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Synthesis and Spin Trapping Stereochemistry of the Chiral Spin Trap, 5,5-Dimethyl-3-phenylpyrroline-1-oxide

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A chiral spin trap, 5,5-dimethyl-3-phenylpyrroline-1-oxide (5), and several hydroxylamines were synthesized. The structures and conformations of these compounds were investigated mainly by ^{1}H NMR spectroscopy. This spin trap was used to trap carbon- and oxygen-centered radicals. The corresponding hydrogen spin adduct was prepared by oxidizing the hydroxylamine to the nitroxide radical. By the combination of ^{1}H NMR and EPR results, it was shown that the conformation with the phenyl group of C-3 at the equatorial position is exclusively populated and its lifetime is long compared with the EPR time-scale. Addition of carbon-centered radicals to 5 leads to *trans* adducts whereas oxygen-centered radicals formed *cis* isomers. These could be confirmed by the investigation of the monovalent oxidation products of the hydroxylamines. The preference for these conformers is explained by the competition between steric and stereoelectronic effects. Despite the racemic nature of 5 and the formation of a new chiral center(s) in the spin adducts, the presence of different diastereomers would not be observed by EPR whereas ^{1}H NMR studies of some of the nitrones showed clear evidence of diastereomeric mixtures. However, the spin adduct of *sec*-hydroxybutyl radical showed some linewidth effects which could be attributed to the presence of two groups of diastereomers that were resolved by ENDOR spectroscopy. In general, the spin adducts of 5 are closely related to those of the well known DMPO, but the presence of a phenyl substituent at the 3-position results in a variation of the β-H coupling constants. In contrast to DMPO, 5 can scavenge short-lived radicals in aqueous and non-aqueous solutions.

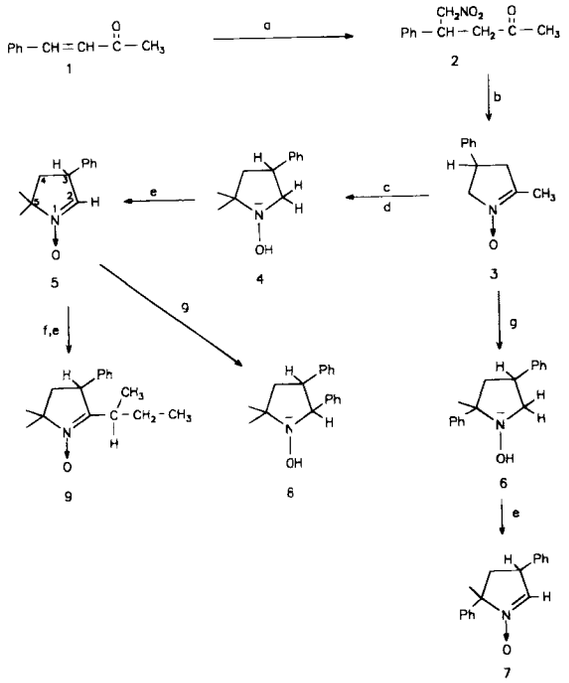
KEY WORDS ESR; spin trap; chiral spin trap

INTRODUCTION

The technique of spin trapping using electron spin resonance continues to attract considerable interest in the detection and identification of short-lived radicals in various systems.[1–3] The identification of the radical trapped is derived from the magnitude of the nitrogen and β-hydrogen coupling constants and the *g*-factor.[4] In recent years, free radicals have been proposed as mediators of cellular responses such as cancer, aging, oxidative stress and ischemia/reperfusion injury.[5,6]

Cyclic nitrones with chiral centers may allow the study of stereochemistry/stereospecificity of radical addition.[7,8] A successful spin trap must be able to scavenge efficiently transient free radicals leading to persistant spin adducts whose hyperfine splittings point to the identity of the species trapped.[9]

We report here the synthesis of the chiral spin trap 5,5-dimethyl-3-phenylpyrroline-1-oxide (**5**) by analogy with the method of Rosen and Turner,[10] as illustrated in Scheme 1. Furthermore, we illustrate its use in a study of spin trapping stereochemistry and its relationship to the stereochemical properties of the incoming



Scheme 1. Reagents: (a) CH_3NO_2–KF–CH_3CN–18-crown-6-ether; (b) Zn–NH_4Cl–60% EtOH; (c) CH_3MgBr; (d) NH_4Cl; (e) PbO_2–benzene; (f) $CH_3CH(MgBr)CH_2CH_3$; (g) PhMgBr.

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radicals. Hydroxylamines produced by the addition of Grignard reagents to **5** were also investigated.

EXPERIMENTAL

Synthesis

The nitrone **5** was synthesized in four steps as shown in Scheme 1. A Michael addition with nitromethane to the benzylideneacetone **1** gave the nitro ketone **2**. Reduction with zinc produced the cyclic nitrone **3**, methylation with methylmagnesium bromide led to the hydroxylamine **4**. Subsequent oxidation with lead dioxide gave the spin trap **5**.

5-Nitro-4-phenylpentan-2-one (2)
Benzylidenacetone (73.0 g, 0.50 mol), nitromethane (270 ml, 5.0 mol), dry potassium fluoride (5.82 g, 0.10 mol) and 18-crown-6-ether (6.60 g, 25 mmol) were dissolved in 300 ml of dry acetonitrile and refluxed for 1 week in a protective atmosphere. Most of the solvent was removed under vacuum to give 96.3 g (93%) of crystalline product **2**.

^{13}C NMR ($CDCl_3$): δ 30.36 ($COCH_3$), 39.08 (CH_2CO), 46.15 (CH_2NO_2), 79.49 (PhCH), 127.40, 127.89, 129.07, 138.90 (Ph), 205.39 ($COCH_3$).

4-Phenyl-2-methylpyrroline-1-oxide (3)
To a solution of **2** (55.2 g, 266 mmol) and ammonium chloride (15.2 g) in 500 ml of 60% aqueous ethanol at 10 °C was added portionwise zinc powder (70.8 g) and the reaction mixture stirred at 10–15 °C for 5 h. The reaction mixture was then filtered and the residue washed with warm water. After evaporation to about 50 ml, the filtrate was extracted with four portions of 50 ml of dichloromethane. The combined extracts were dried over sodium sulfate and the solvent was removed under vacuum to give 28 g of brown oil (60%).

IR (cm^{-1}): 1310 (N—O); 1650 (C=N). ^{13}C NMR ($CDCl_3$): δ 12.65 (N=CCH_3), 36.14 (N=CCH_2), 41.89 (PhCH), 68.55 (CH_2N), 126.63, 127.35, 129.09, 141.98 (Ph), 144.74 (C=N).

5,5-Dimethyl-3-phenyl-*N*-hydroxypyrroline-1-oxide (4)
A diethyl ether solution of crude **3** (6.72 g, 38.6 mmol) was added dropwise to methylmagnesium bromide (3.2 M, 24 ml, 77.2 mmol) (Aldrich) at 0 °C. After refluxing for 2 h, saturated ammonium chloride was added to the reaction mixture and the aqueous phase extracted three times with 50 ml portions of diethyl ether. The combined extracts were dried over $MgSO_4$ and rotary evaporated. The hydroxylamine was then crystallized and recrystallized from light petroleum to give yellowish white crystals with a melting range of 80–84 °C and a yield of 4.43 g (60%).

IR (cm^{-1}): 1364 (N—O), 1456 (C—N), 3226 (O—H). ^{13}C NMR ($CDCl_3$): δ 25.24 ($2CH_3$), 39.52 [$(CH_3)_2CCH_2$], 45.82 (PhCH), 62.75 (CH_2N), 65.52 [$(CH_3)_2C$], 126–146 (Ph). Mass spectrometry (MS): *m/z* 192.2 ($M^+ + 1$, 6%), 191.2 (M^+, 34), 176.2 ($M^+ - 15$, 98), 131.1 ($M^+ - 60$, 100), 91.0 ($M^+ - 100$, 56), 78.0 ($M^+ - 113$, 14). Elemental analysis, $C_{12}H_{17}NO$ (191.2): calculated C 75.41, H 8.89, N 7.32; found C 75.94, H 9.55, N 7.25%.

5,5-Dimethyl-3-phenylpyrroline-1-oxide (5)
To a solution of **4** (1 g, 5.24 mmol) in dry benzene was added portionwise PbO_2 (5 g) under a nitrogen atmosphere and the mixture was stirred for about 2 h. After filtration of the reaction mixture and removal of the solvent under vacuum, the solid residue was recrystallized from cylohexane, giving 1 g (100%) of white crystals of **5** with m.p. 96–98 °C.

IR (cm^{-1}): 1226 (N—O), 1570 (C=N). ^{13}C NMR ($CDCl_3$): δ 25.23, 26.37 ($2CH_3$), 42.99 (CH_2), 44.95 (PhCH), 134 (N=CH), 127–140 (Ph). MS: *m/z* 190.3 ($M^+ + 1$, 5%), 189.3 (M^+, 59), 159.2 ($M^+ - 30$, 59), 117.0 ($M^+ - 72$, 84), 102.9 ($M^+ - 86$, 100), 77.0 ($M^+ - 112$, 46). Elemental analysis, $C_{12}H_{15}NO$ (189.3): calculated C 76.21, H 7.93, N 7.40; found C 76.1, H 8.1, N 7.7%.

The hydroxylamines **6** and **8** were obtained by the reaction of phenylmagnesium bromide with their corresponding nitrones **3** and **5**, respectively. Nitrones **7** and **9** were synthesized analogously to **5**. Compound **9** is a product of the reaction of **5** with 2-butylmagnesium bromide, serving as a chiral anion addend. The analytical data for compounds **6–9** will be published with their corresponding spin adducts elsewhere. Their ^1H NMR data are given in Table 1 for comparison purposes in the discussion of the stereochemistry of nitrone **5** with its spin adducts.

Spin trapping procedure

Hydroxyalkyl radicals were generated by the well known method of hydrogen abstraction by the triplet state of photoexcited benzophenone.[11,12]

The adducts of the anions were prepared by the addition of Grignard compounds (purchased from Aldrich) to the nitrone followed by air oxidation to give the corresponding nitroxide radicals. The method of Makino *et al.*[13] was used for the preparation of the hydroxyl spin adducts. The *tert*-butoxy radicals were generated by irradiating a solution of the spin trap and di-*tert*-butyl peroxide in benzene with UV radiation from a low-pressure mercury lamp. The hydrogen atom spin adducts were derived from the corresponding hydroxylamines by oxidation with either air oxygen or lead dioxide. The trichloromethyl radicals were trapped in rat liver during the metabolism of carbon tetrachloride (more details will be published elsewhere). All samples were deoxygenated by bubbling argon through them for at least 5 min.

Instrumentation

ESR and ENDOR spectra were recorded on a Bruker ESP 300 and a Bruker ER 810 spectrometer, respectively. NMR spectra were obtained on Bruker AC 250 (62.9, 250 MHz) and Bruker WM 400 (400 MHz) spectrometers. Mass spectra were recorded using a TSQ 70 (Finnigan MAT) and MAT 711A (Finnigan) and infrared spectra on a Perkin–Elmer 1600 Series Fourier transform IR spectrometer.

RESULTS

Three cyclic hydroxylamines and their corresponding nitrones were synthesized. Structural and conformational analyses of these compounds were performed using common analytical spectroscopic methods, especially NMR spectroscopy, as indicated in Table 1. The ^1H NMR spectra show two intense singlet signals upfield which are due to the two methyl groups. Methylene protons appear as two doublets of doublets. An assignment of these protons to pseudo-axial and pseudo-equatorial conformations will be discussed later. A triplet of doublets appears at 4.10 ppm and is attributed to the methyne proton. This arises because of the similar coupling constant (8.5 Hz) between the methyne proton and the pseudo-axial and pseudo-equatorial methylene protons. Further downfield is the doublet ($\delta = 7.17$) due to the olefinic proton. All the compounds **2–9** show multiplets ($\delta \approx 7.2$) due to aromatic protons (Table 1).

It is also interesting that ^1H NMR studies of the nitrone **9** showed evidence of diastereomeric mixtures which were not observed in EPR. The spectrum shows, in addition to the usual signals of the heterocyclic ring, two doublets, $\delta = 0.78$ and 1.02 with $^3J_{H,H} = 7.1$ Hz, assigned to the methyl protons at the chiral carbon of the butyl substituent. The high-field signal is superimposed by the triplet of the other methyl group. The

Table 1. ^{1}H NMR data compounds 2–9 in $CDCl_3$ at room temperature

Compound	CH_3	PhCH	CH_2C	CH=N	CH_2N	NCHPh
2	2.09 s	3.99 m; 7.06, 7.07 7.23, 7.31 Hz	2.90 d; 7.02 Hz		4.62 2dd; 6.8, 7.8, 12.8 Hz	
3	2.07 s	3.48 m	2.58, 2.95 2dd; 6.8, 9.7, 17.5 Hz		3.82, 4.23 2dd; 7.9, 8.6, 15.4 Hz	
4	1.23, 1.29 2s	3.42 m; 6.9, 8.9 Hz	1.77, 2.12 2dd; 8.3, 9.6, 12.6 Hz		3.30, 3.52 2dd; 6.5, 10.0, 19.8 Hz	
5	1.48, 1.50 2s	4.10 2t; 2.5, 8.5 Hz	1.99, 2.66 2dd; 8.1, 8.8, 13.0 Hz	6.88 d, 2.45 Hz		
6	1.63 s	3.4 m	2.27, 2.81 2dd; 8.8, 9.0, 13.1 Hz		3.3, 3.57 2dd; 7.0, 8.1, 11.0 Hz	
7	1.91 s	4.01 m; 2.4, 7.9, 9.8 Hz	2.27, 3.01 2dd; 7.9, 9.3, 12.8 Hz	7.17 d; 2.4 Hz		
8	1.33, 1.36 2s	3.12 2t; 7.7, 10.5 Hz	1.99, 2.27 2dd; 7.7, 10.7, 13.3 Hz			4.09 d; 10.28 Hz
9[a]	1.43, 1.55 2s	4.0 m; 8.6, 9.3 Hz	1.92, 2.53 3dd; 7.5, 7.9, 9.4, 13.2 Hz			

[a] Additional absorptions observed for the addend are CH_3 [(0.78, 1.02; 2d, 7.1 Hz); (0.82; t, 7.4 Hz)] and CH (2.68, 2.94; 2m, 7.2, 7.3).

Table 2. Hyperfine splitting constants for nitroxides produced in radical/anion addition reactions to 5,5-dimethyl-3-phenyl pyrroline-1-oxide

No.	Type (293 K)	Solvent	a_N (G)	$a_{\beta\text{-H}}$ (G)	ΔH (G)	g-Factor
I	Radicals	CH_2OH[a]	14.75	24.38	1.00	2.005 63
II		CH_3CHOH[a]	15.00	24.25	1.00	2.005 62
		CH_3CHOH[e]	15.00	24.25	1.63	
III		$CH_3COHCH_2CH_3$[a]	14.75	25.50 (22.45, 21.80)[f]	1.50	2.005 62
		$CH_3COHCH_2CH_3$ [e]	15.13	25.50	1.50	
IV		$CH_3CH_2COHCH_2NH_2$[a]	14.75	23.50	2.00	2.005 72
		$CH_3CH_2COHCH_2NH_2$[e]	15.13	24.75	1.38	
V		$(CH_3)_3CO$[a]	13.21	15.48 ($a_{\gamma\text{-H}}$, 0.421, 0.686)[f]	1.55	2.005 99
VI		HO[c]	12.96	14.16	0.95	2.005 83
VII		CCl_3[d]	13.27	17.76	1.22	—
VIII	Anions[b]	$CH{=}CH_2$	13.91	22.17	1.09	2.005 52
IX		Ph	13.70	22.83	1.09	2.005 81
X		$PhCH_2$	13.91	23.70	1.52	2.005 75
XI		$CH_3\ (CH_2)_6CH_2$	13.91	23.48	1.74	2.005 81
XII		$CH_3CH_2CHCH_3$	14.25	24.50 ($a_{\gamma\text{-H}}$, 0.375)[f]	1.58	2.005 75
XIII		C_6H_{11}	13.91	24.13	1.30	2.005 75
XIV		$(CH_3)_2CH$	13.91	23.91	1.30	2.005 84
XV		H	14.25	14.25 23.38	—	—

[a] Toluene.
[b] Benzene.
[c] Water.
[d] Chloroform.
[e] Methanol.
[f] Obtained by ENDOR in toluene at 213 K.
The spin adduct of **5** with the aminoalkyl radical ($CH_3CH_2CH_2CHNH_2$) obtained from *n*-butylamine gave the following splitting constants: $a_N = 14.16$ G, $a_{\gamma\text{-N}} = 1.07$ G, $a_{\beta\text{-H}} = 22.66$ G. The absence of the γ-N splitting in the EPR spectrum of **IV** with this nitrone indicates the trapping of a hydroxyalkyl radical rather than an aminoalkyl radical.

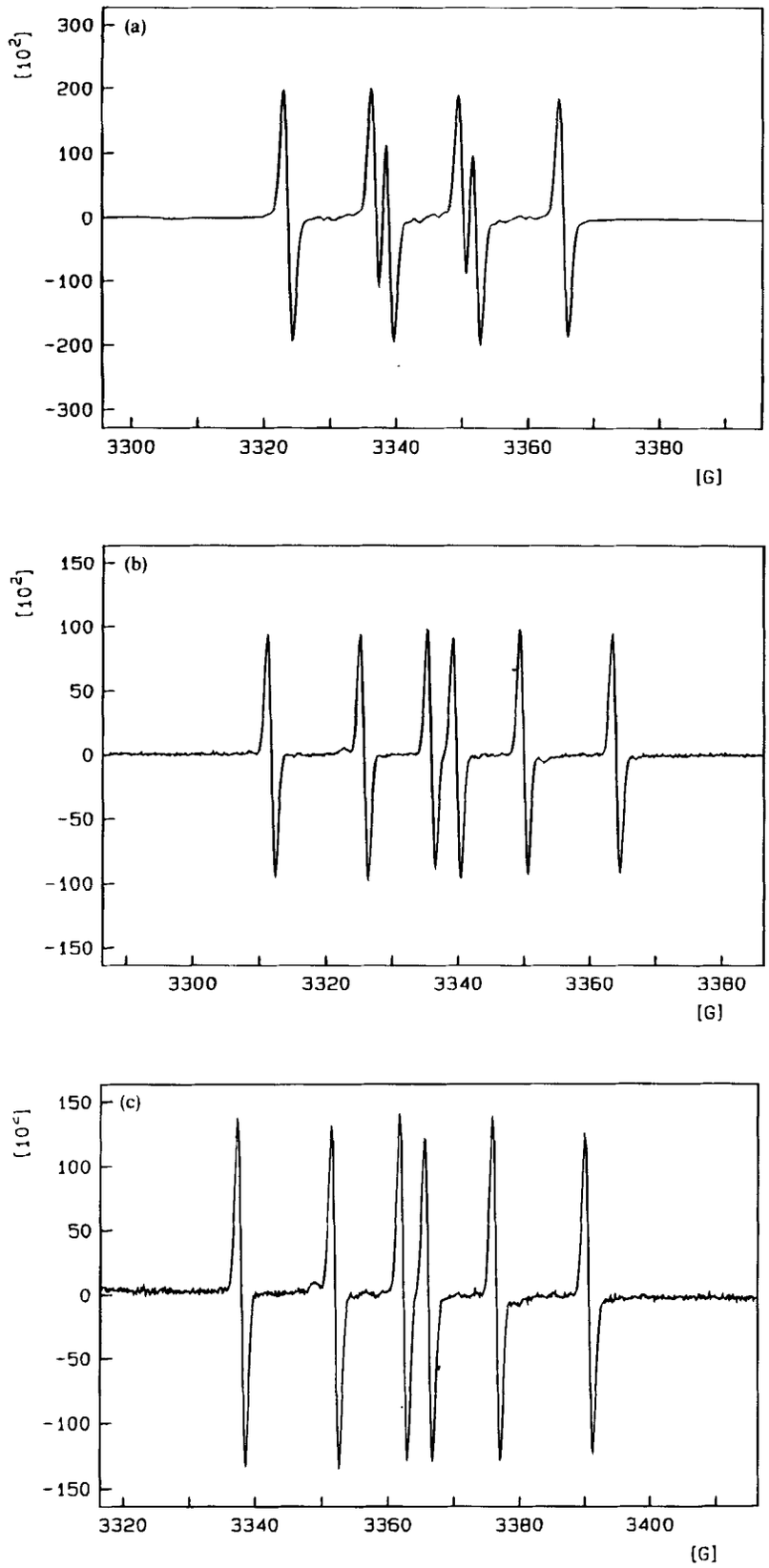


Figure 1. EPR spectrum of the spin adduct of nitrone **5** with (A) *tert*-butoxyl radicals (benzene), (B) hydroxyalkyl radical from 2-butanol (toluene) and (C) $CH_3CH_2CHCH_3$ anion (toluene).

methyne proton is represented by two sextets ($\delta = 2.67$, 2.93). One methylene proton of the ring also showed two doublets of doublets. The other signals do not give evidence for the presence of diastereomers (see Table 1).

The EPR spectroscopic data for the spin adducts (**I–XV** are shown in Table 2. By inspection of these EPR parameters, it is possible to determine whether the radical/anion addend is carbon or oxygen centered from

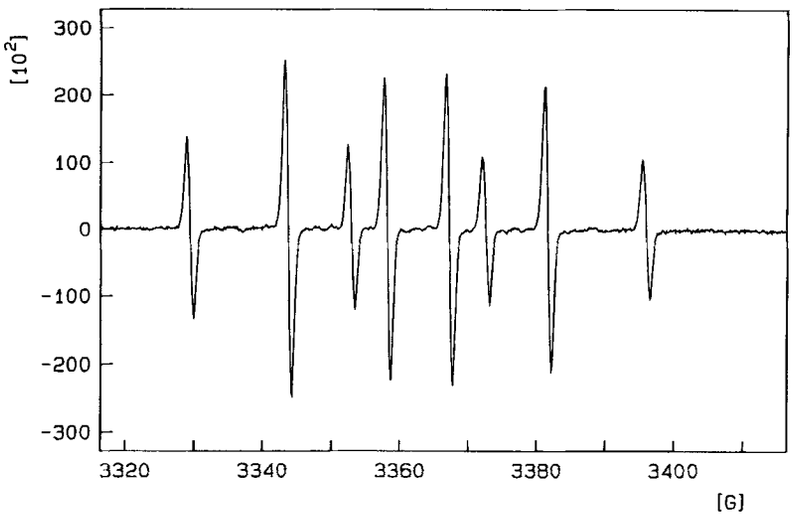


Figure 2. EPR spectrum obtained after air oxidation of hydroxylamine **4** in benzene.

the N and β-hydrogen splitting constants. These N and β-H coupling constants for carbon-centered radicals/anions fall in the ranges 13.5–15 and 22–26 G, respectively, and the oxygen-centered ones in the corresponding ranges 12–13.5 and 14–16 G. These ranges are comparable to those of the corresponding adducts of 5,5-dimethylpyrroline-1-oxide (DMPO).

In Fig. 1, some typical examples of the different EPR spectra obtained with various addends are shown. The different β-proton coupling constants lead to significant alterations of the EPR spectra. Furthermore, it is remarkable that in the spectra of radical **XII** no evidence for the presence of two different paramagnetic species could be detected, even with enhanced resolution.

The H-adduct of **5** obtained when the hydroxylamine **4** was oxidized by air/oxygen to **XV** in benzene exhibits (Fig. 2), in contrast to the corresponding DMPO derivative, two β-proton coupling constants, whose average is 18.8 G, which is observed for the DMPO-H adduct. This observation indicates a rigid conformation of XV.

By ENDOR experiments, γ-H splittings (**V**, **XII**) could be resolved and two signals in the 22 MHz region were observed for the spin adduct **III** (Fig. 3). These lines represent the low-frequency transition according to $\nu_E = (a_H/2) - \nu_H$ of the β-proton with $a_H/2 \approx 25.5$ G, indicating the presence of two radicals with different β-proton splitting constants produced by the scavenging of the hydroxyalkyl radical from 2-butanol.

DISCUSSION

The different ^{1}H NMR values of the 5,5-dimethyl protons in **4**, **5** and **9** indicate that the phenyl substituent on C-3 has different influences on the two methyl groups and the pyrroline ring in general and therefore the compounds should exist in a preferential conformation. Likewise, the different chemical shifts of the methylene protons on either C-2 or C-4 of **4**–**9** confirms the structural rigidity of these compounds.

The coupling constant (10.28 Hz) between the methyne protons of **8** indicate that both occupy pseudo-axial positions. The methyne proton on C-3 can then be assumed to occupy a similar position in **3**–**7** and **9** and

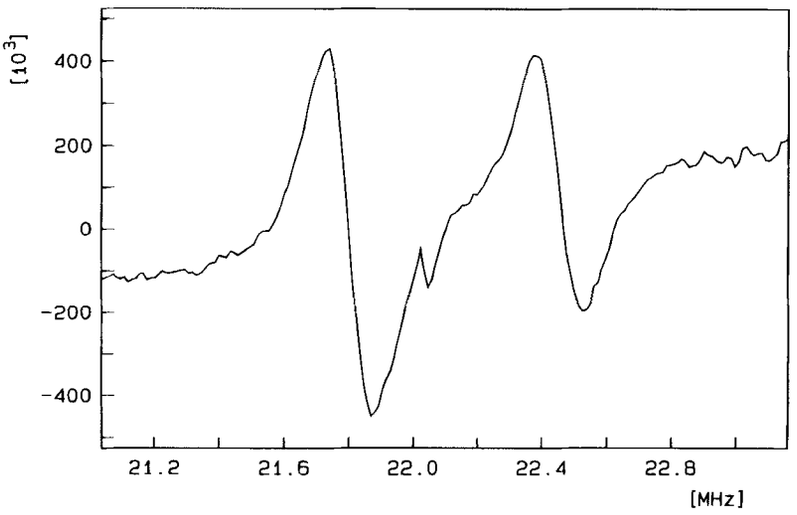


Figure 3. A section of the ENDOR spectrum of the spin adduct **III** in toluene at 213 K.

the phenyl substituent the sterically favourable pseudo-equatorial one. Since the coupling between axial–axial protons leads to higher values than for axial–equatorial protons, the ^{1}H NMR spectra of these compounds showed that the pseudo-axial protons absorbed further downfield than pseudo-equatorial protons. This may be explained by shielding of the latter by the phenyl ring. Compounds **6** and **7**, with a second phenyl substituent on C-5, show the opposite effect, probably resulting from the different orientations of the methylene protons with respect to the second phenyl substituent, but we do not have any reliable evidence on whether it is in a pseudo-axial or equatorial position.

Also, the EPR spectrum obtained by the partial autoxidation of the hydroxylamine **4** leading to radical **XV** show that the β-protons on C-2 are significantly magnetically non-equivalent, indicating the rigid conformation of the five-membered ring even on the faster EPR time-scale. Similar results were obtained with the hydroxylamine **6**, whose oxidation product showed β-proton splittings of 16 and 22 G. One β-hydrogen is in a pseudo-axial and the other in a pseudo-equatorial position. Only the larger β-H splitting constant decreased slightly with decreasing temperature (14 mG/K), also indicating the magnetic non-equivalence of the β-hydrogens and confirming the interpretation given. In principle, different coupling constants for the protons in position 2 can be expected, due either to different hyperconjugation angles or to the presence of the chiral carbon in position 3 leading to diastereotopic protons in positions 2 and 4, but the latter are EPR silent. However, diastereotopic protons known in the literature[14] show merely small differences of their splittings, but the radical **XV** shows β-proton coupling constants of 14.25 and 23.38 G. If the β-protons in radical **XV** and the monovalent oxidation product of **6** are geometrically equivalent, the B values would differ by a factor of approximately two and this is very unlikely for nuclei bound to the same sp^3-hybridized carbon. Using the McConnell–Heller equation, the angles for the large and small splittings can be attributed to the axial and equatorial positions, respectively:

$$a = B \cos^2 \theta\rho_N$$

where B is a constant, ρ_N = spin density on the N atom and θ = dihedral angle. The exact angles cannot be calculated directly because neither ρ_N nor B is known for the compounds under discussion. However, using the expression

$$\frac{a_{Hax}}{a_{Heq}} = \frac{\cos^2 \theta}{\cos^2 (\theta + 120)} = \frac{23.38}{14.25} = 1.64$$

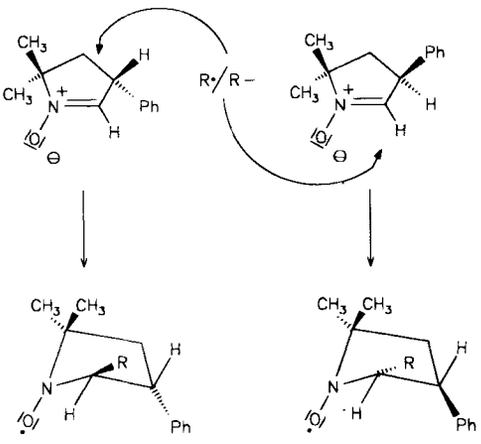
the knowledge of $B\rho_N$ is not required. The solutions yield angles of 18° and 138°, which result in $B\rho_N = 25.8$, which is very close to the value deduced from the dimethylnitroxide radical (24.6).

In contrast to the hydroxylamines, no distinct information concerning the conformation of the nitrones, especially **5**, could be deduced from the NMR spectra. The methylene protons of **5** have different chemical shifts but their coupling constants with the methyne proton show only small differences. This does not influence the problem under consideration because the structure of the spin adducts is identical with that of the hydroxylamines. The synthesis of **5** was done in an achiral environment, leading to a racemic mixture.

The addition of a radical or an anion can proceed from either the *si* or the *re* side of the prochiral carbon in position 2 and therefore a new chiral center is formed, leading to diastereomers which are known to have different chemical and spectral properties (Scheme 2).[15–17] If the chiral centers in the enantiomers of the nitrone are characterized by *R*, *S* and those formed by the addition reaction by *R'*, *S'*, then the products are *RR'*, *SS'* and *RS'*, *R'S*, which means two enantiomeric pairs of diastereomers. In principle, these compounds can exist in different conformations; however, the NMR spectra of **4** and **6** and the EPR spectra of their oxidation products show rigid molecules in which the phenyl substituent in position 3 occupies an equatorial position, as discussed above. The introduction of a third chiral center results in groups of diastereomeric hydroxylamines or nitroxide pairs (Table 3) that are expected to have at least different NMR or EPR spectral parameters. The pairs in each column are enantiomeric to each other but diastereomeric to those in the next column.

Addition of chiral or achiral carbon-centered radicals or anions to nitrone **5** leads exclusively to products in which the new substituent is equatorially bound (*trans* product) according to the coupling constants in the ^{1}H NMR spectra of **8** and the β-proton splittings in the EPR spectra of **VIII–XIV**. This observation is consistent with results obtained by using DMPO and related spin traps.[18]

In contrast, oxygen-centered radicals (**V**, **VI**) were found to give spin adducts with low β-H splittings, indicating an axial position for the substituent. This result is likewise consistent with those for analogous DMPO adducts. However, in the literature[18] there are other



Scheme 2

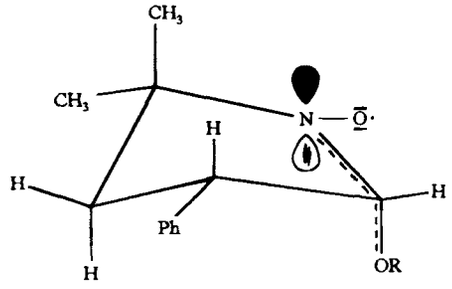
Table 3. Pairs of diastereomers of nitrone 5

Diastereomers A		Diastereomers B	
RR'R"	*RR'S"*	*S'RR"*	*R'SR"*
SS'S"	*SS'R"*	*R'SS"*	*S'RS"*

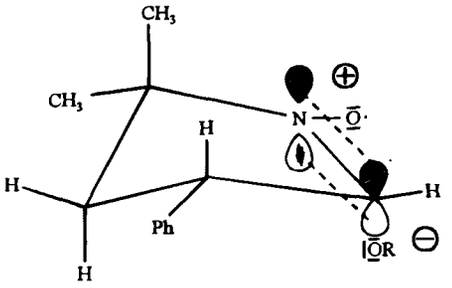
numerous examples of trapping products of sulfur-centered radicals with similar low β-H splitting constants. These observations seem to be general. The equatorial position is, of course, favored by steric interactions and this raises the question of what the driving force is for the location of ether and thioether groups in axial positions even in the case of the bulky *tert*-butoxy group. The most striking difference between the *trans* and *cis* adducts is the lone electron pairs of the ether and thioether moiety. For this reason, we propose a stereoelectronic interaction which favors in this case the axial over the equatorial position, despite the steric interaction advantages of the latter.

Such stereoelectronic effects, referred to as an 'anomeric effect', are considered to stabilize axial positions of hydroxy groups in cyclic acetals by delocalization of bonded and non-bonded electronic pairs in these molecules.[19] Oxygen-centered spin adducts of **5** and other DMPO analogs show the preference for the same conformation observed in acetals, but in this case one oxygen atom is replaced by a nitrogen atom. This interpretation also holds for sulfur-centered spin adducts where the oxygen atom in the polarized bond is now replaced by a sulfur atom.

This stabilization can be illustrated either by the partial transfer of an electron of one heteroatom to another electronegative atom or by the concept of 'double bond–no-bond resonance' whereby this delocalization is due to the overlap of an electron pair orbital of a heteroatom with the antibonding orbital of the polarized sigma bond.[20] The electron pair orbital of the nitrogen atom is oriented antiperiplanar to this bond, as shown in the formulae.



"Partial Delocalization"



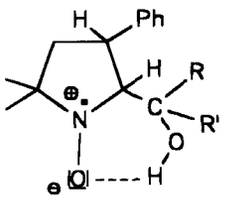
"Double bond - no bond resonance"

Addition of a chiral substituent 2-butyl anion to the nitrone **5** and subsequent monovalent oxidation leads to the radical **XII**. Both the EPR and the ENDOR spectra of this nitroxide radical show only one β-proton constant with the expected value of 24.50 G, but one γ-H splitting could be resolved in the latter. However, the corresponding nitrone **9** obtained by divalent oxidation gives a relatively complex ^{1}H NMR spectrum (see Fig. 2), which can be attributed to an almost equimolar mixture of two diastereomers, as already discussed. Obviously neither the *B* values nor the dihedral angle of the β-H have sufficient differences to result in a variation of the EPR coupling constants of the diastereomers. However, the introduction of a hydroxyl group on the chiral carbon of the butyl substituent shows in the ENDOR spectra the existence of two species attributed to the two diastereomers. The EPR spectra only give some indication of a radical mixture, but no resolution.

The hydrogen bonding between the hydroxyl and nitroxyl functions has been known to enhance the EPR spectral differences between such diastereomers.[12,21] It has been reported by Kotake *et al.*[22] that the formation of a chelate ring due to intramolecular hydrogen bonding (see structure) is expected to strengthen the interactions of substituents because of hindrance to internal rotation, but no resolution was achieved.

EPR temperature variation studies of the hydroxyalkyl spin adducts with toluene as solvent were conducted in order to increase the intramolecular hydrogen bonding and probably achieve a better resolution of the spectra. This was not successful, but linewidth broadening with decreasing temperature was observed, probably resulting from the anisotropic contributions and the increasing EPR spectral differences between the two groups of diastereomers as the intramolecular hydrogen bonding strengthens. Similar results have been observed by several other investigators.[23,24]

A change of solvent from the aprotic toluene to the protic methanol for the hydroxyalkyl spin adducts (see Table 2) showed a slight increase in the N and β-hydrogen splitting constants, which could be a result of the enhanced contribution of the polar resonance form (see structure) in the high dielectric methanol solvent.[24–26]



A general comparison of β-H splitting constants given in the literature of various DMPO spin adducts of carbon-centered radicals/anions with the corresponding adducts of nitrone **5** show an increase of 2–4 G for the latter. These relatively large differences are definitely due to their structural variation and dynamic properties. The most interesting comparison involves the β-H coupling constants of the *tert*-butoxy spin adducts of **5** ($a_{\beta\text{-H}} = 15.48$ G) and DMPO ($a_{\beta\text{-H}} = 8.16$ G)[27] in benzene. The steric repulsions between the pseudo-equatorial phenyl substituent and the pseudo-axial *tert*-butoxy adduct results in a lowering of the β-H dihedral angle in the case of **5**. The determination of the β-CH

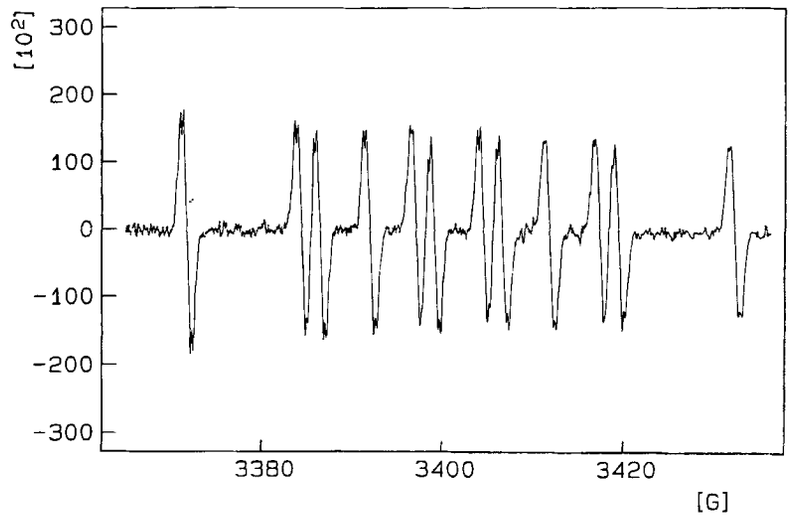


Figure 4. EPR spectrum of a hydrogen spin adduct of nitrone **7** (a_N = 13.75 G, $a_{\beta\text{-H}}$ = 17.5 G, $a_{\beta\text{-H}}$ = 22.13 G; for $a_{\gamma\text{-H}}$, see Fig. 5).

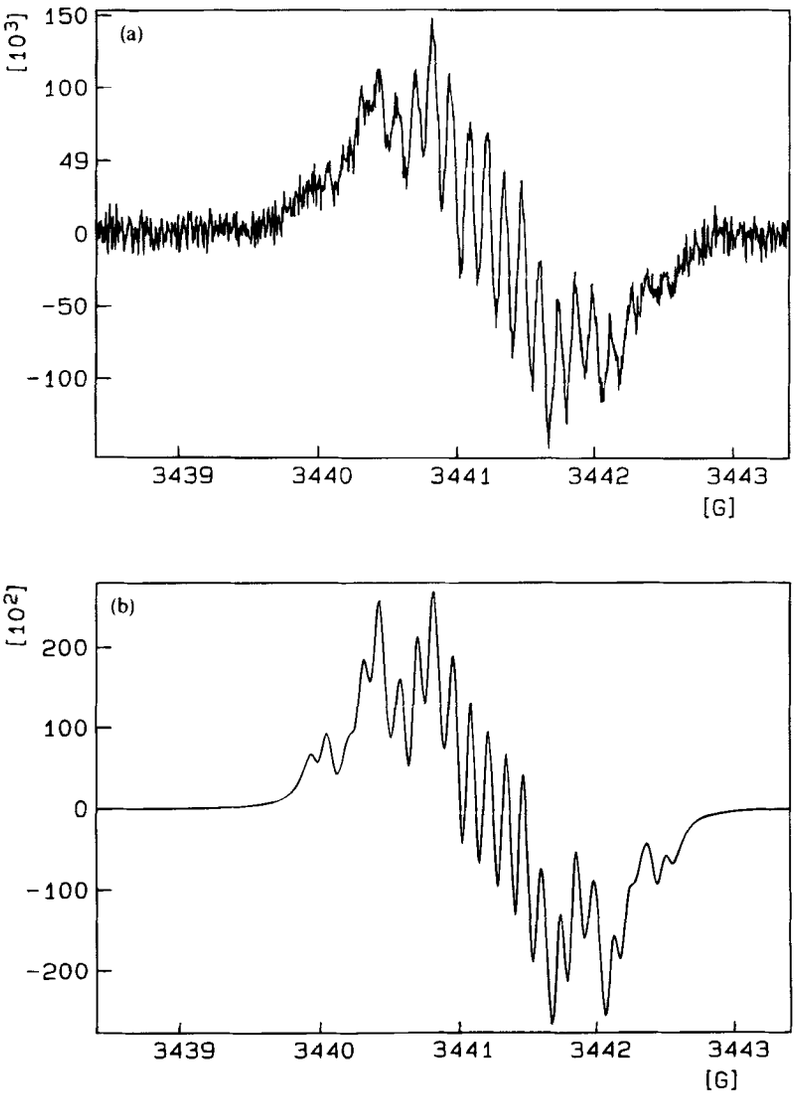


Figure 5. (A) Expanded spectrum of the first peak of the hydrogen spin adduct spectrum of nitrone **7**; (B) simulated (2γH = 0.112 G, 3γH = 0.371 G, 1γH = 0.166 G, 1γH = 0.410 G, 1γH = 0.645 G).

dihedral angle with the semi-occupied p-orbital of the N atom for the nitrone **5** and DMPO using the McConnell–Heller equation (with $B\rho_N = 25.8$) gave 39° and 55°, respectively. The relatively large linewidths which result from small unresolved γ-H splittings decrease the chances of obtaining distinct EPR spectra of diastereomers. To confirm this interpretation of the lack of spectral resolution due to γ-hyperfine splittings, one of the methyl groups of nitrone **5** was substituted with a phenyl group with an aim of reducing the number of γ-hydrogens.

The EPR spectra of the hydrogen spin adduct of this new nitrone, 5-methyl-3,5-diphenylpyrroline-1-oxide (**7**), exhibited relatively smaller linewidths (0.75 G) with well resolved γ-H splittings (see Fig. 4). To resolve completely the γ-H splittings, the first peak of the hydrogen spin adduct spectrum was expanded by recording it on a 5 G scan range, resulting in a 17-line spectrum whose computer simulation confirmed it to be due to the splitting of 8 γ-hydrogens ($2\gamma H = 0.112$ G, $3\gamma H = 0.371$ G, $1\gamma H = 0.166$ G, $1\gamma H = 0.410$ G, $1\gamma H = 0.645$ G) (see Fig. 5). These γ-hydrogens are likely to be those of the methyl group and two from the neighboring phenyl substituent.

Nitrone **5** has several other additional advantages. Its solubility in both aqueous and ordinary organic solvents makes it very ideal for probing short-lived radicals in various systems, including biological media. It can also scavenge hydroxyalkyl radicals in both aprotic and protic solvents similarly to DMPO.[16] *In vivo* spin trapping of trichloromethyl radicals in rat liver (these results will be published elsewhere) was also successful using this nitrone, demonstrating its lipid solubility, a very important property for radical spin trapping in biological systems. This nitrone has been found to be very stable at room temperature. Its crystalline nature and reasonable melting point (96–98 °C) allow good purification to be achieved. Problems with benzylic hydrogen atom abstraction were not experienced in this study.

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