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Sesquiterpene Lactones from the Caribbean Sea Plume *Pseudopterogorgia americana*

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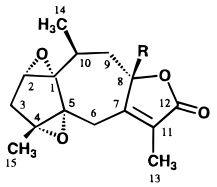
In continuation of our chemical investigation of the Caribbean sea plume *Pseudopterogorgia americana* from Puerto Rico, six previously undescribed guaiane lactones were recently discovered. The chemical structures of metabolites **1**−**6**, including their relative stereochemistry, were established largely by NMR. The constitution and relative configurations of **1** were confirmed by a single-crystal X-ray diffraction study. In some instances, the new compounds generated NMR spectra characterized by an abundance of broad signals of very low intensity, suggesting that the coalescence temperature for interconversion of different conformations was near the NMR probe temperature.

Gorgonian coral species of the genus *Pseudopterogorgia* continue to yield a variety of structurally interesting bioactive sesquiterpenes and diterpenes.[1] *Pseudopterogorgia americana* (Gmelin) (phylum Cnidaria, class Anthozoa, subclass Alcyonaria, order Gorgonaceae, family Gorgoniidae) is widespread in the Caribbean region and has been studied extensively from the chemical point of view. A summary of the results through 1994 has recently appeared.[2] Our previous work with *P. americana* has established that guaianolides and related sesquiterpenes are common metabolites of this species of gorgonian.[3−5] During the course of our investigation of the biologically active constituents of this gorgonian species, we have encountered six highly oxygenated metabolites belonging to the guaiane class of sesquiterpene lactones. The present paper concerns the isolation and structure elucidation of these new compounds. The structures of **1**−**6** were established by spectroscopic methods, mainly NMR and mass spectrometry, and by detailed NMR spectral comparisons with guaianolide sesquiterpene models previously isolated from *P. americana*. The molecular structure of guaianolide **1**, including relative stereochemistry, was confirmed by single-crystal X-ray crystallography. Interestingly, most of the new compounds described here (**1**−**4**) gave rise to NMR spectra characterized by an abundance of broad signals of very low intensity, suggesting rapid intramolecular mobility near the NMR probe temperature.



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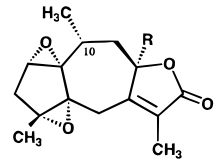
2 R= H



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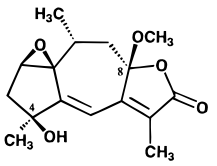

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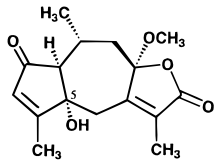

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Results and Discussion

The residue from the MeOH−$CHCl_3$ extract obtained from freeze-dried specimens of *P. americana* (2.3 kg) was extracted as previously described.[3] The residue obtained from the hexane extract upon adsorption chromatography on Si gel gave a group of fractions that were separated by repeated column chromatography and normal-phase HPLC. Two-dimensional NMR experiments (COSY, long-range COSY, and NOESY) were used to establish scalar and dipolar $^1H-^1H$ connectivities. $^1H-^{13}C$ correlations were obtained with HMQC and HMBC experiments.

10-Epimethoxyamericanolide A (**1**) was isolated as a UV active (λ_{max} = 214 nm, ϵ 8350) white solid. The HREIMS of **1** displayed a molecular ion peak at *m*/*z* 292.1315 indicating its molecular composition as $C_{16}H_{20}O_5$. Analysis of the NMR data (Tables 1 and 2) indicated the presence of one lactone carbonyl carbon and one tetrasubstituted double bond; and because the molecular formula suggested seven degrees of unsaturation, compound **1** must, therefore, be pentacyclic. A combined $^1H-^{13}C$ COSY (CSCM) and APT experiment established that the ^{13}C spectrum was composed of four CH_3, three CH_2, two CH, and seven nonprotonated carbon signals. Strong IR absorptions at 1756 and 1682 cm^{-1} corroborated the presence of an α,β-unsaturated ester, and the presence of epoxy groups was indicated by a strong, sharp band at 1263 cm^{-1}. A series of ^{13}C NMR resonances at δ 64.5 (s, C-5), 64.7 (d, C-2), 66.5 (s, C-1), and 75.7 (s, C-4) suggested the presence in **1** of the same α,α-cyclopentane-diepoxide ring moiety found in methoxyamericanolide A, a known metabolite previously isolated from the same specimen of *P. americana*.[3] The presence of a C-8 methoxy group was deduced from the ^1H NMR spectrum, which showed a methoxy signal at δ 3.17, and the ^{13}C NMR spectrum in which a

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Table 1. ^{1}H NMR (500 MHz) Spectral Data for Guaianolides **1**−**6** in $CDCl_3$[a]

	1	**2**	**3**	**4**	**5**	**6**
position	δ_H, mult. J (Hz)	δ_H, mult. J (Hz)	δ_H, mult. J (Hz)	δ_H, mult. J (Hz)	δ_H, mult. J (Hz)	δ_H, mult. J (Hz)
1						1.83, br s
2	3.50, br s	3.54, d (3.1)	3.54, br s	3.58, br s	3.76, br d (1.5)	
3α	2.12, d (16.0)	2.17, d (16.0)	2.14, d (16.0)	2.15, d (16.2)	2.03, dd (1.5, 15.2)	5.90, br d (1.3)
β	1.65, dd (2.3, 16.0)	1.68,dd (3.1, 16.0)	1.63, dd (3.1, 16.0)	1.67, dd (3.0, 16.2)	2.23, d (15.2)	
6α	2.80, br m[b]	2.89, br m[b]	2.52, br m[b]	3.06, br m[b]	6.50, s	3.00, d (18.1)
β	2.80, br m[b]	2.89, br m[b]	3.25, br m[b]	2.75, br m[b]		2.90, d (18.1)
8		4.98, br m		5.21, br m		
9α	2.13, br m[b]	1.82, ddd (4.1, 11.1, 13.4)	2.40, br m[b]	2.36, br m[b]	2.32, dd (9.5, 14.5)	2.57, dd (3.4, 14.6)
β	2.34, br m[b]	2.35, br m	2.06, br m[b]	1.67, br m[b]	2.44, m	1.43, dd (7.8, 14.6)
10	2.20, br m[b]	2.21, br m[b]	2.44, br m[b]	2.06, br m[b]	2.42, m	2.38, m
13	1.89, s	1.85, d (0.7)	1.89, s	1.83, d (0.8)	1.98, s	1.86, s
14	1.20, br m[b]	1.19, br m[b]	0.82, br m[b]	0.88, br m[b]	0.87, d (6.3)	1.42, d (6.6)
15	1.38, s	1.39, s	1.39, s	1.40, s	1.46, s	2.16, d (1.3)
OH					2.69, br s	4.98, br s
8-OCH_3	3.17, s		3.21, s		3.01, s	3.35, s

[a] ^{1}H NMR spectra were recorded at 25 °C. Chemical shifts are given in ppm downfield from TMS. Assignments were aided by ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments. [b] Value taken at the center of a broad and very weak signal. Therefore, the precise location of this signal is uncertain.

Table 2. ^{13}C NMR (125 MHz) (δ_C) Spectral Data for Guaianolides **1**−**6** in $CDCl_3$[a]

position	**1**	**2**	**3**	**4**	**5**	**6**
1	66.5[b]	66.3[b]	66.1[b]	66.3[b]	68.9	59.5
2	64.7	64.1	64.4[b]	66.7	62.9	204.3
3	32.5	32.4	32.2	32.3	41.9	130.9
4	75.7	75.1	74.6[b]	75.8	77.3	171.9
5	64.5[b]	64.1[b]	63.6[b]	64.9	157.4	77.8
6	25.4	26.7	26.5[b]	26.4	114.7	36.3
7	153.8	157.2	154.5[b]	158.1[b]	152.0	155.6
8	108.8	79.9	109.3[b]	79.5	110.2	108.1
9	42.5[b]	37.0[b]	42.8[b]	37.1	43.5	48.0
10	29.5[b]	32.3[b]	28.2[b]	32.2	28.6	26.1
11	128.9[b]	125.5[b]	128.6[b]	125.1[b]	128.8	129.4
12	170.6	173.6	170.7	173.7	171.1	169.9
13	8.6	8.5	8.7[b]	8.9	9.0	8.7
14	16.1[b]	16.1	15.4[b]	15.2	15.6	18.5
15	16.8	16.7	17.0[b]	16.9	24.0	13.5
8-OCH_3	50.6		51.0[b]		50.2	51.2

[a] ^{13}C NMR spectra were recorded at 25 °C. Chemical shift values are in ppm downfield from TMS. Number of attached protons were determined by APT experiments. Assignments were aided by ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b] Due to intramolecular mobility at 25 °C this resonance line appeared as an extremely broad, low intensity, averaged signal. Therefore, the precise location of this signal is uncertain.

hemiketal carbon (C-8) appeared at δ 108.8. From the NMR spectra and the data obtained from ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY experiments, it soon became apparent that compound **1** had the same molecular constitution as methoxyamericanolide A. A side-by-side comparison of the ^{13}C NMR spectra of these compounds revealed many striking similarities in chemical shifts suggesting identical relative stereochemistries at positions C-1, C-2, C-4, C-5, and C-8. These orientations bring H-3*β* within close NOE distance of H-2 and Me-15, in accord with the observed NOEs, confirming that these protons lie on the same molecular face. However, distinctive variations in signal high stability were observed specifically for six of the 16 carbon resonances [i.e., δ 16.1 (q, C-14), 29.5 (d, C-10), 42.5 (t, C-9), 64.5 (s, C-5), 66.5 (s, C-1), and 128.9 (s, C-11)] suggesting that compound **1** differed from methoxyamericanolide A only in its relative stereochemistry at C-10. As a result, the coalescence temperature for interconversion of different conformations in epimer **1** is now near the NMR probe temperature, causing many resonance lines in the ^{13}C NMR spectrum and as many signals in the

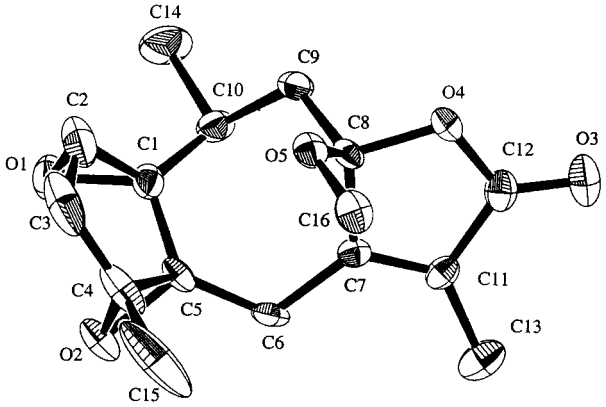

Figure 1. Computer-generated perspective drawing of the final X-ray model of 10-epimethoxyamericanolide A (**1**). The hydrogen atoms have been omitted for clarity.

^{1}H NMR spectrum to be broad and of low intensity (see Tables 1 and 2).[6] Fortunately, slow evaporation of a solution of **1** in $CHCl_3$−MeOH mixtures produced crystals that subsequently allowed confirmation of our structure assignment by X-ray crystallography (Figure 1).

10-Epiamericanolide C (**2**) was obtained as a colorless oil, and the molecular formula $C_{15}H_{18}O_4$ obtained from HREIMS indicated that **2** was an isomer of americanolide C, a known metabolite found previously in this gorgonian.[3] Moreover, comparison of the NMR spectra of **2** with those of **1** confirmed the overall similarity between their structures (Tables 1 and 2). A proton rather than a methoxy group at C-8 was deduced from the ^{1}H NMR spectrum, which showed a broad signal at δ 4.98, together with its corresponding signal in the ^{13}C NMR spectrum at δ 79.9 compared to δ 108.8 in **1**. Many of the remaining spectral features (IR, MS, UV, NMR, and NOESY data) indicated no further differences between the structures of these compounds. The orientation of H-8 followed from NOESY experiments and coupling constant analyses. The observation of a strong NOE between the H-14 methyl protons and H-8 allowed assignment of the relative stereochemistry at C-8/C-10 with the C-10 methyl and H-8 on the same face of the molecule. A weak NOE between H-2 at δ 3.54 and the H-14 methyl protons was also observed, allowing assignment of the relative stereochemistry at

C-2. The coupling constants for H-9α ($J_{8,9\alpha}$ = 11.1 Hz; $J_{9\alpha,10}$ = 4.1 Hz) required a dihedral angle between H-10 and H-9α close to 60° and of almost 180° between H-8 and H-9α. These orientations bring H-8 within 2.5 and 2.8 Å, respectively, of the H-9β and H-6β protons, in accord with the observed NOE (angles and distance estimates come from a molecular modeling study). In addition, strong NOEs between H-6α and methyl protons H-13 and H-15 were also observed. All these geometric constraints dictated by the observed NOEs and coupling constants are compatible with H-8 having the β-orientation. On the basis of the above spectroscopic evidence we propose that **2** is the C-10 epimer of americanolide C. Interestingly, like in **1**, many of the resonance lines in the ^{13}C NMR spectrum of **2** were low intensity broad signals suggesting significant intramolecular mobility at 25 °C (see Table 2).[6] Conceivably, allylic oxidation at C-8 of 10-epiamericanolide C (**2**) followed by methylation gave rise to guaianolide lactone **1**.

8-Epimethoxyamericanolide A (**3**) had a molecular formula of $C_{16}H_{20}O_5$ (*m/z* 292.1317) and an MS that was essentially identical to that of **1**. A 2D ^{1}H−^{1}H COSY, a ^{1}H−^{13}C COSY, and a HMBC experiment allowed the assignment of all ^{1}H and ^{13}C NMR signals. The NMR data, which closely resembled those of 10-epimethoxyamericanolide A (**1**), quickly revealed the presence of the same α,α-cyclopentane-diepoxide ring moiety and suggested that **3** was the bisepimer of **1** at C-8 and C-10 (see Tables 1 and 2). In comparison to **1**, the most obvious difference in the ^{1}H NMR spectrum of **3** was that the signals for H-10 and Me-14 had shifted from δ 2.20 and 1.20 in compound **1**, to δ 2.44 and 0.82 in **3**, respectively. Likewise, in the ^{13}C NMR spectrum the signal for Me-14 also shifted from δ 16.1 in **1**, to δ 15.4 in compound **3**. As predicted on the basis of the difference in their NMR data, the relative stereochemistry at C-10 in 8-epimethoxyamericanolide A (**3**) [C-10 (*R**)] was shown to be opposite to that in **1** by the intense correlation suggested by NOESY H-2/Me-14. Inasmuch as this metabolite was not methoxyamericanolide A, an inversion of relative configuration at C-8 was compulsory. Accordingly, the remaining NOEs observed correlated with a Dreiding model representing the relative stereochemistry shown in the structure of 8-epimethoxyamericanolide A (**3**). Interestingly, the inversion of relative stereochemistry in **3** at C-8 and C-10 had a profound effect in the general appearance of its ^{13}C NMR spectrum causing all but two resonance lines [δ 170.7 (s, C-12) and 32.2 (t, C-3)] to appear as extremely broad low-intensity signals at 25 °C (see Table 2).[6]

8-Epiamericanolide C (**4**) was also a sesquiterpene as suggested by the HREIMS (262.1198, M^+, calcd for $C_{15}H_{18}O_4$, 262.1205) and ^{13}C NMR spectrum, which revealed 15 carbons several of which appeared also as low-intensity broad signals. Compound **4** shared many spectral features in common with **3** excepting its NMR spectra, which lacked the absorptions for a methoxy group, and its mass spectral molecular ion, which was 30 Da lower than that of **3**. These differences were consistent with replacement of the methoxy group in **3** with a hydrogen. The appearance of a lowfield ^{1}H NMR signal at δ 5.21 (br m, 1H) combined with its corresponding resonance in the ^{13}C NMR at δ 79.5 (d) confirmed the presence in this compound of a γ-lactonic methine. Because the NMR (Tables 1 and 2) and NOESY spectra of **3** and **4** were otherwise remarkably similar, it was concluded that these compounds had the same relative stereochemistry at all the ring junctures and at chiral center C-10. Furthermore, H-8 was shown to be on the same molecular face as H-9α and Me-14 by the strong dipolar couplings observed among these protons. Biogenetically, it is likely that methyl ether **3** arises from oxidation at C-8 of 8-epiamericanolide C (**4**) followed by methylation.

Methoxyamericanolide H (**5**) was obtained as a strongly UV active [λ_{max} = 216 (ϵ 5030) and 276 (ϵ 3420) nm] colorless oil, and the molecular formula $C_{16}H_{20}O_5$ obtained from HREIMS indicated that **5** was an isomer of metabolites **1** and **3**. A sharp 3H singlet at δ 3.01 in the ^{1}H NMR spectrum, together with its corresponding signal in the ^{13}C NMR spectrum at δ 50.2, suggested that **5**, too, contained a methoxy group at C-8. A ^{1}H NMR signal at δ 3.76 (br d, J = 1.5 Hz, H-2) and ^{13}C NMR signals δ 68.9 (s, C-1) and 62.9 (d, C-2) also indicated an epoxy functionality in **5**. However, further comparison of the IR, UV, ^{1}H NMR, and ^{13}C NMR spectra of **5** with those of **1** and **3** rapidly established significant differences between their structures. For instance, while the presence of an epoxy group in **5** was corroborated by a strong, sharp IR band at 1263 cm^{-1}, a strong absorption at 3450 cm^{-1} established the presence of a hydroxyl group, a structural feature not found in neither **1** or **3**.

The gross structure of **5** was determined by a detailed analysis of 1D and 2D NMR spectra (Tables 1 and 2). The structures of the two five-membered ring units and cycloheptane unit were determined by ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY experiments, and the three units were connected together in the proper sequence by long-range COSY and HMBC experiments. Critically, the oxygen-bearing quaternary carbon at δ 68.9 (C-1) was correlated by HMBC experiments to H-3β, H-9α, and Me-14. The other oxygen-bearing quaternary carbon in the ^{13}C NMR spectrum (δ 77.3; C-4) was, in turn, correlated to H-2, H-3αβ, Me-15, and H-6. The vinylic proton H-6, resonating at δ 6.50, in turn correlated with resonances ascribable to C-1 (δ 68.9), C-8 (δ 110.2), C-11 (δ 128.8), C-7 (δ 152.0), and C-12 (δ 171.1). These combined data, the two absorption maxima observed in the UV spectrum, the strong IR absorption at 1749 cm^{-1}, and the fact that the typical C-6 methylene multiplets were absent, clearly indicated the presence of a double bond across C-5,6 and a tertiary carbinol moiety at C-4.

The stereochemistries about the C-1,2 epoxide ring moiety, the tertiary carbinol group at C-4, and C-10 were resolved by a combination of NOE and coupling constant data (Table 1). The cycloheptene ring in **5** is in a twisted-chair conformation in order to compensate for the planarity of the flanking five-membered rings. NOEs of the H-2 epoxymethine with H-3α and Me-14, and most importantly, between H-3α and Me-15 were observed. These geometric constraints require the relative stereochemistry to be as shown. In molecular modeling studies it proved to be impossible to simultaneously bring these protons, especially H-2 and Me-14,

within observable NOE distances in the C-8α epimer. The distances between protons experiencing these NOEs in **5** all lie within 2.0−2.6 Å according to molecular modeling studies, while the distance between H-10 and H-2 was calculated to be 3.9 Å (there was no NOE observed between the latter protons). Moreover, the coupling constants between H-9 and H-10 ($J_{9\alpha,10} = 9.5$ Hz; $J_{9\beta,10} < 1$ Hz) required a dihedral angle between H-10 and H-9β close to 90° and of almost 180° between H-10 and H-9α. These orientations bring H-6 within 2.4−2.9 Å of the Me-15 and Me-13 protons, in accord with the observed NOE. The C-8 methoxy group was predicted to be on the same molecular face as the C-10 methine based upon the large coupling between H-10 and H-9α and the strong NOEs between Me-14 and H9αβ. All these geometric constraints dictated by the observed NOEs and coupling constants are incompatible with a C-8 methoxy substituent having the α-orientation. Therefore, the following relative stereochemistry for **5** was inferred: C-1 (*S**), C-2 (*R**), C-4 (*S**), C-8 (*S**), and C-10 (*R**). These results suggest a possible biogenetic relationship between methoxyamericanolide H (**5**) and methoxyamericanolide B, another known metabolite previously isolated from this specimen of *P. americana*.[3]

The last sesquiterpene lactone, named methoxyamericanolide I (**6**), also analyzed for $C_{16}H_{20}O_5$ by HREIMS and ^{13}C NMR spectroscopy. The ^1H and ^{13}C NMR spectral data for **6** (Tables 1 and 2) differed considerably from those of guaianolide **3**, except for the presence of signals for an α-methyl butenolide moiety and the typical methoxy group at C-8. The ^1H NMR spectrum of **6** contained signals at δ 5.90 (br d, $J = 1.3$ Hz, 1H, H-3) and 2.16 (d, $J = 1.3$, 3H, Me-15) due to a β-methyl-α,β-unsaturated cyclopentenone unit. This was further confirmed by the ^{13}C NMR chemical shift values at δ 204.3 (s, C-2), 130.9 (d, C-3), 171.9 (s, C-4), and 13.5 (q, C-15). The placement of the C-5 hydroxyl group was established by a $^1H-^{13}C$ long-range HMBC experiment, which demonstrated the key three-bond correlation between δ 5.90 (H-3), δ_{C-5} 77.8, and δ_{C-1} 59.5 and two-bond correlation between H-1 and C-5 (δ 77.8). In addition, the HMBC experiment showed the two-bond correlation between H-6α (δ 3.00) and δ_{C-5} 77.8. On the basis of the foregoing data and by comparing the remaining ^1H and ^{13}C NMR chemical shift values with those of metabolite **3** (Tables 1 and 2), this guaianolide sesquiterpene was formulated as **6**.

The relative stereochemistry assignment at the centers C-1 (*S**), C-5 (*R**), C-8 (*R**), and C-10 (*R**) was inferred from extensive molecular modeling studies, NOESY experiments, and coupling constant analysis. The observed coupling constant between H-9β and H-10 ($J = 7.8$ Hz), the absence of significant cross peaks in the $^1H-^1H$ COSY spectrum between H-10 and protons H-1 and H-9α, and the relatively large differences in the chemical shifts, coupling constants, and NOEs of the diastereotopic methylene proton pairs (Table 1) suggested that the seven-membered ring in **6** is dominated by a single twisted-chair conformation. In molecular-modeling studies of **6** having the relative stereochemistry shown, it proved possible to bring H-1 and Me-14, H-3 and Me-15, H-10 and H-9β, and Me-14 and H-9α simultaneously within observable NOE distances. Indeed, strong dipolar couplings between these protons were observed. Accordingly, the distances between protons experiencing these NOEs in **6** all lie within 2.2−2.5 Å. Our relative stereochemistry assignments suggest that methoxyamericanolide I (**6**) is likely biosynthesized from epoxide ring openings of 8-epimethoxyamericanolide A (**3**).

The structures of guaiane derivatives **1**−**6** are unique and continue to build on the theme that some species of *Pseudopterogorgia* elaborate complex sesquiterpenes. From the conformational stability point of view, the highly modified cyclopentane units of guaianolides **5** and **6**, though similar to those of **1**−**4**, differ in a fundamental way causing the seven-membered ring to be dominated by a single conformation. Our combined results suggest that the cycloheptane ring of guaianolides similar to **1**−**4**, possessing α,α- (or β,β) cyclopentanediepoxide ring moieties and C-10 methyl and C-8 methoxy (or H-8) groups on the same face of the molecule will likely display significant intramolecular mobility at 25 °C. Due to the small amounts of material available, no bioassays have yet been performed on metabolites **1**−**6**. In view of the new and interesting structures of these compounds, we plan to recollect a larger sample of *P. americana*, so as to be able to completely evaluate their biological properties.

Experimental Section

General Experimental Procedures. For general experimental procedures, see Rodríguez and Boulanger.[3,4] Calculations in MM+ were performed on SPARTAN 4.1 (Wavefunction, Inc., Irvine, CA 92715) and implemented on a Silicon Graphics IRIS−INDIGO XS24−4000 workstation.

Collection and Extraction of *Pseudopterogorgia americana*. The gorgonian was collected at La Parguera, Puerto Rico, in December 1994. A voucher specimen (No. PALP-01) is stored at the Chemistry Department of the University of Puerto Rico. Extraction was carried out as described previously.[3] The $CHCl_3$ extract was filtered and concentrated to leave a dark oily residue (17.7 g) that was chromatographed over Si gel (800 g) with $CHCl_3$ containing increasing proportions of MeOH. Combination of like fractions on the basis of TLC analyses afforded 27 fractions. Fraction 10 (788 mg) was rechromatographed over Si gel with hexane−iPrOH (85:15) to give subfractions 10G (39.1 mg), 10I (59.4 mg), and 10K (25.6 mg). HPLC analysis [Partisil 10 M9/10 with hexane−iPrOH (65:35)] of subfraction 10G yielded methoxyamericanolide H (**5**) (2.6 mg, 0.00012% dry wt). Subfraction 10I was in turn chromatographed over Si gel using hexane−EtOAc (55:45) followed by HPLC [Partisil-10 M9/10 using hexane−iPrOH (75:25)] to yield methoxyamericanolide I (**6**) (6.1 mg, 0.00026% dry wt) and with hexane−iPrOH (65:35) to yield 10-epimethoxyamericanolide A (**1**) (27.9 mg, 0.0012% dry wt). 8-Epimethoxyamericanolide A (**3**) (10 mg, 0.00043% dry wt), 8-epiamericanolide C (**4**) (8.8 mg, 0.00037% dry wt), and 10-epiamericanolide C (**2**) (1.0 mg, 0.00004% dry wt) were isolated from subfraction 10K after column chromatography over Si gel with hexane−iPrOH (55:45) followed by HPLC [Partisil 10 M9/10 with hexane−iPrOH (55:45)].

10-Epimethoxyamericanolide A (1): white solid; IR (neat) 3010, 2984, 2970, 2914, 2850, 1756, 1682,

1463, 1405, 1289, 1263, 1209, 1152, 1120, 1105, 1071, 1033, 938, 765 cm^{-1}; UV (MeOH) λ_{max} 214 nm (ϵ 8350); $[\alpha]^{26}_D$ +151.6° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 292 (5), 274 (100), 260 (20), 243 (50), 242 (54), 227 (46), 218 (56), 216 (26), 199 (22), 187 (21), 173 (29), 137 (67), 128 (25), 124 (44), 115 (24), 95 (36), 77 (17), 69 (18), 53 (21); HREIMS *m/z* [M^+] 292.1315 (calcd for $C_{16}H_{20}O_5$, 292.1311).

X-Ray Structure Determination. Crystallization of 10-epimethoxyamericanolide A (**1**) from $CHCl_3$–MeOH mixtures at room temperature yielded clear needles of marginal quality. X-ray diffraction data were collected on a Siemens SMART CCD system at 23 ± 1 °C to a maximum 2θ of 54.1°, using Mo Kα radiation (λ = 0.71069 Å). The structure, which was solved by direct methods and completed by successive Fourier calculations, was refined by full-matrix least-squares methods, with anisotropic thermal parameters for all non-H atoms. Following initial refinement, H atoms were located from a difference Fourier map. HO8 was refined with a fixed isotropic thermal parameter, and all remaining H atoms were included in the final model at calculated positions, riding on the connected atoms. The diffraction data were weak and exhibited wide mosaic spread, limiting the quality of the refinement, which converged to $R = 0.050$ and $Rw = 0.060$ for 8000 observed reflections [$I > 3.0\sigma(I)$], of a total of 1786 unique measured intensities. The final difference map had a maximum peak of 0.23 $e^-Å^{-3}$ and a minimum peak of −0.18 $e^-Å^{-3}$. All calculations were performed with the NRCVAX package of crystallographic programs.[7] Scattering factors were taken from the *International Tables for X-ray Crystallography*.[8,9]

10-Epiamericanolide C (2): colorless oil; IR (neat) 2960, 2922, 2850, 1750, 1684, 1458, 1264, 1091, 1030, 804 cm^{-1}; UV (MeOH) λ_{max} 218 (ϵ 6300) nm; $[\alpha]^{26}_D$ −179.7° (*c* 6.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 262 (4), 247 (18), 244 (30), 229 (10), 220 (77), 201 (31), 192 (26), 175 (19), 163 (36), 151 (97), 138 (100), 137 (79), 125 (44), 119 (26), 110 (35), 105 (35), 95 (24), 91 (40), 77 (34), 69 (33), 53 (29); HREIMS *m/z* [M^+] 262.1202 (calcd for $C_{15}H_{18}O_4$, 262.1205).

8-Epimethoxyamericanolide A (3): colorless oil; IR (neat) 2959, 2924, 2870, 2852, 1761, 1458, 1282, 1159, 1106, 1009, 941 cm^{-1}; UV (MeOH) λ_{max} 214 nm (ϵ 7560); $[\alpha]^{26}_D$ −66.8° (*c* 2.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 292 (5), 274 (50), 260 (35), 244 (31), 243 (26), 242 (36), 231 (16), 218 (72), 191 (39), 175 (28), 173 (22), 163 (28), 147 (27), 137 (100), 124 (42), 109 (23), 105 (19), 97 (35), 95 (63), 91 (44), 77 (35), 69 (30), 53 (31); HREIMS *m/z* [M^+] 292.1317 (calcd for $C_{16}H_{20}O_5$, 292.1311).

8-Epiamericanolide C (4): colorless oil; IR (neat) 3018, 2962, 2923, 2853, 1761, 1453, 1097, 1020, 939 cm^{-1}; UV (MeOH) λ_{max} 218 nm (ϵ 7560); $[\alpha]^{26}_D$ +26.3° (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 262 (8), 244 (67), 220 (62), 218 (43), 201 (30), 191 (30), 175 (27), 163 (43), 151 (70), 138 (75), 137 (100), 124 (43), 110 (30), 95 (46), 91 (44), 77 (36), 69 (38), 53 (52); HREIMS *m/z* [M^+] 262.1198 (calcd for $C_{15}H_{18}O_4$, 262.1205).

Methoxyamericanolide H (5): colorless oil; IR (neat) 3450, 2963, 2932, 2849, 1749, 1698, 1681, 1454, 1263, 1154, 1101, 1024, 945, 800 cm^{-1}; UV (MeOH) λ_{max} 216 (ϵ 5030), 276 (ϵ 3420) nm; $[\alpha]^{26}_D$ −39.7° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 292 (16), 277 (8), 274 (10), 261 (32), 260 (100), 246 (29), 242 (64), 232 (19), 217 (43), 214 (29), 205 (16), 186 (42), 175 (31), 147 (22), 140 (53), 115 (16), 105 (18), 91 (35), 77 (27), 69 (23), 57 (26); HREIMS *m/z* [$M]^+$ 292.1316 (calcd for $C_{16}H_{20}O_5$, 292.1311).

Methoxyamericanolide I (6): colorless oil; IR (neat) 3450, 2956, 2921, 2869, 2846, 1769, 1714, 1682, 1630, 1105, 960 cm^{-1}; UV (MeOH) λ_{max} 218 (ϵ 14 400) nm; $[\alpha]^{26}_D$ −150.0° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 292 (5), 274 (2), 260 (45), 242 (9), 232 (18), 215 (17), 214 (14), 187 (10), 163 (10), 150 (34), 137 (100), 135 (17), 124 (11), 109 (14), 91 (17), 81 (13), 69 (25); HREIMS *m/z* [$M]^+$ 292.1318 (calcd for $C_{16}H_{20}O_5$, 292.1311).

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References and Notes

(1) Fenical, W. *J. Nat. Prod.* **1987**, *50*, 1001–1008.
(2) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571–4618.
(3) Rodríguez, A. D.; Boulanger, A. *J. Nat. Prod.* **1996**, *59*, 653–657.
(4) Rodríguez, A. D.; Boulanger, A. *J. Nat. Prod.* **1997**, *60*, 207–211.
(5) Richardson, P. L.; Rodríguez, A. D.; Boulanger, A.; Huang, S. D. *Acta Crystallogr., Sect. C* **1998**, *C54*, 66–68.
(6) Sharp and strong NMR signals may be obtained at ultralow temperature.
(7) Rogers, D. *Acta Cryst.* **1981**, A37, 734–741.
(8) Cromer, D. T.; Waber, J. T. *International Tables for X-ray Crystallography*; The Kynoch Press: Birmingham, England, 1974; Vol. 4, Tables 2.3.1 and 2.2A.
(9) Hydrogen coordinates, thermal parameters, and bond distances and angles have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, U.K.

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