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Hyrtioseragamines A and B, New Alkaloids from the Sponge *Hyrtios* Species

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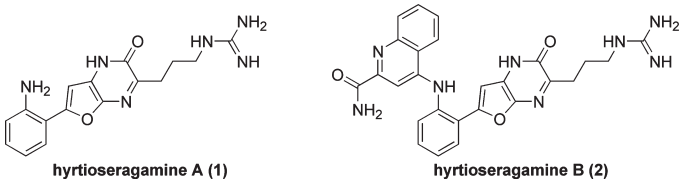
ABSTRACT



Two novel alkaloids with a furo[2,3-*b*]pyrazin-2(1*H*)-one moiety and a guanidino group, hyrtioseragamines A (1) and B (2), have been isolated from an Okinawan marine sponge *Hyrtios* species. The structures of 1 and 2 were elucidated on the basis of spectroscopic data and chemical conversions. Compounds 1 and 2 are the first natural products possessing a furo[2,3-*b*]pyrazine-related moiety.

Marine sponges have been recognized as a rich source of bioactive secondary metabolites with unprecedented skeletons.[1] During our continuing search for bioactive substances from marine sponges,[2] we have investigated extracts of an Okinawan marine sponge *Hyrtios* sp. (SS-985) and isolated two novel alkaloids, hyrtioseragamines A (**1**) and B (**2**), possessing a furo[2,3-*b*]pyrazin-2(1*H*)-one and a guanidino group. Here we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Hyrtios* sp. (SS-985, 2.85 kg) collected off Seragaki, Okinawa was extracted with MeOH. The MeOH extract was partitioned between *n*-hexane and H_2O, and the aqueous layer was successively extracted with $CHCl_3$, EtOAc, and *n*-BuOH. $CHCl_3$- and *n*-BuOH-soluble materials of the extract were subjected to C_{18} column chromatography followed by repeated C_{18} HPLC to afford hyrtioseragamines A (**1**, 12.6 mg, 4.4×10^{-4}% wet weight)[3] and B (**2**, 2.2 mg, 7.7×10^{-5}%)[4] together with known β-carboline alkaloids, gesashidine A[5] and dragmacidonamines A and B.[6]

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(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165–237.

(2) Kura, K.; Kubota, T.; Fromont, J.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 267–270.

(3) Hyrtioseragamine A (**1**): yellow amorphous solid; UV (MeOH) λ_{max} 214 (log ε 4.3), 238 (4.1 sh), 270 (3.9), and 389 nm (4.1); IR (film) ν_{max} 3343, 3171, 2922, 2604, 1645, 1548, and 1470 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) see Table 1; ESIMS (pos.) *m*/*z* 327 $[M+H]^+$; HRESIMS (pos.) *m*/*z* 327.15700 ($[M+H]^+$, calcd for $C_{16}H_{19}N_6O_2$, 327.15640).

(4) Hyrtioseragamine B (**2**): yellow amorphous solid; UV (MeOH) λ_{max} 222 (log ε 4.4), 250 (4.1 sh), 280 (3.8 sh), and 373 nm (4.0); IR (film) ν_{max} 3353, 3152, 2936, 2722, 1680, 1631, 1554, and 1453 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) see Table 1; ESIMS (pos.) *m*/*z* 497 $[M+H]^+$; HRESIMS (pos.) *m*/*z* 497.20467 ($[M+H]^+$, calcd for $C_{26}H_{25}N_8O_3$, 497.20441).

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Table 1. ^{1}H and ^{13}C NMR Data of Hyrtioseragamines A (**1**) and B (**2**) in CD_3OD at 300 K[a]

	1				**2**			
position	δ_C (mult.)		δ_H (mult., J in Hz)	HMBC (H to C)	position	δ_C (mult.)	δ_H (mult., J in Hz)	HMBC (H to C)
1	144.6	C			1	135.4	C	
2	120.6	CH	7.03 (brd, 8.0)	4, 6	2	131.2	CH	7.67 (m)	1, 4, 6
3	132.6	CH	7.25 (ddd, 8.0, 7.8, 1.4)	1, 2, 5	3	133.3	CH	7.72 (m)	1
4	121.8	CH	6.91 (ddd, 7.8, 7.6, 1.0)	2, 3, 5, 6	4	131.6	CH	7.71 (m)	6
5	129.9	CH	7.64 (dd, 7.8, 1.4)	1, 3, 7	5	130.9	CH	8.16 (m)	1, 3, 7
6	117.4	C			6	129.1	C		
7	158.5	C			7	154.8	C		
8	100.3	CH	6.94 (s)	7, 8a, 12a	8	103.2	CH	6.88 (s)	7, 12a
8a	127.8	C			8a	127.3	C		
10	159.1	C			10	158.9	C		
11	148.7	C			11	150.9	C		
12a	148.2	C			12a	148.6	C		
14	31.3	CH_2	2.91[b] (t, 7.2)	10, 11, 15, 16	14	31.3	CH_2	2.82[b] (t, 7.2)	10, 11, 15, 16
15	27.8	CH_2	2.08[b] (quin, 7.2)	11, 14, 16	15	27.6	CH_2	1.97[b] (quin, 7.2)	11, 14, 16
16	42.7	CH_2	3.34[b] (t, 7.2)	14, 15, 18	16	42.6	CH_2	3.25[b] (t, 7.2)	14, 15, 18
18	159.6	C			18	159.6[c]	C		
					2′	146.3	C		
					3′	100.3	CH	7.15 (s)	2′, 4′, 4′a, 2′-*C*O
					4′	159.5[c]	C		
					4′a	119.6	C		
					5′	124.8	CH	8.71 (d, 8.2)	4′, 4′a, 7′, 8′a
					6′	130.4	CH	7.91 (dd, (8.2, 7.8)	4′a, 5′, 6′, 8′
					7′	136.9	CH	8.12 (dd, 7.8, 8.4)	5′, 8′a
					8′	123.1	CH	8.25 (d, 8.4)	4′,[d] 4′a, 6′, 7′
					8′a	140.9	C		
					2′-*C*O	164.0	C		

[a] ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. [b] 2H. [c] Interchangeable. [d] The correlation observed through four bonds due to a long-range coupling.

The molecular formula, $C_{16}H_{18}N_6O_2$, of hyrtioseragamine A (**1**) was established by HRESIMS (m/z 327.15700 $[M+H]^+$, Δ +0.60 mmu). ESIMS (m/z 335 $[M+D]^+$) using CD_3OD as a mobile phase revealed that 7 out of 18 hydrogen atoms were H/D exchangeable. IR absorptions indicated the existence of OH and/or NH (ν_{max} 3343 and 3171 cm^{-1}) groups and a carbonyl group (1645 cm^{-1}), while UV absorptions (λ_{max} 270 and 389 nm) were attributed to a conjugated aromatic functionality.

The ^{1}H NMR (Table 1) spectrum of **1** measured in CD_3OD contained eight proton signals, three of which were sp^3 methylene protons and five of which were sp^2 methine protons. In the ^{13}C NMR (Table 1) spectrum of **1**, three sp^3 methylenes were observed in the aliphatic carbon region from δ_C 20 to 50. On the other hand, five sp^2 methines and eight sp^2 quaternary carbons were observed in aromatic and carbonyl carbons region from δ_C 100 to 160. The sp^2 quaternary carbon observed in the low-field region (C-18, δ_C 159.6) and a positive coloration in the Sakaguchi test implied the presence of a guanidino group.

The $^{1}H-^{1}H$ COSY and HMBC spectrum of **1** in CD_3OD at 300 K revealed the presence of a 1,2-disubstituted benzene ring (C-1−C-6) (Figure 1a). The chemical shift at C-1 (δ_C 144.6) suggested that an amino group was attached to C-1. The connectivity of C-6 to C-8 was elucidated from HMBC cross-peaks of H-5/C-7 and H-8/C-7 and the ROESY correlation of H-5/H-8. The chemical shift of C-7 (δ_C 158.5) indicated that C-7 was connected to an oxygen atom (O-13). H-8 showed a large $^{1}J_{CH}$ value (180.0 Hz) and HMBC cross-peaks to C-8a (δ_C 127.8) and C-12a (δ_C 148.2), indicating the existence of a 2,3,5-trisubstituted furan ring. On the other hand, $^{1}H-^{1}H$ COSY correlations and the HMBC correlation of H-16/C-18 suggested the existence of a 1-propylguanidino group (Figure 1b). The connection of C-14 to C-10 via C-11 was disclosed by HMBC correlations of H_2-14/C-10, H_2-14/C-11, and H_2-15/C-11. The strong IR absorption at 1645 cm^{-1} and the chemical shift of C-10 (δ_C 159.1) and C-11 (δ_C 148.7) indicated that C-10 and C-11 were an amide carbonyl and an imino carbon, respectively.

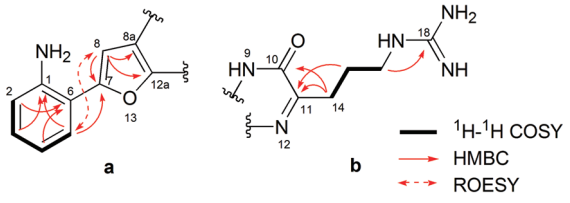

Figure 1. Selected 2D NMR correlations for two partial structures (**a** and **b**) of hyrtioseragamine A (**1**) in CD_3OD at 300 K.

Furthermore, it was implied by the molecular formula of hyrtioseragamine A (**1**) that C-10 and C-11 in the partial structure **b** were connected to C-8a or C-12a in the partial structure **a** via N-9 and N-12 (Figure 1).

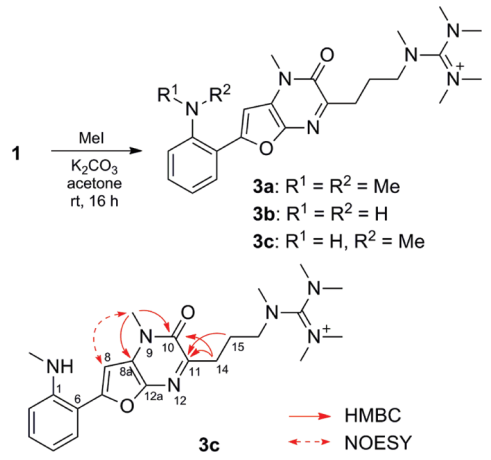

Figure 2. Methylation of hyrtioseragamine A (**1**) and key 2D NMR correlations for septamethyl derivative (**3c**) of hyrtioseragamine A (**1**) in DMSO-d_6 at 350 K.

To elucidate the structure of hyrtioseragamine A, **1** was methylated by methyl iodide under basic conditions to afford three methylated derivatives **3a**−**3c** (Figure 2). The HMBC spectrum of **3c** recorded in DMSO-d_6 at 350 K showed cross-peaks of N-9-Me/C-8a, N-9-Me/C-10, H_2-14/C-10, H_2-14/C-11, and H_2-15/C-11 (Figure 2). In addition, N-9-Me showed the NOESY correlation to H-8. These correlations disclosed that C-11 was connected to C-8a and C-12a via an amide bond (N-9 and C-10) and an imine nitrogen atom (N-12), respectively. Thus, the structure of hyrtioseragamine A was elucidated to be **1**.

IR and UV spectra of hyrtioseragamine B (**2**) were similar to those of **1**, implying the existence of OH and/or NH (ν_{max} 3353 and 3152 cm^{-1}), carbonyl group(s) (ν_{max} 1631 cm^{-1}), and a conjugated aromatic system (λ_{max} 280 and 373 nm). The HRESIMS of **2** showed the molecular formula to be $C_{26}H_{24}N_8O_3$ (m/z 497.20467 $[M+H]^+$, Δ +0.26 mmu).

The ^{1}H NMR (Table 1) spectrum of **2** included 10 sp^2 methine and 3 sp^3 methylene protons, while the ^{13}C NMR (Table 1) spectrum of **2** showed 13 sp^2 quaternary carbons, 10 sp^2 methines, and 3 sp^3 methylenes. These data indicated that **2** had another aromatic ring in addition to the same partial structure as that of **1**.

Inspection of the ^{1}H−^{1}H-COSY, HMBC, and ROESY spectra of **2** in CD_3OD at 300 K and comparison of ^{1}H and ^{13}C NMR data of **2** with those of **1** disclosed that **2** had the same partial structure as that of **1** (C-1−C-18) (Figure 3). Another 1,2-disubstituted benzene ring (C-4′a−C-8′a) in **2** was revealed by the ^{1}H−^{1}H COSY and HMBC spectra. The presence of a 2,4-disubstituted quinoline ring (C-1′−C-8′) was deduced by HMBC correlations of H-5′/C-4′, H-3′/C-2′,

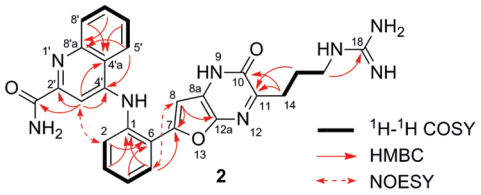

Figure 3. Selected 2D NMR correlations for hyrtioseragamine B (**2**) in CD_3OD at 300 K.

H-3′/C-4′, and H-3′/C-4′a, the chemical shifts of C-2′ (δ_C 146.3) and C-8′a (δ_C 140.9), and the $^1J_{CH}$ value of CH-3′ (169.8 Hz). Given the molecular formula of **2**, chemical shifts of C-2′ (δ_C 146.3) and C-4′ (δ_C 159.5), and the HMBC cross-peak of H-3′ to a carbonyl carbon (C-2′-*C*O, δ_C 164.0), it was indicated that an amino carbonyl group and an amino group were attached to C-2′ and C-4′, respectively. Additionally, the ROESY correlation of H-2/H-3′ implied the connectivity of C-1 and C-4′ via a secondary amino group (Figure 3).

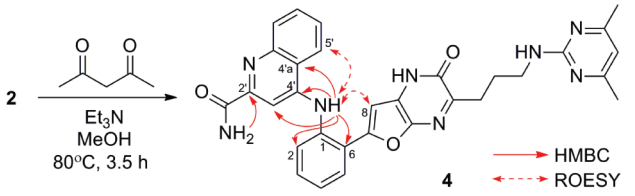

Figure 4. Chemical conversion of hyrtioseragamine B (**2**) into pyrimidine derivative (**4**) and key 2D NMR correlations for **4** in C_5D_5N at 300 K.

To confirm the structure of hyrtioseragamine B (**2**) by 2D NMR cross-peaks from exchangeable protons, **2** was treated with 2,4-pentanedione under basic conditions to give a pyrimidine derivative (**4**) of **2** (Figure 4). Detailed analysis of 1D and 2D NMR spectra of **4** in C_5D_5N at 300 K supported the structure of **2**. The NH proton (δ_H 9.85) in **4** exhibited HMBC correlations to C-2, C-6, C-3′, C-4′, and C-4′a, indicating the connection of C-1 and C-4′ via a secondary amino group. This connectivity was supported by ROESY correlations from C-1-NH to H-8 and H-5′. Furthermore, the presence of two exchangeable protons coupling to each other (δ_H 8.88 and 8.48, J = 3.8 Hz) and the HMBC correlation from one of these (δ_H 8.48) and H-3′ to C-2′ and a carbonyl carbon (C-2′-*C*O, δ_C 168.0), respectively, suggested the existence of a primary amide group at C-2′. Thus, the structure of hyrtioseragamine B was elucidated to be **2**.

To the best of our knowledge, hyrtioseragamines A (**1**) and B (**2**) are the first natural products possessing a furo-[2,3-*b*]pyrazine-related moiety. In addition, quinolinecarboxylic

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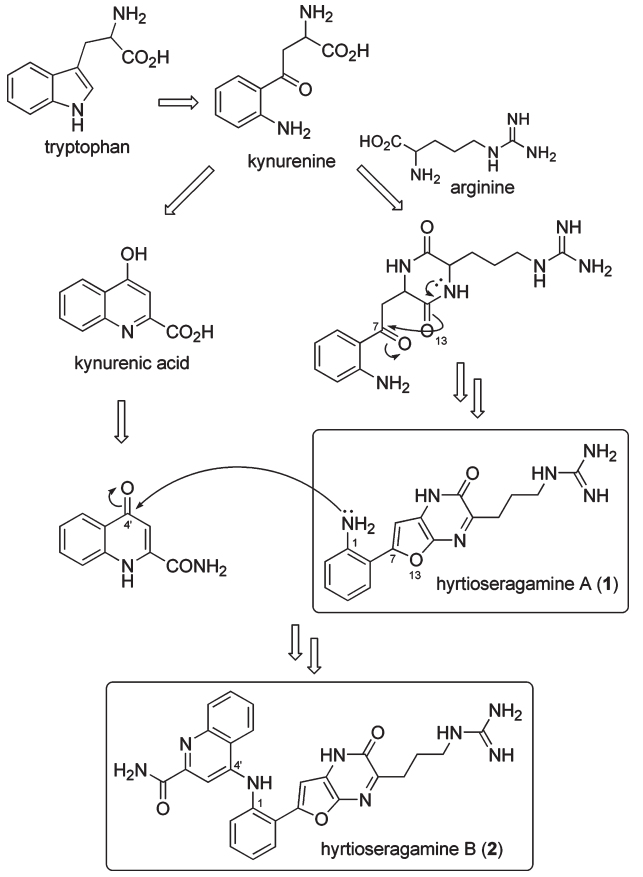
Scheme 1. Possible Biogenetic Path for Hyrtioseragamines A (**1**) and B (**2**)



acid derivatives from marine organisms are very rare.[7] Biogenetically, **1** might be generated from tryptophan and arginine (Scheme 1).

A diketopyperazine ring might be formed by kynurenine, which is a metabolite of tryptophan, and arginine and nucleophilic addition of O-13 to C-7 followed by aromatization to produce **1**. Hyrtioseragamine B (**2**) might be generated by nucleophilic addition of a primary amino group attached to C-1 of **1** to the carbonyl group of C-4 in a quinolone form of the kynurenic acid derivative. Since the diketopyperazine moiety is well-known to be a microbial metabolite, compounds **1** and **2** might be produced by symbiotic microbes of the sponge.

Hyrtioseragamines A (**1**) and B (**2**) showed antimicrobial activities against *Aspergillus niger* (MIC, 8.33 and 16.6 μg/mL, respectively) and *Cryptococcus neoformans* (MIC, 33.3 and 16.6 μg/mL, respectively). Compounds **1** and **2** did not show cytotoxicity (IC_{50} > 10 μg/mL) against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro*.

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Supporting Information Available. Detailed experimental section and 1D and 2D NMR data for hyrtioseragamines A and B and their derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.