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Elisabethamine: a new diterpene alkaloid from *Pseudopterogorgia elisabethae*

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Abstract

Detailed chemical studies of the methanolic extract of *Pseudopterogorgia elisabethae*, collected from the Florida Keys, have resulted in the isolation of elisabethamine (**1**), a new diterpene alkaloid. Its structure was established with the aid of extensive spectroscopic studies. Compound **1** exhibited cytotoxicity against lung and prostate cancer cell lines as determined by an MTT assay.

Keywords: alkaloids; anti-tumor compounds; anti-inflammatory compounds; NMR; mass spectrometry.

The soft coral, *Pseudopterogorgia elisabethae*, is found in the Caribbean region and is a rich source of biomedically useful and structurally novel natural products.[1,2] For instance, pseudopterosins isolated from *P. elisabethae* collected from The Bahamas and *seco*-pseudopterosins purified from *Pseudopterogorgia* sp. exhibit potent anti-inflammatory activity.[3–5] *Pseudopterogorgia elisabethae* of Colombian origin has yielded diterpenes and diterpene alkaloids exhibiting anti-cancer and anti-tuberculosis activities, respectively.[6–8] We have previously reported a new pseudopterane type diterpene from this species collected from the Florida Keys.[9] Our recent detailed chemical studies of the methanolic extract of this species have yielded a minor metabolite, elisabethamine (**1**). Extensive spectroscopic data were used to establish the structure of **1**. Significant anti-cancer activity of **1** against prostate and lung cancer cell lines was observed. To our knowledge, this is the first example of a diterpene alkaloid possessing the serrulatane type skeleton with a methyl amino functionality. The occurrence of nitrogenous diterpenes in soft corals is very rare; only four examples have been reported to date.[1,8,10]

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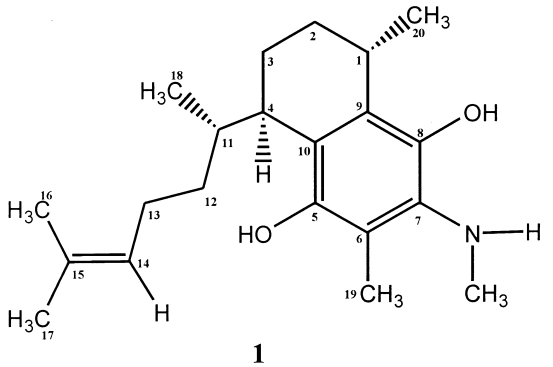

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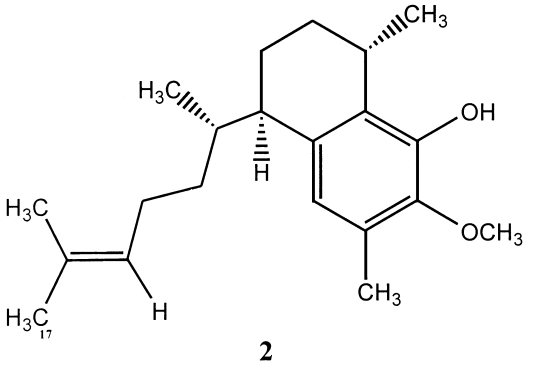

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The methanolic extract of *P. elisabethae*, collected from the Florida Keys, was subjected to column chromatography using silica gel as adsorbent and hexane–ethyl acetate (0–100%) and ethyl acetate–methanol (0–100%) as eluents. The fraction obtained on elution with 80% ethyl acetate/20% hexane was subjected to reverse phase HPLC using a gradient elution of acetonitrile:water (60:40) to 100% acetonitrile, and monitoring at 260 nm yielded elisabethamine (**1**) as a yellow colored gum. The presence of nitrogen in **1** was evident due to a positive test with Dragendroff's reagent. Its UV spectrum showed an absorption maximum at 292 nm, and the IR spectrum showed intense absorption bands at 3543 (OH), 3325 (NH) and 1695 (C=C) cm^{-1}. High-resolution FABMS of **1** showed a molecular ion peak at *m/z* 331.2515, which was consistent with the molecular formula $C_{21}H_{33}NO_2$ (calcd 331.2511) and indicated the presence of six degrees of unsaturation in the molecule. The mass spectrum of **1** showed a base peak at *m/z* 220 (C_8H_{15}), which could arise by the cleavage of the C-4/C-11 bond, representing the loss of the side chain. An ion at *m/z* 316 (M^+–CH_3) was also observed.

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **1** showed two three-proton doublets at δ 0.99 ($J = 6.5$ Hz) and 1.26 ($J = 7.0$ Hz), which have been assigned to the protons of the C-18 and C-20 secondary methyl groups, respectively. Two three-proton singlets at δ 1.66 and 1.80 were assigned to the allylic C-16 and C-17 methyl protons, while another three-proton singlet at δ 2.12 was due to the C-19 methyl protons. Another singlet at δ 2.70 was ascribed to the *N*-methyl protons. The olefinic C-14 methine proton resonated at δ 5.06. Three exchangeable broad signals at δ 4.94, 4.80 and 4.72 for OH and NH were also observed in the ^1H NMR spectrum, which disappeared when the ^1H NMR spectrum was recorded in MeOH-d_4.

The COSY-45° spectrum was used to complete the ^{1}H NMR chemical shift assignments of **1**. The allylic C-13 protons (δ 2.15 and 1.72) showed COSY-45° interactions with the C-12 methylene (δ 1.83 and 1.21) and the olefinic C-14 methine (δ 5.06) protons. The C-12 methylene protons exhibited vicinal couplings with the C-11 methine proton (δ 2.99), which in turn showed a ^{1}H–^{1}H spin correlation with the C-18 methyl (δ 0.99) and C-4 methine (δ 3.15) protons. The latter exhibited cross-peaks with the C-3 methylene protons (δ 1.94 and 1.66), which further exhibited vicinal couplings with the C-2 methylene protons (δ 1.88 and 1.52). Cross-peaks of the C-1 methine proton (δ 3.45) with the C-20 methyl protons (δ 1.26) and C-2 methylene protons were also observed in the COSY-45° spectrum.

The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) showed distinct resonances for all 21 carbon atoms. Intrepretation of ^{1}H, ^{13}C NMR, COSY-45° and HMQC spectral data of **1** revealed that this compound is a serrulatane type diterpene, as the majority of the signals have similar chemical shift values to those of *seco*-pseudopterosin aglycones such as **2**.[5] The DEPT spectrum was also recorded to establish the multiplicity of each carbon signal in the ^{13}C NMR spectrum, which revealed the presence of four CH, four CH_2 and six CH_3 in compound **1**. The subtraction of the DEPT spectrum from the broad band spectrum indicated the presence of seven quaternary carbon atoms in the molecule. Complete ^{13}C NMR chemical shift assignments of **1** are shown in Table 1. The HMQC spectrum was also recorded to determine the ^{1}H and ^{13}C one-bond shift correlations in **1** and is presented in Table 1.

The HMBC spectrum of **1** showed cross-peaks of the C-14 olefinic methine proton (δ 5.06) with C-13 (δ 29.0), C-15 (δ 133.1), C-16 (δ 25.3) and C-17 (δ 18.9). Allylic C-16 (δ 1.66) and C-17 (δ 1.80) methyl protons showed HMBC interactions with C-15 (δ 133.1) and C-14 (δ 124.0). HMBC interactions of C-18 methyl protons (δ 0.99) with C-4 (δ 40.2) and C-11 (δ 39.5) were also observed in the spectrum. These HMBC correlations support the assigned connectivities based on the COSY data. The C-4 methine proton (δ 3.15) showed cross-peaks with C-3 (δ 19.4), C-5 (δ 148.9), C-10 (δ 139.1) and C-11 (δ 39.5), while the C-20 methyl protons (δ 1.26) showed cross-peaks with C-1 (δ 26.9), C-2 (δ 27.7) and C-9 (δ 132.1), locating the aromatic ring. The *N*-methyl protons (δ 2.70) exhibited long-range couplings with C-7 (δ 141.6). Cross-peaks of the C-19 methyl protons (δ 2.12) with C-5 (δ 148.9), C-6 (δ 128.0) and C-7 (δ 141.6) were also observed in the HMBC spectrum. These HMBC observations strongly favor the presence of an *N*-methyl amino moiety at C-7. The NOESY showed NOE cross-peaks of the *N*-methyl protons (δ 2.70) with the C-19 methyl protons (δ 2.12), which further supports the location of the *N*-methyl group at C-7. Significant HMBC interactions are presented in Table 1.

The relative stereochemistry at all chiral centers was established with the aid of optical rotation and NOESY. The $[\alpha]_D^{20}$ of compound **1** was found to be 89.0, nearly identical to that for the *seco*-pseudopterosin A derivative **2** ($[\alpha]_D^{20} = 87.0$), which was prepared by the method established by Roussis et al.[4] This suggested that compound **1** would have the same stereochemistry as **2** at all chiral centers. A *cis* relationship of the C-18 methyl, C-20 methyl and C-4 methine was confirmed by examination of the NOESY spectrum. The C-18 methyl protons (δ 0.99) showed cross-peaks with the C-4 methine proton (δ 3.15), which also showed cross-peaks with the C-20 methyl protons (δ 1.26). Based on these spectral studies, structure **1** was established for this new compound.

The occurrence of a nitrogenous diterpene in soft corals is rare and, in all cases, the biosynthetic origin of the nitrogenous moiety is unknown. It is conceivable that the *N*-methyl amino functionality in **1** is derived from a glycine residue, or from the reduction of the corresponding isonitrile. The latter scenario is particularly intriguing as isonitriles are unknown in soft corals.[11]

Table 1
^{1}H and ^{13}C NMR spectral data of elisabethamine (**1**)

Carbon No	^{1}H-NMR† (δ)	Significant HMBC Interactions	^{13}C-NMR† (δ)
1	3.45 (m)	C-2, C-9 and C-20	26.9 (d)
2	1.88 (m) and 1.52 (m)	C-1, and C-3	27.7 (t)
3	1.94 (m) and 1.66 (m)	C-2 and C-4	19.4 (t)
4	3.15 (m)	C-3, C-5, C-10 and C-11	40.2 (d)
5	---	---	148.9 (s)
6	---	---	128.0 (s)
7	---	---	141.6 (s)
8	---	---	154.9 (s)
9	---	---	132.1 (s)
10	---	---	139.1 (s)
11	2.99 (m)	C-4 and C-12	39.5 (d)
12	1.83 (m) and 1.21 (m)	C-11 and C-13	36.7 ((t)
13	2.15 (m) and 1.72 (m)	C-12, C-13 and C-14	29.0 (t)
14	5.06 (br t)	C-13, C-15, C-16 and C-17	124.0 (d)
15	---	---	133.1 (s)
16	1.66 (s)	C-14, C-15 and C-17	25.3 (q)
17	1.80 (s)	C-14, C-15 and C-16	18.9 (q)
18	0.99 (d, $J = 6.5$ Hz)	C-4, C-11 and C-12	16.2 (q)
19	2.12 (s)	C-5, C-6 and C-7	20.9 (s)
20	1.26 (d, $J = 7.0$ Hz)	C-1 and C-9	16.9 (q)
N-CH_3	2.70 (br s)	C-7	40.6 (q)
OH	4.94 (br s) ‡	---	---
	4.80 (br s) ‡	---	---
NH	4.72 (br s) ‡	---	---

†^{1}H-NMR spectrum was recorded in $CDCl_3$. Multiplicity was determined by DEPT, and C-H one-bond shift correlation was determined by HMQC. ‡Signals disappeared in the ^{1}H-NMR spectrum recorded in CD_3OD.

Compound **1** exhibited activity against lung cancer (LNCap) and prostate cancer (Calu) cell lines, with observed IC_{50} values of 10.35 and 20 μg/ml as determined by using an MTT assay.

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