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A Novel Modified Pterin from a *Eudistoma* Species Ascidian

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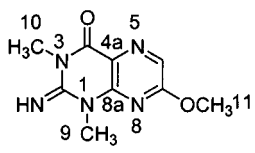
The MeOH extract of an Indonesia *Eudistoma* sp. ascidian contained 1,3,O^7-trimethylisoxanthopterin (**1**), a novel pteridine. The purification of **1** was achieved through flash C_{18} chromatography and cyano HPLC. The structure was determined primarily through the use of $^1H-^{13}C$ and $^1H-^{15}N$ HMBC measurements and comparison with data obtained for 1,3,7-trimethylguanine (**2**).

In a recent report we described the chemical investigation of a previously undescribed *Eudistoma* sp. ascidian which yielded a variety of metabolites including the β-carbolines trypargine, 1-carboxytrypargine, trypargimine, and tetrahydropentoxyline.[1] Further study of this organism led to the isolation of a novel methylated heterocycle. Spectral comparison with 1,3,7-trimethylguanine (**2**),[2] also isolated from the organism, indicated strong structural similarities between the two compounds. However the differences were sufficient to frustrate all attempts to construct a purine-based structure consistent with the data. Thus, this compound was not included in the original report for this ascidian. Identification of **1** as 1,3,O^7-trimethylisoxanthopterin was recently made possible through the measurement of medium-range $^1H-^{15}N$ heteronuclear scalar coupling interactions.



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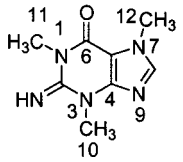

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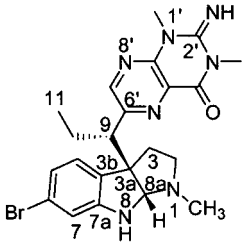

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Solvent−solvent partitioning of the methanolic extract of the ascidian *Eudistoma* sp. led to concentration of **1** in the MeOH/$CHCl_3$ soluble material. Fractionation of this material first by flash C_{18} chromatography and subsequently by cyano HPLC yielded pure **1**. High-resolution mass spectrometry for **1** was consistent with a molecular formula of $C_9H_{11}N_5O_2$, which differs from that of **2** by addition of CO. The ^1H NMR spectrum consists of a low-field aromatic singlet (δ 8.34 ppm) and three low-field methyl singlets (δ 4.20, 3.89, and 3.63 ppm). Single-bond correlation of the singlet at 4.20 ppm with a carbon resonating at 56.2 ppm indicates substitution with oxygen. Further support for this is provided by a $^1H-^{13}C$ HMBC to δ 164.0 ppm, a potentially oxygenated sp^2 carbon. The chemical shifts of the remaining methyl groups are consistent with substitution with nitrogen. The ^{13}C chemical shifts of the heterocyclic portion of the molecule show general similarity with **2** (compare Table 1 to Table 2) with the exception of δ 164.0 ppm, which is absent in **2**. Closer inspection of the data, however, reveals some significant differences. Specifically, the scalar coupling interaction

Table 1. NMR Assignments for 1,3,O^7-Trimethylisoxanthopterin (**1**) in CD_3OD

atom no.	δ ^{13}C	δ ^1H	δ ^{15}N[a]	HMBC
1			115	
2	155.1			
3			143	
4	158.6			
4a	121.2			
5			338	
6	136.3, d, 194 Hz	8.34		4a, 5, 7, 8a (weak)
7	164.0			
8				
8a	147.4			
9	31.9, q, 144 Hz	3.89		1, 2, 8a
10	30.7, q, 145 Hz	3.63		2, 3, 4
11	56.2, q, 149 Hz	4.20		7

[a] ^{15}N shifts are referenced to liquid NH_3 indirectly by CH_3NO_2 present as an internal standard.

Table 2. NMR Assignments for 1,3,7-Trimethylguanine (**2**) in CD_3OD

atom no.	δ ^{13}C	δ ^1H	δ ^{15}N[a]	HMBC
1			143	
2	154			
3			109	
4	149			
5	110			
6	153			
7			159	
8	145[b]	8.04		4, 5, 6 (weak), 7, 9, 12 (weak)
9			230	
10	33[c]	3.79		2, 3, 4
11	30[c]	3.53		1, 2, 6
12	34[c]	4.02		5, 7, 8

[a] ^{15}N shifts are referenced to liquid NH_3 indirectly by CH_3NO_2 present as an internal standard. [b] $^1J_{C-H}$ was measured to be 215 Hz from a nonsuppressed one-bond correlation observed in the gHMBC experiment. [c] ^{13}C shifts for the three methyl groups have been tentatively assigned to δ values in CD_3OD on the basis of correlations observed in an HMQC acquired in DMSO-d_6.

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between C-8 and its directly attached proton in **2** has a magnitude near 210 Hz, consistent with the presence of an imidazole ring.[3] The analogous coupling in **1**, however, is considerably weaker at 194 Hz, suggesting a different ring system. Furthermore, the chemical shift of C-4a at 121.2 ppm is significantly downfield of the value for C-5 (~110 ppm) that often characterizes 1-methylguanines.[2,4] Also, the pattern of long-range $^{1}H-^{13}C$ multiple bond correlations for the aromatic methine is different between **1** and **2**, which again supports a different ring system.

Support for structure **1** is provided by the $^{1}H-^{15}N$ HMBC data. Similar spectra for **2** were also collected for comparison, as the ^{15}N chemical shifts have not yet been reported. For both compounds, only two-bond correlations are observed in experiments optimized for 10 Hz coupling. No additional long-range correlations are apparent in an experiment optimized for 4 Hz coupling. Correlations to N-1 (143 ppm), N-3 (109 ppm), N-7 (159 ppm), and N-9 (230 ppm) are present for **2**. Correlations for the pyrimidine moiety of **1** show agreement for both N-1 (115 ppm) and N-3 (143 ppm) (note the difference in numbering for the two compounds). The aromatic methine, however, has only a single correlation to N-5 at 338 ppm. Such an ^{15}N shift differs drastically from those typically observed in five-membered heterocycles but agrees well with those observed for imines or fused piperazines.[5] No correlations to ^{15}N are seen for the furthest downfield methyl group in **1**.

Additional evidence for **1** is provided by comparison of the ^{13}C NMR data for **1** with that of the pterin moiety of urochordamine A (**3**).[6] All values for both ring carbons and methyl carbons are within 5 ppm of those reported aside from C-6 (15 ppm upfield in **1**) and C-7 (15 ppm downfield in **1**), both differences consistent with methoxy substitution at C-7 in **1** versus alkyl substitution at C-6′ in **3**.[3] The UV−vis spectrum also shows similarity to that reported for urochordamine A. Hypsochromic shift of the lowest energy UV−vis band of **1** upon acidification implies the presence of a base with pK_a near 6 in association with the aromatic chromophore. EIMS fragmentation patterns for **1** show similarity to those of **2**, with which it shares a common pyrimidine moiety, with a positive *m*/*z* offset of 28. FTIR of a film on an NaCl plate indicates the following vibrations (among others) with tentative assignments shown: 3500−2800 cm^{-1} (N−H), 1710−1650 cm^{-1} (C═O, C═N), 1202 cm^{-1} (═C−O−C). In general, the FTIR spectrum of **1** resembles that of its fully demethylated form, isoxanthopterin (7-hydroxypterin).[7]

This report marks only the second occasion of a modified pterin being described as a secondary metabolite in a tunicate. In the first, urochordamines A and B were isolated from the tunic of *Ciona savignyi*.[6] The epimeric pterin-containing bromophysostigmines were assayed for their ability to trigger settlement in *C. savignyi* larvae. Only one of the two epimers, urochordamine A, was found to be active. An earlier report described the presence and secretion of pterin and isoxanthopterin by various ascidian species in the families Styelidae, Molgulidae, Polyclinidae, Cionidae, and Ascidiidae.[8] In general, pteridines are ubiquitous as primary metabolites and catabolites.

Experimental Section

General Experimental Procedures. The ^{1}H, ^{13}C, and ^{15}N NMR data were obtained at 500, 125, and 50 MHz respectively on a Varian Unity 500 spectrometer. Proton chemical shifts are reported in parts per million relative to residual undeuterated solvent. ^{15}N chemical shifts are referenced to liquid ammonia indirectly through nitromethane present as a standard in the sample. $^{1}H-^{15}N$ HMBC data were acquired by optimizing a gHMQC experiment for a 10 Hz coupling. UV−vis spectra were obtained in water using 3.7% HCl and 3.0% NaOH solutions for pH adjustment on a Hewlett-Packard 8452A diode array spectrophotometer. High- and low-resolution EI mass spectral measurements were made on a Finnegan MAT 95 high-resolution spectrometer. HPLC was carried out with a Rainin cyano Dynamax column (4.6 × 250 mm, 5 μm) on a Beckman 126 solvent module and a Beckman 168 diode array detector.

Animal Material. A description of the ascidian *Eudistoma* sp. appeared in our previous report.[1]

Extraction and Isolation. The freeze-dried *Eudistoma* sp. ascidians (~30−40 g dry weight) were extracted repeatedly with MeOH followed by filtration. The combined extracts were concentrated in vacuo and redissolved in a solution of MeOH/H_2O (9:1). This solution was extracted with hexanes prior to dilution with water to a composition of MeOH/H_2O (7:3) and extraction with $CHCl_3$. The $CHCl_3$-soluble material was then subjected to flash C_{18} chromatography using a step gradient from MeOH/H_2O (1:9) to MeOH. The loading solution contained 0.1% TFA to enhance solubility. Compound **1** was found to elute with a mobile phase composition of MeOH/H_2O (1:9). Further purification of **1** was achieved through the use of cyano HPLC and an isocratic mobile phase consisting of MeOH/H_2O/TFA (3:7:0.01). Compound **1** was obtained as a colorless solid (3.3 mg, 0.009%).

1,3,*O*^{7}-Trimethylisoxanthopterin (1): colorless solid, produces violet fluorescence upon irradiation at λ 365 nm of a solution in MeOH; UV (H_2O) (*c* 0.1 mM) λ_{max} (log ϵ) pH < 6 210 (sh) (4.0), 236 (sh) (3.6), 268 (3.4), 314 (3.7), 328 (sh) (3.6); pH = 6.3 208 (sh) (4.0), 236 (sh) (3.7), 268 (3.4), 316 (3.6), 328 (sh) (3.6), 350 (sh) (3.0); pH > 7 208 (sh) (4.0), 240 (sh) (3.7), 270 (3.5), 338 (3.6), 352 (sh) (3.5); IR (film) ν_{max} 3500−2800, 1712, 1671, 1611, 1590, 1562, 1381, 1368, 1202, 1180, 1130 cm^{-1}; ^{1}H, ^{13}C, and gHMBC data, Table 1; EIMS *m*/*z* (abundance) 221 (100), 193 (16), 192 (32), 166 (4.2), 165 (3.9), 164 (3.0), 151 (23), 137 (10), 110 (9.1), 69 (10), 67 (10) 45 (8.8); EIHRMS *m*/*z* 221.0918 (calcd for $C_9H_{11}N_5O_2$, 221.0913).

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