III. (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *m*-**anisidide:** 0–6 hr, 1.190 g*, 102–103°; 6–12 hr, 0.117 g, 102–103°; 12–24 hr, 0.024 g 101–102°; ($[\alpha]^{25}D$ +1.20°, % N calcd 9.51 and found 9.62). (*R*)- **and** (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *m*-**anisidides:** 0–3 hr, 1.037 g*, 78–79°; 3–6 hr, 0.353 g, 76–77°; 6–12 hr, 0.199 g, 76–77°; 12–24 hr, 0.061 g, 76–77°; ($[\alpha]^{25}D$ +0.138°, % N calcd 9.51 and found 9.52, 55.8% *S* enantiomer in 0–3-hr product.

IV. (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *p*-**anisidide:** 0–6 hr, 1.168 g*, 116–118°; 6–12 hr, 0.346 g, 116–118°; 12–24 hr, 0.132 g, 116–118°; ($[\alpha]^{25}D$ +2.40°, % N calcd 9.51 and found 9.70). (*R*)- **and** (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *p*-**anisidides:** 0-3 hr, 0.856 g*, 111–112°; 3–6 hr, 0.456 g, 111–112°; 6–12 hr, 0.397 g, 110–111°; 12–24 hr, 0.174 g, 110–111°; 24–48 hr, 0.130 g, 107–109°; ($[\alpha]^{25}D$ +0.222°, % N calcd 9.51 and found 9.70, 54.6% *S* enantiomer in 0–3 hr product).

Registry No.—*N*-(*tert*-Butoxycarbonyl)glycine, 4530-20-5; *N*-(*tert*-pentyloxycarbonyl)glycine, 3588-44-1; *N*-(*tert*-butoxycarbonyl)-DL-alanine, 3744-87-4; *N*-(*tert*-pentyloxycarbonyl)-DL-alanine, 34885-82-0; (*R,S*)-*N*-(*sec*-butoxycarbonyl)glycine DCHA, 38435-97-1; (*R,S*)-*N*-(1-methylbutoxycarbonyl)glycine DCHA, 38435-98-2; (*R,S*)-*N*-(2-methylbutoxycarbonyl)glycine DCHA, 38435-99-3; (*S*)-*N*-(2-methylbutoxycarbonyl)glycine DCHA, 38436-00-9; (*R*)-*N*-(*sec*-butoxycarbonyl)glycine *m*-anisidide, 38436-01-0; (*S*)-*N*-(*sec*-butoxycarbonyl)glycine *m*-anisidide, 38436-02-1; (*R*)-*N*-(*sec*-butoxycarbonyl)glycine *p*-anisidide, 38436-03-2; (*S*)-*N*-(*sec*-butoxycarbonyl)glycine *p*-anisidide, 38436-04-3; (*R*)-*N*-(1-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-05-4; (*S*)-*N*-(1-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-06-5; (*S*)-*N*-(2-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-07-6; (*R*)-*N*-(2-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-08-7; (*S*)-*N*-(2-methylbutoxycarbonyl)glycine *p*-anisidide, 38436-09-8; (*R*)-*N*-(2-methylbutoxycarbonyl)glycine *p*-anisidide, 38436-10-1; papain, 9001-73-4.

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Purine *N*-Oxides. XLVI. Some Interesting Reactions of 3-Acetoxy-8-methylxanthine[1]

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The reactivities of 3-acetoxy-8-methylxanthine have been compared with corresponding reactivities of 3-acetoxyxanthine. The former undergoes a rearrangement in water to yield 8-hydroxymethylxanthine, and evidence is presented suggesting an intermediate possessing an exocyclic methylene group. In addition, some hydrolysis to 3-hydroxy-8-methylxanthine and some reduction to 8-methylxanthene occur, the latter apparently proceeding through a radical mechanism. 8-Methylguanine 3-oxide can also be rearranged to 8-hydroxymethylguanine. 3-Acetoxyxanthine reacts in aqueous solutions with many nucleophiles to yield a variety of 8-substituted xanthines. Under the same conditions 3-acetoxy-8-methylxanthine reacts only with the water to afford the 8-hydroxymethyl derivative. 3-Acetoxy-8-azaxanthine undergoes only the hydrolysis and reduction reactions.

Esters of the oncogenic 3-hydroxyxanthine react with nucleophiles under mild conditions *in vitro*,[3] and *in vivo*,[4] to form 8-substituted xanthines. Similar nucleophilic substitution reactions with macromolecules of the cell have long been offered as an explanation of the initiation of the cancer process by chemical oncogens.[5] A weak oncogenicity of 3-hydroxy-8-azaxanthine (**16**) and the possible similar activity of 3-hydroxy-8-methylxanthine (**1**)[4] prompted a comparative investigation of the chemical behavior of compounds with these distinct alterations of the 8 position of 3-hydroxyxanthine.

Treatment of 3-hydroxy-8-methylxanthine (**1**) with acetic anhydride in trifluoroacetic acid at room temperature afforded a monoacetyl derivative. The ir and nmr[6] spectra indicated the presence of an O-acetyl group, and the slow development of a purple color with ferric chloride provided further support for the 3-acetoxy-8-methylxanthine structure (**2**). Reaction of 3-hydroxy-8-methylxanthine (**1**) in hot acetic anhydride, followed by treatment with water, gave some 8-hydroxymethylxanthine (**10**) and extensive decomposition. The *O*-acetyl derivative of 3-hydroxy-8-methylguanine (**14**) was not isolable, but in acetic anhydride and trifluoroacetic acid at room temperature, followed

(1) This investigation was supported in part by funds from the National Cancer Institute, Grant No. CA 08748.

(2) D. R. S. is a Damon Runyon Memorial Fellow.

(3) N. J. M. Birdsall, U. Wölcke, T.-C. Lee, and G. B. Brown, *Tetrahedron*, **27**, 5969 (1971).

(4) G. B. Brown, M. N. Teller, I. Smullyan, N. J. M. Birdsall, T.-C. Lee, J. C. Parham, and G. Stöhrer, *Cancer Res.*, in press.

(5) J. A. Miller, *ibid.*, **30**, 559 (1970).

(6) A downfield shift of the nmr signal of the 8-methyl protons in 8-methylxanthine and guanine derivatives, when the solvent was TFA rather than DMSO-d_6, was attributed to protonation of the imidazole ring in the former solvent. This phenomenon has been observed previously in N-methylated xanthines.[7]

(7) D. Lichtenberg, F. Bergmann, and Z. Neiman, *J. Chem. Soc. C*, 1676 (1971).

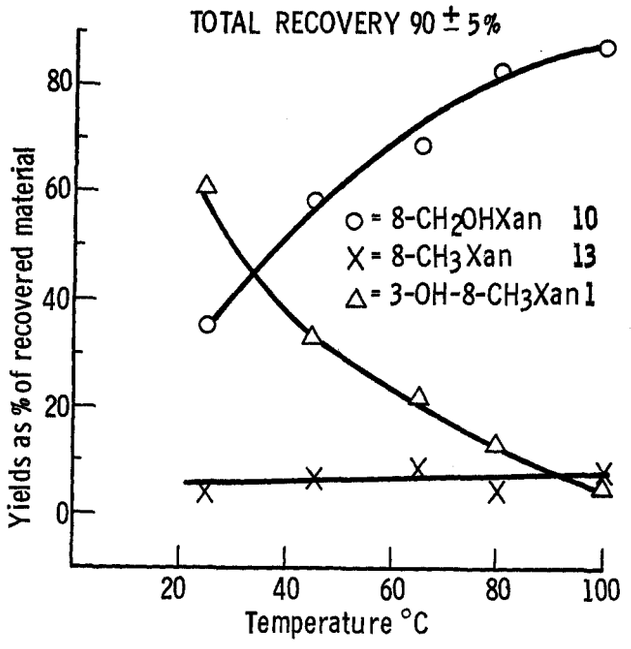
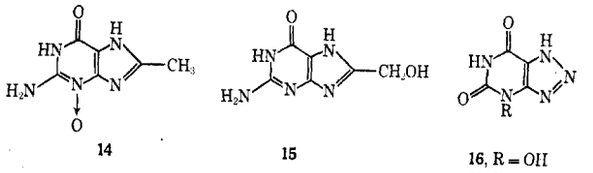


Figure 1.—Effect of temperature on yields of products.

by treatment with water, **14** was converted to 8-hydroxymethylguanine (**15**).



14 **15**

16, R = OH
17, R = OAc
18, R = H

If the treatment of 3-hydroxy-8-methylxanthine (**1**) with acetic anhydride and trifluoroacetic acid was prolonged, a solid could be isolated, the nmr (δ 5.82, two-proton singlet) and ir (carbonyl absorption at 1750 cm^{-1}) spectra of which indicated the presence of a trifluoroacetoxymethyl group.[8] On standing, this compound slowly reacted, presumably with atmospheric moisture, to give 8-hydroxymethylxanthine (**10**), which showed a two-proton singlet at δ 5.38. The difference in chemical shifts of the methylene protons of these two compounds is in agreement with the assigned 8-trifluoroacetoxymethylxanthine structure (**11**).[8] It has previously been observed that esters of this type are readily hydrolyzed by atmospheric moisture.[8]

In water at room temperature 3-acetoxy-8-methylxanthine (**2**) yielded 8-hydroxymethylxanthine (**10**), together with 3-hydroxy-8-methylxanthine (**1**) and 8-methylxanthine (**13**). Increase in temperature of this reaction (Figure 1) resulted in an increase in the amount of rearrangement to 8-hydroxymethylxanthine, with a corresponding decrease in the hydrolysis to 3-hydroxy-8-methylxanthine. The formation of 8-methylxanthine (**13**) showed little sensitivity to changes in reaction temperature.

Examination of the reactions of **2** in aqueous solution at different pH's (Figure 2) showed the preferential formation of the hydrolysis product 3-hydroxy-8-methylxanthine (**1**) below pH 5. Above pH 5 the major product was 8-hydroxymethylxanthine (**10**). There was also a significant increase in the production of 8-methylxanthine (**13**) as the pH increased.

(8) D. R. Sutherland and G. Tennant, *J. Chem. Soc., Perkin Trans. 1*, in press.

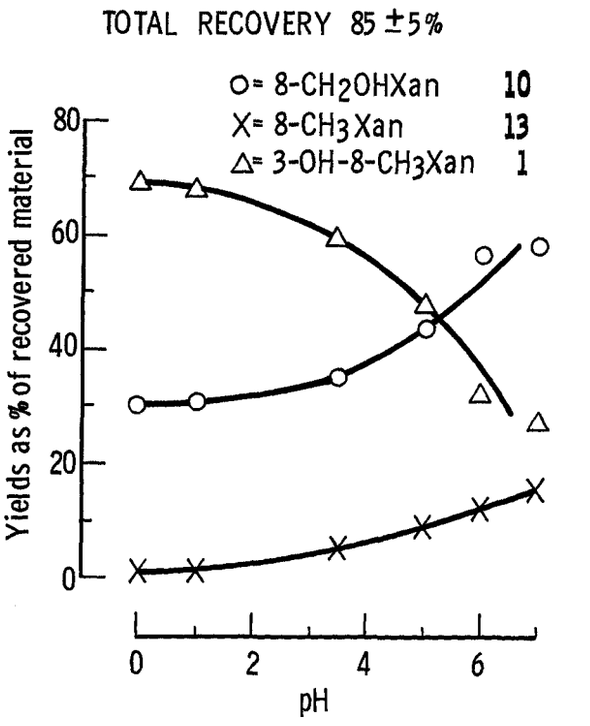


Figure 2.—Effect of pH on yields of products.

Investigation of changes in the polarity of the solvent on the reaction product distribution (Figure 3) showed that the amount of rearrangement to **10** increased, with a corresponding decrease in hydrolysis to **1**, as the polarity of the solvent decreased. In contrast, the solvent polarity had no effect on the production of 8-methylxanthine (**13**).

Treatment of 3-acetoxy-8-methylxanthine (**2**) with methanol or ethanol afforded, after evaporation, a product which was unstable in most solvents. Upon treatment with water it afforded 8-hydroxymethylxanthine (**10**), together with smaller quantities of 3-hydroxy-8-methylxanthine (**1**) and 8-methylxanthine (**13**). When the crude product from methanol or ethanol treatment was dissolved in trifluoroacetic acid, it showed an nmr signal at δ 4.95, attributable to an exocyclic methylene group[9] at C-8. However, this signal rapidly disappeared and a new signal developed at δ 5.82, identical with that assigned to the methylene protons of 8-trifluoroacetoxymethylxanthine (**11**). The latter signal also gradually disappeared and was replaced by the δ 5.38 signal of the hydroxymethyl derivative **10**. The nmr spectrum of the crude product also exhibited signals attributed to **13** and **1**, showing that both the reduction and the hydrolysis also occurred in alcohols. The uv spectrum of 3-acetoxy-8-methylxanthine (**2**) in methanol showed an initial absorption at 268 nm. This absorbance rapidly decreased threefold while shifting to 270 nm. The 270-nm absorption is identical with that exhibited by the

(9) (a) D. Meuche, M. Neuenschwander, H. Schaltegger, and H. U. Schlunegger, *Helv. Chim. Acta*, **47**, 1211 (1964); (b) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "Nmr Spectral Catalog," Vol. 1, Varian Associates, Palo Alto, Calif., 1962, No. 109, 132, and 180; (c) R. F. C. Brown, G. E. Gream, D. E. Peters, and R. K. Solly, *Aust. J. Chem.*, **21**, 2223 (1968).

product obtained from treatment of 2 with refluxing methanol. This product underwent a series of changes when treated with water. The initial absorption at 270 nm increased threefold in intensity within 10 min, and then decreased about fourfold over 2 hr to the final spectrum. That spectrum was the same as the ultimate spectrum of 2 in water, which was reached after a similar series of changes, and was that of a mixture of 8-hydroxymethylxanthine (**10**), 3-hydroxy-8-methylxanthine (**1**), and 8-methylxanthine (**13**). The acetoxy compound 2 in water initially showed a 272-nm maximum, which shifted in 60 sec to 269 nm with a slight increase in intensity. After 90 sec the absorption decreased threefold with a shift to 270 nm, and then showed changes identical with those described for the uv of the product from alcohol treatment.

The similarity of the uv spectra changes observed when 2 was treated with water or with alcohols indicated the presence of a similar intermediate in each solvent. Nmr evidence indicated the presence of an exocyclic methylene group in the product obtained after treatment of 2 with methanol or ethanol, suggesting that an intermediate such as **7** is involved in the rearrangement to **10**. Such intermediates have been invoked to explain the conversions of heterocyclic *N*-oxides to products acyloxylated in the nucleus or on a side chain.[10-12]

In marked contrast to the results with 3-acetoxyxanthine,[3] no reaction of 3-acetoxy-8-methylxanthine (2) with aqueous solutions of nucleophiles such as chloride, nitrite, or azide ion or methionine has been observed. The only products detected were those from the reaction of 2 with water.

There are, however, obvious similarities in the reactivities of 3-acetoxyxanthine[3] and 3-acetoxy-8-methylxanthine (2) in aqueous solutions. Each compound yields products resulting from three reactions: hydrolysis, reduction, and rearrangement. With each compound rearrangement became the major reaction pathway only as the pH increased (Figure 2). At lower pH's ester hydrolysis was the major reaction. These changes in the ratios of the products arising from the different reactions coincide with the pH of formation of the anion of 3-hydroxyxanthine,[13] and the same is apparently true for the 8-methyl compound **3**.[14]

It has been suggested that at lower pH's the rearrangement of 3-acetoxyxanthine to uric acid proceeds *via* a heterolytic cleavage of the *N*-acetoxy bond to give a nitrenium ion at N-3, which undergoes an allylic

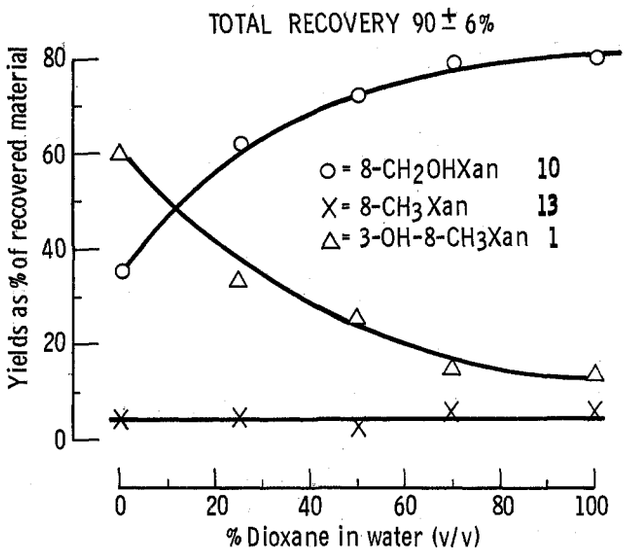
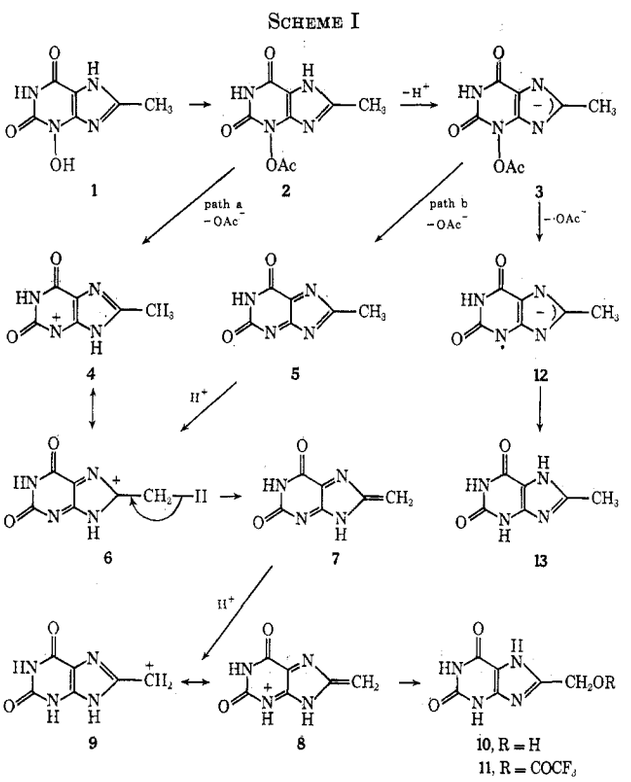


Figure 3.—Effect of solvent composition on yields of products.

shift to afford a more stable secondary carbonium ion at C-8. This intermediate undergoes nucleophilic attack to yield uric acid.[13] If this mechanism were true for the 8-methyl derivative 2 (see Scheme I),

SCHEME I



a more stable tertiary carbonium ion **6** would be formed at C-8 and a greater degree of rearrangement might be expected. In fact, the yield of the rearrangement product, 8-hydroxymethylxanthine, was at least four times greater than that of the uric acid derived from 3-acetoxyxanthine at pH's less than 5.

At higher pH's, where expulsion of the acetate ion was aided by the negative charge on the imidazole ring (**3**), the amount of rearrangement increased noticeably with a simultaneous decrease in the hydrolysis to 3-

(10) E. Ochiai, "Aromatic Amine Oxides," Elsevier, New York, N. Y., 1967.

(11) V. J. Traynellis in "Mechanisms of Molecular Migrations," Vol. 2, B. S. Thyagarajan, Ed., Wiley-Interscience, New York, N. Y., 1969, Chapter 1.

(12) A. R. Katritzky and J. M. Lagowski, "Chemistry of the Heterocyclic N-Oxides," Academic Press, New York, N. Y., 1971.

(13) N. J. M. Birdsall, J. C. Parham, U. Wölcke, and G. B. Brown, *Tetrahedron*, **28**, 3 (1972).

(14) The pK_a of 3-acetoxy-8-methylxanthine was difficult to obtain because of the rapid and complex changes in the uv spectrum of the compound in aqueous solution. However, the influence of the 8-methyl group in similar compounds increased the pK_a of ionization of the imidazole proton by ~0.6 units.[15] Therefore, from the known pK_a of 3-acetoxyxanthine (6.8 ± 0.5)[16] it can be deduced that the pK_a of the 8-methyl derivative is ~7.4 ± 0.5.

(15) J. C. Parham, T. G. Winn, and G. B. Brown, *J. Org. Chem.*, **36**, 2639 (1971).

(16) N. J. M. Birdsall, T.-C. Lee, and U. Wölcke, *Tetrahedron*, **27**, 5961 (1971).

hydroxy-8-methylxanthine (**1**). At these higher pH's the proportion of rearrangement compared to hydrolysis was again higher than that observed in the reactions of 3-acetoxyxanthine. It is probable that the two pathways leading to the tertiary carbonium ion **6** are competitive in aqueous solution, with path b predominating at higher pH's and path a at lower pH's.

The rearrangement product obtained from 3-acetoxy-8-methylxanthine (**2**) was not truly analogous to that obtained from 3-acetoxyxanthine. The latter, uric acid, was formed by direct nucleophilic attack at C-8, whereas the former, 8-hydroxymethylxanthine, must be formed by attack at the carbon atom of the exocyclic methyl group. The absence of any product arising from direct attack at C-8 can be attributed to steric hindrance by the methyl group in the carbonium ion **6**. The nmr evidence has suggested an intermediate with an exocyclic methylene group at C-8 (**7**). Such a species, presumably formed from the tertiary carbonium ion **6**, could be protonated to give the cation **8**, which could then react with water by a Michael addition type process to yield the hydroxymethyl compound **10**. The primary carbonium ion **9** is less likely to be a contributing factor, since it should be less stable than the resonance-stabilized tertiary carbonium ion **6**. If **9** is involved, preferential solvation of it by water molecules could explain the failure to find any evidence for reactions with other nucleophiles in aqueous solution. The absence of a reaction giving 8-methoxymethylxanthine in sodium methoxide in methanol infers that **7** does not undergo a Michael addition under such conditions. However, the strongly basic nature of sodium methoxide may cause ionization of the proton at N-9, thereby rendering the methylene group of **7** very resistant to nucleophilic attack. Treatment of **2** with methanol in the presence of Dowex-50 (H^+), which might have aided nucleophilic attack by protonating **7**, did not result in formation of the methoxymethyl derivative.

At lower pH's (Figure 2) and in solutions of high polarity (Figure 3), there was a significant amount of rearrangement, but hydrolysis to 3-hydroxy-8-methylxanthine (**1**) was favored, which suggests an $A_{Ac}2$ hydrolysis mechanism.[17]

The yield of 8-methylxanthine (**13**) obtained from 3-acetoxy-8-methylxanthine (**2**) in aqueous solutions was found to be relatively insensitive to changes in solvent polarity and temperature. These observations, like those with 3-hydroxyxanthine,[13] suggest that the reduction occurs by a free-radical mechanism. In aqueous solutions of the acetoxy compound **2** the presence of iodide ion, but not chloride ion, caused a large increase in the production of the reduced compound. In another study it has been noted that uv irradiation of 3-hydroxy-8-methylxanthine (**1**) or of its acetoxy derivative **2** in the solid state afforded a free radical.[18]

Since the production of 8-methylxanthine (**13**) in aqueous solution became significant only at higher pH's (Figure 2), it appears that formation of the radical proceeds from the anion **3**. A radical (**12**) derived by homolytic cleavage of the N–O bond of the 8-methylxanthyl anion will abstract hydrogen from water and then protonate to give 8-methylxanthine (**13**).

A by-product in the reactions of 3-acetoxyxanthine in nearly neutral aqueous solutions was an unstable and highly insoluble blue compound.[3,13] It may be significant to the structure of that product that no evidence was found for the formation of such a compound from 3-acetoxy-8-methylxanthine (**2**), in which the 8 position is blocked.

3-Hydroxy-8-azaxanthine (**16**), in which the 8 position is altered in a different manner, was also investigated. It decomposed in refluxing acetic anhydride. The preparation of 3-acetoxy-8-azaxanthine (**17**)[16] from 3-hydroxy-8-azaxanthine was improved by the use of acetic anhydride in trifluoroacetic acid at room temperature. In water at room temperature the 3-acetoxy derivative **17** hydrolyzed quantitatively to the *N*-hydroxy compound **16**. No reactions could be observed with nucleophiles in solution. Treatment of the acetoxy compound **17** with hot methanol gave 3-hydroxy-8-azaxanthine (**16**) as the major product, but it also yielded a small amount of the reduction product, 8-azaxanthine (**18**), in analogy to the reduction of 3-acetoxy-8-methylxanthine (**2**) in methanol under comparable conditions.

Whether these chemical properties of 3-hydroxy-8-methylxanthine and 3-hydroxy-8-azaxanthine are related to the process of tumor induction remains to be seen. The observations that each of these, like 3-acetoxyxanthine, are reduced, in part, by a reaction which may proceed through a radical mechanism add to the evidence encouraging serious consideration of the possibility that a free-radical reaction mechanism may be involved in oncogenicity.[4]

Experimental Section

Analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich. The uv and ir spectra were recorded with Unicam SP800A and Perkin-Elmer Model 137B Infracord (Nujol suspension) spectrophotometers, respectively. Nmr spectra were measured at 60 MHz using a Varian A-60 spectrometer, in dimethyl sulfoxide-d_6 or trifluoroacetic acid, at 35°, with tetramethylsilane as the internal standard.

3-Acetoxy-8-methylxanthine (**2**).—3-Hydroxy-8-methylxanthine[19] (**1**) (250 mg) was stirred in trifluoroacetic acid (15 ml) and acetic anhydride (10 ml) at room temperature for 3 hr. This was poured into dry ether (100 ml) and the resulting solution was concentrated to 10 ml. Treatment of the concentrate with dry ether (100 ml) and overnight refrigeration afforded the acetoxy compound **2**, which was washed with dry ether and dried under vacuum at room temperature overnight: yield 72%; nmr (TFA) δ 2.90 (s, 3, CCH_3), 2.53 (s, 3, $NOCOCH_3$); nmr (DMSO-d_6) δ 13.40 (br, 1, NH), 11.65 (br, 1, NH), 2.42 (s, 3, CCH_3), 2.37 (s, 3, $NOCOCH_3$); ir (Nujol) 3100–3400, 2600–2750 (NH), 1810 ($NOCOCH_3$), 1670 cm^{-1} (CO). The ir and nmr spectra also exhibited absorption attributable to acetic acid and the analysis corresponded to a hemiacetate.

Anal. Calcd for $C_8H_8N_4O_4 \cdot 0.5CH_3CO_2H$: C, 42.3; H, 4.3; N, 19.7. Found: C, 41.9; H, 4.1; N, 19.3.

If the reaction is continued for 3 days before work-up, the nmr spectrum of the crude product shows the presence of 3-acetoxy-8-methylxanthine (**2**), 8-methylxanthine (**13**)[20] [nmr (TFA) δ 2.93 (s, 3, CCH_3)], 3-hydroxy-8-methylxanthine (**1**) [nmr (TFA) δ 2.97 (s, 3, CCH_3)], 8 trifluoroacetoxymethylxanthine (**11**) [nmr (TFA) δ 5.82 (s, 2, CH_2OCOCF_3); ir (Nujol) 1750 cm^{-1} ($OCOCF_3$)], and 8-hydroxymethylxanthine (**10**)[21] [nmr (TFA) δ 5.38 (s, 2, CH_2OH)].

(17) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," 2nd ed, Cornell University Press, Ithaca, N. Y., 1969.

(18) J. C. Parham, I. Pullman, and G. B. Brown, unpublished results.

(19) N. J. M. Birdsall, T.-C. Lee, T. J. Delia, and J. C. Parham, *J. Org. Chem.*, **36**, 2635 (1971).

(20) H. C. Koppel and R. K. Robins, *ibid.*, **23**, 1457 (1958).

(21) H. Bredereck, E. Siegel, and B. Föhlisch, *Chem. Ber.*, **95**, 403 (1962).

Examination of the reaction mixture by nmr spectroscopy at 60-min intervals showed that formation of 8-trifluoroacetoxymethylxanthine (11) began after 4 hr, before the formation of the hydroxymethyl compound 10.

8-Hydroxymethylxanthine (10). A.—3-Acetoxy-8-methylxanthine (2) (250 mg) was refluxed in water (25 ml) for 1 hr, the solution was evaporated, and the residue was crystallized from water to yield 80% of 10, identical with an authentic sample.[21]

B.—3-Hydroxy-8-methylxanthine (1) (250 mg) was refluxed in acetic anhydride (60 ml) for 30 min. The dark solution was evaporated, and the residue was crystallized from water to yield 11% of 10. The nmr spectrum of the crude residue did not show signals assignable to methylene protons. Evaporation of the aqueous crystallization liquors afforded a complex mixture, inseparable by column chromatography, and showing only end absorption in the uv.

8-Hydroxymethylguanine (15).—8-Methylguanine 3-oxide (14)[19] (250 mg) was stirred in trifluoroacetic acid (25 ml) and acetic anhydride (15 ml) for 24 hr at room temperature. The resulting solution was evaporated under vacuum at room temperature to a gum, which was crystallized from water to yield 29% of 15, identical with an authentic sample.[22] Temperatures above 30° caused decomposition to products possessing only end absorption in the uv.

Reactions of 3-Acetoxy-8-methylxanthine (2) in Aqueous Solutions (See Figures 1–3).—3-Acetoxy-8-methylxanthine (2) (11.2 mg) was dissolved in the solvent (25 ml) and at the temperature specified. The solutions were stirred for 16 hr, after which no further reaction occurred as shown by lack of change in optical density of diluted aliquots. Aliquots (5 ml) were analyzed with an 8 × 1 cm column of Dowex-50 [H^+], 200–400 mesh, eluted with 0.1 *N* HCl, and with the uv absorption of the effluent recorded by an ISCO UA2 monitor. The elution volumes follow: 8-hydroxymethylxanthine, 125 ml; 3-hydroxy-8-methylxanthine, 170 ml; 8-methylxanthine, 360 ml. The fractions were evaporated and dissolved in aqueous solutions of the required pH. The yields were calculated from the volume and absorbance of each fraction with the following reference values: 8-hydroxymethylxanthine, pH 13, λ_{max} 286 nm (ϵ 11,000);[21] 3-hydroxy-8-methylxanthine, pH 13, λ_{max} 297 nm (ϵ 8050); 8-methylxanthine, pH 1, λ_{max} 265 nm (ϵ 7800).[20] For reactions in dioxane and water the solvent was evaporated under vacuum, and the residues were dissolved in water for analysis.

3-Acetoxy-8-methylxanthine (2) (11.2 mg) was added to a solution of sodium iodide (15 mg) in water (30 ml) and stirred for 16 hr. The reaction mixture was extracted with chloroform, and the aqueous fraction was analyzed to yield 11% of 3-hydroxy-8-methylxanthine (1), 10% of 8-hydroxymethylxanthine (10), and 46% of 8-methylxanthine (13). In a similar experiment with sodium chloride, the yield of 8-methylxanthine (13) was 8%.

Under conditions that led to numerous 8-substituted products of 3-hydroxyxanthine,[3] 2 was treated with aqueous solutions of sodium azide, sodium nitrite, sodium chloride, or methionine. Upon column chromatography 1, 10, and 13 were found in each case, but no other products representing substitution by these nucleophiles were detected. The nmr spectra of the crude reaction residues showed no signals attributable to 8-substituted products, other than 10.

Treatment of 3-Acetoxy-8-methylxanthine (2) with Methanol and Ethanol.—3-Acetoxy-8-methylxanthine (2) (250 mg) was refluxed in dry methanol or ethanol (100 ml) for 1 hr, and the solution was evaporated. The nmr spectrum (TFA) of the residue initially showed a singlet at δ 4.95, which disappeared with the concomitant appearance of a signal at 5.82, identical with that of 8-trifluoroacetoxymethylxanthine (11). The spectrum also showed signals in the CH_3 region (δ 2–3), that could be assigned to 3-hydroxy-8-methylxanthine (1), 8-methylxanthine (13), and acetic acid. The residue from the methanol reaction upon treatment with water and chromatographic separation afforded 28% of 1, 7% of 13, and 49% of 10. 3-Acetoxy-8-methylxanthine was treated with methanol as above, in the presence of dry Dowex-50 [H^+] (1 g). The Dowex resin was removed by filtration and the filtrate was treated as above to give 33% of 1, 5% of 13, and 42% of 10.

Treatment of 3-Acetoxy-8-methylxanthine (2) with Sodium Methoxide.—3-Acetoxy-8-methylxanthine (2) (225 mg) was stirred with a solution of sodium (115 mg) in methanol (250 ml) for 2 hr at room temperature. The solvent was evaporated, and the residue was dissolved in water. The resulting solution was adjusted to pH 5 with 1 *N* HCl. Column chromatography with Dowex-50 [H^+] afforded 21% of 13, 23% of 1, and 31% of 10, together with some material possessing only end absorption in the uv.

3-Acetoxy-8-azaxanthine (17).—3-Hydroxy-8-azaxanthine (16)[23] (250 mg) was stirred in trifluoroacetic acid (12 ml) and acetic anhydride (5 ml) at room temperature for 17 hr. The solution was evaporated at room temperature, and the residue was stirred in dry methanol (100 ml) for 24 hr to give, after evaporation, 78% of 17, identical with an authentic sample.[16]

Treatment of 16 with refluxing acetic anhydride caused immediate decomposition to a tar, which showed only end absorption in the uv.

Reaction of 3-Acetoxy-8-azaxanthine (17) in Methanol.—3-Acetoxy-8-azaxanthine (17) (50 mg) was refluxed in dry methanol (10 ml) for 4 hr. The solution was evaporated and the residue was dissolved in water. After column chromatography with Dowex-50 [H^+], 91% of 3-hydroxy-8-azaxanthine (16) and 3% of 8-azaxanthine (18) were obtained.

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(22) E. P. Studentsov and V. G. Nemets, *Khim. Geterotsikl. Soedin*, **4**, 732 (1968).

(23) R. M. Cresswell, H. K. Maurer, T. Strauss, and G. B. Brown, *J. Org. Chem.*, **30**, 408 (1965).