

ENDOR DETERMINATION OF PROTON HYPERFINE SPLITTING CONSTANTS OF
AMINOXYL RADICALS FREQUENTLY USED IN SPIN-PROBE-SPIN-LABEL METHOD

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ENDOR technique was applied to the determination of the accurate hyperfine splitting constants of protons in several aminoxyl radicals which are frequently used in spin-probe-spin-label method. The result was compared with that of NMR in the concentrated solution of the radicals. The magnitude of hyperfine splitting constants is shown to be very sensitive to the structure of the radical.

In spin-probe-spin-label method several aminoxyl radicals have been used as probes bearing electron spin.¹⁾ Rotational correlation time is derived from the linewidth of the ESR spectrum, which is a measure of the dynamic property of the system. However it has been pointed out that the unresolved hyperfine splitting (hfs) of protons in aminoxyl radicals contributes as residual linewidth. As a result, it makes accurate determination of the intrinsic linewidth very difficult. Numerous studies neglect this inaccuracy, which spoils the quantitative nature of the correlation time.²⁾

Hwang et al.³⁾ have used a perdeuterated aminoxyl radical to reduce the contribution of the hfs to the linewidth. Poggi and Johnson⁴⁾ have proposed a method of the estimation of the intrinsic linewidth in di-*t*-butylaminoxyl (DTBN) from apparent linewidth by making use of the hfs constant of its *t*-butyl protons. Similar type of analysis should be applied to aminoxyl radicals other than DTBN in spin-probe-spin-label method. The reported values of proton hfs of aminoxyl radicals which are frequently used in spin-probe-spin-label method are those determined by NMR in the highly concentrated solution of the radicals.⁵⁻⁷⁾ In NMR of radicals, the hfs must be calculated from the contact shift of the peak from the

corresponding diamagnetic amine. The choice of such diamagnetic species is not always definite. Also the shift is temperature dependent. Therefore accurate hf coupling constants which are measured under the same condition as in ESR measurement are desirable.

ENDOR determination of the proton hfs was carried out for the dilute toluene solution of five aminoxyl radicals. High resolution ENDOR resolved the small hfs of nonequivalent protons. Table 1 lists the radicals and their ENDOR spectra and hfs constants derived from the spectra.

Table 1. ENDOR Spectra and Hfs Constants of Aminoxyl Radicals

Radical	Structure	ENDOR spectra (in toluene, -70 °C)	ENDOR	Hfsc/ $10^{-4}T$ NMR ⁵⁻⁷⁾
2,2,6,6-Tetramethyl- piperidine-1-oxyl-4-ol (TEMPOL)			0.48 (CH ₂) 0.40 (CH ₃) 0.25 (CH ₂)	-0.48 (βCH ₂) -0.45 (αCH ₃) -0.31 (βCH ₂) +0.07 (γCH) -0.02 (αCH ₃)
2,2,6,6-Tetramethyl- piperidine-1-oxyl-4-one (TEMPONE)			0.13	-0.12 (αCH ₃) -0.02 (βCH ₂)
2,2,6,6-Tetramethyl- piperidine-1-oxyl (TEMPO)			0.37 (CH ₂) 0.18 (CH ₃)	-0.39 (βCH ₂) -0.23 (αCH ₃) +0.18 (γCH ₂)
2,2,5,5-Tetramethyl- pyrrolidine-1-oxyl- 3-carboxamide			0.32	
Di-t-butylaminoxyl (DTBN)			0.10	0.10

In ENDOR spectra it is not possible to assign the peak to each proton based on the relative intensity of each line. However among the protons with similar magnetic environment the intensity of the ENDOR peak is proportional to its number. Protons in the present radicals are all alkyl protons with the iso- and anisotropic hf interactions of similar magnitude. The assignment based upon the relative ENDOR intensity is also shown in Table 1.

Independent NMR studies by Brière et al. and Kreilick have determined hfs of protons at various positions of aminoxyl radicals.^{6,7)} Especially Brière et al.⁶⁾ assigned each NMR peak by the partial deuteration. Their result shows that in cyclic aminoxyl radicals which contain no mirror plane within the ring plane have nonequivalent hfs of methyl and methylene protons. The sensitivity of the magnitude of the long-range hfs on the structure of cyclic aminoxyl radical has been clearly shown by the ENDOR study of doxyl cyclododecane derivative.⁸⁾ In this radical even γ -proton shows the hfs of about 0.2 mT with additional small hfs.

There is no common characteristic in the proton-ENDOR spectra of aminoxyl radicals in Table 1. The hfs obtained from the analysis of the ENDOR spectra shows good accordance with the result of NMR study. The accordance indicates that the concentration of the radical has small effect on the magnitude of hfs. This fact is in line with small solvent effect on the hfs of protons farther than γ -position in an aminoxyl radical.^{6,7)}

In TEMPOL three different hfs were observed in ENDOR spectrum, which shows the nonequivalence of the methyl and methylene protons. The linewidth of the ESR spectrum of TEMPOL is large due to the large hfs of protons. TEMPONE and DTBN have been favorably used as spin probes because of sharp linewidth of ESR spectrum. It is evident that the sharp linewidth derives from small hfs of protons. It should be pointed out that in cyclic aminoxyl radicals the proton hfs is very much dependent on the substituents attached to the carbon atom of γ -position within the ring.

The sample was prepared by packing toluene solution of each aminoxyl radical into an ESR sample tube of 4 mm o.d. and was degassed. The concentration of DTBN could not be adjusted under vacuum by the distillation of the solvent so that it was adjusted before degassing. ENDOR spectrum was observed by using TE₀₁₁ cavity with four-turn rf coil inside the cavity. The microwave and radiofrequency fields were kept comparatively low such as 10 mW and 100 W, respectively. The rf was frequency-modulated at 30 kHz and the amplitude of frequency modulation was 10 kHz. The

temperature of the sample was controlled to obtain optimum signal by the flow of chilled nitrogen gas. The resolution of the spectrum was better at higher temperature where the linewidth of the ENDOR signal is small due to longer nuclear relaxation time.

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