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CRYSTAL STRUCTURE AND CONFORMATIONAL FEATURES OF α-PANOSE

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ABSTRACT

The crystal structure of α-panose [*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-α-D-glucose], ($C_{18}O_{16}H_{32}$), has been established by direct methods from 1491 independent reflections, and refined to a final *R*-value of 0.077. The crystal belongs to the monoclinic system, space group $P2_1$, and has a unit cell of dimensions a = 0.9662(3), b = 0.8505(3), c = 1.2955(5) nm, and β = 102.4(1)°. The three D-glucose residues have the $^4C_1$ pyranose conformation. The orientation of the α-(1→6)-glycosidic linkage is characterized by torsion angles of Φ = 71.4°, Ψ = 165.2°, and ω = 75.7°. The orientation of the α-(1→4)-glycosidic linkage is characterized by torsion angles Φ' = 92.9° and Ψ' = −131.3°, which results in an intramolecular hydrogen-bonding between secondary hydroxyl groups belonging to contiguous residues (O-3″···O-2′ = 299.6 pm). The intramolecular conformation is also stabilized by the occurrence of another intramolecular hydrogen-bond between the nonreducing and the reducing residues (O-2···O-6″ = 283.9 pm). This is made possible because of a unique, eclipsed orientation of the primary hydroxyl group at O-6″. As a result, the trisaccharide molecule adopts a folded conformation. Molecules are held together by a complicated network of hydrogen bonding involving all of the hydroxyl groups; this explains the high density of 1.63 g.cm^{-3} measured for the crystals. The structural features observed have been rationalized through the use of conformational analysis and packing computations.

INTRODUCTION

Panose is a trisaccharide composed only of D-glucopyranosyl residues with successive α-(1→6) and α-(1→4) linkages. The elucidation of the crystal structure presented here is the first report on a carbohydrate moiety having an α-(1→6)

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linkage between two D-glucose residues. Such a linkage is present in the two most important storage polysaccharides in Nature, namely, starch and glycogen. Also, the class of α-(1→6)-linked polysaccharides represents large and naturally occurring macromolecules, important in the biotechnologic field, such as dextran and pullulan. In a continuous dialog between polysaccharide crystallography and oligosaccharide crystallography, the elucidation of crystal structures of oligomers is required. The purpose is to accumulate highly reliable information about the observed crystallographic structural features in order to elucidate the relative contributions of hydrogen bonding and crystal packing, and, ultimately, to refine the potential-energy functions used in conformational analysis studies. Conspicuous by their absences among the reported structures of oligosaccharides are the class of α-(1→6)-linked D-glucose residues.

Panose was first synthesized by the action of a crude transglycosidase from *Aspergillus niger* on maltose[1]. Panose has also been isolated from acid hydrolyzates of amylopectin, glycogen, and pullulan[2–6], and from enzymic hydrolysis of pullulan[7]. It can also be synthesized chemically[8]. This trisaccharide is therefore a pertinent model with which to study the structure of pullulan, as well as the branch point in amylopectin and glycogen. Furthermore, it occurs in some glycoproteins having immunochemical interest[9].

EXPERIMENTAL

Crystallization. — Panose was purchased from Hayashibara Biochemical Laboratories, Inc., Japan. Crystals suitable for X-ray investigations were grown in the following way. Sufficient water was added to the dried solid to make a thick syrup. Four volumes of methanol were added in small portions under gentle stirring. Whenever a precipitate formed, a few drops of water were added. The solution was kept for a few days at ~45°, when crystallization took place. The

TABLE I

CRYSTAL DATA FOR PANOSE

Formula	$C_{18}H_{32}O_{16}$
Mol. wt.	504.44
$F(000)$	536
a(nm)	0.9662(3)
b(nm)	0.8505(3)
c(nm)	1.2955(5)
$\beta(^\circ)$	102.4(1)
Space group	$P2_1$
Z	2
$V(nm^3)$	1.040
d_c	1.612 g.cm^{-3}
d_{obs}	1.63 g.cm^{-3}

crystals were collected, washed with methanol, and stored in a vacuum desiccator over[10] P_2O_5.

X-Ray investigation. — Preliminary cell dimensions and space group were determined from oscillation on Weissenberg photographs recorded with CuKα radiation. Final cell parameters were refined from diffractometer measurements of 15 well-centered, high-angle reflections. These and other pertinent crystal data are given in Table I. Density measurement in carbon tetrachloride–iodoethane indicated the lack of water molecules. Intensity data were collected in the $\Theta - 2\Theta$ scan mode, by using Ni-filtered CuKα radiation, up to a 2Θ limit of 152° ($-8 \leq h \leq 6$, $0 \leq k \leq 9$, $0 \leq 1 \leq 12$).

The intensities of three reference reflections measured every 2 h decreased by ~3% of their initial values over the duration of the data collection. The data were corrected for Lorentz and polarization effects. No absorption correction was employed. Of the 1491 independent reflections measured, 1333 with $Fo/\sigma Fo > 2.5$ were used for structural analysis. The X-ray scattering factors were obtained from the article by Cromer and Waber[11] for C and O atoms, and from that by Stewart *et al.*[12] for H atoms.

The structure was solved by direct methods, using a combination of the Devin and the Multan-80 (ref. 13) programs. The value of $|E| \leq 1.27$ (290 reflections) was used. The *E*-map computed with the best set of phases revealed all the non-hydrogen atoms; the initial residual calculated for these atomic positions was 0.30. Following several cycles of full matrix, anisotropic, least-squares refinement for the non-hydrogen atoms, with the XFLS program[14], the hydrogen atoms were approximately located on successive difference Fourier syntheses. They were then set at a theoretical bond length of 95 pm, and they were given isotropic thermal parameters 0.01 nm^3 greater than the equivalent B_{iso} of the atom to which they were bonded, in order to account for their internal vibrations.

The final *R*-values for all observed and measured reflections were 0.093 and 0.077, respectively. The quantity minimized was $w(Fo - Fc)^2$, each reflection being assigned a weight $w = 1/\sigma^2(F)$ derived from $\sigma(I)$. A final, electron-density map showed no significant residual density, the extreme fluctuations being −0.5, 0.5 eA^{-3}. The final atomic coordinates are listed in Table II. The hydrogen-atom coordinates, a tabulation of observed and calculated structure factors, and the final, anisotropic, thermal parameters are available as supplementary materials*.

Conformational analysis. — The numbering of the atoms, shown in Fig. 1, proceeds (in contrast to that normally employed) from the nonreducing end (unprimed atoms) to the reducing end (double-primed atoms). The potential energy was calculated by including the partitioned contributions arising from the van der Waals, torsional, exo-anomeric, and hydrogen-bond contributions. The van der

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TABLE II

ATOMIC POSITIONAL PARAMETERS (AND THEIR ESD VALUES IN PARENTHESES)

Atom	*x/a* (10^{-4})	*y/b* (10^{-4})	*z/c* (10^{-4})	B_{eq} (10^{-2})
O-1	2893(1)	9569(2)	6318(1)	106
O-2	3068(1)	12444(4)	5417(1)	144
O-3	1303(1)	14263(1)	6435(1)	226
O-4	−359(1)	12500(4)	7690(1)	140
O-5	432(1)	9535(2)	5831(1)	164
O-6	−2354(2)	9699(1)	6132(1)	232
C-1	1733(1)	10110(1)	5587(1)	178
C-2	1694(1)	11893(1)	5553(2)	195
C-3	1417(2)	12595(2)	6600(3)	223
C-4	−36(1)	11912(2)	6697(1)	224
C-5	139(1)	10152(2)	6811(1)	182
C-6	−1196(3)	9283(3)	6953(1)	197
O-1′	6745(1)	8605(1)	8601(1)	119
O-2′	8066(1)	5802(2)	8980(1)	271
O-3′	7208(2)	4177(1)	6956(3)	201
O-4′	5031(1)	5670(3)	5442(2)	233
O-5′	4551(1)	7488(1)	7921(1)	48
C-1′	5833(2)	7280(2)	8606(1)	131
C-2′	6650(1)	5808(6)	8359(2)	161
C-3′	6623(2)	5673(2)	7162(1)	63
C-4′	5100(2)	5861(4)	6535(1)	160
C-5′	4594(2)	7524(3)	6800(1)	141
C-6′	3132(1)	7869(2)	6181(2)	135
O-1″	8869(2)	13029(0)	10099(1)	371
O-2″	9284(1)	11020(1)	11832(1)	197
O-3″	8080(2)	8157(1)	10702(1)	237
O-5″	6455(1)	12509(1)	9708(1)	140
O-6″	5485(2)	11676(1)	7008(1)	240
C-1″	7703(2)	12573(7)	10545(7)	264
C-2″	7981(1)	10993(2)	11082(2)	172
C-3″	8022(2)	9675(2)	10241(1)	180
C-4″	6665(1)	9758(3)	9387(1)	146
C-5″	6492(1)	11346(1)	8864(2)	174
C-6″	5118(2)	11564(2)	8063(1)	212

Waals interactions were evaluated by using 6–12 potential functions with the parameters proposed by Scott and Scheraga[15]. A three-fold, sinusoidal potential was used for rotation about the glycosidic torsion angle Ψ, with a barrier of 4.2 kJ/mol (1.0 kcal/mol) for the eclipsed orientation. For rotation about the glycosidic linkage C-1–O-1, *i.e.*, angle Φ, the intramolecular mechanism responsible for the exo-anomeric effect was taken into account by using the potential function proposed by Tvaroška[16]; a sinusoidal, three-fold, eclipsed, rotational barrier of 1.0 kcal/mol was also included. The hydrogen-bond energy was computed by an empirical expression proposed by Pérez and Vergelati[17]. No electrostatic interaction was taken into account.

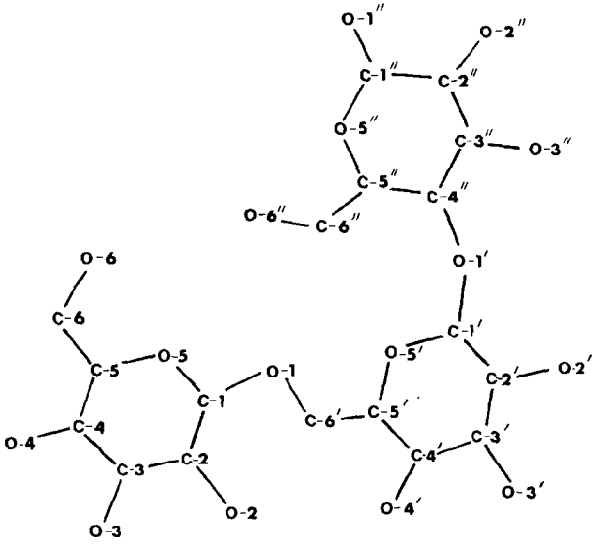

Fig. 1. Schematic representation of the α-panose molecule, along with the atomic numbering.

The crystal coordinates were used, except for the positions of the hydrogen atoms bonded to C atoms; the C–H distance was extended to 109 pm. The hydroxyl hydrogen atoms were omitted. The energy maps were computed as a function of Φ and Ψ at intervals of 5°. With respect to the relative energy minimum, iso-energy contours were drawn by interpolation of 1 kcal/mol. The 10 kcal/mol contour was selected as the outer limit. The sign of the torsional angles is given according to the rules of the IUPAC–IUB Commission on Biochemical Nomenclature[18].

Helical arrangement is customarily described in terms of helical parameters (*n.h.*), *n* being the number of residues per turn of the helix, and *h*, the translation along the helix axis. These parameters can be calculated for any given values of the glycosidic torsion-angles by following an algorithm reported previously[19]. All of the calculations were performed on a Honeywell–Bull computer at the C.I.C.G. (Centre Interuniversitaire de Calcul de Grenoble).

Packing analysis. — In order to arrive at a full description of the packing arrangement, the intermolecular energy between a given molecule, *i.e.*, the reference molecule, and all its neighbors was evaluated by taking into account the intermolecular hydrogen-bonds, as well as the non-bonded interactions. These calculations were performed by using the same parameters as those utilized in the conformational analysis of a single molecule. Moreover, the contributions to the energy were broken down into the pure non-bonded part and the intermolecular hydrogen-bonding stabilization. In addition, the number of "short" contacts corresponding to interatomic distances less than 1.5 times the sum of the van der Waals radii of the interacting atoms were computed.

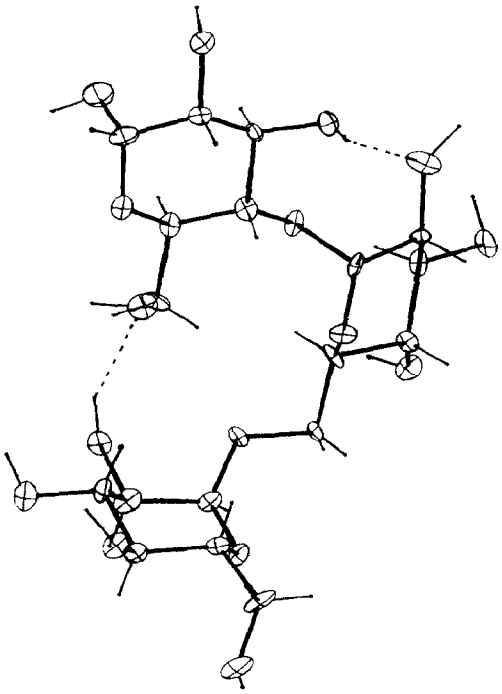

Fig. 2. Molecular conformation of α-panose as found in the crystal structure. Intramolecular hydrogen-bonds are shown as broken lines.

RESULTS AND DISCUSSION

A representation (ORTEP[20]) of the asymmetric unit content of an α-panose crystal is shown in Fig. 2. The bond distances and angles in the molecule are given in Table III and IV, respectively. The present results establish that the glycosidic linkages are α-(1→6) and α-(1→4); the configuration of the reducing glucopyranose residue is α-D. There is no evidence of any α:β configurational disorder at the O-1″ atom. The structure also lacks water molecules.

Molecular features. — The mean C–C distances of 153.1 pm and the mean C–O bond-length of 144.2 pm conform to the tabulated values for carbohydrates[21]. A significant lengthening of the intracyclic C-5–O-5 bonds in all of the residues is observed. As to the anomeric bonds (C-1–O-1 type) involved in the glycosidic junction, their respective values are 138.2(2), 143.1(2) and 142.6(5) pm. Except for the one involved in the (1→6) linkage, the two others fall into the normal range (from 138.5 to 142.5 pm) observed for an axial type of linkage.

The internal, C–C–C ring-angles are close to tetrahedral (mean value, 109.1°), whereas the exocyclic ones exhibit a significant opening, with respective values of 114.2(1), 111.4(2), and 114.6(1)° for the unprimed, primed, and double-primed residues. The endocyclic C–C–O bond-angles average 108.9°, and range

TABLE III

BOND DISTANCES (nm) AND THEIR ESD VALUES

Bond	*Unprimed residue*	*Primed residue*	*Double-primed residue*
C-1–C-2	0.1517(1)	0.1550(5)	0.1511(7)
C-2–C-3	0.1557(3)	0.1550(3)	0.1570(3)
C-3–C-4	0.1549(2)	0.1529(3)	0.1525(2)
C-4–C-5	0.1510(2)	0.1559(4)	0.1504(3)
C-5–C-6	0.1532(3)	0.1496(2)	0.1511(2)
C-1–O-1	0.1382(2)	0.1431(2)	0.1426(5)
C-1–O-5	0.1436(1)	0.1372(2)	0.1439(7)
C-2–O-2	0.1454(2)	0.1431(2)	0.1416(2)
C-3–O-3	0.1435(2)	0.1440(2)	0.1419(2)
C-4–O-4	0.1475(2)	0.1413(3)	0.1428(2)
C-5–O-5	0.1478(2)	0.1462(2)	0.1481(2)
C-6–O-6	0.1413(3)	0.1481(2)	0.1487(2)

TABLE IV

BOND ANGLES (°) AND THEIR ESD VALUES

Bond angles	*Unprimed residue*	*Primed residue*	*Double-primed residue*
O-1–C-1–C-2	111.4(1)	107.1(2)	110.5(4)
O-1–C-1–O-5	111.3(1)	111.5(1)	108.2(4)
C-2–C-1–O-5	109.2(1)	113.6(2)	110.5(4)
C-1–C-2–C-3	111.5(1)	111.5(2)	110.1(3)
C-1–C-2–O-2	107.9(1)	110.8(2)	109.7(1)
C-3–C-2–O-2	108.3(2)	111.8(2)	109.7(1)
C-2–C-3–C-4	104.8(2)	109.3(2)	108.9(1)
C-2–C-3–O-3	105.7(2)	109.1(2)	111.2(1)
C-4–C-3–O-3	109.9(1)	111.4(2)	107.3(1)
C-3–C-4–C-5	107.2(1)	106.8(2)	111.3(1)
C-3–C-4–O-4	108.0(2)	110.7(2)	108.4(1)
C-5–C-4–O-4	106.9(1)	112.1(2)	108.3(1)
C-4–C-5–C-6	114.2(1)	111.4(2)	114.6(1)
C-4–C-5–O-5	107.7(1)	106.1(2)	106.6(1)
C-6–C-5–O-5	105.0(1)	108.1(2)	105.6(1)
C-5–C-6–O-6	109.9(2)	106.3(1)	106.9(1)
C-1–O-5–C-5	113.4(1)	115.5(1)	115.6(3)
Glycosidic angles			
C-1–O-1–C-6′	111.6(1)		
C-1′–O-1′–C-4″	114.2(1)		

TABLE V

TORSION ANGLES (°) AND THEIR ESD VALUES

Torsion angles about the glycosidic bonds		
O-5–C-1–O-1–C-6′	71.4(2)	
H-1–C-1–O-1–C-6′	−45.5(2)	
C-1–O-1–C-6′–C-5′	165.2(2)	
C-1–O-1–C-6′–H-61′	−76.7(2)	α-(1→6) linkage
C-1–O-1–C-6′–H-62′	46.4(2)	
O-1–C-6′–C-5′–O-5′	75.7(3)	
O-1–C-6′–C-5′–H-5′	−49.5(3)	
O-5′–C-1′–O-1′–C-4″	92.9(2)	
H-1–C-1′–O-1′–C-4″	−23.9(2)	
		α-(1→4) linkage
C-1′–O-1′–C-4″–C-5″	−131.3(1)	
C-1′–O-1′–C-4″–H-4″	−12.8(1)	

Endocyclic torsion angles

	Unprimed residue	*Primed residue*	*Double-primed residue*
O-5–C-1–C-2–C-3	56.2(2)	45.0(1)	53.0(1)
C-1–C-2–C-3–C-4	−59.8(1)	−49.3(3)	−53.6(5)
C-2–C-3–C-4–C-5	63.9(1)	60.5(2)	58.3(4)
C-3–C-4–C-5–O-5	−65.7(2)	−65.5(3)	−59.3(5)
C-4–C-5–O-5–C-1	63.2(3)	64.3(3)	60.7(2)
C-5–O-5–C-1–C-2	−57.3(1)	−54.1(2)	−58.9(2)
Exocyclic torsion angles			
C-5–O-5–C-1–O-1	66.1(1)	67.0(3)	62.2(4)
O-1–C-1–C-2–O-2	51.7(1)	46.8(1)	54.4(2)
O-2–C-2–C-3–O-3	65.5(2)	63.8(3)	66.8(4)
O-3–C-3–C-4–O-4	−68.0(1)	−56.5(2)	−62.2(5)
O-4–C-4–C-5–C-6	62.6(2)	55.6(2)	65.2(5)
O-5–C-5–C-6–O-6	−64.7(2)	75.7(3)	131.1(3)
C-4–C-5–C-6–O-6	53.1(2)	−168.1(3)	−111.9(2)

from 106.1 to 113.6°. The endocyclic C–O–C ring-angles vary little, and average 114.8°. The exocyclic C–O–C angles average 109.3, and range from 105.7 to 112.2°.

The torsional angles about the various skeletal bonds of the pyranose rings are given in Table V. The expected $^4C_1$(D) conformation is found for each of the three residues. For the middle and the reducing residues, the absolute values of the ring torsion angles average 56.4 and 57.3°, respectively, whereas an average absolute value of 61.0° is found for the nonreducing ring, indicating a severe alteration of the D-glucosyl ring conformation. Alterations occurring within α-D-glucose residues have already been noted[22,23].

The orientation of the primary hydroxyl group at C-6 is usually defined as *gauche-gauche*, *gauche-trans*, or *trans-gauche*[24]. In this terminology, the torsion

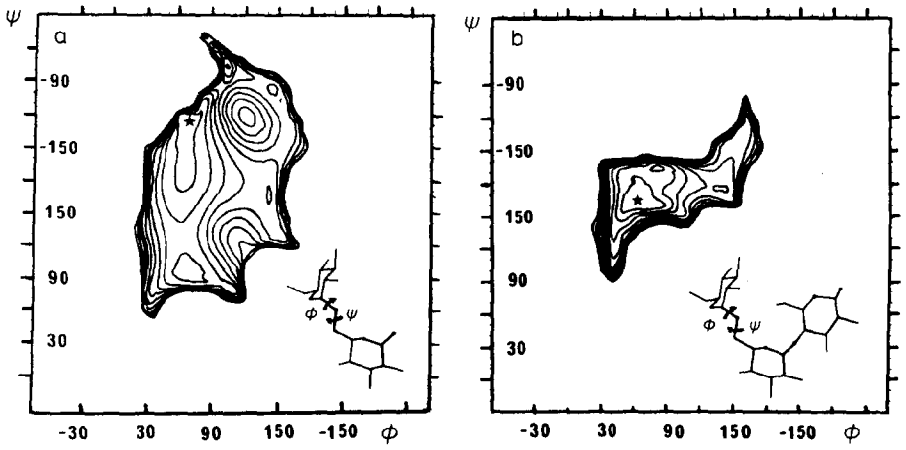

Fig. 3. Iso-energy map (Φ,Ψ) about the α-(1→6) linkage (torsion angle ω is left in the conformation found in the crystal). Relative iso-energy contours are drawn at intervals of 1 kcal/mol. The symbol * indicates the occurrence of the calculated minimum. (a) (Φ,Ψ) map for glucose-α-(1→6)-glucose molecule; (b) (Φ,Ψ) map for glucose-α-(1→6)-maltose molecule.

angle (O-5–C-5–C-6–O-6) is stated first, and the torsion angle (C-4–C-5–C-6–O-6), second. For the *gluco* configuration, the orientations observed are usually almost equally distributed between the *gauche-gauche* and the *gauche-trans*[24]. This situation is again found for the primary hydroxyl group of the nonreducing residue (*gauche-gauche*) and for the one involved in the (1→6) linkage (*gauche-trans*). As regards the primary hydroxyl group of the reducing residue, a very unusual situation, corresponding to an eclipsed orientation (O-5″–C-5″–C-6″–O-6″ = 131.1°, C-4″–C-5″–C-6″–O-6″ = −111.9°), is found. The existence of such an energetically unstable conformation is, in fact, accompanied by the occurrence of an intramolecular hydrogen-bond involving the O-2 and O-6″ atoms. To the best of our knowledge, such a conformational feature has never been observed in any other oligosaccharide structure.

Conformational analysis. — The relative orientation of contiguous pyranosides is customarily described by a set of torsion angles around the glycosidic linkage. The present work provides the first experimental evidence for the solid-state orientation between two α-(1→6)-linked D-glucopyranose residues. For this particular linkage, three torsion angles, Φ, Ψ, and ω, have to be considered, where $\Phi = \Theta$(O-5–C-1–O-1–C-6′), $\Psi = \Theta$(C-1–O-1–C-6′–C-5′), and $\omega = \Theta$(O-1–C-6′–C-5′–O-5′). The values of these torsional angles are $\Phi = 71.4(2)°$, $\Psi = 165.2(2)°$, and $\omega = 75.7(3)°$. Another important parameter is the valence angle τ (C-1–O-1–C-6′), which is 111.6(1)°. Such a value is in agreement with those found for α-(1→6) linkages, as in α-melibiose (111.5, 111.8°)[25–27], raffinose (111.4°)[28], stachyose (110.9, 113.5°)[29], planteose (111.2°)[30], and isomaltulose (115.5°)[31].

In order to rationalize the solid-state conformation observed, a set of iso-

energy maps was computed and plotted. They correspond to intramolecular-energy variations as a function of rotations about Φ and Ψ torsion angles, the orientation about ω being assigned its crystallographic value. Calculations were performed for an isomaltose moiety [glucose-α-(1→6)-glucose] (see Fig. 3a), and for the trisaccharide [glucose-α-(1→6)-maltose] (see Fig. 3b). As regards the isomaltose oligomer, the overall shape of the iso-contour map is very reminiscent of that reported by Tvaroška *et al.*[32]. The allowed values for Φ (centered about 80°) are much more restricted than the allowed values for Ψ (centered about 180°), which span some 200°. The calculated minimum occurs for $\Phi = 70°$, $\Psi = -120°$, whereas the observed crystallographic conformation ($\Phi = 71.4°$ and $\Psi = 165.2°$) is only 1.3 kcal/mol higher in energy. Both conformations belong to the same low energy domain. When a similar study is performed on the glucose-α-(1→6)-maltose entity, quite a different situation is found. It is clear from Fig. 3b that the allowed conformational space has undergone a drastic shrinkage especially for rotation about the angle Ψ. A single energy-well is now being dealt with, the absolute minimum occurs at $\Phi = 70°$ and $\Psi = 165°$. This finding illustrates the amount of restriction that the orientation about an α-(1→6) linkage can undergo when a bulky substituent is attached at the reducing end. Such a change is mainly due to steric conflicts between the nonreducing and the reducing residues of the panose molecule, with, in addition, a deepening of the energy well due to the occurrence of an intramolecular hydrogen-bond between the O-2 and O-6″ atoms. Interestingly, the observed arrangement of the two D-glucose residues about the α-(1→6) linkage would generate helical parameters $n = 2.3$ and $h = -355$ pm if it were extrapolated to the solid structure of the homopolymer, *i.e.*, the linear dextran molecule. Recent work on single crystals of dextran[33,34] grown *in vitro*, proposed $n = 2$ and $h = 390$ pm.

The relative orientation about the α-(1→4) linkage is governed by a set of two torsion angles, $\Phi' = \Theta$(O-5′–C-1′–O-1′–C-4″) and $\Psi' = \Theta$(C-1′–O-1′–C-4″–C-5″). In the present work, the values of the torsion angles are $\Phi' = 92.9(2)°$ and $\Psi' = -131.3(1)°$. The valence angle C-1′–O-1′–C-4″ has a magnitude of 114.2(1)°. The distance between the H-1′ and H-4″ atoms about the glycosidic bridge is 204 pm. Such a conformation results in a non-bonded distance of 299 pm between O-3″ and O-2′, indicating an intramolecular hydrogen-bond in which O-3″ is the donor. Recent work on the conformational analysis of amylose and amylosic fragments established that the stable orientations about the glycosidic linkage could be classified into four families, designated[17] C1, C2, C3, and C4. C1 has been shown to correspond to conformations of V-amylose and cyclodextrins, and C3 is closely related to the conformation found in A-amylose allomorph[35]. C2 has not yet been observed for an amylose polymorph, although it is preponderant in maltoheptaose bound to phosphorylase[36]. Only C4 has not been found in crystal structures. As in the case of the α-(1→6) linkage, the observed solid-state conformation of panose was rationalized through computation of iso-energy contour maps. Calculations were performed for a maltose entity [glucose-α-(1→4)-glucose] (see Fig. 4a) and

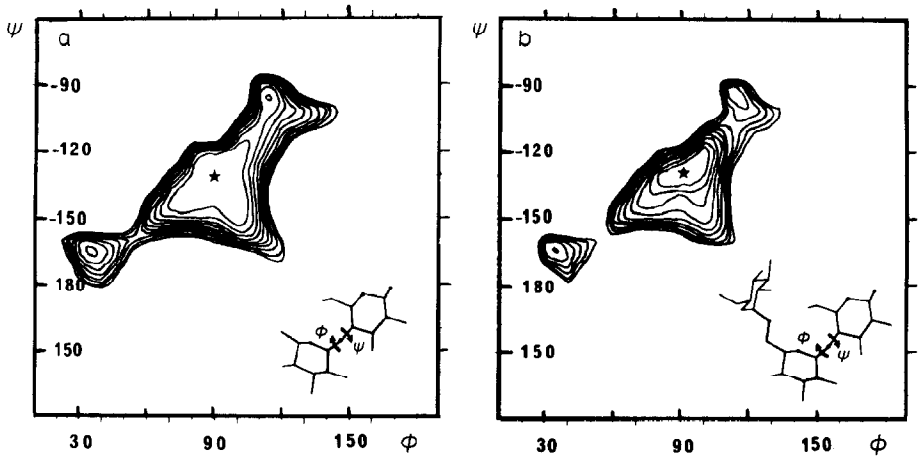

Fig. 4. Iso-energy map (Φ,Ψ) about the α-(1→4) linkage. Relative iso-energy contours are drawn at intervals of 1 kcal/mol. The symbol * indicates the occurrence of the calculated minimum. (a) (Φ,Ψ) map for glucose-α-(1→4)-glucose molecule; (b) (Φ,Ψ) map for isomaltose-α-(1→4)-glucose molecule.

for the trisaccharide [isomaltose-α-(1→4)-glucose] (see Fig. 4b). For the maltose case, the overall shape of the low-energy domain is similar to those reported previously[17]. The absolute minimum calculated is found for $\Phi = 90°$ and $\Psi = -130°$, which is indeed very close to the observed minimum ($\Phi = 92.9°$, $\Psi = -131.3°$). In contrast to what was found for the α-(1→6) case, there is no detectable restriction which occurs when an extra substituent is added at the reducing end. As already noted, the occurrence of this stable conformation is accompanied by a weak, intermolecular hydrogen-bond between secondary hydroxyl groups belonging to contiguous α-(1→4) residues. The orientation observed for the α-(1→4) bridge in panose belongs to the C2 family already described. Extrapolated to an amylose

TABLE VI

HYDROGEN BONDING

i *j* *k*		*Distance i–k (nm)*	*Distance j–k (nm)*	*Angle i–j–k (°)*
O-2–HO-2···O-6″	I[a]	0.2839	0.2003	145.9
O-3–HO-3···O-2″	II + a + 2c	0.2853	0.2419	107.6
O-4–HO-4···O-3″	II + a + 2c	0.2744	0.1959	139.0
O-6–HO-6···O-2	II − b + c	0.2754	0.2037	131.2
O-2′–HO-2′···O-1″	I − b	0.2790	0.2062	132.1
O-3′–HO-3′···O-4	I + a − b	0.2739	0.2257	110.5
O-4′–HO-4′···O-2	II + a − b + c	0.2787	0.1902	153.5
O-2″–HO-2″···O-2′	II + 2a + 2c	0.2975	0.2054	163.4
O-3″–HO-3″···O-2′	I	0.2996	0.2084	159.0
O-6″–HO-6″···O-3′	I + b	0.2711	0.2032	111.2

[a]I: x, y, z; II: −x, 1/2 + y, −z.

chain, it would generate a left-handed helical structure characterized by a set of parameters $n = 9$ and $h = -326$ pm.

Hydrogen bonding, and packing features. — The crystal structure of panose is stabilized by an extensive system of intermolecular hydrogen-bonds, described in Table VI. The network of these hydrogen bonds is visibilized in a projection (a,c) of the packing (see Fig. 5). None of the glycosidic-bridge oxygen atoms and none of the ring-oxygen atoms are hydrogen bonded. The lack of hydrogen bonding of the bridge-oxygen atoms appears to be common to all di- and higher oligo-saccharides thus far characterized. All of the hydroxyl oxygen atoms participate at least

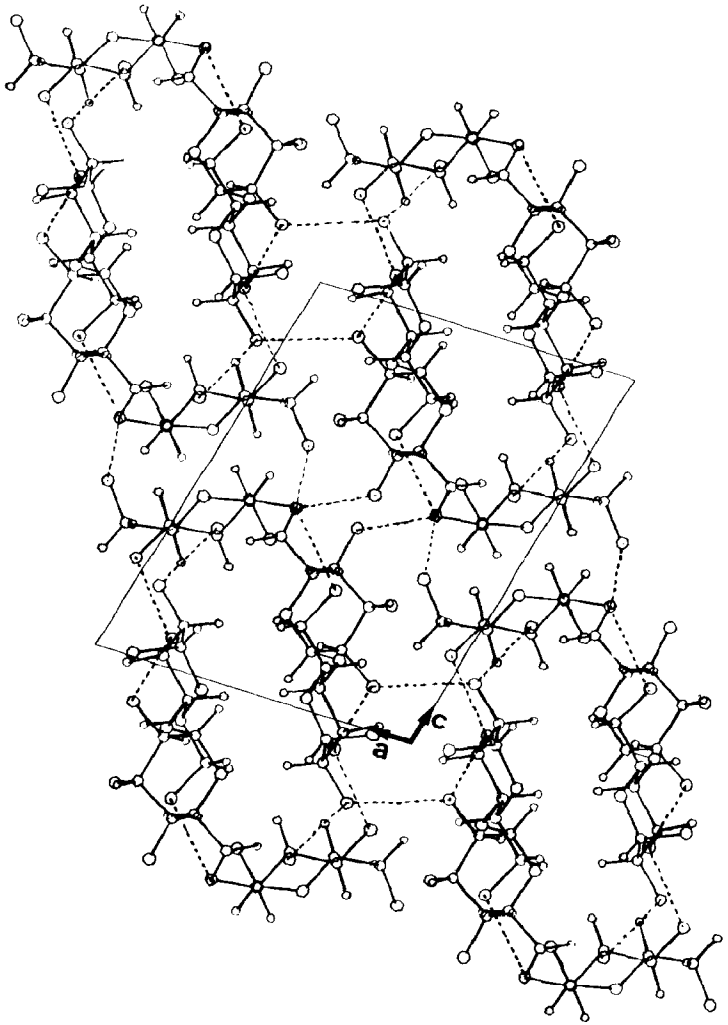

Fig. 5. Projected view (PITMOS[41]) of the packing of α-panose in the crystallographic (a,c) plane. Hydrogen bonds are shown as broken lines.

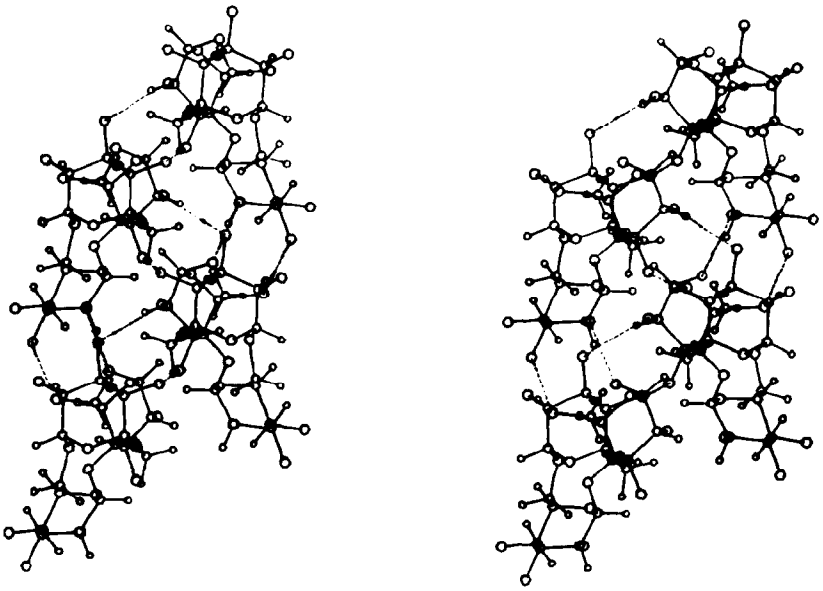

Fig. 6. Stereoscopic representation (PITMOS[41]) of the three-dimensional arrangement of α-panose molecules around the two-fold screw-axis of symmetry in the crystal. Hydrogen bonds are shown as broken lines.

once in hydrogen bonds. Most of them act as both donor and acceptor. Only the O-2 and O-2′ atoms participate in three hydrogen bonds, in one as a donor, and in two as an acceptor. Such behavior had already been observed in several cases, such as α-lactose[37], methyl α-D-galactoside[38], an aldotriouronic acid[39], and methyl α-maltotrioside[23].

The crystal structure is highly dense, each panose molecule being surrounded

TABLE VII

PACKING ENERGY

Molecule	*Non-bonded energy (kcal/mol)*	*Intermolecular hydrogen bond (kcal/mol)*	*Total energy (kcal/mol)*	*Number of short contacts*
Strong interactions[a]				
II + a + 2c/II + a − b + 2c	−7.31	−3.12	−10.43	146
I − b/I + b	−4.74	−3.22	−7.96	82
Weak interactions				
I + a/I − a	−4.14	0	−4.14	77
I + a − b/I − a + b	−0.69	−1.64	−2.33	13
II + a + c/II + a − b + c	−2.44	−1.67	−4.11	47
II + c/II − b + c	−3.39	−1.66	−5.65	71
II + 2a + 2c/II + 2a − b + 2c	−1.37	−0.35	−1.71	24

[a]I: x, y, z; II: −x, 1/2 + y, −z.

by 14 neighbors. Among the 14 neighbors, which occur in pairs, a very energetically stable "molecular chain", is centered about the two-fold screw-axis of symmetry of the $P2_1$ space group (for a stereoscopic representation see Fig. 6). The energy values of the interactions of a panose molecule with all its neighbors are given in Table VII. The strongest interactions occur between molecule I and its two-fold screw-axis-related neighbors (II + a − b + 2c and II + a + 2c). The stabilizing energy involved in this association emanates more from non-bonded energy (2/3) than from intermolecular-hydrogen-bond energy (1/3). An indicator of the intermolecular interactions is given by the large number of van der Waals contacts (146) occurring for each pairing. A complementary, favorable organization is achieved through interactions between molecule I and its translationally related homologs along the *b* axis (I − b, I + b). Again, stabilizing energy arises from both van der Waals and hydrogen-bond interactions. As a net result, 50% of the lattice energy is tied up in the formation of this "molecular chain". Therefore, the three-dimensional arrangement of panose can be best described in terms of chain–chain interactions, in which those related by translation along the *a* axis result in the formation of layers of "molecular chains". Intermolecular interactions resulting from translation along the crystallographic *c* axis are very weak.

CONCLUSIONS

The present work has established the detailed, three-dimensional features of crystalline α-panose, and has provided the first experimental characterization of the α-(1→6) linkage between two contiguous D-glucopyranosyl residues. It has been found that the observed orientations at the glycosidic bridges correspond to low-energy arrangements which can be rationalized through the use of "empirical" potential-energy calculations. However, a very unusual orientation of one primary hydroxyl group has been found; this shows that energetically disfavored conformations can be trapped in the solid state. This is not unlikely to happen provided that other stabilizing interactions, such as hydrogen bonding and van der Waals contacts, yield a more-favorable energy balance. The overall combination of the torsional angles about the α-(1→6) linkages results in a folded trisaccharide structure and a concomitant, highly packed structure. It may be reasoned that, in the case of starch, where the concentration of such branching points is only a few percent, similar types of arrangement are essential for obtaining a dense packing of macromolecular chains. This is especially true in the case of the proposed "cluster" structure[40].

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