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Nagelamides X–Z, Dimeric Bromopyrrole Alkaloids from a Marine Sponge *Agelas* sp.

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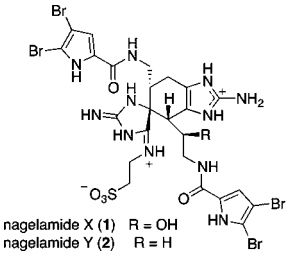
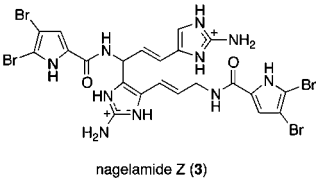
ABSTRACT





Three new dimeric bromopyrrole alkaloids, nagelamides X–Z (1–3), were isolated from a marine sponge *Agelas* sp. Nagelamides X (1) and Y (2) possess a novel tricyclic skeleton consisting of spiro-bonded tetrahydrobenzaminoimidazole and aminoimidazolidine moieties. Nagelamide Z (3) is the first dimeric bromopyrrole alkaloid involving the C-8 position in dimerization. The structures of 1–3 were elucidated on the basis of spectroscopic data. Nagelamides X–Z (1–3) exhibited antimicrobial activity.

Bromopyrrole alkaloids are known to be one of the most common metabolites contained in marine sponges. Among them, dimeric bromopyrrole alkaloids derived from monomers such as oroidin, hymenidin, and clathrodin have attracted widespread interest due to their fascinating complex chemical structures with a high N to C ratio (~1:2) and intriguing biological activities.[1] In our continuing search for structurally unique metabolites from Okinawan marine sponges, we have previously reported the isolation of a series of dimeric bromopyrrole alkaloids with unique cyclic skeletons, nagelamides[2] and benzosceptrin C,[3] from *Agelas* spp. Recently, we have isolated three new monomeric bromopyrrole alkaloids, nagelamides U–W,[4] from the extracts of a marine sponge *Agelas* sp. (SS-162). Further investigation of the extracts resulted in the isolation of three new dimeric bromopyrrole alkaloids, nagelamides X–Z (**1**–**3**). In this letter, we describe the isolation and structure elucidation of **1**–**3**.

The sponge *Agelas* sp. (SS-162, 3.9 kg, wet weight) collected off the Kerama Islands, Okinawa, was extracted with MeOH. The extracts were partitioned successively with EtOAc, *n*-BuOH, and water, and the EtOAc-soluble materials were partitioned with *n*-hexane and 10% aq MeOH. The 10% aq MeOH-soluble materials were subjected to silica gel column, C_{18} column, and Sephadex LH-20

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column chromatographies to give fractions containing bromopyrrole alkaloids. Purification of the fractions using reverse-phase HPLC afforded nagelamides X (**1**, 0.000057%, wet weight), Y (**2**, 0.000074%), and Z (**3**, 0.00035%).

Nagelamide X (**1**)[5] was obtained as a colorless amorphous solid, and the ESIMS showed the pseudomolecular ion peak at *m*/*z* 914, 916, 918, 920, and 922 (1:4:6:4:1), suggesting the existence of four bromine atoms in the molecule. The molecular formula of **1**, $C_{24}H_{28}N_{11}O_6Br_4S$, was established by the HRESIMS (*m*/*z* 913.86795 $[M]^+$, Δ+0.67 mmu). An IR absorption at 1684 cm^{-1} and a UV absorption at 278 nm were indicative of a pyrrole amide moiety, a common unit on bromopyrrole alkaloids.[1,2] Comparison of the 1D NMR data for **1** (Table 1) with those for known bromopyrrole alkaloids[2] implied that **1** has two dibromopyrrole amide moieties (N-1−N-7 and N-1′−N-7′) and one 2-aminoethanesulfonic acid moiety (N-1″−C-3″). These partial structures were confirmed by 2D NMR analysis (Figure 1).

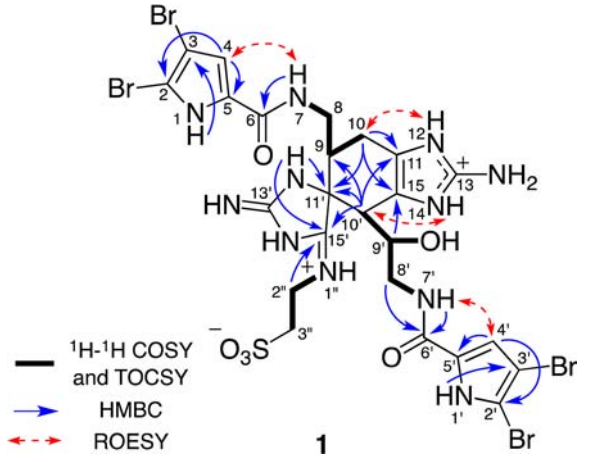

Figure 1. Selected 2D NMR correlations for nagelamide X (**1**).

Interpretation of the $^1H-^1H$ COSY and HMBC spectra disclosed the existence of a cylohexene ring (C-9−C-11, C-15, C-10′, and C-11′) in **1** (Figure 1). On the other hand, an aminoimidazole ring (C-11−C-15) was suggested by resemblance of the chemical shifts for C-11, C-13, and C-15 with the corresponding position of nagelamide G.[2e] ROESY correlations for H_2-10/12-NH and H-10′/14-NH indicated that the aminoimidazole ring was fused to the cyclohexene ring. Similarly, the existence of an aminoimidazolidine ring with 2-aminoethanesulfonic acid (C-11′−C-15′ and N-1″−C-3″) was deduced by comparison of their chemical shifts with those of nagelamide H.[2e] The connectivity of the aminoimidazolidine moiety to the cyclohexene ring through a spiro-linkage (C-11′) was disclosed by HMBC correlations for H-10′/C-15′ and 12′-NH/C-11′. Thus, the tricyclic core of **1** was elucidated as shown in Figure 1.

The connectivities of one dibromopyrrole amide moiety (N-7) to C-9 via an sp^3 methylene (C-8) was revealed by $^1H-^1H$ COSY and TOCSY cross-peaks of 7-NH/H_2-8 and H_2-8/H-9, while $^1H-^1H$ COSY cross-peaks of 7′-NH to H-10′ and H-9′/9′-OH and an HMBC correlation for H-9′/C-15 suggested the connectivities of C-10′ to C-8′ through an sp^3 oxymethine (C-9′) and C-8′ to the other dibromopyrrole amide moiety (N-7′). Thus, the gross structure of nagelamide X (**1**) was assigned as shown in Figure 1.

ROESY cross-peaks of H-8a/12′-NH, 12′-NH/H-9′, and H-10′/1″-NH suggested the relative configurations for C-9, C-10′, and C-11′ to be *S**, *R**, and *R**, respectively (Figure 2A). The 9′*R** configuration was deduced by ROESY correlations for H-9′/12′-NH, H-9′/H-10′, H-10′/7′-NH, and H-8′a/14-NH (Figure 2B). The 9′*R** configuration was also supported by the coupling constant of H-10′ (br s), implying that the dihedral angle of H-10′/H-9′ was close to 90°. Accordingly, the relative stereochemistry of nagelamide X (**1**) was assigned as shown in Figure 2.

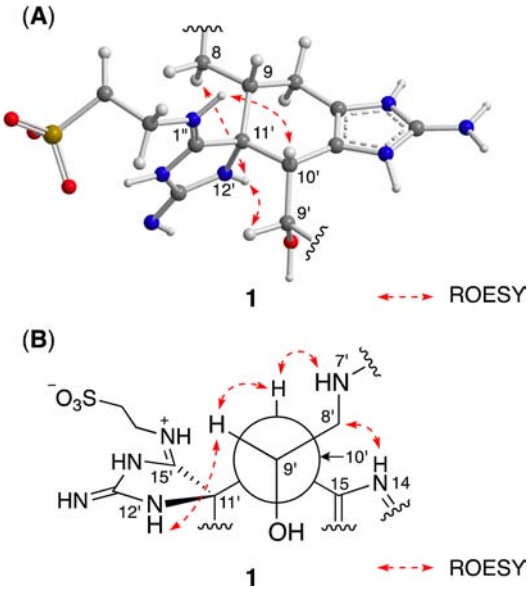

Figure 2. (A) Selected ROESY correlations and the relative stereochemsitry for the tricyclic core and (B) projection for the C-9′ to C-10′ bond and the relative configuration for C-9′ of nagelamide X (**1**).

Nagelamide Y (**2**)[6] was isolated as a colorless amorphous solid. The molecular formula, $C_{24}H_{28}N_{11}O_5Br_4S$, was indicated by the HRESIMS (*m*/*z* 897.87359 $[M]^+$,

(5) Nagelamide X (**1**): colorless amorphous solid; $[\alpha]_D^{21} \approx 0$ (*c* 0.25, MeOH); UV (MeOH) λ_{max} 212 (ε 18800, sh) and 278 (16300) nm; IR (KBr) ν_{max} 3581, 1684, 1206, and 1138 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m*/*z* 914, 916, 918, 920, and 922 (1:4:6:4:1) $[M]^+$; HRESIMS *m*/*z* 913.86795 $[M]^+$ (calcd for $C_{24}H_{28}N_{11}O_6{}^{79}Br_4S$, 913.86728).

(6) Nagelamide Y (**2**): colorless amorphous solid; $[\alpha]_D^{22} \approx 0$ (*c* 0.25, MeOH); UV (MeOH) λ_{max} 212 (ε 22900, sh) and 278 (18400) nm; IR (KBr) ν_{max} 3390, 1684, 1205, and 1136 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m*/*z* 898, 900, 902, 904, and 906 (1:4:6:4:1) $[M]^+$; HRESIMS *m*/*z* 897.87359 $[M]^+$ (calcd for $C_{24}H_{28}N_{11}O_5{}^{79}Br_4S$, 897.87236).

Table 1. ^{1}H and ^{13}C NMR Data for Nagelamides X–Z (**1**–**3**) in DMSO-d_6

	1		**2**		**3**	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	–	12.75 (1H, brs)[f]	–	12.76 (1H, brs)	–	12.79 (1H, brs)
2	104.9[b]	–	104.9	–	105.4	–
3	97.9[e]	–	97.9[h]	–	98.2[m]	–
4	113.0[d]	6.95 (1H, s)	112.9	6.93 (1H, d, J = 2.2)	113.8	7.03 (1H, s)
5	127.6[e]	–	127.8[i]	–	127.0	–
6	159.2	–	159.2[j]	–	158.2	–
7	–	8.21 (1H, brt, J = 5.6)	–	8.23 (1H, brt, J = 5.4)	–	8.61 (1H, d, J = 7.4)
8	37.8	3.14, 2.97 (1H each, m)	37.3	3.28, 3.08 (1H each, m)	44.6	5.84 (1H, dd, J = 7.4, 4.6)
9	36.0	3.21(1H, m)	37.0	2.55 (1H, m)	125.6	6.19 (1H, dd, J = 16.5, 4.6)
10	20.4	2.72 (1H, m) 2.39 (1H, dd, J = 17.3, 10.0)	20.9	2.72, 2.50 (1H each, m)	117.8	6.24 (1H, d, J = 16.5)
11	122.0	–	119.1[k]	–	124.4	–
12	–	12.06 (1H, brs)	–	12.21 (1H, brs)	–	12.63 (1H, brs)[n]
13	147.7	–	147.9	–	148.0	–
13-NH_2	–	7.63 (2H, brs)	–	7.66 (2H, brs)	–	7.64 (2H, brs)
14	–	11.90 (1H, brs)	–	12.15 (1H, brs)	–	12.17 (1H, brs)[n]
15	114.3	–	118.9[k]	–	112.9	6.97 (1H, s)
1'	-	12.87 (1H, brs)[f]	–	12.76 (1H, brs)	–	12.70 (1H, brs)
2'	105.4[b]	–	104.9	–	104.7	–
3'	98.1[e]	–	98.0[h]	–	98.0[m]	–
4'	113.5[d]	6.96 (1H, s)	112.9	6.91 (1H, d, J = 1.9)	112.5	6.93 (1H, s)
5'	127.8[e]	–	127.9[i]	–	128.0	–
6'	159.8	–	159.4[j]	–	158.8	–
7'	–	8.53 (1H, brt, J = 5.8)	–	8.32 (1H, t, J = 5.6)	–	8.44 (1H, t, J = 5.7)
8'	43.2	3.46, 3.14 (1H each, m)	36.7	3.28 (2H, m)	40.5	3.96 (2H, t, J = 5.7)
9'	70.1	3.91(1H, m)	32.0	1.83, 1.66 (1H each, m)	127.3	6.09 (1H, dt, J = 16.1, 5.7)
9'-OH	–	5.26 (1H, brd, J = 5.6)				
10'	40.3	2.99 (1H, brs)	34.9	3.06 (1H, m)	115.9	6.49 (1H, d, J = 16.1)
11'	70.9	–	71.5	–	121.1	–
12'	–	9.76 (1H, brs)	–	10.04 (1H, brs)	–	12.59 (1H, brs)[n]
13'	167.6	–	167.3	–	148.3	–
13'-NH	–	8.25 (1H, brs)[g]	–	8.51 (1H, brs)[l]	–	7.64 (2H, brs)
14'	–	8.97 (1H, brs)[g]	–	8.94 (1H, brs)[l]	–	12.02 (1H, brs)[n]
15'	179.1	–	179.1	–	122.2	–
1"	–	8.38 (1H, brs)	–	8.70 (1H, brs)		
2"	40.0[a]	3.74, 3.47 (1H each, m)	40.5	3.71, 3.53 (1H each, m)		
3"	48.5	2.70 (2H, m)	48.5	2.75 (2H, t, J = 6.2)		

[a] Overlapped with signal of DMSO-d_6, [b–n] Interchangeable.

Δ+1.23 mmu), smaller by 16 mass unit as compared with that of **1**. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, and the resonances of an sp^2 methylene in **2** were discerned in place of the signals of an oxymethine (C-9′) in **1** (Table 1). Therefore, nagelamide Y (**2**) was deduced to be a dehydroxy derivative of **1**. The structure of **2** including the relative stereochemistry was confirmed based on analysis of the 2D NMR spectra (Supporting Information).

Nagelamides X (**1**) and Y (**2**) were optically inactive, which prompted us to perform the optical resolution of **1** and **2** on chiral HPLC.[7] The analysis of **1** showed the separation of enantiomers, the ratio of which was approximately 1:1. Therefore, nagelamide X (**1**) was concluded to be a racemate. Similarly, the enantiomers of nagelamide Y (**2**) was separated by chiral HPLC under the same condition, suggesting that **2** was also a racemate.

Nagelamide Z (**3**)[8] was yielded as an optically active pale yellow amorphous solid {$[\alpha]_D^{22}$ −3.0 (*c* 0.25, MeOH)}. The HRESIMS revealed the molecular formula of **3** to be $C_{22}H_{22}N_{10}O_2Br_4$ (*m*/*z* 772.86011 $[M - H]^+$, Δ+2.41 mmu). The ^{1}H and ^{13}C NMR spectra showed the signals of two dibromopyrrole amide moieties (N-1–N-7 and N-1′–N-7′) and two aminoimidazole moieties (C-11–C-15 and C-11′–C-15′) as well as two *trans*-1,2-disubstituted olefins, one sp^3 methine, and one sp^3 methylene. These data implied that **3** is a dimer of oroidin.[9] The connectivity (C-8 to C-15′)

(7) Racemic forms of **1** and **2** were analyzed using CHIRALPAK ZWIX(+) (DAICEL Corp., 250 × 4.0 mm, flow rate 0.3 mL/min, UV detection 254 nm) with eluent THF/MeOH/H_2O (40:40:20, with 100 mM formic acid and 50 mM diethylamine) to separate each enantiomer (t_R 28.7 and 29.6 min in **1**; 30.2 and 31.4 min in **2**).

(8) Nagelamide Z (**3**): pale yellow amorphous solid; $[\alpha]_D^{22}$ −3.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 280 (ε 21400) nm; IR (KBr) ν_{max} 3175, 1685, 1203, and 1138 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS *m*/*z* 773, 775, 777, 779, and 781 (1:4:6:4:1) $[M-H]^+$; HRESIMS *m*/*z* 772.86011 $[M - H]^+$ (calcd for $C_{22}H_{21}N_{10}O_2{}^{79}Br_4$, 772.85770).

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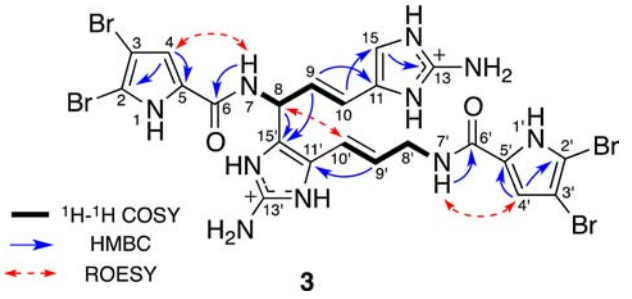
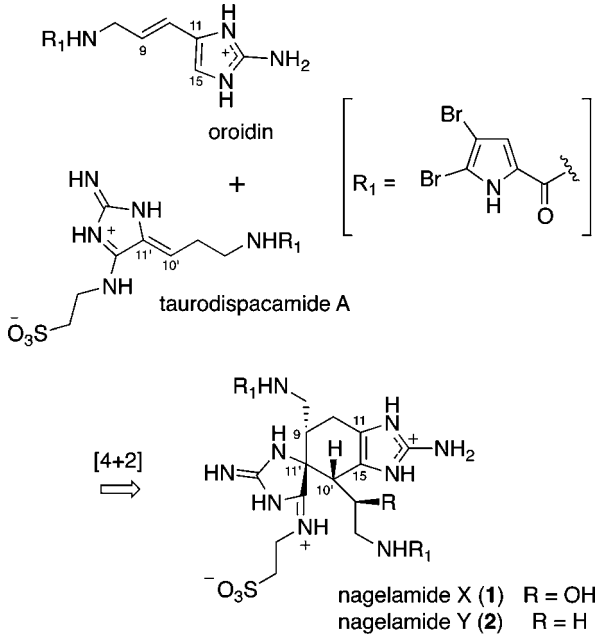


Figure 3. Selected 2D NMR correlations for nagelamide Z (**3**).

Scheme 1. Possible Biogenetic Path of Nagelamides X (**1**) and Y (**2**)



between each oroidin unit was confirmed by HMBC cross-peaks of H-8/C-15′ and H-9/C-15′ and a ROESY correlation for H-8/H-10′ (Figure 3). Thus, the gross structure of nagelamide Z (**3**) was assigned as shown in Figure 3. The absolute stereochemistry of C-8 remains unsolved.

Nagelamides X (**1**) and Y (**2**) are dimeric bromopyrrole alkaloids with a novel tricyclic skeleton, which consists of spiro-bonded tetrahydrobenzaminoimidazole and aminoimidazolidine moieties. Nagelamides X (**1**) and Y (**2**) seem to be derived from oroidin[9] and taurodispacamide A[10] by [4 + 2] cycloaddition (Scheme 1). Nagelamide Z (**3**) is the first dimeric bromopyrrole alkaloid involving the C-8 position in dimerization.

Nagelamides X–Z (**1**–**3**) exhibited antimicrobial activities against some bacteria and fungi (Table 2). In particular, nagelamide Z (**3**) showed potent inhibitory activity against *Candida albicans* (IC_{50}, 0.25 μg/mL).

Table 2. Antimicrobial Activities of Nagelamides X–Z (**1**–**3**)

strain	**1**	**2**	**3**
Escherichia coli[a]	32<	32<	32<
Staphylococcus aureus[a]	8.0	32<	16
Bacillus subtilis[a]	32<	32<	32<
Micrococcus luteus[a]	8.0	32<	8.0
Aspergillus niger[b]	32	32<	4.0
Trichophyton mentagrophytes[b]	16	32<	4.0
Candida albicans[b]	2.0	2.0	0.25
Cryptococcus neoformans[b]	32<	32<	2.0

[a] MIC value (μg/mL). [b] IC_{50} value (μg/mL).

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Supporting Information Available. Experimental section, 1D and 2D NMR spectra of nagelamides X–Z. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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