Use of a phosphorylated spin trap to discriminate between the hydroxyl radical and other oxidising species

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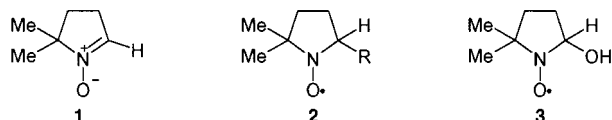
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The spin-trap 5-diethoxyphosphoryl-5-methyl-1-pyrroline N-oxide (DEPMPO) is shown to provide a simple method to distinguish free-radical reactions which lead to the production of ˙OH from those which proceed *via* one-electron transfer followed by hydration or nucleophilic substitution of another adduct. In the first case, only an ˙OH adduct is detected; in the latter, signals from the hydroxyl adduct (labelled with ^{17}O when $H_2{}^{17}O$ is employed) are accompanied by a second signal believed to result *via* internal trapping of a radical formed from an intermediate radical cation. This provides a potentially useful mechanistic probe, for example for use in biochemical systems.

Fig. 1 EPR spectrum of the HO˙ adduct of DEPMPO (**7**) obtained on photolysis of a solution of H_2O_2 (10 mM) in the presence of DEPMPO (50 mM)

Introduction

Developments in the design of spin-traps have substantially increased the range and nature of the potential applications of EPR spectroscopy to free-radical chemistry. This is particularly true for biochemical systems and other experiments in which only minute amounts of material are available and for which only particularly low steady-state radical concentrations are generated. Nitrones are particularly effective spin-traps[1–3] and the cyclic nitrone 5,5-dimethyl-1-pyrroline N-oxide, DMPO (**1**)

especially, has proved invaluable *via* its ability to react with short-lived free-radicals (R˙) to give relatively long-lived adducts (**2**) whose parameters [especially $a(\beta\text{-H})$] are characteristic of the type of initial radical (*i.e.* oxygen-, sulfur- or carbon-centred species). The hydroxyl adduct (**3**) is particularly well characterised [with, in water, $a(N) = a(H) = 1.495$ mT].

However, it is also now very well established that caution must be exercised when interpreting the results of EPR experiments involving these (and other) spin-traps since several other routes exist whereby apparent radical-adducts may arise. These include, for example, the result of *nucleophilic attack* on the nitrone followed by one-electron oxidation (the so-called Forrester–Hepburn mechanism[4]) and the '*inverse*' *spin-trapping* process whereby one-electron oxidation of a nitrone to a radical cation is followed by nucleophilic attack [see *e.g.* reactions (1) and (2), respectively which have been recently described by Eberson and co-workers[5,6]].

Following preparation of a new range of phosphorylated spin-traps [see *e.g.* DEPMPO (**4**), for which longer-lived
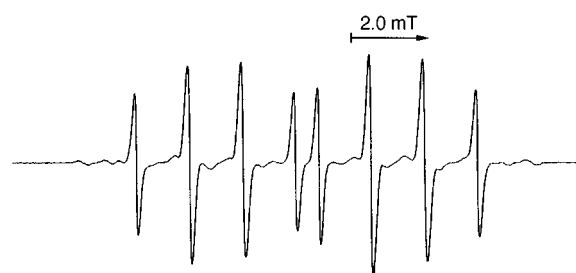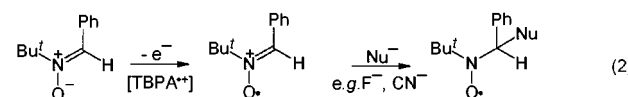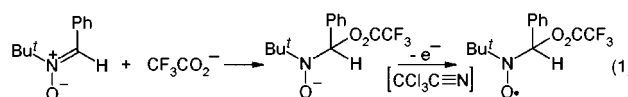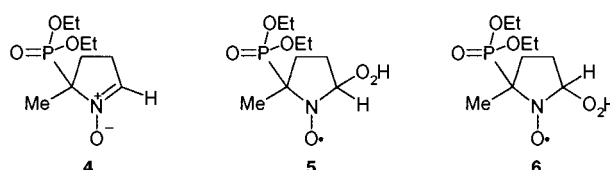

$$TBPA^{+} = \text{Tris(4-bromophenyl)aminium radical cation}$$

hydroperoxyl ($HO_2{}^{\bullet}$) adducts have been described][7] we have examined their potential ability for distinguishing different types of reaction in a series of experiments designed to differentiate between spin-adducts formed from free-radical pathways, electron-transfer chemistry or spin-adduct substitution reactions. It should be noted that traps such as **4** also have the potential to provide extra information as a result of expected differences which should arise in hyperfine splittings for the discrete *cis* and *trans* isomers [*e.g.* (**5**) and (**6**) which have been reported for the DEPMPO adducts of $HO_2{}^{\bullet}$].[7]

In situ photolysis ($\lambda > 250$ nm) of a hydrogen peroxide solution (10 mM) in the presence of **4** (50 mM) is found to give rise to a very strong and characteristic EPR spectrum (shown in Fig. 1) with hyperfine splittings of $a(N)$ 1.35, $a(H)$ 1.35, $a(P)$ 4.75 mT; g 2.0061, and assigned to the *trans* hydroxyl adduct **7** (*cf.* also ref. 8). The same spectrum was also obtained in experiments in the presence of **4** (10 mM) involving the Fenton or related reactions (with $Fe^{II}SO_4$ 1 mM, H_2O_2 5 mM, pH 7.4) and the related $Ti^{III}–H_2O_2$ system ($Ti^{III}Cl_3$ 1 mM, H_2O_2 5 mM, pH 7.4), as well as experiments with $Cu^{II}–H_2O_2$ and ascorbate (Cu^{II}-

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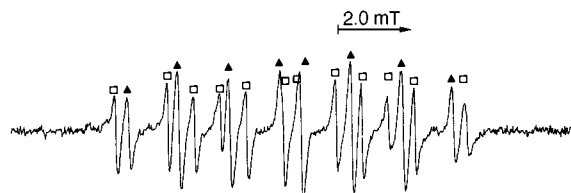
Fig. 2 EPR spectrum obtained on reaction of DEPMPO (4 mM) with $Cu^{II}SO_4$ (1 mM). Signals are assigned to the 'HO'' adduct (**7**) (▲) and a carbon-centred radical adduct (**10**) (□).


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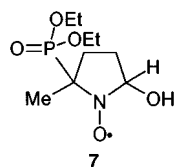
SO_4 bis(1,10-phenanthroline) 1 mM, H_2O_2 2 mM, ascorbate 2 mM, pH 7.0) in which Cu^I is believed to be produced.[9] We believe that in all cases, this provides good evidence for the production of the hydroxyl radical.

In a number of other systems (in which DMPO is found to give simply the 'HO' adduct') a different spectrum was obtained from DEPMPO, comprising a mixture of the adduct **7** and a second species with parameters a(N) 1.45, a(H) 2.20, a(P) 4.60 mT and g 2.0061 which is evidently characteristic of the trapping of a carbon-centred radical [for which a(H) values are typically in the range 2.0–2.4 mT[2,3]]. In all experiments a ratio of *ca.* 1:1 was observed. This mixture of signals was obtained in experiments with DEPMPO (typically 4 mM) in the presence of $Fe^{III}Cl_3$ (4 mM) or $Cu^{II}SO_4$ (1 mM) or the high-valent manganese species **8** (12 µM); an example is given in Fig. 2. When the


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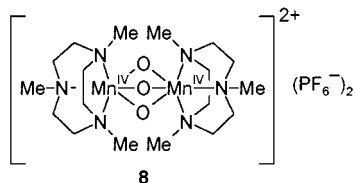
same experiments were carried out in [17]O enriched water, only the adduct **7** showed evidence of extra splitting [a([17]O) 0.42 mT] associated with coupling to a β-[17]O (*i.e.* an apparent $H^{17}O$' adduct). This observation suggests that hydration (with $H_2^{17}O$) of a DEPMPO radical cation intermediate has occurred. In contrast, the lack of an observable [17]O splitting in the second radical-adduct (any splitting must be less than 0.05 mT) supports our conclusion that this is not a *cis*-hydroxyl adduct, but a carbon-centred adduct.

When $SO_4^{\cdot-}$ was generated in the presence of DEPMPO (50 mM) [either by photolytic decomposition of sodium peroxydisulfate (10 mM) ($\lambda > 250$ nm) or reaction of potassium peroxymonosulfate (10 mM) with 2 mM $Fe^{II}SO_4$ or $Ti^{III}Cl_3$] a first-formed adduct was observed [a(N) 1.35, a(H) 0.92, a(P) 4.58, a(γ-H) 0.16, a(γ-H) 0.04, a(γ-H) 0.02 mT; g 2.0061, see Fig. 3] which is attributed to the appropriate (*trans*) adduct (**9**) of the sulfate radical anion (*cf.* our detection of $SO_4^{\cdot-}$ adducts of DMPO[10]). This adduct decayed ($t_{\frac{1}{2}}$ *ca.* 280 s at 293 K), to be replaced by a mixture of the two radicals noted above for the high-valent metal-ion species. When this experiment was carried out in [17]O-enriched water only the hydroxyl adduct **7** showed evidence of enrichment. The behaviour of the $SO_4^{\cdot-}$ adduct can be understood in terms of the formation of a *radical cation* and its hydration to give the 'HO'' adduct (*trans*) **7** and, in addition, the second species. Since the HO-adduct **7** is evidently generated with *trans* stereochemistry, the mechanism for conversion of the $SO_4^{\cdot-}$-adduct **9** to the HO-adduct **7** must be *via* an S_N1 process.
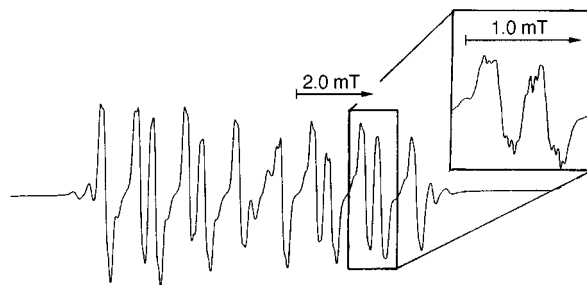
Fig. 3 EPR spectrum obtained immediately on photolysis of $Na_2S_2O_8$ (10 mM) in the presence of DEPMPO (50 mM). Signals are assigned to the $SO_4^{\cdot-}$ adduct of DEPMPO (**9**). The inset shows a portion of the spectrum recorded at lower modulation to reveal extra splittings due to the γ-protons.
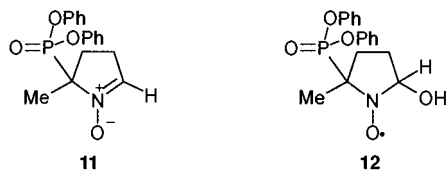

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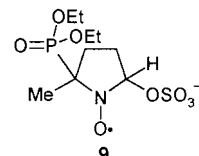
Since the final spectrum obtained *via* reaction of $SO_4^{\cdot-}$ matches precisely those obtained for the high-valent metal ions described above, we conclude that in all of these examples, radical cation formation is involved and the detection of the two radicals is diagnostic of whether a reaction system produces HO' or a one-electron oxidant.‡ We also observed that the ratio of 'HO'' to carbon-centred radical adducts is independent of the concentration of DEPMPO and also the nature of the oxidant. Hence, the formation of the carbon-centred radical is also believed to be a unimolecular process and therefore presumably involves an intramolecular rearrangement. We propose this proceeds *via* intramolecular electron-transfer to give a phosphorus/oxygen-centred radical cation which would be expected to deprotonate readily from the α-carbon of the ethyl group (see *e.g.* ref. 11). This generates a species which has both a carbon-centred radical and a nitrone double bond and therefore might undergo a self-trapping reaction to yield a bicyclic species. Given that the spectrum observed is inconsistent with the bicyclic aminoxyl (as this would be expected to have a very small β-proton splitting since this proton is constrained to lie almost in the plane of the N–O' bond) but is characteristic of a *cis* carbon-centred adduct (*cf.* ref. 12 in which splittings for *cis* aminoxyls are compared with those of the *trans*), we ascribe it to **10** formed by hydrolysis of the bicyclic aminoxyl species (see Scheme 1).
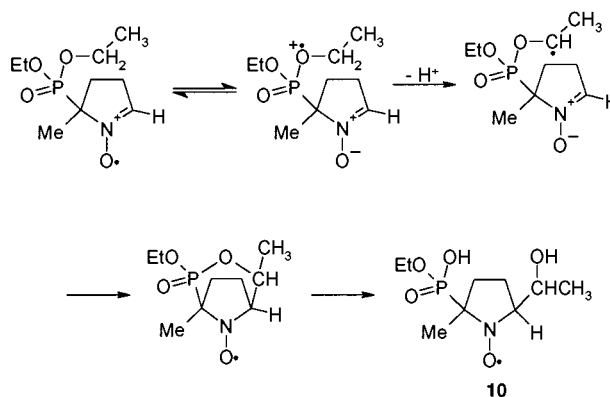
Scheme 1

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‡ Note that this behaviour does not reflect a relative ease of oxidation of DEPMPO given the anodic peak potentials as determined electrochemically in CH_3CN: DMPO $E_a^{ox} = 1.87$ V; DEPMPO $E_a^{ox} = 2.24$ V (*vs.* NHE).[7]

Reactions under similar conditions using the closely related phosphorylated spin-trap **11** (OPMPO), for which such intra-

molecular reaction is precluded, gave only the HO• adduct **12** under all sets of conditions referred to above. This is as expected on the basis of the proposed mechanism described above.

Acknowledgements

References

1 E. G. Janzen, *Acc. Chem. Res.*, 1971, **4**, 31.
2 G. R. Buettner, *Free Radicals Biol. Med.*, 1987, **3**, 259.
3 M. J. Davies and G. S. Timmins, *Adv. Spectrosc.*, 1996, **25**, 217.
4 A. R. Forrester and S. P. Hepburn, *J. Chem. Soc. (C)*, 1971, 701.
5 L. Eberson, J. J. MacCullough and O. Persson, *J. Chem. Soc., Perkin Trans. 2*, 1997, 133.
6 L. Eberson, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1807.
7 B. Tuccio, R. Lauricella, C. Fréjaville, J.-C. Bouteiller and P. Tordo, *J. Chem. Soc., Perkin Trans. 2*, 1995, 295.
8 C. Fréjaville, H. Karoui, B. Tuccio, F. Le Moigne, M. Culcasi, S. Pietri, R. Lauricella and P. Tordo, *J. Chem. Soc., Chem. Commun.*, 1994, 1793.
9 B. C. Gilbert, G. Harrington, G. Scrivens and S. Silvester, in *Free Radicals in Biology and Environment*, ed. F. Minisci, Kluwer Academic Publishers, Netherlands, 1997, pp. 49–62.
10 M. J. Davies, B. C. Gilbert, J. K. Stell and A. C. Whitwood, *J. Chem. Soc., Perkin Trans. 2*, 1992, 333.
11 B. C. Gilbert, P. A. Kelsall, M. D. Sexton, G. D. G. McConnacchie and M. C. R. Symons, *J. Chem. Soc., Perkin Trans. 2*, 1984, 629.
12 F. Le Moigne and P. Tordo, *J. Org. Chem.*, 1994, **59**, 3365.