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PURINE N-OXIDES—XLI
THE 3-ACYLOXYPURINE 8-SUBSTITUTION REACTION: ON THE MECHANISM OF THE REACTION*

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Abstract—The 3-acyloxypurine 8-substitution reaction involves elimination of the 3-acyloxy group and nucleophilic substitution at C-8 to yield 8-substituted xanthines or guanines, In aqueous solutions the reaction of 3-acetoxyxanthine proceeds slowly below pH 2, but is greatly accelerated with an increase of the pH from 3 to 7. It is proposed that the slow reaction involves heterolytic cleavage of the 3-acetoxy moiety from 3-acetoxyxanthine to yield a nitrenium ion at N-3 followed by intermolecular nucleophilic substitution of the incipient carbonium ion at the allylic C-8 position, also the most probable mechanism in polar aprotic solvents. The beginning of the fast reaction coincides with the beginning of ionization of the imidazole hydrogen of 3-acetoxyxanthine. It is proposed that this ionization induces a similar but more rapid departure of the 3-acetoxy group from the anion of 3-acetoxyxanthine to produce dehydroxanthine. The latter, upon protonation, yields the same reactive carbonium ion at C-8 that is formed in the slow reaction. Some reduction of 3-acetoxyxanthine to xanthine accompanies the fast reaction. That reduction has the characteristics of a free-radical mediated reaction. It is proposed that reduction results from a homolytic cleavage of the N—O bond in the 3-acetoxyxanthine anion to produce a radical-anion, which abstracts hydrogen from water to yield xanthine. These reaction mechanisms and possible alternatives are evaluated.

STUDIES OF THE SCOPE of the 3-acyloxypurine 8-substitution reaction[1] have shown that 3-acetoxyxanthine (**1**)[2] can react readily with many inorganic and organic nucleophiles to furnish good yields of 8-substituted xanthines. Those studies also showed that the production of some xanthine, previously[3] noted only occasionally, invariably accompanied the substitution reaction in water. The facile nucleophilic substitution of a purine at C-8 is unusual since the π-excessive imidazole ring normally undergoes electrophilic attack.[4] In this investigation of the mechanism of the reaction, we have studied the influence of various parameters on the overall rate of reaction of 3-acetoxyxanthine and on the production of each of the major products from **1**. In aqueous solutions water reacts with 3-acetoxyxanthine in competition with ionic nucleophiles such as chloride or nitrite, and we have investigated the reaction in water alone to avoid the complication of reaction with a second nucleophile and to emphasize the secondary reactions. The products in water include 3-hydroxyxanthine, uric acid, xanthine and an unidentified blue compound.[1] The reactions are competitive and proceed only from 3-acetoxyxanthine; 3-hydroxyxanthine is not altered under the reaction conditions.

RESULTS

Study of the kinetics for the formation of each product was complicated by the multi-

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plicity of products, the rapidity of the reactions, and the inability to quench all of them under any one condition. The similar UV absorption of **1** and its reaction products precluded measurement of the relative rates of the competing reactions from changes in the spectrum of the reaction mixture.

Reactions were carried to completion under standardized conditions, with one parameter varied at a time, and each mixture was then chromatographed to determine quantitatively the major products. The recoveries accounted for 80 to 97% of the starting material, with duplicates within $\pm 5\%$. A blue product,[1] which is highly insoluble, unstable, and is not recovered unchanged from Dowex-50, may represent the unaccounted for material. The final yields of the products were determined from 2×10^{-3} M solutions of **1**, while the half-times for the disappearance of **1** were determined spectrophotometrically with 10^{-4} M solutions.

The seven experiments plotted in Fig. 1 illustrate the influence of changes of

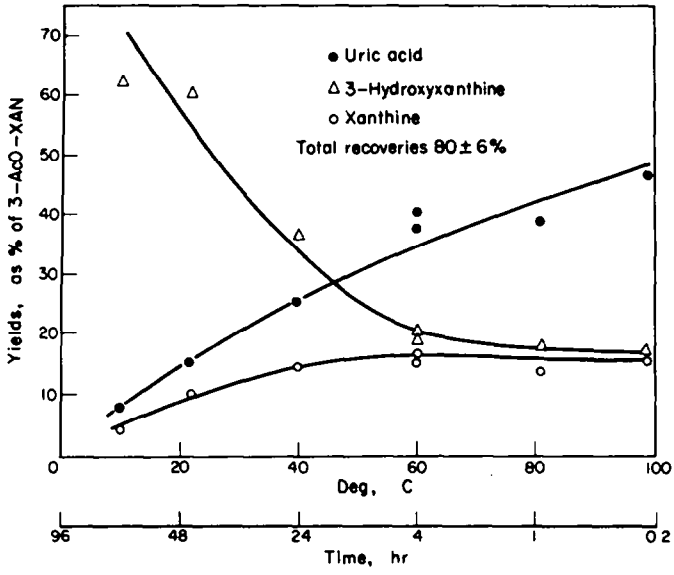


FIG 1. Effects of temperature on yields of products

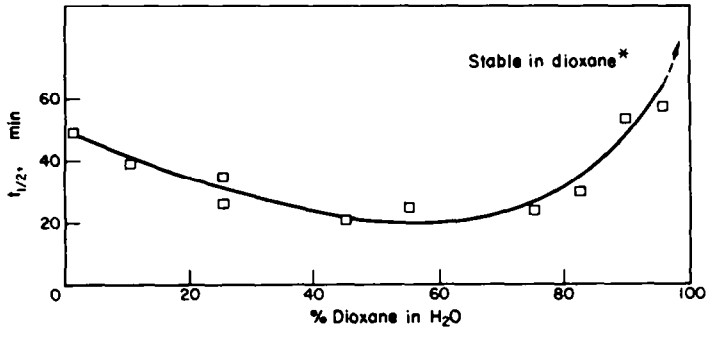


FIG 2. Effect of solvent composition on half time of overall reaction

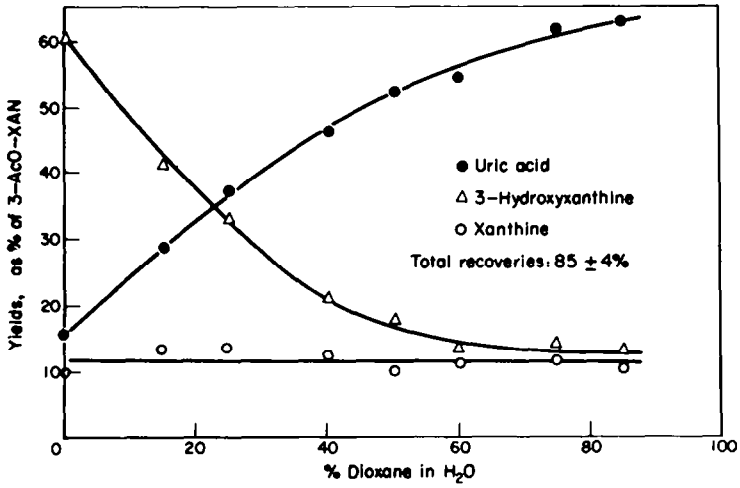


FIG 3. Effect of solvent composition on yields of the products

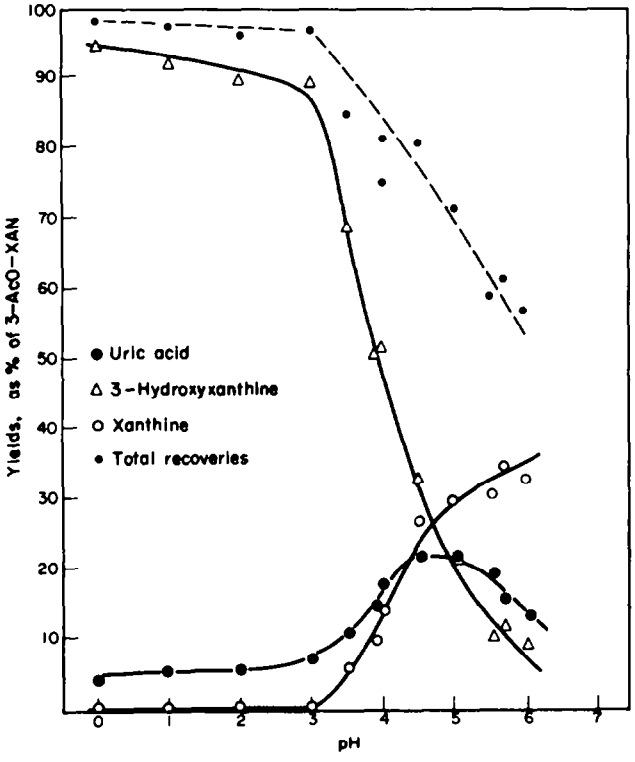


FIG 4. Effect of pH on the yields of products

temperature on the relative yields of the products from an unbuffered 2 mM solution of 3-acetoxy-xanthine. The pH decreased as AcOH was liberated, and from comparison with Fig. 4 was effectively 3·5 to 4. With increasing temp the rate of decomposition of 3-acetoxyxanthine increased rapidly. The times given (Fig. 1) are those at which

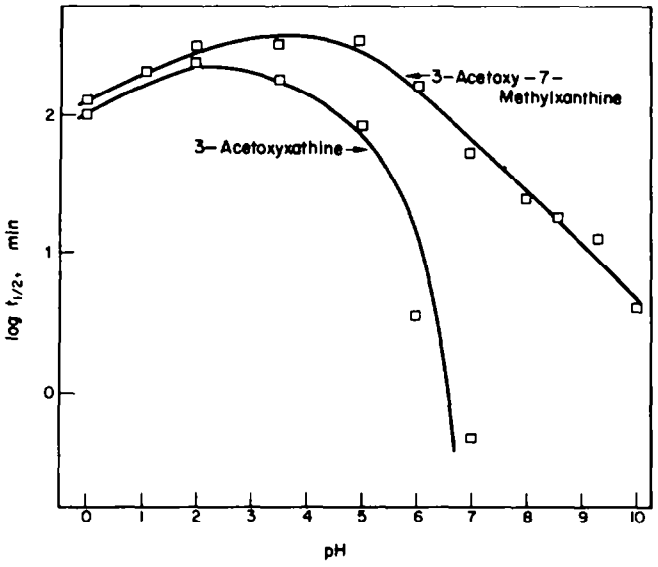


FIG 5. Effect of pH on the rates of reaction in water

each reaction mixture was analyzed and are roughly indicative of the increase in the overall reaction rate with increasing temperature. At low temp hydrolysis of 3-acetoxyxanthine to 3-hydroxyxanthine predominated, but this decreased rapidly as the temp was raised. Uric acid production increased almost linearly from 10 to 100° (Fig. 1), which indicates that the rate of the 8-substitution reaction increases faster than do the rates of the hydrolysis and the reduction reactions. The yield of xanthine increased somewhat between 10 and about 40° but further temp increases to 100° had no effect on its yield.

In dioxane-water mixtures (Fig. 2) the half-time for the decomposition of **1** (10^{-4} M) decreased with increasing dioxane concentration to about 60%, and then increased toward infinity at higher concentrations of dioxane; in pure dioxane **1** was stable. These changes in the rate of reaction of **1** were accompanied by changes in the ratios of the products. In Fig. 3 the yields of the products, determined from 2 mM solutions, are plotted for the same range of dioxane-water concentrations. Hydrolysis to 3-hydroxyxanthine decreased progressively as the water concentration decreased, and there was a reciprocal increase in the yield of uric acid (Fig. 3). Most notable in Fig. 3 is the constant yield of xanthine, 12 to 14%.

The complex changes in the yields of the products over a pH range of 1 to 7 are shown in Fig. 4. Below pH 3 the primary reaction was hydrolysis to 3-hydroxyxanthine; conversion to uric acid remained almost constant at about 5% and no xanthine was formed. Above pH 3 abrupt changes in the courses of the reactions began, marked by a sharp decrease of the hydrolysis reaction and an increase in the substitution reaction. The initiation of xanthine production was also correlated with this change of pH, and increased with the increasing uric acid production to pH 4·5. Above pH 5 the yields of uric acid and xanthine diverge, again indicating that there is no stoichiometric relationship between the formation of uric acid and xanthine.

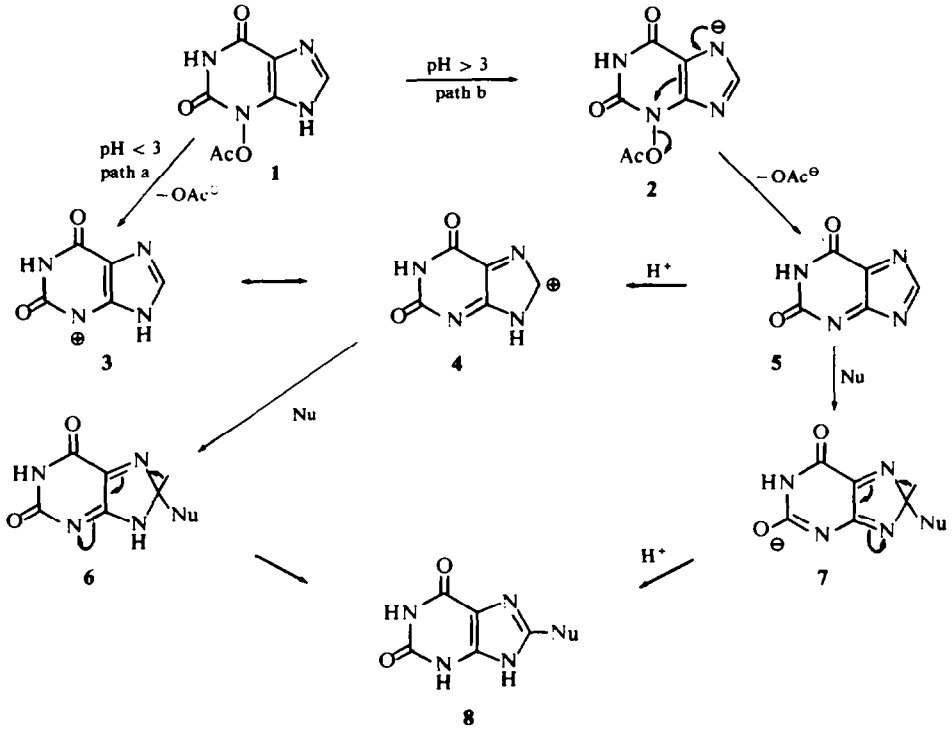
The total recovery was essentially quantitative below pH 3. The decrease as the pH was increased further coincides with the appearance of the insoluble blue compound.

Fig. 5 illustrates the influence of pH on the half-times for the overall reactions of 3-acetoxyxanthine and 3-acetoxy-7-methylxanthine. The half-time for the disappearance of 3-acetoxyxanthine (10^{-4} M), over 100 min at pH 3, decreased rapidly, to about 4 min at pH 6 and to a fraction of a minute at pH 7. The pH's at which the overall reaction rate accelerated were those associated with both the increased extent of the substitution reaction and the initiation of the production of xanthine (Fig. 4). Under these conditions 3-acetoxy-7-methylxanthine undergoes only hydrolysis to 3-hydroxy-7-methylxanthine[1] and particularly above pH 3, it serves as a model for the kinetics of hydrolysis of 3-acetoxyxanthine.

DISCUSSION

Ionic mechanisms for the 8-substitution reaction. In the first report of this 8-substitution reaction,[3] a mechanism was proposed that included heterolytic cleavage of **1** to an ion pair, **3**, nucleophilic attack at the allylic C-8 position, as in **4**, (Scheme 1, path a)

SCHEME 1.



and shift of the C-8 hydrogen in **6** to yield **8**. This suggested sequence for the apparent rearrangement of the 3-oxygen to the 8-position has analogies both to an S_N1' substitution-rearrangement reaction[5a, 6a] and to a $B_{AL}1'$ ester hydrolysis.[5b] In reactions of either of those types an ion pair is generated which reacts at an allylic

position to yield a rearranged product. The initial cation suggested for **1** is the nitrenium ion,* **3**, but the positive charge could be delocalized to the allylic C-8 position. The instability of a nitrenium ion at N-3 should favour the formation of a carbonium ion at C-8. The reactivity of a carbonium ion can account for the facile reactivity with weak nucleophiles, such as chloride ion and alcohols,[1] that is characteristic of the 3-acyloxypurine 8-substitution reaction.

The allylic shift of the cation from N-3, as in **4**, requires the participation of N-7 and alkyl substitution at N-7 should block the 8-substitution reaction by preventing this shift of the positive charge. In accord with this interpretation, 3-acetoxy-7-methylxanthine does not undergo the 8-substitution reaction under mild conditions.†,‡

In solutions of pH 2 or below the primary reaction of 3-acetoxyxanthine is hydrolysis to 3-hydroxyxanthine. This is accompanied by a small and almost constant amount of the 8-substitution reaction, with the two products accounting for 97% or more of the 3-acetoxyxanthine (Fig. 4). In this pH range, and in polar aprotic solvents, the scheme originally proposed[3] (path *a*, Scheme 1) is the probable route for 8-substitution.

The fact that the reaction of 3-acetoxy-7-methylxanthine in water is not complicated by the formation of either 7-methyluric acid or 7-methylxanthine has implications for each of the reactions of **1**. Its hydrolysis to 3-hydroxy-7-methylxanthine provides a model for the hydrolysis reaction of **1** at all pH's. In the pH range from 2 to 0 (Fig. 5), the similar increases of the reaction rates of both esters suggest an $A_{Ac}2$ hydrolysis for each.

At pH's from 3 to 5, the greatly accelerated reaction rate of **1** (Fig. 5), as well as the appearance of xanthine and the increased formation of uric acid (Fig. 4), indicate that some other reaction pathway is operative. The constant rate of hydrolysis of 3-acetoxy-7-methylxanthine is indicative of little change in the rate of hydrolysis of **1** to 3-hydroxyxanthine in this pH range,§ and the accelerated rate of reaction of **1** at pH's above 3 must be attributed to a change in the mechanism of the other reactions.

The first ionization of 3-acetoxyxanthine, at pK_a 6·8 ± 0·5,[2,]‖ must be associated with the change in reaction mechanism. This ionization should occur in the imidazole ring, as does that of 3-methylxanthine.[9,10] The 7-methyl derivative of 3-acetoxyxanthine cannot ionize from the imidazole moiety and does not undergo the facile nucleophilic substitution at C-8. In contrast, 3-acetoxy-1-methylxanthine, with its only ionizable proton on the imidazole ring, undergoes 8-substitution and reduction reactions similar to **1**. The behaviours of these methyl derivatives thus support the arguments that the first ionization of **1** takes place from the imidazole ring and that this ionization is involved in accelerating the reactions of 3-acetoxyxanthine at pH's above 3.

* Nitrenium ions, which have resemblances to carbonium ions,[7] have but recently received intensive study. They have been proposed as short-lived intermediates in rearrangements of isoquinuclidine[7] and in the solvolysis of N-chloramines.[8]

† In polar aprotic solvents, with heat, 3-acyloxy-7-methylxanthine can undergo 8-substitution.[1]

‡ The 8-substitution reaction should not be blocked by alkylation at N-9. No 3-acetoxy-9-methylxanthine has been isolated, but, even at 20°, some 9-methyluric acid was formed from 3-hydroxy-9-methylxanthine.[2] It was suggested[2] that steric hindrance may reduce both the rate of formation and the stability of 3-acetoxy-9-methylxanthine such that, once formed, it is rapidly converted to 9-methyluric acid.

§ A decreased half-life of the 7-methyl derivative, attributable to base catalyzed hydrolysis, became significant only above pH 5.

‖ A more accurate determination was not possible because of the short half-life of 3-acetoxyxanthine near the pK_a.

The apparent paradox that the anion of **1** shows a greater reactivity to nucleophilic substitution at C-8 than does **1** is explicable if the negative charge on the imidazole accelerates the substitution reaction by facilitating the departure of acetate ion from **2**. That elimination, probably through a mechanism comparable to an Elcb elimination,[5c, 6b, 11] would lead to the neutral dehydroxanthine, **5**. Protonation of **5** on an imidazole nitrogen would yield **4**, the same intermediate arising from **3** in path *a*, and reaction of it with a nucleophile would lead through **6** to **8**. Alternatively, a direct nucleophilic attack on the neutral **5**, a reaction comparable to Michael addition, would lead to **7** and thence to **8**. It is most probable that the dehydroxanthine, **5**, would be rapidly protonated at pH 3 to 7, and the path via **4** and **6** would be preferred in this pH range.* Preference is given to a carbonium ion intermediate, as it was for path *a*, because it most readily explains the rapid reactions with a weak nucleophile such as water.

It is probable that the two pathways, *a* and *b*, are competitive in aqueous solutions with path *a* predominating from pH 0 to 3 and with path *b* soon predominating as the pH increases further. Since the pK_a of **1** is near 6·5, about 0·1% of the anion **2** is present at pH 3·5, and the decrease in $t_{1/2}$ near pH 3 must correspond to the increasing contribution of the rapid path *b* from low concentrations of **2**.

No xanthine is produced below pH 3 where only path *a* is operative (Fig. 4). From the initiation of the fast reaction the yields of uric acid and xanthine show a nearly parallel increase with further increase in the pH (Fig. 4), which suggests that both arise from a common intermediate on path *b*. The production of xanthine may therefore be accepted as an indication that some of the 8-substitution reaction is proceeding via path *b*.

An increase in temp decreases the reaction times markedly (Fig. 1) and favours the 8-substitution reaction at the expense of hydrolysis. The reduction reaction shows little sensitivity to temperature changes.

A change in solvent polarity alters both the overall reaction rate (Fig. 2) and the product distribution (Fig. 3). As the percentage of dioxane increases from 0 to 100%, the dielectric constant of the medium decreases from 80 to 2·2 and two effects on the $t_{1/2}$ are discernible. From 0 to about 50% dioxane, the half-time for decomposition of **1** (10^{-4} M) shows a linear decrease from 50 to 20 min (Fig. 2). This range of $t_{1/2}$ is comparable to that near pH 5·5 in 10^{-4} M aqueous solutions (Fig. 5). In 2 mM solutions the same increase in dioxane concentration leads to an increase in the yield of uric acid and a corresponding decrease in the yield of 3-hydroxyxanthine. The yields of the products in water alone in Fig. 3 correspond to those at about pH 4 in Fig. 4, both at 2 mM.

The increase in reaction rate with decrease in solvent polarity (Fig. 2) is particularly noteworthy, since a decrease in the polarity of the medium should be unfavourable to both acid-catalyzed ester hydrolysis[12] and S_N1' nucleophilic substitution.[5d] The decrease in yield of 3-hydroxyxanthine as the concentration of dioxane increases is in agreement with the expected solvent effect. Since the yield of xanthine remains constant and the increase in reaction rate is correlated with a higher yield of uric acid, it is apparent that decrease of solvent polarity favours the 8-substitution reaction.

Both the range for $t_{1/2}$ and the production of xanthine show that path *b* predominates in dioxane-water mixtures (Fig. 3). Since the yield of xanthine is pH sensitive (Fig. 4)

* At higher pH's or with stronger nucleophiles, the Michael addition pathway *via* **7** to **8** could contribute.

but is nearly constant in dioxane-water (Fig. 3), the effective pH and hence the concentration of **2** must be little changed from 0–85% dioxane. As the polarity decreases the ion **2** should be increasingly less stabilized by the medium; this should favour the formation of an unionized intermediate such as **5**. Although **5** should be rapidly protonated in the pH range of 4 to 5, the charge of the resulting cation **3–4** is dispersed over several atoms of the purine ring and further stabilized by the electron cloud of the imidazole ring. Thus the cation **3–4**, generated by either reaction pathway, should be less dependent on solvation than the protonated intermediates associated with $A_{AC}2$ ester hydrolysis. The latter, which are unable to effect charge dispersal and must rely on solvation for stabilization,[12] should be more susceptible to a decrease in solvent polarity than **3**. The increase in overall reaction rate and increase in uric acid formation via path *b* with a decrease in solvent polarity (0 to 50% dioxane) can be attributed primarily to this facilitation of the formation of the un-ionized dehydroxanthine **5** rather than to internal stabilization of the carbonium ion **4**.*

As the percentage of dioxane is increased from 50 to 100%, the half-time for the decomposition of **1** increases rapidly (Fig. 2) but it is accompanied by only small additional changes in the ratios of products. This suggests that the dielectric constant of the medium has decreased to the point that ionization is retarded and that the reactions which produce ionized species, *i.e.*, **1** → **2** and **1** → **3** now become rate-limiting. The reaction **1** → **3** should be rate-limiting for path *a* under all conditions and obviously proceeds very slowly, since only a small amount of uric acid is formed when this is the only path yielding uric acid, *e.g.*, below pH 2.

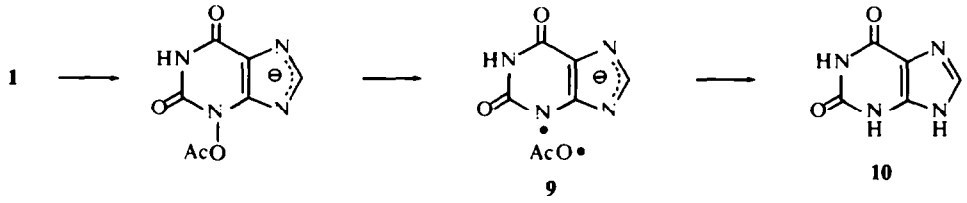
It can be deduced that reaction **2** → **5** must be rate-limiting for path *b* in 0 to 50% dioxane. Xanthine **10** arises simultaneously with the appearance of **2**. The parallel increases in the yields of uric acid and of xanthine *via* path *b*, associated with the increasing concentrations of **2** above pH 3, indicate that both products arise from **2** *via* competing reactions. Both the ionization **1** → **2** and the protonation **5** → **4** reactions on path *b* should be rapid. The cation **3–4** is common to both paths *a* and *b*, and the considerable difference in the rates of the two pathways require that the rate determining steps precede the common intermediate **4**, in each case. Thus **2** → **5** must be rate determining for path *b* under these conditions, and **1**–**3** remains that for path *a*. The rate determining step for the reduction must also be **2** → **9**. Thus as the ionization of **1** to **2** becomes rate-limiting in high concentrations of dioxane no further change should be observed in the ratios of uric acid and xanthine, as is observed in Fig. 3.

A free-radical mechanism for the reduction to xanthine. The relative insensitivity of the yield of xanthine to changes in temp (Fig. 1) or in solvent polarity (Fig. 2) suggests that xanthine may arise *via* a radical reaction. Support for this interpretation can be found from the reactions of **1** with halide ions.[1] While chloride and bromide ions readily react with 3-acetoxyxanthine as nucleophiles, iodide ion quantitatively reduces **1** to xanthine,[1] which suggests that iodide ion is behaving as a radical scavanger. The changes in the course (Fig. 4) and rate (Fig. 5) of the reaction are associated with ionization of the imidazole hydrogen of **1** and are correlated with the appearance of xanthine. The similar increases in the yields of uric acid and xanthine above that pH have been attributed to an origin of each from a common intermediate on path *b*. Ionization, to **2**, must precede radical formation because xanthine is not produced

* Another example of rapid reaction in solvents of low dielectric constant, probably also *via* path *b*, is the formation of 8-alkoxyxanthines from **1** in alcohols.[1]

below pH 3 and because the 7-methyl derivative of **1** is not reduced to 7-methylxanthine. Since NaI is unreactive toward 3-hydroxyxanthine, even at elevated temps.[1] the acetoxy group must also be essential for the dissociation to a radical. A radical anion **9** (Scheme 2), derived from **2** by thermal homolysis of the N—O bond, could be the radical intermediate. That xanthyl anion radical, **9**, once formed should readily abstract hydrogen from water to yield the anion of xanthine.

SCHEME 2.



This postulation of a radical mechanism for the reduction observed in aqueous reaction media is reinforced by the knowledge that a free-radical can be derived from 3-hydroxyxanthine. We have observed [13] that UV irradiation of 3-hydroxyxanthine or 3-acetoxyxanthine in the solid state leads to a stable free-radical, and is accompanied by a change in colour from white to purple. In water this solid loses both its colour and ESR spectrum and yields xanthine. This sequence which proceeds through a photochemically generated free-radical may be analogous to the reduction of **1** in solution.

The production of xanthine from **1** observed in solutions is not due to a photochemical effect. Identical amounts of xanthine were produced in reactions carried out in pyrex or quartz flasks under fluorescent room light, as well as in low actinic glassware with complete light exclusion. No coloration corresponding to that observed with the radical formed in the dry state was observed during the aqueous reactions of **1**, although the blue precipitate, which is not a free-radical, could have obscured any coloured intermediate. Radicals were not detected by ESR during the reaction but this could well be due to a concentration of **9** below the limit of detection.

It is conceivable that xanthine could arise from the nitrenium ion, **3**. Spin inversion of nitrenium ions from the singlet to the triplet state produces a hydrogen abstractor which can yield the parent amine.[7] Since heavy atom solvents promote such a spin inversion,[14] the addition of CCl_4 to a dioxane-water solution of 3-acetoxyxanthine should increase the amount of nitrenium triplet present and raise the yield of xanthine, at the expense of uric acid, if reduction proceeds through the triplet state of **3**. The maximum amount of CCl_4 which could be dissolved in a dioxane-water mixture did not significantly affect the yield of xanthine (Table 1). This, with the fact that xanthine

TABLE 1. THE EFFECT OF ADDITION OF CARBON TETRACHLORIDE

Solvent Composition	% Yields		
	Uric acid	3-Hydroxyxanthine	Xanthine
20% H_2O, 80% dioxane	62	14	12
20% H_2O, 72·5% dioxane, 7·5% CCl_4	54	21	13

is not produced below pH 3 where the nitrenium ion is a postulated intermediate in uric acid formation. indicates that xanthine is unlikely to originate from spin inversion of **3** and supports the proposed radical ion mechanism.

Unidentified blue product. The structure and role of the highly insoluble blue compound[1] remains to be determined. It can represent an appreciable proportion of the product in some cases. and appears to be produced by additional reactions of an intermediate on path *b*. probably at the expense of uric acid. It is not a free radical.

Analogies to similar reactions. The 3-acyloxypurine 8-substitution reaction has partial analogies to several known reactions. The N.O-diacyl derivatives of several oncogenic arylhydroxylamines. notably that of hydroxylaminofluorene.[15] undergo deoxygenation with concomitant nucleophilic substitution at an allylic position.[16] Those diacylderivatives are structurally comparable to 3-acetoxyxanthine in that they are substituted acylhydroxamates. Their reaction with methionine[17] parallels that of 3-acetoxyxanthine. but reactions with very weak nucleophiles such as water and chloride have not been reported.

Studies on the reactions of nitrosobenzene with trimethylphosphite have demonstrated nucleophilic aromatic substitution with accompanying deoxygenation.[18] It was suggested that that reaction could have proceeded *via* a nitrene, which was protonated to a phenyl nitrenium ion that then underwent nucleophilic substitution. That sequence is analogous to **5** → **4** → **6** in Scheme 1.

The Bamberger reaction[5e, 19, 20] in which phenylhydroxylamine is converted to *p*-aminophenol is also similar. This reaction is acid catalyzed and is believed to occur by an S_N1' reaction, which involves protonation of the hydroxylamine oxygen, loss of water to generate a phenyl nitrenium intermediate, and nucleophilic substitution at the *para* position.

The only reaction in the purine series with any resemblance to the 3-acyloxypurine 8-substitution reaction is the conversion of hypoxanthine 3-oxide to 8-chlorohypoxanthine or to 6.8-dihydroxypurine in HCl[21] or HCOOH.[22] However. that reaction more closely resembles the Bamberger reaction in that it requires acid catalysis. while the 3-acyloxypurine 8-substitution reaction is base catalyzed. In Ac_2O hypoxanthine 3-oxide reacts as do pyridine N-oxides[23, 24] and only the adjacent 2-position is substituted.[22, 25]

The reactions of 3-acetoxyxanthine are unique in the facile nucleophilic substitution of the purine ring. While the mechanisms suggested provide a consistent interpretation of the observations they must be considered tentative without direct evidence for the presence of a radical-ion (**9**) or of the dehydroxanthine (**5**) intermediates.

Biological implications. Nucleophilic substitution reactions with macromolecules of the cell have long been offered as an explanation of the initiation of the cancer process by chemical oncogens.[15] However, free-radicals are also assumed to be capable of initiating cancer. With two modes of formation of a reactive intermediate. one ionic and the other a radical. decision should be reserved as to which may be responsible for tumor induction by metabolically derived esters[26] of 3-hydroxyxanthine or 3-hydroxyguanine.

EXPERIMENTAL

The 3-acetoxyxanthine and 3-acetoxy-7-methylxanthine were synthesized as described.[2] Glass distilled water and spectroquality dioxane (Eastman Organic Chemicals) were used. Buffers used were 0·01 M

succinate or acetate. and below pH 3 HCl was used. The pH quoted for each reaction is the average of the initial and final readings. which generally differed less than 0·1 pH unit. Reactions were stirred magnetically in a thermostated (± 2°) water bath. At pH's 0 to 2, in HCl, some 8-chloroxanthine was produced, and is included in the uric acid values.

The general procedure was to dissolve 8·40 mg. 40 μmoles. of 3-acetoxyxanthine in 20 ml of the solvent of the composition or pH specified. and at the temp specified. The progress of the reaction was followed spectrophotometrically or by chromatography. When no more starting material remained. as measured by the lack of further OD change in diluted aliquots, or sometimes by chromatography, a 2·00 ml aliquot was taken for analysis for the products. These were separated by chromatography over an 8 × 1 cm column of Dowex-50 [H^+], 8 ×, 200 to 400 mesh (Bio Rad Laboratories) and the UV absorption of the effluent was recorded by an ISCO-UA2 monitor. Uric acid and 3-hydroxyxanthine were eluted with water as to well separated bands; then xanthine was eluted by 1 N HCl. The retention volumes of these compounds on a standardized column are recorded for 0·05 N HCl and 1·0 N HCl.[1] The yield of each product was calculated from the volume and absorbance of each fraction. with the use of the reference values: uric acid. pH 2 to 4. λ_{max} 285. ε 12.000;[27] 3-hydroxyxanthine. pH 2 to 4. λ_{max} 272. ε 10.100;[27] xanthine. pH 0. λ_{max} 260. ε 9.200. Dioxane and water mixtures were concentrated to dryness *in vacuo*. the residue was dissolved in 20 ml of water and an aliquot analyzed as above. The results are plotted in Figs. 1. 3 and 4.

The half-times. Figs 2 and 5. were determined by following the absorption of an about 10^{-4} M solution at a given wavelength. usually 290 nm. which was read at intervals. The half-times were determined from a plot of log (OD − OD_f) against time. For slower reactions a family of curves was also plotted at given time intervals with a Unicam SP800 recording spectrophotometer.

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