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AGGREGATION WITH CHANGE OF CONFORMATION IN SOLUTIONS OF HEMICELLULOSE XYLANS

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ABSTRACT

Arabinoxylan preparations from sugar cane show temperature-induced shifts of optical rotation in aqueous methyl sulphoxide solution, the sign and magnitude of which depend on the content of arabinofuranose side-groups. The shift has a sigmoidal form and shows a distinct hysteresis loop on heating and cooling. This evidence, together with the sign and magnitude, is interpreted in terms of a conformation change of the backbone of β-(1→4)-linked D-xylose residues, from the random coil to an ordered, ribbon-like conformation similar to that which is known to exist in the solid state. The driving force for this change is not intramolecular but derives from an aggregation that occurs simultaneously and can be detected by other methods. The arabinofuranose side-groups can be incorporated into the ordered assembly and contribute to the optical rotation shift, and they therefore have an unusual role compared with other polysaccharide side-chains. We conclude that the optical rotation shifts show a "melting" and re-formation of ordered associations which may imitate the natural biological cohesion between hemicellulose chains.

INTRODUCTION

In earlier work[1], it was shown that xylans from several plant sources, in aqueous solution, underwent changes in viscosity, turbidity, sedimentation behaviour, and optical rotation, with change in temperature. These phenomena were caused by molecular aggregation, but the details and the mechanism of this process were not explained. We have now examined the sign and magnitude of the optical rotation shifts that occur in these systems, in an attempt to understand the nature of the process. This analysis is based on the methods by which optical rotation has recently been correlated with the conformation of other oligosaccharides and polysaccharides[2–5].

RESULTS AND DISCUSSION

The earlier studies of aggregation behaviour[1] were mostly with a hemicellulose fraction from guinea grass. We have now found that a polysaccharide from sugar cane, having xylose and arabinose residues in the ratio ~5:1, shows a gradual negative

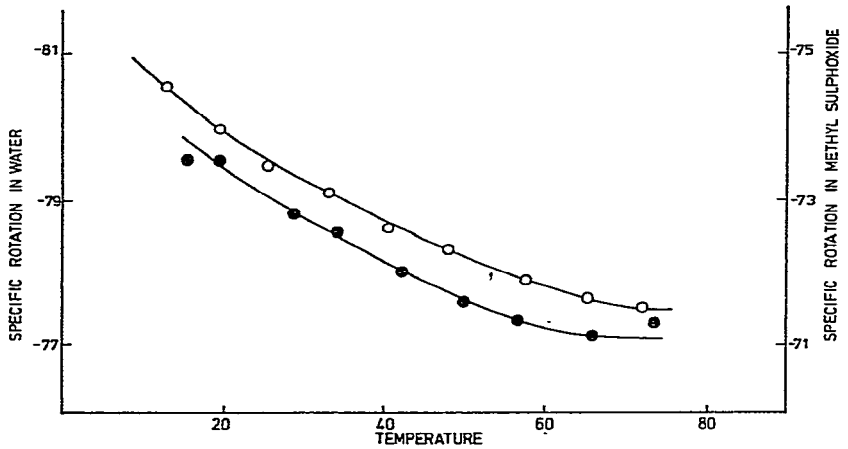

Fig. 1. Changes in optical rotation with stepwise cooling of solutions of sugar-cane hemicellulose (Fraction *A*; 0.5%) in water (○) and in methyl sulphoxide (●). The temperature and the specific rotation are in degrees.

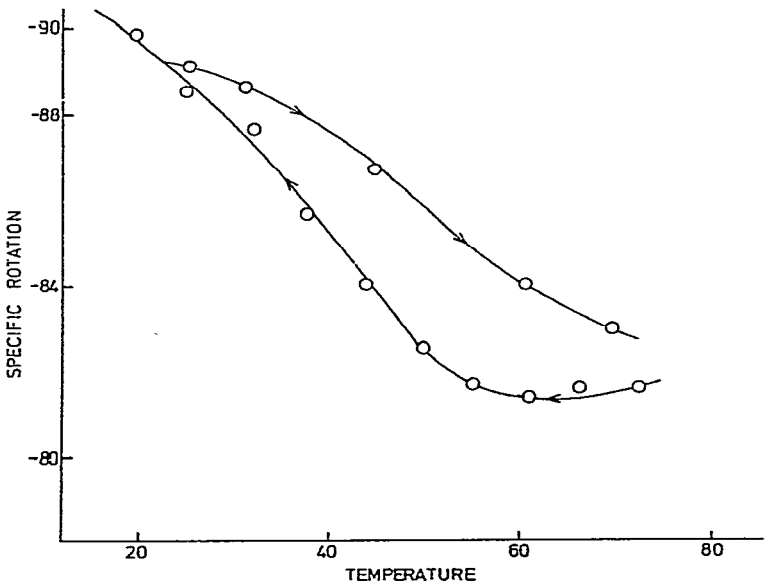

Fig. 2. Changes in optical rotation with stepwise cooling followed by stepwise heating of sugar-cane hemicellulose (Fraction *A*; 0.5%) in aqueous methyl sulphoxide (1:1, v/v). The heating and cooling curves are distinguished by the arrows. The temperature and the specific rotation are in degrees.

drift (Fig. 1) rather than the sharp shift that was seen before. The drift has a similar form for solutions in water and in methyl sulphoxide (Fig. 1), but in a mixture of these solvents, the change becomes sharper and is larger in magnitude (Fig. 2). After very mild hydrolysis to remove some of the arabinofuranosyl substituents, there was a remarkable change: a sharp shift was observed at about the same temperature, but it occurred in the opposite direction (Fig. 3).

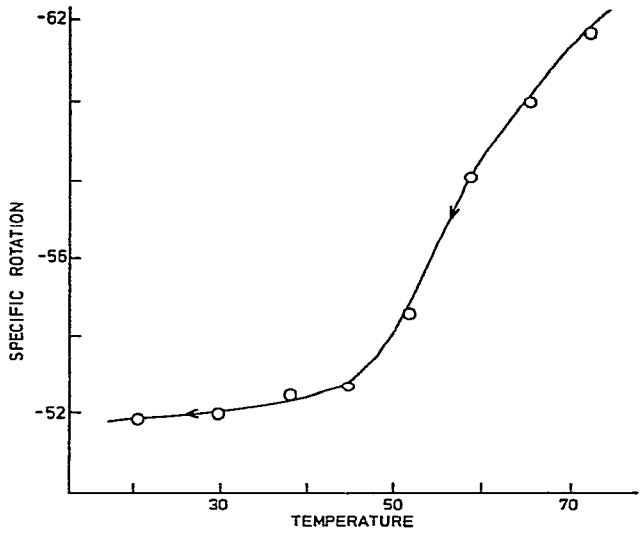

Fig. 3. Changes in optical rotation with stepwise cooling of partially hydrolysed, sugar-cane hemicellulose (Fraction *E*; 0.25%) in aqueous methyl sulphoxide (1:1, v/v). The temperature and the specific rotation are in degrees.

The general character of the process can be deduced from certain qualitative aspects of behaviour. The sigmoidal form of both shifts (Figs. 2 and 3) is clear evidence for a co-operative transition[6]. This conclusion is supported by the observation that the forward and reverse paths do not follow the same path (*i.e.* there is hysteresis) and, for reasons discussed below and elsewhere[7,8], a nucleation step must be involved. However, we know from computer model-building[3,4], as well as from diffraction evidence for the solid state[9–11], that any ordered conformation of xylan will be of the linear, extended type in which it is unlikely that intramolecular attractions and repulsions can be arranged in a way that could by themselves lead to such effects[12]. Thus, although a change in conformation is indicated by the optical rotation shift, the co-operative character and nucleation step are likely to be associated with the aggregation that has been demonstrated by a variety of techniques for the guinea-grass polysaccharide[1] and which probably therefore drives the conformation switch. X-Ray powder diagrams do indeed show that related but less densely substituted xylans can separate from aqueous solution as crystalline aggregates in which the chain has an ordered conformation[13].

Although material does separate from solution and turbidity does develop, we believe that our measurements show genuine shifts in optical rotation rather than "concentration obscuring[14]" or machine artefacts from insufficient transmission of light. To avoid artefacts, the solution was clarified in the cold before heating; we used centrifugation at 2,000g or filtration through a Millipore filter (pore size, 5 μm). The absence of artefacts was then shown by proportionality of optical rotation to path length of the cell for the same solution, by a lack of correlation between absorbance and optical rotation, and by the optical rotatory dispersion (Perkin–Elmer spectropolarimeter). This last test is used routinely because it is very convenient and sensitive: the instrument has different light sources for different parts of the spectrum, and these vary very much in intensity and therefore in sensitivity to artefacts. Artefacts can then be detected by a distortion of the usual plain curve that is to be expected for neutral carbohydrates. Finally, the fact that the direction of shift differs with different samples (Figs. 2 and 3) and changes smoothly with varying composition (see below) would indicate that it cannot be totally an artefact.

The concentrations used in this work were chosen to give the biggest shift in optical rotation that was consistent with the minimisation of artefacts, *i.e.* to maximise the accuracy. However, the behaviour was shown to be qualitatively similar for a wide range of concentrations (0.2 to 2.0%); similar molecular processes could occur at even higher concentrations, although they could not easily be detected by optical rotation.

To interpret the optical rotation shift, we will suppose that the perturbations that cause it are within the chains of bonded atoms rather than within the packing arrangement. The justification is that intramolecular effects have consistently appeared to be the more important in other systems[2–5,15], and that an internally consistent explanation of the xylan system can be reached in this way, as follows.

The shift in rotation to be expected

It is reasonable to suppose that the xylan backbone would convert to the ordered conformation that exists[9,10] in the crystalline state at 100% humidity, which is a twisted ribbon with three residues in one turn of 15.0 Å, having values for the glycosidic torsion angles[2] ($\Delta\phi$; $\Delta\psi$) of ($+54°$, $+12°$)[3,4] or ($+70°$, $0°$)[16] depending on the assumptions about bond lengths and bond angles. The chain-conformational contribution to optical rotation (the "linkage rotation", $[A]_D$ as defined elsewhere[2]) is therefore estimated at $\sim -13°$. Extrapolation of the optical rotations of the oligosaccharide series, according to the classical relationship[17], should lead to the value that corresponds to the polysaccharide in the random coil form. Using the published values[18], this procedure leads to $[A]_D = -41°$. Hence, we would predict that a transition between ordered and random coil forms would cause a shift of $\sim +28°$ in molecular rotation or $\sim +21°$ in specific rotation.

The magnitude of the observed shift in rotation

The experimental measurements were not with a pure homoxylan, but rather with hemicellulose preparations that contained a proportion of other sugar residues,

notably arabinofuranose. The series of products, obtained by progressive removal of arabinose residues by mild hydrolysis with acid, showed a gradation in optical rotation properties (Fig. 4), and the intercept at zero content of arabinose (Fig. 5) was $\sim +10°$ in specific rotation. Comparison with the prediction in the preceding paragraph shows that this is in the expected direction and corresponds to a conformational conversion of $\sim 50\%$ for the arabinose-free backbone. The change in magnitude of the optical rotation shift with hydrolysis of arabinose side-chains could arise from either or all of the following causes: (*i*) arabinose side-chains participate in the conformation change and, when removed, there is a loss of the corresponding contribution to the shift; (*ii*) arabinose side-chains block the aggregation of regions of backbone, hence they block the driving force for conformation change, and the change is facilitated by their removal; (*iii*) some hydrolysis of the xylan backbone occurs as a side reaction in the hydrolytic removal of arabinose side-chains, to give soluble segments which are too short for co-operative aggregation. We shall argue below that (*i*) is certainly involved. However, it cannot be the only influence because the extrapolation (Fig. 5) would then be linear. The two other factors would each introduce curvature but in opposite directions. For (*ii*), removal of successive arabinose residues would expose (on average) a longer chain segment for conformational change, and therefore the curve would be convex to the axes. In contrast, if (*iii*) were over-riding, the magnitude of the shift would diminish with degree of hydrolysis as more short chains were formed and unable to participate; this indeed corresponds to the behaviour that is observed.

It follows that, rather than making the extrapolation by a smooth curve through all points (Fig. 5), a better estimate for the arabinose-free backbone would be obtained by a tangent at the point which corresponds to undegraded polymer. The result ($+24°$ in molecular rotation) is indeed closer to the predicted value. We conclude that conformational conversion of the native polymer can approach completion, but that this is impaired by degradation of the backbone which occurs as a side reaction in removal of arabinofuranose residues.

Role of arabinofuranose side-chains

Reversal of the sign of the shift with removal of arabinofuranose residues (Fig. 4) shows clearly that these residues make a direct contribution to the shift rather than merely influencing the extent of the contribution of the backbone residues. This in turn implies that they participate in the conformation change and aggregation. Surprising though this is, it is entirely consistent with the analogy that has already been made with the structure of the crystalline, solid state. It appears[10,19,20] that such side-chain residues can be accommodated in the lattice to form columns that are otherwise occupied by water molecules. The negative contribution to the shift may therefore be attributed to changes in glycosidic torsion angles in the side chains as these units become incorporated in the lattice, perhaps together with a contribution from a change in furanose ring conformation if this occurs. Making allowance for the contribution of the backbone residues, we estimate that, when the slope is steepest on

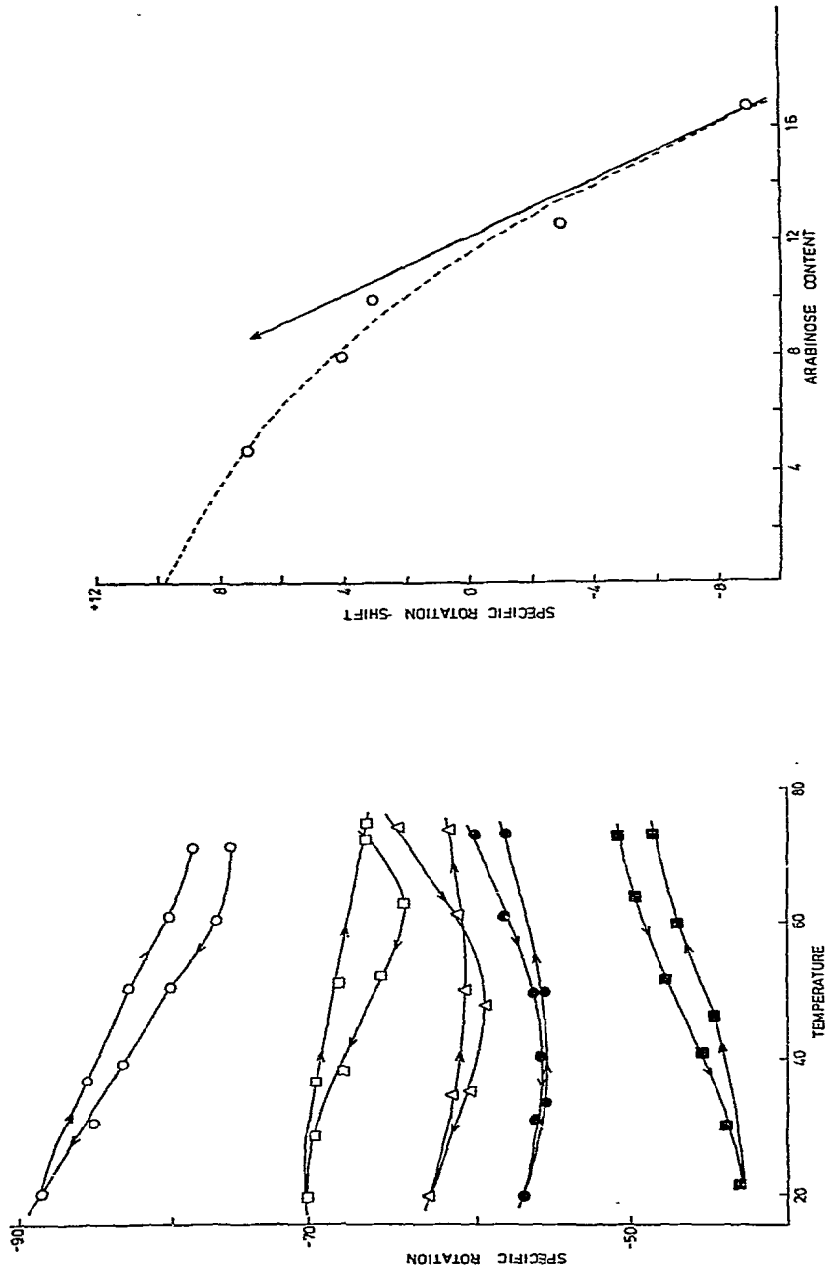

Fig. 4. Changes in optical rotation with stepwise cooling of sugar-cane hemicellulose and preparations derived from it by partial hydrolysis. The solvent was aqueous methyl sulphoxide (1:1, v/v) and the concentrations, as determined by the method described in the Experimental Section, were 0.63% (Fraction *A*, ○), 0.58% (*B*, □), 0.63% (*C*, △), 0.63% (*D*, ●), 0.50% (*E*, ■). Heating and cooling curves are distinguished by arrows. The temperature and the specific rotation are in degrees.

Fig. 5. Variation of the optical rotation shift with the proportion of arabinofuranose residues in preparations of sugar-cane hemicellulose. The shift in specific rotation that is observed when each preparation is cooled in solution from 75→20°, is plotted against the weight percentage of arabinose residues in the arabinoxylan. The simple extrapolation is shown by the broken line, and the extrapolation by a tangent at the point which corresponds to the undegraded polymer is shown by the full line.

Fig. 5 and we suspect for reasons given above that conformational conversion approaches completion, the arabinofuranose residues make an average contribution of $\sim -250°$ to the shift in molecular rotation. If this were caused by conformation changes at the glycosidic linkage alone, it would imply[2–4] that both torsion angles change by 60° at the very least. Although this is possible, it is equally likely that some change occurs in furanose ring conformation to make an additional contribution to optical rotation; as a precedent, we may cite, for example, the change in ring conformation of "2-deoxyribofuranose" residues in DNA with interconversion between A and B forms[21].

Nucleation and hysteresis

One of the arabinoxylan preparations shows a complex hysteresis form in which the cooling and heating curves cross (Fig. 4, Fraction *C*). This is similar to the "butterfly" hysteresis phenomenon that is observed in agar-galactomannan systems[5], and is to be explained in essentially the same way. Two processes occur with change of temperature which make opposite contributions to optical rotation in temperature ranges that overlap but do not coincide. In the arabinoxylan system, the two processes are, of course, the association of branched and unbranched parts of the xylan backbone, and the curves show that it is the latter that tends to occur first. The galactomannan component of the agar-galactomannan system[5] also carries side chains containing single units, but they prevent association rather than participating therein. Hysteresis arises in both systems because, to bring about nucleation[7,8], cooling is necessary below the equilibrium temperature for association. The retrogradation of starch is a more familiar polysaccharide system in which such temperature effects on nucleation are very noticeable.

Possible biological relevance

The hemicellulose xylans are natural components of plant cell-walls, in which they exist in noncovalent association with each other and with polysaccharide chains of other types. What we have observed is probably a "renaturation" process or an imitation thereof. The effect is enhanced artificially by use of the mixed solvent, whereas, in the biological state, the water activity may be depressed or the association enhanced in other ways. Although both methyl sulphoxide and water are good solvents for many polysaccharides, the mixture of the two is often a poor solvent. The biological analogy would suggest an unusual function for the arabinofuranosyl side-chains because they do not cause the termination of "binding sites" involved in association, which is the usual role of side chains[22]. Rather, the associations remain, but in modified form. Perhaps they cause the alteration of the "equilibrium constant" for association, as for example do sulphate groups in carrageenans.

It is likely that many other xylans show behaviour of the type that we report. For example, we have found that the temperature dependence of optical rotation shown by the corm-sac polysaccharide of *Watsonia pyrimidata* [a β-(1→4)-linked D-xylan which is heavily substituted with L-arabinose[23]] shows a large negative

transition, whereas Sapote gum [a β-(1→4)-linked D-xylan which is lightly substituted with L-arabinose[24]] shows a small positive transition.

EXPERIMENTAL

Optical rotations were measured, using a Perkin–Elmer Model 141 polarimeter and a 10-cm jacketed cell, at 589 nm unless stated otherwise. The temperature was decreased in steps, and measurements were made after the stated equilibration times. Paper chromatography was performed on Whatman No. 1 paper with 1-butanol–ethanol–water (4:1:5, upper layer), using the *p*-anisidine hydrochloride spray reagent[25]. Hemicelluloses were analysed by hydrolysis and reduction to form alditol acetates[26], followed by g.l.c. with 0.4% silicone oil XF-1150 and 0.4% ethylene glycol succinate liquid phases on Gas-Chrom P (100–120 mesh) in a glass column (210 × 0.7 cm). An Aerograph Autoprep 705 equipped with a flame-ionization detector was operated isothermally at a column temperature of 185° and a nitrogen flow-rate of *ca.* 45 ml/min.

Fractionation of sugar-cane hemicellulose. — Hemicellulose B from sugar cane (strain Q-57; 2.47 g)[27], in water (100 ml), was heated on a boiling water-bath (5 min) and then kept overnight at room temperature. After centrifugation (2000 *g*), the precipitate was washed twice with water and freeze-dried (500 mg). To the remaining solution (pH 3.4), a solution of cetyltrimethylammonium hydroxide [prepared by passing a 4% aqueous solution of the bromide through Amberlite IRA-400(HO^-) resin] was added dropwise. At pH 7.0, the solution was centrifuged (2000*g*) and a small amount of precipitate was discarded. Addition of the hydroxide was continued with stirring until pH 10.5 was reached, to give a precipitate that was collected by centrifugation and washed twice with distilled water. This product was dissolved in 10% aqueous sodium chloride (15 ml), and ethanol (3 vol.) was added to reprecipitate the product which was redissolved in 10% aqueous sodium chloride and reprecipitated in the same way. It was then dissolved in distilled water (15 ml) and precipitated with ethanol (3 vol.), and this process was repeated twice. The final precipitate was dissolved in, and dialysed against, distilled water (3 changes for 24 h each), and the polysaccharide product was isolated by freeze-drying to give Fraction *A* (0.80 g). Analysis of the sugars present in a hydrolysate of *A* showed xylose, arabinose, glucose, and galactose in the ratios 74:15:9:2.

After dialysis of Fraction *A* (1% solution) against 50mM sodium tetraborate for 24 h, free-boundary electrophoresis on a Tiselius apparatus (Perkin–Elmer Model 38-A) at 150 volts and 20 mamp indicated the presence of a minor, fast-moving component and a major, slower-moving component having a mobility of 1.83×10^{-6} $cm^3.sec^{-1}.volt^{-1}$.

Partial hydrolysis of Fraction A. — A solution of Fraction *A* (1.3 g) in 5mM sulphuric acid (100 ml) was heated in a boiling water-bath. Samples (25 ml) were removed at intervals of 1, 2, 3, and 7 h, each was dialysed against distilled water, and then freeze-dried to give Fractions *B*, *C*, *D*, and *E*, respectively. The composition of the part of each Fraction that was soluble in aqueous methyl sulphoxide was determined as described below, and the results are shown in Table I.

TABLE I

COMPOSITION OF HEMICELLULOSE PREPARATIONS[a]

Fraction	*Yields of sugars*[b] (%)			
	Xylose	*Arabinose*	*Glucose*	*Galactose*
A	74	15	9	2
B	78	11	7	4
C	81	9	7	3
D	83	7	7	3
E	81	4	10	5

[a]These determinations were for the part of each fraction that was soluble in aqueous methyl sulphoxide and was therefore used for optical rotation measurements (see Experimental); this part represented at least 90% of each fraction. [b]After complete acid hydrolysis, as estimated by g.l.c. of the alditol acetates. The results are expressed in terms of "anhydrosugars".

Optical rotation changes with temperature for solutions of Fraction A *in water and in methyl sulphoxide.* — A solution (0.5%) was prepared in water and heated in a boiling water-bath (10 min) before cooling to room temperature and centrifugation 2000*g*; 10 min). This was re-heated in the water bath for 10 min and introduced into the hot polarimeter cell, and measurements were made in the usual way with equilibration for 1 h after the temperature had become steady. For measurements in methyl sulphoxide, the solution (0.5%) was prepared in anhydrous solvent and heated in an oven at 100° for 20 min before introducing it into the hot polarimeter cell and making measurements as described above. Both sets of results are shown in Fig. 1.

Optical rotation changes in the mixed solvent system. — Solutions of each of Fractions *A* to *E* (~60 mg) in methyl sulphoxide (5 ml) were heated in a boiling water-bath for 10 min before cooling to room temperature and dilution with water (5 ml). Precipitated material was removed by centrifugation (2000 *g* for 10 min), washed thrice with ethanol, and dried; the weights were subtracted from the respective starting weights of the polysaccharides when the specific rotations were calculated. A sample of each supernatant solution (2 ml) was dialysed against distilled water and then freeze-dried, for analysis of the composition in terms of sugar residues (see Table I).

Each polysaccharide solution was heated in a boiling water-bath for 15 min before being introduced into the polarimeter cell. The solution was allowed to equilibrate for 1.5 h after each temperature had become steady, before making the measurement.

Possible influence of artefacts on optical rotation measurements. — Measurements of absorbance were made at 589 nm under essentially the same conditions as for optical rotation. All fractions showed a slight increase in absorbance with cooling, but their behaviour was very similar and therefore could not account for differences in optical rotation behaviour. The absorbance in a cell of path length 1 cm was always <0.1. Other experiments, using the plain o.r.d. curve as a criterion, are described in

the Discussion. The measurements for these were made at 589, 578, 546, 436, and 365 nm. Readings at 589 and 365 nm were the most sensitive to interference.

In some experiments, the measurement did not become steady for up to 5 h, and long equilibration times would therefore be required to reach the true values.

Care was taken that measurements to be used for quantitative interpretation (Fig. 5) were made in the complete absence of all artefacts, *i.e.* by the use of 1-cm cells, with long equilibration times and scrupulous use of the checking procedures given above. The actual results given in Figs. 2–4 are slightly distorted by artefacts, but the general features (those to which attention is drawn in the Discussion) are genuine.

REFERENCES

1 J. D. BLAKE AND G. N. RICHARDS, *Carbohyd. Res.*, 18 (1971) 11.
2 D. A. REES, *J. Chem. Soc., B*, (1970) 877.
3 D. A. REES AND W. E. SCOTT, *J. Chem. Soc., B*, (1971) 469.
4 D. A. REES, W. E. SCOTT, AND F. B. WILLIAMSON, *Nature (London)*, 227 (1970) 390.
5 I. C. M. DEA, A. A. MCKINNON, AND D. A. REES, *J. Mol. Biol.*, 68 (1972) 153.
6 J. ENGEL AND G. SCHWARZ, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 389.
7 D. A. REES, F. B. WILLIAMSON, AND I. W. STEELE, *J. Polym. Sci., Part C*, 28 (1969) 261.
8 D. A. REES, *Advan. Carbohyd. Chem. Biochem.*, 24 (1969) 267.
9 W. J. SETTINERI AND R. H. MARCHESSAULT, *J. Polym. Sci., Part C*, 11 (1965) 253.
10 I. A. NIEDUSZYNSKI AND R. H. MARCHESSAULT, *Biopolymers*, 11 (1972) 1335.
11 S. M. GABBAY, P. R. SUNDARAJAN, AND R. H. MARCHESSAULT, *Biopolymers*, 11 (1972) 79.
12 D. A. REES, in G. O. ASPINALL (Ed.), *MTP International Review of Science: Organic Chemistry Series One: Volume 7, Carbohydrates*, Butterworth, London, 1973, p. 251.
13 R. H. MARCHESSAULT, W. J. SETTINERI, F. F. MOREHEAD, N. M. WALTER, C. P. J. GLAUDEMANS, AND T. E. TIMELL, *J. Polym. Sci.*, 51 (1961) S66.
14 D. W. URRY, *Biochim. Biophys. Acta*, 265 (1972) 115.
15 A. A. MCKINNON, D. A. REES, AND F. B. WILLIAMSON, *Chem. Commun.*, (1969) 701.
16 D. A. REES AND R. J. SKERRETT, *Carbohyd. Res.*, 7 (1968) 334.
17 K. FREUDENBERG, W. KUHN, W. DÜRR, F. BOLZ, AND G. STEINBRUNN, *Ber.*, 63 (1930) 1510.
18 P. J. GUTMANN AND T. E. TIMELL, *J. Polym. Sci., Part C*, 11 (1965) 281.
19 I. G. FOWIE, J. F. W. JURITZ, A. KLUG, AND A. M. STEPHEN, *S. African Med. J.*, 44 (1970) 152.
20 I. G. FOWIE, J. W. F. JURITZ, AND A. M. STEPHEN, *S. African Med. J.*, 45 (1971) 1209.
21 S. ARNOTT, *Progr. Biophys. Mol. Biol.*, 6 (1970) 267.
22 D. A. REES, *Biochem. J.*, 126 (1972) 257.
23 D. H. SHAW AND A. M. STEPHEN, *Carbohyd. Res.*, 1 (1966) 400.
24 R. D. LAMBERT, E. E. DICKEY, AND N. S. THOMPSON, *Carbohyd. Res.*, 6 (1968) 43.
25 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702.
26 D. G. LANCE AND J. K. N. JONES, *Can. J. Chem.*, 45 (1967) 1995.
27 P. T. MURPHY, Ph. D. Thesis, James Cook University of North Queensland, Townsville, Australia, 1970.