Crystal Structure of Nystose Trihydrate

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A tetrasaccharide, *O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranosyl-(1→2)-*O*-β-D-fructofuranosyl-(1→2)-*O*-β-D-fructofuranoside (nystose) trihydrate ($C_{24}H_{42}O_{21}\cdot 3H_2O$), was crystallized from its aqueous solution by using seed crystals. The crystal structure has been determined by an X-ray diffraction method. The crystal data are as follows: orthorhombic, $P2_12_12_1$, $Z=4$, $a=13.582(3)$, $b=23.385(6)$, and $c=10.198(2)$ Å. The α-D-glucopyranose ring has the normal type $^4C_1$ chair conformation, and three fructofuranose rings have the $^4T_3$, E_4, and $^4T_3$ (or E_3) conformations. As in the 1-kestose crystal, all of the hydrogen bonds found made connections between adjacent molecules. Although the linkage conformation ($\angle C_1O_1C_{1-2}C_{1-1}$, $\angle C_2C_1O_1C_{1-2}$) of the sucrose component in nystose (100.3°, −137.1°) was different from that in 1-kestose (53.3°, −152.9°), the linkage conformation (ϕ,ψ,ω) of the inulobiose component at the end of nystose (71.3°, −165.9°, 68.0°) was very similar to that of 1-kestose (80.5°, −169.6°, 56.5°).

Inulin, poly(2→1)-β-D-linked fructose with a sucrose component at the chain end, is a plant polysaccharide which serves as a major reserve carbohydrate in the plant kingdom. It is synthesized by the successive addition of D-fructofuranose residues to sucrose, *O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranose. The tri- and tetrasaccharide, which are products after one or two additions of D-fructofuranose, are known as 1-kestose and nystose, respectively. 1-Kestose was synthesized through the enzymatic action of invertase on sucrose; its crystal structure was analyzed in detail.[1] On the other hand, the titled compound, β-D-fructofuranosyl-1-kestose trihydrate (nystose trihydrate) crystals, were identified from the products obtained from sucrose by *Dermatium pullulants*;[2] recently, its crystal structure was briefly reported.[3] In this study, the molecular and crystal structures of nystose trihydrate were analyzed in detail and compared with that of 1-kestose for a better understanding of the flexible linkage conformation in fructofuranans and the fructofuranosyl ring conformation. This stereochemical information concerning oligosaccharide single crystals will be very useful for structural investigations of the polyfructans.

Experimental and Structure Determination

Sample Preparation. *A. pullulans* AHU 9549 cells were cultured in the presence of 10% sucrose at 30°C for 72 h in order to accumulate β-fructofuranosidase in *fungi*. After filtration, the *fungi* were added to a sucrose solution with a concentration of 55°Brix, and then incubated at 50°C for 16 h. Nystose was produced as a syrup through the enzymatic action of β-fructofuranosidase on sucrose by transfructosylation. This syrup was separated chromatographically by the CG-6000 ion-exchange resin, which gave a nystose concentration of about 45%. After a further concentration of this syrup, seed crystals with a size of about 5—10 μm were added. The mixture solution was slowly stirred and the concentration adjusted as the crystal grew. Nystose crystals with a purity of about 89% were separated from the syrup when the crystal size reached to about 0.5 mm. By performing recrystallization with a similar procedure, 99.8% pure crystals were obtained. One of these crystals was used for the X-ray diffraction study.

Thermal Analysis. Crystalline powder of a nystose trihydrate specimen (5.84 mg) was examined regarding thermal gravimetry as well as differential thermal calorimetry (THERMOFLEX TG 8110, Rigaku Co.) at a scanning rate of 10°C min^{-1} from room temperature to 250°C.

X-Ray Diffraction. A determination of the unit-cell dimensions and collection of the X-ray intensity data were carried out using a four-circle diffractometer (RASA 5R-II, Rigaku Co.) with graphite monochromatized Cu $K\alpha$ radiation ($\lambda=1.5418$ Å). A crystal with dimensions of $0.4\times0.2\times0.1$ mm^3 was used for the X-ray measurement. The lattice constants were obtained from 20 reflections with a 2θ range of 40°—50°. The intensity data were collected in the ω-scan mode with a scanning speed of 6° min^{-1} and a scanning width of $\Delta\omega=(2.6+0.14\tan\theta)°$. Three reference reflections (measured after every 100 reflections) showed no significant intensity change during data collection. Although the observed intensities were corrected for Lorentz-polarization factors, they were not corrected for the absorption effect. A total of 2983 reflections were measured up to $2\theta=130°$, of which 2148 reflections with $F_o\geq2\sigma(F_o)$ were used for the following calculations. The density of the crystal was measured by a flotation method using a mixture of carbon tetrachloride and toluene. The observed density (1.476 g cm^{-3}) agreed very well with the calculated density (1.477 g cm^{-3}), where the unit cell contains four nystose molecules and 12 water molecules.

Crystal Data: $C_{24}H_{42}O_{21}\cdot 3H_2O$, F.W. = 720.6, orthorhombic, $P2_12_12_1$, $Z=4$, $a=13.582(3)$, $b=23.385(6)$, and $c=10.198(2)$ Å, $V=3239(1)$ $Å^3$, $D_x=1.477$ g cm^{-3}, $D_m=1.476$ g cm^{-3}, $\mu(CuK\alpha)=11.8$ cm^{-1}.

Determination and Refinement of the Structure The initial atomic coordinates were obtained by a direct

method using the SHELX 86 program.[4] Three oxygen atoms of water molecules were obtained by a weighted Fourier procedure. After several cycles of a full-matrix least-squares calculation, the temperature factors of two of these oxygen atoms became large, and two additional peaks, which were located in the vicinity of the above oxygen atoms, appeared in the difference Fourier map. These peaks were also assigned as oxygen atoms of the water molecules and their coordinates were included in the refinement along with the occupancy factors. Since the distance between a pair of oxygen atoms became very small after several refinements, these were again assumed to be one atom (O_{w2}) with a rather large temperature factor. The locations of 35 hydrogen atoms were found in the difference Fourier map. An additional refinement was performed which included the hydrogen atoms with equivalent temperature factors (B_{eq}) of the corresponding parent atom. The quantity minimized in the refinement was $\Sigma\omega(|F_o|-|F_c|)^2$, with $\omega=1/\sigma^2(F_o)$, where $\sigma(F_o)$ was the standard deviation of F_o estimated from counting statistics. The final discrepancy factor (R value) was 0.074 and the R_w value was 0.071 for all of the non-hydrogen atoms with anisotropic temperature factors and 35 hydrogen atoms for the observed 2148 reflections. The occupancy factors of two oxygen atoms (O_{w3} and O_{w4}) were 0.7 and 0.3, respectively. The final atomic coordinates for the non-hydrogen atoms are given in Table 1.[5]

The atomic scattering factors were taken from the International Tables for X-Ray Crystallography, Vol. IV.[6] Computations were performed on an A-70 minicomputer with the help of the CRYSTAN program in a RASA-5RII system (Rigaku Co.).

Results and Discussion

Molecular Conformation. The molecular conformation of nystose is shown in Fig. 1 together with the atomic numberings. According to the structure analysis reported for the 1-kestose, oxygen atom O_6 is located at two positions with occupancies of 0.66 and 0.34, respectively.[1] Although no such statistical structure was found in this study, a fairly large temperature factor (B_{eq}=6.1) of O_6 atom was found (Fig. 1 and Table 1). The bond distances and angles are shown in Figs. 2(a) and 2(b). These values are very similar to those found in the related compounds.[1]

Various dihedral angles in the nystose molecule are given in Table 2. The glucopyranose conformation was found to be of the $^4C_1$ chair form, which has been commonly observed in many other oligosaccharide molecules. (The notation of the ring conformation in this study follows the rules approved by the British Carbohydrate Nomenclature Committee and by the U. S. Carbohydrate Nomenclature Committee.[7]) The values of the endocyclic dihedral angles of the pyranose ring are within the range of |54°|—|62°|, which is usually observed for the other pyranose crystal structures having the normal chair conformation. The ring conformations of three fructose residues in the molecule are different from each other. The deviation of atoms from the various least-squares best planes, which determine the ring conformation, is given in Table 3 for each fructose residue. For Fructose 1, a 'twist' conformation ($^4T_3$), with C_{1-4} displaced by −0.293 Å and C_{1-3} displaced by 0.317 Å out of the plane of C_{1-2}, C_{1-5}, and O_{1-2}, is clear. The above-mentioned displacement of

Table 1. Fractional Coordinates and Equivalent Isotropic Temperature Factors (B_{eq}) for Non-Hydrogen Atoms of Nystose Trihydrate with Estimated Standard Deviations in Parentheses. $B_{eq}=(4/3)\times\{B_{11}a^2+B_{22}b^2+B_{33}c^2+2(B_{12}ab+B_{13}ac+B_{23}bc)\}$

Atom	x	y	z	B_{eq}/Å^2
C_1	0.5095(6)	0.0766(4)	1.0437(8)	3.0
C_2	0.4926(7)	0.1056(4)	1.1760(9)	3.1
C_3	0.5320(7)	0.0693(4)	1.2870(9)	3.5
C_4	0.4856(6)	0.0098(4)	1.2781(9)	2.9
C_5	0.5057(7)	−0.0160(4)	1.1431(9)	3.3
C_6	0.4540(9)	−0.0732(5)	1.1201(11)	4.6
C_{1-1}	0.6877(7)	0.1447(4)	0.8928(10)	3.3
C_{1-2}	0.6477(6)	0.0854(4)	0.8856(8)	2.9
C_{1-3}	0.7211(7)	0.0382(4)	0.8489(9)	3.4
C_{1-4}	0.6528(7)	−0.0064(4)	0.7882(8)	2.9
C_{1-5}	0.5777(6)	0.0309(4)	0.7176(9)	2.9
C_{1-6}	0.4782(8)	0.0048(4)	0.7028(10)	4.0
C_{2-1}	0.8515(7)	0.1870(4)	0.6236(10)	3.6
C_{2-2}	0.8185(6)	0.1862(4)	0.7677(8)	2.9
C_{2-3}	0.8236(7)	0.2464(4)	0.8304(9)	3.2
C_{2-4}	0.8798(7)	0.2364(4)	0.9552(9)	3.4
C_{2-5}	0.9497(7)	0.1882(4)	0.9150(9)	3.4
C_{2-6}	0.9938(7)	0.1544(4)	1.0298(10)	4.4
C_{3-1}	0.7474(7)	0.1621(5)	0.3708(10)	3.6
C_{3-2}	0.7935(6)	0.2191(4)	0.4130(8)	2.7
C_{3-3}	0.7482(6)	0.2734(4)	0.3567(9)	2.9
C_{3-4}	0.8308(6)	0.3156(4)	0.3741(9)	3.1
C_{3-5}	0.9202(7)	0.2783(4)	0.3345(9)	3.4
C_{3-6}	1.0173(7)	0.2921(5)	0.3969(10)	4.1
O_1	0.6116(4)	0.0725(2)	1.0166(5)	2.7
O_2	0.5389(5)	0.1616(2)	1.1770(6)	3.5
O_3	0.5023(5)	0.0971(3)	1.4073(6)	4.7
O_4	0.5371(5)	−0.0230(3)	1.3767(8)	4.0
O_5	0.4659(4)	0.0220(2)	1.0439(6)	3.3
O_6	0.3510(5)	−0.0662(3)	1.1396(9)	6.1
O_{1-1}	0.7280(4)	0.1585(2)	0.7677(5)	3.0
O_{1-2}	0.5719(4)	0.0824(2)	0.7929(5)	3.1
O_{1-3}	0.7774(4)	0.0179(3)	0.9513(7)	3.9
O_{1-4}	0.7002(5)	−0.0475(2)	0.7077(6)	3.9
O_{1-6}	0.4173(5)	0.0396(3)	0.6208(6)	3.7
O_{2-1}	0.7881(4)	0.2236(3)	0.5520(5)	3.4
O_{2-2}	0.8894(4)	0.1522(2)	0.8360(5)	3.2
O_{2-3}	0.7300(5)	0.2714(3)	0.8584(7)	4.4
O_{2-4}	0.9285(6)	0.2853(3)	1.0083(7)	4.6
O_{2-6}	1.0570(5)	0.1104(3)	0.9856(7)	4.5
O_{3-1}	0.7370(4)	0.1583(3)	0.2333(6)	3.7
O_{3-2}	0.8908(4)	0.2194(2)	0.3681(5)	3.2
O_{3-3}	0.6624(6)	0.2942(4)	0.4155(10)	4.5
O_{3-4}	0.8185(5)	0.3644(2)	0.2908(6)	4.1
O_{3-6}	1.0160(5)	0.2859(3)	0.5364(6)	4.0
O_{W1}	0.7493(5)	0.3673(3)	0.0376(7)	5.4
O_{W2}	0.2602(6)	−0.0160(4)	0.5240(11)	11.2
O_{W3}	0.6706(9)	0.3428(4)	0.6718(10)	6.6
O_{W4}	0.7428(13)	0.4394(10)	0.6969(29)	5.9

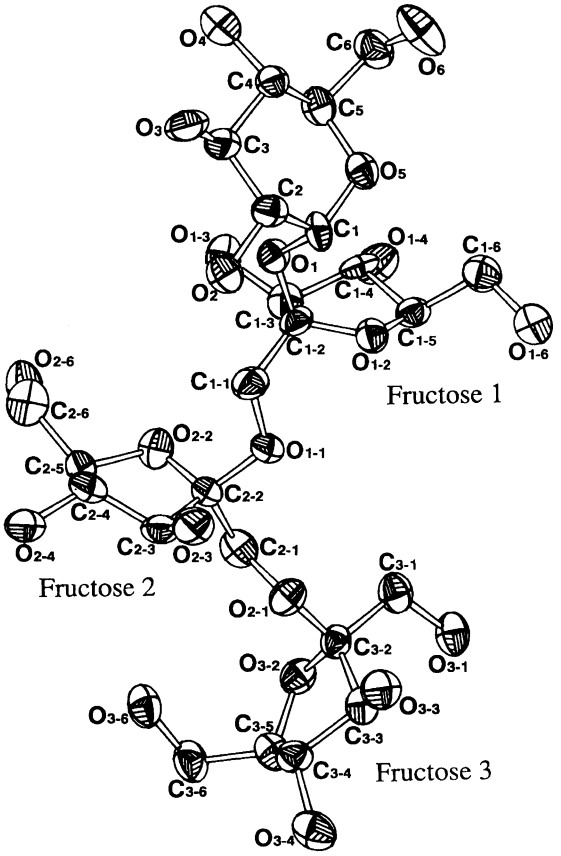

Fig. 1. Molecular conformation and atomic numbering in nystose. Hydrogen atoms were excluded in order to avoid a complex presentation. The 50% probability thermal ellipsoids[8] are shown for the carbon and oxygen atoms.

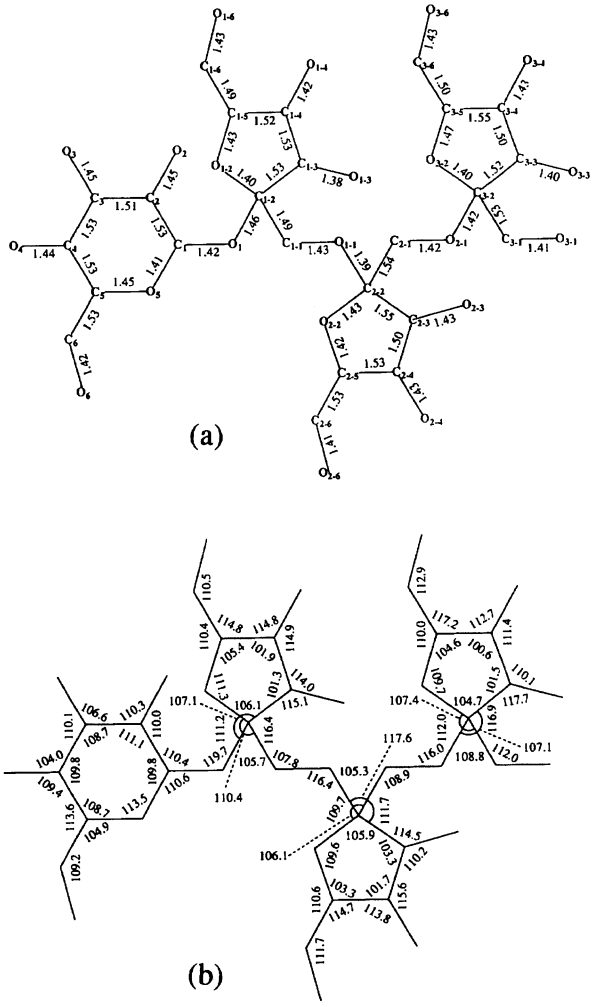

Fig. 2. (a) Bond distances (Å) and (b) angles (°) in nystose. The average estimated standard deviations for the bond distances and angles are 0.01 Å and 0.7°, respectively.

C_{1-3} and C_{1-4} relative to the C_{1-6} is *exo* and *endo*, respectively. On the other hand, Fructose 2 takes an 'envelop' conformation (E_4), with C_{2-4} being displaced by −0.578 Å out of the plane of C_{2-2}, C_{2-3}, C_{2-5}, and O_{2-2}. There are two alternate descriptions of the Fructose 3 conformation. One is a 'twist' conformation ($^4T_3$), with C_{3-3} being displaced by −0.560 Å and C_{3-4} displaced by 0.107 Å out of the plane of C_{3-2}, C_{3-5}, and O_{3-2}. The displacement of C_{3-3} and C_{3-4} relative to C_{3-6} is *exo* and *endo*, respectively. The other is an 'envelop' conformation (E_3), with C_{3-3} being displaced by −0.643 Å out of the plane of C_{3-2}, C_{3-4}, C_{3-5}, and O_{3-2}. The ring conformation of Fructose 1 had the 'twist' conformation ($^4T_3$) similar to that of the corresponding residue in the 1-kestose molecule. On the other hand, the conformations of Fructose 2 (E_4) and Fructose 3 ($^4T_3$ or E_3) were different from those of the other fructose residue in 1-kestose (^3E).

The dihedral angles at the linkage between the Glucose and Fructose 1 residues are listed in Table 2, together with those at the other linkages. Although the dihedral angle around C_1–O_1 (−137.1°) is similar to that of 1-kestose (−152.9°), the other linkage angle around O_1–C_{1-2} (100.3°) is fairly different from the corresponding angle (53.5°) in 1-kestose. As a result, the overall conformational shapes of the sucrose components in nystose and 1-kestose are different from each other. The dihedral angles, (ϕ, ψ, ω),[9] which determine the inulobiose conformation, are (171.2°, −136.6°, 177.6°) and (71.3°, −165.9°, 68.0°) for linkages at (Fructose 1 → Fructose 2) and (Fructose 2 → Fructose 3), respectively. Here, (ϕ, ψ, ω) were taken along the main chain. For example, $\phi = \angle C_{3-1}C_{3-2}O_{2-1}C_{2-1}$, $\psi = \angle C_{3-2}O_{2-1}C_{2-1}C_{2-2}$, and $\omega = \angle O_{2-1}C_{2-1}C_{2-2}O_{1-1}$. The latter linkage conformation of the inulobiose in nystose molecule is very similar to that found in the 1-kestose crystal (80.5°, −169.7°, 56.5°). This (*gauche*, *trans*, *gauche*) type of conformation is also found in cycloinulohexaose.[10] The proposed structure of inulin, based on a conformational analysis coupled with X-ray and electron diffraction data, had a main chain con-

Table 2. Endocyclic Dihedral Angles (°) and Linkage Dihedral Angles (°)

Endocyclic			
Glucose			
O_5–C_1–C_2–C_3		56.7	(10)
C_1–C_2–C_3–C_4		−54.8	(9)
C_2–C_3–C_4–C_5		56.0	(14)
C_3–C_4–C_5–O_5		−58.2	(10)
C_4–C_5–O_5–C_1		61.9	(10)
C_5–O_5–C_1–C_2		−60.6	(11)
Fructose 1			
O_{1-2}–C_{1-2}–C_{1-3}–C_{1-4}		−30.8	(9)
C_{1-2}–C_{1-3}–C_{1-4}–C_{1-5}		36.4	(7)
C_{1-3}–C_{1-4}–C_{1-5}–O_{1-2}		−30.4	(6)
C_{1-4}–C_{1-5}–O_{1-2}–C_{1-2}		11.5	(10)
C_{1-5}–O_{1-2}–C_{1-2}–C_{1-3}		12.4	(10)
Fructose 2			
O_{2-2}–C_{2-2}–C_{2-3}–C_{2-4}		−14.8	(10)
C_{2-2}–C_{2-3}–C_{2-4}–C_{2-5}		32.9	(7)
C_{2-3}–C_{2-4}–C_{2-5}–O_{2-2}		−40.3	(8)
C_{2-4}–C_{2-5}–O_{2-2}–C_{2-2}		32.4	(7)
C_{2-5}–O_{2-2}–C_{2-2}–C_{2-3}		−11.3	(11)
Fructose 3			
O_{3-2}–C_{3-2}–C_{3-3}–C_{3-4}		−40.5	(6)
C_{3-2}–C_{3-3}–C_{3-4}–C_{3-5}		41.2	(8)
C_{3-3}–C_{3-4}–C_{3-5}–O_{3-2}		−28.7	(11)
C_{3-4}–C_{3-5}–O_{3-2}–C_{3-2}		4.1	(8)
C_{3-5}–O_{3-2}–C_{3-2}–C_{3-3}		22.3	(9)
Linkage			
Glucose → Fructose 1			
C_2–C_1–O_1–C_{1-2}		−137.1	(6)
O_5–C_1–O_1–C_{1-2}		101.2	(13)
C_1–O_1–C_{1-2}–C_{1-1}		100.3	(8)
C_1–O_1–C_{1-2}–C_{1-3}		−135.0	(6)
C_1–O_1–C_{1-2}–O_{1-2}		−19.5	(5)
Fructose 1 → Fructose 2			
C_{1-3}–C_{1-2}–C_{1-1}–O_{1-1}		58.9	(12)
O_1–C_{1-2}–C_{1-1}–O_{1-1}	(ω)	177.6	(9)
O_{1-2}–C_{1-2}–C_{1-1}–O_{1-1}		−62.1	(14)
C_{1-2}–C_{1-1}–O_{1-1}–C_{2-2}	(ψ)	−136.6	(11)
C_{1-1}–O_{1-1}–C_{2-2}–C_{2-1}	(ϕ)	171.2	(9)
C_{1-1}–O_{1-1}–C_{2-2}–C_{2-3}		−63.7	(15)
C_{1-1}–O_{1-1}–C_{2-2}–O_{2-2}		57.4	(10)
Fructose 2 → Fructose 3			
C_{2-3}–C_{2-2}–C_{2-1}–O_{2-1}		−60.7	(9)
O_{1-1}–C_{2-2}–C_{2-1}–O_{2-1}	(ω)	68.0	(8)
O_{2-2}–C_{2-2}–C_{2-1}–O_{2-1}		−175.7	(8)
C_{2-2}–C_{2-1}–O_{2-1}–C_{3-2}	(ψ)	−165.9	(13)
C_{2-1}–O_{2-1}–C_{3-2}–C_{3-1}	(ϕ)	71.3	(9)
C_{2-1}–O_{2-1}–C_{3-2}–C_{3-3}		−161.4	(8)
C_{2-1}–O_{2-1}–C_{3-2}–O_{3-2}		−47.2	(6)

Table 3. Displacements (Å) from Various Least-Squares Best Planes for the Fructose Rings in Nystose Atoms whose displacements are in bold-face type were excluded from the calculation of the best plane. The coefficients of each equation of the plane, $Ax+By+Cz+D=0$ where x, y, z are in Å, are also listed.

Fructose 1		(a)	(b)	(c)
	C_{1-1}	**0.911**	**−0.913**	**0.851**
	C_{1-2}	0.000	0.064	0.039
	C_{1-3}	**0.317**	−0.037	**0.559**
	C_{1-4}	**−0.293**	**0.552**	−0.035
	C_{1-5}	0.000	0.041	0.059
	C_{1-6}	**−0.969**	**0.906**	**−0.969**
	O_{1-2}	0.000	−0.068	−0.064
Fructose 2		(d)	(e)	(f)
	C_{2-1}	**1.407**	**1.442**	**1.340**
	C_{2-2}	0.000	0.060	0.108
	C_{2-3}	**−0.291**	−0.035	**0.415**
	C_{2-4}	**−0.797**	**−0.578**	−0.095
	C_{2-5}	0.000	0.039	0.170
	C_{2-6}	**−0.593**	**−0.662**	**−0.639**
	O_{2-2}	0.000	−0.064	−0.182
Fructose 3		(g)	(h)	(i)
	C_{3-1}	**−0.791**	**−0.816**	**−0.766**
	C_{3-2}	0.000	0.120	−0.015
	C_{3-3}	**−0.560**	−0.069	**−0.643**
	C_{3-4}	**0.107**	**0.581**	0.013
	C_{3-5}	0.000	0.074	−0.022
	C_{3-6}	**1.067**	**0.955**	**1.066**
	O_{3-2}	0.000	−0.125	0.023

Plane	A	B	C	D
(a)	0.5814	0.4644	−0.6681	−0.0083
(b)	−0.4632	−0.5597	0.6871	−0.9479
(c)	0.6716	0.3365	−0.6496	−0.7148
(d)	0.6394	0.1075	−0.7613	−1.6161
(e)	0.6150	0.2428	−0.7502	−1.9617
(f)	0.5756	0.4875	−0.6565	−3.2738
(g)	0.3235	0.1393	0.9359	−8.1417
(h)	0.1352	0.3378	0.9315	−6.9913
(i)	0.3512	0.0974	0.9312	−8.2206

formation of (75°, 130°, 180°),[9] which is very different from those found in 1-kestose and nystose crystals. However, it is very interesting that the linkage conformation (*gauche,trans, gauche*) found in 1-kestose and nystose crystals is very similar to that of the second candidate for the molecular structure of inulin (60°, 135°, 60°).[9]

Crystal Structure. The packing structure of nystose trihydrate is shown in Fig. 3. All of the possible candidates for hydrogen bonding, except for ether oxygens, play the role as a donor or an acceptor atom for hydrogen bonds. These oxygen atoms, except for O_6 in the glucopyranose residue, are responsible for more than one hydrogen bonding. Details concerning the hydrogen bond network are given in Table 4. There are two water molecules (W_1 and W_2) with full occupancies, and another two (W_3 and W_4) with partial occupancies. The oxygen atom (O_{w1}) of water molecule W_1 is tightly bonded to four adjacent oxygen atoms (O_{2-3}, O_{2-6}, O_{3-4}, and O_{w2}) with hydrogen bond distances of 2.90, 2.675, 2.748, and 2.81 Å, respectively. Similarly, O_{w2} is bonded to four oxygen atoms of O_{1-6}, O_6, O_{w1}, and O_{w4} with distances of 2.69, 2.71, 2.81, and 2.89 Å, respectively. On the other hand, O_{w3} makes two hydrogen bonds with O_{2-3} (2.66 Å) and O_{3-3}(2.85 Å), and O_{w4} makes only one hydrogen bond with O_{w2}(2.89

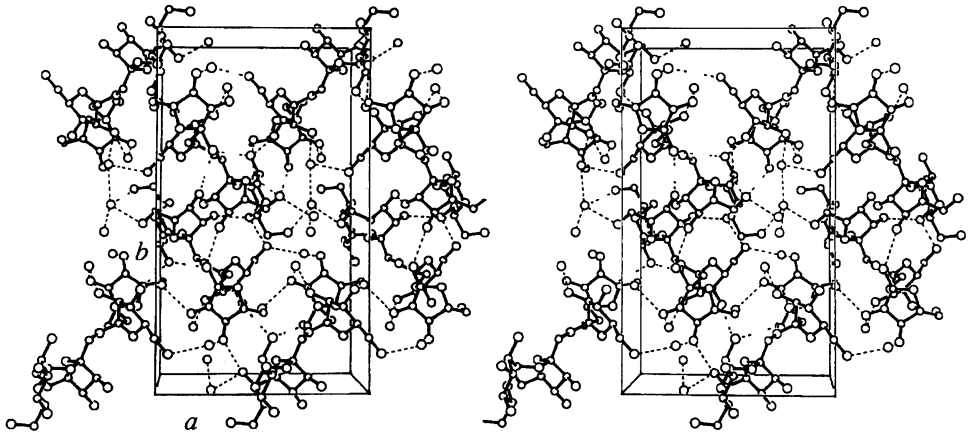

Fig. 3. Stereogram of nystose trihydrate viewed down the c-axis. Hydrogen bonding is indicated by dotted lines.

Table 4. Hydrogen Bonds in a Nystose Trihydrate Crystal

Donor	Acceptor	Distance(Å)	$\angle O_D H O_A$(°)	Symmetry operation of an acceptor atom [a]
O_2	O_{3-1}	2.751(9)	174	556.1
O_3	O_{1-6}	2.808(9)	—	556.1
O_4	O_{2-6}	2.653(9)	160	655.2
O_6*	O_{w2}	2.71(1)	—	555.2
O_{1-3}	O_4	2.635(9)	174	654.2
O_{1-4}	O_{1-3}	2.722(9)	—	655.2
O_{1-6}	O_{w2}	2.69(1)	164	555.1
O_{2-3}*	O_{w1}	2.90(1)	—	556.1
O_{2-3}*	O_{w3}	2.66(1)	—	555.1
O_{2-4}	O_2	2.713(9)	174	557.3
O_{2-6}*	O_{w1}	2.675(9)	—	556.3
O_{3-3}	O_{3-6}	2.78(1)	143	456.3
O_{3-1}	O_{1-4}	2.740(8)	—	654.2
O_{3-4}	O_{1-6}	2.767(8)	—	556.3
O_{3-6}	O_3	2.803(9)	151	557.3
O_{w1}	O_{3-4}	2.748(9)	—	555.1
O_{w1}*	O_{w2}	2.81(1)	—	655.4
O_{w2}*	O_{w4}	2.89(3)	—	456.3
O_{w3}	O_{3-3}	2.85(1)	—	555.1

* In case of the hydrogen bonds with *, the definition of a donor or acceptor atom is uncertain. a) The first three digits code denotes a lattice translation code, e.g. 466.1 is $-a+b+c$ from the reference point, 555.1. The last digit indicates one of the following operations: (1) x y z, (2) $0.5-x$ $-y$ $0.5+z$, (3) $0.5+x$ $0.5-y$ $-z$, (4) $-x$ $0.5+y$ $0.5-z$.

Å). It can therefore be said that the stability of the water molecules may decrease in this order. As in the 1-kestose crystal, no intramolecular hydrogen bond was found. Instead, one hydrogen bond bridge (O_{2-3} ⋯ O_{w3} ⋯ O_{3-3}) was found. Since the occupancy of O_{w3} is 0.7, however, 30% of nystose molecules could not have this hydrogen bond bridge.

The thermal gravimetry (TG) and differential thermal calorimetry (DTA) of nystose trihydrate crystals are given in Fig. 4. Three steps of dehydration were observed before thermal decomposition of the specimen.

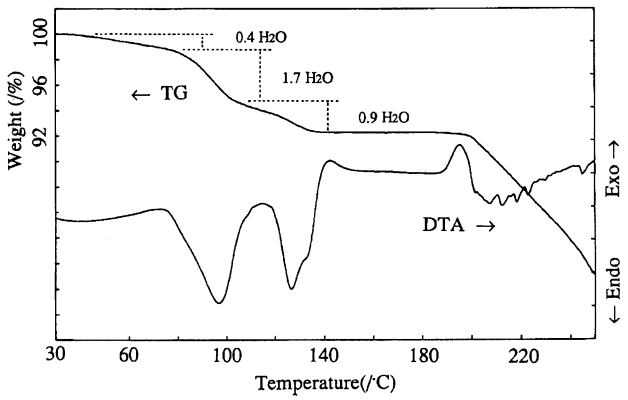

Fig. 4. Thermal gravimetry (TG) and differential thermal calorimetry (DTA) curves of the nystose trihydrate crystal.

The first dehydration starts from 40°C and stops at 80°C with a 1% weight loss, which corresponds to 0.4 water molecules per one nystose molecule. The second dehydration step follows the first one up to 100°C. The weight loss of this dehydration corresponds to 1.7 water molecules. The last dehydration takes place at between 100 and 140°C with a weight loss corresponding to 0.9 water molecules. As a result, three water molecules (0.4+1.7+0.9) per one nystose molecule were removed from the crystals. From the viewpoint of stability of the water molecules, the most weakly bonded oxygen atom (O_{w4}) seems to be removed during the first dehydration step. During the second dehydration step, O_{w2} and O_{w3}, having two hydrogen bonds with nystose molecules, may be removed from the crystals. Oxygen atom O_{w1}, which is most tightly fixed to nystose molecules, can be removed during the last step. Therefore, the number of water molecules removed during each step seems to be 0.3 (W_4), 1.7 (W_3+W_2), and 1.0 (W_1) per one nystose molecule respectively; this agrees very well with the values calculated from the weight loss in the corresponding temperature region.

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