Pibocin B, the First *N-O*-Methylindole Marine Alkaloid, a Metabolite from the Far-Eastern Ascidian *Eudistoma* Species

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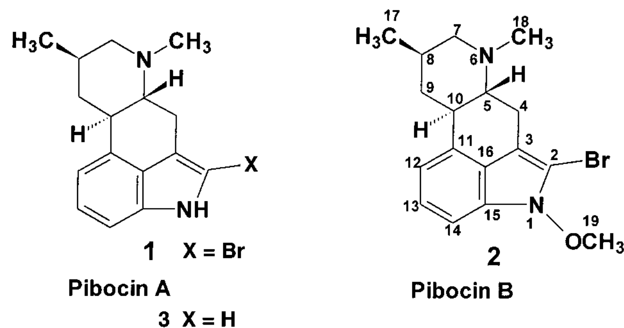
Pibocin B (**2**), the first representative of marine alkaloids with a unique structural feature, an *N-O*-methylindole group, was isolated from the Far-Eastern ascidian *Eudistoma* sp. Its structure has been established as (8β)-2-bromo-*N-O*-methyl-6,8-dimethylergoline on the basis of NMR data, FAB and MALDI-TOF MS, and chemical correlations. Pibocin B showed moderate cytotoxic activity against mouse Ehrlich carcinoma cells.

Ascidians belonging to the genus *Eudistoma* have proven to be a rich source of biologically active secondary metabolites, for example, β-carboline derivatives,[1–3] 24-membered macrolides,[4] indoles,[5] pyridoacridine alkaloids,[6] and aromatic polysulfides.[7]

In the course of our continuing studies on biologically active marine natural products we earlier isolated pibocin A (**1**),[8] the first representative of marine ergoline alkaloids, from an ascidian, *Eudistoma* sp. Further investigation of this ascidian led us to the isolation of a new cytotoxic ergoline alkaloid, pibocin B (**2**). In this paper we describe structure elucidation of **2**, including its correlation with ergoline alkaloids of terrestrial origin.

The colonial ascidian *Eudistoma* sp. (phylum Chordata, subphylum Urochordata, class Ascidiaceae, suborder Aplousobranchia, family Polycitoridae), was collected using scuba during the 18th scientific cruise of R/V "Academik Oparin" in the Sea of Japan and immediately after collection was extracted with EtOH. The obtained extracts were antimicrobial against *Bacillus subtilus*, *Candida albicans*, and *Staphylococcus aureus* and cytotoxic against mouse Ehrlich carcinoma cells. These extracts were concentrated in vacuo, and the residue was partitioned between aqueous EtOH and hexane. The EtOH-soluble materials were repeatedly chromatographed on Polychrome-1, Sephadex LH-20, and Si gel and after crystallization from MeOH gave pibocin A (**1**) and pibocin B (**2**; 0.0005%, based on dry weight of ascidian). The ^{1}H and ^{13}C NMR data of **2** as well as those of **1** in C_5D_5N, which were not reported earlier,[8] are given in the Table 1.



Pibocin B (**2**) was obtained as colorless, thin crystals. FABMS showed pseudo molecular ion $[M + H]^+$ peaks at m/z 351/349 (1:1), indicating the presence of one bromine atom in the molecule. The molecular formula $C_{17}H_{21}N_2OBr$ was obtained for **2** from a high-resolution mass measurement of the $[M + H]^+$ ion in MALDI-TOF MS and ^{1}H and ^{13}C NMR data. The UV spectrum (λ_{max} 231 and 283 nm) indicated a substituted indole chromophore in **2**. The ^{1}H and ^{13}C NMR spectra revealed signals at δ 106.8 (C-14, 7.37, dd), 124.0 (C-13, 7.33, dd), 115.2 (C-12, 6.99, dt), characteristic of 2-bromo-3,4-trisubstituted indoles. Further interpretation of NMR data revealed three CH_2, three CH, one CH_3, one N–CH_3, and one OCH_3 group (Table 1). The ^{1}H NMR data for the coupling constants of the nine-proton spin system from H-4 to H-10 (see Table 1) in **2** were essentially identical to those of **1**. An exception was that the singlet at δ 12.76 (N–H) observed for **1** was absent in the ^{1}H NMR spectrum of **2**. Instead, a three-proton singlet at δ 4.02 (CH_3-19) was observed. Consistent with this, the ^{13}C NMR spectrum of **2** showed a signal at δ 65.5 (*C*H_3O). In addition, the difference of 30 mass units between **2** and **1** suggested **2** to be an OCH_3 analogue of **1**. The position of the OCH_3 group in **2** (see structure) was deduced by analysis of MS and NMR data. In particular, a strong $[M - 31]^+$ (100%) peak in EIMS spectrum of **2** at m/z 319/317 corresponding to the facile loss of OCH_3 suggested the attachment of methoxy group to nitrogen.[9] The observation of NOESY correlations between the signals of the methoxyl protons at δ 4.02 and H-14 at δ 7.37 also supported the position of the OCH_3 group at N-1.

Pibocins B and A were hydrogenated over PtO_2 to give the same compound **3**, identified as festuclavine[10] by comparison of ^{1}H NMR data and physical constants. Therefore, the structure of pibocin B (**2**) was confirmed and the stereochemistry assigned by chemical correlation with pibocin A (**1**), whose structure was established by single-crystal X-ray diffraction studies[8] and with festuclavine (**3**), a known fungal metabolite of terrestrial origin. On the basis of all the above-mentioned data the structure for pibocin B (**2**) was established as the (8β)-2-bromo-*N-O*-methyl-6,8-dimethylergoline.

Pibocin A and pibocin B gave no precipitate with $AgNO_3$ (absence of Cl^-), so we formulated **1** and **2** as free bases. Pibocin B (**2**) and pibocin A (**1**) are closely related and differ from each other only by the occurrence of the OCH_3 group in **2** instead of H in **1**. The ^{13}C NMR data for C-4, C-5, C-7, C-8, C-10, and CH_3-18 of **2** were identical with those of **1**. However, the chemical shifts of 4α-H, 5β-H, 7α-H, 7β-H, 8α-H, 10α-H, and 18-CH_3 in the ^{1}H NMR spectrum of

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Table 1. ^{1}H and ^{13}C NMR Data (C_5D_5N) for Pibocins A (**1**) and B (**2**)

position	**1** $\delta^{13}C$	**2** $\delta^{13}C$	**1** δ ^{1}H	**2** δ ^{1}H	m, J (Hz)	NOESY
2	110.0	108.0				
3	103.6	105.0				
4α	25.6	25.5	2.72	2.94	dd, 15.0; 11.0	
4β			3.40	3.32	dd, 15.0; 4.5	CH_3-18, H-5β
5β	66.6	66.2	2.07	2.38	ddd, 10.5; 11.0; 4.5	
7α	63.9	64.1	2.86	3.10	dq, 11.5; 1.9	7β, CH_3-18
7β			1.75	2.06	t, 11.5	CH_3-18, 7α
8α	29.4	29.5	1.97	2.24	m	7α, CH_3-17
9α	35.8	35.8	2.56	2.50	ddd, 12.5; 3.8; 5.7	9β
9			1.03	1.03	q, 12.5	
10α	39.6	39.7	3.01	3.29	ddd, 12.5; 10.5; 3.8	8α, 9α
11	131.0	132.5				9α, 13
12	113.7	115.2	7.04	6.99	dt, 6.4; 1.5	10α
13	123.0	124.0	7.32	7.33	dd, 7.9; 6.4	H-12
14	108.6	106.8	7.41	7.37	dd, 7.9; 1.5	CH_3-19
15	134.5	110.0				
16	126.7	131.1				
17	18.8	19.0	0.89	0.86	d, 6.5	
18	41.6	42.0	2.37	2.59	s	
19		65.5		4.02	s	H-14
NH			12.71		brs	

pibocin B (**2**) were shifted downfield (about 0.2 ppm) in comparison with those of pibocin A (**1**) at equal concentrations of **1** and **2** in solution. That is why we believe that the N−OCH_3 group affects chemical shifts of protons neighboring the N-6 atom.

Pibocin B contains a rare indole N−OCH_3 fragment that previously was found only in metabolites of a few terrestrial plants[11,12] and those of the fungus *Penicillium oxalicum.*[13,14] This structural feature has not been previously found in marine alkaloids or in ergoline alkaloids from terrestrial sources. Methoxyaminoalkyl pyridines with a similar, but acyclic, N−OCH_3 fragment have been isolated recently from the marine sponges.[9,15,16] However, biosynthetic pathways of acyclic N−OCH_3 and indole N−OCH_3 fragments in marine alkaloids may be different. It is possibly that the acyclic N−OCH_3 fragments originate from oximes,[16] while an indole N−OCH_3-containing fragment of **2** was derived from unknown marine *N*-oxides.

Pibocin B is cytotoxic against mouse Ehrlich carcinoma cells (ED_{50} 25 μg/mL).

Experimental Section

General Experimental Procedures. Melting points (mp) were determined on a Boethius apparatus. Optical rotations ($[\alpha]_D$) were measured using a Perkin-Elmer 141 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer at 300 and 75 MHz, respectively, with tetramethylsilane as an internal standard. EIMS were measured on a LKB 9000S spectrometer (ionizing energy 70 eV), FABMS were obtained using a LKB 9091 instrument, and MALDI-TOF mass spectra were measured on a Bruker Biflex III laser desorption mass spectrometer coupled with delayed extraction using an N_2 laser (337 nm) on α-cyano-4-hydroxycinnamic acid as matrix.

Low-pressure column liquid chromatography was performed using Polichrom-1 (powder Teflon, Biolar, Latvia), and Sephadex LH-20 (Sigma, Chemical Co), Si gel L (40/100 μm, Chemapol, Praha, Czech Republic), and Si gel plates 4.5 × 6.0 cm (5−17 μ, Sorbfil, Russia) were used for thin-layer chromatography.

Animal Material. The ascidian *Eudistoma* sp. was collected using scuba during the 18th scientific cruise of R/V "Academik Oparin", September 1995, 43°05′7″ N, 134°18′4″ E, 3−12 m. A voucher specimen (018−228) is on deposit in the collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia. The colonies are characterized by a massive round shape and brick red color. Maximum dimension of a colony is 11 × 5 × 3 cm. Zooids are completely enclosed in a common test and have no visible systems. Zooid lengths are from 5 to 10 mm. Appertures of both short siphons are six-lobed. The branchial sac has three rows of stigmata.

Extraction and Isolation. Animal material was extracted with EtOH immediately after collection. The crude EtOH extract of the ascidian *Eudistoma* sp. was evaporated in vacuo at 50 °C to give a brown oil. This oil was dissolved in 200 mL of EtOH−H_2O (9:1). The hexane-solubles were extracted three times by partitioning with equal volumes of hexane. The EtOH−H_2O solubles were concentrated to give 30 g of brown oil. This residue was chromatographed over a Polichrome I column (powder Teflon, Biolar, Latvia), using H_2O → 50% EtOH → EtOH as eluents, to give an active fraction (433 mg) eluted with 50% EtOH. The latter was chromatographed on a column with Si gel, using $CHCl_3$/MeOH mixtures as eluents, to give an active crude pibocin B (88 mg) and known pibocin A (**1**) (119 mg) eluted with $CHCl_3$/MeOH (2:1). The crude pibocin B was chromatographed three times on a column with Sephadex LH-20 (elution with $CHCl_3$/MeOH, 1:1; 3:1, and 0:1), and after crystallization of part of crude pibocin B from MeOH 1.9 mg of pibocin B was obtained.

Pibocin B (1): $C_{17}H_{21}N_2OBr$, thin colorless crystals (MeOH); mp > 358 °C, $[\alpha]_D$ −51° (c 0.19, EtOH); IR (KBr) γ_{max} 3063, 2956, 2929, 2854, 2781, 1606, 1554, 1461, 1438, 1366, 1282, 1155 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 231 (4410), 283 (2450) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRMS [MALDI-TOF (positive ion mode)] m/z 349.0942 $[M + H]^+$ (calcd for $C_{17}H_{22}N_2OBr$, 349.0910); FABMS m/z (%) 351 ($[M + H],^+$ 21), 349 ($[M + H],^+$ 23), 320 ($[M + H]^+ - 31,^+$ 18), 318 ($[M + H]^+ - 31,^+$ 21), 237 (5), 225 (7), 195 (6), 181 (2), 166 (8), 154 (8), 153 (8), 133 (100), 127 (6), 115 (6); EIMS (70 eV) m/z (%): 350/348 (1:1, $[M^+]$, 6), 335/333 (1:1, $[M - 15]^+$, 5), 319/317 (1:1, $[M - 31]^+$, 100), 305/303 (1:1, 7), 238 (35), 195 (14), 155 (25), 128 (30), 82, 80, 57, 57, 55, 45, 41, 36, 31.

Festuclavine (3) from 1 and 2. PtO_2 was added to a solution of **1** (1 mg) or **2** (0.5 mg) in MeOH (1 mL) and stirred under H_2 at 25 °C for 1 h. Removal of the catalyst by filtration and evaporation of solvent gave **3** (1 mg) from **1**; 0.5 mg from **2**, colorless needles (MeOH), mp 249−252 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.88 (1H, brt, J = 1.8 Hz, H-2), 2.68 (1H, ddd, J = 14.7, 11.1, 1.8 Hz, H-4α), 3.42 (1H, dd, J = 14.7, 4.3, Hz, H-4β), 2.10 (1H, ddd, J = 11.1, 9.6, 4.3, Hz, H-5β), 2.98 (1H, m, H-7α), 1.88 (1H, t, J = 11.1 Hz, H-7β), 2.02 (1H, m, H-8α), 2.63 (1H, m, H-9α), 1.09 (1H, q, J = 12.3 Hz, H-9β), 2.95 (1H, m, H-10α), 6.94 (1H, m, H-12), 7.17 (2H, m, H-13, 14); HRMS [MALDI-TOF (positive ion mode)] m/z 241 $[M + H]^+$; identical by ^{1}H NMR (300 MHz) and mp with festuclavine.[10]

Biological Assays. The in vitro cytotoxic assays on Ehrlich ascites carcinoma cells were performed according to the methods of Sasaki et al.[17]

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