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Studies on the photolytic breakdown of hydroperoxides and peroxidized fatty acids by using electron spin resonance spectroscopy

Spin trapping of alkoxyl and peroxyl radicals in organic solvents

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Spin trapping using 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) has been used to detect and distinguish between the carbon-centred, alkoxyl, and peroxyl radicals produced during the photolytic decomposition of hydroperoxides. Photolysis of *tert*-butyl and cumene hydroperoxides, and peroxidized fatty acids, in toluene, with low levels of u.v. light, is shown to lead to the initial production of alkoxyl radicals by homolysis of the oxygen–oxygen bond. Subsequent reaction of these radicals with excess hydroperoxide leads, by hydrogen abstraction, to the production of peroxyl radicals that can be detected as their corresponding adducts with the spin trap. Subsequent breakdown of these adducts produces alkoxyl radicals and a further species that is believed to be the oxidized spin-trap radical 5,5-dimethyl-1-pyrrolidone-2-oxyl. No evidence was obtained at low hydroperoxide concentrations, with either the cumene or lipid alkoxyl radicals, for the occurrence of β-scission reactions; the production of low levels of carbon-centred radicals is believed to be due to the alternative reactions of hydrogen abstraction, ring closure, and/or 1,2 hydrogen shifts. Analogous experiments with 3,3,5,5-tetramethyl-1-pyrroline *N*-oxide (TMPO) led only to the trapping of alkoxyl radicals with no evidence for peroxyl radical adducts; this is presumably due to a decreased rate of radical addition because of increased steric hindrance.

INTRODUCTION

Peroxyl free radicals, $RO_2^{\bullet}$, have been implicated in many oxidative metabolic and degradation reactions and have been shown to be the main chain-carrying species in autoxidation reactions (Howard, 1973). Autoperoxidation, chemically (Witting, 1980) and biologically induced lipid peroxidation (Recknagel *et al.*, 1977; Slater, 1984) and the breakdown of preformed lipid hydroperoxides by metal complexes (Dix & Marnett, 1985; Griffin & Ramirez, 1981; Hrycay & O'Brien, 1971; Kalyanaraman *et al.*, 1983) are believed to proceed via the production and subsequent reactions of peroxyl radicals, as are the aerobic production of hydroperoxides by lipoxygenases (De Groot *et al.*, 1975; Vliegenthart & Veldink, 1982) and formation of prostaglandin H_2 by prostaglandin endoperoxide synthase (Porter, 1980; Rahimtula & O'Brien, 1976).

Unfortunately, unequivocal evidence for the production of peroxyl free radicals in many biological systems has been difficult to obtain; direct detection is usually not possible, and although electron spin resonance studies using the spin traps PBN and MNP have been attempted (for review see Mason, 1984) the results have been disappointing due to the photochemical and thermal instability of the resultant spin adducts (Janzen *et al.*, 1978; Howard & Tait, 1978; Pfab, 1978), though Niki and his co-workers have shown that it is possible to trap both lipid peroxyl ($RO_2^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals by using chemical methods for the generation of $RO_2^{\bullet}$ at room temperature in the dark and the spin traps PBN and MDN (Niki *et al.*, 1983; Ohto *et al.*, 1977; Yamada *et al.*, 1984). Rosen and co-workers have detected a signal, which they assigned to the cumene peroxyl radical adduct, on reaction of cumene hydroperoxide with haematin in the presence of POBN at pH 3, and by use of such model systems have identified a similar signal in rat liver microsomes incubated with CCl_4 and NADPH as being due to lipid peroxyl radicals (Rosen *et al.*, 1980; Rosen & Rauckman, 1981). However, the serious problem of unambiguous assignment of the observed signals to specific species remains, as these particular spin traps are relatively insensitive to changes in the nature of the added radical (Mason, 1984; Niki *et al.*, 1983) and the usefulness of these spin traps in biological systems is limited, especially in processes such as lipid peroxidation where several different types of radicals ($R^{\bullet}$, $RO^{\bullet}$ and $RO_2^{\bullet}$) have been implicated.

To study such systems systematically it is desirable to use a spin trap which can not only give detectable spin adducts with each of the types of radical mentioned above but also characteristic spectra (i.e. have different hyperfine splitting constants allowing unambiguous detection of these types of radicals). To achieve this aim the cyclic nitrones DMPO and TMPO were chosen for further study. Though these traps, like PBN, POBN, and MDN, react with radicals to produce spin adducts where the added radical is β to the nitroxide function, and hence

Abbreviations used: DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; TMPO, 3,3,5,5-tetramethyl-1-pyrroline *N*-oxide; DMPOX, 5,5-dimethyl-1-pyrrolidone-2-oxyl radical; PBN, α-phenyl-*N*-*tert*-butylnitrone; MNP, 2-methyl-2-nitrosopropane; MDN, methyl-*N*-durylnitrone; POBN, α-(4-pyridyl 1-oxide)-*N*-*tert*-butylnitrone.

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gives no additional hyperfine splittings from the added radical (unlike spin adducts formed with MNP where the added radical is α to the nitroxide moiety and usually gives quite considerable fine structure to the spectrum), useful information about the nature of the added radical can be obtained from the size of the β-hydrogen splitting because of the cyclic nature of these traps. For instance, it is known that the spectral parameters of cyclic nitroxide radicals can be markedly affected by the size and nature of substituents, due to their influence on the degree of overlap between the singly-occupied orbital and, in this case, the β C–H bond (Janzen *et al.*, 1973); thus on changing the added radical from $RO_2^{\bullet}$ to $R^{\bullet}$ it might be expected that the size of the β-hydrogen splitting would vary markedly, resulting in a useful way of distinguishing between each of these types of radical.

Previous work with these spin traps has shown that alkoxyl and carbon-centred radicals, produced by using model systems in organic solvents, do indeed give spin adducts with markedly different hyperfine coupling constants (Janzen & Liu, 1973; Janzen *et al.*, 1981; Schaich & Borg, 1980), and recent reports on the spin trapping of the cumene peroxyl radical (at pH 3) and the ethyl peroxyl radical in aqueous solution with DMPO suggest that this might also be the case with peroxyl radical adducts (Kalyanaraman *et al.*, 1983; Rosen & Rauckman, 1980). However, it is impossible to compare directly the coupling constants obtained in these studies due to the different solvents used, and thus, because of the paucity of data, further work has been done to clarify the situation.

MATERIALS AND METHODS

E.s.r. spectra were recorded on an X-band Bruker ER200D spectrometer equipped with 100 kHz modulation, an ER 4102 ST universal cavity, and a Bruker ERO35M Gaussmeter for field calibration. Hyperfine coupling constants ($\pm$0.005 mT) were measured directly from the spectra using 1 mT Gaussmeter marker signals for calibration. Spectra were recorded at room temperature using solutions of the spin trap (final concentration 50 mM) and the hydroperoxide or peroxide in thoroughly degassed toluene (20 min bubbling with O_2-free N_2), contained in e.s.r. Suprasil tubes, and prepared immediately before use. Photolysis of the samples was carried out by using the focused, but unfiltered, output of a Heraeus 200 W mercury/xenon arc (for wavelength profile see Kaiser *et al.*, 1978) incident through a 50% transmission grating on the sample tube inserted into the cavity of the spectrometer. Spectral simulations were carried out on a BBC microcomputer equipped with a Torch Z80 disc pack and Watanabe MP1000 plotter using programs kindly supplied by Dr. E. L. Short and Dr. K. A. K. Lott (Chemistry Department, Brunel University).

Chemicals were obtained from Fluka (cumene hydroperoxide), Sigma Chemical Co. (oleic, linoleic, linolenic and arachidonic acids), Aldrich Chemical Co. (*tert*-butyl hydroperoxide, DMPO, TMPO), Pfalz and Baeur Inc., Stamford, CT, U.S.A. (dicumyl peroxide), and BDH (all other chemicals) and used as supplied with the exception of the fatty acids, which were allowed to peroxidize before use by exposure to air for 72 h at room temperature (O'Brien, 1969). No experimental differences were observed between using partially and highly purified (using the method of O'Brien, 1969) fatty acid hydroperoxide samples.

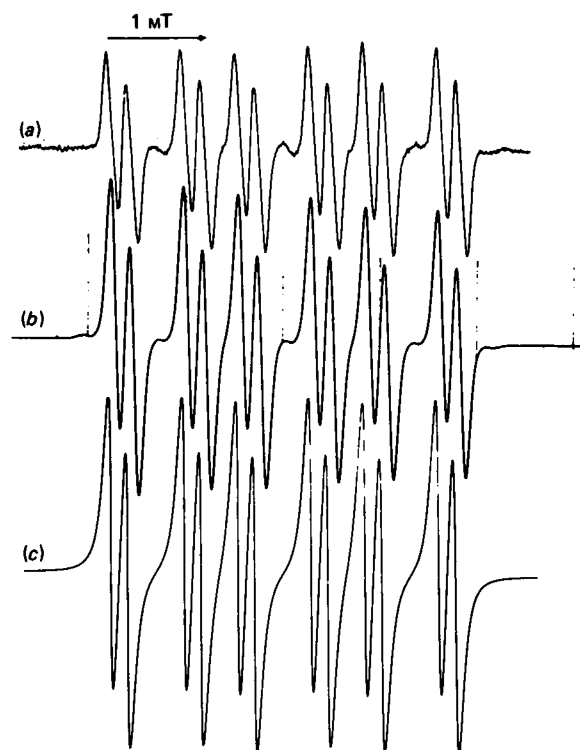


Fig. 1. E.s.r. spectra observed on photolysis of *tert*-butyl hydroperoxide and di-*tert*-butyl peroxide in the presence of DMPO

(*a*) Spectrum observed on photolysis of *tert*-butyl hydroperoxide (1 mM) with a mercury/xenon arc in deoxygenated toluene in the presence of DMPO (50 mM) and assigned to the alkoxyl radical adduct. The instrumental parameters were: gain 4×10^5, modulation amplitude 0.0125 mT, time constant 0.5 s, scan time 500 s, field 347 mT, scan range 6 mT, microwave power 13 dB, frequency 9.71 GHz. (*b*) As (*a*) but with di-*tert*-butyl peroxide in place of *tert*-butyl hydroperoxide. Spectrum assigned to the alkoxyl radical adduct. (*c*) Simulation of the alkoxyl radical adduct using the parameters listed in Table 1.

RESULTS

DMPO as spin trap

***tert*-Butyl hydroperoxide.** Exposure of dilute solutions of *tert*-butyl hydroperoxide (50 μM) and DMPO (50 mM) in deoxygenated toluene to low levels of u.v. light resulted in the detection of what appears to be a single radical adduct whose spectrum is shown in Fig. 1(*a*). The e.s.r. parameters of this radical (a_N 1.308, a_H 0.744, a_H 0.168 mT: see Table 1 for list of all e.s.r. parameters) are inconsistent with the trapping of a carbon-centred species such as the methyl radical (which might be formed by β-scission of initially formed alkoxyl radicals) or benzyl radical (from reaction of a substrate derived radical with the solvent), as the size of the β-hydrogen splitting in this case (0.744 mT) is markedly different from the parameters expected for these species where

Table 1. E.s.r. parameters of oxygen-centred radical adducts to DMPO and TMPO

Spin trap	Radical added to trap	Solvent	Hyperfine coupling constants (mT) (±0.005 mT)			Reference*
			a_N	a_H	a_H	
DMPO	MeO˙	Benzene	1.358	0.761	0.185	*a*
DMPO	EtO˙	Benzene	1.322	0.696	0.189	*a*
DMPO	EtO_2˙	Water (pH 7.4)	1.46	1.10	0.125	*b*
DMPO	BuO˙	Benzene	1.361	0.683	0.206	*a*
DMPO	*tert*-BuO˙	Benzene	1.311	0.793	0.197	*a*
DMPO	*tert*-BuO˙	Toluene	1.308	0.744	0.168	*c*
DMPO	*tert*-BuO_2˙	Toluene	1.272	0.936	0.144	*c*
DMPO	$PhC(CH_3)_2O$˙	Toluene	1.308	0.888	0.168	*c*
DMPO	$PhC(CH_3)_2O_2$˙	Toluene	1.392	1.120		*c*
DMPO	$PhC(CH_3)_2O_2$˙	Water (pH 3.0)	1.45	1.075	0.175	*d*
DMPO	Oleic alkoxyl	Toluene	1.284	0.648	0.168	*c*
DMPO	Linoleic alkoxyl	Toluene				
DMPO	Linolenic alkoxyl	Toluene				
DMPO	Arachidonic alkoxyl	Toluene				
DMPO	Oleic peroxyl	Toluene	1.480	1.260		*c*
DMPO	Linoleic peroxyl	Toluene				
DMPO	Linolenic peroxyl	Toluene				
DMPO	Arachidonic peroxyl	Toluene				
TMPO	*tert*-BuO˙	Benzene	1.331	0.581		*e*
TMPO	*tert*-BuO˙	Toluene	1.328	0.542		*c*
TMPO	$PhC(CH_3)_2O$˙	Toluene	1.312	0.456		*c*
TMPO	Oleic alkoxyl	Toluene	1.312	0.432		*c*
TMPO	Linoleic alkoxyl	Toluene	1.328	0.432		*c*
TMPO	Linolenic alkoxyl	Toluene	1.328	0.432		*c*
TMPO	Arachidonic alkoxyl	Toluene	1.328	0.456		*c*

(The values for the four DMPO alkoxyl entries and the four DMPO peroxyl entries are printed once, bracketed for each group.)

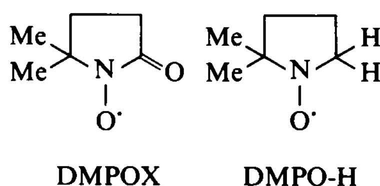
* References: *a*, Janzen & Liu (1973); *b*, Kalyanaraman *et al.* (1983); *c*, this work; *d*, Rosen & Rauckman (1980); *e*, Janzen *et al.* (1981).

β-hydrogen splittings of approx. 2.0 mT have been obtained in benzene (Janzen & Liu, 1973). The parameters for the adduct in Fig. 1(*a*) are similar, however, to those observed previously on addition of ^{t}BuO˙ and EtO˙ radicals to DMPO in benzene [which give adducts with β-hydrogen splittings of 0.793 and 0.696 mT respectively (Janzen & Liu, 1973)], and this would suggest that the species observed in this case is also the alkoxyl radical adduct formed by homolytic cleavage of the O–O bond in the hydroperoxide and subsequent addition of the alkoxyl radical to the trap. The lack of signals from a hydroxyl radical adduct to the trap, which would be expected if homolytic O–O cleavage is occurring, is not unexpected in view of the very high rate constant for addition of the hydroxyl radical to toluene ($5 \times 10^9\ dm^3 \cdot mol^{-1} \cdot s^{-1}$; Farhataziz & Ross, 1977) and the relative concentrations of the spin trap and solvent. In contrast, alkoxyl radicals such as ^{t}BuO˙are known to add only slowly to aromatic rings (Kochi, 1973). Evidence consistent with the identity of this radical adduct as being due to the interaction of DMPO with the alkoxyl radical $(CH_3)_3CO$˙ and not the peroxyl species $(CH_3)_3CO_2$˙ was obtained from studying the photolysis of di-*tert*-butyl peroxide [where only $(CH_3)_3CO$˙ is formed to any significant extent] and the effects of varying the concentration of the hydroperoxide on the observed signals. Photolysis of di-*tert*-butyl peroxide (1 mM) in degassed toluene in the presence of DMPO (50 mM) led to the observation of an identical spectrum (see Fig. 1*b*) to that observed with *tert*-butyl hydroperoxide at a concentration of 50 μM, supporting the identification of this species as the *tert*-butoxyl radical adduct.

Increasing the concentration of the hydroperoxide had a marked affect on the observed signals: when the concentration of the hydroperoxide was increased to 80 mM additional lines in the e.s.r. spectrum were observed (see Figs. 2*a* and 2*b*), and these new lines became more pronounced as the hydroperoxide concentration was further increased until at hydroperoxide concentrations greater than 500 mM only the second species was observed (Fig. 2*c*). These lines are components of a spectrum with parameters a_N 1.272, a_H 0.936 and a_H 0.144 mT, which are consistent with the added radical having a heteroatom centre (Janzen & Liu, 1973). This species is believed to be the previously unobserved peroxyl radical adduct formed by reaction of the initially formed alkoxyl radicals with more *tert*-butyl hydroperoxide by hydrogen abstraction, a process known to occur at high hydroperoxide concentrations (Howard, 1973; Bors *et al.*, 1984). These values (with toluene as solvent) are smaller than those previously reported for the ethyl and cumene peroxyl radical adducts in water, as would be expected from the decreased solvent polarity.

Unlike the alkoxyl radical adduct, which decayed only slowly on removal of the initiating light source and could be detected for several days when kept in darkness, the peroxyl radical adduct proved to be relatively unstable and decayed rapidly when the u.v. light beam was

interrupted; despite this it could still be detected for up to 1 h after generation. The decay of this adduct produced two further signals (see Fig. 3), the first of which could be easily identified as being due to the alkoxyl radical adduct. The second species, which could not be definitively assigned due to the overlapping nature of the signals in the centre of the spectrum, is characterized by having an odd number of spectral lines (i.e. it is symmetrical around a single central line), showing that the unpaired electron must be interacting with either a single nitrogen or a single nitrogen and an even number of hydrogens. The two most likely candidates for this species are the oxidized and reduced forms of the spin trap (DMPOX and DMPO-H respectively):



DMPOX DMPO-H

The latter of these two species is known to have large splittings from the two β-hydrogens (of approx. 1.889 mT; Janzen & Liu, 1973) producing spectra with very large overall spectral widths (5.22 mT; Janzen & Liu, 1973); the absence of such widely spaced lines, which would appear at magnetic field positions far removed from the alkoxyl and peroxyl radical adduct signals, suggests that the observed signal is due to the oxidized form of the trap DMPOX.

The observation of these two further signals supports the assignment of the initially observed signal as being due to the peroxyl radical adduct, in that it is known that the cumene peroxyl radical adduct to DMPO in water decomposes to give DMPOX (Rosen & Rauckman, 1980) and that peroxyl radical adducts to other nitroso and nitrone spin traps decompose to yield further alkoxyl radicals and oxidized spin trap species presumably via homolytic cleavage (photolytically or thermally induced) of the O–O bond in the adduct (Howard & Tait, 1978; Pfab, 1978; Niki *et al.*, 1983; Davies & Slater, 1986). In this respect the peroxyl radical adducts appear to behave in a similar manner to the superoxide adduct to the same trap which is known to decompose to give the hydroxyl radical adduct (Finkelstein *et al.*, 1979).

Cumene hydroperoxide. Similar behaviour to that observed with *tert*-butyl hydroperoxide was observed with, at low concentrations of the hydroperoxide, a strong signal being observed whose parameters (see Table 1) are similar to those observed for the *tert*-butoxyl radical adduct. An identical signal was observed on photolysis of dicumyl peroxide (1 mM) where only $PhC(CH_3)_2O^{\cdot}$ radicals are expected to be formed, suggesting that this species is the alkoxyl radical adduct. Increasing the concentration of the hydroperoxide above 200 mM led to a decrease in the intensity of these lines and the appearance of a further signal whose parameters (see Table 1) suggest, by analogy with the *tert*-butyl hydroperoxide system, the trapping of the corresponding peroxyl radical [$PhC(CH_3)_2O_2^{\cdot}$], which subsequently breaks down to give the alkoxyl radical and a further signal which may be due to DMPOX.

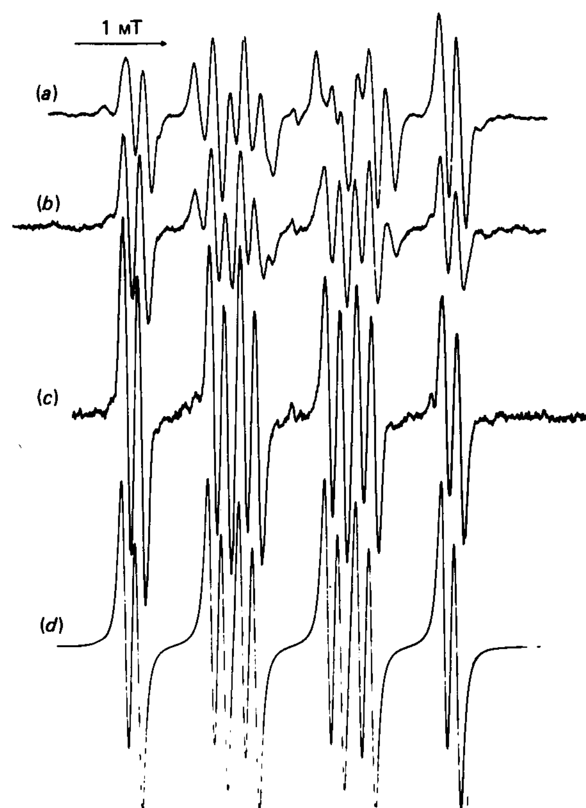


Fig. 2. E.s.r. spectra observed on photolysis of high concentrations of *tert*-butyl hydroperoxide in the presence of DMPO

(*a*) Spectrum observed on photolysis of *tert*-butyl hydroperoxide (100 mM) with a mercury/xenon arc in deoxygenated toluene in the presence of DMPO (50 mM). The instrumental parameters were the same as in Fig. 1(*a*) except gain 1.6×10^5, and time constant 0.2 s. (*b*) As (*a*) except with *tert*-butyl hydroperoxide (220 mM) and gain 2.5×10^5, time constant 0.2 s. (*c*) As (*a*) except with *tert*-butyl hydroperoxide (500 mM) and gain 5×10^5, time constant 0.2 s. The additional lines to those in Figs. 1(*a*) and 1(*b*) which grow in as the hydroperoxide concentration is increased, are assigned to the peroxyl radical adduct. (*d*) Simulation of the peroxyl radical adduct using the parameters listed in Table 1.

Peroxidized fatty acids. Analogous reactions to those observed with both *tert*-butyl and cumene hydroperoxides were detected with each of the peroxidized fatty acids studied (oleic, linoleic, linolenic and arachidonic acids) though additional radicals were also detected. Thus when either peroxidized oleic acid (10 μl) or the pure hydroperoxide (50 μM) was added to 0.8 cm³ of a degassed solution of DMPO (50 mM) in toluene and exposed to low levels of u.v. light, the e.s.r. spectra of three different radicals were obtained (see Fig. 4*a*). The major spectrum present has e.s.r. hyperfine coupling constants similar to those observed for the *tert*-butoxyl, ethoxyl and cumene alkoxyl radical adducts, suggesting that this species is the corresponding alkoxyl radical species. The second species has parameters of a_N 1.480 and a_H 1.260 mT and, as with the other hydroperoxides studied, the relative concentration of this radical increased markedly as the amount of peroxidized fatty acid added was increased (Fig. 4*b*) and on the basis of

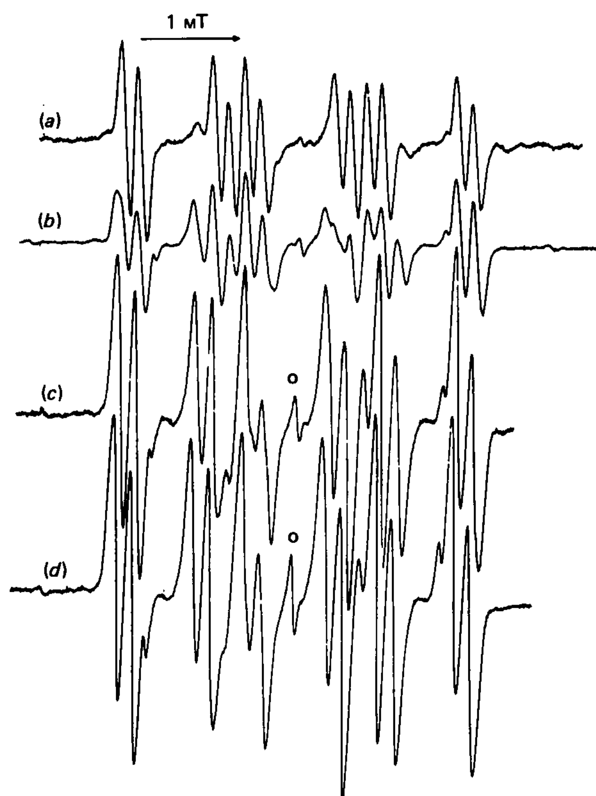


Fig. 3. Decay of the signal assigned to the *tert*-butyl peroxyl radical adduct

(*a*) E.s.r. spectrum observed on photolysis (see the Materials and methods section) of *tert*-butyl hydroperoxide (350 mM) in the presence of DMPO (50 mM), and assigned to the *tert*-butyl peroxyl radical adduct. Instrumental parameters were as in Fig. 1(*a*) except gain 2×10^5 and time constant 0.2 s. (*b*) Identical to (*a*) except scan started 8 min after cessation of photolysis and gain 1.25×10^5. (*c*) As (*a*) except scan started 16 min after cessation of photolysis and gain 2×10^5. (*d*) As (*c*) except that scan started 90 min after photolysis stopped. Additional lines which appear on decay of the peroxyl radical adduct are assigned to the alkoxyl radical adduct (simulated in Fig. 1*c* using the parameters in Table 1) and possibly DMPOX (line marked O and further lines obscured by lines from the alkoxyl radical adduct).

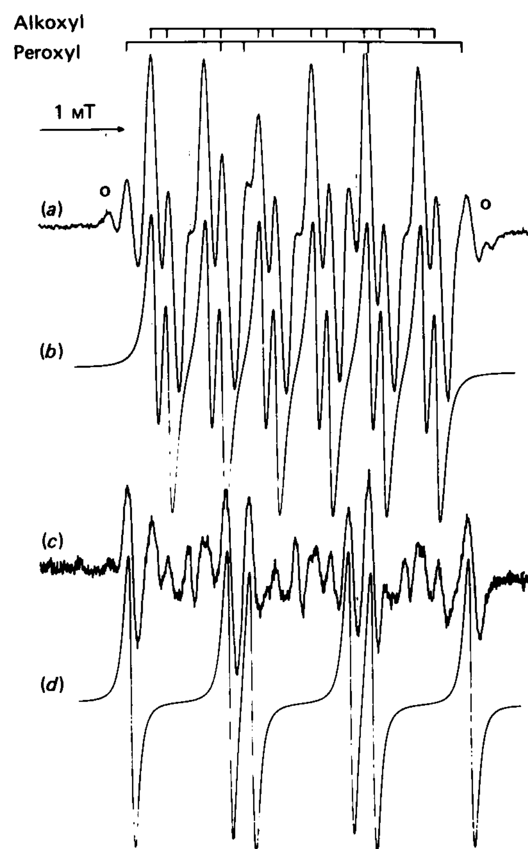


Fig. 4. E.s.r. spectra observed on photolysis of oleic acid hydroperoxide

(*a*) Spectrum obtained on photolysis of 10 μl of peroxidized oleic acid in deoxygenated toluene in the presence of DMPO (50 mM) and assigned to a mixture of alkoxyl, peroxyl and carbon-centred radical adducts. Analysis of the spectrum in terms of lines from the alkoxyl and peroxyl radical species is as shown by the stick diagram; lines marked O are assigned to a carbon-centred radical adduct. Instrumental settings were as in Fig. 1(*a*) except gain 5×10^5 and modulation amplitude 0.1 mT. (*b*) Simulation of the spectrum assigned to the alkoxyl radical adduct using the parameters in Table 1. (*c*) As (*a*) except 100 μl of peroxidized oleic acid and gain 2×10^6, time constant 1.0 s, and scan time 1000 s. (*d*) Simulation of the peroxyl radical adduct using the parameters listed in Table 1.

this evidence and the similarity in parameters with the cumene peroxyl radical adduct, this signal is assigned to the oleic acid peroxyl radical adduct. The third spectrum, which was apparent only at a very low intensity in the wings of the total combined spectrum, had an overall width of approx. 4.8 mT. Complete analysis of this spectrum was not possible due to the overlapping nature of the lines in the centre of the spectrum, but if, as is usual with nitroxides derived from this trap, it has a nitrogen hyperfine coupling constant of approx. 1.4 mT then it would have a hydrogen splitting constant of approx. 2.0 mT which would identify it as a carbon-centred radical.

Very similar behaviours were observed with peroxidized linoleic, linolenic and arachidonic acids (or the pure hydroperoxides) where, in each case, signals assignable to the corresponding alkoxyl and peroxyl radical adducts to the spin trap were observed as well as weak signals from unidentified carbon-centred radicals. The parameters of the alkoxyl and peroxyl radical adducts (see Table 1) were, within experimental error, identical with those observed with peroxidized oleic acid and this is as expected in view of the similarity of the carbon chains in each case. A similar consistency of e.s.r. parameters has been observed by Yamada *et al.* (1984) with other trapped peroxyl and alkoxyl radicals using the spin traps PBN and MDN.

TMPO as spin trap

***tert*-Butyl hydroperoxide.** Photolysis of either *tert*-butyl hydroperoxide in the concentration range 20 μM–500 mM or di-*tert*-butyl hydroperoxide (1 mM) in toluene in the presence of TMPO (50 mM) produced identical spectra of a single species with hyperfine

coupling constants of a_N 1.328 and a_H 0.542 mT; the observation of identical spectra with both of these precursors and the similarity of the parameters with those previously reported for the *tert*-butoxyl radical adduct in benzene (Janzen *et al.*, 1981) suggest that this species is the alkoxyl radical adduct. The intensity of the signal obtained from photolysis of the hydroperoxide decreased at high hydroperoxide concentrations (greater than 100 mM) suggesting that further reactions of the initially generated alkoxyl radical are occurring (hence lowering the spin adduct concentration), and this is believed to be due to reaction of these species with more hydroperoxide to give the peroxyl radical (Howard & Tait, 1978; Bors *et al.*, 1984) in the same manner as observed with DMPO as the spin trap. However, even at very high hydroperoxide concentrations (up to 2 M; higher than those needed to observe the peroxyl radical adduct in analogous experiments with DMPO) no further signals were observed. This suggests that the decrease in rate of trapping of the peroxyl radicals that occurs with this trap due to increased steric interactions with the two additional methyl groups in the 3-position on the ring outweighs any increase in stability of the radical adduct due to the decreased rate of disproportionation (Ingold, 1973; Janzen *et al.*, 1981), leading to a steady state concentration of the peroxyl radical adduct that is too low to detect.

Cumene hydroperoxide. Analogous behaviour to that determined for *tert*-butyl hydroperoxide was observed, with both the hydroperoxide (in the concentration range 20 μM–1 M) and dicumyl peroxide (1 mM) giving rise to a single detectable species whose parameters (a_N 1.312 and a_H 0.456 mT) are similar to those measured for the *tert*-butoxyl radical adduct with the same trap, and is therefore believed to be due to the corresponding alkoxyl radical species. Increasing the hydroperoxide concentration led to a similar decrease in signal intensity, but no further signals which could be assigned to a peroxyl radical adduct were observed, presumably for similar reasons.

Fatty acid hydroperoxides. Photolysis of any of the pure hydroperoxides or peroxidized fatty acids (10μl or 50 μM) in toluene in the presence of TMPO (50 mM) gave only weak signals from a single species in each case whose parameters (see Table 1) are similar to those observed for the corresponding alkoxyl radical adducts from *tert*-butyl and cumene hydroperoxides, and a similar assignment is therefore made. No evidence was obtained for peroxyl radical adducts as expected on the basis of the experiments conducted with the model hydroperoxides, as the steric hindrance to addition of a peroxyl radical will be of even greater significance with these substrates.

DISCUSSION

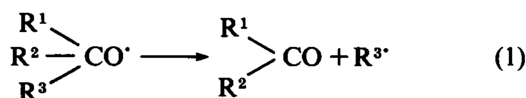
The results obtained in this study show that the spin trap DMPO is not only a good trap for alkoxyl and peroxyl radicals produced by photolysis at room temperature in toluene, but also, because of the cyclic nature of the trap, produces different spectra with each of the three main types of radical studied ($R^{\cdot}$, $RO^{\cdot}$, $RO_2^{\cdot}$). Thus addition of carbon-centred radicals to the spin trap produces adducts with β-hydrogen splittings of the order of 2.0 mT, whereas alkoxyl and peroxyl radical adducts give spectra that have β-hydrogen splittings of 0.6–0.9 mT and 0.9–1.2 mT respectively. This spin trap should prove to be of some considerable use in specifically identifying these different radicals in peroxidation reactions, though care must still be taken in attempting to distinguish between alkoxyl and peroxyl radical adducts because of the relatively small difference between the values of the β-hydrogen splitting for these two types of adduct; this difference is however much more marked than that seen with other spin traps such as PBN and MDN (Niki *et al.*, 1983; Ohto *et al.*, 1977; Yamada *et al.*, 1984) and would therefore appear to be the spin trap of choice for trapping these species. Results obtained with aqueous and microsomal systems back-extracted into toluene (M. J. Davies & T. F. Slater, unpublished work) show that this is indeed the case. This trap has the added advantages over spin traps such as PBN, POBN, and MNP in being far less inhibitory on certain enzyme systems, such as cytochrome P_{450} (Augusto *et al.*, 1982), and of being readily taken up by whole cell systems (Morgan *et al.*, 1985), thus allowing greater concentrations of the spin trap to be present during the reaction(s) under study.

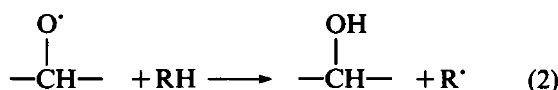
The tetramethylated analogue TMPO has also been shown to trap alkoxyl radicals, though its usefulness as a trap for studying peroxidation reactions will, presumably, be more limited due to its apparent inability to trap peroxyl radical species rapidly, which results in steady state concentrations of peroxyl radical adducts which are too low to detect with present instrumentation.

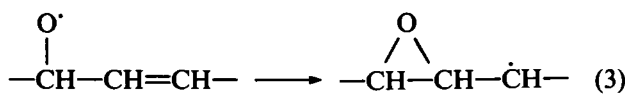
The increase in the β-hydrogen splitting on going from the alkoxyl radical adduct to the peroxyl radical adduct is the opposite of what might have been expected from consideration of the parameters of the corresponding unsubstituted species, namely the hydroxyl and the hydroperoxyl radical adducts which have a_H values of 1.17 mT and 0.69 mT respectively (Janzen, 1980). This reversal is understandable in terms of the ability of these latter species to hydrogen-bond to the nitroxide function, a process that will markedly affect the conformation of the radical adduct and presumably result in increased overlap between the nitroxide group and the β-hydrogen atom, yielding the larger splitting constants for these substituents.

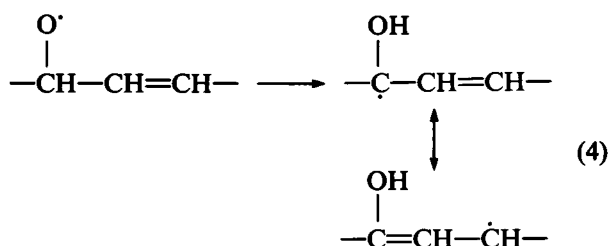
The absence of any signal assignable to the methyl radical adduct in the breakdown of cumene hydroperoxide suggests, since this species is known to add readily to DMPO and is easily identifiable, that there is little propensity for the formation of this radical by β-scission of the tertiary alkoxyl radical (reaction 1). The cumene alkoxyl radical therefore appears to behave in a similar fashion to the $^{t}BuO^{\cdot}$ radical, which fragments rapidly to the methyl radical and propanone in aqueous but not organic solvents (Bors *et al.*, 1984). β-Fragmentation of secondary alkoxyl radicals is known to be even less favourable than with the tertiary species (Gray & Williams, 1959), suggesting that the carbon-centred radicals detected with the peroxidized fatty acids (which will yield secondary alkoxyl radicals) do not arise via a β-fragmentation process, and this would appear to suggest that this type of reaction mechanism does not occur rapidly in lipid membranes, though the observed products suggest that this does occur to at least some extent (Schaich, 1980). The identity of these carbon-centred radicals remains unknown, though it is possible that they are formed by intra- or inter-molecular

hydrogen abstraction from other lipid molecules by alkoxyl radicals (reaction 2), or for the polyunsaturated species by ring closure reactions (reaction 3; Dix & Marnett, 1981) or 1,2-hydrogen shifts (reaction 4), a process known to occur very rapidly with this type of radical (Gilbert *et al.*, 1976). This last process would result in the production of a highly stabilized α-hydroxyl-substituted allyl radical; the stability of this species together with the unimolecular nature of its formation suggests that this last reaction would be highly favoured.









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