(11) J. P. Marsh, Jr., R. H. Iwamoto, and L. Goodman, *Chem. Commun.*, 589 (1968).
(12) F. Farina and J. C. Vega, *Tetrahedron Lett.*, 1655 (1972).
(13) A. N. Fujiwara, E. M. Acton, and D. W. Henry, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1974, Abstract No. MEDI-59.
(14) A. N. Fujiwara, E. M. Acton, and D. W. Henry, *J. Med. Chem.*, **17**, 659 (1974).
(15) C. E. Lewis, *J. Org. Chem.*, **35**, 7938 (1970).
(16) C. M. Wong, D. Popien, R. Schwenk, and J. Te Raa, *Can. J. Chem.*, **49**, 2712 (1971), have reported the synthesis of 4-demethoxy-7-*O*-methyldaunomycinone.
(17) C. D. Snyder and H. Rapoport, *J. Am. Chem. Soc.*, **94**, 227 (1972); **96**, 8046 (1974).
(18) We thank Professor Leon Goodman, University of Rhode Island, for suggesting this application of the oxidative demethylation procedure.
(19) δ 9.83 is found for the hydrogen-bonded protons in 1,4-dimethoxy-9,10-dihydroxyanthracene: S. M. Blood and R. F. Hutton, *Tetrahedron Lett.*, 1993 (1963).
(20) G. T. Rodeweaver and D. F. Hunt, *Chem. Commun.*, 818 (1971).
(21) H. C. Brown and C. P. Garg, *J. Am. Chem. Soc.*, **83**, 2951 (1961).
(22) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5670 (1965).
(23) J. DeGraw, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1156 (1961).
(24) R. P. Fleger and F. Reinhardt, *Chem. Ber.*, **90**, 2404 (1957); C. Delchler and C. Weizmann, *ibid.*, **36**, 547 (1903).
(25) H. Brockmann and W. Müller, *Chem. Ber.*, **92**, 1164 (1959).

Chemical Transformations of 7,9-Disubstituted Purines and Related Heterocycles. Selective Reduction of Imines and Immonium Salts

Sidney M. Hecht,*[1] Benjamin L. Adams, and John W. Kozarich[2]

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received October 17, 1975

Ten 7,9-disubstituted purines and related heterocycles were reduced with borohydride to afford the corresponding 7,8-dihydro species, a type of heterocycle which has not previously been studied in any detail. The reduced species were found to reoxidize quantitatively at rates characteristic of and predictable for the individual heterocycles; the reoxidation phenomenon was investigated and shown to involve reaction with water or oxygen. In solutions containing mixtures of reduced and oxidized heterocycles, the two species were found to be in rapid equilibrium, undoubtedly via hydride transfer. This observation prompted the utilization of the 7,8-dihydro-7,9-disubstituted heterocycles for the novel and selective reduction of imines and immonium salts. Benzylideneaniline, benzylidenebenzylamine, and cyclohexylidenepyrrolidinium perchlorate, e.g., were converted to their respective amines in excellent yields. Over the range of conditions employed for these transformations, no significant reduction of aldehydes, ketones, or several other common organic functional groups was observed, so the heterocycles may prove useful as selective reducing agents. The reactivity of the disubstituted heterocycles with nucleophiles was also studied. Certain of the oxidized compounds, e.g., were found to undergo ring opening at high pH, as has been observed previously. Treatment of the reduced, ribosylated species with aniline at pH 4.5 resulted in deribosylation of the nucleosides. This transformation, which previously has been possible only after opening of the imidazole moiety at high pH, should be of considerable utility to biochemists for the depurination of 7-methylguanosine moieties in transfer and messenger RNA's.

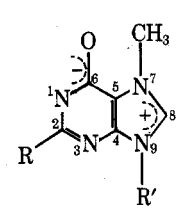
Many reports concerned with alkylated purines and their derivatives and analogues have appeared in recent years. These investigations have dealt with the synthesis of substituted heterocycles and with the effects of specific alkylating agents and reaction conditions on the position and extent of alkylation. The relevance of these alkylations as models for mutagenic change at the nucleic acid level has also been considered, as have the biological and physical properties of the alkylated species. However, substantially less work descriptive of the chemistry of the individual alkylated compounds themselves is available.

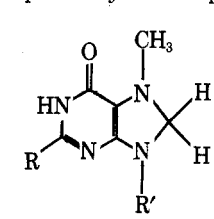
One alkylated nucleoside of special interest in this regard is 7-methylguanosine, which occurs in unique positions in certain transfer[3] and messenger[4] RNA's, and is the only naturally occurring nucleoside known to exist as a zwitterion at physiological pH. Owing to its zwitterionic character, 7-methylguanosine may undergo facile and reversible reduction,[5] a transformation first noted[6] for 1,3-dimethylbenzimidazolium iodide and 9-methylcaffeine perchlorate. 7-Methylguanosine also undergoes ring opening in strong aqueous base,[7] depurination in strong acid,[8] and selective demethylation in the presence of a powerful nucleophile.[9]

To determine the possible generality of these transformations for related heterocycles, as well as additional reaction pathways which may be available, we have studied the chemistry of ten 7,9-disubstituted purines and related compounds and report on the nature of the pH-dependent reoxidation of the reduced heterocycles, the utilization of the reduced species in the selective reduction of imines and immonium salts, and the interaction of certain of the oxidized and reduced heterocycles with strong bases and nucleophiles.

Results and Discussion

Synthesis of Disubstituted Heterocycles. The 7,9-disubstituted purines **1a–8a** were prepared by known proce-



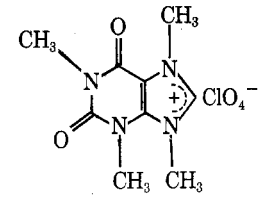


	R	R′	
1a	NH_2	β-D-ribofuranosyl	**1b**
2a	NH_2	CH_3	**2b**
3a	H	β-D-ribofuranosyl	**3b**
4a	H	CH_3	**4b**
5a	OH	β-D-ribofuranosyl	**5b**
6a	OH	CH_3	**6b**

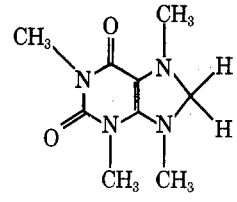
dures[10] and converted to the corresponding 7,9-disubstituted 7,8-dihydropurines by reduction with sodium borohydride in water. Isolation of the reduced purines free from boron hydrides was accomplished by concentration of the reaction mixtures to a small volume, treatment with acetone, and concentration to dryness under diminished pressure. This

procedure afforded the reduced compounds as their respective sodium salts.

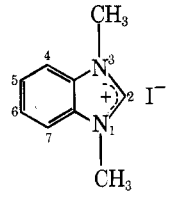
1-Benzyl-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine



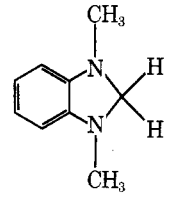
7a



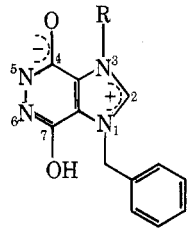
7b



8a

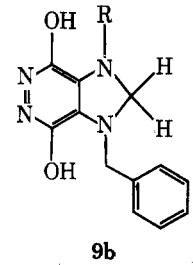


8b

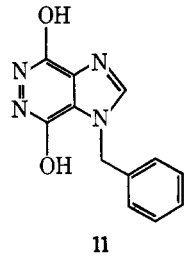


9a
10a

R
CH_3
$C_6H_5CH_2$



9b
10b



11

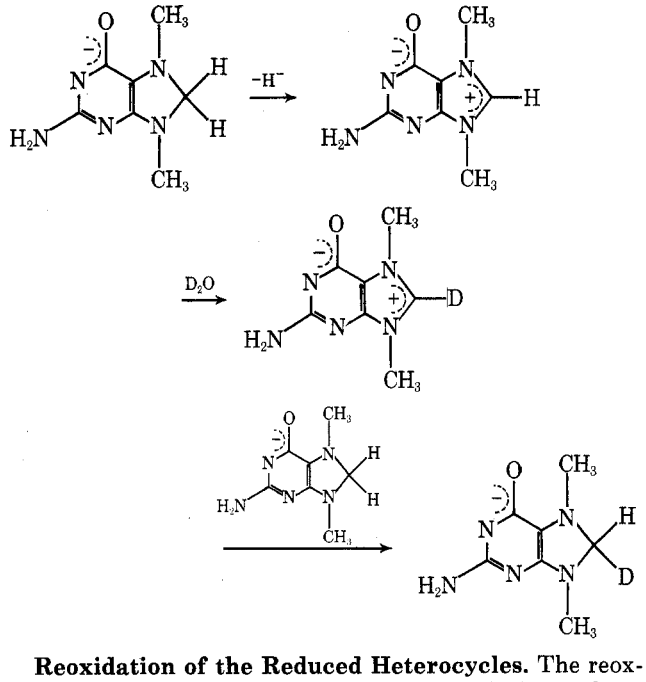
(**9a**) was obtained in 95% yield by methylation of 1-benzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**11**)[11] with methyl iodide at room temperature. The ultraviolet spectrum, high- and low-resolution mass spectra, and NMR data of **9a** were consistent with the assigned structure. 1,3-Dibenzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**10a**) was produced in similar fashion, by treatment of **11** with benzyl bromide in DMF at reflux.[12] Compounds **9a** and **10a** were converted to the respective 3-substituted 1-benzyl-2,3-dihydro-4,7-dihydroxyimidazo[4,5-*d*]pyridazines by treatment with sodium borohydride and could be isolated as the sodium salts by the evaporative procedure outlined above for the 7,9-disubstituted 7,8-dihydropurines. Alternatively, the reduced imidazo[4,5-*d*]pyridazines (**9b** and **10b**) could be obtained in protonated form rather than as the sodium salts by treatment of concentrated aqueous solutions of **9a** and **10a** with excess borohydride and then with acetic acid. The resulting suspensions of **9b** and **10b** were filtered, washed with water and tetrahydrofuran, and then dried under vacuum.

Physical and Spectral Properties of the Disubstituted Heterocycles. All of the disubstituted heterocycles studied, as well as the corresponding disubstituted dihydro species, were found to be soluble in water and to a lesser extent in ethanol, methanol, and dimethylformamide. Some of the reduced species, particularly **4b, 5b, 7b,** and **9b,** were also soluble in ethyl acetate, which permitted their extraction from aqueous media in pure form after borohydride reduction of the individual oxidized heterocycles.[13]

The ultraviolet spectra of the 7,9-dialkyl-7,8-dihydropurines and related analogues all had longer wavelength absorption than did the respective oxidized heterocycles (Table I). Thus 7,9-dimethyl-7,8-dihydroguanine (**2b**) had an absorption maximum in the ultraviolet at 304 nm at neutral pH. This absorption was characteristic of the reduced species (λ_{max} for **2a** itself were at 281 and 251 nm) and permitted the course of borohydride reduction of purine **2a,** or reoxidation of dihydropurine **2b,** to be followed conveniently by changes in the ultraviolet.[14]

The ^1H NMR spectra of the heterocycles were also characteristic of oxidation state, as was apparent for **9a** and **9b** (Table II). For 9-methylcaffeine perchlorate (**7a**), e.g., the NMR spectrum determined in D_2O (external Me_4Si) revealed singlets of equal intensity at δ 3.34, 3.78, 4.11, and 4.17, corresponding to the four methyl groups. The C-8 proton was not observed, owing presumably to rapid exchange with solvent.

This type of exchange has been noted previously[15] and parallels the exchange of tritium observed between T_2O and the C-8 proton of adenosine or guanosine.[16] The exchange suggested that the C-8 position in the disubstituted heterocycles possessed both positive and negative character. In fact, when the spectrum of 9-methylcaffeine perchlorate was recorded in dimethyl sulfoxide-d_6, in which proton exchange would be expected to be much slower, the C-8 proton was observed to resonate at δ 9.29. As expected, reduction of 9-methylcaffeine perchlorate with sodium borohydride to afford **7b** resulted in the shielding of the four methyl groups, which gave signals at δ 2.57, 3.09, 3.17, and 3.45 when measured in D_2O. The C-8 methylene protons resonated as a singlet at δ 4.35. These protons did not exchange readily in D_2O at room temperature or at 45 °C, although under more favorable conditions the corresponding methylene protons in **2b** were observed to undergo some exchange with solvent at 45 °C. This may be attributed to exchange of the following type:



Reoxidation of the Reduced Heterocycles. The reoxidation of 8,9-dihydro-7-methylguanosine (**1b**) has been studied as a model for the reversible reduction of the 7-methylguanosine moiety (which occurs in certain transfer RNA's.)[5] It was reported that the reduced species reoxidized to 7-methylguanosine with a $t_{1/2}$ of 20–35 min in 0.5 M sodium phosphate buffer at pH 7.0 and the conversion was attributed to reaction of **1b** with O_2 dissolved in the aqueous medium, although neither the experimental basis for this conclusion nor its generality were described. Investigation of reduced

Table I. Ultraviolet Absorption Maxima of the Reduced, Disubstituted Heterocycles

Compd	pH 2, λ_{max}, nm	pH 7, λ_{max}, nm	pH 10, λ_{max}, nm
7,8-Dihydro-7-methylguanosine (**1b**)	274	302	293
7,8-Dihydro-7,9-dimethylguanine (**2b**)	272	304	292
7,8-Dihydro-7-methylinosine (**3b**)	286	289	287
7,8-Dihydro-7,9-dimethylhypoxanthine (**4b**)	289	292	285
7,8-Dihydro-7-methylxanthosine (**5b**)	305, 271	300, 246	297
7,8-Dihydro-7,9-dimethylxanthine (**6b**)	291	292	288
7,8-Dihydro-9-methylcaffeine (**7b**)	277	309	310
1,2-Dihydro-1,3-dimethylbenzimidazole (**8b**)	293	302	302
1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (**9b**)	361	340	342
1,3-Dibenzyl-1,2-dihydro-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**10b**)	372	342	342

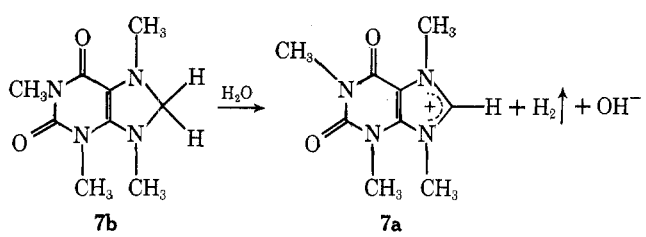
Table II. Rates of Reoxidation of the Reduced Heterocycles in Aqueous Solution at Variable pH[a]

Compd	$t_{1/2}$ values for reoxidation, min						
	pH 2	pH 3.5	pH 5	pH 6	pH 7	pH 8	pH 10
7,8-Dihydro-7-methylguanosine (**1b**)	7	8	6	13	12	22	*b*
7,8-Dihydro-7,9-dimethylguanine (**2b**)	4	3	7	10	11	6	3
7,8-Dihydro-7-methylinosine (**3b**)	35	40	52	42	50	65	*b*
7,8-Dihydro-7,9-dimethylhypoxanthine (**4b**)	18	42	50	47	50	45	40
7,8-Dihydro-7-methylxanthosine (**5b**)	1	1.5	1.5	1.5	5	12	*b*
7,8-Dihydro-7,9-dimethylxanthine (**6b**)	1	1	1	1	2	4	2
7,8-Dihydro-9-methylcaffeine (**7b**)	6	10	15	17	55	86	160
1,2-Dihydro-1,3-dimethylbenzimidazole (**8b**)	46	75	61	95	230	220	500
1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo[4,5-*d*]-pyridazine (**9b**)	10	19	17	24	12	21	*b*
1,3-Dibenzyl-1,2-dihydro-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**10b**)	2	3	12	22	24	19	*b*

[a] The pH was maintained within 0.2 unit by the use of acetate buffer at pH 2, 3.5, 5, and 6 and borate buffer at pH 7, 8, and 10. [b] Unstable at pH 10.

heterocycles **1b–10b** revealed that reoxidation in aqueous solution was a general phenomenon. Each compound was reduced in aqueous solution with sodium borohydride and, after destruction of excess borohydride, the individual solutions were adjusted to appropriate concentrations and pH values by the addition of aliquots of the concentrated solutions containing the heterocycles to acetate or borate buffer solutions. The ultraviolet spectra of the reduced heterocycles were then recorded at predetermined time intervals, as illustrated in Figure 1 for the reoxidation of 7,8-dihydro-9-methylcaffeine (**7b**) in aqueous solution at pH 4.5. The ultraviolet spectra were then utilized to calculate the $t_{1/2}$ values for reoxidation at individual pH values and the results are given in Table II. For 8,9-dihydro-7-methylguanosine (**1b**), e.g., the $t_{1/2}$ value at pH 7.0 was found to be 13 min, which was the same as the rate determined in unbuffered solution but somewhat less than the reported[5] value of 20–35 min. However, when the experiment was repeated in the presence of 0.1 M phosphate buffer, the value of $t_{1/2}$ changed to approximately 25 min, in agreement with the published value obtained under similar conditions.

If the reversible reduction of compounds **1a–10a** were to involve the type of transformation previously proposed for compounds **1a**, **7a**, and **8a**, then oxidation of the reduced



7b **7a**

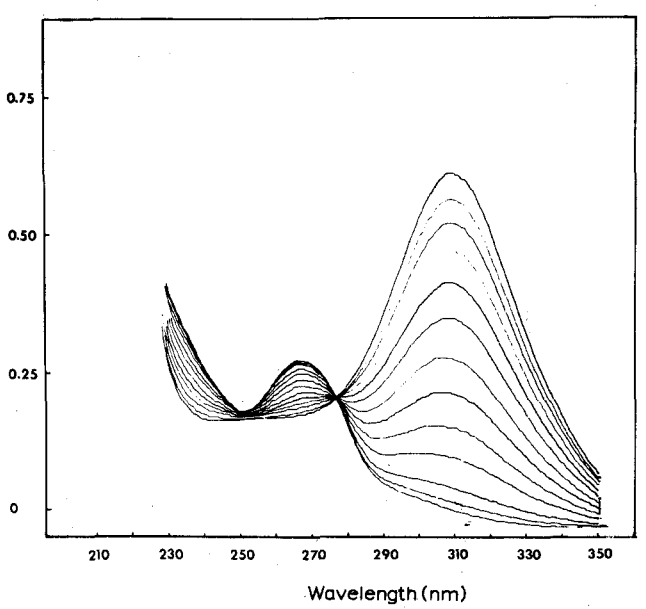


Figure 1. Reoxidation profile of 7,8-dihydro-9-methylcaffeine (**7b**) at pH 4.5. The ultraviolet spectrum of **7b** (λ_{max} 309 nm) was recorded at regular time intervals. The increase in absorbance at 266 nm was due to the formation of **7a**.

species via reaction with water or oxygen would necessarily result in the formation of equivalent molar amounts of hydroxide ion, hydrogen, and the oxidized heterocycles (**1a–10a**). Consistent with this scheme was the change in pH from 4.5 to 8.5 which accompanied the reoxidation of a 0.3 M aqueous solution of 7,8-dihydro-9-methylcaffeine (**7b**). Also of interest in this regard was the recovery in quantitative yield of a

Table III. Approximate Rates of Reoxidation of the Reduced Heterocycles in Degassed, Aqueous Solution at Variable pH[a]

Compd	$t_{1/2}$, min						
	pH 2	pH 3.5	pH 5	pH 6	pH 7	pH 8	pH 10
7,8-Dihydro-7-methylguanosine (**1b**)	7	55	100	82	20	27	*b*
7,8-Dihydro-7,9-dimethylguanine (**2b**)	18	30	24	13	6	3	4
7,8-Dihydro-7-methylxanthosine (**5b**)	12	47	68	22	15	8	*b*
7,8-Dihydro-7,9-dimethylxanthine (**6b**)	11	50	70	9	13	5	11
1,2-Dihydro-1,3-dimethylbenzimidazole (**8b**)	30	105	110	95	155	300	>600
1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo[4,5-*d*]-pyridazine (**9b**)	30	35	25	40	35	80	*b*

[a] The pH was maintained within 0.2 unit by the use of acetate buffer at pH 2, 3.5, 4, 5, and 6 and borate buffer at pH 7, 8, and 10. [b] Unstable at pH 10.

sample of 1-benzyl-4,7-dihydroxy-3-methylimidazo[4,5-*d*]-pyridazine (**9a**) which had been reduced with borohydride and allowed to reoxidize in aqueous solution. The recovered sample was identical with authentic **9a** as judged by its melting point and ultraviolet spectrum, as well as its chromatographic properties (TLC on silica gel). An experiment was also carried out in which a sample of 7,8-dihydro-7,9-dimethylxanthine (**6b**) was treated with 3 M aqueous hydrochloric acid in a sealed system and the evolved gas was trapped in a gas collection apparatus and subsequently shown to be flammable.

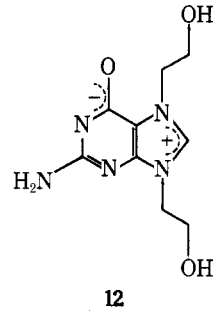
To determine if reoxidation of the reduced heterocycles did indeed involve reaction with molecular oxygen, the reoxidation experiment with 7,8-dihydro-7,9-dimethylxanthine (**6b**) was repeated in an aqueous buffer (pH 3.5) which had been carefully degassed to remove dissolved oxygen. The measured $t_{1/2}$ value in the degassed solution was 50 min (as compared with the original value of 1 min in oxygen-saturated solution) and brief agitation of the degassed solution in the presence of air reestablished reoxidation at the faster rate.[17,18] Since a substantial portion of the observed reoxidation of **6b** at pH 3.5 had obviously occurred via reaction with oxygen, the $t_{1/2}$ values for several of the heterocycles were also determined in degassed aqueous solutions to provide an approximate measure of the rate of reoxidation in the absence of oxygen (Table III). Comparison of the $t_{1/2}$ values for reoxidation obtained in the presence and absence of oxygen (Tables II and III) indicated that most of the reoxidation occurred via reaction with oxygen, especially at moderate pH values.

To demonstrate that reaction could occur between the reduced heterocycles and water at neutral pH, a sample of 7,8-dihydro-7,9-dimethylguanine (**2b**) was dissolved in degassed, aqueous solution and a portion was sealed in a tube whose volume was such that it could have contained no more than 13% of the oxygen required to effect oxidation of **2b** to **2a**. The sealed tube was maintained at room temperature for 2 weeks, during which time **2a** was formed in quantitative yield. In experiments in degassed aqueous solutions at lower pH values, all of the compounds studied oxidized faster than at neutral pH, presumably reflecting reaction of the reduced species with hydronium ions, according to the scheme

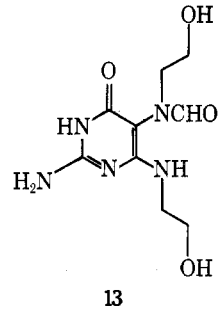
$$\mathbf{7b} + \overset{+}{H_3O} \longrightarrow \mathbf{7a} + H_2O + H_2\uparrow$$

Consistent with this scheme were the rates of reoxidation of species **7b** and **8b**, which varied inversely with pH (Tables II and III). However, a more complicated pH profile was observed for several other species, notably **1b, 2b, 5b,** and **6b** (Table III). For these compounds, the rate of reoxidation was also faster at high pH. The difference in the shape of these rate curves is presumably due to the presence of ionizable hydrogens in compounds **1b, 2b, 5b,** and **6b**. At high pH the pyrimidine ring could thus have substantial negative character. Since **1a, 2a, 5a,** and **6a** are resonance stabilized, with considerable delocalization of the net positive and negative charges formally written for each, the presence of negative charge in the pyrimidine moiety of **1b, 2b, 5b,** and **6b** would be expected to facilitate the development of positive charge in the imidazole moiety, e.g., by promoting the net loss of hydride.

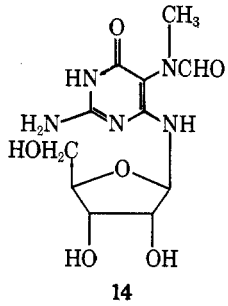
Ring Opening of the Oxidized Heterocycles. At high pH, an additional effect is operative which affects the observation of the reoxidation phenomenon but not its kinetics, namely hydrolysis of the imidazolium ring in the oxidized heterocycles. Hydrolytic opening of the imidazolium ring has been reported for several 7,9-disubstituted purines and for the 1,3-dimethylbenzimidazolium ion. Brooks and Lawley, e.g., studied the base-promoted hydrolysis of 7,9-di(2-hydroxyethyl)guanine (**12**) and reported the product as 2-amino-4-hydroxy-5-[*N*-(2-hydroxyethyl)formamido]-6-*N*-(1-hydroxyethyl)pyrimidine (**13**).[19] No evidence was presented in support of the position assigned to the formyl group, however, in spite of earlier evidence for the formation of two products from the ring opening of 1,2,3,5-tetramethylbenzimidazole.[20] Similarly, Haines et al.[7] studied the decomposition of 7-methylguanosine (**1a**) in aqueous ammonium hydroxide at pH



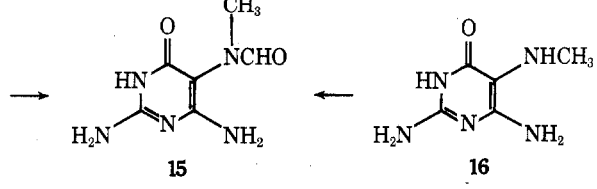
12



13



14



15 **16**

Table IV. Rates of Ring Opening of Disubstituted Heterocycles[a]

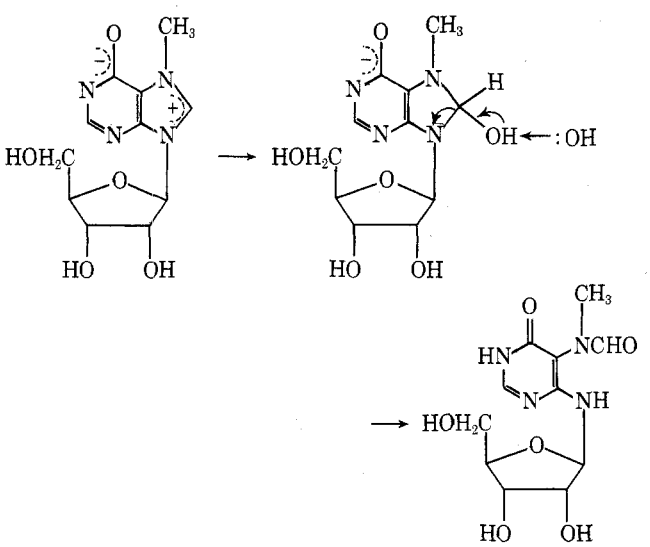
Compd	$t_{1/2}$, min
7-Methylguanosine (**1a**)	25
7,9-Dimethylguanine (**2a**)	>120
7-Methylinosine (**3a**)	5
7,9-Dimethylhypoxanthine (**4a**)	*b*
7-Methylxanthosine (**5a**)	10
7,9-Dimethylxanthine (**6a**)	*b*
9-Methylcaffeine percholorate (**7a**)	1.5

[a] Aqueous Tris–HCl, pH 10.0. [b] Stable under the reaction conditions.

11 and observed the formation of a pyrimidine which was homogeneous as judged by paper chromatography. This species was assigned the N^5-formyl structure **14** by virtue of the fact that a compound identical with its aglycone (**15**) could be obtained by formylation of **16**.[21] By analogy with the earlier work, the 5-formyl isomers have also been reported as the exclusive products resulting from the ring opening of 7-methylxanthosine[22] and 6-thio-7-methylguanosine.[23]

We have monitored the ring opening of several 7,9-disubstituted purines by ultraviolet and NMR spectrometry. For 7-methylguanosine (**1a**), e.g., treatment with 0.1 M Tris–HCl at pH 10.0 effected ring opening, which was monitored by a decrease in λ_{max} 283 nm and an increase in λ_{max} 266 nm (Figure 2), and by the appearance of the formyl proton in the NMR. For 7-methylguanosine (**1a**), the $t_{1/2}$ value for ring opening was approximately 25 min (Table IV). Rapid hydrolytic cleavage was also observed for 7-methylinosine (**3a**, $t_{1/2}$ = 5 min) and 7-methylxanthosine (**5a**, $t_{1/2}$ = 10 min) in 0.1 M Tris–HCl, pH 10.0, while the corresponding 9-methyl analogues (**2a, 4a,** and **6a,** respectively) were much more stable. The rate differences may be attributed to the ribosyl substituents in compounds **1a, 3a,** and **5a,** which may facilitate the ring opening via inductive stabilization of reaction intermediates and which undoubtedly cause some distortion of the imidazole ring, resulting in ring strain which can be relieved by ring opening. No comparable effects would be anticipated for compounds **2a, 4a,** or **6a.**

The mechanism of ring opening is postulated to involve addition of hydroxide ion at C-8 (numbered as in the purine series), followed by base-catalyzed displacement of one of the two possible anionic aminopyrimidine species. This scheme would predict that to the extent that it is delocalized into the imidazole ring, a negative charge in the six-membered ring would substantially diminish the rate of ring opening.[24] In fact



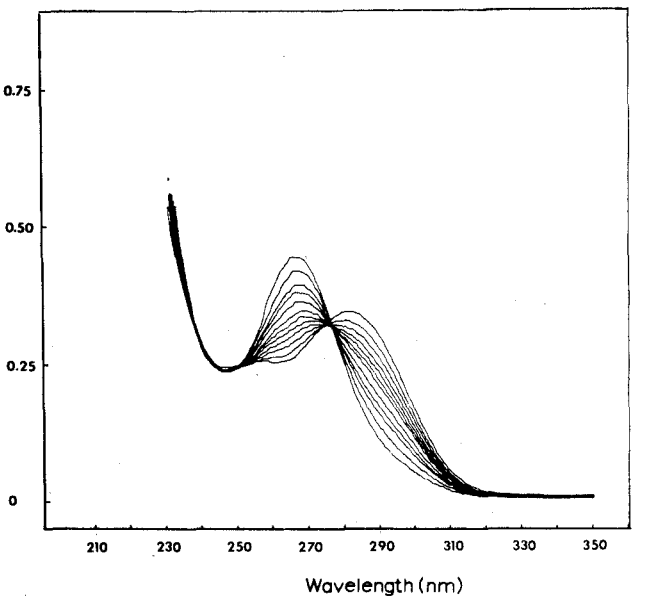
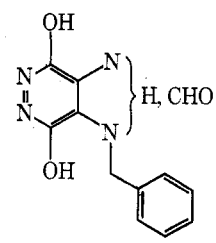


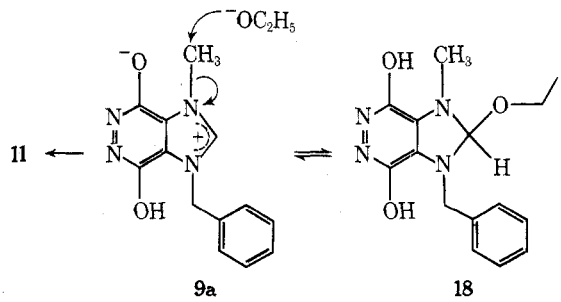
Figure 2. Base-induced ring opening of 7-methylguanosine (**1a**) in 0.1 M Tris–HCl, pH 10.0. The ultraviolet spectrum of **1a** (λ_{max} 283 nm) was recorded at 5-min time intervals. The increase in absorbance at 266 nm corresponded to the formation of ring-opened product.

treatment of 9-methylcaffeine perchlorate (**7a**) with 0.1 M Tris–HCl at pH 10.0 resulted in extremely rapid cleavage of the imidazole ring, consistent with the absence of negative charge in the pyrimidine moiety.

Treatment of the Disubstituted Heterocycles with Nucleophiles Other Than Hydroxide Ion. In analogy with the results obtained for compounds **1a, 5a,** and **7a,** treatment of 1-benzyl-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (**9a**) with aqueous base afforded N-formylated, ring-opened product **17.** In contrast, treatment of **9a** with ethoxide under anhydrous conditions afforded no *N*-formylpyridazine. Obtained instead in 44% yield was 1-benzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**11**). The formation of **11** presumably



17



9a **18**

reflected the fact that the expected addition of ethoxide to C-2 in **9a** would afford species **18,** which cannot undergo ring opening in the same sense as the hydrate of **9a.** It is anticipated that formation of **18** would be reversible, as is observed for imino esters[25] and for the addition of hydride to C-2 in **9a,** and that the slower, but irreversible displacement of the 3-methyl substituent may therefore occur.[26]

Table V. Reduction of Imines with 1,2-Dihydro-1,3-Disubstituted Imidazo[4,5-*d*]pyridazines

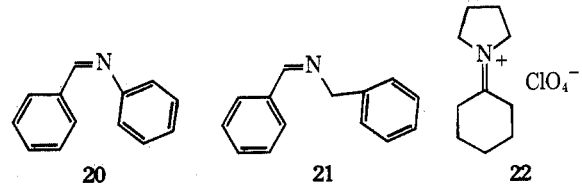
Compd[a]	Solvent[b]	Percent reduction: Benzylideneaniline (20) 8 h, room temp	Benzylideneaniline (20) 3 h, 55 °C	Benzylidenebenzylamine (21) 8 h, room temp	Benzylidenebenzylamine (21) 3 h, 55 °C
1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo-[4,5-*d*]pyridazine (**9b**)	CH_3OH	1	35	6	11
9b	DMF		0		
9b	CH_3OH + 10 μl CH_3COOH		87		
9b	CH_3OH + 25 μl CH_3COOH		97		
9b	2:1 DMF–CH_3COOH		80		
1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo-[4,5-*d*]pyridazine (**9b**) Na salt	CH_3OH		0		
9b Na^+ salt	CH_3OH + 25 μl CH_3COOH	43	67	15	31
9b Na^+ salt	CH_3OH + 40 μl CH_3COOH	88	95	70	69
1,3-Dibenzyl-1,2-dihydro-4,7-dihydroxylimidazo[4,5-*d*]pyridazine (**10b**)	CH_3OH	15	34	12	40
1,3-Dibenzyl-1,2-dihydro-4,7-dihydroxylimidazo[4,5-*d*]pyridazine (**10b**) Na^+ salt	CH_3OH		0		0
10b Na^+ salt	CH_3OH + 25 μl CH_3COOH	47	49	42	51
10b Na^+ salt	CH_3OH + 40 μl CH_3COOH	56	53	58	69

[a] The reductions utilized reducing agent and imine in a ratio of 4:1. [b] The reactions were carried out in a total volume of 200 μl (excluding added acetic acid, where indicated).

Certain of the reduced heterocycles also underwent nucleophilic displacement reactions. For example, treatment of 7,8-dihydro-7-methylguanosine (**1b**) with an aqueous 1 M solution of anilinium acetate, pH 4.5, afforded several products, including 7-methylguanine,[27] which was obtained in 68% yield and identified by comparison of ultraviolet and chromatographic properties with those of an authentic sample.

The mild conditions utilized for the reduction of **1a** with sodium borohydride or other reducing agents (e.g., **1b**), suggests the possible utility of a reducing agent–aniline treatment for depurination of the 7-methylguanosine moieties in tRNA and mRNA.

Hydride Transfer Experiments. While the reoxidation of 7,9-dialkyl-7,8-dihydropurines and related compounds apparently proceeds primarily via reaction with oxygen, the reduced compounds can effect the net transfer of hydride ions to other organic compounds. For example, mass spectrometric analysis of a sample of 7,9-dimethylguanine isolated after incubation in aqueous solution with reduced [8-^{2}H]-7-methylguanosine (**1b**) revealed 12% incorporation of deuterium.[29] Similarly, treatment of $NADP^+$ with an excess of reduced 7,8-dihydro-7,9-dimethylxanthine (**6b**) at pH 10 for 5 min afforded 25% reduction to NADPH, as judged by the change in A_{340}.[30] The reduced heterocycles were also capable of the reduction of aliphatic and aromatic imines and immonium ions. Thus treatment of a methanolic solution of benzylideneaniline (**20**) or benzylidenebenzylamine (**21**) with a



20 **21** **22**

fourfold excess of reduced heterocycles **9b** or **10b** at 55 °C for 3 h gave reduction in moderate yields to *N*-benzylaniline and dibenzylamine, respectively.

Certain factors were found to affect the extent of reduction of imines and immonium ions. For example, while treatment of a methanolic solution of benzylideneaniline with a sample of **9b** afforded *N*-benzylaniline in 35% yield (Table V), the addition of acetic acid to the reaction mixture increased the yield to 97%, presumably via protonation of the imine.[31,32] The extent of reduction of imines and immonium salts was also found to depend on the specific heterocycle utilized for the transformation. Thus reduction of benzylideneaniline with 1,2-dihydro-1,3-dimethylbenzimidazole (**8b**) in methanol–acetic acid afforded 16% of *N*-benzylaniline after 8 h at room temperature and 20% of the product after 3 h at 55 °C. Under the same conditions 7,8-dihydro-7,9-dimethylguanine (**2b**) gave 91 and 94% reduction, respectively. As shown in Table VI, the extent of reduction of **21** effected by individual compounds was generally consistent with the rate at which those

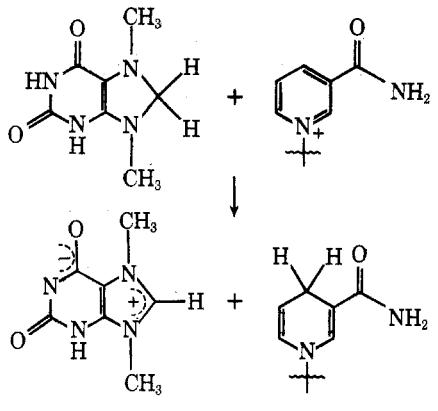


Table VI. Reduction of Benzylideneaniline, Benzylidenebenzylamine, and Cyclohexylidenepyrrolidinium Perchlorate[a]

Compd	Percent reduction: Benzylideneaniline (20) 8 h, room temp	Benzylideneaniline (20) 3 h, 55 °C	Benzylidenebenzylamine (21) 8 h, room temp	Benzylidenebenzylamine (21) 3 h, 55 °C	Cyclohexylidenepyrrolidinium perchlorate (22) 1 h, room temp
7,8-Dihydro-7,9-dimethylguanine (**2b**)	91	94	46	62	36
7,8-Dihydro-7-methylinosine (**3b**)	70	77	25	34	31
7,8-Dihydro-7,9-dimethylhypoxanthine (**4b**)	81	82	49	59	71
7,8-Dihydro-7-methylxanthosine (**5b**)	50	42	15	39	38 (96)[b]
7,8-Dihydro-7,9-dimethylxanthine (**6b**)	76	84	38	40	58
7,8-Dihydro-9-methylcaffeine (**7b**)	95	89	42	49	64
1,2-Dihydro-1,3-dimethylbenzimidazole (**8b**)	16	20	28	22	51 (76)[b]
1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (**9b**)	43	67	15	31	25 (55)[c]
1,3-Dibenzyl-1,2-dihydro-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**10b**)	47	49	42	51	(48)[c]

[a] The reductions were carried out with a fourfold excess of reducing agent in 200 μl of methanol and 25 μl of acetic acid. [b] This reduction was carried out with a fivefold excess of reducing agent in 200 μl of ethyl acetate at 40 °C for 16 h. [c] This reduction was carried out with a fivefold excess of reducing agent in 200 μl of ethyl acetate at 40 °C for 3 h.

Table VII. Reduction of Benzylideneaniline and Benzylidenebenzylamine under Optimized Conditions[a]

Compd	Percent reduction: Benzylideneaniline (20)	Benzylidenebenzylamine (21)
7,8-Dihydro-7,9-dimethylguanine (**2b**)	89	86
7,8-Dihydro-7,9-dimethylhypoxanthine (**4b**)	96 (81)[b]	85 (76)[b]
7,8-Dihydro-7-methylxanthosine (**5b**)	94	80
7,8-Dihydro-7,9-dimethylxanthine (**6b**)	98 (84)[b]	97 (82)[b]
1,2-Dihydro-1,3-dimethylbenzimidazole (**8b**)	42	40
1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (**9b**)	98	94
1,3-Dibenzyl-1,2-dihydro-4,7-dihydroxylimidazo-[4,5-*d*]pyridazine (**10b**)	79	77

[a] The reductions were carried out with an eightfold excess of reducing agent in 250 μl of 20% acetic acid in methanol at reflux for 1 h. [b] Isolated yield.

compounds were found to reoxidize in aqueous solution.[33,34]

The reductions shown in Table VI were carried out for comparative purposes and do not represent the best yields which can be obtained with the individual reducing agents. For example, the low yields of *N*-cyclohexylpyrrolidine were due in part to the instability of cyclohexylidenepyrrolidinium perchlorate in methanol–acetic acid. The same reduction can be effected in ethyl acetate, utilizing reduced heterocycles which were isolated after borohydride reduction by extraction into ethyl acetate. Thus treatment of the immonium salt with 5 equiv of 7,8-dihydro-7-methylxanthosine (**5b**) in ethyl acetate at 40 °C for 16 h afforded *N*-cyclohexylpyrrolidine in 96% yield. When the same reduction was carried out on a larger scale to permit isolation of *N*-cyclohexylpyrrolidine, the use of 1.7 equiv of **5b** for each equivalent of **22** afforded the pyrrolidine in 75% isolated yield (90% by GLC). As shown in Table VII, higher yields of dibenzylamine and *N*-benzylaniline were also obtained by treatment of imines **20** and **21** with a greater excess of reducing agent and acetic acid. These were verified by isolating the products from larger scale reactions in similar yield.

It is of interest that the reduced compounds reported here are very specific in their reduction of imines and immonium salts as compared with other common functional groups such as aldehydes, ketones, etc., and may prove useful as selective reducing agents. This is particularly true since the rate and extent of reductions obtained with these reduced heterocycles varies substantially from compound to compound and according to the reaction conditions.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are corrected. Ultraviolet spectra were recorded on a Cary 15 spectrophotometer, fluorescence spectra on a Turner Model 430 spectrofluorometer, and low-resolution mass spectra on a Perkin-Elmer Hitachi RMU-6 spectrometer using a direct inlet. The high-resolution mass spectra were obtained by Professor K. Biemann and Dr. C. Costello on a CEC-21-110B high-resolution mass spectrometer. NMR spectra were recorded on a Perkin-Elmer Hitachi R22 spectrometer at 90 MHz.

1-Benzyl-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (9a). A solution of 10.0 g (41 mmol) of 1-benzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**11**)[11] and 10.0 g (70 mmol) of methyl iodide in 250 ml of dimethylformamide was stirred at room temperature for 48 h. The solution was concentrated to afford a viscous oil which was treated with 100 ml of absolute ethanol and 400 ml of petroleum ether and allowed to stand overnight. The resulting solid was filtered, redissolved in aqueous ammonium hydroxide, and treated with hydrochloric acid to adjust to pH 5. The white precipitate was filtered and dried to afford **9a:** yield 10.0 g (95%); mp 222–224 °C dec; $C_{13}H_{12}N_4O_2$ (M^+ calcd, 256.0960; found, 256.095); λ_{max} (H_2O) (pH 2) 273 nm (ε 3400), λ_{min} 248 (2400); λ_{max} (H_2O) (pH 7) 299 (3500), λ_{min} 251 (1100); λ_{max} (H_2O) (pH 12) 307 (3800), λ_{min} 253 (1100); fluorescence spectrum $\lambda_{excitation}$ 355 nm, $\lambda_{emission}$ 470 nm; ir (KBr) 3510, 3440, 3110, 3015, 1700, 1400, 1225, 1165, and 710 cm^{-1}; NMR (Me_2SO-d_6) δ 3.97 (3 H, s), 5.65 (2 H, s), 7.22 (5 H, m) and 9.58 (1 H, s); mass spectrum *m/e* 256.095 (M^+), 242.077, 180.063, 166.048, and 134.037.

1-Benzyl-1,2-dihydro-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (9b). To 50 mg (0.20 mmol) of 1-benzyl-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (**9a**) in 0.5 ml of water was added 50 mg (1.3 mmol) of sodium borohydride. Glacial acetic acid (ca. 0.5 ml) was added dropwise to destroy the excess borohydride and the yellow precipitate was filtered, washed with water and THF, and dried in vacuo to afford **9b** as a yellow solid: yield 25 mg (50%); mp 196–200 °C dec; λ_{max} (H_2O) (pH 7) 340 nm (ε 4000), λ_{min} 279 (1900);

λ_{max} (H_2O) (pH 10) 342 (4100), λ_{min} 278 (1600); NMR (Me_2SO-d_6) δ 2.77 (3 H, s), 4.26 (2 H, s), 4.56 (2 H, s) and 7.39 (5 H, s); mass spectrum *m/e* 258 (M^+) 257, 242, 181, 180, 167, 166, 109, 108, 91, and 90.

1,3-Dibenzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (10a). A solution of 0.51 g (2.1 mmol) of 1-benzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**11**)[11] and 0.72 g (4.2 mmol) of benzyl bromide in 25 ml of DMF was heated at reflux for 24 h. The cooled solution was concentrated and the residue was treated with 10 ml of ethanol and 150 ml of petroleum ether. The viscous oil which separated was dried in vacuo to afford an off-white solid which was recrystallized from methanol–aqueous ammonium hydroxide to give **10a** as white crystals: yield 0.38 g (54%); mp 110 °C dec; $C_{19}H_{16}N_4O_2$ (M^+ calcd 332.1273; found 332.125); λ_{max} (H_2O) (pH 2) 277 nm (ϵ 3400), 268 (3400), and 264 (3200), λ_{min} 270 (3300) and 247 (2400); λ_{max} (H_2O) (pH 7) 299 (3300), λ_{min} 252 (1500); λ_{max} (H_2O) (pH 12) 306 (3400), λ_{min} 253 (1300); fluorescence spectrum $\lambda_{excitation}$ 358 nm, $\lambda_{emission}$ 471 nm; NMR (Me_2SO-d_6) δ 5.77 (4 H, s), 7.33 (10 H, m) and 10.18 (1 H, s); mass spectrum *m/e* 332.125 (M^+), 242.079, 183.055, and 129.058.

1,3-Dibenzyl-1,2-dihydro-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (10b). 1,3-Dibenzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**10a**) was reduced with borohydride and precipitated with acetic acid as described above for **9a**: yield 40%; mp 193–197 °C dec; λ_{max} (H_2O) (pH 2) 372 nm, λ_{min} 329; λ_{max} (H_2O) (pH 7) 342, λ_{min} 279; λ_{max} (H_2O) (pH 10) 342, λ_{min} 281; mass spectrum *m/e* 334, 333, 332, 243, 242, 183, 106, 92, 91, and 65.

Preparation of Reduced Dialkylated Heterocycles. Individual dialkylated heterocycles (100 mg, **1a–10a**) were dissolved in 25 ml of water and treated with 50 mg (1.3 mmol) of sodium borohydride. The reaction mixture was maintained at room temperature for several minutes and then concentrated to 1 ml, treated with 2 ml of acetone, and evaporated to dryness to afford approximately 130 mg of a white solid, approximately 75% of which consisted of the reduced dialkylated heterocycle. In several cases, the pure heterocycle could be separated from the inorganic salt by trituration with hot ethyl acetate and concentration of the ethyl acetate extract under diminished pressure.

Exchange of the C-8 Protons in 7,8-Dihydro-7,9-dimethylguanine (2b) in D_2O. A sample of 7,9-dimethylguanine (100 mg, 0.56 mmol) was reduced with borohydride and isolated as the sodium salt. The reduced heterocycle (**2b**) was dissolved in 0.5 ml of degassed D_2O and sealed in an NMR tube. After an initial NMR spectrum was recorded, the sample was heated at 50 °C for 4 h and then maintained at room temperature for an additional 6 h. An NMR spectrum was again recorded and revealed that the total intensity of resonances at δ 2.78 and 2.60 (*N*-methyl peaks) relative to that at δ 4.31 (C-8 protons) had changed from approximately 3:1 to 6:1, reflecting net exchange of deuterium into the C-8 position.

Measurement of $t_{1/2}$ Values for Reoxidation. Stock stolutions of the individual heterocycles (**1a–10a**) were prepared by dissolving approximately 5 mg of each compound and 5 mg of $NaBH_4$ in 1 ml of water. A 10-μl aliquot was withdrawn, treated with 20 μl of glacial acetic acid or 1 M hydrochloric acid to quench excess borohydride, and then combined with 1 ml of (degassed or aerated) 0.1 M acetate or 0.1 M borate buffer at the appropriate pH. Ultraviolet spectra were taken at predetermined time intervals and the spectra were used to obtain $t_{1/2}$ values for reoxidation.

Reoxidation of 7,8-Dihydro-7,9-dimethylguanine in a Sealed Tube. A solution of 21 mg (0.21 mmol) of 7,8-dihydro-7,9-dimethylguanine in 1.5 ml of degassed water was sealed in a tube (under N_2) having a total volume of 2.5 ml. The tube was maintained at room temperature for 2 weeks, then opened and immediately analyzed by TLC on silica gel which gave a single spot with R_f 0.14 (development with methanol) corresponding to 7,9-dimethylguanine (**2a**), and by its ultraviolet spectrum which had λ_{max} (H_2O) (pH 7) 252 and 279 nm, characteristic of **2a**.

Ring-Opening Reactions. The individual disubstituted heterocycles were dissolved in 2 M ammonium hydroxide and maintained at room temperature for 2 h. The solution was evaporated to dryness, redissolved in D_2O, and concentrated. This process was repeated and the final white solid was dissolved in D_2O and used to record an NMR spectrum. Descending paper chromatography (Whatman No. 1 paper) of the products derived from **1a, 5a,** and **7a** gave only one spot in each of three solvent systems (A, 6:4 5% aqueous ammonium carbonate–methanol; B, 65:16.7:18.3 2-propanol–concentrated hydrochloric acid–water; C, 7:3 ethanol–water.) The product(s) derived from the ring opening of **1a** had R_f values of 0.86 (solvent A), 0.68 (B), and 0.80 (C). The product(s) derived from **5a** had values of 0.84 (A), 0.74 (B), and 0.84 (C), while the values for the product(s) from **7a** were 0.89 (A), 0.98 (B), and 1.00 (C).

Treatment of 1-Benzyl-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine (9a) with Sodium Ethoxide. To a stirred solution of 1.02 g (4.0 mmol) of 1-benzyl-4,7-dihydroxy-3-methylimidazo[4,5-*d*]pyridazine in 50 ml of absolute ethanol was added 2 g of sodium metal at a rate which maintained the temperature of the solution at about 55 °C. The resulting solution was stirred overnight and the precipitate which formed was filtered, washed with ethanol, and dried, affording 0.57 g of a white solid. The solid was dissolved in 5 ml of water and treated with acetic acid to afford a white precipitate which was filtered and dried, giving 0.42 g (44%) of pure 1-benzyl-4,7-dihydroxyimidazo[4,5-*d*]pyridazine (**11**), identical in all respects with an authentic sample.

Treatment of 7,8-Dihydro-7-methylguanosine with Aniline. A solution of 119 mg (0.40 mmol) of 7-methylguanosine in 2 ml of water was treated with 50 mg (1.3 mmol) of sodium borohydride at room temperature for 10 min. Acetic acid (0.5 ml) was added to quench excess borohydride and to adjust the pH to 4.5. The solution was treated with 5 ml of 1 M aniline–HCl, pH 4.5, and maintained at room temperature for 5 h, during which time the solution became dark in color. An aliquot of the reaction mixture was analyzed by ascending paper chromatography on Whatman No. 1 paper in 60:40 5% aqueous ammonium carbonate–methanol. Several spots were observed and one of these (R_f 0.63) was believed to be the ribose–aniline adduct **19** on the basis of its mass spectrum [*m/e* 225 (M^+), 224, 106, 93, 73, 64, and 30] and ultraviolet spectrum [λ_{max} (H_2O) (pH 1) 248 nm; λ_{max} (H_2O) (pH 7) 274; λ_{max} (H_2O) (pH 12) 274]. Another aliquot was analyzed by paper chromatography in 5:1:4 1-butanol–acetic acid–water and several products were observed, one of which had an R_f (0.60) identical with that of 7-methylguanine. The original reaction mixture was maintained at 0 °C for 48 h and the precipitate which had formed was filtered after treatment of the reaction mixture with 20 ml of ethanol. The solid was washed with ethanol and dried to afford 7-methylguanine as a tan solid, yield 45 mg (68%), identical with authentic 7-methylguanine, as judged by comparison of their ultraviolet spectra and paper chromatographic characteristics.

Condensation of Ribose and Aniline. Ribose (135 mg, 0.90 mmol) and aniline (84 mg, 0.90 mmol) were heated at 70 °C in a solution of 5 ml of absolute ethanol and 5 ml of benzene containing 0.5 ml of 6 M hydrochloric acid. After 15 min the solution had turned reddish-brown and the cooled reaction mixture was concentrated to 2 ml. Petroleum ether (30 ml) was added and the dark oil which formed was separated and washed with petroleum ether (15 ml). The crude product (235 mg) was purified by ascending paper chromatography on Whatman No. 1 paper (60:40 5% aqueous ammonium carbonate–methanol). Three bands were observed (R_f 0.0, 0.68, and 0.80) and the band at R_f 0.68 was fluorescent and contained material whose ultraviolet and mass spectra were identical with those of the material isolated from the decomposition of **1b** in the presence of aniline.

Hydride Transfer from 7,8-Dihydro-7,9-dimethylxanthine (6b) to $NADP^+$. To 11 mg (62 μmol) of 7,9-dimethylxanthine (**6a**) in 3 ml of water was added an excess of sodium borohydride. The excess borohydride was decomposed by the addition of aqueous acetic acid and the pH was adjusted from 3.6 to 4.5 by the addition of aqueous ammonia. Nicotinamide adenine dinucleotide phosphate ($NADP^+$) (0.74 mg, 1.1 μmol) was added and the pH was quickly readjusted to 10.0 with aqueous ammonia. After 5 min, the absorbance at 340 nm was measured by dilution of the sample with 0.1 M Tris–HCl, pH 10.0. The net A_{340} value was 0.10, after correction for a control which contained no $NADP^+$, corresponding to 25% reduction of $NADP^+$ to NADPH. An additional control was run in the absence of **6b** to verify that the formation of NADPH was not due to incomplete decomposition of sodium borohydride.

Reduction of Imines with Reduced Disubstituted Heterocycles. To 50 mg (~0.2 mmol) of the reduced disubstituted heterocycles in 200 μl of solvent was added 10 mg (~0.05 mmol) of the appropriate imine. The reaction was maintained at room temperature for 8 h or heated at 55 °C for 3 h. The reaction mixture was then examined by GLC on a 6 ft × 0.25 in. copper column packed with 20% Carbowax 20M on Chromosorb W. The percent reduction was determined by comparison of the imine and secondary amine peaks. External standards of authentic products were used to verify the absolute recovery.

Reduction of Cyclohexylidenepyrrolidinium Perchlorate (22) with Reduced Disubstituted Heterocycles. To 50 mg (~0.2 mmol) of the reduced disubstituted heterocycles in 200 μl of solvent was added 10 mg (0.04 mmol) of cyclohexylidenepyrrolidinium perchlorate. The reaction mixture was warmed at 40 °C for 3 h and analyzed by GLC relative to a sample of *N*-cyclohexylpyrrolidine prepared under identical conditions using borohydride as the reducing agent.

Dibenzylamine. To 2.0 g (11 mmol) of 7,8-dihydro-7,9-dimethylxanthine in a solution consisting of 20 ml of methanol and 2

ml of acetic acid was added 0.50 g (2.5 mmol) of benzylidenebenzylamine (**21**). The reaction mixture was heated at reflux for 3 h and maintained overnight at room temperature. The reaction mixture was concentrated and the residue was partitioned between ether and 10% aqueous sodium hydroxide. The aqueous layer was extracted with two additional portions of ether and the combined ether extract was dried and concentrated to afford dibenzylamine as a clear liquid, yield 0.41 g (82%), identified by comparison of its GLC retention time and NMR spectrum with those of an authentic sample. Utilization of 7,8-dihydro-7,9-dimethylhypoxanthine as the reducing agent afforded dibenzylamine in 76% isolated yield.

***N*-Benzylaniline Hydrochloride.** To a solution of 4.0 g (22 mmol) of 7,8-dihydro-7,9-dimethylxanthine in 25 ml of methanol and 3 ml of acetic acid was added 1.0 g (5.4 mmol) of benzylideneaniline (**20**). The reaction mixture was heated at 55 °C for 1 h and maintained overnight at room temperature. The mixture was concentrated to remove excess methanol, neutralized with 10% aqueous sodium hydroxide, and extracted with benzene. Saturation of the benzene extract with hydrogen chloride effected precipitation of *N*-benzylaniline hydrochloride, which was isolated as white crystals by filtration: yield 1.0 g (84%); mp 210–212 °C (lit.[35] mp 210–212 °C); NMR ($CDCl_3$, Me_4Si) δ 4.35 (2 H, s) and 7.25 (12 H, s). Also utilized for the reduction was 7,8-dihydro-7,9-dimethylhypoxanthine. The reaction mixture was worked up as indicated above for dibenzylamine and afforded *N*-benzylaniline (identical with an authentic sample as judged by NMR spectra and GLC retention times) in 81% yield.

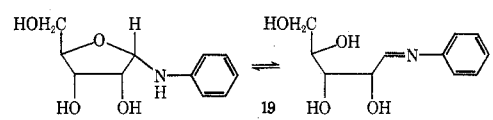
***N*-Cyclohexylpyrrolidine.** A solution of 0.50 g (2.0 mmol) of cyclohexylidenepyrrolidinium perchlorate[36] and 1.0 g (3.3 mmol) of 7,8-dihydro-7-methylxanthosine (**5b**) in 20 ml of ethyl acetate was heated at 40 °C for 16 h. The mixture was filtered and the residue was washed with ether. The combined organic solution was concentrated to afford 0.40 g (75%) of *N*-cyclohexylpyrrolidine, which was purified further by chromatography on alumina and elution with chloroform. The identity of the product was verified by comparison of NMR and mass spectral data with published values.[37]

Acknowledgment. We thank Miss Virginia Lightner for the preparation of several synthetic intermediates and Professor George M. Whitesides for the use of his polarographic analyzer and for a helpful discussion during the course of this work. This investigation was supported by Public Health Service Research Grant CA-15535 from the National Cancer Institute.

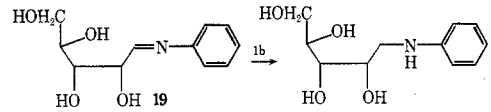
Registry No.—**1a,** 22164-16-5; **1b,** 15313-37-8; **2a,** 524-35-6; **2b,** 55235-22-8; **3a,** 22163-89-9; **3b,** 58526-72-0; **4a,** 5752-16-9; **4b,** 25472-81-5; **5a,** 58526-73-1; **5b,** 58526-74-2; **6a,** 5752-21-6; **6b,** 58526-75-3; **7a,** 17749-99-4; **7b,** 17749-90-5; **8b,** 3204-31-7; **9a,** 58526-76-4; **9b,** 58526-77-5; **9b** Na salt, 58958-41-1; **10a,** 58526-78-6; **10b,** 58526-79-7; **10b** Na salt, 58944-52-8; **11,** 5424-28-2; **19,** 55782-50-8; **20,** 538-51-2; methyl iodide, 74-88-4; benzyl bromide, 100-39-0; 7-methylguanine, 578-76-7; ribose, 50-69-1; aniline, 62-53-3.

References and Notes

(1) Alfred P. Sloan Fellow, 1975–1977. Research Career Development Awardee of the National Cancer Institute, 1975–1980.
(2) National Science Foundation Predoctoral Fellow, 1972–1975.
(3) B. G. Barrell and B. F. C. Clark, "Handbook of Nucleic Acid Sequences", Joynson-Bruvvers Ltd., Oxford, 1974.
(4) (a) C. M. Wei and B. Moss, *Proc. Natl. Acad. Sci. U.S.A.*, **72,** 318 (1975); (b) Y. Furuichi, M. Morgan, S. Muthukrishnan, and A. J. Shatkin, *ibid.*, **72,** 362 (1975); (c) J. M. Adams and S. Cory, *Nature, (London)*, **255,** 28 (1975); (d) S. Muthukrishnan, G. W. Both, Y. Furuichi, and A. J. Shatkin, *ibid.*, **255,** 33 (1975).
(5) T. Igo-Kemenes and H. G. Zachau, *Eur. J. Biochem.*, **18,** 292 (1971).
(6) A. V. El'tsov and Kh. L. Muravich-Alexander, *Tetrahedron Lett.*, 739 (1968).
(7) J. A. Haines, C. B. Reese, and Lord Todd, *J. Chem. Soc.*, 5281 (1962).
(8) D. B. Dunn, *Biochim. Biophys. Acta*, **46,** 198 (1961).
(9) S. M. Hecht and J. W. Kozarich, *J. Chem. Soc., Chem. Commun.*, 387 (1973).
(10) (a) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **84,** 1914 (1962); (b) *ibid.*, **85,** 193 (1963); (c) O. Fischer and E. Fussenegger, *Chem. Ber.*, **34,** 936 (1901); (d) H. Bredereck, G. Kupsch, and H. Wieland, *ibid.*, **92,** 566 (1959).
(11) J. A. Carbon, *J. Am. Chem. Soc.*, **80,** 6083 (1958).
(12) The compound was also characterized by its NMR, ultraviolet, and high- and low-resolution mass spectra, all of which were consistent with the assigned structure and similar to the analogous data obtained for **9a.**
(13) Analysis of the reduced species could be effected by thin layer chromatography on silica gel, development with methanol. In this system the less polar reduced heterocycles had larger R_f values than their respective oxidized forms, all of which exist as charged species.
(14) Compounds **1a–6a,** as well as **8a,** were also found to be fluorescent while the respective reduced species were not, so that the course of reduction or reoxidation could also be monitored by the disappearance or appearance of fluorescence. A similar characteristic was noted for the 1,3-disubstituted 4,7-dihydroxyimidazo[4,5-*d*]pyridazines **9a** and **10a,** which were fluorescent only in their respective reduced forms (i.e., **9b** and **10b**).
(15) M. Tomasz, *Biochim. Biophys. Acta*, **199,** 18 (1970).
(16) K. R. Shelton and J. M. Clark, Jr., *Biochemistry*, **6,** 2735 (1967).
(17) Although the addition of metal ions, such as copper and silver, to solutions of **6b** markedly increased its rate of reoxidation (as did the presence of benzoyl peroxide), treatment of **6b** with 0.05 M EDTA at pH 5.5 (in degassed or oxygenated solutions) had essentially no effect on the rate of reoxidation. It was also found that reoxidation of **6b** at pH 3.5 in the presence of 6.5 mM dithiothreitol (in degassed or oxygenated solutions) had no effect on the rate of reoxidation.
(18) Since reoxidation of the heterocycles via reaction with water would result in the formation of hydrogen peroxide, the reactivity of hydrogen peroxide with the reduced heterocycles was tested. It was found that treatment of a degassed solution (pH 7.0) of 7,8-dihydro-7,9-dimethylhypoxanthine (**4b**) with a large molar excess of hydrogen peroxide increased the rate of reoxidation by a factor of 10.
(19) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 3923 (1961).
(20) C. W. Smith, R. S. Rasmussen, and S. A. Ballard, *J. Am. Chem. Soc.*, **71,** 1082 (1949).
(21) Compound **19** was assumed to formylate on N^5 by analogy with earlier work on 2,5,6-triamino-4-pyrimidone. See W. Traube and H. W. Dudley, *Chem. Ber.*, **46,** 3839 (1913).
(22) L. B. Townsend and R. K. Robins, *J. Am. Chem. Soc.*, **85,** 242 (1963).
(23) A. D. Broom and R. K. Robins, *J. Med. Chem.*, **1,** 113 (1964).
(24) This would be expected both through diminution of the ability of the negatively charged aminopyrimidine to act as a leaving group and through a decrease in the electrophilic character at C-8.
(25) G. Simchen, H. Hoffmann, and H. Bredereck, *Chem. Ber.*, **101,** 51 (1968).
(26) This transformation parallels the demethylation of 7-methylguanosine by lithium *tert*-butylmercaptide in hexamethylphosphoramide[9] and is analogous to the SN2 displacement of methyl groups from quaternary ammonium salts [M. Shamma, N. C. Deno, and J. F. Remar, *Tetrahedron Lett.*, 1375 (1966)].
(27) Also obtained was the imine derived from ribose and aniline (**19**).[28] The latter was identified by its low-resolution mass spectrum (*m/e* 225) and by its ultraviolet and chromatographic properties, all of which were identical with those of an authentic sample derived from a 1:1 ethanol–benzene



solution of ribose and aniline which was heated in the presence of a catalytic amount of concentrated hydrochloric acid for 15 min.
(28) Although **1b** should certainly be capable of reducing imine **19** to the corresponding amine, the formation of **19** was effected under mild conditions



which disfavor its reduction and no significant amount of the amine has been observed.
(29) The theoretical incorporation of deuterium into 7,9-dimethylguanine by a pathway involving reoxidation of [8-^{2}H]-7,8-dihydro-7-methylguanosine (**1b**), deuterium exchange of [8-^{2}H]-7-methylguanosine (**1a**) with water, and subsequent exchange of deuterated water with 7,9-dimethylguanine would give <0.05% deuterium incorporation. A control reaction, run with sodium borodeuteride that had been pretreated with acetic acid, gave no detectable [8-^{2}H]-7,9-dimethylguanine.
(30) Control reactions were run without $NADP^+$ and without **6b.**
(31) In those cases in which the reduced heterocycles were utilized as their sodium salts, no reduction of **20** or **21** was observed in alcoholic media owing to the low solubility of the reducing agents. In these cases, the addition of acetic acid served to solubilize the reduced heterocycles in addition to protonating the imines.
(32) The extent of reduction was also affected by the choice of solvent. Although some reduction of **21** by **9b** was observed in neutral ethanolic or methanolic solutions, none was observed when 2-propanol, dimethylacetamide, dimethyl sulfoxide, or dimethylformamide–methanol (2:1 or 1:1) were employed as solvents. Moreover, while the addition of acetic acid did facilitate the reduction in some of these cases, as may be appreciated from the 80% yield of *N*-benzylaniline obtained by reduction in 2:1 dimethylformamide–acetic acid, no *N*-benzylaniline was formed in the presence of 4:1 dimethyl sulfoxide–acetic acid. (In the last case a portion of the reducing agent was utilized in the conversion of dimethyl sulfoxide to dimethyl sulfide, but this transformation did not consume all of the available reducing agent.)
(33) As would be expected, reduction of benzylidenebenzylamine proceeded in lower yield than reduction of benzylideneaniline in almost every case, although the extent of reduction was generally still in agreement with the relative rates of reoxidation for individual heterocycles. The few exceptions evident in Table VI can probably be attributed to phenomena such as side reactions between the heterocyclic species and reduced imines, the instability of cyclohexylidenepyrrolidinium perchlorate (**22**) to the reaction conditions, and differences in solubility of the heterocyclic species in the reaction media.
(34) Preliminary polarographic data indicated that the order of half-wave potentials paralleled the order of reoxidation and hydride transfer rates.
(35) W. S. Emerson, U.S. Patent 2 380 420 (1945); *Chem. Abstr.*, **40,** 93^7 (1946).
(36) N. J. Leonard and J. V. Paukstelis, *J. Org. Chem.*, **28,** 3021 (1963).
(37) E. L. Patmore and H. Chafetz, *J. Org. Chem.*, **32,** 1254 (1967).