Gesashidine A, a β-Carboline Alkaloid with an Imidazole Ring from a Thorectidae Sponge

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Received February 28, 2005

A new β-carboline alkaloid with an imidazole ring, gesashidine A (**1**), has been isolated from an Okinawan marine sponge of the family Thorectidae (SS-1035), and the structure was elucidated from the spectroscopic data.

β-Carboline alkaloids are known to be one of the most common metabolites contained in marine sponges.[1] During our search for new metabolites from marine organisms,[2] we previously isolated several β-carboline alkaloids with unique polycyclic ring systems from *Amphimedon* sponges.[3,4] More recently, we have isolated a new β-carboline alkaloid with an imidazole ring, gesashidine A (**1**), from an Okinawan marine sponge of the family Thorectidae (SS-1035). Here we describe the isolation and structure elucidation of **1**.

The sponge of the family Thorectidae (SS-1035) collected off Gesashi, Okinawa, was extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O, and subsequently the aqueous layer was extracted with *n*-BuOH. *n*-BuOH-soluble materials of the extract were subjected to gel filtration on Sephadex LH-20 and C_{18} column chromatographies followed by C_{18} HPLC to yield gesashidine A (**1**, 0.0006%, wet weight) together with known related alkaloids, dragmacidonamines A (**2**) and B (**3**).[5] The molecular formula, $C_{18}H_{19}N_4OS$, of **1** was established by HRESIMS [*m/z* 339.1260 $(M)^+$, Δ −1.9 mmu]. IR absorptions indicated the existence of OH and/or NH (3420 cm^{-1}) and amide carbonyl (1680 cm^{-1}) groups. The ^1H and ^{13}C NMR (Table 1) spectra of **1** were similar to those of hyrtiomanzamine,[6] previously isolated from the sponge *Hyrtios erecta*, except for the resonance at C-10 in **1**. The molecular weight of **1** was smaller than that of hyrtiomanzamine by 14 mass units, indicating that a ketone group at C-10 in hyrtiomanzamine was replaced by a methylene in **1**. HMBC correlations (Figure 1) of H-4 to C-4a (δ 129.6) and C-9a (δ 135.7), H-5 to C-4b (δ 121.2), H-8 to C-4a and C-6 (δ 151.8), and NH to C-4b indicated the presence of a β-carboline moiety (C-1−C-4, C-4a−C-4b, C-5−C-8, C-8a−N-9, and N-9−C-9a). The presence of an imidazolium unit (C-11−C-15) was deduced from HMBC correlations of 12-*N*-Me to C-11 (δ 136.1) and C-13 (δ 138.6), 14-*N*-Me to C-15 (δ 125.9), *S*-Me to C-15, and H-13 to C-11 and C-15. HMBC correlations of H-10 to C-1 (δ 137.4), C-9a, C-11, and C-15 suggested that the imidazolium unit was attached to C-1. Thus, the structure of gesashidine A was assigned as **1**.

Gesashidine A (**1**) showed antibacterial activity against *Micrococcus luteus* (MIC, 16.6 μg/mL), while dragmaci-

Table 1. ^1H and ^{13}C NMR Data of Gesashidine A (**1**) in DMSO-d_6

position	^1H[a]	^{13}C[a]	H coupled with C[b]
1		137.4	H-10
3	8.80 (s)	134.5	
4	8.80 (s)	115.1	
4a		129.6	H-4
4b		121.2	H-5, H-8, N-H
5	7.62 (s)	106.0	H-7
6		151.8	H-8
7	7.21 (d, 7.6)	120.4	
8	7.56 (d, 7.6)	113.2	
8a		133.5	
9a		135.7	H-4, H-10
10	4.86 (s)	26.5	
11		136.1	H-10, 12N-Me, H-13
13	9.33 (s)	138.6	12N-Me
15		125.9	H-10, H-13, 14N-Me, S-Me
S-Me	2.04 (s)	18.6	
12N-Me	3.84 (s)	34.6	
14N-Me	3.82 (s)	33.7	
OH			
NH	12.19 (s)		

[a] δ in ppm. [b] HMBC correlations.

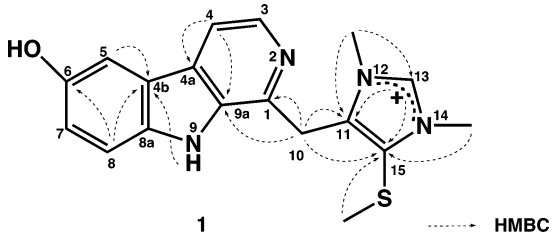

Figure 1. Selected HMBC correlations for gesashidine A (**1**).

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10.1021/np050070p CCC: $30.25
Published on Web 06/28/2005

donamines A (**2**) and B (**3**) did not exhibit such antibacterial activity. Gesashidine A (**1**) did not show cytotoxicity (IC_{50} > 10 μg/mL) against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro

Experimental Section

General Experimental Procedures. IR and UV spectra were recorded on a JASCO FT/IR-5300 and a Shimadzu UV-1600PC spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells for DMSO (Shigemi Co., Ltd). The 2.49 and 39.5 ppm resonances of residual DMSO-d_6 were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-SX102A spectrometer.

Sponge Description. The sponge (SS-1035, family Thorectidae, order Dictyoceratida)[7] was collected off Gesashi, Okinawa, and kept frozen until used. The voucher specimen (SS-1035) was deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (0.5 kg, wet weight) was extracted with MeOH (0.5 L × 3), and the extract (19.1 g) was partitioned between EtOAc (200 mL × 3) and H_2O (200 mL). Subsequently, the aqueous layer was extracted with *n*-BuOH (200 mL × 3). The *n*-BuOH-soluble materials (2.1 g) were subjected to gel filtration on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to give an alkaloid-containing fraction, which was separated by C_{18} column chromatography (MeOH/H_2O/TFA, 40:60:1) and then C_{18} HPLC (Hydrosphere C18, YMC Co., Ltd., 10 × 250 mm; eluent, MeOH/H2O/TFA, 40:60:0.1; flow rate, 1.5 mL/min; UV detection at 220 nm) to afford gesashidine A (**1**, 2.9 mg, 0.0006%, wet weight, t_R 12.8 min) and dragmacidonamines A (**2**, 23.1 mg) and B (**3**, 9.9 mg).

Gesashidine A (1): yellow amorphous solid; UV (MeOH) λ_{max} 212 (ϵ 25 000), 246 (7300), 289 (4400), 297 (5600), and 364 (1400) nm; IR (film) ν_{max} 3420 and 1680 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS (pos.) *m/z* 339 [M$^+$]; HRESIMS *m/z* 339.1260 [M$^+$] (calcd for $C_{18}H_{19}N_4OS$, 339.1279).

Acknowledgment. We thank Z. Nagahama and S. Furugen for their help with collection of the sponge. This work was partly supported by grants from the Akiyama Foundation and Takeda Science Foundation, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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(7) Due to problems with preservation, this sponge was difficult to determine to the level of genus, but appears to have affinities to the genus *Fasciospongia*.

NP050070P