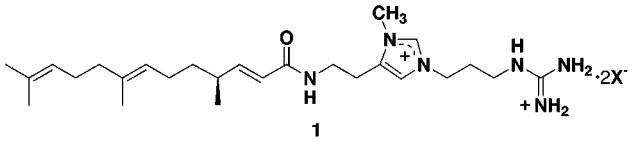
Bistellettadines A and B: Two Bioactive Dimeric Stellettadines from a Marine Sponge *Stelletta* sp.[1]

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

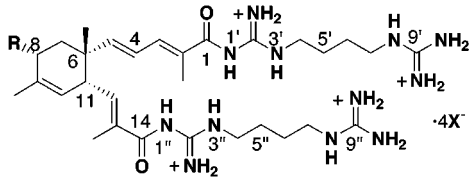
Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

Received February 22, 1999

We recently have reported isolation of stellettazole A (**1**), an antibacterial homosesquiterpene amide of 1-guanidinopropyl-3-methylhistamine, from a *Stelletta* sponge collected from Shikine-jima island, 200 km south of Tokyo.[2] Interestingly, it inhibited Ca^{2+}/calmodulin-dependent phosphodiesterase. Therefore, we continued to examine the sponge extract, which resulted in the isolation of two dimeric stellettadines, bistellettadines A (**2**) and B (**3**). Stellettadine A (**4**) is a sesquiterpene amide of 1,4-diguanidinobutane possessing larval metamorphosis-inducing activity in an ascidian, which we had isolated from another *Stelletta* sponge.[3] In this paper, we describe isolation, structure determination, and biological activities of these new metabolites.

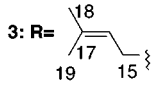


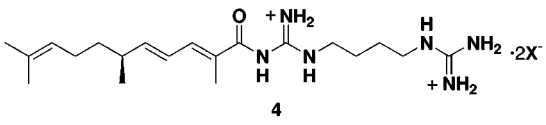
1



2: R=H

3: R=





4

The frozen sponge (100 g)[4] was extracted with MeOH, and the concentrated aqueous residue was extracted with ether and then with *n*-BuOH. The *n*-BuOH layer was successively fractionated by ODS column chromatography ($MeOH/H_2O$), gel filtration on Sephadex LH-20 (MeOH), and ODS HPLC (*n*-$PrOH/H_2O$/TFA and CH_3CN/H_2O/TFA) to afford bistellettadines A (**2**, 15.6 mg, 1.6×10^{-2}%, wet wt)[5] and B (**3**, 0.9 mg, 0.9×10^{-3}%).[6]

Table 1. ^1H and ^{13}C NMR Data for 2[a]

no.	^1H[b]	^{13}C[c]	HMBC
1		169.6 s	
2		127.0 s	
3	7.06 d 10.8	138.5 d	1, 5, 2-Me
4	6.42 dd 16.2, 10.8	123.5 d	3, 5, 6
5	6.29 d 16.2	150.3 d	3, 4, 6, 6-Me
6		38.2 s	
7	a′ 1.80 m	31.2 t	6
	e′ 1.60 m		6
8	1.98 (2H) m	26.9 t	6, 7, 9, 10
9		134.5 s	
10	5.12 s	119.7 d	6, 8, 9-Me
11	3.05 m	44.7 d	12
12	6.32 d 10.2	141.7 d	6, 10, 14, 13-Me
13		129.6 s	
14		169.6 s	
2-Me	1.90 (3H) s	12.2 q	1, 2, 3
6-Me	1.08 (3H) s	24.3 q	5, 6, 7, 11
9-Me	1.65 (3H) s	22.8 q	8, 9, 10
13-Me	1.86 (3H) s	12.3 q	12, 13, 14
1′, 1″	11.09 br s		
	11.17 br s		
2′, 2″		156.8 (2C) s	
3′, 3″	7.85 (2H) br s		
4′, 4″	3.11 (4H) m	40.1 (2C) t	2′, 5′, 6′, 2″, 5″, 6″
5′, 5″	1.50 (4H) m	25.5 (2C) t	4′, 4″, 6′, 6″
6′, 6″	1.53 (4H) m	24.8 (2C) t	5′, 5″, 7′, 7″
7′, 7″	3.30 (4H) m	40.5 (2C) t	5′, 6′, 9′, 5″, 6″, 9″
8′, 8″	9.31 br s		
	9.41 br s		
9′, 9″		153.5 (2C) s	
2′-NH_2, 2″-NH_2	8.84 (3H) br s		
	9.01 br s		
9′-NH_2, 9″-NH_2	7.25 (8H) br s		

[a] Data recorded in DMSO-d_6. [b] Coupling constants in Hz are given. [c] Multiplicities were determined by an HMQC spectrum.

Bistellettadine A (**2**) had a molecular formula of $C_{30}H_{53}N_{12}O_2$, established by HRFABMS. The ^1H NMR spectrum recorded in DMSO-d_6 exhibited the presence of 18 exchangeable protons in the low-field region, one aliphatic methyl, three vinylic singlet methyls, and five olefinic protons (Table 1). Interpretation of the COSY and HMBC

* To whom correspondence should be addressed. Tel: +81-3-5841-5299. Fax: +81-3-5841-8166. E-mail: anobu@hongo.ecc.u-tokyo.ac.jp.

(1) Bioactive marine metabolites. 92. Part 91: Sata, N. U.; Sugano, M.; Matsunaga, S.; Fusetani, N.; van Soest, R. W. M. *J. Nat. Prod.*, in press.

(2) Tsukamoto, S.; Yamashita, T.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1999**, *40*, 737−738.

(3) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1996**, *37*, 5555−5556.

(4) A voucher specimen (ZMA POR. 13010) was deposited at the Institute for Systematics and Population Biology, University of Amsterdam, The Netherlands.

(5) Experimental data: $[\alpha]^{21}_D$ +2.0° (*c* 0.72, MeOH); UV λ_{max} (MeOH) 255 nm (ϵ 13 000); ^1H and ^{13}C NMR (DMSO-d_6) see Table 1; FABMS (positive, glycerol) *m*/*z* 613 $(M + H)^+$; HRFABMS (positive, PEG600 in NBA) *m*/*z* 613.4400 (calcd for $C_{30}H_{53}N_{12}O_2$, Δ −1.4 mmu).

(6) Experimental data: $[\alpha]^{21}_D$ +3.9° (*c* 0.10, MeOH); UV λ_{max} (MeOH) 255 nm (ϵ 11 000); ^1H NMR (DMSO-d_6) δ 1.10 (3H, s, 6-Me), 1.47 (1H, m, H-7e′), 1.49 (4H, m, H_2-5′ and H_2-5″), 1.51 (4H, m, H_2-6′ and H_2-6″), 1.58 (3H, s, H_3-19), 1.65 (1H, m, H-7a′), 1.66 (3H, s, H_3-18), 1.67 (3H, s, 9-Me), 1.84 (3H, s, 13-Me), 1.90 (3H, s, 2-Me), 2.04 (1H, m, H-15), 2.14 (1H, m, H-8), 2.27 (1H, m, H-15), 2.94 (1H, m, H-11), 3.10 (4H, m, H_2-4′ and H_2-4″), 3.28 (4H, m, H_2-7′ and H_2-7″), 5.07 (1H, t, J = 6.0 Hz, H-16), 5.22 (1H, d, J = 4.2 Hz, H-10), 6.22 (1H, d, J = 15.0 Hz, H-5), 6.33 (1H, d, J = 10.8 Hz, H-12), 6.37 (1H, dd, J = 15.0, 10.8 Hz, H-4), 7.01 (1H, d, J = 10.8 Hz, H-3), 7.20 (8H, br s, 2 × 9′-NH_2 and 2 × 9″-NH_2), 7.76 (2H, br s, H-3′ and H-3″), [8.80 (3H, br s) and 9.01 (1H, br s), 2′-NH_2 and 2″-NH_2], 9.25 (1H, br s, H-8′[b]), 9.41 (1H, br s, H-8″[b]), 10.95 (1H, br s, H-1′[a]), and 11.06 (1H, br s, H-1″[a]) ([a,b]may be interchangeable); ^{13}C NMR (DMSO-d_6) δ 12.2 (q, 2-Me), 12.3 (q, 13-Me), 17.8 (q, C19), 21.0 (q, 9-Me), 23.8 (q, 6-Me), 24.9 (2C, t, C6′ and C6″), 25.6 (q, C18), 25.6 (2C, t, C5′ and C5″), 30.1 (t, C15), 34.2 (t, C2), 36.1 (d, C8), 38.7 (s, C6), 40.2 (2C, t, C4′ and C4″), 40.5 (2C, t, C7′ and C7″), 44.6 (d, C11), 121.4 (d, C10), 121.7 (d, C16), 122.3 (d, C4), 126.8 (s, C2), 128.1 (s, C13), 132.3 (s, C17), 136.8 (s, C9), 138.7 (d, C3), 142.0 (d, C12), 153.5 (d, C5), 153.5 (2C, s, C9′ and C9″), 156.8 (2C, s, C2′ and C2″), and 169.4 (2C, s, C1 and C14); HMBC cross peaks: H-4/C6; H-10/C8 and 9-Me; H-12/C14 and 13-Me; H-16/C18 and C19; H18/C16, C17, and C19; H-19/C16, C17, and C18; 2-Me/C1, C2, and C3; 6-Me/C5, C6, C7, and C11; 9-Me/C8 and C9; 13-Me/C12, C13, and C14; H_2-4′ and H_2-4″/C2′, C5′, C6′, C2″, C5″, and C6″; H_2-5′ and H_2-5″/C6′ and C6″; H_2-6′ and H_2-6″/C5′ and C5″; H_2-7′ and H_2-7″/C5′, C6′, C9′, C5″, C6″, and C9″; FABMS (positive, glycerol) *m*/*z* 681 $(M + H)^+$; HRFABMS (positive, PEG600 in NBA) *m*/*z* 681.5076 (calcd for $C_{35}H_{61}N_{12}O_2$, Δ +3.6 mmu).

10.1021/jo9903252 CCC: $18.00
Published on Web 05/12/1999

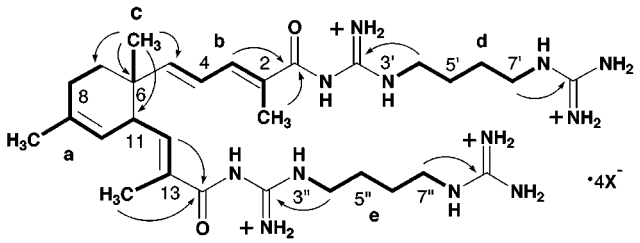


Figure 1. Partial structures **a**−**e** of bistellettadine A (**2**); bold lines and arrows show COSY and HMBC connectivities, respectively.

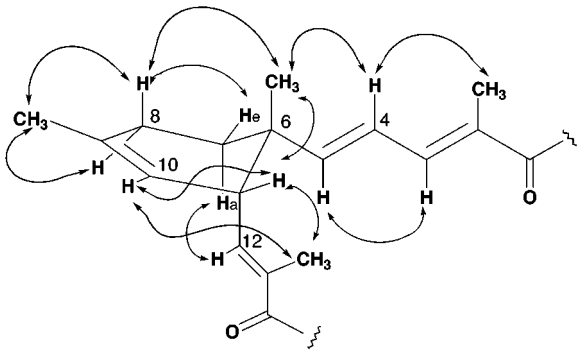


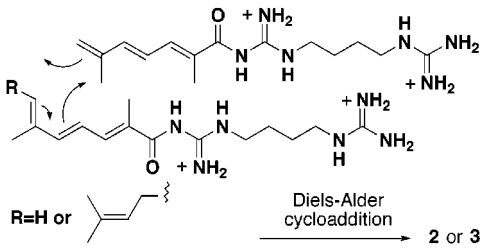
Figure 2. NOE correlations observed for bistellettadine A (**2**).

spectra led to partial structures **a**−**e** (Figure 1). Unit **a** had two trisubstituted olefins, one of which was polarized as judged from the chemical shift of δ 6.32 (H-12), while unit **b** was comprised of a polarized butadiene. Unit **c** was a methyl on a quaternary carbon, whereas units **d** and **e** shared the same 1,4-diaminobutane moiety. Analysis of the HMBC spectrum resulted in formulation of a cyclohexene ring, which incorporated units **a**−**c** (Table 1 and Figure 1); the termini of units **a** and **b** were connected to either amide or ester carbons (δ 169.6). As to the remaining portion, the presence of guanidino carbons (δ 156.8 and 153.5) together with the exchangeable proton signals and the molecular formula of **2** suggested that two 1,4-diguanidinobutane comprising units **d** and **e** formed amide linkages with units **a** and **b** as in the case of stellettadine A (**4**).[3]

12*E*-Geometry was evident from a chemical shift for 13-Me (δ 12.3)[7] and NOE cross peaks, 13-Me/H-10 and H-11; H-7_a/H-12 (Figure 2). The coupling constant ($J_{4,5}$ = 16.2 Hz), chemical shift for 2-Me (δ 12.2),[7] and NOE cross peaks, 2-Me/H-4; H-3/H-5 (Figure 2), implied 2*E*,4*E* geometry. The relative stereochemistry of the cyclohexene ring was deduced

(7) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy: High Resolution Methods and Applications in Organic Chemistry and Biochemistry*, 3rd completely revised ed.; VCH: New York, 1987; p 192.

Scheme 1



by NOE correlations, 6-Me/H-8 and H-11; H-7_a/H-12 (Figure 2), thus revealing that cyclohexene ring adopted the half-chair conformation; 6-Me and the 11-substituent were axially and pseudoaxially oriented, respectively.

Bistellettadine B (**3**) has a molecular formula of $C_{35}H_{61}N_{12}O_2$ [*m*/*z* 681 $(M + H)^+$] as established by HR-FABMS, which is larger than **2** by a C_5H_8 unit. NMR data were similar to those of **2** except for the presence of an additional isoprene unit, which could be placed at C-8 on the basis of COSY data. The remaining part of **3** was identical with **2**. NOE cross peaks, H-8/6-Me, H-7_e, and 9-Me; 6-Me/H-11 and H-4; H-4/2-Me; H-3/H-5; 13-Me/H-11 and H-10; 9-Me/H-10, H_2-15; H-7_a/H-12; H-16/H_3-18, indicated the pseudoequatorial orientation of the isopentenyl group.

Bistellettadines A (**2**) and B (**3**) are presumably generated by [4π + 2π] Diels−Alder cycloaddition of two units related to stellettadine A (**4**)[3] (Scheme 1). Relative stereochemistry of the cyclohexene moieties in **2** and **3** indicated that the reaction takes place with endo orientation. Since **2** and **3** are optically active, enzyme systems may be involved in the biosynthesis.

Bistellettadines A (**2**) and B (**3**) moderately inhibited Ca^{2+}/calmodulin-dependent phosphodiesterase (40 % inhibition at 100 μM). They inhibited the growth of yeast and of the bacterium *Escherichia coli* at 10 μg/disk.

Acknowledgment. We thank Prof. P. J. Scheuer of the University of Hawaii for reading this manuscript and Prof. A. Toh-e of the University of Tokyo for a generous supply of the yeast. Thanks are also due to Prof. H. Karaki and Dr. M. Hori, the University of Tokyo, for enzyme assays and to Dr. R. van Soest, University of Amsterdam, for identification of the sponge. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan and the Japan Society for the promoting of Science, "Research for the Future Program" (JSPS-RFTF96I00301).

Supporting Information Available: 1D and 2D NMR spectra for compounds **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9903252