(1968).
(10) One referee has called in question of the conversion of **2a** to **5** since, he believes, **2a** should rapidly react to give an *N*-diazonium hydroxide. Whatever intermediate may have formed (*N*-diazonium hydroxide or *N*-nitroso compound), the thermodynamic product obtained by us must be **5** on the basis of the combustion data, UV, CD (the positive Cotton effect), NMR, and mass spectral data (abudant M^+ ion etc.). The guanidin type structure seems to particularly stabilize the $C_{5'}$–N–C_8 bond of our compounds, in contrast to the oxidation or diazotization reactions of many *N*-amino alicyclic amines.
(11) The crystallization process was hampered by strong solvation with protic solvents to form a gelatine.
(12) Some spectral comparisons between **9** and **10: 9** absorbs at 256 (ε 21 400) and 279 nm (ε 13 400, sh), while **10** absorbs at 260 (ε 19 100) and 283 nm (ε 10 600, sh) (see Experimental Section). The ^{1}H NMR spectrum of **9** indicated the resonance of the lactam NH at 12.21 ppm and that of the phthaloyl group at 7.93 ppm (overlayed on the H_2 signal) as a sharp singlet (accidentally conditioned by steric and electronic factors in the nucleoside molecule). On the other hand, the spectrum of **10** exhibited the phthaloyl resonance at 7.76–8.17 ppm as a complex multiplet and no lactam resonances for both inosine base and phthalazin-1,4-dione under the same measurement conditions (60 MHz, Me_2SO-d_6). This latter finding seems to suggest complex formation by hydrogen bonding between the molecules.
(13) G. A. Ivanovics, R. J. Rousseau, M. Kawana, P. C. Strivastava, and R. K. Robins, *J. Org. Chem.*, **39**, 3651 (1974).
(14) This is also the case with adenine or adenine *N*-oxide base. For example, see M. A. Stevens and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2759 (1958).
(15) The CD spectra were recorded by the Japan Spectroscopic Co., Ltd., using a JASCO Model J-20 recording spectropolarimeter, for which we are grateful.
(16) The mass spectra were measured using a JMS-OISG2 mass spectrometer in the laboratory of the Daiichi Pharmaceutical Co., Ltd., to whom we are grateful. The ionization potential was 75 eV and ionizing current 200 μA for all the compounds; temperature 205 °C for **4a** and 230 °C for **4b** and **6**. The direct insertion probe was used.
(17) M. Ikeda, Y. Tamura, and M. Ikehara, *J. Heterocycl. Chem.*, **7**, 1377 (1970).
(18) E. G. Lovett and D. Lipkin, *J. Am. Chem. Soc.*, **95**, 2312 (1973).
(19) S. Tsuboyama and J. A. McCloskey, *J. Org. Chem.*, **37**, 166 (1972).
(20) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds", Holden-Day, San Francisco, Calif., 1967, pp 297, 382.
(21) For an example of intramolecular N^3,3′-cyclization see: (a) E. J. Reist, V. J. Bartuska, D. F. Calkins, and L. Goodman, *J. Org. Chem.*, **30**, 3401 (1965); (b) A. P. Matinez, W. W. Lee, and L. Goodman, *ibid.*, **31**, 3263 (1966); (c) E. J. Reist, D. F. Calkins, and L. Goodman, *ibid.*, **32**, 2538 (1967).

Purine *N*-Oxides. 67. Redox and Rearrangement Reactions of 1,7-Dimethylguanine 3-Oxide with Anhydrides[1]

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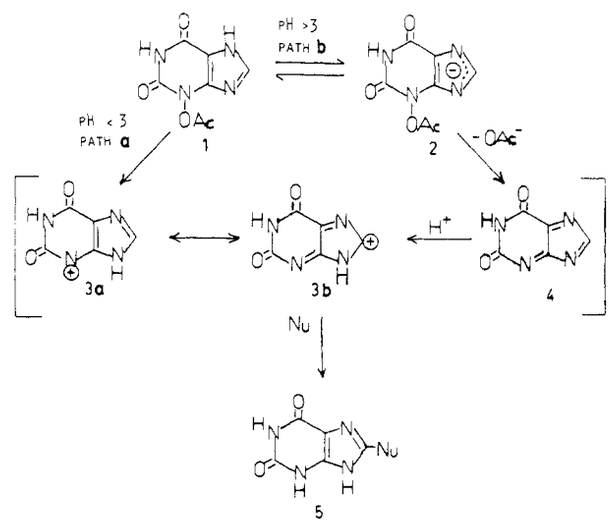
Acetylation of 1,7-dimethylguanine 3-oxide in aqueous or methanolic solution produces an intermediate that undergoes an extremely rapid intermolecular reaction with the solvent under ambient conditions to yield 8-substitution products of 1,7-dimethylguanine. This reaction occurs despite the presence of an alkyl group at N-7 that prevents delocalization of a positive charge generated at N-3 to the C-8 position. Added nucleophiles, even at high concentrations, do not react to yield 8-substitution products. Iodide and bromide ions undergo a redox reaction with the intermediate to afford 1,7-dimethylguanine and iodine or bromine. The extent of 8 substitution with water and the reduction by bromide ion are inversely affected by variations in the concentrations of bromide ion, indicating that the two reactions are competitive and proceed from a single intermediate. A delocalized nitrenium ion is proposed as the common intermediate. Accompanying the 8-substitution reaction is a competitive, slower reaction that results in loss of UV absorption. This reaction can be enhanced at the expense of the 8-substitution reaction by the use of trifluoroacetic anhydride. Oncogenicity assays in rats show that 1,7-dimethylguanine 3-oxide does not induce tumors.

A number of *O*-acyl esters of purine 3-oxides[2-6] undergo a spontaneous N-3 elimination–C-8 substitution reaction that parallels those observed with some oncogenic *N,O*-diacyl aromatic hydroxylamines.[7-12] As part of studies to elucidate the mechanism of tumor induction by N-oxidized purines, the reactions of one ester, 3-acetoxyxanthine (**1**, Scheme I), were examined in detail. Those studies[13] indicated that the 8-substitution reaction of **1** can proceed by either of two routes (Scheme I) depending upon the pH of the medium. A relatively slow S_N1' reaction (path a) is observed in the pH range 0 to 3, while the faster path b, requiring ionization of the imidazole proton, predominates at pH's above 3. Interference with delocalization of the positive charge in the common intermediate **3** by a substituent at N-7 was found to inhibit the 8-substitution reaction by both routes.[3,13] A second spontaneous reaction of **1**, reduction to xanthine, was observed only in conjunction with the 8-substitution reaction via path b. The presence of iodide ion greatly enhanced the reduction of **1**, and the enhanced reduction was accompanied by oxidation of iodide to iodine. It was suggested[13] that the redox reaction with iodide ion was correlated with the spontaneous reduction of **1** and proceeded via the same intermediate. A radical anion, presumed to form by homolysis of the N–O bond of **2**, was suggested as the common intermediate. However, recent evidence[14] indicates that oxidation of iodide can also occur in conjunction with path a and thus cannot proceed solely via a radical from **2**.

Scheme I



One member of the purine *N*-oxide series, 1,7-dimethylguanine 3-oxide (**6**, Scheme II), appeared to react anoma-

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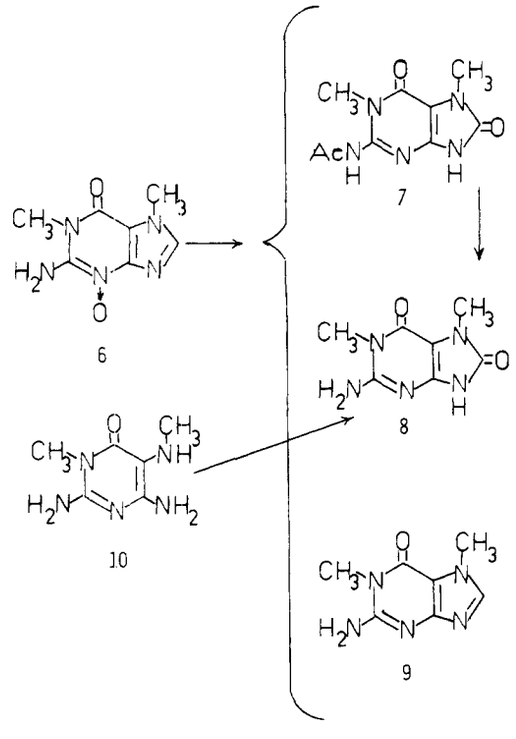
Table I. Reaction Parameters for the Rearrangement of 1,7-Dimethylguanine 3-Oxide with Acetic Anhydride

pH	7, %	9, %	Recovery, %	λ_{max},[a] nm	Apparent pseudo-first-order rate constant, min^{-1}	
					k_1	k_2
2				265		
3	21		21	265	2×10^{-1}	
4	18		18	242	4×10^{-2}	6×10^{-2}
5	50	5	55	244	2×10^{-2}	5×10^{-4}
6				242	8×10^{-2}	3×10^{-4}
7	45	6	51	260	6×10^{-2}	7×10^{-3}
8				255	3×10^{-2}	3×10^{-4}

[a] Absorption maximum of the intermediate immediately after addition of acetic anhydride.

Scheme II



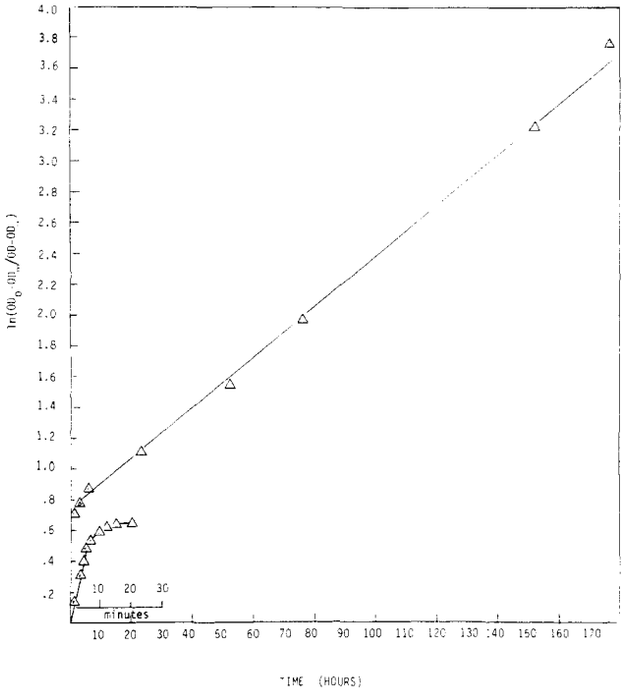


Figure 1. Pseudo-first-order plot for the reaction of 1,7-dimethylguanine 3-oxide with acetic anhydride at pH 6.

lously. Formation of its ester in situ was reported to generate a species that could oxidize iodide ion readily, but it did not yield an 8-substitution product with other nucleophiles.[5] Since a 3-*O*-acyl derivative of **6** could not ionize in the imidazole ring to afford a radical anion comparable to that proposed as an intermediate in the redox reaction of **1**, it appeared that a study of the reactions of acylated **6** would be uniquely helpful in delineating the mechanism of the redox reaction of esters of purine *N*-oxides. We now report that in the presence of acetic anhydride, **6**, although possessing a substituent at N-7, nevertheless reacts very rapidly under ambient conditions with water or with methanol to afford 8-substitution products. We present proof of structures of the products of the reaction, data on the mechanism, and evidence on the redox properties of the reaction intermediate.

Results

The addition of acetic anhydride to a solution of **6** yielded 2-*N*-acetyl-1,7-dimethyl-8-oxo-9*H*-guanine (**7**; 29%) (Scheme II), 1,7-dimethyl-8-oxo-9*H*-guanine (**8**; 2.5%), a small amount of 1,7-dimethylguanine (**9**), and traces of a fourth, unidentified, product. The assignments of **7** and **8** were confirmed by independent synthesis of **8** from 2,6-diamino-3-methyl-5-methylamino-4-oxopyrimidine (**10**). To examine the kinetics of the reaction, the UV spectral changes of the reaction of **6** in buffered solutions at pH's 2–8 were monitored. Upon addition of acetic anhydride to each of the solutions, there was an immediate change in the UV spectrum to that of an intermediate, the absorption maximum of which varied with pH (Table I). At pH 2 only reversion to the spectrum of **6** was observed. At pH's above 2 there was a gradual change in the spectrum to that of **7**, but with significant loss (~50%) of optical density. Treatment of the spectral changes as a pseudo-first-order decomposition gave plots that were linear only for the first few minutes of reaction. This was followed by a period of nonlinear change, and then at pH's 4–8 the change became linear again and remained so for the rest of the reaction (Figure 1). Apparent pseudo-first-order rate constants were calculated for the two linear portions of the plots (Table I). When the course of the reaction at pH 7 was monitored chromatographically, the reaction of **6** to **7** was found to be nearly complete within the first few minutes of reaction. No reaction was observed between **7** and acetic anhydride under the conditions for rearrangement, but **8** reacted to yield an unstable acetyl derivative with UV absorption different from that of **7**. It quickly reverted to **8** with no loss of optical density. Treatment of the spectral changes for that reaction, as a pseudo-first-order reaction, gave good linear plots for the entire course of the reaction and apparent pseudo-first-order

Table II. NMR Spectral Parameters (δ) during the Reaction of 1,7-Dimethylguanine 3-Oxide (6) with Anhydrides

Time	C-8		NCH_3		NCH_3					
T_0[a]	8.04		4.00		3.54					
30 s[b]	↓8.07[c]	4.09[d]	↓4.00	3.66[d]	↑3.57[c]		3.42[d]			
3 min[b]	↓8.09	↓4.09	↓4.03	↓3.66	↑3.57		3.43		3.12[d]	
10 min[b]					↑3.54			3.15[d]	↑3.11	2.95[d]
4 h[b]					3.55			↑3.16	3.13	2.95
5 days[b]					3.55	3.46[d]	3.41[d]		3.12	
7[a]			3.55		3.55					
8[a]			3.32		3.27					

Time	C-8				NCH_3			NCH_3		
T_0[e]	7.99				3.88			3.37		
10 min[f]		4.09	4.03	3.98		3.43	3.39		3.33	
7[g]					3.40			3.37		
8[g]					3.30			3.25		

[a] Spectrum taken in D_2O containing 2 drops of CD_3CO_2D with DSS as a reference. [b] Reaction of 5 mg of 7 in D_2O containing 2 drops of CD_3CO_2D and 20 μL of acetic-d_6 anhydride with DSS as a reference. [c] Increase (↑) or decrease (↓) in relative intensity of the band from previous reading. [d] New band. [e] Spectrum taken in Me_2SO-d_6 with Me_4Si as a reference. [f] Reaction of 5 mg of 7 in Me_2SO-d_6 with 50 μL of $(CF_3CO_2)_2O$ with Me_4Si as a reference. [g] Spectrum taken in Me_2SO-d_6 after addition of 50 μL of $(CF_3CO_2)_2O$ with Me_4Si as a reference.

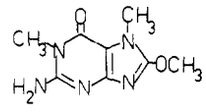
rate constants of 0.15, 0.2, and 0.3 min^{-1} for pH's 2, 5, and 8, respectively.

The kinetic data indicate that **6** undergoes two competing reactions with acetic anhydride: (a) a very rapid ($k \sim 10^{-2}$ min^{-1}) rearrangement to **7** (and **8**), and (b) a slower reaction ($k \sim 10^{-4}$ min^{-1}) that leads to the decomposition of **6** to non-UV-absorbing compounds. These conclusions were supported by an NMR study of the reaction of **6** with acetic-d_6 anhydride in D_2O containing CD_3CO_2D (pD ~ 5). Addition of acetic-d_6 anhydride to a solution of **6** in D_2O caused a rapid decrease in the signal intensities of the C-8 hydrogen and one of the NCH_3 groups of **6** (Table II). The signal of the second NCH_3 group of **6** increased in intensity since it appeared at the same position as the two coincident NCH_3 signals of **7**. Three signals at δ 4.01, 3.66, and 3.42 appeared briefly, but they were absent by 10 min. These bands are probably attributable to *N*-methyl groups of acetylated intermediates in the rearrangement of **6** to **7**. Paralleling the slower changes in UV absorption, changes in several bands were apparent for several days in the NMR spectra; these must reflect changes in the NCH_3 bands of intermediates associated with the slow reaction leading to loss of UV absorption.

The reaction of **6** with $(CF_3CO)_2O$ did not yield **7** or **8**, but instead induced an extremely rapid reaction that destroyed the purine chromophore. When the reaction of **6** with $(CF_3CO)_2$ in Me_2SO-d_6 was followed by NMR spectroscopy, the reaction was complete in 10 min (Table II) and two groups of signals were present. Little absorbance due to the NCH_3 groups of **7** or **8** was evident.

Under the conditions for rearrangement of **6**, the structurally related compounds 1,7-dimethyl-3-hydroxyxanthine,[15] 3-hydroxy-7-methylguanine,[15] and 2-amino-6-methoxypurine 3-oxide[15] failed to yield 8-substitution products. The 3-acetoxy-7-methylguanine (λ_{max} 267 nm) generated in situ did afford a second product (λ_{max} 282 nm), but this slowly hydrolyzed to 3-hydroxy-7-methylguanine and is presumably the 2-*N*-acetyl derivative.

Reaction of Acetylated 6 with Other Nucleophiles. In agreement with an earlier report using pyridine,[5] **6** in the presence of aqueous acetic anhydride showed no evidence of undergoing 8 substitution with azide ion (1 M) or with methionine (0.05 M), both of which are quite reactive with 1.[3] However, when **6** was reacted with acetic anhydride in methanol, 1,7-dimethyl-8-methoxyguanine (**11**) and its 2-*N*-acetyl derivative were obtained.



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The reaction of acetylated **6** at pH 7 in the presence of 3 M NaBr afforded a large quantity of **9** and some 2-*N*-acetyl-1,7-dimethylguanine, but no **7** and no 8-bromo derivative of **9** were present. The absence of the latter was confirmed by synthesis and characterization of 8-bromo-1,7-dimethylguanine and careful chromatographic examination of the reaction mixture for it. At pH 5 there was an inverse correlation of the yields of **7** and **9** which was directly related to changes in the bromide ion concentration (Table III). In the absence of bromide ion little reduction of **6** to **9** occurs, but in the presence of 3 M NaBr no 8-substitution product (**7**) was detectable and **9** was obtained in 55% yield. There was little variation in the overall recovery over the range of 0 to 3 M NaBr. This indicates that only the portion of **6** that yields **7** is diverted to **9** in the presence of bromide ion. Bromide ion apparently has no effect on the slower reaction that results in destruction of **6**. Addition of acetic anhydride to a concentrated solution of **6** in 3 M NaBr caused the transient appearance of a red color. The solution showed absorption maxima at 405 and 470 nm, which correspond to the maxima for bromine and tribromide ion. These results demonstrate that the enhanced reduction of **6** to **9** in the presence of bromide ion is part of a redox reaction that also results in oxidation of bromide ion to bromine. This is the first evidence that esters of purine *N*-oxides can oxidize bromide ion.

The reaction of acetylated **6** in the presence of 1 M iodide ion at pH 7 also resulted in diversion from the formation of **7** to a mixture of **9** (33%) and its 2-*N*-acetyl derivative (19%). This recovery (52%) is slightly less than the yield of triiodide ion (81%) produced in the reaction but is comparable to the yield of **9** obtained from the reaction of **6** in 3 M NaBr (Table III). These data thus demonstrate that the oxidation of iodide ion is correlated with the reduction of **6** to **9**.

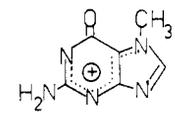
Discussion

The rapid reaction of acetylated 1,7-dimethylguanine 3-oxide under ambient conditions to yield the 8-substituted derivatives **7** and **11** is unusual since such reactions have been observed with esters of other 7-substituted purine 3-oxides

Table III. The Effect of Bromide Ion on the Reaction of 1,7-Dimethylguanine 3-Oxide (6) with Acetic Anhydride at pH 5

	Yield of products, %			
	[Br⁻], M			
Product	0	0.1	0.5	3
2-*N*-Acetyl-1,7-dimethyl-8-oxo-9*H*-guanine (7)	50	41	18	0
1,7-Dimethylguanine (9)	6	21	34	55
Total recovery	56	62	52	55

only under more vigorous conditions, if at all.[16] The rate of reaction of acetylated **6** to **7** is comparable to that for the reaction of 3-acetoxyxanthine (**1**) via path b.[13] However, the mechanism for the reaction of **6** must differ somewhat from either of those proposed[13] for the elimination–substitution reactions of **1** (paths a and b, Scheme I) since a delocalized cation (**3**) which is common to both paths is precluded by the 7-methyl group of **6**. The presence of both the 1-methyl and 2-amino groups of **6** is essential for rearrangement. Both 1,7-dimethyl-3-hydroxyxanthine and 3-hydroxy-7-methylguanine readily form neutral 3-acetoxy derivatives,[5] but neither undergoes a 3 to 8 rearrangement. The formation of **11** from the reaction of **6** with acetic anhydride in methanol clearly demonstrates that the reaction of **6** is intermolecular. A plausible mechanism for the rearrangement of **6** that accommodates the accumulated observations involves acetylation to **12** (Scheme III), ionization and tautomerization of **12** to the neutral **13**, and ionization of **13** to the delocalized cation **14 (a–c)**. The electron-withdrawing effect of the positive charge in the imidazole ring of **14a** must render the 8 position susceptible to attack by basic solvents to afford the Michael addition product **15**, but not susceptible to attack by nucleophiles in general. Ionization of the hydrogen at C-8 of **15** would permit rearomatization of **15** to **16**, a tautomer of **7**. The rearrangement of **13** but not the structurally related 1,7-dimethyl-3-acetoxyxanthine indicates that the 2-amino group plays a significant role in stabilizing the cationic intermediate **14** and encouraging ionization of **13**. Resonance contributors analogous to **14** are conceivable for a cation from 3-acetoxy-7-methylguanine, i.e., **17**, but there are two significant differences between **14** and **17**. (a) The extension in conjugation provided by **14c** is linear to that of the other resonance contributors of **14**. This contrasts with the nearly perpendicular relationship of any charge density at N-1, the resonance contributor to **17** comparable to **14c**, to the remainder of the resonance system of **17**. This suggests that the former cation should be more stable than the latter, and the difference in resonance stabilization may be sufficient enough to permit ionization to **14**, but not to **17**. (b) Only **14a** contains an imine function at C-2 that is also in linear conjugation with C-8 and is oriented appropriately to provide stabilization to a Michael adduct by a base at C-8 of **14a**. These two factors, both unique to a cation from **6**, must play a significant role in determining whether an N-3 elimination–C-8 substitution reaction can occur under mild conditions in a compound for which direct allylic delocalization of the positive charge is blocked by a 7 substituent.[17] The observation that acetylation appears to occur at pH 2, but rearrangement to **7** does not follow, suggests that an ionization that is essential for the reaction does occur, but only at pH's above 3. This agrees with the proposed mechanism.



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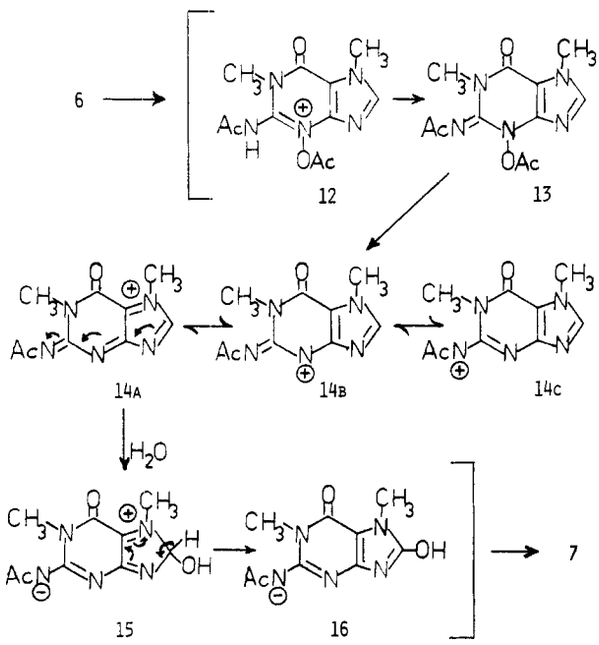
Scheme III



With the demonstration that acetylated **6** can undergo an elimination–substitution reaction, a consistent picture emerges on the reactions of *O*-esters of purine *N*-oxides: they either undergo a 3 to 8 rearrangement *and* oxidize iodide ion or exhibit neither reaction. The high reactivity with a variety of nucleophiles at C-8 of all esters in the series that do rearrange,[3–5] with the single exception of **13**, provides strong support for the participation of a carbonium ion, e.g., **3b**, in the 8-substitution reaction. Recent evidence[14] indicates that there is sufficient electron deficiency at the allylic N-3 position to permit certain nucleophiles to react at N-3 of **3a**. It was proposed[14] that oxidation of iodide ion by **1**, with concomitant formation of xanthine, occurs by formation of a reactive *N*-iodo intermediate at the nitrenium ion and subsequent reaction of this with a second iodide ion to afford iodine and the parent purine. The demonstration that acetylated **6** can undergo both 8-substitution and redox reactions and that the two reactions are competitive (Table III) provides strong evidence that they occur from a single delocalized cation, viz., **14**. The observation of redox reactivity with acetylated **6** indicates that there must be sufficient contribution from **14b** or **14c** to permit iodide and bromide ions to react at an electron-deficient nitrogen to form reactive *N*-halo intermediates that undergo subsequent reaction to **9** and iodine or bromine. The fact that there is a correlation between the yields of I_3^- and of **9** indicates that the iodide-mediated reduction is part of a redox reaction and is not due to a heavy atom induced spin inversion to the nitrenium triplet[18] and reduction of that species by hydrogen abstraction.[19] Acetylated intermediates of **6** cannot form anions comparable to **2** (Scheme I) from **1**, and in contrast to the ~25% reduction of **1** via path b,[13,14] little spontaneous reduction of **6** accompanies its rearrangement to **7**. The inability of intermediates from **6** to form an anion comparable to **2** precludes the formation of a radical anion, such as that previously suggested[13] as an intermediate in the spontaneous reduction of **1** to xanthine, and the possibility of such a species being an intermediate in the redox reaction with iodide ion. Thus, data on the reactions of acetylated **6** complement those from recent studies on the reactions of **1**[14] and support the conclusion that redox reactions observed with *O*-esters of purine *N*-oxides occur via reactions at nitrenium ions and not via radical intermediates. This conclusion may

also explain the reduction reactions observed with acylated intermediates from other heterocyclic *N*-oxides, e.g., 1,5-naphthyridine 1-oxide,[20] in the presence of bromide or iodide ions.

In parallel with the chemical studies, **6** was assayed in vivo for its oncogenic potential. Administration of 1.2 mg of **6** subcutaneously three times per week for 8 weeks induced no tumors. By comparison, the tumor incidence was 35% (7/20) in rats treated similarly with approximately the same molar equiv of 3-hydroxyguanine (1.0 mg).[21]

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., or Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. An ISCO UA-2 analyzer with a coordinated fraction collector was used to monitor all column eluates.

Reaction of 1,7-Dimethylguanine 3-Oxide (6) with Acetic Anhydride. A 1.52-g sample of **6**, purified as described,[15] was dissolved in 34 mL of H_2O, 6 mL of acetic anhydride was added, and the pH of the solution was adjusted from 2.5 to 4.5 with 1 N NaOH. The reaction was allowed to proceed for 2 weeks, and then the precipitate that had formed was collected, washed with acetone, and air dried; yield 420 mg. This was chromatographically homogeneous 2-*N*-acetyl-1,7-dimethyl-8-oxo-9*H*-guanine (**7**): mp, gradual decomposition at >180 °C; mass spectrum (CI), *m/e* 238 (M + 1), 194, 166, 153; UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 265 nm (7.8), 296 (5.5); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 12, 228 nm (22.3), 284 (9.2); NMR (Me_2SO-d_6) δ 2.12 (s, 3, $NCOCH_3$), 3.40 (s, 6, $2NCH_3$), 10.62 (s, 1, NH), 11.62 (s, 1, NH).

An analytical sample was recrystallized from CH_3OH–EtOH and dried at 120 °C over P_2O_5 for 4 h under vacuum.

Anal. Calcd for $C_9H_{11}H_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.54; H, 4.67; N, 29.05.

The filtrate was evaporated to dryness under vacuum. A solution of the residue was applied to a 25 × 200 mm Dowex 50 (H^+), 200–400 mesh, column that was eluted with H_2O, 1 N HCl, and finally 2 N HCl in 60% aqueous CH_3OH; 25-mL fractions were collected. Fractions showing similar UV absorption spectra were combined and the solvents removed under vacuum. Water (fractions 1–12) eluted an additional 75 mg of **7**; the total yield of **7** was 495 mg (29%).

Fractions 73–79 (1 N HCl) contained a small amount, too little for structure identification, of an unknown material: UV λ_{max} pH 1, 240, 285 nm; UV λ_{max} pH 12, 292 nm. Fractions 82–92 contained 35 mg (2.5%) of 1,7-dimethyl-8-oxo-9*H*-guanine (**8**): mp, gradual decomposition above 240 °C; mass spectrum (CI), *m/e* 197 (M + 2), 196 (M + 1); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 250 nm (10.9), 291 (9.9); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 7, 250 nm (10.6), 293 (9.9); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 13, 225 nm (37.2), 260 sh (7.2), 294 (11.6); NMR (Me_2SO-d_6) δ 3.27 (s, 3, NCH_3), 3.32 (s, 3, NCH_3), 6.94 (s, 2, NH_2), 10.99 (s, 1, NH).

An analytical sample was recrystallized from H_2O and dried at 80 °C over P_2O_5 under vacuum for 2 h.

Anal. Calcd for $C_7H_9N_5O_2$: C, 43.07; H, 4.65; N, 35.88. Found: C, 42.75; H, 4.63; N, 35.68.

This product, **8**, was also obtained by treatment of **7** with $NaOCH_3$ in CH_3OH.

Continued elution of the column with 2 N HCl eluted **9** (2%), which was identified from its UV spectra.[22]

Synthesis of 1,7-Dimethyl-8-oxo-9*H*-guanine (8). 2,6-Diamino-3-methyl-5-methylamino-4-oxopyrimidine (**10**) was prepared from 1,7-dimethylguanosinium iodide by a sequence of reactions similar to that reported for the synthesis of 2,6-diamino-4-hydroxy-5-methylaminopyrimidine·2HCl from 7-methylguanosine.[23] 1,7-Dimethylguanosinium iodide[24] (10 g) was dissolved in 50 mL of 28% NH_4OH. The solvent was removed under vacuum, and the flask was left under vacuum for several days. The residue was then dissolved in methanolic HCl, and the solution was warmed slightly. Progress of the reaction was monitored by UV spectra of aliquots. The solvents were removed, and the residue was chromatographed over a 2.5 × 15 cm Dowex 50 (H^+) column that was eluted first with 60% aqueous CH_3OH and then with 1 N HCl in 60% aqueous CH_3OH to elute **10** as the dihydrochloride. The product was recrystallized from CH_3OH–EtOAc: yield 2.6 g (32%); mp 250–251 °C dec; NMR (Me_2SO-d_6) δ 2.64 (s, 3, NCH_3), 3.22 (s, 3, NCH_3), 6.56, 7.64, 11.31 (broad, exchangeable, NH's); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 223 sh nm (13.5), 262 (13.9); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 7, 212 nm (22.5), 277 (9.7); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 12, 211 nm (22.7), 279 (11.0).

An analytical sample was recrystallized from CH_3OH–EtOAc and dried at 120 °C for 3 h over P_2O_5.

Anal. Calcd for $C_6H_{11}N_5O{\cdot}2HCl{\cdot}0.5H_2O$: C, 28.70; H, 5.61; N, 27.89. Found: C, 28.82; H, 5.32; N, 27.62.

A mixture of **10**·2HCl (100 mg, 10 mmol) and urea (130 mg) was heated at 180 °C for 4 days; progress of the reaction was monitored by UV spectra of aliquots. A solution of the reaction mixture was chromatographed over Dowex 50 (H^+), 200–400 mesh, to obtain **8**; yield 55 mg (68%). The chromatographic and NMR, UV, and mass spectral properties of the synthetic sample were identical with those of the product from the reaction of **6**.

Reaction of 6 with Acetic Anhydride in Methanol. To a solution of **6** (470 mg, 2.2 mmol) in 900 mL of CH_3OH was added 18 mL of acetic anhydride. Progress of the reaction was followed by TLC (silica gel; $CHCl_3$–CH_3OH, 9:1). After 5 h no **6** remained, and three new components (R_f 0.4, 0.47, and 0.56) were present. Removal of ~300 mL of solvent under reduced pressure induced precipitation of material which was collected (130 mg) and found to contain a single chromatographically homogeneous component (R_f 0.47). Further reduction in volume to ~200 mL yielded more (75 mg) of the same product, which was identified as 1,7-dimethyl-8-methoxyguanine (**11**): mp, gradual decomposition at >240 °C; mass spectrum (CI), *m/e* 210 (M + 1); NMR (Me_2SO-d_6) δ 3.31 (s, 3, NCH_3), 3.57 (s, 3, NCH_3), 3.77 (s, 3, OCH_3), 4.06 (s, 2, NH_2); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 212 sh nm (15.2), 251 sh (8.1), 274 (12.1); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 7, 217 nm (25), 245 (6.7), 285 (9.5).

An analytical sample was recrystallized from CH_3OH–EtOAc and dried at 120 °C for 3 h over P_2O_5.

Anal. Calcd for $C_8H_{11}N_5O_2{\cdot}H_2O$: C, 42.28; H, 5.76; N, 30.80. Found: C, 42.75; H, 5.28; N, 31.16.

The structure of **11** was confirmed by heating a sample in 0.1 N HCl for 2 h, which converted the sample into **8**, as shown by comparison of the UV and NMR spectra and chromatographic properties in three solvent systems of the hydrolysis product with those of an authentic sample of **8**.

Reduction of the remaining reaction solution to a small volume, application of the solution to preparative silica gel TLC plates (developed in $CHCl_3$–CH_3OH, 4:1, v/v), and elution of the three bands (R_f 0.35, 0.45, and 0.7) yielded additional 1,7-dimethyl-8-methoxyguanine (R_f 0.45) (23 mg); the total yield of **11** was 228 mg (51%).

Elution of the band at R_f 0.7 gave 2-*N*-acetyl-1,7-dimethyl-8-methoxyguanine: yield 8.5 mg (1.5%); mp 219–220 °C; NMR (Me_2SO-d_6) δ 2.099 (s, 3, $COCH_3$), 3.38 (s, 3, NCH_3), 3.66 (s, 3, NCH_3), 4.08 (s, 3, OCH_3), 10.51 (s, 1, exchangeable, NH); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 217 nm (26.5), 261 (10.6), 278 sh (7.9); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 7, 220 nm (32.8), 263 (10.7), 277 sh (7.5); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 12, 218 nm (28.3), 261–277 (10.7).

An analytical sample was recrystallized from CH_3OH–EtOAc and dried at 120 °C over P_2O_5 for 3 h.

Anal. Calcd for $C_{10}H_{13}N_5O_3{\cdot}H_2O$: C, 44.60; H, 5.62; N, 26.01. Found: C, 44.77; H, 5.22; N, 26.15.

The band at R_f 0.35 was **9**, 6.7 mg (2%); the overall recovery from **6** was 54%.

Reaction of 6 with Acetic Anhydride in the Presence of 3 M NaBr. To a solution of **6** (600 mg) in 40 mL of 0.5 N pH 7.0 phosphate buffer containing 12.3 g of NaBr was added 2.4 mL of acetic anhydride. The reaction was allowed to proceed overnight, and then the solvents were removed. A solution of the residue was applied to a 25 × 200 mm Dowex 50, 200–400 mesh, column. Elution with H_2O afforded a product, the UV absorption of which was not identical with that of **7**. The fractions containing the product were combined, reduced in volume, and then neutralized with BioRad AG-3 in the basic form. The eluate was reduced to a small volume and applied to a preparative silica gel TLC plate that was developed in $CHCl_3$–CH_3OH (19:1). Two UV-absorbing bands were present at R_f 0.1 and 0.2. The former was **9**. Elution of the latter afforded 95 mg of 2-*N*-acetyl-1,7-dimethylguanine: mp 252–254 °C; NMR (Me_2SO-d_6) δ 2.10 (s, 3, $COCH_3$), 3.39 (s, 3, NCH_3), 3.95 (s, 3, NCH_3), 8.15 (s, 1, C-8–H), 10.59 (s, 1, NH); mass spectrum (CI), *m/e* 222 (M + 1), 206 (M + 1 − O), 179 (M + 1 − $COCH_3$), 163 (M + 1 − O, $COCH_3$); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1.0, 260 nm (11.2); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 7.0, 216 nm (23), 261 (9.7); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 13, 265–270 nm (10.1).

Anal. Calcd for $C_9H_{11}N_5O_2$: C, 48.86; H, 5.01; N, 31.66. Found: C, 48.88; H, 4.94; N, 31.69.

The structure was confirmed by acetylating a sample of 1,7-dimethylguanine. The synthetic and isolated samples showed identical chromatographic and UV, NMR, and mass spectral properties.

Synthesis of 8-Bromo-1,7-dimethylguanine. A solution of 8-bromoguanosine (1.0 g, 2.8 mM) and 0.75 mL of $(CH_3)_2SO_4$ (8 mM) in 5 mL of Me_2SO was allowed to stir in a sealed flask at room tem-

perature; progress of the reaction was monitored by TLC (silica gel; CH_3CN–H_2O–28% NH_4OH, 7:2:1). After 5 days $KHCO_3$ (975 mg, 8.4 mM) and 0.7 mL of $(CH_3)_2SO_4$ were added, and the reaction was continued for another week. The reaction mixture was then diluted with 10 mL of 50% aqueous CF_3COOH and allowed to stir for 4 days. It was then applied to a 20 × 150 mm Dowex 50 (H^+) column that was eluted with water, 60% aqueous CH_3OH, 1 N HCl in 60% aqueous CH_3OH, and finally with 2 N HCl in 60% aqueous CH_3OH, which eluted the major product; 25-mL fractions were collected. Fractions (72–83) containing the major product were combined, and the solvents were removed under vacuum. The residue was dissolved in CH_3OH, neutralized with AG-1 (HCO_3^-), and recrystallized (charcoal) to afford 8-bromo-1,7-dimethylguanine, yield 390 mg (50%, calculated as hydrate): mp, gradual decomposition at >257 °C; NMR (Me_2SO-d_6) δ 3.46 (s, 3, NCH_3), 3.84 (s, 3, NCH_3), 7.03 (s, 2, exchangeable, NH_2); mass spectrum (CI), *m/e* 260 (M + 1, Br = 81), 258 (M + 1, Br = 79); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 1, 217 nm (19.1), 253 sh (8.2), 272 (12.8); UV λ_{max} ($\epsilon \times 10^{-3}$) pH 7, 216 nm (23.5), 237 sh (8.8), 276 (14.2).

An analytical sample was recrystallized from CH_3OH–H_2O and dried over P_2O_5 at 120 °C under vacuum for 3 h.

Anal. Calcd for $C_7H_8N_5BrO \cdot 0.5H_2O$: C, 31.47; H, 3.39; N, 26.22; Br, 29.91. Found: C, 31.40; H, 3.26; N, 25.77; Br, 29.05.

8-Bromo-1,7-dimethylguanine was eluted from a 9 × 250 mm Dowex 50 (H^+) column by 2 N HCl in 60% CH_3OH as a discrete band well before **9**. It could be readily identified and distinguished from **7**, **8**, and **9** by its UV spectra and was not detectable in the mixture of products from the reaction of **6** with acetic anhydride in the presence of 3 M NaBr at pH 7.

Kinetic Analyses. Aliquots were removed from solutions containing weighed samples of **6** (2.5 mg, 11 μmol), dissolved in 9.9 mL of buffer immediately after the addition of 0.1 mL (1.05 mmol) of acetic anhydride, and added to 1 mm path-length cells that were then stoppered. The UV spectral changes were monitored under ambient conditions (23 °C) with a Unicam SP800A spectrophotometer. Buffers (pH) employed were 10^{-2} M HCl (2), 10^{-3} M HCl (3), 0.1 M formate (4), 0.1 M acetate (5), 0.1 M succinate (6), and 0.5 M phosphate (7 and 8). Rate constants in Table I were obtained from plots of $\ln(OD_0 - OD_\infty)/(OD - OD_\infty)$ vs. time using data from the spectral changes at the maximum of the acetylated intermediate at each pH (Table I). After reactions were complete, the product compositions in the remaining solutions were determined for the pH's shown in Table I by chromatography over a 9 × 100 mm column containing Dowex 50 (H^+), 200–400 mesh. Yields were calculated from elution volumes and known ϵ values.

The product composition of aliquots withdrawn at various times from a reacting solution of **6** at pH 7 was determined by the same method. The initial aliquot, taken immediately after adding acetic anhydride to the reaction, contained 40% of **7**. The yield of **7** increased slightly in later aliquots, e.g., 46% after 2 h, but was little changed after 7 days of reaction (48%). Small amounts (3–8%) of **8** and **9**, which were often poorly resolved, were present throughout the later phase of the reaction. The fourth, unidentified, UV-absorbing product was still detectable after a week of reaction.

Reaction of 6 with Acetic Anhydride in the Presence of 1 M KI. This reaction was performed, as described above, using 0.1 M phosphate buffer (pH 7) containing 1 M KI. The quantity of I_3^- was determined spectrophotometrically at 352 nm (ϵ 26 500)[5] in a 0.1 mm path-length cell. Quantities of the products were determined chromatographically.

Assays for oncogenicity were carried out as described[21] using groups of 20 CD rats (Charles River Breeding Laboratories, Wilmington, Mass.). Compounds were homogenized in 0.5% carboxymethylcellulose in 0.85% NaCl and injected subcutaneously in the intrascapular area. The experiment was terminated after the 18th month. Autopsies were performed on all animals, and suspicious masses were prepared histologically and examined microscopically.

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Registry No.—**6**, 30345-29-0; **7**, 65879-10-9; **8**, 65879-11-0; **9**, 26758-00-9; **10**·2HCl, 65879-12-1; **11**, 65879-13-2; 1,7-dimethylguanosinium iodide, 26758-44-1; urea, 57-13-6; 2-*N*-acetyl-1,7-dimethyl-8-methoxyguanine, 65879-14-3; 2-*N*-acetyl-1,7-dimethylguanine, 65879-15-4; 8-bromoguanosine, 4016-63-1; 8-bromo-1,7-dimethylguanine, 65879-16-5.

References and Notes

(1) This investigation was supported in part by funds from Public Health Service Research Grants CA 15274, CA 8748, and CA 17085 from the National Cancer Institute, DHEW.
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(16) The 3-acetyl ester of 3-hydroxy-7-methylxanthine only undergoes hydrolysis in water.[13] However, reaction of 3-hydroxy-7-methylxanthine with methane–sulfonyl chloride in dimethylformamide does afford 8-substitution products.[3] The 3-methylsulfate ester of 7,9-dimethyl-3-hydroxyxanthine will also rearrange to 7,9-dimethyluric acid, but only upon heating.[3]
(17) The fact that the 3-methylsulfate ester of 3-hydroxy-7-methylxanthine undergoes an 8-substitution reaction in dimethylformamide suggests that the presence of a more effective leaving group than acetate and a highly polar solvent can also facilitate the formation of a cation from an ester of a 7-substituted 3-hydroxypurine and permit an 8-substitution reaction.
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