Notes

Homopseudopteroxazole, a New Antimycobacterial Diterpene Alkaloid from *Pseudopterogorgia elisabethae*

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In the course of our study to find novel antimycobacterial secondary metabolites from Caribbean gorgonian octocorals, we have isolated a new diterpene alkaloid, namely, homopseudopteroxazole (**1**), as a minor constituent of the hexane extract from the sea plume *Pseudopterogorgia elisabethae*. Its structure was deduced by interpretation of combined spectroscopic data, including extensive 1D and 2D NMR measurements, and NMR spectral comparisons with known amphilectane models. Biological screening studies indicate that homopseudopteroxazole (**1**) is a strong growth inhibitor of *Mycobacterium tuberculosis* $H_{37}Rv$.

The discovery of new anti-infective drug molecules continues to be the subject of intense research activity among chemists and biochemists alike.[1] Among infectious diseases, tuberculosis still constitutes the leading killer disease.[2] It is estimated that seven million new cases and three million deaths occur every year due to tuberculosis.[3] To make matters worse, this rate of mortality is likely to increase dramatically as AIDS reaches epidemic proportions in many developing countries. As part of our ongoing screening program to identify secondary metabolites with antimycobacterial activity from Caribbean gorgonian octocorals, we have continued our chemical investigation of the sea plume *Pseudopterogorgia elisabethae* (Bayer). Herein, we report the isolation, structure elucidation, and antitubercular properties of a new diterpene alkaloid from this sea plume, namely, homopseudopteroxazole (**1**). While the occurrence of diterpene alkaloids in soft corals is typically very rare, several examples (**2**−**4**) of such metabolites have been previously described from this animal.[4,5]

Several large colonies of *P. elisabethae* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Gorgonacea, family Gorgoniidae) were collected by scuba during an expedition to San Andrés Island, Colombia, in May 1996. After homogenization, the dry organism (1.0 kg) was exhaustively extracted with MeOH−$CHCl_3$, 1:1. The crude extract was partitioned between hexane and water, and then a large portion of the organic extract was subjected to chromatography over a column packed with Si gel and eluted with a system of solvents of increasing polarity from hexane to acetone. Repeated chromatography of the more polar fractions eluted with hexane−$CHCl_3$ mixtures led to the isolation of a new diterpene alkaloid, homopseudopteroxazole (**1**).

Compound **1** was isolated as a yellowish oil, $[\alpha]_D^{25}$ +103.2° (*c* 0.9, $CHCl_3$), of composition $C_{26}H_{37}NO$ on the basis of the HREI-MS ($[M]^+$ *m/z* 379.2884, calcd for $C_{26}H_{37}NO$, 379.2875) and the LRFAB-MS data of the monoprotonated species ($[M + 1]^+$, *m/z* 380). The lack of IR absorption bands for a hydroxyl or carbonyl group suggested that the only oxygen atom in the molecular formula of **1** was ethereal in nature. The UV (MeOH) spectrum with maxima at λ_{max} 204 (ϵ 25 500), 244 (ϵ 9000), and 279 (ϵ 3500) nm was reminiscent of that of known pseudopteroxazole (**2**), suggesting a five-membered heteroaromatic functionality. The ^1H NMR spectrum of **1** (Table 1) showed a doublet of quartets at δ 4.97 (J = 9.3 and 1.1 Hz) and two vinyl methyl doublets (each J = 1.1 Hz) at δ 1.66 and 1.77 indicative of an isobutenyl group, two three-proton doublets at δ 1.04 (J = 5.9 Hz) and 1.44 (J = 6.9 Hz), and one three-proton triplet at δ 0.90 (J = 7.0 Hz), indicating a pair of secondary methyls and a terminal methyl group, respectively, a three-protons singlet at δ 2.41 suggesting an aromatic methyl, a one-proton doublet of triplets at δ 3.93 (J = 9.3, 8.8 Hz), ascribable to a bis-allyl hydrogen atom, and two one-proton multiplets at δ 3.22 (qdd, J = 6.8, 6.4, 4.9 Hz) and 2.23 assigned to benzylic hydrogens. For the most part, the ^1H NMR spectra of **1** and pseudopteroxazole (**2**) were quite similar, but the former compound did not display the telltale benzoxazole methine singlet at δ 7.98 present in **2**. The ^{13}C NMR spectrum exhibited 26 signals (6 CH_3, 7 CH_2, 5 CH, and 8 C) whose

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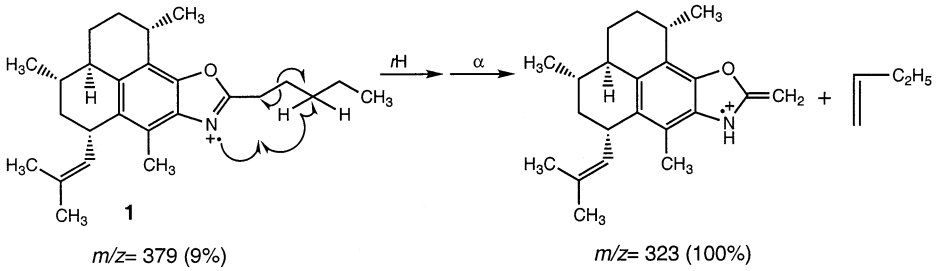
Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of **1** in $CDCl_3$[a]

position	δ_H, mult, intgr (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}HCOSY	NOESY	HMBC[c]
1	3.93, td, 1H (9.3, 8.8)	36.4 (d)	H2α, H2*β*, H14	H2*β*, H3, H_3-17, H_3-20	H2α*β*, H3
2α	1.27, m, 1H	40.1 (t)	H1, H2*β*, H3	H4, H14	H1, H4, H_3-18
2*β*	2.13, m, 1H		H1, H2α, H3	H1	
3	1.25, m, 1H	34.6 (d)	H2α, H2*β*, H4, H_3-18	H1, H5*β*, H_3-18	H2α*β*, H_3-18
4	2.23, br t, 1H (9.9)	44.7 (d)	H3, H5α, H5*β*	H2α, H_3-18, H_3-19	H2*β*, H5*β*, H6*β*, H_3-18
5α	1.08, m, 1H	28.1 (t)	H4, H5*β*, H6α*β*	H5*β*	
5*β*	2.15, m, 1H		H4, H5α, H6α*β*	H3, H5α	
6α	1.30, m, 1H	32.3 (t)	H5α*β*, H6*β*, H7	H6*β*, H_3-19	H_3-19
6*β*	2.17, m, 1H		H5α*β*, H6α, H7	H6α, H7	
7	3.22, qdd, 1H (6.8, 6.4, 4.9)	30.3 (d)	H6α*β*, H_3-19	H6*β*, H_3-19	H6α*β*, H_3-19
8		121.7 (s)			H6*β*, H_3-19
9		147.8 (s)			H7
10		139.3 (s)			H_3-20
11		125.6 (s)			H_3-20
12		134.0 (s)			H1, H2*β*, H_3-20
13		135.3 (s)			H1
14	4.97, dq, 1H (9.3, 1.1)	131.0 (d)	H1, H_3-16, H_3-17	H2α, H_3-16, H_3-20	H1, H_3-16, H_3-17
15		128.6 (s)			H1, H_3-16, H_3-17
16	1.66, d, 3H (1.1)	25.4 (q)	H14	H14, H_3-17	H14, H_3-17
17	1.77, d, 3H (1.1)	17.6 (q)	H14	H1, H_3-16	H14, H_3-16
18	1.04, d, 3H (5.9)	19.8 (q)	H3	H3, H4	
19	1.44, d, 3H (6.9)	22.2 (q)	H7	H4, H6α, H7	
20	2.41, s, 3H	13.5 (q)		H1, H14	
21		165.7 (s)			H22
22	2.89, br t, 2H (7.7)	28.8 (t)	H23		H23
23	1.85, m, 2H	27.0 (t)	H22, H24		H22
24	1.38, m, 2H	31.4 (t)	H23, H25		H22, H23, H25, H26
25	1.41, m, 2H	22.3 (t)	H24, H_3-26		H23, H24, H_3-26
26	0.90, t, 3H (7.0)	13.9 (q)	H25		

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from a DEPT-135 experiment. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz.

Scheme 1



chemical shift values and multiplicity confirmed the presence of a fully substituted benzoxazole moiety [δ 165.7 (s), 147.8 (s), 139.3 (s), 135.3 (s), 134.0 (s), 125.6 (s), 121.7 (s)] and a trisubstituted olefin [δ 131.0 (d), 128.6 (s)]. Spectral evidence thus demanded that compound **1** was tetracyclic with one C=N and four C=C double bonds. 2D NMR studies (COSY, HMQC, HMBC) revealed the connectivity and thus the gross structure of **1**. Thus, in common with pseudopteroxazole (**2**), it appeared that compound **1** contained the same amphilectane skeleton found in the aglycon portion of the pseudopterosins.[6] Assignments of the NMR signals are given in Table 1. Comparison of the molecular formulas of **1** and **2** showed that homopseudopteroxazole possessed the additional element of C_5H_{10} ascribable to a *n*-pentyl alkyl chain connected to the benzoxazole moiety through the C-21 position. The benzoxazole ring and the *n*-pentyl moiety were connected as depicted based on the following evidence. The quaternary aromatic carbon at δ 165.7 assigned to C21 was connected to a two-proton triplet at δ 2.89 (J = 7.7 Hz) assigned to H_2-22. In addition, the mass spectrum of homopseudopteroxazole displayed the base peak at *m/z* 323 through loss of C_4H_8 from the molecular ion via a McLafferty rearrangement. Scheme 1 shows a possible rationalization of the electron shifts involved. The oxygen and nitrogen atoms of the oxazole ring are attached at C9 (δ_C 147.8) and C10 (δ_C 139.3), respectively, by comparison of the carbon chemical shifts with those of known benzoxazoles, including **2**.[7] Since HMBC correlations connected C10 to the aromatic methyl (δ_H 2.41) and C9 to the methine proton H7 (δ_H 3.22), the locus of each heteroatom about the oxazole ring was established unambiguously.

The relative stereochemistry of **1** was elucidated from analysis of the NOESY spectrum, coupling constant analysis, and comparisons of the NMR chemical shifts with those of **2**. A half-chair conformation of ring A[4b] was deduced from NOESY correlations of H1/H2*β*, H1/H3, H1/H_3-17, H1/H_3-20, and H4/H_3-18, suggesting that H_3-18 and the isobutenyl side chain are both pseudoequatorial. Thus, H3 and H1 must be pseudoaxial. A relatively large coupling constant (9.3 Hz) between the protons at C1 and C14 of **1** indicates that the two protons are nearly *trans* in a preferred conformation. A broad triplet (δ 2.23) with a large coupling constant (J = 9.9 Hz) ascribable to H4 suggests that the latter methine is *trans* diaxial to H3 and H5*β*. Additional NOESY correlations of H2α/H4, H6*β*/H7, and H4/H_3-19 revealed that ring B adopts a skew boat conformation with H4 and the methyl group at C7 in a *cis* 1,4-pseudodiaxial conformation.[4b] Therefore, the isobutenyl group at C1, the methyl groups at C3 and C7, and H-4 are all α-oriented. Except for the C21−C26 substructure, the ^{13}C NMR resonances of **1** and **2** were highly comparable. We,

therefore, conclude that homopseudopteroxazole (**1**) and pseudopteroxazole (**2**) must possess the same relative stereochemistry.

Compounds **1**−**3** inhibited 80, 97, and 66% of the growth of *Mycobacterium tuberculosis* H_{37}Rv with a MIC of 12.5 μg/mL, respectively, thus suggesting that gorgonian-derived secondary metabolites indeed represent a plausible unexplored resource of novel antimycobacterial leads.

Experimental Section

The optical rotation was measured with a Perkin-Elmer 241 polarimeter. The UV spectrum was recorded with a Hewlett-Packard 8453 spectrophotometer and the infrared spectrum with a Nicolet Magna 750 FT-IR spectrophotometer. ^{1}H NMR spectral data were generated with a 500 MHz Bruker DPX-500 FT-NMR spectrometer, and the ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, NOESY, DEPT-135, HMQC, and HMBC experiments were measured with a 300 MHz Bruker DPX-300 FT-NMR spectrometer. FABMS were carried out in a VG AutoSpec (Fisons). Lowest energy conformers were searched using MMFF force field implemented in the McSpartan *Pro* program (Wavefunction, Inc.). Column chromatography was performed on Si gel (35−75 mesh). TLC analyses were carried out using glass Si gel plates, and spots were visualized by exposure to I_2 vapors or heating Si gel plates sprayed with 5% H_2SO_4 in EtOH. All solvents used were spectral grade or were distilled from glass prior to use. The percentage yield of **1** is based on the weight of the dry gorgonian MeOH−$CHCl_3$ extract (284 g).

Collection and Extraction of *P. elisabethae*. Healthy colonies of the sea plume were removed by scuba from their natural habitat at depths of 24−28 m near the Island of San Andrés, Colombia. A taxonomic reference specimen is deposited at the Chemistry Department of the University of Puerto Rico, sample number SAI-I-PE-1996. Extraction procedures have been described elsewhere.[8] A large portion of the hexane extract (128 g) was loaded onto a Si gel flash chromatography column and eluted with mixtures of hexane−acetone of increasing polarity (100%−0%) to afford eight fractions (I−VIII). Fraction III (4.6 g) was dissolved in toluene and purified by size exclusion chromatography (Bio-Beads SX-3) to yield nine subfractions (A−I). Fraction F (140 mg) was chromatographed over Si gel (7.8 g) using mixtures of hexane−$CHCl_3$ of increasing polarity (95%−65%) to yield pure homopseudopteroxazole (**1**) (4.0 mg, 0.002%).

Homopseudopteroxazole (1): yellowish oil; $[\alpha]_D^{25}$ +103.2° (*c* 0.9, $CHCl_3$); IR (film) 2953, 2925, 2853, 1604, 1575, 1454, 1373, 1117, 1061 cm^{-1}; UV (MeOH) λ_{max} 204 (ϵ 25 500), 244 (ϵ 9000), 279 (ϵ 3500) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 1); LRFAB-MS (glycerol) *m*/*z* $[M + H]^+$ 380 (calcd for $C_{26}H_{38}NO$, 380); HREI-MS *m*/*z* $[M]^+$ calcd for $C_{26}H_{37}NO$ 379.2875, found 379.2884 (9), 323.2234 (100, $C_{22}H_{29}NO$), 309.2078 (29, $C_{21}H_{27}NO$).

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