Carbohydrate Research, 206 (1990) 173–182
Elsevier Science Publishers B.V., Amsterdam

The hydrogen bonding in the crystal structure of raffinose pentahydrate

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(Received December 11th, 1989; accepted for publication March 14th, 1990)

ABSTRACT

The crystal structure of raffinose pentahydrate, *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1↔2)-β-D-fructofuranose pentahydrate, $C_{18}H_{32}O_{16}\cdot 5H_2O$, has been redetermined using low-temperature, 119 K, CuKα X-ray data. All hydrogen atoms were unambiguously located on difference syntheses. The final *R*-factor is 0.036 for 2423 observed structure amplitudes. The hydrogen bonding is composed of infinite chains, which are linked through the water molecules to form a three-dimensional network containing a chain of five linked water molecules. Three of the infinite chains extend in the directions of the crystallographic axis of the space group $P2_12_12_1$. Four of the water molecules accept two hydrogen bonds and one accepts one. All the hydroxyls and the ring and glycosidic oxygen atoms are involved in the hydrogen bonding. With one exception, the ring and glycosidic oxygens are hydrogen-bonded by means of the minor components of unsymmetrical three-center bonds.

INTRODUCTION

Raffinose pentahydrate, $C_{18}H_{32}O_{16}\cdot 5H_2O$, is one of the few highly hydrated oligosaccharides that have been crystallized. Others are stachyose pentahydrate[1], the cyclodextrins[2], and a *p*-nitrophenylmaltohexaoside–tri-iodide complex[3]. A room-temperature crystal structure analysis[4] of raffinose hydrate revealed that the five water molecules are linked in chains in channels between the trisaccharide molecules. The purpose of this low-temperature study was to better define the hydrogen-bonding geometry and compare the overall scheme with those observed in the anhydrous and low-hydrated mono- and di-saccharides[5], and the high-hydrated cyclodextrins[6].

EXPERIMENTAL

Raffinose pentahydrate (Sigma Chemical Co.) was recrystallized from water–methanol at 4°. The X-ray diffraction data given in Table I were obtained on a CAD-4 diffractometer at 119 K with Ni-filtered CuKα radiation and corrected for absorption. The crystal structure was redetermined using MITHRIL[7]. All hydrogen atoms were located on difference maps. A full-matrix least-squares refinement was carried out using UPALS[8], with anisotropic thermal parameters for the carbon and oxygen atoms and positional parameters only for hydrogen atoms. The hydrogen atoms were assigned the

0008-6215/90/$ 03.50

TABLE I

Crystal data and structure refinement data for raffinose pentahydrate

Crystal data

$C_{18}H_{32}O_{16}\cdot 5H_2O$; mol. wt. 594.5; m.p. 79.1°; $P2_12_12_1$; $Z = 4$
Cell dimensions at 119 K [295][a], $a = 8.9045(4)$ [8.966(10)],
$b = 12.2727(6)$ [12.327(15)], $c = 23.795(1)$ [23.837(24)] Å,
$V = 2600.6$ [2634.6] Å^3; $D_x = 1.516$ [1.496] g.cm^{-3}

Structure refinement data

Crystal dimensions, $0.15 \times 0.17 \times 0.40$ mm^3
Absorption, max 1.311 cm^{-1}; min 1.140 cm^{-1}
2885 intensities measured, 2443 observed [$F_o > 2\sigma(F_o)$]
Number of parameters, 478
Final agreement factors, $R(F) = 0.036$; $R_w(F) = 0.038$

[a] Values in square brackets are from ref. 4.

TABLE II

Fractional atomic positional parameters and equivalent isotropic thermal parameters for raffinose pentahydrate at 119 K[a]

Atom	x/a	y/b	z/c	B_{eq}
C-1	2772(4)	3182(3)	1426(1)	76(8)
C-2	3918(5)	3943(3)	1154(1)	89(8)
C-3	3598(4)	5136(3)	1308(1)	74(8)
C-4	1972(4)	5421(3)	1164(1)	75(8)
C-5	880(4)	4598(3)	1426(1)	72(8)
C-6	−722(4)	4785(3)	1250(1)	82(8)
C-1′	−2225(5)	5057(3)	450(2)	100(8)
C-2′	−2347(4)	4721(3)	−170(2)	83(8)
C-3′	−1252(4)	5372(3)	−532(1)	79(8)
C-4′	−1484(4)	6596(3)	−432(1)	89(8)
C-5′	−1328(4)	6829(3)	194(1)	76(8)
C-6′	−1645(5)	8014(3)	350(2)	108(8)
C-1″	3842(4)	2117(3)	2745(1)	93(8)
C-2″	2488(4)	2390(3)	2379(1)	73(8)
C-3″	1088(5)	2743(3)	2700(1)	107(9)
C-4″	−180(4)	2374(3)	2314(1)	96(9)
C-5″	441(4)	1276(3)	2112(2)	95(8)
C-6″	−91(5)	919(3)	1540(2)	117(9)
O-1	2992(3)	3251(2)	2009(1)	82(6)
O-2	5414(3)	3622(2)	1293(1)	105(6)
O-3	4549(3)	5861(2)	1000(1)	105(6)
O-4	1570(3)	6462(2)	1384(1)	101(6)
O-5	1295(3)	3510(2)	1260(1)	75(5)
O-6	−812(3)	4724(2)	651(1)	81(6)
O-2′	−2158(3)	3574(2)	−237(1)	118(6)
O-3′	−1485(3)	5142(2)	−1110(1)	122(6)
O-4′	−2887(3)	6956(2)	−645(1)	106(6)
O-5′	−2423(3)	6211(2)	503(1)	86(5)
O-6′	−521(3)	8736(2)	138(1)	96(6)

O-1″	3468(3)	1269(2)	3133(1)	112(6)
O-2″	2072(3)	1444(2)	2081(1)	89(6)
O-3″	1128(3)	3878(2)	2815(1)	113(6)
O-4″	−1631(3)	2327(2)	2556(1)	117(6)
O-6″	444(3)	−147(2)	1393(1)	134(6)
O-W-1	7962(3)	848(2)	354(1)	207(7)
O-W-2	5878(3)	1353(2)	1204(1)	144(6)
O-W-3	6477(3)	4164(2)	2333(1)	133(6)
O-W-4	191(3)	2514(2)	245(1)	176(6)
O-W-5	6023(3)	3(2)	2168(1)	170(7)
H-1	286(4)	240(3)	130(1)	
H-2	378(4)	388(3)	76(1)	
H-3	378(4)	522(3)	172(1)	
H-4	185(4)	542(3)	74(1)	
H-5	95(5)	463(3)	187(2)	
H-6	−141(4)	424(3)	142(1)	
H′-6	−110(5)	548(3)	139(2)	
H-1′	−304(4)	473(3)	69(1)	
H-2′	−340(4)	491(3)	−27(1)	
H-3′	−26(4)	513(3)	−41(1)	
H-4′	−73(4)	696(3)	−67(1)	
H-5′	−21(4)	664(3)	32(1)	
H-6′	−163(4)	808(3)	79(1)	
H′-6′	−270(5)	825(3)	18(2)	
H-1″	417(4)	274(3)	298(1)	
H′-1″	477(5)	193(3)	249(2)	
H-3″	107(4)	229(3)	310(1)	
H-4″	−23(4)	288(3)	199(1)	
H-5″	20(4)	72(3)	243(1)	
H-6″	−122(4)	88(3)	154(1)	
H′-6″	13(5)	148(3)	126(2)	
H-2-O	561(5)	376(3)	165(2)	
H-3-O	515(5)	606(4)	126(2)	
H-4-O	183(6)	693(4)	113(2)	
H-2′-O	−149(5)	340(4)	−5(2)	
H-3′-O	−100(5)	460(3)	−122(2)	
H-4′-O	−350(5)	669(3)	−47(2)	
H-6′-O	−57(5)	877(3)	−23(2)	
H-1″-O	321(6)	73(4)	295(2)	
H-3″-O	66(5)	405(4)	311(2)	
H-4″-O	−157(5)	201(3)	287(2)	
H-6″-O	126(4)	− 11(3)	132(1)	
H-1-W-1	851(7)	152(5)	42(2)	
H-2-W-1	868(10)	10(7)	36(4)	
H-1-W-2	584(6)	198(4)	120(2)	
H-2-W-2	636(5)	131(3)	90(2)	
H-1-W-3	699(5)	371(4)	256(2)	
H-2-W-3	589(5)	435(3)	261(2)	
H-1-W-4	53(6)	308(4)	56(2)	
H-2-W-4	109(5)	213(3)	8(2)	
H-1-W-5	613(5)	43(3)	185(2)	
H-2-W-5	679(6)	−36(4)	226(2)	

[a] Fractional coordinates × 10^4 for non-hydrogen atoms, × 10^3 for hydrogen atoms. E.s.d. values given in parentheses refer to the least significant digit. $B_{eq} = 4/3(\Sigma_{ij} B_{ij} a_i a_j)$, calculated in $Å^2$ (× 10^{-2}) from the refined, anisotropic, thermal parameters.

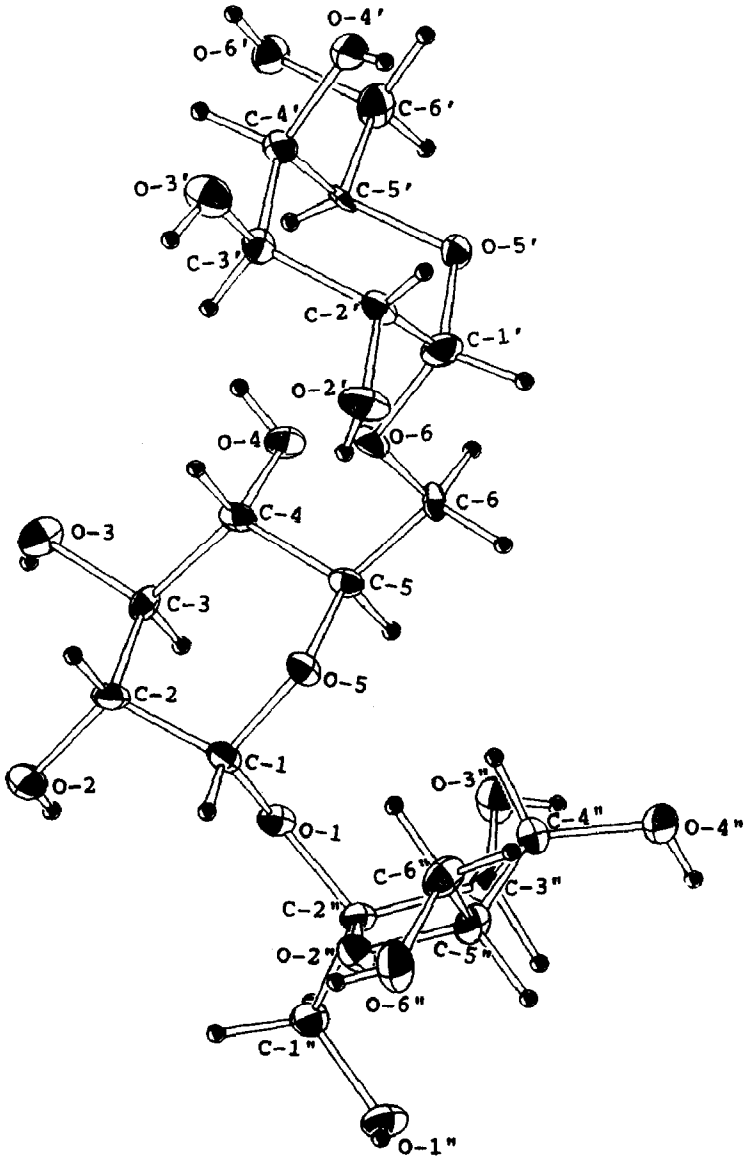


Fig. 1. Molecular conformation and atomic notation for raffinose in the crystal structure of raffinose pentahydrate.

isotropic temperature factors of the atoms to which they are bonded, and these were fixed during the refinement. The final atomic parameters are given in Table II*. The atomic notation is shown in Fig. 1.

* Lists of structure factors and anisotropic temperature factors for the non-hydrogen atoms 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/439/Carbohydr. Res., 206 (1990) 173–182.

DISCUSSION

The structure of the raffinose molecule at 119 K is in good agreement with the results from the earlier room-temperature study. The bond lengths, selected bond angles and torsion angles, and the Cremer-Pople ring puckering parameters[9,10], are reported in Table III. A comparison with the results from the room-temperature analysis showed

TABLE III

Molecular geometry of raffinose pentahydrate at 119 K[a]

Bond lengths

C-1–C-2	1.527(5)	C-1′–C-2′	1.534(5)	C-1″–C-2″	1.525(5)
C-1–O-1	1.404(4)	C-1′–O-6	1.407(4)	C-1″–O-1″	1.430(4)
C-1–O-5	1.431(4)	C-1′–O-5′	1.433(4)	C-2″–O-1	1.447(4)
C-2–C-3	1.536(5)	C-2′–C-3′	1.527(5)	C-2″–C-3″	1.524(5)
C-2–O-2	1.428(4)	C-2′–O-2′	1.427(4)	C-2″–O-2″	1.410(4)
C-3–C-4	1.529(5)	C-3′–C-4′	1.534(5)	C-3″–C-4″	1.524(5)
C-3–O-3	1.431(4)	C-3′–O-3′	1.421(4)	C-3″–O-3″	1.421(4)
C-4–C-5	1.534(5)	C-4′–C-5′	1.523(5)	C-4″–C-5″	1.535(5)
C-4–O-4	1.427(4)	C-4′–O-4′	1.419(4)	C-4″–O-4″	1.416(4)
C-5–C-6	1.506(5)	C-5′–C-6′	1.528(5)	C-5″–C-6″	1.507(5)
C-5–O-5	1.441(4)	C-5′–O-5′	1.438(4)	C-5″–O-2″	1.469(4)
C-6–O-6	1.430(4)	C-6′–O-6′	1.429(4)	C-6″–O-6″	1.436(5)

Valence angles differing from 110 ± 1°

O-1–C-1–C-2	106.8(3)	O-6–C-1′–C-2′	108.1(3)	C-1″–C-2″–O-2″	108.3(3)
O-1–C-1–O-5	112.6(3)	O-6–C-1′–O-5′	111.6(3)	C-1″–C-2″–C-3″	115.1(3)
C-1–C-3–O-3	111.1(3)	C-1′–C-2′–O-2′	111.4(3)	C-1″–C-2″–O-1	105.2(3)
O-2–C-2–C-3	112.4(3)	O-2′–C-2′–C-3′	112.2(3)	O-1–C-2″–O-2″	112.2(3)
O-3–C-3–C-4	107.6(3)	O-3′–C-3′–C-4′	108.9(3)	O-2″–C-2″–C-3″	105.7(3)
C-3–C-4–O-4	111.1(3)	C-3′–C-4′–O-4′	111.6(3)	C-2″–C-3″–C-4″	102.7(3)
O-4–C-4–C-5	106.4(3)	C-3′–C-4′–C-5′	108.8(3)	O-3″–C-3″–C-4″	115.3(3)
C-4–C-5–C-6	112.7(3)	O-4′–C-4′–C-5″	111.8(3)	C-3″–C-4″–C-5″	100.5(3)
O-5–C-5–C-6	107.9(3)	C-4′–C-5′–C-6′	113.6(3)	O-4″–C-4″–C-5″	114.9(3)
C-5–C-6–O-6	108.9(3)	O-5′–C-5′–C-6′	104.6(3)	C-4″–C-5″–O-2″	104.4(3)
C-4–O-1–C-2″	120.9(3)	C-5′–C-6′–O-6′	112.0(3)	O-2″–C-5″–C-6″	107.8(3)
C-6–O-6–C-1′	112.0(3)	C-5″–C-6″–O-6″	112.4(3)	C-4″–C-5″–C-6″	115.2(3)

Selected torsion angles

C-2″–O-1–C-1–O-5	82.1(3)	O-1″–C-1″–C-2″–O-1	179.7(3)
O-5–C-5–C-6–O-6	−63.1(3)	O-1″–C-1″–C-2″–O-2″	−60.1(3)
C-5–C-6–O-6–C-1′	−170.8(3)	O-2″–C-5″–C-6″–O-6″	68.1(4)
C-6–O-6–C-1′–O-5′	71.7(3)	C-2″–C-3″–C-4″–C-5″	39.3(3)
O-5′–C-5′–C-6′–O-6′	172.0(3)	C-1–C-2–C-3–C-4	−53.7(4)
C-1–O-1–C-2″–O-2″	12.0(4)	C-1′–C-2′–C-3′–C-4′	−53.5(4)

Cremer–Pople puckering parameters[9,10]

Ring	*Q(Å)*	*Θ(deg)*	*φ(deg)*
Galactose	0.566	1.8	42
Glucose	0.581	3.3	286
Fructose	0.395	-	274

[a] Bond lengths in Å, valence and torsion angles in degrees. Estimated standard deviations, given in parentheses, refer to the least significant digit.

no significant changes in molecular conformation at the two temperatures, except for a mean expansion of 0.007 Å in bond lengths at the lower temperature due to the lesser thermal motion. The greatest difference in torsion angles is 1.7° and the mean difference is 0.7°. There is an average decrease of 1.43 $Å^2$ in the isotropic equivalent temperature factors between the room and low temperature results for the carbon atoms, and of 1.79 $Å^2$ for the hydroxyl oxygen atoms.

The hydrogen bonding. — All hydroxyls, glycosidic and ring oxygen atoms, and water molecules are involved in the hydrogen bonding, which is completely ordered. The hydrogen bonding scheme is a three-dimensional network which can be decomposed into a series of infinite chains intersecting at the water molecules. These chains, with their geometry, are shown schematically in Fig. 2. As may be seen in the figure, three of the infinite chains extend in the directions of the crystallographic axes. Those along [*b*] and [*c*] intersect at W-3. The chain along [*a*] is linked to the chains along [*b*] and [*c*] through W-1, which is a single acceptor. There is a fourth infinite chain through W-5, and a single link from W-4 to the ring oxygen O-5. The hydrogen bonding in these infinite chains is ordered and sequential[11], thereby maximizing the cooperative energy[12]. The infinite chains correspond to the homodromic cyclic patterns observed in the cyclodextrin hydrates[13]. Since the crystal structure is nonpolar, there are pairs of identical chains in which the donor–acceptor directions are opposite. The sequential symmetry relations of the oxygen atoms in these chains are shown in Fig. 3.

In addition to the principal hydrogen bonds there are a number of weaker interactions, which are the minor components of three-center bonds. These bond the ring oxygens, O-5′ and O-2″, and the glycosidic oxygens, O-1 and O-6. There is also a weak link between two of the infinite chains through O-3′-H→O-W-5.

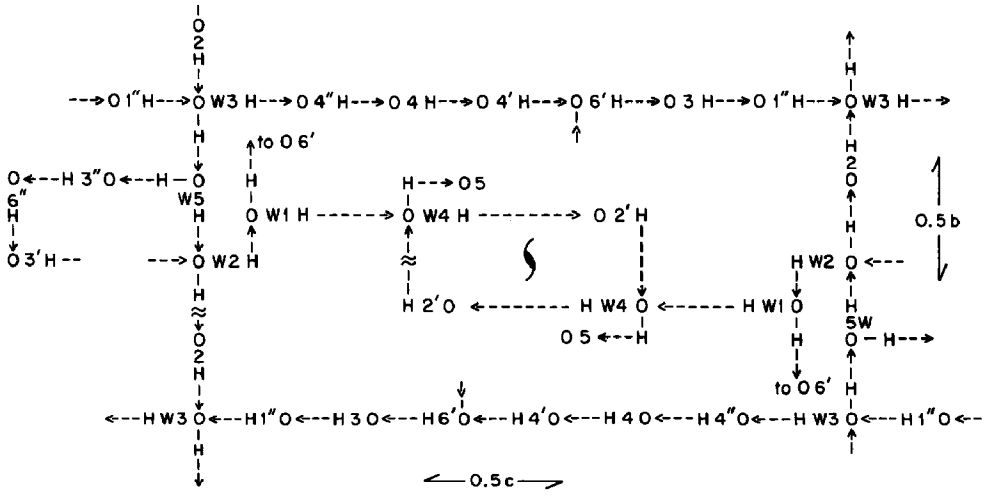


Fig. 2. Hydrogen-bonding scheme in the crystal structure of raffinose pentahydrate.

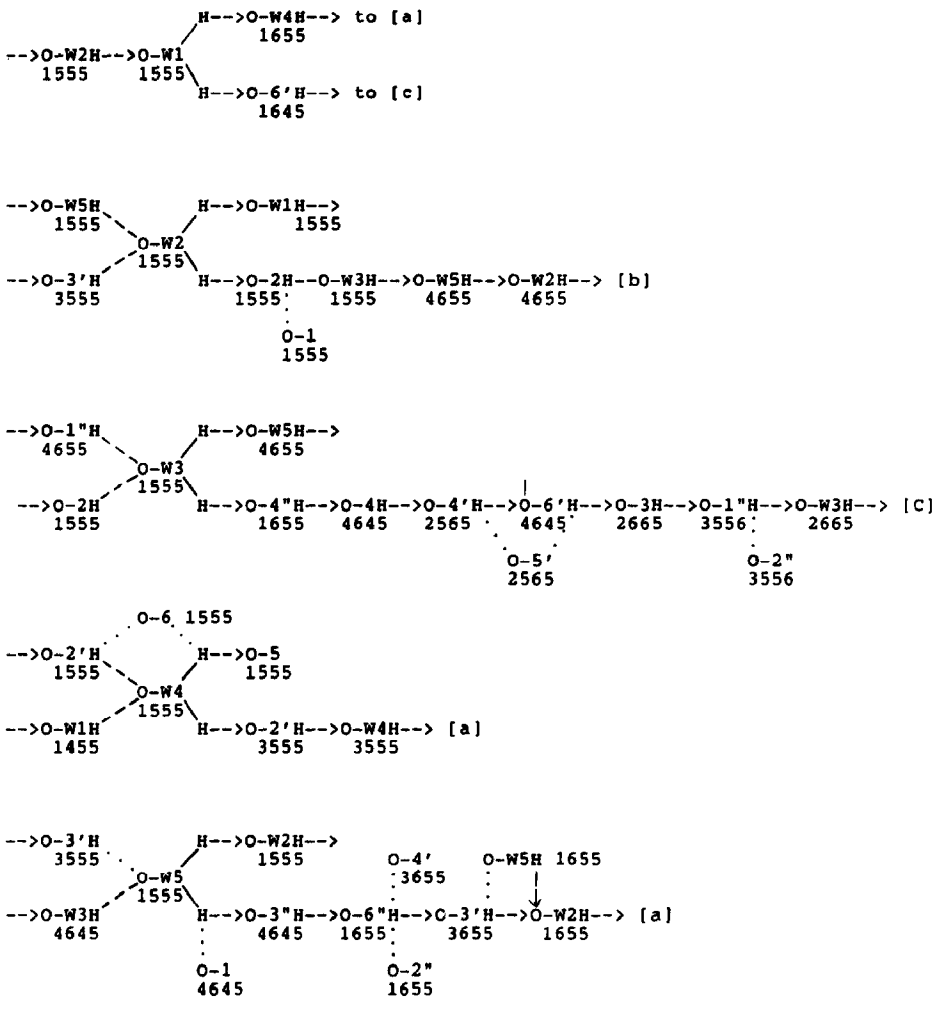


Fig. 3. Hydrogen-bonding scheme with respect to the water molecules in the crystal structure of raffinose pentahydrate. Stronger bonds, dashed lines; weaker bonds, dotted lines. Symmetry code as in Table IV.

The individual hydrogen bond lengths and angles are given in Table IV, with the covalent O–H bond lengths normalized to 0.97 Å to correct for the bonding charge density[14,15]. The C–O–H angles range from 105 to 110(3)° and the H–O–H angles from 91 to 115(5)°. A comparison between X-ray and neutron diffraction data from the same crystal structures indicated no systematic differences in angles[15].

There are no primary inter-residue intramolecular hydrogen bonds, but the minor components to the ring oxygens are intramolecular, *i.e.*, O-4′-H→O-5′, O-1″-H→O-2″, O-6″-H→O-2″, and (to the linkage oxygens) O-2-H→O-1 and O-2′-H→O-6 (see Table IV). Analyzing the hydrogen bond geometry in more detail, there are four two-center bonds, with H→O distances 1.703 to 1.802 Å and OH—O angles 158.0 to 171.8°. There

are six three-center bonds, all of which are unsymmetrical. The hydrogen atom in the O-6″-H group is linked to three oxygen atoms, with one strong and two weaker interactions, to form a four-center bond. As noted elsewhere[16], the water molecules are stronger acceptors than donors, and the O-H→O-W bonds tend to be shorter than O-W-H→O-H. The mean value for the O-H→O-W bonds is 1.86 Å, whereas those for the O-W-H→O-W and the O-W-H→O-H bonds are 1.90 Å and 1.95 Å, respectively. The range of hydrogen bond lengths and their mean values are consistent with those reported from surveys of O-H—O bonds in the crystal structures of small biological molecules[17] and their hydrates[16].

TABLE IV

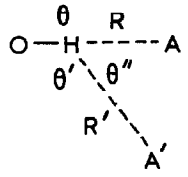
The structural parameters of hydrogen bonds in raffinose pentahydrate[a]

	Acceptor symmetry code	R(Å)	*Θ(deg)*	*ΣΘ(deg)*
Two-center bonds				
O-3-H ··· O-1″	4 655	1.802(3)	170.2(1)	
O-3″-H···O-6″	4 555	1.709	158.0	
O-4-H ··· O-4′	3 565	1.703	169.4	
O-4″-H···O-4	4 545	1.774	171.8	
Three-center bonds				
O-2-H··· O-W-3	1 555	1.784	164.8	359.9
O-1	1 555	2.550	93.7	
O-2′-H···O-W-4	1 555	1.795	157.7	360.0
O-6	1 555	2.333	109.4	
O-3′-H···O-W-2	3 455	1.920	149.8	331.8
O-W-5	3 455	2.873	112.8	
O-4′-H···O-6′	3 465	1.835	161.0	357.3
O-5′	1 555	2.510	104.6	
O-6′-H···O-3	3 465	1.802	166.0	339.6
O-5′	3 565	2.865	98.2	
O-1″-H···O-W-3	4 645	1.928	150.4	354.6
O-2″	1 555	2.423	102.7	
Four-center bond				
O-2″	1 555	2.694	94.5	359.1
O-6″-H···O-3′	3 555	1.850	174.0	
O-4′	3 555	2.823	104.8	348.4

Water molecules				
W-1-H-1--O-4-W	1 655	1.979	150.3	
W-1-H-2--O-6′	1 465	2.033	161.4	
W-2-H-1--O-2	1 555	1.867	168.5	
W-2-H-2--O-1-W	1 555	1.891	158.0	
W-3-H-1--O-4″	1 655	2.039	141.6	
W-3-H-2--O-5-W	4 655	1.870	145.3	
W-4-H-1--O-5	1 555	1.961	156.9	356.7
\O-6	1 555	2.402	120.6	
W-4-H-2--O-2′	3 555	1.857	145.4	
W-5-H-1--O-2-W	1 555	1.880	167.1	
W-5-H-2--O-3″	4 645	1.964	158.7	358.5
\O-1	4 645	2.373	125.1	

[a] O–H bond lengths are normalized to 0.97 Å. Symmetry code: The three digits give the unit cell translation in the *a*, *b*, and *c* directions with respect to 555; the first digit of the symmetry code 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: $-x, 1/2 + y, 1/2 - z$

$\sum\Theta = \Theta + \Theta' + \Theta''$ in the bifurcated bonds, as shown in:



The hydrogen bonding in this trisaccharide hydrate is similar in principle to that of the monosaccharides and disaccharides[4], where the predominant theme is finite and infinite chains with the ring and glycosidic oxygens linked by the minor components of three-center bonds[18]. The five- and six-membered cyclic systems present in the cyclodextrin hydrates are not observed.

ACKNOWLEDGMENT

This research was supported by National Institutes of Health Grant No. GM-24526.

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