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A GENERAL SURVEY OF THE PROTON SPIN–LATTICE RELAXATION-TIMES OF SOME OLIGO- AND POLY-SACCHARIDE DERIVATIVES*†

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ABSTRACT

A Fourier-transform method has been used to measure the spin–lattice relaxation-times (T_1 values) of the anomeric protons of a selection of oligo- and poly-saccharide derivatives. Although systematic variations are found for the substances of lower molecular weight, these variations are essentially non-existent at higher molecular weights. Data for the disaccharides cellobiose, maltose, lactose, gentiobiose, and melibiose demonstrate that proton T_1-values may provide a powerful method for evaluating conformations of oligosaccharides.

INTRODUCTION

In a previous survey, we reported[4] the stereospecific dependencies of the spin–lattice relaxation-times of the anomeric protons of a range of monosaccharide derivatives. We now present a direct extension of that survey to include some oligo- and poly-saccharide systems.

The dipole–dipole mechanism for spin–lattice relaxation between a receptor nucleus (R) and a donor nucleus (D) can be approximated[3,4] for nuclides of spin 1/2 by the expression,

$$(R_1) \propto \frac{\gamma_D^2 \gamma_R^2}{(r_{D-R})^6} \cdot \tau_c(D\text{–}R),$$

where γ_D and γ_R are the magnetogyric ratios of the donor and receptor nuclei, respectively, (r_{D-R}) is the distance between those two nuclei, and $\tau_c(D\text{–}R)$ is the correlation time of the (D–R) vector. For most carbohydrate derivatives, the relaxation of any receptor proton will be derived from other protons and, in suitably dilute solutions, intermolecular contributions will be small.

*Dedicated to the memory of Professor Edward J. Bourne.
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Studies of relaxation between the protons of a single substance can lead to valuable information concerning the spatial arrangement of those protons involved in the relaxation, and follows the expression,

$$\frac{(R_1)(D1\text{–}R)}{(R_1)(D2\text{–}R)} = \frac{(r_{D2\text{–}R})^6}{(r_{D1\text{–}R})^6}.$$

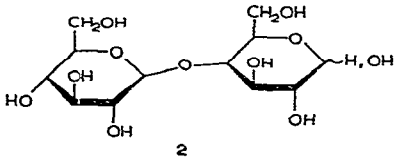
This formulation presupposes that the protons are all relaxing exclusively *via* the *intra*molecular dipole–dipole mechanism, and that the molecules are tumbling isotropically in solution, that is, that τ_c(D–R) is the same for all interproton vectors.

A further, rather stringent requirement must be satisfied if comparisons are to be made between the relaxation rates of the protons of *two different* molecules; it is then also necessary that τ_c(D–R) be numerically identical for all internuclear vectors of *both* molecules. This effectively requires that the solution microviscosity be identical for both molecules.

Fortunately, it seems that an *approximation* to all of the above conditions is feasible without great expenditures of effort. Simply confining attention to closely related molecules in solutions of identical concentration, in a common solvent at a constant temperature, appears to suffice[4,5]. As will be seen in the following discussion, the same approach appears to be valid for small oligosaccharides having the same nominal molecular weight, but not for substances having widely differing, or very long, chain-lengths.

RESULTS AND DISCUSSION

The data for the disaccharides **2–6** are summarised in Table I; also included for reference purposes are the data for D-glucose (**1**). Measurements were generally made at two temperatures. The normal probe temperature was 42°; when this left the



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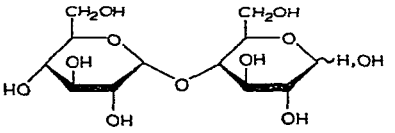

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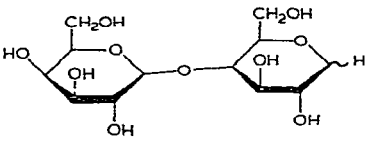

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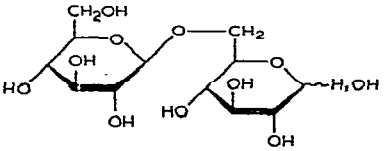

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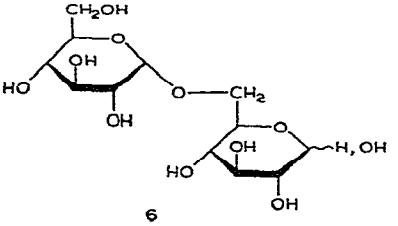

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residual HOD peak sufficiently close to one or other of the anomeric resonances, a second set of measurements was made at different temperatures, each selected to shift the HOD peak to a convenient position. In every instance, the T_1-values increased with increase in solution temperature but the ratio (H-1α/H-1β) and the ratio [H-1 (reducing residue)/H-1′ (non-reducing residue)] remained identical *within experimental error*. This result is in accord with the behaviour previously observed[4] for monosaccharides, and the following discussion will be based solely on the data measured at 42°.

TABLE I

PROTON SPIN–LATTICE RELAXATION-TIMES (SEC) FOR THE ANOMERIC PROTONS OF DISACCHARIDES IN DEUTERIUM OXIDE (99.96%) SOLUTION (5% w/v)

Compound	*Temperature (degrees)*	*H-1 (reducing residue)*			*H-1′ (non-reducing residue)*			*Acetone*
		H-1α	*H-1β*	*Ratio H-1α/H-1β*	*H-1′α*	*H-1′β*	*Ratio H-1/H-1′*	
D-Glucose (**1**)	42[a]	4.2	2.3	1.9	—	—	—	—
	42[b]	5.3	2.6	2.0	—	—	—	—
	46[a]	6.2	3.4	1.8	—	—	—	—
Cellobiose (**2**)	33	1.6	0.84	1.9	—	0.36	2.3	13.8
	42	2.1	1.1[c]	1.9	—	0.52[c]	2.1	17.2
Maltose (**3**)	42	2.5	1.1[c]	2.2	0.86	—	2.9	16.3
	47	2.7	1.2	2.3	1.00	—	2.7	21.0[d]
Lactose (**4**)	42	2.1	1.1	1.8	—	0.54	2.1	16.9
	47	2.5	1.3	1.9	—	0.60	2.2	23.9[d]
Gentiobiose (**5**)	42	2.3[e]	1.2	1.9	—	0.59	2.0	15.9
	65[f]	4.7	2.5	1.9	—	1.4	1.7	23.6
Melibiose (**6**)	42	2.6[g]	1.4	1.85	1.1	—	2.3	17.0
	42	2.6	1.3	1.9	1.1	—	2.3	16.0

[a]Concentration, 10% w/v. [b]Obtained by interpolation of Fig. 2 of Ref. 4. [c]Only one transition visible. [d]Unreliable decay curve. [e]Some cross-relaxation, as evidenced by differences in the relaxation times of the two transitions. [f]Concentration, 3.3% w/v. [g]Much cross-relaxation; initial slope of transitions, 2.4 and 2.6 sec.

Consider first the relaxation times for the anomeric protons (H-1) at the reducing end of each compound. There is a large, systematic differential between the T_1-values of the equatorially oriented proton (H-1α) of the α anomer and the axially oriented proton of the β anomer (H-1β), which parallels that documented for D-glucose (**1**) and for the other D-hexopyranoses. This difference simply reflects the close spatial proximity of an axial proton at C-1 with the axial protons at C-3 and C-5, which causes H-1β to relax more rapidly than H-1α. It is encouraging to note the close internal consistency of these data, since this shows that comparisons can be made

between different disaccharides. Consider, for example, the values for H-1 in compounds **2**–**6** measured at 42°; these are essentially identical within experimental error, as they should be. In like fashion, the values for H-1′ of **2** and **4** are identical, which accords with a previous observation[4] that the relaxation of an anomeric proton is little affected by the orientation of the proton at C-4.

The most important aspect of the data given in Table I is the differential that exists between the relaxation times of the anomeric protons (H-1 and H-1′, respectively) of the reducing and non-reducing moieties of the disaccharides; for every molecule, H-1′ relaxes more rapidly (shorter T_1-value) than H-1. For cellobiose, for example, H-1β at the reducing end has a T_1-value of 0.84 sec, whereas H-1β at the non-reducing end has a T_1-value of 0.36 sec. The precise reason for this differential is not yet known with certainty, but may be due to a difference in the correlation times at the two anomeric sites of the disaccharide. A more probable and very exciting explanation is that *the anomeric proton of the non-reducing moiety receives relaxation contributions from the protons on both sugar rings.*

There is substantial precedent for this explanation. Hall and Preston have previously shown[4] that the anomeric proton of methyl α-D-glucopyranoside and that of the β anomer each receives an appreciable relaxation contribution from the methoxyl protons, and a more-recent, quantitative study[6] has shown that this contribution is higher for the β-glucoside than for the α-glucoside. The data in Table I imply that H-1′ of cellobiose also receives a greater relaxation contribution from the glycosidic ring than does H-1′ of maltose. That is, the β-glycosidic substituent again makes a larger relaxation contribution. There seems little merit in pursuing this discussion further at this juncture, as we have recently developed[7,8] two methods to quantitatively evaluate this point and a detailed study is now in progress[9]. For the present, it suffices to note that this differential between the T_1-values of H-1 and H-1′ provides a facile method for assigning such resonances, and has some important diagnostic potential.

TABLE II

PROTON SPIN–LATTICE RELAXATION-TIMES (SEC) FOR THE ANOMERIC PROTONS OF SOME OLIGOMERS OF D-GLUCOSE IN DEUTERIUM OXIDE (99.96%) SOLUTION (5% W/V) AT 42°

Compound	*Concentration* (*w*/*v*, %)	*H-1 (reducing residue)*			*H-1′ (non-reducing residue)*			*Acetone*
		H-1α	*H-1β*	*Ratio H-1α/H-1β*	*H-1′α*	*H-1′β*	*Ratio H-1/H-1′*	
D-Glucose (**1**)	5	5.3	2.6	2.0	—	—	—	—
Maltose (**3**)	5	2.5	1.1	2.2	0.86	—	2.9	16.3
Maltotriose (**7**)	5	1.7	0.73	2.3	0.53	—	3.2	16.3
α-Schardinger dextrin (**8**)	1	—	—	—	0.27	—	—	16.8
β-Schardinger dextrin (**9**)	1	—	—	—	0.23	—	—	19.8

Further indication of the enhancement of relaxation with increase in the length of the oligosaccharide chain comes from comparison of the relaxation times of D-glucose (**1**), with those of maltose (**3**), maltotriose (**7**), and the α- and β-Schardinger dextrins (**8** and **9**, respectively), all of which are (1→4)-linked oligomers of D-glucose (Table II). This is almost certainly associated with the increase in motional correlation time τ_c, corresponding to slower molecular tumbling as molecular size increases. It is important to note that the near-constancy of the T_1-value of the acetone added as an internal reference implies, but does not prove conclusively, that the observed effects do not simply reflect changes in bulk viscosity.

The series sucrose (**10**), raffinose (**11**), and stachyose (**12**), which only have anomeric protons for non-reducing residues, shows a similar, progressive enhancement of relaxation with increasing molecular weight (Fig. 1). There is also a differential of 15% between the T_1 values of the two chemically distinct, anomeric protons, which is maintained as the number of sugar units increases from three to four.

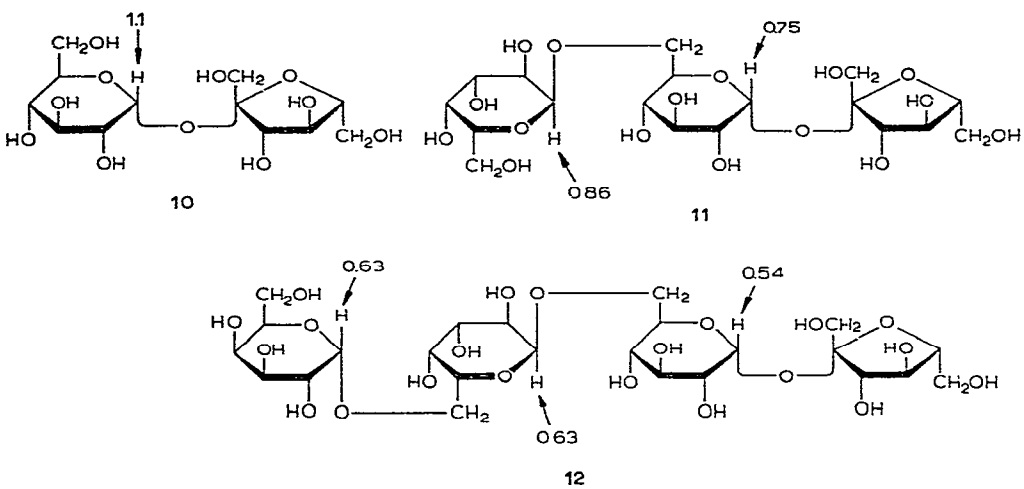

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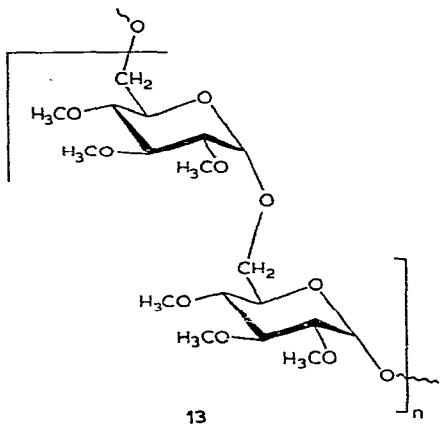
Fig. 1. Proton spin–lattice relaxation-times (sec) for the anomeric protons of three oligosaccharides as 5% w/v solutions in 99.96% deuterium oxide, measured at 42°.

TABLE III

PROTON SPIN–LATTICE RELAXATION-TIMES (SEC) FOR THE ANOMERIC PROTONS OF SOME POLYSACCHARIDES IN DEUTERIUM OXIDE (99.96%) SOLUTIONS (5% W/V)

Compound	*Anomeric proton*	*Acetone*
2,3,4-Tri-*O*-methyldextran (**13**)	0.26	19.2
2,3,6-Tri-*O*-methylamylose (**14**)	0.22	21.1
2,3,6-Tri-*O*-methylcellulose (**15**)	0.25	18.7

Intrigued by the above demonstrations that specific, intramolecular interactions still lead to T_1 differences in short oligosaccharides, we were prompted to see if the same was true for polysaccharides. Table III lists anomeric spin–lattice relaxation-times for three fully methylated glucans, namely, dextran (**13**), amylose (**14**), and cellulose (**15**). The T_1 values are identical within experimental error [although the bulk viscosity of the cellulose polymer (**15**) was much greater]. We also studied the



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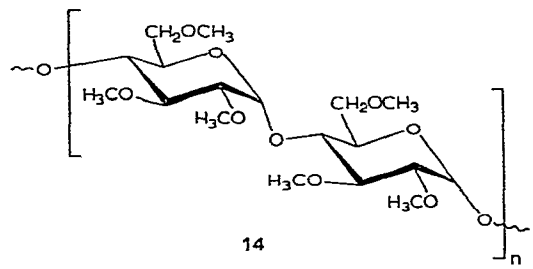

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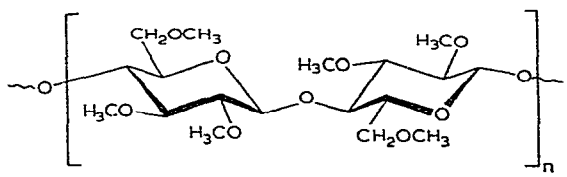

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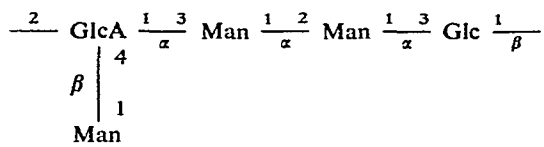
capsular polysaccharide of the bacterium *Klebsiella* 24, which has molecular weight $(5–9) \times 10^5$ and contains the five-sugar repeating-unit[10] shown in Table IV. Fig. 2 shows the 100-MHz n.m.r. spectrum of a solution of the K-24 polysaccharide in D_2O at 95°, while the relaxation times are given in Table IV. Again, the results lack diagnostic potential; the small differences between the relaxation times of the anomeric protons are less than those for mono- or di-saccharides.

Whilst the relaxation data for the anomeric protons of the polysaccharides were disappointing, the data for the acetate peaks of K-24 showed some interesting features. The K-24 polysaccharide contains one *O*-acetyl group (its precise location is unknown) to seven or eight sugar units[10]. This group is represented by peak 4 in the n.m.r. spectrum of K-24, while peak 5 is from free acetate, resulting from decomposition of the sample, which had been held at 95° several times. There is a four-fold difference in their relaxation times, which are 2.4 sec for the free-acetate methyl protons and

TABLE IV

SPIN–LATTICE RELAXATION-TIMES FOR BACTERIAL CAPSULAR POLYSACCHARIDE FROM *Klebsiella* 24, UNDEGASSED, IN D_2O AT 95°



Peak number[a]	*Assignment*[a]	T_1 (*sec*)
1	α-GlcA $\frac{1\ 3}{}$ Man α-Man $\frac{1\ 3}{}$ Glc	0.23
2	α-Man $\frac{1\ 2}{}$ Man	0.31
3	β-Man $\frac{1\ 4}{}$ GlcA β-Glc $\frac{1\ 2}{}$ GlcA	0.22
4	*O*-Acetyl	0.55[b]
5	Free acetate	2.4

[a]See Fig. 2. [b]Based on initial slope.

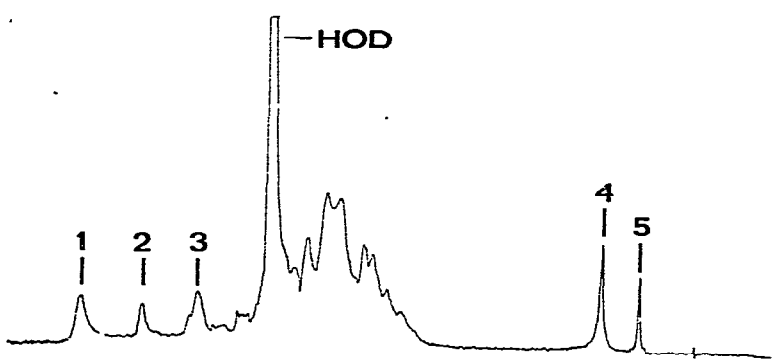


Fig. 2. Partial 100-MHz ^{1}H-n.m.r. spectrum of *Klebsiella* 24 polysaccharide at 95° in D_2O. Peaks 1, 2, and 3 are from the anomeric protons; their assignments are given in Table IV. Peaks 4 and 5 are from free acetate (4) and *O*-acetyl groups (5).

~0.55 sec (based on initial slope) for the *O*-acetyl methyl protons. Typical decay plots for the two peaks are shown in Fig. 3, and it can be seen that the decay for the *O*-acetyl group is decidedly non-exponential. This behaviour, which is similar to, but more pronounced than, the non-exponentiality observed for three of the four methyl groups of the alkaloid vindoline[11], may be due to cross-correlation effects, which

depend on the degree of hindrance of the methyl group rotation, the degree of anisotropy of the overall motion of the large polymer, and the difference in the correlation times for the two motions[12,13]. It is also possible that at least part of the non-exponentiality may be due to the different relaxation times of *O*-acetyl groups in two or more sites in the molecule, but with the same chemical shift. Until the position(s) of the *O*-acetyl group have been clarified, this point cannot be settled, but it clearly merits further study.

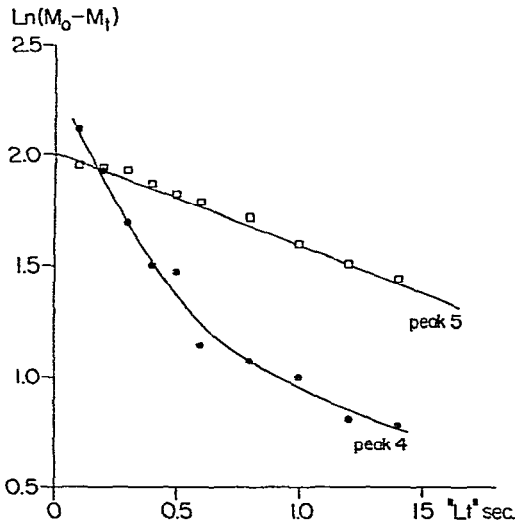


Fig. 3. Plots of ln $(M_0 - M_t)$ *vs.* delay time "t" for the acetate peaks in the 100-MHz n.m.r. spectrum of the capsular polysaccharide from *Klebsiella* 24 in D_2O at 95°; peak 4, free acetate; peak 5, *O*-acetyl groups.

CONCLUSIONS

From the rather small number of di- and poly-saccharides surveyed, it is difficult to draw final conclusions. For disaccharides, at least, T_1 values seem to have the same general stereospecificity and diagnostic potential as for monosaccharides, with the very important, additional feature that inter-ring relaxation contributions appear to be detectable and thereby offer a powerful new method for studying the conformation of oligosaccharides. We are studying[9] this possibility in detail.

The situation for higher polymers is less clear. Obviously, the concepts of "correlation time" and "intramolecular" become more complex. In addition to the possible anisotropic motion of the whole molecule, there may be faster local motions of short segments of the main chain, and of branching sugar-units. This aspect is best studied *via* carbon-13 spin–lattice relaxation-times; we have already described[14] data for monosaccharides, and a detailed evaluation of oligosaccharides is now in progress and will shortly be reported elsewhere[15].

EXPERIMENTAL

Previous papers in this series[1,4] have summarised the experimental methods used to acquire the n.m.r. data and to calculate the spin–lattice relaxation-times.

Most of the substances used in this study were commercially available; the remaining samples were gifts.

ACKNOWLEDGMENTS

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