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Hydrogen-bond network in cyclodecaamylose hydrate at 20 K; neutron diffraction study of novel structural motifs band-flip and kink in α-(1→4)-D-glucoside oligosaccharides

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A single-crystal neutron diffraction study of cyclodecaamylose (CA10) was carried out at 20 K. CA10 crystallizes with 27.18 water molecules [$(C_6H_{10}O_5)_{10}\cdot 27.18H_2O$] in space group $C2$ with unit-cell constants a = 29.31 (5), b = 9.976 (10), c = 19.34 (2) Å, β = 121.07 (2)°. The asymmetric unit contains a half molecule of CA10 and 13.59 water molecules, the other half being related by a crystallographic twofold rotation axis. All H atoms except two water H atoms could be located from difference neutron-density maps; structure refinement converged at R = 0.635. Two of the five CH_2—O6 groups and one of the 15 O2, O3 hydroxyl groups of CA10 are twofold orientationally disordered. A total of 13.59 water molecules in the asymmetric unit are distributed over 23 positions; 20 of which are in the CA10 cavity, and the other three occupy intermolecular interstices. Of the 123 symmetry-independent hydrogen bonds, 25 (= 20%) are three-centered and 7 (= 6%) are four-centered. Water molecules and O—H groups of CA10 form an extended network with cooperative O—H···O—H···O—H hydrogen bonds. They are arranged in 11 polygons with three, four, five, six and eight O—H bonds and in homodromic, antidromic and heterodromic arrangements. Nine polygons are located within the cavity and the others are outside.

1. Introduction

A number of neutron diffraction studies of hydrates of the smaller cycloamyloses (CAs) with six, seven and eight glucoside units have revealed detailed geometrical characteristics of glucoside–water interactions (Steiner & Saenger, 1994). All these CAs resemble hollow, truncated cones in which the glucosides adopt the $4C_1$ chair conformation and are in *syn* orientation: their O2—H and O3—H hydroxyl groups are on the wider side of the cone and the O6–H hydroxyls on the narrower side. The main interaction stabilizing the structure of the cone is a system of $O3_n\cdots O2_{n+1}$ hydrogen bonds between the adjacent glucosides.

In recent room-temperature X-ray diffraction studies we reported the crystal structure of cycloamylose with ten glucoside residues (CA10; Jacob *et al.*, 1998, 1999). In contrast to the round shape of smaller CAs, CA10 has an elliptical shape with a narrow, groove-like cavity because two diametrically opposed glucosides are flipped by *ca* 180° (*anti* orientation) to relieve steric strain. This novel 'band-flip' conformation is stabilized by three-center hydrogen bonds in which the O3—H donor of one glucoside faces two acceptor atoms, O5 and O6, of the adjacent glucoside. The hydrogen

Table 1
Experimental details.

Crystal data	
Chemical formula	$C_{60}H_{100}O_{50}\cdot 27.18H_2O$
Chemical formula weight	2108.3
Cell setting, space group	Monoclinic, C_2
a, b, c (Å)	29.31 (5), 9.976 (10), 19.34 (2)
β (°)	121.07 (2)
V (Å^3)	4843 (11)
Z	2
D_x (Mg m^{-3})	1.446
Radiation type	Neutron
Wavelength (Å)	1.53200
No. of reflections for cell parameters	2193
θ range (°)	3.55–50.85
μ (mm^{-1})	2.31
Temperature (K)	20 (2)
Crystal form, color	Plate, colorless
Crystal size (mm)	1.0 × 0.6 × 0.2
Data collection	
Diffractometer	D19
Data collection method	4 × 64 ° position-sensitive detector (Thomas *et al.*, 1983)
Absorption correction	Analytical
T_{min}	0.816
T_{max}	0.948
No. of measured, independent and observed parameters	3929, 2494, 2355
Criterion for observed reflections	$I > 2\sigma(I)$
R_{int}	0.0480
θ_{max} (°)	50.77
Range of h, k, l	$-29 \rightarrow h \rightarrow 29$ $-4 \rightarrow k \rightarrow 10$ $-19 \rightarrow l \rightarrow 19$
No. and frequency of standard reflections	3 every 50 reflections
Refinement	
Refinement on	F^2
$R[F^2>2\sigma(F^2)]$, $wR(F^2)$, S	0.0635, 0.1477, 1.056
No. of reflections and parameters used in refinement	2494, 736
H-atom treatment	Mixed
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0540P)^2 + 409.4507P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.073
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	1.035, −1.082

Computer programs used: ILL programs *Hklgen* and *Mad*, *SHELXL*97 (Sheldrick & Schneider, 1997).

bonds can be either symmetrical with comparable H···O distances or asymmetrical with shorter and longer H···O distances for the major and minor components, respectively. The 'band-flip' motif was also found in CA14 and in CA26, which is folded like a figure of eight with two left-handed helical turns as in V-amylose (Gessler *et al.*, 1999).

The H-atom positions involved in the hydrogen-bonding network of CA10 remained undetermined in the X-ray study (Jacob *et al.*, 1998, 1999) even with synchrotron radiation (EMBL DESY outstation Hamburg) at a resolution of 0.92 Å, which should have been sufficient to resolve H atoms. Here we present the neutron diffraction study of $CA10\cdot 27.18H_2O$ at 20 K and clarify the hydrogen-bonding interactions in this complex.

2. Experimental

2.1. Sample preparation and neutron diffraction experiment

CA10 was prepared, purified and crystallized as described (Takaha *et al.*, 1996; Jacob *et al.*, 1998, 1999). A crystal of $CA10\cdot 27.18H_2O$ was mounted in a quartz capillary and equilibrated against a drop of mother liquor of 10% PEG400 in H_2O. D_2O was not used to avoid complications arising due to the disorder of O—H groups and water molecules. The capillary was glued to an aluminium pin using kwikfill resin and put on a Displex cryorefrigerator (Archer & Lehmann, 1986). The spherical outer cryostat can was of aluminium rather than vanadium to reduce background scattering and mounted on the thermal beam diffractometer D19 equipped with a 4 × 64° position-sensitive detector. The crystal was cooled at 0.5 K min^{-1} to 180 K and then at 0.25 K min^{-1} to a final temperature of 20 K while monitoring the strong 200 reflection at a wavelength of 1.532 Å. There was no significant change in intensity or mosaic spread during cooling, although there was an apparent rotation of the crystal in the tube by ∼0.5° in the temperature range 295–250 K, presumably associated with freezing of the mother liquor.

Reflections were measured in equatorial or normal-beam geometry, the exposure time increasing with scattering angle from 9.4 to 18.8 s per step in omega (ILL programs *Hklgen* and *Mad*). Most of the unique reflections up to 2θ of 100° (*i.e.* d_{min} = 1.0 Å) were recorded and three standard reflections were measured daily. The unit-cell dimensions at 20 K were determined from positions of 2193 strong reflections (ILL program RAFD19) in the 2θ range from 7.1 to 101.7° (Table 1). Bragg intensities were integrated in three-dimensions using the ILL program *Retreat* (Wilkinson *et al.*, 1988). They were corrected for absorption by the crystal (Table 1) with the program *D19abs*, based on the ILL version of the *CCSL* system (Matthewman *et al.*, 1982), and for attenuation by the cryostat cans (Table 1); the crystal shape was described by six faces. The aluminium powder lines from the spherical outer cryostat can were accounted for with an empirical powder line correction (ILL program). A total of 3929 reflections were recorded, yielding 2494 unique reflections: R(int) = 0.0480, R(sigma) = 0.0499. The coherent neutron scattering lengths were those tabulated by Sears (1992).

2.2. Structure determination and refinement

The crystal structure was determined using the X-ray structure of $CA10\cdot 23.5H_2O$ as the starting model (Jacob *et al.*, 1999). After several cycles of full-matrix least-squares refinement with *SHELXL*97 (Sheldrick & Schneider, 1997), in which the 1,2 and 1,3 interatomic distances of glucosides were restrained and thermal displacement factors were treated isotropically, the H atoms and water molecules were located from Fermi density maps using *Xtalview* (McRee, 1993).

The asymmetric unit contains half a CA10 molecule $(C_6H_{10}O_5)_5$ and a total of 13.59 water molecules distributed over 23 positions, of which six are fully occupied. Three glucoside O6—H groups are twofold disordered; during refinement, the sums of their occupation factors were fixed at

Table 2
Selected geometrical parameters (Å, °) of CA10·27.18H_2O.

Residue	(1)	(2)	(3)	(4)	(5)
Q†	0.596	0.569	0.572	0.569	0.586
θ‡	5.74	4.44	10.13	1.74	4.08
φ§	76.5	103.4	94.1	103.9	84.2
ψ§	82.4	126.5	101.3	98.5	−63.0
O4 angle¶	125.6	142.9	141.5	135.0	146.2
C4(n)−O4(n)−C1(n + 1)††	116.1	118.9	114.6	116.6	119.0
O4(n + 1)···O4(n) (Å)	4.52	4.47	4.38	4.49	4.59
O3(n − 1)···O2(n) (Å)	5.60	3.97	2.93	2.99	2.78
O(5)−C(5)−C(6)−O(6)	58.9/−59.6	−68.4	−65.3/72.0	−56.1	−72.7/43.8
Occupation of O6	0.77/0.23	1.00	0.56/0.44	1.00	0.75/0.25

† Puckering amplitude (Å) defined by Cremer & Pople (1975). ‡ Deviation (°) from theoretical chair conformation (ideal value: θ = 0°). § Torsion angles φ and ψ at glycosidic O4, defined as O5(n)−C1(n)−O4(n − 1)−C4(n − 1) and C1(n)−O4(n − 1)−C4(n − 1)−C3(n − 1), respectively. ¶ Angle between neighboring O4 atoms, O4(n + 1)−O4(n)−O4(n − 1). †† Angle at the glycosidic bond, C4(n)−O4(n)−C1(n + 1).

1.0. All H atoms of CA10 could be located, 12 of the C−H hydrogen atoms refined poorly and were fixed at C–H 1.1 Å. The severe disorder of water molecules in the CA10 cavity made refinement difficult; 37 of the water H atoms were restrained to chemically reasonable water geometry. A total of 736 atomic parameters were isotropically refined against 2355 data with $F_o^2 > 2\sigma(F_o^2)$ and against all 2494 data (Table 1). The geometrical parameters collected in Table 2 were calculated using *PARST*96 (Nardelli, 1995).

3. Results and discussion

The crystallographic data for the neutron study of CA10·27.18H_2O (at 20 K) are given in Table 1. Fractional atomic coordinates, isotropic thermal displacement factors, occupation factors, bond angles and distances, torsion angles, and glucoside puckering parameters of CA10 are given as supplementary materials.[1] The atom labeling is as generally used for carbohydrates, *i.e.* C32 denotes C3 of the glucoside residue 2 in CA10. An additional letter, *A*, *B* or *C*, is used to distinguish partially occupied atom sites or if two atoms are bound to a central atom, *e.g.* H_2O or CH_2.

3.1. Molecular structure of CA10·27.18H_2O

In the asymmetric unit half a CA10 molecule and 13.59 water molecules are located. The other half is related by a crystallographic twofold rotation axis and the atom names in the second half are distinguished by a prime (Fig. 1). As described by Jacob *et al.* (1998, 1999), CA10 adopts an elliptical shape with two diametrically opposed glucoside units G1 and G5′ flipped by ∼180°, see Fig. 2. This conformation is stabilized by hydrogen bonds, as described in §3.3.1.

The neutron structure of CA10 is isomorphous to the X-ray structure (Jacob *et al.*, 1999), as shown by the small r.m.s. deviation of 0.072 Å when two CA10s are superimposed (all H atoms and O6 were excluded from the calculation). However, there are differences in the disorder of O6−H groups, and the number and distribution of water molecules in the crystal lattice (discussed in §3.2).

All glucosides in CA10 are in the $^4C_1$ chair conformation and O5−C5−C6−O6 torsion angles are in the −*gauche* and +*gauche* ranges, see Table 2. Two C6−O6 groups (G2 and G4) are ordered and oriented −*gauche* (−68.4°, −56.7°); the other three C6−O6 groups (G1, G3 and G5) are disordered and −*gauche*/+*gauche*, with the main disorder component +*gauche* for G1 (occupancy = 0.77) 58.9/−59.6 and −*gauche* in G3 (occupancy = 0.56) −65.3/72.0 and G5 (occupancy = 0.75) −72.7/43.8.

3.2. Disordered water molecules occupy 17 positions

In the asymmetric unit six water sites are fully occupied and the remaining 7.59 water molecules are distributed over 17 sites, see Figs. 1(*b*) and (*c*). This contrasts with the room-temperature X-ray structure of CA10·23.50H_2O (Jacob *et al.*, 1999), which contains 11.75 water molecules in the asymmetric unit; they are distributed over 20 sites, of which only three are fully occupied (Fig. 1*a*). In the 20 K neutron structure, 16 water sites are at the same positions as in the X-ray structure (but with different occupancies), namely *W*1–*W*5, *W*7–*W*14, *W*17–*W*19. At 20 K, of the three water sites outside the CA10 cavity two are fully occupied (*W*2 and *W*3) and one is partially occupied (*W*4; occupancy factor 0.6). The 10.99 water molecules located in the CA10 cavity are distributed over 20 sites, of which four are fully occupied (*W*1, *W*5, *W*7, *W*9) and the other 16 (*W*6, *W*8*A*, *W*8*B*, *W*10*A*, *W*10*B*, *W*11*A*, *W*11*B*, *W*12, *W*13*A*, *W*13*B*, *W*13*C*, *W*14, *W*17, *W*18*A*, *W*18*B*, W19) are partially occupied in the range 0.23–0.76, average 0.44, see Figs. 1(*b*) and (*c*).

Five water molecules (*W*8, *W*10, *W*11, *W*13, *W*18) are twofold or threefold disordered with the sum of occupancies equal to 1.0. The major site occupancies of *W*8, *W*10, *W*11 and *W*18 are 0.76, 0.76, 0.6 and 0.65, respectively. The major site *W*10*A* is rotationally disordered and shows three H-atom positions. *W*13 is threefold disordered with occupancy = 0.45, 0.32, 0.23 and one H atom of *W*13*C* could not be determined. Since the distance between *W*14 and O61*A* (occupancy = 0.77) is only 1.90 Å, these positions cannot be occupied simultaneously (Fig. 2).

3.3. Hydrogen bonds

All hydroxyl and water H atoms take part in hydrogen-bonding interactions with geometrical parameters listed in Table 3. In this paper we use cutoffs for the O···O distance of less than 3.50 Å and H···O distance of less than 2.85 Å (slightly larger than the sum of the van der Waals radii

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: JZ0013). Services for accessing these data are described at the back of the journal.

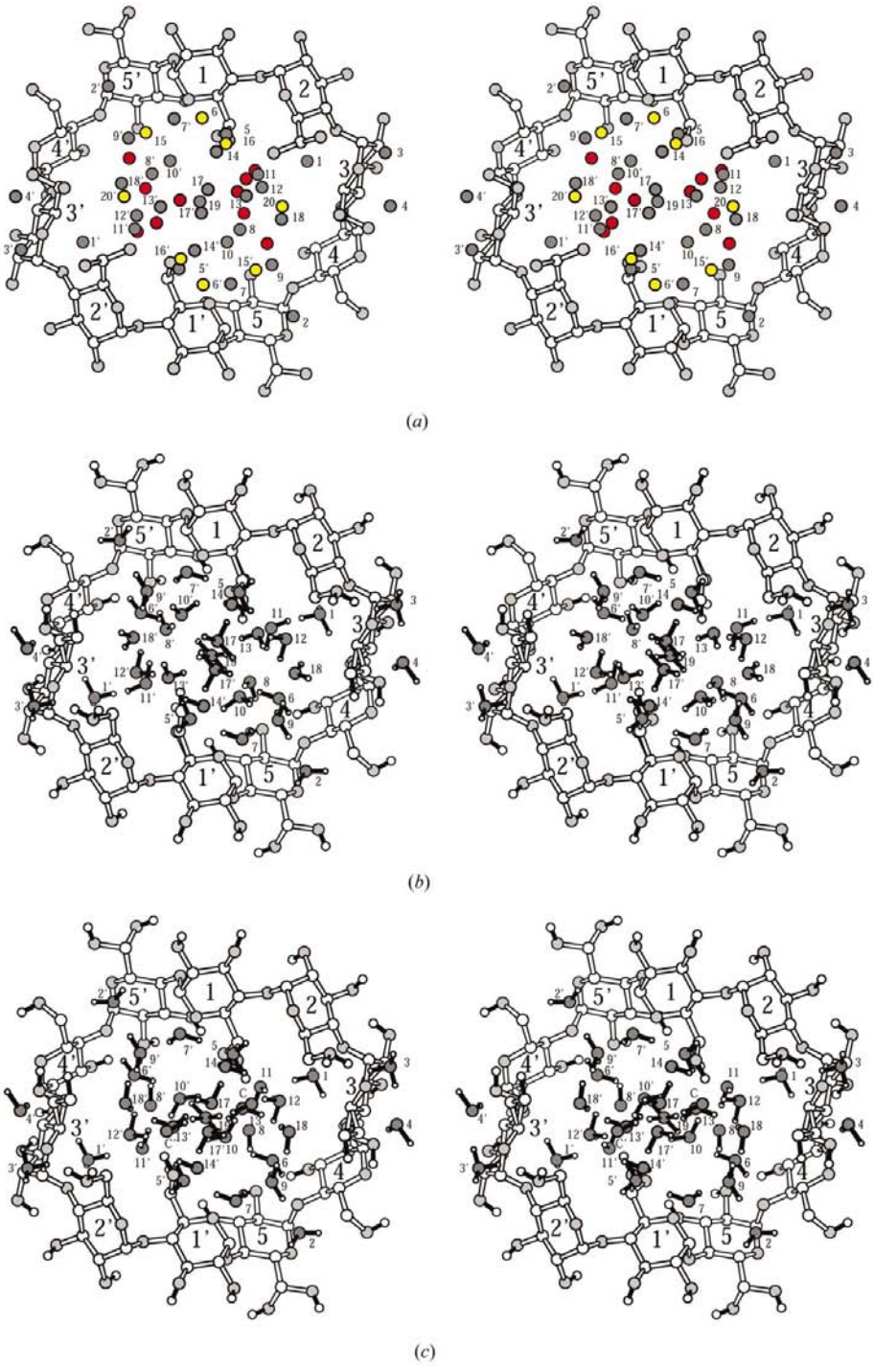

Figure 1
Stereo plot of (*a*) X-ray structure of CA10·23.50H_2O at room temperature (Jacob *et al.*, 1998, 1999), (*b*) and (*c*) neutron structure of CA10·27.18H_2O at 20 K. (*a*) shows the position of water in CA10·27.18H_2O and CA10·23.50H_2O for comparison. Dark gray circles show identical water positions in both CA10 structures. Yellow and red circles present different atom positions in CA10·23.50H_2O at room temperature and in CA10·27.18H_2O at 20 K, respectively. For clarity, the major and minor sites of water molecules are separately drawn in (*b*) and (*c*), respectively; C—H hydrogen atoms not shown. Unfilled, light and dark gray circles represent the CA10 C, O and water O atoms, respectively; O—H bonds are illustrated with black sticks. Small and large italic numbers indicate the water molecules and glucoside residues, respectively. Primed (′) atoms belong to the asymmetric unit related to the unprimed atoms of CA10 hydrate by the crystallographic twofold rotation axis (passing through W19 and normal to the paper plane). Drawn with the program *MOLSCRIPT* (Kraulis, 1991).

2.60 Å), and angles O—H···O greater than 90°. Nine of the 18 hydroxyl groups accept one hydrogen bond; O24, O25, O32, O33 and O61*A* accept two, O34, O61*B* and O62 accept three, and O63*B* accepts four hydrogen bonds (see Fig. 3).

3.3.1. Band-flips and kinks. CA10 has two symmetrically equivalent 'band-flips', between glucosides G5′ and G1, G5 and G1′. The flipped glucoside is stabilized in an *anti* orientation by intramolecular three-center hydrogen bonds with $O3_n$—H as donors and $O5_{n+1}/O6_{n+1}$ as acceptors; O35′—H···O61*A* (occupancy = 0.77) being major and O35′—H···O51 being minor components (see Fig. 2). In addition, the 'band-flip' is stabilized by *W*3, which mediates hydrogen bonding between O65*A*′ and O21, whose separation of 3.86 Å is too long to permit direct hydrogen bonding. No intramolecular hydrogen bond is formed between glucosides G1 and G2, as evidenced by a long distance between O22···O31 of 3.97 Å. This is due to a 'kink' between glucosides 2 and 3 (see φ and ψ angles; Table 2) that is found associated with a 'band-flip' to relieve steric strain (Gessler *et al.*, 1999).

Except for the 'band-flip' and 'kink', all other glucosides in CA10 are stabilized in the *syn* orientation by typical intramolecular $O2_n\cdots O3_{n-1}$ hydrogen bonds between adjacent glucosides. As commonly observed in CA crystal structures, they may be of the form $O2_n$—H···$O3_{n-1}$ or $O2_n$···H—$O3_{n-1}$ and are of the three–center type, with minor component H···$O4_{n-1}$ to the associated glycosidic link. There are one $O2_n$—H···$O3_{n-1}$ and two $O2_n$···H—$O3_{n-1}$ interglucoside hydrogen bonds.

3.3.2. Three-center hydrogen bonds. Of the 123 symmetry-independent hydrogen bonds in the asymmetric unit of CA10·27.18H_2O, 25 (= 20%) are found to be three-center (Table 4 and Fig. 3) and 6 (= 5%) are four-center. All others are of the 'conventional' two-center type (O—H···O; Table 3). Of the 25 three-center hydrogen bonds, O61*B* and

Table 3
O—H···O hydrogen-bond geometry (Å, °) in CA10·27.18H_2O (neutron structure).

	O—H	O···O	H···O	O—H···O	Sym.
O21—Ho21···O23	0.98 (2)	2.86 (2)	2.01 (2)	144 (2)	i
O31—Ho31···*OW1*	0.97 (2)	2.86 (2)	1.89 (2)	175 (2)	i
O61*A*—Ho61···*OW11B*	0.95 (4)	2.80 (3)	1.86 (5)	175 (4)	ii
O61*B*—HB61···*OW19*	0.97 (4)	3.02 (5)	2.33 (1)	128 (3)	ii
O22—Ho22···O52	0.91 (3)	2.89 (2)	2.06 (2)	152 (2)	iii
O32—Ho32···O35	0.95 (2)	2.77 (2)	1.82 (2)	176 (2)	iv
O62—Ho62···*OW1*	0.95 (4)	2.69 (2)	1.75 (4)	173 (4)	v
O62—HB62···O53	1.05 (2)	3.33 (1)	2.45 (3)	141 (1)	ii
O23—Ho23···O32	0.98 (2)	2.93 (1)	2.01 (2)	155 (2)	ii
O33—Ho33···O24	0.96 (3)	2.99 (2)	2.05 (3)	166 (2)	ii
O63*A*—Ho63···O65*A*	1.04 (3)	2.77 (3)	1.75 (3)	164 (3)	vi
O63*B*—HB63···O65*A*	1.14 (6)	3.31 (3)	2.38 (5)	138 (5)	vi
O24—Ho24···*OW4*	0.90 (4)	2.69 (2)	1.79 (5)	176 (3)	vii
O24—HB24···O63*B*	1.14 (4)	2.98 (3)	1.86 (5)	166 (4)	vii
O34—Ho34···O25	0.92 (3)	2.78 (2)	1.87 (3)	175 (2)	ii
O64—Ho64···O23	0.96 (3)	2.84 (2)	1.88 (3)	172 (2)	viii
O25—Ho25···*OW7*	1.01 (2)	2.61 (2)	1.61 (2)	172 (2)	vii
O35—Ho35···O61*A*	0.92 (2)	2.77 (2)	1.86 (2)	169 (2)	ix
O65*A*—Ho65···*OW3*	0.92 (4)	2.71 (3)	1.79 (3)	177 (3)	x
O65*B*—HB65···O34	0.97 (9)	2.72 (5)	1.77 (9)	165 (10)	vi
OW1—H1*W1*···O24	0.99 (2)	2.86 (1)	1.91 (2)	160 (2)	ii
OW1—H2*W1*···O62	0.96 (2)	2.69 (2)	1.75 (3)	170 (2)	vii
OW1—H3*W1*···*OW11B*	0.96 (5)	2.71 (4)	1.75 (6)	178 (3)	ii
OW2—H1*W2*···O64	0.92 (3)	2.78 (2)	1.87 (2)	166 (2)	vi
OW2—H2*W2*···O55	0.95 (3)	2.91 (2)	2.02 (2)	157 (2)	vi
OW3—H1*W3*···O21	0.94 (2)	2.78 (2)	1.84 (2)	174 (2)	i
OW3—H2*W3*···O62	0.97 (4)	2.95 (2)	2.12 (3)	142 (2)	ii
OW3—H3*W3*···*OW4*	0.93 (1)	2.79 (2)	1.89 (2)	163 (2)	ii
OW4—H1*W4*···O63*A*	0.96 (4)	2.71 (3)	1.76 (4)	169 (3)	ii
OW4—H2*W4*···*OW3*	1.13 (3)	3.00 (2)	1.91 (2)	161 (3)	viii
OW5—H1*W5*···O22	0.95 (3)	2.79 (2)	1.84 (3)	174 (2)	i
OW5—H2*W5*···*OW13A*	0.95 (2)	2.79 (2)	1.85 (3)	166 (3)	ii
OW6—H1*W6*···O44	0.96 (7)	2.86 (4)	1.91 (6)	170 (6)	ii
OW6—H2*W6*···*OW10B*	0.96 (9)	2.67 (6)	1.72 (9)	170 (6)	ii
OW7—H1*W7*···*OW5*	0.95 (3)	2.73 (2)	1.78 (3)	172 (2)	ix
OW7—H2*W7*···*OW9*	0.95 (3)	2.76 (2)	1.85 (3)	160 (3)	ii
OW8A—H1*W8*···O25	0.92 (3)	2.77 (2)	1.86 (3)	177 (3)	ii
OW8A—H2*W8*···*OW10A*	0.96 (3)	2.73 (2)	1.78 (3)	169 (2)	ii
OW8B—H3*W8*···O25	0.96 (6)	2.93 (3)	2.02 (4)	159 (7)	ii
OW9—H1*W9*···*OW2*	0.95 (2)	2.79 (1)	1.85 (1)	174 (2)	ii
OW9—H2*W9*···*OW18A*	0.95 (3)	2.91 (2)	1.97 (4)	171 (3)	ii
OW9—H3*W9*···*OW6*	0.95 (3)	2.71 (5)	1.76 (6)	176 (3)	ii
OW10A—H101···*OW9*	0.99 (3)	2.81 (2)	1.86 (3)	159 (3)	ii
OW10A—H102···O61*A*	0.97 (10)	2.89 (3)	1.95 (9)	163 (6)	ix
OW10A—H103···*OW8A*	0.99 (4)	2.73 (2)	1.99 (4)	130 (3)	ii
OW10B—H104···*OW11A*	0.96 (8)	3.00 (7)	2.39 (8)	121 (5)	ii
OW10B—H105···*OW19*	1.00 (7)	3.03 (5)	2.33 (6)	126 (4)	ii
OW11A—H111···*OW8A*	1.05 (5)	2.84 (3)	1.81 (4)	169 (4)	ii
OW11A—H112···*OW1*	1.03 (7)	2.78 (4)	1.77 (6)	164 (5)	0
OW11B—H113···*OW8A*	0.96 (4)	2.83 (3)	1.97 (4)	149 (4)	0
OW11B—H114···*OW12*	0.97 (5)	3.30 (5)	2.39 (6)	157 (4)	0
OW12—H121···O61*B*	0.96 (9)	2.92 (5)	2.24 (7)	127 (5)	ii
OW12—H122···*OW11B*	0.96 (6)	3.30 (5)	2.34 (6)	175 (4)	ii
OW12—H123···*OW6*	1.10 (9)	3.14 (6)	2.08 (9)	161 (6)	ii
OW13A—H131···*OW18A*	0.96 (4)	2.79 (2)	2.02 (3)	136 (3)	ii
OW13A—H132···*OW19*	0.96 (6)	2.69 (3)	1.76 (6)	161 (5)	ii
OW13B—H133···*OW17*	0.96 (7)	2.87 (4)	1.95 (6)	161 (5)	ix
OW13B—H134···*OW18A*	1.07 (10)	2.60 (3)	1.60 (8)	154 (7)	ii
OW13C—H135···*OW17*	0.96 (12)	2.73 (8)	1.81 (10)	159 (9)	ix
OW14—H141···*OW11A*	0.99 (11)	2.69 (8)	1.72 (8)	168 (7)	v
OW14—H142···*OW5*	1.06 (8)	2.91 (6)	1.94 (8)	150 (5)	ii
OW17—H117···*OW8A*	0.96 (5)	2.73 (3)	1.80 (6)	161 (4)	xi
OW17—H217···*OW5*	1.05 (7)	3.00 (3)	2.00 (6)	157 (6)	ii
OW17—H317···*OW11B*	1.05 (5)	3.33 (4)	2.43 (7)	144 (4)	v
OW18A—H181···O34	0.96 (4)	2.87 (2)	2.01 (3)	149 (3)	v
OW18A—H182···*OW12*	0.95 (4)	3.48 (4)	2.65 (4)	146 (2)	ii
OW18B—H183···*OW9*	0.95 (4)	2.70 (3)	1.97 (4)	132 (4)	ii
OW18B—H184···*OW12*	0.95 (6)	2.78 (4)	1.95 (6)	144 (4)	ii
OW19—H119···O13*B*	0.96 (4)	2.89 (4)	1.95 (5)	166 (3)	ii
OW19—H219···*OW10A*	1.11 (3)	2.75 (2)	1.67 (3)	165 (2)	ii

Symmetry codes: (i) $-x+\frac{1}{2}, y-\frac{1}{2}, -z$; (ii) x, y, z; (iii) $-x+\frac{1}{2}, y+\frac{1}{2}, -z$; (iv) $x-\frac{1}{2}, y-\frac{1}{2}, z$; (v) $x, y-1, z$; (vi) $-x+\frac{3}{2}, y-\frac{1}{2}, -z+1$; (vii) $x, y+1, z$; (viii) $-x+1, y, -z+1$; (ix) $-x+1, y, -z$; (x) $x+\frac{1}{2}, y+\frac{1}{2}, z$; (xi) $-x+1, y-1, -z$.

W10B donate symmetric three-center bonds to O*W12*, O*W19* and O*W11B*, O*W19*; the others are asymmetric (see Table 4). The three intramolecular hydrogen bonds $O2_n\cdots O3_{n-1}$ donate to the corresponding glycosidic O4 atoms as a minor component with H···O4 distances in the range 2.32–2.60 Å, with an average of 2.50 Å.

The minor site hydroxyl group O62—H*B* donates an intramolecular interglucosidic three–center hydrogen bond to O53, with major component to W3′ and minor component to O53 (Table 5 and Fig. 3). The O54 atom is an acceptor of a three–center hydrogen bond from O64—H in the same residue (Fig. 3).

3.3.3. Hydrogen bonds in the CA10 cavity. The CA10 cavity differs from those of smaller CAs as it is not 'round', but 'elliptical' and slit-like. In the cavity a total of 10.99 water molecules are distributed over 14 sites. Three of these sites are fully occupied (*W*1, *W*5 and *W*7) and the remaining 11 are partially occupied (*W*6, *W*8–*W*14 and *W*17–*W*19). Together with the hydroxyl groups of CA10, they form an extended hydrogen-bonding network. A homodromic cyclic octagonal pattern formed by O61*A* → *W11A* → *W8A* → *W10A* → O61*A*′ → *W11A*′ → *W8A*′ → *W10A*′ → O61*A* connects the two 'band-flipped' glucosides G1 and G1′ in the same CA10 (Fig. 4). *W10A* and *W10A*′ are bridged by and hydrogen bonded to *W19*, whose O atom is placed on the *C*2 axis located in the center of the octagon. (The O···O distances in the hydrogen bonds of this octagon vary from 2.73 to 2.89 Å and the O—H···O angles from 161 to 169°.) The partly occupied minor sites O61*B* (occupancy = 0.23) and O61*B*′ donate hydrogen bonds to O*W19* (O61*B*—H···*W19*···O61*B*′—H; Fig. 5*b*).

W3 and *W4* are located outside the cavity and are connected to CA10 hydroxyl groups directly (Table 4 and Fig. 5). They

Table 4
Three-center hydrogen bonds (H···A < 2.85 Å, angle O—H···A > 90°) and sum of angles (sum), involving O and H atoms of CA10, see Fig. 3 (all water···water interactions omitted).

A = acceptor O atoms; sum = sum of the angles around H.

	O—H	O···A	H···A	O—H···A	A_1···A_2	A_1···H···A_2	Sum	Symmetry
O21—Ho21···O23	0.98 (2)	2.86 (2)	2.01 (2)	144 (2)	2.93 (2)	86 (1)	360	i
O21—Ho21···O33		2.95 (1)	2.21 (2)	130 (2)				i
O61*B*—HB61···O*W*12	0.97 (4)	2.92 (5)	2.33 (3)	118 (3)	3.75 (3)	107	353	ii
O61*B*—HB61···O*W*19		3.02 (5)	2.33 (1)	128 (3)				ii
O22—Ho22···O52	0.91 (3)	2.89 (2)	2.06 (2)	152 (2)	3.46 (1)	104 (1)	359	iii
O22—Ho22···O41		2.68 (2)	2.32 (3)	103 (2)				ii
O62—HB62···O53	1.05 (2)	3.33 (1)	2.45 (3)	141 (1)	2.96 (1)	81 (1)	359	ii
O62—HB62···O*W*3		2.95 (2)	2.09 (3)	137 (1)				ii
O63*B*—HB63···O34	1.14 (6)	3.11 (4)	2.56 (6)	108 (4)	4.01 (2)	108 (2)	354	iv
O63*B*—HB63···O65*A*		3.31 (3)	2.38 (5)	138 (5)				v
O24—Ho24···O63*B*	0.90 (4)	2.98 (3)	2.57 (5)	108 (3)	2.72 (3)	75 (2)	359	vi
O24—Ho24···O*W*4		2.69 (2)	1.79 (5)	176 (3)				vi
O24—HB24···O63*B*	1.14 (4)	2.98 (3)	1.86 (5)	166 (4)	2.72 (3)	83 (2)	349	vi
O24—HB24···O*W*4		2.69 (2)	2.24 (5)	100 (3)				vi
O34—Ho34···O44	0.92 (3)	2.91 (2)	2.60 (2)	101 (2)	2.76 (1)	74 (1)	350	ii
O34—Ho34···O25		2.78 (2)	1.87 (3)	175 (2)				ii
O64—Ho64···O54	0.96 (3)	2.79 (1)	2.52 (1)	96 (1)	3.20 (1)	92 (1)	360	ii
O64—Ho64···O23		2.84 (2)	1.88 (3)	172 (2)				vii
O35—Ho35···O51	0.92 (2)	3.21 (2)	2.85 (2)	104 (2)				viii
O35—Ho35···O61*A*		2.77 (2)	1.86 (2)	169 (2)	2.81 (2)	69 (1)	342	viii
O35—Ho35···O*W*14		2.66 (5)	1.99 (5)	129 (3)	4.32 (6)	125 (2)	358	ix
O*W*2—H2*W*2···O55	0.95 (3)	2.91 (2)	2.02 (2)	157 (2)	2.60 (5)	74 (1)	353	v
O*W*2—H2*W*2···O65*B*		2.90 (7)	2.28 (7)	122 (2)				v
O*W*3—H1*W*3···O21	0.94 (2)	2.78 (2)	1.84 (2)	174 (2)	2.70 (1)	68 (1)	351	i
O*W*3—H1*W*3···O45		3.20 (1)	2.77 (2)	109 (1)				x
O*W*3—H2*W*3···O62	0.97 (4)	2.95 (2)	2.12 (3)	142 (2)	3.33 (1)	93 (1)	347	ii
O*W*3—H2*W*3···O53		2.97 (2)	2.46 (3)	112 (2)				0
O*W*4—H1*W*4···O53	0.96 (4)	3.31 (2)	2.77 (3)	117 (2)				ii
O*W*4—H1*W*4···O63*A*		2.71 (3)	1.76 (4)	169 (3)	2.77 (2)	72 (1)	358	ii
O*W*4—H1*W*4···O63*B*		2.72 (4)	2.14 (5)	117 (3)	2.89 (3)	71 (1)	305	ii
O*W*10*A*—H102···O51	0.97 (10)	3.06 (2)	2.47 (6)	119 (6)				viii
O*W*10*A*—H102···O61*A*		2.89 (3)	1.95 (9)	163 (6)	2.81 (2)	78 (1)	360	viii
O*W*10*A*—H102···O61*B*		3.23 (6)	2.63 (9)	120 (5)	2.98 (4)	71 (1)	310	viii
O*W*18*A*—H181···O63*B*	0.96 (4)	2.71 (4)	2.34 (6)	103 (3)	3.11 (3)	91 (1)	343	ii
O*W*18*A*—H181···O34		2.87 (2)	2.01 (3)	149 (3)				iv

Symmetry codes: (i) $-x+\frac{1}{2}, y-\frac{1}{2}, -z$; (ii) x, y, z; (iii) $-x+\frac{1}{2}, y+\frac{1}{2}, -z$; (iv) $x, y-1, z$; (v) $-x+\frac{3}{2}, y-\frac{1}{2}, -z+1$; (vi) $x, y+1, z$; (vii) $-x+1, y, -z+1$; (viii) $-x+1, y, -z$; (ix) $-x+1, y+1, -z$; (x) $x-\frac{1}{2}, y-\frac{1}{2}, z$.

are involved in two different hydrogen bonding cycles (*J* and *K*) outside the cavity (Figs. 5 and 6).

3.3.4. Hydrogen-bonding network. In the crystal lattice CA10 molecules are stacked on top of each other like coins in a roll, leading to infinite channels parallel to the *b* axis. Within the channels, water molecules and CA10 O—H groups form an infinite hydrogen-bond network composed of nine repeating polygons. Outside the CA10 cavity, *W*3 and *W*4 form two polygons involving secondary hydroxyl groups (see *J* and *K* in Figs. 5 and 6). A total of 11 polygons have homodromic, antidromic and heterodromic directional arrangements. A well defined hexagon (Fig. 6, *A*) is found in the center of the channel. Additionally, one triangle (*B*), one quadrilateral (*C*), three pentagons (*D*, *E* and *F*), two hexagons (*G* and *H*) and

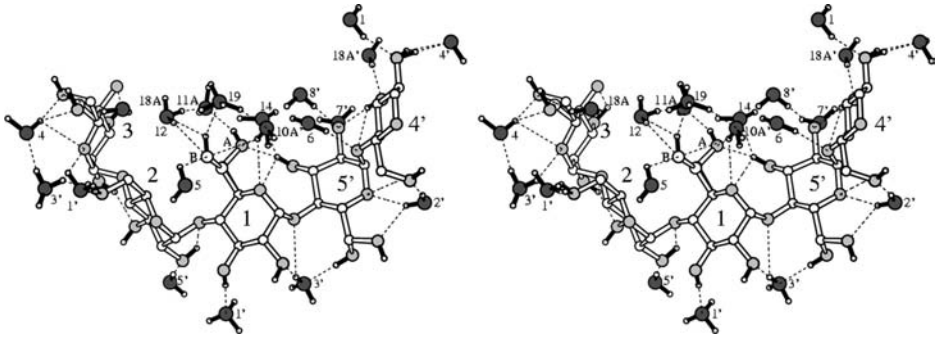

Figure 2
Stereo plot of the asymmetric unit of CA10·27.18H_2O showing the 'band–flip'. C—H hydrogen atoms of CA10 are omitted for clarity. Hydrogen bonds are indicated by dashed lines. Glucosides and water O atoms in different asymmetric units distinguished by ′.

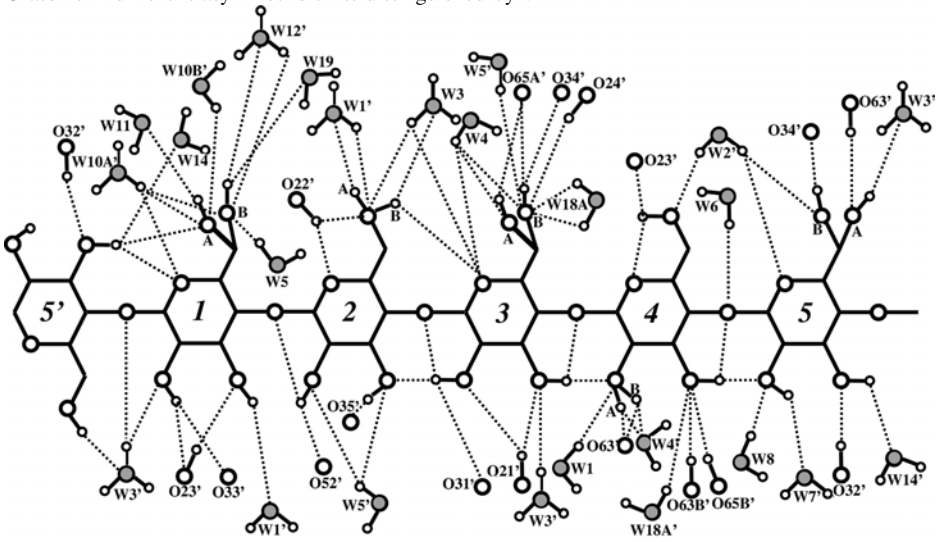

Figure 3
Schematic representation of the hydrogen-bonding arrangement around CA10. Large white and gray circles represent O atoms of CA10 and water molecules, respectively, and small open circles H atoms. Hydrogen bonds are indicated by dotted lines. Glucoside numbering in italics, partially occupied atom sites indicated by *A* and *B*.

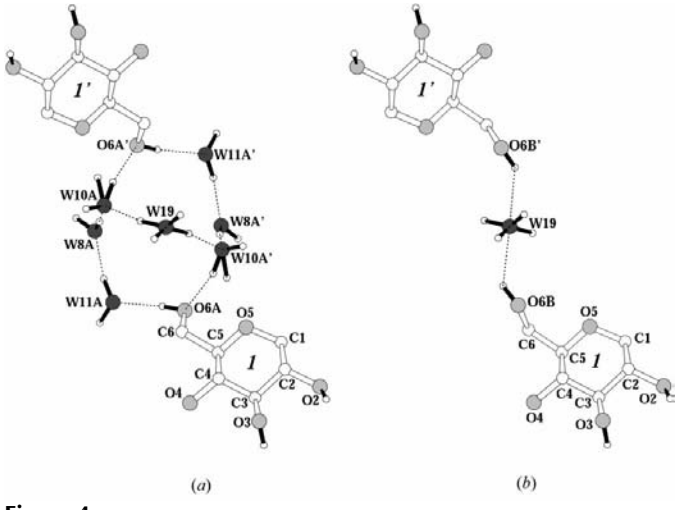

Figure 4
(*a*) Octagonal, homodromic cycle and (*b*) bridge found between the 'band-flipped' glucosides G1 and G1′ of the CA10 molecule. (*a*) and (*b*) indicate the major and minor site of O61—H, respectively. The crystallographic twofold rotation axis is normal to the paper plane and passes through *W*19, whose H-atom sites are occupied at 0.5 each.

one nonagon (*I*) are formed around the hexagon (*A*). One quadrilateral (*J*) and one pentagon (*K*) are next to the nonagon (*I*), with antidromic arrangements.

In the following we describe three of the polygons as representative examples; see Fig. 6. The numbers in parentheses indicate equivalent positions. The well defined hexagon *A* (Fig. 6) is formed exclusively by water molecules *W*17(3), *W*8*A*, *W*10*A*, *W*19, *W*10*A*(1) and *W*8*A*(1), and is located in the CA10 cavity. This hexagon has the two water sites *W*17(2) and *W*17(3) in its ring, which are related by the *C*2 axis and cannot be occupied simultaneously as they are separated by only 1.35 Å, hence their occupancy is 0.5. *W*19 is located on the twofold rotation axis and features four half-occupied H atoms. Pentagon *F* (Fig. 6) is formed by two hydroxyl groups (O34 and O25) and three water molecules [*W*7(2), *W*9(2) and *W*18*A*(2)] and is located next to another pentagon *D* [two O—H groups are common, O25 and *W*7(2), Fig. 5]. The O—H groups in pentagon *F* are in a homodromic hydrogen-bond orientation; this is the energetically most stable state because of the cooperative effect which, as shown by quantum chemical calculations, contributes ∼25% additional energy to an individual hydrogen bond (Lesyng & Saenger, 1981*a*,*b*). Pentagon *K* (Fig. 6) is formed by two water molecules, *W*3 and *W*4, located outside the cavity and by the three secondary hydroxyl groups, O24(7), O33(7) and O21(6).

3.4. Comparison with the X-ray structure at room temperature

The crystal structures of CA10 determined with neutron data at 20 K and with X-ray data at room temperature are isomorphous, although the numbers of water molecules are different, 27.18 and 23.50 H_2O, respectively. A comparison of the hydroxyl groups of CA10 and disordered water positions is appropriate.

In the two crystal structures the coordinates of the C and O atoms of CA10 are almost identical, except for the disordered O61, O63 and O62. In the X-ray structure O62 is disordered and in the neutron structure O62 is well ordered, but O61 and O63 are disordered. This we associate with sample preparation and/or data collection at 20 and 298 K, respectively. Of the water molecules, 16 are located at identical positions in the

Table 5
Elements of the hydrogen-bonding network in CA10·27.18H_2O.

Equivalent positions as given in the caption to Fig. 5.

A	*W*17(3) → *W*8*A* → *W*10*A* ← *W*19 → *W*10*A*(1) → *W*8*A*(1) ← *W*17(3) Hexagon: heterodromic
B	*W*5 → *W*13*B* → *W*17 → *W*5 Triangle: homodromic
C	O61*B* → *W*19 → *W*13*B* ← *W*5 → O61*B* Tetragon: antidromic
D	*W*17(3) → *W*8*A* → O25 → *W*7(2) → *W*5(3) ← *W*17(3) Pentagon: antidromic
E	*W*10*A* → *W*9 → *W*18*A* ← *W*13*B* ← *W*19 → *W*10*A* Pentagon: antidromic
F	O34 → O25 → *W*7(2) → *W*9(2) → *W*18*A*(2) → O34 Pentagon: homodromic
G	*W*7(1) → *W*5 ← *W*17 ← *W*13*B*(1) → *W*18*A*(1) ← *W*9(1) ← *W*7(1) Hexagon: heterodromic
H	*W*8*A* → *W*10*A* ← *W*19 ← O61*B*(1) ← *W*12 → *W*11*A* → *W*8*A* Hexagon: antidromic
I	*W*4(2) → O63*B*(2) ← *W*18*A*(2) → O34 → O25 ← *W*8*A* ← *W*11*A* → *W*1 → O24 → *W*4(2) Nonagon: heterodromic
J	O63*A* → O65*A*(5) → *W*3(4) ← *W*4 → O63*A* Tetragon: antidromic
K	O24(7) → *W*4 ← *W*3 → O21(6) →O33(7) → O24(7) Pentagon: antidromic

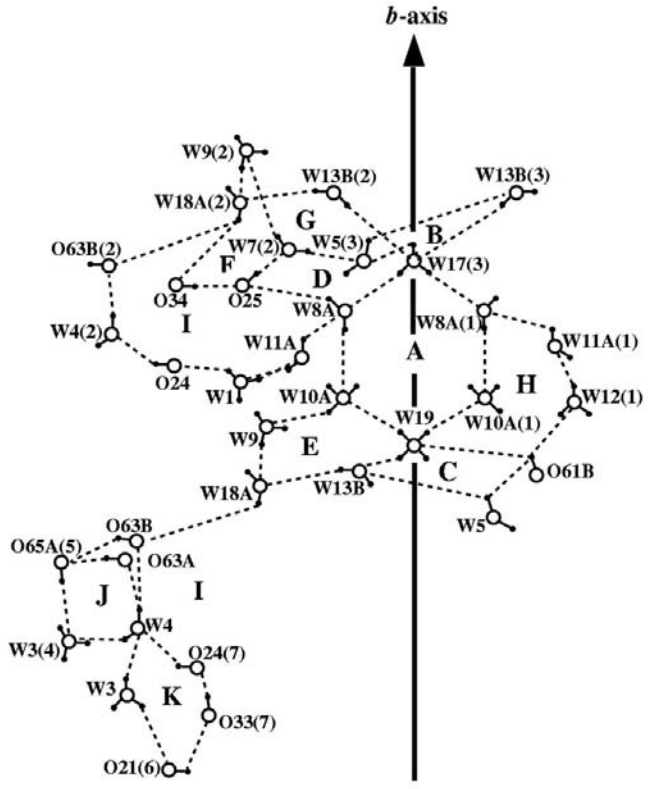

Figure 5
Schematic description of the infinite hydrogen-bonding network located in the channel-like cavity formed by stacking of CA10 molecules along the *b* axis (arrow). *W*2, *W*6 and *W*14 are not shown as they are not involved in hydrogen-bonded cycles. Polygons *A*–*K* are cyclic hydrogen-bonded structures shown in detail in Fig. 6. The numbers in parentheses show equivalent positions: (1) $-x+1, y, -z$; (2) $x, y+1, z$; (3) $-x+1, y+1, -z$; (4) $-x+1, y, -z+1$; (5) $-x+\frac{1}{2}, y-\frac{1}{2}, -z+1$; (6) $-x+\frac{1}{2}, y-\frac{1}{2}, -z$; (7) $x, y-1, z$.

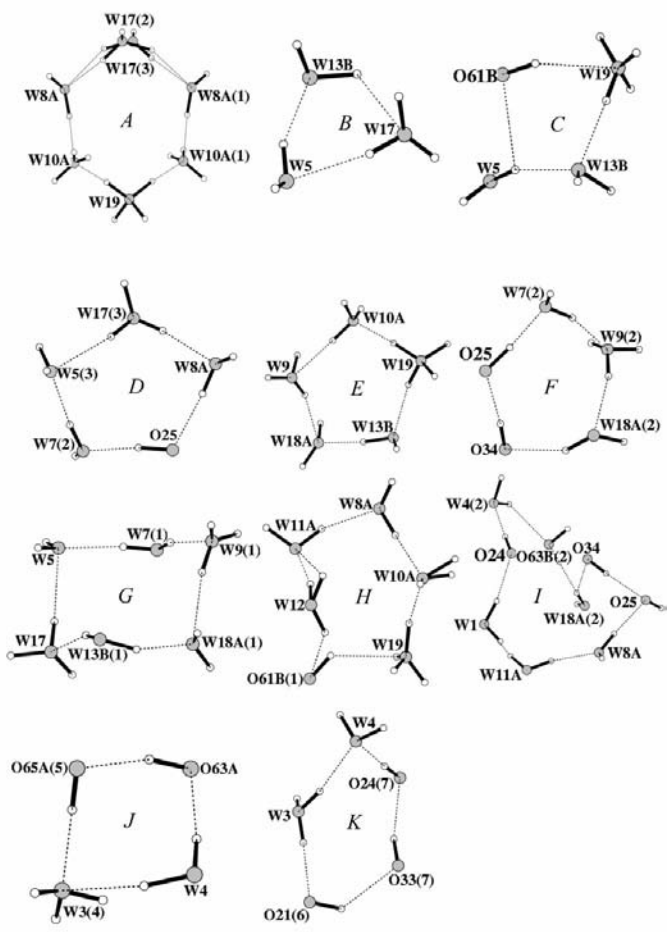

Figure 6
Cyclic elements of the hydrogen-bonding network in CA10·27.18H_2O. *B* triangle; *C*, *J* tetragons; *D*–*F*, *K* pentagons; *A*, *G*, *H* hexagons, and *I* nonagon. Hydrogen bonds are shown as dashed lines. See Fig. 5 for connection of these cycles. Equivalent positions as given in the caption to Fig. 5.

asymmetric units of both crystal structures. The occupation average of each water site in the cavity of the neutron structure is 1.21 times higher than in that of the X-ray structure.

4. Conclusions

This study shows that the crystal structures of room-temperature X-ray and 20 K neutron diffraction studies of hydrated CA10 are comparable but not identical. The differences between them are disordered C atoms and hydroxyl group C61, C63 and O62, and 10 water site positions, *W*6, *W*8*B*, *W*10*B*, *W*11*B*, *W*13*A*, *W*13*B*, *W*15, *W*16, *W*18*B* and *W*20. This could be due to slight variations in the conditions of crystallization or to the different temperature of data collection. There is no indication of the originally anticipated dynamically disordered flip-flop hydrogen bonds (Jacob *et al.*, 1998, 1999). The reason is probably that the disorder changes to order at low temperatures, as was observed for CA7·11H_2O at room temperature (disordered; Betzel *et al.*, 1984) and at 120 K (ordered; Zabel *et al.*, 1986).

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