Purine *N*-Oxides. LVI. Photoisomerization of 1-Hydroxy- to 3-Hydroxyxanthine. Photochemistry of Related 1-Hydroxypurines[1]

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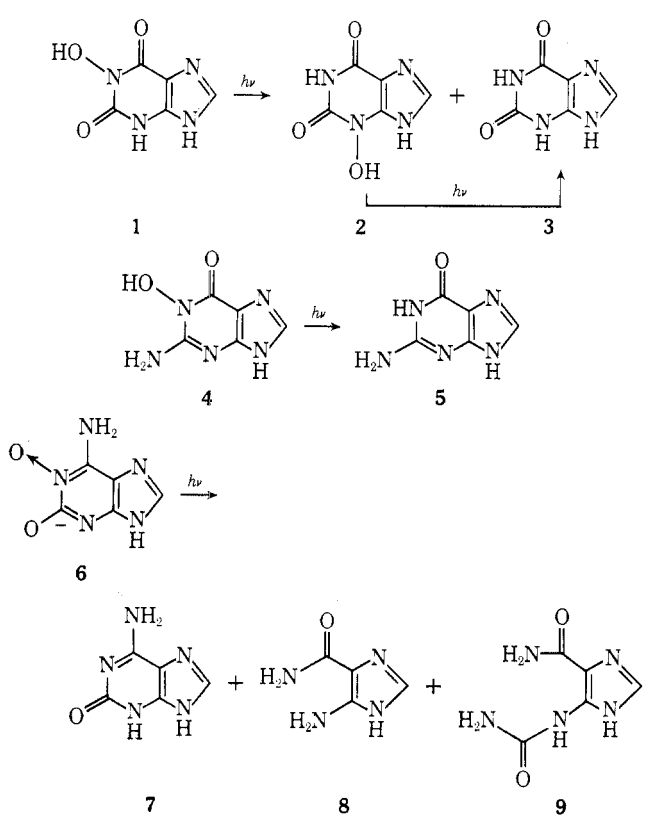
Ultraviolet irradiation of solutions of 1-hydroxyxanthine causes extensive photoreduction. Concomitantly, there is some photoisomerization to 3-hydroxyxanthine that is less rapidly photoreduced. This novel rearrangement of a hydroxyl from N-1 to N-3 occurs in either the neutral species of 1-hydroxyxanthine or its anion. Two structurally related purines, 1-hydroxyguanine and 1-hydroxyisoguanine, showed no evidence of comparable photoisomerization of the *N*-hydroxyls. The former undergoes photoreduction only, regardless of the ionic state. Irradiation of the cation of 1-hydroxyisoguanine yielded isoguanine and its 8-hydroxy derivative, while irradiation of the anion induced photoreduction and ring opening to two imidazoles, 4(5)-amino- and 4(5)-ureidoimidazole-5(4)-carboxamides.

Previous studies on the reactions of esters[2] of the oncogen[3] 3-hydroxyxanthine demonstrated that at certain pH's spontaneous reduction to xanthine is one mode of its reactivity. A comparable reduction of 3-hydroxyxanthine, or of 3-acetoxyxanthine, can be accomplished photochemically, either by direct uv irradiation in solution or by irradiation of the dry solid, to produce a free radical that is reduced instantly upon reaction with water.[4] These observations prompted a more detailed study of the photoinduced reactions of N-oxidized purines in solution.[5] Photoreduction and photorearrangements of oxygen from N to C are usually observed.[5-11] We now report that photoreduction of 1-hydroxyxanthine (**1**) (Scheme I) in solution is accompanied by a novel photoisomerization of the *N*-hydroxyl to form 3-hydroxyxanthine (**2**). This isomerization is of interest from both chemical and biological respects, since **2** is a potent carcinogen,[3] while **1** is not.[12] The photochemical reactivities of two structurally related derivatives, 1-hydroxyguanine (**4**) and 1-hydroxyisoguanine (**6**), are also examined.

Scheme I



Results

Each of the *N*-hydroxypurines was irradiated in deaerated solutions with a Corex filter at pH values selected to maximize the amount of a single ionic species. The pK_a's associated with the protonation and first two ionizations of 3-hydroxyxanthine (**2**) are 0.35, 6.71, and 9.65.[13] The neutral species of **2** was irradiated at pH 3 and the anion at pH 9; **2** was also irradiated at pH 0, where it is partially protonated. Xanthine (**3**) was the only uv-absorbing product in each case. The rate of photodecomposition of **2** increased significantly with increased pH. In Figure 1 the rates of the disappearance of **2** and the yields of **3** are plotted for the three pH's as a function of time.

The pK_a's for the protonation and first two ionizations of **1** are 0.85, 6.54, and 9.94.[14] Irradiation of **1** at pH's 0, 3, and 9 induced photoreduction to **3** (14–20%) and rearrangement to **2** (2–7%). Prolonged irradiation of **1** gave **3** only. The amounts of **1**, **2**, and **3** were determined following irradiation of **1** for various periods of time and the values are plotted as a function of time in Figure 2.

Irradiation of **4** (pK_a's 3.49, 6.73, and 11.51[15]) in solutions at either pH 2 for the cation, or at pH 5.5, where the neutral species should predominate, gave only the photoreduction product, guanine (**5**) (24–28%). The irradiation of the anion at pH 10 yielded mainly **5** (23%) with traces of two unidentified uv-absorbing compounds and an insoluble precipitate.

Because of its low solubility 1-hydroxyisoguanine (**6**) could be irradiated in a sufficiently concentrated solution only as its cation at pH's 0–3 or as its monoanion at pH 10 (pK_a's 3.64, 6.41, and 11.48).[14] The irradiation of **6** at pH 3 gave only the reduction product, isoguanine (**7**, 36%), and a trace of an unknown whose uv absorption suggests an imidazole. The irradiation at pH 0 gave **7** (36%), traces of an unidentified product, and the 8-hydroxy derivative of **7**, 6-amino-2,8-dihydroxypurine (1%). The last was identified by comparison of its uv spectra at three pH's with those of an authentic sample.[16] Comparable photooxidation at C-8 under acid conditions was noted previously.[5]

The first ionization of **6** was deduced[13] to occur from the *N*-hydroxyl group, and the species at pH 10 should be the enolate anion shown as **6** (Scheme I). Upon irradiation of the anion three uv-absorbing products were obtained, all in low yield. These include **7** (8%) and two products resulting from ring opening, 4(5)-aminoimidazole-5(4)-carboxamide (**8**, 3%) and 4(5)-ureidoimidazole-5(4)-carboxamide (**9**, 8%). The structure of **9**, which has not previously been reported, was deduced from its uv, nmr, and mass spectral properties. It was authenticated by comparison of

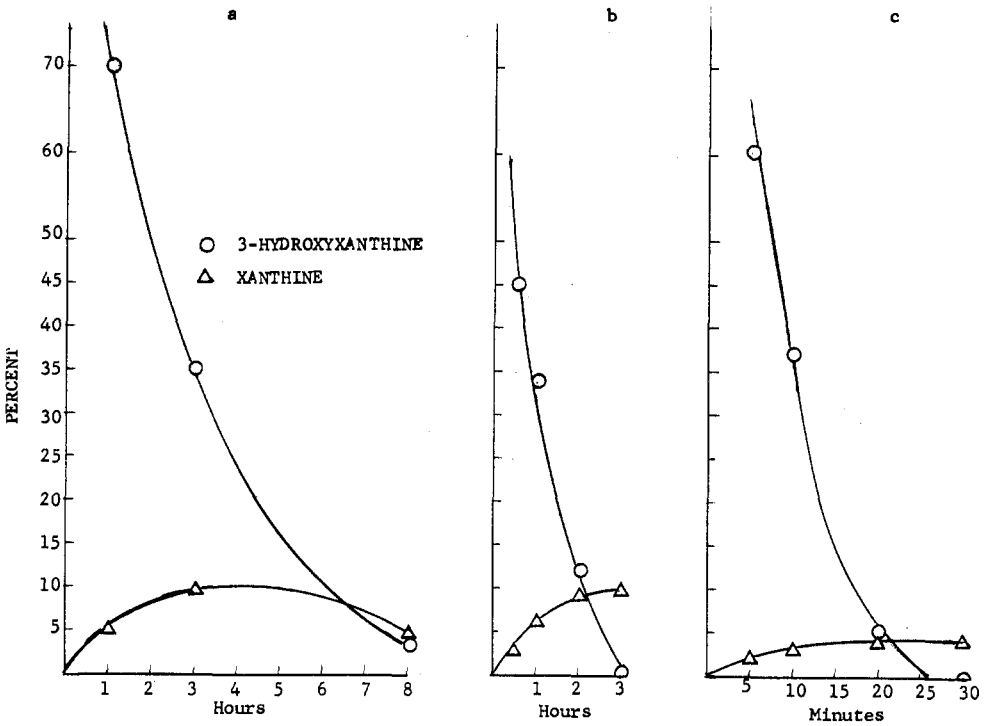


Figure 1. Irradiations of 3-hydroxyxanthine: (a) pH 0; (b) pH 3; (c) pH 9.

these and other properties with those of a sample synthesized from 8 and KCNO.

Discussion

An initial study[5] examined the influence of ionic and tautomeric states on the photochemical reactivity of 1-hydroxyhypoxanthine. That compound, with a single isolated hydroxamate function, was selected for its minimal tautomeric possibilities. Photoreduction was observed both from the neutral *N*-hydroxy species and from its conjugate enolate anion, but was favored when the neutral form predominated. Ionization was a prerequisite for photorearrangement, which was the predominant photoreaction of the anion.

The state of ionization also exerts a strong influence on the photochemistry of the more complex *N*-hydroxyxanthines. The several pK_a's of 1-hydroxy- (1) and 3-hydroxyxanthine (2) have been determined and the sequence of ionization of 3-hydroxyxanthine has been assigned as 3-OH, 9-H, 1-H.[13] This sequence parallels that of the parent xanthine.[17,18] For 1-hydroxyxanthine, ionization of the 1-hydroxyl group is not associated with the first pK_a, but with the second pK_a of 1.[14] These data and the known sequence of xanthine indicate that the ionization

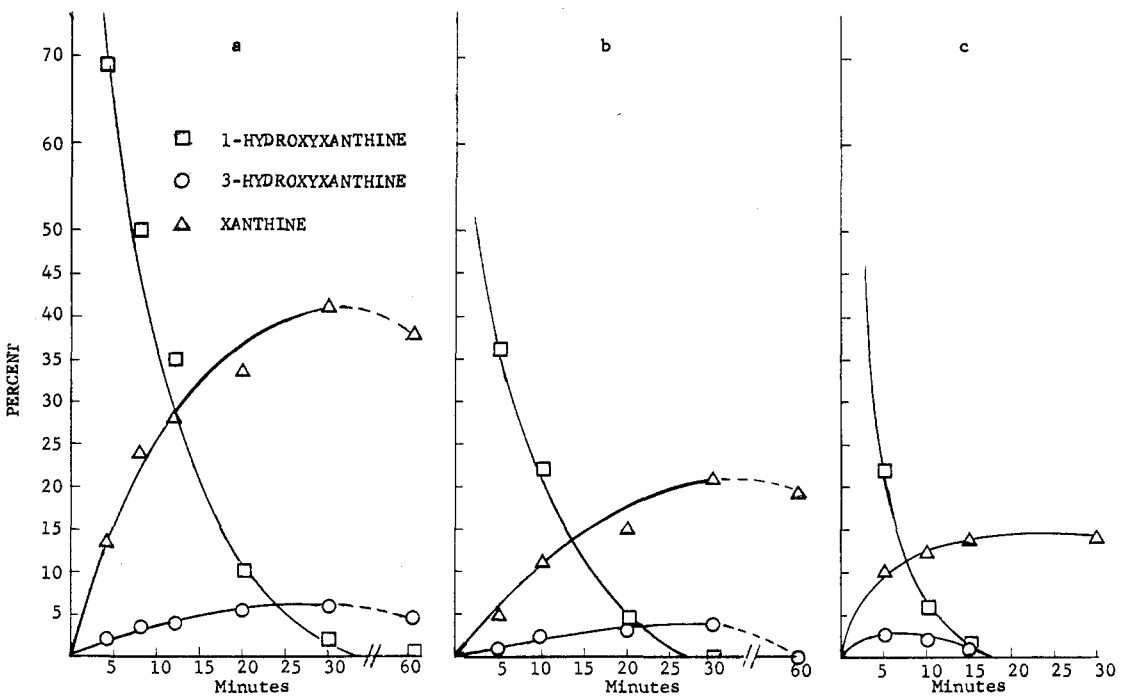


Figure 2. Irradiations of 1-hydroxyxanthine: (a) pH 0; (b) pH 3; (c) pH 9.

sequence of 1 is 3-H, 1-OH, 7,9-H. In general[13] an *N*-hydroxyl substituent lowers all of the ionization pK_a's for a compound. The digression of 1 from the usual ionization sequence, *i.e.*, N-1 ionization following that of N-3, can be attributed to the greater acid-strengthening effect of the 1-hydroxyl group on the pyrimidine moiety. Both 1 and 2 exist as the *N*-hydroxyl form in the neutral species.[13] The monoanions, however, must differ if each ionizes from N_3. The monoanion of 2 contains a nitrone group,[13] comparable to that of 1-hydroxyhypoxanthine, while the monoanion of 1-hydroxyxanthine should have no interaction with the N_1 hydroxyl, leaving it in the nonionized *N*-hydroxy form. Although the closeness of the second pK_a (9.94) of 1, that of the N_1 hydroxyl, makes it impossible to achieve a "pure" monoanion of 1, it is evident from uv spectra that there are different states of ionization of the *N*-hydroxyl groups in the monoanions of 1 and 2.

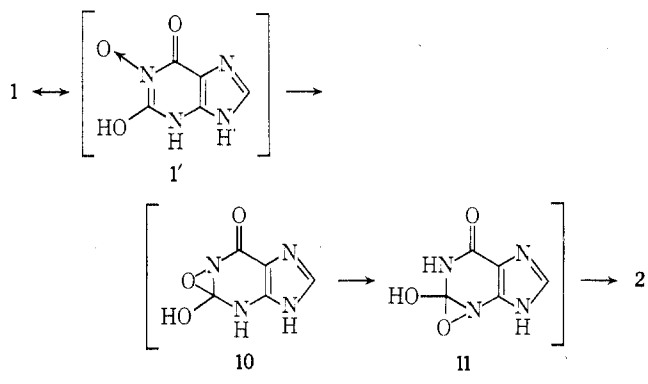
Photolysis of any ionic species of 2 (Figure 1) gave 3 as the only uv-absorbing product. The higher photodecomposition rate and poorer material balance with increasing ionization are analogous to results from the irradiations of 1-hydroxyhypoxanthine. Irradiation of 1, either as the neutral species (pH 3) or primarily as the monoanion (pH 9.0), gave qualitatively similar results (Figure 2), a complete loss of 1 in 30 min and comparable yields of 2 (4 and 2%) and of 3 (20 and 14%). These similarities agree with the deduction that the extent of ionization of the *N*-hydroxyl group is approximately the same for both the neutral species and the monoanion of 1. The small differences in rates of decomposition and yields might initially be attributed to a small degree of ionization of the N_1 hydroxyl at pH 9 to form some dianion. An alternative interpretation is discussed below.

The data for 1-hydroxyhypoxanthine indicated that the pK_a for its *N*-hydroxyl group was lowered 2–3 pH units in the excited state.[5] Therefore, 1 and 2 were each irradiated at pH 0, where ionization of the *N*-hydroxyl function should be suppressed even if the pK_a's of their excited states (pK_a*'s) are shifted to lower values. Should the pK_a*'s be lower than those of the ground states, a difference in the photochemical reactivities at pH 0, compared to those at pH 3, would be expected. The rate of photolysis of 3-hydroxyxanthine was decidedly slower at pH 0 (Figure 1a), but the rate of formation and apparent maximum yield of xanthine were identical with those from the irradiation at pH 3 (Figure 1b). The rate of photolysis of 1 at pH 0 (Figure 2a) was only slightly lower than that at pH 3, but the yield of xanthine at pH 0 (43%) was twice that obtained at pH 3 (21%). This difference suggests that the pK_a of the *N*-hydroxyl proton of 1 is lowered in the excited state, probably to below pH 3. This pK_a* would then be below that of the ground-state N_3-H pK_a (6.54) and consequently in the excited state both N_3 H and N_1 OH should be completely ionized at pH 9. This deduction clarifies the observation that ionization of the N-3 proton has little effect on the photoreactivity of 1. The small differences in the data at pH's 3 and 9 correspond to a completion of ionization of the *N*-hydroxyl at pH 9 and not to the partial ionization indicated by ground-state pK_a's. These data indicate that photoreduction of 1 is favored by the presence of the nonionized *N*-hydroxyl species, predominant at pH 0, but that it can occur to a smaller extent from the ionized form.

The unexpected photoisomerization of 1-hydroxy- to 3-hydroxyxanthine was observed at all pH's studied (Figure 2). The yield of 2 was maximal at pH 0 and decreased with increasing pH. This is partially due to the greater photolability of 2 at higher pH's (Figure 1). The 6% yield of 2 after irradiation of 1 at pH 0 for 30 min (Figure 2a) is essentially a maximum formation of 2 under these conditions, since 2 was not significantly degraded within 30 min under comparable conditions (Figure 1a). By contrast, the 4% of 2 formed after irradiation of 1 for 30 min at pH 3 (Figure 2b) does not represent a maximum yield, since over half of any 2 formed would have been decomposed during this period (Figure 1b). The corrected yield of 2 may be estimated as ~8%. Similarly, the maximum yield of 2 isolated after irradiation of 1 at pH 9 for 5 min was 2.5%, but at that time ~40% of 2 would have been decomposed, and the corrected value of 2 is ~4%. The high photolability of both 1 and 2 at pH 9 reduces the accuracy of this estimated yield, but it is certainly less than that at pH 3.

A plausible mechanism for the rearrangement of an *N*-hydroxyl group from N-1 to N-3 can be suggested based upon mechanisms proposed for other photoisomerizations. Ionization of the *N*-hydroxyl was shown to be necessary for N to C photorearrangement of 1-hydroxyhypoxanthine, and it was postulated that the nitrone component of the anion rearranged *via* an intermediate oxazirane.[5] If 1 to 3 photoisomerization is a comparable intramolecular process, it should also occur preferentially from a nitrone-containing species. It would thus be dependent upon ionization of the *N*-hydroxyl group and should increase with increasing pH, as noted for N to C photorearrangement of 1-hydroxyhypoxanthine. The increased photolability of 2 at higher pH's makes it difficult to evaluate this accurately, but the estimated corrected values for maximum yield of 2 show that ionization of the *N*-hydroxyl of 1 does not enhance its migration. Although little difference was noted in yields of 2 between pH's 0 and 3, the large change in yields of xanthine indicates that in this pH range there is some change in the form of 1 that influences its photochemical reactivity. This was interpreted to indicate that the nonionized *N*-hydroxyl species was present to a greater extent at pH 0 and that 1 must have a pK_a* in this range. The absence of a parallel change in the yield of 2 suggests that formation of 2 is not associated with ionization of 1 in this range. One plausible intramolecular[19] mechanism that is consistent with rearrangement *via* a nitrone intermediate without ionization involves a photoinduced enolization of 1. If 1 is converted to an enol, *e.g.*, 1′ (Scheme II), as a primary photochemical

Scheme II

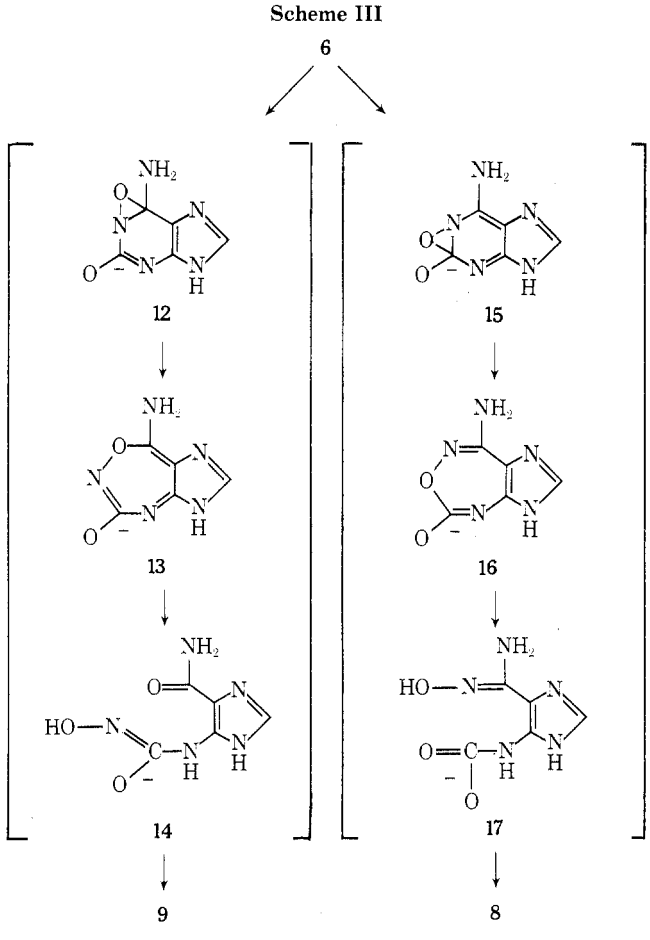


process,[20] the nitrone thus formed could then be photochemically converted to an oxazirane (10) comparable to that proposed for N to C rearrangements.[21] Since the adjacent position is substituted, 10 might then undergo a subsequent rearrangement to the isomeric oxazirane (11) and thence to 2. Sequential oxazirane migrations have been proposed previously in the photochemical isomerizations of *N*-oxides,[22] but this is the first example of a photoinduced allylic N to N migration.

Two other 1-hydroxypurines structurally related to **1**, 1-hydroxyguanine[15] (**4**) and 1-hydroxyisoguanine[14] (**6**), were studied as possible additional examples of such an *N*-hydroxyl rearrangement. Irradiation of **4** at selected pH's yielded none of the known[30] 3-hydroxyguanine, but produced guanine (**5**) as the only, or the predominant, uv-absorbing product.

The possible rearrangement product from 1-hydroxyisoguanine (**6**) would be 3-hydroxyisoguanine. That compound is not reported, but certain of its properties can be predicted by analogy to those of other known purine 3-oxides.[8,9,31] There was no evidence of such a product. The photoproducts obtained from the irradiation of **6** in acidic solution were isoguanine (**7**, 36%) and 6-amino-2,8-dihydroxypurine (1%). Irradiation of the anion produced isoguanine (**7**, 8%), 4(5)-aminoimidazole-5(4)-carboxamide (**8**, 3%), and 4(5)-ureidoimidazole-5(4)-carboxamide (**9**, 8%). Comparable products resulting from ring opening of intermediates have been isolated from irradiations of heterocyclic *N*-oxides.[32] One suggested[33] route for formation of such products involves initial rearrangement of the *N*-oxide to an oxazirane, ring expansion, followed by hydrolytic ring cleavage of the ring-expansion product. Since the first ionization of **6** produces a nitrone-containing enolate anion, the parallels previously noted[5] between the photochemical reactivity of such anions and heterocyclic *N*-oxides should also be applicable to that of **6**. Two isomeric oxaziranes, **12** and **15** (Scheme III), could form from **6**. Ring expansion[21] of these would lead to the isomeric imidazolooxadiazepines, **13** and **16**, respectively. Hydrolysis of these would yield initially the two disubstituted imidazoles, **14** and **17**. The ureido derivative isolated, **9**, can only arise from the *N*-hydroxyureide, **14**, or its precursor, **13**.[35] This suggests that the oxazirane **12** is a requisite intermediate from **6**.

Scheme III



No plausible path from **12**, **13**, or **14** to **8** is obvious, nor does **8** arise experimentally from **7** or **9** under the conditions employed. A facile explanation for the formation of **8** is available from reactions of the isomeric oxazirane, **15**, *via* the path **15** → **16** → **17** → **8**. Following ring opening of **16**, both the formate and amidoxime groups of **17** must hydrolyze to lead to **8**. Thus oxazirane **15** apparently undergoes reactions other than rearrangement to 3-hydroxyisoguanine.[36]

These studies demonstrate that only certain structural systems permit migration of the hydroxyl from N-1 to N-3. Under some conditions oxazirane formation occurs in a direction unfavorable for 1 to 3 migration, as suggested by the formation of **9** from **6** *via* **12** (Scheme III). Even the appropriate oxazirane intermediates can be diverted to other reactions, as shown by the production of **8**. The hydroxyl isomerization apparently requires both carbonyl groups, since replacement of either carbonyl of the pyrimidine moiety prevents rearrangement. No comparable 1 to 3 rearrangement was observed with 1-hydroxyhypoxanthine,[5] nor has any reverse 3 to 1 hydroxyl migration been noted from **2**, although the relative photochemical sensitivities of **1** and **2** would make detection of **1** from **2** difficult. The requisite structural features for the rearrangement have thus far been found only in 1-hydroxyxanthine.

Experimental Section

The uv spectra were determined with a Unicam SP800A recording spectrophotometer and the nmr spectra with a Varian A-60 spectrometer, using TMS as an internal standard. An ISCO UA-2 uv analyzer was used to monitor column eluates, except as noted for values in Figures 1 and 2. The λ_{max} and ϵ values were determined with a Cary 15 spectrophotometer. Elemental analyses were performed by Spang Microanalytical Laboratories. Ann Arbor, Mich. Paper chromatograms were developed, ascending, on Whatman No. 1 paper using the following solvents: (A) CH_3CN–H_2O–28% NH_4OH (7:2:1 v/v); (B) 3% NH_4Cl; (C) 5% Na_2HPO_4–isoamyl alcohol (3:2); and were viewed under uv light (253.7 nm). Samples of **7** and **8** were obtained from Cyclo Chemical Co. for comparison with photoproducts from **6**.

Irradiation Procedures. Samples were irradiated in 1.2×10^{-3} *M* solutions that had been adjusted to pH 3.0 or 9.0 with 1 *N* HCl or 28% NH_4OH; 3 *N* CF_3COOH was used for pH 0. Nitrogen was bubbled through solutions for 2 hr prior to irradiations that were then carried out in an immersion apparatus with a 450-W Hanovia high-pressure mercury lamp with a Corex filter, as described.[5] Aliquots were withdrawn periodically and the photoproducts were analyzed by ion exchange chromatography. For identification the solutions were concentrated *in vacuo* to a small volume when the reactions were complete and the products were separated by chromatography.

Chromatography. Photolysis products were separated with a Bio-Rad AG-50, X8 [H^+], 200–400 mesh column (9 × 220 mm) that was monitored with an ISCO uv analyzer. Yields of reaction products were calculated from their known ϵ_{max} values. The λ_{max} and ϵ_{max} values at pH 0 were determined to be 267 nm (7.0×10^3) for **8** and 255 nm (11.4×10^3) for **9**. The quantities of **1**, **2**, and **3** in the mixture of products following the irradiation of **1** for various times were determined with a standardized AG-50 [H^+] column (9 × 150 mm) that was pumped at 60 ml/hr and was monitored at 240, 260, and 290 nm with a Beckman DB spectrophotometer. The column was eluted with 0.05 *N* HCl, and the products were isolated in the sequence (ml) **2** (85),[37] **1** (185), **3** (340).[37] Linear plots of known concentrations of **1**, **2**, and **3** against their OD values at 260 nm were used as calibration curves to calculate the yields shown in Figures 1 and 2. Values were reproducible within ±5%.

Identification of 3-Hydroxyxanthine (2). This photoproduct from **1** was unambiguously identified by comparisons of it with an authentic sample[13,30] of **2**. The uv absorption at selected pH's of a sample of the photoproduct isolated from a Bio-Rad AG-50 [H^+] column was identical with values reported[13] for **2** at those pH's. The R_f values of both were identical in three solvents: A, (R_f), **1** (0.09), **2** (0.09), **3**, (0.28); B, **1** (0.57), **2** (0.56), **3** (0.34); C, **1** (0.58), **2** (0.60), **3** (0.47). While the R_f values of **1** and **2** are close in all solvents, they are easily distinguished when the paper is viewed

under uv light; **1** appears as a dark purple spot, but **2** has blue fluorescence. The photoproduct also manifested blue fluorescence identical with that of **2** under uv light. The photoproduct and authentic **2** had identical positions of elution from two standardized columns. From the AG-50 [H^+] column both appeared at 85 ml. From a Bio-Rad A-6,[38] 6 × 400 mm column, eluted at 50° with 0.4 *M* ammonium formate (pH 4.7) at 20 ml/hr and monitored with the Beckman DB spectrophotometer, authentic **2** and the photoproduct were eluted at 17.8 ml, **3** at 20 ml, and **1** at 22 ml.

4(5)-Ureidoimidazole-5(4)-carboxamide (9). A solution of 267 mg (3.3 mmol) of KCNO and 198 mg (3.3 mmol) of HOAc in 20 ml of H_2O was added to a solution of 340 mg (3.3 mmol) of **8** in 20 ml of water. The clear solution was stirred at room temperature overnight, the solvent was then evaporated to dryness *in vacuo*, and the brown residue was dissolved in ~40 ml of methanol. After filtration the solvent was removed *in vacuo* and the residue was chromatographed on a 2.4 × 24 cm AG-50 [H^+] column by elution with 1 *N* HCl to yield first **9** (35 mg) and then **8** (150 mg). The crude HCl salt of **9** was neutralized by passing an aqueous solution of it through a Bio-Rad AG-3 [OH^-] column and eluting with H_2O. The eluate was evaporated *in vacuo* to give 30 mg (11%) of pure 4-ureidoimidazole-5-carboxamide: mp 230° dec; nmr (CF_3CO_2H) δ 6.96 (s); nmr (Me_2SO-d_6) a broad, unresolved multiplet centered near δ 7.0 (The addition of D_2O caused collapse to a singlet at δ 7.35. The multiplet integral was seven times that of the singlet.): mass spectra (chemical ionization) *m/e* 170 (M + 1), 153, 127, and 109 (major peaks); uv λ_{max} (H_2O) (pH) 240, 255 (2), 232, 267 (6), and 281 nm (12).

Anal. Calcd for $C_5H_7N_5O_2$: C, 35.51; H, 4.17; N, 41.40. Found: C, 35.35; H, 4.23; N, 41.47.

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Registry No.—**1**, 1932-15-6; **2**, 13479-29-3; **8**, 360-97-4; **9**, 51022-63-0.

References and Notes

(1) This investigation was supported in part by funds from the National Cancer Institute (Grants No. CA 08748 and 15274) and from the Atomic Energy Commission (Contract No. AT[11-1]-3521).
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(19) An intermolecular mechanism seems unlikely. It would require an oxygen species, possibly a hydroxyl radical, to attack N_3 initially and be followed either by loss of hydrogen to lead directly to **2** or by loss of HOH to yield a radical at N_3 that would then combine with a second hydroxyl radical. Both lack precedents. Hydroxyl radicals have been reported to add only at C_6 or C_8 of purines: C. Nofre, A. Lefier, and A. Cier, *C. R. Acad. Sci.*, **253**, 687 (1961), and ref 5. To test such unlikely routes, xanthine and H_2O_2 were irradiated under the usual conditions. Hydroxyl radicals have been thus generated from H_2O_2 previously: R. O. C. Norman and G. K. Radda, *Proc. Chem. Soc.*, 138 (1962). No *N*-hydroxyxanthine and no uric acid were detected. In fact, no uric acid was detected in any of the irradiations of **1** or **2**, although 6,8-dihydroxypurine was formed at low pH's by irradiation of 1-hydroxyhypoxanthine.[5] These considerations enhance the probability of an intramolecular mechanism.
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(22) Sequential migrations of oxazirane intermediates have been suggested to explain the rearrangements of 2-cyano-4-methoxyquinoline *N*-oxide to 2-cyano-3-hydroxy-4-methoxyquinoline;[23] of 1-phenyl- and 1-cyanoisoquinoline *N*-oxides to benz[*f*][1,3]oxazepines;[24] and of 3-phenylquinoline *N*-oxide to 4-phenylbenz[*d*][1,3]oxazepine.[21,25] Other examples have been proposed in the photoinduced rearrangements of tetrahydroacridine 10-oxide derivatives,[26] acridine 10-oxides,[27] phenazine 5-oxide,[28] and quinoline *N*-oxide-2-*d*.[29]
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(35) It is not apparent whether photolytic N–O cleavage in **13** leads directly to **9** or whether **13** is hydrolyzed to **14** which is then subject to photoreduction.
(36) Several possibilities might explain this: (a) 3-hydroxyisoguanine is much more photolabile than might be expected from comparison of the rates of photoreaction of **1** and **2**; (b) ring expansion of the 1,2-oxazirane, **15**, is more facile than conversion to the isomeric 2,3-oxazirane comparable to **11**; or (c) ring expansion of the 2,3-oxazirane is favored over stabilization as 3-hydroxyisoguanine. The latter two are more likely; the third also represents an alternative path to **8**, since the imidazolooxadiazepine from the 2,3-oxazirane might also be expected, after ring opening, to lead to **8**.
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