# Synthesis of a Novel Nitrone, 2-Phenyl-5,5-dimethyl-1-pyrroline *N*-Oxide (*nitronyl*-$^{13}C$), for Enhanced Radical Addend Recognition and Spin Adduct Persistence

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**Abstract:** Synthesis and spin trapping chemistry of the new $^{13}C$-labeled cyclic nitrone, 2-phenyl-5,5-dimethyl-1-pyrroline *N*-oxide (*nitronyl*-$^{13}C$), is described. A total of 15 carbon- and oxygen-centered radical adducts were prepared and their electron paramagnetic resonance (EPR) spectral parameters examined in organic as well as aqueous solutions. All the radical spin adducts derived from the novel nitrone displayed a well-resolved triplet of doublets in the EPR spectra. The $\alpha$-$^{13}C$ hyperfine splitting is a good indicator for the chemical structure of the added radical and varies from around 3.2 to 6.2 G. Free radicals are spin trapped at rates that are quite comparable to those observed with *C*-phenyl-*N*-*tert*-butylnitrone (PBN). The main advantage, however, is the enhanced persistence of its radical spin adducts. For this reason the title nitrone holds promise as a biological spin trapping agent because spin adduct decay during metabolism or isolation often governs the success or failure of a given experiment.

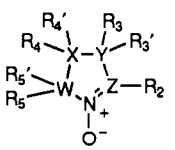
## Introduction

Free radicals are well-established transient intermediates in chemical reactions.[1,2] In addition, free radicals very likely play a role in a number of biological processes.[2,3] Recent *in vitro* and *in vivo* studies[2,3] have strongly pointed to the view that free radicals are responsible for some of the deleterious effects associated with drug metabolism, ischemia reperfusion, high-energy radiation, and lipid peroxidation. Interestingly some of these same intermediates (e.g. the hydroxyl radical or related species) may act beneficially in various catabolic/metabolic reactions as well as cellular phenomena such as phagocytosis.

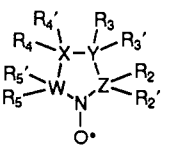
The prevalent feature of most free radicals is their extremely high chemical reactivity. Consequently, they are often difficult to detect directly because only low concentrations of these short-lived species can accumulate. One widely applicable strategy to overcome the detection dilemma is spin trapping.[4-6] Here, reactive free radicals add to unsaturated acceptor molecules (known as spin traps) to yield persistent addition products (called spin adducts) (eq 1). Spin adducts are longer-lived free radicals and as such may readily be observed by electron paramagnetic resonance (EPR) spectroscopy.

$$\underset{\text{free radical}}{\underset{\mathbf{I}}{{}^{\bullet}\text{FR}}} + \underset{\text{spin trap}}{\underset{\mathbf{II}}{\text{ST}}} \rightarrow \underset{\text{spin trap–radical addend (or spin adduct)}}{\underset{\mathbf{III}}{\text{ST–RA}^{\bullet}}} \qquad (1)$$

Over the past decade, spin trapping has opened numerous new avenues for the investigation of free radicals in biological systems. The many reviews on the subject as well as the computer data bases devoted to spin adduct EPR parameters[7,8] are evidence of this. Even though the parent cyclic nitrone spin trap DMPO (IV) was introduced some time ago,[9,10] it is only recently that a family of improved or modified analogues has appeared:[11-24]



DMPO-type nitrone spin traps



DMPO-type nitrone spin adducts

where in the parent nitrone, $R_2 = H$, $R_3 = R_3' = R_4 = R_4' = H$, $R_5 = R_5' = CH_3$, W = X = Y = Z = $^{12}C$ (IV); in the title nitrone,

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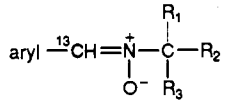
$R_2 = C_6H_5$, $R_3 = R_3' = R_4 = R_4' = H$, $R_5 = R_5' = CH_3$, W = X = Y = $^{12}C$, Z = $^{13}C$ (V); in the parent aminoxyl, $R_2 = H$, $R_3 = R_3' = R_4 = R_4' = H$, $R_5 = R_5' = CH_3$, W = X = Y = Z = $^{12}C$ (VI); and in the title aminoxyl, $R_2 = C_6H_5$, $R_3 = R_3' = R_4 = R_4' = H$, $R_5 = R_5' = CH_3$, W = X = Y = $^{12}C$, Z = $^{13}C$ (VII). Some new nitrones were created for specific applications. For instance, lipophilic nitrones[11-15] are suitable for probing model membrane systems (e.g. micelles and phospholipid vesicles) or actual intracellular processes. Conversely, hydrophilic analogues[16,17] appear to be made to investigate extracellular phenomena. Cyclic nitrones with chiral centers allow one to probe the stereochemistry/stereoselectivity of the radical addition.[24] Deuterium and nitrogen-15 labeled nitrones provide increased EPR sensitivity.[19,22,25,26] The development of a special low-frequency (250 MHz) EPR spectrometer coupled with the implementation of perdeuterated spin traps[26] has illustrated that spin trapped oxygen-centered radicals can be identified and imaged in model heterogeneous systems.

In contrast to the above-mentioned studies where exclusively *aldo-nitrones* (i.e. $R_2 = H$, or $^2H$) (IV) are introduced, we propose the use of a novel *keto-nitrone* (i.e. $R_2$ = carbon-centered group = $C_6H_5$) (V). The reasoning behind this choice relates to the corresponding aminoxyl spin adducts. These are expected to exhibit *enhanced longevity* (VII vs VI) because disproportionation is prevented due to the lack of an abstractable $\beta$-hydrogen atom. To our knowledge only *one* other cyclic keto-nitrone, 2,5,5-trimethyl-1-pyrroline *N*-oxide (also known as 2,5,5-TMPO, 2,5,5-$M_3$PO, or 2-methyl-DMPO), has been tested as a spin trap.[9,10,27-29] The problem with 2-methyl-DMPO as a spin trap is that its spin adducts exhibit *little EPR spectral uniqueness* with respect to the trapped radical. Attempts to alleviate this feature by incorporating a $^{13}C$ atom into the methyl position have been questionable as dimerization reactions (apparently via enamine intermediates) produce strong control signals that cannot be eliminated.[30]

Recently, we[31] (as well as Motten et al.[32]) have found that $^{13}C$-labeling of the *nitronyl* carbon of PBN-type nitrones (VII) leads to spin adducts with three valuable hyperfine splittings ($a^N$, $a_\alpha^{^{13}C}$, and $a_\beta^H$) to help better reflect the nature of the of the added radical (e.g. carbon- vs oxygen-centered):

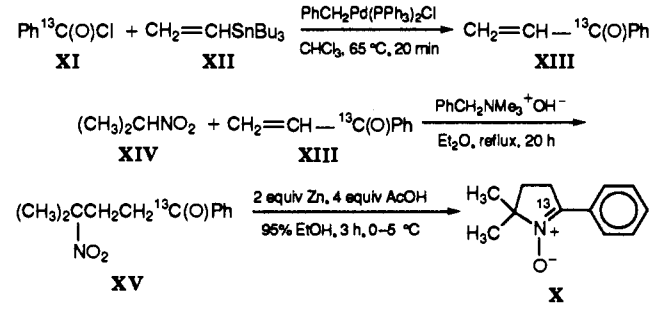


**VIII**

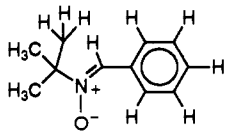
PBN-type spin trap (*nitronyl*-$^{13}C$-labeled)

This is the notion that led to the design of 2-phenyl-DMPO-*nitronyl*-$^{13}C$. It was anticipated that the absence of the $\beta$-H atom in the spin adduct should lead to enhanced chemical persistence (VII vs VI). It was hoped that loss of the informative $\beta$-H hyperfine splitting (or hfs) would be recovered (to some degree) by the introduction of a new diagnostic hyperfine splitting (i.e. the $\alpha$-$^{13}C$ hfs). It should be noted that, despite the similarity to DMPO (IV), 2-phenyl-DMPO (V) may resemble *C*-phenyl-*N*-*tert*-butylnitrone (PBN) even more. The only differences are that 2-phenyl-DMPO has one carbon atom more than PBN and that the nitrone function is confined to a ring structure. The X-ray crystal structure of PBN has been reported,[33] and the *tert*-butyl and phenyl groups are *trans* (or *E*) and the phenyl ring is slightly tilted (13.4°) from the plane of the nitronyl group. The crystal structure of 2-phenyl-DMPO is not known.

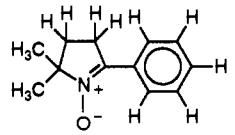
**Scheme 1.** Synthesis of 2-Phenyl-5,5-dimethyl-1-pyrroline *N*-Oxide (*nitronyl*-$^{13}C$)




**XI** **XII** **XIII** **XIV** **XIII** **XV** **X**



PBN (**IX**)



2-Ph-DMPO-*nitronyl*-$^{13}C$ (**X**)

## Synthesis

The title nitrone was prepared in three steps according to Scheme 1. The $\alpha,\beta$-unsaturated carbonyl compound was synthesized by the method of Labadie et al.[34] by reacting benzoyl chloride (*carbonyl*-$^{13}C$) with vinyltributyltin. A Michael addition[35] with 2-nitropropane afforded the nitroketone. Reduction with zinc[35] gave the cyclic nitrone. The $^{12}C$ analogue is known in the literature; however, its synthesis is quite different.[36]

**1-Phenyl-2-propen-1-al-*1*-$^{13}C$ (XIII).** This compound was prepared from benzoyl-*carbonyl*-$^{13}C$ chloride by the same procedure as that used for the preparation of the corresponding normal unsaturated ketone.[34] Purification was carried out by flash column chromatography on silica gel eluted with $C_5H_{12}/CH_2Cl_2$ (50/50, v/v, $R_f = 0.30$) in 99% yield: $^1H$ NMR ($CDCl_3$) $\delta$ 7.97–7.93 (m, 2H, H-Ar), 7.55–7.46 (m, 2H, H-Ar), 7.16 (ddd, $J_H$ = 17.1, 10.5 Hz, $J_{^{13}C}$ = 5.4 Hz, 1H, 2-CH=), 6.44 (ddd, $J_H$ = 17.1, 1.8 Hz, $J_{^{13}C}$ = 6.8 Hz, 1H, *cis*-CH=), 5.94 (ddd, $J_H$ = 10.5 Hz, 1.4 Hz, $J_{^{13}C}$ = 10.5 Hz, 1H, *trans*-CH=) ppm.

**4-Methyl-4-nitro-1-phenylpentan-1al-*1*-$^{13}C$ (XV).** To a solution of 2-nitropropane (4.68 g, 52.6 mmol) and benzyltrimethylammonium hydroxide (40%, 0.15 mL, 0.3 mmol) in diethyl ether (50 mL) was added dropwise a solution of freshly prepared 1-phenyl-2-propen-1-al-*1*-$^{13}C$ (1.4 g, 10.5 mmol) in diethyl ether (20 mL) with stirring at refluxing temperature. The mixture was refluxed for 20 h. After neutralization with 5 drops of concentrated HCl, the solution was washed with a NaCl-saturated aqueous solution (70 mL), dried over $Na_2SO_4$, and then filtered. The solvent was rotary evaporated. Vacuum pumping (room temperature at 1 Torr) gave 2.15 g (92%) of clear liquid which was chromatographed on silica gel. It eluted with $C_5H_{12}/CH_2Cl_2$ (50/50, v/v, $R_f$ = 0.26), providing 1.9 g of the new $^{13}C$-labeled nitroketone compound in 82% yield: $^1H$ NMR ($CDCl_3$) $\delta$ 7.95–7.91 (m, 2H, H-Ar), 7.58–7.55 (t, 1H,

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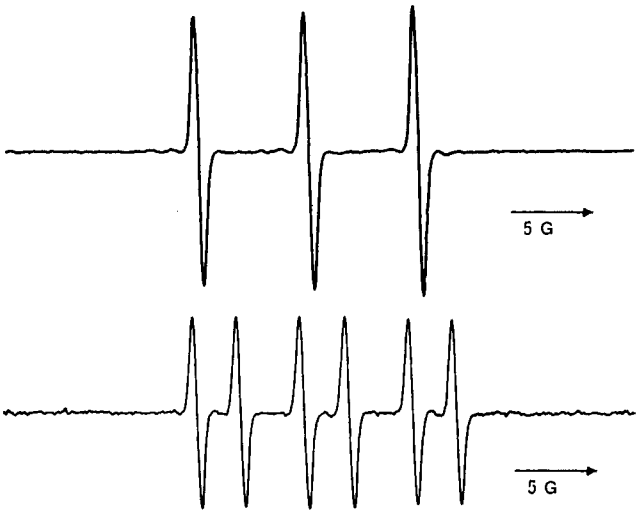


**Figure 1.** EPR spectra of the methyl spin adduct of (top) 2-phenyl-DMPO-*nitronyl*-$^{12}C$ and (bottom) 2-phenyl-DMPO-*nitronyl*-$^{13}C$ in $C_6H_6$.

H-Ar), 7.49–7.44 (t, 2H, H-Ar), 2.98 (q, $J_H = J_{^{13}C} = 7.2$ Hz, 2H, 2-$CH_2$), 2.38 (dt, $J_H = 5.2$ Hz, $J_{^{13}C} = 2.7$ Hz, 2H, 3-$CH_2$), 1.65 (s, 6H, 4-$CH_3$) ppm.

**2-Phenyl-5,5-dimethyl-1-pyrroline *N*-Oxide (*nitronyl*-$^{13}C$) (X).** Zinc dust (1.20 g, 18.3 mmol) was added to a solution of 4-methyl-4-nitro-1-phenylpentan-1-al-*1*-$^{13}C$ (1.9 g, 8.56 mmol) in 95% ethanol (40 mL) which had been precooled to –5 °C. Under brisk mechanical stirring, another solution of acetic acid (2.05 g, 34.2 mmol) in 95% ethanol (20 mL) was added dropwise for 1 h. The mixture was stirred for an additional 1 h at 0 °C and 3 h at 0–3 °C and then set in a refrigerator overnight. Filtration was followed by rotary evaporation. The residue was dissolved in $CHCl_3$ (100 mL) and then washed with a NaCl-saturated aqueous solution (100 mL). The usual workup gave 1.6 g of crude crystals which were chromatographed on silica gel with ethyl acetate to afford 1.0 g of white crystals (61% yield). Further purification by sublimation provided 1.0 g of the novel $^{13}C$-labeled nitrone, mp = 99 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 8.40–8.36 (m, 2H, H-Ar), 7.44–7.42 (m, 3H, H-Ar), 3.04 (q, $J_H = J_{^{13}C} = 7.4$ Hz, 2H, 3-$CH_2$), 2.12 (dt, $J_H = 7.4$ Hz, $J_{^{13}C} = 2.6$ Hz, 2H, 4-$CH_2$), 1.50 (s, 6H, 5-$CH_3$) ppm. These $^1H$ NMR values agree well with those reported by Black and Boscacci[36] for the $^{12}C$ analogue. $^1H$ NMR ($CCl_4$) $\delta$ 8.50–8.33 (m, 2H, H-Ar), 7.53–7.35 (m, 3H, H-Ar), 3.15–2.92 (m, 2H, 3-$CH_2$), 2.23–1.97 (m, 2H, 4-$CH_2$), 1.48 (s, 6H, 5-$CH_3$).

**EPR.** EPR spectra were recorded using a Bruker ER-200D spectrometer (ST 4102 X-band cavity) and Bruker ER-140 (Aspect 2000) data system. Some spectra are computer accumulated to improve signal/noise. EPR spectra were simulated with a BASIC program developed by Oehler et al.[37] All EPR spectra were recorded at low resolution unless otherwise noted. Low resolution means a microwave power of 20 mW, modulation amplitude of 1.0 G, modulation frequency of 100 kHz, and microwave frequency of 9.81 GHz. High resolution refers to the same settings except that the microwave power and modulation frequency were 1 mW and 0.1 G, respectively.

## Results and Discussion

The EPR spectra of spin adducts of the unlabeled parent spin trap (i.e. 2-phenyl-DMPO-*nitronyl*-$^{12}C$ (**IV**)) exhibit simple three line spectra[9,10,27-30,38] due to the hyperfine splitting of the aminoxyl nitrogen atom (e.g. the methyl adduct, Figure 1 (top)). While it is true that the nitrogen hfs's of spin adducts of 2-phenyl-DMPO-*nitronyl*-$^{12}C$ may be able to distinguish carbon- from oxygen-centered radicals, the ability to disclose the detailed structure of the added radical is lacking. It is for this reason we synthesized 2-phenyl-DMPO-*nitronyl*-$^{13}C$. The basic EPR pattern for spin adducts of 2-phenyl-DMPO-*nitronyl*-$^{13}C$ is a well-resolved six-line spectrum (triplet of doublets) (e.g. the methyl adduct, Figure 1 (bottom)) that resembles that of PBN spin adduct spectra. The difference, of course, is the origin of the doublet splitting. For 2-phenyl-DMPO-*nitronyl*-$^{13}C$, it is due to the $\alpha$-$^{13}C$ hfs; for PBN, it is the $\beta$-H hfs. Improved EPR spectral uniqueness is achieved via the $\alpha$-$^{13}C$ hfs.

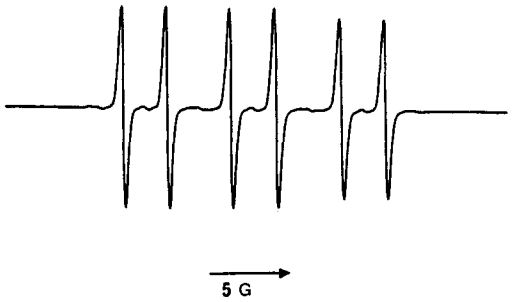


**Figure 2.** EPR spectrum of the phenyl spin adduct of 2-phenyl-DMPO-*nitronyl*-$^{13}C$ • from (phenylazo)triphenylmethane in $C_6H_6$.

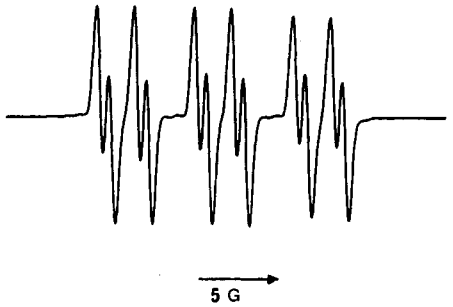


**Figure 3.** EPR spectrum of the (2-cyano-2-propyl)oxyl spin adduct of 2-phenyl-DMPO-*nitronyl*-$^{13}C$ from 2,2′-azobis(isobutyronitrile) (AIBN) in $C_6H_6$.

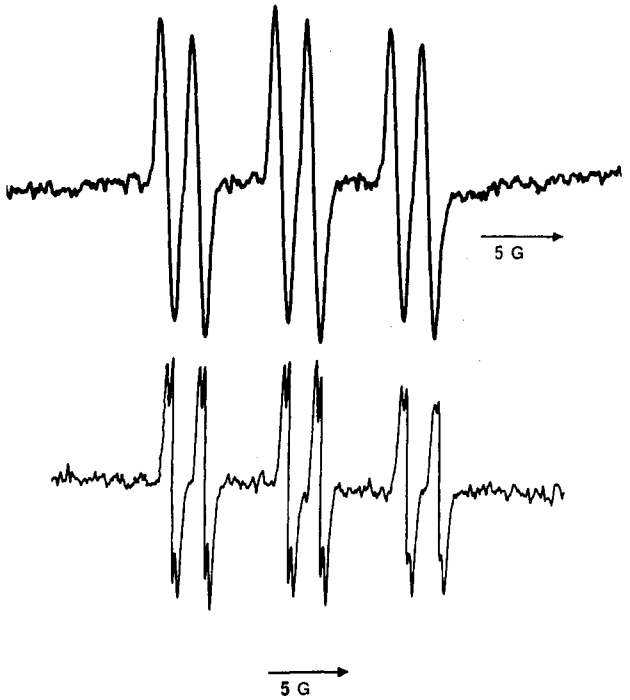


**Figure 4.** (top) EPR spectrum of the hydroxyl spin adduct of 2-phenyl-DMPO-*nitronyl*-$^{13}C$ from sodium persulfate oxidation in water at low resolution. (bottom) EPR spectrum of the hydroxyl spin adduct of 2-phenyl-DMPO-*nitronyl*-$^{13}C$ at high resolution.

**The Phenyl Radical Adduct.** Thermolysis of (phenylazo)-triphenylmethane in the presence of 2-Ph-DMPO-*nitronyl*-$^{13}C$ in $C_6H_6$ produces an EPR spectrum (Figure 2) that is typical of carbon-centered radical adducts. A triplet of doublets pattern is seen due to the aminoxyl nitrogen and $\alpha$-$^{13}C$ nuclei. The N hfs is 13.60 G and the $\alpha$-$^{13}C$ hfs is 5.58 G.

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**Table 1.** EPR Hyperfine Splittings for the Radical Spin Adducts of 2-Phenyl-DMPO-*nitronyl*-$^{13}C$ in $C_6H_6$[a]

| | radical addend | $a^N$ | $a_\alpha^{13C}$ | $a^{other}$ | generation conditions |
|---|---|---|---|---|---|
| 1 | $CH_3$ | 13.80 | 5.67 | | Grignard |
| 2 | $C(CH_3)_3$[b] | 13.91 | 5.76 | | Grignard |
| 3 | H | 13.70 | 5.91 | $a_\beta^H$ = 19.37 | $NaBH_4$ |
| 4 | $C(CH_3)_2CN$ | 13.50 | 6.12 | | AIBN |
| 5 | $CH(CH_3)_2$ | 13.94 | 5.94 | | Grignard |
| 6 | $CH_2C_6H_5$ | 13.77 | 6.03 | | Grignard |
| 7 | $CH(OH)CH_3$ | 14.55 | 5.90 | | EtOH, BP* [d] |
| 8 | $CH_2OH$ | 14.28 | 6.03 | | MeOH, BP* [d] |
| 9 | $CH{=}CH_2$ | 13.71 | 5.81 | | Grignard |
| 10 | $C_6H_5$[c] | 13.70 | 5.58 | | Grignard |
| 11 | $C(O)CH_3$ | 13.26 | 6.17 | | $CH_3CHO$, BP* [d] |
| 12 | $CCl_3$ | 13.20 | 6.03 | | $CBrCl_3$, $h\nu$ |
| 13 | $OC(CH_3)_3$ | 12.76 | 4.79 | $a_\gamma^H$ = 1.69 | $((CH_3)_3CO)_2$ |
| 14 | $OC(CH_3)_2CN$ | 12.32 | 4.64 | $a_\gamma^H$ = 1.53 | AIBN, $O_2$ |
| 15 | $OC(O)C_6H_5$ | 12.66 | 3.20 | $a_\gamma^{2H}$ = 0.61 | $(C_6H_5C(O)O)_2$ |

[a] The EPR spectra were recorded at ambient temperature, and the hyperfine splittings ($a$) are given in gauss (1 G = 0.1 mT). [b] The hfs's for the $C(CH_3)_3$ spin adduct (from 2-azo-2-methylpropane) are $a^N$ = 13.80 and $a_\alpha^{13C}$ = 5.75 G. [c] The hfs's for the $C_6H_5$ spin adduct (from (phenylazo)triphenylmethane) are $\alpha^N$ = 13.60 and $a_\alpha^{13C}$ = 5.58 G. [d] BP* represents photoexcited benzophenone.

**The (2-Cyano-2-propyl)oxyl Radical Adduct.** The (2-cyano-2-propyl)oxyl radical spin adduct from the thermolysis of 2,2'-azobis(isobutyronitrile) in the presence of $O_2$ in $C_6H_6$ gives the usual triplet of doublets pattern plus one resolvable $\gamma$-H hfs. The result is a 12-line spectrum with a N hfs of 12.32 G, $a_\alpha^{13C}$ of 4.64 G, and a $\gamma$-H hfs of 1.53 G (Figure 3). The pattern of long-range hfs's from the $\gamma$-position(s) appears to be the norm for oxygen-centered radical spin adducts of 2-Ph-DMPO-*nitronyl*-$^{13}C$.

**The Hydroxyl Radical Adduct.** At ordinary resolution (e.g. microwave power = 20 mW, modulation = 1G) $\gamma$-H hfs is not seen (Figure 4 (top)). However, at high resolution (e.g. microwave power = 1 mW, modulation = 0.1 G) the trend of long-range $\gamma$-H hfs for oxygen-centered radical adducts is followed by the hydroxyl radical adduct in water (Figure 4 (bottom)). The standard triplet of doublets pattern with additional triplet structure is observed due to two $\gamma$-H's with an hfs of 0.74 G.

Collected in Tables 1 and 2 are the EPR hfs's for representative examples of carbon- and oxygen-centered radical adducts of 2-Ph-DMPO-*nitronyl*-$^{13}C$ in $C_6H_6$ and $H_2O$, respectively. The ranges (from Tables 1 and 2) of EPR hfs's of various carbon- and oxygen-centered radical adducts in $C_6H_6$ and $H_2O$ are as follows:

| Carbon-Centered Radical Adducts | |
|---|---|
| N hfs (G) | $\alpha$-$^{13}C$ hfs (G) |
| 13.20–14.55 | 5.58–6.17 (in $C_6H_6$) |
| 14.71–15.84 | 6.06–6.57 (in $H_2O$) |

| Oxygen-Centered Radical Adducts | |
|---|---|
| N hfs (G) | $\alpha$-$^{13}C$ hfs (G) |
| 12.32–12.76 | 3.20–4.79 (in $C_6H_6$) |
| 14.07–14.72 | 4.05–5.04 (in $H_2O$) |

**Uniqueness of the $\alpha$-$^{13}C$ hfs and Predictive Correlations.** To evaluate whether or not the $\alpha$-$^{13}C$ hfs is a good indicator to distinguish various added radicals, a plot of $a_\alpha^{13C}$ hfs vs the N hfs was constructed (Figure 5). The observed *scatter plot* is evidence that the new EPR parameter ($\alpha$-$^{13}C$ hfs) generates some EPR spectral uniqueness.[10]

When the N hfs of spin adducts of 2-Ph-DMPO-*nitronyl*-$^{13}C$ are plotted vs the N hfs's of the corresponding PBN adducts (in $C_6H_6$) (Table 3, Figure 6), some linear character is observed ($r^2$ = 0.71). The greater the linearity in these types of correlations the better the predictive power to discern the identity of an unknown radical addend. Interestingly, a plot of the $\alpha$-$^{13}C$ hfs's of spin adducts of 2-Ph-DMPO-*nitronyl*-$^{13}C$ vs those [31] for PBN-*nitronyl*-$^{13}C$ (in $C_6H_6$) (Table 3) shows significantly higher linearity ($r^2$ = 0.94, Figure 7). A plot of the N hfs's of spin adducts of 2-Ph-DMPO-*nitronyl*-$^{13}C$ vs those for DMPO (in

**Table 2.** EPR Hyperfine Splittings for the Radical Spin Adducts of 2-Phenyl-DMPO-*nitronyl*-$^{13}C$ in $H_2O$[a]

| | radical addend (R) | $a^N$ | $a_\alpha^{13C}$ | $a^{other}$ | generation conditions |
|---|---|---|---|---|---|
| 1 | $CH_3$[b] | 15.76 | 6.07 | | Grignard |
| 2 | $C(CH_3)_3$ | 15.29 | 6.26 | | Grignard |
| 3 | H | 15.84 | 6.56 | $a_\beta^H$ = 24.50 | $NaBH_4$ |
| 4 | $CH(CH_3)_2$ | 15.59 | 6.09 | | Grignard |
| 5 | $CH_2C_6H_5$ | 15.28 | 6.35 | | Grignard |
| 6 | $CH(OH)CH_3$ | 15.39 | 6.26 | | EtOH, BP* |
| 7 | $CH_2OH$ | 15.16 | 6.30 | | MeOH, BP* |
| 8 | $CH{=}CH_2$ | 15.48 | 6.08 | | Grignard |
| 9 | $C_6H_5$[c] | 15.39 | 6.06 | | Grignard |
| 10 | $CO_2^-$ | 15.36 | 6.57 | | $HCO_2^-$, $S_2O_8^{2-}$ |
| 11 | $C(O)CH_3$ | 14.71 | 6.53 | | $CH_3CHO$, BP* |
| 12 | OH | 14.72 | 4.05 | $a_\gamma^{2H}$ = 0.74 | $S_2O_8^{2-}$ |
| 13 | $OC(CH_3)_3$ | 14.68 | 5.04 | not resolveable | $((CH_3)_3CO)_2$ |
| 14 | $OC(CH_3)_2CN$ | 14.07 | 4.68 | $a_\gamma^{2H}$ = 0.81 | AIBN |

[a] The EPR spectra were recorded at ambient temperature, and the hyperfine splittings ($a$) are given in gauss (1 G = 0.1 mT). [b] The hfs's for the $CH_3$ spin adduct (from the photolysis of hydrogen peroxide in the presence of dimethyl sulfoxide) are $a^N$ = 15.80 and $a_\alpha^{13C}$ = 6.03 G. [c] The hfs's for the $C_6H_5$ adduct (from (phenylazo)triphenylmethane) are $a^N$ = 15.49 and $a_\alpha^{13C}$ = 6.07 G.

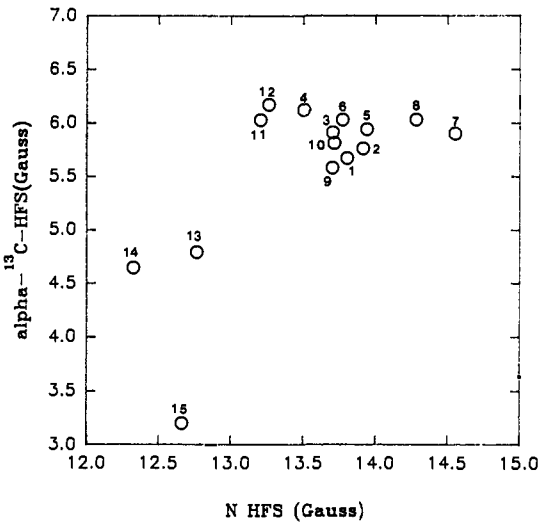



**Figure 5.** Scatter plot of the $\alpha$-$^{13}C$ hyperfine splittings vs the N hyperfine splittings of 2-phenyl-DMPO-*nitronyl*-$^{13}C$–R$^\bullet$ in $C_6H_6$.

**Table 3.** EPR Hyperfine Splittings for the Radical Spin Adducts of PBN-*nitronyl*-$^{13}C$ in $C_6H_6$[a,b]

| | radical addend (R) | $a^N$ | $a_\beta^H$ | $a_\alpha^{13C}$ | generation conditions |
|---|---|---|---|---|---|
| 1 | $CH_3$ | 14.85 | 3.53 | 5.29 | cf. Table 1 |
| 2 | $C(CH_3)_3$ | 14.66 | 2.32 | 5.49 | |
| 3 | H | 14.87 | 7.41 | 5.38 | |
| 4 | $C(CH_3)_2CN$ | 14.28 | 3.29 | 5.78 | |
| 5 | $CH(CH_3)_2$ | 14.67 | 2.59 | 5.30 | |
| 6 | $CH_2C_6H_5$ | 14.45 | 2.54 | 5.68 | |
| 7 | $CH(OH)CH_3$[c] | 15.10 | 5.00 | 5.60 | |
| 8 | $CH_2OH$ | 14.88 | 6.51 | 5.74 | |
| 9 | $CH{=}CH_2$ | 14.85 | 2.68 | 5.31 | |
| 10 | $C_6H_5$ | 14.37 | 2.18 | 5.53 | |
| 11 | $C(O)R^d$ | 14.27 | 3.14 | 5.90 | |
| 12 | $CCl_3$ | 14.01 | 1.77 | 5.82 | |
| 13 | $OC(CH_3)_3$ | 14.28 | 2.03 | 4.91 | |
| 14 | $OC(CH_3)_2CN$ | 13.93 | 2.16 | 4.70 | |
| 15 | $OC(O)C_6H_5$ | 13.29 | 2.16 | 3.17 | |

[a] The EPR spectra were recorded at ambient temperature, and the hyperfine splittings ($a$) are given in gauss (1 G = 0.1 mT). [b] Unless otherwise noted, the EPR data are from ref 31. [c] Average values for the diastereomeric $\beta$-H. [d] R = $C_2H_5$.

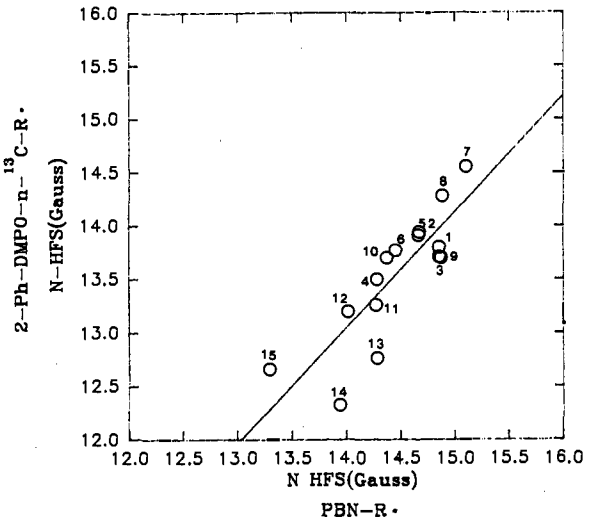



**Figure 6.** Plot of the N hyperfine splittings of 2-phenyl-DMPO-*nitronyl*-$^{13}C$–R• vs PBN–R• in $C_6H_6$.

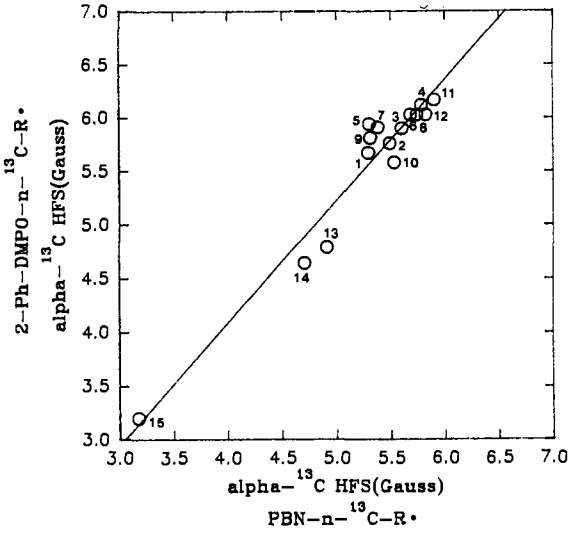
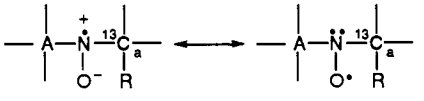



**Figure 7.** Plot of the $\alpha$-$^{13}C$ hyperfine splittings of 2-phenyl-DMPO-*nitronyl*-$^{13}C$–R• vs PBN-*nitronyl*-$^{13}C$–R• in $C_6H_6$.

$C_6H_6$) (Table 4, Figure 8) exhibits a correlation ($r^2$ = 0.80) intermediate to those observed in Figure 5 ($r^2$ = 0.71) and Figure 7 ($r^2$ = 0.94).

The aminoxyl function can be described as a hybrid of two resonance structures:



**XVI** **XVII**

The larger N and $\alpha$-$^{13}C$ hfs's (in water (Table 2) vs $C_6H_6$ (Table 1)) may be explained by the stabilization of the polar form (**XVI**), which places the unpaired electron directly on the N atom. The consistently lower N and $\alpha$-$^{13}C$ hfs's for oxygen-centered radicals (with respect to carbon-centered radicals) are likely due to unfavorable dipole–dipole interactions. Specifically, if the radical addend (R) is an electron withdrawing group (e.g. an oxygen-centered addend), the polar canonical form (**XVI**) would be disfavored.

**Spin Adduct Kinetics—Preliminary Data. Formation Kinetics.** In Figure 9 is shown the EPR mixture spectrum derived from equimolar (0.034 M) amounts of 2-phenyl-DMPO-*nitronyl*-$^{13}C$ and PBN. The concentration of the phenyl radical source

**Table 4.** EPR Hyperfine Splittings for the Radical Spin Adducts of DMPO in $C_6H_6$[a,b]

| | radical addend | $a^N$ | $a_\beta^H$ | $a^{other}$ | generation conditions |
|---|---|---|---|---|---|
| 1 | $CH_3$ | 14.25 | 20.64 | | cf. Table 1 |
| 2 | $C(CH_3)_3$ | 14.23 | 20.88 | | |
| 3 | H | 14.30 | 18.80 | | |
| 4 | $C(CH_3)_2CN$[c] | 14.60 | 20.40 | | |
| 5 | $CH(CH_3)_2$[d] | 14.11 | 21.42 | | |
| 6 | $CH_2C_6H_5$[e] | 14.16 | 20.66 | | |
| 7 | $CH(OH)CH_3$[e] | 15.03 | 22.53 | | |
| 8 | $CH_2OH$ | 14.71 | 21.66 | | |
| 9 | $CH{=}CH_2$[d] | 14.07 | 18.68 | | |
| 10 | $C_6H_5$ | 13.78 | 19.21 | | |
| 11 | $CCl_3$ | 13.17 | 15.28 | | |
| 12 | $C(O)CH_3$[e] | 14.03 | 17.87 | | |
| 13 | $OC(CH_3)_3$ | 13.19 | 8.16 | $a_\gamma^H$ = 1.97 | |
| 14 | $OC(CH_3)_2CN$[f] | 12.66 | 8.37 | $a_\gamma^H$ = 1.89 | |
| 15 | $OC(O)C_6H_5$ | 12.24 | 9.63 | $a_\gamma^{2H}$ = 0.87 | |

[a] The EPR spectra were recorded at ambient temperature, and the hyperfine splittings ($a$) are given in gauss (1 G = 0.1 mT). [b] Unless otherwise noted, the EPR data are from ref 31. [c] These hyperfine splittings were measured in xylene, cf. ref 39. [d] This work. [e] These hyperfine splittings were collected from ref 9. [f] See ref 40.

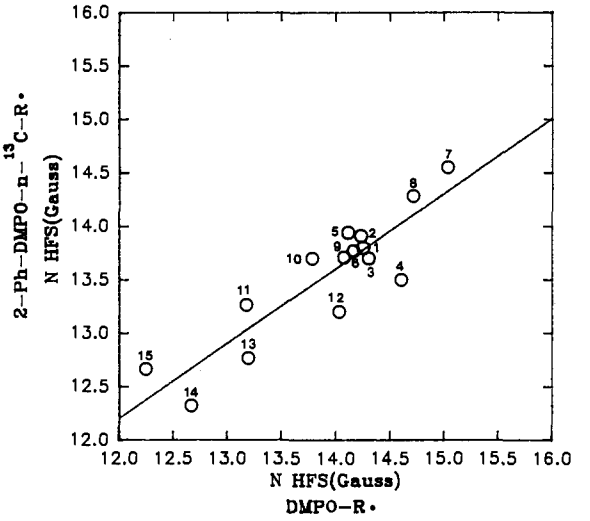



**Figure 8.** Plot of the N hyperfine splittings of 2-phenyl-DMPO-*nitronyl*-$^{13}C$–R• vs DMPO–R• in $C_6H_6$.

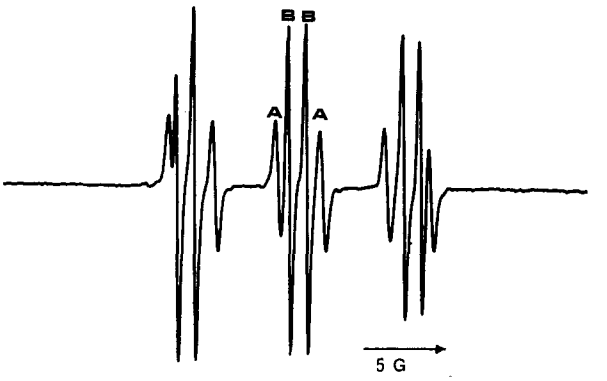



**Figure 9.** EPR mixture spectrum of the phenyl adducts of 2-phenyl-DMPO-*nitronyl*-$^{13}C$ (labeled A) and PBN (labeled B) in $C_6H_6$ at room temperature.

((phenylazo)triphenylmethane) was 0.003 M. Since both spin traps each produce a triplet of doublets spectrum, the EPR mixture spectrum displays a 12-line pattern. The middle multiplet was chosen for kinetics measurements because it displays the least spectral overlap. The two outermost lines of this multiplet are due to 2-phenyl-DMPO-*nitronyl*-$^{13}C$–$C_6H_5$• (labeled A), while the two innermost lines are due to PBN–$C_6H_5$• (labeled B).

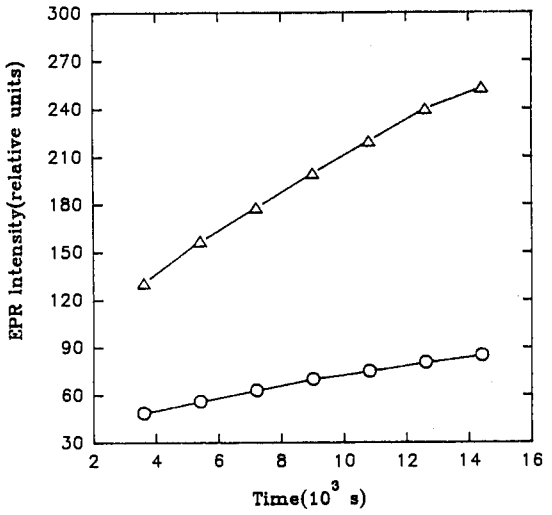



**Figure 10.** Comparison of the rates for spin trapping of phenyl radicals by 2-phenyl-DMPO-*nitronyl*-$^{13}C$ (circles) and PBN (triangles) in $C_6H_6$ at room temperature.

Examination of the initial slopes for phenyl spin adduct production (Figure 10) shows that 2-Ph-DMPO-*nitronyl*-$^{13}C$ traps phenyl radicals at approximately one-fifth the rate for PBN (or $\sim 2.0 \times 10^6$ $M^{-1}$ $s^{-1}$). The slower rate of trapping may be a reflection of the increased steric hindrance (keto-nitrone vs aldo-nitrone). Alternatively, the tilt of the phenyl group with respect to the plane of the nitronyl group may be different than that observed with PBN.[33] Curvature in the plots (Figure 10) is likely due to double-trapping at the aminoxyl oxygen.[40]

In a similar way, the rate constant of spin trapping an oxygen-centered radical was determined. Thus benzoyloxyl ($^{\bullet}OC(O)C_6H_5$) was found to be approximately one-half that of PBN or $2.5 \times 10^5$ $M^{-1}$ $s^{-1}$.

**Persistence Kinetics.** To study the persistence of 2-Ph-DMPO-*nitronyl*-$^{13}C$ spin adducts, oxygen-centered radicals (*tert*-butoxyl) were prepared by photolysis of di-*tert*-butyl peroxide in $C_6H_6$ at 25 °C. Carbon-centered adducts (methyl) were prepared by Grignard addition also in $C_6H_6$ at 25 °C. The *tert*-butoxyl adduct of PBN exhibited a half-life of about *6 days*. In contrast, the *tert*-butoxyl adduct of 2-Ph-DMPO-*nitronyl*-$^{13}C$ exhibits a $t_{1/2}$ estimated to be in *weeks*. The methyl adduct of PBN displayed a half-life of around *9 days*, while the corresponding 2-Ph-DMPO-*nitronyl*-$^{13}C$ adduct again showed no change after 1 week. It is possible that this spin adduct is stable indefinitely.

Persistence of the methyl and *tert*-butoxyl spin adducts of 2-Ph-DMPO-*nitronyl*-$^{13}C$ and PBN in aqueous solution was also examined. The half-life of the EPR signal for the methyl radical adduct of the title nitrone is estimated to be in *months*. The oxygen-centered radical adduct (*tert*-butoxyl) exhibited a $t_{1/2}$ of around *1 week*. The methyl and *tert*-butoxyl PBN adducts on the other hand showed half-lives of 9 and 2 days, respectively.

**Table 5**

Radical Addition Rate Constants

| R$^{\bullet}$ | 2-Ph-DMPO-*nitronyl*-$^{13}C$ | PBN |
|---|---|---|
| $C_6H_5^{\bullet}$ [a] | $2.4 \times 10^6$ | $1.20 \times 10^7$ |
| $C_6H_5C(O)O^{\bullet}$ [a] | $2.5 \times 10^5$ | $5.5 \times 10^5$ |

Spin Adduct Persistence (Time Profiles)

| R$^{\bullet}$ | 2-Ph-DMPO-*nitronyl*-$^{13}C$ | PBN |
|---|---|---|
| $H_3C^{\bullet}$ [b] | ~months | 9 |
| $(CH_3)_3CO^{\bullet}$ [b] | weeks | 6 |
| $H_3C^{\bullet}$ [c] | ~months | 9 |
| $(CH_3)_3CO^{\bullet}$ [c] | ~1 week | 2 |

[a] These measurements were recorded in $C_6H_6$ at room temperature and the units are $M^{-1}$ $s^{-1}$. [b] These measurements were recorded in $C_6H_6$ at room temperature and the units for the half-lives ($t_{1/2}$) are in days if not otherwise noted. [c] These measurements were recorded in $H_2O$ at room temperature and the units are likewise in days.

## Summary

The new $^{13}C$-labeled cyclic nitrone, 2-Ph-DMPO-*nitronyl*-$^{13}C$, is shown to be an improved spin trap for a variety of carbon- and oxygen-centered radicals. It is noteworthy that 2-Ph-DMPO-*nitronyl*-$^{13}C$ traps radicals only a little slower than PBN. Unlike the radical spin adducts of the related spin trap PBN, those derived from 2-Ph-DMPO-*nitronyl*-$^{13}C$ possess no $\beta$-H. Spin adducts therefore decay more slowly and are more chemically persistent because the primary decay route ($\beta$-H abstraction and subsequent disproportionation) is not a factor. Substitution at the *nitronyl* 2-position also prevents oxidative degradation to a carbonyl at this position. The title nitrone appears to be well suited to biological spin trapping applications where decay of spin adducts during isolation is often problematic. Although the use of 2-Ph-DMPO-*nitronyl*-$^{13}C$ is still in the preliminary stages, it is worth noting that this nitrone has already proven to be a superior spin trap (compared to PBN) for scavenging trichloromethyl radicals during the *in vivo* metabolism of tetrachloromethane.[41]

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(41) Poyer, J. L., Oklahoma Medical Research Foundation (OMRF), Personal communication, 1993.