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PHOTOCHEMISTRY OF PURINE 3-OXIDES IN HYDROXYLIC SOLVENTS

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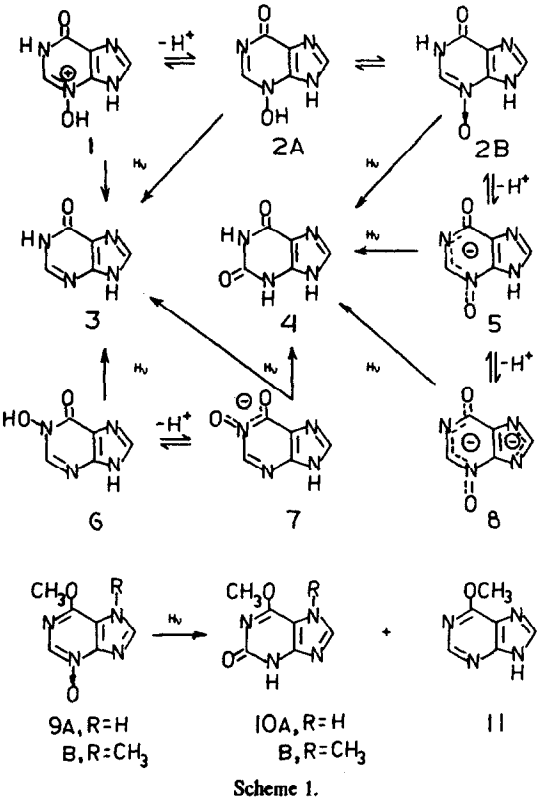
Abstract—UV irradiation of the potent oncogen hypoxanthine 3-oxide in aqueous solution induces elimination of and rearrangement of the nitrogen-bound oxygen. The extent of each reaction shows a complex variation over the pH range 0–7. The variations in quantum yield for product formation are shown to result from the presence in the neutral molecule of tautomeric species with differing photochemistries that ionize in the excited state ($pK_a^* \sim 3.5$) just above the protonation pK_a (1.2). The photochemical reactivity of each ionic and each tautomeric form was assigned by comparing the effect of pH changes between 0 and 11 on the quantum yields for formation of each photoproduct from hypoxanthine 3-oxide with those of two model compounds, 1-hydroxyhypoxanthine and 6-methoxypurine 3-oxide. Photoreduction of the 3-oxides occurs *via* the triplet state. This process has a relatively consistent low quantum yield ($\Phi = 0.005$ to 0.04) for most ionic and tautomeric forms of both purine 1-oxides and purine 3-oxides. Photorearrangement is a much more efficient process for purine 3-oxides ($\Phi = 0.3$) than for purine 1-oxides ($\Phi = 0.04$).

One aspect of coordinated investigations[1,2] to elucidate the chemical and metabolic events leading to tumour induction by oncogenic N-oxidized purines has been studies of their photochemical reactions in solution.[3–5] Since UV irradiation of the solids of these oncogens readily induces radicals[6,7] that are highly reactive in solution, we sought to obtain a basic understanding of the reactions and properties of photochemically-produced species. Such studies proved indispensable in evaluating the possible role of radical intermediates in the spontaneous reaction *in vitro* of purine N-oxide esters.[8] These were models for the "activated" form produced metabolically *in vivo* and thought to be essential intermediates for the initiation of oncogenesis by purine *N*-oxides.[1,9–11] Within the series of *N*-oxidized heterocycles examined as oncogens, purines *N*-oxidized at the 3-position have exhibited the strongest ability to induce tumors in experimental animals.[12]

Earlier photochemical studies of purine 3-oxides showed that 3-hydroxyxanthine, one of the most potent oncogens in the purine *N*-oxides series,[13] was readily photoreduced[4] while purine 3-oxide and its 6-methyl derivative underwent rearrangement of the oxygen at *N*(3) to the adjacent carbon with negligible photoreduction.[14] We now report a study of the photoreactions of the potent oncogen,[15] hypoxanthine 3-oxide, **2A,B** (Scheme 1). This *N*-oxide affords two main photoproducts. The proportion of these shows an unusually high dependence on pH. Comparison of the product ratios and product quantum yields from **2A,B** to those of model compounds that are known to exist only in specific *N*-oxide or *N*-hydroxy forms indicated that the unusual variation in product ratios with changes in pH is attributable to the photoreactions of several tautomeric and ionic contributors that are present in overlapping pH regions. From the variation in quantum yields with changes in pH and reference to comparable values for compounds of known structure, the quantum yield for formation of each photoproduct from each tautomeric and each ionic form of hypoxanthine 3-oxide were determined over the pH range 0 to 11.



Scheme 1.

EXPERIMENTAL

Materials. 1-Hydroxyhypoxanthine,[16] hypoxanthine 3-oxide and 6-methoxypurine 3-oxide[17] were prepared by reported procedures and were purified by chromatography and recrystallization prior to irradiation. The purified samples exhibited single peaks when examined by analytical HPLC. The UV absorption values (ϵ) of 1-hydroxyhypoxanthine, **6**, are pH 3, 250 nm (8300); pH 8, 228 (36,000), 259 (5600); and pH 13, 229 (44,000), 264 (6400).[16] UV absorption values for hypoxanthine 3-oxide, **2**, and 6-methoxypurine 3-oxide, **9A**, are listed in Table 2. NMR spectra were determined in $(CD_3)_2SO$ using Me_4Si as an internal standard with a JEOL PFT-100 NMR spectrometer, UV spectra were deter-

mined with a Cary 15 Recording Spectrophotometer and melting points were determined with a Mel-Temp Apparatus and are uncorrected. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, Michigan. Deionized H_2O was glass distilled and reagent grade CH_3OH was dried over Lind Type 4A molecular sieves and glass distilled prior to use.

6-Methoxy-7-methylpurine 3-oxide, **9B**. 6-Methoxy-7-methylpurine (1.0 g. 6.1 mmol), prepared from 6-chloro-7-methylpurine[18] by reaction with $NaOCH_3/CH_3OH$, was dissolved in CF_3CO_2H (8 ml) and 30% H_2O_2 (4 ml) and allowed to react at room temperature for 12 days. The solvents were removed under reduced pressure and unreacted starting material was removed by extraction of the residue with two 200 ml volumes of ether. The product was recrystallized from CH_3OH–EtOAc to afford 0.44 g (49%) of **9B**: mp 162–164°; NMR δ 8.62 (s, 1, CH), 8.50 (s, 1, CH), 4.10 (s, 3, OCH_3), 4.05 (s, 3, NCH_3); UV λ_{max} (ϵ) pH 0, 257 (7300); pH 7, 225 (10,200), 262 (5240), 287 (4260). Calc. for $C_7H_8N_4O_2H_2O$: C, 42.42; H, 5.08; N. 28.27. Found: C, 42.80; H, 5.08; N, 28.26%.

Procedures for irradiations

(a) *Preparative scale irradiations.* Solutions of hypoxanthine 3-oxide, **2** (15.5 mg/350 ml H_2O, pH 6), 6-methoxypurine 3-oxide, **9A** (27 mg/350 H_2O, pH 6), and 7-methyl-6-methoxypurine 3-oxide, **9B** (32 mg/250 ml CH_3OH), were deoxygenated with N_2 for 30 min, then were irradiated with an uncalibrated 450 W Hanovia Type L high pressure Hg lamp (major emission wavelengths [watts]: 360 nm [25.6], 313 (13.2], 302 [7.2], 296 [4.3]) through a 1 mm Corex 9700 filter (< 10% transmission at λ < 260 nm). Progress of the photolyses was monitored by inspection of the UV spectra of aliquots withdrawn periodically. When no further spectral changes occurred, the solvents were removed and the products were isolated as described below; yields are based on weight of starting material.

Products from hypoxanthine 3-oxide were separated by chromatography over a 9 × 230 mm column packed with Dowex 50 [H^+], 200–400 mesh, 8X resin that was eluted with H_2O and 1N HCl. The yield of isolated xanthine, **4**, (Scheme 1) was 65% and of hypoxanthine, **3**, 8%. Product identities were confirmed by comparison of UV spectral values of the photoproducts to those reported for hypoxanthine[19] and xanthine[20] at several pH's.

Products from 6-methoxypurine 3-oxide, **9A**, were isolated by washing the residue with CH_3OH to remove starting material and traces of 6-methoxy-purine, **11**, then recrystallizing the remaining 2-hydroxy-6-methoxypurine, **10A**, from H_2O; yield 12 mg (44%): mp > 250°; UV λ_{max} (ϵ) pH 0, 280 nm (9500); pH 7.0, 283 (10,300); pH 11, 286 (10,400). Calc. for $C_6H_8N_4O_2$: C, 43.37; H, 3.61. Found: C, 43.28, H, 3.73%.

NMR δ 8.02 (s, 1, CH), 3.96 (s, 3, OCH_3). The identity of **10A** was also confirmed by the fact that it could be readily hydrolyzed to xanthine, **4**, by heating a solution of **10A** in dilute acid.

The irradiation of 6-methoxy-7-methylpurine 3-oxide, **9B**, in CH_3OH afforded essentially pure 7-methyl-2-hydroxy-6-methoxypurine, **10B**, which was washed with EtOH and dried; yield 17 mg (56%): mp > 140° (dec); UV λ_{max} (ϵ) pH 2.0, 230 sh (4770), 282 nm (15,300); pH 7.0, 230 sh (5150), 284 (10,700); pH 11, 233 sh (6500), 287 (11,760); NMR δ 11.96 (b, s, 1, NH), 8.60 (s, 1, CH), 3.96 (s, 3, OCH), 3.81 (s, 3, NCH_3). Calc. for $C_7H_8N_4O_2$: C, 46.66; H, 4.67; N, 31.09. Found: C, 46.49; H, 4.62; N, 30.57%.

The identity of **10B** was similarly confirmed by hydrolysis to 7-methyl-xanthine in acid.

(b) *Analytical scale irradiations.* Values in Fig. 1–3 were determined from irradiations of solutions containing 1.3×10^{-3} *M* of the compounds in sealed, deoxygenated, aqueous solutions of the designated pH's in 100 ml quartz flasks. Appropriate concentrations of HCl were used for pH's 0 to 2 and 0.02 *M* chloroacetate (pH 2.0–3.0), formate (3.0–4.0), acetate (4.0–5.0), succinate (5.0–6.0), citrate (6.0–6.5), phosphate (7.0–7.5), *Tris* (8.0–8.5), borate (9.0–9.5), carbonate (10.0–10.5) and butyl amine (11.0) buffers[21] were used for higher pH's. Solutions were deoxygenated for 30 min with N_2 prior to irradiation. Photolyses were performed for 2 min in a Rayonet photochemical chamber reactor (Model RPR-100) equipped with low pressure Hg lamps

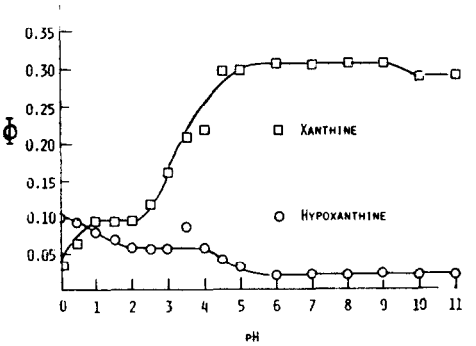


Fig. 1. Effect of pH on quantum yields of products from hypoxanthine 3-oxide.

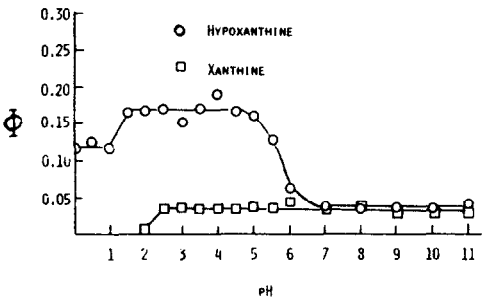


Fig. 2. Effect of pH on quantum yields of products from 1-hydroxyhypoxanthine.

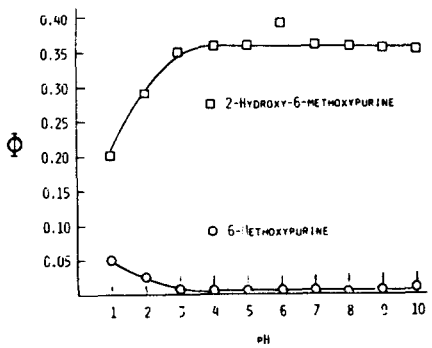


Fig. 3. Effect of pH on quantum yields of products from 6-methoxypurine 3-oxide.

(> 90% emission at 254 nm) that had been calibrated by potassium ferrioxalate actinometry.[22]

Flasks were placed on a magnetic stirrer in the centre of the reactor which positioned the centre of the flask 12 cm from the lamps. The light intensity at the surface of the flask was then 13 mW/cm². Aliquots (50 μl) were removed by syringe and chromatographed over a 9 × 450 mm column packed with Aminex (NH_4+) resin and eluted at 50° and 100 psi with 0.02 *M* ammonium formate buffer (pH 4.7). The eluate was monitored at 254 nm with an ISCO Model UA-5 UV monitor. Quantum yields were determined from peak heights using calibration curves obtained with purified samples of the products. Other UV-absorbing products formed in trace amounts could be detected with the analytical column, but these were not formed in sufficient amounts to identify.

(c) Sensitized irradiations were performed with the Hanovia high pressure Hg lamp described above. Solutions of **2** and **9A** in CH_3OH (~ 0.3×10^{-3} *mM*) containing 2.2×10^{-3} *mM* sensitizer were placed in a quartz flask, deoxygenated with N_2, and the

flask was placed in a wood box fitted with a Corning 0-52 filter (< 0.1% transmission below 340 nm) on one side so that the centre of the flask was 7 cm from the light source. Solutions were then irradiated for the times indicated in Table 1). After irradiation the solvent was removed under vacuum and the residue was washed with ether (2 × 200 ml). The ethereal extracts were then washed with the H_2O and the aqueous layers were combined and evaporated to dryness with the residue from the irradiation. That residue was then dissolved in 10 ml of H_2O and the product yields were obtained by chromatography using calibration curves as described above.

RESULTS

Ultraviolet irradiation of hypoxanthine 3-oxide, 2. Irradiation of **2** (Scheme 1) in unbuffered aqueous solution afforded two UV-absorbing products, xanthine, **4** (65%), arising by migration of the nitrogen-bound oxygen at N-3 to the adjacent carbon, and hypoxanthine, **3** (8%). The variation in the quantum yield of formation for each of these products over the pH range 0 to 11 is shown in Fig. 1. Similar studies over the same pH range were then performed with two model compounds. One can exist only in the *N*-oxide form while the other is present in the *N*-hydroxyl tautomer in the neutral species, but ionizes to an aromatic hydroxamate anion.

Irradiation of model compounds. 1-Hydroxyhypoxanthine, **6** (Scheme 1), was previously shown to exist solely in the *N*-hydroxyl form in the neutral species and to ionize (pK_a 5.65) initially to the nitrone-containing hydroxamate anion, **7**. UV irradiation of **6** in buffered aqueous solutions afforded mixtures of hypoxanthine, **3**, and xanthine, **4**. The effect of changes of pH on the quantum yield for formation of **3** and **4** from **6** was determined over the pH range 0–11 (Fig. 2).

The compounds selected as models for the *N*-oxide tautomer were 6-methoxy-purine 3-oxide, **9A**, and its 7-methyl derivative, **9B**. Irradiation of **9A** in unbuffered aqueous solution afforded primarily the rearrangement product, 2-hydroxy-6-methoxypurine, **10A**, and a small amount of 6-methoxypurine, **11**. Irradiation of 6-methoxy-7-methylpurine 3-oxide, **9B**, in methanol afforded essentially pure 2-hydroxy-6-methoxy-7-methylpurine, **10B**. A study of the effect of changes of pH on the quantum yields for formation of 2-hydroxy-6-methoxypurine, **10A**, and 6-methoxypurine, **11**, from 6-methoxypurine 3-oxide (Figure 3) showed that ionization of the imidazole hydrogen (pK_a 6.75)[17] did not affect the photoreactions of **9A**. However, protonation of **9A** (pK_a 1.47) Greatly reduced rearrangement to **10A** and enhanced photoreduction to **11**. These changes were closely correlated with the protonation pK_a of **9A**.

Table 2. Ultraviolet absorption properties of the ionic species of purine 3-oxides[a]

	λ_{max}, nm (ε × 10^{-3})			
Charge:	+	0	-1	-2
6-Methoxypurine 3-oxide[17]	263(6.9)	224(24.1) 283(10.1)	227(28.2) 276(9.1)	
Hypoxanthine 3-oxide[17]	212(14.4) 257(8.0)[b]	223(17.2) 271(9.4)	218(19.5) 286(12.1)	224(22) 285(10.3)
3-Hydroxy-guanine[26]	213(12.5) 245(7.8) 267(9.5)	217(23) 270(8.8)	224(31) 254(5.2) 292(6.6)	226(31) 283(9.7)

a/ + = cation; 0 = neutral species; -1 and -2 represent the mono- and dianion, respectively.

b/ The value was incorrectly reported originally as 275 nm.[17]

The similarity of the results with 6-methoxypurine 3-oxide, **9A**, and its 7-methyl derivative, **9B**, as well as the absence of any effect on the photoproduct composition from **9A** upon ionization of the imidazole hydrogen indicate that the imidazole hydrogen does not influence the photoreactions of **9A**. Thus the presence of any *N*-hydroxy tautomer or the possible effect of hydrogen bonding between the 3-oxide and 9-H on the photochemistry of **9A** can be discounted.

Sensitization studies. Studies by several groups to determine the nature of the excited state that produces each of the photoproducts from various aromatic amine *N*-oxides have occasionally reached conflicting conclusions. This was often due to experimental ambiguities (reviewed in Reference 5). Previous studies using triplet sensitizers with an *N*-hydroxy-purine[3] and triplet quenchers with purine *N*-oxides[5] demonstrated that photoreduction occurs from the triplet state in *N*-hydroxypurines, their hydroxamate anions, and in purine *N*-oxides. Rearrangement of the nitrogen-bound oxygen is associated with the singlet state of *N*-hydroxypurine anions and purine *N*-oxides.

Table 1. Results from triplet photosensitization of 6-methoxypurine 3-oxide and hypoxanthine 3-oxide in methanol

6-Methoxypurine 3-oxide, 9A

Expt No	Sensitizer	Time hr	9A%	6-Methoxy-2-hydroxy-purine, 10A	6-Methoxy-purine, 11%	Recovery, %
1	-	5	100	-	-	100
2	Phenanthrene[a]	5	70	-	4.6	74

Hypoxanthine 3-Oxide, 2

	Sensitizer	Time	2%	Xanthine, 4%	Hypoxanthine, 3%	Recovery, %
3	-	1	94	-	-	94
4	Phenanthrene[a]	1	44	-	39	83

a/ E_T = 62 kcal/mole

Hypoxanthine 3-oxide, **2**, and the 3-oxide, **9A**, were irradiated in the presence of the triplet sensitizer phenanthrene. Since the UV absorption bands of purine 3-oxides extend beyond 300 mm, the selection of a sensitizer had to be restricted to those of relatively low energy to avoid direct excitation. A Corning 0-52 filter (< 0.1% transmission < 340 mm) was used to exclude absorption by the purine 3-oxides and assured that light absorption occurred only by the sensitizer. In the absence of sensitizer (Control Expts 1 and 3, Table 1) no photochemical transformations were observed.

Irradiation of 6-methoxypurine 3-oxide, **9A**, in methanol in the presence of phenanthrene (E_T = 62 kcal/mole) afforded only the reduction product, 6-methoxypurine, **11**, in low yield (Expt. 2). Irradiation of hypoxanthine 3-oxide, **2**, in the presence of phenanthrene afforded a moderate yield of hypoxanthine and no rearrangement product (Expt. 4). The formation of reduction products only from both **9A** and **2** in the presence of phenanthrene indicates that loss of oxygen occurs from the excited triplet state in purine 3-oxides. This accords with previous studies of purines *N*-oxidized at the 1-position.

DISCUSSION

Previous investigations demonstrated that the ionic and tautomeric form play a significant role in determining the nature of photoreactions of purine *N*-oxides.[3,5] Compounds existing in the nonionized form as the *N*-hydroxy tautomer undergo photo-deoxygenation only. Ionization of these, however, produces aromatic hydroxamate anions that have many features in common with aromatic amine oxides. Such anions, as well as purine oxides existing exclusively as the *N*-oxide tautomer, undergo reactions typically observed with heterocyclic amine oxides,[23] *viz.* deoxygenation and 1,2-migration of the oxygen.†

The effect of changing ionic form on the photoproducts from a simple *N*-hydroxypurine, 1-hydroxyhypoxanthine, **6**, can be seen in Fig. 2. This illustrates the effect of changes in pH on the quantum yields of formation of the two main photoproducts, hypoxanthine, **3**, and zanthine, **4**, from **6**. Photoreduction of the neutral *N*-hydroxyl species of **6** to **3** (eqn i) is observed as the sole process below pH 2. At higher pH's the hydroxamate anion of 1-hydroxyhypoxanthine, **7**, undergoes rearrangement to xanthine, **4**, via the excited singlet (eqn ii) and photoreduction to **3** via the triplet state (eqn iii). Xanthine formation occurs well below the ground state pK_a for ionization of the *N*-hydroxyl proton (5.65). Consequently, the inflection in the curve for formation of **4** indicates that the pK_a* for ionization of the excited singlet of the neutral species, ^{1}**6***, to the singlet of the monoanion, ^{1}**7***, is approximately 2.2. As a result photoreduction of the pure nonionized *N*-hydroxyl form, **6**, can only be observed in the pH range 1.5–2.0. Below that range **6** is protonated, while above it the anion, ^{1}**7***, is formed either by direct excitation (eqn ii) or by ionization of excited **6** (^{1}**6***).

$$^1\mathbf{6}_o \rightarrow [^3\mathbf{6}^*] \rightarrow \mathbf{3} \quad \text{(i)}$$

$$^1\mathbf{7}_o \rightarrow [^1\mathbf{7}^*] \rightarrow \mathbf{4} \quad \text{(ii)}$$

$$^1\mathbf{7}^* \rightarrow [^3\mathbf{7}^*] \rightarrow \mathbf{3}. \quad \text{(iii)}$$

In the pH range 2.5 to 5.0 hypoxanthine, **3**, is produced largely by deoxygenation of the triplet of the neutral species, ^{3}**6***, (eqn i) and to a small extent via the triplet of the anion, ^{3}**7***, (eqn iii). Xanthine, **4**, is produced via the excited singlet, ^{1}**7***, formed by ionization of the excited singlet of the neutral species, ^{1}**6***. The inflection in the Φ for formation of hypoxanthine, **3**, near pH 5.5 does coincide with the ground state pK_a for ionization of **6** to **7** (5.65). Accordingly, above pH 7 only the photoreactions of the hydroxamate anion, **7**, are observed; direct excitation of **7** leads to xanthine, **4**, via the singlet ^{1}**7*** and to hypoxanthine, **3**, via the triplet ^{3}**7*** (Φ = 0.04 for both reactions).

6-Methoxypurine 3-oxide, **9A**, showed a very low Φ for photoreduction (0.005) at most pH's, but had a high Φ for rearrangement to the 2-hydroxy-derivative, **10A**. These values were not affected by ionization of the imidazole proton, but were altered by protonation. In contrast to the slight decrease in Φ for photoreduction produced by acidification of the neutral species of the *N*-hydroxyl derivative, **6**, acidification of the *N*-oxide, **9A**, caused an increase in Φ for photoreduction.

These studies demonstrate that a compound possessing an *N*-hydroxyl tautomeric configuration shows an increase in rearrangement with increases in pH near its Pk_a* as the hydroxamate anion is formed by ionization of the excited *N*-hydroxyl species and a decrease in photoreduction near the ground state *PK*'s for protonation and for ionization of the *N*-hydroxyl proton. Photoprocesses of compounds in an *N*-oxide form are unaffected by ionization of the molecule, but near the protonation pK_a rearrangement decreases and photo reduction increases.

The effect of changes in pH on the photoproducts from hypoxanthine 3-oxide, **2**, (Fig. 1) did not correspond exactly to those of the isomeric 1-hydroxyhypoxanthine, **6**, (Fig. 2) or of the 3-oxide, **9A**, (Fig. 3). Instead, the photoproduct pH profile of hypoxanthine 3-oxide appeared to be a composite of the two tautomeric types, **6** and **9A**. The high Φ for formation of the rearrangement product, **4**, from **2** in neutral and basic solution parallels the behavior of the 3-oxide, **9A**, but the decrease in yield of this product between pH 3 and 5 is not correlated with the protonation pK_a of **2** (1.2) and is not associated with an increase in photoreduction. However, near the protonation *pK* of **2** there are changes in the product quantum yields that do correspond to those of the *N*-oxide, **9A**, in acid. Conversely, the increase in photorearrangement of **2** near 3.5 and decrease in photoreduction near the pK_a of the *N*-hydroxyl proton resemble the actions of 1-hydroxy-hypoxanthine (Figure 2).

Assignment of tautomeric structure and ionization sequence of hypoxanthine 3-oxide. The photochemical results (Fig. 1) suggested that the neutral species of **2** consists of an equilibrium mixture of *N*-oxide and *N*-hydroxyl tautomers. To confirm this, we examined the UV spectral changes associated with the *pK* of protonation (1.2) and the pK_a's of ionization (5.08 and 9.3) of **2**. All of the required *N* and *O*-methyl derivatives of **2** are not available for a definitive assignment of the tautomeric structure and positions of protonation and of

†These are usually the only products that can be detected by UV absorption following irradiation of *N*-oxidized purines and the only ones that are readily isolable. The poor material balance for these reactions indicates that other processes leading to non-UV-absorbing degradation products also occur. 4,5-Disubstituted imidazoles have been isolated occasionally.[5,24]

ionization of **2**. However, a number of UV spectral studies on the tautomeric structure and order of ionization of several related *N*-oxidized purines have been reported.[14,16,17,25,26] The comprehensive study of the 2-amino-derivative of **2**, i.e. 3-hydroxy-guanine,[26] for which all of the requisite alkyl derivatives were available, was particularly useful in assigning the position of protonation and the order of ionization of **2** with reasonable confidence. This compound was shown to exist as a nearly equal mixture of *N*-oxide and *N*-hydroxyl tautomers in the neutral species and to ionize first from the pyrimidine ring.

The presence of a high extinction UV absorption band at low wavelength (223 nm, $\epsilon = 17{,}200$) (Table 2) in the neutral species of **2** is comparable in position and intensity to those of other *N*-oxidized purines that are present primarily or exclusively as the *N*-oxide tautomer,[25,26] e.g. **9A** (224 nm, $\epsilon = 24{,}100$). This indicates that the *N*-oxide tautomer, **2B**, is a significant contributor to the neutral species of **2**.

Protonation of purine *N*-oxides is often accompanied by a decrease in intensity of the high extinction band near 225 nm.[14,16,17,25–29] This behavior has been interpreted to indicate that protonation occurs on the *N*-oxide function.[25,26] Protonation of both **2** and **9A** is accompanied by loss of the UV band near 230 nm that appears to be a characteristic of purine *N*-oxides. The cation of **2** absorbs (257 nm, pH-1) near the absorption band of the cation of 3-methylhypoxanthine (253 nm).[19] This indicates that protonation of **2** must occur largely on the oxygen at N(3) to afford **1** as a main contributor. The evidence indicated that 3-hydroxyguanine is also protonated on the 3-position.[26]

The spectral changes that accompany the first dissociation pK_a of **2** (271→286 nm) parallel those for its 2-amino derivative, 3-hydroxyguanine (270→290) nm).[26] The spectral similarities between the neutral species of **2** and 3-hydroxyguanine (Table 2) indicate that some *N*-hydroxy tautomer, **2A**, must be present in the neutral species of **2**. Similarities between the spectral shifts that accompany the first ionization of the two compounds indicate that the hydrogen of the pyrimidine ring of **2** is the first to ionize to afford **5** as the monoanion. The second ionization of **2** then occurs from the imidazole ring to yield **8**.

Interpretation of hypoxanthine 3-oxide photochemical data. The spectral and pK_a data indicated that below pH 1 hypoxanthine 3-oxide, **2**, is present largely as the protonated *N*-oxide cation, **1**. Between pH's 2 and 4 the neutral species of hypoxanthine 3-oxide consists of a mixture of the *N*-hydroxy, **2A**, and *N*-oxide, **2B**, tautomers. Above pH 4 ionization to the monoanion, **5**, occurs (pK_a 5.08). Each of these changes is reflected by changes in the Φ of formation of the two major photoproducts from **2** (Fig. 1).

As the cation of hypoxanthine 3-oxide, **1**, was deprotonated between pH's 0 and 2 (pK_a 1.2), there was a gradual decrease in Φ of hypoxanthine, **3**, formation (Fig. 1). An increase in the ϕ of xanthine was observed up to pH 1. These changes parallel those observed as the cation of the model *N*-oxide, 6-methoxypurine 3-oxide, **9A**, was deprotonated (Fig. 3). Since the nonionized *N*-hydroxyl form of 1-hydroxyhypoxanthine, **6**, did not photoarrange to xanthine, **4**, under similar conditions (Fig. 2), the data are strongly indicative (1) that protonation of **2** to the cation, **1**, favors deoxygenation, most likely via the triplet state (eqn 4) and (2) that deprotonation of the cation, **1**, leads to the gradual appearance of photo-rearrangement via the singlet of the *N*-oxide tautomer, **2B**, (eqn 5). The fact that the inflection in the increase of Φ of xanthine from **2B** (pH 0.5) does not coincide with that for the decrease in ϕ of hypoxanthine or with the ground state pK_a of protonation of **2** (1.2) suggests that the pK^* for deprotonation of the singlet of the cation, **1**, is slightly lower than that of the ground state.

The photoreactions of the nonionized tautomers, **2A** and **2B**, of hypoxanthine 3-oxide were observable together only at pH 2. The plateau in the Φ for the formation of xanthine, **4**, between pH's 1.0 and 2.0 in Fig. 1 corresponds to the rearrangement of the neutral *N*-oxide tautomer, **2B**, to xanthine, **4**. By analogy to studies of other *N*-oxidized purines and the triplet sensitizer studies of **2**, this must be a singlet process (eqn 5). Above pH 2 there was a substantial increase in Φ of xanthine, **4**, which must be associated with ionization of the excited singlet of the neutral species, **2A,B**, to the singlet of the monoanion, ^{1}**5***, (eqn 8). The inflection at pH 3.5 in Fig. 1 defines the pK_a* for that process. The *N*-oxide anion, **5**, whether formed by ionization of excited **2** (^{1}**2A,B***) (eqn 8) or by direct excitation of **5** (at pH's > pK_a, 5.08) (eqn 9), rearranges to xanthine, **4**, with Φ = 0.30. This Φ is comparable to that for rearrangement of the model for the *N*-oxide tautomer, 6-methoxypurine 3-oxide, **9A**, to the 2-hydroxyderivative, **10A**, (Fig. 3).

The Φ for photoreduction of the neutral species of **2** (Φ = 0.06) is unchanged between pH's 2 and 4 (Fig. 1) and is considerably higher than that of the neutral form of 6-methoxypurine 3-oxide, **9A**, (0.005) (Fig. 3). The greater extent of photoreduction of **2** than of **9A** would be consistent with photoreduction of the neutral species of **2** occurring largely via the *N*-hydroxy tautomer, **2A**, (eqn 7), which is not present in **9A**. By analogy to 1-hydroxy-hypoxanthine, **1**, (Figure 2) the Φ for deoxygenation of the *N*-hydroxy tautomer, **2A**, (eqn 7) should be greater than that of the *N*-oxide tautomer, **2B**, (eqn 6). The Φ for deoxygenation of **2B** might be expected to be close to that of 6-methoxypurine 3-oxide, **9A**, (0.005), but could be as large as that of the anion, **5**, (eqn 10, Φ = 0.02). The decrease in Φ for photoreduction of the neutral form of **2** near the ground state ionization pK_a of the *N*-hydroxyl proton (5.08) parallels the behavior of 1-hydroxyhypoxanthine near its *N*-hydroxyl pK_a (Fig. 2). This behavior is also in accord with the presence of some *N*-hydroxyl tautomer, **2A**.

$$^1\mathbf{1}_o \rightarrow [^3\mathbf{1}^*] \rightarrow \mathbf{3}\ (\Phi = 0.1) \quad (4)$$

$$\mathbf{2B} \rightarrow [^1\mathbf{2B}^*] \rightarrow \mathbf{4}\ (\Phi = 0.095) \quad (5)$$

$$[^1\mathbf{2B}^*] \rightarrow [^3\mathbf{2B}^*] \rightarrow \mathbf{3}\ \Phi = ? \quad (6)$$

$$^1\mathbf{2A}_O \rightarrow [^3\mathbf{2A}^*] \rightarrow \mathbf{3}\ (\Phi = 0.06) \quad (7)$$

$$\mathbf{2A/B}_o \rightarrow [^1\mathbf{2A/B}^*] \rightarrow [^1\mathbf{5}^*] \rightarrow \mathbf{4}\ (\Phi = 0.30) \quad (8)$$

$$^1\mathbf{5}_o \rightarrow [^1\mathbf{5}^*] \rightarrow \mathbf{4}\ (\Phi = 0.30) \quad (9)$$

$$[^1\mathbf{5}^* \rightarrow [^3\mathbf{5}^*] \rightarrow \mathbf{3}\ (\Phi = 0.02). \quad (10)$$

The quantum yields for photoreduction and photorearrangement of 1-hydroxyhypoxanthine, **6**; of the neutral *N*-hydroxy tautomer of hypoxanthine 3-oxide, **2A**; of their conjugate anions, **7** and **5**; as well as of 6-methoxy-purine 3-oxide, **9A**, and the *N*-oxide tautomer of hypoxanthine 3-oxide, **2B**, are summarized in Fig. 4. Most members of the *N*-oxidized purine series have a

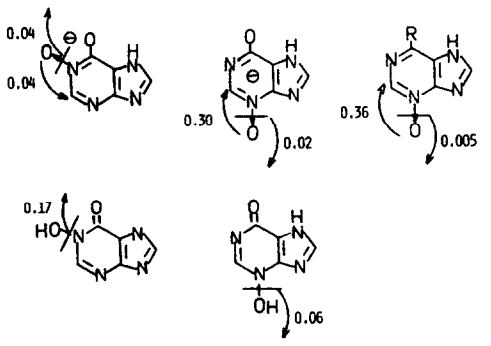


Fig. 4. Quantum yields for photoreduction and photorearrangement of purine 3-oxides and of 1- and 3-hydroxypurine neutral species and enolate anions.

relatively low Φ for photoreduction, regardless of the tautomeric form (Φ = 0.005 to 0.04) with the exception of the neutral 1-hydroxy derivative, **6**. Photorearrangement is a much more efficient process for purine 3-oxides (Φ = 0.3) than for the hydroxamate anion or the *N*-oxide group at the 1-position of purines (Φ = 0.04). Adenine 1-oxide, which exists in the *N*-oxide form,[25] has a Φ for disappearance of 0.1[30] and affords nearly equal amounts of rearrangement and reduction products (5:4).[31] This indicates that the quantum yields for the two individual processes are nearly equal and are comparable to those for the two photoreactions of the aromatic hydroxamate anion, **5**, (Φ = 0.04). Thus the much greater extent of photorearrangement of purine 3-oxides than of purine 1-oxides can be attributed to the relative inefficiency of the rearrangement process in the purine 1-oxide series, rather than to an inherently greater tendency to undergo photoreduction.

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