Marine Alkaloids. 11. Flustramide B and Flustrarine B from the Marine Bryozoan *Flustra foliacea*. Synthesis of Flustrarine B

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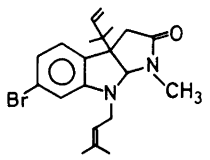
Two bromosubstituted indole alkaloids, flustramide B and flustrarine B, were isolated from the marine bryozoan *Flustra foliacea* (L.). The structural determinations result from spectroscopic studies. Flustrarine B was synthesized by oxidation of flustramine B.

So far, ten alkaloids have been isolated from the marine bryozoan *Flustra foliacea* (L.).[1] Except for one, 7-bromo-4-(2-ethoxyethyl)quinoline, they were all formally derived from a 6-bromotryptamine skeleton. We now report the isolation and structural elucidation of two new alkaloids derived from 6-bromotryptamine, flustramide B and flustrarine B, and the synthesis of the latter from flustramine B.



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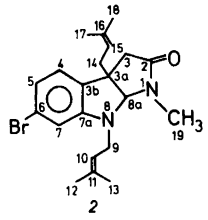

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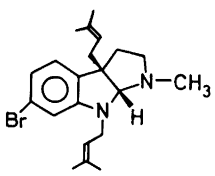
Results and discussion

One of the minor alkaloids isolated (~5–6×10^{-5} % of dry weight), named flustramide B, had spectroscopic properties comparable to those recorded for flustramide A (*1*).[2] The molecular ion (*m/z* 404/402) gave rise to *m/z* 267/265 by loss of two isoprene units in close analogy to the findings for *1*. A strong IR absorption band at 1700 cm^{-1} (KBr) suggested the presence of a 5-membered lactam. Comparison of the ^1H NMR spectra of flustramide A (*1*) and B (Table 1) in combination with the data mentioned above left little doubt that flustramide A (*1*) differed from B only in the structure of the isoprene group at position 3a. We therefore concluded that flustramide B is 1-methyl-2-oxo-3a,8-bis(3-methyl-2-butenyl)-6-bromo-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*] indole (*2*). Flustramide B (*2*) was optically active ($[\alpha]^{20}_{589}$ −53.79°, 1.45 mg/0.6 ml EtOH); the absolute stereochemistry was not determined.

The other alkaloid, flustrarine B, isolated in 4.4×10^{-3} % of dry weight had spectroscopic properties reminiscent of those found for flustramine B (*3*).[3] The mass spectrum was indicative of a structure where flustramine has acquired an additonal oxygen atom (M^+ *m/z* 406/404). Loss of M^+-16, M^+-17, or M^+-18, as might be expected for an *N*-oxide, was not observed. Instead the



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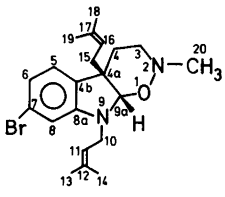

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Table 1. ^{1}H NMR data of flustramide A and flustramide B[a].

Position	Flustramide A δ/ppm	$J_{H/H}$ Hz	Flustramide B δ/ppm	$J_{H/H}$ Hz
3	2.61 (2H,s)		2.63 (2H,s)	
4	6.94 (1H,d)	$^3J_{H(C-4)/H(C-5)}=7.8$	6.85 (1H,d)	
		$^3J_{H(C-5)/H(C-4)}=7.8$		
5	6.80 (1H,dd)		6.84(1H,dd)	$^4J_{H(C-5)/H(C-7)}=1.5$
		$^4J_{H(C-5)/H(C-7)}=1.5$		
7	6.56 (1H,d)	$^4J_{H(C-7)/H(C-5)}=1.5$	6.60 (1H,d)	$^4J_{H(C-7)/H(C-5)}=1.5$
8a	4.83 (1H,s)		4.72 (1H,s)	
9	3.93 (2H,d)	$^3J_{H(C-9)/H(C-10)}=6.6$	3.92 (2H,t)	$^3J_{H(C-9)/H(C-10)}=6.6$
10	5.14 (1H,m)	$^3J_{H(C-10)/H(C-9)}=6.6$	5.18 (1H,t)	$^3J_{H(C-10)/H(C-9)}=6.6$
12	1.76 (3H,s)		1.70 (3H,s)*	
13	1.76 (3H,s)		1.74 (3H,s)*	
14			2.34 (2H,d)	$^3J_{H(C-14)/H(C-15)}=6.9$
15	0.94* (3H,s)		4.95 (1H,t)	$^3J_{H(C-15)/H(C-14)}=6.9$
16	1.03* (3H,s)			
17	5.8 (1H,m)		1.55 (3H,s)	
18	5.1 (2H,m)		1.55 (3H,s)	
19	2.85 (3H,s)		2.87 (3H,s)	

[a]Spectra measured at 90 MHz (flustramide A) and 270 MHz (flustramide B) in $CDCl_3$ relative to internal TMS δ 0.00 ppm. Assignments for values marked with an asterisk may be interchanged.

fragmentation of the molecular ion gave rise to *m/z* 347/345 (M^+-C_2H_5NO) and *m/z* 346/344 (M^+-C_2H_6NO) in close analogy to the findings reported for geneserine.[4] The ^{1}H NMR spectrum (Table 2) accounted for all the protons of the flustramine B (*3*) skeleton, thus excluding the presence of a hydroxyl group. Consequently, we assigned the structure 2-methyl-4a,9-bis(3-methyl-2-butenyl)-7-bromo-3,4,4a,9a-tetrahydro-2*H*-1,2-oxazino[5,6-*b*]indole (*4*) to flustrarine B. This assignment was supported by ^{13}C NMR (Table 2).

The position of the bromo substituent and, hence, the assignment of the proton resonance originating from H-8 were clarified by ^{1}H-[^{1}H] nuclear Overhauser enhancement (NOE) difference spectroscopy. Irradiation of the signal originating from H-10 (δ 3.79 ppm) gave rise to a 23 % enhancement of the signal at δ 6.59 ppm identifying this signal as originating from H-8. In the same experiment, enhancements of 14 %, 4.5 %, and 3 % were observed for the signals appearing at δ 5.28, 4.87, and 1.75 ppm, respectively, attesting to the spatial closeness of H-11, H-9a, and H-13(14) to H-10. Irradiation at δ 4.87 ppm resulted in a 1.5 % enhancement of 2.09 (H-15) demonstrating the *cis* junction of the indoline and tetrahydro-1,2-oxazino ring systems.

Flustrarine B was optically active ($[\alpha]_{589}^{20}$ −180.0°, 4.7 mg/ml in EtOH) but only the relative stereochemistry was determined. These findings closely parallel those reported for the physostigmine alkaloid, geneserine (*5*)[5], and reveal the same relationship of flustrarine B to the flustramine family as geneserine to the physostigmine family of alkaloids.

The synthesis of flustrarine B (*4*) was affected in 88.6 % yield by oxidation of flustramine B (*3*) with hydrogen peroxide in acetone. The synthetic product exhibited spectroscopic properties identical to those of natural *4* except for a somewhat lower specific optical rotation ($[\alpha]_{589}^{20}$ −113.2°, EtOH, c = 10.4 mg/ml) reflecting presumably a cumulative error from handling these small amounts and the uncertainty of the effect of trace impurities. We can not exclude the possibility, however, that part of either the starting material

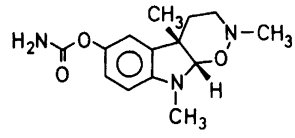

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Table 2. NMR data of flustrarine B.

Position	^{13}C δ/ppm[a]	^{1}H δ/ppm/J/Hz[b]
3	53.8	2.36 (1H(a),m) 2.71(1H(e),ddd) $^{2}J_{H(C-3)/H(C-3)}$=12.0; $^{3}J_{H(C-3)/H(C-4)}$=3.3
4	29.3*	2.07 (2H,m)
4a	44.9	
4b	133.0+	
5	123.6	6.78 (1H,d) $^{3}J_{H(C-5)/H(C-6)}$=7.6
6	120.1°	6.82 (1H,dd) $^{3}J_{H(C-6)/H(C-5)}$=7.6
7	121.2	$^{4}J_{H(C-6)/H(C-8)}$=1.5
8	109.8	6.59 (1H,d) $^{4}J_{H(C-8)/H(C-6)}$=1.5
8a	151.5	
9a	97.7	4.87 (1H,s)
10	41.9	3.78 (2H,m)
11	120.1°	5.28 (1H,t) $^{3}J_{H(C-11)/H(C-10)}$=7.1
12	134.6+	
13	25.8+	1.75 (3H,s)*
14	17.9*f	1.76 (3H,s)*
15	37.0*	2.07 (2H,m)
16	119.0°	5.10 (1H,t) $^{3}J_{H(C-16)/H(C-15)}$=7.8
17	135.7+	
18	25.9+	1.68 (3H,s)+
19	17.8*	1.40 (3H,s)+
20	46.1	2.49 (3H,s)

[a]Spectra measured at 22.63 MHz in $CDCl_3$.
[b]Spectra measured at 270 MHz in $CDCl_3$.
[c]Assignments for values marked with the same symbols may be interchanged.

or the product had undergone racemization during the synthesis and subsequent purification.

Experimental

The optical rotations were recorded on a Perkin-Elmer 141 polarimeter. For other spectroscopic equipment used see Ref. 3.

Isolation of flustramide B. Lyophilized *Flustra foliacea* (4.48 kg) was extracted at room temperature with, successively, twice distilled petroleum ether (b.p. 60–80 °C) and twice distilled ethyl acetate. The latter extract gave after evaporation 4.27 g (0.095 %) solid material. Repeated column chromatography (silica gel A 60, Lobar size C, Merck, eluent ethyl acetate) of 2.48 g of the ethyl acetate extract monitored by UV absorption (279 nm) gave seven fractions. One of the most polar fractions had a high bromine content (17.88 % Br) and was subjected to HPLC separation (silica gel, Hibar, 7 μ, Merck, in 99 % ethyl acetate/1 % ethanol) yielding 1.45 mg flustramide B as a colorless oil.

Mass spectrum: *m/z* (%); 404/402 (10) M^+, 335/333 (6), 278/276 (8), 267/265 (22), 253/251 (10), 210/208 (14),172 (8), 129 (8), 69 (100). UV, λ_{max}, nm, EtOH (log ε), 216 (2.36), 260 (3.89), 310 (3.55). IR (KBr), cm^{-1}; 1492 m, 1605 m, 1700 s, 5865 w, 2935 s, 2970 w. Optical rotation; $[\alpha]^{20}_{589}$ −180.0°, $[\alpha]^{20}_{578}$ −195.0°, $[\alpha]^{20}_{546}$ −225.0°, $[\alpha]^{20}_{436}$ −490.7°, $[\alpha]^{20}_{365}$ −310.7°.

Isolation of flustrarine B. The petroleum ether extract mentioned above was subjected to repeated chromatographic separation (silica gel, Lobar Si-60, Merck, size C and B, eluent ethyl acetate and ethyl acetate/methylene chloride, 1/3) followed by HPLC separation (Hibar Si-60, $d = 10$ mm, Merck, hexane/ethyl acetate 3/1). The yield was 10.2 mg (4.4×10^{-3} % of dry animal weight).

Mass spectrum: *m/z* (%); 406/404 (100) M^+, 347/345 (100 %) M^+-C_2H_5NO, 346/344 (79) M^+-C_2H_6NO, 279/277 (33), 278/276 (43), 210/208 (45). UV, λ_{max}, nm, EtOH (log ε); 216 (4.34), 255 (3.59), 304 (3.28). IR (KBr), cm^{-1}; 800 m, 992 m, 1378 m, 1440 m, 1480 s, 1582 m, 1605 s, 2855 m, 2928 s.

Flustrarine B was synthesized by oxidation[6] of flustramine B obtained from *F. foliacea.*[3] A solution of hydrogen peroxide (0.25 ml 3 % diluted with 0.6 ml water followed by neutralization with calcium and filtration) was added to a solution of flustramine B (*3*) (80.0 mg) dissolved in acetone (1 ml) and stirred at room temperature for 10 days, at which time, TLC (silica gel 60 F_{254}, Merck, eluent ethyl acetate) failed to show any *3* in the reaction mixture. After extraction with ether (4×10 ml), evaporation of the ether phase left 73.5 mg (88.6 %) flustrarine B. The identity of the product was inferred from the ^{1}H NMR data (largest deviation 0.02 ppm) and ^{13}C data (largest deviation 0.225 ppm).

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