Three New Antibacterial Alkaloids from a Marine Sponge *Stelletta* Species[1]

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From a marine sponge of the genus *Stelletta* we isolated three alkaloids, stellettazole B (**3**), stellettazole C (**4**), and stellettamide C (**5**), which were antibacterial against *Escherichia coli*. Their structures were determined on the basis of spectral and chemical methods.

Secondary metabolites of sponges of the genus *Stelletta* can be classified into two groups: less polar isomalabaricane triterpenes[2−5] and highly polar nitrogenous terpenoids.[6−11] We found antimicrobial activity in the polar fraction of the MeOH extract of a *Stelletta* sp. collected off Shikine-jima Island (34°19′25″ N, 139°13′19″ E) from which we isolated stellettazole A (**1**)[9] and bistellettadines,[10] together with stellettamide A (**2**).[6] Further fractionation of the extract afforded three related alkaloids, two of which possess a geranylgeranoyl moiety.

The MeOH extract of the sponge was subjected to solvent partitioning to furnish a *n*-BuOH fraction that inhibited growth of *E. coli*. Fractionation by ODS flash chromatography, gel-permeation chromatography, and ODS HPLC afforded stellettazole B (**3**), stellettazole C (**4**), and stellettamide C (**5**) in yields of 0.04%, 0.02%, and 0.01%, respectively.

Stellettazole B (**3**), having a molecular formula of $C_{30}H_{52}N_6O$ as established by HRFABMS, displayed a ^{1}H NMR spectrum similar to that of stellettazole A (**1**),[9] except for methyl and olefinic signals; the secondary methyl in **1** was missing, although two additional olefinic methyls were observed. Instead of mutually coupled olefinic protons in **1**, a singlet olefinic proton (δ 5.60) was detected (Table 1). The ^{13}C NMR spectrum also reflected the changes in the terpenoid portion (Table 1). Interpretation of 2D NMR data, including COSY, HMQC, and HMBC spectra, revealed that stellettazole B (**3**) contained the imidazole-containing nitrogenous unit in stellettazole A (**1**) connected to a geranylgeranoyl group through an amide linkage.

Stellettazole C (**4**) had a molecular formula of $C_{29}H_{49}N_4O$ as established by HRFABMS. The ^{1}H NMR spectrum was almost superimposable on that of **3**, except for the absence of the exchangeable signals arising from the guanidino group. The molecular formula together with 2D NMR data implied that stellettazole C (**4**) had an aminopropyl group in place of the guanidinopropyl unit in **3**. When stellettazole C (**4**) was acetylated with Ac_2O it yielded an acetamide (**6**), in which a newly generated amide proton at 7.98 ppm was coupled to H-11′ in the COSY spectrum.

Stellettamide C (**5**) not only exhibited the ^{1}H NMR spectrum quite similar to that of stellettamide A (**2**), but also had the same molecular formula. Interpretation of 2D NMR data disclosed that stellettamide C (**5**) differed from stellettamide A (**2**) only in the location of a double bond; $\Delta^{3,4}$ in **5** as opposed to $\Delta^{2,3}$ in **2**. NMR data for the relevant portion are as follows: δ_C 170.8 (C-1′), 35.0 (C-2′), 117.9 (C-3′), 136.8 (C-4′), 39.1 (C-5′), 16.1 (C-4′-Me); δ_H 2.80 (2H, d, J = 7.3 Hz; H_2-2), 5.24 (1H, t, J = 7.3 Hz; H-3′), 1.58 (3H, s; 4′-Me). The similarity of ^{1}H and ^{13}C NMR signals for the *N*-methylindolizidine moiety in both compounds suggested identical relative stereochemistry of the ring portion. This was supported by the NOESY spectrum (Figure 1), which showed cross-peaks between the *N*-methyl signal and protons at δ 2.86 (H-1) and 3.58 (H-8a). Therefore, the *N*-methyl group, H-1, and H-8a could be placed on the same face of the bicyclic system. The absolute stereochemistry of **5** was assigned by chemical transformation. Hydrogenation of stellettamide A (**2**) followed by acid hydrolysis yielded **7**, whose absolute stereochemistry had been secured by total synthesis of **2**.[14] Compound **7** was converted to **8** and **9** by reacting with L-FDAA and D-FDAA, respectively[15,16] (FDAA = 1-fluoro-2,4-dinitrophenyl-5-L-

Table 1. ^{1}H and ^{13}C NMR Data of Stellettazole B (**3**) and Stellettazole C (**4**) in DMSO-d_6

	3		**4**	
atom	δ_C	δ_H	δ_C	δ_H
1	166.4		166.4	
2	118.2	5.60 s	118.2	5.60 s
3	152.1		152.4	
4	40.0	2.04 m	40.0	2.04 m
5	25.6	2.09 m	25.7	2.08 m
6	123.2	5.08 t (6.9)[a]	123.2	5.08 t (6.9)
7	135.0		135.0	
8	39.0	1.94 m	39.3	1.94 m
9	26.1	2.02 m	25.9	2.03 m
10	123.6	5.06 m	123.8	5.06 m
11	134.0		134.4	
12	39.0	1.92 m	39.3	1.93 m
13	26.1	2.01 m	26.5	2.01 m
14	124.0	5.05 m	124.2	5.05 m
15	130.8		130.7	
16	25.2	1.62 s	25.5	1.62 s
3-Me	17.5	2.04 s	17.8	2.04 s
7-Me	15.6	1.57 s	15.8	1.56 s
11-Me	15.6	1.55 s	15.8	1.54 s
15-Me	17.4	1.55 s	17.5	1.54 s
1′		7.89 t (5.8)		7.94 t (5.8)
2′	36.2	3.35 m	36.2	3.34 m
3′	23.5	2.78 t (6.9)	23.5	2.78 t (6.9)
4′	133.2		133.4	
5′	119.1	7.54 s	119.1	7.55 s
6′				
7′	136.2	8.98 s	136.4	9.00 s
8′				
9′	46.2	4.12 t (7.1)	46.0	4.18 t (6.9)
10′	28.8	1.98 m	27.7	2.03 m
11′	37.8	3.12 dq (6.5,6.5)	35.8	2.78 t (6.9)
12′		7.47 s		
13′	156.8			
8′-Me	33.2	3.77 s	33.3	3.77 s

[a] Coupling constant in Hertz.

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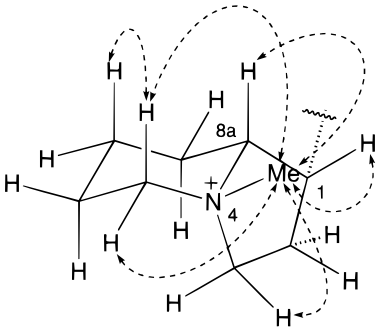
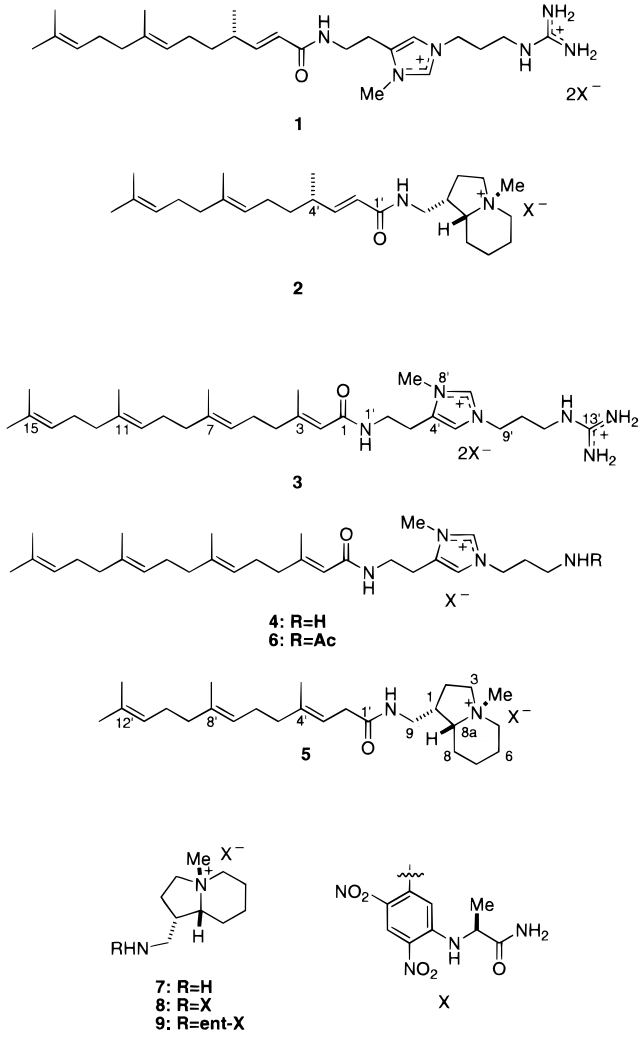


Figure 1. NOESY correlations of indolizidine ring of stellettamide C (**5**).

alaninamide). Compounds **8** and **9** exhibited different retention times in reversed-phase HPLC, thereby confirming that the enantiomers of **7** can be distinguished by this strategy. Stellettamide C (**5**) was hydrogenated, followed by acid hydrolysis, to afford **7**, which gave rise to the same ^{1}H NMR spectrum as authentic **7** prepared from **2**. Compound **7** from **5** was converted to the L-FDAA derivative, which exhibited a peak coeluting with **8** in HPLC. Consequently, the absolute stereochemistry of the indolizidine moiety in stellettamide C (**5**) is identical with that in stellettamide A (**2**).



Stellettazole B (**3**), stellettazole C (**4**), and stellettamide C (**5**) were marginally antibacterial against *Escherichia coli* (6.1, 6.2, and 10.5 mm inhibitory zones at 20 μg/6 mm ϕ disk, respectively).

Experimental Section[17]

Animal Material. The sponge sample was collected off Shikine-jima Island, 200 km south of Tokyo. The sponge was identified as a *Stelletta* sp. by Dr. Rob van Soest.[10] A voucher specimen (ZMA POR. 13010) was deposited at the Institute for Systematics and Ecology, University of Amsterdam, The Netherlands.

Extraction and Isolation. The frozen sponge (100 g) was extracted with MeOH, and the combined extracts were concentrated and partitioned between ether and H_2O. The aqueous phase was further extracted with *n*-BuOH; the *n*-BuOH layer was subjected to ODS column chromatography using stepwise elution from MeOH−H_2O (4:6) to 100% MeOH. The fractions eluted with MeOH−H_2O (4:6) and MeOH−H_2O (6:4) were combined and separated by gel permeation on Sephadex LH-20 with MeOH, followed by ODS HPLC with MeCN−300 mM $NaClO_4$ in H_2O (64:36) to yield stellettazole B (**3**; 40.0 mg, 0.04% of wet wt), stellettazole C (**4**; 20.0 mg, 0.02% of wet wt), and stellettamide C (**5**; 10.0 mg, 0.01% of wet wt).

Stellettazole B (3): colorless amorphous solid; UV (MeOH) no absorption above 220 nm; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 511.4140 [M + H]$^+$ ($C_{30}H_{51}N_6O$ Δ +1.6 mmu).

Stellettazole C (4): colorless amorphous solid; UV (MeOH) no absorption above 220 nm; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 469.3899 [M + H]$^+$ ($C_{29}H_{49}N_4O$ Δ −0.8 mmu).

Stellettamide C (5): colorless amorphous solid; $[\alpha]^{23}_D$ +1.1° (*c* 0.32, MeOH); UV (MeOH) no absorption above 220 nm; ^{1}H and ^{13}C NMR data, see Table 1 of Supporting Information; HRFABMS *m/z* 401.3531 [M]$^+$ ($C_{26}H_{45}N_2O$ Δ −0.1 mmu).

Acetylation of Stellettazole C (4). A 1-mg portion of stellettazole C (**4**) was dissolved in a mixture of Ac_2O and pyridine (1:1, 1 mL) and stirred at room temperature for 1 h. The solvent was removed in vacuo to afford acetamide **6** in quantitative yield. Selected ^{1}H NMR signals for **6** (DMSO-d_6): δ 9.06 (1H, s), 7.99 (1H, m), 7.98 (1H, m), 7.59 (1H, s), 5.60 (1H, s), 5.06 (3H, m), 3.76 (3H, s), 2.06 (3H, s), 1.81 (3H, s), 1.62 (3H, s), 1.56 (3H, s), 1.54 (6H, s).

Degradation of Stellettamide A (1) and Stellettamide C (5). To a solution of stellettamide A (**1**) (9.0 mg) in EtOH (1.0 mL) was added Pd−C (5.0 mg), and the mixture was stirred under a H_2 atmosphere at room temperature for 1 h. After removal of Pd−C by filtration the solution was evaporated, redissolved in 3N HCl (0.6 mL), and heated at 105 °C for 4 h. The reaction mixture was extracted with EtOAc to remove the lipophilic carboxylic acid, and the aqueous layer was evaporated to afford **7**.

Compound 7: ^{1}H NMR (D_2O) δ 3.82 (1H, m), 3.68 (1H, m), 3.51 (1H, m), 3.30 (2H, m), 3.24 (1H, m), 3.16 (1H, m), 3.05 (3H, s), 3.02 (2H, m), 2.45 (1H, m), 2.00 (1H, m), 1.7−1.8 (3H, m), 1.45 (1H, m), 1.40 (1H, m).

A one-tenth portion of **7** was reacted with a 0.1% solution of L-FDAA (0.1 mL) at 50 °C for 30 min to furnish **8**. Another portion was reacted with D-FDAA to give **9**. Each product was diluted with H_2O−MeCN (1:1) and analyzed by ODS−HPLC: stationary phase, ODS, 5 μm, 4.6 × 250 mm; mobile phase MeCN−H_2O−TFA (10:90:0.05) for 10 min and linear gradient to MeCN−H_2O−TFA (30:70:0.05) in 25 min. Compounds **8** and **9** gave retention times of 32.2 and 32.4 min, respectively. Stellettamide C (1.0 mg) was reacted in the same way to provide **7** indistinguishable from that prepared from **2**; derivatization with L-FDAA showed a peak at 32.2 min in the HPLC. This was further confirmed by co-injection analysis.

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Supporting Information Available: Table of the ^{1}H and ^{13}C NMR data of stelletamide C. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) For general experimental procedures, see Tsukamoto et al.[10]

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