HETEROCYCLES, Vol. 98, No. 4, 2019, pp. 558 - 563. © 2019 The Japan Institute of Heterocyclic Chemistry
Received, 5th February, 2019, Accepted, 12th March, 2019, Published online, 14th March, 2019
DOI: 10.3987/COM-19-14054

AGESAMINES A AND B, NEW DIBROMOPYRROLE ALKALOIDS FROM THE SPONGE *AGELAS* sp.

Ayako Katsuki,[1] Hikaru Kato,[1] Yuji Ise,[2] Fitje Losung,[3] Remy E. P. Mangindaan,[3] and Sachiko Tsukamoto[1]*

[1]Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan. [2]Centre for Marine & Coastal Studies (CEMACS), Universiti Sains Malaysia, 11800 USM, Penang, Malaysia. [3]Faculty of Fisheries and Marine Science, Sam Ratulangi University, Kampus Bahu, Manado 95115, Indonesia. E-mail: sachiko@kumamoto-u.ac.jp

Abstract – New dibromopyrrole alkaloids, agesamines A (**1**) and B (**2**), were obtained as an inseparable epimeric mixture from a marine sponge *Agelas* sp., collected in Indonesia. The planar structures were elucidated by analysis of spectroscopic data, and the absolute configuration was determined by the calculated ECD spectrum.

Marine sponges contain structurally diverse metabolites with interesting biological activities.[1] In our research to discover new biologically active compounds from natural sources, an extract of the marine sponge *Agelas* sp., collected in Indonesia, showed cytotoxicity in HeLa cells. We here describe the elucidation of the structures of agesamines A (**1**) and B (**2**).

The sponge was extracted with EtOH and the extract was partitioned between EtOAc and H_2O. The EtOAc fraction was partitioned between *n*-hexane and 90% MeOH, and the H_2O fraction was partitioned between *n*-BuOH and H_2O. The 90% MeOH and *n*-BuOH fractions showed cytotoxicity toward HeLa cells. The fractions were combined and subjected to column chromatography and HPLC, yielding new dibromopyrrole alkaloids (**1** and **2**) as an inseparable epimeric mixture, along with the known bromopyrrole alkaloids oroidin[2,3] (**3**) and manzacidin C[4,5] (**4**) (Figure 1). Despite repeated purification by HPLC using various columns, the ^{1}H NMR spectrum of the mixture of **1** and **2** revealed nine pairs of signals with the same intensity in the ratio of 1:1 (Table 1). This indicated that **1**/**2** was an inseparable mixture of structurally similar compounds or a dimeric compound composed of similar structures. ESIMS of **1**/**2** showed protonated ion peaks at *m/z* 403, 405, and 407 (1:2:1), indicating the presence of two bromine atoms, and HRESIMS revealed the molecular formula to be $C_{11}H_{11}Br_2N_5O_2$. These data

suggested that **1**/**2** was likely an inseparable mixture rather than a dimeric compound. Because the amount of **1**/**2** was small, the structure was elucidated without separation. The ^{1}H-^{1}H COSY experiment showed a sequence terminated with two amide protons: H-7 (δ_H 7.84, 7.85)/H_2-8 (δ_H 3.56/3.71, 3.47/3.73)/H-9 (δ_H 4.51, 4.65)/H-10 (δ_H 1.36/2.08, 1.70/1.99)/H-11 (δ_H 3.83, 3.76)/H-12 (δ_H 7.80, 7.62) (Figure 2). The HMBC experiment showed correlations from a relatively lower field signal: H-4 (δ_H 6.84, 6.83) to two olefinic carbons C-2 (δ_C 105.6, 105.5) and C-5 (δ_C 125.72, 125.69), from the amide proton H-7 to C-5 and C-9 (δ_C 51.5, 50.8), and from H_2-8 to an amide carbon C-6 (δ_C 157.7, 157.6) and C-9. The chemical shifts for C-9 (δ_H 4.51, 4.65; δ_C 51.5, 50.8) suggested that C-9 was attached to a nitrogen atom. These results and the presence of two bromine atoms in **1**/**2** together with the chemical shift of a quaternary carbon C-3 (δ_C 99.3, 99.2) indicated that **1** contained a pyrroloketopiperazine unit. The HMBC experiment showed correlations between H-12 and a carbonyl carbon C-15 (δ_C 188.2, 188.4), between H-11 and C-13 (δ_C 177.2, 177.1) and C-15, and between H_2-10 and C-15, indicating that **1**/**2** possessed a 2-amino-1,5-dihydro-4*H*-imidazol-4-one unit, and the planar structure was determined as shown in Figure 2.

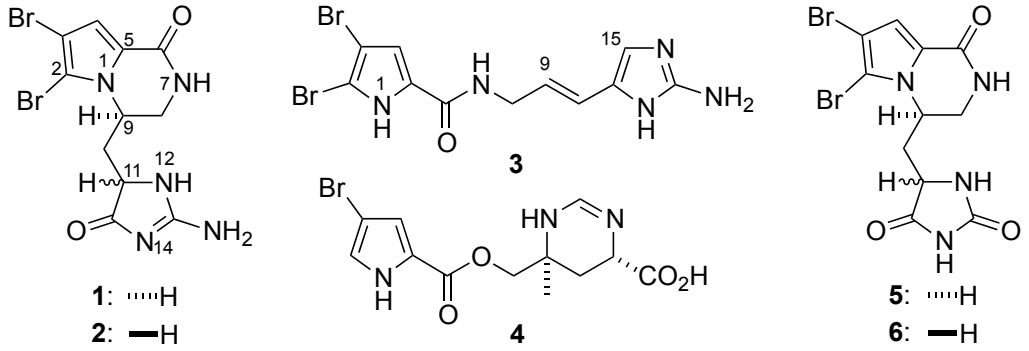


Figure 1. Structures of **1**-**6**

Table 1. ^{1}H and ^{13}C NMR data (DMSO-d_6) for **1** and **2**

	1			**2**		
No.	δ_C, type	δ_H, mult (*J* in Hz)	HMBC[f]	δ_C, type	δ_H, mult (*J* in Hz)	HMBC[f]
2	105.6,[a] C			105.5,[a] C		
3	99.3,[b] C			99.2,[b] C		
4	114.0,[c] CH	6.84, s	2, 5	113.9,[c] CH	6.83, s	2, 5
5	125.72,[d] C			125.69,[d] C		
6	157.7, C			157.6, C		
7		7.84, brs	5, 6, 8, 9		7.85, brs	5, 6, 8, 9
8	40.5, CH_2	3.56, dd (13.6, 5.4)	6, 9	42.0, CH_2	3.47, dd (13.5, 5.2)	6, 9
		3.71, dd (13.6, 3.6)	10		3.73, dd (13.5, 4.6)	10
9	51.5, CH	4.51, m		50.8, CH	4.65, m	

10	35.4, CH_2	1.36, ddd (13.7, 11.3, 3.0)		34.2, CH_2	1.70, ddd (13.9, 7.1, 5.8)	8, 9, 11, 15
		2.08, ddd (13.7, 10.9, 3.4)			1.99, ddd (13.9, 7.5, 7.1)	8, 9, 11, 15
11	57.0, CH	3.83, dd (10.9, 3.0)	15	57.3, CH	3.76, dd (7.1, 7.1)	9, 10, 13, 15
12		7.80, s	11, 15		7.62, s	11, 15
13	177.2,[e] C			177.1,[e] C		
15	188.2, C			188.4, C		

[a–e] May be interchangeable.
[f] HMBC correlations are from proton(s) stated for the indicated carbon(s).

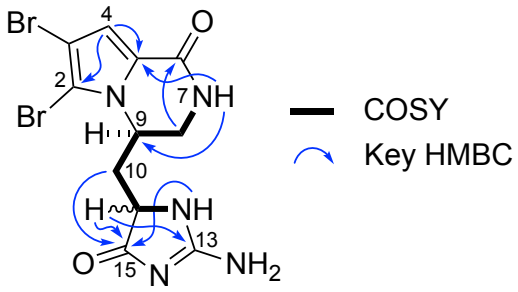


Figure 2. COSY and key HMBC correlations of **1/2**

The structures of **1/2** were found to be congeners of agesamides A[6] (**5**) and B (**6**) isolated from the Okinawan marine sponge *Agelas* sp. The 2-amino-1,5-dihydro-4*H*-imidazol-4-one rings in **1/2** were replaced with the hydantoin rings in **5/6**. The relative configurations of **5/6** were determined by ^{1}H-^{1}H coupling constants and ROESY correlations, but their absolute configurations remained unknown. The *J* values of **5/6** were reported to be 4.0 Hz (H-10a/H-11)/8.0 Hz (H-10b/H-11) and 5.0 Hz (H-10a/H-11)/5.5 Hz (H-10b/H-11), respectively. Although the corresponding NOESY correlations were not observed for **1/2**, the respective *J* values of **1** (3.0 and 10.9 Hz)/**2** (7.1 and 7.1 Hz) matched those of **5/6** well, suggesting that the relative configurations were the same (*syn* (**1**)/*anti* (**2**)). To elucidate the absolute configurations at C-9 of **1/2**, the ECD spectra of 9*R*,11*R*-**1** and 9*R*,11*S*-**2** together with their enantiomers, 9*S*,11*S*-**1** and 9*S*,11*R*-**2**, were calculated (Figure 3). The spectra of 9*R*,11*R*-**1** and 9*R*,11*S*-**2** matched the experimental spectrum of a mixture of **1** and **2** (left), which indicated that the configurations of C-11 in **1/2** did not affect their ECD data and that they possessed the 9*R*-configuration. Therefore, absolute configurations of 9*R*,11*R* and 9*R*,11*S* were suggested for **1** and **2**, respectively.

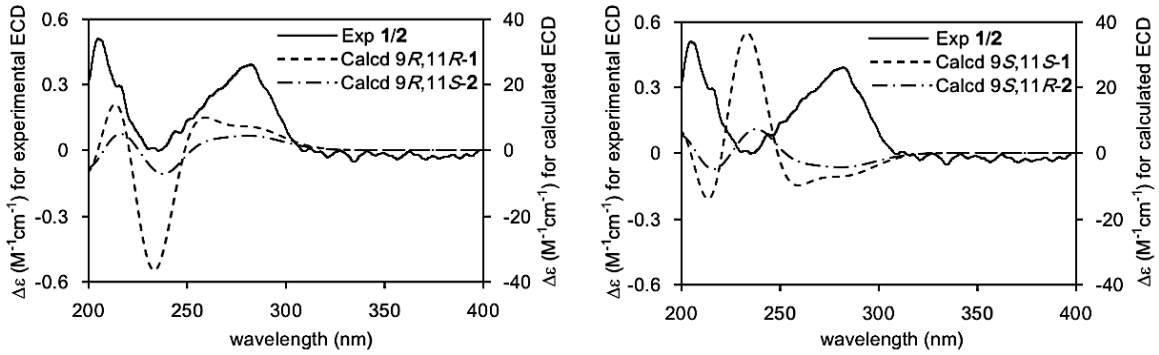
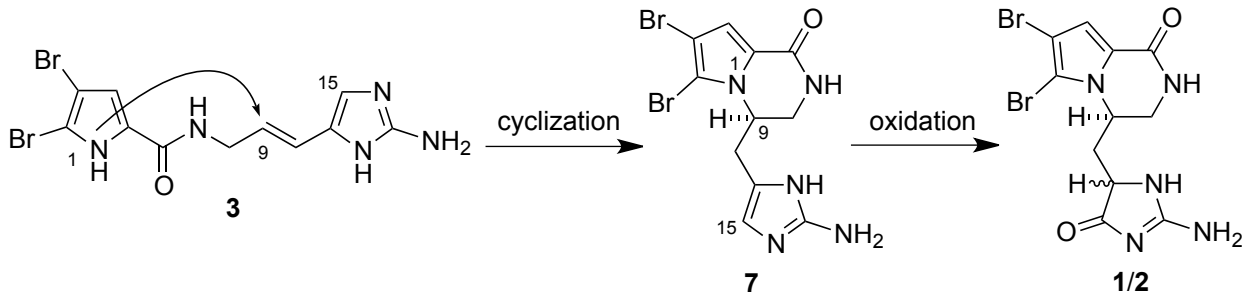


Figure 3. Experimental ECD spectrum of **1**/**2** and calculated ECD spectra of 9*R*,11*R*-**1** and 9*R*,11*S*-**2** (left) and 9*S*,11*S*-**1** and 9*S*,11*R*-**2** (right)

A number of oroidin (**3**) derivatives have been isolated from marine sponges in the orders of Agelasida, Axinellida, and Halichondrida.[1] Agesamines A/B (**1**/**2**) may be biosynthesized from **3** by cyclization from N-1 to C-9 followed by oxidation at C-15 (Scheme 1). The biological activity of **1**/**2** could not be evaluated, because the sample degraded before an assay could be performed.



Scheme 1. Possible formation of **1**/**2** from **3**

EXPERIMENTAL

General Experimental Procedures.

UV spectra were measured on a JASCO V-550 spectrophotometer in MeOH. ECD spectra were measured on a JASCO J-820 spectropolarimeter in MeOH. IR spectra were recorded on a PerkinElmer Frontier FT-IR spectrophotometer. NMR spectra were recorded on a Bruker AVANCE III 600 NMR spectrometer in DMSO-d_6. Chemical shifts were referenced to the residual solvent peaks (δ_H 2.49 and δ_C 39.5 for DMSO-d_6). ESIMS spectrum was measured on a Bruker amaZon speed mass spectrometer. HRESIMS spectrum was measured on a Waters Xevo G2-XS Qtof mass spectrometer.

Animal Material.

The marine sponge was collected at a depth of 10 m in Likpan, Indonesia, in September 2007 and immediately soaked in EtOH. The sponge was identified as *Agelas* sp. by one of the authors (Y.I.). A voucher specimen (07M042) has been deposited at the Department of Natural Medicines, Graduate School of Pharmaceutical Sciences, Kumamoto University, Japan.

Extraction and Isolation.

The sponge (wet weight: 384 g) was extracted with EtOH. The extract was partitioned between EtOAc and H_2O. The EtOAc fraction was partitioned between *n*-hexane and 90% MeOH, and the H_2O fraction was partitioned between *n*-BuOH and H_2O. The 90% MeOH (2.3 g) and *n*-BuOH (9.6 g) fractions were combined and subjected to silica gel column chromatography with a stepwise gradient of CH_2Cl_2/MeOH (8:2), CH_2Cl_2/MeOH (7:3) (Fr. A), CH_2Cl_2/MeOH/H_2O (6:4:1) (Fr. B), and MeOH. Fr. A (3.7 g) was subjected to NH_2 column chromatography with 95, 90, 80, and 50% MeCN-H_2O. The second fraction was identified as **3** (627 mg). The third fraction (193 mg) was purified by gel-filtration HPLC (Asahipak GS-310P column, Asahi Chemical Industry Co., Ltd., 21.5 x 500 mm) with CH_2Cl_2/MeOH (1:1) to yield seven fractions. The fourth fraction (15.1 mg) was purified by NH_2 HPLC (Inertsil NH_2, GL Sciences Inc., 20 x 250 mm) with 90% MeCN-H_2O to afford a mixture of **1** and **2** (1.62 mg). A part (180 mg) of Fr. B (1.27 g) was purified by gel-filtration HPLC (Asahipak GS-310P column, 21.5 x 500 mm) with MeOH to yield eight fractions. The fifth fraction (23.9 mg) was subjected to NH_2 column chromatography with 90, 80, and 50% MeCN-H_2O to afford **4** (4.32 mg) with 80% MeCN-H_2O.

Agesamines A/B (1/2) (1:1 ratio): Yellow amorphous solid; UV (MeOH) λ_{max} (log ε) 226 (3.8), 284 (3.5) nm; ECD (100 μM, MeOH) λ_{max} (Δε) 207 (0.53), 237 (-0.01), 282 (0.39) nm; IR (film) ν_{max} 3344, 1683, 1617, 1055 cm^{-1}; NMR data, see Table 1; ESIMS $[M + H]^+$ *m/z* 403, 405, 407 (1:2:1). HRESIMS $[M + H]^+$ *m/z* 403.9337 (calcd for $C_{11}H_{12}Br_2N_5O_2$, 403.9352).

Conformational Analyses and ECD Calculations for 1/2.

These experiments were performed as previously described.[7] ECD calculations were performed at the B3LYP/TZVP level for **1**/**2**. The wavelength was corrected (+13 nm).

ACKNOWLEDGEMENTS

This work was partially supported by JSPS KAKENHI Grants 18406002 (S.T.), 17H03994 (S.T.), and 18K0671900 (H.K.) of Japan. We thank Prof. M. Namikoshi and Dr. K. Ukai of Tohoku Pharmaceutical University and Dr. H. Kobayashi of the University of Tokyo for collection of the sponge.

REFERENCES

1. J. W. Blunt, A. R. Carroll, B. R. Copp, R. A. Davis, R. A. Keyzers, and M. R. Prinsep, *Nat. Prod. Rep.*, 2018, **35**, 8.

2. S. Forenza, L. Minale, R. Riccio, and E. Fattorusso, *Chem. Commun.*, 1971, 1129.
3. E. E. Garcia, L. E. Benjamin, and R. I. Fryer, *Chem. Commun.*, 1973, 78.
4. J. Kobayashi, F. Kanda, M. Ishibashi, and H. Shigemori, *J. Org. Chem.*, 1991, **56**, 4574.
5. K. Namba, T. Shinada, T. Teramoto, and Y. Ohfune, *J. Am. Chem. Soc.*, 2000, **122**, 10708.
6. M. Tsuda, T. Yasuda, E. Fukushi, J. Kawabata, M. Sekiguchi, J. Fromont, and J. Kobayashi, *Org. Lett.*, 2006, **8**, 4235.
7. M. Torii, H. Kato, Y. Hitora, E. D. Angkouw, R. E. P. Mangindaan, N. J. de Voogd, and S. Tsukamoto, *J. Nat. Prod.*, 2017, **80**, 253.