

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

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A new cytotoxic imidazole alkaloid, stelletazole D, containing norsesquiterpene and aminopropylimidazolium units, has been isolated from the marine sponge *Jaspis duoaster*. The structure of stelletazole D was assigned on the basis of NMR, mass spectrometry, and IR spectral data. Stelletazole 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 stelletazole-type alkaloid, stelletazole D (**1**),⁵ together with the known compounds stelletadine A (**2**)⁶ and stelletamide B (**3**) (Figure 1).⁷ Stelletazole D features an imidazole-containing nitrogenous unit similar to those of other stelletazoles,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 stelletazole D (**1**), a new stelletazole-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 HPLC⁹ with MeOH/H₂O/HCO₂H (9/1/0.002) to obtain impure stelletazole D (retention time: 8.60–9.32 min). Finally, preparative TLC with CHCl₃–MeOH (4/1) as the eluent yielded pure stelletazole D (**1**, 15.1 mg) as yellow amorphous solid.

Stelletazole D (**1**), [α]_D²⁵ +6.1° (*c* 0.10, MeOH), has a molecular formula of C₂₂H₃₇N₄O, as established by HRESI-TOF-MS¹⁰ [*m/z* 373.2957 (M)⁺, Δ –0.5 mmu] and ¹³C NMR spectroscopy. The presence of an amide functionality was readily recognized by the quaternary carbon signal at δ 171.0 in the ¹³C 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 stelletazole D (**1**)

No.	$\delta_{\text{H}}^{\text{a}}$, H, mult (J^{b})	$\delta_{\text{C}}^{\text{c}}$	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'

^a400 MHz. ^bCoupling constants in Hz. ^c100 MHz. ^dCH assignments by gsHSQC-edited experiments. ^eMeasured in DMSO-*d*₆.

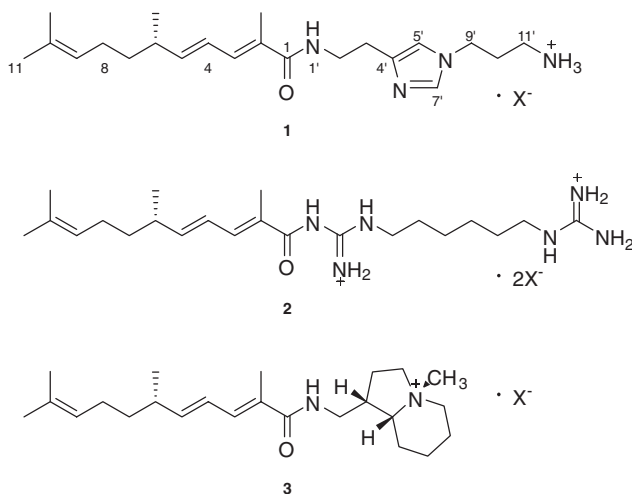


Figure 1. Bioactive alkaloids from the marine sponge *Jaspis duoaster*.

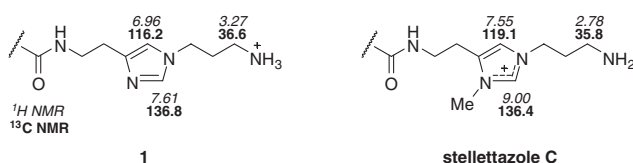


Figure 2. NMR chemical shifts of cationic moiety.

The ^1H 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 stelletazole 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 stelletazole D (**1**) has an aminopropyl group.

The remaining norsesquiterpene unit was identified by a combination of 2D NMR techniques including ^1H - ^1H COSY, edited-gsHSQC,¹² and gHMBC experiments, the same as for stelletamide B (**3**) (Table 1). The geometry of the two asymmetric double bonds at C-2 and C-4 was assigned as *2E,4E* 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, stelletazole D (**1**) was treated with NaO_4 in the presence of RuCl_3 ¹³ to generate (*S*)-2-methylglutaric acid¹⁴ ($[\alpha]_{\text{D}}^{25}$ +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 stelletazole D (**1**) was different from stelletazole 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

Stelletazole D (**1**) was moderately cytotoxic against the murine leukemia cell line P388 and against HeLa cells (IC_{50} : 29.1 and 83.6 $\mu\text{g mL}^{-1}$, respectively). To our knowledge, this is the first report of a stelletazole-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 stelletazoles, stelletadines, and stelletamides. Further studies on the biosynthetic pathway and biological activity of stelletazole D (**1**) are in progress.

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- Stelletazole D (**1**): yellow amorphous solid; $[\alpha]_{\text{D}}^{25}$ +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: $[\text{M}]^+$ *m/z* 373.2957, calculated for $\text{C}_{22}\text{H}_{37}\text{N}_4\text{O}$ 373.2962.
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- Preparative HPLC was performed using a JAI LC-9104 recycling preparative HPLC system.
- HRESI-TOF-MS data were recorded on a Waters LCT-Premier XE mass spectrometer using positive electrospray ionization with a Waters ACQUITY Ultra Performance LC system. NMR spectra were recorded on a Bruker Avance DPX400 spectrometer. The ^1H 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 $\text{DMSO}-d_6$ 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.
- Stelletazole 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. ^1H NMR signals for **4** ($\text{DMSO}-d_6$): 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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