Nitroxides. LIX. Rotational Correlation Time Determination of Nitroxide Biradical Application to Solvation Studies

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Abstract: Nitroxide biradicals with large dipolar splitting can be used to study the molecular rotational correlation times in the range 4×10^{-11} sec $< \tau_c < 10^{-7}$ sec. Peak-to-peak line width can be used to estimate solvation.

Detailed information on surrounding, orientation, and motion of biological molecules can be obtained by electron spin resonance (esr) using nitroxide monoradicals[1] as spin label.[2-4]

In these studies, the spectra allow distinction between isotropic and anisotropic motion of the paramagnetic species in solution. Analysis of these spectra can be made in terms of a characteristic parameter, the rotational correlation time τ_c.[5,6]

Another correlation time τ_s can be obtained for a sphere of radius r flowing in a liquid of viscosity η at temperature T.[5]

$$\tau_s = 4\pi\eta r^3/3kT$$

Information on solvation of the paramagnetic molecule in solution can be obtained by comparing these two correlation times.[7,8]

Determination of τ_c for nitroxide monoradicals in solution has recently been made,[9-16] in connection with spin label studies.

Except for some publications,[17-20] nitroxide bi-radicals[21-23] have not received much attention as spin labels in biological systems. However, their spectra should be useful since they contain information derived from two new parameters: scalar exchange interaction J, and dipolar tensor D.[24,25]

In most nitroxide spin labels, the difference between anisotropic principal values is *ca.* 3×10^7 Hz for the *g* tensor (at X band) and 10^8 Hz for nitrogen hyperfine splitting tensor A_N. It is possible to prepare biradicals[26,27] in which the distance between the two unpaired electrons is sufficiently short (*ca.* 5 Å) and in which anisotropic dipolar splitting reaches a value of *ca.* 10^9 Hz.[28] We may then expect large anisotropic effects.

For instance, a large dipolar splitting may simplify the analysis of line shape in solution because the main contribution to line width is of dipolar origin.[29] This suggests that the use of such a biradical could allow a simple determination of its correlation time τ_c without making the usual detailed analysis[30,31] of the esr spectrum.

Furthermore, the anisotropic parameter being of *ca.* 10^9 Hz, we may expect important variations in the esr spectrum for $\tau_c < 10^{-9}$ sec, *i.e.*, in a range where the monoradicals spectra are not very sensitive to τ_c variation.

Experimental Section

Stable biradical **2** (mp 101°) has been prepared from biradical spin label **1**[26] and cyclohexanone.

(1) A. R. Forrester, J. M. Hay, and R. H. Thomson, "Organic Chemistry of Stable Free Radicals," Academic Press, London, 1968.

(2) C. L. Hamilton and H. M. McConnell, "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968.

(3) H. M. McConnell and B. G. McFarland, *Quart. Rev. Biophys.*, 3, 91 (1970).

(4) I. C. P. Smith, "Biological Applications of Electron Spin Resonance Spectroscopy," J. R. Bolton, D. Borg, and H. Schwartz, Ed., Wiley-Interscience, New York, N. Y., 1971.

(5) N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, 73, 679 (1948).

(6) H. M. McConnell, *J. Chem. Phys.*, 25, 709 (1956).

(7) For a general review see A. Hudson and G. R. Luckhurst, *Chem. Rev.*, 69 (2), 191 (1969).

(8) R. Wilson and D. Kivelson, *J. Chem. Phys.*, 44, 154, 4440, 4445 (1966).

(9) M. S. Itzkowitz, *J. Chem. Phys.*, 46, 3048 (1967).

(10) M. D. Barratt, D. K. Green, and D. Chapman, *Biochim. Biophys. Acta*, 152, 20 (1968).

(11) L. Ya. Gendel', M. G. Gol'dfel'd, V. K. Kol'tover, E. G. Rozantsev, and V. I. Suskina, *Biophysics (USSR)*, 13, 1293 (1968).

(12) I. V. Alexandrov, A. N. Ivanova, N. N. Korst, A. V. Lazarev, A. I. Prikhozhenko, and V. B. Stryukov, *Mol. Phys.*, 18, 681 (1970).

(13) P. Törmälä, K. Silvennoinen, and J. J. Lindberg, *Acta Chem. Scand.*, 25, 2659 (1971).

(14) A. N. Kuznetsov, A. M. Wasserman, A. U. Volkov, and N. N. Korst, *Chem. Phys. Lett.*, 12, 103 (1971).

(15) R. C. McCalley, E. J. Shimshick, and H. M. McConnell, *Chem. Phys. Lett.*, 13, 115 (1972).

(16) J. H. Freed, *Annu. Rev. Phys. Chem.*, 23, 265 (1972).

(17) M. Calvin, H. H. Wang, G. Entine, D. Gill, P. Ferruti, M. A. Harpold, and M. P. Klein, *Proc. Nat. Acad. Sci. U. S.*, 63, 1 (1969).

(18) P. Ferruti, D. Gill, M. P. Klein, H. H. Wang, G. Entine, and M. Calvin, *J. Amer. Chem. Soc.*, 92, 3704 (1970).

(19) J. C. Hsia, D. J. Kosman, and L. H. Piette, *Biochem. Biophys. Res. Commun.*, 36, 75 (1969).

(20) S. Ohnishi, T. J. R. Cyr, and H. Fukushima, *Bull. Chem. Soc. Jap.*, 43, 673 (1970).

(21) R. Briere, R. M. Dupeyre, H. Lemaire, C. Morat, A. Rassat, and P. Rey, *Bull. Soc. Chim. Fr.*, 11, 3290 (1965).

(22) R. M. Dupeyre, H. Lemaire, and A. Rassat, *J. Amer. Chem. Soc.*, 87, 3771 (1965).

(23) E. G. Rozantsev, V. A. Golubev, M. B. Neiman, and Yu. V. Kokhanov, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 559 (1965).

(24) *Cf.* H. Lemaire, Thèse, Grenoble, 1966; Rapport CEA No. R 3119.

(25) Biradical may also be useful in reduction studies; conversion of a monoradical to the hydroxylamine induces a loss of information, while reduction of a biradical to a monoradical produces a new information by a drastic change of esr signal.

(26) J. F. W. Keana and R. J. Dinerstein, *J. Amer. Chem. Soc.*, 93, 2808 (1971); J. Michon and A. Rassat, French Patent EN 7115999.

(27) *Cf.* A. Rassat, *Pure Appl. Chem.*, 25, 623 (1971); P. Michon, Thèse, Grenoble, 1970; Rapport CEA No. R 4021.

(28) N. Hirota and S. I. Weissman, *J. Amer. Chem. Soc.*, 86, 2538 (1964).

(29) A. Carrington and A. D. McLachlan, "Introduction to Magnetic Resonance," Harper and Row, New York, N. Y., 1967, p 201.

(30) J. R. Norris and S. I. Weissman, *J. Phys. Chem.*, 73, 3119 (1969).

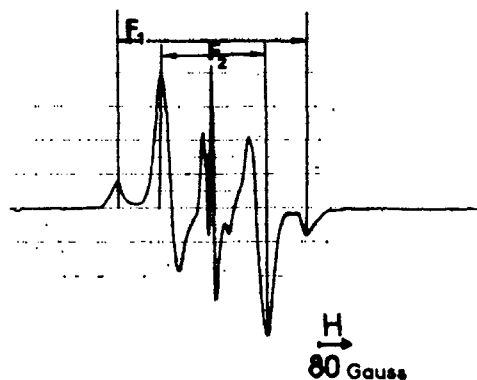(31) J. H. Freed, G. V. Bruno, and C. Polnaszek, *J. Chem. Phys.*, 55, 5270 (1971).

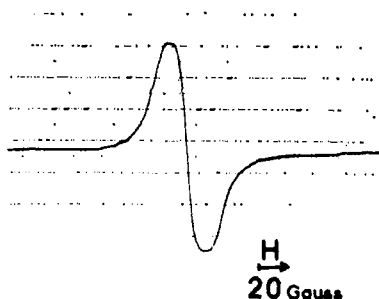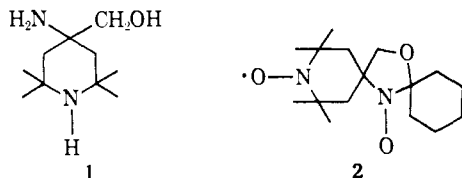**Figure 1.** Spectrum of biradical 2 in Nujol oil at −20°.

Figure 2. Spectrum of biradical 2 in dilute acetone solution.

In frozen (at −120°) ethyl alcohol solution or in Nujol oil (at −20°),[32] "fully immobilized" spectrum of biradical 2 is characteristic of large dipolar electron–electron interaction D (Figure 1). A standard analysis[33] gives $D = 230$ G[34] and $E \ll D$ (tentatively $E = 18$ G).

At room temperature, 10^{-3} M solutions in benzene, ethyl alcohol, ethyl ether, or acetone show an esr spectrum consisting of a single broad line of ca. 30 G width (Figure 2). Measurements in different solvents show that the peak-to-peak line width ΔH_{pp} increases as viscosity increases and temperature decreases. For large viscosities and low temperature, the spectrum changes continuously to the fully immobilized spectrum. Following Weissman,[30] we characterize our spectra by line width parameters F_1 and F_2 (Figure 1). Figure 3 gives F_1 and F_2 as a function of log η/T for biradical 2 in n-butyl alcohol.

Discussion

1. Immobilization Ratio. This graph (Figure 3) contains the information relative to the motion of biradical 2.[14, 15, 30] Although this information is usually summarized in a language which uses correlation time τ_c, it may be simpler to express it in term of the immobilization ratio (I) defined[35] as

$$I = \frac{F_1 - F_{1(min)}}{F_{1(max)} - F_{1(min)}}$$

(32) In Nujol oil at −80° and in frozen dimethylformamide at −100°, a slightly different spectrum is obtained in which a shoulder is observed corresponding to $D' = 254$ G. This may be assigned to two different conformations of biradical 2.[26]

(33) E. Wasserman, L. C. Snyder, and W. A. Yager, *J. Chem. Phys.*, 41, 1763 (1964).

(34) In the point dipole approximation[28] this dipolar interaction D gives an average distance[27] between the two unpaired electrons $\langle r \rangle$ = 4.95 Å, in agreement with measurement on molecular models.

(35) A. N. Kuznetsov and V. A. Radtsig, *Chem. Phys. Lett.*, 17, 377 (1972).

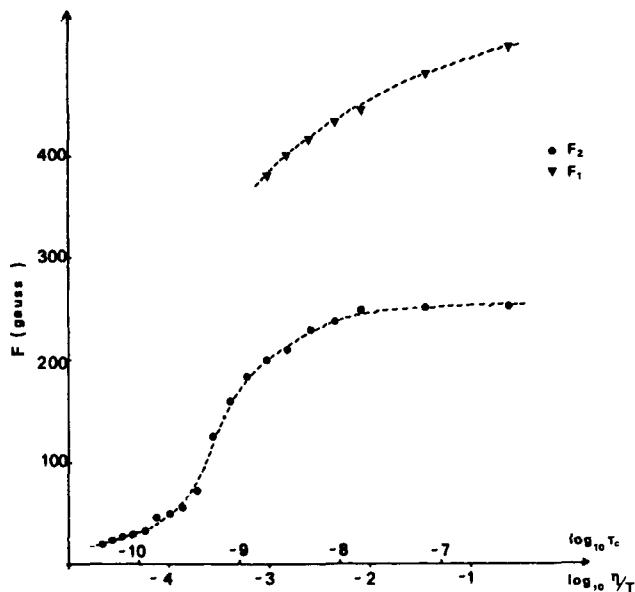**Figure 3.** Variation of parameters F_1 and F_2 of biradical 2 as a function of log η/T for n-butyl alcohol.

This immobilization ratio is of course dependent on the nature of the spectrum used for its determination. For instance, at −120° in n-butyl alcohol, biradical 2 is "100% immobilized" or at −40° biradical 2 is 77% immobilized, while the corresponding monoradical is only "weakly immobilized."

2. Correlation Time Determination. These results on the immobilization ratio can be used to calculate rotational correlation times τ_c.

A. The standard approach is to analyze the esr line shape in order to obtain the relaxation time T_2. For a Lorentzian line shape[29, 36]

$$\frac{1}{T_2} = \frac{D^2}{30\hbar^2}\left(6\tau_c + \frac{10\tau_c}{1 + \omega_0^2\tau_c^2} + \frac{4\tau_c}{1 + 4\omega_0^2\tau_c^2}\right) \quad (1)$$

where $\omega_0 = 2\pi\nu_0$, ν_0 being the resonance frequency.

Assuming that the observed spectrum is the superposition of individual lines of ΔH_i line width (in a 1:2:3:2:1 ratio, ($J \gg a_N = 15$ G)[24]), this individual line width ΔH_i can be obtained by comparison with known spectra[37] for Lorentzian (L) or Gaussian (G) line shape.

Figure 4 gives a plot of computed ΔH_{pp} as a function of ΔH_i for L or G line shape; ΔH_{pp} can be taken as ΔH_i for $\Delta H_{pp} > 40$ G, with an error less than 10%. The experimental curves are found to be the superposition of individual Lorentzian lines for $\Delta H_{pp} < 60$ G and of individual lines which are intermediate between Lorentzian and Gaussian for $\Delta H_{pp} > 60$ G. Using formula 1, τ_c can be calculated for Lorentzian line shape.

According to the Stokes model, τ_c is proportional to η/T

$$\tau_c = V\eta/kT \quad (2)$$

V being an "effective volume" including solvation of the species in solution, but V may be temperature de-

(36) D. Kivelson, "Electron Spin Relaxation in Liquids," L. T. Muus and P. W. Atkins, Ed., Plenum Press, New York, N. Y., 1972.

(37) Ya. S. Lebedev, V. V. Voevodskii, and N. N. Tikhomirova, "Atlas of Electron Spin Resonance Spectra, "Consultants Bureau, New York, N. Y., 1964.
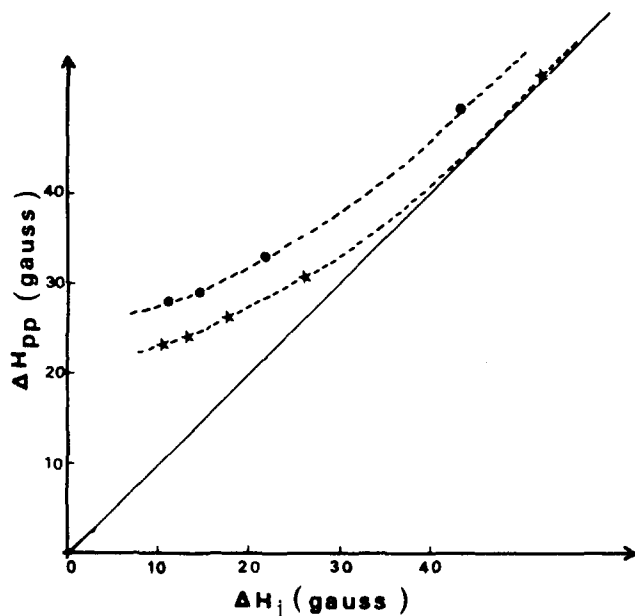
Figure 4. Computed ΔH_{pp} as a function of ΔH_i for (●) Lorentzian (L) or (★) Gaussian (G) line shape.

Figure 5. Variation of τ_c as a function of η/T for biradical 2 in n-butyl alcohol.

Figure 6. Line width of biradical 2 as a function of η/T for (□) ethanol, (●) methanol, (∗) n-butyl alchol, and (☆) n-octyl alcohol.

pendent. Figure 5 gives a plot of τ_c as a function of η/T; a proportionality relationship is verified for 4×10^{-11} sec $< \tau_c < 3 \times 10^{-10}$ sec. The proportionality constant measured on this graph may be used for correlation time calibration in the same solvent.

B. Correlation Time Calibration. Since it has been verified for nitroxide monoradicals that τ_c is proportional to η/T for 2×10^{-8} sec $< \tau_c < 2 \times 10^{-7}$ sec,[15] we shall assume that relation 2 is valid in the whole range of our measurements.

Figure 3 gives the relationship between the "immobilization ratio" and correlation time τ_c by adding $\log V/k = -5.73$ to $\log \eta/T$.

This example shows that biradical spin label may be useful for τ_c determination in the range 4×10^{-11} sec $< \tau_c < 10^{-7}$ sec.

3. Solvation Studies. It is possible to get information on the solvation of biradical 2 with some simplifying assumptions; we have seen that $\Delta H_{pp} \sim \Delta H_i$ for $\Delta H_i > 40$ G; i.e., $\tau_c > \tau_0 = 1 \times 5 \cdot 10^{-10}$ sec. Since the main contribution to line width is dipolar relaxation, $\Delta H_i = k\tau_c$ for $\omega_0^{-1} < \tau_c < D^{-1}$,[36,38] i.e., 10^{-11} sec $< \tau_c < 7 \times 10^{-10}$ sec.

In this range, ΔH_{pp} should be proportional to τ_c. If we now plot ΔH_{pp} (Figure 6) as a function of η/T for the same biradical in different solvents, there is an interval in which ΔH_{pp} has a linear variation in η/T. In this interval, the ratios of the slopes can be taken as the ratios of the variation of the effective volume V, including solvation of this biradical in solution. Figure 6 gives the variation of ΔH_{pp} as a function of η/T for biradical 2 in methanol, ethanol, n-butyl alcohol, and n-octyl alcohol.

(38) R. Kubo, "Fluctuation, Relaxation and Resonance in Magnetic Systems," D. Ter Haar, Ed., Oliver and Boyd, Edinburgh, 1962.
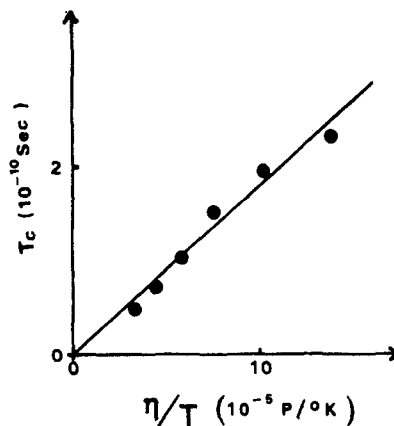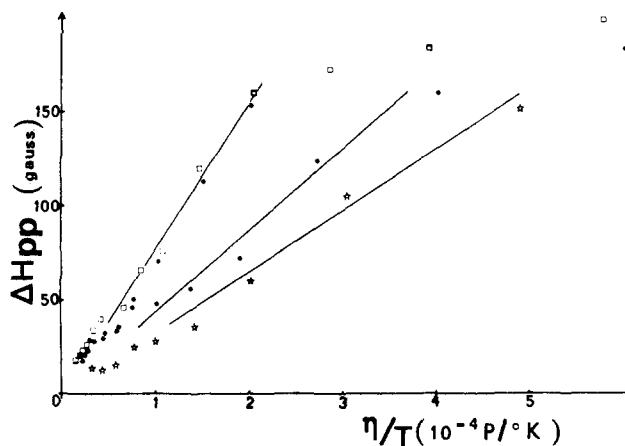
The following effective volumes can be measured.

$$V_{MeOH} \sim V_{EtOH} = 2V_{n\text{-BuOH}} = 2.3V_{n\text{-octOH}}$$

These results are consistent with a solvation equilibrium B + ROH $\leftrightarrows$ (B, ROH) in which the smaller the alcohol ROH the stronger the solvation but the smaller the volume of the H-bonded complex (B, ROH).

Conclusion

1. For a biradical spin label (as well as for monoradicals[35]), the total width of the spectrum gives a measure of its immobilization ratio, as already shown by Norris and Weissman.[30]

2. If the fast tumbling limit is available, the correlation time can be calculated in this case and extrapolated using $\tau_c = k\eta/T$. If not, a calibration must be made for a smaller spin labeled molecule and used to translate immobilizaton ratio into correlation time.

3. For solvation studies, it is not necessary to calculate τ_c since a plot of ΔH_{pp} vs. η/T can give effective volume ratios.