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Elisapterosins D and E: complex polycyclic diterpenes of the rare elisapterane class of natural products from the Caribbean sea whip *Pseudopterogorgia elisabethae* (Bayer)

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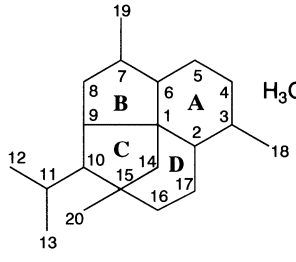
Abstract—From the hexane extracts of a Colombian specimen of *Pseudopterogorgia elisabethae* (Bayer) we have isolated elisapterosin D (**2**) and elisapterosin E (**3**), two structurally complex polycyclic diterpenes based on the rare elisapterane carbon skeleton. The structures of these scanty compounds were elucidated after interpretation of their combined spectroscopic data and NMR spectral comparisons with known elisapterane models.

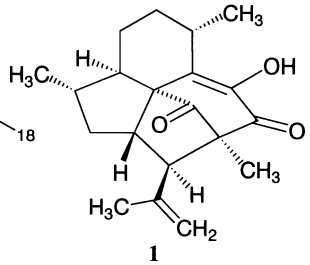
Since 1996 the Caribbean gorgonian octocoral (sea whip) *P. elisabethae* (Bayer) has been the subject of several chemical investigations in this laboratory. This effort has led to the discovery of many structurally novel and biologically active compounds.[1] The structural variety found among the many terpenoid natural products isolated from *P. elisabethae*, as well as the ample spectrum of biological activities exhibited by many of these compounds, is indeed quite remarkable.[1,2] Therefore, the natural products chemistry of this chemically prolific marine animal continues to capture our attention.

In 2000, we reported the discovery of elisapterosins A–C, an intriguing new class of natural products possessing the intricate elisapterane carbon skeleton. The structures of these metabolites, which until now have remained as the only known examples of this rare family of marine natural products, were established by spectroscopic and chemical methods.[3] Subsequently, the structure of elisapterosin B (**1**) was confirmed by a single-crystal X-ray diffraction experiment. From the same specimen of *P. elisabethae*, we have now isolated two novel elisapterane diterpenes, namely elisapterosin D (**2**) and elisapterosin E (**3**). In addition to their complex carbocyclic array, **2** and **3** possess several unusual structural features. For instance, in **2** there exists a fully substituted 1,2,4-cyclohexatrione ring moiety, whereas in **2** and **3**, the carbonyl functionality about the cyclopentanone unit is flanked by quaternary carbons. This paper describes the isolation and structural characterization of **2** and **3** which was based exclusively on the results of spectroscopic analyses and comparisons to the spectral properties of known elisapterane diterpenes **1** and **4**.

After extraction with MeOH–$CHCl_3$ (1:1) of the sun-dried *P. elisabethae* (1.0 kg) collected at depths of 80–100 ft off San Andrés Island, Colombia, the *n*-hexane extract (178 g) was fractionated as two separate portions by successive size exclusion chromatography (Bio-Beads SX-3 in toluene) and SiO_2 chromatography leading to the isolation of pure diterpenes **2** (2.1 mg, 0.001%) and **3** (1.1 mg, 0.0005%). Their structures were elucidated by interpretation of the data obtained from 1D and 2D NMR experiments, IR, UV, and HREI-MS spectral determinations.



elisapterane skeleton



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Elisapterosin D (**2**) was obtained as a yellowish oil; $[\alpha]_D^{25}$ +248 (*c* 0.5, $CHCl_3$). Eight degrees of unsaturation

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were deduced from its molecular formula $C_{20}H_{26}O_4$, established from HREI-MS m/z $[M]^{+\bullet}$ 330.1828 (calcd 330.1831) and ^{13}C NMR measurements. The UV spectrum of **2** (MeOH) displayed broad absorptions at $\lambda_{max}=204$ (ε 5300) and 252 (ε 2000) nm and the IR spectrum indicated the presence of hydroxyl (3492 cm^{-1}), carbonyl (1753, 1710, 1707 cm^{-1}) and olefin (3072, 1643 cm^{-1}) groups. One of the ketone functions (1753 cm^{-1}) was tentatively assigned to a cyclopentanone moiety. These observations were supported by ^{13}C and DEPT NMR experiments ($CDCl_3$; 75 MHz) which, in addition to a 1,1-disubstituted olefin [δ_C 114.5 (CH_2) and 141.7 (C)], exhibited three sp^2 quaternary carbon signals [δ_C 207.6, 204.5, and 195.5] ascribable to ketone carbonyl functionalities. These functional groups accounted for all the oxygen atoms present in the molecular formula of **2**. After subtraction of all the unsaturations due to carbon–carbon and carbon–oxygen double bonds, we concluded that elisapterosin D (**2**) must be tetracyclic.

As was the case with elisapterosin B (**1**), the ^1H NMR spectrum ($CDCl_3$, 300 MHz) of **2** showed four methyl signals, two of which were singlets displaced at δ_H 1.63 and 1.36, designated as H_3-13 and H_3-20, respectively (Table 1). Two secondary methyl groups observed at δ_H 1.01 (d, $J=6.4$ Hz) and 0.57 (d, $J=7.2$ Hz) were ascribed to H_3-19 and H_3-18, respectively. Two broad singlets at δ_H 4.87 and 4.66 (each 1H) were readily assigned to a terminal methylene and a sharp one-proton singlet at 3.46 ppm (D_2O exchangeable) confirmed the presence in **2** of a hydroxyl group. Other features of the ^1H NMR spectrum of **2** included two complex multiplets at δ 2.91 (ddd, 1H, $J=7.2$, 8.7, 10.7 Hz, H-9) and 0.87 (br dd, 1H, $J=10.7$, 11.7 Hz, H-8α), and a sharp doublet at 2.32 ppm (1H, $J=8.7$ Hz, H-10).

In the ^{13}C NMR spectrum, which contained signals for all 20 carbons, there was an oxygen-bearing tertiary carbon at δ_C 80.6 (C-2) and two conspicuous quaternary carbons at 63.9 (C-1) and 76.6 (C-15) ppm. The latter signals, as well as the overall similarity between the ^{13}C NMR spectra of **1** and **2**, strongly suggested that the structure of elisapterosin D was based on the elisapterane carbon skeleton.[3] After assignments between all the direct C–H bonds were made by HMQC, the main connectivities allowing the entire structure elucidation of the carbocyclic framework of **2** were established by ^1H–^1H COSY and HMBC NMR experiments (Table 1).

Connectivities from C-3 to C-10 were inferred from the ^1H–^1H COSY cross-peaks, including correlations from H-3 to H_3-18 and H-7 to H_3-19. Allylic couplings between both H_2-12 at δ_H 4.87 and 4.66 with H-10 (δ_H 2.32) and H_3-13 (δ_H 1.63) led connectivity from C-10 to C-12 and C-13, thus allowing placement of the isopropenyl side chain at C-10. 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). Thus, the structure elucidation of these spin systems (rings A–C) proceeded

Table 1. ^1H NMR (300 MHz), ^{13}C NMR (75 MHz), ^1H–^1H COSY, and HMBC spectral data for elisapterosin D (**2**)[a]

Position	δ_H, mult, intgrt (J in Hz)	δ_C (mult)[b]	^1H–^1H COSY	HMBC[c]
1		63.9 (C)		2-OH, H-6, H-8αβ
2		80.6 (C)		2-OH, H-9, H_3-18
3	1.96, m, 1H	40.8 (CH)	H-4α, H-4β, H_3-18	H_3-18
4α	1.28, m, 1H	29.1 (CH_2)	H-3, H-4β, H-5αβ	H_3-18
4β	1.93, m, 1H		H-3, H-4α, H-5αβ	
5α	1.53, m, 1H	23.3 (CH_2)	H-4αβ, H-5β, H-6	
5β	1.87, m, 1H		H-4αβ, H-5α, H-6	
6	2.10, m, 1H	44.3 (CH)	H-5αβ, H-7	H-8αβ, H_3-19
7	1.93, m, 1H	44.5 (CH)	H-6, H-8αβ, H_3-19	H-8αβ, H_3-19
8α	0.87, br dd, 1H (10.7, 11.7)	43.4 (CH_2)	H-7, H-8β, H-9	H-10, H_3-19
8β	2.16, m, 1H		H-7, H-8α, H-9	
9	2.91, ddd, 1H (7.2, 8.7, 10.7)	43.9 (CH)	H-8αβ, H-10	H-10
10	2.32, d, 1H (8.7)	59.6 (CH)	H-9, H-12αβ	H-8α, H-12αβ, H_3-13, H_3-20
11		141.7 (C)		H-10, H_3-13
12α	4.87, br s, 1H	114.5 (CH_2)	H-10, H-12β, H_3-13	H-10, H_3-13
12β	4.66, br s, 1H		H-10, H-12α, H_3-13	
13	1.63, br s, 3H	22.0 (CH_3)	H-12αβ	H-10, H-12αβ
14		207.6 (C)		H-6, H-9, H_3-20
15		76.6 (C)		H-10, H_3-20
16		195.5 (C)		H-10, H_3-20
17		204.5 (C)		2-OH
18	0.57, d, 3H (7.2)	18.2 (CH_3)	H-3	
19	1.01, d, 3H (6.4)	17.6 (CH_3)	H-7	
20	1.36, s, 3H	12.8 (CH_3)		
2-OH	3.46, s, 1H			

[a] NMR spectra were recorded in $CDCl_3$ at 25°C. ^1H and ^{13}C NMR chemical shift values are in ppm and are referenced to the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals.

[b] ^{13}C NMR multiplicities were obtained by a DEPT experiment.

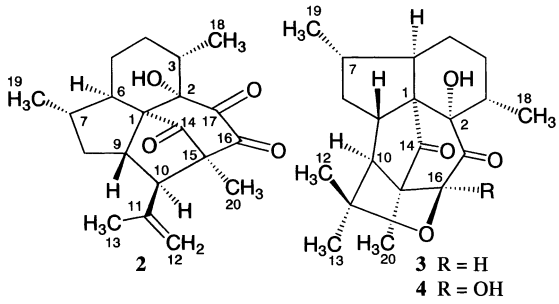
[c] Protons correlated to carbon resonances in ^{13}C column.

in a smooth fashion with none of the difficulties found for the remaining partial structure (ring D). This substructure, which was comprised by carbons C-1, C-2, C-14, C-15, C-16, C-17 and C-20, contained all the oxygen atoms present in **2** and thus, was subsequently formulated as a hydroxyl-bearing 1,2,4-cyclohexatrione moiety on the basis of HMBC correlations.

The HMBC experiment (HMBC experiments optimized for $^{2,3}J_{CH}=6$ and 8 Hz) showed connectivity between the C-1 carbon [δ_C 63.9 (C)] and the protons of 2-OH, C-6, and C-8. The hydroxyl-bearing tertiary carbon at position 2 (δ_C 80.6) showed strong HMBC correlations to 2-OH, H-9, and H_3-18. Thus, the pivotal C-1 quaternary carbon must be attached to C-2, C-6, and C-9 thereby establishing the substituted perhydroindan substructure within **2** (rings A and B). Furthermore, rings B and C were linked by a strong correlation between C-14 [δ_C 207.6 (C)] and protons H-6, H-9, and H_3-20. Since C-10 [δ_C 59.6 (CH)] correlated strongly with H-8α, H_2-12, H_3-13, and H_3-20, and C-15 [δ_C 76.6 (C)] was strongly coupled to H-10 and H_3-20, 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 H_3-20 and to ring A from the correlations of C-17 [δ_C 204.5 (C)] to the C-2 hydroxyl proton. This strongly implied that the carbonyl carbons C-16 and C-17 (the last connecting points remaining) must themselves be linked to one another. As the UV spectrum of **2** showed absorptions indicative of conjugation this connection allowed the complete planar structure for **2** to be assigned.

The relative stereochemistry of elisapterosin D (**2**) was established by a combination of NOESY data and coupling constant analysis. Comparisons of these data with those of elisapterosin B (**1**) suggested that these compounds possess identical relative stereochemistry. For instance, the 9*S**, 10*R** relative configuration in **2** was supported by the large 8.7 Hz coupling constant observed between H-9 and H-10, consistent with the *trans* configuration shown. Weak, but very diagnostic NOEs between H-10 with H_3-20 and H-8α indicated that these protons were on the same face of the molecule and were assigned as the α protons. Likewise, H_3-19 showed NOE responses with H-5α, H-6, and H-8α, but not with H-9, consistent with C-6 and C-7 having the *R** and *S** configurations, respectively. Similarly, weak NOESY correlations between tertiary protons H-9 and H-3 as well as those between H-9 and H-12β established the spatial proximities of these protons. Due to the inherent skeletal rigidity of the cage-like geometry within **2**, the aforementioned correlations were sufficient to establish the identity of the stereocenters at C-1 and C-2 as *S**, which allowed elimination of numerous inconsistent possibilities. Thus, the overall relative stereochemistry of **2** was assigned as 1*S**, 2*S**, 3*S**, 6*R**, 7*S**, 9*S**, 10*R** and 15*S**.

The structure elucidation of elisapterosin E (**3**), isolated as a colorless oil; $[\alpha]_D^{25}$ +51.5 (*c* 0.34, $CHCl_3$), commenced when its molecular formula of $C_{20}H_{28}O_4$ was established on the basis of the HREI-MS data *m*/*z* $[M]^{+\bullet}$ 332.1985 (calcd 332.1987) and overall NMR information.



In addition to a strong IR absorption at 3454 cm^{-1} indicative of a hydroxyl group, intense bands at 1748 and 1728 cm^{-1} indicated two ketone functions, one of which (1748 cm^{-1}) was tentatively assigned to a cyclopentanone moiety on the basis of the ^{13}C NMR signal at δ_C 212.1 (Table 2). As segments of the ^1H and ^{13}C NMR spectra of **3** were quite similar to those of known elisapterosin A (**4**), it appeared that **3** also contained an elisapterane skeleton. Thus, the ^1H NMR spectrum ($CDCl_3$, 500 MHz) showed five methyl groups, three of which were singlets at δ_H 1.47, 1.40, and 1.34, designated as H_3-20, H_3-13, and H_3-12, respectively. Two secondary methyl groups observed at δ_H 1.08 (d, $J=6.6$ Hz) and 0.82 (d, $J=7.3$ Hz) were ascribed to H_3-18 and H_3-19, respectively. Moreover, a sharp one-proton doublet at δ 4.19 (1H, $J=1.4$ Hz, H-16) and eleven complex proton resonances between δ 0.85 and 2.44 (H-3 through H-10) were suggestive of a polycyclic terpenoid structure.

Table 2. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectral data for elisapterosin E (**3**)[a]

Position	δ_H, mult, intgrt (*J* in Hz)	δ_C (mult)[b]
1		63.2 (C)
2		82.5 (C)
3	1.99, ddq, 1H (5.1, 6.6, 6.9)	31.6 (CH)
4α	1.23, m, 1H	27.5 (CH_2)
4β	1.54, m, 1H	
5α	1.13, m, 1H	26.8 (CH_2)
5β	1.78, m, 1H	
6	2.39, m, 1H	42.4 (CH)
7	1.88, m, 1H	40.7 (CH)
8α	0.96, dt, 1H (4.5, 13.3)	41.2 (CH_2)
8β	2.33, ddd, 1H (7.5, 10.0, 13.3)	
9	2.41, dt, 1H (4.4, 10.0)	40.2 (CH)
10	1.86, dd, 1H (1.4, 4.4)	62.7 (CH)
11		86.9 (C)
12	1.34, s, 3H	26.5 (CH_3)
13	1.40, s, 3H	31.4 (CH_3)
14		212.1 (C)
15		62.2 (C)
16	4.19, d, 1H (1.4)	89.8 (CH)
17		204.5 (C)
18	1.08, d, 3H (6.6)	16.7 (CH_3)
19	0.82, d, 3H (7.3)	21.2 (CH_3)
20	1.47, s, 3H	17.3 (CH_3)

[a] NMR spectra recorded in $CDCl_3$ at 25°C.
[b] ^{13}C NMR multiplicities were obtained by a DEPT experiment.

The ^{13}C NMR spectrum of **3** ($CDCl_3$, 125 MHz) contained signals for all 20 carbons, including the following: two ketone carbonyls (δ 212.1, 204.5); three oxygenated carbons: two tertiary (δ 86.9, 82.5) and one secondary (δ 89.8); three methylenes (δ 41.2, 27.5, 26.8); five methine carbons (δ 62.7, 42.4, 40.7, 40.2, 31.6); and five methyl groups (δ 31.4, 26.5, 21.2, 17.3, 16.7). The remaining signals were ascribable to two quaternary sp^3 carbons (δ 63.2, 62.2). The absence of UV absorption, when considered with the lack of olefinic carbons, indicated that the two carbonyls in compound **3** were present as two nonconjugated ketones. The remaining five degrees of unsaturation required that the molecule possess five rings.

The molecular structure of elisapterosin E (**3**) was defined on the basis of a standard series of one- and two-dimensional NMR experiments, which included ^1H–^1H COSY, TOCSY, NOESY, HMQC, and HMBC. Analysis of the ^1H–^1H COSY spectrum suggested the presence in **3** of many of the same partial structures present in **4**. These fragments could also be defined using data obtained from HMBC experiments, in particular, correlations from methyl protons which led to the confident assignment of all the carbon atoms of **3** (Table 2). Long-range homonuclear couplings, especially via four bonds, provided additional connectivities within rings A–D. A detailed side-by-side comparison of the NMR data of **3** and **4** suggested the presence in **3** of a five-membered cyclic ether substructure whereby an ether oxygen bridged carbons C-11 and C-16. This contention was evident from the strong downfield chemical shift experienced by C-11 [δ_C 86.9 (C)] and C-16 [δ_C 89.8 (CH)] and the HMBC experiment, which showed the following key correlations: H-16 to C-10, C-11, C-17, and C-20; H_3-12 and H_3-13 to C-10 and C-11; and H_3-20 to C-10, C-14, C-15, and C-16. Thus, in order to account for the few significant spectral differences between **3** and **4**, the C-16 hydroxyl in **4** needs to be replaced by a hydrogen atom in **3**. The planar structure of elisapterosin E, therefore, was determined as depicted in formula **3**.

The relative stereochemistry of **3** was shown to be the same as that of **4** using a combination of NMR methods (NOESY and ^1H–^1H coupling constants) and molecular modeling studies. The configurations at C-10, C-15, and C-16 were defined as follows: the C-10 proton showed a NOESY correlation with the C-13 methyl protons, which were themselves placed in the α face of the molecule by a NOESY interaction with H_3-20. Since H_3-20 in turn showed a strong NOESY correlation to H-16, consequently, we have assigned the configurations of C-10, C-15, and C-16 as *S**, *R**, and *S**, respectively. Most informative was a pronounced NOESY correlation between H-9 and H_3-12, consistent with their *cis* orientation on the opposite (top) face of the molecule. Both C-9 and C-10 having the *S** configuration was supported by the small 4.4 Hz coupling constant observed between H-9 and H-10, consistent with the *trans* orientation shown.[4] NOESY correlations between H-9 and tertiary protons H-3 and H-7 were also observed; thus the stereocenters at C-3 and C-7 were defined as *S**. On the other hand, NOESY correlations between H_3-19 and H-6 established the spatial proximities of these protons on the bottom face of the molecule. Thus, the overall relative stereochemistry for elisapterosin E (**3**) was assigned as 1*S**, 2*S**, 3*S**, 6*R**, 7*S**, 9*S**, 10*S**, 15*R**, and 16*S**.

Although the biological properties of compounds **2** and **3** could not be ascertained due to the scarcity of material, elisapterosin B (**1**),[3] a plausible precursor to **2** and **3**, exhibited strong antiplasmodial activity (IC_{50} 10 μg/mL) against *Plasmodium falciparum*, the parasite responsible for the most severe forms of malaria.[5] This result suggests that elisapterane diterpenes could represent a novel family of antimalarial leads. Biological screening of elisapterosin A (**4**) in the NCI's 60 cell-line tumor panel indicated moderate (non-selective) in vitro cancer cell cytotoxicity.

Acknowledgements

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