1,9-Dimethylhypoxanthine from a Southern Australian Marine Sponge *Spongosorites* Species

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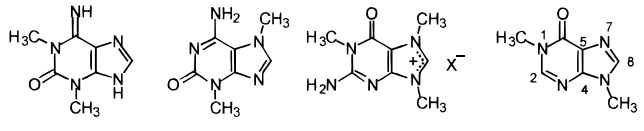
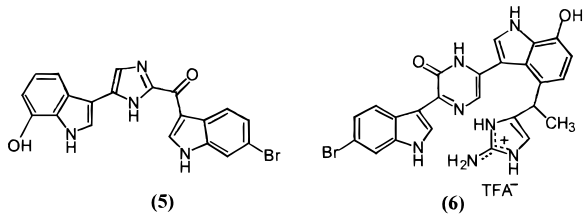
A *Spongosorites* sp. collected off southern Australia has yielded 1,9-dimethylhypoxanthine (**4**). The structure for **4** was solved by spectroscopic analysis.

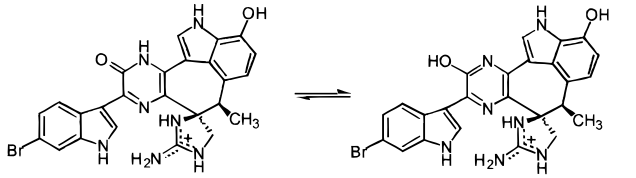
Methylated purine bases with a range of medicinal properties have been isolated from a variety of marine and terrestrial sources. Those isolated from marine sponges include 1,3-dimethylisoguanine (**1**)[1] from a Bermudan sponge *Amphimedon viridis*, which was active against an ovarian cancer cell line (IC_{50} 2.1 μg/mL); 3,7-dimethylisoguanine (**2**)[2] from a Caribbean sponge *Agelas longissima*, which displayed mild antibacterial properties; and 1-methylherbipoline (**3**)[3] from a Japanese sponge *Jaspis* sp., which was reported to be a collagenase inhibitor. In this report we describe the isolation and identification of a new example of this structure class, 1,9-dimethylhypoxanthine (**4**), from a *Spongosorites* sp. collected off southern Australia.



(1) (2) (3) (4)



(5) (6)



(7)

The aqueous ethanol extract of a *Spongosorites* sp. was decanted and concentrated in vacuo, after which it was subjected to solvent partitioning and chromatographic fractionation. In earlier reports we described the isolation of isobromotopsentin (**5**)[4] as well as dragmacidin D (**6**)[5] and dragmacidin E (**7**)[6] from the methanol-soluble portion of this extract. In the present report we describe our studies into the H_2O-soluble material from this same sponge.

The H_2O-soluble residue was subjected to gel permeation (Sephadex G-10) chromatography and C_{18} HPLC to yield **4** as a white solid. HREIMS gave a molecular formula ($C_7H_8ON_4$ Δ 0.2 mmu) requiring six double-bond equivalents. The ^{1}H NMR data for **4** revealed resonances consistent with two N−CH_3 groups (δ 3.87 and 3.88) and two deshielded methines (δ 8.25 and 8.48). Particularly significant was the absence of exchangeable signals when the ^{1}H NMR data were acquired in DMSO-d_6, together with the lack of OH or NH absorptions in the IR spectrum. In addition to two N−CH_3 resonances, the ^{13}C NMR spectrum for **4** featured five deshielded sp^2 hybridized carbons (see Table 1). Together with characteristic UV absorptions (260 and 209 nm), the data described above are consistent with a dimethylhypoxanthine. Examination of COSY and ^{1}H−^{13}C gHMBC NMR data (see Table 1) failed to distinguish between a number of possible substitution patterns; however, NOE difference analysis did confirm a 12% NOE to N(1)−CH_3 on irradiation of H-2 (δ 8.48) and a 10% NOE to N(9)−CH_3 on irradiation of H-8 (δ 8.25). Final confirmation of the 1,9-methylation pattern was achieved by interpretation of the ^{1}H−^{15}N gHMBC NMR data for **4**, which revealed correlations from (a) H-2 to N-1 and N-3, (b) N(1)−CH_3 to N-1, (c) H-8 to N-7 and N-9, and (d) N(9)−CH_3 to N-9. To the best of our knowledge this is the first account of 1,9-dimethylhypoxanthine (**4**).

The assignment of structures to highly substituted heterocycles such as **4** is not trivial, and this analysis serves to highlight the valuable role that ^{1}H−^{15}N inverse NMR experiments can play in this process.

Experimental Section

General Experimental Procedures. Procedures were as performed by Urban et al.[7]

Animal Material. A *Spongosorites* sp. (432 g dry wt, Demospongiae, Halichondriidae) was used. Its growth form was massive; live color, texture, and surface features unknown; aerophobic dark green pigments in ethanol and producing a dark eosinic pigment; texture very hard (stony), arenaceous; ectosomal skeleton with embedded detritus and producing erect, slightly larger oxeas from ascending choanosomal tracts, surmounted by a paratangential felt-like network of slightly smaller oxeas; choanosome with a criss-cross halichondroid reticulation of both smaller and larger oxeas forming vaguely directionless tracts, eventually ascending to the surface, with large sand grains and other detritus throughout the skeleton; oxeas moderately small, slender, sharply pointed, fusiform, some with centrangulate swellings, more or less divided into two size classes but with numerous intermediates (85−160 × 3−5 μm); no microscleres were present. The *Spongosorites* sp.

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Table 1. NMR (D_2O, 400 MHz) Data for 1,9-Dimethylhypoxanthine (**4**)[a]

number	^1H (δ, m)	^{13}C (ppm)	$^1H-^{13}C$ gHMBC	$^1H-^{15}N$ gHMBC
2	8.48, s	148.5	C-4, C-5, N(1)−**C**H_3	N-1, N-3
4		149.0		
5		120.1		
6		158.8		
8	8.25, s	146.2	C-4, C-5, N(9)−**C**H_3	N-7, N-9
N(1)−CH_3	3.87, s	38.1	C-2, C-6	N-1
N(9)−CH_3	3.88, s	30.6	C-4, C-8	N-9

[a] ^{13}C NMR assignments supported by gHMQC, DEPT 90° and 135° NMR experiments; referenced to dioxane (δ 66.5).

was collected by epibenthic sled at a depth of 90 m off the coast of South Australia during a scientific cruise aboard the *R. V. Franklin* in May 1991. A voucher specimen was deposited with the Queensland Museum (registry number QMG301315).

Extraction and Isolation. After transportation to the laboratory, the sponge was diced, steeped in EtOH, and stored at −18 °C. The EtOH extract was decanted, filtered through Celite, and partitioned into BuOH and H_2O soluble fractions. The H_2O soluble fraction (1.45 g, 0.34%) was concentrated to a white solid that was fractionated by gel permeation chromatography (elution with H_2O through a 2 × 40 cm Sephadex G-10 column equipped with an ISCO fraction collector and ISCO UV/vis detector) and C_{18} HPLC (2 mL/min, 10% MeOH−H_2O through a 25 × 1 cm Phenomenex 5 μ ODS column) to yield 1,9-dimethylhypoxanthine (**4**) (42 mg, 0.01% dry wt).

1,9-Dimethylhypoxanthine (4): white powder, mp > 300 °C; UV (H_2O) λ_{max} (log ϵ) 209 (3.7), 260 (3.4) nm; IR (film) ν_{max} 1692, 1649, 1579 cm^{-1}; ^1H and ^{13}C NMR (400 MHz, D_2O), see Table 1; EIMS *m*/*z* 164 (M, 10), 163 (M − H, 100), 162 (97), 142 (10), 135 (15), 121 (16), 108 (20), 107 (10), 82 (28); HREIMS *m*/*z* 164.0700 (calcd for $C_7H_8N_4O$, 164.0698); 163.0618 (calcd for $C_7H_7N_4O$, 163.0620).

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References and Notes

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