Tetrahedron 64 (2008) 10810–10813

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Nagelamides M and N, new bromopyrrole alkaloids from sponge *Agelas* species

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ARTICLE INFO

Article history:
Received 11 August 2008
Received in revised form
15 September 2008
Accepted 15 September 2008
Available online 24 September 2008

ABSTRACT

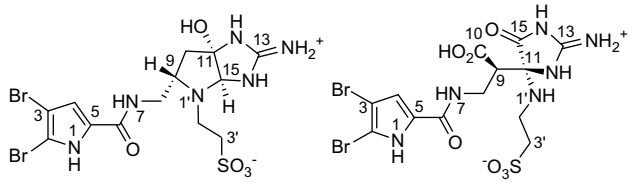
Two new bromopyrrole alkaloids, nagelamides M (**1**) and N (**2**), have been isolated from an Okinawan marine sponge *Agelas* species, and the structures and stereochemistry were elucidated from the spectroscopic data. Nagelamide M (**1**) is a novel bromopyrrole alkaloid possessing a 2-amino-octahydropyrrolo[2,3-*d*]imidazole ring with a taurine unit, while nagelamide N (**2**) is a new bromopyrrole alkaloid possessing a 2-amino-tetrahydroimidazole-4-one ring with a taurine unit and 3-(dibromopyrrole-2-carboxamido)propanoic acid moiety. Nagelamides M (**1**) and N (**2**) exhibited antimicrobial activity.

1. Introduction

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 *Agelas* or *Hymeniacidon* sp.[2] More recently, two new bromopyrrole alkaloids, nagelamides M (**1**) and N (**2**), have been isolated from an Okinawan marine sponge *Agelas* sp. (SS-1134). Here we describe the isolation and structure elucidation of **1** and **2**.

2. Results and discussion

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} column chromatographies followed by C_{18} HPLC to yield nagelamides M (**1**, 0.00069%, wet weight) and N (**2**, 0.0016%) together with known related alkaloids, tauroacidin A,[3] taurodispacamide A,[4] and nagelamides C[5] and K.[2]



1 **2**

The ESIMS spectrum of nagelamide M (**1**) showed the pseudomolecular ion peaks at *m*/*z* 527, 529, and 531 (1:2:1), indicating the presence of two bromine atoms, and the molecular formula of **1** was revealed to be $C_{13}H_{18}N_6O_5Br_2S_1$ by HRESIMS data [*m*/*z* 526.9340 $(M-H)^-$, Δ −0.8 mmu]. The UV absorption [λ_{max} 275 nm (ε 18,000)] was attributed to a pyrrole chromophore,[6] while the IR absorption (1684 cm^{-1}) indicated the existence of amide carbonyl functionality.

The ^1H NMR (Table 1) spectrum included five D_2O-exchangeable signals (δ_H 12.65, 9.02, 8.64, 8.30, and 7.95) attributed to amino and/or amide protons. The ^{13}C NMR (Table 1) spectrum disclosed 13 signals due to one amide carbonyl carbon, four sp^2 quaternary carbons, one sp^2 methine, one sp^3 quaternary carbon, two sp^3 methine, and four sp^3 methylenes. Among the ^{13}C signals of **1**, one amide carbonyl (159.10), three sp^2 quaternary carbons (127.98, 104.41, and 97.91), and one sp^2 methine (δ_C 113.29) were ascribed to a 2,3-dibromopyrrole carbonyl moiety (N-1–C-6) by comparison with those of known bromopyrrole alkaloids,[2] while one sp^3 quaternary carbon (δ_C 94.36) and one sp^3 methine (δ_C 81.26) were assigned as those bearing two hetero atoms such as oxygen and nitrogen atoms.

Detailed analyses of the ^1H–^1H COSY and HMQC spectra disclosed three structural fragments, N-7 to C-10, C-2′ to C-3′, and N-14 to C-15. The presence of a 2,3-dibromopyrrole moiety was suggested by HMBC cross-peaks of NH-1 to C-3 and C-4, and H-4 to C-2 and C-5 (Fig. 1). The NOESY correlation for NH-7/H-4 indicated that the 2,3-dibromopyrrole moiety was attached to N-7 through an amide bond. The presence of a 2-amino-octahydropyrrolo[2,3-*d*]imidazole ring was deduced from analysis of the HMBC spectrum of **1**. Connections among C-10, N-12, and C-15 via C-11 were implied by HMBC cross-peaks for H_2-10 and H-15 to C-11, and NH-12 to C-15. HMBC correlations for NH-12, NH-14, and H-15 to C-13, and

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doi:10.1016/j.tet.2008.09.039

Table 1
^{1}H and ^{13}C NMR data of nagelamide M (**1**) in DMSO-d_6

Position	δ_H		δ_C
1	12.65	brs	—
2	—		104.41
3	—		97.91
4	7.02	brs	113.29
5	—		127.98
6	—		159.10
7	8.30	brdd 7.7, 4.2	—
8a	3.67	m	37.40
8b	3.18	m	—
9	2.94	m	59.50
10a	2.11	dd 12.1, 4.7	40.67
10b	1.77	brt 11.5	
11	—		94.36
12	9.02	brs	—
13	—		157.54
13-NH_2	7.95 (2H)	brs	—
14	8.64	brs	—
15	4.93	brs	81.26
2′a	3.16	m	48.34
2′b	2.86	m	—
3′a	2.82	m	40.78
3′b	2.72	m	

NH-12 to C-15 indicated the connection of N-12 and N-14 via C-13. The connectivity of C-9 and C-15 through N-1′ was implied by the HMBC cross-peak of H-15 to C-9, while HMBC correlations for NH-14 to C-11 indicated the connection of C-11 and N-14 via C-15. In addition, NOESY correlations for H_2-8/H-2′ and H-15/H_2-2′ suggested that a taurine unit was attached to N-1′. Thus, the gross structure of nagelamide M was elucidated to be **1**.

Relative stereochemistry of the bicyclic system in **1** was deduced from *J*-values and NOESY correlations as shown in Figure 2. The NOESY correlation for H-10b/H-15 indicated that H-10b and H-15 was α-oriented, while NOESY cross-peaks of H-9/H-10a, and H-10a/NH-12 suggested that these hydrogen atoms were β-oriented. The cis ring junction of the bicyclic ring system and an α-orientation of 11-OH were implied by data described above.

The ESIMS spectrum of nagelamide N (**2**) showed the pseudomolecular ion peaks at *m*/*z* 557, 559, and 561 (1:2:1), suggesting the presence of two bromine atoms. The molecular formula of **2** was revealed to be $C_{13}H_{15}N_6O_7Br_2S_1$ from HRESIMS data [*m*/*z* 556.9105 $(M-H)^-$, Δ +1.5 mmu]. The UV absorption [λ_{max} 275 nm (ε 10,000)] indicated the presence of pyrrole chromophore, while IR absorptions (3388, 1700, and 1678 cm^{-1}) suggested the existence of hydroxyl and carbonyl functionalities.

The ^{1}H NMR (Table 2) spectrum included six D_2O-exchangeable signals (δ_H 12.75, 9.84, 9.15, 9.07, 8.31, and 7.92) attributed to amino and/or amide protons. The ^{13}C NMR (Table 2) spectrum disclosed 13 signals due to seven sp^2 quaternary carbons, one sp^2 methine, one sp^3 methine, one sp^3 quaternary carbon, and three sp^3 methylenes.

Inspection of the ^{1}H–^{1}H COSY and HMQC spectra of **2** revealed two structural fragments, N-7 to C-9 and N-1′ to C-3′. The presence

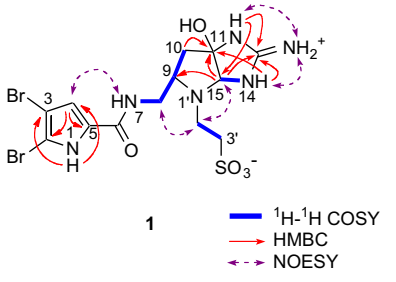


Figure 1. Selected 2D NMR correlations for nagelamide M (**1**).

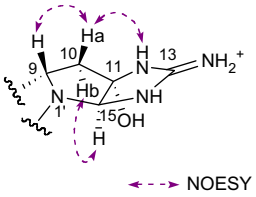


Figure 2. Selected NOESY correlations and relative stereochemistry for the bicyclic core in nagelamide M (**1**).

of 2,3-dibromopyrrole moiety was suggested by HMBC cross-peaks of NH-1 to C-3 and C-4, and H-4 to C-2 and C-5. The ROESY correlation for NH-7/H-4 indicated that the 2,3-dibromopyrrole moiety was attached to NH-7 through an amide bond. The HMBC correlation for H-9 to C-10 indicated that a carboxy group was attached to C-9. The presence of an aminoimidazole ring was deduced from HMBC correlations for NH-12 to C-11, C-13, and NH-12 and NH-14 to C-15, and ROESY cross-peaks for 13-NH_2/NH-12 and NH-14. The HMBC correlation for H-9 to C-11 and the ROESY cross-peak of NH-1′/H-9 revealed that both C-9 and NH-1′ were attached to C-11. Thus, the gross structure of nagelamide N was assigned as **2** (Fig. 3).

The relative stereochemistry of **2** was deduced from ROESY data. The relative stereochemistry for C-9 and C-11 in **2** was elucidated by ROESY correlations of NH-1′/H-8a and H-9, and NH-12/H-8b as shown in Figure 4.

A plausible biogenetic path for nagelamides M (**1**) and N (**2**) is proposed as shown in Scheme 1. Nagelamide M (**1**) could be produced by oxidation of intermediate **A**, which might be derived from taurodispacamide A[4] through cyclization, while nagelamide N (**2**) could be generated from hydrolysis and oxidation of intermediate **B**, which might be derived from taurodispacamide A through Baeyer–Villiger oxidation and cyclization.

Nagelamide M (**1**) is a novel bromopyrrole alkaloid possessing a 2-amino-octahydropyrrolo[2,3-*d*]imidazole ring with a taurine unit, while nagelamide N (**2**) is a new bromopyrrole alkaloid consisting of a 2-amino-tetrahydroimidazole-4-one ring with a taurine unit and 3-(dibromopyrrole-2-carboxamido)propanoic acid moiety. Nagelamides M (**1**) and N (**2**) showed inhibitory activity against *Aspergillus niger* (MIC, 33.3 μg/mL, each).

Table 2
^{1}H and ^{13}C NMR data of nagelamide N (**2**) in DMSO-d_6

Position	δ_H		δ_C
1	12.75	brs	—
2	—		105.07
3	—		98.05
4	6.89	s	113.12
5	—		127.91
6	—		159.09
7	8.31	brt	—
8a	3.02	m	36.57
8b	3.29	m	—
9	3.12	dd 12.2, 4.0	51.91
10	—		172.13
11	—		90.40
12	9.07	brs	—
13	—		167.55
13-NH_2	7.92 (2H)	brs	—
14	9.15	brs	—
15	—	brs	178.60
1′	9.84	brt	—
2′a	3.58		40.24
2′b	3.68	s	—
3′	3.82 (2H)	t 7.6	49.17

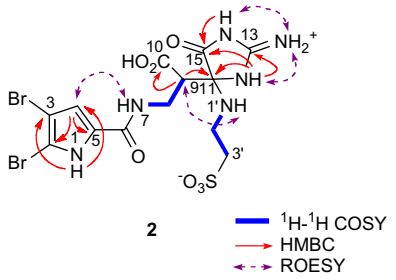


Figure 3. Selected 2D NMR correlations for nagelamide N (**2**).

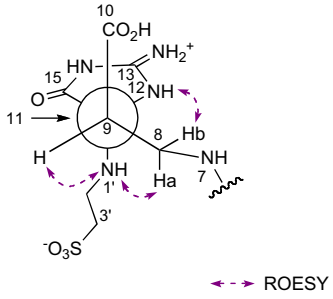


Figure 4. Rotation model for C-9/C-11 bond of nagelamide N (**2**).

3. Experimental section

3.1. General experimental procedures

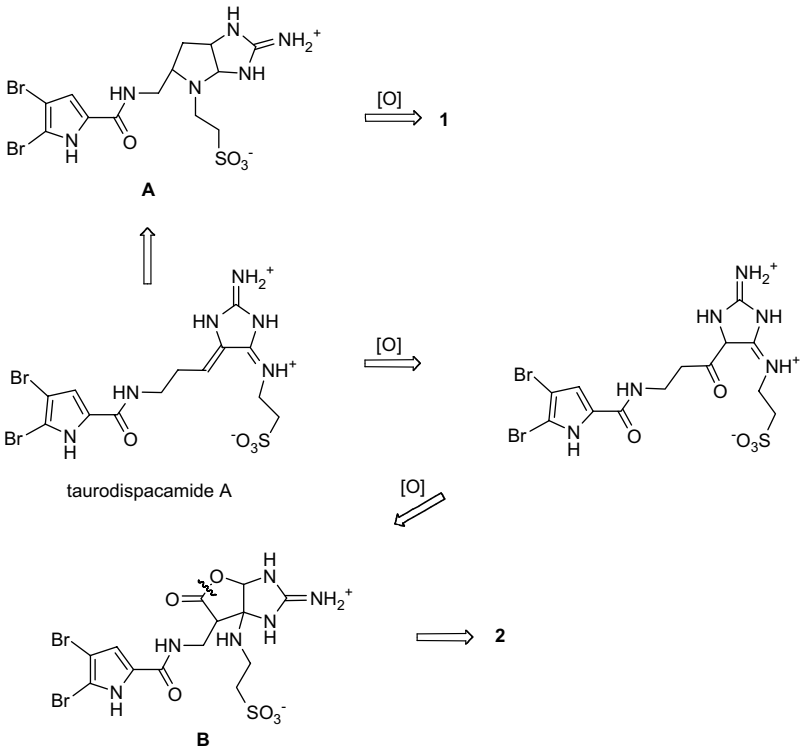
Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on a Shimadzu UV-1600PC and a JASCO FT/IR-5300 spectrophotometers, respectively. ^{1}H, ^{13}C and 2D NMR spectra were measured on a JEOL ramuda-400 spectrometer and Bruker AMX-600 spectrometer using 5 and 2.5 mm micro cells (Shigemi Co., Ltd.) for DMSO-d_6. Positive mode ESIMS spectra were measured at −80 V as a focus voltage using a sample dissolved in MeOH with flow rate of 200 μL/min. Antimicrobial activities were determined by a microbroth dilution method using BHI medium.

3.2. Sponge description

The sponge (SS-1134) *Agelas* sp. (order, Agelasida, family Agelasiidae) was collected off Seragaki, Okinawa, and kept frozen until used. The sponge was open textured sponge with convoluted surface with numerous superficial depressions and occasional oscules. The sponge has a fine ridged pattern over the surface with large apical oscules approximately 5 mm wide. The surface patterning gives a 'brain-like' appearance. The sponge has a dense internal structure and is firm and compressible. Skeleton is reticulate with fiber development, with primary fibers cored by verticillate acanthostyles, 4 spicules across, fibers are 130 μm wide and mesh spaces are approximately 500 μm wide. Secondary fibers form a tight mesh and are either cored or unispicular. Spicules are verticillate spined acanthostyles, thick, slightly curved 250×20 μm, some thin forms occur. The voucher specimen was deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

3.3. Extraction and isolation

The sponge (SS-1134, 0.70 kg, wet weight) was extracted with MeOH (2L×2), and the extract was partitioned between BuOH (500 mL×3) and H_2O (500 mL). Part (7.63 g) of BuOH-soluble materials (10.63 g) were subjected to a silica gel column ($CHCl_3$/MeOH/AcOH) and then a C_{18} column (Cosmosil 140C_{18} PREP, Nakarai Tesque Inc.; eluent, MeOH/H_2O/CF_3CO_2H, 80:20:0.1) to give a crude fraction of alkaloids. This fraction was separated by C_{18} MPLC (KUSANO C.I.G.; Kusano Science Corp.; eluent, MeOH/H_2O/CF_3CO_2H, 45:55:0.1 to 90:10:0.1 in 90 min; flow rate, 3.5 mL/min). A part of the fraction containing **1** and **2** were purified by C_{18} HPLC



Scheme 1. Plausible biogenetic path for nagelamides M (**1**) and N (**2**).

[YMC Hydrospere C18, YMC Co., Inc., 10×250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 20:80:0.1; flow rate, 2.5 mL/min; UV detection at 255 nm] to afford nagelamide N (**2**, 3.5 mg, 0.0016%, wet weight) and a fraction containing **1**. Further purification of the fraction containing **1** by C_{18} HPLC [YMC Hydrospere C18, YMC Co., Inc., 10×250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 15:85:0.1 to 25:75:0.1 in 20 min; flow rate, 2.5 mL/min; UV detection at 255 nm] afforded nagelamide M (**1**, 1.5 mg, 0.00069%).

3.4. Nagelamide M (1)

Colorless amorphous solid; $[\alpha]_D^{24}$ −7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 275 nm (ε 18,000); IR (KBr) ν_{max} 3404, 1684, 1635, 1200, and 1044 cm^{-1}; ESIMS (neg.) *m*/*z* 527, 529, and 531 [1:2:1, $(M-H)^-$]; HRESIMS (neg.) *m*/*z* 526.9340 [$(M-H)^-$, calcd for $C_{13}H_{17}N_6O_5^{79}Br_2S$, 526.9348].

3.5. Nagelamide N (2)

Colorless amorphous solid; $[\alpha]_D^{24}$ −1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 275 nm (ε 10,000); IR (KBr) ν_{max} 3388, 1700, 1678, 1638, 1206, and 1045 cm^{-1}; ESIMS (neg.) *m*/*z* 557, 559, and 561 [1:2:1, $(M-H)^-$]; HRESIMS (neg.) *m*/*z* 556.9105 [$(M-H)^-$, calcd for $C_{12}H_{15}N_6O_7^{79}Br_2S$, 556.9090].

Acknowledgements

Authors thank Ms. S. Oka and Mr. T. Hirose, Center for Instrumental Analysis, Hokkaido University, for measurements of ESIMS and NMR spectra, respectively. This work was partly supported by grants from the Uehara Memorial Foundation, the Yakult Bio-Science Foundation, and Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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