Tautomerism of 4-Hydroxy- and 4,6-Dihydroxypyrimidine

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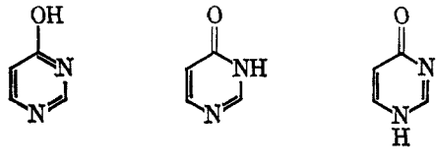
An n.m.r. study of the tautomerism of 4-hydroxy- and 4,6-dihydroxypyrimidine has shown that, in the former case, the main species present in the aqueous solution is the 3,4-dihydro-4-oxo form **2**, and, for the latter case, in dimethyl sulfoxide and 50% aqueous dimethyl sulfoxide, the compound exists predominantly in the hydroxyoxo form **5** which is in equilibrium with much smaller amounts of the dioxo form **7**. This latter conclusion is in disagreement with that drawn recently by Brown and Teitei from a comparison of the ultraviolet spectra of the parent compound and its methylated derivatives with fixed structures.

4-Hydroxypyrimidine could exist in any of the three forms, **1** (lactim), **2** (lactam), or **3** (lactam). The n.m.r. spectrum of 4-hydroxypyrimidine in D_2O consists of three signals of equal intensities, two groups of four closely spaced lines centered at 6.51 and 7.98 p.p.m., and a rather broad band at 8.37 p.p.m. These signals can be assigned unequivocally to H-5, H-6, and H-2, respectively, by comparing the chemical shifts with those of 4-hydroxy-6-methylpyrimidine. The broadening of the peak at lowest field can be attributed to the nuclear quadrupole moment effect of the two adjacent nitrogen atoms and small long-range couplings to H-5 and H-6 (Table I). Before entering into a discussion on tautomeric equilibria of 4-hydroxypyrimidine, some findings about the coupling constants will be mentioned.



1 **2** **3**

Firstly, in 4-methoxypyrimidine the value of $J_{2,5}$ (*para*) is larger than $J_{2,6}$ (across the nitrogen), a trend which is contrary to that observed for phenyl compounds where J_{para} (0–1 c.p.s.) is always smaller than J_{meta} (1–3 c.p.s.). This must be partly due to a larger dipolar separation between the two *para* carbon atoms than that between the *meta* carbon atoms. The trend is also clearly shown in the n.m.r. spectrum of pyrimidine itself[1]: $J_{2,4} = J_{2,6} = 0.6$ c.p.s., $J_{2,5} = 1.4$ c.p.s., $J_{4,5} = J_{5,6} = 5.0$ c.p.s., $J_{4,6} = 2.5$ c.p.s.

Secondly, the *ortho* coupling constants, $J_{5,6}$, increase in the order of pyrimidine (5.0 c.p.s.), 4-methoxypyrimidine (6.2 c.p.s.), 3-methyl-4-oxopyrimidine (6.8 c.p.s.), 4-hydroxypyrimidine (7.15 c.p.s.), and 1-methyl-4-oxopyrimidine (7.5 c.p.s.). Since in the present type of compounds the magnitude of the *ortho* coupling constant is determined by the contributions from π-electron interactions and σ-electron interactions,[2] a correlation between $J_{5,6}$ and the C-5–C-6 bond order may be expected. That this indeed is the case is clear from the calculations of the C-5–C-6 bond order by the simple LCAO–MO treatment for pyrimidine and the three tautomeric forms of 4-hydroxypyrimidine: $p_{4,5} = p_{5,6} = 0.667$ for pyrimidine, $p_{5,6} = 0.676$ for **1**, $p_{5,6} = 0.725$ for **2**, and $p_{5,6} = 0.784$ for **3**.

When an aromatic methoxyl group is replaced by a phenolic hydroxyl, it has been shown that the chemical shifts of the *ortho* and *meta* protons are shifted upfield by the extent of 0.11 ± 0.04 and 0.07 ± 0.04 p.p.m., respectively.[3] Thus, the predicted δ_5 and δ_6 values for the 4-hydroxypyrimidine structure **1** would be 6.84 and 8.38 p.p.m., respectively, which does not agree with the observed values of 6.51 and 7.98 p.p.m. The observed values instead are in much better agreement with the values for 1-methyl- and 3-methyl-4-oxopyrimidine. In order to estimate which of the two tautomers is predominant in aqueous solution, the chemical shifts of C-5 protons were compared. Comparison of the chemical shifts of the C-2 or C-6 protons is less suited for this purpose because the chemical shifts would be more sensitive to the electronic and/or anisotropic effects of the methyl groups introduced at C-1 or C-3. From the values 6.51, 6.33, and 6.55 p.p.m. for 4-hydroxypyrimidine (**2** and **3**), and its methyl derivatives, it is concluded that the main species present is **2** and that this conclusion supports previous estimations based on Raman and infrared spectroscopy.[4] However, this ratio naturally depends on the dielectric constant of the solvent, and as expected, the shape of the ultraviolet spectra in water–ethanol solutions is progressively shifted toward the isomer **2** as the ethanol content is increased. It was not possible for us to examine the temperature dependence of the tautomeric equilibrium.

Cation formation decreases the local charge density of the ring carbon atoms, and consequently the protons become less shielded and a marked paramagnetic shift is observed. Anion formation, on the other hand, causes small but definite diamagnetic shifts of the chemical shifts; in the case of anion, the diamagnetic shift caused by the negative charge is partly counterbalanced by a paramagnetic ring-current effect. In 4-hydroxypyrimidine, a plot of the δ_2 and $J_{2,5}$ values, which changed significantly with variation of acidity of the media, against pH and H_0 showed curves typical for acid–base titration (Figure 1). From the inflection points of these curves,[5] the apparent pK values were obtained, pK_1 = 1.8 and pK_2 = 8.5, being in fairly good agreement with those obtained by potentiometric and spectrophotometric methods,[6] pK_1 = 1.69 and pK_2 = 8.60.

4,6-Dihydroxypyrimidine could also exist in any of five forms, namely, the dihydroxy form **4**, the three hydroxyoxo forms **5**, **6**, and **8**, and the dioxo form **7**.

(1) S. Gronowitz and R. A. Hoffman, *Arkiv Kemi*, **16**, 459 (1960).
(2) *Cf.* R. A. Hoffman and S. Gronowitz, *ibid.*, **16**, 471 (1960).
(3) P. Diehl, *Helv. Chim. Acta*, **44**, 829 (1961); G. W. Smith, quoted in A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, 1523 (1964).
(4) A. Albert and E. Spinner, *ibid.*, 1221 (1960); in this paper, it is concluded that the *o*-quinonoid form **2** is present in aqueous solution in more than 80%.
(5) R. W. Taft and P. L. Levins, *Anal. Chem.*, **34**, 436 (1962).
(6) D. J. Brown and L. N. Short, *J. Chem. Soc.*, 331 (1953).

TABLE I

CHEMICAL SHIFTS AND COUPLING CONSTANTS OF 4-HYDROXYPYRIMIDINE AND RELATED COMPOUNDS[a]

Pyrimidine	Solvent[b]	δ_2	δ_5	δ_6	δ_{CH_3}	$J_{2,5}$	$J_{2,6}$	$J_{5,6}$	J_{5,CH_3}
4-Hydroxy	N	8.37	6.51	7.98	...	1.0	1.2	7.15	...
	A	9.26	6.85	8.13	...	0.8	1.7	7.7	...
	B	8.22	6.30	7.91	...	1.1	1.0	6.30	...
4-Hydroxy-6-methyl	N	8.21	6.29	...	2.30		...	...	0.84
	A	8.90	6.56	...	2.46	0.8	...	...	1.0
	B	8.14	6.16	...	2.19	1.0	...	...	0.7
1-Methyl-4-oxo	N	8.36	6.33	7.79	3.75	0.6	2.5	7.5	...
	A	9.18	6.88	8.09	3.99	0.7	2.3	7.9	...
3-Methyl-4-oxo	N	8.42	6.55	7.99	3.56	0.8	0.6	6.8	...
	A	9.46	6.92	8.19	5.01	0.6	1.7	7.7	...
4-Methoxy	N	8.68	6.95	8.45	3.99	1.2	0.8	6.2	...
	A	9.23	7.44	8.74	4.28	1.0	1.6	7.2	...

[a] Chemical shift (δ) in parts per million; coupling constant in cycles per second. [b] N, D_2O; A, 2.13 *N* D_2SO_4; and B, 1.44 *N* NaOD.

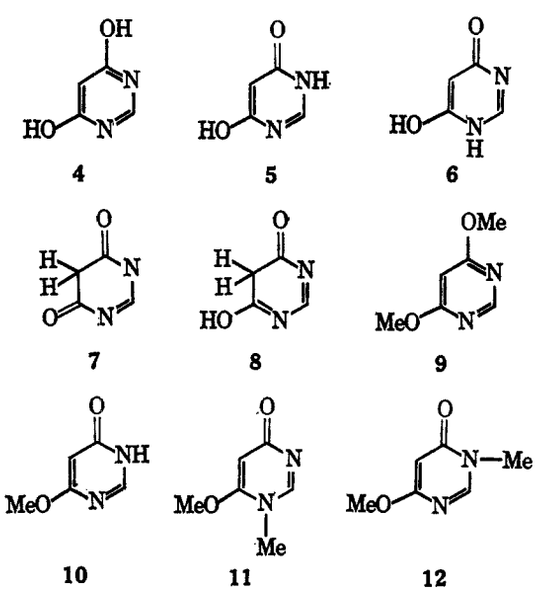
TABLE II

CHEMICAL SHIFTS OF 4,6-DIHYDROXYPYRIMIDINE AND ITS METHYL DERIVATIVES UNDER VARIOUS CONDITIONS

Entry	Pyrimidine	Solvent	NH and OH	C-2 H	C-5 H	O-CH_3	N-CH_3
a	4,6-Dihydroxy	DMSO	11.8	8.09	5.32	...	...
b		*Ca.* 50% DMSO–D_2O	...	8.09	5.34	...	...
c[a]		DMSO + 1 drop of D_2O (after 7 days)	...	8.10	5.34	...	...
d[b]		DMSO + 1 drop of NaOD (after 3 min.)	...	8.09	5.34	...	...
e		1.44 *N* NaOD	...	7.88	...	...	...
f		14.1 *N* D_2SO_4	...	8.84	...	...	...
g	4,6-Dimethoxy	D_2O	...	8.32	6.22	3.96	...
h	4-Hydroxy-6-methoxy	DMSO	12.0	8.12	5.57	3.80	...
i		D_2O	...	8.19	5.83	3.90	...
j		1.44 *N* NaOD	...	8.06	5.70	3.82	...
k		14.1 *N* D_2SO_4	...	9.00	6.17	4.11	...
l	1-Methyl-4-oxo-6-methoxy	D_2O	...	9.15	6.04	3.53	2.86
m	3-Methyl-4-oxo-6-methoxy	D_2O	...	8.31	5.88	3.91	3.53

[a] Intensity ratio of C-5 H to C-2 H is less than 1:2. [b] Intensity ratio of C-5 H to C-2 H is about 1:4.

Although some attempts have been made to clarify the tautomerism of this compound, no definite conclusion has been drawn until recently. Early infrared studies in the solid state suggested that it existed in the hydroxyoxo form **5**, **6**, or **8**,[7,8] and the relatively strong acidity (pK = 5.4) was considered to be consistent with such a structure.[9] More recently,[10] on the other hand, comparison of its ultraviolet spectrum in aqueous buffer with the spectra of N-alkyl, O-alkyl, and 5,5-dialkyl derivatives having fixed structures led to the conclusion that, "beyond reasonable doubt," 4,6-dihydroxypyrimidine exists in aqueous solution essentially as the dioxo form **7** in equilibrium with a smaller amount of the hydroxyoxo form **6**.



4 **5** **6**

7 **8** **9**

10 **11** **12**

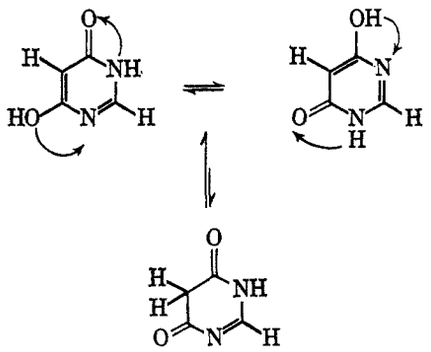
However, the present n.m.r. measurements are not in agreement with the latter view and establish that 4,6-dihydroxypyrimidine exists predominantly as an equilibrium mixture of the two identical hydroxyoxo forms **5** with a minor contribution from at least the dioxo form **7** (Chart I, in which the curved arrows denote rapid proton exchange).[11]

The n.m.r. spectra of 4,6-dihydroxypyrimidine and several methyl derivatives are summarized in Table II. The spectrum of the parent compound in dry dimethyl sulfoxide (DMSO) consists of two sharp doublets (J = 0.8 c.p.s.) of equal intensity at 5.32 and 8.09 p.p.m. [chemical shift from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS)] and a very broad signal at 11.8 p.p.m. with twice the intensity of each of the doublet signals. The low-field signal is due to the NH and OH protons. This n.m.r. spectrum alone enables one to conclude that forms **7** and **8**, carrying an active methylene group, are not the major species in dry DMSO.

(7) L. N. Short and H. W. Thompson, *J. Chem. Soc.*, 168 (1952).
(8) E. M. Tanner, *Spectrochim. Acta*, **8**, 9 (1956).
(9) A. Albert and J. N. Phillips, *J. Chem. Soc.*, 1294 (1956).
(10) D. J. Brown and T. Teitei, *Australian J. Chem.*, **17**, 567 (1964).
(11) A similar study has been made on the structure of maleic hydrazide by O. Ohashi, M. Mashima, and M. Kubo, *Can. J. Chem.*, **42**, 970 (1964).

CHART I

TAUTOMERISM OF 4,6-DIHYDROXYPRIMIDINE



This conclusion can also be extended to aqueous solutions, since stepwise addition of D_2O to the DMSO solution up to a concentration of 50% resulted in no change of the n.m.r. spectrum apart from the disappearance of the low-field NH and OH signal (Table II, b); the sample started to precipitate at higher concentrations of D_2O. When a DMSO solution containing 1 drop of D_2O was left for 1 week, the 5.34-p.p.m. peak intensity decreased to less than one-half of the 8.10-p.p.m. intensity (Table II, c). The rate of this proton and deuterium exchange was greatly enhanced by the presence of acid or base, so that the spectra of the compound in aqueous D_2SO_4 or NaOD consisted of only one peak around 8 p.p.m. (Table II, d–f). This phenomenon can only be understood if 4,6-dihydroxypyrimidine exists predominantly in one of the forms **4**, **5**, or **6**, or a mixture of them, and only if the major species is assumed to be in equilibrium with a small amount of one or both of the tautomers **7** and **8** in which the protons attached to C-5 could readily undergo exchange with protons from protic solvents.

The tautomer **4** cannot be present to any significant extent, since solutions of 4,6-dihydroxypyrimidine in 50% DMSO–D_2O show the C-5 proton resonance at 5.34 p.p.m. which is considerably less than that (6.00 p.p.m.) calculated[3] from the resonance of the C-5 proton (at 6.22 p.p.m.; Table II, g) in 4,6-dimethoxypyrimidine **9** with a fixed structure. Assignments of the 5.32- and 8.09-p.p.m. signals, in the n.m.r. spectrum of 4,6-dihydroxypyrimidine as indicated in Table II, a, to the C-5 proton and the C-2 proton, respectively, is based on a comparison with the spectrum of 4-hydroxy-6-methoxypyrimidine **10** (Table II, h); as in the case of 4-hydroxypyrimidine, the n.m.r. peaks of compound **10** have been unambiguously assigned. In order to determine which of the remaining two forms, the cyclic amide form **5** or its vinylog **6**, is the more favored, the spectra of the two dimethyl derivatives **11** and **12** were compared with that of **10** and then with that of the parent compound (Table II, b, i, l, and m) and this indicated that the major species of 4,6-dihydroxypyrimidine is **5**.

As mentioned above, the fact that the C-5 proton is readily exchanged with protons from the solvent showed that tautomers **7** and/or **8** carrying an active methylene group should be involved, although to a minor extent, in the equilibrium. However, 4-hydroxy-6-methoxypyrimidine **10** was not deuterated at C-5, even under strongly basic or acidic conditions, as can be seen from Table II, j and k, and consequently

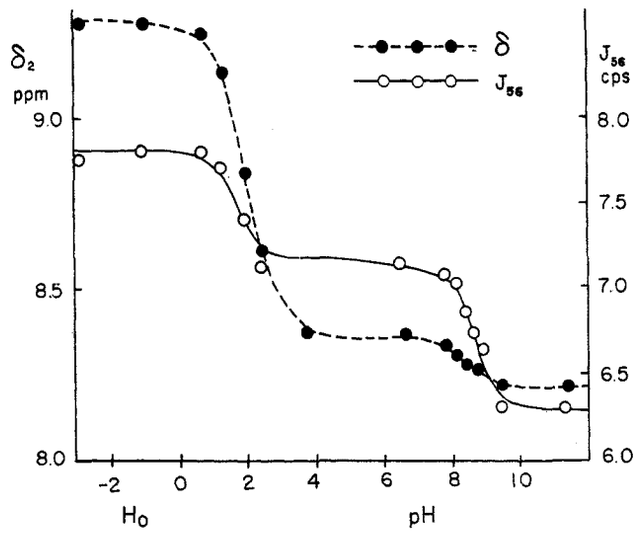


Figure 1.—Acid–base equilibria of 4-hydroxypyrimidine: δ_2 (p.p.m.) and $J_{5,6}$ (c.p.s.) *vs.* pH and H_0.

it is apparent that the dioxo form is a necessary intermediate for deuterium exchange at C-5.

The fact that 4,6-dihydroxypyrimidine in aqueous solution readily undergoes condensation with benzaldehyde[10] shows that significant amounts of a tautomer possessing an active 5-methylene grouping must exist in rapid equilibrium with **5**, but does not prove that the former tautomer is the predominant one.

Thus, the structure of 4,6-dihydroxypyrimidine seems best expressed by the equilibrium scheme shown in Chart I.[12]

Experimental Section

Materials.—4-Hydroxypyrimidine was kindly supplied by Dr. S. Matsuura of Nagoya University. Other materials used in the present study were prepared by standard methods: 1-methyl-4-oxo-pyrimidine,[13] 3-methyl-4-oxo-pyrimidine,[13] 4-methoxypyrimidine,[6] 4,6-dihydroxypyrimidine,[14] 4,6-dimethoxypyrimidine,[15] 4-hydroxy-6-methoxypyrimidine,[10] 1-methyl-4-oxo-6-methoxypyrimidine,[10] and 3-methyl-4-oxo-6-methoxypyrimidine.[10]

Instrumentation.—All n.m.r. spectra were measured with a Varian A-60 spectrometer. Chemical shifts were measured from an internal reference signal of sodium 2,2-dimethyl-2-silapentane-5-sulfonate in parts per million. Ultraviolet spectra were recorded on either a Shimadzu Model RS 27 or a Jasco Model ORD/UV-5 recording spectrophotometer and the extinction coefficients and maxima were checked on a Hitachi Model EPU-2 photoelectric spectrophotometer. The results are summarized in Table III.

TABLE III

LIGHT-ABSORPTION SPECTRA IN WATER–ETHANOL MIXTURES

Compd.	Medium	λ_{max}, mμ (ε)
4-Hydroxypyrimidine	Water	223 (6180), 240 (4100), 260 (3280)
	25% ethanol	222 (6350), 240 (3720), 262 (3270)
	50% ethanol	222 (6570), 241 (3060), 265 (3220)
	75% ethanol	221 (6670), 241 (2440), 268 (3170)
	Ethanol	221 (6570), 271 (3060)
1-Methyl-4-oxopyrimidine	Water	240 (12,300)
3-Methyl-4-oxopyrimidine	Water	221 (5470), 270 (3030)

(12) Although the electronic spectrum is one of the useful methods available to study tautomerism, this method may lead to erroneous conclusions if the spectra of the potentially tautomeric compound and various alkylated derivatives are not markedly different as in the case of present compound.

(13) D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, 221 (1955).

(14) R. Hull, *ibid.*, 2214 (1951).

(15) D. J. Brown and J. S. Harper, *ibid.*, 1298 (1961).

MO Calculations.—In the simple LCAO–MO calculations of π-bond orders, the recommended figures for the coulomb and the exchange integral parameters were used, and a factor of 0.10 was employed for the "auxiliary inductive parameter" of the carbon atoms vicinal to heteroatoms.[16] Actual calculations were made with a Model NEAC-2230 electronic computor at the Computor Center of Tohoku University.

(16) A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemists," John Wiley and Sons, Inc., New York, 1961, p. 135.

Purine N-Oxides. XVII. The Oxidation of Guanine at Position 7[1]

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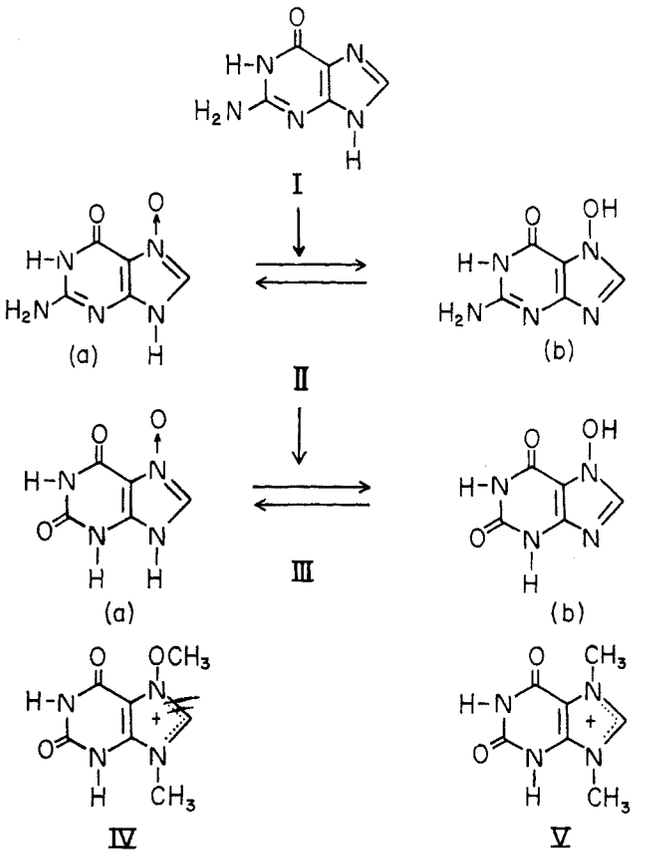
The 7-nitrogen of guanine was oxidized by trifluoroperoxyacetic acid. Upon hydrolysis only the corresponding xanthine derivative results. The position of the oxygen atom was proven by hydrolysis to N-hydroxyglycine.

The oxidation of a nitrogen of guanine was accomplished[2] and the product, designated there as guanine x,N-oxide, and its hydrolysis product, designated as xanthine x,N-oxide, were found to be potent carcinogenic agents.[2] We present here the synthesis, structure, and the characterization of several derivatives of the oxidized guanine.

A mixture of trifluoroacetic acid and hydrogen peroxide was used to oxidize guanine (I). Oxidations of purines with acetic acid–hydrogen peroxide mixtures have been successful when amino[3] or methyl substituents,[4] but not oxygen, are present on the 2- or 6-carbons of the pyrimidine ring, although the presence of oxygen on the imidazole carbon does not interfere.[4,5] Many unsuccessful attempts have been made, including at higher temperatures and with the presence of minimal amounts of water, to oxidize guanine, xanthine, and similar purines. The failures could be attributed to either the existence of the oxypurines in the lactam form, or to their insolubility in acetic acid–hydrogen peroxide mixtures. However, in mineral acid solutions guanine is readily oxidized by hydrogen peroxide to products without a specific absorption in the ultraviolet region.

Despite its insolubility in acetic acid, guanine is quite soluble in trifluoroacetic acid. From a hot solution complexes with trifluoroacetic acid can be obtained; there is no evidence that these are acyl derivatives. With hydrogen peroxide present there results a facile oxidation to one major product. Upon recrystallization from hydrochloric acid, this product is obtained as a hemihydrochloride, and from sodium hydroxide the anhydrous free base, $C_5H_5N_5O_2$ (II), is precipitated by acetic acid. With Raney nickel II consumed 1 mole of hydrogen to yield guanine.[2]

This guanine oxide is stable in moderate concentrations of hydrochloric acid, in contrast to adenine 1-N-oxide which is easily hydrolyzed in 0.1 *N* HCl with the loss of C-2.[6] With hot 6 *N* HCl overnight, conditions comparable to the hydrolysis of guanine to xanthine,[7] II yields a xanthine oxide (III). This separates as a hydrochloride hydrate from 6 *N* HCl and as a hemihydrochloride from 2 *N* HCl. That the xanthine N-oxide is more basic than xanthine is shown by the fact that the latter forms a hydrochloride only in concentrated hydrochloric acid, whereas the xanthine hydrochloride yields free xanthine when recrystallized from 2 *N* HCl. A mono- and a dihydrate of the xanthine N-oxide are obtained from either neutral or acetic acid solutions.



Xanthine oxide is but slowly reduced to xanthine by Raney nickel and hydrogen,[2] in analogy to the difficult reduction of xanthine 3-N-oxide,[8] and of 1-hydroxy-7-benzylxanthine.[9]

(1) This investigation was supported in part by Public Health Service Research Grant No. CA-03190-08, and from the Atomic Energy Commission, Contract AT(30-1)-910.

(2) G. B. Brown, K. Sugiura, and R. M. Cresswell, *Cancer Res.*, in press.

(3) M. A. Stevens, D. I. Magrath, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958).

(4) M. A. Stevens, A. Giner-Sorolla, H. W. Smith, and G. B. Brown, *J. Org. Chem.*, **27**, 567 (1962).

(5) G. B. Brown, M. A. Stevens, and H. W. Smith, *J. Biol. Chem.*, **233**, 1513 (1958).

(6) M. A. Stevens and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2759 (1958).

(7) E. Fischer, *Ber.*, **43**, 805 (1910).

(8) R. M. Cresswell, H. K. Maurer, T. Strauss, and G. B. Brown, *J. Org. Chem.*, **30**, 408 (1965).

(9) L. Bauer, *J. Heterocyclic Chem.*, **1**, 275 (1964).