1,3-Dimethylisoguanine, a New Purine from the Marine Sponge *Amphimedon viridis*

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A new purine, 1,3-dimethylisoguanine (**1**), has been isolated from the marine sponge *Amphimedon viridis* and identified by analysis of spectroscopic data. Compound **1** increased the contractions obtained by transmural electrical stimulation in the guinea pig longitudinal muscle/myenteric plexus in a dose-dependent manner.

During the past few decades, marine sponges have been shown to be one of the most prolific sources of natural bioactive compounds with unusual structural features.[1−4] In particular, sponges of the genus *Amphimedon* (Niphatidae, Haplosclerida, Demospongiae) have provided different types of natural products, such as alkylpyridine- and alkylpiperidine-type alkaloids,[5] a polycyclic heteroaromatic alkaloid,[6] an unusual terpene,[7] several unusual fatty acids,[8] and steroids.[9] Many authors have observed that aqueous and alcoholic extracts of *Amphimedon viridis* Duchassaing & Michelotti 1864 (previously known as *Haliclona viridis*) presented several potent biological activities, mostly due to halitoxin,[10,11] but also due to the presence of other biologically active compounds.[12] We have recently initiated a program in search of biologically active compounds from marine sponges and observed that polar extracts of *A. viridis* were the most active in antimicrobial, hemolytic, and antimitotic bioassays.[11,13] We now wish to report the structure of a new purine isolated from *A. viridis*, 1,3-dimethylisoguanine (**1**).

1,3-Dimethylisoguanine was isolated from both aqueous and *n*-BuOH extracts of *A. viridis*[11] by a series of chromatographies on XAD-2 styrene-divinylbenzene copolymer, Sephadex LH20, BIOGEL P-2, C-18 reversed-phase and Si gel Lobar columns and purification by C-18 reversed-phase HPLC (3:7 MeOH−0.1% TFA), giving **1** as a white powder (a total yield of 0.0043% of wet sponge).

HREIMS analysis of compound **1** indicated the formula $C_7H_9N_5O$, consistent with a purine derivative isomeric with longamide (3,7-dimethylisoguanine) from *Agelas longissima*,[14] 1,9-dimethyl-6-imino-8-oxopurine from *Hymeniacidon sanguinea*,[15,16] and herbipoline (7,9-dimethylguanine) from *Geodia gigas*.[17] The IR and UV (MeOH) spectra of 1,3-dimethylisoguanine showed bands at 3300, 3100, 2970, 2918, 2870, 1694, 1644, and 1609 cm^{-1} and at λ_{max} 206.8 and 294.6 nm, respectively. The ^{1}H-NMR spectrum of 1,3-dimethylisoguanine (**1**) was deceptively simple, showing two methyl signals (δ 3.60 and 3.64) and a methine proton at δ 8.12 (MeOH-d_4) and two very broad NH protons (δ 8.62) in DMSO-d_6.

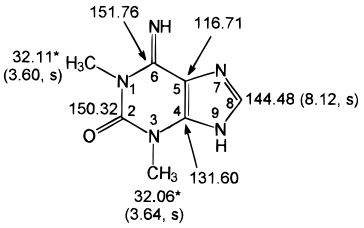

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Figure 1. Structure and ^{1}H- (400.35 MHz) and ^{13}C-NMR data (100.1 MHz, MeOH-d_4) of 1,3-dimethylisoguanine. *Assignments may be interchanged.

The ^{13}C-NMR spectrum of **1** showed four quaternary carbons (δ 151.76, 150.32, 131.60, and 116.71), one methine (δ 144.48), and two methyl carbons (δ 32.11 and 32.06). All these data indicated it to have a dimethylisoguanine-like structure. In several HMBC spectra (8, 5, 4, and 3 Hz), long-range couplings were observed between both methyl protons and the carbon at δ 150.32, but a coupling between the methyl protons at δ 3.60 and the carbon at δ 151.76 could only be evidenced in the 3-Hz HMBC spectrum. Comparison of the ^{13}C- and ^{1}H-NMR data for the above-mentioned purine derivatives,[14−16] as well as those of doridosine,[18] 1-methylisoguanosine,[19] caissarone,[20] and several other purines,[21] excluded the possibility of methyl substitution at nitrogens 7 or 9. Indeed, in the latter cases, the methyl signals would be expected to resonate at lower field. 1,6- or 3,6-Dimethyl-substitution is incompatible with the spectroscopic data, due to the tautomerization of the C=N imino double bond and the subsequent bathochromic shift of the UV absorption, and also the shielding of C-5[19] in the case of 1,6-dimethyl substitution. All the above results clearly indicated the structure of **1** to be 1,3-dimethylisoguanine (Figure 1). In the HREIMS compound **1** exhibited fragment ions corresponding to $[M - CHO]^+$ and $[M - MeNCO - H]^+$ caused by cleavage of N-1/C-6 and C-2/N-3 bonds or N-1/C-2 and N-3/C-4 bonds from the isoguanosine skeleton attached to two methyl groups at *N*-1 and *N*-3.[14,19]

1,3-Dimethylisoguanine (**1**) was essentially inactive in hemolytic and antimitotic bioassays but caused an increase in the contractions obtained by transmural electrical stimulation in the guinea pig longitudinal muscle/myenteric plexus, a bioactivity also reported for caissarone, an iminopurine isolated from the sea

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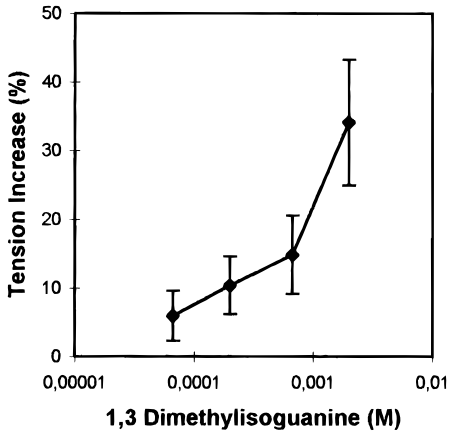

Figure 2. Increments in tension developed by transmurally stimulated guinea pig longitudinal muscle/myenteric plexus with increasing 1,3-dimethylisoguanine concentration. Each point shows the mean and standard error of three experiments.

anemone *Bunodosoma caissarum*.[20] Caissarone also increases the gut motility by antagonism to the adenosine receptor;[22,23] 1,3-dimethylisoguanine had a similar but weaker response. While caissarone acts in a concentration of 3×10^{-4}M, enhancing by 80% the tension in this preparation, 1,3-dimethylisoguanine requires a concentration of 2×10^{-3}M and gave only a $34 \pm 9.17\%$ enhancement (Figure 2).

The structure of **1** is, to the best of our knowledge, new in nature. During the submission of this paper, we became aware of the isolation of **1** from the same sponge by Mitchell *et al*.[24] Modified purine and pyrimidine bases as well as unusual nucleosides are of widespread occurrence in marine organisms;[2,21] 1,3-dimethylisoguanine (**1**) is an additional member of this interesting class of compounds.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a FT-IR Bomem MB102 infrared spectrometer. UV spectra were recorded in MeOH on a Shimadzu UV-180 spectrophotometer. NMR spectra were run either on a Bruker AC-4.7 T spectrometer, operating at 200.1 MHz for ^{1}H- and 50.3 MHz for ^{13}C-NMR spectra, or on Bruker DRX or ARX 9.4 T instruments, operating at 400.35 MHz for ^{1}H and 100.10 MHz for ^{13}C channels. All the NMR spectra were obtained at 28 °C using TMS as internal reference. HREIMS were obtained on a Fisons Autospec Instrument. HPLC was performed with a Shimadzu LC-9A system, monitoring at 254 nm. Solvents employed for extraction and gel permeation chromatography were glass distilled prior to use. HPLC-grade solvents were utilized without further purification in HPLC separations. TLC analyses were performed with Aldrich precoated TLC sheets of Si gel on polyester with a 254-nm fluorescent indicator eluting with two mixtures: EtOH−NH_4OH 8:2 (eluent 1) and CH_2Cl_2−MeOH 8:2 with a few drops of HOAc (eluent 2). Plates were developed by observing at 254 nm and subsequently by spraying with ninhydrin reagent in EtOH and further heating at 120 °C.

Sponge Material. The sponge *A. viridis* was collected in the São Sebastião channel (southeastern Brazilian coast) in January 1994, at depths of 3−5 m, and stored in EtOH. Vouchers of *A. viridis* were deposited in the Porifera collections of the Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJPOR 4459) and Zoological Museum, University of Amsterdam (ZMAPOR 10985).

Extraction and Isolation of 1,3-Dimethylisoguanine 1. (a) From the MeOH-Soluble Aqueous Extract. The sponge *A. viridis* (600 g wet) was processed as previously described,[11] giving 11.5 g of the MeOH-soluble aqueous extract. XAD-2 (ca. 200 g) was added to the extract dissolved in 500 mL of distilled H_2O, which was mechanically stirred in a shaker during 14 h. The aqueous extract was then filtered, and the XAD-2 copolymer was washed with 50 mL of distilled H_2O. The XAD-2 copolymer was extracted with MeOH (1L) for 2 h and then with MeOH-Me_2CO 1:1 (1 L) for 2 h with occasional stirring. The organic fractions were combined and evaporated to give 1.25 g of a brownish gum that was divided into two portions. Both portions were applied to a Sephadex LH20 column (170.0 × 2.0 cm) and eluted with CH_2Cl_2−MeOH 1:1 to give three fractions, F1 (0.3372 g), F2 (0.6520 g), and F3 (0.0559 g). F2 was submitted to further chromatography on Sephadex LH20 with the same eluent, with a further separation into two fractions, F2.1 (0.0218 g) and F2.2 (0.4235 g). F2.2 was applied to a previously equilibrated column (170.0 × 2.0 cm) of BIOGEL P2 in H_2O−EtOH (8:2) with elution at 0.5 mL/min flow rate. This separation afforded 94 fractions, which were analyzed by TLC (eluents 1 and 2) and combined according to their chromatographic pattern, yielding eight fractions: F2.2.1−F2.2.8. ^{1}H-NMR, TLC (eluents 1 and 2), and reversed-phase HPLC analysis of fraction F2.2.7 (0.0115 g) indicated an almost pure compound, which was purified on a C-18 reversed-phase HPLC column (Waters Microbondapak 300.0 × 4.0 mm; 10.0 μm), eluting with 3:7 MeOH−0.1% TFA, to give pure 1,3-dimethylisoguanine (**1**) (0.0060 g).

(b) From the *n*-BuOH Extract. The second fraction (5.59 g), obtained after chromatography of the *n*-BuOH extract on Sephadex LH20,[11] was applied in portions of ca. 0.2 g to a BIOGEL P-2 column as above. After TLC evaluation (eluent 1), nine fractions were obtained, Fbg1−Fbg9. The Fbg2 fraction (2.4303 g) was submitted to a reversed-phase chromatography on a Merck Lobar Lichroprep RP-18 column (310 × 25 mm; 40−63 μm) and eluted with a gradient of MeOH in H_2O, giving six fractions Fbg2.1−Fbg2.6. The two last fractions, Fbg2.5 (0.2440 g) and Fbg2.6 (0.1490 g), were separated by chromatography on a Merck Lobar Lichroprep Si-60 column (310 × 25 mm; 40−63 μm) with a gradient of MeOH in CH_2Cl_2. Crude **1** was obtained from both fractions. It was further purified by HPLC as described above, giving a total amount of 0.0200 g of the pure compound.

1,3-Dimethylisoguanine (1): white powder; UV (MeOH) λ_{max} (log ϵ) 206.8 (4.5), 294.6 nm (3.9); IR (liquid film on silicon plate) 3300 (broad, NH), 3100 (CH, aromatic), 2970−2870 (CH, aliphatic), 1694 (C=O, urea), 1644 (C=N) and 1609 (C=C) cm^{-1}; HREIMS *m/z* 179.080 20 (calcd for $C_7H_9N_5O$, 179.080 71) (100), 150.077 84 (calcd for $C_6H_8N_5$, 150.077 97) (57), 121.051 21 (calcd. for $C_5H_5N_4$, 121.051 42) (27), 94.040 27 (calcd for $C_4H_4N_3$, 94.040 52) (17); ^{1}H-NMR (400.35 MHz, MeOH-d_4), see Figure 1; ^{13}C-NMR (100.1 MHz, MeOH-d_4), see Figure 1.

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