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CHEMICAL AND BIOLOGICAL STUDIES ON NUCLEIC ACIDS AND DERIVATIVES

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ABSTRACT

Irradiation of N-hydroxyxanthines in the solid state induces a radical that has been assigned as an acyl amidogen radical in which the unpaired electron density is partially delocalized throughout the π-cloud of the purine ring. The radical is reduced in the presence of water. In the presence of methanol this process is also accompanied by some 8-substitution. Photochemical deoxygenation in solution has been shown to proceed from either the nonionized N-hydroxyl tautomer or the conjugate anion of N-hydroxypurines. Reduction is the sole photoreaction of the former, while intramolecular migration of the oxygen is the main photoprocess of the latter with photoreduction a minor result. The comparison of the photochemistry of the N-hydroxypurine anion to that of the corresponding purine N-oxide has been documented with a study of suitable model purine 1-oxides. A unique photorearrangement of 1-hydroxyxanthine to the oncogenic 3-isomer has been examined and is deduced to be a two step process.

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TITLE: "Chemical and Biological Studies on Nucleic Acid Derivatives"

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 of 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 450W 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 Aliquots are withdrawn periodically and the photoproducts are analyzed by ion-exchange chromatography.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 Bio-Rad AG-50, X8 (H^+) or with silica thin layer plates. Yields of reaction products are calculated from their known 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-hydroxypurines[20-25-43-44-48-57] and assays to determine their oncogenic potential[19-32-38-50] 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] and pyrollopyrimidines[56] 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. It is hoped that derivatives can also be prepared that will react preferentially via either the ionic or the radical-like reaction of path b, discussed in section c, and that such compounds may permit a decision regarding the role and importance of each pathway to the oncogenic process. 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-hydroxypurines[27-30-33-42-45-53] 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.

b) Background of a photochemically produced radical and a "radical-like" redox reaction in solutions. The photochemistry of N-hydroxypurines in solution is also being examined[47-54-60] 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 particularly helpful in understanding the redox chemistry of acetoxyxanthine. It has not been possible to demonstrate directly with ESR the presence of radicals in aqueous solutions of 3-acetoxyxanthine undergoing reaction,[41] even with the use of radical trapping agents that should afford more stable radical species.[101] Attempts to demonstrate the presence of radicals indirectly by inducing polymerization of acrylamide with radicals formed from the reaction of acetoxyxanthine gave no evidence for the presence of radicals. Parallel attempts to initiate polymerization of acrylamide with UV irradiated 3-hydroxyxanthine known by ESR determination to contain 12-15% radical were also unsuccessful. In general, nitrogen radicals, especially those that are largely delocalized, often fail to initiate polymerization.[102-103] An alternate method to demonstrate the presence of radicals, the reaction of acetoxyxanthine under N_2 in dioxane in the presence of nitric oxide, was also unsuccessful. Since the radical photoinduced in solid 3-hydroxyxanthine has too short a life in aqueous solution to be observed by ESR,[52] it is probable that any radical formed during the decomposition of 3-acetoxyxanthine in solution would have a similar short lifetime. 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, are essential for understanding the redox chemistry of 3-acetoxyxanthine in solution.

c) Background of the studies on the mechanism of N-acetoxypurine reactions and their relations to the oncogenicity of such derivatives. 3-Acetoxyxanthine reacts with water at room temperature to yield uric acid (9,Nu=OH) (Scheme I) 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, 6. Redistribution of the positive charge produces the carbonium ion, 7, which forms 8-substituted xanthines by reactions with weak nucleophiles such as water and chloride ion.

The 3-acetoxyxanthine anion, 2, which is predominant at physiological pH's, is much more reactive than the neutral molecule 1.[41] As the proportion of the anion increases, the half-time for the reaction of 3-acetoxyxanthine 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: hydrolysis to 3-hydroxyxanthine, 5, becomes negligible; the extent of the 8-substitution reaction leading to 9 increases manyfold; another product, xanthine, 4, appears in increasing amounts;

and a blue, insoluble precipitate of undetermined structure is formed. The transient neutral intermediate, 8, can protonate to yield the same carbonium ion, 7, and thence the 8-substitution products, 9, that arise via path a. It was suggested[41] that the acetoxyxanthine anion, 2, can also participate in a second reaction that results in the reduction of a portion of the anion, 2, to xanthine, 4. The reduction reaction manifests some properties that are characteristic of radical reactions, and it was proposed that a radical anion, 3, may result from homolytic cleavage of an acetoxy radical from the anion, 2.

Since ionization of the imidazole proton is essential for the fast reaction via path b, the 7-methyl- derivative of 3-acetoxyxanthine will not participate in that reaction. 3-Acetoxy-7-methylxanthine in water at pH's from 1 to 9 is only hydrolyzed to 3-hydroxy-7-methylxanthine.[39-41]

In contrast, the hydrogen on N-1 plays no role in path b, and 3-acetoxy-1--methylxanthine undergoes both the 8-substitution and the reduction reactions as readily as does 3-acetoxyxanthine.

The assay results for oncogenicity were found to correlate with the chemical reactivities of the various methyl derivatives[50] 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 vivo*[50-42] leads to either the 8-substitution reaction via 7 to 9 (Scheme I), or to the reduction to xanthine, 4. *In vitro* the reaction can be diverted to *ca.* 100% reduction by radical scavengers (I^-, Vit. C, hydroquinone, HSO_3^-, etc.[41-59]), or it can be diverted to *ca.* 80% 8-substitution with highly effective nucleophiles.[40-41-59] In water alone at pH *ca.* 7 about 1/3 proceeds via the reduction pathway to xanthine, about 1/3 to uric acid by 8-substitution (by water) and about 1/3 goes to the yet uncharacterized "blue compound"[41] (and previous reports). In related work it has been found that uric acid "disappearance" by air (or radical?) oxidation accounts for the decreased uric acid recovery above pH 5.[59] This oxidative mode of reactivity of 3-acetoxyxanthine has also been found to extend to oxidation of tryptophan,[54] tyrosine[59] and cysteine[59] in solution. The oxidation of iodide ion to iodine has been shown to be a general type of reactivity of many N-acetoxypurines.[59]

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. Information on the character of stable free radicals photochemically induced in solid purine N-oxide derivatives[52] and, on the mechanism of photochemical reduction of N-oxidized purines in solution,[47-60] is providing essential data on the redox chemistry of these compounds and on the types of ancillary reactions that may accompany 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.

Recent Work Completed and Published*

1. F. L. Lam and J. C. Parham, Purine N-oxides. XLVII. Photochemistry of 1-hydroxy- and 1-methoxyhypoxanthines, J. Org. Chem. 38:2397-2403 (1973).

2. J. C. Parham, I. Pullman and G. B. Brown, Purine N-oxides. LII. ESR studies on photochemically induced radicals from N-hydroxyxanthines, Tetrahedron 29: 3329-3336 (1973).

3. F. L. Lam, G. B. Brown and J. C. Parham, Purine N-oxides. LVI. Photoisomerization of 1-hydroxy- to 3-hydroxyxanthine. Photochemistry of related 1-hydroxypurines, J. Org. Chem. 39:1391-1395 (1974).

4. F. L. Lam and J. C. Parham, Purine N-oxides. LX. The photoreactions of 6-methyl- and 6,9-dimethylpurine 1-oxides, J. Amer. Chem. Soc. 97:2839-2844 (1975).

5. T.-C. Lee, F. L. Lam and G. B. Brown, Purine N-oxides. LXI. 3-Hydroxy-2,3--dihydro-2-oxopurine, J. Org. Chem. 40:1547-1549 (1975).

(* Supported, in part, by AEC and reported 1972-1975)

RECENT RESULTS ON PROJECTS NOW IN PROGRESS AND SUPPORTED, IN PART, BY AEC AND CURRENTLY BY ERDA.

a) Structural studies on the solid state radical of 3-hydroxyxanthine. The ESR data from UV irradiated microcrystalline powders of 3-hydroxyxanthine and derivatives of it did not permit an unambiguous distinction between an amidyl and an acyl nitroxyl radical.[52] The nitroxyl was generated by chemical oxidation in solutions of 3-hydroxyxanthine and of its 8-methyl- derivative. The acyl nitroxyl of the latter showed little interaction with the 8-methyl group, in contrast to the radical formed photochemically in the solid state of 8-CH_3-3-hydroxyxanthine. This evidence contributed to the conclusion that the photoinduced radical was an amidyl.

To substantiate the amidyl assignment, syntheses of derivatives of 3-hydroxyxanthine disubstituted at positions 1- and 7- with long chain alkyl groups were initiated for use in radical trapping studies. Such derivatives were desirable because they are expected to show some solubility in inert nonhydroxylic solvents and because they lack easily abstractable protons on the purine molecule. It proved impractical to prepare the initial target compounds by the conventional route of alkylation of guanosine because O-6 alkylation was found to predominate over N-1 alkylation with long-chain alkyl halides. It was possible to prepare 7-butyl-1-methyl-3-hydroxyxanthine by this route and that compound proved to be reasonably soluble in benzene. In collaboration with Dr. Chester Alexander of the University of Alabama, the ESR of the irradiated solid of this compound was examined. The irradiated solid showed a very weak three line spectrum. However, when the sample was dissolved in benzene containing the spin trapping agents nitroso-t-butane or phenyl-t-butyl nitrone, no signal could be detected. Examination of the ESR of the irradiated solid at both X- and K-band frequencies showed that the splitting between the three lines of the spectrum was frequency dependent. This suggests that the three lines of the spectrum are not due to a triplet hyperfine interaction, but are instead due to the presence of three radical

species with different g-values in the solid. There are suggestions of additional hyperfine structure, but the weakness of the signal makes it difficult to analyze the spectrum. The presence of several radical species, perhaps all different from that formed in 3-hydroxyxanthine, is consistent with results from irradiations of 1,7-dimethyl-3-hydroxyxanthine in methanol solution and with the reactions of the irradiated solid with methanol which are described below in section b.

Dr. Alexander has been successful in growing crystals of 3-hydroxyxanthine sufficiently large for single crystal work. ESR data from irradiated single crystals indicate a spin density of about 0.3 on a nitrogen atom, probably N-3, and a large spin density on an oxygen atom or atoms. The nitrogen hyperfine splitting is 6.1 G, which is nearly identical to that reported for the irradiated powder. The data indicate that there are two magnetically in-equivalent molecular sites in the crystal unit cell. The unpaired electron density appears to be delocalized over the π-cloud of the purine ring. It is not apparent at this time whether the unpaired electron density on the oxygen atom(s) is located only on the oxygen at N-3, which would be conclusive evidence for an acyl nitroxyl assignment or whether it is delocalized on to one or both of the carbonyl oxygens. The latter would not provide a conclusive distinction between the two radical possibilities. Studies are still in progress.

b) Reactions of photoinduced radicals of N-hydroxypurines with organic solvents. The radical photoinduced in solid 3-hydroxyxanthine reacts in refluxing methanol to yield 8-OCH_3-xanthine, in addition to 3-hydroxyxanthine and xanthine. The formation of 8-OCH_3-xanthine is unusual, since no uric acid is produced from the reaction of the radical in water, and the photoinitiated addition of alcohols to purines occurs on the carbon of CH_3OH to yield 8-CH_2OH purines,[104-6] and not 8-OCH_3- derivatives. When 3-hydroxyxanthine was irradiated in CH_3OH solution, either in the presence of O_2 or under N_2, xanthine was the major product and 8-OCH_3-xanthine could not be detected. The 1-methyl derivative of 3-hydroxyxanthine, when irradiated as a solid and allowed to react with CH_3OH, gave 1-methyl uric acid (spectral identification), 1-CH_3-xanthine and 1-CH_3-3-hydroxyxanthine, but it formed no 8-OCH_3 derivative. UV irradiation of solid 1,7-dimethyl-3-hydroxyxanthine followed by reaction of it with CH_3OH gave a mixture of four products, two minor and two major. In contrast to all of the other N-hydroxypurines, none of the reduction product that might be anticipated, 1,7-dimethylxanthine, was detected. Irradiation of 1,7-dimethyl--3-hydroxyxanthine in CH_3OH solution under N_2 also gave no photoreduction, but instead yielded a mixture of products that are as yet unidentified.

8-Substitution products were not detected from the reaction of the photoinduced radical of solid 3-hydroxyxanthine with isopropanol, pyridine or mercaptoethanol. In general, only xanthine and unreacted 3-hydroxyxanthine were present. In 12 N HCl the radical gave a small amount of an unidentified new product (UV: pH 1, 277 nm; pH 12, 296 nm) that is eluted by water from AG-50 (H^+). Its UV spectral properties do not agree with those of either uric acid (pH 1, 230, 284; pH 12, 235, 291) or 8-chloroxanthine (pH 1, 273; pH 12, 285) either of which might have been expected and are eluted under similar conditions.

When the radical of 3-hydroxyxanthine is placed in either methanolic HCl or concentrated H_2SO_4, a gas is evolved. That evolved from H_2SO_4 has been identified by mass spectrometry as CO_2. At this stage the origin of that CO_2 could probably only be traced with an isotope.

The isolation of 8-CF_3-xanthine from irradiations of 3-hydroxyxanthine in CF_3COOH is described in section g. It is reasonable to suggest that this mode of reactivity might be expected from the reaction of the radical photoinduced in solid 3-hydroxyxanthine with CF_3COOH, and that such a reactivity might also be a means to document the extent of radical participation in the reactions of 3-acetoxyxanthine

in solution. The reaction of the solid state radical with CF_3COOH at 25° gave a trace of a product, tentatively identified as uric acid, and xanthine but no 8-CF_3--xanthine could be detected. In refluxing CF_3COOH uric acid and xanthine were obtained, but again, no 8-CF_3-xanthine could be detected. The presence of uric acid may well result from esterification and rearrangement of the ester. Because 3-acetoxyxanthine does not undergo spontaneous reduction in acid solution,[41] its reactions with trifluoroacetate were examined in 5 M potassium trifluoroacetate adjusted to ca. pH 3. In this system neither 3-acetoxyxanthine nor the radical of 3-hydroxyxanthine formed 8-CF_3-xanthine.

c) Comparison of the redox ability of the radical of 3-hydroxyxanthine to that of 3-acetoxyxanthine. The ability of the photoinduced radical of 3-hydroxyxanthine to oxidize iodide ion in aqueous solution was examined to determine whether this radical could act as an oxidizing agent comparable to 3-acetoxyxanthine.[40,41,59] Within the limitations of the experimental difficulties (discussed in our last report), there was no evidence for the oxidation of iodide by the radical.

d) Attempts to simulate photoreactions with radical generating systems. Experiments were undertaken to determine whether the effects manifested by irradiation of 3-hydroxyxanthine in CF_3COOH solution or by irradiation of the solid and reaction with CH_3OH to yield 8-OCH_3 (or 8-CH_2OH) xanthine could be simulated by known radical generating systems. Persulfate has been reported to react with amides to yield amidyl radicals,[107] hence the reaction of xanthine with persulfate should simulate the reactions of any amidyl radicals that might be formed by UV irradiation of 3-hydroxyxanthine in solution. However, following the reaction of xanthine in CF_3COOH under reflux with excess potassium persulfate, no reaction products, 8-CF_3-xanthine in particular, could be detected by ion-exchange chromatography. The presence of radical species in solution was not documented, however. Persulfate has also been reported to react with CH_3OH to yield initially the radical cation of CH_3OH ($CH_3\overset{+}{O}H$).[108] This species rapidly abstracts a hydrogen from CH_3OH to produce $\cdot CH_2OH$. The reaction of xanthine with potassium persulfate in refluxing CH_3OH thus might be expected to yield either 8-OCH_3- or 8-CH_2OH xanthine. However, no products could be detected from the reaction. From these experiments persulfate does not appear to be a useful model for simulating reactions of the photoinduced radical or photoexcited species from 3-hydroxyxanthine in solution.

ESR data from irradiated single crystals of 3-hydroxyxanthine indicate the presence of some unpaired electron density on an oxygen atom(s) (section a, p. 4) and one interpretation of this data is that the photoinduced radical is a cyclic acyl nitroxyl. As a test of this hypothesis and to determine whether the acyl nitroxyl of 3-hydroxyxanthine would react in CH_3OH to yield either 8-OCH_3- or 8-CH_2OH xanthine, that radical was generated chemically in CH_3OH solution by reaction of 3-hydroxyxanthine with $Ce(SO_4)_2$. Neither of the 8-substitution products was detected, but a significant amount of the 3-hydroxyxanthine was reduced to xanthine. This is the first indication that the acyl nitroxyl, if formed, can undergo reduction in an hydroxylic solvent. When the nitroxyl of 3-hydroxyxanthine was generated previously in aqueous solution, only a trace of xanthine was detectable.

e) Photochemical studies of purine 3-N-oxides. The photochemistry of 6-substituted purine 3-oxides differs from that of purine 1-oxides. 6-Methyl- and 6-methylmercaptopurine 3-oxides (1 and 2, Table I) undergo only rearrangement

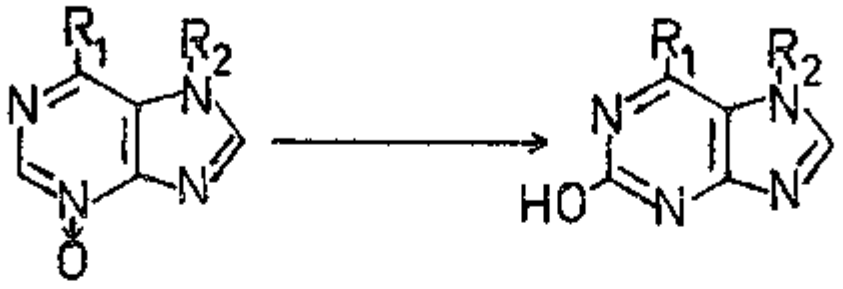
to the corresponding 2-hydroxy derivatives, 3 and 4, upon UV irradiation in solution.[15-34] The lack of deoxygenation products from these N-oxides is unusual since the photolysis of purine 1-oxides[12-13-60] and of most aromatic amine oxides[109] is almost invariably accompanied by some deoxygenation. However, 6-mercaptopurine 3-oxide, 3, is almost exclusively photoreduced by UV light.[15] In order to explore the photochemistry of purine 3-oxides, we selected 6-methoxypurine 3-oxide,[25] 4, 7-methyl--6-methoxypurine 3-oxide, 5, and hypoxanthine 3-oxide,[25] 10, (Scheme I) for study. These compounds have the advantages of being more accessible than the thio analogs and are not susceptible to photochemical modification by fluorescent room lighting, as is 6-mercaptopurine 3-oxide,[15] since they absorb only at shorter wavelengths.

6-Methoxypurine 3-oxide, 4, has a photochemistry similar to that of the 6-methyl- and 6-methylmercaptopurine 3-oxides. In methanol solution it is rearranged by UV light exclusively to 2-hydroxy-6-methoxypurine, 8. In acid this product is slowly hydrolyzed in the absence of light to xanthine, 12. For increased solubility in nonaqueous solvents and to exclude the possibility of tautomerism, the 6-methoxy--7-methylpurine 3-oxide, 5, was prepared by peracid oxidation of the parent purine. Initial attempts to prepare the corresponding 9-methyl derivative by the same method were unsuccessful. Irradiation of 7-methyl-6-methoxypurine 3-oxide produced 2-hydroxy-6-methoxy-7-methylpurine, 9, in quantitative yield. These studies confirm that 4 and 5 are satisfactory models for studies on the photochemistry of purine 3-oxides.

Initial studies with hypoxanthine 3-oxide, 10, showed that its photochemistry was more complex. Both rearrangement and photoreduction products were obtained. Hypoxanthine 3-oxide is structurally similar to 3-hydroxyguanine, i.e., the former lacks only the 2-amino group of the latter. It was shown earlier that the neutral species of 3-hydroxyguanine is an equilibrium mixture of the 3-hydroxy and 3-N-oxide tautomers, with the former predominating slightly.[36] The structural similarities of these two compounds suggest that the neutral species of hypoxanthine 3-oxide should also exist as an equilibrium mixture of N-hydroxyl, 10a, and N-oxide, 10b, tautomers at N-3 and have the carbonyl group, rather than a lactim function, at C-6. In further analogy to 3-hydroxyguanine the first ionization of hypoxanthine 3-oxide, pK 5.08, should occur from the pyrimidine ring and this ionization should afford a nitrone-containing anion at the 3-position, as in 13. The requisite alkyl derivatives of 10 are unfortunately not available to document these deductions. However, the yields of the two photoproducts, hypoxanthine, 11, and xanthine, 12, and the quantum efficiency for loss of the starting material are pH-dependent and vary in a manner that is consistent with these deductions on the tautomeric structure of hypoxanthine 3-oxide. Previously we demonstrated that the isomeric 1-hydroxyhypoxanthine existed solely in the N-hydroxy form in the neutral species and that UV irradiation of that species induced only photoreduction to the parent purine.[47] Ionization of the N-hydroxyl group yielded a nitrone-containing enolate anion and photolysis of that species caused rearrangement of the oxygen as a major photoreaction, with reduction a minor result.

The products from irradiation of hypoxanthine 3-oxide for 2 min and 10 min in a series of buffered solutions from pH 2 to pH 11 are illustrated in Figures 1 and 2. Hypoxanthine 3-oxide is too acid-sensitive for irradiations to be done below pH 2.[25-110] After 2 min 40-70% of the 3-oxide has reacted, depending on the pH, while after 10 min 70-95% has reacted. From the two series of values several trends can be discerned. The yield of the photoreduction product hypoxanthine is constant between pH's 1 to 3, then decreases to a constant value at pH's above 6 with an inflection point for the change near pH 5. That value is close to the first ionization pK of 10, which is deduced, by analogy to 3-hydroxyguanine, to occur from the pyrimidine ring. Thus

TABLE I



	R_1	R_2		R_1	R_2
1	CH_3	H	6	CH_3	H
2	SCH_3	H	7	SCH_3	H
3	SH	H			
4	OCH_3	H	8	OCH_3	H
5	OCH_3	CH_3	9	OCH_3	CH_3

SCHEME I

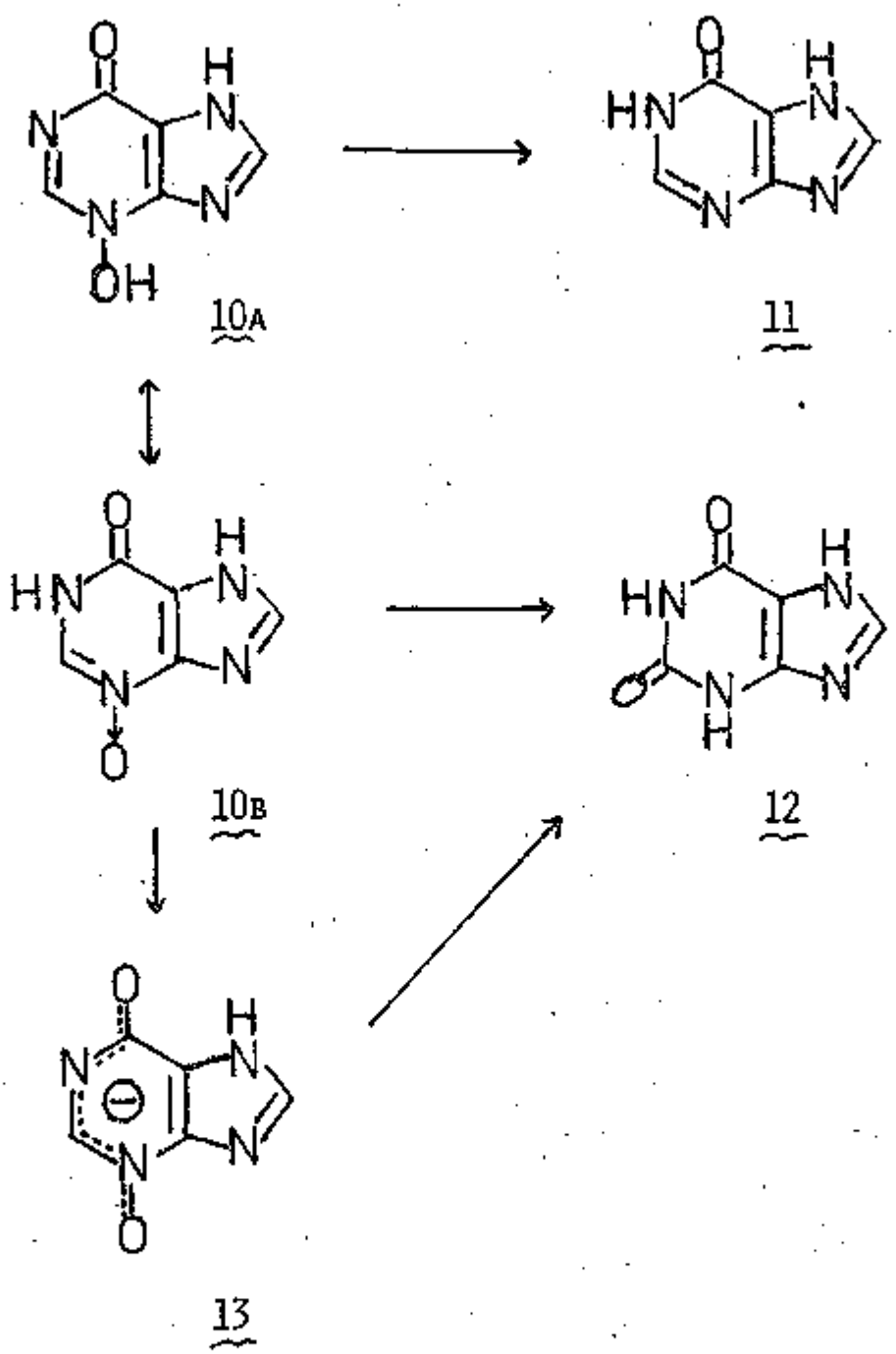


FIGURE 1

Irradiation of Hypoxanthine 3-Oxide for 2 min[a]

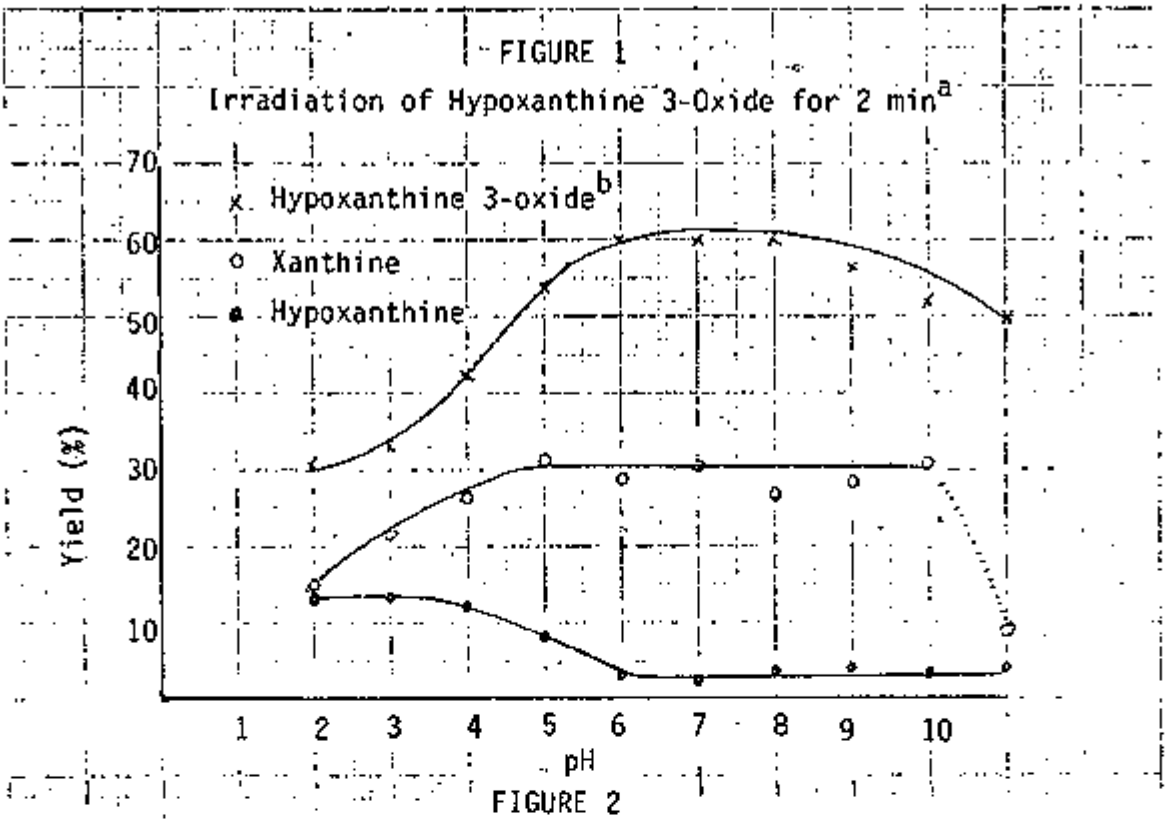
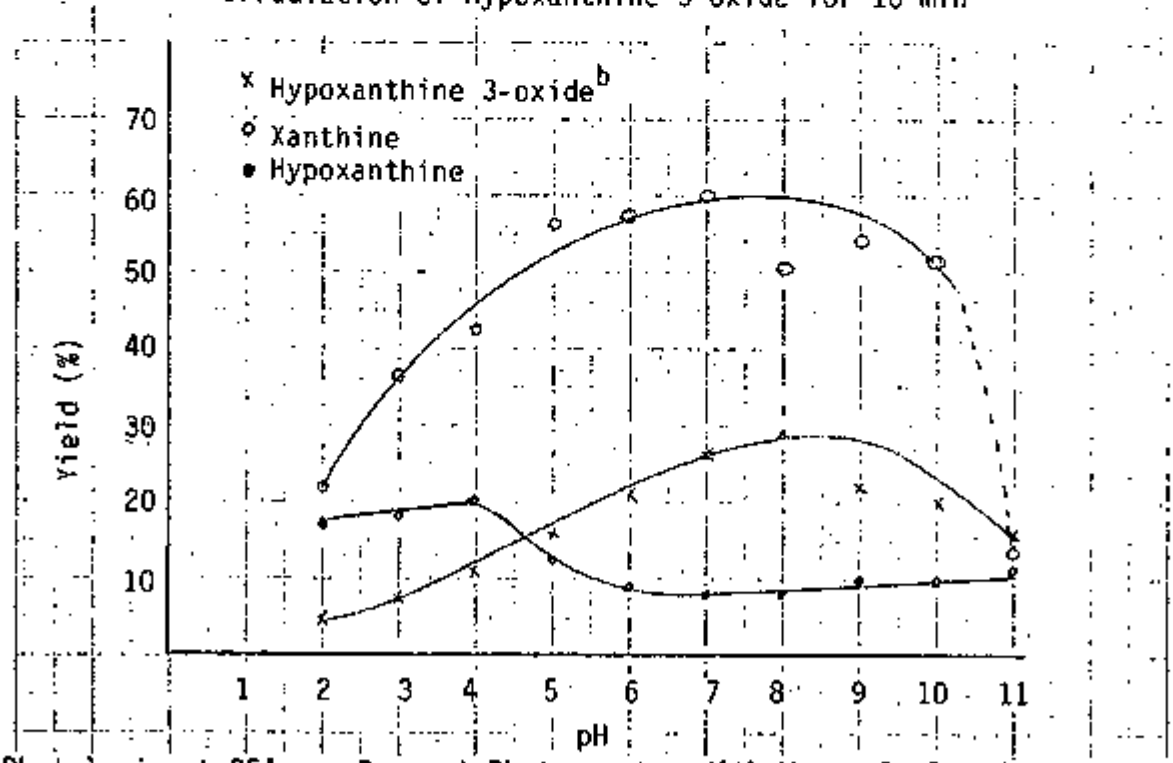


FIGURE 2

Irradiation of Hypoxanthine 3-Oxide for 10 min[a]



a) Photolysis at 254 nm, Rayonet Photoreactor with Merry-Go-Round apparatus.

b) The % of recovery.

the curve for the yield of hypoxanthine parallels that of the first ionization pK of hypoxanthine 3-oxide. The close correspondence of the two curves is consistent with the presence of some N-hydroxy tautomer in the neutral species which undergoes photoreduction as a sole or predominant process at pH's below 4. Above pH 6, the nitrone-containing anion, 13, is photoreduced to a small extent only and the main photoreaction of that species is rearrangement to xanthine, 12. The yield of xanthine increased nearly linearly from pH 2 to pH 5, then remained constant until pH 11. Above pH 11 the recovery of xanthine drops precipitously which is probably related to ionization of the imidazole proton of xanthine, pK 11.94. The much greater extent of formation of xanthine than of hypoxanthine at all pH values is in accord with the interpretation that there is a significant contribution by the N-oxide tautomer, 10b, in the neutral species of 10 and that photoreduction is a minor process of the anion, 13. The curve for formation of xanthine does not correspond to that for formation of hypoxanthine, but instead levels off at a lower pH value, which suggests that the former process is associated with an ionization with a lower pK value than the ground state pK. Previously, it was calculated that the pK for the N-hydroxyl proton in the excited singlet state [pK*] of 1-hydroxyhypoxanthine was 2.7.[47] From the values of xanthine formation in Figures 1 and 2, a value of ca. 3.5 can be calculated for the inflection point of the curve by the use of a least squares analysis. This should correspond to the pK for the pyrimidine proton in the excited singlet state. This value is considerably higher than one calculated, 0.85, with procedures used previously to estimate the pK* of 1-hydroxyhypoxanthine.[47] The values for unreacted hypoxanthine 3-oxide after 2 min also follow a sigmoid curve between pH's 2 and 6, with an inflection point near 4.2. This curve represents a composite of the quantum efficiencies of the photolysis reactions of hypoxanthine 3-oxide. The "end-point" of this curve corresponds well with that for the formation of hypoxanthine, 11. This correlation supports the conclusion above that there is a tautomeric equilibrium in the neutral species of 10 and that ionization of 10 destroys this equilibrium and produces a new species, 13, that undergoes mainly rearrangement. It is apparent from this curve that photoreduction has a higher quantum efficiency than photorearrangement. When rearrangement is the major process, eg,at pH 7, 60% of the starting material is recovered, while at pH 2, when the two processes are nearly equal, only 30% of 10 remains. The lower yield of photoreduction product at low pH's is due to the relatively smaller amount of the requisite N-hydroxy tautomer.

We reported previously that the use of paramagnetic inorganic ions as triplet quenchers was a particularly useful tool with compounds that are poorly soluble in nonaqueous solvents.[60] Unfortunately, hypoxanthine 3-oxide reacts with these ions and forms insoluble metal complexes. However, it is sufficiently soluble in methanol for studies with triplet sensitizers. Preliminary studies indicated that a Corning glass filter with cut off below 340 nm (No. 0-52) was necessary to eliminate all absorption by 10. The results with several triplet sensitizers are listed in Table II. The formation of photoproducts only in the presence of the sensitizers is consistent with energy transfer in solution, but the formation of significant amounts of the rearrangement product xanthine, 12, suggest that some singlet-singlet energy transfer may be occurring. Previous results with 1-hydroxyhypoxanthine indicated that photoreduction was a triplet process and that with some triplet sensitizers, particularly aromatic ketones with lowest n-π^* triplets, chemical reduction by ketyl radicals could be a significant complication.[47] Studies on the sensitized irradiation of 10 are still in progress.

TABLE II

Sensitization Studies of Hypoxanthine 3-Oxide, 10[a]

Expt.	Sensitizer[b]	Time (min)	Recovery of 10, %	Xanthine % yield	Hypo-xanthine % yield	% Recovery
1	None	60	100	-	-	100
2	Benzophenone	60	59	12	29	100
3	Acetophenone	60	4	38	45	87[c]
4	m-Methoxy-acetophenone	60	77	2	4	83
5	m-Methoxy-acetophenone	300	48	7	9	63[c]

a) 1.64×10^{-4} M in CH_3OH solution.

b) ca. 3.3×10^{-3} M.

c) With an unidentified product.

f) Action of peroxides on purines. The action of ionizing radiation on nucleic acid components in aqueous solution has been reported to produce several hydroperoxides.[111] One major product was identified as cis-5-hydroxy-6-hydroxyperoxy-5,6-dihydrothymine (TOOH).[112] This product has been reported to oxidize cytosine to its 3-N-oxide and to yield reaction products with guanine and thymine, but not with adenine.[113] Since adenine is more readily oxidized by peroxyacids than is cytosine, we have examined the reaction of TOOH with adenine. In a preliminary experiment adenine 1-oxide was formed in low yield in citrate buffer at pH 4. In nonbuffered aqueous solution, however, no adenine 1-oxide was detectable. It is difficult to prepare pure samples of TOOH, and it is possible that H_2O_2 is generated by decomposition of it in solution. As a control we have examined the effect of changes of the medium on the reaction of adenine with 3% H_2O_2 (Figure III).[Pg 10] It was found that at acid pH's, especially those in which buffers containing organic acids are used, adenine 1-oxide was formed in up to 13% yield (pH 4) after 3 days under ambient conditions. At higher or lower pH's the yield decreased markedly. At pH 7 adenine 1-oxide was formed in 0.2% yield. These results are qualitatively similar to those from earlier studies on the oxidation of adenosine with m-chloroperoxybenzoic acid.[114]

g) A "radical mediated" 8-substitution reaction of N-hydroxypurines. 8-Trifluoromethylhypoxanthine was formed in low yield by UV irradiation of 1-hydroxyhypoxanthine in 3 N CF_3COOH.[47] We have now examined the photochemistry of 1-, 3- and 7-hydroxyxanthines in 3 N CF_3COOH and find that in each instance a new photoproduct, identified by independent synthesis as 8-CF_3-xanthine is formed in 1-2% yield. 8-Trifluoromethylguanine is formed from 3-hydroxyguanine under the same conditions. In contrast, no 8-trifluoromethyl derivatives were detected from irradiations of 1-methylguanine 3-oxide, 1,7-dimethylguanine 3-oxide, 6-methylpurine 1-oxide[60] or 6,9-dimethylpurine 1-oxide[60] in 3 N CF_3COOH. The latter compounds are constrained in the N-oxide tautomeric form, in contrast to the hydroxyxanthines.[36] The fact that none of them affords an 8-CF_3-derivative suggests that the reaction to yield 8-CF_3-purines must be associated with photochemical loss of the N-hydroxyl group and may be radical mediated.

h) Photolyses of 7- and 9-hydroxyxanthines. 7-Hydroxyxanthine is readily esterified and its ester undergoes reactions identical to those of 3-hydroxyxanthine.[44] It is also an oncogen (unpublished). In contrast, 9-hydroxyxanthine is not easily esterified and undergoes rearrangement to uric acid only under vigorous conditions.[57] Their photochemical reactivities also differ (Table III). 7-Hydroxyxanthine undergoes rearrangement and reduction to the same extent at both pH 3 and pH 10. Its first ionization is from the 7-hydroxy group,[44] and the similarity of results at pH's above and below this ionization suggests that the excited state pK is near or below pH 3.

The first ionization of 9-hydroxyxanthine, however, was deduced to occur from both the N_3-H and the N_9-OH.[57] Even as the dianion at pH 10 it yielded only 10% rearrangement and this was almost eliminated at pH 3. It is possible that in this compound the excited state pK would be associated with ionization at N-3, the usual position for the first ionization for xanthine, rather than at the N_9-OH.

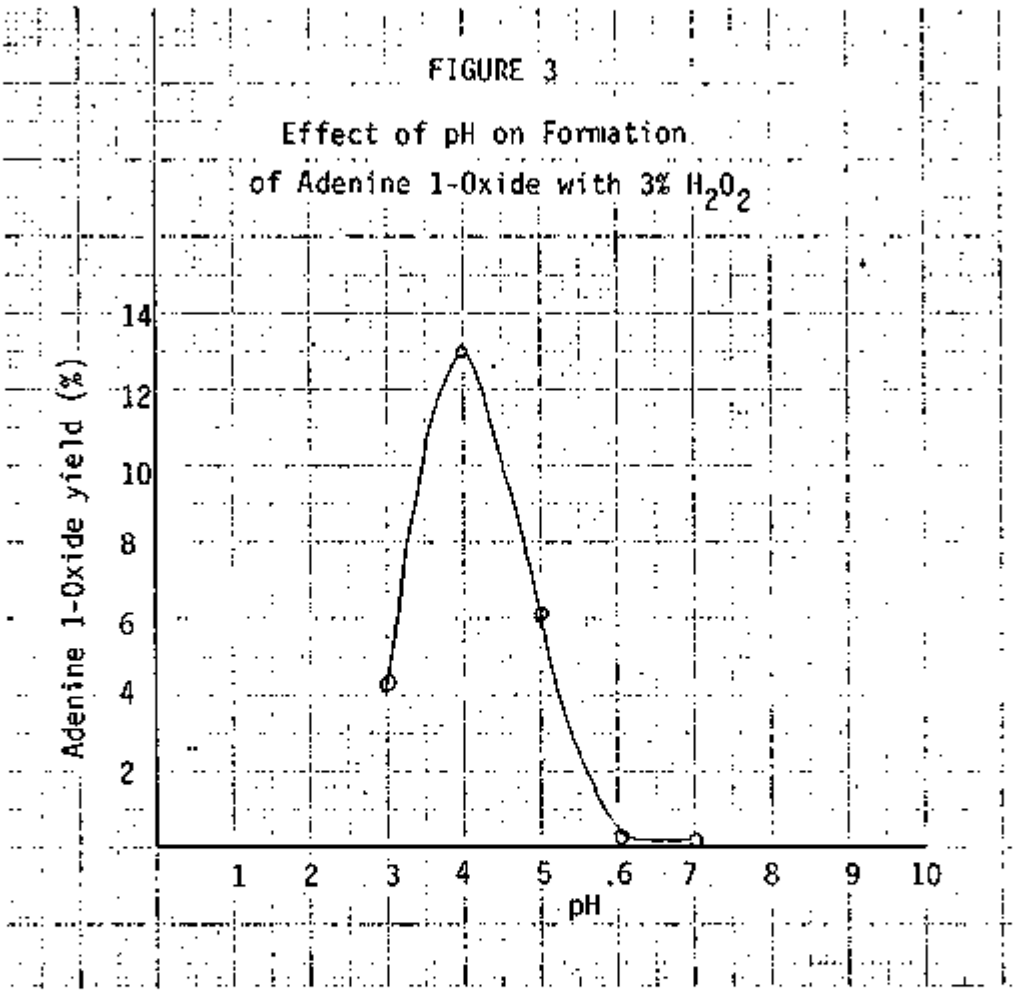


FIGURE 3
Effect of pH on Formation
of Adenine 1-Oxide with 3% H_2O_2
Adenine 1-Oxide yield (%)
14
12
10
8
6
4
2
1
2
3
4
5
6
7
8
9
10
pH

TABLE III

Photolysis of 7- and 9-Hydroxyxanthines

Starting Material	pH	Products[a] Uric Acid,% 6	Xanthine,% 5	Unreacted Starting Material,%
	0	20	7	0
7-Hydroxyxanthine[b]	3.0	20	21	45
	10.0	23	20	37
9-Hydroxyxanthine[c]	3.6	0.2	27	0
	10.0	10	27	0

a) 254 nm lamps. b) pK_a 5.04 and 9.64. c) pK_a 5.06 and 8.41.

i) Mass spectra of purine N-oxides and related compounds. (In collaboration with Dr. Frank Field et al. at the Rockefeller University). Although the mass spectra of some purines have been reported,[115] no such studies of purine N-oxides have been published. The major fragments (m/e) from all N-hydroxyxanthines and N-hydroxypteridines are M-16, rather than the M-17 peak expected for loss of OH. In contrast, 6-substituted purine 1-oxides are found to have two major molecular ions, M-16 and M-17 (Table IV).

TABLE IV

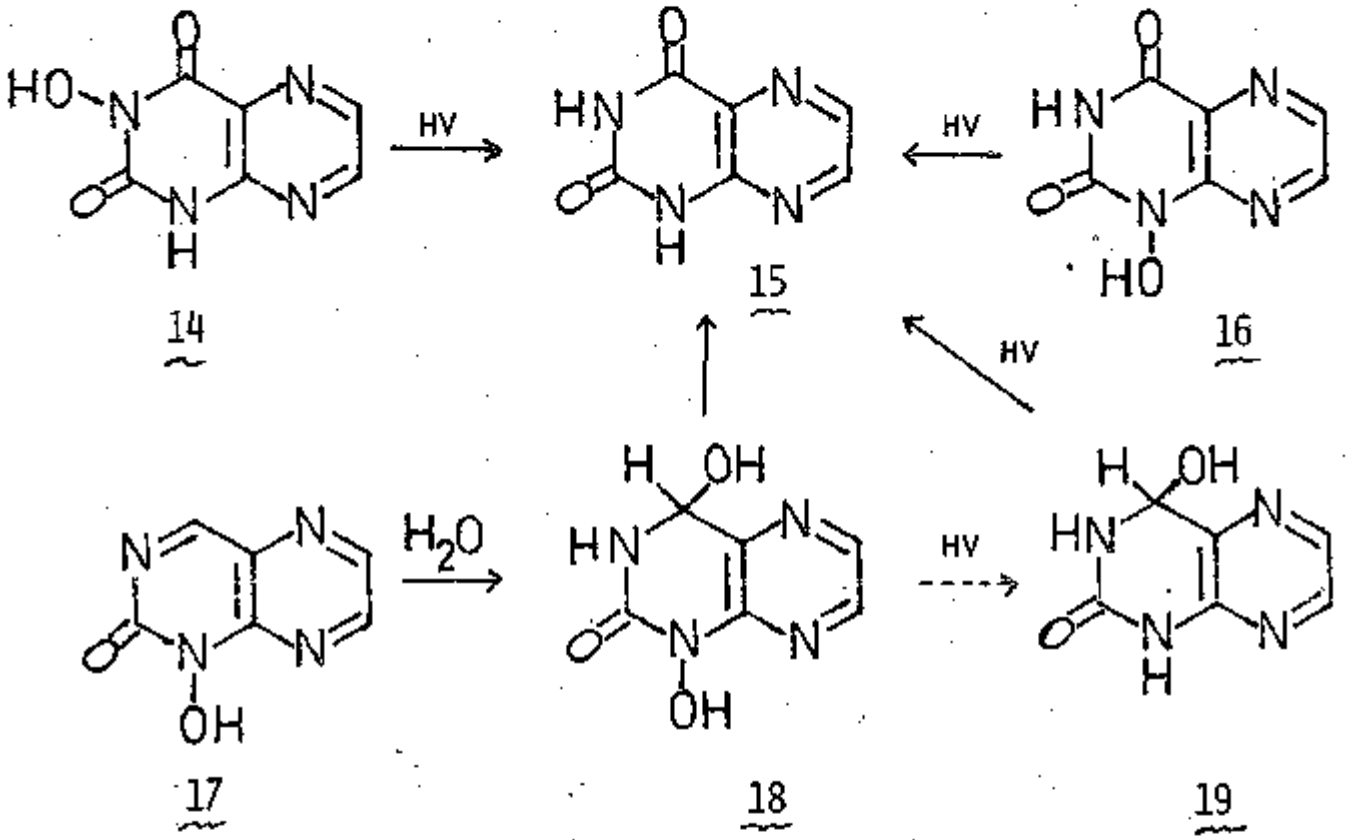
Mass Spectra of 6-Substituted Purine N-Oxides

Compound	M,% Abundance	M-16,% Abundance	M-17,% Abundance
6-Methylpurine 1-oxide	150(89.6)	134(73.0)	133(44.2)
6,9-Dimethylpurine--1-N-oxide	164(100)	148(41.7)	147(36.1)
6-Methylpurine-3-N-oxide	150(45.3)	134(100)	133(2.5)
6-Methoxypurine-3-N-oxide	166(18.0)	150(41.2)	149(13.7)
6-Methoxy-7-methylpurine--3-N-oxide	180(10.8)	164(25.7)	163(9.5)

Mass spectral data will continue to be accumulated, at least as an adjunct to the characterization of these compounds.

j) Photochemistry of heterocyclic N-oxides. A comparison of the photochemical reactivity of π-deficient and π-excessive systems. In a continuation of our study of the novel 1:3 N-hydroxylphotoisomerization reported earlier for 1-hydroxyxanthine,[46] 3-hydroxy-2,4-dioxopteridine,[49] 14, a structural analog of 1-hydroxyxanthine, was irradiated to determine its ability to undergo N-hydroxyl isomerization. Only the photoreduction product, 2,4-dioxopteridine, 15, was observed at both pH's 3 and 10. Under the same conditions the anticipated rearrangement product, 1-hydroxy-2,4-dioxopteridine, 16, showed little change. Prolonged irradiation of 16 did yield some 15. (Scheme II).

SCHEME II



These indicate that even the presence of two adjacent carbonyl groups is not sufficient criterion for the allylic photoisomerization of N-hydroxy groups, which is observed with 1-hydroxyxanthine, and suggest that the π-rich cloud of the imidazole ring plays an important role in the 1- to 3-hydroxyl rearrangement.

Another π-deficient compound, 2-oxo-1-hydroxypteridine, 17, has also been synthesized in this laboratory.[59] Its photoreactivity was examined in comparison with that of its structural analog, 2-oxo-3-hydroxypurine[61] (containing a π-excessive imidazole ring). The irradiation of either the anion or the neutral molecule of 17 did not yield the anticipated photoreduction product, 19, but instead produced 2,4-dioxopteridine, 15, in 9% and 38%, respectively, as the only UV-absorbing product. Irradiation of 2-oxopteridine, under the same conditions also yielded 15. Since this compound is known to form a hydrate in aqueous solution,[116] the photolysis pathway presumably involves the hydrate, 19. Although air oxidation of 19 to 15 can occur, the photolyses have only been performed under N_2 and no 15 was found under the reaction conditions without irradiation. The mechanism for the unexpected formation of 15 from the photolysis of 17 or 19 will require additional study.

k) Actions of ionizing radiation on purine derivatives. With the use of the ion exchange columns, we have, over several years, reinvestigated the ^{60}Co-γ irradiation of adenine. All of the products reported by others have been separated on the A-6 resin column with pH 4.7 buffer. We have repeated the experiments of Van Hemmen[117] with his Sephadex G-10 column and pH 7, 0.05 M phosphate. The compound which he claims is 7-hydroxy-7,8-dihydroadenine is present in freshly irradiated samples. It reverts to adenine at pH 7.0, with a t ½ of less than a day. In acid it reverts to adenine with a t ½ of min. It is not seen in the pH 4.7 or 0.05 N Dowex 50 columns. Its possible identity with the 7-hydroxyadenine claimed by Rhaese[118] was investigated. Repetition of Rhaese's experiments yielded mixtures in all cases. His "7-hydroxyadenine" fraction proved to be a complex mixture, possibly impure adenine 1-oxide. No compound comparable to Van Hemmen's was observed at pH 7.0 on Sephadex.

As previously reported we have repeated Wacker's observation that 5'-AMP-1-oxide is obtained from 5'-AMP when it is irradiated in the presence of organic material which can form peroxides (0.1 N acetic acid). The trace of 2-hydroxyadenine produced from adenine (Conlay[119]) is confirmed. It could arise via a small amount of adenine 1-oxide, since adenine 1-oxide is more sensitive than is adenine to ionizing radiation.

l) Unpublished work supported by AEC but not directly related to the current efforts.

Dr. I. Scheinfeld, who, with Dr. Parham, synthesized the 3-N-oxides of adenine and hypoxanthine[25] also synthesized 2,6-diaminopurine 3-N-oxide. That is anticipated to be oncogenic, in contrast to adenine 3-N-oxide which has proven to be non-oncogenic as predicted (unpublished). When it is possible to assay the 2,6-diaminopurine 3-N-oxide, the work on its synthesis and chemistry will be published.

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