Purines. LI.[1] Synthesis and Biological Activity of Hypoxanthine 7-*N*-Oxide and Related Compounds

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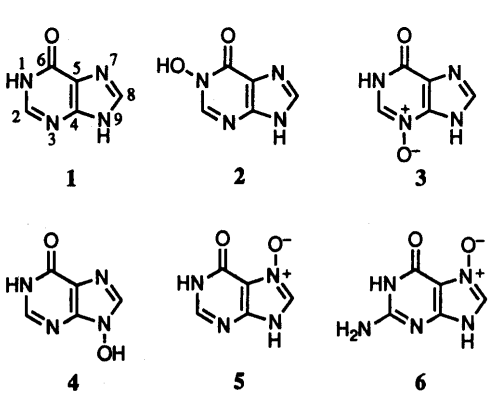
A detailed account is given of the first chemical synthesis of hypoxanthine 7-*N*-oxide (5), which started from coupling of 6-chloro-5-nitro-4(3*H*)-pyrimidinone (7) with *N*-(4-methoxybenzyl)phenacylamine, generated *in situ* from the hydrochloride (8), and proceeded through cyclization of the resulting phenacylaminopyrimidinone (9) and removal of the 4-methoxybenzyl group. The results of catalytic hydrogenolysis, methylation followed by catalytic hydrogenolysis, and rearrangement under acidic conditions of 5 supported the correctness of the assigned structure. An ultraviolet spectroscopic approach suggested that the neutral species of 5 exists in H_2O mainly as the N(7)-OH tautomer (21). In the *in vitro* bioassay of antileukemic activity against murine L5178Y cells, 5 was weakly cytotoxic, with IC_{50} of 100 μg/ml. It did not show any antimicrobial activity even at 1000 μg/ml. None of the 9-(4-methoxybenzyl) (11) and *O*-methyl (12, 13, and 14) derivatives was found to be antileukemic or antimicrobial.

Keywords condensation chloropyrimidinone–phenacylamine; cyclization nitro–phenacylamino; hypoxanthine 7-*N*-oxide 9-substituted; hypoxanthine 7-*N*-oxide synthesis; debenzylation benzyl carbenium ion; *N*-oxide methylation; *N*-oxide rearrangement; *N*-oxide hydrogenolysis; tautomerism *N*-oxide–*N*-hydroxy; antitumor activity

Hypoxanthine (**1**) is a biologically significant oxopurine, which occurs in the animal body during the breakdown of nucleic acids and in the plant kingdom as well.[2] It also occurs as the 9-β-D-ribofuranoside inosine and the related nucleotide inosine 5'-phosphate, the former having been isolated as a minor component of more than 30 species of transfer ribonucleic acid[3] and the latter being an important precursor in the *de novo* biosynthesis of purine nucleotides such as adenosine 5'-phosphate and guanosine 5'-phosphate.[2b,4] Because the oxopurine **1** carries four endocyclic nitrogen atoms, four kinds of mono-*N*-oxide should be possible theoretically. Among the four possible *N*-oxides,[5] 1-hydroxyhypoxanthine (**2**),[6] hypoxanthine 3-oxide (**3**),[7] and 9-hydroxyhypoxanthine (**4**)[8] have been prepared by chemical synthesis, but the 7-*N*-oxide (**5**) is hitherto unknown. Such a blank in chemical synthesis is reminiscent of a similar situation experienced recently in the guanine *N*-oxide series,[1b,9] leading to a design for a synthesis of this new purine 7-*N*-oxide at the hypoxanthine level. This paper details the results of an extension of the "phenacylamine route",[1b,9] developed in our laboratories for the chemical synthesis of guanine 7-oxide (**6**) and 9-substituted derivatives, to the synthesis of hypoxanthine 7-*N*-oxide (**5**). We also report the chemical behavior observed for **5** and some biological evaluations of **5** and related compounds. A brief account of the chemical results presented here has been published in a preliminary form.[10]



1 **2** **3**

4 **5** **6**

This article is dedicated to Emeritus Professor Dr. Nelson J. Leonard (University of Illinois) on the occasion of his 75th birthday.

The first step for the synthesis of **5** was coupling of *N*-(4-methoxybenzyl)phenacylamine, generated *in situ* from the hydrochloride **8**[1b,9] (2 molar eq) and 1 N aqueous NaOH (2 molar eq), with the chloropyrimidinone **7**,[11] which was effected in EtOH at room temperature for 6 h to furnish the phenacylaminopyrimidinone **9** in 59% yield. On treatment with 2 N aqueous NaOH at room temperature for 1 h, **9** gave the *N*-oxide **11** and benzoic acid in 57% and 59% yields, respectively. It seemed likely that the cyclization had proceeded *via* the tetrahedral intermediate **10** (which would have formed through nucleophilic attack by the phenacyl carbanion on the NO_2 nitrogen atom in **9**), as in the cases of the guanine 7-oxide series.[1b,9,12] Characterization of **11** as the 7-oxide was readily achieved by elemental analysis; measurements of its ultraviolet (UV) spectrum in acid, neutral, and basic media (see Table I for a strong absorption in the 230 nm region in neutral medium[1b]) and of its proton nuclear magnetic resonance (^{1}H-NMR) spectrum in 1 N D_2SO_4/D_2O [δ 8.39 (C(2)-H) and 9.35 (C(8)-H)]; and the above and the following self-consistent reaction sequences.

Removal of the 4-methoxybenzyl group from **11** was then effected with 90% aqueous H_2SO_4 at 30 °C for 1 h in the presence of toluene, affording the target hypoxanthine 7-*N*-oxide (**5**) [mp >300 °C; ^{1}H-NMR (1 N D_2SO_4/D_2O) δ: 8.39 (C(2)-H) and 9.20 (C(8)-H)] in 77% yield. This deblocking procedure, analogous to that employed for the guanine 7-oxide series,[1b,9] was based on the previously reported specific debenzylation of 3-benzyladenine[13] and 7-alkyl-3-benzyladenines[14] that proceeds through benzyl carbenium ion formation and trapping of the cation by transbenzylation with toluene. In order to accumulate proofs of the correctness of structure **5**, the chemical behavior of **5** was studied in the following manner.

On hydrogenolysis using Raney Ni catalyst and H_2 in H_2O (1 atm, 50 °C, 4 h), **5** produced hypoxanthine (**1**) in 82% yield (Chart 1). Treatment of **5** with hot AcOH (95—100 °C) for 20 h or with boiling 2 N aqueous HCl for

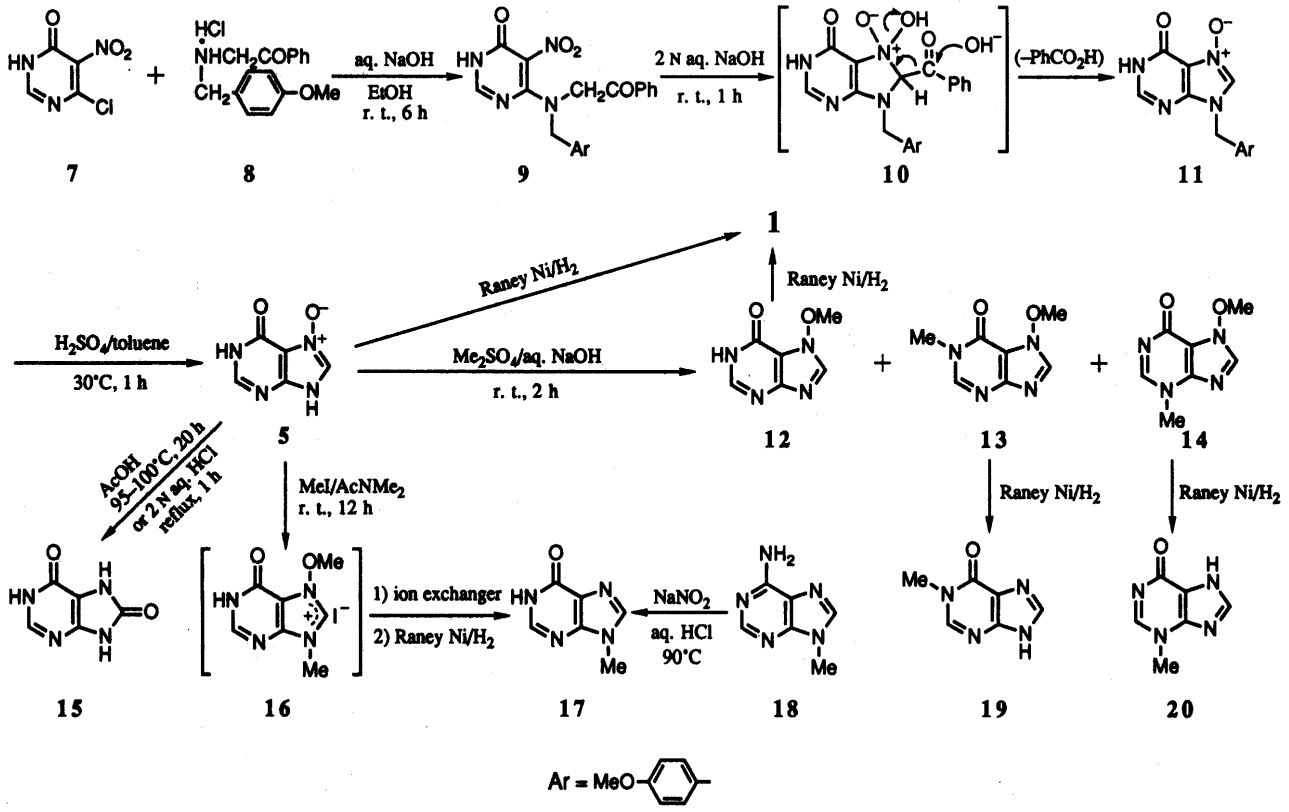


Chart 1

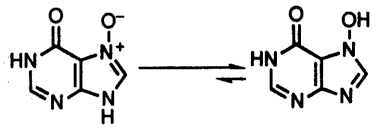
TABLE I. UV Spectral Data for N(7)-Oxygenated Hypoxanthines

Compound	UV spectra							
	95% (v/v) aq. EtOH		H_2O (pH 1)[a]		H_2O (pH 7)[b]		H_2O (pH 13)[c]	
	λ_{max} (nm)	$\varepsilon \times 10^{-3}$	λ_{max} (nm)	$\varepsilon \times 10^{-3}$	λ_{max} (nm)	$\varepsilon \times 10^{-3}$	λ_{max} (nm)	$\varepsilon \times 10^{-3}$
5	258	8.1	254	9.5	234	15.8	230	13.2
					276	7.1	277	7.6
11	230	22.1	227	16.4	228	23.0	225	18.1
	274	9.9	256	11.8	272	10.5	275	12.0
12	256	8.3	256	9.1	257	8.9	263	9.7
13	257	7.3	256	8.1	257[d]	7.9	257[e]	7.4[e]
14	268	11.6	256	9.8	268	12.1	268[e]	10.2[e]

a) Measured in 0.1 N aqueous HCl. *b*) Measured in 0.005 M phosphate buffer (pH 7). *c*) Measured in 0.1 N aqueous NaOH. *d*) λ_{min} 231 nm (ε 3400). *e*) Not accurate because of a slow change of the spectrum with time.

1 h gave 6,8-dioxopurine (**15**)[15] in 95% or 50% yield, respectively. The apparent migration of the oxygen function from N(7) to C(8) under acidic conditions is analogous to that observed for guanine 7-oxide (**6**) (in boiling AcOH)[16] and theophylline 7-oxide (in boiling Ac_2O).[17] Methylation of **5** with dimethyl sulfate in 0.2 N aqueous NaOH at room temperature afforded 7-methoxyhypoxanthine (**12**) (28% yield), 7-methoxy-1-methylhypoxanthine (**13**) (7%), and 7-methoxy-3-methylhypoxanthine (**14**) (3%). The locations of the methyl groups were established by reductive demethoxylations of **12**, **13**, and **14** (Raney Ni/H_2, MeOH, 1 atm, 40 °C—room temp., 4—8 h), which led to the formation of hypoxanthine (**1**), 1-methylhypoxanthine (**19**),[18] and 3-methylhypoxanthine (**20**)[19,20] in 81%, 83%, and 66% yields, respectively. On the other hand, methylation of **5** with MeI in $AcNMe_2$ at room temperature in the absence of alkali gave a complex mixture of products presumed to contain the 7-methoxy-9-methyl derivative (**16**), and the mixture yielded 9-methylhypoxanthine (**17**)[21] when subjected to hydrogenolysis [Raney Ni/H_2, 50% (v/v) aqueous MeOH, 1 atm, 40 °C, 3 h] after removal of iodide ion by the use of Dowex 50W-X8 (H^+). Compound **17** was identical with a sample prepared in 79% yield from 9-methyladenine (**18**) by deamination with $NaNO_2$ in aqueous HCl at 90 °C.[22]

In approaching the problem of the tautomeric form of hypoxanthine 7-*N*-oxide (**5**) in solution, we next determined its pK_a values spectrophotometrically, obtaining three values of < 1.4 (basic) (for protonated form⇌neutral form), 5.02 (acidic) (for neutral form⇌monoanion), and 10.23 (acidic) (for monoanion⇌dianion). The strong UV absorption of purine *N*-oxides in the 215—240 nm region is considered to be due to >N→O or the enol anion >N-O$^-$.[23] Although a fairly strong absorption band (ε



5 21

Chart 2

15800) is observed at 234 nm in the UV spectrum of hypoxanthine 7-*N*-oxide in H_2O at pH 7 (Table I), it may be regarded as that arising from the monoanionic species in view of the above pK_a values.[24] The spectrum in H_2O at pH 3.20 [λ_{min} 233 nm (ε 5100); λ_{max} 257 (8500)] should reflect that of the neutral species, and it not only lacks a strong absorption in the 230 nm region but also bears a certain overall similarity to the neutral species spectrum of **13**, a fixed model for the N(7)-OH form (**21**), at pH 7 (Table I). Thus, this observation suggests that the neutral species of hypoxanthine 7-*N*-oxide exists in H_2O mainly as the N(7)-OH tautomer (**21**) (Chart 2), contrary to our previous suggestion.[10]

Finally, in view of the significant anticancer[16,25-27] and weak anticandidal[16] activities of guanine 7-oxide (**6**), some of the above synthetic compounds were subjected to biological evaluations. In a bioassay of cytotoxicity against murine L5178Y leukemia cell line *in vitro*,[28] compounds **11**, **12**, **13**, and **14** were completely inactive at 50 μg/ml concentration, but hypoxanthine 7-*N*-oxide (**5**) was weakly cytotoxic, with IC_{50} of 100 μg/ml.[29] This IC_{50} value is much higher than that (1.10 μg/ml) reported for **6**, indicating the importance of attachment of an amino group at the 2-position in **6**. It is interesting in this connection that xanthine 7-*N*-oxide,[30] a 2-oxo analogue of **5**, is a potent carcinogen.[31] In tests for antibacterial activity against *Staphylococcus aureus* 209P, *Escherichia coli* NIHJ, and *Pseudomonas aeruginosa* and for antifungal activity against *Candida albicans*, *Trychophyton mentagrophytes*, and *T. rubrum* using the conventional paper disk method, none of **5**, **11**, **12**, **13**, and **14** showed any activity even at a concentration as high as 1000 μg/ml.

In conclusion, hypoxanthine 7-*N*-oxide (**5**), the only remaining isomer of the four possible hypoxanthine mono-*N*-oxides, has now become known as a result of the above three-step synthesis starting from the chloropyrimidinone **7** and the *N*-substituted phenacylamine derivative **8** and proceeding through the intermediates **9** and **11**. The *N*-oxide **5** showed a weak antileukemic activity and no antimicrobial activity, while the 9-(4-methoxybenzyl) derivative **11** and the 7-methoxy derivatives **12**, **13**, and **14** showed neither antileukemic nor antimicrobial activity.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Mass spectra (MS) were recorded on a JEOL JMS-D-300 mass spectrometer. Internal standards used for the measurements of ^{1}H-NMR spectra were Me_4Si (for Me_2SO-d_6 solutions) and sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (for solutions in 1 N D_2SO_4/D_2O). Spectrophotometric determination of acid dissociation constants was carried out in a manner similar to that described previously.[32] See refs. 1*b* and 32*b* for details of other instrumentation and measurements and of chromatographies. Elemental analyses were performed mainly by Mr. Y. Itatani and his associates at Kanazawa University and partly by Mr. T. Sugino at Ikeda Mohando Co. The following abbreviations are used: br=broad, d=doublet, m=multiplet, s=singlet.

6-[(4-Methoxybenzyl)(2-oxo-2-phenylethyl)amino]-5-nitro-4(3*H*)-pyrimidinone (9) 2-[(4-Methoxybenzyl)amino]-1-phenylethanone hydrochloride (**8**)[1b,9] (2.91 g, 9.97 mmol) was dissolved in a stirred mixture of EtOH (20 ml) and 1 N aqueous NaOH (10 ml), and then 6-chloro-5-nitro-4(3*H*)-pyrimidinone (**7**)[11] (875 mg, 4.98 mmol) was added in small portions. The resulting mixture was stirred at room temperature for 6 h. The precipitate that resulted was filtered off, washed successively with H_2O (2×1 ml), EtOH (2×1 ml), and ether (2×1 ml), and dried to afford **9** (1.16 g, 59%) as a pale yellow solid, mp 161—163 °C (dec.); IR ν_{max}^{Nujol} cm^{-1}: 1700 (COAr), 1650 (CONH); ^{1}H-NMR (Me_2SO-d_6) δ: 3.73 (3H, s, OMe), 4.68 (2H, s, C$\underline{H}_2$Ar), 4.96 (2H, s, C$\underline{H}_2$COPh), 6.88 [2H, d, *J*=9 Hz, C(3′)-H and C(5′)-H],[33] 7.25 [2H, d, *J*=9 Hz, C(2′)-H and C(6′)-H],[33] 7.4—8.0 (5H, m, Ph), 8.06 [1H, s, C(2)-H], 12.65 (1H, br, NH). This sample was homogeneous on thin-layer chromatographic (TLC) analysis and was used in the next cyclization step without further purification.

9-(4-Methoxybenzyl)hypoxanthine 7-*N*-Oxide (11) Compound **9** (7.89 g, 20 mmol) was added in small portions to 2 N aqueous NaOH (100 ml) with stirring. Stirring was continued at room temperature for 1 h, and the crystals that deposited were filtered off. The filtrate was brought to pH 1 by addition of 10% aqueous HCl and extracted with ether. The ethereal extracts were combined, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave benzoic acid (1.44 g, 59%), mp 120—122 °C. This sample was identical (by mixture melting point test and comparison of the IR spectrum) with authentic benzoic acid.

On the other hand, the above crystals, isolated by filtration of the reaction mixture, were dissolved in H_2O (200 ml). The aqueous solution was, after removal of an insoluble material by filtration, adjusted to pH 1 with 10% aqueous HCl, extracted with ether, brought to pH 7 with 28% aqueous NH_3, and then concentrated to a volume of *ca.* 50 ml *in vacuo*. The pale yellow crystals that resulted were filtered off, washed successively with small amounts of H_2O, EtOH, and ether, and dried to give **11**·4/5H_2O (3.26 g, 57%). Recrystallization from 50% (v/v) aqueous MeOH and drying over P_2O_5 at 2 mmHg and 50 °C for 12 h yielded an analytical sample of **11**·4/5H_2O as colorless needles, mp 205—225 °C (dec.); UV (Table I); ^{1}H-NMR (1 N D_2SO_4/D_2O) δ: 3.83 (3H, s, OMe), 5.52 (2H, s, C$\underline{H}_2$Ar), 7.03 [2H, d, *J*=9 Hz, C(3′)-H and C(5′)-H],[33] 7.46 [2H, d, *J*=9 Hz, C(2′)-H and C(6′)-H],[33] 8.39 [1H, s, C(2)-H], 9.35 [1H, s, C(8)-H]. *Anal.* Calcd for $C_{13}H_{12}N_4O_3$·4/5H_2O: C, 54.46; H, 4.78; N, 19.54. Found: C, 54.29; H, 4.67; N, 19.70.

Hypoxanthine 7-*N*-Oxide (5) To a stirred suspension of **11**·4/5H_2O (4.60 g, 16 mmol) in toluene (32 ml) was added dropwise 90% aqueous H_2SO_4 (17.4 g, *ca.* 160 mmol) at 30 °C, and the resulting reddish purple mixture was stirred vigorously at room temperature for 1 h. The reaction mixture was cooled in an ice bath, and H_2O (200 ml) and ether (200 ml) were added. The yellow precipitate that resulted was filtered off and washed successively with ether (100 ml) and H_2O (100 ml). The ethereal and aqueous washings were combined with the above filtrate, and the aqueous layer was separated from the ethereal layer and then passed through a column of Dowex 50W-X8 (H^+) (200 ml). After having been eluted with H_2O until the eluate became neutral, the column was eluted with 5% aqueous NH_3 (*ca.* 1000 ml). The ammoniacal eluate was concentrated to dryness *in vacuo*, and the residue was washed with EtOH (10 ml) and dried over P_2O_5 at 18 mmHg and room temperature overnight and then at 2 mmHg and 110 °C for 15 h, giving **5** (1.88 g, 77%) as a colorless solid, mp >300 °C. Recrystallization from H_2O and drying in the same manner as described above provided an analytical sample as colorless needles, mp >300 °C; pK_a (at 30 °C and ionic strength 1.0): <1.4, 5.02, 10.23; UV (Table I); UV $\lambda_{max}^{H_2O}$ (pH 3.20) 257 nm (ε 8500); $\lambda_{min}^{H_2O}$ (pH 3.20) 233 (5100); ^{1}H-NMR (1 N D_2SO_4/D_2O) δ: 8.39 [1H, s, C(2)-H], 9.20 [1H, s, C(8)-H]. *Anal.* Calcd for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.83. Found: C, 39.51; H, 2.61; N, 36.56.

Hydrogenolysis of 5 Leading to Hypoxanthine (1) A solution of **5** (106 mg, 0.697 mmol) in H_2O (20 ml) was hydrogenated over Raney Ni W-2 catalyst[34] (0.3 ml) at atmospheric pressure and 50 °C for 4 h. After cooling, the reaction mixture was mixed with H_2O (30 ml) and warmed in order to dissolve the crystals that had deposited. The catalyst was filtered off while hot and washed with hot H_2O (2×20 ml). The filtrate and washings were combined and concentrated to dryness *in vacuo*, leaving a colorless solid. The solid was washed with a little EtOH and dried to give hypoxanthine (**1**) (78.2 mg, 82%), mp >300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **1**.

6,8-Dioxopurine (15) i) A stirred mixture of **5** (106 mg, 0.697 mmol) and AcOH (5 ml) was heated at 95—100 °C for 20 h. The reaction mixture was concentrated *in vacuo*, and the residue was washed successively with EtOH (1 ml) and ether (2×1 ml), and dried to give **15** (101 mg, 95%) as

a colorless solid, mp > 300 °C. Recrystallization from H_2O and drying over P_2O_5 at 2 mmHg and 150 °C for 10 h yielded an analytical sample of **15** as colorless, fine needles, mp > 300 °C; UV $\lambda_{max}^{H_2O}$ (pH 1) 256 nm (ε 11600); $\lambda_{max}^{H_2O}$ (pH 7) 256 (11300); $\lambda_{max}^{H_2O}$ (pH 13) 271 (13200); IR ν_{max}^{Nujol} cm^{-1}: 3250—3050 (NH), 1760 (NHCONH), 1658 (CONH); ^{1}H-NMR (Me_2SO-d_6) δ: 7.86 [1H, s, C(2)-H], 10.94, 11.39, and 12.38 (1H each, br, NH's). *Anal.* Calcd for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.83. Found: C, 39.25; H, 2.52; N, 36.56. This sample was identical with one prepared according to the literature.[15]

ii) A stirred mixture of **5** (304 mg, 2 mmol) and 2 N aqueous HCl (20 ml) was heated under reflux for 1 h. The reaction mixture was cooled to room temperature, and the colorless needles that resulted were filtered off, washed with a little H_2O, and dried over P_2O_5 at 2 mmHg and 100 °C for 8 h to furnish **15** (151 mg, 50%), mp > 300 °C. This sample was identified by spectral comparison with authentic **15**.[15]

Methylation of 5 Leading to 7-Methoxyhypoxanthine (12), 7-Methoxy-1-methylhypoxanthine (13), and 7-Methoxy-3-methylhypoxanthine (14) A solution of **5** (913 mg, 6 mmol) in 0.2 N aqueous NaOH (30 ml) was stirred at room temperature, and Me_2SO_4 (757 mg, 6 mmol) was added. The mixture was stirred at room temperature for 2 h and then concentrated to dryness *in vacuo*. The residual semisolid was triturated with MeOH (*ca.* 10 ml), and the insoluble solid that resulted was removed by filtration and washed with a little MeOH. The methanolic filtrate and washings were combined and concentrated *in vacuo* to leave a colorless oil. The oil was chromatographed on a column packed with silica gel (120 g) using $CHCl_3$–EtOH (8 : 1, v/v). Earlier fractions gave **13** (78 mg, 7%) as a colorless solid. Recrystallization of the solid from EtOH produced an analytical sample of **13** as colorless needles, mp 179—180 °C; MS *m/z*: 180 (M^+); UV (Table I); ^{1}H-NMR (Me_2SO-d_6) δ: 3.51 [3H, s, N(1)-Me], 4.19 (3H, s, OMe), 8.33 [1H, s, C(2)-H], 8.52 [1H, s, C(8)-H]. *Anal.* Calcd for $C_7H_8N_4O_2$: C, 46.67; H, 4.48; N, 31.10. Found: C, 46.57; H, 4.42; N, 31.12.

Middle fractions of the above chromatography provided **12** (275 mg, 28%) as a colorless solid. Recrystallization from EtOH yielded an analytical sample of **12** as colorless prisms, mp 215—218 °C (dec.); MS *m/z*: 166 (M^+); UV (Table I); ^{1}H-NMR (Me_2SO-d_6) δ: 4.19 (3H, s, OMe), 8.00 [1H, br, C(2)-H], 8.51 [1H, s, C(8)-H]. 12.45 (1H, br, NH). *Anal.* Calcd for $C_6H_6N_4O_2$: C, 43.38; H, 3.64; N, 33.72. Found: C, 43.62; H, 3.52; N, 33.63.

Later fractions of the above chromatography afforded **14** (36 mg, 3%) as a colorless solid. Recrystallization from EtOH gave an analytical sample of **14** as colorless prisms, mp 175—178 °C (dec.); MS *m/z*: 180 (M^+); UV (Table I); ^{1}H-NMR (Me_2SO-d_6) δ: 3.73 [3H, s, N(3)-Me], 4.20 (3H, s, OMe), 8.31 [1H, s, C(2)-H], 8.52 [1H, s, C(8)-H]. *Anal.* Calcd for $C_7H_8N_4O_2$: C, 46.67; H, 4.48; N, 31.10. Found: C, 46.92; H, 4.36; N, 30.88.

Hydrogenolysis of 12 Leading to Hypoxanthine (1) A solution of **12** (100 mg, 0.602 mmol) in MeOH (20 ml) was hydrogenated over Raney Ni W-2 catalyst[34] (0.3 ml) at atmospheric pressure and 40 °C for 5 h. After cooling, the reaction mixture was mixed with H_2O (20 ml) and warmed in order to dissolve the crystals that had deposited. The catalyst was then removed by filtration while hot and washed with hot H_2O (*ca.* 10 ml). The filtrate and washings were combined and concentrated to dryness *in vacuo*. The residue was washed with a little EtOH and dried to give **1** (66 mg, 81%) as a colorless solid, mp > 300 °C. This sample was identical (by comparison of the IR spectrum and TLC behavior) with authentic hypoxanthine.

Hydrogenolysis of 13 Leading to 1-Methylhypoxanthine (19) A solution of **13** (36 mg, 0.2 mmol) in MeOH (15 ml) was hydrogenated over Raney Ni W-2 catalyst[34] (0.2 ml) at atmospheric pressure and room temperature for 8 h. The catalyst was removed by filtration and washed with MeOH (10 ml). The methanolic filtrate and washings were combined and concentrated *in vacuo* to leave a colorless solid. Recrystallization of the solid from EtOH furnished **19** (25 mg, 83%), mp > 300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **19** prepared according to the literature.[18]

Hydrogenolysis of 14 Leading to 3-Methylhypoxanthine (20) A solution of **14** (20 mg, 0.11 mmol) in MeOH (10 ml) was hydrogenated over Raney Ni W-2 catalyst[34] (0.2 ml) at atmospheric pressure and 40 °C for 4 h. The reaction mixture was worked up in a manner similar to that described above for the hydrogenolysis of **13**, furnishing **20**·3/2H_2O (13 mg, 66%), mp > 300 °C. This sample was identified by spectral and TLC comparison with authentic **20**·3/2H_2O.[19b]

9-Methylhypoxanthine (17) i) From **5**: A mixture of **5** (304 mg, 2 mmol) and MeI (1.42 g, 10 mmol) in $AcNMe_2$ (6 ml) was stirred at room temperature for 12 h. The reaction mixture was concentrated *in vacuo* to leave a reddish oil. The oil was triturated with acetone (5 ml), and the precipitate that resulted was filtered off and washed with acetone. The filtrate and washings were combined and concentrated *in vacuo*. The residual reddish oil, presumed to contain **16**, was dissolved in H_2O (5 ml), and the resulting aqueous solution was passed through a column of Dowex 50W-X8 (H^+) (3 ml), which was further eluted with H_2O until the eluate became neutral. The column was then eluted with 5% aqueous NH_3 (*ca.* 150 ml), and the ammoniacal eluate was concentrated to dryness *in vacuo*. The residue was washed with EtOH to leave a colorless solid (100 mg). A small amount (30 mg) of the solid was dissolved in 50% (v/v) aqueous MeOH (15 ml), and the solution was hydrogenated over Raney Ni W-2 catalyst[34] (0.2 ml) at atmospheric pressure and 40 °C for 3 h. The catalyst was then removed by filtration, and the filtrate was concentrated *in vacuo*. The residual solid was washed with a little EtOH and recrystallized from H_2O to yield **17** (7 mg) as colorless prisms, mp > 300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with the one prepared by method (ii).

ii) From 9-Methyladenine (**18**): A solution of **18**[35] (224 mg, 1.5 mmol) in 0.8 N aqueous HCl (7 ml) was heated to 90 °C, and a solution of $NaNO_2$ (124 mg, 1.8 mmol) in H_2O (4 ml) was added dropwise over a period of 30 min. After the addition, the temperature was maintained at 90 °C for 50 min. The reaction mixture was then concentrated to dryness *in vacuo*. The residue was dissolved in H_2O (1 ml), and the aqueous solution was brought to pH 7 with 10% aqueous NaOH. After the mixture had been cooled in an ice bath, the precipitate that resulted was filtered off, washed with a little H_2O, and dried to give **17** (178 mg, 79%) as a slightly pinkish solid, mp > 300 °C. Recrystallizations from H_2O (including decoloration with activated charcoal powder) yielded an analytical sample as colorless prisms, mp > 300 °C (lit.[21] mp > 300 °C); UV $\lambda_{max}^{H_2O}$ (pH 1) 249.5 nm (ε 10900); $\lambda_{max}^{H_2O}$ (pH 7) 250 (11800); $\lambda_{max}^{H_2O}$ (pH 13) 254 (12400); ^{1}H-NMR (Me_2SO-d_6) δ: 3.73 [3H, s, N(9)-Me], 8.04 (2H, s, ring protons), 12.26 (1H, br, NH). *Anal.* Calcd for $C_6H_6N_4O$: C, 48.00; H, 4.03; N, 37.32. Found: C, 47.88; H, 3.94; N, 37.41.

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References and Notes

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