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A NEW ANTIBIOTIC FROM THE MARINE BRYOZOAN *FLUSTRA FOLIACEAE*[1]

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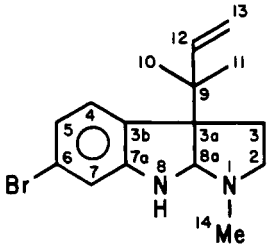
Recent investigations of the bryozoan *Flustra foliaceae* L from Scandinavian waters have revealed the presence of a series of bromoalkaloids belonging to the physostigmine group (1,2).

Antibacterial testing of the CH_2Cl_2 extract from *F. foliaceae* collected in the Minas Basin, Nova Scotia, showed strong activity against *Bacillus subtilis*. Assay guided fractionation of the crude extract by open column chromatography resulted in several active fractions. Further separation of the combined fractions by thick layer chromatography and final purification by hplc yielded the bromoalkaloid dihydroflustramine C (**1**) as the active principle. Structure elucidation was based on the following evidence.



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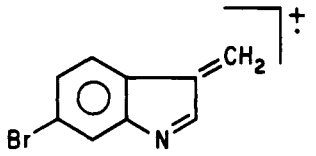

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The optically active alkaloid ($[\alpha]^{25}D$ $-110°$) was obtained as colorless crystals mp 82-84°. The uv spectrum was characteristic of an indole nucleus [λ max (MeOH) 213, 250, 309 nm]. From hrms data the elemental composition was determined as $C_{16}N_{21}N_2Br$ (calcd, *m/z* 322.0869 found *m/z* 322.0875). The presence of an isoprene unit was suggested by the characteristic loss of 69 mass units from the parent ion, and further loss of 43 mass units (CH_3-$N{=}CH_2$) gave the fragment ion at *m/z* 210/208 corresponding to the brominated analogue of the characteristic methylene indole ion (**2**). This supports the placement of bromine on the benzene nucleus.

All sixteen carbons of the alkaloid could be accounted for in the cmr spectrum (Table 1), and the chemical shifts were entirely compatible with a brominated physostigmine skeleton (3, 4) containing an isoprene unit. The aromatic carbons bearing hydrogen were assigned from consideration of their multiplicities, respective $^1J_{CH}$ coupling values (Table 1) and by single frequency decoupling experiments. These decoupling experiments also helped in the assignment of resonances for the remaining quaternary aromatic carbons C-3b, C-6, and C-7a. The methine carbon C-8a (δ_c 84.49) was identified from its large $^1J_{CH}$ coupling value (156.8Hz) and by long-range coupling to the *N*-methyl protons. From another single frequency decoupling experiment, the resonance at δ_c36.89 was assigned to the *N*-methyl carbon C-14. The high field quaternary resonance at δ_c 64.0 ppm was assigned to C-3a.

From the pmr chemical shift and coupling data (Table 1) it was established that the isoprene unit was present as a

[1]NRCC 23608.

TABLE 1. Nmr Data for Dihydroflustramine C: ^{13}C (δ_c)[a] and ^1H (δ_H)[a] Chemical Shifts and ^{13}C-^1H, ^1H-^1H Coupling Constants (^nJ Hz)

Carbon Atom	Value	Hydrogen Atom	Value
C2	53.1 (t) ^1J 137.0	H2	2.53 (m)[c]
C3	34.78 (t) ^1J 134.2	H3(a,e)	2.27 (m),[c] 1.77 (m)[c]
C3a	64.0 (s)	H4	6.94 (d) ^3J 8.0
C3b	132.53 (bs)	H5	6.76 (dd) ^3J 8.0, ^4J 1.8
C4	126.3 (d) ^1J 159.8	H7	6.66 (d) ^4J 1.8
C5	120.82 (dd)	H8a	4.37 (s)
C6	121.38 (bdt) ^2J~3.0, ^3J~11.0	H10, 11	1.02 (s), 0.96 (s)
C7	111.63 (dd) ^1J 164.5, ^3J 5.7	H12	5.94 (dd) $^3J_{C15}$ 10.8, $^3J_{trans}$ 17.3
C7a	152.19 (dd) ^2J~3.1, ^3J~6.2	H13 (*trans*)	4.99 (dd) ^2J 1.3, ^3J 17.3
C8a	84.49 (bs) ^1J 156.8	H13 (*cis*)	5.05 (dd) ^2J 1.3, ^3J 10.8
C9	41.30 (bs)	H14	2.36 (s)
C10	23.21 (q) ^1J 125.7		
C11	22.44 (q) ^1J 125.7		
C12	144.6 (bd) ^1J 151.1		
C13	113.2 (t) ^1J 156.8		
C14	36.89 (q) ^1J 131.5		

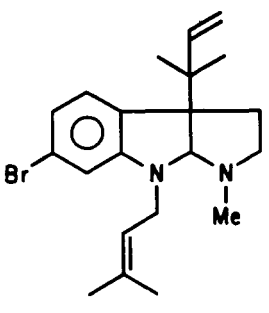
[a]Samples dissolved in $CDCl_3$. Chemical shifts are given in ppm relative to TMS.

[b]Shifts and coupling constants for H-4 to H-13 were derived by first order analysis of the sub-spectra. The J_{CH} values are measured spacings only.

[c]H-2 and H-3 protons form a complex ABMX system. No attempt was made to analyse the system and shift values in Table 1 correspond to the centres of the multiplets.

1,1-dimethylallyl system (5). The signals for the H-2 and H-3 protons appeared as a complex ABMX pattern, while the *N*-methyl protons and H-8a appeared as sharp singlets at δ_H 2.36 ppm and δ_H 4.37 ppm, respectively. The coupling pattern for the aromatic protons (Table 1) placed the bromine at C-5 or C-6. However, irradiation of the methyl protons at C-10 (δ_H 0.96 ppm) resulted in nOe (9.8%) of the H-4 doublet and hence placed the bromine at C-6. Irradiation of both C-10 and C-11 methyl groups, resulted in a substantial nOe (35 and 36%) of H-8a confirming the *cis*-fusion of the two pyrrolidine rings. Finally irradiation of the *N*-methyl protons gave a nOe (30%) of H-8a but not H-7, and so the methyl group is placed at N-1. Further structure proof was obtained by comparison of the published pmr and cmr data for the flustramines A(**3**), B(**4**), and C(**5**) previously isolated from *F. foliaceae* collected in the North Sea (1,2).

The physostigmine alkaloids originally isolated from the Calabar bean (*Physostigma venenosum* Balf., Leguminosae) display potent biological activity (6). The variety of biological activity



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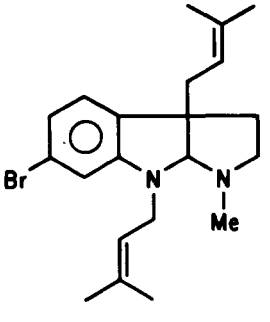

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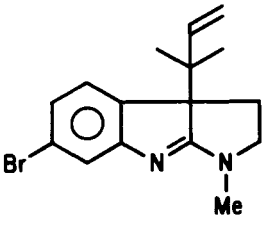

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displayed by this brominated marine analogue is currently under investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The melting point was measured with a Kofler apparatus and is uncorrected. Optical rotation was determined with a Perkin-Elmer polarimeter. The uv spectrum was obtained with a GCA-MacPherson Series 700 and the ir spectrum was obtained in KBr using a Perkin-Elmer 283B spectrophotometer. Pmr and cmr spectra were recorded in $CDCl_3$ with TMS as internal standard on the Nicolet 360 NB spectrometer of the Atlantic Region Magnetic Resonance Centre. The mass spectrum was obtained with a Dupont model 21-110B double-focusing spectrometer used in the electrical detection mode; 8 kV accelerating voltage, 70 eV ionising energy. Accurate mass measurement was made by peak matching against a perfluorokerosene reference. Hplc was performed with a Waters Associates system; model 6000 pump, U6K injector and a model 450 variable wave-length detector.

The bryozoan, *F. foliaceae* was collected in the Minas Basin, Nova Scotia. A voucher specimen is on deposit at the Department of Biology, Acadia University, Wolfville, N.S.

EXTRACTION AND ISOLATION.—The collection (500 g) was frozen (−20°) until extraction with MeOH at ambient temperature for 3 days. The MeOH fraction (5 liters) was concentrated under reduced pressure and shaken against CH_2Cl_2 (3×400 ml). The CH_2Cl_2 solubles (3.3g) were chromatographed on a pad (9.5×9×3.8 cm) of silica gel (Merck, Kieselgel GF_{254}, Type 60) and eluted with a solvent gradient system starting with hexane, then Et_2O, CH_2Cl_2, and EtOAc. Fraction 11 (93 mg) displaying antibiotic activity was further refined by preparative tlc (Merck GF_{254}; 20×20×0.1 cm) using 10% MeOH-CH_2Cl_2 as eluent. The plates were divided into bands based on fluorescence under short-wavelength (254 nm) uv light, and the organic material was eluted from the silica using 20% MeOH-EtOAc. The band (24 mg) displaying antibiotic activity was further purified by hplc using spherisorb (5μ, CSC; 9.4×50 cm) and elution with 4.5% MeOH-CH_2Cl_2. The biologically active (**1**) was eluted as the major fraction (16 mg).

Dihydroflustramine C (**1**): crystallized from Et_2O-petroleum ether mixtures, mp 82-84°; $[\alpha]^{25}D$ −110° (c=1.5, CH_2Cl_2); uv λ max (MeOH) 213 (ξ 2×10^4), 250 (5.6×10^3), 309 (3.4×10^3) nm; ir ν max (KBr) 3200, 1600, 1590, 1475, 1350, 1150, 1050 cm^{-1}. Pmr (360 MHz) and cmr (98.2 MHz) (see Table 1); ms *m/z* (rel. int.) 322/320 (M^+ 50%) 305/307 (8%) 227/229 (15%) 251/253 (100%) 208/210 (66%) 172 (90%) 129 (40%).

ACKNOWLEDGMENTS

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