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Chemical Models and Radiation Damage [and Discussion]

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Chemical models and radiation damage

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E.s.r. spectroscopy has been used in conjunction with an aqueous flow system to investigate both the metal-catalysed decomposition of hydrogen peroxide to $OH^{\cdot}$ and the subsequent reactions of this radical with a variety of biomolecules. Particular emphasis is placed on the effects of pH and ligand on the Fe^{II}–H_2O_2 reaction and on the sites of attack by $OH^{\cdot}$ in its reaction with pyranose and furanose sugars, sugar phosphates, nucleosides and nucleotides. Attention is focused on subsequent reactions (for example, of radicals formed by attack in the ribofuranose moiety of adenosine) which may be involved in radiation damage.

Introduction

Free radicals are increasingly implicated in the initiation and progression of diseases and the toxic action of drugs, chemicals, and radiation (Willson 1977). Oxygen-centred radicals, in particular, are thought to be involved in the irreversible damage of vitally important biomolecules, as in the degeneration of synovial membranes (McCord 1974), and in many radiation-induced reactions (see, for example, Adams & Wardman 1978). Radiation damage to genetic material is thought to arise both via direct interaction of the ionizing radiation with DNA, giving radical cations and radical anions localized on the polynucleotide bases (Boon *et al.* 1984), and a secondary process in which the polynucleotide components react with $OH^{\cdot}$, $H^{\cdot}$ and e^{-}_{aq} (formed by the interaction of the radiation with water) (see, for example, von Sonntag & Schulte-Frohlinde 1978).

The radiation chemistry of aqueous solutions of polynucleotide components has been studied extensively, mainly by product studies following attack by $OH^{\cdot}$ (generated from pulse radiolysis) on model compounds (von Sonntag *et al.* 1981). E.s.r. spectroscopy, which allows the direct detection of free radicals, has previously been used in studies of the reaction of $OH^{\cdot}$ with pyrimidines (Nicolau *et al.* 1969) and purines (Schmidt & Borg 1976) as well as some models for sugar phosphates (Steenken *et al.* 1974; Samuni & Neta 1973). It has subsequently been suggested that radical formation from the *sugar* moiety of DNA is responsible for strand breakage (Behrens *et al.* 1982; Bothe *et al.* 1984), and pulse radiolysis experiments indicate that these radicals may be derived via secondary reactions of base-derived radicals both in the absence (Deeble & von Sonntag 1984) and presence of oxygen (Schulte-Frohlinde & Bothe 1984).

The Ti^{III}–H_2O_2 redox couple can be used in conjunction with e.s.r. spectroscopy (see, for example, Norman 1979) to study the complex mixture of radicals formed from the reaction of $OH^{\cdot}$ with a variety of carbohydrates (Gilbert *et al.* 1981), and subsequent acid- and base-catalysed transformations of these first-formed radicals can be characterized. This relatively direct approach to the study of radical reactions in biomimetic and radiomimetic systems has now been extended to an investigation of the corresponding Fenton system

(Fe^{II}–H_2O_2) and to the reaction of $OH^{\cdot}$ with compounds chosen to provide information on the damage inflicted by radiation on nucleic acids and their components. Particular emphasis has been placed on the selectivity of attack and on reactions which lead to fragmentation.

E.S.R. STUDIES OF THE Ti^{III}–H_2O_2 AND Fe^{II}–H_2O_2 COUPLES

(*a*) Ti^{III}–H_2O_2

The experiments described here are based on previous extensive use of the Ti^{III}–H_2O_2 couple as a source of $OH^{\cdot}$ (reaction (1); see, for example, Norman 1979). We have used a continous-flow system in which three streams (containing, respectively, Ti^{III} (typically 5×10^{-3} mol dm^{-3}), hydrogen peroxide (4×10^{-2} mol dm^{-3}), and the organic substrate (typically in the range 0.1–1 mol dm^{-3})) are mixed a short time (20–50 ms) before their passage through the cavity of an e.s.r. spectrometer. The radicals observed are produced by a reaction of the first-formed hydroxyl radical with the organic substrate; the relatively simple reaction scheme shown in reactions (1)–(3) is applicable under circumstances where [RH] is sufficiently high to prevent further reaction of $OH^{\cdot}$ with Ti^{III} or H_2O_2 (i.e. to 'scavenge' $OH^{\cdot}$) and in which the radicals decay by bimolecular termination (typically at a diffusion-controlled rate, $2k_3 \approx 10^9$ dm^3 mol^{-1} s^{-1}).

$$Ti^{III} + H_2O_2 \rightarrow Ti^{IV} + OH^{\cdot} + HO^{-}, \tag{1}$$

$$OH^{\cdot} + RH \rightarrow R^{\cdot} + H_2O, \tag{2}$$

$$2R^{\cdot} \rightarrow \text{molecular products}. \tag{3}$$

It has been shown that under these conditions a pseudo-steady-state is achieved in the cavity (Czapski 1971; Samuni *et al.* 1972; Gilbert *et al.* 1973) and it follows that $k_1[Ti^{III}]_t[H_2O_2]_t = 2k_3[R^{\cdot}]^2$ (where the subscript t refers to concentrations in the cavity at time t (in seconds after mixing). Because $[H_2O_2] \gg [Ti^{III}]$, the concentration of the latter falls exponentially, and

$$[R^{\cdot}]^2 = k_1[Ti^{III}]_0[H_2O_2]_0 \exp(-k_1[H_2O_2]_0 t)/2k_3 \tag{4}$$

so that, for given t and $[H_2O_2]_0$, $[R^{\cdot}]$ depends on $[Ti^{III}]^{\frac{1}{2}}$ (and, of course, on k_1 and k_3). It can also be shown that the concentration of $R^{\cdot}$ as a function of $[H_2O_2]_0$ for given t should have a maximum given by (5), from which it follows that if t is known then k_1 can be obtained. For several simple radicals in the Ti^{III}–H_2O_2 system the behaviour expected on the basis of (4) and (5) is observed: these equations form the basis for determining k_1 and k_3 and also rate constants for other processes which compete with bimolecular termination.

$$[H_2O_2]_0 = 1/k_1 t. \tag{5}$$

When ethanol is used as a substrate with the Ti^{III}–H_2O_2 couple (with $[H_2O_2]_0 \approx 10^{-2}$ mol dm^{-3}) at *ca.* pH 2, signals from both $^{\cdot}CHMeOH$ (α) and $^{\cdot}CH_2CH_2OH$ (β) are observed, with relative concentrations (as determined by computer simulation of spectra) of *ca.* 10:1. This ratio reflects the relative rates of attack of $OH^{\cdot}$ at the two positions (and highlights the electrophilic character of $OH^{\cdot}$), though the ratio decreases as $[H_2O_2]$ is increased

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(for example, to *ca.* 4.5:1 for $[H_2O_2]_0 = 0.07$ mol dm^{-3}); this is evidently the result of the reaction of the oxygen-conjugated α-radical with H_2O_2 (reaction (6)) (Gilbert *et al.* 1974),

$$\cdot CHMeOH + H_2O_2 \rightarrow {}^{+}CHMeOH + OH^{\cdot} + OH^{-}. \quad (6)$$

To confirm this and to obtain more detailed kinetic information we have used a kinetic simulation program (kindly provided by Professor D. J. Waddington) to compute steady-state values of $[R^{\cdot}]$, $[H_2O_2]$, etc., as a function of rate constants, concentrations of reagent, and time after mixing. (When some competing secondary reactions are involved (such as reaction (6)) the appropriate steady-state equations can be derived and solved, but simulation allows more complex systems to be treated (see later).) In a set of preliminary experiments we oxidized Me_3COH to $\cdot CH_2CMe_2OH$, a radical for which only simple bimolecular decay would be expected. We observed both the predicted dependence of $[\cdot CH_2CMe_2OH]$ on $[Ti^{III}]^{\frac{1}{2}}$ (with $[H_2O_2]$ and t constant) and the maximum in $[\cdot CH_2CMe_2OH]$ as $[H_2O_2]$ alone was varied (see equation (5)): measurement of the mixing-time t (by using a spectrophotometric method) leads to a value for k_1 of 2250 dm^3 mol^{-1} at 24 °C.

Use of this value for k_1 in the kinetic simulation program, together with reported rate constants for the $OH^{\cdot}$ reaction with ethanol (at C_α and C_β) and for radical termination (Gilbert *et al.* 1982) leads (when a value $k_6 = 1.6 \times 10^5$ dm^3 mol^{-1} s^{-1} is incorporated) to exact agreement of the observed and calculated values of the α:β ratio and its variation with $[H_2O_2]$.

$(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2$

1

$(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)CH_2CH_2N(CH_2CO_2H)_2$

2

While the effects of pH and ligands on k_1 remain to be fully explored, we have found that use of ethylenediaminetetraacetic acid (EDTA, **1**) as a ligand makes relatively little difference to k_1 (though Ti^{IV}–EDTA itself appears to be a relatively good oxidant for $\cdot CHMeOH$); on the other hand the ligand diethylenetriaminepentaacetic acid (DTPA, **2**) retards the initiation reaction dramatically.

(*b*) Fe^{II}–H_2O_2

The Fenton system is reported to give e.s.r. signals from ethanol (but with $[\beta] > [\alpha]$) under certain circumstances (Shiga 1965). The use of phosphate buffer (at pH 7) as previously employed is found to be unnecessary; greater radical concentrations with a range of substrates are obtained when EDTA, for example, is used to complex Fe^{II}, particularly at the upper end of the pH range 1.5–8 (with the use of typical conditions as follows: $[Fe^{II}] = [EDTA] = 2.5 \times 10^{-3}$ mol dm^{-3} and $[H_2O_2] = 2.5 \times 10^{-2}$ mol dm^{-3}). $[H_2O_2]$ has to be significantly increased (to *ca.* 0.1 mol dm^{-3}) for the successful detection of radicals in experiments (at pH 1.5–2) in which EDTA is omitted.

Kinetic analysis of experiments with $\cdot CH_2CMe_2OH$ (from t-butyl alcohol) formed in the Fe^{II}–EDTA–H_2O_2 system (based on the parallel reactions and equations for Ti^{III}) leads to a value of $k_7 = 9 \times 10^3$ dm^3 mol^{-1} s^{-1} (at pH 7). In contrast, at pH 1.85 in the absence of EDTA the initiation reaction has a value $k_7 \approx 200$ dm^3 mol^{-1} s^{-1}. We conclude that complexing, and a change in pH, can have a dramatic effect on the initiation rate, and preliminary

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investigations indicate that use of DTPA between pH 2 and 7 leads to an increase in rate similar to that observed for EDTA.

$$Fe^{II} + H_2O_2 \rightarrow Fe^{III} + OH^{\cdot} + OH^{-}. \quad (7)$$

As with the previous report of Fenton-system oxidation of ethanol (Shiga 1965), we find that under all conditions [$^{\cdot}CH_2CH_2OH$] > [$^{\cdot}CHMeOH$] (typically *ca.* 5:1), in marked contrast with the result for Ti^{III}–H_2O_2 (similar results are obtained for related radicals from other substrates, such as propan-2-ol). By the use of the kinetic simulation program we find that this behaviour is reproduced if rapid oxidation of the α radical by Fe^{III} is incorporated (see, for example, Walling 1975). In experiments with Fe^{II}, EDTA, H_2O_2 and ethanol at pH 7, for example, both the α:β ratio and the absolute radical concentration of each is reproduced with a value of $k_8 = 10^9$ dm^3 mol^{-1} s^{-1}.

$$CH_3\dot{C}HOH + Fe^{III}\text{–EDTA} \rightarrow CH_3CHO + H^+ + Fe^{II}\text{–EDTA}. \quad (8)$$

While the role of pH and ligand remains to be investigated further, it is clear that, at least under the conditions described above, the Fenton system is an excellent source of the hydroxyl radical; it is also confirmed that oxygen-conjugated radicals are rapidly oxidized by Fe^{III} under these conditions, to perpetuate a chain process.

Reactions of OH$^{\cdot}$ with biomolecules

The extent of damage sustained, and by what part of the molecule, is governed both by the initial site of attack (and hence the relative reactivities of different moieties) and the subsequent fate of first-formed radicals. We have been particularly interested in those processes which compete with the commonly encountered radical 'repair' mechanisms (for example, by hydrogen-abstraction from a thiol) or dimerization and which, via fragmentation or other reactions, serve to 'fix' the initial damage. These processes include reaction with, for example, metal ions, hydrogen peroxide (see above) or oxygen, but also a variety of fragmentation reactions, many of which are related to the much simpler and well established decomposition of $^{\cdot}CH(OH)CH_2OH$ to $^{\cdot}CH_2CHO$ (see, for example, Gilbert *et al.* 1972); this is subject to both acid and base catalysis, and at least in the former case is believed to involve the formation of an incipient radical cation (reaction (9)). This reaction appears to be encouraged by the mesomeric effect of oxygen at the α-radical centre and the possession of a good β-leaving group (H_2O in this case); we have investigated (for radicals from a variety of biomolecules) variations of this process which involve other leaving groups (such as phosphate and nucleotide bases), and in which steric and electronic features are revealed, and other reactions involving long-range electron transfer in the incipient radical cations.

$$^{\cdot}\underset{\text{OH}}{\text{CH}}\text{—}\underset{\text{OH}}{\text{CH}_2} \xrightarrow[-H_2O]{H^+} \left[\dot{\underset{\text{OH}}{\text{C}}}\text{H—}\overset{+}{\text{C}}\text{H}_2\right] \xrightarrow{-H^+} \text{O=CH—}\dot{\text{C}}\text{H}_2 \quad (9)$$

(*a*) *Sugars and sugar phosphates*

As with the α-hydrogen in ethanol, the —CH(OH)— fragments in sugars are reactive targets for OH$^{\cdot}$ attack, and, at least for pyranose sugars, there appears to be little selectivity between the different positions. For example, when ice-cooled solutions of β-D-fructose (at a concentration

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after mixing of *ca.* 0.05 mol dm^{-3}) are reacted at *ca.* pH 4 with the Ti^{III}–H_2O_2 couples (generally preferred to Fe^{II}–H_2O_2 so that radical oxidation by Fe^{III} is avoided), attack at OH$^{\cdot}$ gives all five possible radicals from the *pyranose* form (present almost exclusively under these conditions, and in the $^2C_5$ conformation). Details of just one of these are given here (see structure **3**). As the pH is lowered, changes occur which indicate that the first-formed radicals are susceptible to acid-catalysed rearrangement (cf. reaction(**9**)) and it is significant that the conversion of **3** into **4** occurs much faster than the corresponding reaction of the other hydroxyalkyl radicals: the reaction evidently has a stereoelectronic requirement and is facilitated when, as in **3**, the (axial) leaving group eclipses the orbital containing the unpaired electron at the radical centre.

Solutions of fructose that have been allowed to stand for a few minutes before flowing show new features (see figure 1) which are consistent with the oxidation of a significant proportion

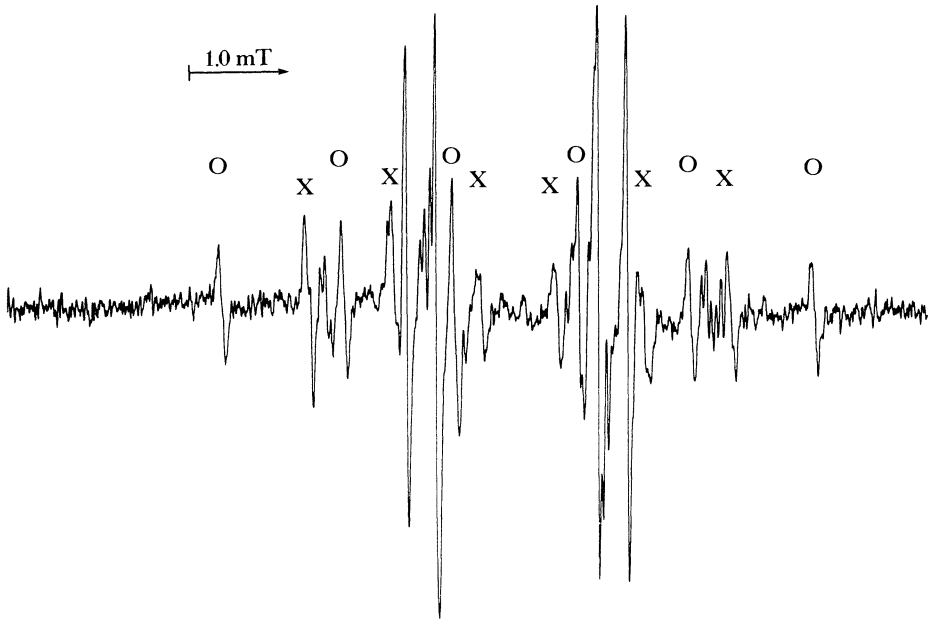
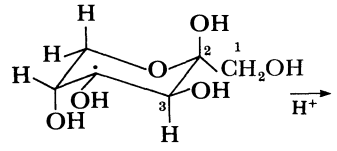


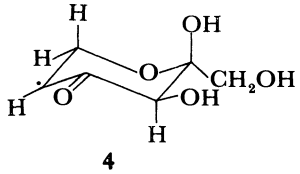
FIGURE 1. E.s.r. spectrum obtained from β-D-fructose (after mutarotation) and OH$^{\cdot}$ at pH 4, showing (○) signals assigned to the C-5 (pyranose) radical, and (×) the C-5 radical (**5**) from the minor furanose structure.

of β-D-fructo*furanose*, formed by mutarotation (the predominant forms of fructose in aqueous solution at room temperature are β-D-fructopyranose (*ca.* 75%) and β-D-fructofuranose (*ca.* 20%); Angyal & Bethell (1976)). The detection of a significant concentration of the furanose C-5 radical **5**, rather than other radicals from this ring, is consistent with a preference



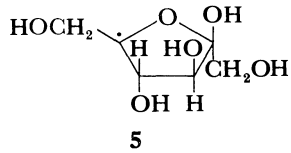
3

a(β-H) = {3.360 mT, 0.785} g = 2.0031



4

a(α-H) = 1.763 a(β-H) = {4.010 mT, 3.520} a(γ-H) = 0.370 g = 2.0042

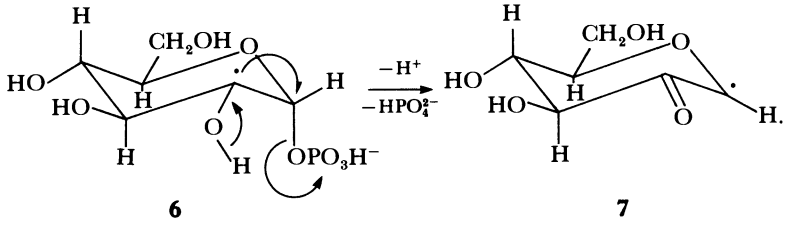


5

a(β-H) = {2.520 mT, 0.887 (2)} g = 2.0031

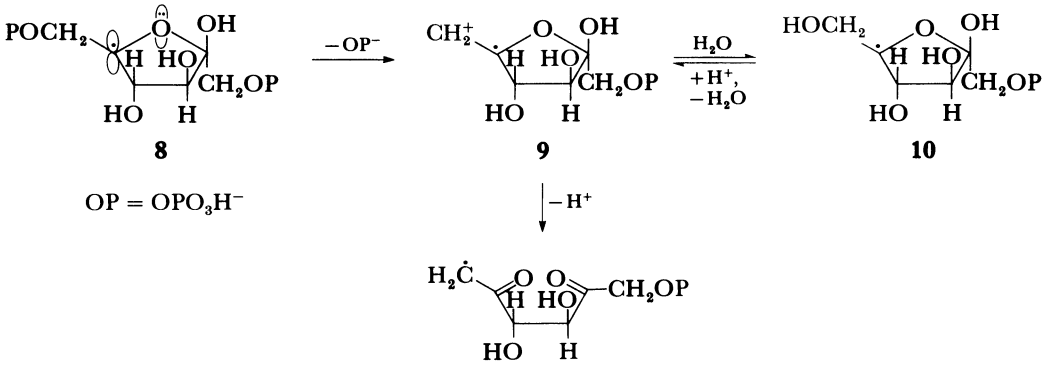
for attack by $OH^{\cdot}$ at the hydrogen on the carbon adjacent to the ring-oxygen in the five-membered ring (see a comparable result for sucrose, in which pyranose and furanose rings are linked together (Gilbert *et al.* 1983)). This is interpreted in terms of the stabilization offered in the transition state for hydrogen abstraction by the optimum overlap between the unpaired electron and the lone pair of electrons on oxygen (Malatesta & Ingold 1981). This may be particularly relevant for the ribofuranose rings in nucleic acid components (see later).

The effect of incorporation of a phosphate group has been investigated through the study of both pyranose and furanose substrates. For example, reaction of $OH^{\cdot}$ with α-D-glucose-1-phosphate at pH 4 gives complex spectra corresponding closely to those from α-D-glucose under similar conditions (Gilbert *et al.* 1981), except that signals from **7** replace those anticipated for the C-2-derived radical **6**. The detection of **7** rather than **6** indicates that the loss of the β-phosphate group, reaction (10), is much faster than the corresponding loss of β-OH or β-alkoxy groups (in, say, glucose or dextran (Gilbert *et al.* 1984)), for which acid catalysis is required. The rate constant k_{10} for the *overall* process, obtained from pulse radiolysis experiments performed in collaboration with Professor R. L. Willson, is 1.2×10^4 dm^3 mol^{-1} s^{-1} (the loss of phosphate and a proton may not be concerted, and a radical cation may be involved).



6 **7** (10)

Related fragmentation pathways are revealed for fructose-6-phosphate and fructose-1,6-diphosphate, in both of which the sugar is furanose. For fructose-1,6-diphosphate, for example, reaction of $OH^{\cdot}$ leads to e.s.r. signals which indicate that there is preferential attack at C-5 (as with the formation of **5** from fructose itself) and that there is also a significant extent of reaction at the C-1 hydroxymethyl group. Though **8** is itself detected, the additional observation of the signal attributed to **10** is interpreted (scheme 1) in terms of attack at C-5 followed by rapid loss of phosphate and subsequent hydration of the resulting radical cation (loss of phosphate, with $k \approx 10^4$ s^{-1}, is evidently somewhat faster than for some related acyclic



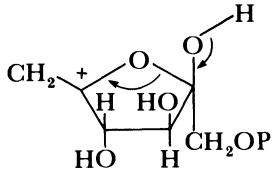
8 **9** **10**

OP = OPO_3H^-

11

Scheme 1

radicals; this is *ca.* 10^3 s^{-1} for $^{\cdot}CH(OMe)CH_2OPO_3H^-$ (Behrens *et al.* 1978)). As the pH is lowered (to *ca.* 1.5), signals from **8** and **10** are themselves replaced by those attributed to a ring-opened carbonyl-conjugated radical **11**, which evidently derives from **9**; the role of acid is presumably to accelerate the regeneration of **9** via catalysis of the loss of β-OH in **10**. (Such acid-catalysed rearrangements are revealed by e.s.r. when the rates of the appropriate reactions become faster than radical–radical termination rates (which in the systems described here limit radical lifetimes to several milliseconds). When radical concentrations are lower and lifetimes longer (as under physiological conditions), these fragmentation processes can play an important role at higher pH values.) The ring-opening must involve the loss of the proton on the C_2-hydroxyl group (possibly in concerted fashion, see **12**), because the corresponding radical from sucrose, **13**, fails to undergo cleavage even at pH 0.25.



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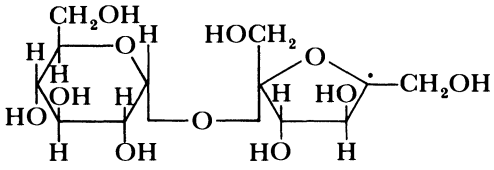
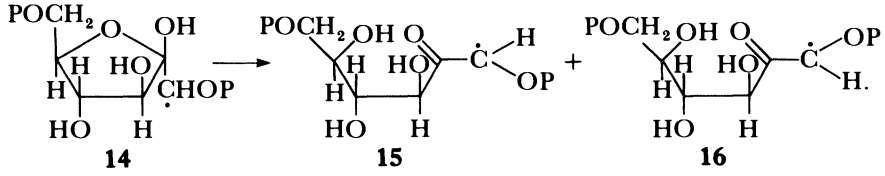


13

Another rearrangement revealed for a radical derived from fructose-1,6-diphosphate is reaction (11); the detection of the *cis* and *trans* isomers of the α-hydroxyketo-substituted radical (**15** and **16**) is attributed to breakage of the β C—O bond in the C_1-derived radical (**14**), with, presumably, encouragement from the remaining (β) OH group in stabilizing the intermediate radical cation.



14 **15** **16** (11)

Ribose-5-phosphate reacts with $OH^{\cdot}$ at *ca.* pH 4 in a slightly less selective fashion, giving rise to signals from **17**, **18**, **19** (R = H), **20** and **24** (see table 1). Radical **19** (R = H) evidently arises (scheme 1) via loss of phosphate and rehydration. As the pH is lowered, radical **19** is replaced by the ring-opened radical **22** (pH < 2) (*cf.* **11**) and acid-catalysed dehydration accounts for the conversion of **20** into **23** (pH < 1.5). The observation of **24** under all conditions implies that the dehydration of its precursor, **21**, is extremely rapid even at *ca.* pH 4; the rapidity of this fragmentation may reflect strain in the ring, or (as with reaction of **8**, scheme 1) the overlap between the β C—O bond and the orbital of the unpaired electron, or both.

The results described here for furanose phosphates provide crucial direct evidence to support the suggestion (Behrens *et al.* 1978, 1982) that attack by $OH^{\cdot}$ in the ribofuranose rings of DNA, and subsequent loss of phosphate are important components of a mechanism for radiation-induced strand breakage.

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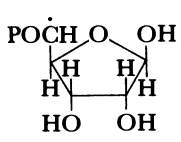
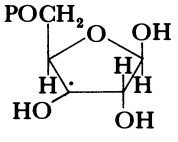
TABLE 1. RADICALS FORMED FROM THE REACTION BETWEEN OH· AND RIBOSE-5-PHOSPHATE AT pH 4 AND BELOW



17



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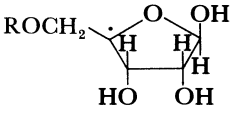
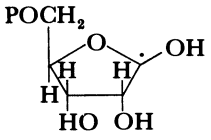
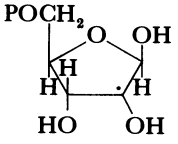


19†

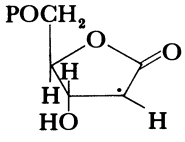


20

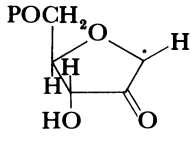


21‡

22§



23‖



24

† Radical **19** (**R**=H) is evidently derived by rehydration of the radical cation from **19** (R=PO_3H^-); see scheme 1.

‡ This radical, the precursor of **24**, is not detected.

§ Replaces **19** (R=H) below pH 2.

‖ Replaces **20** below pH 1.5.

(*b*) *Pyrimidines, purines and nucleosides*

Reaction of OH· (from the Ti^{III}–H_2O_2 couple) with the pyrimidine nucleosides uridine **25** and thymidine gives signals attributable to the radicals formed by addition to the C=C bond in the base (for example, **26** for uridine; see figure 2). This pattern of behaviour resembles the reactions of the pyrimidines themselves (Nicolau *et al.* 1979), though steric effects are evidently more important here, and reflects the greater reactivity of alkenic bonds compared with sugar moieties.

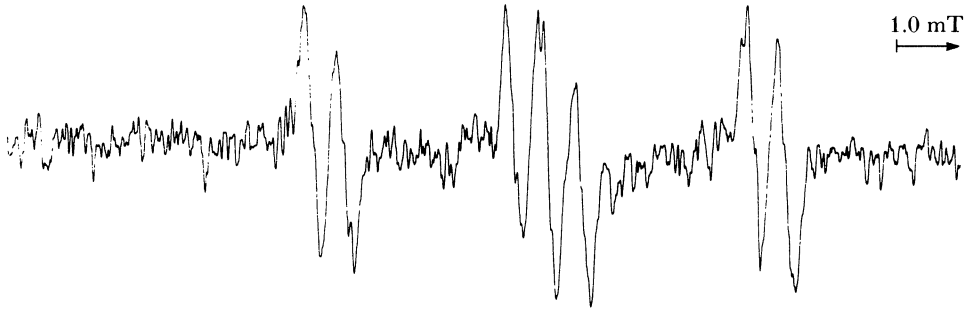


FIGURE 2. E.s.r. spectrum of the radical **26**, obtained from uridine and OH· at *ca.* pH 4.

These findings contrast markedly with results for the purine nucleosides adenosine (**27**, R = H) and inosine (**28**), and their respective monophosphates (for example, **27**, R = HPO_3^-)). Although it has previously been suggested that the (weak) signals from purine nucleosides may be assigned to OH· adducts at the purine bases themselves (Schmidt & Borg 1976), our spectra have significantly higher signal:noise ratios and allow a different interpretation. We have been unable to detect any significant signals in experiments in which the reactions of purines themselves were studied (which may reflect, at least in part, some deactivation of the aromatic ring towards attack by the electrophilic hydroxyl radical). However, inosine and adenosine give identical spectra, which closely resemble those obtained

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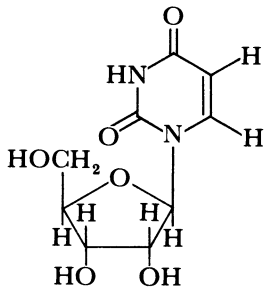

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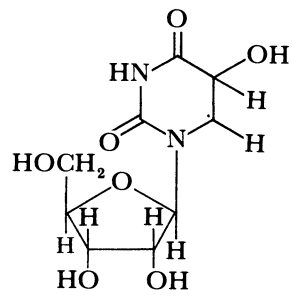

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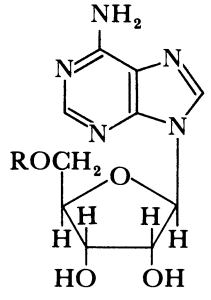
from ribose-5-phosphate and which hence characterize attack in the ribofuranose ring. (The contrasting reactivities observed for purine and pyrimidine nucleosides reflect the difference in rate constants for the addition of $OH^{\cdot}$ to the bases (9×10^8 dm^3 mol^{-1} s^{-1} for adenine, 5×10^9 dm^3 mol^{-1} s^{-1} for uracil, both at pH 2; Anbar (1977).) This indicates a substantial proportion of attack on the sugar under these conditions (though product studies (Scholes *et al.* 1960) suggest that this route represents only *ca.* 10–20% of all products from reaction of $OH^{\cdot}$ with polynucleotides).

The most striking feature of the e.s.r. results for adenosine, inosine, and their 5′-monophosphates is the detection at all pH values in the range 2–8 of a signal from a radical that is both carbonyl and oxygen conjugated (cf. **24** from ribose-5-phosphate). This is assigned in each case to the radical derived by rapid fragmentation ($k > ca.\ 10^4$ s^{-1}) of the radical formed via attack at C-2′ (see, for example, **29** formed from adenosine; reaction (12)).



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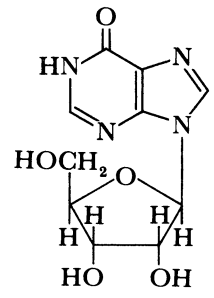

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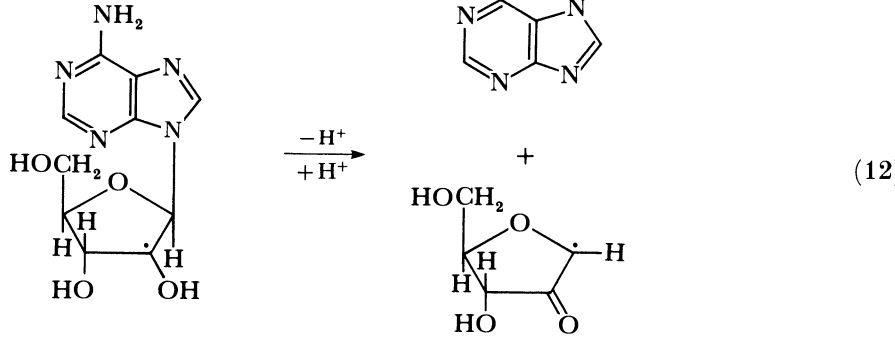
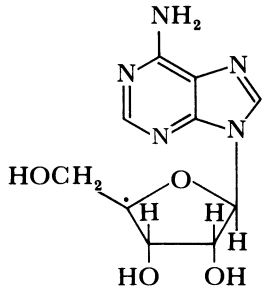


(12)

29

Signals are also observed from radicals formed by initial attack at positions 2′–4′ in the sugar ring, and it is particularly significant that for both adenosine and adenosine-5′-phosphate the radical **30** is clearly seen. This confirms not only that there is significant attack at C-4′ but also that in the latter substrate, loss of phosphate (and rehydration of a radical cation) has also occurred. Our results thus not only provide a mechanism for the loss of base in radiolysed nucleotides and polynucleotides (Ullrich & Hagen 1971; Deeble & von Sonntag 1984), but also provide strong support for the suggestions that radiation-induced strand breakage in DNA and RNA involves attack at C_4' (either directly by $OH^{\cdot}$ (Behrens *et al.* 1982) or via secondary reaction of a peroxy radical derived from initial base attack (Schulte-Frohlinde & Bothe 1984)), followed by loss of the C-5′ (or C-3′) phosphate group.



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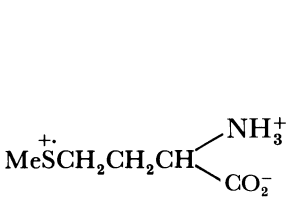
(*c*) *Aromatic and sulphur-containing amino acids*

Finally, we draw attention to other ways in which initial $OH^{\cdot}$ attack can be followed by novel, irreversible fragmentation reactions. The substrates concerned are amino acids possessing either an aromatic ring or a sulphur substituent, both of which provide a reactive 'target' for $OH^{\cdot}$.

Radicals that are detected from methionine, $^{\cdot}CH_2SCH_2CH_2CH(CO_2^-)NH_3^+$, $^{\cdot}CH(SMe)CH_2CH(CO_2^-)NH_3^+$ (and their protonated counterparts) and, perhaps more surprisingly, $^{\cdot}CH(NH_2)CH_2CH_2SMe$ (above *ca.* pH 2) are derived by subsequent reaction of an adduct (formed by attack of $OH^{\cdot}$ at sulphur) and a sulphur-centred radical cation, **31**. The decarboxylation step implicated in the formation of these radicals evidently involves overall one-electron transfer either from CO_2^- (Davies *et al.* 1983) or NH_2 (Asmus *et al.* 1985). A similar and perhaps parallel example is provided by the observation that whereas $OH^{\cdot}$ reacts readily with phenylalanine to give cyclohexadienyl adducts (such as **32**) above pH 4, spin-trapping experiments with t-BuNO show that in the pH range 2–4 the decarboxylated radical



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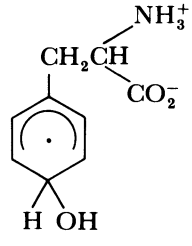

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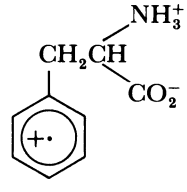

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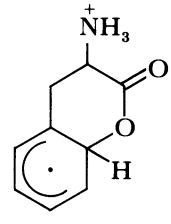

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$^{\cdot}CH(NH_2)CH_2C_6H_5$ is formed (the corresponding adduct with t-BuNO has $a = 1.45$ mT (1N), 0.250 mT (1N), 0.105 mT (1H), 0.0375 mT (2H) (see, for example, Davies *et al.* 1983). As with the similar behaviour of simpler aromatic carboxylic acids ($Ph(CH_2)_nCO_2H$, where $n = 2$–4 (Davies *et al.* 1984)), this is believed to involve the acid-catalysed formation of an aromatic radical cation **33** (*cf.* **31**) and effective overall one-electron transfer in which a discrete but short-lived cyclic intermediate, **34**, may be formed.

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Discussion

B. J. Parsons (*Research Division, North East Wales Institute, Clwyd*). I would like to comment that it is not always necessary to deal with a mixture of free radicals. We have shown, for example (B. J. Parsons *et al.* 1978) that $Br_3^{2-\cdot}$ reacts selectively at C-1 on 2-deoxy-D-ribose. Such selectivity would make analysis of Dr Gilbert's e.s.r. experiments more straightforward.

Reference

Parsons, B. J., Schulte-Frohlinde, D. & von Sonntag, C. 1978 *Z. Naturf.* **33** b, 666.

B. C. Gilbert. We have attempted to study the reaction of carbohydrates with, for example, the radicals $Cl_2^{-\cdot}$ and $Br_2^{-\cdot}$, which would be expected to be more selective than $OH^{\cdot}$. We suspect that it is their comparative lack of reactivity that precludes the detection of e.s.r. signals from radicals derived from their reaction.

Catherine Rice-Evans (*Department of Biochemistry, Royal Free Hospital School of Medicine*). Dr Gilbert has described the attack of hydroxyl radicals on purine and pyrimidine nucleotides at pH 4. Could he explain the relevance of his observations to radiation-induced hydroxyl radical attack on nucleic acids at physiological pH values in vivo?

B. C. Gilbert. In our experiments, signals are generally unchanged in the pH range 4–7 (except for a slight decrease in intensity). The choice of *ca.* pH 4 (in contrast to pH $\lesssim 2$ for the investigation of the acid-catalysed processes described) reflects the optimum conditions for $OH^{\cdot}$ generation (as well as the need to avoid interference from rapid base-catalysed reactions above *ca.* pH 7).

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R. L. Willson (*Department of Biochemistry, Brunel University*). I feel that attack must occur, with uridine and adenosine, at both the base and the sugar. Dr G. Scholes many years ago showed this elegantly by product analysis of irradiated solutions. Is it that Dr Gilbert is just not observing a particular radical because it is oxidized or reduced by other species present in the flowing system? Pulse radiolysis studies show that the different radicals from uracil and adenine and have a wide range of redox properties.

B. C. Gilbert. It is clear from the results that Professor Willson mentions that there must be a significant amount of attack by $^{\bullet}OH$ at the base in purine nucleosides: it seems likely that the appropriate adducts have e.s.r. spectra possessing many weak lines (so that detection and recognition is hampered), rather than that they are destroyed by further reactions. On the other hand, the e.s.r. results clearly indicate both the importance of attack at identifiable positions in the sugar ring and the reactivity of the pyrimidine ring (cf. figure 2).

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