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PURINE N-OXIDES—LII

ESR STUDIES ON PHOTOCHEMICALLY INDUCED RADICALS FROM N-HYDROXYXANTHINES*†

J. C. PARHAM,‡ I. PULLMAN and G. B. BROWN
Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

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Abstract—UV, *gamma*-, or X-irradiation of several N-hydroxyxanthines as powdered solids produces radicals that are indefinitely stable in the solid state at room temperature, but are highly unstable in protic solvents. The ESR spectra are not sufficiently resolved to be definitive but are compatible with an amidogen radical, the unpaired electron of which is partially delocalized through the aromatic π system. Structural characterization was obtained by comparing the UV induced radicals from 3-hydroxyxanthine and 3-hydroxy-8-methylxanthine with chemically generated nitroxyl radicals from the same compounds. These two radical species show differences in their ESR spectra, in the extent of interaction of the unpaired electron with the methyl group at position 8, and in the products resulting upon reaction in water.

The amidogen radical reacts instantaneously with water to yield the parent xanthines. Parallels are drawn between this reduction of the amidogen radical, the photoreduction of 3-hydroxyxanthine when solutions of it are irradiated with UV light, and the reduction of 3-acetoxyxanthine in aqueous solution in the absence of light.

The synthesis of a requisite derivative, 3-hydroxy-7,8-dimethylxanthine, is reported.

Stable radicals have been produced in several purines in the solid state by the direct action of ionizing radiation, including X^2 and γ[3,4] rays and atomic hydrogen and deuterium.[4,5] We have previously mentioned[1,6-8] that a stable radical can be produced in powdered crystals of 3-hydroxyxanthine by the milder action of UV light. The potential significance of this observation is heightened by recent chemical evidence that suggest a free-radical intermediate for one of the pathways by which esters of the potent oncogen[9,10] 3-hydroxyxanthine (**1**) (Scheme 1) react in neutral aqueous solutions.[7,8,11,12] We now report a study on the structure of the radical produced from **1** and on related radicals derived from several derivatives and isomers of **1**.

RESULTS AND DISCUSSION

Irradiation of **1** as a dry solid with UV light at room temperature induced a progressive color change from white to deep purple. This was accompanied by the appearance of an ESR signal, the intensity of which increased with the duration of irradiation. With the maximum irradiation employed the yield of radical increased almost linearly for the first 100 hr and then leveled off at 12 ± 3% with little further change to 300 hr (Fig 1). The radical is indefinitely stable in the solid state; there was no significant decay in 1 yr. It is immediately lost when dissolved in water, acid, base or organic solvents such as DMF or DMSO. This rapid decomposition in all solvents of sufficient polarity to dissolve **1** or its radical derivative precluded ESR spectra of solutions, in which better resolution of hyperfine interactions would be expected. It has not been possible to grow crystals of 3-hydroxyxanthine large enough for irradiation to attempt single crystal ESR spectra.

The ESR spectrum of a powdered sample of **1**, after a 3 min irradiation with a low UV dose which induced less than 0·01% conversion to the radical, is illustrated in Fig 2. It shows an anisotropic triplet with a separation between the outer lines of 33 G and with individual line widths of 6 G. Additional weak lines can be noted on the side of the low field main line. The g value at the center of the spectrum was 2·006 ± 0·001. With further irradiation the concentration of radicals increased and the spectrum collapsed to an anisotropic spectrum with about a 10 G spread. The width of the main line was 3 G and the high field side was split into a second, unresolved line (Fig 2). The collapsing is presumably due to exchange narrowing. To determine whether the lines in the spectrum were due to hyperfine splitting or to g factor anisotropy, the 8 min irradiation spectrum was measured at two different frequencies in cavities resonating at 9641·34 MHz and at 8855·15 MHz, a difference of 8·9%. The separations

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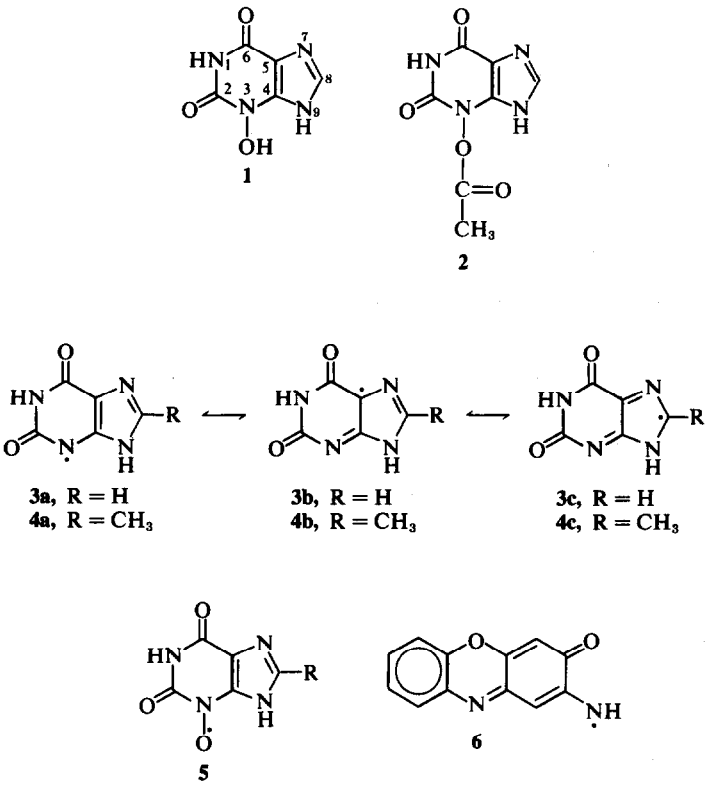
‡To whom correspondence should be addressed.



SCHEME 1

of the three lines changed by 2% or less, which indicates that they belong to the same hyperfine multiplet. Energy saturation studies showed that all parts of the spectrum saturated at the same rate, which is also consistent with the presence of a single radical species.

X- or γ-irradiations (~200,000 Rads) of solid samples of **1** gave a radical with an ESR spectrum identical to that produced by a low dose of UV light (3 min, Fig 2). A radical has previously been produced in xanthine in the solid state by X-irradiation and was reported[2] to have a g-factor of 2·0040. It was deduced[2] to be a π-delocalized radical resulting from the addition of an electron to the lowest unoccupied orbital of the neutral molecule.

The lack of hyperfine resolution severely compli-

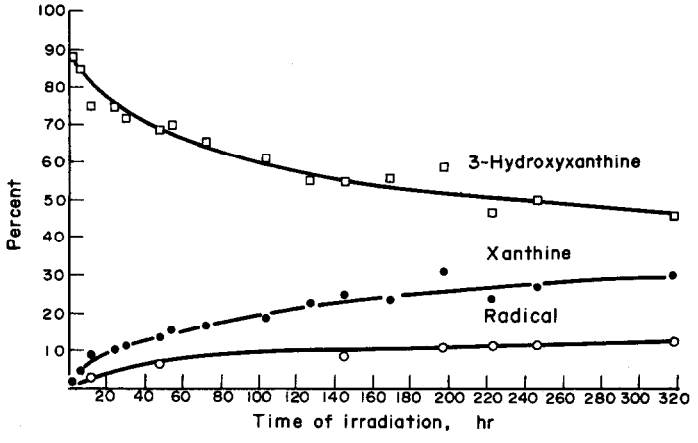


Fig 1. Comparison of radical formation with product composition from aqueous solutions of irradiated 3-hydroxyxanthine.

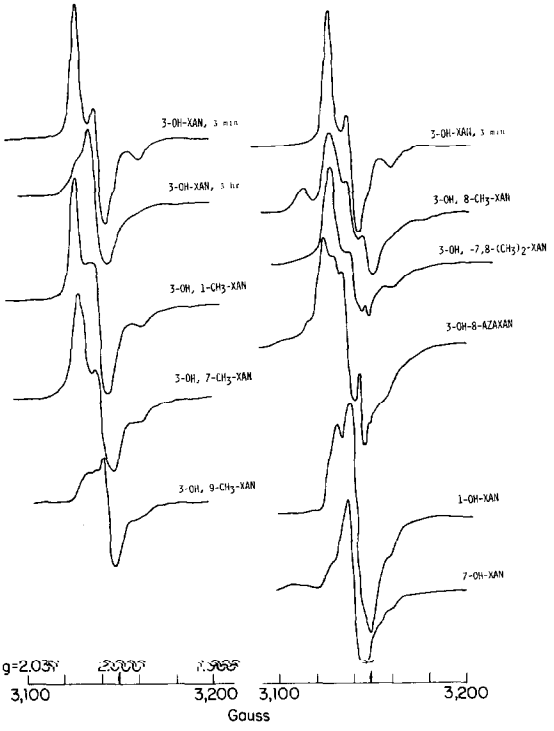


Fig 2. ESR spectra of UV irradiated N-hydroxyxanthines.

cates the determination of the structure of the photochemically induced radical. We have partially circumvented this difficulty by examining the ESR of radicals photo-induced in a series of methyl-substituted derivatives[13] of **1**, as well as its 3-acetoxyderivative (**2**). In each case, a radical was generated with a g value of $2{\cdot}005 \pm 0{\cdot}001$ at the center of the spectrum (Fig 2). The irradiated 3-acetoxyxanthine gave an exchange-narrowed spectrum identical to that of **1** and a comparable yield of a radical. The yields obtained from the irradiated Me derivatives were considerably lower, ~2 to 4% of the radical yield from **1**, but the times of irradiation of maximum yield of radical have not been investigated in each case. Development of the purple color was much less evident in these compounds. The spectra of the radicals from 1- and 7-methyl-3-hydroxyxanthine were very similar to that of low concentrations of the radical of 3-hydroxyxanthine. Each also had a g value of $2{\cdot}006 \pm 0{\cdot}001$ at the center of the spectrum. The close correspondence in the ESR spectra of radicals from **1**, from its 1- and 7-Me derivatives and from 3-acetoxyxanthine indicate similar radicals are formed. These must all be associated with the N-OH group at N-3 since no radical was produced by irradiation of xanthine under the same conditions.

The ESR spectra of radicals from the 8-Me and 9-Me derivatives of **1** differed from that **1** (Fig 2), but the presence of lines at positions corresponding to those in the spectrum of **1** indicate that these radicals have some similarity to that derived from **1**. While all other spectra were about 33 G in width, the spectrum of the radical from 3-hydroxy-8-methylxanthine contained additional bands and the separation between the outermost lines was 65 G. It was centered at g equal to $2{\cdot}004 \pm 0{\cdot}001$. Both the presence of the additional lines in the spectrum of the 8-Me derivative and the greater width of the spectrum suggest that the unpaired electron interacts with the protons of the C-8 Me group. This implies a π radical delocalized over both the pyrimidine and imidazole rings, with canonical forms such as **3a–c** (Scheme 1) contributing to the structure. If so, the resonance contribution that permits interaction with the methyl group at C-8, **4c**, should be prevented by a substituent at N-7. To test this interpretation 3-hydroxy-7,8-dimethylxanthine was prepared and irradiated. Its spectrum more closely resembled that of irradiated **1**, with little indication of interaction of the free electron with the 8-Me group.

Without ESR spectra of the radical in solution, nor of oriented single crystals, it is difficult to determine whether the radical is an imidogen (N–), (**3**), or a nitroxyl (N—O) (**5**). Since 3-hydroxyxanthines are cyclic hydroxamic acids and nitroxyls of hydroxamic acids can be produced in solution by oxidation with ceric ions,[14–16] **1** and its 8-Me derivative were reacted with $Ce(SO_4)_2$ to generate the respective nitroxyls, **5** and its 8-Me derivative (**5**, R = Me), for comparison with the radicals induced photochemically in the solids. Reaction of 10^{-3} M solutions of **1** (colorless) and of $Ce(SO_4)_2$ (yellow), both in M H_2SO_4, gave a transient purple color which faded to a colorless solution. For ESR determinations flowing solutions were mixed just prior to measurement. Radicals with similar ESR spectra were produced from both **1** and its 8-Me derivative. In each case, a three line spectrum was obtained with equal spacings and equal amplitude, which corresponds to an interaction with a single nitrogen nucleus and is consistent with the formation of the nitroxyl, **5**. No other spectral lines were observed to within 2% of the derivative height for **1** or to within 5% for 3-hydroxy-8-methylxanthine. The values for g and for a_N are in Table 1. The g values for these nitroxyl radicals from 3-hydroxyxanthines are comparable to those of stable nitroxyls in solution, $2{\cdot}0060 \pm 0{\cdot}0002$[17] and to those of unstable nitroxyls derived from hydroxamic acids by ceric oxidation (2·0065 – 7).[16] The nitrogen coupling constants are quite close to that for the similarly generated benzohydroxamic acid nitroxyl (6·0 G),[14] and near those for nitroxyls derived from substituted hydroxamic acids by oxidation with $Ce(SO_4)_2$ (7·5–7·9 G),[16] nickel peroxide (7·5 G)[18] or silver oxide (7·4–7·5 G).[19,20]

The slightly lower g value of the nitroxyl from 3-hydroxy-8-methylxanthine than that of the nitroxyl

Table 1. ESR values of solutions of 3-hydroxyxanthines oxidized by ceric sulfate

	g	a_N, gauss	Line width, gauss
3-Hydroxyxanthine	2·0061 ± 0·0002	6·1 ± 0·1	2·2 ± 0·1
3-Hydroxy-8-methylxanthine	2·0053 ± 0·0002	5·9 ± 0·1	~2

of **1** (Table 1) indicates a greater extent of delocalization of the unpaired electron in the presence of the 8-Me group.* Unlike the spectrum of the radical generated photochemically from 3-hydroxy-8-methylxanthine, the spectrum of its nitroxyl does not show additional structure attributable to an interaction with the 8-Me group.

The differences in the ESR spectra of the chemically generated nitroxyl, **5**, and the radical photoinduced in the solid state were accompanied by differences in their decomposition products in solution. The photo-induced radical, which is stable in the solid state, lost its ESR signal and the purple color when dissolved in water and gave a yellow solution. Ion exchange chromatography of this solution showed that the primary product from reaction with water was xanthine and that much unchanged **1** remained. In a time study of **1** irradiated as a suspension in ethyl acetate (Fig 1), the increase in yield of xanthine roughly paralleled that of the radical. The yield of xanthine increased rapidly to 11% in the first 24 hr, then gradually approached a maximum value of 28 ± 3%. The radical yield approached its maximum after 100 hr and remained constant to 300 hr. At this time there was still nearly 50% of unreacted **1**. The initial rapid formation of xanthine and the difference in the yields of the radical and xanthine suggest that traces of water were present.† The continued presence of **1** suggests that radical production on the particle surfaces protects a core of unreacted **1**. In contrast to the production of nearly 30% of xanthine from the photo-induced radical, the oxidation of **1** to the nitroxyl **5** yielded only traces of xanthine (1·4%) and incomplete recovery of **1** (29%). The decrease in UV absorption accompanying the oxidation of **1** by ceric sulfate and the low recovery of UV-absorbing material indicate that reactions other than nitroxyl production must result in destruction of the chromophore of **1**. Xanthine was unaffected by ceric sulfate under similar conditions.

The UV irradiation of solutions of either **1** or its 3-0-acetyl derivative, **2**, showed similarities to the behavior of these compounds when irradiated as solids and then dissolved. Irradiation of **1** in aqueous solution caused a loss of UV absorption which was linear with time. Xanthine was the primary photolysis product. Since 3-acetoxyxanthine, **2**, is highly reactive in water, but is stable in dioxane,[7] it was irradiated in dioxane. This irradiation caused some loss of UV absorption and gave xanthine as the main product. Similar behavior was noted when **2** was irradiated in the solid state, then reacted in 1 N HCl.‡ The irradiated sample of **2** yielded 20% xanthine, while an unirradiated sample yielded no xanthine when dissolved in 1 N HCl.

Photoreduction of **1** or **2** can thus be accomplished in either one or two steps, upon irradiation of solutions, or when the solid is irradiated and then allowed to react in solution. Very little reduction to xanthine occurs when **1** is oxidized with ceric sulfate to the nitroxyl, **5**, and it, in turn, decomposes in solution.

Collectively, the available evidence favors the amidogen structure, **3**, rather than the nitroxyl, **5**, for the radical induced with UV or ionizing radiation in the solid state of **1**.§ While there are differences in the ESR spectra of **3** and **5**, the poorly resolved spectra of **3** and the availability of few examples of unambiguous acyl amidogen (amidyl) radicals in the literature prevent a definitive assignment on this basis alone.‖ The triplet observed in the ESR of the photoinduced radicals must be due

*The small effect of the 8-Me substituent on a_N of these nitroxyls, little more than experimental error, is in accord with the negligible effect exerted by substituents on a_N in substituted benzoyl nitroxides.[22]

†Less than 0·01% of water in the ethyl acetate could account for the difference in yields of the radical and xanthine.

‡3-Acetoxyxanthine, **2**, has been demonstrated[7] to react in aqueous solution at pH's above 3 to yield xanthine in the absence of light. At pH's below 3 the reaction leading to xanthine does not occur,[7] hence the irradiated sample of solid **2** was allowed to react at pH 0 (1 N HCl).

§This assignment contrasts with evidence which indicates that a nitroxyl is formed by X-irradiation of solid N-hydroxyurea.[23]

‖For recent commentaries on literature reporting ESR data of radicals considered to be amidyls, see M. C. R. Symons, *J. chem. Phys.* **55**, 1493 (1971) and G. A. Helcké and R. Fantechi.[24] Contradictory evidence plagued attempts to characterize amidyl radicals. The structures of two radicals initially assigned as amidyls, $HCO\dot{N}H$[25] and $NH_2COCH_2C\dot{O}NH$,[26] were subsequently reassigned as $\cdot CONH_2$[24,27-29] and as $\dot{N}{=}CHCH_2CONH_2$.[30] Another example, $CF_3CF_2C\dot{O}NH$, was inconclusively characterized.[31] The structure of a radical from CF_3CONH_2, tentatively assigned as $NH_2\dot{C}O$,[32] has lately been questioned and an amidyl structure, $CF_3C\dot{O}NH$, has been suggested.[24]

Recently ESR parameters have been reported for amidyl radicals generated by UV irradiation of N-nitrosoamides[33] or diacyltetrazenes[34] in solution and by γ-irradiation of solid urea.[35] In addition, values for N-t-butoxyamido radicals, generated in solution by several routes, have also been reported.[35]

to hyperfine interaction of the radical with the nitrogen at position 3. The g values of about 2·006 for these radicals in the polycrystalline state, which can only be approximated due to the asymmetry and the broadened lines in the ESR spectra, are approximately those reported for both acyl nitroxyls[16,21] and for amidyls.[33-35] However, the differences both in the extent of interaction with the 8-Me substituent in the 8-Me derivatives of **3** and **5** and in the formation of xanthine indicate that **3** and **5** differ. In addition, the width of most nitroxyl ESR spectra is broadened to about 65 G in the solid state or in solution in viscous media.[37,38] The narrowness of the spectrum, 33 G, thus favors the amidogen structure, **3**. This spectral width of **3** is more comparable to the 25·7 G width observed for the vinyl amino radical from Questiomycin A, **6**,[39] which is also stable in the solid and has a g value of 2·009. Thus, while diphenylamine radicals can usually be distinguished from diphenylnitroxyls by their smaller g values, 2·003 compared to 2·0055–2·0068,[40] the higher g value of 2·009 for **6** demonstrates that vinylamine radicals can have even higher g values than nitroxyls. Structure **3**, with its adjacent carbonyl function is an acyl amidogen, but the 3-nitrogen and the 4–5 double bond also represent a vinylamine system. This dual substitution of the nitrogen radical makes its character complex.* The g value of 2·006 for the photoinduced radical is thus compatible with the assignment as an amidogen. The rapid reaction in solution with accompanying loss of ESR indicates that **3** is an extremely efficient hydrogen abstractor. Such behavior would agree with the amidogen assignment since it is characteristic of the amidyl radicals that have been generated photochemically in solution from N-halo or N-nitroso amides.[42] The narrow spectrum width of **3** and the presence of a single hyperfine multiplet are consistent with a small interaction with the 3-nitrogen and some π-delocalization. The similarity of the ESR spectrum of **3** to those of its 1-Me and 7-Me derivatives indicates that in the radical from **1** there is no interaction of the unpaired electron with the hydrogen at N-1 and little or no interaction with the hydrogen of the imidazole ring. In **3** the delocalization must be limited primarily to the pyrimidine ring, as in **3a** and **3b** (Fig 1).† Some delocalization of the odd electron in **3** accords with the behavior of other amine radicals‡ and agrees with the conclusions[24,34-36] that amidyls are π-radicals. The electron donating capacity of the 8-Me group of 3-hydroxy-8-methylxanthine must support stabilization at C-8 of the unpaired electron of the amidogen radical, **4a**, and thus promote a significant contribution of resonance form **4c**.§

Although free radicals are usually high colored, the intense purple color which develops with UV irradiation of **1** is apparently not that of **3**. Irradiated **1** dissolves in di-n-butylsulfoxide with loss of the ESR signal, but without loss of the purple color. The yellow color of aqueous solutions of the irradiated material, as well as the minor product noted on ion-exchange chromatography, may be associated with the decomposition of the colored material. The presence of the colored component complicates interpretations of the IR‖ and visible¶ spectra of the samples.

Stable radicals could also be induced with UV light in solid samples of 3-hydroxy-8-azaxanthine,[47] 1-hydroxyxanthine,[48] and 7-hydroxyxanthine[49] (Fig 2). That from 3-hydroxy-8-azaxanthine showed an anisotropic spectrum with unresolved fine structure that may be due to some interaction of the odd electron with the nitrogen of the triazole ring, comparable to that shown with the methyl group in the radical from 3-hydroxy-8-methylxanthine. The radical from 7-hydroxyxanthine also showed an anisotropic spectrum which resembled that of **3** after prolonged (3 hr) irradiation.

The similarity of ESR spectra of the radicals from **1** and from 7-hydroxyxanthine is paralleled by the similar reactivities of their esters in aqueous solutio , for which a common intermediate has been proposed.[49] Esters of each react in solution at pH's above 3 to yield not only 8-substitution products, but also the reduction product, xanthine, and a blue product.[7,49] Evidence suggesting a radical inter-

*Hedaya *et al.*[41] considered the influence of vinyl or acyl substituents on amidogen radicals and concluded that vinylamine radicals are π-radicals, while acylamine radicals will be σ-radicals if the carbonyl oxygen is sufficiently more electronegative than the nitrogen. Most reports conclude that acylamino radicals have a π-ground state.[24,34-36]

†The ESR of these radicals resembles that of the radical generated by thermal homolysis of N-(4′-pyridyloxy)4 (1H)-pyridone. That radical is also deduced to be π-delocalized and reacts to yield the 3,3′-(4,4′-dihydroxy)-bipyridine dimer.[43]

‡In diphenylaminyl radicals the unpaired electron is calculated to be delocalized into the aromatic substituents to the extent of about 60%.[44]

§Such a substituent effect is observed in other amidogen radicals; delocalization of the unpaired electron is enhanced by electron-donating substituents in diphenylamine radical cations[44] and in diphenylamine radicals.[45]

‖The IR spectrum of an irradiated sample of **1** containing about 12% of **3** showed a strong resemblance to a spectrum of unirradiated **1**. One difference was a new but very weak band at 1320 cm^{-1} that is close to a frequency characteristic of nitroxyls with aromatic substituents (1342–1370 cm^{-1}).[46a] The presence of the purple contaminant does not permit a definitive assignment of this absorption.

¶Nitroxyls in solution absorb in the visible at 490–570 nm.[46b] A di-n-butylsulfoxide solution of irradiated **1** showed broad absorption, centered at 550 nm in the visible spectrum, but from the loss of ESR signal in the solution this absorption cannot be due to the radical. This absorption band is near that of a nonradical blue product which arises from **2** in aqueous solutions,[7] and which absorbs at 540–560 nm in DMSO solution (G. Zvilichovsky, unpublished data).

mediate in the reduction of 2 to xanthine in solution has been reported.[7] The blue compound shows no ESR signal and is unstable in solution, but does not react to yield xanthine.[7] This correlation in the production of colored products from 7-acetoxy and 3-acetoxyxanthines in solution, and from solid samples of 7-hydroxyxanthine, 1 or 2 by UV irradiation, suggests that the colored product arising from the acetoxy derivatives in aqueous solutions may be formed in association with, or be a secondary reaction product from, comparable radical intermediates.

Both 1 and 2 yield radicals with similar ESR spectra upon UV irradiation in the solid state and these radicals each decompose in water to yield xanthine. This evidence parallels that suggesting[7,8] that a radical thermally induced from 3-acetoxyxanthine in solution also leads to the xanthine which is experimentally observed. The radicals induced by photochemical excitation of solid 3-hydroxyxanthine and arising in solution from 3-acetoxyxanthine may or may not be identical. Further studies of photochemically induced reductions of N-hydroxypurines in solution, some of which are underway,[50] may clarify the character of the probable radical intermediates, and may aid the assessment of the biological importance of radicals[12] which may arise *in vivo* from esters[51] of such oncogenic compounds.

EXPERIMENTAL

The ESR spectra were determined with an X-band spectrometer, with 30 MHz superheterodyne phase detection and 212 Hz magnetic field modulation, which has been described.[52] A 60 k volt X-ray source[52] was used for X-irradiation and a ^{60}Co source for γ-irradiation of 1. IR spectra were determined with an Infracord spectrometer and UV spectra with a Unicam SP-800 recording spectrophotometer. Analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich. An ISCO UA-2 UV analyzer was used to monitor column eluates. A Nester Faust NFUV-300 low pressure Hg light source or a Spectroline R-51 lamp (2537 nm) was used for UV irradiations.

ESR spectra. First derivative ESR spectra of solid samples were measured in the presence of air at room temperature. Intensities were determined by double integration and comparison with diphenylpicrylhydrazyl standards. The microwave power level in the cavity was 30 microwatts and the modulation amplitude chosen was between one and four gauss.

Irradiation of 3-hydroxyxanthine in the solid state

A sample of 3-hydroxyxanthine[53,54] was allowed to stir overnight as a suspension in 0·1 N HCl to remove traces of guanine 3-oxide and metal contaminants. It was collected and washed with water. Chromatographic examination (BioRad 50 [H$^+$]) showed that the sample contained ~0·5% of xanthine, but no other UV absorbing component. The sample of 1 was then ground to a fine powder and dried for 18 hr over P_2O_5 at 80° under vacuum to remove the water of hydration.[53] In a 2 l., 3-neck, round bottom flask equipped for magnetic stirring, a 1·0 g portion was suspended in 2 l. freshly distilled EtOAc previously dried over Type 4A molecular sieve. The unfiltered NFUV-300 light source, primarily 253·7 nm, (λ_{max} neutral species of 1 is 273 nm)[54] was inserted in the center well and glass stoppers sealed the other openings. The flask was partially immersed in a H_2O bath which was maintained at 26 ± 2° during irradiation by a Cold Finger portable immersion cooler. The sample was stirred vigorously and irradiated at 75% intensity of the UV source (~34 W total energy output). Aliquots were withdrawn at periodic intervals and the solid was collected and air dried.

A portion of aliquots from the irradiation of 1 suspended in EtOAc was dried as described, weighed, then dissolved in dilute NH_4OH and chromatographed over a 1 × 10 cm BioRad 50 [H$^+$], X8, 200–400 mesh column. 3-Hydroxyxanthine was eluted with H_2O and xanthine with N HCl. The molar quantities in each fraction were calculated from known ϵ_{max} values at 273 nm (ϵ, 10,100)[13] for 1 and at 261 (ϵ, 9200)[7] for xanthine. Duplicate determinations varied by 2% or less. The results, expressed as weight %, are plotted in Fig 1 along with the corresponding % by weight of free radical.

A second product from the irradiation was detected when the solns were fractionated over BioRad-50. It was eluted with H_2O before 1 and increased with increasing time of irradiation, but was still only a trace after 300 hr. Its UV spectrum showed broad absorption bands, λ_{max}: pH 1, 266 and 325; pH 5, 267 and 325; pH 12, 255 nm. It proved unstable in attempts to isolate it. In solns of the crude irradiation mixture, a band of low intensity near 350 nm in H_2O and at 330 nm in MeOH was probably attributable to this product.

In solns of di-n-butylsulfoxide (technical grade, Aldrich Chem. Co.) the purple color of the irradiation product remained and the spectrum of the soln showed a broad absorption band from 400 to 700 nm, centered at 550 nm.

A portion of the irradiated product was dried at 80° over P_2O_5 under vacuum for 18 hr. Although C, H and N were all high, the analysis is within the experimental limits for 3-hydroxyxanthine. (Found: C, 35·81; H, 2·48; N, 33·37. Calcd for $C_5H_4N_4O_3$: C, 35·72; H, 2·39; N, 33·32%).

Irradiation of 3-hydroxyxanthine in solution

A 250 ml aqueous, unbuffered soln of 1 ($1·5 \times 10^{-4}$ M) in a quartz flask was stirred vigorously and irradiated with the spectroline UV lamp. Progress of the photolysis was monitored by UV spectra of aliquots. The changing spectra showed a slight hypsochromic shift from 273 to 270 nm and a continuous decrease in optical density that was linear with time. After 13 hr of irradiation ~50% of the original optical density had been lost and chromatographic analysis with Biorex AG-50 [H$^+$] showed the soln contained equal amounts of 1 and of xanthine, plus a small amount of one other component which was eluted prior to 1 and produced only end absorption in the UV.

Irradiation of thin layers of solid samples

Samples of 3-acetoxyxanthine[55] and the 1-; 7-, 8-, and 9-methyl and 7,8-dimethyl derivatives of 3-hydroxyxanthine,[13] of 1-[48] and 7-hydroxyxanthine,[49] and of 3-hydroxy-8-azaxanthine[47] were irradiated, as finely ground powders at room temp, with a Spectroline UV lamp. The face of the lamp was ~2 to 3 cm from the surface of thin layers of the compounds, which were mixed periodically and were irradiated for 6 to 12 hr.

Examination of unirradiated samples of 3-hydroxyxanthine

Several laboratory samples, with varied exposures to light, showed ESR responses of 10^{18} to 10^{19} spins/mol, or about 0·0001 to 0·001 mol% of radicals, while a sample prepared in essentially complete darkness showed 10^{13} spins/mol, or 10^{-9} mol%.

Irradiation of 3-acetoxyxanthine

(a) *Solid state.* A sample of finely powdered 3-acetoxyxanthine[55]·$\frac{1}{3}$ AcOH (as determined from an NMR integration) was irradiated for 24 hr. A 3·0 mg (13 μ mol) sample was dissolved in 10 ml of 1 N HCl and the soln was stirred for 2 days at 25°. The solvent was then removed under reduced pressure and the residue was dissolved in 5·0 ml water. A 2·0 ml aliquot was applied to a 1 × 15 cm column of BioRad AG-50 [H^+] that was eluted first with water to remove 8-chloroxanthine[56] (1·5%) then with 0·4 N HCl to elute 3-hydroxyxanthine (67%) and xanthine (20%).

A control sample of unirradiated 3-acetoxyxanthine·$\frac{1}{3}$ AcOH treated with 1 N HCl and chromatographed similarly gave 1·7% of 8-chloroxanthine, 81% of 3-hydroxyxanthine and traces of two unidentified products, but no xanthine.

(b) *In dioxane solution.* A soln of 3·0 mg 3-acetoxyxanthine·$\frac{1}{3}$ AcOH in 100 ml spectroquality dioxane in a quartz flask was stirred vigorously and irradiated with the Spectroline lamp. The reaction was monitored until there was no change in the UV spectrum of aliquots. There was a 2 nm bathochromic shift from the original 270 nm band and a loss of ~ 30% in optical density as the photolysis proceeded. The dioxane was removed under reduced pressure, the residue dissolved in 5·0 ml water and a 2·0 ml aliquot was chromatographed as described. Elution with water gave an unidentified product with UV spectra; λ_{max} (pH): 267 (2); 273 (5); 265 nm (11). Elution with 0·4 N HCl gave xanthine (12%). No other products were obtained with further elution.

3-Hydroxyxanthine nitroxyl (4)

(a) *ESR analysis.* Solns (10^{-3} M) 3-hydroxyxanthine or 3-hydroxy-8-methylxanthine in 1 M H_2SO_4 and $Ce(SO_4)_2$ (10^{-3} M) in 1 M H_2SO_4 were placed in separate separatory funnels. The solns were allowed to flow by gravity through flow meters and then into a Varian 4-jet lucite mixing chamber. The chamber exist was 1 cm from the top edge of a cylindrical cavity and the mixed solution flowed through a thin wall 1 mm (i.d.) pyrex tube. Flow rates of about 15–20 cc/min of ceric sulfate and 9–12 cc/min of the 3-hydroxyxanthine produced maximum signal intensities.

ESR parameters. The amplitude of magnetic field modulation ranged from 0·75 to 3·8 G. Power levels were set from 0·06 to 1·25 mw. Sweep rates ranged from 20–50 G/min and time constants from 0·1–1·0 sec. Signal averaging on a Varian C-1024 CAT was used when necessary. Magnetic fields were measured to ±0·05 G with a proton NMR probe and microwave frequencies were measured to ±0·02 MHz with a transfer oscillator and counter. The ESR parameters are in Table 1.

(b) *Chromatographic analysis.* Equal vol (50 ml) of 10^{-3} N H_2SO_4, were mixed and stirred at 25°. Reaction was monitored spectrally from diluted (1–3) aliquots until no further changes were noted in the UV spectrum. The soln was concentrated under reduced pressure to ~ 3 ml, then applied to a 1 × 15 cm BioRad-50 [H^+] column. This was first eluted with water, which removed 3-hydroxyxanthine (29%), then with 0·4 N HCl to remove xanthine (1·4%). An unidentified product was eluted by 3 N HCl and showed UV absorption; λ_{max} (pH 1 and 5): 221, 238, 252 nm. It precipitated upon the addition of base to the cuvette.

When equal volumes of xanthine (10^{-4} M in 1 M H_2SO_4) and $Ce(SO_4)_2$ (10^{-4} M in M H_2SO_4) were mixed, there was no change in the UV spectrum from that of xanthine and no loss in optical density over a 2-day period. Xanthine could be recovered quantitatively by chromatography over Dowex-50 [H^+], as described

3-Hydroxy-7,8-dimethylxanthine. A soln of 0·52 g (2·9 mmole) 7,8-dimethylguanine[57,58] dissolved in 4 ml CF_3CO_2H and 2 ml 30% H_2O_2 was stirred at 25° for 4 days. Ether (100 ml) was added and the flask chilled. The solvents were then decanted and discarded. The ppt was dissolved in 4 ml NH_4OH and heated at 70–80° for 20 min. The soln was treated with charcoal and filtered, and the filtrate was acidified with AcOH. The ppt was collected and washed with acetone, then ether and finally air dried to yield 130 mg of 3-hydroxy-7,8-dimethylguanine; NMR (CF_3CO_2H): δ 2·86 (s, 3, 8-CH_3); 4·19 (s, 3, 7-CH_3). Its UV spectra at pH's 1, 5 and 12 closely resembled those of 3-hydroxy-7methylguanine.[59]

The product was dissolved in 15 ml of 4 N HCl and refluxed for 2 days. The solvent was removed in vacuum, the residue dissolved in hot NH_4OH; the soln was treated with charcoal, filtered, and the filtrate was acidified with AcOH and chilled. The ppt was collected and washed with EtOH, then Et_2O and air dried, yield 40 mg.

The remaining soln was reduced in volume and applied to a BioRad 50 [H^+] column (1 × 6 cm) which was eluted with H_2O to yield an additional 5 mg, total yield 45 mg (7% overall).

The analytical sample crystallized from H_2O as fine colorless needles and was dried at 100° in vacuum over P_2O_5 for 6 hr. (Found: C, 39·25; H, 4·58; N, 26·24. Calcd for $C_7H_8N_4O_3 \cdot H_2O$: C, 39·26; H, 4·70; N, 26·16%). The NMR spectrum showed (DMSO-d_6): δ 2·40 (s, 3, 8-CH_3); 3·80 (s, 3, N-CH_3); 10·75 (s, 2, N-H and O-H). The UV spectra, λ_{max} (pH): 204, 274 (3); 221, 250 sh, 305 (11), were nearly identical to those of 3-hydroxy-7-methylxanthine.[59]

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