Americanolides A−C, New Guaianolide Sesquiterpenes from the Caribbean Sea Plume *Pseudopterogorgia americana*

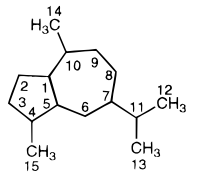
Abimael D. Rodríguez* and Anna Boulanger

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

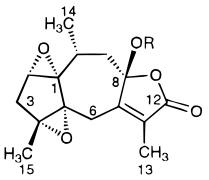
Received February 26, 1996[⊗]

A chemical reinvestigation of the common sea plume *Pseudopterogorgia americana* from Puerto Rico has revealed for the first time the presence of a new family of sesquiterpenoid lactones possessing the guaiane skeleton. The structures of guaianolides **1**−**5**, including relative stereochemistries, were elucidated by NMR, NOE, and MS experiments. The proposed structures were further corroborated by molecular modeling studies.

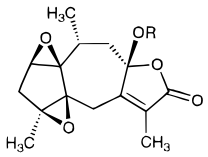
Many gorgonian species of the genus *Pseudopterogorgia* from the Caribbean region have been the object of chemical investigations.[1,2] Diterpene glucopyranosides (pseudopterosins) and lactones (pseudopterolides) are the most usual metabolites of this genus, but sesquiterpene hydrocarbon mixtures are also common.[2] *Pseudopterogorgia americana* Gmelin (phylum Cnidaria, class Anthozoa, subclass Alcyonaria, order Gorgonaceae), which is widespread in the Caribbean zone of the West Indies and which contains some pharmacologically active compounds, has been repeatedly studied from the chemical point of view: an unusual betaine,[3] gorgosterol, and several interesting secosterols,[4−7] bisabolenes,[8] sesquiterpene hydrocarbons,[9,10] and some strained sesquiterpenoid furans of the germacrene class[11,12] have all been isolated from specimens of diverse origins. In the course of a continuing search for tumor inhibitors of marine origin and for chemical contributions to taxonomy and phylogeny in the *Pseudopterogorgia*, we have now reinvestigated specimens of *P. americana* growing in the southwest of Puerto Rico. In addition to the known carotenoid peridinin[13] and a recently described 9,11-secosterol,[7] we have isolated three new guaianolide sesquiterpenes, designated americanolides A (**1**), B (**3**), and C (**5**), along with two 8β-methoxy derivatives **2** and **4**. Guaiane sesquiterpenes, which constitute one of the largest families within the sesquiterpene lactones, are mainly isolated from Compositae plants.[14−18] Because germacrene-type sesquiterpenoids have been found in *P. americana*, it is likely that they are involved in the biogenesis of guaiane sesquiterpenoids. Therefore, we believe that the present series of compounds originates from the rearrangement and further oxidation of germacrene-derived sesquiterpenes. The known compounds were identified by spectral comparison with authentic samples, and the structures of the new compounds were established by spectroscopic methods, mainly NMR and MS.



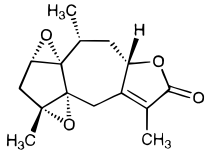
Guaiane skeleton



1 R = H
2 R = CH_3



3 R = H
4 R = CH_3



5

Results and Discussion

The residue from the MeOH−$CHCl_3$ extract obtained from freeze-dried specimens of *P. americana* (2.3 kg) was extracted with hexane followed by $CHCl_3$. The residue obtained from the $CHCl_3$ extract upon adsorption chromatography on Si gel gave a group of fractions containing a complex mixture of several major and minor metabolites. These were separated by repeated column chromatography and normal-phase HPLC to yield five new guaianolides, **1**−**5**, along with the known compounds. As the isolation of guaianolides from any Caribbean species of gorgonian is without precedent, we begin our chemical study of *P. americana* with a rigorous proof of the structure of americanolide A (**1**). Because **1** could not be obtained in a crystalline state suitable for X-ray crystal structure determination, unequivocal assignment of its structure was achieved by ^{1}H- and ^{13}C-NMR studies (Table 1) and supported by molecular modeling studies. Two-dimensional NMR experiments (COSY, long-range COSY, and NOESY) were used to establish scalar and dipolar ^{1}H−^{1}H connectivities. ^{1}H−^{13}C correlations were obtained with ^{1}H−^{13}C COSY and HMBC experiments.

Americanolide A (**1**) was isolated from the gorgonian as a UV-active (λ_{max} = 212 nm, MeOH) colorless semisolid. The EIMS of **1** displayed a molecular ion peak at *m*/*z* 278, and a high resolution measurement established its molecular composition as $C_{15}H_{18}O_5$. Analysis of the NMR data (Table 1) indicated the presence of one ester or lactone carbonyl carbon and one tetrasubstituted double bond. Hence, americanolide A possessed seven degrees of unsaturation, two of which were due to double bonds and five due to rings. A combined ^{1}H−^{13}C COSY and APT experiment established that the ^{13}C spectrum was composed of three

* To whom correspondence should be addressed. Phone: (787) 764-0000, ext-4799. FAX: (787) 751-0625. E-mail: A_Rodriguez@UPR1.UPR.CLU. EDU.

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1996.

S0163-3864(96)00326-6 CCC: $12.00

Table 1. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) Spectral Data for the Americanolides **1**−**5** in $CDCl_3$[a]

	Americanolide A (**1**)		Methoxyamericanolide A (**2**)		Americanolide B (**3**)		Methoxyamericanolide B (**4**)		Americanolide C (**5**)	
Position	^{1}H, mult (*J* (Hz), intgr	^{13}C, m	^{1}H, mult (*J* (Hz), intgr	^{13}C, m	^{1}H, mult (*J* (Hz), intgr	^{13}C, m	^{1}H, mult (*J* (Hz), intgr	^{13}C, m	^{1}H, mult (*J* (Hz), intgr	^{13}C, m
1		67.5, s		67.2, s		67.4, s		67.2, s		67.2, s
2	3.56, d (3.0), 1H	65.4, d	3.55, d (3.0), 1H	65.2, d	3.50, d (3.0), 1H	61.3, d	3.50, d (3.0), 1H	61.0, d	3.60, d (3.0), 1H	65.6, d
3α	2.06, d (16.2), 1H	32.3, t	2.05, d (15.9), 1H	32.3, t	1.58, dd (3.0, 16.2), 1H	31.9, t	1.57, dd (3.0, 15.9), 1H	31.9, t	2.09, d (15.9), 1H	32.3, t
β	1.64, dd (3.0, 16.2), 1H		1.62, dd (3.0, 15.9), 1H		2.09, d (16.2), 1H		2.09, d (15.9), 1H		1.63, dd (3.0, 15.9), 1H	
4		76.1, s		75.9, s		75.0, s		74.8, s		75.7, s
5		66.1, s		65.8, s		66.0, s		65.8, s		65.4, s
6α	2.40, d (13.8), 1H	24.5, t	2.42, d (13.8), 1H	24.4, t	3.25, br d (13.5), 1H	24.6, t	3.04, br d (13.5), 1H	24.7, t	2.58, d (14.1), 1H	26.0, t
β	3.10, br d (13.8), 1H		2.90, dd (0.6, 13.8), 1H		2.47, d (13.5), 1H		2.48, d (13.2), 1H		2.92, br d (14.1), 1H	
7		156.2, s		154.0, s		156.1, s		154.1, s		156.9, s
8		105.4, s		107.6, s		105.3, s		107.3, s	4.89, dd (6.0, 11.1), 1H	81.6, d
9α	1.90, dd (11.4, 14.1), 1H	43.4, t	1.90, dd (11.4, 14.4), 1H	42.5, t	1.65, dd (14.4, 11.1), 1H	43.8, t	1.61, dd (11.1, 14.4), 1H	42.7, t	1.74, ddd (11.1,11.4,13.2), 1H	38.4, t
β	2.22, d (14.1), 1H		2.21, d (14.1), 1H		2.43, d (13.2), 1H		2.44, d (12.9), 1H		2.24, dd (6.0,13.2), 1H	
10	2.73, m, 1H	26.7, d	2.68, m, 1H	26.4, d	2.81, m, 1H	26.6, d	2.77, m, 1H	26.2, d	2.46, m, 1H	27.0, d
11		126.9, s		129.4, s		126.1, s		128.2, s		126.1, s
12		172.2, s		170.9, s		172.0, s		170.9, s		173.7, s
13	1.77, br s, 3H	8.2, q	1.85, br s, 3H	8.4, q	1.80, br s, 3H	9.2, q	1.87, br s, 3H	9.3, q	1.83, br s, 3H	8.3, q
14	0.83, d (6.9), 3H	16.4, q	0.82, d (6.9), 3H	16.4, q	0.85, d (7.2), 3H	16.4, q	0.85, d (6.9), 3H	16.5, q	0.89, d (6.9), 3H	16.7, q
15	1.41, s, 3H	16.9, q	1.40, s, 3H	16.9, q	1.38, s, 3H	17.1, q	1.39, s, 3H	17.3, q	1.43, s, 3H	17.0, q
8-OH	4.86, br s, 1H				5.14, br s, 1H					
8-OCH_3			3.16, s, 3H	50.4, q			3.19, s, 3H	50.4, q		

[a] The *δ* values are in ppm from TMS. Assignments were made on the basis of homonuclear and heteronuclear chemical shift correlation methods. ^{13}C-NMR multiplicities were determined by Attached Proton Test (APT) sequences.

Figure 1. The partial structures of americanolides A (1) and B (3) and major HMBC correlations in NMR (arrowhead; carbon; tail of arrow; proton).

methyl, three methylene, two methine, and seven nonprotonated carbon signals, indicating that **1** contained 15 carbons and 17 carbon-bonded hydrogens. The presence of an exchangeable hydrogen was supported by a strong absorption at 3358 cm^{-1} in the IR spectrum and the fact that a signal at *δ* 4.86 (br s, 1H) in the ^{1}H-NMR spectrum in $CDCl_3$ lacked a ^1J-correlation with a ^{13}C signal in a ^{1}H−^{13}C COSY experiment. Strong IR absorptions at 1758 and 1695 cm^{-1} corroborated the presence of an α,*β*-unsaturated ester, and the presence of epoxy groups was indicated by a strong and sharp band at 1154 cm^{-1} (−COC−).

Data from ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY were used to generate four partial structures (**a**−**d**) for **1**. From the HMBC and ^{1}H−^{1}H COSY spectra, partial structure **a** contained a cyclopentane skeleton with two epoxides (attached to the C-1,2 and C-4,5 positions) adjacent to the same methylene group (Figure 1). Neighboring this methylene was a deshielded quaternary carbon on one side, which was shown by HMBC to bear a methyl group, and a deshielded methine on the opposite side, the latter of which was adjacent to another oxygen-bearing quaternary carbon. Partial structure **b** possessed a terminal methylene group that was adjacent to a methine carbon bearing a methyl group, and substructure **c** consisted of a methylene flanked by quaternary carbons. Partial structure **d** was readily formulated by ^{13}C-NMR, IR, UV, and HMBC information as a fully substituted α-methyl-*γ*-hydroxy butenolide ring.

HMBC data were used to connect these four partial structures as well as to confirm the above structural assignments (Figure 1). Critically, the oxygen-bearing quaternary carbon at *δ* 67.5 (C-1) in partial structure **a** was correlated to the methyl (C-14) and methylene (C-9) groups of partial structure **b**. The other oxygen-bearing quaternary carbon in the former substructure (*δ* 66.1, C-5) was in turn correlated to the methylene protons (C-6) of partial structure **c**. Moreover, partial structures **a** and **c** were connected by observing long-range coupling between the methylene proton at *δ* 3.10 (H-6*β*) and the quaternary carbon at *δ* 67.5 (C-1). Similarly, partial structure **d** was readily connected to subunits **b** and **c** by observing long-range couplings between the proton pairs at *δ* 2.40/3.10 (H6α*β*) and 1.90/2.22 (H9α*β*) with the hemiketal carbon at *δ* 105.4 (C-8) and the vinyl carbon at *δ*156.2 (C-7). The connectivity of partial substructures **c** and **d** was also argued on the basis of long-range proton−proton coupling between H6*β* and the more remote H-13 methyl protons. Confirmation of the structures of the four units as well as the sequencing was provided by HREIMS data.

The relative stereochemical assignments were accomplished by extensive NOE (both 2D NOE and difference NOEs) and molecular modeling studies. The observation of a strong NOE between the H-14 methyl protons and H-2 allowed assignment of the relative stereochemistry at C-2/C-10 with the C-10 methyl in the α-equatorial position and H-2 in the β-pseudoequatorial position. A strong NOE between H-9β at δ 2.22 and the H-14 methyl protons was also observed. The coupling constants between H-9 and H-10 ($J_{9\alpha,10} = 11.4$ Hz; $J_{9\beta,10} \leq 1$ 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-10 within 2.7 Å of the H-6β proton, in accord with the observed NOE (all distance estimates come from molecular modeling studies as discussed below). The C-8 hydroxyl 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 conspicuous absence of NOEs between H-10 and H9αβ. In addition, strong NOEs between H-6α and the H-13 and H-15 methyl protons, as well as between H-2 and H-3β, were also observed. All these geometric constraints dictated by the observed NOEs and coupling constants are incompatible with a C-8 hydroxyl substituent having the α-orientation.

The stereochemistry about the cyclopentane−diepoxide ring moiety was also resolved by a combination of NOE and coupling constant data supported by distance calculations using the QUANTA/CHARMm molecular mechanics program. The four carbons C-1,2 and C-4,5 of the cyclopentane ring containing the epoxide rings are predicted to have shorter C−C single bond lengths (≤1.48 Å) than the standard 1.54 Å of the sp^3 C−C bond.[19] The net result is a planar cyclopentane ring. The lactone ring moiety is also planar, with the C-11,12 bond being somewhat shortened. Thus, the cycloheptane ring is in a chair conformation in order to compensate for the planarity of the two five-membered rings. Strong dipolar coupling (NOEs) between the H-2 epoximethine with H-3β and H-14, H6α with H-13 and H-15, and most importantly, between H-6β and H-10 were observed. These geometric constraints require the relative stereochemistry to be as shown. In molecular modeling studies it proved to be impossible to bring these protons, especially H-6β and H-10, simultaneously within observable NOE distances in the β,β cyclopentane−diepoxy epimer (see discussion below). The distances between protons experiencing these NOEs in **1** all lie within 2.2−2.7 Å according to molecular modeling studies, while the distance between H-10 and H-6β was calculated to be 2.7 Å (an NOE was observed between these two latter protons). Moreover, the ^{1}H-NMR signals of **1** at δ 2.06 (d, $J = 16.2$ Hz) and 1.64 (dd, $J =$ 3.0, 16.2 Hz) were assigned to H-3α and H-3β in agreement with the relevant signals assigned for fusicorrugatol, a related fusicoccane-type structure previously established by X-ray analysis as having the same partial substructure **a** as **1**.[20] These observations require a α,α cyclopentane−diepoxy system.

The structure of compound **2** was deduced from its NMR (Table 1) and EIMS spectra, which were closely related to those of americanolide A (**1**). The presence of an 8β-methoxy group was deduced from the MS (*m/z* 292, $C_{16}H_{20}O_5$); the ^{1}H-NMR spectrum, which showed a methoxy signal at δ 3.16; and the ^{13}C-NMR spectrum in which the hemiketal carbon (C-8) now appeared at δ 107.6, compared to δ 105.4 in **1**. The orientation of the methoxy substituent followed from NOESY experiments and coupling constant analyses. On the basis of the above spectroscopic evidence we propose that **2** is the 8β-methoxy derivative of americanolide A.

The proposed structures of **1** and **2** were further substantiated by detailed examination of their mass spectral data inasmuch as the HREIMS spectra of both compounds produced significant fragmentation. Americanolides **1** and **2** gave a molecular ion at *m/z* 278 and 292, and each produced a fragment ion corresponding to the loss of one molecule of H_2O at *m/z* 260 and 274, respectively. Only americanolide A (**1**), however, loses a second molecule of H_2O to give an ion at *m/z* 242. If elimination of H_2O from *m/z* 274 in **2** could occur, one would observe an ion at *m/z* 256, not at *m/z* 242 (due to the loss of one molecule of MeOH). These results suggest that there is loss of H_2O in **1** occurring at the cyclopentane−diepoxide ring region, which is similar in both structures. A critical fragment came from the carbonyl oxygen-driven cleavage of the lactone linkage in both compounds to give a common major fragment ion at *m/z* 218. It appears that **1** eliminates a molecule of HOAc, while **2** loses methyl acetate to give the same fragment ion. Other major fragment ions found simultaneously in **1** and **2** that support a common fragmentation pattern occur at *m/z* 137 (base peak), 124, and 95.

Americanolide B (**3**) had a molecular formula of $C_{15}H_{18}O_5$ (*m/z* 278.1149) and a MS essentially identical to that of **1**. The NMR data, which closely resembled those of americanolide A (**1**), suggested that **3** was the epimer of **1** at C-1,2 and C-4,5 (see Table 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 (Figure 1). The only significant differences between the ^{1}H-NMR spectra of **1** and **3** were the signals assigned to the methylene proton pairs (H-3αβ, H-6αβ, and H-9αβ). These differences suggested changes both in the stereochemistry about the epoxides and in the conformation of the cycloheptane ring.

In molecular modeling studies of **3**, it also proved possible to bring H-2 and H-14, H-6β and H-15, as well as H-6β and H-13 simultaneously within observable NOE distances. Indeed, weaker dipolar couplings between these protons were observed. Accordingly, the distances between protons experiencing these NOEs in **3** all lie within 2.5−3.5 Å as revealed by molecular modeling studies, while the distance between H-10 and H-6β was calculated to be 5.3 Å (an NOE was not observed between these two latter protons). These NOE studies supported by molecular modeling calculations for a quantitative estimation of dihedral angles predict a "twist-boat-like" conformation for the cycloheptane ring in **3**. Moreover, a strong NOE between H-9β and the H-14 methyl protons (also seen in **1**) and the large coupling between H-10 and one of the C-9 protons (H-9α, 11.1 Hz) combined with the lack of NOEs between H-10 and H9αβ, is in accord with this contention. These observations suggested that americanolide B is the β,β cyclopentane−diepoxy epimer of **1**.

Methoxyamericanolide B (**4**) was obtained as a colorless semisolid, and the molecular formula $C_{16}H_{20}O_5$ obtained from HREIMS indicated that **4** was an isomer

of **2**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** with those of **3** confirmed the overall similarity between their structures (Table 1). However, a sharp 3H singlet at δ 3.19 in the ^{1}H-NMR spectrum, together with its corresponding signal in the ^{13}C-NMR spectrum at δ 50.4, suggested that **4** contained a methoxy group at C-8. The remaining spectral features (IR, MS, UV, NMR, and NOESY data) indicated no further differences between the structures of these compounds.

The least polar compound isolated, americanolide C (**5**), was also a sesquiterpene as suggested by the HREIMS (262.1210, M^+, calcd for $C_{15}H_{18}O_4$ 262.1205) and ^{13}C-NMR spectrum, which revealed 15 carbons. Compound **5** shared many spectral features with compound **1**, except its IR spectrum, which lacked the absorption for a hydroxyl group, and its mass spectral molecular ion, which was 16 Da lower than that of **1**. These differences were consistent with replacement of the hydroxyl group in **1** with a hydrogen. The appearance of a lowfield ^{1}H-NMR signal at δ 4.89 (dd, $J = 6.0$, 11.1 Hz, 1H) combined with its corresponding resonance in the ^{13}C-NMR at δ 81.6 (d) confirmed the presence in this compound of a γ-lactonic methine. Because the NMR and NOESY spectra of **1** and **5** were otherwise remarkably similar (Table 1), it was concluded that these compounds have the same 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 H6β and H-10 by the strong dipolar couplings observed among these protons. These observations, the coupling constants between the H-9 methylene protons and H-8 ($J_{8,9\alpha} = 11.1$ Hz; $J_{8,9\beta} = 6.0$ Hz), and molecular modeling studies suggest that the seven-membered ring in **5** may also be dominated by a single chair conformation. The dominance of one conformation of **5** in solution is supported by the relatively large differences in the chemical shifts, coupling constants, and NOEs of the diastereotopic methylene proton pairs (Table 1).

An interesting characteristic of some Caribbean gorgonian species has been their conformity to chemotaxonomic patterns. Prime examples of consistency lie within the genera *Eunicea* and *Pseudopterogorgia*.[2] The prevalence of sesquiterpenes in *P. americana* and the absence of diterpenes single out this gorgonian from other *Pseudopterogorgia* species previously investigated. Compounds **1**-**5** represent the first examples of guaiane sesquiterpenes isolated from a Caribbean gorgonian species. It is likely that methyl ethers **2** and **4** originate from americanolides A (**1**) and B (**3**), respectively. Compound **1**, in turn, could arise from the oxidation at C-8 of americanolide C (**5**). Whether these parallel transformations take place *in vivo* or during workup, however, is not known.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi 535 capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin−Elmer System 2000 FT-IR spectrophotometer and UV spectra on a Hewlett-Packard diode array spectrophotometer Model 8452A. Optical rotations were determined on a Perkin-Elmer polarimeter Model 243B. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a General Electric QE-300 in $CDCl_3$ (^{1}H-NMR and ^{13}C-NMR chemical shifts 7.26 and 77.0 ppm, respectively). Column chromatography was performed on Si gel (35−75 mesh), and TLC analyses were carried out using glass precoated Si gel plates. HPLC was done using columns of 10-μm Si gel. All solvents used were spectral grade.

Collection and Extraction of *P. americana*. The gorgonian was collected at a depth of 3 m at La Parguera, Lajas, Puerto Rico, in December 1994. A voucher specimen (no. PALP-01) is stored at the Chemistry Department of the University of Puerto Rico. A frozen sample of *P. americana* (2.3 kg) was freeze-dried and blended with MeOH−$CHCl_3$ (1:1). After filtration, the solvent was removed *in vacuo* to yield a crude extract that was taken up with H_2O and extracted successively with hexane (6 × 4 L) and $CHCl_3$ (6 × 4 L). 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 was rechromatographed over Si gel with hexane−2-propanol (85:15) to give americanolide C (**5**) (9.3 mg, 0.0004% dry wt) plus subfractions 10G (39.1 mg) and 10J (89.5 mg). HPLC analysis [Partisil 10 M9/10 with hexane−2-propanol (65:35)] of subfraction 10G yielded methoxyamericanolide B (**4**) (13.3 mg, 0.0006% dry wt), and HPLC of subfraction 10J afforded methoxyamericanolide A (**2**) (50.8 mg, 0.0022% dry wt). Americanolide B (**3**) (25.6 mg, 0.0011% dry wt) and americanolide A (**1**) (27.1 mg, 0.0012% dry wt) were isolated from fraction 15 after column chromatography over Si gel with CH_2Cl_2−$(CH_3)_2CO$ (85:15) followed by HPLC [Partisil-10 M9/10 using hexane−2-propanol (75:25)].

Americanolide A (1): colorless semisolid; mp 82 °C; IR (neat) 3358, 3014, 2972, 2925, 2804, 2853, 1758, 1695, 1154, 1114, 947, 877 cm^{-1}; UV (MeOH) λ_{max} 212 nm (ϵ 8365); $[\alpha]^{28}$D −70.0° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m*/*z* [M^+] calcd for $C_{15}H_{18}O_5$ 278.1154, found 278.1152 (1.5), 263 (6), 260 (13), 245 (8), 242 (11), 232 (6), 218 (29), 203 (8), 190 (13), 175 (14), 163 (16), 151 (17), 150 (16), 137 (100), 124 (52), 109 (17), 97 (40), 95 (45), 77 (16), 69 (34), 53 (29).

Methoxyamericanolide A (2): colorless semisolid; mp 97 °C; IR (neat) 3017, 2971, 2928, 2882, 2838, 1768, 1760, 1695, 1154, 1118, 952, 856 cm^{-1}; UV (MeOH) λ_{max} 216 nm (ϵ 10859); $[\alpha]^{28}$D −73.0° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m*/*z* [M^+] calcd for $C_{16}H_{20}O_5$ 292.1311, found 292.1319 (0.3), 277 (0.7), 274 (2), 261 (7), 260 (8), 245 (5), 242 (3), 232 (7), 218 (38), 203 (7), 191 (19), 175 (14), 163 (14), 151 (14), 150 (14), 137 (100), 124 (36), 109 (19), 97 (33), 95 (48), 77 (27), 69 (28), 53 (64).

Americanolide B (3): yellowish oil; IR (neat) 3362, 3012, 2992, 2968, 2927, 1761, 1694, 1152, 1113, 941, 853 cm^{-1}; UV (MeOH) λ_{max} 212 nm (ϵ 7113); $[\alpha]^{28}$D −45.0° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m*/*z* [M^+] calcd for $C_{15}H_{18}O_5$ 278.1154, found 278.1149 (1.1), 263 (5), 260 (10), 245 (7), 242 (8), 232 (4), 218 (31), 203 (8), 190 (15), 175 (14), 163 (14), 151 (19), 150 (17), 137 (100), 124 (60), 109 (18), 97 (54), 95 (60), 77 (18), 69 (38), 53 (31).

Methoxyamericanolide B (4): colorless semisolid; mp 96 °C; IR (neat) 3018, 2986, 2966, 2937, 2921, 2877, 1754, 1694, 1152, 1111, 958, 854 cm^{-1}; UV (MeOH) λ_{max} 214 nm (ϵ 9857); $[\alpha]^{28}D$ −79.0° (*c* 1.0, $CHCl_3$); ^1H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m/z* $[M^+]$ calcd for $C_{16}H_{20}O_5$ 292.1311, found 292.1307 (0.1), 277 (0.7), 274 (2), 261 (10), 260 (5), 245 (6), 232 (4), 218 (49), 203 (8), 191 (27), 175 (17), 163 (19), 151 (7), 150 (14), 137 (100), 124 (39), 109 (16), 97 (38), 95 (52), 77 (20), 69 (22), 53 (36).

Americanolide C (5):[21,22] yellowish oil; IR (neat) 3014, 2973, 2927, 2881, 2854, 1758, 1696, 1154, 1114, 945, 856 cm^{-1}; UV (MeOH) λ_{max} 218 nm (ϵ 10 356); $[\alpha]^{28}D$ −29.0° (*c* 1.0, $CHCl_3$); ^1H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m/z* $[M]^+$ calcd for $C_{15}H_{18}O_4$ 262.1205, found 262.1210 (3), 247 (9), 244 (10), 219 (30), 201 (12), 192 (15), 191 (11), 175 (11), 163 (18), 151 (53), 138 (44), 137 (32), 125 (18), 124 (14), 110 (20), 109 (11), 95 (13), 91 (25), 77 (21), 69 (19), 53 (36), 43 (100).

Acknowledgment. We thank the NSF Minority Research Center of Excellence (MRCE) Program (Grant No. R11-8802961) and the NSF-EPSCoR Tropical Marine Biotechnology Center (Grant No. R118610677) at the University of Puerto Rico for financial support of this work. The assistance of Dr. Paul Yoshioka, Mr. Anthony Sostre, Javier J. Soto and Miss Ana E. Pomales in specimen collection is gratefully acknowledged. The authors thank Dr. Bernard Banaigs from University of Perpignan, France for the HMBC spectra (400 MHz) of **1** and **3**. HREIMS spectral determinations were performed by the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln, a National Science Foundation Regional Facility (Grant No. CHE8211164). We are also indebted to A. L. Acosta, E. D. Reyes, O. M. Cóbar, C. Ramírez, M. Pagán, and A. Sostre for valuable discussions during the interpretation of the EIMS spectrum of **1**.

References and Notes

(1) Fenical, W. *J. Nat. Prod.* **1987**, *50*, 1001−1008.
(2) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618.
(3) Weinheimer, A. J.; Metzner, E. K.; Mole, M. L., Jr. *Tetrahedron* **1973**, *29*, 3135−3136.
(4) Hale, R. L.; Leclerq, J.; Tursch, B.; Djerassi, C.; Gross, R. A., Jr.; Weinheimer, A. J.; Gupta, K.; Scheuer, P. J. *J. Am. Chem. Soc.* **1970**, *92*, 2179−2180.
(5) Enwall, E. L.; Ven der Helm, D.; Hsu, I. N.; Pattabhiraman, T.; Schmitz, F. J.; Spraggins, R. L.; Weinheimer, A. J. *J. Chem. Soc., Chem. Commun.* **1972**, 215−216.
(6) Miller, S. L.; Tinto, W. F.; Yang, J.; McLean, S.; Reynolds, W. F. *Tetrahedron Lett.* **1995**, *36*, 1227−1228.
(7) He, H.; Kulanthaivel, P.; Baker, B. J.; Kalter, K.; Darges, J.; Cofield, D.; Wolff, L.; Adams, L. *Tetrahedron* **1995**, *51*, 51−58.
(8) Miller, S. L.; Tinto, W. F.; McLean, S.; Reynolds, W. F.; Yu, M. *J. Nat. Prod.* **1995**, *58*, 1116−1119.
(9) Weinheimer, A. J.; Washecheck, P. H.; Van der Helm, D.; Hossain, M. B. *J. Chem. Soc., Chem. Commun.* **1968**, 1070−1071.
(10) Rivero, R. B.; Pérez, A. R.; Castro, H. V.; Argilagos, C. S.; Henríquez, R. D. *Z. Naturforsch.* **1990**, *45B*, 1571−1572.
(11) Izac, R. R.; Bandurraga, M. M.; Wasylyk, J. M.; Dunn, F. W.; Fenical, W. *Tetrahedron* **1982**, *38*, 301−304.
(12) Chan, W. R.; Tinto, W. F.; Moore, R. *Tetrahedron* **1990**, *46*, 1499−1502.
(13) McLean, S.; Reynolds, W. F.; John, L. M. D.; Tinto, W. F. *Magn. Reson. Chem.* **1992**, *30*, 362−363.
(14) Lee, K. H.; Simpson, R. F.; Geissman, T. A. *Phytochemistry* **1969**, *8*, 1515−1521.
(15) Liu, Y.; Mabry, T. J. *J. Nat. Prod.* **1981**, *44*, 722−728.
(16) Jakupovic, J.; Boeker, R.; Grenz, M.; Paredes, L.; Bohlmann, F.; El-Din, A. S. *Phytochemistry* **1988**, *27*, 1135−1140.
(17) Gao, F.; Wang, H.; Mabry, T. J.; Watson, W. H.; Kashyap, R. P. *Phytochemistry* **1990**, *29*, 551−560.
(18) Marco, J. A.; Sanz-Cervera, J. F.; Manglano, E.; Sancenon, F.; Rustaiyan, A.; Kardar, M. *Phytochemistry* **1993**, *34*, 1561−1564.
(19) Kelsey, R. G.; Shafizadeh, F.; Campbell, J. A.; Craig, A. C.; Campana, C. F.; Craig, R. E. R. *J. Org. Chem.* **1983**, *48*, 125−127.
(20) Tori, M.; Nakashima, K.; Takaoka, S.; Asakawa, Y. *Chem. Pharm. Bull.* **1994**, *42*, 2650−2652.
(21) After prolonged exposure to solvents for spectral measurements, it was evident that the sample of **5** was not pure. Unfortunately, it is conceivable that the optical rotation may be inaccurate because it was not taken at the outset.
(22) The HREIMS analysis of **5** was recorded at the Mass Spectrometry Facility of the Industry/University Materials Characterization Center, University of Puerto Rico.

NP960326E