Polysaccharide Conformation. Part VI.[1,2]† Computer Model-Building for Linear and Branched Pyranoglycans. Correlations with Biological Function. Preliminary Assessment of Inter-Residue Forces in Aqueous Solution. Further Interpretation of Optical Rotation in Terms of Chain Conformation

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Homopolymers of glucopyranose, galactopyranose, mannopyranose, xylopyranose, and arabinopyranose, with various positions and configurations of linkage, have been compared by model-building in the computer in an attempt to formulate simple rules for conformational analysis. Regular conformations are very restricted by steric forces alone, and each polymer has one of four characteristic shapes: *Type A*—extended and ribbon-like, *Type B*—flexible and helical, *Type C*—rigid and crumpled, or *Type D*—very flexible indeed but, on the average, rather extended. Chain branching leads to increased steric restriction for many examples which involve two secondary positions.

Correlations exist, but cannot always be explained, between the chain Type and the biological function: skeletal polysaccharides are usually of Type A, reserve and network polysaccharides are often of Type B, whereas loosely jointed polysaccharides have linkages of Type D, and chains of Type C are unnatural and rare.

More evidence is given to support the method set forth in Part V for the interpretation of optical rotations of carbohydrate polymers in terms of chain conformations. When used with model-building calculations, this method shows that the flexibility of the glycosidic system is determined by the equatorial substituents on both residues which are next to the glycosidic oxygen; libratory freedom increases with the number of hydrogen atoms in these positions. For an equatorial–equatorial linkage, A → B, the conformation about the glycosidic bond, C(1)–O, is controlled by the steric bulk of neighbouring substituents on ring A, and the O–C conformation is similarly controlled by neighbouring substituents on ring B. Polar and hydrogen bonding influences seem to be small for most equatorial–equatorial linkages in aqueous solution. For α-linked disaccharides, however, the exo-anomeric effect seems to dominate the conformation about C(1)–O, and to override steric considerations which are suggested by model-building calculations. In α-1,6- and β-1,6-linkages, the conformations about C(1)–O and C(5′)–C(6′) resemble those in corresponding methyl glycosides, and the conformation about O–C(6′) is, on the average, *anti*.

In earlier Parts of this Series, the use of model-building in the computer was described for exploration of polysaccharide conformations that are sterically possible[3,4] and energetically likely,[5,6] and optical rotation measurements were proposed for the partial characterisation of solution conformations.[2] In other investigations,[7] we

† Previous parts of this series are to be found under the title 'Conformational Analysis of Polysaccharides'.

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[1] Preliminary communication: D. A. Rees and W. E. Scott, *Chem. Comm.*, 1969, 1037.

[2] Part V, D. A. Rees, *J. Chem. Soc.* (*B*), 1970, 877.

[3] D. A. Rees, *J. Chem. Soc.* (*B*), 1969, 217.

[4] D. A. Rees, I. W. Steele, and F. B. Williamson, *J. Polymer Sci., Part C, Polymer Symposia*, 1969, **28**, 261.

[5] D. A. Rees and R. J. Skerret,t *J. Chem. Soc.* (*B*), 1970, 189.

[6] D. A. Rees and R. J. Skerrett, *Carbohydrate Res.*, 1968, **7**, 334.

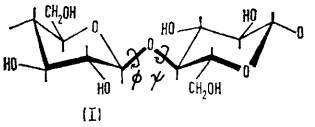
[7] N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, 1969, **45**, 85.

used the model-building method to derive polysaccharide conformations to fit diffraction evidence. We now report its application in a survey of polysaccharide types, to establish generalisations which may guide future thinking about polysaccharide conformations, and to cross-check further with the optical rotation method.

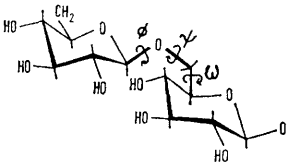
Steric Constraints on Regular Shapes

Introduction and Definitions.—We have found that correlations can be drawn with biological function (see below), by considering the *allowed regular conformations* of homopolysaccharides. '*Allowed* conformations' are those which are free, or almost free, from van der Waals compression. *Regular* conformations are considered at present but we do not suggest that these are the only ones of interest for polysaccharides—merely that (i) they are expected to occur in some circumstances and the possibilities are easy to explore, (ii) co-operative effects may occur in polysaccharide chains to favour the same relation between each residue and the next [2,4,8] and the regular conformation is then an extreme to which the polymer tends, even in aqueous solution, (iii) correlations emerge which seem to be genuine, even if their full significance cannot be grasped at present. The investigation of irregular conformations is in progress [9] and, indeed, since this paper was submitted, the interesting suggestion has been made and substantiated for special cases,[9b] that the properties of a polysaccharide in the random coil conformation can be predicted qualitatively from its regular conformation. In our terms, this suggestion would mean that random coil dimensions are small for polysaccharides of Type A (see below) and large for Type B.

Linear Chains.—For most polysaccharides there is enough evidence to define the geometry of the chain (see Methods) except for two torsion angles, ϕ and ψ, which are shown for cellulose as an example (I). For 1,5- and 1,6-linkages, there is a third angle, ω (II). We use the term [2] *linkage conformation* to describe a set of values for these angles, (ϕ, ψ) or (ϕ, ψ, ω). Each of the angles was stepped systematically * through all values from 0 to 360° in the usual way, [3-7,10-12] with calculation of interatomic distances at each stage. Those linkage conformations which brought any pair of atoms well within the van der Waals distance (see Methods) were rejected. To examine the regular conformations associated with those that remain, we invoke the theorem [10,11] that any polymer with the same linkage conformation throughout can be referred to helical geometry. These conformations could therefore be characterised in terms of n, the number of residues in one turn of the helix—which need not be integral, and h, the *projected* length of the residue on the helix axis. All van der Waals contacts were then checked within a segment corresponding to one turn of each helix plus one residue. For some polysaccharides, this test of



(I)



(II)

remote contacts led to rejection of more linkage conformations. Distinct conformation-types could be recognised from the results. *Type A*, or extended ribbon-like chains, have values of h close to the (non-projected) distance between each pair of adjacent glycosidic oxygens, and n values which can be close to 2. *Type B*, or wide helices with a shape like a wire spring or 'spiral' staircase, have low values of h and high values of n. *Type C*, or crumpled and contorted chains have low values of h, and n values which can be close to 2; they are also recognised by the failure of many linkage conformations at the test of remote contacts.

In principle, there are $36^2 = 1296$ possible linkage conformations for 1,2-, 1,3-, and 1,4-linked polysaccharides, if the angles are taken in steps of 10°. It turns out that the number actually allowed for each polysaccharide (Table 1) corresponds to less than 10% of this total. Because the allowed conformations always fell in a single zone when plotted on a conformational map (*e.g.* Figure 1), and n and h are continuous functions of ϕ and ψ,[6,10-12] there are well-defined ranges for these parameters (Table 1). It is for these reasons that each 1,2-, 1,3-, and 1,4-linked pyranoglycan can be recognised quite clearly as one conformation-type or another, as shown in Table 1. The distinctions are also clear from the computed co-ordinates for each chain in a representative allowed conformation, when plotted

* For analyses of linear polysaccharide conformations which use physical models rather than computer methods, see M. Sundaralingam, *Biopolymers*, 1968, **6**, 189 and A. L. Stone, in 'Structure and Stability of Biological Macromolecules', eds. S. N. Timasheff and G. D. Fasman, Dekker, New York, 1969, vol. 2, p. 353. Where comparison can be made with our conclusions in this and earlier papers in our series, the results are similar.

[8] A. A. McKinnon, D. A. Rees, and F. B. Williamson, *Chem. Comm.*, 1969, 701.

[9] (*a*) C. T. Greenwood, D. A. Rees, and P. J. C. Smith, unpublished work; S. G. Whittington, *Biopolymers*, in the press; V. S. R. Rao, M. Yathindra and P. R. Sundararajan, *ibid.*, 1969, **8**, 325; N. Yathindra and V. S. R. Rao, *ibid.*, 1970, **9**, 783; (*b*) D. A. Brant and W. L. Dimpfl, *Macromolecules*, 1970, **3**, 655.

[10] G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, 1968, **23**, 283.

[11] C. Ramakrishnan, *Proc. Indian Acad. Sci.*, 1964, **12**, *A*, 327.

[12] G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, in 'Aspects of Protein Structure,' ed. G. N. Ramachandran, Academic Press, New York, 1963, p. 121; V. S. R. Rao, P. R. Sundararajan, C. Ramakrishnan, and G. N. Ramachandran, in 'Conformation of Biopolymers,' ed. G. N. Ramachandran, Academic Press, New York, 1967, Volume 2, p. 721.

TABLE 1

Model-building calculations for 1,2-, 1,3-, and 1,4-linked polysaccharides

Polysaccharide	Number of allowed conformations [a]: Before remote contacts test	Number of allowed conformations [a]: After remote contacts test	Range of allowed h values	Range of allowed n values	Conformation type [b]	Ring twist, τ (degrees)	Linkage type [c]	Average allowed conformation [d]: $\overline{\Delta\phi}$	Average allowed conformation [d]: $\overline{\Delta\psi}$
β-1,4-glucan	46	46	4—6	−4 → +3	A	172	*e, e*	24	−11
β-1,3-glucan	63	59	0—5	±15 → +2	B	15	*e, e*	27	−1
β-1,2-glucan	54	35	2—3	−4 → −2	C	−61	*e, e*	26	2
α-1,4-glucan	32	23	1—4	±7 → ±3	B	−3	*a, e*	−20	−10
α-1,3-glucan	53	53	3—5	−4 → +3	A	141	*a, e*	−16	3
α-1,2-glucan	74	33	1—3	+2 → +4	C	59	*a, e*	−12	−4
β-1,4-galactan	35	32	1—5	±9 → ±4	B	0	*e, a*	24	−14
β-1,3-galactan	86	82	0—5	±30 → ±2	B	15	*e, e*	28	−10
β-1,2-galactan	54	35	2—3	−4 → −2	C	−61	*e, e*	27	2
α-1,4-galactan	28	28	4—5	−3 → +3	A	−175	*a, a*	−5	0
α-1,3-galactan	74	74	3—5	−4 → +3	A	141	*a, e*	−11	−3
α-1,2-galactan	74	34	1—3	+2 → +4	C	59	*a, e*	−11	−4
β-1,4-mannan	62	62	4—6	−4 → +3	A	171	*e, e*	11	−8
β-1,3-mannan	100	84	0—5	±15 → ±3	B	15	*e, e*	14	8
β-1,2-mannan	63	46	1—3	+2 → +4	C	59	*e, a*	13	1
α-1,4-mannan	55	38	1—4	±7 → ±3	B	−3	*a, e*	−5	−10
α-1,3-mannan	97	97	2—5	−4 → +3	A	141	*a, e*	−8	3
α-1,2-mannan	82	73	1—4	−6 → +2	B	179	*a, a*	−8	11
β-1,4-xylan	75	75	4—6	−4 → +3	A	171	*e, e*	16	1
β-1,3-xylan	63	59	0—5	±12 → +2	B	15	*e, e*	27	−1
β-1,2-xylan	54	35	2—3	−4 → −2	C	−61	*e, e*	26	2
α-1,4-xylan	71	52	1—4	±7 → ±3	B	−3	*a, e*	−20	11
α-1,3-xylan	53	53	3—5	−4 → +3	A	141	*a, e*	−16	3
α-1,2-xylan	74	27	1—3	+2 → +4	C	59	*a, e*	−12	−4
α-1,4-arabinan	61	51	0—5	±9 → ±4	B	0	*e, a*	14	−28
α-1,3-arabinan	88	84	0—5	±25 → ±3	B	15	*e, e*	28	−10
α-1,2-arabinan	54	35	2—3	−4 → −2	C	−61	*e, e*	26	2

[a] Calculations were at intervals of 10° in $\Delta\phi$ and $\Delta\psi$. [b] A ≡ extended and ribbon-like, B ≡ flexible and helical, C ≡ rigid and crumpled. [c] These symbols indicate the orientation of the glycosidic and aglycone bonds, *e* ≡ equatorial and *a* ≡ axial. [d] The values are numerical averages over all the conformations which do not show intolerable steric compression, sampled at intervals of 10°.

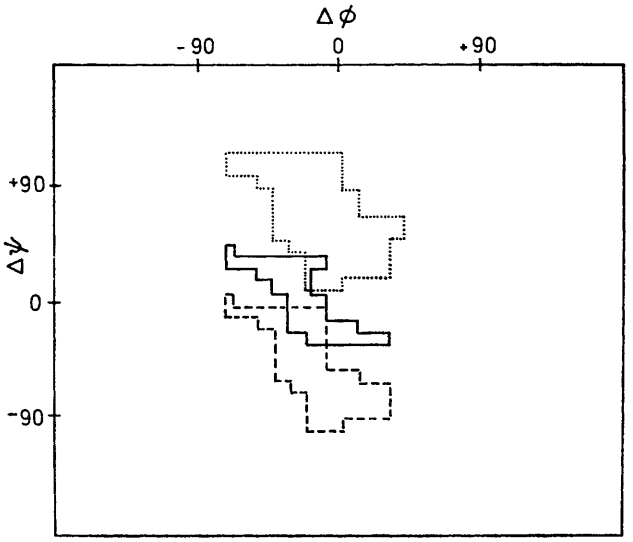
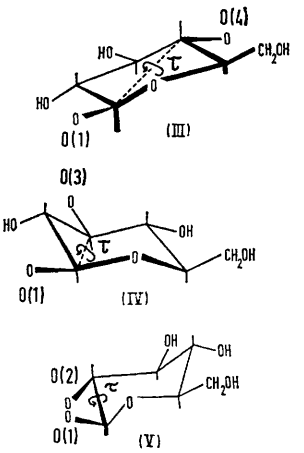


FIGURE 1 Conformational maps for α-1,4-, α-1,3-, and α-1,2-glucans. Allowed conformations are enclosed by continuous, broken, and dotted lines, respectively. Note that, even though the polysaccharides represents three different conformation-types (Table 1 and Figure 2) and there are distinct shifts in the allowed areas, these areas have similar shapes and overlap when the angles are plotted as $\Delta\phi$ and $\Delta\psi$ rather than ϕ and ψ, and each average conformation $(\Delta\phi, \Delta\psi)$ is near to (0, 0).

in projection; these results are shown for α-glucans and β-glucans in Figures 2 and 3 respectively.

The reason *why*, for example, α-1,2-, α-1,3-, and α-1,4-linked glucans correspond to different conformation-types is not primarily that the steric constraints differ; indeed the maps (Figure 1) are rather similar. The difference is more an outcome of the different *linkage positions* and is conveniently rationalised in terms of the idea of 'ring twist'. The angle of twist, τ, is the dihedral angle between the glycosidic and aglycone bonds on the same residue, viewing from C-1.



As shown in the formulae, it is close to 180° for a β-1,4-glucose residue (III), close to 0° for β-1,3- (IV), and close to +300° (*i.e.* −60°) for β-1,2- (V). The precise values for these and other residues, according to the co-ordinates that were assumed in this work, are listed in Table 1. They depend on the *position* of glycosidic

TABLE 2

Model building calculations for 1,6-linked polysaccharides

Polysaccharide	Number of allowed conformations [a] Before remote contacts test	After remote contacts test	Range of allowed values for ϕ	ψ	ω	n	h
α-1,6-glucan	3087	2377	80—190	80—280	40—160, 270—300	$\pm7 \longrightarrow \pm2$	$0 \longrightarrow 6$
β-1,6-glucan	3078	2864	210—310	80—280	40—160, 270—300	$\pm8 \longrightarrow \pm2$	$0 \longrightarrow 6$
α-1,6-galactan	2953	*b*	80—190	80—280	40—190	*b*	*b*
β-1,6-galactan	3140	*b*	210—310	80—280	40—190	*b*	*b*
α-1,6-mannan	3156	*b*	80—190	80—280	40—160, 270—300	*b*	*b*
β-1,6-mannan	3041	*b*	210—310	80—280	40—160, 270—300	*b*	*b*

[a] Calculations were at intervals of 10° in ϕ, ψ, and ω. [b] These calculations were omitted because long computing times would have been required, and the results would be very similar to those for the corresponding glucan.

substitution, and the *configuration* at *both* carbon atoms in the linkage. With one exception, the following relationships hold: τ values close to 180° lead to conformation-type A, τ values around 0° to type B, and τ values around $\pm60°$ to type C. The relationship is not fundamental, because shape is really a function of various interatomic distances and angles,[13] including the angles which are influenced by the steric constraints. Since however, the constraints usually have the similar effect of keeping the conformation around $\Delta\phi = \Delta\psi = 0$ (Table 1), the main variable in backbone geometry is the 'ring twist'. Exceptions are to be expected, but the only one encountered so far is the α-1,2-mannan, which has $\tau \approx 180°$ and corresponds to conformation-type B (Table 1).

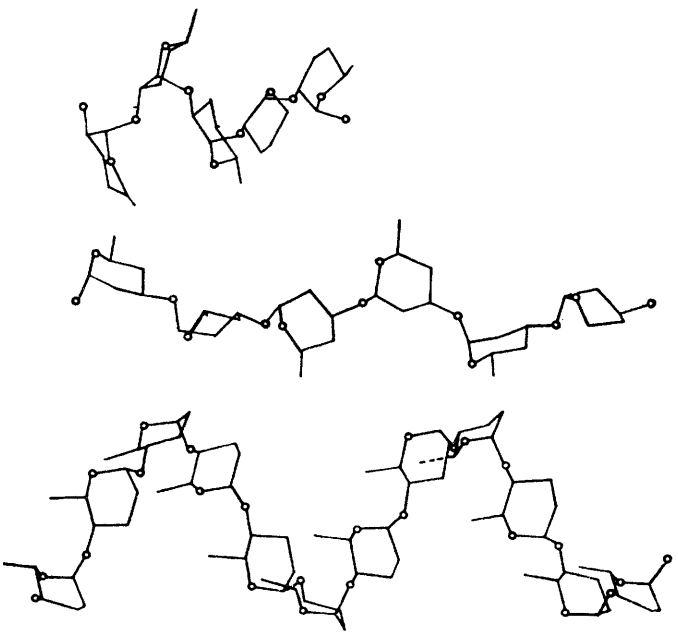
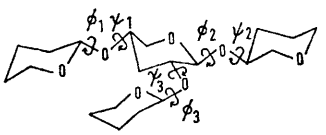


FIGURE 2 Regular α-glucan conformations, in projection, to correspond to 'average conformations,' deduced from the computer output. From top to bottom, the drawings show: α-1,2-glucan ($\phi = 260°$, $\psi = 140°$, $n = 2{\cdot}48$, $h = 2{\cdot}25$ Å; conformation-type C), α-1,3-glucan ($\phi = 210°$, $\psi = 250°$, $n = -2{\cdot}70$, $h = 4{\cdot}11$ Å; conformation-type A), and α-1,4-glucan ($\phi = 150°$, $\psi = 170°$, $n = -5{\cdot}50$, $h = 2{\cdot}25$ Å; conformation-type B). For each polysaccharide, the conformation was chosen to correspond to median values of all four parameters, ϕ, ψ, n and h. Such conformations were more truly representative for Types A and C than for Type B, because the latter can show a wider variety of shapes.

Polysaccharides with 1,6-linkages must be considered separately because they have three variable angles, ϕ, ψ, and ω. For steps of 10°, there is a greatly increased number ($36^3 = 46{,}656$) of conformations to examine, of which a higher *proportion* is expected to be sterically allowed because of the increased separation of the rings. The results (Table 2) confirm that this type of chain is more flexible, as shown by the large number of allowed conformations, by the range allowed for ψ, and by the range of n and h values. The distribution of values for n and h, did, however, suggest a bias towards extended conformations; for the β-1,6-glucan, over 70% of the allowed conformations were in the range $n = 2 \longrightarrow \pm3$, and almost 70% had $h = 4 \longrightarrow 6$ Å. For the α-1,6-glucan, the same bias existed but was less striking (corresponding figures were 55% and 50% respectively). The 1,6-polysaccharides therefore represent a fourth conformation-type, *Type D*, which is very flexible but, on the average, rather extended.

The clear distinction between conformation-types, and the classification of each polysaccharide, are conveniently checked by programming to output the atomic co-ordinates of representative conformations (Figures 2 and 3).

Branching.—Steric interactions were examined around various types of glucose branching unit having linkages through secondary positions. There were six angles to be stepped through the usual increments, as shown schematically in (VI). The results (Table 3) show that



(VI)

the side and main chains restrict each other considerably. Therefore, it is unlikely that linkage contributions to

[13] H. Sugeta and T. Miyazawa, *Biopolymers*, 1967, **5**, 673.

optical rotation [2] would be additive in such systems. For some examples, about 80% or more of the conformations were found to involve steric compression; if such branches are found in Nature, they would represent very 'stiff joints'. The least stiff arrangements are those which involve positions 2 and 4 (Table 3).

Branching through position 6 was not examined because the residues are further apart and the 1,6-linkage has been shown to be very flexible (above). Thus, any restriction at the branch was considered unlikely to be important enough to justify the long computing times to characterise it.

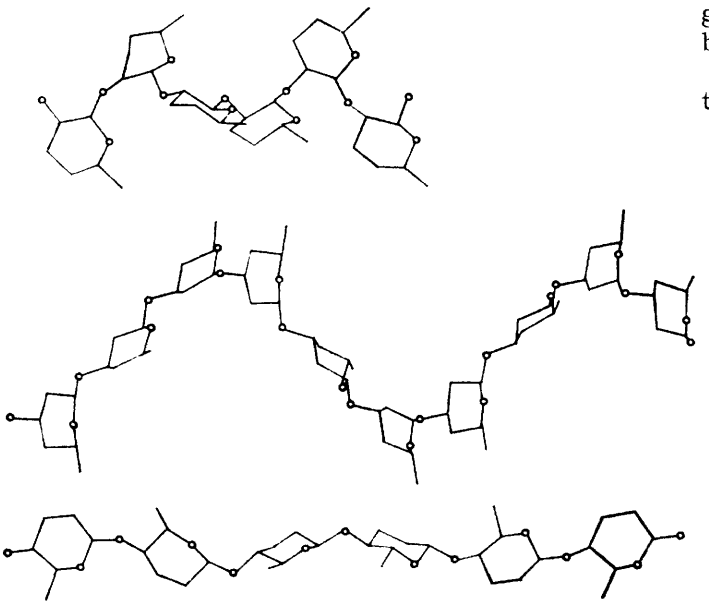


FIGURE 3 Regular β-glucan conformations, in projection, to correspond to 'average conformations' deduced from the computer output. From top to bottom, the drawings show: β-1,2-glucan ($\phi = 160°, \psi = 130°, n = -2{\cdot}62, h = 2{\cdot}79$ Å; conformation-type C), β-1,3-glucan ($\phi = 150°, \psi = 240°, n = 5{\cdot}64, h = 3{\cdot}16$ Å; conformation-type B), and β-1,4-glucan ($\phi = 225°, \psi = 190°, n = -2{\cdot}55, h = 5{\cdot}13$ Å; conformation-type A). The conformations were chosen as for Figure 2.

Biological Correlations

Although it is unlikely [2,14,15] that *isolated* polysaccharide chains can have distinct 'frozen' conformations as polypeptides may, the various chain-shape types (Table 1) do seem to correlate with particular biological functions. For some, these correlations can be explained in terms of the shapes required to interact specifically with other chains.

Skeletal Polysaccharides.—These polysaccharides share the ability to form fibrous aggregates by an efficient packing and strong bonding which derives from an extended and ribbon-like shape of Type A (Figures 2 and 3). Such shapes are shown by model-building calculations (Table 1) to arise from chains of three sub-types: 1*e*,4*e*-linkages, 1*a*,4*a*-linkages, and 1*a*,3*e*-linkages.

Those with 1*e*,4*e*-linkages are important structure materials in Nature—for example, cellulose and chitin. The structure material of bacterial cell walls, peptidoglycan, is also of this type, although the chains are held by covalent cross-linkage rather than lattice forces.

When there is an equatorial hydrogen atom adjacent to the glycosidic linkage, as in mannan, xylan, and poly(mannuronic acid), the rigid chain is 'loosened' and this may serve to weaken the packing and organisation.[16,17] Similar functions seem to be performed by irregularities in sequence, for example in glucomannans and alginic acid. Hydrophilic groups such as arabinofuranose residues * or ionic residues are often incorporated to bind water at surfaces or within gels, as in poly(mannuronic acid), arabinoxylans, and glucuronoxylans.

TABLE 3

Steric restriction by branching in glucans

Main linkage	Branch linkage	Total restriction (%) [a]	Restriction of main chain (%) [b]
α-1,2-	α-1,3	89	
	β-1,3	75	
	α-1,4	0	
	β-1,4	0	0
β-1,2-	α-1,3	83	
	β-1,3	80	
	α-1,4	0	0
	β-1,4	0	0
α-1,3-	α-1,2	89	80
	β-1,2	83	65
	α-1,4	97	
	β-1,4	72	
β-1,3-	α-1,2	75	63
	β-1,2	80	65
	α-1,4	76	
	β-1,4	61	
α-1,4-	α-1,2	57	16
	β-1,2	10	0
	α-1,3	97	95
	β-1,3	76	30
β-1,4-	α-1,2	67	26
	β-1,2	18	8
	α-1,3	72	48
	β-1,3	61	21

[a] The six angles [see (VI)] were stepped in 10° increments over the values allowed for each linkage in isolation. The result is the percentage of conformations ($\phi_1, \psi_1, \phi_2, \psi_2, \phi_3, \psi_3$) which were found to be disallowed. [b] This is the proportion of main chain conformations ($\phi_1, \psi_1, \phi_2, \psi_2$) for which very few branched chain conformations (ϕ_3, ψ_3) are possible (less than 10% of those allowed for the branch linkage in isolation). Not all branching combinations were examined in this way, because of the long computer time that would have been required.

* These residues have a striking hydrophilic character, shown by the deliquescent properties of the glycosides and homopolymers, for which there is at present no satisfactory explanation.

[14] D. A. Rees, 'The Shapes of Molecules: Carbohydrate Polymers,' Oliver and Boyd Limited, Edinburgh, 1967.

[15] D. A. Rees, *Adv. Carbohydrate Chem. Biochem.*, 1969, **24**, 267.

[16] For tabulation of polysaccharides according to structure and occurrence, see G. O. Aspinall, 'Polysaccharides,' Pergamon, Oxford, 1970; see also ref. 17.

[17] G. O. Aspinall, E. Percival, D. A. Rees, and M. Rennie, in 'Rodd's Chemistry of Carbon Compounds,' ed. S. Coffey, Elsevier, Amsterdam, 1967, vol. IF, p. 596.

The other linkages which can give shapes of Type A, namely 1*a*,3*e* and 1*a*,4*a*, are much less common in Nature perhaps because their packing properties are inferior. Models and projection drawings from the computed co-ordinates show a puckering of the chain which increases with the number of axial bonds in the glycosidic linkage. Thus, 1*a*,3*e*-linkages are very rare. Examples of 1*a*,4*a*-polymers are found only for uronic acid residues which are required to combine the ability to pack with the ability to complex cations. Complex formation is enhanced by this increase in the number of axial oxygen functions in each residue,[18,19] as in pectic acid and poly(guluronic acid), and this might override the usual preference for 1*e*, 4*e*.[20]

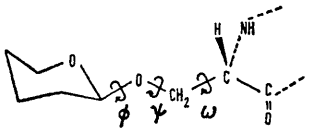
Reserve Polysaccharides.—Many important reserve polysaccharides have chains of Type B: amylose, amylopectin, glycogen, floridean starch (α-1,4-glucans), paramylon, laminarin (β-1,3-glucans), and snail galactan (β-1,3-galactan). We can suggest no convincing reason for this correlation but its existence is quite striking. Branching often occurs, perhaps to make each molecule more compact, allowing solid deposits to be utilised molecule-by-molecule, and giving more end-groups from which degradation may start by exo-enzymes.

The β-glucans of cereal grains [16] have a structural function during dormancy and are then reserve materials during germination. To correspond to both functions, they contain linkages of Type A (β-1,4-) and Type B (β-1,3-). Polysaccharides with such dual functions can also be of Type A only, as shown by β-1,4-mannan and amyloid.[21] Other examples of reserve polysaccharides having ' mixed-linkage type ' are lichenin (β-1,3- and β-1,4-glucan) and isolichenin (α-1,3- and α-1,4-glucan).

Network Polysaccharides.—Although, as a group, these are usefully distinguished from the skeletal type, there is no strict and obvious demarcation. Alginates and pectates, for example, can occur in lattices that are so swollen that they could be considered as networks [15] rather than fibrous components. Similarly, the peptidoglycan (see above) occurs in a covalent network. However, we regard the more characteristic members of the network group as having chains of Type B, with regular conformations that are wide helices which would be difficult to pack in the solid state unless the central cavity were filled, such as by formation of an inclusion complex or a multiple helix.* A β-1,3-xylan, which appears to provide cohesion within algal cell walls, does indeed exist naturally as triple helices.[22] Such twisting of chains in multiple helices would provide a likely mechanism for cross-linkage to form a network, as in carrageenan and gelatin gels.[15] Other examples of Type B polysaccharides in biological networks are the β-1,3-glucans of yeast cell walls [23] and the wound-protection network in higher plants (callose), and the β-1,4-galactans which occur in compression wood [24] and in pectic side-chains. However, the chain-interactions in these latter systems have not yet been characterised experimentally. β-1,3-Galactan chains are common in gums and arabinogalactans,[21] but are usually substituted so densely that specific interactions with other chains might be inhibited.

Many network polysaccharides have chains in which the residues are linked alternately 1*e*,4*e* and 1*e*,3*e*. The stereochemistry retains many characteristics of Type B [3,4] and, indeed, the ' tie points ' in the networks formed by some examples are definitely known to be double helices.[7] The properties and the stereochemistry have been fully discussed elsewhere.[3,4,7,15]

Loosely Jointed Polysaccharides.—1,6-Linkages are widespread in Nature, especially where there is branching. Indeed, it is difficult to find examples of multi-chain polysaccharides which do not have 1,6- (or 1,5-) linkages in their structure. It has already been shown (above) that branching without these linkages can increase the steric crowding very much. The characteristic freedom of rotation between each 1,6-linked residue and the next (Type D properties) would introduce ' flexible joints ' into bush-like macrostructures, and lead to a sponge-like rather than stiff texture which might faciltate biological interactions. Examples [16] are amylopectin, glycogen, various plant gums, pectic arabinan, levans, yeast mannan, and arabinogalactans. In animal systems, multi-chain polysaccharide structures may be



(VII)

attached to a polypeptide backbone [15,25] through linkages which are commonly *O*-glycosidic to serine and stereochemically similar to the 1,6-interglycosidic type [see (VII)]. Other known branch points to polypeptide, such as through threonine and asparagine, also have at least three bonds to allow rotation. Branched polysaccharides without 1,6-linkages are rarely, if ever, true

* Although starch is a reserve rather than a network material, it is the most familiar polysaccharide of Type B and, as such, forms inclusion complexes with many different substrates *e.g.* V-amylose complexes. Double-helix structures are also possible, however, and have been suggested for certain crystal forms of amylose (D. French, in ' Symposium on Foods: Carbohydrates and their Roles ', ed. H. W. Schulz, AVI Publishing Co., Westport, Connecticutt, 1969, p. 26).

[18] R. O. Gould and A. F. Rankin, *Chem. Comm.*, 1970, 489.

[19] R. Kohn, I. Furda, A. Haug, and O. Smidsrød, *Acta Chem. Scand.*, 1968, **22**, 3098.

[20] The α-L-guluronic acid residues have the 1*C* conformation, at least in the solid state: E. D. T. Atkins, W. Mackie, and E. E. Smolko, *Nature*, 1970, **225**, 626.

[21] G. O. Aspinall, *Adv. Carbohydrate Chem. Biochem.*, 1969, **24**, 333.

[22] E. D. T. Atkins, K. D. Parker, and R. D. Preston, *Proc. Roy. Soc.*, 1969, *B*, **173**, 209; E. D. T. Atkins and K. D. Parker, *J. Polymer Sci., Part C, Polymer Symposia*, 1969, **28**, 69.

[23] J. S. D. Bacon, V. C. Farmer, D. Jones, and I. F. Taylor, *Biochem. J.*, 1969, **114**, 557; D. J. Manners and A. J. Masson, *FEBS Letters*, 1969, **4**, 122.

[24] H. R. Schreuder, W. A. Cote, and T. E. Timell, *Svensk. Papperstidn.*, 1966, **69**, 641.

[25] ' Glycoproteins; their Composition, Structure and Function,' ed. A. Gottschalk, Elsevier, Amsterdam, 1966.

multi-chain structures: the branches may be few in number, as in wood xylan,[26] or short in length as in certain mucilages.[21] In plant gums with very dense multi-chain structures, the proportion of 1,6-linkages is correspondingly high.[21]

1,6-Linkages also occur in linear, or essentially linear, polysaccharides, but are not common. Perhaps, for most polysaccharide functions, their shapes are too indefinite. Most examples, such as dextran, pustulan, and pullulan, are from micro-organisms.[16]

Unnatural Polysaccharides.—Type C chains are crumpled and contorted, and have clashes between non-adjacent residues to cause even more stiffening than is found in chains of Type A or B. It is not surprising that the linkages which lead to this type are very rare. Indeed, the common examples of 1,2-linkages involve rhamnose or mannose residues, as in [16,21] pectic substances, yeast mannans, and gums of the Ghatti and Khaya families; this might relate to the conclusion that α-1,2-mannan is the only 1,2-linked chain which is *not* of Type C (Table 1).

1,2-Linkages also occur, as in substituted xylans and gum tragacanth, in side chains [16] which are so short that there is no long-range stereochemistry to be affected. In some of these examples, position 6 is not available for the branch point and position 2 represents the least hindered of other possibilities (see above).

Intramolecular Forces and Optical Rotation

Rules Suggested by the Calculations.—Table 1 shows that the number of allowed conformations before the test of remote contacts is related in a simple way to the substitution pattern: smaller equatorial groups adjacent to the glycosidic oxygen cause a loosening of the chain—that is, an increase in the number of allowed conformations. This we shall call the First Rule. Such an increase in libratory amplitude is illustrated in Figure 4. To investigate whether this is likely to occur by an adjustment in $\Delta\phi$, in $\Delta\psi$, or in both, the angles were averaged numerically for each polysaccharide over all allowed conformations (Table 1). These averages are denoted $\overline{\Delta\phi}$ and $\overline{\Delta\psi}$.

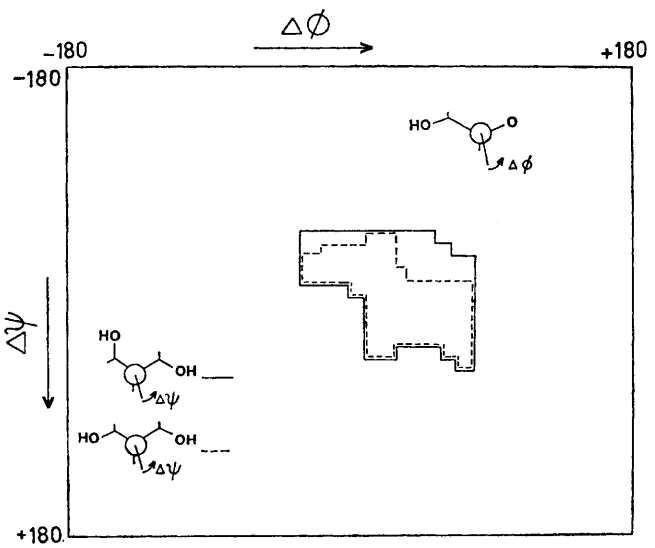
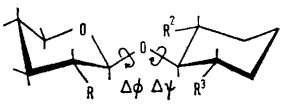


FIGURE 4 Conformational maps for galactose and glucose disaccharides linked β-1,3-; allowed conformations are enclosed for each, and the signs of the angles, are shown in the insets.

The pattern to be seen in the results changes with the number of equatorial bonds to the glycosidic oxygen atom. For equatorial–equatorial glycosidic systems, it turns out that $\overline{\Delta\phi}$ and $\overline{\Delta\psi}$ are almost independent; $\overline{\Delta\phi}$ is controlled by the nature of the substituent R on the glycosyl residue (VIII) and $\overline{\Delta\psi}$ is similarly controlled



(VIII)

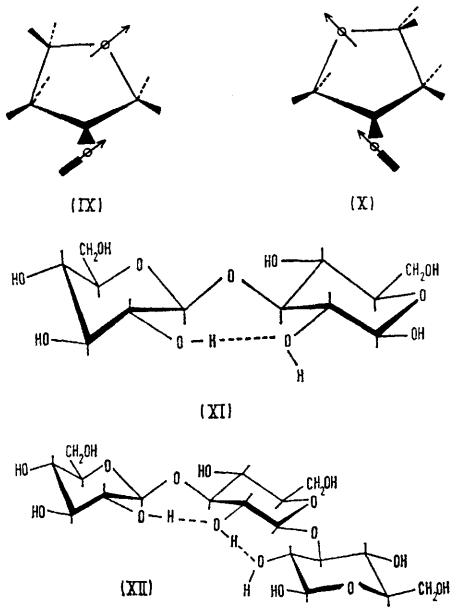
by R^2 and R^3 (VIII). Any change in the size of any of these groups causes a shift in the average value of the appropriate angle towards a smaller substituent or away from a larger one. This will be called the Second Rule. In contrast, for axial–equatorial linkages, the calculations (Table 1) suggest that from one compound to another, changes occur in $\overline{\Delta\phi}$ whatever the changes in structure. Only three equatorial–axial examples were examined and they resembled the axial–equatorial type in showing larger variation in $\overline{\Delta\phi}$ than in $\overline{\Delta\psi}$. Because axial–axial linkages are so crowded, both angles would be expected to be involved in any adjustment; only two examples were examined and were indeed found to differ in both averages (Table 1).

Prediction and Experiment for β-Linkages.—Disaccharides and their derivatives can differ very much in optical rotation, even when they contain the same sugar residues. For example, methyl β-cellobioside and methyl β-laminaribioside are both dimers of β-D-glucose but they have $[\alpha]_D$ −19° and −28° respectively. If such differences are caused by the values of $\overline{\Delta\phi}$ and $\overline{\Delta\psi}$ in solution (as argued elsewhere [2]), they might correlate with the Rules described above. These Rules are based on model-building calculations which attempt, crudely, to assess the influence of van der Waals repulsion on linkage conformations. Although other constraints such as polar interactions and hydrogen bonding exist, they are probably quenched in aqueous solution. If so, the Second Rule would suggest that $\overline{\Delta\phi}$ should be the same for glucose and galactose di- and oligo-saccharides which are linked equatorial–equatorial. It turns out that the optical rotations of cellobiose and lactose, for example, do indeed suggest very similar conformations.[2] The slight differences are explained in terms of vestigial polar interactions.[2] We assume therefore that the value for $\overline{\Delta\phi}$ in cellobiose (42°) will apply to β-glucosyl disaccharides, and that the value for

[26] M. Zinbo and T. E. Timell, *Svensk. Papperstidn.*, 1965, **68**, 647.

lactose (31°) will apply to β-galactosyl disaccharides. It is then possible to calculate $\overline{\Delta\psi}$ for a series of β-glucosyl and β-galactosyl disaccharides, by using optical rotations from the literature with equations (1) and (9) of a previous paper.[2] This gave spectacular support for our assumptions,* in that (i) all values fell within the bounds of steric possibility ($-60° \leqslant \Delta\psi \leqslant +60°$), and (ii) the values were distributed exactly as expected from the First and Second Rules (Figure 5). Thus, compounds which are similar with respect to R^2 and R^3 [*cf.* (VIII)] turn out to have similar values of $\overline{\Delta\psi}$ (Figure 5), and the distinct shifts from one family to another are easily rationalised. Replacement of R^3 by a smaller group leads (Figure 5) to a shift to the right as shown by A → C, C → E, and B → D; similar replacement of R^2 causes a shift to the left, C → B. When two equatorial hydrogen atoms are present, as on arc D, there is a maximum of libratory freedom which leads to a wide range of observed values of $\overline{\Delta\psi}$.

Some of the variations within each family can also be explained. The distribution on arc D (Figure 5) would be explained if polar interactions were to prefer conformation (IX) for carrabiose derivatives and (X) for agarobiose derivatives. On arc A there is a tendency for reducing sugars and methyl α-glycosides to correspond to more negative $\overline{\Delta\psi}$, perhaps because of polar interactions in the α form.[2] Finally, as pointed out by



Sundaralingam,[27] an O(2) · · · O(2′) hydrogen bond (XI) is favoured between β-1,3-linked residues. Because of the co-operative nature of hydrogen bonding, this influence would be stronger in oligomers (XII) and would explain why they, compared with the dimer, are at distinctly more positive values of $\overline{\Delta\psi}$ on arc C (Figure 5). This is a different type of weakly co-operative hydrogen bonding from that which seems to occur in the α-series.[2] The hydrogen bond (XI) is also

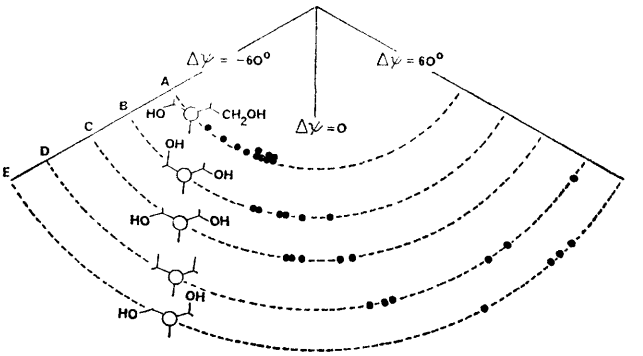


FIGURE 5 Values of $\overline{\Delta\psi}$, calculated from the optical rotations of β-glucosyl and β-galactosyl di- and oligo-saccharides as described in the text. On each arc are plotted the results for a group of compounds having similar stereochemistry about the aglycone bond; this stereochemistry is shown as a Newman projection down the bond, viewing from the glycosidic oxygen.

This method of plotting allowed each result to be translated easily into models, thus: a framework or Dreiding model of the disaccharide with $\Delta\phi$ fixed according to the assumption used (see text), is held so that the glycosidic oxygen is at the apex of the diagram, the aglycone bond is perpendicular to and pointing behind the diagram, and C–H is eclipsed by the line shown for $\Delta\psi = 0$; C(1) is then rotated about the aglycone bond until O(1)–C(1), projected onto the diagram and produced, passes through the point which is plotted for the compound.

From left to right on arc A, results are those calculated from the specific optical rotations of methyl α-cellobioside, cellobiose, 4-*O*-β-D-glucopyranosyl-D-mannose, lactose, methyl β-lactoside, methyl 4-*O*-β-D-galactopyranosyl-α-D-mannopyranoside, methyl β-cellobioside, a second value for 4-*O*-β-D-glucopyranosyl-D-mannose, 4-*O*-β-D-galactopyranosyl-D-mannose (2 independent values), and the cellodextrin glycitols.

From left to right on arc B: 3-*O*-β-D-galactopyranosyl-D-galactose, 3-*O*-β-D-glucopyranosyl-D-galactose, other reported values for the same two sugars, and two independent values for 3-*O*-β-D-galactopyranosyl-L-arabinose.

Arc C: laminaribiose (two independent values), methyl β-laminaribioside, the homologous laminaridextrins, and the derived glycitols.

Arc D: carrabiitol (two independent values), carrabiose dimethyl acetal, agarbiitol, and the limits of the range reported for agarobiose dimethyl acetal.

Arc E: 3-*O*-β-D-galactopyranosyl-D-arabinose, 3-*O*-β-D-glucopyranosyl-D-arabinose, and two more independent values for galactosyl arabinose.

The sources of the optical rotation values are given in the Methods Section. When more than two different values were given for the same compound, with no indication that any value was likely to be more accurate, the limits of the range were plotted.

required to explain the relative sizes of the C → B, C → E, and C → A shifts (Figure 5). Although these polar and hydrogen bonding effects only have a secondary role, they could possibly influence $\overline{\Delta\phi}$ which we have supposed to be invariant. A corresponding error would then be introduced into $\overline{\Delta\psi}$ as well. Therefore, we claim only to have deduced the trends in the conformation angles, rather than absolute values.

* *Added in proof:* Further strong evidence to support this treatment is that it predicted the sign and magnitude of the optical rotations which accompanies the coil ⇌ helix transition for ι-carrageenan (D. A. Rees, W. E. Scott, and F. B. Williamson, *Nature*, 1970, **227**, 390).

[27] M. Sundaralingam, *Biopolymers*, 1968, **6**, 189.

Prediction and Experiment for α-Linkages.—The optical rotations of α-linked disaccharides cannot be interpreted in quite the same way as for β-linkages, because there are no 'standard conformations' from which to start. The crystal conformations of several relevant compounds have, of course, been determined, but they do not seem to survive in solution.[2] We therefore consider two alternative starting assumptions.

(*a*) We assume that, as in the β-series, $\overline{\Delta\phi}$ is the same for related compounds, and we remember that model-building calculations have shown that steric compression can be avoided only if $-60° \leqslant \overline{\Delta\phi} \leqslant +60°$ *and* $-60° \leqslant \overline{\Delta\psi} \leqslant +60°$. It turns out that the only value for $\overline{\Delta\phi}$ which will fit all the published optical rotations without causing any compression, is then about −60°. The corresponding values of $\overline{\Delta\psi}$ are plotted in Figure 6.

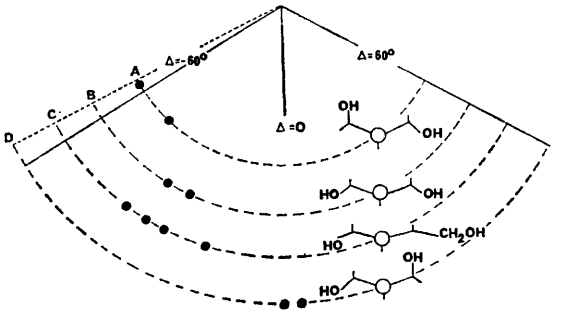


FIGURE 6 Values for torsion angles calculated from the optical rotations of α-linked disaccharides. The diagram may read either as a distribution of $\overline{\Delta\phi}$ values, or of $\overline{\Delta\psi}$ values, depending on the starting assumptions (see text); the symbol Δ represents whichever angle is appropriate. The results are plotted in modified Newman projection, as in Figure 5. (The four small formulae can, of course, be read only as projections down the aglycone bond).

From left to right on arc A, results are those calculated from the specific optical rotations of 1-*O*-α-D-galactopyranosyl-D-*myo*-inositol, and 3-*O*-α-D-galactopyranosyl-L-arabinose. On arc B: nigerose (two independent values). On arc C; methyl β-maltoside, maltose, 4-*O*-α-D-glucopyranosyl-D-mannose, methyl α-maltoside. Arc D: 3-*O*-α-D-glucopyranosyl-D-arabinose (two independent values).

The sources of the optical rotation values are given in the Methods Section.

(*b*) We assume that $\overline{\Delta\psi}$ is the same for all compounds because the model-building calculations suggested that this is the less sensitive angle. Because all the relationships involved in optical rotation are symmetrical with respect to $\Delta\phi$ and $\Delta\psi$,[2] Figure 6 can now be taken to show the distribution of values of $\overline{\Delta\phi}$ which follow from this assumption.

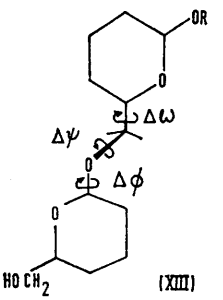
Molecular models or the formulae on Figure 6, show that the results are easily rationalised as a distribution of $\overline{\Delta\psi}$ values [assumption (*a*)]; the shifts evidently occur from one family to another to minimise steric crowding. On the other hand, as a distribution of $\overline{\Delta\phi}$ values, they would imply a tendency to *maximise* steric crowding which is so unlikely that it can be rejected. Of course, these arguments do not show that (*a*) is actually correct—merely that it is a much better approximation than (*b*). No doubt the second assumption fails because it is unsound to suppose, as it implicitly does, that the conformation angles in solution partition evenly over all sterically possible values. Indeed, there are well-known interactions (the *exo*-anomeric effect[28,29]) to cause an important energy bias in favour of conformations with $\Delta\phi \approx -60°$. The need to assign a dominant role to this effect before an intelligible trend can be seen in the results, is further evidence for its importance for conformations of α-linkages in polymeric carbohydrates, even in aqueous solution. Its influence on β-linkages cannot so easily be deduced from our data—although, for methyl glycosides, there is evidence to suggest that it is less important for the β- than the α-series.[29]

Prediction and Experiment for 1,6-*Linkages.*—Because 1,6-linked polysaccharides have three variable angles instead of two, the linkage rotation, $[\Lambda]_D$, is a more complicated term. The expressions (1) and (2) are derived in the usual way,[2] except that an extra empirical

$$[\Lambda^{\beta}_{calc}]_D = 80 - 120 \sin \Delta\phi + 120 \sin \Delta\psi - 55 \sin \Delta\omega \quad (1)$$

$$[\Lambda^{\alpha}_{calc}]_D = -130 - 120 \sin \Delta\phi + 120 \sin \Delta\psi - 55 \sin \Delta\omega \quad (2)$$

parameter [see (XIII) in ref. 30)] is required. A considerable simplification is suggested by the model-



(XIII)

building calculations which show that sterically allowed conformations have $\Delta\psi$ in the range $180° \pm 100°$ (Table 2) because, with the C(1)–O–C(6′)–C(5′) system extended thus, as in (XIII) for example, the interactions which determine $\overline{\Delta\phi}$ and $\overline{\Delta\omega}$ are likely to be similar to those in monosaccharide derivatives. To check this hypothesis, rotation was explored about the C(1)–O(1) bond in methyl α-D-glucoside and in methyl β-D-glucoside, by hard-sphere calculations; the allowed ranges of $\Delta\phi$ were found to correspond exactly to those in α- and β-1,6-linked polymers respectively (Table 2). Similar calculations for the C(5)–C(6) bond in β-D-glucose showed that the allowed range for $\Delta\omega$ corresponded exactly with those for 1,6-linked mannans and glucans.

[28] A. J. de Hoog, H. R. Buys, C. Altona and E. Havinga, *Tetrahedron*, 1969, **25**, 3365; R. U. Lemieux, A. A. Pavia, J. C. Martin, and K. A. Watanabe, *Canad. J. Chem.*, 1969, **47**, 4427.

[29] R. U. Lemieux and J. C. Martin, *Carbohydrate Res.*, 1970, **13**, 139.

[30] J. H. Brewster, *J. Amer. Chem. Soc.*, 1959, **81**, 5483.

As expected, 1,6-linked galactans showed a different range of $\Delta\omega$ because of the changed configuration at C(4). These results therefore confirm the suggestion [5] that sugar residues in 1,6-linked polysaccharides act as independent entities. This hypothesis that $\overline{\Delta\phi}$ and $\overline{\Delta\omega}$ have the same values as in isolated monomers leads to (3) for both α- and β-linkages, and hence to the direct

$$[\Lambda_{\text{calc D}}] = 120 \sin \Delta\psi \qquad (3)$$

calculation of $\overline{\Delta\psi}$ from optical rotations. Agreement is good with estimates from the conformational maps (Table 4) and therefore it is confirmed that: (i) $\overline{\Delta\phi}$ and $\overline{\Delta\omega}$ are perturbed very little in bringing together two residues to form a 1,6-linkage, (ii) the average conformation about O–C(6′) is approximately *anti*, (iii) van der Waals repulsion is the dominant interaction across the linkage. This information, taken with the new knowledge of C(1)–O(1) and C(5)–C(6) conformations in methyl glycosides,[29] and the exceptional flexibility shown by hard-sphere calculations, gives a good qualitative picture of the conformations of 1,6-linkages in solution.

TABLE 4

Estimates of $\overline{\Delta\psi}$ for 1,6-linkages

Compound	$[\alpha]_D$	$[\Lambda]_D$	Data from optical rotation [a] $\overline{\Delta\psi}$	Mean $\overline{\Delta\psi}$	Estimate from the hard sphere map.[b] $\overline{\Delta\psi}$
Gentiobiose	10·5°	7·3°	177°		
	9·6	4·2	178		
Methyl α-gentiobioside	65·5	−10·0	185	181	178
Methyl β-gentiobioside	−36	5·2	178		
Gentiodextrins		−12·7	186		
β-1,6-Glucan	−46	−8·2	184		
Isomaltose	120	7·2	177		
	122	14·0	173		
Methyl α-isomaltoside	182·3	28·7	166		
Isomaltodextrins		−8·3	184	175	171
Isomaltodextrin glycosides		12·7	174		
α-1,6-Glucan	194·5	6·8	177		
β-1,6-Galactobiose	31	−35·4	197		
	29	−42·2	201	194	177
β-1,6-Galactodextrins		−11	185		
α-1,6-Galactobiose	149	−6·6	183		
	142	−30·6	195		
	154	10·5	175	184	186
Raffinose series		−12	188		
Lychnose series		1	180		
α-1,6-Mannobiose	52	−1·4	181		
	57	15·7	172		
	62	32·8	164	176	179
α-1,6-Mannodextrins		−18·6	189		

[a] See Methods Section for the source of these data. [b] Numerical averages from hard sphere maps calculated at intervals of 10°, for all conformations which do not show intolerable steric compression.

Conclusions.—Given the need, in complex systems such as polysaccharides, to treat torsion angles as continuously variable, we have used the crudest possible approach to conformational analysis. The first aim has been to decide whether it would be worthwhile to proceed to more sophisticated theory. It has turned out that the trends in optical rotation can be explained for a large number of compounds and, further, that these trends give qualitative insight into the various attractions and repulsions that need to be treated. Thus, the prospects would seem to be very good. In future papers, we shall therefore describe our attempts to treat the problem in a more fundamental way by use of quantitative energy functions within the framework of statistical mechanics, and by considering the individual contributions to optical rotatory power which can be characterised by circular dichroism spectroscopy.

TABLE 5

Standard co-ordinates for β-D-glucose [a]

C(1)	−1·3900	0·0000	0·0000
C(2)	−1·9030	0·6008	1·3221
C(3)	−3·4427	0·6232	1·3121
C(4)	−3·9385	1·2589	0·0000
C(5)	−3·2270	0·5940	−1·1931
C(6)	−3·7046	1·2464	−2·5038
O(1)	0·0000	0·0000	0·0000
O(2)	−1·4451	−0·1965	2·4173
O(3)	−3·9155	1·3908	2·4220
O(4)	−5·3404	1·0674	−0·1185
O(5)	−1·8532	0·7625	−1·0667
H(1)	−1·7386	−0·9802	−0·0954
H(2)	−1·5411	1·5756	1·4252
H(3)	−3·8058	−0·3539	1·3846
H(4)	−3·7255	2·2819	0·0064
H(5)	−3·4530	−0·4260	−1·2088

[a] The origin of axes is on O(1), with Ox defined by C(1)–O(1) produced, and the Oxy plane defined by C(4)–C(1)–O(1); Oz is drawn to complete a right-handed system. These co-ordinates are derived from those published for β-D-xylose by Settineri and Marchessault,[31] by change of axes and the addition of C(6) as described in the text.

Methods

Dihedral Angles.—Both ϕ and ψ, and the related angles $\Delta\phi$ and $\Delta\psi$, have already been defined for glycosidic linkages to secondary positions; [2,3] strictly analogous definitions are used for ϕ, ψ, ω, $\Delta\phi$, $\Delta\psi$, and $\Delta\omega$ at 1,6-linkages [see also (I), (II), and (XIII)]. All the angles have the same [2] sense. For the 1,6-linkage, the conformation $\Delta\phi = \Delta\psi = \Delta\omega = 0$, has C(1)–H, O(1)–C(6′), C(6′)–C(5′), and C(5′)–H all eclipsed. For 1,6-linkages only, $\psi = \Delta\psi$.

Residue Co-ordinates.—In this paper, we have departed from our practice [3-7] of using co-ordinates from crystal-structure determinations. Because so many sugar residues and positions of linkage were to be compared, it was more important to make assumptions consistent within the set than to achieve high precision for a few examples. The results in Tables 1—4 were obtained with the idealised co-ordinates of Settineri and Marchessault,[31] modified as necessary to generate various residues by use of the standard bond-lengths and angles suggested by the same authors. This was easily done by hand calculation, using the listed atomic positions to calculate direction cosines for appropriate bonds, and hence to change the lengths as required. The co-

[31] W. J. Settineri and R. H. Marchessault, *J. Polymer Sci., Part C, Polymer Symposia*, 1965, **11**, 253.

ordinates for β-D-glucose are given, on convenient axes, in Table 5. Most calculations were actually repeated with β-residue co-ordinates [3,5] from the crystal structure of cellobiose [32] or α-residue co-ordinates derived from methyl β-maltoside,[33] but the results differed only in small details. Any change in the allowed areas shown on conformational maps was always at the edge and usually involved a magnitude of only 10°, although a few differences were as great as 20°. The ranges of n and h for a given polysaccharide were always characteristic of the same Conformation-Type, whatever residue co-ordinates were assumed in the calculations.

Computations.—The methods of calculation have been described before.[3-7] Completely new programs were written in Fortran IV, based on the principles described in Part II,[3] with checking of typical results against the earlier programs in Atlas Autocode. The maps for 1,6-linkages required an additional torsion angle, about C(5)–C(6), to be treated. All maps were computed at intervals of 10°. The results given here assume 'outer limit' contact distances: C–C, 3·00 Å; C–O, 2·70 Å; C–H, 2·20 Å; O–O, 2·70 Å; O–H, 2·20 Å; H–H, 1·90 Å. [The computer program did actually sub-classify into 'fully allowed' and 'marginally allowed (= outer limit)' conformations, but the distinction is not used in the discussion in this paper]. The glycosidic bond-angles for 1,2-, 1,3-, and 1,4-linkages were chosen on the basis of crystal structures published for 1,4-glucosyl-glucose linkages; the values were 117° when the C(1)–O bond was equatorial and 118° when this bond was axial. For 1,6-linkages, the glycosidic bond angle was 113°—a value which corresponds to those reported for several methyl glycosides and is quite close to the value (111°) in the α-1,6-linkage in raffinose.[34] The C(5)–C(6)–O(6) angle was taken as 112°, the average of the values from several crystal structures.

Calculations based on Optical Rotations.—Except for 3,6-anhydrides,[35] α-1,6-glucan,[36] and one value for 3-*O*-α-D-glucopyranosyl-D-arabinose,[37] the optical rotation values were taken from reference volumes [17,38,39] after checking against the original literature.

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