Isolation and Structure Elucidation of Deformylflustrabromine from the North Sea Bryozoan *Flustra foliacea*

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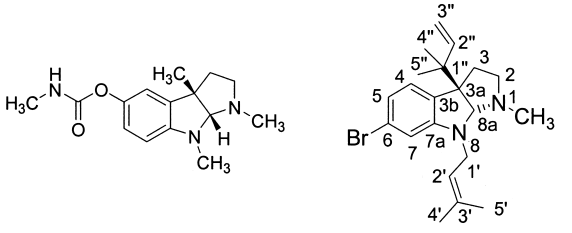
Bromoindole, Bryozoan, Inverse Prenylation

The brominated pyrrolo[2,3-b]indole deformylflustrabromine was isolated as a new natural product from the bryozoan *Flustra foliacea*, collected in the North Sea. Deformylflustrabromine appears to be the missing link in the biosynthetic sequence from flustrabromine to flustraminol A. Flustramines A, D, and dihydroflustramine C were determined as other major constituents of the investigated sample. Deformylflustrabromine is cytotoxic against the human colon cancer cell line HCT-116 (IC_{50} 5.8 μM).

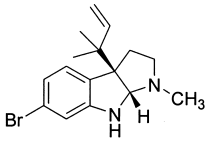
Introduction

The bryozoan *Flustra foliacea* (Flustridae) is the source of unique brominated pyrrolo[2,3-b]indoles sharing their condensed heterocyclic system with the potent acetylcholine esterase inhibitor physostigmine (**1**; Fig. 1), a plant secondary metabolite being a candidate for the treatment of Alzheimer's disease (Witkop, 1998). More than 15 tryptophan-derived alkaloids have been so far isolated from *Flustra* (Holst *et al.*, 1994, and references cited therein). Searching for possible biosynthetic intermediates of the *Flustra* secondary metabolites we re-investigated the organism. In a specimen collected in the southern North Sea, we detected a new monobrominated secondary metabolite with a relative mass of 320/322 which may represent the missing link towards flustraminol A (**8**, Fig. 3; Carlé and Christophersen, 1981).

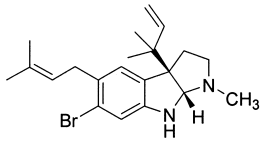


1: physostigmine

2: flustramine A



3: dihydroflustramine C



4: flustramine D

Fig. 1. The potent acetylcholine esterase inhibitor physostigmine (**1**) and three known bromoindole alkaloids (**2**, **3**, **4**) re-isolated from the bryozoan *Flustra foliacea.*

Experimental Section

General

Column chromatography was carried out on Sephadex LH-20 (Pharmacia) and on silica gel (particle size 230–400 mesh, Merck). Thin-layer chromatography (TLC) was performed on silica gel (precoated silica gel plate F_{254} Merck). Preparative HPLC separation columns (25 × 250 mm) were prefilled with LiChroprep RP-8 (25–40 μm, Merck) or LiChroprep Si-60 (25–40 μm, Merck). The peaks were detected at 254 nm. NMR spectra were recorded on Bruker WM 250, WM 300, AM 360 or Varian INOVA-400 spectrometers. The NMR shifts were calibrated using the NMR solvent as internal reference. All infrared spectra were recorded on a Perkin Elmer 1600 series

FT-IR spectrometer. The UV/Vis-spectra were recorded using a Hewlett-Packard UV-spectrometer HP 8452 Diode Array System. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-700 mass spectrometer with nitrobenzyl alcohol as matrix. The optical rotation was recorded using a Perkin-Elmer 241 A polarimeter with a 10 cm cell.

Collection

The material was collected with an otter trawl from the sea floor along the margin of the deep Helgoland trench in the southeastern North Sea at about 54 °12′ N and 7 °47′ E and a water depth of 33 m–45 m during the expedition no. 116 of RV "Heincke" on 12 February 1999. When the haul was on board, *Flustra foliacea* was selected from the catch, washed with sea water and deep-frozen in a PE bag.

Isolation

The bryozoan *Flustra foliacea* (82.5 g, fresh weight) was lyophilized and extracted with $MeOH/CH_2Cl_2$ (1:1 v/v, 750 ml, 3 times). After concentration the crude extract (13.9 g) was partitioned between isooctane and MeOH. The MeOH phase was washed with isooctane (3 times) and concentrated. The residue was partitioned between *n*-BuOH and water. The *n*-BuOH phase was washed with water and the residue (4.08 g) was fractioned by gel chromatography (Sephadex LH-20, MeOH).

Flustramine A (**2**)

Obtained from fraction 4 of the Sephadex LH-20 column via flash chromatography (silica, gradient $CHCl_3$ to $CHCl_3$/MeOH 70:30 v/v). 16 mg (0.02% of the dry weight); ^{1}H NMR (250 MHz, [D_1]chloroform): δ = 6.90 (d, *J* = 8.5 Hz, 1H, 4-H), 6.69 (dd, *J* = 1.5, 8.5 Hz, 1H, 5-H), 6.48 (d, *J* = 1.5 Hz, 1H, 7-H), 5.94 (dd, *J* = 10.5, 17.0 Hz, 1H, 2″-H), 5.22 (t, *J* = 6.3 Hz, 1H, 2′-H), 5.06 (dd, *J* = 1.5, 10.5 Hz, 1H, 3″-H_Z), 4.98 (dd, *J* = 1.5, 17 Hz, 1H, 3″-H_E), 4.35 (s, 1H, 8a-H), 3.84 (d, *J* = 6.3 Hz, 2H, 1′-H), 2.67 (m, 2H, 2-H), 2.42 (s, 3H, NCH_3), 2.22 (m, 2H, 3-H), 1.73 (s, 6H, 4′-H, 5′-H), 1.01 (s, 3H, 4″-H), 0.94 (s, 3H, 5″-H); ^{13}C NMR (90.6 MHz, [D_1]chloroform): δ = 153.6 (C-7a), 144.9 (C-2″), 134.6 (C-3′), 132.6 (C-3b), 125.8 (C-4), 121.7 (C-6), 120.9 (C-2′), 119.1 (C-5), 113.1 (C-3″), 109.3 (C-7), 89.3 (C-8a), 63.4 (C-3a), 53.2 (C-2), 45.9 (C-1′), 41.3 (C-1″), 37.8 (NCH_3), 34.4 (C-3), 25.6 (C-4′), 23.5 (C-4″), 22.3 (C-5″), 18.1 (C-5′); IR (NaCl): $\tilde{\nu}$ = 2965, 2972, 2855, 1674, 1594, 1487 cm^{-1}; UV (EtOH): λ_{max} (ε) = 214 (18248), 262 (6626), 316 nm (2645 mol^{-1} · cm^{-1} ·l); $[\alpha]_D^{20}$ = – 76.92 (*c* = 6.6 mM in EtOH); FABMS *m/z* (%) = 389/391 (49/40) [M^+], 319/321 (100/98); HRFABMS calcd. for $C_{21}H_{30}{}^{81}BrN_2$ 391.1572, found 391.1579.

Deformylflustrabromine (**5**)

Fraction 5 of the Sephadex column was purified by HPLC (flow 11.5 ml · min^{-1}; RP-8, H_2O/MeOH/HOAc (50:50:0.1 v/v/v, t_R 10.06 min)); then (flow 11.5 ml · min^{-1}; silica, gradient $CHCl_3$ to $CHCl_3/CH_3OH$ (70:30, over 30 min, t_R 25.00 min)). 59 mg (0.072% of the dry weight); ^{1}H NMR (360 MHz, [D_1]chloroform/[D_4]methanol (7:3 v/v)): δ = 7.53 (s, 1H, 7-H), 7.45 (d, *J* = 8.3 Hz, 1H, 4-H), 7.15 (d, *J* = 8.3 Hz, 1H, 5-H), 6.15 (dd, *J* = 17.3, 10.4 Hz, 1H, 2″-H), 5.17 (d, *J* = 17.3 Hz, 1H, 3″-H_E), 5.16 (d, *J* = 10.4 Hz, 1H, 3″-H_Z), 3.23 (m, 2H, 1′-H), 3.08 (m, 2H, 2′-H), 2.71 (s, 3H, NCH_3), 1.53 (s, 6H, 4″,5″-H); ^{13}C NMR (90.6 MHz, [D_6]DMSO): δ = 145.5 (C-2″), 141.8 (C-2), 135.5 (C-7a), 127.6 (C-3a), 121.2 (C-5), 119.4 (C-4), 113.5 (C-6), 113.4 (C-7), 111.6 (C-3″), 104.9 (C-3), 48.5 (C-2′), 45.4 (NCH_3), 38.7 (C-1″), 27.6 (C-4″, C-5″), 21.6 (C-1′); IR (NaCl): $\tilde{\nu}$ = 3279, 2978, 2760, 2433, 1710, 1591, 1466 cm^{-1}; UV (MeOH): λ_{max} (ε) = 204 (30190), 230 (22731), 282 nm (4768 mol^{-1} · cm^{-1} · l); FABMS *m/z* (rel. intensity): 321/323 (100/96) [M^+]; HRFABMS calcd. for $C_{16}H_{22}{}^{79}BrN_2$ 321.0966, found 321.0974.

Results and Discussion

The purification protocol stepwise applied solvent partitioning, gel gravity chromatography (Sephadex LH-20) and preparative HPLC using RP-8 and silica stationary phases. For a graphical representation of the isolation procedure see Fig. 2. The molecular formula of the new compound **5** was determined as $C_{16}H_{22}BrN_2$ by HRFABMS. On the basis of connectivity information derived from HSQC, HMBC, and COSY NMR experiments, the computer program COCON (Lindel *et al.*, 1999; Köck *et al.*, 1999) calculated

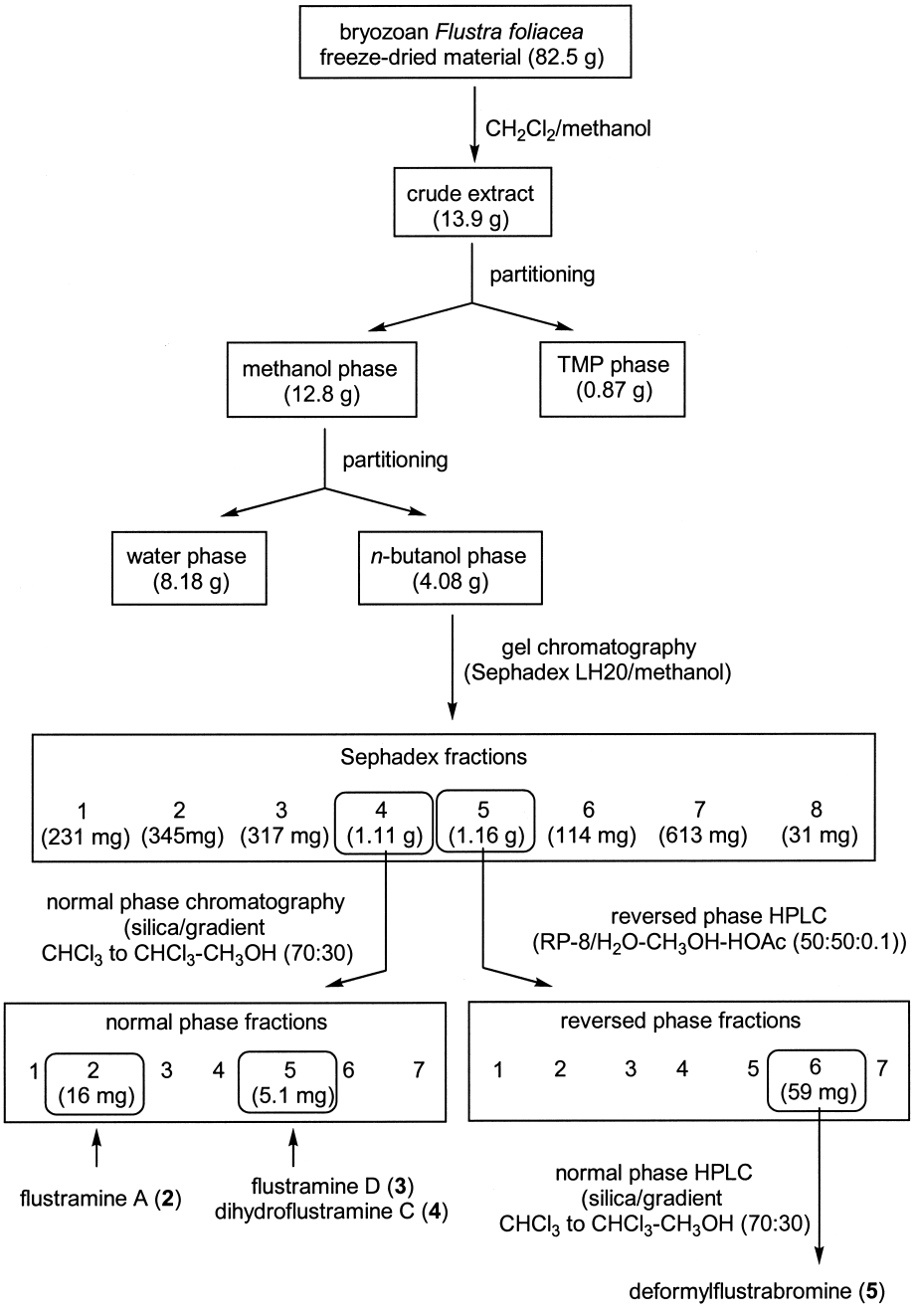


Fig. 2. Isolation protocol leading to the new natural product deformylflustrabromine (**5**) and the known metabolites flustramine A (**2**), dihydroflustramine C (**3**) and flustramine D (**4**).

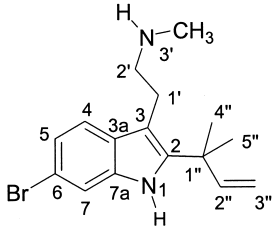
two constitutions fulfilling all required constraints. Due to the absence of experimental HMBC correlations of the methyl protons in [D_6]DMSO (see Table I), an alternative constitution was generated in which the bromo and methylamino substituents have changed places. An increment calculation for C-6 of the indole ring and biosynthetic considerations strongly favor constitution **5**. The ^{1}H-NMR-spectrum of **5** shows the patterns of a 6-substituted indole. In addition to the new compound **5**, the known brominated pyrrolo[2,3-b]indoles flustramine A (**2**; Carlé and Christophersen, 1979, 1980),

dihydroflustramine C (**3**; Wright, 1984; Laycock *et al.*, 1986), and flustramine D (**4**; Laycock *et al.*, 1986) were isolated. Very recently, the isolation of **5** was independently described by Peters *et al.* (2002).

Given the close relationship of the new compound **5** to flustrabromine (**7**; Wulff *et al.*, 1981), **5** should be named deformylflustrabromine. Deformylflustrabromine (**5**) is no artefact, because the ^{1}H-NMR-signals of this major secondary metabolite were clearly visible in the *n*-butanol phase of the investigated specimen of *Flustra foliacea*, before any step of chromatography. There was no signal of any *N*-formyl proton (to be expected at about δ 8.0 in the ^{1}H-NMR-spectrum) present at any stage of the extraction. The content of deformylflustrabromine (0.072% of the dry weight) compares to that of other constituents, in particular of flustrabromine (**7**; Wulff *et al.*, 1981, 1982), 6-bromoformyltryptamine (**6**; Wulff *et al.*, 1982), and flustramine A (**2**; *e.g.*, 0.035% of the dry weight, Carlé and Christophersen, 1980).

The inverse prenylation of the 2-position of indole alkaloids is under intense investigation (for a review, see Williams *et al.*, 2000). From the lack of stereospecifity observed in the construction of the quaternary center at the indole 2-position, Stocking *et al.* (1999, 2000) concluded that the olefinic π-system of dimethylallylpyrophosphate is introduced by a "reverse" prenyl transferase presenting both faces of the π-system to the 2-position of the indole. In all of the investigated cases, the α-amino function of the tryptophan unit was acylated, *e.g.*, as a diketopiperazine.

In the secondary metabolism of *Flustra*, a formyl group may play the analogous role. Flustrabromine (**7**; isolated as a mixture of *E*-/*Z*-isomers) would be formed from 6-bromo-N_b-methyl-N_b-formyltryptamine (**6**) via inverse prenylation. Both **6** and **7** have been isolated as natural products from *Flustra foliacea* (Wulff *et al.*, 1982). In the continued sequence from flustrabromine (**7**) to the natural product flustraminol A (**8**; Carlé and Christophersen, 1981), the new metabolite defor-



5: deformylflustrabromine

Table I. NMR spectroscopical data of deformylflustrabromine (**5**).

Position	^{1}H NMR[a]	^{13}C NMR[b]	HMBC correlations[c]
2	–	141.8	
3	–	104.9	
3a	–	127.6	
4	7.45 (1H, d, 8.3 Hz)	119.4	3, 3a, 6, 7a
5	7.15 (1H, d, 8.3 Hz)	121.2	7
6	–	113.5	
7	7.53 (1H, s)	113.4	3a, 5, 6
7a	–	135.5	
1′	3.23 (2H, m)	21.6	2, 3, 3a
2′	3.08 (2H, m)	48.5	
1″	–	38.7	
2″	6.15; dd; 17.3, 10.4	145.5	2, 1″, 4″(5″)
3″	5.17 (1H, d, 17.3 Hz; *E*); 5.16 (1H, d, 10.4 Hz; *Z*)	111.6	1″, 2″
4″	1.53 (3H, s)	27.6	2, 1″, 2″, 5″
5″	1.53 (3H, s)	27.6	2, 1″, 2″, 4″
NCH_3	2.71 (3H, s)	45.4	

[a] 360 MHz ([D_1]chloroform/[D_4]methanol (7:3)). [b] 90.6 MHz ([D_6]DMSO). [c] Position numbers are given.

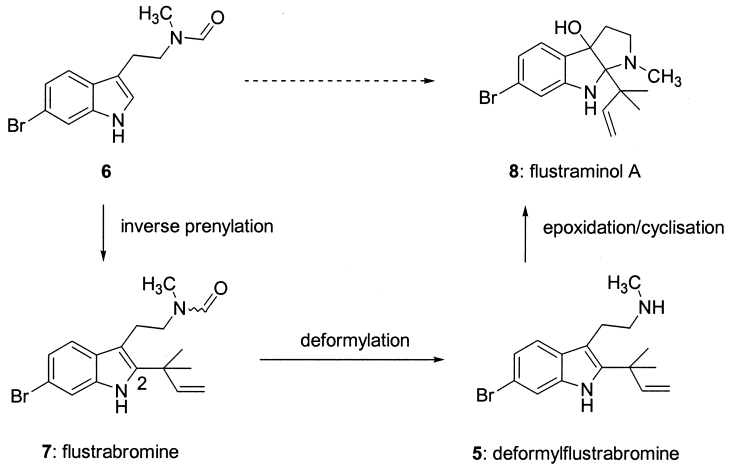


Fig. 3. Deformylflustrabromine (**5**) as a possible missing link in the biosynthesis of flustraminol A (**8**), starting from the natural product **6**.

mylflustrabromine (**5**) may be the missing link. Epoxidation of **5**, followed by ring opening of the epoxide ring would lead to flustraminol A (**8**), as outlined in Fig. 3. Indeed, a biomimetic cyclisation was recently induced by treatment of *N*-Boc-tryptophan methyl ester, inversely prenylated at the 2-position of the indole ring, with dimethyldioxirane (Schkeryantz *et al.*, 1999). The stereochemistry of the natural product **8** was not determined.

While the crude extract of *Flustra foliacea* was not cytotoxic, the purified compounds showed activity against the human colon cancer cell line HCT-116. The new natural product deformylflustrabromine (**5**) showed the highest, but still moderate, cytotoxicity of 1.87 $\mu g \cdot ml^{-1}$ (5.8 μM, IC_{50}, HCT-116). Flustramine A (**2**), D (**4**), and dihydroflustramine C (**3**) were weakly cytotoxic in the range of 10 $\mu g \cdot ml^{-1}$ (26 μM, IC_{50}).

Acknowledgements

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