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STELLETTAMIDE A, AN ANTIFUNGAL ALKALOID FROM A MARINE SPONGE OF THE GENUS *STELLETTA*[1)]

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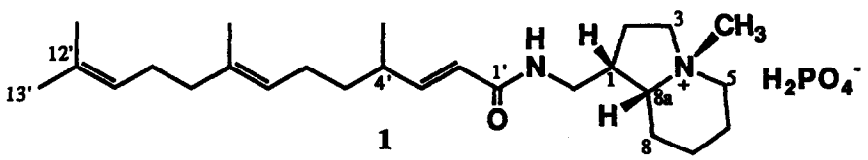
Abstract: A novel antifungal alkaloid, stellettamide A (1), has been isolated from a marine sponge *Stelletta* sp., and its structure was elucidated mainly by spectroscopic methods including extensive 2D NMR experiments.

In the course of our search for bioactive metabolites from Japanese marine invertebrates, we found that a marine sponge[2)] of the genus *Stelletta*, collected in Shikine-jima Island of the Izu Archipelago, showed antifungal activity against *Mortierella remannianus*. The bioassay-guided isolation led to the isolation of an active compound designated stellettamide A and its structure (1) was a novel alkaloid containing a farnesyl moiety and an indolizidine (= octahydroindolizine) skeleton[3)], which were connected through an amide bond.

The CH_2Cl_2 soluble portion of the 70% ethanol extract of the frozen sponge (650 g) was subjected to flash chromatography (E. Merck silica gel 60) with CH_2Cl_2/MeOH/H_2O. The active fractions (1.4 g) eluted with CH_2Cl_2/MeOH/H_2O (8 : 2 : 0.15) were successively fractionated by ODS column chromatography (YMC 70/230 mesh) with H_2O/MeOH. The eluent with 75% MeOH (1.2 g) was further purified by HPLC on ODS (YMC 005-AM) with 28% n-PrOH / 72% 20mM KH_2PO_4 followed by desalting on an ODS column to afford stellettamide A (1; 515 mg) as a semisolid.

Stellettamide A (1) had a molecular formula of $(C_{26}H_{45}N_2O)H_2PO_4$ which was established by elemental analysis,[4)] FAB-MS [*m/z* 401 $(C_{26}H_{45}N_2O)^+$], ^{13}C NMR, and ^{31}P NMR data.[5)] IR (3600-3200, 1670, 1630, 1550, 1275, 1225 cm^{-1}) and ^{13}C NMR (δ 168.9) spectra implied an amide linkage, while the other nitrogen atom was deduced to form an indolizidine ring by NMR analyses including HH-COSY, CH-COSY, HMBC, HOHAHA, and INADEQUATE experiments. The N-methyl proton signal at δ 3.16 showed strong correlation signals with δ_C 57.6, 61.0,



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and 73.6 in HMBC spectrum, which could be assignable to C-3, C-5, and C-8a of indolizidine skeleton, respectively, from INADEQUATE experiments. Substitution of a methylene group at C-1 was also secured by the NMR experiments; the methylene protons (δ ~3.4) showed a strong correlation signals with the amide carbonyl carbon (δ 168.9) in HMBC spectrum. Similarly, the linkage of a modified farnesyl moiety to the amide carbon was also substantiated from NMR data.

The stereochemistry of the indolizidine portion was deduced by NOESY spectra; the N-methyl proton (δ 3.16) correlated with signals at δ 2.43 (2β-H), 3.32 (3β-H), 3.43 (5β-H), 3.61 (5α-H), and 3.70 (8aβ-H), while the C-8a proton (δ 3.70) with protons at δ 1.55 (7β-H), ~1.95 (8β-H), 3.13 (1β-H), 3.16 (N-CH_3), and 3.43 (5β-H). Thus, the N-methyl group had equatorial configuration with respect to the six-membered ring and both hydrogens at C-1 and C-8a were on the same side as this N-methyl group. The stereochemistry of the C-4' methyl group together with the absolute configuration of 1 is under investigation.

Stellettamide A (1) was not only antifungal, but also cytotoxic against K562 epithelium cells (IC_{50} 5.1 μg/mL). To our knowledge this is the first report of an indolizine metabolite from marine sponges.[6]

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References and Notes.

1) Bioactive marine metabolites Part 32. Part 31: N. Fusetani, K. Yasumuro, H. Kawai, T. Natori, L. Brinen, and J. Clardy, *Tetrahedron Lett.*, in press.
2) The sponge had a thin crust of an epizooic sponge of the genus *Desmacella*.
3) Reviews on indolizidine alkaloids: M. F. Grundon, *Nat. Prod. Rep.*, 6, 523 (1989); 4, 415 (1987); 2, 235 (1985); 1, 245 (1984); E. Gellert, *J. Nat. Prod.*, 45, 50 (1982).
4) Trace amount (0.57%) of chlorine was also detected. The counter anion of stellettamide A might be chlorine as the original natural form, but most of chlorine might be substituted by phosphate, which was used for purification. The presence of phosphate was confirmed by ion chromatography and ^{31}P NMR.
5) Stellettamide A (1): semisolid; Found: C, 56.92; H, 9.27; N, 5.00%; $[\alpha]_D$ +23.1° (*c* 0.3, EtOH); UV (EtOH) end absorption; IR (neat) 3600-3200, 1670, 1630, 1550, 1275, 1225 cm^{-1}; FAB-MS (glycerol, positive) *m/z* 401 $[(C_{26}H_{45}N_2O)^+$; base peak]; ^{1}H NMR (CD_3OD) δ 1.05(3H, d, J = 7 Hz; 4'-CH_3), ~1.4 (2H, m; 5'-H_2), 1.55 (1H, m; 7β-H), 1.59 (3H, s; 8'-CH_3), 1.60 (3H, s; 12'-CH_3), 1.63 (1H, m; 8α-H), 1.66 (3H, s; 13'-H_3), ~1.85 (2H, m; 6-H_2), ~1.9 (1H, m; 7α-H), ~1.95 (1H, m; 8β-H), ~2.0 (5H, m; 2α-H, 6'-H_2, 9'-H_2), 2.07 (2H, m; 10'-H_2), 2.32 (1H, m; 4'-H), 2.43 (1H, dddd, J = 14, 9, 9, 7 Hz; 2β-H), 3.13 (1H, m; 1β-H), 3.16 (3H, s; N-CH_3), 3.32 (1H, ddd, J = 12, 9, 5 Hz; 3β-H), ~3.4 (2H, m; 1-CH_2), 3.43 (1H, m; 5β-H), 3.61 (1H, br d, J = 14 Hz; 5α-H), 3.70 (1H, ddd, J = 12, 6, 4 Hz; 8aβ-H), 3.90 (1H, ddd, J = 12, 12, 7 Hz; 3α-H), ~5.1 (2H, m; 7'-H and 11'-H), 5.92 (1H, dd, J = 15.5, 1 Hz; 2'-H), 6.70 (1H, dd, J = 15.5, 8 Hz; 3'-H); ^{13}C NMR (CD_3OD) δ 16.1 (q; 8'-CH_3), 17.8 (q; 12'-CH_3), 20.0 (q; 4'-CH_3), 21.1 (t; C-6), 21.6 (t; C-7), 23.3 (t; C-8), 25.2 (t; C-2), 25.9 (q; C-13'), 26.6 (t; C-6'), 27.7 (t; C-10'), 37.1 (d; C-4'), 37.5 (t; C-5'), 39.7 (t; 1-CH_2), 40.4 (d; C-1), 40.6 (t; C-9'), 54.4 (q; N-CH_3), 57.6 (t; C-3), 61.0 (t; C-5), 73.6 (d; C-8a), 122.9 (d; C-2'), 125.3 (d; C-11'), 125.4 (d; C-7'), 132.1 (s; C-12'), 136.4 (s; C-8'), 151.5 (d; C-3'), 168.9 (s; C-1'). ^{31}P NMR (CD_3OD) δ 6.28 (s).
6) D. J. Faulkner, *Nat. Prod. Rep.*, 5, 613 (1988); 4, 539 (1987); 3, 1 (1986); 1, 551 (1984).

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