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Reaction of $SO_4^{-\bullet}$ with Methylated Uracils. An Electron Spin Resonance Study in Aqueous Solution

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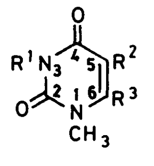
Radicals obtained by reaction of photolytically generated $SO_4^{-\bullet}$ with 1-methyluracil (**1**), 1,3-dimethyluracil (**2**), 1-methylthymine (**3**), 1,3-dimethylthymine (**4**), and 1,3,6-trimethyluracil (**5**) were studied by e.s.r. spectroscopy in aqueous solutions. *In situ* photolysis of neutral and acidic solutions containing (**1**) and persulphate in low concentrations (3mM) resulted in the e.s.r. spectrum of the C(5)-OH adduct radical. E.s.r. spectra obtained from (**1**) and (**2**) with high persulphate concentrations (30mM) were assigned to the C(6)-OH adduct radicals. It is proposed that this change in radical population is due to a persulphate-induced chain reaction which results in selective oxidation of the C(5)-OH radicals and simultaneous accumulation of the C(6)-OH adducts generated in side reactions. When the persulphate concentration was raised to 60mM, 5-oxo-6-yl radicals were formed in secondary processes from (**1**) and (**2**) besides the OH adducts. In contrast to these results the thymines (**3**) and (**4**) yielded only C(6)-OH adduct radicals. Addition of phosphate dianions to the photolysis solutions containing persulphate and the pyrimidine bases (**1**)—(**4**) resulted in the e.s.r. spectra of the C(6)-phosphate adduct radicals (pH 6.5—9.5). Identical spectra were obtained by reaction of (**1**)—(**4**) with $HPO_4^{-\bullet}$ radical anions generated by photolysis of $Li_4P_2O_8$. The results of the experiments with 1,3,6-trimethyluracil (**5**) were completely different. First, reaction of $SO_4^{-\bullet}$ with (**5**), even at low persulphate concentration (3mM), did not lead to an OH adduct but to a 5-oxo-6-yl secondary radical. Secondly it was not possible to generate phosphate adduct radicals from (**5**) either with a mixture of persulphate and phosphate or with peroxodiphosphate. The spectral parameters of the radicals derived from (**1**)—(**5**) are given and possible pathways for the $SO_4^{-\bullet}$-induced radical formation are discussed.

Radical intermediates play a key role in the mechanisms of radiolytically and, to some extent, of the photochemically induced damage in nucleic acids. Therefore, the radical chemistry of pyrimidine bases as model compounds has attracted considerable attention.[1] Numerous reports have been published on e.s.r. spectroscopy of uracil and its derivatives in the solid state[2–5] and a series of N(1)-substituted uracils and thymines has been investigated in aqueous acidic glasses.[6] E.s.r. experiments in aqueous solutions showed that ˙OH radicals are added to the olefinic double bond of pyrimidine bases.[7–9] In alkaline solutions the reducing C(5)-OH adduct of uracil is converted into an oxidizing radical[10] with high spin density[11] at N(1) and C(5). The same species was formed by reaction of $SO_4^{-\bullet}$ with uracil and subsequent deprotonation[11,12] at N(1).

In nucleosides and nucleotides, deprotonation at N(1) is not possible which means that uracil is a rather poor model for the radical chemistry of nucleic acids. A much better approach is expected from a series of N(1)-methylated pyrimidine bases (1)—(5).



(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = CH_3, R^2 = R^3 = H$
(3) $R^1 = R^3 = H, R^2 = CH_3$
(4) $R^1 = R^2 = CH_3, R^3 = H$
(5) $R^1 = R^3 = CH_3, R^2 = H$

Radicals generated from (1)—(3) with ˙OH in aqueous solution could be spin-trapped by 2-methyl-2-nitrosopropane[13] whereas direct e.s.r. detection was not possible. However, $SO_4^{-\bullet}$ anion radicals generated by *in situ* photolysis of $K_2S_2O_8$ yielded rather intense spectra which were assigned to OH adduct radicals or to secondary radicals.

Addition of phosphate dianions to the photolysis solutions of persulphate and the pyrimidine bases (1)—(4) yielded the e.s.r. spectra of phosphate adduct radicals, a result which yielded valuable information on the $SO_4^{-\bullet}$-induced formation of radicals derived from the title compounds and which is reminiscent of the scavenging of 1,1-dialkoxyalkene radical cations[14] by HPO_4^{2-}. The purpose of this paper was to present the $SO_4^{-\bullet}$-induced e.s.r. spectra of the title compounds in aqueous solutions and to discuss the chemical pathways of radical formation.

Results

(I) *Radicals generated from* (1) *and* (2) *by Reaction with* $SO_4^{-\bullet}$.—The e.s.r. spectra obtained by *in situ* photolysis of solutions containing (1) or (2) and $K_2S_2O_8$ depended on persulphate concentration. With 30mM persulphate the intensity reached an optimum and all the signals observed could be assigned to a single radical. In Figure 1 we show the e.s.r. spectrum obtained from (1) in presence of 30 mM-$K_2S_2O_8$ as an example. It consists of four groups of lines two of which are strongly overlapping. This is consistent with the idea that we deal with a radical of the ˙CH-CHR type with two large proton couplings of similar value (1.82 and 2.07 mT). The corresponding spectrum obtained from (2) was of the same type but the structure of the individual groups of lines was more complex due to an additional quartet splitting [a(3CH_3) 0.255 mT]. Values of the spectral parameters are listed in the Table

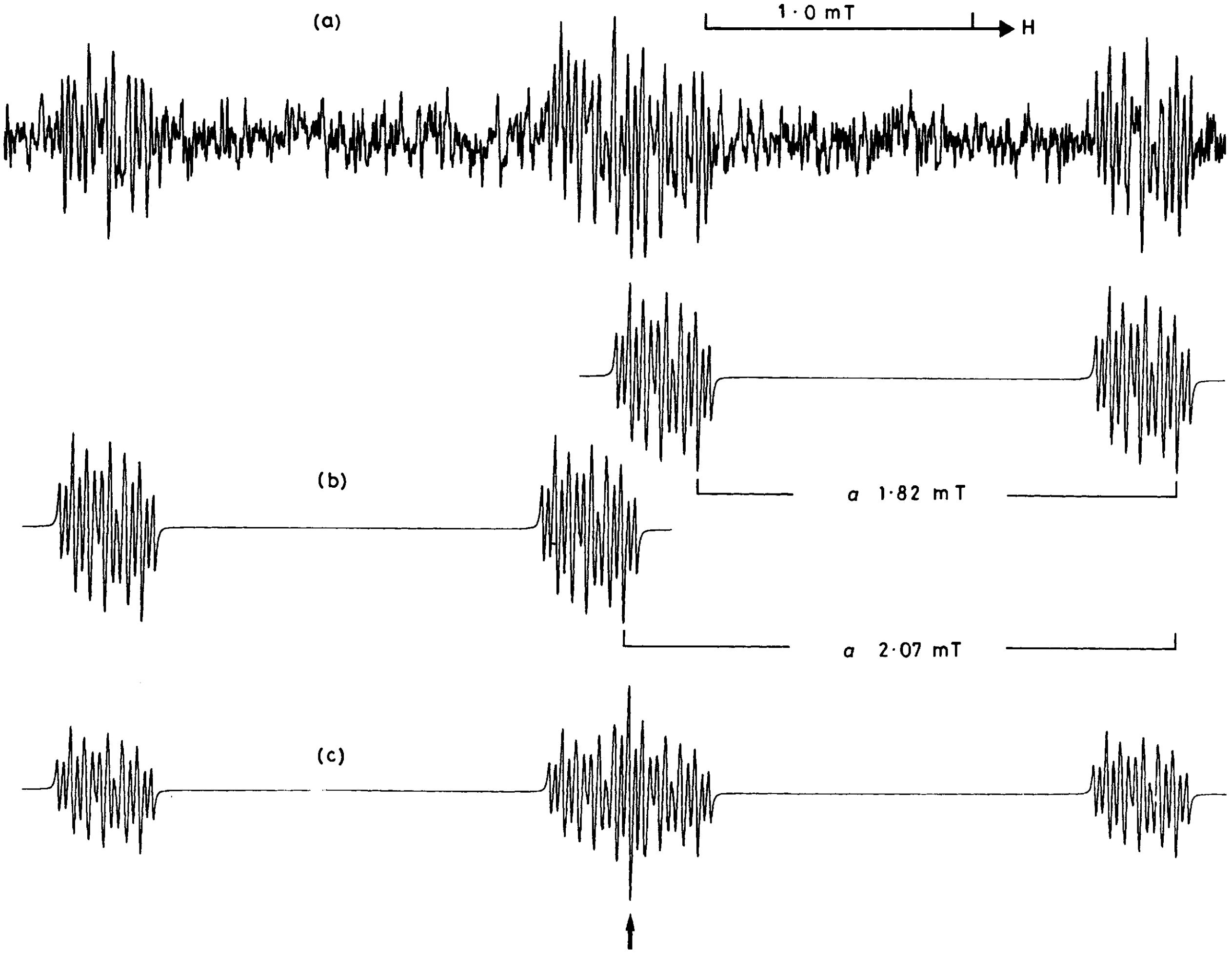


Figure 1. E.s.r. spectrum (first derivative) of the C(6)-OH radical (**6**) obtained by *in situ* photolysis of a solution containing 1-methyluracil (**1**) (2mM) and $K_2S_2O_8$ (30mM), pH 7.5, buffered with 10mM-sodium borate at 4 °C. (a) Experimental. (b) Subspectra simulated with *a*(1N) 0.024, *a*(3N) 0.056, *a*(3H) 0.143, *a*(OH) 0.050, and *a*(β-H) 1.82 mT. Due to *a*(α-H) 2.07 mT the central part of spectrum (a) consists of an overlap of two groups of signals as shown by the addition of the two subspectra in (c). The vertical arrow indicates the centre of the spectrum

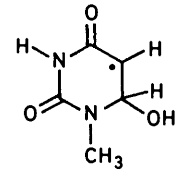
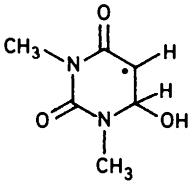
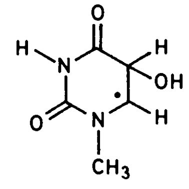
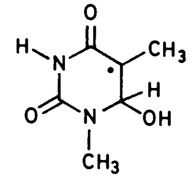
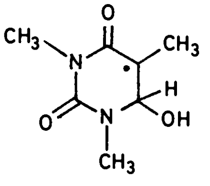
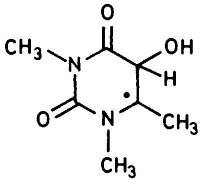
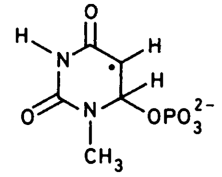
[radicals (**6**) and (**7**)]. The OH couplings of 0.050 and 0.095 mT, respectively, were clearly resolved. The assignment of *a*(3H) 0.143 mT in (**6**) and of the OH couplings in (**6**) and (**7**) was confirmed by using D_2O as solvent. Deuteriation yielded 1:1:1 triplets with reduced couplings of 0.03 mT for *a*(3D) in (**6**) (Figure 2b) and 0.014 mT for *a*(OD) in (**7**) according to the lower gyromagnetic ratio of D compared with that of H (γ_D/γ_H 0.153). The OD coupling in (**6**) was too small to be resolved.

On reducing the persulphate concentration the spectral intensity decreased and contributions from more than one radical were detected in the spectra. In the case of (**2**), due to the low signal:noise ratio and due to the complexity of the spectrum satisfactory analysis was not possible. However, in the experiments with (**1**) e.s.r. signals of reasonable intensity were observed. In Figure 2a the high-field wing of the spectrum obtained from (**1**) with 3mM-$K_2S_2O_8$ in D_2O is shown. The intensities of the simulated spectrum (lower trace) did not fit perfectly due to perturbation by additional signals some of which could be assigned to radical (**6**). Essentially the same spectrum was recorded in H_2O although the contribution of perturbing signals was higher than in D_2O. The spectrum is assigned to the C(5)-OH adduct radical (**8**) (Table). It is reasonable to assume slightly higher *g* factors for 5-yl as compared with 6-yl radicals of pyrimidine derivatives because of delocalization of the unpaired electron to an oxygen atom in the 5-yl structures. In fact, experimental evidence for higher *g* factors of 5-yl radicals has been provided by Nicolau *et al.*[7] on the basis of model studies. In agreement with this idea *g* factors of 2.0032 have been reported for 5-yl radicals of uracil,[8] thymine,[7] thymidine,[15] and iso-orotic acid[8] whereas the 6-yl radicals derived from these substrates had *g* factors of 2.0028.[7,8,15] The hyperfine splittings of 0.143 mT and 0.255 mT due to *a*(3H) and *a*($3CH_3$) in (**6**) and (**7**), respectively, are in good agreement with the 5-yl structures whereas for *a*(3H) in the 6-yl radicals values close to zero are expected.[8]

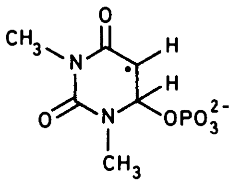
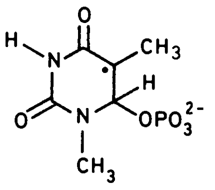
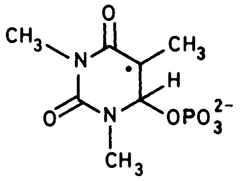
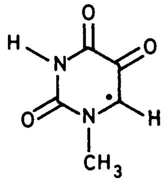
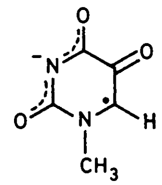
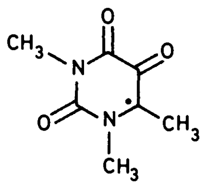
The spectral intensities in the experiments with (**1**) and (**2**) were similar at pH 4 and 7.5 but decreased drastically in the alkaline region.

(II) *Radicals generated by Reaction of* $SO_4^{-\bullet}$ *with* (**3**) *and* (**4**) *in Aqueous Solution.*—E.s.r. spectra obtained by reaction of $SO_4^{-\bullet}$ with the thymine derivatives (**3**) and (**4**) were independent of persulphate concentrations. They consisted of eight groups of lines with an intensity ratio of 1:1:3:3:3:3:1:1 due to a large methyl and a large proton coupling. The values of 2.26 and 2.24 mT prove the 5-yl structures of radicals (**9**) and (**10**). This is in agreement with results reported for the radicals obtained by reaction of Fenton's reagent with thymine[7] and thymidine.[15] The small couplings were derived from the analysis of expanded spectra of individual groups of lines. As an example the high-

Structures and e.s.r. parameters of the radicals detected in the reaction of substrates (1)—(4) with $SO_4^{-\cdot}$ and of (5) with ·OH

		Hyperfine splittings (mT)[a]				
Substrate	Radical	*a*(α-H)	*a*(β-H)	*a*(N)	*a*(other)	*g*[b]
(1)[c]	 **(6)**	2.07	1.82	0.056 0.024	0.143 (3-H) 0.05 (6-OH)	2.0032
(2)[c]	 **(7)**	2.06	1.83	0.05 0.02	0.255 (3-CH_3) 0.095 (6-OH)	2.0032
(1)[d]	 **(8)**	2.04	1.83	0.09 0.048	0.09 (1-CH_3)	2.0028
(3)	 **(9)**	2.26[e]	1.51	0.058 0.011	0.150 (3-H) 0.037 (6-OH)	2.0031
(4)	 **(10)**	2.24[e]	1.48	0.09 0.024	0.200 (3-CH_3) 0.026 (6-OH)	2.0032
(5)	 **(11)**	2.06[e]	1.16	0.07 0.025	0.205 (1-CH_3)	2.0029
(1)	 **(12)**	2.05	1.56	0.051 0.025	0.138 (3-H) 0.13 (6-P)	2.0032

(*continued*)

		Hyperfine splittings (mT)[a]				
Substrate	Radical	*a*(α-H)	*a*(β-H)	*a*(N)	*a*(other)	g^b
(2)	 **(13)**	2.05	1.58	0.026 0.012	0.204 (3-CH_3) 0.128 (6-P)	2.0032
(3)	 **(14)**	2.29[e]	1.18	0.045 0.045	0.140 (3-H) 0.038 (6-P)	2.0032
(4)	 **(15)**	2.27[e]	1.14	0.08 0.023	0.186 (3-CH_3) 0.024 (6-P)	2.0032
(1)[f]	 **(16a)**	0.922		0.249 0.073	0.019 (3-H) 0.328 (1-CH_3)	2.0048
(1)[g]	 **(16b)**	0.835		0.300 0.065	0.360 (1-CH_3)	2.0047
(5)	 **(17)**	0.100		0.22 0.09	0.189 (1-CH_3)	2.0046

[a] ±0.005 mT. [b] ±0.0001. [c] 30mM-Persulphate. [d] 3mM-Persulphate. [e] α-CH_3 coupling. [f] pH 3—4. [g] pH 8—10.

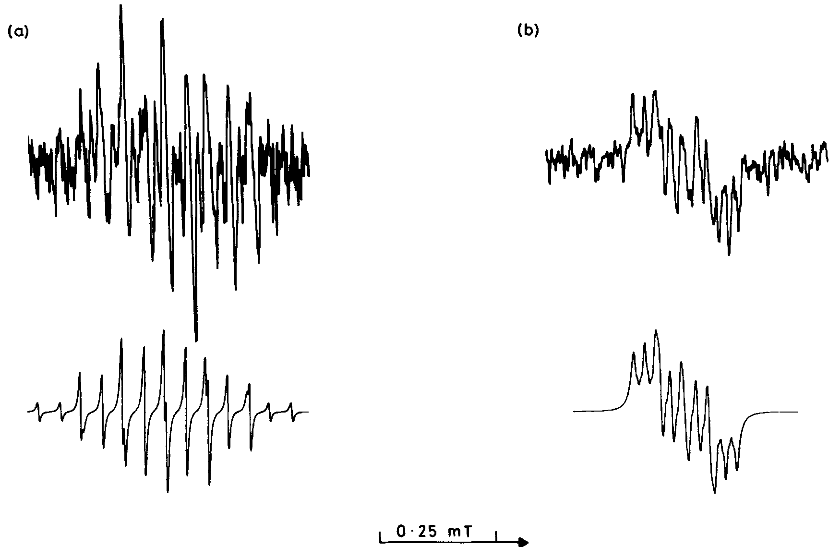


Figure 2. (a) High-field wing of the e.s.r. spectrum of the C(5)-OH radical (**8**) obtained by *in situ* photolysis of a solution containing 2mM-1-methyluracil (**1**), 3mM-$K_2S_2O_8$, and 1% acetone in D_2O; pD 3, *T* 277 K. (b) High-field wing of the e.s.r. spectrum of the C(6)-OH adduct radical (**6**) obtained by *in situ* photolysis of a solution containing 2mM-1-methyluracil (**1**) and 30mM-$K_2S_2O_8$ in D_2O; pD 3, *T* 277 K. Upper traces: experimental spectra. Lower traces: simulated spectra [hyperfine splittings are listed in Table; a value of 0.03 mT was used for *a*(3D)]

field group of the spectrum derived from (**3**) is shown in Figure 3. Assignment of *a*(3H) and *a*(OH) in (**9**) was confirmed by using D_2O as solvent. A doublet splitting of 0.026 mT in the rather complex and weak spectrum of (**10**) was tentatively assigned to the OH coupling.

The intensity of the spectra of both radicals (**9**) and (**10**) was highest at pH 4 and decreased in the neutral range. No signals were obtained at pH 9. Whereas addition of acetone in the experiments with (**4**) did not give rise to new signals this was not true for (**3**). At pH 7 in the presence of 1% acetone an additional spectrum was observed [*g* 2.0034, *a*(CH_3) 2.06, *a*(H) 0.19, *a*(H) 0.66, *a*(N) 0.04, *a*(N) 0.015 mT]. The structure and origin of this radical are not known.

Radical (**11**) was obtained by photolysis of a solution containing 1,3,6-trimethyluracil (**5**) and H_2O_2. The methyl coupling of 2.06 mT proves the 6-yl structure. Reaction of $SO_4^{-\bullet}$ with (**5**) did not yield an OH-adduct radical.

(III) *Radicals generated from* (**1**)—(**4**) *with* $SO_4^{-\bullet}$ *in Presence of Phosphate Dianions.*—The e.s.r. spectra obtained from (**1**) (Figure 4) and from (**2**) by reaction with $SO_4^{-\bullet}$ in the presence of phosphate dianions were composed of four symmetrically arrayed groups of lines. From the separation between these groups two large proton couplings were obtained. The smaller couplings were evaluated from the analysis of individual groups of lines. We assign the spectra to phosphate adduct radicals (**12**) and (**13**). The ^{31}P couplings were clearly resolved, showing values of 0.130 mT for (**12**) and 0.128 mT for (**13**).

From the spectroscopic data for radicals (**12**) and (**13**) alone it would not be possible to decide whether the free spin is located at C(5) of C(6). However, comparison of the parameter sets for radicals (**12**) and (**13**) with those of the other radicals described in this paper and with those of adduct radicals in refs. 7, 8, and 15 strongly supports the structures proposed.

The e.s.r. spectra generated by reaction of $SO_4^{-\bullet}$ with (**3**) and (**4**) in presence of HPO_4^{2-} are assigned to C(6)-phosphate adduct radicals (**14**) and (**15**). They consist of a doublet of quartets [*a*($5CH_3$) 2.29 mT for (**14**) and 2.27 mT for (**15**), *a*(6H) 1.18 mT for (**14**) and 1.14 mT for (**15**)]. Assignment of the H(3) coupling of 0.140 mT in (**14**) was achieved by deuteriation as described above.

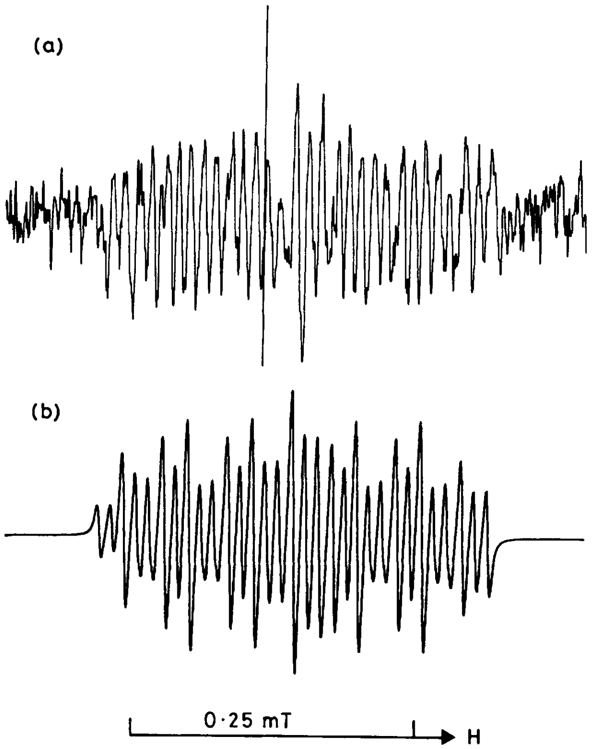


Figure 3. (a) E.s.r. spectra (one of the inner groups of lines) of the C(6)-OH adduct radical (**9**) obtained from a solution containing 3mM-$K_2S_2O_8$, 1% acetone, and 3mM-1-methylthymine (**3**) at pH 4, *T* 277 K. (b) Simulation with the couplings listed in the Table for radical (**9**). A second-order correction for the three methyl protons has been included

The large quartet splittings in radicals (**14**) and (**15**) clearly indicate that we deal with C(6)-phosphate adduct radicals. The *g* factors of 2.0032 and the values of *a*(3H) and *a*($3CH_3$) are in agreement with these structures.

All phosphate adduct radicals are observed at pH 6.5—9.5 with maximal intensity at pH 7.25. The radical concentration

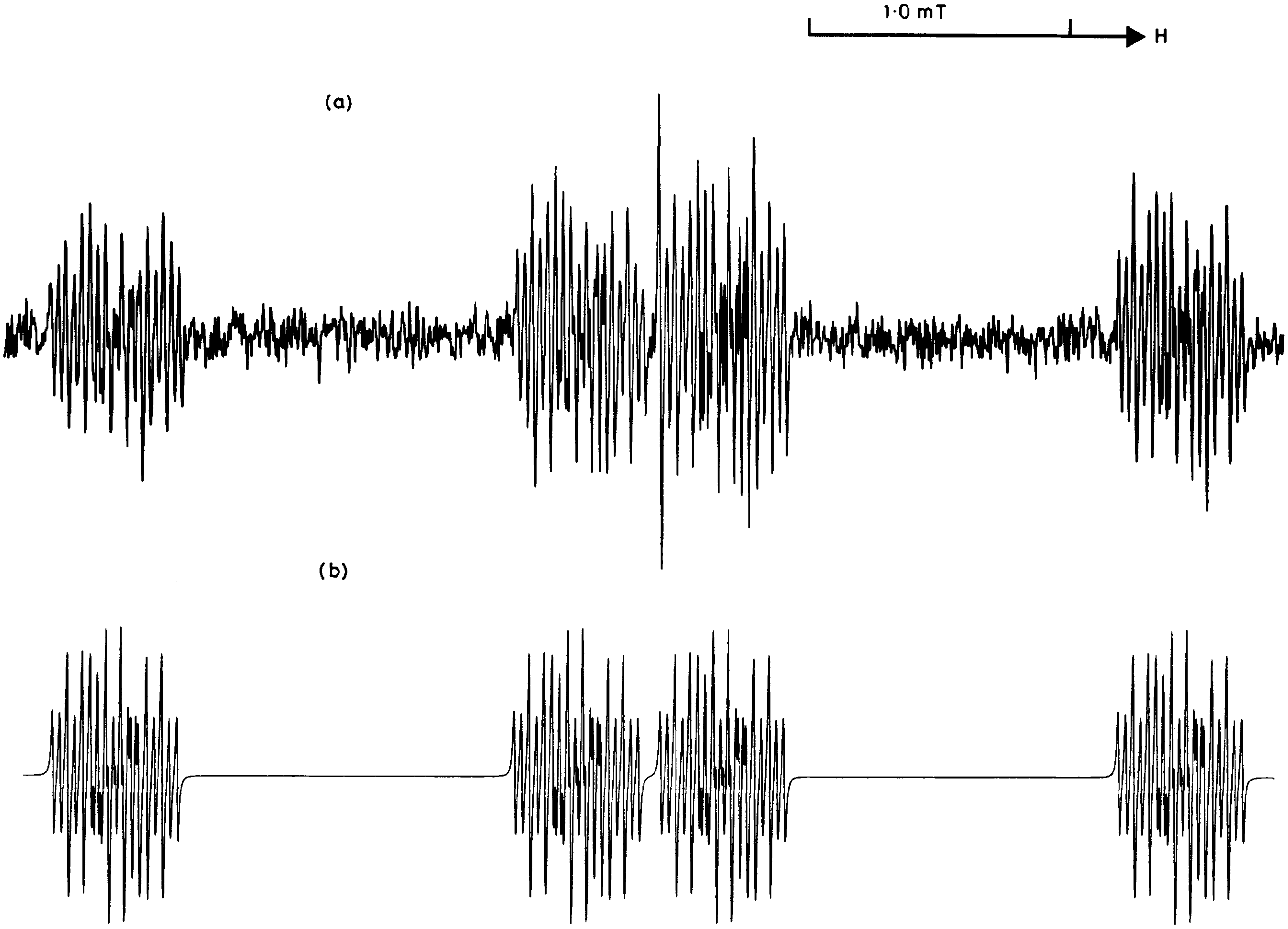


Figure 4. E.s.r. spectrum of the C(6)-phosphate adduct radical (**12**) obtained by *in situ* photolysis of a solution containing 2mM-1-methyluracil (**1**), 30mM-$K_2S_2O_8$, and 30mM-KH_2PO_4 at pH 7.5, *T* 277 K. (a) Experimental. (b) Simulated with the couplings listed in the Table for radical (**12**)

increases with increasing amount of phosphate ions reaching a plateau at *ca.* 20mM-phosphate, with a half-value of saturating intensity attained at *ca.* 1.5mM-phosphate for formation of (**12**)—(**14**) and 0.8mM for (**15**).

In situ photolysis of $Li_4P_2O_8$ in presence of substrates (**1**)—(**4**) at pH 6.5—11.0 yielded spectra identical with those obtained from mixtures of persulphate and phosphate dianions.

(IV) *Secondary Radicals.*—On increasing the persulphate concentration to 60mM or on adding 1—2% acetone to the photolysis solutions the spectrum of an additional radical was observed in the experiments with (**1**). Its intensity decreased upon increasing the flow rate and increased upon further photoirradiation of the sample. This implies that the spectrum is obtained from products of secondary processes. The spectral parameters were similar to those of the 5-oxo-6-yl radical detected by Neta[16,*] in radiolysis experiments of 5-halogenouracils. This is strong evidence for structure (**16a**). Deprotonation of (**16a**) in alkaline solutions led to a slight change in spectral parameters. The spectrum of (**16b**) at pH 8.8 is shown in Figure 5. Evaluation of the spectral parameters of the corresponding spectrum obtained from (**2**) was not possible because of intensity problems. However, an intense spectrum of similar type was obtained by photolysing solutions of (**5**) and persulphate (3—30mM) [radical (**17**)]. The e.s.r. spectrum of a secondary radical with different structure was detected upon photolysing (**2**) and 60mM-persulphate at pH 7.5. It was characterized by *g* 2.0038, a large doublet splitting of 1.95, a small one of 0.013, and two nitrogen couplings of 0.115 and 0.105 mT. The spectrum might be due to a radical formed on ring opening with the proposed structure $NH(CH_3)$-CO-$N(CH_3)$-CO-$^\bullet$CH-CHO.

(V) *Radicals generated from* (**5**).—The results of the experiments with (**5**) can be summarized as follows.

(i) Reaction of OH with (**5**) resulted in an e.s.r. spectrum of reasonable signal : noise ratio consisting of eight groups of lines with an intensity ratio of 1:1:3:3:3:3:1:1. The large value of $a(6CH_3)$ of 2.06 mT and the low *g* factor of 2.0029 allows unambiguous assignment to the C(5)-OH adduct radical (**11**).

(ii) Photolysis of solutions containing (**5**) and persulphate did not yield the OH adduct radical but the secondary radical (**17**).

(iii) No phosphate adduct radicals from substrate (**5**) could be detected. In mixtures of persulphate, phosphate dianions, and (**5**) the secondary radical (**17**) was formed and photolysis of $Li_4P_2O_8$ in presence of (**5**) yielded the C(5)-OH adduct radical (**11**).

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[*] The spectral parameters for the 5-oxo-6-yl radical obtained by reaction of $^\bullet$OH with 5-halogenouracils at pH 2—3 are: *g* 2.00482, $a(\alpha\text{-H})$ 0.967, $a(1N)$ 0.188, $a(3N)$ 0.077, $a(1H)$ 0.341, $a(3H)$ 0.019 mT.

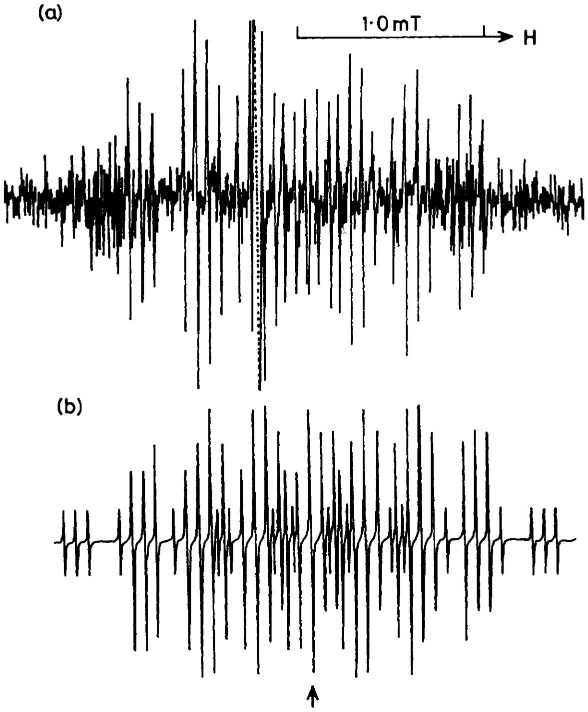


Figure 5. E.s.r. spectra of the secondary radical (**16b**) obtained by photolysing a solution containing 3mM-1-methyluracil (**1**), 3mM-$K_2S_2O_8$, and 1% acetone, pH 8.8, *T* 277 K. (a) Experimental. (b) Simulated with the couplings listed in the Table for radical (**16b**). The vertical arrow indicates the centre of the spectrum

Discussion

The radicals investigated in this paper were generated by $SO_4^{-\bullet}$ and in some cases by $HPO_4^{-\bullet}$ (p*K* values of the phosphate radical[17] are 5.7 and 8.9). Only from substrate (**5**) was an e.s.r. spectrum of reasonable intensity obtained, by attack of $^{\bullet}OH$ radicals. Reactions of sulphate and phosphate radicals with organic and inorganic compounds in aqueous solutions have been studied by pulse radiolysis and by e.s.r. spectroscopy.[17–19] Both radical anions have been shown to add to unsaturated aliphatic carboxylic acids, giving rise to the sulphate[20] and phosphate adducts.[18] Aromatic compounds are oxidized in a one-electron-transfer process[21] by $SO_4^{-\bullet}$ but not by $HPO_4^{-\bullet}$.

A spectacular result demonstrating the high oxidizing power of $SO_4^{-\bullet}$ was revealed by the e.s.r. spectra of radical zwitterions from methoxybenzoic acids[22] and of radical cations[23] from methoxybenzenes.

Pyrimidine bases are not truly aromatic because of the depletion of the π-electron system by the electron-withdrawing nitrogen atoms.[24] This is seen, for example, in the rapid bromination in water.[25] Therefore, in the primary step the electrophilic $SO_4^{-\bullet}$ radical is considered to add to the olefinic double bond [reaction (1)] forming the sulphate adduct (**18**) rather than the radical cation (**19**) by one-electron oxidation.

(I) *Formation of* OH *Adducts from* (**1**) *and* (**2**).—(a) *Experiments with low persulphate concentration.* In substrates (**1**) and (**2**) $SO_4^{-\bullet}$ adds to C(5), the location of highest π-electron density.[26] As indicated in Scheme 1 there are several ways of forming OH adducts from the C(5)-sulphate adduct radical (**18a**).

(i) The most obvious one is hydrolysis [S_N2 reaction (3a)] of the carbon–sulphur bond yielding the C(5)-OH radical.

(ii) A second, more complex pathway is based upon the observation of rapid intramolecular 1,2-migration of electronegative β-substituents such as chlorine,[27] the acetoxy group,[28] or the sulphate group[29] in radicals. This suggests that the thermodynamically more stable C(6)-sulphate adduct radical (**18b**) is formed *via* 1,2-shift of the sulphate group [reaction (2)]. The leaving sulphate directs the attack of water to position C(5) of (**18b**) [reaction (4b)] yielding the C(5)-OH adduct radical.

(iii) The C(6)-sulphate radical (**18b**) may hydrolyse in reaction (3b) to form the C(6)-OH radical.

(iv) By sulphate elimination [reaction (5)] the free radical cation (**19**) may be formed. Taking into account electrostatic interactions only, nucleophilic addition of H_2O to the base radical cation should take place at C(6), the site of highest positive charge[30] [reaction (6b)]. However, as was pointed out by Schuchmann *et al.*[30] not only charge distribution but also the shape of the HOMO might be a decisive factor for the course of hydrolysis of the radical cation accounting for formation of the C(5)-OH adduct in reaction (6a).

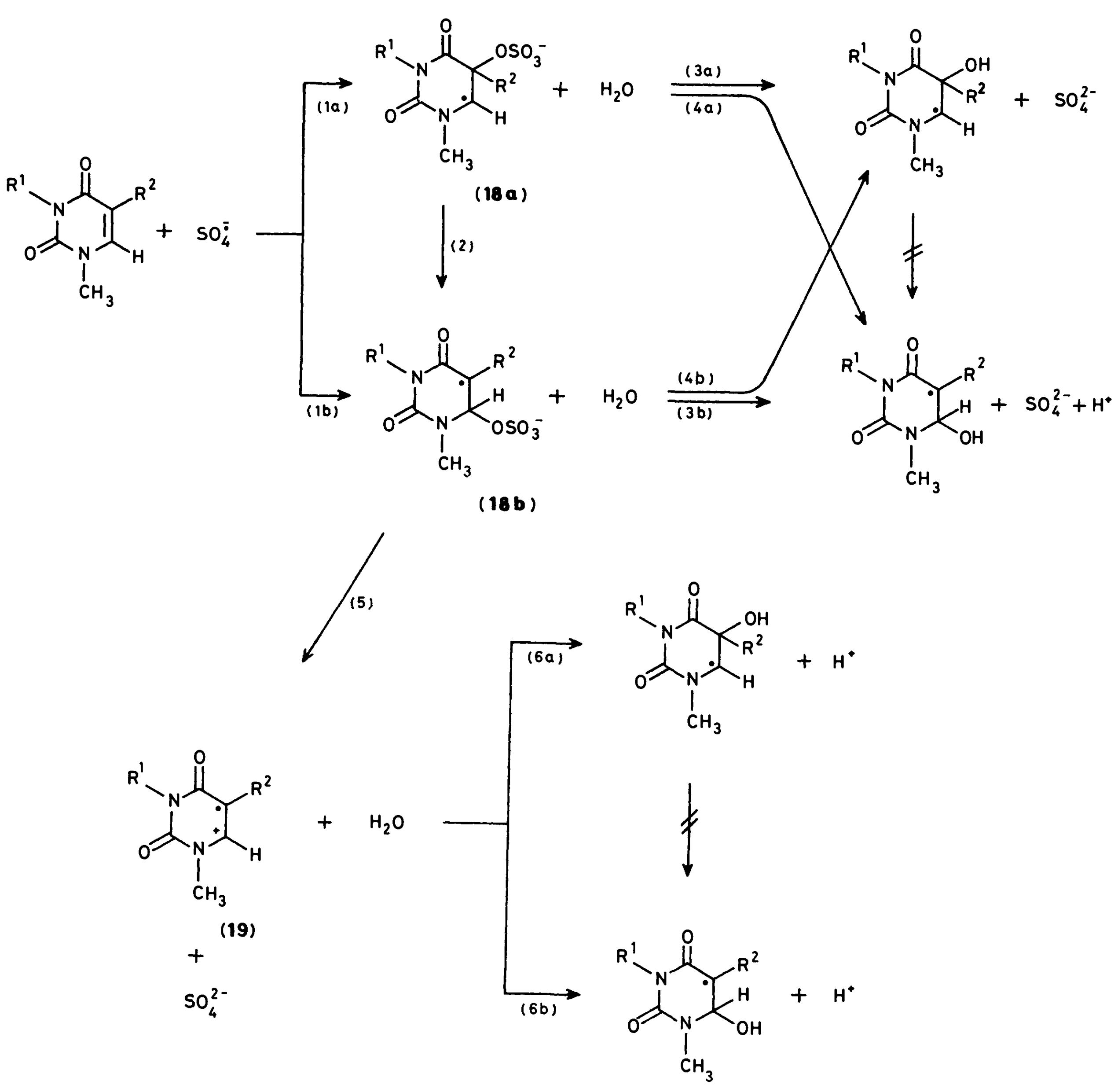
Our results indicate that reactions (3b) and (6b) are side reactions. The lifetimes $\tau_{\frac{1}{2}}$ of the intermediate states, *i.e.* either of the sulphate adducts (**18**) or of the radical cation (**19**), have been determined by pulse radiolysis or by laser flash experiments in aqueous solutions. Values of < 1 μs[30] and *ca.* 3 μs[31] for $\tau_{\frac{1}{2}}$ were obtained in the experiments with (**2**) and (**4**), respectively. Obviously, the intermediates are too short-lived to be characterized by e.s.r. spectroscopy. At first sight one might conclude from the detection of the uracil radical cation in sulphuric acid glasses[6] at 77 K that reactions in the aqueous phase involve the radical cation also as an intermediate. However, the sulphate adduct may be rapidly attacked and hydrolysed by the mobile water molecules whereas the glass matrix is rather inert allowing elimination of sulphate and thus formation of the radical cation.

(b) *Experiments with high persulphate concentrations.* Results of γ-radiolysis of (**1**) and (**2**) in presence of $K_2S_2O_8$ indicated the formation of glycols in a chain reaction.[30] The peculiarities of this chain reaction, *i.e.* a strong effect of substrate concentration and smaller effect of persulphate concentration, cannot be explained by a simple mechanism which yields exclusively the sulphate adduct (**21**), glycol (**22**), and a new $SO_4^{-\bullet}$ radical [reactions (7a—c), Scheme 2]. It was suggested that the less reactive $O\text{-}SO_3^{\bullet}$ adduct (**20**) is formed in reaction (7d) and becomes predominant as the peroxodisulphate concentration is increased [for details of the mechanism of the chain reaction see ref. 30].

We expect that species (**20**) possesses similar properties to $SO_4^{-\bullet}$ and reacts with substrates (**1**) and (**2**) yielding C(5)-OH radicals as the main products [reaction (8)] and minor amounts of C(6)-OH adduct radicals in side reactions. As a result of the chain reaction the C(5)-OH radicals are selectively oxidized and C(6)-OH radicals accumulate in solution.

Pulse radiolysis studies showed[30] that by reaction of $SO_4^{-\bullet}$ with (**2**) the C(5)-OH adduct was formed in yields of $>90\%$ independent of persulphate concentration. Obviously at low persulphate concentration disappearance of the C(5)-OH radicals is slow and thus on the ms time-scale of the e.s.r. experiment the same radical population is observed as in the μs range of pulse radiolysis. However, at high persulphate concentrations the change in radical population is fast enough to lead to differences between e.s.r. and pulse radiolysis results.

(II) *Formation of* OH *Adducts from* (**3**) *and* (**4**).—The e.s.r. spectra obtained by reaction of $SO_4^{-\bullet}$ with the thymine derivatives (**3**) and (**4**) were assigned to C(6)-OH adduct radicals. The failure to detect C(5)-OH radicals cannot be explained in the way described for 1-methyluracil (**1**) because of the absence of the chain reaction.[30] Therefore, we have to assume that position C(5) is not accessible to electrophilic

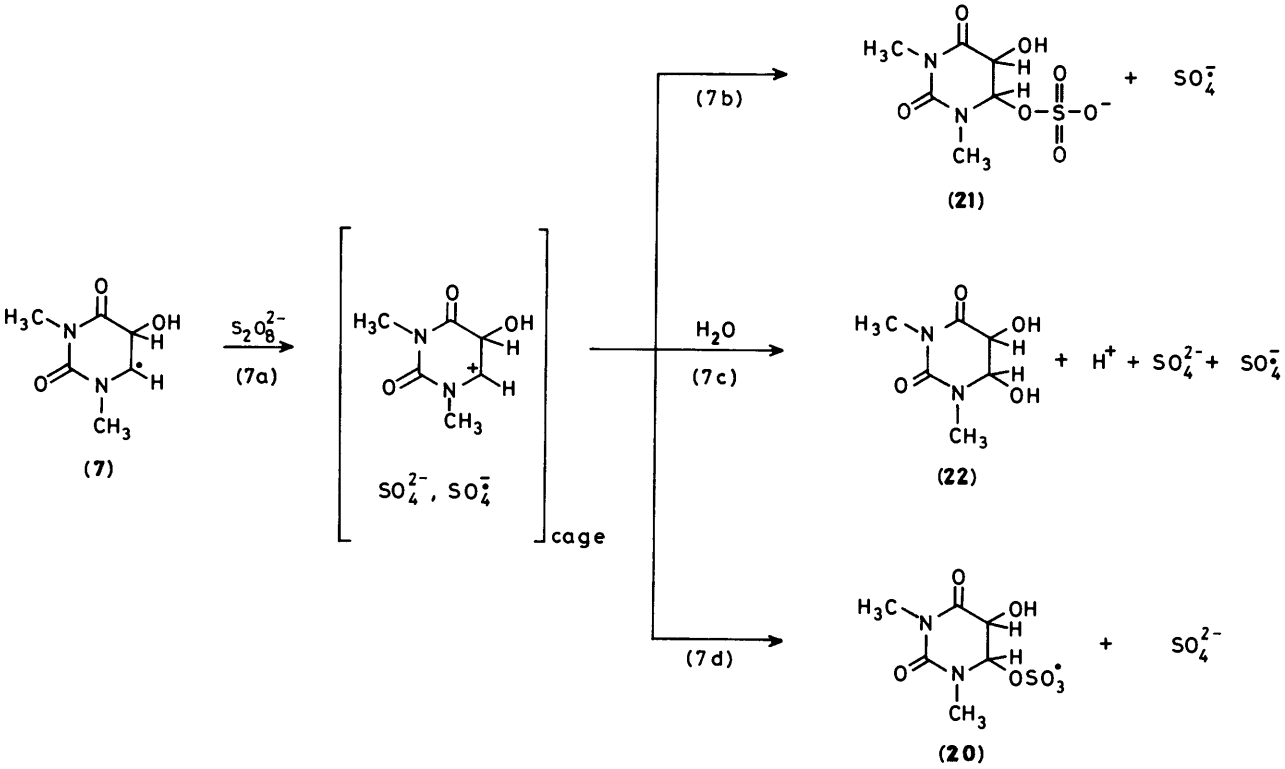


Scheme 1.

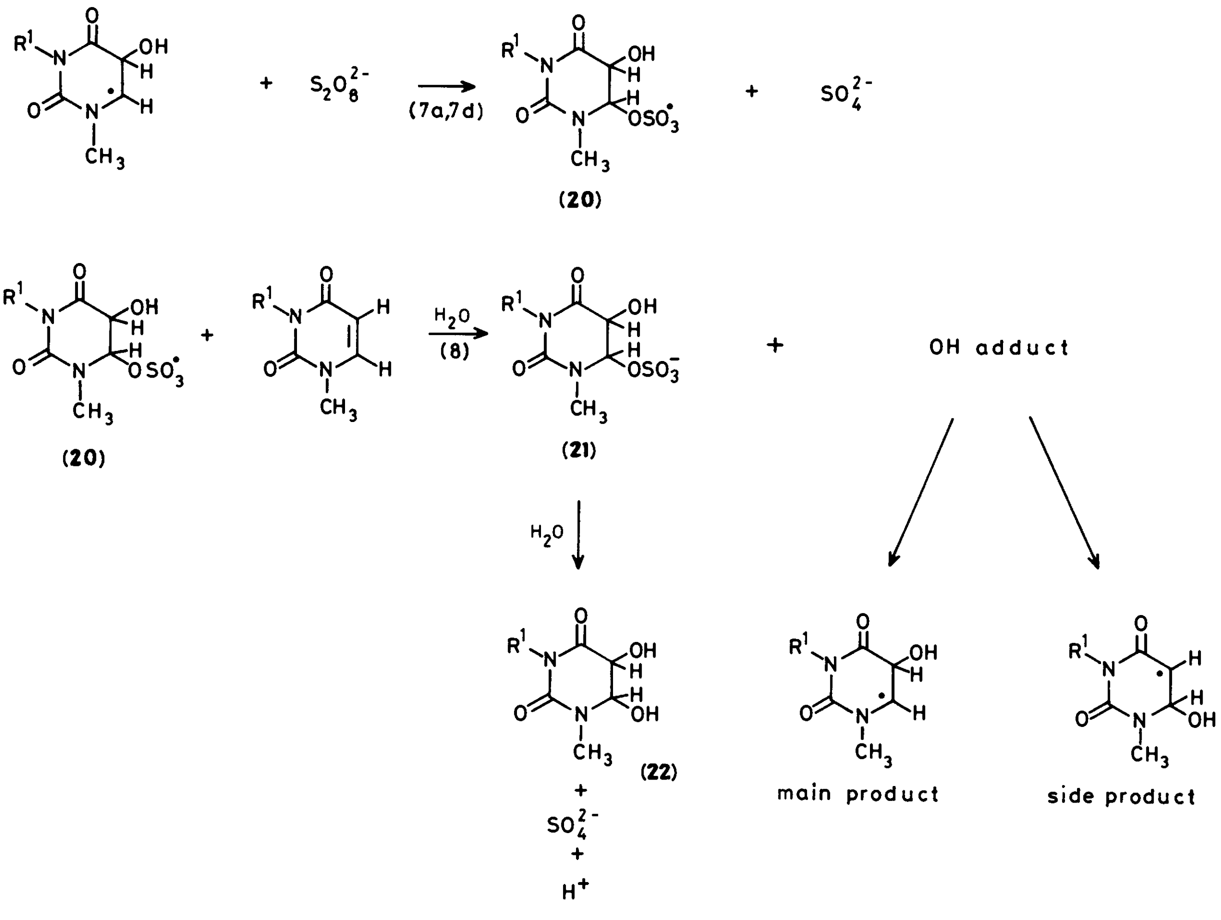
addition of $SO_4^{-\bullet}$ due to the steric effect of the methyl group. We suggest that $SO_4^{-\bullet}$ adds to C(6), instead. Subsequent hydrolysis either *via* reaction (3b) or *via* the radical cation [reactions (5) and (6b)] leads to the C(6)-OH adduct radicals (**9**) and (**10**). Both pathways should be favoured by the electron-releasing effect of the methyl group stabilizing an intermediate ion pair in reaction (3b) or the radical cation (**19**).

The e.s.r. spectra of radicals (**9**) and (**10**) were of rather low intensity in agreement with the fact that addition of $SO_4^{-\bullet}$ to C(6) is rather unfavourable.

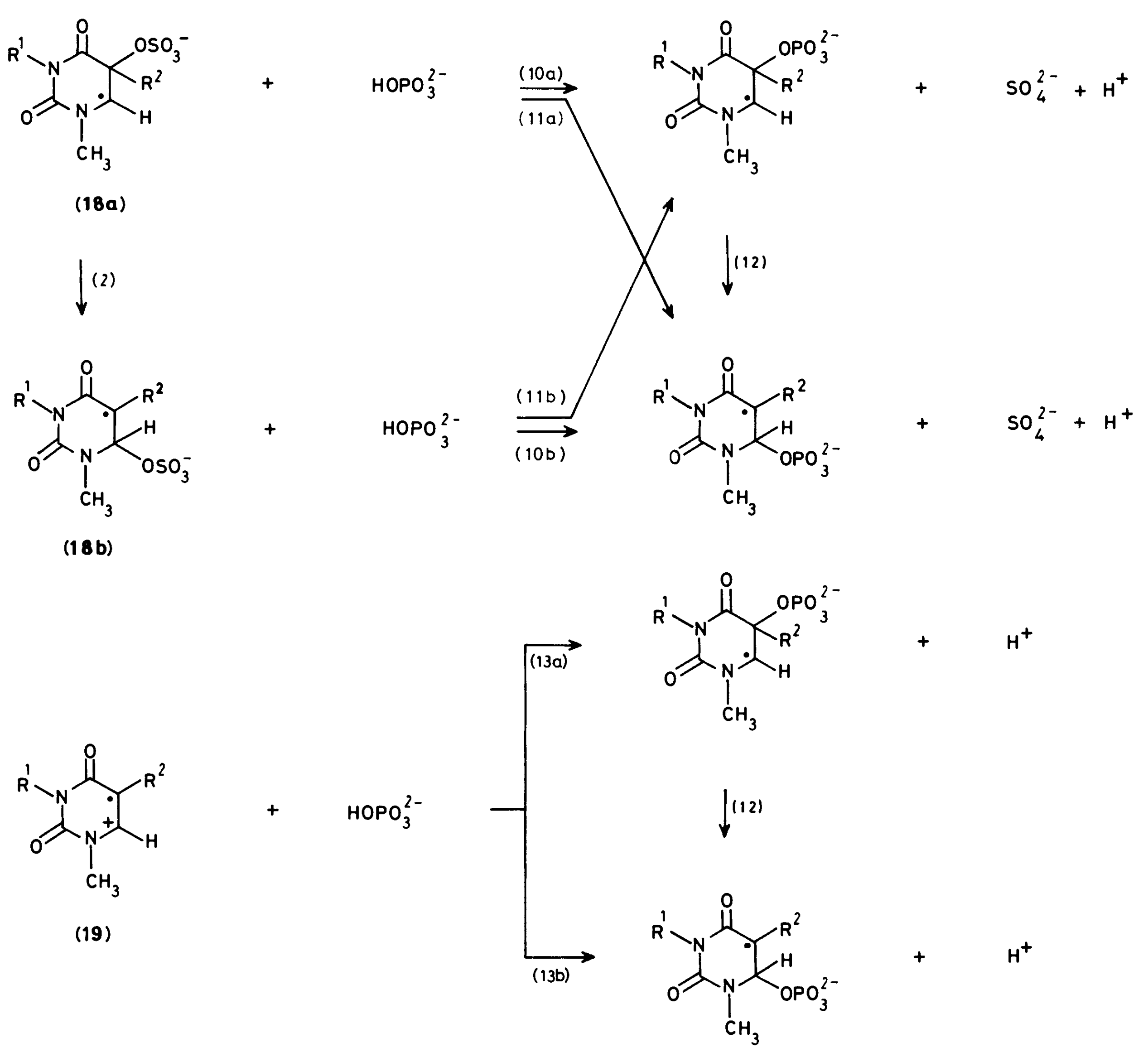
(III) *Comparison of Reaction of* ˙OH *and of* $SO_4^{-\bullet}$ *with* N(1)-*Methylated Pyrimidine Bases*.—Direct e.s.r. detection of the OH-adduct radicals formed by reaction of ˙OH with substrates (**1**)—(**4**) was not possible. However, it emerged from spin-trapping experiments[13] that ˙OH adds to C(5) of (**1**), (**2**), and 6-methyluracil. A mixture of C(5)- and C(6)-OH adducts was observed for (**3**). These results were confirmed by pulse radiolysis studies.[1,10] By *in situ* photolysis of solutions containing persulphate and substrates (**1**)—(**4**), 5-hydroxy-6-yl as well as 6-hydroxy-5-yl adducts were formed, depending on persulphate concentration (see Table). From Schemes 1—3 it is obvious that $SO_4^{-\bullet}$-induced formation of OH adducts is too complex to be compared with the addition of ˙OH to the title compounds. It is not only the persulphate-induced chain reaction [reactions (7) and (8)] which has to be considered but also the possibility of S_N2 *versus* S_N1 hydrolysis [reactions (3) *versus* (6)] as well as 1,2-shift of the sulphate group [reaction (2)]. The results for the thymine compounds (**3**) and (**4**) indicate higher selectivity in the experiments with $SO_4^{-\bullet}$ than with ˙OH. One might speculate that this difference indicates either preference for S_N1 hydrolysis [reaction (6)] for substrates (**3**) and (**4**) or that it reflects the increasing contribution of steric effects of the C(5)-methyl group of the substrate in reactions with $SO_4^{-\bullet}$ compared with those with ˙OH. Hydrogen abstraction from N(1)- and N(3)-methyl groups was not observed in the experiments with $SO_4^{-\bullet}$ which is possibly due to the high reactivity of the products.



Scheme 2.



Scheme 3.



Scheme 4.

(IV) *Formation of Phosphate Adduct Radicals.*—E.s.r. spectra of phosphate adduct radical dianions (**12**)—(**15**) were generated either with $SO_4^{-\bullet}$ in presence of HPO_4^{2-} or with $HPO_4^{-\bullet}$

There is no doubt that in the second type of experiments the electrophilic $HPO_4^{-\bullet}$ radical anion adds to C(5) in substrates (**1**) and (**2**). The reducing C(5)-phosphate adduct radicals may disappear from the solution in the course of a chain reaction[32] as discussed above or by 1,2-shifts [reactions (2) or (12), Scheme 4]. The fact that there was no evidence for formation of a C(5)-phosphate adduct radical even at low $Li_4P_2O_8$ concentrations (3mM) seems to favour the second explanation.

We assume that addition of $HPO_4^{-\bullet}$ to C(5) of the thymine derivatives (**3**) and (**4**) is prevented by the steric effect of C(5)-methyl. Radicals (**14**) and (**15**) are formed by the less favoured addition of $HPO_4^{-\bullet}$ to C(6).

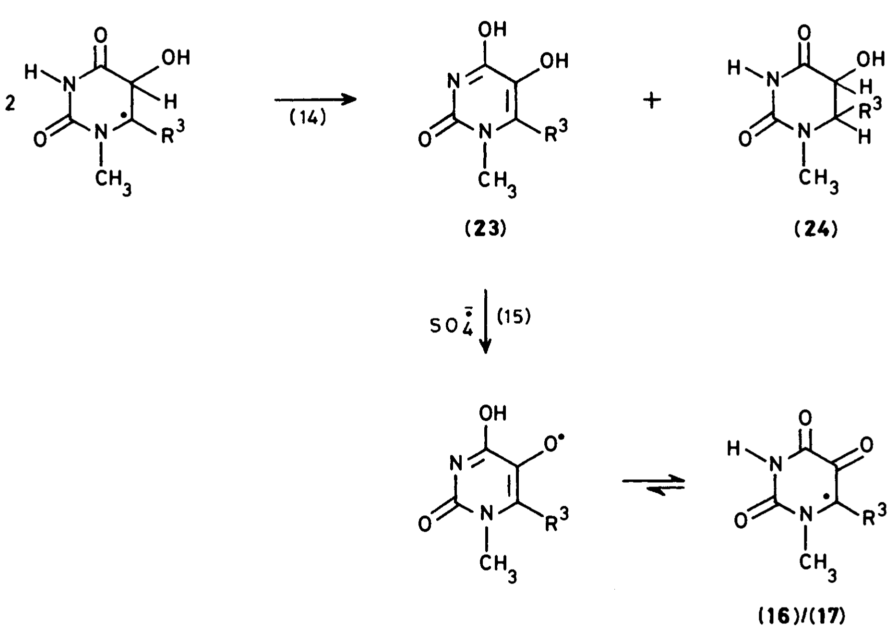
In mixtures of $S_2O_8^{2-}$ and HPO_4^{2-} phosphate radicals may be formed according to equation (9).

$$SO_4^{-\bullet} + HPO_4^{2-} \longrightarrow SO_4^{2-} + HPO_4^{-\bullet} \qquad (9)$$

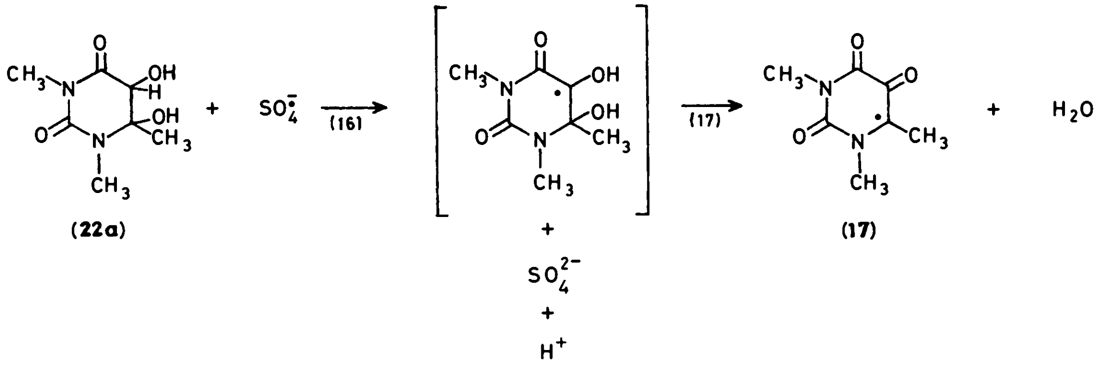
However, comparison of rate constants for reaction (9)[19] ($k_9 = 1.2 \times 10^6$ l mol^{-1} s^{-1}) and for reaction (1) (Scheme 1)[30,33] ($k_1 > 10^9$ l mol^{-1} s^{-1}) shows that $SO_4^{-\bullet}$ reacts faster with the pyrimidine bases than with the phosphate dianions by three orders of magnitude.

The kinetic data were supported by an e.s.r. experiment. It is known that $SO_4^{-\bullet}$ and $HPO_4^{-\bullet}$ add to maleic acid. The sulphate and phosphate adducts give rise to characteristic e.s.r. spectra.[18,20] Photolysis of a solution containing maleic acid (3mM), persulphate (30mM), and phosphate dianions (30mM) yielded the signals of the sulphate and phosphate adduct radicals with an intensity ratio of *ca.* 20:1. Therefore, we exclude that upon photolysing mixtures of $S_2O_8^{2-}$, HPO_4^{2-}, and olefins considerable amounts of $HPO_4^{-\bullet}$ radicals are formed.

We expect C(5)-phosphate radicals formed as main products either in reaction (10a) from the C(5)-sulphate adduct (**18a**) or *via* rearrangement (2) and subsequent nucleophilic attack of C(5) by phosphate [reaction (11b)]. At the same time in side-reactions, either by S_N2 reaction (10b) or *via* the radical cation (**19**) in reaction (13b) minor amounts of the C(6)-phosphate adduct radical may be formed. As was already shown, the C(5)-phosphate adduct may disappear from the solution either by reaction with peroxodisphosphate or by 1,2-phosphate



Scheme 5.



Scheme 6.

migration [reaction (12)] at the expense of the C(6)-phosphate adduct. In pulse radiolysis studies[30] no evidence for the oxidizing 6-phosphate-5-yl radicals was obtained which may mean that oxidation of the C(5)-phosphate adduct or 1,2-shift occurs on timescales of *ca.* 10^{-5}—10^{-3} s.

We propose that in the experiments with the thymines (**3**) and (**4**) the C(6)-phosphate adduct radicals (**14**) and (**15**) are formed in reaction (10b) from the C(6)-sulphate adduct (**18b**) or in reaction (13b) from the radical cation (**19**). Formation of the C(5)-sulphate adduct and subsequent photolysis [reaction (10a)] is prevented by the C(5)-methyl group as discussed above.

From the half-times, $\tau_{\frac{1}{2}}$, of formation of the OH adduct radicals it is possible to estimate the rate constants k_{Ph} for phosphate entry.[14]

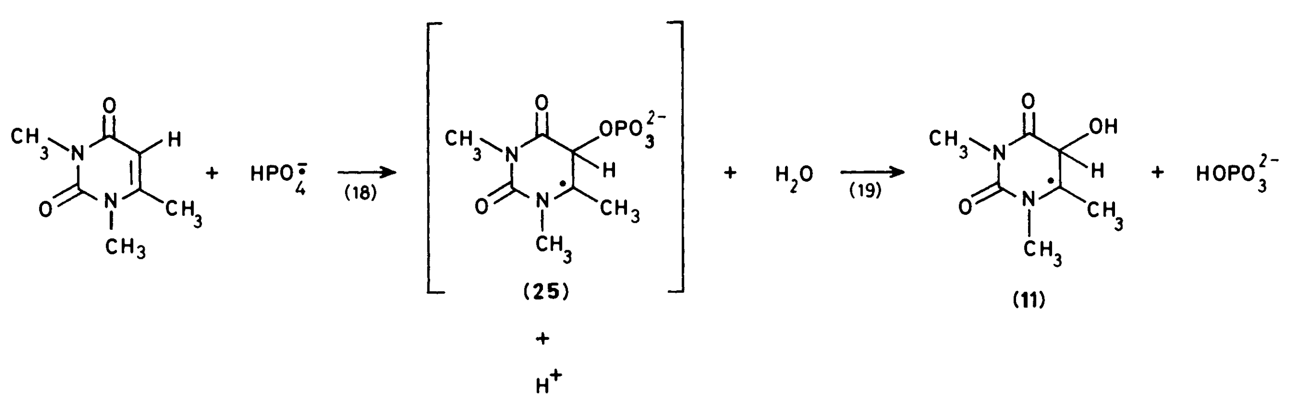
Under conditions such that the intermediate reacts equally fast with water and with phosphate, *i.e.* at half values of saturating concentration of phosphate, $c_{\frac{1}{2}\text{Ph}}$, we obtain $k_w c_w c_i = k_{\text{Ph}}\, c_{\frac{1}{2}\text{Ph}}\, c_i$ where c_i is the concentration of the intermediate (**18**) and/or (**19**) and $k_w c_w = 1/\tau_{\frac{1}{2}}$ is the pseudo-first-order rate constant for reaction of the intermediate with water. With $1/\tau_{\frac{1}{2}} > 10^6$ s^{-1} [30] and *ca.* 3×10^5 s^{-1} [31] and $c_{\frac{1}{2}\text{Ph}}$ *ca.* 1.5×10^{-3} and 0.8×10^{-3}M for formation of the phosphate adducts (**13**) and (**15**), respectively, we obtain rate constants k_{Ph} of $>6.6 \times 10^8$ and 4×10^8 l mol^{-1} s^{-1} for the entry of phosphate into substrates (**2**) and (**4**). These values are higher than rate constants for the entry of phosphate into 1,1-dialkoxyalkene radical cations (2×10^6—3×10^8 l mol^{-1} s^{-1}).[14] They are indicative of the extremely high reactivity of the intermediates formed by $SO_4^{-\bullet}$ from the pyrimidine bases.

(V) *Formation of the Secondary Radicals.*—Formation of the secondary radicals (**16**) and (**17**) requires the presence of a hydrogen at C(5). We suggest that in analogy to results reported on 4,5,6-trihydroxypyrimidines[34] the C(5)-OH adduct undergoes disproportionation [reaction (14)].

1-Methylisobarbituric acid (**23**) is oxidized by $SO_4^{-\bullet}$ [reaction (15)] to yield radicals (**16**) (R^3 = H) and (**17**) (R^3 = CH_3). Photolysis of persulphate in presence of isobarbituric acid yielded the e.s.r. spectrum of radical (**16**) which is strong evidence for the postulated mechanism. An additional pathway for formation of radical (**17**) involves hydrogen abstraction from C(5) of the glycol (**22a**) [reaction (16)] with subsequent water elimination (17).

(VI) *Radicals obtained from* (**5**).—As noted above neither the OH adduct nor the phosphate adduct radical was generated from (**5**) in the experiments with persulphate.

The failure to observe the e.s.r. spectrum of (**11**) may be explained by the high reducing power of this radical favouring its fast oxidation and formation of the secondary radical (**17**).



Scheme 7.

As far as reaction of $HPO_4^{-\bullet}$ with (**5**) is concerned we may conceive that rearrangement [reaction (12)] of the C(5)-phosphate adduct formed in the primary step is not possible because of the steric effect of the C(6)-methyl group. Instead, the labile C(5)-phosphate adduct (**25**) hydrolyses [reaction (19)].

Upon photolysing mixtures of persulphate and phosphate dianions in the presence of (**5**) no phosphate adduct radical was formed. Instead, the secondary radical (**17**) was detected. This indicates that the C(5)-OH adduct radical (**11**) was formed [reaction (19)] and immediately converted into (**17**) in reactions (14)—(17) (Scheme 5 and 6).

It is interesting to note that the e.s.r. spectrum of the C(5)-OH adduct (**11**) was observed in presence of H_2O_2 or peroxidiphosphate but not in the experiments with persulphate. This clearly demonstrates that persulphate has the highest oxidative power of the three radical-inducing agents used in this work.

(VII) *Conclusions*.—E.s.r. spectra of OH-adduct radicals were obtained from (**1**)—(**4**) by reaction with photolytically generated $SO_4^{-\bullet}$. So far it is not clarified whether the OH adducts were formed by S_N2 hydrolysis of the sulphate adducts (**18**) or by S_N1 reaction of water with the free radical cations (**19**).

When photolysis of persulphate was carried out in presence of phosphate dianions and substrates (**1**)—(**4**), C(6)-phosphate adduct radicals were formed in reactions similar to those postulated for formation of OH-adducts.

Identical e.s.r. spectra were observed on reaction of (**1**)—(**4**) with photolytically generated $HPO_4^{-\bullet}$.

In secondary reactions 5-oxo-6-yl radicals were formed from (**1**) and from (**5**).

Experimental

Chemicals.—Substrates (**1**)—(**3**) were commercially available and used without further purification. The sources were Chemical Dynamics Corp., for (**1**), Fluka for (**2**), and Sigma for (**3**). Compounds (**4**) and (**5**) were prepared by methylation of thymine (Sigma) and 6-methyluracil (Sigma) with methyl iodide according to the following procedure. The pyrimidine base (0.08 mol) was dissolved in NaOH (6 g) in H_2O (70 ml). CH_3I (0.3 mol) was added dropwise at 35 °C. The solution was stirred at 35 °C for 12 h and CH_3I was removed by vacuum evaporation. The product was extracted with $CHCl_3$. The extract was dried with Na_2SO_4 and purified by column chromatography [SiO_2; $CHCl_3$–MeOH (19:1 v/v)]. The m.p.s were 111.5 °C and 155 °C for (**4**) and (**5**), respectively. Purity was checked by ^1H n.m.r. and m.s. $K_2S_2O_8$ (p.a.) was from Merck. $Li_4P_2O_8$ was prepared by electrolysis according to ref. 35. The preparation contained $<1\%$ inorganic phosphate as determined by ion chromatography.

E.s.r. measurements were performed at 277 K on aqueous solutions containing the substrates (1—3mM) and either H_2O_2 or $K_2S_2O_8$ or $K_2S_2O_8$–KH_2PO_4 or $Li_4P_2O_8$. By a continuous flow arrangement the solutions were pumped through the e.s.r. cavity. The standard flow rate was 0.1 ml s^{-1} for a 0.3×8 mm^2 cross-section of the cell. *In situ* photolysis was achieved by irradiation with a high-pressure mercury lamp (Philips SP 1000). 0.5 h Prior to the measurement and during the measurement the solutions were gassed with argon. The pH values were adjusted with KOH or $HClO_4$. At pH >7 sodium borate (10mM) was used as a buffer. In some cases, in order to assign certain proton coupling constants, D_2O was used as solvent. In the experiments with low persulphate concentrations, 1% acetone was added to the solutions to increase the spectral intensity. Unless otherwise noted addition of acetone did not give rise to any signals which were not detected in the absence of the photosensitizer. Spectra were measured at the X-band with 100 kHz modulation. *g* Factors were determined with a n.m.r. sideband technique[36] taking into account the field offset of the n.m.r. probe that was attached to the side-wall of the cavity.

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