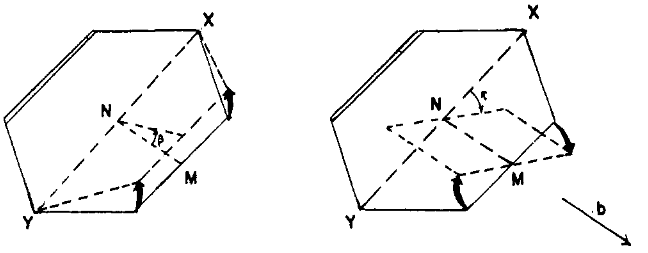

Figure 4. Definition of the ring-bending coordinate β and ring-twisting coordinate τ.[10]

$$J_{a,a} = A[\cos^2(\omega + \phi) + n\cos(\omega + \phi)]$$
$$J_{e,e} = A[\cos^2(\omega - \phi) + n\cos(\omega - \phi)] \quad (2)$$
$$J_{a,e} = A[\cos^2\phi + n\cos\phi]$$

Assuming a tetrahedral H–C–H angle, $\omega = 120°$, the equations yield $A = 8.73$, $n = -0.15$, and $\phi = 55.8°$.

The potential surface shown in Figure 1 was calculated as a function of the bending angle β and the twisting angle τ. These angles are defined in Figure 4. The equilibrium angle of twist for the half-chair form was calculated to be 48.54° from far-infrared data. With the help of geometric relationships this angle can also be computed from the dihedral angle obtained from the proton coupling constants. If tetrahedral angles around the saturated carbon atoms and bond distances of 1.43 and 1.54 Å for the C–O and C–C bonds, respectively, are assumed, the equilibrium angle of twist τ is found to be 39.3°.

The discrepancy of 9° between this value and that obtained from the potential function is not surprising in view of the approximate nature of eq 2, the assumptions about the geometrical parameters needed in the calculation, and the method of obtaining the angle of twist from the potential surface itself. The last is the result of "substantial extrapolation of the potential surface as determined from eigenvalues near the potential minimum"[10] and may be in error by several degrees.

When the present paper was submitted for publication, a referee called to our attention that Bushweller and O'Neil[19] have examined the temperature dependence of the nmr spectrum of $\Delta^{2,3}$-dihydropyran and have estimated a barrier (identified with $\Delta G^{\neq}$) to inversion for the ring of 6.6 ± 0.3 kcal/mol at −140°. The potential surface for this molecule has also been computed from far-infrared data in ref 10. Thus, we have a second example of a barrier height determined by both spectroscopic methods. The far-infrared spectrum and potential surface for $\Delta^{2,3}$-dihydropyran are qualitatively quite similar to those for dioxene. The height of the saddle point above the potential minimum[10] is 6.94 ± 0.7 kcal/mol. Correction for the zero-point energy yields a barrier height above the zero-point plant of 6.31 ± 0.7 kcal/mol.

The results of these two investigations[10,19] cannot be directly compared since only $\Delta G^{\neq}$ was determined rather than ΔE_a in the nmr study. However, if $\Delta G^{\neq}$ for $\Delta^{2,3}$-dihydropyran is compared with the corresponding quantity of 7.32 kcal/mol for dioxene (both at −140°), the difference of 0.7 kcal/mol is in fair agreement with the difference in far-infrared barriers of 1.27 kcal/mol. The disagreement is probably the result of the approximate nature of the treatment of the nmr data for $\Delta^{2,3}$-dihydropyran.

To examine further the question of the measurement of the inversion barrier by far-infrared and nmr methods, we are studying both kinds of spectra for $\Delta^{3,4}$-dihydropyran in collaboration with Professor T. B. Malloy, Jr.

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Internal Rotation and Methyl Proton Magnetic Relaxation for Macromolecules

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Abstract: Two simplified treatments for proton magnetic relaxation for a methyl group on a macromolecule are analyzed. An error in a previous Solomon-type treatment is explained and rectified. Various ways of using T_1 and T_2 values, either separately or in conjunction or at different magnetic fields, are considered. It is concluded that T_1 provides a good approximate estimate of the internal rotation *rate*, which may then be combined with the approximate rotational correlation time of the macromolecule as a whole (based on its molecular weight) to obtain an estimate of the internal rotation *angle* from T_2.

Many attempts to study the flexibility at specific sites on macromolecules from measurement of nuclear relaxation times have been based on use of $-COCH_3$ or $-COCF_3$ groups, on account of their superior signal-to-noise (compared with $-CH$ or $-CH_2$ groups) and lack of scalar coupling (resulting in a single resonance). The experiment typically involves either covalent "labeling" at the desired site[1] or use of a methyl-containing small molecule which binds and exchanges rapidly and reversibly to the macromolecule;[2] in either case, the magnetic relaxation times, T_1 and T_2, for the methyl group attached to the macromolecule are readily extracted from the data.[3] Unfortunately, while a methyl group offers practical advantages in *measurement* of T_1 and T_2, the *interpretation* in terms of gross and internal molecular motion is complicated by two factors peculiar to methyl groups: first, the motion of any one methyl proton is clearly correlated to that of the other two, leading to rather involved expressions for the (sometimes nonexponential) decay;[4] second, if the internal rotation of the methyl group is sufficiently fast, then spin–rotation effects may dominate the relaxation.[5] Based on the recently worked-out detailed relaxation behavior for a methyl group on a macromolecule,[6] we can now critically compare two simplified treatments and establish their respective compromises between rigor and practical utility in obtaining internal motional information from measured methyl relaxation data.

Theory. In principle, the effect of cross correlation between motions of the protons in a given methyl group should be incorporated into calculation of methyl magnetic relaxation rates. Although the resultant relaxation following a radiofrequency pulse can be markedly nonexponential, the *initial* decay of either $\langle M_z \rangle$ or $\langle M_x \rangle$ is well-approximated by the single exponential which is obtained when cross correlations are completely neglected.[6] Thus for practical purposes, one need consider just the relaxation from a two-spin system, where the total relaxation rate for a given proton is the sum of all pairwise interactions: $[1/T_1] = (1/n)\Sigma_{i>j}[2/T_{ij}]$ for n identical spins.[7] There are basically two ways to treat this simpler problem, according to whether the presence of internal rotation is included[8] or not.[9]

Solomon[9] calculated the relaxation rate due to intramolecular dipole–dipole interaction, based on inclusion of all possible transitions between the four energy levels of a system of two spins of one-half. Recently, Nowak and Mildvan[10] tried to extend the Solomon method to the case of three equivalent spins of one-half, by including all possible transitions between the eight levels of the three-spin system (omitting only the triple-quantum transition), and obtained the following equations

$$\frac{1}{T_1} = \frac{3\gamma^4\hbar^2}{10r^6}\left(\frac{\tau_c}{1 + \omega^2\tau_c^2} + \frac{8\tau_c}{1 + 4\omega^2\tau_c^2}\right) \tag{1}$$

$$\frac{1}{T_2} = \frac{9\gamma^4\hbar^2}{20r^6}\left(2\tau_c + \frac{3\tau_c}{1 + \omega^2\tau_c^2} + \frac{\tau_c}{1 + 4\omega^2\tau_c^2}\right) \tag{2}$$

where γ is the proton magnetogyric ratio, r is the proton–proton distance in a methyl group (about 1.8 Å), ω is the proton Larmor frequency, and $\tau_c = (1/6D)$ is the rotational correlation time in terms of the (single) rotational diffusion constant for a sphere. While the algebra leading to eq 1 and 2 is correct, the set-up is not; Solomon's analysis was based on a two-spin system and its extension to a three-spin system is by no means obvious.[11] The most general approach is to solve the three-spin system, including the effect of cross correlation, and then take the limit that cross correlation goes to zero; the correct results then take the form

$$\frac{1}{T_1} = \frac{3\gamma^4\hbar^2}{10r^6}\left(\frac{2\tau_c}{1 + \omega^2\tau_c^2} + \frac{8\tau_c}{1 + 4\omega^2\tau_c^2}\right) \tag{3}$$

$$\frac{1}{T_2} = \frac{3\gamma^4\hbar^2}{10r^6}\left(3\tau_c + \frac{5\tau_c}{1 + \omega^2\tau_c^2} + \frac{2\tau_c}{1 + 4\omega^2\tau_c^2}\right) \tag{4}$$

It may be noted that the relaxation rates (eq 3 and 4) are exactly twice those obtained for a two-spin system, in agreement with intuition.[12] By "counting" only certain of the possible transitions, it is possible to obtain eq 3 and 4 (the error in ref 9 was in "counting" all the transitions), but this approach is not readily generalized and is certainly more complicated than simply considering an "uncorrelated" Hamiltonian, $\mathcal{H} = \mathcal{H}_{12} + \mathcal{H}_{13} + \mathcal{H}_{23}$. It is essential to recognize that eq 3 and 4 are valid only for a methyl group rigidly attached to a spherical molecule (see Discussion).

Explicit inclusion of internal rotation for molecules in liquids derives from basic work by Woessner.[8] Expressions for T_1 and T_2 may be derived directly from the correlation function in ref 13, and although the equations are lengthy (eq 5 and 6), only two additional

$$\begin{aligned}\frac{1}{T_1} = \frac{3\gamma^4\hbar^2}{20r^6}\Bigg[&\frac{3\sin^4\theta\tau_2}{1 + \omega^2\tau_2^2} + \frac{12\sin^2\theta\cos^2\theta\tau_1}{1 + \omega^2\tau_1^2} + \\ &\frac{4[(3\cos^2\theta - 1)/2]^2\tau_0}{1 + \omega^2\tau_0^2} + \frac{12\sin^4\theta\tau_2}{1 + 4\omega^2\tau_2^2} + \\ &\frac{48\sin^2\theta\cos^2\theta\tau_1}{1 + 4\omega^2\tau_1^2} + \frac{16[(3\cos^2\theta - 1)/2]^2\tau_0}{1 + 4\omega^2\tau_0^2}\Bigg]\end{aligned} \tag{5}$$

$$\begin{aligned}\frac{1}{T_2} = \frac{3\gamma^4\hbar^2}{20r^6}\Bigg[&(9/2)\sin^4\theta\tau_2 + 18\sin^2\theta\cos^2\theta\tau_1 + \\ &6[(3\cos^2\theta - 1)/2]^2\tau_0 + \frac{(15/2)\sin^4\theta\tau_2}{\omega^2\tau_2^2} + \\ &\frac{30\sin^2\theta\cos^2\theta\tau_1}{1 + \omega^2\tau_1^2} + \frac{10[(3\cos^2\theta - 1)/2]^2\tau_0}{1 + \omega^2\tau_0^2} + \\ &\frac{3\sin^4\theta\tau_2}{1 + 4\omega^2\tau_2^2} + \frac{12\sin^2\theta\cos^2\theta\tau_1}{1 + 4\omega^2\tau_1^2} + \\ &\frac{4[(3\cos^2\theta - 1)/2]^2\tau_0}{1 + 4\omega^2\tau_0^2}\Bigg]\end{aligned} \tag{6}$$

parameters appear: θ, the angle between the proton–proton axis and an axis about which internal rotation occurs ($\theta = 90°$ for internal rotation of a methyl group about the R–CH_3 axis), and D_{int}, the diffusion constant

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(12) Naive extension of Solomon's method (counting all the transitions involving two quanta or less) to n identical spins leads to $(1/T_1) = 2[W_1 - (n + 1)W_2]$, so that for large n the relaxation would depend mainly on the double-quantum transitions, again contrary to intuition.

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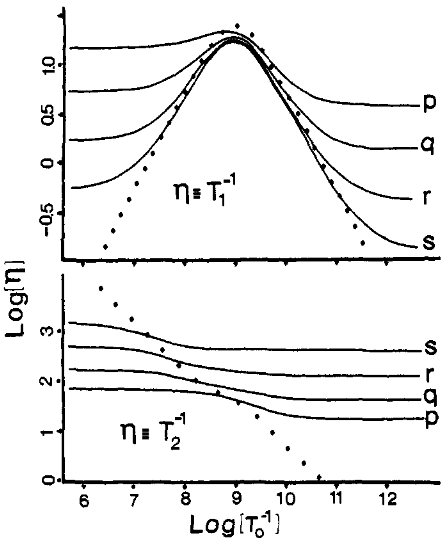

Figure 1. Plots of proton $1/T_1$ (top graph) or $1/T_2$ (bottom graph) *vs.* reciprocal rotational correlation time for a methyl group on a macromolecule. Broken curves are obtained from eq 3 (top graph) and eq 4 (bottom graph); the x axis corresponds to $1/\tau_0$ in those equations. For the solid curves, eq 5 (top graph) and eq 6 (bottom graph) yield separate lines (p, q, r, s) for macromolecules of respective τ_{macro} = 3.16, 10, 31.6, and 100 nsec; the x axis for the solid curves is the value of $1/\tau_{\mathrm{int}} = 4D_{\mathrm{int}}$ for rotation of the methyl group about the R-CH$_3$ axis. In all plots, $\omega = 2\pi \times 10^8$ sec^{-1}, r = 1.78 Å.

for diffusional internal rotation, where $\tau_0 = (1/6D_{\mathrm{macro}})$; $\tau_1 = [1/(6D_{\mathrm{macro}} + D_{\mathrm{int}})]$; $\tau_2 = [1/(6D_{\mathrm{macro}} + 4D_{\mathrm{int}})]$. These results are most readily understood from graphical display.

Discussion

Figure 1 shows the behavior of the two motional models treated in the Theory in terms of their effect on T_1 or T_2. The simplest model is to try to fit the experimental T_1 or T_2 data with a single "effective" rotational correlation time, using eq 3 and 4 (these results are shown as the broken lines in the two graphs). The solid lines in each graph correspond to the more detailed model (eq 5 and 6) in which internal rotation rate and angle appears explicity (different solid lines correspond to molecules of different size, as indicated).

The most interesting feature of the T_1 curves is that for macromolecules of molecular weight $\geq$30,000 (curves r and s) the (single, "effective") correlation time obtained from eq 3 is a good approximation to the actual correlation time for internal rotation, $\tau_{\mathrm{int}} = (1/4D_{\mathrm{int}})$. However, for a given experimental T_1 value, there are in general two possible "effective" correlation times. But an increase in magnetic field will effectively shift the left-hand portion of the $1/T_1$ curves to the right, while leaving the right-hand portion fixed; thus by observing whether the experimental $1/T_1$ decreases or stays the same on increasing the magnetic field, one could decide whether the longer or shorter (respectively) effective correlation time were the correct one. Finally, it may be noted from the figure that the usual idea that faster internal rotation makes relaxation *less* efficient, though valid for T_2, is valid for T_1 only when $6D_{\mathrm{macro}} \gg 2\omega$; internal rotation clearly makes T_1 relaxation *more* efficient in the regions of positive slope of the upper graph of Figure 1.[14]

An immediate important feature of the T_2 curves is that the simplified model (eq 4) gives a misleading picture; for a given macromolecular size, the figure clearly shows that the "effective" correlation time deduced from the broken line will never deviate very much from the rotational correlation time of the macromolecule itself, in contrast to the T_1 result above. This demonstrates that T_1 and T_2 data alone lead to quite different "effective" correlation times and suggests that treatment of $[T_1/T_2]$ ratios according to eq 3 and 4 is likely to give a confused picture of the actual situation. Secondly, it has been suggested that an estimate of the degree of internal flexibility might be obtained by comparing the T_2's at two different magnetic fields,[10] since a change in magnetic field will shift the $1/T_2$ curves with respect to the x axis. However, numerical calculations for a methyl group quickly show that the ratio T_2(220 MHz)/T_2(100 MHz) shows a total range of variation of between 1.00 and 1.52 and is double valued, as may be seen qualitatively from consideration of Figure 1. Since the experimental uncertaintly in this T_2 ratio is likely to be 0.2, this method is too insensitive to be of use. The final interesting feature of the T_2 results is that the ratio of $1/T_2$ in the *presence* of internal rotation to $1/T_2$ in the *absence* of internal rotation appears to be approximately $[(3\cos^2\theta - 1)/2]^2$ for macromolecules of *any* size (the statement is rigorously true for very large or very small molecules). Thus if τ_{macro} and τ_{int} are known, the appropriate solid line and known x value for the bottom graph in Figure 1 can be used to find the angle at which internal rotation occurs.

Conclusions

If T_1 for a methyl group on a macromolecule is measured, eq 3 may be used to obtain a good estimate of the *rate* of internal rotation of that methyl group. From the molecular weight of the macromolecule and the approximate corresponding rotational correlation time for reorientation of the large molecule as a whole [calculated from the relation τ_{macro} = (molecular weight in 1000's) nsec], the graphical display from eq 6 shown in Figure 1 may be used to obtain the average *angle* at which the internal rotation occurs, using T_2 measurement and the internal rotation rate calculated from T_1.

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