Marine Alkaloids. Part 4.† A Formamide, Flustrabromine, from the Marine Bryozoan *Flustra foliacea*

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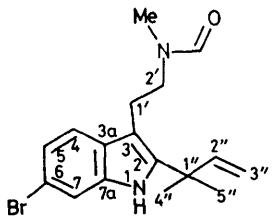
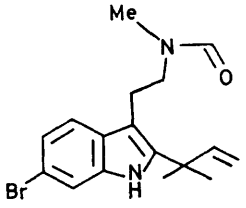
The isolation and structure elucidation of a marine alkaloid from the bryozoan *Flustra foliacea* is described. The alkaloid, 6-bromo-2-(1,1-dimethylallyl)-N^b-formyl-N^b-methyltryptamine (1), has been shown to consist of two rotameric forms reflecting hindered rotation around the carbon–nitrogen bond of the formamide function. Furthermore, each rotamer exists in equilibrium with an intramolecularly associated form where the amide nitrogen is associated with the aromatic system.

DURING an investigation of the tricyclic bromo-alkaloids, the flustramines, from the marine bryozoan *Flustra foliacea* (L.),[1-3] we became interested in unravelling the limits of structural diversity of this new class of compounds. We now wish to report the isolation and structure determination of a unique bicyclic 6-bromotryptamine derivative flustrabromine (1), 6-bromo-2-(1,1-dimethylallyl)-N^b-formyl-N^b-methyltryptamine,



(*E*)-(1a)



(*Z*)-(1b)

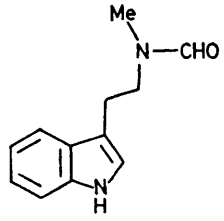
Flustrabromine (1)

which occurs as two rotational isomers as evidenced by n.m.r. analysis.

RESULTS AND DISCUSSION

Isolation and Structure Determination.—From the light petroleum extract of lyophilized *Flustra foliacea*, flustramine A, B, and C, and flustraminol A and B, were isolated.[1-3] The methylene chloride extract (1.14% based on dry weight) was subjected to cellulose column chromatography with toluene as eluant and the least polar fraction collected. Reverse-phase (RP8, Merck) column chromatography with ethyl acetate yielded a fraction which, after repeated silica gel chromatography with ethyl acetate and ethyl acetate–chloroform (15 : 85), gave pure flustrabromine (4.5 mg amorphous solid, 6.3 p.p.m. based on dry weight).

High-resolution mass spectrometry established the elemental composition as $C_{17}H_{21}BrN_2O$ (Found: M^+, 350.0790. Calc. for $C_{17}H_{21}{}^{81}BrN_2O$: M, 350.0818). Prominent loss of C_2H_5NO [NH(Me)CHO] and C_3H_6NO [CH_2N(Me)CHO] from the molecular ion, by analogy with the results reported for N^b-formyl-N^b-methyltryptamine (2),[4] indicated the presence of a bromo-substituted N^b-formyl-N^b-methyltryptamine. Absence of the characteristic signal from the C-2 proton in the ^{1}H n.m.r. spectrum suggested a 2-alkylated tryptamine. The u.v. spectrum ($\lambda_{max.}$ 232 and 290 nm) lent further support to the hypothesis that flustrabromine is a bromo-substituted 2,3-dialkylindole (*cf.* ibogamine,[5] $\lambda_{max.}$ 227 and 291.5 nm). The tertiary amide function, as expected, gives rise to a strong i.r. absorption band at 1 670 cm^{-1}.



(2)

Inspection of the ^{1}H and ^{13}C n.m.r. spectra revealed that the sample was a mixture of two closely related compounds, since all signals were duplicated (see Table 1). All subsequent attempts to isolate the pure compounds failed. From a detailed investigation of solvent shifts in the ^{1}H n.m.r. spectra of the components, and also model compounds, it was concluded (see below) that flustrabromine is an intimate mixture of two stereoisomeric compounds. Having reached this conclusion, the gross structure of the natural products yields to conventional spectroscopic analyses.

The structure of the 2-alkyl group reveals itself as the inverted isoprene unit (1,1-dimethylallyl) by comparison with the corresponding ^{1}H n.m.r. data of, *e.g.*, 2-(1,1-dimethylallyl)-3-methylindole [Me, 1.48(s); CH_2=, 5.10 and 5.09 (dd, J_{cis} 10, J_{trans} 17 Hz); CH=, 6.07(dd)].[6] The ^{13}C n.m.r. data favour a 6-bromosubstituted indole. The position of the bromine atom was finally secured by evidence from ^{1}H-[^{1}H] nuclear Overhauser enhancement (n.O.e.) difference spectroscopy. Alternate irradiations of resonances at δ 8.10 and 8.19 due to the indole N–H resulted in a 17% enhancement of the resonances at δ 7.45 and 7.47, thus defining these signals as originating from the proton at position 7 in the indole nucleus. Taking into account the coupling pattern of the remaining

† Part 3 is ref. 3.

TABLE 1

N.m.r. data of flustrabromine

Atom number [c]	^{13}C N.m.r.[a]	^{1}H N.m.r.[b] (*Z*)-Isomer	*J*/Hz	(*E*)-Isomer
1		8.10 (1 H, br, s)		8.19
2	Not observed			
3	107.8, 107.0 (s)			
3a	127.9, 128.4 (s)			
4	118.7,* 119.4 * (d)	7.51 (1 H, d)	$^{3}J_{4,5}$ 8.4	7.33 (1 H, d)
5	122.6,* 126.7 * (d)	7.16 (1 H, dd)	$^{4}J_{5,7}$ 1.5	7.17 (1 H, dd)
6	114.9 (s)			
7	113.3, 113.6 (d)	7.45 (1 H, d)		7.47 (1 H, d)
7a	Not observed			
N-Me	30.0, 34.9 (q)	2.93 (3 H, s)		2.91 (3 H, s)
N-CHO	162.3, 162.4 (d)	8.02 (1 H, s)		7.95 (1 H, s)
1′	22.4, 24.7 (t)	2.94—3.05 (4 H, m) [d]		2.94—3.05 (4 H, m) [d]
2′	45.3, 50.0 (t)	3.36—3.47 (4 H, m) [d]		3.36—3.47 (4 H, m) [d]
1″	38.7, 38.8 (s)			
2″	145.4, 145.3 (d)	6.15 (1 H, dd)	$^{3}J_{cis}$ 10.8, $^{3}J_{trans}$ 17.6	6.13 (1 H, dd)
3″	112.2 (t)	5.12—5.22 (4 H) [d]		5.12—5.22 (4 H, m) [d]
4″, 5″	27.5, 27.4 (q)	1.52 (6 H, s)		1.55 (6 H, s)

[a] Spectra measured at 63.89 MHz in $CDCl_3$ (11.3 mg ml^{-1}); chemical shifts are in δ relative to internal $SiMe_4$. Signal multiplicities (in parentheses) obtained from the off-resonance decoupled spectrum. [b] Spectra measured at 270 MHz in $CDCl_3$ (11.3 mg ml^{-1}); chemical shifts are given in δ relative to internal $SiMe_4$. [c] Atoms of the aminoethyl side-chain are denoted by a single prime, those of the dimethylallyl unit by a double prime. [d] These signals, appearing as multiplets, could not be separately assigned to the individual isomers; thus the intervals are given for both isomers.

* Assignments for these values may be interchanged.

aromatic protons leaves only position 6 available for the bromo-substituent. The implication of the sum of data mentioned above allows the gross structure to be formulated as 6-bromo-2-(1,1-dimethylallyl)-N^b-formyl-N^b-methyltryptamine (1).

The duplication of signals is analogous to the findings reported for N^b-acetyl-N^b-methyltryptamine, where the (*Z*) and (*E*) rotational isomers cause splitting of the C-2 proton, the N^b-acetyl, and the N^b-methyl resonances.[7] In the latter case coalescence occurs below 100 °C. A coalescence temperature of 128 ± 2 °C was observed for the N^b-methyl protons, the α-protons, and the formyl proton of the rotamers of N^b-formyl-N^a-N^b-dimethyltryptamine.[8] In contrast to these examples, flustrabromine exhibits splittings for all signals, indicating that beside the hindered rotation around the amide bond an inter- or intra-molecular association is involved as well. In order to determine the nature of the phenomenon giving rise to the duplication of all the n.m.r. resonances, a variable-temperature experiment was performed. Comparison of data recorded at 35 and 50 °C {(1) (3 mg) in [$^2H_6$]DMSO (400 μl, 270 MHz)} showed that coalescence was beginning to occur, most pronounced in the aromatic region. Further variable-temperature experiments were not carried out due to the instability of the sample.

Association between formamides and aromatic compounds is a well known phenomenon.[9] In the case of dimethylformamide and an aromatic compound, the most stable associate is thought to be the one with the nitrogen of the amide group as near to, and the oxygen as far from, the aromatic centre as possible.[9] Furthermore, on addition of an aromatic solvent to DMF, the chemical shifts of the *N*-methyl groups move upfield, but the methyl *trans* to the oxygen atoms experiences a larger upfield shift.[9] On this basis, a 270-MHz spectrum of flustrabromine (1 mg in 350 μl CD_2Cl_2 plus 150 μl C_6D_6) revealed the most abundant rotamer to be the one with the N^b-methyl group *cis* to the oxygen atom (1a), since the least intense signals of the N^b-methyl group and the most intense signals of the α-protons are found to highest field when deuteriobenzene is added, in contrast to the situation observed without deuteriobenzene. Addition of the aromatic solvent separates out the two triplets from the α-protons, which in deuteriomethylene chloride appear as an unresolved multiplet. The triplet signal of lowest intensity is considerably broader than the one with highest intensity, reflecting presumably the larger long-range coupling constant of the *trans* configuration (1b) of the methylene group and the formyl proton. Independent evidence of the correctness of this assignment was gained from ^{1}H-[^{1}H] n.O.e. difference spectroscopic measurements. Saturation of the N^b-methyl groups at δ 2.91 and 2.93 gave rise to enhancement of the formyl proton at δ 8.02 (12%), revealing the spatial proximity of the latter proton to the *N*-methyl group (1b), thus identifying the most abundant isomer as the (*E*)-rotamer (1a). The latter experiment also showed small but significant enhancements of all the aromatic protons [both (*Z*)- and (*E*)-forms] (total >2%), the effect declining from H-4 to H-7 to H-5 in good agreement with the expectation for an intramolecularly associated structure. Support for this proposition was also gained from the concentration-dependence of the resonances originating from the two isomers (see Table 2). A sevenfold change

TABLE 2

Concentration- and solvent-dependence of the chemical-shift-differences between selected resonances of the (*Z*)- and (*E*)-isomers

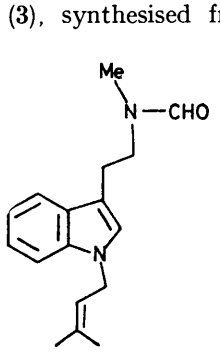
Compound	Solvent (concentration)	Δδ (N^b–Me) [a]	Δδ (N^b–CHO) [a]	Δδ (4-H) [a]
	$CDCl_3$ (7 mM)	−3.6	19.9	55.0
	$CDCl_3$ (50 mM)	−3.6	20.0	55.0
(1)	CD_2Cl_2 (32 mM)	5.0	20.5	50.0
	CD_2Cl_2–C_6D_6 (7 : 3) (6 mM)	−40.0		
(4)	$CDCl_3$ (*ca.* 500 mM)	±26.0 [b]	±90.0 [b]	±33.0 [b]
	$CDCl_3$ (*ca.* 300 mM)	−13	61.2	26.5
	$CDCl_3$–C_6D_6 (8 : 3) (*ca.* 200 mM)	−63	50.8	41.0
(3)	$(CD_3)_2CO$ (95 mM)	25.5	60.0	12.9
	$(CD_3)_2CO$ (370 mM)	24.3	59.5	13.0
	$(CD_3)_2CO$ (930 mM)	15.8	57.5	17.0
(2)	$CDCl_3$ (*ca.* 370 mM)	−13.5	83.2	24.1

[a] Δδ = δ[(*Z*)-isomer] − δ[(*E*)-isomer]. [b] The two set of signals were not unambiguously assigned between the (*Z*)- and (*E*)-isomers.

in concentration (from 7 mM to 50 mM) seemingly has no effect on the amount of association, as measured by the frequency separation between the (*Z*)- and (*E*)-rotamer signals of selected resonances, whereas solvent composition has a pronounced influence.

At higher concentrations the duplication is influenced as observed in compound (3). This may be caused by intermolecular dipolar amide–amide association,[9] and probably also by intermolecular amide–aromatic associations at higher concentrations.

The type of intramolecular association described above is presumably of general occurrence in N^b-formyl-N^b-methyltryptamine derivatives, since *e.g.* the reported ^{13}C n.m.r. data of N^b-formyl-N^b-methyltryptamine (2) show duplication for all resonances except C-5, C-7, and C-7a.[10] We have synthesised the latter compound and find all resonances duplicated except C-7a (see Table 3). The compound 1-(3-methylbut-2-enyl)-N^b-formyl-N^b-methyltryptamine (3), synthesised from (2) and the



(3)

corresponding alkenyl bromide, likewise shows duplication of resonances except for the carbon at position 6 and the alkenyl carbons (see Table 3).

Because of its instability the properties of 2-(3-methylbut-2-enyl)-N^b-formyl-N^b-methyltryptamine (4) were not investigated in detail. However the ^{13}C and ^1H n.m.r. spectra clearly shows that the effects described above are also operative in this case. Compound (4) was isolated in a series of experiments aimed at the synthesis of debromoflustrabromine. By analogy with the reported acid-catalysed rearrangement of 1-(3-methylbut-2-enyl)-3-methylindole and 1-(3-methylbut-2-enyl)-3-ethylindole to the corresponding 2-(1,1-dimethylallyl) isomers,[6] 1-(3-methylbut-2-enyl)-N^b-formyl-N^b-methyltryptamine (3) was expected to give debromoflustrabromine. However, n.m.r. and m.s. analysis of

TABLE 3

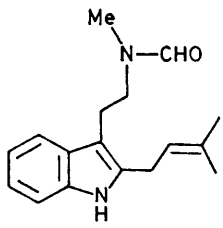
^{13}C N.m.r. data of (2) and (3)

Atom	(2)	(3) [a]
2	122.3 *, 121.8 *	125.5 *, 125.3 *
3	111.2, 110.2	111.2, 110.2
3a	126.7, 126.3	128.0, 127.6
4	117.7 †, 117.4 †	118.8 †, 118.4 †
5	118.5 †, 118.0 †	119.9 †, 119.0 †
6	121.1 *, 121.0 *	121.4 *
7	111.1, 110.9	109.8, 109.5
7a	136.0	136.4, 136.3
N-Me	34.1, 30.0	34.9, 29.6
N-CHO	162.4, 162.2	162.6, 162.4
1′ [b]	23.6, 22.1	24.6, 22.8
2′ [b]	49.4, 44.4	50.2, 45.1

*,† Assignments for these values may be interchanged.

[a] The signals from the isoprenyl group at position 1 appeared at δ 44.0 (C-1), 121.6 (C-2), 136.2 (C-3), 18.0 and 25.6 (C-4 and C-5). [b] The numbering is as explained in Table 1.

reaction mixtures only revealed the presence of 2-(3-methylbut-2-enyl)-N^b-formyl-N^b-methyltryptamine (4)



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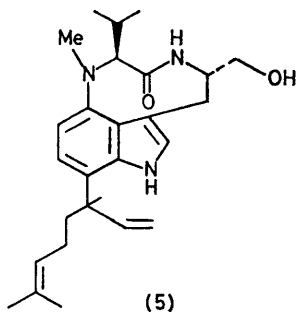

(5)

in any significant yield. It has been reported that the rearrangement of 1-(3-methylbut-2-enyl)-3-t-butylindole under the same conditions fails to give any rearranged product with an inverted isoprene group.[6]

Finally, we wish to point out the phenomenological similarity between the n.m.r. data reported here and

the corresponding data reported for the alkaloid lyngbyatoxin A (5), isolated from the marine cyanophyte *Lyngbya majuscula*.[11] The investigators of this toxin conclude that the observed doubling of ^1H and ^{13}C resonances suggest either two conformational or two positional isomers. Inspection of molecular models of lyngbyatoxin A reveals one of the rotamers [(*Z*)-configuration around the amide C–N bond] to be less strained than the other, where the amide nitrogen is closer to the aromatic ring but also the carbonyl oxygen is closer to the ring. More data are clearly needed to settle this question.

EXPERIMENTAL

Mass spectra were recorded at 70 eV on an AEI-MS 902 instrument; precise mass measurements were obtained by the peak matching method. U.v. spectra were recorded on a Unicam SP 18 instrument and i.r. spectra on a Perkin-Elmer 580 spectrometer. ^{13}C and ^1H N.m.r. spectra were recorded at 63.89 MHz and 270 MHz, respectively, on a Bruker HX 270 instrument, or at 22.5 MHz and 90 MHz on a JEOL FX 90 Q instrument. In the case of n.O.e. difference measurements, the method described in ref. 2 was used, on oxygen-free and dust-free samples. All chemical shifts are reported in δ downfield from $SiMe_4$.

Flustrabromine.—Flustrabromine was isolated as given in the text, R_F 0.37 [silica gel 60F254 (Merck) eluted with ethyl acetate]; *m/z* 348/350 (35%), 319/321 (10), 289/291 (35), 276/278 (100), and 261/263 (25); $\lambda_{max.}$ (EtOH) 232 (ε 1.8×10^4) and 290 nm (5.8×10^3).

N^b-*Formyl*-N^b-*methyltryptamine*.—*N*^{b}-Formyl-*N*^{b}-methyltryptamine was prepared from *N*^{b}-methyltryptamine[12] by treatment of the latter compound with an excess of acetic anhydride–formic acid (1 : 1) in dry ether;[13] *m/z* 202 (12%), 143 (71), 130 (100), 115 (5), 103 (10), 77 (20), and 72 (6); δ 8.30 and 8.24 (1 H, br, indole NH), 8.07 and 7.76 (1 H, s, CHO), 7.65 and 7.56 (1 H, d, 4-H), 7.39—7.10 (3 H, m, 5-, 6-, and 7-H), 7.04 and 6.95 (1 H, d, 2-H), 3.66 and 3.53 (2 H, t, $N^bCH_2CH_2$), 3.05—2.96 (2 H, m, $N^bCH_2CH_2$), 2.93 and 2.88 (3 H, s, NMe).

N^b-*Formyl*-N^b-*methyl*-1-(3-*methylbut*-2-*enyl*)*tryptamine*.—To a suspension of NaH (4.5 mmol) in DMF (2 ml) was added *N*^{b}-formyl-*N*^{b}-methyltryptamine (3 mmol) in DMF (2 ml). After hydrogen evolution had ceased γγ-dimethylallyl bromide (4.5 mmol) in DMF (2 ml) was added and the mixture stirred for 2 h in a closed flask.[6] The complex was destroyed with water and the mixture extracted with chloroform. The chloroform extract was evaporated and the residue purified by column chromatography on silica gel with ethyl acetate as eluant giving a 50% yield of *N*^{b}-formyl-*N*^{b}-methyl-1-(3-methylbut-2-enyl)tryptamine as a bright oil. Since the sample proved extremely difficult to obtain in an analytically pure state a slightly impure sample was used to obtain the spectroscopic data reported below (Found: C, 74.7; H, 8.25; N, 9.9. Calc. for $C_{17}H_{22}N_2O$: C, 75.52; H, 8.20; N, 10.35%); *m/z* 270 (35%), 211 (40), 198 (40), 143 (30), 130 (100), and 69 (32); δ 8.04 and 7.82 (1 H, s, CHO), 7.63 and 7.53 (1 H, d, 4-H), 7.32—7.08 (3 H, m, 5-, 6-, and 7-H), 6.96 and 6.86 (1 H, s, 2-H), 5.33 (1 H, t, CH_2CHCMe_2), 4.63 (2 H, d, CH_2CHCMe_2), 3.64 and 3.50 (2 H, t, $N^bCH_2CH_2$), 3.02—2.93 (2 H, m, $N^bCH_2CH_2$), 2.92, 2.87 (3 H, s, NMe), and 1.86 and 1.74 (6 H, s, CH_2CHCMe_2).

Rearrangement of N^b-*Formyl*-N^b-*methyl*-1-(3-*methylbut*-2-*enyl*)*tryptamine*.[6]—Rearrangement was effected by stirring a solution of (3) (100 mg) in trifluoroacetic acid (TFA) (8 ml) for 2 h under nitrogen, or simply by leaving the solution for two or three days under nitrogen at 2—4 °C. After treatment with TFA the mixture was neutralized with $NaHCO_3$ and extracted with ether. The main products obtained by both methods was the rather unstable *N*^{b}-formyl-*N*^{b}-methyl-2-(3-methylbut-2-enyl)tryptamine identified by ^1H n.m.r. and mass spectrometry; *m/z* 270 (44%), 211 (24), 198 (100), 182 (16), 168 (23), 156 (18), 143 (23), and 130 (20); δ 8.16 and 8.10 (1 H, br, s, NH), 8.02 and 7.70 (1 H, s, CHO), 7.56 and 7.43 (1 H, d, 4-H), 7.29—7.05 (3 H, m, 5-, 6-, and 7-H), 5.27 (1 H, t, CH_2CHCMe_2), 3.56—3.41 (2 H, m, $N^bCH_2CH_2$), 3.36 (1 H, d, CH_2CHCMe_2), 3.00—2.85 (m, $N^bCH_2CH_2$), 2.92 and 2.82 (s, NMe) (δ 3.00 to 2.82 integrates for 5 H), and 1.76 and 1.73 (6 H, s, CH_2CHCMe_2).

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