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Physical chemistry of small carbohydrates—equilibrium solution properties

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Experimental techniques are now available which can probe the intricacies of carbohydrate structures, equilibria and dynamics. The development of theoretical tools has not kept pace with this progress; the reliance on hard sphere models and orientational averaging is unlikely to reveal the true nature of the phenomena involved. Computer simulation of PHCs in solution may, in the hands of experts, provide valuable assistance to the experimenter but is only in the beginning stages. It is becoming clear that several of the "rules" which were postulated in earlier days as governing PHC behaviour will have to be jettisoned. Although solvation interactions of PHCs are weak - they can be no stronger than hydrogen bonds - evidence is accumulating that solvation nevertheless plays a part in determining the positions of sugar equilibria and saccharide shapes. This calls into question the undue reliance on crystal structures in investigations of solution behaviour. There are hopeful signs that the long neglect of carbohydrates by physical chemists is now coming to an end.

INTRODUCTION

Sugars and polyols, along with amino acids, lipids and the nucleotide bases form the chemical building blocks of living matter. Their involvement as monomers, oligomers and polymers ranges from such well-understood attributes as sources of metabolic energy (starch) and load-supporting structures (cellulose), to rather more obscure functions, such as recognition (immune responses) and freeze avoidance (Antarctic fish antifreeze glycoproteins). They appear as adjuncts to proteins and lipids and as major components in nucleic acids. Many different sugar derivatives occur in natural products, the commonest being deoxy- and aminosugars, and sugar phosphates, sulphates and carboxylates. A vast literature describes the chemistry of sugars, their derivatives and polymers. Most of it is devoted to organic and biochemistry: synthesis, derivatization, structures and kinetics. Recently attention has shifted to mechanisms which govern biological recognition and also to the shapes of polysaccharides which are responsible for the remarkable rheological properties of materials such as mucous gels, synovial and oil drilling fluids.

This review is of necessity limited in its scope. It covers recent progress in the physical chemistry of low molecular weight carbohydrates in solution, with emphasis on the manner in which solvents can influence the shapes of carbohydrate molecules, the composition of their equilibrium mixtures (anomers and tautomers) and the interactions between polyhydroxy (PHC) molecules. For the most part, attention will be focussed on very dilute solutions in which the observed behaviour reflects solvation effects and on semi-dilute solutions for which solute-solute interactions can be expressed in terms of second virial coefficients, i.e. molecular pair interactions only are considered. The discussions will be restricted to equilibrium situations. Concentrated solutions and low moisture systems form the subjects of the accompanying review by Slade. Such systems cannot be treated by equilibrium approaches, at least not without stringent tests, because their properties, as measured, are usually time-dependent and dominated by slow relaxation processes. Considerable confusion exists in the published literature, because steady-state behaviour is frequently mistaken for true equilibrium.

HISTORY AND CURRENT POSITION

Sugars and small oligosaccharides were largely neglected by physical chemists until about 10 years ago, when Suggett reviewed the state-of-the-art (ref. 1). Except for their crystal structures, only scant information was at that time available about interactions between sugar molecules, the thermodynamics and kinetics governing interconversions between different isomers and conformers, solvent effects on such processes and the detailed shapes of di- and oligosaccharides in solution. Schallenberger has provided a more up-to-date monograph (ref. 2) in which he discusses such limited progress in the solution chemistry of sugars which was made during the intervening years. One consequence of the neglect of

solvent effects on shapes, equilibria and interactions is the very rudimentary state of our understanding of the molecular basis which underlies the remarkably varied types of rheological behaviour exhibited by polysaccharide solutions, gels and glasses. Such very important but complex subjects are still described in terms of simplistic pictures, e.g. "hairy" versus "smooth" polymer segments, or "egg boxes" (ref. 3) which have not always been tested for consistency with diffraction data.

Early vapour pressure and freezing point measurements on dilute aqueous solutions of some of the common sugars led to the conclusion that deviations from ideal solution behaviour (Henry's law) were marginal and could be well accounted for by simple hydration models. Hydration was expressed by the assignment of one or more "bound" water molecules to each hydration site, i.e. -OH group. The colligative properties of an aqueous solution of a sugar S with n hydration sites could then be expressed in terms of equilibria of the type

$$S_{i-1} + H_2O \overset{K_{i-1}}{\rightleftharpoons} S_i \qquad i = 1, 2, \ldots\ldots, n \qquad (1)$$

Assuming all hydration sites (and therefore all equilibrium constants K_{i-1}) to be equivalent, an average hydration number could be derived which depended only on the water chemical potential (water activity). Remarkably, with such a simple representation of sugars in aqueous solution at 298.2 K, Stokes and Robinson were able to account for the water activity of solutions of glucose (n = 6, K_n = 0.789) up to saturation and for sucrose (n = 11, K_n = 0.994) up to 6M (ref. 4).

The flaws in the treatment become apparent when the effects of temperature and/or pressure on K_n are considered. Thus, for ethane diol, glucose and sucrose, the temperature dependence of the water activity predicts that with increasing temperature, the concentrations of the hydrated species increase (refs. 5,6), a result which must surely be at odds with physical reality. The apparently satisfactory fit of eqn. (1) to the experimental data is due largely to enthalpy/entropy compensation which allows free energies to be adequately fitted with one-parameter models (ref. 7).

Added to the non-discriminatory nature of the Gibbs free energy G is the observation that enthalpies and entropies of solvation and interconversion tend to be small with only minor heat capacity effects compared to those observed for hydrophobic solutes (ref. 8). It therefore requires experimental measurements of very high precision and theoretical models of a high degree of refinement to probe sugar solvation and the effects (if any) of different solvents on the details of sugar conformation. The experimental techniques have become available during the past decade, but theory has hardly kept pace with experiment. Sugars are still commonly treated as hard spheres, so that the distinguishing features, i.e. the orientations of the peripheral -OH dipoles, are averaged out in calculations. Another weakness in the available theoretical approaches depends on the fact that conformational energy calculations provide internal energies, whereas experimental measurements (usually) yield Gibbs free energies. Moreover, the calculations are based on vacuum atom-atom potential functions. Solvation effects are sometimes introduced (with hindsight) as empirical corrections, because no rigorous methods exist for their calculation.

SOLVENT EFFECTS OF CONFORMATIONAL EQUILIBRIA OF MONOSACCHARIDES

The experimental complexities facing the student of sugar conformations and interactions in solution result from the chemical heterogeneity of the solutions. Figure 1 graphically illustrates the many possible conformations of the ϕ-pyranoid sugar ring and their interconversion paths (ref. 9). Only a limited number of the conformers shown occur in simple sugars, although it is not certain whether some of the rarer shapes play a significant role in determining polysaccharide conformations.

The advent of high field ^1H and ^{13}C nmr methods have greatly facilitated the analysis of chemically complex mixtures. Both the nature of the epimers, tautomers and anomers and their respective concentrations in an equilibrium mixture can be estimated from a combination of chemical shift data, coupling constants, nuclear Overhauser effects (n.O.e.) and nuclear magnetic relaxation rates.

In the simplest cases, e.g. glucose, the equilibrium mixture only contains the two anomeric forms of one of the possible ring conformers; in this case it is the $^1C_4$ pyranoid ring. In many other cases several tautomers and their anomeric forms coexist. The equilibrium mixture of ribose contains at least six distinct species: the two anomeric forms of each of the $^1C_4$ and $^4C_1$ pyranoses and of the furanose ring. The composition of the equilibrium mixture depends on the solvent. This is shown in Fig. 2 which compares the effects of solvent [D_2O and dimethyl sulphoxide (DMSO)] and temperature on the equilibrium mixtures as they exist in solutions of glucose and ribose (ref. 10). For tabulated data of the equilibrium compositions of some representative sugars in solution, see ref. 11. Table 1 provides an abstract of Angyal's compilation which illustrates that the nature of the epimer as well as the solvent determine the equilibrium composition. Actually the situation is somewhat more complex than shown in Table 1, because several pyranose conformers might

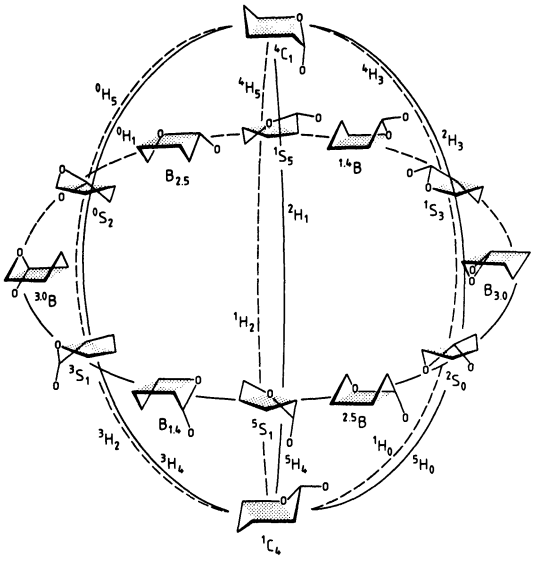


Fig. 1. Ring conformations of α-pyranoses and their positions on the conformational sphere. The 12 half-chain conformations and the interconversion paths are also indicated. Reproduced, with permission from ref. 9.

TABLE 1. Tautomeric and anomeric equilibrium compositions of sugars in solution. D = D_2O, P = pyridine-d_5, DMSO = dimethyl sulphoxide-d_6. Data from ref. 11.

Sugar	Temp. °C	Solvent	Pyranose α	Pyranose β	Furanose α	Furanose β	
Allose	31	D	14	77.5	3.5	5	
	50	P	23	61	5	11	
Altrose	22	D	27	43	17	13	
	25	P	27	36	24	13	
	25	DMSO			44		
Galactose	31	D	30	64	2.5	3.5	
	25	P	33	48	7	12	
Mannose	44	D	65.5	34.5	0.6	0.3	
	116	P	78	22			
	116	DMSO	86	14			
Arabinose	31	D	60	35.5	2.5	2.0	
	80	P	33	33	21	13	
Xylose	31	D	36.5	63	<1		
	25	P	45	53	1	1	
Fructose	30	D	2	70	5	23	
	80	D	2	53	10	32	keto: 3
	33	P	5	43	15	35	
	33	DMSO	5	26	21	48	

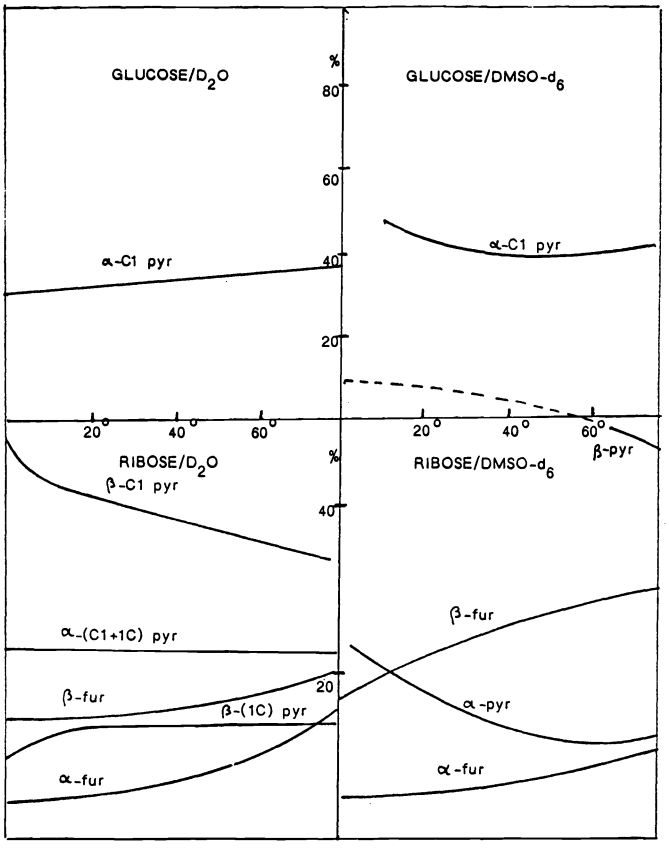


Fig. 2. Composition of anomeric equilibrium mixtures of glucose and ribose in dilute solutions of D_2O and DMSO-d_6, according to ref. 10. Equilibrium compositions, as functions of temperature, were obtained from ^1H nmr chemical shifts and coupling constants (J_{HH}). $^4C_1$ and $^1C_4$ tautomers of α-pyranoses could not be resolved because of their identical coupling constants. No species other than $^4C_1$ pyranoses were detected in solutions of glucose, at any concentration.

coexist, as demonstrated for ribose in Fig. 2. Furthermore, the sugar concentration also affects the equilibrium composition, as well as its temperature dependence (ref. 10).

In order to estimate the solvent contribution to the expected population densities of the various species, the data for different solvents can be compared with one another and/or with calculated estimates. The latter procedure was first adopted by Angyal (ref. 12). A major problem concerns the parameterization of the various non-bonded atom-atom (or atomic group) potential functions. These were obtained from experimental results on model compounds in aqueous solution (ref. 13). The compilation of the glucose data in Table 2 (ref. 9) illustrates a common finding (see also Table 1): experimental (solution) and calculated (vacuum) equilibrium compositions and associated thermodynamic functions agree reasonably well except for aqueous solutions. In this context the results cited by Angyal (refs. 12, 13) cannot really be classified as "calculated" because the parameterization was made to fit the experimental data for aqueous solutions. The arbitrary solvation free energy "corrections" of -1.13 kJ mol^{-1} for equatorial -OH groups only, introduced by Dunfield and Whittington into their Monte Carlo simulations of anomeric equilibria (ref. 14) also cannot be considered as rigorously based. There exists no theoretical or experimental basis for the assumption that only equatorial -OH groups interact with water (ref. 15). It must also be emphasized that no solvation "corrections" are required to achieve agreement between calculated and experimentally determined anomeric equilibria in non-aqueous solvents. This would suggest that the potential functions employed provide a reasonable description of the actual interactions which give rise to the particular position of the equilibrium.

Table 2. Thermodynamic data for the α- and β- anomers of glucose; units are kJ mol^{-1}.

	α	β	α	β	Comments
	Experimental		Calculated		
Ring conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	Ref. 12
Per cent anomer H_2O	37				Ref. 15
D_2O	34				do.
DMSO	44				do.
Pyridine	45				do.
in vacuo			47		Ref. 14
Eq. -OH hydrated			33		do.
Free energy $^4C_1$	10	8.6			Ref. 12
$^1C_4$	27.4	33.5			do.
Internal energy			12.8	13.3	Ref. 14
Entropy at 298 K (TΔS)			18.4	19.2	do.
Free energy (eq. -OH hydrated)			-5.6	-5.9	do.
ΔH (aq. α⇌β, 298 K)	-1.17				
ΔG (α⇌β)	-1.42		-0.54		
TΔS	0.25				Ref. 12

Schallenberger has discussed the factors associated with different types of mutarotation (ref. 2). Thus, all the aldohexoses (with the exception of D-altrose) which exhibit complex mutarotation, i.e. where more than one tautomer is involved, have an axial -OH on C(4). This is also the case for the two pentapyranoses which exhibit complex mutarotation. These observations have led to the generalization that an axial -OH group on C(4) of a pyranose leads to appreciable proportions of furanose forms. The mutarotation of β-D-fructopyranose in water does not produce the corresponding α-anomer but a mixture of α- and β-fructofuranose (ref. 16). This has been rationalized with the aid of a comparison of the favoured $^5C_2$ conformations of the α-D-pyranose forms of allulose, tagatose and sorbose which do not have an axial -OH at C(5) and β-D-fructose which does have an axial -OH(5) group in its favoured tautomer, the $^2C_5$ pyranose.

In aqueous solution the pyranose ring of reducing sugars appears to be more stable than the furanose ring, although this situation is reversed for some sugar derivatives. In non-aqueous solvents appreciable proportions of furanoses exist in the equilibrium mixture; the reason is not immediately obvious but must reside in the solvation contributions to the conformational free energies of the various species and the ease of interconversion. Accepting then, that water stabilizes the pyranose ring, the next question concerns the relative stabilities of the two anomers. Despite several efforts over the past two decades to calculate conformational free energies, it is still not obvious why the α/β ratio for glucose is 36:64, whereas for mannose it is 67:33. Edward first postulated the existence of an interaction to account for differences in anomeric ratios (ref. 17). The effect, which came to be known as the "anomeric" effect, has been discussed at length in the carbohydrate literature (refs. 12, 18). Basically it is postulated that an equatorial -OH group at the anomeric site produces a repulsive dipole-dipole interaction with the ring oxygen atom; hence the α-anomer is favoured. Since the effect is of an electrostatic nature, it is assumed to vary inversely with the dielectric permittivity of the solvent. In water, therefore, the effect is said to be small, so that the β-anomers usually dominate. Such an argument does not explain the anomeric ratios observed for mannose, talose, altrose or lyxose, for all of which α/β exceeds unity.

According to Angyal (ref. 12) "the evaluation of the anomeric effect is the least satisfactory part of the calculations of the free energies of sugars, because the effect varies considerably not only with the nature of the solvent and of the anomeric group but it is affected even by the presence and the configuration of substituents in other parts of the pyranose molecule." In fact, the anomeric effect has been used (at times quite indiscriminately) as an adjustable quantity to account for discrepancies between calculated and experimental conformational free energies. For instance, to reconcile the conformational free energies of the mannose and deoxyarabinose anomers it has beeen assumed that the magnitude of the anomeric effect depends on the presence and configuration of the -OH group on C(2) and also on the presence or absence of an -OH group on C(6), "and probably in other positions" (ref. 20). Although the anomeric effect has become firmly established in carbohydrate folklore, its actual physical significance must be open to doubt, as already foreshadowed by Angyal in his detailed critique (ref. 12).

Alternative explanations of anomeric and tautomeric equilibria take explicit account of solvation as a determining factor. Given that the intrinsic conformational free energy differences between coexisitng species, as calculated from vacuum-based potential functions, are small, of the order of $\underline{kT}$, it is reasonable to postulate that the solvation contribution plays an important role in the determination of the equilibrium composition. Schallenberger has discussed the changes in the specific optical rotation of sugars induced by different solvents. Thus, the replacement of water by pyridine produces optical rotation increases in α- and β-glucose of 36 and 42% respectively (ref. 20). Since the specific rotation is related to the free energy of the sugar in solution, such changes, different for the two anomers, should result in a shift in the equilibrium. Similar changes in tautomeric equilibria are expected. Most experimental effort on mutarotation has gone to kinetic studies. Here again certain "rules" have been established. Thus, mutarotation involving only furanoses is very fast, whereas mutarotation of pyranose sugars is slow, with furanose/pyranose conversions taking an intermediate place. Since the α/β conversion is acid/base catalysed, the solvent would be expected to affect the kinetics. In aqueous solution minimum rate constants (k_{min}) for pyranose/pyranose mutarotations are observed at $2.5<pH<6.5$. For processes involving pyranose/furanose tautomerism (e.g. fructose) k_{min} occurs at pH 4.0. The pH dependence of k is particularly marked during the initial stages of the reaction. The mechanism involves proton transfer from the acid catalyst to the sugar, followed by the transfer of a proton to the base catalyst. The proton attack causes a slow ring opening to yield an intermediate species; ring closure is then rapid. Alternatively, the mechanism could be viewed as the stabilization of a pseudo-acyclic transition state by the solvent. The "polarity" of the solvent is said to affect k, although the definition of polarity is problematical. Salt effects on k have also been observed but cannot be explained by any conventional mechanism. Similarly, the catalytic effects of many amino acids on mutarotation still await an explanation.

Mutarotation in mixed solvents is particularly complex, because k does not always exhibit a monotonic dependence on the solvent composition, especially in mixed aqueous solvents. On the other hand, the effects observed closely resemble the well-established dependence of the physical properties of many mixed aqueous solvents (e.g. alcohol-water mixtures) on solvent composition which does not follow "polarity", as expressed by the dielectric permittivity (ref. 21).

Several attempts have been made to gain a more fundamental insight into the molecular details of sugar solvation. Water is of greatest interest, not only because of its ubiquity or its biological and technological importance, but because aqueous solutions of PHCs seldom conform to the behaviour predicted by theory. The main features that set water apart from other liquids are its spatial and orientational intermolecular correlations which are dominated by labile hydrogen bond interactions, and its time-averaged tetrahedral geometry. PHCs contain the same chemical groups (-OH) and are able to interact with water such that, from an energetic point of view, differences between solute-water and water-water interactions are expected to be marginal, unless cooperative effects (about which little is known) act so as to favour water-water interactions. Configurationally the situation is complex because the mutual spacings and orientations of the -OH vectors in different solute molecules can match those in water to varying degrees. If a maximum degree of hydrogen bonding is to be maintained, then in the neighbourhood of the solute the water molecules will suffer a disturbance. Bearing in mind the wide range of hydrogen bond lengths encountered in different systems and the ease with which the HOH angle can accommodate minor distortions, such hydration disturbances are likely to be of a short-range nature.

Kabayama and Patterson first drew attention to the spatial compatibility of the -OH topology in water with that of equatorial -OH groups on pyranose sugars (ref. 22). In the sugars the spacings of oxygen atoms linked to next-nearest carbon neighbours are 0.485 nm which is also the distance between next-nearest neighbour oxygen atoms in liquid water (ref. 23). Figure 3 illustrates how the glucose anomers could interact with an idealized, unperturbed water lattice, without causing any strain in the hydrogen bonding network.

What is the evidence in favour of such a hydration scheme? Indirect, though convincing support has been provided by Harvey and Symons who realized that, under conditions of slow exchange, the -OH proton nmr signals of sugars could be resolved in H_2O solvent (ref. 24). This enabled them to avoid the usual D_2O exchange procedure which simplifies the nmr spectrum but also removes all potential information about solute-water interactions which would be contained in the -OH resonances. By careful pH adjustment and operating at low enough temperatures they were able to record and assign some of the -OH signals in anomeric mixtures of aldohexopyranoses. Their results are reproduced in diagrammatic form in Fig. 4. Two important features emerge: the anomeric β -OH resonance is always found downfield from the corresponding α -OH signal and for equivalent -OH groups on different sugars, equatorial -OH groups show up downfield from the corresponding axial -OH signal in a different isomer.

The spectra were not well enough resolved to permit estimates of proton exchange rates to be made. However, the spectral assignments made by Harvey and Symons formed the basis of the glucose-H_2O proton exchange studies of Bociek and Franks in which the temperature range was extended down to 238 K in undercooled solutions (ref.25). The line widths of the anomeric -OH protons indicate that proton exchange between β -glucose and water is significantly faster with a lower activation energy that that between α -glucose and water. It is regrettable that such -OH proton studies have not been further pursued, probably because of the obsession of nmr spectroscopists with narrow lines and averaged motions which features simplify the spectra but also reduce their information content.

Further evidence for a specific hydration model comes from ^{13}C and ^{1}H nuclear magnetic relaxation rate measurements (ref. 26) which show the C(1)α ring proton to be extremely susceptible to intermolecular interactions. In β-glucose this is the only proton in the

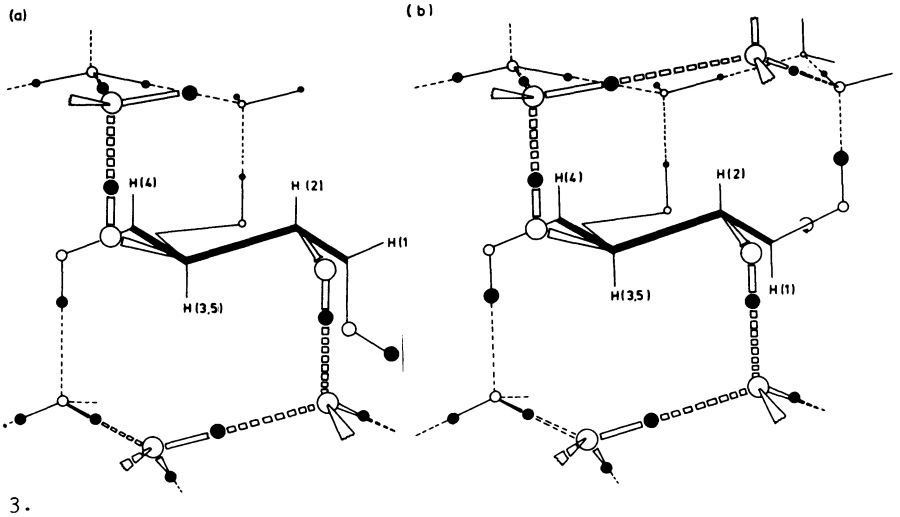


Fig. 3.
D-glucose hydrogen bonded into a hypothetical tehrahedral water structure, according to the specific hydration model. Water molecules below and above the plane of the sugar ring are shown: (a) α-form, (b) β-form. The pyranose ring is indicated by the prominent filled-in line. Oxygen and hydrogen atoms are represented by open and filled-in circles; covalent and hydrogen bonds by solid and broken lines. The hydroxymethyl protons [H(6)] are omitted for sake of clarity. Reproduced, with permission, from ref. 27.

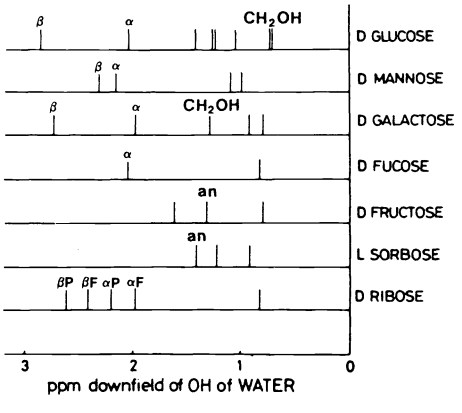


Fig. 4. -OH proton chemical shifts of sugars in aqueous (H_2O) solutions at -6°C, according to data from ref. 24. α and β refer to anomeric -OH protons, an = anomeric, where the assignment is uncertain; P = pyranose, F = furanose. Unmarked signals are due to ring -OH groups, but the assignment is uncertain.

equatorial configuration. Suggett has explained this susceptibility in terms of the exposed nature of this proton in β-glucose, while in the α-anomer it is more shielded by the hydration interactions of the -OH groups on C(1) and C(2), as shown in Fig. 3 (ref.27).

The model is also supported by dielectric relaxation measurements which have already been reviewed (ref. 15). The model explains the observation that the water solvent effect on anomeric and tautomeric equilibria favours the species with the largest number of equatorial -OH groups, especially at low temperatures. Mannose appears to be an exception to this rule, with an α/β ratio in excess of unity. On the other hand, the anomeric ratio in non-aqueous solvents has not yet been reported and may possibly be even higher.

THERMODYNAMICS OF MONOSACCHARIDES IN AQUEOUS SOLUTION

Systematic thermodynamic studies on simple sugars in solution only date from the 70s when my colleagues and I first attempted to differentiate between different types of molecular hydration, but even now the available information is fragmentary, nearly all of it confined to aqueous solutions. The major aspects of interest are 1) sugar hydration as affected by stereochemistry and 2) sugar-sugar interactions, as calculated from thermodynamic excess functions. Wadsö and his colleagues generated high quality calorimetric data capable of being extrapolated to infinite dilution. The laboratories of Wood, Lilley and Barone have concentrated on the problems posed by solute interactions in solution. Yet another approach has been adopted by Goldberg who has studied the enzymatically catalysed interconversion thermodynamics of sugars.

The weakness of all thermodynamic studies lies in the chemical heterogeneity of sugar solutions, so that all measured quantities are suitably weighted averages. One method of inhibiting equilibration is to block some or all of the -OH groups by derivatization. On the other hand, such procedures also reduce the information that might be derived from thermodynamic measurements, because hydration interactions are very susceptible to the -OH group topology of a particular stereoisomer, and derivatization (e.g. acetylation or esterification) destroys this sensitivity. Nevertheless, useful information has been obtained from judicious derivatization, e.g. comparisons of α and β 1-methyl pyranosides (refs. 28, 29).

For comprehensive and reasonably up-to-date tabulations thermodynamic properties of simple carbohydrates in aqueous solution the reader is referred to ref. 9. The selected values collected in Tables 3 and 4 illustrate certain general points. The first caveat concerns the accuracy of the results. The data included in the tables are those from laboratories with reputations for very high quality experimentation. Only in a few cases can the data be compared with corresponding results from another source. Where this is possible, e.g. enthalpic pair interaction parameters (h_{xx}), derived from heats of dilution, discrepancies become apparent. Whatever the reasons for non-agreement between the results from different laboratories, it is advisable not to be too dogmatic in the establishment of universal rules governing the behaviour of sugars. The carbohydrate literature abounds in such "rules" (ref. 2), several of which have subsequently had to be discarded or severely qualified in the light of new experimental evidence (*vide infra*).

Although there is a wealth of limiting partial molar volume ($\bar{V}^o$), expansibility and compressibility data, they have not been included in the tables (but can be found in ref. 9), because differences between isomeric sugars are so small that not too much significance can be read into them as regards the influence of sugar stereochemistry on hydration. A general observation is that $\bar{V}^o$ values of PHCs are surprisingly small compared to their non-hydroxylated counterparts. This is illustrated in Table 5 for some furanose and pyranose derivatives. The results suggest that the density measurement does not "see" the -OH groups as part of the solute molecule but as water molecules (refs. 7 and 30).

As regards thermodynamic information about hydration interactions, the limiting partial molar excess heat capacity $\bar{C}_p^o$ is probably the most sensitive indicator. The available data are included in column 5 of Table 3. The numbers given are the "preferred" values of Lian et al (ref. 31). Quoted uncertainties range from 0.5 to 3%. It must be remembered that, among other factors, $\bar{C}_p^o$ depends on the accuracy of the heat capacity of the crystalline sugar. $\bar{C}_p^o$ measures a combination of the effects of hydration (solute-solvent interactions) and changes in the internal degrees of freedom of the solute molecule when it is removed from the crystal. Thus, $\bar{C}_p^o$ is a measure of the effect of a change in environment on the number of degrees of freedom (mainly intermolecular) of the system. Apart from a general molecular weight dependence, the $\bar{C}_p^o$ data in Table 3 are insufficient in number to allow any conclusions to be drawn, bearing in mind also the chemical heterogeneities of the equilibrium mixtures.

Turning now to solute-solute interactions between like molecules (homotactic), a study of the data in Table 3 reveals some interesting trends. The molecular pair interaction parameters g_{xx} and h_{xx} are the second virial coefficients in the series expansions of the excess free energy and enthalpy. The methods used in the estimation of the virial

Table 3. Second virial coefficients describing homotactic interactions between pairs of monosaccharide molecules [J $mol^{-1}(mol\ kg^{-1})^{-1}$] and limiting partial excess heat capacities (J $mol^{-1}K^{-1}$) of monosaccharides, all measured at 298.2 K. All data refer to D-compounds, unless otherwise stated. For primary references, see ref. 9.

	g_{xx}	h_{xx}	Ts_{xx}	$\bar{C}_p^o$	
Aldopentoses					
D-arabinose		196		278	
L-arabinose	34	192	158	270	
D-xylose	56	332	276	281	
L-xylose		336			
Ribose	20	202	182	271	
Lyxose	31	243	212		
Aldohexoses					
Glucose	92	343	251	347(α)	
Mannose	43	207	164	337	
Galactose	65	133	68	324	
Ketohexoses					
Fructose	83	264	181	352	
L-sorbose	148	395	247		
1-Methyl aldopyranosides					
α-Xylose	114	11261	926		
β-Xylose	100	1098	998		
α-Glucose	171	1097	926		
β-Glucose	82	1048	976		
α-Mannose	144	1206	1062		
α-Galactose	33	900	867		
β-Galactose	134	1081	948		
Deoxysugars					
2-Deoxyribose	80	468	388		$^4C_1$
2-Deoxyglucose	88	592	504		do.
2-Deoxygalactose	58	442	384		do.
6-Deoxy-D-galactose (fucose)		669			do.
6-Deoxy-L-galactose	153	665	512		$^1C_4$
6-Deoxy-L-mannose (rhamnose)	162		523		do.

Table 4. Heterotactic enthalpic second virial coefficients h_{xy} of chiral and anomeric mixtures at 298.2 K [J $mol^{-1}(mol\ kg^{-1})^{-1}$], compared to the arithmetic mean of h_{xx} and h_{yy}.

Chiral mixtures (L + D)	h_{xy}	$h_{xy}-(h_{xx}+h_{yy})/2$
Xylose	317	-17
Arabinose	213	+19
Fucose	664	-3
Arabinitol	195	
Anomeric mixtures (α + β 1-methyl pyranosides)		
Galactose	880	-110
Xylose	1071	-51
Glucose	1026	-46

Table 5. Limiting partial molar volumes of PHCs and their hydrophobic analogues in aqueous solution at 278.2 K; after ref. 7.

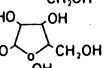
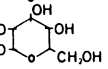
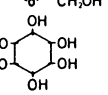
		Molecular weight	ϕ_v^o $cm^3\ mol^{-1}$
Tetrahydrofuran (THF)		72	76
Tetrahydropyran (THP)		86	91
Tetahydrofuran-1-carbinol (THFC)		102	92
Tetrahydropyran-1-carbinol (THPC)		116	107
Ribose		150	93
Galactose		180	108
Scyllo-Inositol		180	97

Table 6. Thermodynamics of sugar conversion at 298.2 K, according to ref. 37.

	ΔG^o	ΔH^o	$T\Delta S^o$	ΔC_p
Xylose-xylulose	4389	16,090	-11,710	40
Glucose-fructose	349	2780	2431	76
Ribose-ribulose	2850	11,000	8150	75 +/- 59
Ribose-arabinose	-3440	-9800	-6360	ca. 0
Allose-allulose (psicose)	-1410	7420	8830	67
Glucose-mannose	3070	2780	290	-10
Allose-altrose	-2000			

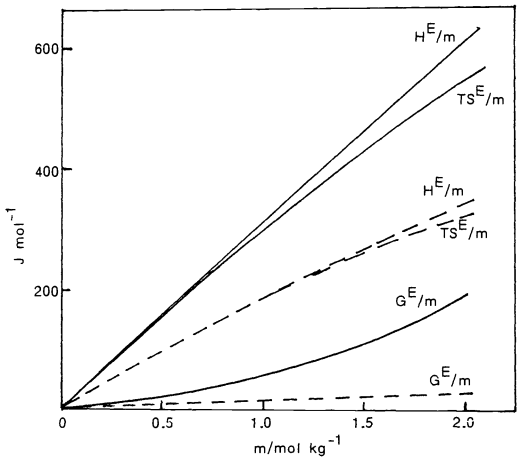


Fig. 5. Excess thermodynamic quantities at 298.2 K of aqueous glucose (———) and mannose (- - -) solutions.

coefficients have been described in detail (refs. 32, 33). Figure 5 shows the concentration trends in the excess functions for glucose and mannose (ref. 34) which are typical of PHCs, with the exception of inositol. Thus, G^E is positive and small, with little concentration dependence even up to 2 molal (27% w/w). Both H^E and TS^E are positive and almost cancel each other (compensation), but $|H^E| > T|S^E|$. The above trends are quantified in terms of the homotactic pair interaction parameters which are, however, once again weighted averages. Significant differences between axial (ax) and equatorial (eq) substituents show up in the 1-methyl aldopyranosides, although the available results are not sufficient for any definite trends to be identified. In general h_{xx} and Ts_{xx} are much larger than for the parent sugars and the H/S compensation is less marked. The deoxy sugars occupy intermediate positions. It should be noted that 6-deoxy-L-galactose and 6-deoxy-L-mannose (rhamnose) preferentially adopt the $^1C_4$ ring form, whereas the other four sugars listed exist mainly as $^4C_1$ tautomers.

The extreme sensitivity of sugar interactions to stereochemical detail is illustrated in Table 4 which summarises h_{xy}, the heterotactic pair interaction parameters between different PHCs; the experimental h_{xy} are compared with arithmetic mean values for the corresponding homotactic parameters h_{xx}, h_{yy}. Even chiral mixtures appear to deviate from the mean, with the deviations increasing for mixtures of anomers.

The g_{xx}, etc. coefficients are related to certain integrals of the potential of mean force $W_{xx}(\underline{r}, \Omega)$. Here $\underline{r}$ is the intermolecular distance of separation and Ω the orientational contribution. For PHCs this latter contribution is important and is likely to show up differences in the interactions of stereoisomers which is indeed found to be the case experimentally. No attempts have yet been made to calculate the orientation-dependent contributions to W_{xx} or W_{xy}. Herrington et al have attempted to estimate repulsive and attractive contributions by treating sugar moleculaes as hard ellipsoids the dimensions of which are estimated from models based on the conformations adopted in the crystal state (ref. 35). Unfortunately this procedure averages out the very orientation-dependent details (e.g. hydrogen bonding) which are likely to differentiate isomeric sugars.

Several atomic group additivity schemes for the calculation of second virial coefficients have been proposed and enjoy a degree of popularity (e.g. ref. 36). The schemes rely on the assignment of constant contributions by chemical groups, such as $>CH_2$, >CHOH, >C=O, etc. and their summation to produce the g_{xx} (h_{xx}, Ts_{xx}, etc) parameters. Whatever might be the practical value of such schemes when applied to other types of compounds, they are quite unable to discriminate between different sugar (or polyol) epimers. For instance, the calculated h_{xx} for <u>all</u> aldopentoses is 159 and for <u>all</u> aldohexoses 279 J mol^{-1}(mol kg^{-1})$^{-1}$, values which can be compared with the diverse experimental values shown in Table 3.

ISOMERIZATION EQUILIBRIA OF MONOSACCHARIDES

The <u>relative</u> stabilities of isomers in solution can be estimated from sugar interconversion studies performed under suitable standard conditions. Goldberg and his colleagues have investigated several aldo-keto isomerizations and also the equilibrium between ribose and arabinose (e.g. ref. 37). They employed the enzyme glucose isomerase (EC 5.3.1.5) to effect the conversions and determined the equilibrium compositions by HPLC and the enthalpy changes by microcalorimetry. The standard thermodynamic quantities are summarized in Table 6; the data refer to a standard state based on a hypothetical unit molality ideal solution.

A detailed analysis of the experimental results is difficult because ΔH for furanose-pyranose transitions is large, typically of the order of 12 kJ mol^{-1}, whereas H accompanying the sugar anomerization in aqueous solution is small, of the order of 1 kJ mol^{-1}. Nevertheless, Tewari and Goldberg comment on the similarities in $T\Delta S^o$ between the

ribose-ribulose and allose-psicose isomerizations. However, they also emphasize the large difference in $T\Delta S^o$ between the corrresponding pair of processes involving glucose/fructose and xylose/xylulose. Although their studies constitute the most complete set of sugar isomerization data, a more rigorous analysis will not become possible until more information becomes available on tautomeric and anomeric equilibria.

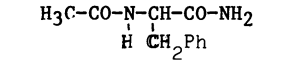
CONFORMATIONAL PROPERTIES OF ALDITOLS IN SOLUTION

As has been repeatedly stressed, the weakness of most physicochemical measurements on unmodified sugars derives from the compositional heterogeneity of the solutions. The chemical modifications of sugars which might prevent anomerization and/or tautomerization may also obscure or distort the very properties which are of interest. One way of studying the effects of stereochemical detail on solvation, conformation and weak solute-solute interactions is with the aid of sugar alcohols. Experimental results can then be related unambiguously to unique chemical species.

Crystal structures serve as starting points for most investigations of solutions and, as was the case for the sugars, certain "rules" have been established as governing the conformational properties of alditols. One such rule states that the carbon chain will adopt a planar zig-zag conformation, unless this extended chain contains parallel C_n-O and $C_{(n+2)}$-O bonds (O//O) which are considered to be unfavourable. In that case rotation about the C_n-$C_{(n+1)}$ bond takes place, giving rise to a sickle-shaped configuration (ref. 38). Aqueous solutions of most alditols up to the heptitols have been examined by ^1H and ^{13}C nmr and such studies have led to a second rule, namely that the molecular configurations in solution and in the crystal are closely similar, if not identical (ref. 39). The rule implies a) that distinguishable conformations exist in solution and b) that such conformations are insensitive to solvent effects. In the crystal each alditol molecule is hydrogen-bonded to several other molecules, each -OH group participating in two such bonds. The crystals therefore resemble infinite hydrogen-bonded networks, not unlike that of ice. The transfer of a molecule, say a pentitol, from the crystal to an aqueous solution takes place with the substitution of 10 solute-solute hydrogen bonds by 10 (?) solute-water bonds. It is not obvious why this transfer should not affect the stability of the particular molecular configuration as it exists in the crystal. Solution conformations are estimated from nmr measurements, usually of the ^{13}C type, because ^1H spectra of alditols are complex. At 300 MHz several signals cannot be assigned with any degree of confidence, and even at 400 MHz the ^1H spectra are very second order and require extensive computer simulation and deuterium labelling. The 600 MHz spectra of the isomeric pentitols xylitol, arabinitol and ribitol in D_2O and pyridine-d_5 have recently been resolved and all signals unambiguously assigned (F. Franks, R.L. Kay, K. Watson and J. Dadok; this meeting). The $^3J_{HH}$ couplings permit comparisons to be made between the molecular conformations in the crystal and those in different solvents. Clear conformational differences are indicated between crystal and solution shapes, but also more subtle differences between hydrated and pyridine solvated molecules. Even the addition of small amounts of D_2O to pyridine produces changes in some coupling constants. The evaluation of the molecular details is not yet complete, but it is certain that the rule relating the molecular configuration to the presence or absence of O//O interactions cannot apply to solutions.

In a detailed reexamination of heptitol conformations, Angyal et al state that the "rule" that 1,3-parallel C-C and C-O bonds (C//O) are so unfavourable that they need not be coinsidered at all, is also incorrect and that this tacit assumption has led to wrong assignments of nmr signals (ref. 40). They also emphasise the important points that a) ^{13}C nmr spectra, taken on their own, can lead to erroneous conclusions (ref. 39) and b) potential energy calculations based on the summation of group interactions derived from six-membered rings are not necessarily applicable to acyclic molecules. In addition, J_{HH} or J_{CH} values alone do not necessarily define the alditol shape in solution (or in the crystal), if alternative molecular configurations are also present. Intermediate J_{HH} values between 9 Hz (antiperiplanar) and 2 Hz (gauche) are usually taken to mean that more than one configuration is present, but they may also arise from a conformation in which the torsional angles differ from the conventional geometry.

In a recent study of model peptide conformations in aqueous and dimethylformamide (DMF) solutions, Leslie reported J_{HH} data on N-acetylphenylalanine amide



She found that three rotamers coexist in equilibrium in both solvents and their mixtures, but marked differences show up in the compositions of the equilibrium mixtures and their temperature dependences (ref. 41). In aqueous solution a four-parameter polynomial was required to fit the equilibrium data, indicating a significant heat capacity contribution which is absent in DMF solutions. The behaviour of the amide in mixed solvents was not a monotonic function of the solvent composition. No equally detailed studies have yet been performed on alditol derivatives, but the recent report by Angyal et al (ref. 40) must cast doubt on previous interpretations of experimental results and on the reliability of potential energy calculations and undue reliance on crystal structure analogies.

Grigera has performed molecular dynamics simulations on sorbitol and mannitol as isolated molecules in vacuo and in aqueous solution (R. Grigera; this meeting). No preferred configurations can be identified for the isolated molecules, i.e. all regions of configurational spaces are explored during a 20 ps simulation, although different mean end-to-end distances ($\langle r \rangle$) are found for the two molecules. In the presence of water, however, rotational freedom is severely restricted, leading to distinct $\langle r \rangle$ values: 0.59 nm for mannitol and 0.35 nm for sorbitol. Qualitatively these values are consistent with the rule governing 0//0 interactions, but quantitatively $\langle r \rangle$ does not correspond to the crystal dimensions.

Thermodynamic information on polyols is sketchy and confined mainly to $\bar{C}_p^o$ and h_{xx} data. This is regrettable because of the interesting role that PHCs play in the stabilization of native protein structures and lipid phases against the disruptive effects caused by freezing, salt and desiccation. Polyols are implicated in heat/cold survival mechanisms of plants, insects and microorganisms, so that a study of their interactions with one another and with proteins and lipids should prove rewarding.

In contrast to monosaccharides, the h_{xx} coefficients in Table 7 exhibit obvious trends, becoming less endothermic with increasing chain length. Inositol, a cyclitol, shows very different behaviour: the large negative virial coefficients reflect the internal symmetry of the molecule which is also the origin of the low solubility of inositol. For details of the primary references, see ref. 9. The h_{xx} values of the pentitol isomers reveal similarities between ribitol and arabinitol, with xylitol apparently out of place. The $\bar{C}_p^o$ data show the same pattern which is not related to the molecular configurations in the crystalline states. Ribitol and xylitol which in the planar zig-zag configuration would have 0//0 interactions occur in the sickle form, whereas arabinitol occurs in the planar geometry. Although no g_{xx} data are available, it appears that the repulsions between xylitol are of a shorter range than those between the other two pentitols. Similarly, the lower $\bar{C}_p^o$ suggests a more compact hydration shell.

Table 7. Second virial coefficients and limiting partial excess heat capacities of alditols at 298.2 K. All data refer to D-compounds, unless otherwise stated. For primary reference sources, see ref. 9. Units as for Table 3.

Sugar alcohol	g_{xx}	h_{xx}	Ts_{xx}	$\bar{C}_p^o$	Conformn. of non-terminal -OH groups
Ethane diol	15	362	374	193	
Glycerol	37	251	214	240	
Erythritol		358		310	dd
Pentaerythritol		395		301	
D-arabinitol		187		375	ddl
L-arabinitol		185		373	
Xylitol		80		346	dld
Ribitol		295		376	ddd
Mannitol	18	66	20	452	lldd
Glucitol (sorbitol)		-11		415	dldd
Galactitol		-132		445	dlld
Perseitol		-299		ddlld	
Myo-inositol	-260	-800	-540	340	

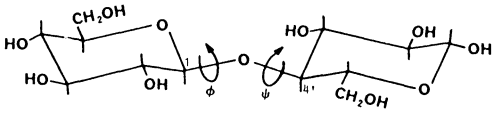


Fig. 6. The dihedral angles for oligosaccharides with a 1-4' glycosidic linkage.

DISACCHARIDES AND OLIGOSACCHARIDES IN SOLUTION

In disaccharides and higher oligomers conformational complexity is due more to the inherent flexibility of such molecules than to the coexistence of anomeric and tautomeric species. The configurational degrees of freedom are expressed in terms of the torsional angles which measure the rotation of two sugar residues about the glycosidic linkage. Increasing attention is being paid to oligosaccharide conformations in the belief that structural detail has important implications in the biological functions of such sugar clusters, especially where they are linked to peptides. Although pyranose residues usually adopt the same conformations which exist in the monomeric sugars, this is probably not true for furanose rings, because of the small energy differences between alternative ring conformers.

For 1-4' linked sugars the significant angles are φ (C-1→O) and ψ (C-4'→O), as shown in Fig. 6. Until the development of very high resolution nmr methods, these angles were estimated from optical rotation measurements. Despite the interpretative problems, already referred to, Rees and his colleagues used such methods to good effect (e.g. ref. 42). They described the optical rotatory power of a disaccharide as a sum of contributions from the two sugar residues and the glycosidic linkage. The latter contribution is related to the

particular values adopted by φ and ψ. A comparison of the experimentally determined linkage rotations for trehalose, cellobiose and methyl-β-D-maltoside in water, DMSO and dioxan with those calculated from crystal structure data and from conformational energy calculations on isolated molecules clearly shows up major differences between crystal and solution conformations. The calculated conformations agree with the experimental values for non-aqueous solvents, but the disaccharide conformations in aqueous solution (and also the temperature dependence of the linkage rotation) differ markedly from those calculated from crystal structures and those experimentally determined for organic solvents, as shown in Table 8. Where crystal and solution data diverge, this may be due to inter- and/or intramolecular hydrogen bonds in the crystal which are replaced in solution by sugar-water bonds (but see below for claims to the contrary).

Direct spectroscopic measurements of sugar-water interactions have demonstrated that the diffusive motions of water molecules in the proximity of a sugar molecule suffer a perturbation (refs. 25, 27; for a summary, see ref. 15). In particular, the dielectric and nuclear magnetic correlation times of water are lengthened. Such observations have led to the assignment of "hydration numbers" and the notion of "bound water". The latter concept, in particular, has become very popular and is often invoked to explain biological and technological phenomena. It should, however, be remembered that the effects are marginal and of a time-averaged nature. The applicability of such concepts is explored in more detail by Slade (this volume).

Nowadays direct methods for establishing rotamer conformations are provided by nmr coupling constants and n.O.e. measurements. In a recent review, Barker and Serianni have described the potency of nmr methods applied to ^{13}C-enriched sugars (ref. 43).

Of the disaccharides, sucrose has received most attention. There is as yet no universal agreement about a preferred solution conformation or the degree of rotational freedom about the glycosidic bond. It has been claimed that in concentrated solution two intramolecular hydrogen bonds are formed and the molecule takes up the conformation which exists in the crystal (ref. 44); in dilute solution these bonds are believed to be absent. In contrast, Bock and Lemieux claim that the sucrose molecule, even in dilute solution, is characterized by its rigidity due to an intermolecular hydrogen bond between O-1(f) and O-2(g) (f = fructose, g = glucose) (ref. 45). The φ,ψ map, calculated on the basis of hard sphere potential functions, exhibits two deep energy wells differing by no more than 10 kJ mol^{-1}, and corresponding to two possible conformational states both of which exist in the crystal.

Despite the undoubted thoroughness of their experimental work, the conclusions reached by Bock and Lemieux cannot be accepted as the last word. The persistence of intramolecular hydrogen bonds in a small molecule in water solvent is unlikely. Also the claim that the conformation of sucrose is unaffected by the nature of the solvent runs counter to earlier experience with other disaccharides and needs further investigation.

The available thermodynamic information for di- and trisaccharides is summarized in Table 9. The g_{xx} coefficients are positive. Of interest is the marked dissimilarity in h_{xx} of the three diglucose isomers. Such subtle differences in sugar-sugar interactions, depending on molecular shape, demonstrate the futility of hard sphere models and orientational averaging procedures as devices to obtain a better understanding of such interactions.

An interesting example of the combination of nmr and energy calculations is the investigation of the conformation of Antarctic fish antifreeze glycoprotein (AFGP) (ref. 46). By assigning all peptide and carbohydrate proton resonances and n.O.e. enhancements and testing the resulting possible torsional angles against calculated conformations, the

Table 8. Glycosidic linkage optical rotation [Λ] (deg) at 298.2 K and its temperature dependence (deg K^{-1}) of disaccharides; after Rees and Thom (ref. 42).

Sugar	State	[Λ]	d[Λ]/dT
Me-β-maltoside	Crystal	-110	
	In vacuo min. energy	-12 and +85	
	Water soln.	+46	-0.12
	Dioxan soln.	-18	+0.22
	DMSO soln.	-24	+0.27
α,α-Trehalose	Water soln.	+18	-0.18
	DMSO soln.	-15	+0.15

Table 9. Virial coefficients and limiting partial excess molar heat capacities of di- and trisaccharides at 298.2 K. For primary reference sources, see ref. 9. Units as for Table 3.

Sugar	g_{xx}	h_{xx}	Ts_{xx}	$\bar{C}_p^o$
Sucrose	181	577	396	650
Cellobiose	138	764 (684)	686	
Maltose	100	483 (571)	383	614
Trehalose	140	595 (795)	455	
Lactose	195	506	311	619
Raffinose	333	811	478	931

Note: Numbers in parentheses are the results of Lilley (reported at this meeting). The discrepancy is probably due to the method of data processing.

authors conclude that the polymer which is based on a tripeptide repeat with threonine O-linked disaccharide units takes up a polyproline-II type helix, "coated" with a carbohydrate layer the -OH groups of which face the aqueous phase. This configuration, so they believe, is responsible for the ability of AFGPs to inhibit ice crystal growth.

More complex saccharide structures are also receiving increasing attention by theoreticians and experimentalists alike. Particular interest centres on the N-asparagine-linked high mannose clusters of the blood group glycoproteins. In this context it must be emphasized that most so-called proteins are in fact glycoproteins, even though the functions of the oligosaccharide residues are not yet fully understood in many cases. A typical example is provided by the blood group A tetrasaccharide above which has been studied by this combination of methods (ref. 47). Model calculations were performed on the two disaccharides Fuc(α1→2)Galβ-OMe and GalNAc(α1→3)Galβ-OMe. Figure 7 shows the non-bonded potential energy map for the former model disaccharide. The three contours (17 kJ mol^{-1} above the minimum energy) were arrived at by three different sets of potential functions. Points marked A, A' and A" represent the global energy minima according to the three calculations, with other local energy minima designated B, C and D. Although the three methods used in the calculations differ markedly in the types of interactions included, the global minima coincide reasonably well. On the other hand, the fact that subsidiary minima are identified raises questions whether a unique "native" conformation exists in vivo. When the energy data are combined with those for the other model disaccharide, the conformation of the trisaccharide Fuc(α1→2)[GalNAc(α1→3)]Galβ-OMe can be deduced. Here the different model potentials yield different low-energy conformations; the authors conclude that "...it is difficult to judge what is the conformation, if indeed there is a unique fixed conformation."

Fuc(α1→2)
Gal(β1→3)-GalNAc-ol
GalNAc(α1→3)

With the aid of n.O.e. and T_1 measurements the area on the φ/ψ map can be delineated where the experimental results agree with those computed from the calculated conformations. The cross-hatched area in Fig. 8 corresponds to the conformation of the fucosidic linkage for which all the computed and experimental values agree. The oligosaccharide residue is thus seen to be rigid, but the various empirical methods used in the calculations of molecular shapes are inadequate and give rise to uncertainties amounting to $\frac{3}{8}$3kT. Many questions remain: which energy contributions are important in determining molecular shape; is there a unique conformation or are the results diagnostic of an averaging over several rotamer conformations; does saccharide function depend on unique "native" configurations and are they subject to cooperative transitions, analogous to those of proteins?

The complexity of the problems posed by small oligosaccharides is well illustrated by Homans et al (ref. 48) in their structural analysis of high mannose derivatives such as occur in cell-surface glycoproteins, e.g. as illustrated alongside:

Manα1→3
Manα1→3 Manα1→4GlcNAcβ1→4GlcNAc
Manα1→6

By a combination of several refined nmr techniques and quantum mechanical calculations they determined the configurations at the Manα1→6Manβ- linkages. In some cases unique conformations were established but other derivatives (e.g. the one shown above) exist as mixtures of rotamers. The studies also showed that some sequence alterations do not greatly

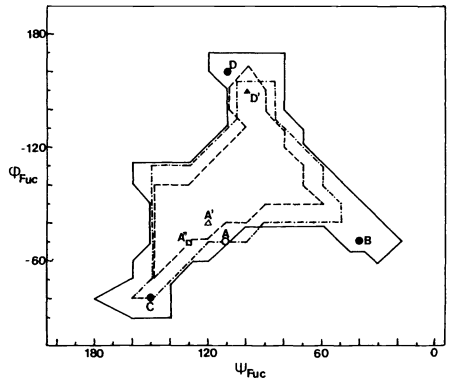


Fig. 7. Nonbonded energy map for Fuc(α1-2)Galβ-OMe. Contours at 17 kJ mol^{-1} above the minimum are drawn for three different types of potential energy functions. Global minima are shown as A, with subdiary minima at B, C and D. For details see ref. 47.

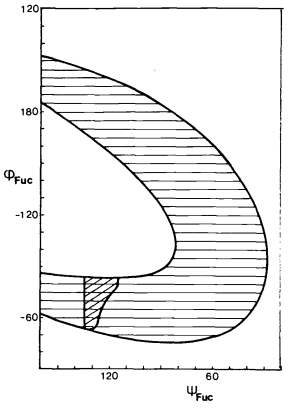


Fig. 8. Regions of the energy map for the Fuc(α1-2) linkage. Computed and experimental nOe values agree in the shaded area. In the cross-hatched area computed and experimental T_1 values agree.

affect the orientation of the α1-6 chain, but certain "key" residue substitutions cause major conformational changes.

The biological relevance of secondary oligosaccharide structures is difficult to assess, and no correlation appears to exist between the primary structural type and the secondary structure, although it is likely that minor primary sequence changes can produce significant changes in shape (viz. protein mutants) and/or biological activity.

Acknowledgements

I wish to thank many colleagues, past and present, for their help in shaping my ideas about the right questions to be addressed in studies of carbohydrates in solutions. Many of the answers still elude us. I apologize for the sketchy referencing of many important contributions; this was imposed by editorial constraint. Finally, I thank G. Barone, R. Grigera and T.H. Lilley for making available as yet unpublished material.

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