Four New Bromotryptamine Derivatives from the Marine Bryozoan *Flustra foliacea*

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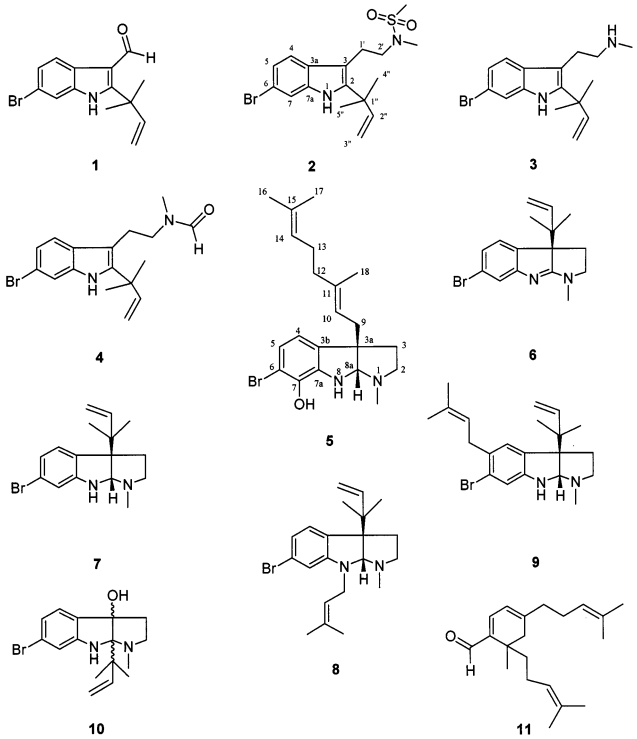
Ten brominated alkaloids, 6-bromo-2-(1,1-dimethyl-2-propenyl)-1*H*-indole-3-carbaldehyde (**1**), *N*-(2-[6-bromo-2-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]ethyl)-*N*-methylmethanesulfonamide (**2**), deformylflustrabromine (**3**), flustrabromine (**4**), (3a*R**,8a*S**)-6-bromo-3a-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]-indol-7-ol (**5**), flustramine C (**6**), dihydroflustramine C (**7**), flustramine A (**8**), flustramine D (**9**), and flustraminol A (**10**), and the diterpene 4,6-bis(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-carbaldehyde (**11**) were isolated from the dichloromethane extract of the North Sea bryozoan *Flustra foliacea*. Of the 10, four (**1**, **2**, **3**, and **5**) represent new natural products. The structures of all isolates were elucidated by interpretation of their spectroscopic data (NMR, MS, UV, and IR). For compound **4** complete ^{13}C NMR data are reported for the first time. Compounds **3** and **6**−**8** were tested on voltage-activated potassium and sodium channels. Flustramine A (**8**) shows an unspecific blocking activity on Kv1.4 potassium-mediated currents.

The marine bryozoan *Flustra foliacea* (L.), which is abundant in various parts of the North Sea, has been shown to contain biologically active brominated alkaloids[1] and monoterpenes.[2] To date 13 alkaloids have been described from North Sea collections of *F. foliacea*[3,4] and five indole alkaloids from *F. foliacea*, collected in Canadian waters.[4−7] Many of the isolated *Flustra* metabolites possess the unusual pyrrolo(2,3-*b*)indole skeleton. Compounds with such a ring system exhibit interesting biological activities, e.g., physostigmin and phenserine, both of which inhibit the enzyme acetylcholine esterase. Phenserine is currently in clinical trials as a drug for the treatment of Alzheimer's disease.[8] Other pyrroloindoles such as pseudophrynaminol, from the Australian frog *Pseudophryne coriacea*,[9] bind to nicotinic acetylcholine receptors (J. W. Daly personal communication). Earlier studies with *Flustra* extracts showed they have muscle relaxant effects, which could be partly attributed to flustramines A (**8**) and B.[10] The current study aimed at a detailed investigation of the secondary metabolite chemistry of *F. foliacea* followed by an assessment of the biological activity of the pure compounds.

A sample of *F. foliacea*, collected at Steingrund, North Sea, Germany, was extracted with CH_2Cl_2. Chromatographic separation of this extract yielded 10 bromotryptamine-based alkaloids (**1**−**10**) and a cyclohexadienecarbaldehyde diterpene (**11**). The isolation and structure elucidation of the four new brominated alkaloids, 6-bromo-2-(1,1-dimethyl-2-propenyl)-1*H*-indole-3-carbaldehyde (**1**), *N*-(2-[6-bromo-2-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]-ethyl)-*N*-methylmethanesulfonamide (**2**), deformylflustrabromine (**3**), and (3a*R**,8a*R**)-6-bromo-3a-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]-indol-7-ol (**5**), are described.



Results and Discussion

Mass spectral analysis of compound **1** showed it to have the molecular formula $C_{14}H_{14}NOBr$. Of the eight degrees of unsaturation indicated by the molecular formula of **1**, six were present as multiple bonds (5 × C=C, and 1 × C=O), as deduced from the ^1H and ^{13}C NMR data; the molecule must be bicyclic. An IR absorption at 1716 cm^{-1}, a weak ^{13}C NMR signal at 186.2 ppm, and a singlet ^1H NMR resonance at 10.44 ppm indicated the presence of an α,β-unsaturated aldehyde. The absorption maxima in the UV spectrum of **1** at 222, 274, and 300 nm and ^1H NMR resonances at 7.37, 7.51, and 8.23 ppm showed the presence of an aromatic moiety, possibly in the form of a quinoline or an indole,[11] and thus accounted for the two rings within **1**, and a further four of the carbon−carbon double bonds. The coupling pattern of the aromatic protons, one ortho- and meta-coupled, one ortho-coupled, and one meta-coupled, revealed the aromatic ring to be 1,2,4-substituted.

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Table 1. ^{1}H NMR Data for Compounds **1**, **2**, **3**, and **5**[a]

proton	**1**[b]	**2**[c]	**3**[d]	proton	**5**[b]
4	8.23 (1H, d, J = 8.4)[e]	7.43 (1H, d, J = 8.4)	7.41 (1H, d, J = 8.4)	2	2.66 (1H, m), 2.80 (1H, m)
5	7.37 (1H, dd, J = 1.8, 8.4)	7.12 (1H, dd, J = 1.7, 8.4)	7.10 (1H, dd, J = 1.8, 8.4)	3	2.11 (1H, m), 2.25 (1H, m)
7	7.51 (1H, d, J = 1.8)	7.50 (1H, d, J = 1.7)	7.50 (1H, d, J = 1.8)	4	6.09 (1H, d, J = 8.5)
2″	6.21 (1H, dd, J = 11.0, 17.6)	6.23 (1H, dd, J = 10.7, 17.4)	6.19 (1H, dd, J = 10.6, 17.5)	5	6.96 (1H, d, J = 8.5)
3″	5.30 (2H, m)	5.17 (1H, dd, J = 1.2, 10.7)	5.11 (1H, dd, J = 1.1, 10.6)	8a	4.49 (1H, brs)
		5.20 (1H, dd, J = 1.2, 17.4)	5.16 (1H, dd, J = 1.1, 17.5)		
4″	1.68 (3H, s)	1.58 (3H, s)	1.54 (3H, s)	N−CH_3	2.49 (3H, s)
5″	1.68 (3H, s)	1.58 (3H, s)	1.54 (3H, s)	9	2.56 (1H, m), 2.66 (1H, m)
1′	10.44 (1H, s)	3.14 (2H, m)	3.03 (2H, m)	10	5.00 (1H, m)
2′		3.34 (2H, m)	2.80 (2H, m)	12	1.93 (2H, m)
N-CH_3		2.95 (3H, s)	3.39 (3H, s)	13	1.93 (2H, m)
S-CH_3		2.85 (3H, s)		14	5.00 (1H, m)
				16	1.65 (3H, s)
				17	1.55 (3H, s)
				18	1.58 (3H, s)

[a] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] $CDCl_3$, 300 MHz. [c] CD_3OD, 500 MHz. [d] CD_3OD, 300 MHz. [e] J in Hz.

The ^{13}C NMR data showed the presence of a further five resonances for 1 × C, 1 × =CH, 1 × =CH_2, and 2 × CH_3 groups, for a total of 14 carbon atoms within **1**. From the HMQC (^{1}H−^{13}C one-bond 2D shift-correlated NMR), HMBC (^{1}H−^{13}C multiple-bond shift-correlated 2D NMR), and COSY (^{1}H−^{1}H shift-correlated 2D NMR) spectra of **1** it was possible to complete its structure elucidation. Thus, the HMBC spectrum correlations observed between CH_3-4″/CH_3-5″, and C-2, C-1″, C-2″, and C-3″, and the ^{1}H−^{1}H COSY couplings observed between H_2-3″ and H-2″, clearly revealed both methyl groups to bond directly to C-1″, which also bonded to C-2 and C-2″. HMBC correlations (see Table 3) also enabled the identity of all of the aromatic carbons to be established, and left C-3, the aldehyde moiety, the bromo function, and the amino group to be positioned. The large HMBC correlation observed between H-1′ and C-3 enabled the bond between C-1′ and C-3 to be deduced. A further HMBC correlation, this time between H-1′ and C-3a, showed C-3 and C-3a to bond directly and left C-3 to bond with C-2, to complete the remaining carbon−carbon double bond, which meant C-2 and C-3 were part of the indole and the α,β-unsaturated aldehyde. This left the amino group to lie between C-2 and C-7a and the Br to be placed at C-6. The new natural product, **1**, is best described as 6-bromo-2(1,1-dimethyl-2-propenyl)-1*H*-indole-3-carbaldehyde.

Compound **2** analyzed for $C_{17}H_{23}N_2O_2SBr$ by MS. Close comparison of all of the spectroscopic data of **2** with those of **1** showed them both to contain the same 6-bromo-2(1,1-dimethyl-2-propenyl)-1*H*-indole moiety, but to have a different substituent at C-3. The elemental composition of the molecular fragment at C-3 was thus $C_4H_{10}NO_2S$. From the NMR data of **2** it was evident that the C_4H_{10} part of this fragment was present as two adjacent methylene groups (whose protons coupled ([a 1,2-disubstituted ethyl moiety]), one of which was adjacent to nitrogen (3.34, 51.9 ppm), and two methyl groups, one of which was also attached to the nitrogen (2.95, 35.5 ppm). As the remaining methyl group was not bonded to oxygen, nitrogen, C-3, or C-1′, it had to be attached to the sulfur atom. The sulfur itself, on the basis of two strong IR absorptions at 1329 and 1149 cm^{-1}, the ^{1}H NMR singlet resonance at 2.85 ppm for −N-SO_2-CH_3 (cf. 2.82 ppm for Phe-NH-SO_2-CH_3), and the ^{13}C NMR signal at 35.7 ppm, also for the −N-SO_2-CH_3, had to be present as part of a sulfonamide, in this case an *N*-methylmethanesulfonamide. This left the unassigned end of the ethyl group to bond to C-3 and so completed the structure of **2**. The new natural product, **2**, is best described as *N*-(2-[6-bromo-2-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]ethyl)-*N*-methylmethanesulfonamide.

Table 2. ^{13}C NMR Data for Compounds **1**−**5**

carbon	**1**[a]	**2**[b]	**3**[c]	**4**[a]	carbon	**5**[a]
2	154.9 s[d]	142.7 s	142.3 s	140.8 s 139.8 s	2	52.7 t
3	113.9 s	108.0 s	108.8 s	107.7 s 106.9 s	3	35.9 t
3a	126.1 s	129.5 s	129.6 s	128.4 s 127.9 s	3a	59.4 s
4	123.5 d	120.0 d	120.0 d	119.4 d 118.7 d	3b	119.3 s
5	126.3 d	122.8 d	122.6 d	122.7 d 122.6 d	4	108.8 d
6	116.8 s	115.2 s	115.0 s	114.9 s 114.8 s	5	131.1 d
7	113.8 d	114.6 d	114.6 d	113.7 d 113.3 d	6	93.3 s
7a	134.5 s	137.3 s	137.2 s	135.0 s	7	152.5 s
1′	186.2 d	25.6 t	25.5 t	24.8 t 22.5 t	7a	148.9 s
2′		51.9 t	53.2 t	50.2 t 45.4 t	8a	85.9 brs
1″	39.8 s	40.0 s	40.2 s	38.9 s 38.8 s	9	35.7 t
2″	144.6 d	147.4 d	147.4 d	145.5 d	10	120.0 d
3″	114.4 t	112.0 t	111.9 t	112.2 t	11	138.6 s
4″	28.8 q	28.3 q	28.4 q	27.5 q	12	39.8 t
5″	28.8 q	28.3 q	28.4 q	27.6 q	13	26.6 t
N-CH_3		35.5 q	35.8 q	35.1 q 30.1 q	14 15	124.1 d 131.4 s
S-CH_3		35.7 q			16	25.7 q
CHO				162.6 d	17	17.7 q
					18	16.4 q
					N-CH_3	36.5 q

[a] $CDCl_3$ 75.5 MHz. [b] CD_3OD 125.5 MHz. [c] CD_3OD 75.5 MHz. [d] Implied multiplicities determined by DEPT (C = s, CH = d, CH_2 = t, CH_3 = q).

The third new brominated alkaloid, deformylflustrabromine {*N*-(2-[6-bromo-2(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]ethyl)-*N*-methylamine} was assigned structure **3** on the basis of the following data. Accurate mass measurement showed it to have the molecular formula $C_{16}H_{21}N_2$-Br. The UV, IR, and NMR data, both ^{1}H and ^{13}C NMR (Tables 1 and 2), clearly showed compounds **1**, **2**, and **3** all to possess the same 6-bromo-2(1,1-dimethyl-2-propenyl)-

Table 3. $^{1}H-^{13}C$ HMBC 2D NMR Data for Compounds **1**−**3** and **5**

proton	**1**[a]	**2**[b]	**3**[c]	proton	**5**[c]
4	3, 5, 6, 7a[d]	3, 6, 7a	6, 7a	2	
5	7, 3a	6, 7, 3a	3, 4, 6, 7, 3a, 7a	3	
7	3a, 6	5, 6, 3a	5, 6, 3a, 7a	4	6, 7, 3a, 3b, 7a
1′	3, 3a	2, 3, 3a, 2′	2, 3, 3a, 2′	5	6, 7, 3b, 7a
2′		N-CH_3, 3, 1′	N-CH_3, 3, 1′	8a	2, 3, 3b, 7a, N-CH_3
2″	2, 1″, 4″, 5″	1″,3″, 4″, 5″	2, 1″,3″, 4″, 5″	9	10, 11, 3a, 8a
3″	1″, 2″	2″, 1″	2, 2″, 1″, 4″	10	
4″	2, 2″, 1″, 5″	2, 2″, 1″, 5″	2, 2″, 1″, 5″	12	
5″	2, 2″, 1″, 5″	2, 2″, 1″, 4″	2, 2″, 1″, 4″	13	
N-CH_3		2′	2′	14	
S-CH_3				16	14, 15
				17	14, 15
				18	10, 11, 12
				N-CH_3	2, 8a

[a] Basic recording frequencies; $CDCl_3$, 300 MHz. [b] CD_3OD, 500 MHz. [c] CD_3OD, 300 MHz. [d] Correlations reported are from proton resonance to carbon resonance. Hence, the numbers in the columns refer to carbon resonances.

1*H*-indole moiety. In the case of **3** it also contained the ethyl-*N*-methyl part of the ethyl-*N*-methylmethanesulfonamide residue found in **2**, but lacked the sulfur-containing part of this group.

A fourth compound, the known substance flustrabromine (**4**), which is structurally very similar to compounds **1**−**3**, was also isolated in this investigation. In contrast to the NMR data for the first three isolates the equivalent data for **4** are relatively complicated (nearly all ^{1}H and ^{13}C NMR resonances are doubled), due to the two isomeric forms of the amide. In the published ^{13}C NMR data for **4**,[12] the resonances for C-2 and C-7a were not reported. In Table 2 the complete ^{13}C NMR data for **4** are now given.

The final new compound isolated in this study, **5**, came from a bromo-alkaloid-rich VLC fraction that was also found to contain flustramine D (**9**) and dihydroflustramine C (**7**). Its mass spectral data showed it to have the molecular formula $C_{21}H_{29}N_2BrO$. These data also suggested the presence of two possible isoprene units; molecular ion less *m*/*z* 69 (C_5H_9) followed by a loss of *m*/*z* 68 (C_5H_8). This fragment then lost *m*/*z* 43 to generate the peaks at *m*/*z* 224 and 226, consistent with the loss of $CH_3-N{=}CH_2$ from the remaining major part of the molecule. This mass spectral fragmentation pattern was quite similar to those reported for flustramine D (**9**)[6] and dihydroflustramine C (**7**),[5] as were the IR and UV data. This information, together with the NMR data (Tables 1, 2, and 3), clearly showed **5** to be a prenylated, hydroxyl, 6-bromo-1-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole derivative. When all of the spectroscopic data for **5** and **9** were taken into account, it was evident that, like **9**, **5** was prenylated at C-3a and that the aromatic ring was tetrasubstituted. In **5**, the two aromatic protons were ortho-coupled, which showed the aromatic ring to be 1,2,3,4-substituted, meaning the Br and OH functions had to be at either C-6 and C-7 or C-4 and C-5. The isoprene moiety was not attached to the aromatic ring, as was the case in **9**, but was deduced from $^{1}H-^{1}H$ and $^{1}H-^{13}C$ NMR correlations (unreported data and Table 3) to be a 3,7-dimethyl-2,6-octadienyl group, i.e., a geranyl moiety attached to C-3a.

With the basic structure of **5** established, the regiochemistry of the aromatic ring, the geometry of the $\Delta^{10,11}$ double bond, and the relative configurations at C-3a and C-8a needed to be established. From a NOESY spectrum of **5**, interactions observed between H-8a and H-10 showed the pyrrolidine and indoline rings to be *cis*-fused, and those between H-4 and at least one of the methyl groups in the side-chain confirmed the positions of H-4 and H-5 to be as shown. From the ^{13}C NMR chemical shift of CH_3-18 the $\Delta^{10,11}$ double bond was assigned the *E*-geometry.[13] Because it was known where C-3b, C-4, C-5, and C-7a were located, and it was evident from the chemical shifts of C-6 (93.7 ppm) that the Br group was at this position, the OH function had to be placed at C-7 (152.0 ppm). Compound **5** is best described as (3a*R**,8a*S**)-6-bromo-3a-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-1-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-7-ol.

Together with the four new metabolites the known compounds flustrabromine (**4**),[12] flustramine C (**6**),[14] dihydroflustramine C (**7**),[4] flustramine A (**8**),[1,15] flustramine D (**9**),[6] flustraminol A (**10**),[14] and 4,6-bis(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-carbaldehyde (**11**)[16] were also isolated and characterized. As our sample of **11** was essentially optically inactive, we concluded that it resulted from the condensation of citral as proposed by Thomas and Guntz-Dubini.[17]

Biological Activity. To study the potential effects of *Flustra* metabolites on proteins important for the electrical excitability of cells, compounds **3** and **6**−**8** were tested on voltage-activated potassium and sodium channels. Electrophysiological measurements were performed using the oocyte expression system. Two-electrode voltage clamp recordings of Shaker-, Kv1.1-, and Kv1.4-mediated potassium currents showed that flustramine A (**8**), flustramine C (**6**), deformylflustrabromine (**3**), and dihydroflustrabromine C (**7**) had no specific effect on the evoked currents for concentrations up to 10 μM. Also, whole cell currents recorded from oocytes expressing rat brain $Na_v1.2$ channels were not affected by these *Flustra* metabolites (data not shown). Higher concentrations of these substances have not been used so as to avoid potential effects of DMSO on the cells. Interestingly, the presence of flustramine A (**8**) in the bath solution in some oocytes resulted in a slight blocking of Kv1.4-mediated currents. Since this effect was not reproducible, it is most likely not due to a direct binding of flustramine A on the channel protein. Instead, it is more likely that the blocking action might be an indirect effect which could be mediated by an interaction of flustramine A with the cell membrane and/or endogenous receptor molecules present in the cytoplasmic membrane of the oocyte. Further evaluation of *Flustra* metabolites, especially on different types of nACh receptors, is in progress.

Experimental Section

General Experimental Procedures. HPLC was carried out using a Merck-Hitachi system consisting of an L-6200A pump, an L-4500A photodiode array detector, and a D-6000A interface. All NMR spectra were recorded on Avance 500-DMX, AM-400, Avance 300-DPX, and AC-200 Bruker spectrometers.

Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 7.26/77.0 ($CDCl_3$) and 3.35/49.0 (CD_3OD), respectively. UV and IR spectra were obtained employing Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. Optical rotations were measured on a Jasco DIP 140 polarimeter. HREIMS were recorded on a Kratos MS 50 spectrometer. All other experimental details were as previously reported.[19]

Electrophysiological Measurements. Oocytes from *Xenopus laevis* were prepared as described previously.[18] cRNA coding for *Shaker*, rat Kv1.1, rat Kv1.4, or rat brain Typ II Na-channels was injected into oocytes, and the cells were incubated 1−5 days to allow expression of the protein. The vitelline membranes of the oocytes were removed mechanically with fine forceps prior to the electrophysiological measurements. Whole cell currents were recorded under two-electrode voltage clamp control using a Turbo-Tec amplifier (npi electronic, Tamm, Germany). The intracellular electrodes were filled with 2 M KCl and had a resistance between 0.6 and 1.0 MΩ. Current records were low-pass filtered at 1 kHz (−3 dB) and sampled at 4 kHz. Current records were corrected on-line for leak and capacitive transients by using a P/n method. The bath solution was normal frog Ringer's (NFR) containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 10 mM Hepes at pH 7.2 (NaOH). Due to the hydrophobicity of the substances, a stock solution of the *Flustra* metabolites in DMSO was prepared. Stock solutions were dissolved in NFR to the final concentration of these substances of up to 10 μM and then applied to the recording chamber. DMSO dissolved alone in NFR was also tested and showed no effects on the evoked currents at the dilution used. All electrophysiological experiments were performed at room temperature (19−22 °C).

Animal Material. Samples of the bryozoan *Flustra foliacea* were collected from "Steingrund" in the North Sea, 10 km northeast of Helgoland, September 1998, from depths of 8−12 m, and maintained frozen until used. Samples were taxonomically identified by P. Haywood, School of Biological Sciences, University of Wales, Swansea, Wales. A voucher sample is retained at the Department for Pharmaceutical Biology, Bonn.

Extraction and Isolation. Frozen *F. foliacea* (wet weight ≈ 5 kg) was extracted with CH_2Cl_2 (3 × 5 L), followed by MeOH (3 × 5 L). The CH_2Cl_2 extract, 12 g of brown oil, was fractionated by vacuum liquid chromatography (VLC) over Si gel (5−40 μm), employing a step gradient from petroleum ether to $(CH_3)_2CO$ to CH_2Cl_2 to MeOH, to yield 14 fractions, each of approximately 90 mL. TLC and ^{1}H NMR control of these fractions indicated fractions 2−12 to be of further interest, mainly on the basis of Dragendorff-positive areas on the TLC plates and ^{1}H NMR resonances characteristic of aromatic protons. VLC fractions 2 (56 mg), 4 (440 mg), 9 (5652 mg), and 12 (910 mg) were purified by solid-phase extraction (SPE, Bakerbond Octadecyl C_{18}) and by normal- and reversed-phase HPLC. Fraction 2 (Eurospher 100-C_{18}, 250 × 8 mm, 5 μm; MeOH/H_2O gradient, from 80 to 90% MeOH in 15 min, 2 mL/min) gave 3.0 mg of **1** and 2.0 mg of **2**. SPE (Bakerbond Octadecyl C_{18}) of fraction 4, employing a step gradient from H_2O to MeOH to CH_2Cl_2, yielded 10 further fractions. HPLC of fraction 4.5 (193 mg, Eurospher 100-Diol 5 μm, 250 × 8 mm, petroleum ether/CH_2Cl_2, gradient, from 10 to 90% CH_2Cl_2 in 12 min, 2 mL/min) gave 5.7 mg of **11**. HPLC of fraction 4.10 (244 mg, LiChrospher Si60 5 μm 250 × 8 mm, CH_2Cl_2/EtOAc gradient, from 1 to 8% EtOAc in 20 min, 2 mL/min) yielded 6.1 mg of **10**. Fraction 9 was separated by HPLC (LiChrospher Si60 10 μm, 250 × 10 mm, CH_2Cl_2/MeOH + 0.05% NH_4OH gradient, from 85/15 to 40/60 in 10 min, 3 mL/min) to yield a further six fractions. Purification of fraction 9.1 (50 mg, Eurospher 100-C_{18} 5 μm, 250 × 8 mm, H_2O/MeOH/CH_2Cl_2 gradient, from 5 to 0% H_2O in 5 min/95 to 50% MeOH in 20 min/0 to 50% CH_2Cl_2 in 20 min; 1.5 mL/min) gave 16.8 mg of **4**, 19.6 mg of **6**, and 7.3 mg of **8**. HPLC separation of fraction 9.3 (150 mg, Eurospher 100-C_{18} 5 μm, 250 × 8 mm, MeOH/CH_2Cl_2 gradient, from 100 to 70% MeOH in 15 min, 1.5 mL/min) gave 43.2 mg of **5**, 51.3 mg of **7**, and 26.8 mg of **9**. SPE (Bakerbond SiOH) of fraction 12 with MeOH yielded 17.3 mg of **3**.

6-Bromo-2(1,1-dimethyl-2-propenyl)-1*H*-indole-3-carbaldehyde (1): yellow oil (3.0 mg, <0.001%); UV (MeOH) λ_{max} (log ϵ) 222 (4.6), 274 (4.0), 300 (3.9) nm; IR (ATR) ν_{max} 3155, 2923, 1716, 1623, 1574, 1456, 1377 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* 291, 293 (93, 88), 276, 278 (100, 95), 248, 250 (66, 62), 235, 237 (42, 41) 168 (49); HREIMS *m*/*z* 291.0263 (calcd for $C_{14}H_{14}NO^{79}Br$, 291.0288).

***N*-(2-[6-Bromo-2-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]ethyl)-*N*-methylmethanesulfonamide (2):** yellow oil (2.0 mg, <0.001%); UV (MeOH) λ_{max} (log ϵ) 227 (4.8), 288 (4.3) nm; IR (ATR) ν_{max} 3354, 2926, 1665, 1458, 1377, 1329, 1149, 966, 909 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* 398, 400 (15, 17), 334, 336 (7, 7), 292, 294 (15, 14), 276, 278 (100, 96), 167 (18), 69 (19); HREIMS *m*/*z* 398.0667 (calcd for $C_{17}H_{23}N_2O_2S^{79}Br$, 398.0664).

Deformylflustrabromine, {*N*-(2-[6-bromo-2(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]ethyl)-*N*-methylamine} (3): yellow oil (17.3 mg, <0.001%); UV (MeOH) λ_{max} (ϵ) 231 (4.6), 289 (3.9) nm; IR (ATR) ν_{max} 3220, 2900, 1710, 1600, 1450, 1220, 1045, 905 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* 320, 322 (5, 4), 277, 279 (70, 69), 262, 264 (27, 24), 181 (20), 167 (20); HREIMS *m*/*z* 320.0893 (calcd for $C_{16}H_{21}N_2Br$, 320.0888).

Flustrabromine (4): yellow oil (16.8 mg, <0.001%); with all spectroscopic data in good agreement with those previously published.[12]

(3a*R,8a*S**)-6-Bromo-3a-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*}indol-7-ol (5):** yellow oil (43.2 mg, <0.001%); $[\alpha]_D^{22}$ −22.0° (*c* 0.1 $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 251 (4.7), 309 (4.5) nm; IR (ATR) ν_{max} 3304, 2964, 2930, 2869, 1596, 1482, 1380, 1364, 1349, 1312, 1244, 1151, 1006, 913 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* 404, 406 (40, 39), 335, 337 (62, 58), 267, 269 (100, 98), 224, 226 (33, 36), 188 (38), 69 (54); HREIMS *m*/*z* 404.1463 (calcd for $C_{21}H_{29}N_2O^{79}Br$, 404.1463).

Flustramine C (6): yellow oil (19.6 mg, <0.001%); $[\alpha]_D^{22}$ −10.1° (*c* 0.1 $CHCl_3$), lit.[14] $[\alpha]_D^{22}$ not reported; all remaining spectroscopic data in good agreement with those previously published.

Dihydroflustramine C (7): yellow oil (43.2 mg, <0.001%); $[\alpha]_D^{22}$ −53.1° (*c* 0.1 $CHCl_3$), lit.[5] $[\alpha]_D^{22}$ −110° (*c* 1.5, CH_2Cl_2); all remaining spectroscopic data in good agreement with those previously published.[5]

Flustramine A (8): yellow oil (7.3 mg, <0.001%); $[\alpha]_D^{22}$ −40.0° (*c* 0.1 $CHCl_3$), lit.[15] $[\alpha]_D^{22}$ not reported; all remaining spectroscopic data in good agreement with those previously published.[1,15]

Flustramine D (9): yellow oil (51.3 mg, <0.001%); $[\alpha]_D^{22}$ −17.4° (*c* 0.1 $CHCl_3$); lit.[6] $[\alpha]_D^{22}$ −86.5° (*c* 1.03, CH_2Cl_2); all remaining spectroscopic data in good agreement with those previously published.[6]

Flustaminol A (10): yellow oil (6.1 mg, <0.001%), with spectroscopic data in good agreement with those previously published.[14]

4,6-Bis(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-dienecarbaldehyde (11): yellow oil (5.7 mg, <0.001%); $[\alpha]_D^{22}$ −0.2° (*c* 0 0.1 $CHCl_3$), lit.[16] $[\alpha]_D$ 0°; all remaining spectroscopic data in good agreement with those previously published.[16]

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References and Notes

(1) Carlé, J. S.; Christophersen, C. *J. Am. Chem. Soc.* **1979**, *101*, 4012−4013.
(2) Christophersen, C.; Carlé, J. S. *Naturwissenschaften* **1978**, *65*, 440−441.

(3) Holst, P. B.; Anthoni, U.; Christophersen, C.; Nielsen, P. H. *J. Nat. Prod.* **1994**, *57*, 997−1000.
(4) Anthoni, U.; Nielsen, P. H.; Pereira, M.; Christophersen, C. *Comp. Biochem. Physiol.* **1990**, *96B*, 431−437.
(5) Wright, J. L. C. *J. Nat. Prod.* **1984**, *47*, 893−895.
(6) Laycock, M. V.; Wright, J. L. C.; Findlay, J. A.; Patil, A. D. *Can. J. Chem.* **1986**, *64*, 1312−1316.
(7) Christophersen, C. *Comp. Biochem. Physiol.* **1991**, *98B*, 427−432.
(8) Evans, J. *Chem. Br.* **2001**, 47−51.
(9) Spande, T. F.; Edwards, M. W.; Pannell, L. K.; Daly, J. W.; Erspamer, V.; Melchiorri, P. *J. Org. Chem.* **1988**, *53*, 1222−1226.
(10) Sjöblom, T.; Bohlin, L.; Christophersen, C. *Acta Pharm. Suec.* **1983**, *20*, 415−419.
(11) Itokawa, H.; Akita, Y.; Yamazaki, M. *Yakugaku Zasshi* **1973**, *93*, 1251−1252.
(12) Wulff, P.; Carlé, J. S.; Christophersen, C. *J. Chem. Soc., Perkin Trans.* **1981**, *1*, 2895−2898.
(13) Höller, U.; König, G. M.; Wright, A. D. *J. Nat. Prod.* **1997**, *60*, 832−835.
(14) Carlé J. S.; Christophersen, C. *J. Org. Chem.* **1981**, *46*, 3440−3443.
(15) Carlé, J. S.; Christophersen, C. *J. Org. Chem.* **1980**, *45*, 1586−1589.
(16) Bachmann-Holst, P.; Anthoni U.; Christophersen, C.; Nielsen, P. H.; Bock, K. *Acta Chem. Scand.* **1994**, *48*, 765−768, and references therein.
(17) Thomas, A. F.; Guntz-Dubini, R. *Helv. Chim. Acta* **1976**, *59*, 2261−2267.
(18) Stühmer, W. *Methods Enzymol.* **1992**, *207*, 319−339.
(19) Wright, A. D.; König, G. M.; Angerhofer, C. K.; Greenidge, P.; Linden, A.; Desqueyroux-Faundez, R. J. *J. Nat. Prod.* **1996**, *59*, 710−716.

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