Variation in the Hydrophilicity of Hexapyranose Sugars Explains Features of the Anomeric Effect

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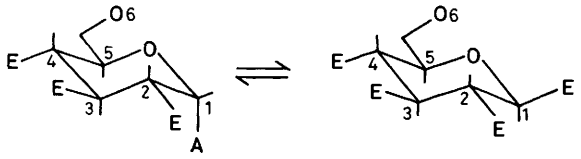
For the complete family of aldopyranoses, the relative amounts of α and β anomers present in aqueous solution can be explained solely in terms of the relative hydrophilicities of the anomeric pairs. Hydrophilicity is defined here as the probability that a water • • • solute hydrogen bond will form, and a method for its calculation is described. The hydrophilicities of the sugars also correlate with a number of, previously uninterpretable, thermodynamic data which shows the structure-breaking effect of sugar solutions follows the order glucose > mannose > galactose.

D-Glucose, D-galactose, and D-mannose are the commonest members of the family of 16 aldohexapyranose sugars. Their configurations are given in the Table. In aqueous solution, these sugars mutarotate and set up an equilibrium between the α-anomer (I), with O(1) axial, and the β-anomer (II), with O(1)



(I) (II)

equatorial to the chair-shaped pyranose ring. A set of empirical energy parameters has been derived to rationalise the experimentally observed α:β ratios found in solution.[1] Subsequent molecular mechanics calculations on aldopentapyranoses [2] and aldohexapyranoses [3] did not provide a straightforward explanation of the observed α:β ratios, and the suggestion was made that specific solvation effects were likely to be important, particularly for isomers with a large number of equatorial groups.

The anomeric effect was a term introduced to describe the surprising preference, shown by a variety of substituted hexapyranoses, for a polar substituent on C(1) to adopt the axial configuration. The effect has been extensively reviewed and discussed.[4–11] In general there is an increase in the proportion of α-anomer with (i) decreasing dielectric constant of the solvent, (ii) presence of an axial group on C(2), and (iii) successive methylation of hydroxy groups. There is a considerable amount of theoretical and experimental evidence [8] to show that the α-anomer is stabilised by an electronic effect which increases with the electronegativity of the C(1) substituent. *Ab initio* calculations on the model compound dimethoxymethane [9] show that this stabilisation comes from a preferential delocalisation of the lone-pair electrons of the ring oxygen atom for a molecular conformation corresponding to the α-anomer.

The conventional rationalisation of the α:β anomeric ratio is

Table.

Name	Configuration at carbon[a] 1 2 3 4 5	%α[b]	Hydrophilicity (Å³)[c]	$H(\alpha) - H(\beta)$/ Å³[d]	Energy PIFF (kJ mol⁻¹)[e]	$E(\alpha) - E(\beta)$/ kJ mol⁻¹[f]	Energy MM2 (kJ mol⁻¹)[g]	$E(\alpha) - E(\beta)$/ kJ mol⁻¹[h]	A.E. nodes[i]
1 α-Glucose	A E E E E	37	235.9	−6.0	−1.31	−4.63	38.82	−4.58	1
β-Glucose	E E E E E		241.9		3.32		43.40		0
2 α-Galactose	A E E A E	38	226.4	−5.8	−3.23	−4.30	40.27	−2.67	3
β-Galactose	E E E A E		232.2		1.07		42.94		2
3 α-Mannose	A A E E E	66	236.9	+5.5	1.42	−0.67	39.90	+0.45	1
β-Mannose	E A E E E		231.4		2.09		39.45		2
4 α-Talose	A A E A E	56	219.1	+5.1	−2.63	+4.12	37.03	+1.31	3
β-Talose	E A E A E		214.0		−6.75		35.72		4
5 α-Allose	A E A E E	19	221.3	−13.9	−5.40	−5.33	40.13	−2.93	3
β-Allose	E E A E E		235.2		−0.07		43.06		2
6 α-Altrose	A A A E E	43	227.9	−6.6	1.38	+5.92	44.08	+10.30	1
β-Altrose	E A A E E		234.5		−4.54		33.78		2
7 α-Gulose	A E A A E	17	221.8	−13.8	−3.28	−5.54	42.33	+0.56	3
β-Gulose	E E A A E		235.6		2.26		41.77		2
8 α-Idose (*C*1)	A A A A E	55	215.0		−8.05	−7.27	44.84	+0.03	1
β-Idose (*C*1)	E A A A E		224.4		−0.78		44.81		2
α-Idose (1*C*)	E E E E A	(55)	238.8		0.24	+2.07	40.82	−3.46	
β-Idose (1*C*)	A E E E A		223.1		−1.83		44.29		

[a] A = axial, E = equatorial for hydroxy groups on (I) or (II). [b] % α-anomer in aqueous solution as determined by n.m.r. studies.[2,3] [c] Hydrophilicity is the volume (Å³) round the sugar in which a water molecule can form a hydrogen bond stronger than −6 kJ mol⁻¹. [d] $H(\alpha) - H(\beta)$ is the difference in hydrophilicities of a pair of anomers. [e] Energies calculated using the molecular mechanics package PIFF.[12] [f] $E(\alpha) - E(\beta)$ is the difference in energy of a pair of anomers calculated by PIFF. [g] Energies calculated using the molecular mechanics package MM2.[13] [h] The difference in energy of a pair of anomers calculated by MM2. [i] The number of A.E and E.A (equatorial, axial) nodes in the isomer; from inspection of column *a* in Table.

that there is a balance between the electronic effect, which favours the α-anomer, and a (presumably) steric effect which favours the β-anomer. This description however does not explain changes in the α:β ratio on changing solvent, on methylation or on change in configuration of other atoms in the pyranose ring. An alternative description is given here by showing that it is the energy of interaction with the solvent which provides the driving force for glucopyranoses to adopt a given anomeric configuration.

Methods

Models of each of the 16 isomers were derived from molecular mechanics refinements of a standard hexapyranose group. For each of the models a value of its hydrophilicity was calculated, as described below, by determining the volume round each molecule in which a water molecule may form a hydrogen bond to the sugar.

Energy-minimum Conformations.—Models of each of the 16 aldohexapyranoses were constructed with O(6) in the energetically favoured conformation [O(5)–C(5)–C(6)–O(6) = 60°]. These models were then refined by two molecular mechanics programs, PIFF[12] and MM2,[13] each using a quite different force field. There are no statistically significant differences in bond lengths and angles between the energy-refined structures and average *X*-ray crystal structure values.[14] Bond lengths differ by up to 0.05 Å and bond angles by *ca.* 2° from these average experimental values. Energies for each structure are given in the Table.

The main purpose of the molecular mechanics procedures used here was to provide reasonable starting geometries for the subsequent determination of hydrophilic volumes and it was indeed shown that the calculated hydrophilic volumes obtained using models based on the refinements of either force field gave very similar results. It is however worth noting that both force fields show only altrose and talose to have a significant (> 1 kJ mol^{-1}) preference for the β-configuration. There also appears to be no correlation between the observed α:β ratio and $E(\alpha - \beta)$ for either force field, and furthermore, isomers in which the C(2) substituent is equatorial show a lower energy for the α-anomer, resulting from more favourable van der Waals terms.

Molecular Hydrophilicity.—The 'molecular interaction potential' E(ip)[15] between the sugar and a probe atom is defined as the sum of van der Waals E(vdw) and hydrogen bond E(hb) energy terms [equation (1)].

$$E(\text{ip}) = E(\text{vdw}) + E(\text{hb}) \qquad (1)$$

In this case the probe atom mimics the properties of a water molecule acting as a hydrogen-bond donor or acceptor, and E(ip) is calculated at all points of a finely spaced (0.2 Å) cubic lattice. The van der Waals energy contribution at each lattice point is given by equation (2) where g are all atoms not

$$E(\text{vdw}) = \sum_g (A/r_{\text{gp}}^{12} - B/r_{\text{gp}}^{6}) \qquad (2)$$

forming a hydrogen bond to the probe, r(ip) is the distance between the probe and the sugar atom g. A and B are coefficients taken from the program AMBER.[16]

The hydrogen-bond function provides an angular dependence on bond strength and is based on the empirical function derived by Vedani and Dunitz[17] [equation (3) where h are all atoms

$$E(\text{hb}) = \sum_h (C/r_{\text{hp}}^{12} - D/r_{\text{hp}}^{10}) \cdot \cos^2(115 - \chi) \qquad (3)$$

forming a hydrogen bond to the probe and C and D are coefficients taken from ref. 17]. In an ideal geometry where probe · · · O distance is 2.8 Å and the probe · · · O–C angle is 115°, the hydrogen-bond energy is −18.5 kJ mol^{-1}.

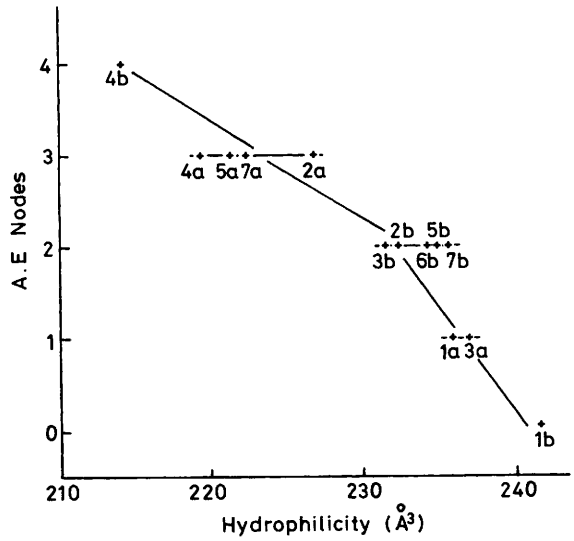


Figure 1. A plot of the hydrophilicity (column *c*, Table) against the number of A.E nodes (column *i*, Table). Key in Table

The three-dimensional lattice of E(ip) values can be contoured at any given energy value and the volume enclosed by the contour is then a measure of the hydrogen-bonding capacity of the molecule. Molecular hydrophilicity of a given molecule is defined here as the total volume in which a water molecule probe has an interaction energy of < −6 kJ mol^{-1} with the sugar molecule. The value of −6 kJ mol^{-1} was chosen to eliminate the effects of favourable water · · · H or · · · C van der Waals contacts, interactions which could also occur in lipophilic solvents. Results did not vary appreciably on choosing different cut-off limits, though for high interaction energies (< −10 kJ mol^{-1}), the contribution to the hydrogen-bonding term from axial oxygen atoms was reduced. This indicates that equatorial oxygen atoms have a bigger probability of forming a strong hydrogen bond.

The Table shows the hydrophilicities for each of the aldopyranose isomers. It is interesting to note that for those isomers which do not have three consecutive axial substituents (*i.e.* excluding α,β-idose and α-altrose), the hydrophilicity is a function of the number of axial and equatorial nodes (Figure 1).

This method, described above, of evaluating the effect of solvent interaction is quite different from the methods used in protein studies in which properties of the portions of molecular surface are ascribed to particular atoms in the molecule,[18,19] an approach which has also been used to explain partition coefficients of a number of monosaccharides.[20]

Discussion

α:β *Equilibrium and the Anomeric Effect.*—Anomeric ratios for each of the aldopyranoses have been experimentally determined from n.m.r. work.[21–24] The difference in hydrophilic volume between each anomeric pair [$H(\alpha) - H(\beta)$] is clearly correlated with the experimentally observed anomeric ratios in aqueous solution (Figure 2), and gives a correlation coefficient of 0.974. There is also good correlation of 0.966 between log (α/β) and the difference in hydrophilic volume.

Some spectra are complicated by the presence of significant (*ca.* 30%) amounts of furanose in solution, as is the case for talose, altrose, and idose. The n.m.r. analysis of idose is further complicated by the presence of $C1$ and $1C$ conformers. Recent

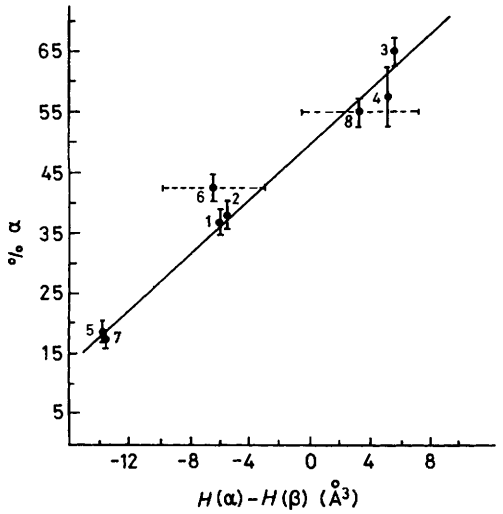


Figure 2. A plot of the $\%\alpha$ isomer in aqueous solution (column *b*, Table) against the difference in hydrophilicities of the α,β anomeric pair (column *d*, Table). The error bar for 4 is larger because of overlapping signals in the n.m.r. spectrum.[23] There is a large uncertainty in the hydrophilicities of 8 and 6 because of the presence of 1*C* and *C*1 conformers in solution

high-field analysis[28] has made use of assigned ^1H–^1H coupling constants to suggest that the β-idose anomer exists 75% as the *C*1 conformer and 25% as the 1*C* conformer. The weighted mean hydrophilic volume of both conformers of β-idose (Table) is then 223.8 Å^3. It is likely, however, that the α-idose anomer adopts a skew conformation[25,26] which is taken here to have the average hydrophilic volume of the α-*C*1 and the α-1*C* conformers and gives a hydrophilicity of 226.9 Å^3 for the α-idose anomer. The difference in hydrophilicity between the idose anomers $[H(\alpha) - H(\beta)]$ is then 3.1 Å^3. There is also some experimental evidence to show that altrose may also partially adopt the 1*C* conformation, though to a lesser extent than iodose.[23] The calculated hydrophilicities for both altrose and idose show a greater hydrophilic volume for the α-anomer over the β-anomer in the 1*C* conformation, but a greater hydrophilic volume for the β-anomer over the α-anomer in the *C*1 conformation.

The correlation between greater hydrophilicity and percentage α-anomer shown in Figure 2 indicates that the overriding factor in determining the anomeric equilibrium is the relative hydrophilicity of the two anomeric isomers. The energy gain in forming sugar • • • water hydrogen bonds outweighs both the intramolecular van der Waals and electronic effects. A strong hydrogen bond has an energy of *ca.* −18 kJ mol^{-1}, so it is not surprising that the formation of even one weak hydrogen bond will outweigh the van der Waals and electronic terms which are likely to be smaller than −10 kJ mol^{-1}.

The observation that the anomeric effect increases with decreasing dielectric constant is now simply explained as a reduction of the importance of sugar • • • solvent interaction and an increase in the importance of van der Waals and electronic effects. This interpretation fits with results from some recent PCILO quantum chemical calculations which have been used to determine the anomeric composition of glucose in a number of solvents.[27] It was shown that, *in vacuo*, the α-anomer is preferred (in agreement with the molecular mechanics results in the Table), but by incorporating a solvent effect, the proportion of β-anomer increases, particularly with water as solvent.

The anomeric effect has also been found to depend on the configuration of other ring substituents, especially C(2) where there is a stronger effect with an axial substituent (*e.g.* mannose). This can gain be explained as a solvent effect in that an equatorial O(2) impinges slightly on the hydrogen-bonding volumes of both an equatorial and axial O(1) (as implied by Figure 1). With O(2) axial, however, only the hydrogen-bonding capacity of O(1) in an equatorial configuration is affected and there will be an additional push towards the α-anomer.

Thermodynamic Results.—Calorimetric studies on selected mono- and oligo-saccharides[28] have provided values of 'excess enthalpy' which gives a measure of the effect of solvent–solute interaction. For one mol of sugar in 1 kg water, the excess enthalpies were found to decrease in the order D-glucose (330) > D-mannose (193) > D-galactose (133) J. The total hydrophilicity of each aldopyranose can be calculated as a weighted average of the separate α and β values to give values D-glucose 239.7, D-mannose 235.0, and D-galactose 230.4 Å^3, showing a good correlation between hydrophilicity and excess enthalpy.

Activity coefficients for glucose, mannose, and galactose in aqueous solution have also been experimentally determined from osmotic measurements.[29] These coefficients combined with the excess enthalpy values give values for the 'excess partial molar entropy of water'. Essentially the more negative this number, the more ordered the water. These experimental results show that the structure-making ability of the monosaccharides lies in the order D-glucose > D-mannose > D-galactose. An attempt to fit these data[29] to specific hydration models[30] was not successful. However, the non-specific model provided by molecular hydrophilicity predicts exactly this order.

Molecular hydrophilicity as defined here is a measure of the probability that a solute • • • water hydrogen bond will form. The approach outlined in this paper seems to provide a good quantitative method for evaluating the importance of hydrogen bonding in solvent • • • solute interactions.

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