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Pibocin, the First Ergoline Marine Alkaloid from the Far-Eastern Ascidian *Eudistoma sp.*

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Abstract: Pibocin[1] (**1**), the first representative of marine ergoline alkaloids, was isolated from the Far-Eastern ascidian *Eudistoma sp.* Its structure and absolute stereochemistry have been established as (8β)-2-bromo-6,8-dimethylergoline on the basis of spectroscopic and X-Ray analysis data. Pibocin showed moderate cytotoxic activity against mouse Ehrlich carcinoma cells.

Keywords: Marine metabolites; Alkaloids; Cytotoxins; X-Ray crystal structures

Ergoline alkaloids are well-known as a group of natural products exhibiting extraordinary biological activities [1]. Some of these secondary metabolites such as lysergic acid derivatives show hallucinogenic effects [2], while other compounds with the tetracyclic ergoline ring system demonstrate antibiotic and cytotoxic activities [3,4]. So far the ergoline alkaloids were found in the fungi belonging to the genera *Claviceps*, *Aspergillus*, *Rhizopus*, and *Penicillium* as well as in the higher plants of the family Convolvulaceae.

In the course of our continuing studies on marine natural products [5,6], we have found that extracts from the Far-Eastern ascidian *Eudistoma sp.*[2] were antimicrobial against *Bacillus subtilus*, *Candida albicans* and *Staphylococcus aureus* and cytotoxic against mouse Ehrlich carcinoma cells. Bioassay-guided isolation afforded several active compounds, of which pibocin

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[1] The name is coined from PIBOC (Pacific Institute of Bioorganic Chemistry).

[2] The ascidian was collected using SCUBA during 18-th scientific cruise of R/V "Academik Oparin", September 1995, 43°05′7″N, 134°18′4″E, 3-12m.

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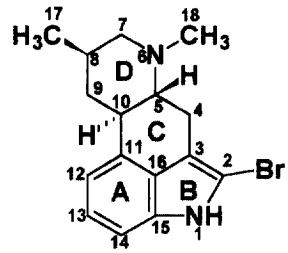
(1) was the first representative of marine ergoline alkaloids. In this report we describe the isolation and structure elucidation of **1**.



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The EtOH extract of the fresh ascidian was partitioned between aqueous EtOH and hexane. EtOH-Soluble materials were further separated by column chromatography on Polychrome-1 (powder Teflon, Biolar, Latvia) in the system EtOH:H_2O, 1:1, Sephadex LH-20 ($CHCl_3$:MeOH, 1:1; 3:1 and 0:1) and Silica gel ($CHCl_3$:EtOH, 3:1) to give **1**[3] (0,0006 %, based on dry weight of ascidian).

Pibocin (**1**) showed m.p. 226-228°C, $[\alpha]_D$ -36° (c 0.14, EtOH) and was analyzed for $C_{16}H_{19}N_2Br$ by EIMS, ^1H and ^{13}C NMR (Table 1).

Table 1. NMR Data for Pibocin*

Position	$\delta^{13}C$	δ ^1H	m	J (Hz)
2	110.0	-	-	-
3	103.6	-	-	-
4α	25.6	2.57	dd	15.0; 11.0
4β		3.24	dd	15.0; 4.5
5	66.6	1.88	ddd	11.0; 4.5; 9.5
7α	63.9	2.70	ddd	11.0; 4.0; 1.8
7β		1.59	t	11.0; 11.0
8	29.4	1.83	m	
9α	35.8	2.38	ddt	12.5; 4.0; 4.0; 1.8
9β		0.92	q	12.5; 12.5; 12.5 12.5
10	39.6	2.86	dddd	4.0; 12.5; 9.5; 1.8
11	131.0	-	-	-
12	113.7	6.82	dt	0.9; 6.7; 1.8
13	123.0	7.19	dd	6.7; 8.0;
14	108.6	6.93	dd	8.0; 1.8
15	134.5	-	-	-
16	126.7	-	-	-
17	18.8	0.80	d	6.4
18	41.6	2.17	s	
NH	-	6.77	bs	

*All ^1H NMR experiments were performed at 250 MHz in C_6D_6 and $\delta^{13}C$ experiments were performed at 62.3 MHz in C_5D_5N: CD_3OD, 9:1.

[3] Compound **1**: UV (EtOH): 225 (ε 27400), 281 (ε 7000); EIMS (m/z, rel.int.,%): 320/318 (1:1, 100); 277/275 (1:1, 16); 234/232 (1:1, 22); 224/222 (1:1, 23); 154 (30).

Absorption bands at 225 and 281 nm in the UV spectrum along with NMR signals at δ 108.6 (6.93, dd), 123.0 (7.19, dd), 113.7 (6.82, dt), confirmed **1** as a derivative of a 2-bromo-3,4-disubstituted indole. Further NMR data showed the compounds to contain additionally three CH_2, three CH, one CH_3 and one N-CH_3 groups.

The molecular structure of **1** was established by X-Ray analysis. Crystal data: $C_{16}H_{19}N_2Br \times CH_3OH$, monoclinic, space group $P2_1$, Z=2, a=6.439(2), b=15.259(5), c=8.640(2) Å, β=97.83(2)°, V=840.9(4) Å^3, D_c=1.39 g/cm^3, μ=2.445 mm^{-1}, MoKα radiation, λ=0.71073 Å, t= -120°C. Intensities of 1683 reflections were measured on a Siemens P3 diffractometer (θ-2θ scanning technique, 2θ<55°).The Lorentz, polarization and absorption corrections were applied. The structure[4] was solved by direct methods and refined by full matrix least-squares procedure of the SHELXL-93 system [7] to R=0.0537, S=1.051 for the 1541 reflections with I>2σ(I). The absolute configuration was determined from comparison of the Flack parameter [8] value [-0.01(2)] with expected values equal 0 for correct and 1 for inverted absolute structures.

Figure 1 is a computer generated perspective drawing of the final X-Ray model. The pyrrole ring B of the molecule has a planar conformation. The conformation of the six-membered ring C is a sofa. The piperidine ring D has a chair conformation. The rings C and D have a *trans* junction.The (5R,8R,10S) absolute stereochemistry of **1** coincided with that of the terrestrial origin ergot alkaloids [9].

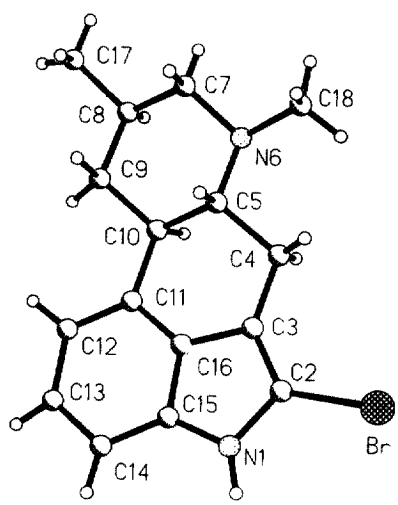

Figure 1. The computer-generated perspective drawing for pibocin (**1**).

In contrast with all the known ergoline alkaloids, pibocin contains a rare 2-bromo-indole fragment previously found only in metabolites of marine organisms [10-12]. The presence of this fragment suggests that **1** is biosynthesized by the ascidian itself but not by symbiotic or epiphytic fungi derived from the coastal discharge. Isolation of ergoline alkaloids from such

[4] The final atomic coordinates have been deposited with the Cambridge Crystallographic Data Center.

phylogenetically distant organisms as fungi, higher plants and ascidians represents, presumably, a case of accidental biochemical parallelism. Pibocin is cytotoxic against mouse Ehrlich carcinoma cells (ED_{50} 12.5 μg/mL).

Acknowledgements

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