Synthesis and Properties of 7-Hydroxyxanthine and its Derivatives

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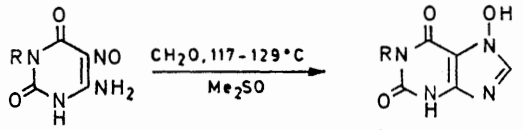
Use of an improved procedure for the synthesis of 7-hydroxyxanthine has enabled the synthesis of 7-hydroxy-1-methylxanthine and several 8-alkyl derivatives. The reactivity of 7-hydroxyxanthine and 7-hydroxy-1-methylxanthine towards acetic anhydride has been compared with that of 3-hydroxyxanthine. The 8-alkyl derivatives, which have the same electronic and tautomeric properties as the 8-unsubstituted compounds were more soluble in organic solvents. Reduction of the *N*-hydroxy-group in these derivatives gave xanthines soluble in organic solvents. U.v. and n.m.r. spectra and dissociation constants are discussed.

PURINE *N*-OXIDES exhibit various biological activities.[1-5] For compounds where the dominant tautomer is the *N*-hydroxy-form the name *N*-hydroxypurine is preferred. 3-Hydroxyxanthine is a potent oncogen in mice.[4] The ready reaction of its 3-*O*-acyl derivatives with nucleophiles to yield 8-substituted xanthines has been correlated with its biological activity.[5]

The compound reported in 1966 as 7-hydroxyxanthine [6] was shown later [7] to be the 3-hydroxy-isomer. 7-Hydroxyxanthine (1a) was first isolated in low yield from the reaction of 4-amino-5-nitrosouracil (2a) with formaldehyde in slightly acidic aqueous media.[8] In the present work moderate yields (40%) of 7-hydroxyxanthine have been obtained in the reaction of compound (2a) with formaldehyde in dimethyl sulphoxide at 117—119 °C. Higher temperatures cause decomposition of the 7-hydroxyxanthine, mostly to uric acid. 7-Hydroxy-1,3-dimethylxanthine (7-hydroxytheophylline) has been reported,[9] but the monomethyl derivatives have not been described hitherto. Application of the present method to 4-amino-1-methyl-5-nitrosouracil (2b) afforded 7-hydroxy-1-methylxanthine (1b) in 35% yield. Attempts to synthesize compound (1b) in aqueous media resulted in lower yields. In dimethyl sulphoxide the tautomer of (2) which is appropriate for this reaction is probably

[1] G. B. Brown, K. Sugiura, and R. M. Creswell, *Cancer Res.*, 1965, **25**, 986.
[2] G. B. Brown, G. Levin, S. Murphy, A. Sele, H. C. Reilly, G. S. Tarnowski, F. A. Schmid, M. N. Teller, and C. C. Stock, *J. Medicin. Chem.*, 1965, **8**, 190.
[3] G. B. Brown and M. N. Teller, *Proc. Amer. Assoc. ibid.*, 1971, **12**, 41.
[4] M. N. Teller, G. Stohr, and H. Dienst, *Cancer Res.*, 1970, **30**, 179.
[5] G. Stöhrer and G. B. Brown, *Science*, 1970, **167**, 1622; K. Sugiura, M. N. Teller, J. C. Parham, and G. B. Brown, *Cancer Res.*, 1970, **30**, 184; G. B. Brown, M. N. Teller, I. Smullyan, N. J. M. Birdsall, T. C. Lee, J. C. Parham, and G. Stöhrer, *ibid*, 1973, **33**, 1113.
[6] T. J. Delia and G. B. Brown, *J. Org. Chem.*, 1966, **31**, 178.
[7] U. Wölke and G. B. Brown, *J. Org. Chem.*, 1969, **34**, 978; U. Wölcke, N. J. M. Birdsall, and G. B. Brown, *Tetrahedron Letters*, 1969, 785.
[8] G. Zvilichovsky and G. B. Brown, *J. Org. Chem.*, 1972, **37**, 1871.
[9] H. Goldner, G. Dietz, and E. Carstens, *Annalen*, 1966, **691**, 142, 233.

the predominant form. The solution at these temperatures has a blue colour.



(2) a; R = H
b; R = Me

(1) a; R = H
b; R = Me

In addition the present method is more convenient because the product is obtained in relatively pure solid form by precipitation in a two-phase system (dilute hydrochloric acid–chloroform). This solid needs only one passage through a Dowex-50 ion-exchange column for further purification, whereas the earlier procedure [8] required two or more such passages. The two-phase technique enables slow crystallization of the product, as the dimethyl sulphoxide is extracted gradually into the aqueous layer. The acid is needed to hydrolyse unchanged starting material (2a). Treatment with ammonia removes any ring *N*-hydroxymethyl groups.

Attempts to cyclise 4-amino-3-methyl-5-nitrosouracil (3) [10] with formaldehyde in the same manner did not lead to 7-hydroxy-3-methylxanthine (4); only 3-methyluric acid was identified in the reaction mixture. This may result either from (4) by rearrangement or from (3) *via* an intermediate 4-*N*-hydroxymethyl derivative.

[10] T. Ukai, Y. Yakamoto, and S. Kanemoto, *J. Pharm. Soc. Japan*, 1954, **74**, 674.

The u.v. absorption and pK_a data of 7-hydroxy-1-methylxanthine (Table 1), and those of 7-hydroxyxanthine and the 1,3-dimethyl derivative, indicate that in these compounds the *N*-hydroxy-form is predominant because the typical absorption at 226 nm appears only at the first ionization, as shown for 3-hydroxyxanthine.[11]

(3) (4)

The spectral changes at the first ionization are similar in the three derivatives. The spectral change at the second ionization is the same in 7-hydroxyxanthine and its 1-methyl derivative and does not occur in the 1,3-disubstituted derivative. These facts indicate that N-3 is deprotonated at the second ionization.

Comparison of the n.m.r. spectrum of 7-hydroxy-1-methylxanthine (1b) with that of 7-hydroxyxanthine (1a) (Table 2) shows that the 1-proton absorbs at δ 10.75 (the highest field of the exchangeable protons). This agrees with previous findings that this is also the least acidic proton.

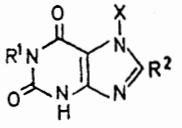
7-Acetoxyxanthine,[8] like 3-acetoxyxanthine,[11,12]

[11] J. C. Parham, T. G. Winn, and G. B. Brown, *J. Org. Chem.*, 1971, **36**, 2639.

[12] N. J. M. Birdsall, U. Wölke, J. C. Parham, and G. B. Brown, *Tetrahedron*, 1972, **28**, 3.

TABLE 1

Dissociation constants and u.v. absorptions of alkyl derivatives of 7-hydroxyxanthines and xanthines



(6)

R^1	R^2	X	Species	pH	$\lambda_{max.}$/nm (ε × 10^{-3})	$\lambda_{min.}$/nm (ε × 10^{-3})	pK_a
Me	H	OH	Neutral	3.1	204 (24.8), 268 (9.0)	242 (3.6)	
			Monoanion	7.2	222 (15.0), 255 (8.5), 275 (5.7)	244 (7.6), 272 (5.6)	4.99
			Dianion	12.0	226 (20.7), 295 (6.9)	267 (3.5)	9.54
H	Pr	OH	Neutral	3.0	204 (21.5), 270 (11.2)	243 (3.5)	
			Monoanion	7.6	226 (15.9), 256 (8.4), 277 (6.3)	247 (8.2), 272 (6.3)	5.40
			Dianion	11.9	228 (20.2), 294 (6.1)	267 (3.7)	9.60
H	Hexyl [a]	OH	Neutral	3.2	204 (21.1), 270 (11.2)	243 (3.5)	
			Monoanion	7.6	226 (17.0), 256 (9.0), 278 (6.9)	247 (8.6), 272 (6.9)	5.40
			Dianion	11.9	228 (21.2), 294 (6.5)	267 (3.7)	9.75
H	Pr [b]	H	Cation	0.0	230 (7.2), 261 (9.4)	217 (5.2), 243 (5.2)	
			Neutral	5.0	201 (16.7), 269 (11.0)	242 (3.0)	1.90
			Monoanion	10.5	240 (9.0), 279 (8.7)	222 (6.7), 259 (4.9)	8.25
			Dianion	14.0	217 (14.2), 283 (8.0)	258 (5.0)	12.6

[a] Other 8-alkyl-7-hydroxyxanthines (R^2 = pentyl, heptyl, or undecyl) have similar properties. [b] 8-Hexyl- and 8-undecylxanthine have similar properties.

TABLE 2

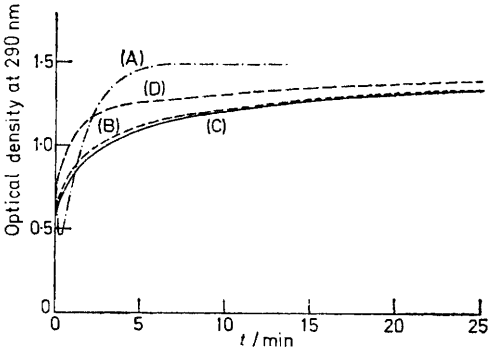
^{1}H N.m.r. data of derivatives of 7-hydroxyxanthine in $(CD_3)SO_2$ and xanthine (6) (δ) values)

R^1	R^2	X	H-1	H-3	NOH	H-7	H-8	8-CH_2
H	H	OH	10.75	11.45	12.2		7.97	
Me	H	OH	3.12 (CH_3)	11.90	12.3		7.90	
H	Pr	OH	10.80	11.50	12.12			2.40 (t)
H	Hexyl [a]	OH	10.75	11.45	12.06			2.40 (t)
H	Hexyl [b]	H	10.66	11.45		13.2		2.40 (t)

[a] Other 8-alkyl derivatives of 7-hydroxyxanthine have similar properties. [b] 8-Propyl- and 8-undecylxanthine have similar properties.

undergoes rearrangement and 8-substitution reactions. We have observed that in the presence of a slight excess of acetic anhydride, as was shown for 3-hydroxyxanthine [13] in buffered solutions (pH 3 and above), 7-hydroxyxanthine undergoes the same reactions. The acylation reaction is considerably faster than rearrangement or 8-substitution. The acylation step in neutral aqueous solution is immediate and faster than in 3-hydroxyxanthine. A comparison of the course of the reaction in the two isomers is shown in the Figure. The initial decline in the curve of 3-hydroxyxanthine results from transformation into the *N*-acetoxy-derivative. The ensuing decomposition of 3-acetoxyxanthine is then a pseudo-first-order reaction with k_1 *ca.* 0.67 min^{-1}. However 7-hydroxyxanthine shows second-order behaviour with an apparent k_2 of *ca.* 320 l mol^{-1} min^{-1}. Another difference between the two isomers is that in the case of 3-hydroxyxanthine it is possible to interrupt the rearrangement by acidification to pH 0 by addition of hydrochloric acid and then to isolate unchanged 3-hydroxy- and 3-acetoxy-xanthine; in the 7-hydroxy-isomer the rearrangement to uric acid is accelerated by hydrochloric acid.

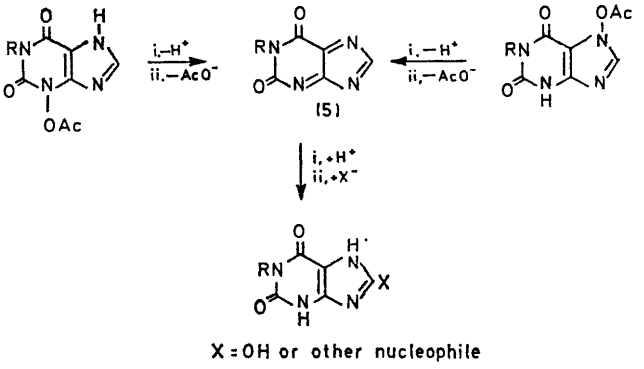
The reaction of 7-hydroxyxanthine in 75% dioxan–water proceeded more rapidly (Figure), suggesting a non-polar intermediate and thus supporting an earlier



Course of reaction of *N*-hydroxyxanthines (1.33×10^{-4}M) with acetic anhydride (1.6×10^{-4}M) at 32 °C and pH 7.0, followed at 290 nm: (A) 3-hydroxyxanthine, (B) 7-hydroxyxanthine, (C) 7-hydroxy-1-methylxanthine, (D) 7-hydroxyxanthine in 75% dioxan

theory [8,12] involving an intermediate didehydro-derivative (5). The difference in the rates of the reaction of the two isomeric compounds is probably due to the occurrence of two different mechanisms leading to the same intermediate (5). The fact that the product compositions in the reactions of both isomers with acetic anhydride are similar suggests a common intermediate.

The course of the reaction of 7-hydroxy-1-methylxanthine (1b) with acetic anhydride is similar to that of the unsubstituted compound (1a), with an apparent k_2 of *ca.* 340 l mol^{-1} min^{-1}. Here the major product was 1-methyluric acid, with various amounts of 1-methylxanthine, depending on the pH. Once again, in the presence of nucleophiles such as NO_2^-, pyridine *etc.*, or methionine the corresponding 8-substituted products were identified. In the presence of I^- the only product



obtained was 1-methylxanthine, in analogy to the reactions with the unsubstituted 7-hydroxyxanthine [8] and 3-hydroxyxanthine.[7,12] However, unlike the reactions of the latter compounds, the reaction of 7-hydroxy-1-methylxanthine with acetic anhydride does not involve the formation of the ' blue compound '.[7,8]

We considered that replacement of formaldehyde by an aliphatic aldehyde in the reaction with 4-amino-5-nitrosouracil (2a) could serve as a general method for introduction of an 8-alkyl group. The 8-position is the only one, in purine derivatives like guanine and xanthine, where the introduction of an alkyl group will not alter the tautomeric and electronic properties of the parent purine. On the other hand it may change other properties such as solubility and surface interactions. A long aliphatic chain would be expected to increase solubility in organic solvents, and might assist in determining some molecular properties of purines such as dipole moments, where dissolution in a non-polar solvent is essential. The biological character of the purines might also be modified by the introduction of a long-chain 8-alkyl group as it could increase its rate of transfer through lipid membranes.

Long-chain 8-alkyl derivatives of purines have rarely been described. A method involving a malonic ester synthesis [14] led to 8-alkyl derivatives of caffeine. We have studied the reactions of aliphatic aldehydes of various chain lengths with compound (2a) in dimethyl sulphoxide at controlled temperatures. The properties of the 8-alkyl-7-hydroxyxanthines thus obtained are summarized in Table 3. The higher yield in some of these reactions as compared with the reaction of formaldehyde is probably due to the impossibility of the formation of uric acid.

8-Methyl derivatives of 3-, 7-, and 9-hydroxyxanthines were prepared by radical substitution of the corresponding hydroxyxanthines by means of t-butyl hydroperoxide.[15]

Various reducing agents, including hydrogen–Raney

[13] G. Stohrer and G. Salemnick, *Cancer Res.*, 1975, **35**, 122.

[14] E. S. Golovchinskaya, *Sbornik. Statei obshchei Khim., Akad. Nauk, S.S.S.R.*, 1953, **1**, 692.

[15] D. R. Sutherland and G. B. Brown, *J. Org. Chem.*, 1973, **38**, 1291, and unpublished results of these authors.

TABLE 3

Experimental data for derivatives of 7-hydroxyxanthine and xanthine (6)

R¹	R	X	Reaction solvent	Reaction temp.[a] (°C)	Yield (%)[b]	M.p. (°C) (solvent)	Formula	Found (Reqd.) %C	%H	%N
H	H	OH	Me_2SO	117—119	40	>300 (H_2O)	$C_5H_4N_4O_3,0.5H_2O$	33.7 (33.9	2.7 2.8	31.7 31.7)
Me	H	OH	Me_2SO	117—119	35	>300 (H_2O)	$C_6H_6N_4O_3$	39.5 (39.55	3.65 3.3	31.0 30.75)
H	Pr	OH	Me_2SO	123—125	65	255 (EtOH)	$C_8H_{10}N_4O_3,0.5H_2O$	44.0 (43.9	5.3 5.05	25.5 25.55)
H	Pentyl	OH	Me_2SO	123—125	30	201 (EtOH–H_2O)	$C_{10}H_{14}N_4O_3,H_2O$	47.35 (46.85	5.9 6.3	21.8 21.85)
H	Hexyl	OH	Me_2SO	121—123	70	195 (EtOH)	$C_{11}H_{16}N_4O_3,H_2O$	48.6 (48.9	6.65 6.7	20.9 20.75)
H	Heptyl	OH	Me_2SO	127—129	32	193 (EtOH)	$C_{12}H_{18}N_4O_3,EtOH$	53.95 (53.85	7.75 7.75	17.95 17.95)
H	Undecyl	OH	Me_2SO	125—127	60	206 (EtOH–H_2O)	$C_{16}H_{26}N_4O_3,H_2O$	56.9 (56.8	8.25 7.7	16.85 16.55)
H	Pr	H	Buffer (H_2O)	25	35	>300 (EtOH)	$C_8H_{10}N_4O_2$	49.6 (50.0	5.35 5.2	29.15 28.85)
H	Hexyl	H	Buffer (EtOH–H_2O)	45	45	277 (EtOH)	$C_{11}H_{16}N_4O_2$	53.3 (53.55	7.25 7.2	24.1 25.0)
H	Undecyl	H	EtOH	65	30	295 (EtOH–H_2O)	$C_{16}H_{26}N_4O_2$	62.7 (62.55	8.55 8.35	18.3 18.4)

[a] The reaction is followed by the disappearance of the blue colour of the solution or by the change in u.v. absorption. The time of reaction depends on the aldehyde (15 min for formaldehyde and 30—60 min for others). [b] Yields of derivatives of 7-hydroxyxanthine are calculated on the basis of the pyrimidine. Excess of aldehyde is always used.

nickel, were unsuitable for reducing the *N*-hydroxy-group in 8-alkyl-7-hydroxyxanthines. However these compounds could be reduced to the parent 8-alkylxanthines (Table 3) by using the previously described method of introducing acetic anhydride in the presence of potassium iodide (see Experimental section). As expected, the 8-alkylxanthines, like the 8-alkyl-7-hydroxyxanthines, showed little variation in u.v. absorptions and dissociation constants (Table 1). They are more soluble in organic solvents and their solubility increases with increase in the chain length.

EXPERIMENTAL

Dissociation constants were determined as described earlier.[16] pH Values were measured with Radiometer-Copenhagen pH-meter-29 with a combined electrode. U.v. spectra were taken with a Varian-Techtron 635 spectrophotometer. N.m.r. spectra were measured with a Varian T-60 instrument. Fluka Dowex-50-X8 resin (200—400 mesh) was used for column chromatography. Elution was monitored with an ISCO UA-4 u.v. analyser. Dimethyl sulphoxide was dried by azeotropic distillation with benzene.

4-Amino-5-nitrosouracil (2a).—The nitrosation of 4-aminouracil is carried out by a slight modification of a previous procedure.[8] 4-Aminouracil (6.35 g) was stirred in water (350 ml) at 15 °C and a solution of sodium nitrite (3.85 g) in water (50 ml) was added, followed by N-hydrochloric acid (120 ml). The purple solid was collected after 5 min, resuspended in water (150 ml), and boiled with stirring for 2 min. The mixture was allowed to cool to 50 °C, and the product filtered off and dried for 2 h at 100 °C (yield 6.8 g).

4-Amino-1-methyl-5-nitrosouracil (2b).—This product was prepared by a slight modification of a route described earlier.[17] *O*-Methylisourea hydrochloride (27.5 g) and methyl cyanoacetate (30 g) were dissolved in sodium methoxide [from sodium (12 g) and methanol (200 ml)]. The solution was refluxed for 4 h and concentrated under vacuum. The viscous residue was dissolved in hot water (75 ml) and acidified with acetic acid. The 6-amino-2-methoxypyrimidin-4-one was collected after cooling and recrystallized from water (charcoal). This product was treated as shown earlier [17] with dimethyl sulphate and then hydrolysed in the same manner [17] to yield 4-amino-1-methyluracil.

The latter product (5.6 g) was stirred at 80 °C with sodium nitrite (5.0 g) in water (100 ml), and glacial acetic acid (20 ml) was added dropwise during 30 min. The mixture was cooled and the violet 4-amino-1-methyl-5-nitrosouracil was collected (5.0 g) (overall yield from methylisourea *ca.* 15%).

7-Hydroxyxanthine (1a).—Dried dimethyl sulphoxide (375 ml) was heated to 120 °C and 4-amino-5-nitrosouracil (2a) (6.24 g) was added in portions with stirring during 10 min. After all the solid had dissolved, aqueous 36% formaldehyde (10 ml) was added in one portion, the internal temperature was kept at 117—119 °C and stirring was continued for 15 min. The reaction was followed by u.v. spectroscopy and by the fading of the blue colour. The solution was concentrated at 75—80 °C to 27.5 ml. Chloroform (130 ml) was added with shaking. If a solid precipitated (precipitate A) it was filtered off. 0.5N-Hydrochloric acid (50 ml) was added and the two layers were shaken for 1 min and left at room temperature for 3 days. If precipitate A (which is bulky and hygroscopic) contained, in addition to uric acid, considerable amounts of 7-hydroxyxanthine it was redissolved in not more than 12 ml of dimethyl sulphoxide and treated with chloroform (60 ml) as above; the mixture was then filtered and the solution shaken with 0.5N-hydrochloric acid (25 ml) and left for 3 days at room temperature.

The precipitate which accumulated in the two-phase mixture was collected (3.5 g) and redissolved in boiling water (100 ml). Concentrated ammonia (7.5 ml) was added and the solution was boiled with stirring for an additional 15 min. After cooling and acidification, white amorphous 7-hydroxyxanthine was precipitated. It could be crystallized from water (with active charcoal) but if a very pure product

[16] G. Zvilichovsky, *Tetrahedron*, 1975, **31**, 1861.
[17] W. Pfleiderer, *Chem. Ber.*, 1957, **90**, 2272.

was needed it had to be purified by column chromatography on Dowex-50 resin. The product was dissolved in boiling water and loaded onto a warm column (at least 100 mm long) in order to avoid precipitation. If precipitation occurred, the upper layer of the column was mixed with the precipitated crystals. Elution with water gave the desired fraction after an initial fraction containing uric and violuric acids. Recrystallization from water gave white *needles* (2.7 g) (see Tables).

7-*Hydroxy*-1-*methylxanthine* (1b).—4-Amino-1-methyl-5-nitrosouracil (2b) was treated with formaldehyde solution in dried dimethyl sulphoxide as above. Similar isolation and purification gave 7-*hydroxy*-1-*methylxanthine* (25%) (see Tables).

8-*Alkyl*-7-*hydroxyxanthines*.—These derivatives are prepared from 4-amino-5-nitrosouracil and freshly distilled aliphatic aldehydes as in the preparation of the parent compound (1a). Here a larger excess of the aldehyde was necessary as large amounts of the aliphatic aldehydes are oxidized during the reaction. With the more volatile aldehydes (*e.g.* butyraldehyde and valeraldehyde) a reflux condenser was attached to the reaction flask. The aldehyde was added in 5 ml portions at 5 min intervals until the reaction was complete, as indicated by the disappearance of the blue colour.

Isolation in two phases was carried out as above, as well as treatment with ammonia and acid. Crystallization from ethanol proved more efficient than from water. If purification on a column was needed the product was dissolved in ethanol (sometimes only suspended and mixed with the top layer of the Dowex-50 column). The column was then washed with water and the *product* eluted with another solvent. For 8-propyl derivatives hot 50% aqueous ethanol was used for elution. The column was kept warm (50—60 °C). With longer alkyl groups the proportion of ethanol had to be increased. 8-Hexyl-7-hydroxyxanthine was eluted with warm 95% ethanol. 7-Hydroxy-8-undecylxanthine had to be eluted with warm dioxan. Further data are given in the Tables.

Conversion of 8-*Alkyl*-7-*hydroxyxanthines into* 8-*Alkylxanthines*.—The starting material (1.0 g) was dissolved in 70 ml of solvent. The nature of the solvent depends on the length of the alkyl group (ranging aqueous buffer when R^2 = propyl to 50% ethanol–buffer when R^2 = hexyl to pure ethanol when R^2 = undecyl; the buffer consists of 0.1M–Na_2HPO_4 at pH 9.4). Potassium iodide (2.0 g) was dissolved in the mixture, which was heated, if necessary, and then acetic anhydride (0.9 ml) was added. The mixture was kept for 15 min at the same temperature then cooled in ice for a few h. The precipitate was collected and redissolved in boiling ethanol (100 ml), and the solution was filtered hot. The filtrate was evaporated under vacuum and the residue recrystallized from ethanol (with active charcoal). Additional data are given in the Tables.

Reactions of N-*Hydroxyxanthines with Acetic Anhydride*.—In order to follow the reaction in a u.v. cell, *ca.* 1.5×10^{-4}M-solutions were prepared in 0.1M-phosphate buffer. A standard 10^{-2}M-solution of acetic anhydride in dioxan was prepared and a micropipette was used to introduce the desired amounts of acetic anhydride. The reaction was followed either by repeated u.v. scanning every 2 min or by looking at the change at a constant wavelength (*e.g.* 290 nm). It was assumed that if the major product was uric acid the change of optical density (optical density at the end of reaction minus the optical density at a specific time), was proportional to the conversion of 7-hydroxyxanthine into its rearrangement products.

For studying the constitution of the products *ca.* 3×10^{-3}M-solutions were prepared and pure acetic anhydride was added with a micropipette. At the end of the reaction the mixture was chromatographed on a Dowex-50 column and eluted with water or dilute hydrochloric acid. The products were identified by their retention times and u.v. spectra. Data for uric acid, xanthine, and 8-substitution products (when NO_2^-, pyridine, or methionine were present) are given in earlier reports.[7,8] In cases when the blue compound was formed it remained on the top of the column.

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