*Carbohydrate Research*, 61 (1978) 97–106
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# CONFORMATIONAL ANALYSIS OF (1 → 6)-α-D-GLUCAN*

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(Received June 27th, 1977; accepted for publication in revised form, September 12th, 1977)

## ABSTRACT

The three-dimensional conformational space available to α-D-(1 → 6)-linked glycans is reported. The isoenergy conformational maps were prepared by defining three rotatable bonds between rigid glucopyranose residues. The calculation of internal energy considered van der Waals, torsional, and hydrogen-bond factors. The results of the analysis may be used to predict properties of (1 → 6)-α-D-homoglycans having either an axial or equatorial hydroxyl group at C-4. The theoretical isoenergy-maps are in good agreement with experimental data from crystal structures of oligosaccharides having the same linkage. The results are discussed in terms of the biological role of polysaccharides containing the α-D-(1 → 6) linkage.

## INTRODUCTION

The conformations of most of the α-D-linked homoglucans have been examined by surveying the conformational space available to two contiguous sugar residues in terms of isoenergy contour maps[1]. Only dextran[2], the glucan related to isomaltose, and such other, related homopolysaccharides as pullulan[3] remain to be studied. A preliminary study[4] of the problem was a "hard-sphere" calculation that led to the qualitative conclusion[4] that (1 → 6) (α- or β-linked) polysaccharides are "very flexible but, on the average, rather extended, in their minimum-energy conformation". In more-quantitative terms, the allowed values for $\phi$ and $\psi$ (compare Fig. 1) are 80–190° and 80–280°, respectively, with $\omega$ extending over a range of 120°. This variation would constitute greater flexibility of at least two orders of magnitude for (1 → 6)-linked polysaccharides as compared with the more-restricted (1 → 2)- or (1 → 4)-linked species. However, on the basis of "available" conformational space,

*Dedicated to Professor Dexter French on the occasion of his 60th birthday; his contributions to the chemistry and biochemistry of starch have opened the "Pandora's box" of the native granule.

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a (1 → 6)-linked polysaccharide, with its three rotatable bonds between residues, occupies the same fraction of the total space as for the other linkage-types.

Whereas dextrans yield powder diffraction-data, the preparation of crystalline, oriented fibers has not been successful, even with synthetic samples[5]. One single report of a successful single-crystal preparation, of tri-*O*-benzyldextran, is in the literature[6]. A number of crystalline structures of oligomers having (1 → 6)-α-linkages have been determined by single-crystal X-ray methods, namely for α-melibiose[7–9], isomaltulose[10], raffinose[11], planteose[12], and stachyose[13,14].

The present analysis was undertaken to provide a more-quantitative exploration of the three-dimensional conformational space available for α-D-(1 → 6)-linked linear glucans, as well as to compare the energy minima calculated theoretically with those found experimentally in related, crystalline oligosaccharides.

## METHOD OF CALCULATION

The relative orientation of two contiguous (1 → 6)-linked α-D-glucose residues is described by the three torsion angles: $\phi_i$, $\psi_i$, and $\omega_{i+1}$, as depicted in Fig. 1, where:

$$\phi_i = \theta\ (H_i\text{-}1,\ C_i\text{-}1,\ O_i\text{-}1,\ C_{i+1}\text{-}6),$$
$$\psi_i = \theta\ (C_i\text{-}1,\ O_i\text{-}1,\ C_{i+1}\text{-}6,\ C_{i+1}\text{-}5),$$
$$\omega_{i+1} = \theta\ (O_i\text{-}1,\ C_{i+1}\text{-}6,\ C_{i+1}\text{-}5,\ H_{i+1}\text{-}5).$$

In order to avoid confusion in defining one of the methylene hydrogen atoms as reference, the angle $\psi$ is referred to $C_{i+1}$-5 rather than to a hydrogen atom on $C_{i+1}$-6. For the sake of simplicity, the three torsion angles will be referred to as $\phi$, $\psi$, and $\omega$. Fig. 1 shows the origin and positive sense of $\phi$, $\psi$, and $\omega$ where the sign of the dihedral

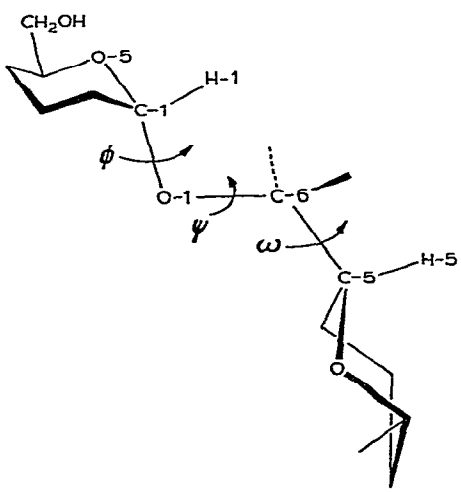


Fig. 1. Schematic diagram of a pair of (1 → 6)-linked α-D-glucose residues. The directions of positive rotation for $\phi$, $\psi$, and $\omega$ are indicated; the conformation shown in the drawing corresponds to $\phi = 0°$, $\psi = 180°$, and $\omega = 180°$.

angle is defined according to the rules recommended by the IUPAC–IUB Commission of Biochemical Nomenclature[15].

Another angular parameter to be used is $\omega$(O-5), the same torsional angle as the defined angle $\omega$, but referred to O-5, the oxygen atom of the ring. Thus $\omega$(O-5) and $\omega$ for the ideal tetrahedral system differ by 120°.

The geometry of the α-D-glucose residue was based on the average values from Arnott and Scott[16]. The (1 → 6)-α-D-glucan was constructed in the conventional fashion for-rigid body transformations[1], by using a glycosidic valence-angle ($\tau$) of 111.5° (compare Table II). The potential energy was computed by taking into account the van der Waals, torsional, and hydrogen-bond contributions. The van der Waals interactions between nonbonded atoms were evaluated by using 6–12 potential functions, with the parameters proposed by Scott and Scheraga[17,18]. A three-fold, intrinsic torsional-potential was used for rotations around the $C_i$-1–$O_i$-1, $O_i$-1–$C_{i+1}$-6, and $C_{i+1}$-6–$C_{i+1}$-5 bonds with barriers of 0.9, 2.7, and 2.7 kcal/mol, respectively[19,20]. Hydrogen-bond energies were calculated by an empirical[19], inverse third-power expression $V_{hb} = -55.0/R^3$, where $R$ is the distance between oxygen atoms, which should lie between 2.6 and 3.2 Å. The hydrogen-bond search and its related energy calculations were limited to the atoms in contiguous residues. The total potential-energy for different values of $\phi$, $\psi$, and $\omega$ was calculated by using the same fundamental approach as for the two-dimensional ($\phi$,$\psi$) maps, except that a series of maps was constructed, each one corresponding to a different value of $\omega$. These calculations were made at 30° intervals in the value of $\omega$, over the range $0 < \omega < 360°$. Each two-dimensional ($\phi$,$\psi$) map was constructed for the full angular range over 10° intervals. The calculations were performed on a CDC Cyber "74" computer at the Université de Montréal.

## RESULTS AND DISCUSSION

Conformations of (1 → 6)-α-D-glucans were plotted in terms of isoenergy contours covering the range 0–12 kcal/mol of relative energies. The results may be presented by a three-dimensional drawing, with $\phi$, $\psi$, and $\omega$ as variables defining a general, asymmetric volume whose principal axis would be extended parallel to $\omega$. The calculations indicate that the asymmetric volume is best described as a cylinder having three favored levels of $\omega$, corresponding to the non-eclipsing conformations: $\omega = -60°$, $\omega = 180°$, and $\omega = 60°$. For these three values of $\omega$, in the energy range assumed, the ($\phi$, $\psi$) isoenergy contour maps are shown in Figs. 2, 3, and 4.

To obtain some notion of the three-dimensional shape of the asymmetric volume, a section has been drawn for $\psi = 180°$. The surface so defined is shown in Fig. 5, together with the corresponding energy-contours. The values of $\phi$, $\psi$, and $\omega$ for all of the energy minima are given in Table I.

*General features of isoenergy maps for (1 → 6)-α-D-glucan.* — The use of energy calculations instead of a "hard-sphere" method better defines the range of allowed conformations for the (1 → 6)-α-D-glucan. It should be noted that $\omega$ describes the

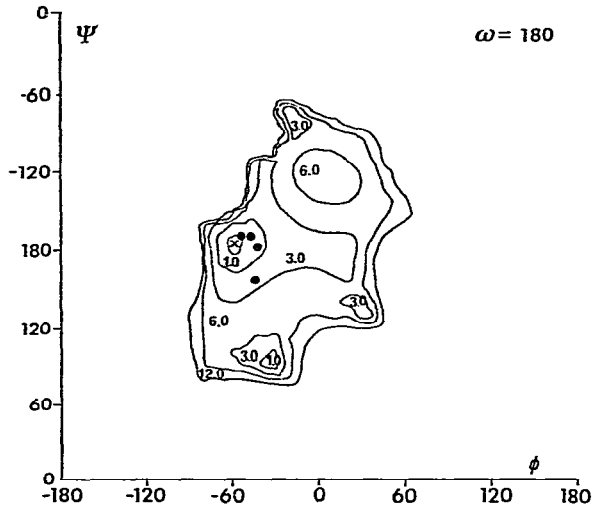


Fig. 2. A ($\phi$, $\psi$) section having $\omega = 180°$ (gg form) for (1 → 6)-linked α-D-glucose residues. Relative isoenergy contours are indicated in kcal/mol; (X) indicates the lowest calculated minimum in the sections; ● indicate the crystallographic conformations observed for stachyose, raffinose, α-melibiose, and isomaltulose, (see Table II).

conformation around the exocyclic ($C_{i+1}$-5–$C_{i+1}$-6) bond. Sundaralingam[21] found that, of the non-eclipsing conformations: gauche–trans* ($\omega = -60°$), gauche–gauche ($\omega = 180°$), and trans–gauche ($\omega = 60°$), only two occur for glucosides. A recent survey[22] of the rotameric dispositions about the $C_{i+1}$-5–$C_{i+1}$-6 bond in crystalline glucosides showed that, among 101 conformations examined, 40% exist in the gauche–trans form, whereas the remaining ones (60%) adopt the gauche–gauche arrangement. It was also shown[22] that semi-empirical functions, such as the ones described in this paper, were inadequate to explain the non-occurrence of the trans–gauche orientations in crystalline glucosides. No suitable potential-energy expression is available at this time to account for this factor.

For these reasons, the minima located on the section corresponding to $\omega = 60°$ (trans–gauche form) have to be eliminated from the set of probable conformations for (1 → 6)-linked α-D-glucose residues. There remain two sets of probable conformations corresponding to $\omega$ fixed at 180° or −60°. The available area in $\phi$ and $\psi$ space is comparable to that found for other α-D-glucans[1]. The section containing the conformation of lowest energy corresponds to $\omega = 180°$ (Fig. 2). The relative energies of the four minima recorded in this section differ little in energy. The three possible minima recorded in the section corresponding to $\omega = -60°$ (Fig. 3) are energetically comparable to the previous ones. Table I summarizes the foregoing conclusions.

*This terminology expresses the steric disposition of O-6 with reference to O-5 and C-4. For a detailed definition, see ref. 25.

In terms of helical parameters (n and h), the allowed area corresponds to: n = 2 − 10; h = ∓4 Å, in the section having $\omega = 180°$, and
n = 2 − 5.4; h = ∓5 Å, in the section having $\omega = -60°$.

Because the favored areas in Figs. 2 and 3 show more than one low-energy region, these n and h values correspond to a broad range of helical dimensions and

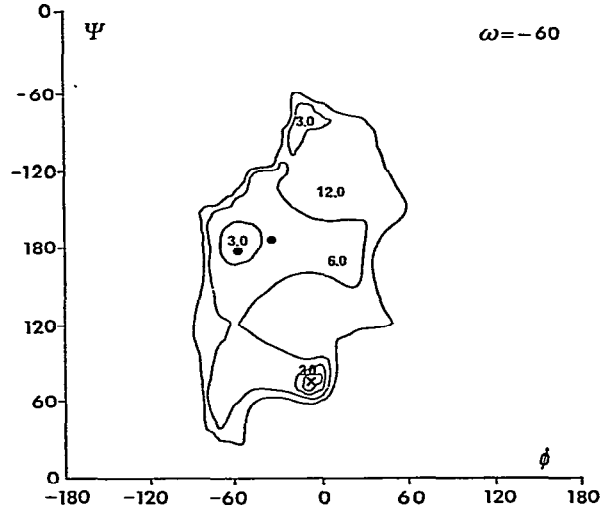


Fig. 3. Two-dimensional isoenergy map: ($\phi$, $\psi$) section having $\omega = -60°$ (gt form) for (1 → 6)-linked α-D-glucose residues. Relative isoenergy contours indicate energy values in kcal/mol; (X) indicates the lowest calculated minimum in the section; ● indicate the crystallographic conformations observed for planteose and stachyose (see Table II).

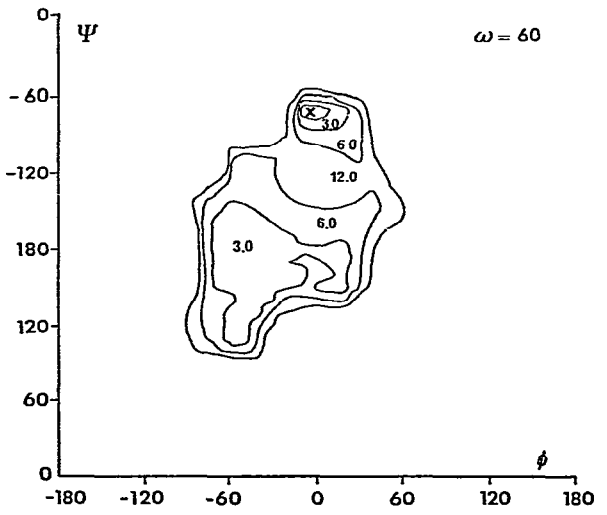


Fig. 4. Two-dimensional isoenergy maps: ($\phi$, $\psi$) section having $\omega = 60°$ (tg form) for (1 → 6)-linked α-D-glucose residues. Relative isoenergy contours indicate energy values in kcal/mol; (X) indicates the lowest calculated minimum in the section.

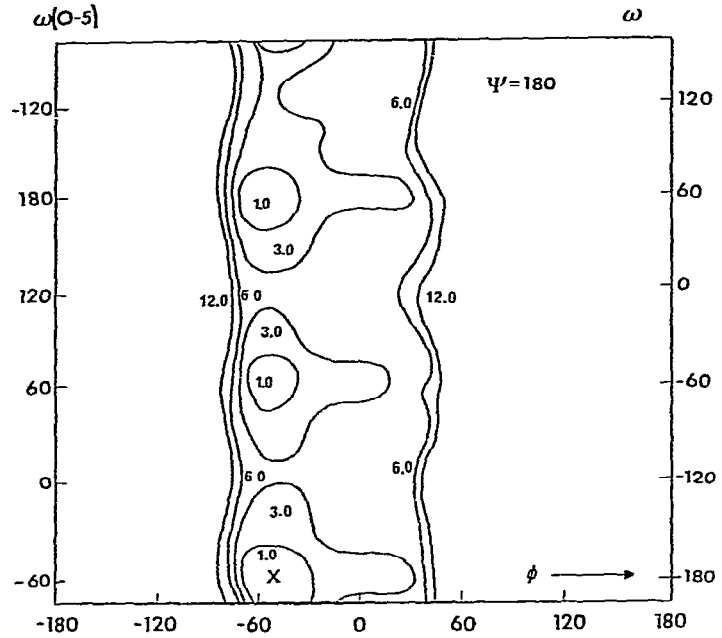


Fig. 5. Section of the three-dimensional ($\phi$, $\psi$, $\omega$) volume through $\psi = 180°$.

TABLE I

FAVORED CONFORMATIONS, IN DEGREES, FOR (1 → 6)-α-D-GLYCANS

| $\omega$ | $\phi$ | $\psi$ | |
|---|---|---|---|
| | −60 | 180 | Favored $\omega$ sections for glucans |
| 180 | −30 | 90 | |
| (*gauche–gauche*) | 30 | 130 | |
| | −20 | 270 | |
| −60 | −60 | 180 | Favored $\omega$ sections for galactans |
| (*gauche–trans*) | −10 | 70 | |
| | 0 | 270 | |
| 60 | −50 | 80 | |
| (*trans–gauche*) | 0 | 280 | |

symmetry, all having rather similar energy. This explains why it has been experimentally difficult to obtain oriented, crystalline fibers of (1 → 6)-linked polysaccharides, as many crystalline conformations have similar free-energies for given crystallization conditions.

*Comparison with experimental results.* — Among the energy minima in Table I, only two are found in the crystal structures of oligosaccharides having α-D-(1 → 6)-linked residues (Table II). In agreement with the foregoing calculations, the most-restricted conformational parameter is the rotation ($\omega$) about the exocyclic $C_{i+1}$-6–

$C_{i+1}$-5 bond. Conformations of type *A* are found in the three structures having a (1 → 6)-α-D-galactosyl-glucose linkage, and in isomaltulose, which is a (1 → 6)-α-D-galactosyl-fructose disaccharide. The gauche–gauche form characterizes the type-*A* conformation. The range of $\omega$ is very narrow (from 174 to 176°), as is also the range of $\phi$ (−41 to −52°). The basic conformation about the glycosidic ($O_i$-1–$C_{i+1}$-6) bond is trans in the four structures, and the extreme limits of $\psi$ (144° to 190°)* fall in the area of lowest energy of the map shown in Fig. 2. The same conformation of the α-D-(1 → 6) linkage is maintained in α-melibiose, raffinose, and stachyose, despite the fact that they have different intermolecular environments. A similar observation may be made for isomaltulose.

A conformation of type *B* is found in planteose and in the nanninotriose moiety of stachyose, where a gauche–trans arrangement about the $C_{i+1}$-6–$C_{i+1}$-5 bond is found. The conformation about the glycosidic ($O_i$-1–$C_{i+1}$-6) bond is trans, whereas the $\phi$ angle assumes a value somewhat similar to that found in type *A*. These conformations belong clearly to one of the low-energy regions of the map shown in Fig. 3.

The common features found for the two types of conformations are the limited range of $\phi$ and the conformation around the central bond ($\psi$) of the linkage. The first point is reminiscent of the exo-anomeric effect and its resulting conformational bias at the glycosidic linkage, which governs the value of $\phi$. A survey of the conformations characterized by $\phi$, in 21 α-D-linked sugars whose crystal structures have been reported, showed that the range of $\phi$ was narrow, ranging[23] from −60 to 0°. If the dihedral angles $\phi$ and $\omega$ are restricted, then adjustments in $\psi$ reflect most typically intra-residue interactions. These interactions limit the freedom of rotation, as depicted in the different isoenergy contour-maps, or as found in the crystal structures of oligosaccharides (Table II) in which the range of $\psi$ is relatively narrow and close to 180°. Nevertheless, the approximate ellipsoidal shape of the energy contours in Figs. 2, 3, and 4, with major axes parallel to $\psi$, indicates that this is the torsional angle that imparts most of the flexibility in solution.

*Significance with respect to other α-D-(1 → 6)-linked polysaccharides and their biological role.* — The agreement between the energy minima calculated for an α-D-(1 → 6)-linked homoglucan and the minima found experimentally in crystal structures of oligosaccharides made up of different type of sugars containing the same linkage suggests that these results may be extended to such other polysaccharides as (1 → 6)-α-D-mannan or (1 → 6)-α-D-galactan. In doing so, the configuration of the substituent at $C_{i+1}$-4 must be considered. When the latter is equatorial with respect to a $^4C_1$ (D) ring, isoenergy maps corresponding to $\omega = 180°$ and $\omega = -60°$ (Figs. 2 and 3) have to be used. The maps corresponding to $\omega = 60°$ and $\omega = -60°$ (Figs. 3 and 4) denote the two probable sections for an axially oriented substituent at $C_{i+1}$-4, as in galactans, as recent studies[22] have shown that the gauche–gauche disposition at C-6 is seldom observed in the crystal structures of small-molecule galactopyranosides.

*Note: In the IUPAC–IUB nomenclature, torsion angles are defined in the range of −180° to +180°, so that 190° is equivalent to −170°.

TABLE II

STRUCTURAL AND CONFORMATIONAL DATA FOR THE α-D (1 → 6) LINKAGE AS FOUND IN SOME CRYSTALLINE OLIGOSACCHARIDES

| *Compound* | *Ref.* | *Linkages* | $C_1$-*1*-$O_1$-*1*-$C_{1+1}$-*6* $\tau$ (*degrees*) | $H_1$-*1*-$C_1$-*1*-$O_1$-*1*-$C_{1+1}$-*6* $\phi$ (*degrees*) | $O_1$-*5*-$C_1$-*1*-$O_1$-*1*-$C_{1+1}$-*6* (*degrees*) | $C_1$-*1*-$O_1$-*1*-$C_{1+1}$-*6*-$C_{1+1}$-*5* $\psi$ (*degrees*) | $O_1$-*1*-$C_{1+1}$-*6*-$C_{1+1}$-*5*-$H_{1+1}$-*5* $\omega$ (*degrees*) | $O_1$-*1*-$C_{1+1}$-*6*-$C_{1+1}$-*5*-$O_{1+1}$-*5* $\omega$(*O*-*5*) (*degrees*) | $O_1$-*1*-$C_{1+1}$-*6*-$C_{1+1}$-*5*-$C_{1+1}$-*4* (*degrees*) |
|---|---|---|---|---|---|---|---|---|---|
| α-Melibiose | 7<br>8 | α-D-(1 → 6)<br>Gal → Glc | 111.5<br>111.8 | −41 | 76 | −174 | 176 | −63 | 60 |
| Raffinose | 11 | α-D-(1 → 6)<br>Gal → Glc | 111.4 | −47 | 72 | −170 | 174 | −65 | 58 |
| Stachyose | 14 | α-D-(1 → 6)<br>Gal → Glc | 110.9 | −52 | 66 | −175 | 176 | −63 | 60 |
| Isomaltulose | 10 | α-D-(1 → 6)<br>Glc → Fru | 115.5 | −41 | 77 | 144 | 174 | −64 | 55 |
| Planteose | 12 | α-D-(1 → 6)<br>Glc → Fru | 111.2 | −61 | 58 | 172 | −59 | 63 | 180 |
| Stachyose | 14 | α-D-(1 → 6)<br>Gal → Gal | 113.5 | −36 | 84 | −172 | −33 | 87 | −151 |

From Figs. 2, 3, and 4 it may be deduced that there are 7 and 5 favored conformations for dextran and (1 → 6)-α-D-galactan, respectively. This flexibility is in contrast to the energy maps for most β-linked homopolysaccharides, which show a single energy-minimum inside the favored area[4]. For the α-D-linked glycans studied by Santhyanarayana and Rao[1], a single energy-minimum was found, except for amylose where right- and left-handed regions are equally possible. Clearly, the additional rotatable bond in α-D-(1 → 6)-linked glycans adds to the possible range of conformations corresponding to energy minima. This factor is bound to make crystallization more difficult, except in dilute solutions and under conditions of slow crystallization. Conversely, the plethora of minimum-energy conformations makes it easy to understand why polysaccharides having the α-D-(1 → 6) linkage are usually water soluble. These linkages are also found to impart partial or complete water solubility to such normally crystalline polysaccharides as mannans; thus the galactomannans are water soluble. This factor may be thought of as a steric–conformational driving force.

The α-D-(1 → 6) linkage is present in the two most important storage polysaccharides in Nature, namely starch and glycogen. It may be reasoned that, in the case of starch where the concentration of such links is at most a few percent, the flexibility imparted by the α-D-(1 → 6) link is essential for obtaining a dense packing of molecules, especially in recently proposed structures having "cluster" branching[26]. Furthermore, the α-D-(1 → 6) linkage, with its greater flexibility, may be envisaged as introducing "local solubility" or "accessible" regions inside the carbon-storage domain. The crystalline matrix nevertheless maintains the overall granular shape, leading to enzyme-attacked granules that have a sponge-like or microporous appearance[27,28].

ACKNOWLEDGMENTS

This work was supported by grants from the National Research Council of Canada and the Ministère de l'Education du Québec. Thanks are extended to G. A. Jeffrey who provided data on stachyose before publication.

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