Synthesis and Spin-Trapping Chemistry of 5,5-Dimethyl-2-(trifluoromethyl)-1-pyrroline *N*-Oxide[||]

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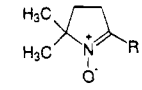
A new five-membered ring nitrone, 5,5-dimethyl-2-(trifluoromethyl)-1-pyrroline *N*-oxide (2-TFDMPO), is synthesized for the purpose of spin trapping in free radical biology. Most of the spin adducts of 2-TFDMPO are persistent, and EPR, ENDOR, and MS spectra can be obtained. A variety of radicals give characteristic spectral signatures, among which is a rare type of line width alternation pattern due to hindered rotation of the trifluoromethyl group.

Introduction

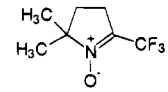
Free radicals may be involved in a variety of biological processes which are associated with certain diseases, such as cancer, heart attack, and Alzheimer's, and aging. With the intense increase in attention being paid to biological free radical research,[1] the development of better techniques, which can be conveniently used to detect and identify free radicals in biological systems, has become more urgent. The spin-trapping technique[2] is a unique method which uses a small compound called a spin trap to react with short-lived free radicals to generate relatively persistent addition products called spin adducts. Spin adducts are characterized by modern analytical instrumentations such as electron paramagnetic resonance (EPR) spectroscopy[3] and mass spectrometry (MS).[4] Spin trapping has been widely used to identify biological free radicals,[1] but recent concerns with this application suggest that better spin traps should be developed so that the spin adducts are less susceptible to oxidation, reduction, hydrolysis, or other degradation processes prevalent in biological systems. Also, spin traps and spin adducts should be resistent to thermal decomposition and have the desired lipophilicities so that the spin trap molecules are available to interested bioradicals located in different regions of a living system.

Over recent years, many cyclic DMPO (5,5-dimethyl-1-pyrroline *N*-oxide, **I**) type spin traps have been prepared[3,5–7] for the purposes mentioned above. More recent work on 5,5-dimethyl-2-phenyl-1-pyrroline *N*-oxide (2-Ph-DMPO, **II**) indicates that 2-Ph-DMPO *in vivo* spin trapping of $Cl_3C^{\bullet}$ radicals in rat liver gives an EPR signal of twice the intensity as compared to that of PBN (*C*-phenyl-*N*-*tert*-butylnitrone) under the same conditions,[8] although PBN traps phenyl radicals 5 times faster than 2-Ph-DMPO.[3] In order to increase spin-trapping rates,



R = H (I), C_6H_5 (II)

replacement of the phenyl substituent in 2-Ph-DMPO with a less bulky and more inert group was considered. The trifluoromethyl group was our choice because of its superior stability, lipophilicity, and small volume (comparable with CH_3). Eventually, results obtained from EPR spin-trapping experiments, ENDOR (electron nuclear double resonance) measurements, mass spectrometric tests, and *in vivo* observation with rats as an animal model demonstrated that 5,5-dimethyl-2-(trifluoromethyl)-1-pyrroline *N*-oxide (2-TFDMPO, **III**) is a promising nitrone spin trap.



(III)

This article describes the first synthesis of 2-TFDMPO and reports on the evaluation of its spin-trapping ability with EPR, ENDOR, and MS spin-trapping methodologies. Details about MS investigations of carbon-centered radical adducts are presented because mass spectrometry is

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|| Dedicated to Professor Glen A. Russell on the occasion of his 70th birthday.

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(1) (a) Janzen, E. G.; Makino, K.; Yoshikawa, T. *Free Radical Res. Commun. Supplement* **1993**, *19*, S1. (b) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*; Clarenden: Oxford, U.K., 1989.

(2) Janzen, E. G.; Haire, D. L. In *Advances in Free Radical Chemistry*; Tanner, D. D., Ed.; JAI Press Inc.: Greenwich, CT, 1990; pp 253–295.

(3) Janzen, E. G.; Zhang, Y.-K.; Haire, D. L. *J. Am. Chem. Soc.* **1994**, *116*, 3738.

(4) (a) Janzen, E. G.; Wever, J. R.; Haire, D. L.; Fung, D. M. *Anal. Lett.* **1985**, *18*, 1749. (b) Iwahashi, H.; Parker, C. E.; Mason, R. P.; Tomer, K. B. *Anal. Chem.* **1992**, *64*, 224 and references therein.

(5) (a) Pou, S.; Rosen, G. M.; Wu, Y.; Keana, J. F. W. *J. Org. Chem.* **1990**, *55*, 4438. (b) Dehnel, A.; Griller, D.; Kanabus-Kaminska, J. M. *J. Org. Chem.* **1988**, *53*, 1566. (c) Turner, M. J.; Rosen, G. M. *J. Med. Chem.* **1986**, *29*, 2439. (d) Barker, P.; Beckwith, A. L. J.; Cherry, W. R.; Huie, R. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1147.

(6) (a) Janzen, E. G.; Zhang, Y.-K. *J. Magn. Reson.* **1993**, *101B*, 91. (b) Haire, D. L.; Oehler, U. M.; Goldman, H. D.; Dudley, R. L.; Janzen, E. G. *Can. J. Chem.* **1988**, *66*, 2395. (c) Haire, D. L.; Hilborn, J. W.; Janzen, E. G. *J. Org. Chem.* **1986**, *51*, 4298.

(7) (a) Zhang, Y.-K.; Lu, D. H.; Xu, G. Z. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1855. (b) Zhang, Y.-K.; Lu, D.-H.; Xu, G.-Z. *Z. Naturforsch.* **1990**, *45B*, 1075. (c) Zhang, Y.-K.; Xu, G.-Z. *Magn. Reson. Chem.* **1989**, *27*, 846.

(8) Poyer, J. L., Oklahoma Medical Research Foundation (OMRF), personal communication, 1993.

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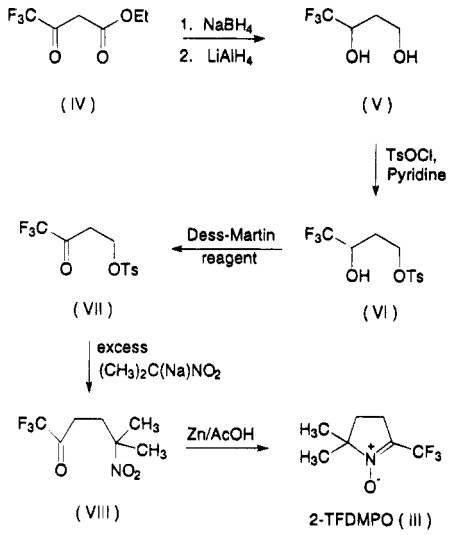
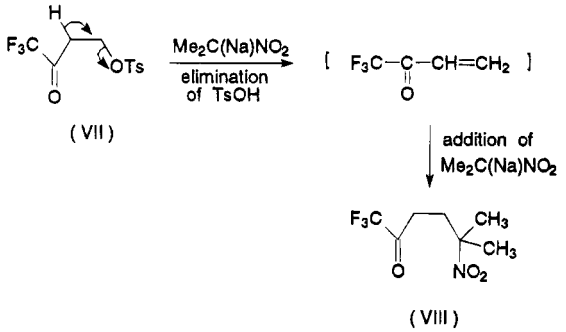
Scheme 1



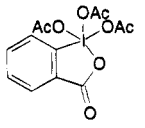
Scheme 2



becoming an important method for characterizing structures of radical adducts.[4]

Results and Discussion

The synthetic route for the preparation of 2-TFDMPO is illustrated in Scheme 1. Ethyl 4,4,4-trifluoroacetoacetate (**IV**) was reduced successively with $NaBH_4$ and $LiAlH_4$ to produce 2,4-dihydroxy-1,1,1-trifluorobutane (**V**) by the procedure of Tordeux et al.[9] Selective monotosylation of **V** gave 2-hydroxy-4-(tosyloxy)-1,1,1-trifluorobutane (**VI**).[9] The oxidation of the $CF_3CH(OH)$ moiety required a specific periodinane oxidant (Dess–Martin reagent).[10] Monotosylate (**VI**) was oxidized with Dess–



Dess-Martin reagent

Martin reagent[10] by the method of Linderman et al.[11] to provide 4-(tosyloxy)-1,1,1-trifluoro-2-butanone (**VII**) which was treated with 10-fold $Me_2C(Na)NO_2$ in ethanol to generate 5-methyl-5-nitro-1,1,1-trifluoro-2-hexanone (**VIII**). The latter process involves an elimination–addition reaction (Scheme 2). Compound **VIII** cannot be generated by direct substitution reaction on the TsO-linked carbon of **VII** because dimethylnitrocarboanion is a poor nucleophile but a good Michael addition reactant. For instance, no substitution product resulted when the carboanion is allowed to react with $CF_3CH(OH)CH_2CH_2I$. Nitro ketone **VIII** was reduced with zinc and acetic acid in 95% EtOH at 0–5 °C to provide the title spin trap **III**.

EPR and ENDOR Spectra of Spin Adducts of 2-TFDMPO. The EPR spectra of 25 different spin adducts have been recorded. These spectra can be organized into four different types depending on the nature of the splitting patterns obtained and the magnitudes of the hyperfine splitting constants (hfsc's) measured (see Table 1).

Oxyl adducts give EPR spectra consisting of three groups of 1:3:3:1 quartets (Figures 1 and 2). The hydroxyl, hydrodioxyl (hydroperoxyl), and butoxyl adducts all give relatively small N-hfsc's (Table 1) in keeping with two electronegative groups attached to carbon-2. The quartets come from γ-F hfs as found in the trifluoromethyl adduct of PBN.[12] In addition, γ-H hfs is found for the hydroperoxyl adduct. It is assumed that this additional hfs comes from the methylene hydrogens in the 3-position as found for DMPO adducts.[6b] Spectra could be simulated by a computer.[13]

A variety of carbon-centered radical adducts (but not all, see later) also give EPR spectra composed of triplets of 1:3:3:1 quartets. A typical example is shown in Figure 2 from the methyl adduct. When the hfsc's for carbon-centered spin adducts are compared to those from oxyl adducts, the main differences are larger *N*-hfsc's for carbon-centered adducts (by about 1 G, compared in the same solvent) and no γ-H hfs (within the line width of the peaks, $\approx$1.0 G). There is the usual increase in the N-hfsc with an increase in polarity of the solvent, although surprisingly little change is found in the γ-F hfsc with different radical addend structure or solvent.

The phenyl adduct of 2-TFDMPO exemplifies an entirely different type of spectrum obtained from certain carbon-centered adducts (Figure 3). At first glance, the spectrum at room temperature seems to consist of three groups of doublets, but the unresolved peaks between the doublets are important. As the temperature is raised (from 23 to 110 °C), the intensity of the second and third peaks of the 1:3:3:1 quartet reluctantly develop and approach the intensity they should have if free rotation of the trifluoromethyl group were allowed. When the second and third peaks of the trifluoromethyl hyperfine splitting pattern experience broadening as a function of temperature (or neighboring substituent[14]), the effect is known as line width alternation (LWA) and comes from hindered rotation of the trifluoromethyl group. At room temperature, the three γ-F hfsc's are different (Table 1). At 110 °C, the γ-F hfsc's are averaged and equal to 1.96 G in toluene. At 200 K in toluene, the EPR spectrum again looks like a triplet of doublets with additional splitting. From ENDOR spectroscopy, three different γ-F hfsc's can be resolved plus one γ-H hfsc (see Table 1). The very large F-hfsc for one γ-F (4.92 G) may be the largest γ-F hfsc ever recorded for an aminoxyl radical.[15,16] The EPR spectra of the vinyl, benzyl, and *n*-hexanoyl adducts show features similar to that of the phenyl

(9) Tordeux, M.; Wakselman, C. *J. Fluorine Chem.* **1982**, *20*, 301.
(10) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
(11) Linderman, R. J.; Graves, D. M. *J. Org. Chem.* **1989**, *54*, 661.
(12) Janzen, E. G.; Lopp, I. G.; Morgan, T. V. *J. Phys. Chem.* **1973**, *77*, 139.
(13) Oehler, U. M.; Janzen, E. G. *Can. J. Chem.* **1982**, *60*, 1542.
(14) Janzen, E. G.; Gerlock, J. L. *J. Am. Chem. Soc.* **1967**, *89*, 4902.
(15) Janzen, E. G. *Top. Stereochem.* **1971**, *6*, 177.
(16) Janzen, E. G. In *Creation and Detection of the Excited State*; Ware, W. R., Ed.; Marcel Dekker, Inc.: New York, 1976; pp 83–138.

Table 1. Hyperfine Splitting Constants (hfsc's) for Spin Adducts of 2-Trifluoromethyl DMPO

radical	source	solvent	N-hfsc[a]	F-hfsc[a]	H-hfsc[a]
n-BuO•	*n*-BuONO photolysis (*hν*)	C6H6	11.88	2.74	0.79 (2H)
i-BuO•	*i*-BuONO (*hν*)	C_6H_6	11.83	2.69	0.79 (2H)
i-amyl O•	*i*-amylono (*hν*)	C_6H_6	11.83	2.74	0.75 (2H)
HO•	1% H_2O_2 (*hν*)	H_2O	13.98	2.70	–
HO_2•	30% H_2O_2	H_2O	13.14	2.80	0.83 (2H)
^-O_3SO•	$Na_2S_2O_8$	H_2O	12.97	3.21	–
$HOCH_2$	1% H_2O_2, 10% v MeOH (*hν*)	H_2O	14.42	2.33	–
HOCH(Me)	1% H_2O_2, 10% v ETOH (*hν*)	H_2O	14.47	2.87	–
HOCH(Et)	1% H_2O_2, 10% v *n*-PrOH (*hν*)	H_2O	14.52	2.74	–
$HOC(Me)_2$	1% H_2O_2, 10% v *i*-PrOH (*hν*)	H_2O	14.32	2.93	–
$HOCH_2CH(OH)$	1% H_2O_2, $(HOCH_2)_2$ (*hν*)	H_2O	14.37	2.64	–
THF•	1% H_2O_2, THF (*hν*)	H_2O	14.37	2.64	–
Me•	1% H_2O_2, DMSO (*hν*)	H_2O	14.90	2.05	–
Et•	ethyl Grignard	H_2O/C_6H_6	14.66/12.93	2.51/2.64	–
i-Pr•	isopropyl Grignard	H_2O/C_6H_6	14.69/12.95	2.97/3.19	–
Cl_312C•	rat liver/CCl_4	$CHCl_3$	12.26	3.66	–
Cl_313C•	rat liver/CCl_4	$CHCl_3$	12.54	3.41	12.66[b]
HC≡C•	ethynyl Grignard	H_2O/C_6H_6	14.91/13.44	1.76/1.66	–
CH_2=$CHCH_2$•	allyl Grignard	H_2O/C_6H_6	14.59/12.90	2.44/2.53	–
H•	2.5% *n*-Bu_3SnH (*hν*)	C_6H_6	13.20	1.14	15.40
Ph•	phenyl Grignard	C_6H_6	13.05	3.02, 1.41, 0.8	0.3
Ph•	phenyl Grignard	H_2O	14.54	3.05, 1.44, 0.82	0.3
Ph•	phenyl Grignard	$C_6H_5CH_3$ (110 °C)	13.07	1.96	–
Ph•	phenyl Grignard	$C_6H_5CH_3$ (−73 °C)	from ENDOR	4.92, 1.12, 0.81	0.31
CH_2=CH•	vinyl Grignard	H_2O	14.74	2.6, 1.7, 1.3	0.92, 0.69, 0.34
CH_2=CH•	vinyl Grignard	C_6H_6	13.25	2.7, 1.8, 1.4	–
CH_2=CH•	vinyl Grignard	$C_6H_5CH_3$	from ENDOR	4.94, 1.12, 0.81	1.0, 0.51, 0.19
$C_6H_5CH_2$•	benzyl Grignard	H_2O	14.47	2.99, 2.69, 2.39	0.88
$C_6H_5CH_2$•	benzyl Grignard	C_6H_6	12.76	3.15, 2.84, 2.53	0.88
$C_6H_5CH_2$•	benzyl Grignard	$C_6H_5CH_3$ (110 °C)	12.81	2.82	–
$C_6H_5CH_2$•	benzyl Grignard	$C_6H_5CH_3$ (−73 °C)	from ENDOR	not found	0.81, 0.20
$C_5H_{11}C$=O	hexanal, $(t\text{-BuO})_2$ (*hν*)	H_2O	13.88	2.65, 2.15, 1.4	–
O=CO^-	1% H_2O_2, sodium formate	H_2O	14.57	3.25, 1.7, 0.76	–

[a] The error is estimated to be ±0.05 G. Units are gauss obtained at *X*-Band. [b] ^{13}C-hfsc.

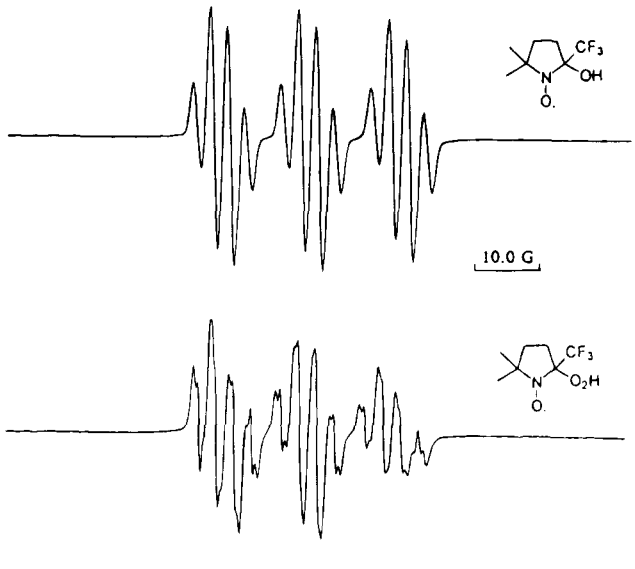


Figure 1. EPR spectra at room temperature of 2-TFDMPO spin adducts: hydroxyl adduct in water from photolysis of 1% H_2O_2 (top), and hydrodioxyl adduct in water from photolysis of 30% H_2O_2 (bottom).

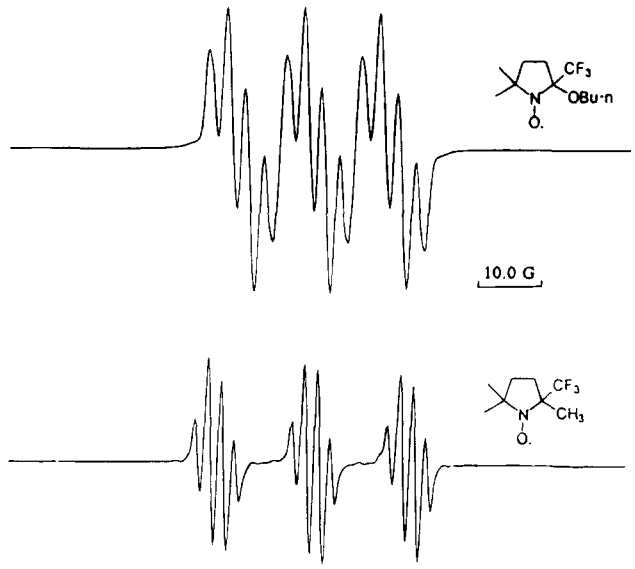


Figure 2. EPR spectra at room temperature of 2-TFDMPO spin adducts: *n*-butoxyl adduct in benzene from photolysis of n-butyl nitrite (top), and methyl adduct in 1:1 water/DMSO from photolysis of 1% H_2O_2 (bottom).

adduct, namely, LWA at room temperature (although slight) which disappears with an increase in temperature and becomes more pronounced at lower temperatures. In the case of the vinyl adduct, the ENDOR spectrum shows numerous additional proton splittings as has been observed before for the vinyl adduct of PBN.[17] The benzyl adduct, however, gave no ENDOR fluorine doublets at 200 K, indicating that hindered rotation of the trifluoromethyl group in this case does not involve discrete jumps between equivalent positions of the C−F bond at this temperature. The carboxylate adduct produced by addition of the carbon dioxide anion radical also gave EPR spectra with LWA at room temperature in aqueous solutions.

Clearly, there are subtle differences in the influence provided by the radical addend to free rotation of the

(17) Janzen, E. G.; Oehler, U. M.; Haire, D. L.; Kotake, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6858.

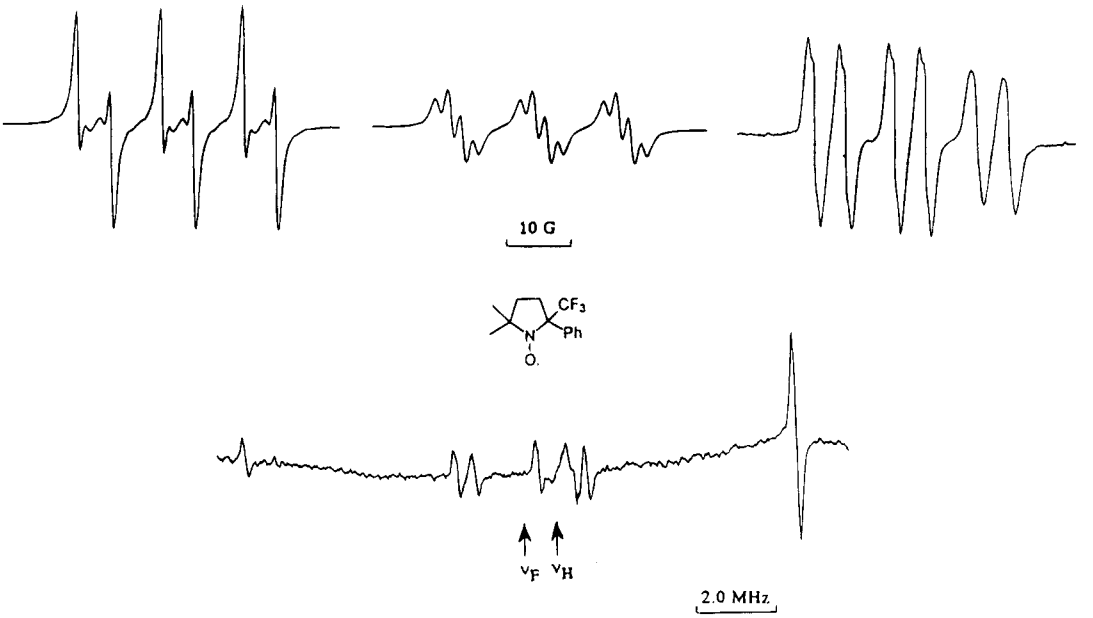


Figure 3. EPR and ENDOR spectra of the phenyl adduct of 2-TFDMPO in toluene: room temperature (top left), 110 °C (top middle), −73 °C (200 K) (top right), and ENDOR at 200 K (bottom).

trifluoromethyl group attached to the same carbon atom. The presence of a contiguous π-bond in the radical addend seems to strongly hinder free rotation of the trifluoromethyl group, although the ethynyl adduct (no LWA but a π-bond on a contiguous atom) and the benzyl adduct (LWA but the π-bond is one atom removed) appear to be exceptions to this rule. Steric bulk alone as in the 1-methylethyl or trichloromethyl group does not seem to be enough to produce LWA at room temperature (see Table 1).

The hydrogen atom adduct gives a unique spectrum with a relatively small γ-F-hfsc and a large β-H-hfsc which is slightly larger than the N-hfsc (see Table 1). This adduct has been reported before by addition of trifluoromethyl radicals to DMPO.[18]

Mass Spectra and the Fragmentation Mechanism of Carbon-Centered Radical Spin Adducts. The assignment of EPR spectra of spin adducts is usually based on the production of the same adduct from known radical sources. However, the available spectral parameters sometimes do not vary enough to provide structural information about groups attached further than two or three bonds from the nitroxyl function. In order to provide this information, MS methods are under development.[4] It is desirable to have an intense molecular ion peak and a significant intensity pattern due to fragment ions still retaining the radical addend. The relative intensity of a molecular ion peak is strongly related to the volatility of the molecule, the stability of the molecular ion, and the spectrometric conditions.[19]

The trifluoromethyl functionality makes the spin trap, 2-TFDMPO, and its adducts more volatile and more stable. This nitrone appears to be a very applicable spin trap when the MS method is chosen to characterize spin adducts. The alkyl adducts of 2-TFDMPO (R = methyl, ethyl, or 1-methylethyl) give molecular ion peaks as do the vinyl and phenyl adduct (in Figure 4, see m/z = 224 for 1-methylethyl and m/z = 258 for phenyl). However,

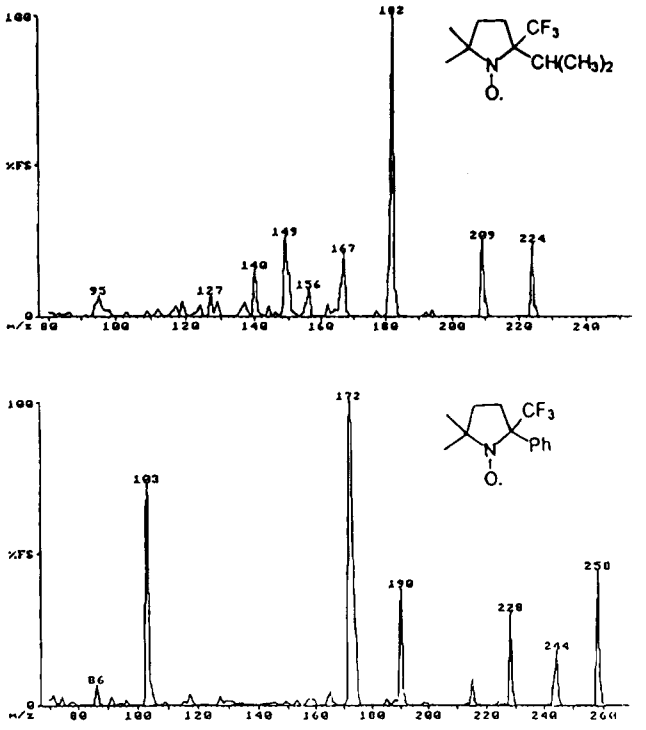
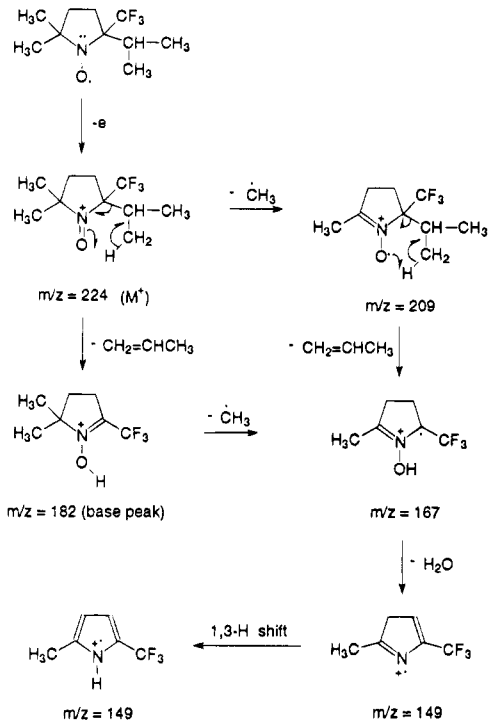


Figure 4. Mass spectra (GC/MS: injector, 230 °C; column, 180 °C) obtained in EI+ mode for the isopropyl and phenyl spin adducts (top and bottom, respectively) showing molecular ion peaks (m/z = 196, 210, 224, and 258) and some fragments.

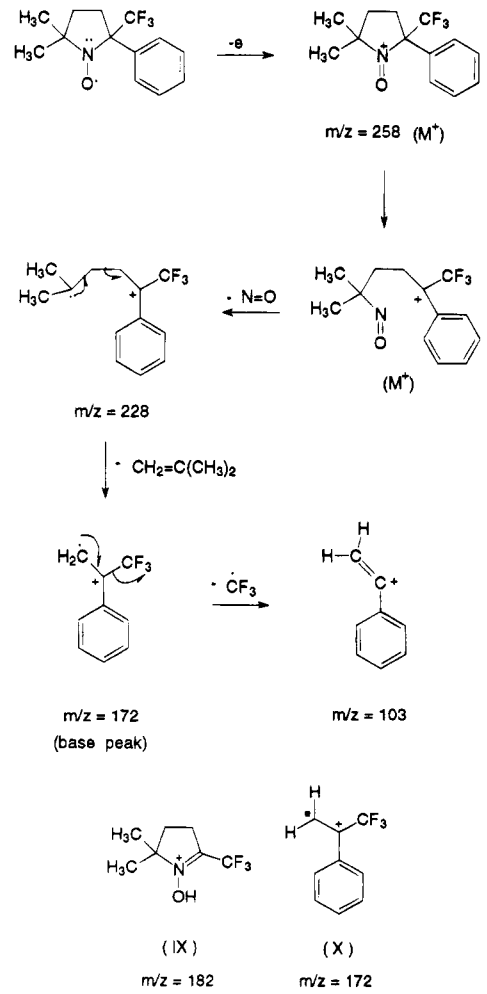
the base peak may be a fragment which does not always retain the radical addend group; compare for example m/z = 182 (structure **IX**) from the 1-methylethyl adduct with m/z = 172 (structure **X**) from the phenyl adduct. A possible mechanism for the major fragmentation of the 1-methylethyl adduct and the phenyl adduct is given in Schemes 3 and 4, respectively. Rearrangement, which involves a six-membered ring and the loss of a molecule of CH_2=$CHCH_3$ (see Scheme 3), is an important approach for the fragmentation of the 1-methylethyl adduct. In the case of the ethyl adduct, the loss of a CH_2=CH_2 molecule is the consequence of such a rearrangement. For

(18) Janzen, E. G.; Liu, J. I.-P. *J. Magn. Reson.* **1973**, *9*, 510.
(19) Watson, J. T. *Introduction to Mass Spectrometry*; Raven Press: New York, 1985; pp 153–242.

Scheme 3



Scheme 4



the phenyl adduct, major fragments usually contain the phenyl ring because this ring can conjugate with a positive charge, thus stablizing these ions (see Scheme 4). Also, the double adduct of 2-TFDMPO can sometimes be detected, for example, when the addend group is ethyl (**XI**).

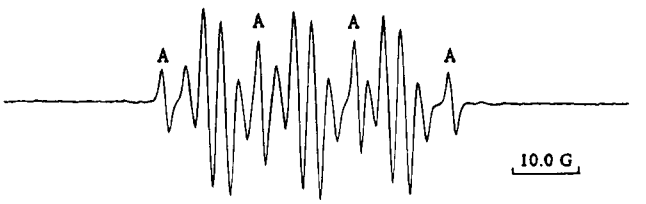
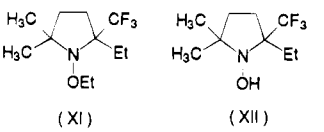


Figure 5. Room-temperature EPR spectrum of the hydroxyl adducts of 2-TFDMPO (without a label) and DMPO (marked with label A) in 1% H_2O_2 water solution.

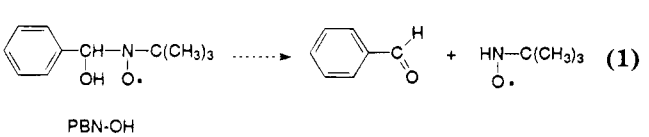


The reduced form of a spin adduct may also be stable as the hydroxylamine (**XII**). It is important to know the mass spectra of these species since fragmentation patterns of the hydroxylamine may be different from that of the parent spin adduct or the double spin adduct. For example, when the ethyl adduct hydroxylamine was studied, the base peak is the molecular ion (m/z = 211) but a major fragment results from the loss of the trifluoromethyl group. In comparison, when the ethyl adduct itself is investigated, one of the major fragments is due to the loss of the ethylene molecule giving **IX** (m/z = 182). Further work will be needed to provide the MS rules of fragmentation of these types of products potentially formed in spin trapping.

Competition Spin Trapping of 2-TFDMPO with DMPO and Stabilities of 2-TFDMPO Adducts. The most important factors influencing the success of a spin-trapping experiment are the rate of spin trapping and the persistence of the spin adduct. In fact, the rate of hydrodioxyl radical addition to 2-TFDMPO is faster than in the case of DMPO from measurement of spin adduct production in a solution containing both spin traps and photolysis of 30% H_2O_2 (see Table 1). Also, EPR signal-decaying experiments (Figure 5) show that the hydroxyl adduct of 2-TFDMPO is more persistent than that of DMPO in 1% H_2O_2 (half-life time ($t_{1/2}$): 72 vs 15 min).

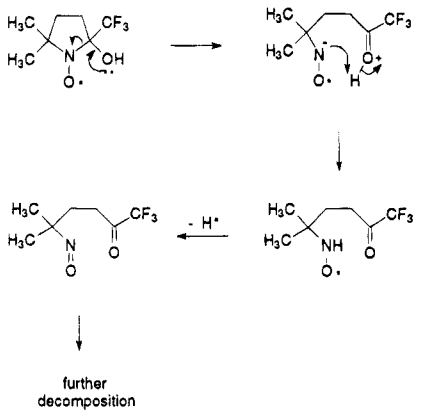
Both 2-TFDMPO and its spin adducts are more stable at higher temperatures. Thus, the phenyl and benzyl adducts can be heated to 110 °C and returned to room temperature with no loss in EPR signal. The *n*-hexanoyl adduct is stable to 80 °C and can be isolated and purified by chromatography. This is one of the most remarkable observations since no other spin traps of either PBN- or DMPO-type have ever given stable acyl adducts before.

Decomposition Mechanism of the Hydroxyl Adduct of 2-TFDMPO. The strong reactivity of the hydroxyl radical and its wide involvement in free radical biology make this reactive intermediate of great interest. For the hydroxyl adduct of PBN-type spin traps, the lack of stabilities seems to be associated with β-cleavage reactions at the CH–N bond (eq 1):[20-23] From the

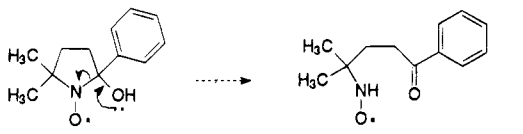


(1)

Scheme 5

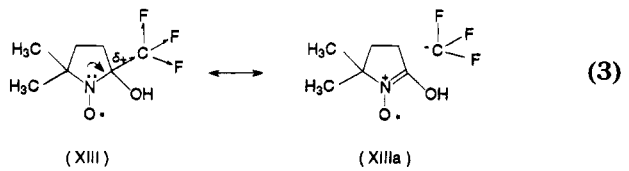


structural point of view, PBN-OH tends to decompose rapidly because a much more stable molecule, benzaldehyde, results with conjugation between the benzene ring and the carbonyl function. This explanation may be applicable to the hydroxyl adduct of 2-Ph-DMPO (eq 2):[24] These results deliver an important message that, if



(2)

the hydroxyl radical adduct is of interest, a phenyl group should not be attached to the carbon of the nitronyl C=N$^+$(O$^-$) function when a spin trap for detecting hydroxyl radicals is the ultimate destination. Thus, spin traps should be designed under the guidance of known or reasonably predicted mechanisms, which might be called mechanistic design of spin traps. The title nitrone is an example of this concept. As mentioned before, the hydroxyl adduct of 2-TFDMPO is more persistent than the DMPO hydroxyl adduct in 1% H_2O_2. A possible approach for the decomposition of 2-TFDMPO-OH adducts is proposed in Scheme 5. The adduct is stabilized by the electron-attracting inductive and field effects and the polarizability of the CF_3 substituent (eq 3):[25,26] The



(XIII) (XIIIa) (3)

C−N bond in **XIII** should be stronger than the bond in the DMPO-OH adduct. Hyperconjugation between the CF_3 group and the lone pair of electrons on the N atom may also contribute to the stabilization of this adduct.[27]

Conclusions

The cyclic nitrone spin trap, 2-TFDMPO, has been prepared for the first time. It appears that 2-TFDMPO shows promise as a new spin trap with good persistence of its spin adducts. The spin trap contains a lipophilic and inert CF_3 functionality and would be useful for spin trappings of biological free radicals. Much work needs to be done, however, whenever a new nitrone is considered for spin-trapping studies. Eventually, the advantages and disadvantages of all spin traps will be available and the investigator will be able to select the features most suitable for the experiment in question.

Experimental Section

EPR spectra were recorded on Bruker ESP-300E or Bruker ESP-300 EPR spectrometers. The sample solution was bubbled for several minutes with pure nitrogen immediately before the measurements were taken. The spectrometer settings were normally at a modulation amplitude of 1.0 G, modulation frequency of 100 kHz, microwave power of 19.9 mW, and microwave frequency of 9.65 GHz. When photolysis was the method used to generate free radicals, the ultraviolet light beam from a 75 W high-pressure mercury UV lamp was focused into an EPR cavity for several seconds. EPR spectra were computer-simulated with an in-house simulation program.[13]

ENDOR spectra were determined on a Bruker ESP-300E EPR spectrometer combined with an ENDOR accessory. A radio frequency modulation of 12.5 kHz and an attenuation of 6 dB were used.

Mass spectra were obtained using a FISONS/VG QUATTRO MS/MS triple quadrupole spectrometer. Spin adduct samples were separated by gas chromatography before entering the mass chamber. Carbon-centered spin adducts of 2-TFDMPO used for GC/MS spectroscopic determination were produced by Grignard additions of RMgX followed by oxidation of the resulting hydroxylamines with MnO_2.

^{1}H NMR spectra of $CDCl_3$ solutions were recorded at room temperature on a Varian XL-300 NMR spectrometer using tetramethylsilane (TMS) as internal standard.

2,4-Dihydroxy-1,1,1-trifluorobutane (V).[9] To a solution of **IV** (18.4 g, 100 mmol) in Et_2O (200 mL) was added $NaBH_4$ (4.0 g, 105 mmol) in several portions over 30 min at 0−5 °C. The mixture was stirred at this temperature for 1 h and at room temperature overnight. A solution of HCl (10%, 100 mL) was carefully added, and the solid was removed by filtration. The aqueous layer was extracted with Et_2O (150 mL), and the combination was dried over Na_2SO_4, filtered, and evaporated to give 18.0 g of liquid (98%). The solution of the liquid in Et_2O (50 mL) was added to a suspension of $LiAlH_4$ (6.0 g, 0.157 mol) in Et_2O (50 mL) at 0−5 °C during 80 min. After the mixture was stirred overnight at room temperature, 100 mL of 10% HCl was very carefully added to decompose excess $LiAlH_4$. The aqueous layer was extracted with Et_2O or CH_3CO_2Et (2 × 150 mL), and the combination was dried, filtered, and evaporated to afford 12.6 g of a clear liquid **V** (88% yield). The product was purified by distillation. Bp: 51.5−54 °C/0.32 Torr. ^{1}H NMR: δ 6.05 (d, J = 6.6 Hz, 1H), 4.60 (s, 1H), 4.03 (m, 1H), 3.56 (s, 2H), 1.59 (m, 2H).

2-Hydroxy-4-(tosyloxy)-1,1,1-trifluorobutane (VI). To a solution of **V** (12.6 g, 87.5 mmol) and pyridine (30 mL) in CH_2Cl_2 was added solid p-$CH_3C_6H_4SO_2Cl$ (20.5 g, 108 mmol) over 30 min. The solution was stirred for 4 days at 4 °C and then poured into a mixture of 10% HCl (100 mL) and ice. The organic layer was washed successively with 10% HCl (100 mL) and NaCl-saturated dilute Na_2CO_3 aqueous solution (100 mL). The solution was dried, filtered, and evaporated to give a mixture which was chromatographed on silica gel eluted with CH_2Cl_2 (R_f = 0.22), affording 14.25 g of clear liquid **VI** in 55% yield. MS: m/z (relative intensity) 298 (M^+, 49), 297 (M^+ − 1, 21), 278 (M^+ − HF, 23), 277 (9), 229 (M^+ − CF_3, 2), 173 (100), 172 (32), 155 (12), 106 (24), 93 (13). ^{1}H NMR: δ 7.80 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 4.30 (dt, J_1 = 3.9 Hz, J_2 = 10.2 Hz, 1H), 4.15 (m, 2H), 3.20 (d, J = 5.7 Hz, 1H), 2.44 (s, 3H), 2.06 and 1.84 (m, 2H).

4-(Tosyloxy)-1,1,1-trifluoro-2-butanone (VII). The previous procedure[11] was adapted. A dry N_2 flow was passed

(20) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1991**, *113*, 9503.
(21) Janzen, E. G.; Kotake, Y.; Hinton, R. D. *Free Radical Biol. Med.* **1992**, *12*, 169.
(22) Janzen, E. G.; Hinton, R. D.; Kotake, Y. *Tetrahedron Lett.* **1992**, *33*, 1257.
(23) Hinton, R. D.; Janzen, E. G. *J. Org. Chem.* **1992**, *57*, 2646.
(24) Zhang, Y.-K.; Janzen, E. G. Unpublished results.
(25) Holtz, D. *Chem. Rev.* **1971**, *71*, 139.
(26) Streitwieser, A., Jr.; Holtz, D.; Ziegler, G. R.; Stroffer, J. O.; Brokaw, M. L.; Guibe, F. *J. Am. Chem. Soc.* **1976**, *98*, 5229.
(27) Koppel, I. A.; Pihl, V.; Koppel, J.; Anvia, F.; Taft, R. W. *J. Am. Chem. Soc.* **1994**, *116*, 8654 and references therein.

through a flask containing Dess–Martin reagent[10] (10.0 g, 23.6 mmol) to remove possible traces of Ac_2O. After a few minutes, CH_2Cl_2 (100 mL) was added to dissolve the solid. A solution of **VI** (1.9 g, 6.3 mmol) in CH_2Cl_2 (40 mL) was added, and the flask was sealed with a stopper. After being stirred at room temperature for 4.5 h, the mixture was poured into aqueous $NaHSO_3$ solution and stirred. A Na_2CO_3-saturated aqueous solution (100 mL) was slowly added, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic solutions were washed with H_2O (100 mL) and dried over $MgSO_4$. Filtration was followed by rota-evaporation, giving 1.9 g of clear liquid **VII** in 100% yield. The liquid was pure enough for the further preparation. ^1H NMR: δ 7.76 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 8.5 Hz, 2H), 4.32 (t, J = 5.8 Hz, 2H), 3.11 (t, J = 5.8 Hz, 2H), 2.45 (s, 3H).

5-Methyl-5-nitro-1,1,1-trifluoro-2-hexanone (VIII). A solution of 2-nitropropane (17.5 g, 196.5 mmol) and sodium ethoxide (42.4 g of 21 wt % EtOH solution, 131 mmol) in EtOH (150 mL) was cooled to 0–5 °C under a N_2 atmosphere. The crude ketone **VII** (3.9 g, 13.1 mmol) in 50 mL of EtOH was added. In about 2 min, solid was generated from the homogeneous solution. The mixture was stirred for 2 h at 0–5 °C and then for 6 h at room temperature. Concentrated hydrochloric acid (15 mL) was slowly added while the mixture was cooled. The solid was removed by filtration, and the solution was evaporated. A solution of the residue in $CHCl_3$ (150 mL) was washed with NaCl-saturated water (2 × 100 mL), dried over $MgSO_4$, filtered, and evaporated to give 2.7 g of a brown liquid (96%). Distillation afforded 2.1 g of colorless liquid **VIII** in 75% yield. Bp: 46 °C/0.18 Torr. ^1H NMR: δ 2.77 (t, J = 7.5 Hz, 2H), 2.28 (t, J = 7.5 Hz, 2H), 1.61 (s, 6H).

5,5-Dimethyl-2-(trifluoromethyl)-1-pyrroline *N*-Oxide (III). Zinc dust (1.26 g, 19.2 mmol) was added to a solution of **VIII** (2.05 g, 9.6 mmol) in 95% EtOH (60 mL) which had been precooled to 3 °C. With vigorous mechanical stirring, CH_3COOH (2.31 g, 38.5 mmol) in 95% EtOH (20 mL) was added dropwise during 10 min at ≤7 °C. The mixture was stirred for an additional 3 h at 3 °C and then filtered. The solid was washed with 95% EtOH (50 mL). The residue after evaporation was dissolved in $CHCl_3$ (150 mL), and the solution was washed with NaCl-saturated H_2O (2 × 50 mL), dried over $MgSO_4$, filtered, and evaporated to give 1.22 g (70% yield) of a clear liquid. TLC analysis with a silica gel plate showed a single spot (R_f = 0.22, CH_2Cl_2). Distillation afforded 1.1 g of a colorless liquid in 63% yield. Bp: 44 °C/0.18 Torr. MS: m/z (relative intensity) 181 (M^+, 84), 166 ($M^+ - CH_3$, 25), 162 (20), 152 (8), 149 (37), 69 (CF_3^+, 100). ^1H NMR: δ 2.78 (tm, J_t = 7.4 Hz, 2H), 2.13 (t, J = 7.4 Hz, 2H), 1.40 (s, 3H), 1.31 (s, 3H). These data are consistent with the structure of 2-TFDMPO (**III**).

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