Isolation and Characterization of the New Purine 1,3,7-Trimethylisoguanine from the New Zealand Ascidian *Pseudodistoma cereum*

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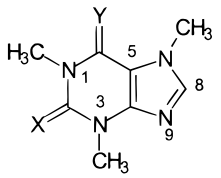
Received April 12, 2000

The new purine 1,3,7-trimethylisoguanine (**1**) has been isolated from the ascidian *Pseudodistoma cereum*. The structure of **1** was elucidated by analysis of NMR spectroscopic and mass spectrometric data and by comparison with the regioisomeric purine 1,3,7-trimethylguanine (**2**).

Ascidians are proving to be a valuable source of novel modified purines.[1] As part of our program to screen extracts of New Zealand ascidians for novel secondary metabolites, we have studied the organism *Pseudodistoma cereum* Michaelson, 1924 (Polyclinidae), collected in the far north of New Zealand. Reversed-phase C_{18} chromatography of the crude MeOH extract of the ascidian afforded the known purine 1,3-dimethylisoguanine[2−4] as well as a light tan oil, which was identified as the new purine 1,3,7-trimethylisoguanine (**1**). Previous studies of ascidians of the genus *Pseudodistoma* have led to the discovery of cytotoxic amines,[5] aminols,[6] and alkaloids.[7]



1 X = O, Y = NH

2 X = NH, Y = O

A molecular formula for **1** of $C_8H_{11}N_5O$, obtained by high-resolution EIMS in conjunction with ^1H and ^{13}C NMR data, was suggestive of a trimethylated guanine or isoguanine. This was confirmed by the observation in the ^1H NMR spectrum (DMSO-d_6) of three *N*-methyl singlets (δ 4.05, 3.52, and 3.51), a methine (δ 8.34), and an exchangeable proton as a broad singlet (δ 9.60). Similarly, the ^{13}C NMR spectrum of **1** showed signals for four quaternary carbons (δ 150.5, 149.0, 148.3, and 102.7), one methine (δ 147.3, d, J = 216 Hz), and three methyl carbons (δ 34.5, 31.6, and 30.3), indicative of a trimethylated guanine or isoguanine. A gHMBC experiment located the methyl groups on *N*-1, *N*-3, and *N*-7, with correlations observed from *N*-7-Me (δ 4.05) to C-5 (δ 102.7) and C-8 (δ 147.3), from *N*-3-Me (δ 3.51) to C-2 (δ 148.3) and C-4 (δ 150.5), and from *N*-1-Me (δ 3.52) to C-2 (δ 148.3) and C-6 (δ 149.0). The assignments of C-4, C-5, and C-6 were supported by the observation of correlations with H-8 (δ 8.34). Distinction between the two possible structures, isoguanine **1** and guanine **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[8] afforded two peaks (**1** t_R 15.1 min, **2** t_R 15.8 min). In the HREIMS spectrum of **1**, fragments were observed at $[M - CHO]^+$ (*m*/*z* 164) and $[M - MeNCO - H]^+$ (*m*/*z* 135), the latter fragment being diagnostic of an *N*-1-methylated isoguanine purine.[9] The corresponding retro Diels−Alder fragmentation of isomeric guanine **2** affords an ion at *m*/*z* 138 ($C_6H_8N_3O$).[10]

Our screening of New Zealand ascidian extracts by diode-array-detected analytical HPLC has revealed dimethylated purines (such as 1,3-dimethylguanine and 1,3-dimethylisoguanine) to be relatively common metabolites (present in 15% of our collection, N = 122). In contrast to this however, we have detected trimethylated purines in only three ascidians: *Botryllus schlosseri*, *Pseudodistoma aureum* (1,3,7-trimethylguanine, **2**), and *Pseudodistoma cereum* (1,3,7-trimethylisoguanine, **1**). It is interesting to note also that in each case to date where we have detected a 1,3,7-trimethylated purine in an organism the corresponding 1,3-dimethylated purine was also present.

1,3,7-Trimethylisoguanine had no detectable activity toward tumor cell lines (tested at 100 μM concentration against P-388 murine leukemia, NCI-H460 human lung, MCF7 human breast, and SF-268 human CNS), nonmalignant cells (BSC-1), virii (HSV-1 and PV1), and microorganisms (*E. coli*, *B. subtilis*, *C. albicans*, *T. mentagrophytes*). 1,3,7-Trimethylisoguanine also failed to inhibit the cell cycle regulating enzyme cdc2/cyclin B kinase (no detectable activity at 10 μM).[12]

Experimental Section

General Experimental Procedures. Details of general procedures and analytical HPLC conditions have been reported previously.[1b]

Animal Material. Specimens of the lobed colonial ascidian *P. cereum* (Polyclinidae)[13] were collected using scuba (−13 m) from Irishman's Garden in the Three Kings Island Group, New Zealand, in April 1999 and kept frozen until used. Voucher specimens are held at the University of Auckland, Chemistry Department (99MNP0088), and at the NIWA Museum, Wellington (MNP0088).

Extraction and Isolation. The ascidians (wet weight 98 g) were 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. The crude extract (5.9 g) was subjected to C_{18} flash chromatography (aqueous through to MeOH) with the 0−25% MeOH fractions containing the compound of interest. Compound **1** was further purified by C_{18} semipreparative HPLC

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10.1021/np000172+ CCC: $19.00
Published on Web 07/22/2000

(15% MeCN−aqueous TFA (0.05%), 5.0 mL/min), yielding **1** as a light tan oil (16.7 mg).

1,3,7-Trimethylisoguanine (1): UV (MeOH) λ_{max} (log ϵ) 214 (3.6), 288 (3.4) nm; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.60 (1H, br s), 8.34 (1H, s, H-8, HMBC C-4, C-5, C-6, N-7-Me), 4.05 (3H, s, N-7-Me, HMBC C-5, C-8), 3.52 (3H, s, N-1-Me, HMBC C-2, C-6), 3.51 (3H, s, N-3-Me, HMBC C-2, C-4); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 150.5 (C-4), 149.0 (C-6), 148.3 (C-2), 147.3 (d, J = 216 Hz, C-8), 102.7 (C-5), 34.5 (N-7-Me), 31.6 (N-1-Me), 30.3 (N-3-Me); EIMS m/z [M]$^+$ 193 (45), 192 (100), 164 (25), 135 (20), 108 (15), 97 (10), 83 (15), 67 (22), 57 (25), 55 (30), 45 (30), 40 (35); HREIMS m/z 193.0953 (calcd for $C_8H_{11}N_5O$, 193.0964), 192.0882 (calcd for $C_8H_{10}N_5O$, 192.0885), 164.0933 (calcd for $C_7H_{10}N_5$, 164.0936), 135.0674 (calcd for $C_6H_7N_4$, 135.0671).

Acknowledgment. We wish to thank the Coral Reef Research Foundation (under contract from the NCI) for assistance with organism collection, M. Walker for mass spectral and NMR data acquisition, Dr. V. Narayanan (NCI, Bethesda) for some of the antitumor results, Dr. Laurent Meijer (CNRS, France) for the cdc2/cyclin B kinase assay result, and the University of Auckland and Auckland Medical Research Foundation for funding.

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(8) As part of our ongoing studies, we have previously isolated a sample of the known purine 1,3,7-trimethylguanine (**2**)[10,11] from the common encrusting ascidian *Botryllus schlosseri* (Styelidae) (identified by Dr Chris N. Battershill, AIMS, Australia). The ^{1}H and ^{13}C NMR and mass spectroscopic data observed for our sample were identical to those reported originally by Munro et al.[10] We have also been able to fully assign the ^{1}H and ^{13}C NMR spectra of **2**: ^{1}H NMR (MeOH-d_4, 400 MHz) δ 8.02 (1H, s, H-8), 4.01 (3H, s, *N*-7-Me), 3.79 (3H, s, *N*-3-Me), 3.53 (3H, s, *N*-1-Me); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 153.6 (C-2), 153.1 (C-6), 148.4 (C-4), 145.4 (C-8), 109.8 (C-5), 34.3 (*N*-7-Me), 33.1 (*N*-3-Me), 30.0 (*N*-1-Me).

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NP000172+