Full Papers

New Guaiane Metabolites from the Caribbean Gorgonian Coral, *Pseudopterogorgia americana*

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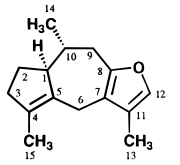
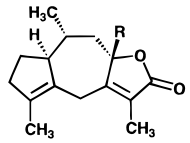
Six new sesquiterpenes having the guaiane skeleton were isolated from the hexane extract of the Caribbean gorgonian coral *Pseudopterogorgia americana* collected in Puerto Rico. Their structure assignments and relative stereochemistries were based on interpretation of spectroscopic data, molecular modeling studies, and comparisons with known compounds. Most of the new metabolites were found to be quite labile and decomposed slowly under normal spectral measurement conditions.

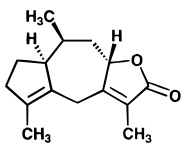
The gorgonian octocoral *Pseudopterogorgia americana* Gmelin (phylum Cnidaria, class Anthozoa, subclass Alcyonaria, order Gorgonacea) has been the subject of several chemical investigations.[1−11] In continuation of our studies of a Puerto Rican specimen of *P. americana*, the guaiane sesquiterpene mixture reported recently by us has been examined in more detail and found to consist primarily of six new components.[12] In this paper, we report the isolation and structure elucidation of guaiane metabolites **1**−**6** from the same gorgonian specimen collected in La Parguera, Puerto Rico. Guaiazulenes and related sesquiterpenes are recognized metabolites of some species of gorgonians.[13] Five of the components are guaiane sesquiterpene lactones having either a cyclopentene or cyclopentadiene residue (**2**−**6**), and one component, furanoguaian-4-ene (**1**), is a short-lived furanoguaiane metabolite. The majority of these metabolites were isolated as unstable oils that gradually decomposed on standing.



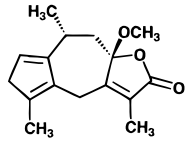
Furanoguaian-4-ene (**1**)



Americanolide D (**2**) R = H
Americanolide E (**3**) R = OH
Methoxyamericanolide E (**4**) R = OCH_3



Americanolide F (**5**)



Methoxyamericanolide G (**6**)

Results and Discussion

The sea plume *P. americana*, collected in December 1994, along the southwest coast of Puerto Rico, was extracted as described previously.[12] The guaiane sesquiterpene mixture was isolated by chromatography (Si gel) of the hexane extract of the total organism.

A molecular formula of $C_{15}H_{20}O$ was estimated from ^{1}H- and ^{13}C-NMR data for furanoguaian-4-ene (**1**). An APT experiment established that the ^{13}C spectrum was composed of three methyl, four methylene, three methine, and five non-protonated carbon signals, indicating that **1** contained 15 carbons and 20 carbon-bonded hydrogens. The lack of ^{13}C signals in the 55−115 ppm and 155−220 ppm regions suggested the absence in **1** of hydroxyl, ether, and carbonyl functionalities. The only oxygen atom in the molecular formula was accounted for by a trisubstituted furan ring, as shown by the ^{1}H signal at δ 7.03 (br s, 1H, H12) and the four ^{13}C signals at δ 117.8 (s, C11), 120.1 (s, C7), 135.6 (d, C12), and 151.0 (s, C8).[5,7] Three of the six units of unsaturation required by the molecular formula could be assigned to three double bonds. The remaining units had to be due to three rings. Unfortunately, we could not confirm the proposed molecular formula as the sample decomposed before high-resolution mass determination could be recorded.

The gross structure of **1** 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 the 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 selective INEPT experiments (the peculiar abundance in **1** of proton−proton couplings across four single and one double bond, *i.e.*, homoallylic couplings, greatly simplified this task). In general, the abundance of homoallylic couplings in compounds **1**−**6** might explain the excessive number of signals in Table 1 whose multiplicities and coupling constants were not able to be determined even at 300 MHz.

One of the five-membered rings was a furan residue. The ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY spectra revealed that an oxygen-bearing sp^2 methine [δ 7.03 (H12), 135.6 (C12)] was connected to a nonprotonated sp^2 carbon (δ 117.8) possessing one methyl group [δ 2.02 (H13), 8.3 (C13)]. Two methylene groups were directly attached to the furan ring unit: one to the oxygen-bearing

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Table 1. ^{1}H-NMR (300-MHz) Spectral Data of Guaiane Metabolites **1**−**6** in $CDCl_3$[a]

	furanoguaian-4-ene (**1**)	americanolide D (**2**)	americanolide E (**3**)	MeO-americanolide E (**4**)	americanolide F (**5**)	MeO-americanolide G (**6**)
position	1H, mult, *J* (Hz),intg	1H, mult, *J* (Hz),intg	1H, mult, *J* (Hz),intg	1H, mult, *J* (Hz),intg	1H, mult, *J* (Hz),intg	1H, mult, *J* (Hz),intg
1	2.98, m, 1H	2.87, br m, 1H	2.86, m, 1H	2.84, br m, 1H	2.65, m, 1H	
2α	1.97, m, 1H	1.79, m, 1H	1.75, m, 1H	1.76, m, 1H	1.95, m, 1H	5.81, br s, 1H
β	1.57, m, 1H	1.27, m, 1H	1.25, m, 1H	1.25, m, 1H	1.49, m, 1H	
3α	2.29, m, 1H	2.11, m, 1H	2.12, m, 1H	2.10, m, 1H	2.25, m, 1H	2.80, br s, 1H
β	2.29, m, 1H	2.20, m, 1H	2.18, m, 1H	2.19, m, 1H	2.30, m, 1H	2.80, br s, 1H
6α	2.87, br d, 15.9, 1H	2.62, br d, 13.5, 1H	2.79, br d, 12.9, 1H	2.63, br d, 12.8, 1H	3.28, br s, 1H	2.94, br d, 13.5, 1H
β	3.35, d, 15.9, 1H	3.56, d, 13.3, 1H	3.45, d, 12.9, 1H	3.44, d, 12.8, 1H	3.28, br s, 1H	3.59, d, 13.5, 1H
8		4.68, dd, 3.6, 11.4, 1H			4.84, m, 1H	
9α	2.68, m, 1H	1.39, m, 1H	1.69, m, 1H	1.61, dd, 10.6, 14.3,1H	1.45, m, 1H	1.41, dd, 12.0, 13.8,1H
β	2.68, m, 1H	1.82, m, 1H	1.83, m, 1H	1.87, br d, 14.3, 1H	2.26, m, 1H	2.40, br d, 13.8, 1H
10	2.30, m, 1H	2.08, m, 1H	2.49, m, 1H	2.44, m, 1H	2.01, m, 1H	2.80, m, 1H
12	7.03, br s, 1H					
13	2.02, d, 1.2, 3H	1.84, d, 1.1, 3H	1.85, br s, 3H	1.88, d, 0.6, 3H	1.81, d, 1.2, 3H	1.84, d, 0.9, 3H
14	0.92, d, 6.9, 3H	0.99, d, 7.1, 3H	0.98, d, 7.2, 3H	0.96, d, 7.2, 3H	0.91, d, 6.9, 3H	1.26, d, 6.3, 3H
15	1.71, dd, 0.9, 2.1, 3H	1.68, br s, 3H	1.69, br s, 3H	1.70, d, 1.0, 3H	1.67, d, 0.6, 3H	2.04, d, 1.2, 3H
8-OH			3.24, br s, 1H			
8-OCH_3				3.19, s, 3H		3.21, s, 3H

[a] The *δ* values are in ppm downfield from TMS. Assignments were made on the basis of ^{1}H−^{1}H−COSY, ^{1}H−^{13}C COSY, NOESY, spin splitting patterns, and comparison of *J* values.

Table 2. ^{13}C-NMR (75-MHz) Spectral Data of Guaiane Metabolites **1**−**6** in $CDCl_3$[a]

	compound (^{13}C, m)					
position	**1**	**2**	**3**	**4**	**5**	**6**
1	54.3, d	52.4, d	52.5, d	52.5, d	53.5, d	152.0, s
2	26.3, t	24.9, t	25.1, t	25.2, t	27.8, t	121.4, d
3	36.7, t	36.1, t	38.6, t	36.0, t	36.9, t	43.9, t
4	132.4, s	131.3, s	132.1, s	132.1, s	129.1, s	133.2, s
5	133.8, s	136.8, s	136.8, s	136.6, s	136.7, s	137.5, s
6	21.5, t	24.8, t	23.8, t	23.9, t	26.4, t	23.2, t
7	120.1, s	162.0, s	160.3, s	159.1, s	163.2, s	157.6, s
8	151.0, s	83.8, d	105.7, s	108.3, s	80.7, d	108.7, s
9	32.1, t	33.6, t	36.0, t	37.0, t	40.1, t	45.9, t
10	34.6, d	29.3, d	27.8, d	27.2, d	34.1, d	27.7, d
11	117.8, s	119.7, s	120.9, s	122.6, s	122.8, s	125.4, s
12	135.6, d	175.0, s	172.6, s	172.4, s	174.6, s	171.5, s
13	8.3, q	8.4, q	8.6, q	8.6, q	8.5, q	8.3, q
14	16.3, q	19.9 q	19.8, q	19.7, q	14.1, q	19.6, q
15	13.9, q	14.3, q	14.6, q	14.6, q	13.9, q	13.4, q
8-OCH_3				50.3, q		50.4, q

[a] The *δ* values are in ppm downfield from TMS. ^{13}C-NMR multiplicities were determined by attached proton test (APT) sequences. Assignments were made on the basis of homonuclear and heteronuclear chemical shift correlation experiments and comparisons to known models.

quaternary carbon and one to the olefin carbon signal at 120.1 ppm, since selective INEPT correlations could be seen from the *δ* 2.68 (H9α*β*) and *δ* 3.35 (H6*β*) signals to the carbon signals at *δ* 151.0 (C8) and 120.1 (C7). Moreover, interpretation of the ^{1}H−^{1}H COSY spectrum allowed to link these methylene groups (C6 and C9) by long-range couplings.

The second five-membered ring unit was deduced to be a 4-methyl-cyclopent-4-ene residue. ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY analyses indicated that this unit possessed a methine group [*δ* 2.98 (H1), 54.3 (C1)] that was connected successively to two contiguous methylenes [*δ* 1.97 (H2α), 1.57 (H2*β*), 26.3 (C2) and 2.29 (H3α*β*), 36.7 (C3)] as well as to a methyl-bearing methine [*δ* 2.30 (H10), 34.6 (C10)]. The latter methine was, in turn, joined to the methylene terminated by the oxygen-bearing quaternary carbon, as shown by selective INEPT correlations from the proton signal at *δ* 2.68 (H9α*β*) to the ^{13}C signals at 151.0 ppm (C8) and 34.6 (C10). The chain of two contiguous methylenes was terminated by a nonprotonated methyl-bearing vinyl carbon, as the proton signal at 2.29 ppm (H3α*β*) displayed selective INEPT correlations to the carbon signals at *δ* 132.4 (C4), 133.8 (C5), and 13.9 (C15). The connectivity of these partial substructures was also argued on the basis of long-range ($^{4,5}J$) proton−proton couplings between H3α*β*/Me-15, H1/Me-15, and H6α/Me-15. Finally, long-range $^2J_{CH}$ correlations from the methylene proton signals at *δ* 3.35 (H6*β*) and 2.87 (H6α) to the olefinic carbon signals at 133.8 (C5) and 120.1 (C7) ppm as well as their ^{1}H chemical shift values, coupling constants, and spin−spin splitting patterns (AB system), showed that the latter proton resonances belong to an isolated bisallyl methylene unit. This sequencing established the cycloheptane ring unit and confirmed that furanoguaian-4-ene (**1**) was a tricyclic sesquiterpene.

Concerning the carbons C1 (*S**) and C10 (*R**), the stereochemistry was determined by the correlations suggested by NOESY H1/H6α, H9*β*/Me-14, H2*β*/Me-14, H2α/H1, H6α/H9α, H6*β*/Me-13, and H6*β*/Me-15. The lack of a cross-peak correlation in the NOESY between H1 and H10 suggests that the dihedral angle between these protons is near 180° (*trans*). These stereochemical assignments were confirmed by molecular modeling studies, which revealed that the cycloheptane ring in **1** adopts a chairlike conformation in order to compensate for the partial planarity of the two five-membered rings. The geometric constraints dictated by the molecular modeling studies and the observed NOEs are compatible with the contention that H1 and H10 must be on opposite faces of the molecule in 1,2-diaxial fashion (Figure 1).[14] Because **1** was found to decompose in a matter of days on standing, it could not be examined for biological activity.[15]

The EIMS of americanolide D (**2**) displayed a molecular ion peak at *m*/*z* 232, and a high resolution measurement established its molecular composition as $C_{15}H_{20}O_2$. The gross structure of **2** was likewise determined by a detailed analysis of 1D and 2D NMR spectra (Tables 1 and 2). When the ^{1}H- and ^{13}C-NMR data of **2** in $CDCl_3$ and the ^{1}H−^{1}H COSY, and ^{1}H−^{13}C COSY spectra were compared with those recorded for furanoguaian-4-ene (**1**), it was concluded that three of the six units of unsaturation required by the molecular formula could be assigned to the same cyclopentene and cycloheptane ring units found in **1**. The remaining three unsaturations were accounted for by an α-methyl buteno-

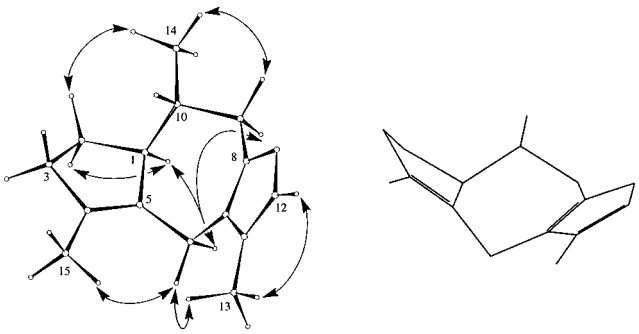

Figure 1. MMX-optimized models for the major conformation of furanoguaian-4-ene (**1**) generated by computer modeling together with selected NOE correlations. The stick drawing of **1** (with the hydrogen atoms removed for clarity) clearly reveals that at the MMX minimum energy the cycloheptane ring is dominated by a single chairlike conformation.

lide group, as shown by the ^{1}H signals at δ 4.68 (dd, 1H, H8) and 1.84 (d, 3H, Me-13) and the five ^{13}C signals at δ 175.0 (s, C12), 162.0 (s, C7), 119.7 (s, C11), 83.8 (d, C8), and 8.4 (q, Me-13). The presence of a substituted butenolide moiety was supported by a strong absorption at 1754 cm^{-1} in the IR spectrum and at λ_{max} 208 nm (ϵ 7467) in the UV spectrum. Confirmation of the structures of the three ring units as well as their sequencing was provided by HMBC data. The carbonyl C12 (δ 175.0) exhibited correlation with Me-13, whereas the quaternary ethylenic C11 (δ 119.7) correlated with H6$\alpha\beta$ and H8, and C7 (δ 162.0) showed crosspeaks with H6$\alpha\beta$, H9$\alpha\beta$, and H8, too.

The stereochemical assignments were accomplished by extensive NOE and molecular modeling studies. The observation of an NOE between the H14 methyl protons and H1 allowed assignment of the relative stereochemistry at C1/C10 with the C10 methyl and H1 on the same face of the molecule. A weak NOE between the H8 lactone oxymethine and H10 was also observed, showing these protons to be on the same molecular face. Interestingly, although no coupling was observed between H1 and H10 during a COSY experiment on **2**, an NOE between these protons was detected. These data combined with NMR information suggest a conformational change in the C8-C9-C10-C1 fragment of the seven-membered ring of **2** rather than a stereochemical change.

The next two components isolated from the lipid extract, americanolide E (**3**) and methoxyamericanolide E (**4**), differed from **2** only in the functionality at C8. Absorptions for the ester and hydroxyl functionalities were apparent in the IR spectrum of **3**. Compound **3**, which possesses the same UV chromophore as **2**, has a molecular formula of $C_{15}H_{20}O_3$, established from HREIMS and ^{13}C-NMR spectrometry. Further consideration of the NMR data for this compound suggested the absence of a lactonic oxymethine at position C8. In its place, a resonance line corresponding to a hemiketal carbon in the ^{13}C-NMR spectrum (δ 105.7) was observed. Once the gross structure of **3** was fully defined, the structure of the closely related metabolite, methoxyamericanolide E (**4**), could be assigned based on spectral comparisons to **3**. Compound **4** lacked the IR absorption that clearly defined the presence of the hydroxyl functionality in **3**, and its molecular formula, $C_{16}H_{22}O_3$, was established by HREIMS. The NMR (Tables 1 and 2) features of methoxyamericanolide E were also analogous to those observed for compound **3**. Derivative **4**, however, possessed a methoxy group in place of the hydroxyl at C8. The stereochemistries of the chiral positions in metabolites **3** and **4** were established in the same manner as **2**, using NOE measurements and molecular modeling studies. In the case of **4**, coupling-constant analysis was also used to assign the stereochemistry at C10 as the 10.6 Hz coupling observed between H9α and H10 suggest axial−axial-type coupling, thus confirming the equatorial orientation of Me-14. Because our extraction procedures required the use of MeOH, it is possible that **4** is an artifact arising through transacetalization of **3**.

Americanolide F (**5**), also an unstable metabolite, showed many spectral features in common with americanolide D (**2**). A molecular formula of $C_{15}H_{20}O_2$ was established for **5** from HREIMS and ^{13}C-NMR spectrometry (Table 2). The presence of a butenolide ester functionality was confirmed by examination of the IR and UV spectra of this molecule. Many of the salient NMR spectral features recorded for this yellow oil were reminiscent of those described earlier for americanolide D (**2**). However, in comparison to compound **2**, the most obvious difference in the ^{13}C-NMR spectrum of **5** was that the signals for C10 and C14 had shifted considerably from δ 29.3 and 19.9 in compound **2**, to δ 34.1 and 14.1 in **5**, respectively. Although in fact many of the ^{13}C signals have shifted slightly when data for **2** and **5** are compared, americanolide F (**5**) was nevertheless perceived to be the C10 epimer of americanolide D (**2**). After further examination of the ^{1}H- and ^{13}C-NMR spectra (see Tables 1 and 2), it was concluded that compounds **2** and **5** were otherwise identical. As predicted on the basis of the difference in their ^{13}C-NMR data, the relative stereochemistry at C10 in americanolide F (**5**) [C10 (*S**)] was shown to be opposite to that in **2** by the correlations suggested by NOESY H8/Me-14 and H6β/Me-14. These features, which are not found in **2**, are difficult to rationalize if the stereochemistry at C1 (or C8) rather than C10 was changed. After being placed in a refrigerator (−10 °C) for several weeks without solvent, compound **5**, like compound **2**, was completely consumed as determined by TLC and NMR.

Compound **6** displayed a strong IR lactone band at 1766 cm^{-1} and a strong UV band at λ_{max} 212 nm (ϵ 10458) with minor absorbances at λ_{max} 254 nm (ϵ 2679) and 282 nm (ϵ 1132). The HREIMS showed an intense molecular peak at *m*/*z* 260.1382, which suggested the molecular formula $C_{16}H_{20}O_3$. This was confirmed by high resolution measurement of the $[M - CH_3OH]^+$ peak at *m*/*z* 228. The ^{1}H-NMR spectrum was very similar to that of methoxyamericanolide E (**4**), but the typical C2 methylene multiplets were absent. The presence of a new olefinic methine signal (δ 5.81, br s) and the changes in other signals, particularly those concerning the five-membered carbocyclic ring, clearly pointed to **6** as having a cyclopentadiene moiety, a conclusion supported by the ^{13}C-NMR and UV spectra. The remaining features of the NMR spectra of compound **6** (Tables 1 and 2) indicated a close structural similarity to methoxyamericanolide E. Linking the various proton spin systems and completing the skeleton of methoxyamericanolide G (**6**) were accomplished by the observation of long-range homonuclear couplings in the long-range ^{1}H−^{1}H COSY spectrum and long-range

heteronuclear couplings in HMBC experiments. Simultaneous coupling from the most downfield methine (δ 5.81, H2) to the ^{13}C signals at δ 152.0 (C1), 137.5 (C5), 133.2 (C4), and 43.9 (C3), could be discerned in the HMBC spectrum. In turn, the overlapped methylene pair of protons at δ 2.80 (H3$\alpha\beta$) showed observable couplings with the ^{13}C signals assigned to C2, C4, and C5. These observations, along with strong cross-peak correlations in the NOESY between H2 and Me-14 and in the long-range COSY between H3/H6α, Me-13/H6α, and Me-15/H6α, require a 1,4-cyclopentadiene ring as shown.

The relative stereochemistry shown was also confirmed by NOEs: H10 with H9β, H9β with the C8 methoxy group, Me-14 with H9α and H2, and H6β with Me-13 and Me-15. These NOEs suggest that the cycloheptane ring in **6** may also be dominated by a single chairlike conformation. Molecular modeling studies indicated that in the lowest energy conformation, whereby the C8 methoxy group is oriented in axial fashion on the β-face, the H10 proton, too, is axially oriented on the same face. In such conformation the interproton distances for which NOEs were observed are 3.0 Å or less. These modeling experiments, in accord with the NOE and coupling constant observations, indicate that methoxyamericanolide G has the relative stereochemistry depicted in structure **6**. It is likely that methyl ether **6** arises from transacetalization of the parent hemiketal. Whether this transformation takes place in vivo or during workup, however, is uncertain.

Although americanolide D (**2**) exhibited very modest levels of cytotoxicity to HeLa and CHO-K1 cells (ED_{50}'s of 30 and 100 μg/mL, respectively), it showed strong cytotoxicity against a human colon (KM-12) cancer cell line (IC_{50} = 0.1 μg/mL). Methoxyamericanolide G (**6**), on the other hand, was not cytotoxic to any of the human cancer cell lines in the NCI panel. Interestingly, methoxyamericanolide A (the α,α-cyclopentane-diepoxy derivative of **6** reported earlier by us)[12] is a strong and selective in vitro inhibitor of MOLT-4 leukemia cells with an IC_{50} of 0.1 μg/mL.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a MAGNA-IR 750 Nicolet spectrophotometer and UV spectra on a Hewlett-Packard diode array spectrophotometer Model 8452A. Optical rotations were determined on a Perkin-Elmer polarimeter Model 243B. ^1H- (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a General Electric QE-300 in $CDCl_3$ (^1H NMR and ^{13}C NMR chemical shifts 7.26 and 77.0 ppm, respectively). HMBC and HMQC data were recorded on a Bruker Avance DRX-500 spectrometer. 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 Si gel columns of 10 μm. All solvents used were spectral grade.

Collection and Extraction of *P. americana*. The sea plume was collected at a depth of 3 m near La Parguera Marine Station (Lajas, 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.[12] The hexane extract (302 g) was loaded on a Si gel gravity column and eluted in succession with: (1) 100% hexane, (2) 2% Me_2CO in hexane, (3) 5% Me_2CO in hexane, (4) 10% Me_2CO in hexane, (5) 20% Me_2CO in hexane, (6) 30% Me_2CO in hexane, (7) 50% Me_2CO in hexane, (8) 100% Me_2CO, (9) 30% MeOH in Me_2CO, and (10) 100% MeOH. This led to fractions 1−10. Fraction 2 [15.3 g; eluted with hexane−Me_2CO (98:2)] was chromatographed on a Si gel column (800 g, dry packing eluting with 0−30% Me_2CO−hexane mixtures) to yield furanoguaian-4-ene (**1**) (149.2 mg, 0.0065% dry wt) and two subfractions. One of the latter (425 mg) was rechromatographed successively over Si gel (9:1 hexane−*i*-PrOH) and HPLC (Partisil-10 M9/10 with 97:3 hexane−*i*-PrOH) leading to pure americanolide D (**2**) (21.0 mg, 0.0009% dry wt) and americanolide F (**5**) (13.3 mg, 0.0006% dry wt). The remaining subfraction (543 mg) was rechromatographed over Si gel with 97:3 hexane−*i*-PrOH followed by HPLC (Partisil-10 M9/10 with 98:2 hexane−*i*-PrOH) to afford americanolide E (**3**) (3.4 mg, 0.00015% dry wt). Fraction 4 [48.1 g; eluted with hexane−Me_2CO (9:1)] was loaded onto a Si gel column and eluted with hexane−Me_2CO mixtures leading to the isolation of subfractions A−X. Subfraction F [2.5 g; eluted with hexane−Me_2CO (9:1)] was, in turn, rechromatographed over Si gel using as eluent a mixture of hexane−*i*-PrOH (98:2). Two main fractions were isolated: from the first (403 mg) methoxyamericanolide E (**4**) (17.6 mg, 0.0008% dry wt) was isolated after successive column chromatography over Si gel (9:1 hexane−Me_2CO) followed by HPLC (Partisil 10 M9/10 with 98.5:1.5 hexane−*i*-PrOH), and from the second (131 mg) methoxyamericanolide G (**6**) (27.5 mg, 0.0012% dry wt) was isolated using identical purification conditions.

Furanoguaian-4-ene (1): unstable yellow oil; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Tables 1 and 2). Unfortunately, compound **1** decomposed before optical rotation, IR, UV, and MS data could be recorded.[15]

Americanolide D (2):[16] unstable yellow oil; IR (neat) 2962, 2931, 2875, 1754, 1454, 1379, 1330, 1216, 1169, 1102, 1021, 756 cm^{-1}; UV (MeOH) λ_{max} 208 nm (ϵ 7467); $[\alpha]^{23}_D$ −15.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Tables 1 and 2); HREIMS *m/z* [M^+] 232.1461, calcd for $C_{15}H_{20}O_2$ 232.1463; EIMS *m/z* [M^+] 232 (100), 217 (22), 203 (31), 190 (16), 175 (11), 161 (20), 150 (22), 147 (19), 133 (29), 121 (48), 119 (28), 109 (52), 107 (48), 105 (34), 95 (30), 93 (27), 91 (51), 81 (26), 79 (36), 77 (35).

Americanolide E (3): unstable yellow oil; IR (neat) 3388, 2965, 2926, 2872, 2852, 1765, 1744, 1599, 1453, 1380, 1325, 1260, 1243, 1170, 1120, 1020, 952 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Tables 1 and 2); HREIMS *m/z* [M^+] 248.1401, calcd for $C_{15}H_{20}O_3$ 248.1412; EIMS *m/z* [M^+] 248 (5), 233 (10), 230 (10), 218 (24), 204 (24), 201 (21), 174 (66), 151 (39), 137 (29), 135 (29), 124 (44), 109 (54), 107 (55), 105 (51), 91 (92), 79 (62), 77 (75), 69 (100). Compound **3** decomposed before optical rotation and UV data could be recorded.

Methoxyamericanolide E (4): stable yellow oil; IR (neat) 3008, 2958, 2925, 2854, 1767, 1743, 1456, 1378, 1309, 1261, 1164, 1089, 1021, 961 cm^{-1}; UV (MeOH) λ_{max} 208 nm (ϵ 10066); $[\alpha]^{23}_D$ −44.0° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz)

(see Tables 1 and 2); HREIMS *m/z* [M^+] 262.1572, calcd for $C_{16}H_{22}O_3$ 262.1569; EIMS *m/z* [M^+] 262 (13), 230 (100), 215 (57), 201 (65), 187 (47), 174 (25), 160 (24), 159 (25), 145 (16), 133 (16), 121 (10), 119 (16), 117 (17), 115 (15), 107 (31), 105 (25), 93 (21), 91 (39), 79 (26), 77 (26).

Americanolide F (5):[16] unstable yellow oil; IR (neat) 2960, 2921, 2850, 1752, 1597, 1461, 1453, 1378, 1100,1025, 752 cm^{-1}; UV (MeOH) λ_{max} 208 nm (ϵ 6670); $[\alpha]^{23}_D$ −2.0° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Tables 1 and 2); GC-HREIMS *m/z* [M^+] 232.1439, calcd for $C_{15}H_{20}O_2$ 232.1463; EIMS *m/z* [M^+] 232 (14), 217 (17), 190 (10), 164 (10), 121 (100), 107 (80), 105 (32), 95 (20), 93 (64), 91 (61), 81 (25), 79 (32), 77 (35).

Methoxyamericanolide G (6): stable yellow oil; IR (neat) 2960, 2927, 2874, 2854, 1766, 1692, 1454, 1383, 1311, 1276, 1163, 1110, 1054, 947, 761 cm^{-1}; UV (MeOH) λ_{max} 212 nm (ϵ 10458), 254 nm (ϵ 2679) and 282 nm (ϵ 1132); $[\alpha]^{23}_D$ −60.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Tables 1 and 2); HREIMS *m/z* [M^+] 260.1382, calcd for $C_{16}H_{20}O_3$ 260.1412; EIMS *m/z* [M^+] 260 (82), 228 (100), 213 (96), 200 (63), 199 (64), 185 (81), 171 (46), 157 (78), 145 (29), 142 (47), 141 (47), 128 (65), 119 (59), 115 (67), 105 (82), 91 (29), 79 (45), 77 (70).

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References and Notes

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(14) Interestingly, the absence of cross-peak correlation in the COSY allows us to consider the coupling constant of $^3J_{1,10}$ to be near 0, suggesting that the dihedral angle between H1 and H10 is instead near 90°. Excluding other factors besides ϕ that affect coupling constants, the $J_{1,10}$ value found is inconsistent with the conformation predicted by molecular modeling studies (coupling near 12 Hz expected for a dihedral angle of 178°). On the other hand, because the lack of coupling between C1 and C10 protons has been observed in related guaianolides with identical relative stereochemistry at these sites, the dependence of this vicinal coupling constant on factors *other than the dihedral angle* is implicit: see (a) Hernández, L. R.; Catalán, C. A. N.; Cerda-García-Rojas, C. M.; Joseph-Nathan, P. *Phytochemistry* **1994**, *37*, 1331−1335 and (b) Gao, F.; Wang, H.; Mabry, T. J.; Watson, W. H.; Kashyap, R. P. *Phytochemistry* **1990**, *29*, 551−560. This peculiarity, which must be taken into account in the conformational analysis of the seven-membered ring, is common to all the metabolites reported here except in americanolide F (**5**).
(15) Attempts to reisolate compound **1** from specimens of *P. americana* from the same collections, but stored at −10 °C for longer periods of time, have been unsuccessful.
(16) After prolonged exposure to light, air, and solvents for spectral measurements, it was evident that this sample was not pure. Thus, it is conceivable that the UV and optical rotation data may be inaccurate because they were not taken at the outset.

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