

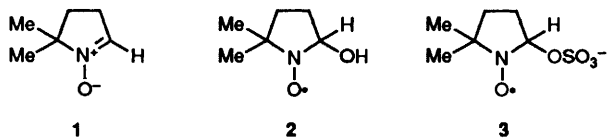
## Nucleophilic Substitution Reactions of Spin Adducts. Implications for the Correct Identification of Reaction Intermediates by EPR/Spin Trapping

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Radical adducts to nitron spin traps have been shown, in aqueous solution, to undergo conversion to the hydroxyl radical adduct *via* nucleophilic substitution reactions; these observations have important consequences for the interpretation of data from EPR/spin-trapping experiments.

The technique of spin trapping in conjunction with EPR spectroscopy has proved to be of considerable use for detecting and identifying free-radical species produced in both chemical and biological systems.<sup>1</sup> In such experiments a short-lived free radical typically reacts with a spin-trap (customarily a nitron or nitroso compound) to give a longer-lived nitroxide whose EPR splittings provide crucial information about the structure of the addend.

However, a draw-back of this approach is that while a given set of parameters may clearly characterize a particular spin adduct, the appropriate mechanism for its formation may not necessarily be unambiguously revealed. For example, while the hydroxyl-radical adduct (**2**) of DMPO [5,5-dimethyl-1-pyrroline *N*-oxide, (**1**)] is detected when  $\cdot\text{OH}$  is generated from  $\text{H}_2\text{O}_2$  (e.g. by photolysis<sup>2</sup> or reaction with  $^3\text{Fe}^{\text{II}}$ ), **2** also results from other types of reaction, including the hydration of the radical-cation of **1**, formed by irradiation,<sup>4</sup> and *via* the decomposition of the superoxide ( $\text{O}_2^{\cdot-}$ ) and hydroperoxyl ( $\text{HO}_2^{\cdot}$ ) radical adducts (see e.g. ref. 5). As a result of EPR investigations<sup>6</sup> of alternative decomposition pathways for unsymmetric peroxides (in which, for example, either  $\text{SO}_4^{\cdot-}$  or  $\cdot\text{OH}$  may be formed from one-electron reduction of  $\text{HOOSO}_3^-$ ) we have discovered a range of reactions in which nucleophilic substitution reactions also bring about transformations of initial spin adducts: our results are reported here.



For example, spin-trapping experiments involving the reaction between  $\text{HOOSO}_3^-$  ( $0.001 \text{ mol dm}^{-3}$ ) and either  $\text{Ti}^{\text{III}}$  or  $\text{Fe}^{\text{II}}$  (both  $0.0017 \text{ mol dm}^{-3}$ ) in the presence of the spin-trap **1** ( $0.017 \text{ mol dm}^{-3}$ ) led to the immediate detection of the adduct **3** and to the steady build-up of the signal from **2** as **3** decayed (with  $t_{1/2}$  ca. 95 s) (for EPR parameters, see Table 1). To obtain confirmation of assignments and mechanistic information, a variety of photochemical experiments were carried out with several different radicals and a variety of traps.

The radical anion  $\text{SO}_4^{\cdot-}$  was subsequently generated by *in situ* photolysis of  $\text{S}_2\text{O}_8^{2-}$  (with the unfiltered radiation from a 100 W mercury/xenon arc lamp),  $\text{PO}_4^{2-}$ ,  $\text{HPO}_4^-$  and  $\text{H}_2\text{PO}_4^+$  by photolysis of the peroxydiphosphate anion (at pH 7, 4 and 1.5 respectively) and  $\text{Cl}_2^{\cdot-}$  and  $\text{Br}_2^{\cdot-}$  by photolysis of  $\text{S}_2\text{O}_8^{2-}$  in the presence of an excess of the appropriate halide ion (*cf.* refs. 7 and 8: typical concentrations are given in Table 1). Also included in the reaction mixtures were the appropriate spin traps: DMPO (**1**),  $\text{M}_4\text{PO}$  [3,3,5,5-tetramethyl-1-pyrroline *N*-oxide, (**4**)], PBN [benzylidene-*tert*-butylamine *N*-oxide (**5**)] and POBN [1-oxido-4-pyridylmethylene-*tert*-butylamine *N*-oxide (**6**)]. The radical species detected by EPR spectroscopy during photolysis

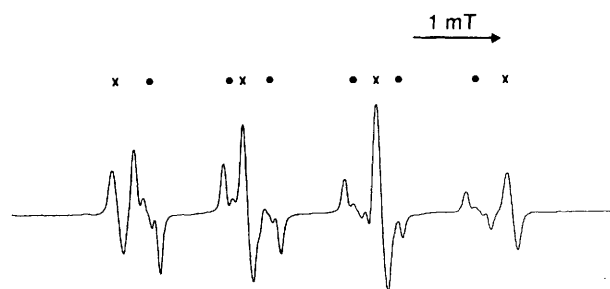
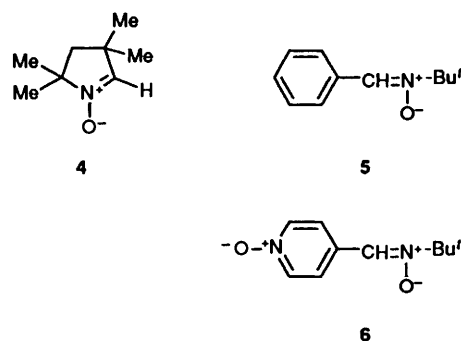


Fig. 1 EPR spectrum obtained from photolysis of the peroxydisulphate anion ( $0.025 \text{ mol dm}^{-3}$ ) in aqueous solution in the presence of DMPO ( $0.050 \text{ mol dm}^{-3}$ ) at pH 7: ●  $\text{SO}_4^{\cdot-}$  radical adduct; ×  $\text{HO}^{\cdot}$  radical adduct

have parameters which are listed in Table 1 (assignments are based on literature values where these are known<sup>9</sup>): authentic spectra of  $\text{HO}^{\cdot}$  adducts were obtained *via* experiments in which dilute solutions of hydrogen peroxide ( $0.025 \text{ mol dm}^{-3}$ ) were irradiated in the presence of the spin trap.



The reaction of both  $\text{Cl}_2^{\cdot-}$  and  $\text{Br}_2^{\cdot-}$  with all of the traps investigated gave rise to *hydroxyl* adducts with, in only one case [that of  $\text{Cl}_2^{\cdot-}$  with  $\text{M}_4\text{PO}$  (**4**)] evidence for a halogen-atom adduct. With  $\text{SO}_4^{\cdot-}$ , trapping with PBN (**5**) and POBN (**6**) led to the detection solely of the appropriate *hydroxyl* adducts and use of DMPO (**1**) and  $\text{M}_4\text{PO}$  (**4**) led to the detection of mixtures of adducts typical of those of  $\text{SO}_4^{\cdot-}$  and  $\cdot\text{OH}$  (see Fig. 1), the latter building up with time: when photolysis was interrupted the build up of, for example, **3** at the expense of **2** could be followed (see Fig. 2). Again, in the peroxydiphosphate experiments, adducts of  $\text{HPO}_4^-$  and  $\text{H}_2\text{PO}_4^+$  were accompanied in some cases by the spectra of hydroxyl adducts (see Table 1).

The detection of hydroxyl adducts in the reactions of  $\text{SO}_4^{\cdot-}$ ,  $\text{H}_2\text{PO}_4^+$  (and its analogues) and  $\text{X}_2^{\cdot-}$  ( $\text{X} = \text{Br}, \text{Cl}$ ) suggests either that the initially-formed radicals oxidize the nitrones to radical-cations, which undergo subsequent hydration [see reaction (1): Scheme 1] or that nucleophilic substitution of the incipient leaving group in the initial radical adduct has occurred

Table 1

Spin trap	Attacking radical <sup>a</sup>	Adduct detected	Hyperfine coupling constants of adducts/mT <sup>b</sup>		
			<i>a<sub>N</sub></i>	<i>a</i> (β-H)	Other
DMPO	HO <sup>•</sup> , Cl <sub>2</sub> <sup>-</sup>	HO <sup>•</sup>	1.49	1.49	0.14, <sup>c</sup> 0.08 <sup>c</sup>
		SO <sub>4</sub> <sup>-</sup>	1.38	1.02	
	Br <sub>2</sub> <sup>-</sup> , PO <sub>4</sub> <sup>2-</sup> , HPO <sub>4</sub> <sup>-</sup> , H <sub>2</sub> PO <sub>4</sub> <sup>•</sup>	HO <sup>•</sup> <sup>d</sup>	—	—	0.10, <sup>e</sup> 0.53 <sup>e</sup> 0.10, <sup>e</sup> 0.53 <sup>e</sup> 0.27, <sup>e</sup> 0.16 <sup>e</sup>
		HO <sup>•</sup>	1.42	1.47	
		HPO <sub>4</sub> <sup>-</sup>	1.42	1.47	
		H <sub>2</sub> PO <sub>4</sub> <sup>•</sup>	1.42	1.16	
M <sub>4</sub> PO	HO <sup>•</sup>	HO <sup>•</sup>	1.53	1.66	—
		SO <sub>4</sub> <sup>-</sup>	1.39	0.84	
	Cl <sub>2</sub> <sup>-</sup> , Br <sub>2</sub> <sup>-</sup> , PO <sub>4</sub> <sup>2-</sup>	HO <sup>•</sup> <sup>g</sup>	—	—	—
		h	—	—	
	HPO <sub>4</sub> <sup>-</sup>	PO <sub>4</sub> <sup>2-</sup>	1.50	1.35	—
		HPO <sub>4</sub> <sup>-</sup> <sup>i</sup>	1.51	1.33	
	H <sub>2</sub> PO <sub>4</sub> <sup>•</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>•</sup>	1.44	1.04	—
		HO <sup>•</sup>	—	—	
PBN	HO <sup>•</sup> , SO <sub>4</sub> <sup>-</sup> , Br <sub>2</sub> <sup>-</sup>	HO <sup>•</sup>	1.54	0.26	—
		HO <sup>•</sup> <sup>j</sup>	—	—	
	PO <sub>4</sub> <sup>2-</sup> , HPO <sub>4</sub> <sup>-</sup> , H <sub>2</sub> PO <sub>4</sub> <sup>•</sup>	PO <sub>4</sub> <sup>2-</sup>	1.54	0.17	—
		HPO <sub>4</sub> <sup>-</sup>	1.53	0.22	
		HO <sup>•</sup> <sup>k</sup>	—	—	
POBN	HO <sup>•</sup> , SO <sub>4</sub> <sup>-</sup> , Cl <sub>2</sub> <sup>-</sup> , H <sub>2</sub> PO <sub>4</sub> <sup>•</sup>	HO <sup>•</sup>	1.49	0.17	0.03 <sup>m</sup>
		n	—	—	
	Br <sub>2</sub> <sup>-</sup> , PO <sub>4</sub> <sup>2-</sup> , HPO <sub>4</sub> <sup>-</sup>	PO <sub>4</sub> <sup>2-</sup>	1.50	0.14	—
		HPO <sub>4</sub> <sup>-</sup>	1.50	0.14	

<sup>a</sup> HO<sup>•</sup> generated by photolysis of H<sub>2</sub>O<sub>2</sub>; SO<sub>4</sub><sup>-</sup> generated by photolysis of S<sub>2</sub>O<sub>8</sub><sup>2-</sup> at pH 7; Cl<sub>2</sub><sup>-</sup> generated by photolysis of S<sub>2</sub>O<sub>8</sub><sup>2-</sup> in presence of 1 mol dm<sup>-3</sup> NaCl at pH 7; Br<sub>2</sub><sup>-</sup> generated by photolysis of S<sub>2</sub>O<sub>8</sub><sup>2-</sup> in presence of 1 mol dm<sup>-3</sup> NaBr at pH 7; PO<sub>4</sub><sup>2-</sup>, HPO<sub>4</sub><sup>-</sup> and H<sub>2</sub>PO<sub>4</sub><sup>•</sup> generated by photolysis of P<sub>2</sub>O<sub>8</sub><sup>4-</sup> at pH 7, 4 and 1.5, respectively. <sup>b</sup> ±0.025 mT. <sup>c</sup> From the two inequivalent γ-hydrogens in the trap. <sup>d</sup> Plus small amounts of the oxidised spin trap radical DMPOX (*a<sub>N</sub>* 0.72, *a<sub>2H</sub>* 0.41 mT). <sup>e</sup> From one γ-hydrogen on the trap and the <sup>32</sup>P nucleus. <sup>f</sup> Plus small amounts of the CO<sub>2</sub><sup>-</sup> adduct (*a<sub>N</sub>* 1.56, *a<sub>H</sub>* 1.87 mT) arising from reaction with methanoate buffer. <sup>g</sup> Complex, weak, spectra consisting of the HO<sup>•</sup> adduct plus further signals tentatively assigned to the <sup>35</sup>Cl and <sup>37</sup>Cl adducts. <sup>h</sup> Only signals from the oxidised spin trap M<sub>4</sub>POX observed (*a<sub>N</sub>* 0.693 mT). <sup>i</sup> Plus small amounts of the CO<sub>2</sub><sup>-</sup> adduct (*a<sub>N</sub>* 1.57, *a<sub>H</sub>* 1.97 mT) arising from reaction with methanoate buffer. <sup>j</sup> Weak signals. <sup>k</sup> Plus small amounts of the CO<sub>2</sub><sup>-</sup> adduct (*a<sub>N</sub>* 1.57, *a<sub>H</sub>* 0.46 mT) arising from reaction with methanoate buffer. <sup>l</sup> Tentative assignment; may be due to the corresponding phosphate adduct with identical parameters to the HO<sup>•</sup> adduct. <sup>m</sup> From the hydroxyl hydrogen. <sup>n</sup> No signals observed.

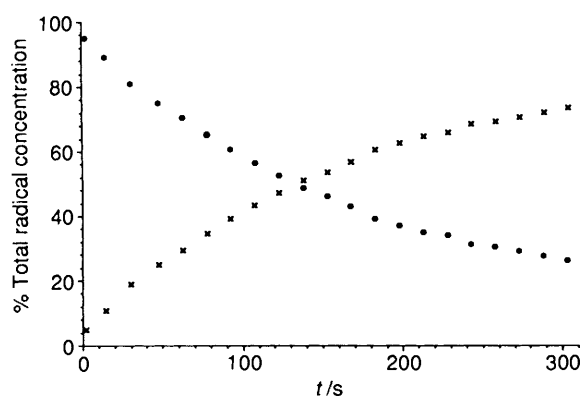


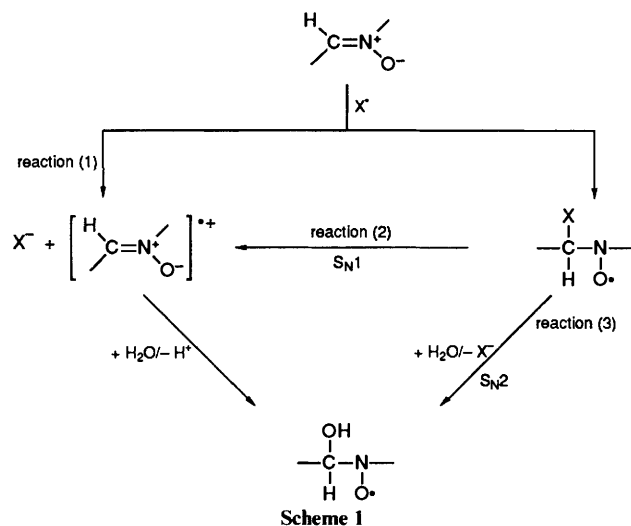
Fig. 2 Variation with time (after cessation of photolysis) of the concentrations of the SO<sub>4</sub><sup>-</sup> (●) and HO<sup>•</sup> (×) radical adducts expressed as a percentage of the total radical adduct concentration (conditions as in Fig. 1)

[reactions (2) and (3); Scheme 1]. Precedents for these types of processes have been claimed following EPR and pulse-radiolysis experiments involving the reactions of SO<sub>4</sub><sup>-</sup> or Cl<sub>2</sub><sup>-</sup> with alkenes<sup>7</sup> and the generation of β-chloro, -bromo, and -phosphate radicals in α-hydrogen-atom abstraction reactions.<sup>10,11</sup>

A mechanism involving direct oxidation to the nitron's radical-cation is clearly not generally appropriate, given the

detection in several instances of the first-formed adduct. We believe instead that these observations provide evidence for addition-elimination sequences involving either S<sub>N</sub>1 or S<sub>N</sub>2 reaction of the initial adducts (see Scheme 1). The rates of the hydrolysis reaction appear to be governed by leaving-group ability, with Cl<sup>-</sup> better than SO<sub>4</sub><sup>2-</sup> (as judged by the results obtained with 1 and 4), rather than the expected efficacy in one-electron oxidation (*cf.* *E*<sup>o</sup> values of 3.08, 2.55 and 2.0 V for SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup> and Br<sup>-</sup>, respectively<sup>12,13</sup>). Similarly, the difference in behaviour of the phosphate adducts observed in this study (loss of the β-group in the order H<sub>2</sub>PO<sub>4</sub><sup>-</sup> > HPO<sub>4</sub><sup>2-</sup> > PO<sub>4</sub><sup>3-</sup>) reflects their leaving-group ability and also the order of loss of β-phosphate-derived substituents in the hydrolysis of radicals of the type <sup>•</sup>CH(OMe)CH<sub>2</sub>OP (P = PO<sub>3</sub><sup>2-</sup>, HPO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>3</sub>).<sup>11</sup>

It is unclear at this stage whether the hydrolysis of the radical-adducts occurs *via* S<sub>N</sub>1- or S<sub>N</sub>2-type processes. Certainly the greater facility of loss of SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup> from the *acyclic* nitron adducts could well be explained in terms of an S<sub>N</sub>1 process (to give a short-lived radical-cation), favoured both by conjugation of spin and charge with the aromatic ring in the transition state and the ease of achieving an eclipsing geometry between the unpaired electron and the β-leaving group (a factor generally believed to increase the rate of these processes<sup>14</sup>). Similarly the more ready loss of HPO<sub>4</sub><sup>2-</sup> from the adducts formed with 5 (compared with 6), and 4 (compared with 1) can also be explained in terms of the increased stability of a radical-cation intermediate formed in an S<sub>N</sub>1 process, with in addition a



decrease in steric crowding. In contrast the presence of the extra methyl groups in **4** relative to **1** would have been expected to decrease dramatically the rate of an  $S_N2$  process.

Further kinetic, stereochemical and solvent effects remain to be explored if this novel reaction chemistry is to be fully elucidated; it is nevertheless clear at this stage that account must be taken of these interconversion reactions (and other possible analogous processes) in the analysis and interpretation of spectra obtained from spin-trapping experiments.

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