New Pseudopterosin and *seco*-Pseudopterosin Diterpene Glycosides from Two Colombian Isolates of *Pseudopterogorgia elisabethae* and Their Diverse Biological Activities

Ileana I. Rodríguez,[†] Yan-Ping Shi,[†] Oscar J. García,[†] Abimael D. Rodríguez,*[,†] Alejandro M. S. Mayer,[‡] Juan A. Sánchez,[§] Eduardo Ortega-Barria,[⊥] and José González[⊥]

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U.P.R. Station, San Juan, Puerto Rico 00931-3346, Department of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern University, 555 31st Street, Downers Grove, Illinois 60515, Department of Systematic Biology & Laboratories of Analytical Biology, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013-7012, and Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología, Centro de Estudios Biomédicos, Clayton, Building 175, P.O. Box 7250, Panama 5, Republic of Panama

Received June 10, 2004

As part of an ongoing program to explore the chemical constituents of Caribbean marine invertebrates, a family of 13 new diterpene glycosides, pseudopterosins P−Z (**1**−**11**) and *seco*-pseudopterosins H (**12**) and I (**13**), have been isolated from the organic extracts of two collections of the sea whip *Pseudopterogorgia elisabethae* procured near the Colombian Southwestern Caribbean Sea. The structures of compounds **1**−**13**, including absolute stereochemistry, have been proposed on the basis of comprehensive spectral analyses, chemical transformations, specific rotation, and TLC chromatographic analyses. Pseudopterosin Q (**2**) inhibited thromboxane B_2 (TXB_2) (IC_{50} = 4.7 μM) and superoxide anion (O_2^-) (IC_{50} = 11.2 μM) generation from *E. coli* lipopolysaccharide (LPS) activated rat neonatal microglia in vitro. In contrast, pseudopterosins P (**1**), U (**6**), V (**7**), W (**8**), and X (**9**) as well as *seco*-pseudopterosins H (**12**) and I (**13**) demonstrated minimal effects on both TXB_2 and O_2^- release. In addition, some of the new compounds displayed strong antituberculosis, antiviral, antimalarial, and anticancer activity.

Gorgonian octocorals of the genus *Pseudopterogorgia* are quite abundant throughout the Caribbean Sea, and previous chemical investigations have demonstrated that this genus constitutes an important resource of pharmacologically active secondary metabolites.[1] For instance, the pseudopterosins A−L are a well-known family of diterpene glycosides isolated by the Fenical group in the late 1980s from specimens of the gorgonian *Pseudopterogorgia elisabethae* Bayer (order Gorgonacea, family Gorgoniidae, phylum Cnidaria) collected from Grand Bahama Island, the central Bahamas, Bermuda, and Great Abaco Island.[2,3] The pseudopterosins exhibit superior anti-inflammatory properties when compared to some topical anti-inflammatory drugs currently in use.[4] Cell studies with human neutrophils have indicated that pseudopterosin E, for example, inhibits leukotriene synthesis, suggesting that the latter compound is an antagonist of lipooxygenases or enzymes higher in the arachidonic acid cascade.[3,5] Recent studies suggest that pseudopterosins block the release of eicosanoids without interrupting biosynthesis.[6] On the other hand, *seco*-pseudopterosins A−D, also isolated by the Fenical group from the closely related *P. kallos* collected near the Florida Keys, are potent anti-inflammatory and analgesic compounds related to the pseudopterosins by bond cleavage at the C-5−C-13 positions.[7] Recently, a 2003 report by Ata and collaborators described the co-occurrence of new anti-inflammatory pseudopterosins (named pseudopterosins M−O) and *seco*-pseudopterosins (named *seco*-pseudopterosins E−G) in a *P. elisabethae* specimen collected off Long Key, Florida.[8] As it appears that distinct members of the pseudopterosin and *seco*-pseudopterosin families of diterpene glycosides are found in *P. elisabethae* obtained from different regions, we undertook an extensive investigation of this animal with particular reference to the distribution of these families of compounds in specimens collected at two distinct geographic locations near the Colombian Southwestern Caribbean Sea.

Results and Discussion

Metabolite Isolation and Identification. Freshly collected specimens from Old Providence Island were frozen and subsequently freeze-dried, cut in small pieces, blended with 1:1 MeOH−CH_2Cl_2, filtered, and concentrated. The crude extract was subjected to our standard solvent partitioning scheme, and the hexane extract was purified by flash chromatography over silica gel followed by polar-bonded-phase HPLC to afford pseudopterosins P−Z (**1**−**11**). *P. elisabethae* collected in San Andrés Island was air-dried, stored frozen, and subsequently extracted with 50% MeOH−$CHCl_3$. *seco*-Pseudopterosins H (**12**) and I (**13**) were isolated by gradient flash chromatography and size-exclusion chromatography (Bio-Beads SX-3 in toluene) of the hexane extract and were finally purified by successive normal-phase silica gel chromatography. The molecular structures of these compounds, including absolute stereochemistry, were determined on the basis of spectral data interpretation, chemical evidence, specific rotation, and TLC chromatographic analyses. Interestingly, each isolate of *P. elisabethae* was found to contain exclusively new diterpene glycosides without any trace of the known pseudopterosins A−O or *seco*-pseudopterosins A−G. These findings support the generally accepted notion that the terpenoid secondary metabolite chemistry of *Pseudoptero-*

* To whom correspondence should be addressed. Tel: (787)-764-0000, ext. 4799. Fax: (787)-751-0625. E-mail: arodrig@cnnet.upr.edu.
[†] University of Puerto Rico.
[‡] Midwestern University.
[§] Smithsonian Institution.
[⊥] Instituto de Investigaciones Científicas, Panamá.

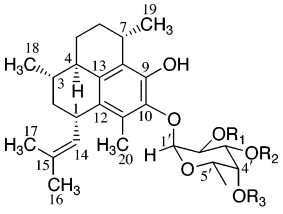
10.1021/np049802o CCC: $27.50
Published on Web 09/11/2004

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for Pseudopterosins P−S (**1**−**4**) in $CDCl_3$[a]

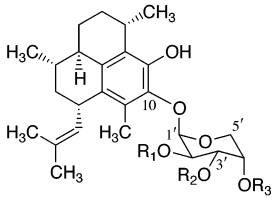
	pseudopterosin P (**1**)		pseudopterosin Q (**2**)		pseudopterosin R (**3**)		pseudopterosin S (**4**)	
atom	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)
1	3.65, dd, 9.1, 8.0	37.3 (d)	3.66, dd, 9.1, 8.0	37.3 (d)	3.67, dd, 9.0, 7.9	37.3 (d)	3.65, dd, 9.0, 7.9	37.3 (d)
2α	1.20, d, 10.0	40.2 (t)	1.18, m	40.1 (t)	1.22, m	40.2 (t)	1.20, m	40.1 (t)
2*β*	1.92, br dd, 10.5, 7.9		1.93, ddd, 12.3, 7.9, 1.9		1.93, dd, 10.5, 7.8		1.92, m	
3	1.26, m	33.9 (d)	1.21, m	33.9 (d)	1.23, m	33.9 (d)	1.23, m	33.8 (d)
4	2.05, m	44.7 (d)	2.01, m	44.6 (d)	2.04, m	44.6 (d)	2.03, m	44.7 (d)
5α	0.91, m	27.8 (t)	0.92, m	27.8 (t)	0.92, m	27.8 (t)	0.89, m	27.8 (t)
5*β*	2.00, m		2.05, m		2.02, m		2.04, m	
6α	2.15, m	31.8 (t)	2.13, ddd, 13.1, 8.8, 4.3	31.8 (t)	2.13, m	31.8 (t)	2.15, m	31.9 (t)
6*β*	1.32, m		1.29, m		1.33, m		1.30, m	
7	3.19, qdd, 7.6, 7.0, 6.9	28.4 (d)	3.18, m	28.4 (d)	3.19, m	28.4 (d)	3.18, m	28.6 (d)
8		127.0 (s)		126.9 (s)		127.0 (s)		126.6 (s)
9		145.2 (s)		145.0 (s)		145.0 (s)		144.5 (s)
10		143.3 (s)		143.0 (s)		143.1 (s)		143.0 (s)
11		127.7 (s)		127.7 (s)		127.6 (s)		127.2 (s)
12		129.3 (s)		129.1 (s)		129.3 (s)		129.4 (s)
13		136.7 (s)		136.6 (s)		136.6 (s)		136.7 (s)
14	4.97, br d, 9.2	131.4 (d)	4.98, br d, 9.1	131.4 (d)	4.97, br d, 9.2	131.4 (d)	4.92, br d, 9.4	131.1 (d)
15		128.2 (s)		128.2 (s)		128.2 (s)		128.4 (s)
16	1.66, br s	25.4 (q)	1.67, br s	25.4 (q)	1.67, br s	25.4 (q)	1.66, br s	25.4 (q)
17	1.71, br s	17.5 (q)	1.72, br s	17.5 (q)	1.72, br s	17.5 (q)	1.71, br s	17.5 (q)
18	1.02, d, 5.9	20.1 (q)	1.02, d, 6.0	20.1 (q)	1.02, d, 6.1	20.1 (q)	1.02, d, 5.8	20.0 (q)
19	1.26, d, 6.8	23.2 (q)	1.25, d, 6.7	23.2 (q)	1.19, d, 6.6	23.2 (q)	1.23, d, 6.0	23.1 (q)
20	2.04, s	13.6 (q)	2.01, s	13.5 (q)	2.07, s	13.6 (q)	2.08, s	13.8 (q)
1′	5.13, d, 3.8	103.0 (d)	5.17, d, 4.0	102.8 (d)	5.20, d, 3.9	102.8 (d)	5.14, d, 3.6	101.4 (d)
2′	4.03, dd, 10.1, 3.8	69.5 (d)	4.29, dd, 10.4, 4.1	67.6 (d)	4.25, dd, 10.2, 3.8	67.6 (d)	5.26, dd, 10.6, 3.7	71.1 (d)
3′	4.35, dd, 10.1, 3.4	69.1 (d)	5.28, dd, 10.5, 3.0	74.3 (d)	5.38, dd, 10.2, 3.2	71.3 (d)	4.45, dd, 10.7, 3.5	67.2 (d)
4′	5.20, d, 2.3	73.6 (d)	4.05, d, 2.5	70.4 (d)	5.40, d, 3.1	71.0 (d)	5.38, br d, 2.4	73.6 (d)
5′	4.52, br q, 6.5	66.4 (d)	4.57, q, 6.5	67.1 (d)	4.66, q, 6.7	66.2 (d)	4.62, q, 6.4	66.7 (d)
6′	1.16, br d, 6.5	16.3 (q)	1.31, d, 6.6	16.1 (q)	1.26, d, 6.8	16.1 (q)	1.25, d, 6.0	16.3 (q)
−OAc	2.19, s	20.8 (q)	2.21, s	21.1 (q)	2.17, s	20.8 (q)	2.23, s	21.0 (q)
		171.9 (s)		171.7 (s)		170.5 (s)		170.6 (s)
−OAc					2.09, s	20.6 (q)	2.23, s	20.8 (q)
						170.9 (s)		171.2 (s)

[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.

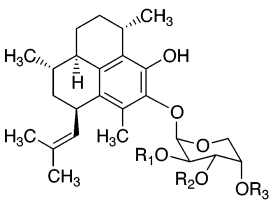
gorgia species is highly species-specific and, hence, useful in the identification of morphologically similar species.[9]



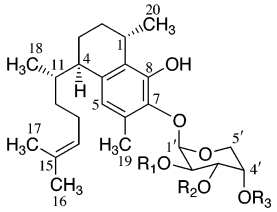
1 R_3 = Ac; R_1 = R_2 = H
2 R_2 = Ac; R_1 = R_3 = H
3 R_2 = R_3 = Ac; R_1 = H
4 R_1 = R_3 = Ac; R_2 = H
5 R_1 = R_2 = R_3 = H
14 R_1 = R_2 = R_3 = Ac



6 R_3 = Ac; R_1 = R_2 = H
7 R_2 = Ac; R_1 = R_3 = H
8 R_2 = R_3 = Ac; R_1 = H
9 R_1 = R_3 = Ac; R_2 = H
10 R_1 = R_2 = R_3 = H
15 R_1 = R_2 = R_3 = Ac



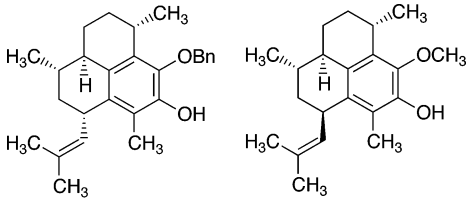
11 R_2 = R_3 = Ac; R_1 = H
16 R_1 = R_2 = R_3 = Ac



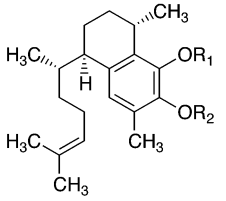
12 R_3 = Ac; R_1 = R_2 = H
13 R_1 = Ac; R_2 = R_3 = H

Pseudopterosin P (**1**), $[\alpha]^{20}_D$ −107.2° (*c* 1.3, $CHCl_3$), a major constituent of the organic extract of the Providence Island collection of *P. elisabethae*, showed M^+ *m*/*z* = 488.2774, suggesting a molecular formula of $C_{28}H_{40}O_7$. The ^{1}H NMR spectrum of **1** (Table 1) showed three singlet methyls (*δ* 1.66, 1.71, 2.04) and two doublet methyls (*δ* 1.02 and 1.26) along with an olefinic doublet resonance (*δ* 4.97, d, *J* = 9.2 Hz), all of which are characteristic of the pseudopterosin diterpene skeleton. A D_2O-exchangeable proton observed at *δ* 7.78 (1H, br s) in the ^{1}H NMR spectrum of pseudopterosin P was assigned to the phenolic hydroxyl group. The ^{1}H NMR spectrum also contained one methyl signal at *δ* 2.19 (3H, s), which together with an IR absorption of 1725 cm^{-1} suggested that **1** was a monoacetate. Acetylation of **1** with excess acetic anhydride in pyridine yielded the triacetate **14** in high yield.[10] In the ^{1}H NMR spectrum of **14**, two new methyl resonances were observed at *δ* 2.02 and 2.16 that were assigned to the newly formed esters. Also, two oxymethine protons were observed to be shifted downfield in the ^{1}H NMR spectrum of triacetate **14**. Therefore, in addition to the phenolic hydroxyl group, pseudopterosin P was proposed to have two secondary alcohols. Further interpretation of ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, and HMBC spectral data revealed that **1** has the amphilectane-based diterpene skeleton with a monoacetylated α-fucose attached at C-10. Thus, it was established that diterpene glycoside **1** is a new member of the pseudopterosin family of marine natural products. The complete ^{1}H and ^{13}C NMR chemical shift assignments of pseudopterosin P (**1**) are depicted in Table 1.

The relative stereochemistry of the aglycon substructure of **1** was elucidated from analysis of the NOESY spectrum, coupling constant analysis, and comparisons of the NMR chemical shifts with those of known models.[2,3] For instance,



17 **18**



19 $R_1 = CH_3$; $R_2 = H$
20 $R_1 = H$; $R_2 = CH_3$
21 $R_1 = R_2 = CH_3$

the 2-methyl-1-propenyl side chain was confidently assigned with the α-orientation since H-14 resonated at *δ* 4.97.[11] NOESY correlations of H-1/H-3 and H-4/H_3-18 indicated that H-1 and H-3 must be pseudoaxial and that the isobutenyl side chain and H_3-18 are both pseudoequatorial. A broad triplet (observed in C_6D_6) at *δ* 2.12 with a large coupling constant (J = 10.0 Hz) ascribable to H-4 suggested that the latter methine is *trans*-diaxial to H-3 and H-5*β*. Additional NOESY correlations of H-2α/H-4, H-4/H-5α, H-5α/H-6α, H-6α/H_3-19, and H-6*β*/H-7 revealed that H-4 and the methyl group at C-7 are in a *cis* 1,4-pseudodiaxial conformation. Therefore, the isobutenyl group at C-1, the methyl groups at C-3 and C-7, and H-4 are all α-oriented. To provide conclusive proof for the stereochemical assignment for pseudopterosin P aglycon, *O*-benzylation of pseudopterosin P with benzyl bromide and anhydrous K_2CO_3 in acetone followed by acid hydrolysis afforded in excellent yield *O*-benzyl ether **17**, a known compound whose absolute stereochemistry has been described as shown. The spectral data and specific rotation of **17** were essentially identical with values already published.[11] These chemical conversion data confirmed that the sugar component of pseudopterosin P (**1**) was indeed appended through the C-10 hydroxyl and established the absolute stereochemistry of the aglycon as shown, namely, 1*S*,3*S*,4*R*,7*S*. Thus, the tricyclic core of pseudopterosin P must correspond stereochemically with that of the recently revised pseudopterosins G−J.[11,12]

The hexose portion of pseudopterosin P (**1**) was shown to be the C-4′ monoacetate derivative of fucose by $^1H-^1H$ COSY NMR, coupling constant analysis, 2D-NOESY NMR, and comparison of ^1H NMR chemical shift data with literature values.[13] Analysis of the coupling constant for the H-1′ (*δ* 5.13, d) anomeric proton revealed a J value of 3.8 Hz, assignable to an equatorial−axial configuration between the H-1′ and H-2′ protons. The small axial−equatorial coupling between H-1′ and H-2′ confirmed that compound **1** is an α-glycoside. The large axial−axial coupling between H-2′ and H-3′ (10.1 Hz) confirmed that H-2′ is axial. Proton H-3′ (*δ* 4.35, dd) showed further coupling with H-4′ (*δ* 5.20, d) with J = 3.4 Hz, and the latter proton barely coupled with H-5′ (J < 1 Hz). The small couplings of H-4′ with H-3′ and H-5′ suggested axial−equatorial orientations between these proton pairs. Furthermore, NOESY correlations observed from H-3′ to H-5′ confirmed the axial orientation for H-5′. Isolation and identification of the carbohydrate component of pseudopterosin P (**1**) and L-fucose upon acid hydrolysis were confirmed by its chromatographic mobility on TLC in three solvent systems and by the strong negative rotation observed in the aqueous sugar hydrolysate. The sugar moiety was, therefore, identified as 4′-*O*-acetyl-L-fucose.

The NMR features of the isomeric monoacetate pseudopterosin Q (**2**), $[\alpha]^{20}_D$ −43.6° (*c* 1.0, $CHCl_3$), compared favorably (Table 1) to **1**, except that the ester appeared to be located at another site on the sugar. The structure of the sugar component of pseudopterosin Q, including the site of acetylation, was determined by $^1H-^1H$ COSY NMR and coupling constant and ^1H NMR chemical shift analysis. On the other hand, pseudopterosins R (**3**) and S (**4**) were identified as diacetate glycosides from their ^1H NMR spectral characteristics (Table 1) as well as data from high-resolution mass spectrometry and ^{13}C NMR spectroscopy. Similarly, pseudopterosin T (**5**) was recognized as the unacetylated glycoside from its polarity and NMR spectral characteristics (Table 2). To confirm these conclusions, pseudopterosins Q, R, S, and T (**2**−**5**) were acetylated to yield the same triacetate, **14**, produced earlier from **1**. Furthermore, base hydrolysis of pseudopterosins P−S (**1**−**4**) (5% KOH−MeOH) yielded pseudopterosin T (**5**). From a chemotaxonomic point of view, it should be noted that pseudopterosins R (**3**) and S (**4**), as well as pseudopterosins W (**8**), X (**9**), and Z (**11**), represent the first diacetylated pseudopterosin glycosides to be isolated and, thus comprise metabolites unique to the Old Providence Island collection of *P. elisabethae*.

Comparison of spectral as well as high-resolution mass spectrometry data, showed that pseudopterosins U (**6**) and V (**7**) are isomeric monoacetates of molecular formula $C_{27}H_{38}O_7$. IR and UV absorptions defined the same hydroxyl, phenolic, and ester functional groups to be present in pseudopterosins P and Q (**1** and **2**). Furthermore, the NMR features (Table 2) of the isomeric metabolites suggested that the sugar moieties were attached at the C-10 hydroxyl and that the aglycon component of pseudopterosins U and V is identical with that from monoacetates **1** and **2**. To determine if the only difference between these pairs of monoacetylated glycosides resided in the sugar component, a comparison of their aglycons was desirable. Thus, *O*-benzylation of pseudopterosin U (**6**) with benzyl bromide, followed by acid hydrolysis, yielded the same *O*-benzyl ether aglycon, **17**, derived from pseudopterosin P (**1**). Once the identity of the aglycon residue in pseudopterosins U and V was established, attention was directed toward determining the complete structures of the sugars. ^1H NMR analysis of pseudopterosins U (**6**) and V (**7**), with particular emphasis on the midfield pentose methine region, confirmed that both metabolites possess the same α-arabinose component and showed the exact locations of acetylation in each glycoside. The latter contention was confirmed upon acetylation, wherein compounds **6** and **7** produced the same triacetate derivative, **15**, in high yield.[10] The sugar was next isolated in order to determine its absolute configuration. Hydrolysis of **6** was carried out with 1 N HCl at 48−50 °C. Optical rotation of the aqueous sugar hydrolysate was then measured, showing that the sugar component of pseudopterosin U (**6**) is D-arabinose. Thus, the pentose portions of pseudopterosins U and V were 4′-*O*-acetyl-α-D-arabinose and 3′-*O*-acetyl-α-D-arabinose, respectively.

As was the case with diterpene glycosides **3** and **4**, pseudopterosins W (**8**) and X (**9**) were diacetate positional isomers. The differences in their ^1H NMR spectra (Tables 2 and 3) were in the carbohydrate region, which indicated that pseudopterosin W (**8**) is the C-3′,C-4′ diacetoxyl derivative whereas pseudopterosin X (**9**) is the C-2′,C-4′ diacetoxyl positional isomer. Similarly, pseudopterosin Y

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for Pseudopterosins T−W (**5**−**8**) in $CDCl_3$[a]

	pseudopterosin T (**5**)		pseudopterosin U (**6**)		pseudopterosin V (**7**)		pseudopterosin W (**8**)	
atom	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)
1	3.65, dd, 9.2, 8.2	37.3 (d)	3.65, dd, 9.4, 8.5	37.3 (d)	3.66, dd, 9.3, 8.3	37.3 (d)	3.66, dd, 9.3, 8.4	37.3 (d)
2α	1.20, m	40.2 (t)	1.20, m	40.2 (t)	1.18, m	40.2 (t)	1.18, m	40.1 (t)
2*β*	1.92, br dd, 11.3, 8.8		1.92, br dd, 10.7, 8.0		1.93, ddd, 12.3, 7.9, 1.9		1.93, dd, 10.8, 8.0	
3	1.20, m	33.9 (d)	1.20, m	33.9 (d)	1.21, m	33.9 (d)	1.24, m	33.9 (d)
4	2.03, m	44.6 (d)	2.00, m	44.7 (d)	2.01, m	44.7 (d)	2.05, m	44.6 (d)
5α	0.88, m	27.8 (t)	0.90, ddd, 14.0, 7.0, 4.5	27.8 (t)	0.92, m	27.8 (t)	0.92, m	27.8 (t)
5*β*	2.06, m		2.03, m		2.05, m		2.01, m	
6α	2.13, m	31.8 (t)	2.14, m	31.8 (t)	2.15, m	31.8 (t)	2.07, m	31.7 (t)
6*β*	1.29, m		1.30, ddd, 13.3, 8.5, 4.2		1.29, m		1.30, m	
7	3.18, m	28.5 (d)	3.18, m	28.5 (d)	3.19, m	28.5 (d)	3.18, m	28.4 (d)
8		126.9 (s)		127.0 (s)		127.1 (s)		127.0 (s)
9		145.2 (s)		145.2 (s)		145.0 (s)		145.0 (s)
10		143.3 (s)		143.1 (s)		142.9 (s)		143.0 (s)
11		127.7 (s)		127.8 (s)		127.6 (s)		127.8 (s)
12		129.2 (s)		129.3 (s)		129.2 (s)		129.3 (s)
13		136.6 (s)		136.8 (s)		136.7 (s)		136.7 (s)
14	4.97, br d, 9.1	131.4 (d)	4.95, br d, 9.2	131.3 (d)	4.97, br d, 9.1	131.4 (d)	4.96, br d, 9.2	131.3 (d)
15		128.2 (s)		128.2 (s)		128.2 (s)		128.2 (s)
16	1.67, br s	25.4 (q)	1.66, br s	25.4 (q)	1.67, br s	25.5 (q)	1.66, br s	25.4 (q)
17	1.72, br s	17.5 (q)	1.71, br s	17.5 (q)	1.72, br s	17.5 (q)	1.71, br s	17.5 (q)
18	1.01, d, 5.8	20.1 (q)	1.02, d, 6.0	20.1 (q)	1.02, d, 5.9	20.1 (q)	1.02, d, 6.0	20.0 (q)
19	1.25, d, 6.8	23.2 (q)	1.26, d, 6.7	23.4 (q)	1.26, d, 6.8	23.2 (q)	1.26, d, 6.8	23.3 (q)
20	2.06, s	13.5 (q)	2.05, s	13.9 (q)	2.08, s	13.8 (q)	2.07, s	13.9 (q)
1′	5.11, d, 3.6	103.0 (d)	5.17, d, 3.6	103.1 (d)	5.21, d, 3.8	103.1 (d)	5.23, d, 3.7	103.1 (d)
2′	4.03, dd, 9.8, 3.5	69.8 (d)	4.10, dd, 9.9, 3.6	70.0 (d)	4.32, dd, 10.0, 3.8	68.0 (d)	4.30, dd, 10.3, 3.8	67.7 (d)
3′	4.13, br d, 9.8	70.5 (d)	4.33, dd, 9.9, 3.5	68.2 (d)	5.31, dd, 10.0, 3.1	73.3 (d)	5.39, dd, 10.3, 3.2	70.3 (d)
4′	3.90, br d, 2.2	72.2 (d)	5.23, br s	71.5 (d)	4.26, d, 1.4	67.5 (d)	5.44, br d, 1.1	68.8 (d)
5α′	4.51, br q, 6.7	67.3 (d)	4.36, br d, 13.2	61.9 (t)	4.40, d, 11.7	63.7 (t)	4.45, d, 12.9	61.9 (t)
5*β*′			3.88, br d, 13.2		3.86, dd, 12.6, 2.5		3.85, dd, 13.0, 2.0	
6′	1.32, d, 6.6	16.2 (q)						
−OAc			2.16, s	21.1 (q)	2.21, s	21.1 (q)	2.10, s	20.9 (q)
				171.3 (s)		171.6 (s)		171.0 (s)
−OAc							2.14, s	20.8 (q)
								170.3 (s)

[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.

(**10**) was recognized as the unacetylated glycoside from its limited chromatographic mobility on TLC analysis and from its NMR spectral features (Table 3). The structural relationship of compounds **8**−**10** was decisively demonstrated when all three metabolites, upon acetylation, yielded the same triacetate, **15**, produced earlier from **6** and **7**.[10] In addition, base hydrolysis (5% KOH−MeOH) of a mixture of pseudopterosins U−X (**6**−**9**) yielded pseudopterosin Y (**10**) as the sole product.

The high-resolution mass spectrum of pseudopterosin Z (**11**), $[\alpha]^{20}_D$ −142.6° (*c* 0.9, $CHCl_3$), suggested a molecular formula of $C_{29}H_{40}O_8$, indicating that **11** is isomeric with pseudopterosins W (**8**) and X (**9**). Comprehensive analysis of the ^{1}H and ^{13}C NMR spectra of pseudopterosin Z indicated that this natural product is a diacetylated diterpene glycoside related to known pseudopterosin F.[3] The sugar component of pseudopterosin Z (**11**) was shown to be identical with that of pseudopterosin W (**8**) by ^{1}H−^{1}H COSY and by ^{1}H and ^{13}C NMR chemical shift comparisons. Hydrolysis of pseudopterosin Z yielded an arabinose solution with a strong negative rotation, thus indicating the sugar to be of the D configuration. Acetylation of pseudopterosin Z yielded the triacetate **16**, whose spectral data were similar to but not identical with those of the triacetate **15** derived from pseudopterosin W (**8**).[10] A side-by-side comparison of the ^{1}H NMR spectra of pseudopterosins W and Z showed that the most striking differences between the two aglycons were the chemical shifts ascribable to the H-14 protons: in **11** H-14 resonated at *δ* 5.10, while in **8** H-14 appeared at *δ* 4.96. These observations strongly suggested that the 2-methyl-1-propenyl side chain in pseudopterosin Z (**11**) has the *β*-orientation.[11] At this point, while the spectral data were in full accord with the proposed structure for the molecule, an unambiguous proof of the aglycon structure was desirable. Methylation and hydrolysis of pseudopterosin Z yielded an *O*-methyl ether aglycon, which, from its spectral properties and optical rotation, was identical to the aglycon derivative (**18**) produced from pseudopterosin F.[3]

seco-Pseudopterosins H (**12**) and I (**13**) were found to have similar IR, MS, UV, and ^{1}H and ^{13}C NMR spectra, as well as comparable optical rotations, to those of previously reported *seco*-pseudopterosins A−D.[7] The high-resolution mass spectra of *seco*-pseudopterosins H and I suggested molecular formulas of $C_{27}H_{40}O_7$, thus indicating 8 degrees of unsaturation in these molecules. The ^{13}C NMR spectra displayed distinct resonances for all 27 carbon atoms, and since they contained eight aromatic and olefinic carbon resonances in addition to one carbonyl, the molecules were judged to be tricyclic. Interpretation of ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, and HMBC spectral data revealed that these metabolites have the serrulatane-based diterpene skeleton characteristic of the *seco*-pseudopterosins, with an acetylated α-arabinose attached at the C-7 hydroxyl. Cross-peaks in the HMBC spectra for the H_3-19 methyl protons with C-7, which in turn showed cross-peaks with H-1′, placed the sugar moieties at C-7. As in the *seco*-pseudopterosin B−D series, these related diterpene glycosides were easily recognized as monoacetate positional isomers by ^{1}H NMR. Thus, it was realized that **12** and **13** are new members of the *seco*-pseudopterosin class of marine natural products. The complete NMR chemical shift

Table 3. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for Pseudopterosins X−Z (**9**−**11**)and *seco*-Pseudopterosin H (**12**) in $CDCl_3$[a]

	pseudopterosin X (**9**)		pseudopterosin Y (**10**)		pseudopterosin Z (**11**)		*seco*-pseudopterosin H (**12**)	
atom	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)	δ_H, mult, *J* (Hz)	δ_C (mult)
1	3.65, dd, 9.0, 8.4	37.3 (d)	3.65, dd, 9.0, 8.2	37.3 (d)	3.58, br m	35.5 (d)	3.14, m	27.0 (d)
2α	1.21, m	40.1 (t)	1.23, m	40.2 (t)	1.65, m	39.4 (t)	1.48, m	27.8 (t)
2*β*	1.94, m		1.92, br dd, 10.6, 8.0				1.81, m	
3	1.22, m	33.9 (d)	1.20, m	33.9 (d)	1.62, m	29.9 (d)	1.66, m	18.4 (t)
4	2.05, m	44.7 (d)	2.03, m	44.7 (d)	2.04, m	43.2 (d)	2.64, m	39.5 (d)
5α	0.89, m	27.8 (t)	0.87, m	27.8 (t)	1.12, m	28.2 (t)	6.50, br s	121.2 (d)
5*β*	2.07, m		2.05, m		2.12, m			
6α	2.16, m	31.9 (t)	2.15, m	31.8 (t)	2.10, m	30.7 (t)		127.9 (s)
6*β*	1.32, m		1.30, m		1.46, m			
7	3.18, m	28.6 (d)	3.17, m	28.5 (d)	3.35, m	27.3 (d)		141.9 (s)
8		126.7 (s)		127.1 (s)		127.8 (s)		146.5 (s)
9		144.6 (s)		145.2 (s)		144.6 (s)		129.2 (s)
10		142.9 (s)		143.1 (s)		142.6 (s)		137.1 (s)
11		127.3 (s)		127.8 (s)		127.1 (s)	1.99, m	38.5 (d)
12		129.9 (s)		129.2 (s)		129.6 (s)	1.46, m	35.7 (t)
13		136.8 (s)		136.7 (s)		135.1 (s)	2.05, m	26.2 (t)
14	4.91, br d, 9.3	131.1 (d)	4.95, br d, 9.0	131.3 (d)	5.10, br d, 9.1	130.2 (d)	5.14, br t	124.9 (d)
15		128.4 (s)		128.2 (s)		128.8 (s)		131.2 (s)
16	1.65, d, 1.0	25.4 (q)	1.66, br s	25.5 (q)	1.66, br s	25.7 (q)	1.71, s	25.7 (q)
17	1.71, d, 0.9	17.5 (q)	1.71, br s	17.5 (q)	1.73, br s	17.6 (q)	1.62, s	17.7 (q)
18	1.02, d, 6.0	20.0 (q)	1.01, d, 5.9	20.1 (q)	1.03, d, 6.0	21.1 (q)	0.71, d, 6.9	16.4 (q)
19	1.24, d, 6.7	23.1 (q)	1.25, d, 6.7	23.3 (q)	1.23, d, 7.0	22.9 (q)	2.27, s	17.2 (q)
20	2.08, s	13.9 (q)	2.07, s	13.9 (q)	2.01, s	12.1 (q)	1.17, d, 6.9	20.9 (q)
1′	5.15, d, 3.4	101.5 (d)	5.15, d, 3.2	103.3 (d)	5.24, d, 3.6	102.9 (d)	5.16, d, 3.4	103.5 (d)
2′	5.32, dd, 10.0, 3.4	71.2 (d)	4.15, m	69.9 (d)	4.31, dd, 10.1, 3.7	68.0 (d)	4.10, dd, 9.9, 3.5	69.6 (d)
3′	4.38, dd, 10.2, 3.5	66.6 (d)	4.13, m	69.1 (d)	5.39, dd, 10.1, 3.3	70.4 (d)	4.32, br d, 9.7	68.2 (d)
4′	5.27, br m	71.6 (d)	4.11, m	69.6 (d)	5.44, br s	68.7 (d)	5.24, br s	71.5 (d)
5α′	4.36, dd, 14.3, 1.5	61.7 (t)	4.35, d, 12.6	63.9 (t)	4.47, d, 13.1	62.0 (t)	4.37, br d, 13.0	61.8 (t)
5*β*′	3.98, dd, 13.0, 2.6		3.87, br d, 12.0		3.85, dd, 13.1, 2.0		3.88, br d, 13.0	
−OAc	2.19, s	20.9 (q)			2.14, s	20.9 (q)	2.17, s	21.1 (q)
		170.5 (s)				170.2 (s)		171.5 (s)
−OAc	2.24, s	21.0 (q)			2.11, s	20.9 (q)		
		170.7 (s)				171.0 (s)		

[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.

assignments of **12** and **13** are shown in Table 3 and in the Experimental Section, respectively.

As the NOESY spectra for **12** and **13** contained very little useful information, the relative stereochemistry of the aglycon component of *seco*-pseudopterosins H and I was determined by NMR chemical shift comparisons and from chemical interconversion experiments. The ^{1}H NMR chemical shifts of H-4, H_3-18, and H_3-20 were essentially identical to those of *seco*-pseudopterosins A−D, as were the ^{13}C NMR chemical shifts of C-1, C-4, C-11, C-18, and C-20, suggesting that the aglycon portion of **12** and **13** might correspond stereochemically to that of *seco*-pseudopterosins A−D.[14] Furthermore, methylation of *seco*-pseudopterosin H (**12**) followed by acid hydrolysis yielded the *O*-methyl ether **19**, which from its spectral properties was evidently similar to the known *O*-methyl ether aglycon **20** derived from *seco*-pseudopterosin A.[7] Subsequent methylation of **19** generated the known *O*-dimethyl ether **21**.[7] On the basis of these chemical conversions, the aglycon component of *seco*-pseudopterosins H and I was concluded to be identical with that from *seco*-pseudopterosins A−D. The identities of the pentose portions in compounds **12** and **13** were determined to be 4′-*O*-acetyl-α-arabinose and 2′-*O*-acetyl-α-arabinose, respectively, by ^{1}H−^{1}H COSY and NOESY NMR experiments. Unfortunately, attempts to isolate the arabinose in **12** and **13** and determine its absolute configuration were unsuccessful, presumably due to decomposition of the sugar under the strong hydrolytic conditions needed for glycoside cleavage.

Biological Evaluation of the New Diterpene Glycosides. Activation of brain microglia and concomitant release of both O_2^- and TXB_2 have been reported in neurodegenerative disorders and neuroinflammation.[15] Table 4 shows the concentration-dependent effect of pseudopterosins P (**1**), Q (**2**), U (**6**), V (**7**), W (**8**), and X (**9**) and *seco*-pseudopterosins H (**12**) and I (**13**) on the release of O_2^-, TXB_2, and lactate dehydrogenase (LDH), a marker for cell toxicity. Pseudopterosins P, U, V, W, and X, as well as *seco*-pseudopterosins H and I, demonstrated minimal effects on both TXB_2 and O_2^- release, although they also appeared to be rather toxic (i.e., LDH_{50} 0.6−10 μM) to microglia cells in vitro. In contrast, although pseudopterosin Q (**2**) inhibited TXB_2 (IC_{50} = 4.7 μM), LDH release was observed to increase above 50% of that observed in Triton X-100 (0.1%)-treated microglia (maximal LDH release) at 3.4 μM, thus suggesting that the inhibition of TXB_2 by **2** could have resulted, at least in part, from a toxic rather than a pharmacological effect on the microglia cells under our experimental conditions. Taken together, our results suggest that pseudopterosin Q (**2**) could become an anti-inflammatory lead compound if its current cytotoxic effects could be reduced while maintaining or perhaps enhancing its pharmacological effect on both TXB_2 and O_2^- generation by activated brain microglia. This possibility remains to be determined by future studies. Considering the potential ability of these diterpene glycosides to inhibit inflammation, pseudopterosins P, Q, U, V, and W were also evaluated in a three-cell line panel consisting of MCF-7 (breast cancer), NCI-H460 (non-small-cell lung cancer), and SF-268 (CNS) cells. Results from the one dose primary anticancer assay showed pseudopterosins P (**1**), U (**6**), V (**7**), and W (**8**) to have significant cytotoxicity. In each case, the percent of growth of the treated cells when compared to the untreated control cells was 0%. In the NCI 60-cell

line screen pseudopterosin P (**1**) showed noticeable differential cytotoxicity, but it lacked potency (GI_{50}'s = 1.7−5.8 μM). In the same screen, pseudopterosins U and V were generally much less toxic (GI_{50}'s = 20−100 μM). Curiously, pseudopterosin Q (**2**) indicated a lack of significant cytotoxicity in the three-cell line panel, as the percent of growth of the treated cells when compared to the untreated control cells was approximately 103%, 101%, and 107%, respectively. Compounds **1**, **2**, **6**, **7**, **12**, and **13** were tested as well for their inhibitory activity toward the growth of *Mycobacterium tuberculosis* $H_{37}Rv$ at a concentration of 6.25 μg/mL. Pseudopterosin P (**1**) exhibited the strongest inhibitory activity (76%), whereas pseudopterosins Q (**2**), U (**6**), and V (**7**) marginally inhibited 37%, 43%, and 25% of mycobacterial growth, respectively. On the other hand, *seco*-pseudopterosins H (**12**) and I (**13**) induced, respectively, 25% and 37% of mycobacterial growth at the same concentration. Pseudopterosin P (**1**) was also subjected to antiviral testing against Herpes simplex viruses HSV-1 and HSV-2. Acyclovir (ACV) was used as a control. The tests showed pseudopterosin P to be very toxic against each virus (EC_{50} = 2.9 μM), with SI values of <2.4 (ACV EC_{50} = 0.95 μM). Compound **1** also showed strong antiviral activity against HCMV and VZV with EC_{50} values of 2.9 and 2.6 μM (SI values = <3.6 and <3.2, respectively). Finally, pseudopterosins P (**1**), Q (**2**), U (**6**), and V (**7**) were tested against the *Plasmodium falciparum* W2 (chloroquine-resistant) strain. Compounds **1**, **2**, and **6** were very active, with IC_{50} values of 12, 15, and 19 μg/mL, respectively, whereas **7** showed the most potent in vitro antimalarial activity, with an IC_{50} of 1 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Rudolph Autopol IV automatic polarimeter. IR and UV spectra were measured with a Nicolet Magna FT-IR 750 spectrophotometer and a Shimadzu UV−vis spectrophotometer (UV-2401PC), respectively. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500 and 125 MHz, respectively, with a Bruker Avance DRX-500 spectrometer with TMS as internal standard. Silica gel (35−75 mesh) and Bio-Beads SX-3 were used for column chromatography, and precoated silica gel GF_{254} plates were used for TLC and were revealed by heating the plates sprayed with 5% H_2SO_4 in EtOH. HPLC separations were carried out on a 10 mm × 25 cm Ultrasphere-Cyano Polar-Bonded column, 5 μm, eluted isocratically with hexane−2-propanol mixtures at 1.7 mL/min, with UV detection at 220 nm. All solvents used either were spectral grade or were distilled from glass prior to use.

Animal Material. Healthy specimens of the gorgonian coral *Pseudopterogorgia elisabethae* Bayer (order Gorgonacea, family Gorgoniidae, phylum Cnidaria) were collected by scuba at depths of 80−100 ft from the coral reefs off San Andrés Island (May 1996) and Old Providence Island (March 2002), Colombia, located off the Nicaraguan shelf in the Southwestern Caribbean Sea. Voucher specimens (No. PESAI-01 and PEPI-02-1, respectively) have been deposited at the Chemistry Department of the University of Puerto Rico, Río Piedras campus.

Extraction and Isolation Procedures. The extraction scheme used for *P. elisabethae* specimens from San Andrés Island has been previously described.[16] A large portion of the hexane extract (128 g) was chromatographed on a silica gel column (stepwise elution with 0−100% acetone in hexane, then 100% MeOH). Fractions were pooled on the basis of their TLC and NMR profiles to yield seven primary fractions, I−VII. The fraction obtained from 60% acetone in hexane [fraction IV (83.3 g)] was further purified by column chromatography over silica gel using a step gradient of ethyl acetate−hexane mixtures. Sixteen tertiary fractions were obtained (a−p). Fraction n (14.9 g) was dissolved in a small volume of toluene and purified by size-exclusion chromatography (Bio-Beads SX-3, toluene). The penultimate fraction was further purified by successive column chromatography over silica gel with 50% EtOAc in $CHCl_3$ and then 3% 2-propanol in hexane to afford *seco*-pseudopterosin H (**12**) (6.7 mg). *seco*-Pseudopterosin I (**13**) (10.5 mg) was obtained after repeated chromatography of the last fraction eluting from the size-exclusion column on a silica gel column run with 50% EtOAc in $CHCl_3$ and then 3% 2-propanol in hexane. *P. elisabethae* specimens from Old Providence Island were partially air-dried (1.8 kg), freeze-dried, and then kept frozen prior to homogenization in a mixture of 1:1 CH_2Cl_2−MeOH (5 × 4 L). After filtration and concentration the crude gummy extract (400 g) was suspended in water (1 L) and extracted successively with hexane (4 × 2 L), CH_2Cl_2 (4 × 2 L), and EtOAc (3 × 2 L). The hexane extract (323 g) was chromatographed on a large silica gel column by stepwise elution with 9:1 hexane−CH_2Cl_2, CH_2Cl_2, and MeOH. A total of 26 fractions, designated I−XXVI, were obtained. Pseudopterosin diterpene glycosides **1**−**11** were found among the more polar fractions (XXII−XXVI). Fractions XXII (12 g) and XXIII (8.4 g) were dissolved in toluene, filtered over sand and Celite, and chromatographed individually on a Bio-Beads SX-3 column eluted with toluene. After size-exclusion chromatography, a small portion (150 mg) of the mixture of glycosides (3.6 g) stemming from fraction XXII was further purified by HPLC (95:5 hexane−2-propanol) to afford pseudopterosins R (**3**) (5.2 mg), S (**4**) (2.0 mg), W (**8**) (81.0 mg), X (**9**) (16.3 mg), and Z (**11**) (5.5 mg). Further separation and purification of fraction XXIII using repeated flash silica gel columns [eluted with hexane−acetone (85:15)] led to a small subfraction (82 mg) that was subsequently purified by HPLC (95:5 hexane−2-propanol) to afford pure pseudopterosins T (**5**) (2.0 mg) and Y (**10**) (2.2 mg). Purification of a small portion (500 mg) of fraction XXV (60 g) by size-exclusion on a Bio-Beads SX-3 column eluted with toluene led to a mixture that was ultimately separated by HPLC (9:1 hexane−2-propanol), thus leading to pseudopterosins P (**1**) (17.3 mg), Q (**2**) (14.1 mg), U (**6**) (10.6 mg), and V (**7**) (9.4 mg).

Pseudopterosin P (1): ivory amorphous solid; $[\alpha]^{20}_D$ −107.2° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3341, 2969, 2926, 2858, 1725, 1449, 1372, 1238 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 35 000), 277 (ϵ 7000), 284 (ϵ 9000) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRFABMS *m/z* $[M]^+$ 488.2774 (calcd for $C_{28}H_{40}O_7$, 488.2774).

Pseudopterosin Q (2): ivory powder; $[\alpha]^{20}_D$ −43.6° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3462, 3312, 2963, 2929, 2862, 1726, 1463, 1454, 1376, 1260 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 31 500), 278 (ϵ 9800), 284 (ϵ 7500) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRFABMS *m/z* $[M]^+$ 488.2774 (calcd for $C_{28}H_{40}O_7$, 488.2774).

Pseudopterosin R (3): ivory amorphous solid; $[\alpha]^{20}_D$ −45.0° (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 3340, 2964, 2923, 2862, 1751, 1448, 1373, 1238 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 36 700), 277 (ϵ 8500), 284 (ϵ 11 000) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRESIMS *m/z* $[M + 1]^+$ 531.2946 (calcd for $C_{30}H_{43}O_8$, 531.2958).

Pseudopterosin S (4): ivory amorphous solid; $[\alpha]^{20}_D$ −11.2° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3375, 2948, 2924, 2865, 1742, 1451, 1371, 1234 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 29 300), 277 (ϵ 6500), 284 (ϵ 7600) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRESIMS *m/z* $[M + 1]^+$ 531.2945 (calcd for $C_{30}H_{43}O_8$, 531.2958).

Pseudopterosin T (5): ivory amorphous solid; $[\alpha]^{20}_D$ −31.8° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3389, 2958, 2929, 2858, 1448, 1378, 1255 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 32 800), 277 (ϵ 9400), 284 (ϵ 7400) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 2); HRESIMS *m/z* $[M + 1]^+$ 447.2748 (calcd for $C_{26}H_{39}O_6$, 447.2747).

Pseudopterosin U (6): light yellow oil; $[\alpha]^{20}_D$ −78.3° (*c* 1.2, $CHCl_3$); IR (film) ν_{max} 3337, 2923, 2962, 2856, 1745, 1449, 1375, 1242 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 37 000), 277 (ϵ 6800), 284 (ϵ 7500) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 2); HRFABMS *m/z* $[M - 1]^+$ 473.2339 (calcd for $C_{27}H_{37}O_7$, 473.2339).

Pseudopterosin V (7): white powder; $[\alpha]^{20}_D$ −32.9° (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 3363, 2964, 2929, 2863, 1746, 1445, 1377, 1247 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 22 300), 277 (ϵ 6500), 284 (ϵ 6300) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 2); HRFABMS *m/z* $[M - 1]^+$ 473.2339 (calcd for $C_{27}H_{37}O_7$, 473.2339).

Pseudopterosin W (8): white amorphous solid; $[\alpha]^{20}_D$ −54.8° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3351, 2948, 2924, 2857, 1747, 1448, 1372, 1237 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 35 000), 277 (ϵ 13 000), 284 (ϵ 9000) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 2); HRESIMS *m/z* $[M + 1]^+$ 517.2795 (calcd for $C_{29}H_{41}O_8$, 517.2801).

Pseudopterosin X (9): light yellow oil; $[\alpha]^{20}_D$ −39.0° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3477, 2958, 2928, 2858, 1743, 1450, 1372, 1233 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 33 800), 277 (ϵ 6800), 284 (ϵ 5100) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 3); HRFABMS *m/z* $[M + Na]^+$ 539.2614 (calcd for $C_{29}H_{40}O_8Na$, 539.2621).

Pseudopterosin Y (10): white powder; $[\alpha]^{20}_D$ −54.2° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3363, 2962, 2925, 2868, 1452, 1376, 1238, 1083 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 28 400), 278 (ϵ 7500), 284 (ϵ 6800) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 3); HRESIMS *m/z* $[M + 1]^+$ 433.2590 (calcd for $C_{25}H_{37}O_6$, 433.2590).

Pseudopterosin Z (11): white powder; $[\alpha]^{20}_D$ −142.6° (*c* 0.9, $CHCl_3$); IR (film) ν_{max} 3343, 2947, 2924, 2868, 1747, 1451, 1430, 1374, 1237 cm^{-1}; UV (MeOH) λ_{max} 228 (ϵ 21 700), 277 (ϵ 18 700), 284 (ϵ 4500) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 3); HRESIMS *m/z* $[M + 1]^+$ 517.2778 (calcd for $C_{29}H_{41}O_8$, 517.2801).

***seco*-Pseudopterosin H (12):** white amorphous powder; $[\alpha]^{25}_D$ −142.3° (*c* 0.8, $CHCl_3$); IR (film) ν_{max} 3339, 2954, 2928, 2871, 1743, 1451, 1376, 1228, 1084 cm^{-1}; UV (MeOH) λ_{max} 208 (ϵ 54 600), 228 (ϵ 17 000), 280 (ϵ 7900) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 3); HRFABMS *m/z* $[M]^+$ 476.2771 (calcd for $C_{27}H_{40}O_7$, 476.2774).

***seco*-Pseudopterosin I (13):** white amorphous powder; $[\alpha]^{25}_D$ −92.4° (*c* 2.1, $CHCl_3$); IR (film) ν_{max} 3472, 3363, 2957, 2927, 2851, 1746, 1498, 1448, 1376, 1232 cm^{-1}; UV (MeOH) λ_{max} 208 (ϵ 57 500), 228 (ϵ 17 300), 278 (ϵ 4200) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 7.06 (1H, br s, C-8 OH), 6.51 (1H, br s, H-5), 5.30 (1H, dd, *J* = 9.7, 3.4 Hz, H-2′), 5.15 (1H, d, *J* = 3.5 Hz, H-1′), 4.39, (1H, dd, *J* = 12.6, 1.6 Hz, H-5′ax), 4.27 (1H, br d, *J* = 9.5 Hz, H-3′), 4.18 (1H, br s, H-4′), 3.97 (1H, dd, *J* = 12.6, 2.5 Hz, H-5′eq), 3.14 (1H, m, H-1), 2.63 (1H, m, H-4), 2.29 (3H, s, Me-19), 2.25 (3H, s, COC*H*$_3$), 2.04 (2H, m, H-13), 1.99 (1H, m, H-11), 1.80 (1H, m, H-2β), 1.71 (3H, br s, Me-16), 1.62 (3H, br s, Me-17), 1.49−1.16 (3H, m, H-12, H-2α), 1.17 (3H, d, *J* = 6.9 Hz, Me-20), 0.71 (3H, d, *J* = 6.9 Hz, Me-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9 (s, *C*OCH$_3$), 146.0 (s, C-8), 141.6 (s, C-7), 137.1 (s, C-10), 131.2 (s, C-15), 128.9 (s, C-9), 127.3 (s, C-6), 124.8 (d, C-14), 121.6 (d, C-5), 101.5 (d, C-1′), 71.7 (d, C-2′), 68.9 (d, C-4′), 68.1 (d, C-3′), 63.5 (t, C-5′), 39.5 (d, C-4), 38.4 (d, C-11), 35.7 (t, C-12), 28.0 (t, C-2), 27.7 (d, C-1), 26.2 (t, C-13), 25.8 (q, C-16), 21.0 (q, C-20), 20.9 (q, CO*C*H$_3$), 18.5 (t, C-3), 17.7 (q, C-17), 17.2 (q, C-19), 16.4 (q, C-18); HRFABMS *m/z* $[M]^+$ 476.2797 (calcd for $C_{27}H_{40}O_7$, 476.2774).

Peracetylation of Pseudopterosins P−T (1−5). Individual samples of approximately 3−4 mg of each pseudopterosin were dissolved in a 1:1 mixture of acetic anhydride−pyridine (1 mL) and stirred at 20 °C overnight. Evaporation in vacuo of the volatile materials followed by silica gel column chromatography of the oily residue isolated afforded each time the same triacetate **14** (average yield = 69%): colorless viscous oil; $[\alpha]^{20}_D$ −45.7° (*c* 0.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.85 (1H, s, C-9 OH), 5.59 (1H, dd, *J* = 11.0, 3.1 Hz, H-3′), 5.43 (2H, m, H-2′, H-4′), 5.18 (1H, d, *J* = 3.5 Hz, H-1′), 4.92 (1H, d, *J* = 9.2 Hz, H-14), 4.69 (1H, q, *J* = 6.6 Hz, H-5′), 3.66 (1H, dd, *J* = 9.2, 8.8 Hz, H-1), 3.18 (1H, m, H-7), 2.20 (3H, s), 2.16 (3H, s), 2.09 (3H, s, Me-20), 2.02 (3H, s), 1.92 (1H, m, H-2β), 1.71 (3H, br s, Me-17), 1.66 (3H, br s, Me-16), 1.32−1.23 (2H, m, H-6β, H-3), 1.24 (3H, d, *J* = 6.5 Hz, Me-6′), 1.23 (3H, d, *J* = 6.8 Hz, Me-19), 1.02 (3H, d, *J* = 6.0 Hz, Me-18), 0.90 (1H, m, H-5α); LRESIMS *m/z* $(M + H)^+$ 573.

Peracetylation of Pseudopterosins U−Y (6−10). Individual samples of approximately 2−3 mg of each pseudopterosin were dissolved in a 1:1 mixture of acetic anhydride−pyridine (1 mL) and stirred at 20 °C overnight. Evaporation in vacuo of the volatile materials followed by silica gel column chromatography of the oily residue isolated afforded each time the same triacetate **15** (average yield = 59%): colorless viscous oil; $[\alpha]^{20}_D$ −107.3° (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.78 (1H, s, C-9 OH), 5.59 (1H, dd, *J* = 10.5, 3.4 Hz, H-3′), 5.47 (1H, dd, *J* = 10.7, 3.2 Hz, H-2′), 5.46 (1H, br s, H-4′), 5.19 (1H, d, *J* = 3.3 Hz, H-1′), 4.91 (1H, d, *J* = 9.5 Hz, H-14), 4.45 (1H, d, *J* = 12.5 Hz, H-5′ax), 3.90 (1H, dd, *J* = 13.0, 2.4 Hz, H-5′eq), 3.65 (1H, dd, *J* = 9.6, 8.1 Hz, H-1), 3.18 (1H, m, H-7), 2.17 (3H, s), 2.16 (3H, s), 2.09 (3H, s, Me-20), 2.05 (3H, s), 1.93 (1H, m, H-2β), 1.71 (3H, br s, Me-17), 1.65 (3H, br s, Me-16), 1.32−1.23 (2H, m, H-6β, H-3), 1.23 (3H, d, *J* = 6.7 Hz, Me-19), 1.02 (3H, d, *J* = 6.0 Hz, Me-18), 0.91 (1H, m, H-5α); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (s, −*C*OCH$_3$), 170.0 (s, −*C*OCH$_3$), 169.5 (s, −*C*OCH$_3$), 144.5 (s, C-9), 142.9 (s, C-10), 136.8 (s, C-13), 131.0 (d, C-14), 129.9 (s, C-12), 128.4 (s, C-15), 127.3 (s, C-11), 126.6 (s, C-8), 101.5 (d, C-1′), 68.6 (d), 68.0 (d), 67.0 (d), 61.9 (t, C-5′), 44.7 (d, C-4), 40.1 (t, C-2), 37.3 (d, C-1), 33.8 (d, C-3), 31.9 (t, C-6), 28.5 (d, C-7), 27.8 (t, C-5), 25.4 (q, C-16), 23.1 (q, C-19), 20.9 (q, CO*C*H$_3$), 20.7 (q, CO*C*H$_3$), 20.6 (q, CO*C*H$_3$), 20.0 (q, C-18), 17.5 (q, C-17), 14.0 (q, C-20); LRESIMS *m/z* $(M + H)^+$ 559.

Peracetylation of Pseudopterosin Z (11). Pseudopterosin Z (3 mg, 0.006 mmol) was dissolved in a 1:1 mixture of acetic anhydride−pyridine (1 mL) and stirred at 20 °C overnight. Evaporation in vacuo of the volatile materials followed by silica gel column chromatography of the oily residue isolated afforded triacetate **16** (2.5 mg, 66%): colorless viscous oil; $[\alpha]^{20}_D$ −193.5° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.50 (1H, s, C-9 OH), 5.58 (1H, dd, *J* = 10.3, 3.2 Hz, H-3′), 5.42 (1H, br s, H-4′), 5.40 (1H, dd, *J* = 10.2, 3.7 Hz, H-2′), 5.23 (1H, d, *J* = 3.6 Hz, H-1′), 5.10 (1H, d, *J* = 8.8 Hz, H-14), 4.47 (1H, d, *J* = 13.0 Hz, H-5′ax), 3.75 (1H, dd, *J* = 13.1, 2.5 Hz, H-5′eq), 3.57 (1H, br m, H-1), 3.34 (1H, m, H-7), 2.17 (3H, s), 2.14 (3H, s), 2.09 (3H, s, Me-20), 2.09 (3H, s), 2.02 (1H, m, H-4), 1.72 (3H, br s, Me-17), 1.66 (3H, br s, Me-16), 1.64−1.50 (2H, m, H-2α, H-3), 1.45 (1H, m, H-6β), 1.22 (3H, d, *J* = 6.9 Hz, Me-19), 1.14 (1H, m, H-5α), 1.03 (3H, d, *J* = 6.0 Hz, Me-18); LRESIMS *m/z* $(M + H)^+$ 559.

Basic Hydrolysis of Pseudopterosins P−S (1−4). Individual samples of approximately 5−6 mg of each pseudopterosin were treated with 4 mL of 5% KOH/MeOH, and the resulting suspension was stirred overnight at 20 °C. The solution was diluted with H_2O (5 mL), acidified to neutrality with 0.5 N HCl, and extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield each time pseudopterosin T (**5**). The ^1H and ^{13}C NMR data, TLC retention time, and optical rotation were identical with those of the natural product.

Basic Hydrolysis of Pseudopterosins U−X (6−9). An artificially prepared mixture of pseudopterosins U−X (∼8 mg) was treated with 4 mL of 5% KOH−MeOH, and after the suspension was stirred overnight at 20 °C the solution was poured onto H_2O (8 mL), acidified to neutrality, and extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield pseudopterosin Y (**10**) as the sole product. The ^1H and ^{13}C NMR data, TLC retention time, and optical rotation were identical with those of the natural product.

Aglycon Derivative from Pseudopterosin P (1). A stirred mixture of pseudopterosin P (100 mg, 0.205 mmol), benzyl bromide (100 μL, 0.72 mmol), and anhydrous K_2CO_3 (99 mg, 0.72 mmol) in dry acetone (15 mL) was refluxed under N_2 for 5 h and then allowed to cool to room temperature. After solvent removal, the residue was taken up with water (30 mL) and the aqueous layer was repeatedly extracted with $CHCl_3$ (3 × 10 mL). The combined organic layer was dried ($MgSO_4$), filtered, and concentrated to afford a yellowish oily residue. Without further purification, this residue was dissolved in MeOH (15 mL) and treated with 1 N HCl (10 mL), and the mixture was carefully warmed to 55 °C for 12 h. After allowing

the solution to cool to room temperature, it was diluted with H_2O (10 mL) and extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were then washed with 5% $NaHCO_3$ (1 × 15 mL), dried ($MgSO_4$), filtered, and concentrated to afford an oily residue. The sole $CHCl_3$-soluble product from the reaction was identified as the known *O*-benzyl ether aglycon **17**, 72 mg (90% from **1** after silica gel column chromatography). The ^1H and ^{13}C NMR data, TLC retention time, and optical rotation were identical with values reported elsewhere.[11]

Aglycon Derivative from Pseudopterosin U (6). Compound **6** (10 mg, 0.021 mmol) was *O*-benzylated and then treated with 1 N HCl using the same procedure outlined above for pseudopterosin P. The known *O*-benzyl ether **17**, produced in quantitative yield [8.1 mg (98% from **6**)], was the sole $CHCl_3$-soluble product from this reaction.

Aglycon Derivative from Pseudopterosin Z (11). A stirred mixture of pseudopterosin Z (10 mg, 0.020 mmol), excess CH_3I, and anhydrous K_2CO_3 in dry acetone (20 mL) was refluxed for 5 h and then allowed to cool to room temperature. After solvent removal, the residue was taken up with water (20 mL) and the aqueous layer was repeatedly extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to afford a yellowish oily residue. The sole $CHCl_3$-soluble product from this reaction was identified as the known *O*-methyl ether aglycon **18**, 5.9 mg (97% from **11** after silica gel column chromatography). The spectral and optical rotation data were identical with those reported for the same aglycon derivative (**18**) produced from pseudopterosin F.[3]

Aglycon Derivative from *seco*-Pseudopterosin H (12). A stirred mixture of *seco*-pseudopterosin H (5 mg, 0.010 mmol), excess CH_3I, and anhydrous K_2CO_3 in dry acetone (10 mL) was refluxed for 5 h and then allowed to cool to room temperature. After solvent removal, the residue was taken up with water (10 mL) and the aqueous layer was repeatedly extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to yield *seco*-pseudopterosin H *O*-methyl ether (6 mg). Without further purification, the *O*-methyl ether derivative was dissolved in MeOH (1 mL) and treated with 1 N HCl (0.25 mL), and the mixture was carefully warmed to 48−50 °C for 3 h. After allowing the solution to cool to room temperature, it was diluted with H_2O (5 mL) and extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were then washed with 5% $NaHCO_3$ (1 × 10 mL), dried ($MgSO_4$), filtered, and concentrated to afford an oily residue, identified as the *O*-methyl ether aglycon **19**, 3.2 mg (97% from **12** after silica gel column chromatography). *O*-Methyl ether aglycon **19**: light yellowish film; $[\alpha]^{20}_D$ −4.7° (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.53 (1H, s, H-5), 5.72 (1H, s, C-7 OH), 5.15 (1H, tdd, J = 7.1, 2.5, 1.3 Hz, H-14), 3.78 (3H, s, C-8 OCH_3), 3.14 (1H, m, H-1), 2.63 (1H, m, H-4), 2.26 (3H, s, Me-19), 2.09−1.75 (5H, m, H-13αβ, H-11, H-2β, H-3α), 1.71 (3H, br s, Me-16), 1.67 (1H, br m, H-3β), 1.63 (3H, br s, Me-17), 1.50−1.45 (3H, m, H-12αβ, H-2α), 1.21 (3H, d, J = 7.0 Hz, Me-20), 0.73 (3H, d, J = 7.0 Hz, Me-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.8 (s, C-7), 142.6 (s, C-8), 136.2 (s, C-10), 131.2 (s, C-15), 127.9 (s, C-9), 126.5 (s, C-6), 124.9 (d, C-14), 121.7 (d, C-5), 60.6 (q, C-8 OCH_3), 39.7 (d, C-4), 38.5 (d, C-11), 35.6 (t, C-12), 27.9 (t, C-2), 27.1 (d, C-1), 26.2 (t, C-13), 25.8 (q, C-16), 20.9 (q, C-20), 18.6 (t, C-3), 17.7 (q, C-17), 16.4 (q, C-18), 15.9 (q, C-19); EIMS *m*/*z* 316 $[M]^+$ (23), 232 (8), 206 (18), 205 (100), 173 (16), 145 (10); HREIMS *m*/*z* $[M]^+$ 316.2396 (calcd for $C_{21}H_{32}O_2$, 316.2402).

Methylation of *O*-Methyl Ether Aglycon 19. A stirred mixture of *O*-methyl ether **19** (~3 mg, 0.009 mmol), excess CH_3I, and anhydrous K_2CO_3 in dry acetone (2 mL) was refluxed under N_2 for 12 h and then allowed to cool to room temperature. After solvent removal, the residue was taken up with water (5 mL) and the aqueous layer was repeatedly extracted with $CHCl_3$ (3 × 5 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to yield the known dimethyl ether **21** (3 mg, 96%): yellowish film; $[\alpha]^{20}_D$ −21.7° (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.72 (1H, s, H-5), 5.15 (1H, tdd, 7.1, 1.3, 1.2 Hz, H-14), 3.87 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.14 (1H, m, H-1), 2.65 (1H, m, H-4), 2.22 (3H, s, Me-19), 2.09−1.77 (5H, m, H-13αβ, H-11, H-2β, H-3α), 1.71 (3H, br s, Me-16), 1.63 (3H, br s, Me-17), 1.50−1.40 (3H, m, H12αβ, H-2α), 1.16 (3H, d, J = 6.9 Hz, Me-20), 0.71 (3H, d, J = 6.9 Hz, Me-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.4 (s), 148.8 (s), 135.6 (s), 134.9 (s), 131.2 (s), 128.6 (s), 125.4 (d), 124.9 (d), 60.4 (q), 59.8 (q), 39.3 (d), 38.8 (d), 35.7 (t), 28.0 (t), 27.2 (d), 26.3 (t), 25.8 (q), 22.3 (q), 18.3 (t), 17.7 (q), 16.4 (q), 15.9 (q); EIMS *m*/*z* 330 $[M]^+$ (15), 246 (11), 220 (15), 219 (100), 204 (7), 189 (8), 188 (13). The ^1H NMR spectrum of **21** was almost identical with values reported in the literature.[7]

Table 4. Anti-inflammatory Activity of Diterpene Glycosides **1**, **2**, **6**−**9**, **12**, and **13**[a]

compound	O_2^- IC_{50}, μM	TXB_2 IC_{50}, μM	LDH_{50},[b] μM
Ps P (**1**)	>10	>10	0.6
Ps Q (**2**)	11.2	4.7	3.4
Ps U (**6**)	14.7	>10	0.7
Ps V (**7**)	>10	>10	2.2
Ps W (**8**)	>10	>10	10
Ps X (**9**)	10	>10	2.35
seco-Ps H (**12**)	>10	>10	3.6
seco-Ps I (**13**)	>10	>10	10

[a] Effect on rat microglia PMA [1 μM] − stimulated release of O_2^-, TXB_2, and LDH. Data shown correspond to two independent experiments. The experiment protocol used is described in the Experimental Section. [b] LDH_{50} represents the concentration of the compound that caused release of 50% of the total LDH from Triton X-100(0.1%)-treated microglia cells. LDH was measured as described in the Experimental Section.

Acid Hydrolysis of Pseudopterosins P (1), U (6), and Z (11). The L- configuration of the hexose portion of pseudopterosins P−T (**1**−**5**) and the D-configuration of the pentose portion of pseudopterosins U−Z (**6**−**11**) were established by comparing the optical rotation of the sugar solution that was obtained by hydrolysis of three representative glycosides according to an established protocol.[2] Thus, compounds **1**, **6**, and **11** (~3 mg, each) were individually dissolved in a 1:1 mixture of MeOH and 1 N HCl (10 mL), and the solution was carefully warmed to 48−50 °C for 4−5 h until all the starting material had disappeared (established by TLC analysis). The MeOH was removed under vacuo, and the aqueous layer remaining was extracted with CH_2Cl_2 (3 × 10 mL) to remove the aglycon portion and any traces of unreacted starting glycoside. The strong negative optical rotations of the aqueous hydrolysates indicated the presence of L-fucose in pseudopterosin P (**1**) and D-arabinose in pseudopterosins U (**6**) and Z (**11**), respectively. Unfortunately, several attempts to isolate arabinose and to determine its optical rotation from *seco*-pseudopterosin H (**12**) using minor variations of this protocol failed.

Anti-inflammatory Assay. Rat neonatal microglia (2 × 10^5 cells) were seeded into each well of 24-well flat-bottom culture clusters and stimulated with bacterial lipopolysaccharide (LPS) (0.3 ng/mL) in Dulbecco's modified Eagle medium + 10% fetal bovine serum + penicillin + streptomycin for 17 h in a humidified 5% CO_2 incubator at 35.9 °C.[15] Media were then removed, and microglia were washed with warm (37 °C) Hanks' balanced salt solution (HBSS) and then incubated with compounds **1**, **2**, **6**, **7**, **8**, **9**, **12**, and **13** (0.01−10 μM) or vehicle (DMSO) for 15 min prior to stimulation with phorbol 12-myristate 13-acetate (PMA) (1 μM). All experimental treatments were run in triplicate and in a final volume of 1 mL. Seventy minutes after PMA stimulation, HBSS was aspirated from each well and O_2^-, TXB_2, and LDH release were determined as described elsewhere.[15] Table 4 shows the data for each compound from two representative experiments, expressed as the compound's 50% inhibitory concentration (IC_{50}) for either O_2^- or TXB_2. Lactate dehydrogenase (LDH) release from microglia was determined spectrophotometrically, as described previously.[15] Microglia LDH release was expressed as percent of LDH release from Triton X-100 (0.1%)-treated (and 100% lysed) microglia cells.

Antimalaria Inhibition Bioassay. Compounds **1**, **2**, **6**, and **7** were tested for antimalarial activity against a chloro-

quine-resistant *Plasmodium falciparum* strain using a novel fluorometric method, based on the intercalation of the fluorochrome PicoGreen in the parasite DNA, as described by Corbett et al.[17]

Acknowledgment. Fieldwork for this project was supported, in part, by funding from the NIH-SCORE Program (Grant S06GM08102) of the University of Puerto Rico. Mrs. M. Cano Correa (Parque Nacional Natural Old Providence McBean Lagoon) and the staff of the Ministerio del Medio Ambiente (Bogotá, Colombia) provided logistic support. The National Cancer Institute (NCI), the National Institute of Allergy and Infectious Diseases (NIAID), and the Tuberculosis/Antimicrobial Acquisition & Coordinating Facility (TAACF) provided in vitro cytotoxicity, antiviral, and antituberculosis activity data, respectively. High-resolution and low-resolution mass spectral determinations were provided by the Mass Spectroscopy Laboratory of the University of Illinois at Urbana−Champaign. I.I.R. and O.J.G. thank the UPR-GAANN Program and the UPR-Pfizer Fellowship Program, respectively, for financial support. This research was made possible, in part, by NIH grant 1 R15 ES12654-01 (to A.M.S.M.) from the National Institute of Environmental Health Sciences. Additional support from Midwestern University and the excellent technical assistance of Ms. M. Hall are gratefully acknowledged. This work was partially supported by the International Cooperative Biodiversity Groups Program, award 1U01 TW01021-01 (to E.O.B. and J.G.).

Supporting Information Available: ^{1}H and ^{13}C NMR spectra ($CDCl_3$) of pseudopterosins P (**1**), U (**6**), and Z (**11**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Fenical, W. *J. Nat. Prod*. **1987**, *50*, 1001−1008. (b) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618.
(2) Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem*. **1986**, *51*, 5140−5145.
(3) Roussis, V.; Wu, Z.; Fenical, W.; Strobel, S. A.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem*. **1990**, *55*, 4916−4922.
(4) Look, S. A.; Fenical, W.; Jacobs, R. S.; Clardy, J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6238−6240.
(5) Ettuati, W. S.; Jacobs, R. S. *Mol. Pharmacol*. **1987**, *31*, 500−505.
(6) Although the molecular mechanism of action is not known yet, there exists evidence in culture cells to suggest that pseudopterosin A stabilizes nuclear lamina in dividing sea urchin embryos; see refs 5 and 8.
(7) Look, S. A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363−3370.
(8) Ata, A.; Kerr, R. G.; Moya, C. E.; Jacobs, R. S. *Tetrahedron* **2003**, *59*, 4215−4222.
(9) Burch, M. T. A Chemosystematic Study of the Caribbean Gorgonian Genus *Pseudopterogorgia* (Octocorallia, Cnidaria). M.S. Thesis, Scripps Institute of Oceanography, San Diego, CA, 1986.
(10) Curiously, the peracetylation of the new pseudopterosins under standard conditions afforded the corresponding triacetate derivatives, leaving the phenolic hydroxyl group unacetylated. Even after prolonged mild heating, the triacetates, not the tetraacetates, were always obtained.
(11) Lazerwith, S. E.; Johnson, T. W.; Corey, E. J. *Org. Lett*. **2000**, *2*, 2389−2392.
(12) As expected, the ^{1}H and ^{13}C NMR chemical shifts ascribable to the aglycon component of pseudopterosins P−Y (**1**−**10**) were essentially identical to those of the revised pseudopterosin G−J aglycon, suggesting that the aglycon of compounds **1**−**10** corresponds stereochemically to that of pseudopterosins G−J. Interestingly, the same aglycon has been proposed for the recently discovered pseudopterosins M−O (see ref 8), but the corresponding NMR chemical shifts (in $CDCl_3$) do not compare so favorably, suggesting that it is possible that the structures for pseudopterosins M−O may require revision.
(13) Walker, T. E.; Londoin, R. E.; Whaley, T. W.; Barker, R.; Matwiyoff, N. A. *J. Am. Chem. Soc.* **1976**, *98*, 5807−5813.
(14) The ^{1}H and ^{13}C NMR chemical shifts assigned to the aglycon component of the recently described *seco*-pseudopterosin E−G (see ref 8) do not correspond well with the NMR values reported for the aglycon portion of *seco*-pseudopterosins A−D nor the new glycosides **12** and **13**. Therefore, the proposed structures for *seco*-pseudopterosins E−G may also require revision.
(15) Mayer, A. M. S.; Oh, S.; Ramsey, K. H.; Jacobson, P. B.; Glaser, K. B.; Romanic, A. M. *Shock* **1999**, *11*, 180−186.
(16) Rodríguez, A. D.; González, E.; Huang, S. D. *J. Org. Chem*. **1998**, *63*, 7083−7091.
(17) Corbett, Y.; Herrera, L.; González, J.; Cubilla, L.; Capson, T.; Colley, P. D.; Kursar, T. A.; Romero, L. I.; Ortega-Barria, E. *J. Trop. Med. Hyg*. **2004**, *70*, 119−124.

NP049802O