

# The Structure of 6-O- $\alpha$ -D-Galactopyranosyl-( $\alpha,\beta$ )-D-glucopyranose ( $\alpha,\beta$ -Melibiose) Monohydrate

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This structure analysis of  $C_{12}H_{22}O_{11} \cdot H_2O$  [orthorhombic,  $P2_12_12_1$ ,  $a = 8.900$  (2),  $b = 10.894$  (1),  $c = 15.868$  (3) Å] differs from two recently reported independent analyses in that the component of the  $\beta$  anomer is 28% instead of 15% and 20%. There are significant differences in the cell parameters of the three structures and a minor difference in the interpretation of the hydrogen bonding.

## Introduction

Crystal structure analyses of  $\alpha,\beta$ -melibiose monohydrate,  $C_{12}H_{22}O_{11} \cdot H_2O$ , have been reported in a preliminary communication by Gress, Jeffrey & Rohrer (1976), and by Hirotsu & Higuchi (1976) (H&H) and Kanters, Roelofsen, Doesburg & Koops (1976) (KRD&K). The compositions of the crystals used differed in the three analyses. That of H&H was 85%  $\alpha$ , 15%  $\beta$ , and that of KRD&K was 80%  $\alpha$ , 20%  $\beta$ . The structure described herein is an anomeric mixture consisting of 72%  $\alpha$  and 28%  $\beta$ .

The crystals were grown by slow evaporation from an ethanol/ $H_2O$  solution. The cell parameters, given in Table 1, and the intensity data were measured at 19.5°C on a crystal of dimensions 0.25 × 0.25 × 0.16 mm using an Enraf-Nonius CAD-4 diffractometer. The cell parameters were obtained by least-squares refinement of 23 high-order reflection angles centered at  $\pm 2\theta$  using graphite-monochromated Cu  $K\alpha_1$  radiation

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Table 1. Crystal data

	72% $\alpha$ :28% $\beta$ (this work)	80% $\alpha$ :20% $\beta$ (KRD & K)	85% $\alpha$ :15% $\beta$ (H & H)†
$C_{12}H_{22}O_{11} \cdot H_2O$ , $M_r = 360.32$			
Space group $P2_12_12_1$ , $Z = 4$			
$\mu$ (Cu $K\alpha$ ) = 12.5 cm <sup>-1</sup>			
$a$ (Å)	8.900 (2)	8.878 (5)	8.903 (4)
$b$ (Å)	10.894 (1)	10.920 (6)	10.924 (5)
$c$ (Å)	15.868 (3)	15.730 (10)	15.814 (5)
$V$ (Å <sup>3</sup> )	1538.5	1525.0	1538.0
$D_c$ (g cm <sup>-3</sup> )	1.556	1.570	1.556
$D_m$ (g cm <sup>-3</sup> )	1.554	1.552	1.54

† Axes are interchanged for comparison with this work and that of KRD&K.

( $\lambda = 1.5405$  Å). The biggest differences are between our values and those reported by KRD&K, which correspond to  $4\sigma$ ,  $6\sigma$ , and  $13\sigma$  in  $a$ ,  $b$ , and  $c$  respectively. The difference in the  $c$  parameter should be significant, but the fact that the value for the 80:20 mixture is not intermediate between the other two suggests that the errors in this cell parameter have been underestimated.

Table 2. Positional parameters ( $\times 10^4$ ) for the non-hydrogen atoms in  $\alpha,\beta$ -melibiose monohydrate

Estimated standard deviations are given in parentheses for the least significant figures.

	$x$	$y$	$z$
C(1)	4180 (3)	7123 (3)	-93 (2)
C(2)	4296 (3)	8458 (3)	171 (2)
C(3)	4901 (3)	8602 (2)	1068 (2)
C(4)	4105 (3)	7752 (3)	1687 (1)
C(5)	4101 (3)	6449 (2)	1331 (2)
C(6)	3368 (3)	5499 (3)	1882 (2)
O(1 $\alpha$ )†	5554 (3)	6652 (3)	-253 (2)
O(1 $\beta$ )†	3675 (9)	7028 (7)	-839 (4)
O(2)	5204 (2)	9140 (2)	-401 (1)
O(3)	4737 (3)	9832 (2)	1341 (2)
O(4)	4812 (3)	7775 (2)	2493 (1)
O(5)	3370 (2)	6448 (2)	526 (1)
O(6)	1825 (2)	5783 (2)	2045 (1)
C(1')	1188 (3)	4966 (2)	2621 (2)
C(2')	-512 (3)	5025 (3)	2551 (2)
C(3')	-1097 (3)	6262 (3)	2849 (2)
C(4')	-515 (3)	6525 (3)	3736 (2)
C(5')	1198 (3)	6409 (3)	3745 (2)
C(6')	1868 (4)	6559 (4)	4620 (2)
O(2')	-962 (3)	4697 (3)	1729 (1)
O(3')	-2691 (2)	6326 (3)	2813 (2)
O(4')	-1186 (3)	5653 (3)	4295 (2)
O(5')	1643 (2)	5214 (2)	3465 (1)
O(6')	3470 (3)	6604 (3)	4583 (2)
O(W)	3703 (3)	8982 (2)	3900 (2)

† Refined occupancy factors for O(1 $\alpha$ ) and O(1 $\beta$ ) were 0.72 (1) and 0.28 (1) respectively. These factors were unconstrained, but their sum was normalized to unity after each cycle of refinement.

The intensity data were measured with graphite-monochromated Cu K $\alpha$  radiation ( $\lambda = 1.5418 \text{ \AA}$ ) by  $\theta$ - $2\theta$  scans out to  $2\theta = 156^\circ$ . The 7633 data (four octants) were corrected for Lorentz-polarization effects and for decomposition, which was 2.5% over the period of the measurement. The agreement between the symmetry-equivalent reflections in different octants was 2.4%. The averaged intensities gave 1874 independent structure amplitudes, of which 69 had  $|F_o|^2 < 0$ . No absorption correction was made, since the minimum and maximum transmission factors were 0.73 and 0.82 respectively. The initial structure solution was based on a set of intensity data collected at the University of Pittsburgh in 1972, but the data were recollected as described above and the solution was repeated on the new data using the *MULTAN* program of Germain, Main & Woolfson (1971). The refinement was by full-matrix least squares in two blocks using a local version of the program *ORFLS* (Busing, Martin & Levy, 1962). The two blocks consisted of the unprimed C atoms and the remaining atoms in the final cycle; and the primed C atoms and the remainder in the preceding cycle. The function minimized was  $\sum w(|F_o| - k|F_c|)^2$ , where  $w^{-1}$  is defined as  $[\sigma_c^2(F_o^2) + (aF_o^2)^2]/4F_o^2$ ;  $\sigma_c$  was from the counting statistics and  $a = 0.03$ . The atomic scattering factors for C and O were those tabulated by

Table 3. Fractional coordinates ( $\times 10^3$ ), isotropic temperature factors ( $\text{\AA}^2$ ), and bond distances ( $\text{\AA}$ ) for the hydrogen atoms in  $\alpha,\beta$ -melibiose monohydrate

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i>	C,O-H
H(C1 $\alpha$ )*	357	711	-63	3.0	1.00
H(C1 $\beta$ )*	521	680	-11	3.0	1.00
H(C2)	326 (3)	884 (3)	14 (2)	2.4 (6)	1.01 (3)
H(C3)	600 (4)	842 (3)	113 (2)	2.8 (6)	1.00 (3)
H(C4)	300 (3)	807 (2)	175 (2)	2.0 (5)	1.04 (3)
H(C5)	507 (4)	617 (3)	121 (3)	3.1 (6)	0.94 (4)
H(C6A)	392 (4)	543 (3)	234 (2)	3.3 (7)	0.89 (4)
H(C6B)	341 (4)	476 (3)	165 (2)	3.1 (6)	0.88 (3)
H(O1 $\alpha$ )*	560 (6)	576 (5)	-40 (3)	4.1 (11)	1.00 (5)
H(O2)	552 (9)	858 (7)	-75 (5)	11.3 (22)	0.87 (7)
H(O3)	514 (5)	1015 (4)	119 (3)	3.9 (12)	0.55 (4)
H(O4)	564 (6)	742 (5)	250 (3)	6.2 (12)	0.83 (5)
H(C1')	149 (3)	415 (3)	246 (2)	2.7 (6)	0.96 (3)
H(C2')	-82 (4)	436 (3)	301 (2)	3.4 (6)	1.07 (3)
H(C3')	-75 (4)	689 (3)	245 (2)	2.7 (6)	0.98 (3)
H(C4')	-67 (5)	744 (4)	397 (3)	5.9 (10)	1.07 (4)
H(C5')	158 (3)	703 (3)	342 (2)	2.5 (6)	0.91 (3)
H(C6A')	168 (4)	581 (3)	491 (2)	3.5 (7)	0.94 (4)
H(C6B')	148 (4)	743 (3)	486 (2)	3.8 (7)	1.08 (4)
H(O2')	-68 (8)	518 (6)	128 (4)	9.6 (17)	0.92 (6)
H(O3')	-307 (5)	580 (3)	326 (2)	4.4 (8)	0.98 (4)
H(O4')	-117 (6)	580 (5)	473 (3)	6.5 (13)	0.71 (5)
H(O6')	362 (4)	749 (3)	428 (2)	3.2 (7)	1.09 (3)
H(1W)	273 (8)	937 (5)	383 (3)	9.2 (17)	0.97 (7)
H(2W)	411 (8)	842 (6)	339 (4)	10.2 (18)	1.08 (7)

\* H(C1 $\alpha$ ) and H(C1 $\beta$ ) were fixed during the refinement at their calculated positions with the occupancy factors of O(1 $\alpha$ ) and O(1 $\beta$ ), respectively. H(O1 $\alpha$ ) was assigned the occupancy parameter of O(1 $\alpha$ ).

Table 4. Puckering parameters (Cremer & Pople, 1975) for the glucopyranose and galactopyranose rings in  $\alpha,\beta$ -melibiose monohydrate

	$\alpha,\beta$ -Glucose	$\alpha$ -Galactose
$q_2$	0.083 $\text{\AA}$	0.016 $\text{\AA}$
$q_3$	0.557 $\text{\AA}$	0.576 $\text{\AA}$
$\theta$	8.5 $^\circ$	1.6 $^\circ$
$\phi_2$	33.0 $^\circ$	39.5 $^\circ$
$Q$	0.563 $\text{\AA}$	0.576 $\text{\AA}$

Doyle & Turner (1968) and for H by Stewart, Davidson & Simpson (1965). The final agreement factors based on anisotropic refinement of all non-hydrogen atoms and isotropic refinement of all hydrogens except H(C1) were  $R = \sum |F_o - kF_c| / \sum |F_o| = 0.044$  and  $R_w = \{\sum [w(F_o - kF_c)]^2 / \sum wF_o^2\}^{1/2} = 0.047$ . One hydroxyl H atom, H(O2), moved 0.57  $\text{\AA}$  during least-squares refinement from its original position on the difference electron density map (0.601, 0.870, -0.053). This shift corresponded to a movement from a weak hydrogen-bonding situation with respect to O(5) to a bifurcated-bond situation between O(5) and O(6). The final positional parameters are given in Tables 2 and 3.\* The ring puckering parameters are given in Table 4.

## Discussion

The final coordinates agree well with those of H&H and KRD&K, as shown by the half-normal probability plots given in Fig. 1 (Abrahams & Keve, 1971). The slopes of the curves for the positional and thermal parameters of the non-hydrogen atoms indicate that standard deviations in all three structure analyses were underestimated by a factor of about 3.

The bond lengths are shown in Fig. 2. The average C-C bond lengths for the glucose and galactose rings are 1.520 and 1.523  $\text{\AA}$ , whereas in the structure determinations of H&H and KRD&K these values are 1.513 and 1.526  $\text{\AA}$ , and 1.509 and 1.522  $\text{\AA}$  respectively. The average C-O bond lengths are 1.426 and 1.424  $\text{\AA}$  in the glucose and galactose rings, as compared with 1.428 and 1.423  $\text{\AA}$  observed by H&H and 1.426 and 1.423  $\text{\AA}$  by KRD&K. These averages exclude the anomeric C(1)-O(1) bond. That of the galactose residue has the normal short value of 1.396  $\text{\AA}$ ; that of the glucose residue is foreshortened owing to

\* Lists of structure factors, anisotropic thermal parameters, bond lengths, bond angles and torsion angles have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32976 (16 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

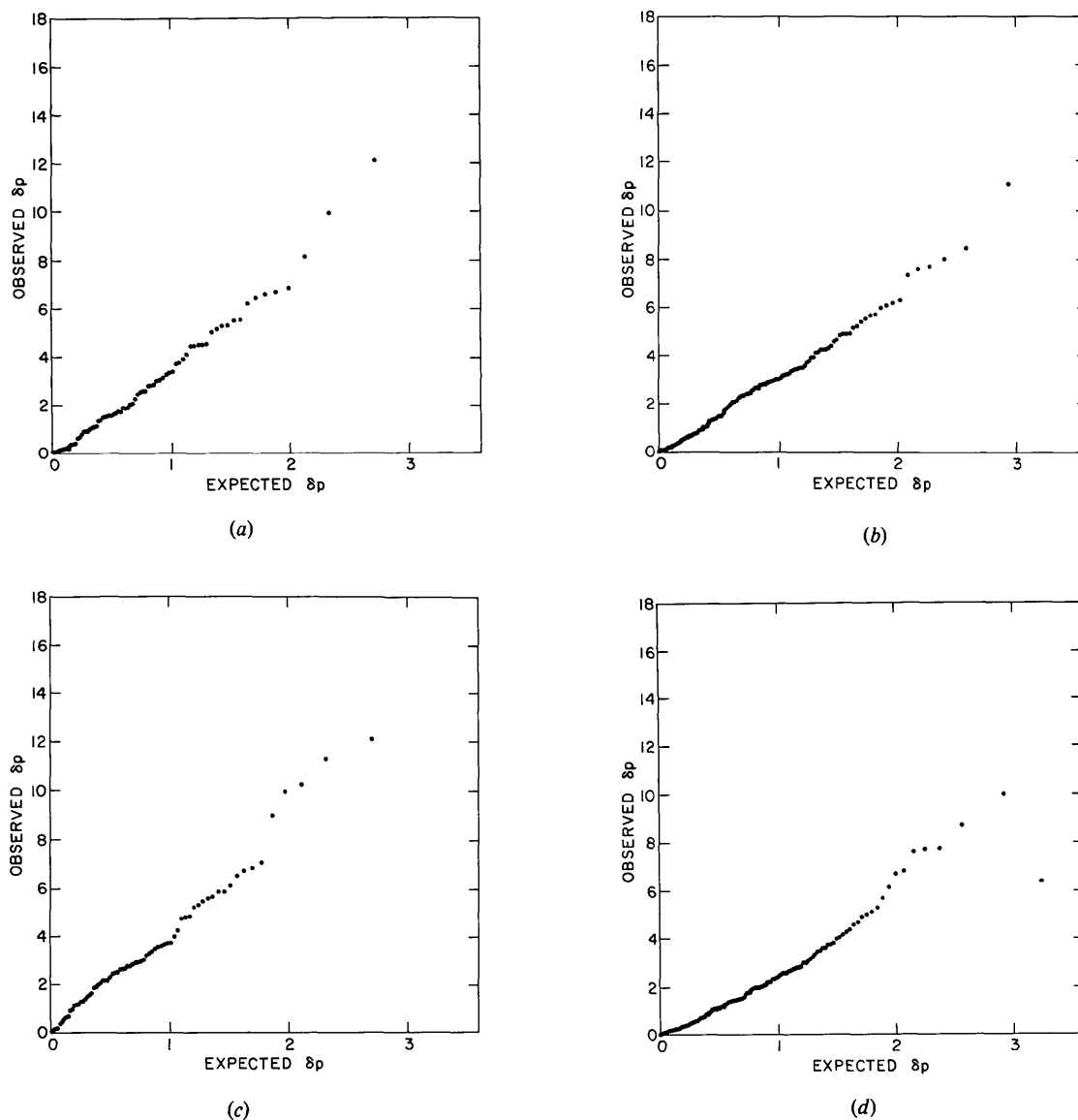


Fig. 1. Half-normal probability plots. This work *versus* KRD&K: (a) C and O positional parameters; slope 3.4, (b) C and O thermal parameters; slope 3.1. This work *versus* H&H: (c) C and O positional parameters; slope 4.0, (d) C and O thermal parameters; slope 2.5.

the anomeric disorder, which results in an overlap of the O(1) and H(1) electron density peaks.

The valence bond angles and torsion angles are not significantly different from the values reported for the H&H and KRD&K structures, except for those involving hydroxyl H atoms which are discussed below.

The hydrogen bonding is illustrated in Fig. 3, and the hydrogen-bond distances and angles are given in Table 5. The hydrogen-bond structure consists of a finite chain, which originates at the anomeric hydroxyl and terminates at a bifurcated bond to a ring oxygen and an O(6) oxygen. This chain passes twice through the water oxygen, forming a closed loop and providing a tetra-

hedral hydrogen-bond environment for the water molecule. Both anomers can form good hydrogen bonds without any major changes in other parts of the hydrogen-bond structure. This is probably an important condition for *anomeric co-crystallization*, as in the structure of  $\alpha,\beta$ -D-lactose monohydrate (Fries, Rao & Sundaralingam, 1971),  $\alpha$ -lactose-calcium chloride and  $\alpha$ -lactose-calcium bromide (Cook & Bugg, 1973; Bugg, 1973), and O- $\alpha,\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranose monohydrate (Takeda, Yasuoka & Kasai, 1977). The stereochemistry around the water molecule is shown in Fig. 4(a). As described above, the H(O2) position derived from the difference map corre-

sponded to a weak hydrogen bond to the ring oxygen O(5) with  $H\cdots O = 2.2 \text{ \AA}$  and an abnormally acute  $O-H\cdots O$  angle of  $129^\circ$ . This is the interpretation given in both the H&H and KRDK&K structure analyses. After least-squares refinement, the H(O2) position moved to a bifurcated bonding position with respect to O(5) and the linkage acetal oxygen O(6). The geometry of this bifurcated interaction is shown in Fig. 4(b). It agrees well with the bifurcated hydrogen bonds

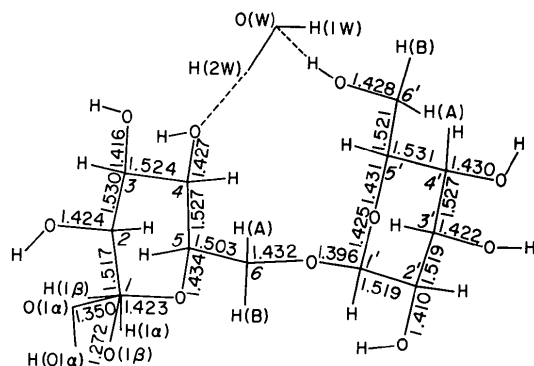


Fig. 2. C-C and C-O bond lengths in  $\alpha,\beta$ -melibiose monohydrate. The standard deviations are C-C 0.004 Å, C-O 0.003 Å, except for C-O(1 $\alpha$ ) and C-O(1 $\beta$ ).

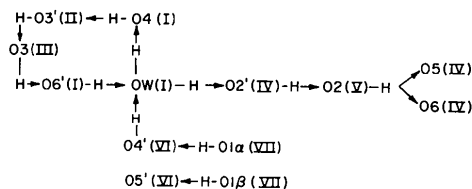
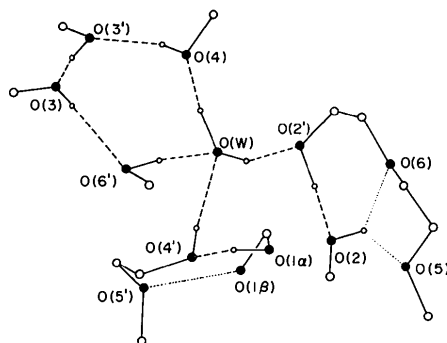


Fig. 3. Hydrogen bonding in  $\alpha,\beta$ -melibiose monohydrate. Top, actual; bottom, schematic. ● Oxygen, ○ hydrogen, ○ carbon. Dashed lines are hydrogen bonds, dotted lines refer to the bifurcated hydrogen bond and the bond inferred for the  $\beta$  anomer. Symmetry code: (I)  $x, y, z$ , (II)  $1 + x, y, z$ , (III)  $1 - x, -\frac{1}{2} + y, \frac{1}{2} - z$ , (IV)  $-x, \frac{1}{2} + y, \frac{1}{2} - z$ , (V)  $\frac{1}{2} - x, 2 - y, \frac{1}{2} + z$ , (VI)  $\frac{1}{2} + x, \frac{3}{2} - y, 1 - z$ , (VII)  $1 - x, \frac{1}{2} + y, \frac{1}{2} - z$ .

Table 5. Hydrogen-bond distances (Å) and angles ( $^\circ$ ) in the crystal structure of  $\alpha,\beta$ -melibiose monohydrate

Type of interaction	O...O	H...O	H...O <sub>corr</sub> *	O-H...O	O-H...O <sub>corr</sub> *	H...O (KRDK&K)	H...O (H&H)
...O-H...O(W)(H)							
O(4')-H...O(W) <sup>a</sup>	2.894 (4)	2.18 (5)	1.94	173 (6)	172	2.28	2.08
O(6')-H...O(W)	2.815 (4)	1.73 (3)	1.86	172 (3)	172	1.88	1.82
...O(W)-H...O(H)							
O(W)-H...O(4)	2.773 (4)	1.71 (7)	1.82	170 (6)	170	1.84	1.80
O(W)-H...O(2') <sup>b</sup>	2.748 (3)	1.84 (7)	1.85	155 (5)	155	1.74	1.91
...O-H...O(H)							
O(3)-H...O(6') <sup>c</sup>	2.902 (4)	2.36 (4)	1.96	171 (6)	169	2.20	2.19
O(4)-H...O(3') <sup>d</sup>	2.773 (3)	1.97 (5)	1.85	162 (5)	161	1.92	2.02
O(2')-H...O(2) <sup>e</sup>	2.670 (3)	1.77 (6)	1.73	167 (6)	166	2.02	1.90
O(3')-H...O(3) <sup>f</sup>	2.787 (3)	1.92 (4)	1.94	146 (3)	146	1.97	2.08
O-H...O(H)							
O(1 $\alpha$ )-H...O(4') <sup>g</sup>	2.672 (4)	1.70 (6)	1.74	164 (5)	164	1.79	2.03
...O-H...O							
O(2)-H...O(5) <sup>h</sup>	2.896 (2)	2.56 (8)	2.54	104 (6)	102	2.20	2.20
O(2)-H...O(6) <sup>h</sup>	2.982 (3)	2.47 (7)	2.42	119 (6)	117	2.72	2.73
O-H...O							
O(1 $\beta$ )-H...O(5') <sup>†</sup>	2.695 (7)						
Symmetry code							
(a)	$-\frac{1}{2} + x, \frac{3}{2} - y, 1 - z$	(e)	$-\frac{1}{2} + x, \frac{3}{2} - y, -z$				
(b)	$-x, \frac{1}{2} + y, \frac{1}{2} - z$	(f)	$-x, -\frac{1}{2} + y, \frac{1}{2} - z$				
(c)	$1 - x, \frac{1}{2} + y, \frac{1}{2} - z$	(g)	$\frac{1}{2} - x, 1 - y, -\frac{1}{2} + z$				
(d)	$1 + x, y, z$	(h)	$\frac{1}{2} + x, \frac{3}{2} - y, -z$				

\* Corrected by expanding the covalent O-H bond distances to the neutron diffraction value of 0.96 Å in the direction of the bond.

† H position not determined.

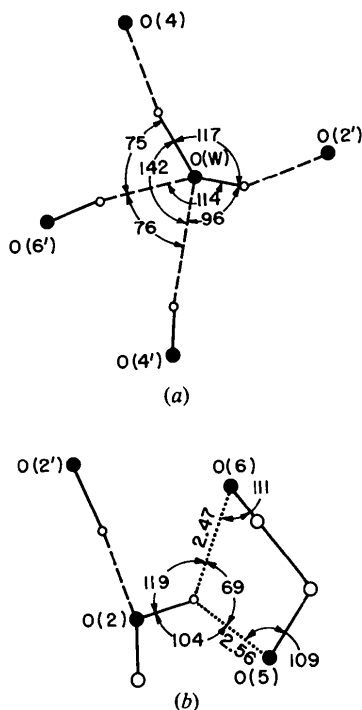


Fig. 4. Hydrogen-bond structural dimensions. (a) The water molecule, (b) the bifurcated interactions. ● Oxygen, ○ hydrogen, ○ carbon.

observed by neutron diffraction in methyl  $\alpha$ -D-altropyranoside by Poppleton, Jeffrey & Williams (1975). Resolution of this question will require a neutron diffraction study. The H attached to O(1) of the  $\beta$  anomer was not observed. The presence of a hydrogen bond is inferred from the O(1 $\beta$ )...O(5') distance of 2.695 Å.

All the hydroxyl groups are both hydrogen-bond donors and hydrogen-bond acceptors, with the exception of the anomeric hydroxyl O(1)—H, which donates only, to form one of the two shortest hydrogen bonds in the structure. This is in agreement with the experimental observations by Jeffrey, Gress & Takagi (1977), Jeffrey & Lewis (1977), and Jeffrey & Takagi (1977), and with the theoretical predictions (Tse & Newton,

1977) that anomeric hydroxyls are exceptional, being strong hydrogen-bond donors, but weak acceptors.

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