Organic LETTERS

Letter

pubs.acs.org/OrgLett

Agelamadins A and B, Dimeric Bromopyrrole Alkaloids from a Marine Sponge *Agelas* sp.

Taishi Kusama,[†] Naonobu Tanaka,[‡,†] Kanae Sakai,[§] Tohru Gonoi,[§] Jane Fromont,[∥] Yoshiki Kashiwada,[‡] and Jun'ichi Kobayashi*[,†]

[†]Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

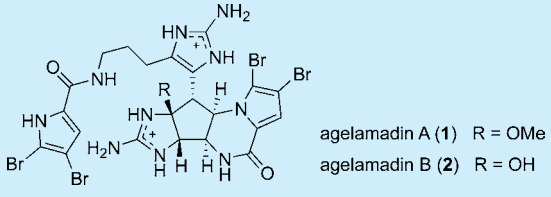
[‡]Graduate School of Pharmaceutical Sciences, The University of Tokushima, Tokushima 770-8505, Japan

[§]Mycology Research Center, Chiba University, Chiba 260-8673, Japan

[∥]Western Australian Museum, Locked Bag 49, Welshpool DC, Washington 6986, Australia

Supporting Information

ABSTRACT: Two structurally unique dimeric bromopyrrole alkaloids, agelamadins A (**1**) and B (**2**), were isolated from a marine sponge *Agelas* sp. Agelamadins A (**1**) and B (**2**) have a structure consisting of an agelastatin-like tetracyclic moiety and an oroidin-like linear moiety in common. The structures of **1** and **2** were elucidated on the basis of spectroscopic analysis. The antimicrobial activity and cytotoxicity of agelamadins A (**1**) and B (**2**) were evaluated.



Bromopyrrole alkaloids are one of the most common metabolites contained in marine sponges. Various bromopyrrole alkaloids possessing monomeric, dimeric, and tetrameric structures have been reported.[1] Agelastatins, a member of monomeric bromopyrrole alkaloids, possess a unique 5/5/6/5 tetracyclic ring system. Six agelastatin congeners, agelastatins A–F,[2] have been isolated from marine sponges *Agelas* sp. and *Cymbastela* sp. Among them, agelastatin A[2a] has attracted widespread interest due to its potent cytotoxicity against various cancer cell lines and has been regarded as a challenging target for total synthesis.[3] In our continuing search for structurally unique metabolites from marine organisms, we have isolated several bromopyrrole alkaloids from the extract of an Okinawan marine sponge *Agelas* sp. (SS-162).[4] Further investigation of the extract has resulted in the isolation of two structurally unique dimeric bromopyrrole alkaloids, agelamadins A (**1**) and B (**2**), which have an agelastatin-like tetracyclic moiety. In this Letter, we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Agelas* sp. (SS-162) collected off Kerama Islands, Okinawa, was extracted with MeOH. The extract was partitioned with *n*-hexane and 90% MeOH aq. The 90% MeOH aq.-soluble materials were partitioned with *n*-BuOH and water. The *n*-BuOH-soluble materials were subjected to passage over silica gel and ODS to give fractions containing bromopyrrole alkaloids. The fractions were purified using ODS HPLC to afford agelamadins A (**1**, 0.000039%, wet weight) and B (**2**, 0.000041%).

Agelamadin A (**1**)[5] was obtained as a colorless amorphous solid. The ESIMS displayed pseudomolecular ion peaks at *m*/*z* 805, 807, 809, 811, and 813 (1:4:6:4:1), suggesting the presence of four bromine atoms in **1**. The molecular formula of **1**, $C_{23}H_{26}N_{10}O_3Br_4$, was revealed by the HRESIMS (*m*/*z* 804.88553 $[M-H]^+$, Δ+1.62 mmu). The IR and UV spectra showed typical absorptions of a pyrrole amide moiety, a common unit on bromopyrrole alkaloids {ν_{max} 1685 cm^{-1} (IR); λ_{max} 277 nm (UV)}. These observations suggested **1** to be a dimeric bromopyrrole alkaloid. Analysis of the NMR spectra (Table 1), including the $^1H-^1H$ COSY, HMBC, and ROESY spectra, implied that **1** consisted of two partial structures (units A and B), which were presumed to be structurally related to oroidin[6] and agelastatins,[2] respectively. The gross structures of units A and B were elucidated as follows.

In unit A (N-1′–C-15′), the proton resonances of two sp^3 methylenes (CH_2-9′ and CH_2-10′) were observed in place of the signals of a 1,2-disubstituted olefin in oroidin, suggesting unit A to be 9,10-dihydrooroidin. This was confirmed by $^1H-^1H$ COSY cross-peaks of H_2-8′/H_2-9′ and H_2-9′/H_2-10′ and an HMBC correlation for H_2-9′ with C-11′ (Figure 1).

In unit B (N-1–C-15), the existence of a dibromopyrrole amide moiety (N-1–N-7) was deduced by the ^1H and ^{13}C NMR data {δ_H 8.21 (brs) and 6.90 (s); δ_C 156.1, 124.2, 115.5, 106.1, and 100.6}, while four D_2O-exchangeable proton signals at δ_H 9.50, 9.14, and 8.37 (2H) and a carbon signal at δ_C 158.1 implied the presence of a guanidino moiety (N-12, C-13, and N-14). Analysis of the $^1H-^1H$ COSY and HMBC spectra (Figure 1) revealed the existence of a 5/5/6/5 tetracyclic ring system including dihydro-2-aminoimidazole, cyclopentane, piperazine-2-one, and 2,3-dibromopyrrole rings as well as the presence of a methoxy group at C-11. Unit B is structurally related to agelastatin F,[2c] whereas agelastatin F has a 2-imidazolidinone moiety in place of a dihydro-2-aminoimidazole moiety in unit B. The connectivity between units A and B

Received: June 10, 2014
Published: July 14, 2014

dx.doi.org/10.1021/ol501664b | *Org. Lett.* 2014, 16, 3916–3918

Table 1. ^{1}H and ^{13}C NMR Data for Agelamadins A (1) and B (2) in DMSO-d_6

	1		2	
position	^{13}C	^{1}H	^{13}C	^{1}H
2	106.1	–	106.4	–
3	100.6	–	100.4	–
4	115.5	6.90 (1H, s)	115.3	6.89 (1H, s)
5	124.2	–	124.2	–
6	156.1	–	156.1	–
7	–	8.21 (1H, brs)	–	8.29 (1H, brs)
8	57.4	4.43 (1H, d, J = 5.1 Hz)	57.5	4.36 (1H, d, J = 5.1)
9	56.1	5.06 (1H, dd, J = 11.5, 5.1 Hz)	56.3	4.94 (1H, dd, J = 11.5, 5.1)
10	49.1	3.58 (1H, d, J = 11.5 Hz)	49.4	3.53 (1H, d, J = 11.5)
11	100.0	–	95.6	–
11-OMe	50.4	3.14 (3H, s)		
11-OH				7.34 (1H, brs)
12	–	9.50 (1H, brs)	–	9.21 (1H, brs)
13	158.1	–	157.7	–
13-NH$_2$		8.37 (2H, brs)		8.13 (2H, brs)
14	–	9.14 (1H, brs)	–	9.02 (1H, brs)
15	62.5	4.41 (1H, brs)	68.7	4.16 (1H, brs)
1′	–	12.59 (1H, brs)	–	12.59 (1H, brs)
2′	104.3	–	104.2	–
3′	97.7	–	97.6	–
4′	112.5	6.87 (1H, d, J = 2.0)	112.4	6.87 (1H, d, J = 1.9)
5′	128.2	–	128.1	–
6′	158.8	–	158.6	–
7′	–	8.02 (1H, brt, J = 5.6)	–	8.02 (1H, brt, J = 5.6)
8′	37.4	2.84 (2H, m)	37.3	2.87, 2.80 (1H each, m)
9′	29.0	1.35, 1.26 (1H each, m)	28.9	1.32, 1.23 (1H each, m)
10′	20.4	2.16 (2H, m)	20.2	2.16 (2H, t, J = 7.2)
11′	126.5	–	126.2	–
12′	–	12.40 (1H, brs)	–	12.39 (1H, brs)
13′	147.3	–	147.2	
13′-NH$_2$		7.56 (2H, brs)		7.53 (2H, brs)
14′	–	12.70 (1H, brs)	–	12.64 (1H, brs)
15′	113.3	–	113.5	–

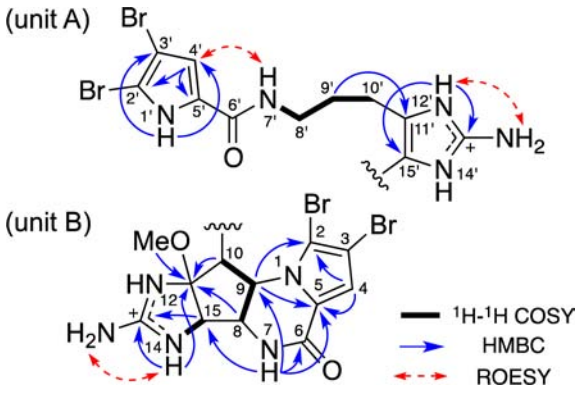


Figure 1. Selected 2D NMR correlations for two partial structures (units A and B) of agelamadin A (**1**).

through a C−C bond (C-15′−C-10) was revealed by HMBC cross-peaks of H-10 to C-11′ and C-15′ and of H-9 to C-15′ (Figure 2). Therefore, the gross structure of agelamadin A (**1**) was concluded as shown in Figure 2.

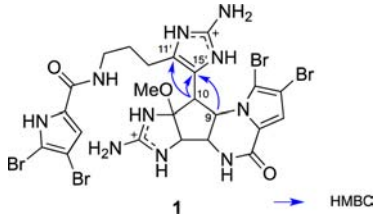


Figure 2. Gross structure of agelamadin A (**1**).

Agelamadin B (**2**)[7] was isolated as a colorless amorphous solid, and the molecular formula of **2**, $C_{22}H_{24}N_{10}O_3Br_4$, was elucidated by the HRESIMS (m/z 790.86975 $[M-H]^+$, Δ+1.49 mmu) being smaller by 14 mass units as compared with that of **1**. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, and the resonance of a D_2O-exchangeable proton in **2** {δ_H 7.34 (1H, brs)} was discerned in place of the signal due to a methoxy group at C-10 in **1** (Table 1). Therefore, agelamadin B (**2**) was deduced to be a demethyl derivative of **1**.

The relative stereochemistry of agelamadins A (**1**) and B (**2**) was assigned as follows. In the ROESY spectrum of **2**, correlations for H-9/H-14′, H-9/H-8, and H-8/H-14 were observed, implying that these protons were present on the same α-side (Figure 3). This was underpinned by ROESY cross-

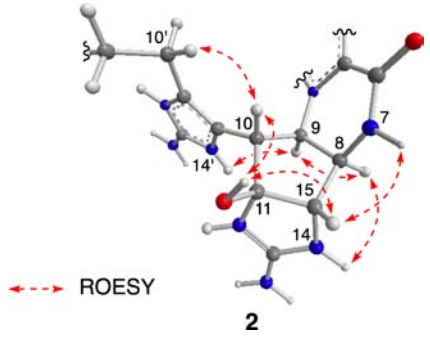


Figure 3. Selected ROESY correlations and the relative stereochemsitry for agelamadin B (**2**).

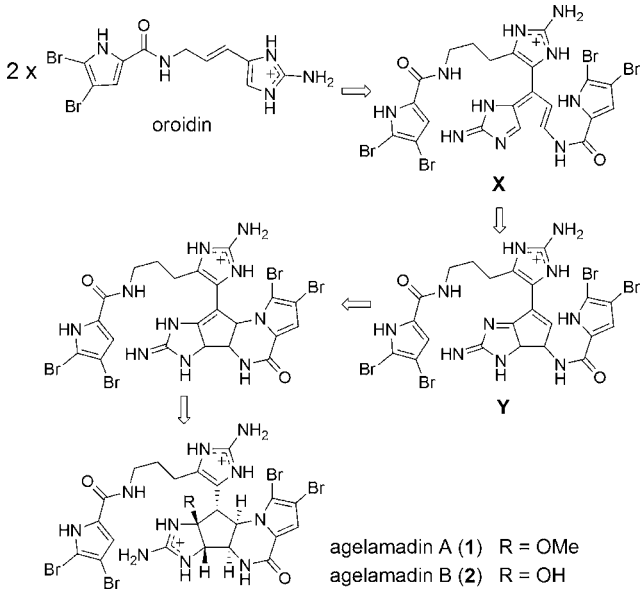
peaks observed among protons on the β-side in the molecule. Therefore, the relative stereochemistry of **2** was deduced as shown in Figure 3. The resemblance of ^3J values for H-9/H-10, H-8/H-9, and H-8/H-15 in **2** with those corresponding positions in agelastatin A,[2a] whose stereochemistry was confirmed by total synthesis,[8] supported this assignment. The coupling constants of H-8, H-9, H-10, and H-15 in agelamadin A (**1**) were similar to those in **2**, suggesting that **1** had the same relative stereochemistry as that of **2**.

Since agelamadins A (**1**) and B (**2**) were optically incative and showed no Cotton effects in the CD spectra, the optical resolutions on chiral HPLC were carried out.[9] The analyses showed the separation of enantiomers, indicating that **1** and **2** were both racemates.

Agelamadins A (**1**) and B (**2**) are structurally unique dimeric bromopyrrole alkaloids consisting of an agelastatin-like tetracyclic moiety and an oroidin-like linear moiety in common. A possible biogenetic pathway for **1** and **2** is shown in Scheme 1. Condensation of two molecules of oroidin would give a plausible biosynthetic precursor **X**, and subsequent intra-

dx.doi.org/10.1021/ol501664b | Org. Lett. 2014, 16, 3916−3918

Scheme 1. Possible Biogenetic Pathway of Agelamadins A (1) and B (2)



molecular cyclization of **X** yields racemic **Y**. Agelamadins A (**1**) and B (**2**) might be derived by intramolecular cyclization and oxidation of **Y**. Alternatively, **1** and **2** also could be derived by intramolecular cyclization of nagelamide J,[10] a dimeric bromopyrrole alkaloid isolated from a sponge *Agelas* sp.

Agelamadins A (**1**) and B (**2**) exhibited antimicrobial activity against *Bacillus subtilis* (MIC, 16 μg/mL, each), *Micrococcus luteus* (MIC, 4.0 and 8.0 μg/mL, respectively), and *Cryptococcus neoformans* (IFM 62681, IC_{50}, 8.0 and 4.0 μg/mL, respectively). While **1** and **2** did not show cytotoxicity (IC_{50}, >10 μg/mL) against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro.

■ ASSOCIATED CONTENT

Supporting Information

Experimental section, 1D and 2D NMR spectra, CD spectra, and charts of chiral HPLC analyses of agelamadins A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jkobay@pharm.hokudai.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Ms. S. Oka and Ms. A. Tokumitsu, Equipment Management Center, Hokkaido University, for measurements of MS spectra, and Mr. Z. Nagahama for help with sponge collection. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

■ REFERENCES

(1) (a) Aiello, A.; Fattorusso, E.; Menna, M.; Taglialatela-Scafati, O. In *Modern Alkaloids: Structure, Isolation, Synthesis and Biology*; Fattorusso, E., Taglialatela-Scafati, O., Eds.; Wiley-VCH Verlag GmbH & Co. KgaA: Weinheim, 2008; pp 271−304. (b) Forte, B.; Malgesini, B.; Piutti, C.; Quartieri, F.; Scolaro, A.; Papeo, G. *Mar. Drugs* **2009**, *7*, 705−753. (c) Al-Mourabit, A.; Zancanella, M. A.; Tilvi, S.; Romo, D. *Nat. Prod. Rep.* **2011**, *28*, 1229−1260.

(2) (a) D' Ambrosio, M.; Guerriero, A.; Debitus, C.; Ribes, O.; Pusset, J.; Leroy, S.; Pietra, F. *J. Chem. Soc., Chem. Commun.* **1993**, 1305−1306. (b) Hong, T. W.; Jímenez, D. R.; Molinski, T. F. *J. Nat. Prod.* **1998**, *61*, 158−161. (c) Tilvi, S.; Moriou, C.; Martin, M.-T.; Gallard, J.-F.; Sorres, J.; Patel, K.; Petek, S.; Debitus, C.; Ermolenko, L.; Al-Mourabit, A. *J. Nat. Prod.* **2010**, *73*, 720−723.

(3) Dong, G. *Pure Appl. Chem.* **2010**, *82*, 2231−2246.

(4) (a) Tanaka, N.; Kusama, T.; Takahashi-Nakaguchi, A.; Gonoi, T.; Fromont, J.; Kobayashi, J. *Tetrahedron Lett.* **2013**, *54*, 3794−3796. (b) Tanaka, N.; Kusama, T.; Takahashi-Nakaguchi, A.; Gonoi, T.; Fromont, J.; Kobayashi, J. *Org. Lett.* **2013**, *15*, 3262−3265. (c) Kusama, T.; Tanaka, N.; Takahashi-Nakaguchi, A.; Gonoi, T.; Fromont, J.; Kobayashi, J. *Chem. Pharm. Bull.* **2014**, *62*, 499−503.

(5) Agelamadin A (**1**): colorless amorphous solid; $[\alpha]^{25}_{D} \approx 0$ (*c* 0.25, MeOH); UV (MeOH) λ_{max} 209 (ε 42 200, sh) and 277 (16 300) nm; IR (KBr) ν_{max} 3370 and 1685 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS: *m/z* 805, 807, 809, 811, and 813 (1:4:6:4:1) $[M-H]^+$; HRESIMS: *m/z* 804.88553 $[M-H]^+$ (calcd for $C_{23}H_{25}N_{10}O_3{}^{79}Br_4$, 804.88391).

(6) (a) Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.* **1971**, 1129−1130. (b) Garcia, E. E.; Benjamin, L. E.; Fryer, R. I. *J. Chem. Soc., Chem. Commun.* **1973**, 78−79.

(7) Agelamadin B (**2**): colorless amorphous solid; $[\alpha]^{25}_{D} \approx 0$ (*c* 0.25, MeOH); UV (MeOH) λ_{max} 209 (ε 36 000, sh) and 277 (12 100) nm; IR (KBr) ν_{max} 3308 and 1686 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS: *m/z* 791, 793, 795, 797, and 799 (1:4:6:4:1) $[M-H]^+$; HRESIMS: *m/z* 790.86975 $[M-H]^+$ (calcd for $C_{22}H_{23}N_{10}O_3{}^{79}Br_4$, 790.86826).

(8) (a) Stien, D.; Anderson, G. T.; Chase, C. E.; Koh, Y.; Weinreb, S. M. *J. Am. Chem. Soc.* **1999**, *121*, 9574−9579. (b) Feldman, K. S.; Saunders, J. C. *J. Am. Chem. Soc.* **2002**, *124*, 9060−9061.

(9) Racemic forms of **1** and **2** were analyzed using chiral HPLC {CHIRALPAK ZWIX (+), DAICEL Corp., 4.0 mm × 250 mm, flow rate 0.3 mL/min, UV (254 nm) and OR detections} with eluent THF/MeOH/H_2O (60:20:20 with 100 μM formic acid and 50 μM diethylamine) to separate each enantiomer {t_R 39.1 (+) min and 40.5 (−) min for **1**; 46.5 (+) min and 50.9 (−) min for **2**}.

(10) Araki, A.; Tsuda, M.; Kubota, T.; Mikami, Y.; Fromont, J.; Kobayashi, J. *Org. Lett.* **2007**, *9*, 2369−2371.