Elisabatins A and B: New Amphilectane-Type Diterpenes from the West Indian Sea Whip *Pseudopterogorgia elisabethae*

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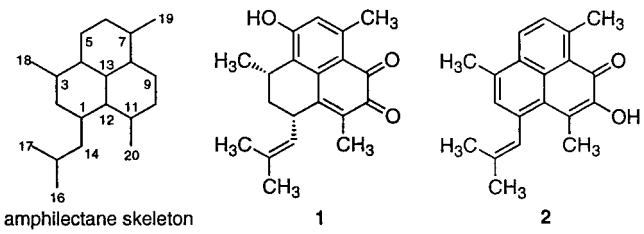
A chemical study of the hexane extracts of the gorgonian octocoral *Pseudopterogorgia elisabethae* collected in San Andrés Island, Colombia, led to the isolation of two highly conjugated amphilectane-type diterpenes, compounds **1** and **2**. Their structures were established by spectroscopic studies, which included 2D NMR correlation methods, IR, UV, and accurate mass measurements (HREIMS).

Sea whips of the genus *Pseudopterogorgia* are conspicuous dwellers of the Caribbean reefs, and previous chemical studies have shown that this genus constitutes a remarkable source of a large variety of secondary metabolites, mainly diterpenoids, sesquiterpenoids, and steroids.[3] As part of our continuing interest in the biomedical potential of these abundant marine invertebrates, we have recently focused our attention on one representative of this genus, *Pseudopterogorgia elisabethae* Bayer (order Gorgonacea, family Gorgoniidae), collected in deep waters off San Andrés Island, Colombia (1 kg of dry wt). In an earlier investigation of the biomedical applications of secondary metabolites from this source, we encountered a family of cancer cell cytotoxins possessing novel carbocyclic skeletons.[4] In this paper, we report the isolation and structure elucidation of two compounds, elisabatin A (**1**) and elisabatin B (**2**), which have the same amphilectane skeleton found in the aglycon portion of the pseudopterosins, a class of potent antiinflammatory glycosides isolated from *P. elisabethae*.[5] Diterpenoids having the amphilectane skeleton have also been found in sponges of the order Halichondrida[6a−c] and in the Japanese corals *Heliopora coerulea*[6d] and *Sinularia nanolobata*.[6e] Compounds **1** and **2**, however, are unique among the amphilectane-type diterpenoids because they possess an unusually high unsaturation number that leads to extended aromatic conjugation. Moreover, the α configuration is assigned to the isobutenyl side chain at C1 in elisabatin A, making **1** distinctly different from other amphilectanes previously isolated from *P. elisabethae*, having the opposite configuration at that center.[5]



amphilectane skeleton **1** **2**

The major metabolite, elisabatin A (**1**) (2.97×10^{-2}% of the crude extract), was isolated from the hexane extracts as a yellow oil after size exclusion (Bio Beads SX-3 in toluene) and replicate column chromatography. HREIMS and ^{13}C NMR spectral analyses of elisabatin A suggested a molecular formula of $C_{20}H_{22}O_3$, thus indicating 10 degrees of unsaturation. Because the ^{13}C NMR spectrum contained only 10 aromatic and olefinic carbon resonances, in addition to two carbonyls, the molecule was judged to be tricyclic. The ^{1}H NMR spectrum of compound **1** contained five methyl resonances [δ 2.58 (3H, s), 1.93 (3H, s), 1.78 (3H, s), 1.71 (3H, s), 1.36 (3H, d, J = 7.2 Hz)], which was consistent with a diterpenoid skeleton. The presence of a pentasubstituted aromatic ring and a trisubstituted double bond was indicated from resonances in the aromatic [δ 6.75 (1H, br s)] and olefinic [δ 5.13 (1H, d, J = 9.1 Hz)] region in the ^{1}H NMR spectrum. This was confirmed when the multiplicities of the corresponding carbons were obtained (see Table 1).

The detection of three absorption maxima in the UV (MeOH) spectrum of **1** at λ_{max} = 218 (ϵ 17 000), 276 (ϵ 10 000), and 406 (ϵ 3000) nm suggested that the phenol ring had extended conjugation. An absorption at 3600−3030 cm^{-1} in the IR spectrum, as well as a pronounced bathochromic shift (from 276 to 308 nm) in the UV spectrum upon addition of base (one drop 5% KOH−MeOH) confirmed the presence of a phenol in the molecule. A D_2O-exchangeable proton observed at δ 8.10 (1H, br s) in the ^{1}H NMR spectrum was assigned to the phenolic hydroxyl group. One substituent on the phenol was proposed to be an aromatic methyl on the basis of a deshielded three-proton singlet resonance at δ 2.58. Furthermore, a ^{1}H−^{1}H COSY experiment showed allylic coupling between the latter and the aromatic resonance at δ 6.75 (1H, br s), thus establishing their vicinal relationship.

With the aid of spin-decoupling and ^{1}H−^{1}H COSY experiments, considerable connectivity could be established, and most resonances in the ^{1}H NMR spectrum of **1** were subsequently assigned. Further consideration of the ^{13}C NMR spectral data showed elisabatin A to possess an additional trisubstituted olefin [125.8 (d), 132.5 (s)] that was nonconjugated. Irradiation of a lowfield resonance in the ^{1}H NMR spectrum of **1** [δ 5.13 (1H, d, J = 9.1 Hz)] assigned to the olefinic proton sharpened the signals at δ 1.78 (3H, br s) and 1.71 (3H, br s) and decoupled a band at midfield [δ 3.85 (1H, dd)]. These data suggested that an isobutenyl group was present in the molecule.

Irradiation of a complex one-proton signal at δ 3.33 caused the doublet methyl group signal at δ 1.36 to collapse to a singlet resonance and also decoupled a diastereotopic proton at δ 2.12 (1H, ddd) to a doublet of doublets [J = 5.4, 13.5 Hz; the other diastereotopic proton at δ 1.89 (1H, d) remained unchanged]. In turn, irradiation of the band at δ 2.12, assigned to H2β, caused two methine signals at δ 3.85 (H1) and 3.33 (H3) to collapse into a broad doublet

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Table 1. ^{1}H NMR (500 MHz), ^{13}C (125 MHz) NMR, and HMBC Spectral Data for Elisabatin A (**1**) and Elisabatin B (**2**)[a]

	elisabatin A (**1**)			elisabatin B (**2**)		
position	δ, mult (J in Hz)	^{13}C	HMBC	δ, mult (J in Hz)	^{13}C	HMBC
1	3.85, dd (5.4, 9.1)	36.1, d	H2α*β*, H3, H14		131.1, s	
2α	1.89, d (13.5)	33.7, t	H1, H3, H18	7.16, br s	131.8, d	H18
2*β*	2.12,ddd (5.4,5.7,13.5)					
3	3.33, dq (5.7, 7.2)	26.3, d	H1, H2α*β*, H18		125.7, s	H2, H18
4		130.9, s	H3, H6, H18		129.4, s	H2, H6, H18
5		160.5, s	H3, H6	8.29, d (8.4)	131.3, d	
6	6.75, br s	119.2, d	H19	7.57, d (8.4)	130.6, d	H19
7		146.8, s	H19		147.5, s	H5, H19
8		122.0, s	H6, H19		124.2, s	H6, H19
9		180.8, s			179.3, s	
10		182.6, s	H20		147.9, s	H20
11		132.2, s	H1, H20		141.2, s	
12		153.0, s	H1, H14, H20		122.9, s	H20
13		133.6, s	H1, H3		136.1, s	H5
14	5.13, d (9.1)	125.8, d	H1, H2α*β*, H16, H17	6.68, br s	128.6, d	H16, H17
15		132.5, s	H1, H16, H17		134.7, s	H16, H17
16	1.71, br s	25.5, q	H14, H17	1.97, d (1.2)	25.7, q	H14, H17
17	1.78, br s	17.6, q	H14, H16	1.62, d (0.9)	19.5, q	H14, H16
18	1.36, d (7.2)	22.2, q	H2α*β*, H3	2.76, d (0.6)	19.4, q	H2
19	2.58, s	23.8, q	H6	3.05, br s	25.1, q	H6
20	1.93, s	10.8, q		2.51, s	16.7, q	
−OH	8.10, br s, exchangeable			7.56, br s, exchangeable		

[a] Recorded in $CDCl_3$. Assignments were aided by ^{1}H−^{1}H COSY, spin splitting patterns, analysis of J values, HMBC and HMQC experiments, numbers of attached protons as measured from DEPT spectra, and chemical shift values. The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals.

(J = 9.1 Hz) and a sharp quartet (J = 7.2 Hz) resonance, respectively. Furthermore, irradiation of the signal assigned to H18 [δ 1.36 (3H, d, J = 7.2 Hz)] decoupled the signal at δ 3.33 (H3) to a broad doublet (J = 5.7 Hz). Thus, the signal at δ 5.13 (H14) is coupled to a signal at δ 3.85 (H1), which was assigned to a pseudoequatorial proton on a six-membered ring that is, in turn, coupled only to one pseudoaxial proton (H2*β*). This is exactly the situation for the isobutenyl side chain in an amphilectane ring system lacking hydrogens at positions 4 and 12. These data, together with the UV spectrum and the absence of further spin systems, indicated that the remaining substructure of **1** must be a highly conjugated bicyclic system.

Further examination of the spectral data suggested such substructure to be a *o*-naphthoquinone. By HREIMS, the reduced form of elisabatin A (**1**) was analyzed for $C_{20}H_{24}O_3$ $[M + 2]^+$, a molecular formula consistent with this assignment. The UV absorption at 350−450 nm (br s, ϵ 3000) and IR absorptions at 1653 and 1646 cm^{-1}, in conjunction with the observed $[M + 2]^+$ ion observed in the mass spectrum of **1**, were all consistent with this assignment. Hence, for these data to be compatible with the proposed amphilectane-type structure, a phenolic hydroxyl and two methyl groups were proposed to be at positions 5, 7, and 11, respectively, around the *o*-naphthoquinone component of **1**.[7] Connectivities of all the partial structures in elisabatin A were determined by a detailed analysis of the HMBC spectrum of **1**, in particular, those depicted in Table 1. The relative stereochemistry of **1** was revealed by NOE correlations and J values for the ^{1}H NMR spectrum, that is, $J_{1-2\beta}$ = 5.4 Hz and $J_{2\beta-3}$ = 5.7 Hz (pseudoaxial−pseudoequatorial couplings). In particular, the very intense NOE correlation of δ_H 5.13 (H14) and δ_H 1.36 (H18) together with the conspicuous absence of NOE between δ_H 3.85 (H1) and δ_H 3.33 (H3) suggested that the methyl group at C3 and the isobutenyl side chain at C1 are both pseudoaxial.[8] Thus, H1 and H3 must be pseudoequatorial. These observations also proved to be consistent with the apparent lack of coupling between H1/H2α and H3/H2α, which according to estimates from a molecular modeling study, should have dihedral angles between them approaching 90°.

Elisabatin B (**2**) (3.76 × 10^{-3}% of the crude extract) analyzed for $C_{20}H_{20}O_2$ by HREIMS and ^{13}C NMR methods and suggested that the molecule was highly unsaturated. This orange oil possessed ^{1}H and ^{13}C NMR features similar to **1**, including one hydroxyl group, but was subsequently recognized as a pentasubstituted perinaphthenone by its spectral properties [IR: 1602 cm^{-1}; ^{13}C NMR: 179.3 ppm; UV (MeOH): λ_{max}= 246 (vs), 286 (sh), 348 (w), 374 (sh), 450 (br); the HREIMS gave a $[M + 1]^+$ ion at m/z 293.1495 (24%) ascribable to the reduced form of **2**, which analyzed for $C_{20}H_{21}O_2$]. These latter functional groups accounted for all the oxygen atoms in **2**. Further, HMBC (Table 1), NOE,[9] spin-decoupling, and long-range ^{1}H−^{1}H COSY[10] experiments positioned a vinylic alcohol at C10 and defined three aromatic protons [δ 7.16 (1H, br s, H2), 8.29 (1H, d, J = 8.4 Hz, H5), 7.57 (1H, d, J = 8.4 Hz, H6)], three methyl groups [δ 2.76 (3H, d, J = 0.6 Hz, H18), 3.05 (3H, br s, H19), 2.51 (3H, s, H20)], and a conjugated isobutenyl side chain [δ 6.68 (1H, br s, H14), 1.97 (3H, d, J = 1.2 Hz, H16), 1.62 (3H, d, J = 0.9 Hz, H17)] along the 12-carbon backbone between C1 and C12. Comparison of these data with those of **1** showed that **2** possessed the same amphilectane skeleton. Notwithstanding, an alternative structure to **2** was carefully considered in which the −OH is placed at C2 and the carbonyl is placed at C5. Although such an isomer could not be dismissed readily by NMR (1D and 2D), IR, or UV methods, structure **2**, on the other hand, was strongly favored by the mass spectral data.[11] Elisabatin B (**2**) decomposed slowly upon prolonged storage in $CDCl_3$.

Biological screening of elisabatin A (**1**) in the NCI's 60-cell-line tumor panel indicated weak in vitro cancer cell cytotoxicity. Compound **1** also proved inactive in the NCI test for agents with anti-HIV activity. In vitro antituberculosis screening of **1** against *Mycobacterium tuberculosis* at 12.5 μg/mL showed a mere 21% inhibition.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively, with

a Bruker Avance DRX-500 spectrometer. IR spectra were determined with a Nicolet Magna FT-IR 750 spectrophotometer and UV spectra were recorded with a Hewlett−Packard Chem Station 8452A spectrometer. Optical rotations were recorded on a Perkin−Elmer polarimeter (model 243B). Column chromatography was performed on Si gel (35−75 mesh) and TLC analyses were carried out using glass precoated Si gel plates. HPLC was performed using a 10 μm Si gel Partisil 10 semipreparative column (9.4 mm × 50 cm). All solvents used were either spectral grade or were distilled from glass prior to use.

Collection and Extraction Procedures. The Caribbean sea whip *P. elisabethae* Bayer (order Gorgonacea, family Gorgoniidae) was collected by hand using scuba at depths of 80−100 ft in May 1996, off San Andrés Island, Colombia. A voucher specimen (no. PESAI-01) is stored at the Chemistry Department of the University of Puerto Rico. The gorgonian was sun-dried and kept frozen prior to its extraction. The dry animal (1.0 kg) was blended with MeOH−$CHCl_3$ (1:1) (11 × 1 L), and after filtration, the crude extract was evaporated under vacuum to yield a green residue (284 g). After partitioning the crude extract between hexane and H_2O, the resulting extract was concentrated in vacuo to yield 178 g of an oil, a portion of which (50 g) was dissolved in a small volume of toluene, filtered, and loaded onto a large Bio-Beads SX-3 column with toluene as eluant. Four fractions were obtained: fraction 1 (24.1 g), fraction 2 (9.2 g), fraction 3 (15.1 g), and fraction 4 (1.57 g). After preliminary NMR analyses, fraction 3 was separated into 18 subfractions by Si gel (270 g) column chromatography using 10% EtOAc in hexane as eluant. Fraction 3.17 (588 mg), which was subsequently purified by successive column chromatography [(a) Si gel (20.5 g) with 5% 2-propanol in hexane and (b) Si gel (5.0 g) with 1% 2-propanol in hexane], afforded elisabatin A (**1**) (23.7 mg; 2.97 × 10^{-2}% yield). Elisabatin B (**2**) (3.0 mg; 3.76 × 10^{-3}% yield) was obtained pure after purification of fraction 4 by column chromatography on Si gel (20.0 g) using a step gradient of EtOAc−hexane (0−25%) as eluant followed by normal-phase HPLC [Partisil 10 M9/50 Si gel with 3% 2-propanol in hexane].

Elisabatin A (1): yellow oil; $[\alpha]^{24}_D$ +59° (*c* 0.56, $CHCl_3$); IR (film) 3600−3030, 2925, 1653, 1646, 1635, 1558 cm^{-1}; UV λ_{max} (MeOH) 218 (ϵ 17 000), 276 (ϵ 10 000), and 406 (ϵ 3000) nm; ^1H NMR (500 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* 312 $[M + 2]^+$ (2), 310 $[M]^+$ (17), 295 (23), 282 (100), 267 (44), 259 (19), 239 (59), 225 (28), 211 (29), 183 (23), 165 (34), 152 (22), 128 (31), 115 (33), 91 (25), 55 (41); HREIMS *m*/*z* 312.1726 $[M + 2]^+$ (calcd for $C_{20}H_{24}O_3$, 312.1725), 310.1579 $[M]^+$ (calcd for $C_{20}H_{22}O_3$, 310.1569).

Elisabatin B (2): orange oil; IR (film) 3360, 2926, 1602, 1378, 1336, 1256, 1137 cm^{-1}; UV λ_{max} (MeOH) 246 (vs), 286 (sh), 348 (w), 374 (sh), 450 (br); ^1H NMR (500 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* 293 $[M + 1]^+$ (24), 292 $[M]^+$ (100), 277 (99), 262 (21), 254 (19), 249 (24), 231 (16), 224 (9), 202 (10); HREIMS *m*/*z* 293.1495 $[M + 1]^+$ (calcd for $C_{20}H_{21}O_2$, 293.1541), 292.1463 $[M]^+$ (calcd for $C_{20}H_{20}O_2$, 292.1463).

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(7) Another conceivable structure for **1** was carefully considered in which the phenolic −OH was placed at C9 and the two carbonyls of the *o*-naphthoquinone were placed at C5 and C6. Structure **1**, however, was strongly favored as the HMBC, NOE, and $^1H-^1H$ COSY data fully agreed only with this isomer.
(8) Selected NOEs for **1**: H6/H19, H14/H16, H14/H18, H2β/H3, H1/H17, H1/H2β, H14/H20, H1/H20.
(9) Selected NOEs for **2**: H2/H18, H5/H6, H5/H18, H6/H19, H14/H16, H14/H20, H16/H17.
(10) Long-range $^1H-^1H$ COSY couplings in **2** were observed between H2/H18, H6/H19, H14/H16, and H14/H17.
(11) For instance, an ion peak at *m*/*z* 231.1165 (16%; $C_{18}H_{15}$) could arise by loss of $C_2H_5O_2^{\bullet}$ from $M^{\bullet+}$ or loss of CH_2O_2 from the $[M - CH_3]^+$ ion. Whichever way, loss of a small fragment containing both oxygen atoms is likely to occur only in structure **2**.

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