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Hyperfine Interactions of Narrow-line Trityl Radical with Solvent Molecules

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Abstract

The electron nuclear dipolar interactions responsible for some dynamic nuclear polarization (DNP) mechanisms also are responsible for the presence formally in CW EPR spectra of forbidden satellite lines in which both the electron spin and a nuclear spin flip. Such lines arising from ^1H nuclei are easily resolved in CW EPR measurements of trityl radicals, a popular family of DNP reagents. The satellite lines overlap some of the hyperfine features from ^{13}C in natural abundance in the trityl radical, but their intensity can be easily determined by simple simulations of the EPR spectra using the hyperfine parameters of the trityl radical. Isotopic substitution of ^2H for ^1H among the hydrogens of the trityl radical and/or the solvent allows the dipolar interactions from the ^1H on the trityl radical and from the solvent to be determined. The intensity of the dipolar interactions, integrated over all the ^1H in the system, is characterized by the traditional parameter

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called r_{eff} . For the so-called Finland trityl in methanol, the r_{eff} values indicate that collectively the ^1H in the unlabeled solvent have a stronger integrated dipolar interaction with the unpaired electron spin of the Finland trityl than do the ^1H in the radical and consequently will be a more important DNP route. Although r_{eff} has the dimensions of distance, it does not correspond to any simple physical dimension in the trityl radical because the details of the unpaired electron spin distribution and the hydrogen distribution are important in the case of trityls.

Keywords

DNP; Trityl Radical; ENDOR

Introduction

The recent advances in dynamic nuclear polarization of frozen solutions for solid-state NMR or production of hyperpolarized molecules rely on transfer of spin polarization between free radicals and solvent molecules. The magnetic dipolar interactions between the unpaired electron spin of the free radical and the spins, typically of ^1H or ^{13}C nuclei, on solvent molecules near the radical or on the radicals themselves, play an important role in the generation and diffusion of polarization through the sample [1-4]. Yet, measurement of these dipole interactions by conventional magnetic resonance spectroscopy is complicated in frozen solutions. Typically, each electron spin interacts with several ^1H in the radical and many in the solvent, so that the sample responds to an average interaction (the square of the principal values of the dipolar interaction tensor summed over all nuclei outside a so-called diffusion barrier) [1-4]. Such an average is difficult to obtain from EPR spectra. Experimental spectra of frozen solutions typically have poorly-resolved features because of overlapping contributions from multiple conformations of radicals and/or solvent. In addition, weak isotropic hyperfine couplings, also known as Fermi-contact interactions, combine with the dipolar interactions to produce the hyperfine interactions (HFI) that helps produce the EPR lineshapes.

One distinct consequence of the dipolar interaction for $I=1/2$ nuclei is the production of formally-forbidden spin-flip satellite transitions in the EPR spectrum [5-7]. The satellite lines are a manifestation of the Solid Effect in which both electron and nuclear spins change during the EPR transition. The satellite line intensity in frozen solution is proportional to the square of the dipolar interactions summed over all nuclei and is directly relevant to DNP from the Solid Effect and the Cross Effect. These forbidden transitions are split from the allowed transition by the Larmor frequency of the nucleus but are typically buried under the wings of the allowed transitions.

The recently-designed, persistent triarylmethyl radicals (trityls, TAMs) [8-11] have found extensive application as extremely-efficient spin probes. These radicals have high stability towards typical biological reduction and oxidation processes, high solubility in polar solvents including water, and a very sharp, narrow, single EPR line in dilute deoxygenated water solutions with a linewidth in the range of 0.003-0.012 mT. This combination of properties stimulated their widespread use in: 3D, high-resolution EPR imaging of molecular oxygen [12-14], dynamic nuclear polarization [15-17], detection of reactive oxygen species [18, 19], distance measurements in spin-labeled proteins [20], and techniques for the study of molecular diffusion in micro-heterogeneous systems [21].

The narrow, single EPR line is retained even in frozen solutions because of the small g-factor anisotropy typical of TAMs. This allows use of the weak satellite transitions to characterize the anisotropic dipole-dipole HFI with nearby nuclei. Trammel, Zeldes and

Livingston [22] defined an r_{eff} parameter as an effective distance to the nearest magnetic nucleus (a hydrogen or ^1H in this case) as: $r_{\text{eff}}^{-6} = \langle 1/r^6 \rangle$, where $\langle \dots \rangle$ indicates an average over all conformations and r is the distance between the hydrogen and the electron spin. TAM solutions are expected to have several hydrogens at a similar distance and the definition can be generalized [5, 22] to $r_{\text{eff}}^{-6} = \sum_i n_i / r_i^6$, where the summation index i runs over the N nearest ^1H at distances r_i . The r_{eff} is easily calculated from the relative intensity of the satellite line and has served as a convenient characterization of the net dipolar interaction strength with nearby hydrogens in crystals, frozen solutions and glasses.

In disordered solids, EPR spectra generally exhibit a significant inhomogeneous broadening that conceals details in the spectra. Hence, the satellite lines are observed only in systems with very narrow lines. Most experimental reports in non-single crystals are for systems in which the unpaired electron spin interacts with ^1H . The splitting between the main and satellite lines is approximately the ^1H Larmor frequency in the applied external magnetic field, ~ 15 MHz (or 0.55 mT) at the ~ 350 mT field for X-band. The satellite lines from nearby hydrogen nuclei were observed in EPR spectra of hydrogen atoms resulting from γ -irradiation of frozen acids H_2SO_4 [5, 6, 23], H_3PO_4 [5, 6], HClO_4 [24], in aluminum hydroxides [25] and in solid hydrogen [26]. The satellite lines from ^1H were also observed in the spectra of trapped electrons formed by γ -irradiation of frozen methanol [27] and 10 M NaOH [7]. Recently, the satellite lines from the neighboring ^1H were observed in EPR spectra of hydrogen and deuterium atoms in solid mixtures of ^4He and hydrogen [28]. In polycrystalline diamond films, prepared by the CVD method, hydrogen trapped in the lattice also gives rise to satellite lines against a background of the carbon EPR spectrum which has the typical width of about 0.5 mT in X-band [29].

Despite the many papers on satellite lines in EPR spectra in disordered solids, reports from stable organic radicals are virtually absent. The reason is that most organic radicals in frozen solutions have a reasonably broad linewidth from g -factor anisotropy and hyperfine broadening that covers up the satellite lines. The TAM radical commonly known as Finland trityl, tris(8-carboxyl-2,2,6,6-tetramethylbenzo[1,2-*d*;4,5-*d'*]bis[1,3]dithiol-4-yl)methyl [30-32], Scheme 1, does have an EPR spectrum that is sharp enough that the satellite lines are resolved from the main line in X-band EPR. Replacement of its twelve methyl groups by trideuteromethyls gives Finland trityl- d_{36} with substantial narrowing of its main EPR line [21] allowing even better resolution.

Early EPR measurements and *ab initio* calculations of Finland trityl in a broad temperature range in liquid and frozen water and methanol solution allowed experimental assignment of ^{13}C hyperfine couplings at virtually every position in the radical [33]. The ^1H satellite lines in its EPR spectra were reported at X-band for frozen water-glycerol mixed solution [34], flanking a sharp central line with peak-to-peak linewidth $\Delta H_{\text{pp}} \sim 0.15$ mT. The intensity of each satellite line was reported as about 2.5% of that of the central line. That value includes intensity from natural-abundance ^{13}C hyperfine lines along with the spin-flip satellites, so the unique information on the dipolar interaction with hydrogens in and around the radical was not obtained. The importance of TAMs for DNP led us to determine the satellite line intensities for Finland trityl in methanol. Deuteration of the solvent allows us to separate the contributions from solvent and the methyl groups. The results are compared to ENDOR measurements of the same hydrogens.

Results

EPR

EPR spectra for samples of frozen Finland trityl in methanol solution with differently deuterated solvent and radical, Table 1, were measured. The EPR spectrum of sample FT7 in

liquid solution is an intense narrow line with $g=2.0027$ and $\Delta H_{pp} \sim 0.01$ mT, Fig. 1. Weaker absorption lines, split symmetrically about the main spectral line, are due to the HFI of the unpaired electron with natural-abundance ^{13}C nuclei. The observed isotropic HFI, A_{iso} , Table 2, for each ^{13}C agrees with literature data [33] within 1%. The A_{iso} values in the FT1-FT7 samples coincide within the experimental error.

Fig. 2(FT7) shows the EPR spectrum of the frozen FT7 sample at 130 K. The main, central line with $\Delta H_{pp} = 0.117$ mT is flanked by additional, weak absorption lines. The additional lines are ~ 1.0 mT from each other and are symmetric around the central line. Those lines have two sources: 1) the satellite lines caused by the HFI interaction of the unpaired electron with nearby ^1H spins; and 2) the $A_{||}$ and $A_{\perp}$ hyperfine features from natural-abundance C in the Finland trityl. Many of the ^{13}C in Finland trityl have $A_{||}$ and/or $A_{\perp}$ hyperfine splittings in the range of 18-40 MHz (0.6-1.4 mT) [33] which strongly overlap the satellite lines in X-band EPR.

The main line and ^{13}C hyperfine lines are easily saturated, unlike the satellite lines [23]. Because of this, the relative intensities of satellite spectrum lines must be measured at low microwave power where all spectral components are unsaturated. The spectra in Fig. 2 were measured with microwave power of 0.02 mW. A decrease in power by 20 dB alters the relative line intensity by no more than 5%, while a 20 dB increase results in an increase of the satellite to main line intensity ratio by an order of magnitude because the satellite lines are more difficult to saturate. The satellite lines make a detectable contribution to the absorption spectrum.

The EPR spectra of frozen samples with varying degrees of ^1H labeling were registered, Fig. 2. As the degree of deuteration of the radical and/or the solvent increases, the relative intensity of the flanking lines decreases but does not go to zero because of significant contributions from the ^{13}C hyperfine lines. The line width of the central line decreases by as much as 35%, and its g -tensor anisotropy becomes noticeable [34].

ENDOR

The stochastic Mims ENDOR spectra, even with rf pulses 72 μs long, were exceptionally intense with excellent resolution, Fig. 3A. The sharp features in the ENDOR spectrum means that it is unlikely that two hydrogen nuclei near the same radical are ever in resonance with the ENDOR rf pulse except for ^1H within a few kHz of the ^1H Larmor frequency. Consequently, the normalized ENDOR spectrum is a simple sum of the normalized contributions of the individual ^1H . In other words, ^1H in and around the trityl make independent contributions to the total ENDOR intensity except at the matrix ENDOR peak which is largely suppressed in Mims ENDOR [35]. This means that the Mims ENDOR spectrum of hydrogens from the trityl and from the solvent are independent and that subtraction of normalized spectra in methanol and deuterated d^4 -methanol, without any scaling or adjustable parameters, yields a Mims Difference ENDOR spectrum of just the solvent hydrogens, Fig. 3(B). The prominent features from the methyl hydrogens of Finland trityl disappear, leaving a spectrum from the solvent resembling that reported previously using Finland- d_{36} [33] but showing some residual structure unresolved in the earlier spectrum.

Discussion

Satellite Line Intensity

In disordered matrices, the ratio of the satellite peak intensity, I_{sat} , relative to the main line intensity, I_{main} , is given by the expression

$$I_{sat}/I_{main} = \frac{3}{20} \left(\frac{g_e \beta_e}{B_0} \right)^2 \sum_i \frac{n_i}{r_i^6} \quad 1$$

where r_i is the distance to the i -th ^1H of the radical or the solvent [22]. Unfortunately, the I_{sat}/I_{main} ratios needed to determine r_{eff} or $\sum_i n_i/r_i^6$ are difficult to obtain directly from the EPR spectra because of the overlap of the satellite peaks and the ^{13}C HFI lines, and because of the noticeable g -factor anisotropy in deuterated samples. Increasing the magnetic field and EPR frequency would proportionately increase the splitting of the satellite lines and separate them from the ^{13}C hyperfine lines. But the intensity ratio drops proportionally to the square of the field causing the satellite lines to disappear in the noise. For instance, going from X-band to Q-band would resolve the ^1H satellite lines from the ^{13}C hyperfine lines, but would decrease their intensity roughly 10-fold. In going to W-band the intensity would drop 100-fold.

The relative peak intensity of the satellite lines can best be determined by spectral simulations including the HFI with ^{13}C nuclear spins in the radical, the g -anisotropy and the forbidden transitions caused by the anisotropic dipolar HFI [36]. Calculated spectra for radicals with no ^{13}C and with one ^{13}C nucleus at the different positions in the molecule were combined to simulate spectra at the ^{13}C natural abundance of 1.1%. The principal values of the ^{13}C hyperfine tensors were taken as: $A_{\perp} = A_{\text{iso}} - T_{\perp}$, $A_{\parallel} = A_{\text{iso}} + 2T_{\perp}$, Table 2, where A_{iso} are obtained from the spectrum of the radical in liquid methanol, and $T_{\perp}$ from [33]. The g_{iso} value for all samples was 2.0032 ± 0.0002 that agrees well with those in [34]. The width of the main line and $g_{\parallel} - g_{\perp}$ from the best fits are reported in Table 1.

The calculated spectra simulate the observed spectra reasonably well. Fig. 4 shows the observed, Fig. 4(1), and calculated, Fig. 4(2), spectra of the frozen FT7 sample in CH_3OH . For comparison, the calculated spectrum without satellite lines, Fig. 4(3), shows that an acceptable agreement occurs only when the satellite lines are included.

Comparison of the Finland trityl radical with Finland- d_{36} in the same solvent, that is, FT1 vs. FT5, FT2 vs. FT6, and FT3 vs. FT7, show practically the same increase in I_{sat}/I_{main} of $0.5\% \pm 0.1\%$. This consistent change in satellite peak intensity provides an internal check showing the accuracy of the simulations and revealing the satellite peak intensity from the methyl hydrogens of the Finland trityl. The I_{sat}/I_{main} ratio of $0.5\% \pm 0.1\%$ (or 0.55% when corrected for the 90% isotopic purity of Finland- d_{36}) attributable to ^1H on Finland trityl gives an $r_{\text{eff}}(\text{Radical}) = 3.1 \pm 0.2 \text{ \AA}$. However, the methyl groups of Finland trityl contain $N=36$ hydrogens. If the hydrogens are all at the same distance from the unpaired electron spin, essentially a single shell, they are a factor of $(36)^{1/6}$ farther away than this r_{eff} . This gives a distance, $R(\text{Radical}) = 5.6 \pm 0.2 \text{ \AA}$, that agrees well with the average distance calculated from the Finland trityl structure in Scheme 1 [37].

The I_{sat}/I_{main} ratio also depends on the deuteration of the methanol solvent, showing that the unpaired electron has significant dipolar interactions with hydrogens in the solvent. In fact, as the ^1H content of the methanol solvent increases in the series CD_3OD (d_4 -methanol), CH_3OD (d_1 -methanol) and CH_3OH , the I_{sat}/I_{main} ratio increases linearly with the ^1H content, Fig. 5. The -OH proton contributes $0.26 \pm 0.03\%$ of the I_{sat}/I_{main} ratio, the three - CH_3 protons $0.74 \pm 0.07\%$, and all four protons $1.0 \pm 0.1\%$. The solvent component of the I_{sat}/I_{main} ratio is proportional to the total ^1H content of the methanol and not to their position in the methanol. While this suggests that the solvent -OH and - CH_3 hydrogens have identical distributions around the Finland trityl, this result could arise from a fortuitous combination of unequal interactions and preferential solvation of the radical.

The I_{sat}/I_{main} ratio from methanol hydrogens is about twice that from the 36 hydrogens of the radical and gives an effective dipolar distance even shorter than that to hydrogens of the Finland trityl, $r_{eff}(\text{MeOH}) = 2.8 \pm 0.1 \text{ \AA}$. The solvent hydrogens cannot be treated as lying in a single shell as the hydrogens of the Finland trityl were. Consequently, we model the solvent as a continuum with the same hydrogen concentration as methanol, with the radical inside a spherical cavity of radius R_0 . This allows the summation in Eqn. 1 to be replaced by integration from R_0 to infinity. The average concentration of hydrogens in methanol is $[H] = 4\rho N_A/\mu = 0.06802 \text{ \AA}^{-3}$, where $\rho = 904.6 \text{ kg/m}^3$ is the density of frozen methanol [38], N_A is Avogadro's number, and $\mu = 32.05 \text{ g/mol}$ is the molar mass of methanol, so that

$$\begin{aligned} I_{sat}/I_{main} &= \frac{3}{20} \left(\frac{g\beta_e}{B_0} \right)^2 [H] \int_{R_0}^{\infty} \frac{4\pi}{r^6} r^2 dr \\ &= \frac{3}{20} \left(\frac{g\beta_e}{B_0} \right)^2 [H] \frac{4\pi}{3R_0^3} \end{aligned} \quad 2$$

The experimental ratio of $I_{sat}/I_{main} = 1.0 \pm 0.1 \%$ for solvent indicates $R_0 = 4.9 \pm 0.2 \text{ \AA}$.

The solvent hydrogens seem to approach closer to the unpaired electron than do the hydrogens of the radical. This is not as strange as it first seems. A close examination of the solvent-accessible surface, Scheme I, shows that the surface of Finland trityl is not smooth and that small solvent molecules can come closer to the center than the trityl hydrogens [33, 37]. The spherical approximation used to derive Eqn. 2 is unrealistic for Finland trityl and so R_0 does not represent a physical distance. Neither does r_{eff} represent a physical or geometric distance. However, the r_{eff} and I_{sat}/I_{main} parameters are very relevant to DNP because each reflects the number of ^1H and the square of the dipolar interaction in the same relation as they contribute to Solid Effect and Cross Effect DNP.

ENDOR Analysis

The ^1H of methanol have a larger I_{sat}/I_{main} and a smaller r_{eff} than those of the methyl groups of the Finland trityl, suggesting that the solvent ^1H will be more effective in generating DNP than will the ^1H of the Finland trityl. Smith, *et al.* [4] reached the same conclusion based, in part, on earlier ENDOR spectra [33]. We fit the experimental Mims ENDOR spectrum of the methyl hydrogens of Finland trityl in d_4 -methanol in order to determine the ^1H hyperfine tensors and independently estimate r_{eff} . Finland trityl has two groups of six equivalent methyl groups. Each methyl group is non-rotating on the ENDOR timescale [33] and does not rotate rapidly at temperatures below 130 K [39]. We first fit the ENDOR spectrum in d_4 -methanol based on these facts using six inequivalent rhombic hyperfine tensors; but two of the hydrogens consistently gave identical HFI tensors and four consistently gave axial HFI tensors. It was also clear that the shoulders with a hyperfine splitting of about 1.3 MHz had a greater distribution of couplings (A-strain) than the other features, presumably from a distribution of conformations of the methyl group.

The final round of fitting used a spectral model with two equivalent axial hydrogens, two inequivalent axial hydrogens, one rhombic hydrogen and two rhombic hydrogens but with half occupancy to simulate the A-strain. The best fit with this model, Fig. 3(C), has an rms deviation of less than 1.3% although it is clear that the fit could be improved with a more sophisticated model of A-strain. The fit converged from multiple starting points to a single minimum with the HFI tensors in Table 3. The isotropic hyperfine couplings are small.

The satellite line intensity from each ^1H in an unordered powder or glassy sample is proportional to the sum of the squares of the principal values of the dipole-dipole part of the HFI when the HFI is much less than the nuclear Zeeman interaction. Consequently we

define an effective dipolar coupling $T_{\perp,eff} = \sqrt{(T_1^2 + T_2^2 + T_3^2)/6}$ where the T_i are principal

values of the dipole-dipole HFI tensor. For an axial HFI tensor, $T_{\perp, \text{eff}} = T_{\perp}$. In the point-dipole approximation, the dipolar interaction has axial symmetry. However, the distance from the hydrogens to the center of the unpaired electron spin distribution is comparable to the dimensions of that distribution. In addition, the unpaired electron spin distribution is far from having spherical symmetry. These two key requirements of the point-dipole approximation are not met and the dipolar HFI tensors are expected to have some degree of rhombicity, as found. The sum of the squares of the $T_{\perp, \text{eff}}$ from all 36 hydrogens in the 12 methyl groups of Finland trityl is proportional to the satellite peak intensity, and allows us to estimate $r_{\text{eff}}(\text{Radical}) = 3.27 \pm 0.15 \text{ \AA}$, in good agreement with the $3.1 \pm 0.2 \text{ \AA}$ from the satellite line intensity. The inequivalent dipolar tensors for the methyl hydrogens confirm that the methyl groups are not rotating and are at different distances from the unpaired electron spin. The assumption of a common distance to all methyl hydrogens that was used in calculation of $R(\text{Radical})$ is not valid and so $R(\text{Radical})$ is not a valid physical distance.

ENDOR spectra from frozen solutions rarely have the resolution or S/N ratio of those in Fig. 3 and it is rare to obtain six consistent hyperfine tensors from such highly-overlapped spectra. If we had merely taken the outer shoulders of the ENDOR spectrum as the $T_{\parallel}$ of the strongest dipole-dipole HFI, we would have obtained $2.7 \text{ \AA} < r_{\text{eff}} < 3.7 \text{ \AA}$ depending on assumptions about the number of ^1H with that splitting. This range of r_{eff} corresponds to a factor of six uncertainty in the intensity of the satellite lines and also in the limiting rate of DNP production by Solid Effect and Cross Effect mechanisms.

ENDOR also is not useful in determining R_0 for the solvent hydrogen. The difference Mims ENDOR spectrum of the solvent hydrogens in Fig. 3(C) cannot be fit with any confidence because the center of the spectrum arises from large numbers of distant solvent hydrogens. The lineshape of this matrix ENDOR line is poorly understood [35]. The inflections on the ENDOR spectrum of the solvent ^1H are reminiscent of an axial HFI with $T_{\perp} \sim 0.4 \text{ MHz}$, which would place the closest hydrogens $\sim 5.8 \text{ \AA}$ from the center of the electron spin in the point dipole approximation. This would place the closest solvent hydrogens slightly farther from the electron spin than some of the methyl hydrogens of the Finland trityl, but still closer than the most distant methyl hydrogens. The $r_{\text{eff}}(\text{MeOH}) = 2.8 \pm 0.1 \text{ \AA}$ from the satellite line intensity is consistent with the closest solvent hydrogens being at $\sim 5.8 \text{ \AA}$ if there are many solvent hydrogens at that distance. For example, the continuum model of eqn. 2 could be modified to include preferential orientation of the first shell of solvent placing large numbers of solvent hydrogens at $5.8 \text{ \AA}$. The main drawbacks for using ENDOR of bulk solvent ^1H are the inability to simulate the matrix line and the inability to determine the number of nuclei.

Conclusion

The total dipolar interaction relevant for DNP, for both the hydrogens of the Finland trityl radical and the methanol solvent, is readily measured by the parameter r_{eff} from the intensity of the spin-flip satellite lines if the weak hyperfine features from natural abundance ^{13}C in the trityl are taken into account. Attempts to obtain detailed structural information about actual distances by using simple spherical shell or continuum models for the ^1H distribution fail because the detailed molecular structure is important on this distance scale. The trityl methyl groups are not rotating at temperatures used for DNP and their dipolar HFI varies as much as a factor of four. Although the methyl hydrogens of the Finland trityl are chemically equivalent, under conditions used for DNP they are conformationally and magnetically inequivalent.

The integrated effect of the dipole-dipole interactions, as reflected in r_{eff} , are actually stronger for the ^1H in methanol than in the Finland trityl methyl groups even though most or

all of the solvent hydrogens are more distant than the Finland trityl hydrogens. The larger density of ^1H in the solvent makes up for their greater distance. This result indicates that the polarization of bulk protons by the Solid Effect and Cross Effect mechanisms proceeds more by polarization of solvent protons near a trityl radical followed by polarization transfer to bulk protons than by the parallel route through the trityl methyl groups to the bulk protons, at least for Finland trityl in methanol.

The ^{13}C features observed in the spectra are fundamentally different from proton spin-flip lines measured here. The ^{13}C features are ‘allowed’ hyperfine transitions and the ‘forbidden’ ^{13}C spin-flip transitions are both broadened and suppressed by the large ^{13}C hyperfine anisotropy. The proton hyperfine structure in the spectrum is unresolved and the spin-flip lines are resolved, while for ^{13}C the hyperfine structure is resolved while the spin-flip lines, particularly those from the solvent, are not. The large ^{13}C hyperfine anisotropy shifts the NMR frequencies of the ^{13}C in the trityl, so that they are ‘out-of-resonance’ with ^{13}C in the solvent, preventing polarization transfer from ^{13}C in the trityl radicals to solvent ^{13}C . The ^{13}C in the trityl may still play a role in DNP, by shifting the EPR frequency of a few trityls by half the hyperfine splitting. The few trityl radicals where this shift equals the nuclear Zeeman frequency of bulk ^{13}C might make a dominant contribution in the Cross Effect.

The largest ^{13}C hyperfine coupling in Finland trityl is about 160 MHz at the center carbon, so that the largest frequency difference with the major trityl line is about 80 MHz. This situation would support polarization of any nucleus in the bulk that has an NMR frequency less than 80 MHz, which at W-band or $\sim 3.4\text{T}$ includes ^{13}C (nominally 35 MHz) and ^{31}P (60 MHz) but not ^{19}F (130 MHz) and ^1H (140 MHz). This may help explain the common observation that trityls are quite effective in polarization of ^{13}C but not ^1H , although the large differences in the heat capacity of the proton spins versus carbon spins may also contribute.

The integrated effect of dipolar interactions to ^1H (or other abundant nuclei), as reflected by r_{eff} , is a more useful parameter for understanding and controlling DNP than is the strongest interaction indicated by a hyperfine splitting. The r from both the trityl and the solvent ^1H is readily measureable from CW X-band EPR spectra as long as the overlapping ^{13}C HFI features are taken into account.

Experiment

Finland trityl and its deuterated analogue Finland- d_{36} were prepared by known methods [21, 37]. Samples were made from the tri-sodium salts of the appropriate trityl in methanol, d_1 - or d_4 -methanol with a nominal concentration of $2 \cdot 10^{-3}\text{ M}$. The EPR spectra were registered with a Bruker EleXsys-E500 radiospectrometer in X-band at 130 K or room temperature. Stochastic Mims ENDOR spectra [40] were measured at 85 K with a Bruker EleXsys-E680 with EN4118X-MD4 resonator, DICE ENDOR accessory, a 50W ENI 550L RF amplifier and an ER4118CF cryostat with ITC503 controller. Simulation and least-squares fitting of Mims ENDOR spectra were made using the EasySpin package [41]. The ENDOR spectra are plotted in terms of the normalized ENDOR effect, defined as $1 - V(\text{rf}_{\text{on}})/V(\text{rf}_{\text{off}})$ where $V(\text{rf}_{\text{on/off}})$ is the echo amplitude with the ENDOR rf pulse on/off resonance.

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Highlights

- Spin-flip satellite line intensity measures total dipolar interactions with nuclei
- Trityl radicals have stronger interaction with solvent ^1H than internal ^1H
- Natural-abundance ^{13}C hyperfine features overlap ^1H spin-flip satellite lines
- ENDOR confirms dipolar coupling strength measured from spin-flip satellites

Fig. 1. EPR spectrum of 2.0 mM Finland trityl at 293 K. $\Delta H_{pp} \sim 0.01$ mT. The lines with least intensity are due to the hyperfine interactions in molecules containing two ^{13}C atoms.

Fig. 2.

EPR spectra of frozen samples with different extents of deuteration. The measurement temperature is 130 K, microwave power 0.02 mW. The spectra were normalized by the integrated intensity. The spectra from top to bottom are from: FT3, Finland-d₃₆ (90%) / CH₃OH; FT2, Finland-d₃₆ (90%) / CH₃OD; FT1, Finland-d₃₆ (90%) / CD₃OD; FT7, Finland trityl unlabeled / CH₃OH; FT6, Finland trityl unlabeled / CH₃OD; FT5, Finland trityl unlabeled / CD₃OD; FT4, Finland -d₃₆ (75%) / CD₃OD.

Fig. 3. Mims ENDOR spectra of 2.0 mM Finland trityl at 85 K: **A)** in methanol (black trace) and deuterated d_4 -methanol (blue trace); **B)** Difference Mims ENDOR spectrum showing the solvent ^1H , produced by subtracting the spectra in A); and **C)** Mims ENDOR spectrum of the methyl ^1H of the Finland trityl in d_4 -methanol (black) with the best least-squares fit (red), the parameters are given in Table 3. The vertical axis is the normalized change in echo amplitude caused by the 72 μs rf pulse.

Fig. 4. The observed (1) and calculated (2) spectra of the frozen sample FT7 in CH_3OH , as well as the spectrum calculated with the satellite lines neglected (3).

Fig. 5.
The ratio of the integral intensities of satellite and main lines ($I_{\text{sat}}/I_{\text{main}}$) obtained from fitting of spectra simulations versus the number of ^1H per molecule in the methanol solvent.

Scheme 1.

Finland trityl: Chemical structure; 3D ball and stick structure; and solvent accessible surface using a 1.4 Å probe. The molecule is viewed along its 3-fold axis. The hydrogens are colored cyan for emphasis. The largest diameter of the Finland trityl is approximately 11 Å.

Table 1

Characteristics of the studied samples of Finland trityl solution in methanol: deuteration extent of the radical, the solvent used, and the parameters of the calculated spectra for frozen samples such that the calculated and observed spectra are in the best agreement. The linewidth on the half-height is w , g $\perp$ and g - are the values of perpendicular and parallel components of the g-tensor, respectively, and $I_{\text{sat}}/I_{\text{main}}$ - is the integral intensity ratios of the satellite and the main lines obtained from our calculations. In all cases, the deuteration extent of methanol in the given position was more than 99%.

Sample	Radical	Deuteration of Radical, %	Solvent	w , mT	$g_{\parallel}$ - g $\perp$	$I_{\text{sat}}/I_{\text{main}}$ % (± 0.1)
FT1	Finland-d ₃₆	90	CD ₃ OD	0.155	0.00078	0.0
FT2	Finland-d ₃₆	90	CH ₃ OD	0.20	0.00087	0.8
FT3	Finland-d ₃₆	90	CH ₃ OH	0.21	0.00084	1.0
FT4	Finland-d ₃₆	75	CD ₃ OD	0.16	0.00078	0.0
FT5	Finland trityl unlabeled	0	CD ₃ OD	0.185	0.00093	0.5
FT6	Finland trityl unlabeled	0	CH ₃ OD	0.235	0.00090	1.3
FT7	Finland trityl unlabeled	0	CH ₃ OH	0.24	0.00084	1.7

Table 2

The ^{13}C hyperfine parameters used in simulations of the CW EPR spectra. The A_{iso} are the isotropic hyperfine splittings obtained from the spectra at room temperature. The A_{aniso} are the perpendicular components of anisotropic HFI estimated from ref. [33] and were used to calculate the $A_{\perp}$ and $A_{\parallel}$ values used in the simulations. The parameter, D , is the number of atoms or relative degeneracy at that position in each Finland triyl molecule.

^{13}C position	A_{iso} mT	A_{iso} MHz	A_{aniso} MHz	$A_{\perp}$ MHz	$A_{\parallel}$ MHz	D
Central	2.37	66.4	46.5	159	20	1
1-Phenyl	1.12	31.4	4.2	39.8	27.2	3
2,6-Phenyl	0.906	25.4	6.2	37.8	19.2	6
4-Phenyl	0.334	9.36	1.7	12.8	7.6	3
3,5-Phenyl	0.237	6.64	1.7	10.0	4.5	6
Carboxyl	0.128	3.59	0.15	3.9	3.5	3
Methylene bridge	-0.018	-0.50	0.15	-0.2	-0.65	6
Methyl	0.002	0.05	0.05	0.15	0	12

Table 3

HFI parameters from the least-squares fitting of the ENDOR spectrum from Finland trityl in d_4 -methanol. The spectral model is based on inequivalent pairs of non-rotating methyl groups with two equivalent hydrogens having axial HFI tensors (starting at the bottom of this table), two inequivalent axial hydrogens, one rhombic hydrogen and two rhombic hydrogens with half occupancy to simulate the A-strain. The principal values of the hyperfine tensors are given the second column. Parentheses indicate that the HFI tensor was forced to have axial symmetry. The third column is the values of the isotropic HFI and the last column shows the effective value of the dipolar HFI tensor.

Number of hydrogens	Principal Values (MHz)	A_{iso} (MHz)	$ T_{eff} $ (MHz)
0.5	-0.796, -0.432, +1.411	+0.061	0.683
0.5	-0.800, -0.443, +1.196	-0.016	0.615
1	-0.744, -0.270, +0.930	-0.028	0.498
1	-0.162, (-0.162), +0.647	+0.108	0.270
1	-0.386, (-0.386), +0.326	-0.149	0.237
2	-0.318, (-0.318), +0.186	-0.150	0.168