Polysaccharide Conformation. Part 10.[1] Solvent and Temperature Effects on the Optical Rotation and Conformation of Model Carbohydrates

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The optical rotations of simple carbohydrates vary with temperature and solvent even after allowance for mutarotaton and local field effects. The variation can be as large as 70° in molecular rotation. For monosaccharides we show that the sign and magnitude can be estimated by an extension of Brewster's treatment of the optical activity of sugars. The mechanism of solvent action is probably through direct spectroscopic perturbation of the optically active transitions. The conformation of the glycosidic linkage in crystalline di- and oligo-saccharides is often but not always preferred in solution. At least for the examples we have studied, change of solvent does not have a major influence on the conformation at equatorial–equatorial linkages. In the series of di- and oligo-saccharides that have an axial glycosidic bond, however, it seems that change of solvent can induce a redistribution between conformational states at the glycosidic linkage. For methyl β-maltoside, α,α-trehalose, and cyclohexa-amylose, transfer from non-aqueous to aqueous solvent appears to favour 'folded' conformations in which adjacent sugar rings shield C–H bonds from water and expose hydroxy-groups in an array that is more compatible with the structured component of liquid water. For all the solvent influences that we have observed, the largest effects are seen between the least polar solvent (dioxan) and water. Water differs from other solvents in its influence more than other solvents differ between themselves and shows its most extreme behaviour when it contains a maximum of hydrogen bonded structure. Breakdown of this structure by heating or addition of alkali, moves the properties towards those of, for example, dimethyl sulphoxide.

In conformation studies of biologically interesting polysaccharides,[2-6] we have frequently found it useful to monitor such events as helix–coil transitions by measurement of monochromatic optical rotation. This method is particularly useful when interfering chromophores are absent, because measurements can then be accurately correlated [3,6-8] with the torsion angles that define the conformational geometry. The quantitative treatment of optical rotation that is used for such interpretation is based on the earlier approaches of Whiffen [9] and Brewster,[10] modified to allow for continuously rather than discretely variable conformation angles, as is necessary for polymers. In parallel with our own work has been a revival of interest in the applicability of this type of analysis of monochromatic optical rotations, to problems in the conformational analysis of simple sugars.[11-14] We have now chosen some mono-, di-, and oligo-saccharide derivatives in which optical rotation behaviour cannot be complicated by mutarotation, to study the influence of temperature and solvent. The initial objective was to accumulate background information which could be used to help recognize long range effects when they occur in polymers in solution, by avoiding confusion with local conformation changes in the individual residues. We have found that the nature of the solvent can have rather specific effects on the optical rotatory behaviour of these model compounds. For the monosaccharides the effects appear to operate directly on the spectroscopic parameters of optically active transitions. For two disaccharides having an axial glycosidic bond, it seems that replacement of an organic solvent by water favours the shift to a conformation in which the two sugar residues fold together to form a structure which is more compatible with the structured component of liquid water.

Only dioxan, dimethyl sulphoxide, and water have been systematically studied as solvents here. The extremes of behaviour in both type of effect (spectroscopic and conformational) are seen in the least polar solvent (dioxan) on the one hand and water on the other. The properties of water differ more from dioxan, and dimethyl sulphoxide, than these two solvents do from each other. With increase of temperature or addition of alkali, the properties of water move towards those of dimethyl sulphoxide. The special properties of water would therefore seem to be favoured by a maximum degree of hydrogen bonding between solvent molecules.

EXPERIMENTAL

General Methods.—M.p.s were determined on a Kofler hot stage and are uncorrected. Paper chromatograms were doubly developed (2 × 10 h) on Whatman No. 1 paper. The descending solvent system used was butan-1-ol–ethanol–water–ammonia (40 : 10 : 49 : 1 v/v). The methyl glycosides were detected by spraying with potassium periodatocuprate [15] followed after 1 min by spraying with

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a solution of rosaniline hydrochloride (0.3 g) dissolved in acetic acid (100 ml) and made up to 1 l with acetone.

Materials.—Methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, and methyl β-D-galactopyranoside were commercial samples (Koch–Light). Methyl β-L-arabinopyranoside was a sample previously prepared at Edinburgh University. These samples were recrystallised twice from ethanol and possessed m.p.s and specific rotations in close agreement with literature values.

Methyl β-cellobioside was a gift from Dr. I. C. M. Dea and had m.p. 187—191 °C; $[\alpha]_D^{25°}$ −18.1° (lit.,[16] 193 °C; $[\alpha]_D$ −19.1°). The methyl α-sophoroside was prepared by Mr. W. G. Blann and had m.p. 251—255 °C; $[\alpha]_D^{25}$ 63.7°. These samples were used without recrystallisation.

Methyl α-D-galactopyranoside monohydrate was purchased from Koch–Light, dissolved in ethanol, a small volume of benzene added, and the solvent removed by rotary evaporation. This was repeated twice and was followed by twice repeating the process using ethanol alone. The sample was recrystallised from ethanol, dried overnight in a vacuum desiccator and stored tightly sealed, m.p. 117—119 °C (lit.,[16] for the anhydrous form 114—116 °C). Anhydrous 1-*O*-(α-D-glucopyranosyl)-*O*-α-D-glucopyranoside (α,α-trehalose) was prepared from α,α-trehalose crystals (Nutritional Biochemicals Corporation) by the above procedure. The crystals were stored as above, m.p. 207—211 °C (lit.,[16] 203 °C). Methyl 3,6-anhydro-α-D-galactopyranoside was prepared from methyl 6-*O*-toluene-*p*-sulphonyl-α-D-galactopyranoside [17] using the method of Haworth *et al.*[18] and had m.p. 137—141 °C (lit.,[16] 140 °C). Methyl β-maltoside was prepared as described by Wolfrom *et al.*[19] The product was recrystallised twice from ethanol, m.p. 107—110 °C; $[\alpha]_D^{25}$ 77.1° (lit.,[2] 110—111 °C; $[\alpha]_D$ 78.8°).

Methyl α- and β-D-Xylopyranosides.—Acetyl chloride (4 ml) was added dropwise to anhydrous methanol (200 ml) to give a 1% solution of hydrogen chloride in methanol. D-Xylose (20 g) was boiled under reflux with this solution for 6 h and then the acid was neutralised overnight with silver carbonate. The solution was decolourised by filtration through a pad of active charcoal and concentrated to a thick syrup. Paper chromatography showed the presence of three spots, one of which was unchanged xylose. Ethyl acetate was carefully added to the syrupy methyl xyloside mixture and the resultant white, oily semi-solid filtered off. Crude methyl β-D-xylopyranoside was crystallised by careful precipitation from ethanol using ethyl acetate. The crystals (1.5 g), m.p. 150—158 °C were applied to a column (2.5 × 40 cm) of Dowex 1 × 2 resin (200—400 mesh) in the hydroxide form.[20] Elution (20 ml h^{-1}) was with carbonate free water and was monitored by the phenol–sulphuric acid test.[21] One sharply defined peak was observed and appropriate fractions were combined and concentrated. The sample was recrystallised twice from ethanol–ethyl acetate and had m.p. 156—159 °C (lit.,[16] 156—157 °C). The ethyl acetate mother liquor from fractional crystallisation was concentrated to a thick syrup. Paper chromatography showed three distinct spots: unchanged xylose, methyl β-D-xylopyranoside, and a third more prominent spot which was attributed to methyl α-D-xylopyranoside. The syrupy mixture (5 g) was fractionated [20] on a column (2.5 × 40 cm) of Dowex 1 × 2 resin (200—400 mesh). Elution (20 ml h^{-1}) was with carbon dioxide free water and fractions (10 ml) were collected. The elution pattern was determined using the phenol–sulphuric acid test and the extent of overlap of the methyl xyloside peaks was determined by paper chromatography. D-Xylose was not eluted from the column. Appropriate fractions were combined and concentrated, using small volumes of benzene to azeotrope off all the water. Methyl α-D-xylopyranoside was recrystallised twice from ethyl acetate and had m.p. 89—91 °C (lit.,[16] 91—92 °C).

Solvents used in the Optical Rotation Measurements.—The measurement of the optical rotation against temperature was carried out in three solvents: distilled water, dioxan, and dimethyl sulphoxide. AnalaR dioxan (B.D.H.) was redistilled before use and stored in a dark bottle away from the light. The dimethyl sulphoxide (B.D.H.) was used either directly from a new bottle or it was dried by vacuum distillation over calcium hydride, having refluxed over calcium hydride for 4 h. It was stored, tightly sealed, over molecular sieve (4A) in a vacuum desiccator. The variation of the refractive index and density of water and dimethyl sulphoxide with temperature and the variation of dioxan density with temperature were obtained from the literature.[22] The refractive index of dioxan was measured at intervals of 7—8 °C from 17.5 to 87.5 °C on an Abbé refractometer thermostatted using a Haake thermocirculator. The graph of refractive index against temperature showed a linear relationship.

Preparation of Solutions and Measurement of Optical Rotations.—Accurately prepared volumetric solutions were used for the optical rotation measurements. The methyl glycosides and α,α-trehalose had solution concentrations calculated to give a polarimeter reading close to 1°. The remaining disaccharides had concentrations of 1—1.5 g per 100 ml. In dioxan solutions these high concentrations could not normally be achieved and the maximum amount which could be dissolved was used. These solutions were stored overnight at low temperature and visually examined for precipitation before the measurements were recorded. All solutions were millipore filtered and transferred to the polarimeter cell in one operation using a syringe.

Optical rotation measurements were made on a Perkin-Elmer 141 polarimeter fitted with an automatic digital readout. The measurements were recorded at the sodium D line, 589 nm, and at 546 nm. The measurements were recorded in a 1 dm polarimeter cell thermostatted by a Haake thermocirculator. The deviation from the thermostatted temperature was ±0.25 °C. The average (±0.001°) of four recordings of the optical rotation was noted, for both wavelengths, at intervals of 7—8 °C from 20—90 °C and at three points when the cell was recooled. The cell was allowed 20 min to equilibrate when it reached each new temperature. All readings were corrected for the cell blank of the cell containing the appropriate solvent at the

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TABLE 1

The variation in compensated molecular rotation [Δ c.m.r.] with change of temperature from 25 to 80 °C

$[\Delta \text{ c.m.r.}]_{25\,°C}^{80\,°C}$ in the solvent indicated (°) at the given concentration (g l^{-1})

Sample	$[M]_{H_2O}^{25°C}$	Water		Dimethyl sulphoxide		Dioxan	
		Concentration	Δ c.m.r.	Concentration	Δ c.m.r.	Concentration	Δ c.m.r.
Methyl α-D-glucopyranoside	304	6.3	3	6.2	−1	1.6	3
Methyl α-D-xylopyranoside	249	6.9	2	5.4	−3	1.5	−2
Methyl α-D-mannopyranoside	149	12.5	5	12.6	13	0.4	−22
Methyl α-D-galactopyranoside	373	6.3	18	5.1	−8	0.5	7
Methyl β-L-arabinopyranoside	390	4.1	−8	4.1	−13	1.4	−4
Methyl 3,6-anhydro-α-D-galactopyranoside	138	8.5	−8	13.0	1	1.4	−2
Methyl β-D-glucopyranoside	−62	29.2	3	15.9	8	2.9	2
Methyl β-D-xylopyranoside	−106	15.7	3	14.6	1	1.5	−8
Methyl β-D-galactopyranoside	−1	100	−5	10.6	0	1.2	0

appropriate temperature. The cell blanks, in all solvents, were very small (±0.002° up to 50 °C and ≤0.006° from 50 to 90 °C).

The molecular rotations were calculated from equation (1) in contrast to the normal carbohydrate convention

$$[M] = \text{molecular weight} \times [\alpha]/100 \qquad (1)$$

which omits the denominator. For comparison of values obtained under different conditions of solvent and temperature, the molecular rotations were compensated for the change in solvent refractive index and density with temperature using equation (2) [23] where $[M]_s^t$ is the molecular

$$[\text{compensated molecular rotation}]_s^t = \frac{[M]_s^t (n_{H_2O}^{25\,°C})^2 + 2}{(n_s^t)^2 + 2} \cdot \frac{\rho_{H_2O}^{25\,°C}}{\rho_s^t} \qquad (2)$$

rotation measured at temperature t/°C in the solvent (s) of refractive index (n_s^t) and density (ρ_s^t). The compensated molecular rotation (c.m.r.) is, therefore, the molecular rotation referred to the refractive index and density of water at 25 °C.

RESULTS AND DISCUSSION

The c.m.r. of each methyl glycoside was plotted against temperature and the best curve was drawn through the points. Observed variations were always linear or followed shallow curves and examples of monosaccharide changes in water are given in Figure 1. The magnitudes of variations in dimethyl sulphoxide and dioxan are similar although dioxan values are less accurate because of measurement errors caused by limited solubility. Only the results at 589 nm will be considered in detail since these can be correlated with previous measurements. The rotations of disaccharide glycosides will be discussed in terms of the ' linkage rotations ' $[\Lambda_{obs}]_D$ as defined previously.[7]

Monosaccharides.—The temperature-induced changes in compensated molecular rotation of some methyl pyranosides are shown in Table 1 and are designated $[\Delta \text{ c.m.r.}]_{25\,°C}^{80\,°C}$. Such temperature dependent effects are extremely small compared with rotational differences associated with large conformational changes such as in the interconversion of pyranose ring conformations or in helix–coil transitions of polysaccharides, such as carrageenan or agarose. As shown in Table 2 the variation in c.m.r. with solvent, given in equation (3), can be much

$$[\Delta \text{ c.m.r.}]_{\text{solvent B}}^{\text{solvent A}} = [\text{c.m.r.}]_{\text{solvent A}}^{25\,°C} - [\text{c.m.r.}]_{\text{solvent B}}^{25\,°C} \qquad (3)$$

greater although, in general, they also remain small compared with major conformational transitions. Rotational variations calculated from the observations of others [11,24] are also included in Table 2.

For methyl pyranosides of the type considered, the

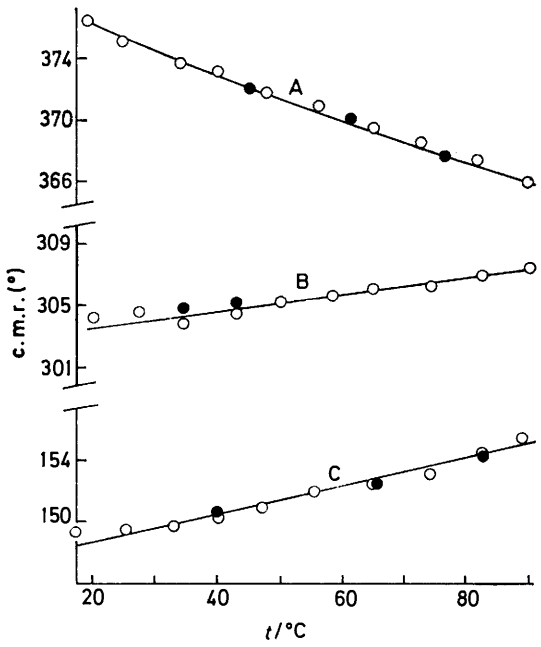

FIGURE 1 The variation of optical rotation (589 nm) with temperature for some methyl glycosides in aqueous solution: A, methyl α-D-galactopyranoside; B, methyl α-D-glucopyranoside; C, methyl α-D-mannopyranoside. The open and closed circles represent measurements on heating and cooling respectively

chair shape of the pyranoid ring is undoubtedly [25] the most stable and there is a preference for the $^4C_1$ chair conformation. Variations of solvent and temperature would not be expected to alter this conformational

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[24] R. E. Reeves and F. A. Blouin, *J. Amer. Chem. Soc.*, 1957, **79**, 2261.

[25] E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, ' Conformational Analysis,' Interscience, New York, 1965.

TABLE 2

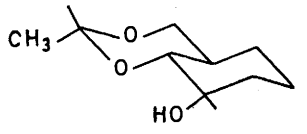
The change in compensated molecular rotation $[\Delta \text{ c.m.r.}]^{\text{solvent A}}_{\text{solvent B}}$ with change of solvent measured at 589 nm

Sample	Δ c.m.r. $(CH_3)_2SO$ H_2O	Dioxan H_2O	Dioxan $(CH_3)_2SO$	$CHCl_3$ H_2O	CCl_4 H_2O	NaOH [a] H_2O
Methyl α-D-glucopyranoside	−5 (3)	3 (−15)	8 (−18)			0 (3)
Methyl α-D-xylopyranoside	−8	18	26			−3
Methyl α-D-mannopyranoside	−9	63	72			1
Methyl α-D-galactopyranoside	−38	−69	−32			−16
Methyl β-L-arabinopyranoside	−59	−56	3			−16
Methyl 3,6-anhydro-α-D-galactopyranoside	−9	10	19			
Methyl β-D-glucopyranoside	−1 (−12)	−5 (2)	−4 (15)			3 (5)
Methyl β-D-xylopyranoside	11	−7	−19			−2
Methyl β-D-galactopyranoside	−22	−47	−25			−19
Methyl β-D-mannopyranoside						19
(1a) [b]	12			8	9	
(1b) [b]	−17			3		
(1c) [b]	17			20	21	
(1d) [b]	13			1	9	

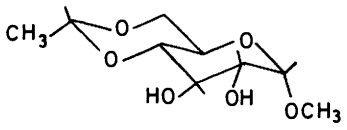
The values in parentheses are the contributions to $[\Delta \text{ c.m.r.}]^{\text{solvent A}}_{\text{solvent B}}$ from the hydroxymethyl groups and they were calculated as described in the text.

[a] From the measurements of Reeves and Blouin [24] at 27.5 ± 2.5 °C. [b] From Lemieux and Pavia [12] approximately corrected for solvent refractive index.[22,23]

preference (see below), with the possible exception of methyl β-L-arabinopyranoside where the free energy difference between $^4C_1$(L) and $^1C_4$(L) chair forms is smaller. However, this ring form has some very limited flexibility in that bond angles can adjust to accommodate local non-bonded interactions. Such changes are well characterized for crystal structures [26] and we might expect similar effects to be caused by change of solvation energy and to be reflected in the measured optical rotation.



(1a)



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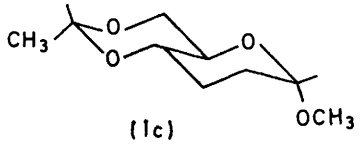

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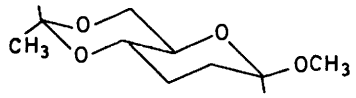

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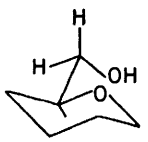
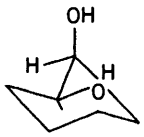
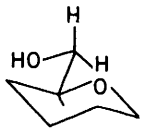


(2a)



(2b)



(2c)

$k(O-H)(O-H) = 45°$

Forbidden [18] but would have the contribution $k(C-H)(O-H)$ $-k(O-H)(O-H)$ $= 5°$

$-k(C-H)(O-H) = -50°$

The possibility that the dependence of optical rotation on solvent and temperature could result from changes in the conformational equilibria of pendant side groups has recently been discussed by Lemieux.[12,14] Another contributing factor could be the alteration of the

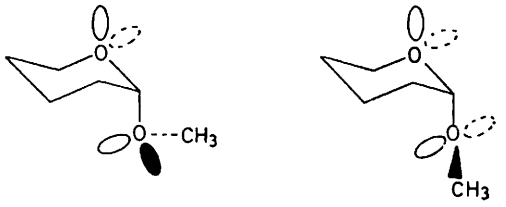
[26] S. Arnott and W. E. Scott, *J.C.S. Perkin II*, 1972, 324.

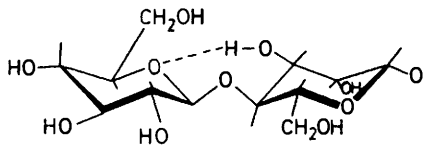
behaviour of chromophores in a changed solvent environment. The two possibilities will be considered in turn.

Influence of the conformations of side groups. It now seems highly unlikely that the optical rotation changes could be caused by conformational effects as drastic as was suggested when they were first discussed in conformational terms; it was proposed that the ring form of certain glycosides underwent a conversion from chair to flexible boat when taken from neutral to alkaline aqueous solution.[24] This has since been refuted by n.m.r. studies.[27] In any case, we now know that, for most of the compounds under study, the free energy difference between ring conformations is too large for such complete transitions to be plausible.[25] We may also note that conformationally constrained molecules, such as methyl 3,6-anhydro-α-D-galactopyranoside and the 4,6-*O*-ethylidenehexopyranosides (1a—d), show solvent-induced changes in optical rotation which are comparable in magnitude to those of the other compounds. For methyl hexopyranosides conformational equilibria involving side groups could however contribute significantly; these involve rotation of the hydroxymethyl and glycosidic groups.[12,14]

An assessment of the maximum likely influence of the hydroxymethyl groups is possible by subtraction of the observed shifts in optical rotation of each methyl pentopyranoside from that of the corresponding hexopyranoside. We use this approach here only for the glucosides and xylosides because instability of the ring conformation of methyl β-L-arabinoside could have an uncertain influence. The three possible staggered orientations of the hydroxymethyl group are shown in (2a—c) with their calculated contributions to molecular rotation by Brewster's rules.[10] In crystal structures,[28] (2a) is found most commonly, (2b) occasionally, and (2c) has not so far been reported. The hydroxymethyl contribution (25°) assigned in empirical rotational calculations [10,12] indicate a preponderance of (2a) in aqueous solution as might be expected by analogy with the crystal structures. When the glucosides are transferred to a less polar solvent less positive hydroxymethyl contributions might be expected [13] if (2c) can be stabilised by O(4) · · · O(6) hydrogen bonding. However, if this effect occurs at all it clearly does not dominate the optical rotation behaviour of hexopyranosides because the shift is small and is sometimes in the opposite direction. In support of this conclusion, optical rotation changes of similar magnitude to those of the hexopyranosides occur for compounds for which this effect is impossible.

Another conformational equilibrium which might contribute to the variation of optical rotation involves the glycosidic C(1)–OCH_3 bonds. This effect has previously been discussed [11] in relation to the molecular rotational behaviour of methyl 2,3-dideoxy-4,6-*O*-ethylidene-α-D-hexopyranoside (1c) and its corresponding β-anomer (1d). The results suggested that changes in conformation about the glycosidic bond do not occur or are negligible *except* for certain α-glycosides when water was involved as solvent. This special effect of water was then attributed [11] to unique stabilisation of conformation (3b) in aqueous solution. If this were an important





(4)

effect, the transfer of an α-D-glycoside from non-aqueous to aqueous solution should always be accompanied by a significant negative shift of optical rotation, which is not the case (Table 2). Indeed, in rationalising the optical rotations and other properties of α-sugars and α-glycosides in aqueous solution, it has always been quite satisfactory to assign the conformation (3a) exclusively.[8-10,14]

We therefore conclude that the solvent and temperature-induced changes in optical rotation of simple glycosides are not to be explained in terms of redistribution between different conformational states of the pendant groups.

Solvent effects. It seems possible that the physical properties of the solvent environment could modify the dissymetric perturbation of chromophores that gives rise to optical rotation, leading to a different result in each solvent. These influences could change the wavelength of far u.v. o.r.d. extrema and/or change the intensity of their Cotton effects. Such effects have previously been observed directly for the carbonyl group,[29,30] and they make important contributions to the influence of solvents on the Cotton effects of rigid ketones and may indeed be the dominant factor when chromo-

[27] V. S. R. Rao and J. F. Foster, *J. Phys. Chem.*, 1965, **69**, 636.
[28] G. A. Jeffrey and M. Sundaralingam, *Adv. Carbohydrate Chem. Biochem.*, 1974, **30**, 445; 1975, **31**, 347, and references therein.
[29] L. Velluz, M. Legrand, and M. Grosjean, 'Optical Circular Dichorism: Principles, Measurements and Applications,' Verlag Chemie, Weinheim, 1965.
[30] P. Crabbe, 'An Introduction to the Chiroptical Methods in Chemistry,' Mexico, 1971.

phores are not excessively restricted.[31] If such influences do modify optically active transitions in sugar glycosides, their effect could be represented by changes in the values assigned to Brewster's parameters [10] for the various terms which contribute to observed optical rotations. To consider from this point of view the largest of the solvent-induced shifts in molecular rotation, namely those that occur when water is replaced as solvent by the least polar liquids (dioxan, chloroform, and carbon tetrachloride), we have derived new values for Brewster's constants to give the best fit to the optical rotations in these solvents. At the carbohydrate concentrations being used no significant contributions would be expected from intermolecular hydrogen bonding in non-hydrogen bonding solvents and this has not been considered. The proposed alterations are shown in Table 3. The values, rounded to the nearest 5°, were deduced using the minimum number of compounds as a guide before adjusting to give the best overall fit with experimental observations. This leads to a comparison of the calculated and observed shifts in Table 4 which is generally satisfactory.

In cases where significant rotational shifts are predicted after deduction of hydroxymethyl group contributions as described in Table 4, the effect on optical

TABLE 3

Changes in empirical parameters to fit solvent-induced optical rotation shifts

Brewster's empirical constants [10]	Brewster's values (°)	Proposed alternation in these values (°)
k(O–H)(O–H)	+45	−15
k(C–H)(O–H)	+50	0
Permolecular effect C(1)/C(5) (XXXIX)	+100	+20
Permolecular effect C(2)/C(4) (XL)	+60	−25
6-Hydroxymethyl (XLII)	+25	0 [a]
1-*O*-Methyl (XLV)	±105	0

[a] Table 4, note *a*.

rotation of replacing water by other solvents is almost invariably greater than interchanging such solvents. This agrees with previous observations [11] and it is tempting to ask whether this results from the special ability of carbohydrates to interact with the 'structured' component of liquid water. The spacing of hydroxy-groups in a glucopyranose ring corresponds exactly to tetrahedrally co-ordinated oxygen atoms hydrogen bonded into the dynamic tridymite arrays suggested for water [32] and mutual stabilisation of this water structure and particular, predominantly equatorial pyranose sugar forms have been proposed.[33] This reasoning is consistent with the shift from furanose to pyranose forms when dimethyl sulphoxide is replaced by water [34] and with the relative rates of hydrolysis of anomeric glycosides.[35] More recent thermodynamic, dielectric, and n.m.r. studies [36] of simple sugar solutions, and conformational energy calculations,[37] also support this view. If adjacent equatorial hydroxy-groups were hydrogen bonded to different layers of 'lattice' water [33,36] they might acquire some increased tendency to the ring flattening by steric

TABLE 4

Solvent-induced shifts in optical rotation as predicted with the modified Brewster parameters

Compound	Observed shift (°) [a]	Calculated shift (°)
Methyl α-D-glucopyranoside–methyl α-D-xylopyranoside	+18 [b]	+5
Methyl α-D-mannopyranoside	+78 [b]	+75
Methyl α-D-galactopyranoside–methyl β-L-arabino-pyranoside	−56 [b]	−50
Methyl β-D-glucopyranoside–methyl β-D-xylopyranoside	−6 [b]	+15
Methyl β-D-galactopyranoside	−34 [b]	−40
(1a)	+9 [c]	+15
(1b)	+3 [d]	+5
(1c)	+21 [c]	+20
(1d)	+5 [c]	0

[a] To avoid complications from any solvent-induced redistribution of conformations about C(5)–C(6), the part of the shift that was associated with the hydroxymethyl group was estimated by consideration of hexoside and pentoside values and substracted from Δ c.m.r. for the hexoside. For methyl α-D-mannopyranoside and methyl β-D-galactopyranoside equivalent contributions were assumed to those of the methyl α-D-glucoside and α-D-galactoside respectively. [b] $(\Delta \text{ c.m.r.})^{\text{Dioxan}}_{H_2O}$. [c] Average value of $(\Delta \text{ c.m.r.})^{CHCl_3}_{H_2O}$ and $(\Delta \text{ c.m.r.})^{CCl_4}_{H_2O}$. [d] $(\Delta \text{ c.m.r.})^{CHCl_3}_{H_2O}$.

repulsions that are observed in the solid state,[26] and also some resistance to free rotation of pendant hydroxy-groups. We note from Tables 1 and 2 that the changes in optical rotation caused by alkali and by heating aqueous solutions, are generally in the same direction as from aqueous to non-aqueous solution. This would correlate with the expectation that the influence of hydroxide ions [38] or of increased temperatures [36,39] would be to diminish the proportion of the structured component of liquid water.

This line of reasoning would lead to the expectation that, in a nonpolar solvent compared with water, ring atoms would be closer, torsion angles subtended by diequatorial hydroxy-groups would be smaller, and torsion angles subtended by axial–equatorial hydroxy-group systems would be larger. Although these conformational distortions might directly influence the optical activity, the directions of the adjustments needed in the parameters (Table 3) do not correlate with them in any simple way. Therefore it appears the solvent

[31] D. N. Kirk, W. Klyne, and S. R. Wallis, *J. Chem. Soc.* (*C*), 1970, 350.
[32] M. D. Danford and H. A. Levy, *J. Amer. Chem. Soc.*, 1962, **84**, 3965; J. A. Pople, *Proc. Roy. Soc., Ser. A*, 1951, **205**, 163.
[33] M. A. Kabayama and D. Patterson, *Canad. J. Chem.*, 1958, **36**, 563.
[34] A. S. Perlin, *Canad. J. Chem.*, 1966, **44**, 539; W. Mackie and A. S. Perlin, *ibid.*, p. 2039.
[35] T. J. Painter, *Acta Chem. Scand.*, 1973, **27**, 2463.
[36] F. Franks, J. R. Ravenhill, and D. S. Reid, *J. Solution Chem.*, 1972, **1**, 3; M. J. Tait, A. Suggett, F. Franks, S. Ablett, and P. A. Quickenden, *ibid.*, p. 131; F. Franks, D. S. Reid, and A. Suggett, *ibid.*, 1973, **2**, 99.
[37] D. A. Rees and P. J. C. Smith, *J.C.S. Perkin II*, 1975, 830.
[38] W. Good, *Electrochim. Acta*, 1967, **12**, 1031.
[39] A. H. Narton, M. D. Danford, and H. A. Levy, *Discuss. Faraday Soc.*, 1967, **43**, 97.

perturbations may operate on electronic transitions directly rather than on the intramolecular geometry which affects their optical activity. These solvent perturbations are of course additional to those that have been removed by the refractive index compensation. The fact that a consistent rationalisation of them can be achieved through the Brewster parameters, would suggest that these parameters do express real properties of the ' invisible ' optically active transitions. Evidently these transitions are perturbed in some systematic way as the solvent environment changes in polarity; the most extreme polar environment is provided by an aqueous solvent having the water molecules involved in the maximum degree of hydrogen bonded structure.

β-Linked Disaccharides.—In earlier publications we concluded that the conformation at the glycosidic linkage in crystalline disaccharides and oligosaccharides is often but not always preferred in solution. For example, for most of the cellobiose derivatives for which evidence exists and for α-lactose in water, and also for cyclohexa-amylose in dimethyl sulphoxide, the measured value of the optical rotation corresponds closely to predictions made from crystal structures.[7] On the other hand, the values for methyl β-cellobioside, maltose, and cyclohexa-amylose in water, do not correspond with predictions from the crystal structures of these compounds.[7] However, the value for methyl β-cellobioside does correspond with the cellobiose crystal structure, indicating, as discussed elsewhere,[7] that the conformation of this glycoside, but not that of the parent disaccharide, alters with crystallization to facilitate packing. We now describe the influence of solvent and temperature changes on methyl β-cellobioside and methyl α-sophoroside conformations in solution.

In discussing the optical rotations of disaccharides we shall use the concept [7] of ' linkage rotation ', [Λ], and thereby remove the solvent and temperature influences of the type discussed above for monosaccharides. The linkage rotation is essentially the residual optical activity after the contributions of constituent sugar residues have been removed by subtraction.

The variations in linkage rotation $[\Lambda_{obs}]_D$ with temperature for the β-linked disaccharides, methyl β-cellobioside, and methyl α-sophoroside, in water and dimethyl sulphoxide are shown in Figure 2. For methyl β-cellobioside at 25 °C, the optical rotations would suggest that the average linkage conformations are similar in both solvents and, as we have mentioned above, similar to the crystal conformation of cellobiose.[40] This crystal conformation has [41] $\Delta\phi$ 42°, $\Delta\psi$ −18° corresponding to $[\Lambda_{calc}]_D$ 62° in good agreement with the observed values (59°). The solid state conformation is close to the overall minimum of conformational energy calculations [40-42] and is stabilised by hydrogen bonding between O(5) on the non-reducing residue and O(3′) on the reducing residue (4). Independent evidence that the solution and crystal conformations are similar is provided by the observation of hydrogen bonding to O(5) in dimethyl sulphoxide solution.[43] This agreement between observed and calculated rotations does not mean that the disaccharide is locked in the crystal conformation in solution but it does suggest an oscillation of $\Delta\phi$ and $\Delta\psi$ in its neighbourhood. The energy wells seem to have different shapes in each solvent because the optical rotation shows significant temperature dependence in dimethyl sulphoxide but negligible change in aqueous solution (Figure 2). Perhaps the conformation of minimum energy has additional

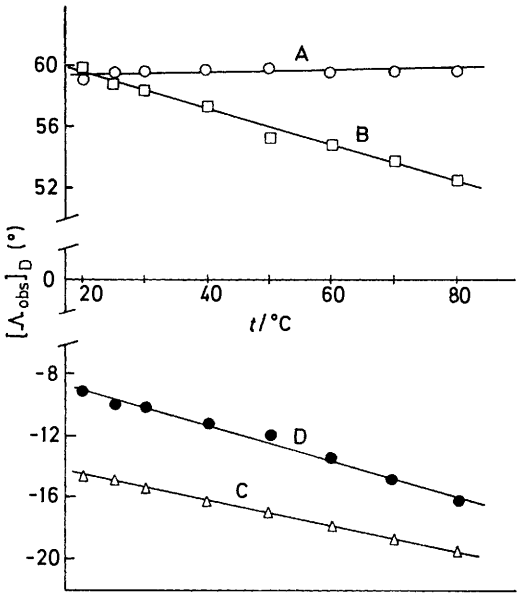


FIGURE 2 The variation of linkage rotation $[\Lambda_{obs}]_D$ with temperature for methyl-β-cellobioside in A, water; and, B, dimethyl sulphoxide; and for methyl α-sophoroside in C, water and D, dimethyl sulphoxide

stabilisation such as from ' special ' hydration (see below) in aqueous solution.

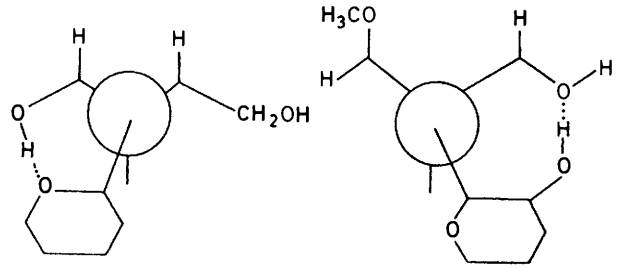
The diequatorial 1,2-linked methyl α-sophoroside also has very similar optical rotations in the two solvents although, as would be expected, the actual values differ from those for methyl β-cellobioside. The linkage rotation, $[\Lambda_{obs}]_D$ −15°, does not correspond to the crystal conformation [$\Delta\phi$ 41°, $\Delta\psi$ −17° and hence $[\Lambda_{calc}]_D$ +61°].[44] This compound therefore provides another example of a disaccharide derivative of the type for which solution and crystal conformations differ. This difference can, however, be rationalised to some extent as follows.

We note that the values of $\Delta\phi$ are similar for α-cellobiose and α-sophorose in the crystal state (+42 and +41° respectively), as expected if the nature of the glycosyl residue has the dominant influence on $\Delta\phi$ that

[40] S. S. C. Chu and G. A. Jeffrey, *Acta Cryst.*, 1968, **B24**, 830.
[41] D. A. Rees and R. J. Skerrett, *Carbohyd. Res.*, 1968, **7**, 334.
[42] D. A. Rees, *M.T.P. Internat. Rev. Sci., Org. Chem., Series One*, 1973, **7**, 251.
[43] B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, *Tetrahedron*, 1966, **22**, 3061; B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, *ibid.*, 1968, **24**, 803; B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, *Carbohydrate Res.*, 1970, **12**, 157.
[44] R. O. Gould and M. Walkinshaw, in preparation; M. Walkinshaw, Ph.D. Thesis, University of Edinburgh, 1975.

has been suggested [8] for diequatorially linked disaccharides. Assuming that $\Delta\phi$ is roughly determined in this way, it has already been shown that several disaccharides analogous to α-sophorose in all configurations adjacent to the glycosidic linkage, show a positive change in $\Delta\psi$ of up to +20° relative to β-cellobiose (see arc B in Figure 8 of ref. 8). This is believed to be the resultant of the changed steric factors around the glycosidic linkage and changed hydrogen bonding possibilities, as shown schematically in (5a and b) for cellobiose and methyl-α-sophoroside. When we now interpret the optical rotation of methyl α-sophoroside in the same way, the change in $\Delta\psi$ relative to cellobiose is +34°. The fact that this value and the value for the crystal conformation of α-sophorose lie at either extreme of the range for analogous compounds,[8] suggests to us that the two conformations represent different points in the same broad energy minimum.

α-Linked Disaccharides.—Unlike the diequatorially linked disaccharides, the α-linked disaccharides show marked solvent and temperature dependence in their



(5a) (5b)

optical rotations. We shall propose that additional conformational effects must therefore be considered. The optical rotation of methyl β-maltoside is similar in dimethyl sulphoxide and dioxan, and the temperature dependence is also similar in each of these solvents. In aqueous solution, however, drastically different behaviour is seen, both in the magnitude of optical rotation and in the sign of variation with temperature (Figure 3). Analogous, but less marked differences are observed for α,α-trehalose (Figure 3).

Maltose.—For methyl β-maltoside, the crystal conformation [45] ($\Delta\phi$ −11°, $\Delta\psi$ 13°, corresponding [7] to $[\Lambda_{calc}]_D$ −109°) cannot account for the optical rotation in any solvent. This is not surprising because conformational energy calculations [1,46] show that the energy minimum in which the crystal conformation occurs, allows a wide variation of optical rotation within it; also, it does not represent the only important energy minimum. In the region of the crystal conformation, the molecule is stabilised by the O(2) · · · O(3′) hydrogen bond (6) but is destabilised by torsion strain in the glycosidic bridge; a second minimum exists in which this torsion is relieved at the expense of the hydrogen bond. In solution, we would expect the molecule to distribute within and between these states. The linkage rotations $[\Lambda_{calc}]_D$ of the two important minima [1] are −14° (taking $\Delta\phi$ −30°, $\Delta\psi$ −15° as the lowest point in the energy well in which

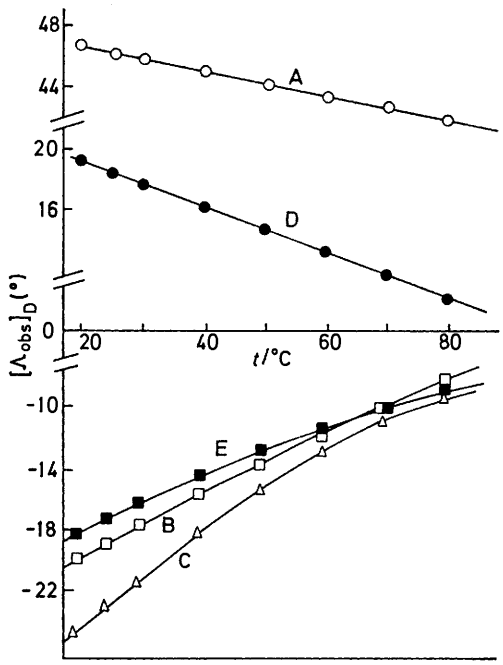
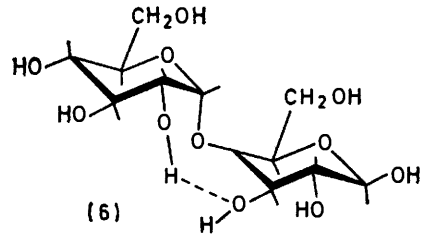


FIGURE 3 The variation of linkage rotation $[\Lambda_{obs}]_D$ with temperature for methyl β-maltoside in A, water; B, dimethyl sulphoxide; and C, dioxan, and for α,α-trehalose in D, water and E, dimethyl sulphoxide

the crystal conformation occurs) and +85° ($\Delta\phi$ −70°, $\Delta\psi$ −40°).

In dimethyl sulphoxide and in dioxan solution, the optical rotations ($[\Lambda_{obs}]_D$ −19 and −23° respectively) are taken to indicate that, even though the time-averaged conformation is significantly displaced from the crystal form, a high proportion of the molecules exist in the



(6)

region in which the inter-residue hydrogen bond is possible. We cannot distinguish between possibilities such as (i) most of the molecules exist in this energy well and, at any given time, are quite close to the calculated energy minimum and quite far from the crystal form, (ii) over half of the molecules, at any given time, exist close to the crystal position with a further substantial proportion in the second energy well. Consistent with

[45] A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, 1965, **87**, 2779.

[46] V. S. R. Rao, P. R. Sundararajan, C. Ramakrishnan, and G. N. Ramachandran, in 'Conformation of Biopolymers,' ed. G. N. Ramachandran, Academic Press, London, 1967, vol. 2, p. 721; J. Blackwell, A. Sarko, and R. H. Marchessault, *J. Mol. Biol.*, 1969, **42**, 379.

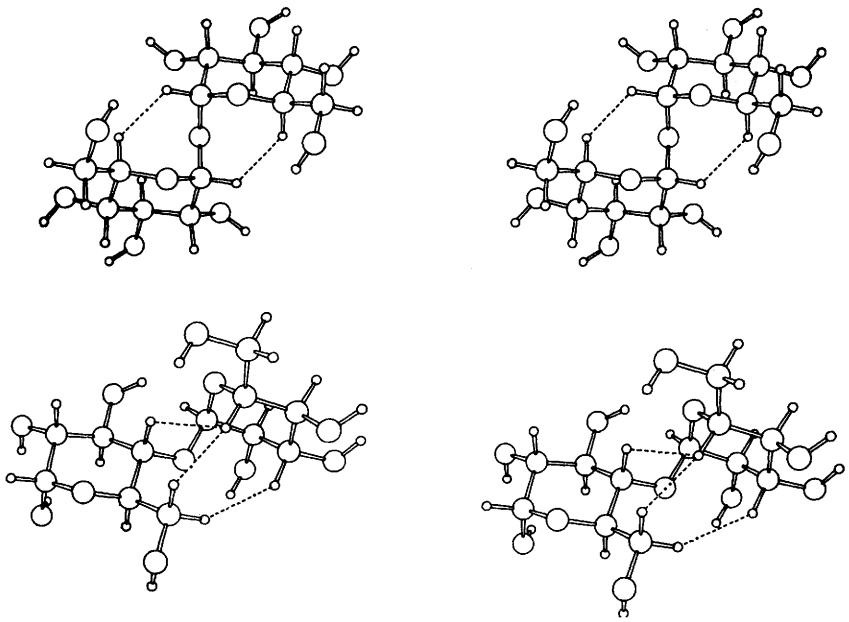


FIGURE 4 Stereoviews of suggested conformations in aqueous solution for (lower) maltose in the ' folded ' state ($\Delta\phi$ −70°, $\Delta\psi$ −40°, *i.e.* $[\Lambda]_{obs}$ 85°); and (upper) α,α-trehalose. The orientations of hydroxy-groups around C–O bonds have been arbitrarily assigned. Hydrophobic contacts between juxtaposed hydrogen atoms are shown by dotted bonds. The stereoviews are drawn by the computer program ORTEP (C. K. Johnson, report ORNL–3794, Oak Ridge National Laboratory, Oak Ridge, 1975)

either interpretation would be the ^{1}H n.m.r. evidence:[43] (i) the chemical shift of the anomeric proton suggests that the H(1)–C(1)–O–C(4) system is not very close to the eclipsed position on average, (ii) the downfield shift of O(2) and O(3) protons indicates that the molecule exists in the hydrogen bonded conformation but not exclusively so. The positive shifts in optical rotation with temperature in both nonaqueous solvents are consistent with the progressive equalisation of the population of molecules in the various possible states.

In aqueous solution, the situation is quite different. The optical rotation ($[\Lambda_{obs}]_D$ +46°) suggests that the molecule must spend a much larger proportion of its time in the conformation with $[\Lambda_{calc}]_D$ 85°. Such a conformation has been independently suggested[47] to account for the apparent specific expansibility of maltose in aqueous solution and the residues were shown in a photograph of a molecular model to have folded in a way that screens hydrophobic surfaces thus increasing the net hydrophilic character of the molecule. A computer-drawn stereoview is shown in Figure 4. Recent ^{13}C n.m.r. observations confirm that, in contrast to cellobiose, steric or proximity effects occur between the sugar residues.[48] We have considered the possibility that this conformation is also favoured because it increases the accessibility of the hydroxy-groups around the glycosidic linkage to solvation by the ' ordered ' component of liquid water. Molecular model building suggests that such a conformation has the planes of the non-reducing and reducing residues almost vertically aligned and this spacial arrangement of hydroxy-groups could maximise hydrogen bonding interactions with the dynamic tridymite arrays. This could explain the large shift in optical rotation when water is replaced by other solvents and the negative shifts in $[\Lambda_{obs}]_D$ with increasing temperature (Figure 3) or alkali concentration.[49] The linkage rotation (5°) calculated from Reeves' measurements in 5N-NaOH[49] (using the 1N-NaOH value[24] for methyl β-D-glucopyranoside) underlines the point made in discussion of the monosaccharide observations above, that aqueous alkali has solvent properties intermediate between water and dimethyl sulphoxide.

Trehalose.—The optical rotatory behaviour of another disaccharide with an axial glycosidic bond, α,α-trehalose (Figure 3), shows clear analogies with methyl β-maltoside and provides further evidence for the conformation changes we propose because the interpretation of optical rotation is considerably simplified. The two glucose residues are related by a two-fold rotation axis that passes through the glycosidic oxygen so that $\Delta\psi = \Delta\phi$ and equation (4) may now be solved directly for $\Delta\phi$. Using

$$[\Lambda]_D = -105 - 120(\sin\Delta\phi + \sin\Delta\psi) \qquad (4)$$

this approach and the measured molecular rotations, $\Delta\phi$ is calculated for dimethyl sulphoxide and water solutions at 25°, to be −53 and −72° respectively. The former value is in excellent agreement with the torsion angles in the crystal structure of α,α-trehalose dihydrate[50] ($\Delta\phi = 57.7°, \Delta\phi' = 45.1°$). The molecule therefore seems to exist in this solvent predominantly in the conformation found in the crystal. In aqueous solution, however,

[47] J. L. Neal and D. A. I. Goring, *Canad. J. Chem.*, 1970, **48**, 3745.

[48] D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 1971, **93**, 4463.

[49] R. E. Reeves, *J. Amer. Chem. Soc.*, 1954, **76**, 4595.

[50] G. M. Brown, D. C. Rohrer, B. Berking, C. A. Beevers, R. O. Gould, and R. Simpson, *Acta Cryst.*, 1972, **B28**, 3145.

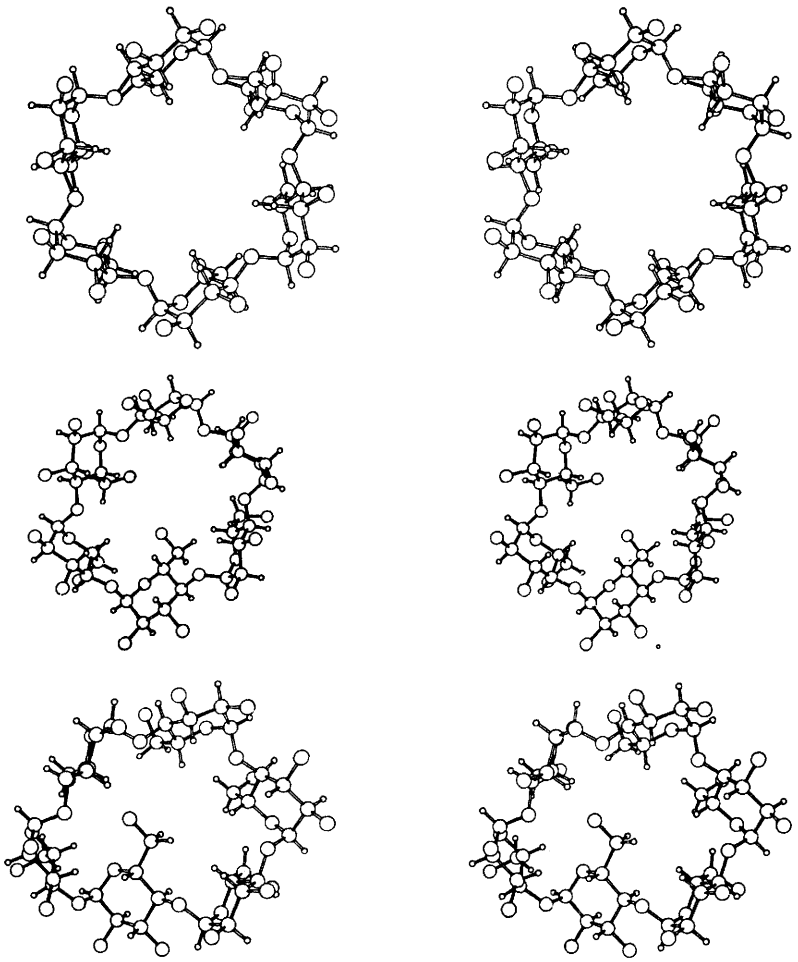


FIGURE 5 Comparison of cyclohexa-amylose in (upper) the hydrogen bonded potassium acetate crystal conformation;[45] (middle) the hexahydrate crystal conformation;[54,55] (lower) the distorted conformation in aqueous solution with one linkage conformation in the folded state and the remainder in their hexahydrate crystal conformations. The models are drawn as described in Figure 4. Hydrophobic contacts are not shown

the torsion angles are displaced by a further 20°. Molecular models show that for $\Delta\phi$ 72° the disaccharide has H(1) on the one residue juxtaposed with H(5′) on the other, and *vice versa* (Figure 4). In this case the dominant effect is probably the increased availability of HO(2) and HO(6) for hydrogen bonding to solvent and such a conformation is thereby stabilised by ' special ' hydration in an analogous way to the ' hydrophobically bonded ' conformation of the maltoside. This reasoning is consistent with the observed temperature dependent variation of $[\Lambda_{obs}]_D$.

Cyclohexa-amylose.—In dimethyl sulphoxide the observed linkage rotation (−99°) of the looped hexasaccharide cyclohexa-amylose agrees closely with $[\Lambda_{calc}]_D$ (−105°) for the hydrogen bonded potassium acetate complex [45] and this is supported by ^{1}H n.m.r. observations [43] of the O(2) · · · O(3′) hydrogen bonding. In a previous publication [7] it was pointed out that inter-residue hydrogen bonding probably occurs at the expense of angle strain, at each glycosidic oxygen. This would be relieved in aqueous solution where inter-residue hydrogen bonding is weakened by competition involving solvent, and calculation shows that the conformation could relax to $\Delta\phi$ −20°, $\Delta\psi$ 11°, *i.e.* $[\Lambda_{calc}]_D$ −87° [46] or (using different assumptions) $\Delta\phi$ −15 to −25°, $\Delta\psi$ 11—18°, *i.e.* $[\Lambda_{calc}]_D$ −94°.[51] However, the optical rotation observed in aqueous solution ($[\Lambda_{obs}]_D$ −65°) is in poor agreement with both the crystal structure and these calculated values. We suggest that there is some further distortion of the molecule in aqueous solution which arises because the intra- and inter-molecular interactions in the cavity are unfavourable in this solvent. As expected on this basis, relief of this distortion by formation of inclusion complexes with non-polar substrates [52,53] shifts the optical rotation to values which agree much better.[7]

Molecular model building coupled with the conclusions from the α-linked disaccharides, suggest a possible explanation of this distortion. We have found that the

[51] P. R. Sundararajan and V. S. R. Rao, *Carbohydrate Res.*, 1970, **13**, 351.

[52] J. A. Thoma and L. Stewart in ' Starch: Chemistry and Technology,' eds. E. L. Whistler and E. F. Paschall, Academic Press, New York, 1965, vol. 1, p. 209.

[53] H. Schlenk and D. M. Sand, *J. Amer. Chem. Soc.*, 1961, **83**, 2312.

strain in the hexagonally symmetrical conformation can be relieved by rotation of a single residue about its glycosidic linkages to give an orientation close to the ' folded ' state for methyl β-maltoside. In addition this conformation increases the accessibility of O(2) and O(3) hydroxy-groups around the altered glycosidic linkages for hydrogen bonding with the solvent.

A similar model has been suggested from recent *X*-ray analysis of the cyclohexa-amylose hexahydrate [54,55] which shows that, in contrast to the symmetrical potassium acetate complex, one of the glucosyl residues, designated number 5, is more nearly normal to the ring axis (Figure 5). However, although the conformation at the glycosidic linkage in which C(4) of this residue is involved, tends towards the folded state that we have discussed, the optical rotation calculated from the crystal co-ordinates ($[\Lambda_{calc}]_D$ −85°) is significantly different from that observed ($[\Lambda_{obs}]_D$ −65°) for aqueous solution. Better agreement ($[\Lambda_{calc}]_D$ −66°), assuming that other linkage conformations are unaltered, needs this linkage conformation to be adjusted further in the same direction to adopt the folded state. This can only be considered an approximation to the actual conformation since closure of the cyclohexa-amylose ring would require secondary adjustment of glycosidic torsion and/or bond angles. This has been done in an arbitrary way in the model shown (Figure 5). Although it is sterically possible for only one residue at a time to twist in this way, it is likely that different residues would adopt this form in turn. The optical rotations of higher cyclodextrins suggest [7] increasing contributions from this form as the ring increases in size and becomes more flexible.

Note added in proof: Since this manuscript was submitted, several of our conclusions have received important confirmation. (i) The proposal that the maltose residue can exist in the ' hydrophobically folded ' conformation is supported [56] by the *X*-ray structure of 6′-iodophenyl α-maltoside which shows that this conformation ($\Delta\phi$ −52°, $\Delta\psi$ −34°) has actually been captured in the crystal. (ii) The hydrogen bonded conformation of methyl β-maltoside in dimethyl sulphoxide solution is,[57] as we have supposed, close to the crystal form—except that the direction of the inter-residue hydrogen bond is reversed. (iii) Analysis [58] of the n.m.r. chemical shifts and coupling constants confirms, at least for gluco- and manno-pyranosides, that conformation 2(c) is unimportant in aqueous solution.

One of us (D. T.) acknowledges the award of an S.R.C. studentship. We thank Dr. W Saenger for crystal structure information and Dr. P. J. C. Smith for the stereoviews. The first part of this work was carried out at the Chemistry Department, University of Edinburgh.

[6/259 *Received*, 6*th February*, 1976]

[54] P. C. Manor and W. Saenger, *Nature*, 1972, **237**, 392; P. C. Manor and W. Saenger, *J. Amer. Chem. Soc.*, 1974, **96**, 3630.
[55] W. Saenger, personal communication.
[56] I. Tanaka, N. Tanaka, T. Ashida, and M. Kakudo, *Acta Cryst.*, 1976, **B32**, 155.
[57] M. St.-Jacques, P. R. Sundararajan, K. J. Taylor, and R. H. Marehessault, *J. Amer. Chem. Soc.*, 1976, **98**, 4386.
[58] A. De Bruyn and M. Anteunis, *Carbohydrate Res.*, 1976, **47**, 311.