

Isolation and Characterization of 1,3-Dimethylisoguanine from the Bermudian Sponge *Amphimedon viridis*

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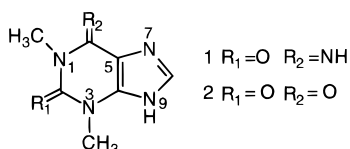
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The new compound 1,3-dimethylisoguanine has been isolated and characterized from the Bermudian sponge *Amphimedon viridis*. Chemical conversion of the natural product to theophylline and 2D NMR methods were used to determine the position of the methyl groups on the purine ring. Analysis of the mass spectral fragmentation pattern allowed assignment of the purine ring as isoguanine.

Marine sponges have proven to be an exceptionally rich source of modified nucleosides. The isolation of spongouridine and spongothymidine from *Cryptothia crypta*¹ and subsequent development of antiviral analogues demonstrated the potential medicinal importance of these compounds. More recently, several groups have reported the isolation of methylated guanine base analogues from sponges, including 7,9-dimethylguanine (herbipoline),² 1,7,9-trimethylguanine,³ 1,3,7-trimethylguanine,⁴ and 3,7-dimethylisoguanine.⁵ No physiological role for the myriad of methylated guanine analogues isolated from sponges is apparent.

The crude MeOH extract of *Amphimedon viridis* (Duchassaing and Michelotti, 1864, formerly known as *Haliclona viridis*) was found to have potent activity in an HCT 116 cytotoxicity assay. The majority of this activity was traced to meta-substituted pyridinium compounds similar to the halitoxins.⁶ Further examination of the extract led to the isolation of 1,3-dimethylisoguanine (**1**), theophylline (**2**), and thymine. To the best of our knowledge, this is the first report of 1,3-dimethylisoguanine as either a natural or synthetic product.



The freeze-dried sponge was extracted repeatedly with MeOH and the resulting crude extract partitioned according to a modified Kupchan fractionation protocol.⁷ The $CHCl_3$ -soluble material was subjected to counter-current chromatography (CCC) using a $CHCl_3$, MeOH, and H_2O solvent system to yield pure compound **1** (24 mg).

A molecular formula of $C_7H_9N_5O$ established by HRFABMS⁺, and ^{13}C -NMR chemical shifts suggested compound **1** was a purine heterocycle with two *N*-methyl substituents. A very broad exchangeable peak was observed centered about 7.60 ppm in the 1H -NMR spectrum, and a deuterium exchange experiment using

ESIMS with deuterated electrospray solvent demonstrated the presence of two exchangeable protons in the neutral molecule. Both methyl proton signals showed HMBC correlations to a quaternary carbon at δ 150.8 ppm, while the δ 3.60 ppm methyl proton and the δ 7.62 ppm proton both showed HMBC correlations to a carbon at δ 152.7 ppm. This HMBC pattern was consistent with several substitution patterns on the purine base. Both guanines and isoguanines are known to deaminate when heated with HCl, and the methylation positions may be inferred by comparing the product with a commercially available methylated xanthine analogue. Conversion of the natural product to **2** by refluxing with HCl demonstrated the positions of the methyl substituents on the purine base. The reaction product was found to be identical to commercial theophylline by 1H NMR, ^{13}C NMR, UV spectroscopy, MS/MS, and EIMS.

Fragmentation patterns for purines are relatively well characterized,^{8,9} and EIMS has been used previously to distinguish between guanines and isoguanines.^{4,9} A characteristic mode of fragmentation for some guanines via EIMS,^{4,8,9} but especially for xanthines and isoguanines,⁹ is the expulsion of neutral cyanamide fragments consisting of N1, C2, and their substituents. Guanines and isoguanines can be distinguished by MS due to a one-mass-unit difference in this fragment; isoguanines contain an oxygen substituent on C2, while guanines have an imino substituent in the same position. The EIMS of **1** displays an abundant ion of m/z 122, corresponding to the loss of CH_3NCO , whereas no loss of CH_3NCNH is observed. HREIMS of the m/z 122 ion gave an exact mass of 122.0589, corresponding to a composition of $C_5H_6N_4$ (+0.3 mmu error).¹⁰ This fragmentation is particularly clear in the negative ion collision-induced dissociation mass spectrum of **1** (Figure 1). The loss of the CH_3NCO moiety proves the purine heterocycle is an isoguanine and unambiguously assigns **1** as 1,3-dimethylisoguanine.

Compound **1** was tested in an assay of 26 human cancer cell lines showing highest cytotoxicity to an ovarian cancer cell line (IC₅₀ 2.1 $\mu g/mL$).

Experimental Section

General Experimental Procedures. 1H - and ^{13}C -NMR experiments were performed on a Varian Unity 500 MHz spectrometer. Spectra were referenced to residual undeuterated solvent peaks or solvent ^{13}C signals. HREIMS and LREIMS measurements were

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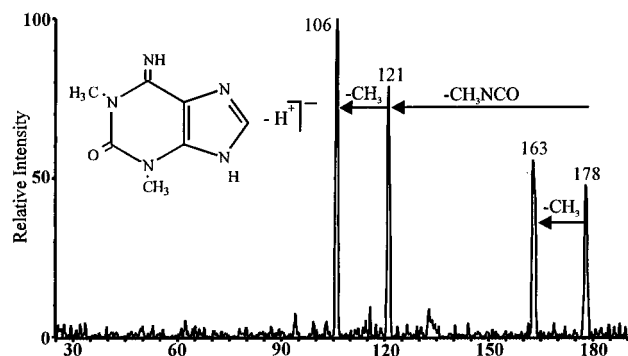


Figure 1. Collisionally induced dissociation spectrum from 1 (ESI-).

performed on a Finnegan MAT 95 high-resolution mass spectrometer. ESIMS were collected on a SCIEX (Thornhill, Ontario, CA) API III+ triple quadrupole mass spectrometer. Samples were infused via a syringe pump at 1.5 μ L/min in a 90:10 v/v CH₃OH–H₂O solution. Deuterium exchange was accomplished by evaporating the sample to dryness and redissolving the sample in a 90:10 CD₃OD–D₂O solution, which was then directly electrosprayed. A high-speed countercurrent chromatograph (P. C. Inc., model HSCCC) with a column volume of 380 mL was used for countercurrent chromatographic separations. All solvents for ccc were freshly distilled or HPLC grade, and mixtures were equilibrated overnight with stirring.

The organism was collected while snorkeling at depths from 1–3 m in Shark Hole, Harrington Sound, Bermuda. The sponge (300 g dry wt) was freeze dried and extracted repeatedly with MeOH, and the resulting crude extract was dried and resuspended in 90% MeOH–10% H₂O (300 mL). This solution was extracted with hexane (1 L), then 60 mL H₂O was added to the aqueous MeOH extract, and this solution extracted with CHCl₃ (600 mL). The CHCl₃-soluble material was then subjected to ccc using 40% CHCl₃–30% MeOH: 30% H₂O with normal-phase elution. Fractions containing **1** were combined, and this material was resubjected to ccc using the same conditions to yield pure compound **1** (24 mg).

1,3-Dimethylisoguanine: white solid; ¹H NMR (DMSO, 500 MHz) N1-Me 3.56 (s, 3H; HMBC C2, C6), N3-Me 3.60 (s, 3H; HMBC C2, C4), N6H, 8.1 (br s), N9H, 8.1 (br s), H8, 7.62 (s, 1H; HMBC C4, C5), ¹³C NMR (CD₃OD, 125 MHz) N1-Me 30.4, C2 150.8, N3-Me 30.6, C4 152.7, C5 111.2, C6 152.3; UV (MeOH) $\lambda_{\max}$

210, 292; EIMS (70 eV) m/z [M⁺] 179 (34), 122 (100), 121 (78), 94 (65), 57 (9).

The acid hydrolysis of compound **1** to compound **2** was performed by dissolving compound **1** (6 mg) in 5 mL HCl. The solution was heated at reflux for 24 h and the HCl removed *in vacuo*. ¹H NMR of the residue showed a 50/50 mixture of starting material and product. The residue was dissolved in concentrated HCl (5 mL) and heated at reflux for an additional 24 h. The dried residue was then washed through a plug of Si gel (10 cm³) using 10% MeOH–90% CHCl₃ to give compound **2** (4 mg).

Compound 2: white solid; UV $\lambda_{\max}$ 210, 220, 262, 278 nm. ¹H NMR (CD₃OD, 500 MHz) δ 8.69 (s, 1H), 3.65 (s, 3H), 3.38 (s, 3H); ¹³C NMR (CD₃OD, 125 MHz) δ 159.5, 156.5, 153.4, 149.2, 141.3, 30.5, 28.5.

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