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Ileabethoxazole: a novel benzoxazole alkaloid with antimycobacterial activity

Ileana I. Rodríguez,[a] Abimael D. Rodríguez,[a,*] Yuehong Wang[b] and Scott G. Franzblau[b]

[a]*Department of Chemistry, University of Puerto Rico, PO Box 23346, U.P.R. Station, San Juan 00931-3346, PR, USA*
[b]*Institute for Tuberculosis Research, College of Pharmacy, The University of Illinois at Chicago, Chicago, IL 60612-7231, USA*

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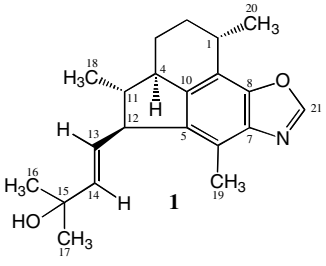
Abstract—Ileabethoxazole (**1**), a new perhydroacenaphthene-type diterpene alkaloid containing the uncommon benzoxazole moiety, was isolated from the Caribbean sea whip *Pseudopterogorgia elisabethae*. The structure of **1** was elucidated by extensive spectroscopic data interpretation. Ileabethoxazole showed 92% inhibition of *Mycobacterium tuberculosis* ($H_{37}Rv$) at the concentration range of 128–64 μg/mL.

Benzoxazoles represent a rare category of heterocyclic natural compounds having a variety of pharmacological properties, including antitubercular activity.[1] One very interesting subclass of this category is the marine benzoxazoles.[2] The Caribbean sea whip *Pseudopterogorgia elisabethae* is a known source of the aforementioned subclass of benzoxazoles, and it contains several analogs that have been described as strongly antimycobacterial.[3] Having as target the investigation of the extensive chemodiversity of *P. elisabethae* and the assessment of its secondary metabolites as potential antitubercular agents, we studied a small fraction from the hexane extract that had not been previously investigated. Our specimens of *P. elisabethae* were removed by scuba from their natural habitat at depths of 25–30 m near the Island of Providencia (Old Providence), Colombia. The present paper reports the isolation, characterization, and anti-bacterial activity of a new diterpene alkaloid named ileabethoxazole (**1**) from *P. elisabethae*. After extensive use of two-dimensional NMR techniques, it was found that the molecule is based on the same ileabethane diterpenoid skeleton as ileabethin (**2**), a scanty secondary metabolite previously isolated by us from specimens of *P. elisabethae* collected elsewhere.[4]

Although ileabethoxazole (**1**) represents the second example of a naturally occurring substance based on this intriguing carbon framework, it is, however, the first example of a benzoxazole-type alkaloid based on this very rare skeletal class. Since ileabethoxazole is present only in very low concentration (yield 9.4×10^{-5}% on dried gorgonian weight basis), it is quite conceivable that **1** actually originates from the symbiotic bacteria of *P. elisabethae* and not the host coral.



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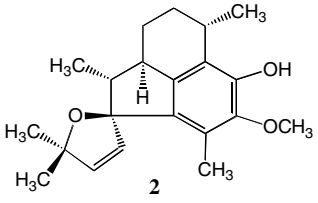

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The 1:1 CH_2Cl_2/MeOH extract of the freeze-dried gorgonian coral *P. elisabethae* (1.8 kg) was subjected to our usual solvent partitioning scheme,[5] and the hexane extract (323 g) was purified by a combination of flash silica gel chromatography using stepwise elution with hexane/CH_2Cl_2 mixtures, gel filtration chromatography on Bio-Beads SX-3 (toluene), silica gel chromatography eluting with hexane/acetone mixtures, and HPLC to afford ileabethoxazole (1.7 mg) as a light yellowish oil.[6]

Keywords: *Pseudopterogorgia elisabethae*; Tuberculosis; Caribbean gorgonian octocorals; Ileabethane skeleton; Benzoxazole; Mycobacterium.
* Corresponding author. Tel.: +1 787 764 0000x4799; fax: +1 787 756 8242; e-mail: arodrig@cnnet.upr.edu

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The ESIMS of compound **1** in the positive mode exhibited a quasi-molecular ion peak at m/z 326 $[M+H]^+$, and an accurate mass measurement indicated the molecular formula $C_{21}H_{28}NO_2$. Nine degrees of unsaturation were deduced from the actual molecular formula $C_{21}H_{27}NO_2$ and ^{13}C NMR measurements. The IR spectrum indicated the presence of a hydroxyl group (3550–3100 cm^{-1}) and the UV spectrum (MeOH) displayed a broad absorption between $\lambda_{max} = 225$–288 nm that was particularly informative, as it was reminiscent of a five-membered heteroaromatic functionality.[3] Full NMR data confirmed its distinctive benzoxazole moiety (Table 1). The ^1H NMR spectrum of **1** showed a sharp one-proton singlet at δ 7.99 that correlated in the HMQC spectrum with an off-resonance doublet carbon at 150.9 ppm (assigned to C-21), suggesting an aromatic hydrogen atom on the carbon bearing the heteroatoms. This observation was supported by ^{13}C NMR and DEPT-135 NMR experiments ($CDCl_3$; 125 MHz), which in addition to a 1,2-disubstituted olefin [δ_C 129.6 (CH) and 139.4 (CH)], exhibited six quaternary signals [δ_C 119.5 (C), 124.4 (C), 138.0 (C), 139.2 (C), 142.1 (C), and 147.2 (C)] ascribable to a fully substituted benzene ring. After subtraction of all the unsaturations due to carbon–carbon double bonds, we concluded that ileabethoxazole (**1**) must be tetracyclic with one C=N and four C=C double bonds.

Additional salient features of the ^1H NMR spectrum included a sharp six-proton singlet at δ 1.39 and two mutually coupled vinyl signals at δ 5.86 (d, 1H, $J = 15.6$ Hz) and 5.60 (dd, 1H, $J = 15.6$, 9.5 Hz) indicative of a *trans*-3-hydroxy-3-methyl-1-butenyl group, two three-proton doublets at δ 1.16 ($J = 6.6$ Hz) and 1.50 ($J = 6.9$ Hz), indicating a pair of secondary methyl groups, a three-proton singlet at δ 2.51, suggesting an aromatic methyl, a one-proton doublet of doublets at δ 3.37 ($J = 9.7$, 9.5 Hz), ascribable to a bis-allyl hydrogen atom, and two complex multiplets at δ 3.11 and 2.50 (each 1H) suggesting two benzylic hydrogens. After assignment between all the direct C–H bonds were made by HMQC, the main connectivities allowing the entire structure elucidation of the carbocyclic framework of **1** were established by ^1H–^1H COSY and HMBC NMR experiments (Table 1).

Only one scalarly coupled spin system was detected from the COSY NMR spectrum for the partial structure shown in Figure 1. In this large spin system there is a terminal olefinic CH (δ_H 5.86) resonating as a doublet (15.6 Hz). Its partner has a chemical shift of δ 5.60, but it is also coupled (9.5 Hz) to an sp^3 carbon-attached proton at δ 3.37 that has a triplet-like appearance. This methine signal has a CH neighbor, resonating as a complex multiplet at δ 1.63, which is coupled to both a doublet methyl at δ 1.16 and a methine proton (δ 2.50) that is assumed to be at a bridgehead position. The latter CH

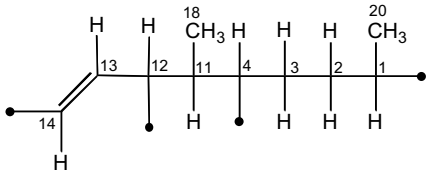


Figure 1. Coupled spin system detected from the COSY NMR spectrum of ileabethoxazole (**1**).

Table 1. ^1H NMR (500 MHz), ^{13}C NMR (125 MHz), ^1H–^1H COSY, HMBC, and NOESY spectral data for ileabethoxazole (**1**)[a]

Position	δ_H, mult, intrgt (J in Hz)	δ_C (mult)[b]	^1H–^1H COSY	HMBC[c]	NOESY
1	3.11, m, 1H	29.5 (CH)	H-2α, H_3-20	H_3-20	H-2α, H-3β, H_3-20
2α	2.24, m, 1H	33.5 (CH_2)	H-1, H-2β	H_3-20	H-1, H-3β
2β	1.46, m, 1H		H-2α		H-3α
3α	2.16, m, 1H	28.3 (CH_2)	H-3β		H-2β
3β	1.25, m 1H		H-3α, H-4		H-1, H-2α
4	2.50, m, 1H	48.0 (CH)	H-3β, H-11	H_3-18	H-2β, H-12, H_3-18, H_3-20
5		138.0 (C)		H-4, H-12, H_3-19	
6		124.4 (C)		H_3-19	
7		139.2 (C)		H_3-19, H-21	
8		147.2 (C)		H-1, H-21	
9		119.5 (C)		H-4, H_3-20	
10		142.1 (C)		H-4	
11	1.63, m, 1H	51.2 (CH)	H-4, H-12, H_3-18	H-12, H_3-18	H-13
12	3.37, dd, 1H (9.7, 9.5)	55.5 (CH)	H-11, H-13	H-14, H_3-18	H-4, H-14, H_3-18
13	5.60, dd, 1H (15.6, 9.5)	129.6 (CH)	H-12, H-14		H-11
14	5.86, d, 1H (15.6)	139.4 (CH)	H-13	H-12, H_3-16, H_3-17	H-12, H_3-18
15		70.8 (C)		H-13, H-14, H_3-16, H_3-17	
16	1.39, s, 3H	29.7 (CH_3)		H_3-17	
17	1.39, s, 3H	29.8 (CH_3)		H_3-16	
18	1.16, d, 3H (6.6)	15.2 (CH_3)	H-11		H-4, H-12
19	2.51, s, 3H	13.5 (CH_3)			
20	1.50, d, 3H (6.9)	20.7 (CH_3)	H-1		H-4
21	7.99, s, 1H	150.9 (CH)			

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in parts per million relative to TMS.
[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 $J_{CH} = 6$–8 Hz.

proton has a CH_2 neighbor, resonating as two nonequivalent protons at δ 2.16 and 1.25, which in turn show strong couplings to two protons at δ 2.24 and 1.46 due to nonequivalent methylene protons. This spin system terminates with a benzylic sp^3 CH proton with a chemical shift of 3.11 ppm (strong couplings to the previous CH_2 group) that has a CH_3 neighbor, resonating as a doublet (6.9 Hz) at δ 1.50.

In addition, three partial structures (**A**–**C**) were deduced from extensive analyses of the 2D NMR data of **1** including COSY, HMQC, and HMBC spectra in $CDCl_3$ (Fig. 2). The HMBC experiment showed connectivity between the C14 carbon [δ_C 139.4 (CH)] and the protons of C16 and C17. The oxygen-bearing quaternary carbon at position 15 (δ_C 70.8) showed strong HMBC correlations to H_3-16 and H_3-17, and to the olefinic protons H-13 and H-14. Thus, the geminal methyls must be attached to C15 thereby linking units **A** and **B**. The methine protons (δ_H 3.37 and 2.50) at two ends of unit A have long-range correlations to the same sp^2 ^{13}C signals at δ_C 142.1 (C10) and 138.0 (C5), indicating that fragments **A** and **C** form a five-membered ring through the C4–C10 and C5–C12 bonds. The strong downfield displacements observed for C4 (δ_C 48.0), C11 (δ_C 51.2), and C12 (δ_C 55.5) alluded in part to such ring size. Likewise, the methyl protons (δ 1.50) at the remaining end of unit **A** has long-range correlations to the sp^2 ^{13}C signal at δ_C 119.5 (C9) indicating that units **A** and **C** also form a six-membered ring through C1–C9. The protons of the **A** moiety have additional correlations to the aromatic function consistent with the proposed constitution of the molecule (Table 1). Furthermore, the oxazole ring and the ileabethane moiety were connected as depicted based on the following evidence. The isolated aromatic methine at δ 7.99 assigned to C21 was connected to C7 and C8 from HMBC correlations (Table 1). In turn, the oxygen and nitrogen atoms of the oxazole ring were attached at C8 (δ 147.2) and C7 (δ 139.2), respectively, by comparison of the carbon chemical shifts with those of known benzoxazoles.[3] Since HMBC correlations connected C7 to the aromatic methyl (δ_H 2.51) and C8 to the methine proton H1 (δ_H 3.11), the locus of each heteroatom about the benzoxazole ring was established unambiguously. This allowed the complete planar structure for **1** to be assigned.

The solution conformation of the molecule and the relative configurations were obtained from the analysis of the scalar coupling constants and the NOESY spectrum

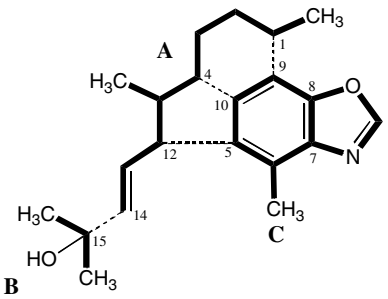


Figure 2. Partial structures of ileabethoxazole (**1**) deduced from the ^1H–^1H COSY, HMQC, and HMBC experiments.

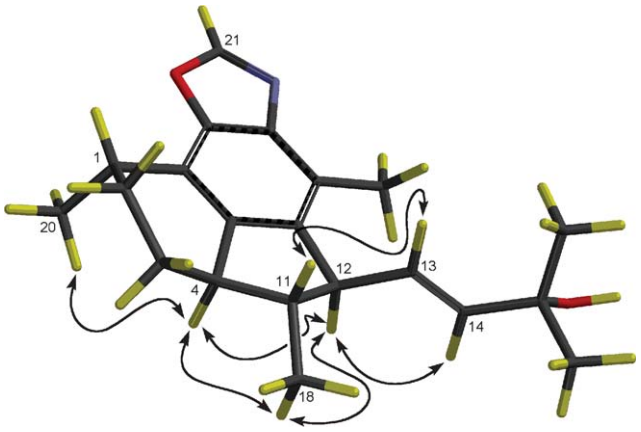
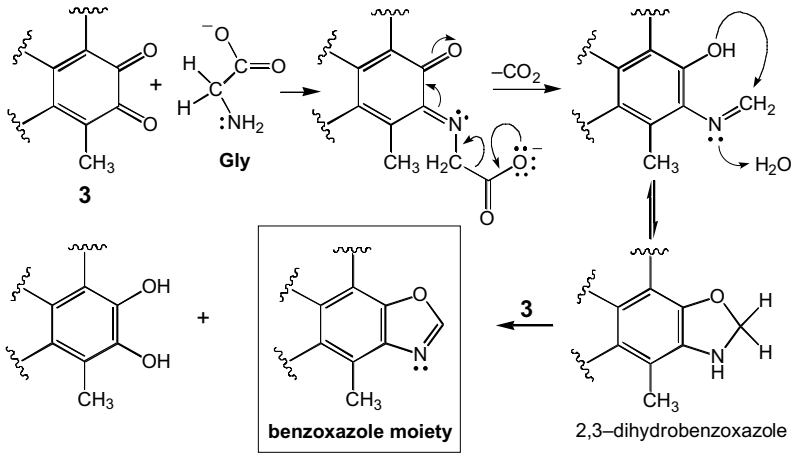


Figure 3. Energy-minimized molecular model of ileabethoxazole (**1**) with diagnostic NOESY correlations observed.

and by performing molecular modeling calculations (Fig. 3).[7] The conformation of the carbocyclic five-membered ring of **1** was first examined. The H-12 doublet of doublets shows two large couplings to the neighboring H-11 and H-13 protons. These large scalar interactions (9.7 and 9.5 Hz) indicate that the dihedral angles between H-12 and its two neighboring protons H-11 and H-13 are close to 180° (calculations showed 162.2° and 175.4°, respectively) and thus, the relative orientations of these protons are anti-periplanar (clearly, the protons at C-12 and C-13 of **1** are nearly trans in a preferred conformation). These conformations are further supported by the NOESY spectrum where H-12 shows strong correlations to both H_3-18 and H-14, but not with H-11 or H-13, confirming the α-orientation of the H_3-18 protons. Similarly, strong NOESY correlations between H-12 and H-4, and those of H_3-18 and H-4, established the spatial proximities of these protons. In agreement with the proposed relative stereochemistry at these stereocenters H-11 shows strong correlations to the vinyl proton H-13. Furthermore, the proposed geometrical arrangement for ileabethoxazole (**1**) proves the α-orientation of the substituent at the more remote C-1 stereocenter. The overall conformation of the saturated six-membered ring can be described as a half-chair (Fig. 3). In this conformation the α-proton at 2.50 ppm (H-4) has a strong NOESY interaction with the C-20 methyl protons. Moreover, the ^{13}C NMR resonances of **1** ascribable to C-1 (δ_C 29.5) and C-20 (δ_C 20.7) are highly comparable with those of ileabethin (**2**) (29.4 and 21.0 ppm, respectively),[4] suggesting that these compounds must possess the same relative stereochemistry at C-1. These data provided the basis for our assignment of stereocenters 1*S**, 4*R**, 11*R**, and 12*S** for compound **1**.

The biosynthetic origin of the benzoxazole moiety remains uncertain.[1–3] Nevertheless, a condensation between an amino acid (such as glycine) and a *o*-benzoquinone residue like **3** should not be excluded as a possible biogenetic pathway to the benzoxazole functionality in **1** (Scheme 1). This likely scenario is particularly appealing as metabolites possessing the proposed *o*-benzoquinone and catechol moieties have been reported from *P. elisabethae*.[8]



Scheme 1. Plausible biogenesis of the benzoxazole moiety in **1**.

Ileabethoxazole (**1**) was submitted to the Institute for Tuberculosis Research of the University of Illinois at Chicago for biological studies with *Mycobacterium tuberculosis* ($H_{37}Rv$) (ATCC 27294), in 7H12 medium using the Microplate Alamar Blue Assay (MABA).[9] Thus, compound **1** was found to have potent inhibitory activity (92%) against *M. tuberculosis* ($H_{37}Rv$) at the concentration range of 128–64 μg/mL. At these concentrations the inhibitory activity of ileabethoxazole lies within the same range as that of the very active rifampin (100%). At lower concentrations (32, 16, 8, and 4 μg/mL) **1** displayed 73%, 54%, 38%, and 29% inhibition, respectively. From these results it was determined that ileabethoxazole has an MIC of 61 μg/mL. The rifampin control yielded an expected MIC of 0.1 μg/mL.

Acknowledgments

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6. Ileabethoxazole (**1**): light yellowish oil; $[\alpha]_D^{20}$ +6.8 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3550–3100, 2926, 2855, 1455, 1376, 1260, 802 cm^{-1}; UV (MeOH) λ_{max} 225 (ε 22 200), 277 (ε 2300), 288 (ε 1800) nm; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRESIMS *m/z* $[M+1]^+$ 326.2116 (calcd for $C_{21}H_{28}NO_2$, 326.2120).
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