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The tetrasaccharide nystose trihydrate: Crystal structure analysis and hydrogen bonding

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ABSTRACT

The crystal structure of nystose trihydrate, *O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl α-D-glucopyranoside trihydrate, $C_{24}H_{42}O_{21}\cdot 3H_2O$, has been determined using Cu$K\alpha$ X-ray data at 121 K. The space group is $P2_12_12_1$, with 4 molecules in a unit cell of dimensions $a = 10.155(1)$, $b = 13.506(1)$, and $c = 23.278(2)$ Å. The structure was refined to $R = 0.052$ and $R_w = 0.048$ for 2734 observed structure amplitudes. The α-D-glucopyranose unit of the molecule has the normal $^4C_1$ chair conformation and the three fructofuranose units have twist conformations lying between E_3 and $^4T_5$. One of the three water molecules is distributed over two sites: W-3 with occupancy 0.80 and W-3′ with occupancy 0.20. All the hydrogen atoms were located on the difference synthesis with the exception of those attached to the low-occupancy water site. All hydroxyls, two of the three linkage oxygen atoms, and the water molecules are involved in a complex three-dimensional network which can be decomposed into a series of infinite chains intersecting at the water molecules to form homo- and hetero-dromic cycles.

INTRODUCTION

Nystose, *O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-α-D-glucopyranoside, crystallizes as a trihydrate. It is only the second tetrasaccharide for which crystals were obtained suitable for X-ray structure analysis. The other example is stachyose tetrahydrate, for which large crystals have been available since 1910[1–3]. With the exception of the cyclodextrin hydrates and complexes, crystals of higher oligosaccharides suitable for X-ray analysis are obtained only by blocking the reducing end with a *p*-nitrophenyl group and forming a BaI_3 complex[4].

The linkage sequence of the fructose residues in nystose, and their furanose nature, was elucidated by methylation analysis[5] and confirmed by NMR examination of nystose tetradeca-acetate[6]. The β configuration of the outer fructofuranosyl units was inferred from the specificity of an invertase that yielded nystose by action on sucrose[5].

Related trisaccharides containing the interesting three-bond (2 → 1) or (2 → 6) interresidue linkages and having crystal structure analyses available are 1-kestose[7]

TABLE I

Crystal data, and structure determination and refinement data for nystose trihydrate at 121 K

Crystal data

$C_{24}H_{42}O_{21}\cdot 3H_2O$, mol wt 720.6, $P2_12_12_1$, $Z = 4$

Cell dimensions at 121 K [295 K]: $a = 10.155(1)$ [10.187(1)], $b = 13.506(1)$ [13.579(2)], $c = 23.278(2)$ [23.391(2)] Å

$V = 3192.7$ [3235.8] Å^3, $D_X = 1.499$ [1.479] g cm^{-3}

Structure determination and refinement data at 121 K

Crystal dimensions, $0.24 \times 0.13 \times 0.30$ mm

3402 intensities measured on a CAD-4-diffractometer, by ω-2θ scans to $\theta < 70°$; 2734 with $I > 2\sigma(I)$

Radiation: Cu$K\alpha$ ($\lambda = 1.5418$ Å), Ni-filtered, μ(Cu$K\alpha$) = 11.99 cm^{-1}

Absorption correction: max 1.355 cm^{-1}, min 1.149 cm^{-1}

No extinction corrections applied

586 parameters; refinement using UPALS[14], minimizing $w(k|F_0| - |F_c|)^2$, where $w = 1/[\sigma^2(F) + (0.01F_0)^2]$

Observation to parameter ratio: 4.7

Final agreement factors: $R(F) = 0.052$, $R_w(F) = 0.048$

and 6-kestose[8]. There is a molecular mechanics study of the (2 → 1)-linked disaccharide inulobiose, for which there is no crystallographic data[9], and a combined molecular mechanics and NMR study of 1-kestose[10].

EXPERIMENTAL

A single crystal of nystose trihydrate was provided by Drs. S. Pérez and N. Mouhous-Riou * of the Institut National de la Recherche Agronomique, Nantes, France, whose work on the computer modeling of the structure is described in the immediately following paper[11]. X-ray diffraction data collected at room temperature with Cu$K\alpha$ radiation failed to provide a solution to the phase problem. The data collection was repeated at 121 K, and this led to a satisfactory structure analysis, the experimental details of which are given in Table I. The crystal structure determination was by the direct method MITHRIL[12], using as input a group of 20 carbon and oxygen atoms of known stereochemistry from the crystal structure of 1-kestose. The resulting E-map revealed all 45 carbon and oxygen atoms of the tetrasaccharide molecule. Difference synthesis revealed the presence of 3 water molecules, one of which was unequally distributed over two sites 2.3 Å apart. Subsequent refinement gave occupancy factors for the disordered water oxygen atom of 0.80 and 0.20 and all the hydrogen positions except those of the low-occupancy water site. An R-factor ratio test[13] showed that the disordered water model was superior to an ordered model at the 99.5% level. Final refinement using UPALS[14] gave the atomic parameters listed in Table II. The hydrogen

* Crystals of the sugar were grown by slow evaporation of an ethanol–water solution.

atoms were assigned fixed isotropic temperature factors equal to the isotropic equivalent temperature factors of the carbon or oxygen atoms to which they were covalently bonded. The refinement data are given in Table I and the atomic notation and thermal elliposids are shown in Fig. 1 *. With the crystal structure solved, an anisotropic refinement based on the room temperature data gave thermal parameters which showed no evidence of disorder, such as was observed in the fructofuranose rings in the crystal structure of stachyose tetrahydrate[3].

RESULTS AND DISCUSSION

The molecular geometry of the nystose molecule in the crystal at 121 K is given in Table III. With the exception of the C-2″–C-3″ bond, the C–C bond lengths range from 1.492 to 1.543 Å (6σ). The C–O bond lengths range from 1.399 to 1.475 Å (10σ). These ranges are wider than normally observed in carbohydrate crystal structure analyses. They are, however, comparable to those observed in stachyose tetrahydrate (C–C, 1.505 to 1.558 Å; C–O, 1.401 to 1.476 Å). The short lengths (1.492–1.516 Å) of the primary alcohol C–C bonds can be ascribed to their thermal motion. There is no explanation for the long C-2″–C-3″ bond, which we believe to be an outlying experimental error.

The glucopyranose ring conformation is a slightly distorted $^4C_1$ with Cremer–Pople puckering parameters[15] of $Q = 0.57$ Å, $\theta = 4°$, consistent with the narrow range of values observed in other oligosaccharides containing glucose and fructose moieties (see Table III of ref 3).

The first (primed) and third (triple-primed) fructofuranose rings have conformations within the "Northern" range most commonly observed in oligosaccharide crystal structures[27], i.e., ${}_3E$ to ${}^4_3T$, $\Psi = 250–276°$. The inner (double-primed) ring is outside this range, having a ${}^4_5T$ conformation, $\Psi = 304°$. The only example of a fructofuranose conformation in the "Southern" range is found in 1-kestose. The linkage-bond torsion angles are given in Table III and are compared with observations of comparable bonds in other oligosaccharides in Table IV.

For the α-Glc*p*-(1 ↔ 2)-β-Fru*f* (sucrose) type linkages, the observed O-5–C-1–O-1–C-2′ angles lie in a relatively narrow range of 28°, with the nystose value 3° from the mean value. The C-1–O-1–C-2′–O-2′ angles are less constrained. Excluding the raffinose value, the range is over 47° with a mean value of −41°. The nystose value of −19° lies at the extreme of this range. In all the sucrose moieties, except in raffinose, the two ring oxygens lie on the same side of the long axis of the dissacharide. In the three-bond linkages, all three staggered conformations (+*sc*, −*sc*, and *ap*) are observed for the two outer bonds, but the *gauche* conformations

* Tables of anisotropic temperature factors and observed and calculated structure amplitudes have been deposited with, and can be obtained from, Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/635/*Carbohydr. Res.*, 247 (1993) 37–50.

TABLE II

Atomic positional parameters and equivalent isotropic thermal parameters for nystose tetrahydrate at 121 K [a]

Atom	x/a	y/b	z/c	B_{eq} (Å^2)
O-1	10193(3)×10^{-4}	8892(3)×10^{-4}	4275(2)×10^{-4}	78(9)×10^{-2}
O-2	11788(4)	9635(3)	3381(2)	118(9)
O-3	14094(4)	9999(4)	4014(2)	179(12)
O-4	13811(4)	9672(3)	5228(2)	131(9)
O-5	10447(4)	10359(3)	4787(2)	98(9)
O-6	11411(5)	11490(3)	5671(2)	247(13)
O-1′	7682(3)	7711(3)	3405(2)	111(10)
O-2′	7935(4)	9295(3)	4170(2)	98(9)
O-3′	9534(4)	7215(3)	4827(2)	138(10)
O-4′	7090(4)	8012(3)	5482(2)	148(11)
O-6′	6207(4)	10841(3)	4600(2)	125(10)
O-1″	5524(4)	7100(3)	2764(2)	157(11)
O-2″	8380(4)	6080(3)	3487(2)	108(9)
O-3″	8590(5)	7658(4)	2275(2)	202(12)
O-4″	10104(4)	5681(4)	2145(2)	173(12)
O-6″	9909(5)	4423(3)	3908(2)	157(10)
O-1‴	2327(4)	7663(3)	3414(2)	137(10)
O-2‴	3663(4)	6075(3)	2822(2)	131(10)
O-3‴	4163(5)	8379(3)	2051(2)	184(11)
O-4‴	2879(4)	6783(3)	1364(2)	178(11)
O-6‴	5381(4)	4815(3)	2155(2)	173(11)
C-1	10428(5)	9915(4)	4234(2)	80(12)
C-2	11769(6)	10087(5)	3939(2)	111(13)
C-3	12901(6)	9729(5)	4297(2)	103(13)
C-4	12819(6)	10157(4)	4900(3)	112(13)
C-5	11451(6)	9958(4)	5160(2)	105(13)
C-6	11259(7)	10451(5)	5739(3)	173(15)
C-1′	8951(6)	8137(5)	3539(3)	131(14)
C-2′	8883(5)	8529(4)	4145(2)	93(13)
C-3′	8491(6)	7802(5)	4619(3)	124(14)
C-4′	7903(6)	8487(5)	5068(2)	111(13)
C-5′	7166(6)	9243(4)	4699(2)	95(13)
C-6′	7034(6)	10247(5)	4961(3)	117(14)
C-1″	6238(6)	6446(5)	3142(2)	110(13)
C-2″	7677(5)	6775(4)	3146(2)	110(13)
C-3″	8339(6)	6723(5)	2536(3)	117(14)
C-4″	9589(6)	6157(5)	2636(2)	124(14)
C-5″	9186(6)	5470(5)	3123(2)	113(13)
C-6″	10326(6)	5059(5)	3460(3)	146(14)
C-1‴	3746(6)	7552(5)	3389(3)	160(15)
C-2‴	4125(6)	7063(5)	2816(3)	151(15)
C-3‴	3563(6)	7516(5)	2263(3)	118(14)
C-4‴	3720(6)	6650(5)	1854(3)	146(14)
C-5‴	3331(6)	5787(5)	2229(3)	144(14)
C-6‴	3960(6)	4804(5)	2103(3)	167(15)
O-W-1	9658(5)	2502(4)	3682(2)	216(12)
O-W-2	5105(7)	12361(4)	5125(3)	496(19)
O-W-3 (0.80)	6679(7)	8249(8)	1562(4)	489(25)
O-W-3′ (0.20)	3056(39)	2554(20)	4350(13)	65(11)

TABLE II (continued)

Atom	x/a	y/b	z/c	B_{eq} (Å^2)
H-C-1	976(5)×10^{-3}	1021(4)×10^{-3}	404(2)×10^{-3}	
H-C-2	1175(5)	1083(4)	387(2)	
H-C-3	1283(5)	895(4)	434(2)	
H-C-4	1297(5)	1095(4)	489(2)	
H-C-5	1116(6)	920(5)	520(3)	
H-C-6	1190(5)	1021(4)	603(2)	
H′-C-6	1037(6)	1028(4)	585(2)	
H-C-1′	961(6)	759(4)	358(2)	
H′-C-1′	911(8)	871(5)	331(3)	
H-C-3′	778(6)	735(4)	449(2)	
H-C-4′	858(5)	876(4)	528(2)	
H-C-5′	623(6)	890(4)	462(2)	
H-C-6′	799(6)	1053(5)	500(3)	
H′-C-6′	659(5)	1021(4)	536(2)	
H-C-1″	604(5)	561(4)	300(2)	
H′-C-1″	585(5)	650(4)	355(2)	
H-C-3″	789(7)	641(6)	225(3)	
H-C-4″	1018(5)	673(4)	277(2)	
H-C-5″	877(5)	494(4)	297(2)	
H-C-6″	1066(5)	569(4)	363(2)	
H′-C-6″	1077(5)	466(4)	321(2)	
H-C-1‴	410(5)	704(4)	376(2)	
H′-C-1‴	440(7)	822(5)	346(3)	
H-C-3‴	262(7)	764(5)	232(3)	
H-C-4‴	474(6)	657(5)	178(2)	
H-C-5‴	228(6)	576(4)	215(3)	
H-C-6‴	376(5)	470(4)	164(3)	
H′-C-6‴	367(6)	415(4)	247(2)	
H-O-2	1150(9)	913(6)	341(4)	
H-O-3	1455(7)	1028(6)	423(3)	
H-O-4	1408(10)	1001(7)	550(4)	
H-O-6	1143(6)	1178(5)	608(2)	
H-O-3′	948(8)	659(6)	485(3)	
H-O-4′	638(7)	782(5)	537(3)	
H-O-6′	590(6)	1124(5)	487(3)	
H-O-3″	772(10)	780(8)	210(5)	
H-O-4″	966(6)	540(5)	205(3)	
H-O-6″	979(7)	382(5)	387(3)	
H-O-1‴	221(6)	752(5)	375(3)	
H-O-3‴	421(5)	874(4)	234(2)	
H-O-4‴	332(7)	653(6)	98(3)	
H-O-6‴	548(6)	515(4)	175(2)	
H-1-W-1	887(7)	216(5)	366(3)	
H-2-W-1	997(9)	222(6)	332(4)	
H-1-W-2	572(13)	1266(10)	491(5)	
H-2-W-2	515(3)	1254(3)	547(1)	
H-1-W-3	611(7)	814(6)	168(4)	
H-2-W-3	695(11)	810(8)	124(5)	

[a] Esd values given in parentheses refer to the least significant digit. $B_{eq} = 4/3(\Sigma_{ij} B_{ij} a_i a_j)$, calculated from the refined, anisotropic thermal parameters.

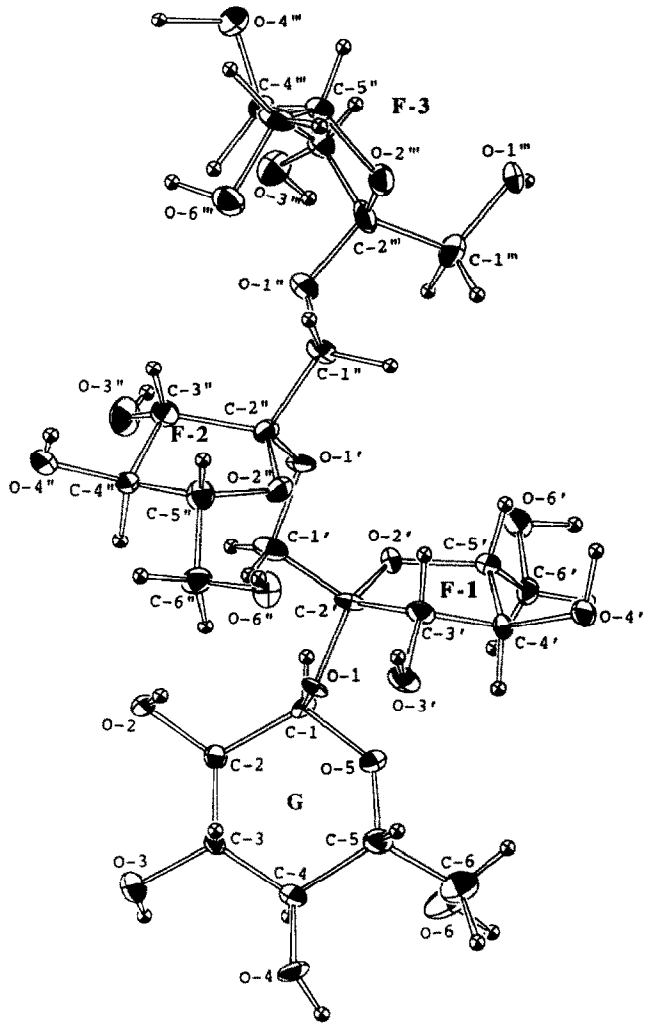


Fig. 1. The atomic notation and thermal ellipsoids at 50% probability for the nystose molecule at 121 K.

are more frequent. The inner bonds show a definite tendency to be close to 180°, irrespective of the nature of the monosaccharide components. This conclusion was also derived from the molecular mechanics study of the disaccharide inulobiose[9]. The β-Fru*f*″-(2 → 1)-β-Fru*f*′ linkage in nystose, at −133°, is however an exception to this rule.

The other variable conformational angles are those of the primary alcohol groups. The pyranoside orientation is *gg*, which is one of the two that are preferred[28]. For the fructofuranosides, all three staggered orientations are possible and two of the three are observed in the nystose structure, *gt* and *gg*. With their three-bond linkages the fructofuranose residues are well separated, and as sug-

gested by the molecular mechanics calculations on inulobiose[9], differences in the three staggered orientations of these groups result in relatively small energy differences.

TABLE III

Molecular geometry of nystose at 121 K [a]

Bond(s)	Values found			
	Glc*p*(G) (unprimed)	Fru*f* (F-1) (primed)	Fru*f* (F-2) (double-primed)	Fru*f* (F-3) (triple-primed)
Bond lengths (Å)				
C-1–C-2	1.543(8)	1.508(8)	1.527(8)	1.537(9)
C-2–C-3	1.502(8)	1.530(8)	1.573(8)	1.534(9)
C-3–C-4	1.520(8)	1.517(8)	1.500(8)	1.517(9)
C-4–C-5	1.538(8)	1.530(8)	1.521(8)	1.508(9)
C-5–C-6	1.516(9)	1.492(8)	1.505(8)	1.503(9)
C-1–O-5	1.420(7)			
C-1–O-1	1.406(7)	1.446(7)	1.441(7)	1.450(8)
C-2–O-2	1.435(7)	1.414(6)	1.422(7)	1.415(7)
C-3–O-3	1.425(7)	1.409(7)	1.424(8)	1.405(7)
C-4–O-4	1.424(7)	1.422(7)	1.411(7)	1.437(8)
C-5–O-5 [b]	1.445(7)	1.461(7)	1.437(7)	1.475(7)
C-6–O-6	1.420(8)			
C-5–O-2		1.434(7)	1.417(7)	1.449(8)
C-2′–O-1		1.451(7)	1.399(7)	1.426(7)
Bond angles (deg)				
C-2–C-1–O-5	109.2(4)			
O-1–C-1–O-5	110.9(4)			
O-1–C-1–C-2	109.1(4)	107.5(5)	107.8(4)	109.1(5)
C-1–C-2–C-3	112.3(5)	117.4(5)	113.0(5)	117.6(5)
C-1–C-2–O-2	110.5(4)	109.0(4)	106.9(4)	108.2(5)
C-3–C-2–O-2	110.8(5)	105.3(4)	105.0(4)	105.2(5)
C-2–C-3–C-4	110.3(5)	101.9(5)	104.2(4)	100.4(5)
C-2–C-3–O-3	108.1(5)	114.4(5)	115.0(4)	117.7(5)
C-4–C-3–O-3	112.1(5)	113.7(5)	111.5(5)	111.9(5)
C-3–C-4–C-5	110.3(5)	102.3(4)	101.5(5)	101.8(5)
C-3–C-4–O-4	106.3(4)	114.8(5)	114.9(5)	109.9(5)
C-5–C-4–O-4	110.3(5)	113.4(5)	115.2(5)	113.6(5)
C-4–C-5–C-6	112.9(5)	114.9(5)	114.0(5)	117.3(5)
C-4–C-5–O-5	109.6(4)			
C-4–C-5–O-2		104.1(4)	104.1(4)	106.2(5)
C-6–C-5–O-5	106.1(5)			
C-6–C-5–O-2		110.6(4)	110.0(5)	108.6(5)
C-5–C-6–O-6	108.8(5)	108.7(5)	112.2(5)	113.4(5)
C-2–O-2–C-5		111.3(4)	109.6(4)	108.3(4)
C-1–O-5–C-5	113.3(4)			

Linkage bond angles (deg)			
C-1–O-1–C-2′	118.3(4)	O-1–C-2′–C-1′	105.7(4)
C-1′–O-1′–C-2″	117.1(3)	O-1′–C-2″–C-1″	105.6(4)
C-1″–O-1″–C-2‴	115.3(4)	O-1″–C-2‴–C-1‴	107.9(4)

TABLE III (continued)

Selected torsion angles (deg)				
Furanose rings		F-1	F-2	F-3
C-2–C-3–C-4–C-5		38.3(5)	31.6(5)	40.5(6)
C-3–C-4–C-5–O-2		−30.8(5)	−39.8(5)	−28.7(6)
C-4–C-5–O-2–C-2		10.9(5)	32.2(5)	4.1(6)
C-5–O-2–C-2–C-3		13.6(5)	−12.5(5)	22.2(6)
O-2–C-2–C-3–C-4		−32.6(5)	−13.1(6)	−39.4(6)
Pyranose ring [(1 ↔ 2)glucose – fructose linkage]				
C-1–C-2–C-3–C-4	−51.9(6)		O-5–C-1–O-1–C-2′	102.2(5)
C-2–C-3–C-4–C-5	52.3(6)		C-2–C-1–O-1–C-2′	−137.5(4)
C-3–C-4–C-5–O-5	−56.4(6)		C-1–O-1–C-2′–O-2′	−18.5(6)
C-4–C-5–O-5–C-1	62.2(5)		C-1–O-1–C-2′–C-1′	99.9(5)
C-5–O-5–C-1–C-2	−60.4(5)		O-1–C-2′–C-1′–O-1′	176.5(4)
O-5–C-1–C-2–C-3	54.9(6)			
(2 → 1)Fructose – fructose linkages		F-3 → F-2	F-2 → F-1	
O-2′–C-2′–C-1′–O-1′		−63.3(5)	−175.9(4)	
C-2′–C-1′–O-1′–C-2″		−133.4(5)	−165.1(4)	
C-1′–O-1′–C-2″–O-2″		56.0(6)	−47.0(6)	
C-1′–O-1′–C-2″–C-1″		171.3(4)	71.6(6)	
O-1′–C-2″–C-1″–O-1″		66.2(5)	171.3(4)	
Primary alcohol groups		G	F-1	F-2 F-3
C-4–C-5–C-6–O-6		59.7(6)	−173.8(5)	180.0(5) 60.3(7)
O(ring)[b]–C-5–C-6–O-6		−60.3(6)	68.8(5)	63.6(6) −59.9(6)

[a] Esd values given in parentheses refer to the least significant digit. [b] The ring oxygens are O-5 in the pyranose ring and O-2′, O-2″, O-2‴ in the furanose rings.

THE HYDROGEN BONDING

The hydrogen bonding scheme consists of a series of infinite chains, shown in Fig. 2, which intersect at the water molecules to form *homodromic* and *antidromic* cycles[29] (Fig. 3). As shown in Fig. 3, three of the chains extend in the directions of the crystallographic screw axes and there are pairs of identical chains with opposite donor–acceptor directions in this nonpolar space group. The chain in the *a*-axis direction contains highly cooperative four-link *homodromic* cycles[30] similar to that observed in stachyose tetrahydrate[3] (see Fig. 4).

The individual hydrogen bond lengths and angles are given in Table V, with the covalent O–H bond lengths normalized to 0.97 Å to correct for the bonding electron charge density[31,32]. This is a procedure that significantly improves the agreement with the more precise O–H bond lengths derived from neutron diffraction data[33]. The C–O–H angles range from 97 to 124°, the H–O–H angles from 92° to 111°.

All the hydroxy groups, water molecules, and linkage oxygen atoms are involved in the hydrogen bonding, except for linkage oxygen O-1′. Of the four ring oxygens, O-5 accepts a hydrogen bond which is the minor component of the three-center bond from W-2–H-1; O-2″ accepts a minor component from O-4-H.

TABLE IV

Related linkage torsion angles (deg) in oligosaccharide crystal structures

Oligosaccharide	Sucrose type linkage [a] (α-Glc$p$-(1 → 2)-β-Fru$f$) Torsion angles		Three-bond linkages				Ref
			Residues coupled	Torsion angles			
	O_r–C–O_l–C	C–O_l–C–O_r		O_r–C–C(H_2)–O_l	C–C(H_2)–O_l–C	C(H_2)–O_l–C–O_r	
Nystose·3H_2O	+102	−19	β-Fru$f$-(2 → 1)-β-Fru$f$	−63, −176	−133, −165	+171, +72	
Stachyose·4H_2O	+109	−47	α-Gal$p$-(1 → 6)-α-Gal$p$	+87	−172	+84	3
			α-Gal$p$-(1 → 6)-α-Glc$p$	−62	−175	+65	
1-Kestose	+85	−66	β-Fru$f$-(2 ↔ 1)-β-Fru$f$	+179	−170	−41	7
6-Kestose	+90	−55	β-Fru$f$-(2 → 6)-β-Fru$f$	+68	+146	−56	8
Raffinose·5H_2O	+82	+12	α-Gal$p$-(1 → 6)-α-Glc$p$	−65	−170	+72	16
Planteose·2H_2O	+109	−27	α-Gal$p$-(1 → 6)-β-Fru$f$	+64	+173	+59	19
Melezitose 1	+100	−31					17
Melezitose 2	+110	−43					18
Sucrose	+108	−44					20
Panose			α-Glc$p$-(1 → 6)-α-Glc$p$	+64	+173	+59	22, 23
Gentiobiose			β-Glc$p$-(1 → 6)-β-Glc$p$	−62	−156	−58	24
Isomaltulose·H_2O			α-Glc$p$-(1 → 6)-β-Fru$f$	−65	+144	+77	21
Melibiose·H_2O			α-Gal$p$-(1 → 6)-β-Glc$p$	−64	−174	+76	25
Cycloinulohexaose			β-Fru$f$-(2 → 1)-β-Fru$f$	−73, −72	+178, +178	−71, −67	26

[a] O_r is the ring oxygen, O_l is the glycosidic linkage oxygen.

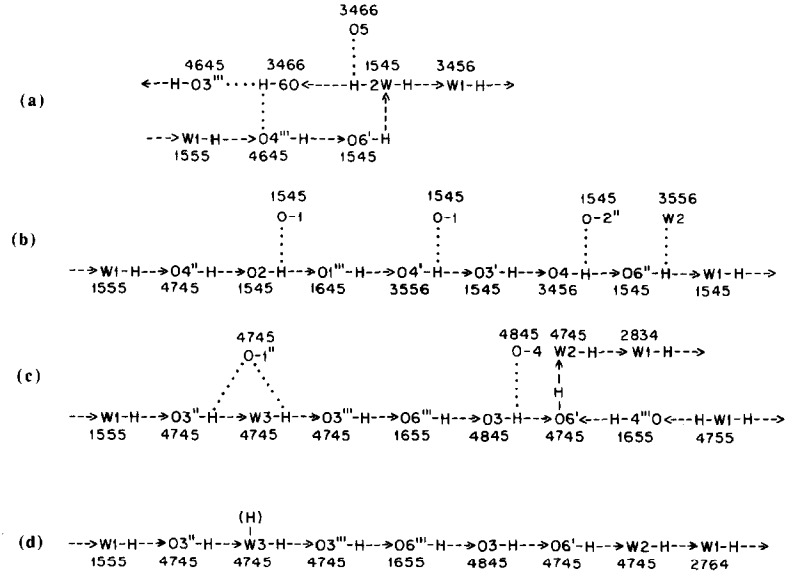


Fig. 2. The symmetry relations in the infinite hydrogen-bond chains with respect to W-1 in the crystal structure of nystose trihydrate. (a) The chain in *a* direction; (b) The chain extends in the *b* direction through translation operation; (c) The chain extends in the *b* direction through two-fold screw axis; (d) The chain in the *c* direction. Symmetry code as in Table V.

Of the fourteen hydroxyl groups, seven form three-center bonds; these include all four hydroxyls from the glucopyranose component. With one exception, that from O-6-H, the three-center bonds are unsymmetrical, with a major and a minor component. In three cases, the minor interaction is intramolecular.

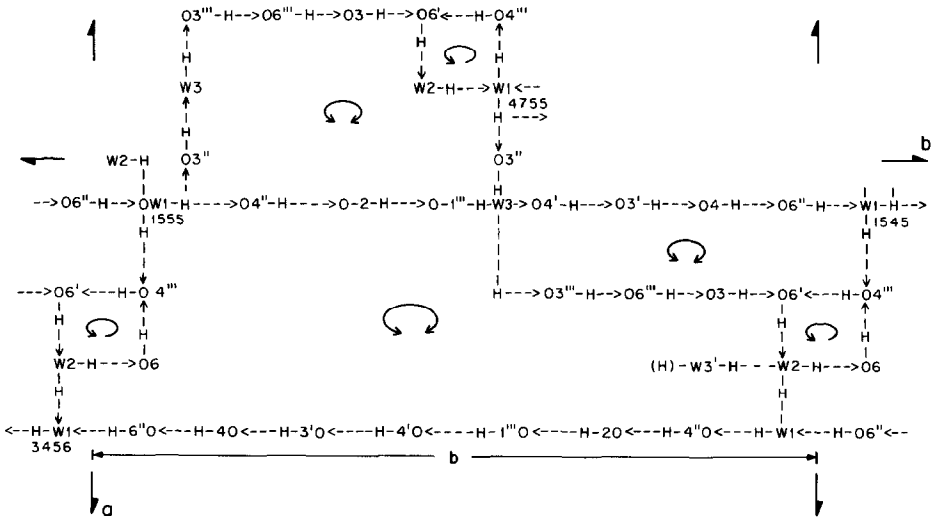


Fig. 3. The hydrogen-bonding scheme in the crystal structure of nystose trihydrate.

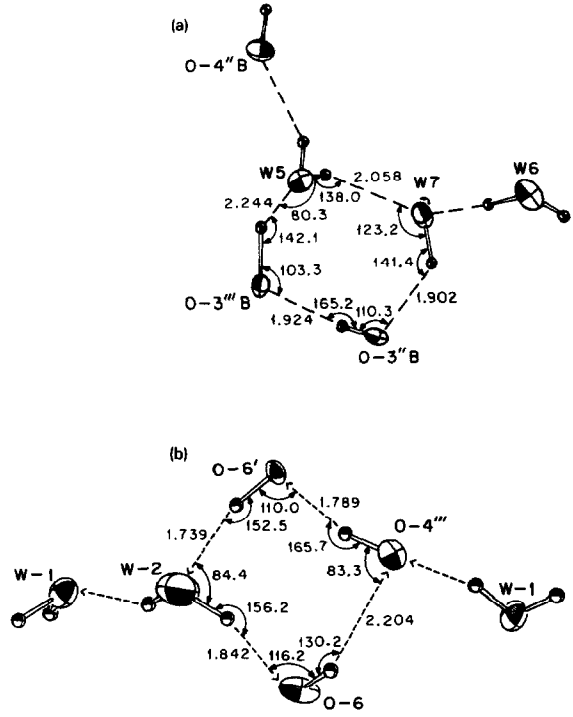


Fig. 4. The geometry of the four-membered homodromic cycles: (a) in stachyose tetrahydrate; (b) in nystose trihydrate.

The water molecules W-1 and W-2 form a hydrogen-bonded dimer. This bonds to the glucose primary hydroxyl oxygen O-6, and to O-3″, O-4″, and O-4‴ of the second and third fructofuranoses. Since these oxygens are in different molecules,

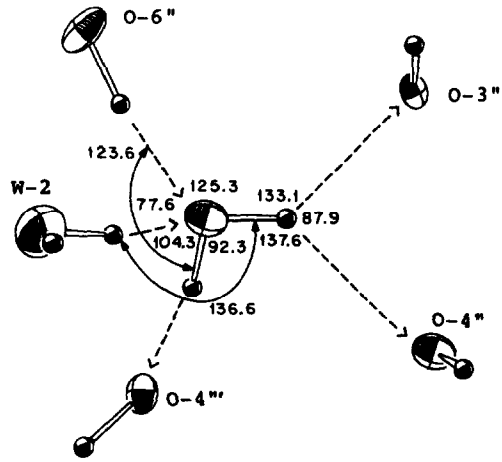
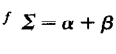


Fig. 5. The configuration of the five-coordinated water molecule, W-1.

TABLE V

Hydrogen-bond lengths and angles in nystose trihydrate

Donor	Acceptor	Acceptor symmetry code [a]	Lengths (Å)			Angle (deg)	Σ [f]
			H---O		O---O [d]	O—H---O	
			Observed [b]	Normalized [c]		Observed [e]	
Two-center							
O-3′-H	O-4	3466	1.84	1.73	2.655	158	341
O-6′-H	O-W-2	1555	1.81	1.74	2.636	153	
O-4″-H	O-2	4745	2.06	1.73	2.675	168	
O-1‴-H	O-4′	3466	1.93	1.78	2.737	170	
O-3‴-H	O-6‴	4655	1.91	1.78	2.719	162	
O-4‴-H	O-6′	4645	1.71	1.79	2.740	166	
O-6‴-H	O-3	4745	1.84	1.91	2.784	149	
Three-center							
O-2-H	O-1‴	1655	2.15	2.01	2.720	129	341
	O-1	1555 [g]	2.43	2.34	2.898	110	
O-3-H	O-6′	1655	2.04	1.86	2.779	158	353
	O-4	1555 [g]	2.58	2.53	2.875	101	
O-4-H	O-6″	3566	1.79	1.65	2.602	168	346
	O-2″	3566	2.87	2.84	3.189	103	
O-6-H	O-4‴	2675	2.17	2.20	2.926	130	360
	O-3‴	2675	2.35	2.40	3.269	149	
O-4′-H	O-3′	3466	1.93	1.78	2.701	161	353
	O-1	3466	2.73	2.65	3.258	122	
O-3″-H	O-W-3	1555	1.75	1.77	2.701	154	360
	O-1″	1555 [b]	2.87	2.88	3.388	115	
O-6″-H	O-W-1	1555	1.84	1.70	2.660	170	358
	O-W-2	3566	2.77	2.78	3.303	115	
From water donors							
W-1-H-1	O-4‴	4646	1.85	1.80	2.746	165	
W-1-H-2	O-3″	4745	2.10	2.11	2.854	133	359
	O-4″	4745	2.34	2.35	3.133	138	
W-2-H-1	O-6	3476		1.84	2.754	156	328
	O-5	3476		2.76	3.106	102	
W-2-H-2	O-W-1	3466		1.92	2.802	153	
W-3-H-1	O-3‴	1555	2.18	1.89	2.793	155	349
	O-1″	1555		2.77	3.406	124	

[a] Symmetry code. The last three digits give the unit cell translation in the a, b, and c directions with respect to 555; the first digit specifies one of the following operations: 1; x, y, z; 2: $1/2-x$, $-y$, $1/2+z$; 3: $1/2+x$, $1/2-y$, $-z$; 4: $-x$, $1/2+y$, $1/2-z$. [b] Estimated standard deviations ±0.05 Å. [c] O–H bond distance normalized to internuclear value of 0.97 Å. [d] Estimated standard deviations ±0.005 Å. [e] Estimated standard deviation ±1°. Those involving O—H---O angles of less than 90° were excluded. [f] $\Sigma = \alpha + \beta + \gamma$ in  ; when $\Sigma = 360°$, H is in the plane of the three oxygens of the three-center bond. [g] Intramolecular hydrogen bonds.

there is no indirect intramolecular bonding through a water molecule, as is observed in the crystal structures of some disaccharides[33]. The water molecule W-1 has five oxygen nearest neighbours, accepting two two-center bonds and donating one two-center and one three-center bond, as shown in Fig. 5. W-2 has the more common four-fold environment, except when there is a hydrogen bond from the low occupancy site of W-3. There are only two oxygens within 3.4 Å of W-3, which accepts a bond from H-O-3″ and donates to O-3‴. The other hydrogen of W-3 is not involved in H-bonding. The location of the low occupancy site W-3′ suggests that it forms a hydrogen bond with O-W-2 at an O–O distance of 2.77 Å.

The hydrogen bonding in this structure is similar in principle to that observed in the hydrates of the disaccharide lactulose[34], the trisaccharide raffinose[16], the tetrasaccharide stachyose[3], and of the β-cyclodextrins[35], where infinite chains intersect at water molecules to form a three-dimensional network, not unlike that of the less symmetrical high pressure ices[33,36].

NOTE ADDED IN PROOF

A room temperature X-ray crystal structure analysis of nystose trihydrate appeared in *Bull. Chem. Soc. Jpn.*, 66 (1993) 374–379.

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