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ISOLATION OF 1-METHYLHERBIPOLINE, A PURINE BASE, FROM A MARINE SPONGE, *JASPIS* SP.[1]

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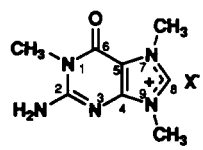
ABSTRACT.—1-Methylherbipoline [**1**], a purine base active as a collagenase inhibitor, was isolated from the marine sponge *Jaspis* sp. The structure was determined by spectral data interpretation and chemical synthesis.

Only limited numbers of unusual nucleic acid bases have been isolated from marine sponges, since pioneering work by Ackermann *et al.* (2,3) on the isolation of herbipoline (7,9-dimethylguanidium salt) and 1-methyladenine from *Geodia gigas*. Two other examples have been reported, namely, 1,9-dimethyl-6-imino-8-oxopurine from *Hymeniacidon sanguinea* (4) and 1,3,7-trimethylguanine from *Latrunculia brevis* (5). In the course of screening for collagenase inhibitors from Japanese marine invertebrates, the H_2O-soluble portion of the sponge *Jaspis* sp. (Coppatiidae) collected off Hachijo-jima Island showed modest activity. From the extract we isolated 1-methylherbipoline [**1**], whose structure was assigned spectroscopically and confirmed by synthesis. This is the first isolation of **1** as a natural product.

The MeOH extract of the sponge was partitioned between H_2O and Et_2O, and the aqueous phase was subsequently extracted with *n*-BuOH. The MeOH-soluble portion of the *n*-BuOH extract was fractionated by Sephadex LH-20 gel filtration, octadecylsilyl Si gel flash chromatography, and reversed-phase hplc to yield 1-methylherbipoline [**1**] as a colorless solid (3×10^{-3}% yield).



1

Compound **1** gave a molecular ion $C_8H_{12}N_5O$ by hrfabms (*m/z* 194.1049, Δ 0.7 mmu). The ^{1}H-nmr spectrum displayed three methyl singlets (δ 3.44, 3.79, and 4.11) and a partially exchanged aromatic signal [δ 8.96 (0.2 H, s)], while the ^{13}C-nmr spectrum exhibited five sp^2 carbons (δ 158.1, 155.0, 150.1, 140.2, and 108.1) and three *N*-methyls (δ 35.9, 31.4, and 28.9). The carbon at δ 140.2 was actually composed of four signals: one with a hydrogen and a 1:1:1 triplet coupled with deuterium. The uv absorption at 260 nm (ε 7600) together with the molecular formula suggested that **1** was a purine base. The HMBC nmr spectrum revealed cross-peaks between N-1-Me/C-2, C-6; N-7-Me/C-5, C-8; N-9-Me/C-4, C-8, which were compatible with either 1,7,9-trimethylguanium or 1,7,9-trimethylisoguanium structures.

Although the synthesis of 1,7,9-trimethylguanium iodide was reported in 1961 (6), no nmr spectral data have been reported for this compound. Therefore, the structure of compound **1** was established by direct comparison with a synthetically prepared material. To effect this, 1-methylguanine was treated with methyl *p*-toluenesulfonate to afford 1,7,9-trimethylguanine *p*-toluenesulfonate (7), which was converted to the chloride salt. The ^{1}H- and ^{13}C-nmr data of the synthetic material were identical with those of natural **1**. We have not characterized the counter anion in natural 1-methylherbipoline.

[1]Bioactive Marine Metabolites, 58. For part 57, see Sata *et al.* (1).

Compound **1** exhibited moderate inhibitory activity against *Clostridium histolyticum* collagenase at 1.25 mg/ml (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on either a Bruker AC-300P or a JEOL GSX-500 instrument. Chemical shifts are referenced to solvent peaks: δ_H 3.30 (residual CHD_2OD) and δ_C 49.0 for CD_3OD. Fabms were measured on a VG70-250S mass spectrometer. Uv spectra were recorded on a Hitachi 330 spectrophotometer in MeOH and ir spectra were recorded on a Jasco Ft-ir-5300 spectrometer.

SPONGE MATERIAL.—The sponge *Jaspis* sp. was collected off Hachijo-jima Island and kept frozen until extracted. A voucher specimen was deposited at the Institute for Taxonomic Zoology, University of Amsterdam (registration No. ZMA POR. 10485).

The sponge specimens were collected from a marine cave at a depth of 10 m off Hachijo-jima Island (33° 3.80N 139° 47.80E). This was an erected sponge reaching a height of 40 cm. The color of the major portion was brown, while the color around the osculum was bluish gray. This sponge had coarse architecture and big spicules mostly arranged perpendicular to each other, characteristic of this genus.

EXTRACTION AND ISOLATION.—The sponge (1 kg) was extracted with MeOH (3 liters×3) and the concentrated extract was partitioned between Et_2O and H_2O. The aqueous phase was further extracted with *n*-BuOH, and the *n*-BuOH phase was evaporated under reduced pressure to give a brown gum which was triturated with MeOH. The MeOH-soluble portion was fractionated by Sephadex LH-20 (5×80 cm, MeOH) and oDs flash (2×5 cm, H_2O) columns followed by oDs-hplc (H_2O) to yield 50 mg of **1**.

1-Methylherbipoline [**1**].—Ir (film) ν max 3390, 3330, 3190, 1700, 1652, 1620, 1580, 1550, 1530, 1460, 1220, 1070, 1020, and 760 cm^{-1}; uv (MeOH) λ max 260 (ϵ 7600) and 282 (4460) nm; ^{1}H nmr (CD_3OD) δ 3.44 (3H, s, N-1-Me), 3.79 (3H, s, N-9-Me), 4.11 (3H, s, N-7-Me), and 8.96 (0.2H, s, H-8); ^{13}C nmr (CD_3OD) δ 158.1 (C-2), 155.0 (C-6), 150.1 (C-4), 140.2 (C-8), 108.1 (C-5), 35.9 (N-1-Me), 31.4 (N-9-Me), and 28.9 (N-1-Me).

Synthesis of **1**.—1-Methyladenine (50 mg) and methyl *p*-toluenesulfonate (1.0 g) were heated at 125° for 1 h (7). Crystals deposited upon cooling to room temperature were collected and crystallized twice from EtOH to give 1-methylherbipoline *p*-toluenesulfonate, which was passed through a column of Dowex 1-X8 (Cl^- form, 100–200 mesh, 1×2 cm) to obtain 1-methylherbipoline chloride salt.

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