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Novel Antimycobacterial Benzoxazole Alkaloids, from the West Indian Sea Whip *Pseudopterogorgia elisabethae*

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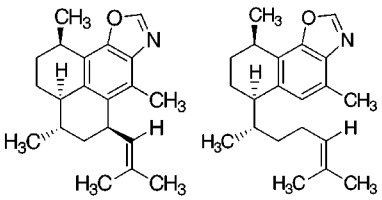
ABSTRACT



Our screening for marine natural products with anti-tuberculosis activity from the West Indian gorgonian coral *Pseudopterogorgia elisabethae* resulted in the isolation of two active diterpenoid alkaloids, pseudopteroxazole (1) and *seco*-pseudopteroxazole (2). Their structures were elucidated by NMR spectral analysis, including a variety of two-dimensional techniques. Compounds 1 and 2 are previously undescribed diterpenoids containing the uncommon benzoxazole moiety. Biological screening studies indicated that pseudopteroxazole (1) is a potent growth inhibitor of *Mycobacterium tuberculosis* H37Rv, while *seco*-pseudopteroxazole (2) shows moderate to strong inhibitorial activity.

Worldwide, tuberculosis causes more deaths than any other infectious disease. An estimated 2 billion people (including at least 15 million Americans) carry the tuberculosis bacterium.[1] Over the past decade, significant advances have been made in the discovery and development of antimicrobial agents in general. However, the need for safe, microbially selective, and effective drugs is apparent, and certainly no ideal agents have yet been developed. The recent emergence of drug resistant strains of tuberculosis is also of special concern.[2] In connection with our continuing interest in the development of new agents for the treatment of TB, our screening for such antitubercular substances resulted in the isolation of two active metabolites, pseudopteroxazole (**1**) and *seco*-pseudopteroxazole (**2**), from the hexane extracts of the West Indian gorgonian coral *Pseudopterogorgia elisabethae* (Bayer) collected near San Andrés Island, Colombia. *seco*-Pseudopteroxazole (**2**), which was isolated as a minor constituent, is assumed to be a logical biogenetic precursor to **1** (or vice versa) upon undergoing further dehydrogen-coupling at the C5−C13 positions. We report herein the isolation and structure elucidation of compounds **1** and **2** as well as their biological activities.[3] From a chemotaxonomic viewpoint, these metabolites are so unusual that we discuss their possible origin.

Bioassay-guided fractionation of a portion of the crude hexane extracts of *P. elisabethae* (ca. 1 kg of dry wt) involving Bio-Beads SX-3 chromatography and successive silica gel and reversed-phase C-18 flash column chromatography afforded pseudopteroxazole (**1**, 15.0 mg) and *seco*-pseudopteroxazole (**2**, 2.0 mg). The structures of benzox-

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(2) (a) DiGiacomo, M. A Deadly Return. *Newsweek* **1992**, *March 16*, 53−57. (b) Lemonick, M. D. The Killer All Around. *Time Magazine* **1994**, *Sept 12*, 62−68.

(3) Taken in part from the M.S. Dissertation of E. González, University of Puerto Rico, 1997 and the Ph.D. Dissertation of C. Ramírez, University of Puerto Rico, in preparation.

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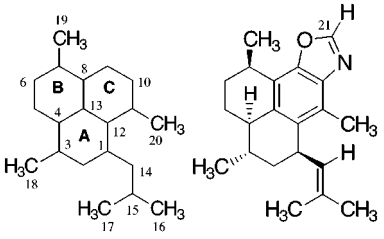
Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), and HMBC Spectral Data of Pseudopteroxazole (**1**) and *seco*-Pseudopteroxazole (**2**) in $CDCl_3$[a]

	pseudopteroxazole (**1**)			*seco*-pseudopteroxazole (**2**)		
atom	δ_H, mult, intgrt (*J* in Hz)	δ_C (mult)[b]	HMBC[c]	δ_H, mult, intgrt (*J* in Hz)	δ_C (mult)[b]	HMBC[c]
1	3.90, br dd, 1H (8.9, 9.1)	36.4 (d)	H2α	3.22, m, 1H	28.8 (d)	H20
2α	2.22, m, 1H	40.0 (t)	H1, H18	2.05, m, 1H	29.5 (t)	H20
2*β*	1.27, m, 1H			1.47, m, 1H		
3α				1.64, m, 1H	20.2 (t)	
3*β*	1.22, m, 1H	34.5 (d)	H1, H2α, H18	1.88, m, 1H		
4	2.25, br t, 1H (9.5)	44.7 (d)	H2α, H18	2.87, m, 1H	40.7 (d)	H5, H18
5α	1.10, m, 1H	28.0 (t)	H4	7.03, br s, 1H	125.1 (d)	H19
5*β*	2.15, m, 1H					
6α	2.17, m, 1H	32.2 (t)	H7, H19		126.9 (s)	H19
6*β*	1.29, m, 1H					
7	3.20, m, 1H	30.2 (d)	H19		136.8 (s)	H5, H19, H21
8		122.4 (s)	H7, H6α, H19		148.6 (s)	H21
9		147.1 (s)	H7, H21		124.4 (s)	H1, H5, H20
10		137.9 (s)	H20, H21		137.5 (s)	H1, H4, H5
11		126.6 (s)	H1, H20	2.10, m, 1H	37.5 (d)	H18
12		134.7 (s)	H1, H14, H20	1.46, m, 2H	35.4 (t)	H18
13		136.7 (s)	H1, H7	2.08, m, 2H	26.2 (t)	H14
14	5.02, d, 1H (9.1)	130.7 (d)	H1, H16, H17	5.16, br t, 1H (8.1)	124.6 (d)	H16, H17
15		128.8 (s)	H1, H16, H17		131.3 (s)	H16, H17
16	1.68, s, 3H	25.4 (q)	H14, H17	1.72, s, 3H	25.6 (q)	H14, H17
17	1.78, s, 3H	17.6 (q)	H14, H16	1.64, s, 3H	17.6 (q)	H14, H16
18	1.05, d, 3H (5.4 Hz)	19.8 (q)	H2α	0.68, d, 3H (6.9)	15.4 (q)	H4
19	1.45, d, 3H (6.7 Hz)	22.2 (q)	H7	2.57, s, 3H	16.2 (q)	H5
20	2.44, s, 3H	13.5 (q)		1.38, d, 3H (6.9)	21.3 (q)	H1
21	7.98, s, 1H	151.0 (d)		8.02, s, 1H	151.0 (d)	

[a] Spectra were recorded at room temperature. Chemical shift values are in ppm relative to TMS. [b] ^{13}C NMR multiplicities were obtained by an Attached Proton Test (APT) experiment. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 and 8 Hz.

azoles **1** and **2** were proposed on the basis of comprehensive spectral analyses which included 1D and 2D NMR data (Table 1), IR, UV, and MS analyses.



amphilectane skeleton **1**

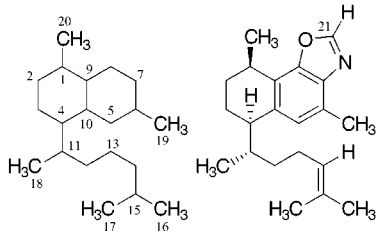
Pseudopteroxazole (**1**) was isolated as a yellowish oil, $[\alpha]^{25}_D$ +101° (*c* 1.0, $CHCl_3$), of composition $C_{21}H_{27}NO$ based on the HRFABMS of the monoprotonated species [(M + 1)$^+$, *m*/*z* 310.2168, calcd for $C_{21}H_{28}NO$, 310.2171]. The lack of IR absorption bands for a hydroxyl or carbonyl group suggested that the only oxygen atom in the molecular formula of **1** was ethereal in nature. Infrared bands at 3125 cm^{-1} (w, br), 2000−1700 cm^{-1} (overtones and combinations), and 1665−1430 cm^{-1} (m−s), pointed to oxazole, benzene, and C═N and C═C functions.[4] The UV (MeOH) spectrum, with maxima at λ_{max} 220 (ϵ 15400), 250 (ϵ 5000), and 284 (ϵ 2000) nm, was particularly informative, as it was reminiscent of a five-membered heteroaromatic functionality. Full NMR data confirmed its distinctive benzoxazole moiety.[5] The ^{1}H NMR spectrum of **1** showed a sharp one-proton singlet at δ 7.98, suggesting an aromatic hydrogen atom on the carbon bearing the heteroatoms. Other features of the spectrum included a doublet at δ 5.02 (J = 9.1 Hz) and two vinyl methyl singlets at δ 1.68 and 1.78 indicative of an isobutenyl group, two three-proton doublets at δ 1.05 (J = 5.4 Hz) and 1.45 (J = 6.7 Hz), indicating a pair of secondary methyl groups, a three-proton singlet at δ 2.44, suggesting an aromatic methyl, a one-proton doublet of doublets at δ 3.90 (J = 8.9, 9.1 Hz), ascribable to a bis-allyl hydrogen atom, and a one-proton multiplet at δ 3.20 assigned to a benzylic hydrogen. The ^{13}C NMR spectrum exhibited 21 signals (5 CH_3, 3 CH_2, 6 CH, and 7 C), whose chemical shift values and multiplicity confirmed the presence of a fully substituted benzoxazole moiety [δ 151.0 (d), 147.1 (s), 137.9 (s), 136.7 (s), 134.7 (s), 126.6 (s), 122.4 (s)] and a trisubstituted olefin [δ 130.7 (d), 128.8 (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,

(4) Pouchert, C. J. *The Aldrich Library of Infrared Spectra*, 2nd ed.; Aldrich Chemical Co.: Milwaukee, WI, 1975; pp 1041−1075.

(5) (a) Kobayashi, J.; Madono, T.; Shigemori, H. *Tetrahedron Lett.* **1995**, *36*, 5589−5590. (b) Stewart, M.; Fell, P. M.; Blunt, J. W.; Munro, M. H. G. *Aust. J. Chem.* **1997**, *50*, 341−347.

HMBC) revealed the connectivity and thus the gross structure of **1**. Assignments of the NMR signals are given in Table 1. Thus, in common with the majority of *P. elisabethae* metabolites, it appeared that compound **1** contained the same amphilectane skeleton found in the aglycon portion of the pseudopterosins.[6] Notwithstanding, comparison of their molecular formulas showed that pseudopteroxazole possessed the additional element of HCN. The oxazole ring and the amphilectane moiety were connected as depicted based on the following evidence. The isolated aromatic methine at δ 7.98 assigned to C21 was connected to C9 and C10 from HMBC correlations. In turn, the oxygen and nitrogen atoms of the oxazole ring were attached at C9 (δ_C 147.1) and C10 (δ_C 137.9), respectively, by comparison of the carbon chemical shifts with those of known benzoxazoles.[7] Since HMBC correlations connected C10 to the aromatic methyl (δ_H 2.44) and C9 to the methine proton H7 (δ_H 3.20), 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, from coupling constant analysis, and by comparisons of the NMR chemical shifts with those of known amphilectanes. A half-chair conformation of ring A was deduced from NOESY correlations of H1/H2α, H1/H17, H1/H20, and H4/H18, suggesting that H3 and the isobutenyl side chain are both pseudoaxial. Thus, the methyl group at C3 and H1 must be pseudoequatorial. A relatively large coupling constant (9.1 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 2.25) with a large coupling constant (J = 9.5 Hz) ascribable to H4 suggests that the latter methine is trans diaxial to H3 and H5β. Additional NOESY correlations of H5α/H18, H5β/H19, and H6α/H7 revealed that ring B adopts also a half-chair conformation with H4 and the methyl group at C7 in a near trans pseudodiaxial conformation. Therefore, H1, H4, H7, and the methyl group at C3 are all α-oriented. Except for the benzoxazole substructure, the ^{13}C NMR resonances of **1** were highly comparable with those of helioporin E (**3**), a structurally similar diterpene isolated from the Okinawan blue coral *Heliopora coerulea*.[8] We therefore conclude that pseudopteroxazole (**1**) and helioporin E (**3**) must possess the same relative stereochemistry. As expected, minor variations were observed only in those resonances assigned to protons in benzylic positions (H1, H4, and H7).

seco-Pseudopteroxazole (**2**), a yellowish oil, $[\alpha]^{25}_D$ +28.2° (*c* 0.85, $CHCl_3$),[9] was analyzed for $C_{21}H_{29}NO$ by HREIMS [$(M)^+$, *m*/*z* 311.2258, calcd for $C_{21}H_{29}NO$, 311.2249] and by ^{13}C NMR (Table 1). The mass spectrum of this compound showed a base peak ion at *m*/*z* 200.1076 representing loss of C_8H_{15} due to the terpenoid eight-carbon side chain. This behavior, coupled with appropriate proton and carbon NMR bands, indicated the presence in *seco*-pseudopteroxazole (**2**) of a bicyclic diterpenoid component containing a pentasubstituted aromatic ring. Interpretation of the NMR spectral features indicated that this skeleton was similar to that of the aglycon portion of a related series of antiinflammatory glycosides, the *seco*-pseudopterosins.[10] The complete structure determination of **2** was facilitated by further interpretation of NMR data and by consideration of the results of our earlier work with pseudopteroxazole (**1**). Compound **2** possessed ^{13}C NMR features which were highly characteristic of its bicyclic diterpenoid skeleton and suggested, as in **1**, the presence of a benzoxazole moiety. Excluding the sp^2 resonances due to the alkenyl side chain, seven aromatic resonances were observed between 151 and 124 ppm, diagnostic of the benzoxazole constellation. Unlike the ^{13}C NMR features of **1**, the *seco* compound possessed an off-resonance doublet carbon at 125.1 ppm (assigned to C5) and a triplet carbon at 26.2 ppm (assigned to C13), indicative that bond cleavage had occurred at the C5−C13 positions. One (δ_H 8.02; δ_C 151.0) of the two aromatic methines was assigned to C21 of the oxazole ring, while an oxygen and nitrogen atoms of the oxazole ring were attached at C8 (δ_C 148.6) and C7 (δ_C 136.8), respectively. The other aromatic proton (δ_H 7.03; δ_C 125.1) was assigned to C5 from HMBC correlations of the methine proton (H5) to C4, C7, C10, and C19. Thus, the gross structure of *seco*-pseudopteroxazole (**2**) was proposed as shown. NMR data for **2** and their assignments are given in Table 1. Except for the aromatic portion, the ^{13}C NMR spectrum of **2** correlated exceedingly well with that of helioporin D (**4**), a metabolite from *H. coerulea*, which like **2**, belongs to the serrulatane class of diterpenes.[8] On the basis of this comparison, compounds **2** and **4** were concluded to have the same relative stereochemistry.



serrulatane skeleton **2**

The $[\alpha]_D$ values of **1** (+101°) and **3** (+111°) suggest that the absolute configuration of (+)-pseudopteroxazole (**1**) is probably identical to that of (+)-helioporin E (**3**) since their specific rotations are similar both in sign and order of magnitude and because in both instances their optical rotation can be attributed to the same isolated tricyclic chiral unit.

(6) (a) Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5140−5145. (b) Roussis, V.; Wu, Z.; Fenical, W.; Strobel, S. A.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 4916−4922. (c) Harvis, C. A.; Burch, M. T.; Fenical, W. *Tetrahedron Lett.* **1988**, *29*, 4361−4364.

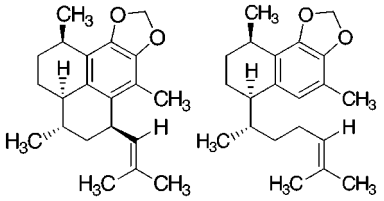
(7) (a) Ueki, M.; Ueno, K.; Miyadoh, S.; Abe, K.; Shibata, K.; Taniguchi, M.; Oi, S. *J. Antibiot.* **1993**, *46*, 1089−1100. (b) Chaney, M. O.; Demarco, P. V.; Jones, N. D.; Occolowitz, J. L. *J. Am. Chem. Soc.* **1974**, *96*, 1932−1933.

(8) Tanaka, J.; Ogawa, N.; Liang, J.; Higa, T.; Gravalos, D. G. *Tetrahedron* **1993**, *49*, 811−822.

(9) After isolation and routine spectral measurements, it was evident that our sample of **2** was contaminated with traces of known elisabethin A, a strongly dextrorotatory substance. Thus, it is conceivable that the actual specific rotation of **2** is significantly lower than the value shown here; see: Rodríguez, A. D.; González, E.; Huang, S. D. *J. Org. Chem.* **1998**, *63*, 7083−7091.

(10) Look, S. A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363−3370.

Likewise, the similarity of $[\alpha]_D$ values of **2** (+28.2°) and **4** (+6.3°) suggests that their absolute configuration is probably identical.[9]



3 **4**

Occurrence of the benzoxazole group in marine biota is exceedingly rare. So far, only two such marine natural products, the sesquiterpene nakijinol and a closely related analog, have been reported from an Okinawan sponge and a New Zealand specimen of *Dysidea* sp., respectively.[5] The benzoxazole function in compounds **1** and **2** could arise by reaction of HCN with an intermediate arene epoxide, whether directly from isomerization and dehydrogenation of a betaine species[11] or from dehydrogenation of an arenocyanohydrin species followed by adventitious rearrangement of an intermediate 2-cyanophenol.[12] On the other hand, the fact that amphilectane-based isonitriles have been found in several marine sponges of the order Halichondrida lends credence to the contention that transformation of a 2-isocyanophenol precursor may lead to the benzoxazole functionality.[13] That amphilectane-based isonitriles appear to be present at such low concentrations in marine organisms belonging to different phyla suggests that their biogenesis could be microbial in nature.

Biological Activity. Pseudopteroxazole (**1**) and *seco*-pseudopteroxazole (**2**) were submitted to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) of the Southern Research Institute for biological studies with *M. tuberculosis*. Compound **1** was found to effect potent inhibitory activity (97%) against *M. tuberculosis* H37Rv at a concentration of 12.5 μg/mL, whereas **2** inhibited 66% of mycobacterial growth. Thus, it appears that the potent activity of **1** might be attributed, at least in part, to the benzoxazole function. Interestingly, biological screening of pseudopteroxazole (**1**) in the National Cancer Institute's (NCI) 60-cell-line tumor panel indicated insignificant in vitro cancer cell cytotoxicity.

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