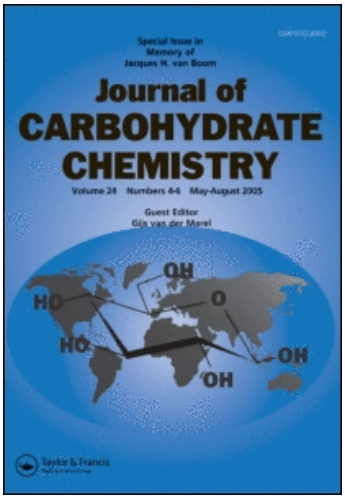

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Yui, Toshifumi , Ogawa, Kozo , Kakuta, Mariko and Misaki, Akira(1995) 'Chain Conformation of a Glucurono-xylo-mannan Isolated from Fruit Body of *Tremella fuciformis* Berk', Journal of Carbohydrate Chemistry, 14: 2, 255 — 263
To link to this Article: DOI: 10.1080/07328309508002068
URL: http://dx.doi.org/10.1080/07328309508002068

J. CARBOHYDRATE CHEMISTRY, 14(2), 255-263 (1995)

CHAIN CONFORMATION OF A GLUCURONO-XYLO-MANNAN ISOLATED FROM FRUIT BODY OF *TREMELLA FUCIFORMIS* BERK

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Received August 22, 1994 - Final Form November 11, 1994

ABSTRACT

A possible chain conformation of the acidic heteropolysaccharide isolated from the fruit body of *Tremella fuciformis* Berk (Shirokikurage) was proposed by the X-ray diffraction study combined with the computational model building technique. The polysaccharide consists of a linear backbone of 1,3-linked α-D-mannose which is highly substituted with β-D-xylose, β-D-glucuronic acid, and 1,2-linked β-D-xylobiose units at the C2 position of the mannose residue. It was suggested from the X-ray data, together with the knowledge of the chain conformations proposed for other 1,3-linked α-D-glycans, that the backbone conformation has the left-handed, three-fold helical symmetry. Orientations of the side group residues on the mannan backbone were suggested from the theoretical calculations. The proposed conformation of a repeating unit of the helical model consisted of six mannose residues and three side group residues in the 2.42 nm distance along the fiber axis. Two interresidue hydrogen bonds were observed over each 1,2-linkage between the mannose residue and the side group residue, while no hydrogen bond was observed over the backbone linkage.

INTRODUCTION

It is known that some acidic heteropolysaccharides isolated from edible mushrooms decrease cholesterol level in the blood serum.[1,2] D-Glucurono-D-xylo-D-mannan, an

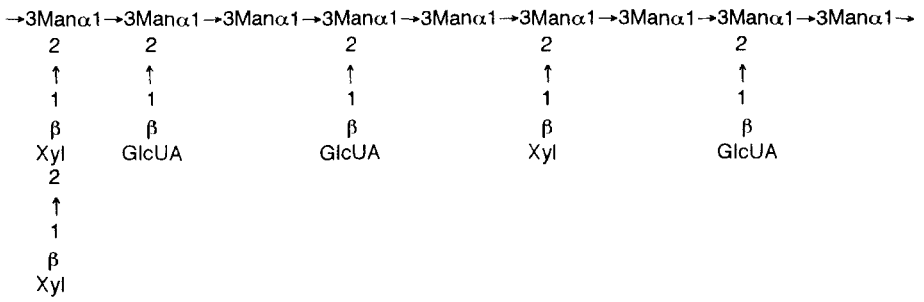

Figure 1. Proposed chemical repearing unit of D-glucurono-D-xylo-D-mannan.

acidic heteropolysaccharide, was isolated from the fruit body of *Tremella fuciformis* Berk (Shirokikurage) by extraction with water and aqueous alkali. The polysaccharide consists of repeating units containing nine 1,3-linked mannose residues as a backbone sequence to which are attached five side groups; three β-D-glucuronyl groups, a β-D-xylosyl group, and a 1,2-linked β-D-xylobiosyl group (**Figure 1**).[3] In relation to the biological functions of this fungal polysaccharide, its three dimensional structure would be of primary interest.

In the present paper, we wish to report a backbone conformation of the D-glucurono-D-xylo-D-mannan and possible orientations of the side groups elucidated from X-ray diffraction data coupled with molecular modeling data.

RESULTS AND DISCUSSION

Figure 2 shows the X-ray diffraction pattern obtained from the glucurono-xylo-mannan. The four reflections with significant intensities appeared on the meridional region in the pattern, each having the *d*-spacings of 1.236 nm, 0.752 nm, 0.612 nm, and 0.481 nm. At a first glance, a poor orientation of the diffraction pattern prevented us from distinguishing the real meridional reflections from the near meridional ones. After examination of the diffraction pattern by tilting of the fiber sample, we selected the two reflections of 0.612 nm and 1.236 nm as meridional data. These values for the *d*-spacings were larger than the virtual bond length of 0.447 nm proposed for the α-(1→3)-linked mannose residue. This obviously suggested that the crystallographic asymmetrical unit was composed of more than one mannose residue. In our previous studies on the molecular and crystal structure analyses for the three α-1,3-linked glycans, namely (1→3)-α-D-mannan,[4] (1→3)-α-D-glucan,[5] and (1→3)-α-D-glucan triacetate,[6] it was observed that the former two molecules adopted the two-fold helical conformation with the fiber repeat of 0.825 nm for the mannan and 0.844 nm for the glucan, whereas the backbone conformation of the

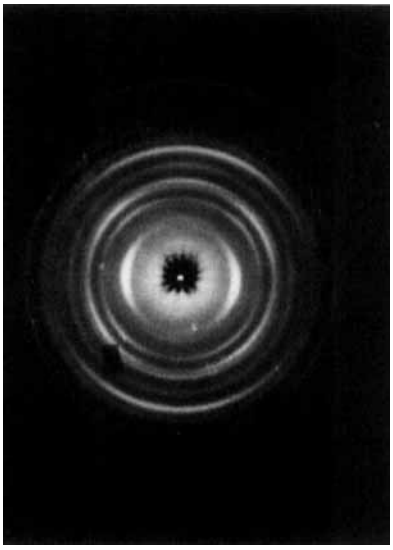

Figure 2. The X-ray diffraction pattern of D-glucurono-D-xylo-D-mannan recorded at a high relative humidity. The fiber axis is vertical.

glucan triacetate were the left-handed, three-fold (the 3_2-fold) helix with the 1.211 nm fiber repeat as a consequence of the three acetyl groups introduced to each glucose residue. The observed *d*-spacings of the two meridional reflections for the glucurono-xylo-mannan in the present study are consistent with the presence of the three-fold, probably left-handed, helix symmetry in the mannan backbone. It should be noted that, when mannan backbone has a two-fold helix symmetry, the side group residues which are connected to every second mannose align on one side of the mannan backbone. Such an asymmetrical form of the entire molecule is obviously unrealistic as a polymer crystal structure.

Preferred orientations of the side group residues were studied by calculating the energy map of the di- and the tetrasaccharide fragments of the present polysaccharide. The energy map represents the variation of steric energy with respect to two glycosidic bond rotations, each designated as Φ and Ψ. The glycosidic bonds of current interest are those of the branching positions. Φ and Ψ along with the atom labeling of the fragment in the polysaccharide are depicted in **Figure 3.** The map (**Figure 4-a**) shows the potential energy surface of the disaccharide unit, D-xylose-β-(1→2)-D-mannose. It comprises the global minimum at (Φ, Ψ)=(-110°, 170°) along with the two local minima at (-110°, 80°) and (40°, 140°) with the relative energy levels of 2.5 kcal/mol and 4.5 kcal/mol, respectively, from the global minimum. An interresidue hydrogen bond is formed between

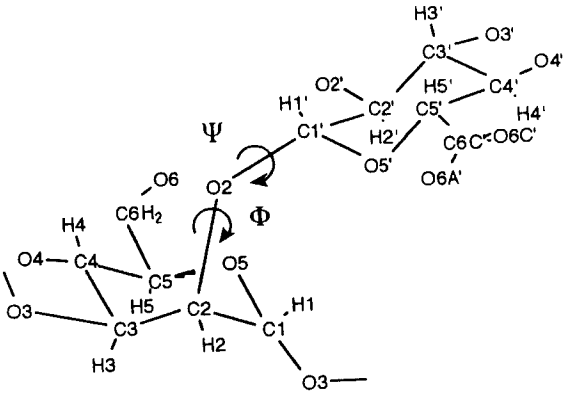

Figure 3. Schematic representation of the branching fragment of the D-glucurono-D-xylo-D-mannan. The side group residue is D-glucuronic acid. The atom labeling and the Φ and Ψ rotations are shown. In the case of the 1,2-linkage, Φ is defined by the atom sequence, O5'-C1'-O2-C2, and Ψ, C1'-O2-C2-C3. A similar representation is applied to a mannose chain unit with the D-xylose side group.

the O2' and O5 atoms over the glycosidic linkage. The energy map in **Figure 4-b** was calculated for the tetrasaccharide molecule, D-xylose-β-(1→2)-[α-(1→3)-D-mannotriose]. The conformation of the mannotriose unit was fixed at that corresponding to the fragment of the 3_2-fold helix and a D-xylose residue is connected to the C2 position of the middle mannose residue. Comparing with the potential energy surface of the preceding map, the area inside the external contour line is considerably restricted due to additional steric interactions between the xylose and the terminal mannose residues. No distinct local minimum is found in the potential well and the global minimum position has not been changed from that of the disaccharide map. The energy map of D-glucuronic acid-β-(1→2)-D-mannose is depicted in **Figure 5-a**. The resulting potential energy surface, having the global minimum at (-110°, 70°), is essentially similar to that of the D-xylose-β-(1→2)-D-mannose. In the case of the energy map for the D-glucuronic acid-β-(1→2)-D-[α-(1→3)-D-mannotriose] given in **Figure 5-b**, the potential energy surface comprises the two small potential wells, whose minimum positions are located at (-120°, 170°) and (-70°, 160°). The latter position corresponds to the global minimum being 3.9 kcal/mol lower in the steric energy than the former. Part of this stabilization at the global minimum is contributed from the formation of hydrogen bond between O6 and O6C' atoms.

On the basis of above discussion, helical models of the glucurono-xylo-mannan were established. The backbone structure of a stereochemical repeating unit was generated by the 3_2 screw symmetry operation of the asymmetric unit that consisted of a mannobiose backbone unit and a side group residue of either xylose or glucuronic acid. The fiber repeat

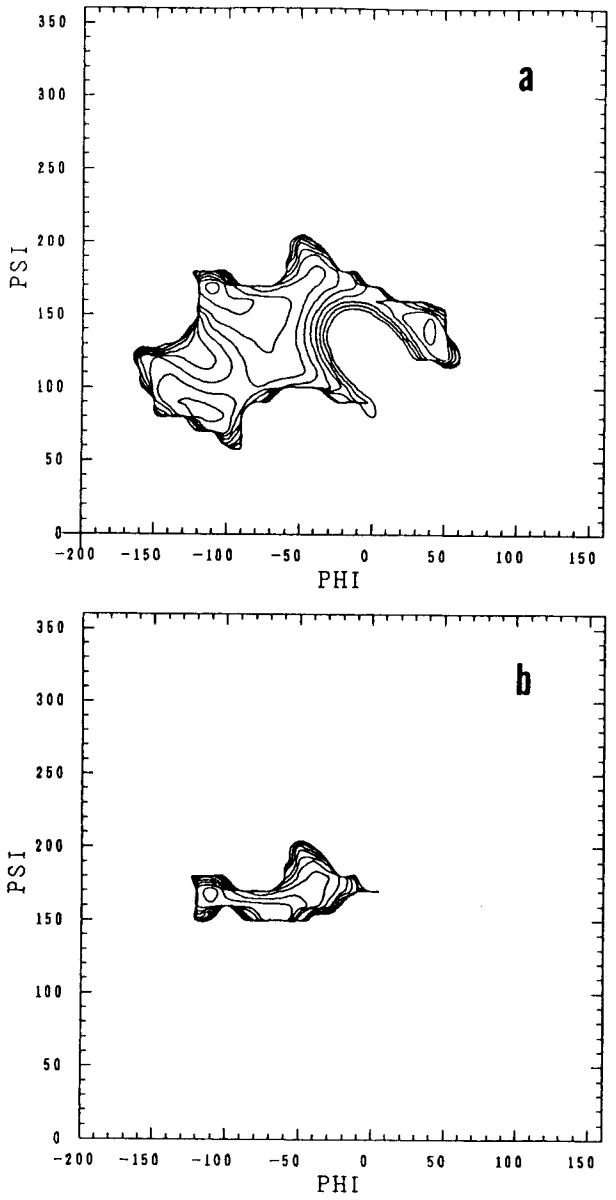

Figure 4. The potential energy surfaces of the di- and the tetrasaccharide molecules as model compounds of D-glucurono-D-xylo-D-mannan: (a) D-xylose-β-(1→2)-D-mannose, (b) D-xylose-β-(1→2)-[α-(1→3)-D-mannotriose]. The iso-energy contours are drawn by extrapolation of 1 kcal/mol with respect to the absolute minimum of each map. The external contour is 10 kcal/mol.

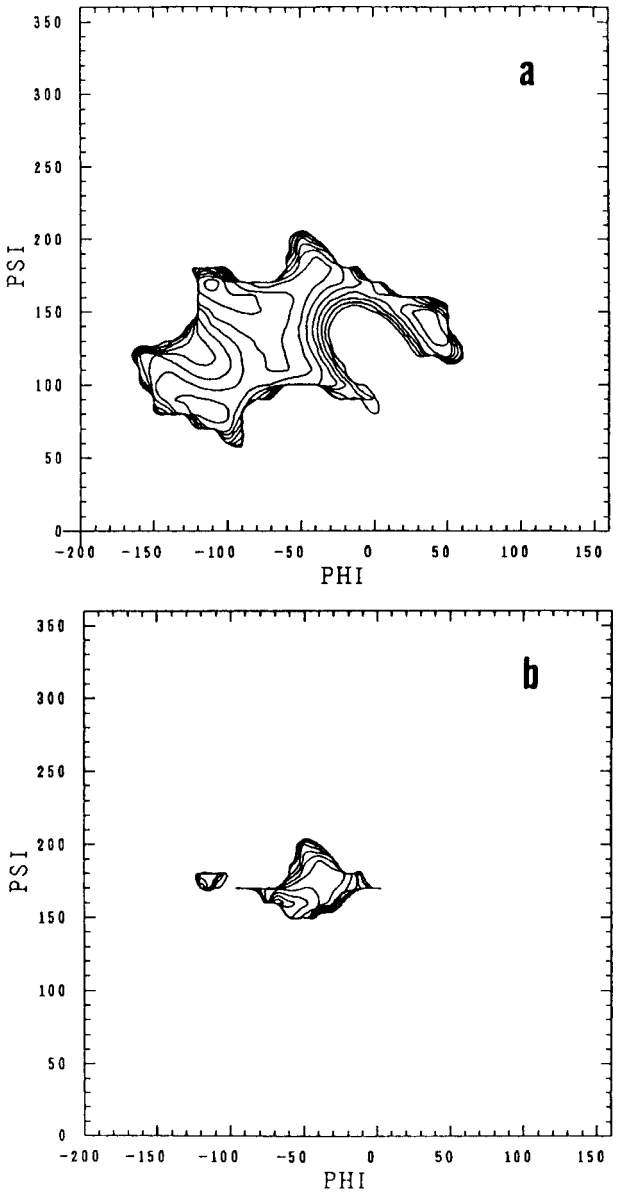

Figure 5. The potential energy surfaces of: (a) D-glucuronic acid-β-(1→2)-D-mannose, (b) D-glucuronic acid-β-(1→2)-[α-(1→3)-D-mannotriose]. The legend is the same as in Figure 4.

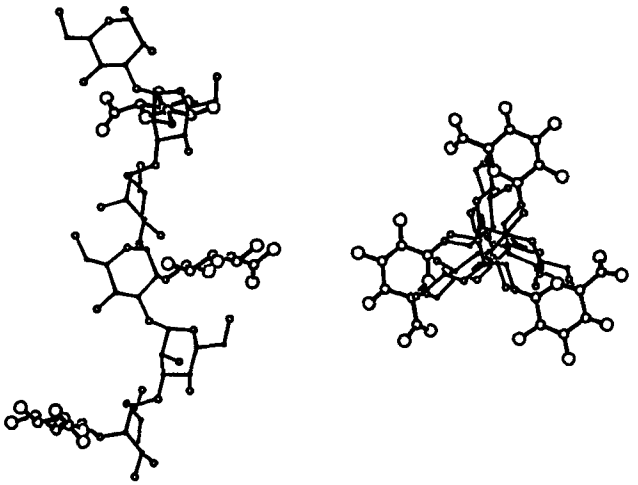

Figure 6. A proposed helix model of D-glucurono-D-xylo-D-mannan projected perpendicular (left) and parallel to the chain axis (right). The mannan backbone conformation is the left handed, three-fold helix and only the glucuronic acid residues are drawn as a side group. Small circles represent oxygen atoms of the backbone mannan, middle, carbon atoms, large, oxygen atoms of the side group residue. Hydrogen atoms are omitted for clarity.

distance was assumed to be 2.460 nm estimated from the *d*-spacings of the meridional reflections. The mannobiose unit adopted the dimer part of the 3_2-fold helical structure and, hence, the entire mannan backbone conformed to the near 6_4-fold helical symmetry within the fiber repeat. The initial orientation of each side group residue was taken from the global minimum found in the energy map of either **Figure 4-b** or **5-b**. During the conformational refinement, the Φ, Ψ rotations and the bond angles of both the backbone and the side group glycosidic linkages, and a rotation of O6 atom of the mannose were allowed to vary. The resulting, refined chain models are characterized by formations of some hydrogen bonds between the mannose and the side group residues. For the model with the xylose side group, the atoms O5 and O2' involve a hydrogen bond as has been predicted from the energy map, and an additional bond occurs between the atoms O6 and O5' after the conformational refinement. The O6---O5' and the O6---O6C' hydrogen bonds are formed in the model with the glucuronic acid. In either model, no hydrogen bond over the backbone linkage is detected. This is also the case for the crystal structure of (1→3)-α-D-mannan having the two-fold helical conformation.[4] The projections of the chain conformation proposed for the latter model are drawn in **Figure 6**.

The present conformational analysis of the glucurono-xylo-mannan has revealed its three dimensional structure which is characterized in the following two respects; a relatively flexible mannan backbone without any interresidue hydrogen bond involved and restricted motion of the side group orientations which is represented by the small conformational space along with the hydrogen bond formations over the side group linkage. Flexibility of the 1,3-α-linkage present in mannose sequence was suggested by the energy map study of the corresponding mannobiose molecule using the relaxed residue scheme,[7] which provided the result consistent with the previously observed ^{1}H NMR NOE data.[8] The backbone conformation suggested for the present glucurono-xylo-mannan indicates a Φ ranging from 99-110°, and a Ψ ranging from 164-173°.[9] These values are reasonably approximate to the global minimum found in the relaxed map of mannobiose (Φ=75-84°, Ψ=171-180°), whereas the conformation of (1→3)-α-D-mannan with the two-fold helical symmetry (Φ=72°, Ψ=116°)[4] corresponds to the second lowest minimum in the map (Φ=63-66°, Ψ=97-118°, 0.9 kcal/mol above the global minimum). As for the solution conformation of the glucurono-xylo-mannan, fragments of the two-fold helix conformation may arise in the mannan backbone, which accompanies a significant change in the molecular structure of the fragment, especially, in terms of orientation of the side group residue. A biological activity of the glucurono-xylo-mannan, such as affinity to some acceptor molecules, would considerably change between these two conformational states.

EXPERIMENTAL

The acidic heteropolysaccharide, D-glucurono-D-xylo-D-mannan was isolated from the water and alkaline extract of the fruit body of *Tremella fuciformis* Berk (Shirokikurage). The polysaccharide (2.6 g in DMSO 100 mL) was deposited onto a polyethylene terephthalate film and drying under vacuum at 70 °C. The resultant film was stretched 1.5 times in a 75% aqueous methanol. The crystallinity of the stretched film was improved by annealing in the aqueous methanol solution at 170 °C, followed by washing with methanol and drying in air. X-ray diffraction patterns were recorded at high relative humidity under a helium atmosphere in a flat-film camera with a Rigaku Geigerflex X-ray diffractometer using Ni-filtered CuKα radiation at 40 kV and 15 mA.

The energy maps were calculated by stepping Φ, Ψ values in a systematic fashion in 10° increments over the whole angular range. For each Φ, Ψ conformation, the total steric energy arising from a disaccharide or a tetrasaccharide fragment was computed by using the PFOS force field which consists of the three partitioned contributions; the van der Waals interaction,[10] the torsional potential for the Φ, Ψ rotations, and the hydrogen

bond energy.[11] The second contribution involves an additional contribution from the exo-anomeric effect[12] about the Φ rotation. The residue geometries used in the energy map calculation were generated by the structure optimizations using MM2CARB force field[13] and the rotation of the O6 atom was fixed at the *gt* position[14] in the course of the calculations. Helical structures of the polysaccharide chain were established by the PS79 program.[15] The latter program optimizes conformation of helical molecules with imposition of a given helical symmetry by evaluating the nonbonding and the hydrogen bond interactions. All calculations for the conformational analysis were carried out on FACOM-M1600 computer at Miyazaki University Computer Center.

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