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2-DEBROMONAGELAMIDE U, 2-DEBROMOMUKANADIN G, AND 2-DEBROMONAGELAMIDE P FROM MARINE SPONGE *AGELAS* SP.

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Abstract – Two new monomeric bromopyrrole alkaloids (**1** and **2**) and one new dimeric bromopyrrole alkaloid (**3**) were isolated from an Okinawan marine sponge *Agelas* sp. Spectroscopic analyses of these compounds revealed the structures of **1**–**3** to be 2-debromonagelamide U, 2-debromomukanadin G, and 2-debromonagelamide P, respectively. Antimicrobial activity of **1**–**3** was evaluated.

INTRODUCTION

Marine sponges have been recognized as a rich source of bioactive metabolites with unique chemical structures.[1] Among them, bromopyrrole alkaloids are one of the most common metabolites contained in marine sponges. These alkaloids have attracted widespread interest due to their fascinating chemical structures with high N to C ratio (ca 1:2).[2-4] During our search for new metabolites from Okinawan marine sponges, we have reported many bromopyrrole alkaloids with a variety of chemical structures from sponges *Agelas* spp.[5-8] Recently, we isolated three new bromopyrrole alkaloids, 2-debromonagelamide U (**1**), 2-debromomukanadin G (**2**), and 2-debromonagelamide P (**3**) (Chart 1) from a sponge *Agelas* sp. (SS-156). In this article, we describe the isolation, structure elucidation, and antimicrobial activity of **1**–**3**.

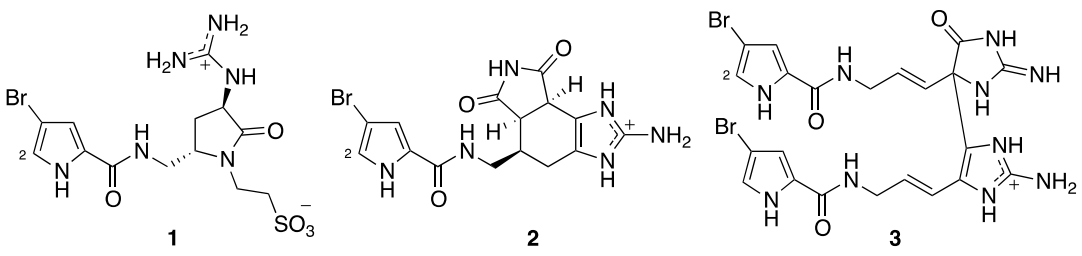

Chart 1. Structures of 2-debromonagelamide U (**1**), 2-debromomukanadin G (**2**), and 2-debromonagelamide P (**3**)

RESULTS AND DISCUSSION

The sponge *Agelas* sp. (SS-156, 6.3 kg, wet weight) collected at Kerama Islands, Okinawa, was extracted with MeOH to give the extract, a part of which was partitioned with *n*-hexane, *n*-BuOH, and water. Chromatographic separations of the *n*-BuOH-soluble materials gave 2-debromonagelamide U (**1**, 0.00003%, wet weight), 2-debromomukanadin G (**2**, 0.00017%), and 2-debromonagelamide P (**3**, 0.00010%), together with six known bromopyrrole alkaloids, mukanadin A,[9] tauroacidin B,[10] laughine,[11] 2-debromotaurodispacamide A,[12] ageliferin,[13] and 2,2'-didebromonagelamide B.[7]

2-Debromonagelamide U (**1**) was isolated as an optically active colorless amorphous solid. The pseudomolecular ion peaks at *m/z* 449 and 451 (1:1) in the ESIMS suggested the existence of one bromine atom in the molecule. The molecular formula of **1**, $C_{13}H_{19}N_6O_5BrS$, was established by the HRESIMS (*m/z* 449.02591 $[M-H]^-$, Δ+1.09 mmu). The presence of a pyrrole amide moiety, a common unit on bromopyrrole alkaloids, was disclosed by an IR absorption at 1684 cm^{-1} and a UV absorption at 268 nm. The ^1H and ^{13}C NMR spectra (Table 1) showed the signals from a 3-bromopyrrole amide moiety (N-1–N-7), a guanidine moiety (N-13–N-16), an ethanesulfonic acid moiety (C-2' and C-3'), two sp^3 methylenes, and two sp^3 methines. The 1D NMR spectra resembled those of nagelamide U,[6] except for the resonances due to the bromopyrrole amide moiety. From these observations, **1** was presumed to be a 2-debromo form of nagelamide U. The gross structure of **1** was confirmed by analysis of the ^1H-^1H COSY and HMBC spectra (Figure 1). The *anti* relationship for H-9/H-11 was implied by a ROESY correlation for H-8a/H-11. Thus, the structure of 2-debromonagelamide U (**1**) was assigned as shown in Chart 1.

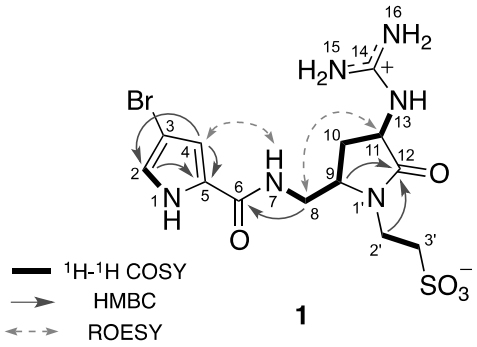

Figure 1. Selected 2D NMR correlations for 2-debromonagelamide U (**1**)

The molecular formula of 2-debromomukanadin G (**2**) was assigned by the HRESIMS (*m/z* 407.04590 $[M]^+$, Δ–0.28 mmu). The ^1H and ^{13}C NMR data for **2** (Table 1) were similar to those for mukanadin G,[8] a bromopyrrole alkaloid with the tricyclic skeleton consisting of a tetrahydrobenzaminoimidazole and a 2,5-dioxopyrrolidine moieties, and the resonances due to a 3-bromopyrrole amide moiety (N-1–N-7) in **2** were discerned in place of the signals of a dibromopyrrole amide moiety in mukanadin G. The relative configurations of C-9, C-16, and C-20 were deduced to be the same as those of mukanadin G by

resemblance of their ^{1}H coupling constants. Given correlations observed in the 2D NMR spectra (Figure 2), **2** was assigned as 2-debromomukanadin G.

Table 1. ^{1}H and ^{13}C NMR data for 2-debromonagelamide U (**1**) and 2-debromomukanadin G (**2**) in DMSO-$_{6}$

	1		**2**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	–	11.84 (1H, brs)	–	11.82 (1H, brs)
2	121.4	6.98 (1H, s)	121.3	6.98 (1H, s)
3	95.0	–	94.9	–
4	111.8	6.86 (1H, s)	111.7	6.86 (1H, s)
5	126.6	–	126.8	–
6	160.0	–	160.0	–
7	–	8.25 (1H, t, 5.7)	–	8.27 (1H, t, 5.7)
8	40.2	3.36 (1H, m) 3.46 (1H, m)	40.6	3.57 (1H, m) 3.83 (1H, m)
9	54.7	3.87 (1H, m)	34.7	2.19 (1H, m)
10	29.9	1.92 (1H, m) 2.37(1H, dd, 11.9, 8.4)	21.2	2.14 (1H, t, 14.4) 2.54 (1H, d, 14.4)
11	51.3	4.31 (1H, q, 8.4)	121.5	–
12	170.4	–	–	12.45 (1H, brs)
13	–	7.81 (1H, d, 8.4)	147.8	–
13-NH_2			–	7.60 (2H, brs)
14	157.1	–	–	12.45 (1H, brs)
15	–	7.26 (2H, brs)	115.5	–
16	–	7.26 (2H, brs)	40.3	3.92 (1H, d, 7.3)
17			176.1	–
18			–	11.30 (1H, brs)
19			178.5	–
20			42.7	3.62 (1H, dd, 7.3, 3.7)
1’	–	–		
2’	38.5	3.30 (1H, m) 3.72 (1H, dt, 12.4, 6.2)		
3’	48.1	2.68 (1H, dt, 12.4, 6.2) 2.80 (1H, dt, 12.4, 6.2)		

2-Debromonagelamide P (**3**) was obtained as a colorless amorphous solid. The ESIMS showed the pseudomolecular ion peaks at *m/z* 633, 635, and 637 (1:2:1), indicating the existence of two bromine atoms in the molecule. The HRESIMS suggested the molecular formula of **3** to be $C_{22}H_{23}N_{10}O_3Br_2$ (*m/z* 633.03191 $[M]^+$, Δ+0.32 mmu). Comparison of the 1D NMR data for **3** (Table 2) with those for bromopyrrole alklaoids from sponges of *Agelas* spp. implied **3** to be a 2-debromo derivative of nagelamide P.[14] This was confirmed based on analysis of the 2D NMR spectra (Figure 2).

2-Debromomukanadin G (**2**) and 2-debromonagelamide P (**3**) were both optically inactive, implying that they are racemates, while mukanadin G[8] and nagelamide P[14] were also assigned as racemates by analysis of the chiral HPLC and the CD spectrum, respectively.

Antimicrobial activities of **1**–**3** against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Aspergillus niger*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Cryptococcus neoformans* were evaluated. As a result, 2-debromonagelamide U (**1**) and

2-debromonagelamide P (**3**) showed antimicrobial activity against *Trichophyton mentagrophytes* (IC_{50} 16 and 32 μg/mL, respectively), while **1** and 2-debromomukanadin G (**2**) exhibited activity against *Cryptococcus neoformans* (IC_{50} 32 μg/mL each). **1**–**3** showed no cytotoxicity against human epidermoid carcinoma KB and murine lymphoma L1210 cells (IC_{50} >10 μg/mL) in vitro.

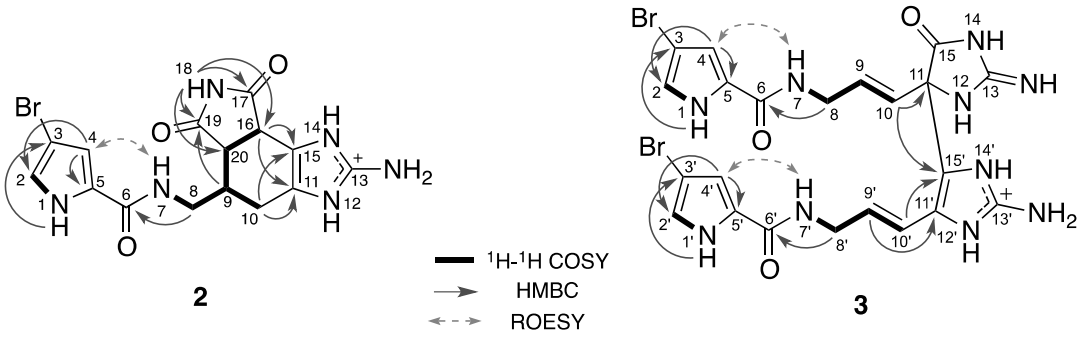

Figure 2. Selected 2D NMR correlations for 2-debromomukanadin G (**2**) and 2-debromonagelamide P (**3**)

Table 2 ^{1}H and ^{13}C NMR data for 2-debromonagelamide P (**3**) in DMSO-d_6

position	δ_C	δ_H (*J* in Hz)	position	δ_C	δ_H (*J* in Hz)
1	–	11.82 (1H, brs)	1’	–	11.82 (1H, brs)
2	121.3	7.02[c] (1H, s)	2’	121.1	6.98[c] (1H, s)
3	94.8	–	3’	94.8	–
4	111.5	6.87[d] (1H, s)	4’	111.5	6.88[d] (1H, s)
5	126.2	–	5’	126.4	–
6	159.3	–	6’	159.4	–
7	–	8.43 (1H, t, 5.1)	7’	–	8.45 (1H, t, 5.3)
8	39.6	3.89[b] (2H, m)	8’	39.5[a]	4.00[b] (2H, m)
9	130.4	5.91 (1H, dt, 15.4, 4.5)	9’	128.6	6.11 (1H, dt, 15.9, 5.9)
10	124.9	5.81 (1H, d, 15.4)	10’	115.8	6.44 (1H, d, 15.9)
11	64.9	–	11’	122.4	–
12	–	9.13[e] (1H, brs)	12’	–	12.89 (1H, brs)
13	147.6	–	13’	147.6	–
13-NH	–	8.99[e] (1H, brs)	13’-NH_2	–	7.65 (2H, brs)
14	–	10.27[e] (1H, brs)	14’	–	12.89 (1H, brs)
15	nd[g]	–	15’	118.8	–

[a] Overlapped with the signals of DMSO-d_6, [b] Signal was overlapped with that of water,
[c-f] Signals may be interchangeable, [g] not detected.

CONCLUSION

The investigation of the extract from an Okinawan marine sponge *Agelas* sp. (SS-156) resulted in the isolation of two new monomeric bromopyrrole alkaloids (**1** and **2**) and one new dimeric bromopyrrole alkaloid (**3**). The structures of **1**–**3** were assigned as 2-debromonagelamide U, 2-debromomukanadin G, and 2-debromonagelamide P, respectively, on the basis of spectroscopic analyses.

EXPERIMENTAL

General procedures: Optical rotations were recorded on a JASCO P-1030 digital polarimeter. IR and UV spectra were recorded on a JASCO FT/IR-230 and a Shimadzu UV-1600PC spectrophotometers,

respectively. NMR spectra were measured by a Bruker Avance 600 NMR spectrometer. The resonances of CHD_2SOCD_3 (δ_H 2.49) and DMSO-d_6 (δ_C 39.5) were used as internal references for ^1H and ^{13}C NMR spectra, respectively. Mass spectra were recorded on a Thermo Scientific Exactive spectrometer.

Sponge description: The sponge (SS-156: Order Agelasida, Family Agelasidae, *Agelas* sp.) with smooth, finely conulose surface collected at Kerama Islands, Okinawa, was kept frozen until used. Mesohyl is compact with large internal canals. Sponges are firm, springy and compressible. Skeleton is dense, reticulate fibre skeleton, with grainy texture over fibres, echinated by verticillate spined acanthostyles. Fibres sparsely cored by spicules, more coring of spicules towards surface of sponge. Spicules are verticillate, regularly spined acanthostyles, 170x15 μm.

Isolation of 2-debromonagelamide U (1), 2-debromomukanadin G (2), and 2-debromonagelamide P (3): The sponge *Agelas* sp. (6.3 kg, wet weight) was extracted with MeOH (6 L x 3) to give the extract (424.0 g). A part of the extract (205.6 g) was partitioned successively with *n*-hexane (800 mL x 3), *n*-BuOH (800 mL x 3), and water (800 mL). The *n*-BuOH-soluble materials (77.1 g) were subjected to a silica gel column ($CHCl_3$/MeOH/AcOH, 80:20:2→0:100:2) to give six fractions (frs. 1–6). Separation of fr. 3 on a TOYOPEARL HW-40 column (MeOH/H_2O/TFA, 30:70:0.1→100:0:0.1) afforded six fractions (frs. 3.1–6), and fr. 3.4 was separated by a C_{18} column (MeOH/H_2O/TFA, 20:80:0.1→100:0:0.1) to give seven fractions (frs. 3.4.1–7). Fr. 3.4.2 was purified using C_{18} HPLC (YMC ODS-AQ, 20 x 250 mm; flow rate 5.0 mL/min; UV detection at 254 nm; eluent MeCN/H_2O/TFA, 25:75:0.1) to isolate 2-debromomukanadin G (**2**, 10.9 mg). Fr. 3.4.5 was loaded on a Sephadex LH-20 column (MeOH/H_2O/TFA, 20:80:0.1→100:0:0.1) chromatography to give five fractions (frs. 3.4.5.1–5). Fr. 3.4.5.4 was purified by C_{18} HPLC (YMC ODS-AQ, 20 x 250 mm; 5.0 mL/min; 254 nm; MeCN/H_2O/TFA, 30:70:0.1, and then YMC Hydrosphere C18, 10 x 250 mm; 2.5 mL/min; 254 nm; MeCN/H_2O/TFA, 23:77:0.1) to afford 2-debromonagelamide P (**3**, 6.7 mg). Separation of fr. 4 using a TOYOPEARL HW-40 column (MeOH/H_2O/TFA, 10:90:0.1→100:0:0.1) and a Sephadex LH-20 column (MeOH/H_2O/TFA, 10:90:0.1→100:0:0.1) gave a fraction containing bromopyrrole alkaloids. The fraction was purified by C_{18} HPLC (YMC ODS-AQ, 20 × 250 mm, 5.0 mL/min, 254 nm, MeCN/H_2O/TFA, 15:85:0.1) and HILIC HPLC (Cosmosil HILIC, 10 × 250 mm, 3.0 mL/min, 254 nm, MeCN/H_2O, 87:13) to isolate 2-debromonagelamide U (**1**, 1.7 mg).

2-Debromonagelamide U (1). Colorless amorphous solid; $[\alpha]^{21}_D$ +14.3 (*c* 0.025, MeOH); UV (MeOH) ν_{max} 209 (ε 17200 sh) and 268 (4200) nm; IR (KBr) λ_{max} 3444, 1684, and 1673 cm^{-1}; ESIMS *m/z* 449 and

451 (1:1) $[M–H]^-$; HRESIMS: *m/z* 449.02591 $[M–H]^-$ (calcd for $C_{13}H_{18}N_6O_5{}^{79}BrS$, 449.02482; ^1H and ^{13}C NMR (Table 1).

2-Debromomukanadin G (2). Colorless amorphous solid; $[\alpha]^{21}{}_D$ ≈0 (*c* 0.25, MeOH); UV (MeOH) ν_{max} 221 (ε 7100 sh) and 268 (6900) nm; IR (KBr) λ_{max} 3181, 1717, and 1683 cm^{-1}; ESIMS *m/z* 407 and 409 (1:1) $[M]^+$; HRESIMS: *m/z* 407.04590 $[M]^+$ (calcd for $C_{15}H_{16}N_6O_3{}^{79}Br$, 407.04618); ^1H and ^{13}C NMR (Table 1).

2-Debromonagelamide P (3). Colorless amorphous solid; $[\alpha]^{21}{}_D$ ≈0 (*c* 0.25, MeOH); UV (MeOH) ν_{max} 221 (ε 12200 sh) and 271 (13800) nm; IR (KBr) λ_{max} 3181, 1690, and 1637 cm^{-1}; ESIMS *m/z* 633, 635, and 637 (1:2:1) $[M]^+$; HRESIMS: *m/z* 633.03191 $[M]^+$ (calcd for $C_{22}H_{23}N_{10}O_3{}^{79}Br_2$, 633.03159; ^1H and ^{13}C NMR (Table 2).

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