doi:10.1246/cl.2011.186

Published on the web January 15, 2011

Stellettazole D, a Cytotoxic Imidazole Alkaloid from the Marine Sponge *Jaspis duoaster*

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A new cytotoxic imidazole alkaloid, stellettazole D, containing norsesquiterpene and aminopropylimidazolium units, has been isolated from the marine sponge *Jaspis duoaster*. The structure of stellettazole D was assigned on the basis of NMR, mass spectrometry, and IR spectral data. Stellettazole D has moderate cytotoxicity against a limited number of tumor cells.

Marine benthic invertebrates, mainly sponges, are a rich source of natural products with unique structures and interesting biological properties.[1] In our continuing search for biologically active metabolites from Japanese marine invertebrates,[2–4] we found a response to the ninhydrin reagent on TLC of the methanolic extract of a marine sponge, *Jaspis duoaster*, collected at Cape Sada in Ehime prefecture, Japan. Ninhydrin response-guided isolation yielded a new stellettazole-type alkaloid, stellettazole D (**1**),[5] together with the known compounds stellettadine A (**2**)[6] and stellettamide B (**3**) (Figure 1).[7] Stellettazole D features an imidazole-containing nitrogenous unit similar to those of other stellettazoles,[8] previously reported from another sponge *Stelletta* sp. This unit is connected to the norsesquiterpene structure through an amide linkage. We report the isolation and structural elucidation of stellettazole D (**1**), a new stellettazole-type alkaloid with a norsesquiterpene group.

Frozen sponges (1.8 kg) were cut into pieces and extracted with MeOH for five hours at room temperature. After evaporation of the solvent, the resulting aqueous residue was extracted with EtOAc. The EtOAc layer was concentrated by vacuum evaporation, and the organic residue was partitioned between 75% aq. MeOH and *n*-hexane. The aqueous MeOH layer was fractionated by C-18 reverse-phase HPLC[9] with MeOH/H_2O/HCO_2H (9/1/0.002) to obtain impure stellettazole D (retention time: 8.60–9.32 min). Finally, preparative TLC with $CHCl_3$–MeOH (4/1) as the eluent yielded pure stellettazole D (**1**, 15.1 mg) as yellow amorphous solid.

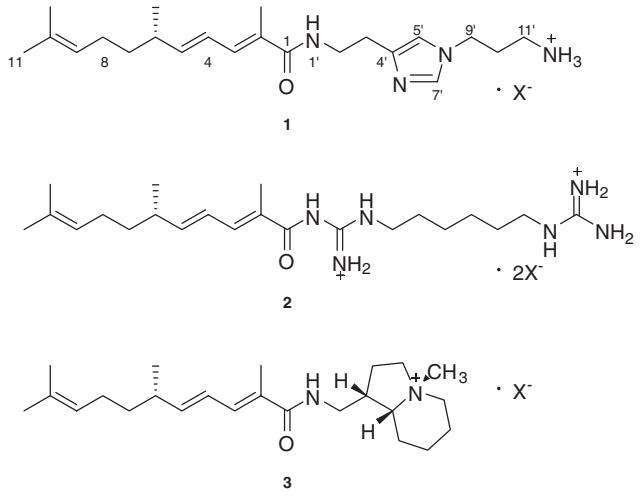

Figure 1. Bioactive alkaloids from the marine sponge *Jaspis duoaster*.

Stellettazole D (**1**), $[\alpha]_D^{25}$ +6.1° (*c* 0.10, MeOH), has a molecular formula of $C_{22}H_{37}N_4O$, as established by HRESI-TOF-MS[10] [*m*/*z* 373.2957 $(M)^+$, Δ −0.5 mmu] and ^{13}C NMR spectroscopy. The presence of an amide functionality was readily recognized by the quaternary carbon signal at δ 171.0 in the ^{13}C NMR spectrum (Table 1) and by the characteristic absorption bands at 3346 (br) and 1652 cm^{-1} in the IR spectrum. The UV maximum absorption at 263 nm revealed at least one set of conjugated double bonds in **1**.[7]

Table 1. NMR assignments for stellettazole D (**1**)

No.	δ_H[a], H, mult (*J*[b])	δ_C[c]	CH[d]	HMBC
1	—	171.0		
2	—	127.9		
3	6.84, 1H, d (11.0)	134.0	CH	1, 5, 2-Me
4	6.44, 1H, dd (15.0, 11.0)	124.2	CH	2, 3, 6
5	5.93, 1H, dd (15.0, 8.3)	146.9	CH	3, 7, 6-Me
6	2.32, 1H, dtq (8.3, 7.1, 6.7)	36.9	CH	4, 5, 7, 8, 6-Me
7	1.39, 2H, dt (7.1, 6.2)	36.7	CH_2	
8	2.04, 2H, td (6.2, 7.2)	25.5	CH_2	10
9	5.12, 1H, t (7.2)	124.1	CH	10, 11, 11-Me
10	—	139.3		
11	1.68, 3H, s	24.5	CH_3	
2-Me	1.93, 3H, s	11.5	CH_3	2, 3
6-Me	1.06, 3H, d (6.7)	19.4	CH_3	5, 6
10-Me	1.70, 3H, s	22.4	CH_3	9, 10, 11
1′	7.91[e], 1H, br s			1
2′	2.88, 2H, m	41.0	CH_2	
3′	2.69, 2H, t (6.7)	30.8	CH_2	2′, 4′, 5′
4′	—	131.0		
5′	6.96, 1H, s	116.2	CH	4′, 7′
6′	—	—		
7′	7.61, 1H, s	136.8	CH	4′, 5′
8′	—	—		
9′	4.02, 2H, t (6.7)	44.4	CH_2	5′, 7′, 10′, 11′
10′	2.07, 2H, tt (6.7, 6.7)	30.5	CH_2	9′, 11′
11′	3.27, 2H, t (6.7)	36.6	CH_2	9′, 10′

[a]400 MHz. [b]Coupling constants in Hz. [c]100 MHz. [d]CH assignments by gsHSQC-edited experiments. [e]Measured in DMSO-d_6.

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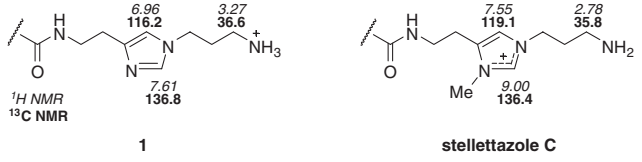

Figure 2. NMR chemical shifts of cationic moiety.

The ^{1}H NMR spectrum exhibited a spin system corresponding to disubstituted ethylamine (from H-1′ to H_2-3′), monosubstituted propylamine (from H_2-9′ to H_2-12′), and mutually coupled heteroaromatic protons (H-5′ and H-7′). HMBC cross peaks (Table 1) revealed that the heteroaromatic protons are incorporated into an imidazolium ring, which is substituted by both ethyl and propyl groups [H-5′ (δ 6.96)/C-4′ (δ 131.0) and C-7′ (δ 136.8); H-7′ (δ 7.61)/C-4′ and C-5′ (δ 116.2); H_2-3′/C-4′ and C-5′; H_2-9′ (δ 4.02)/C-5′ and C-7′]. Acetylation of stellettazole D (**1**) with Ac_2O yielded acetamide **4**.[11] A newly generated amide proton at δ 7.99 (H-12′) coupled to H-11′ (δ 3.25) indicated that stellettazole D (**1**) has an aminopropyl group.

The remaining norsesquiterpene unit was identified by a combination of 2D NMR techniques including ^{1}H–^{1}H COSY, edited-gsHSQC,[12] and gHMBC experiments, the same as for stellettamide B (**3**) (Table 1). The geometry of the two asymmetric double bonds at C-2 and C-4 was assigned as 2*E*,4*E* by NOESY correlations [H-3 (δ 6.84)/H-5 (δ 5.93), H-4 (δ 6.44)/2-Me (δ 1.93)] and the proton–proton coupling constant ($J_{4,5}$ = 15.0 Hz). To elucidate the stereochemistry at C-6, stellettazole D (**1**) was treated with $NaIO_4$ in the presence of $RuCl_3$[13] to generate (*S*)-2-methylglutaric acid[14] ($[\alpha]_D^{25}$ +24°, *c* 0.042), thereby determining 6*S*-stereochemistry. Finally, the norsesquiterpene and aminopropylimidazolium units were determined to be connected via an amide linkage on the basis of HMBC cross peaks [H-3 (δ 6.84), 2-Me (δ 1.93) and H-1′ (δ 7.91)/C1 (δ 171.0)].

Interestingly, the cationic position of stellettazole D (**1**) was different from stellettazole C, on the basis of the chemical shifts of the imidazole and the terminal amide showing a typical change by cation influence (Figure 2).[15]

Stellettazole D (**1**) was moderately cytotoxic against the murine leukemia cell line P388 and against HeLa cells (IC_{50}: 29.1 and 83.6 μg mL^{-1}, respectively). To our knowledge, this is the first report of a stellettazole-type alkaloid from a marine sponge other than *Stelletta* spp.[16] This may suggest that symbiotic bacteria in the marine sponge are the true producers of stelletta-type alkaloids, such as stellettazoles, stellettadines, and stellettamides. Further studies on the biosynthetic pathway and biological activity of stellettazole D (**1**) are in progress.

We are grateful to Dr. K. Yamada (Keio University) for biological testing. We thank Dr. P. R. Bergquist (University of Auckland) for the identification of the sponge. We wish to thank Ms. W. Seki (Nippon Suisan Kaisha, Ltd.) for her technical support.

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5 Stellettazole D (**1**): yellow amorphous solid; $[\alpha]_D^{25}$ +6.1° (*c* 0.10, MeOH), UV (MeOH): λ_{max} (log ε) 263 (4.03) nm; IR (KBr): ν_{max} 3346, 2964, 2928, 2871, 1652, 1598, 1537, 1449, 1386, 1310, 1247, 1135, 973 cm^{-1}; HRESI-TOF-MS: $[M]^+$ *m*/*z* 373.2957, calculated for $C_{22}H_{37}N_4O$ 373.2962.
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10 HRESI-TOF-MS data were recorded on a Waters LCT-Premier XE mass spectrometer using positive electronspray ionization with a Waters ACQUITY Ultra Performance LC system. NMR spectra were recorded on a Bruker Avance DPX400 spectrometer. The ^{1}H and ^{13}C chemical shifts were referenced to the CD_3OD solvent signals at δ_H 3.33 and δ_C 49.0 or the DMSO-d_6 solvent signals at δ_H 2.50 and δ_C 39.5. IR and UV spectra were recorded on a JASCO FTIR VALOR-III spectrometer and a JASCO V-560 spectrometer, respectively.
11 Stellettazole D (**1**, 0.5 mg) 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 generate the acetamide **4** in quantitative yield. ^{1}H NMR signals for **4** (DMSO-d_6): δ 7.99 (1H, br s, H-12′), 7.95 (1H, br s, H-1′), 7.67 (1H, s, H-7′), 7.01 (1H, s, H-5′), 6.84 (1H, d, *J* = 11.1 Hz, H-3), 6.44 (1H, dd, *J* = 15.0, 11.1 Hz, H-4), 5.93 (1H, dd, *J* = 15.0, 8.3 Hz, H-5), 5.12 (1H, *J* = 7.2 Hz, H-9), 4.01 (2H, t, *J* = 6.7 Hz, H_2-9′), 3.25 (2H, br t, *J* = 6.7 Hz, H_2-11′), 2.88 (2H, m, H_2-2′), 2.69 (2H, t, *J* = 6.7 Hz, H_2-3′), 2.32 (1H, dtq, *J* = 8.3, 7.1, 6.7 Hz, H-6), 2.28 (3H, s), 2.08 (2H, tt, *J* = 6.7, 6.7 Hz, H_2-10′), 2.04 (2H, dt, *J* = 7.2, 6.2 Hz, H_2-8), 1.94 (3H, s, 2-Me), 1.74 (3H, s, 10-Me), 1.68 (3H, s, H_3-11), 1.39 (2H, dt, *J* = 7.1, 6.2 Hz, H_2-7), 1.07 (3H, d, *J* = 6.7 Hz, 6-Me).
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