5-Bromo-8-methoxy-1-methyl-β-carboline, an Alkaloid from the New Zealand Marine Bryozoan *Pterocella vesiculosa*

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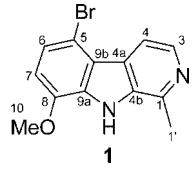
A new alkaloid, 5-bromo-8-methoxy-1-methyl-β-carboline (**1**), has been isolated from the New Zealand marine bryozoan *Pterocella vesiculosa*. Structural elucidation was achieved through NMR spectroscopic and mass spectrometric analysis, and a single-crystal X-ray diffraction analysis of **1** was performed. The biological activity of **1** was assessed against P388 murine leukemia cells and three microorganisms and compared with that of a number of related β-carboline alkaloids.

Comparatively little research has been undertaken into the chemistry of bryozoans as compared with that of other marine invertebrates; nevertheless bryozoans have proven to be an excellent source of novel and/or biologically active compounds.[1] The vast majority of bryozoan metabolites isolated to date have been alkaloids[2] such as the euthyroideones,[3] the amathaspiramides,[4] and the pterocellins.[5,6]

As part of our ongoing search for bioactive and/or novel compounds from New Zealand marine bryozoans, we undertook an investigation of an extract of *Pterocella vesiculosa* (Lamarck, 1816) (order Cheilostomatida, suborder Ascophorina, family Catenicellidae). We have previously reported the isolation and structural elucidation of the alkaloids pterocellins A−F from this species.[1,5,6] We now report the isolation, structural elucidation, and bioactivity determination of a new β-carboline alkaloid, 5-bromo-8-methoxy-1-methyl-β-carboline (**1**), from the bryozoan.



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P. vesiculosa is currently known only from waters off the North Island of New Zealand and South Eastern Australia.[5] The bryozoan was collected by scuba from the Alderman Islands, off the North Island of New Zealand, and identified as *P. vesiculosa*. An extract of the bryozoan (CH_2Cl_2) was subjected to reversed-phase flash column chromatography and gel permeation chromatography to give the orange-yellow 5-bromo-8-methoxy-1-methyl-β-carboline (**1**) in 3.0×10^{-3} % yield (based on bryozoan wet weight).

The structure of the new alkaloid was determined by analysis of its ^{1}H NMR, ^{13}C NMR, and mass spectra and by use of 2-D NMR techniques such as COSY, NOESY, HSQC, and HMBC and confirmed with the aid of a single-crystal X-ray diffraction study. Positive ion ESIMS of **1** contained peaks at 291/293 $[M + H]^+$ and 313/315 $[M + Na]^+$. HRESIMS of **1** contained a pair of peaks at *m/z* 291.0147 and 293.0049 $[M + H]^+$ and a further pair of peaks at *m/z* 312.9886 and 314.9866 $[M + Na]^+$, consistent with a molecular formula of $C_{13}H_{12}BrN_2O$.

The ^{1}H NMR spectrum of **1** in $CDCl_3$ (Table 1) contained five resonances. Three signals represent aromatic protons (a two proton singlet at 8.33 ppm and two mutually coupled doublets at 7.25 and 6.80 ppm). The spectrum also contained a methoxy signal at 3.92 ppm and a methyl signal at 2.77 ppm. The ^{13}C NMR spectrum of **1** (Table 1) contained 13 signals, including six protonated carbon resonances (two aliphatic and four aromatic) and seven quaternary aromatic carbons. Atom connectivities were established by NOESY, ^{1}H−^{13}C HSQC, and ^{1}H−^{13}C HMBC NMR experiments. The results of these experiments confirmed the presence of the β-carboline skeleton with methyl, methoxy, and bromo substituents.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data (400 MHz, $CDCl_3$) for 5-Bromo-8-methoxy-1-methyl-β-carboline (**1**)

position	δ_C, mult.	δ_H (*J* in Hz)	NOE	HMBC[a]
1	142.4, qC			
3	114.7, CH	8.33, s		1, 4, 4a,
4	139.0, CH	8.33, s		3, 4a, 4b, 9b
4a	128.5, qC			
4b	134.1, qC			
5	108.1, qC			
6	123.6, CH	7.25, d (8.3)	H-7	5, 7, 8, 9b
7	109.0, CH	6.80, d (8.3)	H-6, H-10	5, 6, 8, 9a
8	145.8, qC			
9a	131.5, qC			
9b	121.3, qC			
10	55.6, CH_3	3.92, s	H-7	8
1′	20.4, CH_3	2.77, s		1, 3, 4b

[a] HMBC correlations, optimized for 6 Hz, are from proton(s) stated to the indicated carbon.

The substituents were located through analysis of the ^{1}H−^{13}C HSQC and HMBC results (Table 1) and through NOE analyses (Table 1). For example, analysis of the HSQC NMR spectrum indicated that the resonance at 8.33 ppm in the ^{1}H NMR spectrum represented two coincidental aromatic protons, since it showed correlations to two different carbon atoms, C-3 and C-4 (114.7 and 139.0 ppm, respectively). The NOESY NMR experiment showed correlations between H-6 at 7.25 ppm and H-7 at 6.80 ppm and between H-7 and the methoxy group at 3.92 ppm. The methoxy signal correlated to a quaternary carbon resonance at 145.8 ppm in the HMBC NMR experiment, which was assigned as C-8, thus confirming the site of attachment. In the HMBC NMR experiment, the methyl proton resonance at 2.77 ppm was correlated to C-1, C-4b, and C-3, indicating that it was attached at C-1. ^{1}H NMR and NOESY spectroscopic experiments indicated that there was no proton at C-5; therefore the bromine is attached at this position. Full NMR data are listed in Table 1.

The ^{1}H NMR signals of H-3 and H-4 are coincidental, although their chemical environments are different. It has been previously observed in 5-bromo-β-carbolines that H-3 and H-4 resonate closer together than is typical of other β-carboline alkaloids. In the reported NMR spectroscopic data for eudistomin D (5-bromo-6-hydroxy-β-carboline),[7] H-3 and H-4 resonate at 8.55 and 8.34 ppm, respectively, and for the acetylated derivative, 6-acetoxy-9-acetyl-5-bromo-β-carboline, the H-3 and H-4 resonances were even closer

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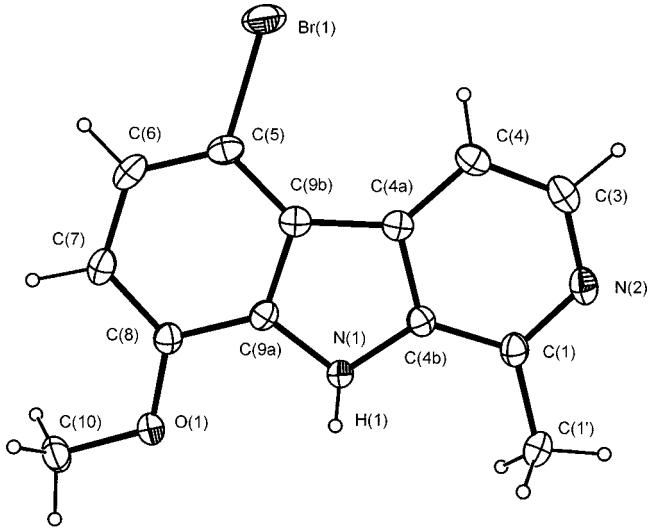

Figure 1. Structure of one independent molecule of 5-bromo-8-methoxy-1-methyl-β-carboline (**1**) showing the numbering system.

together at 8.71 and 8.67 ppm, respectively.[7] This is in contrast to a β-carboline lacking a 5-bromo substituent such as harman (1-methyl-β-carboline), in which H-3 and H-4 resonate at 8.43 and 7.83 ppm, respectively.[8] These data indicate that the effect of a bromine substituent at C-5 on a β-carboline ring system is to move the H-4 resonance significantly downfield, and for 5-bromo-8-methoxy-1-methyl-β-carboline (**1**), this results in the H-3 and H-4 signals becoming coincident.

The structure of **1** was confirmed with the aid of an X-ray crystal structure on the methanol solvate. An ORTEP plot of the crystal structure of **1** is shown in Figure 1. Crystal structure data (Tables S1−S3, Supporting Information) indicated that **1** is planar to within ±0.06 Å except for the methoxy group, which is twisted a little from the molecular plane. All of the bond lengths and the bond angles are within previously observed limits for these types of molecules. Methanol is an integral part of the crystal structure, acting as an H-bond donor to N(2) and as an H-bond acceptor from the N(1)−H(1) group of an adjacent molecule.[9]

5-Bromo-8-methoxy-1-methyl-β-carboline (**1**) was assayed in P388 murine leukemia and antimicrobial assay systems[10,11] and exhibited moderate activity against the P388 murine leukemia cell line with an IC_{50} value of 5089 ng/mL and also displayed inhibitory action toward the Gram-positive bacterium *Bacillus subtilis* and the fungi *Candida albicans* and *Trichophyton mentagrophytes* with MID ranges of 2−4, 4−5, and 4−5 μg/mL, respectively. β-Carboline alkaloids have been found previously from the family Catenicellidae,[10,12] to which *P. vesiculosa* belongs, although this is the first example of a β-carboline alkaloid reported from the genus *Pterocella*. Our previous investigation of *Cribricellina cribraria* (Catenicellidae) included a structure−activity relationship study of a number of 1-substituted β-carboline alkaloids.[10] Of the compounds tested, only those with a vinyl substituent at C-1 such as pavettine (1-vinyl-β-carboline) displayed significant activity in the P388 biological assay. Others such as harman (1-methyl-β-carboline) and 1-ethyl-8-methoxy-β-carboline were inactive.[10] These data indicate that it is the bromine substituent at C-5 that confers some activity in this assay system for **1**. In the previous study, most of the 1-substituted β-carboline alkaloids displayed some degree of antimicrobial activity.[10] All compounds were less active than pavettine (1-vinyl-β-carboline) against *B. subtilis*, *C. albicans*, and *T. mentagrophytes* with only one exception; harman displayed the same level of activity as pavettine against *Candida albicans*.[10] In general, the addition of an 8-methoxy substituent decreased activity against the range of microorganisms as compared with pavettine, as did the presence of an alkyl group at C-1.[10] The minimum inhibitory doses for **1** against these organisms are intermediate between those of pavettine and the 8-methoxy or 1-alkyl compounds, indicating that it is the 5-bromo substituent that improves the antimicrobial activity somewhat compared to that of 1-alkyl- or 8-methoxy-substituted β-carbolines in general. The biological assay results for **1** (along with those of selected β-carboline alkaloids for comparative purposes) are listed in Tables S4 and S5, Supporting Information.

Experimental Section

General Experimental Procedures. The melting point was determined on a Reichert Thermovar hot-stage microscope and was uncorrected. UV−visible spectra were acquired in methanol on a Varian CARY 100 UV/visible spectrophotometer. All NMR spectra were determined on a Bruker Avance 9.4 T instrument, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Spectra were acquired in $CDCl_3$. ^{1}H NMR and ^{13}C NMR chemical shifts were referenced to residual chloroform (7.26 and 77.0 ppm, respectively). Standard pulse sequences were used for HMBC, HSQC, COSY, and NOESY experiments. ESIMS mass spectrometric data were measured on a Fisons VG Platform II machine operating in positive ion mode with the cone voltage set between 20 and 80 eV. High-resolution mass spectrometric data were obtained in the ES mode on a Bruker Daltonics MicrOTOF mass spectrometer. Details of the assay procedures have been reported elsewhere.[10,11]

Collection of *P. vesiculosa*. Colonies of bryozoans (2772 g wet weight) were collected by scuba at the Alderman Islands off the North Island of New Zealand and stored frozen. A voucher specimen, 01-AI201-03, is held at the Department of Chemistry, University of Waikato. The bryozoan was identified by Dr. Dennis Gordon.

Extraction, Isolation, and Characterization. The bryozoan (1440 g wet weight) was macerated in a blender and exhaustively extracted with dichloromethane (4 L). The combined extracts were filtered, and the solvent was removed in vacuo. The CH_2Cl_2 extract (1.42 g) was fractionated by reversed-phase flash column chromatography on C_{18} silica using a stepped gradient from H_2O to MeOH to CH_2Cl_2. A fraction from this separation (240.8 mg) contained an intense orange spot by TLC (silica, EtOAc/MeOH, (5:1)) and was subjected to gel permeation on Sephadex LH-20 in MeOH to yield 5-bromo-8-methoxy-1-methyl-β-carboline (**1**) (42.6 mg).

5-Bromo-8-methoxy-1-methyl-β-carboline (1): square, yellow crystals (methanol/toluene/heptane); mp 204 °C; UV (MeOH) λ_{max} (ϵ) 288 (4.72), 293 (4.60), 340 (4.66), 353 (4.61), 487 (3.27) nm, (KOH/MeOH) λ_{max} (ϵ) 290 (4.72), 298 (4.74), 357 (4.43), 373 (4.48), 388 (4.39) nm; ^{1}H and ^{13}C NMR data, see Table 1; COSY H-6↔H-7; HRESIMS *m/z* 291.0142 and 293.0124 $[MH]^+$ (calcd for $C_{13}H_{12}{}^{79}BrN_2O$, 291.0128 and $C_{13}H_{12}{}^{81}BrN_2O$, 293.0108), 312.9971 and 314.9989 $[MNa]^+$ (calcd for $C_{13}H_{11}{}^{79}BrN_2ONa$, 312.9947 and $C_{13}H_{11}{}^{81}BrN_2ONa$, 314.9927).

X-ray Crystallography of 5-Bromo-8-methoxy-1-methyl-β-carboline (1·MeOH). Small block crystals suitable for data collection were grown by diffusion of heptane into a methanolic toluene solution of **1** at room temperature. The unit cell dimensions and intensity data were obtained on a Bruker-Nonius Apex II CCD diffractometer at 93 K. The structure was solved by direct methods and refined by full matrix least-squares refinement (on F_o^2) using the SHELX programs.[13]

Crystal data: $C_{13}H_{12}BrN_2O\cdot CH_3OH$, $M = 323.19$, monoclinic, $a = 9.6025(5)$ Å, $b = 10.9301(5)$ Å, $c = 13.3928(6)$ Å, $\beta = 107.645(2)°$, $U = 1339.53(11)$ Å^3, $T = 93$ K, space group $P2_1/c$, $Z = 4$, μ(Mo Kα) = 3.068 mm^{-1}, 11 505 reflections collected, 3236 unique (R_{int} 0.0382) used after correction for absorption ($T_{max,\ min} = 0.4596, 0.2604$). Crystal dimensions 0.60 × 0.56 × 0.30 mm^3. Refinement gave $R_1 = 0.0316$ [2871 data with $I > 2\sigma(I)$] and $wR_2 = 0.0844$ (all data). Hydrogen atoms were located in a penultimate difference map and were refined with isotropic temperature factors, except for the methyl hydrogens on the main molecule and on the lattice methanol molecule, which were included in calculated positions.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**. Crystal data and structure refinement details, tabulations of atomic coordinates and equivalent isotropic displacement parameters, and bond lengths and angles for **1**, P388 murine leukemia and antimicrobial assay results for **1** and selected β-carboline alkaloids. This material is available free of charge via the Internet at http://pubs.acs.org.

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