CARBON-13 MAGNETIC RESONANCE IN STUDIES OF MOLECULAR DYNAMICS AND STRUCTURE

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ABSTRACT

The application of computer technology in carbon-13 magnetic resonance spectroscopy has resulted in much improved signal detection through Fourier transform and time averaging techniques. These advances have compensated for the less favourable gyromagnetic ratio and relatively low (1.1 per cent) natural abundance of the carbon-13 isotope, and carbon-13 magnetic resonance methodology may now be employed in a variety of chemical studies. Two very different applications are reviewed in this paper to illustrate the diversity of the method.

Aside from the privilege of participating in this excellent international conference it is of considerable pleasure to have the conference here in Tallinn which ranks as one of the major carbon-13 magnetic resonance research centres in the world. For many years E. T. Lippmaa of the Estonian Academy of Sciences has pioneered in the study of the magnetic properties of this important isotope of carbon. Ten years ago, carbon-13 spectra were obtained only with tedious and tenacious effort. This resulted primarily from the relatively low isotopic abundance (1.1 per cent) of the carbon-13 isotope. In addition, the magnetogyric ratio of carbon is such as to reduce by 63-fold the relative sensitivity of detecting an equal number of carbon-13 nuclei compared with the more common proton whose magnetic properties are well known. These two factors combine to give a 5700-fold decrease in detectability for carbon-13 as compared with hydrogen, and thus the experimental problems associated with this area of magnetic resonance have obviously been severe. Dr Lippmaa and his associates were among those who faced this challenge very early and have contributed greatly to the resolution of the problem.

Here the limitations of the method end as carbon, which appears as the fundamental atomic building block of organic chemistry, offers a much more sensitive magnetic probe for studying certain molecular properties of organic systems. The greatest benefits of the technique have come from studies dealing with chemical shielding parameters and with nuclear spin lattice relaxation times. These two areas yield valuable information on two significant but somewhat unrelated aspects of organic or biological molecules. Shielding data for carbon-13 have been especially useful in studies of molecular and electronic structure. With a total shielding range which is about 50 times

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greater than that found for protons, carbon-13 data offer a much more powerful and descriminating technique for characterizing minor structural variations. Furthermore, as the carbon nucleus is seldom at the periphery of a molecule, intermolecular shielding effects tend to be minimal. Also, shielding fields arising from electronic currents in remote molecular moieties will affect all magnetic nuclei to the same extent and as such, the several ppm shifts observed for this effect in protons sets the upper limit of remote field effects at less than 1 per cent of the total range in carbon shifts. Thus, it turns out for the above reasons and a few others for which there is not time to discuss, that carbon-13 chemical shift data more faithfully reflect a given structural feature which may appear in a variety of different molecules than the other more common magnetic nuclei. Linear additive relationships have been observed to exist for substitiuents and special structural features, and these provide a powerful basis for characterizing the molecular structure of new compounds.

Unlike the shielding parameter the carbon-13 nuclear relaxation rate provides information on the dynamical properties of a molecule. Rotational diffusion, segmental motion and internal rotation or torsional vibrations in a large variety of molecules all provide temporal modulation of magnetic fields associated with the molecular system, and these fluctuating fields lead to nuclear spin relaxation. The principal random magnetic field which has sufficient magnitude and proper frequency dependence to dominate most carbon relaxation processes arises from coupling between pairs of nuclear magnetic dipoles, and is referred to as dipolar or dipole–dipole relaxation. In some smaller molecules the presence of local magnetic fields due to moving electrons in rapidly rotating molecules can couple to the nuclear magnetic moments and also contribute significantly to spin relaxation. This process is the spin-rotation mechanism. The two relaxation processes are characterized by different correlation times, the characteristic time associated with the random fluctuating fields. Both mechanisms reveal important, but different details of the dynamical processes involved in the liquid state. The correlation time governing dipole–dipole relaxation measures a characteristic time for the molecule to undergo reorientation relative to the static magnetic field. This reorientation results from overall molecular diffusion or various other types of internal motion of the molecule. The spin-rotation correlation time, on the other hand, provides a measure of the time of motional persistency as opposed to a reorientation period. In liquids, it may be thought of as the time between intermolecular interactions which interrupt a persistent mode of molecular rotation; either of an overall or segmental type. These two correlation times together provide details of molecular motion in liquid samples of a specific character not always obtainable by other techniques. This is especially the case when several correlation times are appropriate to describe the motion about the principal axes of a rigid molecule which is involved in *anisotropic* molecular diffusion. We shall return to examples of this later in this paper.

The experimental developments required to overcome the sensitivity problems intrinsic to carbon-13 magnetic resonance spectroscopy could in themselves consume the whole time allotted to this talk. I will, therefore, enumerate only the highlights. One of the earliest developments came with

the use of proton decoupling to eliminate the very large number of proton splittings common in even the simplest organic molecules. Even in benzene which has a single proton line in its spectrum, Roberts and Weigert have observed more than two dozen lines in the carbon-13 spectrum arising from proton splittings. While elimination of the proton splitting constituted an extensive loss of information, it was a necessary sacrifice in order to obtain single sweep spectra. The chemical shift is easily obtained from the proton decoupled singlets observed in these spectra. An additional benefit was realized with proton decoupling in that the signal was enhanced by almost three-fold in most carbons bearing directly bond protons. This enhancement due to a nuclear Overhauser effect arises only when the proton–carbon dipolar relaxation mechanism is a significant part of spin relaxation. Thus, the nuclear Overhauser enhancement (NOE) factor becomes a valuable parameter for separating out quantitatively the contribution of the dipolar mechanism in the overall T_1 value. Altogether, proton decoupling methods have gained a factor of 6- to 50-fold for us in signal to noise. This depends, of course, on the extent of proton splittings in the signal. The construction of field-frequency lock spectrometers offered the next significant advance as these methods permitted time averaging techniques to be employed. The eventual use of pulse techniques along with minicomputers employing Fourier transform methods have further extended signal to noise by a factor approaching 100. Thus, routine spectra of reasonably simple molecules can now be secured in a few minutes, while spectra of fairly complex biological systems can even be obtained provided several hours are dedicated to spectral acquisition. The spectra used in this paper are all of the proton decoupled variety where each carbon-13 is observed as a single resonance peak. The chemical shift of a given carbon may then be measured directly from its line position.

We now present a typical study of molecular conformation and structure using the cyclic alkanes to illustrate the type of information which can be derived from carbon-13 chemical shift data. It has been observed that the range in chemical shifts for the methyl cyclohexanes alone covers a span of about 35 ppm. At first glance, there appears to be little order or system in the 100 or so odd chemical shifts available for this class of compounds, and one has great difficulty assigning the many resonance lines. In a few instances the symmetry of the molecule is such that some of the peaks can be assigned on the basis of their intensities. For instance the peak of greatest intensity in trans-1,4-dimethylcyclohexane is assignable to the 2, 3, 4 and 6 carbons. This example is, of course, typical of some of the other molecules where intensities may be used to unequivocally assign the peaks, but the great majority of lines cannot be assigned in this manner. Using partial or off-resonance proton decoupling allows one to differentiate between carbons bearing one, two or three directly bonded protons as residual splittings appear in the resonances of each carbon. This will increase the number of definite assignments, but still many ambiguities are left. Recognizing that the methylcyclohexanes fall into at least three general categories is necessary if one is to employ linear, additive substituent parameters for sorting out the remaining chemical shifts. There are those methyl cyclohexanes which may exist equally in one of two chair conformations each of the same energy
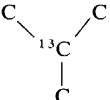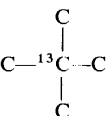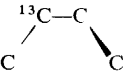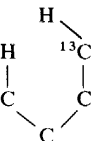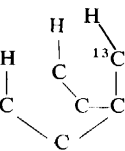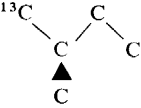
because of the intrinsic symmetry possessed by the molecule. *Trans*-1,3-dimethylcyclohexane is cited as an example. Both conformations exhibit one axial and one equatorial methyl with the conformation exchange between the stable chair structures merely reversing the roles of the two separate methyl groups. It becomes clear that one must weight equally the geometric features found in each equivalent form when using a linear regression analysis to establish structural parameters. The second category of compounds includes all of those in which one conformational isomer is much more stable. Here the *cis*-1,3-dimethylcyclohexane is illustrative. The two methyls in the equatorial position give a conformation which is more stable by several kcal mol^{-1} than the corresponding diaxial form. This energy difference is sufficient to decrease the population of the less stable form below the level where it can significantly affect the carbon-13 magnetic resonance spectrum, and only the structural parameters of the low-energy conformation need be included in the analysis. The third category includes those compounds in which the two more stable conformations are not equivalent but sufficiently close in energy to permit the population of the less stable form to affect the carbon-13 shift parameters. There were only a few of these molecules and they were excluded from a factor analysis of the chemical shifts as the exact populational weighting factors for the two non-equivalent forms are not available from an independent source.

Some of the structural parameters important in the methylcyclohexanes are suggested by a comparison of the shifts in cyclohexane, methylcyclohexane and 1,1-dimethylcyclohexane. It is observed that the addition of a methyl group to cyclohexane is associated with a downfield shift at both the α- and β-carbons while the remaining three carbons are only slightly affected. With the addition of the second methyl in the 1,1-dimethylcyclohexane it becomes essential for one of the two methyls to occupy an axial position, and this group sterically perturbs the axial hydrogens at C-3,5. This single substituent upfield shift of better than $+5$ ppm is about one-half of the normal proton chemical shift range indicating clearly that carbon-13 magnetic resonance techniques provide a very sensitive method for studying molecular coiling, which is a very important part of determining conformational and structural features. If one characterizes the various structural features found in the methylcyclohexanes with the set of parameters given in *Table 1*, a correlation of chemical shifts between predicted and observed is obtained, upon which peak assignments can be made with a high degree of confidence.

These same parameters were then used to characterize the carbon-13 spectra of the *cis* and *trans* decalins and many of their methyl derivatives. The parent decalins, first reported by the Tallinn group under Dr Lippmaa, provide a reference compound from which the spectra of the various methyl analogues can be predicted. In the short time available, we focus on only one of the structural parameters—the upfield steric shift associated with 1,4-carbon atoms in the *gauche* configuration. This is the structure which exists between axial methyl groups and the ring carbons at positions 3 or 5 in the six-membered ring. Comparison of the 9-methyl-*trans*-decalin spectrum with that of *trans*-decalin indicates that the 2,7-carbons and the 4,5-carbons each appear $+5.1 \pm 0.05$ ppm upfield from the corresponding β- and α-carbons in the parent compound. This shift is almost identical with that found

Table 1. Linear additive parameters for chemical shifts in cyclic alkanes

Structural feature		Parametric value (ppm)
α-carbon	^{13}C—C	−9.94
β-carbon	(structure)	−8.49
Trigonal	(structure)	+2.91
Quaternary	(structure)	+9.04
Vicinal	(structure)	+3.50
γ-gauche	(structure)	+4.56
γ_2-gauche	(structure)	+8.51
β-gauche-γ-trans	(structure)	−1.91

for axial methyls in the cyclohexane. As reproducible features of this type provide information which is at the very basis of conformation analysis the power of carbon-13 magnetic resonance techniques is again clearly indicated. One could in turn point out the highly reproducible character of all of the structural parameters derived from the methylcyclohexane if time permitted, but we content ourselves with an indication of the success of the shift predictions for a series of methyl decalins, both *cis* and *trans*, and of the perhydro-anthracenes. In these cases the compound's structure had been verified by other means, and it is to be noted that in every case the spectrum predicted

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for each isomer much more closely matches the pattern of the experimental spectrum than that of any of the other isomeric forms.

Armed with the confidence which these data on known systems provided, we turned to an investigation of the six isomers of perhydrophenanthrene whose characterization had not been made. The six isomers can be specified with the symbols TST, TAT, TSC, TAC, CSC and CAC where the letters are T ≡ *trans*, C ≡ *cis* for hydrogens at the ring junctures and the letters A ≡ *anti*, S ≡ *syn* specify the relationship of closest hydrogens between the two ring junctures. The *trans* ring juncture locks the conformation of each of the first four isomers listed, with the possible exception of the TAT which undoubtedly has a boat conformation for the middle ring. These four compounds further break down into those having seven peaks due to the inherent symmetry of the TST and TAT molecules and those with fourteen different resonances (barring some overlapping of closely positioned lines) found in the TSC and TAC compounds where the *trans* juncture locks the compound and the *cis* juncture destroys the symmetry. The two *cis–cis* isomers CSC and CAC can undergo rapid conformational interconversions because the *cis* juncture does allow the three six-membered rings to pass from one all-chair conformation through into the opposite all-chair form. In the CSC case both forms exist in equal population and the rapid inter-conversion averages the 14 peaks into 7 peaks in the high-temperature limit while at low temperature the motion is frozen out on the n.m.r. time scale and 14 peaks should be observed. For the CAC molecule the two all-chair conformations at each extreme are of different energies and while rapid interconversion is possible the less stable form will not contribute as significantly to the carbon-13 spectrum. A C_2 symmetry axis for both conformations in CAC perhydrophenanthrene will reduce the number of observable peaks to 7 in each conformation, although the chemical shift for each carbon can be expected to change from form to form. Hydrogenation of phenanthrene and isomer separation yielded 4 of the above 6 isomers, i.e. TST, TSC, TAC and CSC. These were selected on the basis of the alkane substituent parameters, which gave predicted patterns for the six possible isomers that were identical with four of the observed spectra. In each instance the spectrum observed for one of the isomers was markedly different from that of the other isomers. Comparison of the carbon-13 spectra of these compounds with the more common hydrogen spectrum clearly exhibits the superiority of the information obtained with the carbon-13 technique. The carbon spectra exhibit sharp lines over a 30 ppm range while the proton spectra are broad bands lacking significant details and only cover about 1.5 ppm range. The contrast is dramatic and illustrates the power of the carbon-13 method.

In the final part of this paper, I should like to illustrate the types of liquid state dynamics that can be obtained from carbon-13 spin-relaxation information. Using the NOE (Nuclear Overhauser enhancement) factor the overall T_1 can be separated into the dipolar relaxation time T_{1d}, and a part due to all other processes, T_{10}. However T_{10} is usually the spin-rotation relaxation time, T_{1sr}. The following two equations can be used to effect the separation:

$$\frac{1}{T_1} = \frac{1}{T_{1d}} + \frac{1}{T_{10}}; \qquad \eta = 1 + \frac{\gamma_H T_1}{2\gamma_C T_{1d}}$$

where $\eta \equiv$ NOE. Thus, for $\eta = 2.98$, $T_{1d} = T_1$ and the dipolar term dominates. When $\eta \cong 1$, T_{1d} is very long and other mechanisms dominate the T_1 processes. This separation permits one to use theoretical formulations of the various relaxation mechanisms to calculate a reorientation correlation time, τ_r from the T_{1d}, and a motional persistency correlation time τ_{sr}, from the relaxation time T_{1sr} due to the spin-rotation effect. The relationships between the two relaxation times and the corresponding correlation times for small molecules and low viscosity are given as follows for a molecule undergoing isotropic tumbling:

$$\frac{1}{T_{1d}} = \frac{n_H \hbar^2 \gamma_C^2 \gamma_H^2 \tau_r}{r_{CH}^6}$$

$$\frac{1}{T_{1sr}} = \frac{2kT(I_m C)^2 \tau_{sr}}{\hbar^2 I_m}$$

where n_H is the number of directly bonded H's, I_m the moment of inertia, C the spin-rotation interaction constant, and r_{CH} the carbon–hydrogen internuclear distance. We draw special attention to the inverse sixth power dependence of the dipolar mechanism upon the C–H distance and the inverse relationship found between the spin-rotation relaxation rate and the moment of inertia (N.B. the manner in which C, the spin-rotation interaction constant, is defined makes the product $(I_m C)$ fairly constant from molecule to molecule even though I_m may vary by a large ratio). Thus, as the C–H distance increases the efficiency of the dipolar time attenuates very rapidly. Hydrogen atoms on an adjacent carbon atom contribute less than 2 per cent to the relaxation of a carbon with a directly bonded proton and therefore it is usually sufficient to consider only directly bonded hydrogen atoms in carbon relaxation as indicated by the equation for T_{1d}. On the other hand, the spin-rotation mechanism will only be efficient for molecules with low moments of inertia such as found in very low molecular weight compounds or in rapidly moving moieties such as a freely rotating methyl group. High barriers to methyl rotation may make the effective moment of inertia of the CH_3 group the same as that for the whole molecule instead of the very small value dictated by the 3 hydrogens off the rotation axis. In the methyl group, when the molecule lacks spherical symmetry then anisotropic motion may become significant, and a rotational diffusion tensor is needed to characterize the molecular dynamics of such a species in the liquid state. For symmetrical tops the expressions for T_{1d} and T_{1sr} become:

$$\frac{1}{T_{1d}} = \frac{n_H \hbar^2 \gamma_C^2 \gamma_H^2}{r_{CH}^6} \left[\frac{A}{6R_\perp} + \frac{B}{5R_\perp + R_\parallel} + \frac{C}{2R_\perp + 4R_\parallel} \right]$$

$$\frac{1}{T_{1d}} \cong \frac{n_H \hbar^2 \gamma_C^2 \gamma_H^2 A}{6 r_{CH}^6 R_\perp}, \qquad \text{if } R_\parallel \gg R_\perp$$

where A, B and C are geometric constants given by Woessner, and the corresponding spin-rotation term is

$$\frac{1}{T_{1sr}} \cong \frac{2kT\left(\sum_i^{x,y,z} I_{m,i} C_i\right)^2}{3\hbar^2}\left\{\frac{\tau_{sr,\parallel}}{I_{m,\parallel}} + \frac{\tau_{sr,\perp}}{I_{m,\perp}}\right\}$$

where $R_\perp$ and $R_\parallel$ are components in the rotational diffusion tensors (N.B. $\tau_{r,\perp} = 1/6R_\perp$). For compounds such as CH_3X where X is some linear substituent (e.g., CN, Cl, I, etc.) it is to be noted that $R_\parallel \gg R_\perp$. When the inequality is very great $R_\parallel$ drops out of the expression for T_{1d} and only $R_\perp$ will significantly affect the dipolar relaxation rate (see second expression for $1/T_{1d}$ above). Likewise, when $I_{m,\parallel} \ll I_{m,\perp}$ only the $\tau_{sr,\parallel}$ is important in the spin-rotation mechanism. We now look at T_1 and η(NOE) data for CH_3I given in *Table 2* from which T_{1d} and T_{1sr} can be determined, and consequently $\tau_{r,\perp}$ and $\tau_{sr,\parallel}$ may be calculated, the estimated values for $\tau_{sr,\perp}$ can be derived from $\tau_{r,\perp}$ using the Hubbard relationship as follows:

$$\tau_{r,i}\tau_{sr,i} = \frac{I_{m,i}}{6kT}$$

This equation only holds for a diffusion controlled small angular process, and thus cannot be used to calculate the parallel relationships which are obviously inertial controlled. It may be observed from the following *Table 2* that motion persists on the average in the parallel mode for a time which is comparable to free rotation through 1 radian as determined by the equipartition of energy principle. On the other hand, the motion persists about the perpendicular axis only long enough to rotate through about 4°. Thus, from the relaxation data we derive quantitative details of rotation diffusion in the liquid state even to the point of characterizing the anisotropy of the various characteristic motions.

Table 2. Relaxation data for CH_3I

	Neat liquid	Dissolved in nematic phase
T_1	11.1 ± 0.4 s	6.8 ± 0.5 s
NOE	1.4_2	2.0_6
T_{1d}	52.5 ± 6 s	12.8 ± 2 s
T_{1sr}	14.1 ± 2 s	14.6 ± 2 s
$\tau_{r\perp}$	1.2 ps	4.9 ps
$\tau_{sr,\parallel}$	0.13 ps	0.13 ps
$\tau_{sr,\perp}$	0.036 ps	†
Free rotation through 1 radian		
$\tau_{fr,\parallel}$	0.11 ps	0.11 ps
$\tau_{fr,\perp}$	0.51 ps	0.51 ps
Average angle/jump		
$\theta_\parallel$	~68°	68°
$\theta_\perp$	~4°	†
$I_{m,\parallel}$	5.5×10^{-40} g cm^2	
$I_{m,\perp}$	111.9×10^{-4} g cm^2	

† The Hubbard relation would not be relevant to $\tau_{sr,\perp}$ if director of nematic phase governs $\tau_{r,\perp}$.

An interesting extension to this work on CH_3I comes from work in the nematic phase. In this medium CH_3I exhibits a $T_{1d} = 12.8$ s and a $T_{1sr} = 14.6$ s. It is noted that T_{1sr} has not changed within the experimental errors suggesting that rotational motion about the parallel axis is not greatly affected. The T_{1d} on the other hand has shortened by slightly better than four-fold, indicating either that the $R_\perp$ motion has been slowed down by four-fold or that other oscillatory motions such as slower temporal variations in the director of the liquid crystal are affecting the movement of the CH_3I molecule and consequently are altering the T_1 relaxation time. Detailed discussions of symmetric top motions such as delineated here are of considerable importance in formulating dynamic models of liquids and it is anticipated that carbon-13 relaxation studies will continue to provide a wealth of information in the coming months.

Woessner has derived expressions for T_{1d} in asymmetric molecules in terms of three different rotational diffusion constants with each of these parameters associated with one of the molecular principal axes appropriate for rotational diffusion. Although this formulation is too extensive to reproduce here in the time available, it is easy to appreciate the consequences of the method. If one has at least three different carbon atoms in a rigid molecule with attached protons whose directional cosines are not identical, it becomes easy to obtain these simultaneous equations from which three R's can be obtained. A molecule meeting these specifications is *trans*-decalin which because of its ellipsoidal shape can be expected to tumble anisotropically about the C_2 axis and about two additional axes lying in the plane normal to the C_2 axis. As all axial hydrogens, that is those perpendicular to the plane of the ring, manifest the same directional cosines they will contribute equally to the dipolar relaxation of each of the three different carbon atoms. Thus, the variation in relaxation times noted at the several positions follows entirely from the effectiveness of the equatorial protons attached to the methylene carbons. As these no longer have the same directional cosines, the anisotropic behaviour of the molecular diffusion is revealed. The anisotropy is fairly large as can be observed from the three rotational diffusion constants $R_1 = 11 \pm 3 \times 10^{10}$ rad s^{-1}, $R_2 = 6 \pm 2 \times 10^{10}$ rad s^{-1} and $R_3 = 2.5 \pm 1 \times 10^{10}$ rad s^{-1}. It is interesting to note that the three constants do not exhibit the inverse relationship with the corresponding moments of inertia (645, 206 and 488×10^{40} g cm^2, respectively) which would have been found if the process had been inertial controlled and in the gas phase. Conversely, the rotational diffusion constants do parallel the degree of ellipticity estimated from the ratio of perpendicular molecular dimensions in the plane normal to the axis of rotation. This suggests that the process is diffusion controlled with an emphasis being placed on the general shape of the molecule.

Norbornane is another anisotropic tumbler that manifests three rotational diffusion constants ($R_1 = 17 \times 10^{10}$, $R_2 = 10 \times 10^{10}$ and $R_3 = 7 \times 10^{10}$ rad s^{-1}) which are determinable again in this molecule from the three uniquely different carbon atoms. In this instance the deviation from isotropic behaviour is not quite as great as for *trans*-decalin, but this might be expected from the spherical nature of the molecule. It is interesting to compare the anisotropic rotational diffusion constants ($R_\parallel = 17 \times 10^{10}$ and $R_\perp = 9 \times 10^{10}$ rad s^{-1}) found in the symmetric top molecule, bicyclooctane, and

with the single rotational diffusion constant ($R = 8 \times 10^{10}\,\text{rad s}^{-1}$) determined in the spherical adamantane top. In each case a combination of shape factors and/or moments of inertia are sufficient to rationalize the observed results. That anisotropic behaviour is found for these systems is not surprising, but the ability to estimate the magnitude of such variations is felt to be of considerable importance in formulating a better picture of molecular reorientational processes in liquids.

When rotation is partially restricted about the three-fold axis of a methyl group due to the presence of a rotation barrier, the T_{1d} and T_{1sr} of the methyl carbon will be affected significantly. We noted in the CH_3I study how extremely rapid motion about the principal axis gave rise to an efficient T_{1sr} process and how this same rapid motion drops out of the T_{1d} expressions in the limit of $R_{\parallel} \gg R_{\perp}$. Restriction of methyl rotation in a class of compounds such as the methyl ethylenes can be expected to modify these conclusions. As the barrier increases, the rotation rates will decrease and the spin-rotation process must of necessity become less efficient. Conversely, as the methyl top slows down, the reorientation rate about the methyl axis will decrease and $R_{\parallel} \approx R_{\perp}$ and $R_{\parallel}$ will begin to make a contribution to the relaxation. The dipolar process will increase in its efficiency and T_{1d} will shorten. Using both of these parameters T_{1d} and T_{1sr} along with a simple Arrhenius expression it becomes possible to predict energy barriers from the effective spin-rotation and dipolar correlation times. Both parameters actually corroborate each other and because of their reciprocal character supplement one another in the extreme limits where one or the other process is described by expressions which mathematically may be ill-conditioned. Comparison of the energy barriers determined by this means and more standard optical methods indicates the reasonableness of the approach in the case of the simpler molecules in the series. For the more complicated molecules having more than one energy barrier, the n.m.r. approach continued to yield information where analysis of the optical data may be prohibitively difficult. Thus, even the energetics of segmental molecular motion can be determined if sufficient relaxation data can be obtained and if the various relaxation mechanisms can be separated so that appropriate mathematical formulations can be invoked.

The availability of information on segmental motion in very large molecules becomes of considerable interest when one considers biological systems. The use of x-ray techniques has excited the scientific community for several years now with their three-dimensional pictures of biological substances such as important polypeptides, etc. However, these data have all been accumulated in the solid state and one might ask justifiably what information exists in the liquid state where enzymatic activity is found. One approach to this question would be to secure a dynamical map to be used along with the three-dimension structure determined from x-rays. Now, here is where carbon-13 methods become important as many polypeptides may exhibit upwards of 50 carbon-13 resonance lines. It then becomes possible to secure 50 pieces of dynamical data provided relaxation times can be secured. Only the problem of low signal to noise of carbon-13 resonance lines in molecules with a molecular weight of 30 000 stands in the way of pursuing this goal. One solution immediately suggests itself, that of carbon-13 isotopic enrichment which is possible

up to the 90 per cent level. Such enriched samples in the 30 000 molecular weight range have signals which can be studied at reasonable concentration levels, and one can expect to see this type of work in the near future. We conducted an exploratory experiment to determine if this technique would yield information of sufficient value to justify the rather difficult and tedious labelling work required. We converted most of the free amino groups in horse heart cytochrome-C into carbon-13 labelled urea type terminal groups. All but one of the free amines are derived from the 19 lysines in this molecule, which generally are found at the surface of the peptide. Treatment of the polypeptide with enriched potassium cyanate for several hours was sufficient to give better than 85 per cent incorporation of the isotope at all of the lysines considered collectively. It is still not known whether or not the label is uniformly distributed among the 19 lysines, however. Evenso, we see at least 8 different carbon-13 bands, spread about 20 Hz in the substituted urea region of the carbon-13 spectrum. This in itself was considered to be significant as structural differences would be minimal at the end of the lysine chain, and one might expect even greater conformational and structural difference if the substitution had been closer to the backbone of the peptide where secondary structural features would perturb the carbon-13 of the peptide to a greater extent. The most exciting aspect, however, is with the T_1 relaxation times data obtained on these 8 different bands. It is observed that their effective relaxation time varies by almost three-fold between peaks. This tells us that the segmental motion from lysine to lysine is not uniform, but instead reflects variations in the local structural features as they modify the nature and extent of segmental reorientation of the lysines side chain. While this result still falls in the exploratory category it none-the-less illustrates the type of dynamical information which ultimately may be obtainable from enriched macromolecules. Reduction of the heme in the cytochrome-C resulted in considerable change in the spectral pattern, but the lack of uniformity in relaxation times persisted. Reoxidation of the sample indicated that the changes affecting the spectral pattern and the relaxation times were reversible. The differences in spectral features are assumed to be due to conformation changes between the reduced and oxidized forms.

By way of summary, this paper has surveyed several different applications of the fairly new carbon-13 magnetic resonance technique. In general, its use in structural analysis and liquid dynamical studies has been exhibited. Furthermore, potential application in the study of macromolecular systems appears to be very promising.

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