New Terpenoid Constituents from *Eunicea pinta*

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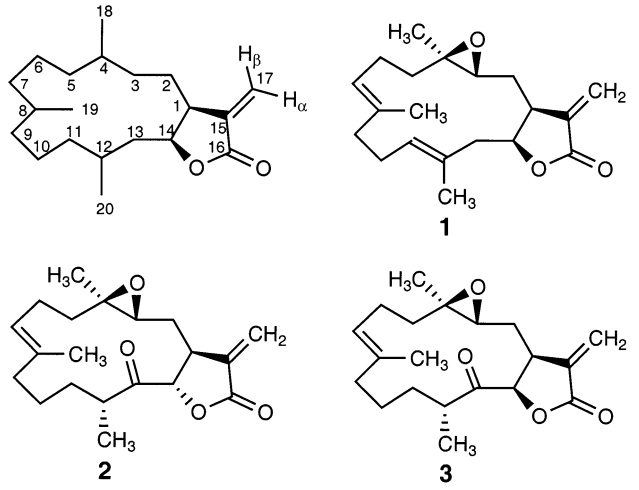
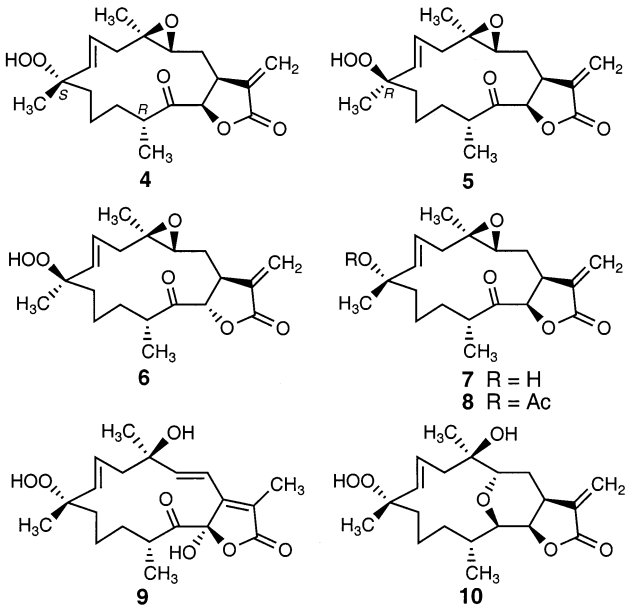
The secondary metabolite composition of the gorgonian octocoral *Eunicea pinta* from San Andrés Island, Colombia, is described for the first time. This investigation has resulted in the isolation of eight new γ-cembranolide-type diterpenes, namely, compounds **3**−**10**, and a new saponin, **13**, possessing a pregnene-derived aglycon. In addition, two previously known α-methylene-γ-lactone cembranolides, euniolide (**1**) and succinolide (**2**), were also isolated. Their structures were determined on the basis of the results of spectroscopic analysis, X-ray diffraction analysis, and chemical conversions. The discovery of cembranolides **3**−**10** has prompted us to conclude that the structures proposed earlier for several uprolide-type cembranolides need revision and that a minor stereochemical correction is also in order for succinolide. Cytotoxicity of compounds **3**, **4**, and **13** toward various cancer cell lines and results of an antimycobacterial screening for **2**, **4**, and **13** also are described.

Gorgonian octocorals (order Gorgonacea, phylum Cnidaria) of the genus *Eunicea* were among the first marine invertebrates to be chemically investigated.[1] Thus far, more than 15 species have been documented throughout the West Indian region.[2] The search for new natural products from Caribbean gorgonian specimens belonging to this genus has been an ongoing project in our laboratory for the past decade. This effort has led to the isolation of several new natural products of diverse molecular architecture.[3]

Previous work in this laboratory on other species of the genus *Eunicea*, namely, *E. mammosa* and *E. succinea*, resulted in the isolation of several highly functionalized α-methylene-γ-cembranolides such as euniolide (**1**),[4] succinolide (**2**),[5] and a series of structural congeners characterized by the presence of a Δ^6 olefin known collectively as "uprolides".[6] In this report, we describe the first chemical investigation of the gorgonian octocoral *Eunicea pinta* (Bayer & Deichmann, 1958), leading to the isolation and structure determination of 10 cembranolides, including 12-epieupalmerone (**3**) and uprolides H−M (**4**−**10**), which are new natural products. These compounds were found to be related to the uprolide class of cembranoids isolated previously from *E. mammosa* and *E. succinea*.[7,8] This investigation also led to the isolation of known γ-lactones **1** and **2** as well as a new saponin, namely, 3β-pregna-5,-20-diene-β-D-xylopyranoside (**13**). On the basis of the extensive spectroscopic and X-ray data gathered from this investigation, we now conclude that the structures previously reported for a number of uprolide-type cembranolides need revision and that the original stereochemistry at C-12 for succinolide needs to be changed as that shown in structure **2**.





Results and Discussion

A bountiful MeOH−$CHCl_3$ extract of freeze-dried *E. pinta* collected in May 1996 off San Andrés Island, Colombia, accounted for 11.2% of the total dry weight of the gorgonian. On TLC- and NMR-guided fractionation of this extract by conventional liquid−liquid partition, the most promising compounds were found in the $CHCl_3$ extract.

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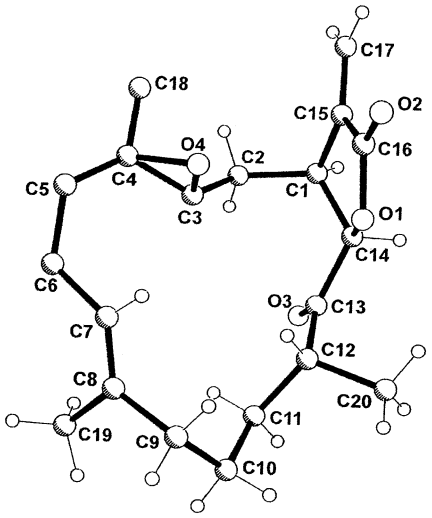
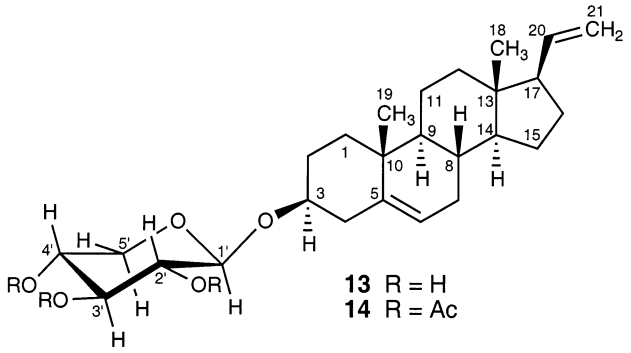


Figure 1. X-ray structure of **3** showing relative configuration.

Purification of the $CHCl_3$-soluble fraction using Bio-Beads SX-3, RP_{18}, and Si gel columns resulted in the isolation of cembranolides **1**−**10** and saponin **13**.



Cembranolides **1** and **2** were identified from chemical and spectroscopic data and by comparison with literature data as euniolide and succinolide, respectively.[4,5] Although 12-epieupalmerone (**3**) is in fact a new natural product, its physical and spectroscopic data had been reported earlier by us upon its synthesis from known 12,13-bisepieupalmerin.[5] Nonetheless, a single-crystal X-ray structure analysis was carried out, to confirm its molecular structure (Figure 1). Thus, the structure of diterpene **3**, including the α orientation of the secondary methyl group at C-12, was elucidated and found to be closely related to the new uprolides herein described. Interestingly, the co-occurrence of **2** and **3** within the same *Eunicea* species prompted us to reexamine our original *β* stereochemical assignment for the C-12 methyl group in succinolide.[5] Indeed, reinterpretation of the NOESY data, this time supported by distance calculations using the MacSpartan Pro molecular mechanics program, clearly indicated that the C-12 methyl group in succinolide, like in **3**, has the α-orientation. Strong and very diagnostic NOEs between H-14 (δ_H 4.92) with H-3 (δ_H 2.70) and H-12 (δ_H 2.98) indicated that these protons were on the same *β*-face of the molecule. The distances between protons experiencing these NOEs in succinolide all lie within 2.09−2.37 Å according to the molecular modeling study. Thus, we now conclude that **2** is the correct structure for succinolide.

The structures of metabolites **4**−**10** and that of saponin **13** were elucidated by a series of spectral analyses and by comparison with the spectral and physical data from other known compounds. Several pregnene glycosides have been isolated from other Caribbean gorgonian octocorals, including one from an *Eunicea* species.[9,10] The structure of uprolide H (**4**) and that of the acetylated derivative of **13** were subsequently confirmed by X-ray diffraction analysis.

Uprolide H (**4**) was isolated as colorless needles, mp 161 °C. Its molecular formula, $C_{20}H_{28}O_6$, was established by HRFABMS *m*/*z* 387.1794 $[M + Na]^+$ and ^{13}C NMR data implying seven degrees of unsaturation. Its IR spectrum showed a broad absorption between 3000 and 3500 cm^{-1} (OH stretching) and strong absorptions at 1764 and 1707 cm^{-1}, consistent with the presence of an α,*β*-unsaturated ester and saturated ketone functions, respectively. ^1H and ^{13}C NMR spectra, combined with 2D NMR experiments, allowed the chemical structure of **4** to be established (Table 1).

The presence in the ^1H NMR spectrum of two sharp doublets at *δ* 6.35 and 5.73 (each 1H, $J = 2.1$ Hz) suggested an exocyclic α-methylene lactone ring. This was supported by the ^{13}C NMR [*δ* 168.9 (s), 138.2 (s), 123.3 (t)] and IR spectra (1764 cm^{-1}). A carbon signal at *δ* 211.4 confirmed the presence of a saturated ketone in **4**, and a secondary oxygenated carbon signal at *δ* 79.7 hinted at a *γ*-lactone, a contention that was subsequently confirmed by the $^1H-^1H$ COSY, HMQC, and HMBC experiment.[11] Carbon signals were observed at *δ* 60.0 (s) and 55.8 (d), and a proton signal at *δ* 2.33 (1H, t, $J = 9.6$ Hz) indicated a trisubstituted epoxide. These combined NMR measurements accounted for five of the six oxygen atoms present in the molecular formula. Therefore, the remaining oxygenated carbon signal at *δ* 85.5 (s) implied that **4** most likely possessed a hydroperoxide group. Two olefinic methine carbons were further identified, and their corresponding mutually coupled proton signals at *δ* 5.52 (1H, dd, $J = 0.9$, 16.2 Hz) and 5.21 (1H, ddd, $J = 4.8$, 9.9, 16.2 Hz) indicated the presence in **4** of a *trans* ethylenic double bond. Signals for a secondary methyl group at *δ* 1.19 (3H, d, $J = 7.2$ Hz) and two tertiary oxygenated methyl groups at *δ* 1.32 (3H, s) and 1.23 (3H, s) were observed in the ^1H NMR spectrum. $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC NMR techniques were employed extensively to position the epoxy, olefin, hydroperoxide, and ketone moieties about the α-methylene-*γ*-cembranolide ring.

The relative stereochemistry of **4** was determined by a NOESY experiment.[12] The NOE correlations from H-14 to H-1 and H_3-20 indicated that these protons were on the same face of the 14-membered ring and were assigned as the α-protons. The relative stereochemistry of H-3 was deduced from the $^3J_{H2\alpha\beta,H3}$ values (∼9.6 Hz) and the absence of a NOESY cross-peak between H-3 and H_3-18. On the other hand, H-6 showed NOE responses with H-3, H-5*β*, and H_3-19, but not with H-7, conforming the *β*-orientation of H-3 and the C-8 methyl group as well as the *trans* orientation of the Δ^6 double bond. Conformational space for **4** was searched using MMFF force field implemented in the MacSpartan Pro program, and in the lowest energy conformer, H-5*β*, H-6, and the H_3-19 group were nearly coplanar and faced each other, which was consistent with the conformation deduced from the NOESY data. This conformation was also implied by the strong NOESY correlation between H_3-18 and H-5α and, in particular, the absence of an NOE response between H-7 and H_3-19. In this geometry, the vinylic H-7 proton (δ_H 5.52), which lies close to the −OOH group, is significantly more deshielded than H-6 (δ_H 5.21). A single-crystal X-ray structure analysis confirmed the molecular structure and relative stereochemistry of uprolide H (**4**) (Figure 2). The absolute stereochemistry was not determined.

Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Spectral Data for Uprolides **4**−**7** in $CDCl_3$ [δ_H, mult, J (Hz); δ_C (mult)][a]

	uprolide H (**4**)		uprolide I (**5**)		uprolide J (**6**)		uprolide K (**7**)	
atom	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.62, m	40.6 (d)	3.62, m	40.4 (d)	3.56, m	37.7 (d)	3.63, m	40.5 (d)
2α	2.72, ddd, 2.1, 3.9,14.7	29.0 (t)	2.63, ddd, 2.7,3.9, 14.7	29.3 (t)	2.17, m	30.7 (t)	2.67, ddd, 2.4, 3.9,14.7	29.2 (t)
2*β*	1.55, m		1.54, m		1.87, ddd, 5.1, 8.4,14.1		1.56, m	
3	2.33, t, 9.6	55.8 (d)	2.33, m	56.0 (d)	2.53, m	57.1 (d)	2.34, m	56.0 (d)
4		60.0 (s)		60.2 (s)		60.2 (s)		60.2 (s)
5α	2.51, ddd, 1.5, 4.8,15.0	38.7 (t)	2.42, br dd, 4.8, 14.4	39.1 (t)	2.53, m	39.4 (t)	2.36, m	38.9 (t)
5*β*	2.32, dd, 3.6, 9.9		2.34, br dd, 7.2, 14.4		2.23, br dd, 6.0, 14.4		2.31, m	
6	5.21, ddd, 4.8, 9.9,16.2	125.0 (d)	5.42, ddd, 5.4,7.8, 15.9	126.4 (d)	5.40, m	125.1 (d)	5.17, ddd, 6.0, 8.7,15.9	122.6 (d)
7	5.52, dd, 0.9,16.2	137.6 (d)	5.34, d, 15.9	136.4 (d)	5.42, m	137.2 (d)	5.48, d, 15.9	141.5 (d)
8		85.5 (s)		84.2 (s)		85.1 (s)		73.4 (s)
9α	1.53, m	38.2 (t)	1.65, m	37.6 (t)	1.63, m	36.8 (t)	1.52, m	42.4 (t)
9*β*	1.53, m		1.42, m		1.46, m		1.45, m	
10α	1.30, m	22.1 (t)	1.32, m	22.1 (t)	1.25, m	21.5 (t)	1.41, m	22.2 (t)
10*β*	1.15, m		1.16, m		0.89, m		1.11, m	
11α	1.96, m	30.8 (t)	2.02, m	30.3 (t)	1.68, m	33.2 (t)	2.02, m	30.3 (t)
11*β*	1.14, m		1.00, m		1.68, m		1.15, m	
12	3.21, m	42.3 (d)	3.23, m	42.3 (d)	3.18, m	44.0 (d)	3.19, m	42.5 (d)
13		211.4 (s)		211.2 (s)		209.1 (s)		211.3 (s)
14	4.86, d, 7.8	79.7 (d)	4.87, d, 7.5	79.8 (d)	4.76, d, 6.0	81.8 (d)	4.87, d, 7.8	79.8 (d)
15		138.2 (s)		138.4 (s)		137.1 (s)		138.2 (s)
16		168.9 (s)		168.9 (s)		168.4 (s)		169.0 (s)
17α	6.35, d, 2.1	123.3 (t)	6.34, d, 1.8	123.3 (t)	6.37, d, 3.3	123.1 (t)	6.35, d, 1.8	123.4 (t)
17*β*	5.73, d, 2.1		5.71, d, 1.5		5.70, d, 2.7		5.72, d, 1.8	
H_3-18	1.23, s	18.9 (q)	1.22, s	18.6 (q)	1.29, s	17.9 (q)	1.22, s	18.7 (q)
H_3-19	1.32, s	22.5 (q)	1.30, s	22.2 (q)	1.30, s	21.3 (q)	1.26, s	29.2 (q)
H_3-20	1.19, d, 7.2	18.9 (q)	1.23, d, 6.9	18.9 (q)	1.11, d, 6.9	15.9 (q)	1.21, d, 7.2	18.9 (q)

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. ^{13}C NMR multiplicities were obtained from APT experiments.

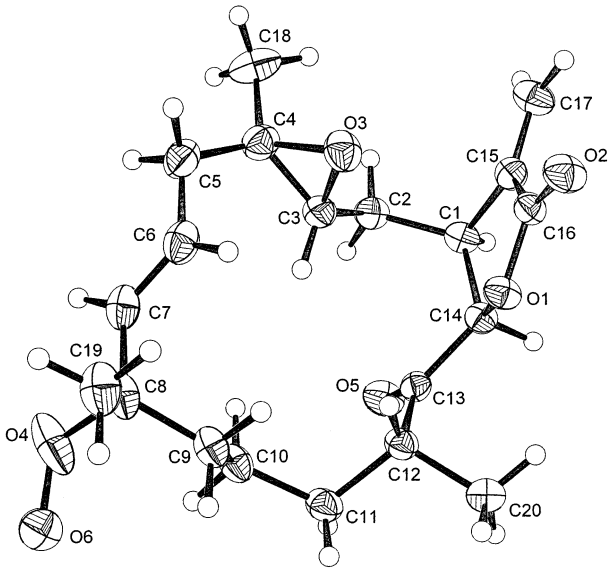


Figure 2. Computer-generated ORTEP plot of **4** showing relative configuration.

Uprolide I (**5**) was isolated as a colorless gum. Its molecular formula of $C_{20}H_{28}O_6$, the same as that of **4**, was deduced from the pseudomolecular ion at *m*/*z* 387.1789 [M + Na]$^+$ in its HRFABMS, which indicated seven degrees of unsaturation. The IR, UV, and particularly the ^{1}H and ^{13}C NMR data of **5** were strikingly similar to those recorded for uprolide H (**4**) (Table 1). These similarities, together with the fact that **4** and **5** possess identical molecular formulas, suggested a close structural relationship between these compounds. The ^{1}H−^{1}H COSY spectrum allowed us to establish the proton sequences from H-1 to H-3, H-5 to H-7, and H-9 to H-12. COSY correlations were also observed between H-1 and H-14 and between H-12 and H_3-20. The positions of a saturated ketone at C-13 and a hydroperoxide at C-8 were supported by HMBC connectivities from H_3-20 to C-13 and H-6 to C-8, respectively.[13] A detailed comparison of the ^{1}H and ^{13}C NMR spectra of **4** and **5** revealed that the few differences observed between these compounds were in fact consistent with a change in the relative stereochemical orientation of the methyl group at C-8. The change at C-8 from *S** in **4** to *R** in **5** was clearly indicated by the difference in ^{1}H NMR chemical shift, multiplicity, and coupling constant data of the ethylenic protons H-6 [δ 5.21 (ddd, J = 4.8, 9.9, 16.2 Hz) in **4** vs 5.42 (ddd, J = 5.4, 7.8, 15.9 Hz) in **5**] and H-7 [δ 5.52 (dd, J = 0.9, 16.2 Hz) in **4** vs 5.34 (d, J = 15.9 Hz) in **5**]. The C-8(*R**) stereochemistry was also evident from the key NOESY correlations of H-3/H-6, H-5α/H_3-18, H-5α/H-7, and H-7/H_3-19, as shown in Figure 3. The ^{1}H and ^{13}C NMR data for **5**, along with the analysis of J values, ^{1}H−^{1}H COSY, and NOESY experiments, showed that all common stereochemistry elements remaining were unchanged.

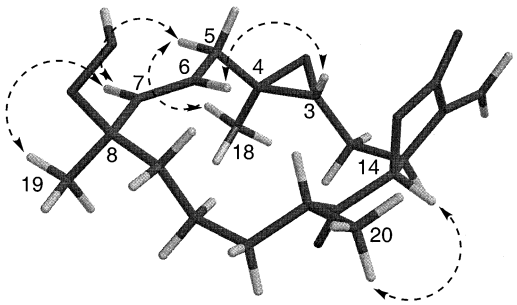


Figure 3. Selected NOESY correlations (dotted arrows) and relative configurations for uprolide I (**5**).

Uprolide J (**6**) also had the molecular formula $C_{20}H_{28}O_6$, *m/z* 387.1790, $[M + Na]^+$, and showed a UV absorption at λ_{max} 210 nm (ϵ 3550) and IR absorptions at ν 3411, 1775, 1662, and 1265 cm^{-1}, typical for hydroxyl, γ-lactone, olefin, and epoxide functionalities. The ^{13}C NMR spectrum (Table 1) exhibited all 20 signals divided by APT into five quaternary carbons, six methine carbons, six methylenes, and three methyl groups. The ^1H NMR spectrum (Table 1) contained a proton signal at δ 2.53 (1H, m), which could be assigned to an α-epoxy proton, and showed additional signals at δ 6.37 (1H, d, $J = 3.3$ Hz) and 5.70 (1H, d, $J = 2.7$ Hz) due to a pair of terminal methylene protons, two nearly overlapped ethylenic signals around δ 5.42 (each 1H, m), three key signals at δ 4.76 (1H, d, $J = 6.0$ Hz), 3.56 (1H, m), and 3.18 (1H, m), and three methyl signals: one as a doublet at δ 1.11 (3H, $J = 6.9$ Hz) and two as partially overlapped singlets at δ 1.30 and 1.29 (each 3H). After assignment of all the direct C−H bonds based on HMQC, the gross structure was determined by HMBC analysis.[14] A side-by-side comparison of the ^1H and ^{13}C NMR spectra of **6** with those of **5** suggested for **6** the same substituted cembranolide ring as in compound **5** and, in addition, suggested that the two lactone protons H-1 and H-14 were now *trans* to each other as in succinolide (**2**).[5] The change in relative configuration about C-14 in **6** was confirmed by the absence of a NOESY correlation between H-1 and H-14, a significant change in coupling constant between these protons ($J_{1,14} = 6.0$ Hz), and the ^{13}C chemical shift value for the γ-lactone oxymethine carbon signal, which in **6** appears at δ 81.8. The relative stereochemistry of the remaining chiral centers was assumed to be the same as that of uprolide I (**5**) due to the similarity of proton−proton coupling constants and ^1H and ^{13}C chemical shifts and from the following NOE analysis: NOE correlations between H_3-18 and H-5α, H-7 and H-5α, and H-7 and H_3-19 indicated that these protons were oriented to the same side in a manner similar to that found in compound **5**.

Compound **7**, which was named uprolide K, was isolated as a UV-active (λ_{max} 212 nm) substance that analyzed for $C_{20}H_{28}O_5$ by combined HRFABMS and ^{13}C NMR methods. The compound showed IR bands consistent with hydroxyl (3356 cm^{-1}), γ-lactone (1775 cm^{-1}), ketone (1709 cm^{-1}), olefin (1659 cm^{-1}), and epoxide ring (1270 and 820 cm^{-1}) functions. The ^1H NMR spectrum (Table 1) showed two sharp doublets at δ 6.35 and 5.72 (each 1H, $J = 1.8$ Hz), suggesting a γ-lactone conjugated to an exomethylene group, and two multiplets at δ 5.48 (1H, d, $J = 15.9$ Hz) and 5.17 (1H, ddd, $J = 6.0, 8.7, 15.9$ Hz) due to a 1,2-disubstituted *trans* olefin. Moreover, two signals centered near δ 4.87 (1H, d, $J = 7.8$ Hz) and 2.34 (1H, m) were attributed to methine protons on carbon atoms bearing oxygen (H-14 and H-3, respectively). The two 3H singlets at δ 1.26 and 1.22 were ascribed to the methyl groups placed, respectively, at C-8 and C-4, while the doublet at δ 1.21 (3H, $J = 7.2$ Hz) was ascribable to the methyl group attached to C-12. The ^{13}C NMR spectrum of uprolide K exhibited all 20 signals separated by APT into five quaternary carbons, six CH groups, six methylenes, and three methyl groups. The striking similarity of the chemical and spectral data of uprolide H (**4**) and uprolide K (**7**), including their optical rotations, revealed that these compounds shared many common structural features (i.e., the same gross structure and identical relative configuration at all chiral centers). Likewise, comparison of the 2D NMR spectra of these compounds (including $^1H-^1H$ COSY, HMQC, HMBC,[15] and NOESY) suggested that the structure of **7** was essentially identical to that of **4**, except that in **7** there lies a hydroxyl group at C-8 in lieu of a hydroperoxide functionality. This contention was in full agreement with the observation that the signal for C-19 had shifted from δ_C 22.5 in **4** to δ_C 29.2 in **7**. The relative configuration of **7** (1*S**, 3*R**, 4*R**, 8*S**, 12*R**, 14*R**) was established from the NOESY spectrum, which exhibited correlations between H-1 and H-14 and H_3-18, and H-14 and H_3-20, to disclose their α-configuration. Simultaneous NOE correlations between H-6 and H-5β and H_3-19 demonstrated that these protons were oriented in the opposite direction, establishing the C-8(*S**) configuration in **7**. From these results, it was concluded that **7** was the 8-hydroxyl analogue of **4** with the same relative (and absolute) configuration.

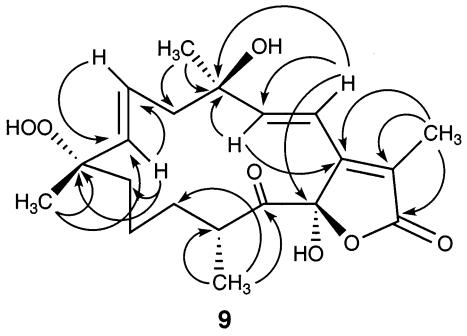
Uprolide K acetate (**8**) had a molecular formula of $C_{22}H_{30}O_6$ deduced from the pseudomolecular ion *m/z* 391 $[M + H]^+$ in its LRFABMS and the ^1H and ^{13}C NMR data. The ^1H NMR spectrum of **8** was almost identical with that of uprolide H (**4**), with the exception that it contained an acetate signal at δ 1.98 (3H, s) and that the signal for H_3-19 had shifted from δ_H 1.32 in **4** to δ_H 1.52 in compound **8** (Table 2). The ^{13}C NMR spectrum in $CDCl_3$, which also showed a striking resemblance to that of uprolide H (**4**), exhibited signals for 22 carbon atoms, suggesting a diterpene acetate: three were carbonyls (δ 211.0, 170.9, and 168.4) and four were methyl carbons (δ 25.1, 22.2, 19.4, and 18.9). These spectral features suggested that **8** had a close similarity to uprolide H (**4**) and that the C-8 hydroperoxide moiety in **4** had been replaced by an acetate group in **8**. That the stereochemistry at the Δ^6 double bond and chiral centers C-1, C-3, C-4, C-8, C-12, and C-14 was identical in uprolides **4** and **8** could be shown by the striking similarity of proton−proton coupling constants and ^1H and ^{13}C NMR chemical shift values involving these sites (Table 2). Thus, uprolide K acetate was assigned as **8**.

Uprolide L (**9**) was obtained as a UV-active colorless oil with a λ_{max} value of 270 nm (ϵ 8600). HRFABMS measurements of uprolide L presented a pseudomolecular $[M + Na]^+$ ion at *m/z* 403.1735, which fits the molecular formula $C_{20}H_{28}O_7$. The IR absorptions at ν_{max} 3399, 1742, 1712, and 1645 cm^{-1} suggested that **9** possessed hydroxyl, α,β-unsaturated ester and ketone carbonyls, and olefin functionalities. The ^1H NMR spectrum indicated the presence of two sets of *trans* ethylenic double bonds from signals at δ 6.55 and 6.03 (each 1H, d, $J = 16.2$ Hz) and δ 5.51 (1H, ddd, $J = 3.3, 10.2, 15.9$ Hz) and 5.34 (1H, dd, $J = 1.2, 15.9$ Hz) and also contained three methyl singlets at δ 2.05, 1.39, and 1.28 and one methyl doublet at δ 1.18 ($J = 6.9$ Hz). An APT experiment indicated four methyl, four methylene, five methine, and seven quaternary carbons. The ^{13}C NMR spectrum indicated the presence of one tetrasubstituted double bond [δ 149.6 (s) and 128.8 (s)], two 1,2-disubstituted double bonds [δ 146.8 (d) and 116.2 (d); δ 135.1 (d) and 128.2 (d)], two carbonyl groups [δ 209.0 (s) and 171.5 (s)], two carbons bearing one oxygen [δ 85.3 (s) and 74.1 (s)], and one carbon bearing two oxygens [δ 103.5 (s)]. The HMBC spectrum showed that the H-2 methine proton was coupled to C-1, C-3, C-4, C-14, and C-15, H-3 was coupled to C-1, C-2, and C-4, and H_3-17 was coupled to the butenolide moiety carbons C-1, C-15, and C-16 (Figure 4). The ketone and hemiacetal groups were located at C-13 and C-14, respectively, on the basis of HMBC correlations which showed that the H_3-20 methyl protons were coupled to C-13 (δ_C 209.0) and the H-2 methine proton to C-14 (δ_C 103.5). Thus, the planar structure of **9** was determined. Strong NOESY correlations were observed between H-2 and H_3-17, H-5α and H_3-18, H-3 and H-5α, H-5α and H-7,

Table 2. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Spectral Data for Uprolides **8**−**10** and Compound **11** in $CDCl_3$ [δ_H, mult, J (Hz); δ_C (mult)][a]

	uprolide K acetate (**8**)		uprolide L (**9**)		uprolide M (**10**)		compound **11**	
atom	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.62, m	40.5 (d)		149.6 (s)	3.48, ddd, 2.4,5.4, 9.3	34.3 (d)	2.80, m 37.7 (d)	
2α	2.67, ddd, 2.7, 4.2, 14.4	29.2 (t)	6.55, d, 16.2	116.2 (d)	2.03, ddd, 5.4,12.9, 14.1	25.9	1.69, m	26.4 (t)
2β	1.53, m				1.85, ddd, 2.4,3.9, 14.1		1.42, m	
3	2.30, m	55.7 (d)	6.03, d, 16.2	146.8 (d)	3.67, dd, 3.9, 12.9	76.8 (d)	3.47, m	75.2 (d)
4		60.3 (s)		74.1 (s)		74.3 (s)		80.5 (s)
5α	2.50, ddd, 1.5, 4.8,15.0	38.5 (t)	2.34, dd, 10.2, 14.4	45.5 (t)	2.38, br dd, 7.8,14.4	45.6 (t)	2.10, m	31.2 (t)
5β	2.29, m		2.50, dd, 3.3, 14.4		2.27, br dd, 7.2,14.4		1.25, m	
6α	5.14, ddd, 5.1, 9.9,16.2	122.2 (d)	5.51, ddd, 3.3, 10.2,15.9	128.2 (d)	5.79, dt, 7.5,16.2	128.3 (d)	2.28, m	22.9 (t)
6β							2.16, m	
7	5.60, dd, 0.6, 16.2	139.4 (d)	5.34, dd, 1.2, 15.9	135.1 (d)	5.50, d, 16.2	134.2 (d)	5.46, br t, 6.3	123.8 (d)
8		83.4 (s)		85.3 (s)		85.5 (s)		135.4 (s)
9α	1.87, m	41.3 (t)	1.35, m	37.3 (t)	1.73, m	34.4 (t)	1.99, m	39.3 (t)
9β	1.41, m		1.30, m		1.50, m		1.72, m	
10α	1.43, m	21.3 (t)	1.34, m	24.5 (t)	1.47, m	20.0 (t)	1.86, m	25.8 (t)
10β	1.05, m		0.86, m		1.35, m		1.05, m	
11α	2.07, m	30.1 (t)	1.50, m	34.2 (t)	1.68, m	32.4 (t)	1.26, m	29.6 (t)
11β	1.12, m		1.90, m		1.26, m		1.42, m	
12	3.25, m	41.8 (d)	2.57, m	39.4 (d)	1.83, m	32.7 (d)	1.43, m	42.7 (d)
13		211.0 (s)		209.0 (s)	3.37, dd, 1.5,9.6	73.7 (d)	3.78, dd, 1.2,4.5	72.2 (d)
14	4.87, d, 7.5	79.5 (d)		103.5 (s)	4.73, dd, 1.5,9.3	75.7 (d)	4.24, br t, 4.5	86.9 (d)
15		138.7 (s)		128.8 (s)		137.8 (s)	2.81, m	41.3 (d)
16		168.4 (s)		171.5 (s)		170.1 (s)		178.8 (s)
17α	6.33, d, 2.1	123.1 (t)	2.05, s	9.2 (q)	6.45, d, 3.0	123.7 (t)	1.15, d, 6.9	10.0 (q)
17β	5.69, d, 1.8				5.70, d, 2.7			
H_3-18	1.21, s	19.4 (q)	1.39, s	30.6 (q)	1.17, s	22.2 (q)	1.30, s	22.0 (q)
H_3-19	1.52, s	25.1 (q)	1.28, s	19.6 (q)	1.34, s	21.7 (q)	1.71, s	16.5 (q)
H_3-20	1.22, d, 6.9	18.9 (q)	1.18, d, 6.9	20.2 (q)	0.89, d, 6.9	15.9 (q)	0.89, d, 6.6	14.9 (q)
21		170.9 (s)						
H_3-22	1.98, s	22.2 (q)						

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. ^{13}C NMR multiplicities were obtained from APT experiments.



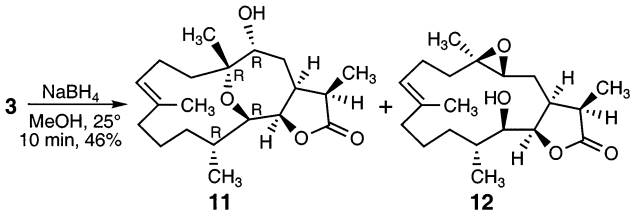
9

Figure 4. Selected HMBC correlations (^{1}H → ^{13}C) for uprolide L (**9**).

and H_3-19 and H-6, which required that the relative configuration of the molecule be 4*R**, 8*S**, 12*R**, and 14*R**. Conformational MMFF calculations of uprolide L suggested that such NOESY correlations were compatible only in the most stable conformation of structure **9**. Contrary to uprolide H (**4**), which also has the C-8(*S**) configuration, the geometry in **9** is such that H-7 and the −OOH group now lie nearly orthogonally from each other, thus causing H-6 to resonate at lower field than H-7.

The molecular formula of uprolide M (**10**) was determined to be $C_{20}H_{30}O_6$ from both HRFABMS and ^{13}C NMR data (Table 2). The absorptions observed in the IR and UV spectra suggested the presence of an α-methylene-γ-lactone [ν_{max} 1760, 1660 cm^{-1}; λ_{max} 208 nm (ϵ 5000)] plus hydroxyl (3409 cm^{-1}) and ether (1272 cm^{-1}) functionalities. The ^{1}H NMR spectrum showed three characteristic signals at δ 1.34 (3H, s), 1.17 (3H, s), and 0.89 (3H, d, J = 6.9 Hz) due to tertiary or secondary methyl groups, two well-resolved signals at δ 5.79 (1H, dt, J = 7.5, 16.2 Hz) and 5.50 (1H, d, J = 16.2 Hz) ascribable to a *trans* 1,2-disubstituted double bond, and three oxymethine protons bearing ethers or in the γ-lactone group at δ 3.37 (1H, dd, J = 1.5, 9.6 Hz), 3.67 (1H, dd, J = 3.9, 12.9 Hz), and 4.73 (1H, dd, J = 1.5, 9.3 Hz). The ^{1}H−^{1}H COSY spectrum displayed correlations from H-1 to H-3, H-5 to H-7, H-9 to H-14, H-12 to H_3-20, and H-1 to H-14. ^{1}H−^{1}H long-range correlations were also observed between H-1 and the H_2-17 protons and H-5α and H-7. The ^{13}C NMR spectrum showed one carbonyl resonance at δ 170.1 (s), confirming the presence of a γ-lactone, four olefin resonances at δ 123.7 (t), 128.3 (d), 134.2 (d), and 137.8 (s), and five carbons bearing oxygen at δ 73.7 (d), 74.3 (s), 75.7 (d), 76.8 (d), and 85.5 (s). The latter carbon signal was ascribable to a tertiary allylic carbon bearing a hydroperoxide function. These spectroscopic findings and the degree of unsaturation (six) showed **10** to have the characteristic 14-membered α-methylene-γ-cembranolide framework with a pyranether linkage across C-3 and C-13. After assignments for all direct C−H bonds were made based on HMQC, the gross structure of **10** was determined by HMBC analysis.[16] The relative configuration of the stereocenters about the tricyclic ring system in **10** was assigned on the basis of correlations observed in the NOESY NMR spectrum and through interpretation of NMR coupling constant data (Table 2). Strong NOESY

Scheme 1



correlations observed between H-14 and H-1, H-13, and H_3-20 established that these protons were all spatially oriented on the bottom face of the molecule and allowed the C-1/C-14 ring junction to be assigned as *cis*. The noticeably small coupling constant between H-13 and H-14 ($J_{13,14}$ = 1.5 Hz) and the large coupling constant between H-1 and H-14 ($J_{1,14}$ = 9.3 Hz) supported in part these stereochemical assignments.[17] Likewise, strong NOESY cross-peaks between H-1 and H-2α, between H-5α and H_3-18, and between H-13, H-2α, and H_3-18 provided evidence that these protons must all be α-oriented. Conversely, strong NOESY correlations between H-2*β* and H-3 and between H_3-19 and H-6 required that H2*β*, H-3, and H_3-19 be positioned upward on the top face of the molecule. Thus, the stereochemical descriptors for uprolide M (**10**) were shown to be 1*S**, 3*S**, 4*R**, 8*S**, 12*R**, 13*R**, 14*R**.

To support our stereochemical assignment at C-13 in uprolide M (**10**), we sought the chemical conversion of **3** into suitable cembranolide analogues.[17] As shown in Scheme 1, reduction of the C-13 ketone in **3** with $NaBH_4$ in MeOH at 25 °C accompanied by net addition of H_2 to the α-methylene-*γ*-lactone moiety gave analogues **11** and **12** in 46% yield as a 1:1.3 mixture after chromatographic separation. The complete structural assignment of these analogues was accomplished on the basis of 1D and 2D NMR experiments involving $^1H-^1H$ COSY, APT, NOESY, HMQC, and HMBC measurements. In each instance, these 2D NMR spectra provided both the structure and the complete proton and carbon atom assignments. Thus, in support of our proposed relative configuration at C-13 in **10**, reduction of **3** by $NaBH_4$ appears to take place exclusively from the least hindered α-face of the molecule. The structure of analogue **11** was supported by the HMBC correlation of the C-13 proton signal (δ_H 3.78) with C-4 across the cyclic ether linkage and the NOE correlations of protons H-14 with H-1, H-13, H-15, and H_3-20, H_3-18 with H-3, H-6*β*, and H-7, and H-13 with H-2α, H-5α, and H-14. On the other hand, the proposed relative stereochemistry for analogue **12** was supported by strong NOESY correlations between H-3 and H-13 and those between H-14 and H-1, H-13, and H_3-20. We surmise that in this reaction the formation of **12** is accompanied by concomitant transannular attack of the C-13 hydroxyl group at C-4 of the epoxide with overall retention of configuration leading to cyclic ether **11**.[17]

Compound **13** was isolated as colorless needles, mp 210 °C, that had a molecular formula of $C_{26}H_{40}O_5$ from the pseudomolecular ion *m*/*z* 455.2842 $[M + Na]^+$ in its HRFABMS. The seven degrees of unsaturation inherent in the molecular formula could be accounted for in part by two carbon−carbon double bonds; hence, **13** possessed five rings. A conspicuous group of methine resonances between *δ* 2.84 and 4.13 (6H) and the presence of a ^{13}C NMR acetal resonance (δ_C 101.8, d) suggested the presence of a cyclized pentose unit. Comparison of the ^{13}C NMR bands from relevant pentose-glycoside models showed that **13** contained a pentose ring in the pyranose form.[18] Evaluation of the FAB mass spectral fragmentation pattern of the saponin showing $C_{21}H_{31}$ (*m*/*z* 283) [M + H − $C_5H_{10}O_5$, aglycon unit] and $C_5H_9O_4$ (*m*/*z* 133) [M + H − $C_{21}H_{32}O$, sugar unit] supported this assignment. The complete structural details of the tetracyclic diene and pentose portion of the molecule were subsequently determined by NMR analysis. The ^1H and ^{13} C NMR spectra of **13** (in DMSO-d_6) immediately suggested that the aglycon was the degraded sterol pregna-5,20-dien-3*β*-ol (pregnadienol), the same aglycon present in several gorgonian-derived steroidal glycosides.[19] Although **13** was soluble in DMSO-d_6, the sugar's ^1H NMR signals in this solvent were broad and poorly dispersed. Therefore, the complete assignment of the pentose unit was accomplished on its peracetylated derivative **14** in $CDCl_3$ by coupling constant analysis and NOE experiments (see Experimental Section). Through these experiments, the sugar unit in **13** was identified as xylose in a *β*-pyranoside configuration. The anomeric proton at C-1′ in **14** appeared at *δ* 4.54. This proton was a doublet with *J* = 7.2 Hz, which confirmed it as an axial proton, thus showing the acetal linkage as equatorial or *β*. Because H-3′ was split into a triplet (*J* = 8.9 Hz), it was clearly an axial proton. From this we concluded that H-2′ was *trans axial* to both H-1′ and H-3′; therefore the C-2′ and C-3′ hydroxyls must be equatorial. Furthermore, H-4′ (a doublet of doublets of doublets with *J* = 9.5, 8.9, and 5.2 Hz) had to be axial, *trans* to both H-3′ and H-5′$_{ax}$. Strong NOESY correlations from H-1′ to H-3′ and H-5′$_{ax}$ confirmed the 1,3-diaxial relationship of the latter protons. The relative configuration of **14** was subsequently confirmed by X-ray diffraction analysis (Figure 5). Assuming that the pregnadiene aglycon in **13** possesses the same absolute configuration as found in other *Eunicea* species,[9] by the use of Hudson's rules of isorotation, the positive molecular rotation difference between glycoside **13** ($[\alpha]^{25}_D$ −57.3° in MeOH) and the suspected aglycon ($[\alpha]^{25}_D$ −62.1° in $CDCl_3$) indicates that the sugar component in saponin **13** must be *β*-D-xylopyranose.[20]

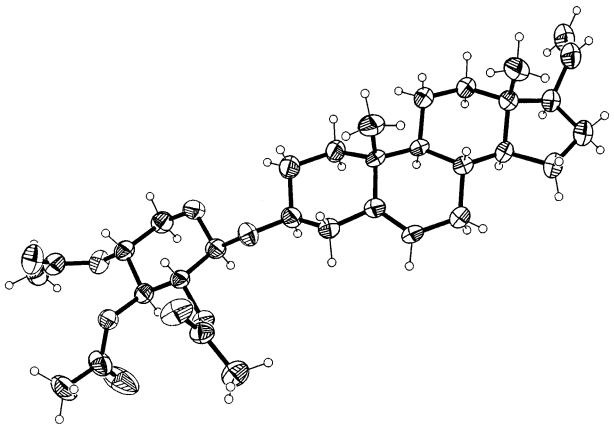
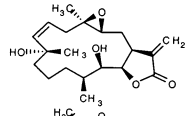
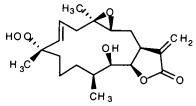
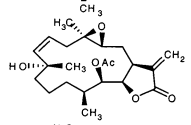
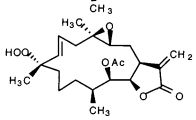
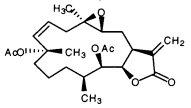
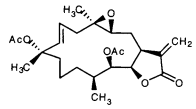
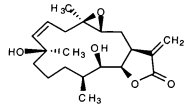
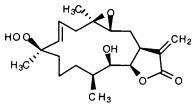
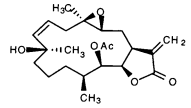
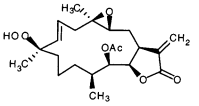
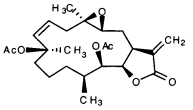
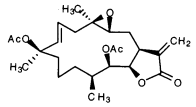
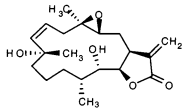
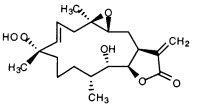
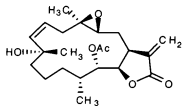
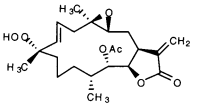
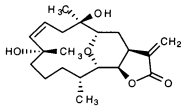
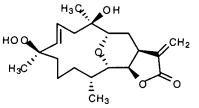
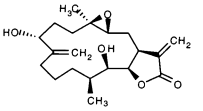
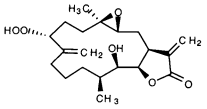
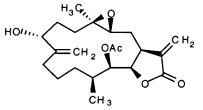
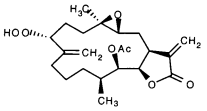
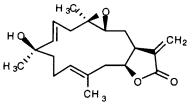
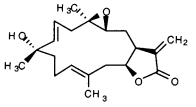


Figure 5. Computer-generated ORTEP plot of **14** showing relative configuration.

Conclusions

Eight new cembranolide diterpenoids, 12-epieupalmerone (**3**) and uprolides **4**−**10**, and a new saponin, **13**, have been isolated from the gorgonian *Eunicea pinta*. As far as we are able to ascertain, this report constitutes the first chemical investigation of this marine animal. Cembranolides **4**−**10** are likely autoxidation products of **2** and **3** upon addition of singlet oxygen to the Δ^7 double bond to give a perepoxide followed by internal proton transfer.[22] Except for the C-13 ketone function and, to a certain extent, the relative configuration of the C-12 methyl group, compounds **4**−**10** are structurally similar to the uprolide series of

Table 3. Original and Revised Structures for Some Previously Isolated Uprolide-Type Cembranolides

compound's name	original structure	revised structure	revised MS data	reference
uprolide B			*m/z* $[M + Na]^+$ 389 for $C_{20}H_{30}O_6Na$	7
uprolide B acetate			*m/z* $[M + H]^+$ 409 for $C_{22}H_{33}O_7$	7
uprolide B diacetate			no change	7
8-epi-uprolide B			*m/z* $[M + Na]^+$ 389 for $C_{20}H_{30}O_6Na$	7
8-epi-uprolide B acetate			*m/z* $[M + Na]^+$ 431 for $C_{22}H_{32}O_7Na$	7
8-epi-uprolide B diacetate			no change	7
12,13-bisepiuprolide B			*m/z* $[M + Li]^+$ 373.2201 for $C_{20}H_{30}O_6Li$	8b
12,13-bisepiuprolide B acetate			*m/z* $[M - OH]^+$ 391.2123 for $C_{22}H_{31}O_6$	8b
uproeunicin			*m/z* $[M + Li]^+$ 373.2193 for $C_{20}H_{30}O_6Li$	8b
uprolide C			*m/z* $[M + Na]^+$ 389 for $C_{20}H_{30}O_6Na$	7
uprolide C acetate			*m/z* $[M + H]^+$ 409 for $C_{22}H_{33}O_7$	7
uproeuniolide			no change	8b

cembranolides isolated from *E. mammosa* and *E. succinea*.[7,8] From the structure of uprolide H (**4**), which was unambiguously secured by spectral and crystallographic methods, it now appears obvious that the strong downfield shift experienced by the C-8 resonance in many uprolides can be attributed to the presence of a hydroperoxide unit at that position and not a change in stereochemical orientation about the Δ^6 olefin as previously thought.[7] If the hydroperoxide is replaced by a hydroxyl function, a significant upfield shift is experienced by the C-8 resonance (i.e., 85.5 ppm in **4** vs 73.4 ppm in **7**). Such replacement is always accompanied by a downfield shift of the C-19 resonance (i.e., 22.5 ppm in **4** vs 29.2 ppm in **7**). From these data we can now conclude that all uprolides isolated thus far have the *trans* configuration about the Δ^6 double bond, as suggested by the large $J_{6,7}$ coupling constant (typically 12−16 Hz).

Moreover, our results clearly show that most *Eunicea* cembranolides belonging to the so-called uprolide-B series bear a hydroperoxide function, not a hydroxyl, at C-8.[7] Thus, the structures for the entire uprolide-B series (eight in total) require revision, as do uproeunicin, uproeuniolide, and two analogues of the uprolide-C series (Table 3).[21] Also, there seems to be an empirical relationship between the ^1H NMR chemical shift values of H-6 and H-7 and the relative configuration at C-8. Thus, when C-8 has the *S**

configuration, H-7 typically appears downfield from H-6. If, on the other hand, C-8 has the *R** configuration, H-7 resonates upfield from H-6.[23]

Like most α-methylene-γ-cembranolides, compounds **3** and **4** showed strong cytotoxic activity (in vitro testing) when tested in the NCI 60 cell-line tumor panel. Specifically, compound **3** showed strong growth inhibition against non-small cell lung cancer cells (NCI-H322M, IC_{50} 0.90 μg/mL) and renal cancer cells (TK-10, IC_{50} 0.13 μg/mL), whereas compound **4** displayed strong growth inhibition against human T lymphocytic leukemia cells (MOLT-4, IC_{50} 0.01 μg/mL; SR, IC_{50} 0.07 μg/mL). On the other hand, saponin **13** was strongly toxic only to renal cancer cells (A498, IC_{50} 4.2 μg/mL; ACHN, IC_{50} 2.8 μg/mL; CAKI-1, IC_{50} 6.6 μg/mL). In vitro antituberculosis screening of succinolide (**2**) and uprolide H (**4**) against *Mycobacterium tuberculosis* $H_{37}Rv$ at a concentration of 6.25 μg/mL showed no inhibitory activity, whereas 3β-pregna-5,20-diene-β-D-xylopyranoside (**13**) inhibited 52% of mycobacterial growth.

Experimental Section

General Experimental Procedures. Melting points were determined with a Büchi 535 capillary apparatus and are uncorrected, and optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded with a Nicolet Magna 750 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectral data and $^1H-^1H$ COSY, NOESY, APT, HMQC, CSCMBB, and HMBC experiments were measured with a 300 MHz Bruker DPX-300 FT-NMR spectrometer. FABMS were carried out in a VG AutoSpec (Fisons). Column chromatography was performed on Si gel (35−75 mesh) or bonded C_{18} Si gel (35−75 mesh). Analytical TLC analyses were carried out on Analtech Si gel glass plates, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH. Lowest energy conformers were searched using MMFF force field implemented in the MacSpartan Pro program (Wavefunction, Inc.). All solvents used were either spectral grade or were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the gorgonian $CHCl_3$ extract.

Animal Material. The specimens analyzed in this paper belong to the species *Eunicea pinta* Bayer & Deichmann, 1958. A voucher sample (EPSM # 01) is kept at the Department of Chemistry, University of Puerto Rico, Río Piedras Campus. The specimens were collected in May 1996 along the southern coast of San Andrés Island in Colombia between 25 and 30 m in water depth. The specimens externally resembled congeners of *Plexaurella* or *Pseudoplexaura* because of their flexible and slimy long branches. Nevertheless, microscopic observations of their calcite sclerites revealed that the specimens belonged to *Eunicea*. Specimens exhibited an inner sclerite layer with small purple and finely ornate spindles, a middle layer filled with midsize (~1 mm) robust spindles, and an external layer covered with particular torch club-like sclerites, which are particularities from *Eunicea* species. The torch club-like sclerite is a characteristic trait from the *Eunicea* subgenus (species: *E. mammosa*, *E. succinea*, *E. laxispica*, *E. palmeri*, and *E. pinta*). Specimens presented anthocodial sclerites, which were flat rods of several sizes and including some deformations. These polyp sclerites are distinctive characteristics of *E. pinta* Bayer & Deichmann, 1959, over the rest of the subgenus, locating *E. pinta* at the basal part of the group when observing phylogenetic reconstructions made with sclerite morphology. Sclerites from two different voucher specimens of *E. pinta* from San Andrés Island were carefully compared with the sclerites of the type specimen of *E. pinta* (USNM 50563). The torch club-like sclerites, flat rods from the polyp as well as the purple spindles of the middle layer, were nearly identical in both shape and size. The spindle sclerites from the middle layer, on the other hand, were reduced in size in specimens from San Andrés, but conserving the typical form and ornamentation as in the type specimen. This finding expands considerably the geographical range of *E. pinta* since it was known to be distributed at Florida, Bahamas, and Bermuda, but not at localities in the Southwestern Caribbean such as San Andrés Island. This is also the most shallow record of this species (25 m), as it was reported between 40 and 70 m in water depth in previous collections.[24]

Extraction and Isolation of Cembranolides 1−10 and Saponin 13. The gorgonian *E. pinta* was immediately frozen after collection and subsequently freeze-dried (2.2 kg). All of the freeze-dried animal was extracted with MeOH−$CHCl_3$ (1:1) (4 × 2 L) to give, after filtration and concentration to dryness under a vacuum, a dark brown syrup (247 g) that was partitioned between *n*-hexane and H_2O. The H_2O-soluble fraction was further extracted with $CHCl_3$. The $CHCl_3$-soluble fraction (28.7 g) was chromatographed over Si gel 60 (500 g) using *n*-hexane−EtOAc and then $CHCl_3$−acetone mixtures of increasing polarity to yield 11 primary fractions, designated as I−XI. Purification of fraction II (0.47 g) by Si gel (13 g) chromatography using *n*-hexane−EtOAc (5:1) afforded known cembranolides euniolide (**1**, 27.6 mg, 9.62 × 10^{-2}%) and succinolide (**2**, 13.3 mg, 4.63 × 10^{-2}%). Compounds **1** and **2** were identified by comparison with reference compounds and literature data.[4,5] For isolation of compound **3**, fraction IV (1.37 g) was chromatographed on Si gel (37 g) with *n*-hexane−EtOAc (5:1), yielding 12-epieupalmerone as colorless needles (**3**, 77.4 mg, 0.27%) and a more polar fraction containing compounds **4** and **5**. Uprolide H (**4**, 25.3 mg, 8.81 × 10^{-2}%) and uprolide I (**5**, 8.3 mg, 2.89 × 10^{-2}%) were purified by Si gel chromatography using $CHCl_3$−acetone (30:1). From fraction V (1.46 g) uprolide K acetate (**8**, 1.2 mg, 4.18 × 10^{-3}%) and uprolide J (**6**, 9.7 mg, 3.38 × 10^{-2}%) were obtained by repetitive Si gel column chromatography eluting first with *n*-hexane−EtOAc (15:1) and then $CHCl_3$−acetone (30:1). Fraction VI (4.6 g) was dissolved in a small volume of toluene, filtered, concentrated in vacuo, and loaded onto a Bio-Beads SX-3 (110 g) column with toluene as eluent. Five secondary fractions, designated as VI(A)−VI(E), were obtained. Fraction D (0.5 g) was initially flash chromatographed on Si gel (gradient elution; 40:1 and 4:1 $CHCl_3$−acetone, and 100% acetone) yielding 11 tertiary fractions. Several of the flash chromatography fractions were purified further over Si gel using either $CHCl_3$−MeOH or $CHCl_3$−acetone mixtures of increasing polarity, and further flash column chromatography on C_{18} Si gel (gradient elution; 100% H_2O, 5:3, 20:1, and 100% MeOH) was necessary before selected fractions yielded uprolide K (**7**, 3.2 mg, 1.11 × 10^{-2}%), uprolide L (**9**, 6.0 mg, 2.09 × 10^{-2}%), and uprolide M (**10**, 4.8 mg, 1.67 × 10^{-2}%). Fraction IX (5.83 g) was stored at 25 °C for several days before it crystallized. Recrystallization from hot EtOAc followed by filtration led to the isolation of pure saponin **13** (43.5 mg, 0.15%) as colorless needles.

12-Epieupalmerone (3): colorless needles, mp 92 °C; $[\alpha]^{25}_D$ −25.9° (*c* 1.0, $CHCl_3$); IR, UV, ^1H NMR, ^{13}C NMR, HREIMS, as previously reported.[5] X-ray crystallographic analysis confirmed the structure of **3** (relative stereochemistry; molecular structure, Figure 1).

Single-Crystal X-ray Crystallography of 3.[25] Colorless prisms of **3** were obtained from a solution in 5:1 hexane−EtOAc. The crystal belongs to the monoclinic space group $P2_1$ with a = 8.9000(10) Å, b = 6.1380(10)Å, c = 16.9670(20) Å, V = 926 (3) Å^3, λ(Cu Kα) = 1.54178 Å, μ(Cu Kα) = 0.577 mm^{-1}. Intensity data were measured on a Bruker P4 diffractometer. All atoms were given isotropic thermal parameters and refined to an R value of 0.15.

Uprolide H (4): colorless needles; mp 161 °C; $[\alpha]^{25}_D$ −25.6° (*c* 1.0, $CHCl_3$); IR (film) 3411, 3116, 1764, 1707, 1668, 1376, 1279, 1094, 747 cm^{-1}; UV (MeOH) λ_{max} 216 nm (ϵ 4900); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HRFABMS (3-NBA) *m/z* $[M + Na]^+$ 387.1794 (calcd for $C_{20}H_{28}O_6Na$, 387.1783). X-ray crystallographic analysis confirmed the structure of **4** (relative stereochemistry; ORTEP diagram, Figure 2).

Single-Crystal X-ray Crystallography of 4.[26] Suitable colorless prisms of **4** were obtained from a mixture of 30:1

$CHCl_3$−acetone; $C_{20}H_{28}O_6$; fw 364.42. The crystal (0.50 × 0.15 × 0.10 mm) belongs to the monoclinic system, space group $P2_1$ with a = 8.6890(15) Å, b = 5.9748(11) Å, c = 17.934(3) Å, V = 927.8(3) Å^3, Z = 2, D_{cal} = 1.305 g/cm^3, λ(Mo Kα) = 0.71073 Å. Intensity data were measured on a Siemens SMART CCD diffractometer up to 2.28θ of 27.12°. All 5770 unique reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.0452, R_w = 0.1112 for 3696 observed reflections [$I > 2.00\sigma(I)$] and 248 variable parameters. Values of the neutral atom scattering factors and real and imaginary dispersion corrections were taken from the *International Tables for X-ray Crystallography*.[27]

Uprolide I (5): colorless gum; $[\alpha]^{25}_D$ −15.6° (*c* 0.9, $CHCl_3$); IR (film) 3414, 1771, 1716, 1459, 1375, 1100, 756 cm^{-1}; UV (MeOH) λ_{max} 214 nm (ϵ 3100); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 387.1789 (calcd for $C_{20}H_{28}O_6Na$, 387.1783).

Uprolide J (6): colorless gum; $[\alpha]^{25}_D$ +12.0° (*c* 0.25, $CHCl_3$); IR (film) 3411, 3101, 1775, 1724, 1662, 1631, 1455, 1378, 1265, 1098, 758 cm^{-1}; UV (MeOH) λ_{max} 210 nm (ϵ 3550); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 387.1790 (calcd for $C_{20}H_{28}O_6Na$, 387.1783).

Uprolide K (7): colorless gum; $[\alpha]^{25}_D$ −22.0° (*c* 1.0, $CHCl_3$); IR (film) 3356, 3113, 1775, 1709, 1659, 1458, 1370, 1270, 1094, 820 cm^{-1}; UV (MeOH) λ_{max} 212 nm (ϵ 3500); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 371.1823 (calcd for $C_{20}H_{28}O_5Na$, 371.1834).

Uprolide K acetate (8): colorless gum; $[\alpha]^{25}_D$ −4.0° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 206 nm (ϵ 2425); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); LRFABMS (glycerol) *m/z* [M + H]$^+$ 391 (calcd for $C_{22}H_{31}O_6$, 391).

Uprolide L (9): colorless oil; $[\alpha]^{25}_D$ +42.5° (*c* 0.4, $CHCl_3$); IR (film) 3399, 2927, 2856, 1742, 1712, 1645, 1460, 1373, 1270, 1091, 1024, 809 cm^{-1}; UV (MeOH) λ_{max} 270 nm (ϵ 8600); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRFABMS (glycerol/thioglycerol) *m/z* [M + Na]$^+$ 403.1735 (calcd for $C_{20}H_{28}O_7Na$, 403.1733).

Uprolide M (10): colorless gum; IR (film) 3409, 2937, 1760, 1660, 1460, 1379, 1272, 984, 759 cm^{-1}; UV (MeOH) λ_{max} 208 nm (ϵ 5000); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 389.1952 (calcd for $C_{20}H_{30}O_6Na$, 389.1940).

Reduction of 12-Epieupalmerone (3) with $NaBH_4$. A mixture of **3** (10.7 mg, 0.032 mmol) and $NaBH_4$ (7.2 mg, 0.19 mmol) in MeOH (2 mL) was stirred at 25 °C for 10 min. The reaction mixture was quenched with 1 N HCl (1 mL) and water (5 mL), extracted with $CHCl_3$ (3 × 10 mL), and concentrated under reduced pressure. Purification of the crude residue by Si gel (2.5 g) column chromatography using a 2:1 mixture of hexane and EtOAc yielded lactones **11** (2.2 mg, 20% yield) and **12** (2.8 mg, 26% yield).

15(*R),17-Dihydro-4,13-bisepijeunicin (11):** colorless oil; $[\alpha]^{25}_D$ −30.0° (*c* 0.2, $CHCl_3$); IR (film) 3462, 2963, 2928, 2856, 1770, 1452, 1381, 1185, 1166, 1048 cm^{-1}; UV (MeOH) λ_{max} 206 nm (ϵ 4100); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRFABMS (glycerol) *m/z* [M + H]$^+$ 337.2387 (calcd for $C_{20}H_{33}O_4$, 337.2379).

15(*R),17-Dihydro-12-epieupalmerin (12):** colorless oil; $[\alpha]^{25}_D$ −9.0° (*c* 0.9, $CHCl_3$); IR (film) 3482, 2932, 2860, 1756, 1463, 1387, 1186, 1025, 989 cm^{-1}; UV (MeOH) λ_{max} 206 nm (ϵ 1400); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.83 (m, H-1), 1.62 (m, H-2α), 1.43 (m, H-2β), 2.60 (m, H-3), 2.09 (m, H-5α), 1.20 (m, H-5β), 2.15 (m, H-6αβ), 5.01 (br t, J = 5.7 Hz, H-7), 2.15 (m, H-9α), 2.03 (m, H-9β), 1.63 (m, H-10α), 1.43 (m, H-10β), 1.34 (m, H-11αβ), 2.05 (m, H-12), 3.72 (br d, J = 10.2 Hz, H-13), 4.24 (dd, J = 4.8, 10.2 Hz, H-14), 2.88 (br q, J = 7.8 Hz, H-15), 1.38 (d, J = 6.9 Hz, Me-17), 1.24 (s, Me-18), 1.57 (br s, Me-19), 0.90 (d, J = 6.9 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.6 (d, C-1), 25.1 (t, C-2), 59.3 (d, C-3), 61.0 (s, C-4), 38.3 (t, C-5), 23.5 (t, C-6), 124.7 (d, C-7), 136.0 (s, C-8), 36.9 (t, C-9), 22.9 (t, C-10), 31.4 (t, C-11), 31.0 (d, C-12), 71.8 (d, C-13), 79.4 (d, C-14), 39.2 (d, C-15), 175.4 (s, C-16), 9.6 (q, C-17), 16.2 (q, C-18), 15.4 (q, C-19), 11.8 (q, C-20); HRFABMS (glycerol) *m/z* [M + H]$^+$ 337.2380 (calcd for $C_{20}H_{33}O_4$, 337.2379).

3β-Pregna-5,20-diene-β-D-xylopyranoside (13): colorless needles; mp 210 °C; $[\alpha]^{25}_D$ −57.3° (*c* 0.75, MeOH); IR (film) 3468, 3309, 3070, 2959, 2935, 2882, 1635, 1370, 1082, 1053, 1017, 983 cm^{-1}; ^{1}H NMR (selected data, DMSO-d_6, 300 MHz) δ 3.34 (m, H-3), 5.28 (br s, H-6), 0.51 (s, Me-18), 0.90 (s, Me-19), 5.70 (ddd, J = 7.5, 10.8, 16.2 Hz, H-20), 4.93 (br d, J = 10.8 Hz, H-21α); 4.86 (br d, J = 16.2 Hz, H-21β), 4.13 (d, J = 7.5 Hz, H-1′), 2.84 (m, H-2′), 3.02 (m, H-3′), 3.19 (m, H-4′), 3.58 (dd, J = 5.1, 11.1 Hz, H-5′$_{eq}$), 2.96 (br t, J = 10.8 Hz, H-5′$_{ax}$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 36.8 (t, C-1), 29.4 (t, C-2), 77.2 (d, C-3), 38.3 (t, C-4), 140.4 (s, C-5), 121.1 (d, C-6), 31.4 (t, C-7), 31.5 (d, C-8), 49.8 (d, C-9), 36.3 (s, C-10), 20.2 (t, C-11), 36.8 (t, C-12), 42.9 (s, C-13), 55.2 (d, C-14), 24.4 (t, C-15), 26.5 (t, C-16), 54.6 (d, C-17), 12.6 (q, C-18), 19.1 (q, C-19), 139.4 (d, C-20), 114.9 (t, C-21), 101.8 (d, C-1′), 73.2 (d, C-2′), 76.6 (d, C-3′), 69.5 (d, C-4′), 65.5 (t, C-5′); HRFABMS (glycerol) *m/z* [M + Na]$^+$ 455.2842 (calcd for $C_{26}H_{40}O_5Na$, 455.2773).

Peracetylation of 3β-Pregna-5,20-diene-β-D-xylopyranoside (13). Compound **13** (25 mg, 0.058 mmol) was treated with a mixture of pyridine (0.5 mL) and Ac_2O (0.5 mL) at 25 °C overnight. After concentration in vacuo, the residue was subjected to passage over a Si gel (1.0 g) column eluting with hexane−EtOAc (6:1) to afford 3β-pregna-5,20-diene-(2,3,4-triacetyl-O)-β-D-xylopyranoside (**14**) (31 mg, 96% yield) as colorless needles; mp 164 °C; $[\alpha]^{25}_D$ −60.0° (*c* 1.0, $CHCl_3$); IR (film) 3073, 2945, 2893, 1752, 1637, 1254, 1081, 1061, 982 cm^{-1}; ^{1}H NMR (partial data for the sugar moiety, $CDCl_3$, 300 MHz) δ 4.54 (d, 1H, J = 7.2 Hz, H-1′), 4.90 (dd, 1H, J = 7.2, 8.9 Hz, H-2′), 5.16 (t, 1H, J = 8.9 Hz, H-3′), 4.93 (ddd, 1H, J = 5.2, 8.9, 9.5 Hz, H-4′), 4.08 (dd, 1H, J = 5.2, 11.5 Hz, H-5′$_{eq}$), 3.31 (dd, 1H, J = 9.5, 11.5 Hz, H-5′$_{ax}$), 2.02 (s, 9H, OCOCH_3); ^{13}C NMR (partial data for the sugar moiety, $CDCl_3$, 75 MHz) δ 99.5 (d, C-1′), 71.1 (d, C-2′), 71.7 (d, C-3′), 68.9 (d, C-4′), 62.1 (t, C-5′), 170.2 (s, O*C*OCH$_3$), 169.8 (s, O*C*OCH$_3$), 169.4 (s, O*C*OCH$_3$), 20.7 (q, 3C, OC*O*CH$_3$); HRFABMS (glycerol) *m/z* [M + Na]$^+$ 581.3112 (calcd for $C_{32}H_{46}O_8Na$, 581.3090). X-ray crystallographic analysis confirmed the structure of **14** (relative stereochemistry; ORTEP diagram, Figure 5).

Single-Crystal X-ray Crystallography of 14.[26] Suitable colorless prisms of **14** were obtained from a solution in 6:1 hexane−EtOAc; $C_{32}H_{46}O_8$; fw 558.69. The crystal (0.24 × 0.22 × 0.22 mm) belongs to the monoclinic system, space group $P2_1$ with a = 7.343(7) Å, b = 40.322(4) Å, c = 10.913(1) Å, β = 95.147(2)°, V = 3218.1(5) Å^3, Z = 2, D_{cal} = 1.153 g/cm^3, λ(Mo Kα) = 0.71073 Å. Intensity data were measured on a Siemens SMART CCD diffractometer up to 1.87θ of 23.3°. A total of 14 208 reflections were collected, and 8047 were unique. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.0628, R_w = 0.1540 for 5364 observed reflections [$I > 2.00\sigma(I)$] and 731 variable parameters. Values of the neutral atom scattering factors and real and imaginary dispersion corrections were taken from the *International Tables for X-ray Crystallography*.[27]

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Supporting Information Available: Description of the X-ray crystal structure data including tables of intramolecular distances, torsion angles, positional parameters, and intramolecular bond angles for 12-epieupalmerone (**3**), uprolide H (**4**), and peracetylated saponin **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Burkholder, P. R.; Burkholder, L. M. *Science* **1958**, *127*, 1174−1175.
(2) Bayer, F. M. *The Shallow-Water Octocorallia of the West Indian Region*; Martinus Nijhoff: The Hague, 1961.
(3) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618.
(4) Morales, J. J.; Espina, J. R.; Rodríguez, A. D. *Tetrahedron* **1990**, *46*, 5889−5894.
(5) Rodríguez, A. D.; Dhasmana, H. *J. Nat. Prod*. **1993**, *56*, 564−570.
(6) Uprolide is an acronym of University of Puerto Rico. Formally, the uprolides are *Eunicea* cembranolides characterized by the presence of a Δ^6 1,2-disubstituted olefin (or a terminal Δ^8 olefin), a hydroxyl or hydroperoxide group at C-8 (or C-7), and usually (but not always) an epoxy group at C-3, -4 and an α-methylene-γ-lactone moiety between C-1, -14.
(7) Rodríguez, A. D.; Piña, I. C.; Soto, J. J.; Rojas, D. R.; Barnes, C. L. *Can. J. Chem*. **1995**, *73*, 643−654.
(8) (a) Rodriguez, A. D.; Soto, J. J.; Piña, I. C. *J. Nat. Prod*. **1995**, *58*, 1209−1216. (b) Rodríguez, A. D.; Acosta, A. L. *J. Nat Prod*. **1998**, *61*, 40−45. (c) Rodríguez, A. D.; Acosta, A. L. *J. Nat Prod*. **1997**, *60*, 1134−1138. (d) Rodríguez, A. D.; Soto, J. J.; Barnes, C. L. *J. Org. Chem*. **2000**, *65*, 7700−7702.
(9) Cobár, O. M.; Rodríguez, A. D.; Padilla, O. L. *J. Nat. Prod*. **1997**, *60*, 1186−1188.
(10) Wasylyk, J. M.; Martin, G. E.; Weinheimer, A. J.; Alam, M. *J. Nat. Prod*. **1989**, *52*, 391−394.
(11) Selected correlations observed in the HMBC spectrum of **4**: C-1 [H-2α*β*, H-14, H-17α*β*], C-2 [H-3, H-14], C-3 [H-2*β*, H-5α*β*, H_3-18], C-4 [H-2*β*, H-5α, H_3-18], C-5 [H-6, H-7, H_3-18], C-6 [H-5α*β*], C-7 [H-5α*β*], C-8 [H-6, H-9α*β*, H_3-19], C-9 [H_3-19], C-12 [H-11α*β*, H_3-20], C-13 [H-11α*β*, H-12, H-14, H_3-20], C-14 [H-2*β*], C-15 [H-2α*β*, H-17α], C-16 [H-14, H-17α*β*], C-19 [H-7], C-20 [H-12].
(12) Selected NOEs for **4**: H-1/H-14, H-3/H-6, H-5*β*/H-6, H-5α/Me-18, H-6/Me-19, H-12/Me-20.
(13) Selected correlations observed in the HMBC spectrum of **5**: C-1 [H-2α*β*, H-14, H-17α*β*], C-2 [H-3, H-14], C-3 [H-1, H-2α*β*, H-5α*β*], C-4 [H-3, H-5α*β*], C-5 [H-6, H-7], C-6 [H-5α*β*, H-7], C-7 [H-5α*β*, H-6, H-9α*β*, H_3-19], C-8 [H-6, H-7, H-9α*β*, H_3-19], C-9 [H-10α*β*, H_3-19], C-10 [H-12], C-11 [H-9α*β*, H-12, H_3-20], C-12 [H-9α*β*, H-10, H_3-20], C-13 [H-11α*β*, H-12, H-14, H_3-20], C-14 [H-2α*β*], C-15 [H-2α*β*, H-17α*β*], C-16 [H-17α*β*], C-18 [H-5α*β*], C-19 [H-7, H-9α*β*], C-20 [H-12].
(14) Selected correlations observed in the HMBC spectrum of **6**: C-1 [H-17α*β*], C-2 [H-3, H-14], C-3 [H-2*β*, H-5α*β*, H_3-18], C-4 [H-2*β*, H-5α, H_3-18], C-5 [H-6, H-7, H_3-18], C-6 [H-7], C-7 [H-6, H-9α*β*, H_3-19], C-8 [H-7, H-9α*β*, H_3-19], C-9 [H_3-19], C-10 [H-12], C-11 [H-12, H_3-20], C-12 [H_3-20], C-13 [H-11α*β*, H-12, H_3-20], C-14 [H-1, H-2*β*], C-15 [H-2α*β*, H-17α*β*], C-16 [H-17α*β*], C-19 [H-7], C-20 [H-12].
(15) Selected correlations observed in the HMBC spectrum of **7**: C-1 [H-14, H-17α*β*], C-2 [H-3, H-14], C-3 [H-2*β*, H-5α*β*, H_3-18], C-4 [H-2*β*, H-5α, H_3-18], C-5 [H-7, H_3-18], C-6 [H-5α*β*], C-7 [H-5α*β*, H_3-19], C-8 [H-6, H_3-19], C-11 [H_3-20], C-12 [H_3-20], C-13 [H-12, H-14, H_3-20], C-14 [H-2*β*], C-15 [H-2α*β*, H-17α], C-16 [H-17α*β*], C-20 [H-12].
(16) Selected correlations observed in the HMBC spectrum of **10**: C-1 [H-17α*β*], C-3 [H-5α*β*, H_3-18], C-4 [H-5α, H_3-18], C-5 [H-7, H_3-18], C-6 [H-5α*β*], C-7 [H-5α*β*, H-6, H_3-19], C-8 [H-6, H_3-19], C-9 [H_3-19], C-12 [H_3-20], C-13 [H_3-20], C-14 [H-13], C-16 [H-17α*β*].
(17) Rodríguez, A. D.; Piña, I. C.; Acosta, A. L.; Barnes, C. L. *Tetrahedron* **2001**, *57*, 93−107.
(18) (a) Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem*. **1986**, *51*, 5140−5145. (b) Look, S. A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363−3370.
(19) (a) Kingston, J. F.; Gregory, B.; Fallis, A. G.; *J. Chem. Soc., Perkin Trans. 1* **1979**, 2064−2068. (b) Bandurraga, M. M.; Fenical, W. *Tetrahedron* **1985**, *41*, 1057−1065.
(20) According to the Hudson's Rules of isorotation the molecular rotation [*M*] of **13** can be defined as the sum of $[M]_{\text{pregnadienol}} + [M]_{\text{sugar}}$. For references see: (a) Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933−1944. (b) Ripperger, H.; Preiss, A.; Schmidt, J. *Phytochemistry* **1981**, *20*, 2434−2435.
(21) Specifically, we propose that the original structures for uprolide B, uprolide B acetate, uprolide B diacetate, 8-epi-uprolide B, 8-epi-uprolide B acetate, 8-epi-uprolide B diacetate, 12,13-bisepiuprolide B, 12,13-bisepiuprolide B acetate, and uproeunicin (i.e., the "uprolide-B series") be revised as depicting a Δ^6 *trans* ethylenic double bond. Moreover, in uprolide B, uprolide B acetate, 8-epi-uprolide B, 8-epi-uprolide B acetate, 12,13-bisepiuprolide B, 12,13-bisepiuprolide B acetate, and uproeunicin there should be a hydroperoxide, not a hydroxyl group, at C-8. By the same token, in uprolide C (δ_{C-7} 85.9) and uprolide C acetate (δ_{C-7} 89.8) (i.e., members of the "uprolide-C series") there should appear a hydroperoxide at C-7 and not a hydroxyl group (see Table 3). Reinterpretation of the spectral data (in particular the mass spectra) for the above-mentioned compounds supported these revisions. The structural revision of some of these analogues also includes an inversion of configuration at the C-8 position.
(22) For a review of perepoxides as intermediates in organic reactions, see: Mitchell, J. C. *Chem. Soc. Rev*. **1985**, *14*, 399−419, 401−406.
(23) These trends seem to hold whether there is a hydroxyl, a hydroperoxyl, or an acetyl group attached to C-8. Seemingly, uprolide L (**9**) and uprolide M (**10**), each having the C-8(*S**) configuration, represent exceptions to this empirical rule.
(24) Bayer, F. M.; Deichmann, E. *Bull. Mar. Sci. Gulf. Carib*. **1958**, *8*, 224−235.
(25) The poor quality of the crystals of **3** have only allowed a preliminary structural study. However, the stereochemistry and the atom coordinates of **3**, as depicted in Figure 1, are established with certainty.
(26) Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: 44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(27) *International Tables for Crystallography*; Kluwer Academic Publishers: Dordrecht, 1992; Vol. C.

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