Mukanadins A−C, New Bromopyrrole Alkaloids from Marine Sponge *Agelas nakamurai*

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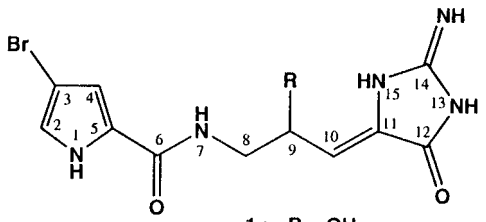
Three new bromopyrrole alkaloids, mukanadins A−C (**1**−**3**), have been isolated from the Okinawan marine sponge *Agelas nakamurai*, and the structures were elucidated from spectroscopic data.

Marine sponges are a rich source of bromopyrrole alkaloids with unique biological activity.[1] During our search for bioactive substances from marine organisms,[2] we previously isolated several bromopyrrole alkaloids from sponges of the genus *Agelas* or *Hymeniacidon*.[3] Further investigation of extracts of the Okinawan sponge *Agelas nakamurai* resulted in the isolation of three new bromopyrrole alkaloids, mukanadins A−C (**1**−**3**). Here we describe the isolation and structure elucidation of **1**−**3**.

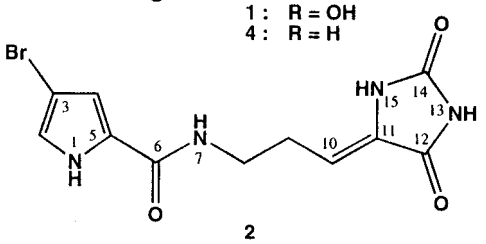
The sponge *Agelas nakamurai* Hoshino (family Agelasidae) collected off Ie Island, Okinawa, was extracted with MeOH. Ethyl acetate-soluble materials of the extract were subjected to Sephadex LH-20 ($CHCl_3$−MeOH) column chromatography and repeated C_{18} HPLC (MeOH−H_2O−CF_3CO_2H) to yield mukanadins A (**1**, 0.0005%, wet wt), B (**2**, 0.005%), and C (**3**, 0.0021%) as colorless amorphous solids, together with a known bromopyrrole alkaloid, monobromodispacamide[4] (**4**, 0.0032%).

Mukanadin A {**1**, $[\alpha]^{22}_D$ +5° (*c*, 0.3, MeOH)} showed pseudomolecular ion peaks at *m*/*z* 342 and 344 [M + H^+] in the FABMS, and the molecular formula was deduced to be $C_{11}H_{13}N_5O_3Br$ from HRFABMS [*m*/*z* 342.0211 $(M + H)^+$, Δ +1.0 mmu]. IR absorptions indicated the presence of OH and/or NH (3430 cm^{-1}) and amide carbonyl (1685 and 1640 cm^{-1}) groups. The UV absorption [λ_{max} 274 nm (ϵ 6000)] was attributable to a substituted pyrrole chromophore.[5] The ^1H and ^{13}C NMR (Table 1) spectra showed signals due to a 3-bromopyrrole carbonyl moiety (N-1−C-6).[6] The ^{13}C NMR data, including DEPT experiments, disclosed the presence of two carbonyls, four sp^2 quaternary and three sp^2 methine carbons, one oxymethine, and one methylene carbon. Although ^1H NMR data of **1** were close to those of monobromodispacamide (**4**),[4] **1** differed from **4** in the presence of an oxymethine [H-9, δ_H 4.62 (m)] and a hydroxy [9-OH, δ_H 5.98 (m)] signals. The ^1H−^1H COSY spectrum showed the connectivity from NH-7 to H-10, indicating that the hydroxyl group was attached to C-9. The carbon chemical shifts of C-10 (δ_C 117.4), C-11 (δ_C 127.6), C-12 (δ_C 163.1), and C-14 (δ_C 155.1) corresponded to those of the aminoimidazolinone moiety in **4**. The $\Delta^{10,11}$ double bond was assigned *Z*-geometry from the NOESY correlation for H-9/NH-15. Thus, the structure of **1** was concluded to be the 9-hydroxy form of monobromodispacamide (**4**). To determine the absolute configuration at C-9, **1** was treated with ozone and then formic acid and hydroperoxide. Amino acid analysis of the hydrolysate of the ozonolysis products showed the presence of one molar equivalent of isoserine. Chiral HPLC analyses of the hydrolysate revealed the presence of (*S*)- and (*R*)-isoserines in the ratio of about 7:3. Therefore, mukanadin A (**1**) was determined to be a 7:3 mixture of (9*R*)- and (9*S*)-isomers.

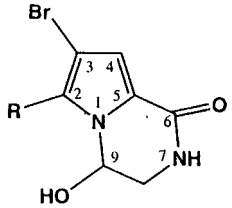
The molecular formula of mukanadin B (**2**) was revealed to be $C_{11}H_{11}N_4O_3Br$ by HRFABMS [*m*/*z* 327.0101 $(M + H)^+$, Δ +0.8 mmu]. The ^{13}C NMR data (Table 1), including DEPT data, disclosed the presence of three carbonyls, three sp^2 quaternary carbons, and three sp^2 methine and two methylene carbons, suggesting that the structure of **2** was analogous to that of monobromodispacamide (**4**).[4] The ^1H NMR spectrum of **2** showed signals due to four D_2O-exchangeable protons [NH-1, δ_H 11.80 (br s); NH-7, δ_H 8.18 (t); NH-13, δ_H 10.15 (br s); NH-15, δ_H 10.95 (br s)], while that of **4** included five D_2O-exchangeable proton signals [NH-1, δ_H 11.80 (b rs); NH-7, δ_H 8.44 (m); NH-13, δ_H 9.42 (br s); NH-15, δ_H 9.34 (br s), NH-16, δ_H 7.02 (b rs)]. The carbon chemical shifts of C-10 (δ_C 108.9), C-11 (δ_C 131.2), C-12 (δ_C 164.3), and C-14 (δ_C 154.8) of **2** differed from those of monobromodispacamide (**4**) {C-10, δ_C 117.9; C-11, δ_C 129.2; C-12, δ_C 162.8; C-14, δ_C 155.3], suggesting the presence of a hydantoin ring in **2**. The $\Delta^{10,11}$ double bond was assigned *Z*-geometry from the NOESY correlation for H_2-9/NH-12. Thus, the structure of mukanadin B (**2**) was elucidated to be the 14-keto form of monobromodispacamide (**4**).



1 : R = OH
4 : R = H



2



3 : R = H
5 : R = Br

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Table 1. ^{1}H and ^{13}C NMR Data of Mukanadins A (**1**) and B (**2**) and Monobromodispacamide (**4**)

	1		**2**		**4**	
position	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[b]	δ_H[c]	δ_C[b]
1	11.75 br s		11.80 br s		11.80 br s	
2	6.98 br s	121.3 d	6.96 br s	121.2 d	6.96 br s	121.4 d
3		94.9 s		94.9 s		95.2 s
4	6.87 br s	111.7 d	6.85 br s	111.4 d	6.87 br s	112.0 d
5		126.6 s		126.8 s		126.9 s
6		159.9 s		159.6 s		160.0 s
7	8.23 t, 5.7		8.18 t, 5.7		8.44 m	
8	3.38 m	44.4 t	3.34[d] m	37.6 t	3.35[d] m	37.5 t
	3.32 m					
9	4.62 m	67.3 d	2.37[d] br q, 6.7	26.8 t	2.46[d] m	27.6 t
9-OH	5.98 m					
10	5.97 d, 4.3	117.4 d	5.54 t, 7.6	108.9 d	5.98 t, 7.5	117.9 d
11		127.6 s		131.2 s		129.2 s
12		163.1 s		164.3 s		162.8 s
13	11.05 br s		10.15 br s		9.42[e] br s	
14		155.1 s		154.8 s		155.3 s
15	9.50 br s		10.95 br s		9.34[e] br s	
16-NH	8.82 br s				7.02 br s	

[a] 600 MHz, DMSO-d_6. [b] 100 MHz, DMSO-d_6. [c] 500 MHz, DMSO-d_6 containing one drop of 1 N HCl. [d] 2H. [e] These signals may be interchangeable.

The molecular formula of mukanadin C (**3**) was established as $C_7H_7O_2N_2Br$ by HRFABMS [*m/z* 230.9754 (M + H)$^+$, Δ −1.5 mmu]. ^{1}H and ^{13}C NMR data of **3** were reminiscent of those of longamide (**5**).[7,8] The ^{1}H NMR spectrum of **3** revealed two sp^2 methine signals [H-2, δ_H 7.17 (d, J = 1.5 Hz); H-4 δ_H 6.85 (d, J = 1.5 Hz)], while that of **5** showed one sp^2 methine signal. Optical rotation {[α]$_D$ 0° (*c* 1.0, MeOH)} implied **3** to be a racemic mixture at C-9. Thus, the structure of mukanadin C (**3**) was the 2-debromo form of longamide (**5**).

Mukanadins A (**1**) and B (**2**) are new bromopyrrole alkaloids with an aminoimidazolinone and a hydantoin moiety, respectively. Compound **1** differs from monobromodispacamide[4] (**4**) in possessing a hydroxy group at C-9. Bromopyrrole alkaloids possessing a hydantoin ring are rare, and only four such examples—axinohydantoin,[9] spongiacidins C and D,[10] and midpacamide[11]—have been reported. Mukanadin C (**3**) is 2-debromo analogue of longamide[5] (**5**).

Experimental Section

General Experimental Procedures. IR and UV spectra were recorded on JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometers, respectively. Optical rotations were measured on JASCO DIP-370 polarimeter. FABMS were obtained on a JEOL JMX HX-110 spectrometer using glycerol as a matrix.

Sponge Materials. The red-brown sponge *Agelas nakanurai* Hoshino (order Axinellida; family Agelasidae) was collected off Ie Island, Okinawa. Morphology was smooth mound shaped. The voucher specimen (SS-933) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Separation. The sponge (770 g, wet wt) was extracted with MeOH (1 L × 2). The MeOH extract (54.9 g) was partitioned between EtOAc (500 mL × 3) and H_2O (500 mL). Part (2 g) of the EtOAc-soluble material (7.87 g) was subjected to a Sephadex LH-20 column ($CHCl_3$−MeOH, 1:1) and C_{18} HPLC (Develosil-HG-5, Nomura Chemical, 10 × 250 mm; MeOH−H_2O−CF_3CO_2H, 40:60:0.1; flow rate, 1.5 mL/min; UV detection at 260 nm) to afford mukanadins B (**2**, 0.005% wet wt, t_R 30.8 min) and C (**3**, 0.0021%, t_R 18.0 min), monobromodispacamide (**4**, 0.0032%, t_R 24.8 min), and fraction **a**. Fraction **a** was purified by C_{18} HPLC (Develosil-HG-5; MeOH−H_2O−CF_3CO_2H, 20:80:0.1; flow rate, 3.0 mL/min; UV detection at 260 nm) to yield mukanadin A (**1**, 0.0005%, t_R 46.0 min).

Mukanadin A (1): colorless amorphous solid; [α]$^{22}_D$ +5° (*c* 0.3, MeOH); UV (MeOH) λ_{max} 274 nm (ϵ 6000); IR (KBr) ν_{max} 3430, 1685, and 1640 cm^{-1}; ^{1}H and ^{13}C NMR, Table 1; FABMS *m/z* 342 and 344 [(M + H)$^+$, 1:1]; HRFABMS *m/z* 342.0211 [M + H]$^+$, calcd for $C_{11}H_{14}N_5O_3{}^{79}Br$ 342.0201.

Mukanadin B (2): colorless amorphous solid; UV (MeOH) λ_{max} 274 nm (ϵ 6700); IR (KBr) ν_{max} 3430, 1685, and 1635 cm^{-1}; ^{1}H and ^{13}C NMR, Table 1; FABMS *m/z* 327 and 329 [(M + H)$^+$, 1:1]; HRFABMS *m/z* 327.0101 [M + H]$^+$, calcd for $C_{11}H_{12}N_4O_3{}^{79}Br$ 327.0093.

Mukanadin C (3): colorless amorphous solid; [α]$^{22}_D$ 0° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 275 (ϵ 4300) and 235 nm (4000); IR (KBr) ν_{max} 3400, 1680, and 1645 cm^{-1}; ^{1}H NMR (MeOH-d_4) δ 3.56 (1H, dd, J = 3.5, 13.4 Hz, H-8), 3.78 (1H, dd, J = 3.5, 13.4 Hz, H-8), 5.68 (1H, t, J = 3.5 Hz, H-9), 6.85 (1H, d, J = 1.5 Hz, H-4), and 7.17 (1H, d, J = 1.5 Hz, H-2); ^{13}C NMR (MeOH-d_4) δ 77.0 (d, C-9), 99.6 (s, C-3), 117.0 (d, C-4), 124.5 (d, C-2), 125.6 (s, C-5), and 162.3 (s, C-6); FABMS *m/z* 231 and 233 [(M + H)$^+$, 1:1]; HRFABMS *m/z* 230.9754 [M + H]$^+$, calcd for $C_7H_8O_2N_2{}^{79}Br$ 230.9769.

Absolute Stereochemistry at C-9 of Mukanadin A (1). A solution of compound **1** (0.1 mg) in MeOH (100 μL) was treated with ozone at −78 °C for 1 min. After excess ozone was removed by N_2 gas, to the residue were added HCO_2H (100 μL) and 35% aqueous H_2O_2 (100 μL). The mixture was stirred at 0 °C for 1 h and then at room temperature for 5 h. After evaporation, the residue was hydrolyzed with 6 N HCl (100 μL) at 110 °C for 24 h in a sealed tube. Standard amino acid analysis of the hydrolysate showed the presence of 1 mole of isoserine. The hydrolysate was subjected to chiral HPLC analyses [SUMICHIRAL OA-5000, 4 × 150 mm; 24 °C, flow rate, 2.0 mL/min; eluent: *i*-PrOH−H_2O (15:85) containing 2.0 mM $CuSO_4$]. The retention times of authentic (*S*)- and (*R*)-isoserine were found to be 15.6 and 23.6 min, respectively. The hydrolysate of compound **1** was subjected to chiral HPLC analysis under the same condition as described above, and both (*S*)- and (*R*)-isoserine were detected in the ratio of about 7:3.

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