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

Nicola Lysek^a^, Eike Rachor^b^ and Thomas Lindel^c^*

^a^ Pharmazeutisch-chemisches Institut der Universität, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany
^b^ Alfred-Wegener-Institut für Polar- und Meeresforschung, Columbusstraße, D-27568 Bremerhaven
^c^ Department Chemie der Universität, Butenandtstr. 5–13, D-81377 München, Germany.
Fax: (+49)89/21 80-77 34. E-mail: thomas.lindel@cup.uni-muenchen.de

* Author for correspondence and reprint requests

Z. Naturforsch. **57c**, 1056–1061 (2002); received February 6, 2002

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 (IC₅₀ 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).

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 F₂₅₄ 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

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![Fig. 1.](image-url) Fig. 1. The potent acetylcholine esterase inhibitor physostigmine (1) and three known bromoindole alkaloids (2, 3, 4) re-isolated from the bryozoan *Flustra foliacea.*
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/CH2Cl2 (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 CHCl3 to CHCl3/MeOH 70:30 v/v), 16 mg (0.02% of the dry weight); 1H NMR (250 MHz, [D1]chloroform): $\delta = 6.90$ (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), 4.98 (dd, $J = 1.5, 17$ Hz, 1H, 3''-H), 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, NCH3), 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’m-H); 13C NMR (90.6 MHz, [D1]chloroform): $\delta = 153.6$ (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 (NCH3), 34.4 (C-3), 25.6 (C-4’), 23.5 (C-4’’), 22.3 (C-5’'), 18.1 (C-5’); IR (NaCl): $\bar{\nu} = 2965$, 2972, 2855, 1674, 1594, 1487 cm$^{-1}$; UV (EtOH): $\lambda_{max}$ ($\epsilon$) = 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$_{30}$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$_2$O/MeOH/HOAc (50:50:0.1 v/v/v, $t_R$ 10.06 min); then (flow 11.5 ml·min$^{-1}$; silica, gradient CHCl3 to CHCl3/CH3OH (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)): $\delta = 7.53$ (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 (dd, $J = 10.4$ 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$_4$]DMSO): $\delta = 145.5$ (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): $\bar{\nu} = 3279$, 2978, 2760, 2433, 1710, 1591, 1466 cm$^{-1}$; UV (MeOH): $\lambda_{max}$ ($\epsilon$) = 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$_{22}$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
two constitutions fulfilling all required constraints. Due to the absence of experimental HMBC correlations of the methyl protons in [D₆]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 ¹H-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),

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).
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 1H-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 1H-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 stereospecificity 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-Nb-methyl-Nb-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-
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 µg·ml⁻¹ (5.8 µM, IC₅₀, HCT-116). Flustramine A (2), D (4), and dihydroflustramine C (3) were weakly cytotoxic in the range of 10 µg·ml⁻¹ (26 µM, IC₅₀).

Acknowledgements

This work was funded in part by the Bundesministerium für Bildung und Forschung (BMBF grant V-258, cooperation with the BASF AG, Ludwigshafen). We are grateful to Tanja Mülhaupt and Prof. Dr. William H. Fenical (Scripps Institution of Oceanography, San Diego, USA) for performing the cytotoxicity assays.


