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ON NUCLEIC ACIDS AND DERIVATIVES

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ABSTRACT

The photochemical products from N-oxidized purines in solution are strongly dependent on the tautomeric and ionic state of the compounds. Studies have been done on the effect of pH on the quantum efficiencies of photoreduction and photorearrangement of a 1-hydroxypurine, a purine 3-oxide and hypoxanthine 3-oxide, a compound that exists as a tautomeric N-oxide-N-hydroxyl equilibrium. The quantum yields for each photoreaction from each tautomer of the nonionized species and from the anions have been determined. In every case photoreduction is promoted in the presence of triplet sensilizers.

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OBJECTIVE: The purpose of these studies is to elucidate the chemistry of purine N-oxides in the excited state, to evaluate their potential as sources of radicals and to consider possible routes to their natural formation; and to assess the importance of this to oncogenesis. These are a fundamental adjunct to, and are correlated with, a larger program that seeks to determine the chemical events that lead to oncogenesis by some purine N-oxides.

MATERIALS AND METHODS: Samples are irradiated in degassed solutions (N_2) that have been adjusted to appropriate pH values, based on known pK_a values. Irradiations are carried out either in an immersion apparatus with a 450 W Hanovia high-pressure mercury lamp with a Corex filter or in a Rayonet Photochemical Reactor equipped with 254 or 300 nm lamps and a Merry-Go-Around apparatus. Aliquots are withdrawn periodically and the photoproducts are analyzed by ion-exchange chromatography. For identification the solutions are concentrated in vacuo to a small volume when the reactions are complete and the products are separated by chromatography, usually with BioRad AG-50, X8 (H^+) or with silica thin layer plates. Yields of reaction products are calculated from their own ϵ_{max} values. The structure of unknown products is established by appropriate techniques, including UV, IR, NMR, and mass spectral and elemental analyses, and by total synthesis when the compound has not been reported previously.

These studies have the advantage of the availability of a variety of N-oxide derivatives of purines, and other ring systems, synthesized in connection with the program on the relationships of structure to chemical and biological activities.

RESULTS:

a) General background of the program on oncogenesis by purine derivatives. Purines in which a nitrogen is oxidized are a special class of chemical oncogens, the only ones derived from compounds normally present in all cells. The oncogenicity of these purine derivatives suggests that chemical oncogenesis may not be limited to nonbiological compounds from the environment. At least AMP-1-oxide can arise in vivo through the secondary peroxide effects of ionizing radiation.

Continuing parallel organic, biochemical, metabolic, and biological studies in this laboratory are attempting to elucidate the chemical events leading to oncogenesis. Syntheses of all of the isomeric N-hydroxypurines²⁰⁻²⁵⁻⁴³⁻⁴⁴⁻⁴⁸⁻⁵⁷ and assays to determine their oncogenic potential¹⁹⁻³²⁻³⁸⁻⁵⁰ constitute one portion of this program. The synthesis of structural analogs of oncogenic N-hydroxypurines, such as N-hydroxy- derivatives of pteridines,⁴⁹ quinazolines,⁵¹ pyrrolopyrimidines⁵⁶ and pyridopyrimidines⁶² are also in progress. Comparative studies of the chemistry, metabolism and oncogenic ability of these compounds with similar work on the corresponding N-hydroxypurines and selectively alkylated derivatives of them is assisting in the correlation of structural features with oncogenic requirements. Candidate compounds, selected by organic and physico-chemical studies, are subjected to assays in vivo and to metabolic studies. Studies on the metabolism of oncogenic N-hydroxypurines²⁷⁻³⁰⁻³³⁻⁴²⁻⁴⁵⁻⁵³ and on the mutagenicity,⁵⁵ and reactivity of their activated form with possible targets in vivo,⁵⁴⁻⁵⁹ are also in progress in related studies.

and

b) Background of a photochemically produced radical/an apparent "radical-like" redox reaction in solutions. The photochemistry of N-hydroxypurines in solution is also being examined⁴⁷⁻⁵⁴⁻⁶⁰ in more detail than that reported earlier,¹²⁻¹³⁻³⁴ with particular attention to the structural features and excited states that are involved in photoreduction.⁴⁷⁻⁶⁰ This work is correlated with studies on the structure and reactions of radicals photoinduced in N-hydroxypurines in the solid state,⁵² and with studies on the mechanism of the spontaneous reduction of esterified derivatives of N-oxidized purines in solution. The photochemical studies of N-oxidized purines have been a useful adjunct for studies on the redox chemistry of 3-acetoxanthine. It has not been possible to demonstrate with ESR the presence of radicals in aqueous solutions of 3-acetoxanthine undergoing reaction,⁴¹ even with the use of radical trapping agents that should afford more stable radical species.¹⁰¹ Attempts to detect radicals, using other methods, e.g., polymerization of acrylonitrile or reaction with nitric oxide (see Progress Report, 1975) were also unsuccessful. Thus fundamental studies on the mechanism of reactions involving the reduction of N-oxidized purines and on associated ancillary reactions initiated by that reduction, particularly by demonstrable radical species, have been valuable in understanding the redox chemistry of 3-acetoxanthine in solution.

c) Background of the studies on the mechanism of N-acetoxypurine reactions and their relations to the oncogenicity of such derivatives. 3-Acetoxanthine reacts with water at room temperature to yield uric acid (9a, Nu = OH) (Scheme 1) as one of the products.⁴¹ The addition of other nucleophiles to the solution also yields 8-substituted xanthines, 9.²⁴⁻⁴⁰ In this respect, these N-hydroxypurines have many features in common with the oncogenic N-hydroxyarylamines, and with certain other oncogens thought to react with nucleophiles through an electrophilic carbonium ion intermediate.

At pH's below 3, acid hydrolysis to 3-hydroxyxanthine, 5, is extensive but some 8-substitution does occur via path a, which involves ionization to acetate ion and the unstable nitrenium ion, 6a. Redistribution of the positive charge produces the carbonium ion, 6b, which forms 8-substituted xanthines by reactions with nucleophiles.

The 3-acetoxanthine anion, 2, which is predominant at physiological pH's, is much more reactive than the neutral molecule 1.⁴¹ The half-time for the reaction of 3-acetoxanthine decreases from over 100 min at pH 3 to 20 sec at pH 7. This fast reaction (path b) is accompanied by major changes in the proportions of the products. As the pH is increased from 3 hydrolysis to 3-hydroxyxanthine, 5, becomes negligible; the extent of the 8-substitution reaction leading to 9 increases manyfold and another product, xanthine, 4, appears in increasing amounts and a blue, insoluble precipitate of undetermined structure is formed. The transient neutral intermediate, 7, can protonate to yield the same carbonium ion, 6b and thence the 8-substitution products, 9, that arise via path a. Since ionization of the imidazole proton is essential for the fast reaction via path b, the 7-methyl- derivative of 3-acetoxanthine will not participate in that reaction. In contrast, the hydrogen on N-1 plays no role in path b, and 3-acetoxy-1-methylxanthine reacts similarly to 3-acetoxanthine.

The assay results for oncogenicity were found to correlate with the chemical reactivities of the various methyl derivatives⁵⁰ and are consistent with the operation of path b in vivo during the process of induction of cancer by these compounds. The ionization of the imidazole proton to the anion, 2, is an essential intermediate step in the "fast" path b reaction, and prevention of formation of such an anion (as in 3-hydroxy-7- (or 9) methylxanthine (or guanine) abolishes oncogenicity. The H on N-1 does not participate in this reaction, and replacement of it by a methyl (as in 3-hydroxy-1-methylxanthine (or guanine)) does not decrease the oncogenicity. Substitution at the 8-position with a methyl group does abolish the oncogenicity. The ester anion (the acetoxy derivative in vitro,³⁹⁻⁴⁰⁻⁴¹ or the sulfate in vivo⁵⁰⁻⁴²) leads to either the 8-substitution reaction via 7 to 9 (Scheme 1), or to the reduction to

SCHEME 1

FIGURE 1

Products from 3-Acetoxyxanthine in the Presence
of Two Equivalents of Iodide Ion

FIGURE 2

Effect of Variation in Iodide Ion Concentration
on the Product Composition from 3-Acetoxanthine

FIGURE 3

Effect of pH Changes on the Product Composition from 3-Acetoxyxanthine
in the Presence of Two Equivalents of Thiourea

xanthine, 4.

Since the reduction product, xanthine, 4, was only formed under the conditions associated with path b, it was deduced that 4 must arise from an intermediate formed only on path b. It was suggested⁴¹ that 2 might undergo not only elimination to 7 but also homolysis to 3 and that 4 arose from the radical anion, 3. This deduction was supported by several observations: (a) The yield of xanthine was not affected by changes in the polarity of the reaction medium, a characteristic of radical reactions; (b) UV irradiation of either 1 or 5 in the solid state induces a radical species with the same ESR spectrum.⁵² When dissolved in water, the radical, deduced to be an amidyl radical comparable to 3, was immediately reduced to xanthine, 4. Since no ESR signals could be detected from solutions of the photo-generated radical,⁵² it was assumed that the inability to detect an ESR signal in solutions of reacting 1⁴¹ meant that 3 was as rapidly destroyed; (c) In the presence of iodide ion, 1 was quantitatively reduced to xanthine and one-half an equivalent of I₂ was produced. Bromide ion and chloride ion, however, reacted with the carbonium ion, 6b, to afford 8-substitution products, 9b or 9d. It was then concluded⁴¹ that iodide ion, but not bromide or chloride ion, acts as a radical scavenger and promotes the reduction reaction. This oxidative mode of reactivity has also been found to extend to oxidation of tryptophan,⁵⁴ tyrosine⁵⁹ and cysteine⁵⁹ in solution. The oxidation of iodide ion to iodine has been shown to be a general type of reactivity of many, but not all, N-acetoxypurines.⁵⁹

d) Recent work on the mechanism of reduction of acetoxyxanthine by iodide ion.

We have now found that the amount of reduction of 1 in the presence of iodide ion is quite pH dependent and is quantitative only at pH's above 5.⁶⁵ As the pH is decreased below 5, there is a steep decline in the yield of 4 (Figure 1), but no 8-substitution product, 9, is formed. At pH's below 3 the yield of xanthine equals that of the 8-substitution product normally formed in the absence of iodide. In this pH region path a, involving only the ionic intermediate, 6, is operative and xanthine is not usually observed. No anion 2 or radical anion 3 from path b should be present. The observation of quantitative reduction of only that portion of 1 that normally is associated with 8-substitution in a pH region where only one reaction intermediate, 6, is evident indicates that iodide must be reacting with the cation 6.

3-Acetoxyxanthine is a cyclic N,O-diacylaromatic hydroxylamine and O-acylhydroxylamines are known to liberate I₂ from acidic iodide solutions.¹⁰² A double displacement reaction has been proposed for the reaction of hydroxylamine-O-sulfonic acid with iodide.¹⁰²⁻¹⁰³

The evidence presented in Figure 2 is not consistent with the reaction of iodide with 3-acetoxyxanthine itself. Increases in the concentration of iodide beyond two equivalents did not result in a greater extent of reduction of 1. Instead, only that portion of 1 that normally reacts via 6 to yield the 8-substitution product is reduced by iodide. It is proposed that the reduction of 1 to 4 in the presence of iodide occurs by a mechanism somewhat comparable to that for the reduction of hydroxylamine-O-sulfonic acid by iodide ion,¹⁰²⁻¹⁰³ viz., nucleophilic addition of iodide at N-3 of the cation 6 to form the intermediate 8a followed by nucleophilic displacement by a second iodide ion to yield the anion of 4 and I₂. This mechanism accommodates the observations that only that amount of 1 that normally leads to 9, via 6, is reduced

at low pH's and that this reduction and 8-substitution reactions are competitive when less than two equivalents of iodide are present. The facts that the total recovery from 1 and the yield of 3-hydroxyxanthine are unaffected by the presence of iodide or by the changes in the concentration of it confirm that iodide ion is not reacting with either 1 or 5.

To test the hypothesis that the nitrenium ion is the active intermediate in the oxidation of iodide ion, we examined the reactivity of 1 toward thiourea since there are several examples of the reaction of thiourea with electron-deficient nitrogen centers. These include the nitrosonium ion¹⁰⁴ and the N-methyl-N-nitrosoanilium cation.¹⁰⁵

The reaction of 1 in the presence of 5 equivalents of thiourea did not lead to quantitative reduction. Instead, a mixture of the reduction product 4 and the 8-substitution product, 8-thiouric acid, 9c, was obtained (Figure 3). Increases in the concentration of thiourea at pH 3 did not significantly alter the ratio of the two products. As the pH was increased to 5, the yields of the two products showed parallel increases, while the yield of 5 declined to zero. Further increases in the pH to 7 did not affect the yields of the products. The fact that in the presence of thiourea 10% of the reaction of 1 can be diverted to the reduction reaction at pH 3, where path a predominates and there is normally no reduction, indicates that thiourea is reacting similarly to iodide. We interpret this to indicate that thiourea can react at the nitrenium ion, 6a, to form an S-(3-xanthyl)-isothiuronium intermediate 8b, and that this intermediate then decomposes to 4. These data thus accord with the conclusion that oxidation of iodide ion is mediated by the nitrenium ion, 6a, rather than by a radical intermediate. The concomitant formation of some 8-thiouric acid is not surprising because of the known high reactivity of 6b with nucleophiles.⁴⁰

The observations that iodide reacts quantitatively at the nitrenium ion at N-3 of 6a, while thiourea reacts at either N-3 of 6a or C-8 of 6b and other nucleophiles react only at C-8 of 6b are explicable in terms of "soft" and "hard" nucleophiles advanced by Pearson and co-workers.¹⁰⁶⁻¹⁰⁷ It is proposed that "soft" nucleophiles react at the "soft" nitrenium ion of 6a and lead to reduction of 6 to 4 with oxidation of the nucleophile while "hard" nucleophiles react at C-8 and yield 8-substitution products. Further studies to document these proposals are planned.

These studies indicate that the reactions *in vivo* which lead to the demonstrated substitution of proteins via a carbonium ion intermediate are paralleled by oxidation reactions. This demonstration reinforces our interest in the parallel redox reaction pathway and any role it may play in the initiation of the cancer process. While the reduction by iodide ion may be explicable as a process of the nitrenium ion, there is yet no explanation for the spontaneous formation of 4 from 1 in solution in the absence of nucleophiles. In water alone at pH ~7 about one-third proceeds via the reduction pathway to xanthine, about one third to uric acid by 8-substitution (by water) and about one-third goes to the yet uncharacterized "blue compound"⁴¹ (and previous reports). In related work it has been found that uric acid "disappearance" by an oxidative process over and above that found for simple air oxidation accounts for the decreased uric acid recovery above pH 5.⁵⁹ This process, as well as the oxidation of tryptophan⁵⁴ and tyrosine,⁵⁹ has not been shown to be correlated with a reaction at the nitrenium ion and might instead be associated with the spontaneous reduction of 1 in solution.

Information on the chemistry of stable free radicals photochemically induced in solid purine N-oxide derivatives and on the mechanism of photochemical reduction of N-oxidized purines in solution⁴⁷⁻⁶⁰ consequently provides fundamental information on processes leading to reduction. The comparison of these reactivities with those of acetoxyxanthine in solution are essential adjuncts to studies on the redox activity of 3-acetoxyxanthine in solution.

e) Current results on projects in progress and supported, in part, by AEC and currently by ERDA.

1. Photochemical studies of purine 3-oxides. A major emphasis in the past year has been given to completing these studies. As discussed in our last report, the purine 3-oxides are unusual in that they undergo photodeoxygenation to a very small extent. Hypoxanthine 3-oxide, 10, (Scheme 2) was reported to be the single exception to this. It was proposed that 10 can exist as an equilibrium of the 3-hydroxy, 10a, (Scheme 2) and 3-oxide, 10b, tautomers, analogous to those that have been demonstrated for 3-hydroxyguanine, and that the photochemistry of 10 could be interpreted in terms of such an equilibrium.

To document these deductions, we have now determined the quantum yields for the appearance of photo-product as a function of pH from 1-hydroxyhypoxanthine, 14, (Scheme 2) a model compound for the N-hydroxyl tautomer, 10a; from 6-methoxypurine 3-oxide, 16, a model for the N-oxide tautomer, 10b; and for hypoxanthine 3-oxide, 10, (Figures 4, 5, and 6). The yield of the photoreduction product of 1-hydroxyhypoxanthine, hypoxanthine, 11, was unchanged over the pH range 2 to 5. It decreased from pH 5 to 8, then remained essentially unchanged with further increases in pH. The inflection in the curve for formation of hypoxanthine in Figure 4, near pH 5.5, is close to the ground state pK_a , 5.65¹⁸ and must reflect the ionization of 14 to 15. Below pH 2.0 there was no rearrangement of 1-hydroxyhypoxanthine to xanthine, 12. Above that pH there was a small amount of xanthine formed and its yield showed little variation with pH. The pH dependence for the appearance of xanthine is in agreement with the previous calculation of ~3.5 for the singlet pK_a and with the interpretation that rearrangement occurs only from the excited singlet of the anion of 1-hydroxyhypoxanthine, 15. The quantum yield for that process is 0.04. The data in Figure 4 indicate that the quantum yield for photoreduction of the nonionized N-hydroxyl tautomer of 1-hydroxyhypoxanthine, 14, is 0.17, while that for photoreduction of the enolate anion, 15, is 0.04.

The effect of pH on the photo-products from 6-methoxypurine 3-oxide is shown in Figure 5. Above pH 3 changes in pH had no effect on the formation of the main photo-product, 2-hydroxy-6-methoxypurine, 17. We have now detected a small amount of the reduction product, 6-methoxypurine, and this yield of the product also remained constant above pH 3. As the pH was decreased below 3, there was a decrease in the yield of 6-methoxy-2-hydroxypurine and an increase in the amount of photoreduction. These changes appear to be well correlated with the pK of protonation, 1.47.²⁵ It was previously deduced that protonation of 6-methoxypurine 3-oxide occurs on the N-3 oxide function and the data in Figure 5 indicate that this protonation reduces photorearrangement and enhances photoreduction. The quantum yield for reduction of 6-methoxypurine 3-oxide is quite low, 0.05, while that for rearrangement is 0.35. In the presence of the triplet sensitizer benzophenone rearrangement was still the major process (Table I). With phenanthrene (E_T 62 kcal/mole) only photoreduction was observed. These data indicate that photoreduction occurs from the triplet of 16. Photoreduction of 10 is also a triplet process (Table I). The triplet sensitizers acetophenone and benzophenone sensitize both reduction and rearrangement.

SCHEME 2

SCHEME 3

FIGURE 4

Effect of pH on Quantum Yields of Products from 1-Hydroxyhypoxanthine

FIGURE 5

Effect of pH on Quantum Yields of Products from 6-Methoxypurine 3-oxide

FIGURE 6

Effect of pH on Quantum Yields of Formation of Products from Hypoxanthine 3-oxide

TABLE I

Results from Irradiations of 6-Methoxypurine 3-oxide
and Hypoxanthine 3-oxide with Triplet Sensitizers

6-Methoxypurine 3-oxide, 16

Sensitizer	Time hr	<u>16</u> , %	6-Methoxy-2- hydroxypurine, %	6-Methoxy- purine, %	Recovery, %
-	5	100	-	-	100
Benzophenone ^a	5	2.2	70	-	72
Phenanthrene ^b	5	70	-	4.6	74

Hypoxanthine 3-oxide, 10

Sensitizer	Time hr	<u>10</u> , %	Xanthine, <u>12</u> , %	Hypoxan- thine, <u>11</u> , %	Recovery, %
-	1	94	-	-	94
Acetophenone ^c	1	15	8.3	10	33
Benzophenone ^a	1	< 1	33.4	44	77
Phenanthrene ^b	1	44	-	39	83

a) $E_T = 68.5$ kcal/mole

b) $E_T = 62$ kcal/mole

c) $E_T = 73.6$ kcal/mole

Since there was no photochemical change in the absence of these sensitizers with the filter utilized, these results suggest that following triplet energy transfer by these higher energy ketones, intersystem crossing to the singlet can occur. In the presence of a lower energy triplet sensitizer, phenanthrene, E_T 62 kcal/mole, xanthine was not formed. Hypoxanthine was the sole product; these results confirm that photoreduction occurs from the triplet in hypoxanthine 3-oxide, as shown previously for other purine N-oxides,⁴⁷⁻⁶⁰ and indicate that rearrangement is a singlet process. At pH 2 or below the N-oxide, 10b, and N-hydroxyl, 10a, tautomers must exist in equilibrium. The N-hydroxyl tautomer 10a may predominate slightly by analogy to the equilibrium of tautomers in guanine 3-oxide. Rearrangement occurs from the singlet of the N-oxide tautomer, 10b, with a quantum yield of 0.10. Most of the photoreduction observed in this pH range must occur from the triplet of the N-hydroxyl tautomer, 10a. Although it is conceivable that, as in 6-methoxypurine 3-oxide (Figure 5), there is a very small contribution of deoxygenation from the triplet of the N-oxide tautomer, 10b. The combined processes have a quantum yield of 0.07. Above pH 2 the increase in yield of xanthine with increase in pK reflects the pK_a^* of the singlet, ~3.5, and the increasing contribution to photorearrangement of the excited singlet of the anion, 13. The anion

has a higher quantum efficiency for rearrangement which at its maximal value above pH 5 was 0.31. This quantum yield is comparable to that for rearrangement of 6-methoxypurine 3-oxide, 0.35 (Figure 5). The slight drop in quantum yield for formation of xanthine from pH 8 to 9 may reflect the ionization of the imidazole proton of 10, pK 9.3.²⁵ The inflection point in the curve for the formation of hypoxanthine at pH 5 is near that of the ground state pK , 5.08²⁵ and must reflect the ionization to 13. The quantum yield for photoreduction observed below pH 4, 0.07, must be that for the N-hydroxyl tautomer, 10a, while that at pH 6 and above, 0.05, must be the quantum yield for photoreduction solely via the triplet of the enolate anion, 13.

The present data permit an assessment of the quantum yields for deoxygenation from a purine 3-N-oxide, from enolate anions at both the 1- and 3-position, and from N-hydroxyl groups at the 1- and 3-position as well as the quantum yields of rearrangement for the N-3-oxide group and for the enolate anions at the 1- and 3-positions.

2. Action of peroxides on purines. In our last report we examined the formation of A-1-oxide with 3% peroxide in a series of buffers as a control study for the reaction of adenine with 5-bromo-6-hydroperoxy-5,6-dihydrothymine. To minimize variation in experimental conditions, we have repeated the experiment using only citrate as a buffer over the pH range 2 to 7. The results are qualitatively similar to the previous data, i.e., maximum formation of adenine 1-oxide after several days at pH 4 and steep declines above and below pH 4. However, in contrast to our previous work using succinate buffer, in citrate buffer there was still a significant formation of N-oxide at pH 6. At pH 7 in either phosphate or citrate buffer, there was less than half a percent of adenine 1-oxide formed after a week of reaction. Adenine 1-oxide was identified by comparison of the elution position of the product to that of an authentic sample of adenine 1-oxide on a standard A-6 column and by comparison of the UV spectrum of a sample obtained at pH 4 with the spectrum of adenine 1-oxide. Adenine 1-oxide is well resolved A-6 from the known adenine 3-oxide and from adenine 9-oxide,¹⁰⁹ which has recently been synthesized in this laboratory. These results suggest that if adenine 7-oxide were formed at pH 7, as previously reported,¹⁰⁸ then it should be well resolved on the A-6 column from the other N-oxides of adenine. Our data thus suggest that the 1-oxide of adenine and not the 7-oxide is formed with peroxide at pH 7. 5-Bromo-6-hydroperoxy-5,6-dihydrothymine, an intermediate in the synthesis of the 5-hydroxy derivatives, is a reasonably stable compound that can be obtained free of any peroxide contaminant. It has been reacted with adenine in citrate buffer over the pH range 2 to 7 and we find that no oxidation of adenine occurs. This suggests that if oxidation products are formed only in the presence of unstable hydroperoxides, it is conceivable that the products result from the action of peroxide released by decomposition of the hydroperoxide, rather than by a direct reaction with the hydroperoxide. Accordingly, appropriate controls with peroxide are necessary for an unambiguous interpretation of results from reactions with hydroperoxides. Reactions with adenine and 5-hydroxy-6-hydroperoxy-5,6-dihydrothymine are in progress.

3. Solid state radical. We are continuing to provide Dr. Chester Alexander, U. of Alabama, with purified samples of purine N-oxides for his ESR studies of radicals photoinduced in the solid state. Crystal structure determination by X-ray diffraction techniques of crystals obtained by slow evaporation is currently in progress at the Departments of Physics and Chemistry at the U. of Alabama. There is now evidence for the formation both of a monoradical and radical pair species in irradiated solid 3-hydroxyxanthine. It has also been discovered that thermal gradient induced electric discharges take place in the single crystals and experiments are planned to determine whether radicals can be generated by these internal electric discharges.

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