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Pseudopterosins P–V, new compounds from the gorgonian octocoral *Pseudopterogorgia elisabethae* from Providencia island, Colombian Caribbean

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Abstract—Seven new diterpene glycosides, pseudopterosins P (**1**), Q (**2**), R (**3**), S (**4**), T (**5**), U (**6**) and V (**7**) along with two known compounds PsG and PsK have been isolated from the methanol/dichloromethane extract of the gorgonian octocoral *Pseudopterogorgia elisabethae* collected off Providencia Island, Colombian Caribbean. The structures of the new metabolites, including their relative and absolute configuration, were established by MS and NMR spectroscopic studies as well as their conversion to known compounds.

1. Introduction

Pseudopterosins are an interesting group of diterpene glycosides first discovered by Fenical and collaborators[1] about 15 years ago from specimens of the gorgonian coral *Pseudopterogorgia elisabethae*. So far, 15 pseudopterosins (PsA–PsO) isolated from specimens collected in the Bahamas,[1] Bermuda,[2] and the Florida Keys[3] have been reported. The structurally related secopseudopterosins A–D have also been identified in *Pseudopterogorgia kallos* collected near the Marquesas Keys in Florida.[4] These pseudopterosins and secopseudopterosins exhibit more potent antiinflammatory and analgesic activities than the common market drug indomethacin.[5,6] It is suggested that the mechanism of action of the pseudopterosins may involve membrane stabilization, different from inhibition of eicosanoid release from inflammatory cells mediated by traditional non-steroidal drugs.[6] Due to their excellent antiinflammatory and analgesic activity, partially purified extracts containing pseudopterosins are currently incorporated into skin care preparations.[7] The availability of pseudopterosins however, is limited by the actual supply of organic extracts of *Pseudopterogorgia elisabethae* which currently only comes from the Bahamas islands. The complex and expensive chemical synthesis of these compounds makes these animals an attractive study target in other areas of the Caribbean.

As part of our continuous search for biologically active compounds from Colombian marine invertebrates,[8,9] we have recently examined *Pseudopterogorgia elisabethae* specimens from San Andrés and Providencia islands from the Colombian Caribbean, finding distinct chemotypes regarding to the pseudopterosin content, in each island.[10] Samples from Providencia island afforded seven new pseudopterosins, together with the known pseudopterosins G and K. In this paper, we report the isolation and structure elucidation of the seven new pseudopterosins.

2. Results and discussion

Pseudopterogorgia elisabethae specimens were collected at Providencia island, Colombian Caribbean, and air-dried. Animal tissue was extracted with a $MeOH/CH_2Cl_2$ (1:1) mixture and the extract was separated on silica gel CC and reversed phase HPLC to yield seven new compounds which we named pseudopterosins P–V (**1**–**7**), along with the known compounds pseudopterosins G[2] and K.[2]

Pseudopterosin-P (**1**) showed a molecular ion at *m/z* 446 and intense fragment ions at *m/z* 300 and 244. The former fragment ion corresponds to the pseudopterosin aglycone, suggesting the loss of a deoxyhexose (146 mass unit) and

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Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) data for compounds **1–7**

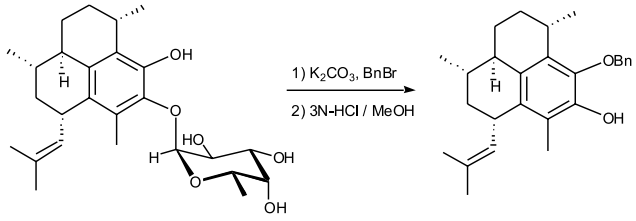
H No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	3.66 q-like (8.5)	3.66 q-like (8.6)	3.67 q-like (8.5)	3.66 q-like (8.7)	3.62 q-like (8.7)	3.67 m	3.67 q (8.7)
2	1.93 dd (10.5, 7.8)	1.93 m	1.93 m	1.94 m	1.90 dd (10.2, 6.3)	1.23 m	1.22 m
	1.21 m	1.22 m	ca. 1.22 m	ca. 1.21 m	ca. 1.22 m	2.25 m	1.93 m
3	1.22 m	1.22 m	ca. 1.22 m	ca. 1.23 m	ca. 1.23 m	1.22 m	1.20 m
4	2.05 m	2.06 m	ca. 2.05 m	ca. 2.05 m	ca. 2.02 m	2.05 m	2.05 m
5	0.92 qd (12.7, 3.2)	0.93 qd (12.7, 3.2)	0.93 qd (12.7, 3.2)	0.93 qd (12.7, 3.0)	0.88 qd (12.7, 3.2)	0.92 qd (12.7, 3.2)	0.93 qd (12.7, 3.3)
						2.02 m	2.05 m
6	1.31 m	1.32 m	1.32 m	1.32 m	1.30 m	1.31 m	1.33 m
	2.13 m	2.14 m	2.14 m	2.15 m	2.08 m	2.13 m	2.15 m
7	3.19 sextet-like (7.0)	3.20 sextet-like (7.2)	3.20 sextet-like (7.0)	3.19 sextet-like (7.2)	3.12 sextet-like (7.2)	3.19 m	3.20 sextet-like (7.1)
14	4.98 br d (9.3)	4.97 d (9.1)	4.99 br d (9.2)	4.93 d (9.2)	4.93 br d (9.3)	4.96 d (9.1)	4.98 br d (11.5)
16	1.67 s	1.67 s	1.67 s	1.67 s	1.64 s	1.66 s	1.67 d (1.0)
17	1.71 s	1.71 s	1.72 s	1.72 s	1.68 s	1.72 s	1.72 d (1.5)
18	1.02 d (6.0)	1.02 d (5.9)	1.02 d (6.0)	1.03 d (5.9)	1.00 d (5.6)	1.02 d (5.9)	1.02 d (7.5)
19	1.25 d (6.8)	1.27 d (6.8)	1.26 d (6.8)	1.25 d (6.8)	1.18 d (6.6)	1.27 d (1.8)	1.26 d (8.5)
20	2.07 s	2.05 s	2.08 s	2.10 s	1.97 s	2.06 s	2.09 s
1′	5.11 d (3.7)	5.14 d (3.8)	5.18 d (4.0)	5.12 d (3.7)	5.10 d (2.5)	5.18 d (3.6)	5.22 d (3.6)
2′	4.03 dd (9.5, 3.7)	4.04 dd (10.1, 3.8)	4.30 ddd (10.4, 4.0, 3.0[a])	5.24 dd (10.4, 3.7)	4.17 br d (7.3)	4.10 dd (9.9, 3.6)	4.33 dd (10.0, 3.8)
3′	4.13 br d (9.5)	4.35 dd (10.1, 3.3)	5.29 dd (10.4, 3.0)	4.26 dd (10.4, 3.2)	4.12 br d (7.3)	4.32 dd (9.9, 3.4)	5.31 dd (10.0, 3.8)
4′	3.89 br	5.21 br d (3.3)	4.06 br s	3.97 br d (3.2)	4.08 br s	5.24 m	4.27 m
5′	4.52 q (6.7)	4.55 q (6.6)	4.58 q (6.6)	4.56 q (6.6)	4.25 d (12.6)	4.36 br d (13.0)	4.42 dd (16.0, 1.5)
					3.78 d (12.6)	3.89 dd (13.0, 1.9)	3.87 dd (16.0, 3.0)
6′	1.33 d (6.7)	1.17 d (6.6)	1.33 d (6.6)	1.39 d (6.6)			
Ac		2.19 s	2.21 s	2.23 s		2.16 s	2.22 s

[a] The coupling is due to hydroxyl group present at C-2′.

m/z 244 the aglycone after loosing an isopropylidene unit. Its molecular formula, $C_{26}H_{38}O_6$, was determined by HREIMS. The UV spectrum showed maximum absorptions at 213, 232, 276, and 285 nm due to the presence of a substituted benzene ring. The ^{1}H and ^{13}C NMR data of **1** (Tables 1 and 2) suggested that **1** belongs to pseudopterosin family.[1–4] The ^{1}H NMR spectrum of **1** showed signals of two singlet methyls at δ 1.67 (H_3-16) and 1.71 (H_3-17), a singlet methyl substituted on an aromatic ring at δ 2.07 (H_3-20), two doublet methyls at δ 1.02 (H_3-18) and 1.25 (H_3-19) and an olefinic proton at δ 4.98 (H-14) for the aglycone part. The ^{13}C NMR spectrum of **1** showed 26 signals, among which 20 resonances including six aromatic carbons at δ 126.8, 127.8, 129.2, 136.6, 143.2, and 145.2 were assigned to the aglycone. Further analysis of ^{1}H signals, assisted with H–H COSY spectra, allowed assigning all proton signals. In particular, the chemical shifts and coupling patterns of H-1 (δ 3.66, q-like, J=8.5 Hz), H-7 (δ 3.19, sextet-like, J=7.0 Hz) and H-14 (δ 4.98, br d, J=9.3 Hz) suggested 1S^*,3S^*,4R^*,7S^* configuration for the aglycone, since the data were close to the values reported for the aglycone derivative with the known configuration.[2,11]

The sugar portion showed signals of an anomeric proton, H-1′ (δ 5.11), a doublet methyl (δ 1.33) assignable to H_3-6′ and four oxymethine protons. Decoupling experiments and correlations in the H–H COSY spectrum established the assignments of H-2′ (δ 4.03), H-3′ (δ 4.13), H-4′ (δ 3.89), and H-5′ (δ 4.52). $J_{H\text{-}2',H\text{-}3'}$ and $J_{H\text{-}2',H\text{-}3'}$ values were 3.7 and 9.5 Hz, respectively, and H-4′ was observed as a broad singlet. These data evidenced axial–axial relationships for H-2′ and H-3′ and equatorial orientation of H-1′ and H-4′, suggesting α-fucose as a sugar structure. Furthermore, the ^{13}C data for the sugar moiety of **1** were in good agreement with those of a fucose-substituted pseudopterosin, pseudopterosin-E[2] which is a C-1 epimer of **1**. Thus, the sugar moiety was determined as α-fucopyranoside. That the sugar was linked to C-10 rather than C-9 was initially deduced from comparison of the ^{13}C data of **1** with those of compound **2**.

We then investigated the absolute configuration of the aglycone and fucose moieties of **1**, since **1** was available in good quantity and not acetylated. It should be noted that the occurrence of an antipode of pseudopterosin aglycone was reported in pseudopterosins K and L (1*S*,3*R*,4*S*,7*R*-aglycone, compared to an usual 1*R*,3*S*,4*R*,7*S*-aglycone).[2] For this purpose, **1** was benzylated and the resulting 9-benzyl ether derivative was hydrolyzed under acidic conditions to give the aglycone 9-benzyl ether (Scheme 1). Fortunately, Lazerwith et al.[11] reported the synthesis of the same ether of the aglycone with known 1*S*,3*S*,4*R*,7*S* absolute configuration together with the NMR and optical data. The NMR data of the aglycone 9-benzyl ether derived from **1** were in



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Scheme 1. Conversion of pseudopterosin-P (**1**) to the C-9 benzyl ether derivative.

Table 2. ^{13}C NMR (500 MHz, $CDCl_3$) spectral data of compounds **1–7**

Carbon No.	1	2	3	4	5	6	7
1	37.3	37.3	37.3	37.3	37.3	37.3	37.3
2	40.2	40.2	40.2	40.1	40.2	40.2	40.2
3	33.9	33.9	33.9	33.9	33.9	33.9	33.9
4	44.6	44.7	44.6	44.7	44.6	44.7	44.7
5	27.8	27.8	27.8	27.8	27.8	27.8	27.8
6	31.7	31.8	31.8	31.9	31.7	31.8	31.8
7	28.4	28.4	28.5	28.6	28.4	28.5	28.5
8	126.8	126.9	127.0	126.7	126.8	126.9	127.1
9	145.2	145.2	145.0	144.5	145.0	145.2	145.0
10	143.2	143.2	143.1	143.0	143.1	143.1	142.9
11	127.8	127.8	127.6	127.3	127.9	127.8	127.6
12	136.6	136.7	136.6	136.6	136.6	136.8	136.7
13	129.2	129.3	129.2	129.8	129.3	129.2	129.2
14	131.3	131.4	131.4	131.2	131.3	131.3	131.4
15	128.1	128.2	128.2	128.4	128.1	128.2	128.2
16	25.4	25.4	25.4	25.4	25.4	25.4	25.5
17	17.5	17.5	17.5	17.5	17.5	17.5	17.5
18	20.1	20.1	20.1	20.0	20.0	20.1	20.1
19	23.3	23.3	23.2	23.1	23.3	23.2	23.2
20	13.5	13.6	13.5	13.7	13.8	13.9	13.8
1′	103.0	103.0	102.8	101.3	103.8	103.1	103.1
2′	70.4	69.5	70.4	72.3	69.5[a]	70.0	68.1
3′	69.6	69.1	74.3	68.8	69.5[a]	68.2	73.3
4′	72.2	73.6	67.7	71.4	69.4[a]	71.5	67.5
5′	67.3	66.3	67.1	67.4	64.0	61.9	63.7
6′	16.2	16.3	16.1	16.1			
Ac		20.8	20.8	21.0		21.1	21.1
		171.9	171.7	170.9		171.3	171.6

Assignments for compound **2** and **6** were based on ^{1}H–^{1}H COSY, DEPT, HMQC and HMBC spectra, while the other compounds were assigned from their analogy and based on ^{1}H–^{1}H COSY and DEPT spectra.

[a] Assignments may be reversed.

excellent agreement with those reported.[11] This unequivocally determined the relative stereochemistry at the chiral centers of **1**. Further, the sign and magnitude of the α value for the ether were similar to the reported values, thus establishing the absolute configuration of the aglycone as 1*S*,3*S*,4*R*,7*S*. Conversion of **1** to the known benzyl ether also proved the position of glycosylation.

Fucose was identified by TLC in the aqueous phase of the above acidic hydrolysis. Chirality of the fucose sample was analyzed by the method of Hara et al.[12] GLC analysis of the trimethylsilyl ether of the corresponding methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylate derivative revealed that the fucose is in L-form. The results agree with earlier findings in which fucose-bearing pseudopterosins utilize the L-form of fucose.[2,3,11] The structure of compound **1** was, therefore, established to be as shown in Figure 1, which depicts the correct absolute configuration.

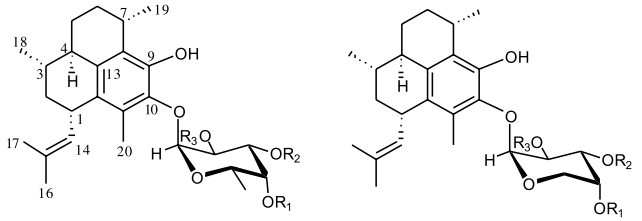

Figure 1. New pseudopterosins isolated from *Pseudopterogorgia elisabethae* from Providencia island.

Pseudopterosin Q (**2**) showed a molecular ion at *m*/*z* 488 and intense fragment ions at *m*/*z* 300 and 244 in EI-MS. The former fragment ion corresponds to the pseudopterosin aglycone, suggesting a loss (188 mass unit) of a monoacetylated deoxyhexose. The ion at *m*/*z* 244 could be due to a loss of an isopropylidene unit from the aglycone. The molecular formula of **2** was established as $C_{28}H_{40}O_7$ by HREIMS. The general characteristics of the UV and NMR data (Tables 1 and 2) suggested that **2** belongs to the pseudopterosin family too. The ^{1}H and ^{13}C NMR spectra of **2** resembled those of **1**, but with an additional signal for an acetyl group. It is obvious from the similarity of the NMR data for the aglycone part of **1** and **2** that compound **2** has the aglycone with the same stereochemistry as in compound **1**.

The ^{1}H NMR spectrum of **2** exhibited signals of an anomeric proton (δ 5.14), a doublet methyl assignable to H_3-6′ (δ_H 1.17) and an acetyl methyl (δ 2.19) along with four oxymethine proton signals for the sugar moiety. The anomeric proton was coupled to H-2′ (δ 4.04) with J=3.8 Hz, which in turn coupled to H-3′ (δ 4.35) with J=10.1 Hz. H-3′ further coupled to H-4′ (δ 5.21) with J=3.3 Hz. The downfield shift of H-4′ evidenced that the acetoxy group was attached to C-4 of the sugar. The large coupling constant between H-2′ and H-3′ indicated axial–axial relationships for these protons, while H-1′ and H-4′ must bear equatorial orientation. These data established an α-glycosidic linkage. H-5′ (δ 4.55, q, J=6.6 Hz), coupled to H_3-6, was sharpened by a decoupling experiment irradiating H-4′, although the J value between H-4′ and H-5′ was nearly zero. Further, an NOE correlation was observed from H-3′ to H-5′, indicating an axial orientation of H-5′. Thus, the sugar moiety was unambiguously established as 4-*O*-acetyl-α-L-fucopyranoside (L configuration of the sugar was assigned by converting **2** to peracetylated derivative (vide infra)). The ^{13}C NMR signals for the sugar, assigned by HMQC spectrum, were in good agreement with those of pseudopterosin J which has an 4-*O*-acetyl-α-fucopyranoside moiety.[2] Finally, the sugar was linked to the C-10 position on the basis of an HMBC correlation from the anomeric proton to C-10 (δ_C 143.2). The structure of compound **2** was established to be as shown in Figure 1, which depicts the correct absolute configuration. The ^{13}C signals were completely assigned with the aid of the HMBC spectrum (Fig. 2). Pseudopterosin-Q is a regioisomer of pseudopterosin-J[2] which is glycosylated at C-9. Originally assigned 1*R*,3*S*,4*R*,7*R* stereochemistry of pseudopterosin-J was revised to 1*S*,3*S*,4*R*,7*S* by synthetic work.[11]

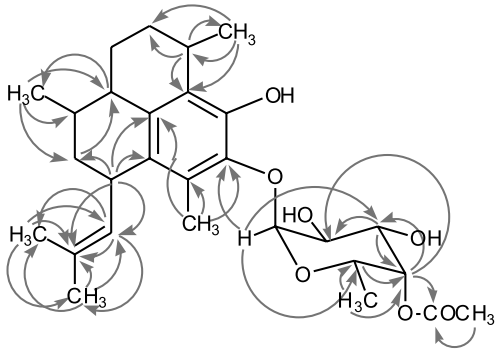

Figure 2. HMBC correlations from H to C for pseudopterosin-Q (**2**).

Pseudopterosin-R (**3**) showed a molecular ion at *m*/*z* 488 and intense fragment ions at *m*/*z* 300 and 244 in EI-MS. The molecular formula, $C_{28}H_{40}O_7$, was deduced from HREIMS. Analysis of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) indicated that the aglycone structure of **3** is identical to that of **2**. It became also clear from ^{13}C NMR data that the C-10 phenol is glycosidated. Compound **3** showed an anomeric proton at δ 5.18 (d) which was coupled to H-2′ (δ 4.30) with J=3.0 Hz. The latter was coupled to H-3′ (δ 5.29) with J=10.4 Hz, which was then coupled to H-4′ (δ 4.06) with J=3.0 Hz. H-5′ (δ 4.58, q, 6.6 Hz) was coupled to a doublet methyl at δ 1.33. $J_{H\text{-}4',H\text{-}5'}$ value was nearly zero. The acetyl methyl signal resonated at δ 2.21. These data established that the sugar moiety at C-10 of **3** is 3-*O*-acetyl-α-fucopyranoside. Chirality of 3-*O*-acetyl-fucose was determined to be L, since **3** and **1** furnished the same peracetate (vide infra). Hence, the structure of **3** was established to be as shown in Figure 1. Pseudopterosin-R is a regioisomer of pseudopterosin-I[2] which is glycosylated at C-9 (initially reported 1*R*,3*S*,4*R*,7*R* configuration was revised to 1*S*,3*S*,4*R*,7*S*[11]).

Pseudopterosin-S (**4**) showed a molecular ion at *m*/*z* 488 in EI-MS and its molecular formula was determined to be $C_{28}H_{40}O_7$ by HREIMS. The NMR data revealed that compound **4** is an isomer of **2** and **3** in terms of the position of the acetyl group. Signals of an anomeric proton, a doublet methyl and acetyl methyl were observed at δ 5.12

(d, J=3.7 Hz), 1.39 and 2.23, in that order. The H–H COSY spectrum allowed us to connect oxymethine protons to form the network: H-1′–H-2′ (δ 5.24, dd, J=10.4, 3.7 Hz)–H-3′ (δ 4.26, dd, J=10.4, 3.2 Hz)–H-4′ (δ 3.97, J=3.2 Hz). H-5′ (δ 4.56, q, J=6.6 Hz) was coupled to the methyl doublet. It is therefore clear that the sugar moiety at C-10 of **4** is 2-*O*-acetyl-α-L-fucopyranoside (L configuration of the sugar was assigned by converting **3** to peracetylated derivative (vide infra)). Hence, compound **4** was elucidated to be as shown in Figure 1. Pseudopterosin-S is a regioisomer of pseudopterosin-H[2] which is glycosylated at C-9 (initially assigned 1*R*,3*S*,4*R*,7*R* configuration was revised to 1*S*,3*S*,4*R*,7*S*[11]).

Pseudopterosin-T (**5**) showed a molecular ion peak at *m*/*z* 432 and intense fragment ions at *m*/*z* 300 and 244. The loss of 132 mass units suggested the presence of a pentose. Its molecular formula, $C_{25}H_{36}O_6$, was determined by HREIMS. Comparison of the NMR data of **5** with those of **1** indicated that **5** has the same aglycone as in **1**, but the structure of the sugar moiety substituted at C-10 is different from **1**. The ^{1}H NMR spectrum of **5** exhibited an anomeric proton at δ 5.10 in addition to the five signals assignable to the sugar moiety. The H–H COSY spectrum correlated these signals: H-1′ (d, J=2.5 Hz)–H-2′ (δ 4.17, br d, J=7.3 Hz)–H-3′ (δ 4.12, br d, J=7.3 Hz)–H-4′ (δ 4.08, br s)–H-5′a (δ 4.25, d, J=12.5 Hz)/H-5′b (δ 3.78, d, J=12.5 Hz). These data clearly indicated that H-2′ and H-3′ have an axial orientation while H-1′ and H-4′ must take an equatorial position and that the anomeric configuration is β. The data further indicated that sugar is β-arabinopyranoside. In order to establish the chirality of arabinose, **5** was subjected to acidic hydrolysis. Arabinose was identified by TLC in the hydrolysate. Analysis of the sample in the same manner as described for fucose revealed that the arabinose is in D-form. Hence, the structure of **5** was established to be as shown in Figure 1.

Pseudopterosin-U (**6**) showed a molecular ion at *m*/*z* 474 and intense fragment ions at *m*/*z* 300 and 244. The loss of 174 mass units could be due to a mono-acetylated pentose. The molecular formula of **6** was determined to be $C_{27}H_{38}O_7$ by HREIMS. Comparison of the NMR data of **6** and **5** indicated that **6** is a mono-acetyl derivative in the sugar moiety of **5**.

The ^{1}H signals for the sugar moiety of **6** were correlated by H–H COSY spectrum: H-1′ (5.18, d, J=3.6 Hz)–H-2′ (δ 4.10, dd, J=9.9, 3.6 Hz)–H-3′ (δ 4.32, dd, J=9.9, 3.4 Hz)–H-4′ (δ 5.24, m)–H-5′a (δ 4.36, br d, J=13.0 Hz)/H-5′b (δ 3.89, dd, J=13.0, 1.9 Hz). These data clearly indicated that sugar is 4-*O*-acetyl-β-D-arabinopyranoside. D configuration of the sugar was assigned by converting **6** to peracetylated derivative (vide infra). HMBC correlations (Fig. 3) corroborated the sugar structure and C-10 substitution of the sugar. Hence, the structure of compound **6** was established to be as shown in Figure 1.

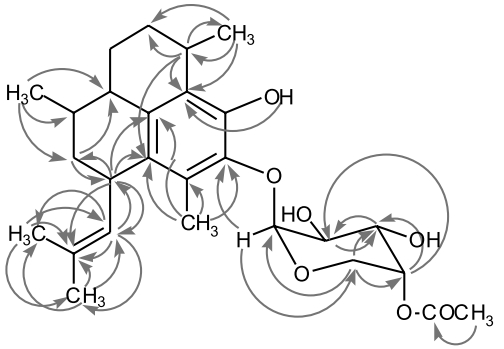

Figure 3. HMBC correlations from H to C for pseudopterosin-U (**6**).

Pseudopterosin-V (**7**) had the same molecular formula ($C_{27}H_{38}O_7$) as for **6**, and the mass spectral pattern was essentially identical to that of **6**. These data, together with ^{1}H NMR data, indicated that **7** is an isomer in terms of the position of acetyl group. The H–H COSY spectrum of **7** showed coupling networks: H-1′ (δ 5.22)–H-2′ (δ 4.33)–H-3′ (δ 5.31)–H-4′ (δ 4.27)–H-5′a (δ 4.42)/H-5′b (δ 3.87). These data clearly indicated that the sugar structure is 3-*O*-acetyl-β-D-arabinopyranoside. D configuration of the sugar was assigned by converting **6** to peracetylated derivative (vide infra). Hence, the structure of **7** was established to be as depicted in Figure 1.

It should be mentioned that Jacobs et al. reported in their patent the isolation of pseudopterosins M, N and O,[13] which are listed in SciFinder as 2-*O*-acetyl-xyloside, 3-*O*-acetyl-xyloside and 4-*O*-acetyl-xyloside at C-10 of 1*R**,3*S**,4*R**,7*S**-aglycone. They recently published details of these compounds, reporting that pseudopterosins M, N and O are acetyl-arabinoside derivatives (the structures shown in the paper are not acetyl-arabinoside but acetyl-xyloside derivatives).[3] Our ^{1}H and ^{13}C NMR data for compounds **6** and **7** are not consistent with the values reported for pseudopterosins M and O.[14]

Compounds **1**–**4** yielded a common peracetylated derivative upon acetylation, which secured the stereochemical (absolute configuration) identity for the aglycone and sugar moieties (L-fucose derivatives) among the four pseudopterosins. The ^{1}H NMR spectrum of the tetra-acetate showed a unique pattern due to the presence of two conformers as illustrated in Figure 4. When recorded at 20 °C, the spectrum showed two sets (ca. 45:55 ratio) of signals in some peaks which might correspond to two conformers. For example, the signal for H-7 was clearly observed at δ 2.70 and δ 3.18 as broad peaks. When measured at 60 °C,[15] the two H-7 signals turned to one very broad signal, and one of the acetate signals was similarly very broad, suggesting that the two conformers were not rapidly interchanged at this temperature. Compounds **5**–**7** furnished the other common peracetylated derivative, confirming the absolute stereochemistry of the aglycone and sugar moieties (D-arabinose derivatives). When the ^{1}H NMR spectrum of the acetate was recorded at 20 °C, the signal for H-7 appeared very broad around at δ 2.8–3.1, also the H_3-19 and one of the acetates H_3 were not clear due to their broad and complex signals. At 60 °C, the spectrum became clearer except for the some broadness of H-7 (Fig. 4). The 9-benzyl ether of pseudopterosin-P (see Scheme 1) did not show such broadening of the ^{1}H NMR signals. It is, therefore, conceivable that these ^{1}H NMR behaviors are due to restricted rotation of the glycosidic bond caused by acetylation of the C-10 hydroxyl as well as the hydroxyl groups at the sugar moiety. Furthermore, higher energy

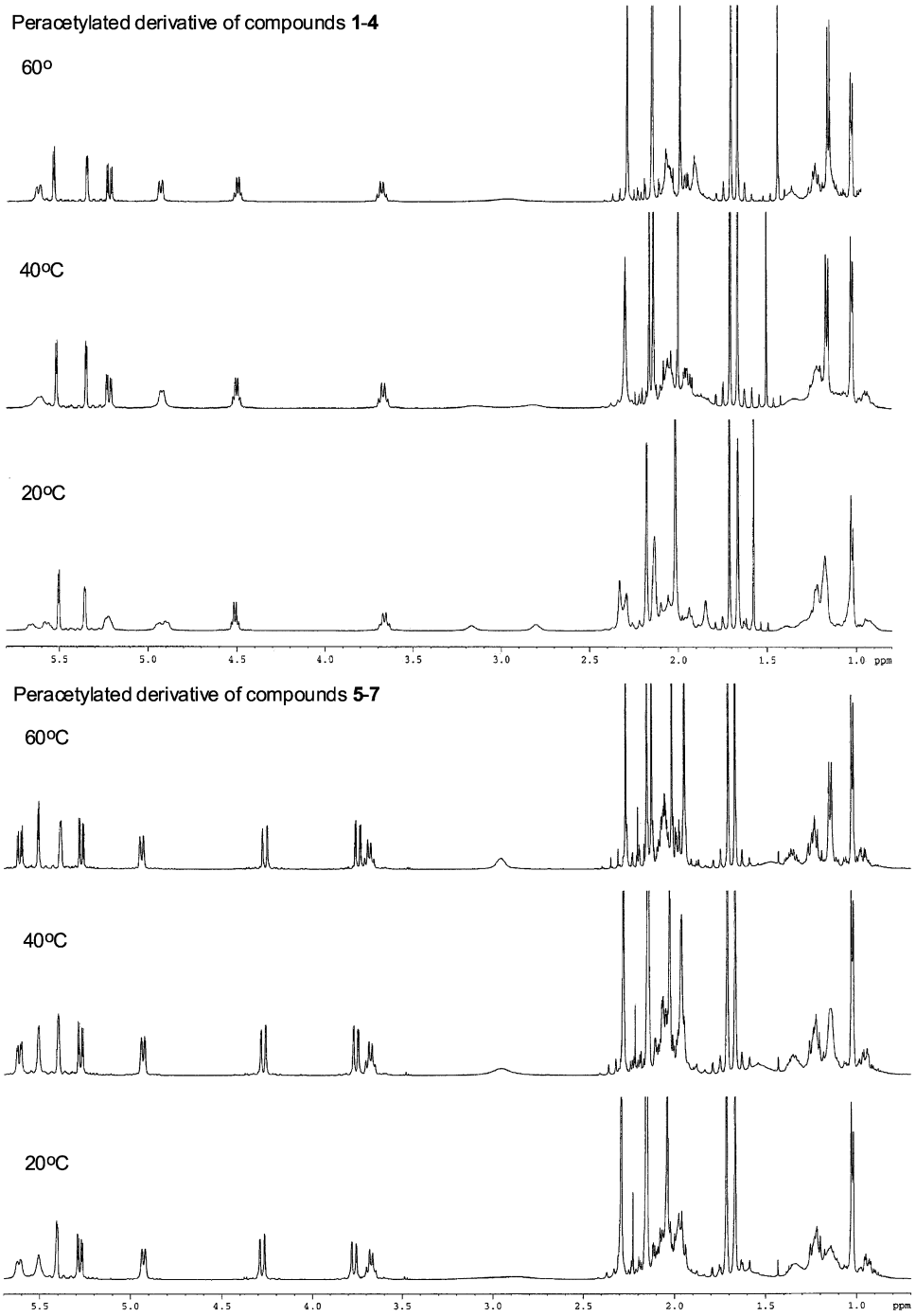

Figure 4. ^{1}H NMR spectra of the peracetylated derivatives of compounds **1**–**4** and of compounds **5**–**7**, at different temperature.

barrier for the rotation of the fucose derivative, compared to that of the arabinose derivative, allowed us to detect two distinct conformers at 20 °C.

Cumulative NMR data for pseudopterosins including the present study provided a simple clue to differentiate C-10 glycosylation from C-9 glycosylation. The chemical shift for C-11 in ^{13}C NMR spectra showed the most diagnostic difference: C-11 appears at δ 121–122 for C-9 glycosylated compounds, whereas it resonates at δ 126–127 for C-10 glycosylated pseudopterosins. Fortunately, there are no disturbing ^{13}C signals in this area in pseudopterosin molecules, and this rule seems to be applicable, irrespective of C-1 stereochemistry of the aglycone.

In addition to the new pseudopterosins reported in this paper, new *seco*-type pseudopterosins were isolated as minor constituents. Further isolation and structure analysis of these compounds are in progress in our laboratory.

Our preliminary results in the neutrophil degranulation inhibition assay have revealed interesting activity of the new pseudopterosins reported here, but varying effects in neutrophil activation or inhibition depending on

concentration tested. Therefore, determination of antiinflammatory activity will be matter of further research and results will be published elsewhere.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were recorded on a Shimadzu UV-1600PC spectrophotometer. ^{1}H NMR and ^{13}C NMR (one- and two-dimensional) spectra were recorded on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ solution. ^{1}H chemical shifts are expressed with tetramethylsilane as an internal standard ($\delta=0.00$), while ^{13}C chemical shifts are referenced to the solvent signal ($\delta=77.0$). EI-MS and HREIMS were obtained on a JEOL JMS-700 spectrometer. HPLC-MS on APCI mode was carried out on a Shimadzu QP-8000α spectrometer with a Thermo Hypersil-Keystone RP-18 (100×2 mm i.d., 3 μm) column. Preparative HPLC was conducted with a Merck-Hitachi instrument with a UV/VIS L-4250 detector (detected at 230 nm) using a Nucleosil 120 10 C-18 (300×8 mm i.d., 10 μm) column with a 30 min gradient of acetonitrile–water (70–100%) to 100% acetonitrile as mobile phase at a flow rate of 1 ml/min. Final HPLC purification was performed with a Shimadzu LC-6A apparatus equipped with a UV detector (detected at 230 nm) under a Shimadzu Shim-Pack CLC-ODS (15×6 mm i.d., 5 μm) using MeOH/water (9:1) as mobile phase at a flow rate of 1 ml/min. The following adsorbents were used for purification: column chromatography, Merck Kieselgel 60; preparative TLC, Merck Kieselgel 60 F_{254}. TLC plates were visualized by dipping with 5% phosphomolybdic acid in EtOH followed by heating.

3.2. Animal material

Fragments of several *Pseudopterogorgia elisabethae* colonies were collected by SCUBA at a range depth of 20–30 m at various sites around the island of Providencia. Sample collection never implied removing whole colonies, only a terminal fragment of each individual colony was cut off the main gorgonian axis with sharp scissors. Gorgonian fragments were air-dried and stored in the freezer. Samples were kept frozen until the moment of extraction. Animals were identified as *Pseudopterogorgia elisabethae* by M. Puyana, and voucher specimens were deposited at the invertebrate collection of Museo de Historia Natural Marina Colombiano (MHNMC) at Instituto de Investigaciones Marinas de Punta de Betín (INVEMAR), coded as INV CNI 1612, INV CNI 1613 and INV CNI 1614.

3.3. Extraction and separation of pseudopterosins

Dried gorgonian tissue (10 g) were repeatedly extracted with a CH_2Cl_2/MeOH (1:1) mixture. The resultant extract was filtered and concentrated by rotary evaporation obtaining a dark green oily extract (4 g). This crude extract was subjected to silica gel column chromatography using CH_2Cl_2 and EtOAc mixtures of increasing polarity, and finally pure EtOAc, to yield eight fractions. Pseudopterosins were present, as shown by HPLC-MS in APCI mode, in fraction 6 which eluted with EtOAc/CH_2Cl_2 (6:4) and in fraction 8 which eluted with EtOAc. Fraction 6 was subjected first to preparative HPLC using a Nucleosil 120 10 C-18 column with a solvent gradient of acetonitrile–water to yield pure compounds **2** (6 mg), **3** (2 mg), **4** (1.5 mg), **6** (4.5 mg) and **7** (1.5 mg). On the other hand, fraction 8 was subjected to preparative HPLC, as mentioned above, and then for final purification to HPLC on a Shimadzu Shim-Pack CLC-ODS with MeO–H_2O as solvent system to yield compounds **1** (5 mg) and **5** (6 mg) and pseudopterosins G (4 mg), and K (7 mg). Structures of the known three compounds were determined by comparison of the spectral data with reported values.[2,4]

3.3.1. Pseudopterosin P (1). White powder; $[\alpha]_D^{25}$ −29° (*c*, 0.52, MeOH); UV λ_{max} (MeOH) 213 (ε 24,300), 232 (shoulder), 276 (shoulder), 285 (ε 1400) nm; EI-MS *m/z* (relative intensity): 446 (M^+, 2), 300 (4.7), 285 (8), 244 (100), 229 (78); HREIMS *m/z*: 446.2669 (M^+), $C_{26}H_{38}O_6$ requires 446.2668; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.3.2. Pseudopterosin Q (2). White powder; $[\alpha]_D^{25}$ −53° (*c*, 0.56, MeOH); UV λ_{max} (MeOH) 214 (ε 23,700), 232 (shoulder), 276 (shoulder), 285 (ε 1670) nm; EI-MS *m/z* (relative intensity): 488 (M^+, 8), 300 (71), 285 (9), 244 (100), 229 (38); HREIMS 488.2762 (M^+), $C_{28}H_{40}O_7$ requires 488.2774; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.3.3. Pseudopterosin R (3). White powder; $[\alpha]_D^{25}$ −34° (*c*, 0.28, MeOH); UV λ_{max} (MeOH) 208 (ε 25,900), 234 (shoulder), 276 (shoulder), 285 (ε 1150) nm; EI-MS *m/z* (relative intensity): 488 (M^+, 2), 300 (40), 285 (10), 244 (100), 229 (56); HREIMS *m/z*: 488.2779 (M^+), $C_{28}H_{40}O_7$ requires 488.2774; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.3.4. Pseudopterosin S (4). White powder; $[\alpha]_D^{25}$ −48° (*c*, 0.18, MeOH); UV λ_{max} (MeOH) 208 (ε 20,500), 232 (shoulder), 276 (shoulder), 284 (ε 1200) nm; EI-MS *m/z* (relative intensity): 488 (M^+, 1), 300 (30), 285 (7), 244 (100), 229 (48); HREIMS *m/z*: 488.2773 (M^+), $C_{28}H_{40}O_7$ requires 488.2774; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.3.5. Pseudopterosin T (5). White powder; $[\alpha]_D^{25}$ −38° (*c*, 0.89, MeOH); UV λ_{max} (MeOH) 212 (ε 15,100), 232 (shoulder), 276 (shoulder), 285 (ε 1770) nm; EI-MS *m/z* (relative intensity): 432 (M^+, 22), 300 (94), 285 (32), 244 (100), 229 (88); HREIMS *m/z*: 432.2544 (M^+), $C_{25}H_{36}O_6$ requires 432.2512; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.3.6. Pseudopterosin U (6). White powder; $[\alpha]_D^{25}$ −90° (*c*, 0.88, MeOH); UV λ_{max} (MeOH) 211 (ε 21,900), 232 (shoulder), 276 (shoulder), 285 (ε 2020) nm; EI-MS *m/z* (relative intensity): 474 (M^+, 13), 300 (91), 285 (11), 244 (100), 229 (32); HREIMS *m/z*: 474.2626 (M^+), $C_{27}H_{38}O_7$ requires 474.2618; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.3.7. Pseudopterosin V (7). White powder; $[\alpha]_D^{25}$ −63°

(*c*, 0.31, MeOH); UV λ_{max} (MeOH) 207 (ε 21,100), 232 (shoulder), 274 (shoulder), 285 (ε 2130) nm; EI-MS *m/z* (relative intensity): 474 (M^+, 15), 300 (91), 285 (8), 244 (92), 229 (13); HREIMS *m/z*: 474.2582 (M^+), $C_{27}H_{38}O_7$ requires 474.2618; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.4. Conversion of Pseudopterosin P (1) to the C-9 benzyl ether derivative

Potassium carbonate (8.8 mg) was added to a solution of compound **1** (8 mg, combined amount from two extraction processes) in dry acetone (0.5 ml). The mixture was stirred at room temperature under nitrogen for 10 min. Benzyl bromide (5.0 µl) was added to this suspension and the mixture was heated at reflux for 7 h. Solvent was removed by flushing nitrogen and the residue was partitioned between $CHCl_3$ and water. The water layer was washed with $CHCl_3$ once more, and the combined $CHCl_3$ layer was dried over Na_2SO_4 and concentrated to dryness. The crude benzyl ether (8 mg) was dissolved in MeOH (0.5 ml) and 3 N-HCl (0.5 ml) and the solution was allowed to react at 50 °C under nitrogen for 3 h. The solution was cooled to room temperature, diluted with water, and extracted with $CHCl_3$ (3×10 ml). The $CHCl_3$ layer was washed with 5% aq $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated to give a crude aglycone benzyl ether, which was chromatographed on silica gel with hexane/ether (10:1) to furnish pure aglycone benzyl ether (3 mg). $[\alpha]_D = +83$ (*c*, 0.24, $CHCl_3$) [lit. +103[11]]; ^{1}H NMR δ: 0.97, 1.03 (d, 3H), 1.25 (m, 2H), 1.34 (d, 3H), 1.36 (m, 1H), 1.67 (s, 3H), 1.74 (s, 3H), 2.01 (m, 1H), 2.06 (s, 3H), 2.10 (m, 3H), 3.24 (sextet, J=7.3 Hz, 1H), 3.74 (m, 1H), 4.73 (d, J=11.2 Hz, 1H), 4.97 (d, J=11.2 Hz, 2H), 5.51 (s, 1H), 7.42 (m, 5H); ^{13}C NMR δ: 12.1, 17.7, 20.1, 23.8, 25.6, 27.8, 29.3, 31.6, 36.4, 37.1, 40.2, 43.8, 75.3, 121.0, 127.9, 128.3, 128.5, 128.7, 131.0, 131.2, 131.8, 135.1, 137.3, 142.3, 145.3. These data were in good agreement with reported values.[11]

3.5. Analysis of the sugar portion of Pseudopterosins P (1) and T (5)

The water layer of the acidic treatment of the C-9 benzyl ether of compound **1** was freeze-dried. Fucose was identified by TLC (CH_2Cl_2/MeOH/H_2O, 8:7:1 and visualized with *p*-anisaldehyde reagent) analysis of the residue. Chirality of the sugar was determined according to the protocol of Hara et al.[12] To the dried sugar sample dissolved in pyridine (0.2 ml) was added L-cysteine methyl ester hydrochloride (2 mg), and the mixture was allowed to react at 60 °C for 1.5 h. The solvent was evaporated by flushing nitrogen and the residue was treated with hexamethyldisilazane–trimethylchlorosilane (HMDS–TMCS) (0.1 ml) at 60 °C for 1 h. The mixture was partitioned between hexane (0.3 ml) and water (0.3 ml) and an aliquot of the hexane layer was injected to GC [column, DB-1, 0.25 mm×30 m, oven temp 200 °C]. The derivatives from D-fucose and L-fucose had retention times of 13.4 and 15.2 min, respectively, and the sample from compound **1** eluted at 15.2 min.

Compound **5** (4 mg) was hydrolyzed in MeOH (0.5 ml) and 3 N-HCl (0.5 ml) at 50 °C under nitrogen for 3 h. The mixture was diluted with chloroform and water. The water layer was washed with chloroform once more and then lyophilized. TLC analysis of the residue indicated the presence of arabinose. Chirality of the sugar was examined as described for fucose. Retention times of the D- and L-arabinose derivatives were 11.5 and 10.4 min in the same GC conditions as described above. The derivative of compound **5** was detected at 11.5 min.

3.5.1. Conversion of compounds 1–4 and compounds 5–7 to the peracetylated derivatives. Each compound (1–2 mg) was treated with pyridine (40 µl) and acetic anhydride (20 µl) at room temperature overnight. Addition of methanol followed by evaporation of the solvent gave an oily residue, which was partitioned with ether and water. The ether layer was washed with 2 N-HCl, satd $NaHCO_3$ and brine, dried over $MgSO_4$, evaporated and analyzed by ^{1}H NMR. The peracetylated derivatives of compounds **1**–**4** showed identical behavior on TLC (hexane/EtOAc, 4:1). ^{1}H NMR (recorded at 60 °C) δ: 1.03 (d, J=7.0 Hz, H_3-18), 1.16 (d, J=6.5 Hz, H_3-19 and H_3-6′), 1.67 (d, J=1.0 Hz, H_3-16), 1.70 (d, J=1.1 Hz, H_3-17), 1.90 (broad, Ac), 1.99 (s, Ac), 2.146 (s, Ac), 2.151 (s, Ac), 2.29 (s, H_3-20), 2.96 (very broad, H-7), 3.68 (q-like, J=8.5 Hz, H-1), 4.50 (q-like, J=6.6 Hz, H-5′), 4.93 (br d, J=8.9 Hz, H-14), 5.20 (dd, J=10.9, 3.4 Hz, H-2′), 5.35 (d, J=3.3 Hz, H-4′), 5.54 (d, J=3.4 Hz, H-1′), 5.62 (br d, J=10.9 Hz, H-3′). HRFABMS *m/z*: 615.3156 (MH^+), $C_{34}H_{47}O_{10}$ requires 615.3169.

Compounds **5**–**7** were similarly converted to the peracetylated derivatives. Identity of them was confirmed by TLC (hexane/EtOAc 4:1) and ^{1}H NMR. ^{1}H NMR (recorded at 60 °C) δ: 1.03 (d, J=5.9 Hz, H_3-18), 1.15 (d, J=6.8 Hz, H_3-19), 1.67 (d, J=1.0 Hz, H_3-16), 1.70 (d, J=1.1 Hz, H_3-17), 1.95 (s, Ac), 2.02 (s, Ac), 2.13 (s, Ac), 2.15 (s, Ac), 2.27 (s, H_3-20), 2.96 (br, H-7), 3.69 (q-like, J=8.7 Hz, H-1), 3.75 (dd, J=14.2, 2.3 Hz, H-5a), 4.27 (dd, J=14.2, 1.5 Hz, H-5b), 4.94 (d, J=9.1 Hz, H-14), 5.28 (dd, J=10.6, 3.2 Hz, H-2′), 5.39 (m, H-4′), 5.51 (d, J=3.2 Hz, H-1′), 5.61 (dd, J=10.5, 3.5 Hz, H-3′). HRFABMS *m/z*: 623.2864 (MNa^+), $C_{33}H_{44}O_{10}Na$ requires 623.2832.

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14. Direct comparison of compounds **6** and **7** with pseudopterosins M and O were not possible, since authentic samples were not available to us.
15. Look and Fenical (Ref. 4) reported on the ^{1}H NMR measurement of *seco*-pseudopterosin peracetate derivatives at 60 °C ($CDCl_3$), probably due to the broadening of the signals at room temperature. Their results may rule out the possibility that the presently observed two conformers are associated with the aglycone moiety.