Comparative Reactivities of Esters of Oncogenic and Nononcogenic Purine N-Oxides and Evidence of the Oxidation-Reduction Reactivity of Aromatic Nitrenium Ions

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ABSTRACT

Studies on the relative reactivities of esters of oncogenic and nononcogenic members of the purine N-oxide series indicate that, despite similarities in rates of reaction with the solvent, electrophilic cations from oncogenic derivatives are 10- to 100-fold more reactive toward added nucleophiles in vitro than are cations from nononcogenic compounds. The studies provide strong confirmation of an earlier proposal that nitrenium ion contributors of delocalized aromatic cations from 3-acetylpyridines, rather than radical intermediates, are the agents responsible for the oxidizing reactivity of these esters. They demonstrate further that delocalized aromatic nitrenium ions are highly susceptible to reduction by common nucleophiles that are not usually associated with oxidation-reduction reactions. Examples of such behavior with “soft” bases and other oncogenic arylamines indicate the generality of this little recognized property of aromatic nitrenium ions.

INTRODUCTION

Ionization of esters of oncogenic arylhydroxylamines produces delocalized cations that show a high reactivity toward a number of electrophilic sites in vivo (15, 16). Their reaction with nucleic acids is widely regarded as an essential step in the initiation of oncogenesis by aromatic amines (15, 16). However, cations derived from esters of oncogenic (11, 28–31) purine N-oxides have shown little reactivity toward nucleic acids in vivo or in vitro, although they did exhibit some reactivity as alkylating agents in vivo toward a few amino acids (7, 14, 23, 24, 27). This encouraged fundamental studies on other modes of reactivity that might have potential relevance to the initiation of oncogenesis by these compounds.

Studies of the reactions of a purine N-oxide ester, 3-acetoxyxanthine (Chart 1), a model for the ester formed metabolically in vivo (23, 25), indicated that the chemistry of these esters was complex (5). At physiological pH’s, formation of the delocalized cation (6) associated with electrophilic substitution required the anion (5), involved several events (Chart 1, Path B), and was quite rapid. Direct ionization of the ester to the cation (6) was a slow process that was observed only under acidic conditions (Path A). Other types of reactions observed with 3-acetoxyxanthine included spontaneous reduction and an oxidation-reduction reaction with certain nucleophiles. The potential biological importance of the latter reaction was suggested by the evidence that mutagenesis by purine N-oxide esters appeared to resemble that produced by a radical oxidant (12).

Alkylation of the 7- or 9-nitrogen of 3-acetoxyxanthine prevented ionization of the imidazole proton and greatly reduced or abolished oncogenicity (7). This was understandable for the 7-alkyl derivatives, since alkylation would interfere with delocalization of the charge in the cation. The diminished oncogenicity of the 9-alkyl derivatives was not anticipated, since alkylation at that position should not interfere with delocalization of the charge in the cation (e.g., Chart 2, 11 and 12). In addition, 9-methyluric acid (15A) was observed in attempts to prepare 3-acetoxy-9-methylxanthine (10) (3). The presence of the 9-substituent would, however, prevent the formation of intermediates associated with Path B, which was the one implicated in the initiation of oncogenesis by the unsubstituted members of the series.

The 3-acetoxy-9-methyl esters (10 and 16B) thus provided a unique opportunity to study at pH’s near neutrality the reactions of delocalized cations comparable to 6 in the absence of intermediates from Path B. It was of interest to elucidate differences between the reactivities of esters of oncogenic and nononcogenic members of the series and in particular to examine the oxidation-reduction reactivity of nononcogenic compounds that react via delocalized aromatic cations possessing a nitrenium ion resonance contributor.

MATERIALS AND METHODS

Chemicals. 3-Hydroxyguanine and 3-hydroxy-9-methylguanine were prepared as described (2, 6). Acid hydrolysis of those afforded the corresponding 3-hydroxyxanthine derivatives (2, 6). Although the ester, 3-acetoxyxanthine (4) can be isolated (3, 32), attempts to prepare the acetoxy esters (70 or 76) have not been successful (3). Generation of the esters in situ (26), however, by the addition of small quantities of acetic anhydride to solutions of the parent purine N-oxides proved quite satisfactory. 8-Thioguanine was purchased from Alfred Bader, Division of Aldrich Chemical Co., and was precipitated from a hot ammoniacal solution with acetic acid and dried under vacuum over P2O5 for 3 hr at 120° prior to determining ε values, $\lambda_{\text{max}}$ ($\epsilon \times 10^3$): pH 1, 228 nm (11.4), 269 (16.1), 304 (16.3); pH 7, 243 (15.9), 303 (15.5); pH 13, 234 (19.1), 299 (17.1).

8-Mercapto-9-methylguanine (21D). 8-Bromo-9-methylguanine (9) (500 mg, 2 mmol) and 300 mg of thiouracil were dissolved in 50 ml of ethanol, and the solution was heated under reflux overnight. The solvent was removed under re-

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The filtrate was acidified with acetic acid and chilled to reduced pressure; the residue was dissolved in hot NH₄OH and (13.3), 270 (15.7), 303 (16.7); pH 7, 233 (13.0), 270 (15.5), (with gradual decomposition); Aₘₐₓ (e x 10⁻³): pH 1, 236 nm

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were dissolved in 100 ml of ethanol, and the solution was (150 mg, 0.6 mmol) and 100 mg of thiourea (1.2 mmol) were dissolved in 100 ml of ethanol, and the solution was heated under reflux overnight. Progress of the reaction was monitored by UV spectra of aliquots. When it was complete, the solution was applied to a 20-x 500-mm Dowex 50 (H⁺) column. Water eluted 8-thio-9-methyluric acid (1) which was reprecipitated as described above; the yield was 55 mg (42%); m.p. >252° (with gradual decomposition) UV Aₘₐₓ (e x 10⁻³): 15A, X=OH

Conditions for Reactions. Weighed samples of 10 μmol, approximately 2.0 to 2.5 mg, of the purine N-oxide were dissolved with heating in 5 ml of 0.5 M NaH₂PO₄·H₂O buffer (pH 7.0) containing 0.01 M (5 equivalents), 0.1 M (50 equivalents), or 1 M (500 equivalents) of thiourea or KI. 3-Hydroxyguanine required 10 ml of buffer to dissolve 2 mg; the ratio of nucleophile to reactant was retained by halving the concentration of nucleophile added. When the solutions were cool, 20 μl of acetic anhydride were added to solutions containing 2, 3-hydroxy-9-methylxanthine, and 3-hydroxy-9-methylguanine, and 50 μl were added to solutions containing 3-hydroxyxan-9ine. Reactions were stirred in the dark overnight and then evaporated under reduced pressure to approximately 2 ml and applied to columns of lengths described below containing Dowex 50 (H⁺)-X8, 200 to 400 mesh resin. Column eluates were monitored as described previously (32). Reactions containing no nucleophile or KI were applied to 9- x 150-mm columns; H₂O eluted iodide and the 8-oxo derivatives, 9A, 15A, 21A, or 27C; 1 N HCI eluted the reduction products 7, 13, or 19. Reactions containing thiourea were applied to 9- x 250-mm columns. Water eluted thiourea, the 8-oxo derivatives, 9A, 15A, 21A, or 21C, and then the 8-thio derivatives, 9B, 15B, or 21D, in that order with good resolution; 0.1 N HCl eluted 2, if present, and 21B; 1 n HCl eluted the xanthines, 1 and 13; and 2 n HCl was required to elute the guanines (19). In reactions containing 1 μl thiourea, the yields of 19B had to be determined in separate experiments using 9- x 150-mm columns. Products were identified by the position of elution on the columns, the acid strength required for elution, and by comparison of UV spectra of products in acid and in base with those of authentic samples, where possible, or reported values. Molar quantities were calculated from the elution volumes and from the e values that were reported (32) previously for 1, 2, 9A, and 9B. The e (λₘₐₓ) used for 15A was 12,000 (285 nm, pH 2) (21), and for 21C it was 8400 (250 nm, pH 2) (20). Values for the e of 15B, 21B, and 21D are reported in this paper. All yields and recoveries are based on the initial weight of the N-oxides used. Values in Tables 1 and 2 are the average of 2 or more reactions with maximum derivations indicated. Yields of triiodide formed were determined at 352 nm (e = 26,500) (26) following the addition of 50 μl of acetic anhydride to solutions of the purine N-oxides in 0.5 M phosphate buffer (pH 7.0) containing 1 μl KI.

Values for t¹/₂ in Chart 4 were determined from pseudo first-order rate constants. Those were determined at 23° by diluting 1.5 ml of a solution of 3-hydroxy-9-methylxanthine (4.492 mg/100 ml H₂O) or of 3-hydroxyxanthine, 2 (23.29 mg/100 ml H₂O), with 1.5 ml of buffer in a 1-cm cuvet and then adding 10 μl of acetic anhydride (500 equivalents) or 2 μl of a solution of 10% acetic anhydride in dioxane (10 equivalents) to the reaction mixture. Formation of the esters, 1 and 10, was immediate. Product formation from the esters was monitored by the in-

- 15A, X=OH
- B, X=SH

C₆H₇N₆OS

Calculated: C 36.11, H 3.57, N 35.07, S 15.87

Found: C 36.11, H 3.57, N 35.07, S 15.87

8-Thio-9-methyluric Acid (15B). 8-Bromo-9-methylxanthine (1) (150 mg, 0.6 mmol) and 100 mg of thiourea (1.2 mmol) were dissolved in 100 ml of ethanol, and the solution was heated under reflux overnight. Progress of the reaction was monitored by UV spectra of aliquots. When it was complete, the solution was applied to a 20-x 500-mm Dowex 50 (H⁺) column. Water eluted 8-thio-9-methyluric acid (1) which was precipitated as described above; the yield was 55 mg (42%); m.p. >252° (with gradual decomposition) UV λₘₐₓ (e x 10⁻³):

- 15A, X=OH
- B, X=SH

C₆H₇N₆OS

Calculated: C 36.54, H 3.58, N 35.51, S 16.26

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- 15A, X=OH
- B, X=SH

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- 15A, X=OH
- B, X=SH

C₆H₇N₆OS

Calculated: C 36.54, H 3.58, N 35.51, S 16.26

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Reactions of Aromatic Nitrenium Ions

### Table 1

Effect of iodide ion on the product composition from 3-acetoxypurines generated in situ

<table>
<thead>
<tr>
<th>3-Acloyxypurine Products</th>
<th>0 KI equivalents</th>
<th>5 KI equivalents</th>
<th>50 KI equivalents</th>
<th>500 KI equivalents</th>
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<tr>
<td>Uric acid</td>
<td>50 ± 5</td>
<td>53 ± 1</td>
<td>68 ± 8</td>
<td>90 ± 6</td>
</tr>
<tr>
<td>Xanthine</td>
<td>19 ± 1</td>
<td></td>
<td>68 ± 8</td>
<td>90 ± 6</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>53</td>
<td>68</td>
<td>90</td>
</tr>
<tr>
<td>9-Methyl-9-methyl-xanthine</td>
<td>68 ± 2</td>
<td>63 ± 1</td>
<td>57 ± 2</td>
<td>63 ± 5</td>
</tr>
<tr>
<td>9-Methylxanthine</td>
<td>5 ± 4</td>
<td>10 ± 1</td>
<td>19 ± 2</td>
<td>63 ± 5</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>73</td>
<td>76</td>
<td>63</td>
</tr>
<tr>
<td>8-Hydroxyguanine</td>
<td>24 ± 4</td>
<td></td>
<td></td>
<td>76 ± 9</td>
</tr>
<tr>
<td>Guanine</td>
<td>24 ± 7</td>
<td>66 ± 6</td>
<td>78 ± 6</td>
<td>76 ± 6</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>66</td>
<td>78</td>
<td>76</td>
</tr>
<tr>
<td>9-Methyl-8-hydroxyguanine</td>
<td>55 ± 4</td>
<td>50 ± 5</td>
<td>43 ± 1</td>
<td>2</td>
</tr>
<tr>
<td>9-Methylguanine</td>
<td></td>
<td></td>
<td>20 ± 4</td>
<td>80 ± 1</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>50</td>
<td>63</td>
<td>82</td>
</tr>
</tbody>
</table>

*A* Acetic anhydride (20 μ) added to 10 μmol of compound dissolved in 5 ml of 0.5 M phosphate buffer (pH 7.0) containing 0.01, 0.1, or 1 mM KI.

*b* Mean ± S.D.

### Table 2

Effect of thiourea on the product composition from 3-acetoxypurines generated in situ

<table>
<thead>
<tr>
<th>3-Acloyxypurine Products</th>
<th>0 thiourea equivalents</th>
<th>5 thiourea equivalents</th>
<th>50 thiourea equivalents</th>
<th>500 thiourea equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid</td>
<td>50 ± 5</td>
<td>8 ± 1</td>
<td>16 ± 5</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>8-Thioric acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Hydroxyxanthine</td>
<td>19 ± 1</td>
<td>56 ± 1</td>
<td>55 ± 5</td>
<td>52 ± 3</td>
</tr>
<tr>
<td>Xanthine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>66</td>
<td>74</td>
<td>70</td>
</tr>
<tr>
<td>9-Methyl-9-methyl-xanthine</td>
<td>68 ± 2</td>
<td>63 ± 2</td>
<td>68 ± 3</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>9-Methyl-8-thioric acid</td>
<td>5 ± 4</td>
<td>6 ± 1</td>
<td>9 ± 1</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Xanthine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>69</td>
<td>77</td>
<td>71</td>
</tr>
<tr>
<td>8-Hydroxyguanine</td>
<td>24 ± 4</td>
<td>3 ± 1</td>
<td>10 ± 1</td>
<td>67 ± 1</td>
</tr>
<tr>
<td>Guanine</td>
<td>24 ± 7</td>
<td>63 ± 1</td>
<td>70 ± 6</td>
<td>67 ± 1</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>66</td>
<td>70</td>
<td>77</td>
</tr>
<tr>
<td>9-Methyl-8-hydroxyguanine</td>
<td>55 ± 4</td>
<td>50 ± 5</td>
<td>44 ± 2</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>9-Methyl-8-mercaptopurine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-Methylguanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>50</td>
<td>44</td>
<td>49</td>
</tr>
</tbody>
</table>

*a* Acetic anhydride (20 μ) added to 10 μmol of compound dissolved in 5 ml of 0.5 M phosphate buffer (pH 7.0) containing 0.005, 0.05, or 0.5 M thiourea.

*b* Mean ± S.D.

### RESULTS

The spontaneous reaction of esters of the oncogenic purine N-oxides, 3-hydroxyxanthine, 2 (Chart 1), and guanine 3-oxide
at pH 7 in the absence of nucleophile (Tables 1 and 2) included products arising by 8-substitution with the solvent, i.e., uric acid (9A), or 8-hydroxyguanine (21A) (Chart 3), as well as by spontaneous reduction, i.e., xanthine and guanine (19A). The product composition from 1 generated in situ is comparable to that formed from a sample of synthetic 7 at pH 7. The extent of spontaneous reduction of both esters was comparable and about 20 to 25%. The extent of 8-substitution was much higher for 3-acetoxyxanthine (45%) than for 3-acetoxyguanine (24%). The esters (4 and 16A) of both oncogenic purine N-oxides proved to be highly susceptible to the addition of the "soft" bases, iodide, and thiourea. In the presence of 5 equivalents of iodide (Table 1), no substitution occurred with either while the yield of the reduction products was significantly enhanced and became comparable to the sum of both types of products formed in the absence of added nucleophiles. Further increases in the concentration of iodide ion (Table 1) to 500 equivalents increased the overall recoveries. No 8-iodo derivatives were detected at any concentration of iodide.

In the presence of 5 equivalents of thiourea (Table 2), the yield of the reduction product, xanthine, from 4 was greatly increased. The extent of the formation of uric acid (9A) was almost correspondingly decreased. No 8-thiouric acid (9B) (Chart 1), was formed. This was unexpected because at pH 7 and with 5 equivalents of thiourea, synthetic 4 affords 8-thiouric acid in ~30% yield (32). Further increases in the concentration of thiourea produced 8-thiouric acid in small amounts, 10 to 20% but did not affect the yield of xanthine or the overall recovery. In the presence of thiourea, the reactivity of 3-acetoxyxanthine, (Table 2, 16A) was comparable to that of the ester in the presence of iodide (Table 1), except in 1 M thiourea, where a small amount (10%) of 8-mercaptopurine (21B) was formed.

3-Acetoxy-9-methylxanthine (Chart 2, 10) in the absence of nucleophiles afforded a high yield of the 8-substitution product, 9-methyluric acid (19A) and a lower yield of the reduction product, 9-methylxanthine (Table 1, 13). There was little effect on the product composition with lower concentrations of iodide or thiourea (Tables 1 and 2). Only in the presence of 500 equivalents of iodide was 8-substitution eliminated and reduced the sole observable process. With 500 equivalents of thiourea, reduction to 9-methylxanthine (13) was enhanced only slightly, and some 8-substitution with thiourea to 9-methyl-8-thiouric acid (15B) was observed (10%).

3-Acetoxy-9-methylguanine (Chart 3, 16B) underwent no spontaneous reduction but did afford 8-hydroxy-9-methylguanine (21C) (Table 1). Five equivalents of iodide did not affect the product composition from 16B, but 50 equivalents decreased the extent of 8-substitution and afforded the reduction product, 9-methylguanine (19B) (20%). With 500 equivalents of iodide, little 8-substitution occurred (2% of 21C), and the primary reaction was reduction to 19B. Reduction of the esters (10 and 16B) in the presence of 1 M KI was accompanied by the formation of triiodide ion (I$_3^-$) in yields of 40 ± (S.D.) 3% for each of the 3 derivatives.

The presence of 5 or 50 equivalents of thiourea did not substantially change the extent of 8-substitution of 16B to 8-hydroxy-9-methylguanine (21C) (Table 2). The addition of 500 equivalents of thiourea reduced 8-substitution to 21C, gave a small amount (5%) of 8-mercaptopurine (21D), and gave a 20% yield of the reduction product, 9-methylguanine (19B).

The rate of reaction of synthetic 3-acetoxyxanthine (4), as well as the product composition from it, are highly dependent on the pH of the medium (5). The rate of reaction and product composition (Table 3) of 3-acetoxyxanthine generated in situ demonstrated a similar pH dependence (Chart 4). In contrast, the rate of reaction of 3-acetoxy-9-methylxanthine (10) that was generated in situ as well as the product composition from it (Table 3) were nearly independent of pH over the range of 1.5 to 7. These data indicate that, unlike 3-acetoxyxanthine, the 9-methyl derivative (10) reacts by the same mechanism over that entire pH range.

**DISCUSSION**

Earlier studies (5, 26, 32–34) on the reactions of 3-acetoxyxanthine, (Chart 1, 4) noted not only the facile reactivity of 4 with nucleophiles but also that a portion of it would undergo

<table>
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<tr>
<th>3-Acetoxypurine</th>
<th>Product</th>
<th>Product yield at pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Acetoxyxanthine (1)</td>
<td>9-Methyluric acid</td>
<td>95%</td>
</tr>
<tr>
<td>3-Acetoxy-9-methylxanthine (10)</td>
<td>9-Methylxanthine</td>
<td>5%</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>Product</th>
<th>1.5</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid (9A)</td>
<td>20</td>
<td>46</td>
<td>56</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Xanthine (4)</td>
<td>10</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Hydroxyxanthine (5)</td>
<td>76</td>
<td>38</td>
<td>20</td>
<td></td>
<td></td>
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<tr>
<td>Total recovery</td>
<td>96</td>
<td>94</td>
<td>89</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

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![Chart 3. Reaction pathways of 3-acetoxy-9-methylxanthine.](chart3.png)
spontaneous reduction in solution to xanthine (1). This reduction could be enhanced by the addition of iodide ion with the concomitant oxidation of xanthine to iodide. It was suggested (5) that the 2 reactions, spontaneous reduction and oxidation-reduction with iodide, might proceed via a common intermediate, and the radical (Chart 1, 3) was proposed. However, subsequent efforts to substantiate that proposal found no evidence for a radical intermediate from 4 (17, 32). A recent study of the oxidation-reduction reaction between 3-acycloxyxanthine and iodide ion (32) considered a number of possible alternative mechanisms, including direct reduction of the ester (4), reduction of undetected 8-iodoxanthine, and reduction via heavy atom-induced spin inversion to the nitrenium triplet of 6A and reduction of that. The results were not consistent with any of those mechanisms but instead implicated the electron-deficient nitrogen of the cation (6) as the oxidizing agent.

Delocalized aromatic nitrenium-carbocation ions from arylamine oncogens, such as N-acetoxy-AAF, can form covalent adducts with nucleophiles at either electron-deficient center (15, 16). For example, guanine and derivatives of it react at the 8-position with the nitrenium ion of N-acetoxy-AAF, whereas methionine forms adducts at a carbonium ion (15, 16). However, only C-substitution products have been isolated from esters of oncogenic purine N-oxides (4, 7, 14, 23, 24, 27, 33), and little consideration was given to the possible reactivity of the electron-deficient nitrogen center of the cations arising from them.

We recently observed (18, 32) that nitrenium ions from N-acyloxypurines display all of the properties of extremely "soft" acids in the Pearson hard and soft acid and base classification (19). A unifying mechanism was proposed (32) in which competitive oxidation-reduction and C-substitution reactions could occur from the cation (6) depending upon which electron-deficient site of the cation reacted with a nucleophile. By that interpretation, "soft" bases, such as iodide, would be expected (19) to react selectively at N-3 of 6A. Those adducts (8) were not stable but reacted further to afford the parent purine and the oxidized nucleophile. The lack of formation of 8-iodoxanthine was then apparent because only "hard" bases, such as water, amines, or chlorine ion should react at the "harder" acid site of 6, the carbonium ion at C-8, to yield stable C-substitution products. Nucleophiles of intermediate "soft" base character, e.g., thiourea, appeared to react at both positions and afford enhanced yields of xanthine (1) and a nucleophilic substitution product, 8-thiouric acid (9B).

Because the cation (6) could be obtained from 4 free of other possible intermediates only at pH’s below 3, it was desirable to examine the reactions of N-acyloxypurines that produced uncontaminated cations at pH’s near neutrality. The present studies demonstrate that the 3-acycloxy-9-methylpurines (Charts 2 and 3, 10 and 16B) undergo facile substitution at the 8-position with the "hard" base water to afford the 8-oxo derivatives, 9-methyluric acid (Chart 2, 15A), or 8-hydroxy-9-methyluric acid (Chart 3, 21C). Since the esters cannot ionize to produce intermediates via Path B, the cations, (Chart 2, 11 and 12 and Chart 3, 17 and 18), formed by direct ionization, should be the sole intermediates from them. In confirmation of that conclusion, 3-acetoxy-9-methylxanthine (10), unlike 4, reacted with the solvent at the same rate (Chart 1) and afforded essentially the same product composition over the pH range 1.5 to 7 (Table 3).

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The 8-substitution reaction between the 9-methyl esters (10 and 16B) with the solvent to afford 9-methyluric acid (15A) and 8-hydroxy-9-methyluric acid (21C), however, could be diverted completely by the presence of 1 m iodide to yield 9-methylxanthine (13) and 9-methylguanine (19B). That this reduction is part of an oxidation-reduction reaction was demonstrated by the concomitant formation of the oxidized nucleophile, iodine. Similarly, thiourea diverted the reaction of the esters from one with the solvent to a combination of oxidation-reduction, affording the parent purines (13 and 19B) and C-substitution to form 9-methyl-8-thiouric acid (15B) and 8-mercaptopo-9-methylguanine (21D). Reduction of the 9-methyl esters with iodide and thiourea would be consistent with the initial formation of an unstable intermediate (Chart 2, 14, or Chart 3, 20B);

\[
\begin{align*}
\text{NH}_2 & \\
Y = \text{I or S—C} & + \text{ } \\
\text{NH}_2 & 
\end{align*}
\]

which then reacts to afford the oxidized nucleophile, iodine in the case of iodide, and the reduction product, the 9-methylpurines (13 or 19B). 8-Substitution occurs with "hard" and "intermediate" bases via the carbonium ions, (Chart 2) 12 and (Chart 3) 18B. As observed in the earlier study, iodide, which is one of the "softest" bases, showed no tendency to react at the "harder" carbonium ion site to afford 8-iodopurines. This would be entirely consistent (19) with selective reaction of the "soft" base iodide with the "soft" acid nitrenium ion contributor in the delocalized cations, 11–12 and 17–18. Thiourea, a base slightly less "soft" than iodide was nearly as effective as iodide in affecting the reaction course of the unhindered esters, 3-acetoxyxanthine (4- and 3-acetoxyguanine (16A) but was much less effective than iodide in diverting the course of the hindered 9-methyl esters (10 and 16B). The higher yields of xanthine (1) and guanine (19A) than of 8-thiouric acid (9B) and 8-mercaptopuaguanine (21B) from 3-acetoxyxanthine and 3-acetoxyguanine, respectively (Table 2), indicate that the "soft" base thiourea reacts preferentially, although not exclusively, at the nitrenium site in the cations (6A and 17). Thus, the present studies with the 9-methyl esters (10 and 16B) provide strong substantiation for the earlier proposal that aromatic nitrenium ions undergo competitive substitution and oxidation-reduction reactions (Tables 1 and 2).

The esters of the nononcogenic 3-hydroxy-9-methylpurines required much higher concentrations of nucleophile to divert their course of reaction than did the esters of oncogenic members of the series. A possible explanation for the poorer reactivity of 10 and 16B with iodide ion could conceivably be steric interference to the approach by the bulky iodide ion at the electron-deficient nitrogen in 11 and 17B by the 9-methyl group. However, thiourea can react at either the sterically hindered electron-deficient nitrogens or the relatively unhindered electron-deficient carbons of the cations 11–12, 17–18, and it too required high levels to alter the course of reaction of 10 and 16B. The "soft" base thiourea reacted noticeably more at the "softer" acid site of the cation (17B), even though that is the more sterically hindered position. Thus, the energy levels of the reacting orbitals can regulate the site of reaction and thus define the reaction type, even in the presence of a steric constraint.
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The high rate of reactivity (Chart 4) of the 9-methyl esters (10 and 16B) was not expected. The reaction of 3-acetoxyxanthine (4) via Path A is quite slow (t_{1/2} = 120 min\(^{-1}\)) (5) and a comparable rate of reaction might have been expected for 16B. However, at pH 7, the rates of reaction of 3-acetoxyxanthine and of the 9-methyl homolog with the solvent differed little. The reaction of the former via Path B was slightly faster (t_{1/2} = 0.66 min\(^{-1}\)) than that of 3-acetoxy-9-methylxanthine (10) (t_{1/2} = 1.14 min\(^{-1}\)) reacting via the equivalent of Path A for 3-acetoxyxanthine. The high rate of reactivity of 3-acetoxy-9-methylxanthine by a process that is slow for 3-acetoxyxanthine suggests that there is a driving force present in the 9-methyl esters that is not present in 3-acetoxyxanthine. This force is undoubtedly a steric strain between the 9-methyl and 3-acetoxy groups which can be relieved by ionization to acetate ion and cation (11–12).

The present studies suggest that the lack of oncogenicity of the 9-methylpurine 3-oxides is probably related to the tendency of the cations derived from their esters to react preferentially with the solvent even in the presence of strong nucleophiles. Ester formation was not found to be a limiting condition for the reactivity of these oncogenic intermediates with nucleophiles. This property must be taken into consideration in future studies on the mechanism of oncogenesis by arylamines. Studies on the possible contribution of this reactivity to the mechanism of action of reducing agents that exhibit protective activity against tumorigenesis are now in progress.

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### REFERENCES


4 The results in these studies parallel those on the reactivity of oncogenic N-acetoxy-2-AAF and the nononcogenic 4-isomer (35). Under identical conditions, N-acetoxy-4-AAF reacted with all cellular nucleophiles that N-acetoxy-2-AAF did. In that case, the reactivity of the nononcogenic 2-isomer exceeded that of the 4-isomer by a factor of 10, but the nononcogenic N-acetoxy-4-AAF showed a greater tendency to react with noncritical nucleophiles, such as water and chloride ion, than did the oncogenic 2-derivative.
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Comparative Reactivities of Esters of Oncogenic and Nononcogenic Purine N-Oxides and Evidence of the Oxidation-Reduction Reactivity of Aromatic Nitrenium Ions

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