Hydration of Spin-trap Cations as a Source of Hydroxyl Adducts

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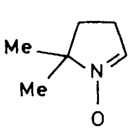
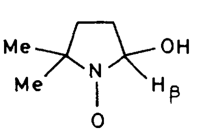
Exposure of dilute solutions of the spin-trap 5,5-dimethyl-1-pyrroline 1-oxide (DMPO) in fluorotrichloromethane containing traces of water to ^{60}Co γ-rays at 77 K gave the DMPO$^{\cdot+}$ cation: annealing gave the four-line e.s.r. spectrum characteristic of the hydroxyl adduct.

Extensive research has established that the best spin-trap for hydroxyl radicals is 5,5-dimethyl-1-pyrroline 1-oxide (DMPO) (**1**).[1,2] The adduct (**2**) is relatively stable and is well characterised by a four-line e.s.r. spectrum (Figure 1) which gives $A(\mathrm{H}_\beta) = A(^{14}\mathrm{N}) = 14.9$ G.†[1,2] This is important since relaxation effects are very efficient for ·OH radicals, and hence they cannot be detected directly by e.s.r. spectroscopy in the liquid-phase. Also, they are so reactive and their solid-state spectra so poorly defined[3] that they are also not detected by rapid-freeze techniques.

Whilst not wishing to question these concepts, our aim is to support a suggestion[4] that ionization followed by reaction with water might also form adduct (**2**), thereby misleading workers into believing that ·OH radicals had been scavenged. We stress that redox chains are of great importance, for example, in mitochondria, and, in view of the low ionization potentials of nitroxide forming spin-traps, involvement of the traps in the redox chain is quite reasonable and hydroxylation would be expected as the major decay pathway, equations (1) and (2), where X$^+$ is part of a biological redox system.



(**1**)



(**2**)

$$\mathrm{DMPO} + \mathrm{X}^+ \leftrightarrows \mathrm{DMPO}^{\cdot+} + \mathrm{X} \qquad (1)$$

$$\mathrm{DMPO}^{\cdot+} + \mathrm{H_2O} \rightarrow \underset{(\mathbf{2})}{\mathrm{DMPO\text{-}OH}} + (\mathrm{H}^+) \qquad (2)$$

It is now well established that dilute solutions of substrates (S) in solvents such as $\mathrm{CFCl_3}$ on exposure to ionizing radiation at low temperatures gives selectively the corresponding radical-cations, (S$^{\cdot+}$) in good yield.[5] This proved to be the case for a range of spin-traps including DMPO.[6] In particular, for DMPO the spectrum of the radical-cation was intense and well defined, being readily interpreted in terms of the expected structure. This spectrum was essentially the same in the presence or absence of traces of water. (In most studies, [DMPO] was ≤ 0.01 mole-fraction, and water was in the same range.) When the system was scrupulously dried, annealing to the melting point gave some weak, complex features due to unidentified nitroxides but no four-line species of the type shown in Figure 1. However, in the presence of traces of water, well-defined quartet features with $A(^1\mathrm{H}) = A(^{14}\mathrm{N}) = 14.9$ G were obtained, together with the other weaker features

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† 1 G = 10^{-4} T.

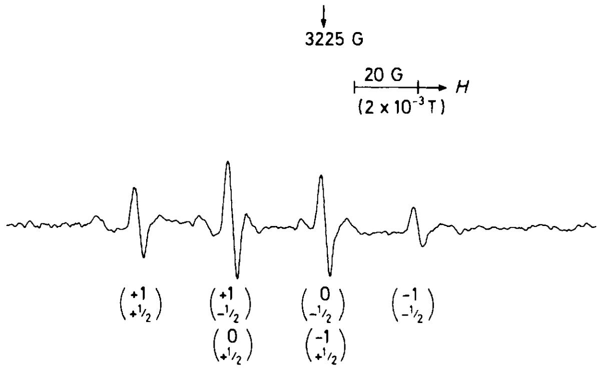

Figure 1. First derivative X-band e.s.r. spectrum for a dilute solution of DMPO in $CFCl_3$ containing water in low concentration after exposure to ^{60}Co γ-rays at 77 K and annealing to *ca.* 270 K. [The quartet, having A_{iso} = 14.9 G is assigned to the OH adduct, (**2**).]

on melting. On warming to room temperature, only the OH adduct was detected but $A(^1H,^{14}N)$ had fallen to *ca.* 13 G. When water was added in higher concentrations, this fall in A_{iso} did not occur.

We interpret this change in A_{iso} in terms of solvation by water of the NO group of the adduct radical. Thus at low temperature, the group is solvated by water, but when $[H_2O]$ is low, this solvation is lost at room temperature and the spectrum is characteristic of the unsolvated adduct.[7,8]

The alternative, that $^{\bullet}OH$ radicals are formed from traces of water, and that these react with the spin-trap is considered to be far less likely. This is because $^{\bullet}OH$ radicals were not detected in the presence or absence of DMPO and, since H_2O has a higher ionization potential than the solvent, formation of $H_2O^{\bullet+}$ (and hence $^{\bullet}OH$) is not possible in $CFCl_3$. (When $[H_2O]$ was so high that phase separation occurred, $^{\bullet}OH$ radicals were detected.) However, these were lost in the range *ca.* 120—130 K, as usual for $^{\bullet}OH$ in ice, the product being H_2O_2.

We conclude that this alternative reaction route to OH adducts must be added to other[9] routes which also do not involve attack by free $^{\bullet}OH$ radicals.

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