Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 62 (2006) 4205–4213

Further studies on the constituents of the gorgonian octocoral *Pseudopterogorgia elisabethae* collected in San Andrés and Providencia islands, Colombian Caribbean: isolation of a putative biosynthetic intermediate leading to erogorgiaene

Carmenza Duque,[a,*] Mónica Puyana,[a] Leonardo Castellanos,[a] Andrea Arias,[a] Hebelin Correa,[a] Oscar Osorno,[a] Teigo Asai,[b] Noriyuki Hara[b] and Yoshinori Fujimoto[b,*]

[a]*Departamento de Química, Universidad Nacional de Colombia, AA 14490 Bogotá, Colombia*
[b]*Department of Chemistry and Materials Science, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan*

Received 15 September 2005; accepted 2 February 2006

Available online 9 March 2006

Abstract—Chemical investigations of the MeOH–CH_2Cl_2 extract of *Pseudopterogorgia elisabethae* specimens collected in the islands of San Andrés and Providencia, Colombian Caribbean, yielded four new diterpenes (**1**, **3**, **5**, **7**) along with seco-pseudopterosin J (**8**), and amphilectosins A (**9**) and B (**10**). The structures of the new compounds were established through spectral studies as an elisabethatriene analog named elisabethatrienol (**1**), 10-acetoxy-9-hydroxy- and 9-acetoxy-10-hydroxy-amphilecta-8,10,12,14-tetraenes (isolated as an interconverting mixture) (**3**), amphilecta-8(13),11,14-triene-9,10-dione (**5**), and a *seco*-pseudopterosin 7-*O*-α-L-fucopyranoside named *seco*-pseudopterosin K (**7**). Elisabethatrienol can be regarded as a biosynthetic intermediate leading to erogorgiaene.

1. Introduction

The purple sea feather, *Pseudopterogorgia elisabethae*, is a gorgonian octocoral found in protected reef environments throughout the Caribbean Sea. This species has been the subject of numerous chemical and biological studies (SciFinder search revealed more than 50 papers published on this matter up to date), particularly in regard of its diterpene and diterpene glycoside content. Among the latter compounds, the pseudopterosins and *seco*-pseudopterosins are of particular interest due to their excellent anti-inflammatory properties superior even to the activity shown by the commercial drug indomethacin.

There are several studies showing the great degree of variability in pseudopterosin (Ps) composition from specimens of *P. elisabethae* collected at different Caribbean locations. For instance, PsA–PsD, PsE–PsL and PsM–PsO were obtained from animals collected at the Bahamas,[1] Bermuda[2] and in the Florida Keys,[3] respectively. Recently, as a part of our continuous search for biologically active compounds from marine organisms,[4,5] we examined the extracts of *P. elisabethae* collected in San Andrés and Providencia islands (SW Caribbean) by LC–MS, finding two distinct chemotypes that were characterized based on their pseudopterosin and related compounds composition and correlated quite well with the geographical distribution.[6] Chemotype 1, found almost exclusively in Providencia island, was mainly characterized by the presence of PsP–PsV,[7] PsG and PsK and two *seco*-pseudopterosins (compounds **7** and **8** in this text). Chemotype 2, found in San Andrés island, was revealed to contain several non-glycosylated diterpenes (compounds **1**, **3** and **5** in this text) structurally related to pseudopterosins, along with much smaller amounts of the pseudopterosins found in Chemotype 1. Recently, Rodríguez et al. reported the isolation of PsP–PsZ from *P. elisabethae* collected at San Andrés and Providencia islands.[8,9]

The structurally related *seco*-pseudopterosins, named *seco*-PsA–*seco*-PsD, were isolated from *P. kallos* collected in the Florida Keys.[10] Subsequently, the isolation of *seco*-PsE–*seco*-PsG from *P. elisabethae* collected in the Florida Keys was reported.[3] Recently, *seco*-PsH and *seco*-PsI were found in *P. elisabethae* specimens collected at Providencia island.[8]

Keywords: Pseudopterosin; Seco-pseudopterosin; *Pseudopterogorgia elisabethae*; Elisabethatrienol; Seco-pseudopterosin K.
* Corresponding authors. Tel.: +57 1 3165000x14472; fax: +57 1 3165220 (C.D); tel./fax: +81 3 5734 2241 (Y.F);
e-mail addresses: cduqueb@unal.edu.co; fujimoto@cms.titech.ac.jp

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.02.032

In continuation of our recent work on the constituents of *P. elisabethae* from San Andrés and Providencia islands, Colombian Caribbean,[6,7] we report here the isolation and structure elucidation of the new non-glycosylated diterpenes (**1**, **3**, **5**) and one new *seco*-pseudopterosin (*seco*-pseudopterosin K) (**7**) together with the known *seco*-pseudopterosin J (**8**) and amphilectosins A (**9**) and B (**10**).

2. Results and discussion

The MeOH–CH_2Cl_2 (1/1) extract of the animal tissue was separated on silica gel column chromatography and reversed-phase HPLC to yield compounds **1**, **3**, **5** from chemotype 2 specimens from San Andrés island, and compounds **7**–**10** from chemotype 1 specimens from Providencia island. The structures of these compounds were elucidated by spectral means including 2D NMR experiments. Four compounds, out of seven, were characterized as new diterpene derivatives: elisabethatrienol (**1**), 10-acetoxy-9-hydroxy- and 9-acetoxy-10-hydroxy-amphilecta-8,10,12,14-tetraenes (isolated as an interconverting mixture) (**3**), amphilecta-8(13),11,14-triene-9,10-dione (**5**), and a *seco*-pseudopterosin 7-*O*-α-L-fucopyranoside named *seco*-pseudopterosin K (**7**), whereas the other compounds were identified as the recently reported *seco*-pseudopterosin J (**8**),[11] and amphilectosins A (**9**) and B (**10**) (Fig. 1).[11]

Compound **1** was isolated as a white amorphous solid. The molecular formula of **1** was determined to be $C_{20}H_{32}O$ by HREIMS. The UV absorption was found at 236 nm probably due to a conjugated diene. The ^{1}H NMR of **1** exhibited signals for two doublet methyls (δ 0.91 and 0.84), two olefinic methyls (δ 1.67 and 1.57), two olefinic protons (δ 5.92 and 5.01), exomethylene protons (δ 5.06 and 4.80), and one oxymethine proton (δ 4.20). The ^{13}C NMR data, assisted by the DEPT spectrum, indicated the presence of three double bonds [δ 147.6 (C), 143.9 (C), 131.2 (C), 125.6 (CH), 124.9 (CH) and 105.0 (CH_2)]. These spectral features were reminiscent of (+)-elisabethatriene (**2**),[12] a bicyclic, biosynthetic intermediate of pseudopterosin arising from geranylgeranyl diphosphate. Compound **1** is thus suggested to be a hydroxylated derivative of **2**.

The H–H COSY spectrum of **1** indicated that the oxymethine proton was correlated with the methylene protons at δ 1.52 and 1.91, and the exomethylene protons. The methylene protons were further connected to a methine proton at δ 2.56 (H-9), which in turn showed allylic coupling with the olefinic proton at δ 5.92 (H-5). The olefinic proton was further correlated with the exomethylene protons. These COSY correlations clearly showed the C-7 location of the hydroxyl group. HMBC correlations (Fig. 2) fully supported the structure formulation. Coupling patterns of 8α-H at δ 1.52 (q-like, J=12.0 Hz) and 8β-H at δ 1.91 (dt, J=12.0, 6.0 Hz) helped us to assign the pseudo-axial β-orientation of H-7 ($J_{7\text{-H},8\alpha\text{-H}}$=12.0 Hz) and pseudo-equatorial α-orientation of the C-7 hydroxyl group. The β-axial orientation of H-9 was also evident from the coupling constants of $J_{\text{H-9,H-8}\alpha}$=12.0 Hz and $J_{\text{H-9,H-8}\beta}$=6.0 Hz. A small coupling constant of $J_{\text{H-1,H-9}}$ (ca. 4 Hz), estimated from the shape of H-9, further indicated that H-1 is β-equatorially oriented. It was difficult to assign the orientation of H-4 on the basis of decoupling studies, although the H-4 resonance appeared at δ 1.82 as a broad doublet with J=ca. 10.0 Hz. Extensive NOE studies afforded useful information on the stereochemistry at the chiral centers. The pertinent NOE correlations are depicted in Figure 3. In particular, H-9 and H-5 showed NOE correlations with H-11 and H-4, respectively. The findings established the 4α-orientation of H-4. The C-4/C-11 relative stereochemistry was assumed from the biogenesis of pseudopterosins and the similarity of the ^{13}C data (C-11, C-12 and C-18) of **1** with those of **2**,[12,13] serrulatane

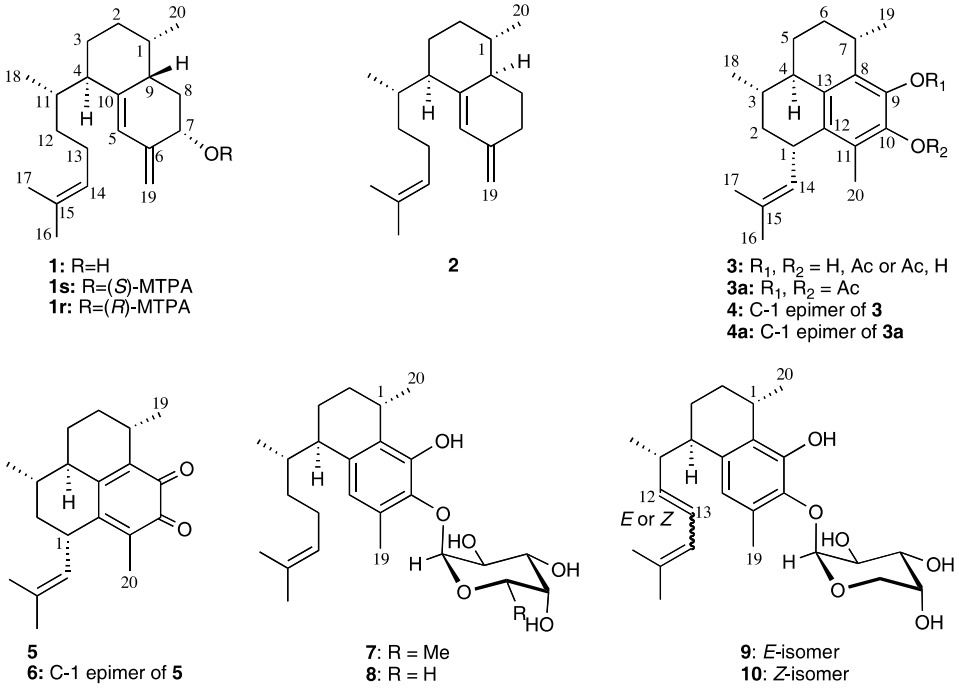
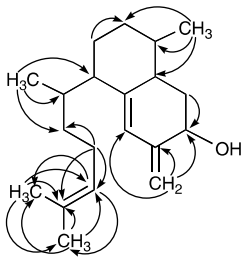


Figure 1. Chemical structures of compounds.



1

Figure 2. HMBC correlations from H to C for elisabethatrienol **1**.

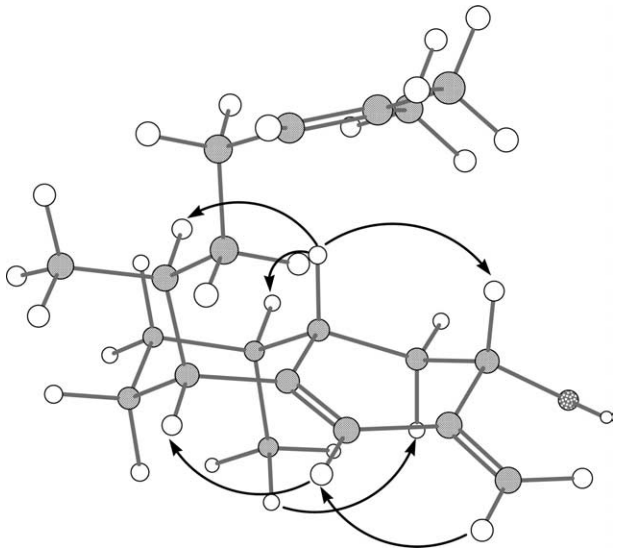


Figure 3. Pertinent NOE correlations and most stable conformation of elisabethatrienol **1**.

diterpenes,[14] and seco-pseudopterosins.[3] On the basis of these data the structure of **1** was determined as shown in Figure 1. The most stable conformation of the molecule, deduced by MM2 calculation, is also illustrated in Figure 3. It should be noted that the olefinic bond-containing six-membered ring adopts a half-chair conformation. Assignments of the NMR signals are listed in Table 1.

Mosher's ester method[15] was applied to determine the absolute configuration of this molecule. The ^{1}H NMR data for the (*S*)- and (*R*)-MTPA esters of **1** (**1s** and **1r**) are summarized in Table 2. The data clearly established the 7*S* configuration of **1**. Hence, the absolute configuration of the chiral centers in **1** was determined to be 1*S*,4*R*,7*S*,9*S*,11*S*. The new diterpene was named elisabethatrienol.[16] The possibility that compound **1** is 9-*epi*-elisabethatrienol, which is suggested from the structure of elisabethatriene **2**, was ruled out, since the observed NOE data was incompatible with the structure. Elisabethatrienol is an interesting molecule from a biosynthetic point of view and this will be discussed later.

Compound **3** was isolated as a yellow amorphous solid. Interestingly, it showed two distinct peaks when analyzed on reversed-phase HPLC. However, the samples separated by HPLC again showed the original two peaks in HPLC analysis. The phenomenon suggested that compound **3** should be a readily interconverting mixture of two components. The molecular formula of **3** was determined to be $C_{22}H_{30}O_3$ (MW 342) on the basis of HREIMS. The UV absorption maxima at 230 and 276 nm were similar to those of pseudopterosins. The ^{1}H NMR of **3** displayed pairs of signals in a 54:46 ratio, suggesting a mixture of two structurally related constituents. The ^{13}C NMR spectrum also showed a series of paired signals, including an acetoxy group (δ_C 20.6/20.9, 169.2/169.6). The HMBC spectrum (Fig. 4) helped us to propose that compound **3** is a mixture of 10-acetoxy-9-hydroxy- and 9-acetoxy-10-hydroxy-amphilecta-8,10,12,14-tetraenes[17] with the former being a major constituent. For the purpose of rigorous identification, **3** was acetylated to furnish a single compound **3a** having two acetyl groups (EIMS, M^+, 384), which was fully characterized by spectral methods, including 2D NMR. The coupling patterns of H-1 and H-7 of **3a** were closely similar to those of the pseudopterosin aglycone with 1β-H configuration, and different from those of 1α-H epimers.[18] Furthermore, the ^{13}C NMR chemical shifts of C-1–C-7 and C-14–C-19 in **3a** were in excellent agreement with those of pseudopterosins with 1β-H configuration,[7] thus indicating 1*S**,3*S**,4*R**,7*S** relative configuration of **3a**. Compound **3a** was therefore determined to be 9,10-diacetoxyamphilecta-8,10,12,14-tetraene. Accordingly, compound **3** was established to be a mixture of 10-acetoxy-9-hydroxy- and 9-acetoxy-10-hydroxy-amphilecta-8,10,12,14-tetraenes. It is likely that **3** has the 1*S*,3*S*,4*R*,7*S* absolute stereochemistry, since compound **3** ($[\alpha]_D^{25}$ +77.5) and known 9-methoxy-10-hydroxy-amphilecta-8,10,12,14-tetraene (lit.[18] +90) have the positive sign of optical rotation. The C-1 epimer of **3** (**4**) was previously isolated, also as an interconverting mono-acetate mixture, from the same species collected in the Bahamas islands and characterized in the form of the acetyl derivative, 9,10-diacetoxy-1-*epi*-amphilecta-8,10,12,14-tetraene (**4a**).[19] The ^{1}H NMR data of **3a** showed diagnostic difference from those reported for **4a**: H-14, δ 4.99 for **3a** versus δ 5.18 for **4a**.[18,19] The diacetate **4a** was also prepared from pseudopterosin C[20] and chemically synthesized.[21]

Compound **5** was isolated as an orange oil. Its molecular formula was determined as $C_{20}H_{26}O_2$ on the basis of HREIMS, which requires eight degree of unsaturation, one additional unsaturation expected for pseudopterosin aglycones. The ^{1}H NMR spectrum showed signals of two doublet methyls assignable to H_3-18 (δ 1.02) and H_3-19 (δ 1.14), two singlet methyls and an olefinic proton due to the isobutenyl group, and two methine protons assignable to H-1 (δ 3.70, td, J=9.1, 4.9 Hz) and H-7 (δ 2.77, sextet-like, J=6.4 Hz). The singlet methyl at δ 1.84, presumably due to H_3-20, was appreciably shifted upfield compared to that of pseudopterosins (ca. δ 2.2–2.3), suggesting that the aromatic ring is modified. In the ^{13}C NMR spectrum compound **5** exhibited eight sp^2 carbons, two of which (δ 181.0 and 180.8) could be assigned to carbonyl groups, most likely α,β-unsaturated carbonyls. In the HMBC spectrum, H_3-20 (δ 1.84) showed correlations to the carbonyl (δ 180.8) and two other sp^2 carbons (δ 132.2, 151.8), while H_3-19 was correlated with an sp^2 carbon (δ 138.8), C-6 and C-7. These NMR data agreed with the structure in which the catechol aromatic ring of a pseudopterosin aglycone was oxidized to an *ortho*-quinone. HMBC correlations depicted in Figure 5

Table 1. NMR ($CDCl_3$) data for compounds **1**, **3**, **3a** and **5**

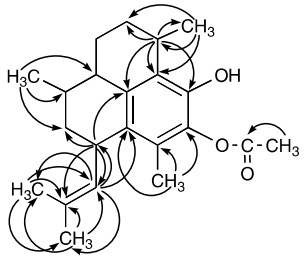
No.[a]	**1**		**3**		**3a**		**5**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1/7	1.96 (m)	32.6	3.18 (sextet-like, 7.2)/2.94 (sextet-like, 7.2)	28.8/28.4	2.95 (sextet-like, 7.2)	28.7	2.77 (sextet-like, 6.4)	28.7
2/6	1.35 (m) 1.75 (m)	28.5	1.33 (m) 2.13 (m)/2.07 (m)	31.7/31.5	1.36 (m) 2.10 (m)	31.3	1.23 (m) 1.95 (m)	29.8
3/5	1.60 (m) 1.65 (m)	21.8	0.92 (m) 2.03 (m)	27.5	0.97 (m) 2.07 (m)	27.2	1.13 (m) 1.96 (m)	25.4
4/4	1.82 (br d, 10.0)	47.8	2.05 (m)	44.6/44.0	2.05 (m)	44.2	2.04 (m)	43.0
5/12	5.92 (s)	125.6	—	130.6/136.9	—	137.0	—	151.8
6/11	—	147.6	—	126.7/122.8	—	128.0	—	132.2
7/10	4.20 (m)	69.7	—	136.0/143.4	—	139.5	—	181.0
8/9	1.52 (q-like, 12.0) 1.91 (dt, 12.0, 6.0)	36.4	—	143.0/135.6	—	138.8	—	180.8
9/8	2.56 (m)	38.7	—	127.2/131.4	—	132.4	—	138.8
10/13	—	143.9	—	137.9/132.2	—	137.9	—	152.3
11/3	1.65 (m)	32.4	1.20 (m)	33.7/33.9	1.33 (m)	33.6	1.40 (m)	31.8
12/2	1.31 (m) 0.88 (m)	34.6	1.18 (m) 1.96 (m)	40.0/39.9	1.23 (dd, 13.0, 3.3) 1.97 (ddd, 13.0, 8.1, 2.8)	39.9	1.28 (dd, 13.6, 4.9) 2.14 (ddd, 13.6, 9.0, 6.0)	37.7
13/1	2.02 (m) 1.82 (m)	25.5	3.68 (q-like, 8.8)/ 3.72 (q-like, 8.8)	36.9/37.3	3.71 (q-like, 8.8)	37.4	3.70 (td, 9.1, 4.9)	36.0
14/14	5.01 (t, 7.1)	124.9	4.97 (d, 9.2)/4.95 (d, 9.2)	130.9/130.6	4.99 (dt, 9.3, 1.2)	130.2	5.11 (dt, 9.2, 1.3)	126.5
15/15	—	131.2	—	128.6/128.8	—	129.2	—	132.0
16/16	1.67 (s)	25.7	1.71 (s)/173 (s)	25.4	1.71 (d, 1.2)	25.4	1.72 (br s)	25.6
17/17	1.57 (s)	17.7	1.66 (s)/1.67 (s)	17.5	1.66 (d, 1.2)	17.5	1.72 (br s)	17.9
18/18	0.91 (d, 6.6)	17.5	1.02 (d, 6.6)/ 1.01 (d, 6.6)	20.0/19.9	1.03 (d, 6.3)	19.9	1.02 (d, 6.6)	20.5
19/20	4.80 (s), 5.06 (s)	105.0	1.92 (s)/2.05 (s)	13.0/12.1	1.93 (s)	12.9	1.84 (s)	11.0
20/19	0.84 (d, 7.1)	14.6	1.26 (d, 6.8)/ 1.19 (d, 6.8)	23.1/23.4	1.19 (d, 6.9)	23.3	1.14 (d, 7.0)	21.3
Ac			2.34 (s)/2.35 (s)	20.6/20.9 169.2/169.6	2.27 (s) 2.29 (s)	20.4 20.6 168.6 168.3		

[a] Seco-type numbering/pseudopterosin numbering.

supported the *o*-quinone structure. The assignments of ^{1}H and ^{13}C signals are listed in Table 2. The shape (septet-like) of H-7 was similar to those of pseudopterosins with 1β-H,[7] thus indicating the β orientation of 7-H. NOE correlation between H_3-18 and H-4 evidenced the *syn*-orientation of the two groups. This compound was thus suggested to be 1-amphilecta-8(13),11,14-triene-9,10-dione (**5**) or its C-1 epimer **6**. However, the coupling pattern of H-1 was somewhat different from those of pseudopterosins with 1β-H (typically ddd, J=7.2, 7.2, 7.2 Hz).[7] Fortunately, 1-*epi*-amphilecta-8(13),11,14-triene-9,10-dione (**6**), was previously prepared from natural pseudopterosin C[20] and chemically synthesized.[21] Comparison of the NMR data of **5** and **6** showed considerable differences (e.g., H-14: δ 5.06 for **6**[21] vs δ 5.11 for **5**, H-1: δ 3.61 for **6**[21] vs δ 3.70 for **5**). Hence, compound **5** was determined to be amphilecta-8(13),11,14-triene-9,10-dione. Definitive proof of the structure came from the chemical correlation of **5** with **3**. Compound **3** was reduced by $LiAlH_4$ in ether and the reaction mixture was worked up in the usual extractive

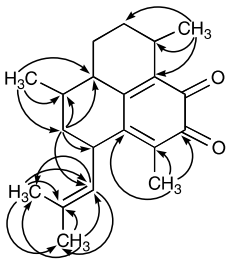
Table 2. ^{1}H NMR ($CDCl_3$) data for the MTPA esters **1s** and **1r**

No.	**1s**	**1r**	Δ (δ**1s**–δ**1r**)
1	1.94 (m)	1.97 (m)	−0.03
5	5.93 (s)	5.90 (s)	+0.03
7	5.40 (br d, 12.5)	5.56 (br d, 12.3)	
8	1.98 (ddd, 12.0, 6.1, 4.2)	2.02 (ddd, 11.5, 5.6, 4.2)	−0.04
	1.63 (m)	1.72 (m)	−0.09
9	2.64 (m)	2.66 (m)	−0.02
11	1.65 (m)	1.65 (m)	0.00
12	1.37 (m)	1.36 (m)	+0.01
	0.88 (m)	0.87 (m)	+0.01
14	5.06 (br t, 12.4)	5.05 (br t, 12.0)	+0.01
16	1.60 (s)	1.60 (s)	0.00
17	1.69 (s)	1.69 (s)	0.00
18	0.91 (d, 6.6)	0.91 (d, 6.6)	0.00
19	4.87 (s)	4.69 (s)	+0.18
	4.79 (s)	4.60 (s)	+0.19
20	0.79 (d, 7.0)	0.83 (d, 7.1)	−0.04
OMe	3.58 (br s)	3.62 (br s)	



3

Figure 4. HMBC correlations from H to C for the major constituent of compound **3**.



5

Figure 5. HMBC correlations from H to C for compound **5**.

manner to give the *ortho*-quinone **5** (identified by co-TLC and ^{1}H NMR) in 63% yield. It is likely that an air-oxidation of the initially formed diol yielded **5**. The optical rotation ($[\alpha]_D^{25}$ +134) of **5** derived from **3** was practically identical with that of natural sample **5**, thus establishing the 1*S*,3*S*,4*R*,7*S* absolute configuration of **5**. Compound **5** was a sensitive material and gradually decomposed even when stored in a freezer, as reported for **6**.

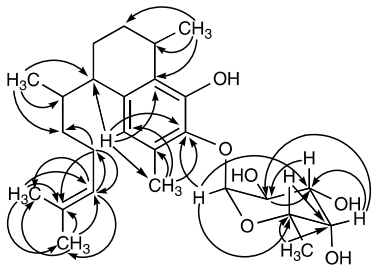
Compound **7** was isolated as a white amorphous solid. The molecular formula of **7** was determined to be $C_{26}H_{40}O_6$ on the basis of HREIMS. The UV spectrum showed absorption maxima at 208 and 280 nm due to a substituted benzene ring. In the EIMS spectrum, an intense fragment ion at *m/z* 302 arising from loss of 146 mass units from the molecular ion peak at *m/z* 448 suggested that **7** could be a seco-type pseudopterosin containing a deoxy-hexose moiety. Interpretation of the ^{1}H and ^{13}C NMR data (Table 3) indicated that the signals for the aglycone moiety were in good agreement with those reported for *seco*-pseudopterosin J,[11] while those for the sugar moiety were essentially identical with the data of α-fucopyranosyl moiety found in pseudopterosin-P,[7] which has recently been isolated from the same organism by us. HMBC correlation between the anomeric proton (δ 5.04) and C-7 (δ 142.1) gave evidence for the C-7 glycosylation (Fig. 6). The C-7 resonance was unequivocally assigned by HMBC cross peaks between C-7 and both H_3-19 (δ 2.29) and H-5 (δ 6.49). Chirality of fucose was assumed to be L, since the L-form of fucose, a sugar moiety of pseudopterosin-P, was unambiguously established in our previous paper.[7] Hence, the structure of **7** was established to be *seco*-pseudopterosin with the α-L-fucopyranosyl moiety linked at C-7, as shown in Figure 1. Compound **7** was named *seco*-pseudopterosin K. Previously reported *seco*-pseudopterosins E-G are 2-*O*-acetyl, 3-*O*-acetyl and 4-*O*-acetyl derivatives at the fucose moiety of **7**.[3]

Compound (**8**), $C_{25}H_{38}O_6$, showed a molecular ion peak at *m/z* 434. Interpretation of the NMR data, including HMQC and HMBC spectra, established that compound **8** is a *seco*-pseudopterosin with a β-D-arabinopyranosyl group attached to C-7 (evidenced by the HMBC correlation from the

Table 3. NMR ($CDCl_3$) data for compounds **7–10**

No.[a]	**7**		**8**		**9**		**10**
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1/7	3.14 (m)	27.0	3.12 (m)	27.1	3.15 (m)	27.0	3.17 (m)
2/6	1.46 (m) 1.87 (tdd, 12.7, 5.9, 3.4)	27.9	1.45 (m) 1.85 (tdd, 12.7, 5.9, 3.3)	27.9	1.46 (m) 1.87 (m)	27.3	1.48 (m) 1.99 (tdd, 12.7, 6.0, 3.5)
3/5	1.66 (m) 1.79 (tdd, 12.7, 6.4, 3.3)	18.5	1.66 (m) 1.77 (tdd, 12.7, 6.3, 3.3)	18.5	N[b] 1.81 (m)	19.4	N 1.87 (tdd, 12.7, 5.8, 3.4)
4/4	2.63 (m)	39.5	2.62 (m)	39.6	2.65 (m)	42.5	2.62 (m)
5/12	6.49 (s)	120.8	6.49 (s)	121.2	6.49 (s)	121.9	6.53 (s)
6/11	—	127.8	—	127.9	—	127.6	—
7/10	—	142.1	—	141.9	—	142.1	—
8/9	—	146.6	—	146.5	—	146.6	—
9/8	—	128.9	—	129.2	—	129.0	—
10/13	—	136.7	—	137.1	—	135.4	—
11/3	1.97 (m)	38.5	1.97 (m)	38.6	2.66 (m)	41.3	2.99 (m)
12/2	1.34 (m) 1.46 (m)	35.6	1.33 (m) 1.46 (m)	35.7	5.61 (dd, 15.2, 6.4)	137.4	5.31 (t, 10.3)
13/1	1.95 (m) 2.07 (m)	26.2	1.95 (m) 2.06 (m)	26.3	6.15 (dd, 15.2, 10.5)	125.2	6.08 (dd, 11.5, 10.3)
14/14	5.15 (t, 7.1)	124.9	5.14 (t, 7.2)	124.9	5.81 (d, 10.5)	125.5	5.91 (d, 11.5)
15/15	—	131.1	—	131.2	—	133.0	—
16/16	1.71 (s)	25.6	1.71 (s)	25.7	1.77 (s)	25.9	1.77 (s)
17/17	1.63 (s)	17.6	1.62 (s)	17.7	1.73 (s)	18.3	1.72 (s)
18/18	0.71 (d, 7.0)	16.4	0.70 (d, 6.9)	16.4	0.89 (d, 6.8)	16.3	0.92 (d, 6.7)
19/20	2.29 (s)	16.9	2.25 (s)	17.2	2.23 (s)	17.1	2.28 (s)
20/19	1.19 (d, 6.8)	20.8	1.15 (d, 6.9)	21.0	1.18 (d, 6.9)	20.9	1.19 (d, 6.5)
1′	5.04 (d, 3.8)	103.9	5.13 (d, 3.4)	103.7	5.14 (d, 3.0)	103.3	5.14 (d, 3.1)
2′	3.96 (dd, 10.0, 3.8)	69.0	4.17 (br d 10.0)	69.7	4.16 (br d, 9.3)	69.6	4.17 (br d, 9.6)
3′	4.07 (dd, 10.0, 3.2)	70.2	4.12 (dd, 10.0, 3.6)	69.8	4.10 (br d, 9.3)	70.1	4.10 (br d, 9.6)
4′	3.86 (br d, 3.2)	67.2	4.10 (br s)	69.3	4.13 (br s)	69.1	4.13 (br s)
5′	4.51 (q, 6.6)	71.9	3.84 (dd, 12.0, 1.7) 4.34 (d, 12.0)	63.8	3.89 (d, 11.9) 4.40 (d, 11.9)	63.7	3.90 (d, 11.9) 4.41 (d, 11.9)
6′	1.34 (d, 6.6)	16.1					
OH	8.33 (br)		8.38 (br)		8.19 (br)		8.11 (br)

[a] Seco-type numbering/pseudopterosin numbering.
[b] ^{1}H NMR resonances not assigned.



7

Figure 6. HMBC correlations from H to C for compound **7**.

anomeric proton to C-7). This *seco*-pseudopterosin (*seco*-pseudopterosin J) was quite recently reported from *P. elisabethae* collected in the Florida Keys (no ^{13}C assignments were reported).[11] The accurate ^{13}C assignments for compounds **7** and **8** are listed in Table 3. It should be noted that the ^{13}C data of the aglycone moieties for 7-*O*-glycosylated seco-pseudopterosins[11] (e.g., **7** and **8**) and 8-*O*-glycosylated congeners (seco-PsA–seco-PsD[10]) are coincidentally closely similar to each other. The C-7 and C-8 signals for the former isomer resonate at ca. 142.0 and 146.5 ppm, respectively,[10] while the C-7 and C-8 for the latter appear at ca. 146.5 and 142.0 ppm, respectively.[8,11] It is therefore essential to assign unambiguously the two carbon chemical shifts on the basis of long-range C–H correlations for distinguishing 7-*O*- and 8-*O*-glycosylated seco-pseudopterosin structures. Otherwise, chemical conversion of glycosylated seco-pseudopterosins to the known 7-hydroxy-8-methoxy- or 8-hydroxy-7-methoxy-serrulat-14-ene is necessary for the structure determination.[8,10,11] The absolute configurations at the chiral centers of most seco-pseudopterosins can be assumed as 1*S*,4*R*,11*S* from biosynthetic consideration. However, no rigorous stereochemical study has been reported until now.

Compound **9** was identified as amphilectosin A, a recently isolated (*E*)-12,13-didehydro derivative of seco-pseudopterosin J,[11] while compound **10** was identified as amphilectosin B,[11] (*Z*)-12,13-didehydro derivative of *seco*-pseudopterosin J. Our ^{1}H and ^{13}C NMR data and the assignments of signals for compound **9** are listed in Table 3.[22] The ^{13}C NMR data of **10** was not available due to the scarcity of the sample.

Kerr and his co-workers have reported a pioneering work on the biosynthesis of pseudopterosins, demonstrating that geranylgeranyl diphosphate is cyclized to give elisabethatriene **2** as the first isolable intermediate.[12,23] The bicyclic intermediate was reported to be converted to erogorgiaene presumably via dehydrogenation and aromatization.[24] Coleman et al. initially proposed a biosynthetic pathway, which involved isomerization of **2** into the isomeric 5(6),9(10)-diene, but failed to obtain evidence supporting the double-bond isomerization pathway.[24] Among the compounds isolated in the present study, elisabethatrienol **1** is interesting, since the hydroxylated structure can be considered as a biosynthetic intermediate leading to erogorgiaene (Fig. 7). If this is the case, 9-*epi*-elisabethatriene must be a precursor of **1** in place of elisabethatriene **2**. We would like to propose the biosynthetic pathway, which involves 9-*epi*-elisabethatriene and elisabethatrienol as shown in Figure 7. Elisabethatrienol can be converted to erogorgiaene by dehydration followed by double bond isomerization (aromatization). As proposed by Kohl et al.,[23] subsequent oxidation of the benzene ring would produce the catechol (*seco*-pseudopterosin aglycone). The co-occurrence of compounds **7** and **8** (both are 7-*O*-glycoside) suggests that the glycosylation should take place at this stage using either D-arabinose, D-xylose or L-fucose. Glycosylation at C-8 would be also possible so that pseudopterosins and *seco*-pseudopterosins glycosylated at the C-8 position can be formed. Acetylation onto the sugar may proceed just after glycosylation, which can explain the co-occurrence of variously acetylated *seco*-pseudopterosins. The glycosylated intermediate will then

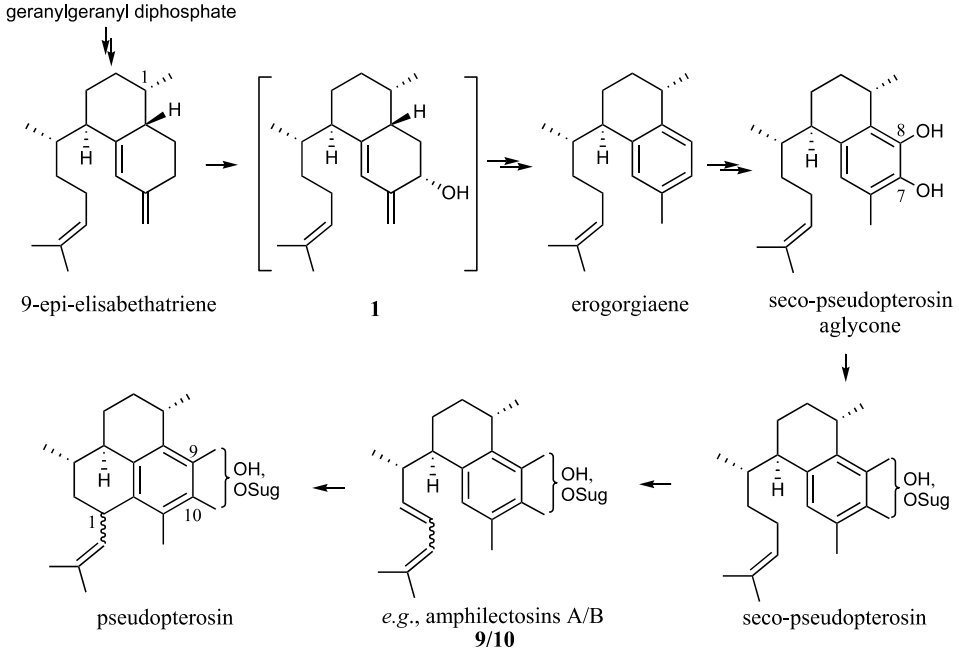


Figure 7. Postulated biosynthetic pathway of pseudopterosins involving elisabethatrienol **1** and 9-epi-elisabethatriene as putative intermediates.

undergo dehydrogenation to give the *E* and *Z* dienes (amphilectosins A/B **9**/**10**).[11] Fern et al.[11] recently described important findings that **8** is converted into **9** and **10** with a cell-free extract of *P. elisabethae*, which are further metabolized into pseudopterosin Y (1β-H) and pseudopterosin F (1α-H), respectively. The formation of the dienes **9** and **10** seems to be an obligatory step, since the activation at the C-13 position is reasonably explained by the presence of the double bond.

It should be noted that the C-9 configuration of elisabethatrienol **1** is different from that of elisabethatriene **2**. An early stage of the biosynthesis of pseudopterosins may involve either 9β-H compounds or 9α-H counterparts, and the discrepancy of the C-9 configuration in **1** and **2** needs to be clarified. A further investigation is highly awaited to see if **1** can be metabolized into erogorgiaene.

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. IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 500 spectrophotometer. ^{1}H 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. ^{13}C chemical shifts are referenced to the solvent signal ($\delta=$ 77.0). EIMS 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 210 nm) using a Nucleosil 120 10 C-18 (300×8 mm i.d., 10 μm) column with a gradient of 70% aq acetonitrile to 100% acetonitrile within 30 min 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 254 nm) under a Shimadzu Shim-Pack CLC-ODS (150×6 mm i.d., 5 μm) using MeOH–water (9/1) as mobile phase at a flow rate of 1.0–1.5 ml/min.

3.2. Animal material

Fragments of individual colonies of *Pseudopterogorgia elisabethae* were collected by SCUBA (ca. 20–30 m depth) at four sites at the leeward portion of San Andrés island (SW Caribbean) and 12 sites at the leeward portion of Providencia island, between June and September, 2002. Colony fragments were cut off along the main gorgonian axis with sharp scissors. Ten replicates were collected on average per site. Gorgonian fragments were air-dried under the shade, and stored in the freezer until the moment of extraction. Animals were identified as *P. elisabethae* by Dr. M. Puyana, and voucher specimens were deposited at the invertebrate collection of MHNMC (Museo de Historia Natural Marina Colombiano) at INVEMAR (Instituto de Investigaciones Marinas de Punta de Betín), coded as INV CNI 1612, INV CNI 1613 and INV CNI 1614.

3.3. Extraction and separation of compounds

Dried colony fragments were cut in small pieces, weighed and repeatedly extracted with a dichloromethane–methanol (1/1) mixture. Resultant extracts were filtered and concentrated by rotary evaporation obtaining a dark green oily extract. HPLC analysis with the aid of HPLC-MS (APCI mode) was carried out for each colony extract from each location in order to determine chemical variability between the different collection sites.[6] Crude extracts obtained from each chemotype (chemotype 1 from Providencia island and chemotype 2 from San Andrés island) were pooled, until substantial amounts of individual extracts were accumulated. The crude extracts thus obtained (4.0 g for each chemotype) were subjected to silica gel column chromatography eluting with a solvent of increasing polarity consisting of hexane/CH_2Cl_2, AcOEt/CH_2Cl_2, and AcOEt/MeOH to yield eight fractions.

Fraction 6 (eluted with AcOEt) from the extract of chemotype 1 contained compounds **7**–**10**. The fraction was further separated on preparative reversed-phase HPLC (solvent gradient acetonitrile/water, 70:100% in 30 min; flow rate 1 ml/min) to give compounds **9** (retention time, 15.5–16.5 min), **10** (16.6–17.8 min), **7** (21–22 min), and **8** (25–26 min). Final HPLC purification of these samples yielded pure compounds **7** (6 mg), **8** (4 mg), **9** (4 mg) and **10** (1 mg).

Fraction 4 [eluted with AcOEt–CH_2Cl_2 (4/1)] from the extract of the chemotype 2 contained compounds **1**, **3** and **5**. The fraction was further chromatographed on a C-18 open column with a discontinuous gradient of acetonitrile–water (50/50, 70/30, 85/15, 90/10) to give a mixture (500 mg) of **1**, **3** and **5** in the fraction eluted with acetonitrile–water (85/15). A part of that mixture (200 mg) was then separated by preparative reversed-phase HPLC (solvent, acetonitrile; flow rate, 1.5 ml/min) to give **3** (50 mg), and a mixture of compounds **1** and **5**, which were further purified by reversed-phase HPLC (solvent, acetonitrile/water, 85:15; flow rate 1 ml/min), to obtain 5 mg of each. Compounds **1** and **5** were purified by *p*-TLC prior to spectral analysis since these compounds decomposed slowly.

3.3.1. Compound 1. White amorphous solid; $[\alpha]_D^{25}$ −44.8 (*c*, 0.14, MeOH); UV λ_{max}^{MeOH} (ε): 236 (16,800) nm; IR ($CHCl_3$) ν_{max} 3600, 3525, 3020, 2970, 2930, 2880, 1675, 1450, 1385, 1070, 890 cm^{-1}; EI-MS *m*/*z* (relative intensity): 288 (M^+, 100), 270 (14), 255 (8), 227 (8), 213 (7), 204 (45), 177 (70), 160 (60), 159 (33), 131 (59), 117 (60), 105 (49), 91 (36), 79 (37); HREIMS *m*/*z*: 288.2415 (M^+), $C_{20}H_{32}O$ requires 288.2453; ^{1}H and ^{13}C NMR: see Table 1.

3.3.2. MTPA esters of 1 (1s and 1r). (*S*)- and (*R*)-MTPA ester derivatives of **1** were prepared by reacting **1** (0.3 mg) in pyridine (30 μl) with (*R*)- and (*S*)-MTPA chlorides (1.5 μl), respectively, at room temperature for 1 h. The reaction mixture was diluted with methanol and directly applied to silica gel *p*-TLC plate. The plate was developed with hexane–AcOEt (20/1) and elution of the major band furnished the desired ester as an oil in a good yield. ^{1}H NMR data, see Table 2. HRFABMS **1s** *m*/*z*: 505.2919

$[M+H]^+$ and **1r** *m/z*: 505.2914 $[M+H]^+$. $C_{30}H_{40}O_3F_3$ requires 505.2930.

3.3.3. Compound 3. Yellow amorphous solid; $[\alpha]_D^{25}$ +77.5 (*c*, 0.21, MeOH); UV λ_{max}^{MeOH} (*ε*): 207 (36,900), 230 (shoulder), 276 (2000) nm; IR ($CHCl_3$) ν_{max} 3590, 3030, 2970, 2930, 2880, 1765, 1450, 1440, 1370, 1040 cm^{-1}; EI-MS *m/z* (relative intensity): 342 (M^+, 10), 300 (9), 286 (39), 244 (100), 229 (35), 187 (13), 109 (14); HREIMS *m/z*: 342.2166 (M^+), $C_{22}H_{30}O_3$ requires 342.2195; ^1H and ^{13}C NMR: see Table 1.

3.3.4. Acetate derivative of 3 (3a). Compound **3** (4 mg) was allowed to stand in pyridine (100 μl) and acetic anhydride (50 μl) at room temperature for 2 h. After addition of 0.2 ml of methanol, the whole mixture was concentrated by flushing nitrogen. The orange oily residue was analyzed by NMR without purification. Yellow oil; $[\alpha]_D^{25}$ +66.4 (*c*, 0.71, MeOH); UV λ_{max}^{MeOH} (*ε*): 206 (35,800), 227 (shoulder) nm; IR ($CHCl_3$) ν_{max} 3030, 2970, 2930, 2880, 1760, 1440, 1370, 1050 cm^{-1}; EI-MS *m/z* (relative intensity): 384 (M^+, 4), 342 (5), 328 (50), 300 (8), 286 (90), 244 (100), 229 (20), 187 (9); HREIMS *m/z*: 384.2293 (M^+), $C_{24}H_{32}O_4$ requires 384.2301; ^1H and ^{13}C NMR: see Table 1.

3.3.5. Conversion of 3–5. $LiAlH_4$ (1 mg) was added to a stirred solution of compound **3** (2.0 mg) in dry ether (1 ml) and the mixture was reacted for 10 min when the starting material disappeared and a polar spot presumably due to a catecol was monitored by TLC analysis. Several drops of water and satd aq NH_4Cl were added to the mixture. TLC analysis of the organic layer showed practically single spot corresponding to the quinone with disappearance of the polar spot. The organic layer was separated and the water layer was re-extracted with ether. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was chromatographed on silica gel with *n*-hexane–AcOEt (4/1) to give **5** (1.1 mg, 63%) as a yellow oil; $[\alpha]_D^{25}$ +134 (*c*, 0.13, MeOH). The compound was identified with compound **5** by ^1H NMR and TLC analysis.

3.3.6. Compound 5. Orange oil; $[\alpha]_D^{25}$ +146.0 (*c*, 0.10, MeOH); UV λ_{max}^{MeOH} (*ε*): 208 (27,700), 285 (2100) nm; IR ($CHCl_3$) ν_{max} 3600, 3525, 3020, 2970, 2930, 2880, 1675, 1450, 1385, 1070, 890 cm^{-1}; EI-MS *m/z* (relative intensity): 298 (M^+, 100), 283 (59), 255 (24), 244 (93), 229 (65), 197 (28), 187 (25); HREIMS *m/z*: 298.1894 (M^+), $C_{20}H_{26}O_2$ requires 298.1933; ^1H and ^{13}C NMR: see Table 1.

3.3.7. Compound 7. White amorphous solid; $[\alpha]_D^{25}$ −121.3 (*c*, 0.43, MeOH); UV λ_{max}^{MeOH} 208 (42,000), 230 (shoulder), 280 (1500) nm; IR ($CHCl_3$) ν_{max} 3330, 3020, 2970, 2930, 2880, 1090 cm^{-1}; EI-MS *m/z* (relative intensity): 448 (M^+, 15), 303 (96), 302 (75), 218 (90), 192 (84), 173 (90), 161 (41), 157 (40), 145 (71), 129 (61), 115 (31), 91 (28), 75 (100), 73 (81), 69 (91); HREIMS *m/z*: 448.2816 (M^+), $C_{26}H_{40}O_6$ requires 446.2825; ^1H and ^{13}C NMR: see Table 3.

3.3.8. Compound 8. White amorphous solid; $[\alpha]_D^{25}$ −120.0 (*c*, 0.70, MeOH); UV λ_{max}^{MeOH} 207 (42,000), 230 (shoulder), 280 (1300) nm; EI-MS *m/z* (relative intensity): 434 (M^+, 37), 303 (100), 302 (51), 244 (66), 218 (95), 192 (84), 173 (78), 157 (35), 145 (53), 129 (40), 115 (37), 91 (19), 73 (90), 69 (65); HREIMS *m/z*: 434.2711 (M^+), $C_{25}H_{38}O_6$ requires 434.2668; ^1H and ^{13}C NMR: see Table 3.

3.3.9. Compound 9. Colorless oil; $[\alpha]_D^{25}$ −102.3 (*c*, 0.19, MeOH); UV λ_{max}^{MeOH} 206 (32,000), 233 (19,500), 280 (1400) nm; EI-MS *m/z* (relative intensity): 432 (M^+, trace), 365 (1), 323 (75), 316 (41), 298 (40), 229 (37), 192 (97), 173 (99), 157 (39), 145 (67), 129 (50), 109 (100), 91 (33), 73 (82), 67 (41); HRFABMS (negative mode) *m/z*: 431.2454 ($M-H^-$), $C_{25}H_{35}O_6$ requires 431.2434; ^1H and ^{13}C NMR: see Table 3.

3.3.10. Compound 10. Colorless oil; $[\alpha]_D^{25}$ −64.4 (*c*, 0.07, MeOH); UV λ_{max}^{MeOH} 205 (29,300), 230 (13,700), 281 (1400) nm; EI-MS *m/z* (relative intensity): 432 (3), 365 (4), 323 (22), 316 (8), 298 (26), 229 (22), 192 (75), 191 (100), 173 (29), 157 (22), 145 (14), 129 (11), 109 (34), 91 (8), 73 (13), 67 (7); HRFABMS (negative mode) *m/z*: 431.2472 ($M-H^-$), $C_{25}H_{35}O_6$ requires 431.2434; ^1H and ^{13}C NMR: see Table 3.

Acknowledgements

This work was financially supported by grants from Colciencias, Fundación para la Promoción de la Investigación y la Tecnología del Banco de la República and DIB (División de Investigaciones-sede Bogotá, de la Universidad Nacional de Colombia). Coralina granted permission to collect samples and perform research at the archipiélago of San Andres and Providencia, Colombian Caribbean.

References and notes

1. Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5140–5145.
2. Roussis, V.; Wu, Z.; Strobel, S. A.; Duyne, G. D. V.; Clardy, J.; Fenical, W. *J. Org. Chem.* **1990**, *55*, 4916–4922.
3. Ata, A.; Kerr, R. G.; Moya, C. E.; Jacobs, R. S. *Tetrahedron* **2003**, *59*, 4215–4222.
4. Petrichtcheva, N. V.; Duque, C.; Dueñas, A.; Zea, S.; Hara, N.; Fujimoto, Y. *J. Nat. Prod.* **2002**, *65*, 851–855.
5. Martínez, A.; Duque, C.; Sato, N.; Fujimoto, Y. *Chem. Pharm. Bull.* **1997**, *45*, 181–184.
6. Puyana, M.; Narvaez, G.; Paz, A.; Osorno, O.; Duque, C. *J. Chem. Ecol.* **2004**, *30*, 1183–1201.
7. Duque, C.; Puyana, M.; Narvaez, G.; Osorno, O.; Hara, N.; Fujimoto, Y. *Tetrahedron* **2004**, *60*, 10627–10635.
8. Rodríguez, I. I.; Shi, Y.-P.; García, O. J.; Rodríguez, A. D.; Mayer, A. M. S.; Sanchez, J. A.; Ortega-Barria, E.; González, J. *J. Nat. Prod.* **2004**, *67*, 1672–1680.
9. Confusion exists in the designation of pseudopterosins. For example, three different compounds were named as PsP (Ata, A.; Win, H. Y.; Holt, D.; Holloway, P.; Segstro, E. P.; Jayatilake, G. S. *Helv. Chim. Acta* **2004**, *87*, 1090–1098. Refs. 6, 7 and 8).
10. Look, S. A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363–3370.
11. Ferns, T. A.; Kerr, R. G. *J. Org. Chem.* **2005**, *70*, 6152–6157.
12. Coleman, A. C.; Kerr, R. G. *Tetrahedron* **2000**, *56*, 9569–9574.

13. It is likely that the assignments of 18-H_3 and 20-H_3 of **2** reported in Ref. 12 need to be reversed. This implies the ^{13}C assignments of **2** based on HMBC correlations from these methyl groups have to be re-assigned.
14. Forster, P. G.; Ghisalberti, E. L.; Jefferies, P. R.; Poletti, V. M.; Whiteside, N. J. *Phytochemistry* **1986**, *25*, 1377–1383.
15. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
16. Compound **1** may be named serrulatane or biflorane diterpene. However, the application of these naming systems suffers serious disadvantage. The serrulatane skeleton (1*R*,4*S*,11*S* absolute configuration) represents a bicyclic diterpene structure in which one of the six-membered ring must be a benzene ring. The biflorane skeleton has a completely different numbering system, compared to serrulatane.
17. The amphilectane skeleton represents a 1β-H,3β-H,4α-H,7β-H tricyclic structure. Pseudopterosins and related compounds can be named as amphilectane or 1-*epi*-amphilectane depending on the orientation of 1α-H and 1β-H.
18. Lazerwith, S. E.; Johnson, T. W.; Corey, E. J. *Org. Lett.* **2000**, *12*, 2389–2392.
19. Harvis, C. A.; Burch, M. T.; Fenical, W. *Tetrahedron Lett.* **1988**, *29*, 4361–4364.
20. Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5140–5145.
21. McCombie, S. W.; Cox, B.; Ganguly, A. K. *Tetrahedron Lett.* **1991**, *32*, 2087–2090.
22. The ^{13}C NMR data of amphilectosin A (**9**) reported in Ref. 11 involves errors: the δ 53.4 signal should be deleted and a sugar oxymethine carbon signal needs to be added.
23. Kohl, A. C.; Ata, A.; Kerr, R. G. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 495–499.
24. Coleman, A. C.; Kerr, R. G. *Mar. Drugs* **2003**, *1*, 54–65.
25. Ghisalberti, E. L.; Jefferies, P. R.; Vu, H. T. N. *Phytochemistry* **1990**, *29*, 316–318.