New Cembranolides from the Gorgonian *Eunicea succinea*[1,2]

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A new chemical study of the Caribbean gorgonian *Eunicea succinea* collected in Puerto Rico afforded seven new cembranolides (**1**−**5**, **7**, and **8**). Excepting **1**, the new compounds described here possess an unusual unsaturation at either position C-1, C-6, or C-8, and all but **8** possess the C-12*R*, C-13*S* stereochemistry usually ascribed to cembranolides from *E. succinea*. Their chemical structures were carefully established by a combination of chemical and spectroscopic methods in addition to detailed NMR spectral comparisons with known cembranolide models.

Gorgonian octocorals are among the most abundant organisms found in the coral reefs, with the genus *Eunicea* being particularly diverse and abundant in the Caribbean region.[4] Despite their abundance, many *Eunicea* species have not been investigated comprehensively due, in part, to difficulties in collecting taxonomically homogeneous samples. Our laboratory has been particularly interested in the study of the gorgonian species *E. succinea* and *E. mammosa*. These organisms are characterized by the biosynthesis of cembranolide-type diterpenes.[5] A previous investigation in this laboratory of the cytotoxic constituents of specimens of *E. mammosa* collected in Puerto Rico resulted in the discovery of a new family of cembranolides known as uprolides.[6,7] These compounds represented the first examples of naturally occurring cembranolides from a Caribbean gorgonian species possessing unsaturation at position C-6 or C-8, and several had an oxo-bridge across the cembrane skeleton between C-4 and C-7. In our continuing search for new bioactive cembranolides from the Caribbean Sea, we have investigated a specimen of *E. succinea* (Pallas) (phylum Coelenterate, class Anthozoa, subclass Octocorallia, order Gorgonacea, family Plexauridae) collected near Mona Island off the west coast of Puerto Rico. We report herein the isolation and structure elucidation of seven previously unreported cembranolides, five of which belong to the "uprolide family" of Caribbean cembranolides.

Results and Discussion

Large specimens of *E. succinea* were frozen shortly after collection and freeze-dried prior to extraction using a mixture of 1:1 MeOH−$CHCl_3$. The main fractions obtained from the hexane extract consisted of several well-known α-methylene-γ-cembranolides such as eunicolide,[8] eupalmerin acetate,[9] eunicin,[10] and 12,13-bisepieupalmerin.[11] From the same hexane extract, cembranolides **1**−**5**, **7**, and **8** were isolated after conventional chromatographic procedures (see the Experimental Section).

A molecular formula of $C_{20}H_{30}O_5$ was established for compound **1** from HREIMS ([M^+] m/z = 350.2095) plus ^{1}H- and ^{13}C-NMR data. The IR spectrum contained a carbonyl band at 1753 cm^{-1} consistent with the presence of an α-methylene-γ-lactone in addition to strong hydroxyl and epoxide stretching bands at 3450 cm^{-1} and 1269 cm^{-1}, respectively. The presence of two epoxide functionalities was indicated by four ^{13}C-NMR signals at δ 58.4 (C-4), 59.5 (C-3), 60.3 (C-8), and 60.3 (C-7). Consideration of ^{1}H- and ^{13}C-NMR data as well as other spectral data allowed the complete structure of this metabolite to be assigned as 12,13-bisepieupalmerin epoxide (**1**). To confirm this observation, we treated the known compound 12,13-bisepieupalmerin[11] with *m*-CPBA in CH_2Cl_2 at 25 °C obtaining roughly a 2:1 mixture of diepoxides, the major one of which, after separation by HPLC, turned out to be identical in all respects to natural product **1**. The C-7*R*, C-8*R* (β) stereochemistry in **1** was argued based on the ^{13}C-NMR chemical shift values for C-7 and C-8.[12] Also, the NOEs detected during a PSNOESY experiment (i.e., H-3/H-13, H-14/Me-20, H-7/H10, H-7/H-3, and H-7/H-13) clearly correlated with a Dreiding model representing the relative stereochemistry shown in structure **1**.

12,13-Bisepiuprolide B (**2**) was isolated as a UV active (λ_{max} = 211 nm) colorless oil, also with a molecular formula of $C_{20}H_{30}O_5$ established from HRFABMS data. Strong IR bands due to hydroxyl (3400 cm^{-1}), epoxide (1263 cm^{-1}), and ester functionalities (1754 cm^{-1}) were clearly detected. The ^{1}H-NMR spectrum showed two partially overlapped signals at δ 5.54 and 5.58 indicative of a 1,2-disubstituted olefin at C-6 and three signals near δ 4.33, 3.73, and 2.52 that could be attributed to methine protons on carbon atoms bearing oxygen (H-14, H-13, and H-3, respectively).[6] The ^{13}C-NMR spectrum exhibited 20 signals (3 CH_3, 6 CH_2, 7 CH, and 4 C) whose chemical shift values and multiplicities hinted the presence of an epoxide [δ 61.3 (s) and 58.0 (d)], a 1,2-disubstituted olefin [δ 136.2 (d) and 128.3 (d)], a terminal olefin [δ 138.7 (s) and 124.7 (t)], two hydroxyl groups [δ 84.5 (s) and 68.0 (d)], and one ester carbonyl [δ 170.1 (s)]. At this stage we realized that the ^{1}H- and ^{13}C-NMR spectra of compound **2** were reminiscent of those reported for the known metabolite uprolide B.[6] Nevertheless, three major differences between these compounds in the ^{13}C-NMR spectra were observed: the signals ascribed to C-12, C-13, and Me-20 in **2** showed considerable differences in chemical shifts and had

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shifted from δ 35.2, 72.5, and 16.7 in uprolide B to δ 30.2, 68.0, and 13.3 in **2**, respectively. Epimerization of **2** at C12 and C13 would account for these spectral differences. Also, a NOESY experiment showed that H-14 and Me-20 were within NOE proximity, which confirmed the relative stereochemistry at C-12. Because there was severe overlapping between H-6 and H-7 in the ^{1}H-NMR spectrum we could not determine their coupling constant. Therefore, the stereochemistry at Δ^6 was deduced by comparing the NMR data of **2** with those of uprolide B. Specifically, the ^{13}C-NMR shifts at δ 84.5 (C-8) and 20.5 (Me-19) distinctively established the *Z* stereochemistry for the 1,2−disubstituted olefin.[6] A strong IR band at 756 cm^{-1} confirmed this contention. In accord with previous findings, the ^{13}C-NMR chemical shift value for C-10 (δ 19.6) and Me-19 (δ 20.5) clearly suggested the *S* relative stereochemistry for C-8.[6]

The HREIMS and ^{13}C-NMR data for compound **3** were consistent with a molecular formula of $C_{22}H_{32}O_6$. An intense IR band at 3405 cm^{-1} suggested a hydroxyl group, and two bands at 1769 and 1741 cm^{-1} were ascribed to ester carbonyl groups. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of 12,13-bisepiuprolide B (**2**) revealed only minor differences, which were consistent with the replacement of the C-13 hydroxyl group in **2** with an acetoxy group in **3**. For instance, in **2**, H-13 resonates at δ 3.73 (1H, br d, J = 9.9 Hz), whereas in **3** the same signal appears at δ 5.18 (1H, br d, J = 10.5 Hz). The presence in the NMR spectra of an acetate signal [δ 2.09 (3H, s); δ 169.4 (s) and 20.8 (q)] along with key long-range ^{1}H−^{13}C correlations obtained from HMBC experiments indicated that compound **3** was indeed 12,13-bisepiuprolide B acetate. Unfortunately, the paucity of **2** hampered our ability to correlate structures **2** and **3** chemically.

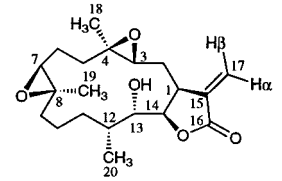
A molecular formula of $C_{20}H_{30}O_5$ was established for uproeunicin (**4**) from HRFABMS ([M+Li]$^+$ m/z= 357.2244) and ^{1}H- and ^{13}C-NMR data. Detailed comparisons of the NMR data of isomers **2** and **4** quickly revealed a close structural relationship between them. For instance, the ^{1}H-NMR spectrum in CD_3OD showed two signals at δ 5.57 (1H, m, H-6) and 5.36 (1H, br d, J = 15.9 Hz, H-7), suggesting the presence in **4** of a 1,2-disubstituted double bond. Other relevant features in the ^{1}H-NMR spectrum were three lowfield signals [δ 3.05 (br d, J = 9.3 Hz, H-13), 3.29 (dd, J = 12.3, 13.8 Hz, H-3), and 3.45 (br m, H-1)], ascribable to protons attached to carbons bearing oxygen and three methyl groups [δ 0.90 (d, J = 6.3 Hz, Me-20), 1.29 (s, Me-19), and 1.16 (s, Me-18)]. The ^{13}C-NMR spectrum of **4** also exhibited 20 signals (3 CH_3, 6 CH_2, 7 CH, and 4 C) indicating an ester carbonyl [δ 172.2 (s, C-16)], five oxygenated carbons [δ 74.0 (s, C-4); 74.4 (d, C-14); 78.9 (d, C-3); 79.8 (d, C-13), 85.2 (s, C-8)], and four olefinic carbons [δ 122.0 (t, C-17), 124.9 (d, C-6), 134.8 (d, C-7), 138.1 (s, C-15)]. From these data, it became apparent that **4** no longer had an epoxide ring between C-3 and C-4 but instead possessed a six-membered ether ring across the cembrane skeleton at C-3 and C-13. A combination of ^{1}H−^{1}H COSY and selective INAPT experiments established the position of many of the functional groups in **4**. Irradiation of H-1 (δ 3.45) enhanced the carbon signals assigned to C-17 (δ 122.0), C-3 (δ 78.9), and C-13 (δ 79.8), establishing unambiguously the position of the tetrahydropyran ring as shown in structure **4.** Although the large coupling constant (15.9 Hz) observed in **4** for the Δ^6 olefinic protons suggested a trans configuration, the strong downfield shift experienced by C-8 (δ 85.2) and the chemical shift of the nearby C-9 methylene (δ 36.2) clearly indicated the *Z* geometry.[6] The cis geometry of the ethylenic double bond in **4** was also supported by a strong NOE between H-6 and H-7. A NOESY experiment revealed that proton pairs H-3/H-13, H-14/Me-20, H-1/H-14, H-5/Me-18, and H-2/Me-18 were also within NOE proximity. Moreover, the 8*S* configuration depicted in **4** was consistent with the ^{13}C-NMR chemical shift values in $CDCl_3$ solution observed for C-10 (δ 20.6) and Me-19 (δ 23.6),[6] suggesting a possible biogenetic relationship between **2** and uproeunicin (**4**). Unfortunately, the paucity of 12,13-bisepiuprolide B (**2**) prevented us from correlating structures **2** and **4** chemically.

The major compound **5**, named 12,13-bisepiuprolide D acetate, had a molecular formula of $C_{22}H_{32}O_6$ as deduced from HREIMS data. The IR spectrum contained absorptions for hydroxyl (3463 cm^{-1}), γ-lactone (1767 cm^{-1}), ester (1744 cm^{-1}), and olefin (1664 cm^{-1}) functionalities. The usual α-methylene γ-lactone functionality was supported by a UV absorption at λ_{max}= 207 nm and two doublets in the ^{1}H-NMR spectrum at δ 6.25 (1H, J = 2.1 Hz, H-17α) and 5.61 (1H, J = 2.1 Hz, H-17β). In addition to these signals, the ^{1}H-NMR spectrum contained two olefinic singlets at δ 4.86 (1H, H-19) and 4.97 (1H, H-19′), four oxygenated signals: two at δ 4.99 (1H, br d, J = 8.7, H-3) and 4.45 (1H, dd, J = 5.7, 9.3 Hz, H-7) and two overlapped at δ 4.34 assigned to H-13 and H-14; and three methyl signals at δ 0.87 (3H, d, J = 6.6 Hz, Me-20), 1.17 (3H, s, Me-18), and 1.99 (3H, s, Me-22). In the ^{13}C-NMR spectrum only four olefinic signals were detected at δ 148.5 (s, C-8), 138.6 (s, C-15), 121.9 (t, C-17), and 114.0 (t, C-19). From these data the presence of an additional exomethylene group in the molecule was deduced. Signals for an acetoxy group [δ 170.3 (s, C-21), 21.2 (q, C-22)] plus two additional oxygenated carbon signals at δ 84.6 (s, C-4) and 84.3 (d, C-7) were also observed. When the molecular formula and the NMR data of **5** were compared with those of the known compound uprolide D acetate,[7] it became evident that these compounds were isomers with very similar structures. The chemical shift values of many ^{13}C-NMR signals in **5** were consistent with the presence of a five-membered cyclic ether, as in uprolide-D acetate. A ^{1}H−H COSY experiment revealed correlations between H-1/H-17α/β and H-1/H-2α/β. The H-2 protons in turn correlated with H-3 (δ 4.99), whose lower than usual chemical shift suggested its proximity to an acetate group. To establish the relative position of the remaining functional groups in **5**, a series of selective INAPT experiments were performed. When H-1 (δ 3.26) was irradiated, C-3 (δ 74.6), C-15 (δ 138.6), and C-16 (δ 169.8) were enhanced again, suggesting C-3 as the locus of the acetoxy group. In turn, irradiation of H-3 enhanced the signals ascribed to C-1 (δ 38.6), C-4 (δ 84.6), C-5 (δ 35.5), C-7 (δ 84.3), and C-21 (δ 170.3), confirming the location of the acetate group at C-3 and the oxolane ring between C-4 and C-7. The location of the exomethylene group at C-8 was established after the irradiation of H-7 (δ 4.45) caused enhancement of

the signal ascribed to C-19 (δ 114.0). A NOESY experiment clearly showed that the methyl groups at C-4 and H-7 were within NOE proximity. Because H-13 and H-14 appear overlapped at δ 4.34 we could not establish unambiguously, from the NMR data alone, whether compound **5** and uprolide D acetate had identical relative stereochemistries at C-12 and C-13. To resolve this issue, we acetylated compound **5** at 25 °C using a mixture of Ac_2O and pyridine to obtain diacetate **6**, a derivative remarkably similar, but not identical, to uprolide D diacetate,[7] thus suggesting that these metabolites differed from each other only in their relative configuration at C-12, C-13, or both. In **6** H-13 now appeared at δ 5.29 and H-14 at δ 4.58. Analysis of the NMR, $^1H-^1H$ COSY, and NOESY spectra of **6** established that **5** indeed had relative stereochemistries at C-12 and C-13 opposite to those found in uprolide D acetate.[7]

Eunicenolide (**7**) was isolated as a UV active (λ_{max} = 229 nm) oily substance that analyzed for $C_{20}H_{30}O_6$ by combined HRFABMS and ^{13}C-NMR methods. The IR spectrum was consistent with the presence of epoxide (1261 cm^{-1}) and ester carbonyl (1745 cm^{-1}) functionalities. An intense broad absorption near 3394 cm^{-1} also suggested several hydroxyl groups. The most striking difference observed in the ^1H-NMR spectrum of **7** was the absence of the signals usually ascribed to H-17$\alpha\beta$. Also, the signal due to H-14 [δ 4.62 (1H, br d, J = 9.0 Hz)] had a different multiplicity and appeared at lower field than usual. In addition, the ^1H-NMR spectrum showed signals for four protons attached to carbons bearing oxygen [δ 4.45 (2H, br s, H-17), 3.34 (1H, dd, J = 2.1, 10.8 Hz, H-3), 3.18 (1H, dd, J = 2.1, 9.3 Hz, H-13), and 2.60 (1H, br d, J = 11.4 Hz, H-7)] and three methyl signals, two overlapped at δ 1.27 (6H, s, Me-18 and Me-19) and one at δ 1.00 (3H, d, J = 6.9 Hz, Me-20). The ^{13}C-NMR spectrum exhibited 20 signals separated by APT into five quaternary carbons (C-1, C-4, C-8, C-15, and C-16), five methines (C-3, C-7, C-12, C-13, and C-14), seven methylenes (C-2, C-5, C-6, C-9, C-10, C-11, and C-17), and three methyl groups (Me-18, Me-19, and Me-20). One of the signals was an ester carbonyl [δ 173.6 (s)], seven were due to oxygenated carbons [δ 81.2 (d), 79.3 (d), 77.3 (d), 73.6 (s), 60.3 (d), 60.1 (s), and 54.7 (t)], and two were olefinic [δ 163.9 (s) and 123.6 (s)]. The resonance at δ 54.7 (t) suggested an allylic primary alcohol, and those at δ 163.9 (s) and 123.6 (s) suggested a double bond in conjugation with the γ-lactone carbonyl. From these data, we concluded that eunicenolide (**7**) possessed an α-hydroxymethylene-γ-butenolide moiety. ^{13}C-NMR resonances at δ 60.3 (d) and 60.1 (s) and those at δ 81.2 (d) and 79.3 (d) clearly indicated epoxide and tetrahydropyran ring systems. The relative positions of the various functional groups in **7** were confirmed by a combination of $^1H-^1H$ COSY, HETCOR, and INAPT NMR experiments. Irradiation of the ^1H-NMR signal at δ 2.38 (H-2) caused enhancement of C-3 (δ 79.3) and C-15 (δ 163.9), while irradiation at δ 4.45 (H-17) enhanced the signal ascribed to C-16 (δ 173.6). The latter result established the locus of the primary alcohol at C-17. The position of the epoxide was established from the $^1H-^1H$ COSY and HETCOR spectra, whereas irradiation at δ 1.00 (Me-20) enhanced C-13 (δ 81.2) and C-11 (δ 32.3), thus allowing us to place the oxo-bridge between C-3 and C-13. The relative stereochemistries at C-3, C-4, C-7, C-8, C-12, C-13, and C-14 were deduced as before from analysis of the NMR, $^1H-^1H$ COSY, and NOESY spectra. Eunicenolide (**7**) is the first example of a naturally occurring cembranolide from a Caribbean gorgonian possessing an α-hydroxymethylene-γ-butenolide moiety.

Compound **8**, named uproeuniolide, was an optically active oil of formula $C_{20}H_{28}O_4$ as determined by HREIMS. The IR spectrum showed absorptions ascribable to hydroxyl, epoxide, and ester functionalities. The ^1H-NMR spectrum showed signals for a 1,2-disubstituted double bond [δ 5.40, (1H, m, J = 6.9, 8.7, 15.9 Hz) and 5.52 (1H, d, J = 15.9 Hz)], a methyl bearing trisubstituted double bond [δ 1.66 (3H, s); 5.24 (1H, br t, J = 6.3 Hz)], two methyl groups on quaternary carbons bearing oxygen [δ 1.31 (3H, s); 1.29 (3H, s)], an allylic methine at δ 3.10 (m), an epoxymethine proton at δ 2.79 (dd, J = 4.8, 7.2 Hz), and a lactonic methine at δ 4.70 (m). The ^{13}C-NMR spectrum showed resonance lines with appropriate multiplicities to support these assignments. $^1H-^1H$ COSY and HMBC experiments established the relative positions of all the functional groups within the cembrane carbon framework. These data showed that compound **8** was structurally related to euniolide,[8] a metabolite usually found in *E. succinea* and *E. mammosa*. However, unlike euniolide, compound **8** had a trans 1,2-disubstituted double bond between C-6 and C-7 and a tertiary hydroxyl group at C-8. The configuration at Δ^6 was assigned as trans and the relative stereochemistry about the tertiary carbinol as C-8*R* after a side-by-side comparison between the NMR data of uproeuniolide (**8**) and those of known 8-epiuprolide A acetate, which rapidly pinpointed their structural similarities and differences.[6]



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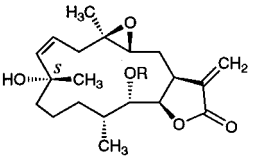

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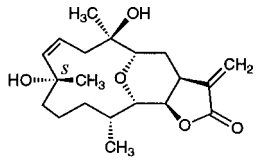

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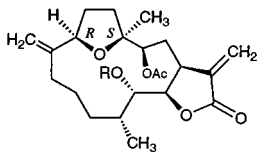

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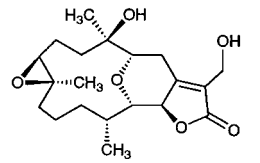

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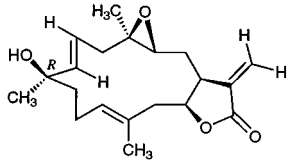

8

In the present work we report the structures of seven new γ-cembranolides from the Caribbean gorgonian *E. succinea*. The structures of these metabolites are unique and continue to build on the theme that Caribbean *Eunicea* species elaborate complex cembranolides.

For instance, the presence in eunicenolide (**7**) of an α-hydroxymethylene-γ-butene lactone together with a tetrahydropyran ring system across the cembrane skeleton is unprecedented when compared to the structures of other gorgonian-derived cembranolides. Excepting **8**, all the new cembranolides described here possessed the C-12*R*, C-13*S* stereochemistry usually ascribed to cembranolides from *E. succinea*. The NCI in vitro primary disease-oriented antitumor screen was used to ascertain the cytotoxic properties of three (**1**, **5**, and **7**) of the cembranolides described here. Of these compounds, 12,13-bisepieupalmerin epoxide (**1**) was the most promising, with concentrations of 10^{-6} M eliciting strong differential responses at the LC_{50} level from nearly all the breast cancer cell lines and from several of the colon cancer cell lines. On the other hand, eunicenolide (**7**) was the least toxic, displaying moderate cytotoxicity against only one ovarian (IGROV1), one nonsmall cell lung (NCI−H522), and two leukemia (CCRF−CEM and RPMI-8226) cancer cell lines at concentrations of 10^{-5} M. Finally, at 10^{-6}−10^{-7} M 12,13-bisepiuprolide D acetate (**5**) displayed strong nonselective cytotoxicity against almost all of the NCI panel cell lines.

Experimental Section

General Experimental Procedures. Experimental details have been reported.[6] Percentage yield of each compound is based on the weight of the dry hexane extract.

Collection, Extraction, and Isolation of Cembranolide Diterpenes. The Caribbean gorgonian *E. succinea* was collected at 25 m depth by scuba in November 1992, from Mona Island, Puerto Rico. The gorgonian was freeze-dried upon arrival and kept frozen until extraction. A voucher specimen (no. MIES-001) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras campus. The dried organism (2.5 kg) was blended with MeOH−$CHCl_3$ (1:1), and after filtration the crude extract was evaporated under vacuum to yield a residue (322.9 g) that was partitioned between hexane and H_2O. The hexane extract was concentrated to yield 170.9 g of a dark green oily residue, which was later dissolved in toluene and filtered. The resulting filtrate was concentrated (168.9 g), loaded onto a large-size exclusion column (Bio-Beads SX-3), and eluted with toluene. The combined terpenoid-rich fractions (TLC guided) were concentrated to a dark yellow oil (118.6 g) and chromatographed over a large Si gel column (3 kg) using 30% EtOAc in hexane. From this column 14 fractions were obtained, the less polar of which consisted of complex mixtures of unidentified sterols and fatty acid derivatives (3.8 g) and the following known cembranoid diterpenes: pseudoplexauric acid methyl ester[13] (29 g), euniolide[8] (24 g), succinolide[14] (150 mg), and eupalmerin acetate[9] (12.9 g). The more polar portion of the extract was divided roughly into fractions 6−14 on the basis of TLC analyses. From some of these fractions five known cembranolides were identified: 12,13-bisepieupalmerin acetate[14] (845 mg), 14-deoxycrassin[15] (225 mg), eunicin[10] (537 mg), 12,13-bisepieupalmerin[11] (3.0 g), and uprolide B acetate[6] (8.3 mg). Fraction 8 (4.7 g) was chromatographed over Si gel (250 g) with 45% EtOAc in hexane to give several fractions. One fraction (F8-J; 441.4 mg) was subjected to column chromatography over Si gel several times (using mixtures of EtOAc in hexane) and finally over Si gel (8 g) using 5% Et_2O in $CHCl_3$ to afford 12,13-bisepiuprolide B acetate (**3**) (13.0 mg, 0.0077%). Column chromatography on Si gel (290 g) of fraction 12 (5.22 g) with 45% EtOAc in hexane also afforded several fractions. One fraction (F12-G; 199.2 mg) was chromatographed successively over Si gel (9 g) with 10% MeOH in $CHCl_3$ and silica gel (5.6 g) with 25% Me_2CO in $CHCl_3$ to yield 12,13-bisepiuprolide B (**2**) (7.4 mg, 0.0044%) and eunicenolide (**7**) (7.7 mg, 0.0045%). Finally, fraction 13 (5.2 g) was chromatographed over Si gel (285 g) using 25% Me_2CO in hexane to give four major fractions. Fraction 13-D (534.6 mg) was purified by column chromatography on Si gel (28 g) with 5% Et_2O in $CHCl_3$, leading to pure uproeuniolide (**8**) (15.4 mg, 0.0091%) and 12,13-bisepiuprolide D acetate (**5**) (54.5 mg, 0.032%). Fraction 13-E (138.8 mg) was chromatographed on Si gel (6 g) with 5% Me_2CO in CH_2Cl_2 to give 12,13-bisepieupalmerin epoxide (**1**) (39 mg, 0.023%). Fraction 13-H (5.2 g) was purified successively by column chromatography on Si gel (35 g) with 5% Me_2CO in CH_2Cl_2 and normal-phase HPLC (Ultrasphere-Si with 10% 2-propanol in hexane) to give uproeunicin (**4**) (14.2 mg, 0.0084%).

12,13-Bisepieupalmerin epoxide (1): colorless oil; $[\alpha]^{25}_D$ −13.2° (*c* 4.4, $CHCl_3$); IR (neat) 3450, 2959, 2937, 2874, 1753, 1663, 1461, 1386, 1269, 1163, 1106, 1065, 982, 838, 811, 753, 667 cm^{-1}; UV λ_{max} (MeOH) 208 nm (ϵ 11 400); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 350 (3), 332 (5), 156 (14), 141 (12), 139 (25), 119 (100), 85 (24), 83 (36), 69 (23); HREIMS *m/z* $[M]^+$ 350.2095 (calcd for $C_{20}H_{30}O_5$, 350.2093).

12,13-Bisepiuprolide B (2): colorless oil; $[\alpha]^{25}_D$ −50.9° (*c* 2.2, $CHCl_3$); IR (neat) 3400, 2960, 2929, 2875, 2856, 1754, 1665, 1377, 1263, 1100, 1019, 800, 756 cm^{-1}; UV λ_{max} (MeOH) 211 nm (ϵ 6200); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$), see Table 2; HRFABMS (*m*-nitrobenzyl alcohol) *m/z* $[M+Li]^+$ 357.2256 (calcd for $C_{20}H_{30}O_5Li$, 357.2253).

12,13-Bisepiuprolide B acetate (3): colorless oil; $[\alpha]^{25}_D$ −52.6° (*c* 4.3, $CHCl_3$); IR (neat): 3405, 2926, 2854, 1769, 1741, 1459, 1373, 1234, 1026, 980, 757, 667 cm^{-1}; UV λ_{max} (MeOH) 218 nm (ϵ 4900); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 392 (3), 375 (32), 315 (14), 297 (11), 177 (19), 163 (24), 151 (38), 135 (36), 121 (38), 111 (96), 109 (90), 95 (96), 81 (100), 71 (73), 69 (69); HREIMS *m/z* $[M]^+$ 392.2121 (calcd for $C_{22}H_{32}O_6$, 392.2199).

Uproeunicin (4): colorless oil; ^{1}H-NMR (300 MHz, CD_3OD), see Table 1; ^{13}C-NMR (75 MHz, CD_3OD), see Table 2; HRFABMS (*m*-nitrobenzyl alcohol) *m/z* $[M + Li]^+$ 357.2244 (calcd for $C_{20}H_{30}O_5Li$, 357.2253). Due to the accidental loss of this sample, we were unable to record all the spectral and physical properties of this metabolite.

12,13-Bisepiuprolide D acetate (5): colorless oil; $[\alpha]^{25}_D$ −38.3° (*c* 9.4, $CHCl_3$); IR (neat) 3463, 2958, 2927, 2872, 2854, 1767, 1744, 1664, 1460, 1374, 1233, 1165, 1101, 1058, 1028, 976, 914, 818, 756, 666 cm^{-1}; UV λ_{max} (MeOH) 207 nm (ϵ 8500); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 392 (10), 332 (14), 306 (19), 209 (8), 151 (20), 137 (21), 133 (30), 121 (37), 109 (65), 95 (73), 93 (83),

Table 1. ^{1}H-NMR (300 MHz) Spectral Data for Compounds **1**−**5**, **7**, and **8** [δ, int., mult., J (Hz)][a]

H	1	2	3	4	5	7	8
1	3.54, 1H, br m	3.44, 1H, br m	3.29, 1H, br m	3.45, 1H, br m	3.26, 1H, br m		3.10, 1H, m
2	1.70, 1H, m	1.79, 1H, m	1.69, 1H, m	1.95, 1H, m	2.02, 1H, m	2.38, 1H, br t, 12.6	1.61, 1H, m
	2.94, 1H, br d, 15.9	2.52, 1H, m	2.17, 1H, m	2.25, 1H, m	2.21, 1H, m	3.24, 1H, dd, 2.1, 13.2	1.91, 1H, m
3	2.64, 1H, br d, 6.6	2.52, 1H, m	2.52, 1H, br t, 3.9	3.29, 1H, dd, 12.3, 13.8	4.99, 1H, br d, 8.7	3.34, 1H, dd, 2.1, 10.8	2.79, 1H, dd, 4.8, 7.2
5	1.05, 1H, m	1.77, 1H, m	1.89, 1H, dd, 6.6, 13.5	2.29, 1H, dd, 5.7, 14.4	1.59, 1H, m	1.68, 1H, dd, 8.7, 14.7	2.30, 2H, m
	2.19, 1H, dt, 3.0, 16.2	2.63, 1H, dd, 6.0, 13.0	2.62, 1H, dd, 6.6, 13.5	2.39, 1H, dd, 7.5, 14.4	1.82, 1H, m	1.91, 1H, m	
6	1.70, 2H, m	5.54, 1H, m	5.55, 1H, m	5.57, 1H, m	1.74, 2H, m	1.32, 1H, m 2.06, 1H, m	5.40, 1H, m, 6.9, 8.7, 15.9
7	2.81, 1H, br t, 5.8	5.58, 1H, m	5.58, 1H, m	5.36, 1H, br d, 15.9	4.45, 1H, dd, 5.7, 9.3	2.60, 1H, br d, 11.4	5.52, 1H, d, 15.9
9	1.58, 1H, m	1.43, 1H, m	1.37, 1H, m	1.12, 1H, m	1.82, 1H, m	1.47, 1H, m	1.72, 2H, m
	1.85, 1H, m	1.74, 1H, m	1.65, 1H, m	1.92, 1H, m	1.99, 1H, m	1.91, 1H, m	
10	1.01, 1H, m	1.22, 1H, m	1.41, 2H, m	1.09, 1H, m	1.45, 1H, m	0.95, 1H, m	1.98, 1H, m
	1.33, 1H, m	1.43, 1H, m		1.24, 1H, m	1.67, 1H, m	1.28, 1H, m	2.11, 1H, m
11	1.20, 1H, m	1.32, 1H, m	1.22, 2H, m	1.03, 1H, m	1.25, 1H, m	1.46, 1H, m	5.24, 1H, br t, 6.3
	1.44, 1H, m	1.58, 1H, m		1.56, 1H, m	1.47, 1H, m	1.37, 1H, m	
12	1.91, 1H, m	2.15, 1H, m	2.15, 1H, m	1.62, 1H, m	1.88, 1H, m	1.90, 1H, m	
13	3.89, 1H, dd, 1.2, 10.5	3.73, 1H, br d, 9.9	5.18, 1H, br d, 10.5	3.05, 1H, br d, 9.3	4.34, 1H, m	3.18, 1H, dd, 2.1, 9.3	2.21, 1H, m 2.46, 1H, m
14	4.41, 1H, dd, 7.5, 10.5	4.33, 1H, br t, 6.9	4.47, 1H, dd, 6.0, 10.2	4.59, 1H, dd, 6.9, 15.0	4.34, 1H, m	4.62, 1H, br d, 9.0	4.70, 1H, m
17α	6.46, 1H, d, 2.1	6.46, 1H, br s	6.43, 1H, br s	6.30, 1H, d, 3.6	6.25, 1H, d, 2.1	4.45, 2H, br s	6.21, 1H, d, 2.1
17β	5.81, 1H, d, 2.1	5.84, 1H, br s	5.80, 1H, br s	5.72, 1H, d, 3.3	5.61, 1H, d, 2.1		5.58, 1H, d, 1.8
18	1.28, 3H, s	1.30, 3H, s	1.32, 3H, s	1.16, 3H, s	1.17, 3H, s	1.27, 3H, s	1.31, 3H, s
19	1.25, 3H, s	1.32, 3H, s	1.32, 3H, s	1.29, 3H, s	4.86, 1H, br s 4.97, 1H, br s	1.27, 3H, s	1.29, 3H, s
20	0.88, 3H, d, 6.9	0.91, 3H, d, 6.9	0.95, 3H, d, 6.6	0.90, 3H, d, 6.3	0.87, 3H, d, 6.6	1.00, 3H, d, 6.9	1.66, 3H, s
22			2.09, 3H, s		1.99, 3H, s		

[a] ^{1}H NMR spectra were recorded at 25 °C in $CDCl_3$ except for **4**, which were recorded in CD_3OD. Chemical shifts are given in δ units downfield from Me_4Si. Assignments were aided by ^{1}H-^{1}H COSY, HETCOR, selective INAPT, HMBC, and NOESY experiments.

Table 2. ^{13}C-NMR (75 MHz) Spectral Data for Compounds **1**−**5**, **7**, and **8** [δ (mult.)][a]

C	1	2	3	4	5	7	8
1	38.9 (d)	39.2 (d)	39.3 (d)	39.2 (d)	38.6 (d)	123.6 (s)	42.2 (d)
2	30.1 (t)	30.3 (t)	30.8 (t)	25.8 (t)	29.1 (t)	28.1 (t)	30.0 (t)
3	59.5 (d)	58.0 (d)	57.1 (d)	78.9 (d)	74.6 (d)	79.3 (d)	57.1 (d)
4	58.4 (s)	61.3 (s)	61.3 (s)	74.0 (s)	84.6 (s)	73.6 (s)	61.0 (s)
5	37.2 (t)	42.7 (t)	42.5 (t)	48.2 (t)	35.5 (t)	35.8 (t)	39.2 (t)
6	24.7 (t)	128.3 (d)	127.8 (d)	124.9 (d)	31.7 (t)	20.9 (t)	120.8 (d)
7	60.3 (d)	136.2 (d)	136.3 (d)	134.8 (d)	84.3 (d)	60.3 (d)	141.7 (d)
8	60.3 (s)	84.5 (s)	84.9 (s)	85.2 (s)	148.5 (s)	60.1 (s)	72.8 (s)
9	33.6 (t)	34.3 (t)	35.0 (t)	36.2 (t)	28.4 (t)	34.2 (t)	42.0 (t)
10	18.6 (t)	19.6 (t)	19.7 (t)	21.1 (t)	28.6 (t)	18.6 (t)	22.8 (t)
11	31.3 (t)	30.9 (t)	31.5 (t)	34.7 (t)	32.3 (t)	32.3 (t)	128.7 (d)
12	32.8 (d)	30.2 (d)	30.7 (d)	33.6 (d)	30.3 (d)	33.4 (d)	129.2 (s)
13	67.4 (d)	68.0 (d)	69.6 (d)	79.8 (d)	67.3 (d)	81.2 (d)	38.6 (t)
14	77.8 (d)	78.6 (d)	77.0 (d)	74.4 (d)	81.6 (d)	77.3 (d)	79.4 (d)
15	137.6 (s)	138.7 (s)	138.7 (s)	138.1 (s)	138.6 (s)	163.9 (s)	139.9 (s)
16	170.2 (s)	170.1 (s)	169.9 (s)	172.2 (s)	169.8 (s)	173.6 (s)	169.6 (s)
17	124.6 (t)	124.7 (t)	124.6 (t)	122.0 (t)	121.9 (t)	54.7 (t)	121.3 (t)
18	16.0 (q)	17.0 (q)	17.3 (q)	22.3 (q)	22.0 (q)	23.6 (q)	18.0 (q)
19	18.7 (q)	20.5 (q)	20.4 (q)	24.6 (q)	114.0 (t)	20.0 (q)	28.8 (q)
20	13.1 (q)	13.3 (q)	14.5 (q)	14.8 (q)	14.2 (q)	15.0 (q)	16.8 (q)
21			169.4 (s)		170.3 (s)		
22			20.8 (q)		21.2 (q)		

[a] ^{13}C-NMR spectra were recorded in $CDCl_3$ at 25 °C except for **4**, which were recorded in CD_3OD. Number of attached protons were determined by APT experiments. Assignments were aided by ^{1}H-^{1}H COSY, selective INAPT, HETCOR, and HMBC experiments.

81 (100), 79 (61), 67 (69); HREIMS m/z $[M]^+$ 392.2206 (calcd for $C_{22}H_{32}O_6$, 392.2199).

Eunicenolide (7): colorless oil; $[\alpha]^{25}_D$ +13.7° (*c* 2.0, $CHCl_3$); IR (neat) 3394, 2962, 2926, 2873, 2854, 1745, 1681, 1632, 1462, 1384, 1261, 1217, 1082, 1035, 1018, 972, 936, 798, 756, 697, 665 cm^{-1}; UV λ_{max} (MeOH) 229 nm (ϵ 8500); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75 MHz, $CDCl_3$), see Table 2; HRFABMS (*m*-nitrobenzyl alcohol) m/z $[M + Li]^+$ 373.2189 (calcd for $C_{20}H_{30}O_6Li$, 373.2202).

Uproeuniolide (8): colorless oil; $[\alpha]^{25}_D$ −30.6° (*c* 5.0, $CHCl_3$); IR (neat) 3448, 2959, 2925, 2871, 2854, 1762, 1741, 1676, 1457, 1375, 1239, 1100, 1026, 961, 815, 757, 666 cm^{-1}; UV λ_{max} (MeOH) 212 nm (ϵ 3900); ^{1}H-NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C-NMR (75 MHz,

$CDCl_3$), see Table 2; EIMS *m/z* 332 (2), 314 (13), 296 (25), 278 (10), 183 (23), 157 (35), 145 (39), 133 (35), 119 (47), 105 (64), 93 (66), 91 (100), 79 (65), 69 (84); HREIMS *m/z* $[M]^+$ 332.1988 (calcd for $C_{20}H_{28}O_4$, 332.1987).

Acetylation of 12,13-Bisepiuprolide D Acetate (5). A solution of **5** (20.3 mg, 51.7 mmol) in a mixture of Ac_2O (5 mL) and pyridine (1 mL) was stirred at 25 °C for 12 h. Excess reagents were removed by rotoevaporation, and, after concentration and storage *in vacuo*, the reaction product was purified by column chromatography on Si gel using 1% Me_2CO in $CHCl_3$ to yield 15.7 mg of diacetate **6**: a colorless oil; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 2.1 Hz, H-17α), 5.73 (1H, d, J = 1.2 Hz, H-17β), 5.29 (1H, br s, H-13), 5.11 (1H, br d, J = 9.3 Hz, H-3), 5.04 (1H, br s, H-19), 4.90 (1H, br s, H-19′), 4.58 (1H, br t, J = 7.5 Hz, H-14), 4.52 (1H, br t, J = 6.9 Hz, H-7), 3.10 (1H, m, H-1), 2.18 (3H, s, 13-OAc), 2.09 (3H, s, 3-OAc), 1.19 (3H, s, Me-18), 1.07 (3H, d, J = 6.6 Hz, Me-20); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.4, 170.8, 169.8, 148.6, 137.8, 122.6, 115.0, 84.4, 83.9, 83.1, 75.3, 72.2, 38.9, 35.3, 30.7, 29.7, 29.6, 28.7, 27.8, 27.7, 23.4, 21.0, 20.9, 17.3; EIMS *m/z* 434 (15), 374 (12), 348 (14), 332 (7), 314 (10), 296 (8), 177 (19), 145 (35), 133 (42), 109 (76), 93 (72), 81 (77), 67 (63), 55 (100); HREIMS *m/z* $[M]^+$ 434.2303 (calcd for $C_{24}H_{34}O_7$, 434.2304).

Synthesis of 12,13-Bisepieupalmerin Epoxide (1). 12,13-Bisepieupalmerin[11] (46 mg, 0.14 mmol) in CH_2Cl_2 (15 mL) was stirred with *m*-chloroperoxybenzoic acid (24 mg, 0.14 mmol) at 25 °C for 3 h. After concentration *in vacuo*, the reaction mixture was subjected to column chromatography on Si gel (3 g) using 40% Me_2CO with hexane, followed by HPLC (Ultrasphere-Si with 20% 2-propanol in hexane) to afford 16 mg of 12,13-bisepieupalmerin epoxide (**1**).

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Supporting Information Available: Structures of known cembranoids from *E. mammosa* and *E. succinea*, side-by-side comparison of ^{1}H- and ^{13}C-NMR data of 12,13-bisepiuprolide D acetate (**5**) and uprolide D acetate (Table S1), and side-by-side comparison of ^{1}H- and ^{13}C-NMR data of uproeuniolide (**8**) and 8-epiuprolide A acetate (Table S2) (3 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Taken in part from the M.S. Dissertation of A. L. Acosta, University of Puerto Rico, 1997.
(2) Presented in part at the 20th Senior Technical Meeting of the American Chemical Society, Puerto Rico Section, Tropimar Beach Club and Convention Center, Isla Verde, Puerto Rico, Nov. 8−9, 1996.
(3) Graduate student supported by the NIH−MBRS Program of the University of Puerto Rico.
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