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Configurational Statistics of 1,6-Linked Glycans

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SUMMARY

We have calculated the unperturbed dimensions of some 1,6-linked glycans by regarding the homopolymer as a 'copolymer' in which the different 'comonomers' are conformers characterized by the dihedral angle about the C5–C6 bond.

We find that the characteristic ratio is small (e.g. for the α-glucan it is about 3·3 to 3·4). From the results of some model calculations in which the dihedral angle about the C5–C6 bond is fixed, we argue that these low values arise from bonding geometry effects, which are at least as important as the additional conformational freedom from rotation about the C5–C6 bond.

1. INTRODUCTION

The use of computer model-building techniques to investigate the flexibility and unperturbed dimensions of polysaccharides in solution is now well established. It is 13 years since Rees & Scott (1971) published a pioneering study of the conformational space available to disaccharides of aldohexopyranoses by rotation about the glycosidic and aglycone bonds. They used a computer model-building approach based on a hard-sphere potential function and categorized the regular conformations of polysaccharides into four classes. Brant & Dimpfl (1970) suggested that solution properties could be predicted (at least qualitatively) from these regular conformations and Whittington & Glover (1972) showed, for

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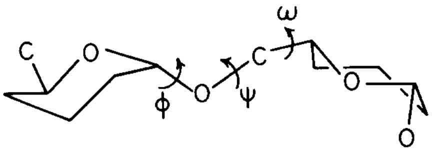

Fig. 1. Schematic structure of a 1,6-linked dimer showing the dihedral angles ϕ, ψ and ω.

1,3- and 1,4-linked homopolysaccharides, that this was generally true although both the freedom of rotation about the glycosidic and aglycone bonds, and the bonding geometry, were important factors in determining the unperturbed dimensions in solution.

Although the factors affecting the dimensions of 1,3- and 1,4-linked systems are now fairly well understood, relatively little work has been carried out on 1,6-linked systems. These are distinctly more difficult to treat because of the additional rotational freedom about the C5–C6 bond; see Fig. 1. Tvaroska *et al.* (1978) have investigated the conformational space available to 1,6-α-glycans, using a model incorporating van der Waals, torsional and hydrogen-bond energy terms. Brant & Burton (1981), have obtained rather similar results for the 1,6-α-glucan, as part of a study of pullulan (a copolymer of 1,4- and 1,6-linked α-glucose) and have also estimated the characteristic ratios of both the homopolymer and the copolymer. They found that the characteristic ratio of the 1,6-linked homopolymer was quite small (1·8 to 5 depending on the assumptions made in the calculation), consistent with the high degree of conformational freedom found by them and also by Tvaroska *et al.* (1978) and by Rees & Scott (1971). Gagnaire *et al.* (1982) have calculated characteristic ratios for α-glucans with various bonding geometries, using a novel approach in which conformational states with 'low' energy are assumed to be equi-probable. Their results for the 1,6-linked polymer are in quite good agreement with the work of Brant & Burton (1981).

Both Brant & Burton (1981) and Gagnaire *et al.* (1982) calculated the chacteristic ratio by generating a sample of polymer conformations by a Monte Carlo method in which the sets of triples (ϕ, ψ, ω) characterizing the polymer conformation are sampled independently from an appropriate tri-variate probability distribution. For 1,3- and 1,4-linked systems it is more convenient to use **T** matrix methods (Flory, 1969) since the length of the virtual bond joining adjacent bridge oxygens is fixed (Rao *et al.*, 1969) and does not depend on the dihedral angles ϕ and ψ about the glycosidic and aglycone bonds. In 1,6-linked systems the additional rotation about the C5–C6 bond complicates matters but **T** matrix methods can still be used if one assumes that only a finite number of discrete values of ω (the dihedral angle about the C5–C6 bond) are allowed. Essentially one can then think of the polymer as a copolymer in which the comonomers correspond to the various conformers defined by the values of ω. (This is related to an idea used by Olson (1975) in a polynucleotide problem.) The sequence distribution can then be generated by Monte Carlo methods (Hallman & Whittington, 1973) and the averaging over the ϕ and ψ variables can be carried out using **G** matrix techniques (Miller *et al.*, 1967; Hallman & Whittington, 1973). This is the approach used in the present paper. We examine three 1,6-linked homopolymers, α-glucan, β-glucan and α-galactan.

2. CONFIGURATIONAL STATISTICS

The treatment of the hexose ring as a rigid entity seems to be quite a good approximation in the configurational statistics of polysaccharides so that the conformation of a 1,6-linked homopolymer can be characterized by the sequence of dihedral angles about the C1–O1 (ϕ), O1–C6′ (ψ) and C6′–C5′ (ω) bonds. The set $\{(\phi_i, \psi_i, \omega_i), i = 1, 2, \ldots, n\}$ specifies the structure of the polymer, where i runs over the first, second, etc., sets of bonds between adjacent dimers making up the polymer.

The rotation about the C5–C6 bond is hindered and one would expect that the staggered forms (tg, gt and gg) would appear as the only likely conformations. In the case of glucosides it appears that only the gt and gg forms are found (Sundaralingham, 1968; Pérez *et al.*, 1978; Marchessault & Pérez, 1979). We have therefore assumed that the 1,6-linked glucan can be regarded as a ‘copolymer’ in which the two comonomers are glucose residues with the C5–C6 bond fixed in the gt and gg conformations, respectively. Marchessault & Pérez (1979), in a survey of crystal structures, found that the frequency of gg and gt forms was about 60% and 40% respectively. We have therefore assumed that the two conformers have a Bernoullian distribution in the polymer with the probability of the gg form being 0·6 and of the gt form being 0·4. Similarly, for the galactan, we have assumed that the only two possible conformers are gt and tg and that these are Bernoullian-distributed with probabilities 0·62 and 0·38 respectively (Marchessault & Pérez, 1979).

In order to survey the conformational space available to each 'dimer' (made up of two conformers) we have assumed a potential function of the Kitaigorodsky type with parameters for each non-bonded pair of atoms given by Rao *et al.* (1969). In addition, a three-fold intrinsic barrier to rotation about C–C and C–O bonds was sometimes included, with barriers equal to those used by Tvaroska *et al.* (1978).

We have used monomer geometries calculated by an optimization method developed by Dunfield (1975) and Dunfield & Whittington (1977) and the monomer coordinates used are taken directly from this work. A bond angle of 111·5° at the bridging (C1–O–C6′) oxygen was assumed (see Tvaroska *et al.*, 1978).

The assumption that the pyranose ring is rigid and that a 'monomer' has a fixed value of the dihedral angle (ω) about the exocyclic C5–C6 bond means that the distances between adjacent glycosidic oxygens are fixed and, moreover, that rotation about each pair of glycosidic and aglycone bonds is essentially independent of rotations about neighbouring pairs. This means that a virtual bond joining an adjacent pair of bridge oxygens can be mapped onto the direction of the virtual bond of the next pair of bridge oxygens by a rotation matrix, **T**, and the value of this **T** matrix can be suitably Boltzmann-averaged over all values of the dihedral angles ϕ and ψ specifying the conformation of the dimer. The independence of rotation about adjacent pairs of these bonds means that the average of a product of **T** matrices is just equal to the product of the averages. (This is of course quite familiar from previous studies of 1,3- and 1,4-linked systems (Whittington & Glover, 1972).) Under these circumstances, the calculation of the unperturbed dimensions of a polymer with a particular sequence of 'comonomers' is relatively straightforward. The characteristic ratio C_n for this monomer sequence is given by (Flory & Jernigan, 1965)

$$\begin{aligned} C_n &= \langle R_n^2 \rangle / nl^2 \\ &= 1 + (2/nl^2).(1\,0\,0\,0\,0) \prod_{j=1}^{n-1} \mathbf{G}_j \begin{pmatrix} 0 \\ 1 \\ 1 \end{pmatrix} \end{aligned}$$

where n is the degree of polymerization, $\mathbf{l}$ is $(l\ \ 0\ \ 0)'$, l^2 is the mean-square virtual bond length and $\mathbf{G}_i$ is the 5×5 matrix defined as

$$\mathbf{G}_i = \begin{pmatrix} 1 & l_i'\langle \mathbf{T}_i \rangle & 0 \\ \mathbf{0} & \langle \mathbf{T}_i \rangle & \mathbf{l}_i \\ 0 & \mathbf{0} & 1 \end{pmatrix}$$

$\langle \mathbf{T}_i \rangle$ is the average **T** matrix for the dimer corresponding to ith virtual bond, $\mathbf{l}_i$ is $(l_i\ 0\ 0)$ and l_i is the length of the ith virtual bond. Assuming only two conformations (defined by the values of ω) this means that four average **T** matrices must be calculated corresponding, for instance, to the gt–gt, gg–gt, gt–gg and gg–gg 'dimers'. All **T** matrices were calculated using sums over 10° intervals of ϕ and ψ.

We average the characteristic ratio C_n over all possible sequences of 'comonomers', consistent with the assumed statistical distribution, using Monte Carlo techniques (Hallman & Whittington, 1973) although other methods are available for this simple Bernoullian case (Flory, 1969). The averaged values of C_n are then extrapolated to $n \rightarrow \infty$, to give the characteristic ratio C_∞.

3. RESULTS

We have calculated conformational energy maps for rotation about the dihedral angles ϕ and ψ, with the values of the dihedral angles about C5–C6 (ω_1) and C5′–C6′ (ω_2) fixed in the dimer. For the 1,6-linked dimer of α-glucose these maps are similar to those reported by Tvaroska *et al.* (1978), though they differ in detail, partly because Tvaroska *et al.* include a hydrogen bonding term, and partly because of differences in the assumed monomer coordinates.

For the 1,6-linked α-glucan, with 60% gg and 40% gt (assumed to be Bernoullian-distributed), we have calculated the characteristic ratio to be between 3·3 and 3·4 (depending on whether or not a three-fold rotational potential is included). This compares quite well with the value of 1·8 to 5, depending on assumptions, reported by Brant & Burton (1981) and about 4·7 reported by Gagnaire *et al.* (1982). Similarly, for the α-galactan, with 62% gt and 38% tg, we find that the characteristic ratio is between 1·4 and 1·8. This is lower than the value for α-glucan, indicating that the α-galactan is less extended in solution.

In both cases it is tempting to ascribe these low values to the additional conformational freedom in 1,6-linked systems, due to rotation about the C5–C6 bond. However, similar (low) values of the characteristic ratio have been predicted (Whittington & Glover, 1972) for some 1e–3e linked systems and for some 1a–4e linked systems. In these cases, the low values are due to effects of bonding geometry rather than additional rotational freedom. If, in the 1,6-linked cases, the rotational freedom about the C5–C6 bond is a dominant factor in determining these rather low values of the characteristic ratios, then we would expect that C_∞ would be lower for the 'copolymer' than for either 'homopolymer'. For example, for the α-glucan we would expect that a hypothetical polymer in which all C5–C6 bonds are fixed in the gg state (or in the gt state) would have a larger value of C_∞. In fact this is not the case. If we assume that all residues have the dihedral angle ω fixed in the gg form, we calculate the value of C_∞ of the α-glucan to be about 25 (not including the inherent rotational potential) or about 40 (including these rotational barriers). However, for the α-glucan in which ω is fixed to give the gt form, the characteristic ratio is only about 1·1 to 1·2, even lower than for the polymer with a distribution of gg and gt conformers. Similar results are found for the α-galactan. In that case the value of C_∞ for the 'poly-gt' case is about 1·4 to 1·5, and for the 'poly-tg' case it is between 7 and 21. In both cases it seems that the bonding geometry in the gt form is an important factor in determining the low characteristic ratios.

We have carried out similar calculations for the 1,6-linked β-glucan, with a range of values for the relative amounts of the two conformers. The results are given in Table 1. The characteristic ratio varies as the amount of the gg conformer increases and this variation is not monotonic but shows a slight dip between zero and 40%, and then an increase as the percentage of gg monomer is further increased. This dip is probably caused by the additional conformational freedom but, once again, bonding geometry seems to play an important role.

4. CONCLUSIONS

We have shown that 1,6-linked polysaccharides can be conveniently treated by regarding the conformers (characterized by the value of the dihedral angle about the C5–C6 bond) as different 'comonomers' and

TABLE 1

Characteristic Ratio of 1,6-β-Glucan Containing Only gg and gt Conformers

Results are for the Kitaigorodsky potential alone and for the Kitaigorodsky potential plus inherent rotational potentials.

Percentage of gg conformers	*Kitaigorodsky*	*Kitaigorodsky + rotation*
0	3·3	6·6
30	3·1	5·4
40	3·6	6·7
60	4·6	10
100	7·0	23

treating the polymer as a copolysaccharide. Effectively, this means that the averaging over dihedral angles is done in two stages, one for ω, and one for ϕ and ψ. This is made possible by the small range of allowed values of ω about two of the three staggered conformations. (Including the third staggered conformation, if necessary, would be perfectly possible.) For the α-glucan, our calculated values are in reasonable agreement with those of other workers using different methods.

We have also emphasized the important role played by bonding geometry in determining the low values of the characteristic ratio in 1,6-linked systems.

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