Further Studies on the Chemistry of the Flustra Alkaloids from the Bryozoan *Flustra foliacea*

Simone J. Rochfort,[†,‡] Stanley Moore,[†,§] Cheryl Craft,[†] Ned H. Martin,[||] Ryan M. Van Wagoner,[⊥] and Jeffrey L. C. Wright*[,†,⊥]

Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford Street, Halifax, NS, Canada B3H 3Z1, Department of Chemistry and Biochemistry, University of North Carolina Wilmington, Wilmington, North Carolina 28403, and Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, North Carolina 28409

Received May 8, 2009

Since 1980, over a dozen novel brominated alkaloids, named flustramines, have been isolated from Scandinavian and Canadian collections of the marine bryozoan *Flustra foliacea.* This paper describes the reisolation of the known compound dihydroflustramine C (**1**) and the isolation of 11 new flustramines (**2**−**4**, **6**−**13**), including two dimers (**12**, **13**) that may be isolation artifacts. Together these compounds, some with an unexpected aryl substitution pattern, reveal an intricate network of metabolites present in the extracts of the bryozoan. The structures of these metabolites were solved using a variety of spectroscopic techniques and chemical derivatization and modification. This work also led to the recognition of an unusual rearrangement reaction that occurred slowly over a number of years.

Early chemical studies of the marine bryozoan *F. foliacea* were notable in several aspects. First, these studies were among the first carried out on a phylum of animals that have since yielded structurally and pharmacologically interesting compounds such as the bryostatins.[1] Second, the family of compounds identified by these studies, the flustramines, were early examples of alkaloids identified from marine sources.[2−4] Moreover, the flustramines were among the first marine natural products found to have a strong structural affinity with terrestrial natural products, in this case the physostigmine alkaloids from legumes and other sources. The marine structures still have distinctive features however, such as bromination of the indole ring, prenylation of the indole ring nitrogen of flustramines A and B, and the presence of a "reverse" prenyl substitution in flustramines A and C, the latter of which lacks prenylation of the indole nitrogen. Subsequent studies led to the identification of dihydroflustramine C[5] and also flustramine D and isoflustramine D,[6] the latter two containing prenylated aromatic rings. Flustramine E,[7] like flustramine B, exhibits "normal" prenylation, but like dihydroflustramine C, lacks prenylation of the indole nitrogen. Variations seen in other flustramine congeners include incorporation of oxygen atoms into the terminal pyrrolidine moiety via ring expansion as in flustrarine B,[8] as an exocyclic *N*-oxide,[6,9] or via lactamization.[8,10]

The flustramines have attracted attention for their biological activities and as synthetic targets. These alkaloids and related compounds have been reported to have antibacterial,[5−7] muscle relaxant,[11] nonspecific voltage-sensitive potassium channel blocking,[12] and subtype-specific nicotinic acetylcholine receptor blocking activities.[13] The first synthetic studies on the flustramines led to the production of racemic flustramine B[14] and debromoflustramine B.[15] The later synthesis of *ent*-debromoflustramine B established the configuration of natural flustramine B as being 3a*S*, 8a*R*.[16] More recently, Austin et al. reported the synthesis of chiral flustramine B identical to the natural product using a cascade addition−cyclization approach.[17] Other studies have targeted racemic debromodihydroflustramine C,[18,19] racemic flustramine C,[20] various racemic flustramines,[21] racemic dihydroflustramine C and flustramine E,[22] racemic flustramines A and C,[23] and scalemic flustramines A and B.[24]

We report here studies building on our previous work with *F. foliacea* collected from Canadian waters. In this latest report, we have been able to identify 11 new flustramines, which differ from those previously reported in bromination patterns and by a new hydroxylation pattern on the aromatic ring. We also observed flustramines that appear to be derived by further cyclization, by isomerization to an alternative ring system, or by dimerization mediated by a single carbon block.

Results and Discussion

Following a large collection of *F. foliacea*, the cells were freshly extracted and the CH_2Cl_2-soluble fraction of the aqueous MeOH extract showed significant antibacterial and antifungal activity. Large-scale, medium-pressure reversed-phase chromatography of the extract produced several fractions containing UV-active compounds. Compounds containing the flustramine skeleton were identified on the basis of their IR and UV spectra and by characteristic fragmentation pathways by EIMS. The most useful EIMS fragmentations were the loss of one or two isoprene subunits followed by the loss of 43 mass units, corresponding to ejection of a $CH_3-N{=}CH_2$ fragment from the pyrrole moiety.[2−7] Further purification of selected fractions by C18 reversed-phase HPLC yielded the known dihydroflustramine C (**1**) and 11 new flustramine derivatives (**2**−**4**, **6**−**13**).

The major metabolite **1**, first reported in this laboratory,[5] was identified by comparison of its spectroscopic data with that originally reported. A new product, designated flustramine F (**2**), possessed a molecular formula of $C_{18}H_{23}BrN_2O$ ($[M]^+$ 362.0987) requiring eight degrees of unsaturation. The spectroscopic data for **2** were very similar to those of **1**, and the difference of 42 mass units between **1** and **2** corresponds to the addition of C_2H_2O. In $CDCl_3$ the ^1H NMR resonances were broad, while in DMSO-d_6 at elevated temperature (367 K) the resonances became sharp. Despite this effect, the NMR data obtained for **2** in $CDCl_3$ (Tables 1 and 2) were very similar to those for **1**, the only significant differences being the appearance of acetate resonances in **2** (δ_H 2.31 ppm, br s; δ_C 23.8 ppm, CH_3; 169.0 ppm, qC) and the absence of the resonance assigned to the benzylic amine proton. Collectively, these data suggested that **2** was the *N*-acetate of **1**. The broad signals observed in the ^1H NMR spectra of **2** at standard temperature were ascribed to the presence of rotational isomers of the *N*-acetate moiety. This was confirmed by acetylation of **1** to yield a product that was spectroscopically identical to **2**.

* To whom correspondence should be addressed. Current address: Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC 28409. Tel: 910-962-2397. Fax: 910-962-2410. E-mail: wrightj@uncw.edu.

† National Research Council of Canada.

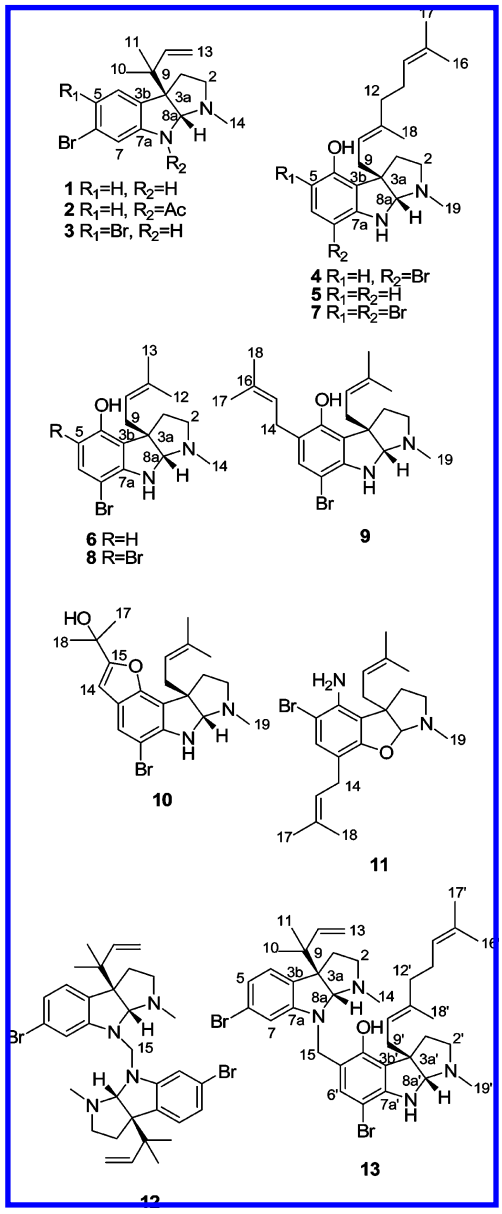
‡ Current address: Biosciences Research Division, Department of Primary Industries, 1 Park Drive, Bundoora 3083, Australia.

§ Current address: Department of Biochemistry, University of Saskatchewan.

|| Department of Chemistry and Biochemistry, UNCW.

⊥ Center for Marine Science, UNCW.

10.1021/np900282j CCC: $40.75
Published on Web 09/28/2009



The isotope pattern and accurate mass for the parent ion observed in the EIMS of flustramine G (**3**) indicated a molecular formula ($C_{16}H_{20}Br_2N_2$; $[M]^+$ 397.9990) related to that of **1** by addition of a bromine atom. The most important differences between the ^{1}H NMR spectroscopic data for **3** and **2** were that the aromatic region of the spectrum of **3** contained only two aromatic singlets (δ_H 7.30 and 6.82 ppm), suggesting a *para* relationship between the protons. Complete assignment of the proton and carbon resonances was possible by detailed 2D NMR studies (Tables 1 and 2) and led to structure **3** for flustramine G.

The molecular formula $C_{21}H_{29}BrN_2O$ ($[M]^+$ 404.1460) obtained for flustramine H (**4**) suggested that **4** contained additional C_5H_8 (isoprene) and OH moieties compared to **1**. Indeed, the EIMS fragmentation pattern confirmed the presence of a geranyl moiety, from which two isoprene subunits are lost sequentially from the molecular ion to give fragment ions at *m*/*z* 335/337 and 267/269. The presence of a geranyl moiety was supported by analysis of the NMR data (Tables 1 and 2), which showed three olefinic methyls (δ_H 1.59, 1.57, and 1.66 ppm) and two olefinic methines (δ_H 5.12 and 5.03 ppm). In addition, 2D COSY and HMBC analysis enabled complete assignment of the geranyl moiety. HMBC correlations were observed from the two olefinic methyls, H_3-16 and H_3-17, to C-14, which in turn was connected by successive COSY correlations to C-13, then C-12. The HMBC correlation between the olefinic methyl resonance H_3-18 and C-12 confirmed the link between the two C_5 isoprene units. The point of attachment of the C_{10} side chain to the aromatic nucleus was established by the HMBC correlation between the H_2-9 protons and C-3b.

The remaining differences between **4** and **1** centered on the nature and substitution pattern of the aromatic nucleus. The ^{1}H NMR spectrum of **4** revealed the presence of two rather than three aromatic protons, suggesting the aromatic ring to be the site of hydroxylation. This was supported by ^{13}C NMR resonances at δ_C 152.2 and 93.4 ppm, consistent with hydroxylated and brominated aromatic carbons, respectively. However, the location of these groups on the aromatic ring was ambiguous. In the ^{1}H NMR spectra, the two aromatic resonances showed a large *ortho* coupling (J = 8.5 Hz), but one proton was significantly more shielded than the other (δ_H 6.02 cf. 6.99 ppm), suggesting that it is *ortho* to either the phenolic moiety or the nitrogen of the indole system. Regardless of chemical shift correlations, six possible substitution patterns can accommodate *ortho* coupling of the aromatic protons, as illustrated in Figure 1 (substructures A−F).

To determine the correct substitution pattern, two derivatives were made and characterized. The first derivative, debromoflustramine H (**5**), was formed by reaction of flustramine H with $LiAlH_4$. EIMS analysis of the product showed a single molecular ion at *m*/*z* 326 with no isotope peaks, confirming the replacement of bromine by hydrogen. Inspection of the ^{1}H NMR spectrum confirmed the presence of three aromatic protons (δ_H 6.14 ppm, d (J = 7.9 Hz); 6.19 ppm, d (J = 7.9 Hz); 6.90 ppm, dd (J = 7.9, 7.9 Hz)). Each aromatic proton showed a large *ortho* coupling, eliminating substructures E and F, as these would have resulted in one *meta*-coupled proton.

It was considered that the methoxy derivative would be useful in identifying which of the four remaining substructures were correct. Treatment of **4** with K_2CO_3 and MeI yielded several quite polar compounds. Surprisingly, the major product was shown to be the rearranged permethylated ammonium cation **19** (Scheme 1). HRFABMS revealed the molecular formula of **19** as $C_{25}H_{38}BrN_2O$ ($[M]^+$ 461/463), suggesting the addition of four methyl groups rather than the expected three. The presence of four additional methyl groups in **19** was supported by the ^{1}H NMR spectra, which showed the presence of three downfield singlet methyl resonances, one of which integrated for nine protons and was more deshielded than the others. The ^{13}C and ^{1}H NMR data (δ_H 3.45 ppm, 9H; δ_C 53.5 ppm) were consistent with the three degenerate methyl groups being attached to a positively charged nitrogen atom. The ^{1}H chemical shift of the remaining *N*-methyl resonance was somewhat deshielded (δ_H 3.99 ppm), whereas the associated carbon resonance was relatively shielded (δ_C 32.7 ppm), values typical of an *N*-methyl indole group (δ_H 3.40−4.20 ppm; δ_C 28.0−34.0 ppm).[25−27] The remaining 3H resonance was assigned as a methoxy signal on the basis of the chemical shift data (δ_H 3.89 ppm, 3H; δ_C 55.6 ppm).

Better resolution of the methyl resonances was achieved by collecting ^{1}H NMR spectroscopic data of the product in a 60% benzene-d_6/$CDCl_3$ solution. Signals for the methyl groups were now observed at δ_H 3.38 ppm (3H), δ_C 55.5 ppm (OMe); δ_H 2.60 ppm (9H), δ_C 52.5 ppm (NMe_3); and δ_H 3.68 ppm (3H), δ_C 33.9 ppm (indole N-Me). HMBC correlations helped to derive the new indole substructure and the attachment points of the side chains. The H_2-8 protons showed a long-range correlation to a carbon at 128.5 ppm (C-3). The H_2-10 protons showed a long-range correlation to a carbon at 139.0 ppm (C-2) as well as correlations into the geranyl side chain. This established that the two side chains are attached to the indole at different positions. The indole N-Me resonance at δ_H 3.68 ppm showed HMBC correlations to two deshielded quaternary carbon resonances at δ_C 135.0 ppm (C-7a) and δ_C 139.0 ppm (C-2), confirming the point of attachment of the geranyl side chain on C-2. This was supported by correlations in the NOESY spectrum from the indole N-Me resonance to the geranyl side-chain

Table 1. ^{13}C NMR Assignments for Flustramines **2**−**4** and **6**−**12** in $CDCl_3$ (δ_C, mult)[a]

pos.	2	3	4	6	7	8	9	10	11	12
2	54.0, CH_2	52.9, CH_2	52.6, CH_2	52.6, CH_2	52.5, CH_2	52.4, CH_2	52.6, CH_2	52.5, CH_2	51.9, CH_2	54.0, CH_2
3	32.6, CH_2	34.5, CH_2	36.5, CH_2	36.2, CH_2	36.7, CH_2	36.8, CH_2	36.2, CH_2	37.2, CH_2	35.3, CH_2	33.9, CH_2
3a	64.0, qC	64.3, qC	59.3, qC	59.4, qC	59.7, qC	59.6, qC	59.2, qC	59.1, qC	57.7, qC	64.9, qC
3b	127.6, qC	134.9, qC	119.9, qC	119.6, qC	120.2, qC	120.1, qC	119.3, qC	122.8, qC	115.8, qC	132.1, qC
4	126.0, CH	129.5, CH	152.2, qC	152.0, qC	147.6, qC	147.5, qC	150.5, qC	150.3, qC	137.8, qC	126.2, CH
5	126.7, CH	123.3, qC	108.3, CH	108.8, CH	99.1, qC	99.1, qC	120.8, qC	117.0, qC	100.6, qC	119.8, CH
6	119.2, qC	111.6, qC	130.9, CH	131.2, CH	132.0, CH	132.0, CH	130.9, CH	121.9, CH	131.3, CH	121.8, qC
7	121.2, CH	113.0, CH	93.4, qC	93.7, qC	93.7, qC	93.7, qC	93.5, qC	99.0, qC	114.9, qC	109.4, CH
7a	144.4, qC	150.9, qC	149.5, qC	149.3, qC	149.6, qC	149.6, qC	148.0, qC	146.0, qC	158.3, qC	152.1, qC
8a	87.3, CH	84.8, CH	86.1, CH	86.3, CH	86.3, CH	86.1, CH	86.8, CH	86.3, CH	106.2, CH	90.5, CH
9	40.8, qC	41.2, qC	35.7, CH_2	35.9, CH_2	35.2, CH_2	35.3, CH_2	35.9, CH_2	36.5, CH_2	34.8, CH_2	41.8, qC
10	22.4, CH_3	22.4, CH_3	120.6, CH	120.3, CH	120.4, CH	120.3, CH	120.5, CH	120.2, CH	119.7, CH	23.8, CH_3
11	23.3, CH_3	23.1, CH_3	138.3, qC	135.5, qC	137.9, qC	134.3, qC	135.1, qC	134.1, qC	134.7, qC	23.1, CH_3
12	144.2, CH	143.9, CH	39.9, CH_2	18.2, CH_3	39.9, CH_2	18.1, CH_3	18.2, CH_3	18.0, CH_3	18.2, CH_3	144.9, CH
13	112.7, CH_2	113.9, CH_2	26.7, CH_2	26.0, CH_3	26.7, CH_2	26.0, CH_3	26.0, CH_3	25.6, CH_3	25.9, CH_3	113.5, CH_2
14	38.9, CH_3	36.7, CH_3	124.2, CH	36.9, CH_3	124.2, CH	37.0, CH_3	29.4, CH_2	100.0, CH	27.4, CH_2	41.8, CH_3
15			131.4, qC		131.3, qC		122.2, CH	161.6, qC	122.2, CH	58.2, CH_2
16			17.7, CH_3		17.7, CH_3		134.8, qC	69.1, qC	132.4, qC	
17			25.7, CH_3		25.7, CH_3		17.9, CH_3	28.7, CH_3	25.8, CH_3	
18			16.4, CH_3		16.3, CH_3		25.8, CH_3	28.7, CH_3	17.7, CH_3	
19			37.0, CH_3		37.1, CH_3		37.0, CH_3	36.9, CH_3	35.1, CH_3	
$\underline{C}OCH_3$	169.0, qC									
$CO\underline{C}H_3$	23.8, CH_3									

[a] Recorded at 125 MHz; referenced to solvent at δ 77.0 ppm.

Table 2. ^1H NMR Assignments for Flustramines **2**−**4** and **6**−**7** in $CDCl_3$ (δ_H, mult, *J* in Hz)[a]

pos.	2	3	4	6	7
2	2.61, m	2.67, m	2.66, m	2.60, m	2.63, m
		2.55, td (8.0, 6.0)		2.80, m	
3	1.89, m	1.82, dt (12.0, 6.0)	2.05, dt (12.4, 7.3)	2.13, m	2.05, dt (12.5, 7.2)
	2.35, m	2.32, dt (12.0, 8.0)	2.20, dt (12.4, 5.4)	2.23, m	2.20, dt (12.5, 5.7)
4	7.08, br d (8)	7.30, s			
5	7.19, dd (8.1, 1.4)		6.02, d (8.5)	6.09, d (8.6)	
6			6.99, d (8.5)	7.00, d (8.6)	7.38, s
7	8.23, br s	6.82, s			
8a	4.77, br s	4.51, br s	4.33, s	4.44, s	4.35, s
9			2.50, dd (14.5, 8.4)	2.60, m	2.44, m
			2.66, m		2.71, dd (14.7, 6.6)
10	1.08, s	1.04, s	5.12, t (7.3)	5.05, br t (7)	4.97, br t (7)
11	0.99, s	1.00, s			
12	5.31, dd (17.4, 10.8)	5.93, dd (17.4, 10.9)	2.00, m	1.61, s	1.96, m
13	5.06, br m	*trans* 5.03, d (17.6)	2.00, m	1.67, s	1.96, m
		cis 5.12, d (10.9)			
14	2.49, s	2.41, s	5.03, br t (7.3)	2.50, s	5.01, m
16			1.66, s		1.67, s
17			1.57, s		1.58, s
18			1.59, s		1.59, s
19			2.45, s		2.43, s
N$\underline{H}$		4.51, br s	4.39, br s	4.49, br s	4.37, br s
O$\underline{H}$					5.30, br s
$CO\underline{C}H_3$	2.31, br s				

[a] Recorded at 500 MHz; referenced to residual solvent at δ 7.24 ppm.

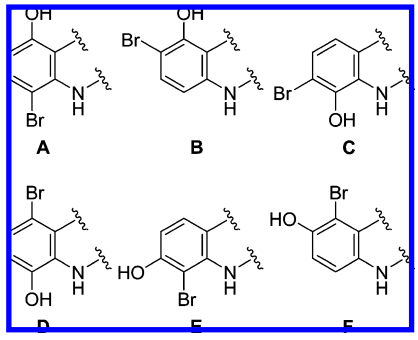
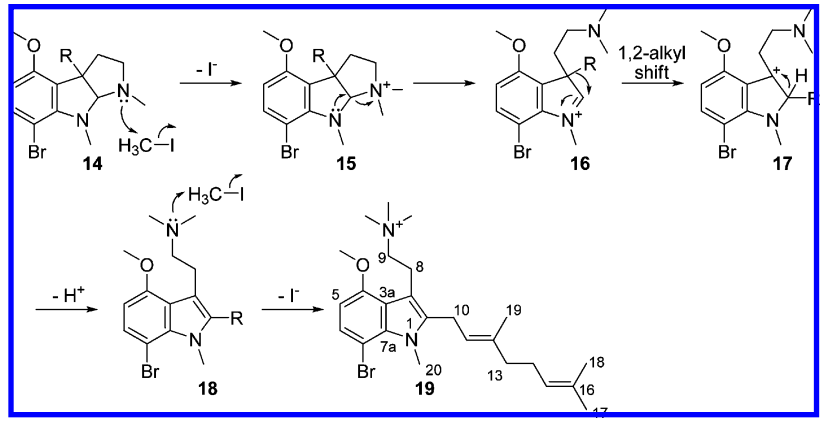


Figure 1. Possible substitution patterns for flustramine H (**4**).

protons H_2-10 and H-11 (weak). NOESY correlations observed between protons in the two side chains (H_2-8 and H_2-10) indicated that the two chains were adjacent on the indole. Structure **19** was fully consistent with the FABMS/MS data, which showed a loss of the N-Me_3 group, followed by sequential losses of side-chain fragments and the bromine atom.

A plausible mechanism by which **19** may be formed through ring-opening and Wagner−Meerwein rearrangement is depicted in Scheme 1. Expected methylation of the phenol group and indole nitrogen, yielding **14**, is followed by further methylation to yield the quaternary ammonium compound **15**. Ring-opening to produce **16** followed by a 1,2-alkyl shift of the geranyl chain and subsequent loss of a proton quenches the positive charge and forms the indole nucleus. Further methylation yields the charged ammonium salt **19**. This variant of the Wagner−Meerwein rearrangement is well known, and it has been demonstrated that the more electron rich R group will generally migrate preferentially,[28] though stability of the product and bond alignment can also affect which R group migrates.[29] Molecular modeling showed that in this case the two possible products had very similar energy minima (approximately

Scheme 1. Methylation and Rearrangement of Flustramine H



50 kJ/mol; Sculpt modeling program).[30] The two R groups were also similarly disposed toward the vacant p-orbital of the carbocation center, implying that the electron-donating ability of the two R groups is the dominant factor in deciding the product of Wagner−Meerwein rearrangement.

Fortunately the unexpected methylation product **19** was able to supply an important piece of information in determining the structure of flustramine H. A strong NOESY correlation from the OMe resonance (δ_H 3.38 ppm) in **19** to the shielded aromatic methine at δ_H 6.05 ppm was observed. This indicated that flustramine H must contain an aromatic methine adjacent to the hydroxyl group, thus eliminating substructures B and C. Of the two remaining substructures, A and D, the latter was disfavored by the absence of a NOESY correlation between the methoxy protons and the indole methyl. Thus, our NMR coupling data and chemical derivatization results were most consistent with substructure A as incorporated in **4**.

To further distinguish between substructures A and D in Figure 1, a series of deuterium exchange experiments were carried out with **4** and four model phenolic compounds, **20**−**23**. In every case, exchange of the phenolic proton with deuterium in the model compounds resulted in an upfield shift of the resonance frequencies of the phenolic carbon and the two *ortho* carbon atoms. The isotope shifts for the four model compounds are summarized in Figure 2. The largest chemical shift changes for all the model compounds occur at the phenolic carbon and its *ortho* carbons, with smaller shifts occurring at the more distant carbons. In **4**, the largest shift occurred at the phenolic carbon C-4 ($\Delta\delta$ −0.19 ppm) and the adjacent *ortho* carbons C-5 ($\Delta\delta$ −0.17 ppm) and C-3b ($\Delta\delta$ + 0.11 ppm). The chemical shift changes for the remaining indole carbon resonances were all less than 0.06 ppm. In the alternative substructure D, significant changes in the chemical shifts of the two most deshielded quaternary carbons (C-7 and C-7a), as well as the shielded aromatic carbon (C-6), would be predicted, but these were not observed. Thus the results of the isotope-shift experiments were consistent with substructure A, and this result in combination with the other spectroscopic and chemical data described above led to the structural assignment of flustramine H as **4**.

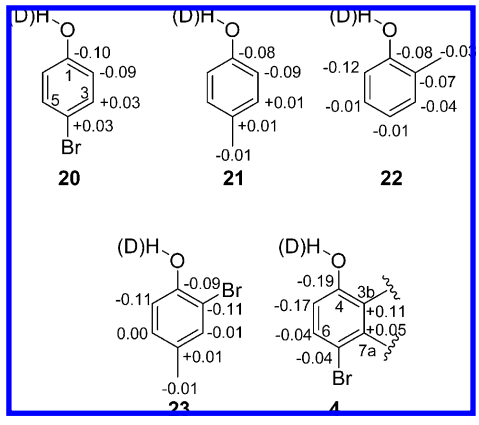
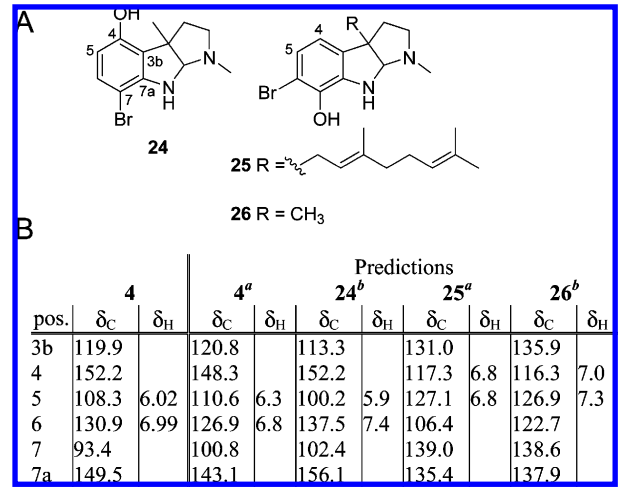


Figure 2. Model compounds for deuterium shift experiments with observed isotope shift ($\Delta\delta$) shown for each carbon.

Substructure A, however, required bromination at a site different from that in previously isolated flustramines, which would be more consistent with substructure C. Chemical shift predictions were calculated using ACD/CNMR Predictor and ACD/HNMR Predictor (both from Advanced Chemistry Development, Inc.) for model compounds (**4**, **24**−**26**) based on substructures A and C to further test our assigned structure (Figure 3). The results of the calculations further confirmed substructure A over substructure C. Specifically, the chemical shift differences for the ^{1}H and ^{13}C resonances predicted for positions 4 and 5 clearly favored a *para* relationship between the Br and OH groups as in substructure A. This was further supported by *ab initio* chemical shift calculations using two model compounds and comparing the shift data as illustrated in Figure 3. Additionally, the model compound with an *ortho* relationship between the Br and OH groups (as in substructure C, Figure 1) cannot account for the shielded aromatic proton (δ_H 6.02 ppm; δ_C 108.3 ppm) observed in the NMR spectra of flustramine H.

It should be mentioned that compound **25**, an isomer of **4**, has been reported along with other novel compounds as constituents of *F. foliacea*.[12] In this study, **25** corresponds to substructure C in



	4		Predictions							
			4[a]		24[b]		25[a]		26[b]	
pos.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3b	119.9		120.8		113.3		131.0		135.9	
4	152.2		148.3		152.2		117.3	6.8	116.3	7.0
5	108.3	6.02	110.6	6.3	100.2	5.9	127.1	6.8	126.9	7.3
6	130.9	6.99	126.9	6.8	137.5	7.4	106.4		122.7	
7	93.4		100.8		102.4		139.0		138.6	
7a	149.5		143.1		156.1		135.4		137.9	

Figure 3. Predicted ^{13}C and ^{1}H NMR shifts for model compounds **4** and **24**−**26**. (A) Structures of the model compounds. (B) Experimental and predicted shifts: (a) calculated using ACD/Laboratories software; (b) calculated *ab initio* using GIAO-HF within Gaussian 03 and the 6-31G** basis set.

Table 3. ^{1}H NMR Assignments for Flustramines **8**−**12** in $CDCl_3$ (δ_H, mult)[a]

pos.	8	9	10	11	12
2	2.62, m	2.66, m	2.70, br m	2.63, ddd (10.4, 8.8, 5.6) 2.85, ddd (8.8, 7.0, 1.8)	2.70, m
3	2.05, dt (12.7, 6.7) 2.19, dt (12.7, 5.9)	2.05, m 2.19, m	1.80, m 2.20, m	1.97, ddd (11.8, 10.4, 7.0) 2.09, ddd (11.8, 5.6, 1.8)	1.62, m 2.39, m
4					7.00, d (8.0)
5					6.80, dd (8.0, 1.6)
6	7.25, s	6.89, s	7.34, s	6.98, s	
7					6.75, d (1.6)
8a	4.39, br s	4.28, s	4.49, br s	5.21, br s	4.56, br s
9	2.47, dd (15.0, 8.1) 2.69, dd (15.0, 6.6)	2.52, m 2.58, m	2.70, br m	2.59, m	
10	4.96, br t (7)	5.05, br t (6.8)	5.06, br t (7)	5.01, br t (7)	0.99, s
11					1.10, s
12	1.61, s	1.60, s	1.63, s	1.67, s	6.03, dd (17.6, 10.5)
13	1.65, s	1.68, s	1.55, s	1.64, s	*trans* 5.10, d (17.6) *cis* 5.14, d (10.5)
14	2.44, s	3.22, br d (7)	6.41, s	3.10, dd (15.9, 7.5) 3.19, dd (15.9, 7.5)	2.52, s
15		5.24, br t (7)		5.23, t (7.5)	4.89, s
17		1.76, s	1.64, s	1.73, s	
18		1.76, s	1.64, s	1.69, s	
19		2.44, s	2.47, s	2.57, s	
OH	5.30, br s				
NH	4.39, br s	4.24, br s	4.41, br s		
NH_2				3.94, br s	

[a] Recorded at 500 MHz; referenced to residual solvent at δ 7.24 ppm.

Figure 1, which is the substitution pattern that one might expect given precedent in the flustramine family. However, the reported spectroscopic data are nearly identical to those of **4**. In light of our studies, the reported structure should be reconsidered.

The detailed structural characterization of **4** revealed an additional substitution pattern in these alkaloids that, once established, permitted straightforward identification of the related metabolite flustramine I (**6**). The molecular formula for **6** was established as $C_{16}H_{21}BrN_2O$ ($[M]^+$ 336.0853), indicating the loss of an isoprene unit (C_5H_8) when compared with the molecular formula of **4**. The ^{1}H NMR data (Table 2) for **6** confirmed this relationship by displaying resonances for only one olefinic methine and two olefinic methyls, consistent with a regular C_5 isoprene unit and unlike the reversed isoprene side chain found in **1**, **2**, and **3**. The ^{1}H and ^{13}C NMR data for the indoline ring system in **4** and **6** were virtually identical, indicating the functional groups and substitution pattern are the same in both molecules. Extensive 2D NMR analysis including HMQC, HMBC, COSY, and NOESY confirmed the structure.

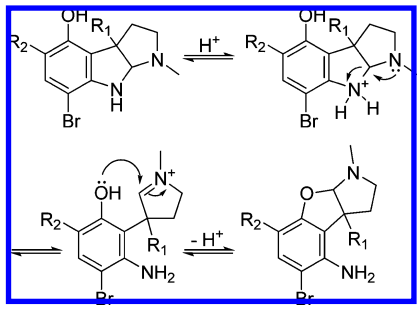
A characteristic triplet pattern for the molecular ion in the mass spectra of two additional compounds, **7** and **8**, indicated they were both dibromo derivatives. The first, flustramine J (**7**), possessed a molecular formula of $C_{21}H_{28}Br_2N_2O$ ($[M + H]^+$ 483.0644), indicating the molecule contained an additional bromine atom compared with **4**. Inspection of the ^{1}H NMR data (Table 2) revealed only one aromatic proton (δ_H 7.38 ppm), hence locating the additional bromine on the aromatic ring. The chemical shift of the aromatic methine suggested that it was *meta* to the phenolic moiety, implying that the two bromines were at C-5 and C-7. The ^{1}H and ^{13}C NMR data for the isoprenoid side chain were very similar to those of **4**, indicating that the only difference between the compounds was in the aromatic portion of the molecule. The structure was fully supported by 2D NMR analysis including HMQC, HMBC, COSY, and NOESY.

The molecular formula $C_{16}H_{20}Br_2N_2O$ ($[M]^+$ 413.9944) for the second dibromo derivative, flustramine K (**8**), suggested the compound was the dibromo derivative of **6**. Indeed, comparison of the ^{1}H NMR data for **4**, **6**, **7**, and **8** (Tables 2 and 3) confirmed that **8** was the 5,7-dibromo analogue of **6** in the same manner that **7** was the 5,7-dibromo analogue of **4**. The ^{1}H and ^{13}C NMR data for the isoprenoid side chain in **8** were also similar to that of **6**, establishing the same C_5 side chain. 2D NMR analysis supported this structure.

Accurate mass measurements on the molecular ion of flustramine L (**9**) ($C_{21}H_{29}BrN_2O$; $[M]^+$ 404.1464) suggested it was an isomer of **4**, although preliminary inspection of the ^{1}H NMR data (Table 3) indicated that **9** was quite different from **4**. The most obvious difference in the ^{1}H NMR data was the presence of only one aromatic methine (δ_H 6.89 ppm) and two separate C5 isoprene units. For example, the ^{1}H NMR spectrum of **9** displayed two olefinic methines (δ_H 5.05 and 5.24 ppm) but four olefinic methyls at δ_H 1.60 (3H), 1.68 (3H), and 1.76 ppm (6H), indicative of two C_5 isoprene units as opposed to a single C_{10} chain. This information, together with the presence of only one aromatic methine, was consistent with one isoprene subunit attached to the aromatic ring, and this was confirmed by HMBC correlations from the benzylic methylene protons (δ_H 3.22 ppm) to C-6 and C-3b. Furthermore, comparison of the ^{13}C NMR data (Table 1) for the physostigmine carbon skeleton of **9** and **6** showed they were very similar (within 1.5 ppm), with the exception of the chemical shift for C-5, suggesting this as the point of attachment. The interconversion of flustramines L and N, described shortly, confirmed this.

The molecular formula ($C_{21}H_{27}BrN_2O_2$; $[M]^+$ 418.1265) of flustramine M (**10**) corresponded to the molecular formula of **9**, plus the addition of H_2O. The ^{1}H and ^{13}C NMR data for **10** revealed the chemical shift resonances for C-2, C-3, C-3a, C-8a, and C-9−C-13 were very similar to the corresponding positions in **9**, suggesting that this portion of both molecules was identical. However, the resonances assigned to the isoprene aromatic substituent in **9** were replaced in **10** by two new olefinic carbon resonances (δ_C 161.6 ppm, qC; and δ_C 100.0 ppm, CH; δ_H 6.41 ppm) and a new deshielded singlet (δ_C 69.1 ppm) in addition to two aliphatic methyl groups. These shifts suggest a five-membered heteroaromatic ring system and a tertiary alcohol. Two coincident deshielded methyl groups (δ_H 1.64 ppm, δ_C 28.7 ppm) showed HMBC correlations with the alcohol carbon and also with the furano singlet C-15 (δ_C 161.6 ppm), suggesting an allylic carbon bearing a hydroxy group and the two methyls. A new aromatic methine (δ_H 6.41 ppm) also showed HMBC correlations with C-15 and C-4 (δ_C 150.3 ppm), which supported the presence of the furanobenzene moiety in **10**. NOESY correlations from H-14 to H_3-17, H_3-18, and H-6 confirmed the substitution pattern as shown. One plausible origin for **10** could

Scheme 2. Rearrangement of **4** ($R_1 = C_{10}H_{17}$, $R_2 = H$) and **9** ($R_1 = C_5H_9$, $R_2 = C_5H_9$)



involve intramolecular cyclization of **9**. This raises the question of whether or not **10** is an isolation artifact produced from **9**. No conversion of **9** into **10** was observed, even after several years of storage or after exposure to mild acid (*p*TSOH in EtOH), making it unlikely that **10** was an artifact. However, **9** does slowly interconvert with its isomer flustramine N (**11**) (see below).

The mass spectrometric data for flustramine N (**11**) ($C_{21}H_{29}BrN_2O$; $[M]^+$ 404.1468) established that it was isomeric with **4** and **9**. The ^{1}H NMR and ^{13}C NMR data for positions C-2, C-9−C-13, and C-14−C-19 in **11** were similar to those for **9**, suggesting that these portions of the molecule were the same. However, by the same comparison, the resonances associated with the methine at position 8a were quite different. For all of the other phenolic flustramines described to date, the H-8a methine resonates between δ_H 4.20 and 4.50 ppm, whereas in **11**, H-8a was considerably deshielded at δ_H 5.21 ppm. Similarly, the ^{13}C resonance for C-8a was also deshielded and now appeared at δ_C 106.2 ppm, consistent with a carbon bonded to at least one oxygen. While the imine proton of the other flustramines usually resonates in the region δ_H 4.2−4.5 ppm, the NMR data of flustramine N showed two protons not bonded to carbon resonating at δ_H 3.94 ppm. This denoted the presence of a primary rather than a secondary amine in flustramine N and, taken with the other data, suggested that the compound did not possess the physostigmine skeleton. Instead, structure **11** was further supported by additional NMR data. The amine protons showed an HMBC correlation with the shielded bromo-substituted carbon C-5 (δ_C 100.6 ppm), as well as C-3b (δ_C 115.8 ppm). The benzylic methylene protons H_2-14 (δ_H 3.10 and 3.19 ppm) of the isoprene substituent showed HMBC correlations with C-7 (δ_C 114.9 ppm), C-6 (δ_C 131.3 ppm), and C-7a (δ_C 158.3 ppm), as well as the olefinic carbons C-15 (δ_C 122.2 ppm) and C-16 (δ_C 132.4 ppm). An additional correlation between H-8a and C-7a helped to further establish the substitution pattern as shown, which was also supported by NOESY correlations between H_2-14 and H-6 and between NH_2 and H_2-9.

Following prolonged storage (~5 y) of **11**, the ^{1}H NMR spectra displayed new resonances, which upon closer inspection matched those for **9**. This prompted investigation of **9**, which had been stored for a similar period of time, and the ^{1}H NMR data revealed an approximate 1:1 ratio of **9** and **11**, confirming interconversion of the two compounds. A possible mechanism is presented in Scheme 2. For such a mechanism to operate, it is essential to have a phenolic group located at position C-4, and so the presence of **9** and **11** and their interconversion adds further indirect support for the proposed structure of **4**. In order for this to be valid, it would be expected that the corresponding interconversion product of **4** would be observed following extended storage. Indeed, careful reinvestigation of the ^{13}C NMR data of **4** suggested that such an interconversion was occurring. The characteristic resonance assigned to the deshielded methine C-8a was particularly distinct. However, the isomer of **4** was present only in trace amounts (<5%), suggesting that the interconversion of **4** must proceed even more slowly than that of **9**.

The isolation of flustramine O (**12**) initially posed an interesting conundrum: the mass spectrometric data showed a molecular formula of $C_{33}H_{42}Br_2N_4$ ($[M]^+$ 652.1769), yet the ^{1}H NMR and ^{13}C NMR data (Tables 1 and 3) suggested that the compound was very similar to the known metabolite **1**.[5] In fact, the only significant differences in the ^{1}H spectra of **1** and **12** were the absence of the amine proton in the spectrum of **12** and the appearance of a new singlet at δ_H 4.89 ppm. The ^{13}C NMR data were also very similar, with the exception of the carbon resonances assigned to C-8a (δ_C 90.5 ppm), C-14 (δ_C 41.8 ppm), and a new resonance at δ_C 58.2 ppm. This latter resonance (δ_H 4.89 ppm/δ_C 58.2 ppm) integrated for only one proton, yet the DEPT spectra showed that it was a methylene. This observation, combined with a molecular formula that corresponded to two **1** units plus an additional methylene, suggested that flustramine O was a dimer of **1** linked through a methylene group. The absence of the imine resonance in the ^{1}H NMR spectrum of **12** implied that the two **1** molecules were linked through their respective imine functions by a single methylene bridge.

The molecular formula of flustramine P (**13**) ($C_{38}H_{50}Br_2N_4O$; $[M]^+$ 736.2370) indicated that it too was substantially larger than most flustramines. On the basis of the example of **12**, it appeared that this latest compound was composed of two flustramine molecules linked in some fashion. However, in contrast to **12**, the ^{1}H and ^{13}C NMR data for **13** clearly showed that this dimer was not symmetrical, nor did it contain two identical subunits (Table 4). Instead, the data displayed resonances for a single methylene group, a unit of **1**, and a unit of a derivative of **4**. Furthermore, the ^{1}H and ^{13}C NMR data for the **1** portion of the molecule were essentially identical with the data for **12**, suggesting that it was similarly linked through the indoline nitrogen. This was supported by the presence of only one non-carbon-bonded proton resonance in the NMR spectra of **13**. The NMR data for the flustramine H subunit established the presence of only one aromatic methine (Table 4), implying a linkage through the aromatic ring of the flustramine H moiety. The deshielded nature of this aromatic methine (δ_H 7.10 ppm) suggested the substitution of flustramine H at C-5′. These conclusions were supported by HMBC analyses, which showed correlations from the bridging methylene protons (H_2-15; δ_H 4.14 and 4.25 ppm) to C-7a and C-8a of the dihydroflustramine C subunit and to C-4′ and C-6′ of the flustramine H subunit, thus confirming the structure of flustramine P as **13**. The isolation of these methylene-linked compounds was surprising. As natural products, they could arise by joining two flustramine units through a one-carbon unit derived from formate. However, while it is interesting to speculate on a biosynthetic origin, it is more likely they are artifacts of the workup process that employed CH_2Cl_2.

The relative configuration of the ring juncture joining C-3a and C-8a was found to be *cis* for the novel flustramine congeners on the basis of the presence of NOE interactions between H-8a and the isoprenyl/reversed-isoprenyl/geranyl side chains.[3] The relative configuration of **11** could not be assigned unambiguously due to overlap of multiple resonances. Furthermore, all of the newly isolated flustramines described here exhibited levorotation. Although we only report the relative configuration for the novel flustramines, it is worth noting that enantioselective syntheses of derivatives of flustramine B[16,17,24] and flustramine A[24] have consistently shown levorotatory species having an absolute configuration equivalent to that shown for **1**−**10**, **12**, and **13**, although the *R* and *S* designations vary with the types of substituents at C-3a and N-8.

The flustramines described here are a family of biologically active, biosynthetically related alkaloids. The antimicrobial potency was determined for a number of flustramines (Table 5), with most of the tested compounds showing broad-spectrum activity. The two dimers **12** and **13** did not exhibit any detectable activity. Acetylation of the indole nitrogen in **2** relative to **1** also reduced the potency

Table 4. Complete NMR Assignments for Flustramine P (**13**) in $CDCl_3$[a]

pos.	δ_C (mult.)	δ_H (*J* in Hz)	pos.	δ_C (mult.)	δ_H (*J* in Hz)
2	52.4, CH_2	2.65, m	2′	52.4, CH_2	2.65, m
3	32.8, CH_2	1.78, dd (12.5, 5.0)	3′	36.3, CH_2	2.04, m
		2.32, m			2.19, m
3a	64.6, qC		3a′	59.4, qC	
3b	130.8, qC		3b′	116.2, qC	
4	125.5, CH	6.90, d (7.9)	4′	153.4, qC	
5	119.1, CH	6.64, dd (7.9, 1.6)	5′	122.3, qC	
6	122.1, qC		6′	132.7, CH	7.10, s
7	108.8, CH	6.47, d (1.6)	7′	91.7, qC	
7a	151.3, qC		7a′	148.2, qC	
8a	93.6, CH	4.40, s	8a′	86.1, CH	4.22, s
9	40.7, qC		9′	35.1, CH_2	2.53, m, 2.70, m
10	23.8, CH_3	0.94, s	10′	121.0, CH	5.00, m
11	23.0, CH_3	1.07, s	11′	137.1, qC	
12	144.7, CH	5.94, dd (17.4, 11.0)	12′	40.0, CH_2	1.97, m
13	114.0, CH_2	*trans* 5.02, d (17.4)	13′	26.8, CH_2	1.97, m
		cis 5.13, d (11.0)	14′	124.4, CH	5.03, m
14	41.7, CH_3	2.48, s	15′	131.2, qC	
15	48.3, CH_2	4.14, d (15.0)	16′	17.7, CH_3	1.67, s
		4.25, d (15.0)	17′	25.8, CH_3	1.58, s
			18′	16.4, CH_3	1.60, s
			19′	37.2, CH_3	2.43, s
			NH		4.30, br s

[a] Recorded at 300 MHz; referenced to residual solvent at δ_H 7.24 ppm and δ_C 77.0 ppm. Assignment supported by HMQC and HMBC experiments obtained at 500 MHz.

Table 5. Antimicrobial Activity of Flustramines: Zone of Inhibition (mm)

	2	**5**	**6**	**9**	**12**	**13**
mol/disk ($\times 10^6$)	1.4	1.3	1.5	1.2	0.9	0.7
E. coli		22	25	24		
M. luteus		23	29	25		
C. utilis	18	28	29	30		
S. cerevisiae		16	15	20		

against most of the tested organisms, suggesting that antimicrobial activity is hindered by substitution of the indoline nitrogen.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Ultraviolet and infrared spectra were recorded with a GCA-MacPherson Series 700 and a Perkin-Elmer 283B spectrophotometer. The ^1H NMR and ^{13}C NMR data were obtained at either 500 and 125 MHz, respectively, or 300 and 75 MHz, as indicated, on a Bruker AMX 500 or Bruker AML 300 spectrometer, respectively. Electron impact mass spectra were obtained with a Dupont model 21-110B double-focusing spectrometer used in electrical detection mode. FAB mass spectra were obtained using a Micromass/AutoSpec oa-TOF hybrid sector/time-of-flight mass spectometer (Manchester, UK). LSIMS (liquid secondary ion mass spectrometry)/MS/MS experiments were performed using a collision energy of 800 eV and Xe collision gas. The LSIMS matrix used was a 1:1 mixture of *m*-nitrobenzyl alcohol and glycerol with 1% trifluoroacetic acid. Exact mass measurements were carried out at a mass resolution of 10 000 using voltage scanning, and the peaks were calibrated using polyethylene glycol reference peaks. TLC was carried out using glass-backed silica TLC plates (Merck) and 100% EtOAc as solvent and were visualized with a vanillin spray reagent (0.5 g vanillin/100 mL H_2SO_4/EtOH, 4:1) and gentle heat. Large-scale HPLC was performed with a Waters Associates Prep System, and semipreparative HPLC was carried out using a Waters Associates model 6000 pump, U6K injector, and a model 450 variable-wavelength detector.

Animal Material. The bryozoan *F. foliacea* was collected in the Minas Basin of the Bay of Fundy off the New Brunswick and Nova Scotian shores and identified by Prof. Sherman Blakeney of the Department of Biology, Acadia University, Wolfville, N.S. A voucher specimen has been retained at the Nova Scotia Museum, Halifax, N.S., under the catalog number 17573.

Antimicrobial Assay. Antimicrobial activity was determined using a standard disk-diffusion assay. A solution of the test compound in MeOH was applied to a 6 mm diameter filter paper and air-dried. The disk was transferred to an agar plate overgrown with the test organism, and the development of zones of growth inhibition was measured from the outer edge of the disk. The strains used for testing were *Escherichia coli* (ATCC 11775), *Micrococcus luteus* (ATCC 49732), *Candida utilis* (ATCC 9950), and *Saccharomyces cerevisiae* (ATCC 9763).

Isolation of Flustramines. The bryozoan (6 kg) was ground to a pulp and extracted with methanol at room temperature for 2 days. The methanolic extract (10 L) was concentrated under reduced pressure until most of the methanol was removed, and the residue was shaken against dichloromethane. Half of the dichloromethane-soluble fraction (15.0 g) was chromatographed on a pad of silica gel (Merck) to yield two indole-rich fractions eluting with 0−25% EtOAc/Et_2O (1.726 and 1.756 g). These fractions were further purified by preparative liquid chromatography (C18 Lobar column, 30 × 2 cm, 100 mL/min, 80−100% MeOH in H_2O; and silica, Merck, TLC grade, 1.5 mL/min, 0−10% MeOH in CH_2Cl_2) to yield a number of fractions including pure dihydroflustramine C (**1**; 455.9 mg). Other fractions contained complex mixtures of the indole alkaloids, which were eventually purified (after several preparative silica and reversed-phase steps involving the pooling of similar fractions) by semipreparative HPLC (Whatman Partisil M9 10/50 ODSII 10% H_2O/MeOH (0.2% Et_3N) or CSC S5W silica column, 25 × 0.9 cm, 4% MeOH/CH_2Cl_2) to yield **1** and flustramines F−P.

Dihydroflustramine C (1): crystalline solid (616 mg), all spectroscopic data in good agreement with literature data.[5]

Acetylation of 1. Magnesium turnings (0.07 g, 2.9 mmol), an iodine crystal, and ethyl bromide (0.25 mL, 0.37 g, 3.3 mmol) were stirred in diethyl ether (5 mL) and gently heated for 20 min. An ethereal solution of **1** (64 mg in 1 mL, 0.2 mmol) and acetyl chloride (150 μL, 2 mmol) was added dropwise, and the solution maintained at reflux for 20 min. After this time the reaction mixture was poured onto crushed ice, acidified (AcOH), and extracted with CH_2Cl_2 (3 × 15 mL). The organic solvent was evaporated under reduced pressure to yield a mixture (44 mg). This mixture was purified by HPLC (Whatman ODSII 10% H_2O/MeOH, 3 mL/min) to yield pure *N*-acetyl dihydroflustramine C (24.1 mg). This product was spectroscopically identical to **2**.

Flustramine F (2): oil (31 mg); $[\alpha]_D$ −22 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 219 (4.41), 252 (3.98), 287 (3.39); IR (film) ν_{max} 3250, 2970, 1660, 1585, 1470, 1410, 1385, 1290, 1110 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 2; HREIMS *m/z* 362.0987 (calcd for $C_{18}H_{23}{}^{79}BrN_2O$ 362.0993); *m/z* 364.0972 (calcd for $C_{18}H_{23}{}^{81}BrN_2O$ 364.0974); TLC R_f 0.36 (gray upon spraying and heating).

Flustramine G (3): oil (7 mg); $[\alpha]_D$ −80 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} 212, 247, 310; IR (film) ν_{max} 3420, 2960, 1590, 1470, 1410, 1350, 1160, 1090 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 2; EIMS *m/z* (%) 398/400/402 (15/30/15), 329/331/333 (60/100/55), 286/

288/290 (15/20/15), 250/252 (20/20), 171 (20), 128 (10), 69 (25); HREIMS *m/z* 397.9990 (calcd for $C_{16}H_{20}{}^{79}Br_2N_2$ 397.9994); *m/z* 401.9960 (calcd for $C_{16}H_{20}{}^{81}Br_2N_2$ 401.9953); TLC R_f 0.17 (yellow upon spraying then gray upon heating).

Flustramine H (4): oil (36 mg); $[\alpha]_D$ −58 (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (4.77), 246 (4.20), 295 (3.84); IR (film) ν_{max} 3415, 2975, 1623, 1593, 1469, 1380, 1100, 1037 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 2; EIMS *m/z* (%) 404/406 (48/50), 335/337 (70/70), 267/269 (98/100), 224/226 (25/28), 188 (22), 117 (12), 69 (32); HREIMS *m/z* 404.1460 (calcd for $C_{21}H_{29}{}^{79}BrN_2O$ 404.1464); TLC R_f 0.31 (purple upon spraying and heating).

Debromoflustramine H (5): 4 (12 mg) and $LiAlH_4$ (2 mg) were heated at reflux in dry diethyl ether (5 mL) for 5 h before quenching with H_2O (100 mL). The product was extracted with diethyl ether and the organic solvent removed under reduced pressure to yield a mixture, which was purified by HPLC (Whatman ODSII 10% H_2O/MeOH (0.3% Et_3N), 3 mL/min) to yield **4** (2.5 mg) and **5** (2.2 mg) as an oil: ^1H NMR ($CDCl_3$, 300 MHz) 6.90 (dd, 7.9 Hz, H-6), 6.19 (d, 7.9 Hz, H-5 or H-7), 6.14 (7.9 Hz, H-5 or H-7), 5.00 (br m, H-10 and H-14), 4.83 (br s, H-8a), 3.09 (m, H_a-2), 2.60 (br m, H_2-9, H_b-2), 2.57 (s, H_3-19), 2.43 (m, H_a-3), 2.24 (m, H_b-3), 1.94 (m, H_2-12 and H_2-13), 1.65 (s, H_3-18 or H_3-16), 1.60 (s, H_3-18 or H_3-16), 1.56 (s, H_3-17); EIMS *m/z* (%) 326 (35), 269 (15), 257 (98), 214 (25), 189 (98), 146 (100), 86 (99); HRFABMS *m/z* 327.2444 (calcd. for $[M + H]^+$ $C_{21}H_{31}N_2O$ 327.2436).

Flustramine I (6): oil (20 mg); $[\alpha]_D$ −87 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (4.79), 297 (3.65); IR (film) ν_{max} 2980, 2940, 1675, 1640, 1520, 1430, 1395, 1200, 1170 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 2; EIMS *m/z* (%) 336/338 (60/50), 279/281 (70/70), 267/269 (100/80), 224/226 (60/55), 188 (40), 171 (10), 159 (6), 146 (5), 84 (70); HREIMS *m/z* 336.0853 (calcd for $C_{16}H_{21}{}^{79}BrN_2O$ 336.0838); *m/z* 338.0823 (calcd for $C_{16}H_{21}{}^{81}BrN_2O$ 338.0817); TLC R_f 0.16 (purple upon spraying and heating).

Flustramine J (7): oil (8 mg); $[\alpha]_D$ −40 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} 217, 293; IR (film) ν_{max} 3410, 2970, 2930, 1720, 1670, 1600, 1450, 1380, 1270, 1160 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 2; EIMS *m/z* (%) 482/484/486 (30/65/30), 413/415/417 (50/100/50), 345/347/349 (15/30/17), 302/304/306 (5/10/5), 69 (20); HRFABMS *m/z* 483.0644 (calcd for $[M + H]^+$ $C_{21}H_{29}{}^{79}Br_2N_2O$ 483.0647); *m/z* 485.0633 (calcd for $[M + H]^+$ $C_{21}H_{29}{}^{79}Br^{81}BrN_2O$ 485.0626); TLC R_f 0.41 (purple upon spraying and heating).

Flustramine K (8): oil (2.7 mg); $[\alpha]_D$ −29 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} 215, 245, 303; IR (film) ν_{max} 3226, 2960, 1720, 1600, 1450, 1300, 1260, 1160 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 3; EIMS *m/z* (%) 414/416/418 (35/65/30), 357/359/361 (50/100/50), 345/347/349 (70/100/50), 302/304/306 (30/55/30), 225 (20), 116 (20), 96 (35), 69 (75); HRFABMS *m/z* 413.9944 (calcd for $[M + H]^+$ $C_{16}H_{20}{}^{79}Br_2N_2O$ 413.9943); *m/z* 415.9926 (calcd for $[M + H]^+$ $C_{16}H_{20}{}^{79}Br^{81}BrN_2O$ 415.9923); TLC R_f 0.28 (mustard upon spraying and heating).

Flustramine L (9): oil (5.4 mg); $[\alpha]_D$ −53 (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (4.82), 302 (3.83); IR (film) ν_{max} 3420, 2970, 2930, 2800, 1620, 1590, 1470, 1460, 1380, 1350, 1270, 1150 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 3; EIMS *m/z* (%) 404/406 (80/100), 361/363 (15/20), 347/349 (45/60), 335/337 (70/70), 291/292 (25/20), 279/280 (35/35), 238 (35), 69 (32); HREIMS *m/z* 404.1464 (calcd for $C_{21}H_{29}{}^{79}BrN_2O$ 404.1464); *m/z* 406.1450 (calcd for $C_{21}H_{29}{}^{81}BrN_2O$ 406.1443); TLC R_f 0.22 (yellow upon spraying and heating).

Flustramine M (10): oil (2.8 mg); $[\alpha]_D$ −159 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (4.50), 209 (4.06), 318 (3.85); IR (film) ν_{max} 3420, 2970, 2940, 2870, 2800, 1640, 1460, 1430, 1380, 1340, 1260, 1140 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 3; EIMS *m/z* (%) 418/420 (25/25), 400/402 (5/5), 349/351 (40/40), 279/281 (10/10), 243 (70), 159 (100), 69 (32); HREIMS *m/z* 418.1265 (calcd for $C_{21}H_{27}{}^{79}BrN_2O_2$ 418.1256); *m/z* 420.1242 (calcd for $C_{21}H_{27}{}^{81}BrN_2O_2$ 420.1236); TLC R_f 0.13 (orange upon spraying and heating).

Flustramine N (11): oil (2.7 mg); $[\alpha]_D$ −61 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (4.73), 299 (3.79); IR (film) ν_{max} 3490, 3390, 2975, 2860, 2815, 1630, 1480, 1430, 1360, 1260, 1150 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 3; EIMS *m/z* (%) 404/406 (35/25), 335/337 (10/05), 279/281 (100/90), 251 (5), 157 (2), 69 (18); EIMS *m/z* 404.1468 (calcd for $C_{21}H_{29}{}^{79}BrN_2O$ 404.1464); *m/z* 406.1454 (calcd for $C_{21}H_{29}{}^{81}BrN_2O$ 406.1443); TLC R_f 0.20 (yellow upon spraying and heating).

Flustramine O (12): oil (46.0 mg); $[\alpha]_D$ −211 (*c* 0.63, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.82), 259 (4.32), 312 (3.89); IR (film) ν_{max} 3090, 2970, 2870, 2800, 1640, 1590, 1480, 1420, 1310, 1270, 1170 cm^{-1}; ^{13}C NMR Table 1, ^1H NMR Table 3; EIMS *m/z* (%) 652/654/656 (20/40/20), 583/585/586 (70/100/40), 320 (80), 251/253 (30/30), 172 (75), 69 (50); HREIMS *m/z* 652.1769 (calcd for $C_{33}H_{42}{}^{79}Br_2N_4$ 652.1777); *m/z* 654.1758 (calcd for $C_{33}H_{42}{}^{81}Br_2N_4$ 654.1758); TLC R_f 0.35 (yellow upon spraying, then gray upon heating).

Flustramine P (13): oil (25.0 mg); $[\alpha]_D$ −5 (*c* 0.44, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 213 (5.06), 259 (4.53), 305 (4.07); IR (film) ν_{max} 3420, 2970, 2930, 2790, 1620, 1590, 1490, 1320, 1160 cm^{-1}; ^{13}C NMR Table 4, ^1H NMR Table 4; EIMS *m/z* (%) 736/738/740 (1/2/1), 667/669/667 (2/4/2), 320/322 (30/35), 251/253 (95/100), 208/210 (30/35), 172 (35), 128 (25), 69 (15); EIMS *m/z* 736.2370 (calcd for $C_{38}H_{50}{}^{79}Br_2N_4O$ 736.2352); *m/z* 738.2347 (calcd for $C_{38}H_{50}{}^{79}Br^{81}BrN_4O$ 738.2331); TLC R_f 0.22 (purple upon spraying and heating).

Permethylflustramine H (19). 4 (1.5 mg) was dissolved in acetone (1 mL). To the stirred solution were added MeI (0.1 mL) and K_2CO_3 (20 mg), and the solution was allowed to stir at room temperature for 12 h. The reaction was diluted with H_2O and extracted with CH_2Cl_2 (3 × 5 mL) to yield, after removal of the organic solvent, a mixture of polar compounds (3 mg). Initial purification by silica flash chromatography yielded one fraction of interest (0.96 mg). Further purification by HPLC (Zorbax RX-C8, 0.46 × 25 cm, gradient elution: 67% ACN/H_2O (0.1% TFA) to 90% ACN/H_2O (0.1% TFA) over 9 min, 0.5 mL/min) yielded a major fraction, **19** (0.40 mg), as an oil: ^1H NMR (60% benzene-d_6/$CDCl_3$, 500 MHz) 7.15 (H-6, under solvent), 6.05 (d, 7.5 Hz, H-5), 5.05 (m, H-15), 4.89 (m, H-11), 3.63 (s, H_3-20), 3.38 (s, OMe), 3.26 (brd, 4 Hz, H_2-10), 2.85 (m, H_2-9), 2.72 (H_2-8), 2.60 (s, NMe_3), 2.06 (m, H_2-14), 1.98 (m, H_2-13), 1.68 (s, H_3-19), 1.62 (s, H_3-18), 1.50 (H_3-17); ^{13}C NMR (60% benzene-d_6/$CDCl_3$, from HMQC and HMBC, not all carbons seen) 153.9 (C-4), 139.0 (C-2), 138.0 (C-12), 135.0 (C-7a), 132.0 (C-16), 128.5 (C-3), 124.5 (C-15), 121.0 (C-11), 120.0 (C-6 or C-3a), 102.0 (C-5), 96.1 (C-7), 67.5 (C-9), 55.5 (OMe), 52.5 (NMe_3), 40.0 (C-13), 33.9 (C-20), 27.0 (C-14), 26.2 (C-17), 23.4 (C-10), 18.2 (C-18), 16.8 (C-19); FABMS *m/z* (%) 461/463 (50/40), 402/404 (15/15), 338 (25), 246 (100), 226 (47); FABMS/MS ion selected 463.50 *m/z* (%) 404 (100), 389 (2), 349 (35), 335 (20), 334 (20), 320 (57), 306 (27), 293 (54), 281 (68), 280 (57), 266 (53), 254 (30), 225 (50); FABMS/MS ion selected 461.50 *m/z* (%) 402 (100), 387 (28), 347 (25), 333 (20), 332 (23), 318 (60), 304 (26), 291 (50), 279 (75), 278 (60), 264 (55), 252 (33), 225 (53); HRFABMS *m/z* 461.2178 (calcd for $[M]^+$ $C_{25}H_{38}{}^{79}BrN_2O$ 461.2162); *m/z* 463.2156 (calcd for $C_{25}H_{38}{}^{81}BrN_2O$ 463.2147).

Acknowledgment. The authors gratefully acknowledge J. M. Curtis and J. A. Walter for providing much of the mass spectrometric and NMR data. We also express gratitude to I. Burton for assistance with the preparation of figures of NMR data. Issued as NRCC publication 51723.

Supporting Information Available: ^1H NMR spectra for **2**−**13**; ^{13}C NMR and HMQC spectra for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6846–6848.
(2) Carlé, J. S.; Christophersen, C. *J. Am. Chem. Soc.* **1979**, *101*, 4012–4013.
(3) Carlé, J. S.; Christophersen, C. *J. Org. Chem.* **1980**, *45*, 1586–1589.
(4) Carlé, J. S.; Christophersen, C. *J. Org. Chem.* **1981**, *46*, 3440–3443.
(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) Holst, P. B.; Anthoni, U.; Christophersen, C.; Nielsen, P. H. *J. Nat. Prod.* **1994**, *57*, 997–1000.
(8) Keil, P.; Nielsen, E. G.; Anthoni, U.; Christophersen, C. *Acta Chem. Scand. B* **1986**, *B40*, 555–558.
(9) Holst, P. B.; Anthoni, U.; Christophersen, C.; Nielsen, P. H. *J. Nat. Prod.* **1994**, *57*, 1310–1312.
(10) Wulff, P.; Carlé, J. S.; Christophersen, C. *Comp. Chem. Physiol., B: Biochem. Mol. Biol.* **1982**, *71*, 523–524.
(11) Sjöblom, T.; Bohlin, L.; Christophersen, C. *Acta Pharm. Suecica* **1983**, *20*, 415–418.
(12) Peters, L.; König, G. M.; Terlau, H.; Wright, A. D. *J. Nat. Prod.* **2002**, *65*, 1633–1637.

(13) Peters, L.; Wright, A. D.; Kehraus, S.; Gündisch, D.; Tilotta, M. C.; König, G. M. *Planta Med.* **2004**, *70*, 883–886.
(14) Hino, T.; Tanaka, T.; Matsuki, K.; Nakagawa, M. *Chem. Pharm. Bull.* **1983**, *31*, 1806–1808.
(15) Muthusubramanian, P.; Carlé, J. S.; Christophersen, C. *Acta Chem. Scand. B* **1983**, *B37*, 803–807.
(16) Bruncko, M.; Crich, D.; Samy, R. *J. Org. Chem.* **1994**, *59*, 5543–5549.
(17) Austin, J. F.; Kim, S.-G.; Sinz, C. J.; Xiao, W.-J.; MacMillan, D. W. C. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5482–5487.
(18) Takase, S.; Uchida, I.; Tanaka, H.; Aoki, H. *Heterocycles* **1984**, *22*, 2491–2494.
(19) Takase, S.; Uchida, I.; Tanaka, H.; Aoki, H. *Tetrahedron* **1986**, *42*, 5879–5886.
(20) Kawasaki, T.; Terashima, R.; Sakaguchi, K.; Sekiguchi, H.; Sakamoto, M. *Tetrahedron Lett.* **1996**, *37*, 7525–7528.
(21) Morales-Ríos, M. S.; Suárez-Castillo, O. R.; Trujillo-Serrato, J. J.; Joseph-Nathan, P. *J. Org. Chem.* **2001**, *66*, 1186–1192.
(22) Morales-Ríos, M. S.; Suárez-Castillo, O. R.; Joseph-Nathan, P. *Tetrahedron* **2002**, *58*, 1479–1484.
(23) Fuchs, J. R. New Strategies for the Synthesis of Polycyclic Natural Products: Total Syntheses of Lennoxamine, Aphanorphine, Flustramine A, Flustramine C, and Perophoramidine. Ph.D. Thesis, Pennsylvania State University, University Park, PA, 2005.
(24) Kawasaki, T.; Shinada, M.; Kamimura, D.; Ohzono, M.; Ogawa, A. *J. Chem. Soc., Chem. Commun.* **2006**, 420–422.
(25) Crews, P.; Farias, J. J.; Emrich, R.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 2932–2934.
(26) Sadtler Research Laboratories, I. 1975; pp 1290C and 21266.
(27) Sato, A.; Fenical, W. *Tetrahedron Lett.* **1983**, *24*, 481–484.
(28) Sykes, P. *A Guidebook to Mechanism in Organic Chemistry*, 6th ed.; Longman Group Ltf: Essex, 1986.
(29) Nickon, A.; Weglein, R. C. *J. Am. Chem. Soc.* **1975**, *97*, 1271–1273.
(30) *Sculpt* modeling program; HyperCube, Inc., 1995.

NP900282J