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Identification of anti-inflammatory diterpenes from the marine gorgonian *Pseudopterogorgia elisabethae*

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Abstract—Analysis of the terpene metabolites of *Pseudopterogorgia elisabethae* collected from the Florida Keys has resulted in the identification of a novel hydroxyquinone, elisabethadione (**1**), as well as new pseudopterosins and seco-pseudopterosins. Anti-inflammatory assays indicate that elisabethadione is more potent than the well characterized pseudopterosin A and E. This report also describes the co-occurrence of pseudopterosins and seco-pseudopterosins, diterpenes with amphilectane and serrulatane skeletons, respectively. This together with our previously described isolation of elisabethatriene as the sole diterpene cyclase product in *P. elisabethae* suggests that the amphilectane and serrulatane families of diterpenes are derived from the same geranylgeranyl diphosphate cyclase product.

1. Introduction

The pseudopterosins are a family of diterpene glycosides isolated from the gorgonian *Pseudopterogorgia elisabethae*. Different members of the pseudopterosin family are found in *P. elisabethae* collected in distinct geographic locations. There are 12 derivatives, pseudopterosins A–L (PsA–PsL) and in most cases, they comprise 2–5% of the crude extract. Pseudopterosins A–D have been isolated from *P. elisabethae* collected from Grand Bahama Island and the central Bahamas,[1] Ps E–J were isolated from collections from Bermuda, and Ps K and L were found in collections from Great Abaco Island.[2] The diterpene skeletons of pseudopterosins A–F are identical and only differ from the diterpene moiety of Ps G–L in the orientation of the alkyl group at C-1. Collections of the closely related *Pseudopterogorgia kallos* from Cosgrove Shoal near the Marquesas Keys in Florida revealed the presence of seco-pseudopterosins A–D.[3] The pseudopterosins exhibit a novel spectrum of activity when compared to existing topical anti-inflammatory drugs. In animal studies they block the edema produced by acute application of phorbol 12-myristate 13-acetate (PMA).[4] In harvested human polymorphonuclear granulocytes (PMNs), the pseudopterosins block calcium ionophore induced degranulation and release of leukotriene B (LTB),[4] neutrophil myeloperoxidase and lactoferrin.[5] Recent studies indicate release of eicosanoids is blocked without interrupting biosynthesis.[6] Although the molecular mechanism of action is not known as yet, we have found evidence in cultured cells that pseudopterosin A stabilizes nuclear lamina in dividing sea urchin embryos[7] and decreases phagosome formation in *Tetrahymena* cultures activated with calcium or zymosan.[8,9]

We have been engaged in a study to elucidate the biosynthetic origin of the pseudopterosins using a combination of radioactivity-guided isolations[10] coupled with a search for putative intermediates from extracts of *P. elisabethae*. As described above, the pseudopterosins have thus far been isolated from this coral collected in regions of the Bahamas, and Bermuda. We have recently located *P. elisabethae* in the Florida Keys and from this sample have identified a number of novel diterpenes. These compounds include a novel serrulatane diterpene (elisabethadione, **1**), a new amphilectane derivative (elisabethol, **2**), three new pseudopterosins (Ps M–O, **3**–**5**), and three new seco-pseudopterosins (seco-E–G, **6**–**8**). Compounds **1** and **2** have been shown to be intermediates in the biosynthesis of pseudopterosins A–D.[11] All new compounds were evaluated in a mouse ear anti-inflammatory assay which indicated that elisabethadione (**1**), pseudopterosin N (**4**) and seco-pseudopterosin E (**6**) exhibit potencies equal to or somewhat greater than PsA and were significantly more potent when compared to PsE ($p<0.05$).[12]

Keywords: marine gorgonian; *Pseudopterogorgia elisabethae*; diterpenes; antiinflammatory activity; biosynthesis.

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2. Results and discussion

Samples of *P. elisabethae* were collected at a depth of 25 m off Long Key, Florida. An extract (360 g) of the freeze-dried material was subjected to solvent partitioning and the chloroform fraction was purified by flash chromatography over silica followed by reversed-phase (C18) HPLC to afford elisabethadione (**1**), elisabethol (**2**), pseudopterosins M–O (**3**–**5**) and seco-pseudopterosins E–G (**6**–**8**).

Elisabethadione (**1**) was isolated as yellow gummy material. Its UV spectrum showed a maximum absorption at 248 nm indicating the presence of an α,β-unsaturated carbonyl and its IR spectrum displayed intense absorptions at 1690 (C═O) and 3395 (OH) cm^{-1}. HREIMS showed a molecular ion peak at *m/z* 316.2034 indicating a molecular formula of $C_{20}H_{28}O_3$ (calcd 316.2038) and thus the presence of seven degrees of unsaturation in the molecule.

The ^1H NMR spectrum of **1** suggested that this compound was terpenoid in origin due to the presence of two three-proton doublets at δ 0.86 (*J*=6.5 Hz) and 1.17 (d, *J*=6.7 Hz), a singlet at δ 1.96 characteristic of an aromatic methyl and resonances at δ 1.68 and 1.72 characteristic of olefinic methyls. A COSY-45° spectrum indicated the presence of a single spin system and an additional, isolated methyl group. An olefinic proton (δ 5.08) showed cross-peaks with both the olefinic methyls as well as a methylene group (δ 1.59 and 2.05). This methylene exhibited cross-peaks with a second methylene (δ 1.29 and 1.98) which in turn showed cross-peaks with a methine proton at δ 2.24. The methine at δ 2.24 had cross-peaks with a methyl at δ 0.86 and a methine at δ 3.10. The latter methine exhibited cross-peaks with a methylene at 1.71 and 1.90 which in turn had cross-peaks with a second methylene (δ 1.51 and 1.99) and this had COSY interactions with a methine at δ 2.98. A final interaction was observed between this last methine signal and a methyl at δ 1.17.

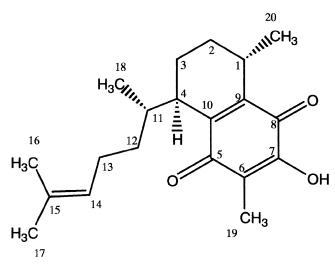
The remainder of the molecule was required to have five degrees of unsaturation to comply with the MS data. This was satisfied by the presence of a quinone which was evident from the UV, IR and ^{13}C NMR spectra. The location of the carbonyls of the quinone was confirmed by a HMBC experiment in which H-4 (δ 3.10) showed cross-peaks with C-3 (δ 26.7), C-10 (δ 150.8) and C-11 (δ 35.6), and the C-1 methine proton (δ 2.98) showed cross-peaks with C-2 (δ 25.4), C-8 (δ 190.0), C-9 (δ 144.6) and C-10 (δ 150.8). The C-19 methyl was assigned to C-6 due to the presence of long-range heteronuclear couplings with C-5 (δ 191.0), C-6 (δ 117.4), and C-7 (δ 159.9). The remaining hydroxyl proton, an exchangeable 1H broad singlet at δ 4.95, was assigned to C-7. The attachment of the hydroxyquinone to the spin system identified from the COSY spectrum completed the gross structure of **1**.

The $[\alpha]_D^{20}$ of compound **1** was found to be 93.3° which was identical to that of elisabethamine, a structurally related metabolite which we previously reported[13] from *P. elisabethae*, suggesting that compound **1** may have the same relative stereochemistry (α-orientation for the C-4 methine, C-18 and C-20 methyl groups). Further, the co-occurrence of compound **1** with the pseudopterosins whose stereochemistry has been rigorously established supports the depicted stereochemistry.

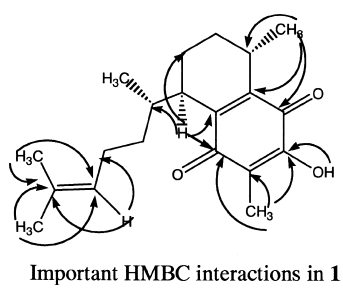
Compound **2**, elisabethol, was isolated as a colorless amorphous solid. Its UV spectrum showed a maximum absorption at 240 nm indicative of an α,β-unsaturated carbonyl group. The IR spectrum displayed strong absorption bands at 1705 (C═O) and 3512 (OH) cm^{-1}. The HREIMS of compound **2** showed a molecular ion peak at *m/z* 316.2032 which provided the molecular formula $C_{20}H_{28}O_3$ (calcd 316.2038) indicating the presence of seven degrees of unsaturation.

The ^1H NMR spectrum of **2** was similar to that of compound **1** (Fig. 1) with the exception that in the former the C-14 olefinic proton appeared as a doublet (at δ 5.21) and two exchangeable signals were observed at δ 5.01 and 5.45 indicating the presence of two hydroxyl groups. The multiplicity of C-14 revealed that compound **2** is an amphilectane-type diterpene, rather than a serrulatane derivative as identified for **1**. This multiplicity difference can be used as an important tool to differentiate between these two classes of diterpenes. The presence of the third ring in **2** was confirmed by the HMBC correlation between the C-1 methine proton and C-12. Additional key HMBC correlations are shown in Figure 2 and complete ^1H and ^{13}C NMR chemical shift assignments of compound **2** are shown in Table 1.

In addition to compounds **1** and **2**, three new pseudopterosins and three new seco-pseudopterosins were isolated from this extract. Pseudopterosin M (**3**) was isolated as a yellow gum. The HREIMS exhibited a molecular ion peak at *m/z* 474.2631 ($C_{27}H_{38}O_7$, calcd 474.2618), indicating the presence of nine degrees of unsaturation in the molecule. The IR spectrum showed intense absorptions at 1675 (C═C), 1710 (ester carbonyl) and 3435 (OH) cm^{-1}, while the UV spectrum exhibited absorption maxima at 282 and 226 nm, indicative of a substituted benzene ring.[1]

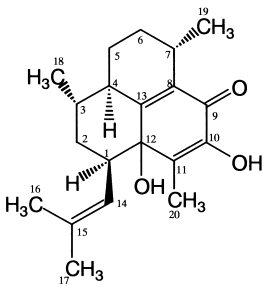


1

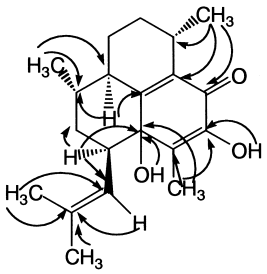


Important HMBC interactions in **1**

Figure 1. Elisabethadione (**1**).



2



Important HMBC interactions in **2**

Figure 2. Elisabethol (**2**).

The ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of **3** suggested that this compound was of terpenoid origin, and comparison with spectra of the known pseudopterosins indicated that **3** was a new member of this family of diterpene glycosides. The ^{1}H NMR spectrum showed two three-proton doublets at δ 1.00 (J=7.3 Hz) and 1.19 (J=6.5 Hz) assigned to the C-18 and C-19 methyl groups, respectively. Another signal, integrating for three protons, at δ 2.09 was assigned to the C-20 methyl, and a methyl singlet at δ 2.21 was assigned to the C-2′ acetoxy group. Two allylic C-16 and C-17 methyl groups appeared as two singlets at δ 1.69 and 1.76 respectively. A downfield resonance for the C-14 methine proton was also observed at δ 4.96 (J=7.9 Hz) as a doublet in the ^{1}H NMR spectrum of **3**. Another doublet at δ 5.10 (1H, J=9.0 Hz) was assigned to the C-1′ proton, while a signal at δ 5.29 (1H) was assigned to the C-2′ proton. Its downfield chemical shift value was indicative of the presence of a geminal acetoxy group. Remaining protons of the sugar moiety resonated at δ 3.83–4.33. Three exchangeable signals at δ 6.23, 7.17 and 8.23 for hydroxyl groups were also observed in the ^{1}H NMR spectrum.

The COSY-45° spectrum of **3** was used to complete ^{1}H NMR chemical shift assignments. One spin system includes the olefinic C-14 methine proton (δ 4.96), which showed cross-peaks with the olefinic methyls C-16 (δ 1.69) and

Table 1. ^{1}H and ^{13}C NMR chemical shift assignments of compounds **1**–**8**

Carbon no	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
	^{1}H, δ	^{13}C, δ	^{1}H, δ	^{13}C, δ	^{1}H, δ	^{13}C, δ	^{1}H, δ	^{13}C, δ	^{1}H, δ	^{13}C, δ	^{1}H, δ	^{13}C, δ	^{1}H, δ	^{13}C, δ	^{1}H, δ	^{13}C, δ
1	2.98	35.1	3.46	30.5	3.55	35.4	3.56	35.2	3.59	34.9	3.33	26.8	3.29	27.0	3.35	27.1
2	1.99	25.4	1.68	37.6	2.23	38.9	2.21	39.0	2.20	40.1	1.90	27.7	1.91	28.0	1.89	28.4
	1.51	–	1.29	–	1.78	–	1.76	–	1.77	–	1.40	–	1.39	–	1.42	–
3	1.90	26.7	2.85	33.9	2.99	35.3	3.01	34.9	3.03	35.1	1.93	19.6	1.90	19.2	1.91	18.9
	1.71	–	–	–	–	–	–	–	–	–	1.67	–	1.64	–	1.65	–
4	3.10	40.9	4.31	40.6	3.39	42.9	3.40	43.0	3.38	42.8	3.01	39.7	2.99	39.8	3.02	40.0
5	–	191.0	1.97	25.9	2.10	27.6	2.05	28.1	2.08	28.0	6.55	123.0	6.54	123.4	6.52	123.1
	–	–	1.39	–	1.51	–	1.49	–	1.53	–	–	–	–	–	–	–
6	–	117.4	1.88	24.3	1.98	30.1	1.95	30.0	1.96	29.9	–	128.0	–	129.9	–	128.1
	–	–	1.40	–	1.43	–	1.45	–	1.42	–	–	–	–	–	–	–
7	–	159.9	3.30	42.8	3.27	26.7	3.29	26.9	3.29	26.5	–	140.1	–	139.9	–	140.1
8	–	190.0	–	134.3	–	127.3	–	127.5	–	127.1	–	144.9	–	144.8	–	145.0
9	–	144.6	–	191.3	–	144.9	–	144.7	–	144.5	–	132.1	–	132.0	–	131.9
10	–	150.8	–	155.9	–	146.1	–	146.0	–	144.9	–	138.9	–	139.0	–	140.4
11	2.24	35.6	–	130.2	–	126.4	–	126.7	–	126.5	2.94	40.1	2.89	39.6	2.93	40.0
12	1.98	41.2	–	72.1	–	128.3	–	128.0	–	128.1	1.80	35.9	1.85	35.7	1.83	36.0
	1.29	–	–	–	–	–	–	–	–	–	1.19	–	1.20	–	1.21	–
13	2.05	39.6	–	–	–	133.9	–	134.1	–	133.8	2.10	27.1	2.08	26.9	2.11	27.0
	1.59	–	–	–	–	–	–	–	–	–	1.68	–	1.65	–	1.69	–
14	5.08	127.4	5.21	124.2	4.96	129.9	4.97	129.8	4.96	130.1	5.15	124.9	5.14	125.1	5.16	125.0
15	–	147.1	–	138.9	–	129.0	–	129.2	–	128.9	–	132.7	–	132.8	–	132.6
16	1.68	25.0	1.61	18.2	1.69	24.9	1.67	25.0	1.67	24.7	1.67	25.4	1.69	25.1	1.70	25.0
17	1.72	20.8	1.70	25.6	1.76	16.9	1.78	16.8	1.78	17.0	1.77	17.8	1.75	18.0	1.79	17.9
18	0.86	16.1	0.91	15.4	1.00	19.9	1.01	20.0	1.03	20.2	0.78	16.1	0.77	16.4	0.80	16.2
19	1.96	13.9	1.10	16.8	1.19	22.3	1.18	22.5	1.16	22.2	2.21	21.9	2.19	21.8	2.20	22.0
20	1.17	17.5	2.00	11.0	2.09	10.9	2.10	11.1	2.12	11.0	1.17	16.8	1.16	16.9	1.15	17.0
1′	–	–	–	–	5.10	105.1	5.09	104.9	5.08	105.3	5.09	103.8	5.10	104.0	5.08	104.5
2′	–	–	–	–	5.29	71.9	4.06	68.9	4.06	70.0	5.37	72.2	4.29	68.9	4.35	67.8
3′	–	–	–	–	4.07	67.8	5.34	70.6	4.02	69.9	4.27	67.9	5.32	71.9	4.26	67.5
4′	–	–	–	–	3.99	69.6	4.00	70.3	5.30	71.0	4.10	70.0	4.14	67.8	5.39	71.9
5′	–	–	–	–	4.33	63.2	4.30	62.9	4.36	63.6	4.50	67.2	4.49	67.0	4.48	66.9
	–	–	–	–	3.83	–	3.79	–	3.89	–	–	–	–	–	–	–
6′	–	–	–	–	2.21	20.9	2.19	21.0	2.20	21.4	1.29	16.1	1.28	15.9	1.30	15.8
7′	–	–	–	–	–	171.9	–	171.5	–	171.8	2.25	21.0	2.24	20.8	2.26	21.3
8′	–	–	–	–	–	–	–	–	–	–	–	170.9	–	170.7	–	171.0

C-17 (δ 1.76), and the C-1 methine (δ 3.55). The latter in turn exhibited vicinal couplings with the C-2 methylene hydrogens (δ 1.78 and 2.23), which showed cross-peaks with the C-3 methine proton (δ 2.99). This methine showed cross-peaks with the C-18 methyl (δ 1.00) and C-4 methine (δ 3.39) protons that in turn showed vicinal couplings with C-5 methylene protons (δ 1.51 and 2.10). The ^{1}H–^{1}H spin correlations of H_2-5 with the C-6 methylene protons (δ 1.43 and 1.98) were also observed in the COSY-45° spectrum. The latter showed vicinal couplings with the C-7 methine proton (δ 3.27), which in turn showed a COSY-45° interaction with the C-19 methyl (δ 1.19). A second spin system in the COSY-45° spectrum was observed for the sugar moiety. The anomeric C-1′ proton (δ 5.10) showed cross-peaks with the C-2′ methine proton (δ 5.29) that showed ^{1}H–^{1}H spin correlations with the C-3 methine (δ 4.07). H-3′ in turn exhibited cross-peaks with the C-4′ methine protons (δ 3.99) which showed cross-peaks with C-5′ methylene proton (δ 3.83 and 4.33). The presence of two isolated spin systems in the molecule was also confirmed by recording the TOCSY spectrum (100 ms).

The ^{13}C NMR spectrum of **3** showed distinct resonances for all 27 carbon atoms. Interpretation of ^{1}H, ^{13}C NMR and COSY-45° and HMQC spectral data of **3** revealed that this compound has the amphilectane-type diterpene skeleton characteristic of the pseudopterosins, with an acetylated arabinose appended at C-10. This compound is therefore a new member of the pseudopterosin family of marine natural products.[1,2] Complete ^{13}C NMR chemical shift assignments of **3** are shown in Table 1.

Key HMBC correlations of **3** are shown in Figure 3. These HMBC correlations support the assigned connectivities based on the COSY data and were used to confirm the location of the carbohydrate moiety on the aromatic ring at C-10. This was further confirmed by examining the NOESY spectrum of the aglycone methyl ether derivative prepared using the method established by Roussis et al.[2] In this spectrum, a cross-peak of the C-9 methoxy with the C-19 methyl was clearly evident. The relative stereochemistry at all chiral centers was established by further examination of the NOESY spectrum in which a *cis* relationship of the C-18 methyl, C-19 methyl and C-4 methine was evident. The C-18 methyl protons (δ 1.00) showed cross-peaks with the C-4 methine proton (δ 3.39), which also showed cross-peaks with the C-19 methyl protons (δ 1.19). The α-orientation of H-4, H_3-18 and H_3-19 was assigned on the basis of chemical shift comparison of C-18, C-19 methyl groups of compound **3** with that of other reported compounds of this series.[1,2] The C-1 methine (δ 3.55) showed cross-peaks with the C-3 methine proton (δ 2.99). It has been reported recently that the aglycone in which the 2-methyl-1-propenyl side chain has the α-orientation, H-14 resonates at δ 4.97 while the aglycone with the β-oriented side chain exhibits a downfield resonance of H-14 at δ 5.11.[14] In compound **3**, the absence of an NOE between H-1 and H_3-18 and the observed chemical shift of H-14 (δ 4.96) suggests the β-stereochemistry for H-1 and thus the α-orientation of the 2-methyl-1-propenyl side chain.

After complete structure determination of the diterpene portion of **3**, the carbohydrate moiety was identified as an arabinose derivative by comparison of the ^{1}H and ^{13}C NMR chemical shifts of the sugar moiety with literature values.[1,2] The D- configuration of the sugar was established by comparing the optical rotation of the sugar solution that was obtained by hydrolysis of compound **3** using an established protocol.[1,2] The negative optical rotation of the arabinose sugar solution generated from **3** was identical to that of a standard sample of D-arabinose which had been treated with a similar amount of acid.

Pseudopterosin N (**4**), was also isolated as a gum. Its UV, IR, and MS spectra were essentially identical to those of pseudopterosin M. The ^{1}H NMR and ^{13}C NMR spectra were also similar to that of compound **3** with the exception of the pentose methyl region. The ^{1}H NMR spectrum of the sugar moiety showed a downfield resonance for the C-3′ methine proton of δ 5.34 which showed cross-peaks with the C-2′ (δ 4.06) and C-4′ (δ 4.00) methine protons in the COSY-45° spectrum. This suggested the presence of an acetoxy group at C-3′ in the sugar moiety. Complete ^{1}H and ^{13}C NMR chemical shift assignments of compound **4** are presented in Table 1. Combination of ^{1}H, ^{13}C, COSY, HMQC, HMBC and NOESY spectral data established structure **4** for this new pseudopterosin.

The third compound in this series, pseudopterosin O (**5**), was isolated as yellow gum. The IR, UV, MS and NMR spectral data of **5** was nearly identical to those of **3** and **4**. Again, the difference in the ^{1}H NMR spectrum was in the

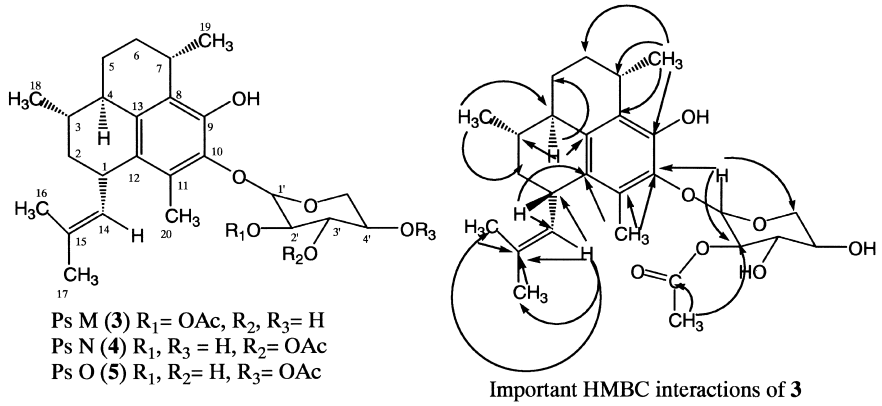


Figure 3. Pseudopterosins M–O (**3**–**5**).

carbohydrate region which indicated that compound **5** was the C-4′ acetoxy derivative. This was evident from the observation of a resonance at δ 5.30 which was assigned to the C-4′ proton geminal to the acetoxy group and showed COSY-45° interactions with C-3′ methine (δ 4.02) and C-5′ methylene (δ 4.36 and 3.89) protons. Complete ^{1}H- and ^{13}C NMR chemical shift assignments for compound **5** are shown in Table 1.

A sixth compound, seco-pseudopterosin E (**6**), was isolated as a colorless gum. Compound **6** showed a molecular ion peak at *m/z* 490.3037, which is in agreement with molecular formula $C_{28}H_{42}O_7$ (calcd 490.3031) and indicated the presence of eight degrees of unsaturation in the molecule. Its UV spectrum exhibited maximum absorptions at 281 and 221 nm. The IR spectrum displayed intense absorption bands at 1680 and 3400 (OH) cm^{-1}.

The ^{1}H NMR spectrum of **6** was similar to that reported for seco-pseudopterosins A–D isolated from *P. kallos*.[3] The COSY-45° spectrum of compound **6** indicated the presence of two isolated spin systems in the molecule and provided confirmation that this compound was a seco-pseudopterosin. One spin system included the C-14 olefinic proton (δ 5.15), which showed cross-peaks with the C-13 methylene (δ 1.68 and 2.10), C-16 methyl (δ 1.67) and C-17 methyl (δ 1.77) protons. The C-13 methylene showed vicinal couplings with the C-12 methylene (δ 1.19 and 1.80), which in turn showed COSY-45° interactions with the C-11 methine proton (δ 2.94). The latter also displayed ^{1}H–^{1}H spin correlations with the C-4 methine (δ 3.01) and the C-18 methyl protons (δ 0.78). The C-4 methine showed vicinal coupling with the C-3 methylene protons (δ 1.67 and 1.93) which in turn showed cross-peaks with the C-2 methylene (δ 1.40 and 1.90). The latter showed cross-peaks with the C-1 methine proton (δ 3.33), which in turn exhibited cross-peaks with the C-20 methyl group (δ 1.17). The second spin system was traced from the C-1′ methine proton (δ 5.09) which showed cross-peaks with the C-2′ methine proton (δ 5.37) which further exhibited vicinal coupling with the C-3′ methine proton (δ 4.27). The C-3′ methine showed cross-peaks with the C-4′ methine proton (δ 4.10) which in turn had a correlation with the C-5′ methine proton (δ 4.50). The latter showed a cross-peak with the C-6′ methyl (δ 1.29).

The HMBC spectrum of **6** showed cross-peaks of the C-14 olefinic proton (δ 5.15) with C-13 (δ 27.1), C-15 (δ 132.7), C-16 (δ 25.4) and C-17 (δ 17.8). HMBC interactions of the C-18 methyl protons (δ 0.78) with C-3 (δ 19.0), C-4 (δ 39.7) and C-11 (δ 40.1) were also observed. These HMBC observations support the assigned connectivities based on the COSY data. The C-4 methine proton (δ 3.01) showed long-range hetero-nuclear couplings with C-3 (δ 19.0), C-5 (δ 123.0), C-10 (δ 138.9), and C-11 (δ 40.1), while the C-20 methyl protons (δ 1.17) showed cross-peaks with C-1 (δ 26.8), C-2 (δ 27.7), and C-9 (δ 132.1), thus locating the aromatic ring. The C-1′ methine proton (δ 5.09) showed cross-peaks with C-2′ (δ 72.2), C-7 (δ 140.1).

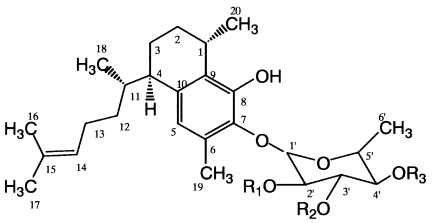
From the above analysis, and comparison with spectroscopic data for previously characterized seco-pseudopterosins,[3] it is evident that compound **6** is a new member of the seco-pseudopterosin family of diterpenes. The location of the carbohydrate moiety on the serrulatane skeleton was addressed using HMBC and NOESY. Cross-peaks for the C-19 methyl protons (δ 2.21) with C-8 (δ 132.1) in the HMBC spectrum suggested the presence of the sugar moiety at C-7. This was further confirmed by a NOESY spectrum of the methyl ether derivative of compound **6**, which was prepared by a previously described method.[2] The strong NOE between the C-20 methyl protons and the C-8 methoxy further confirmed the presence of sugar moiety at C-7.

The relative stereochemistry in compound **6** was determined by analysis of the NOESY spectrum, in which a *cis* relationship between the C-4 methine, C-18 methyl and C-20 methyl groups was evident. The chemical shifts of H-4, H_3-18 and H_3-20 were essentially identical to those of seco-pseudopterosins A–D previously reported.[3] On the basis of these observations and NOESY data, the α-orientation for H-4, H_3-18 and H_3-20 was assigned. The identity of the carbohydrate in compound **6** was determined to be a fucose by comparing the ^{1}H and ^{13}C NMR chemical shifts of the sugar residue with that of pseudopterosins containing a fucose.[2] The diaxial coupling constant (8.9 Hz) of H-1′ and H-2′ indicated the β-linkage between C-1′ and C-7. The L configuration of the fucose was assigned on the basis of a strong negative optical rotation of the sugar obtained by the hydrolysis of **6**.

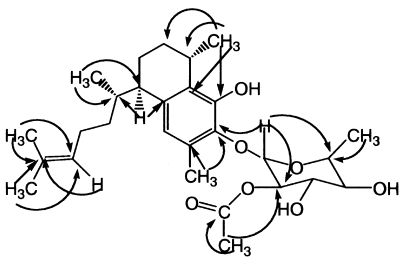
The two additional seco-pseudopterosins F and G (**7**, **8**) were found to have very similar UV, IR, MS, ^{1}H and ^{13}C NMR spectra to those of **6**. Compounds **7** and **8** were found to differ in the point of attachment of the acetoxy group in the fucose residue. In compound **7**, the acetoxy group was found to be present at C-3′, based on the COSY-45° spectrum in which the C-3′ methine proton (δ 5.32), geminal to the acetoxy group, showed cross-peaks with the C-2′ methine (δ 4.29) and the C-4′ methine proton (δ 4.14). In compound **8**, the acetoxy group was assigned to C-4′ of the fucose moiety based on analogous COSY data. Complete ^{1}H and ^{13}C NMR chemical shift assignments of compounds **7** and **8** are shown in Table 1.

Compounds **1**–**8** (Fig. 4) were evaluated in a mouse ear anti-inflammatory assay. Each compound was topically applied in acetone to the inside pinnae of the ear of a mouse in a solution containing the edema-causing irritant, PMA. A solution of 2 μg per ear of PMA alone or 25 μg per ear of test compound in combination with PMA was applied to the left ear of each mouse and acetone (control) was applied to the right ear of each mouse. There were five mice per treatment group including a control group. Following a 3 h and 20 min incubation, the mice were euthanized and 7 mm ear biopsies weighed. Edema (inflammation) was measured by subtracting the weight of the right, untreated biopsy, from the left treated biopsy. Results were recorded as percent decrease (inhibition) or percent increase (potentiation) in edema relative to a concurrent PMA control group. As shown in Table 2, compound **2** produced only a 9% inhibition, whereas compounds **1**, **4** and **6** exhibited the highest percent inhibition (83, 88, and 88%, respectively).

For each of the most active compounds from the screening assay, a dose response curve was obtained and the ED_{50} and



Seco-E (**6**) R_1= OAc, R_2, R_3= H
Seco-F (**7**) R_1, R_3 = H, R_2= OAc
Seco-G (**8**) R_1, R_2= H, R_3= OAc



Important HMBC interactions of **6**

Figure 4. Seco-pseudopterosins E–G (**6**–**8**).

relative potency to PsA and PsE computed. Statistical significance ($p<0.05$) was estimated from the potency ratios using established statistical methods.[15] All three active compounds exhibited potencies equal to or somewhat greater than PsA and were significantly more potent when compared to PsE ($p<0.05$) (Table 3).

3. Conclusions

The activity data indicates that the new pseudopterosins and seco-pseudopterosins identified in this Floridian sample of *P. elisabethae* have similar or slightly superior activity to that of the previously characterized members of these families of marine natural products. It is therefore apparent that the identity and the location of the carbohydrate moiety, as well its degree of acetylation do not greatly affect the anti-inflammatory activity. The potent activity of elisabethadione (**1**) and lack of any significant activity of elisabethol (**2**) are somewhat surprising results. The structure of hydroxyquinone **1** is clearly only distantly related to that of the pseudopterosins and seco-pseudopterosins and thus it is not clear that **1** acts by the same mechanism as metabolites **3**–**8**. Compounds **1** and **2** have both been shown to be biosynthetic intermediates leading to the pseudopterosins.[11] Our anti-inflammatory data thus indicates that the identification of a biosynthetic intermediate, compound **1**, has led to the discovery of a more potent metabolite than the parent pseudopterosins. The structural simplicity of **1**, together with the bioactivity data, indicates that this biosynthetic intermediate may be a useful anti-inflammatory agent.

This report also describes for the first time the co-occurrence of pseudopterosins with seco-pseudopterosins, diterpenes with the amphilectane and serrulatane skeletons, respectively. Their co-occurrence, together with our previously described isolation of elisabethatriene as the sole diterpene cyclase product in *P. elisabethae*,[10] suggests that both the amphilectane and serrulatane families of diterpene are derived from the same geranylgeranyl diphosphate cyclase product. Experiments are currently underway to fully describe the biosynthesis of diterpenes in *P. elisabethae*.

Table 2. Anti-inflammatory screen of diterpenes **1**–**8**

Treatment	Dose	*N*	Edema (mg±sem)	% Inhibition
Control group	2 μg/ear PMA	5	6.7±1.1	–
Compound **1**	25 μg/ear	5	1.2±0.1	83[a]
Compound **2**	25 μg/ear	5	6.2±0.6	9
Compound **3**	25 μg/ear	5	2.1±0.5	68[a]
Compound **4**	25 μg/ear	5	0.8±0.2	88[a]
Compound **5**	25 μg/ear	5	0.9±0.2	69[a]
Compound **6**	25 μg/ear	5	0.8±0.2	88[a]
Compound **7**	25 μg/ear	5	2.4±0.6	65[a]
Compound **8**	25 μg/ear	5	1.8±0.3	74[a]

[a] Statistically significant difference relative to PMA treated group ($p<0.01$) unpaired student's *t* test.

Table 3. ED_{50} and potency of most active diterpenes (**1**, **4**, and **6**)

Compound	*N*	ED_{50} (μg/ear)	95% C.L.[a]		Potency ratio	
			Lower	Upper	PsA	PsE
PsA	25	14.6	12.3	17.3	1.0	2.8[b]
PsE	25	41.0	35.0	49.0	0.4[b]	1.0
1	25	14.6	6.9	30.8	1.0	2.8[b]
4	25	9.7	5.6	17.0	1.5	4.2[b]
6	25	10.4	5.7	18.9	1.4	4.0[b]

[a] C.L.=95% confidence limits of ED_{50}.
[b] Statistically significant difference in potency ratio ($p<0.05$) unpaired probit analysis.

4. Experimental

4.1. General

Optical rotations were measured on a Jasco polarimeter. The UV spectra were recorded on a Shimadzu UV 240 instrument. The IR spectra were recorded on a Galaxy FT-IR spectrophotometer. The ^{1}H NMR spectra (one- and two-dimensional) were recorded in $CDCl_3$ on a Varian 500 NMR spectrometer at 500 MHz, while ^{13}C NMR spectrum was recorded on the same instrument at 125 MHz. Mass spectral measurements were conducted at the Midwest Center for Mass Spectrometry at University of Nebraska-Lincoln. TLC was performed using silica gel, GF_{254} pre-coated Plate and HPLC was performed using a Perkin–Elmer Series 410 pump with a Hitachi UV detector monitoring 265 nm using gradient elution of acetonitrile–water (90–10) to 100% acetonitrile on semipreparative reversed-phase C18 column (Vydac).

4.2. Collections

P. elisabethae was collected off Long Key, Florida during

August 1999 by SCUBA at a depth of 25 m. The organism was identified as *Pseudopeterogorgia elisabethae* by Frederick M. Bayer, Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian. A voucher specimen (USNM100430) has been deposited in this institute.

4.3. Extraction and isolation

P. elisabethae (997 g) was freeze-dried and extracted with methanol and then two times with chloroform. The solvent was evaporated under reduced pressure to prepare a gum (360 g). This gum was then re-dissolved in 60% aqueous methanol. This aqueous alcoholic extract was partitioned to give a hexane extract (201.9 g). The defatted extract was then partitioned between chloroform and water and the resulting organic extract (11.43 g) loaded on to silica gel column and eluted with hexanes-ethyl acetate (0–100%) and ethyl acetate–methanol (0–100%). Three fractions F-1, F-2, and F-3 were obtained on elution of silica gel column with hexane-ethyl acetate (75:25), (10:90), and ethyl acetate–methanol (90:10), respectively. Fraction F-1 was subjected to repeated reversed-phase HPLC using a gradient of acetonitrile–water (80–100%) as mobile phase to purify compounds **1** (4.9 mg), **3** (14.1 mg), **4** (11.2 mg) and **5** (7.9 mg). Fraction F-2 was also chromatographed over reversed-phase HPLC using a gradient of acetonitrile–water (60–100%) to isolate compounds **6** (9.7 mg), **7** (6.7 mg) and **8** (5.9 mg), while compound **2** (4.9 mg) was purified from fraction F-3 using the same conditions as described for compounds **6**–**8**.

4.3.1. Elisabethadione (1). $[\alpha]_D^{20}$=93.3°; UV (MeOH) λ_{max} 248 and 326 nm; IR ($CHCl_3$) ν_{max} cm^{-1}: 1690 (C=O), 3395 (OH); ^1H and ^{13}C NMR (Table 1); HREIMS *m/z* 316.2034 [calcd for $C_{20}H_{28}O_3$ $(M)^+$, 316.2038].

4.3.2. Elisabethol (2). UV (MeOH) λ_{max} 240 nm; IR ($CHCl_3$) ν_{max} cm^{-1}: 1705 (C=O), 3512 (OH); ^1H and ^{13}C NMR (Table 1); HREIMS *m/z* 316.2032 [calcd for $C_{20}H_{28}O_3$ $(M)^+$, 316.2038].

4.3.3. Pseudopterosin M (3). $[\alpha]_D^{20}$=−85°; UV (MeOH) λ_{max} 226 and 282 nm; IR ($CHCl_3$) ν_{max} cm^{-1}: 1675 (C=C), 1710 (ester carbonyl), 3435 (OH); ^1H and ^{13}C NMR (Table 1); HREIMS *m/z* 474.2631 [calcd for $C_{27}H_{38}O_7$ $(M)^+$, 474.2618].

4.3.4. Pseudopterosin N (4). $[\alpha]_D^{20}$=−87°; UV (MeOH) λ_{max} 226 and 281 nm; IR ($CHCl_3$) ν_{max} cm^{-1}: 1673 (C=C), 1707 (ester carbonyl), 3430 (OH); ^1H and ^{13}C NMR (Table 1); HREIMS *m/z* 474.2629 [calcd for $C_{27}H_{38}O_7$ $(M)^+$, 474.2618].

4.3.5. Pseudopterosin O (5). $[\alpha]_D^{20}$=−75°; UV (MeOH) λ_{max} 226 and 279 nm; IR ($CHCl_3$) ν_{max} cm^{-1}: 1675 (C=C), 1701 (ester carbonyl), 3434 (OH); ^1H and ^{13}C NMR (Table 1); HREIMS *m/z* 474.2615 [calcd for $C_{27}H_{38}O_7$ $(M)^+$, 474.2618].

4.3.6. Seco-pseudopterosin E (6). $[\alpha]_D^{20}$=−102°; UV (MeOH) λ_{max} 221 and 281 nm; IR ($CHCl_3$)ν_{max} cm^{-1}: 1680 (C=C), 1701 (ester carbonyl), 3400 (OH); ^1H and ^{13}C NMR ($CDCl_3$, 125 MHz) δ: see Table 1; HREIMS *m/z* 490.3037 [calcd for $C_{28}H_{42}O_7$ $(M)^+$, 490.3031].

4.3.7. Seco-pseudopterosin F (7). $[\alpha]_D^{20}$=−55°; UV (MeOH) λ_{max} 223 and 283 nm; IR ($CHCl_3$) ν_{max} cm^{-1}: 1683 (C=C), 1705 (ester carbonyl), 3402 (OH); ^1H NMR ($CDCl_3$, 500 MHz) δ: see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ: see Table 1; HREIMS *m/z* 490.3139 [calcd for $C_{28}H_{42}O_7$ $(M)^+$, 490.3031].

4.3.8. Seco-pseudopterosin G (8). $[\alpha]_D^{20}$=−124°; UV (MeOH) λ_{max} 224 and 280 nm; IR ($CHCl_3$) ν_{max} cm^{-1}: 1680 (C=C), 1710 (ester carbonyl), 3390 (OH); ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ: see Table 1; HREIMS *m/z* 490.3130 [calcd for $C_{28}H_{42}O_7$ $(M)^+$, 490.3031].

4.4. Hydrolysis of Ps M–O and seco-E–G

Compounds **3**–**8** (3 mg, each) were individually dissolved in 5 mL methanol containing 1N HCl (5 mL). The solution was stirred at 60°C for 4–5 h until the starting material had been completely consumed (TLC). The methanol was removed under reduced pressure and the aqueous layer was extracted with dichloromethane to remove aglycone moiety and any unreacted glycosides. The strong negative optical rotations of the aqueous layers indicated the presence of D-arabinose in pseudopterosins (**3**–**5**) and L-fucose in seco-pseudopterosins (**6**–**8**).

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