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A new 9,11-*seco*sterol, stellettasterol from a marine sponge *Stelletta* sp.[1]

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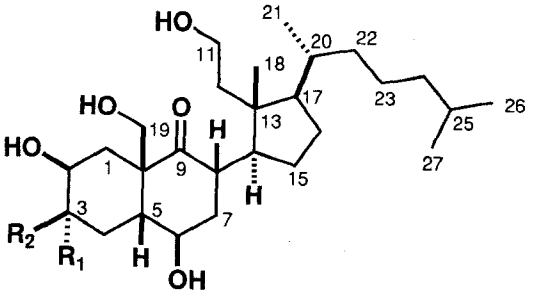
Abstract. A new 9,11-*seco*steroid, stellettasterol (**1**) was isolated from a Japanese marine sponge, *Stelletta* sp.; its structure was determined by spectroscopic analysis. All NMR signals of **1** were unambiguously assigned by application of various 2D NMR techniques. Stellettasterol exhibited antifungal activity against *Mortieralla ramannianus*.
Key words. *Stelletta* sp.; antifungal; *Mortieralla ramannianus*; stellettastreol; 9,11-*seco*sterol.

An increasing number of *seco*sterols have been isolated from Dictyoceratid sponges. These sterols showed a variety of biological activities, e.g. antimicrobial[2], ichthyotoxic[2], cytotoxic[3], antiproliferative[4], cell division inhibitory[5], antihistaminic[6], and toxic to brine shrimps[7]. As part of our continuing studies of antifungal metabolites from Japanese marine invertebrates, we found that the ethanol extract of a marine sponge *Stelletta* sp., collected off Shikine-jima Island, 200 km south of Tokyo was antifungal against *Mortieralla ramannianus*. Bioassay-guided isolation afforded stellettamide A as the major active constituent[8]. Further investigation of the extract led to isolation of a new antifungal sterol, stellettasterol (**1**). This paper deals with the isolation and structure elucidation of stellettasterol.

Materials and methods

Analytical methods. Infrared spectra were measured on a JASCO-IR-G infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AM-600, JEOL GX-500, or a Bruker AC-300 NMR spectrometer. ^1H and ^{13}C NMR chemical shifts are referenced to solvent (CD_3OD) peaks: δ_H 3.30 and δ_C 49.0. Optical rotation was determined on a JASCO DIP-371 digital polarimeter. FAB mass spectra were measured on a JEOL JMX-SX102 mass spectrometer with glycerol as the matrix. The CD spectrum was measured on a JASCO J-20C automatic recording spectropolarimeter.



1 R_1=H, R_2=OH

2 R_1=OH, R_2=H

Sponge samples and isolation. Specimens of *Stelletta* sp. were collected by snorkeling off Shikine-jima Island of the Izu Archipelago, Japan. The frozen sponge (650 g, wet weight) was homogenized and extracted twice with 70% EtOH (3 l). The combined extracts were concentrated and partitioned between dichloromethane and water. The dichloromethane-soluble material, which was antifungal against *M. ramannianus*[9], was subjected to flash column chromatography on silica gel with a $CHCl_3$/MeOH/H_2O system. The antifungal fraction eluted with $CHCl_3$/MeOH/H_2O (7:3:0.5) yielded an oily residue (146 mg), which was further separated by ODS flash chromatography (ODS-230/70 mesh, 5.5 × 2 cm) with increasing amounts of MeOH in H_2O. An active fraction (27.6 mg) eluted with 85% MeOH was subjected to silica gel open column chromatography with a MeOH/$CHCl_3$ system. The fractions eluted with 10% and 20% MeOH/$CHCl_3$ were combined, and finally purified by ODS HPLC with 78% MeOH to yield **1** (7.1 mg). **1:** colorless solid, $[\alpha]_D^{23}$ −18.5° (c = 0.35, MeOH); IR (film) 3350, 1715 cm^{-1}; CD (MeOH) $(\theta)_{295}$ −9100°; FABMS (positive ion) m/z 491 $(M + Na)^+$; HRFABMS m/z 491.3328 (Δ −4.3 mmu) for $C_{27}H_{48}O_6Na$; ^1H and ^{13}C NMR, see table.

Results and discussion

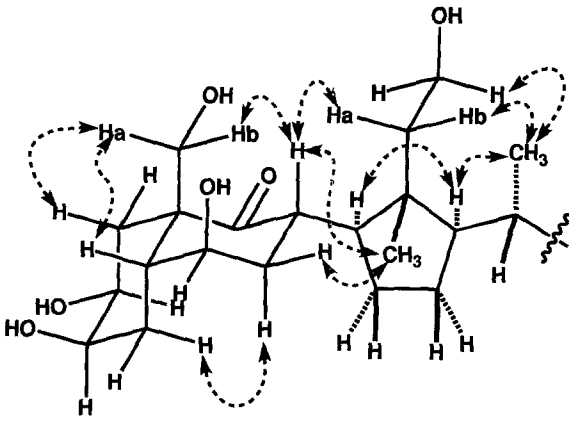
The EtOH extract of the frozen specimens (650 g) was partitioned between dichloromethane and water, and the organic phase was fractionated by flash chromatography on silica gel and ODS columns, followed by reversed-phase HPLC to yield stellettasterol (1.1×10^{-3}% yield based on wet weight). It was antifungal against *Mortieralla ramannianus* with a MIC of 12.5 μg/ml, but not cytotoxic against P388 leukemia cells at 2 μg/ml.

NMR spectral data for stellettasterol (**1**) in CD_3OD

no.	^1H[a]	^{13}C[b]
1α	2.31 (dd, J = 12.8, 4.3 Hz)	33.0 (t)
1β	1.36 (dd, J = 12.8, 11.6 Hz)	
2	3.68 (ddd, J = 11.6, 4.8, 2.9 Hz)	71.6 (d)
3	3.83 (m)	69.7 (d)
4α	1.68 (m)	35.1 (t)
4β	1.14 (m)	
5	2.34 (dddd, J = 13.6, 4.1, 1.9, 1.9 Hz)	42.5 (d)
6	3.73 (brq, J = 2.5 Hz)	69.2 (d)
7α	1.79 (ddd, J = 14.0, 14.0, 3.1 Hz)	36.7 (t)
7β	2.07 (dddd, J = 14.4, 5.4, 2.7, 2.7 Hz)	
8	3.35 (ddd, J = 13.6, 5.5, 3.2 Hz)	40.5 (d)
9		216.2 (s)
10		57.6 (s)
11	3.57 (2H, dt, J = 7.2, 1.1 Hz)	59.1 (t)
12a	1.70 (m)	41.3 (t)
12b	1.57 (m)	
13		46.6 (s)
14	2.52 (ddd, J = 11.3, 8.4, 3.5 Hz)	42.6 (d)
15α	1.16 (m)	23.5 (t)
15β	1.48 (m)	
16α	1.13 (m)	27.0 (t)
16β	1.78 (m)	
17	1.55 (m)	50.8 (d)
18	0.77 (3H, s)	17.8 (q)
19a	4.72 (d, J = 11.1 Hz)	71.9 (t)
19b	3.51 (d, J = 11.1 Hz)	
20	1.49 (m)	35.7 (d)
21	1.00 (3H, d, J = 6.6 Hz)	19.9 (q)
22a	1.42 (m)	36.7 (t)
22b	1.02 (m)	
23a	1.43 (m)	25.6 (t)
23b	1.19 (m)	
24a	1.13 (m)	40.6 (t)
24b	1.16 (m)	
25	1.54 (m)	29.1 (d)
26	0.88 (3H, d, J = 6.6 Hz)	22.9 (q)
27	0.88 (3H, d, J = 6.6 Hz)	23.1 (q)

[a]Recorded at 600 MHz.
[b]Recorded at 75 MHz.

Stellettasterol (**1**) has a molecular formula of $C_{27}H_{48}O_6$ as established by high resolution FAB mass spectrometry and ^{13}C NMR data. The IR bands at 3350 and 1715 cm^{-1} implied the presence of hydroxy and ketone groups, which was supported by ^{13}C NMR signals at δ 71.9, 71.6, 69.7 69.2, 59.1, and 216.2. The ^1H NMR spectrum revealed the presence of a tertiary methyl [δ 0.77, s, 3H], three secondary methyls [δ 0.88 (6H, d, J = 6.6 Hz) and 1.00 (3H, d, J = 6.6 Hz)], two oxygenated methylenes [δ 3.57 (2H, dt, J = 7.2, 1.1 Hz); 4.72 (d, J = 11.1 Hz), 3.51 (d, J = 11.1 Hz)], and three oxygenated methines [δ 3.68 (ddd, J = 11.6, 4.8, 2.9 Hz), 3.83 (m), and 3.73 (brq, J = 2.5 Hz)]. These spectral data were indicative of a polyoxygenated 9,11-*seco*sterol. Interpretation of the COSY spectrum with concomitant analysis of the HMQC spectrum revealed a gross structure identical with that of herbasterol (**2**)[2], an ichthyotoxic *seco*sterol isolated from the marine sponge, *Dysidea herbacea*. However, chemical shift values for ring A differ significantly between the two compounds.



Scheme 1.

A coupling constant of 11.6 Hz between H1β and H2 and that of 2.9 Hz between H2 and H3 indicated that H2 was axial, while H3 was equatorial. Another oxygenated methine proton at C6 was equatorial, because it exhibited small couplings (approximately 2.5 Hz, each) with H5, H7ax, and H7eq. The configurations at C5 and C8 were assigned on the basis of NOESY data. A prominent cross peak between H19a and H5 inferred an A/B *cis* ring junction, which was supported by a cross peak H4α and H7α. A cross peak between H19b and H8 indicated β-orientation of H8, which agreed with a coupling constant of 13.6 Hz between H8 and H7α. Stereochemical correlation between ring B and D was also inferred from NOESY data (scheme 1). A negative Cotton effect at 293 nm in the CD spectrum implied the absolute configuration in ring B as shown in **1**. It was identical with that of herbasterol (**2**)[2, 10], therefore, stellettasterol differs from herbasterol only in the absolute configuration at C3. It would be worthwhile to carry out parallel bioassays with these two sterols in order to assess the significance of the C3 stereochemistry.

Interestingly, taxonomically remote sponges [*Dysidea* (Dyctioceratida) *Stelletta* (Christida)] contained *seco*sterols differing only in the stereochemistry at C3, which poses a biogenetic question.

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