 tan θ) mm were used. A maximum of 50 s was spent on each reflection with 2/3 of the time for scanning the peak and 1/6 of the time for scanning each of the left and right backgrounds. Intensities of a monitor reflection were measured after every 2500 seconds of X-ray exposure and appropriate scaling correction was applied to the data. The orientation matrix was checked after every 200 observations. The intensities of 40 reflections were considered indistinguishable from background on the basis that I was less than 2 σ (I). Lorentz and polarization corrections were applied to the data and an experimental weight, based on counting statistics (6), was assigned to each structure amplitude.

TABLE 1. Crystallographic Data

C ₂₀ H ₃₀ O ₄ MW = 334.5	Space Group P2 ₁ Z = 2
Cell dimensions ^a (-135°)	(25°)
a = 6.083(4) Å	a = 6.1551(7) Å
b = 8.280(4) Å	b = 8.3476(6) Å
c = 18.104(13) Å	c = 18.202(2) Å
β = 96.02(4)°	β = 96.11(1)°
V = 906.8 Å ³	V = 929.9 Å ³
	D _c = 1.195 gms/cc

^aDetermined by a least squares fit of +2 θ and -2 θ values of 48 reflections taken from all octants of reciprocal space, using CuK α radiations (λ = 1.54051 Å).

STRUCTURE DETERMINATION AND REFINEMENT: The structure was solved by direct methods using the computer program MULTAN (18). All nonhydrogen atoms were located from the E-map. Refinement of the structure was carried out using a block-diagonal least squares program (19). Initial refinement was done with isotropic thermal parameters and after a few cycles of isotropic refinement, anisotropic thermal parameters were introduced. All hydrogen atoms were located from a difference Fourier map and were refined isotropically. The standard deviations of atomic parameters were calculated using all reflections and the refinement was stopped when all shifts were less than 0.35 σ . The final discrepancy factor, R, defined as

$$R = \frac{\sum ||kF_o| - |F_c||}{\sum |kF_o|}$$

was 0.050 for 1969 reflections (0.051 for all reflections). A final difference Fourier map showed a maximum residual electron density of $\pm 3e \text{ \AA}^{-3}$. The scattering factors for C and O atoms were taken from the International Tables for X-ray Crystallography (20) while those for H atoms were taken from Stewart, Davidson and Simpson (21).

DETERMINATION OF THE ABSOLUTE CONFIGURATION OF 6: The absolute configuration of 12, 13-bis-epieupalmerin was determined using the anomalous dispersion of CuK α radiation by O atoms. Twenty-four pairs of reflections were selected for the purpose. Selections were made on the basis of the largest values of (F⁺ - F⁻)/ σ (F²) was obtained from counting statistics (6). Values of F⁺ and F⁻ were calculated according to the method of James (22). Intensities I_{hkl} and I $\bar{h}\bar{k}\bar{l}$ of the 24 Friedel pairs were measured 17 times each. The results are summarized in Table 2. Of the 24 pairs of reflections, 22 pairs indicated the absolute configuration of the structure as shown in Figure 1, one pair indicated opposite configuration, and one pair did not show any consistent absolute configuration.

TABLE 2. Comparison of Observed and Calculated Bijvoet Differences^a

h	k	l	DEL (%)		SF	
			obsd	calcd	obsd	calcd
1	6	4	-10.48	-9.59	-3.32	-0.35
1	1	7	2.68	5.57	2.87	0.37
1	3	7	-77.10	-16.02	-16.47	-0.56
1	1	11	-3.80	-6.67	-2.65	-0.38
1	2	11	-6.91	-7.51	-3.60	-0.39
2	1	12	-1.03	-7.57	-0.41	-0.86
2	2	17	1.71	9.50	0.56	0.35
2	4	-18	4.19	8.54	2.10	0.43
3	3	-2	-0.83	6.52	-0.71	0.35
3	4	-5	9.94	-7.07	5.11	-0.35
1	4	-5	-4.01	-7.05	-2.93	-0.34
4	8	-5	-6.93	-6.51	-2.88	-0.34
5	2	14	-1.75	-6.60	-0.90	-0.35
2	2	9	1.90	4.90	0.79	0.33
3	2	16	-1.87	-5.60	-0.91	-0.32
0	8	0	-7.61	-14.30	-2.51	-0.63
1	2	4	-7.64	-10.59	-6.32	-0.61
1	4	8	12.59	27.68	0.44	0.40
3	1	19	-5.89	-12.39	-1.41	-0.36
4	3	16	3.54	6.22	2.39	0.34
7	2	-4	1.83	8.19	0.36	0.33
1	7	-7	22.21	14.77	2.96	0.25
4	8	-1	7.88	19.60	0.88	0.26
6	5	2	18.01	27.23	1.21	0.25

^aThe parameters which are used for the purpose are defined by:

$$\text{DEL} = \{ [F^2(+)-F^2(-)] / \frac{1}{2}[F^2(+)+F^2(-)] \} \times 100 \text{ (calcd)}$$

$$\text{DEL (obsd)} = \{ [I(+)-I(-)] / \frac{1}{2}[I(+)+I(-)] \} \times 100, \text{ where } F^2(-) = F^2(hkL), F^2(-) = F^2(\overline{hkL}), \text{ etc.}$$

$$\text{and SF (calcd)} = [F^2(+)-F^2(-)] / \sigma(F_o^2)$$

SF (obsd) = $[I(+)-I(-)] / \sigma(I)$, where $\sigma(F_o^2)$, obtained from counting statistics (4c), reflects accuracy of a single measurement, and $\sigma(I) = [1/(N-1) \sum_{j=1}^N (I_j - \bar{I})^2]^{1/2}$, \bar{I} is the mean of N measurements.

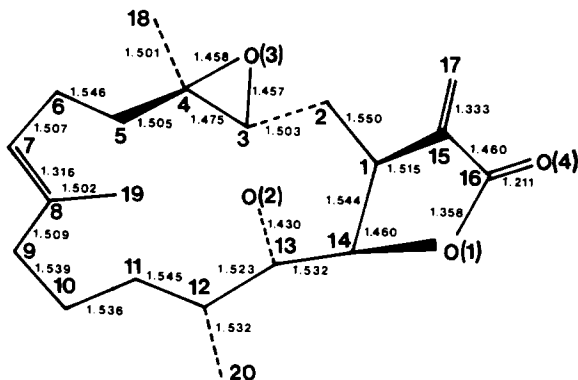


FIGURE 1. Chemical structure, numbering scheme, and bond distances (Å) in bis-epieupalmerin. Estimated standard deviations: 0.004-0.005 Å.

RESULTS AND DISCUSSION

Extracts of *E. succinea* collected at St. Croix, U.S. Virgin Islands, yielded a new cembranolide, mp 144-145°, $[\alpha]_D -158.8^\circ$ with the formula $C_{20}H_{30}O_4$ as determined by mass spectral and combustion analysis. Ir absorptions for hydroxyl (3560

cm^{-1}), carbonyl (1760 cm^{-1}), and exocyclic methylene groups (980 cm^{-1}) were observed for the new compound. The pmr spectrum exhibited signals typical of the known cembranolides: one secondary methyl ($\delta\ 0.91$), one vinyl methyl ($\delta\ 1.57$), one quaternary methyl deshielded by oxygen ($\delta\ 1.24$), protons of an α -methylene- γ -lactone ($\delta\ 5.75\text{ s}, 2\text{ Hz}$ and $6.40\text{ d}, 2\text{ Hz}$) and several oxygen deshielded methine protons ($\delta\ 3.42, 3.75, 4.30$ and 5.01). Careful comparisons of these physical and spectral properties with those of known cembranolides confirmed that this was a new compound. Single crystal X-ray analysis established the structure **6** for this new cembranolide, which is designated 12,13-bisepieupalmerin by comparison to the previously isolated eupalmerin acetate (4-7).

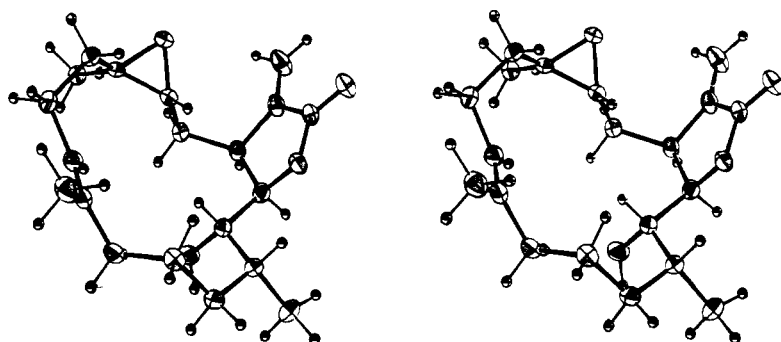


FIGURE 2. Stereoview of a single molecule. Thermal ellipsoids correspond to 50% probability.

Atom numbering scheme (23) for cembranolide **6** and its bond distances are given in Figure 1. An ORTEP (24) stereoview is shown in Figure 2. The molecule has a γ -lactone C(1) and C(14). The C-7,8 double bond has the E configuration. The bond lengths and bond angles are normal and compare well with those in eupalmerin acetate (**3**) with a few exceptions (6). The bonds C(1)-C(2), C(13)-C(14) and C(15)-C(16) in the two structures differ by 0.021, 0.015 and 0.028 Å, respectively, while angles C(1)-C(2)-C(3), C(4)-C(5)-C(6), C(5)-C(6)-C(7) and C(11)-C(12)-C(13) differ by 6.5, 4.7, 5.3 and 5.9°, respectively. The 14-membered ring is characterized by a good degree of planarity with the maximum deviation of 0.770 Å for the atom C(5). Figure 3 shows a comparison of the planarity of the 14-membered ring and the endocyclic torsion angles

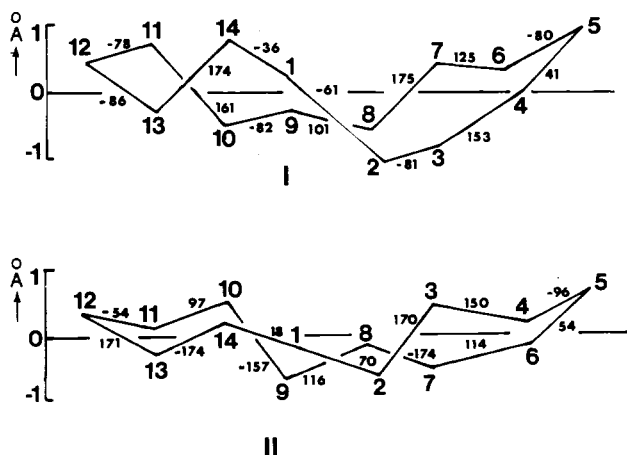


FIGURE 3. Torsion angles ($^{\circ}$) and deviations (\AA) from the least squares plane in the cembrane ring in eupalmerin acetate (I) and bis-epieupalmerin (II).

TABLE 3. Positional Parameters ($\times 10^4$) of Nonhydrogen Atoms

Atom	x	y	z
O(1)	25(4) ^a	5460(3)	1087(1)
O(2)	3917(4)	8242(3)	2089(1)
O(3)	4493(4)	1767(3)	2363(1)
O(4)	-1069(4)	3181(4)	495(1)
C(1)	3976(5)	5199(4)	1177(2)
C(2)	5247(5)	4604(4)	1914(2)
C(3)	4050(5)	3494(4)	2392(2)
C(4)	5222(5)	2662(4)	3038(2)
C(5)	4035(6)	2242(4)	3701(2)
C(6)	4387(6)	3509(5)	4329(2)
C(7)	3801(5)	5186(4)	4051(2)
C(8)	2196(5)	6137(4)	4221(2)
C(9)	1789(5)	7720(4)	3817(2)
C(10)	-470(6)	7808(5)	3349(2)
C(11)	-550(6)	9037(4)	2711(2)
C(12)	-188(5)	8314(4)	1947(2)
C(13)	1925(5)	7325(4)	1955(2)
C(14)	2058(5)	6400(4)	1228(2)
C(15)	2810(5)	3825(4)	744(2)
C(16)	430(5)	4052(5)	742(2)
C(17)	3642(6)	2544(6)	425(2)
C(18)	7683(5)	2836(4)	3196(2)
C(19)	613(6)	5753(5)	4780(2)
C(20)	-369(7)	9675(5)	1370(2)

^aStandard deviations ($\times 10^4$) are given in parentheses.

of eupalmerin acetate and the present structure. The five-membered ring of the γ -lactone is also planar. The maximum deviations occur for C(1) and C(14) (0.107Å and 0.120Å) which lie on the opposite sides of the best plane. The carbonyl oxygen lies very close to this plane (0.033Å), and the methylene carbon lies at a distance of 0.190Å). There is one very weak intermolecular hydrogen bond (2.975Å) between O(2) and O(3) (x,y+1,z), with O(2)-H . . . O(3) distance of 2.219Å and the O(2)-H . . . O(3) angle of 150°.

12,13-Bisepieupalmerin (**6**) has the proper stereochemistry to be a logical biosynthetic precursor to eunicin (**1a**) and jeunicin (**2**). Transannular back-side attack of the C-13 hydroxyl group in **6** at C-3 or C-4 of the epoxide would lead respectively to **1a** and **2**. This hypothetical interconversion derives support from the fact that **1a** and **2** were found along with **6** in extracts of *E. succinea* from St. Croix.

From specimens of *E. succinea* collected at South Caicos Island, the cembranolides eunicin (**1a**) (large amounts) and eunicin acetate (**1b**) (small amounts) were isolated. Acetate **1b** has not previously been reported as a natural product but has been prepared from eunicin (17).

The mother liquors from direct crystallization of 12,13-bisepieupalmerin (**6**) from Fraction A of St. Croix *E. succinea* and the corresponding mother liquors from South Caicos *E. succinea* were each chromatographed on silica gel, using hexane as eluant to obtain sesquiterpene hydrocarbons. The isomeric hydrocarbons (-)-germacrene-A and (+)- β -elemene were the predominant sesquiterpenes isolated from St. Croix specimens. (+)- β -Epibourbonene, (+)- β -copaene, and (+)- α -muurolene were isolated in pure form from the South Caicos specimens and germacrene-A was identified by gc and pmr analysis of a partially purified fraction.

The sesquiterpene content of *E. succinea* from the two different locations was not

studied in sufficient detail to know whether significant differences exist. Germacrene-A has been isolated previously (13) from *E. mammosa* collected at Bimini in the Bahamas and has been identified as the alarm pheromone of the alfalfa aphid *Therioaphis maculata* (25).

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