Stellettamide B, a New Indolizidine Alkaloid from a Sponge of the Genus *Stelletta*

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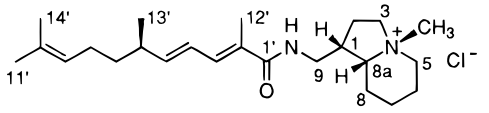
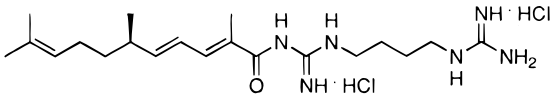
Stellettamide B and stellettadine A, two alkaloids possessing carbon skeletons of mixed biogenetic origins, have been isolated from a sponge of the genus *Stelletta*. The structure of stellettamide B, a novel indolizidine alkaloid, has been determined by combined chemical and spectral methods. These compounds exhibited moderate antifungal and RNA-cleaving activities.

In our search for bioactive substances from Korean water organisms, we encountered a sponge of the genus *Stelletta* whose organic extract exhibited moderate brine-shrimp lethality and antifungal activity. Activity-guided isolation using various chromatographic techniques yielded two alkaloids possessing carbon frameworks of mixed biogenetic origins. In this paper, we report the structure determination and bioactivities of stellettamide B (**1**), a novel compound, and stellettadine A (**2**), a very recently isolated metabolite of the same sponge collected from Japanese waters.[1] Both compounds possessed a linear norsesquiterpene moiety as a common structural feature; however, these compounds were structurally very distinct from each other. Stellettamide B contained an indolizidine skeleton identical with that of stellettamide A (**3**), a previously reported metabolite of another Japanese sponge *Stelletta* sp., while stellettadine A possessed a linear bisguanidinium unit.[2]



1



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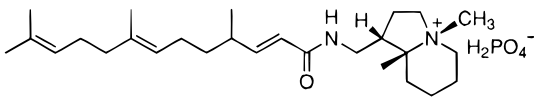

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The brown encrusting sponge (specimen no. 94K-25) was collected off the shore of Keomun Island, South Sea, Korea.[3] The specimens were exhaustively extracted with MeOH and CH_2Cl_2. After removal of salt by a partitioning of the combined crude extracts between *n*-BuOH and H_2O, the *n*-BuOH layer was repartitioned between *n*-hexane and 10% aqueous MeOH. Separation of the aqueous MeOH layer by C_{18} reversed-phase flash chromatography followed by Sephadex LH-20 column chromatography and reversed-phase HPLC of moderately polar fractions yielded pure alkaloids.

Stellettamide B (**1**) was isolated as a yellow gum that analyzed for $C_{24}H_{41}N_2O$ by a combination of HRFABMS and ^{13}C-NMR spectrometry. The presence of an amide functionality was readily recognized by a quaternary carbon signal at δ 172.37 in the ^{13}C-NMR spectrum (Table 1) and characteristic absorption bands at 3440 (br) and 1650 cm^{-1} in the IR spectrum. A UV maximum of 262 nm revealed that the amide contained at least one set of conjugated double bonds.

The structure of the indolizidine part was determined by 2D NMR experiments. Despite partial overlapping of the upfield proton signals, a combination of the ^{1}H COSY and HMQC data enabled us to define a bicyclic system as well as assignments of carbons and protons. In addition, the TOCSY data showed several long-range correlations in which the key correlations were observed between the H-3α (δ 3.90) and H-8a (δ 3.67), H-3 (δ 3.90 and 3.28) and H-9 (δ 3.44), and H-5α (δ 3.58) and H-8a (δ 3.67). This interpretation was further supported by HMBC correlations between the H-3, H-8a, H-9, and 4-Me protons and neighboring carbons (Table 1).

The structure of the remaining norsesquiterpene unit was also determined by a combination of the 2D NMR techniques including ^{1}H-COSY, HMQC, and HMBC experiments. In particular, it was possible to define the spin system throughout the entire chain by the TOCSY data in which overlapping correlations among all of the protons between H-3′ and Me-13′, H-5′ and Me-12′, H-5′ and H-7′, H-6′ and H-9′, and Me-11′ (also Me-14′) and Me-13′ were clearly observed. The geometry of two asymmetric double bonds at C-2′ and C-4′ was assigned as 2′*E*,4′*E* by NOESY correlations (H-3′ and H-5′, H-4′ and Me-12′) and measurement of the proton−proton coupling constant ($J_{4',5'}$ = 15.1 Hz).

Although the connection of the indolizidine with norsesquiterpene parts was not directly observed from the HMBC experiments (i.e., H-9 - C′-1), the downfield chemical shifts of the H-9 (δ 3.44 and 3.32) and the molecular formula suggested the connection of partial structures by an amide bond in **1**. The quaternary ammonium (N-4) of **1** indicated the presence of a counterion. This anion was determined as a chloride

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Table 1. NMR Assignments for Stellettamide B (**1**)[a]

no.	H	C	HMBC[b]
1	3.16 (1 H, m)	40.5	
2	2.40 (1 H, m), 2.00 (1 H, m)	25.1	
3	3.90 (1 H, ddd, 11.7, 11.7, 7.3)	57.6	2, 4-Me
	3.28 (1 H, m)		
5	3.58 (1 H, br d, 13.5)	61.0	
	3.40 (1 H, ddd, 13.5, 13.2, 3.9)		
6	1.89 (1 H, m), 1.85 (1 H, m)	21.1	
7	2.00 (1 H, m), 1.53 (1 H, br d, 13.2)	21.6	
8	1.94 (1 H, m), 1.59 (1 H, m)	23.3	
8a	3.67 (1 H, m)	74.0	2, 3
9	3.44 (1 H, dd, 13.2, 7.3)	40.1	1, 2
	3.32 (1 H, dd, 13.2, 6.9)		8a
4-Me	3.14 (3 H, s)	54.3	3, 5, 8a
1′		172.4	
2′		129.0	
3′	6.83 (1 H, br d, 11.2)	135.6	1′, 5′, 12′
4′	6.35 (1 H, ddd, 15.1, 11.2, 1.0)	125.39	2′, 3′, 6′
5′	5.90 (1 H, dd, 15.1, 8.3)	148.5	3′, 7′, 13′
6′	2.28 (1 H, m)	38.3	4′, 5′, 7′, 8′, 13′
7′	1.36 (2 H, m)	38.0	
8′	1.94 (2 H, m)	26.9	
9′	5.09 (1 H, br t, 7.1)	125.41	
10′		132.3	
11′	1.67 (3 H, br s)	25.9	
12′	1.92 (3 H, d, 1.0)	12.9	2′, 3′
13′	1.03 (3 H, d, 6.4)	20.8	5′, 6′
14′	1.58 (3 H, brs)	17.8	9′, 10′, 11′

[a] ^{1}H- and ^{13}C- NMR spectra were obtained in CD_3OD solutions at 500 and 125 MHz, respectively. Assignments were aided by COSY, TOCSY, DEPT, HMQC, and HMBC experiments. [b] Parameters were optimized for 7 Hz CH coupling constants.

by an energy-dispersive spectroscopic experiment performed on a scanning electron microscope.

The indolizidine system of **1** possessed asymmetric centers at C-1, N-4, and C-8a. Configurations of these centers were determined by NOESY experiments. The NCH_3 protons exhibited correlations with the H-3β (δ 3.28) and H-5β (δ 3.40) protons, while the H-1 proton showed strong correlations with the H-9 protons. Due to the proximity of signals of H-1 (δ 3.16) and NCH_3 (δ 3.14) in the ^{1}H-NMR spectrum, however, the key correlations among the H-1, H-8a, and NCH_3 protons were not clear. This problem was solved by performing the NOESY experiment in Me_2CO-d_6 in which the mutual correlations among these protons were clearly observed (H-1a; δ 3.56, H-8a; δ 4.10, N-CH_3; δ 3.37). Thus, the relative configurations were defined as 1*S**,4*S**,8a*R**.

In addition to the indolizidine system, compound **1** possessed an asymmetric carbon center at C-6′. The absolute configuration of this center was determined using oxidation to 2-methylglutaric acid, a method adopted for the structure determination of stellettadine A.[1,4] Treatment of **1** with $NaIO_4$ in the presence of $RuCl_3 \cdot xH_2O$ as a catalyst yielded (*S*)-2-methylglutaric acid that was confirmed by comparison of the ^{1}H-NMR and GC analysis data with an authentic sample and measurement of optical rotation ($[\alpha]^{25}_D$ +17.5° (lit.[1] $[\alpha]^{25}_D$ +22°)). Thus, the structure of stellettamide B was unambiguously determined as an alkaloid consisting of a norsesquiterpene and an indolizidine unit. Compounds possessing the indolizidine system have been rather rarely isolated from marine organisms. To the best of our knowledge, this indolizidine skeleton of stellettamide B has been precedented only by stellettamide A from the sponge *Stelletta* sp. and piclavines from the tunicate *Clavelina picta*.[6,7]

Compound **2** was isolated as a yellow gum that analyzed for $C_{20}H_{36}N_6O$ by a combination of HRFABMS and ^{13}C-NMR spectrometry. NMR analysis of **2** showed that this compound possessed the identical norsesquiterpene unit as **1**; however, the indolizidine system of **1** is replaced by a highly symmetric C_6 unit, both termini consisting of carbons bearing heteroatoms (δ 158.58 and 155.68 in the ^{13}C-NMR spectrum). The molecular formula revealed that these were carbons of guanidines (or guanidiniums). A 20-nm bathochromic shift of UV maximum in **2** supported the attachment of a guanidine to the carbonyl carbon of the norsesquiterpene unit. Thus, **2** is an alkaloid possessing a linear bisguanidino unit. A literature survey revealed that **2** was identical with stellettadine A recently isolated from the sponge *Stelletta* sp.[1] Comparison of spectral data showed very good correlation with published data for this compound.[8] Stellettadine A and stellettamide A were reported to exhibit various bioactivities; the former induced larval metamorphosis of the ascidian *Halocynthia roretzi*, the latter was cytotoxic and antifungal. In our measurement of antifungal activity using paper-disk method, stellettamide B (**1**) and stellettadine A (**2**) were moderately active against *Candida albicans* (inhibition zone 4 and 9 mm for **1** and **2**, respectively) at the concentration of 25 μg/mL.

In addition to the antifungal activity, a very interesting biochemical action of **1** and **2** was found. In a measurement of reverse-transcriptase-inhibiting activity using agarose-gel electrophoresis, we found that the substrate RNA was totally digested. Subsequently, in a similar test using pure rRNAs and mRNA, stellettadine A and stellettamide B cleaved both single- and double-stranded RNA at the concentration of 50 μg/mL. The mechanism of this activity is currently under investigation, and the results will be published in due course.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in CD_3OD and $(CD_3)_2CO$ solutions on a Varian Unity 500 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me_4Si. UV spectra were obtained in MeOH using a Milton-Roy spectrophotometer. IR spectra were recorded on a Mattson GALAXY spectrophotometer. Mass spectra were obtained by using a VG ZAB-2FHF high resolution mass spectrometer and provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. The FABMS data were obtained by adding the sample dissolved in MeOH to NBA (nitrobenzyl alcohol) matrix. The optical rotations were measured on a JASCO digital polarimeter using a 5-cm cell. Energy dispersive spectra were obtained by electron bombardments at 30 kV on a Philips 515 scanning electron microscope. GC analysis was performed on a Hewlett-Packard HP 5890 II gas chromatograph using an Ω-wax-320 column. Temperatures of injector, detector, and oven were 280, 280, and 180 °C, respectively. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The specimens of *Stelletta* sp. (order Astrophorida, family Stellettidae) were collected by hand using scuba at 20−30 m depth in August 1994, along the offshore of Keomun Island, South Sea, Korea.[3] This sponge was massive (2-cm thick), and the color was

dark brown. The specimens were firm and slightly compressible. The skeleton was formed with large oxea (780−1250 × 15−32 μm) and plagiotriaene (450−750 × 20−48 μm) arranged compactly at the sponge surface, large oxyaster (30−50 μm in diameter), and small oxyaster (7.5−20 μm in diameter). This sponge was similar to *S. grubii* Schmidt in its spicule type;[9] however, our specimens had shorter megascleres than *S. grubii* (oxea 2000−2200 × 60 μm, plagiotriaene 1200 × 60 μm). In addition, large and small oxyasters had microspines at the end of the sharply pointed ray.

Extraction and Isolation. The samples were immediately frozen and kept at −25 °C until chemically investigated. The sponge (0.9 kg, wet wt) was defrosted, macerated, and extracted with MeOH (2 L × 2) and CH_2Cl_2 (2 L × 2). The combined extracts (29.1 g) were partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer was dried under vacuum (6.4 g) and repartitioned between *n*-hexane and 10% aqueous MeOH. The aqueous MeOH layer (3.7 g) was dried and separated by C_{18} reversed-phase vacuum flash chromatography (YMC ODS A 60-I25 gel) by using sequential mixtures of H_2O and MeOH as eluents (10, 20, 30, 50% aqueous MeOH, and MeOH). The fractions eluted with 10% and 20% aqueous MeOH were combined and subjected to Sephadex LH-20 column chromatography (100% MeOH). Fractions containing alkaloids were combined and separated by C_{18} reversed-phase HPLC (Shiseido Capcell column, 25% aqueous MeOH). Final purifications were made by C_{18} reversed-phase HPLC (YMC ODS-H80 column, 35% aqueous MeOH) to yield 106 mg of **1** and 21 mg of **2**.

Stellettamide B (1): a yellow gum; $[\alpha]^{25}_D$ −24.2° (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 262 (4.20) nm; IR (KBr) ν_{max} 3440 (br), 2930, 1650, 1535, 1450, 1380, 975 cm^{-1}; HRFABMS $[M + H]^+$ *m/z* 373.3210, calcd for $C_{24}H_{41}N_2O$ 373.3219.

Stellettadine A (2): a yellow gum; $[\alpha]^{25}_D$ −25.3° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 282 (4.02) nm; IR (KBr) ν_{max} 3400 (br), 2960, 1630, 1460, 1380, 1250, 1100, 980 cm^{-1}; HRFABMS $[M + H]^+$ *m/z* 377.3012, calcd for $C_{20}H_{37}N_6O$ 377.3029.

Oxidative Cleavage of Stellettamide B (1). To a biphasic solution of 6.6 mg (0.016 mmol) of **1** and 41.3 mg (0.192 mmol) of $NaIO_4$ in a mixture of 1 mL of CCl_4, 1 mL of CH_3CN, and 1.5 mL of H_2O was added 21.5 mg (0.104 mmol) of $RuCl_3 \cdot xH_2O$. After vigorous stirring the mixture for 2 h at room temperature, the solvents were removed under vacuum. The residue was redissolved in MeOH and filtered on an ODS Cepak column. The filtrate was dried and separated by reversed-phase HPLC (YMC ODS column, 5% aqueous MeOH) to give 1.1 mg of 2-methylglutaric acid (48% yield): $[\alpha]^{25}_D$ +17.5° (*c* 0.08, MeOH) [lit.[1] $[\alpha]^{25}_D$ +24.4° (*c* 0.5% in $CHCl_3$), lit.[5] $[\alpha]^{25}_D$ +22° (*c* 0.033 in MeOH)]; ^1H NMR ($CDCl_3$) δ 2.45 (1 H, m), 2.32 (2 H, m), 1.89 (1 H, m), 1.72 (1 H, m), 1.16 (3 H, d, J = 6.8 Hz). The synthetic 2-methylglutaric acid was dissolved in 1 mL of 5% HCl−MeOH and left overnight at room temperature. After removing the solvent under vacuum, the residue was redissolved and analyzed on a gas chromatograph. Dimethyl 2-methylglutarate had a retention time of 4.355 min. An authentic compound formed by the same method from (±) 2-methylglutaric acid (Aldrich) gave a peak at 4.357 min.

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