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Nagelamides Q and R, Novel Dimeric Bromopyrrole Alkaloids from Sponges *Agelas* sp.

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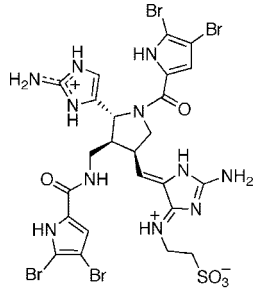
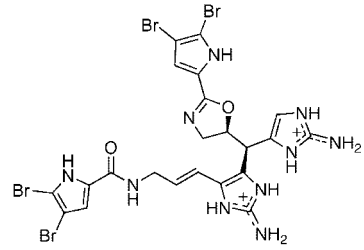
ABSTRACT



nagelamide Q (1)



nagelamide R (2)

Two new dimeric bromopyrrole alkaloids, nagelamides Q (1) and R (2), have been isolated from Okinawan marine sponges of the genus *Agelas* (SS-1134 and SS-956, respectively), and the structures and stereochemistry were elucidated from spectroscopic data. Nagelamide Q (1) is a rare dimeric bromopyrrole alkaloid possessing a pyrrolidine ring, while nagelamide R (2) is the first bromopyrrole alkaloid having an oxazoline ring. Nagelamides Q (1) and R (2) showed antimicrobial activity.

Bromopyrrole alkaloids are known to be one of the most common metabolites contained in marine sponges.[1] During our search for bioactive substances from marine organisms, we previously isolated several bromopyrrole alkaloids with unique cyclic skeletons from sponges of the genus *Agelas*.[2] Further investigation of extracts of Okinawan marine sponges of the genus *Agelas* (SS-1134 and SS-956) resulted in isolation of two new dimeric bromopyrrole alkaloids, nagelamides Q (**1**) and R (**2**), respectively. Herein we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Agelas* sp. (SS-1134) collected off Seragaki beach, Okinawa, was extracted with MeOH. BuOH-soluble materials of the extract were subjected to silica gel and C_{18} columns followed by C_{18} HPLC to yield nagelamide Q (**1**, 0.0012% wet weight) as a colorless amorphous solid together with the known related alkaloids oroidin,[3] ageliferin,[4] tauroacidin A,[5] taurodispacamide A,[6] and nagelamides B,[2a] C,[2a] H,[2a] K,[2c] L,[2c] M,[2d] and N.[2d]

The ESIMS spectrum of nagelamide Q (**1**) showed pseudomolecular ion peaks at *m*/*z* 896, 898, 900, 902, and 904 (1:4:6:4:1), indicating the presence of four bromine atoms, and the molecular formula of **1** was revealed to be $C_{24}H_{26}N_{11}O_5{}^{79}Br_4S$ by HRESIMS data [*m*/*z* 895.85868 $(M)^+$,

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Δ −1.42 mmu]. The UV absorption [λ_{max} 276 nm (ε 18000)] was attributed to a substituted pyrrole chromophore,[2] while IR absorptions indicated the existence of amino (3406 cm^{-1}) and amide carbonyl (1684 cm^{-1}) functionalities.

Two amide carbonyl (δ_C 159.0 and 158.5), six sp^2 quaternary carbons (δ_C 127.7, 126.7, 105.7, 104.5, 98.2, and 97.7), and two sp^2 methine (δ_C 112.7 and 110.1) were assigned to two 2,3-dibromopyrrole carbonyl moieties (N-1−C-6 and N-1′−C-6′) for comparison of ^1H and ^{13}C NMR data of **1** (Table 1) with those of known bromopyrrole

Table 1. ^1H and ^{13}C NMR Data of Nagelamide Q (**1**) in DMSO-d_6

position	δ_H	δ_C
1	12.61 brs	
2		105.7
3		98.2
4	6.77 s	110.1
5		126.7
6		158.5
7		
8	4.23 m	53.1
	3.37 m	
9	3.48 m	38.4
10	6.22 d (10.2)	112.3
11		133.6
12	11.54 brs	
13		166.2
13-NH_2	9.23 brs	
	8.69 brs	
14		
15		167.2
1′	12.61 brs	
2′		104.5
3′		97.7
4′	6.81 s	112.7
5′		127.7
6′		159.0
7′	8.11 brt (5.2)	
8′	3.23 (2H) m	37.3
9′	2.77 m	46.5
10′	4.90 d (6.3)	55.2
11′		126.4
12′	12.47 brs	
13′		147.1
13′-NH_2	7.44 (2H) brs	
14′	11.95 brs	
15′	6.79 s	110.1
1″	9.64 brt (5.6)	
2″	3.64 (2H) m	40.6
3″	2.75 (2H) m	49.3

alkaloids[2] and inspection of 2D NMR spectra of **1** (Figure 1). Two sp^2 quaternary carbons (δ_C 147.1 and 126.4) and one sp^2 methine (δ_C 110.1) were ascribed to a 4-monosubstituted aminoimidazole ring (C-11′−C-15′), while three sp^2 quaternary carbons (δ_C 167.2, 166.2, and 133.6) were attributed to a 4,5-disubstituted 2-aminoimidazole ring (C-11−C-15) connected to a taurine unit (N-1″−C-3″).

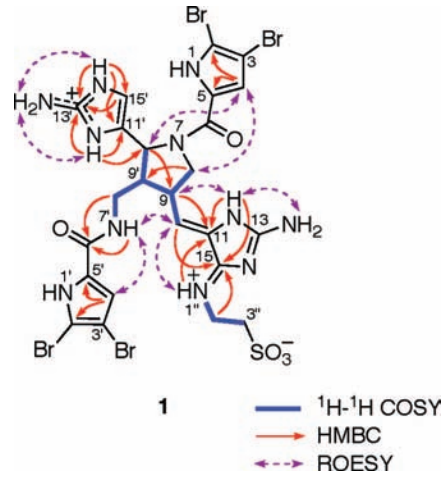


Figure 1. Selected 2D correlations for nagelamide Q (**1**).

Analysis of the $^1H-^1H$ COSY spectrum disclosed connectivities of C-8 to C-10, NH-7′ to C-10′, and C-9 to C-9′, and HMBC correlations for NH-7′ and H-8′ to C-6′ and a ROESY correlation of NH-7′/H-4′ indicated that a 2,3-dibromopyrrole carbonyl group (N-1′−C-6′) was attached to N-7′ in an amide linkage. Connection of a 4-monosubstituted aminoimidazole ring to C-10′ was indicated from HMBC correlations for NH-12′ to C-10′ and C-11′, while HMBC correlations for H-9 to C-11 and H-10 to C-15 revealed that a 4,5-disubstituted 2-aminoimidazole ring (C-11−C-15) was connected to C-10 through a double bond. Geometry of the double bond between C-10−C-11 was assigned as *Z* from ROESY correlations of H-10/NH-7′ and H-10/NH-1″. Considering the molecular formula of **1**, C-6, C-8, and C-10′ were deduced to be connected with each other through N-7. ROESY correlations of H-4/H-8 and H-4/H-10′ also supported this connection. The relative stereochemistry of the pyrrolidine ring (N-7−C-9 and C-9′−C-10′) was deduced from ROESY correlations as shown in Figure 2. Thus, the structure of nagelamide Q was elucidated to be **1**.

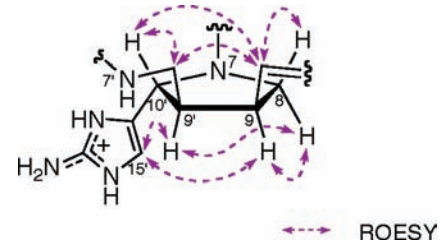


Figure 2. Selected ROESY correlations and relative stereochemistry for C-9, C-9′, and C-10′ in nagelamide Q (**1**).

BuOH-soluble materials of the MeOH extract of another collection of the sponge *Agelas* sp. (SS-956) collected off Unten-Port, Okinawa, were subjected to silica gel and C_{18} column chromatographies followed by C_{18} HPLC to yield

nagelamide R (**2**, 0.0013%, wet weight) as a colorless amorphous solid together with the known related alkaloids oroidin,[3] ageliferin,[4] mauritiamine,[7] and nagelamides B,[2a] C,[2a] and L.[2c]

The ESIMS spectrum of nagelamide R (**2**) showed pseudomolecular ion peaks at *m*/*z* 773, 775, 777, 779, and 781 (1:4:6:4:1), indicating the presence of four bromine atoms, and the molecular formula of nagelamide R (**2**) was established to be $C_{22}H_{22}N_{10}O_2{}^{79}Br_4$ by HRESIMS data [*m*/*z* 772.85649 $(M - H)^+$, Δ −1.76 mmu]. The ^{13}C NMR data disclosed 22 signals due to 13 sp^2 quaternary carbons, five sp^2 methines, two sp^3 methines, and two sp^3 methylenes (Table 2). On the basis of analyses of the HMQC spectrum

Table 2. ^1H and ^{13}C NMR Data of Nagelamide R (**2**) in C_5D_5N

position	δ_H	δ_C
1		
2		106.0
3		99.1
4	7.11 brs	116.1
5		122.7
6		156.7
7		
8	3.98 dd (15.0, 7.3)	59.2
	4.23 m	
9	5.50 m	79.5
10	4.73 d (5.9)	37.3
11		124.4
12		
13		150.2
13-NH_2		
14		
15	7.17 s	112.0
1′		
2′		105.2
3′		99.5
4′	7.21 brs	113.3
5′		129.6
6′		160.2
7′	9.02 brt (5.7)	
8′	4.18 (2H) m	41.5
9′	6.48 dt (15.9, 6.1)	128.1
10′	6.75 d 15.9	116.5
11′		124.2
12′		
13′		149.8
13′-NH_2		
14′		
15′		119.8

as well as ^1H and ^{13}C NMR of **2**, six sp^2 quaternary carbons (δ_C 129.6, 122.7, 106.0, 105.2, 99.5, and 99.1) and two sp^2 methines (δ_C 116.1 and 113.3) were ascribed to two 5-monosubstituted 2,3-dibromopyrrole rings (N-1−C-5 and N-1′−C-5′) (Figure 3). Two sp^2 quaternary carbons (δ_C 150.2 and 124.4) and one sp^2 methine (δ_C 112.0) were attributed to a 4-monosubstituted aminoimidazole ring (C-11−C-15), while three sp^2 quaternary carbons (δ_C 149.8, 124.2, and 119.8) were assigned to a 4,5-disubstituted 2-aminoimidazole

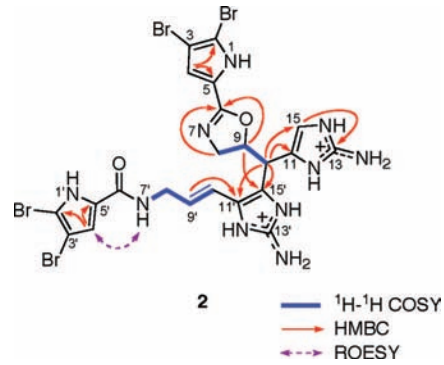


Figure 3. Selected 2D correlations for nagelamide R (**2**).

ring (C-11′−C-15′). The $^1H-^1H$ COSY spectrum of **2** disclosed the connections for C-8 to C-10 and N-7′ to C-10′. HMBC correlations of H-10 to C-11, C-15, C-11′ and C-15′ suggested that two 2-aminoimidazole rings were attached to C-10, while the connection of C-10′ and C-11′ in the 4,5-disubstituted 2-aminoimidazole ring was implied from HMBC correlation for H-9′ to C-11′. The ROESY correlation for H-4′ and an amide proton at N-7′ (δ_H 9.02) indicated that one of two 5-monosubstituted 2,3-dibromopyrrole rings was connected to N-7′ through an amide carbonyl carbon (C-6′, δ_C 160.2). HMBC correlations for protons of a nitrogen-bearing methylene (C-8, δ_C 59.2) and an oxymethine (C-9, δ_C 79.5) to an imidate carbon (C-6, δ_C 156.7) suggested the presence of a 2,5-disubstituted oxazoline ring (C-6, N-7, C-8−C-9, and 9-O),[9,10] and a connection of C-5 and C-6 was deduced from the molecular formula of **2**. The relative stereochemistry of C-9 and C-10 was elucidated on the basis of ROESY correlations as shown in Figure 4. The structure

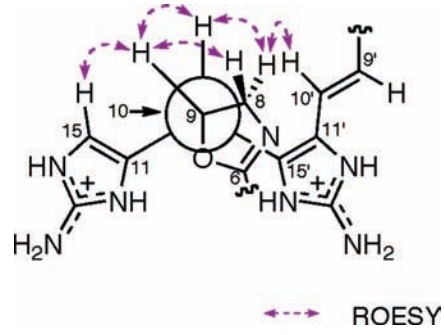
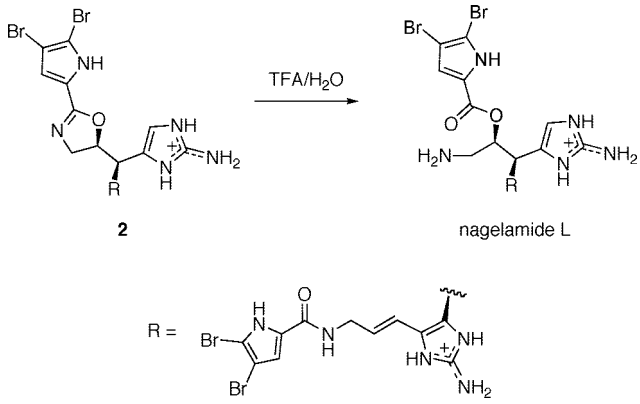


Figure 4. Rotation model for the C-9/C-10 bond of nagelamide R (**2**).

of **2** was confirmed by the chemical correlation with nagelamide L.[2c] Hydrolysis of **2** with trifluoroacetic acid resulted in generation of a reaction compound whose spectral data were identical with those of nagelamide L[2c] (Scheme 1). Thus, the structure of nagelamide R was concluded to be **2**.

Scheme 1. Chemical Correlation of Nagelamide R (**2**) to Nagelamide L



A plausible biogenetic path for nagelamides K[2c] and Q (**1**) is proposed as shown in Figure 5. Several proposals for

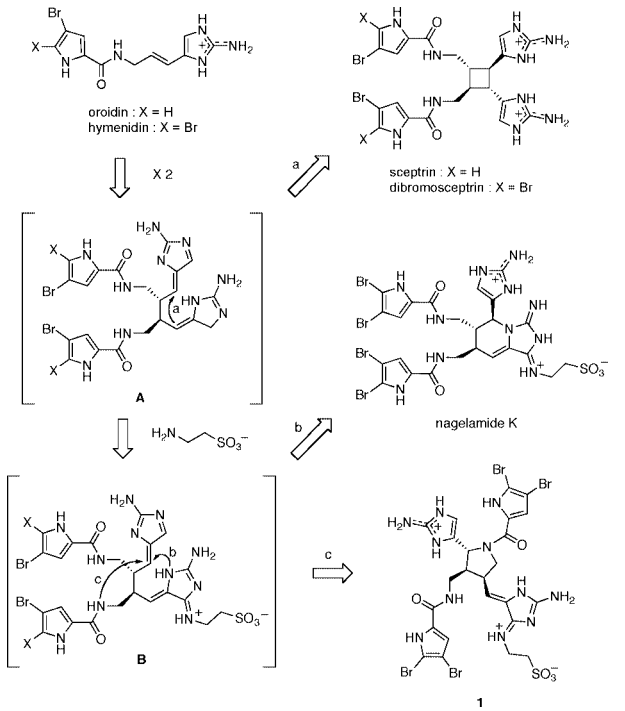


Figure 5. Plausible biogenetic path for nagelamides K and Q (**1**).

the biogenetic path of sceptrin[8] and its related alkaloids have been reported.[9−11] Nagelamides K[2c] and Q (**1**) could be produced from a common intermediate (**B**), which might be derived from a taurine and a hypothetical precursor (**A**) of sceptrin[8] through intramolecular cyclization.

It has been reported that treatment of oroidin with TFA yielded a compound possessing an oxazoline ring.[12,13] This report implied that nagelamide C[2a] could be a candidate for the precursor of nagelamide R (**2**). However, nagelamide C was not converted into **2** under the same conditions. Nagelamide L[2c] might be generated from nagelamide R (**2**) by enzymatic or spontaneous hydrolysis in the organism.

Nagelamide Q (**1**) is a rare dimeric bromopyrrole alkaloid possessing a pyrrolidine ring, while nagelamide R (**2**) is the first bromopyrrole alkaloid having an oxazoline ring. Nagelamides Q (**1**) and R (**2**) showed antimicrobial activities[14] against some bacteria and fungi as shown in Table 3.

Table 3. Antimicrobial Activities of Nagelamides Q (**1**) and R (**2**)

	MIC (μg/mL)	
strain	**1**	**2**
Bacillus subtilis	13.0	13.0
Escherichia coli	>25.0	>25.0
Micrococcus luteus	>25.0	>25.0
Staphylococcus aureus	>25.0	>25.0
Trichophyton mentagrophytes	6.0	6.0
Cryptococcus neoformans	13.0	>25.0
Candida albicans	13.0	13.0
Aspergillus niger	13.0	13.0

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Supporting Information Available: General experimental methods and one- and two-dimensional NMR spectra for nagelamides Q (**1**) and R (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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