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MARINE ALKALOIDS, 15.[1] TWO ALKALOIDS, FLUSTRAMINE E AND DEBROMOFLUSTRAMINE B, FROM THE MARINE BRYOZOAN *FLUSTRA FOLIACEA*

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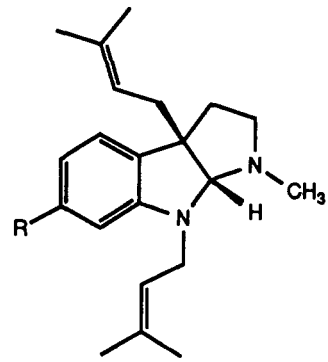
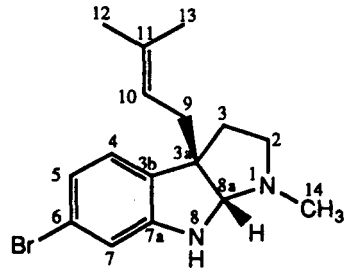
ABSTRACT.—A new alkaloid, flustramine E [**1**], with inhibitory activity towards *Rhizotonia solani* and *Botrytis cinerea* was isolated by gas-phase extraction from the marine bryozoan *Flustra foliacea*. The structure was determined as 3a,8a-*cis*-1-methyl-3a-(3-methyl-2-butenyl)-6-bromo-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole, based on spectroscopic investigations. Another alkaloid, debromoflustramine B [**2a**], was detected in trace amounts and identified by comparison of the mass spectrum with that of a synthetic sample. The extract also contained the previously reported alkaloids flustramine A and B in the ratio 1:7.

So far eleven alkaloids, ten indoles and one quinoline, have been isolated from the marine bryozoan *Flustra foliacea* (L.) (Flustridae) collected in the North Sea (2). The same species from Canadian waters has yielded five different but closely related indole alkaloids (3,4). The alkaloids from the Canadian waters showed strong activity against *Bacillus subtilis*, while those from the North Sea were devoid of such activity. We now report the presence of two alkaloids, flustramine E [**1**] and debromoflustramine B [**2a**], from a North Sea sample of *F. foliacea*. Flustramine E showed only marginal activity when tested against *B. subtilis* but was active against *Botrytis cinerea* and *Rhizotonia solani*.

Column chromatography (Si gel) of the Et_2O gas phase extract of *F. foliacea* led to the isolation of a new optically active bromo-alkaloid which was assigned the structure 3a,8a-*cis*-1-methyl-3a-(3-methyl-2-butenyl)-6-bromo-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole [**1**]. The molecular composition $C_{16}H_{21}N_2Br$ was supported by hreims; the base peak in the mass spectrum (*m/z* 253/251) corresponds to loss of an isoprene unit from the molecular ion. Further loss of 43 mass units ($CH_3-N=CH_2$) generates *m/z* 210/208, indicative of the 6-bromo-3-methylidenindoline ion, characteristic of the ms fragmentation of structurally similar indole alkaloids (2–5).



2a R=H
2b R=Br



1

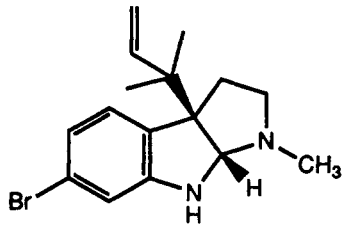
The ^1H- and ^{13}C-nmr data of **1** are presented in Table 1. Comparison of ^1H- and ^{13}C-nmr data (3) of the isomeric 8,8a-dihydroflustramine C [**3**] reveals that flustramine E differs only by a 3-methyl-2-butenyl substituent at the 3a-position.

[1]For part 14, see Nielsen *et al.* (1).

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of Flustramine E [**1**] and 8,8a-Dihydroflustramine C [**3**].

Position	Compound			
	1		**3**[d]	
	δ_C[a]	δ_H (multiplicity, J_{HH} Hz)[b]	δ_C[a]	δ_H
2	52.1	2.58 (m, 9, 9, 6) 2.66 (m, 9, 7, 4)	53.1	2.53
3	38.7	2.06 (m, 12, 9, 7) 1.90 (m, 12, 6, 4)	34.7	2.27 1.77
3a	57.3		64.0	
3b	134.1[c]		132.5	
4	124.4	6.85 (d, 7.8)	126.3	6.94
5	120.9	6.80 (dd, 1.8, 7.8)	120.8	6.76
6	121.1		121.3	
7	111.6	6.67 (d, 1.8)	111.6	6.66
7a	151.4		152.1	
8a	86.9	4.41 (s)	84.4	4.37
9	37.7	2.41 (d, overlapping)		
10	119.8	5.02 (t, 1.3)		
11	134.3[c]			
12	25.8	1.56 (s)		
13	18.1	1.66 (s)		
14	36.7	2.41 (s)		

[a]Spectra measured at 100.6 MHz.
[b]Spectra measured at 400.0 MHz
[c]Assignments may be interchanged.
[d]Ring system signals (3) are included for comparison.



3

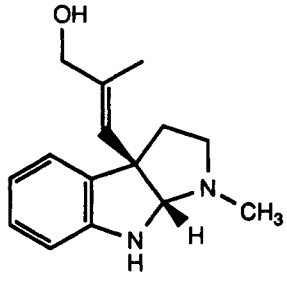
The similarity of the cd spectra of **1** and flustramine A and B [**2b**] clearly indicates the alkaloids to have the same absolute configuration, which is unknown; accordingly formulas **1** and **2b** are not intended to depict absolute configurations.

When tested against various microorganisms (in 1000 ppm, DMSO) **1** was inactive against *Pseudomonas aeruginosa, Pythium ultimum,* and *Aspergillus niger.* At the same dose **1** exhibited marginal activity against *B. subtilis* (12-mm inhibition zone), *Fusarium oxysporum* (10 mm), and *Saccharomyces cerevisiae* (14 mm), but showed no activity against these organisms at 100 ppm. In the case of *Rhizotonia solani* and *Botrytis cinerea* the zones of inhibition at 1000, 100, and 10 ppm were 10, 9, 9, and 27, 25, 21 mm, respectively. The structurally related alkaloids from *F. foliacea* collected in Canadian waters showed strong activity against *B. subtilis* while **1** only showed marginal activity. This is in accordance with the theory of adaptive variance (11). The two populations have been subjected to different ecological conditions and thus produce quantitatively different metabolites with different bioactivity.

A trace alkaloid **2a** was detected in the Et_2O extract in insufficient amounts for nmr data to be collected. The fragmentation pattern was virtually identical with that of flustramine B [**2b**], except for the characteristic bromine isotopic clusters. Reduction of flustramine B with $LiAlH_4$ gave, according to mass spectral

and nmr analysis, debromoflustramine B. The mass spectrum of debromoflustramine B was identical with that of **2a** proving that **2a** is the same compound.

The observation of both **2b** and **2a** present in the same extract suggests a mechanism where the bromine substituent is introduced in the last step in the biosynthesis of **2b**. The alkaloid **1** bears a strong structural resemblance to pseudophrynaminol [**4**] isolated from the skin of the Australian frog, *Pseudophryne coriacea* (5). Although the configuration of the ring system has not been assigned, the similarity of the cd spectra of **1** and **4** strongly indicates that both alkaloids have the same absolute configuration. It is thus conceivable that the biogenesis of the *Flustra* alkaloids is identical to that of the *Pseudophryne* alkaloid except for the last step (bromination vs. hydroxylation). This makes it interesting to speculate on the role of associated microorganisms.



4

The previously reported (6) flustramine A and B [**2b**] were also the main alkaloids in this collection. The yield of flustramine B (0.031% of dry wt) was comparable to that previously determined (0.035% of dry wt), but the yield of flustramine A was considerably smaller (0.0045% vs. 0.035% of dry wt). Several extractions were therefore performed on two other collections of *F. foliacea* available from the North Sea. These also showed different ratios of the two alkaloids. Whether these quantitative differences are caused by different ecological factors will be determined by future experiments.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Gc-ms results were obtained at 70 eV, 250 mA, 1 sec/scan at 250° on a VG 7070F instument and gc traces at 248° on a Hewlett-Packard 5890A instrument with an fid. Both gc-ms and gc were programmed from 40° (1 min) to 150° at 15° min^{-1} and from 150° to 250° (20 min) at 30° min^{-1} using a cross-linked 5% phenylmethyl silicone column (25 m × 0.2mm i.d.) and He as the carrier gas. Ms were obtained on a JEOL JMS-HX110A tandem mass spectrometer. High-resolution ms data were obtained by peak matching. Nmr spectra (including HETCOR and COSY experiments) were recorded ($CDCl_3$, relative to internal TMS) on a Varian 400 FT-nmr spectromeeter at 400.0 MHz and 100.6 MHz for ^1H- and ^{13}C-nmr spectra, respectively. The cd spectra were measured on a Jasco J-710 spectropolarimeter. The ir spectrum was obtained on a Perkin-Elmer 1760 Ft-ir spectrometer and the uv spectrum on a Hewlett-Packard 8452A diode array spectrophotometer. Tlc was performed on Si gel 60 F_{254} Merck plates with petroleum ether-EtOAc (1:1) as solvent.

BIOLOGICAL MATERIAL.—The bryozoan, *Flustra foliacea* (L.) was collected in the North Sea near Harboøre Tange, on the Danish west coast in April 1993, and kept frozen until used. A voucher sample is retained in our laboratory.

EXTRACTION AND ISOLATION.—Frozen *Flustra foliacea* (ca. 6 kg wet wt) was extracted portionwise (10×600 g) with Et_2O (10×15 ml) for 4 h using a Likens and Nickerson apparatus modified as described previously (7). The combined extracts (150 ml) were dried ($MgSO_4$) and concentrated by purging with N_2 to give an oil (ca. 1 g). This was subjected to cc (Si gel, Lobar Merck size B). The column was sequentially eluted with 0.5% EtOAc in petroleum ether (725 ml), EtOAc (610 ml), and EtOH (350 ml), resulting in the separation and isolation of the pure alkaloids: [**1**] (56 mg, 0.0060% of dry wt) obtained as a brown oil, flustramine A (42.6 mg, 0.0045% of dry wt), and **2b** (289.8 mg, 0.031%).

Flustramine B [**2b**].—Cd λ ext (c=0.0039, EtOH) nm, (Δε) 259 (−3.75), 313 (−7.51); $[\alpha]^{20}_D$ −511° (c=0.0039, EtOH).

Flustramine E [**1**].—Ir (KBr) ν max 3400–3200, 2964, 2929, 1602, 1484, 1446 cm^{-1}; uv (EtOH) λ max (log ε) 308 (6.59), 250 (6.82), 210 (7.47) nm; cd λ ext (c=0.0088, EtOH) nm, (Δε) 210 (1.32), 250 (−0.55), 305 (−0.17); $[\alpha]^{20}_D$ −1136° (c=0.0088, EtOH); eims *m/z* $[M]^+$ 322/320 (80), 307/305 (20), 279/277 (10), 265/263

(32), 253/251 (100), 210/208 (33), 172 (37), 129 (14), 69 (21); hreims, found *m/z* 322.0869; calcd for $C_{16}H_{21}N_2Br$ 322.0868; for ^1H- and ^{13}C-nmr data, see Table 1.

Debromoflustramine B [**2a**].—A solution of 8.3 mg (0.021 mmol) **2b** (8) in 1 ml of dry Et_2O was added to a stirred suspension of 5.4 mg (0.142 mmol) $LiAlH_4$ in 1 ml dry Et_2O. Stirring was continued for 1 h at room temperature. The reaction mixture was diluted with H_2O (5 ml) and extracted with Et_2O (5×5 ml). The dried Et_2O extract upon evaporation gave **2a**, 5.45 mg (66%); eims *m/z* $[M]^+$ 310 (60), 241 (100), 210 (50), 198 (60), 173 (65), 130 (57); ^1H- and ^{13}C-nmr data were identical with those of a synthetic sample; cd λ ext (*c*=0.0046, $CHCl_3$) nm, (Δε) 257 (−1.89), 308 (−2.05); $[\alpha]^{20}D$ −98.2° (*c*=0.0153, $CHCl_3$).

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