6-Methoxy-7-methyl-8-oxoguanine, an Unusual Purine from the Ascidian *Symplegma rubra*

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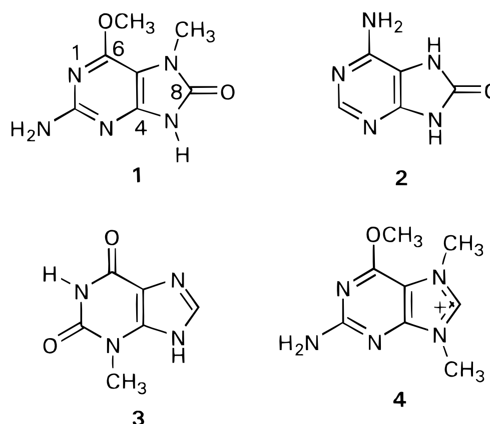
A new purine derivative, 6-methoxy-7-methyl-8-oxoguanine (**1**), along with 8-oxoadenine (**2**) and the human metabolite 3-methylxanthine (**3**), has been isolated from the ascidian *Symplegma rubra* collected on the southeastern coastline of Brazil. The structures of the three purines were established by analysis of spectroscopic data.

Modified purines and nucleosides are commonly encountered in marine organisms, particularly in sponges.[1] They represent an important group of bioactive substances, such as the well-known spongothymidine and spongouridine, which served as models for the development of adenine arabinoside (ARA-A) for treatment of Herpes simplex infection and cytosine arabinoside (ARA-C) for treatment of leukemia, the only marine-derived drugs commercially available.[2] Many other purines and nucleosides isolated from marine organisms display potent bioactivities, such as the antifungal phidolopine from the bryozoan *Phidolopora pacifica*,[3] the hypotensive doridosine (1-methylisoguanosine) isolated from the nudibranch *Anisodoris nobilis*[4] and from the marine sponge *Tedania digitata*,[5] and the cytotoxic mycalisines from the sponge *Mycale* sp.[6] In our continuing search for new bioactive marine natural products,[7,8] we have isolated three purines from the ascidian *Symplegma rubra* (Styelidae) occurring on the southeastern Brazilian coast. Herein we report their isolation and structure elucidation.

S. rubra Monniot, 1972 occurs as three different color variants: yellow, pink, and red morphs. A collection of the differently colored animals was immediately stored in ethanol. After a re-extraction with methanol, the extracts were combined, concentrated, and the aqueous MeOH phase partitioned against hexane. Evaporation of MeOH yielded an aqueous phase that was partitioned against ethyl acetate and CH_2Cl_2−EtOH 3:2. Both the EtOAc and CH_2Cl_2−EtOH extracts showed identical chemical constituents by TLC analysis and were combined. The polar organic extract was subjected to chromatography on a Sep Pak C_{18} reversed-phase column, Sephadex LH20, and finally on C_{18} reversed-phase HPLC, to give **1**, **2**, and **3** in 0.003% (6 mg), 0.002% (3 mg), and 0.0005% (1 mg) wet wt yield, respectively.

HREIMS analysis of **1** gave a molecular formula of $C_7H_9N_5O_2$ (measured: *m/z* 195.0749; $\Delta - 0.7$ mmu), with six degrees of unsaturation. The UV spectrum of **1** showed bands at 210, 246, and 284 nm in methanol. The deceptively simple ^1H NMR spectrum of **1** (DMSO-d_6, 500 MHz) showed four singlets at δ 3.37 (NMe), 3.74 (OMe), 6.56 (NH_2), and 11.38 (NH). These data and the ^{13}C NMR spectrum suggested a purine skeleton, with typical carbon chemical shifts. Analysis of the gHMBC spectrum indicated the position of the *O*-methyl group at C-6 because the singlet at δ 3.74 showed a long-range correlation with C-6 (δ 159.6) and because mass spectroscopic analysis excluded the position of the OMe at either C-2 or C-8 (see below). The *N*-methyl group was located at *N*-7 because it showed long-range correlations with C-5 (δ 103.0) and C-8 (δ 155.1). The carbonyl group was placed at C-8, based of its unusual lowfield resonance at δ 155.1, when compared with common CH-8 purines (usually between δ 140 and 150).[9] Additionally, the EIMS of **1** showed a peak at *m/z* 153 due to the loss of a CH_2N_2 fragment via a retro-Diels−Alder path, as commonly observed for purines,[5,10,11] excluding the location of the carbonyl or *O*-methyl groups at the C-2 position. Finally, comparison of ^1H and ^{13}C NMR data of **1** with data reported for heteromine C (**4**) recently isolated from the terrestrial plant *Heterostemna brownii* (Asclepiadaceae),[12] indicated that the *O*-methyl group was attached at C-6, corroborating our structure proposal and establishing the identity of **1** as 6-methoxy-7-methyl-8-oxoguanine (2-amino-6-methoxy-7-methyl-7,9-dihydro-8*H*-purin-8-one). As far as we know, this is the first report of **1** either as a synthetic or as a natural product.



The molecular formula for **2** of $C_5H_5N_5O$ was derived from HREIMS (measured: *m/z* 151.0525; $\Delta - 0.5$ mmu) and ^1H and ^{13}C NMR data. HREIMS analysis of the retro-Diels−Alder fragment[5,11,12] at *m/z* 124 (measured for $C_4H_4N_4O$: *m/z* 124.0394) confirmed the expulsion of HCN

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as expected for 8-oxoadenine. The UV spectrum of **2** showed absorption bands at 210 and 270 nm in methanol. The ^1H NMR spectrum of **2** (DMSO-d_6, 500 MHz) showed four singlets at δ 6.67 (NH_2), 8.03 (CH) 10.09 (NH), and 11.46 (NH). The ^1H and ^{13}C NMR data, in conjunction with the observed mass spectrum, were in good agreement with those recently reported for synthetic 8-oxoadenine.[13] To the best of our knowledge this is the first report of this compound as a natural product from a marine organism, although it is known as an excretion product of humans suffering from leukemia.[13b]

Compound **3** was assigned a molecular formula of $C_6H_6N_4O_2$ based on HREIMS (measured: 166.0523; Δ +3.2 mmu), ^1H, and ^{13}C NMR data, also suggesting a purine derivative. The UV spectrum of **3** showed bands at 204 and 272 nm in methanol. The ^1H NMR spectrum of **3** (DMSO-d_6, 500 MHz) showed four singlets at δ 3.36 (NMe), 7.99 (CH-8), 11.07 (NH), and 13.5 (NH). In addition to the methyl and methine groups, the ^{13}C NMR spectrum of **3** showed four quaternary carbons, two of which were attributed to carbonyl groups, C-2 at δ 151.1 and C-6 at δ 154.6. Furthermore, the ^{13}C NMR and UV spectra of **3** were characteristic of a methylxanthine.[9] HREIMS analysis of the retro-Diels−Alder fragment at *m*/*z* 123 confirmed the expulsion of HCNO expected for 3-, 7-, or 9-methylxanthine. Key long-range correlations were observed between the *N*-methyl group and C-4 (δ 149.6) and C-2 (δ 151.1), enabling placement of the methyl group at *N*-3, establishing the structure of **3** as 3-methylxanthine (3-methyl-3,9-dihydro-2*H*-6*H*-purine-2,6-dione). 3-Methylxanthine has been previously reported only as a excretion product in humans.[14]

2-Amino-6-methoxy-7-methyl-8-oxopurine (**1**) does belong to the rare class of 8-oxopurines, which also includes caissarone isolated from the endemic Brazilian sea-anemone *Bunodosoma caissarum*[15] and a related 8-oxopurine isolated from the marine sponge *Hymeniacidon sanguinea*.[16] Two modified purines have previously been reported from ascidians of the Styelidae family, namely *Cnemidocarpa bicornuta* and *Botrylloides leachi*: 1,3-dimethylisoguanine and 1,3-dimethylguanine.[17,18] 1,3-Dimethylisoguanine was also isolated from two geographically distinct collections of the marine sponge *Amphimedon viridis*.[10,11] Although 8-oxopurines are considered one of the major products of the oxidative damage of DNA purine bases,[13] the physiological role(s) in the ascidians of these modified purines is currently unknown.

Experimental Section

General Experimental Procedures. UV spectra were obtained in MeOH on a Hewlett−Packard 8452A diode array spectrophotometer. NMR spectra were obtained on a Varian Unity 500 MHz spectrometer, operating at 500 MHz for ^1H and 125 MHz for ^{13}C NMR spectra. All the NMR spectra were obtained at 26 °C using residual signal of nondeuterated solvents as internal reference. HREIMS and LREIMS were obtained on a Finnigan MAT 95 mass spectrometer. HPLC was performed with a Shimadzu LC-9A system, monitoring at 254 nm for preparative separations, and a Beckman 168 diode array system was used for final purification. Solvents employed for extraction and column chromatography were distilled from glass prior to use. HPLC grade solvents were utilized without further purification in HPLC separations. TLC analysis were performed with Aldrich precoated TLC sheets of Si gel on polyester with 254 nm fluorescent indicator eluting with CH_2Cl_2−MeOH 8:2 with a few drops of HOAc acid. Plates were inspected under UV radiation at 254 nm.

Animal Material. *Symplegma rubra* is an encrusting colonial ascidian that presents color patterns varying between yellow and red, and also pink. It is easily recognized by a bright pink ring linking the siphons.[19] The colonies can reach 65 cm of maximum length and are conspicuous on the substrate, though they are more abundant on sites of low-light incidence. The species occurs in the western Atlantic and has been reported from Bermuda (type locality), Guadeloupe, and Brazil (from Rio de Janeiro to Paraná). Both yellow and red color morphs of the ascidian *S. rubra* were collected in the São Sebastião channel (southeastern Brazilian coast) in February 1997, at depths of 2−5 m, and stored in EtOH. Vouchers of *S. rubra* were deposited in the ascidian collection of the Departamento de Zoologia, Setor de Ciências Biológicas, Universidade Federal do Paraná (voucher STY42 − Ponta do Jarobá, São Sebastião).

Extraction and Isolation of Compounds 1, 2, and 3. The ascidian *S. rubra* (190 g wet wt) was separated from the EtOH and blended in MeOH. The EtOH and MeOH extracts were combined and concentrated to ca. 300 mL. The aqueous MeOH suspension was partitioned against hexane. After evaporation of MeOH, H_2O was added to a final volume of 300 mL, and the aqueous phase was partitioned between EtOAc and CH_2Cl_2−EtOH 3:2. The EtOAc and CH_2Cl_2−EtOH extracts were combined based on the similarity of their TLC patterns. The organic material (1.22 g) was subjected to reversed-phase chromatography on a Sep Pak C_{18} column (10 g) with a gradient of MeOH in H_2O. TLC and ^1H NMR analysis (pyridine-d_5, 400 MHz) indicated the presence of purine derivatives in the third fraction obtained from the reversed phase separation. This fraction was subjected to chromatography on Sephadex LH20 (MeOH), yielding five fractions. The fourth fraction (61 mg) contained the purine derivatives, which were further separated by C_{18} reversed-phase HPLC on a Whatman Partisil 10 ODS 3 column (eluent: H_2O−MeOH, 9:1). Fractions containing compounds **1**, **2**, and **3** were finally purified by C_{18} reversed-phase HPLC on a Rainin Dynamax column (10 × 250 mm, 8 μm, eluent: 0.05% aqueous TFA−MeOH 6:1, 5 mL/min), giving pure **1**, **2**, and **3** in 0.003% (6 mg), 0.002% (3 mg), and 0.0005% (1 mg) wet-wt yield, respectively.

6-Methoxy-7-methyl-8-oxoguanine (1): white solid; UV (MeOH) λ max (log ϵ) 210 (4.2), 246 (3.6), 284 (3.9) nm; HREIMS *m*/*z* 195.0749 (calcd for $C_7H_9N_5O_2$, 195.0756); EIMS *m*/*z* (rel int) 195 (100), 166 (28), 153 (11), 117 (3), 69 (23), 42 (5); ^1H NMR (500 MHz, DMSO-d_6) 11.38 (1H, s, NH-9), 6.56 (2H, br s, NH_2), 3.74 (3H, s, OMe), 3.37 (3H, s, NMe); ^{13}C NMR (125 MHz, MeOH-d_4) 159.6 (C-6), 155.1 (C-8), 150.7 (C-2), 147.9 (C-4), 103.0 (C-5), 55.9 (OMe), 28.9 (NMe).

8-Oxoadenine (2): white solid; UV (MeOH) λ max (log ϵ) 210 (3.9), 270 (3.4) nm; HREIMS *m*/*z* 151.0525 (calcd for $C_5H_5N_5O$, 151.0494), 124.0394 (calcd for $C_4H_4N_4O$, 124.0385); EIMS *m*/*z* (rel int) 151 (100), 124 (9), 69 (5), 53 (7); ^1H NMR (500 MHz, DMSO-d_6) 11.46 (1H, s, NH-9), 10.09 (1H, s, NH-7), 8.03 (1H, s, CH-2), 6.67 (2H, br s, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) 152.7 (C-8), 149.6 (C-2), 148.0 (C-6), 145.3 (C-4), 104.4 (C-5).

3-Methylxanthine (3): white solid; UV (MeOH) λ max (log ϵ) 204 (3.7), 272 (3.3) nm; HREIMS *m*/*z* 166.0523 (calcd for $C_6H_6N_4O_2$, 166.0491), 123.0434 (calcd for $C_5H_5N_3O$, 123.0433); EIMS *m*/*z* (rel int) 166 (100), 123 (44), 91 (22), 68 (34), 44 (46); ^1H NMR (500 MHz, DMSO-d_6) 13.50 (1H, br s, NH-1), 11.07 (1H, s, NH-9), 7.99 (1H, s, CH-8), 3.36 (1H, s, NMe); ^{13}C NMR (125 MHz, DMSO-d_6) 154.6 (C-6), 151.1 (C-2), 149.6 (C-4), 140.4 (C-8), 106.7 (C-5), 28.7 (NMe).

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