1,3-Dimethylguanine, a New Purine from the New Zealand Ascidian *Botrylloides leachi*

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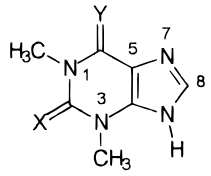
The new purine 1,3-dimethylguanine (**1**) has been isolated from the ascidian *Botrylloides leachi*. The structure of **1** was elucidated by analysis of spectroscopic data, by comparison with the regioisomeric purine 1,3-dimethylisoguanine (**2**), and by hydrolysis to theophylline (**3**).

Although methylated guanine analogues are proving to be common in marine sponges,[1−6] our isolation of 1,3-dimethylisoguanine (**2**) from *Cnemidocarpa bicornuta* was the first report of such a purine from an ascidian.[7] Our ongoing screening program indicated the presence of a related metabolite, present at qualitatively high levels, in numerous ascidians collected from various shallow-water sites around the coast of New Zealand. Reversed-phase C_{18} chromatography of the crude MeOH extract of one such ascidian, of which we had obtained a sufficiently large sample for isolation work, the common subtidal purple compound ascidian *Botrylloides leachi* Savigny, 1816 (Styelidae),[8] afforded a white solid that was identified as the new natural product 1,3-dimethylguanine (**1**). To the best of our knowledge, this is the first report of **1** as either a natural or a synthetic product.

A molecular formula for **1** of $C_7H_9N_5O$, obtained by HREIMS in conjunction with ^{1}H and ^{13}C NMR data, was suggestive of a dimethylated guanine or isoguanine. This was confirmed by the observation of a deceptively simple ^{1}H NMR spectrum (DMSO-d_6) composed of two *N*-methyl singlets (δ 3.65 and 3.39), a methine (δ 8.13), and two exchangeable protons as a broad singlet (δ 4.10). Similarly, the ^{13}C NMR spectrum of **1** showed four quaternary carbons (δ 152.0, 151.4, 147.3, and 107.4), one methine (δ 142.0, d, J = 213 Hz), and two methyl carbons (δ 32.4 and 29.3) indicative of a dimethylated guanine or isoguanine. An HMBC experiment located the methyl groups on N-1 and N-3, with crucial HMBC correlations observed from N-3-Me (δ 3.65) to C-2 (δ 151.4) and C-4 (δ 147.3) and from N-1-Me (δ 3.39) to C-2 (δ 151.4) and C-6 (δ 152.0). The assignments of C-4 and C-5 (δ 107.4) were supported by the observation of correlations with H-8 (δ 8.13). Final confirmation of the *N*-1 and *N*-3 locations of the methyl groups was achieved by acid hydrolysis of **1** to afford theophylline (**3**), which possessed identical analytical HPLC UV spectral data and retention times, in multiple solvent systems, to that of a standard sample (BDH). Distinction between the two possible structures **1** and **2** was made by C_{18} analytical HPLC and by EIMS. Thus, HPLC co-injection of **1** and a sample of **2** previously isolated by us[7] afforded two peaks (**1**, t_R 14.2 min; **2**, t_R 15.6 min), while the LRMS observed for **1** exhibited a mass loss of 55 that is characteristically observed for a guanine-derived purine bearing a methyl substituent on N-1.[4,9] This mass loss corresponds to the expulsion of N-1, C-2, and their attached substituents, with the rearrangement of a hydrogen radical onto the remaining imidazole fragment, to give an observed ion of mass 124.[9] This retro-Diels−Alder fragmentation is quite distinct from that observed for 1,3-dimethylisoguanine (**2**), where the ion generated from this process is observed at 121 mass units under EIMS.[1,2] The molecule was thus determined to be the hitherto unknown 1,3-dimethylguanine (1,3,6,7-tetrahydro-2-imino-1,3-dimethyl-6*H*-purin-6-one).

Using analytical HPLC, we have detected 1,3-dimethylguanine in a wide variety of New Zealand ascidians, including identified organisms from the families Didemnidae (*Didemnum candidum*), Polyclinidae (*Leptoclinides* sp. and *Aplidium scabellum*), and Styelidae (*Botryllus* sp. and *B. schlosseri*). Such a widespread metabolite, present at relatively high levels (usually > 0.2% dry wt) is likely to play a fundamental role in ascidian physiology and, as such, warrants further investigation. Preliminary ecological evaluation of **2** failed to detect any feeding deterrence against important local consumers.[7] Clearly, further studies are required to determine the physiological role(s) of methylated purines in ascidians, and we are endeavoring to address this issue.



1 X = NH, Y = O

2 X = O, Y = NH

3 X = Y = O

Experimental Section

General Experimental Procedures. Details of general procedures and analytical HPLC conditions have been reported previously.[7]

Animal Material. We collected specimens of *Botrylloides leachi* (Styelidae) while snorkeling (−2 m) in Momorangi Bay, Marlborough Sounds, New Zealand, in January 1997. The ascidians were kept frozen until used. Voucher specimens are held at the University of Auckland, Chemistry Department (97MO1−1) and at the NIWA Museum, Wellington (NZOI Stn Z9118).

Extraction and Isolation. The ascidians were freeze-dried (dry wt 39.83 g) and extracted with MeOH−CH_2Cl_2 (10:1, 220 mL) for 72 h, followed by MeOH (250 mL) for 24 h. The combined extracts were filtered, and the solvent was removed under reduced pressure (total extract after desalting once with MeOH: 4.01 g). A portion of the crude extract (200 mg) was subjected to C_{18} flash chromatography (aqueous through to MeOH), with the 30−50% MeOH fractions containing the compound of interest. Compound **1** was further purified by semipreparative HPLC, MeOH−aqueous TFA (0.05%) 1:14, 6.0 mL/min, yielding **1** as a white solid (7.0 mg, equates to 0.35% dry wt).

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1,3-Dimethylguanine (1): UV (MeOH) λ_{max} (log ϵ) 200.8 (3.7), 262.8 (3.3) nm; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.13 (1H, s, H-8, HMBC C-4, C-5), 4.10 (2H, br s), 3.65 (3H, s, N-3-Me, HMBC C-2, C-4), 3.39 (3H, s, N-1-Me, HMBC C-2, C-6); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 152.0 (s, C-6), 151.4 (s, C-2), 147.3 (d, J = 9 Hz, C-4), 142.0 (d, J = 213 Hz, C-8), 107.4 (d, J = 7 Hz, C-5), 32.4 (q, J = 143 Hz, N-3-Me), 29.3 (q, J = 143 Hz, N-1-Me); desorption EIMS m/z $[M]^+$ 179 (100), 150 (20), 137 (5), 124 (20), 110 (5), 95 (20), 82 (5), 68 (25), 57 (10), 53 (10), 45 (20); HREIMS m/z 179.0808 (calcd for $C_7H_9N_5O$, 179.0807).

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References and Notes

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