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THE EFFECTS OF GADOLINIUM IONS ON THE PROTON SPIN–LATTICE RELAXATION-RATES OF SUGARS IN AQUEOUS SOLUTION*

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ABSTRACT

Addition of very small proportions (~1 mmolar equivalent) of gadolinium nitrate to aqueous solutions of several mono- and di-saccharides has been found to cause substantial changes in the proton spin–lattice relaxation-rates of these compounds If the binding of the gadolinium to the sugar is regiospecific, this approach has some interesting diagnostic potential, which is discussed

INTRODUCTION

Williams and co-workers[2] and LaMar and Faller[3] have pointed out‡ that the association of a gadolinium ion with an organic molecule should lead to systematic changes in the spin–lattice relaxation-rates (R_1 values) of the protons of that molecule these changes, ΔR_1, should have the form $\Delta(R_1)\ \alpha/r^6$, where r is the distance between the gadolinium nucleus and the proton under investigation Although this relationship is *apparently*[2] quite simple for organic molecules that have but a single locus for association with the gadolinium ion, its potential for facilitating studies of molecules having several potential binding-sites is much less obvious.

Prompted by an interest both in the association of lanthanide ions with carbohydrate derivatives[5] and in the development of methods whereby spin–lattice relaxation-times can be used to study the conformations of carbohydrate derivatives[1,6], we have examined the effects of very small proportions (typically, 0 1 to 1 0 mmolar equivalent) of gadolinium ions on the proton R_1-values of some sugars in aqueous solution. A subsidiary object of the experiments described here was to increase the differential between the R_1 values of individual protons of a sugar, and

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‡It is worth noting that these studies were preceded by very extensive investigations of other paramagnetic ions[4].

thereby extend the effectiveness of the partial-relaxation approach to spectral assignments

RESULTS AND DISCUSSION

The first measurements were made with sugars not anticipated to have any specific locus for association with a gadolinium ion. The data obtained by measuring the spin–lattice relaxation-rates of the anomeric protons of an aqueous solution (0.2M) of D-glucose are summarized in Table I. In the absence of gadolinium ions, there is a systematic[6] differential between the R_1 values of the two anomeric protons

TABLE I

EFFECT OF GADOLINIUM NITRATE ON THE SPIN–LATTICE RELAXATION-RATE OF THE PROTON ON C-1 OF THE ANOMERS OF D-GLUCOPYRANOSE

Molarity		*Relaxation rate* (sec^{-1})		*H-1α/H-1β*
$[Gd(III)] \times 10^4$	$[Gd(III)]/[\text{D-glucose}] \times 10^3$	*H-1α*	*H-1β*	
0.00	0.00	0.22	0.37	0.60
0.20	0.10	0.24	0.40	0.60
0.40	0.20	0.33	0.50	0.66
0.60	0.30	0.42	0.57	0.74
1.0	0.50	0.57	0.70	0.81
1.6	0.80	0.79	0.83	0.95
2.6	1.3	1.2	1.1	1.1
3.0	1.8	1.4	1.4	1.0
10.0	5.0	3.9	3.9	1.0

(*i.e.*, those on C-1 of the two anomers). However, this differential decreases quite rapidly with increasing molar proportions of gadolinium ions, with the relaxation rates of the two protons becoming identical* at ~1.5 mmolar equivalents of added gadolinium ion. At all concentrations above this value, the two anomeric protons have identical R_1 values, indicating that their spin–lattice relaxation is now dominated by the paramagnetic contribution from the gadolinium ions associating in an essentialy random way with the two (or more) anomers. It should be noted that a progressive increase in the R_1 values occurs as further gadolinium nitrate is added.

In the absence of added gadolinium ions, the nonreducing, anomeric proton of both maltose and cellobiose relaxes faster (see Tables II and III) than the anomeric protons at the reducing end. As before, progressive addition of gadolinium nitrate causes all of the proton relaxation-rates to increase, however, the effects on the protons at the reducing end are more marked. A similar effect is found for maltotriose (see Table IV). There seems to be little point in discussing the numerical values of

*The probable experimental error is of the order of ±5%

TABLE II

EFFECT OF GADOLINIUM NITRATE ON THE SPIN–LATTICE RELAXATION-RATES OF THE ANOMERIC PROTONS OF MALTOSE

Molarity		*Relaxation rate* (sec^{-1})			*H-1α/H-1α*[a]
$[Gd(III)] \times 10^4$	$[Gd(III)]/[maltose] \times 10^3$	*H-1α*	*H-1β*	*H-1α*[a]	
0 0	0 0	0 43	0 81	1 2	0 36
0 2	0 1	0 49	0 94	1 2	0 41
0 5	0 25	0 58	1 1	1 3	0 45
0 8	0 4	0 7	1 0	1 3	0 54
1 5	0 75	1 3	1 7	1 7	0 77
2 2	1 1	1 8	2 1	1 9	0 95
3 0	1 5	2 3	2 4	2 2	1 1
8 0	4 0	5 0	5 8±1 5	4 6	1 1

[a]Proton at nonreducing, anomeric carbon atom

TABLE III

EFFECT OF GADOLINIUM NITRATE ON THE SPIN–LATTICE RELAXATION-RATES OF THE ANOMERIC PROTONS OF CELLOBIOSE

Molarity		*Relaxation rate* (sec^{-1})			*H-1β/H-1β*[a]
$[Gd(III)] \times 10^4$	$[Gd(III)]/[cellobiose] \times 10^3$	*H-1α*	*H-1β*	*H-1β*[a]	
0 0	0 0	0 52	1 1	2 1	0 52
0 2	0 10	0 53	1 0	2 2	0 46
0 5	0 25	0 56	0 92	2 1	0 44
1 0	0 50	0 65	1 1	2 1	0 52
2 0	1 0	1 1	1 5	2 1	0 71
2 7	1 4	1 9	2 0	2 4	0 83
4 0	2 0	2 9	2 9	2 6	1 1
10	5 0	7 1	8±3	4±1	2

[a]Proton at nonreducing, anomeric carbon atom

TABLE IV

EFFECT OF GADOLINIUM NITRATE ON THE SPIN–LATTICE RELAXATION-RATES OF THE ANOMERIC PROTONS OF MALTOTRIOSE

Molarity		*Relaxation rate* (sec^{-1})			*H-1α/H-1α*[a]
$[Gd(III)] \times 10^4$	$[Gd(III)]/[maltotriose] \times 10^3$	*H-1α*	*H-1β*	*H-1α*[a]	
0 0	0 0	0 60	1 3	1 8	0 33
0 4	0 3	0 61	1 3	1 8	0 34
1 6	1 2	1 8	2 4	2 3	0 78
3 0	2 2	2 9	3 6	2 9	1 0
6 0	4 4	4 6	4 6	4 0	1 2
10	7 4	7 1	11 0	5 6	1 3

[a]Protons at nonreducing, anomeric carbon atoms

these data, but it is apparent that nonspecific binding of gadolinium ions by a sugar can produce substantial changes in the proton R_1-values of that sugar. However, these changes do not appear to have any substantial, diagnostic potential, indeed, the intrinsic R_1-differential associated with the intramolecular dipole–dipole relaxation may be destroyed in these experiments

Attention was next directed to D-allose Anpval[7] has observed a highly specific association of α-D-allopyranose with europium and other metal ions, and we anticipated that gadolinium ions should have a most marked effect on the proton R_1-values of that species, with less effect on β-D-allopyranose The data given in Fig 1B bear this out in a most convincing fashion· addition of 1.5 mmolar equivalents of

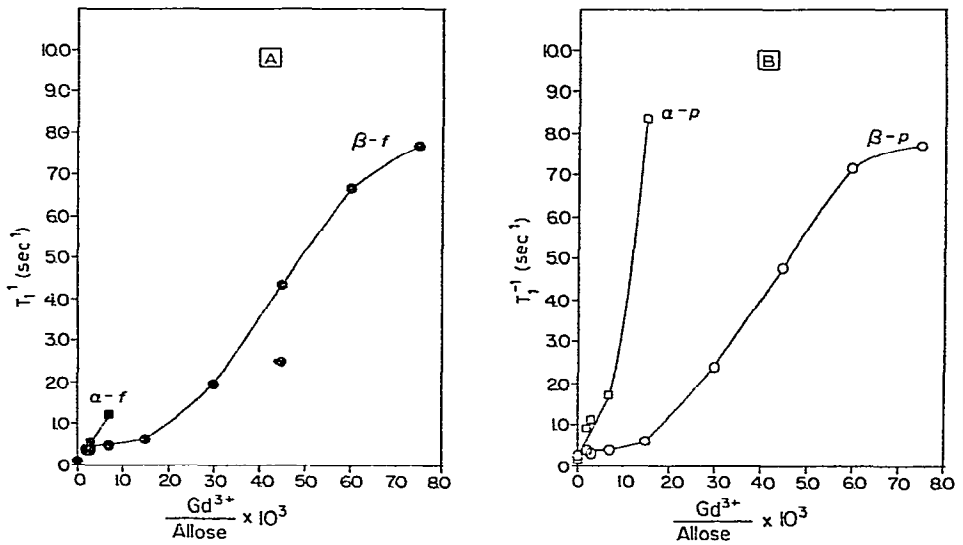

Fig 1 Variation of the relaxation rates (R_1 values) of the anomeric protons of D-allose in D_2O, measured as a function of the molar ratio of added gadolinium nitrate

gadolinium nitrate enhances the relaxation of the α-anomeric proton (the proton on C-1 of the α anomer) more than 50-fold, whereas that of the β-anomeric proton is changed less than 3-fold; this change is accompanied by a rather substantial increase in the line-width of the H-1α resonance, which eventually becomes so broadened that it is effectively removed from the spectrum (see Fig 2)

A similar series of effects was observed for the furanose forms of D-allose (see Fig 1A), and it is important to note that, once again, the α anomer shows favored complexing with the gadolinium

In all of the experiments just described, continued addition of gadolinium nitrate resulted in the increased dominance of paramagnetic relaxation, and the eventual equalization of the R_1 values of all of the identifiable ring-protons In one experiment, addition of a vast excess of gadolinium nitrate *appeared* to lead to a

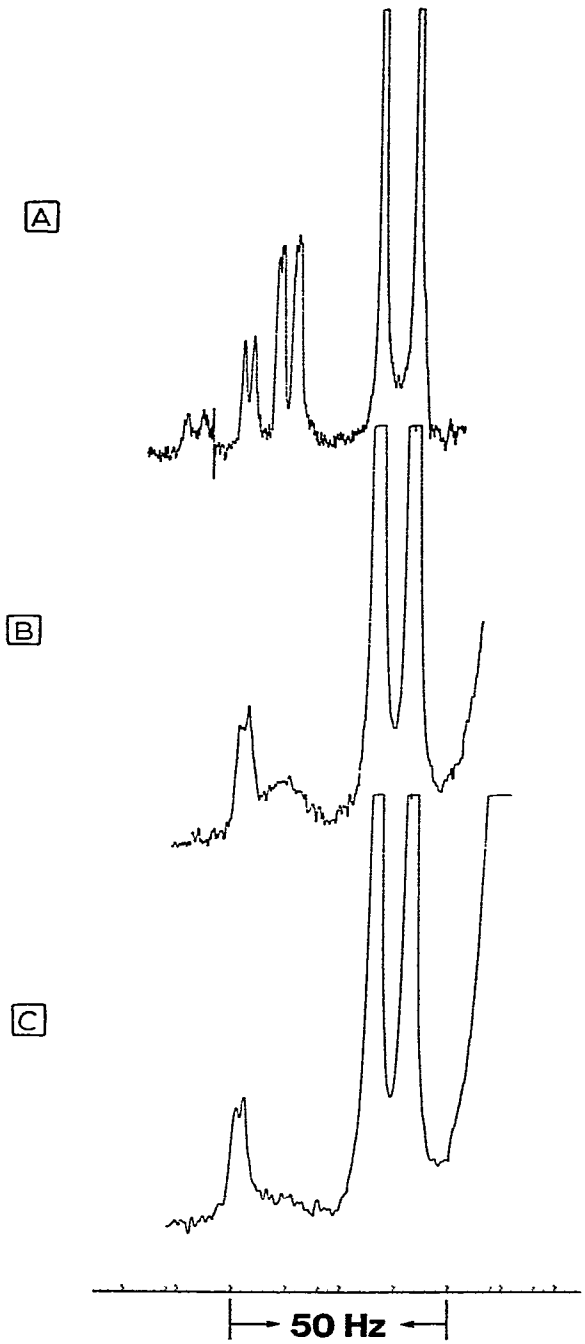

Fig 2 Proton n m r signals (100 MHz) of the anomeric protons of D-allose (0 2M) in D_2O (99 96% deuterium) at 42° [Spectrum A was measured for the pure solution, B was measured after the addition of 1 mmolar equivalent of gadolinium nitrate, C was measured after the addition of 2 mmolar equivalents of gadolinium nitrate See Fig 3 for full assignment.]

further divergence of the R_1 values; however, at very high concentrations of gadolinium ion, the resonance of the residual water becomes so broadened that it precludes accurate measurement of the intensities of the anomeric-proton resonances, and, for that reason, this experiment was not further pursued

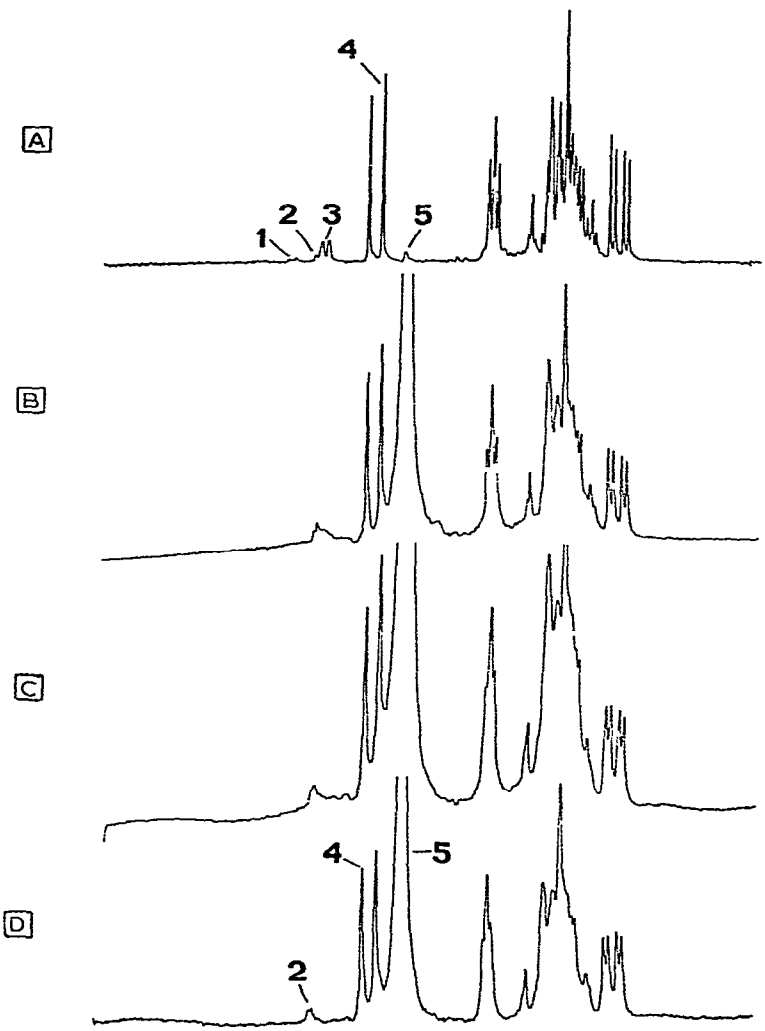

Fig 3 100-MHz, Fourier-transform, ^{1}H n m r spectra of fully mutarotated α-D-allopyranose–calcium chloride, 0 2M, in 99 96% D_2O at 33° [The peak assignments are 1, α-furanose, 2, β-furanose, 3, α-pyranose, 4, β-pyranose, and 5, HOD A shows the normal spectrum, obtained by averaging 16 transients and incorporating a two-pulse sequence with a delay-time of 9 3 sec to null the residual HOD peak In B, 0 5 mmolar equivalent of gadolinium(III) had been added, and the spectrum is the sum of 24 transients In C, the Gd(III) concentration was doubled to 1 0 mmolar equivalent, and 50 scans were used In both B and C, the HOD peak has its full intensity For spectrum D, 50 transients were again used, but the HOD peak was lessened in intensity by using a three-pulse sequence, with a delay-time of 0 3 sec]

Extensive studies by Angyal[7,8] have shown that two types of binding site can lead to strong association between a sugar and an alkaline-earth cation (*1*) an axial–equatorial–axial arrangement of three hydroxyl groups on a pyranose ring, and (*2*) a *cis–cis* arrangement on a furanose ring With these groupings, the association can be so strong as to (*a*) cause a reversal of the normal conformation[7(a)], or (*b*) change the major product of a reaction from one anomeric form to the other[8] It occurred to us that, in the same way that "shifting" and "broadening" lanthanide reagents can be combined[2,9], a concentration of a nonparamagnetic, "binding" cation, such as calcium(II), high enough to produce a large chemical perturbation of a system could be combined with a very small proportion of a freely exchanging, competitive, "broadening" cation, such as gadolinium(III), to effect removal of one set of resonances from the spectrum This type of experiment is illustrated in Fig 3 for the calcium chloride complex of α-D-allopyranose

Fig 3A is a spectrum of the equilibrium mixture, it was obtained by using a two-pulse sequence, to null the HOD peak almost completely, and 16 transients to increase the signal-to-noise ratio Fig 3B shows the effect of the addition of 0 5 mmolar equivalent of Gd(III), and, again, the spectrum was time-averaged In this case, the relaxation rate of water, which also binds to gadolinium, was comparable to that of the sugar protons, so that it was not possible to null the residual HOD peak It may be noted that the H-1 doublet of the α-pyranose form has broadened much more than that of the β-pyranose form, and a small peak, visible as a shoulder on the downfield side of H-1α in spectrum A, is emerging In spectrum 3C, the Gd(III) concentration was doubled, to 1 0 mmolar equivalent, and 50 transients were time-averaged The signal due to H-1 of the α-pyranose has been so broadened that it has been completely removed from the spectrum, while a peak with a smaller, unresolved splitting, probably H-1 of the β-furanose, can readily be distinguished At this concentration of gadolinium ion, the relaxation rate of the HOD peak was higher than that of the sugar protons, so an attempt was made to remove it from the spectrum by using the three-pulse sequence However, the differential was not large, and a great deal of noise and baseline distortion was introduced It was possible to achieve a diminution in the relative intensity of this peak by using a short delay-time, *t*, of 0 3 sec in the three-pulse sequence The result of this procedure is shown in spectrum 3D, which was recorded at the same settings and number of transients as spectrum 3C. Although the signal-to-noise ratio of the sugar peaks is somewhat lower, the overall quality of the spectrum is much better, and it is now possible just to resolve the splitting in the β-furanose peak

CONCLUSIONS

The results of these studies imply that attempts to use gadolinium ions to increase the differential between the spin–lattice relaxation-times of individual protons of sugars in aqueous solution are only likely to be fruitful if there be some dominant locus for the association between the sugar and the gadolinium ion Fortunately, a

number of important carbohydrate systems are likely to satisfy that requirement For such systems, one obvious advantage of this approach over the more conventional one of lanthanide-induced, chemical-shift changes is that significant effects are produced by far smaller molar ratios of added lanthanide; hence, the experiment is accompanied by a much smaller, chemical perturbation of the organic system under study. In that regard, this class of lanthanide experiment may offer significant advantages[9,10] in the area of carbon-13 n m r. spectroscopy.

It is also worth commenting that the effects of other paramagnetic ions are likely to repay investigation For example, it should now be possible to re-investigate some of Reeves's classical studies[11] of cuprammonium complexing, however, it may prove difficult to provide more than a qualitative rationale

EXPERIMENTAL

The Fourier-transform n m r experiments and the subsequent calculations of the spin–lattice relaxation-times were performed as previously described[1,6] As an added convenience, we have now interfaced a tape deck (Linc Tape, Model C0600) to our Varian system, this enables preprogramming of a series of 10 measurements, each being defined by a different value of the pulse delay-time ("little t"), and storage of the output of these experiments as the free-induction decay signals It is then a simple matter to recover the desired spectra as needed The sugars used were commercially available samples cellobiose from Pfanstiehl, and maltotriose and D-allose from Calbiochem Additional samples of D-allose were obtained from Drs Keith N Slessor and John D. Stevens, the latter also provided the α-D-allopyranose–calcium chloride complex

All measurements were made at 42° with 200 mmolar solutions of the particular sugar in D_2O (99 96%), because of solubility problems, the experiments with maltotriose were made at 140 mmolar concentration

Some of the experiments described here are extremely time-consuming; for example, the measurements of the α anomers of D-allose required ~12 h

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