dro-2-deoxy-D-arabinitol (666 mg, 5 mmol) in DMF (50 mL) was slowly added 24 mL of a benzene solution containing 5 mmol of β-ethoxyacryloyl isocyanate[16] at such a rate as to keep the temperature of the reaction solution below −10 °C. The resulting solution was kept for 1 h at −10 °C and then 18 h at ambient temperature before it was evaporated to dryness. The residue was purified by silica gel plates using 9:1 $CHCl_3$–MeOH as the developer. The product was obtained as a glass by elution with MeOH: yield 692 mg (44%).

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Registry No.—1, 53448-53-6; 2, 64332-64-5; 3, 64345-58-0; 4, 64332-65-6; 5, 64332-66-7; 6, 64332-67-8; 7, 64332-68-9; 8, 64332-69-0; 9, 64332-70-3; 10, 64332-71-4; 11, 64395-31-9; 12, 64332-72-5; 14, 57796-78-8; 15, 64332-73-6; 16, 64332-74-7; mesyl chloride, 124-63-0; 5-amino-4,6-dichloropyrimidine, 5413-85-4.

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Purine *N*-Oxides. 65. On the Mechanisms of the Reactions of 3-Acetoxyxanthine[1]

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The redox chemistry of 3-acetoxyxanthine, a model "activated ester" for the proximate form of the oncogen 3-hydroxyxanthine, has been explored. The results indicate that the oxidizing reactivity of the ester, previously attributed to the participation of a radical intermediate, is instead due to reactions at the electron-deficient nitrogen of an intermediate of the S_N1' 8-substitution reaction. A two-step reaction sequence is proposed for the reduction of the nitrenium ion in the presence of iodide and thiourea. 8-Iodoxanthine is shown not to be an intermediate in the reduction by iodide ion. Studies with formate, acetate, and phosphate buffers at pH's 4.0, 5.0, and 7.0, respectively, show that changes in the concentration of each buffer system elicit different responses in the reactions of 3-acetoxyxanthine. The combined studies provide support for a unifying mechanism for competitive redox and C-substitution reactions from a single ambident electrophile. It is proposed that redox reactions are frontier orbital controlled and result from soft–soft interactions at the nitrenium ion, while C-substitution reactions are charge controlled and occur via hard–hard interactions at the carbonium ion. The proposal accommodates the observations that in the presence of 3-acetoxyxanthine certain nucleophiles undergo oxidation only, other nucleophiles lead only to C-8 substitution, while some may participate in both types of reaction.

3-Hydroxyxanthine (**5**) (Scheme I) and certain related purine *N*-oxides are potent oncogens.[2-6] Studies to elucidate the mechanism of cancer induction by **5** have shown that while 3-hydroxyxanthine itself is relatively inert chemically its esters are extremely reactive.[7-11] Esterification in vivo is apparently a prerequisite for the initiation of oncogenesis.[6,12,13] 3-Acetoxyxanthine (**1**) was selected as a model ester for in vitro studies of the presumed "activated" or "proximate" form of 3-hydroxyxanthine.[10,11] Those studies demonstrated the diversity of spontaneous reactions that **1** can undergo, the high reactivity of **1** with nucleophiles,[10] and the strong influence that pH, temperature, and dielectric constant of the medium can exert on the course of these reactions.[11] The reaction with nucleophiles, designated the "3-acyloxypurine 8-substitution reaction",[11] can proceed by either of two routes, paths a and b (Scheme I), depending upon the pH of the medium. A second reaction of **1**, reduction to xanthine (**4**), was observed to be a characteristic only of path b. A radical anion (**3**) was proposed as an intermediate in this reduction, based in part on the quantitative oxidation of iodide ion but not of other halide ions. We now present evidence on the mechanisms of the reactions of 3-acetoxyxanthine that indicates the nitrenium ion (**6a**) rather than the radical anion (**3**) is the agent responsible for the oxidation of iodide ion, and possibly other species as well, and propose an integrated mechanism that accounts for the predominance of redox reactions with certain nucleophiles and of 8-substitution with others.

Results

Syntheses. 3-Acetoxyxanthine (1). The reported preparation of **1**,[14] utilizing equal volumes of acetic acid and acetic anhydride, was found to give incomplete acetylation. Extended reaction times and mild heating did not improve the conversion of **5** to **1**. The addition of acetyl chloride to the reaction medium, however, induced the conversion of almost all of **5** to **1**, which was isolated in 78% yield as the hydrochloride.

8-Iodoxanthine. Attempts to prepare 8-iodoxanthine by

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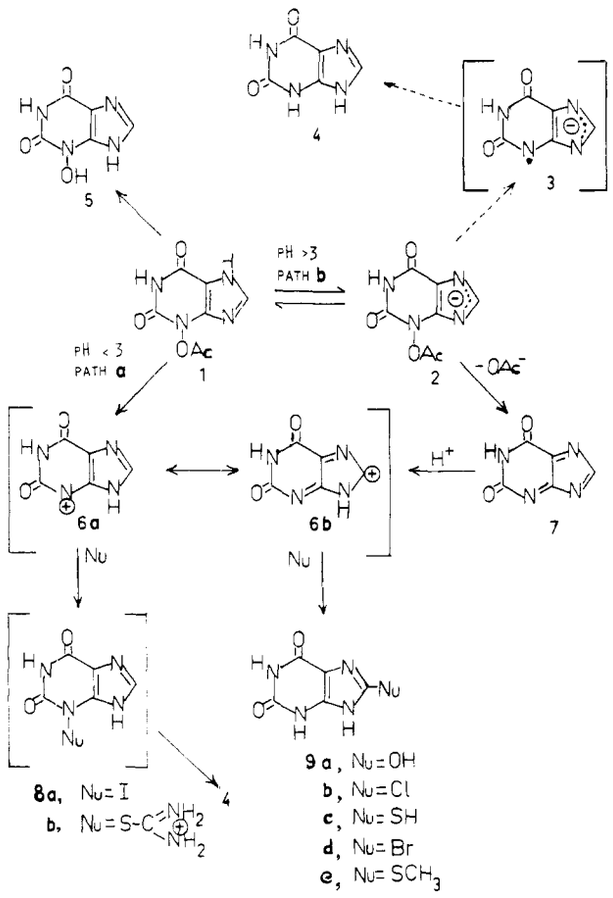
Scheme I



the reported[15] procedure of hydrolysis of 8-iodoxanthosine[15,16] met with limited success. Iodination of xanthosine yielded a gummy solid that rapidly turned to an oil when exposed to air. Hydrolysis of the crude oil in 50% aqueous CF_3COOH at room temperature afforded a low (15%) yield of very impure 8-iodoxanthine. Diazotization of 8-iodoguanine in aqueous CF_3COOH proved to be a superior route to the desired product, although also in low (15%) yield. The structure of 8-iodoxanthine was confirmed by elemental, NMR, and mass spectral analyses and by the similarity to the values reported for UV absorption maxima of it at two pH's.[15]

Reactions of 3-Acetoxyxanthine with Nucleophiles. Studying the mechanisms of reactions from 3-acetoxyxanthine (1) is complicated by the diversity of spontaneous reactions it can undergo and by the high reactivity of 1 with nucleophiles. Consequently the effect of changing conditions on the multiple reactions of 1 was studied by determining the products after all reactions were completed. The effect of varying concentrations of iodide ion on the reduction of 1 to xanthine (4) was studied at pH 1. At that pH the 8-substitution reaction was deduced[11] to proceed solely by path a (Scheme I). The products from 1 at pH 1 (0.1 N HCl) in the absence of KI are 3-hydroxyxanthine, 5 (85%), and 8-chloroxanthine, **9b** (5%) (Figure 1). In the presence of 0.5 equiv of iodide ion (0.001 M) at pH 1, some **9b** was still isolated (~1%), but xanthine was obtained in 5% yield. With ≥2 equiv of iodide ion, no 8-substitution product, **9**, was detectable, the yield of 5 remained unchanged, and ~5% of xanthine was isolated. The effect of varying pH on the products from 1 in the presence of 2 equiv of iodide (4×10^{-3} M) is illustrated in Figure 2. No uric acid (**9a**) or 8-chloroxanthine (**9b**) was obtained at any pH in the presence of 2 equiv of iodide. The yield of xanthine increased slightly from pH 0 to 3 and then rose dramatically from 10 to

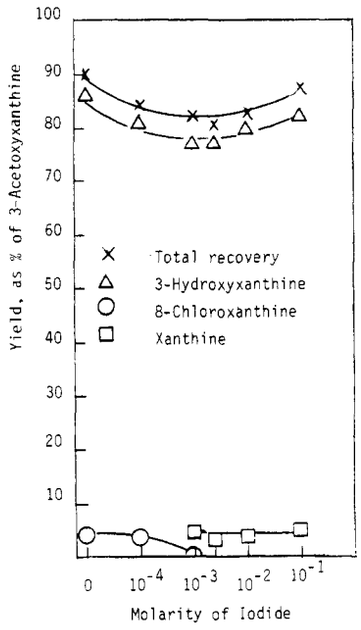


Figure 1. Effect of changes in iodide ion concentration on the composition of products from 3-acetoxyxanthine at pH 1.

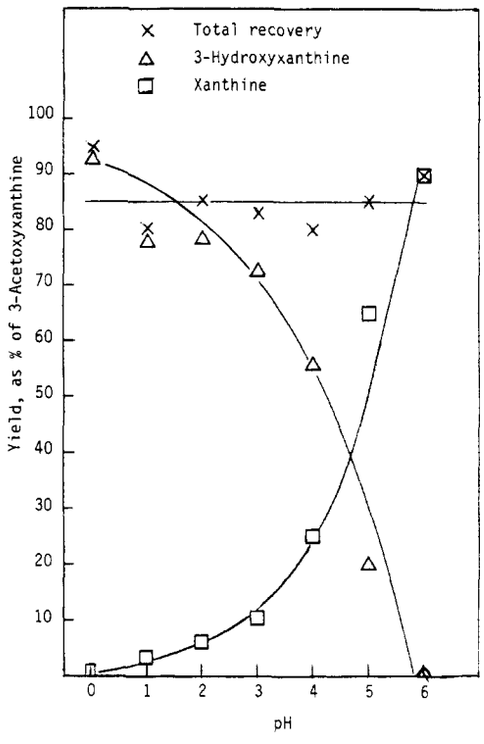


Figure 2. Effect of changes in pH on the composition of products from 3-acetoxyxanthine in the presence of 2 equiv of iodide ion (4×10^{-3} M).

90% over the pH range 3 to 6. The yield of 5 decreased in inverse proportion to that of 4 while the overall recovery remained essentially unchanged.

The effect of varying concentrations of thiourea on the reaction products from 1 was studied at pH 3 (Figure 3). At this pH path a predominates and without added nucleophile only uric acid (**9a**) (~5%) and 5 (85 to 90%) were obtained.[11] With added thiourea over the range of 0.001 to 1 M, no uric acid was detectable, but 8-thiouric acid[17] (**9c**) (4 to 8%), 4 (7 to 19%), and 5 (60 to 70%) were formed (Figure 3). The yields of the

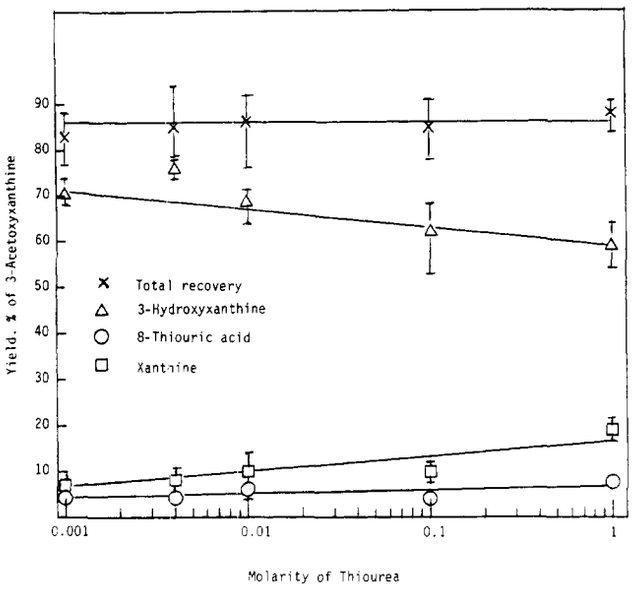


Figure 3. Effect of changes in the concentration of thiourea on the composition of products from 1 at pH 3.

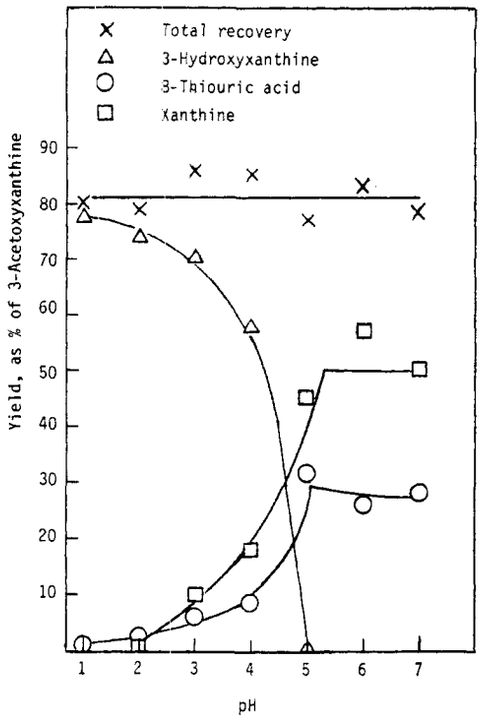


Figure 4. Effect of changes in pH on the composition of products from 1 in the presence of 5 equiv of thiourea.

products were only slightly affected by changes in the concentration of thiourea.

When the pH was varied over the range 1 to 7 in the presence of 5 equiv of thiourea per mol of **1**, the yields of **9c** and **4** showed parallel increases up to pH 5, while that of **5** decreased (Figure 4). Above pH 5 no **5** was detectable and the yields of **9c** and **4** remained unchanged. The overall recovery remained constant over the entire pH range. No uric acid (**9a**) was detected at any pH.

Effect of Varying Buffer Concentrations. Three buffer systems were examined to determine the effect of varying buffer concentration on the reactions of **1**. With an increase in the concentration of formate buffer from 0.01 to 0.1 M, there was little change in the composition of the products from

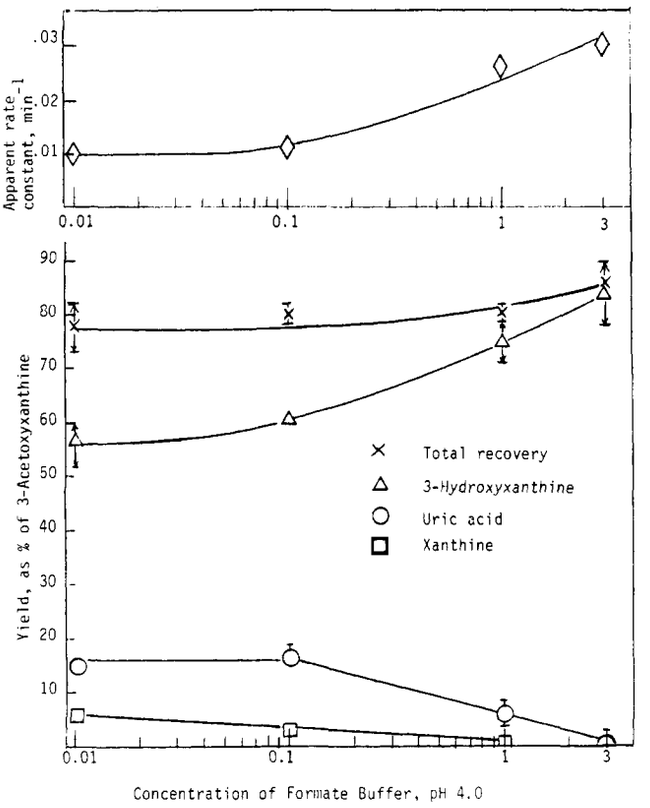


Figure 5. Effect of changes in the concentration of formate buffer at pH 4.0 on the product composition and pseudo-first-order rate constant for the reaction of 3-acetoxyxanthine.

1 (Figure 5). Further increases from 0.1 to 3 M caused an increase in the yield of **5** and a corresponding decrease in the yield of **9a**. The small yield of **4** was reduced to zero. This effect is similar to that obtained by decreasing the pH.[11] Associated with the higher yields of **5** there was an increase in the rate of reaction (Figure 5). These observations suggest that an increased ratio of formic acid to **1** at this low pH effectively acts as though the pH were lowered and hydrolysis of **1** to **5** is favored over other reactions from **1**.

Increases in the concentration of acetate buffer from 0.01 to 3 M produced a large increase in the yield of **9a** from 25% at 0.01 M to 70% at 3 M buffer (Figure 6). The increase in the yield of **9a** was accompanied by a corresponding, but not proportionate, decrease in the yield of **4**. There was little difference in the overall recovery between 0.01 and 0.1 M buffer, but from 0.1 to 3 M there was a significant increase from 60 to 90%. These changes were also accompanied by changes in the reaction rate (Figure 6). Between 0.01 and 2 M acetate buffer there was a gradual increase in the pseudo-first-order rate constant from 0.027 to 0.038 min^{-1}. At higher concentrations of buffer there was a sharp increase in the rate constant to 0.079 min^{-1} in 3 M buffer, which was accompanied by a rise in the yield of **5**. The sudden increase in reaction rate and concomitant rise in formation of **5** suggest that in high concentrations of acetate buffer transacetylation from **5** to acetate ion becomes significant. This conclusion accords with an earlier demonstration that transacetylation can occur between 3-acetoxy- and 3-hydroxyxanthines.[18]

Variation of the phosphate buffer concentration at pH 7.0 from 0.01 to 1 M caused essentially no change in the yield of the reaction products from **1** (Figure 7). A further increase to 1.5 M, i.e., using an almost saturated solution of the buffer, caused an increase in the yield of **9a** and a proportionate decrease in the yield of **4**. Thus only in this narrow concentration range was there an effect of the phosphate buffer and this

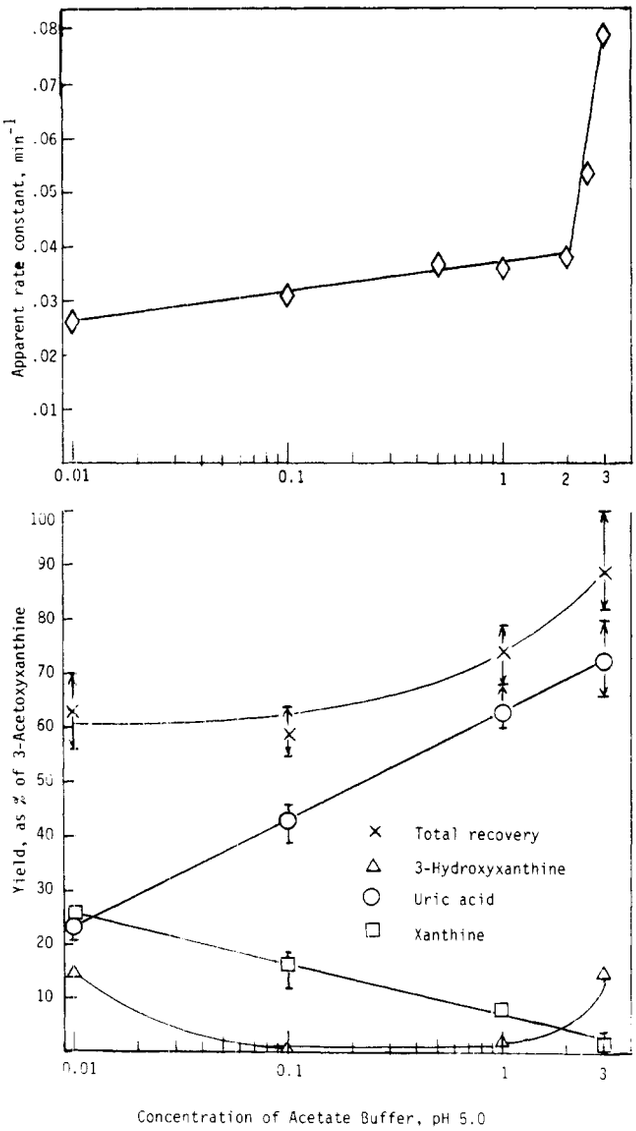


Figure 6. Effect of changes in the concentration of acetate buffer at pH 5.0 on the product composition and pseudo-first-order rate constant for **1**.

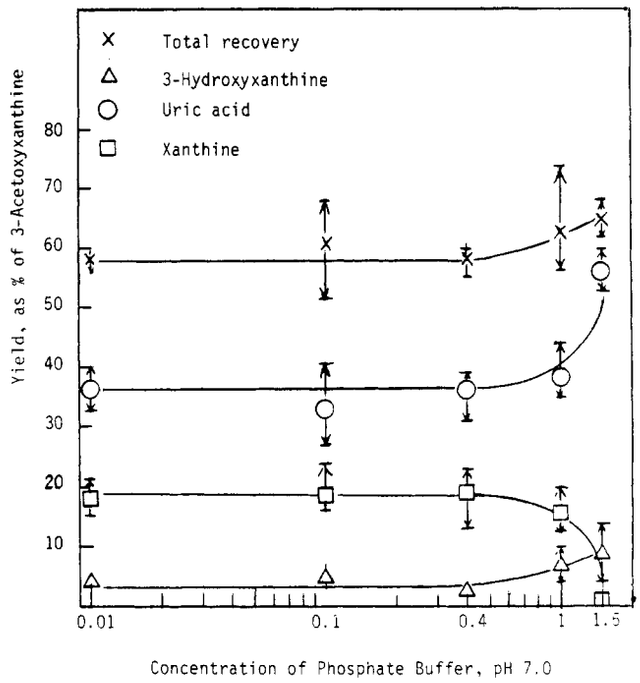


Figure 7. Effect of changes in the concentration of phosphate buffer at pH 7.0 on the product composition from **1**.

Table I. Effect of Variations of pH on the Reaction[a] of 8-Iodoxanthine[b] and Iodide Ion[c]

	Product yields, %, pH =			
	1	3	5	7
8-Iodoxanthine	82 (97)[d]	78	80	92 (98)[e]
Xanthine, **4**	14 (3)[d]	9	5	5 (2)[e]
Recovery	96 (100)[d]	87	85	97 (100)[e]

[a] Reactions were performed for 24 h, except as noted,[e] in the absence of light. Solutions were exposed to room illumination, except as noted,[d] during workup. [b] 2×10^{-3} M. [c] 4×10^{-3} M. [d] The reaction proceeded for 24 h; the reaction and all manipulations, including column chromatography, were performed in the complete absence of light. [e] The reaction was run for 30 min in the absence of light except for room illumination during workup.

effect was similar to the changes induced by increases in the acetate buffer concentration at pH 5.0.

The yields of products from **1** in 0.01 M formate buffer at pH 4 (Figure 5) and in 0.01 M acetate buffer (Figure 6) at pH 5 agree well with the values obtained earlier using 0.01 M buffers.[11] However, in the present experiments we did not observe the drop in yield of **9a** and the increase in yield of **4** at pH 7 that was noted at pH's near neutrality in the previous report.[11] Instead we observed at pH 7 a yield of **4** (20%) that is close to that obtained at pH 5 (0.01 M acetate buffer, Figure 6) and a yield of **9a** (35%) that is higher than that observed at pH 5 (0.01 M acetate buffer) (25%) and higher than the yield of **4** at pH 7 (18%) (Figure 7).

In contrast to earlier reports on the reactions of **1**,[11,18] we did not observe the precipitation of "blue compound" at pH's near neutrality. This may be because the present experiments were performed on a smaller scale (2 mg/5 mL) than that used previously (8 mg/20 mL).[11]

Stability of 8-Iodoxanthine in the Presence of Iodide Ion. Under the conditions used for the reactions of **1**, 8-iodoxanthine afforded a small (10–14%) yield of **4**, which was little affected by the presence of iodide ion, by changes in the concentration of it, or by changes in pH (Table I). The consistent formation of small amounts of **4** was explained when it was found that 8-iodoxanthine is readily reduced to **4** photochemically (Φ = 0.1). Reaction of 8-iodoxanthine with complete light exclusion for all manipulations, including chromatography, reduced the yield of **4** to 3%. Chromatography of a reaction of **1** performed under identical conditions did not reveal the presence of any 8-iodoxanthine. Since the half-life of **1** is only a few minutes at pH 7,[11] **1** and 8-iodoxanthine were each allowed to react for only 30 min with light exclusion in the presence of 2 equiv of iodide. Chromatography of the two solutions showed that under these conditions **1** was completely reduced to **4**, while 8-iodoxanthine was recovered in 98% yield with only a trace (2%) of **4**. No 8-iodoxanthine was detectable from the reaction of **1**. These experiments demonstrate that 8-iodoxanthine is not an intermediate in the reduction of **1** to **4** in the presence of iodide ion.

Discussion

Changes in the pH over the range 0 to 3 exert little effect on the rate of reaction of **1** or on the composition of the reaction products from it.[11] The mechanism proposed for the 8-substitution reaction in this pH range (path a, Scheme I) involves ionization of **1** to acetate ion and the delocalized cation

6 and reaction with a nucleophile at C-8 in the π-rich imidazole ring[19] to yield an 8-substitution product (**9**). At pH's above 3 the effects of changes of pH on the reaction of **1** were found to be complex.[11] As the pH was increased the reaction rate increased, the yield of 3-hydroxyxanthine dropped, the yield of uric acid rose, and a third product, xanthine, was obtained and its yield also increased. Ionization of the imidazole proton, p$K \sim 6.5$,[14] to the anion (**2**) appears to initiate this "fast" reaction. It was proposed that **2** can eliminate acetate ion to yield **7** and that this species upon protonation affords the cation (**6**) that then leads to 8-substitution products. The sequence **2** → **7** → **6** → **9** was designated path b. Since the reduction product, xanthine (**4**), was only formed under the conditions associated with path b, it was deduced that **4** arose from an intermediate formed only on path b. It was suggested that **2** might undergo not only elimination to **7** but also homolysis to **3** and that **4** arose from the radical anion (**3**). This deduction was supported by two observations: that a radical induced photochemically in solid **5**, and deduced to have an amidyl structure comparable to **3**, is rapidly reduced to **4** when dissolved in water,[23] and that in the presence of iodide ion **1** is completely reduced to **4** with the formation of 0.5 equiv of I_2. The inability to detect radicals in solutions either of **1**[11] or of the radical from **5**[23] by ESR suggested that both were too reactive to be detected by this technique. The absence of detectable radicals from **1** with the more sensitive spin-trapping technique,[24] however, suggested that the redox reaction of **1** with iodide merited closer examination for other possible mechanisms.

We now report that the amount of reduction of **1** in the presence of iodide ion is quite pH dependent and is complete only at pH's above 5. As the pH is decreased below 6 in the presence of 2 equiv of iodide ion, there is a steep decline in the yield of **4** (Figure 2), but no 8-substitution product, including 8-iodoxanthine (**9**, Nu = I), is detectable at any pH. At pH's below 3 8-substitution occurs via path a and only the cation **6** is involved. Neither the anion **2** nor a radical derived from it, e.g., **3**, should be formed and in the absence of iodide ion **4** is not observed.[11] However, in the presence of 2 equiv of iodide ion, the amount of **1** normally reacting to form **9a** or **9b** in the pH range 1 to 3 (5%)[11] now yields **4** (Figure 2). The evidence that iodide reduced only the portion of **1** that normally reacts via **6** to afford an 8-substitution product in a pH region where the only intermediate from **1** appears to be **6** suggested that reduction by iodide ion proceeds via **6** or a product derived from it.

Several possible mechanisms have been considered to explain the redox reaction with iodide ion. Refluxing HI containing phosphonium iodide[25] or hypophosphorus acid[26] is reported to reduce 8-halopurines, including 8-chloroxanthine, **9b**. However, under experimental conditions in which ~5% of **1** is reduced at pH 1 **9b** is recovered unchanged. Under the same conditions 8-iodoxanthine did afford a small (10–14%) amount of **4** at pH 1 (Table I), but this arises by photochemical reduction; in the absence of light, the formation of **4** from 8-iodoxanthine is negligible. Under conditions where 8-iodoxanthine could be recovered essentially quantitatively, **1** was reduced to **4** by iodide ion and 8-iodoxanthine could not be detected in the reaction mixture. Thus 8-iodoxanthine is not an intermediate in the reduction of **1** to **4** in the presence of iodide ion. 3-Acetoxyxanthine is a cyclic *N,O*-diacylaromatic hydroxylamine and *O*-acylhydroxylamines are known to liberate I_2 from acidic iodide solution.[27] However, increases in the concentration of iodide beyond 2 equiv did not result in a greater extent of reduction of **1** (Figure 1), indicating that iodide is not reacting directly with **1**.[28] Iodide does not react with **5**[11] and in confirmation of this the yield of **5** from **1** was unaffected by the presence of iodide or by changes in its concentration. It is conceivable that iodide might reduce **6** by promoting spin inversion of the nitrenium ion of the resonance contributor **6a** to the triplet state, a process known to be catalyzed by heavy atoms,[29] and nitrenium triplets are reportedly rapidly reduced to the parent amine.[30] However, this process should be sensitive to changes in the concentration of the heavy atom and that was not observed in the reactions of **1** with iodide (Figure 1). In addition, that process should not induce oxidation of iodide. Thus catalyzed spin inverstion to the nitrenium triplet of **6a** is also excluded.

The proposal most consistent with all of the available data is that the reduction of **1** to **4** in the presence of iodide occurs by nucleophilic addition of iodide at the electron-deficient nitrogen of **6**, i.e., via the resonance contributor **6a**, to form the intermediate **8a** (Scheme I). This is then followed by nucleophilic displacement on **8a** by a second iodide ion to yield the anion of **4** and I_2. This mechanism is analogous to that proposed for the oxidation of iodide by *O*-acyl esters of hydroxylamine[31,32] and the oxidation of iodide by the *N*-iodoamide, **8a**, would correspond to a known reaction of *N*-haloamides.[33,34] The intermediacy of **6a** in the redox reaction with iodide would also be quite consistent with the reduction only of the amount of **1** that normally leads to **9** via **6b** at low pH's.

To test the hypothesis that the electron-deficient nitrogen of **6** is the intermediate in the oxidation of iodide the reaction of **1** with thiourea was examined, since thiourea shows a high reactivity with electron-deficient nitrogen centers.[35] If the nitrenium ion (**6a**) is the active redox intermediate, then thiourea should also produce an enhanced reduction of **6** to **4**. The reaction of **1** with 5 equiv of thiourea at pH 3 did not result in the complete reduction of **1** but instead gave a mixture of **5**, **4**, and 8-thiouric acid, **9c** (Figure 4). However, the formation of a significant amount of xanthine at pH 3, where this product is normally not formed, indicates that thiourea is reacting at least in part in a manner similar to that of iodide. Changes in the concentration of thiourea at pH 3 did not significantly alter the yields of the three products (Figure 3), which indicates that thiourea is not acting directly with **1**. Increases in the pH from 1 to 5 caused parallel increases in the yields of **4** and **9c** and a corresponding decrease in the yield of **5** (Figure 4). By pH 5 all of **1** reacted to yield either **4** or **9c** and further increases to pH 7 did not affect the yields of the products. Significantly, and in contrast to the results in the absence of thiourea (Figures 5, 6, and 7, 0.1 M buffer), the yield of the reduction product, **4**, is greater than that of the 8-substitution product, **9c**, at pH's near neutrality. These data indicate that thiourea, like iodide, can react with an intermediate from **1** and lead to enhanced reduction of **1**. Formation of an *S*-(3-xanthyl)isothiouronium intermediate, **8b**, comparable to the *S*-nitrosoisothiouronium intermediate proposed for the oxidation of thiourea by nitrite[36] and subsequent reaction of **8b** to **4** represent a plausible mechanism for the reduction of **6** to **4** by thiourea. These data thus agree with the conclusion that redox reactions occur via the nitrenium ion, **6a**, rather than via a radical intermediate.

Additional support for that conclusion was provided by studies on the effect of changes in the concentration of buffers at pH's 5.0 and 7.0 on the reaction products from **1** (Figures 6 and 7). At those pH's path b predominates.[11] At a single pH the concentration of **2** should not be significantly affected by changes in the concentration of buffer. If the anion **2** were the common intermediate from which the spontaneous reduction and the 8-substitution reactions of **1** diverge, via **3** and **7** (Scheme I) as previously proposed, then changes in the buffer concentration should have little or no effect on the yields of the products from the two reactions. This was observed over most of the concentration range with the weakly nucleophilic phosphate buffer (Figure 7). However, increases in the concentration of acetate buffer at pH 5.0 caused a dramatic increase in the yield of **9a** and a significant decrease in the yield

of 4 (Figure 6). These data indicate that acetate ion is acting as a nucleophile at C-8[41] and are incompatible with the interpretation that the anion **2** is the central intermediate from which the two reaction paths diverse.[42] It is apparent that acetate ion does not act as a common ion and suppress the 8-substitution reaction. Under those conditions the reaction rate should decrease as the concentration of acetate is increased. However, changes in acetate buffer concentration caused little change in the observed pseudo-first-order rate constant (Figure 6) except in extremely high concentrations of buffer, where it increased. These data are consistent with the conclusion[11] that on path b acetate ion is formed in the course of an elimination reaction, **2** → **7**, rather than through an ionization.

The reactions of 1 with iodide, thiourea, and acetate ion demonstrate that the relative proportion of 8-substitution and reduction products from 1 can vary considerably, depending upon the type of nucleophile added. These results appear explicable in terms of the "hard" and "soft" acid and base (HSAB) proposal of Pearson,[43,44] later extended by Klopman.[45] The present studies indicate that *both* electron-deficient sites of the delocalized cation **6** can react with nucleophiles. The evidence suggests that **6** is an ambident electrophile whose two reactive sites, represented by the nitrenium ion, **6a,** and the carbonium ion, **6b,** differ in their degree of polarizability and that the position and type of reaction with a nucleophile will be determined by the character of the approaching nucleophile. The high reactivity of **6** at N-3 with iodide, the "softest" base of the common anions, suggests that the electron-deficient nitrogen of **6a** must be a very "soft" acid. In agreement, thiourea, a slightly less "soft" base than iodide, reacts preferentially at the nitrenium ion of **6a,** as shown by the higher yield of **4** than of **9c** (Figures 3 and 4), but also reacts at the carbonium ion of **6b.** The preferential reaction of the "borderline" bases, azide, nitrite, and bromide, as well as the "hard" bases, water, amines, acetate, and chloride ions, with the "borderline" carbonium ion of **6b** to yield 8-substitution products[10] accords well with the HSAB interpretation. The available data on the reactions of 1 with nucleophiles indicate that reactions at the nitrenium ion of **6a** are frontier orbital controlled[45] "soft–soft" interactions that result in oxidation of the nucleophile with concomitant reduction of **6** to xanthine while charge controlled[45] interactions occur at the carbonium ion of **6b** and yield 8-substitution products. The single example to date of the reaction of a "soft" base at the C-8 position of **6b,** rather than at N-3 of **6a,** is the reaction of the "soft" thioether function of methionine with **6b** to afford 8-methylmercaptoxanthine, **9e.**[10] That evidence suggests that redox reactions at N-3 of **6a** may be better described in terms of frontier orbital control.[46]

Relationship to Oncogenesis. Although products have been isolated from nucleophilic substitution reactions at nitrenium ions derived from some *N,O*-diacylaromatic hydroxylamines, notably 8-(*N*-2-fluorenylacetamido)guanine from *N*-acetoxy-*N*-acetyl-2-aminofluorene,[48] no such products have been obtained from the cation, **6,** from **1.** The present studies are the first to indicate that reactions can occur at the nitrenium ion of cations from *N*-acetoxypurines. They also indicate that redox and nucleophilic substitution reactions are two manifestations of a single species derived from **1,** a conclusion that is further supported by studies on the reaction of 1,7-dimethylguanine 3-oxide with acetic anhydride.[49] These studies suggest that either mode of reactivity of esters of *N*-oxidized purines could be significant to the initiation of the oncogenic process.

The present work now makes it apparent why only certain[18] acetylated *N*-oxidized purines oxidize iodide ion. The data indicate that only those derivatives that can yield a nitrenium ion should oxidize iodide ion. Since neither the nonacetylated compounds[10] nor the corresponding *O*-acetyl derivatives themselves oxidize iodide, the oxidation of iodide ion represents a convenient method to detect the formation of a nitrenium ion. This technique should have applicability to studies on the mechanism of oncogenesis not only by purine *N*-oxides but also by other *N,O*-diacylaromatic hydroxylamines.

Experimental Section

General. UV spectra were determined with a Unicam SP800A recording spectrophotometer and NMR spectra with a Jeol 100 Hz spectrometer. The elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

The 3 M acetate and formate buffers were prepared by neutralizing solutions of glacial acetic acid (86.2 mL) and 98% formic acid (84.2 mL) with 1 N potassium hydroxide to the desired pH, 5.0 and 4.0, respectively, then diluting to yield 500 mL of the 3 M buffer. The 1.5 M phosphate buffer was prepared by neutralizing a solution of $NaH_2PO_4 \cdot H_2O$ (103.5 g) with 1 N KOH to pH 7.0 and diluting to 500 mL. Dilutions of the concentrated buffers afforded the lower concentrations of the buffers.

All reactions of 3-acetoxyxanthine were performed with $\sim 2 \times 10^{-3}$ M solutions. Weighed samples of 2.0 to 2.5 mg (8.5 to 10 μmol) were dissolved in 5.0 mL of the appropriate buffer in 50-mL round-bottom flasks. The reactions were allowed to proceed overnight with continuous stirring in the absence of light at room temperature, which remained near 20 °C with a maximum variation of ±2 °C. After the reactions were complete the solvent was removed under vacuum and the residue was redissolved in a minimum amount of water with a drop of NH_4OH. The mixtures were analyzed by ion-exchange chromatography with standardized Dowex 50 [H^+], X-8, 200–400 mesh, columns (9 × 120 mm) that had been cleaned by prior washing with 2 N HCl in 60% CH_3OH and water. Column eluates were monitored with an ISCO UA-2 UV analyzer with coordinated fraction collector.

Water eluted **9a–c,** 0.1 N HCl eluted **5,** while **4** required 1 N HCl. Molar quantities were calculated from the elution volumes and ε values: uric acid, **9a** (pH 2 to 4), λ_{max} 285 nm (ε 12 000);[7] 3-hydroxyxanthine, **5** (pH 2 to 4), λ_{max} 272 nm (ε 10 100);[7] 8-chloroxanthine, **9b** (pH 1), λ_{max} 273 nm (ε 11 900);[50] xanthine, **4** (pH 0), λ_{max} 260 nm (ε 9200); 8-thiouric acid, **9c** (pH 2), λ_{max} 303 nm (ε 18 200).[17] 8-Thiouric acid and 8-chloroxanthine were identified by comparison of their UV spectral values with those reported.[17,50] Thiourea was also eluted by water and in the presence of high concentrations of thiourea **9c** and thiourea were eluted together. To determine the yield of **9c,** the fractions containing the mixture were evaporated to dryness and were applied to a longer (9 × 250 mm) Dowex 50 [H^+] column on which **9c** was eluted by water just before thiourea.

All yields and recoveries are based on the initial weight of 1 used. Values in Figures 1, 2, and 4 are the result of single determinations. Those in Figures 3, 5, 6, and 7 are from triplicate determinations and the average values and maximum variations are shown. pH values for the buffered solutions showed only slight changes in most buffers during the course of the reactions. Final values were slightly lower (0.2 pH unit) in formate buffers. The only acetate buffer to show a change was 0.01 M, in which the pH decreased by 0.6 unit. Final pH values in phosphate buffers were 0.2 to 0.4 pH unit higher than the initial value. Iodide ion concentration in the experiments illustrated in Figure 2 was 4×10^{-3} M. The experiments illustrated in Figures 2 and 4 were performed in 0.1 M formate (pH 4), acetate (pH 5), succinate (pH 6), and phosphate (pH 7) buffers. For pH's 3 and below appropriate concentrations of HCl were used.

Pseudo-first-order rate constants in Figures 5 and 6 are for formation of the product mixture and were determined at 23 °C by monitoring the UV spectral changes of aliquots from 2×10^{-3} M solutions in 1-mm path-length cuvettes until the reactions were complete. The rate constants were calculated by a least-squares analysis from a plot of ln (OD_∞ − OD) at 290 nm vs. time. The plots were linear for at least 4 half-lives of the reaction, confirming that the reaction was pseudo-first-order at this wavelength. The major contributor to the UV spectrum of the product mixtues at 290 nm is uric acid; **5** is a minor contributor, while **4** exhibits little absorption at this wavelength.

3-Acetoxyxanthine Hydrochloride. A sample of 3-hydroxyxanthine, stirred overnight as a suspension in 0.01 N HCl, was collected and washed with water, acetone, and ether and then dried over P_2O_5 under vacuum. A portion of the dry, finely ground sample (1 g) was suspended in acetic acid (20 mL), acetic anhydride (20 mL), and

acetyl chloride (8 mL). The reaction mixture was stirred in a sealed flask at room temperature in the dark. Progress of the reaction was monitored by collecting a small sample of the precipitate, dissolving it in 0.05 N HCl, and chromatographing it quickly over a Dowex 50 [H^+], 200–400, mesh, column (9 × 100 mm) eluting with 0.05 N HCl;[14] **1** was eluted first, followed by **5**. After 1 week an additional 1 mL of acetyl chloride was added. The reaction was complete after 2 weeks. The precipitate was collected, washed with acetic acid and then with Et_2O, and then dried under vacuum over KOH: yield 1.14 g (78%); NMR (DMSO-d_6) δ 2.40 (s, 3, $COCH_3$), 8.048 (s, 1, CH), 11.604 (s, 1, NH). Anal. Calcd for $C_7H_6N_4O_4 \cdot HCl$: C, 34.09; H, 2.86; N, 22.72; Cl, 14.38. Found: C, 34.52; H, 2.90; N, 22.26; Cl, 14.24.

The extinction coefficient of **1** was determined by plotting the OD at 267 nm from a family of curves of a reacting solution of **1** at pH 4.0 against time and extrapolating to the initial OD value; λ_{max} (pH 4.0) 267 nm (ϵ 9200).

Chromatography of a weighted sample of **1**, as described previously, and calculation of the weight of **5** in the sample showed that the product contained, at most, 3% of **5**, part of which may be the result of hydrolysis of **1** during the chromatography.

8-Iodoxanthine. 8-Iodoguanine[51] was prepared from 8-iodoguanosine[16] by allowing a solution of the nucleoside in 50% aqueous CF_3CO_2H to be stirred overnight at room temperature, removing the solvents, and recrystallizing from H_2O. To a chilled solution of 8-iodoguanine (450 mg, 1.5 mmol) and 35 mL of CF_3CO_2H was added dropwise over a period of 90 min a solution of $NaNO_2$ (1 g) in 15 mL of water. After addition was complete the reaction was allowed to proceed at room temperature overnight. A silica gel GF TLC of the reaction mixture ($CHCl_3$–CH_3OH, 4:1) indicated the reaction was complete. The solvents were removed under vacuum and a solution of the residue in aqueous CH_3OH was applied to a 2 × 20 cm Dowex 50 [H^+], 200–400 mesh, column. Sixty percent aqueous CH_3OH eluted 8-iodoxanthine as well as a small amount of a second, unidentified product that was eluted just prior to the 8-iodoxanthine. The fractions containing 8-iodoxanthine were combined, the solvents were removed, and the sample was applied to the column and eluted as described. This procedure was repeated three times until all of the contaminant had been removed. The fractions containing 8-iodoxanthine from the final column were combined, the solvents were removed, and the product was recrystallized from CH_3OH/H_2O (C): yield 65 mg (15%); mp >300 °C, grad dec; pK_a's, determined from isosbestic spectra, 6.2 and 10.3; UV λ_{max} ($\epsilon \times 10^{-3}$) (pH 3.0) 215 (14.2), 278 (12.6), (pH 8.0) 213 (19.1), 280 (14.1) (pH 13.0), 245 sh (6.8), 289 (10.2) nm [lit.[15] (pH 1) 278 nm (pH 13), 288 nm]; NMR (DMSO-d_6) δ 11.59 (s, 1, NH), 10.85 (s, 1, NH), both exchangeable in D_2O; mass spectrum *m/e* (CI) 279 (M + 1). The analytical sample was dried over P_2O_5 at 120 °C for 3 h. Anal. Calcd for $C_5H_3N_4O_2I \cdot H_2O$: C, 20.29; H, 1.69; N, 18.93; I, 42.88. Found: C, 20.41; H, 1.55; N, 18.75; I, 42.79.

Photolysis of 8-Iodoxanthine. The quantum yield for photoreduction of 8-iodoxanthine was determined by potassium ferrioxalate actinometry[52] in a Rayonet photochemical reactor equipped with 300-nm lamps with a 1×10^{-3} M solution of 8-iodoxanthine in 60% aqueous CH_3OH; $\Phi = 0.1$. Xanthine formation was determined by column chromatography and its identity was conformed by its characteristic UV absorption spectra[53] at three pH's and by paper chromatography in two solvent systems.

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Registry No.—**1**, 22052-01-3; **1**·HCl, 64761-25-7; 8-iodoguanine, 19690-21-2; 8-iodoxanthine, 64761-27-9.

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$$I^- + H_2NOSO_3H \rightarrow INH_2 + HSO_4^- \quad (I)$$

$$INH_2 + HI \rightarrow NH_3 + I_2 \quad (II)$$

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