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Hydrogen bonding in the crystal structure of the tetrasaccharide stachyose hydrate: a 1:1 complex of two conformers

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ABSTRACT

The crystal structure of stachyose hydrate, *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl β-D-fructofuranoside tetrahydrate, has been refined using X-ray data at 119 K. The crystal structure is a 1:1 complex of conformers which differ in the fructofuranosyl and glucopyranosyl residues. Each conformer has an associated hydrogen-bond structure which includes different sites for the water molecules. When superimposed in the crystal, this gives rise to two sites of 0.5 occupancy for one carbon and one oxygen atom of the fructofuranose component, and for three oxygen atoms of the glucopyranose component. The corresponding three carbon atoms of the glucopyranose component have anomalous thermal motion parameters. The four water molecules are distributed over nine sites, six with occupancy 0.5, two with occupancy 0.4, and one with occupancy 0.2. Four of the water sites are associated with one conformer, and four with the other. The hydrogen bonding includes infinite chains which are linked to form irregular ribbons, extending in the direction of the *a* axis. All hydroxyl, ring, and linkage oxygen atoms are involved in the hydrogen bonding, which includes two-centered, three-centered, and four-centered hydrogen bonds.

INTRODUCTION

Stachyose, together with raffinose, is one of the earliest carbohydrates to be studied crystallographically, since its morphology is described in Groth's *Chemische Kristallographie*[1]. The crystal structure was been previously determined by X-ray diffraction at room temperature[2]. In that analysis, two carbon and two oxygen atoms of the glucopyranosyl unit were found to be disordered. Five water molecules were distributed over seven sites with partial occupanices, but no rational hydrogen-bond scheme could be proposed. This low-temperature structure analysis was undertaken to attempt to resolve the disorder and determine the hydrogen-bonding pattern.

EXPERIMENTAL

Stachyose hydrate (Sigma Chemical Co.) was recrystallized from 90% ethanol in a vacuum dryer. The crystal used for diffraction was protected with vacuum grease. The details of the data collection and structure refinement are given in Table I. Since lower symmetry was a possibility, the data were collected over both hkl and hk$\bar{l}$ quadrants. The comparison of equivalent reflections gave no evidence of other than orthorhombic symmetry, with an agreement index of $R = 0.02$.

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A full-matrix least-squares refinement was started with the coordinates of the ordered atoms from the room temperature analysis. The positions of the disordered carbon and oxygen atoms were determined from successive difference syntheses during the course of the refinement. All the hydrogen atoms were located with the exception of one attached to O-W-8 and those on the low occupancy site O-W-57.

The final atomic parameters are given in Table II*. The atomic notation and the thermal ellipsoids for conformer A are shown in Fig. 1. The disorder in the positions of atoms C-3‴ and O-3‴ gives rise to resolved electron density peaks corresponding to the two fructofuranosyl conformations, A and B, shown in Fig. 2. For the glucopyranosyl component, disorder is shown by the double peaks of O-2″, O-3″, and O-4″, and the anomalous thermal parameters for C-2″, C-3″, and C-4″, shown in Fig. 3. The dual atomic positions assigned for the carbon atoms, given in Table II, were obtained by fitting two $^4C_1$ pyranose rings with standard geometries[4] so as to best fit the double peaks and thermal ellipsoids. Disorder in two of the residues permits four conformers in the crystals, *i.e.* A″A‴, B″B‴, A″B‴, B″A‴. Only the first two combinations provided the rational hydrogen bonding scheme described below without inadmissably short O···O separations. With the exception of the disordered atoms, there is close agreement between the room-temperature and low-temperature atomic positions. On going to the lower temperature there is an average decrease of 1.3 Å^2 in isotropic equivalent temperature factors for the carbon atoms, 2.3 Å^2 for the hydroxyl oxygen atoms, and 7.8

TABLE I

Crystal and structure-refinement data for stachyose tetrahydrate at 119 K

Crystal data		
$C_{24}H_{42}O_{21}\cdot 4\ H_2O$, mol. wt. 738.6, $P2_12_12$, $Z = 4$		
Cell dimensions (Å)	119 K	295 K
a	12.7144(7)	12.801(6)
b	24.033(1)	24.026(5)
c	10.8031(6)	10.856(6)
V (Å^3)	3301.1	3338.8
D_x (g/cm^3)	1.486	1.496 (based on 5 H_2O)
D_m (g/cm^3)		1.479 (this work)

Experimental and refinement data

Crystal dimensions, 0.23 × 0.44 × 0.44 mm

Number of intensities measured on a CAD-4 diffractometer, by ω-2θ scans to $\theta < 72°$, 3653 (3522 with $I > 2\sigma(I)$

Radiation Cu-Kα (λ = 1.5418 Å), Ni-filtered, $\mu_{CuK\alpha}$ = 12.21 cm^{-1}

Refinement using UPALS[3], minimizing $w(k|F_o| - |F_c|)^2$, where $w = 1/\sigma^2(F)$

Number of parameters, 805

Final agreement factors: $R(F) = 0.037$, $R_w(F) = 0.034$, including all reflections

* Lists of anisotropic temperature parameters, bond lengths and valence angles, and observed and calculated structure factors 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/449/*Carbohydr. Res.*, 210 (1991) 89–104.

TABLE II

Fractional atomic positional parameters and equivalent isotropic thermal parameters for stachyose tetrahydrate at 119 K

Atom	x/a[a]	y/b[a]	z/c[a]	B_{eq} ($Å^2$)[a,b]	*Occupancy*[c]
O-1	2142(2) x 10^{-4}	2259(1) x 10^{-4}	5646(2) x 10^{-4}	147(5) x 10^{-2}	
O-2	986(2)	3087(1)	4688(2)	184(5)	
O-3	2725(2)	3738(1)	3687(2)	203(5)	
O-4	3878(2)	3870(1)	5943(2)	170(5)	
O-5	3029(2)	2856(1)	6958(2)	135(5)	
O-6	5856(2)	2958(1)	6495(2)	204(5)	
O-1′	3473(2)	709(1)	3965(2)	131(5)	
O-2′	3233(2)	−356(1)	5073(2)	160(5)	
O-3′	1047(2)	−193(1)	5647(2)	172(5)	
O-4′	1412(2)	701(1)	7296(2)	159(5)	
O-5′	3176(2)	1127(1)	5890(2)	117(4)	
O-1″	4978(2)	1194(1)	−120(2)	255(6)	
O-2″A	3303(3)	850(2)	−1519(3)	194(11)	0.50
O-2″B	3162(3)	1126(2)	−1436(4)	172(10)	0.50
O-3″A	1695(3)	1363(2)	39(5)	250(12)	0.50
O-3″B	1767(3)	1720(2)	232(4)	170(10)	0.50
O-4″A	2701(3)	1937(2)	2326(5)	245(12)	0.50
O-4″B	2844(3)	1994(2)	2499(5)	274(13)	0.50
O-5″	4295(2)	712(1)	1556(2)	156(5)	
O-1‴	5170(2)	1257(1)	−2765(2)	338(7)	
O-2‴	6404(2)	574(1)	−128(2)	318(7)	
O-3‴A	6366(3)	1931(2)	−1025(4)	208(11)	0.50
O-3‴B	6065(3)	2152(2)	−536(4)	192(10)	0.50
O-4‴	8143(2)	1687(1)	716(2)	309(7)	
O-6‴	6438(2)	612(1)	2560(2)	216(6)	
C-1	2083(2)	2761(1)	6295(3)	132(7)	
C-2	1917(2)	3226(1)	5362(3)	146(7)	
C-3	2874(2)	3281(1)	4514(3)	138(7)	
C-4	3876(2)	3357(1)	5289(3)	137(6)	
C-5	3962(2)	2878(1)	6224(3)	137(6)	
C-6	4876(2)	2934(1)	7134(3)	172(7)	
C-1′	3523(2)	644(1)	5263(3)	117(6)	
C-2′	2856(2)	143(1)	5600(3)	126(6)	
C-3′	1700(2)	264(1)	5311(3)	128(6)	
C-4′	1373(2)	793(1)	5984(3)	117(6)	
C-5′	2089(2)	1269(1)	5643(3)	114(6)	
C-6′	1856(2)	1789(1)	6376(3)	138(7)	
C-1″	4273(3)	771(2)	244(3)	228(8)	
C-2″A	3170(5)	846(3)	−232(5)	151(14)	0.50
C-2″B	3180(4)	1007(2)	−110(5)	118(13)	0.50
C-3″A	2786(5)	1360(3)	397(5)	143(13)	0.50
C-3″B	2826(4)	1561(2)	490(5)	128(13)	0.50
C-4″A	2911(5)	1395(3)	1829(6)	191(15)	0.50
C-4″B	2952(5)	1471(3)	1900(6)	246(17)	0.50
C-5″	4044(2)	1223(1)	2181(3)	144(7)	
C-6″	4185(2)	1130(1)	3551(3)	151(7)	
C-1‴	5643(3)	826(2)	−2066(3)	364(12)	
C-2‴	5909(3)	1025(2)	−750(3)	348(11)	
C-3‴A	6870(5)	1407(3)	−804(6)	161(14)	0.50
C-3‴B	6492(4)	1581(3)	−568(5)	136(13)	0.50
C-4‴	7166(3)	1402(2)	577(3)	271(9)	

continued overleaf

TABLE II (continued)

Atom	x/a[a]	y/b[a]	z/c[a]	B_{eq} ($Å^2$)[b,d]	*Occupancy*[c]
C-5‴	7298(3)	773(2)	568(3)	278(9)	
C-6‴	7324(3)	487(2)	1818(3)	263(9)	
O-W-1	3959(4)	2808(2)	1324(5)	274(12)	0.50
O-W-2	4456(3)	3894(2)	2134(4)	295(14)	0.50
O-W-3	6040(4)	2655(2)	873(5)	276(12)	0.50
O-W-4	5706(5)	4661(2)	−167(5)	432(18)	0.50
O-W-5	5206(5)	7488(3)	1742(7)	330(18)	0.40
O-W-6	5477(4)	5412(2)	1833(5)	300(14)	0.50
O-W-7	5195(4)	6431(2)	694(6)	231(14)	0.40
O-W-8	4743(4)	5284(2)	−1451(5)	320(14)	0.50
O-W-57	5336(12)	4473(7)	−2514(13)	492(48)	0.20
H-1	150(2) x 10^{-3}	275(1) x 10^{-3}	695(2) x 10^{-3}	1(1)	
H-2	184(2)	362(1)	586(2)	0(1)	
H-3	295(2)	294(1)	398(2)	0(1)	
H-4	444(2)	333(1)	470(2)	1(1)	
H-5	398(2)	247(1)	573(2)	1(1)	
H-61	492(2)	261(1)	766(2)	1(1)	
H-62	482(2)	332(1)	761(3)	2(1)	
H-1′	427(2)	56(1)	550(2)	0(1)	
H-2′	297(2)	11(1)	635(3)	2(1)	
H-3′	155(2)	33(1)	439(3)	2(1)	
H-4′	61(2)	88(1)	575(2)	1(1)	
H-5′	199(2)	135(1)	480(2)	1(1)	
H-61′	118(2)	179(1)	652(3)	2(1)	
H-62′	229(2)	180(1)	721(3)	2(1)	
H-1″	449(2)	34(1)	−12(3)	3(1)	
H-2″A	283(4)	49(2)	11(5)	0(1)	
H-2″B	262(4)	74(2)	16(5)	0(1)	
H-3″A	317(4)	167(2)	−9(5)	1(1)	
H-3″B	324(4)	191(2)	16(5)	0(1)	
H-4″A	240(4)	108(2)	221(4)	0(1)	
H-4″B	243(4)	113(2)	226(5)	4(2)	
H-5″	451(2)	152(1)	197(2)	0(1)	
H-61″	398(3)	154(1)	398(3)	3(1)	
H-62″	495(2)	101(1)	375(3)	1(1)	
H-11‴	512(3)	45(1)	−212(3)	2(1)	
H-12‴	621(3)	72(1)	−245(3)	3(1)	
H-3‴A	737(4)	134(2)	−131(5)	1(1)	
H-3‴B	694(6)	161(3)	−125(7)	5(2)	
H-4‴	668(3)	150(1)	134(3)	4(1)	
H-5‴	798(2)	68(1)	10(3)	3(1)	
H-6‴	799(3)	62(1)	221(3)	4(1)	
H-62‴	731(2)	−1(1)	177(2)	1(1)	
H-O-2	92(3)	340(2)	414(3)	5(1)	
H-O-3	256(3)	406(2)	412(3)	5(1)	
H-O-4	390(3)	423(2)	539(3)	5(1)	
H-O-6	597(3)	261(1)	618(3)	5(1)	
H-O-2′	330(2)	−30(1)	435(3)	1(1)	
H-O-3′	100(4)	−11(2)	656(4)	7(2)	
H-O-4′	93(3)	74(2)	766(4)	7(1)	
H-O-2″A	272(3)	91(2)	−176(3)	5(1)	
H-O-2″B	265(3)	88(2)	−182(3)	5(1)	
H-O-3″A	142(4)	160(2)	−4(5)	1(1)	
H-O-3″B	166(4)	204(2)	24(5)	1(1)	

TABLE II (continued)

Atom	x/a[a]	y/b[a]	z/c[a]	B_{eq} ($Å^2$)[a,b]	*Occupancy*[c]
H-O-4''A	218(2)	200(1)	267(3)	2(1)	
H-O-4''B	209(2)	198(1)	270(3)	2(1)	
H-O-1'''	464(2)	128(1)	−258(3)	2(1)	
H-O-3'''A	576(7)	191(4)	−164(9)	7(3)	
H-O-3'''B	510(8)	213(4)	33(8)	6(3)	
H-O-4'''	804(4)	192(2)	135(5)	7(2)	
H-O-6'''	593(3)	51(1)	201(3)	3(1)	
H-1-W-1	351(5)	293(3)	79(6)	4(2)	
H-2-W-1	385(7)	244(4)	166(9)	8(3)	
H-1-W-2	442(6)	341(3)	175(7)	6(2)	
H-2-W-2	395(5)	394(3)	262(6)	3(2)	
H-1-W-3	535(19)	258(9)	134(21)	8(4)	
H-2-W-3	619(6)	244(3)	−14(8)	6(2)	
H-1-W-4	601(6)	428(3)	−1(6)	4(2)	
H-2-W-4	505(4)	452(2)	30(4)	0(1)	
H-1-W-5	578(7)	761(3)	230(8)	7(3)	
H-2-W-5	553(4)	725(2)	114(5)	0(1)	
H-1-W-6	540(7)	581(4)	117(9)	10(3)	
H-2-W-6	534(4)	511(2)	125(5)	1(1)	
H-1-W-7	439(7)	650(3)	48(8)	3(2)	
H-2-W-7	554(8)	646(4)	−10(9)	6(3)	
H-1-W-8	524(4)	560(2)	−152(5)	0(1)	

[a] E.s.d. values given in parentheses refer to the last significant digit. [b] $B_{eq} = 4/3\ (\sigma_{ij} B_{ij} a_i a_j)$, calculated in $Å^2$ from the refined anisotropic thermal parameters. [c] The occupancy is 1.0, except where stated otherwise. Hydrogen atoms have the occupancy of the atoms to which they are bonded.

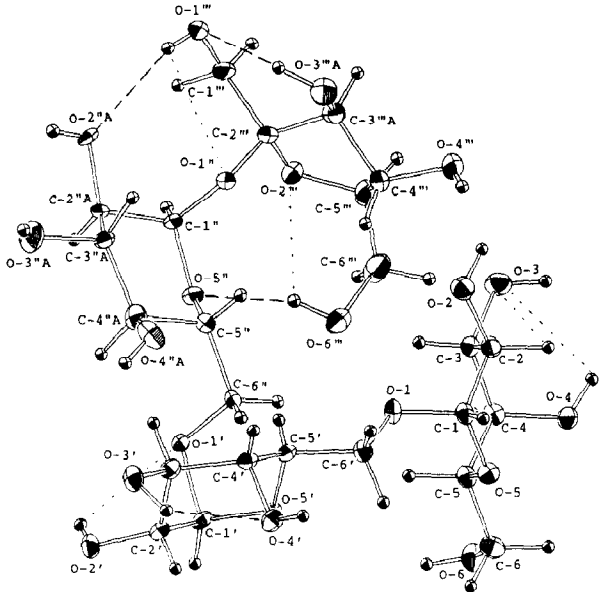

Fig. 1. Atomic notation and thermal ellipsoids (50% probability) for stachyose at 119 K, conformer A. Dotted lines are intramolecular hydrogen bonds.

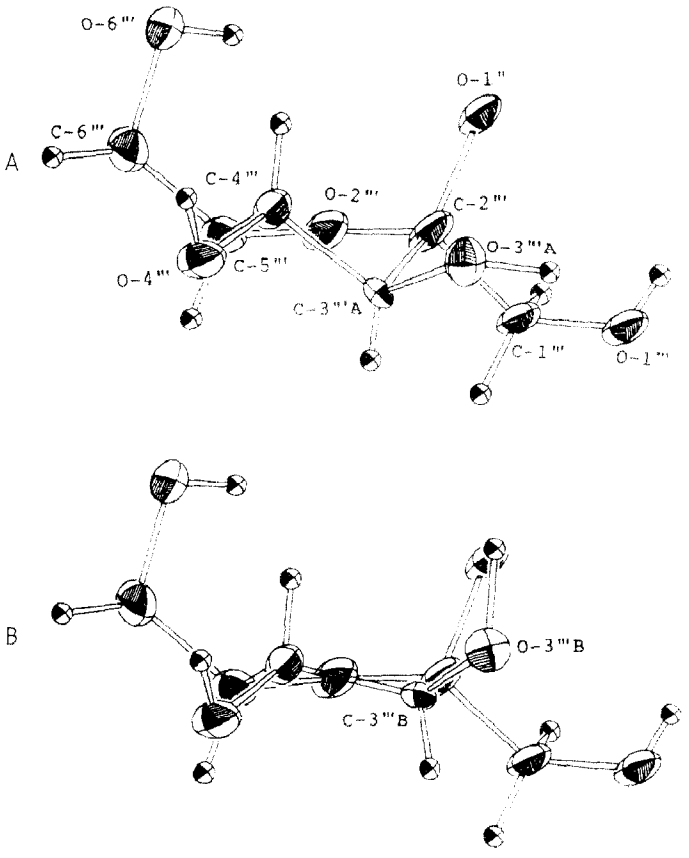

Fig. 2. The fructofuranosyl components of stachyose: upper, conformer A; lower, conformer B.

Å^2 for the water oxygen atoms. The last corresponds to a reduction in r.m.s. amplitude of motion of 0.54 Å.

RESULTS AND DISCUSSION

Conformational differences. — The principal difference between the conformers A and B is in the fructofuranosyl residue. The Cremer and Pople puckering parameters[5] are given in Table III, together with those of some related oligosaccharides. With the exception of one of the fructofuranosyl rings in 1-kestose, the furanose ring conformations are in the $^4T_3$ to $_3T^4$ region of the pseudorotational circle[5].

The fructofuranose rings of the two conformers differ in the degree of puckering, Q, shown in Table III, and in the ring torsion angles, shown in Table IV. The puckering of conformer A is significantly greater than in other oligosaccharides, while that of B is smaller. In the room temperature study, the disorder in the glucopyranosyl ring led to resolved atom positions, but not in the fructofuranose rings. A differential thermal

calorimetry measurement gave no evidence of phase transition. The relaxation of the hydrogen bonds, due to the increased thermal motion at room temperature *versus* that at low temperature, could account for these differences.

The D-Gal*p*-(1→6)-D-Glc*p* and D-Glc*p*-(1↔2)-D-Fru*f* linkages in stachyose occur in several di- and tri-saccharides with known crystal structures. The linkage torsion angles are compared in Table V. With the exception of gentiobiose, the (1→6) torsion angles are $+sc$, ap, $-sc$. The (1↔2) linkages are more variable, with the closest correspondence between stachyose, melezitose-2, and sucrose. Only in stachyose and sucrose are there two intramolecular inter-residue hydrogen bonds, and it would appear that such hydrogen bonding is not a critical factor in determining these inter-residue conformations.

The C–C bond lengths and valence angles are in the normal range[4]. Those in the disordered rings appear to be slightly larger than in the ordered: 1.505–1.558 Å *versus* 1.505–1.536 Å. The exocyclic C–O bond lengths vary more in the disordered residues

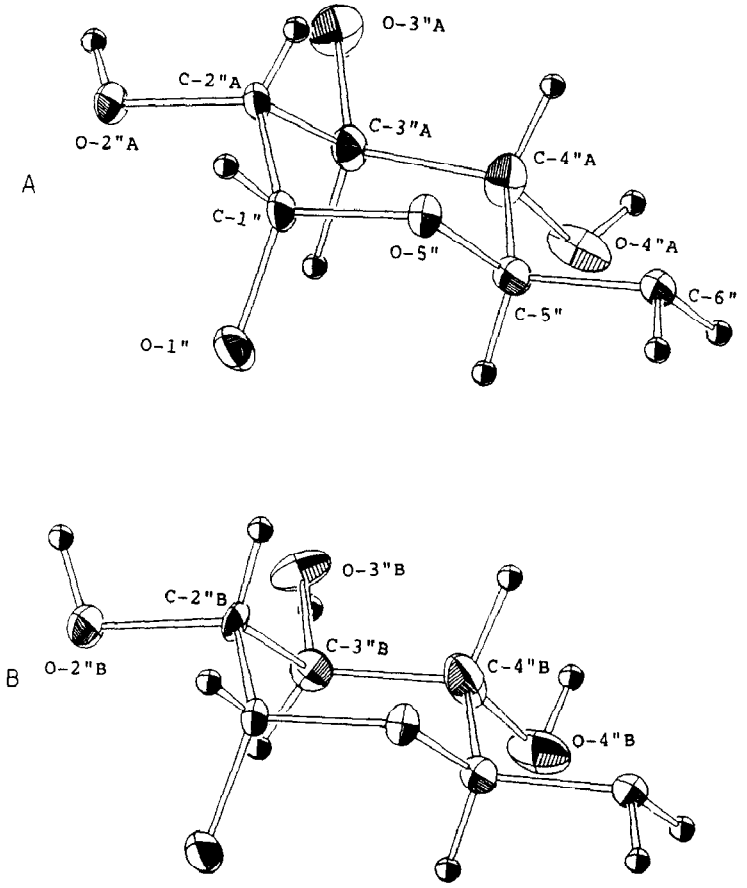

Fig. 3. The glucopyranosyl components of stachyose: upper, conformer A; lower, conformer B.

TABLE III

Cremer-Pople puckering parameters[a] for fructofuranose, galactopyranose, and glucopyranose rings in oligosaccharides[a]

Sugar	*Fructofuranose*		*Galactopyranose*		*Glucopyranose*		*Refcode*[b]	*Lit. ref.*
	Q(Å)	ψ(°)	Q(Å)	ψ(°)	Q(Å)	ψ(°)		
Stachyose 119 K	0.60	265	0.58	1	0.57	10	this work	
	0.26[c]	281[c]	0.57	2	0.56[c]	2[c]		
295 K	0.41	272	0.56	1	0.55	6	STACHY10	2
			0.57	3	0.59	1		
Raffinose 119 K	0.40	274	0.57	2	0.58	3		8
295 K	0.40	274	0.56	1	0.58	4	RAFINO	9
1-Kestose	0.42	254			0.58	9	KESTOS	10
	0.30	101						
6-Kestose	0.35	252			0.55	13	CELGIJ	11
	0.42	273						
Melezitose-1	0.39	276			0.60	3	MELEZT01	12
					0.54	3		
Melezitose-2	0.36	251			0.58	10	MELEZT02	13
					0.55	4		
Planteose	0.39	266	0.56	2	0.55	13	PLANTE10	14
Sucrose	0.35	265			0.56	5	SUCROS04	15

[a] For $^4C_1$ rings when $\theta = 0$, the value of ψ is not significant[6]. [b] From the Cambridge Crystallographic Data Base[7]. [c] Parameters for conformer B.

than in the ordered: 1.401–1.476 Å *versus* 1.411–1.436 Å. The ring C-1–O-5 and C-2‴–O-2‴ bonds are consistently shorter (1.425, 1.426 Å) than the C-5–O-5 and C-5‴–O-2‴ bonds (1.444, 1.445 Å). The orientations of the primary alcohol groups are *t*/*g* and *g*/*g* in the fructofuranosyl residue and *t*/*g* in the terminal galactopyranosyl residue (Table IV).

Hydration and hydrogen bonding. — The four water molecules are distributed over nine sites: W-1 to W-4, W-6, and W-8 have 0.5 occupancy, W-5 and W-7 have 0.4 occupancy, and W-57 has 0.2 occupancy. There is a correspondence with the seven water-oxygen sites reported with occupancies ranging from 0.34 to 0.70 in the room-temperature analysis. The two additional sites in this analysis are O-W-5 and O-W-8.

Sites W-1 and W-2 are hydrogen-bonded with hydroxyls, which include O-2″A and O-4″A, to form infinite chains, shown in Fig. 4 (upper). Sites W-5, W-6, and W-7 are also hydrogen-bonded with hydroxyls, including O-2″B and O-4″B, to form similar infinite chains, shown in Fig. 4 (lower). These infinite chains are linked in pairs to form ribbons which extend in the *a* axis direction of the crystal structure. The A chains are linked through O-3‴A–H and H–O-W-3–H, as shown in Fig. 5. The B chains are linked through O-3‴B–H and the minor components of three-center bonds to the linkage oxygen O-1″, as shown in Fig. 6. The hydrogen bonding in the infinite chains is ordered, and of opposite polarity, in conformers A and B. Individually, their symmetry is $P2_12_12_1$, but together they are $P2_12_12$, so that the polarities cancel. This suggests that the crystal has antiparallel domains containing the conformers A and B. The diffraction experiment does not permit us to distinguish this domain structure from a random array.

TABLE IV

Selected torsion angles (°) of stachyose tetrahydrate at 119 K

Pyranosyl rings	*Galacto C-1—O-5*	*Galacto C-1′—O-5′*	*Gluco C-1″—O-5″*	
			Molecule A	*Molecule B*
C-1–C-2–C-3–C-4	−54.5(3)	−55.5(3)	−50.7(6)	−55.2(6)
C-2–C-3–C-4–C-5	54.4(3)	55.6(3)	47.6(6)	49.8(6)
C-3–C-4–C-5–O-5	−56.5(3)	−55.7(3)	−48.5(5)	−56.3(5)
C-4–C-5–O-5–C-1	60.8(3)	57.5(3)	62.2(4)	60.0(4)
C-5–O-5–C-1–C-2	−60.1(3)	−58.3(3)	−70.4(4)	−55.9(3)
O-5–C-1–C-2–C-3	56.0(3)	56.9(3)	59.5(5)	58.4(5)
Furanosyl ring	*C-2‴—O-2‴*			
	Molecule A	*Molecule B*		
C-2‴–C-3‴–C-4‴–C-5‴	58.4(4)	25.3(4)		
C-3‴–C-4‴–C-5‴–O-2‴	−42.4(3)	−25.1(4)		
C-4‴–C-5‴–O-2‴–C-2‴	13.1(4)	13.1(4)		
C-5‴–O-2‴–C-2‴–C-3‴	24.4(4)	3.5(4)		
O-2‴–C-2‴–C-3‴–C-4‴	−52.8(4)	−17.6(4)		
Linkage bonds				
O-5–C-1–O-1–C-6′	84.3(3)	O-1′–C-6″–C-5″–O-5″		−61.9(2)
C-1–O-1–C-6′–C-5′	−172.4(2)	C-1″–O-1″–C-2″–C-1‴		71.8(4)
O-1–C-6′–C-5′–O-5′	87.2(3)	C-1″–O-1″–C-2″–O-2‴		−47.4(4)
O-5′–C-1′–O-1′–C-6″	64.9(3)	O-5″–C-1″–O-1″–C-2‴		109.3(3)
C-1′–O-1′–C-6″–C-5″	−175.1(2)	O-1′–C-6″–C-5″–O-5″		−61.9(3)
Other exocyclic bonds				
O-5–C-5–C-6–O-6	179.3(2)			
O-2‴–C-2‴–C-1‴–O-1‴	−178.4(4)			
O-2‴–C-5‴–C-6‴–O-6‴	−63.3(4)			

The linkages between the infinite chains result in 14-membered link cycles, which are similar in shape for both conformers but differ in detail, as shown in Fig. 7. The two hydrogen-bonding schemes, shown in Figs. 5 and 6, have as common feature the ordered hydroxyls, and as mutually exclusive features the disordered hydroxyls and the water sites, as shown by the short inter-site distances given in Table VI. These two hydrogen bonding schemes are linked through the water molecules W-4, W-6, and W-8, as shown in Fig. 8.

Only one of the hydrogens of W-8 could be located, and none of the hydrogens of the low occupancy site, W-57. In consequence, this region of the hydrogen bonding scheme is less definitive. A neutron diffraction study at 10 K would be necessary to resolve this feature of the structure.

The hydrogen bonding is similar to that of the trisaccharide raffinose penta-

TABLE V

Comparison of linkage torsion angles in related oligosaccharides

Sugar	α-D-*Galp*-(1→6)-D-*Glcp*[a]			α-D-*Glcp*-(1↔2)-β-D-*Fruf*[b]		*Intramolecular inter-residue hydrogen bonds*	
	1	*2*	*3*	*4*	*5*		
Stachyose	64.9	−175.1	−61.9	−47.4	109.3	O-1‴ H ⋯ O-5′ O-1‴ H ⋯ O-2″ O-1‴ H ⋯ O-1″	O-6‴ H ⋯ O-5″ O-6‴ H ⋯ O-2‴
Raffinose[8]	71.7	−170.8	−63.1	12.0	82.1		O-6′ H ⋯ O-5 O-6′ H ⋯ O-2′
Planteose[14]				26.8	108.7		
Melezitose-1[12]				−30.7	99.8		
Melezitose-2[15]				−43.4	109.5		
1-Kestose[10]				−65.8	84.7		
6-Kestose[11]				−54.5	89.6		
Sucrose[15]				−44.4	107.6	O-1′ H ⋯ O-2 O-1′ H ⋯ O-1	O-6′ H ⋯ O-5 O-6′ H ⋯ O-2′
Melibiose[17]	76.5	−173.9	−63.8				
Gentiobiose[16,c]	−58.3	−156.3	−61.5				

[a] 1, O-5′ C-1′ O-1′ C-6″; 2, C-1′ O-1′ C-6″ C-5″; 3, O-1′ C-6″ C-5″ O-5″. [b] 4, C-1″ O-1″ C-2‴ O-2‴; 5, O-5′ C-1″ O-1″ C-2‴. [c] β-D-Glc*p*-(1→6)-D-Glc*p*.

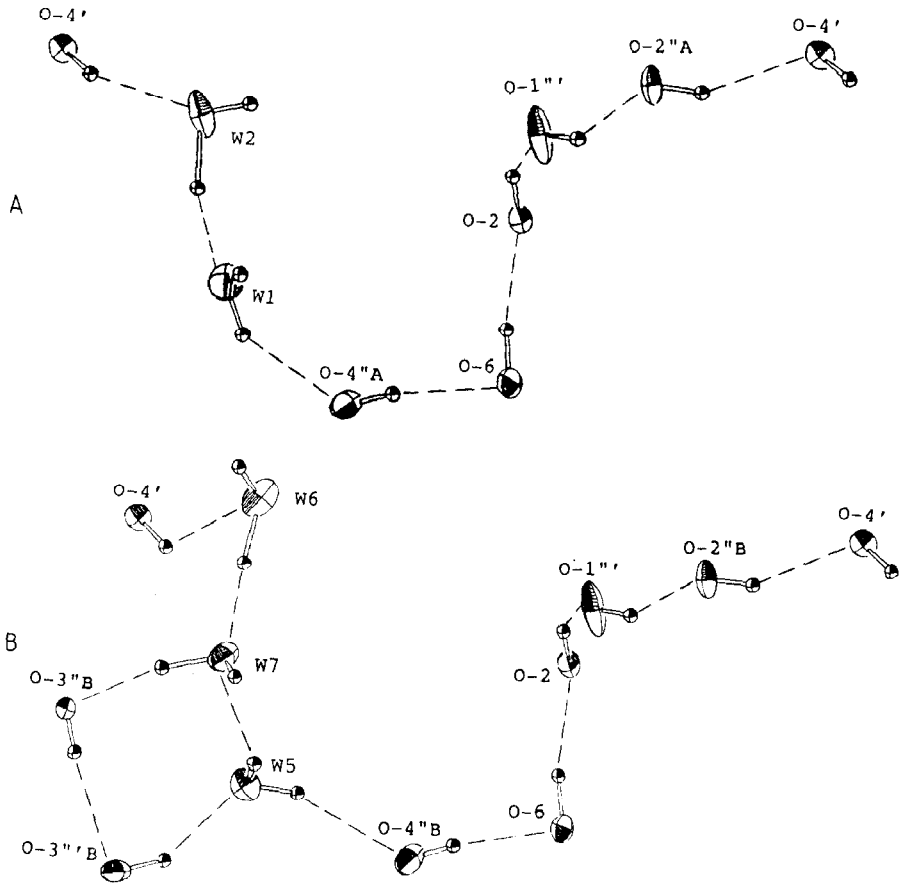

Fig. 4. The infinite chains of hydrogen bonds in stachyose tetrahydrate: upper, associated with conformer A; lower, associated with conformer B.

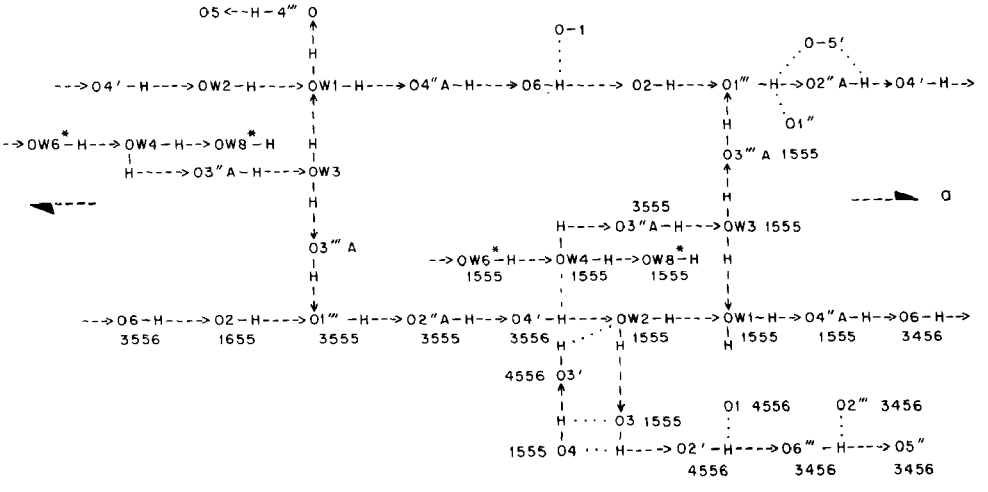

Fig. 5. Schematic of hydrogen bonding associated with conformer A.

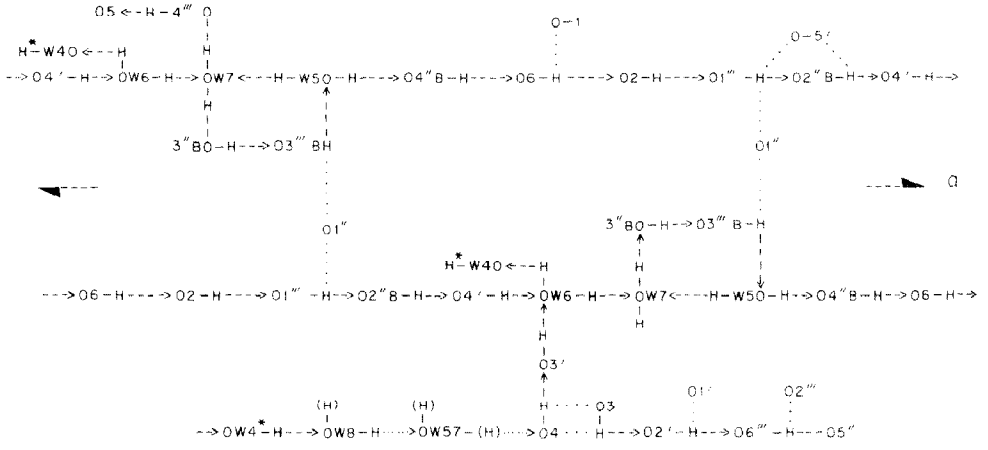

Fig. 6. Schematic of hydrogen bonding associated with conformer B.

hydrate[8] in the predominance of infinite chains, which in that structure intersect at the water molecules to form a three-dimensional network. The details of the hydrogen-bond geometry are given in Table VII. The hydrogen-bond lengths are within the normal range observed in carbohydrate crystal structures[19,20], and in the hydrates of carbohydrates, nucleosides, and nucleotides[21,22]. For the hydroxyl donors, the number of three-center bonds exceeds that of the two-centered, whereas the reverse is observed for the water donors. The majority of the three-center bonds are unsymmetrical, with minor intramolecular components. There is an inverse correlation between bond length and bond angle, which is a consequence of nonbonded van der Waals repulsion, *i.e.* the exclusion effect[23].

The cocrystallization of α and β anomers is common in the crystal structures of disaccharides[24], and lactulose is an example of cocrystallization which involves three different configurations[25]. In laminarabiose quarter-hydrate there is cocrystallization of

TABLE VI

Short interatomic distances (Å) between disordered atomic sites

O-W-1 O-W-5	1.361
O-W-1 O-W-7	2.230
O-W-2 O-W-6	1.697
O-W-2 O-W-7	1.799
O-W-3 O-W-5	1.885
O-W-3 O-3″B	2.133
O-W-3 O-3‴B	1.948
O-W-4 O-W-8	1.749
O-3″A O-2″B	2.531
O-3″A O-W-7	2.539
O-W-8 O-W-8′	1.381[a]

[a] Across the 2-fold axis.

anhydrous and hemihydrated molecules[26]. This crystal structure analysis of stachyose hydrate is the first well-established example of cocrystallization involving two different carbohydrate conformers. It suggests that even for molecules with limited conformational flexibility, a number of slightly different conformers can coexist in a crystal. If the

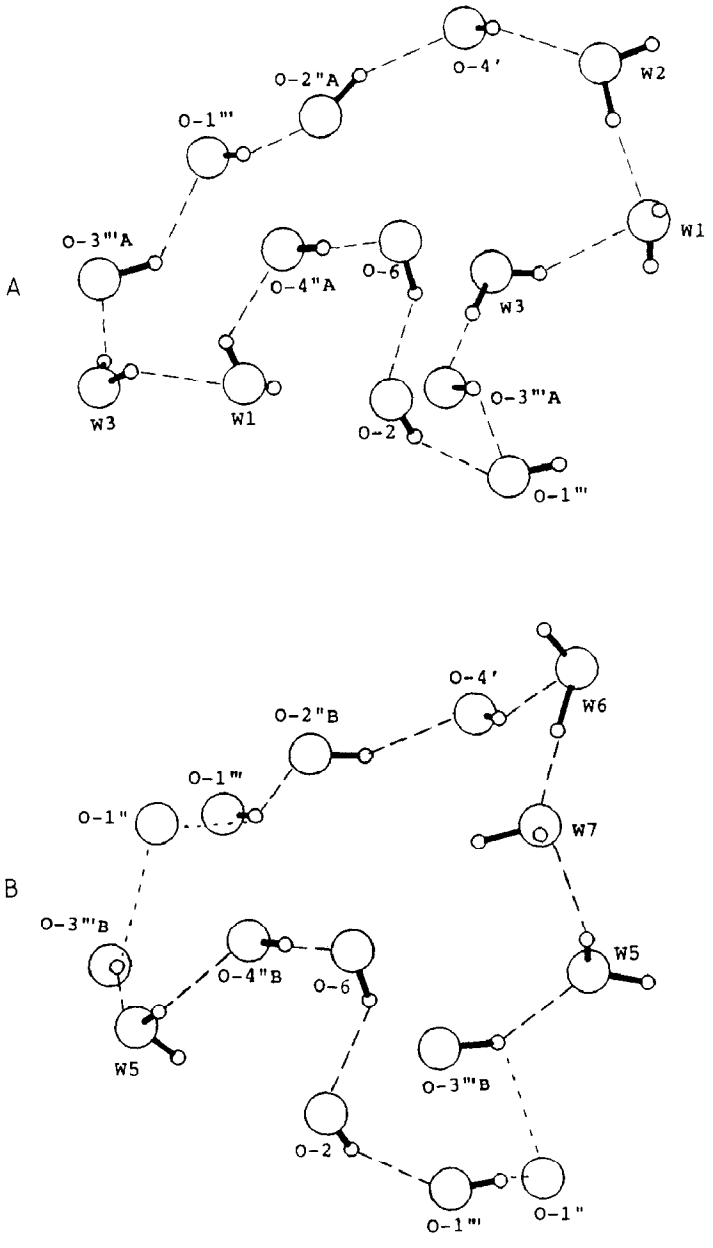

Fig. 7. The 14-membered cycles of hydrogen bonds that link the infinite chains: upper, associated with conformer A; lower, associated with conformer B.

crystals are highly hydrated, each conformer may have associated with it a different hydration hydrogen-bonding scheme. If there are a large number of these conformers in the crystal, the partially occupied water sites will be so numerous as to give the appearance of *unbound* water in a diffraction experiment.

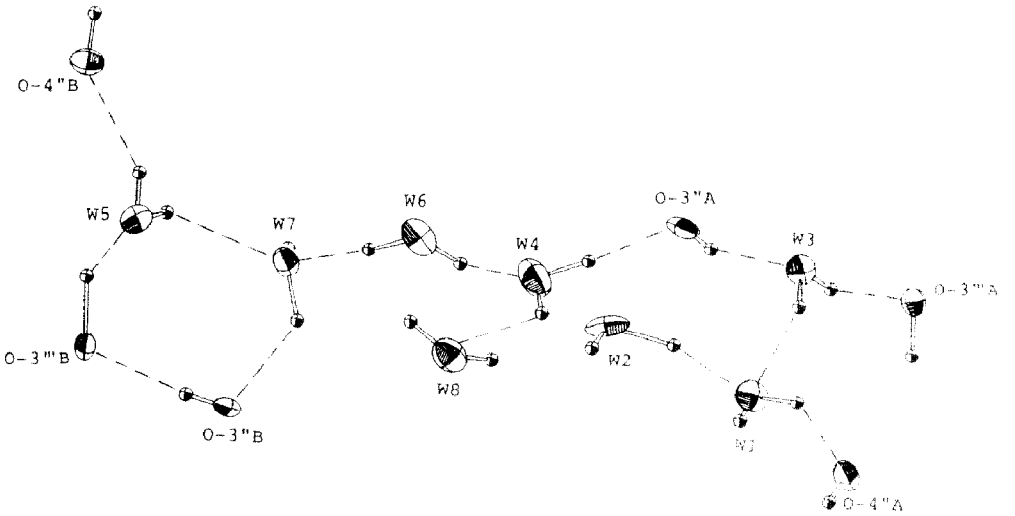

Fig. 8. The linkage between hydrogen-bond systems A and B. Note the 4-member homodromic cycle associated with conformer B, W5 and W7.

TABLE VII

Hydrogen-bond lengths and angles in stachyose tetrahydrate, with O–H bond lengths normalized to 0.97 Å

Donor	*Acceptor*	*Symmetry code*[a]	*H···O* (Å)	*O–H···O* (°)	Σ[b]
Two-centered					
O-3"A-H	O-W-3	3455	1.798	151.5	
O-3"B-H	O-3"'B	3455	1.924	165.2	
O-4"A-H	O-6	3456	1.718	172.9	
O-4"B-H	O-6	3456	1.811	162.9	
O-3"'A-H	O-1"'	4555	2.159	133.3	
O-4"'-H	O-5	3556	1.812	155.3	
Three-centered					
O-2-H	O-1"'	3455	1.942	147.3	354.4
	O-3	1555	2.477	107.3	
O-4-H	O-3'	4556	1.866	176.2	353.7
	O-3	1555	2.645	92.8	
O-6-H	O-2	3556	1.865	168.9	348.8
	O-1	3556	2.473	105.0	
O-2'-H	O-6"'	2655	1.949	147.5	359.6
	O-1'	1555	2.426	105.4	

O-3′-H ⋯ O-4′	1 555	2.189	122.4	359.5
O-W-2	4 546	2.850	110.0	
O-3′-H ⋯ O-W-6	3 456	2.026	143.7	359.7
O-4′	1 555	2.189	122.4	
O-4′-H ⋯ O-W-2	3 456	1.884	145.6	359.2
O-W-4	3 456	2.773	118.4	
O-4′-H ⋯ O-W-6	4 546	18.23	144.0	360.0
O-W-7	4 546	2.644	142.2	
O-2″A-H ⋯ O-4′	1 554	1.868	149.5	323.9
O-5′	1 554	2.627	95.2	
O-2″B-H ⋯ O-4′	1 554	1.896	155.3	328.2
O-5′	1 554	2.633	95.3	
O-3‴B-H ⋯ O-W-5	2 665	2.244	142.1	354.0
O-1″	1 555	2.349	102.1	
O-6‴-H ⋯ O-5″	1 555	2.147	138.2	
O-2‴	1 555	2.369	114.3	
Four-centered				
O-3-H ⋯ O-3′	4 556	2.523	117.4	
O-2′	4 556	1.894	162.0	
O-4	1 555	2.624	94.4	
O-1‴-H ⋯ O-5′	1 554	2.340	115.3	
O-2″A	1 555	2.100	139.2	
O-1″	1 555	2.682	91.0	
O-1‴-H ⋯ O-5′	1 554	2.394	115.3	
O-2″B	1 555	2.020	154.8	
O-1″	1 555	2.682	91.0	
Water donors, two-centered				
W-1-H-2 ⋯ O-4″A	1 555	2.022	141.7	
W-2-H-1 ⋯ O-W-1	1 555	1.881	163.7	
W-3-H-1 ⋯ O-W-1	1 555	1.895	140.7	
W-3-H-2 ⋯ O-3‴A	1 555	1.803	156.9	
W-4-H-1 ⋯ O-3″A	3 555	1.811	168.0	
W-4-H-2 ⋯ O-W-8	1 555	1.714	169.1	
W-5-H-1 ⋯ O-4″B	2 665	2.020	141.6	
W-5-H-2 ⋯ O-W-7	1 555	2.058	138.0	
W-6-H-1 ⋯ O-W-7	1 555	1.822	163.1	
W-7-H-1 ⋯ O-3″B	4 555	1.902	141.4	
W-7-H-2 ⋯ O-4‴	4 655	1.874	138.2	
W-8-H-1 ⋯ O-W-57	1 555	1.699	156.5	
Water donors, three-centered				
W-1-H-1 ⋯ O-4‴	3 455	1.837	150.3	358.8
O-3‴A	3 455	2.689	127.9	
W-2-H-2 ⋯ O-3	1 555	1.873	157.2	357.6
O-3′	4 556	2.737	118.5	
W-6-H-2 ⋯ O-W-4	1 555	1.833	147.8	336.9
O-4′	3 556	2.852	93.9	
		O(H)---O	O---O---O	
O-W-57 ⋯ O-4	1 554	2.668		
O-W-8 ⋯ O-W-57 ⋯ O-4			118.6	
O-W-57 ⋯ O-4 ⋯ O-3′			86.7	

[a] 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 symmetry operations: 1. x, y, z; 2. $-x, -y, z$; 3. $1/2 + x, 1/2 - y, -z$; 4. $1/2 - x, 1/2 + y, -z$. [b] Where $\Sigma = \theta + \theta' + \alpha$ in the three-centered bonds, O-H ⋯ A, A′.

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