The Pseudopterosins: A New Class of Antiinflammatory and Analgesic Diterpene Pentosides from the Marine Sea Whip *Pseudopterogorgia elisabethae* (Octocorallia)

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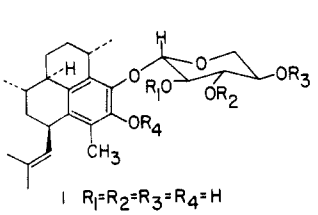
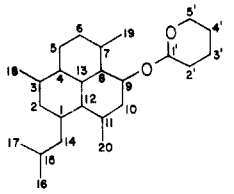
Four new metabolites, the pseudopterosins A–D (**1–4**), are described as natural products from the Caribbean sea whip, *Pseudopterogorgia elisabethae*. The structure of the only crystalline metabolite, pseudopterosin C (**3**), was determined by X-ray crystallography. Isolation and identification of the pentose portion of **3** and D-xylose allowed the assignment of the absolute configuration of all centers in **3**. The structures of pseudopterosins A, B, and D (**1**, **2**, and **4**) were subsequently defined by spectral analyses and by conversion to the peracetylated derivative. The pseudopterosins show antiinflammatory and analgesic activities equivalent in potency to the industrial standard indomethacin.

Sea whips of the family Gorgoniidae (Octocorallia, Gorgonacea, Cnidaria) are among the most important octocorals found in the reef and shallow-water habitats of the West Indian region.[1] Members of the genus *Pseudopterogorgia* are particularly abundant in the Caribbean Sea, and prior chemical studies have shown that this genus is a rich source of terpenoids[2-4] and secosterols.[5,6] As part of our continuing interest in the chemical adaptations and biomedical potential of these abundant marine invertebrates, we have recently focused our attention on the representatives of this genus that are found in deeper water and are less conspicuous. In this paper we report the structures of four new tricyclic diterpene pentosides, pseudopterosins A–D (**1–4**), which have been isolated from





1 $R_1=R_2=R_3=R_4=H$
2 $R_1=Ac$, $R_2=R_3=R_4=H$
3 $R_2=Ac$, $R_1=R_3=R_4=H$
4 $R_3=Ac$, $R_1=R_2=R_4=H$
5 $R_1=R_2=R_3=R_4=Ac$
9 $R_4=CH_3$, $R_1=R_2=R_3=H$

Pseudopterogorgia elisabethae.[7] The pseudopterosins represent a new structural class of antiinflammatory and analgesic metabolites. The pseudopterosins show potencies equivalent to, if not greater than, that of the antiinflammatory drug indomethacin and appear to possess unique, but not yet fully defined, pharmacological mechanisms of action. As analgesic agents, the pseudopterosins show potencies equal to or greater than indomethacin.[8]

P. elisabethae was collected near Crooked Island in the Bahama Islands between −15- and −35-m depth in 1982. Freshly collected animals were stored frozen and subsequently extracted, first with chloroform and then with EtOAc. Pseudopterosins A–D (**1–4**) were isolated by rapid-elution chromatography of the crude extract using TLC-grade Florisil and were finally purified by high-performance liquid chromatography (HPLC). Pseudopterosin C (**3**) was the major component, comprising 7.5% of the lipid extract, while pseudopterosins A, B, and D each represented less than 1% of the organic extract.

The major metabolite, pseudopterosin C (**3**), was crystallized from mixtures of ethyl acetate and absolute ethanol after replicate chromatography by HPLC. Data from high-resolution mass and ^{13}C NMR spectrometry (Table I) established a molecular formula of $C_{27}H_{38}O_7$ for this metabolite and suggested that the molecule was highly unsaturated. The ^1H NMR spectrum (Table I) contained one methyl signal at δ 2.21 (3 H, s) which, together with an infrared absorption at 1725 cm^{-1}, suggested that **3** was a monoacetate. The ^1H NMR spectrum of compound **3** contained five other methyl resonances [δ 2.03 (3 H, s), 1.75 (3 H, s), 1.68 (3 H, s), 1.17 (3 H, d, J = 7.1 Hz), 1.03 (3 H, d, J = 5.9 Hz)] in addition to the acetate methyl. This "methyl count" was consistent with a diterpenoid carbon skeleton. This observation, however, did not correlate with the molecular formula, indicating a larger molecule.

Six ^{13}C NMR resonances at δ 144.6 (s), 140.6 (s), 135.7 (s), 133.9 (s), 128.8 (s), and 121.3 (s), together with a broadened infrared absorption at 3350–3450 cm^{-1} and UV absorptions at 229 nm (ϵ 9600), 275 (ϵ 1500), and 282 (ϵ 1700) that displayed a bathochromic displacement upon addition of base (1 drop, 5% KOH/MeOH), suggested that **3** was a fully substituted phenol.[9] A D_2O-exchangeable proton observed at δ 7.51 (1 H, br s) in the ^1H NMR spectrum of pseudopterosin C was assigned to the phenolic hydroxyl group. One substituent on the phenol was proposed to be an aromatic methyl on the basis of a deshielded three-proton singlet resonance at δ 2.03.

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(7) Gorgonians were identified by Dr. Fredrick M. Bayer, Curator, Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC.
(8) A preliminary account of the discovery of the pseudopterosins has already appeared. See: Look, S. A.; Fenical, W.; Jacobs, R. S.; Clardy, J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6238. We thank Professor Robert S. Jacobs (and his students) for data defining the pharmacological activities of the pseudopterosins.
(9) For a discussion of the ultraviolet absorption of phenols and numerous examples, see: Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon: New York, 1964; pp 91–95.

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Table I. ^{1}H NMR and ^{13}C Assignments for Pseudopterosins A–D (1–4)[a,b]

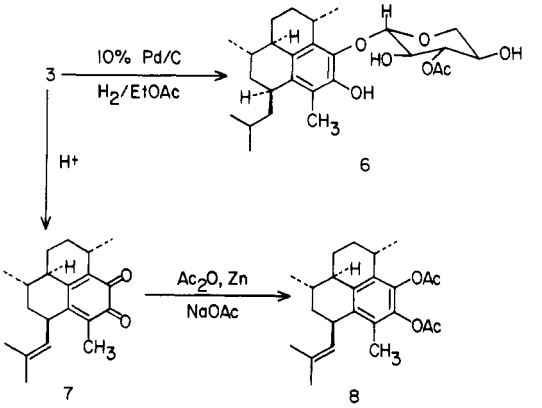
	pseudopterosin A (1)		pseudopterosin B (2)		pseudopterosin C (3)		pseudopterosin D (4)	
C no.	^{1}H	^{19}C	^{1}H	^{13}C	^{1}H	^{1}^{3}C	^{1}H	^{13}C
1	3.56 (1 H, m)	26.8	3.61 (1 H, m)	26.2	3.65 (1 H, m)	26.9	3.62 (1 H, m)	26.9
2	*c*	39.4	*c*	39.2	1.94 (1 H, m) 2.08 (1 H, m)	39.4	2.17 (2 H, m)	39.4
3	1.47 (1 H, m)	30.5	1.61 (1 H, m)	31.4	1.50 (1 H, m)	30.6	1.53 (1 H, m)	30.6
4	3.59 (1 H, br s)	35.6[e]	3.59 (1 H, br s)	35.5[e]	3.80 (1 H, br s)	35.6[e]	3.63 (1 H, br s)	35.6[e]
5	*c*	27.5[f]	*c*	26.4[f]	1.65 (2 H, m)	27.4[f]	*c*	27.5[f]
6	*c*	29.3[f]	1.62 (1 H, m) 1.73 (1 H, m)	27.9[f]	1.66 (2 H, m)	29.2[f]	1.61 (2 H, m)	29.3[f]
7	3.53 (1 H, m)	41.7[e]	3.48 (1 H, m)	39.6[e]	3.63 (1 H, m)	41.6[e]	3.61 (1 H, m)	41.7[e]
8		133.9[g]		133.9[g]		133.9[g]		133.9[g]
9		140.8		139.2		140.6		140.6
10		144.4		145.0		144.6		144.7
11		121.2		121.7		121.3		121.3
12		135.4[g]		136.3[g]		135.7[g]		135.6[g]
13		128.8[h]		128.4[h]		128.8[h]		128.7[h]
14	5.10 (1 H, d, J = 8.2)	129.8	5.08 (1 H, d, J = 9.0)	129.3	5.13 (1 H, d, J = 9.2)	129.5	5.13 (1 H, d, J = 8.5)	129.6
15		129.6[h]		130.1[h]		129.9[h]		129.9[h]
16	1.73 (3 H, s)[e]	25.6	1.74 (3 H, s)[e]	25.7	1.75 (3 H, s)[e]	25.7	1.75 (3 H, s)[e]	25.7
17	1.64 (3 H, s)[e]	10.9	1.67 (3 H, s)[e]	10.9	1.68 (3 H, s)[e]	10.8	1.67 (3 H, s)[e]	10.8
18	1.02 (3 H, d, J = 5.7)	23.0	1.04 (3 H, d, J = 5.4)	21.6	1.03 (3 H, d, J = 5.9)	22.8	1.03 (3 H, d, J = 6.0)	22.9
19	1.12 (3 H, d, J = 7.4)	17.6	1.22 (3 H, d, J = 7.2)	17.6	1.17 (3 H, d, J = 7.1)	17.7	1.17 (3 H, d, J = 7.1)	17.7
20	1.99 (3 H, s)	20.9	2.04 (3 H, s)	20.9	2.03 (3 H, s)	20.9	2.03 (3 H, s)	20.9
1′	4.49 (1 H, d, J = 7.2)	106.0	4.74 (1 H, d, J = 7.7)	103.9	4.59 (1 H, d, J = 7.5)	106.0	4.56 (1 H, d, J = 7.6)	105.6
2′	3.73 (1 H, m)[d]	75.9	5.09 (1 H, dd, J = 7.6, 9.0)	74.0	3.87 (1 H, dd, J = 7.5, 9.0)	72.5[i]	4.87 (1 H, m)	
3′	3.76 (1 H, m)[d]	74.0	3.59 (1 H, dd, J = 9.0, 9.2)	69.8	4.78 (1 H, dd, J = 8.9, 9.0)	79.2	3.81 (1 H, dd, J = 8.9, 9.2)	74.3
4′	3.75 (1 H, m)[d]	69.5	3.86 (1 H, m)	75.6	3.91 (1 H, m)	68.8[i]	3.70 (1 H, dd, J = 9.2, 7.7)	
5′	3.14 (1 H, dd, J = 10.5, 10.2)		4.10 (1 H, dd, J = 5.3, 11.7)		3.24 (1 H, dd, J = 10.4, 11.7)		3.20 (1 H, dd, J = 10.2, 11.4)	
		65.9		65.8		65.8		63.1
	3.95 (1 H, dd, J = 5.4, 10.4)		3.52 (1 H, dd, J = 10.6, 11.5)		4.06 (1 H, dd, J = 5.5, 11.8)		4.09 (1 H, dd, J = 5.4, 11.6)	
				171.0[j]		173.4[j]		170.9[j]
			2.23 (3 H, s)[j]	20.9[j]	2.21 (3 H, s)[j]	21.0[j]	2.10 (3 H, s)[j]	20.9[j]
			6.92 (1 H, exchange with D_2O)		2.62 (1 H, exchange with D_2O) 7.51 (1 H, exchange with D_2O)			

[a] The ^{1}H NMR spectrum was recorded at 360 MHz in $CDCl_3$. Assignments were aided by spin-decoupling experiments. J values are reported in hertz, and chemical shifts are given in δ units (downfield from Me_4Si). [b] The ^{13}C NMR spectrum was recorded in $CDCl_3$ at 50 MHz. Multiplicities were obtained by single-frequency off-resonance decoupling, and assignments were made based on J_R values and/or comparison to models. [c] Nonassignable proton resonances. [d] Peak broadening in the spectrum run at room temperature (20 °C) did not allow for an assignment of J values. [e–j] Signals within a column may be reversed. [j] Resonances associated with an acetate ester group.

Acetylation of pseudopterosin C (excess acetic anhydride in pyridine) yielded tetraacetate **5** in high yield. In the ^{1}H NMR spectrum of **5**, three new methyl resonances were observed at δ 2.09, 2.06, and 1.90 that were assigned to the newly formed esters. Also, two methine protons were observed to be shifted downfield in the ^{1}H NMR spectrum of this tetraacetate. Therefore, in addition to the phenolic hydroxyl group, pseudopterosin C was proposed to possess two secondary alcohols. The remaining two oxygen atoms in **3** were assigned to an acetal functionality on the basis of a high-field resonance [δ 106.0 (d)] in the ^{13}C NMR spectrum. The presence of an acetal and the numerous hydroxyl groups in **3**, together with the molecular formula indicating a five-carbon fragment in excess of a diterpenoid molecule, strongly suggested that pseudopterosin C contained a pentose sugar. A reevaluation of the mass spectral fragmentation pattern of **3**, showing $C_{20}H_{28}O_2$ [$M^+ - (C_5H_8O_5 + Ac)$] as the base peak, supported this assignment.

Further consideration of the ^{13}C NMR spectral data showed pseudopterosin C to possess an additional trisubstituted olefin [δ 129.5 (d), 129.9 (s)] that was nonconjugated. Irradiation of a low-field resonance in the ^{1}H NMR spectrum of **3** [δ 5.13 (1 H, d, J = 9.2 Hz)] assigned to the olefinic proton sharpened two signals at δ 1.75 (3 H, s) and

Scheme I



1.68 (3 H, s) and decoupled a band at midfield [δ 3.65 (1 H, m)]. These data suggested that an isopropylidene group was present in the molecule. Catalytic hydrogenation of pseudopterosin C produced a single product, dihydro derivative **6**. Two new doublet methyl groups were present in the upfield portion of the ^{1}H NMR spectrum of **6**. Irradiation of a complex one-proton signal at δ 1.84 caused both of these methyls to collapse to singlet resonances,

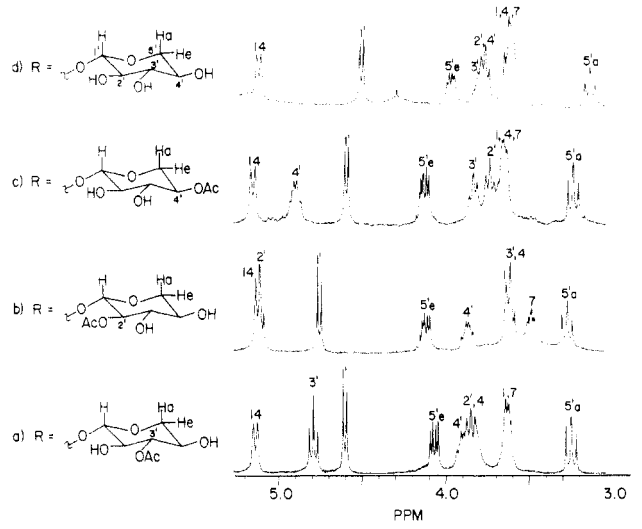


Figure 1. Proton magnetic resonance spectra for pseudopterosins C, B, D, and A (a–d), respectively, showing the midfield regions. Locations of acetylation were determined from the ca. 1 ppm downfield shifts observed for the pentose methine protons.

thereby proving their assignment as part of an isopropyl group.

Acid hydrolysis of pseudopterosin C cleaved the sugar residue, yielding the organish red aglycon **7** as the sole $CHCl_3$-soluble product of the reaction (Scheme I). While five methyl resonances were still observed in the ^1H NMR spectrum of **7**, most of the complex bands in the region between 3.0 and 5.5 ppm were absent. By low-resolution mass spectrometry,[10] the oxidized aglycon **7** was analyzed for $C_{20}H_{28}O_2$ ($M^+ + 2$), a molecular formula consistent with the assignment of a diterpenoid ring system to the aglycon. Further examination of the spectral data suggested that **7** was an *o*-quinone. Absorption at 430–470 nm (br d, ϵ 590) and infrared absorption at 1650–1670 cm^{-1}, in conjunction with the observed intense $M^+ + 2$ ion observed in the mass spectrum of **7**, were all consistent with this latter assignment.[11] Hence, working backward from this product, the oxygen atom forming the acetal linkage to the sugar and the phenolic hydroxyl group were proposed to be ortho substituents on the aromatic ring in the diterpenoid component of **3**.

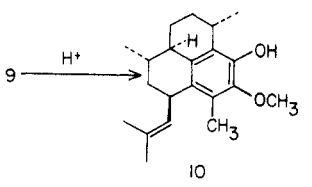
The structure of the sugar portion of pseudopterosin C, including the site of acetylation, was determined by ^1H NMR spin-decoupling experiments, summarized in Figure 1a. Decoupling the pentopyranose ring protons and recognizing the low-field shifts of acetate methine protons identified C3′ as the esterified carbon. Furthermore, the large coupling constants (>7 Hz) observed between the C1′, C2′, C3′, and C4′ methine protons suggested that all protons were axial and all hydroxyl groups equatorial. These results demonstrated that the pentose portion of pseudopterosin C was 3-*O*-acetyl-β-xylopyranose.[12]

(10) The diterpene aglycon **7** was of low stability and could not be subjected to high-resolution mass spectral analysis.

(11) For discussions of the spectral characteristics of *o*-quinones see: Pasto, D. L.; Johnson, C. R. *Organic Structure Determination*; Prentice-Hall: Englewood Cliffs, NJ, 1969; p 99 and reference 156, p 43. For a model, see data for stypoldione: Gerwick, W. H.; Fenical, W. *J. Org. Chem.* **1981**, *46*, 22.

(12) For a general discussion of the application of NMR to conformational analysis in sugars see: Davidson, E. A. *Carbohydrate Chemistry*; Holt, Rinehart and Winston: New York, 1967. Guthrie, R. D.; Honeyman, J. *An Introduction to the Chemistry of Carbohydrates*, 3rd ed. Clarendon: Oxford, 1968. For more specific discussions of conformational analysis see: Barton, D. H. R.; Cookson, R. C. *Q. Rev. Chem. Soc.* **1956**, *10*, 44. Ferrier, R. J.; Overend, W. G. *Q. Rev. Chem. Soc.* **1959**, *13*, 265. Lemieux, R. U.; Kullnig, R. K.; Bernstein, H. J.; Schneider, W. G. *J. Am. Chem. Soc.* **1958**, *80*, 6098.

Scheme II



Although the final structure of the diterpenoid aglycon portion of pseudopterosin C (**3**) was not yet fully defined, comparisons of spectral data showed that pseudopterosins B and D (**2**, and **4**) were isomeric monoacetates. Infrared and UV absorptions defined the same hydroxyl, phenolic, and ester functional groups to be present in **2** and **4**. The NMR features of the isomeric metabolites also compared favorably (Table I) to **3**, except that the ester appeared to be located at another site on the sugar. Similarly, pseudopterosin A (**1**) was recognized as the unacetylated glycoside from its polarity and NMR spectral characteristics (Table I). In order to confirm these conclusion, pseudopterosins A, B, and D (**1**, **2**, and **4**) were acetylated to yield the same tetraacetate, **5**, produced earlier from **3**. Furthermore, base hydrolysis of pseudopterosin C (**3**) (5% KOH/MeOH) yielded pseudopterosin A (**1**).

Proton NMR analysis of all four pseudopterosins, with particular emphasis on the midfield pentose methine region, confirmed that all four metabolites possess the same xylose components and showed the exact locations of acetylation (Figure 1b,c) in metabolites **2** and **4**.

Once the presence of the pentose residue in pseudopterosins **1**–**4** was established, attention was directed toward determining the complete structure of the aglycon. Since two of the nine degrees of unsaturation in the molecular formula of **3** could be accounted for by the acetylated xylopyranose, seven sites of unsaturation were therefore present in the diterpenoid portion of the molecule. The trisubstituted olefin and aromatic ring accounted for five degrees of unsaturation. Hence, the aglycon portion of **3** contained two additional carbocyclic rings. Furthermore, while data from the *o*-quinone **7** established the aglycon of **3** to be a catechol, product **7** was not a suitable aglycon derivative for extensive ^1H NMR analysis because of its instability. Reductive acetylation of **7** produced a stable product that could be purified by HPLC. However, diacetate **8** was produced in such low yield that this series of reactions was not considered practical for continued investigation of the aglycone.

Treatment of pseudopterosin A (**1**) with CH_3I and anhydrous potassium carbonate in acetone produced the methyl ether **9** in good yield. This compound was then extensively investigated by nuclear Overhauser enhancement difference spectroscopy (NOEDS). Irradiation of the signal assigned to the *O*-methyl, observed at δ 3.75 (3 H, s) in the ^1H NMR spectrum of **9**, resulted in enhancement of the aromatic methyl [δ 2.05 (3 H, s)] resonance. From the converse experiment, identical results were obtained. These results suggested that the aromatic methyl and the methyl ether were oriented ortho in derivative **9**, and hence this orientation was present in the pseudopterosins.

Acid hydrolysis of the methyl ether **9** yielded the methyl aglycon **10** (Scheme II). Compound **10** analyzed for $C_{21}H_{30}O_2$ by low-resolution mass spectrometry. With the aid of spin-decoupling experiments and difference decoupling spectroscopy (DDS), considerable connectivity could be established, and most resonances in the ^1H NMR spectrum of **10** were subsequently assigned. Several structures could be constructed that would accommodate these data. But, of the known tricarboxylic diterpenoid

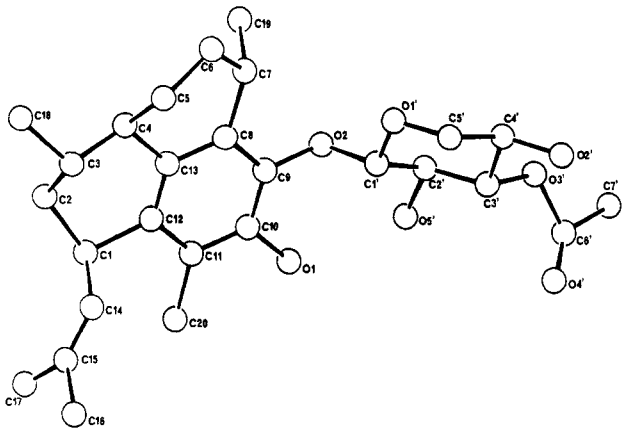
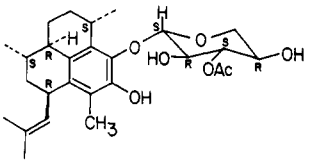


Figure 2. Computer-generated perspective drawing of pseudopterosin C. Hydrogen atoms have been excluded, and the absolute configuration shown was chosen on the basis of chemical results presented in the body of the paper.

ring systems, the rare amphilectane skeleton[13] was the most likely candidate, since it contained the appropriate carbon connectivity.

The relative positions and stereochemistries of the asymmetric centers in the aglycon could not be unambiguously assigned, however. Therefore, the structure of the only crystalline metabolite, pseudopterosin C, was solved by X-ray crystallography. A computer-generated perspective drawing of the final X-ray model of pseudopterosin C is given in Figure 2. Hydrogens are omitted for clarity, and the absolute configuration shown was selected to conform to the presence of D-xylose as the sugar component. The aromatic ring is planar, and the two carbocyclic rings are in the half-chair conformation. The methyl group at C7 is in a pseudoaxial conformation, and the methyl at C3 is pseudoequatorial. The 2-methyl-1-propenyl side chain at C1 and the hydrogen at the bridgehead (C4) are pseudoaxial. The D-xylose is in the pyranose form and adopts a chain conformation with all substituents equatorial. In general, bond distances and angles agree well with generally accepted values.

The sugar was next isolated in order to determine its absolute configuration. Hydrolysis of **1** was carried out with approximately 1 N HCl at 48–50 °C. Samples of D- and L-xylose were simultaneously treated with a 1 N HCl and worked up in the same manner. Optical rotations of each sample were then measured. Results from these experiments showed that the sugar component of **1** was D-xylose; $[\alpha]^{20}_D$ +29° (*c* 0.95, H_2O).[16] Since the relative stereochemistry of all centers was elucidated from the X-ray studies, and the sugar was shown to be 3-*O*-acetyl-β-D-xylopyranose, the absolute configuration of all chiral centers in pseudopterosin C (**3**) could be assigned: C1 (*R*), C3 (*S*), C4 (*R*), C7 (*S*), C1′ (*S*), C2′ (*R*), C3′ (*S*), C4′ (*R*).



3

The pseudopterosins represent a unique new class of marine natural products. The potent antiinflammatory and analgesic properties of these metabolites suggest they represent important new leads in the development of pharmaceuticals for the treatment of disease involving abnormal phospholipid metabolism.[8]

Experimental Section

General Procedures. The instrumentation and general experimental parameters used in this investigation have been recently described.[17]

Collection and Extraction. *P. elisabethae* (spec. nov.) [BS-(56-61)] was collected in July 1982 at French Wells along Crooked Island in the Bahamas at depths of 15–35 m. Organisms were stored frozen. Upon workup, the animals were homogenized and the gorgonian cake was repeatedly extracted with $CHCl_3$ and then EtOAc. The $CHCl_3$ and EtOAc extracts were then combined, filtered, and evaporated under vacuum to give a residue that was partitioned between saturated brine and $CHCl_3$. This $CHCl_3$ extract was subsequently dried over $MgSO_4$, filtered, and evaporated to yield 117 g of crude extract (from 700 g, dry weight of the gorgonian). Pseudopterosins A–D (**1**–**4**) were isolated by rapid elution chromatography of 40 g of crude extract over a column that consisted of a 600-mL Buchner funnel firmly packed with 300 g of thin-layer grade (200-Å mesh) Florisil. Fractions were eluted under vacuum into a 1-L Erlenmeyer suction flask with solvent mixtures (500 mL of each) that ranged from 20% CH_2Cl_2 in isooctane to 100% CH_2Cl_2 and then through mixtures of CH_2Cl_2 with increasing proportions of EtOAc. Pseudopterosins C and D (**3**, **4**) were eluted from the column with 10–30% EtOAc in CH_2Cl_2. Pseudopterosin B (**2**) was eluted from the column with 60–100% EtOAc in CH_2Cl_2. Pseudopterosin A (**1**) eluted from the column with 90–100% EtOAc in CH_2Cl_2.

Pseudopterosin A (1). Pseudopterosin A (**1**) was isolated as an amorphous solid. Extensive purification by HPLC (μ-Porasil, using 85% EtOAc in isooctane) yielded 0.2 g (0.5% of crude extract). Compound **1**: $[\alpha]^{20}_D$ −85° (*c* 0.69, $CHCl_3$); UV (MeOH) λ_{max} 230 nm (ε 11 200), 278 (ε 2060), 283 (ε 2200) (after addition of 1 drop of 5% KOH/MeOH, λ_{max} values shifted to 247 nm, 285, and 295, respectively); IR ($CHCl_3$) 3500, 3030, 2920, 1200 cm^{-1}; HRMS (M^+) *m*/*z* (rel intens) obsd 432.2491 (1.1), $C_{25}H_{36}O_6$ requires 432.2512, 300.2088 ($C_{20}H_{28}O_2$, 100), 285.1852 ($C_{19}H_{25}O_2$, 72).

Pseudopterosin B (2). Pseudopterosin B (**2**) was isolated as an oil from the same fraction as compound **1**. Purification by HPLC (μ-Porasil, using 85% EtOAc in isooctane) gave 0.3 g 0.7% of the crude extract) of **2**. Pseudopterosin B: $[\alpha]^{20}_D$ −55.2° (*c* 2.1, $CHCl_3$); UV (MeOH) λ_{max} 230–235 nm (ε 6000), 274 (ε 1400), 285 (ε 1600) (after addition of 1 drop of 5% KOH/MeOH, λ_{max} values shifted to 250 nm, 288, and 294, respectively); IR ($CHCl_3$) 3500, 3030, 2960, 1745, 1220, 1080, 1040 cm^{-1}; HRMS (M^+) *m*/*z* (rel intens) obsd 474.2656 (1.1), $C_{27}H_{38}O_7$ requires 474.2677, 300.2092 ($C_{20}H_{28}O_2$, 100), 285.1855 ($C_{19}H_{25}O_2$, 61).

Pseudopterosin C (3). Pseudopterosin C (**3**) crystallized from EtOAc and absolute EtOH after purification by HPLC (μ-Porasil,

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using 50% EtOAc in isooctane). Repeated recrystallization gave 3.0 g (7.5% of the crude extract) of **3**, mp 113.5–115 °C. Pseudopterosin C: $[\alpha]^{20}_D$ −77° (*c* 1.09, $CHCl_3$); UV (MeOH) λ_{max} 229 nm (ε 9600), 275 (ε 1500), 282 (ε 1700) (after addition of 1 drop of 5% KOH/MeOH, λ_{max} values shifted to 245 nm, 285, and 295, respectively): IR ($CHCl_3$) 3350–3450, 2920, 1725, 1440, 1370, 1310, 1240, 1070, 1030 cm^{-1}; HRMS (M^+) *m/z* (rel intens) obsd 474.2573 (1.8), $C_{27}H_{38}O_7$ requires 474.2677, 300.2055 ($C_{20}H_{28}O_2$, 100), 285.1849 ($C_{19}H_{25}O_2$, 77), 218.1325 ($C_{14}H_{18}O_2$, 27).

Pseudopterosin D (4). Pseudopterosin D (**4**) was isolated as an oil. Purification by HPLC (μ-Porasil, using 45% and 50% EtOAc in isooctane) yielded 0.75 g (0.1% of the crude extract) of **4**. Pseudopterosin D: $[\alpha]^{20}_D$ −107.3 (*c* 0.55, $CHCl_3$); UV (MeOH) λ_{max} 230 nm (ε 6500), 272 (ε 3300), 282 (ε 3100) (after addition of 1 drop of 5% KOH/MeOH, λ_{max} values shifted to 244 nm, 284, and 294, respectively); IR ($CHCl_3$) 3500, 3030, 2960, 1745, 1220 cm^{-1}; HRMS (M^+) *m/z* (rel intens) obsd 474.2630 (0.91), $C_{27}H_{38}O_7$ requires 474.2677, 300.2097 ($C_{20}H_{28}O_2$, 100) 285.1851 ($C_{19}H_{25}O_2$, 56).

Acetylation of 3 To Yield Tetraacetate 5. Excess acetic anhydride was added to 54 mg of **3** dissolved in 5 mL of dry pyridine with stirring. The reaction mixture was stirred overnight at room temperature, quenched with ice, and extracted with EtOAc (3 × 20 mL). The combined EtOAc layers were then washed with 5% HCl (2 × 20 mL), water, and 5% aqueous $NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated to yield, quantitatively, the crude tetraacetate **5**. Tetraacetate **5** was purified by HPLC (μ-Porasil, using 40% EtOAc in isooctane). Compound **5**: $[\alpha]^{20}_D$ −103° (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} 229 nm (ε 14 000), 260–278 (br, ε 1300); IR ($CHCl_3$) 3020, 2960, 2920, 2400, 1750, 1510, 1420, 1370, 1210 cm^{-1}; HRMS M^+ (*m/z*) (rel intens) obsd 600.2935 (0.3), $C_{33}H_{44}O_{10}$ requires 600.2934, 300.2069 ($C_{20}H_{28}O_2$, 30), 139.0384 ($C_7H_7O_3$, 100); ^1H NMR (360 MHz, $CDCl_3$) δ 5.21 (2 H, m), 5.13 (1 H, br d, *J* = 9.2 Hz), 5.02 (2 H, m), 4.10 (1 H, dd, *J* = 5.0, 11.7 Hz), 3.59 (1 H, br d, *J* = 9.2 Hz), 3.47 (1 H, m), 3.28 (1 H, dd, *J* = 9.1, 11.8 Hz), 2.34 (3 H, s), 2.09 (3 H, s), 2.06 (3 H, s), 2.04 (3 H, s), 1.90 (3 H, s), 1.73 (3 H, s), 1.67 (3 H, s), 1.15 (3 H, d, *J* = 7.2 Hz), 1.03 (3 H, d, *J* = 5.9 Hz).

Catalytic Hydrogenation of Pseudopterosin C (3). Pseudopterosin C (**3**; 17.4 mg, 0.037 mmol) was dissolved in EtOAc (15 mL) and added to a 25-mL Erlenmeyer suction flask containing a catalytic amount of 10% Pd–C and a stir bar. A balloon and septum were secured to the reaction vessel, the flask was purged with hydrogen, and the balloon was filled with dry H_2. After the mixture was stirred at 25 °C for 9 h, excess H_2 was removed and the solution was filtered through Celite. Evaporation of the solvent gave the dihydro derivative **6** [16.5 mg (94% from **3**)] as an amorphous solid. Derivative **6**: $[\alpha]_D^{20}$ −20° (*c* 0.68, $CHCl_3$); UV (MeOH) λ_{max} 226 nm (ε 9600), 244 (ε 2100), 251 (ε 2300), 256 (ε 2400), 263 (ε 2100), 272 (ε 1400), 284 (ε 1700) (following addition of 1 drop of 5% KOH/MeOH, only λ_{max} 272 nm and 284 shifted, to 284 nm and 296, respectively); IR ($CHCl_3$) 3500, 3030, 2960, 2920, 2440, 1745 cm^{-1}; HRMS (M^+) *m/z* (rel intens) obsd 476.2807 (0.10), $C_{27}H_{40}O_7$ requires 476.2774, 302.2212 ($C_{20}H_{30}O_2$, 19), 245.1533 ($C_{16}H_{21}O_2$, 100); ^1H NMR (360 MHz, $CDCl_3$) δ 7.54 (1 H, br s, exchange with D_2O), 4.76 (1 H, dd, *J* = 9.0, 9.1 Hz, C-3′), 4.55 (1 H, d, *J* = 7.7 Hz, C-1′), 4.01 (1 H, dd, *J* = 5.3, 11.6 Hz), 3.85 (1 H, m), 3.84 (1 H, m), 3.59 (1 H, m, C-7), 3.18 (1 H, dd, *J* = 10.5, 11.0 Hz), 2.94 (1 H, br d, *J* = 13.7 Hz), 2.17 (3 H, s), 2.19 (3 H, s), 1.84 (1 H, m, C-15), 1.57 (1 H, m, C-3), 1.11 (3 H, d, *J* = 7.00 Hz, C-19), 1.03 (3 H, d, *J* = 6.2 Hz, (C-18), 0.97 (3 H, d, *J* = 6.5 Hz, C-16), 0.87 (3 H, d, *J* = 6.6 Hz, C-17).

Acid Hydrolysis of Pseudopterosin C. Pseudopterosin C (**3**; 27 mg, 0.057 mmol), dissolved in 1 mL of MeOH, was added to a solution of 3 N HCl (1 mL) in MeOH (1 mL), and the reaction mixture was warmed to 48–50 °C in a loosely sealed Pyrex tube. (If the acid concentration was much higher or if the reaction was heated to higher temperatures, marked decomposition of the aglycon and/or the sugar resulted.) After 3 h, the solution was cooled to room temperature, diluted with H_2O (20 mL), and extracted with $CHCl_3$ (3 × 20 mL). The combined $CHCl_3$ layers were then washed with 5% $NaHCO_3$ (2 × 20 mL), dried over $MgSO_4$, filtered, and evaporated to yield an orangish red oil residue, identified as the *o*-quinone **7**, 13.8 mg (81% from **3**). Attempts to purify this product by HPLC (μ-Porasil or μ-Bondapak C_{18} reversed phase) appeared to result in decomposition. Derivative **7**: UV (EtOH) λ_{max} 430–470 nm (br, ε 590), 338 (ε 2800), 285 (ε 3300), 260 (ε 5900), 237 (ε 9000); IR ($CHCl_3$) 3030, 2960, 1650–1670, 1630, 1500, 1470, 1375, 1280, 1200 cm^{-1}; LRMS (M^+) *m/z* (rel intens) 300 (M^+ + 2, $C_{20}H_{28}O_2$, 2.9), 298 (M^+, $C_{20}H_{26}O_2$, 0.3), 258 (5.7), 243 (10.5), 229 (6.7), 216 (8.4), 203 (20.8); ^1H NMR (360 MHz, $CDCl_3$) δ 5.11 (1 H, br d, *J* = 9.7 Hz, C-14), 3.59 (1 H, br m, C-1), 3.23 (1 H, m, C-7), 2.03 (3 H, s, C-20), 1.75 (3 H, s, C-16), 1.67 (3 H, s, C-17), 1.25 (3 H, d, *J* = 6.8 Hz, C-19), 1.03 (3 H, d, *J* = 6.0 Hz, C-18).

Acetylation of Pseudopterosins A, B, and D (1, 2, 4) To Yield Tetraacetate 5. Acetylation of pseudopterosins A, B, and D was performed as described above for pseudopterosin C. In each case removal of extraction solvents yielded a tetraacetate, in all respects identical with **5**. The tetraacetate obtained from **2** showed $[\alpha]^{20}_D$ −100° (*c* 1.2, $CHCl_3$). Tetraacetate **5**, produced from **4**, showed $[\alpha]^{20}_D$ −105° (*c* 0.65, $CHCl_3$).

Base Hydrolysis of Pseudopterosin C (3). To 22 mg (0.046 mmol) of pseudopterosin C (**3**) was added 15 mL of 5% KOH/MeOH, and the reaction mixture was stirred overnight. Upon workup, ice was added and the solution was poured into 50 mL of H_2O, acidified, and extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ layers were dried ($MgSO_4$), filtered, and evaporated to yield 18 mg (90% from *3*) of pseudopterosin A (**1**) as an amorphous solid. The product of the reaction was in all respects identical with pseudopterosin A (**1**), $[\alpha^{20}_D$ −83° (*c* 0.86, $CHCl_3$).

Reductive Acetylation of *o*-Quinone 7 to Diacetate 8. One part Zn dust and 0.2 part fused NaOAc were added to the *o*-quinone **7** (19.6 mg, 0.066 mmol), suspended in 1 mL of acetic anhydride. The mixture was warmed until the colored material disappeared and the solution was no longer orangish red, but yellowish to colorless. The reaction was then refluxed briefly (15 min), filtered, diluted with water, and extracted with $CHCl_3$ (3 × 20 mL). The combined $CHCl_3$ layers were subsequently washed with 5% $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated to yield 18.8 mg of crude product, 75% from **7**. Purification by HPLC (μ-Porasil, using 30% EtOAc in isooctane), however, resulted in recovery of only 6.6 mg of diacetate **8**, 26% from **7**. Diacetate **8**: UV (MeOH) λ_{max} 222 nm (ε 12 000), 230 (ε 7400), 260 (br d, ε 2900); LRMS (M^+) *m/z* (rel intens) 384 (M^+, $C_{24}H_{32}O_4$, 1.8) 342 (4.6), 300 (11.6), 299 (1.6), 285 (7.1); ^1H NMR (360 MHz, $CDCl_3$) δ 5.15 (1 H, br d, *J* = 9.2 Hz), 3.59 (1 H, m), 3.07 (1 H, m), 2.38 (1 H, br d, *J* = 5.5 Hz), 2.29 (3 H, s), 2.28 (3 H, s), 1.92 (3 H, s), 1.73 (3 H, s), 1.67 (3 H, s), 1.16 (3 H, d, *J* = 7.1 Hz), 1.04 (3 H, d, *J* = 5.8 Hz).

Methylation of Pseudopterosin A (1). To a stirred solution of pseudopterosin A (**1**; 16 mg, 0.037 mmol) in dry acetone (30 mL) was added excess CH_3I and anhydrous K_2CO_3. After refluxing for 5 h, the reaction mixture was allowed to cool to room temperature. The acetone was then evaporated, water (20 mL) was added to the residue, and the aqueous layer was repeatedly extracted with $CHCl_3$ (3 × 20 mL). The $CHCl_3$ layers were then combined, dried ($MgSO_4$), filtered, and evaporated to yield **9** [13.8 mg (85% from **1**)] as a solid. Methyl ether **9**: $[\alpha]^{20}_D$ −68° (*c* 0.48, $CHCl_3$); UV (MeOH) λ_{max} 226 nm (ε 13 200), 268 (ε 1400); IR ($CHCl_3$) 3500, 2960, 1745 cm^{-1}; LRMS (M^+) *m/z* (rel intens) 314 (M^+ − $C_5H_8O_4$, $C_{21}H_{30}O_2$, 100), 300 (1.7), 299 (28); ^1H NMR (360 MHz, $CDCl_3$) δ 5.09 (1 H, br d, *J* = 9.2 Hz), 4.59 (1 H, d, *J* = 7.2 Hz), 4.05 (1 H, dd, *J* = 5.1, 11.5 Hz), 3.82 (1 H, m), 3.75 (3 H, s), 3.67 (2 H), 3.69 (2 H, m), 3.53 (1 H, dd, *J* = 8.7, 8.9 Hz), 3.26 (1 H, dd, *J* = 9.9, 11.5 Hz), 2.05 (3 H, s), 1.74 (3 H, s), 1.67 (8 H, s), 1.19 (3 H, d, *J* = 7.0 Hz), 1.04 (3 H, d, *J* = 5.8 Hz). NOEDS (^1H NMR) irradiation of the *O*-methyl at δ 3.75 (3 H, s) resulted in enhancement of the resonance due to the aromatic methyl [δ 2.05 (3 H, s)]. The converse experiment gave identical results.

Reaction of Derivative 9 with 1 N HCl To Yield 10. Compound **9** (12.1 mg, 0.027 mmol) was treated with 1 N HCl by the procedure outlined above for the acid hydrolysis of **3**. Derivative **10**, produced in quantitative yield [8.2 mg (96% from **9**)] was the sole $CHCl_3$-soluble product from the reaction. Compound **10**: UV (MeOH) λ_{max} 226 nm (ε 7200), 274 (ε 1600), 283 (ε 1500); LRMS (M^+) *m/z* (rel intens) 314 $C_{21}H_{30}O_2$, 53), 300 (26), 299 (100), 259 (17), 257 (10), 244 (10); ^1H NMR (360 MHz, $CDCl_3$) δ 5.65 (1 H, s, exchange with D_2O), 5.10 (1 H, br d, *J* = 8.2 Hz, C-14), 3.74 (3 H, s, OCH_3), 3.55 (1 H, m, C-1), 3.32 (1 H, m, C-7), 2.15 (1 H, m, C-4), 2.07 (3 H, s, C-20), 2.02 (1 H, m, C-2), 1.74

(3 H, s, C-16), 1.67 (3 H, s, C-17), 1.65 (1 H, m, C-2), 1.61 (3 H, m, C-3, C-6), 1.45 (1 H, m, C-5), 1.25 (3 H, d, J = 6.9 Hz, C-19), 1.14 (1 H, m, C-5), 1.04 (3 H, d, J = 5.9 Hz, C-18).

Acid Hydrolysis of Pseudopterosin A (1). Conditions for reaction of 1 with 1 N HCl, which allowed the sugar residue to be isolated, were first worked out with D- and L-xylose alone. If the concentration of HCl was much greater than 1 N, the temperature of the reaction was above 50 °C, and/or if the H_2O was totally removed in the vacuum desiccator prior to measuring the optical rotations, either the values of the rotations were irreproducible or no optical rotation could be measured at all. Pseudopterosin A (1; 63.0 mg, 0.146 mmol) was treated with 1 N HCl by the same procedure outlined above for the acid hydrolysis of **3** and **9**, except that the volume was doubled and the workup was slightly modified. After 3 h, 4.0 mL of water was added, the MeOH was removed by evaporation with a N_2 stream, and the remaining aqueous solution was then repeatedly extracted with EtOAc (3 × 20 mL), until all color was extracted from the aqeous layer. Both the aqueous solution and the combined EtOAc layers were saved. The combined EtOAc layers were washed with $NaHCO_3$ (3 × 20 mL), dried ($MgSO_4$), filtered, and evaporated to yield 39.8 mg (91% from **1**) of the *o*-quinone **7**. Next, any remaining EtOAc was removed from the aqueous layer with a stream of N_2. This solution was then transferred to a small beaker: the HCl was removed, and the H_2O was reduced to a volume slightly less than 1.5 mL in a vacuum desiccator that contained pellets of NaOH. Once the volume was reduced below 1.5 mL (in approximately 48 h), water was added to bring the volume to 2.0 mL. This solution, containing the sugar residue of **1**, showed $[\alpha]^{20}_D$ +29° (*c* 0.95, H_2O). Simutaneously, as controls and for comparison, samples of the pentose sugars D-xylose (32.0 mg) and L-xylose (30.8 mg) (Aldrich Chemical Co.) were also treated with 1 N HCl. Upon completion of the reaction, the acid was removed and the aqueous solution reduced in the same manner as described above. After treatment with 1 N HCl, D-xylose showed $[\alpha]^{20}_D$ +23° (*c* 1.64, H_2O) and L-xylose showed $[\alpha]^{20}_D$ −26° (*c* 1.54, H_2O).

Single-Crystal X-ray Structure Determination of Pseudopterosin C. Preliminary X-ray photographs displayed monoclinic symmetry, and accurate lattice constants of a = 10.177 (1) Å, b = 9.992 (1) Å, c = 17.757 (1) Å, and β = 68.00 (3)° were determined from a least-squares fit of 15 diffractometer measured 2θ values between 35 and 50° [Cu K$\bar{\alpha}$ radiation (1.541 78 Å)]. The presence of chirality, systematic extinctions, and crystal density were uniquely accommodated by space group $P2_1$, with 1 molecule of $C_{26}H_{38}O_5$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using graphite-monochromated Cu K$\bar{\alpha}$ radiation (1.541 78 Å) and variable-speed, 1° ω-scans. A total of 2012 reflections were measured in this fashion, and, after correction for Lorentz, polarization, and background effects, 1799 (89%) were judged observed [$|F_o| \geq 3\sigma(F_o)$].[14] A phasing model was found using 300 normalized structure factors in a multisolution tangent formula approach. The use of the negative quartets figure of merit was decisive in finding the best phase set. The E-synthesis from the best set showed 19 plausible atoms, and this was extended to 24 atoms by tangent formula recycling.[15] The structure was completed by Fourier refinement with $2F_o-F_c$ syntheses. Block-diagonal least-squares refinements with anisotropic non-hydrogen atoms have converged to the present residual of 0.09 for the observed data. Additional crystallographic details are available and are described (supplementary material).

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Supplementary Material Available: Tables of fractional coordinates, bond distances, bond angles, and torsion angles for pseudoterosin C (6 pages); tables of observed and calculated structure factors (12 pages). Ordering information is given on any current masthead page.

Annulins A and B, Metabolites of the Marine Hydroid *Garveia annulata*

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Two new antimicrobial metabolites have been isolated from the marine hydroid *Garveia annulata*. The structure of annulin A (**3**) was determined via X-ray diffraction analysis, and the proposed structure for annulin B (**4**) was inferred from its spectral data.

We have recently shown that the marine hydroid *Garveia annulata* is a rich source of antimicrobial 1-(4*H*)-anthracenone derivatives.[1,2] Garveatin A (**1**) and 2-hydroxygarvin A (**2**) are representative members of the garveatin and garvin families, which encompass all the *Garveia* metabolites described to date. We now report the isolation of two degraded anthracenes, annulins A (**3**) and B (**4**), from *G. annulata* methanol extracts.

G. annulata was collected by hand using SCUBA in Barkley Sound, British Columbia, and its metabolites were extracted and purified as previously described.[2]

Annulin A (**3**), obtained from ethanol as optically inactive bright orange crystals (mp 174–176 °C), showed a parent ion in the HREIMS that was consistent with a molecular formula of $C_{19}H_{20}O_7$ (M^+ 360.1221, calcd 360.1209). The ^1H NMR spectrum of annulin A contained resonances that could be assigned to aromatic methyl (2.44

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