New Bromopyrrole Alkaloids from the Indopacific Sponge *Agelas nakamurai*

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Two new dimeric bromopyrrole alkaloids, nakamuric acid (**1**) and its corresponding methyl ester (**2**), have been isolated from the Indopacific sponge *Agelas nakamurai* along with the known metabolites sceptrin (**3**), debromosceptrin (**4**), and ageliferin (**5**). Their structures were identified by analysis of spectral data. All compounds inhibited the growth of several Gram-positive and Gram-negative bacteria in the agar plate diffusion assay.

Dimeric bromopyrrole alkaloids are secondary metabolites characteristic of sponges of the genus *Agelas*.[1−5] These compounds often show interesting biological properties such as antibiotic and antifouling activity.[2,4,5] From the Indopacific species *Agelas nakamurai* there have been isolated diterpenes possessing a 9-methyladeninium or a hypotaurocyamine moiety as well as monomeric bromopyrrole alkaloids.[6−10] This is the first time that dimeric bromopyrrole alkaloids have been found in the abovementioned species. During our studies of bioactive marine natural products, we observed that the ethanolic extract of *A. nakamurai* collected in 1997 at Ambon, Indonesia, possessed antibacterial activity against Gram-positive and Gram-negative bacteria. In this paper, we describe the isolation of five dimeric bromopyrrole alkaloids, two of which are new, and the antibacterial activity of the isolated metabolites.

Bioassay-guided purification of the alkaloids from *A. nakamurai* was accomplished by vacuum liquid chromatography on Si gel using a step gradient from CH_2Cl_2 to methanol followed by column chromatography on Sephadex LH-20 and RP-18 Si gel and finally by semipreparative HPLC. In addition to the known compounds sceptrin (**3**), debromosceptrin (**4**), and ageliferin (**5**), two new alkaloids (**1** and **2**, Chart 1) were isolated as water-soluble salts of trifluoroacetic acid and identified from their spectroscopic data and by comparison with published data.[1−3,5]

Nakamuric acid (**1**) and its methyl ester (**2**) showed quasi molecular ion peaks $[M + H]^+$ at *m/z* 582, 584, 586 and at 596, 598, 600 in the ESIMS, respectively, suggesting dibrominated metabolites. The difference of 14 mass units indicated that **2** is a $-CH_2-$ analogue of **1**. Molecular formulas of compounds **1** ($C_{20}H_{22}Br_2N_8O_3$) and **2** ($C_{21}H_{24}Br_2N_8O_3$) were established by HRFABMS. Their UV spectra (λ_{max} 269 nm) indicated a substituted pyrrole chromophore. The ^{13}C NMR signals of **1** and **2** closely resemble those of the sceptrins (**3**, **4**). In contrast to the latter, they both have an additional carbonyl signal at about 174 ppm in the ^{13}C NMR spectrum, whereas they lack the signals of one of the two imidazole ring systems present in **3** and **4**. The ^{13}C NMR spectrum of **2** differed from that of **1** only by an additional resonance at 51.6 ppm. From the DEPT spectrum this signal could be identified as a methyl carbon that is directly bound to oxygen. The corresponding characteristic methyl singlet in the ^1H NMR was found at 3.53 ppm. HMBC spectra clearly indicated that the $-COOCH_3$ group of compound **2** is directly bound to the cyclobutane ring instead of an imidazole ring system. The remaining carbon signals for the two substituted pyrrole ring systems were nearly identical with those of sceptrin (**3**). COSY experiments allowed the unambiguous assignment of the signals from H-7 to H-10 and the respective resonances of the other half of the molecule (H-7′ to H-10′). The ^1H NMR chemical shift (H-9, H-9′, H-10, H-10′), coupling pattern, and the corresponding ^{13}C data for the cyclobutane ring system are very similar to those of the sceptrins (**3**, **4**) and support the assignment of an all-trans stereochemistry. This was verified by difference NOE experiments. Irradiation of H-10 (2.89 ppm) revealed a NOE to H-9′ (2.24 ppm) and H-12′ (6.59 ppm). Compound **1** is the free acid of the methyl ester **2** and is probably the true natural product. Compound **2** may have been formed during the isolation procedures involving methanol.

All compounds isolated were analyzed for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. The results of these assays are summarized in Table 3. At a concentration of 0.2 μmol/disk, sceptrin (**3**) and ageliferin (**5**) showed an antibiotic effect against all tested microorganisms. The new compounds (**1**, **2**) only exhibited antibacterial activity against *B. subtilis*, with inhibition zones of 9 mm in diameter. They are less active than sceptrin (**3**) and ageliferin (**5**).

Experimental Section

General Experimental Procedures. ^1H (1D, 2D COSY) and ^{13}C (1D, DEPT-135, 2D HMBC) NMR spectra were recorded on Bruker AM 300 and ARX 400 NMR spectrometers. Mass spectra (ESIMS) were recorded on a Finnigan MAT TSQ-7000 mass spectrometer with a capillary temperature at 220 °C and drift voltage at 3.5 kV. HRFABMS were obtained on a Intectra AMD 402 spectrometer. UV and optical rotations were measured in MeOH on a Perkin−Elmer UV−vis Lambda 2 spectrophotometer and a Perkin−Elmer 241 MC polarimeter, respectively. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC

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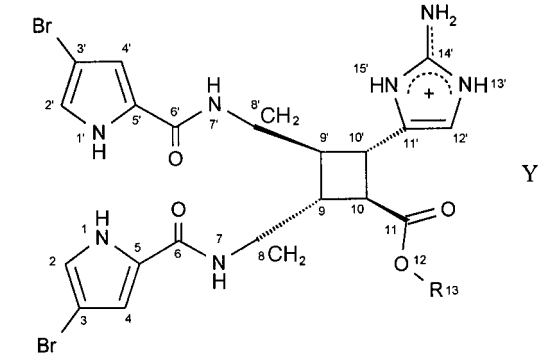
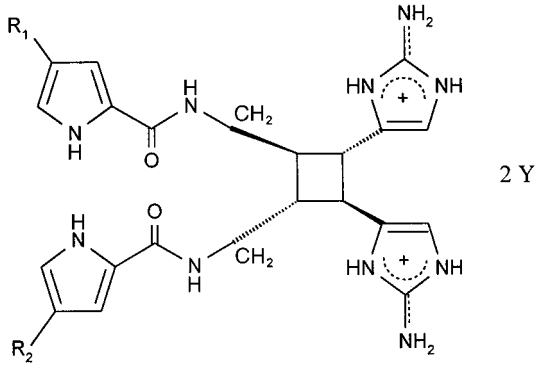
Chart 1



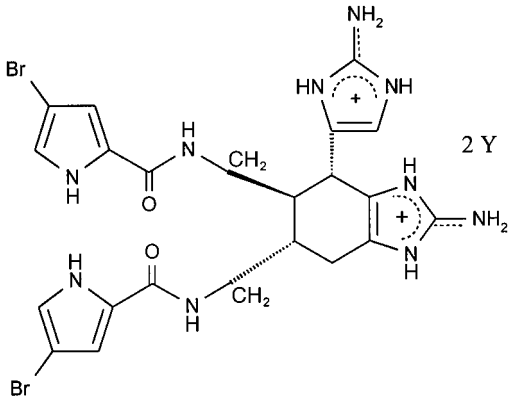
1 R = H, Y = CF_3COO^-

2 R = CH_3, Y = CF_3COO^-



3 $R_1 = R_2 = Br$, Y = CF_3COO^-

4 $R_1 = Br$, $R_2 = H$, Y = CF3COO-



5 Y = CF_3COO^-

was performed on precoated TLC plates with Si gel F_{254} (Merck, Darmstadt, Germany). The compounds were detected by their UV absorbance at 254 and 366 nm. Semipreparative HPLC was performed on a HPLC system (Merck, Darmstadt, Germany) coupled with a UV detector (UV detection at 254 nm). The separation column (8 × 250 mm) was prefilled with Eurospher C_{18} (Knauer, Berlin, Germany), and the compounds were eluted with a solvent system of MeOH−H_2O, containing 0.1% CF_3COOH for improved separation, at a flow rate of 5 mL/min.

Sponge Material. The sponge was collected at a depth of 14 m near the island of Ambon (Seram Seilale), Indonesia, in August 1997. The surface of the orange sponge is pitted and bears irregular grooves and depressions, but in between it is optically smooth. Inside, the sponge is clathrous-cavernous with holes and corridors of up to 5−8 mm in diameter. The skeleton consists of an irregularly anisotropic reticulation of spongin fibers cored and echinated by spicules. The fibers are 20−90 μm in diameter, with little distinction between ascending and interconnecting fibers. They are cored by 1−6 spicules in cross section, with more heavy coring near the surface, and irregularly echinated by single spicules at distances of 100−150 μm. Spicules are verticillatedly spined acanthostyles of unusual range in size: 90−375 × 6−33 μm, with whorls of spines numbering 13−30.

The sponge sample was immersed in EtOH immediately after collection. A voucher specimen is kept in EtOH under the registration number ZMA POR.12957 at the Zoölogisch Museum, Amsterdam.

Table 1. ^{1}H NMR Data of Nakamuric Acid (**1**) and Its Methyl Ester (**2**) in DMSO-d_6

position	**1**	**2**
1 or 1′	11.79 br s, 2H	11.79 br s, 2H
2 or 2′	6.98 m, 2H	6.98 dd, 1.5 and 2.6 Hz, 1H
		6.97 dd, 1.5 and 2.8 Hz, 1H
4 or 4′	6.84 t, 1.9 Hz, 1H	6.85 t, 1.6 Hz, 1H
	6.80 t, 1.7 Hz, 1H	6.81 t, 1.6 Hz, 1H
7	8.10 t, 5.5 Hz, 1H	8.10 t, 5.5 Hz, 1H
7′	8.21 t, 5.8 Hz, 1H	8.22 t, 5.9 Hz, 1H
8 (AB)	under HDO signal	3.40 m, 2H
8′(AB)	under HDO signal	3.38 m, 1H
		3.28 m, 1H
9	2.32 m, 1H	2.33 m, 1H
9′	2.20 m, 1H	2.24 m, 1H
10	2.82 t, 9.5 Hz, 1H	2.89 t, 9.4 Hz, 1H
10′	3.04 t, 9.6 Hz, 1H	3.07 t, 9.6 Hz, 1H
12′	6.58 s, 1H	6.59 s, 1H
13		3.53 s, 3H[a]
13′	11.93 br, 1H[b]	12.14 br, 1H[b]
15′	11.65 br, 1H[b]	11.79 br, 1H[b]
14′-NH_2	7.31 br s, 2H	7.48 br s, 2H

[a] The signal was obtained in MeOD. [b] Signals of H-13′ and H-15′ are interchangeable.

Table 2. ^{13}C NMR Data of Nakamuric Acid (**1**) and Its Methyl Ester (**2**) in DMSO-d_6

position	**1**	**2**
2 or 2′	121.3 d	121.4 d
	121.3 d	121.2 d
3 or 3′	94.9 s	95.0 s
	94.9 s	94.9 s
4 or 4′	111.5 d	111.4 d
	111.5 d	111.5 d
5 or 5′	126.7 s	126.8 s
	126.7 s	126.6 s
6 or 6′	160.1 s	160.2 s
	159.8 s	159.9 s
8	under DMSO	41.2 t
8′	under DMSO	under DMSO
9	42.2 d	42.2 d
9′	33.6 d	33.6 d
10	44.0 d	44.1 d
10′	44.0 d	43.9 d
11	173.9 s	172.6 s
11′	127.5 s	127.2 s
12′	109.0 d	109.1 d
13		51.6 q
14′	146.9 s	147.1 s

Table 3. Antibacterial Activity of the Compounds Isolated from *A. nakamurai*[a]

	B. subtilis	*S. aureus*	*E. coli*	
compound	168	ATCC 25923	ATCC 25922	HB 101
1	9			
2	9			
3	16	16	9	13
4	10			
5	14	11	8	11

[a] 0.2 μmol/disk in the agar plate diffusion assay (zone of inhibition in mm).

Extraction and Isolation. The sponge (ca. 750 g wet wt) was extracted exhaustively with MeOH. The resulting extract was combined with the EtOH storage liquid and evaporated under vacuum. The crude extract (17.6 g) was subjected to vacuum liquid chromatography on Si gel, using a step gradient consisting of different portions of CH_2Cl_2−MeOH. Elution started with 100% CH_2Cl_2, and the MeOH concentrations were increased gradually. Six fractions were obtained. Fraction 80:20 (CH_2Cl_2−MeOH) was further chromatographed on RP-18 Si gel (Lobar, RP-18, Merck, size B; MeOH−H_2O−CF_3COOH, 50:50:0.2), yielding compounds **3** (1.8 g, 10.2%) and **4** (177 mg, 1.0%) in addition to a further antibiotically active fraction. This fraction was further subjected to column chromatography on Sephadex LH-20 (MeOH) followed by RP-18 column chromatography (Lobar, RP-18, Merck, size A; MeCN−H_2O−CF_3COOH, 45:55:0.2), which yielded four fractions. Fraction 3 contained ageliferin (**5**, 7 mg, 0.04%), while the two novel compounds **1** (4 mg, 0.02%) and **2** (10 mg, 0.06%) were isolated from fraction 4. They were finally purified by semi-preparative HPLC with gradient elution starting with a concentration of 27% MeCN and increasing the concentration in a linear manner within 25 min up to 40%. All known compounds were identified from the comparison of their UV, MS, and NMR data with those of the literature.[1−4]

Nakamuric acid (1): brown amorphous solid; $[\alpha]_D$ −9.9° (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.02), 269 (4.08) nm; ^{13}C and ^1H NMR, see Tables 1 and 2; ESIMS *m*/*z* 582, 584 and 586 $[M + H]^+$; HRFABMS *m*/*z* 584.0074 $[M + H]^+$ (calcd for, $C_{20}H_{22}N_8O_3{}^{79}Br_2$ 584.0081).

Nakamuric acid methyl ester (2): brown amorphous solid; $[\alpha]_D$ −4.1° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.04), 269 (4.09) nm; ^{13}C and ^1H NMR, see Tables 1 and 2; ESIMS *m*/*z* 596, 598 and 600 $[M + H]^+$; HRFABMS *m*/*z* 598.0232 $[M + H]^+$ (calcd for $C_{21}H_{24}N_8O_3{}^{79}Br_2$, 598.0237).

Agar Plate Diffusion Assays. Susceptibility disks (5 mm in diameter) were impregnated with 0.2 μmol of the isolated compound and placed on agar plates inoculated with the test organism: *B. subtilis* 168, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. coli* HB 101. The plates were checked for inhibition zones after 24 h of incubation at 37 °C.

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