Spin–Lattice Relaxation of Methyl Protons in Some Compounds of Biological Interest

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Abstract: Proton magnetic relaxation rates of the N-methyl protons of a series of aliphatic ammonium salts in D_2O have been determined by rapid adiabatic passage. Theoretical relaxation rates have been calculated taking into account both asphericity and group rotation around bond axes. Excellent agreement was obtained with calculations which excluded contributions from group rotation. In methoxy and acetoxy derivatives, on the other hand, the relaxation rate of methyl protons in these groups was much lower than predicted on the basis of a rigid model and indicates relatively free group rotation.

Considerable attention has been paid to measurements of the spin–lattice relaxation times of protons in liquid organic compounds and in solutions because of the need for greater understanding of the nature of liquids and solutions. There is a general agreement that provided paramagnetic molecules are absent, relaxation occurs as a result of dipolar interactions the magnitude of which is determined by molecular motion. In the original theory of Bloembergen, Purcell, and Pound,[2] the Stokes frictional coefficients for rotation and translation were used, but a number of authors have noted that the values of the spin–lattice relaxation rate so obtained are too large. Much better agreement has been obtained when the microviscosity coefficients of Gierer and Wirtz[3] which are derived from the exchange theory of diffusion in liquids have been used. The application of this theory for pure liquids and for solutions in which solute and solvent are of comparable size and spherically symmetrical is straightforward. The theory is probably less satisfactory when these conditions are not fulfilled. Pendred, Pritchard, and Richards[4] have found this simple theory, using a fixed microviscosity factor, to give remarkably good predictions of relaxation rates for a wide range of organic molecules.

We have been interested in the molecular motion exhibited by drugs in solution and in particular the group of aliphatic quaternary ammonium compounds which are of prime importance in investigating the pharmacology of the acetylcholine receptor. We are particularly concerned with the question of whether the molecular motion in these compounds relevant to relaxation was tumbling of the whole molecule or whether group rotation about bond axes made an important contribution. This is a question that needs at least a semiquantitative answer before the restriction of molecular motion which occurs in complexes with enzymes, antibodies, or drug receptors can be confidently interpreted. In addition, since many of these compounds depart seriously from sphericity they raise the problem of how far this modifies molecular motion and hence the relaxation rate.

In previous investigations[5,6] we used spin–spin relaxation data obtained from line width broadening to explore drug binding and membrane stabilization. This method is limited, however, insofar as longer T_2 times cannot be determined with accuracy and is also complicated by the uncertainty introduced by line splitting due to spin–spin interaction with ^{14}N in the case of ammonium methyl groups. In the present experiments T_1 has been determined by the adiabatic rapid passage method.[7–9]

Experimental Section

The adiabatic rapid passage method has been used for measurement of T_1. The method is based on the reversal of total nuclear magnetization caused by rapidly sweeping the resonance line "in phase" (*i.e.*, in dispersion mode). The conditions for a complete reversal of magnetization for free spins is

$$\frac{H_1}{T_1} \ll \frac{dH_0}{dt} \ll |\gamma| H_1^2$$

The transverse magnetization will be positive after the passage and negative before, being parallel or antiparallel to H_1.

For relatively long relaxation times the ratio of signal amplitudes is measured in two consecutive fast passages separated by a time interval t, starting from opposite sides of the resonance in a highly saturated field. If time t is short compared to T_1 the second signal will have the same sign as the first. If $t \gg T_1$ the second signal will equal in magnitude but be opposite in sign to the first. The method is similar in principle to that using free precession signals following pulses at 180 and 90°.

The spin–lattice relaxation times were measured on a Varian A-60A nmr spectrometer which had been slightly modified. The internal radiofrequency attenuator was replaced by a step attenuator (Kay Electric Co., Pine Brook, N. J., Model 32-0) and the 0.02-cps filter capacitor (C 824, 1 F) was replaced by one (2200 pF) giving a 10-cps band width, which was used in all measurements. By offsetting the variable-tuning capacitor (NPO 3-12) in the probe the signal was adjusted for the dispersion mode. The positive and negative phases obtained in slow passage were carefully equalized with the detector phase control. With a sweep width of 1000 cps, use of the fast sweep key permits rapid passage at 100

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(2) N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, 73, 679 (1948).
(3) A. Gierer and K. Wirtz, *Z. Naturforsch.*, 8a, 532 (1953).
(4) T. L. Pendred, A. M. Pritchard, and R. C. Richards, *J. Chem. Soc., A*, 1009 (1966).
(5) A. S. V. Burgen, O. Jardetzky, J. C. Metcalfe, and N. G. Wade-Jardetzky, *Proc. Natl. Acad. Sci. U. S.*, 58, 447 (1967).
(6) J. C. Metcalfe, A. S. V. Burgen, and O. Jardetzky in "Molecular Associations in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1968, p 287.
(7) G. Chiarotti, G. Christiani, and L. Giuliotto, *Nuovo Cim.*, [10] 1, 863 (1955).
(8) A. Abragam. "Principles of Nuclear Magnetism," Clarendon Press, Oxford, 1961.
(9) J. G. Powles, *Ber. Bunsenges. Physik. Chem.*, 67, 238 (1963).

cps/sec. Radiofrequency power was at a level sufficient (0.5 *W*) to make the rapid passage signal monophasic.

The signals were recorded on a "Devices" M2R pen recorder driven by a DC6 preamplifier connected to the signal output jack of the A-60A spectrometer.

Sample Preparation. The compounds used were either commercial products purified before use or were prepared by standard synthetic methods. The salts were bromides and in some cases iodides, but there was no evidence that the nature of the anion affected the results. Monoquaternary compounds were measured in a concentration of 250 m*M* in 99.85% D_2O except for *n*-octyltrimethylammonium iodide, where a concentration of 100 m*M* was used to avoid micelle formation. Where single methyl proton signals were measured the concentration was raised to 0.5 or 1.0 *M*.

Since the removal of dissolved paramagnetic oxygen is of extreme importance, the samples (0.5 ml) were degassed by the usual freeze–thaw technique under vacuum, saturated with nitrogen, and kept in a sealed tube.

Calculation of T_1. The maximum amplitudes of two consecutive rapid passages were read from the recorder chart (within ±0.3%) and their difference was substracted from the asymptote value. This in turn could be determined from the difference of average amplitudes of the first passage in a pair of determinations swept in opposite directions. The half-times were read from a semilogarithmic plot against time elapsed between passages. From this $T_1 = t_{1/2}/\ln 2$.

Viscosities were determined in an Ostwald viscometer at 25 ± 0.2° and the viscosities relative to water used to correct the T_1 values. The T_1 values were corrected to infinite dilution in water.

The relaxation times were all measured at 25 ± 0.2° and are mean values of at least four determinations swept alternately in opposite directions. To test the reliability of the method, T_1 times of liquids were determined and compared with values in the literature[4,7] (see Table I).

Table I[a]

	Found	Lit.[4,7]
Dioxane	4.90	5.0
Mesitylene		
methyl	4.90	5.0
phenyl	9.33	10.0
Nitromethane	12.20	12.7
Dichloromethane	19.40	19.7

[a] The standard deviation is 4 abs. %.

Results and Discussion

Ammonium Salts. The values of T_1^{-1} found experimentally for the N-methyl protons of a series of ammonium salts are listed in Table II.

It is obvious that there is a rather steep increase in the relaxation rate with molecular size. This is to be expected if relaxation is dependent on the rate of tumbling of the molecules, whereas if relaxation were mainly due to rotation of the methyl groups around their bond axes it would be expected that relaxation rates ought to be practically independent of the molecular size.

A more precise evaluation of these contributions is possible if relaxation rates are calculated on the assumption that the molecules behave as rigid rotators in solution. Since the experiments have been carried out in dilute solution, the contribution of translational motion may be ignored.

$$T_1^{-1} = {}^3/_2\gamma^4\hbar^2\sum b_{ij}^{-6}\tau_{c_{rot.}}$$

In turn $c_{rot.}$ is obtained from the Stokes–Einstein equation corrected by the microfrictional factor of

Table II. Relaxation Rates of N-Methyl Protons

R	Axial ratio *b/a*	Asphericity corrn	T_1^{-1}, sec^{-1} Exptl	Calcd tumb	Calcd rot.
A. Quaternary Amines, $RN^+(CH_3)_3$					
Methyl	1.00	1.00	0.088	0.203	0.078
Ethyl	0.85	0.96	0.174	0.252	0.083
n-Propyl	0.72	0.90	0.302	0.312	0.088
n-Butyl	0.64	0.85	0.404	0.375	0.093
n-Pentyl	0.57	0.80	0.539	0.450	0.097
n-Hexyl	0.52	0.75	0.595	0.528	0.099
n-Heptyl	0.47	0.71	0.637	0.610	0.102
n-Octyl	0.43	0.67	0.741	0.705	0.105
Isopropyl	0.85	0.96	0.187	0.292	0.086
t-Butyl	0.85	0.96	0.182	0.332	0.090
Cyclohexyl	0.64	0.85	0.427	0.460	0.097
Phenyl	0.63	0.84	0.351	0.452	0.096
Benzyl	0.54	0.77	0.702	0.542	0.100
B. Bis Quaternary Amines, $(CH_3)_3N^+RN^+(CH_3)_3$					
Ethylene	0.52	0.75	0.604	0.536	0.100
Pentamethylene	0.39	0.62	0.849	0.820	0.107
Decamethylene	0.29	0.51	0.987	1.390	0.113
Diethylsuccinyl	0.29	0.45	0.728	1.770	0.115
C. Tertiary Amines, $RN^+D(CH_3)_2$					
Methyl	1.22	1.05	0.123	0.153	
Ethyl	0.93	0.99	0.200	0.202	
n-Butyl	0.57	0.88	0.338	0.338	
n-Hexyl	0.46	0.70	0.492	0.498	
n-Octyl	0.39	0.63	0.581	0.671	
Isopropyl	0.85	0.96	0.310	0.247	
t-Butyl	0.85	0.96	0.232	0.285	
D. Miscellaneous					
Dimethylammonium	0.68	0.88	0.108	0.127	
Methylammonium	0.72	0.90	0.094	0.084	

Gierer and Wirtz[3]

$$c_{rot.} = \frac{4\pi\eta r^3 f_r}{3kT}$$

where *r* is the van der Waal's radius.

The value of f_r is $^1/_6$ for a pure liquid in which site exchange is between molecules of the same size. Because of the difficulties in defining in associated solvent the dimensions of the molecules with which exchange is occurring, we assumed as a working hypothesis that $f_r = {}^1/_6$ is adequate in this situation too.

Calculation of T_1^{-1} in this way provides the right order of result but underestimates the increase in T_1^{-1} with increase in chain length.

The calculations so far have assumed that the molecules under consideration can be considered to be spherical and hence have equal rotational frequencies along all axes. However the real axial ratios for the long chain molecules depart significantly from unity and the molecules are more properly generalized as ellipsoids. The appropriate correction for the mean rotation frequency of an ellipsoid[10] is

$$F_{rel} = \frac{\bar{\tau}_{sphere}}{\tau_{ellipsoid}} = \frac{\frac{r^2(7-2r^2)}{4(1-r^2)^{1/2}}\ln\left(\frac{1+(1-r)^2}{r}\right) + \frac{1-4r^2}{4}}{(1-r^4)}$$

where $r = b/a$, the ratio of minor/major semiaxes of a prolate ellipsoid.

(10) F. Perrin, *J. Phys. Radium*, 5, 497 (1934).

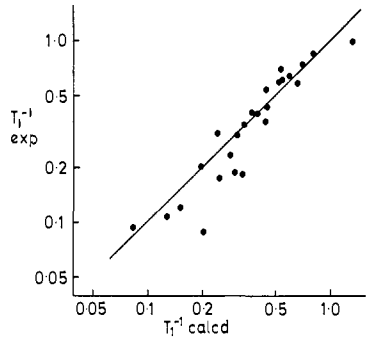

Figure 1. Correlation between experimentally determined values of T_1^{-1} for the substances listed in Table II (excepting succinylcholine) and T_1^{-1} calculated for the molecules. In calculating T_1^{-1} it has been assumed that rotation about C–C and C–N bonds does not contribute significantly to the molecular motion relevant to relaxation.

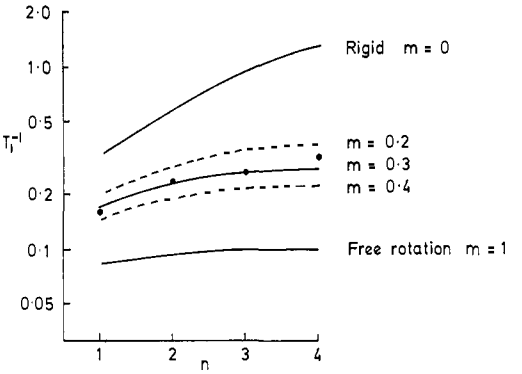

Figure 2. Spin–lattice relaxation rates for the methyl groups of the glycol dimethyl ethers $CH_3O(CH_2CH_2O)_nCH_3$ in dilute solution. The lines are calculated for (a) rotation of the molecule as a rigid unit ($m = 0$), (b) free rotation of methyl groups about the C–O axis ($m = 1$), and (c) intermediate levels of freedom ($m = 0.2$, 0.3, and 0.4).

In calculating the correction for asphericity, it has been assumed that the relevant conformation of the hydrocarbon chain is the extended one. The calculated values of T_1^{-1} are seen in Table II. In Figure 1 the correlation between calculated and experimental values is shown. The agreement for most compounds is good. The main deviations are for tetramethylammonium and the related ethyl, isopropyl, and *t*-butyltrimethylammoniums. In all these cases the experimental values are considerably smaller than the calculated values.

Relaxation rates have also been calculated assuming that free rotation occurs around the C–N bond axes. We will assume that the frequency of rotation of a methyl group follows the molecular dependence of the Stokes–Einstein equation, but since motion is restricted to the plane of the bond axis, this must be divided by three to give the frequency averaged over all orientations. The over-all rotational frequency relevant to relaxation is to a reasonable approximation the sum of the molecular and group rotational frequencies. Thus

$$f_{\text{total}} = f_{\text{molecule}} + mf_{\text{group}}$$

where m is the amount of rotational freedom along the bond axis and takes the value of 1 if rotation is completely unhindered. Relaxation rates calculated for completely free rotation are shown in Table II. It is obvious that for the higher molecular weight compounds in particular the calculated values of T_1^{-1} are far too small.

Since the trimethylammonium group is common to all the compounds in the first two groups, and we would not expect the C–N bond rotational freedom within this group to vary much, we may use the largest discrepancy between the experimental value and that calculated for free rotation (*i.e.*, in the larger molecular weight members) to obtain an estimate of the maximum degree of rotational freedom in these bonds. We find that m is <0.05. Applying the usual Boltzmann considerations, we may take the energy barrier to rotation, $\Delta F \geqslant RT \ln 1/m$, and thus for $m < 0.05$, $F > 1.8$ kcal/mol. Estimates of rotational barriers around C–C bonds mostly obtained by microwave spectroscopy in the gas phase have been 3–4 kcal/mol, and for the C–N bond in methylamine 1.9 kcal/mol.[11–13]

In two compounds, isopropyl- and *t*-butyltrimethylammoniums, the relaxation rate of the side-chain methyl protons was also examined and was found to be the same as that of the N-methyl protons. This is again what is expected for tumbling of the whole molecule. We can conclude therefore that rotation around bond axes makes no significant contribution to the relaxation of the molecules listed in Table II.

The energy barrier around –O– bonds is generally smaller than that around tetrahedral carbons owing to the smaller number of bonding orbitals. It seemed likely therefore that methoxy and acetoxy groups might show evidence of contribution from group rotation to relaxation. We have found evidence that this is the case in two groups of compounds, namely glycol methyl ethers and amino alcohol ethers and esters.

Glycol Ethers. We have examined the relaxation rates in solution in D_2O of the methyl protons in the dimethyl ethers of mono-, di-, tri-, and tetraethylene glycol (Table III).

Table III. Relaxation Rates of Methoxy Protons in Glycol Methyl Ethers $CH_3(-OCH_2CH_2-)_nOCH_3$

		Aspher-	T_1^{-1}, sec^{-1}		
n	Axial ratio	icity corrn	Exptl	Calcd tumb	Calcd rot., $m = 1$
1	0.43	0.67	0.158	0.327	0.082
2	0.32	0.54	0.232	0.604	0.093
3	0.25	0.46	0.261	0.945	0.098
4	0.21	0.41	0.320	1.320	0.100

It can be seen immediately that the increase in relaxation rate with molecular size is much less steep than was the case with the amines. Furthermore, the experimental values of T_1^{-1} are much smaller than those calculated for tumbling of a rigid molecule although larger than expected for completely free motion of the methyl groups. The data fit an average value of $m = 0.3$ (Figure 2) which corresponds to an energy barrier of 0.66 kcal/mol. This is of the expected order.

(11) E. B. Wilson, *Proc. Natl. Acad. Sci. U. S.*, 43, 816 (1957).
(12) D. R. Lide, *J. Chem. Phys.*, 22, 1613 (1954).
(13) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, pp 130–136.

Table IV. Relaxation Rates of Different Methyl Protons in Polyfunctional Amines

Compound	Axial ratio b/a	Asphericity corrn	T_1^{-1}, sec^{-1}: NCH_3 exptl	NCH_3 calcd tumb	CCH_3 exptl	CCH_3 calcd tumb	OCH_3 $AcCH_3$ exptl	OCH_3 $AcCH_3$ calcd tumb	OCH_3 $AcCH_3$ calcd rot.
2-Methoxyethyltrimethylammonium	0.65	0.86	0.330	0.375			0.196	0.335	0.082
3-Methoxypropyltrimethylammonium	0.59	0.82	0.465	0.432			0.212	0.384	0.085
Acetylcholine	0.51	0.74	0.487	0.542			0.293	0.480	0.190
Methacholine	0.48	0.72	0.746	0.608	0.565	0.546	0.305	0.540	0.198

Amino Alcohol Ethers and Esters. In Table IV values for T_1^{-1} of N-methyl and other methyl protons are shown for 2-methoxyethyltrimethylammonium, 3-methoxypropyltrimethylammonium, acetylcholine, and methacholine (acetyl-β-methylcholine). It can be seen that for all these compounds the relaxation rates of the N-methyl protons are reasonably in accord with the prediction based on tumbling of the rigid molecule, as is that of the β-(C)Me of methacholine. However, the relaxation rates of the methoxy and acetyl protons are much below the predicted values. Calculated values of m for the two ethers gave 0.23 for each, therefore of the same order as in the glycol ethers. For the esters it was assumed that the acetyl group rotates as a unit about the ester oxygen. The values of $m = 0.42$ and 0.45, respectively, were found.

In these four molecules one therefore has the interesting conjunction of N-methyl groups that are sufficiently immobilized, so that their relaxation depends on molecular tumbling, and methoxy and acetoxy groups with enough freedom for axial rotation to be a major contribution to relaxation.

Activation Energies. In the case of nine of the compounds measurements of T_1 were carried out at 10, 20, 25, 30, 40, and 50° and Arrhenius plots made. In all cases the data showed a linear regression between ln T_1 and $1/T$. The results are presented in Table V where it can be seen that in six of the nine compounds the activation energy of relaxation of the N-methyl protons fell in the range 3.22–4.60 kcal/mol. This is similar to the activation energy of viscosity of D_2O (3.94 kcal/mol) and is consistent with the molecular motion of the solute being essentially controlled by the motion of the solvent. However, in both tetramethylammonium and ethyltrimethylammonium the activation energy is about 1 kcal/mol smaller. It will be recalled that these were the molecules showing considerably smaller relaxation rates than predicted theoretically. The two pieces of evidence agree in showing that these two molecules can rotate with anomalous facility in solution. It seems likely that because of their nearly spherical shape rotation involves minimal displacement of solvent molecules and hence is only partially restricted by the solvent viscosity.

Table V. Activation Energies of Relaxation (in kcal/mol)

	NCH_3	CCH_3	OCH_3/ $AcCH_3$
Tetramethylammonium	2.70		
Ethyltrimethylammonium	2.84		
n-Propyltrimethylammonium	3.22		
n-Butyltrimethylammonium	4.20		
n-Hexyltrimethylammonium	4.60		
2-Methoxyethyltrimethylammonium	4.16		3.08
Methacholine	4.12	3.36	2.53
Trimethylammonium	3.65		
n-Butyldimethylammonium	3.88		
Activation energy of viscosity for D_2O	3.94		

In methoxyethyltrimethylammonium the activation energy of relaxation of the OMe is also low, as is that of the acetoxy group of methacholine. This suggests that extra facility of rotation also pertains to these groups and that the degree of bond mobility calculated earlier is an overestimate.

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