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Stellettazole A: An Antibacterial Guanidinoimidazole Alkaloid from a Marine Sponge *Stelletta* sp.#

Sachiko Tsukamoto, Takahiro Yamashita, Shigeki Matsunaga, and Nobuhiro Fusetani*

Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

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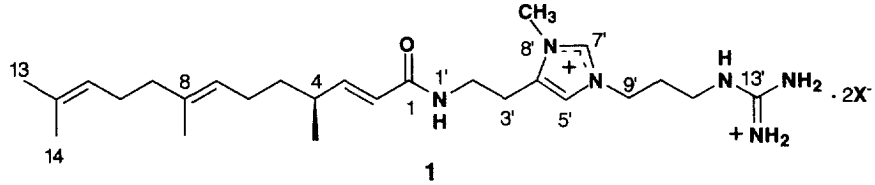
Abstract: A new guanidinoimidazole alkaloid, stellettazole A (**1**), has been isolated from a marine sponge *Stelletta* sp. as an antibacterial constituent. Its structure was determined on the basis of spectral data and chemical degradation. Stellettazole A also inhibited Ca^{2+}/calmodulin-dependent phosphodiesterase.

Keywords: Antibiotics; Alkaloids; Enzyme inhibitors; Sponges

In our continuous search for biologically active metabolites from Japanese marine invertebrates, we found that the methanolic extract of a marine sponge of the genus *Stelletta* collected off Shikine-jima Island, 200 km south of Tokyo inhibited the growth of the bacterium *Escherichia coli*. Bioassay-guided fractionation resulted in the isolation of a new alkaloid, stellettazole A (**1**), together with the known stellettamide A [1] and stellettadine A [2]. We report the isolation and structure elucidation of stellettazole A, which is a homosesquiterpene amide of 1-guanidinopropyl-3-methylhistamine.

The frozen sponge (100 g) was extracted with MeOH. After evaporation of the solvent, the resulting aqueous residue was extracted with ether and *n*-BuOH. The *n*-BuOH fraction was separated by ODS column chromatography (MeOH/H_2O) and gel filtration on Sephadex LH-20 (MeOH), followed by ODS HPLC (*n*-PrOH/H_2O/TFA and CH_3CN/H_2O/TFA) to afford stellettazole A (**1**, 1.0 mg, 1.0×10^{-3} %, wet wt).



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Stellettazole A (**1**), $[\alpha]^{24}_D$ -35° (*c* 0.078, MeOH), had a molecular formula of $C_{26}H_{45}N_6O$ as established by HRFABMS [*m/z* 457.3659 $(M + H)^+$, Δ +0.4 mmu]. The presence of a C_{16}-terpenoid moiety as in the case of stellettamide A was straightforward by comparing ^1H-^1H COSY and HMBC spectra (Table 1). The ^1H NMR spectrum also exhibited spin systems for an *N*,2-disubstituted ethylamine (H-1' ~ H_2-3'), mutually-coupled heteroaromatic protons (H-5' and H-7'), and a propane diamine (H_2-9' ~ H-12'). HMBC cross peaks (Table 1) revealed that the heteroaromatic protons were incorporated into an

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imidazolium ring which was substituted by methyl, ethyl, and propyl groups [H-5' (δ 7.57)/C4' (δ 133.0) and C7' (δ 136.3); H-7'(δ 9.04)/C4' and C5' (δ 119.2); 8'-CH_3 (δ 3.76)/C4' and C7'; H_2-3'(δ 2.80)/C4' and C5'; H_2-9' (δ 4.13)/C5' and C7']. The propyl group was also linked to a guanidino group [δ 156.8 (C13'); δ 7.25 (4H, br s, 13'-NH_2 × 2)] as indicated by an HMBC cross peak H_2-11'/C13'. Finally, the terpenoid and guanidinoimidazolium units were connected via an amide linkage on the basis of HMBC cross peaks [H-2 (δ 5.80), H-3 (δ 6.52), and H-1' (δ 8.15)/C1 (δ 165.3)].

Stereochemistry of C-4 was determined as follows; stellettazole A (**1**) was oxidized with $RuCl_3/NaIO_4$ [3] to afford 2-methylglutaric acid, which was converted to the di-*p*-bromophenacyl ester [4]. A racemic and the (2*S*)-diesters were prepared from the commercially available 2-methylglutaric acid and from stellettamide A [5], respectively. The retention times of the (*S*)- and (*R*)-diesters in HPLC using a chiral column (CHIRALCEL OD, φ4.6 × 250 mm; 10 % hexane-EtOH; 1.0 mL/min) were 10.5 and 13.0 min, respectively. The diester derived from **1** was eluted at a retention time of 10.5 min, thereby indicating 4*S*-configuration for **1**.

Stellettazole A (**1**) was antibacterial against *E. coli* (inhibitory zone of 10 mm; 50 μg/6 mm disk) and inhibitory against Ca^{2+}/calmodulin-dependent phosphodiesterase (45 % inhibition at 100 μM). A few amides consisting of terpenic carboxylic acids and polyamines have been reported from sponges and a soft coral. Their biological profiles included antifungal [1], cytotoxic [1, 6], calmodulin inhibitory [7], H,K-ATPase inhibitory [6], and metamorphosis inducing activities [2].

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

no.	^1H	^{13}C	HMBC	no.	^1H	^{13}C	HMBC
1		165.3 s		1'	8.15 br s		1
2	5.80 d 15.6	122.4 d	1, 4	2'	3.38 (2H) m	36.4 t	
3	6.52 dd 15.6, 7.2	148.1 d	1	3'	2.80 (2H) t 6.6	23.4 t	2', 4', 5'
4	2.24 m	34.8 d	2, 3	4'		133.0 s	
5	1.32 (2H) m	35.7 t	3, 4, 6, 7, 4-Me	5'	7.57 s	119.2 d	4', 7'
6	1.92 (2H) m	25.0 t	4, 5, 7, 8	7'	9.04 s	136.3 d	4', 5'
7	5.06 t 7.2	123.9 d	9, 8-Me	9'	4.13 (2H) t 6.0	46.2 t	5', 7', 10', 11'
8		134.3 s		10'	1.97 (2H) m	28.8 t	9', 11'
9	1.94 (2H) m	39.4 t	7, 8, 10, 11, 8-Me	11'	3.11 (2H) m	37.6 t	9', 10', 13'
10	2.03 (2H) m	26.1 t	9, 11, 12	12'	7.90 br s		
11	5.05 t 7.2	124.0 d		13'		156.8 s	
12		130.6 s		8'-Me	3.76 (3H) s	33.2 q	4', 7'
13	1.62 (3H) s	25.5 q	11, 12, 14	13'-NH_2	7.25 (4H) br s		
14	1.55 (3H) s	17.5 q	11, 12, 13				
4-Me	0.97 (3H) d 6.0	19.4 q	3, 4, 5				
8-Me	1.53 (3H) s	15.8 q	8, 9				

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