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Purine *N*-Oxides. XLIII. 9-Hydroxy-8-methylhypoxanthine, -xanthine, and -guanine[1]

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A total synthesis of 9-hydroxy-8-methylpurine derivatives by way of an imidazole *N*-oxide derivative is described. Closures of 5-amino-1-benzyloxy-2-methylimidazole-4-carboxamide with formate or carbonate esters yielded the hypoxanthine and xanthine analogs and through a guanidino derivative yielded the guanine analog. Triethyl orthoacetate was used in an improved preparation of the acetimidate from aminocyanoacetamide.

While 3-hydroxyxanthine and 3-hydroxyguanine are oncogens comparable in potency to the oncogenic arylamines or hydrocarbons, the isomeric 1-hydroxyxanthine induces inflammations and granulomas which rarely develop into tumors.[2-4] The distinct differences in the biological responses to the 1- and 3-hydroxyxanthine suggest that it would be desirable to investigate the 7 and 9 isomers. The latter bear the oxygen on an imidazole rather than on a pyrimidine nitrogen and no examples of N oxidation of the imidazole portion of a purine have been observed. Numerous 1 or 3 derivatives have been obtained by peroxy acid oxidations of purines or pyrimidines,[5-11] and several pyrimidine *N*-oxides have been obtained by total syntheses.[2,12,13] N oxidation of guanine yields 3-hydroxyguanine[2,14,15] and its hydrolysis yields 3-hydroxyxanthine.[6,14] 3-Hydroxyxanthine has also been obtained by total synthesis from 1-benzyloxy-6-aminouracil.[13] 1-Hydroxyxanthine has been synthesized[16] from an imidazole derivative obtained from adenine 1-oxide.[5]

There have been two reported syntheses of purines with an oxygen on an imidazole nitrogen. Goldner and Deitz[17] obtained 7-hydroxytheophylline (1,3-dimethyl-7-hydroxyxanthine), and they and Taylor and Garcia[18] obtained similar 8-alkyl or aryl derivatives, by ring closures of 1,3-dimethyl-5-nitroso-6-amino- or alkylaminouracils. Timmis reported[19] the synthesis of 8-phenyl-7-hydroxy-2,6-diaminopurine from the adduct of benzaldehyde anil with 2,4,6-triamino-5-nitropyrimidine. An approach to unsubstituted 7-hydroxyxanthine is being described.[20]

Attempts to prepare 9-hydroxypurine derivatives by a classical Traube-type synthesis *via* 4-alkoxyamino-5-aminopyrimidines failed because the reduction of a 5-nitro or 5-phenylazo group was accompanied by reduction of the 4-alkoxyamino group. An alternative approach by the synthetic route elaborated for purines by Shaw[21] has been successful. His studies included 9-alkylpurines from 1-alkyl-5-aminoimidazole-4-carboxamide, and a 9-benzyloxy group has now been incorporated instead of the 9-alkyl group. In this initial study ethyl acetimidate HCl was utilized because of its stability, and also because studies of the mechanism of reactions[22] and the metabolic fates[23] of the 3-hydroxypurines suggest that the 8-methyl-9-hydroxy-

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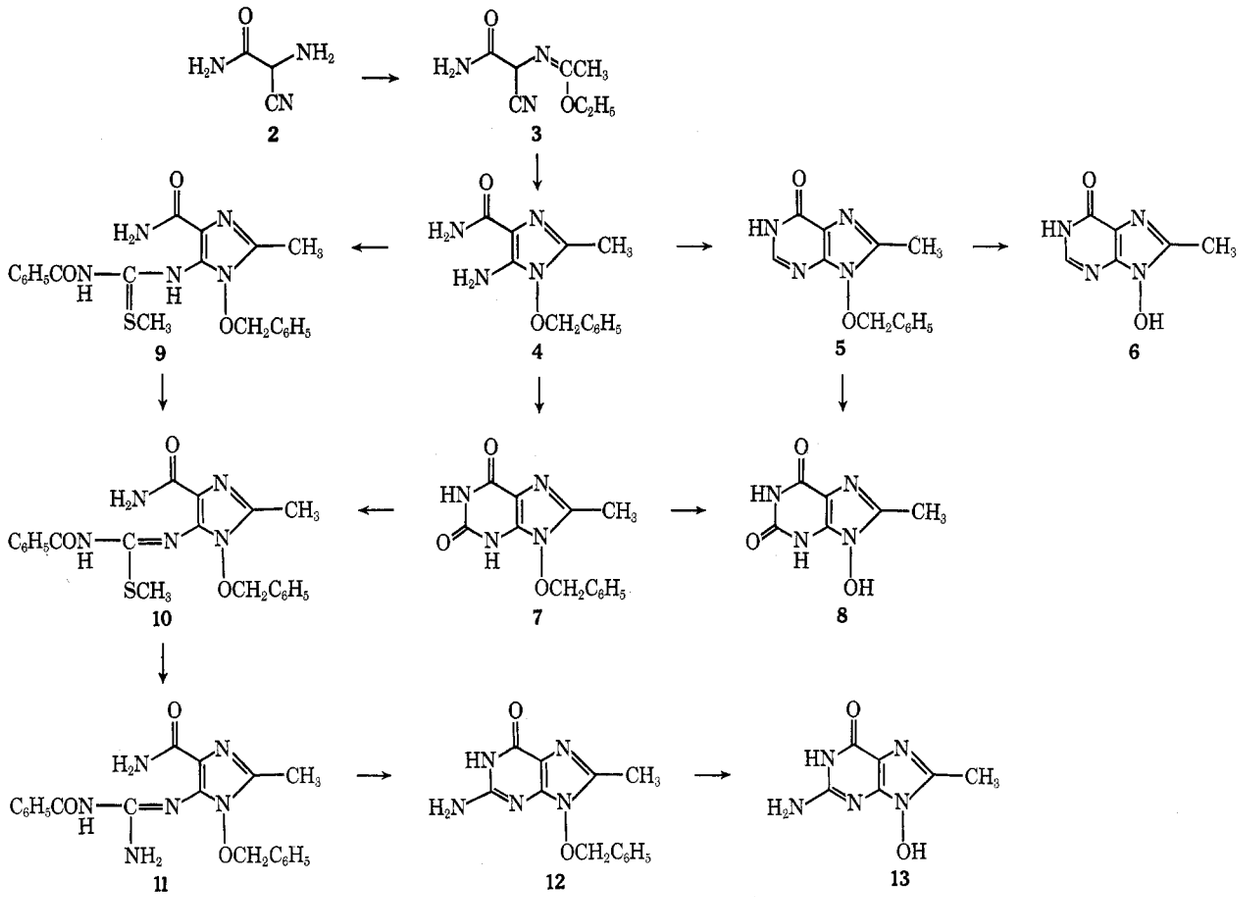
SCHEME I



purines will eventually be desired. Direct repetition of the present sequence of reactions with ethyl orthoformate or ethyl formimidate HCl have failed to yield 9-hydroxypurines without the 8 substituent, but a modification of the Shaw-type synthesis is proving satisfactory.[24]

Results

Ethyl-*N*-[(carbamoylcyano)methyl]acetimidate (**3**) (Scheme I) was prepared by mixing in aqueous solution at room temperature ethyl acetimidate hydrochloride and 2-amino-2-cyanoacetamide (**2**) prepared[21] from cyanoacetamide (**1**). Subsequently better yields of this imino ether were obtained from 2-amino-2-cyanoacetamide and triethyl orthoacetate. The imino ether **3** reacted with benzyloxyamine in methanol to give 5-amino-1-benzyloxy-2-methylimidazole-4-carboxamide (**4**). A comparison of the ir, uv, and nmr spectra with those of 5-amino-1-cyclohexyl-2-methylimidazole-4-carboxamide prepared according to Shaw[21] permitted assignment of the structure **4**.

Several studies in this laboratory have shown that *N*-benzyloxy derivatives may be hydrolyzed to NOH or may lose the complete benzyloxy group on heating at high temperatures in acid media. Procedures involving acid for the closure of the imidazole derivatives to purines were therefore avoided. Yamazaki, Kumashiro, and Takenishi have developed[25] a useful method for the ring closure of ribosylaminoimidazole carboxamide (ribosyl-AICA) to inosine and xanthosine, and it was found to be most applicable for ring closure of 1-benzyloxy-2-methyl-AICA (**4**). This was refluxed in ethanol with ethyl formate in the presence of excess sodium ethoxide to give 9-benzyloxy-8-methylhypoxanthine (**5**) in 78% yield. A similar reaction of **4** with diethyl carbonate gave 9-benzyloxy-8-methylxanthine (**7**) in 20% yield.

Klötzer has shown[15] that debenzylation of *N*-benzyloxypyrimidines to the pyrimidine *N*-oxides can be accomplished in high yields with 32% HBr in glacial acetic acid, thus avoiding the use of hydrogen and metal catalysts, which in many cases give the parent pyrimidines instead of the *N*-oxides. This debenzylating agent was found to yield pure samples of the hydrobromides of both 9-hydroxy-8-methylhypoxanthine (**6**) and -xanthine (**8**), from which the free bases could be obtained.

For cyclization of **4** to a guanine derivative the several methods used by Yamazaki, *et al.*,[26,27] for the preparation of guanosine were investigated. With **4** the optimum conditions involved treatment with benzoyl isothiocyanate[28] in refluxing acetone. This resulted in 5-(*N'*-benzoylthiocarbamoyl)amino-1-benzyloxy-2-methylimidazole-4-carboxamide (**9**) in 88% yield. Methyl iodide and **9** in aqueous sodium hydroxide at room temperature yielded 5-(*N'*-benzoylmethylmercaptocarbamoyl)amino-1-benzyloxy-2-methylimidazole-4-carboxamide (**10**) in 76% yield. In ethanol containing 2% ammonia at 100°, **10** gave 5-*N'*-benzoyl-

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guanidino-1-benzyloxy-2-methylimidazole-4-carboxamide (**11**). This was not isolated, but after removal of ethanol the residue was heated in 1 *N* sodium hydroxide, cooled, and acidified to yield a mixture of 9-benzyloxy-8-methylguanine (**12**) and benzoic acid. The benzoic acid was removed by extraction with hot ethyl ether and **12** was crystallized from ethanol. Debenzylation of this compound with 32% HBr in acetic acid resulted in 9-hydroxy-8-methylguanine HBr, from which the free base **13** was obtained. The intermediates **9**, **10**, and **11** do not give distinctive ultraviolet spectra, but nmr spectra and analysis of **9** and **10** confirmed the assigned structures. The final products, **6**, **8**, and **13**, are not sufficiently soluble in DMSO for nmr measurements. Reductions of each in hot HI yielded the corresponding parent purines, which were identified chromatographically.

The ultraviolet spectra (Table I) of the anions of

TABLE I

UV SPECTRA AND pK_a's OF 9-HYDROXYPURINES

pH	Species	λ_{max}, nm ($\epsilon \times 10^{-3}$)			pK_a
9-Hydroxy-8-methylhypoxanthine					
0.0	+1			252 (11.4)	
					1.73 ± 0.04
3.5	0	221 sh (9.5)	235 (12.3)	252 (11.5)	
					5.73 ± 0.06[a]
8.0	−1		238 (29.3)	265 sh (7.5)	
13.0	−2		232 (24.2)	251 (9.3)	10.75 ± 0.07
9-Hydroxy-8-methylguanine					
−0.6	+1	212 (15.3)	254 (9.7)	277 sh (6.4)	
					2.65 ± 0.07
4.5	0	236 (13.8)	257 sh (7.1)	275 (6.6)	
					6.53 ± 0.08[a]
9.0	−1	238 (22.3)	278 (7.7)		
					11.07 ± 0.07
13.0	−2	231 (18.6)	275 (8.9)		
9-Hydroxy-8-methylxanthine					
−0.6	+1	237 (6.3)	262 (10.1)		
					1.51 ± 0.05
3.0	0	235 (6.2)	266 (10.3)		
					5.14 ± 0.07[a]
6.5	−1	223 (15.8)	275 (11.5)		
					8.14 ± 0.05
13.0	−2	230 (25.6)	280 (8.8)		

[a] Potentiometric titration; others determined by optical methods.

the three 9-hydroxypurines have a strong absorption in the 225–235-nm range—three to four times that of the maxima of their longer wavelength bands. With purines bearing the *N*-oxide group in the pyrimidine ring it was deduced[14] that an $\geqslant N \rightarrow O$ or an enol anion, $-OC^- = N \rightarrow O-$, is associated with strong absorption in the 225–235-nm region. The present spectra extend the evidence, initially made on 7-hydroxytheophylline,[17] that similar interpretations are valid for imidazole *N*-oxide derivatives. In Table I the compounds are designated as *N*-hydroxy derivatives, since that appears to be the predominant tautomer in the neutral species, while the *N*-oxide form predominates in the anions.

The absorptions of the neutral species of 9-hydroxy-8-methylhypoxanthine, ϵ 12.3 × 10^3 at 235 nm, and of 9-hydroxy-8-methylguanine, ϵ 13.8 × 10^3 at 236 nm, compared to the values of ϵ 29.3 and 22.3 × 10^3 at 238 nm, respectively, for the monoanions, indicates that the neutral species of each does have a considerable proportion of the *N*-oxide tautomer with a proton on N-7. With 9-hydroxy-8-methylxanthine the maximum absorption of ϵ 25.6 × 10^3 at 230 nm is fully reached only in the dianion. The lower absorption, ϵ 15.8 × 10^3 at 223 nm at pH 6.5, suggests that the xanthine derivative yields a mixture of monoanions, which must include *N*-hydroxy and *N*-oxide tautomers. The similarity of the neutral and protonated species shows that the neutral species is almost exclusively the *N*-hydroxy form.

The 9-hydroxy-8-methylxanthine and guanine do not show the second absorption band above 300 nm which is observed with the enolate anions of a series of 3-hydroxyxanthines and guanines.[14]

Experimental Section

The uv spectra were determined with a Unicam SP800 spectrometer, and the p*K*'s were determined by methods described[29] at 23 ± 1°, spectrophotometrically with 0.01 *M* buffers[30] with the use of a Beckman DU spectrophotometer. Nmr spectra were determined with a Varian A-60 spectrometer in DMSO-d_6, or in $CDCl_3$ as specified. Analyses were performed by Spang Laboratories, Ann Arbor, Mich.

Ethyl *N*-[(Carbamoylcyano)methyl]acetimidate (3).—A suspension of 2-amino-2-cyanoacetamide[31] (9.9 g, 0.1 mol) in triethyl orthoacetate (100 ml) was heated on a steam bath (with occasional shaking) until all the starting material had gone into solution. A rapidly moving non-uv-absorbing spot began to appear on a thin layer chromatography plate (tlc) run in 9:1 chloroform–ethanol and developed with iodine, and which had the same R_f value as that of the acetimidate prepared by Shaw's method.[21] The heating was continued for about 2 hr, when tlc indicated that all the starting material had reacted. The hot, pale yellow liquid was decanted from a small quantity of brown gum (*i.e.*, decomposed starting material) and cooled at −10° until crystallization was complete. The product **3** was collected and washed with petroleum ether (bp 30–60°). It was crystallized from ethyl acetate–petroleum ether as plates: mp 105° (lit.[21] mp 105°); yield 13 g (77%); nmr δ 7.5 (s, 2, $CONH_2$), 5.3 (s, 1, CHN), 4.2 (q, 2, OCH_2CH_3), 2.2 (s, CCH_3), 1.25 (t, 3, CH_2CH_3).

5-Amino-1-benzyloxy-2-methylimidazole-4-carboxamide (4).—The acetimidate **3** (8.45 g, 0.05 mol) and benzyloxyamine (9.2 g, 0.075 mol) in methanol (20 ml) were heated on a steam bath for about 30 min or until tlc of the solution indicated the absence of the imino ether and a new uv-absorbing spot appeared. This was eluted with ethanol and its uv spectrum, λ_{max} 267 nm, corresponded to that of the known cyclohexyl derivative prepared by Shaw's method.[21] The dark red solution was evaporated to dryness *in vacuo*, and the syrupy residue was chromatographed on a silica gel column (4 × 30 cm) which was eluted with chloroform (which removed the unreacted benzyloxyamine) and then with 9:1 $CHCl_3$–EtOH until the eluent, monitored by tlc, indicated the absence of the required imidazole. The fractions containing the product were evaporated, and the solid residue was triturated with ether and collected. Recrystallization from ethanol gave plates of **4**: mp 208–209° dec; yield 5.04 g (41%); uv λ_{max}^{EtOH} 267 nm (ϵ 13.6 × 10^3), sh 214 (ϵ 12.5 × 10^3); nmr δ 7.5 (s, 5, C_6H_5), 6.67 (s, 2, $CONH_2$), 5.83 (s, 2, NH_2), 5.2 (s, 2, $-OCH_2C_6H_5$), 2.0 (s, 3, CCH_3).

Anal. Calcd for $C_{12}H_{14}N_4O_2$: C, 58.56; H, 5.73; N, 22.77. Found: C, 58.60; H, 5.75; N, 22.87.

9-Benzyloxy-8-methylhypoxanthine (5).—The imidazole **4** (492 mg, 0.002 mol) was dissolved in ethanol (40 ml) containing sodium (460 mg, 0.02 mol); to this was added 1.3 ml (0.016 mol) of ethyl formate. The mixture was heated on a steam bath for 3 hr, during which time a precipitate formed. The dark brown reaction mixture was cooled, and water (50 ml) was added to dissolve the precipitate. Upon acidification with glacial acetic acid the product precipitated and was collected and washed with water. Recrystallization from ethanol with charcoal treatment afforded white plates of the hypoxanthine **5**: yield 403 mg (78%); mp 256° dec; uv $\lambda_{max}^{pH\ 1}$ 251 nm (ϵ 12.9 × 10^3), $\lambda_{max}^{pH\ 13}$ 255 nm (ϵ 14. 2 × 10^3); nmr δ 8.00 (s, 1, CH=N), 7.48 (s, 5, C_6H_5), 5.35 (s, 2, $OCH_2C_6H_5$), 2.2 (s, 3, CCH_3), 11.9 (br, 1, NH).

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Anal. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.94; H, 4.69; N, 21.86. Found: C, 61.03; H, 4.77; N, 21.77.

8-Methyl-9-hydroxyhypoxanthine (**6**).—9-Benzyloxy-8-methylhypoxanthine (**5**) (256 mg, 0.001 mol) was dissolved in 5 ml of warm glacial acetic acid, and 5 ml of 32% HBr in glacial acetic acid was added. The mixture was heated on a steam bath for 3.5 hr, during which time a precipitate formed. The reaction mixture was then cooled and the HBr salt was collected and washed thoroughly with ether. The product was dissolved in hot water containing a few drops of concentrated ammonia, treated with charcoal, and precipitated with glacial acetic acid. The 9-hydroxy-8-methylhypoxanthine was collected, washed with water, ethanol, and ether, and dried *in vacuo* at 78° over P_2O_5, yield 130 mg (78%).

Anal. Calcd for $C_6H_6N_4O_2$: C, 43.37; H, 3.64; N, 33.72. Found: C, 43.29; H, 3.66; N, 33.55.

9-Benzyloxy-8-methylxanthine (**7**).—A solution of the imidazole **4** (492 mg, 0.002 mol), diethyl carbonate (2 ml, 0.016 mol), and metallic sodium (460 mg, 0.02 g-atom) in ethanol was refluxed for 6 hr. The dark brown reaction mixture was cooled, and water (50 ml) was added to dissolve the precipitate. After acidifying with concentrated hydrochloric acid, the reaction mixture was concentrated *in vacuo* to precipitate **7**, which was collected and washed with water, ethanol, and ether: yield 115 mg (21%); uv $\lambda_{max}^{pH\ 1}$ 242, 263 nm; $\lambda_{max}^{pH\ 13}$ 250, 278 nm; nmr δ 7.45 (s, 5, C_6H_5), 5.26 (s, 2, $OCH_2C_6H_5$), 2.3 (s, 3, CCH_3), 10.70 (1, N^1H), 12.3 (br, 1, N^3H).

8-Methyl-9-hydroxyxanthine (**8**).—The debenzylation of **7** (82 mg, 0.003 mol) was carried out as above and the free base **8** was obtained from the hydrobromide salt by dissolving in hot dilute ammonia, treatment with charcoal, and precipitating by addition of glacial acetic acid. The white crystals were collected, washed with water, ethanol, and ether, and dried *in vacuo* over P_2O_5 at 78°, yield 44 mg (80%).

Anal. Calcd for $C_6H_6N_4O_3$: C, 39.56; H, 3.32; N, 30.76. Found: C, 39.39; H, 3.40; N, 30.80.

5-(*N'*-Benzoylthiocarbamoyl)amino-1-benzyloxy-2-methylimidazole-4-carboxamide (**9**).—The imidazole **4** (4.92 g, 0.02 mol) was dissolved in hot acetone (110 ml) and to this was added a 100-ml acetone solution containing 1.1 equiv of benzoyl isothiocyanate.[28] The mixture was refluxed for approximately 2 hr, or until tlc of the solution showed that all the 1-benzyloxyimidiazole (**4**) had reacted. The yellow precipitate that formed during the reaction was collected and washed with acetone. Recrystallization from chloroform–ethanol, after charcoal treatment, afforded the benzoyl thiocarbamoylaminoimidazole derivative (**9**) as pale yellow needles: yield 7.23 g (88%); mp 195–196° dec; λ_{max}^{EtOH} 242, sh 285 nm; nmr δ 8.1 (m, 2, COC_6H_5), 7.7 (m, 3, COC_6H_5), 7.49 (s, 5, $-CH_2C_6H_5$), 5.3 (s, 2, $OCH_2C_6H_5$), 2.18 (s, 3, CCH_3), 7.2 (br, 2, $CONH_2$), 11.93 (s, 2, $-NHCSNH-$).

Anal. Calcd for $C_{20}H_{19}N_5O_3S$: C, 58.67; H, 4.67; N, 17.10; S, 7.83. Found: C, 58.71; H, 4.85; N, 16.78; S, 7.80.

5-(*N'*-Benzoyl-*S*-methylisothiocarbamoyl)amino-1-benzyloxy-2-methylimidazole-4-carboxamide (**10**).—**9** (4.09 g, 0.01 mol) dissolved in 0.1 *N* sodium hydroxide (200 ml) was treated with 1 ml of methyl iodide at room temperature. After being stirred for several hours the solution was adjusted to pH 6 with glacial acetic acid, and then extracted several times with chloroform (100 ml). The combined chloroform extracts were dried (Na_2SO_4), concentrated to a small volume, and chromatographed over a column of silica gel with $CHCl_3$–EtOH (9:1). The eluent was monitored by tlc. Concentration of the appropriate fractions afforded **10**, which was recrystallized from ethanol: yield 3.23 g (76%); mp 165–167° dec; uv λ_{max}^{EtOH} 237 nm; nmr δ 8.80 (m, 2, COC_6H_5), 7.56 (m, 3, COC_6H_5), 7.41 (s, 5, $CH_2C_6H_5$), 7.30 (s, 2, $CONH_2$), 5.29 (s, 2, $OCH_2C_6H_5$), 2.62 (s, 3, SCH_3), 2.04 (s, 3, CCH_3).

Anal. Calcd for $C_{20}H_{21}N_5O_3S$: C, 59.57; H, 4.99; N, 16.54; S, 7.57. Found: C, 59.38; H, 4.99; N, 16.44; S, 7.67.

5-*N'*-Benzoylguanidino-1-benzyloxy-2-methylimidazole-4-carboxamide (**11**).—**10** (2.12 g, 0.005 mol) was treated with 50 ml of 2% NH_3–ethanol at 100° in a steel bomb for 3 hr. At the end of the reaction the odor of methylmercaptan could be recognized. The solvent was removed *in vacuo* to give **11**, which was pure enough for use in the ring closure step, as indicated by its uv and nmr spectra: uv $\lambda_{max}^{pH\ 1}$ 243 nm, $\lambda_{max}^{pH\ 13}$ 260 nm; nmr ($CDCl_3$) 7.9 (m, 2, COC_6H_5), 7.5 (m, 3, COC_6H_5), 7.3 (s, 5, $CH_2C_6H_5$), 5.12 (s, 2, $OCH_2C_6H_5$), 1.98 (s, 3, CCH_3), 7.2 (br, 2, $CONH_2$), 6.3 (br, 2, CNH_2).

9-Benzyloxy-8-methylguanine (**12**).—To the solid residue of **11** was added 200 ml of 0.5 *N* sodium hydroxide and the solution was warmed on a steam bath for 3 hr. The reaction mixture was then cooled and acidified with glacial acetic acid. The concurrent white precipitates of benzoic acid and the 9-benzyloxyguanine were collected and washed with water. The benzoic acid was removed by several extractions, or continuous extraction, with hot ethyl ether (200 ml). The residue remaining was crystallized from ethanol, after charcoal treatment, to afford **12** as white needles: yield 813 mg (60%); uv $\lambda_{max}^{pH\ 1}$ 256 nm (ϵ 13.8 × 10^3), sh 277 (9.29 × 10^3); $\lambda_{max}^{pH\ 12}$ 259 nm sh (ϵ 12.0 × 10^3), 267.5 (12.7 × 10^3); nmr δ 7.5 (s, 5, C_6H_5), 7.05 (s, 2, NH_2), 5.33 (s, 2, $OCH_2C_6H_5$), 2.4 (s, 3, CCH_3).

Anal. Calcd for $C_{13}H_{13}N_5O_2$: C, 57.43; H, 4.83; N, 25.77. Found: C, 57.23; H, 4.80; N, 25.93.

8-Methyl-9-hydroxyguanine (**13**).—The debenzylation of **12** (542 mg, 0.002 mol) was carried out as above. The free base **13** was obtained from the hydrobromide salt by dissolving in hot dilute ammonia, treatment with charcoal, and precipitating by addition of glacial acetic acid. The white crystals were collected, washed with water, ethanol, and ether, and dried *in vacuo* over P_2O_5 at 78°, yield 279 mg (77%).

Anal. Calcd for $C_6H_7N_5O_2$: C, 39.69; H, 3.98; N, 38.57. Found: C, 39.84; H, 3.87; N, 38.25.

Registry No.—**3**, 34407-35-7; **4**, 34407-36-8; **5**, 34407-37-9; **6**, 34407-38-0; **7**, 34407-39-1; **8**, 34407-40-4; **9**, 34417-80-6; **10**, 34417-81-7; **11**, 34407-41-5; **12**, 34407-42-6; **13**, 34407-43-7.

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