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A GENERAL SURVEY OF THE PROTON, SPIN–LATTICE RELAXATION-TIMES OF MONOSACCHARIDE DERIVATIVES*

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ABSTRACT

A Fourier-transform method has been used to determine the spin–lattice relaxation-times (T_1 values) of some of the proton resonances of a selection of carbohydrate derivatives, including pyranoid and furanoid sugars, deoxy sugars, and acetamido sugars in aqueous solution, and some esterified sugars in benzene solution. A discussion is given of the methods used to process the experimental data, and of the chemical relevance of the dependences observed. The use of selective deuteration to identify contributions to intramolecular, dipole–dipole relaxation, and of partial relaxation to remove a water resonance, is each illustrated.

INTRODUCTION

Most practising carbohydrate chemists are familiar with the derivation, and use, of three sets of nuclear magnetic resonance (n.m.r.) parameters, namely, the chemical shifts, coupling constants, and integrated areas. Successful as it is, however, this approach ignores the fact that each high-resolution, n.m.r. spectrum contains, implicitly at least, two further sets of nuclear parameters: the spin–spin and the spin–lattice relaxation-times. To date, carbohydrate chemists have largely ignored the diagnostic potential of these parameters. Of the many good reasons for this neglect, the most cogent is that, until very recently, the experimental methods available for determining relaxation times have been totally incompatible with the complex n.m.r. spectra obtained for a carbohydrate derivative. However, with the advent of Fourier-transform, n.m.r. methods[2], it is now feasible to measure spin–lattice relaxation-times (T_1 values) on a routine basis, and, hence, to evaluate the rather extensive diagnostic potential of this area.

It is not appropriate to attempt to give here a full account[3] of the phenomenon of spin–lattice relaxation; however, some brief statements are mandatory. Spin–

*Part 4 of a series, "Applications of Pulsed Nuclear Magnetic Resonance Spectroscopy". For Part 2, see ref. 1.

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lattice relaxation involves a transfer of energy from the spins of the magnetic nuclei to their surrounding environment (the lattice). The T_1 value of a particular nuclear-relaxation time is actually the inverse of the *rate* of transfer of energy from the "spins" to the "lattice". There are a number of mechanisms that allow this transfer to occur, most of which involve the interaction of the spin with randomly fluctuating magnetic fields arising in the lattice. In general, more than one of these mechanisms can operate simultaneously, and can contribute to the overall relaxation rate (or $1/T_1$) of a particular nucleus. Were it necessary to factor out the individual contributions of several mechanisms to the total relaxation rate prior to any chemical application, then, quite likely, measurements of spin–lattice relaxation would be of little interest to the practising chemist. Fortunately, this is not always the case, and it is often possible to make the measurements under conditions such that one mechanism dominates, sometimes exclusively.

For the protons of a small molecule undergoing isotropic motion in a dilute solution in a magnetically inert solvent, it is reasonable to assume that the dominant contributions to relaxation arise *via* the intramolecular, dipole–dipole (I.D.D.) mechanism. The significance of this is apparent from the form of the equation that defines this mechanism:

$$\left(\frac{1}{T_1}\right)_{\text{I.D.D.}} \propto \frac{\gamma_i^2 \gamma_j^2}{r_{ij}^6} \cdot \tau_c$$

The equation describes the dipole–dipole relaxation between two nuclei (i,j) of magnetogyric ratios γ_i and γ_j, separated by a distance r, and fixed to a molecule undergoing isotropic rotation with a correlation time τ_c. Several important points emerge from this equation. First, the contribution that any nucleus will make to the relaxation of any proton will be proportional to the square of its magnetogyric ratio γ. Because, in an ordinary sugar, protons are the only nuclei having a significant value of γ, it follows that relaxation will arise mainly from the contributions of other protons in the molecule*. Second, the efficiency of this inter-proton relaxation is critically dependent on the distance between the two hydrogen nuclei; hence, it may be anticipated that there will be geometric dependences to inter-proton relaxation contributions and, consequently, to the composite T_1 values. Third, the relaxation is dependent on the correlation time, τ_c, which is related to the rate of rotation of the molecule in solution, a rate dependent on the temperature and the concentration**.

*The other nucleus of high magnetogyric ratio is fluorine-19.

**This rate can be further complicated by anisotropic motion of the whole molecule, and by the freer motion of any side chains and methyl groups. Complications associated with correlation times should not generally be important for most monosaccharides and their derivatives; and, on those occasions when they are, study of the correlation times themselves may provide the chemist with a probe for studying the interactions between (*a*) solvent and solute molecules, or (*b*) pairs of solute molecules on a microscopic level, and also for comparing the strength of associations between a solute and various solvents.

Although the potentiality of relaxation studies of carbohydrates is clear, there are a number of experimental and theoretical limitations that complicate their application. At the experimental level, attempts must be made to ensure the dominance of intramolecular relaxation over intermolecular effects. This is best accomplished by working with dilute solutions in a magnetically inert solvent, that is, a solvent that has neither a proton nor a fluorine substituent; in practice, it is common to use a deuterated solvent. Even if this approach does not entirely eliminate intermolecular contributions, use of a common solvent and a standard concentration is likely to keep those contributions constant.

Even if it is justifiable to assume that the intramolecular, dipole–dipole mechanism is the exclusive mechanism of proton relaxation, it is still not clear that a unique T_1 value for an individual proton-resonance can be defined; this is because the presence of cross-relaxation[4] can cause (*a*) significant differences between the T_1 values measured on the individual components of a spin-coupled multiplet, and (*b*) deviations from a pure, exponential rate of recovery of the equilibrium magnetization. Fortunately, this and several other problems that may have discouraged previous workers from attempting studies in this area do by no means preclude many useful qualitative, and even some quantitative, applications.

In a previous paper of this series[1], we described several geometric dependences for the proton T_1 values of some substituted pyranoses. Those data clearly vindicated earlier assumptions[5] concerning the importance of intramolecular, dipole–dipole relaxation. In the present studies, we have surveyed a wider range of carbohydrates in both aqueous and organic solvents*.

RESULTS AND DISCUSSION

The spin–lattice relaxation-times of all compounds were determined by using a standard, three-pulse sequence; the main advantage claimed[6] for this sequence over the more conventional, two-pulse sequence is that it compensates for small changes in resolution, and so is very useful when time-averaging is required. We have also found it very convenient for complex spectra where multiplets may be broad, or overlapping, or both.

The spectra shown in Fig. 1 are typical of the sets of partially relaxed spectra that constitute the experimental data. Details of the method used to process these spectra and to obtain the spin–lattice relaxation-times are given in the Experimental section.

The data obtained for aqueous solutions of the hexoses and pentoses are summarized in Table I; they indicate several stereospecific dependences for the contributions made by other ring-protons to the spin–lattice relaxation-times of anomeric protons. There are two levels at which the data can be interpreted: by comparison either of the individual numbers, or of ratios. The first approach is valid

*For a preliminary communication, see ref. 5.

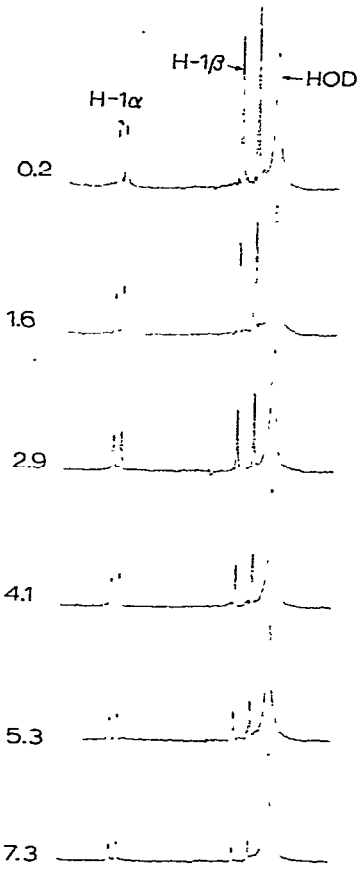


Fig. 1. Partially relaxed, proton n.m.r. spectra (100 MHz) of a degassed solution (10% w/v) of D-glucose in D_2O (99.96%), measured at 51.5°. [The resonances of the anomeric protons and the residual-water peak are shown. The delay time (sec) between the 180° and 90° pulses is indicated to the left of each spectrum. It should be noted that, with progressively longer delay-times, the intensity of H-1 β, the more rapidly relaxing proton, decreases more rapidly than that of H-1 α.]

only if the sugars all have the same rotational-correlation times. For D-glucose (**1**) and D-galactose (**2**), the axially oriented H-1 of the $^4C_1$ conformer of the β anomer has a significantly shorter relaxation-time than the equatorially attached proton of the α anomer in the same conformation. The close similarity of the data for these two sugars implies that the proton at C-4 has little effect on the relaxation of the anomeric proton. Comparison of the data for D-glucose (**1**), on the one hand, with those of D-mannose (**3**), L-rhamnose (**7**), and D-talose (**8**), on the other, suggests that a gauche interaction is very effective in causing relaxation. The ratio of α/β rises from 1.9:1 for D-glucose to ~3.3:1 for **3**, **7**, and **8**. The short relaxation-times for both anomers of 2-deoxy-D-*arabino*-hexose (**4**) confirm the importance of the gauche interaction. Furthermore, evidence that a *trans*-diaxial relationship makes only a

TABLE I

SPIN–LATTICE RELAXATION TIMES (SECONDS) FOR THE H-1 RESONANCES OF GLYCOPYRANOSES IN DEGASSED SOLUTIONS (10%, w/v) IN DEUTERIUM OXIDE (99.96%), MEASURED AT 42°

Sugar	*Relaxation time (seconds)*		*H-1α/H-1β*
	H-1α	*H-1β*	
D-Glucose (1)	4.4	2.3	1.9
D-Galactose (2)	4.0	2.4	1.7
D-Mannose (3)	5.9	1.8	3.4
2-Deoxy-D-*arabino*-hexose (4)	3.8	1.7	2.2
D-Allose (5)	7.0	4.5	1.6
D-Altrose (6)[a]	4.4	2.9	1.5
L-Rhamnose (7)	6.6	2.1	3.1
D-Talose (8)	7.6	2.3	3.3
D-Idose (9)	3.7	2.9	1.3
D-Xylose (10)	5.7	3.3	1.7
D-Ribose (11)	4.6	6.6	0.71
D-Arabinose (12)	4.0	6.2	0.65
D-Lyxose (13)	7.0	3.4	2.0

[a]Concentration, 8%.

small, but significant, contribution to relaxation can be seen in the data for D-allose (5). Removal of one *trans*-diaxial interaction, by inversion of the configuration of C-3, lowers the α/β ratio from 1.9:1 to 1.6:1.

All of these qualitative observations imply a range of useful dependences that might facilitate assignment of anomeric configurations to pyranoses; for example, the three-fold differential between the T_1 values of the anomers of D-mannose clearly provides a rational basis for assigning their anomeric configuration. For all of the aforementioned sugars, both anomers have the $^4C_1$ conformation, and the axial proton has a much shorter relaxation-time than the equatorial one. The data for both D-altrose (6) and D-idose (9) are affected by time-averaging of the two chair conformations. Thus, the possibility of existence of more than one conformation in solution must be considered in applying T_1 data to the assignment of anomeric configuration (*vide infra*).

Continuing now with a qualitative discussion of the other hexoses studied, it is evident, from the data presented in Table II, that 2-acetamido-2-deoxy-D-hexopyranoses and methyl D-hexopyranosides also have pronounced differentials in the T_1 values of their anomeric protons. Although replacement of a 2-hydroxyl group by a 2-acetamido group has little effect on the relaxation time of H-1, the introduction of the anomeric methoxyl group does cause a decrease (of ~50%) in the T_1 value of the anomeric proton. That the latter effect includes the contribution made by the methoxyl protons is clearly demonstrated by the data obtained from the anomeric protons of the corresponding trideuteriomethyl D-glucopyranoside, given in Table II.

It had been hoped that, by working at constant concentration and temperature,

TABLE II

SPIN–LATTICE RELAXATION-TIMES (SECONDS) FOR THE ANOMERIC PROTONS OF SOME 2-ACETAMIDO-2-DEOXY-D-HEXOPYRANOSES AND OF SOME METHYL D-HEXOPYRANOSIDES, IN DEGASSED SOLUTIONS (10%, w/v) IN DEUTERIUM OXIDE (99.96%), MEASURED AT 42°

Compound	*Relaxation time (seconds)*		*H-1α/H-1β*
	H-1α	*H-1β*	
2-Acetamido-2-deoxy-D-glucose (**14**)	4.3	2.0	2.2
2-Acetamido-2-deoxy-D-mannose (**15**)	4.6	1.2	3.9
Methyl D-glucopyranoside (**16**)	2.7	1.6	1.7
Methyl-d_3 D-glucopyranoside[a]	4.7	2.2	2.1
Methyl D-mannopyranoside (**17**)	3.3	1.2[b]	2.8
Methyl D-xylopyranoside (**18**)	3.9	1.7	2.3

[a]Measured as a mixture, total concentration, 10% w/v. [b]Concentration, ~8%, w/v.

variations in intermolecular contributions to T_1 values would be eliminated. However, the observation of some seemingly random variations in the experimental data suggested that this approach had not been completely successful, and, hence, a separate study was made of the concentration and temperature dependences of the T_1 values of the anomeric proton of D-glucose. These results are summarized in Fig. 2 and Table III.

The plot showing the concentration dependence indicated that, over the range of 0.06 to 1.2 molar, the T_1 values change by ~35%. The decrease in T_1 values with increase in concentration is probably due to the change in correlation time with increase in the viscosity of the solution. Unfortunately, the concentration, arbitrarily chosen, at which our measurements were routinely made (~0.5 molar) is sufficiently high for some intermolecular contributions to remain; we now conduct all experiments at significantly lower concentrations. The data in Table III indicate that change in

Fig. 2. Graph showing the variation, with change in concentration, of the value of T_1 for the anomeric protons of D-glucose in degassed D_2O solution. (All measurements were made at 42°.)

TABLE III

SPIN–LATTICE RELAXATION-TIMES (SECONDS) OF THE ANOMERIC PROTONS OF D-GLUCOSE (10%, w/v) AS A FUNCTION OF TEMPERATURE

Temperature (degrees)	*Relaxation time (seconds)*		*H-1α/H-1β*
	H-1α	*H-1β*	
22.6	2.9	1.4	2.0
34.5	4.6	2.3	2.0
42.0[a]	4.4	2.3	1.9
51.5	6.2	3.3	1.9

[a]A redetermination on the same sample gave H-1α = 4.9, H-1β = 2.5; ratio, 1.9:1.

temperature can have a very significant effect on the T_1 value measured; for both of the anomeric protons, a temperature change of $\sim 30°$ causes a greater than twofold change in the T_1 value. Consequently, if data for different compounds are to be intercompared, it is necessary to keep the temperature constant (see the Experimental section). In the light of these two sources of variations, it is interesting that the ratio of the pairs of T_1 values for anomers appears to be essentially independent of the concentration or temperature at which the experiment is conducted*.

Evaluation of the T_1 values for the pentopyranoses (**10–13**), given in Table I, is again complicated by the fact that it is known that, in solution, several of these molecules are conformationally inhomogeneous. For systems undergoing rapid interconversion between the $^4C_1$ and $^1C_4$ conformers, the observed relaxation time, T_1, should be given by the expression

$$\left(\frac{1}{T_1}\right)_{\text{obs.}} = x \cdot \frac{1}{T_1}(^4C_1) + (1-x) \cdot \frac{1}{T_1}(^1C_4),$$

where x is the mole fraction of molecules in the $^4C_1$ conformation. Unfortunately, use of the T_1 values of the configurationally related hexoses as reference data for such calculations is not possible: perhaps, knowledge of the correlation times will prove valuable here. Despite this situation, some interesting comparisons can be made.

The T_1 values for both anomers of D-xylose (**10**) and for β-D-lyxose (**13**) are consistent with exclusive favoring of the $^4C_1$ conformer by these molecules, whereas the data for β-D-ribose (**11**) and β-D-arabinose (**12**) imply an equally marked favoring of the $^1C_4$ conformer; furthermore, it seems that solutions of α-D-ribose (**11**) and α-D-arabinose (**12**) are populated by both the $^4C_1$ and $^1C_4$ conformers, the proportion of the $^1C_4$ conformer being somewhat higher for α-D-arabinose than for α-D-ribose. Although these findings agree well with the evaluations reported by previous workers[7],

*The measurements published in the original preliminary communication[5] were made at a variety of different temperatures, yet, in each case, the ratio $(T_1)_\alpha/(T_1)_\beta$ was identical, within experimental error, to the data given in Tables I and II.

a significant disagreement appears to exist for α-D-lyxose (**13**). Lemieux and Stevens[7] and Rudrum and Shaw[8] postulated that solutions of this sugar should contain equal populations of the two chair conformers, and Angyal and Pickles[9] anticipated some degree of time-averaging. However, the very long T_1 value for α-D-lyxose is more consistent with the predominant favoring of the $^4C_1$ conformation of the molecule. More data and experience are needed before any definitive comments can be made.

Although the principal objective of the present study was to survey the behavior of sugars in aqueous solution, six peracetylated hexoses were also studied; the T_1 values for their anomeric protons are listed in Table IV. As before, there is a clear distinction between the relaxation times of the individual anomers. It is notewor-

TABLE IV

SPIN–LATTICE RELAXATION-TIMES FOR THE ANOMERIC PROTONS OF PERACETYLATED D-HEXOPYRANOSES IN SOLUTION IN C_6D_6 (10%, w/v), MEASURED AT 42°

Compound	*Relaxation time (seconds)*		*H-1α/H-1β*
	H-1α	*H-1β*	
D-Glucose pentaacetate (**19**)	3.3	2.0	1.7
D-Galactose pentaacetate (**20**)	3.7	2.3	1.6
β-D-Galactose penta(acetate-d_3) (**21**)	—	2.6	—
α-D-Idose pentaacetate (**22**)	3.8	—	—

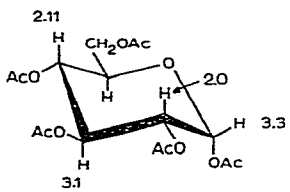
thy that each of these times is significantly shorter than that of the corresponding free sugar, although the ratio is approximately the same as that for the corresponding free sugar.

For clarity, the T_1 values of the other protons that were clearly resolved are given in Fig. 3. The spectrum of the β-D-*galacto* derivative (**20**) provides useful insight into the variations in T_1 values that can occur for protons of a single compound, without the complications that attend intercomparisons of different molecules. The molecule contains four axially oriented protons, whose relaxation times vary from 0.92 to 4.42 sec. The H-2 resonance has the longest T_1 value, because it has no protons optimally oriented for relaxation. The 1,3,5-triaxial arrangement of the bonds to H-1, H-3, and H-5 (on the "lower" face of the molecule) provides a mutual relaxation network, with both H-3 and H-5 benefitting additionally from the *gauche* relaxation with H-4. The H-5 resonance has a characteristically short T_1 value, and this appears to be associated with the particularly effective interactions with the protons on C-6.

Comparison of the T_1 values of derivative **20** with the corresponding values for the per(deuterioacetyl)ated derivative **21** proved interesting (see Table V). The times for the deuterated derivative are between 5 and 25% longer than those of the normal derivative, which indicates that there is a significant relaxational contribution

from the protons of the acetyl groups; evidently, these contributions vary from one group to another.

We have previously alluded to the complications that can arise from cross-



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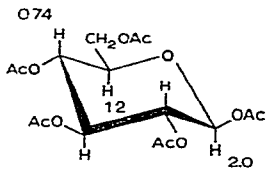

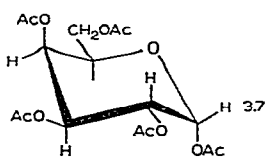

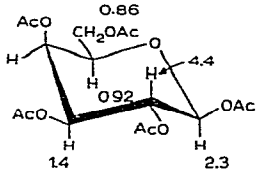

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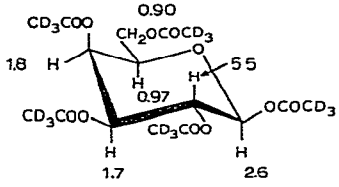

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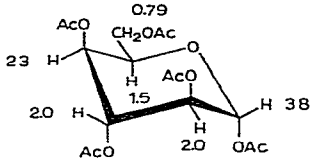

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Fig. 3. Spin–lattice relaxation-times for the assignable protons of peracetylated glycopyranoses in solution (10%, w/v) in C_6D_6.

TABLE V

RATIOS OF THE SPIN–LATTICE RELAXATION-TIMES FOR PENTA-*O*-ACETYL-*β*-D-GALACTOPYRANOSE (**20**) AND ITS PER(TRIDEUTERIOACETYL)ATED COUNTERPART (**21**)

Proton resonance	*Relaxation ratio (deuterated/nondeuterated)*
H-1	1.15:1
H-2	1.25:1
H-3	1.22:1
H-4	1.12:1
H-5	1.05:1
H-6	1.05:1

relaxation[4], and the nicely resolved spectra of **20** and its deuterated analog (**21**) provided an ideal model for making an evaluation of this point. The data are most easily displayed on the original spectra (see Fig. 4), from which it may be seen that the relaxation times of the individual transitions of a multiplet resonance can differ widely (as for H-1) or be identical (as for H-4). Although the general theory of cross-relaxation in spin-coupled systems is not yet available, the theory[4] of the two-

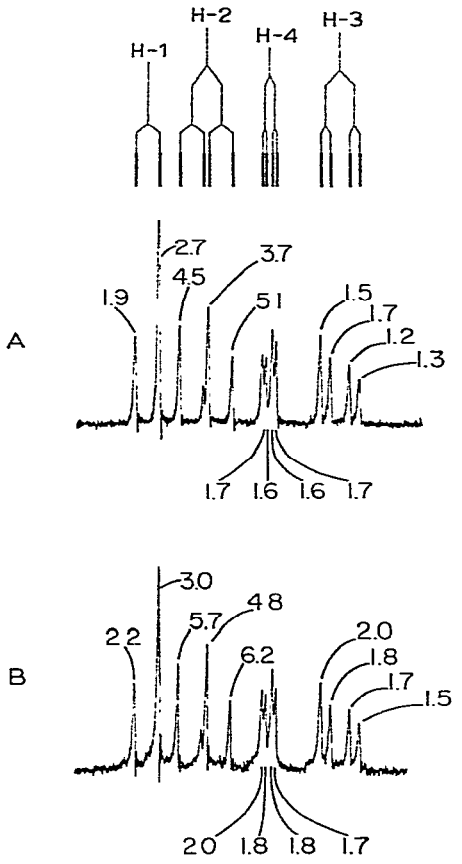

Fig. 4. Partial, 100-MHz proton n.m.r. spectra of solutions of A penta-*O*-acetyl-β-D-galactopyranose (**20**), and B its per(deuterioacetyl)ated analog (**21**) in C_6D_6. [The spin-lattice relaxation-times (in sec) of the individual transitions are as indicated.]

spin system provides some insight; it is evident that cross-relaxation requires that, in addition to the mutual relaxation of the two spins being studied, there be a significant contribution from some external sources; furthermore, the amount of cross-relaxation will depend on how closely coupled the two-spin system is. The proton spin-systems of **20** and **21** both fulfil the first of these conditions, and inspection of the data in Fig. 3 indicate some accord with the latter condition. Thus, the transitions

of H-1 and H-2, which form a closely coupled system (J/δ 0.42), have a larger T_1 value differential than those of H-3 and H-4 (J/δ 0.14). Although these complications add to the difficulties of quantitatively interpreting these experiments, it must be emphasized that they do not significantly prejudice *qualitative* applications, as these variations appear to be smaller in magnitude than the specific variations that are of interest.

CONCLUSIONS

It is obvious from these studies that the proton, spin-lattice relaxation-times of pyranoses show a number of stereospecific dependences. Although explanation of these dependences on a quantitative basis is difficult, it is apparent that they are sufficiently clear cut to provide a useful basis for configurational assignments. That these dependences have generality exceeding the scope of the present study is demonstrated by the results of other studies. For example, we have shown[1] that a series of 2-deoxy-2-halo-D-hexopyranose derivatives exhibits a similar range of dependences. More important, it is possible to distinguish between isomeric pairs of cyclohexane derivatives, as may be seen on comparing the T_1 values of the protons on C-1 of the isomeric 4-*tert*-butylcyclohexanols, shown in Fig. 5.

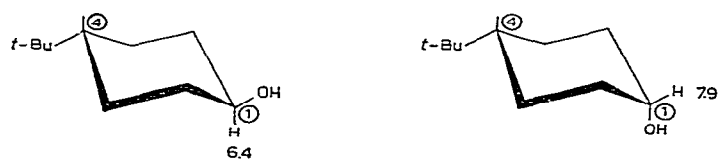

Fig. 5. Proton, spin–lattice relaxation-times for the protons on C-1 of the isomeric 4-*tert*-butylcyclohexanols. [Measured in C_6D_6 at 42° as a mixture (total concentration, 0.2M) having a *cis*:*trans* ratio of 1:3.]

From the standpoint of carbohydrate chemistry, it is important to note that these T_1 dependences extend to oligosaccharides[10], and, if the few data available to us at this time are typical, to furanose systems; the data given in Table VI imply a novel method for assigning the anomeric configuration of ribo-nucleoside and -nucleotide systems, a problem that is notoriously difficult on the basis of "conventional" n.m.r. parameters.

Another very useful application of relaxation studies lies in the removal[11] of the unwanted, residual peaks of deuterated solvents. The HOD peak, for example, has a much longer relaxation-time than the protons of the carbohydrate. To take advantage of this, a standard pulse-sequence of (T... 180° ... t ... 90°), which can be time-averaged if necessary, is used. The delay time (T) is sufficient to allow complete relaxation of the protons of both the solvent and the carbohydrate. The whole spectrum is then inverted by a 180° pulse. which inverts the magnetization from its normal equilibrium value (M_0) to $-M_0$. The second delay-time (t) is the time required for

TABLE VI

SPIN–LATTICE RELAXATION-TIMES OF THE FURANOSE FORMS IN FULLY MUTAROTATED SOLUTIONS OF SEVERAL HEXOSES AND PENTOSES IN SOLUTION (10% w/v) IN 99.96% DEUTERIUM OXIDE, MEASURED AT 42°

Sugar[a]	*Relaxation time (seconds)*		*H-1α/H-1β*
	H-1α	*H-1β*	
D-Allose (5)	10 ±1	9 ±1	~1.1
D-Altrose (6)	7.2	7.5	0.96
D-Talose (8)	8.0	8.4	0.95
D-Idose (9)	9.2	5.9	1.6
D-Ribose (11)	8.5	13.2	0.64

[a]Concentration, 2%.

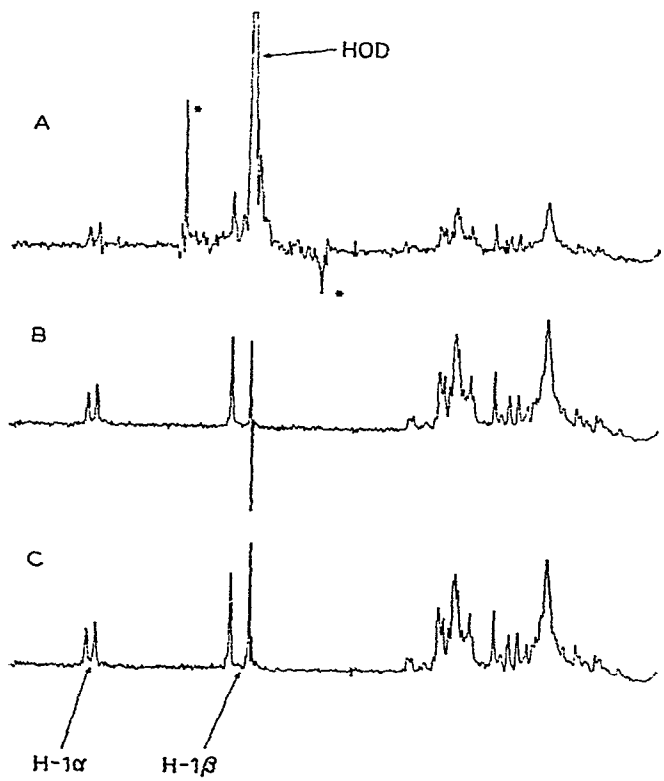

Fig. 6. Partially relaxed spectra of a solution (1% w/v) of D-glucose in D_2O (99.7%) at 42°; the D-glucose was not lyophilized with D_2O prior to this experiment. [A shows a single-pulse, Fourier-transform spectrum. The spectra B and C were the result of 8 acquisitions each, using a two-pulse sequence with pulse delays set at 12.8 and 13.0 sec, respectively. These two spectra were deliberately chosen to show how finely the delay time has to be selected if complete elimination of the water peak is to be achieved.]

the HOD magnetization to decay from "$-M_0$" to "zero", by which time the signals of the faster-relaxing, carbohydrate protons have fully, or nearly, recovered to M_0. The 90° pulse then produces a normal spectrum for the solute, and no response for the HOD peak. For sugars, this procedure can be performed even in undegassed solutions, in which the T_1 differences are lessened. Fig. 6 shows the water-nulled spectrum for a 1% (w/v) solution of D-glucose. Eight transients were time-averaged in order to increase the signal-to-noise ratio, and the total accumulation time was 15 min.

EXPERIMENTAL

Materials. — Most of the free sugars used were commercially available samples that were not further purified. The following samples were gifts from other workers: D-allose (Prof. K. N. Slessor), D-idose pentaacetate (Prof. H. Paulsen), methyl β-D-mannopyranoside (Gwen H. Bebault), and D-galactopyranose pentaacetate as the α and β anomers and as the β-pentakis(trideuterioacetate) (Liane Evelyn). D-Idose was prepared from β-D-glucopyranose pentaacetate by the method of Paulsen and co-workers[12], and D-altrose from methyl 4,6-*O*-benzylidene-α-D-altropyranoside[13] (Paul Steiner). The cyclohexanols were donated by Zinat Akhtar. All samples had melting points in accord with the literature values.

Unless otherwise indicated, the samples were studied as 10% (w/v) solutions in 99.96% D_2O, or as solutions in C_6D_6; acetone (~2 μl) was added to each sample, in a tube, and its resonance provided an internal reference standard both for resolution adjustments and for the presence of paramagnetic impurities. All samples were degassed by six freeze–pump–thaw cycles (in order to remove dissolved oxygen), and the tubes were then sealed in vacuo.

Measurements of nuclear magnetic resonance. — The measurements of T_1 values were made with a Varian XL-100 (15) spectrometer equipped with a Varian 620L (16K) computer and the standard programs provided by Varian Associates. A three-pulse sequence ($T \ldots 180° \ldots t \ldots 90° \ldots T \ldots 90°$) was used; it could be repeated an appropriate number of times to increase the signal-to-noise ratio, but, usually, only one sequence was necessary. The longer delay time (T) was always set to at least $5T_1$ sec, and often longer, to allow for complete return to equilibrium magnetization.

Each experiment was performed by measuring the n.m.r. spectrum as a function of different values of the delay time (t); generally, 10 to 20 separate spectra were recorded. The delay times were usually within the range 0.1 to 10 sec.

Handling and reduction of data. — The three-pulse sequence used in these determinations displays the n.m.r. spectrum in the form* ($M_0 - M_t$), which is always positive, and which decays from an initial value of $2M_0$ to zero as the delay time is increased.

*Where M_0 is the original intensity of the magnetization and M_t is the intensity at time t.

The peak heights of the individual transitions were measured as a function of delay times (t), and these data were then used as the input for a computer* program; this program calculated a first-order, least-squares fit to a straight line, and then plotted the fitted line, together with the individual data-points. The slope of this plot of ln $(M_0 - M_t)$ *versus* t is $-T_1^{-1}$.

As already discussed in this paper, the measurable T_1 value of any transition of a spin-coupled system is a composite value. In addition to other complications, as the delay time t increases, cross-relaxation causes the T_1 decay to proceed more slowly than a purely exponential decay; a typical example is given in Fig. 7. A further complication is that the contribution from cross-relaxation can differ for each

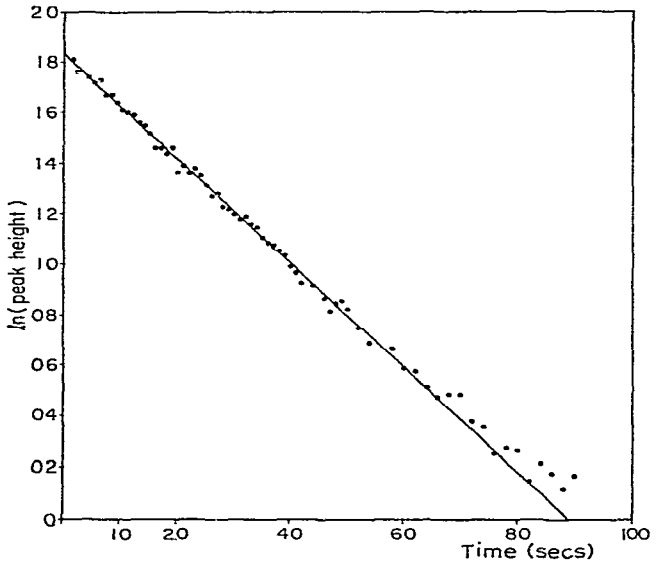

Fig. 7. Typical decay plot of $(M_0 - M_t)$ *versus* t for the upfield transition of H-1α of a solution of D-glucose (10% w/v) in D_2O (99.96%) measured at 42°. [Note the "slowing down" (from purely exponential decay) at the longer delay times of $\sim$1 T_1 period and greater.]

transition of a spin-coupled multiplet. As a result, for evaluating the experimental data, it is necessary to select some method that will minimize the discrepancies that arise from these variations. Unfortunately, there is, as yet, no general theory that can provide an objective basis for any evaluation.

With the foregoing considerations in mind, a series of preliminary experiments were performed that indicated the variations in apparent T_1 values that occur with different values of the delay times.

Fig. 8 shows typical variations in T_1 values obtained when data from successively longer delay-times are included in the calculations. For the H-1α transitions, it may

*All calculations were performed on an IBM 360-67 computer situated in the U.B.C. Computer Centre.

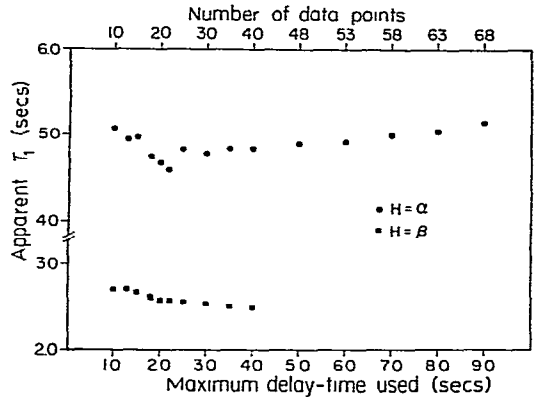

Fig. 8. Plot showing the variation of the apparent T_1 values as a function of the time-period of the plot for solution of D-glucose (10% w/v) in D_2O (99.96%) at 42°. [Upper trace: values for the average of the two H-1α transitions. Lower trace: values for the down-field transition of H-1β; the upfield transition was obscured by the HOD peak.]

be noted that, after an initial decrease, there is a steady increase in the values obtained as t is carried out to nine sec ($\sim 2T_1$), and the total variation is $\sim$20%. In general, we found that apparent T_1 values tend to *increase* as longer times are used, because cross-relaxation increasingly slows the decay from a purely exponential one. However, an increase is not always observed, and, for this example, the values for H-1β show only a steady *decrease*. Similar, detailed evaluations were made for the H-1β resonance of a 10% solution of D-mannose. With plot times varied from 1.6 to 12.0 sec, the apparent T_1 value increased from 5.5 to 6.3 sec.

On the basis of this experience, all T_1 values given in this paper were obtained from plots that were carried out to (one T_1 period +10–20%) sec. This is one of the reasons why some of the data given here differ slightly from the values in the preliminary communication[5]; another is that some of the previous measurements were made at different temperatures. It is important to note that the ratio of $(T_1)_\alpha$:$(T_1)_\beta$ appears to be independent, at least within experimental error, of the time period of the plot.

The T_1 values given here are reproducible to within better than $\pm$5%; however, it seems more realistic to consider that there is an experimental error of $\pm$10%. Even this magnitude of error is small, compared with the stereospecific dependences discussed.

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