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Electron Spin Relaxation of Triarylmethyl Radicals in Fluid Solution[1]

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Electron spin relaxation times of a Nycomed triarylmethyl radical (*sym*-trityl) in water, 1 : 1 water : glycerol, and 1 : 9 water : glycerol were measured at L-band, S-band, and X-band by pulsed EPR methods. In H_2O solution, T_1 is 17 $\pm$ 1 μs at X-band at ambient temperature, is nearly independent of microwave frequency, and exhibits little dependence on viscosity. The temperature dependence of T_1 in 1 : 1 water : glycerol is characteristic of domination by a Raman process between 20 and 80 K. The increased spin–lattice relaxation rates at higher temperatures, including room temperature, are attributed to a local vibrational mode that modulates spin–orbit coupling. In H_2O solution, T_2 is 11 $\pm$ 1 μs at X-band, increasing to 13 $\pm$ 1 μs at L-band. For more viscous solvent mixtures, T_2 is much shorter than T_1 and weakly frequency dependent, which indicates that incomplete motional averaging of hyperfine anisotropy makes a significant contribution to T_2. In water and 1 : 1 water : glycerol solutions continuous wave EPR linewidths are not relaxation determined, but become relaxation determined in the higher viscosity 1 : 9 water : glycerol solutions. The Lorentzian component of the 250-MHz linewidths as a function of viscosity is in good agreement with T_2-determined contributions to the linewidths at higher frequencies.

INTRODUCTION

Collisions with O_2 in solution broaden the EPR spectra of radicals (*1–5*). If sufficiently narrow-line paramagnetic probes are employed (*6*), this broadening provides a means of measuring O_2 concentration *in vivo*. EPR line broadening has been useful for oximetry. Unresolved hyperfine splittings broaden the lines of all of the nitroxyl and trityl species currently available, which decreases the relative contribution of O_2 broadening to the overall linewidth. Relaxation times have the potential of being more sensitive than linewidths to O_2 concentration. However, it is not yet clear whether time domain acquisition can provide signal-to-noise comparable to that of continuous wave acquisition. To interpret changes in relaxation times in terms of O_2 concentration, it is important to understand other factors that also impact relaxation times. Most measurements of electron spin relaxation have been performed at X-band, and there is little information available to extrapolate those results to the RF frequencies needed for *in vivo* EPR (*7, 8*). In this paper we report relaxation times for deoxygenated solutions of trityl radicals provided by Nycomed Innovations AB (*6*). These radicals were designed for imaging with Overhauser magnetic resonance (*6*) and have been used in pulsed RF EPR (*9–15*). Even with a magnetic field gradient applied, the FID of the trityl radical can be observed for several microseconds (*9, 13, 14*). The trityl radical also has been used for continuous wave (CW) EPR imaging (*16*).

In this study, to assign the mechanism of relaxation (*17*) for the trityl radicals, T_1 and T_2 were measured at 21°C at X-band (~9.2 GHz), S-band (~3.1 GHz), and L-band (~1.5 GHz), as a function of viscosity in water : glycerol mixtures at 21°C, and as a function of temperature between 20 and 294 K at X-band. The relaxation-determined contributions to the CW linewidths at 250 MHz at 27°C were determined by lineshape simulation.

EXPERIMENTAL

Samples

The triarylmethyl radicals methyl tris(8-carboxy-2,2,6,6-tetramethyl-benzo[1,2-*d* : 4,5-*d′*]bis(1,3)dithiol-4-yl) trisodium salt (*sym*-trityl-CH_3) and methyl tris(8-carboxy-2,2,6,6-tetramethyl(-d_3)-benzo[1,2-*d* : 4,5-*d′*]bis(1,3)dithiol-4-yl) tripotassium salt (*sym*-trityl-CD_3) used in this study are described in Ardenkjaer-Larsen *et al.* (*6*), and were a gift from Dr. Klaus Golman. Most studies were of the deuterated symmetric trityl (molecular weight 1151, *sym*-trityl-CD_3). Some additional studies were performed with the analog that has CH_3 groups in place of the CD_3 groups (molecular weight 1067, *sym*-trityl-CH_3). Based on the results of Ardenkjaer-Larsen *et al.* (*6*), we chose 0.2 mM as a concentration that is sufficiently low that there is little radical–radical collisional contribution to spin relaxation, but high enough to get good signal-to-noise in the low-frequency saturation-recovery measurements. This choice of concentration was supported by saturation-recovery experiments that found T_1 for *sym*-trityl-CD_3 is 0.5 μs shorter for a 0.4 mM H_2O solution than for a 0.2 mM solution. Samples were contained in thin-wall Teflon tubing with 0.86 mm (water at X-band), 1.15 mm (water : glycerol mixtures at X-band),

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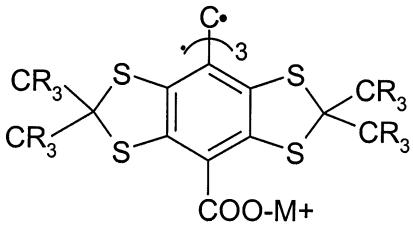
or 1.35 mm i.d. (S-band). The Teflon tubing was placed in a 4-mm-o.d. quartz sample tube and deoxygenation was performed by passing medical-grade nitrogen gas over the sample until the relaxation times reached a limiting value. L-band samples were contained in 4-mm-o.d. quartz tubes. The L-band water sample was bubbled with N_2 to purge out oxygen. L-band samples in 1 : 1 and 1 : 9 H_2O : glycerol were degassed by multiple cycles of freeze–pump–thaw. Mixtures of water and glycerol were prepared by weighing the glycerol, although the compositions are described by v : v ratios. Viscosities are calculated to be 1.0, 8.3, and 384 cP for water, 1 : 1 water : glycerol, and 1 : 9 water : glycerol, respectively, at 20°C (*18*). Note that 1 : 1 water : glycerol by volume corresponds to about 56% glycerol by weight because the density of glycerol (1.26 g/mL) is higher than that of water (1.0 g/mL).



sym-trityl-CH_3 $R = H$, $M = Na^+$
sym-trityl-CD_3 $R = D$, $M = K^+$

Samples of 0.2 mM *sym*-trityl-CD_3 for lineshape measurements at 250 MHz were prepared in 10-mL glass vials with a septum closure mechanically enforced with an aluminum crimp and sealed with epoxy. Mixtures of water and glycerol were prepared by weight. Deoxygenation was performed by passing water-saturated medical-grade N_2 gas through the sample. Samples were weighed before and after bubbling with the water-saturated N_2 and the percentage of glycerol in the sample was recalculated assuming that all weight gain was due to water absorption. The viscosities of the water : glycerol mixtures were calculated by double interpolation using literature data for viscosity as a function of solution composition and temperature (*19*).

EPR Measurements in Denver

X-band two-pulse ESE (electron spin echo) measurements of T_m (T_2) and three-pulse inversion-recovery ESE measurements of T_1 were made on a spectrometer previously described (*20, 21*) using an overcoupled cavity resonator, or on a Bruker E580 with a Bruker split-ring resonator. X-band saturation-recovery (SR) measurements of T_1 used a spectrometer (*22*) and loop-gap resonator (*23*) previously described. The L-band and S-band spectrometers (*24, 25*) include CW, ESE, and SR functions in a single bridge. The ac-coupled final signal amplifier previously described has been replaced with a dc-coupled amplifier with several gain values to avoid the sloping baselines inherent in an ac-coupled amplifier. In the L-band bridge we replaced the General Microwave DM864 detector protection reflective switch with a MiniCircuits ZASWA-2-50DR absorptive switch, which has better impedance match in the on and off states, and hence gave smaller baseline offsets in the signal. The S-band crossed-loop resonator (CLR) has been described (*26, 27*). The L-band LGR was similar to the one described in (*24*), with a 4.3-mm-diameter, 10-mm-long sample region. The power required for a 90° pulse indicated that this LGR has an efficiency of $3.9\ G/\sqrt{W}$. Each of the resonators was designed to work with 4-mm-o.d. tubes.

Saturation-recovery measurements were made at observe powers low enough that the recovery time constant was minimally impacted by the observe power (*28, 29*). The spin relaxation times obtained for the trityl samples by long-pulse SR, three-pulse inversion recovery, and echo-detected SR agreed within experimental uncertainty, which shows that the recovery was not affected by spectral diffusion, and hence gave a true T_1. This result was expected, because the trityl EPR line is so narrow that it was fully excited in the inversion recovery and echo-detected SR experiments. In the following discussion distinctions are not made between values obtained by the three methods.

The time constant for spin echo dephasing is denoted as T_m to encompass all processes that result in dephasing (*17*). As discussed below, in fluid solution the spin echo dephasing for the trityl radicals is dominated by spin–lattice relaxation and incomplete motional averaging and we therefore equate T_m and T_2.

EPR Measurements in Chicago

The CW linewidths for the trityl signal in deoxygenated water : glycerol mixtures were measured on the 250-MHz spectrometer that was previously described (*7*). The spectrometer has been significantly improved by implementing balanced capacitive coupling to the resonator. The sample temperature was maintained at approximately 27°C and monitored with a thermocouple adjacent to the sample. Radiofrequency power of ca. 10 μW incident on a single-loop single-gap resonator 2.5 cm in diameter and 3.2 cm in length containing the 10-ml sample produced a B_1 of about 1 mG. Typical resonator Q was 250. The modulation frequency was 5.06 kHz with a modulation amplitude of 9 mG. Spectra were obtained with 64 points over a field interval of 188 mG. Point dwell time equaled the time constant of 0.3 s. The lineshapes were analyzed with the algorithm of Robinson *et al.* as a convolution of a Gaussian function that includes magnetic inhomogeneity and unresolved nuclear hyperfine and a Lorentzian function that is the spin relaxation contribution (*30, 31*). The algorithm removes the effect of overmodulation. No correction to the Lorentzian widths was made for saturation. This effect is expected to be small, a maximum of 1 mG for the narrowest line. The algorithm also includes correction for a dispersive component introduced when balancing the bridge with finite isolation.

RESULTS AND DISCUSSION

Spin–Lattice Relaxation Time, T_1

The temperature dependence of $1/T_1$ for *sym*-trityl-CD_3 in 1 : 1 water : glycerol is shown in Fig. 1. The fit to the experimental data (*32*) was obtained with a Raman process that dominates at temperatures below about 80 K and a local vibrational mode with an energy of 590 cm^{-1} that dominates at higher temperatures. The IR spectrum of *sym*-trityl-CD_3 in KBr shows a grouping of bands between 518 and 586 cm^{-1} that we tentatively assign to modes with substantial C–S stretching contributions. We propose that these vibrational modes are the local modes that dominate the spin–lattice relaxation near room temperature. The temperature dependence of $1/T_1$ does not exhibit a change of slope in the temperature range where the glass softens and melts (ca. 200 to 250 K), which indicates that relaxation processes that involve molecular rotation do not make dominant contributions to the relaxation. For these trityls in water : glycerol mixtures at room temperature, there is little dependence of T_1 (Table 1) on viscosity, which also indicates minimal contributions to the relaxation from molecular rotation, including spin rotation. The slightly smaller T_1 in water than in the higher viscosity solutions may be due to a small contribution to T_1 from trityl–trityl collisions in water, which decreases as the solution viscosity increases.

Ardenkjaer-Larsen *et al.* (*6*) derived trityl T_1 values from dynamic nuclear polarization enhancement curves at 267 MHz, and obtained values of 8.2 μs at 23°C and 9.4 μs at 37°C, by extrapolation of their concentration-dependent data to infinite dilution. They noted that the longer T_1 at higher temperature rules out spin–rotation interaction as the dominant relaxation mechanism. To determine whether the shorter value of T_1 obtained by DNP at 267 MHz than we obtained at higher frequencies is due to frequency dependence or to differences in measurement techniques will require direct measurement of T_1 at 267 MHz. At 24, 40, and 60 K the S-band values of T_1 for *sym*-trityl-CD_3 in 1 : 1 water : glycerol were the same as at X-band, within experimental uncertainty. Similarly, the values of T_1 at room temperature (Table 1) exhibit little frequency dependence. The minimal frequency dependence of T_1 rules out dominant contributions, either at low temperature or at room temperature, from processes that result in spectral density terms (*17*). The minimal dependence of T_1 on microwave frequency or on molecular tumbling is consistent with assignment of the dominant process in fluid solution to a local vibrational mode.

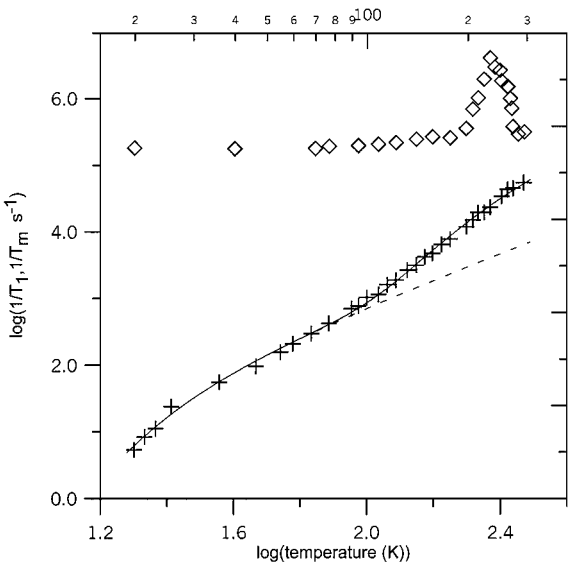


FIG. 1. X-band electron spin relaxation rates for *sym*-trityl-CD_3 in 1 : 1 water : glycerol: (+) $1/T_1$, (◇) $1/T_m$, (—) fit to experimental data calculated as in (*32*), including Raman and local mode relaxation processes, and (- - -) fit to experimental data including only the Raman process.

TABLE 1

Electron Spin Relaxation Times for *sym*-Trityl-CR_3 at 21 ± 1°C[a]

R	Solvent/ frequency	H_2O		1 : 1 H_2O : glycerol (v/v)		1 : 9 H_2O : glycerol (v/v)	
		T_1	T_2	T_1	T_2	T_1	T_2
D	X-band	17	11	17	3.7	19	0.24
D	S-band	16	13	16	3.8		
D	L-band	14	12.5	15	4.2	18	0.21
H	X-band	15	8.7	16	2.2	17	0.18

Note. X-band is ca. 9.2 GHz, S-band is ca. 3.1 GHz, and L-band is ca. 1.5 GHz. Uncertainties based on replicate measurements are ±1 μs for T_1 and about 10% for T_2.

[a] Values are for deoxygenated samples. Relaxation times are in μs.

For nitroxyl radicals it has been proposed that interactions with nuclear spins in the solvent can contribute to spin–lattice relaxation (*33*). To test for this contribution to relaxation, T_1 for *sym*-trityl-CD_3 at X-band was measured in perdeuterated solvents. The values of T_1 in D_2O (17 μs) and in 1 : 9 D_2O : glycerol-d_8 (19 μs) agreed with values obtained in the proton-containing solvents (Table 1), which indicates that solvent nuclei play a negligible role in the relaxation. At X-band the values of T_1 for the *sym*-trityl-CH_3 were consistently slightly smaller than those for *sym*-trityl-CD_3, which suggests that the peripheral methyl groups may play a small role in the relaxation process.

For triphenylmethyl in triphenylamine at X-band Weissman *et al.* (*34*) reported $T_1 = 5$ ms at 77 K and $T_1 = 4$ min at 4.2 K. Their value at 77 K is similar to our value of 3.2 ms at 77 K for *sym*-trityl-CD_3. At 10 K $T_1 = 40$ ms for trityl at 139.5 GHz (*35*). At 9.4 GHz we observed ca. 200 ms T_1 at 22 K (the lowest temperature we studied). The shorter T_1 at higher frequency is consistent with increased importance of a direct process (which results in a relaxation rate proportional to B^4T) at higher frequency and lower temperature.

Spin–Spin Relaxation Time, T_2

The temperature dependence of the spin echo dephasing rates, $1/T_m$, for *sym*-trityl-CD_3 at X-band in 1 : 1 water : glycerol is shown in Fig. 1. At low temperature T_m is approximately

independent of temperature and the shapes of the echo decays are characteristic of dephasing due to spin flips by surrounding nuclei (*36*). As the temperature is increased through the region in which the glassy solvent softens and melts (ca. 200 to 250 K), there is a dramatic increase and then decrease in $1/T_m$. The maximum value in 1 : 1 water : glycerol occurs at about 233 K, which indicates that at about this temperature the tumbling rate is of the same order of magnitude as the anisotropy that is averaged by the tumbling.

For *sym*-trityl-CD_3 in water solution at room temperature, the similarity between T_1 and T_2 indicates that T_2 is largely determined by T_1, and suggests that the trityl is close to the fast tumbling limit. For the more viscous solvents T_2 is significantly shorter than T_1, which indicates that incomplete motional averaging of anisotropy makes a significant contribution to T_2. In the intermediate tumbling regime, $1/T_2$ would be expected to vary approximately linearly with the magnitude of the anisotropy to be averaged. A weaker dependence on the magnitude of the anisotropy is expected near the fast (or slow) tumbling limit. The frequency dependence observed for T_2 in both of the water : glycerol mixtures is substantially less than the factor of ca. 6 change in field/frequency. In 1 : 1 water : glycerol the weaker dependence on frequency may be due to being close to the fast averaging limit. However, the value of $1/T_m$ at 233 K for *sym*-trityl-CD_3 in 1 : 1 water : glycerol is similar to what we observed at room temperature in 1 : 9 water : glycerol. This suggests that in 1 : 9 water : glycerol at room temperature the tumbling correlation time is near the middle of the intermediate regime, where incomplete motional averaging has maximum impact on T_m. The small frequency dependence of T_m in this solvent mixture suggests that much of the anisotropy may be in hyperfine rather than *g* values.

The room temperature T_2 values are systematically shorter for the trityl-CH_3 than for trityl-CD_3, which we attribute to averaging of larger hyperfine anisotropy for the $-CH_3$ derivative.

CW Spectra

A special feature of the trityls is that the only nuclear spins (other than small natural abundance of ^{13}C, ^{17}O, and ^{33}S) are the ^2H and ^1H on the methyl groups five bonds away from the paramagnetic center. The relaxation times are long enough that accurate measurements of the CW EPR linewidths are a challenge to magnet homogeneity and spectrometer stability. Also, the lines are so narrow that the traditional 100-kHz magnetic field modulation would be a significant source of broadening. Therefore CW linewidths reported in this study were based on measurements obtained with 10-kHz magnetic field modulation at X-, S-, and L-band, and 5-kHz magnetic field modulation at 250 MHz. The CW EPR spectrum of a related trityl radical, showing the narrow line and ^{13}C satellite lines, is in Fig. 5 of Ref. (*6*).

In water solutions at room temperature the X-band and L-band CW linewidths for *sym*-trityl-CD_3 were substantially broader than values predicted based on T_2 (Table 2). This broadening may be due in part to magnet inhomogeneity. However, the increase in linewidth between water and 1 : 1 water : glycerol must be due to incomplete motional averaging of anisotropy. The broader CW linewidth in 1 : 9 water : glycerol than that calculated from T_2 suggests that the tumbling rate is slow enough that there are significant additional contributions to the linewidth due to anisotropy. The smaller T_2 for *sym*-trityl-CH_3 than for *sym*-trityl-CD_3 is consistent with the larger CW linewidth. Both observations indicate that the CH_3 groups make a significant contribution to the anisotropy that is only partially motionally averaged in the relatively viscous solvent. The small difference in linewidths between X-band and L-band in the viscous solvents supports the proposal, based on T_2, that much of the anisotropy that is incompletely averaged is in hyperfine rather than *g* values.

The linewidths of the CW spectra at 250 MHz were analyzed to determine the Lorentzian contribution (*30*, *31*), which is substantially smaller than the apparent linewidth (Fig. 2). The Lorentzian contribution increased from 7.4 mG in water to 17 mG in 60% (w/w) glycerol at 27°C. In Ardenkjaer-Larsen *et al.* (*6*), spectral fitting of the CW EPR spectrum yielded 8 ± 1 and 7 ± 1 mG relaxation contribution to the linewidths in isotonic saline at 23 and 37°C at 267 MHz. Furthermore, they noted that the linewidth in water was the same at 267 MHz and at X-band. The measured X-band T_2 value of 11 μs for *sym*-trityl-CD_3 in H_2O at room temperature (Table 1) corresponds to a first derivative peak-to-peak linewidth of ca. 6 mG, again indicating little frequency dependence of T_2 in low-viscosity media.

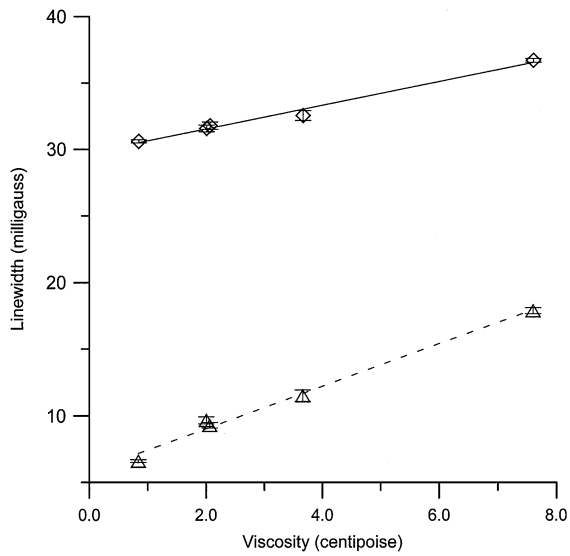


FIG. 2. Viscosity dependence of linewidths for trityl-CD_3 at 250 MHz in deoxygenated solutions: (◇) apparent peak-to-peak linewidths and (△) Lorentzian linewidths determined by fitting to a convolution of Gaussian and Lorentzian functions in water, and water : glycerol mixtures containing 35, 45, and 60% glycerol (v/v) at ca. 27°C. The linear fits to the viscosity dependence were (—) apparent linewidth = 0.90 mG/cP ∗ viscosity + 29.8 mG; (- - -) Lorentzian contribution = 1.6 mG/cP ∗ viscosity + 5.8 mG.

TABLE 2
CW Linewidths for *sym*-Trityl-CR_3[a]

R	Solvent/frequency	H_2O	1 : 1 H_2O : glycerol (v/v)	1 : 9 H_2O : glycerol (v/v)
D	X-band ΔB_{pp}	24	75	380
D	X-band, contribution to linewidth from T_2[b]	6.0	18	273
D	L-band ΔB_{pp}	23	54	350
D	L-band, contribution to linewidth from T_2[b]	5.2	16	312
D	250 MHz ΔB_{pp}	30	32.8	
D	250 MHz, contribution to linewidth from T_2[c]	6.5	11.3	
H	X-band ΔB_{pp}	80	80	360
H	X-band, contribution to linewidth from T_2[b]	7.5	30	364

[a] For deoxygenated samples. Linewidths are in mG.
[b] Calculated using $\Delta B_{pp}\,(\mathrm{mG}) = 6.56 \times 10^{-5}/T_2$ (s).
[c] Calculated by deconvolution of the Lorentzian component.

Our results agree with the conclusion of Ardenjaer-Larsen *et al.* (*6*) that averaging of *g*-tensor anisotropy does not dominate the relaxation in low-viscosity media.

At 5 T (140 GHz) and 10 K, *sym*-trityl-CH_3 has a linewidth of 10 G in 40 : 60 water : glycerol solution. There is a small asymmetry in the lineshape, which indicates small *g* anisotropy (*35*). Scaling to X-band, the linewidth would be ca. 0.7 G, if all of the width at higher frequency were due to *g* anisotropy. We observed frozen-solution linewidths of 1.1 G at S-band (1.5 GHz) and 1.4 G at 9.4 GHz for *sym*-trityl-CD_3, which suggests that a significant portion of the rigid lattice linewidth at the lower frequencies is due to unresolved hyperfine interaction. The inhomogeneity of the magnet used for the S-band measurements contributes less than ca. 50 mG to the CW linewidth. This estimate was based on room temperature CW linewidths and includes broadening due to 100 kHz modulation. Rough comparison among these experiments yields a *g* anisotropy of 1.7×10^{-4} for trityl. For comparison, Δg is about 6×10^{-3} for common nitroxyl radicals (*37*).

The viscosity dependence of the Lorentzian component of the 250-MHz linewidth was 1.6 mG/cP (Fig. 2). A two-point (water to 1 : 1 water : glycerol) estimate of the viscosity dependence of the T_2-determined contribution to the linewidths at higher frequencies gave 1.5, 1.6, and 1.5 mG/cP at L-, S-, and X-band, respectively. The frequency independence of this coefficient is consistent with a predominant contribution from incomplete motional averaging of hyperfine anisotropy. This viscosity dependence of linewidth is more than an order of magnitude less than that for nitroxyls (*5*).

Comparison with Other Radicals

The comparably narrow EPR line due to the solvated electron in metal–ammonia solutions has the same relaxation time ($T_1 = T_2 =$ ca. 3.5 μs) from 8 MHz to 9.3 GHz (*38*). It was concluded that T_2 was due to scalar coupling of the electron to the nitrogen nuclear spin, and T_1 was due to electron spin–orbit interaction with a correlation time of 10^{-14} s. This is the order of magnitude expected for vibrations. For the trityl radicals in fluid solution we similarly interpret T_2 as due to coupling of the electron spin with the ^{2}H or ^{1}H nuclei in the methyl groups in the trityl radical.

The trityl T_1 values increased only slightly with increased viscosity, which is in contrast with observations on nitroxyl radicals (*17*). For the nitroxyl radicals in relatively low-viscosity solution spin rotation makes a significant contribution to T_1, but makes little contribution for the trityl radicals.

CONCLUSION

A local vibrational mode mixing spin and orbital angular momentum would result in electron spin–lattice relaxation with the properties observed here: independent of microwave frequency and of tumbling correlation time, and smoothly temperature dependent through the melting point of the solvent.

Our direct measurements of T_1 at L-, S-, and X-band are within a factor of 2 of those derived from DNP enhancements at 267 MHz. Values of T_2 exhibit little frequency dependence. The negligible viscosity dependence of T_1 compared with the strong dependence on oxygen concentration makes it an attractive metric for oximetry. Although both T_2 and linewidth exhibit a modest dependence on viscosity, these changes are relatively small compared with changes effected by oxygen (*6*). These results lend strong support to the application of trityl radicals to *in vivo* oximetry at low RF frequencies.

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