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Elisapterosin F: a polycyclic gorgonian-derived diterpene with a facially amphiphilic structure

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Abstract

Analysis of the terpene metabolites of *Pseudopterogorgia elisabethae* collected in San Andrés island, Colombia has resulted in the discovery of a novel metabolite, elisapterosin F (**1**). The tangled molecular structure of **1**, which was elucidated after extensive spectroscopic data interpretation, possesses hydrophilic and hydrophobic groups located on two opposite faces, rather than at two ends as in the more conventional head/tail amphiphiles.

Keywords

Pseudopterogorgia elisabethae; Gorgonian coral; Amphiphile; Diterpene; Elisapterosin

The elisapterosins are a small family of structurally interesting diterpenes isolated from the gorgonian octocoral *Pseudopterogorgia elisabethae* (Bayer, 1961).¹ Currently, there are five known elisapterosin derivatives (A–E), all of which have been extracted from collections of *P. elisabethae* from the same geographic location, namely San Andrés island (12°33'N 81°43' W), located at about 775 km (480 miles) northwest of Colombia and 220 km (140 miles) from the coast of Nicaragua.^{2–4} On the other hand, *P. elisabethae* specimens collected from distinct geographic locations, are typically rich in pseudopterogens, a family of 30 or so diterpene glycosides containing an amphilectane diterpene skeleton.⁵ The elisapterane family of marine natural products have attracted considerable interest from the synthetic community, due in significant part to the challenge presented by their fascinating molecular architecture.⁶

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During a recent chemical study of an as yet uninvestigated fraction left over from the hexane extract of *P. elisabethae* collected in San Andrés island, we re-isolated a number of metabolites which had been already described by our research group. The re-isolation of these compounds allowed us to obtain biological activity data not available from previous investigations. In addition, we have isolated a new metabolite containing the rare elisapterane skeleton, namely elisapterosin F (**1**), the subject of this report.

The MeOH–CHCl₃ (1:1) extract of the gorgonian (~ 1 kg of dry wt) was partitioned between hexane and water (3 × 800 mL) to yield a green residue (284 g). A significant portion of the hexane extract (128 g) was loaded onto a large silica gel column (780 g) and separated by stepwise elution with hexane–acetone mixtures (0–100%), and then with 100% MeOH. Fractions were pooled on the basis of their TLC and NMR profiles to yield seven primary fractions (I–VII). Fraction IV (83.3 g) was separated further into 16 subfractions (A–P) after silica gel (600 g) flash column chromatography (CC) using a step gradient eluent system based on hexane–EtOAc mixtures. Subsequent purification of tertiary fraction F (7.0 g) by silica gel (150 g) CC using a 1:1 mixture of hexane–CHCl₃, afforded a series of non polar fractions. Elisapterosin F (**1**) (3.7 mg; 1.3 × 10^{−3}% yield) was obtained pure after the most polar fraction (113 mg) was purified by normal-phase HPLC on a 10 mm × 25 cm Magnum Partisil-10 column, 5 μm, eluted isocratically with 5% 2-propanol in hexane at 2.0 mL/min. The molecular structure of this metabolite was proposed on the basis of comprehensive analysis of the 1D and 2D NMR (¹³C, ¹H, ¹H–¹H COSY, HSQC, HMBC, and NOESY) and IR, UV, and HRESI-MS spectra.

Elisapterosin F (**1**) is a transparent oil, [α]_D²⁵ +4.6 (c 1.3, CHCl₃) that analyzed for C₂₀H₂₈O₅ on the basis of its combined HRESI-MS ([M + H]⁺ *m/z* 349.2024, Δ −0.9 mDa) and ¹³C NMR spectral features (Table 1).⁷ The IR spectrum of **1** showed broad absorptions for hydroxyl (3421 cm^{−1}) and carbonyl (1770 and 1717 cm^{−1}) groups, but showed no significant absorption in the UV spectrum. The ¹H NMR spectrum generated in CDCl₃ (Table 1), showed two sharp exchangeable protons [δ 5.55 (s, 1H), 2.20 (s, 1H)], five methyl groups

[δ 1.56 (s, 3H), 1.54 (s, 3H), 1.18 (s, 3H), 1.06 (d, $J = 7.0$ Hz, 3H), 0.99 (d, $J = 7.0$ Hz, 3H)], and 11 complex proton resonances between δ 0.67 and δ 3.29, suggestive of a polycyclic terpenoid structure.² The ^{13}C NMR spectrum of **1** contained signals for all 20 carbons, including the following: two ketone carbonyls (δ 204.8, 195.5), three oxygenated quaternary carbons, one of which was accounted for by a hemiketal functionality (δ 106.4, 87.5, 76.0), three methylenes (δ 45.4, 27.9, 27.3), five methine carbons (δ 60.9, 43.2, 43.1, 39.3, 30.6), and five methyl groups (δ 25.9, 23.6, 20.6, 17.6, 16.1). The remaining signals were ascribable to two quaternary sp^3 carbons with almost co-incident chemical shifts (δ 67.2 and 67.1). The absence of a UV absorption, when considered with the lack of olefinic carbons in the ^{13}C NMR spectrum, indicated that the two carbonyls in compound **1** were present as two nonconjugated ketones. To account for the 5 degrees of unsaturation remaining (out of the 7 required by the molecular formula), it was assumed that the molecule possessed four carbocyclic rings and a cyclic hemiketal. In spite of some significant differences in the NMR and IR spectra, our preliminary spectroscopic evidence for elisapterosin F (**1**) was slightly reminiscent of that reported for elisapterosins A (**2**) and D (**3**), a pair of congeneric diterpenes isolated previously by us from the same gorgonian extract.^{2,4}

The tangled molecular structure of **1** was defined on the basis of a standard series of 2D NMR experiments, which included ^1H - ^1H COSY, NOESY, HSQC, and HMBC, and NMR spectral comparisons with known elisapterane models.²⁻⁴ Connectivities from C-3 to C-10 were inferred from the ^1H - ^1H COSY cross-peaks, including correlations from H-3 to H₃-18 and H-7 to H₃-19. This lengthy spin system, encompassing half the carbon atoms present in **1** across three rings, was quickly recognized as it is present in all of the previously isolated compounds in this series (i.e. elisapterosins A-E).²⁻⁴ Confirmation of the proton connectivity network already established from the ^1H - ^1H COSY experiment was obtained directly from long range ^1H - ^{13}C couplings (Table 1). The structure elucidation of the remaining substructures, which comprised 10 carbon atoms and all of the oxygens present in **1**, was more difficult to achieve as they deviated substantially from those found in congeners A-E.

The HMBC experiment (HMBC experiments optimized for $2\cdot 3J_{\text{CH}} = 6$ and 8 Hz) showed connectivity between the C-1 carbon [δ_{C} 67.1 (C)] and the protons of 2-OH, C-6, and C-9. The hydroxyl-bearing tertiary carbon at position 2 (δ_{C} 76.0) showed strong HMBC correlations to 2-OH, H-4 β , 17-OH, and H₃-18. Thus the pivotal C-1 quaternary carbon must be attached to C-2, C-6, and C-9 thereby establishing the perhydroindane substructure within **1** (rings A and B). Furthermore, rings B and C were linked by a strong correlation between C-14 [δ_{C} 204.8 (C)] and protons H-6, H-9, and H₃-20. Since C-10 [δ_{C} 60.9 (CH)] correlated strongly with H₃-12, H₃-13, and H₃-20, and C-15 [δ_{C} 67.2 (C)] was strongly coupled to H-10 and H₃-20, C-15 has to be flanked by C-10 and C-14. On the other hand, ring D was connected to ring C by the observation of strong HMBC correlations of C-16 [δ_{C} 195.5 (C)] to H-10 and H₃-20, and to ring A from the mutual correlations of C-17 [δ_{C} 106.4 (C)] to the C-2 hydroxyl proton and of C-2 to the C-17 hydroxyl proton. The observation of a strong HMBC correlation of C-16 and the C-17 hydroxyl proton showed that C-16 and C-17 must themselves be linked to

one another. This strongly implied that the oxygen-bearing carbons C-11 and C-17 (the last connecting points remaining) had to be linked through an ether oxygen atom. Additional correlations supporting this connection were those from C-17 to 17-OH and C-11 [δ_C 87.5 (C)] to H₃-12 and H₃-13. The rather low field chemical shifts of C-11 (δ 87.5) and C-17 (δ 106.4) supported the bridging of these carbons through an oxygen atom as shown. Applying these combined 2D NMR methods resulted in the unambiguous assignment of all protons and carbons as listed in Table 1 and allowed the complete planar structure for **1** to be assigned.

The relative configurations of the stereocenters in the pentacyclic nucleus of elisapterosin F (i.e. C-1, C-2, C-3, C-6, C-7, C-9, C-10, C-15, and C-17) were assigned using a combination of NMR methods (NOESY and ¹H–¹H NMR coupling constants) coupled with NMR spectral comparisons and molecular modeling studies.⁸ Critically, H-9 showed NOE responses with H-3, H-7, H-8 β and H₃-12, which indicated that these protons were on the same face of the molecule and were assigned as the β protons.

Likewise, H₃-19 showed NOE responses with H-5 α , H-6, and H-8 α , but not with H-9, consistent with C-6 and C-7 having the *R** and *S** configurations, respectively. Very diagnostic NOEs between H-10 with H-8 α and H₃-20 established the spatial proximities of these protons, which were themselves placed in the α face of the molecule. Because of the rigid cage-like nature of the pentacyclic framework of elisapterosin F (**1**), the aforementioned correlations were sufficient to establish the identity of the stereocenters at C-1 and C-2 as *S** and that of C-17 as *R**, which quickly allowed the elimination of numerous inconsistent possibilities (Figure 1). Additionally, both C-9 and C-10 having the *S** configuration was supported by the conspicuously small 1.9 Hz vicinal coupling constant observed between H-9 and H-10, which indicated that the dihedral angle between these *trans* oriented protons approached 90° (calculated $\theta = 95.8^\circ$).⁸ Thus, the overall relative stereochemistry of **1** was assigned as 1*S**, 2*S**, 3*S**, 6*R**, 7*S**, 9*S**, 10*S**, 15*S** and 17*R**.

The co-occurrence of **1** with various elisapterane-based diterpenes within the same organism raises the possibility that elisapterosin F represents a further modification of an existing metabolite, thus suggesting the biogenetic pathway outlined in Scheme 1. Although still unproven, elisapterosin F (**1**) might be synthesized in vivo from elisapterosin D (**3**) through acid catalyzed hydration of the $\Delta^{11,12}$ double bond followed by cyclization through the tertiary hydroxyl at C-11 and the C-16 ketone carbonyl to yield a six-membered cyclic hemiketal functionality. A parallel cyclization involving the 11-OH and C-17 carbonyl would account for the five-membered cyclic hemiketal moiety of elisapterosin A (**2**). Interestingly, molecular modeling studies of isomers **1** and **2** revealed that **2** is thermodynamically more stable than **1** by almost 15 kcal/mol, suggesting the possibility of a facile **1**→**2** conversion under slightly acidic conditions. Unfortunately, most of the natural product was consumed during the biological assays leaving us with an inadequate supply of **1** to test this hypothesis.⁹ In point of fact, preliminary screenings revealed that **1** is ineffective against the TB bacillus (MIC > 128 μ g/mL) and only marginally active (IC₅₀ = 18 μ g/mL) against the malaria parasite *Plasmodium falciparum* W2 (chloroquine resistant strain).

The elisapterane class of diterpenes represents a fascinating group of natural products with diverse topologies and intriguing properties.^{1–4} Furthermore, they have only been isolated from a gorgonian coral species found in San Andrés island, Colombia, namely *Pseudopterogorgia elisabethae*. The molecular structure of elisapterosin F is exceptional in several respects. Its rigid polycyclic core is composed of four intimately interwoven carbocyclic ring systems with an additional six-membered cyclic hemiketal across rings C/D. All nine chiral centers in **1** are adjoined, and except for C-3 and C-7, they are located at angular positions. All told, perhaps the most salient feature of **1** is its facially amphiphilic structure.¹⁰ Due to its unique geometry, the hydrophilic and hydrophobic groups in elisapterosin F are

located in two opposite faces (Figure 2), thus representing an intriguing deviation from the more conventional head/tail amphiphiles.¹¹ This feature is fundamental because success in molecular recognition depends on the creation of molecular surfaces that can interact with one another selectively via noncovalent interactions. Therefore, future investigations into the biological properties of this facial amphiphile should take into account the unique geometry and distribution of its hydrophilic and hydrophobic groups, as they constitute key parameters influencing its biological properties.

Because only very small quantities of elisapterosin F were obtained from this coral, it was not possible to screen it further for exciting biological activities. In view of this, we sought to obtain additional quantities of **1** from the remaining gorgonian hexane extract, but no perceptible amounts could be isolated from this source. Thus, it seems likely that total synthesis will be required to access sufficient quantities of this intriguing metabolite for pharmaceutical evaluation.

Supplementary Material

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7. Elisapterosin F (**1**): transparent oil; $[\alpha]_D^{25} = +4.6^\circ$ (c 1.3, CHCl₃); $\nu_{\max}$ (thin film) 3421, 2960, 2930, 2861, 1770, 1717, 1457, 1261, 1099, 1034, 801 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz) (see Table 1); HRESI-MS *m/z* [M + H]⁺ calcd for C₂₀H₂₉O₅ 349. 2015, found 349. 2024.
8. Lowest energy conformers were searched using MMFF force field implemented in the McSpartan '04 program (Wavefunction, Inc.).
9. If indeed elisapterosin A (**2**) is about 15 kcal/mol more stable than elisapterosin F (**1**), and these compounds are formed through a non-enzymatic thermodynamic process, then the yield of **2** from this extract should be several orders of magnitude greater than that of **1**. Interestingly, this is not the case,

perhaps suggesting an enzymatic conversion of 3 to 1 and 2. Alternatively, compound 2 could originate from 1 during isolation upon contact with silica gel.

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Figure 1.
Observed NOEs and conformation for elisapterosin F (1).

Figure 2.
Space filling models of the two faces of elisapterosin F (**1**) depicting the distribution of the hydrophobic (left) and hydrophilic (right) groups. Oxygen atoms are indicated in red.

Scheme 1.

Proposed biogenetic interrelationships between elisapterosins F (1), A (2), and D (3).

Table 1

¹H NMR (500 MHz), ¹³C NMR (125 MHz), ¹H-¹H COSY, HMBC, and NOESY spectral data for elisapterosin F (1) in CDCl₃^a

Atom	δ _H , mult, integr (J in Hz)	δ _C , mult ^b	¹ H- ¹ H COSY	HMBC ^c	NOESY
1		67.1 (C)		H6, H8β, H9, H10, 2-OH	
2		76.0 (C)		H4β, H ₃ -18, 2-OH, 17-OH	
3	2.12, m, 1H	30.6 (CH)	H4αβ, H ₃ -18	H4αβ, H5αβ, H ₃ -18	H4β, H5β, H9, H ₃ -18
4α	1.58, m, 1H	27.9 (CH ₂)	H3, H4β, H5αβ	H3, H ₃ -18	H5α, H ₃ -18
4β	1.43, m, 1H		H3, H4α, H5αβ		H3
5α	1.21, m, 1H	27.3 (CH ₂)	H4αβ, H5α, H6		H4α, H6, H ₃ -19
5β	1.92, m, 1H		H4αβ, H5α, H6		H3, H7
6	2.41, m, 1H	43.2 (CH)	H5αβ, H7	H4αβ, H5αβ, H8αβ, H ₃ -19	H5α, H ₃ -19
7	1.74, m, 1H	39.3 (CH)	H6, H8αβ, H ₃ -19	H8α, H ₃ -19	H5β, H8β, H9, H ₃ -19
8	0.67, ddd, 1H (13.3, 11.1, 8.9)	45.4 (CH ₂)	H7, H8β, H9	H10, H ₃ -19	H10, H ₃ -19
8α	2.36, m, 1H		H7, H8α, H9		H7, H9
9β	3.29, ddd, 1H (8.9, 6.2, 1.9)	43.1 (CH)	H8αβ, H10	H6, H8αβ, H10	H3, H7, H8β, H ₃ -12
10	1.98, d, 1H (1.9)	60.9 (CH)	H9	H8α, H ₃ -12, H ₃ -13, H ₃ -20	H8α, H ₃ -20
11		87.5 (C)		H ₃ -12, H ₃ -13	
12	1.54, s, 3H	23.6 (CH ₃)		H ₃ -13	H9
13	1.18, s, 3H	25.9 (CH ₃)		H10, H ₃ -12	H ₃ -20
14		204.8 (C)		H6, H9, H ₃ -20	
15		67.2 (C)		H10, H ₃ -20	
16		195.5 (C)		H10, 17-OH, H ₃ -20	
17		106.4 (C)		2-OH, 17-OH	
18	1.06, d, 3H (7.0)	17.6 (CH ₃)	H3	H3	H3, H4α
19	0.99, d, 3H (7.0)	20.6 (CH ₃)	H7	H6	H5α, H6, H7, H8α
20	1.56, s, 3H	16.1 (CH ₃)		H10	H10, H ₃ -13
2-OH	2.20, s, 1H				
17-OH	5.55, s, 1H				

^aSpectra were recorded at 25 °C. Chemical shifts are in ppm relative to TMS.

¹³C NMR multiplicities were obtained from a DEPT-135 experiment.

^cProtons correlated to carbon resonances in the ¹³C column. Parameters were optimized for ^{2,3}J_{CH} = 6 and 8 Hz.