Purine N-Oxides. XLIV. The Cyclization of 6-Amino-5-nitrosouracil with Formaldehyde. Preparation and Properties of 7-Hydroxyxanthine[1]

GURY ZVILICHOVSKY[2] AND GEORGE BOSWORTH BROWN*

Division of Biological Chemistry, The Sloan-Kettering Institute for Cancer Research, and The Sloan-Kettering Division of the Graduate School of Medical Sciences of Cornell University, New York, New York 10021

Received November 8, 1971

7-Hydroxyxanthine has been obtained from the reaction of one of the tautomeric forms of 6-amino-5-nitrosouracil and formaldehyde. Its 7-acetoxy derivative reacts rapidly with nucleophiles in neutral aqueous solutions to yield 8-substituted xanthines. Similar facile nucleophilic substitutions at position 8 are observed with 3-acetoxyxanthine, and an analogous mechanism *via* a common intermediate is proposed.

The xanthine *N*-oxide which is derived from the peroxy acid oxidation product of guanine was first designated xanthine-x-*N*-oxide,[3] and later 7-hydroxyxanthine.[4] It was proven to be 3-hydroxyxanthine[5] by both degradation and by total synthesis. The ability of 3-hydroxyxanthine to induce tumors[6] and its chemical properties, including the facile reaction of its 3-*O*-acyl derivative[7] with nucleophiles to yield 8-substituted xanthines,[8,9] have been studied in some detail. 1-Acetoxyxanthine fails to undergo such a reaction,[8] and 1-hydroxyxanthine is very weakly oncogenic.[6] The behaviors of the 7- and 9-hydroxyxanthine derivatives are of particular interest, and syntheses of 9-hydroxy derivatives are in process.[10]

Several synthetic routes to 7-hydroxyxanthine have been explored in this laboratory without success. While 7-hydroxytheophylline and its 8-alkyl derivatives are known,[11,12] the syntheses which led to them have not been successful without blocking groups in the 1 and 3 positions.

We now report the isolation of 7-hydroxyxanthine (**7**) from the mixture resulting from the reaction of 6-amino-5-nitrosouracil (**2**) with aqueous formaldehyde.[13] The yield of pure 7-hydroxyxanthine is low, but the few steps involved make the synthesis of preparative value.

It has been shown that 6-amino-5-nitrosouracil exists in three tautomeric forms (**2a**, **2b**, and **2c**, Scheme I) which differ in their color.[15] The tautomer which is thought to be the nitrosoamino species, **2a**, is apparently the only proper one for this preparation. Samples of 6-amino-5-nitrosouracil (**2**) obtained by a brief nitrosation of 6-aminouracil with $NaNO_2$ in HCl proved satisfactory in the condensation. Recrystallization, or prolonged stirring of 6-amino-5-nitrosouracil in the mother liquor, gave a product which failed to give a satisfactory yield of 7-hydroxyxanthine. The cyclization proceeds at 100° and a pH of 2.5–3.5. Higher pH's prevent the reaction. At lower pH's there is increased hydrolysis of **2** to 5-nitrosobarbituric acid (**1**, violuric acid). A few other solvent systems have been tried: in boiling 4:1 dioxane–H_2O there was formation of a large amount of 6-aminouracil;[16] in 50% ethanol there was negligible reaction.

A major product is uric acid, and, if the reaction is prolonged, little or no 7-hydroxyxanthine (**7**) and much more uric acid (**8**) are obtained. The ability of 7-hydroxyxanthine to rearrange to uric acid in the reaction mixture was demonstrated by boiling **7** in aqueous formaldehyde.

The products are all solubilized in the reaction mixture, presumably as their *N*-hydroxymethyl derivatives. Although most of the *N*-hydroxymethyl groups are lost upon treatment with ammonia, their continued presence complicates the isolation procedures. 1-Hydroxymethyl-7-hydroxyxanthine (**6**) was also isolated and characterized. It is stable at room temperature, but in hot water or hot NH_4OH it is hydrolyzed to **7**.

The separation of the reaction products was carried out by ion-exchange chromatography over Dowex-50, $-H^+$. A fraction containing primarily 7-hydroxyxanthine could be eluted with H_2O and recognized by its characteristic uv absorption, including the appearance of a maximum at 225 nm upon addition of alkali. Two additional fractions which have this spectral characteristic were eluted prior to 7-hydroxyxanthine. One of them contained a methylol derivative isomeric with **6**.

Several possible intermediates are shown in Scheme I. They are probably all hydroxymethylated on some of their nitrogens. It is probable that such hydroxymethyl groups influence the reaction in the same manner as 1- and 3-alkyl groups appear to have influenced previous syntheses of 7-hydroxytheophylline derivatives. It is of interest to consider the mechanisms proposed for similar reactions, although none consider the apparent need of blocking the 1 and 3 nitrogens. Probably the most logical intermediate is **3**, which could yield **7** by a mechanism proposed by Goldner,

(1) This work was supported in part by funds from the National Cancer Institute (Grant No. CA 08748).

(2) David A. Gimbel Memorial Fellow.

(3) G. B. Brown, K. Sugiura, and R. M. Cresswell, *Cancer Res.*, **25**, 986 (1965).

(4) T. J. Delia and G. B. Brown, *J. Org. Chem.*, **31**, 178 (1966).

(5) U. Wölcke and G. B. Brown, *ibid.*, **34**, 378 (1969).

(6) K. Sugiura, M. N. Teller, J. C. Parham, and G. B. Brown, *Cancer Res.*, **30**, 184 (1970).

(7) N. J. M. Birdsall, T. C. Lee, and U. Wölcke, *Tetrahedron*, **27**, 5961 (1971).

(8) N. J. M. Birdsall, U. Wölcke, T. C. Lee, and G. B. Brown, *ibid.*, **27**, 5969 (1971).

(9) N. J. M. Birdsall, U. Wölcke, J. C. Parham, and G. B. Brown, *ibid.*, **28**, 3 (1972).

(10) A. A. Watson and G. B. Brown, *J. Org. Chem.*, **37**, 1867 (1972); A. A. Watson, unpublished work.

(11) (a) H. Goldner, G. Dietz, and E. Carstens, *Justus Liebigs Ann. Chem.*, **691**, 142 (1966); (b) *ibid.*, **693**, 233 (1966).

(12) E. C. Taylor and E. E. Garcia, *J. Amer. Chem. Soc.*, **86**, 4721 (1964).

(13) Taylor and Garcia[12] carried out a condensation of 1,3-dimethyl-4-amino-5-nitrosouracil with benzaldehyde in DMF, and report 8-phenyl-7-hydroxytheophylline as an intermediate in the formation of 8-phenyltheophylline. In other reports[11a,12] benzanil was used as a source for benzaldehyde for formation of an 8-phenylpurine 7-oxide, including one from a 5-nitro pyrimidine.[14]

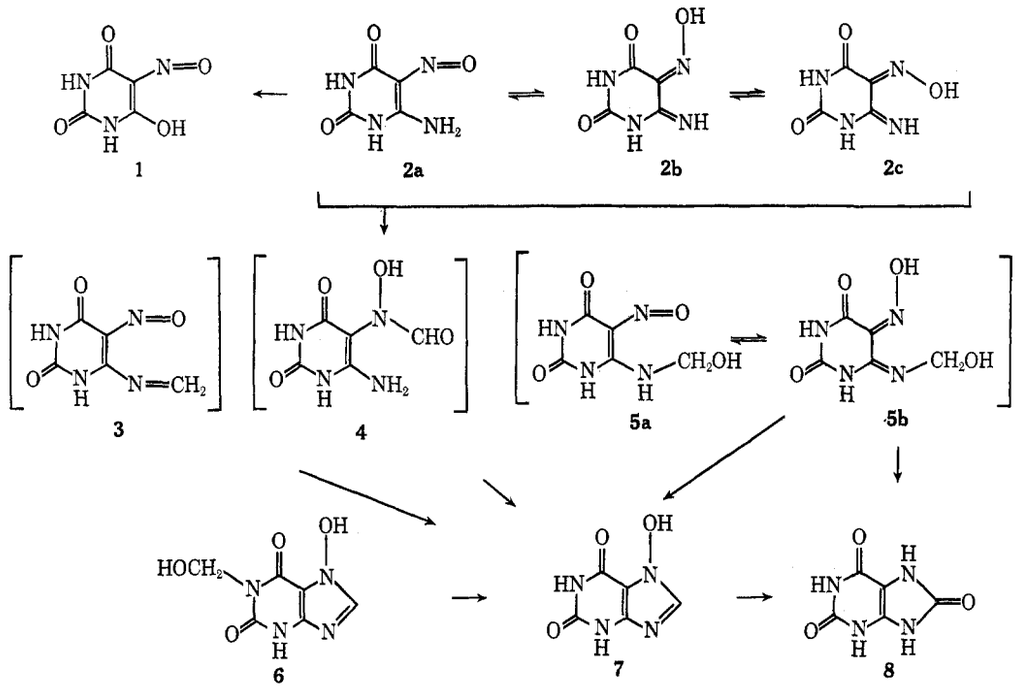
(14) G. M. Timmis, I. Cooke, and R. G. W. Spickett in G. E. W. Wolstenholme and C. M. O'Connor, Ed., "Chemistry and Biology of Purines," J. and A. Churchill, London, 1957, p 134.

(15) I. Lifschitz and L. Kritzman, *Chem. Ber.*, **50**, 1719 (1917).

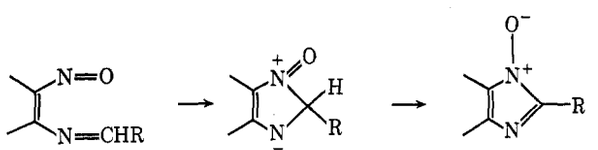
(16) An apparent hydrolytic loss of a 5-nitroso group from a 1,3-disubstituted **2** has previously been noted.[17]

(17) A. D. McNaught and G. B. Brown, *J. Org. Chem.*, **32**, 3689 (1967).

SCHEME I

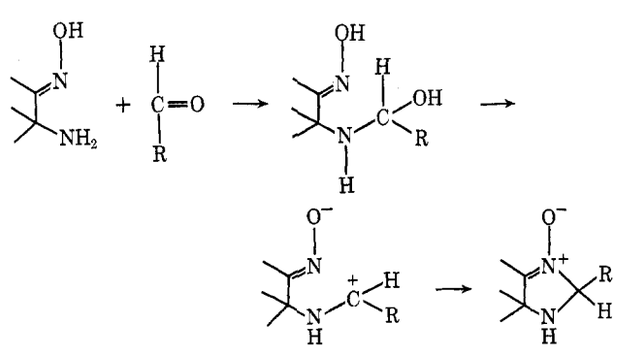


et al.,[11b] and by Taylor and Garcia[12] for similar condensations to 8-methyl- or phenyl-7-hydroxytheophyllines.

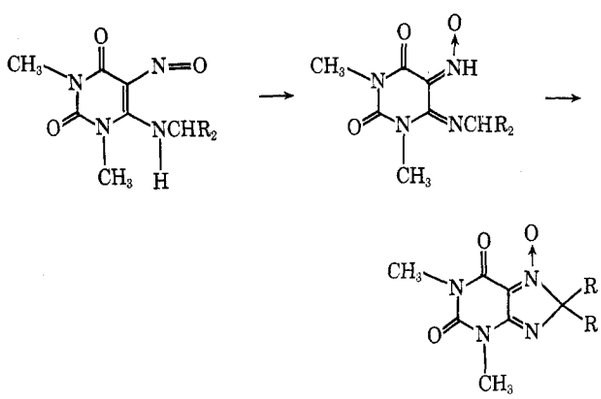


A hydrazone of a structure similar to **4**, the formation of which must involve an oxidation and reduction, was proposed[18] in the cyclization of 1,3-dialkyl-6-amino-5-nitrosouracil with aldehyde hydrazones. If formed, **4** would lead to a classical Traube approach to **7**.

Intermediate **5** could also yield either a uric acid (**8**) or a xanthine derivative (**7**) by loss of one molecule of water, as was proposed by Gnichtel[19] in the case of the formation of imidazoline *N*-oxides from anti-α-amino-oximes and aldehydes. **5a** could also lead to **3** by loss of water.



The formation of the products which are thought to be hydroxymethyl derivatives of 7-hydroxyuric acid and of 8-amino-7-hydroxyxanthine could be explained only by a reaction involving removal of hydrogens from intermediate **5**. An analogous dehydrogenation reaction is known to be involved in the formation of 8,8-dialkyl derivatives of 7-hydroxy-8-*H*-theophyllines, for which Goldner, *et al.*,[20] propose the following.



We have not isolated an intermediate pyrimidine derivative, but intermediates which are apparently basic remain on the Dowex-50 [H^+] during elution by H_2O. After thorough elution of the column with H_2O, elution with HCl yields additional quantities of uric acid, 7-hydroxyxanthine, and also some 8-aminoxanthine. The latter may be the result of ammonia used in the work-up. This suggests that further ring closure may occur during the elution by acid.

When the red-violet tautomer of 6-amino-5-nitrosouracil is used, a yellow product was obtained which does not yield **7**. Its elemental analysis and nmr spectrum suggest a polyhydroxymethyl derivative which is not an *N*-oxide derivative.

A concerted study of the nitroso tautomers, and derivatives of them, will be needed to clarify the mechanism involved, and to improve the yield.

Pure 7-hydroxyxanthine is obtained upon rechroma-

(18) F. Yoneda, K. Ogiwara, M. Kanahori, and S. Nishigaki, *J. Chem. Soc. D*, 1068 (1970).

(19) H. Gnichtel, *Chem. Ber.*, **103**, 2411 (1970).

(20) (a) H. Goldner, G. Dietz, and E. Carstens, *Justus Liebigs Ann. Chem.*, **692**, 134 (1966); (b) **698**, 145 (1966); (c) **699**, 145 (1966).

TABLE I

pK_a'S AND UV SPECTRA OF 7-HYDROXYXANTHINE AND DERIVATIVES

pH	Species	λ_{max}, mm ($\epsilon \times 10^{-3}$)	λ_{min}, mm ($\epsilon \times 10^{-3}$)	pK_a
		7-Hydroxyxanthine		
−2.4	+1	231 (5.7), 262 (8.1)	226 (5.6), 243 (4.5)	−0.25 (±0.08)
2.0	0	201 (22.2), 268 (8.22)	241 (2.77)	5.04[c] (±0.04)
7.3	−1	223 (14.4), 256 (7.11), 276[a] (4.82)	245 (6.41)	9.64[c] (±0.05)
13	−2	227 (19.3), 245, 296 (6.16)	267 (2.96)	
		7-Hydroxy-1-hydroxymethylxanthine		
−2	[+1]	232 (6.40), 262 (9.67)	223 (5.93), 243 (5.37)	−0.1[b]
2	0	201 (26), 268 (10.3)	242 (3.57)	5.0[b]
7	−1	203 (11), 223 (17.1), 256 (8.57), 277[a] (6.0)	245 (7.74)	9.5[b]
12	−2	228 (23.0), 246,[a] 297 (7.4)	212 (11.1), 267 (3.3)	
		7-Hydroxytheophylline[d]		
3	0	208 (23), 230[a] (6.7), 273 (9.1)	247 (3.5)	5.14[c] (±0.05)
8–13	−1	226 (17.2), 257 (7.4), 282 (5.9)	216 (15.5), 248 (7.1) 273 (5.8)	
		7-Hydroxy-8-methyltheophylline[e]		
3	0	208 (24), 230–235[a] (6.7), 275 (10.5)	247 (3.7)	5.59[c] (±0.05)
8–13	−1	228 (17), 250–257[a] (7.8), 280 (6.5)	216 (14.1), 273 (6.4)	

[a] Shoulder. [b] Estimated from isosbestic spectra. [c] Electrometic determination. [d] Reference 11b. [e] Reference 11a.

tography of the main fraction. It can be reduced to xanthine with Raney nickel. The nmr spectrum shows a sharp peak of the imidazole aromatic hydrogen with a chemical shift of δ 7.97 and distinctive peaks for the hydrogens at positions 1 and 3, at δ 10.75 and 11.45, respectively.[21] In the nmr spectrum of compound **6**, the N-1 hydrogen was replaced by the hydroxymethyl group. The uv spectra of the neutral species and of the monoanion of 7-hydroxyxanthine are almost identical with those of the corresponding species of 7-hydroxytheophylline (Table I). The first ionization involves the *N*-hydroxyl group, since it is accompanied by the appearance of the characteristic absorption at 223 nm which is attributed to an N→O group.[22] At a higher pH a dianion is formed from 7-hydroxyxanthine, and this ionization probably involves the proton at N-3; it is accompanied by a shift of the maxima to higher wavelengths. The absence of the absorption at 223 nm in the neutral species provides evidence that the major tautomer in that species is the 7-hydroxy rather than the 7-*N*-oxide structure. The pK_a of protonation of −0.25 compared to that of 3-hydroxyxanthine of 0.35 is in accord with a protonation at N-9, in the same ring with the *N*-OH.

7-Hydroxyxanthine could be converted to uric acid in aqueous thioacetic acid. After gentle treatment with acetic anhydride in acetic acid, 7-acetoxyxanthine (**10**, Scheme II) could be isolated. It is unstable in H_2O, in which it undergoes rearrangement to uric acid. It is also unstable in DMSO, but more stable in dry dioxane.

The reactivities of 7-acetoxyxanthine are remarkably similar to those of 3-acetoxyxanthine, **11**. In aqueous solutions at room temperature in the presence of nucleophiles, it yields a series of 8-substitution products (Scheme II) identical with those obtained from 3-acetoxyxanthine under similar conditions.[8] Thus, it yields 8-chloro-, 8-nitro-, 8-pyridinium, 8-methylmercapto-, and 8-ethoxyxanthines upon treatment with aqueous NaCl, $NaNO_2$, pyridine, methionine, and ethanol, respectively. These reactions involving substitutions by nucleophiles are obviously intermolecular. The products have been characterized after overnight reaction periods, but the early development of color in the reaction with pyridine suggests that the times required may be much less.

At pH's above 4 **10** yields some xanthine in addition to 8-substitution products. With sodium iodide it yields only xanthine, and no 8-substitution product. At pH's between 4.5 and 7.4 it also yields an H_2O-insoluble blue product. This behavior is again directly analogous to that of 3-acetoxyxanthine.[8]

The similarity of the products from 7-acetoxyxanthine and from 3-acetoxyxanthine (**11**) in H_2O suggest that the 8-substitution reactions of each may well proceed *via* the same intermediates. For the facile 3-acyloxypurine 8-substitution reaction,[8] which occurs with increasing rapidity between pH 3 and 7, the anion **12**, dehydroxanthine (**14**), and the carbonium ion **15** were proposed as intermediates.[9] Ionization of **10** to the anion **13** and departure of the 7-acetoxy group would also yield dehydroxanthine (**14**) and thence the carbonium ion **15**.

In further analogy to the deductions made regarding the origin of xanthine from 3-acetoxyxanthine, the reduction of **10** to xanthine could likewise occur through a radical anion arising from homolytic cleavage of the anion **13**.

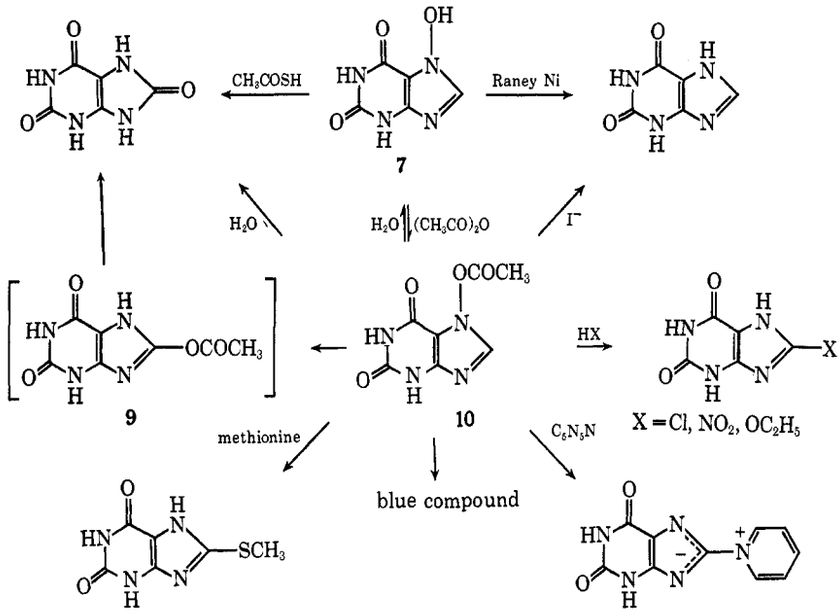
The rearrangement of 7-acetoxyxanthine (**10**) to uric acid involves only a shift to the adjacent atom and could well occur through an intramolecular reaction within a solvent cage, to yield **9** (Scheme II) and thence uric acid. Ir evidence suggested that intermediate **9** was obtained, but it is apparently less stable than the 8-acetoxytheophylline obtained from 7-hydroxytheophylline.[11b]

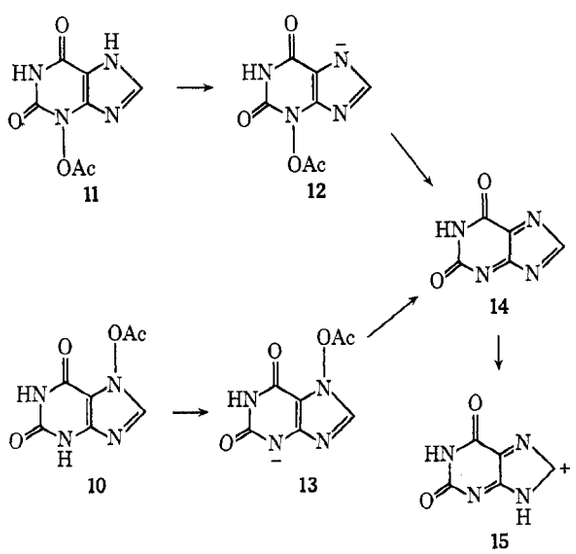
At pH 4.75 the reaction of **10** to yield uric acid, and some xanthine, proceeds about 2.5-fold faster than does that of 3-acetoxyxanthine. This could be due to either the possible intramolecular character of the present reaction, or, if uric acid formation is also an intermolecular reaction, to a pK_a of the ionization of **10** to **13** which is somewhat lower than that of **11** to **12**.

(21) The hydrogen at the 1 position in xanthine has always a lower chemical shift than that in the 3 position: N. J. M. Birdsall, unpublished work.

(22) J. C. Parham, T. G. Winn, and G. B. Brown, *J. Org. Chem.*, **36**, 2639 (1971).

SCHEME II





From the similarities of the reactivities of 7-acetoxyxanthine to those of 3-acetoxyxanthine, it is possible that 7-hydroxyxanthine will, like a 3-hydroxyxanthine, be a potent chemical oncogen. That and further comparisons of the chemical behaviors of the two isomers require study.

In a preliminary experiment 2,4-diamino-6-hydroxy-5-nitrosopyrimidine was condensed with formaldehyde, and 8-hydroxyguanine was obtained. For that reaction it may be possible to obtain the presumed intermediate, 7-hydroxyguanine, for comparison with the behavior of 3-hydroxyguanine.

Experimental Section

The uv spectra were determined with a Unicam SP800A recording spectrophotometer, the ir spectra (KBr or Nujol) with a Perkin-Elmer Model 221 spectrophotometer, and the nmr data with a Varian A-60 spectrometer with DMSO-d_6 as a solvent and TMS as an internal standard. The pK_a's were determined electrometrically or spectrophotometrically by the methods of Albert and Serjeant[23] with a Beckman DU spectrophotometer and buffers of 0.001 M ionic strength.[24] Below pH 2 and above pH 12 pH's were adjusted with KOH and HCl (or H_2SO_4) to give the calculated $H°$ values; pH's 1–13 were measured on a Beckman Research pH meter. For ϵ values a Cary Model 15 spectrophotometer was used. Dowex-50 columns were prepared from BioRad AG-50W-X8, 200–400 mesh, and washed with 3 N HCl before loading. The elution of columns was monitored with an ISCO Model UA-2 uv analyzer.

On a standardized analytical Dowex-50 column similar to that of Uziel and Cohn,[25] 9 × 150 mm, 200–400 mesh, eluted with 1 N HCl at 60 ml/hr and monitored at 240, 260, and 290 nm, these 8-substituted xanthines show the following retention volumes, in milliliters: 8-nitro-, 14; 8-chloro-, 14; 8-ethoxy-, 35; 8-methylmercapto-, 75; 8-amino-, 114; 8-pyridinium-, 205. Uric acid appears at 13, 7-hydroxyxanthine at 35, and xanthine at 67.

From the same column eluted with 0.05 N HCl, uric acid appears at 14, 7-hydroxyxanthine at 48, 3-hydroxyxanthine at 84, 1-hydroxyxanthine at 185, and xanthine at 375 unless the latter is eluted earlier with 1 N HCl.

6-Amino-5-nitrosouracil (2a).—6-Aminouracil (6.35 g) was stirred in H_2O (350 ml) and a solution of $NaNO_2$ (3.85 g) at room temperature in H_2O (50 ml) was added, followed by 1 N HCl (120 ml). After 4–5 min the solid became blue-violet and was quickly collected by filtration (6.8 g). This solid, which can be stored dry for at least a few weeks, was used without further purification.

Anal. Calcd for $C_4H_4N_4O_3$: C, 30.78; H, 2.58; N, 35.89. Found: C, 30.72; H, 2.57; N, 35.98.

The same tautomer could also be prepared by boiling the orange tautomer (0.5 g)[15] in H_2O (50 ml) for 2 min and filtering immediately. The red-purple (**2b**) and the orange (**2c**) tautomers were prepared as described previously.[15] The ir spectrum of the orange tautomer differs considerably from those of the other two. There are smaller differences between the blue-violet and red-purple tautomers. The differences are in the NH(OH) region (3000–3200 cm^{-1}), the C=O region (1600–1800 cm^{-1}), and at 1325, 1275, and 870 cm^{-1}.

7-Hydroxyxanthine (7).—H_2O (400 ml) was brought to the boiling point, formalin solution (55 ml) was added, and the solution was heated again to boiling. 6-Amino-5-nitrosouracil (3.9 g) was added to the boiling solution in small portions, with stirring, during a 10-min period. The stirring and boiling were continued for an additional 20 min, or until the uv maximum, at first near 260 nm, shifted to 273 nm. This was monitored by diluting 0.1-ml aliquots to 25 ml with 0.01 N HCl and recording the spectra. The pH of the mixture, measured at 100°, was an apparent 2.5. When the maximum reached 273 nm, the

(23) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N. Y., 1962.

(24) D. D. Perrin, *Aust. J. Chem.*, **16**, 572 (1963).

(25) M. Uziel, C. K. Koh, and W. E. Cohn, *Anal. Biochem.*, **25**, 77 (1968).

reaction was stopped by cooling the flask to room temperature. The relative intensity of the 273 band to one at 317 nm (the nitroso compound) was then about 3:1. The solution was then concentrated under vacuum below 45° to 50 ml, 1 *N* HCl (25 ml) was added, and the solution was concentrated again to a volume of 25 ml. The pink solution was loaded on a Dowex-50 column (43 × 200 mm) and eluted with 850 ml of H_2O. The first 150 ml, containing violuric acid (1) and uric acid, were neutralized with NH_4OH, concentrated, and separated by chromatography on a smaller column (10 × 150 mm). On paper chromatography with 3% NH_4HCO_3, they had R_f values of 0.7 and 0.4, respectively. The next 700 ml, which had an absorption maximum at 270 nm, were concentrated in vacuum to 25 ml, concentrated NH_4OH (25 ml) was added, and the solution was stirred for 2 hr at room temperature. It was then concentrated under vacuum to 25 ml, a little 1 *N* HCl was added to dissolve any precipitate, and the solution was loaded onto another Dowex-50 column (20 × 290 mm). The column was eluted with H_2O (700 ml), followed by 1 *N* HCl (600 ml). The first fractions, 80–180 ml, contained additional **1** and **8**. The next fraction, 180–260 ml, had a uv spectrum similar to that of 7-hydroxyxanthine, but was unstable on further work-up. The next 260–340 ml yielded, upon evaporation, a hydroxymethyl derivative of **7**, which is apparently a less stable isomer than the one, **6**, described below. It gave an elementary analysis similar to that of **6** but its uv maxima were at about 3–4 nm longer wavelengths. It yielded some 7-hydroxyxanthine upon rechromatography. The next fraction, 340–500 ml, was essentially pure 7-hydroxyxanthine (**7**). Upon evaporation of the solvent and recrystallization twice from H_2O, with charcoal if needed, it yielded 0.5–0.6 g (8–10%) of white crystals. This eluted as a single sharp band at 48 ml from the analytical column. When dried at room temperature under vacuum it gave elementary analyses for a hemihydrate.

Anal. Calcd for $C_5H_4N_4O_3 \cdot {}^1/_2H_2O$: C, 33.89; H, 2.82; N, 31.70. Found: C, 33.89; H, 2.82; N, 31.81.

The anhydrous 7-hydroxyxanthine was obtained on drying over P_2O_5 at 100° under vacuum: nmr (DMSO-d_6) δ 7.97 (8-CH), 10.75 (1-NH), 11.45 (3-NH), 12.2 (7-OH, br); ir (Nujol) 3400 (OH), 3200 (NH), 1680 cm^{-1} (C=O). The uv spectral data and pK_a's are given in Table I.

Anal. Calcd for $C_5H_4N_4O_3$: C, 35.72; H, 2.40; N, 33.33. Found: C, 35.83; H, 2.41; N, 33.19.

Although other fractions contain 7-hydroxyxanthine or derivatives which yield it, this one fraction represents the practical yield.

Nothing was eluted by an additional 200 ml of water. Elution with 1 *N* HCl was then started and the eluate again contained uric acid and **7** in the initial 20–100 ml, and 8-aminoxanthine appeared from 120 to 250 ml. The latter was isolated upon evaporation and was identified by elementary analysis and uv spectrum.[26]

7-Hydroxy-1-hydroxymethylxanthine (**6**).—6-Amino-5-nitrosouracil (**2a**) (0.5 g) was added to a boiling solution of formalin (6 ml) and H_2O (40 ml) as described above. Instead of concentrating and treating with HCl, the reaction mixture was stirred for 4 hr with 1.0 g of Dowex-50 and filtered, and the resin was washed with 100 ml of water. The solution and washings were evaporated to 20 ml under vacuum and loaded on a Dowex-50 column (20 × 290 mm). The column was eluted with 500 ml of H_2O. The fraction from 100 to 300 ml showed an unsymmetrical trailing peak with an absorption maximum near 270 mm. It was concentrated under vacuum to 10 ml and again put on a Dowex-50 column (20 × 290 mm) and eluted with H_2O. The 270-nm absorbing material was largely eluted as a nearly symmetrical peak from 280 to 400 ml. It was evaporated under vacuum and **6** was obtained by crystallization from water (0.05 g). Dried at room temperature, it gave an analysis for a monohydrate.

Anal. Calcd for $C_6H_6N_4O_4 \cdot H_2O$: C, 33.34; H, 3.73; N, 25.92. Found: C, 33.43; H, 3.67; N, 26.03.

The anhydrous product was obtained on drying at 100°: nmr (DMSO-d_6) δ 5.23 (CH_2), 6.0 (COH, broad), 8.01 (8-CH), 11.73 (3-NH), 12.3 (7-OH, broad); ir (Nujol) 3350 (OH), 1680 (C=O), 1640 cm^{-1} (C=O, a band absent in **2**).

Anal. Calcd for $C_6H_6N_4O_4$: C, 36.37; H, 3.05; N, 28.28. Found: C, 36.17; H, 3.10; N, 28.33.

8-Amino-7-hydroxy-x-hydroxymethylxanthine.—A product, which analyses and spectra suggest to be the title compound, was obtained in an attempt to isolate a basic intermediate from the reaction of 6-amino-5-nitrosouracil with formaldehyde. 6-Amino-5-nitrosouracil (0.4 g) was added to a boiling solution of formalin (6 ml) and H_2O (40 ml), and the reaction was followed as described above. After stopping the reaction and concentrating the solution to 10 ml, it was loaded on a Dowex-50 column (20 × 290 mm). After elution of acidic products with H_2O (900 ml), the column was eluted with 0.4 *N* HCl (200 ml). The eluate was concentrated under vacuum to 20 ml, and concentrated NH_4OH (8 ml) was added with cooling. Upon keeping overnight at room temperature the product precipitated. Recrystallized from H_2O it was still brown (0.05 g). It was dried at 100°: uv, pH 2, λ_{max} 206, 275, λ_{min} 245 nm; pH 7, λ_{max} 226, 257, 287, λ_{min} 272 nm; pH 12, λ_{max} 226, 292, λ_{min} 273 nm.

Anal. Calcd for $C_6H_7N_5O_4$: C, 33.80; H, 3.31; N, 32.85. Found: C, 33.86; N, 3.42; C, 32.88.

Reaction of Other Tautomers of 2 with Formaldehyde.—By the procedure described for 7-hydroxyxanthine, the orange tautomer gave a very low yield of **7** and a larger amount of uric acid.

With the red-purple tautomer a trailing peak of a yellow product was obtained from the column. After concentration and treatment with 1 *N* NH_4OH, followed by evaporation to dryness and crystallization from water, it gave an unidentified yellow product. The elementary analyses and nmr spectrum suggest the presence of methylene or hydroxymethyl groups: nmr (DMSO-d_6) δ 4.83 (CH_2, broad peak), 5.24 (CH_2).

Anal. Calcd for $C_7H_8N_4O_4$: C, 39.63; H, 3.80; N, 26.41. Found: C, 39.58; H, 3.81; N, 26.29.

Hydrogenation of 7-Hydroxyxanthine to Xanthine.—7-Hydroxyxanthine (**7**) (24 mg) was dissolved in hot H_2O (5 ml), Raney nickel (150 mg) was added, and the solution was boiled for 30 min and filtered hot. The filtrate was left at room temperature and xanthine, identified through its ir and uv spectra, crystallized (20 mg) (90%).

Reaction of 7-Hydroxyxanthine with Thioacetic Acid.—7-Hydroxyxanthine (**7**) (10 mg) was stirred for 1 week with CH_3COSH (10 mg) in H_2O (2 ml). The solution was loaded on a Dowex-50 column (1 × 10 cm) and eluted with water. The first fraction was evaporated and the residue was crystallized from H_2O. The product was identified as uric acid by its uv and ir spectra.

Acetylation of 7-Hydroxyxanthine. A. 7-Acetoxyxanthine.—Dry 7-hydroxyxanthine (**7**) (0.15 g) was stirred with glacial acetic acid (4.5 ml) and acetic anhydride (4.5 ml) at room temperature until the solution was clear (2–3 hr). Dry ether (150 ml) was added and the solution was kept for 96 hr in the freezer. The product which crystallized was collected by filtration, washed with dry ether, and dried under vacuum overnight at room temperature (0.15 g). The product melts at 155° (with decomposition) when heated rapidly, but does not melt when heated slowly, suggesting the formation of uric acid during slow heating. The elementary analysis corresponded to a hemiacetate of **10**. Drying at elevated temperatures or for a longer period resulted in loss of acetic acid and was accompanied by decomposition.

Anal. Calcd for $C_7H_6N_4O_4$: C, 40.01; H, 3.36; N, 23.33. Found: C, 40.02; H, 3.30; N, 23.35.

When the nmr was taken in DMSO-d_6, a rapid decomposition could be observed. The band of the proton from position 8 (δ 8.21) disappears while at the same time CH_3 protons of the acetoxy group change their chemical shift from δ 2.40 to 2.35 and finally to 1.92, the last corresponding to that of acetic acid.

Ir (KBr) follows: 3200 (NH), 2800 (NH with hydrogen bond), 1800 (C=O, of acetoxy group), 1670 cm^{-1} (C=O).

B. Acetylation under the Conditions for the Preparation of 3-Acetoxyxanthine.—With acetic anhydride and acetic acid at room temperature for 5 days,[7] uric acid and an unstable intermediate, possibly **9**, precipitated. More material could be precipitated by the addition of ether. The ir band at 1820 cm^{-1}, attributed to an *N*-acetoxy group, is weaker and an ester carbonyl absorption at 1750 cm^{-1} is present. This is apparently a mixture containing 8-acetoxyxanthine and 7-acetoxyxanthine. Upon drying most was converted to uric acid. Low nitrogen analyses from the crude product suggest the presence of more than one acetyl group.

Reduction of 7-Acetoxyxanthine with KI.—The acetoxyxanthine (**10**) (6 mg) was stirred for 1 hr with 10% aqueous KI (0.25 ml). The solution turned red and gave a positive starch test for iodine. Only xanthine was detected upon chromatog-

(26) L. F. Cavalieri and A. Bendich, *J. Amer. Chem. Soc.*, **72**, 2587 (1950).

raphy over Dowex-50 with 0.05 N HCl. Its identity was verified by its uv spectrum.

Reactions of 7-Acetoxyxanthine with Nucleophiles.—7-Acetoxyxanthine (**10**) was treated overnight with ethanol or with aqueous solutions of methionine, NaCl, $NaNO_2$, or pyridine as described for 3-acetoxyxanthine.[8] The formation of 8-ethoxy, 8-methylmercapto, 8-chloro, 8-nitro, and 8-pyridinium xanthines, respectively, was accompanied by some formation of uric acid and xanthine. The products were isolated by chromatography and identified by their uv spectra.

The rate of reaction of **10** in acetate buffer at pH 4.75 was compared with that of 3-acetoxyxanthine by repeated scans of the changing spectra at intervals. The half-times for completion of the reactions were approximately 10 and 25 min, respectively.

Blue Compound.—Upon stirring 7-acetoxyxanthine in cold 0.1 N phosphate buffer at pH 7, the solution turns purple followed by precipitation of a blue compound. A similar product is obtained by adding a few drops of 0.01 N NaOH to a cold H_2O solution. Like the blue compound obtained from 3-acetoxyxanthine[8] it is insoluble in H_2O and most organic solvents, and is decomposed in base or acid and by heating in H_2O.

Registry No.—**2a**, 34407-58-4; **6**, 34407-59-5; **7**, 16870-90-9; **10**, 34407-61-9; **11a**, 883-16-9; **11b**, 1012-82-4.

Acknowledgment.—The authors wish to thank Mr. M. J. Olsen for capable assistance with the nmr spectra, Mr. G. Reiser for pK determinations, and Dr. H. Goldner for generously providing samples of 7-hydroxytheophylline and 7-hydroxy-8-methyltheophylline.

Synthesis of 3′ and 5′ Nucleotides Derived from 2′-Amino-2′-deoxyuridine

D. WAGNER,[1] J. P. H. VERHEYDEN, AND J. G. MOFFATT*

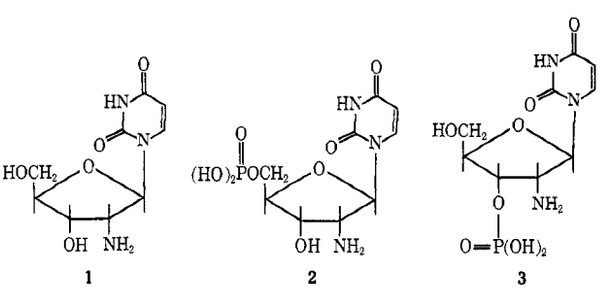
Contribution No. 94 from the Institute of Molecular Biology, Syntex Research, Palo Alto, California 94304

Received November 23, 1971

The previously described 2′-azido-2′-deoxyuridine has been converted into 2′-azido-2′-deoxy-5′-*O*-trityluridine (**5**) and thence to 3′-*O*-acetyl-2′-azido-2′-deoxyuridine (**7**). Phosphorylation of the latter compounds gave, after removal of protecting groups, 2′-azido-2′-deoxyuridine 3′-phosphate (**8**) and 2′-azido-2′-deoxyuridine 5′-phosphate (**9**) which are suitable intermediates for the preparation of 2′-amino-2′-deoxyuridine containing oligonucleotides, etc., for biochemical study. Catalytic reduction of the azido function of **8** and **9** gave 2′-amino-2′-deoxyuridine 3′-phosphate (**3**) and 2′-amino-2′-deoxyuridine 5′-phosphate (**2**) in crystalline form.

Recently we have described the synthesis of 2′-amino-2′-deoxyuridine (**1**) and its conversion into 2′-amino-2′-deoxycytidine.[2] Since these compounds contain free 3′- and 5′-hydroxyl groups, it should be possible to convert them chemically into short oligonucleotides containing amino groups in place of the normal 2′-hydroxyl functions. These compounds would be of interest in order to study the effect of an adjacent amino group on the stability of the phospho diester linkage and also to show whether such compounds would function as messengers in a protein-synthesizing system.[3] Along similar lines, Glinski, *et al.*,[4] have recently described the preparation of phosphate esters derived from 3′-amino-3′-deoxythymidine and 5′-amino-5′-deoxythymidine, while Letsinger and Mungall[5] have prepared short oligonucleotides containing phosphoramidate linkages derived from the latter compounds. These compounds, being derived from 2′-deoxy nucleosides, do not, however, permit one to examine the questions posed above. In this paper we describe the preparation of both 2′-amino-2′-deoxyuridine 5′-phosphate (**2**) and of its isomer 2′-amino-2′-deoxyuridine 3′-phosphate (**3**).

Rather than devising a suitable protecting group for the 2′-amino function of **1**, we have preferred to do the appropriate transformations using, as the starting material, 2′-azido-2′-deoxyuridine (**4**), the immediate precursor of **1**. Thus the selective protection of the 5′-hydroxy group was readily achieved *via* formation of the trityl ether **5**, which was obtained in 86% yield.



1 **2** **3**

Compound **5** could be crystallized only with difficulty, but its homogeneity and structure was readily apparent from its nmr spectrum, which showed, *inter alia*, the presence of a free 3′-hydroxy group at 5.97 ppm. The latter signal was coupled to $C_{3'}H$ which appeared as a pseudoquartet at 4.44 ppm collapsing to a pseudotriplet upon addition of D_2O. Acetylation of **5** gave amorphous, but analytically pure, 3′-*O*-acetyl-2′-azido-2′-deoxy-5′-*O*-trityluridine (**6**) in quantitative yield. Removal of the trityl ether from **6** was achieved by treatment with 80% acetic acid, giving crystalline 3′-*O*-acetyl-2′-azido-2′-deoxyuridine (**7**) in 88% yield.

Phosphorylation of both **5** and **7** was accomplished by reaction with 2-cyanoethyl phosphate and dicyclohexylcarbodiimide (DCC) in pyridine.[6] Following removal of protecting groups by treatment with alkali (and acid in the case of **5**) the corresponding phosphate esters 2′-azido-2′-deoxyuridine 3′-phosphate (**8**) and 2′-azido-2′-deoxyuridine 5′-phosphate (**9**) were isolated by ion exchange chromatography in yields of 60 and 69% respectively.

Catalytic hydrogenation of the free acid forms of **8** and **9** rapidly converts the azido function to the corresponding amines, the reduction being readily followed by paper electrophoresis in 1 M acetic acid. Under

(1) Syntex Postdoctoral Fellow, 1968–1970.

(2) D. Wagner, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, **36**, 250 (1971).

(3) See, *e.g.*, M. W. Moon, S. Nishimura, and H. G. Khorana, *Biochemistry*, **5**, 937 (1966), for a related study involving 2′-deoxy nucleosides.

(4) R. P. Glinski, M. S. Khan, R. L. Kalamas, and C. L. Stevens, *Chem. Commun.*, 915 (1970).

(5) R. L. Letsinger and W. S. Mungall, *J. Org. Chem.*, **35**, 3800 (1970).

(6) G. M. Tener, *J. Amer. Chem. Soc.*, **83**, 159 (1961).