Intramolecular hydrogen bonds in monosaccharides in dimethyl sulfoxide solution

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By the use of the SIMPLE NMR method, strong intramolecular hydrogen bonds were found in the Me_2SO solutions of sugars having two *syn*-axial hydroxy groups, like the talopyranoses. Weaker hydrogen bonds were detected between hydroxy groups in a 1,3-arrangement when one was outside the ring but only weak, although numerous, hydrogen bonds were detected in solutions of other sugars and inositols.

The results described in this paper were obtained, independently, in London and Sydney, and were then correlated, with additional experiments, for publication. The work in London was a continuation of a series of papers[1-5] on hydrogen bonds in Me_2SO solutions of sugars which, however, included only one monosaccharide (D-glucose).[5] The work in Sydney had the aim of establishing whether the substantial changes in the composition[6] of some sugars when going from water to Me_2SO as solvent could be expained by the presence of intramolecular hydrogen bonds in the latter. Dais and Perlin[7] suggested that the sharp increase in the proportion of fructofuranoses on changing the solvent was due to the formation of such a bond in Me_2SO but not in water; this suggestion has now been tested.

The presence of intramolecular hydrogen bonds between the two moieties of disaccharides is well established in Me_2SO solution, *e.g.* in sucrose,[2,8] maltose,[5] methyl β-cellobioside[9] and halobiouronate.[10] In monosaccharides the hydrogen bonds are much weaker but the existence of cooperative hydrogen bonds in the Me_2SO solution of α-glucopyranose has been established,[5] reaching from O-1 through O-2, O-3 and O-4 to O-6. In such a cooperative system, hydroxy groups act as both donors and acceptors of hydrogen bonds.[11,12]

Using NMR spectroscopy, the presence of hydrogen bonds can be established in several different ways:[13] by the study of (i) the magnitude of the chemical shifts, (ii) their temperature coefficients, (iii) the vicinal coupling constant $^3J_{OH.CH}$ and (iv) the rate of exchange with the solvent. None of these methods is sufficiently sensitive to provide definite proof of the existence of weak hydrogen bonds, such as those formed in the Me_2SO solution of glucose. The method which shows their presence is SIMPLE NMR (secondary isotope multiplet NMR spectroscopy of partially labelled entities)[5] in which the hydrogen atoms of hydroxy groups are observed after they have been partially exchanged for deuterium. If a hydrogen bond is present, some of the observed hydrogen atom will be affected by a deuterium and some by a hydrogen atom of the other hydroxy group; hence every line in its spectrum will be duplicated, as first observed by Lemieux and Bock[8] in the spectrum of sucrose.

This method was used to establish the presence of intramolecular hydrogen bonds in Me_2SO solution.[5] By this method we now examined a number of monosaccharides and also some glycosides and inositols as useful model compounds. Particular attention was paid to galactose, talose and fructose which show the greatest differences in composition between solutions in water and in Me_2SO.

Experimental

NMR spectra were recorded, both in London and Sydney, on Bruker-500 spectrometers and the spectra were calculated with resolution enhancement at a data resolution of *ca.* 0.1 Hz per point. The spectra were referenced to the internal Me_2SO signal at 2.49 ppm. In London, mainly pure crystalline samples of sugars were used, whereas in Sydney mostly equilibrium solutions containing all four forms of the sugars were investigated. In London, the instrument was operated at 295 K, in Sydney initially at 300 K. Other spectra were subsequently recorded in Sydney at the lower temperature; in Table 2 the temperature of each experiment is indicated.

Samples were dissolved in deuterium oxide, followed by freezedrying; the residue was then dissolved in $(CD_3)_2SO$. This solution usually showed the correct OH : OD ratio, owing to the water content of commercial Me_2SO. If there was insufficient OH, a small amount of the undeuteriated sample was added. This procedure is better than the one used previously,[3] which involved adding small amounts of D_2O to a solution in Me_2SO. This addition of D_2O (or H_2O) causes a downfield shift of the OH signals, which sometimes leads to an overlap of signals. The OH : OD ratio was obtained from the H-1 signals or from a comparison of the HOH and the HOD signals. The isotope effects are best detected when the OH : OD ratio is close to unity but a different ratio is required for the determination of the sign of the effect.[5] The isotope effects are detected by comparison with the spectra of an undeuteriated sample. Isotope effects smaller than 10×10^{-4} are not detected, except as a broadening of the respective signals. The patterns of various isotope effects are illustrated in ref. 5.

Particular attention has been paid to talose owing to its many strong hydrogen bonds. In the OH region of the spectrum, numerous overlaps occurred when all four forms of the sugar were present: hence we studied its 6-deoxy derivative first. The spectrum of the crystalline compound gave the signals of the α-pyranose form. The equilibrium solution in Me_2SO contains only 9% of the β-D-pyranose; hence the sugar was equilibrated in D_2O (giving a solution with 28% β), freeze-dried and then dissolved in $(CD_3)_2SO$. There was still some overlap with the α-furanose form but fortunately it was found that mutarotation of the crystalline form in Me_2SO produced the α-furanose much faster than the β-pyranose. One of our solutions in Me_2SO, which was left standing for a while before the spectrum was run, was found to contain a lot of the α-furanose but only little β-pyranose.

The OH signals of the talopyranoses, the methyl lyxoside, the

Table 1 Chemical shifts (ppm) and coupling constants (Hz, in parentheses) of hydroxy protons in Me_2SO solution[a]

	OH-1	OH-2	OH-3	OH-4	OH-5	OH-6
D-Xylose, α-pyranose	6.20 (4.6, 1.1)	4.55 (6.8)	4.77 (4.7)	4.81 (5.4)		
D-Xylose, β-pyranose	6.36 (6.5)	4.88[b] (5.2)	4.81[b] (5.5)	4.80[b] (5.8)		
D-Mannose, α-pyranose	6.14 (8.2)	4.41 (4.9)	4.52 (6.0)	4.67 (5.2)		4.43 (6.1, 5.7)
Methyl α-D-allofuranoside		4.78 (4.8)	4.46 (6.0)		4.14 (9.5)	4.51 (6.1, 5.2)
Methyl α-D-lyxofuranoside		5.09 (7.0)	4.74 (5.0)		4.69 (5.4, 5.5)	
D-Galactose, α-pyranose	6.12 (3.7, 1.0)	4.27 (6.5)	4.45 (5.5)	4.26 (4.0)		4.51 (6.5, 5.1)
D-Galactose, β-pyranose	6.50 (6.6)	4.71 (4.4)	4.62 (5.3)	4.30 (4.2)		4.58 (5.5, 5.5)
D-Galactose, α-furanose		4.80 (5.6)	5.17 (5.3)			
D-Galactose, β-furanose	6.16 (5.6)	5.04[b] (6.6)	5.18[b] (5.6)		4.54 (8.0)	4.55 (7.4, 7.0)
L-Arabinose, α-pyranose	6.64 (5.6)	4.72 (4.3)	4.64 (5.6)	4.45 (4.0)		
L-Arabinose, β-pyranose	6.02 (4.6, 0.5)	4.39 (6.2)	4.50 (5.3)	4.40 (4.0, 0.7)		
L-Arabinose, α-furanose	6.13 (4.6)	5.03[b] (5.5)	5.16[b] (5.5)		4.62 (6.2, 5.6)	
L-Arabinose, β-furanose	6.06 (5.6)	4.72 (6.2)	5.10 (5.2)		4.55 (5.4, 5.1)	
D-Talose, α-pyranose **2**	6.37 (4.5)	5.07 (6.6)	4.68 (6.7)	4.57 (7.5)		4.52 (6.3, 5.1)
D-Talose, β-pyranose	6.25 (8.5)	4.75 (6.6)	4.70 (6.8)	4.47 (7.5)		4.59 (5.9, 5.4)
D-Talose, α-furanose	6.17 (5.5)	4.83[b] (5.1)	4.71[b] (6.8)		4.65[b] (6.4)	4.51 (6.3, 5.7)
D-Talose, β-furanose	5.63 (7.8)	4.49[b] (7.8)				4.49 (6.3, 5.1)
Methyl α-D-talopyranoside		5.11 (6.6)	4.78 (6.7)	4.65 (7.5)		4.69 (5.2, 6.2)
6-Deoxy-L-talose, α-pyranose	6.29 (4.3)	5.01 (6.7)	4.65 (7.0)	4.60 (6.8)		
6-Deoxy-L-talose, β-pyranose	6.18 (8.5)	4.70 (6.6)	4.74 (7.0)	4.49 (7.6)		
6-Deoxy-L-talose, α-furanose	6.15 (5.5)	4.85[b] (5.0)	4.65[b] (6.4)	4.45[b] (8.5)		
6-Deoxy-L-talose, β-furanose	5.70 (6.1)		4.57[b] (5.9)	4.43[b] (4.7)		
Methyl 6-deoxy-α-L-talopyranoside		5.06 (6.9)	4.76 (6.5)	4.67 (7.3)		
D-Ribose, β-pyranose	6.35 (5.6)	4.78 (6.7)	4.64 (5.0)	4.74 (6.6)		
Methyl β-D-ribopyranoside		4.88 (7.0)	4.72 (5.4)	4.83 (6.3)		
D-Fructose, α-pyranose	4.58 (5.6, 5.6)	5.98 (1.2)	4.64?[b]	5.03?[b]	4.36[b]	
D-Fructose, α-furanose	4.30 (5.3, 7.2)	5.63	5.10[b] (5.8)	5.09[b] (5.4)		4.68 (4.3, 6.3)
D-Fructose, β-furanose	4.57 (4.7, 6.2)	5.31	4.70 (7.0)	5.10 (5.6)		4.70 (6.4, 6.4)
Methyl β-D-fructopyranoside	4.47 (7.2, 4.9)		4.37 (6.5)	4.43 (6.2)	4.42 (3.7)	
D-Tagatose, α-pyranose	4.48 (5.3, 7.0)	5.34	4.38 (4.5)	4.48 (5.5)	4.63 (4.8)	
myo-Inositol	4.33 (5.6)	4.45 (3.5)	4.33 (5.6)	4.48 (4.5)	4.53 (4.3)	4.48 (4.5)
epi-Inositol **1**	4.37 (6.0)	4.72 (6.3)	4.68 (6.4)	4.72 (6.3)	4.37 (6.0)	4.48 (4.4)
2,7-Anhydro-β-D-*altro*-heptulopyranose	4.58 (5.9, 6.8)		4.47[b] (6.9)	4.71[b] (6.1)	4.39 (4.2)	

[a] Data given in ref. 15 are not listed here. [b] Not assigned.

methyl alloside and the inositols were assigned by decoupling them from the signals of the carbon-bound protons. Those of the arabinopyranoses were assigned by analogy with those of the galactopyranoses. Those of the fructofuranoses were assigned by comparison with the chemical shifts of the methyl furanosides.[14] Most of the signals of the furanoses were not assigned.

Results

Recognition of intramolecular hydrogen bonds

The chemical shifts. The chemical shifts of the hydroxy protons of many aldoses and ketoses in Me_2SO have been listed by Gillet *et al.*[15] The chemical shifts of additional sugars and some inositols are shown in Table 1. The shifts of the hydroxy protons, in contrast to those protons bound to carbon atoms, vary considerably with changes in the temperature.[9] We found that lowering the temperature by 10 °C moves them downfield by *ca.* 0.08 ppm, whereas a 20 °C rise in the temperature moves them upfield by *ca.* 0.13 ppm. The shifts are also considerably affected by the presence of water in the Me_2SO; even traces of water will shift the signals downfield, those of the primary OH groups moving more than those of the secondary ones.

The presence of strong hydrogen bonds causes the chemical shifts of hydroxy groups to be changed from what might be expected by comparison with the chemical shifts of hydroxy groups not partaking in hydrogen bonds. For example, in the spectrum of maltose,[5] the signals of OH-2′ and OH-3 (which are linked by a strong hydrogen bond) are at much lower field than those of OH-2 and OH-3′, respectively. On the other hand, in the spectrum of methyl β-cellobioside,[9] the signal of OH-3 (hydrogen bonded to OH-5′) is at much higher field that that of OH-3 in methyl β-glucopyranoside. However, when the hydrogen bond is weak, the chemical shift becomes much less characteristic; and in most cases it is not known what its exact value would be if there were no hydrogen bonds present.

The vicinal H–C–O–H coupling constant (^3J). Gillet *et al.*[15] have published an extensive list of such coupling constants; our additional data are shown in Table 1. They confirm the previous conclusion that axial hydroxy protons have smaller J values (*ca.* 4 Hz) than equatorial ones (*ca.* 5 Hz), and that a neighbouring axial hydroxy group increases the J value of an equatorial hydroxy group (to *ca.* 6 Hz). Primary hydroxy groups also show values of *ca.* 6 Hz. Secondary hydroxy groups outside the ring have even higher J values (> 8 Hz). The J values are slightly higher for anomeric hydroxy groups. It appears that the hydrogen atom of an equatorial hydroxy group has an approximately equal chance of taking up any of the three staggered conformations: the average value calculated by the Karplus-type equation for the H–C–O–H system[16] is 5.4 Hz. An axial hydroxy group, however, is not likely to assume the *anti* conformation, with the hydrogen atom pointing 'inwards', hence the average J value is smaller. The J values in Table 1 show that the weak hydrogen bonds found in Me_2SO solution do not substantially affect the conformation of the hydroxy groups. However, some exceptions were found, and they point to the presence of stronger hydrogen bonds.

Amongst the compounds listed in Table 1, α- and β-talopyranose, *epi*-inositol, 1,6-anhydro-β-glucose and β-D-ribopyranose show exceptionally high coupling constants. In the talopyranoses (and in their 6-deoxy derivatives) the axial OH-2 and OH-4 have coupling constants (*ca.* 7 Hz) which show that the average H–C–O–H dihedral angle is *ca.* 140°, suggesting the existence of a hydrogen bond between them. Similarly, in *epi*-inositol the axial OH-2 and OH-4 protons have high coupling constants; again, a hydrogen bond appears likely. In the disaccharides where intramolecular hydrogen bonds have been established, similar deviations occur from the norm; *e.g.* in methyl β-cellobioside,[9] OH-3 has $^3J_{OH,CH}$ 1.7 Hz, whereas in

Table 2 Magnitudes ($\times 10^{-4}$ ppm) and sign of isotope effects[a]

	OH-1	OH-2	OH-3	OH-4	OH-5	OH-6
D-Glucose, α-pyr.[b]	—	+22	+20	−15		+9
D-Glucose, β-pyr.	—	+24[d]	*d*	*d*		—
Methyl α-D-glucopyranoside[b]		—	—	−7		+10
Methyl β-D-glucopyranoside[c]		+14	+16	br		br
D-Xylose, α-pyr.[c]	—	+18, +14	+20	+12		
D-Xylose, β-pyr.[c]	—	+23[d]	+25[d]	*d*		
D-Mannose, α-pyr.[c]	—	+32	+30, +9	+5		+16
D-Mannose, β-pyr.[c]	—	+32	—	br		br
α-L-Rhamnopyranose[c]	—	+33	+33	—		
α-D-Allopyranose	—	+17	+15, −15	—		—
Methyl α-D-allofuranoside		—	+27		+69	—
Methyl α-D-lyxofuranoside		+29	—		+26, −26	
D-Galactose, α-pyr.	—	+13	+20, −20	+16		—
D-Galactose, β-pyr.	—	+23	+20, −20	—		—
D-Galactose, α-fur.	—	+24	—		—	—
D-Galactose, β-fur.	—	+24	+26		—	—
L-Arabinose, α-pyr.	—	+22	+35	−20		
L-Arabinose, β-pyr.	—	+20	+18	—		
L-Arabinose, α-fur.	—	+32[d]	+22[d]		—	
L-Arabinose, β-fur.	—	+20[d]	+18[d]		—	
D-Talose, α-pyr. **2**	br	+133, +27	+32, < −10	+130, +21		< +10
		+10		+19		
D-Talose, β-pyr.	+52, −52	+148, +20	br	+160, +22		—
D-Talose, α-fur.	+21	+14, −18[d]	+67[d]		*d*	—
D-Talose, β-fur.	+25	+117?[d]				—
6-Deoxy-L-talose, α-pyr.	+17	+126, +25	+13, −30	+140, +18		
		+11		+18		
6-Deoxy-L-talose, β-pyr.	+51, −51	+150, +15	+17, −17	+189, +21		
6-Deoxy-L-talose, α-fur.	+12	+15, −13[d]	+67[d]		+21, −21[d]	
6-Deoxy-L-talose, β-fur.	+30	*d*	?*d*			
Methyl 6-deoxy α-L-talopyranoside		+136, br	—	+152, +21		
D-Ribose, β-pyr.	—	+38	+22	+66, +16		
Methyl β-D-ribopyranoside[c]		+48	—	+89, +16		
D-Fructose, α-pyr.	—	+35				
D-Fructose, β-pyr.	+32, +10	—	+40, −12	*e*	+21	
	−10					
D-Fructose, α-fur.	+36	—	+149[d]	+28[d]		—
D-Fructose, β-fur.	+34	+11	—	+12		—
Methyl β-D-fructopyranoside	+42		+24, +13	br	+13	
α-L-Sorbopyranose[c]	+28	—	+41	+20	—	
α-D-Tagatopyranose[c]	—	—	+26	+26	—	
MYO-Inositol	+14	br	+14	—	+10	—
EPI-Inositol **1**	+53, −45	+165	—	+165	+53, −45	+16, +16
1,6-Anhydro-β-D-glucopyranose **4**		+22	—	+31		
2,7-Anhydro-β-D-altro-heptulopyranose			br[d]	*d*	+16	

[a] 500 MHz NMR measurements in $(CD_3)_2SO$ solution, *T* 295 K; br = broad, — = no isotope effect. [b] From ref. 5. [c] At 300 K. [d] Not assigned; they are listed in the same order as in Table 1. [e] Owing to virtual coupling, this signal is of irregular shape; it consists of eight lines which suggested two isotope effects but no values could be derived from them.

methyl β-D-glucopyranoside the value is 4.7 Hz. This low value indicates a dihedral angle of *ca.* 90° with little rotational freedom, and therefore the existence of a hydrogen bond to O-5′.

The deuterium isotope effect. This effect, that is, the appearance of additional signals after partial replacement of protons of OH groups by deuterium atoms, shows that the other hydrogen atoms are very close to the observed one, and therefore are linked to it by hydrogen bonds. The magnitude of the isotope effect (given as the separation of the two signals) also indicates the approximate strength of the hydrogen bond. The method is based on the fact that in Me_2SO solution the exchange of hydroxylic protons is slow, and therefore their signals are not averaged; but formation and breaking of hydrogen bonds is fast on the NMR time-scale and only average signals of the bonded and the non-bonded hydroxy protons are observed. Hence a large isotope effect indicates that a high proportion of the observed hydroxy groups are hydrogen-bonded. The isotope effects obtained in this study, together with some previously reported, are shown in Table 2.

These isotope effects, which give the signals the appearance of doublets, triplets, *etc.*, unfortunately do not yield an unequivocal picture of the hydrogen bonds. When several H-bonds occur, some of of the hydroxy groups will participate in two hydrogen bonds, and therefore their hydroxy proton signals should show two isotope effects; however, in most cases only one was observed. The other one is probably too small to be detected. Particularly puzzling is the fact that the anomeric hydroxy proton of aldopyranoses is not split (with some exceptions, see below). It has been proposed[5] that, for example, in α-D-glucopyranose and α-D-xylopyranose, OH-1 is involved in a hydrogen bond with O-2; yet, no isotope effect on OH-1 is found. Calculations[17] suggest that hydrogen bonding with an anomeric hydroxy group should be stronger than with other hydroxy groups. By contrast, OH-2 shows an isotope effect in every aldose; many of these may indicate that there is a hydrogen bond from OH-1. In 3-*O*-methyl-D-glucopyranose, OH-2 shows an isotope effect (25×10^{-4} for the α- and 24×10^{-4} for the β-anomer) but the OH-1 signal shows none; yet, the hydrogen bond can connect OH-2 to no other OH group but OH-1. Anomeric hydroxy groups are poor acceptors of hydrogen bonds; apparently when they act as donors, they produce only small isotope effects.

It has been suggested[2] that the isotope effect is positive (that is, downfield) when the hydroxy group acts as a hydrogen acceptor, and negative when it is a hydrogen donor. However,

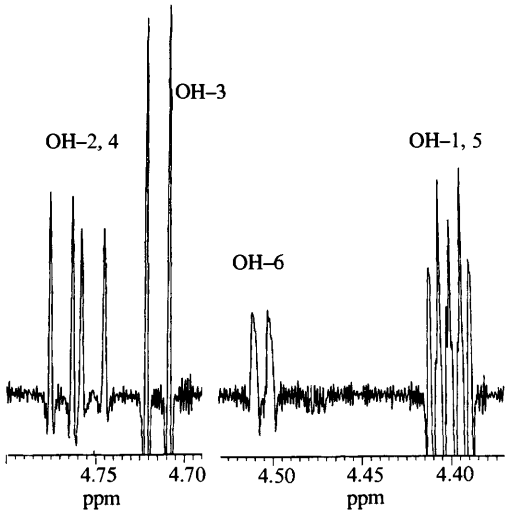


Fig. 1 The hydroxylic proton signals in the SIMPLE NMR spectrum of *epi*-inositol **1**; prior to deuteriation, all the signals were doublets

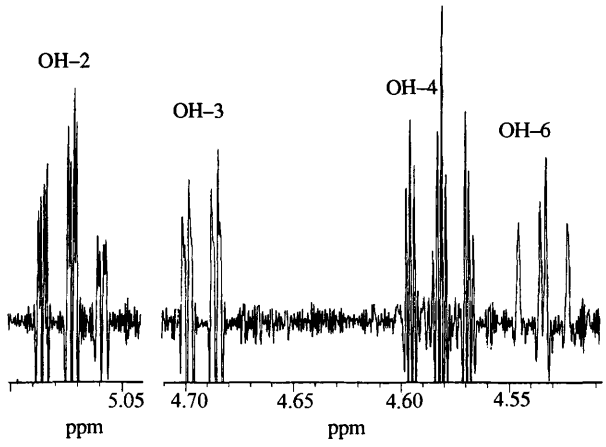


Fig. 2 The hydroxylic proton signals (except OH-1) in the SIMPLE NMR spectrum of α-D-talopyranose **2**; prior to deuteriation, the signal of OH-6 was a pair of doublets, the others were doublets

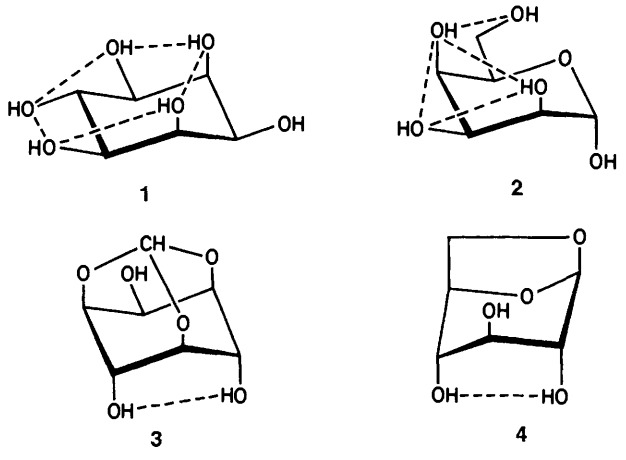
few negative shifts were observed; it appears that this relationship is valid only for strong hydrogen bonds. The isotope effect is stronger for acceptor than for donor hydroxy groups;[5] hence it is possible that in many cases only the isotope effect on acceptor hydroxy protons was observed. Anomeric hydroxy groups are strong donors but weak acceptors;[12] this may be the reason why they do not show any isotope effect.

Owing to these limitations, the assignment of hydrogen bonds to definite hydroxy groups is not always clear; nor is it clear in some cases whether any particular hydroxy group acts as donor or acceptor.

Interpretation of isotope shifts

In many cases—for example, that of α-D-mannopyranose—a satisfactory interpretation is a continuous set of hydrogen bonds from O-2 to O-6, indicated by the fact that every hydroxy group (except O-1) shows an isotope effect. In the galactopyranoses, OH-6 does not take part in the hydrogen bond system, presumably because OH-4 is in an axial position; the conformation required for OH-6 to form a hydrogen bond with it is less favourable. In other instances, only a few OH signals show isotope effects and it is not clear whether the other hydroxy groups are involved in cooperative bonding.

In *epi*-inositol **1**, there seems to be a circular system of



hydrogen bonds from O-2 to O-4 to O-5 to O-6 to O-1 to O-2. The spectrum (Fig. 1) shows that there are isotope effects on all hydroxy groups but OH-3. Clearly, there is a strong hydrogen bond between the *syn*-axial O-2 and O-4 atoms, which induces reasonably strong hydrogen bonds with its neighbours; OH-1 and OH-5 are seen, from their isotope effects, to be both donors and acceptors. This is the system chosen for *epi*-inositol, as optimized by theoretical calculations, by Liang *et al.*[18] Since the molecule is symmetrical, an equal number of molecules must have the same hydrogen bonds in the opposite direction; hence the formula does not show the direction of the bonds. The numerical value obtained for the isotope effects must be the average of the values for the hydroxy group as donor and as acceptor.

In α-D-talopyranose **2**, all hydroxy groups appear to form hydrogen bonds. The isotope effects are particularly numerous and strong, and there seem to be more effects than can be readily accounted for. Hence this sugar was studied in detail, looking not only at the α-pyranose but also at the mixture of all the four forms. Fig. 2 shows the signals of OH-2, OH-3 and OH-4 of the α-pyranose; the full spectrum of the equilibrium mixture, containing all the four forms of the sugar, is too complex to be reduced. To simplify the spectra, the four forms of 6-deoxy-L-talose were also studied, as well as its methyl α-glycoside. In all the pyranose forms there is a strong hydrogen bond between the *syn*-axial OH-2 and OH-4 groups.

In the crystal structure of α-D-talopyranose[19] there is a hydrogen bond between O–2 and O–4. It is of interest to note that Reuben,[20] in his work on the ^{13}C NMR spectra of partially deuteriated monosaccharides in Me_2SO, mentioned the presence of extra effects in the spectrum of α-D-talopyranose, which he attributed to an intramolecular hydrogen bond between O–2 and O–4. Such extra isotope effects on ^{13}C signals will occur only when the hydrogen bond is formed by acceptance;[1] if formed by donation, the deuterium atom would be four bonds away from the carbon atom, too far for an effect to show. Since both C-2 and C-4 of α-D-talopyranose show this extra effect, both OH-2 and OH-4 must act as acceptors of a hydrogen bond; that means that both O2-H···O4 and O2···H-O4 must be present in equilibrium. This explains why the effect is positive at both centres. Recent results of molecular dynamics simulations[21] suggest that in α-D-talopyranose the bond is predominantly from O-2 to O-4, whereas in the β-anomer it is mainly in the opposite direction (this seems to be contradicted by the isotope effects observed on OH-1 of the β-pyranose).

The strong OH-2/OH-4 hydrogen bond in the talopyranoses induces other hydrogen bonds. Both OH-2 and OH-4 are involved in three of them; OH-3 is bonded with OH-2 or OH-4, and OH-1 and OH-6 also have hydrogen bonds. β-D-Talopyranose is remarkable because its OH-1 signal is affected by two isotope effects. There is no way in which this hydroxy group could be bonded to two other oxygen atoms; bonding to the ring oxygen would not account for an isotope effect because that oxygen atom carries no hydrogen. One of the isotope effects is caused by deuterium substitution at O-2; the second

possibly by deuterium on O-4, through the arrangement O-1–H···O-2···H–O-4, which is more likely than a similar arrangement with O-3 because the O-2–O-4 hydrogen bond is the stronger one. This is the only instance encountered in this work where the isotope effect might be transmitted through two hydrogen bonds.

The small isotope effect on OH-1 of 6-deoxy-α-L-talopyranose is surprising; it may be explained by the presence of a small amount of a flexible form in which the O-2/O-4 repulsion is relieved by O-2 moving outwards, thereby coming closer to both O-1 and O-3; hydrogen bonds can then form between these three oxygen atoms. This would explain the presence of a third isotope effect on OH-2. The third isotope effect on OH-4 of 6-deoxy-α-L-talopyranose is not readily explained.

In β-D-ribopyranose and in methyl β-D-ribopyranoside, there is a hydrogen bond between the *syn*-axial O-2 and O-4 atoms and also a bond from O-3 to O-4. The same bonding was recently described by Uhlman and Vasella[22] in benzyl β-D-ribopyranoside. However, the Swiss authors observed an isotope effect (-15×10^{-4}) on OH-3 which we did not detect but we found a second isotope effect ($+18 \times 10^{-4}$) on OH-4 which they missed.

In contrast to the pyranoses, the OH-1 signals of many furanoses show isotope effects. When O-1 and O-2 are *trans*, a hydrogen bond between them appears unlikely; the bond appears to be between O-1 and O-3 or O-5, the flexibility of the furanose ring making such bonds geometrically possible; they are similar to *syn*-axial bonds in the pyranose system. When O-2 and O-3 are *cis* in a five-membered ring, no strong hydrogen bond was detected; the O–H···O angle would be unfavourable for its formation (see methyl α-D-lyxofuranoside and α-D-allofuranoside).

The OH-1 signals of fructose, both pyranose and furanose forms, show isotope effects; these, again, cannot all be explained by bonds between vicinal hydroxy groups. In the β-pyranose, there is a hydrogen bond between O-1 and O-3; the same hydrogen bond is also found in the methyl fructoside. This is a fairly strong bond and appears to extend to OH-4 and OH-5. There are two other isotope effects on OH-1; one of these indicates a bond to OH-2 but the interpretation of the other one is uncertain. There is also a hydrogen bond between OH-1 and OH-3 in α-D-fructofuranose, extending to OH-4, although this involves two *trans* hydroxy groups. A bond between *trans* hydroxy groups in a five-membered ring is expected to be very weak. The distance between O-1 and O-3 is shorter in the α-furanose than in the β-pyranose; hence the polarization induced by this bond may have a stronger cooperative effect on an adjacent hydrogen bond. Formation of the bond between O-1 and O-3 affects the conformation. It it was shown[14] that the predominant $^{\circ}E$ conformation of methyl α-D-fructofuranoside in water changes to E_5 in Me_2SO, presumably to bring O-1 and O-3 closer together for the formation of this bond; this change also reduces the distance between O-3 and O-4.

There appears to be no hydrogen bond between OH-1 and OH-3 of β-D-fructofuranose because they are in a *trans* relationship; instead, there is a bond between OH-1 and the anomeric OH-2. The α-pyranose shows a reasonably strong isotope effect on OH-2 but none on OH-1. The other signals could not be assigned (there is only 5% of the α-pyranose in the equilibrium mixture) but it seems certain that this hydrogen bond is to OH-4. α-D-Fructopyranose is to a considerable extent in the $^4C_1$ form in which OH-2 and OH-4 are *syn*-axial.

Discussion

The polyols listed in Table 2 can be arranged into three groups according to the strength of the intramolecular hydrogen bonds. In the first, there are only four polyols which show strong hydrogen bonds (isotope effect *ca.* 100×10^{-4}), comparable to the inter-residue bonds in disaccharides: α- (**2**) and β-talopyranose (and derivatives thereof), β-D-ribopyranose and *epi*-inositol **1**. All these compounds have two *syn*-axial hydroxy groups and clearly that is where the hydrogen bonding occurs, between O-2 and O-4. To these examples should be added the recently described[13] case of *myo*-inositol 2,4,6-orthoformate (2,4,6-*O*-methanetriyl-*myo*-inositol) **3** with a particularly large isotope effect (253×10^{-4} ppm). This group should also include 1,6-anhydro-β-D-glucopyranose **4**, although the isotope effect is smaller, there clearly is a hydrogen bond between the axial O-2 and O-4 atoms.†

It can be seen that the magnitude of the isotope effect — and the strength of the hydrogen bond — is inversely proportional to the O–O bond distance which is 2.77 Å for *myo*-inositol orthoformate[13] **3**, 2.96 Å for *epi*-inositol[23] **1** and 3.30 Å for the 1,6-anhydride[24] **4** (in crystalline form). The O–O distance is short (2.77 Å) in methyl β-D-ribopyranoside[25] but the isotope effect is smaller because only about half of the molecules are in the $^1C_4$ form — which has the *syn*-axial hydroxy groups — as shown by the coupling constant ($J_{1,2}$ 4.6 Hz).

In the crystal structure of α-D-talopyranose[19] **2**, methyl β-D-ribopyranoside[25] and *myo*-inositol orthoformate[13] **3**, there is a hydrogen bond from O-2 to O-4. However, there is no intramolecular hydrogen bond in the crystal structures of *epi*-inositol **1** or 1,6-anhydro-β-D-glucopyranose.[24] Thus the presence of an intramolecular hydrogen bond in the crystal depends not only on the favourable arrangement of two hydroxy groups but also on the strength of intermolecular hydrogen bonds.

If O-3 in a pyranose form is axial, one would expect to find a hydrogen bond between O-1 and O-3 in one of the anomers. Indeed, the OH-1 signal (at δ 6.05, *J* 8.0 Hz) of α-D-allopyranose shows an isotope effect of $+66 \times 10^{-4}$ with that of the α-furanose (δ 5.66, *J* 7.3) also showing an effect of $+41 \times 10^{-4}$; the signals of the β-anomers are not affected by deuteriation. Similarly, the OH-1 signal of α-D-ribopyranose (δ 6.09, *J* 8.2) shows an isotope effect of $+40 \times 10^{-4}$ and that of the α-furanose form (δ 5.68, *J* 7.7) of $+30 \times 10^{-4}$. These bonds are weaker than those between axial O-2 and O-4 atoms, again showing that the anomeric hydroxy groups form weaker hydrogen bonds than the other oxygen atoms. The hydrogen bonds of ribose are weaker than those of allose because the former is not fully in the $^4C_1$ form; it appears that about one third is in the $^1C_4$ form. Since the α-anomers are only minor components in the equilibrium mixture, their signals could not be assigned but there is an α-allopyranose signal (at δ 4.40, *J* 3.3 Hz) with an isotope effect of $+110 \times 10^{-4}$, probably the signal of OH-3.

The second group of sugars contains somewhat weaker hydrogen bonds formed between a hydroxy group and an adjacent hydroxymethyl group. The relationship between these groups, when the hydrogen bond is formed, is the same as that between *syn*-axial hydroxy groups but the mobility of the hydroxymethyl group means that its hydroxy group will be, for most of the time, in a conformation unsuitable for such a hydrogen bond. Such a bond was found between O-1 and O-3 of β-D-fructopyranose, α-D-fructofuranose, α-L-sorbopyranose, and between O-3 and O-5 of methyl α-D-allofuranoside (but not α-D-tagatopyranose where O-3 is axial). It also occurs between O-4 and O-6 of some aldopyranoses (*e.g.* in glucose and mannose). It is not found in α- or β-D-galactopyranose where O-4 is axial; the conformation required for the hydroxymethyl group would be unfavourable. It is also very weak in the talopyranoses.

By some means the presence of the anomeric oxygen atom of

† The hydroxy proton spectrum of this compound was assigned by Uhlmann and Vasella[13] but, working at 300 MHz, they did not observe an isotope effect.

ketoses strengthens such a hydrogen bond. This is not due to direct participation of the anomeric hydroxy group because it occurs just as readily in methyl β-D-fructopyranoside. The reason must be a steric effect. The methyl group on the anomeric oxygen atom, in its favoured conformation, will clash with OH-1 in its most stable conformation (C-1–O-1 antiparallel to C-2–C-3), thereby making the gauche conformation—required for the hydrogen bond to O-3—less unfavourable than it otherwise would be. 2,7-Anhydro-β-D-*altro*-heptulopyranose has the same configuration of the hydroxy groups as methyl β-D-fructopyranoside but there is no group on the anomeric oxygen atom pointing outwards: no hydrogen bond between O-1 and O-3 was found.

It is well documented[26] that six-membered ring hydrogen bonds between 1,3-diols are much more stable than the five-membered ones formed from 1,2 diols; the latter have rarely been detected in crystal structures of sugars (*e.g.* ref. 27). Calculations[17] indicate that the energy of a hydrogen bond in sugars between vicinal hydroxy groups is 0.85–1.5 kcal mol^{-1},‡ whereas between O-4 and O-6 of pyranoses it is 3.35 kcal mol^{-1} and between *syn*-axial hydroxy groups, 4.2 kcal mol^{-1}.

It is now found that, in the third group of sugars, weak hydrogen bonds occur but their occurrence is irregular and unpredictable. It is not clear whether all hydroxy groups are involved; isotope effects are not detected for every signal but some may be too weak to be observed. A good example is *myo*-inositol: there are six hydroxy groups and weak isotope effects were found on OH-1, OH-3 and OH-5, that is, on hydroxy groups which are not contiguous. Presumably all hydroxy groups have hydrogen bonds but the isotope effects are too small to be detected. The effect on OH-5 is 10×10^{-4} which is just about the limit of detection. Generally the values for the monosaccharides are between 10 and 30×10^{-4}; there seems to be no clear preference, in six-membered rings, of *cis* over *trans* pairs. Initiation of the cooperative bond system by the anomeric hydroxy group has been suggested,[5] yet the hydrogen bonds in α-D-mannopyranose, where this is not possible, are stronger than those in α-D-glucopyranose.

The SIMPLE method has shown that cooperative hydrogen bonds occur, in Me_2SO solution, in all monosaccharides, but how strong are these hydrogen bonds? The hydrogen bonds between the moieties of disaccharides, like maltose, are regarded as strong hydrogen bonds in Me_2SO on the evidence of coupling constants, IR spectra, *etc.* Their isotope effects are of the order of *ca.* 200×10^{-4} ppm, as are also some values of bonds between *syn*-axial hydroxy groups. If these are regarded as being almost completely bonded, then values of 10 or 20×10^{-4}, as found in many sugars, can be taken as being bonded to the extent of 5 and 10%, respectively: that is, at any time, only a small proportion of the molecules is thus bonded. Bonds will occur which are even weaker but are not detected in the NMR spectrum.

In chloroform and similar solvents which cannot form hydrogen bonds, hydroxy groups will be completely hydrogen bonded if there are other hydroxy groups within reach.[28] In Me_2SO these bonds will be much weaker since they have to compete with bonding by the solvent. In aqueous solution, even the inter-moiety disaccharide bonds are undetectable;[9,29] the bonds now discussed between vicinal hydroxyl groups in monosaccharides will not be found in aqueous solution. The suggestion that they may occur in an aqueous solution of glucose[30] is unwarranted.

The hydrogen bonds described herewith clearly do not explain the composition of sugars in Me_2SO solution. For example, there has been some discussion[6] whether formation of an intramolecular hydrogen bond in Me_2SO is responsible for the increase of the proportion of the α- and β-fructofuranoses, compared to that of the β-pyranose, when changing the solvent from water to Me_2SO. However, it is now found that hydrogen bonding of about the same strength occurs in all of the three forms. Similarly, the steep increase[6] in the proportion of α- to β-D-talopyranose on changing the solvent from water to Me_2SO is not caused by intramolecular hydrogen bonding in the latter: the occurrence and strength of this bonding is similar in the two anomers. Also, the hydrogen bonds formed between O-1 and O-3 of α-allo- and ribo-pyranose in Me_2SO solution do not result in an increase in the proportion of the α-anomers. Another explanation will have to be found for the change of composition on changing the solvent.

‡ 1 cal = 4.184 J

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