Marine Alkaloids. 19. Three New Alkaloids, Securamines E−G, from the Marine Bryozoan *Securiflustra securifrons*

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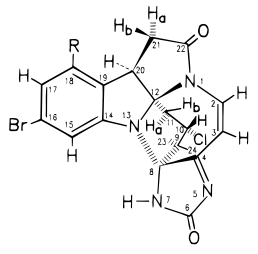
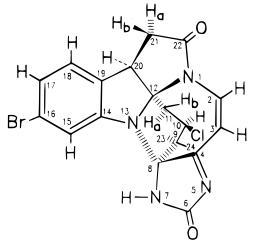
Received August 29, 1996[⊗]

Three new halogenated indole−imidazole alkaloids, securamines E (**1**), F (**2**), and G (**3**), have been isolated from the marine bryozoan *Securiflustra securifrons*. Their structures were determined by NMR and mass spectrometry. The new alkaloids, **1**−**3**, can all be related to the earlier reported securamine C (**4**). Compound **1** has an additional bromine substituent at the indole ring system compared to **4**; **2** and **4** are diastereomers differing in configuration at one of the four asymmetric carbon atoms; and **3** is a hydrogenated form of **4**.

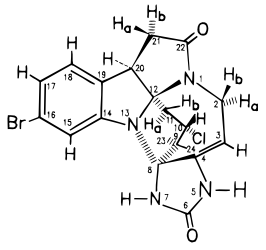
The marine bryozoan family Flustridae has so far yielded indole alkaloids from three genera, *Flustra*,[1] *Chartella*,[2] and *Securiflustra*.[3] We now report the isolation and structure elucidation of three further alkaloids from *Securiflustra securifrons* (Pallas) collected in July 1994. The new halogenated indole−imidazole alkaloids, securamines E (**1**), F (**2**), and G (**3**), are all structural variations of securamine C (**4**). They originate from column chromatography of an EtOAc extract of lyophilized colonies.



R=Br: Securamine E (1)
R=H: Securamine C (4)



Securamine F (2)



Securamine G (3)

The elemental composition of **1** ($C_{20}H_{17}N_4O_2Br_2Cl$) was determined from the isotopic pattern and HRMS of the molecular ion. Those of **2** ($C_{20}H_{18}N_4O_2BrCl$) and **3** ($C_{20}H_{20}N_4O_2BrCl$) were determined from the isotopic pattern of the molecular ion and peak matching of the base peak derived by loss of HCl. ^{1}H- and ^{13}C-NMR spectra of **1** revealed this compound to differ from **4** only by bromine substitution in position 18; for comparison see Tables 1 and 2.

Compound **2** is isomeric with **4** ($C_{20}H_{18}N_4O_2BrCl$), and the ^{13}C-NMR chemical shifts deviate 1.0 ppm or less from the values published for **4**[3] (Table 2), except that the signals assigned to C10, C23, and C24 displaced 7.3, 1.4, and 1.5 ppm, respectively. All δ_H values of **2** deviate 0.09 ppm or less from those of **4**, with the exception of the signal at 2.82 ppm assigned to H11a, which displaced 0.17 ppm, and the signal arising from the exchangeable proton (Table 1). Furthermore, the coupling constants between the protons in one of the ABX systems (H10−H11) have changed. The position of the C9−C11 isoprene fragment at C8 is established by HMBC experiments (long-range couplings between H23 and C8 and between H24 and C8). We conclude that **2** is diastereomeric with **4** and the relative stereochemistry is 8*S*′, 10*R*′, 12*R*′, 20*R*′ and 8*S*′, 10*S*′, 12*R*′, 20*R*′, respectively. This conclusion is confirmed by NOESY experiments. Due to a reduced distance between H10 and H2 in **4** compared to **2**, the NOE effect between H10 and H2 is only observed in the former compound.

According to its elemental composition, **3** is a hydrogenated form of **4**. The structure of **3** was established by comparison of NMR data with those of **4**. The ^{1}H-NMR spectra (Table 1) revealed that **3** lacks the double bond between positions 2 and 3 but contains a new CH_2−CH ABX system (H2−H3). Apart from that of the exchangeable proton, other signals deviate less than 0.35 ppm from the reported positions for compound **4**. Furthermore, **3** exhibits signals from two exchangeable protons instead of one in **4**. According to the ^{13}C-NMR analysis (Table 2) the structure of **3** resembles that of **4**. All values deviate less than 5 ppm except for C2, C4, and C6. The signal from C2 (135.9 ppm) has shifted to 48.3 ppm, C4 (187.5 ppm) to 140.7 ppm, and C6 (166.6 ppm) to 156.8 ppm. The change from an sp^2-hybridized carbon (135.9 ppm) to an sp^3-hybridized carbon (48.3 ppm) and the appearance of a new ABX system indicate a structure with a double bond between positions 3 and 4. Reduction of the double bond between positions 4 and 5 in **4** results in an exchangeable proton at position

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[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1997.

S0163-3864(96)00602-7 CCC: $14.00

Table 1. ^{1}H-NMR Data of Securamine E (**1**), F (**2**), G (**3**), and C (**4**) [δ_H (multiplicity, J_{HH})][a,b]

no.	1	2	3	4
2a	7.51 (d, 10.6)	7.47 (d, 10.4)	3.81 (d, 19.0)	7.49 (d, 10.5)
2b			4.75 (dd, 19.0, 8.6)	
3	5.95 (d, 10.6)	5.90 (d, 10.6)	5.07 (br d, 6.8)	5.93 (d, 10.5)
5			7.69 (br s)	
7	6.21 (br s)	6.70 (br s)	6.23 (br s)	7.25 (br s)
10	4.68 (dd, 11.0, 6.4)	4.83 (dd, 13.2, 4.2)	4.57 (dd, 12.5, 5.3)	4.74 (dd, 11.9, 5.3)
11a	2.62 (br dd, 11.0, 13.2⊙)*	2.82 (dd, 13.2, 13.2)	2.48 (dd, 13.2, 5.3)	2.68 (dd, 13.3, 11.9)
11b	2.61 (br dd, 6.5, 13.3⊙)*	2.72 (dd, 13.2, 4.2)	2.54 (dd, 13.2, 12.5)	2.65 (dd, 13.3, 5.3)
15	6.90 (d, 1.5)	6.93 (d, 1.5)	7.12 (d, 1.3)	6.95 (d, 1.6)
17	7.19 (d, 1.3)	7.02 (dd, 7.9, 1.5)	6.96 (dd, 7.9, 1.5)	7.05 (dd, 7.9, 1.6)
18		6.95 (br d, 7.3)	6.91 (d, 7.9)	6.98 (d, 7.9)
20	3.70 (d, 7.7)	3.64 (d, 7.0)	3.42 (d, 7.3)	3.70 (d, 7.1)
21a	3.62 (d, 18.9)#	3.04 (dd, 18.1, 7.0)	2.78 (dd, 16.8, 7.7)	3.10 (dd, 18.1, 7.1)
21b	3.01 (dd, 18.9, 7.9)#	2.91 (d, 18.1)	2.64 (d, 16.8)	2.96 (d, 18.1)
23	1.35 (s)	1.46 (s)	1.27 (s)	1.45 (s)
24	1.06 (s)	1.08 (s)	1.25 (s)	1.09 (s)

[a] The spectra were recorded at the following frequencies and in the following solvents and the positions of the signals are given relative to the positions of the solvent signals in parentheses: **1** 400 MHz, $CDCl_3$ (7.25); J_{HH} values marked ⊙ are determined in CD_3OD; **2** 400 MHz, $CDCl_3$ (7.25); **3** 400 MHz, $CDCl_3$ (7.26); **4** 500 MHz, $CDCl_3$ (7.30). [b] Values marked * or # are interchangeable.

Table 2. ^{13}C-NMR Data of Securamine E (**1**), F (**2**), G (**3**), and C (**4**) (δ_C)[a,b]

no.	1	2	3	4
2	136.4	135.9	48.3	135.9
3	101.2	101.5	96.3	101.6
4	187.3	187.2	140.7	187.5
6	166.4	166.0	156.8	166.6
8	85.4	84.7	80.7	85.6
9	44.2	43.7	39.2	44.0
10	58.7	52.1	61.4	59.4
11	41.9	42.8	41.0	41.8
12	88.0	89.8	87.9	89.2
14	148.0	146.9	147.5	147.0
15	113.5*	114.7	114.6	114.7
16	120.6#	123.0	122.3	123.1
17	128.0*	124.9	123.5	124.9
18	123.8#	125.5	125.2	125.6
19	126.3#	127.9	130.4	128.0
20	46.8	44.9	45.6	45.0
21	32.6	34.2	33.8	34.2
22	170.0	170.0	171.2	170.3
23	17.1	18.6	15.4	17.2
24	21.1	22.6	21.1	21.1

[a] The spectra were recorded at the following frequencies and in the following solvents and the position of the signals are given relative to the position of the solvent signals in parentheses: **1** 100.6 MHz, $CDCl_3$ (76.9); **2** 100.6 MHz, $CDCl_3$ (76.9); **3** 100.6 MHz, $CDCl_3$ (76.9); **4** 125.7 MHz, $CDCl_3$ (77.0). [b] Values marked * or # are interchangeable.

5 and shifts the δ_C signals for C4 and C6 towards higher field. The structure was confirmed by a NOESY experiment (see experimental part); the NOE effects between H2a and H10, between H3 and H5, and between H7 and H15 are especially significant.

Experimental Section

General Experimental Procedures. NMR spectra were recorded (in $CDCl_3$ or CD_3OD solution) on a Varian 400 FT-NMR spectrometer at 400.0 and 100.6 MHz for ^{1}H- and ^{13}C-NMR spectra, respectively. EIMS spectra originate from a JEOL JMS-HX/HX110A tandem mass spectrometer. High-resolution data were obtained by peak matching. The circular dichroism (CD) spectra were measured on a JASCO J-710 spectropolarimeter and the UV spectrum on a Hewlett-Packard 8452A diode array spectrophotometer.

Biological Material. The bryozoan material was collected in the North Sea (depth 70 m, 57° 5′ north, 43° 5′ east) near Harboøre Tange, at the Danish west coast in July 1994, and kept frozen until used. A voucher specimen (KU1589) is deposited at the H.C. Ørsted Institute, University of Copenhagen, Denmark.

Extraction and Isolation. Frozen *S. securifrons* (1200 g wet wt, 185 g dry wt) was lyophilized and gave, on extraction with EtOAc (4 × 2400 mL), 3.65 g of extract. The extract (3.6 g) was repeatedly separated by Si gel chromatography on a Merck Lobar LiChroprep Si 60 (40−63 μm) size B (EtOAc−heptane 1:1, UV detection at 270 nm) to give 15 fractions. Three fractions were further purified. Fraction 1 was chromatographed with EtOAc−heptane 40:60 on a Merck column [Lobar LiChroprep Si 60 (40−63 μm) size A (240-10), UV detection at 295 nm] followed by HPLC (LiChroCART LichroSper Si 60 250-10, EtOAc−heptane 1:1, 280 nm) to give 2.8 mg **1**. Fraction 2 was separated by HPLC (EtOAc−$CHCl_3$−heptane 50:5:45, 295 nm) giving pure **2** (8 mg). Fraction 3 gave 6 mg of pure **3** by chromatography on a Merck column (Lobar LiChroprep Si 60 (40−63 μm) size A (240-10), EtOAc−CH_3CN−heptane 70:10:20, 295 nm).

Securamine E (1): amorphous yellow solid; $[\alpha]^{20}_D$ −115° (*c* 0.0078, $CHCl_3$); UV ($CHCl_3$) λ max (log ϵ) 303 (3.82) 327 (3.83) nm; CD λ ext (*c* 0.0078) nm ($\Delta\epsilon$) 275 (3.81) 295 (5.08) 319 (−10.69); HREIMS *m/z* 539.9391 $[M]^+$, calcd for $C_{20}H_{17}O_2N_4Br_2Cl$ 539.9386; ^{1}H- and ^{13}C-NMR data (see Tables 1 and 2).

Securamine F (2): amorphous orange solid; $[\alpha]^{20}_D$ −200° (*c* 0.0045, $CHCl_3$); UV ($CHCl_3$) λ max (log ϵ) 299 (3.86) 328 (3.88) nm; CD λ ext (*c* 0.0045) nm ($\Delta\epsilon$) 275 (8.10) 291 (10.85) 319 (−13.35); HREIMS *m/z* 424.0518 $[M - HCl]^+$, calcd for $C_{20}H_{17}O_2N_4Br$ 426.0517; ^{1}H- and ^{13}C-NMR data (see Tables 1 and 2); HMBC data for **2** in $CDCl_3$ (400 MHz), CH_3 (δ_H 1.08) exhibit long-range couplings with δ_C 18.6, 43.7, 52.1, 84.7; CH_3 (1.46) with 22.6, 43.7, 84.7; CH (2.91) with 89.8; CH (6.95) with 123.0, 124.9, 146.9; CH (7.47) with 101.5; NOESY data of **2** in $CDCl_3$ (400 MHz), cross peaks were observed between protons of CH (δ_H 3.04) and δ_H 2.91 (strong); CH (3.64) and 2.82 (s), 3.04 (s); CH (6.95) and 2.91 (s), 3.04 (medium), 3.64 (s); CH (6.93) and 1.08 (m), 1.46 (weak); CH (2.82) and 1.46 (w), 2.72 (s); CH (4.83) and 1.08 (s), 1.46 (w), 2.72 (s); NH (6.70) and 1.08 (m), 1.46 (s); CH (5.90) and 1.08 (w), 1.46 (w), 4.83 (m); CH (7.47) and 4.83 (s), 5.90 (s).

Securamine G (3): amorphous brown solid; $[\alpha]^{20}_D$ −15.6° (*c* 0.032, $CHCl_3$); UV ($CHCl_3$) λ max (log ϵ) 298 (2.81) nm; CD λ ext (*c* 0.032) nm ($\Delta\epsilon$) 269 (0.14) 298 (0.34) 325 (−0.14); HREIMS *m/z* 428.0665 $[M - HCl]^+$, calcd for $C_{20}H_{19}O_2N_4Br$ 428.0673; ^1H- and ^{13}C-NMR data (see Tables 1 and 2); NOESY data of **3** in $CDCl_3$ (400 MHz), cross peaks were observed between protons of CH_3 (δ_H 1.25 or/and 1.27) and δ_H 4.57 (weak), 6.23 (medium); CH (2.54) and 3.42 (w), 4.57 (w); CH (2.64) and 2.78 (strong), 3.42 (w), 6.91 (w); CH (2.78) and 3.42 (w); CH (3.81) and 4.57 (m), 4.75 (s); CH (4.75) and 5.07 (w); CH (5.07) and 7.69 (w); NH (6.23) and 7.12 (w).

Acknowledgments. We are pleased to acknowledge the generous gift of the bryozoan material from Dr. N. Jacobsen and are indebted to Dr. M. Pereira for the taxonomic classification of the material. The project was carried out with financial support from the Danish Biotechnology Programme 1991−1995. We are indepted to Dr. S. E. Harnung for determination of the CD data measured on a modified JASCO 710 instrument financed by the Danish Natural Science Research Council, Grant No.//−0373-1.

References and Notes

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NP960602P