A β-Carboline Alkaloid from the Soft Coral *Lignopsis spongiosum*

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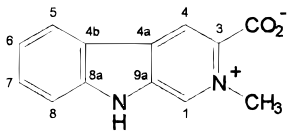
From an EtOH extract of *Lignopsis spongiosum*, a new β-carboline alkaloid, 2-methyl-9H-pyrido[3,4*b*]-indole-3-carboxylic acid (**1**), was isolated and characterized by spectral methods.

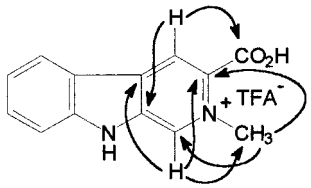
Lignopsis spongiosum, a species belonging to a recently described genus of the family Briareidae,[1] is a soft coral from the South Atlantic, which was collected at a depth of 200 m near the South Georgia Islands. During the present screening program to find compounds with antimicrobial activity, 2-methyl-9H-pyrido[3,4*b*]indole-3-carboxylic acid (**1**) , which possesses a moderate activity against *Escherichia coli*, was isolated. Unlike most soft corals, which are known to produce mainly sesquiterpenoids and diterpenoids,[2] the crude extract of *L. spongiosum* afforded a new alkaloid of the β-carboline type (**1**). β-Carboline alkaloids have been reported previously in the marine environment from tunicates[3] and bryozoans,[4] but have never been detected in a coelenterate.[2]



1

An ethanolic extract of *L. spongiosum* was chromatographed over reversed-phase Si gel and subjected to HPLC, to afford compound **1**. The molecular formula of this compound was established as $C_{13}H_{11}N_2O_2$ by HRFABMS. The ^{1}H NMR (MeOD) spectrum showed an adjacent four-proton system (δ 8.33 d, J = 8 Hz; δ 7.77 m; 7.70 m and δ 7.45 dt, J = 8, 1 Hz) and three singlets, two of them corresponding to heteroaromatic protons (δ 8.90, 1H; δ 8.75, 1H) and one to a methyl group at δ 4.62. Due to low solubility, not all the carbon resonances could be observed in methanol-d_4, and therefore, DMSO-d_6, containing a small addition of trifluoracetic acid, was employed to improve the solubility of compound **1**. In the ^{1}H NMR spectrum of the resulting salt of compound **1**, namely **1a**, another proton signal was observed (δ 13.2 s, exchangeable). The ^{13}C NMR spectrum showed 11 aromatic carbons, one carbonyl carbon at δ 162.9, and a methyl carbon at δ 49.0. The presence of NMR signals at δ 4.62 in the ^{1}H NMR spectrum and at δ 49.0 in the ^{13}C NMR spectrum was attributable to an *N*-methyl group, with the N atom supporting a positive charge. With the aid of HETCOR and COLOC experiments, the structure of compound **1** could be assigned as shown. Correlations observed in the COLOC experiment for the heteroaromatic ring of **1a** are shown in Figure 1.

The spectroscopic data of compound **1** are in good agreement with those reported for the trifluoromethanesulfonate salt of the methyl ester of compound **1**, which was synthesized by Dodd and co-workers, to study the interactions of β-carboline derivatives with benzodiazepine receptors of the mammalian central nervous system.[5]



1a

Figure 1. COLOC correlations observed in the heteroaromatic ring.

Although sesquiterpenoids and diterpenoids are characteristic metabolites of soft corals, these types of compounds were not detected in *L. spongiosum*, even at trace levels. This fact, together with the presence of an unusual class of metabolite in a coelenterate such as the β-carboline alkaloid **1**, may have some chemotaxonomic interest, especially because *L. spongiosum* is a species of a new genus.

Experimental Section

General Experimental Procedures. The UV spectrum was recorded on a Hewlett−Packard 8451A diode-array spectrophotometer, and the IR spectrum was acquired on a Nicolet Magna-IR 550 FT−IR instrument. NMR spectra were recorded on a Bruker AC-200 instrument at 200.1 MHz for ^{1}H and 50.3 MHz for ^{13}C. EIMS and CIMS (CH_4) were obtained on a Trio-2 quadrupole mass spectrometer (VG Biotech). FABMS was performed on a ZAB-SEQ (BEqQ) instrument (VG Analytical, Manchester, UK). HRFABMS was carried at the Washington University Resource for Biomedical and Bioorganic Mass Spectrometry (Washington University, St. Louis, MO).

Animal Material. The soft coral *L. spongiosum* was collected by trawling in March 1996, at depths of between 130 and 280 m, in the ocean west of the San Pedro Islands, South Georgia, in the South Atlantic. The coral was identified by Dr. Carlos D. Perez and Mauricio Zamponi [Laboratorio de Biología de Cnidarios (LABIC) Departamento de Ciencias Marinas, FCEyN, Universidad de Mar del Plata]. A voucher specimen is preserved in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia," accession no. MACN 34110.

Extraction and Isolation. An amount of 600 g of frozen *Lignopsis spongiosum* was extracted sequentially twice with EtOH and twice with CH_2Cl_2 at room temperature, and the extracts were combined. The crude extract was partitioned between EtOAc and H_2O, and the aqueous layer was subjected to vacuum−liquid chromatography on reversed-phase Si gel with an H_2O−MeOH solvent system of decreasing polarity. A fraction eluted with H_2O−MeOH (6:4) was submitted to HPLC (ODS-A YMC column, 20 × 250 mm, 5 μm; CH_3CN−H_2O 4:6, 4 mL/min), yielding pure compound **1** (10 mg).

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **1a**

	1 (MeOD-d_4)	**1a** (DMSO-d_6−TFA)	
position	δ ^{1}H	δ ^{1}H	δ ^{13}C
1	8.90 s	9.40 s	134.4
3			132.8
4	8.75 s	9.21 s	121.6
4a			131.9
4b			120.3
5	8.33 d (J = 8.0 Hz)	8.46 d (J = 8.0 Hz)	124.1
6	7.45 dt (J = 8.0, 8.0, 1.1 Hz)	7.40 m	122.7
7	7.77 m	7.73 m	132.6
8	7.70 m	7.73 m	113.8
8a			144.8
9a			135.6
CO_2H		13.17 s	162.9
CH_3-N^+	4.62 s	4.64 s	49.0

2-Methyl-9H-pyrido[3,4*b*]indole-3-carboxylic acid (1): obtained as a pale yellow solid (MeOH); mp 203−205 °C; UV (MeOH) λ_{max} (log ϵ) 210 (4.16), 236 (4.05), 264 (4.26), 308 (3.88), 384 (3.45) nm; IR (BaF_2) ν_{max} 3400, 2926, 2848, 1630, 1608, 1563, 1386, 1350 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 182 $[M - 44]^+$ (32), 167 (6), 140 (11), 91 (9), 44 (100); CIMS *m*/*z* 183 $[M + H - 44]^+$ (100), 169 (50); FABMS *m*/*z* 265 $[M + K]^+$ (10), 249 $[M + Na]^+$ (10), 227 $[M + H]^+$ (100), 183 (50); HRFABMS *m*/*z* 227.0819 (calcd for $C_{13}H_{11}N_2O_2$, 227.0820).

Antimicrobial Activity. Antimicrobial activity was assessed using an agar diffusion assay. Activity was checked against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. In all cases assays were performed with 50 μg/disk. An inhibition zone of 11 mm was obtained against *E. coli* ATCC 25922.

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References and Notes

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