# Synthesis and Radical-addition Stereochemistry of Two Trimethyl-1-pyrroline 1-Oxides as Studied by EPR Spectroscopy

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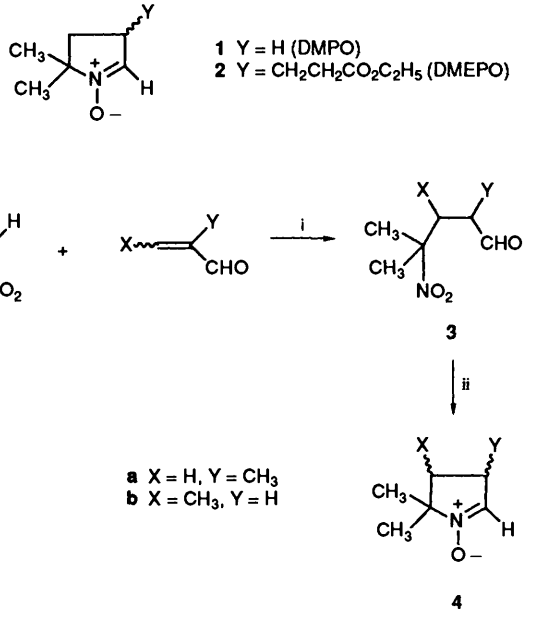
A two-step synthetic route has been applied to the preparation of two five-membered ring nitrone spin traps, 3,5,5-trimethyl-1-pyrroline 1-oxide (3-Me-DMPO) and 4,5,5-trimethyl-1-pyrroline 1-oxide (4-Me-DMPO). A general method has been developed for the purification of the aqueous nitrone solution, which is suitable for the trapping of radicals present in biological processes. The EPR spin-trapping stereochemistry of 3- and 4-Me-DMPO has been investigated. The addition of the radicals *OH, RC*HOH, tetrahydrofuranyl and *OBu$^t$, to Me-DMPO is stereoselective or stereospecific. Hydrogen atom addition to 3-Me-DMPO and 4-Me-DMPO gives aminoxyls **9** and **10**, respectively, which each contain two non-equivalent β-protons.

The spin-trapping technique[1] is now widely used to study transient free radicals in various systems.[2] Since 1973 when 5,5-dimethyl-1-pyrroline 1-oxide (DMPO) was first employed as a spin trap,[3] many DMPO analogues[4–12] have been synthesized and used to detect and identify a large number of transient free radicals. However, care must be exercised in the interpretation of the EPR spectra of the resulting spin adducts when multisubstituted five-membered ring nitrones, containing one or more asymmetric carbons within the ring, are used. Barker *et al.*[9] and Zhang *et al.*[6–8] have previously reported that an asymmetric carbon atom in the ring of some DMPO analogues can induce stereospecific radical-addition. They have studied the spin-trapping stereochemistry of the nitrone in which the asymmetric carbon is at the 3-position[8] or the 5-position of the ring.[6,7,9] However, the radical-addition stereochemistry of a nitrone containing an asymmetric carbon at the 4-position, has not, to our knowledge, been previously reported. In this paper we present the results of a study of the spin-trapping stereochemistry of 3,5,5-trimethyl-1-pyrroline 1-oxide (3-Me-DMPO, **4a**) and 4,5,5-trimethyl-1-pyrroline 1-oxide (4-Me-DMPO, **4b**) A new synthetic method and purification procedure for the two nitrones are also described.




**Scheme 1** *Reagents:* i, $CH_3ONa$ in $CH_3OH$; ii, Zn, AcOH in 95% $C_2H_5OH$.

## Experimental

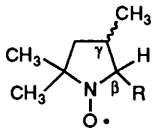
*Instrumentation.*—IR spectra were recorded on a Carl Zeiss Jena Specord-75 spectrometer. Mass spectra were obtained using an AEI MS-50 spectrometer. $^1H$ NMR spectra were determined on a Varian EM-360 (60 MHz) spectrometer using carbon tetrachloride as a solvent and tetramethylsilane as an internal standard. EPR spectra were recorded on a Bruker ESP-300 EPR spectrometer at room temperature. UV spectra were acquired with a Hitachi-340 ultraviolet spectrometer.

*Synthesis.*—The two-step synthetic route illustrated in Scheme 1 has not to our knowledge been previously applied to the preparation of 3-Me-DMPO (**4a**) and 4-Me-DMPO (**4b**). 5,5-Dimethyl-1-pyrroline 1-oxide (DMPO) was obtained as described previously.[13,14]

2,4-Dimethyl-4-nitropentanal (**3a**) was prepared by a procedure similar to that described previously.[15] Yield: 18%, b.p. 123–125 °C (18 mmHg); $\delta_H$ 1.12 (3 H, d, 2-$CH_3$), 1.50 (3 H, s, $CH_3$), 1.58 (3 H, s, $CH_3$), 1.73–2.70 (3 H, m, CH, $CH_2$) and 9.50 (1 H, s, CHO). 3,5,5-Trimethyl-1-pyrroline 1-oxide (**4a**)[15] was prepared by a procedure similar to that used for the synthesis of other DMPO-type nitrones.[8,13] Yield: 64%; b.p. 94–104 °C (3 mmHg); $\nu_{max}$(neat)/cm$^{-1}$ 3060, 2961, 2931, 2867, 1575 (C=N), 1450, 1361 and 1217 (N–O); $\delta_H$ 1.18 (3 H, d, 3-$CH_3$), 1.30 (3 H, s, 5-$CH_3$), 1.35 (3 H, s, 5-$CH_3$), 2.13–2.47 (2 H, q, 4-$CH_2$), 2.60–3.10 (1 H, m, 3-CH) and 6.44 (1 H, d, CH=N); *m/z* 128 ($M^+ + 1$, 10%), 127 ($M^+$, 100), 112 ($M^+ - 15$, 24), 97 ($M^+ - 30$, 22), 71 (87) and 55 (96); $\lambda_{max}(H_2O)$/nm 229.2.

3,4-Dimethyl-4-nitropentanal (**3b**) was obtained by a procedure similar to that reported previously.[16] Yield: 61%; b.p. 110 °C (3 mmHg); $\delta_H$ 0.92 (3 H, d, 3-$CH_3$), 1.50 (6 H, s, 2 × $CH_3$), 2.20–2.83 (3 H, m, CH, $CH_2$) and 9.63 (1 H, s, CHO). The reduction procedure used for the preparation of (**4a**) was undertaken to prepare 4,5,5-trimethyl-1-pyrroline 1-oxide (**4b**).[16] Yield: 62%; b.p. 101 °C (3 mmHg); $\nu_{max}$(neat)/cm$^{-1}$ 3046, 2961, 1571 (C=N), 1449, 1367 and 1220 (N–O); $\delta_H$ 1.07 (3 H, d, 4-$CH_3$), 1.13 (3 H, s, 5-$CH_3$), 1.29 (3 H, s, 5-$CH_3$), 2.00–2.77 (3 H, m, CH, $CH_2$) and 6.72 (1 H, s, CH=N); *m/z* 128 ($M^+ + 1$, 10%), 127 ($M^+$, 100), 112 ($M^+ - 15$, 7), 70 (49), 69 (39) and 55 (95); $\lambda_{max}(H_2O)$/nm 230.2.

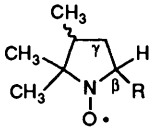
**Table 1** EPR hyperfine splitting constants for spin adducts of 3,5,5-trimethyl-1-pyrroline 1-oxide (3-Me-DMPO)[a]



| Source and solvent | Radical (R•) | *trans*-isomer | | | *cis*-isomer | | Ratio[b] |
|---|---|---|---|---|---|---|---|
| | | $a_N$ | $a_H^\beta$ | $a_H^\gamma$ | $a_N$ | $a_H^\beta$ | |
| 5% $H_2O_2$ + $Fe^{2+}$, $H_2O$[c] | •OH | 14.70 | 8.96 | 0.98 (1 H) | 15.08 | 20.85 | 1.16–1.75:1 |
| 3% $H_2O_2$, *hv* | •OH | 14.61 | 8.77 | 0.89 (1 H) | 14.93 | 20.58 | 2.70:1 |
| $K_2S_2O_8$, $H_2O$ | •OH | 13.60 | 6.56 | 1.49 (1 H) | | | |
| DBPO,[d] $C_6H_6$, *hv* | •OBu$^t$ | 13.26 | 4.89 | 1.33 (1 H) | 13.37 | 15.08 | 1:1.60 |
| DBPO + THF, $C_6H_6$, *hv* | •OBu$^t$ | 12.91 | 4.65 | *e* | | | |
| DBPO + EtOH, $C_6H_6$, *hv* | •OBu$^t$ | 13.43 | 4.82 | *e* | 14.02 | 15.65 | 1.50:1 |
| DBPO + Bu$^n$OH, $C_6H_6$, *hv* | •OBu$^t$ | 13.31 | 4.58 | *e* | 13.75 | 15.59 | 5.10:1 |
| DBPO + Pr$^i$OH, $C_6H_6$, *hv* | •OBu$^t$ | 13.50 | 4.69 | 1.47 (1 H) | 13.62 | 15.78 | 1:1.60 |

[a] The hfs values are given in gauss (1 gauss = 0.1 mT). [b] The ratio is calculated from the EPR signal height of the *trans*-isomer relative to that of the *cis*-isomer. [c] The salt of ammonium iron(II) sulphate was used as the source of iron(II) cation. [d] DBPO is the abbreviation of di-*tert*-butylperoxide. [e] The spectral signal was too broad to accurately determine this parameter.

**Table 2** EPR hyperfine splitting constants for spin adducts of 4,5,5-trimethyl-1-pyrroline 1-oxide (4-Me-DMPO)[a]



| Source and solvent | Radical (R•) | *cis*-isomer | | | *trans*-isomer | | Ratio[b] |
|---|---|---|---|---|---|---|---|
| | | $a_N$ | $a_H^\beta$ | $a_H^\gamma$ | $a_N$ | $a_H^\beta$ | |
| 5% $H_2O_2$ + $Fe^{2+}$, $H_2O$[c] | •OH | 14.77 | 10.72 | 1.14 (1 H) | 14.98 | 18.88 | 1.77–1.94:1 |
| 3% $H_2O_2$, *hv* | •OH | 14.68 | 10.55 | 1.08 (1 H) | 14.68 | 18.80 | 1.70:1 |
| $(NH_4)_2S_2O_8$, $H_2O$ | •OH | 14.58 | 10.65 | 1.27 (1 H) | 14.64 | 18.63 | 4.20:1 |
| $K_2S_2O_8$, $H_2O$ | •OH | 14.61 | 10.74 | 1.27 (1 H) | 14.61 | 18.49 | 2.20:1 |
| 5% $H_2O_2$ + $Fe^{2+}$ + MeOH, $H_2O$ | •$CH_2$OH | 15.88 | 19.45 | | | | |
| 5% $H_2O_2$ + $Fe^{2+}$ + EtOH, $H_2O$ | •CH(OH)Me | 16.00 | 20.41 | | | | |
| 5% $H_2O_2$ + $Fe^{2+}$ + PrOH, $H_2O$ | •CH(OH)Et | 15.87 | 19.83 | | | | |
| 5% $H_2O_2$ + $Fe^{2+}$ + BuOH, $H_2O$ | •CH(OH)Pr | 16.00 | 20.24 | | | | |
| 5% $H_2O_2$ + $Fe^{2+}$ + THF, $H_2O$ | •THF-2-yl | 15.72 | 16.54 | | | | |
| | •THF-3-yl | 15.84 | 19.01 | | | | |
| DBPO,[d] $C_6H_6$, *hv* | •OBu$^t$ | 13.17 | 6.82 | 2.00 (1 H) | 13.65 | 13.65 | 2.70:1 |

[a] The hfs values are given in gauss (1 gauss = 0.1 mT). [b] The ratio is calculated from the EPR signal height of the *cis*-isomer relative to that of the *trans*-isomer. [c] The salt of ammonium iron(II) sulphate was used as the source of iron(II) cation. [d] DBPO is the abbreviation of di-*tert*-butylperoxide.

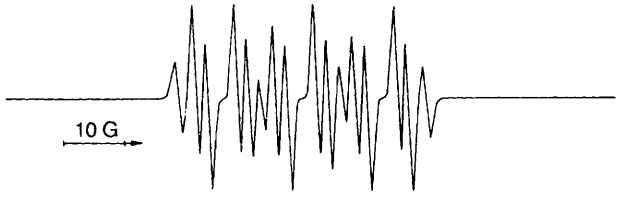



**Fig. 1** EPR spectrum (benzene, room temperature) of the *tert*-butoxyl radical adduct of 4-Me-DMPO (**4b**)

*General Procedure for the Preparation of Hyper-pure Aqueous Solutions of DMPO-type Spin Traps.*—4-Me-DMPO (1.27 g, 10 mmol), which had been prepurified by distillation and chromatography on a silica gel column eluted with chloroform or tetrahydrofuran, was dissolved in doubly-distilled water (50 $cm^3$), and activated charcoal (0.13 g) was added. The solution (0.20 mol $dm^{-3}$) was heated under reflux for 20 min, cooled to room temperature and filtered. The filtrate was treated with activated charcoal (2 × 0.13 g). The concentration of the final solution, as determined by UV spectroscopy, was 0.15 mol $dm^{-3}$. No EPR signal was detected, even after this solution had been scanned ten times with an EPR spectrometer.

*Spin-trapping Procedure.*—The initial concentrations, in mol $dm^{-3}$, of the aqueous nitrone solutions used in this research were 0.11 for DMPO, 0.071 for DMEPO, 0.10 for **4a**, and 0.15 for **4b**. Typically, an aqueous solution of the spin trap (usually 0.10 $cm^3$) was added to an appropriate solution (0.10 $cm^3$) in which the radicals were produced. When benzene was used as a solvent, the appropriate aqueous solution of the spin trap (3 $cm^3$) was saturated with $Na_2SO_4$ and extracted with benzene (6 $cm^3$). The benzene extract (0.25 $cm^3$) was employed in each spin-trapping test.

## Results and Discussion

The values of the hyperfine splitting constants (hfs) for the spin adducts of nitrones 3-Me-DMPO and 4-Me-DMPO are

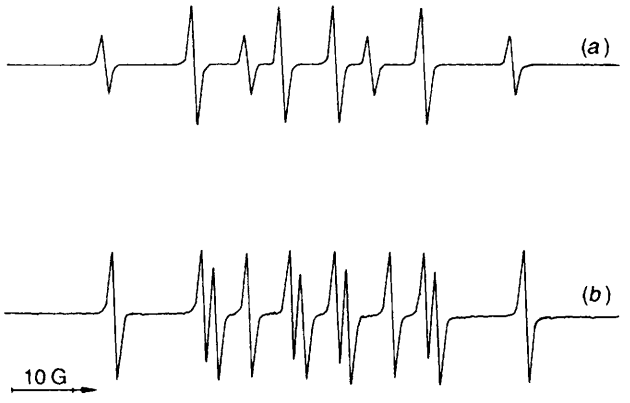



**Fig. 2** EPR spectra (benzene, room temperature) of the hydrogen atom adduct of (*a*) 3-Me-DMPO (**4a**) ($a_N$ = 14.41, $a_H^\beta$ = 14.41 and 23.17 G) and (*b*) 4-Me-DMPO (**4b**) ($a_N$ = 14.50, $a_H^\beta$ = 16.33 and 21.99 G).

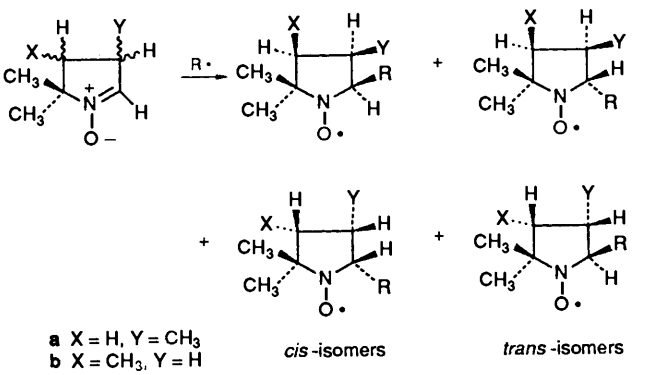
presented in Tables 1 and 2 respectively while some typical spectra for the spin adducts are shown in Figs. 1 and 2.

*Preparation of Hyper-pure Aqueous Solutions of the Spin Trap.*—The spin-trapping technique has been extensively applied as a probe for free radicals in a variety of biochemical systems.[17,18] The study of free radicals in biological processes demands that the aqueous solution of the spin trap must be extremely pure. Frequently the EPR spectrum of a dilute solution of the double-distilled DMPO-type nitrone (>97%) indicates the presence of C-centred radical adducts and hydroxyl adducts. In our laboratory, an improved purification method has been developed for the preparation of hyper-pure aqueous solution of the DMPO-type spin trap and much experience of these systems has been gained

If the aqueous solution (0.15–0.20 mol $dm^{-3}$) was passed through an activated charcoal column or heated under reflux with the charcoal (3 × 10% of the sample weight) for 20 min, there was usually a small amount of the hydroxyl adduct remaining in the solution, although most of the C-centred radical adducts were removed. Sometimes no paramagnetic species were detected in the treated solution. However, the solution may only be used to study radical-rich systems and often fails to detect free radicals in biological processes such as the myocardial ischemia-reperfusion of a rat heart.

TLC analysis indicated that the double-distilled DMPO-type sample always contained a small amount of impurity which may be removed by chromatography on a silica gel column eluted with chloroform or tetrahydrofuran. The distillation–column chromatography–decolourisation method, as described in the experimental section, was employed to prepare the hyper-pure aqueous solutions which have been used successfully to detect the hydroxyl radical generated within the oxygen paradox and myocardial ischemia-reperfusion of the rat heart.[19]

*Spin-trapping Stereochemistry.*—Owing to the presence of a chiral carbon in each Me-DMPO studied, there are two possible spin-trapping approaches, *i.e.*, radical *trans*-addition and *cis*-addition. If the nitrone does not react stereospecifically with a transient free radical, then trapping a radical species should give at least four possible isomers as illustrated in Scheme 2. The two *cis* spin adducts should have the same β-H hfs values, as the chemical and structural environment of the aminoxyl moiety and β-H is the same for each of the two isomers, and similarly for the two *trans*-isomers. However, the β-H hfs value of the *syn*-isomers must be different from that of the *trans*-isomers because the *cis*-isomers are chemically different from the *trans*-isomers and the β-H hfs value is very sensitive to the structural




**Scheme 2**

difference.[8b] Therefore, if the spin-trapping reaction affords both the *cis*- and *trans*-isomers, the observed EPR spectrum should show at least two different β-H hfs values.

*Hydroxyl radicals.* Hydrogen peroxide can generate hydroxyl and hydroperoxyl radicals [eqns. (1) and (2)]. Spin-trapping

$$H_2O_2 \xrightarrow{h\nu} 2\ ^{\bullet}OH \qquad (1)$$

$$^{\bullet}OH + H_2O_2 \longrightarrow {}^{\bullet}OOH + H_2O \qquad (2)$$

with a Me-DMPO nitrone in 3% $H_2O_2$ in this work gave two sets of EPR patterns in each case. The hfs values are $a_N$ = 14.61–14.77, $a_H^\beta$ = 8.77–10.72 and $a_H^\gamma$ = 0.89–1.14 G for one pattern, and $a_N$ = 14.68–15.08 and $a_H^\beta$ = 18.80–20.85 G for the other. The following evidence induced us to attribute these hfs values to the appropriate hydroxyl adducts: (i) the hydroperoxyl adduct of DMPO-type nitrones are usually very unstable, but the EPR signal intensity observed in this work decayed only slowly. For example, the intensity of the 4-Me-DMPO adducts in 3% $H_2O_2$ decayed only slightly in 15 min; (ii) various literature reports have indicated that photolysis of *dilute* $H_2O_2$ in the presence of DMPO-type nitrones only results in the hydroxyl adduct;[20c] (iii) when a Me-DMPO nitrone was replaced by DMPO under the same conditions, a very strong signal due to DMPO'–OH adducts was detected. This result indicates that the solution is rich in 'OH radicals [eqn. (3)]; (iv)

$$DMPO + {}^{\bullet}OH \longrightarrow DMP^{\bullet}O\text{–}OH \qquad (3)$$
$$a_N = a_H^\beta = 14.79\ G$$

when excess methanol was added to the 3% $H_2O_2$ solution before the addition of 4-Me-DMPO, the two original spin adducts were not detected and the hydroxymethyl radical adduct was detected instead. The reaction in equation (4) is

$$CH_3OH + {}^{\bullet}OH \longrightarrow {}^{\bullet}CH_2OH + H_2O \qquad (4)$$

responsible for this observation. This reaction has been widely used to test whether hydroxyl radicals are generated in a reacting system;[20b,21,22] (v) when the $Fe^{2+}$–$H_2O_2$ reagent was used as a radical source, rather than 3% $H_2O_2$, the same hfs values for the corresponding nitrone were obtained. This observation suggests that hydroxyl radicals are trapped, because this radical is the major transient species in this solution, as given in eqn. (5); (vi) when trapping by **4a** or **4b**

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\bullet}OH + OH^- + Fe^{3+} \qquad (5)$$

occurs in aqueous $K_2S_2O_8$ or $(NH_4)_2S_2O_8$, the obtained hfs values are close to those acquired when trapping occurs in the 3% $H_2O_2$ solution. The hydroxyl radical is generated readily in

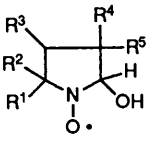
a peroxydisulphate solution [eqns. (6) and (7)] and can easily be

$$S_2O_8^{2-} \longrightarrow 2\,SO_4^{\bullet-} \quad (6)$$

$$SO_4^{\bullet-} + H_2O \longrightarrow {}^{\bullet}OH + SO_4^{2-} + H^+ \quad (7)$$

trapped by DMPO.[20a,23] It is not likely that the resulting signals are due to $SO_4^{\bullet-}$ adducts because these signals did not disappear until more than 9 min had elapsed, which is not consistent with the rather unstable nature of $^-O_3SO$–DMPO$^{\bullet}$ adducts as previously reported [τ(time to 1/e of the initial value), *ca.* 20 s].[23]

The EPR spectra obtained for the Me-DMPO$^{\bullet}$–OH adducts are different from the 1:2:2:1 quartet pattern observed for the OH-adducts of DMPO,[20] DMEPO,[8a] and some other nitrones.[12] This is most probably due to the structural variation of the spin trap used. For instance, the structures of the hydroxyl adducts **5a–d** are obviously different and their hfs values are also dramatically different as shown.

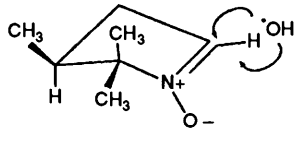


**5**

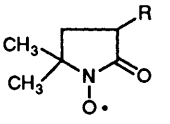
| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $a_N$/G | $a_H^{\beta}$/G |
|---|---|---|---|---|---|---|---|
| **a** | $CH_3$ | $CH_3$ | H | H | H | 15.0 | 15.0[20b] |
| **b** | $-(CH_2)_4-$ | | H | H | H | 15.8 | 22.3[5] |
| **c** | $CH_3$ | $CH_3$ | H | $CH_3$ | $CH_3$ | 15.3 | 16.8[11] |
| **d** | $C_6H_5$ | $CH_3$ | $CO_2C_2H_5$ | $CH_3$ | $CH_3$ | 14.4 | 19.1[12] |

It is apparent, from the observation that their signals decayed at almost equal rates, that the two spin adducts result from the trapping of one kind of radical by each trap. A remaining question is whether the two spin adducts correspond to *cis*- and *trans*-isomers or to one isomer which exists in two different conformations in solution. Although the latter is a possibility, there is no literature evidence for the hydroxyl adduct of a DMPO-type nitrone showing two EPR patterns in one solution. The reason is probably that the hydroxyl adduct, *e.g.*, **5a–d**, assumes one conformation preferentially in solution, or that the difference in hfs values between the various conformations existing in solution for each hydroxyl adduct is not large enough to be detectable, owing to the line-width of the EPR signals. Therefore, the stereoselectively formed *cis*- and *trans*-adducts must be responsible for the two distinct EPR patterns observed. For **4a**, owing to the steric hindrance of the 3-methyl group, the radical should add to the nitrone more readily from the *trans* direction to give the *trans*-isomer. But for **4b**, as illustrated,* the radical should add to the nitrone function more easily in the direction *cis* to the 4-methyl group to afford mainly the *cis*-isomer.

When trapping by **4a** occurs in aqueous $K_2S_2O_8$, an aminoxyl oxidation product **6** [six-line, $a_N$ = 7.05, $a_H^{\gamma}$ = 4.80 (1 H) G] was also detected. This was assigned by comparison of its hfs values with those of its analogue **7** [$a_N$ = 7.1, $a_H^{\gamma}$ = 4.2 (2 H) G].[24]

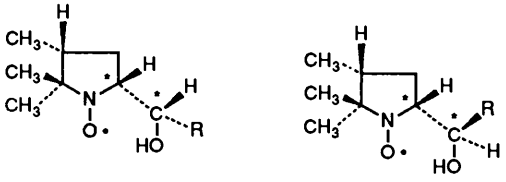


A possible preferential conformation of 4-Me-DMPO



**6** R = $CH_3$
**7** R = H

*Hydroxyalkyl radicals.* Hydroxyalkyl radicals were stereospecifically trapped by **4b** to generate the *cis*-adducts (Table 2). The β-H hfs values (19.45–20.41 G) for the hydroxyalkyl adduct of **4b** are slightly smaller than those for the analogous adduct of DMPO (22.58–23.38 G),[8a] and much smaller than those for DMEPO (24.22–25.58 G).[8a] The differences may be attributed to the structural variation of the spin trap. It should be noted that stereospecific trapping of the hydroxyethyl, 1-hydroxypropyl and 1-hydroxybutyl radicals gives a mixture of diastereomeric aminoxyls, **8′** and **8″**, and their enantiomers. The EPR spectra of **8′** and **8″** should be different because they are chemically different species. In fact, the spectra for these adducts all have slightly asymmetric patterns while the spectrum of the hydroxymethyl adduct consists of one set of reasonably sharp peaks. The relatively high line widths unfortunately decrease the chances of detecting a distinct EPR spectrum for each diastereomer, *i.e.*, **8′** and **8″**.



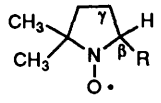
**8′** **8″**

R = $CH_3$, $C_2H_5$, n-$C_3H_7$

*Tetrahydrofuranyl radicals.* The reaction of tetrahydrofuran (THF) with $^{\bullet}OH$ may be expected[25,26] to involve tetrahydrofuran-2- and 3-yl radicals. Only tetrahydrofuran-2-yl radicals have been previously detected, either directly[27,28] or indirectly.[8a,29] The failure to detect tetrahydrofuran-3-yl radicals may be due to the room temperature examination (20 °C)[27,28] and/or the method used for the generation of $^{\bullet}OH$.[30] It occurred to us that tetrahydrofuran-3-yl radicals might be generated in the THF + $Fe^{2+}$ + $H_2O_2$ mixture, since $Et_2O$ in $Fe^{2+}$ + $H_2O_2$ generated $^{\bullet}CH_2CH_2OEt$ radicals.[30] In this work, spin-trapping of the THF + $Fe^{2+}$ + $H_2O_2$ mixture by DMPO gave the HO-DMPO$^{\bullet}$ adducts ($a_N$ = $a_H^{\beta}$ = 14.64 G), the main tetrahydrofuran-2-yl–DMPO$^{\bullet}$ adducts ($a_N$ = 15.57, $a_H^{\beta}$ = 19.54 G) and the tetrahydrofuran-3-yl-DMPO$^{\bullet}$ adducts ($a_N$ = 15.81, $a_H^{\beta}$ = 22.18 G) simultaneously (Table 3), while the tetrahydrofuran-2-yl adducts ($a_N$ = 15.72, $a_H^{\beta}$ = 16.54 G) and the tetrahydrofuran-3-yl adducts ($a_N$ = 15.84, $a_H^{\beta}$ = 19.01 G) of **4b** were also detected when trapping with **4b** (Table 2). The tentative assignment of the hfs values to the tetrahydrofuranyl adducts above is based on three points: (i) proton abstraction from THF by $^{\bullet}OH$ radicals gives tetrahydrofuran-2-yl radicals more readily than tetrahydrofuran-3-yl species;[26] (ii) the hfs values for tetrahydrofuran-2-yl–DMPO$^{\bullet}$ in $H_2O$ were established;[8a] (iii) the values obtained, 15.6–15.8

* The nitrone is a racemic mixture but, for convenience, only one enantiomer is illustrated.

**Table 3** EPR hyperfine splitting constants for spin adducts of 5,5-dimethyl-1-pyrroline 1-oxide (DMPO)[a]



| Source and solvent | Radical (R•) | $a_N$ | $a_H^\beta$ | $a_H^\gamma$ | Ref. |
|---|---|---|---|---|---|
| 5% $H_2O_2$ + $Fe^2$, $H_2O$[b] | •OH | 14.79 | 14.79 | | |
| 5% $H_2O_2$ + $Fe^{2+}$ + THF, $H_2O$ | •OH | 14.64 | 14.64 | | |
| | THF-2-yl | 15.57 | 19.54 | | |
| | THF-3-yl | 15.81 | 22.18 | | |
| ZnO + THF, $H_2O$,*hv* | •OH | 14.80 | 14.80 | | 8*a* |
| | THF-2-yl | 15.71 | 19.59 | | 8*a* |
| DBPO,[c] $C_6H_6$, *hv* | •OBu$^t$ | 13.20 | 7.78 | 2.04 | |
| $Bu_3SnH$, $C_6H_6$ | •H[d] | 14.54 | 19.02 (2 H) | (1 H) | |

[a] The values are given in gauss (1 gauss = 0.1 mT). [b] The salt of ammonium ferrous sulphate was used as the source of ferrous cation. [c] DBPO is the abbreviation of di-*tert*-butylperoxide. [d] The spin adduct was formed spontaneously.

G, are typical of the $^{14}N$ hfs values for carbon-centred radical adducts in aqueous media.[31,32]

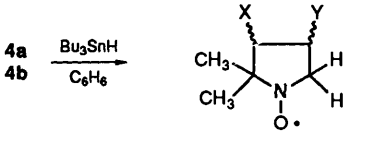
tert-*Butoxyl radical.* Two sets of EPR signals were detected when a mixture of Me-DMPO (**4a** or **4b**) and di-*tert*-butylperoxide (DBPO) in benzene was irradiated. In contrast, only one set of EPR peaks, assigned to DMPO•–OBu$^t$, was observed by using the non-chiral spin trap DMPO. Comparison of the experimental results eliminates the possibility that the two sets of signals observed in the case of **4a** or **4b** result from the trapping of two different kinds of radical. There are still two possible explanations for the appearance of the two sets of EPR signals. One is that a stereoselective spin-trapping reaction has taken place to give *trans*- and *cis*-adducts, which are responsible for the two sets of signals, and the other is that the two sets of EPR patterns correspond to two conformations of one adduct derived from the stereospecific trapping of *tert*-butoxyl radicals by each of **4**. The previous result that the *tert*-butoxyl adduct of 5-propyl(or hexyl or decyl)-5-methyl-1-pyrroline 1-oxide, which also contains a chiral carbon, only shows one set of EPR peaks[4] probably indicates that the adduct preferentially assumes one conformation in benzene. Therefore, the former possibility is probably more likely.

One of the two EPR splitting patterns for 3-Me-DMPO•–OBu$^t$ ($a_N$ = 13.26, $a_H^\beta$ = 4.89, $a_H^\gamma$ = 1.33 G) is similar to that for DMPO•–OBu$^t$ ($a_N$ = 13.20, $a_H^\beta$ = 7.78, $a_H^\gamma$ = 2.04 G) and, therefore, is assigned to the *trans*-isomer since the *trans* species should more closely resemble the analogous DMPO adduct. The other component ($a_N$ = 13.37, $a_H^\beta$ = 15.08 G) is attributed to the corresponding *cis*-isomer. The observation that the half-life of the *cis*-isomer is *ca.* 390 min while the EPR signal intensity of the *trans*-isomer (less perturbation, more stability) is about unchanged over over 390 min supports the above assignment.

The EPR spectrum observed for 4-Me-DMPO•–OBu$^t$ adducts (Fig. 1) is similar to that for 4-Ph-DMPO•–OBu$^t$ species as reported by Haire, Janzen *et al.*[33] They proposed that the *trans*-isomer should exhibit less perturbation than the *cis*-isomer. But, in our opinion, since the pseudo-axial orientation of the Bu$^t$O group is demanded by the stereoelectronic effect,[34] and is further indicated by the very small β-H hfs value,[11] the 4-Ph or 4-Me group in their *cis*-isomers must be in a pseudo-equatorial position, which decreases the strong repulsion with the 5,5-dimethyl groups. Therefore, the *cis*-isomer may exhibit less perturbation than the *trans*-isomer and the hfs values ($a_N$ = 13.17, $a_H^\beta$ = 6.82, $a_H^\gamma$ = 2.00 G) similar to those for DMPO•–OBu$^t$ ($a_N$ = 13.20, $a_H^\beta$ = 7.78, $a_H^\gamma$ = 2.04 G) are assigned to the *cis*-isomer. The other EPR pattern ($a_N$ = $a_H^\beta$ = 13.65 G) is attributed to the *trans*-4-Me-DMPO•–OBu$^t$ isomer.

It is surprising that the presence of THF, MeOH, EtOH, PrOH or BuOH (but not Pr$^i$OH) in the benzene solution of **4** and DBPO dramatically decrease the relative amount of the *cis*-3-Me-DMPO•–OBu$^t$ and the *trans*-4-Me-DMPO•–OBu$^t$ adducts (Table 1). Further studies are planned to probe these reactions.

*Further Evidence for Spin-trapping Stereochemistry.*—The spin-trapping stereochemistry is mainly based on the hypothesis that the *cis*- and *trans*-isomers, derived from a radical and a spin trap, should have different $a_H^\beta$ values. The results given in Table 1 and 2 support this hypothesis. As further substantiation, nitrones **4a** and **4b** were employed to trap hydrogen atoms to give **9** and **10**, respectively (Scheme 3). The EPR spectra of **9** and

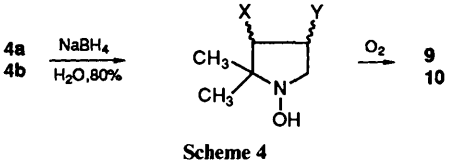


**9** X = H, Y = $CH_3$
**10** X = $CH_3$, Y = H

**Scheme 3**

**10** in Fig. 2 indicate that the two β-hydrogens in each aminoxyl are magnetically non-equivalent. In comparison, the two β-hydrogens in the DMPO•–H adduct have the same hfs value [$a_H^\beta$(2 H) = 19.02 G]. The reason for the non-equivalence of two β-protons is probably that the presence of a chiral carbon in **9** or **10** makes the pyrrolidine ring adopt a preferred conformation at room temperature; one β-proton is in a pseudo-axial position and the other is in a pseudo-equatorial position, and their orientations with respect to the semi-occupied *p*-orbital of the nitrogen atom are different. Similarly, for four possible isomers from a Me-DMPO nitrone and one kind of radical as illustrated in Scheme 2, the β-hydrogen in the *trans*-adduct must be magnetically different from that in the corresponding *cis*-adduct, and different hfs values must be visible in the EPR spectrum if the *trans*- and *cis*-adducts are generated in the course of the same spin-trapping reaction. On the other hand, if only one β-H hfs value is found, the spin-trapping reaction is most probably stereospecific or, in other words, very highly stereoselective.

The aminoxyls **9** and **10** were also prepared by the reduction of the corresponding nitrone with $NaBH_4$ and the auto-oxidation[8] as illustrated in Scheme 4. Their EPR hfs values in



**Scheme 4**

various solvents are collected in Table 4. The values increase with the increase of the solvent electric dipole moment except for acetone. Hydrogen bonding from the aminoxyl oxygen atom to the hydrogen atom of the solvent $H_2O$, MeOH, EtOH or $CHCl_3$ is probably the main contribution to the interaction between the aminoxyl and the solvent molecules.

## Conclusions

Two five-membered cyclic nitrone spin traps, **4a** and **4b**, each

**Table 4** EPR hyperfine splitting constants for the aminoxyls **9** and **10** in various solvents[a]

| Solvent | Aminoxyl **9** | | | | Aminoxyl **10** | | | |
|---|---|---|---|---|---|---|---|---|
| | $a_N$ | $a_{H1}^{\beta}$ | $a_{H2}^{\beta}$ | $\Delta a_H^{\beta}$ | $a_N$ | $a_{H1}^{\beta}$ | $a_{H2}^{\beta}$ | $\Delta a_H^{\beta}$ |
| $H_2O$ | 16.91 | 16.91 | 27.66 | 10.75 | 16.52 | 19.26 | 26.30 | 7.04 |
| MeOH | 15.49 | 15.49 | 25.22 | 9.73 | 15.45 | 17.79 | 24.10 | 6.31 |
| EtOH | 15.50 | 15.50 | 24.98 | 9.48 | 15.40 | 17.50 | 23.66 | 6.16 |
| $CHCl_3$ | 15.25 | 15.25 | 24.49 | 9.24 | 15.05 | 17.25 | 23.17 | 5.92 |
| MeCOMe | 14.76 | 14.76 | 23.80 | 9.04 | 14.71 | 16.57 | 22.48 | 5.91 |
| $C_6H_6$ | 14.57 | 14.57 | 23.31 | 8.74 | 14.47 | 16.42 | 21.90 | 5.48 |
| cyclo-$C_6H_{12}$ | 14.03 | 14.03 | 22.39 | 8.36 | 14.13 | 16.13 | 21.46 | 5.33 |
| $C_5H_{12}$ | 13.98 | 13.98 | 22.39 | 8.41 | 14.03 | 15.49 | 20.77 | 5.28 |

[a] The values are given in gauss (1 G = 0.1 mT).

containing a chiral carbon atom, have been prepared by a two-step synthetic route. The combination of distillation, column chromatography and decolourisation procedures is a general and successful method for the preparation of the hyper-pure DMPO-type aqueous nitrone solution. The nitrone **4** undergoes stereoselective or stereospecific radical addition. Nitrone **4a** stereoselectively scavenges $^{\cdot}OH$ and $^{\cdot}OBu^t$ radicals. Nitrone **4b** stereospecifically traps hydroxyalkyl, tetrahydrofuran-2-yl and tetrahydrofuran-3-yl radicals, and stereoselectively scavenges $^{\cdot}OH$ and $^{\cdot}OBu^t$ radicals. The aminoxyls **9** and **10** have been acquired by two different methods and each of these aminoxyls contains two magnetically non-equivalent β-protons.

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