EPR spectroscopic study of the radical oxidation of hydroxypurines in aqueous solution: acid–base properties of the derived radicals

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EPR spectroscopy has been employed to study the reactions of $SO_4^{\bullet -}$ and $OH^{\bullet}$ radicals with xanthine, 8-methylxanthine, hypoxanthine and 2-hydroxypurine.

Radicals obtained on the reaction of $SO_4^{\bullet -}$ with these hydroxypurines resulted, depending on the pH, from one-electron oxidation of either the parent compound or its anion, followed by deprotonation. For xanthine and 8-methylxanthine the respective radical anion and radical dianion were observed, for hypoxanthine the species detected correspond to the neutral radical and radical anion, whereas for 2-hydroxypurine only its radical anion was observed.

The pK_a values of the radical anion of xanthine and 8-methylxanthine were estimated as 12.0 ± 0.1 and 12.1 ± 0.5, respectively, whereas the pK_a value of the hypoxanthine neutral radical was 7.7 ± 0.1.

In the reaction with $OH^{\bullet}$, only the radical anion of xanthine was detected.

Introduction

The reactions of oxidising radicals with purine bases have been the subject of intense study for several years, principally because of the interest in the chemical changes induced in DNA and its constituents as a result of the exposure of living tissues to ionising radiation. Such exposure results in direct ionisation of DNA leading to the formation of electron loss centres on the guanine moiety (since out of all the DNA bases this has the lowest ionisation potential), and radical anions centred on the pyrimidine bases. Indirect DNA damage may also occur as a result of reaction with water radiolysis products, most importantly with hydroxyl ($OH^{\bullet}$) radicals.[1–4]

Most of the EPR spectroscopic studies of radicals derived from purine bases have been confined to the solid state. Research on the radical cation of guanine has been carried out on single crystals of guanine[5,6] and 2′-deoxyguanosine-5′-phosphate,[7] oriented DNA fibres[8–10] and frozen aqueous DNA samples.[3,11–13] Zehner *et al.*,[14] have studied hydrogen atom addition reactions in single crystals of hypoxanthine and its ribonucleoside and ribonucleotide inosine and inosine-5′-phosphate. Hydrogen atom addition was found to occur either at C(2) or C(8). Nelson and Close[15] have detected two radicals centred on the xanthine base in X-irradiated single crystals of the ribonucleoside xanthosine (at 65 K). One of the radicals resulted from the deprotonation at N(3) of a primary radical cation; the other one resulted from the protonation of a radical anion at O(6).

EPR studies of radicals derived from purines in solution are very limited. Bachler and Hildenbrand[16] have detected guanosyl radicals in liquid aqueous solution at pH < 4, formed by oxidation of 2′-deoxyguanosine-5′-phosphate with $SO_4^{\bullet -}$ radicals generated by *in situ* photolysis. This study provided more information on the molecular structure of these radicals and suggested that the highest spin density is centred on the N(3) and C(8) positions of the purine moiety, concurring with those data reported previously from solid state studies.[5–8,15]

The oxidation of the non-DNA purines hypoxanthine and xanthine to uric acid is biologically significant, since it constitutes the end pathway in purine metabolism in humans and is closely related to chronic inflammatory diseases.[17] It has also been suggested that uric acid protects against oxidative stress damage *in vivo* by acting as a free radical scavenger, although the resulting radical products may not themselves be harmless.[18] The hydroxyl ($OH^{\bullet}$) radical induced oxidation of hypoxanthine and xanthine has been studied by pulse radiolysis.[19,20] The oxidation of hypoxanthine is believed to proceed *via* a radical intermediate which decays by a disproportionation reaction to form xanthine and hypoxanthine. In the case of xanthine oxidation, a transient radical was also proposed, the nature and decay of this radical is less well understood but also proceeds *via* a second-order reaction to yield in part uric acid.

In order to provide further information on the structure and acid–base properties of the radical intermediates involved in the oxidation reactions of xanthines and purines in general in liquid aqueous solution, we have carried out an EPR spectroscopy study of the radical oxidation of hypoxanthine, xanthine, 8-methylxanthine and 2-hydroxypurine under anoxic conditions using $SO_4^{\bullet -}$ and $OH^{\bullet}$ radicals as oxidants.

Results and discussion

Oxidation by $SO_4^{\bullet -}$

Xanthine (2,6-dihydroxypurine). At pH values between 5.6 and 11.6, the reaction of $SO_4^{\bullet -}$ with xanthine, **1**, produced EPR spectra characteristic of a π-radical with hyperfine coupling to four different nitrogen atoms and a single hydrogen atom [Fig. 1(*a*)]. At pH values higher than 12.3 another EPR spectrum was recorded, being again characteristic of a radical with hyperfine coupling to four different nitrogen atoms and a single hydrogen atom, [Fig. 1(*b*)].

In aqueous solution, purines and their anions exist in a variety of tautomeric forms. Xanthine is known to be present essentially as the N(7)H tautomer. Its mono-anion is a mixture of the two tautomers which result from the deprotonation either of the N(3)H or the N(7)H groups.[21] The pK_a values for the deprotonation of xanthine and its mono-anion are 7.74 and 11.86, respectively.[22]

One-electron oxidised purines have been shown to be much more acidic than their parent compounds and the pK_a value for deprotonation of such species has been shown in the case of guanine nucleosides[23] and adenine[24] to be several orders of magnitude lower than the pK_a value for the equivalent deprotonation of the parent compound. Thus, by analogy to the chemically similar DNA purines, the neutral radical formed

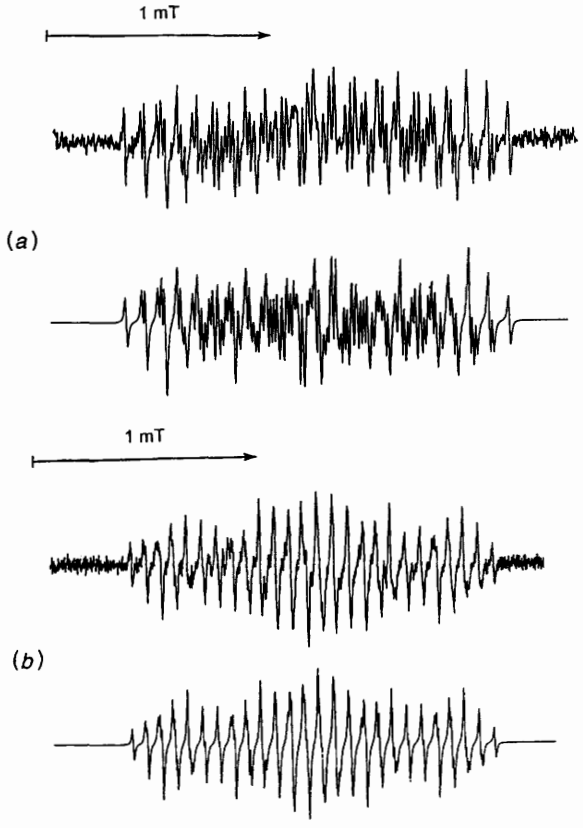

Fig. 1 EPR spectra of the radicals produced by the reaction of $SO_4^{\bullet -}$ with xanthine at 280 K, together with the simulation with the constants of Table 1; (*a*) at pH 7.5, assigned to $\mathbf{1}^{\bullet -}$, (*b*) at pH 13.0, assigned to $\mathbf{1}^{\bullet 2-}$

by the one-electron oxidation of the deprotonated xanthine is expected to be more acidic than the xanthine anion and to deprotonate to give a radical anion. Therefore, the spectra recorded within the pH ranges 5.6–11.6 and >12.3 were assigned to the xanthine radical anion, $\mathbf{1}^{\bullet -}$, and the xanthine radical dianion, $\mathbf{1}^{\bullet 2-}$, respectively (Fig. 2).

At pH values between 11.6 and 12.3 the hyperfine coupling constants depended on the pH value. The largest nitrogen constant increased with the basicity of the solution whereas all the other constants decreased. A plot of the hyperfine coupling constant assigned to the N(3) atom *versus* pH (Fig. 3) clearly demonstrates a pK_a value of 12.0 ± 0.1 for the deprotonation of $\mathbf{1}^{\bullet -}$ to give $\mathbf{1}^{\bullet 2-}$.

The spectrum recorded at pH 12.1 can be simulated with constants that are the average of those corresponding to $\mathbf{1}^{\bullet -}$ and $\mathbf{1}^{\bullet 2-}$, showing that the proton exchange is so fast on the timescale of the EPR experiment that the two different radicals cannot be distinguished. No radicals were detected at pH values below 5.0, which suggests that, at these pH values, the neutral radical may be formed, this radical, however, being too short-lived to be detected. Indeed, there is a significant decrease in the intensity of the spectra assigned to $\mathbf{1}^{\bullet -}$ at pH values approaching 5.4.

Unequivocal assignment of all hyperfine coupling constants (Table 1) was not possible. The results from INDO calculations based on the X-ray structure of the sodium salt of xanthine[25] were not satisfactory, since they overemphasize the nitrogen couplings and yield too small coupling with the proton at C(8). This limitation of INDO calculations for purines has already been reported for radicals derived from hypoxanthine[14] and guanine nucleotides.[26] We carried out McLachlan calculations of spin densities for $\mathbf{1}^{\bullet 2-}$ with parameters identical to those reported for the purine radical anion[27] (γ_{CN} = 1.08, δ_N = 0.9) and those currently used for the carbonyl group[28] (γ_{CO} = 1.6, δ_O = 2.3). The nitrogen hyperfine coupling constants were calculated through the equation $Q_N^N\rho_N + Q_{NC}(\rho_C + \rho_{C'})$ with Q_N^N = 2.72 mT and Q_{NC} = −0.17 mT.[29] Q_{CH}^H = −2.45 mT

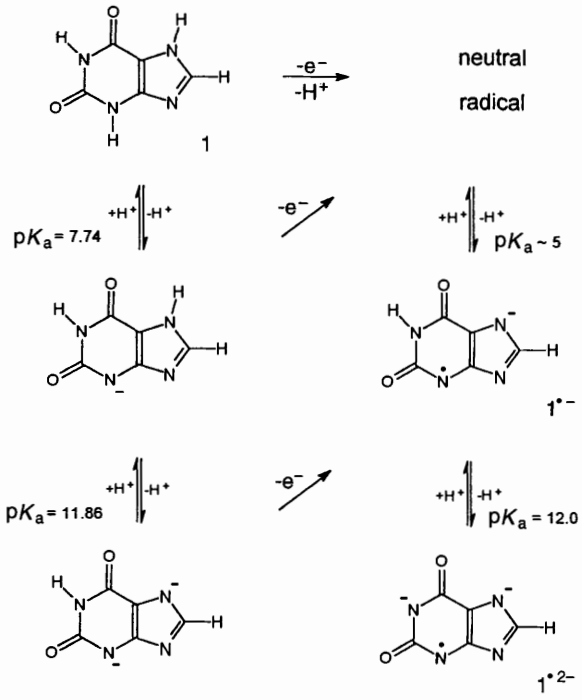

Fig. 2 Acid–base properties of xanthine and the corresponding radicals from one-electron oxidation

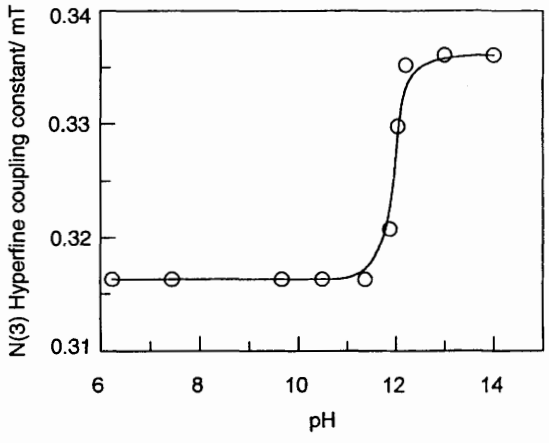

Fig. 3 Plot of the hyperfine coupling constant assigned to the N(3) atom of the xanthine derived radicals *versus* pH

was used in the McConnel equation. The calculated hyperfine coupling constants are included in Table 1. The calculations yield satisfactory results for *a*(N3) and *a*(H8), but a too large value for *a*(N9), in spite of attempts to improve the fitting of the calculated and experimental values. The assignment of the largest couplings to N(3) and H(8) agrees with the results reported for purine radical anion[27] and for the radical centred on the xanthine moiety obtained from xanthosine single crystals.[15] The remaining nitrogen couplings were too small and of so similar a magnitude as to be assigned without speculation. The assignment of the coupling constants of radical $\mathbf{1}^{\bullet -}$ was made by analogy with $\mathbf{1}^{\bullet 2-}$.

Hypoxanthine (6-hydroxypurine). The reaction of $SO_4^{\bullet -}$ with hypoxanthine, **2**, at pH values between 2.0 and 7.5 produced a very weak EPR spectra characteristic of hyperfine coupling to four different nitrogen atoms and three hydrogen atoms [Fig. 4(*a*)]. Different spectra were recorded at pH values above 8.2. The more intense EPR spectra under these conditions were characteristic of hyperfine coupling to three different nitrogen atoms and two hydrogen atoms [Fig. 4(*b*)].

By taking into account the pK_a values reported for hypoxanthine and its monoanion,[22] 8.94 and 12.10, respec-

Table 1 Experimental and calculated EPR parameters and pK_a values for radicals from oxidation of xanthine, **1**, hypoxanthine, **2**, 8-methylxanthine, **3**, and 2-hydroxypurine, **4**

				Hyperfine coupling constants[b]/mT					
Radical	pK_a	g Value[a]		a(N3)	a(H8)	a(N1)	a(N7)	a(N9)	a(H)other
$\mathbf{1}^{\bullet -}$	12.0 ± 0.1	2.004 00		0.316	0.610	0.077[c]	0.091[c]	0.095[c]	
$\mathbf{1}^{\bullet 2-}$	—	2.003 87	exp.	0.336	0.598	0.069[c]	0.060[c]	0.069[c]	
			calc.	0.337	0.549	0.062	0.040	0.236	
$\mathbf{2}^{\bullet}$	7.7 ± 0.1	2.004 58		0.050[d]	0.831	0.099	0.058[d]	0.393	0.199 H(1,2)
$\mathbf{2}^{\bullet -}$	—	2.004 00	exp.	< 0.01	0.548	0.136	0.027	0.351	0.081 H(2)
			calc.	0.003	0.630	0.175	0.013	0.304	0.303 H(2)
$\mathbf{3}^{\bullet -}$	12.1 ± 0.5	2.004 06		0.265	0.685 (CH_3)	0.090[c]	0.102[c]	0.121[c]	
$\mathbf{3}^{\bullet 2-}$	—	2.003 95		0.282	0.640 (CH_3)	0.063[c]	0.098[c]	0.098[c]	
$\mathbf{4}^{\bullet -}$	—	2.004 25	exp.	0.126[d]	0.608	0.042	0.098	0.156[d]	0.370 H(6)
			calc.	0.178	0.469	0.070	0.156	0.185	0.300 H(6)

[a] Error ≤ ±2 × 10^{-5}. [b] Error ≤ ±2 μT. [c] Assignment is ambiguous. [d] Assignment may be reversed.

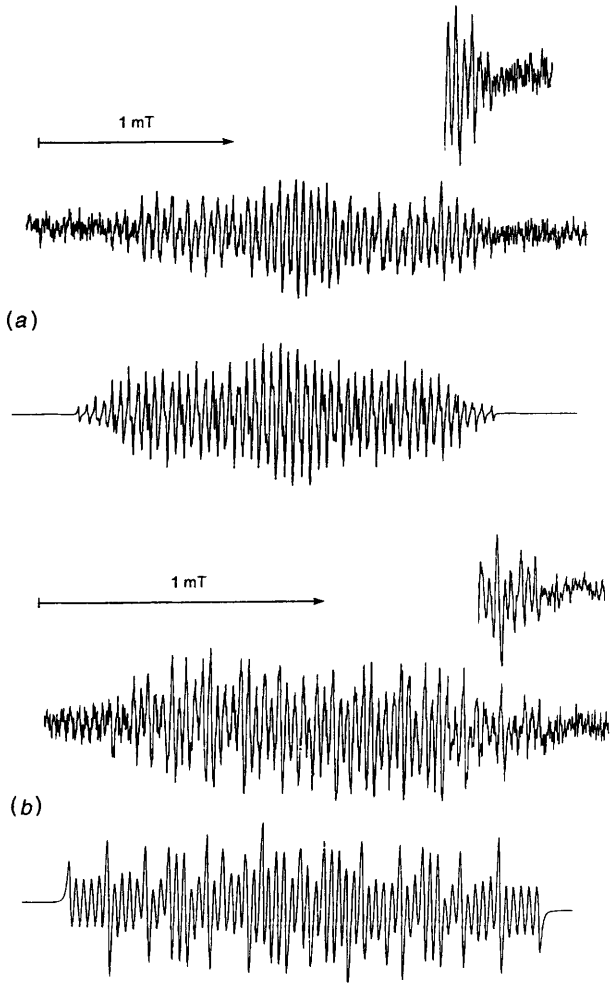

Fig. 4 EPR spectra of the radicals produced by the reaction of $SO_4^{\bullet -}$ with hypoxanthine at 280 K, together with the simulation with the constants of Table 1; (*a*) at pH 3.4, assigned to $\mathbf{2}^{\bullet}$, (*b*) at pH 11.6, assigned to $\mathbf{2}^{\bullet -}$. Inserts show the enhanced high-field wings of the spectra.

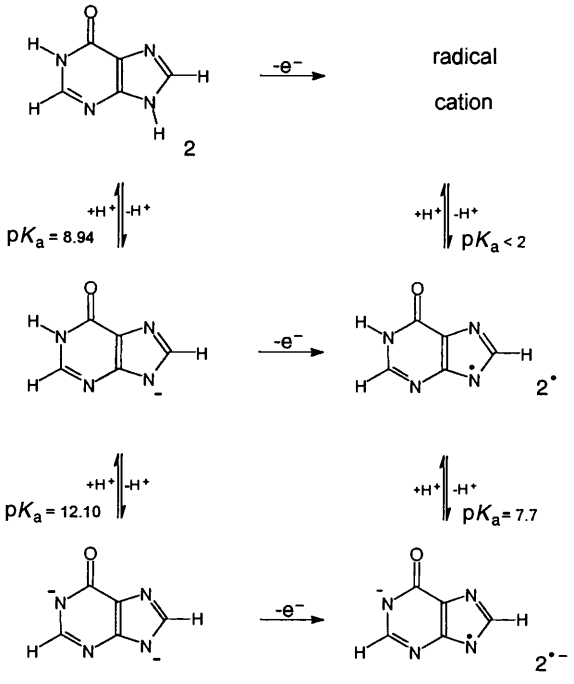

Fig. 5 Acid–base properties of hypoxanthine and the corresponding radicals from one-electron oxidation

tively, and the reported tautomeric forms of these species,[30] the spectra obtained at pH values below 7.0 were assigned to hypoxanthine neutral radical, $\mathbf{2}^{\bullet}$, and the spectra obtained above pH 8.2 were assigned to the radical anion, $\mathbf{2}^{\bullet -}$ (Fig. 5). The assignment of the hyperfine coupling constants for $\mathbf{2}^{\bullet -}$ was made by comparison with the results of McLachlan calculations. Although the calculated coupling constant for the proton in the 2-position is too large, there is no ambiguity in the assignment. Table 1 shows the experimental and calculated coupling constants. The hyperfine coupling constants of $\mathbf{2}^{\bullet}$ were assigned by analogy with $\mathbf{2}^{\bullet -}$.

A pK_a value of 7.7 ± 0.1 for the deprotonation of the neutral radical $\mathbf{2}^{\bullet}$ was estimated from the dependence of the relative concentrations of the two species, $\mathbf{2}^{\bullet}$ and $\mathbf{2}^{\bullet -}$, on the pH. For that purpose, the superimposed spectra of the two radicals were simulated at different pH values arround the pK_a. Unlike the xanthine case, the proton exchange between $\mathbf{2}^{\bullet}$ and $\mathbf{2}^{\bullet -}$ is slow on the timescale of the EPR experiment and the individual spectra may be discerned.

At pH values below 2 no radical was detected. Hypoxanthine is protonated (pK_a = 1.98)[22] at these pH values and the resulting cation is unlikely to be oxidised by $SO_4^{\bullet -}$.

8-Methylxanthine (8-methyl-2,6-dihydroxypurine). The reaction of $SO_4^{\bullet -}$ with 8-methylxanthine, **3**, is very similar to that observed with xanthine. At pH values between 6.0 and 11.5 the EPR spectra [Fig. 6(*a*)] were assigned to the 8-methylxanthine radical monoanion, $\mathbf{3}^{\bullet -}$, and at pH values greater than 12.5 the spectra [Fig. 6(*b*)] were assigned to the 8-methylxanthine radical dianion, $\mathbf{3}^{\bullet 2-}$. We propose for radicals $\mathbf{3}^{\bullet -}$ and $\mathbf{3}^{\bullet 2-}$, structures similar to $\mathbf{1}^{\bullet -}$ and $\mathbf{1}^{\bullet 2-}$, respectively, on the basis of the analogy of the starting compounds and of the EPR parameters of the derived radicals. The pK_a value for the deprotonation of the radical mono-anion, $\mathbf{3}^{\bullet -}$, was estimated from a plot of $a(N_3)$ *vs.* pH, similar to that of Fig. 3. However, due to the complexity of the spectra of radicals derived from **3**

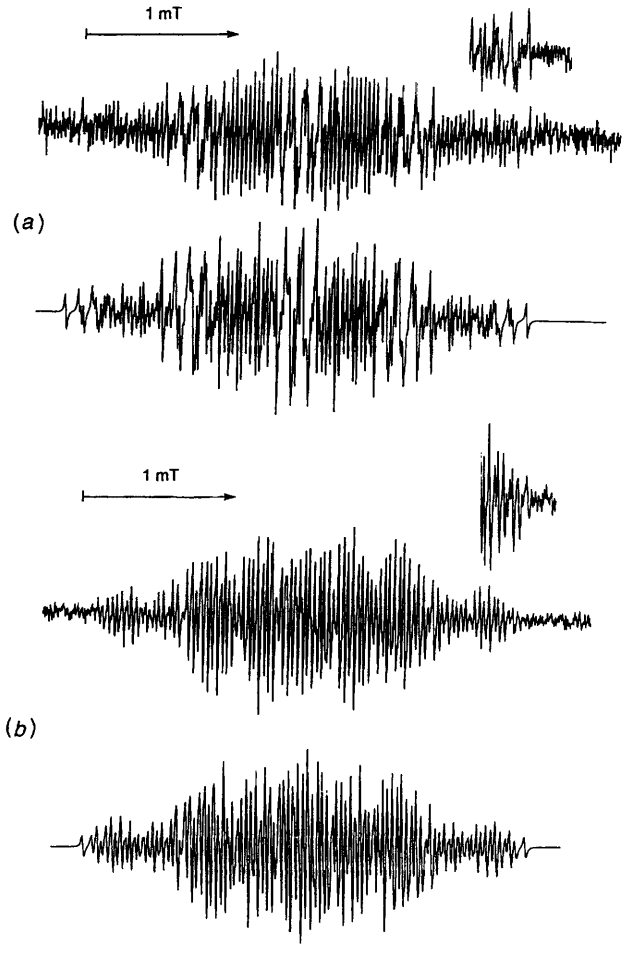

Fig. 6 EPR spectra of the radicals produced by the reaction of $SO_4^{\bullet -}$ with 8-methylxanthine at 280 K, together with the simulation with the constants of Table 1; (*a*) at pH 8.0, assigned to $\mathbf{3}^{\bullet -}$, (*b*) at pH 12.6, assigned to $\mathbf{3}^{\bullet 2-}$. Inserts show the enhanced high-field wings of the spectra.

as compared with those derived from **1**, the value of pK_a = 12.1 ± 0.5 found for $\mathbf{3}^{\bullet -}$ is less precise.

The very complex spectra of both radicals were characteristic of hyperfine coupling to four nitrogen atoms and to the three equivalent hydrogen atoms of the methyl group in the 8-position. In the case of both of these 8-methylxanthine radicals relative to the equivalent xanthine radicals, there is reduced hyperfine coupling assigned to the N(3) (Table 1). This seems reasonable given that the positive inductive effect of the methyl group in the 8-position may increase the stabilisation of the spin density in the imidazole ring.

2-Hydroxypurine. No radicals were detected in the reaction of $SO_4^{\bullet -}$ with 2-hydroxypurine at pH values below 7.5. The spectrum recorded at pH values between 7.5 and 12.0 (Fig. 7) showed a pattern which was interpreted as arising from the coupling of the unpaired electron with four different nitrogen nuclei and two non-equivalent hydrogens.

Taking into account the considerations made for xanthine and the pK_a value of 8.43 reported for 2-hydroxypurine,[31] the spectrum obtained was assigned to the 2-hydroxypurine radical anion $\mathbf{4}^{\bullet -}$, which results from the oxidation of the 2-hydroxypurine anion followed by deprotonation. This radical has a structure analogous to that of the hypoxanthine radical anion.

The coupling constants were assigned by comparison with the results of McLachlan calculations (Table 1).

Oxidation by OH•

No radicals were detected for hypoxanthine, 8-methylxanthine and 2-hydroxypurine when OH• radicals, generated either by

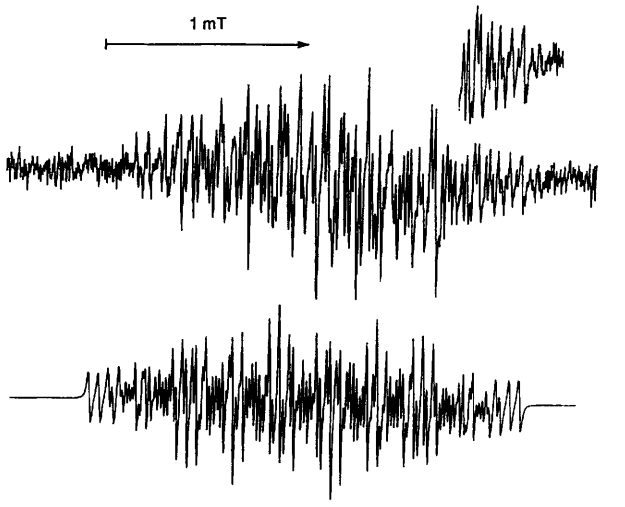

Fig. 7 EPR spectrum of the radical produced by the reaction of $SO_4^{\bullet -}$ with 2-hydroxypurine at 280 K and pH 10.7, together with the simulation with the constants of Table 1, assigned to $\mathbf{4}^{\bullet -}$. Insert shows the enhanced high-field wing of the spectrum.

in situ photolysis of H_2O_2 or by *in situ* mixing of the Fe^{2+}-[EDTA]/H_2O_2 or Ti^{3+}/H_2O_2 couples, were used as the oxidant. Spectra were only obtained with xanthine at pH values close to neutrality (pH 6.8–7.4) by using H_2O_2 photolysis. The radicals detected gave spectra identical to those obtained with $SO_4^{\bullet -}$ at the same pH and were thus assigned to the xanthine radical anion, $\mathbf{1}^{\bullet -}$. This reaction may proceed by direct electron capture by the OH• radical followed by deprotonation; however, in analogy to the reactions of other purines,[4] this may also be the result from OH• addition at the C(4)–C(5) double bond (the dominant reaction in adenine systems), followed by the rapid elimination of water to yield neutral xanthine radicals. These radicals immediately deprotonate at this pH, yielding the observed xanthine radical anion, $\mathbf{1}^{\bullet -}$. Addition of OH• at the C(8)-position (observed in trimethylated xanthines[32]) cannot lead to the formation of the observed radical. However, the radical intermediates possibly formed by OH• addition were not observed under these experimental conditions.

Experimental

Analytical grade xanthine (2,6-dihydroxypurine, Merck), hypoxanthine (6-hydroxypurine, BDH), 8-methylxanthine (8-methyl-2,6-dihydroxypurine, Sigma) and 2-hydroxypurine (Aldrich) were used as purchased, to prepare 1–3 × 10^{-3} mol dm^{-3} solutions, using water purified by a Millipore Milli-Q50 system. Potassium persulfate (0.5–4 × 10^{-2} mol dm^{-3}, Merck) or hydrogen peroxide (1–2 × 10^{-2} mol dm^{-3}, Merck) were added immediately prior to use. No changes, other than in intensity, were observed in the EPR spectra with the variation of the persulfate concentration. Therefore, the spectra shown were recorded with solutions with a typical concentration of 3 × 10^{-2} mol dm^{-3} potassium persulfate.

To avoid undesired superimposition of the features of the acetone radical in the recorded spectra, acetone was usually omitted. However, acetone was used as a photosensitiser when increased intensity was required to enable the recording of poorly defined lines at the extremes of some spectra. However, due to spin polarisation in most of the spectra the wings at low field are poorly resolved. No radical was detected when persulfate or hydrogen peroxide was not present.

The solutions were buffered with phosphate (10 mmol dm^{-3}) in the experiments to determine pK_a values and the relevant pH adjustments were made using KOH and $HClO_4$ solutions. The solutions were then deaerated by argon bubbling and allowed to flow at a constant rate through a quartz flow cell within the

spectrometer cavity. $SO_4^{\bullet -}$ and $OH^{\bullet}$ radicals were generated within the spectrometer cavity by *in situ* photolysis of $S_2O_8^{2-}$ ions and H_2O_2, respectively, using an optically focused pressurised Hg–Xe UV lamp with a stabilised power supply. The experiments where $OH^{\bullet}$ radicals were used as the oxidant were repeated using the Fenton coupled systems, 10^{-2} mol dm^{-3} Fe^{2+}[EDTA] (1:2 ratio)/1–2 × 10^{-2} mol dm^{-3} H_2O_2 and substrate or 10^{-2} mol dm^{-3} $TiCl_3$/1–2 × 10^{-2} mol dm^{-3} H_2O_2 and substrate. The two separate solutions were mixed as they entered the spectrometer cavity in a commercially available mixing cell and pumped through the cavity using a peristaltic pump at flow rates within the range 20–160 cm^3 min^{-1}. The solutions were refrigerated immediately before entering the cavity to a temperature of 280 ± 2.0 K.

X-Band EPR spectra were recorded at a 100 kHz modulation frequency using a Brüker ER200D spectrometer controlled by Brüker AM 300 ESP software. The *g* measurements were made using a microwave frequency counter and a Brüker ER035M gaussmeter, which were calibrated against a standard of known *g* value immediately prior to each experiment.

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References

1 C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor and Francis, London, 1988.
2 *Effects of Ionising Radiation on DNA*, eds. J. Hüttermann, W. Köhnhein, R. Teoule and A. J. Bertinchamps, Springer, Berlin, 1978.
3 M. C. R. Symons, *J. Chem. Soc., Faraday Trans.*, 1987, **83**, 1.
4 S. Steenken, *Chem. Rev.*, 1989, **89**, 503.
5 D. M. Close, E. Sagstuen and W. H. Nelson, *J. Chem. Phys.*, 1985, **82**, 4386.
6 D. M. Close, W. H. Nelson and E. Sagstuen, *Radiat. Res.*, 1987, **112**, 283.
7 B. Rakvin, J. N. Herak, K. Voit and J. Hüttermann, *Radiat. Environ. Biophys.*, 1987, **26**, 1.
8 J. Hüttermann, K. Voit, H. Oloff, W. Köhnhein, A. Gräslund and A. Rupprecht, *Faraday Discuss. Chem. Soc.*, 1984, **78**, 135.
9 A. Gräslund, A. Ehrenberg, A. Rupprecht, G. Ströhm and H. Crespi, *Int. J. Radiat. Biol.*, 1975, **28**, 313.
10 A. Gräslund, A. Ehrenberg, A. Rupprecht and G. Ströhm, *Biochim. Biophys. Acta*, 1971, **254**, 172.
11 M. D. Sevilla, *Excited States in Organic Chemistry and Biochemistry*, eds. B. Pullman and N. Goldblum, Reidel, Dordrecht, 1977, 115.
12 J. Hüttermann, *J. Ultramicroscopy*, 1982, **10**, 25.
13 S. Gregoli, M. Olast and A. Bertinchamps, *Radiat. Res.*, 1982, **89**, 238.
14 H. Zehner, E. Westhof, W. Flossmann and A. Müller, *Int. J. Radiat. Biol.*, 1978, **33**, 121.
15 W. Nelson and D. Close, *J. Phys. Chem.*, 1983, **79**, 3240.
16 V. Bachler and K. Hildenbrand, *Radiat. Phys. Chem.*, 1992, **40**, 59.
17 B. N. Ames, R. Cathcart, E. Schwiers and P. Hochstein, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 6858.
18 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Clarendon Press, Oxford, 2nd edn., 1989.
19 J. Santamaria, C. Pasquier, C. Ferradini and J. Pucheault, *Adv. Exper. Med. Biol.*, 1984, **167A**, 185.
20 J. Santamaria, C. Pasquier, C. Ferradini and J. Pucheault, *Radiat. Res.*, 1984, **97**, 452.
21 D. Lichtenberg, F. Bergmann and Z. Neirmann, *J. Chem. Soc.* (*C*), 1971, 1676.
22 *Handbook of Biochemistry*, ed. H. A. Sosler, Chem. Rubber. Publ. Co., 1968.
23 L. P. Candeias and S. Steenken, *J. Am. Chem. Soc.*, 1989, **111**, 1094.
24 A. J. S. C. Vieira and S. Steenken, unpublished results.
25 H. Mizumo, T. Fujiwara and K. Tormita, *Bull. Chem. Soc. Jpn.*, 1969, **42**, 3099.
26 B. Rakvin and J. N. Herak, *Radiat. Res.*, 1981, **88**, 240.
27 H. Ohya-Nishiguchi, Y. Shimizu, N. Hirota and K. Watanabe, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 1252.
28 A. Streitwieser, Jr., *Molecular Orbital Theory for Organic Chemists*, Wiley, New York, 1961.
29 C. L. Talcott and R. J. Myers, *Mol. Phys.*, 1967, **12**, 549.
30 D. Lichtenberg, F. Bergmann and Z. Neirmann, *Israel J. Chem.*, 1972, **10**, 805.
31 A. R. Katritzky, *Physical Methods in Heterocyclic Chemistry*, vol. 1, Academic Press, New York, 1963.
32 A. J. S. C. Vieira and S. Steenken, *J. Chim. Phys.*, 1996, **93**, 235.

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