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A NOVEL BIMOLECULAR DISPROPORTIONATION IN PURINE-N-OXIDES

A SPONTANEOUS AUTOCATALYTIC AND AN ANHYDRIDE INDUCED DISPROPORTIONATION OF 8-ALKYL-7-HYDROXYXANTHINES

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Abstract—8-Alkyl-7-hydroxyxanthines undergo in aqueous solution, near pH 5, an unusual spontaneous autocatalytic disproportionation. They yield the corresponding 8-alkylxanthines as the reduction product and 6-amino-5-nitrosouracil as the oxidation product. The disproportionation can also be induced by anhydrides at pH 4–12. The alkyl group at position 8 is essential for these reactions. Possible mechanisms are discussed.

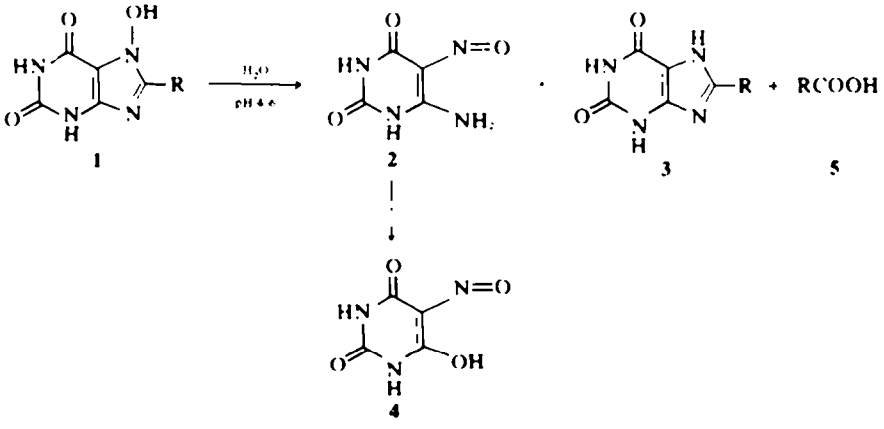
7-Hydroxyxanthine and its alkyl derivatives were described recently.[1,2] The ability of 7-hydroxyxanthine to undergo a rearrangement and 8-substitution similar to that of the potent oncogen 3-hydroxyxanthine[3,4] in the presence of acylating agents was shown earlier.[1,2] The similar chemical reactivity of 7-hydroxyxanthine suggested that it might show comparable biological activity and tests for the oncogenicity of 7-hydroxyxanthine show preliminary positive results.[5] 8-Alkyl derivatives of 7-hydroxyxanthine have UV properties and dissociation properties similar to that of the parent compound.[2] However, the 8-alkyl-7-hydroxyxanthines share some unusual chemical properties which are not encountered in the unsubstituted 7-hydroxyxanthine. They undergo a facile bimolecular disproportionation which is described in this work.

Spontaneous autocatalytic disproportionation

8-Propyl-7-hydroxyxanthine (**1**, R = $(CH_2)_2CH_3$), prepared[2] by the cyclization of 6-amino-5-nitrosouracil with butyraldehyde is a weak acid (pKa = 5.4) and dissolves in base or in neutral or basic buffered solutions. Derivatives with longer alkyl groups tend to precipitate in even mildly acid solutions. Therefore, it was more convenient to study the 8-propyl derivative although all 8-alkyl-7-hydroxyxanthines exhibit the tendency to disproportionate. Upon slight heating of neutral or slightly acid solutions of these compounds a gradual appearance of a purple (lavender) color was observed. The purple solution which is obtained absorbed at a λ_{max} = 314 nm and the product responsible for this absorption was identified as 6-amino-5-nitrosouracil which arises as a decomposition product of 8-alkyl-7-hydroxyxanthines. At concentrations of about 3×10^{-3} M and a pH near 5 the coloration also appeared at room temperature after about 30 min. More dilute solutions (1×10^{-4} M) did not show any decomposition at room temperature even after several weeks.

The additional oxygen in 6-amino-5-nitrosouracil probably comes from another molecule of 8-alkyl-7-hydroxyxanthine (**1**). This assumption is supported by the fact that for each molecule of 6-amino-5-nitrosouracil (**2**) one molecule of 8-alkylxanthine (**3**) is obtained. In addition it was observed that the plot of the appearance of products versus time gave an S shaped curve typical of autocatalytic reactions (Fig. 1). The expression for the rate of such a second order autocatalytic reaction yields eqn (A):[6]

$$k.t = -\frac{1}{A_0 + B_0} \ln \frac{A_0 B}{B_0 A} \qquad (A)$$



1 **2** **3** **5** **4**

Scheme 1.

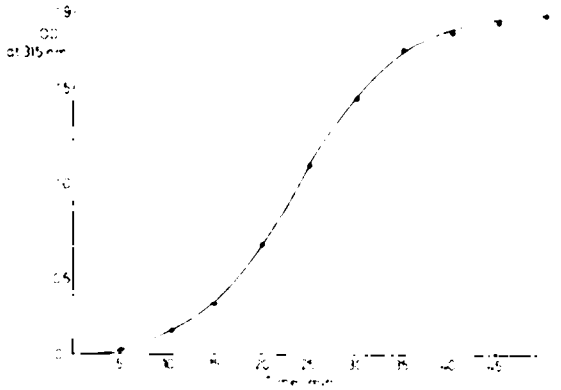


Fig. 1. Spontaneous autocatalytic disproportionation of 3.6×10^{-3} M of 7-hydroxy-8-propylxanthine (1) at 40° and pH 5.0; followed at 315 nm.

Where the reaction is presented by the eqn:

$$A \longrightarrow B + \cdots$$

Where A_0 and B_0 are the initial concentration of the starting material and any one of the products respectively. The initial concentration B_0 is assumed to be 0. The concentration of the catalysing product is assumed to increase at the same rate as other products. The constants for this spontaneous autocatalytic disproportionation (k_{SAD}) were derived from the slope of $\ln OD/(OD_\infty - OD)$ vs time where OD is the optical density at 314 nm (Experimental). The observed k_{SAD} at optimal pH was about 72 min^{-1} M at 50°.

In order to study the mechanism of the autocatalysis, experiments were run in the presence of each of the purified reaction products. None of the products **2**, **3**, **4** nor butyric acid had any catalytic effect. The reaction was found independent on light or oxygen. Fortunately, in the anhydride induced disproportionation reaction which is described below, the unstable intermediate 6-acylamino-5-nitrosouracil (**6**) could be isolated. Upon the addition of about 0.2 equivalents of **6** ($R = (CH_2)_2CH_3$) a considerable increase in the rate of disproportionation was observed (Fig. 3, curve C). The initial slow part of the sigmoid rate curve disappeared and most of the N-oxide decomposed in a few minutes. A reference reaction, without added **6**, was run under the same conditions and the remarkable difference is shown in Fig. 3, curve D. Fortunately **6** absorbs at longer wavelength (334 nm)

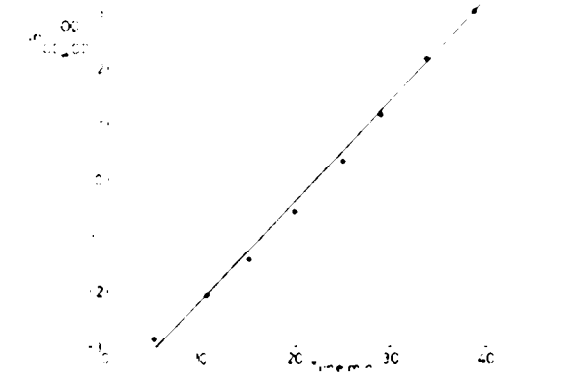


Fig. 2. Logarithmic plot according to eqn (A); conditions are the same as in Fig. 1.

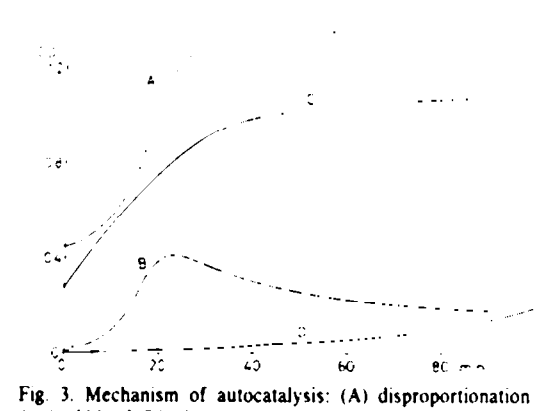
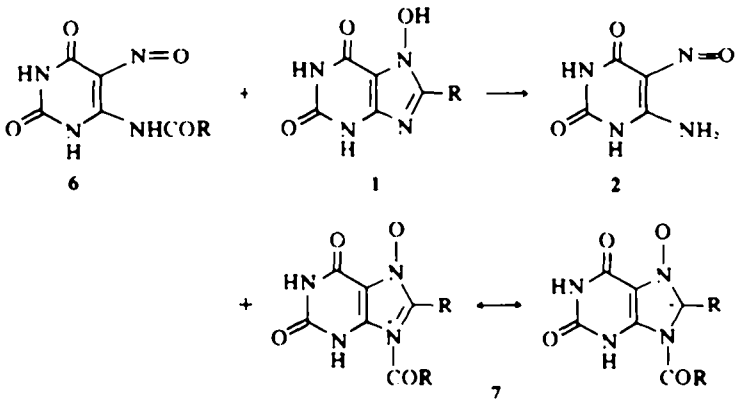


Fig. 3. Mechanism of autocatalysis: (A) disproportionation of 4×10^{-4} M of 7-hydroxy-8-propylxanthine at 42° and pH 5.2, followed at 300 nm (light path 0.1 cm); (B) the same as in A but followed at 350 nm; (C) Disproportionation of 1.7×10^{-3} M 7-hydroxy-8-propylxanthine at 30° and pH 5.2, followed at 315 nm (light path 0.1 cm) in the presence of 0.34×10^{-3} M 6-butyrylamino-5-nitrosouracil; (D) The same as in C but in the absence of 6-butyrylamino-5-nitrosouracil.

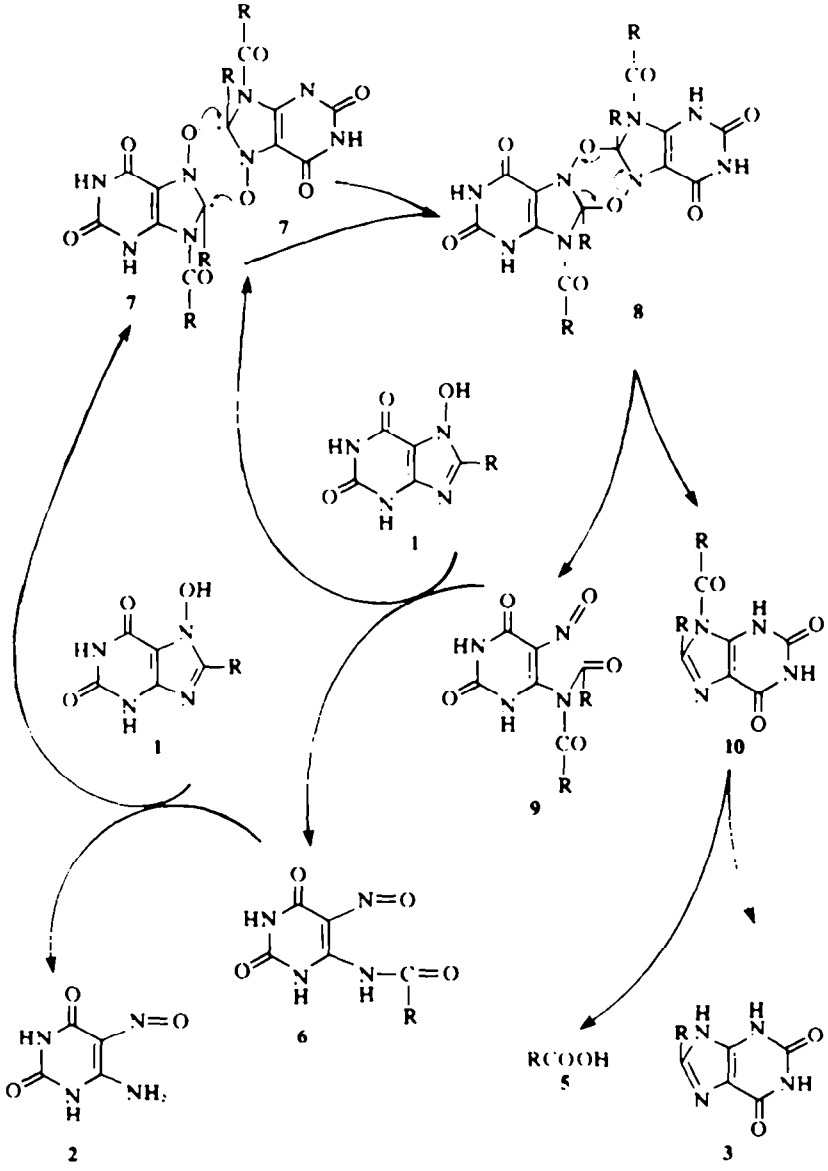
and by following the reaction at 350 nm it was possible to observe the accumulation of about 25% of 6-acylamino-5-nitrosouracil (**6**; Fig. 3, curve B). After the disproportionation reaction is over it undergoes a slow hydrolysis yielding **2**. The fast disappearance of 75% of **6** during the reaction suggests that the catalysis may involve transacylation. It is proposed that the availability of acylating agents determines the rate of this reaction and that acylation of **1** at position 9 may increase the electrophilicity of position 8 as shown in Scheme 2 and thus catalyze the reaction. Acylation on the hydroxyl at position 7 is more plausible, but it is assumed that the various acyl derivatives of **1** are in equilibrium as shown in similar cases.[7] A reasonable explanation of this reaction is given in the cyclic process which is given in Scheme 3. The acylation which is suggested may arise from a transacylation that involves intermediate **6** or some other intermediates as shown in Scheme 3.

Although such an acylation product (**7**) must be unstable it is assumed that once it is formed it dimerizes immediately to a dioxadiazine derivative (**8**) which undergoes a rapid rearrangement, which yields the diacylated nitroso derivative (**9**) and the corresponding acylated xanthine (**10**). The steric possibility of the formation of such an intermediate (**8**) was observed by using a model. The cyclization step of two 1,3 dipoles is assumed to be fast and thus the formation of **8** should be controlled by the presence of acylating agents. The two products (**9** and **10**) which are obtained by a concerted decomposition of **8** as shown by the arrows are both acylating agents. 9-Acyl-8-alkylxanthine (**10**) has acylating ability, being a derivative of N-acylimidazole. 6,6-Diacylamino-5-nitrosouracil (**9**), having two acyl groups on one nitrogen, would be expected to cause acylation even more readily than the mono acyl derivative (**6**). Compound **9** is converted to **6** which may acylate again. If the reaction proceeds through this mechanism there is a continuous formation of active acyl groups and any acyl group which is formed retains its reactivity through the whole process. Although compound **10** was not included in the transacylation cycle in the autocatalytic process it is assumed that it is also reactive.

The initiation of the reaction might proceed by the interaction of two zwitterions which are obtained by the



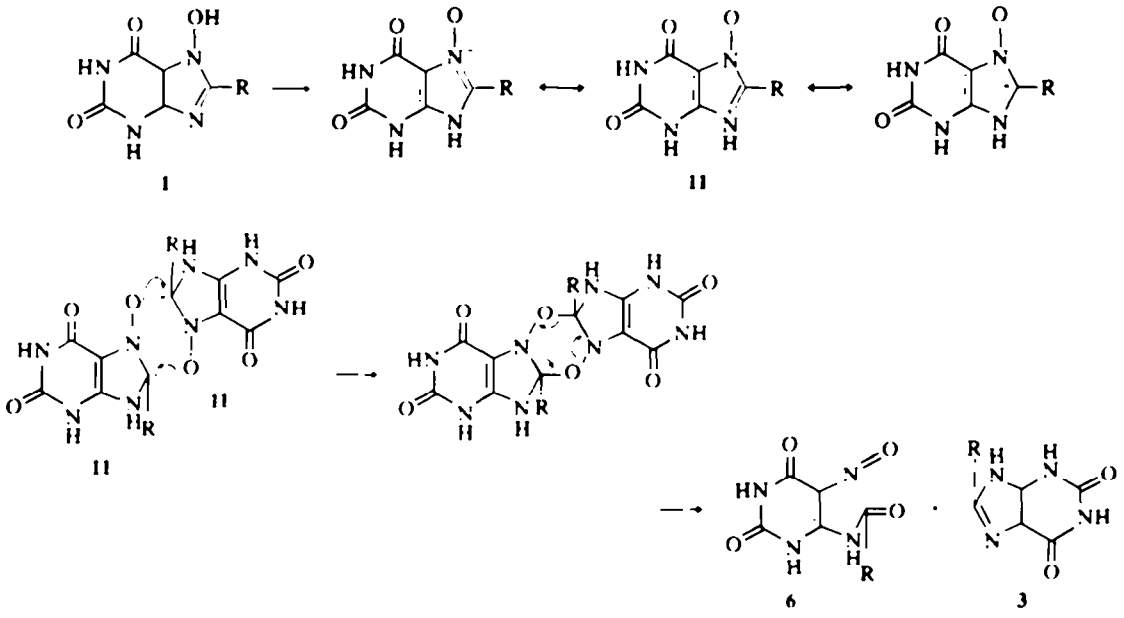
Scheme 2.



Scheme 3.

tautomerization of 1 as shown in Scheme 4. The N-oxide form 11 is a rare tautomer and therefore the initiation is very slow. It requires the interaction of two of these tautomers and that might explain the very slow initiation at low concentrations.

It was observed that this spontaneous autocatalytic



Scheme 4.

disproportionation has a pronounced dependency on the pH (Fig. 4). At pH's above 7 and below 4 the reaction is almost undetectable even at 50°, whereas near pH 5.0, in the same concentration (3×10^{-3} M), the disproportionation is almost complete after 30 min. The observation that the maximum rate of reaction occurs near the pKa of 1, when there are equal amounts of dissociated and undissociated molecules of 1 present, suggests that the initial bimolecular reaction might occur between an anion of 1 and a neutral molecule of 1. The first ionization involves the hydrogen of the N–OH group.[2] An alternative interpretation for the pH dependency is the range of stability of the acylating agents. Hydrolysis probably competes with transacylation. The latter is essential for the autocatalytic process and the optimal pH for transacylation should be, therefore, also optimal for the spontaneous disproportionation. It was indeed observed that the minimal hydrolysis of 6-acylamino-5-nitrosouracil (**6**) is near pH 5.

The temperature dependency of the spontaneous autocatalytic disproportionation reaction is shown in Fig. 5 and allows the calculation of the Arrhenius energy of activation and entropy of activation. The energy of activation was found to be about 10.6 kcal per mole and entropies of activation −19.4 and −11.3 e.u. at 32 and 50° respectively. An irregularity was observed at 70° (Fig. 5), the rate constant of the reaction is smaller than that expected. We assume that at this temperature the hydrolysis of acylating agents competes with the transacylation. 6-Acylamino-5-nitrosouracil, which is one of the acylating agents, was found to undergo rapid hydrolysis at 70°. The simultaneous formation of a nitroso group and a carbonyl group similar to this case has been observed earlier.[8,9]

The unsubstituted compound, e.g. 7-hydroxyxanthine is stable in all pH's and in all concentrations and even in boiling water. Therefore, we may conclude that the 8-alkyl group has a very pronounced influence on the chemical reactivity of the 7-N-oxide and probably enables this unusual reaction which results in reduction

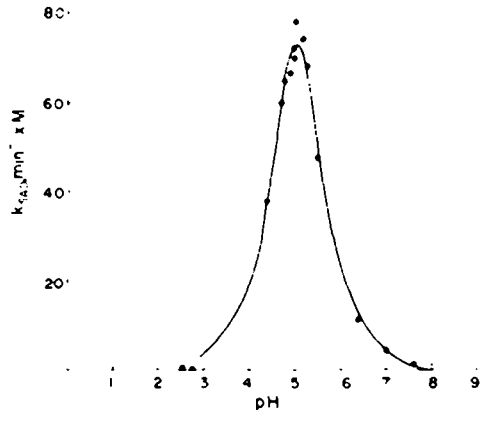


Fig. 4. Dependency of the spontaneous autocatalytic disproportionation on the pH, at 50°.

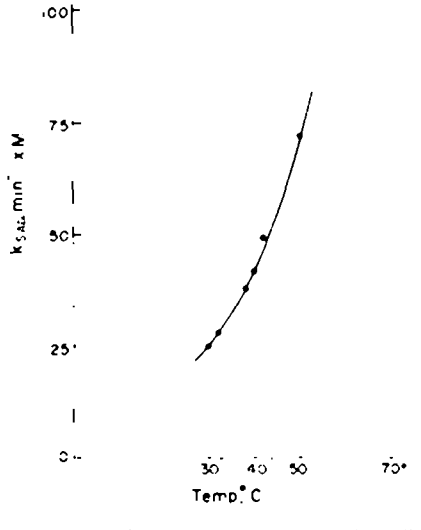


Fig. 5. Dependency of the spontaneous autocatalytic disproportionation on temperature, at pH 5.

on one hand and an oxidative cleavage on the other. One explanation for the effect of the alkyl group could be the stabilization of a positive charge, or better the enhancement of the electrophilicity of the carbon at position 8 by inductive or hyperconjugative effect. However, an alkyl group may also, assist in the approach of the two ends of two molecules by an hydrophobic effect, so that the solution contains head to head dimers linked by hydrophobic forces.

The spontaneous autocatalytic disproportionation described here may provide explanations of some earlier observations,[10,11] where the mechanisms of the "deoxygenation" or reduction of such N-oxides were obscure.

Anhydride induced disproportionation

When 8-alkyl-7-hydroxyxanthines (1) are treated with acetic anhydride, at neutral and basic pH's, an immediate decomposition takes place; even at very low concentrations it is complete within seconds. As shown above, at these concentrations and pH's the spontaneous autocatalytic disproportionation reaction does not occur. However, in the presence of half an equivalent of acetic anhydride the reaction is much faster and 6-acylamino-5-nitrosouracil (6) is the sole oxidized product. The latter's maximum at 334 nm accumulates during the course of the reaction. It is common that N-oxides that have an alkyl group at the adjacent position to the oxygen rearrange in the presence of anhydride to yield alcohols.[12] It was also shown[13] that in 3-hydroxy-8-methylxanthine the hydroxyl group migrates to the side-chain under the influence of acetic anhydride. In our case this kind of rearrangement is of a minor importance and only a small amount of 8-(α-hydroxyalkyl)xanthine is detected (Fig. 6).

In the reaction induced by acetic anhydride there is a large increase of absorption maximum at 334 nm in the first 5 sec, and is not influenced by oxygen nor by light. The initial reaction rate is increased with the increase in concentration of 8-alkyl-7-hydroxyxanthine (1) and is decreased by lowering the pH. However, in all these cases a plot of $\ln(OD_\infty - OD)$, which represents the overall reaction, vs time gave straight lines with apparent first-order constants $k_{AID} = 0.9$ and 1.4 min^{-1} at 32 and 36° respectively. By changing the concentration of acetic anhydride it is observed that the maximum conversion of 1 is when the number of equivalents of acetic anhydride is about half of those of the 8-alkyl-7-hydroxyxanthine (1; Fig. 7). At a lower pH the addition of more than a half equivalent brings about rather a decrease in the yield of the disproportionation products. Actually the introduction of an excess of acetic anhydride at pH lower than 4, a destruction of UV absorbing materials occurs.

The anhydride induced reaction may proceed in the same way as the spontaneous reaction, as shown in

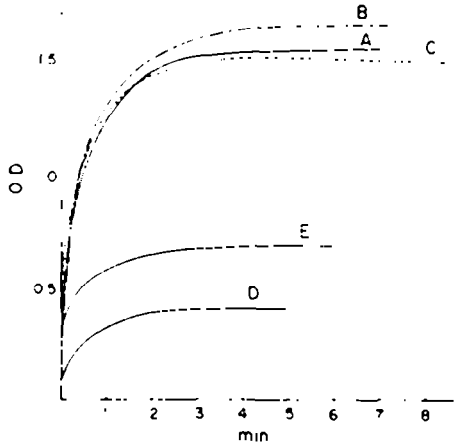


Fig. 7. Anhydride induced disproportionation of 7-hydroxy-8-propylxanthine (A) 2.67×10^{-4} M with 1.34×10^{-4} M of acetic anhydride at 32° and pH 7.0, (B) the same conditions as in A but with 2×10^{-4} M acetic anhydride, (C) the same as in A but at pH 6, (D) the same as in A but with 0.33×10^{-4} M acetic anhydride, (E) 5.3×10^{-4} M and 0.33×10^{-4} M acetic anhydride at 32° and pH 7.0. All experiments were followed at 334 nm (light path 1.0 cm).

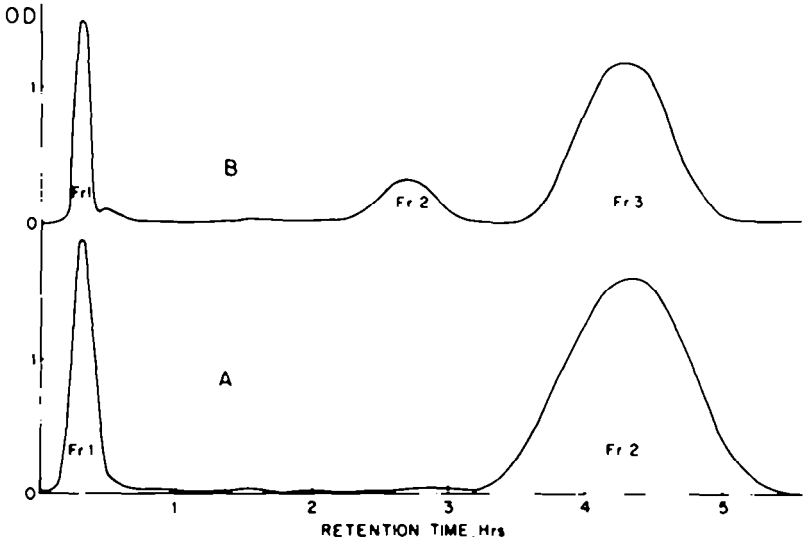


Fig. 6. Ion exchange chromatography of products of disproportionation: Using 40 × 120 mm column of Dowex-50-x8 of 400–300 mesh (H-form). Elution with HCl. Rate of flow 8 ml per min (280 nm). (A) separation of the acidified reaction mixture of the spontaneous autocatalytic disproportionation. (B) Separation of the acidified reaction mixture of the anhydride induced disproportionation.

Scheme 3. However, in the anhydride induced reaction the rate of formation of the bimolecular intermediate does not depend on supply of acylating agents from the reaction itself only. They are provided also externally. The rate determining step is the decomposition of **8** to **3** and **6**. The first order curve which is followed at 334 nm is apparently the decomposition of this intermediate.

Another mechanism that might explain the anhydride induced disproportionation reaction, but which cannot explain the spontaneous autocatalytic reaction, is suggested in Scheme 5. The anhydride may attack the oxygen of the N-oxide group. The next step would be the formation of a nitrene cation, which is assumed to be formed in all rearrangements of purine-N-oxides.[1,2,4] This cation would be attacked by the anion of another molecule of the N-oxide to form intermediate 14. All these steps would be fast and the decomposition of **14**, the rate determining step, would proceed with the aid of both H^+ and OH^- to form compounds **3** and **6** respectively.

In summary the two reactions that are described here, the spontaneous and the anhydride induced disproportionations share many features. Both are dependent upon some particular influence of the alkyl group at position 8. The difference between the two is that the one is spontaneous and the rate determining step is assumed to be the formation of a bimolecular intermediate which involves autocatalysis. In the second reaction the rate determining step is probably the decomposition of an analogous intermediate. These differences are reflected in the apparent orders of the two reactions, an autocatalytic second order in one case and a pseudo first order in the second.

Although 8-alkyl-7-hydroxyxanthines have not been reported to occur in nature, the reactions described here, which take place in aqueous solutions and at moderate temperatures, may have implications to biological processes.

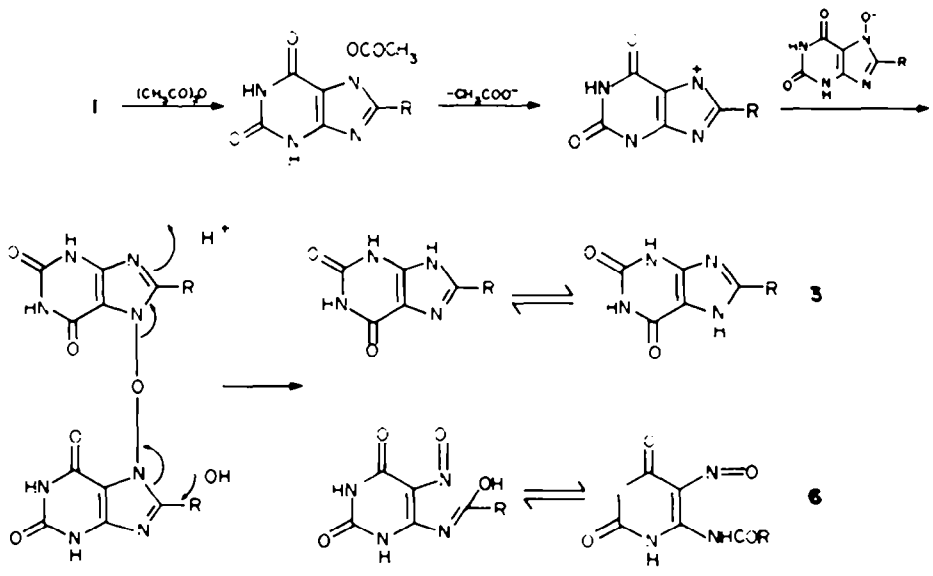
EXPERIMENTAL

UV measurements were carried out in a "Varian Techtrone" spectrophotometer Model 650 with an XY recorder. Adjustments of pH were done with a "Radiometer Copenhagen" pH-meter-29. Buffered solns were of 0.1 M phosphate and 0.1 M acetate. NMR spectra taken with a "Varian" Model T-60. "Fluka" Dowex-50- X8 of 400–300 mesh in the H-form was used as ion exchange resin in column chromatography. Elution of columns monitored with an "ISCO" UA-4 UV analyzer. Columns of 40 × 120 were used for separation and identification of products. 8-Alkyl-7-hydroxyxanthines (**1**) used here (mainly where R = propyl and sometimes hexyl or undecyl) were prepared as previously described.[3] Acetic anhydride of "Riedel-de Haën" was used and its standard solutions were prepared in dioxane.

Isolation and identification of products in the spontaneous autocatalytic disproportionation of 8-propyl-7-hydroxyxanthine

Method A. Compound **1**, (R = propyl; 0.53 g) was refluxed for 1 hr with stirring in 20 ml 0.2 M Na_2HPO_4 which was adjusted to pH 5.1 by the aid of HCl. The purple soln was kept overnight at room temp. The ppt was collected and boiled in 25 ml EtOH and filtered hot. The insoluble residue was boiled in 10 ml water cooled to room temp. and collected by filtration (0.1 g) m.p. > 300°, mixed m.p., and UV showed this product identical with an authentic sample of 6-amino-5-nitrosouracil.[1] The ethanolic soln was cooled overnight (10°) and the ppt which deposited was recrystallized from water and again from EtOH (0.12 g; m.p. ~300° dec.). Mixed m.p., NMR and UV showed this product identical with 8-propylxanthine obtained by method B, below. Additional crops could be obtained by evaporation of the solvents and chromatography as in method B.

Method B. 8-Propyl-7-hydroxyxanthine (0.53 g) was treated with 20 ml of buffer as above. The purple soln was acidified with conc. HCl (5 ml) and kept overnight at room temp. Without treatment with HCl, which causes the hydrolysis of **2** to **4**, the hydrolysis takes place during chromatography and complicates the separation. The resulting yellow soln was loaded on a 40 × 120 mm Dowex 50 (H-form) and eluted with 1N HCl. The elution was monitored at 280 nm and the results are shown in Fig. 6, Curve A. The two fractions were evaporated to dryness. The residue obtained in fraction 1 was recrystallysed from water (0.16 g) m.p. 270° (dec.). Mixed m.p. IR, UV and mixed Dowex-50 chromatography (10 × 120 mm) column) and elementary analysis proved the product identical with an authentic sample of violuric acid.[1] The residue from fraction 2 was recrystallysed from water (0.2 g) m.p. > 300 (dec.), UV (λ_{max}): in 1N HCl 232 and 263 nm, at pH 5 270 nm, at pH 10 280 and 240 nm, at pH 14 285 nm. NMR (δ) 0.85 triplet, 1.65 heptet, 2.50 triplet, 10.75 singlet, 11.45 singlet. The imidazole NH signal is diffused and sometimes



Scheme 5.

unobservable. (Found: C, 45.0; H, 6.0; N, 2.60. Calc. for $C_8H_{10}N_4O_2 \cdot H_2O$: C, 45.3; H, 5.7; N, 26.4%).

In derivatives of **1** with longer alkyl groups, the reaction was followed by UV and the products were identified by UV. Elution from Domex-50 was carried out by 1N HCl in 50–85% EtOH.

Kinetics and pH dependency of the spontaneous autocatalytic disproportionation reaction. 8-Propyl-7-hydroxyxanthine (10 mg) was dissolved in 1N NaOH (1 ml), 0.5 M Na_2HPO_4 (5 ml) and 1 M CH_3COONa (2.5 ml) were added and the soln diluted to 25 ml with water. In this pH the soln could be kept as a stock soln for a few days. Aliquots of 3 ml were brought to the desired pH by adding conc. HCl by means of a micro pippete and transferred immediately to a 1 mm light path UV cell. The UV change was followed at a particular temp. This was carried out either by taking the whole spectrum at intervals (380–200 nm) or by scanning at a constant wavelength, e.g. 314 nm or sometimes at two wavelengths (315 and 350 nm). Buffer solns were used as reference. In the same way temp. and concentration dependencies were studied. Solns of different pH's were prepared and treated as above.

The constant of the reaction was determined by plotting $\ln(OD/OD_\infty - OD)$ vs time, where OD is the optical density at 314 nm and OD_∞ is the optical density at the end of the reaction. The constant of this reaction was then drived from the slope: $k_{SAD} = (\text{slope}/A_0)$ where A_0 is the initial concentration of 1. This expression was derived from eqn (A) by considering the following facts: B_0 is very small and much smaller than A_0 and therefore $k_{SAD} \cdot t = (1/A_0) \ln (A_0/B_0) - (1/A_0) \ln (B/A)$ or $k_{SAD} \cdot t = \text{constant} + (1/A_0) \ln (B/A)$. B is the concentration of the products which absorb at 314 nm. A is the concentration of 1 during the course of the reaction and therefore: $A = A_0 - 2B$, for each equivalent of A only a half equivalent of B is formed. At the end of the reaction $2B_\infty = A_0$ so that $A = 2B_\infty - 2B$, therefore: $k_{SAD} \cdot t = \text{constant} - \ln 2 + (1/A_0) \ln OD/(OD_\infty - OD)$. The slope is $(1/t) \ln OD/(OD_\infty - OD)$ and therefore k_{SAD} (slope/initial concentration).

Energy and entropy of activation. Energy of activation was derived from the plot of $\ln k_{SAD}$ vs 1/T and according to Arhenius equation: slope $= (-E_a/R)$. Entropy of activation was calculated from k_{SAD} by the expression:

$$k_{SAD} = \frac{kT}{h} e^{\Delta S^{\ddagger}/R} \cdot e^{-\Delta H^{\ddagger}/RT} \text{ or } \Delta S^{\ddagger} = R\left(\ln k_{SAD} - \ln \frac{kT}{h} + \frac{\Delta H^{\ddagger}}{RT}\right)$$

where R is the gas constant, k and h are Boltzmann's and Planck's constants respectively, T is the absolute temperature and $\Delta H^{\ddagger} = E_a - RT$ and $\ln k_{SAD}$ is the logarithm of k_{SAD} in $\sec^{-1} \cdot M^{-1}$.

Independency of the spontaneous autocatalytic disproportionation on light or oxygen. Solns of 8-propyl-7-hydroxyxanthine were prepared as above and left in the dark. Eliquots were taken at intervals and the change in UV was examined. No changes from results obtained above were observed. Using oxygen free solutions, by bubbling of N_2, gave the same results as above.

Identification of the autocatalyst in the spontaneous autocatalytic disporportionation reaction. 8-Propyl-7-hydroxyxanthine (10 mg) was dissolved in basic phosphate soln as described above and brought to pH = 7.0 and then diluted to 20 ml with a buffer soln of the same pH (0.1 M phosphate and 0.1 M acetate). Aliquots (4 ml), into which a soln (1 ml) of the tested compound (2×10^{-2} M) in the same buffer was added, were brought to pH = 5.1 by means of conc. HCl. The mixture was transferred to a UV cell of 1 mm light path and the course of the reaction followed as above. Compounds which were thus tested were: **3**, **2**, butyric acid and **6**. Only the last compound (**6**) which was isolated from the AID reaction, described below, acted as catalyst. The results are shown in Fig. 5 and were discussed above.

The stability of 6-butyrylamino-5-nitrosouracil (**6**, R = $(CH_2)_2CH_3$). 6-Butyrylamino-5-nitrosouracil (about 1 mg) was dissolved in phosphate acetate buffer (25 ml) at pH = 7.0. Aliquots were taken and their UV spectra were taken in various pH's and temps. At pH's above 8 and below 4 at 30° a decomposition takes place followed by a change in UV from λ_{max} 334 nm to λ_{max} 314 nm. At 70° the decomposition is rapid also in pH 4–8.

Identification of products in the anhydride induced disproportionation reaction. Compound **1** (0.1 g) was dissolved in 0.5 M phosphate buffer at pH = 9.0 (25 ml) and a soln of 0.1 M AC_2O in dioxane (5 ml) was added with an efficient stirring. After 10–15 min a red product deposited. The purple ppt was **6** (R = propyl). The UV characteristic of this product at neutral solution: λ_{max} 334 nm ($\epsilon = 12000$), 243 nm ($\epsilon = 7700$) and a shoulder at 270 nm ($\epsilon = 4700$); λ_{min} at 290 nm ($\epsilon = 4500$). NMR or protons (δ): 0.90t (CH_3), 2.65 m (CH_2), 2.50 q (CH_2), 10.80 s (NH), 11.52s (NH), 12.5 s (NH). Upon hydrolysis it gave **2** and butyric acid. In a large scale chromatography which was carried out as described above in studying of the spontaneous disproportionation violuric and 8-alkylxanthine were isolated and identified. One additional fraction as shown in Fig. 6B was also isolated. From its UV and NMR data it is assumed to be 8-(α-hydroxypropyl)xanthine. This product has very similar UV properties to those described earlier[13] for 8-(α-hydroxymethyl)xanthine. NMR (δ): 0.88 triplet; 1.65 sextet; 4.45 triplet.

Kinetic study of the anhydride induced disproportionation reaction. A soln of 4×10^{-4} M of **1** (R = propyl; 2 ml) in 0.1 M phosphate and 0.1 M acetate at the desired pH was diluted to 3 ml with the same buffer soln in a UV cell (10 mm light path). A soln of 0.01 M AC_2O in dioxane (0.04 ml) was added rapidly with a micro pippete. The cell was shaken once and the UV change at 334 nm (30°) measured as soon as possible. Zero time was taken at the moment of introduction of the anhydride soln.

By using the same solns of 8-propyl-7-hydroxyxanthine and by varying the amounts of AC_2O the dependency of the reaction on the concentration of the latter could be studied. The range of pH was 5–8. Concentrations of **1** could also be varied by dilution with buffer soln. A soln of 4×10^{-4} M of **1** was stable for a few hours at all pH's.

The rate constant k_{AID} was determined by plotting $\ln(OD_\infty - OD)$ vs time, where OD_∞ and OD are optical density at 334 nm at the end and during the course of the reaction respectively. If the reaction is expressed by: $2A + \cdots \rightarrow B + \cdots$, where A and B are the concentrations of **1** and the product that absorbs at 334 nm respectively, A_0 and B_∞ are concentrations at the beginning and at the end of the reaction respectively. Assuming that the major reaction that occurs is the disproportionation reaction so that $B = 1/2A$ and $A = A_0 - 2B$ and $A_0 = 2B_\infty$, therefore, by using the expression for first order reactions we get: $k_{AID} \cdot t = \ln A_0 - \ln A = \ln A_0 - \ln(2B_\infty - 2B) = \ln A_0 - \ln 2 - \ln(B_\infty - B)$, therefore: $k_{AID} = \text{Slope of } \ln(OD_\infty - OD) \text{ versus time}$.

The independency of the anhydride induced disproportionation on light and oxygen was tested as above for the spontaneous reaction.

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