10. *The Structure and Conformation of Some Monosaccharides in Solution.*

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Nuclear magnetic resonance spectroscopy has been used to study aqueous solutions of some monosaccharides. α-D-Lyxose and β-D-ribose are each in conformational equilibrium. β-D-Lyxose and α-D-ribose exist in the C1 and 1C conformations, respectively. Crystalline D-ribose is in the β-pyranose form.

Mutarotation studies on aqueous solutions of monosaccharides in order to determine their equilibrium composition [1] depend for their success on the assumption that the only species involved are the α- and β-pyranose forms, and on the availability of both of these in the crystalline state. However, crystals of both anomers are available in only a few cases, and in at least one of these the equilibrium solution contains forms other than the two pyranoses.[2]

Measurement of the rates of bromine oxidation of the equilibrium solutions has been used to determine the relative amounts of rapidly and slowly oxidised components. The component with an equatorial anomeric proton is known to be oxidised more slowly than the component with an axial anomeric proton, so this method can also be used to determine the composition of the equilibrium solution of a sugar.[1,3] While it does not require the availability of both anomeric forms, this method cannot succeed if substantial amounts of non-pyranose forms are present or if the pyranose forms are in conformational equilibrium.

Both of the above methods have so far failed to determine the composition of ribose in solution. Infrared examination of the freeze-dried equilibrium solutions has recently [2] been used to determine the number of different species present, and ribose appeared to exist in solution entirely in the same form as the original crystalline sugar.

Nuclear magnetic resonance spectroscopy has been used [4] to determine the structure and conformation of carbohydrate derivatives, but little work on the simple free sugars has been reported. In the present study, the n.m.r. spectra were obtained in deuterium oxide as solvent, and it was also found advantageous to prevent interference by the hydroxyl protons of the sugar by previously replacing these with deuterium atoms.

[1] Isbell and Pigman, *J. Res. Nat. Bur. Stand.*, 1937, **18**, 141.
[2] Tipson and Isbell, *J. Res. Nat. Bur. Stand.*, 1962, **66***A*, 31.
[3] Bentley, *J. Amer. Chem. Soc.*, 1957, **79**, 1720; 1959, **81**, 1952.
[4] Lemieux, *Canad. J. Chem.*, 1961, **39**, 116; Rao and Foster, *J. Phys. Chem.*, 1963, **67**, 951; Pedersen, *Acta Chem. Scand.*, 1963, **17**, 1160.

The peaks in the spectra due to the anomeric protons appeared at low field, owing to the attachment of two oxygen atoms to C-1. In every case they were well separated from the other peaks in the spectrum, and coupling to the C-2 proton caused each anomeric proton to appear as a doublet. Assignment of anomeric proton peaks in the spectrum of an equilibrium solution was made by (i) comparing it with the spectrum obtained immediately after dissolution of the sugar, when only one species was present, (ii) making use of the fact that axial protons appear at higher fields than equatorial protons,[5] and (iii) assuming that the coupling constant (J) between the anomeric proton and the proton on C-2 depends on the dihedral angle between their bonds in the manner determined by Karplus.[6] The relative amounts of each form in solution could then be determined by integration of the area under each peak.

Experimental and Results

All samples were commercially obtained, except β-D-glucose, and were not further purified.

Deuteration.—The sugar (100 mg.) was dissolved in D_2O (0·2 ml.; 99·7%), and the solvent removed under a vacuum. This was repeated twice. In each case the sample crystallised before use and was assumed to have crystallised in the same anomeric form as the original sugar.

β-D-*Glucose.*—This was prepared by recrystallising D-glucose from pyridine.[7] It was not recrystallised from D_2O before running the spectrum.

Spectra.—The sugar (*ca.* 100 mg.) was dissolved in D_2O (0·5 ml.) and the spectrum run as quickly as possible on a Varian A-60 n.m.r. spectrometer at ambient temperature (30°) unless otherwise stated. After 24 hr. the solution was re-examined to obtain the spectrum of the equilibrium mixture. The internal standard was t-butyl alcohol or sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS). Results are quoted on the τ' scale (DSS = 10),[8] and t-butyl alcohol was taken to have $\tau' = 8{\cdot}800$. Coupling constants are given in cycles per second.

In some cases the residual water present (τ' 5·2—5·3) interfered slightly with the anomeric peaks. In such cases it was convenient to move this water peak to higher fields by increasing the temperature, or to lower fields by adding trifluoracetic acid. Either method caused only small differences in the spectra.

Equilibrium Mixture of Methyl D-*Ribosides.*—D-Ribose (5 g.) was dissolved in 2% methanolic hydrochloric acid (100 ml.), and kept at room temperature. The optical rotation of the solution was measured every week; after 11 weeks a constant value had been reached. Hydrochloric acid was removed from the solution by grinding with silver carbonate until neutral. Filtration and removal of the solvent gave the mixture of methyl D-ribosides.

Separation of the α- *and* β-D-*Ribosides.*—The mixture of ribosides (750 mg.), dissolved in a little water, was put on a cellulose column (50 × 5 cm.) and eluted with the top layer of the system n-butanol (40%)–ethanol (10%)–water (50%). The first fraction had a negative rotation and was shown by paper chromatography to contain the β-D-ribosides; the second had a positive rotation and contained the α-D-ribosides.[9] Evaporation of the solvent followed by *O*-deuteration gave the mixtures, which were examined by n.m.r. (Tables 1—3).

Table 1.

Chemical shifts and coupling constants for anomeric pyranose protons.

Original sugar and anomeric form	α-Pyranose form		β-Pyranose form	
	τ'	$J_{1,2}$	τ'	$J_{1,2}$
α- and β-D-Glucose	4·84	3·0	5·42	7·4
α-D-Galactose	4·74	1·8	5·44	6·5
β-D-Mannose	4·82	1·1	5·12	0·9
α-D-Xylose	4·83	2·6	5·44	7·3
β-L-Arabinose	5·48	6·6	4·77	2·0
β-D-Lyxose	5·02	4·0	5·17	1·3
D-Ribose (at 70°)	5·17	2·1	5·06	5·7

[5] Lemieux, Kullnig, Bernstein, and Schneider, *J. Amer. Chem. Soc.*, 1958, **80**, 6098.
[6] Karplus, *J. Chem. Phys.*, 1959, **30**, 11.
[7] Behrend, *Annalen*, 1910, **377**, 220.
[8] Tiers, Abstracts of Papers, 137th A.C.S. Meeting, Cleveland, Ohio, 1960, 17R.
[9] Bishop and Cooper, *Canad. J. Chem.*, 1963, **41**, 2743; Barker and Smith, *J.*, 1954, 2151.

TABLE 2.

Chemical shifts and coupling constants for anomeric protons of methyl ribosides and riboses, and the percentages present at equilibrium.

Sugar		Pyranose α	Pyranose β	Furanose α	Furanose β
Methyl D-riboside	τ'	5·38	5·32	5·01	5·11
	$J_{1,2}$	2·7	5·2	3·9	0
	%	10	71	4	12
D-Ribose (at 70°)	τ'	5·17	5·06	4·66	4·79
	$J_{1,2}$	2·1	5·7	*	1
	%	18	54	12	16
D-Ribose (at 30°)	τ'	5·17	5·08	4·6	4·76
	$J_{1,2}$	—	6·2	*	1·4
	%	77		23	

* Broad peak with separate components unresolved.

TABLE 3.

Percentages of pyranose forms at equilibrium.

Sugar	This work α (%)	This work β (%)	Bromine oxidation [2] Less reactive (%)	Bromine oxidation [2] More reactive (%)
D-Glucose	36	64	37·4	62·6
D-Galactose	35	65	31·4	68·6
D-Mannose	64	36	68·9	31·1
D-Xylose	29	71	32·1	67·9
L-Arabinose	63	37	32·4	67·6
D-Lyxose	69	31	79·7	20·3
D-Ribose (at 70°)	18	54	89·3	10·7

DISCUSSION

The results given in Table 1 for D-glucose, D-galactose, and D-xylose are conveniently discussed together. In each case the spectrum of the equilibrium solution showed two doublets, one centred between τ' 4·74 and 4·84 and the other between τ' 5·44 and 5·42. The former was assigned to an equatorial proton and the latter to an axial proton. These sugars are known to exist in the C1 conformation (*e.g.*, Ia) in which the anomeric proton is equatorial in the α-pyranose, and axial in the β-pyranose. The low- and high-field protons are therefore due to α- and β-pyranoses, respectively. The value of the coupling constant for the three α-sugars is small and is consistent with an equatorial anomeric proton coupled to either an axial or an equatorial proton at C-2. The coupling shown by the β-sugars is larger (6·5—7·4) and supports the conclusion that the protons at both C-1 and C-2 are axial. The above assignments were all confirmed by noting that in the spectra obtained immediately after dissolution of the sugar only one of the doublets was present, and this corresponded with the initial anomeric form of the sugar used.

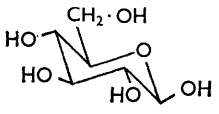
The spectra of both glucose and xylose showed no other peaks in the anomeric region and must consist solely of a mixture of α- and β-pyranoses. The spectrum of galactose showed a small additional doublet ($J = 2·5$) at τ' 4·84 which was incompletely resolved from the doublet due to α-galactopyranose. This probably indicates that there is a small amount of a furanose form present in the equilibrium solution of galactose.[2]

In contrast to most of the other sugars in the D-configurational series, D-arabinose has been shown by bromine oxidation [10] to be in the 1C conformation. The large coupling constant and high τ' value shown by one of the doublets in the spectrum of L-arabinose (Table 1) requires that the sugar responsible for it have axial protons at both C-1 and C-2. This is only possible for α-L-arabinose in the C1 conformation. The other doublet must be due to β-L-arabinose, and the low τ' value requires that this be equatorial. Thus, both

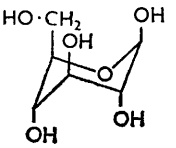
[10] Bentley, *J. Amer. Chem. Soc.*, 1957, **79**, 1720.

pyranose forms of L-arabinose are confirmed as being in the C1 conformation, and D-arabinopyranose must be in the 1C conformation (II). No peaks due to the presence of any other ring form were apparent.

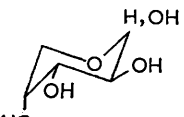
The axial proton in α-galactose and α-arabinose showed a small coupling of 1·1 c./sec. in addition to the larger axial–axial coupling.



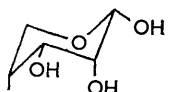
(Ia) C1



(Ib) 1C



(II)



(III)

Although all the above sugars appear to be in one conformation it is not possible on the basis of the individual results to exclude a small contribution from the other chair conformation. However, the closeness of the values of τ' and $J_{1,2}$ for the anomeric protons of the following pairs of compounds is strong evidence that they exist in the same conformation or mixture of conformations: α-D-glucose and α-D-xylose; β-D-glucose and β-D-xylose; β-D-mannose and β-D-lyxose; α-D-galactose and β-L-arabinose; β-D-galactose and α-L-arabinose. If any of these sugars existed as an equilibrium mixture of conformations, for example (Ia) ⇌ (Ib), the equilibrium position could not be the same for the hexose and pentose members of each pair, because the effect of the exocyclic hydroxymethyl group in the hexose would considerably stabilise its C1 conformation and destabilise its 1C conformation relative to the corresponding conformations of the pentose. This would lead to a difference in the values of τ' and J between members of the pair. The absence of any significant difference shows that all these sugars exist in a single conformation which must be C1.

The low value of τ' for the axial anomeric proton in β-D-mannose and β-D-lyxose must be associated with the presence of an axial hydroxyl on C-2.

A similarity in τ' for the anomeric protons is not shown by the pair α-D-mannose and α-D-lyxose. The value for α-D-mannose is about the same as in the case of α-D-glucose and α-D-xylose, and is consistent with its equatorial character. The higher value for α-D-lyxose indicates the presence of a considerable amount of the 1C conformation, in which the anomeric proton is axial. This is supported by the value of $J_{1,2}$ which is roughly half-way between the value for α-D-mannose and the value for compounds with axial protons at C-1 and C-2.

The possibility that the high value of $J_{1,2}$ in α-D-lyxose is due to a distorted ring is ruled out by considering the effect of such a distortion on $J_{1,2}$. The only distortion of the ring which can be expected is the movement of the two axial hydroxyl groups at C-1 and C-2 away from the axis of the ring. This change would reduce any 1,3 interaction with axial hydrogen atoms, but would have the effect of increasing the dihedral angle between the hydrogen atoms on C-1 and C-2 to above the nominal 60°. Such a change would, according to the Karplus equation,[6] result in a decrease and not an increase of $J_{1,2}$.

In the case of ribose the assignment of the peaks was more difficult, first because the anomeric configuration of crystalline ribose is not established with certainty, and secondly, because there were more peaks present than would be expected for a mixture of α- and β-pyranoses, and some peaks were incompletely resolved. If the spectrum was run at 70° the unresolved peaks separated, and the peak due to residual water moved to higher field clear of the anomeric region.

In order to assign these peaks with certainty, an equilibrium mixture of methyl D-ribosides was prepared by treating D-ribose with methanolic hydrogen chloride. The n.m.r. spectrum of this mixture showed overall similarity to the ribose spectrum, and unresolved bands were also present. It has been shown [9] that a mixture of methyl ribosides can be

separated by chromatography into two fractions containing the β-pyranoside and -furanoside in one, and α-pyranoside and -furanoside in the other. Also, the major component of each fraction was the pyranoside.

The n.m.r. spectrum of the fraction containing the β-ribosides had a large doublet (J = 5·2) together with a much smaller single peak at lower field. The large doublet was assigned to the anomeric proton of methyl β-D-ribopyranoside, and the smaller peak to the corresponding furanoside. In the case of the mixture of α-ribosides a large doublet (J = 2·7) which was assigned to methyl α-D-ribopyranoside was accompanied by three small peaks. One of these had the same chemical shift as the small peak in the β-riboside fraction and was probably also due to methyl β-D-ribofuranoside. The remaining two peaks are probably the two parts of a doublet due to the anomeric proton of methyl α-D-ribofuranoside. The furanoside assignments are supported by the coupling constants determined by Hall for 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose ($J_{1,2}$ = 3·9) and for methyl 2,3-anhydro-β-D-ribofuranoside ($J_{1,2}$ = 0).[11]

By comparison of the values of τ', J, and the intensities of the methyl riboside peaks with those of the ribose peaks the assignments shown in Table 2 can be made.

The values of J and τ' shown by the anomeric proton of β-D-ribopyranose (Table 1) shows that it is in equilibrium between the C1 and 1C conformations.

The coupling constant cannot be used to show the conformation of α-D-ribopyranose because in both conformations it would have a low value in the region of 1—3 c./sec. The chemical shift, however, is identical with that in β-D-lyxose and very close to that in β-D-mannose. This value of τ' was shown above to be characteristic of an axial anomeric proton which is associated with an axial hydroxyl group at C-2. This arrangement is also present in the 1C conformation of α-D-ribopyranose (III), and it is therefore concluded that this sugar exists in solution entirely in this conformation.

The conclusions reached in the above discussion are assembled in Table 4.

TABLE 4.

Conformations present in solution.

C1	C1 + 1C	1C
α- and β-D-Glucose	α-D-Lyxose	α-D-Ribose
α- and β-D-Galactose	β-D-Ribose	α- and β-D-Arabinose
α- and β-D-Mannose		
α- and β-D-Xylose		
β-D-Lyxose		

It has been shown [12] that β-D-lyxose forms a relatively stable triester of periodic acid, and for this to occur the sugar must change to the 1C conformation. It is probable, therefore, that although it normally exists in the C1 form the energy difference between the two conformations is small. Such is not the case with β-D-mannose which has the same orientation for its hydroxyl groups as β-D-lyxose. It does not form a triester with periodate presumably because the terminal hydroxymethyl group destabilises the 1C conformation. The presence of some of the C1 conformation in the case of β-D-ribose is consistent with the formation of a triester with periodate of both ribose itself and β-ribopyranosides.[12] It also provides an explanation for the anomalous results obtained from bromine-oxidation studies of ribose and ribopyranosides.[3] Consideration of general principles of conformational analysis would predict that the 1C conformation of α-D-lyxopyranose and α-D-ribopyranose would be more stable than in the case of the β-D-sugars. This prediction is supported by the present results.

Table 3 gives the results obtained in this work for the composition of the equilibrium solutions, compared with the results obtained by bromine oxidation.[2]

There is satisfactory agreement between these results except in the case of D-ribose

[11] Hall, *Chem. and Ind.*, 1963, 950.
[12] Barker and Shaw, *J.*, 1959, 584.

and to a lesser extent D-lyxose. It is significant that these are the two sugars in which mixtures of conformers occur, and the disagreement is probably a result of the inadequacy of the bromine-oxidation method in these circumstances.

The infrared result mentioned earlier also disagrees with the present conclusion, but it was pointed out by Tipson and Isbell that the sugars could crystallise during the freeze-drying of their equilibrium solutions. This must have happened in the case of ribose.

The present estimate of the total amount of non-pyranose forms present in the equilibrium solution of ribose (23%) compares well with the estimate of 20% based on periodate-complexing experiments.[12]

The rapid mutarotation of ribose made it impossible to detect which species was present initially. By the time the first spectrum could be run the equilibrium between α- and β-forms had been established. It was, however, possible to see that there were no furanose sugars present at the start. The initial rapid change which occurs on dissolving ribose in water is therefore an equilibrium between the α- and β-pyranoses only. Furanose forms are formed in a subsequent slower reaction.

It has been pointed out [12] that the direction of the initial mutarotation change can only provide reliable evidence for the configuration of the original sugar when this change is known to be the result of an equilibrium between the two anomeric forms with the same ring size. The above observation, that this is the case for D-ribose, enables the initial mutarotational change, from a negative to a less negative value,[1] to be used to establish that crystalline D-ribose is in fact β-D-ribopyranose.

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