Further Bioactive Cembranolide Diterpenes from the Gorgonian Eunicea succinea

Abimael D. Rodríguez, and Hiresh Dhasmana

J. Nat. Prod., **1993**, 56 (4), 564-570• DOI:
10.1021/np50094a017 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 4, pp. 564-570, April 1993

FURTHER BIOACTIVE CEMBRANOLIDE DITERPENES FROM THE GORGONIAN *EUNICEA SUCCINEA*

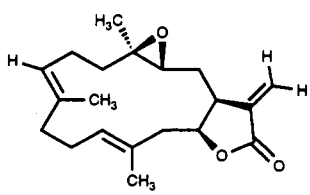
ABIMAEL D. RODRÍGUEZ* and HIRESH DHASMANA

Department of Chemistry, PO Box 23346, U.P.R. Station, University of Puerto Rico, Río Piedras, Puerto Rico 00931-3346

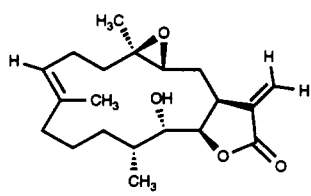
ABSTRACT.—In our continuing comparative study of the diterpenoid content of the Caribbean gorgonian *Eunicea succinea* from different locations around Puerto Rico, three new cembranolide diterpenes, named 12,13-bisepieupalmerin acetate [**5**], 12-*epi*-eupalmerin acetate [**6**], and succinolide [**7**], were isolated from specimens collected off the east coast of Puerto Rico. The structure of each compound was deduced spectroscopically, and those of **5** and **7** were confirmed by chemical correlation with compounds of known structure.

Gorgonians of the genus *Eunicea* (phylum Coelenterata, class Anthozoa, subclass Octocorallia, order Gorgonacea, family Plexauridae) are very common in the shallow waters of the Caribbean region and have yielded a number of sesquiterpenes and a variety of diterpenes which are, with a few exceptions, lactones derived from the cembrane skeleton (1). Studies of the terpenoid content in the gorgonian *Eunicea succinea* (Lamouroux) were initiated by Ciereszko almost forty years ago and have been since continued by Schmitz and his collaborators at the University of Oklahoma (2,3). We have also recently reported the isolation of the cembranolide diterpene euniolide [**1**] from specimens of *E. succinea* and also from *Eunicea mammosa* collected in Puerto Rico (4). In connection with our studies on the distribution of diterpenes across specimens of *E. succinea* collected at various locations around Puerto Rico, we now report the isolation and structure elucidation of three further cembranolide diterpenes isolated in the course of these investigations.

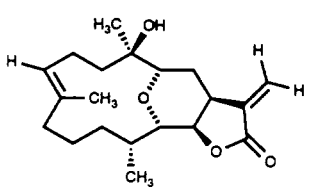
Extraction of a freshly collected sample of the gorgonian *E. succinea* collected near Palomino Island, northeast of Puerto Rico, afforded a rich MeOH/$CHCl_3$ extract (13.3% of the dry wt of the coral), from which the major non-lipid components euniolide [**1**], 12,13-bisepieupalmerin [**2**], eunicin [**3**], and cueunicin [**4**], were isolated after successive size exclusion (Bio-Beads SX-2) and adsorption (Si gel) chromatography of the hexane solubles. These components represented 6.92% of the organic extract and have previously been described in the literature (2–7). The remain-



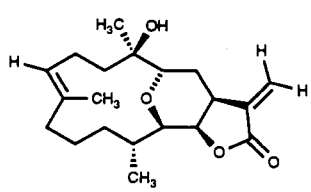
1



2



3

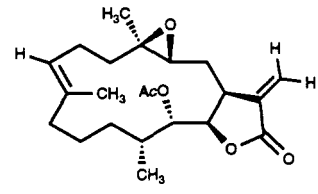


4

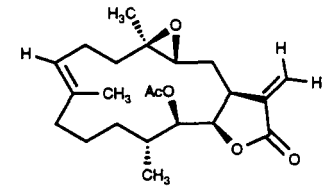
ing and noticeably less abundant components **5**, **6**, and **7** (0.024%; based on the weight of freeze-dried coral) were obtained after repeated chromatography of some of the minor non-lipid fractions.

Among the minor diterpenoid components isolated from *E. succinea*, we found a new compound **5** (*m/z* 376.22501, $C_{22}H_{32}O_5$ by hreims). The ir spectrum indicated the absence of hydroxyl functions and the presence of an unsaturated lactone conjugated to an exomethylene (ir 1769 and 1670 cm^{-1}). Moreover, strong bands at 1742 and 1231 cm^{-1} suggested a second ester carbonyl function. The ^1H nmr of **5** (two 1H broad singlets at δ 6.39 and 5.77) strongly suggested the presence of a γ-lactone conjugated to an exomethylene group. The presence of three 3H singlets at δ 2.00, 1.53, and 1.26 indicated an acetate ester group, a methyl substituted olefin, and a tertiary methyl carbinol, respectively, while the doublet at δ 0.93 is ascribable to the methyl group bonded at C-12. The coupling constant of the broad triplet centered at δ 4.37 (1H, $J = 9.3$ Hz), 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 (8). The ^1H-^1H COSY spectra revealed that the doublet centered at δ 5.13 (1H, $J = 9.3$ Hz) assigned to H-13 is strongly coupled only to H-14. This behavior closely resembles that observed previously for 12,13-bisepieupalmerin [**2**] possessing identical relative stereochemistry at C-13 and C-14; a strong crosspeak connecting H-13 only to H-14 is observed in the contour plot of the ^1H-^1H COSY spectrum of both **2** and **5**. This is consistent with the proposed stereochemical assignment of the acetate ester group at C-13 in **5**. In general, the ^1H-^1H COSY allowed us to establish the chain of couplings in the spin system comprised by the proton sequence H-13 to H-3 around the α-methylene-γ-lactone.

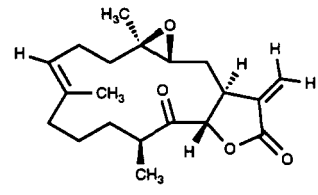
The ^{13}C-nmr spectrum of **5** (see Table 1) showed a striking similarity to that of 12,13-bisepieupalmerin [**2**]. The differences observed could be explained reasonably



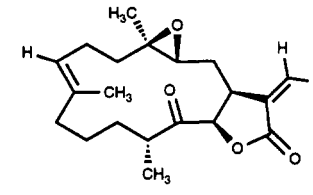
5



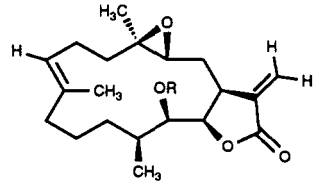
6



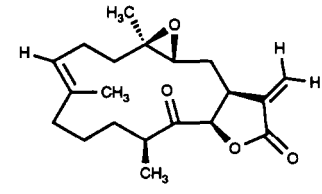
7



8



9 R=H
10 R=Ac



11

TABLE 1. ^{13}C-nmr ($CDCl_3$, 75 MHz) Data for Cembranolide Diterpenes **2**, **5**, **6**, **7**, and **8**.[a]

Carbon	Compound				
	2[b]	**5**	**6**	**7**	**8**
C-1	39.37 (d)	39.41 (d)	41.97 (d)	39.17 (d)	40.72 (d)
C-2	30.79 (t)	31.56 (t)	27.25 (t)	31.96 (t)	30.23 (t)
C-3	60.01 (d)	58.50 (d)	57.40 (d)	59.75 (d)	57.83 (d)
C-4	59.83 (s)	60.18 (s)	60.33 (s)	60.77 (s)	58.95 (s)
C-5	37.98 (t)	37.62 (t)	38.57 (t)	37.58 (t)	37.93 (t)
C-6	23.47 (t)	22.92 (t)	22.63 (t)	23.48 (t)	22.40 (t)[c]
C-7	124.56 (d)	125.44 (d)	126.18 (d)	125.33 (d)	125.98 (d)
C-8	136.01 (s)	135.55 (s)	135.21 (s)	134.12 (s)	135.12 (s)
C-9	37.19 (t)	36.33 (t)	35.99 (t)	36.08 (t)	37.03 (t)
C-10	23.24 (t)	21.76 (t)	24.68 (t)	23.45 (t)	21.28 (t)[c]
C-11	31.43 (t)	31.31 (t)	32.37 (t)	29.99 (t)	28.38 (t)
C-12	31.61 (d)	30.43 (d)	36.28 (d)	43.58 (d)	41.18 (d)
C-13	71.94 (d)	74.21 (d)	71.82 (d)	207.96 (s)	211.62 (s)
C-14	78.52 (d)	77.90 (d)	76.92 (d)	81.17 (d)	81.40 (d)
C-15	138.96 (s)	138.82 (s)	138.68 (s)	137.11 (s)	136.83 (s)
C-16	169.96 (s)	169.48 (s)[c]	169.60 (s)[c]	168.87 (s)	169.03 (s)
C-17	123.59 (t)	124.14 (t)	117.17 (t)	123.02 (t)	123.68 (t)
C-18	16.44 (q)	17.14 (q)	17.42 (q)	17.32 (q)	17.46 (q)
C-19	15.61 (q)	15.66 (q)	16.54 (q)	15.84 (q)	15.53 (q)
C-20	12.19 (q)	12.35 (q)	15.77 (q)	14.32 (q)	14.81 (q)
C-21	—	170.01 (s)[c]	169.64 (s)[c]	—	—
C-22	—	20.79 (q)	20.95 (q)	—	—

[a]^{13}C-nmr multiplicities 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 ppm downfield from TMS.

[b]For the original ^{1}H-nmr and ^{13}C-nmr data of diterpene **2** see Gopichand *et al.* (3) and Morales *et al.* (4).

[c]Signals within a column may be reversed.

upon replacing the hydroxyl group attached to C-13 in **2** with an acetoxy ester function. These observations indicated that diterpene **5** was simply the acetate ester derivative of alcohol **2**. Indeed, when a solution of **2** in pyridine/Ac_2O (each reagent was freshly distilled prior to use) was stirred at 25° overnight, a compound identical to natural product **5** with regard to ^{1}H- and ^{13}C-nmr, ir, and mass spectra was obtained [an earlier attempt to perform the same transformation using undistilled reagents was unsuccessful as previously reported (9)]. These data established the structure of **5**, named 12,13-bisepieupalmerin acetate, as shown, including both the relative and absolute stereochemistry.

The new cembranoid diterpene 12-*epi*-eupalmerin acetate [**6**], showed a molecular ion peak at *m/z* 376.22495 in the hreims spectrum, corresponding to the molecular

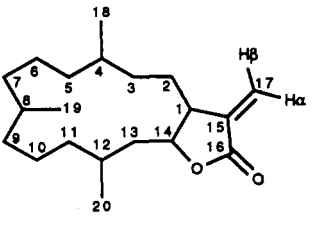


FIGURE 1. γ-Cembranolide skeleton.

composition of $C_{22}H_{32}O_5$ ($[M]^+$ requires 376.22495). The spectral values obtained for diterpene **6** from the ir (ν max 1776, 1738 cm^{-1}), uv (λ max 242, log ε 2.81), and ^1H-nmr spectra [δ 6.10 (1H, d, $J=3.3$ Hz) and 5.31 (1H, d, $J=3.3$ Hz)] were quite similar to the values obtained for 12,13-bisepieupalmerin acetate [**5**]. The remaining ^1H-nmr resonances as well as those observed in the ^{13}C-nmr spectrum of **6** (Table 1) also showed a remarkable similarity to the spectral values recorded for **5**. From these observations, diterpene **6** was suggested to have the same overall structure as 12,13-bisepieupalmerin acetate [**5**] but is epimeric at C-13 bearing the acetate ester group. The proposed stereochemical assignment at C-13 is based on the observation that, contrary to acetate **5**, no cross peaks connecting the H-13 (δ 5.04, d, $J=3.6$ Hz) and H-14 (δ 4.85, d, $J=8.1$ Hz) protons are observed in the contour plot of the ^1H-^1H COSY spectra of **6**. This peculiarity has been observed previously in two closely related cembranoid diterpenes, namely eupalmerin [**9**] and eupalmerin acetate [**10**], isolated from the gorgonian *E. mammosa* and possessing identical relative stereochemistry at C-13 and C-14 (9,10). The absence of coupling ($J<1$ Hz) between H-13 and H-14 in **6**, **9**, and **10** may be attributed to the combined electronegativity effects of vicinal trans-coplanar oxygen atoms on the coupling strengths of the H-13 and H-14 protons (11). The dihedral angles between these protons diminish the coupling strength of each proton, reducing their mutual coupling to less than 1 Hz. Thus, the structure of 12-*epi*-eupalmerin acetate, including its relative stereochemistry, must be as shown in **6**. The new diterpene **6** has the appropriate stereochemistry to make it a logical biosynthetic precursor to cueunicin [**4**], which was found to occur as a minor component in the same specimen of *E. succinea*.

A molecular formula of $C_{20}H_{28}O_4$ was established for diterpene **7** from hreims (332.19893, calcd 332.19874), plus ^1H- and ^{13}C-nmr data (Table 1). The ir spectrum contained two carbonyl bands at 1775 and 1723 cm^{-1}, consistent with the presence of the α-methylene-γ-lactone and ketone functionalities, respectively, and indicated the absence of hydroxyl groups. Consideration of ^1H- and ^{13}C-nmr data, and specifically the results of COSY spectra analyses, allowed the complete structure of **7**, named succinolide, to be assigned. The ^1H-nmr spectrum showed two peaks at δ 6.34 and 5.67 due, respectively, to H-17α and H-17β of an α-methylene-γ-lactone, and signals at δ 5.09 (H-7), 4.92 (H-14), 3.41 (H-1), 2.98 (H-12), and 2.70 (H-3). The two singlets at δ 1.58 and 1.25 have been attributed to the methyl groups placed, respectively, at C-8 and C-4, while the doublet at δ 1.11 is ascribable to the methyl group bonded at C-12. The ^1H-^1H correlation COSY experiment shows that the complex multiplet at δ 3.41 (H-1) has responses to two nonequivalent methylene protons (δ 1.95, 1.80, H-2) and a methine proton (δ 4.92, H-14), which in turn showed no further responses. This latter proton and the chemical shift of its corresponding carbon (δ 81.17, C-14) indicate the location of the attachment of the oxygen of the γ-lactone and that of the new ketone functionality. That the ketone function (δ 207.96) in **7** must indeed be placed at C-13 stems from the overall downfield shift experienced by H-12 now resonating at δ 2.98 (vs. 1.78 ppm in **9**) and C-14 absorbing at δ 81.17 (vs. 79.01 ppm in **9**). The remaining ^1H-^1H COSY responses observed were consistent with the proposed structure for succinolide. The coupling constant for the sharp doublet centered at δ 4.92 (1H, $J=4.8$ Hz), assigned to the hydrogen on the γ-carbon of the lactone, indicated that the two lactone protons (H-1 and H-14) are trans to each other (8). The relative trans spatial arrangement between the protons at chiral centers C-1 and C-14 was also established by nOeds (12) involving the irradiation of the C-14-substituted proton (δ 4.92). This did not result in significant enhancement of the methine proton at C-1 (δ 3.41) but instead caused the enhancement of H-2 resonating upfield at δ 1.80. These results are consistent with the bridgehead transoid juncture proposed in **7**. In order to confirm the trans-

oid stereochemistry of H-1 and H-14 in succinolide [**7**], we oxidized 12,13-bisepieupalmerine [**2**] to the corresponding cis fused α-methylene-γ-lactone **8**, named 12-*epi*-eupalmerone, using the Swern oxidation method (13). After purification, the semisynthetic material **8** was shown to be strikingly similar to natural product **7** with regard to ^{1}H-nmr, ir, uv, and ms analyses. Tlc and ^{13}C-nmr analyses (Table 1), however, demonstrated that the two compounds were not identical. Moreover, the coupling constant for the sharp doublet also centered at δ 4.92 (1H, J = 7.5 Hz) in **8**, assigned likewise to the hydrogen on the γ-carbon of the lactone, indicated that the two lactone protons H-1 and H-14 are now cis to each other (8). The one other noticeable difference between the ^{1}H-nmr spectra of ketones **7** and **8** is the relative position of the Me-20 doublet signal at δ 1.11 in **7** (vs. δ 1.15 in **8**) with respect to that of the Me-18 singlet (δ 1.25 in **7** vs. δ 1.17 in **8**). Mainly owing to the fact that the ^{1}H-nmr spectra of ketones **7** and **8** are otherwise essentially identical, succinolide [**7**] and 12-*epi*-eupalmerone [**8**] must, therefore, be epimers at carbons C-12 and C-14. Furthermore, when we compared the ^{13}C-nmr spectra of ketones **7** and **8**, we observed that the chemical shift values for the C-13 resonances (207.96 vs. 211.62, respectively) were significantly different. This variation in chemical shift may reflect configurational changes at C-12 rather than conformational differences due to the transoid bridgehead stereochemistry in **7**. In order to support this contention, we also synthesized cembranolide ketone **11** by the Swern oxidation of eupalmerine [**9**] which we had isolated earlier in our laboratory from a specimen of *E. mammosa* (9). We found that the chemical shift value for the corresponding C-13 resonance in semisynthetic eupalmerone [**11**] was 208.6 ppm. Based on these arguments, we propose that the relative stereochemistry of the methyl group bonded at C-12 is as depicted in structure **7**. The relative stereochemistry shown at C-12 is also supported by a strong nOe crosspeak between H-12 and H-14 in the NOESY spectrum of **7**. With the possible exception of epipeunicin, an unstable cembranolide diterpene isolated earlier from a specimen of *E. succinea* collected in Panama (14), succinolide [**7**] appears to be the first stable cembranolide diterpene possessing a transoid fused α-methylene-γ-lactone moiety isolated from a Caribbean gorgonian.

All the new compounds reported here exhibited in vitro antitumor activity against the human colon (HCT 116), human breast adenocarcinoma (MCF-7), human T-cell leukemia (CCRF-CEM), or melanoma (SK5-MEL) cell lines within the limited concentration range of 0.01–0.1 μg/ml. The cytotoxic activities of the new compounds were as follows: 12,13-bisepieupalmerin acetate [**5**] [HCT 116 (IC_{50} = 0.4 μg/ml); MCF-7 (IC_{50} = 6.0 μg/ml); SK5-MEL (IC_{50} = 5.0 μg/ml)], 12-*epi*-eupalmerin acetate [**6**] [HCT 116 (IC_{50} = 8.0 μg/ml); MCF-7 (IC_{50} = 8.0 μg/ml); CCRF-CEM (IC_{50} = > 50 μg/ml)], and succinolide [**7**] [HCT 116 (IC_{50} = 2.0 μg/ml); MCF-7 (IC_{50} = 3.0 μg/ml); CCRF-CEM (IC_{50} = >50 μg/ml)].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H nmr and ^{13}C nmr were measured at 300 and 75 MHz, respectively, with a General Electric QE-300 spectrometer. ^{1}H-nmr chemical shifts are reported as δ values in ppm relative to either TMS (0.0 ppm) or $CHCl_3$ (7.26 ppm). Data is reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), and coupling constant (Hz). ^{13}C chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). Ir spectra were recorded on a Nicolet 600 FT-IR spectrophotometer, and uv spectra were recorded on a Hewlett-Packard Chem Station 8452A spectrometer. Analytical tlc was performed using 0.25 mm Analtech Uniplate precoated Si gel plates. Optical rotations were determined on a Perkin-Elmer Polarimeter Model 243B. All solvents used were either spectral grade or were distilled from glass prior to use.

EXTRACTION AND ISOLATION PROCEDURES.—Minced and freeze-dried *E. succinea* (352.6 g), collected at Palomino Island, Puerto Rico in May 1990, was extracted exhaustively with 3.5 liters $CHCl_3$-

MeOH (1:1); a voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The dried residue after filtration and concentration amounted to ca. 47.0 g and was partitioned against hexane and H_2O giving, after subsequent rotaevaporation, 24.0 g of lipids as a viscous dark green oil. A portion of the hexane extract (10.0 g) was dissolved in toluene (5 ml) and passed through a Bio-Beads SX-2 (toluene) column. The fractions eluting last contained all the terpenoid material and were combined on the basis of tlc analyses. After concentration in vacuo, the oily mixture (2.38 g) was purified by cc on Si gel (80 g; 35–75 mesh, Analtech) using EtOAc/hexane of increasing polarity. The less polar portion of the lipids was fractionated roughly into fractions A through G: fraction A (euniolide [**1**], 850 mg, 0.58%); fraction B (succinolide [**7**], 10 mg, 0.007%); fraction C (12-*epi*-eupalmerin acetate [**6**], 12 mg, 0.008%); fraction D (12,13-bisepieupalmerin acetate [**5**], 14 mg, 0.009%); fraction E (eunicin [**3**], 361 mg, 0.24%); fraction F (cueunicin [**4**], 18 mg, 0.012%); and fraction G (12,13-bisepieupalmerin [**2**], 126 mg, 0.085%).

12,13-Bisepieupalmerin acetate [**5**].—Colorless oil: ir (neat) 2985, 2939, 1774, 1769, 1742, 1670, 1268, 1231, 1024, 772 cm^{-1}; uv λ max $CHCl_3$ (log ε) 242 (3.02) nm; $[\alpha]^{27}D$ −170° (*c* = 0.4, $CHCl_3$); ^1H nmr ($CDCl_3$, 300 MHz) δ 6.39 (1H, br s, H-17α), 5.77 (1H, br s, H-17β), 5.13 (1H, d, *J* = 9.3 Hz, H-13), 5.03 (1H, br t, *J* = 6.5 Hz, H-7), 4.37 (1H, br t, *J* = 9.3 Hz, H-14), 3.26 (1H, m, H-1), 2.57 (1H, br t, H-3), 2.35 (1H, m, H-2α), 2.00 (3H, s, Me-22), 1.70 (1H, m, H-2β), 1.53 (3H, s, Me-19), 1.26 (3H, s, Me-18), 0.93 (3H, d, *J* = 6.6 Hz, Me-20); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hreims *m/z* $[M]^+$ 376.22501 (2.6%) ($C_{22}H_{32}O_5$ requires 376.22495), 316 (2.1), 189 (3.3), 151 (14), 121 (16), 113 (15), 107 (24), 95 (31), 81 (37), 67 (24), 55 (34).

Acetylation of 12,13-bisepieupalmerin [**2**].—Treatment of alcohol **2** (20 mg) with freshly distilled Ac_2O (0.2 ml) and pyridine (0.2 ml) afforded, after stirring at 25° for 12–14 h, followed by usual workup and rapid cc on Si gel [eluted with EtOAc-hexane (30:70)], the corresponding acetate (18 mg) identical with regard to tlc and ^1H- and ^{13}C-nmr, ir, uv, and mass spectra to the natural product **5**.

12-epi-*Eupalmerin acetate* [**6**].—Colorless oil: ir (neat) 2923, 2853, 1776, 1738, 1457, 1374, 1261, 1232, 1101, 1031, 992 cm^{-1}; uv λ max $CHCl_3$ (log ε) 242 (2.81) nm; $[\alpha]^{25}D$ +17.8° (*c* = 0.9, $CHCl_3$); ^1H nmr ($CDCl_3$, 300 MHz) δ 6.10 (1H, d, *J* = 3.3 Hz, H-17α), 5.31 (1H, d, *J* = 3.3 Hz, H-17β), 5.08 (1H, m, H-7), 5.04 (1H, d, *J* = 3.6, H-13), 4.85 (1H, d, *J* = 8.1 Hz, H-14), 3.18 (1H, m, H-1), 2.91 (1H, dd, *J* = 6.3, 7.8 Hz, H-3), 2.32 (1H, m, H-6α), 1.91 (3H, s, Me-22), 1.64 (3H, s, Me-19), 1.32 (3H, s, Me-18), 1.04 (3H, d, *J* = 7.2 Hz, Me-20); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hreims *m/z* $[M]^+$ 376.22495 (0.7%) ($C_{22}H_{32}O_5$ requires 376.22495) 316 (1.3), 302 (3), 298 (4), 257 (11), 236 (11), 201 (6), 194 (5), 183 (48), 155 (9), 147 (10), 145 (10), 135 (14), 133 (14), 109 (30), 107 (23), 95 (46), 85 (34), 81 (52), 57 (100).

Succinolide [**7**].—Stable, colorless oil: ir (neat) 2927, 2854, 1775, 1723, 1461, 1383, 1264, 1236, 1099, 993 cm^{-1}; uv λ max MeOH (log ε) 214 (3.72) nm; $[\alpha]^{25}D$ −7.5° (*c* = 0.8, $CHCl_3$); ^1H nmr ($CDCl_3$, 300 MHz) δ 6.34 (1H, d, *J* = 2.7 Hz, H-17α), 5.67 (1H, d, *J* = 2.4 Hz, H-17β), 5.09 (1H, t, *J* = 6.3 Hz, H-7), 4.92 (1H, d, *J* = 4.8 Hz, H-14), 3.41 (1H, m, H-1), 2.98 (1H, m, H-12), 2.70 (1H, dd, *J* = 5.1, 6.3 Hz, H-3), 2.25–1.65 (broad envelope), 1.58 (3H, s, Me-19), 1.25 (3H, s, Me-18), 1.11 (3H, d, *J* = 6.6 Hz, Me-20); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hreims *m/z* $[M]^+$ 332.19893 (2.5%) ($C_{20}H_{28}O_4$ requires 332.19874), 286 (1), 247 (2), 238 (7), 205 (2), 181 (4), 161 (6), 151 (14), 147 (11), 136 (19), 133 (17), 119 (23), 109 (43), 95 (81), 81 (100), 67 (70).

Oxidation of 12,13-bisepieupalmerin [**2**] *to 12*-epi-*eupalmerone* [**8**].—Oxalyl chloride (0.5 ml) dissolved in dry CH_2Cl_2 (5 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_2Cl_2 (3 ml) over a period of 2–3 min, and stirring was continued for 6–8 min. A solution of 12,13-bisepieupalmerin [**2**] (100 mg) dissolved in CH_2Cl_2 (4 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 15–20 min, the cooling bath was removed and distilled H_2O (20 ml) was added at room temperature while stirring was continued for the next 15 min. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (4 × 20 ml). The combined organic extract was washed with distilled H_2O (1 × 30 ml) and dried thoroughly over $MgSO_4$. After removal of the solvent in vacuo the oily residue was loaded on a Si gel column (8 g) and eluted with EtOAc-hexane (8:92). Combination of like fractions on the basis of tlc analyses led to the isolation of semisynthetic 12-*epi*-eupalmerone [**8**] (40 mg): white crystalline solid; mp 95.4°; ir (neat) 2962, 2930, 2856, 1772, 1715, 1457, 1262, 1095, 1017, 800, 758 cm^{-1}; uv λ max MeOH (log ε) 210 (3.58) nm; $[\alpha]^{25}D$ −27.5° (*c* = 0.8, $CHCl_3$); ^1H nmr ($CDCl_3$, 300 MHz) δ 6.39 (1H, d, *J* = 1.8 Hz, H-17α), 5.72 (1H, d, *J* = 1.5 Hz, H-17β), 4.98 (1H, t, *J* = 6.0 Hz, H-7), 4.92 (1H, d, *J* = 7.5 Hz, H-14), 3.53 (1H, m, H-1), 3.03 (1H, m, H-12), 2.51 (1H, dd, *J* = 2.5, 6.3 Hz, H-3), 2.12–1.87 (broad envelope), 1.54 (3H, s, Me-19), 1.17 (3H, s, Me-18), 1.15 (3H, d, *J* = 6.9 Hz, Me-20); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hreims *m/z* $[M]^+$ 332.19916 (8.0%) ($C_{20}H_{28}O_4$ requires 332.19874), 314 (3), 289 (2), 271 (2), 257 (1), 248 (2), 237 (3), 220 (7), 206 (4), 136

(20), 108 (80), 95 (100), 81 (94), 67 (66). A similar experimental procedure was followed during the oxidation of eupalmerin [**9**] (50 mg) into semisynthetic eupalmerone [**11**] (10 mg). However, most of the starting alcohol **9** was recovered unchanged after cc [Si gel; EtOAc-hexane (8:92)] at the end of the reaction.

ACKNOWLEDGMENTS

The assistance of Dr. Paul Yoshioka of La Parguera Marine Biological Station, University of Puerto Rico at Mayagüez for identification of the gorgonian and of Mr. José J. Morales in specimen collection is gratefully acknowledged. The authors also acknowledge the assistance provided by Miss Brendáliz Rivera during the early stages of this work. We also thank Dr. Louis R. Barrows from the University of Utah Center for Drug Discovery for cytotoxicity assays on human tumor cells. Hreims spectral determinations were performed by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Facility (Grant No. CHE8211164). This study was supported in part by the National Science Foundation EPSCoR Program (Grant No. R118610677), the NSF-MRCE Program (Grant No. R11-8802961) and the NIH-MBRS Program (Grant No. S06 RR08102-17).

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **8**, 97 (1991), and previous papers in the series, and references cited therein.
2. L.S. Ciereszko, D.H. Sifford, and A.J. Weinheimer, *Ann. N.Y. Acad. Sci.*, **90**, 917 (1960).
3. Y. Gopichand, L.S. Ciereszko, F.J. Schmitz, D. Switzner, A. Rahman, M.B. Hossain, and D. van der Helm, *J. Nat. Prod.*, **47**, 607 (1984).
4. J.J. Morales, J.R. Espina, and A.D. Rodríguez, *Tetrahedron*, **46**, 5889 (1990).
5. R.A. Gross Jr., "Part I. Cueunicin and Cueunicin Acetate: Two New Marine Cembranolides," Ph.D. Thesis, University of Oklahoma, Norman, 1974.
6. R.T. Gampe Jr., M. Alam, A.J Weinheimer, G.E. Martin, J.A. Matson, M.R. Willcott, R.R. Inners, and R.E. Hurd, *J. Am. Chem. Soc.*, **106**, 1823 (1984).
7. A.J. Weinheimer, R.E Middlebrook, J.O. Bledsoe Jr., W.E. Marsico, and T.K.B. Karns, *Chem. Commun.*, 384 (1968).
8. A. Ahond, B.F. Bowden, J.C. Coll, J.D. Fourneron, and S.J. Mitchell, *Aust. J. Chem.*, **32**, 1273 (1979).
9. L.A. Fontán and A.D. Rodríguez, *J. Nat. Prod.*, **54**, 298 (1991).
10. L.A. Fontán, W.Y. Yoshida, and A.D. Rodríguez, *J. Org. Chem.*, **55**, 4956 (1990).
11. H. Booth, *Tetrahedron Lett.*, **7**, 411 (1965).
12. R. Richard and K. Würthrich, *J. Magn. Reson.*, **30**, 147 (1978).
13. K. Omura and D. Swern, *Tetrahedron*, **34**, 1651 (1978).
14. C.Y. Chang, "Part II. Isolation and Characterization of Two New Cembranolides from the Gorgonian *Eunicea succinea* (Pallas): Peunicin and Epipeunicin," Ph.D. Thesis, University of Oklahoma, Norman, 1977.

Received 28 August 1992