TATE AND LYLE LECTURE*

Spin–Lattice Relaxation: A Fourth Dimension for Proton N.M.R. Spectroscopy

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1 Introduction

The conventional procedure using high-resolution n.m.r. spectroscopy as a tool for studying the structure and stereochemistry of organic molecules in solution is well known: three sets of n.m.r. parameters, the chemical shifts, coupling constants, and integrated areas, are derived from the measured spectrum and these parameters are then used, either singly, or in some suitable combination, as the basis for the structural assignment. Powerful as it is, this approach neglects the fact that each spectrum contains, implicitly at least, two further sets of magnetic resonance parameters; these are the *spin–lattice* relaxation times (T_1-values) and the *spin–spin* relaxation times. Recent instrumental developments have made possible the routine measurement of spin–lattice relaxation times, and it is now appropriate for chemists to begin to evaluate the diagnostic potential of what constitutes, for the organic chemist at least, a new class of n.m.r. parameters.

The phenomenon of spin–lattice relaxation has long attracted the attention of physicists and of some chemical physicists. Largely as a result of their efforts, there is an extensive literature both of methods for measuring T_1-values and of the theory that enables these parameters to be related to a wide variety of molecular properties and, importantly, molecular motions. Until recently most practising chemists, even those with an active interest in n.m.r. spectroscopy, have largely neglected the phenomenon of spin–lattice relaxation.† There have been many reasons for this neglect and of these the most cogent is that until very recently, the methods developed by physicists for measuring T_1-values have been incompatible with studies of the complex molecular species which are of interest to the practising chemist. However, with the development of Fourier transform (FT) techniques it has become possible to measure, on a rout-

* Delivered in Birmingham, April 1974.

† The most notable exceptions to this are the nuclear Overhauser enhancement experiments, the uses of which were first demonstrated by the pioneering studies of Anet and Bourne.[1] For an excellent summary of the literature and of the full theory of this area the reader is referred to the monograph by Noggle and Schirmer.[2]

[1] F. A. L. Anet and A. J. R. Bourne, *J. Amer. Chem. Soc.*, 1965, **87**, 5250.

[2] J. H. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect. Chemical Applications', Academic Press, New York, 1971.

ine basis, the T_1-value of any resonance that can be clearly resolved in an n.m.r. spectrum, and hence to study systems which are chemically quite complex.

It is interesting to note that the majority of the laboratories first equipped with FT n.m.r. spectrometers appear to have directed most attention towards studies of ^{13}C T_1-values;[3] relaxation studies of other magnetic nuclides, particularly of protons, have been somewhat neglected. In practice there are some complications associated with proton T_1-measurements which do not pertain to ^{13}C studies; however, these can be overcome.

This review is principally related to proton studies that have been performed in the author's laboratory. Following a brief introduction to the phenomenon of spin–lattice relaxation and to the pulse n.m.r. methods which are used to measure spin–lattice relaxation times, there is a discussion of experiments which delineate their configurational dependences. This is followed by descriptions of several methods which allow extensive simplifications to be made to certain complex ^1H n.m.r. spectra. The review ends with examples which show that it is a simple matter to manipulate proton spin–lattice relaxation times chemically and thereby to extend the range of molecules which can be studied by FT relaxation techniques.

2 Spin–Lattice Relaxation

What, then, is the phenomenon of spin–lattice relaxation? Basically, we are concerned with the way in which, and the rate at which, magnetic energy is transferred between the magnetic nuclei under study (the 'spins') and their surrounding environment (the 'lattice'). There are a number of distinct mechanisms whereby this energy transfer can be effected, and the names ascribed to these are listed in Table 1. Fortunately we need not be concerned here with details of the important

Table 1 *Mechanisms of spin–lattice relaxation*

1 Dipole–Dipole
  (a) Intramolecular
  (b) Intermolecular
  (c) Paramagnetic
2 Spin Rotation
3 Quadrupolar
4 Scalar Coupling
5 Chemical Shift Anisotropy

differences between these mechanisms; all that need be noted is that in each case a rapidly fluctuating magnetic field, generated and located in the lattice, interacts with the rapidly precessing nuclei of interest and thereby mediates in the energy-transfer process. In principle, and often in practice, several of these different

[3] G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists', Wiley–Interscience, New York, 1972.

relaxation mechanisms operate simultaneously, and in these circumstances the spin–lattice relaxation time measured experimentally $(T_1)_{exp}$ is a composite value and contains contributions from each of the several mechanisms (A, B, *etc.*). Thus

$$\left(\frac{1}{T_1}\right)_{exp} = \left(\frac{1}{T_1}\right)_A + \left(\frac{1}{T_1}\right)_B + \ldots .$$

If this were always the case then it is quite probable that this entire phenomenon would have little, or no, relevance to organic chemists, since although it is a trivial matter to obtain an experimental value for a spin–lattice relaxation time, it is often a more difficult task to derive from that value the individual contributions from several different relaxation mechanisms. Fortunately, it is often possible to conduct experiments in which one of these mechanisms, the intramolecular dipole–dipole mechanism, dominates the relaxation, and under optimum conditions is the only operative mechanism. In practice, this requires that one be interested in molecules which are moving more or less isotropically in the solution. Furthermore, these molecules should be studied as a dilute solution (*ca.* 0.1 mol l^{-1}) in a magnetically inert solvent; both of these requirements minimize contributions from *inter*molecular effects, and both are attainable if one has access to a FT spectrometer. The requirement of a magnetically inert solvent merely means that the solvent should contain neither fluorine nor proton substituents, and in practice it is usual to approximate to this requirement by using a deuteriated substance, which has the added convenience of providing a heteronuclear-lock signal for the spectrometer.

The importance of making experimental measurements under these conditions transcends mere convenience, and this becomes apparent when one studies the mathematical form of the intramolecular dipole–dipole mechanism, which is given below for the contribution that one donor nucleide D makes to the spin–lattice relaxation of a second, receptor nucleus R.

$$\left(\frac{1}{T_1}\right)_{D\rightarrow R} \propto \frac{\gamma_D^2 : \gamma_R^2}{(r_{D\rightarrow R})^6} \cdot \tau_C \qquad (1)$$

In this equation, γ_D and γ_R are the magnetogyric ratios of the two nuclei involved; r, is the internuclear distance between D and R; and τ_C is a motional correlation time (which reflects the memory that the molecule to which the nuclei D and R are attached has of its motion in solution). The first important point is that if one is considering the relaxation of a proton, R, in an organic molecule, the most probable source of relaxation is *another proton*; this follows from the fact that only protons have significantly high values of γ. The second point to note is that the contribution which the donor proton, D, can make to the relaxation of R falls off as the inverse sixth power of the internuclear separation of D and R; hence one can anticipate the possibility that proton spin–lattice relaxation times will show pronounced configurational dependencies. Of course, in most

'real' organic molecules, each individual proton will be relaxed by interactions with several other protons (*D*-1, *D*-2, . . .) in the same molecule, and hence its total relaxation time $\left(\frac{1}{T_1}\right)_R$ will have the form given in equation (2) and the

$$\left(\frac{1}{T_1}\right)_R = \left(\frac{1}{T_1}\right)_{D\text{-}1} + \left(\frac{1}{T_1}\right)_{D\text{-}2} + \left(\frac{1}{T_1}\right)_{D\text{-}3} + \ldots . \qquad (2)$$

magnitude of each of these contributions will depend on the relative magnitudes of the individual internuclear distances. This leads to the third point: if it is possible to measure quantitatively the contributions which any donor proton, *D*-1, makes to the relaxation of two other receptor protons, *R*-1 and *R*-2, then this should provide a direct inter-comparison of the internuclear separations *D*-1 to *R*-1 and *D*-1 to *R*-2. Thus,

$$\frac{\left(\frac{1}{T_1}\right)_{D\text{-}1\rightarrow R\text{-}1}}{\left(\frac{1}{T_1}\right)_{D\text{-}1\rightarrow R\text{-}2}} = \frac{(r_{D\text{-}1\rightarrow R\text{-}2})^6}{(r_{D\text{-}1\rightarrow R\text{-}1})^6} \qquad (3)$$

This potential can be realized by judiciously conducted nuclear Overhauser experiments.[4] In principle it should also be possible to place these distances on an absolute basis by substituting into equation (1) a value for the correlation time, τ_C; however, this presents a number of major problems because it is not easy to obtain values for correlation times which are sufficiently accurate to justify subsequent calculations of the type envisaged here, and alternative approaches may prove to be more useful.

In brief then, under suitable conditions proton spin–lattice relaxation involves through-space interactions between individual protons in the same molecule and, since the efficiency of these interactions falls off rapidly with distance, each proton receives most of its relaxation from its nearer-neighbour protons.

3 Measurement of Spin–Lattice Relaxation Times

The basic experimental technology involves pulse-n.m.r. methods, and the simplest conceptual model upon which a discussion can be based is the 'rotating-reference frame' model.

Consider an ensemble of magnetically equivalent nuclei (spins) subject to the influence of some external magnetic field (H_0). At thermal equilibrium between the spins and the lattice, this ensemble will have a net, macroscopic magnetic moment which will, in the rotating reference frame, be directed along the *z*-axis, which is also the direction of H_0 (see Figure 1). In Figure 1 this magnetic moment is represented by a vector 'arrow', whose length is intended to indicate the total amount of magnetization present; when this vector lies along the *z*-axis, no signal is detected by the spectrometer, which is designed to respond only to that com-

[4] R. Freeman, H. D. W. Hill, B. L. Tomlinson, and L. D. Hall, *J. Chem. Phys.*, 1974, **61**, 4466.

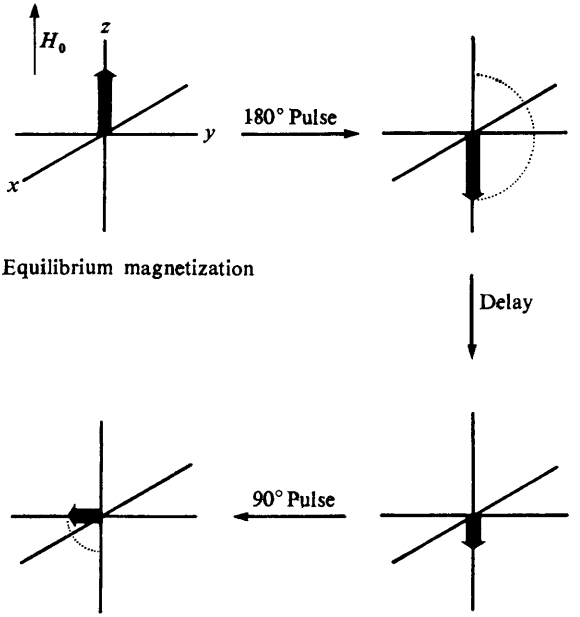


Figure 1 *The rotating reference frame model for the measurement of a spin–lattice relaxation time.*

ponent of the magnetization which lies in the x, y-plane. Thus, to assay the amount of magnetization along the z-axis at any particular time it is necessary to tip the magnetization vector through 90° into the x,y-plane. This is easily accomplished by the simple expedient of applying a suitable amount of radiofrequency power at the appropriate frequency, in the form of a short pulse (a 90°-pulse), and, providing this is done sufficiently rapidly compared with the rate of change of magnetization along the z-axis (see below), this introduces no systematic error. It is important to note that if twice that amount of power is applied say by doubling the length of the pulse, then the original magnetization vector will be tipped through 180° and will lie along the $-z$-axis.

In the rotating reference frame, spin–lattice relaxation refers to the rate at which magnetic energy is transferred from spins which are directed along the z-axis. Initially, in Figure 1 the spin system is at thermal equilibrium with its lattice, and with its macroscopic magnetic moment directed along the z-axis. This equilibrium is then destroyed by applying a 180°-pulse of power which tips the magnetization rapidly into the $-z$-direction. Spin–lattice relaxation then restores the system to thermal equilibrium; this is accompanied initially by a decay in the magnetization intensity along the $-z$-direction and subsequently by a recovery in the $+z$-direction. The amount of magnetization present at any particular instant can be assayed by applying a 90°-pulse and so tipping the

residual component up, or down, into the x,y-plane, where it induces a signal into the receiver of the spectrometer. In practice, then, a 180°-pulse is used to destroy the thermal equilibrium, a known amount of time is allowed to elapse (the delay time, t) and the residual magnetization after that period is assayed by a 90°-pulse. This gives the first point on the decay-recovery curve. The system is then left for 5 or more T_1-periods to recover its equilibrium level of magnetization, and then a second 180°–90° pulse-sequence is applied, this time with a somewhat longer value for t. This gives the second point on the decay-recovery curve, and so on; in normal practice a minimum of at least ten such points must be determined. The spin–lattice relaxation time is the time constant of the curve drawn through the individual points [Figure 2(a)]; this value is more conveniently obtained by making a semi-logarithmic plot of $\log_e$ (peak height) *vs.* t [Figure 2(b)]. The significance of the fact that the decay-recovery curve has zero intensity for a particular value of t is discussed below.

Pulse spectrometers capable of performing the above manipulation have been available for a number of years.[5] Their principle disadvantage is that they lack frequency selectivity and hence their use is mainly confined to molecules having a single set of equivalent nuclei and from which an average T_1-value is obtained.[6] A marked improvement in frequency selectivity has followed from the development of the 'audiofrequency-pulse' technique by Freeman and Wittekoek.[7] We constructed a modified version of that spectrometer[8] and used it in our own first measurements; however, this technique also suffered from a variety of limitations, not the least being the large amount of time needed to measure the T_1-values of a complex proton n.m.r. spectrum, transition by transition.

Fortunately the advent[9] of FT n.m.r. spectrometers has made possible a full realization of proton T_1-measurements for even complex organic molecules. The required 180°- and 90°-pulses are now applied simultaneously to *all* of the proton resonances and hence the spin–lattice relaxation times of each proton resonance, indeed of each individual transition in the spectrum, are obtained simultaneously. The output of the spectrometer following the application of the 90°-pulse is referred to as the 'free-induction decay' signal (F.I.D.) and this is stored, following digitization, in the memory of a small computer. The F.I.D. consists of a series of overlapping sine-waves and contains all the information concerning the n.m.r. spectrum; this is converted to the more-familiar, frequency-domain, n.m.r. spectrum by the mathematical manipulation known as Fourier transformation.

[5] T. C. Farrar and E. D. Becker, 'Pulse and Fourier Transform N.M.R.', Academic Press, New York, 1971.
[6] T. L. Pendred, A. M. Pritchard, and R. E. Richards, *J. Chem. Soc.* (*A*), 1966, 1009.
[7] R. Freeman and S. Wittekoek, *J. Magn. Resonance* 1969, **1**, 238; R. Freeman, S. Wittekoek, and R. R. Ernst, *J. Chem. Phys.*, 1970, **52**, 1529.
[8] R. Burton, C. W. M. Grant, and L. D. Hall, *Canad. J. Chem.*, 1972, **50**, 497.
[9] R. R. Ernst and W. A. Anderson, *Rev. Sci. Instr.*, 1966, **37**, 93; R. R. Ernst, *Adv. Magn. Resonance*, 1966, **2**, 1; R. Freeman and H. D. W. Hill, 'Introduction to Fourier Transform N.M.R.', Varian Associates, Palo Alto, 1970.

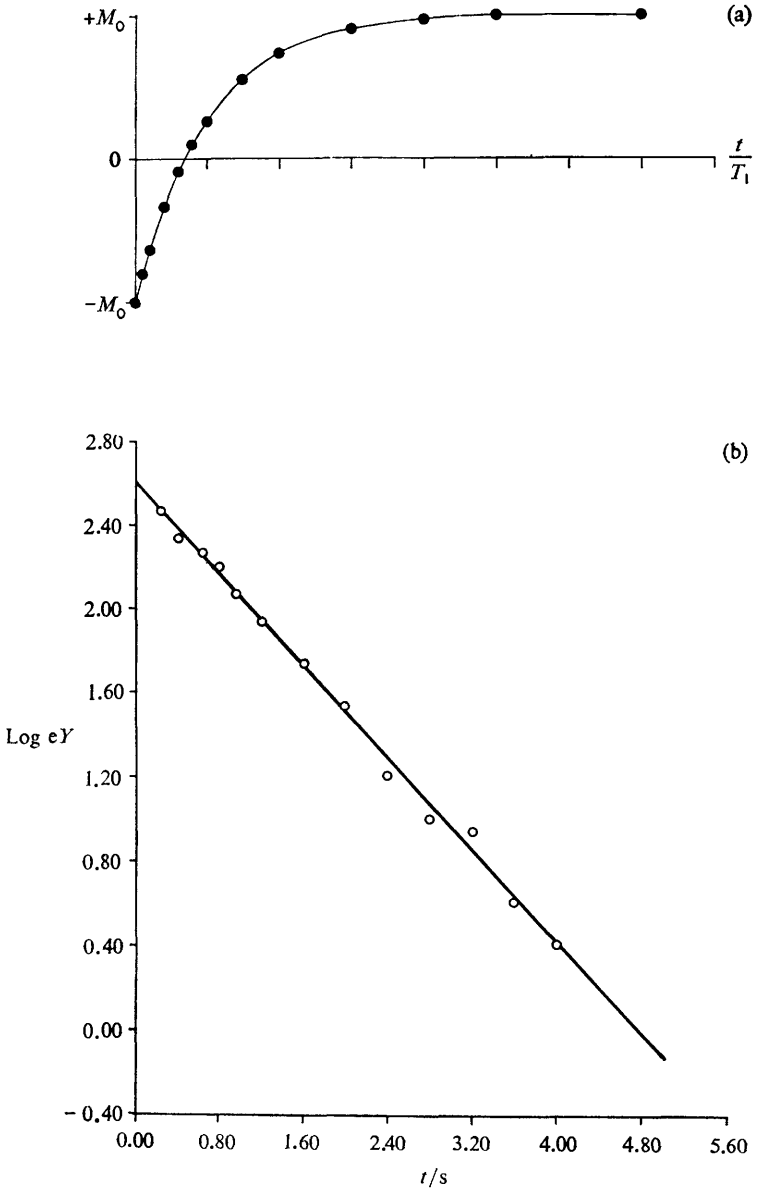


Figure 2 *Representation of the data obtained for a two-pulse determination of a spin–lattice relaxation time.* (a) *The recovery of the magnetization from its inverted value* ($-M_0$) *back to thermal equilibrium* ($+M_0$) *is shown. Note that for short values of t the peak which would be observed in the n.m.r. spectrum would be negative-going, reflecting the fact that the magnetization was still along the* $-z$*-axis when the* 90°*-pulse was applied.* (b) *A typical plot of* $\log_e$ (*peak height*) versus *t is shown.*

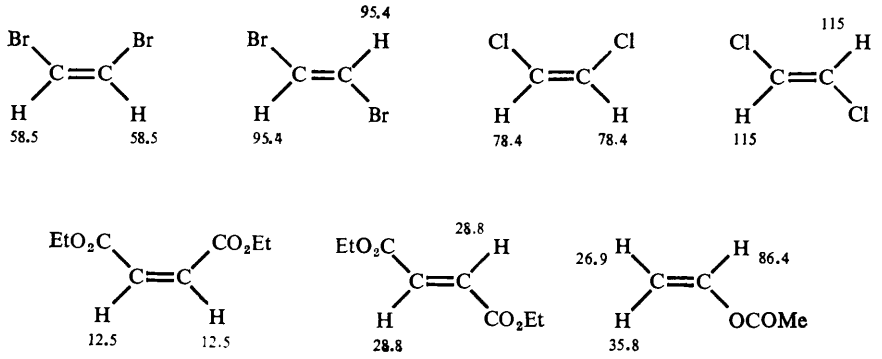


Figure 3 *Values/s of the proton spin-lattice relaxation times for alkenes. The data for the halgeno-ethanes were determined with a selective, audio-pulse spectrometer*[10] *using* ca. 0.5 mol l^{-1} *solutions. The vinyl acetate data were obtained by conventional FT methods, on a* ca. 0.1 mol l^{-1} *solution.*

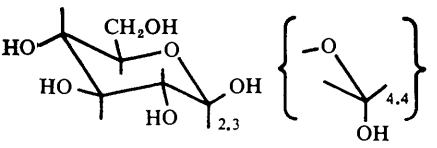
Unpublished[10] experiments involving measurements of *cis*- and *trans*-alkenes with our home-built, audio-pulse spectrometer are summarized in Figure 3. The differential between the T_1-values for the individual pairs of isomers is clear-cut. Although the frequency selectivity of that instrument did not allow a complete study to be made, it was possible to measure[10,11] the T_1-values of some of the protons of 3,4,6-tri-*O*-acetyl-1-*O*-benzoyl-2-chloro-2-deoxy-β-D-glucopyranose (4), and the study was subsequently completed when the FT method became available in our laboratory.

4 Stereospecific Dependence of Spin–Lattice Relaxation Times

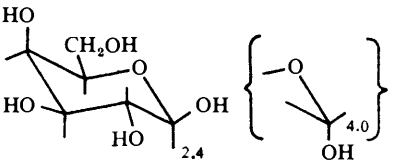
The first observations of a pronounced stereospecific dependence for the proton T_1-values of a sugar derivative were made independently in 1972 by Professor Richards' group at Oxford and in the author's own laboratory in Vancouver. In Oxford,[12] a study of D-glucose-6-phosphate revealed a marked dependence for the T_1-values of the anomeric protons, and a similar study of a series of hexose and pentose sugars in aqueous solution in Vancouver gave the data[13] shown in formulae (1)—(3). The data for D-glucose (1) and D-galactose (2) immediately show that H-4 has little or no influence on the relaxation of the anomeric proton. The difference between the data for D-glucose and D-mannose (3) shows that H-2 makes some contribution to the relaxation of H-1, but forces one to conclude that the dominant source of the T_1-differential between the axial and equatorial anomeric protons must be H-3 and H-5, the implication here being that H-1_{ax}

[10] C. W. M. Grant, Ph.D. Thesis, Department of Chemistry, University of British Columbia, March, 1972.
[11] C. W. M. Grant, L. D. Hall, and C. M. Preston, *J. Amer. Chem. Soc.*, 1973, **95**, 1972.
[12] R. Dwek, 'Nuclear Magnetic Resonance in Biochemistry—Applications to Enzyme Systems', Clarendon Press, Oxford, 1973.
[13] L. D. Hall and C. M. Preston, *J.C.S. Chem. Comm.*, 1972, 1319; *Carbohydrate Res.*, 1974, **37**, 267.

is nearer to H-3$_{ax}$ and H-5$_{ax}$ than is H-1$_{eq}$, and hence H-1$_{ax}$ is relaxed faster by those two axial protons than is H-1$_{eq}$.



(1)



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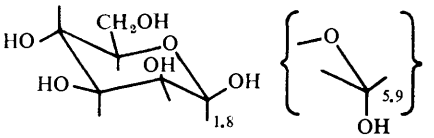
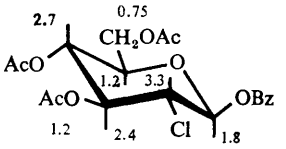


(3)

Unfortunately the complexity of the proton spectra of these sugars precluded any general identification of other proton resonances, and hence further studies of these interesting stereospecific dependencies required selection of derivatives having a greater dispersion in their proton resonance spectra. The proton T_1-data obtained[11] from a set of 3,4,6-tri-*O*-acetyl-1-*O*-benzoyl-2-deoxy-2-halogeno-D-hexopyranose derivatives are shown in formulae (4)—(8). Comparison of data obtained for (4) and (5), and for (6) and (7) immediately demonstrated the same pronounced differential between axial and equatorial anomeric protons that had been observed previously. Furthermore, the reciprocal effect of the contribution of H-1 to the relaxation of H-3 can also be seen. Proton H-5,

however, appears not to show this reciprocity; this is because its relaxation is now dominated by contributions from the C-6 protons.



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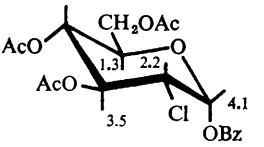

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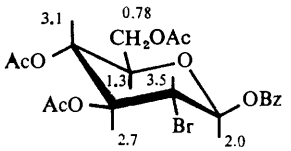

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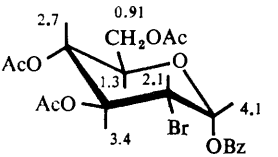

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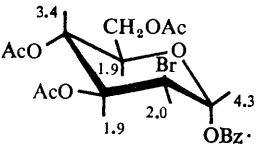

(8)

Data have also been obtained for extensive series of hexose,[13] pentose,[13] inositol,[13] and oligosaccharide[14] derivatives, all in aqueous solutions, and studies have been completed of peracetylated pentopyranoses,[15] and of some septanoside derivatives;[16] in all instances the experimentally determined T_1-values reflect the anticipated geometry and, especially in the case of the septanosides, provide insight as to the likely conformational symmetry of the derivative.

5 Data Manipulation

In this section illustrations are given of the many types of data manipulatior which can be performed once one has access to a FT spectrometer and to the concepts of spin–lattice relaxation. Three distinct opportunities exist; (A) manipulation of the magnetization of the sample by application of suitable sequences of pulses to achieve selective elimination of certain resonances; (B) manipulation of the data contained in the free induction decay signal prior to Fourier transformation, thereby changing either the resolution or the signal-to-noise ratio of the final spectrum; and (C) chemical modification of the relaxation pathways open to the nuclei, thereby obtaining further chemical information about the system under study.

A. Manipulation of Magnetization.—It will be recalled from an earlier discussion [Figure 2(a)] that the recovery of the magnetization along the *z*-axis following application of a 180°-pulse is exponential, and that at a particular time the

[14] L. D. Hall and C. M. Preston, *Carbohydrate Res.*, 1973, **29**, 522.
[15] L. Evelyn, L. D. Hall, and J. D. Stevens, unpublished results.
[16] J. Berry, L. D. Hall and J. D. Stevens, unpublished results.

magnetization decays to zero intensity. Clearly, if the magnetization of the sample is assayed at that time by means of a 90°-pulse, no resonance signal will be detected. Since protons in chemically distinct environments can have significantly different T_1-values, it follows that by choosing a suitable delay time between the 180°- and 90°-pulse the resonances of individual protons can be made to disappear effectively from an n.m.r. spectrum. The use of this experiment to eliminate the residual water peak from the spectrum of an organic compound dissolved in D_2O was first demonstrated by Sykes[17] and subsequently by Feeney,[18] and an example from the author's work[19] is given in Figure 4. The salient point here is that the residual water peak has a far longer relaxation time than the resonances of the sugar, and therefore the more rapidly relaxing sugar protons

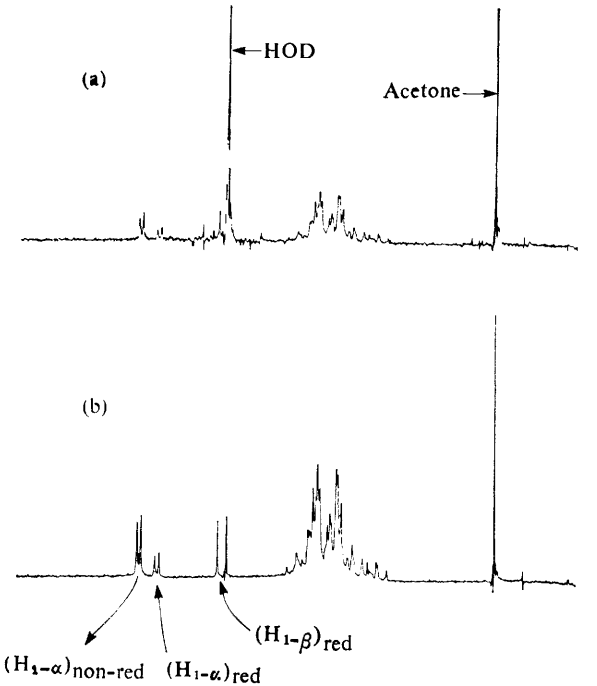

Figure 4 *An example of the nulling of a residual water peak using the partial relaxation method.* ^1H *N.m.r. spectra of a* 5% *solution of gentiobose in* D_2O (99.6%); *the sample had previously been lyophilized once with* D_2O *and then degassed by six freeze–pump–thaw cycles.* (a) *The normal* FT *spectrum (one scan);* (b), *the water-nulled spectrum* (10 *scans*). *In this mode of operation the magnetization is inverted by a* 180°*-pulse, and this is followed, after a delay time of* 0.7 *times the* T_1*-value of water, by a* 90°*-pulse*
(Reproduced by permission from *Carbohydrate Res.*, 1973, **29**, 522)

[17] S. L. Patt and B. D. Sykes, *J. Chem. Phys.*, 1972, **56**, 3182.
[18] F. W. Benz, J. Feeney, and G. C. K. Roberts, *J. Magn. Resonance*, 1972, **8**, 114.
[19] L. D. Hall and C. M. Preston, *Carbohydrate Res.*, 1973, **27**, 286.

recover almost all of their intensity in the *z*-direction during the time required for the water peak to reach its null point.

This experiment provides an invaluable aid to studies of aqueous solutions of polysaccharide derivatives where the residual water peak often obscures some of the resonances of the anomeric protons, and we now use it routinely.

We were intrigued by the possibility that this procedure might also facilitate assignments of complex proton spectra—where the success of the experiment would now depend on the presence of some intramolecular differential in relaxation times. An example which illustrates the exciting potential of this method for solving the hidden resonance problem[20] is given in Figure 5; we had previously

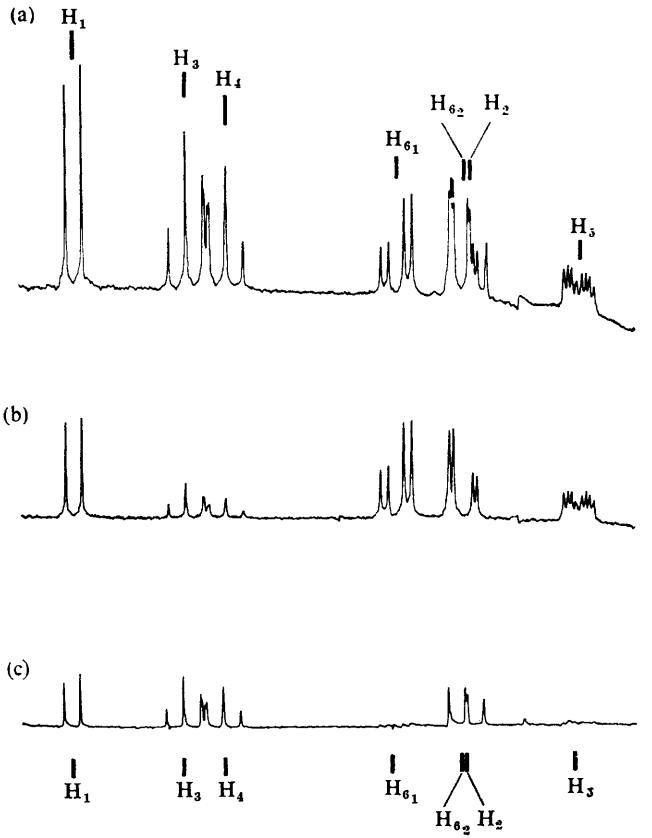

Figure 5 *Partially relaxed* **100 MHz** *proton n.m.r. spectra for* 3,4,6-*tri-O-acetyl*-1-*O-benzoyl*-2-*chloro*-2-*deoxy*-β-D-*glucopyranose* (4) *in* C_6D_6 *solution* (0.1 mol l^{-1}); (a), *the normal spectrum;* (b), *the result of using a two-pulse sequence* (180°–*t*–90°) (c), *the spectrum determined with a three-pulse sequence* (180°–*t*–90° . . . 90°). *For details see text.*

[20] L. D. Hall, *Adv. Carbohydrate Chem.*, **2**, 1974, 911.

shown[11] that the H-2 and H-6_2 resonances of 3,4,6-tri-*O*-acetyl-1-*O*-benzoyl-2-deoxy-β-D-glucopyranose (4) have substantially different T_1-values, and it was interesting to see whether this three-fold differential in T_1-values was sufficient to enable the resonances of H-2 and H-6_2 to be distinguished. The spectrum in Figure 5(a) was obtained in the usual fashion. The resonance of the more rapidly relaxing proton was obtained with the usual two-pulse sequence (180°–t–90°) with t chosen such that the magnetization of the more slowly relaxing proton had decayed to zero intensity at the time when the monitoring pulse was applied; this gives H-6_2 as a positive-going resonance. That of the more slowly relaxing proton could be obtained by two distinct methods. In the first, the two-pulse sequence could be used, with t chosen such that now the magnetization of the more rapidly relaxing proton (H-6_2 in this case) has decayed to zero intensity at the time the 90°-pulse is applied; this would give the more slowly relaxing proton as a negative-going resonance. An alternative and somewhat more convenient method, illustrated in Figure 5(c), uses[21] a three-pulse sequence (180°–t–90° . . . 90°); the second 90°-pulse now provides a reference spectrum which can be automatically subtracted from that obtained by the first 90°-pulse. The final resultant of this procedure is that the resonance signal of every resonance decays to zero intensity for sufficiently long delay times; in the particular example discussed here, the signal of the more rapidly relaxing proton (H-6_2) reaches zero intensity faster than that of the more slowly relaxing proton (H-2). It is interesting to note that the H-6_1, and H-5 resonances, which have closely similar T_1-values to H-6_2, also disappear at the same time as H-6_2.

There are two distinct experimental protocols for the above experiments. If the T_1-values of individual protons are already known, then the value of t required for a resonance to disappear can be calculated. In the normal run of events it is likely that the T_1-values will not be known, and in this case a series of spectra are obtained using arbitrarily chosen values for t; it is then a simple matter to select by interpolation an optimal value of t to ensure the disappearance of any required resonance.

B. Manipulation of F.I.D. Signal.—The second class of data-processing experiment involves mathematical manipulation of the free induction decay signal itself. The salient feature of this experiment is that any F.I.D. signal obtained experimentally is converted *via* Fourier transformation into a n.m.r. spectrum having a specific signal-to-noise ratio and a specific line-width for the individual transitions. This intrinsic relationship between the F.I.D. signal and the final spectrum may be altered simply by mathematically weighting some section of the F.I.D. signal immediately prior to Fourier transformation;[22] increasing the weighting of the early section of the F.I.D. signal improves the effective signal-to-noise ratio of the final spectrum, whereas increasing the weighting of the later part improves the resolution. Each of these alternatives involves a compromise, however, since

[21] R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, 1971, **54**, 3367.
[22] R. R. Ernst, R. Freeman, B. Gestblom, and T. R. Lusebrink, ***Mol. Phys.***, 1967, **13**, 283.

any increase in signal-to-noise is accompanied by some loss of resolution, and *vice-versa.*

A simple example[23] of the way in which this experiment can be used to improve the resolution of an already well dispersed spectrum is given in Figure 6, which

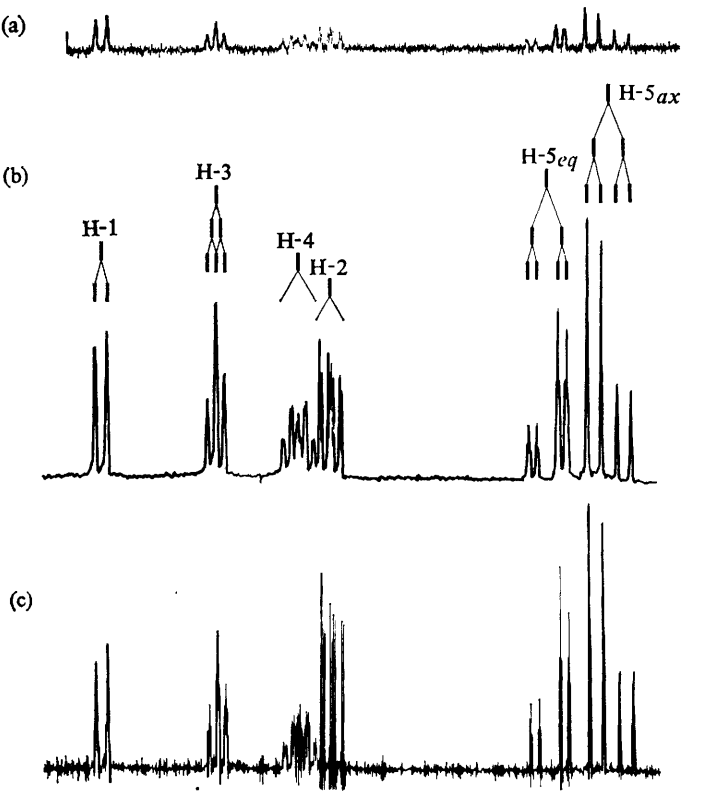

Figure 6 *Partial* 100 MHz ^{1}H *n.m.r. spectra of* 1,2,3,4-*tetra-O-acetyl-β-*D-*ribopyranose in* [$^2H_6$]*acetone* (0.1 mol l^{-1}) at 42°C. (a) *shows the result of a single continuous-wave scan, with a sweep-width of* 250 Hz *and a total scan time of* 1000 s. *The spectrum in* (b) *shows the FT summation of* 100 *transients, each with an acquisition time of* 3.0 s; *the total time used to obtain this spectrum was* 1500 s. *The spectrum given in* (c) *was derived from the same F.I.D. as* (b) *but a resolution-enhancement weighting factor of* 1.0 *units was applied immediately prior to the F.T.*
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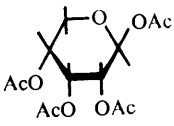
shows spectra of 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose (9). The spectra in Figures 6(b) and (c) were obtained from the same F.I.D. signal, that in (b) by direct Fourier transformation, and that in (c) with prior mathematical weighting

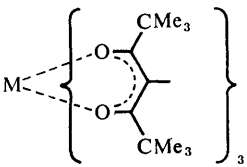
[23] L. D. Hall, C. M. Preston, and J. D. Stevens, *Carbohydrate Res.*, in the press.



(9)

of the latter part of the F.I.D.;* this same result is more clearly visible in Figure 7, which shows an expanded representation of the H-5 resonances. The excellent resolution of the small (*ca.* 0.5 Hz) long-range coupling in the H-5e resonance illustrates the power of this approach; indeed, it now seems sensible to re-examine the entire area of long-range coupling in carbohydrates. It is only necessary to caution here that there is an upper limit to the amount of weighting that can be applied to a F.I.D. signal, beyond which the entire information content of the F.I.D. is destroyed. This feature, together with the systematic relationship between the enhancement of resolution and the accompanying degradation of signal-to-noise ratio, is illustrated in Figure 8.

C. Chemical Manipulation.—There are many ways whereby spin–lattice relaxation times can be chemically manipulated. The example considered here derives from two sources; the known binding of lanthanide shift reagents, such as the europium derivative (10), to an organic molecule *via* a hydroxy-group,[24] and the



(10) M = Eu

(11) M = Gd

equally well documented effect of paramagnetic metal ions on the relaxation times of associated ligands.[25]

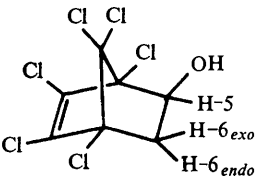
The T_1-values of the bicycloheptenol derivative (12) are shown[26] in Table 2; these accord with the inverse sixth-power dependence; both the geminal protons relax faster than the methine proton, and of these two, that which is nearest to the methine proton relaxes the faster, but barely so. Table 2 shows the effect of adding *ca.* 10^{-4} molar equivalents of tris(dipivalomethanato)gadolinium(III) (11). The relaxation of the protons of the bicycloheptanol derivative is then dominated

* This experiment demonstrates another aspect of the FT method; namely the improvement of the signal-to-noise ratio of an n.m.r. spectrum. This ratio can be improved by repeated sampling of the magnetization of the system, and it increases as the square root of the number of times the spectrum is scanned. The improved signal-to-noise ratio of the spectrum in Figure 6(b) was obtained by averaging 100 transients prior to Fourier transformation.

[24] I. M. Armitage, G. Dunsmore, L. D. Hall, and A. G. Marshall, *Canad. J. Chem.*, 1972, **50**, 2110; A. Arduini, I. M. Armitage, L. D. Hall, and A. G. Marshall, *Carbohydrate Res.*, 1973, **26**, 255.

[25] C. D. Barry, A. C. T. North, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *Nature*, 1971, **232**, 236; G. N. La Mar and J. W. Faller, *J. Amer. Chem. Soc.*, 1973, **95**, 3817.

[26] V. M. Gibb and L. D. Hall, unpublished results.



(12)

by the dipole–dipole relaxation arising from the gadolinium species. This contribution would be expected to fall off with the increase in separation between the gadolinium atom and the individual proton. As expected, the methine proton, which is nearer to the gadolinium ion in the gadolinium–bicycloheptenol complex, now relaxes faster than either of the geminal protons. Furthermore, of these two, it is now the geminal proton which is *cis* to the hydroxy-group which relaxes the faster.

It is important to note that not only has the entire relative ordering of the

Table 2 *Relaxation rates*/s^{-1} *for 1,2,3,4,7,7-hexachloro-5-hydroxynorborn-2-ene as a* 0.1 mol l^{-1} *solution in* $CDCl_3$. *Column A shows the data for a normal solution, without degassing. The data in column B were obtained after the addition of* 1.92×10^{-4} mol l^{-1} *of the gadolinium reagent* (*11*). *Column C gives the numerical difference between columns B and C, and represents the relaxation contributions from* (*11*).

Proton studied	*A*	*B*	*C*
OH	0.211	4.35	4.14
H-5	0.220	1.61	1.39
H-6*exo*	0.341	1.47	1.13
H-6*endo*	0.391	1.04	0.65

proton T_1-values been inverted by the gadolinium complex but also that the differential between the *geminal* protons has been enhanced. This type of manipulation should significantly extend the scope of spin–lattice relaxation studies of more complex substances, at least for those which have a dominant locus of association with a paramagnetic metal.

6 Conclusions

Ever-increasing access to FT n.m.r. spectrometers is clearly going to open up a number of exciting new dimensions to proton n.m.r. studies of carbohydrates and other organic derivatives. It is now possible to obtain routinely spectra of satisfactory quality from the amount of material commonly isolable from a single thin-layer chromatography plate. Providing that a suitable mathematical weighting program is available it is also possible to improve the effective resolution of n.m.r. spectrum at least twofold; this means that it is now practical to measure coupling constants of less than 0.1 Hz with high accuracy, and as a result it is

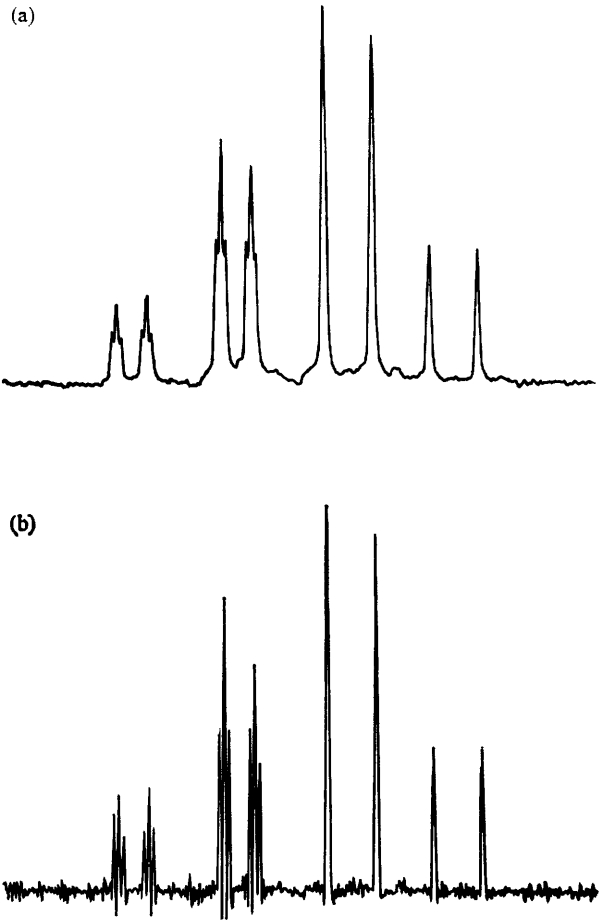


Figure 7 *Expansions of the* H-5_{eq} *and* H-5_{ax} *resonances of* 1,2,3,4-*tetra-O-acetyl-β-*D-*ribopyranose given in Figure* 6. (a) *corresponds to Figure* 6(a) *and* (b) *corresponds to Figure* 6(b). *The half-height width of the H-*5_{eq} *transitions in* (b) *is less than that of the* H-5_{ax} *transitions, implying the presence of a small, but unresolved, coupling into* H-5_{ax}. (Reproduced by permission from *Carbohydrate Res.*, 1975, **41**, 41).

now appropriate to study anew the phenomenon of long-range coupling of carbohydrates. Furthermore, the fact that proton spin–lattice relaxation times can, and do, vary substantially from one site to another in a monosaccharide makes possible the routine simplification of spectra *via* the measurement of partially relaxed spectra. One of the more useful facets of this phenomenon is that it may be possible routinely to eliminate the resonance of methylene protons without the need for specific deuteriation of that site; this has very important implications for studies of nucleosides and nucleotides. Finally, the rapid rate

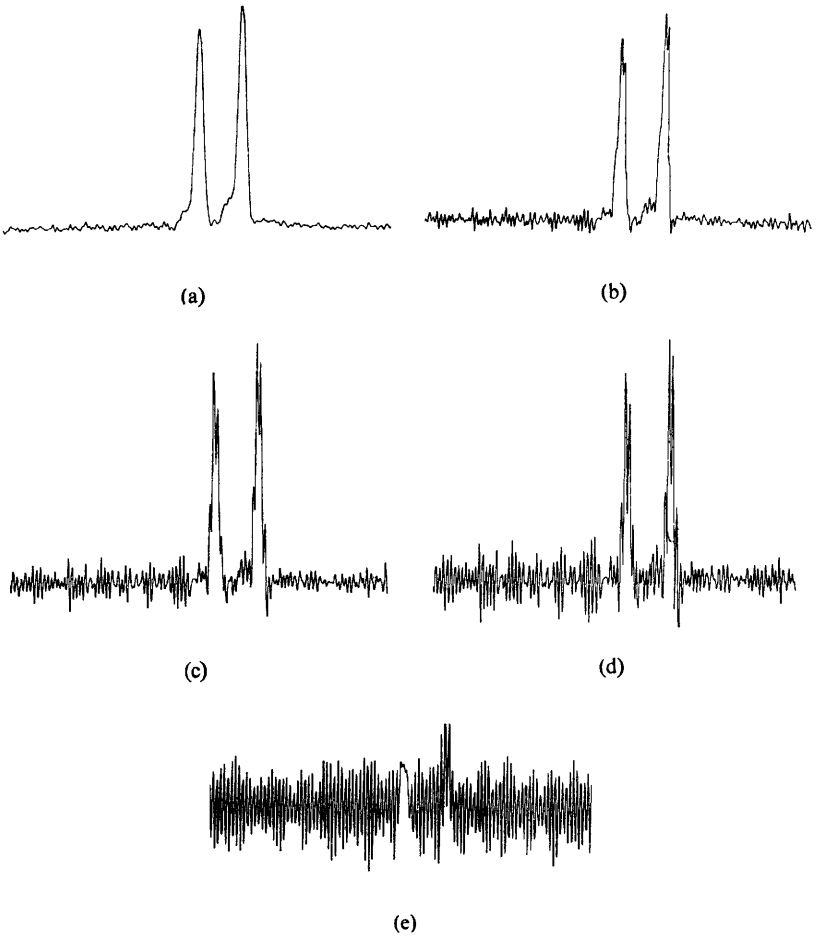


Figure 8 *The* H-1 *resonance of* 1,2,3,4,-*tetra-O-acetyl-β-*D-*ribopyranose in* [$^2H_6$]*acetone solution* (0.1 mol l^{-1}) *at* 42°C. *All the spectra were based on the F.I.D. signal resulting from* 100 *transients with an aquisition time of* 3.0 s *and a pulse-delay time of* 12 s (*total time* 1500 s) (a) *is the result of direct F.T. with no resolution enhancement;* (b) *was obtained by applying a resolution enhancement factor of* 1.5; (c) *a factor of* 1.0; (d) *a factor of* 0.8; *and* (e) *a factor* 0.7. *In* (d) *the* H-1 *resonance is clearly visible as a doubletted quartet with long-range couplings of* ca. 0.5 Hz. *The spectrum shown in* (e) *illustrates the effect of applying too sharp a weighting function.*
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at which spectra can be acquired in the form of a F.I.D. signal opens up important areas for kinetic studies.

The implications of determining the conformations of carbohydrate derivatives by measuring the relative distances between protons are many and varied.

Importantly though, this approach should throw further light on some of the more subtle aspects of carbohydrate conformations, especially of biologically relevant systems such as nucleosides and carbohydrate antibiotics. Beyond that, it is obvious that the methods discussed here have considerable potential for studies of any organic or organometallic system which has a well-dispersed n.m.r. spectrum. In that sense it is to be hoped that the present studies will provide a similar stimulus to that of Lemieux's pioneering investigation[27] of vicinal proton–proton coupling constants.

The author is deeply indebted to his students and co-workers for their enthusiastic and stimulating support and to Professors A. B. Foster, L. Hough, and C. A. McDowell for their steadfast advice and continuing encouragement. The experiments used to illustrate this presentation were performed by Christopher W. M. Grant and Caroline M. Preston, and these studies were supported by operating grants from the National Research Council of Canada and by a Fellowship from the Alfred P. Sloan Foundation.

Notes added in proof:

Since this lecture was presented we have developed[4,28,29] a selective-pulse relaxation experiment which provides direct experimental evidence that the proton spin–lattice relaxation of a monosaccharide generally occurs exclusively *via* the dipole–dipole mechanism. The same type of experiment gives a quantitative measure of specific interproton relaxation contributions and, thereby, of interproton distances. Intercomparisons of the relaxation rates of a specifically deuteriated sugar with its 'normal' counterpart also allow[13,28] a simple evaluation of individual intramolecular relaxation contributions.

These methods have been applied to several series of carbohydrates: (*a*) derivatives of the 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and the allofuranose systems;[28] (*b*) methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside and *arabino*-hexopyranoside;[28] (*c*) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride, its β-anomer, and the corresponding D-xylopyranose derivatives;[30] (*d*) derivatives of uridine;[30] (*e*) all eight isomers of the 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-hexopyranose system;[30] (*f*) derivatives of 1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranose;[31] (*g*) derivatives of methyl 2,3-*O*-isopropylidene-β-L-rhamnopyranoside.[31]

In every instance the proton T_1-values appear to reflect sensibly the solution geometry of the derivatives involved. For the 1,6-anhydro-derivatives it has been proven[30] that the effects on the proton T_1-values of changes in solvent and of solute concentration are associated with changes in τ_c. Preliminary studies[32]

[27] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Amer. Chem. Soc.*, 1958, **80**, 6089.
[28] C. M. Preston, Ph.D. Thesis, Department of Chemistry, University of British Columbia, 1975.
[29] K. Bock, L. D. Hall, T. Marcus, and J. Sallos, unpublished results.
[30] K. Bock and L. D. Hall, unpublished results.
[31] L. D. Hall and K. F. Wong, unpublished results.
[32] K. Bock and L. D. Hall, *Carbohodrate Res.*, 1975, **40**, C3.

of the carbon-13 relaxation times of some sugars in aqueous solutions imply that the derivatives are tumbling isotropically. A study has also been made[33] of the effects of gadolinium ions on the relaxation times of several carbohydrates in aqueous solutions.

[33] C. M. Preston and L. D. Hall, *Carbohydrate Res.*, 1975, 41 53.