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Agesamides A and B, Bromopyrrole Alkaloids from Sponge *Agelas* Species: Application of DOSY for Chemical Screening of New Metabolites

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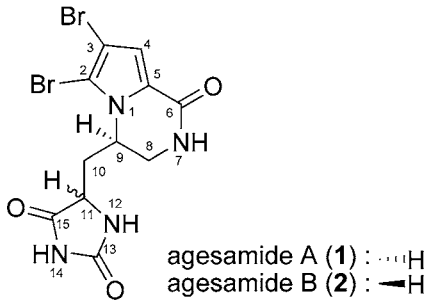
ABSTRACT



Diffusion-ordered NMR spectroscopy (DOSY) is a versatile and powerful NMR technique and a noninvasive analytical method for mixture analysis that does not require prior physical separation of the analytes. In our search for new metabolites from natural resources, DOSY was applied for constituent analysis of crude bromopyrrole fractions separated from an Okinawan marine sponge *Agelas* sp. so that two new bromopyrrole alkaloids, agesamides A (1) and B (2), have been isolated. The structures and relative stereochemistry of 1 and 2 were elucidated from spectroscopic data.

Bromopyrrole alkaloids are well-known to be one group of the most common metabolites contained in marine sponges belonging to the orders Agelasida, Axinellida, and Halichondrida,[1] and over 110 bromopyrrole alkaloids with interesting biological activities have been isolated from these sponges so far. Their molecules are biogenetically generated from the $C_{11}N_5$ scaffold such as oroidin[2] or hymenidin[3] through various metabolic steps, e.g. isomerization, oxidation, reduction, cyclization, or dimerization. In our continuing search for bioactive substances from marine organisms, a number of bromopyrrole alkaloids have been isolated from sponges of the genera *Agelas* or *Hymeniacidon*.[4]

Diffusion-ordered NMR spectroscopy (DOSY) is a versatile and powerful NMR technique that is gaining importance in frontiers of pharmaceutical research.[5] It is a non-

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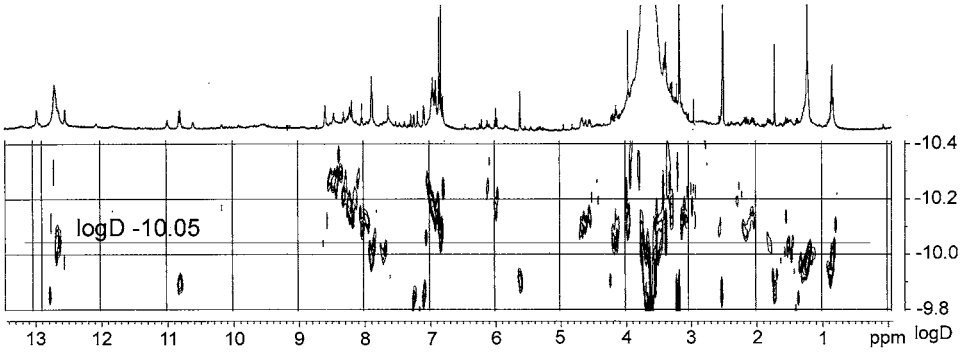
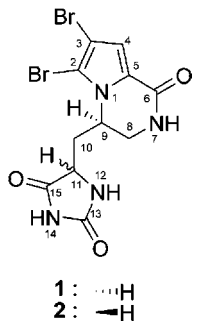


Figure 1. DOSY spectrum (part) of bromopyrrole-containing fraction **A** from a sponge *Agelas* species.

invasive analytical method for mixture analysis that does not require prior physical separation of the analytes. Since we conceived that DOSY analyses are useful for screening of new metabolites from natural resources, the crude bromopyrrole fractions separated from extracts of the Okinawan marine sponges *Agelas* species were applied to the DOSY analyses, resulting in the detection of an unknown bromopyrrole component, which was separated into two new bromopyrrole alkaloids, agesamides A (**1**) and B (**2**), and several known bromopyrrole alkaloids. This paper describes the isolation and structure elucidation of **1** and **2**.



The *Agelas* sponge (SS-1056) collected off Gesashi, Okinawa, was extracted with MeOH. EtOAc soluble materials of the extract were subjected to silica gel and C_{18} chromatographies to afford bromopyrrole-containing fractions, for some of which ^{1}H DOSY spectra were measured. The ^{1}H slice spectra for the logarithm of diffusion constant (log*D*) were analyzed in detail by comparing with literature data of known bromopyrrole alkaloids. It was indicated that fraction **A** contained an unknown component (log*D* −10.05) with a unique ^{1}H NMR profile and three known components such as oroidin (log*D* −10.25), longamide (log*D* −9.92), and dispacamide A (log*D* −9.87). Figures 1 and 2 showed the ^{1}H DOSY spectrum of fraction **A** and a slice spectrum at log*D* −10.05, respectively. Further separation of fraction **A** was carried out with C_{18} HPLC (CH_3CN/H_2O with 0.1% CF_3CO_2H) to give the unknown component with a similar ^{1}H NMR profile to the DOSY slice spectrum at log*D* 10.05, together with oroidin,[2] longamide,[6] dispacamide A,[7] and mauritiamine.[8] Since the unknown component was revealed to be a 1:1 mixture of two compounds, agesamides A (**1**) and B (**2**), from analysis of the spectroscopic data, structure elucidation of **1** and **2** was performed by using this mixture without separation. Finally, agesamides A (**1**) and B (**2**) were separated by reversed-phase HPLC (CH_3CN/H_2O) on a 6-phenylhexylsilyl resin.

Agesamide A[9] (**1**) was obtained as a colorless amorphous solid, and the negative mode ESIMS spectrum showed the pseudomolecular ion peaks at *m*/*z* 403, 405, and 407 (1:2:1), indicating the presence of two bromine atoms in the molecule, and were revealed to possess the molecular formula $C_{11}H_{10}N_4O_3Br_2$ by HRESIMS [*m*/*z* 426.9018 $(M + Na)^+$, Δ +0.1 mmu]. In the ^{1}H NMR (Table 1) spectrum of **1**, three

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

position	**1**		**2**	
4	6.86	s	6.85	s
7	7.87	d, 5.0	7.85	d, 5.1
8a	3.74	dd, 11.5, 4.5	3.76	dd, 11.5, 4.0
8b	3.52	dd, 11.5, 3.5	3.48	dd, 11.5, 3.0
9	4.55	m	4.65	m
10a	2.16	ddd, 12.5, 9.5, 4.0	2.04	ddd, 12.0, 7.5, 5.0
10b	1.56	ddd, 12.5, 2.5, 8.0	1.80	ddd, 12.0, 3.0, 5.5
11	4.21	dd, 4.0, 8.0	4.14	br t, 5.3
12	10.9	br s	10.8	br s
14	8.16	br s	8.00	brs

D_2O-interchangeable proton signals were observed at δ_H 10.9, 8.16, and 7.87. The proton resonance at δ_H 6.86 indicated

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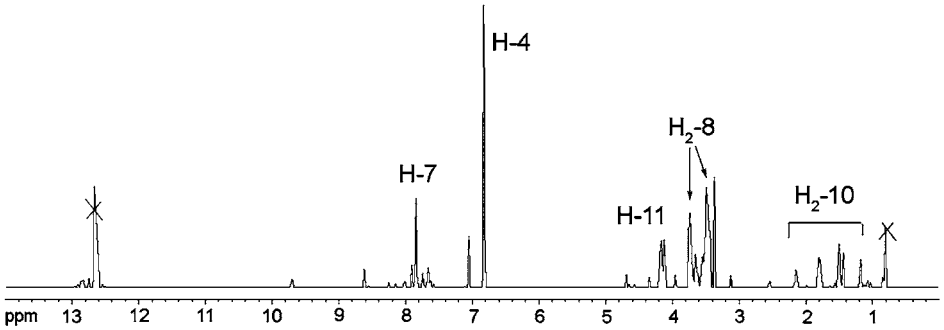

Figure 2. DOSY slice spectrum (log$D$ −10.05) of bromopyrrole-containing fraction **A** from a sponge *Agelas* species.

the presence of 2,3-dibromopyrrole chromophore. The ^{13}C NMR data[10] of **1** disclosed eleven resonances due to six sp^2 quaternary carbons including three amide carbonyl ones, an sp^2 methine, two sp^3 methines, and two sp^3 methylenes. Since five out of eight unsaturations were accounted for, agesamide A (**1**) was inferred to possess three rings.

The structure of **1** was elucidated by detailed analyses of the ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY spectra for the mixture of **1** and **2** (Figure 3). The ^{1}H−^{1}H COSY

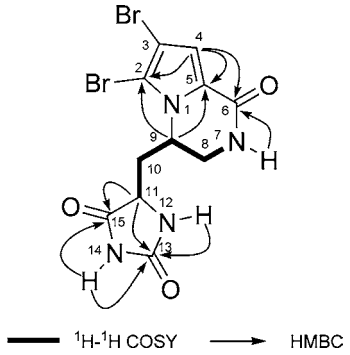

Figure 3. Selected 2D NMR correlations for agesamide A (**1**).

spectrum showed connectivity from NH-7 (δ_H 7.87) to H-11 (δ_H 4.21). The pyrrole proton signal for H-4 showed HMBC correlations for C-2 (δ_C 105.74 or 105.68), C-5 (δ_C 125.38 or 125.36), and C-6 (δ_C 157.62 or 157.56), while the HMBC correlation for an amide NH proton (NH-7) to C-6 was observed, indicating the presence of a 2,3-dibromopyrrole-carbamoyl group. The relatively lower field chemical shifts for C-9 (δ_H 4.55; δ_C 50.61 or 50.63) suggested that C-9 was adjacent to a nitrogen atom. HMBC correlations for H-9/C-2 and H-9/C-5 implied that **1** possessed a pyrroloketopiperazine skeleton such as cyclooroidin.[11] The presence of a hydantoin ring for the C-11−C-15 moiety was suggested by HMBC correlations for H-11/C-13 (δ_C 157.20 or 157.14), H-11/C-15 (δ_C 174.9), NH-12 (δ_H 10.9)/H-13, NH-14 (δ_H 8.16)/C-13, and NH-14/C-15. Thus, the planar structure of agesamide A was assigned as **1**.

The relative stereochemistry of agesamide A (**1**) was elucidated on the basis of ROESY data obtained by using the mixture of **1** and **2** and ^{1}H−^{1}H coupling constants (Figure 4). The J(H-8a/H-9) and J(H-8b/H-9) values (4.5 and 3.5

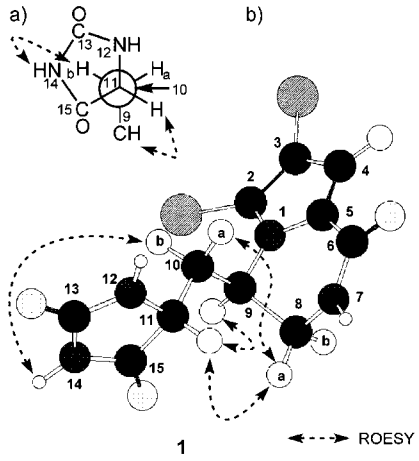

Figure 4. (a) Bond rotation of C-10−C-11 and (b) selected ROESY correlations and relative stereochemistries for agesamide A (**1**).

Hz, respectively) and the ROESY correlation for H-8a/H-10a for **1** suggested that the six-membered ring had a half-boat conformation with an equatorial configuration for H-9. Anti and syn configurations for H-9−H-10a and H-9−

(9) **Agesamide A (1):** colorless amorphous solid; $[\alpha]^{20}_D$ +3.2 ± 1.8 (*c* 0.5, MeOH); IR(KBr) ν_{max} 3418, 1680 and 1637 cm^{-1}; UV (MeOH) λ_{max} 204 (ϵ 12 000) and 282 (2800) nm; ^{1}H and ^{13}C NMR (see Table 1 and ref 10); ESIMS *m*/*z* 403, 405, and 407 [1:2:1, (M − H)$^-$]; HRESIMS *m*/*z* 426.9018 [$C_{11}H_{10}{}^{79}Br_2N_4O_3Na$, (M + Na)$^+$, +0.1 mmu].

(10) ^{13}C NMR data for agesamides A (**1**) and B (**2**) in DMSO-d_6: δ 33.5 (C-10 for **1**), 33.8 (C-10 for **2**), 40.3 (C-8 for **1**), 41.8 (C-8 for **2**), 50.61, 50.63 (C-9), 54.3 (C-11 for **2**), 54.6 (C-11 for **1**), 99.26, 99.29 (C-3), 105.68, 105.74 (C-2), 114.06 (C-4), 125.36, 125.38 (C-5), 157.14, 157.20 (C-13), 157.56, 157.62 (C-6), 174.9 (C-15 for **1**), and 175.2 (C-15 for **2**).

(11) Fattorusso, E.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **2000**, *41*, 9917−9922.

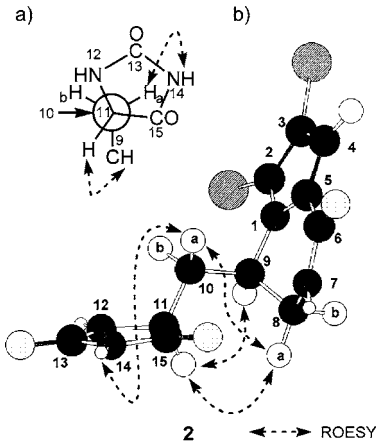

Figure 5. (a) Bond rotation of C-10−C-11 and (b) selected ROESY correlations and relative stereochemistries for agesamide B (**2**).

H-10b, respectively, were deduced from the *J*(H-9/H-10a) and *J*(H-9/H-10b) values (9.5 and 2.5 Hz, respectively), while the C-10−C-11 bond rotation was also elucidated as shown in Figure 4a by small *J*(H-10a/H-11) and large *J*(H-10b/H-11) values (4.0 and 8.0 Hz, respectively) as well as ROESY correlations for H-8a/C-11 and H-9/H-11. Considering the ROESY correlation for H-10b/NH-14, the relative configuration for C-9−C-11 was suggested to be syn.

Agesamide B[12] (**2**) was revealed to have the same molecular formula, $C_{11}H_{10}N_4O_3Br_2$, as **1** by HRESIMS [*m*/*z* 426.9018 $(M + Na)^+$, Δ +0.1 mmu]. ^1H and ^{13}C NMR data of **2** were similar to those of **1**, and the gloss structure of **2** was elucidated to be the same as that of **1** on the basis of analysis of 2D NMR data. The anti configuration for C-9−C-11 of **2** was elucidated as follows. The *J*(H-10a/H-11) and *J*(H-10b/H-11) values (5.0 and 5.5 Hz, respectively) and ROESY correlations for H-9/H-11 and H-10a/NH-14 suggested that the C-10−C-11 bond had an eclipsed conformation as shown in Figure 5a. The bond rotation for C-9−C-10 was deduced to be anti for H-9−H-10a and gauche for H-9−H-10b from $^1H-^1H$ coupling constants [*J*(H-9/H-10a) = 7.5 Hz and *J*(H-9/H-10b) = 3.0 Hz], and ROESY correlations for H-8a/H-10a and H-8a/H-11 indicated the anti configuration of C-9−C-11.

Agesamides A (**1**) and B (**2**) are new bromopyrrole alkaloids possessing pyrroloketopiperazine and hydantoin rings. Absolute stereochemistries of agesamides A (**1**) and B (**2**) were not determined, since CD Cotton curves for the mixture of **1** and **2** were weaker than that of cyclooroidin,[9] which possessed a pyrroloketopiperazine skeleton.

In this study, DOSY analyses were found to be useful for chemical screening of new metabolites from crude fractions of natural sources.

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Supporting Information Available: Spectral data; ^1H NMR spectra for **1** and **2**, ^{13}C and 2D NMR spectra for the mixture of **1** and **2**, and DOSY slice spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) **Agesamide B (2):** colorless amorphous solid; $[\alpha]^{20}_D$ +2.8 ± 1.6 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3418, 1680, and 1637 cm^{-1}; UV (MeOH) λ_{max} 204 (ϵ 12 300) and 282 (3050) nm; ^1H and ^{13}C NMR (see Table 1 and ref 10); ESIMS *m*/*z* 403, 405, and 407 [1:2:1, $(M - H)^-$]; HRESIMS *m*/*z* 426.9018 [$C_{11}H_{10}{}^{79}Br_2N_4O_3Na$, $(M + Na)^+$, +0.1 mmu].