Continuous-flow and spin-trapping EPR studies on the reactions of cytidine induced by the sulfate radical-anion in aqueous solution. Evidence for an intermediate radical-cation

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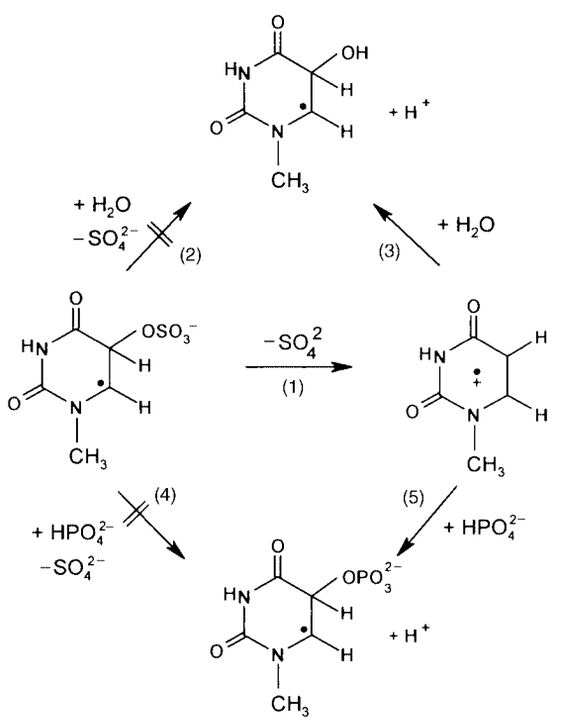
Radicals generated by reaction of the sulfate radical-anion, $SO_4^{\bullet -}$, with cytidine in aqueous solution were characterized by EPR spectroscopy. Two different methods were employed. First, radicals were generated *in situ* in a continuous-flow system by photolysis of solutions containing the substrates and $K_2S_2O_8$. Secondly, $SO_4^{\bullet -}$ was generated either photolytically or by means of the redox couple $Ti(III)/S_2O_8^{2-}$ in presence of the spin trap 2-methyl-2-nitrosopropane (MNP) and EPR spectra were taken from the ensuing persistent spin-adducts. In the continuous-flow experiments the signals of the 2′-oxo-1′-yl sugar radical **1** decreased in intensity upon addition of increasing amounts of phosphate dianions (HPO_4^{2-}, pH > 7.2) and the complex spectrum of base radical **2** appeared. The radicals detected in the spin-trapping experiments were not identical with those observed in the flow-system: in the absence of phosphate the open-chain C(1′) radical **4** was trapped at pH 6–9. It was replaced by spin-trapped 5-yl and 6-yl base radicals **5a/5b** in solutions containing phosphate dianions (pH > 7.2). The results from the *in situ* experiments as well as from the spin-trapping studies are explained by rapid reaction of the intermediate base radical-cation with HPO_4^{2-} competing efficiently with transfer of the radical site to the sugar. From the failure to generate the base radicals **2** and **5** with phosphate monoanions (pH < 7.2) a reaction pathway *via* the negatively charged cytidyl sulfate-adduct radical is excluded.

Introduction

One major event in the interaction of ionizing radiation with nucleic acids is the formation of nucleobase radical-cations. Numerous attempts have been made to generate these species on a model level in aqueous solution from their parent compounds, not only by photoionization,[1] but also by oxidation with a photoexcited quinone (*e.g.* menadione[2] or anthraquinone-2,6-disulfonate[3]), by electron transfer to parent ions of *n*-butyl chloride and acetone[4] and by reaction with other strong electron acceptors such as $Br_2^{\bullet -}$,[5–8] Tl^{2+} [8,9] and $SO_4^{\bullet -}$.[8,10–27] In particular, reaction of $SO_4^{\bullet -}$ with pyrimidines has been studied in detail by γ-radiolysis,[10–14] pulse radiolysis[12,15–19] and by EPR spectroscopy.[20–27] It has been shown that $SO_4^{\bullet -}$ adds to the olefinic carbon–carbon bonds of the nucleobases, the rates being nearly diffusion controlled.[12,15–18]

As shown in Scheme 1 for 1-methyluracil, the emerging sulfate-adduct radical may either hydrolyse [S_N2 reaction (2)] or it may dissociate rapidly to give the base radical-cation and an SO_4^{2-} ion [reaction (1)]. There are contradictory interpretations of pulse radiolysis data on the reactions of $SO_4^{\bullet -}$ with N(1)-methylated pyrimidine bases. Whereas Deeble *et al.*[17] concluded that the sulfate-adduct radicals of these systems dissociate on the nanosecond time scale and that the emerging radical-cations are present in solution for ≈2–20 μs, Lomoth *et al.*[18] claim that the sulfate-adduct radicals are more stable (half-lives of μs) and that the radical-cations, if formed at all, are non-detectable short-lived intermediates ($t_{1/2}$ < 20 ns). Therefore, the question arises as to whether addition of nucleophiles (*e.g.* H_2O or phosphate) to the pyrimidine ring occurs by S_N2 reaction with the sulfate-adduct radical or by a S_N1 mechanism *via* the base radical-cation. Detection of a short-lived species, be it the sulfate adduct or the radical-cation, by steady-state EPR in solution is not possible. However, the EPR spectra are in line with the concept of pyrimidine radical-cation intermediates as



Scheme 1

they indicate neutral radicals formed by deprotonation at N(1) (for uracil and thymine),[28,29] by deprotonation of methyl substituents (for thymidine[25] and thymidine 5′-phosphate[24]), and by the reaction of H_2O at C(5) and C(6).[21–26] This concept was corroborated by the observation that photolysis of solutions containing 1-methyluracil or 1,3-dimethyluracil, $S_2O_8^{2-}$ and phosphate resulted in adduct radicals with a phosphate group

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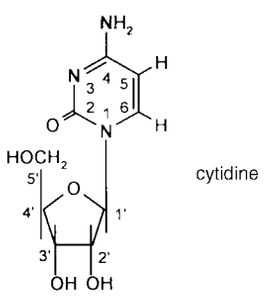
covalently attached to the carbon–carbon bond of the pyrimidine ring.[21] It was found that phosphate monoanions ($H_2PO_4^-$, pH < 7.2) reacted too slowly to compete with hydrolysis [reaction (2) or (3)] but that phosphate dianions (HPO_4^{2-}, pH > 7.2) were rapidly bound to the uracil moiety ($k \approx 5 \times 10^8$ dm^3 mol^{-1} s^{-1}).[21] This may be seen as evidence for a pathway *via* S_N1 reactions (3) and (5) and excludes S_N2 reaction (4) of phosphate with the sulfate-adduct radical. (The regioselectivity of these reactions is discussed in ref. 21.)

EPR results on pyrimidine nucleosides were explained by primary attack at the nucleobase due to the fact that the rate of addition of $SO_4^{\bullet -}$ to pyrimidines is at least an order of magnitude higher than the rate of abstraction of H atoms from the sugar moieties.[22] Subsequent migration of the radical site from the base to the C(2′) position in ribose compounds (but not in their deoxyribose counterparts) resulted in the resonances of sugar-derived radicals.[22–25] Provided that, as in the experiments with N(1)-methylated pyrimidines, base radical-cations are intermediates in the reactions of nucleosides, one would predict an influence of phosphate dianions on the nature of the reaction products. This issue is not only of academic interest for mechanistic details of radiation damage by the 'direct effect'[10] but has also practical implications in so far as phosphate, when employed as a buffer in radiolysis experiments, might become involved in chemical modification of the target molecules. In order to approach this problem by EPR spectroscopy we studied $SO_4^{\bullet -}$-induced radical reactions of cytidine in the absence and presence of phosphate.



cytidine

According to our experience, EPR results obtained from *in situ* photolysis of solutions containing $K_2S_2O_8$ and pyrimidine derivatives suffer from weak intensities and from secondary reactions.[13,21] These problems proved to be less severe in EPR spin-trapping of pyrimidine radicals with 2-methyl-2-nitrosopropane, MNP.[26] However, the EPR parameters of the spin-adducts are less specific than those of directly detected radicals. Therefore, in the present work, both methods were employed in a complementary way with the goal to improve the reliability of the experimental data. Although the radicals detected by the two procedures were not identical, the characteristic influence of phosphate dianions on the radical reactions became evident from continuous-flow and from spin-trapping experiments.

Results and discussion

Continuous-flow experiments

The spectra in Fig. 1 were generated by photolysis of solutions containing cytidine, $K_2S_2O_8$ and traces of acetone-d_6 as a photosensitizer. In line with previous studies,[22,25] sugar-derived radical **1** was detected [Fig. 1(a)]. It is characterized by three doublet splittings [a(1′-H) = 1.36 mT, a(3′-H) = 0.54 mT, a(4′-H) = 0.23 mT, g = 2.0049]. Upon addition of phosphate at pH > 7.2 the intensity of the spectrum of radical **1** decreased [Fig. 1(b) and (c)] and the complex pattern of radical **2** appeared. It was very similar to the spectra of base-derived radicals observed[22] in solutions containing 1-methylcytosine (1-MC) or 2′-deoxycytidine (dC) and $K_2S_2O_8$ in the absence of phosphate and, with higher intensity, in the presence of

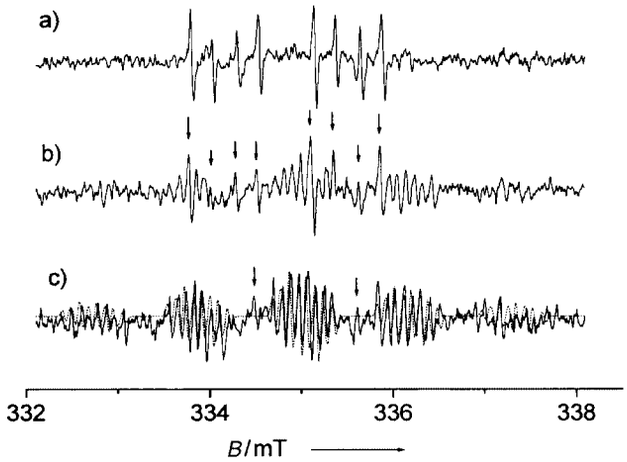


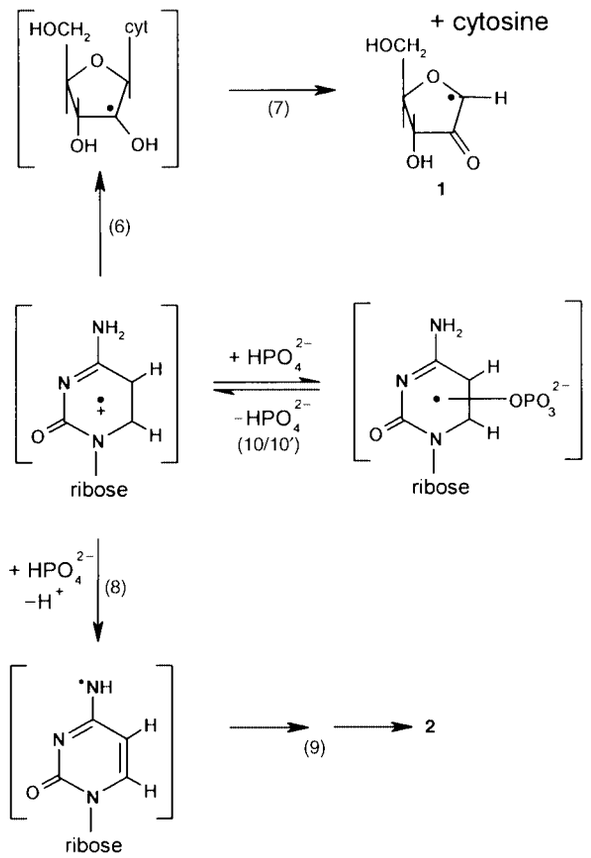
Fig. 1 Continuous-flow EPR spectra obtained by *in situ* photolysis of solutions of a) cytidine, $K_2S_2O_8$, acetone-d_6 (0.3%) and borate (2×10^{-3} mol dm^{-3}); the pH value prior to UV irradiation was 8.0, after irradiation 7.2; b) cytidine, $K_2S_2O_8$, acetone-d_6 (0.3%) and KH_2PO_4 (5×10^{-3} mol dm^{-3}); the pH value dropped from 8.0 to 7.2 during irradiation; c) same as b) but in the presence of 2.5×10^{-2} mol dm^{-3} KH_2PO_4, pH 7.6; the dotted line is a simulation with the parameters given in the text for radical **2**. Signals of radical **1** detected in (b) and (c) are labelled with arrows. Instrument settings: microwave power, 1 mW; modulation amplitude, 0.045 mT; gain, 5×10^3; scan time, 0.45 mT min^{-1}, temperature, 15 °C; number of accumulations, 3.

phosphate. The concentration of the photosensitizer, acetone-d_6, was kept as low as possible (0.3%) in order to minimize the disturbing effect of the acetonyl radical.[22] Under those conditions, upon accumulation of three spectra a signal : noise ratio of ≈2 : 1 for the signal groups in the low and high field wings was achieved. A satisfactory fit [dotted line in Fig. 1(c)] was obtained with two large nitrogen splittings of 1.16 mT, a small nitrogen splitting of 0.18 mT and proton splittings of 0.11 (2 protons) and 0.09 mT. (These parameters are identical within the error limits with those reported for the corresponding radicals of 1-MC and dC).[22]

Interpretation of the results on 1-MC and dC was based on the assumption that by reaction of $SO_4^{\bullet -}$ with the cytidyl ring the base radical-cation is generated.[22,24] The aminyl radical expected[15] from deprotonation of the cytidyl radical-cation [*e.g.* reaction (8) in Scheme 2] with a characteristic doublet splitting of ≈1–1.2 mT due to the $^{\bullet}$NH proton[30,31] was not observed. The spectrum observed instead was proposed to be due either to a tautomeric form of the aminyl radical or to a radical produced in secondary reactions (9).

The situation in cytidine is even more complex because of rapid migration of the radical site from the base radical-cation to the sugar moiety [reaction (6), for mechanistic details of this step see Catterall *et al.*[25]]. It seems that deprotonation of the base radical-cation [reaction (8)] in cytidine is too slow to compete with migration of the unpaired electron to the ribose moiety and therefore by elimination of cytosine from the ensuing C(2′) radical [reaction (7)] sugar radical **1** is generated[22,24,26] instead of the base radical. However, in the presence of increasing amounts of the proton acceptor HPO_4^{2-} the deprotonation rate increases and becomes fast enough to compete with the attack at the sugar moiety. Although the structure of radical **2** is not known and there is only circumstantial evidence for reactions (8) and (9) it is obvious from Fig. 1(b) and (c) that reaction of phosphate dianions in relatively low concentrations in the millimolar range is fast enough to compete with transfer of the radical site from base to sugar.

Whereas the catalytic effect of phosphate in protonation of carbon atoms is well known,[32] evidence for the reverse type of reaction, *i.e.* phosphate-catalysed deprotonation, is rare. An example to be mentioned in this context is the formation of the allyl-type radical of thymidine 5′-phosphate in aqueous



Scheme 2

solution[24] by phosphate-catalysed deprotonation of the methyl group of the thymidyl radical-cation.

It is important to note that at pH < 7.2, *i.e.* below the pK_a value of the equilibrium $H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$, there was no evidence for formation of radical **2** and even in the presence of 5×10^{-2} mol dm^{-3} HPO_4^{2-} the sugar-derived radical **1** was the only species detected. This observation strongly favours the reactions in Scheme 2 *via* a positively charged intermediate and is contradictory to the alternative pathway *via* a 'stable'[18] negatively charged base sulfate-adduct radical [*e.g.* reaction (4)].

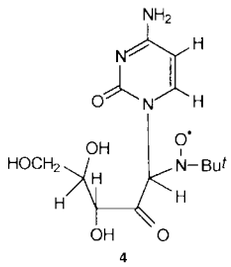
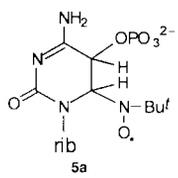
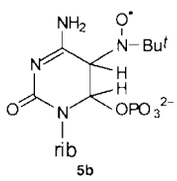
From the continuous-flow experiments there is no evidence for phosphate adducts to the C(5)–C(6) bond of the cytidyl ring with expected large proton splittings of the order of 1.5–2 mT [a(α-H) and a(β-H) = 1.8 mT were observed for the C(5)–OH-adduct radical of cytidine and dC[25]]. The phosphate-adduct radicals of cytidine seem to be less stable than those of 1-methyluracil and 1,3-dimethyluracil,[21] possibly because of the electron donating effect of the exocyclic amino group. However, results from spin-trapping experiments (see below) indicate reaction of phosphate with the carbon–carbon bond of the nucleobase. Therefore, we feel that, besides deprotonation, equilibrium (10/10′) plays an important role for the fate of the radical-cation.

It should be mentioned that $SO_4^{\bullet -}$ may oxidize not only the nucleosides but also phosphate ions according to reaction (11).

$$SO_4^{\bullet -} + HPO_4^{2-} \longrightarrow SO_4^{2-} + HPO_4^{\bullet -} \qquad (11)$$

In principle, the emerging phosphate radical could contribute to the EPR results, *e.g.* by addition–elimination reactions at the pyrimidine ring. However, based on kinetic data, this is excluded. According to Fig. 1(b), upon addition of 5×10^{-3} mol dm^{-3} HPO_4^{2-} to a solution of cytidine (3×10^{-3} mol dm^{-3}) and $K_2S_2O_8$, the intensity of the spectrum of radical **1** decreased to approximately one half of the value determined in the absence of phosphate. With the known rate constant for reaction (11) [$k_{11} = 1.2 \times 10^6$ dm^3 mol^{-1} s^{-1}][33] and the much higher rate constant for reaction of $SO_4^{\bullet -}$ with cytosine nucleosides [k(dC + $SO_4^{\bullet -}$) = 2.5×10^8 dm^3 mol^{-1} s^{-1}][15] it is estimated that under those conditions less than 1% of the $SO_4^{\bullet -}$ radicals react with phosphate.

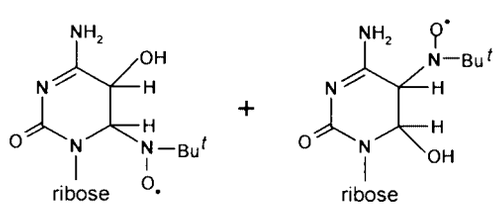
Table 1 Hyperfine couplings a (in mT)[a] for MNP spin-adducts obtained by reaction of $SO_4^{\bullet -}$ with cytidine in the absence (radical **4**) and in presence of HPO_4^{2-} (radicals **5a** and **b**). In parentheses: couplings of MNP spin-adducts **3a** and **3b** produced by reaction of •OH with cytidine (pH 6.5)

Spin adduct	pH	a(N)	a(β-N)	a(β-H)
	6–9	1.450	0.169	0.189
	7.4	1.50 (1.48)	0.243 (0.260)	0.154 (0.161)
	7.4	1.50 (1.50)	— —	0.428 (0.425)

[a] ±0.005 mT.

Spin-trapping

Trapping of OH-adduct radicals. The EPR spectra of MNP spin-adducts produced by reaction of $SO_4^{\bullet -}$ with cytidine in the presence of phosphate (see below) were similar to those of the spin-trapped OH-adduct radicals. Therefore, we first studied the reaction of •OH with the nucleosides in the presence of MNP. The OH radicals were generated either by photolysis of solutions containing H_2O_2 or by γ-irradiation of N_2O-saturated solutions.



The spectra of the spin-adducts **3** from both types of experiments were fitted by assuming a mixture of 6-yl and 5-yl radicals (Table 1). Identical values for hyperfine splittings and linewidths were obtained for spin-adducts generated by γ-irradiation and by photolysis. Differences in relative weights of radicals **3a** and **3b** (values not shown) are probably due to secondary oxidations by H_2O_2 in the photolysis experiments. In line with data reported on MNP spin-trapped OH-adduct radicals of uridine[27] and cytosine derivatives,[34,35] our parameter sets consisted of three coupling constants [a(N), a(β-N) and a(β-H)] for **3a** and two couplings [a(N) and a(β-H)] for **3b**.

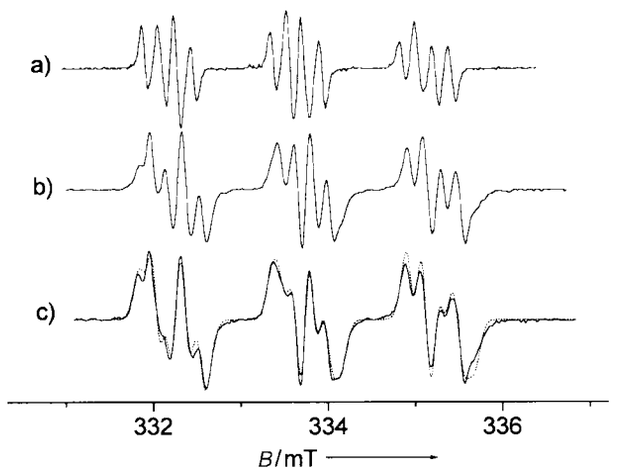


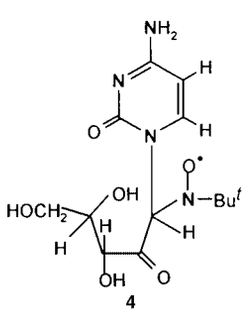
Fig. 2 EPR spectra of MNP spin-adducts obtained from solutions of cytidine, $K_2S_2O_8$, Ti(III)–EDTA and MNP: a) unbuffered, pH dropped from 7.6 to 7.2 during reaction; b) in the presence of 5×10^{-2} mol dm^{-3} HPO_4^{2-}, pH 7.4; c) in the presence of 17.5×10^{-2} mol dm^{-3} HPO_4^{2-}, pH 7.4; the dotted line is a simulation with the hyperfine splittings of **4**, **5a** and **5b** (Table 1) and intensity ratios of **5a**:**4** = 1.6:1 and **5b**:**4** = 1.1:1.

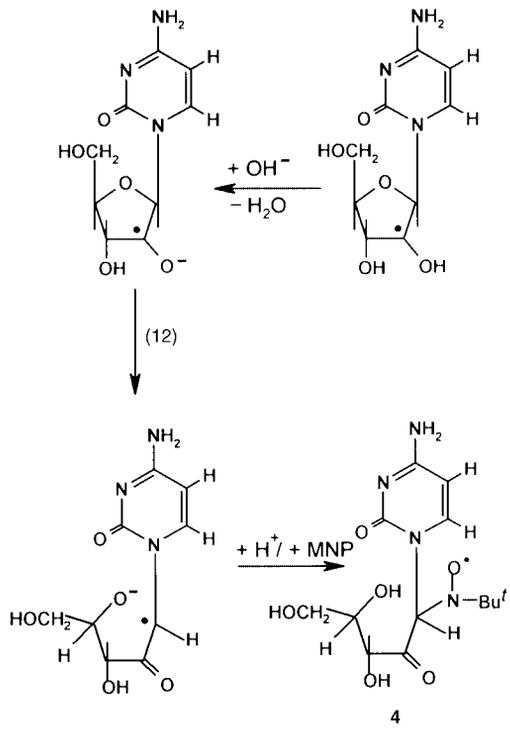
Whereas the parameters recovered from our experiments for **3b** are in good agreement with data in ref. 34 and 35, the values of a(β-H) for **3a** are widely different [a(β-H) = 0.15 mT in our experiments as compared to 0.44 and 0.40 mT reported by Hiraoka *et al.*[34] and Kuwabara *et al.*[35]]. The reasons for these discrepancies are not known.

Trapping of $SO_4^{\bullet -}$-induced radicals. In spin-trapping experiments, $SO_4^{\bullet -}$ was generated from $S_2O_8^{2-}$ either by photolysis or by reduction with Ti(III). In acidic solutions identical results were obtained by the two procedures. At pH ≥ 7 in photolysis experiments the spectra were strongly disturbed by the intense triplet of the di-*tert*-butylaminoxyl radical (DTBN), a photoproduct of MNP. Therefore, in neutral to alkaline solutions radical generation with the Ti(III)/$S_2O_8^{2-}$ redox couple was preferred.

At pH 6–10 a triplet of 1:2:2:1 quartets was detected [a(N) = 1.45 mT, a(β-N) = 0.169 mT, a(β-H) = 0.189 mT, Fig. 2(a)]. A similar spectrum [a(N) = 1.44 mT, a(β-N) = a(β-H) = 0.17 mT] observed by Ho *et al.*[27] upon reaction of $SO_4^{\bullet -}$ with isotopically labeled uridine at pH < 2 was assigned to the spin-adduct of a ring-opened C(1′) radical. It is well known[36] that α-hydroxyalkyl radicals carrying a leaving group in the β-position undergo heterolytic decay in aqueous solution which is catalysed by acids and bases.

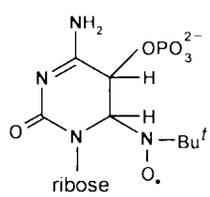
Previous EPR studies on cytidine[22,23,25] have shown that the C(2′) radical generated by transfer of the radical site from the base radical-cation to the sugar (Scheme 2) decays by elimination of cytosine. In a related reaction by cleavage of the C(1′)–oxgen bond [C(4′)–O$^-$ is the leaving group in the base-catalysed reaction (12), Scheme 3] the open chain C(1′) radical is formed which in the presence of MNP gives rise to the spectrum of spin-adduct **4**. The presence of phosphate at pH < 7.2

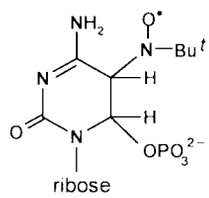




Scheme 3

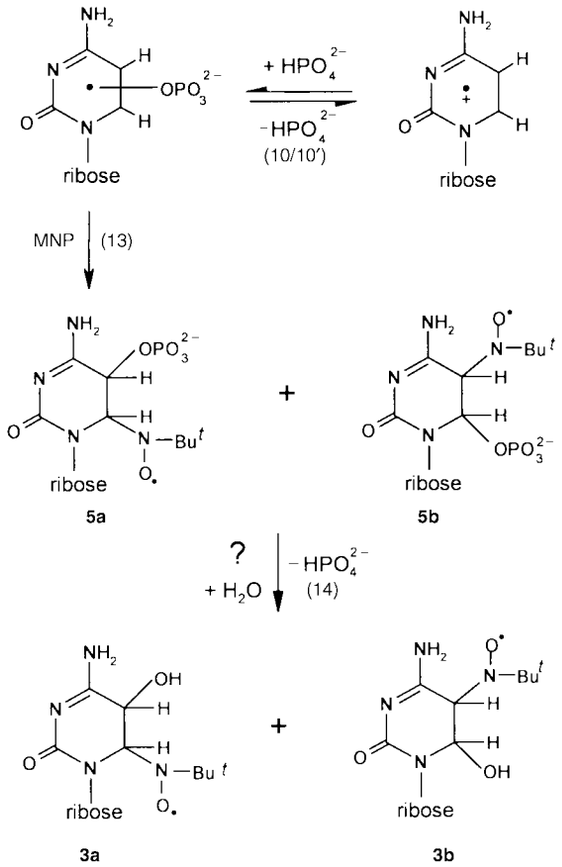
($H_2PO_4^-$) in the reaction mixtures had no influence on the spectrum. However, in the alkaline region (pH > 7.2, HPO_4^{2-}) additional resonances were detected which increased in intensity upon increasing the phosphate concentration [Fig. 2(b,c)]. They are assigned to trapped phosphate-adduct radicals **5a** and **5b**.





Spin-trapped OH- and phosphate-adduct radicals of 1-methyluracil and 1,3-dimethyluracil have been identified by the fine structures of their EPR spectra.[26] The splittings due to the ^{31}P atoms in the γ-position were not resolved. However, the small but characteristic differences in the well resolved β-nitrogen and β-hydrogen couplings allowed us to distinguish between trapped OH- and phosphate-adduct radicals. In contrast, β-couplings were not resolved in the spectra of the spin-adducts derived from cytidine [see Fig. 2(b,c)]. Therefore, from these spectra, one would not expect to get unequivocal evidence for covalent binding of the phosphate group to the base.

The following procedure was applied to analyse the spectra generated in the presence of HPO_4^{2-}: First, for the spectrum taken with the highest phosphate concentration [17.5×10^{-2} mol dm^{-3}, Fig. 2(c)], a simulation was carried out with a data set of 3 components, namely spin-adduct **4**, a spin-trapped 5-yl and a spin-trapped 6-yl radical. The parameters for radical **4** were kept constant and splittings, linewidths and relative peak heights for the two base-centred radicals were fitted by an iterative procedure. The splittings obtained in this way are assigned to trapped 5-phosphate-6-yl (**5a**) and 6-phosphate-5-yl radicals (**5b**) (see Table 1). They are close to the parameters of the spin-adducts generated with OH radicals (**3a** and **3b**). From these data hydrolysis of the carbon–phosphate bonds in the spin-adducts and formation of spin-trapped OH-adducts



Scheme 4

[reaction (14), Scheme 4] cannot be definitely excluded. It is unlikely, however, in view of the stability of trapped 5-phosphate-6-yl radicals of 1-methyluracil and 1,3-dimethyluracil.[26]

After having analysed the spectrum in Fig. 2(c) simulations were carried out for a series of spectra obtained with phosphate concentrations lower than 17.5×10^{-2} mol dm^{-3}. In these calculations all splittings and linewidths were fixed and only the relative peak heights were iterated. In Fig. 3 ratios of the intensities of the spectra of **5a** and **5b**, both divided by the intensity of **4** are plotted. It should be pointed out that for calculation of relative radical concentrations comparison of the double integrals of the subspectra would be necessary which is not possible in view of the heavy overlap of the spectral components. However, due to the fact that the linewidths did not change upon phosphate addition, changes in the peak intensities are directly related to changes in spin-adduct concentrations.

The increase in relative amounts of base- to sugar-derived radicals shown in Fig. 3 is ascribed to reaction of HPO_4^{2-} with the C(5)–C(6) bond of the cytosine ring [reaction (10)] which competes with formation of the open-chain C(1′) radical **4** [reactions (6) and (12)] and which becomes more prominent at higher phosphate concentrations. The effect of pH changes as an explanation for the data in Fig. 3 is excluded because radical **4** was observed as a single component throughout the pH range 6–9 and moreover phosphate concentrations of 2.5×10^{-2} mol dm^{-3} (the lowest value in Fig. 3) proved sufficiently high to keep the pH value at a constant level. The fact that the phosphate monoanion (pH < 7.2) was completely ineffective in producing base-centred radicals strongly supports the S_N1 pathway *via* the base radical-cation intermediate [reaction (10)] and is in contradiction to S_N2 reaction of the sulfate-adduct radical.

The concentration of phosphate required to induce spectral changes was higher in the spin-trapping experiments than in the continuous-flow studies. To make sure that phosphate radicals [see reaction (11)] are not involved in the formation of

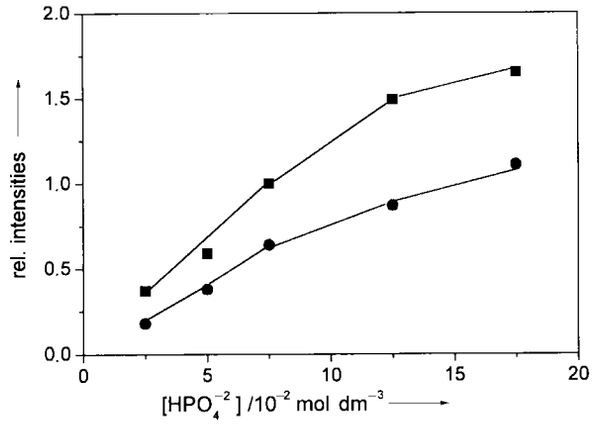


Fig. 3 Ratios of EPR intensities (peak heights) of spin-adducts plotted *vs.* concentration of phosphate dianions; a) squares: intensity of **5a** divided by intensity of **4**; circles: intensity of **5b** divided by intensity of **4**.

spin-adducts **5**, measurements were carried out on a series of samples with identical phosphate concentration (*e.g.* 12.5×10^{-2} mol dm^{-3}) and cytidine concentrations varying by more than an order of magnitude [$(10.5–0.7) \times 10^{-2}$ mol dm^{-3}]. From kinetic data (rate constants for reaction of $SO_4^{\bullet -}$ with cytidine and HPO_4^{2-} are given above) it was estimated that in those experiments the amount of $SO_4^{\bullet -}$ reacting with phosphate instead of the nucleobase increased from 0.6 to 8%. From the fact that the relative yields of spin-adducts **4** and **5** were constant in these experiments it is concluded that reactions of phosphate radicals $HPO_4^{\bullet -}$ are not responsible for the spectral changes shown in Fig. 2 and 3.

Comparison of continuous-flow and spin-trapping EPR

Striking differences were encountered concerning the nature of the radicals detected by the two methods. Whereas in continuous-flow experiments sugar radical **1** and base radical **2** were observed, spin-adducts **4** and **5** were derived from the open-chain C(1′) radical and from phosphate-adduct radicals of the base. Generally speaking, the reasons responsible for these differences may be seen in different timescales of the experiments (radical lifetimes of several milliseconds, at least, are required for *in situ* detection,[37] whereas spin-trapping may occur in less than a millisecond after radical generation[38]), in different rates of reaction of free radicals with the spin-trap[38] and in different rates of decay of the spin-adducts in combination with the fact that the initially generated radical population undergoes time-dependent changes in the strongly oxidizing solutions.[13,21] Madden and Taniguchi[38] have shown by time-resolved EPR that the rate of spin trapping by the electrophilic MNP is directly related to the electron releasing ability of the organic radicals. The high *g* factor of 2.0049 and the low value for the α-hydrogen hyperfine splitting [a(1′-H) = 1.36 mT] indicate that in the sugar-derived radical **1** the unpaired electron is partially delocalized onto the two oxygen atoms adjacent to C(1′). Obviously, the reducing power of **1** is too low for efficient trapping. On the other hand, the stationary concentrations of the parent radicals of spin-adducts **4** and **5** seem to be too low for *in situ* detection. Despite these differences, the two types of experiments provide complementary information on a complex scheme of $SO_4^{\bullet -}$-induced radical reactions without offering the possibility to differentiate between main and side reactions. However, common to both types of experiments and most remarkable is the influence of HPO_4^{2-} on the nature of the radical products.

Conclusion

Reaction of $SO_4^{\bullet -}$ with N(1)-substituted pyrimidines initially results in sulfate-adduct radicals of the nucleobases. Formation

of neutral radicals from these adducts may, in principle, occur either directly in S_N2 reactions or *via* base radical-cation intermediates in S_N1 reactions. In the present work, reaction of $SO_4^{\bullet -}$ with cytidine was studied by continuous-flow and spin-trapping EPR. In the absence of phosphate, sugar-derived radicals were detected. Upon addition of increasing amounts of phosphate dianions (pH > 7.2) but not of the monoanions (pH < 7.2), the concentration of those radicals decreased and base-derived radicals (either phosphate-adducts or OH-adducts resulting from their hydrolysis) were detected instead. These observations are in favour of a base radical-cation intermediate. Reaction of HPO_4^{2-} with the intermediate competes with transfer of the radical site to the sugar moiety and results in base-centred radicals. Formation of the base-derived radicals in S_N2 reactions of HPO_4^{2-} with the base sulfate-adduct radical is excluded.

Experimental

Continuous-flow EPR spectra were recorded on a laboratory-built X-band spectrometer, EPR spectra of the MNP spin-adducts on a Varian E-9 X-band instrument. Both spectrometers were equipped with interfaces from Stelar s.n.c., Mede (PV), Italy and IBM compatible PC's. Spectra simulation was carried out by iterating hyperfine couplings, *g* factors, linewidths and relative peak heights of subspectra with a least squares fitting program.[39] For *in situ* EPR experiments the solutions contained cytidine [$(3–6) \times 10^{-3}$ mol dm^{-3}], $K_2S_2O_8$ [$(3–30) \times 10^{-3}$ mol dm^{-3}] and 0.3% acetone-d_6 as a photosensitizer.[22] The solutions were degassed with argon, pumped through the aqueous solution quartz cell and irradiated in the cavity of the EPR instrument with unfiltered UV light from an argon plasma light source (GAT, Bremerhaven, Germany). In the spin trapping experiments $SO_4^{\bullet -}$ or $^{\bullet}OH$ was generated from $K_2S_2O_8$ or H_2O_2 either by photolysis or by adding $TiCl_3$–EDTA to the solutions. In the photolysis experiments the solutions contained the substrate [$(5–15) \times 10^{-3}$ mol dm^{-3}], $K_2S_2O_8$ (1.5×10^{-2} mol dm^{-3}) or H_2O_2 (0.1 mol dm^{-3}) and MNP (5×10^{-3} mol dm^{-3}) from a stock solution (5×10^{-2} mol dm^{-3} in H_2O–acetonitrile 5 : 1). The solutions were degassed for 20 min with N_2, transferred to an aqueous solution quartz cell and irradiated in the cavity of the EPR instrument with unfiltered and unfocussed light from a LX300UV Cermax lamp (ICL Technology, Sunnyvale, CA). The concentrations of the spin-adducts increased with time and reached a maximum after ≈4 min of irradiation. For radical generation with Ti(III), samples were prepared by adding an aqueous solution (A) of $TiCl_3$–EDTA (5×10^{-3} mol dm^{-3}) to a solution (B) containing the substrates (1×10^{-2} mol dm^{-3}), $K_2S_2O_8$ (3×10^{-2} mol dm^{-3}) or H_2O_2 (5×10^{-3} mol dm^{-3}), KH_2PO_4 and MNP (5×10^{-3} mol dm^{-3}) (numbers in brackets are final concentrations). The pH values of solutions (A) and (B) were separately adjusted with NH_3 or $HClO_4$ before mixing. In experiments carried out in the absence of phosphate, *i.e.* in unbuffered solutions, the pH values dropped by ≈0.4 units upon reaction. In some experiments OH radicals were generated by irradiation of N_2O-saturated solutions of cytidine with a ^{60}Co-γ source (dose rate: 0.3 kGy min^{-1}). Chemicals were supplied by Sigma, Aldrich or Merck, Darmstadt and used without further purification.

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