Comparison of the Reaction of $^{\bullet}OH$ and of $SO_4^{-\bullet}$ Radicals with Pyrimidine Nucleosides. An Electron Spin Resonance Study in Aqueous Solution

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Reactions of photolytically generated $^{\bullet}OH$ and $SO_4^{-\bullet}$ radicals with uridine, cytidine, 2′-deoxyuridine (dU) 2′-deoxycytidine (dC), and thymidine have been studied by e.s.r. spectroscopy under anoxic conditions. In the experiments with $^{\bullet}OH$, the spectra of the uracil compounds were dominated by the signals of radicals originating from $^{\bullet}OH$ addition at the alkenic double bond of the nucleobase. No spectra were observed for the cytosine derivatives and thymidine. With $SO_4^{-\bullet}$, base radicals were generated from the deoxyribonucleosides [C(5)-OH-6-yl from dU, C(6)-OH-5-yl from thymidine, and a nitrogen-centred radical from dC] whereas the ribonucleosides lead to two different types of sugar radical. One of them is derived from the 2′-hydroxyalkyl radical by heterolytic elimination of the nucleobase and the other is the 3′-hydroxyalkyl radical which undergoes ring-opening by heterolytic cleavage of the C(4′)–oxygen bond at neutral and alkaline pH. Both the $^{\bullet}OH$ and $SO_4^{-\bullet}$ radicals add to the base moieties in the primary step. The adduct radicals formed with $^{\bullet}OH$ from uridine and dU are stable on the millisecond time-scale of the e.s.r. experiment whereas the sulphate adducts are too short-lived to be detected by e.s.r. In the deoxyribose derivatives they either hydrolyse (dU and thymidine) or eliminate SO_4^{2-} and a proton (dC) whereas in the ribonucleosides they induce intramolecular H abstraction from positions 2′ and 3′ of the sugar residues.

Damage of DNA is the main cause of cell deactivation by ionizing radiation. The incident radiation may either be absorbed by the macromolecule ('direct effect') or by the mainly aqueous environment ('indirect effect'). Direct interaction of radiation with DNA leads to ionization of the nucleobases and the sugar–phosphate residues. The indirect damage of DNA is caused by reactive radiolysis products of the environment; in aqueous phase at neutral pH the main damaging species is the $^{\bullet}OH$ radical.[1]

Reactions of $^{\bullet}OH$ radicals with DNA and with DNA models, *i.e.* nucleotides, nucleosides, nucleobases, and sugars, have been studied extensively.[2] Investigations of the 'direct effect' in aqueous systems are hampered by the fact that the absorption of ionizing radiation is always accompanied by the formation of the intermediates of the 'indirect effect.' However, in model systems, conditions can be met which favour the 'direct effect' and suppress the 'indirect effect.' For example, irradiation of dilute solutions of polyuridylic acid [poly(U)] with u.v. light from a laser (λ = 248 nm) yields radical cationic sites at the uracil moiety in the primary step.[3] These act as intermediates in a reaction sequence which leads to radical formation at the sugar–phosphate backbone and to subsequent cleavage of the phosphate ester bonds which constitutes strand break formation. In principle, it should be possible to mimic the formation of base radical cations by chemical methods. It is well known that the $SO_4^{-\bullet}$ radical anion is able to generate radical cations from aromatic compounds by one-electron transfer.[4–7] In agreement with these results pulse radiolysis data on the reaction of $SO_4^{-\bullet}$ with purine and pyrimidine deoxyribonucleosides [8] were interpreted in terms of cationic intermediates which undergo either deprotonation or hydration to yield OH-adducts. Therefore, one might expect that with $SO_4^{-\bullet}$ reactions of nucleosides, nucleotides, and nucleic acids are initiated which may yield information on the intermediates of the 'direct effect.'

E.s.r. methods have been used to study reactions of $^{\bullet}OH$ and of $SO_4^{-\bullet}$ with sugars and with pyrimidine bases. It was shown that both $^{\bullet}OH$ and $SO_4^{-\bullet}$ react with carbohydrates in a rather unselective way.[9–11] In acidic solutions the α,β-dihydroxy-substituted radicals originally formed by H abstraction are transformed into carbonyl conjugated radicals by elimination of water.[9–13] In alkaline solutions $^{\bullet}OH$-induced H abstraction from six-membered sugar rings was followed by ring opening and formation of semidiones.[14] E.s.r. studies of uracil indicated that $^{\bullet}OH$ radicals add to the olefinic double bond of the base to yield the OH-adduct radicals [15] whereas with $SO_4^{-\bullet}$ the uracil-1-yl radical with high spin density at N(1) and C(5) was generated.[16] With $^{\bullet}OH$ from N(1)-methylated uracils and thymines extremely weak e.s.r. spectra were obtained, whereas reaction with $SO_4^{-\bullet}$ resulted in more intense signals of OH-adduct radicals.[17] It was assumed that in the primary step base–sulphate adduct radicals are formed which may react with H_2O either *via* an S_N2 mechanism or *via* a radical-cation intermediate in an S_N1 reaction.

In the present study we compared the reactions of $^{\bullet}OH$ and $SO_4^{-\bullet}$ with ribonucleosides (uridine and cytidine) and with deoxyribonucleosides (dU, dC, and thymidine) of the pyrimidine series.

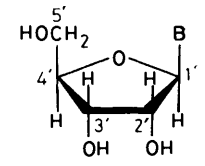
With $^{\bullet}OH$, e.s.r. signals were generated only from uridine and dU, whereas with $SO_4^{-\bullet}$ spectra were obtained from all the substrates under investigation. The structures of the radicals and the pathways of their formation are discussed.

Results

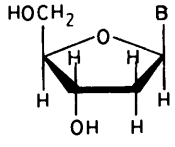
$^{\bullet}OH$-*Induced Radical Formation.*—Rather intense e.s.r. spectra were generated upon reaction of $^{\bullet}OH$ with uridine and dU. In agreement with results reported by Gilbert and co-workers [13,18] they are characterized by g = 2.0028, and by one small and two large doublet splittings (Figure 1 and Table 1).

Table 1. Hyperfine splittings (mT) [±0.02 mT] of the 5-OH adduct radicals (**1**) of uridine and 2′-deoxyuridine formed in reaction (1) at pH 3—7. The *g* factors of both radicals are 2.0028

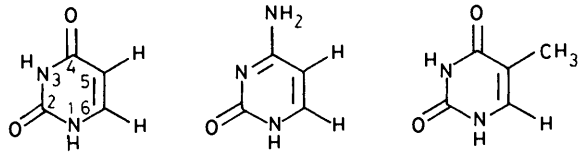
Substrate	Radical	$a(H_\alpha)$	$a(H_\beta)$	a(1′-H)
Uridine	(**1a**)	2.15	1.85	0.3
2′-Deoxyuridine	(**1b**)	2.20	1.88	0.28



B = Uracil; uridine
B = Cytosine: cytidine



B = Uracil: 2′-deoxyuridine (dU)
B = Cytosine: 2′-deoxycytidine (dC)
B = Thymine: thymidine



Uracil Cytosine Thymine

Scheme 1.

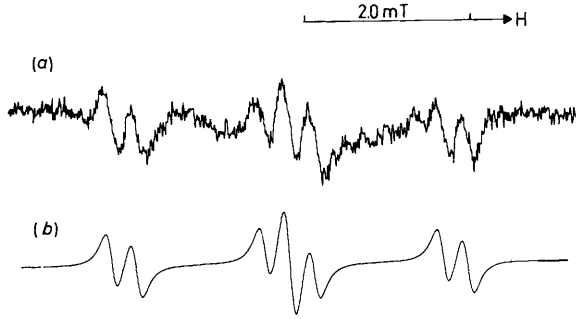
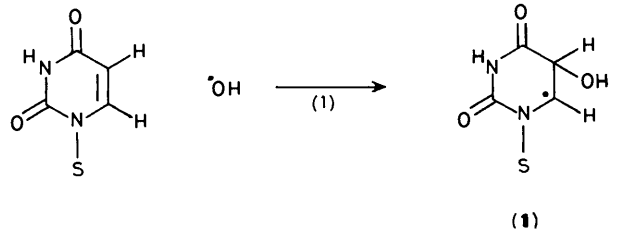


Figure 1. E.s.r. spectra obtained by *in situ* photolysis of a solution containing uridine (1 mmol l^{-1}) and H_2O_2 (1 mmol l^{-1}) at 277 K and pH 7.5. (*a*) Experimental and (*b*) simulation using the parameters listed in Table 1 for radical (**1a**)



Scheme 2.

The two large couplings are assigned [15,19] to a(5-H) and a(6-H), the small one to a(1′-H).[18] No change in the spectra was observed when D_2O was used as the solvent instead of H_2O.

* The following abbreviations are used: 5-hydroxy-5,6-dihydrouracil-6-yl = 5-OH(-6-yl) adduct of uracil; 6-hydroxy-5,6-dihydrouracil-5-yl = 6-OH(-5-yl) adduct of uracil; 6-hydroxy-5,6-dihydrothymin-5-yl = 6-OH(-5-yl) adduct of thymine.

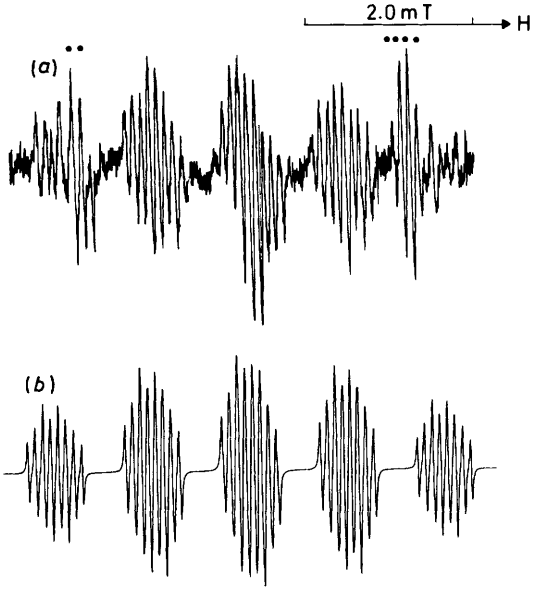


Figure 2. E.s.r. spectra obtained by *in situ* photolysis of a solution containing 2′-deoxycytidine (1 mmol l^{-1}), $K_2S_2O_8$ (6 mmol l^{-1}), and 1% acetone; pH 9; 277 K. (*a*) experimental; some of the signals of the acetonyl radical, $^{\bullet}CH_2COCH_3$, could be identified (●), some of them were obscured by the spectrum of radical (**5**) and (*b*) simulation using the parameters listed in Table 2 for radical (**5**). The same spectrum was obtained from 1-methylcytosine and from IPC

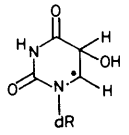
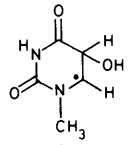
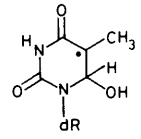
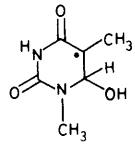
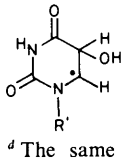
The detection of the 5-OH-6-yl adduct radicals * (**1a**) and (**1b**) is expected on the basis of scavenger experiments on pyrimidine bases [20,21] and on poly(U).[22]

The contribution of sugar radicals to the e.s.r. spectra of the uracilyl nucleosides was negligible. No signals were detected upon reaction of •OH with cytidyl nucleosides and with thymidine.

$SO_4^{-\bullet}$-Induced Radical Formation.—(*a*) *2′-Deoxyribose derivatives.* Reaction of $SO_4^{-\bullet}$ with dU also gave rise to the spectrum of the 5-OH adduct (**1b**), which was of lower intensity than in the experiments with •OH. The spectrum of (**1b**) and that of the OH adduct (**3**), generated from thymidine, resembled those derived from 1-methyluracil [radical (**2**)] and 1-methylthymine [radical (**4**)], respectively.[17] A comparison of the spectral parameters is given in Table 2. The large quartet splittings of 2.23 and 2.26 mT of the radicals derived from thymidine and 1-methylthymine allow unambiguous assignment of the 6-OH-5-yl structures (**3**) and (**4**). In agreement with the results on the *N*(1)-methylated pyrimidines [17] the *g* factor of the 6-OH adduct (**3**) (g = 2.0032) is slightly higher than that of the 5-OH adducts (**1a**) and (**1b**) (g = 2.0028). The β-coupling in the thymidine OH adduct (**3**) is lower than that in the corresponding radical (**4**) formed from 1-methylthymine. This effect is probably related to conformational differences. The signals of radicals (**1b**) and (**3**) showed an intensity maximum at pH ~4 and disappeared at pH > 7. From both substrates, dU and thymidine, in neutral and alkaline solutions spectra of unidentified radicals were detected which might be formed in secondary reactions.

in situ Photolysis of a solution containing dC, peroxodisulphate, and 1% acetone gave rise to five equidistant groups of signals with g = 2.0035 in the pH range 4—9 (Figure 2). The spectral intensity increased at high pH values and upon addition of phosphate dianions. The spectrum was consistent with two similar triplets of 1.175 and 1.16 mT, each of them due either to a nitrogen or to two equivalent protons. The pattern of the individual groups of lines indicated two further small

Table 2. Comparison of spectral parameters of radicals obtained by reaction of $SO_4^{-\bullet}$ with pyrimidine nucleosides at pH 3—7 [radical (**5**) at pH 4—9]

Substrate		Radical	Hyperfine splittings/mT[a] $a(H_\alpha)$	$a(H_\beta)$	a(other)	g[b]
2′-Deoxyuridine[c]	(**1b**)		2.20	1.88	0.28 (1′-H)	2.0028
1-Methyluracil[17]	(**2**)		2.04	1.83	0.09 (1N) 0.048 (1N) 0.09 (1-CH_3)	2.0028
Thymidine[c]	(**3**)		2.23 (α-CH_3)	1.125		2.0032
1-Methylthymine[17]	(**4**)		2.26 (α-CH_3)	1.51	0.058 (1N) 0.011 (1N) 0.037 (6-OH) 0.15 (3-H)	2.0031
2′-Deoxycytidine[d]	(**5**)				1.175[f] 1.16[f] 0.18[f] 0.11[f] 0.09 (1H)	2.0035
IPU[e]	(**11**)		2.13	2.13	0.5 (1′-H)	2.0028

[a] ±0.005 mT. [b] ±0.0001. [c] dR = 2′-deoxyribose. [d] The same spectrum was obtained from IPC and 1-methylcytosine. [e] R′ = 2,3-*O*-isopropylideneribose. [f] Triplets due to one nitrogen or two equivalent protons.

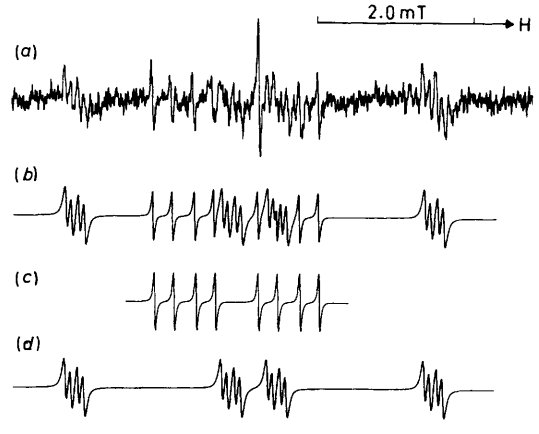


Figure 3. E.s.r. spectra obtained by *in situ* photolysis of a solution containing uridine (2 mmol l^{-1}), $K_2S_2O_8$ (3 mmol l^{-1}), and 1% acetone; pH 4; 277 K. (*a*) experimental, (*b*) overlap of the simulated spectra (*c*) and (*d*) with an intensity ratio of 1:1, (*c*) simulated spectrum of radical (**7**) and (*d*) simulated spectrum of radical (**8**)

triplets of 0.18 and 0.11 mT and a proton coupling of 0.09 mT. Deuteriation with D_2O caused only the small splitting of 0.09 mT to be removed. From the fact that an identical spectrum was generated from 1-methylcytosine it is obvious that we are dealing with a base radical; the spectral parameters suggest a nitrogen-centred species [radical (**5**)]. It is known that anilino radicals[23] show proton couplings (a_{NH}) of 1.2—1.3 mT. Accordingly, the 4-aminyl structure expected upon deprotonation of the cytidyl radical cation[8] should give rise to a large doublet splitting (a_{NH} > 1 mT), which was not found. It may be that the spectrum describes either a tautomeric form of the aminyl radical or a radical produced in secondary reactions.

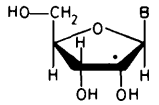
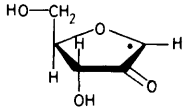
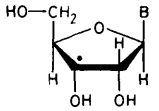
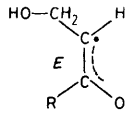
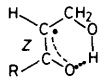
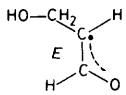
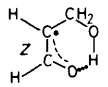
(*b*) *Ribose derivatives.* In contrast with the results obtained with the 2′-deoxyribose derivatives the reactions of $SO_4^{-\bullet}$ with uridine and cytidine yielded signals of sugar radicals.

Structures and spectral parameters of the radicals are given in Table 3. As will be shown in the Discussion section, the e.s.r. spectra originate from heterolytic decay of the 2′-(not detected in the e.s.r. experiment) and the 3′-hydroxyalkyl radicals [(**6**) and (**8**), respectively].

(*i*) Sugar radicals formed by heterolytic decay of the 2′-hydroxyalkyl radical (**6**). Reaction of $SO_4^{-\bullet}$ with uridine and cytidine in the pH range 2—9 yielded an e.s.r. spectrum with g = 2.0049 and three doublet splittings of 1.36, 0.54, and 0.25 mT (see Figures 3 and 4). The g factor is typical of the free spin adjacent to both carbonyl and oxygen functions[24] [radical (**7**)]. Similar spectra have been detected upon reaction of $^{\bullet}OH$ with α-D-glucose,[9] ribose 5-phosphate,[13] inosine,[13] adenosine,[13] and their 5′-monophosphates[13] and upon reaction of $SO_4^{-\bullet}$ with D-ribose.[11]

(*ii*) Sugar radicals formed by heterolytic decay of the 3′-hydroxyalkyl radical (**8**): sugar-ring opening. In acidic solutions (pH 1—4) of uridine, besides the signals due to (**7**), a spectrum

Table 3. Spectral parameters of sugar radicals obtained by reaction of $SO_4^{-\bullet}$ with uridine and cytidine and radicals derived from glycerol, for comparison

Substrate		Radical	pH	Hyperfine splittings/mT[a] $a(H_\alpha)$	$a(H_\beta)$	a(other)	g[b]
Uridine Cytidine	(6)			Not detected			
Uridine Cytidine	(7)		2—9	1.36 (1H)		0.54 (1H) 0.25 (1H)	2.0049
Uridine	(8)		1—4		2.65 (1H) 2.00 (1H)	0.175 (1H) 0.07 (1H)	2.0033
Uridine[c] Cytidine	(9a)		7—11	1.87 (1H)	2.62 (2H)	0.13 (1H)	2.0043
Uridine[c] Cytidine	(9b)		7—11	1.81 (1H)	2.83 (2H)	0.14 (1H)	2.0045
Glycerol[25]	(10a)			1.81 (1H)	2.56 (2H)	0.15 (CHO)	2.0042
Glycerol[25]	(10b)			1.71 (1H)	2.78 (2H)	0.12 (CHO)	2.0045

[a] ±0.01 mT. [b] ±0.0001. [c] R = CH(OH)CH(OH)B, B = uracil or cytosine.

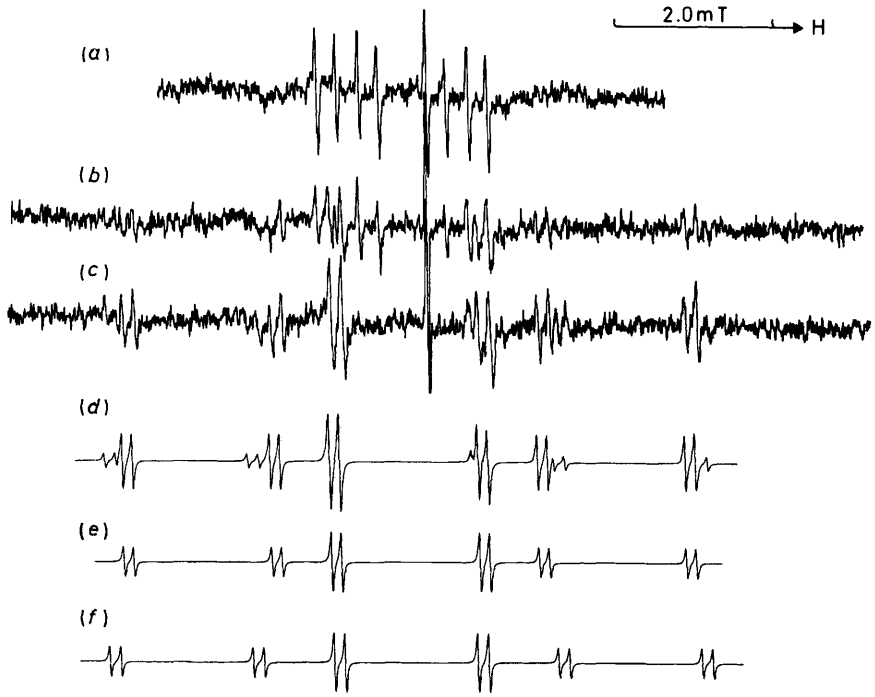
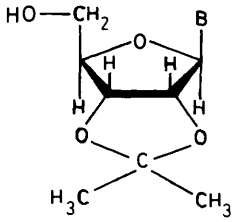


Figure 4. E.s.r. spectra obtained after *in situ* photolysis of a solution containing cytidine (2 mmol l^{-1}), $K_2S_2O_8$ (3 mmol l^{-1}), and 1% acetone. (*a*) pH 4.0, (*b*) pH 7.6, (*c*) pH 9.2; 277 K; (*d*) overlap of (*e*) and (*f*) with an intensity ratio of 1 : 1; (*e*) and (*f*) are the simulated spectra of radicals (**9a**) and (**9b**), respectively

with doublets of 2.00, 2.65, 0.175, and 0.07 mT and g = 2.0033 was identified (Figure 3). These parameters are similar to those of α-hydroxyalkyl radicals formed by H abstraction from six-membered sugar rings.[9,11] Therefore, the spectrum was assigned to the 3′-hydroxyalkyl radical (**8**). In neutral and alkaline solutions of uridine and cytidine two new, strongly overlapping spectra with much wider spread appeared (Figure 4b,c). These are consistent with radicals possessing one α-proton, 2 equivalent β-protons, and one γ-proton [radical (**9**)]. The spectrum is almost identical with that observed by Steenken *et al.*[25] for the radical $CH_2(OH)$–$\dot{C}HCHO$ [radical (**10**)]. It was suggested[25] that the partial double-bond character of the $\dot{C}HCHO$ fragment gives rise to *E* and *Z* isomers. In agreement with ref. 25, in our case, the spectrum with g = 2.0045 shows larger β-couplings and broader lines than that with g = 2.0043. Therefore, it is assigned the *Z* structure (**9b**) whereas that with g = 2.0043 is attributed to the *E* isomer (**9a**). Ring opening of the sugar moieties is responsible for formation of these radicals.

(*iii*) Radicals generated from 2′,3′-*O*-isopropylidene-uridine (IPU) and -cytidine (IPC). In contrast with uridine and cytidine the cyclic ether derivatives IPU and IPC yielded base radicals upon interaction with $SO_4^{-\bullet}$. The 5-OH adduct (**11**) generated



B = Uracil:
2′,3′-*O*-isopropylidene uridine (IPU)
B = cytosine:
2′,3′-*O*-isopropylidene cytidine (IPC)

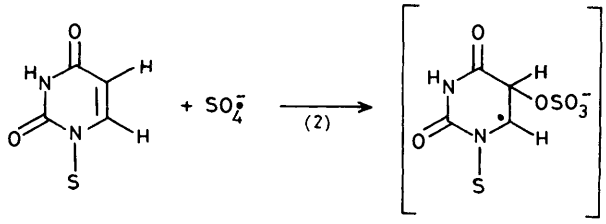
Scheme 3.

from IPU was characterized by g = 2.0028 (see Table 2). The H_α and H_β splittings showed identical values of 2.13 mT and the value of 0.5 mT for a(1′-H) was slightly larger than in the corresponding OH adducts (**1a**) and (**1b**) of uridine and dU. As in the experiments with dU, the intensity maximum was at pH ~4. The spectrum obtained from IPC at pH 4—9 was identical with those of 1-methylcytosine and of dC [radical (**5**), Figure 2].

Discussion

Sites of Reactions.—It is known that $^{\bullet}OH$ radicals add to the base moieties of pyrimidine nucleosides whereas H abstraction from the sugar residues is much less favoured. According to Deeble *et al.*[22] 93% of the $^{\bullet}OH$ radicals add to the uracil residues of poly(U) and 7% only abstract H atoms from the sugar residues.

As far as the site of primary attack of nucleosides by $SO_4^{-\bullet}$ is

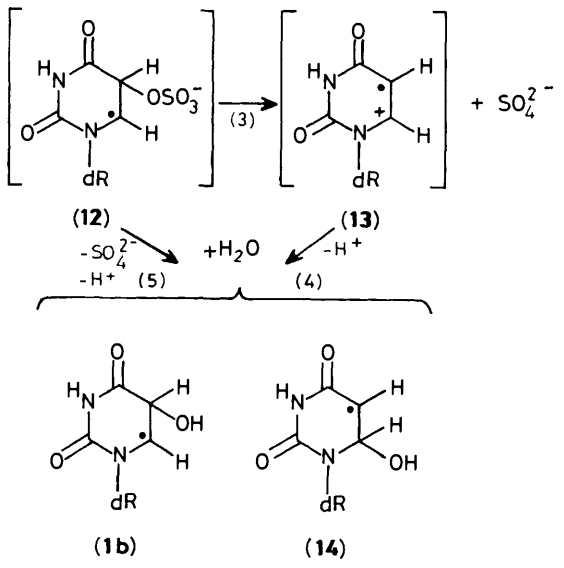


Scheme 4.

concerned we have to rely on kinetic data. The rate constants for reaction of $SO_4^{-\bullet}$ with nucleobases[26,27] ($k \sim 10^9$ l mol^{-1} s^{-1}) are significantly larger than those for abstraction of H atoms from alcohols or ethers[28] ($k_{abs} \sim 10^6$—10^8 l mol^{-1} s^{-1}). Because of the electron-withdrawing effect of the nucleobases even lower rates are expected for H abstraction from the sugar residues of nucleosides. Therefore, we conclude that $SO_4^{-\bullet}$ also reacts predominantly with the nucleobases [e.g. reaction (2)].

Accordingly, in mixtures of 1-methyluracil and ribose 5-phosphate no sugar signals but only the e.s.r. signals of the OH adduct radical of the nucleobase were detected.

The results presented in this paper clearly demonstrate that the reactivities of the sulphate adduct and of the OH-adduct of the nucleobases are widely different. As shown for uridine and dU the 5-OH-6-yl radicals (**1a**) and (**1b**) are stable on the time-scale of the e.s.r. experiment and can be readily detected. The sulphate adducts, on the other hand, are too short-lived to give rise to e.s.r. signals. Their reaction products are base radicals [(**1b**), (**3**), (**5**), and (**11**)] in the case of the deoxyribonucleosides and of the 2′,3′-*O*-isopropylidene derivatives, and sugar radicals (**6**)—(**9**) in the case of the ribonucleosides. Hydrolysis of the sulphate adduct (**12**) of dU may either involve the radical cation (**13**) as intermediate [S_N1 mechanism; reactions (3) and (4)] or or it may occur by an S_N2 mechanism [reaction (5)].

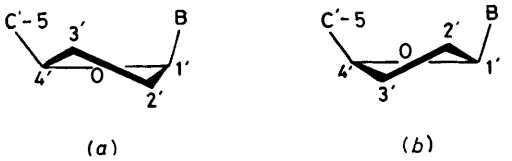


Scheme 5.

According to redox titration with tetranitromethane,[8] the main product upon reaction of $SO_4^{-\bullet}$ with dU is the 5-OH-6-yl radical (**1b**) whereas the isomeric 6-OH-5-yl radical (**14**) is formed as a side product. The same is probably true for IPU. This result is strongly reminiscent of the $SO_4^{-\bullet}$-induced formation of OH adduct radicals from *N*(1)-methylated uracils.[17] Pulse conductivity measurements showed that hydrolysis of (**12**) occurs in less than 1 μs, *i.e.* is equally as fast as the corresonding raction of 1,3-dimethyluracil.[26]

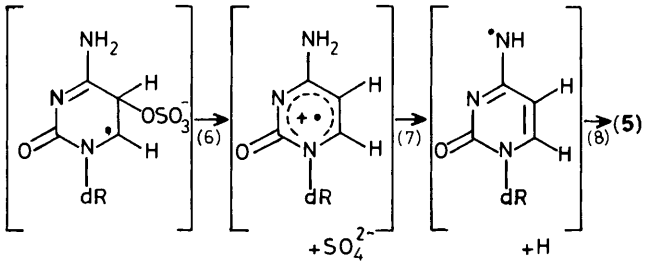
Hydrolysis of the sulphate adduct of thymidine may be described by a similar scheme. However, because of the presence of the 5-methyl group the main hydrolysis product is the 6-OH-5-yl radical (**3**).

In the experiments with dC and IPC we have to take into account formation of the aminyl radical (**17**) either *via* a cationic intermediate (**16**) [reactions (6) and (7)] or by direct H abstraction from the C-4 amino group. As mentioned above, the hyperfine splittings favour either a tautomeric form of (**17**) or a secondary radical formed from (**17**). For final assignment



(a) (b)

Figure 5. Schematic drawings of average sugar ring conformations favoured in (*a*) ribonucleosides and (*b*) in 2′-deoxyribonucleosides or in the 2,3-isopropylidene derivatives IPU and IPC



(15) (16) (17)

Scheme 6.

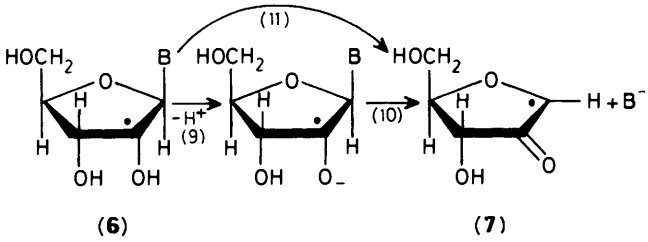
further details will be necessary on the couplings expected for (**17**) and its tautomers.

Reaction of $SO_4^{-\bullet}$ with the ribonucleosides is more complex. If we accept that the primary attack of $SO_4^{-\bullet}$ is directed towards the base moieties we then have to explain how the site of the free spin is transferred from the base radical to the sugar moieties and why this reaction takes place in the ribose- but not in the deoxyribose-derivatives. The most obvious reason for formation of sugar radicals from ribonucleosides seems to originate from the activation of 2′-H. This explains, for example, why $SO_4^{-\bullet}$ abstracts 2′-H from D-ribose but not from 2′-deoxy-D-ribose.[11] Accordingly, reactive base radicals formed with $SO_4^{-\bullet}$ might be able to attack 2′-H from ribose compounds during the formation of radical (**6**). In agreement with the experimental results this pathway is not feasible in the deoxyribose derivatives. The reasons for abstraction of 3′-H in the ribose- but not in the deoxyribose-derivatives are less evident. The electronic environment of 3′-H is similar in the two classes of compounds. It should be mentioned, however, that there are conformational differences which may be relevant to the H-transfer. It is known that the furanose rings are not planar but exist in a variety of non-planar conformations which interconvert by pseudorotation. N.m.r. data[29] show that in ribonucleosides, the furanose rings prefer conformations with C-3′ in an *endo* position relative to C-5′. [A schematic drawing of the average conformation characteristic for ribonucleosides is given in Figure 5(*a*)].

In the deoxyribonucleosides and in the isopropylidene-derivatives IPU and IPC, the conformational equilibrium is shifted towards structures with C-3′ in the *exo* position[29] [Figure 5(*b*)]. From this situation it is conceivable that fast intramolecular H abstraction from C-3′ is more favourable in the ribose- than in the deoxyribose- and isopropylidene-derivatives.

It might be argued that, for the same reasons, H abstraction from C-2′ should be hindered in the ribonucleosies. This was not observed. Possibly the effect of sugar-ring puckering is not strong enough to overcompensate the favourable spatial conditions allowing rapid intramolecular H transfer from C-2′ to the base moiety.

As shown for dU reaction of $SO_4^{-\bullet}$ with the uracil moiety may result in four different base radicals, namely the sulphate adduct, the radical cation, and the two OH adducts. Upon reaction of $^{\bullet}OH$ with uridine, no sugar signals were detected other than the spectrum of the 5-OH-6-yl radical (**1a**) (Figure 1) which means that this radical is not able to abstract H atoms from the ribose residue on the ms time-scale of the e.s.r. experiment. The same is probably true for the isomeric 6-OH-5-yl radical which may be formed in minor amounts with $^{\bullet}OH$ and $SO_4^{-\bullet}$. Both of the remaining species, the sulphate adduct and the base radical cation, are too short-lived to be identified by e.s.r. spectroscopy or even by pulse radiolysis. We would expect the H transfer to the sulphate adduct to be too slow to account for the experimental results which would indicate the radical cation as the reactive intermediate.

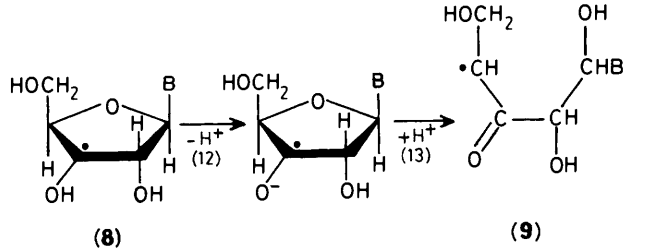


(6) (7)

Scheme 7.

Decay of the Hydroxyalkyl Radicals (**6**) *and* (**8**).—Sugar radical (**7**) is formed by heterolytic elimination of the nucleobase from of the α-hydroxyalkyl radical (**6**) (not detected by e.s.r. spectroscopy).

Sugar-ring opening during the formation of radicals (**9a**) and (**9b**) was detected in the experiments with uridine and cytidine. This can be explained by heterolytic decay of the α-hydroxyalkyl radical (**8**) after deprotonation. The $^{-}$OHCH(B)CH(OH) moiety can be regarded as the leaving group. Rates of



(8) (9)

Scheme 8.

heterolytic decay of α-hydroxyalkyl radicals are strongly influenced by electronic properties of the leaving groups L. A measure for the nucleofugacity is the pK_a(HL) of the conjugate acid.[30] Therefore, the pK_a values of *ca.* 9.5 for N(1)-H of the nucleobases[31] and of 15.7 for H_2O[32] explain why elimination of the nucleobase from radical (**6**) is faster than elimination of the 3′-OH^- group. For the same reasons, in radical (**8**), owing to the electron-withdrawing effect of the nucleobase B, elimination of the $^{-}$O-CH(B)-CH(OH) moiety, *i.e.* ring opening, is faster than that of the OH^- group at C-2′.

Important information on elimination mechanisms can be obtained from pH variation. In pulse radiolysis studies on ethylene glycol, Bansal *et al.*[33] showed that the 1,2-dihydroxyethyl radical is stable at pH 3—7. In alkaline and acidic solutions (pH <3 and >7) dehydration was observed, leading to the formylmethyl radical. Similar behaviour was found in e.s.r. studies on the α-hydroxyalkyl radicals of six-membered sugar rings. These are stabilized at pH 4, whereas at pH 1 cyclic carbonyl-conjugated radicals are formed by dehydration[9–13] and at pH > 7 opening of the sugar rings at the O—C(1′) bond and subsequent formation of semidiones was observed.[14] Ring opening of α-hydroxyalkyl radicals derived from ribose and glucose in alkaline solutions was also observed by pulse radiolysis.[34] Fragmentation of the 3′-hydroxyalkyl radical (**8**) at pH > 7 is in line with these reports. Heterolytic cleavage of the C(4′)–oxygen bond [reaction (13)] occurs following

deprotonation of the 3′-OH group [reaction (12)]. It is interesting to note that, in contrast to the results on the six-membered sugar rings, acid-catalysed water elimination from radical (**8**) was not observed at pH 1. This might be due to the electron-withdrawing effect of the nucleobase resulting in lower basicity of the 2′-OH group and therefore in a decrease in the rate of protonation and in a delay of acid-catalysed dehydration.

In contrast to ring opening, elimination of the base moiety from radical (**6**) was independent of pH values in the region 2—9. This is in agreement with results by Fitchett *et al.*[13] who reported rapid fragmentation of the same type of radical derived from ribose 5-phosphate, adenosine, and inosine at pH 2—8. Reasons for the rapidity of the elimination might arise from strain of the ring or overlap of the β-C–O bond and the orbital of the unpaired electron or both. Presumably, in neutral and alkaline solutions fragmentation of radical (**6**) takes place on the deprotonated form [reaction (10)] whereas in acidic solutions the reaction may occur directly at the α-hydroxyalkyl radical stage [reaction (11)].

Comparison with Spin-trapping Results.—Reactions of ·OH with nucleosides and nucleotides of the pyrimidine bases have been extensively studied by spin-trapping with organic nitroso compounds.[35–37] In agreement with our results, the main products were OH adducts of the alkenic double bonds of the bases. In some cases, H abstraction from C-1′ and C-5′ of the carbohydrate residues was observed by spin-trapping. These radicals were not detected in the present study, possibly owing to their low abundance.

Only few data are available on spin-trapping of the radicals generated by interaction of $SO_4^{-\bullet}$ with nucleosides.[38] In contrast with our studies, the radicals derived from uridine and dC were assigned as C-5′ radicals. These disagreements between the spin-trapping method and the direct detection of radicals might be the result of differences in the yields of formation of nitroxide radicals and to differences in their stability.

Experimental

Chemicals.—All nucleosides with exception of 2′-deoxyuridine (EGA, gold label, >99%) and cytidine (Boehringer, Mannheim) were from Sigma. The purity was checked by h.p.l.c. and found to be >98% for all compounds under investigation. Boric acid, p.a. and H_2O_2 (Perhydrol, p.a.) were from Merck, Darmstadt. All compounds were used without further purification.

E.s.r. Measurements.—E.s.r. spectra were recorded under conditions of *in situ* photolysis as described by Behrens *et al.*[17] $SO_4^{-\bullet}$ was generated from $K_2S_2O_8$ (1—3 mmol l^{-1}) and ·OH from H_2O_2 (1—50 mmol l^{-1}). The solutions were gassed with argon (99.997%). Signal intensities of the spectra detected upon photolysis of peroxodisulphate were generally increased by addition of 1—2% acetone. Control experiments without acetone were performed to ensure that no additional signals were induced by the sensitizer. The cytidyl derivatives (dC, IPC, and 1-methylcytosine) were the only substrates under investigation which showed no e.s.r. signals in the absence of acetone. The pH values were adjusted using $HClO_4$ or NaOH. In the experiments with $K_2S_2O_8$, borate (10 mmol l^{-1}) was used to buffer the solutions in the pH region 7—10.

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