respectively. In parallel experiments, **15a** (3.2 mg), **16a** (2.6 mg), and **17a** (2.2 mg), each dissolved in isooctane (2 mL), were heated at reflux (~100 °C; oil bath temperature 110 °C) under $N_2$ for 40 h. Analysis of the product mixture from each thermolysis experiment by high-pressure LC (Partisil; 40% diisopropyl ether/skellysolve B) revealed the presence of the same equilibrium mixture (integration of the refractive index detector trace) of **15a**, **16a**, and **17a**: 8, 17, and 75% from **15a**; 7, 19, and 74% from **16a**; 7, 17, and 76% from **17a** (average: 7, 18, and 75% of **15a**, **16a**, and **17a**, respectively, with an average deviation of ±1%). In the case of the **17a** thermolysis, the products were collected preparatively by high-pressure LC, and then the absolute yields of products were estimated by UV calculations. The results were as follows: **15a** (0.12 mg), **16a** (0.75 mg), and **17a** (1.1 mg) for a mass balance of ~91%. The relative amounts of the three products correspond to 8, 17, and 75%, respectively, which agree well with the uncorrected refractive index detector trace integration.

**(10) Thermal Equilibration of 15b, 17b, and 16b.** A solution of 1*S*,10*S* isomer **17b** (64 mg, 0.15 mmol) in isooctane (16 mL, freshly distilled from $LiAlH_4$ under argon) was heated (100 °C) under argon for 40 h. NMR analysis of the residue after solvent removal revealed the following composition: **15b** (9%), **17b** (27%), and **16b** (64%). The ratio of **15b** to **17b**/**16b** was obtained by integrating the $\tau$ 4.16 ($H_{6,7}$ of **15b**) and 4.55 ($H_{15}$ of **17b** and **16b**) signals; the ratio of **17b**/**16b** to **16b** was obtained by integrating the $\tau$ 6.1–6.4 ($H_1$ of **17b** and **16b**) and ~6.8 ($H_{10}$ of **16b**) signals.

Similar thermolysis of **16b** (59 mg in 15 mL of isooctane, 40 h reflux, ~100 °C) revealed the following composition of products (analyzed by NMR as for **17b**): **15b** (7%), **17b** (26%), and **16b** (67%).

A solution of **15b** (~10 mg) in isooctane (3 mL) was heated as above for 32 h. Concentration and then preparative high-pressure LC (Partisil M9 10/50 column; 40% diisopropyl ether/skellysolve B; refractive index detector) afforded **15b** (0.6 mg, 10%; assuming $\epsilon_{253}$ ~14000), **17b** (1.4 mg, ~23%; assuming $\epsilon_{274}$ ~20000), and **16b** (4 mg, ~67%; assuming $\epsilon_{273}$ ~20000). Direct integration of the high-pressure LC trace (refractive index detector without correction) afforded the values 14, 25, and 62%, respectively. The average of the two determinations were 12% **15b**, 24% **17b**, and 65% **16b**.

The overall average equilibrium product distributions for the three separate thermolyses were 9 ± 2% **15b**, 26 ± 1% **17b**, and 65 ± 1% **16b** (average deviations are given).

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**Registry No. 3a**, 74398-18-8; **3b**, 74398-19-9; **4a**, 74398-20-2; **4b**, 74398-21-3; **5**, 1125-11-7; **6**, 74398-22-4; **7a**, 74398-23-5; **7b**, 74398-24-6; **9**, 74398-25-7; **10a**, 67632-48-8; **10b**, 67670-78-4; **10c**, 74398-26-8; **11a**, 67670-79-5; **11b**, 67670-80-8; **11c**, 74398-27-9; **12**, 74398-28-0; **15a**, 74431-69-9; **15b**, 74398-29-1; **16a**, 74398-30-4; **16b**, 74398-31-5; **17a**, 74431-20-2; **17b**, 74431-21-3.

**Supplementary Material Available:** Spectral and analytical data (7 pages). Ordering information is given on any current masthead page.

# 1-Methylisoguanosine, a Pharmacologically Active Agent from a Marine Sponge

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Aqueous ethanolic extracts of the marine sponge *Tedania digitata* possessed a number of pharmacological properties. The active constituent, a new N-methylated purine nucleoside, was isolated by ion-exchange chromatography and the structure elucidated by spectral methods, particularly $^{13}C$ NMR and mass spectroscopy, combined with chemical degradations. Mild acid hydrolysis of the glycosyl bond gave D-ribose while strong acid hydrolysis gave 1-methylxanthine (**3**), establishing the position of methylation of the purine nucleus. Synthesis by two routes confirmed the structure as 1-methylisoguanosine (**1**). Synthesis from 5-amino-4-carbamoyl-1β-D-ribofuranosylimidazole (**5**) proceeded in high overall yield, employing cyclization of the protected 5-amino-4-cyanoimidazole (**7**) with methylisocyanate to form the correctly substituted pyrimidine ring of **1**.

Methylated nucleosides have been isolated from a variety of natural sources. Transfer ribonucleic acid (tRNA) has been shown to contain a number of methylated components, over 20 of which have been isolated and identified.[1] Messenger RNA has been shown to possess several methylated species, associated primarily with the 5′-terminal region.[2] Bacterial sources have provided methylated nucleosides such as puromycin[3] and 5,6-dihydro-5-azathymidine,[4] and 2-methoxyadenosine (spongosine) has been isolated from a sponge.[5] Recently a new methylated purine nucleoside, 1-methylisoguanosine (**1**), has been isolated from the marine sponge *Tedania digitata*[6] and

(1) S. Nishimura, *Prog. Nucleic Acid. Res. Mol. Biol.*, **12**, 49–85 (1972).
(2) F. M. Rottman, R. C. Desrosiers, and K. Friderici, *Prog. Nucleic Acid Res. Mol. Biol.*, **19**, 21–38 (1976).
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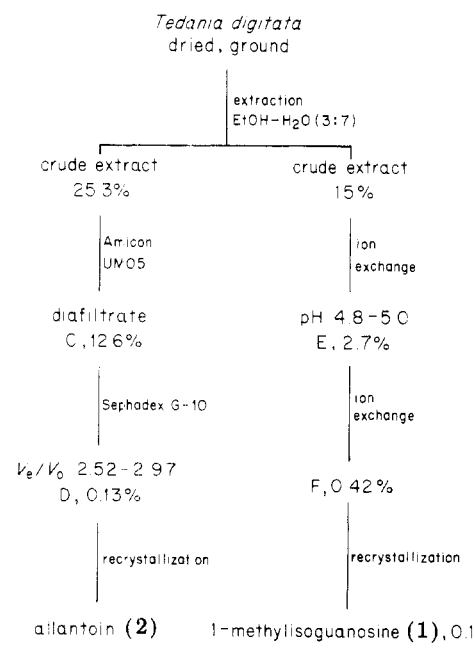
Scheme I




shown to possess potent pharmacological activity.[7-9] We herein present a detailed description of the isolation, structural elucidation and two syntheses of 1-methylisoguanosine from readily available starting materials. The isolation of doridosine, a new *N*-methylpurine nucleoside tentatively assigned the structure of 1-methylisoguanosine, has also recently been reported from the marine nudibranch *Anisodoris nobilis*.[10]

Fractionation (Scheme I) of the aqueous ethanolic extract of *T. digitata*, guided by pharmacological assay, revealed that the active constituent was a water-soluble component of small molecular weight which passed through a membrane of 500 Daltons nominal exclusion limit. Chromatography of the diafiltrate on Sephadex G-10 gave the active fraction as a retarded peak. A pharmacologically inactive constituent crystallized from an aqueous solution of the active Sephadex G-10 fraction and was shown by X-ray analysis to be allantoin (**2**),[11] a product of purine metabolism. Investigation of the Sephadex G-10 fraction showed that the active constituent could be adsorbed onto cation-exchange resins from dilute acid solutions and eluted with ammonia solutions. Thus, it was possible to develop a method to chromatograph the crude extract directly on the sulfonic acid cation-exchange resin Bio-Rad AG 50W-X8, using the lyophilizable buffer ammonium formate. Adsorption of the active constituent onto the resin was achieved from a solution of the crude extract buffered at pH 3.5. After being thoroughly washed with ammonium formate (pH 3.5, 0.1 M formate), the column was eluted with ammonium formate (pH 5.3, 0.1 M formate). It was found that the activity corresponded to a large UV-absorbing peak and that the pH of the active fractions had been maintained between pH 4.8 and 5.0, due to the buffering capacity of the active constituent. The

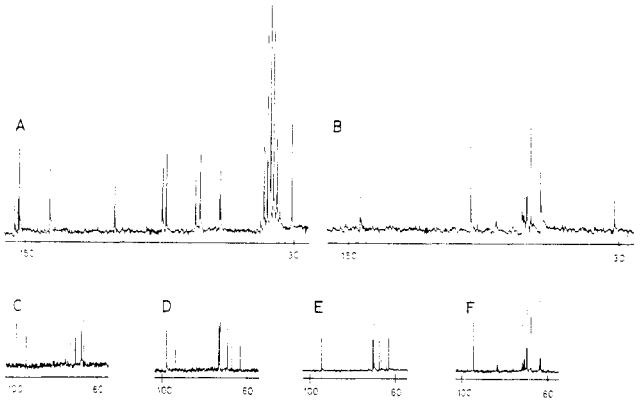



**Figure 1.** $^{13}C$ NMR spectra of (A) 1-methylisoguanosine in $Me_2SO$-$d_6$, (B) 1-methylisoguanosine in $HCl/D_2O$ after 7 h, (C) D(+)-xylose in $D_2O$, (D) D(−)-arabinose in $D_2O$, (E) D(−)-lyxose in $D_2O$, (F) D(−)-ribose in $D_2O$; 1% dioxane at 67.4 ppm was used as internal reference for spectra run in $D_2O$.

active fractions from two runs were combined and rechromatographed under the same conditions. After lyophilization, the active fraction was recrystallised from water to give 1-methylisoguanosine (**1**). This method had a high capacity, allowing the isolation of 2.75 g of **1** (0.71% of the crude extract, 0.11% of the dry organism). **1** was responsible for all the pharmacological effects of the crude extract.

The $^{13}C$ NMR spectrum of **1** revealed that it contained 11 carbon atoms. The presence of a $C_5$ sugar as a furanoside was apparent from doublets at 87.6 ($C_{1'}$), 86.0 ($C_{4'}$), 72.9 ($C_{2'}$), and 70.7 ($C_{3'}$) and a triplet at 61.8 ($C_{5'}$) ppm.[12,13] The high-resolution electron-impact mass spectrum showed a molecular ion, which was confirmed by chemical ionization and field desorption, at *m/e* 297 for $C_{11}H_{15}N_5O_5$. A fragmentation pattern characteristic of nucleosides[14] was observed with the base peak at *m/e* 165 corresponding to the nucleoside base (B) + H and ions at *m/e* 267 (M − $CH_2O$), 208 (B + 44), 194 (B + 30), 166 (B + 2H). This data, together with singlets at 153.8, 152.1, 151.5, and 108.9 assignable to $C_2$, $C_4$, $C_6$, and $C_5$ and a doublet at 138.0 ppm for $C_8$ in the $^{13}C$ NMR spectrum, suggested that **1** contained a purine moiety[11,12] with the composition $C_6H_6N_5O$. A $^{13}C$ resonance (quartet) at 30.0 ppm was assigned to an *N*-methyl group.

Correlation of the $^{13}C$ NMR and mass spectral data suggested that **1** was a pentoside of an *N*-methylguanine or *N*-methylisoguanine. The $C_5$ resonance of **1** occurred at 108.9 ppm while $C_5$ in 1- and $N^2$-methylguanosine occurred at 115.4 and 116.8 ppm, respectively. 7-Methylguanosine is zwitterionic and, although its $C_5$ resonance (107.4 ppm) corresponded to $C_5$ in **1**, the *N*-methyl group resonated 5.5 ppm downfield from that of **1**. The upfield shift of $C_5$ in **1** of approximately 7 ppm relative to 1- and $N^2$-methylguanosine favors an *N*-methylisoguanine as the partial structure for **1**. During synthetic work, described later, isoguanosine was synthesized and $C_5$ resonated at 111.1 ppm. The isoguanine structure was further supported by the UV spectra, since a comparison of the spectra of **1** with published data for isoguanosine[15,19] under acidic, neutral, and alkaline conditions revealed close similarities in each case.

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(7) J. Baird-Lambert, J. F. Marwood, L. P. Davies, and K. M. Taylor, *Life Sci.*, **26**, 1069–1077 (1980).
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Dilute acid hydrolysis of the glycosyl bond of 1 gave the sugar and the nucleoside base. The hydrolysis was monitored by $^{13}C$ NMR spectroscopy and this provided a convenient method for the identification of the sugar moiety. A comparison of the $^{13}C$ NMR spectra of the four aldopentoses with that of the hydrolysis reaction mixture (Figure 1) established that ribose was the sugar. After removal of the insoluble base, the hydrolysate was deionized, using a mixed-bed ion-exchange resin, to give ribose in 91% yield. Paper chromatography confirmed the identity of ribose and the optical rotation established that it was the D enantiomer. The hydrochloride of the nucleoside base, obtained by centrifugation of the hydrolysis mixture, gave a molecular ion at $m/e$ 165 as the base peak, and fragmentation ions occurred at $m/e$ 148, 136, 109, 108, and 81. The position of methylation in xanthines can be readily obtained from their mass spectral fragmentation.[16] A major fragmentation is via a retro-Diels–Alder reaction involving the $N_1$ and $C_2$ atoms such that 1-methylxanthine gives a peak at $m/e$ 109 ($M^+$ (166) - $CH_3NCO$) while 3-methylxanthine gives a corresponding peak at $m/e$ 123 ($M^+$ (166) - HNCO). The loss of $CH_3NCO$ provided further evidence for 1-methylisoguanosine as the structure of 1.

The position of methylation in 1 was established by conversion to the corresponding methylxanthine. Reaction of 1 with nitrous acid gave recovered starting material and the resistance of an amino group at $C_6$ to hydrolysis by nitrous acid in the presence of a $C_2$-oxo group is well-documented.[17,18] However, isoguanine derivatives can be converted to xanthine analogues by mineral acid hydrolyses[17,18] and thus reaction of 1 with concentrated hydrochloric acid under reflux gave 1-methylxanthine (3), establishing the position of methylation.

The $^1H$ NMR spectrum of 1 revealed the presence of an N–$CH_3$ group at $\delta$ 3.36. The anomeric hydrogen occurred as a doublet at $\delta$ 5.62, but the coupling constant (6 Hz) did not provide an indication of the configuration at the $C_{1'}$ atom.

Thus it was established that 1 was a D-ribofuranosyl-1-methylisoguanine. While 9$\beta$-substitution may be expected by analogy with the common naturally occurring nucleosides, the 9$\alpha$, 7$\alpha$, and 7$\beta$ isomers could not be excluded. Previous studies on purines[13] have demonstrated that, when the site of attachment of a $\beta$-D-ribofuranosyl moiety is changed from $N_9$ to $N_7$, a downfield shift of the $C_4$ signal approximately equal to an upfield shift of the $C_5$ resonance is expected. The corresponding resonances of the unsubstituted purine then depend upon the prototropic tautomerism of the imidazole moiety [N(7)H ⇌ N(9)H]. Thus, the site of substitution can be obtained from the nucleoside and the corresponding purine with the N(7)H isomer having $C_4$ downfield and $C_5$ upfield and the N(9)H isomer having $C_4$ upfield and $C_5$ downfield of the corresponding resonances of the purine. In 1 $C_4$ was at 152.1 and $C_5$ at 108.9 ppm while the corresponding purine (4) gave $C_4$ at 153.2 and $C_5$ at 108.6 ppm. While the magnitudes of the shifts are small, the directions are consistent with $N_9$ substitution. As an internal reference the N–$CH_3$ group occurred at 30.0 ppm in both compounds. As the observed shifts between $N_7$ and $N_9$ isomers are of the order of 10 ppm,[13] the small observed shifts indicate that the purine, 1-methylisoguanine, exists predominantly in the tautomer bearing hydrogen at the same position as the ribofuranosyl moiety and (vide infra) is predominantly the N(9)H tautomer.

Table I. Mass Spectral Data of 1

| | mass | | | |
|---|---|---|---|---|
| $m/e$ | measured | calcd | fragment | assignment |
| 297 | 297.1071 | 297.1073 | $C_{11}H_{15}N_5O_5$ | $M^+$ |
| 279 | 279.0968 | 279.0967 | $C_{11}H_{13}N_5O_4$ | $M^+ - H_2O$ |
| 267 | 267.0969 | 267.0967 | $C_{10}H_{13}N_5O_4$ | $M^+ - CH_2O$ |
| 208 | 208.0835 | 208.0834 | $C_8H_{12}N_5O_2$ | B + 44 (C1′, C2′) |
| 194 | 194.0676 | 194.0678 | $C_7H_8N_5O_2$ | B + 30 (C1′ + O) |
| 166 | 166.0726 | 166.0729 | $C_6H_8N_5O$ | B + 2H |
| | 166.0570 | | | |
| 165 | 165.0653 | 165.0651 | $C_6H_7N_5O$ | B + H |
| 148 | 148.0389 | 148.0385 | $C_6H_4N_4O$ | BH - $NH_3$ |
| 136 | 136.0383 | 136.0385 | $C_5H_4N_4O$ | BH - [$CH_2$=NH] |
| 108 | 108.0439 | 108.0436 | $C_4H_4N_4$ | BH - $CH_3NCO$ |
| 81 | 81.0342 | 81.0327 | $C_3H_3N_3$ | 108 - HCN |
| | 81.0710 | | | |

Further discussion of the mass spectral fragmentation pattern of 1 is appropriate to delineate the behavior of isoguanosines in relation to other purine nucleosides. The mass spectral fragmentation pattern was consistent with that obtained for other nucleosides and high-resolution data and assignments[13] are given in Table I. Elimination of the elements of formaldehyde from the 5′-hydroxyl group is an important structural indicator of the 5′ position. The mechanistic model for fragmentation[13] requires steric accessibility of the 5′-hydroxyl hydrogen to the base so that the 9$\beta$ series gives the fragmentation while in the 9$\alpha$ series the ion is absent or markedly reduced in intensity. The ion at $m/e$ 267 was shown to be M - $CH_2O$, although of low intensity (~1%), and a linked scan such that the magnetic ($B$) and electrostatic ($E$) fields were scanned with the ratio $B/E$ constant confirmed that the ion occurred by direct fragmentation of the molecular ion. Of particular relevance to the structural elucidation of 1 was the loss of the N(1)–C(2) unit from the base peak ($m/e$ 165). The direct loss was demonstrated by a linked $B/E$ scan and high-resolution measurements confirmed the structure BH–$CH_3NCO$ so that, as is the case with the methylxanthines,[16] this loss can establish the position of methylation in the isoguanosine series. A sample of doridosine[10] was obtained for direct comparison with 1. These samples proved to be identical when compared by spectral methods (mass spectra, $^{13}C$ NMR) as well as by comparison of $[\alpha]_D$ values and high-performance LC retention times.

Synthesis of 1-methylisoguanosine was achieved via two routes. One method involved cyclization to form the pyrimidine ring from an imidazole precursor already bearing a 9$\beta$-D-ribofuranosyl group. The second method involved direct alkylation of the parent nucleoside, isoguanosine. 5-Amino-4-carbamoyl-1$\beta$-D-ribofuranosylimidazole-5′-phosphate (AICAR) is an intermediate in the biosynthetic pathway to inosine and thence the other naturally occurring purine nucleosides, and thus compound 5, produced by fermentation, was readily available. Elaboration of 1-methylisoguanosine from 5 required insertion of the $CH_3$—N(1)—C(2)=O entity, in effect the reverse of the mass spectral fragmentation, and this could be visualized as accessible via methyl isocyanate addition to a 5-amino-4-cyanoimidazole.

5-Amino-4-carbamoyl-1$\beta$-D-ribofuranosylimidazole (5) was converted into its tri-*O*-acetyl derivative (6), which was then transformed into the nitrile (7) by treatment with phosphorus oxychloride, according to the method of Suzuki and Kumashiro.[19] The crude nitrile was contaminated

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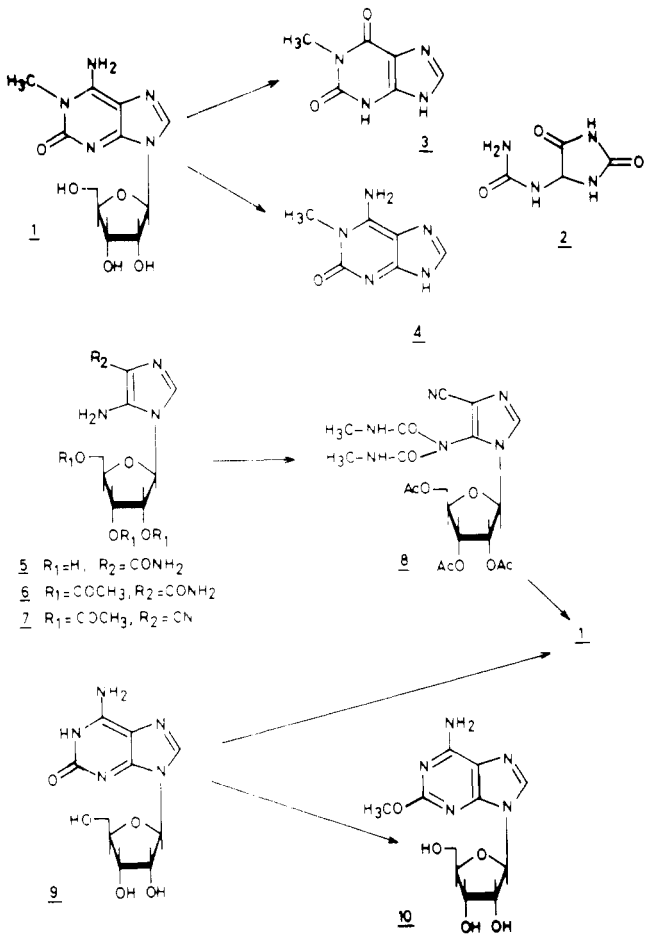
Scheme II



with highly colored materials, but passage through a silica column removed most of these to give almost pure **7** as a pale yellow foam (Scheme II).

Cyclization of **7** with methyl isocyanate was studied under a wide variety of conditions. Reaction in dioxane at 100 °C led to recovery of starting material, and addition of triethylamine to the reaction mixture only induced partial deacetylation. No reaction was detected in pyridine at room temperature, and decomposition of **7** with the formation of fluorescent products was observed when the reaction was run at 100 °C. No reaction was detected in the absence of solvent, or in chloroform at room temperature, in either the presence or absence of triethylamine.

In a reaction of the nitrile **7** with methyl isocyanate in dimethylformamide at 100 °C, the starting material was slowly consumed with the formation of a new, weakly UV-absorbing product. The product was isolated as a foam after silica column chromatography and determined to be the bis(methylcarbamoyl) adduct **8**. The IR spectrum of **8** clearly indicated the presence of the nitrile substituent (2245 $cm^{-1}$), and the NMR spectrum revealed three-proton doublets at $\delta$ 2.72 and 2.63 which coalesced into singlets on addition of $D_2O$, indicating the presence of two $CH_3NH$ groups.

NMR measurements at higher temperature demonstrated that these two signals coalesced into one doublet, their nonequivalence at lower temperature possibly being due to stabilization by hydrogen bonding. Treatment of **8** with aqueous ammonia induced cyclization with concomitant deacetylation to produce 1-methylisoguanosine (**1**) in crystalline form and in high yield. For routine purposes it was not necessary to isolate the intermediate **8**; after reaction of **7** with methyl isocyanate, the reaction mixture was evaporated to dryness and treated directly with methanolic ammonia. Crystals of **1** could be obtained by evaporation and recrystallization. The synthetic sample of **1** was identical with the natural product as determined by mixture melting point, IR, UV, NMR ($^1H$, $^{13}C$), and mass spectra, and elemental analysis, as well as chromatographic properties.

An alternate method for the synthesis of **1** involved the direct methylation of isoguanosine (**9**). Synthesis of the latter compound was achieved by the method of Davoll[20] by partial deamination of 1$\beta$-D-ribofuranosyl-2,6-diaminopurine. Reaction of **9** with methyl iodide and potassium carbonate in dimethyl sulfoxide yielded a mixture of two compounds which were separated by silica column chromatography. These were identified as 1-methylisoguanosine (**1**) and 2-methoxyadenosine (**10**), both of which were obtained in relatively poor yields. 2-Methoxyadenosine (**10**) has previously been synthesized by reaction of 2-methoxyadenine with a suitably protected ribofuranose derivative,[21] and although these authors also obtained chromatographic evidence of **10** by methylation of isoguanosine, **1** was not identified from this reaction.

The use of other methylating agents for the methylation of **9** was not investigated since the cyclization route provided a much more efficient route for the synthesis of **1**. The cyclization route was particularly suitable for large scale synthesis of **1**. Thus, the intermediate **7** (800 g) was converted to **1** (279 g, 43% yield after two recrystallizations). **1** was available for evaluation of its pharmacological properties and as an intermediate for the synthesis of analogues. The synthesis and pharmacological activities of these analogues and other analogues accessible via the use of other isocyanates in the cyclization step will be reported elsewhere.

## Experimental Section

**General.** Water used in fractionations was distilled and saturated with chloroform. An Amicon high-performance filtration system TCF 10 was employed for diafiltration. Melting points were determined by using a Mettler FP52/FP5 and a Thomas Hoover apparatus and are uncorrected. Thin-layer chromatography was performed on precoated Merck plates of silica gel 60 F254 of 0.25-mm layer thickness and visualization was achieved by using short-wavelength UV light. Paper chromatography was performed on Whatman No. 1, using descending flow, and visualization was achieved by using anisidine hydrochloride. Column eluates were monitored, via a series of flow cells, by UV absorption. An Isco Model UA-5 absorbance monitor coupled to a Model 1132 multiplexer-expander was employed to monitor column eluates at 254 and 280 nm. Optical rotations were measured with a Jasco Model DIP-4 digital polarimeter. $^{13}C$ nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-FX60 and a Varian XL-100 spectrometer. Samples for NMR were dissolved in $Me_2SO$-$d_6$ unless otherwise stated; carbon chemical shifts were calculated relative to the internal reference ($Me_2SO$-$d_6$) and converted to the $Me_4Si$ scale, by using $\delta_{Me_4Si} = \delta_{Me_2SO} + 39.5$. $^1H$ NMR spectra were recorded on a JEOL JNM-MH-100 or a Varian XL-100 or HA-100 spectrometer. Low-resolution mass spectra were recorded with a Varian MAT-CH7 mass spectrometer. High-resolution mass spectra were recorded with a VG Micromass 7070 coupled to a VG2035 data system and with an AEI MS902/DS-30. IR spectra were recorded with a Perkin-Elmer 621 or a Beckman IR-9 instrument. UV spectra were obtained by using a Cary Model 14 recording spectrometer.

**Bioassay.** Intraperitoneal injection of mice, at a dose equivalent to 1 g/kg of the crude extract, gave pronounced muscle relaxation and hypothermia which acted as a decisive, reproducible assay for individual fractions during the purification of **1**. Every

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(21) W. Bergmann and M. F. Stempien, Jr., *J. Org. Chem.*, **22**, 1575–1577 (1957).

fraction of each chromatographic separation was assayed and a histogram of activity was obtained.

**Collection.** The orange sponge *Tedania digitata* (Schmitz) (RRIMP Museum Specimens FN 1050 and FN 1248) was collected at a depth of 5–10 m on the southern side of Newport Reef, 33°39′55″S, 151°19′50″E, near Sydney, Australia. A small quantity was also collected at Fairlight, 33°48′10″S, 151°16′15″E, at a depth of 2 m. The organism was frozen upon collection and stored at −20 °C.

**Extraction.** The frozen organism was lyophilized and ground. The resultant powder (300 g) was stirred with ethanol–water (3:7 v/v, 2 L) at 4 °C for 24 h and filtered, and then the residue was extracted again. The combined filtrates were concentrated (35 °C, 7 mm), the aqueous suspension (1.2 L) was centrifuged (9000 rpm, 13000*g*, 0.3 h), and then the supernatant was lyophilized to yield the crude extract as an orange powder A (75.7 g, 25.3%). Other collections gave from 1460 g of dry powder 205 g (14.0%) and from 950 g of dry powder 180 g (16.0%) of crude extract A.

**Diafiltration.** The crude extract A (30 g) was suspended in water (400 mL), sonicated (0.2 h), and then centrifuged (17 000 rpm, 35000*g*, 0.75 h). The supernatant was diafiltered in a 500-mL cell (UMO5 filter, 200 kPa). Ultrafiltration was performed to reduce the cell volume to 100 mL and then continuous diafiltration, at constant cell volume, was performed until the volume of the diafiltrate was 680 mL. The diafiltrate was lyophilized to yield the active material as a pale yellow solid B (15 g, 50% A) and the retentate (100 mL) was lyophilized to yield a brown solid C (12 g, 40% A).

**Gel Permeation Chromatography.** The active diafiltrate B (4.0 g) was dissolved in water (2.0 mL) and chromatographed on Sephadex G-10 (52 × 5 cm, $V_0$ = 330 mL). The column was equilibrated and developed with water (flow rate 66 mL $h^{-1}$) and the eluate monitored in flow cells by percent transmission (254, 280 nm) and conductivity. Individual fractions were subjected to bioassay and the active constituent was found to be eluted between $V_e/V_0$ 2.52 and $V_e/V_0$ 2.97, corresponding to a relatively distinct UV absorption. The active fractions were combined and lyophilized to yield a cream colored powder D (42 mg, 0.53% A). Crystallization from a dilute aqueous solution gave colorless needles. X-ray analysis determined the structure to be allantoin (**3**).[11] The $^{13}C$ NMR spectrum of authentic allantoin (Fluka) was identical with four signals observed in the spectrum of D at 174.3 (s), 158.2 (s), 157.7 (s), 63.4 (d) ppm.

**Ion-Exchange Chromatography. i.** The crude extract A (205 g) was suspended in ammonium formate (pH 3.5, 0.1 M with respect to formate) and then it was sonicated (0.25 h) and stirred on a boiling water bath (0.7 h). The suspension was cooled to 22 °C, its pH was lowered from 4.2 to 3.5 with formic acid, and then it was centrifuged (9000 rpm, 13000*g*, 0.5 h). The supernatant was applied to a column (40 × 5 cm) of Bio Rad AG50W-X8 ($NH_4^+$ form, 200–400 mesh) equilibrated in ammonium formate (pH 3.5, 0.1 M with respect to formate) and the eluate (flow rate 150 mL $h^{-1}$) was monitored in flow cells by percent transmission (254, 280 nm). After 10 L of the starting buffer was eluted, ammonium formate (pH 5.3, 0.1 M with respect to formate) was applied to the column. Fractions of 1.2 (pH 3.5–4.8), 4.5 (pH 4.8–5.0), and 5 L (pH 5.0–5.2) were collected. Only the fraction of pH 4.8–5.0 was active and it was lyophilized to yield a pale yellow solid (31.6 g). This procedure was repeated to give a further 37.3 g from A (180 g) and overall 68.9 g of E (from 385 g of A, 17.9%).

**ii.** A solution of E (68.9 g) in ammonium formate (pH 3.5, 0.1 M with respect to formate, 1.25 L) was centrifuged (9000 rpm, 13000*g*, 0.3 h), then the supernatant was diluted to 4 L with ammonium formate (pH 3.5, 0.1 M with respect to formate), and the pH was adjusted to 3.5 with formic acid. The solution was applied to a column (41.5 × 5 cm) of Bio Rad AG50W-X8 (200–400 mesh, $NH_4^+$ form) and the procedure outlined in i was followed. After 5.1 L of pH 3.5 buffer was eluted, pH 5.3 buffer was applied and the active material eluted between 7.85 and 10.85 L. Lyophilization of the active fraction gave F (10.8 g, 2.8% A).

**iii.** Recrystallization of F (10.8 g) from boiling water (2 × 60 mL) gave **1-methylisoguanosine (1)** (2.75 g, 0.71% A) as a colorless, crystalline solid: mp 262–263 °C; $[\alpha]^{24}_{589}$ −65.4° (*c* 1.0, $Me_2SO$); $[\alpha]^{22}_{589}$ −54.6° (*c* 1.0, $H_2O$); UV $\lambda_{max}$ (pH 1.5) 237 nm ($\epsilon$ 5600), 283 (12 900); UV (pH 6.3) 250 (8600), 294 (11 400); UV (pH 11.5) 253 (8400), 292 (11 200); mass spectrum, *m/e* 297.1067 + 0.0009 ($M^+$, calcd for $C_{11}H_{15}N_5O_5$ 297.1073), 279.0967 ± 0.0009 (M − $H_2O$, calcd for $C_{11}H_{13}N_5O_4$ 279.0967), 267 (M − $CH_2O$), 208 (B + 44), 194 (B + 30), 166 (B + 2H), 165 (B + H), 148 (BH − 17), 136 (BH − [$CH_2$=NH]), 108 (BH − $CH_3NCO$), 81 (108 − HCN) ($M^+$ 297 confirmed by field desorption and chemical ionization); $^{13}C$ NMR 153.8 (s), 152.1 (s), 151.5 (s), 138.0 (C8, d), 108.9 (C5, s), 87.6 (C1′, d), 86.0 (C4′, d), 72.9 (C2′, d), 70.7 (C3′, d), 61.8 (C5′, t), 30.0 ($N^1CH_3$, q) ppm; $^1H$ NMR $\delta$ 3.36 (s, $CH_3$), 3.60 (m, 2 H, H5′), 3.92 (m, 1 H, H4′), 4.10 (dd, $J_{3',4'}$ = 4.5 Hz, $J_{2',3'}$ = 5 Hz, H3′), 4.56 (dd, $J_{2',3'}$ = 5 Hz, $J_{2',1'}$ = 6 Hz, H2′), 5.04 (m, 1 H), 5.30 (m, 1 H), 5.62 (d, *J* = 6 Hz, H1′), 5.72 (m, 1 H), 7.84 (s, H8), 8.08 (m, 2 H, NH) ($^1H$–$^1H$ coupling constants were obtained from double-resonance experiments with a sample containing $D_2O$; resonances at $\delta$ 5.04, 5.30, 5.72, and 8.08 disappeared upon the addition of $D_2O$ to the sample); TLC 2-propanol–ammonia–water (20:6:3), $R_f$ 0.68; TLC *n*-butanol–acetic acid–water (3:1:1), $R_f$ 0.50. Anal. Calcd for $C_{11}H_{15}N_5O_5$: C, 44.45; H, 5.09; N, 23.56. Found: C, 44.40; H, 5.05; N, 23.64.

**Hydrolyses of 1-Methylisoguanosine (1). i. 1-Methylxanthine (3).** A solution of **1** (1.5 mg) in concentrated hydrochloric acid (5 mL) was refluxed for 18 h (bath temperature 160 °C). The solution was evaporated to dryness, water (3 mL) was added, and the solution was evaporated to give an insoluble solid (**3**). A mass spectrum of this solid and 1-methylxanthine were superimposable.

**ii. D-Ribose + 1-Methylisoguanine (4).** A suspension of **1** (200 mg, 0.67 mmol) in hydrochloric acid (1.0 mL, 1.0 M) and deuterium oxide (0.2 mL) was prepared in a 10-mm NMR tube. The suspension was warmed (60 °C) and became homogeneous after 0.1 h. Colorless needles began to form after 1 h and after 7 h the $^{13}C$ NMR spectrum of the heterogeneous sample revealed that ribose was the only species present in solution. The reaction mixture was centrifuged (2000 rpm, 0.05 h), the supernatant was decanted, and then the residue suspended in water (5.0 mL, 4 °C) and centrifuged (2000 rpm, 0.05 h). This procedure was repeated, the residue was retained (see below), and the combined supernatants were applied to a column of Amberlite MB3 (25 mL, 2 × 10 cm). The column was washed with water and the eluate (50 mL) was lyophilized to yield D-ribose (90.8 mg, 91%) as a colorless syrup, with $[\alpha]^{22}_D$ −19.5° (c 0.87, $H_2O$) and $^{13}C$ NMR identical with an authentic sample of D-ribose (Sigma): paper chromatography in *n*-propanol–ethyl acetate–water (7:1:1) (with reference to glucose) gave ribose (1.69), hydrolysis product (1.63), arabinose (1.17), xylose (1.45) and in ethyl acetate–pyridine–water (12:5:4) (with reference to glucose) gave ribose (1.77), hydrolysis product, (1.75), lyxose (1.35), arabinose (1.20), xylose (1.55). The residue was lyophilized to yield 1-methylisoguanine hydrochloride (**4**, 84.1 mg, 63%) as a colorless, insoluble solid: mp 290 °C dec, mass spectrum, *m/e* 165 (M, 100%), 148 (M − $NH_3$), 136 (M − $CH_3N$), 108 (M − $CH_3NCO$), 81 (108 − HCN). The free base was prepared by dissolving **4** in NaOH (1 N), adjusting the pH to 11 with HCl (1 N), collecting the precipitate, and washing with $H_2O$; $^{13}C$ NMR 153.9, 153.2, 151.2, 138.6, 108.6, 30.0 ppm.

**5-Amino-4-carbamoyl-1β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)imidazole (6).** A suspension of 5-amino-4-carbamoyl-1β-D-ribofuranosylimidazole (**5**) (100 g, 387 mmol, Ajinomoto) in pyridine (1.2 L, dried over 4-Å molecular sieves) was cooled to 0 °C and treated with acetic anhydride (330 mL). The mixture was stirred and allowed to warm to room temperature and after 2 h all of the starting material had dissolved. The solution was cooled to 0 °C, treated with methanol (500 mL), and after 30 min evaporated to an oil. This material was evacuated overnight and dissolved in methylene chloride (1.6 L). The methylene chloride solution was extracted with water (3 × 1.6 L), evaporated to a foam, and evacuated overnight to give the triacetyl derivative **6** (140 g, 364 mmol, 94%). This material was sufficiently pure for direct conversion into the triacetyl cyanide **7**.

**5-Amino-4-cyano-1β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)imidazole (7).**[19] A solution of **6** (70 g, 182 mmol) in chloroform (1400 mL) and triethylamine (127 mL) was cooled to 0 °C and phosphorus oxychloride (18.4 mL, 201 mmol) was added dropwise with stirring over 2 h. The solution was stored at 5 °C overnight, and the solution was poured into ice–water (1500 mL) with vigorous stirring. After 30 min the chloroform layer was collected and the aqueous layer was extracted with chloroform

(1 L). The chloroform layers were combined and washed with water (1 × 1.5 L) and 0.055 N aqueous hydrochloric acid (2 × 2 L), followed by water (2 × 2 L). (During the hydrochloric acid extractions an emulsion was formed, which dispersed on storage overnight.) The chloroform solution was evaporated to dryness to give a dark brown foam (55 g), which was dissolved in chloroform (500 mL) and applied to a silica column (Merck, 70–230 mesh, 550 g) which had been packed in the same solvent. The column was eluted with chloroform (2 L) followed by chloroform/ethyl acetate (1:1), and 20-mL fractions were collected. Tubes 180–450, which contained almost pure material, were evaporated to a pale yellow foam, which was dissolved in methanol (200 mL) and filtered through Celite to remove small amounts of insoluble impurities. The filtrate was evaporated to dryness and pumped in vacuo overnight to give **7** as a pale yellow foam, 36.6 g, 100 mmol (55%). This material was of sufficient purity to be used directly in the subsequent (cyclization) step.

**1-Methylisoguanosine (1).** A solution of **7** (10 g, 27.3 mmol) and methyl isocyanate (16.1 mL, 273 mmol) in DMF (100 mL, dried over 4-Å sieves) was heated with stirring at 100 °C (oil bath temperature) for 5 h. The reaction was cooled and evaporated to a froth which was dissolved in ethanol (277 mL) and treated with concentrated aqueous ammonium hydroxide (277 mL, 14.8 N, 4.1 mol). After storage at room temperature overnight, the solution was evaporated to dryness, coevaporated with ethanol (100 mL), and pumped in vacuo for 2 h. The residue was treated with ethanol (250 mL) and after storage overnight at 5 °C the white amorphous solid was collected, washed with ethanol, and dissolved in boiling water (120 mL). The solution was filtered through glass wool, ethanol (120 mL) was added, and the solution was stored at 5 °C overnight. The crystals were collected, washed with ethanol, and dried in vacuo to give 1-methylisoguanosine (**1**; 5.24 g, 17.6 mmol, 64%), mp 266–267 °C dec. The liquors were evaporated to dryness and the residue was recrystallized twice from ethanol/water to give a second crop (1 g, 3.37 mmol, 12%), mp 265–267 °C dec, with a total yield of 6.24 g (76%), mp 266–267 °C dec. IR, UV, $^1$H NMR, $^{13}$C NMR, rotation, TLC, and high-performance LC were identical with those of the natural product. Anal. Calcd for $C_{11}H_{15}N_5O_5$: C, 44.45; H, 5.09; N, 23.56. Found: C, 44.25; H, 5.14; N, 23.68.

**4-Cyano-5-[bis(methylcarbamoyl)amino]-1β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)imidazole (8).** A solution of **7** (5 g, 13.7 mmol) in DMF (50 mL, dried over 4-Å molecular sieves) was treated with methyl isocyanate (8.05 mL, 137 mmol) with stirring at 100 °C (bath temperatures) for 23 h, using a reflux condenser. The solution was evaporated to dryness, coevaporated with methanol (2 × 50 mL), and evacuated overnight to give a froth (6.63 g). A portion (4.76 g) of this material was dissolved in ethyl acetate/chloroform (3:1, 48 mL) and applied to a silica gel column (4.4 × 98 cm) which had been packed in the same solvent. The column was eluted with ethyl acetate/chloroform (3:1) and 20-mL fractions were collected. Fractions 122–160 were combined and evaporated to a white foam. Since thin-layer chromatography on silica gel (chloroform/methanol, 50:1) indicated that this material was impure, the foam was dissolved in chloroform/methanol (50:1, 10 mL) and applied to another silica column (4 × 65 cm) which had been packed in, and was eluted with chloroform/methanol (50:1). Fractions 100–140 (20 mL each) were evaporated to dryness to give 4-cyano-5-[bis(methylcarbamoyl)amino]-1β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)-imidazole as a white foam, 1.51 g, 3.14 mmol (32%): IR 2245 (C≡N), 1753 (ester), 1728, 1680 (urea carbonyl) cm$^{-1}$; UV (methanol) 227 nm (sh, ε 11 360); UV (0.1 N HCl) 226 nm (sh, ε 9540); NMR ($Me_2SO$-$d_6$) δ 8.33 (d, 1 NHCH$_3$), 7.65 (d, 1 NHCH$_3$), 5.49 (d, 1, H$_1$′), 5.3 (m, 2, H$_2$′, H$_3$′), 4.29 (s, 3, H$_4$′, 2 × H$_5$′), 2.72 (d, 3, $J$ = 4 Hz, NHCH$_3$), 2.63 (d, 3, $J$ = 4 Hz, NHCH$_3$), 2.04 (s, 6, CH$_3$CO), 1.98 (s, 3, CH$_3$CO). Anal. Calcd for $C_{19}H_{24}N_6O_9$: C, 47.49; H, 5.03 N, 17.49. Found: C, 47.19; H, 5.43; N, 17.70.

**1 from 8.** A solution of **8** (480 mg) in methanol (5 mL) and concentrated ammonium hydroxide (5 mL) was stored at 5 °C for 18 h. The product was evaporated to dryness and recrystallized from ethanol/water to give **1** (258 mg, 87%).

**Methylation of Isoguanosine.** A solution of isoguanosine[20] (200 mg, 0.71 mmol) in dimethyl sulfoxide (6 mL, dried over 4-Å molecular sieves) was stirred with potassium carbonate (215 mg) and methyl iodide (0.088 mL, 1.4 mmol) for 18 h at room temperature. The mixture was filtered through Celite and evaporated to an oil which on dissolution in methanol (5 mL) deposited crude **1** (63 mg, 30%). The liquors were evaporated to dryness, dissolved in methanol/water (9:1), and impregnated onto silica (7 g). The silica was dried by repeated evaporations of ethanol (3 × 100 mL), evacuated overnight, and applied to the top of a silica column (2.3 × 30 cm). The column was eluted with chloroform/methanol (5:1, 750 mL) followed by chloroform/methanol (3:1). Fractions of 20 mL were collected, and tubes 45–60 were evaporated to give crude 2-methoxyadenosine,[21] 0.11 g (0.37 mmol, 52%). Recrystallization from water gave analytically pure material, 15 mg (7%), mp 189–192 °C (lit.[21] mp 191–191.5 °C).

Fractions 100–140 were evaporated to dryness to give crude 1-methylisoguanosine (**1**), 40 mg (19%). A sample was purified by preparative thin-layer chromatography on silica (chloroform/methanol (2:1) as developing solvent). The appropriate band was extracted with water, and the extract was evaporated to dryness and redissolved in hot water. Filtration was necessary to remove small amounts of silica. The filtrate was evaporated to 0.1 mL and ethanol (0.2 mL) was added. On cooling to 5 °C overnight, crystals of **1** were deposited, mp 265 °C dec. The IR (KBr) spectrum was identical with that of authentic material.

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