Organic LETTERS

Letter

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Agelamadins C–E, Bromopyrrole Alkaloids Comprising Oroidin and 3-Hydroxykynurenine from a Marine Sponge *Agelas* sp.

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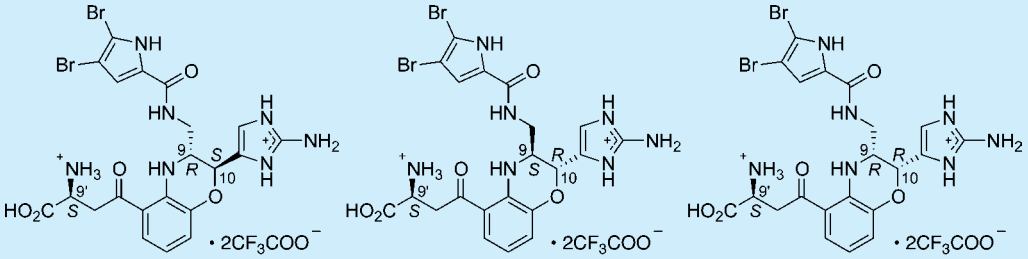
Supporting Information



agelamadin C (**1**) agelamadin D (**2**) agelamadin E (**3**)

ABSTRACT: Three structurally unique bromopyrrole alkaloids, agelamadins C–E (**1**–**3**), were isolated from a marine sponge *Agelas* sp. Agelamadin C (**1**) possesses a hybrid structure of oroidin and 3-hydroxykynurenine connected through a dihydro-1,4-oxazine moiety. Agelamadins D (**2**) and E (**3**) are a C-9/C-10 diastereomer and a 10-epimer of **1**, respectively. The structures of **1**–**3** were elucidated on the basis of spectroscopic analysis as well as application of a PGME method and a TDDFT ECD calculation. Antimicrobial activity of **1**–**3** was evaluated.

Marine sponges have been recognized as a rich source of interesting bioactive metabolites with various chemical structures.[1] Among them, sponges belonging to the genus *Agelas* are known to contain bromopyrrole alkaloids, which have attracted widespread interest due to their fascinating complex chemical structures with a high N to C ratio (~1:2) and intriguing biological activities.[2] In our continuing search for structurally unique metabolites from Okinawan marine sponges, we have reported the isolation of several bromopyrrole alkaloids, nagelamides U–Z and agelamadins A and B, from the extract of a sponge *Agelas* sp. (SS-162).[3] Further investigation of the extract afforded three new bromopyrrole alkaloids, agelamadins C–E (**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 Kerama Islands, Okinawa, was extracted with MeOH, and a part of the extract was partitioned with *n*-hexane and 90% aq MeOH. The 90% aq MeOH-soluble materials were further partitioned with *n*-BuOH and H_2O. The *n*-BuOH-soluble materials were chromatographed on a silica gel column, Toyopearl HW-40 column, and C_{18} column to give fractions containing bromopyrrole alkaloids, which were purified using reverse-phase HPLC to afford agelamadins C (**1**, 0.000095%, wet weight), D (**2**, 0.000072%), and E (**3**, 0.000054%).

Agelamadin C (**1**)[4] was obtained as a yellow amorphous solid {$[\alpha]_D^{23}$ +71.8 (*c* 0.25, MeOH)}, and the ESIMS showed the pseudomolecular ion peaks at *m*/*z* 610, 612, and 614 (1:2:1), suggesting the existence of two bromine atoms in the molecule. The molecular formula of **1**, $C_{21}H_{23}N_7O_5Br_2$, was established by the HRESIMS (*m*/*z* 610.00531 $[M - H]^+$, Δ +0.94 mmu). The IR spectrum implied the presence of carbonyl functionalities (1685 and 1636 cm^{-1}). The gross structure of **1** consisting of two partial units {units A (N-1–C-15) and B (C-1′–C-10′)} (Figure 1) was assigned as follows. Comparison of ^1H and ^{13}C NMR data for **1** (Table 1) and those for known bromopyrrole alkaloids[3] suggested that **1** has one bromopyrrole amide moiety (N-1–N-7) and one aminoimidazole moiety (C-11–C-15). Interpretation of the ^1H–^1H COSY spectrum revealed the connectivities of N-7 to C-10 and C-9 to 9-NH (Figure 1). The presence of an amino group at C-9 was confirmed by an HMBC cross-peak of 9-NH to C-10 (Figure 1). The chemical shifts of CH-10 (δ_H 5.11; δ_C 65.9) were indicative of the existence of an oxygen atom at C-10. The connectivity of C-10 to the aminoimidazole moiety (C-11) was disclosed by HMBC correlations for H-10 to C-11 and C-15

Received: August 27, 2014
Published: September 23, 2014

dx.doi.org/10.1021/ol502528m | *Org. Lett.* 2014, 16, 5176–5179

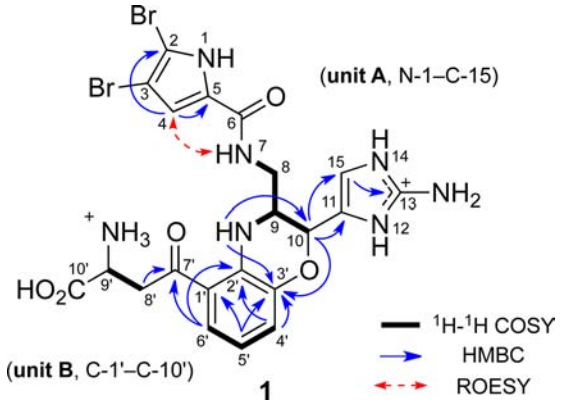


Figure 1. Selected 2D NMR correlations of agelamadin C (**1**).

(Figure 1). Therefore, unit A was elucidated to be 9-amino-10-hydroxyoroidin.

The existence of a 1,2,3-trisubstituted benzene ring (C-1′–C-6′) in unit B was deduced from six aromatic carbon signals (δ_C 141.0, 136.5, 124.5, 121.1, 116.8, and 114.7) as well as the coupling patterns of three aromatic protons {δ_H 7.44 (d, J = 7.8 Hz), 7.00 (d, J = 7.8 Hz), and 6.55 (t, J = 7.8 Hz)}. The carbon chemical shifts of C-2′ (δ_C 136.5) and C-3′ (δ_C 141.0) implied that an amino group and a hydroxy group were located at C-2′ and C-3′, respectively. The presence of a 3-amino-3-carboxypropanoyl group (C-7′–C-10′) was indicated by the remaining four carbon signals (δ_C 197.5, 171.1, 48.4, and 39.2) together with the 2D NMR correlations (Figure 1). The connectivity of C-1′ to C-7′ as well as the presence of an amino group at C-2′ and a hydroxy group at C-3′ were confirmed by HMBC correlations shown in Figure 1. Thus, unit B was suggested to be 3-hydroxykynurenine, which was further confirmed by comparison of the ^{1}H and ^{13}C resonances for unit B with those for literature data.[5] HMBC correlations for 9-NH to C-3′ and H-10 to C-3′ revealed the connectivities between units A and B forming a dihydro-1,4-oxazine ring. Accordingly, the gross structure of agelamadin C (**1**) was assigned as shown in Figure 1.

In the ROESY spectrum of **1**, correlations for NH-7/H-10 and H-9/H-15 were observed (Figure 2), indicating the *anti* relationship for H-9 and H-10.

Agelamadins D (**2**)[6] and E (**3**)[7] were individually isolated as optically active yellow amorphous solids {$[\alpha]_D^{23}$ −85.6 (c 0.25, MeOH) for **2**; $[\alpha]_D^{24}$ −66.6 (c 0.25, MeOH) for **3**}. The HRESIMS spectra revealed that **2** and **3** had the same molecular formula, $C_{21}H_{23}N_7O_5Br_2$, as that of **1** (m/z 610.00562 $[M - H]^+$, Δ + 1.25 mmu for **2**; m/z 610.00486 $[M - H]^+$, Δ + 0.49 mmu for **3**). Resemblance of the ^{1}H and ^{13}C NMR data for **2** and **3** (Table 1) to those for **1** indicated that **2** and **3** are diastereomers of **1**. A similar value of $^3J_{H\text{-}9/H\text{-}10}$ in **2** (J = 2.5 Hz) with that of **1** (J = 2.7 Hz) suggested the H-9/H-10 *anti* relationship in **2**, which was supported by analysis of the ROESY spectrum (Figure S2). In contrast, the H-9/H-10 *syn* relationship in **3** was deduced on the basis of a small value of $^3J_{H\text{-}9/H\text{-}10}$ in **3** ($J \approx 0$ Hz) as well as differences between the chemical shifts for CH_2-8, CH-10, and C-11 in **3** and those corresponding positions in **1**.

Table 1. ^{1}H and ^{13}C NMR Data for Agelamadins C–E (1–3) in DMSO-d_6

	1		2		3	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1		12.72 (1H, br)		12.71 (1H, br)		12.69 (1H, br)
2	104.8		104.9		104.9	
3	97.9		98.0		97.9	
4	113.1	6.95 (1H, s)	113.2	6.95 (1H, s)	113.1	6.91 (1H, s)
5	127.8		127.8		127.8	
6	159.5		159.6		159.4	
7		8.48 (1H, br t, 5.6 Hz)		8.48 (1H, br t, 5.0 Hz)		8.41 (1H, br t, 5.4 Hz)
8	41.2	3.35 (2H, m)	41.4	3.33 (2H, m)	39.4[a]	3.28 (2H, m)
9	49.7	4.01 (1H, m)	49.9	3.97 (1H, m)	50.5	4.03 (1H, m)
9-NH		8.70 (1H, br s)		8.64 (1H, br s)		8.53 (1H, br s)
10	65.9	5.11 (1H, d, 2.7 Hz)	65.9	5.08 (1H, d, 2.5 Hz)	67.8	5.26 (1H, br s)
11	123.1		123.2		121.9	
12		nd[b]		nd[b]		nd[b]
13	148.1		148.3		147.9	
13-NH_2		7.79 (2H, br s)		7.84 (2H, br s)		7.78 (2H, br s)
14		nd[b]		nd[b]		nd[b]
15	112.1	6.99 (1H, s)	112.3	6.86 (1H, s)	111.4	6.82 (1H, s)
1′	116.8		117.3		117.9	
2′	136.5		136.4		136.4	
3′	141.0		141.1		141.7	
4′	121.1	7.00 (1H, d, 7.8 Hz)	121.0	6.96 (1H, d, 7.8 Hz)	121.0	7.00 (1H, d, 7.8 Hz)
5′	114.7	6.55 (1H, t, 7.8 Hz)	114.8	6.53 (1H, t, 7.8 Hz)	115.4	6.61 (1H, t, 7.8 Hz)
6′	124.5	7.44 (1H, d, 7.8 Hz)	124.5	7.40 (1H, d, 7.8 Hz)	124.4	7.46 (1H, d, 7.8 Hz)
7′	197.5		198.1		198.1	
8′	39.2[a]	3.57 (2H, m)	40.2	3.53, 3.41 (1H each, m)	39.7[a]	3.54 (2H, m)
9′	48.4	4.19 (1H, m)	49.1	4.04 (1H, m)	48.6	4.12 (1H, m)
10′	171.1		171.9		171.2	

[a]Overlapped with signal of DMSO-d_6. [b]Not detected.

dx.doi.org/10.1021/ol502528m | Org. Lett. 2014, 16, 5176–5179

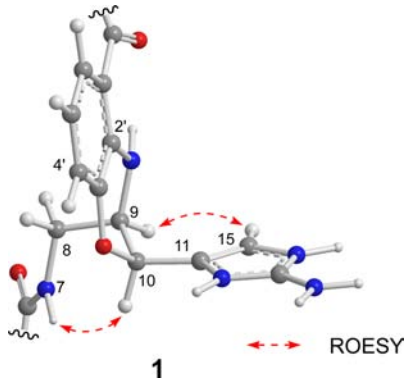


Figure 2. Selected ROESY correlations and the relative configurations at C-9 and C-10 for agelamadin C (**1**).

The absolute configurations at C-9′ in **1**−**3** were assigned by a phenylglycine methyl ester (PGME) method.[8] Since application of a PGME method to α-amino acids was not reported, three authentic α-amino acids, L-kynurenine (**4**), L-tyrosine (**5**), and D-tryptophan (**6**), were converted into the (*S*)- and (*R*)-PGME amides (**4a**/**4b**, **5a**/**5b**, and **6a**/**6b**), respectively. The Δδ values ($\Delta\delta = \delta_S - \delta_R$) obtained from the ^{1}H NMR spectra of **4a**/**4b**, **5a**/**5b**, and **6a**/**6b** measured in CD_3OD and DMSO-d_6 are shown in Figure 3A. Distributions of positive Δδ values were observed in side chains of the PGME amides (**4a**/**4b** and **5a**/**5b**) from L-amino acids, whereas a distribution of negative Δδ values was found in the PGME amides (**5a**/**5b**) of D-amino acids. These observations suggested that a PGME method was applicable to agelamadins C−E (**1**−**3**). The application to **1**−**3** (Figure 3B) indicated that the absolute configurations at C-9′ in **1**−**3** were all *S*.

The absolute configurations at C-9 and C-10 of **1** and **2** were elucidated by comparison of the ECD spectra with those calculated spectra. In the ECD spectrum of **1**, a positive Cotton effect was observed at 242 nm, whereas **2** showed a negative Cotton effect at 242 nm (Figure 4). The calculated ECD spectra of two possible diastereomers (9*R*,10*S*,9′*S* for **1** and 9*S*,10*R*,9′*S* for **2**) by TDDFT method[9] agreed well with the experimental spectra of **1** and **2**, respectively (Figure 4). Accordingly, the 9*R* and 10*S* configurations for **1** and the 9*S* and 10*R* configurations for **2** were assigned. Similarly, the 9*R* and 10*R* configurations for **3** were elucidated by comparison of the experimental and calculated ECD spectra (Figure 5).

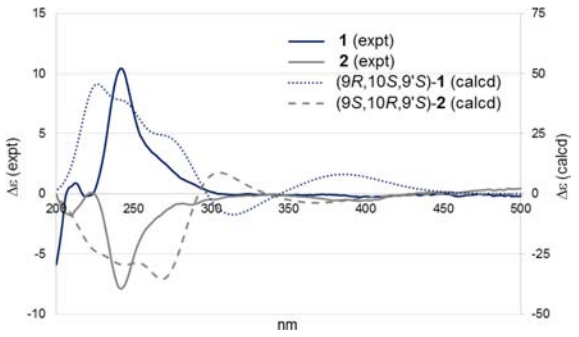


Figure 4. Experimental and calculated ECD spectra of agelamadins C (**1**) and D (**2**).

Agelamadins C−E (**1**−**3**) are structurally unique bromopyrrole alkaloids possessing hybrid structures of oroidin and 3-hydroxykynurenine connected through a dihydro-1,4-oxazine moiety. A possible biogenetic pathway of agelamadins C−E (**1**−**3**) is proposed in Scheme 1. Condensation of oroidin[10] and 3-hydroxykynurenine derived from L-tryptophan would give **1**−**3**. To the best of our knowledge, agelamadins C−E (**1**−**3**) are

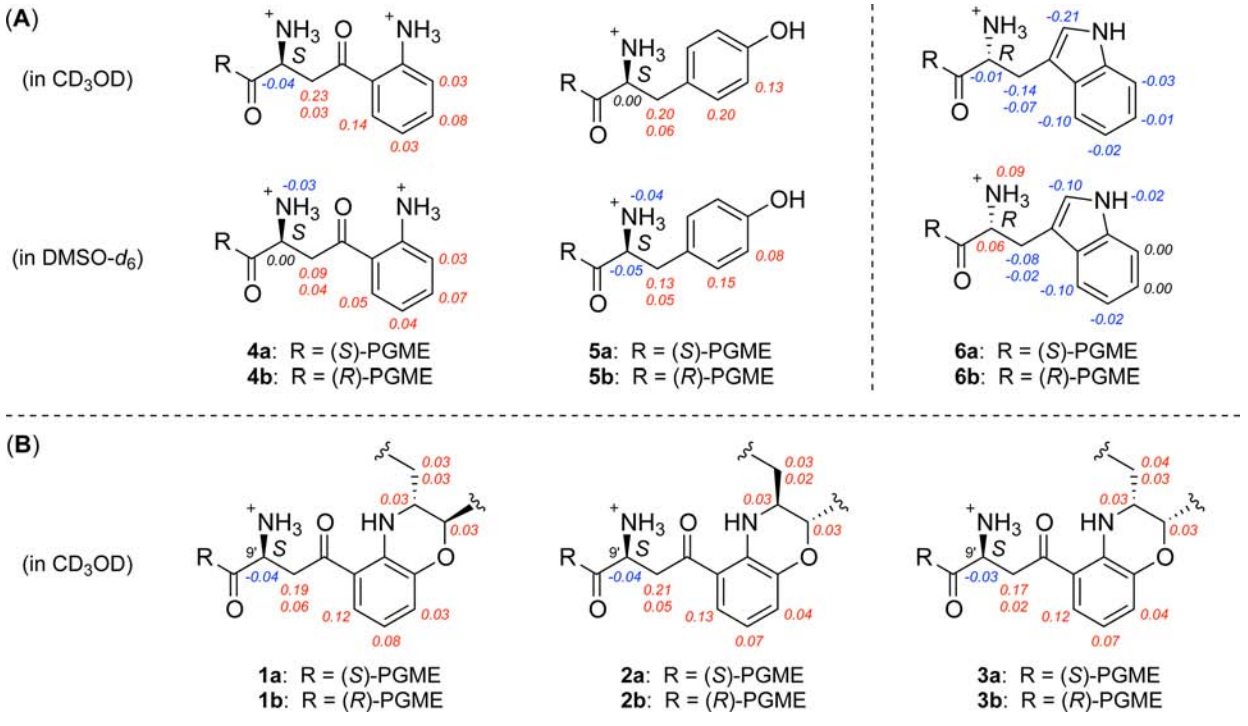


Figure 3. Δδ values [Δδ (in ppm) = $\delta_S - \delta_R$] obtained for (A) (*S*)- and (*R*)-PGME amides (**4a**/**4b**, **5a**/**5b**, and **6a**/**6b**) of L-kynurenine (**4**), L-tyrosine (**5**), and D-tryptophan (**6**); (B) (*S*)- and (*R*)-PGME amides (**1a**/**1b**, **2a**/**2b**, and **3a**/**3b**) of agelamadins C−E (**1**−**3**).

dx.doi.org/10.1021/ol502528m | *Org. Lett.* 2014, 16, 5176−5179

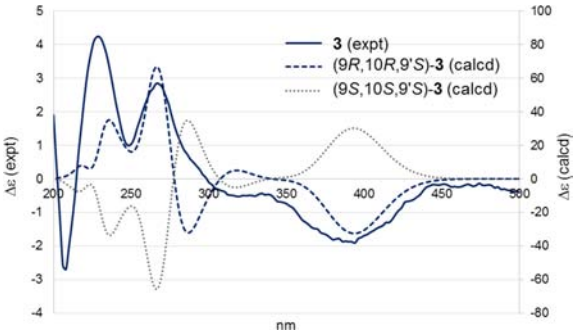
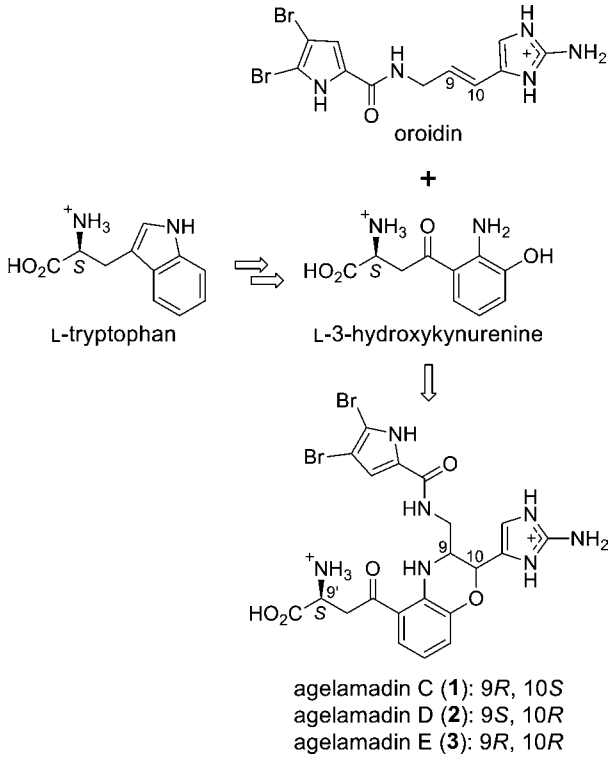


Figure 5. Experimental and calculated ECD spectra of agelamadin E (**3**).

Scheme 1. Possible Biogenetic Pathway of Agelamadins C–E (1–3)



the first example for bromopyrrole alkaloids comprising oroidin and a tryptophan derivative.

In antimicrobial screening of agelamadins C–E (**1**–**3**), **1** and **3** showed a weak inhibitory activity against *Cryptococcus neoformans* (IC_{50}, 32 μg/mL each).

■ ASSOCIATED CONTENT

Supporting Information

Experimental section, and 1D and 2D NMR spectra of agelamadins C–E. This material is available free of charge via the Internet at http://pubs.acs.org.

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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 the 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.

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(4) Agelamadin C (**1**): yellow amorphous solid; $[\alpha]_D^{23}$ +71.8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 233 (ε 15300, sh) 275 (13400), and 387 (3300) nm; IR (KBr) ν_{max} 3337, 1685, and 1636 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m*/*z* 610, 612, and 614 (1:2:1) $[M - H]^+$; HRESIMS *m*/*z* 610.00531 $[M - H]^+$ (calcd for $C_{21}H_{22}N_7O_5{}^{79}Br_2$, 610.00437).

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(6) Agelamadin D (**2**): yellow amorphous solid; $[\alpha]_D^{22}$ −85.6 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 233 (ε 14600, sh), 275 (13100), and 388 (3200) nm; IR (KBr) ν_{max} 3434, 1684, and 1636 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m*/*z* 610, 612, and 614 (1:2:1) $[M - H]^+$; HRESIMS: *m*/*z* 610.00562 $[M - H]^+$ (calcd for $C_{21}H_{22}N_7O_5{}^{79}Br_2$, 610.00437).

(7) Agelamadin E (**3**): yellow amorphous solid; $[\alpha]_D^{24}$ −66.6 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 234 (ε 12800, sh), 275 (10800), and 386 (2700) nm; IR (KBr) ν_{max} 3127, 1683, and 1638 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m*/*z* 610, 612, and 614 (1:2:1) $[M - H]^+$; HRESIMS *m*/*z* 610.00486 $[M - H]^+$ (calcd for $C_{21}H_{22}N_7O_5{}^{79}Br_2$, 610.00437).

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