Crystal and Molecular Structure of β-D-Cellotetraose Hemihydrate as a Model of Cellulose II

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Received September 30, 1994
Revised Manuscript Received December 30, 1994

The current structure of cellulose II—established independently by the two groups of Blackwell[1] and Sarko[2]—consists of a two antiparallel chains of cellulose packed within a monoclinic unit cell with $P2_1$ symmetry. In this structure, the two chains are located on two independent 2_1 screw axes. They have nearly identical conformation with the exception of the hydroxymethyl group which is in the *tg* situation in the "up" chain as opposed to *gt* in the "down" chain. The existence of two types of hydroxymethyl conformations for cellulose II has, however, been challenged. For instance, the ^{13}C solid-state NMR spectrum of this cellulose polymorph displays only a singlet for the C6 resonance as opposed to the two that should be expected if the two conformations *tg* and *gt* were present in the same structure of cellulose.[3] In addition, the polarized infrared data recorded on cellulose II point toward only one type of intramolecular hydrogen bond as opposed to two if half of the chains had their hydroxymethyl groups in the *tg* conformation.[4]

In order to clarify the fine details of the structure of cellulose II, we have undertaken a crystallographic study of cellodextrins with the goal of obtaining a set of accurate coordinates that could be transposed to cellulose II. This paper describes the molecular and crystal structure of β-D-cellotetraose that is known to give diffraction patterns[5] and infrared spectra[6] very similar to those of cellulose II. Several attempts have been made in the past to solve the crystal structure of β-D-cellotetraose[7−9] that appears to be a key structure for understanding cellulose II. Unfortunately, crystals of β-D-cellotetraose of a size suitable for a conventional X-ray study have proven impossible to grow. In the best case, they consist of very thin laths having only about 10 μm in thickness.[7] With such small crystals, a reliable data collection was not possible so far, even with powerful rotating-anode X-ray generators. These small crystals can now be processed by using the intense X-ray beam of a synchrotron. In this work we have used the new European Synchrotron Facility (ESRF) recently opened at Grenoble and report the crystal and molecular structure of β-D-cellotetraose hemihydrate.

Cellotetraose was prepared by acetolysis of cotton linters followed by deacetylation and fractionation with a high-performance liquid chromatograph (HPLC).[10] The purity of the sample was characterized by fast atom bombardment (FAB) mass spectroscopy.[11] Lathlike crystals of β-D-cellotetraose were grown by slow evaporation of a solution of this product dissolved in a water/ethanol mixture. The best crystal which had dimensions of 0.40 × 0.15 × 0.015 mm was mounted on a glass pin with the long dimension of the crystal along the pin axis (Figure 1). X-ray diffraction data were collected at room temperature, using the beam line 2(ID11) at ESRF.[12] Monochromatic radiation with a wavelength of 0.925 Å was used, and the diffraction data were recorded between θ of 1.18° and 26.15° during successive oscillations of the crystal. The data collection was achieved on image plate detectors which were scanned with a Molecular Dynamics Phosphor Imager 400E. The data were corrected using the program FIT2D[13] to minimize the distortion of the detector. The sets of intensities recorded in each plate were then scaled and corrected for Lorentz and polarization using the DENZO (Copyright Zbyszek Otwinowski) program which was also used to index the patterns and refine the unit cell parameters.

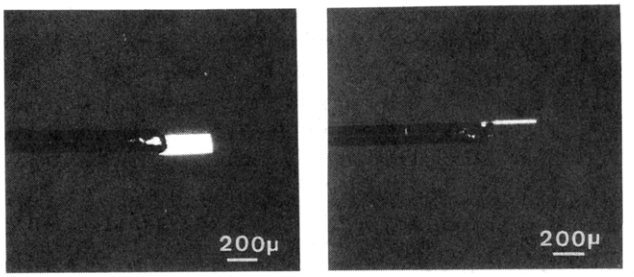

Figure 1. (A) Photomicrographs taken with polarized light between crossed nicols of the lathlike crystal of β-D-cellotetraose hemihydrate that was used for the synchrotron data collection. The crystal was mounted on a glass pin. (B) Same as in A but after a rotation of 90° about the glass pin axis.

The structure of β-D-cellotetraose hemihydrate was solved by a molecular replacement method, using the coordinates of a model deduced from a previous determination of the structure of methyl β-D-cellotrioside.[14] Consecutive cycles of full-matrix least-squares refinements and difference Fourier syntheses with the SHELX-93 program[15] revealed the positions of the four pyranose rings of the two independent β-D-cellotetraose molecules together with one water molecule. The positions and the anisotropic thermal factor of all non-hydrogen atoms were then refined. The position of the hydrogen atoms of the glucose rings were computed and constrained according to their well-known geometry. The hydrogen atoms of the hydroxyl groups were positioned at the maximum electron density on the circle that represents the loci of possible hydrogen positions for a fixed O−H distance of 0.98 Å and a C−O−H angle of 110°. The isotropic thermal factors of all the hydrogens were fixed at 1.2 times the equivalent isotropic value of their connecting atom.

The structure of β-D-cellotetraose hemihydrate is presented in Figures 2 and 3, while its atomic coordinates are listed in Tables 1 and 2. Its unit cell parameters were refined to $a = 8.045$ (12) Å, $b = 9.003$ (9) Å, $c = 22.51$ (2) Å, $\alpha = 89.66$ (7)°, $\beta = 94.83$ (13)°, $\gamma = 115.80$ (4)°. The crystal is triclinic with a $P1$ space group. Its structure consists of a pair of two independent antiparallel β-D-cellotetraose molecules and one water molecule linked by hydrogen bonds to the two β-D-cellotetraose residues. In the structure which was refined to a residual R factor of 0.048 for 3793 indepen-

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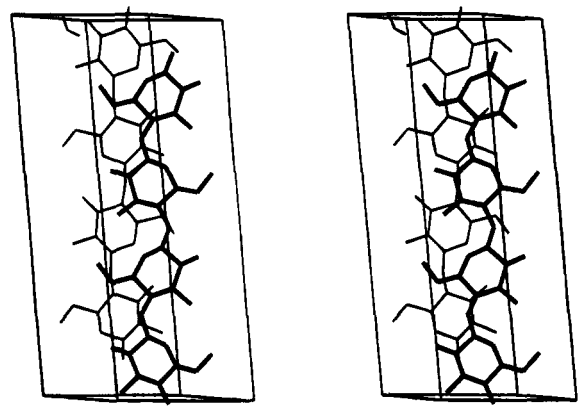
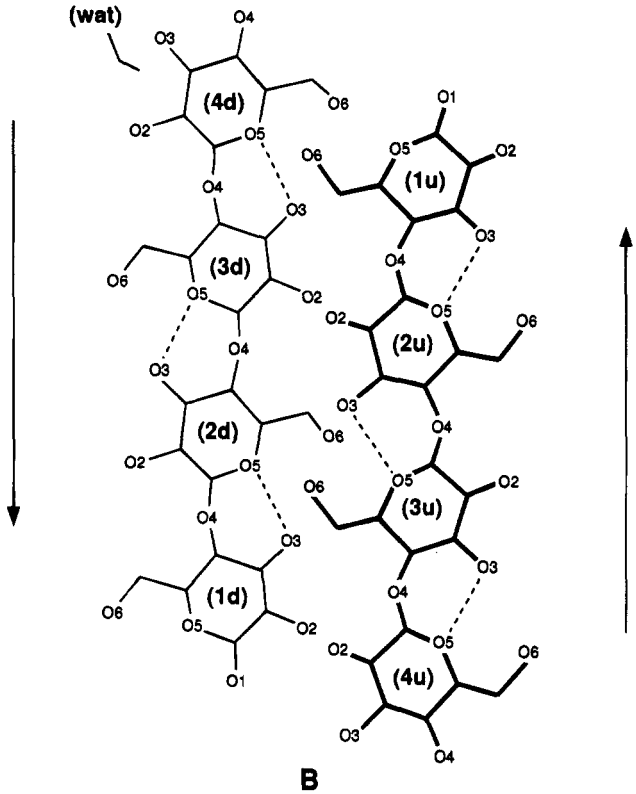


A



B

Figure 2. (A) Molecular packing (stereoviews) of the two molecules of β-D-cellotetraose, together with one water molecule. Projection nearly parallel to the long axis of the molecules. (B) Schematic diagram showing the labeling of the two cellotetraose molecules: the "up" molecules are indicated by bold lines, whereas the ones that are "down" are indicated by thin lines. In each residue, only the oxygen atoms have been labeled for clarity. The intramolecular hydrogen bonds are indicated by dashed lines.

dent reflections, the two β-D-cellotetraose molecules are nearly parallel to the *c* axis and are shifted along this axis with respect to one another by roughly 2.5 Å. The two molecules have almost the same geometry. In particular, all primary hydroxyl groups have the *gt* conformation, and the two OH moieties located at the anomeric ends are in the expected equatorial position. In each molecule, the four β-D-glucose residues have the $^4C_1$ pyranose conformation. Following the definition of Cremer and Pople,[16] all their puckering parameters Q

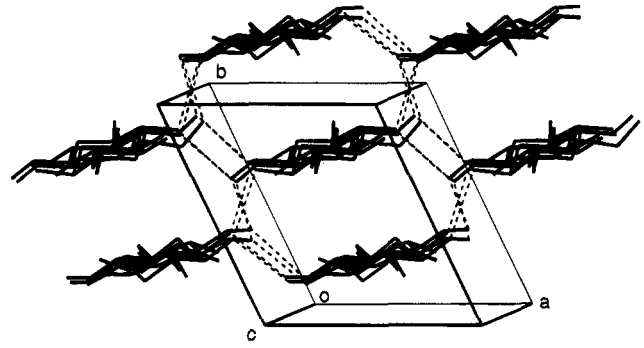
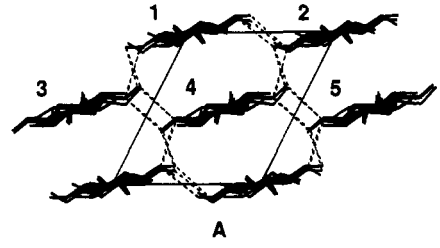
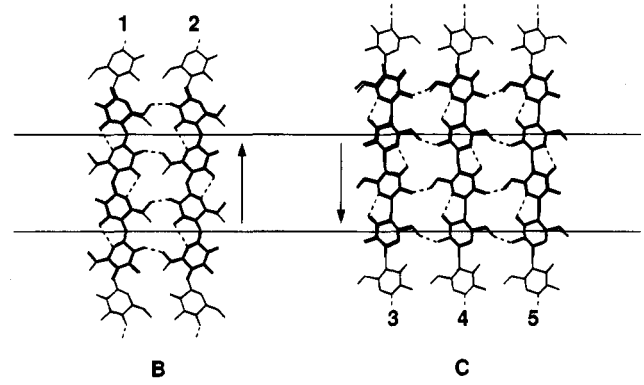


Figure 3. Projection of seven molecules of β-D-cellotetraose nearly perpendicular to the long axis of the molecules. The dashed lines correspond to intermolecular hydrogen bonding.



A



B C

Figure 4. Superposition of the antiparallel β-D-cellotetraose packing over that of cellulose II, taken from the structure of Stipanovic and Sarko.[2] (A) Projection perpendicular to the long direction of the molecules. (B) Molecules 1 and 2 projected parallel to the long direction of the molecules. (C) Molecules 3–5 projected parallel to the long direction of the molecules. The dashed lines correspond to the system of hydrogen bonding in β-D-cellotetraose. In B and C, the cellulose molecules are drawn in thin lines, whereas those of β-D-cellotetraose are in bold.

are slightly inferior to those of an ideal cyclohexane chair. In addition, the puckering angles θ of all the glucose moieties of the up molecule are slightly larger than those of the down molecule: the θ values of the sugar moieties of the down molecule range from 2.2° to 4.8°, whereas those of the up molecule are between 6.7° and 18.9°. Thus, the sugar rings of the up molecule appear more strained than those of the down one. The loss in energy resulting from the strain in the up molecule must be compensated by the gain of a better packing of the two antiparallel tetrasaccharide residues.

The structure of β-D-cellotetraose is held by a tight network of hydrogen bonds. Since all the primary hydroxyl groups are in the *gt* conformation, only one type of intramolecular hydrogen bond is possible, namely, O3–HO3···O5. All the other hydrogen bonds define an infinite network of linear intermolecular bonds linking

Table 1. Fractional Atomic Coordinates of the Non-Hydrogen Atoms ($\times 10^4$)[a] and Equivalent Isotropic (U_{eq})[b] Thermal Factors (Å^2 $\times$ 10^3) for β-D-Cellotetraose Hemihydrate

atom[c]	x	y	z	U_{eq}	atom[c]	x	y	z	U_{eq}
C1(1u)	7041 (13)	2929 (12)	8579 (4)	96 (3)	C1(1d)	4848 (11)	7580 (10)	1499 (3)	85 (3)
C2(1u)	8190 (10)	2765 (11)	8149 (3)	80 (3)	C2(1d)	6468 (8)	8252 (8)	1926 (3)	65 (2)
C3(1u)	7659 (9)	3165 (10)	7518 (3)	70 (2)	C3(1d)	6040 (8)	7289 (9)	2511 (3)	69 (2)
C4(1u)	5622 (9)	2422 (8)	7366 (2)	57 (2)	C4(1d)	4340 (8)	7409 (7)	2728 (2)	53 (2)
C5(1u)	4498 (10)	2654 (11)	7875 (3)	76 (3)	C5(1d)	2755 (8)	6711 (9)	2247 (3)	66 (2)
C6(1u)	2534 (12)	1786 (13)	7758 (4)	92 (3)	C6(1d)	1046 (9)	6807 (9)	2425 (3)	75 (2)
O1(1u)	7284 (9)	2108 (11)	9122 (3)	131 (2)	O1(1d)	5167 (10)	8425 (8)	975 (3)	122 (2)
O2(1u)	10028 (7)	3789 (7)	8329 (2)	97 (2)	O2(1d)	8040 (6)	8104 (6)	1685 (2)	86 (2)
O3(1u)	8558 (9)	2659 (8)	7135 (3)	110 (2)	O3(1d)	7634 (6)	7997 (7)	2914 (2)	88 (2)
O4(1u)	4991 (6)	3029 (5)	6842 (2)	59 (1)	O4(1d)	3811 (5)	6556 (5)	3259 (2)	57 (1)
O5(1u)	5056 (7)	1856 (7)	8388 (2)	89 (2)	O5(1d)	3282 (6)	7623 (6)	1723 (2)	74 (1)
O6(1u)	1608 (7)	1804 (8)	8280 (3)	99 (2)	O6(1d)	−503 (6)	5948 (6)	1988 (2)	81 (1)
C1(2u)	5004 (10)	2384 (10)	6298 (3)	65 (2)	C1(2d)	4426 (7)	7424 (7)	3796 (2)	42 (2)
C2(2u)	3576 (8)	2537 (8)	5857 (3)	49 (2)	C2(2d)	3013 (9)	6713 (8)	4234 (2)	59 (2)
C3(2u)	3684 (8)	2056 (9)	5211 (3)	52 (2)	C3(2d)	3803 (9)	7646 (8)	4852 (3)	60 (2)
C4(2u)	5654 (10)	2815 (9)	5066 (3)	60 (2)	C4(2d)	5599 (8)	7604 (9)	5042 (2)	60 (2)
C5(2u)	6999 (9)	2610 (11)	5537 (3)	67 (2)	C5(2d)	6968 (7)	8313 (8)	4553 (2)	50 (2)
C6(2u)	8978 (9)	3507 (10)	5427 (3)	75 (2)	C6(2d)	8783 (9)	8202 (10)	4720 (3)	69 (2)
O2(2u)	1725 (6)	1486 (6)	6028 (2)	67 (1)	O2(2d)	1340 (5)	6802 (5)	4034 (2)	59 (1)
O3(2u)	2508 (6)	2542 (6)	4833 (2)	72 (1)	O3(2d)	2431 (6)	6966 (6)	5252 (2)	75 (2)
O4(2u)	5934 (6)	2064 (5)	4513 (2)	57 (1)	O4(2d)	6478 (5)	8552 (4)	5579 (2)	49 (1)
O5(2u)	6779 (6)	3342 (5)	6084 (2)	60 (1)	O5(2d)	6100 (5)	7337 (5)	4014 (2)	57 (1)
O6(2u)	10184 (6)	3485 (6)	5932 (2)	79 (2)	O6(2d)	9950 (5)	8970 (5)	4255 (2)	68 (1)
C1(3u)	5645 (9)	2765 (8)	3977 (3)	58 (2)	C1(3d)	6182 (9)	7752 (9)	6107 (3)	63 (2)
C2(3u)	6869 (8)	2611 (8)	3536 (3)	47 (2)	C2(3d)	7896 (7)	8510 (8)	6560 (2)	52 (2)
C3(3u)	6423 (8)	3111 (9)	2911 (3)	51 (2)	C3(3d)	7431 (9)	7573 (9)	7137 (3)	64 (2)
C4(3u)	4346 (9)	2297 (9)	2728 (3)	55 (2)	C4(3d)	5726 (8)	7665 (8)	7357 (2)	54 (2)
C5(3u)	3247 (10)	2484 (10)	3222 (3)	65 (2)	C5(3d)	4154 (8)	6950 (8)	6881 (3)	55 (2)
C6(3u)	1190 (9)	1534 (9)	3096 (3)	64 (2)	C6(3d)	2403 (8)	7032 (9)	7040 (3)	59 (2)
O2(3u)	8739 (6)	3625 (6)	3738 (2)	67 (1)	O2(3d)	9403 (5)	8331 (5)	6329 (2)	61 (1)
O3(3u)	7409 (7)	2610 (7)	2516 (2)	80 (2)	O3(3d)	9028 (6)	8296 (7)	7557 (2)	87 (2)
O4(3u)	3807 (6)	2908 (5)	2205 (2)	62 (2)	O4(3d)	5169 (5)	6731 (5)	7887 (2)	58 (1)
O5(3u)	3762 (5)	1809 (5)	3758 (2)	51 (1)	O5(3d)	4639 (5)	7817 (5)	6339 (2)	53 (1)
O6(3u)	244 (6)	1564 (6)	3595 (2)	75 (1)	O6(3d)	953 (6)	6242 (6)	6602 (2)	72 (1)
C1(4u)	3763 (11)	2100 (11)	1666 (3)	69 (2)	C1(4d)	5762 (9)	7558 (8)	8434 (3)	55 (2)
C2(4u)	2329 (9)	2197 (10)	1209 (3)	65 (2)	C2(4d)	4373 (9)	6803 (9)	8879 (3)	65 (2)
C3(4u)	2425 (10)	1417 (9)	630 (3)	62 (2)	C3(4d)	5092 (10)	7574 (10)	9476 (3)	71 (2)
C4(4u)	4381 (12)	2358 (12)	411 (3)	85 (3)	C4(4d)	6960 (11)	7577 (12)	9688 (3)	88 (3)
C5(4u)	5757 (11)	2396 (12)	896 (3)	79 (3)	C5(4d)	8288 (9)	8344 (9)	9202 (3)	65 (2)
C6(4u)	7739 (13)	3417 (14)	732 (4)	101 (3)	C6(4d)	10035 (9)	8103 (9)	9352 (3)	73 (2)
O2(4u)	522 (7)	1274 (6)	1419 (2)	82 (2)	O2(4d)	2698 (6)	6975 (6)	8681 (2)	81 (2)
O3(4u)	1050 (7)	1406 (7)	181 (2)	83 (2)	O3(4d)	3841 (8)	6869 (10)	9913 (2)	122 (2)
O4(4u)	4537 (8)	1528 (9)	−105 (2)	102 (2)	O4(4d)	7816 (7)	8560 (8)	10207 (2)	100 (2)
O5(4u)	5544 (7)	3141 (7)	1431 (2)	77 (2)	O5(4d)	7474 (5)	7450 (6)	8646 (2)	69 (1)
O6(4u)	9071 (8)	3525 (9)	1201 (3)	109 (2)	O6(4d)	11494 (7)	9197 (7)	9023 (2)	91 (2)
					O1(wat)	875 (15)	4145 (11)	9679 (4)	146 (3)

[a] Standard deviations are given in parentheses. [b] U_{eq} is defined as one-third of the trace of the orthogonalized tensor. [c] As shown in Figure 2B, the sugar residues of each tetrasaccharide are numbered 1−4 starting from the reducing end; u and d correspond respectively to the tetrasaccharide that is "up" and the one that is "down".

O2 and O6. In addition, along the *c* direction, the successive up or down β-D-cellotetraose molecules are linked to one another by a series of intermolecular hydrogen bonding linking the anomeric O1 of one molecule with the nonreducing O4 of the next one. The water molecule is also located at the tip of the β-D-cellotetraose molecules. It is in particular linked to the anomeric O1 of one β-D-cellotetraose molecule and O3 of the nonreducing sugar moiety of an other.

One of the main interests of the structure of β-D-cellotetraose is that it presents many common features with the crystalline structure of cellulose II. Indeed the unit cell parameters of the base plane of cellulose II are given after conversion to the same convention as a = 8.01 Å, b = 9.04 Å, and γ = 117.1° by Kolpak and Blackwell[1] or a = 7.96 Å, b = 9.09 Å, and γ = 117.3° by Stipanovic and Sarko.[2] These values compare quite closely with the parameters a = 8.045 Å, b = 9.003 Å, and γ = 115.8° obtained in this study. Both cellulose II and cellotetraose have nearly the same orientation with respect to the long *c* axis of the unit cell, and both structures result from the packing of antiparallel molecules. Thus, it is likely that the molecular geometry, the packing, and the hydrogen bond system of β-D-cellotetraose can be directly used to model the structure of cellulose II. In fact, an excellent fit is observed when an antiparallel pair of β-D-cellotetraose is superimposed on the structure of cellulose II given by Stipanovic and Sarko[2] or that of Kolpak and Blackwell.[1] The superposition, achieved by displacement minimization is shown in Figure 4A−C where the β-D-cellotetraose molecules are positioned on the 2_1 screw axes of cellulose. In Figure 4A, perpendicular to the chain direction, seven molecules of cellulose and seven molecules of β-D-cellotetraose are so well superimposed that they are indistinguishable. When the molecules labeled 1 and 2 are observed along the chain direction (Figure 4B), the backbone of cellulose and that of β-D-cellotetraose match almost perfectly. On the other hand, the orientation of the O6 is different: *tg* for cellulose and *gt* for β-D-cellotetraose. This situation does not exist any more for molecules 3−5 (Figure 4C) as, for both the cellulose and the tetrasaccharide, the match is almost perfect not only for the backbone but also for the

Table 2. Fractional Atomic Coordinates of the Hydrogen Atoms ($\times 10^4$)[a] and Isotropic (U_{iso})[b] Thermal Factors (Å^2 $\times$ 10^3) for β-D-Cellotetraose Hemihydrate

atom[c]	x	y	z	U_{iso}	atom[c]	x	y	z	U_{iso}
H1(1u)	7288 (13)	4079 (12)	8657 (4)	115	H1(1d)	4559 (11)	6426 (10)	1406 (3)	102
H2(1u)	8028 (10)	1622 (11)	8144 (3)	96	H2(1d)	6804 (8)	9418 (8)	2013 (3)	77
H3(1u)	8137 (9)	4367 (10)	7497 (3)	84	H3(1d)	5744 (8)	6130 (9)	2428 (3)	83
H4(1u)	5198 (9)	1233 (8)	7299 (2)	69	H4(1d)	4647 (8)	8574 (7)	2803 (2)	63
H5(1u)	4880 (10)	3827 (11)	7966 (3)	91	H5(1d)	2469 (8)	5555 (9)	2160 (3)	79
H6A(1u)	2122 (12)	2293 (13)	7433 (4)	110	H6A(1d)	753 (9)	6319 (9)	2810 (3)	90
H6B(1u)	2202 (12)	653 (13)	7636 (4)	110	H6B(1d)	1273 (9)	7954 (9)	2461 (3)	90
HO1(1u)	6499 (190)	1145 (73)	9105 (43)	197	HO1(1d)	5864 (197)	9402 (42)	1050 (8)	183
HO2(1u)	10097 (17)	4617 (68)	8502 (47)	145	HO2(1d)	7647 (7)	7351 (78)	1432 (31)	129
HO3(1u)	8608 (191)	1818 (108)	7257 (37)	165	HO3(1d)	7864 (86)	8960 (40)	2990 (37)	132
HO6(1u)	1351 (163)	941 (69)	8456 (32)	149	HO6(1d)	−325 (69)	6448 (72)	1676 (15)	121
H1(2u)	4776 (10)	1226 (10)	6329 (3)	78	H1(2d)	4680 (7)	8580 (7)	3736 (2)	50
H2(2u)	3755 (8)	3685 (8)	5869 (3)	59	H2(2d)	2746 (9)	5550 (8)	4281 (2)	71
H3(2u)	3204 (8)	852 (9)	5176 (3)	63	H3(2d)	4045 (9)	8802 (8)	4801 (3)	72
H4(2u)	6049 (10)	3996 (9)	5009 (3)	72	H4(2d)	5373 (8)	6456 (9)	5105 (2)	71
H5(2u)	6668 (9)	1435 (11)	5587 (3)	81	H5(2d)	7215 (7)	9465 (8)	4488 (2)	60
H6A(2u)	9207 (9)	3004 (10)	5082 (3)	90	H6A(2d)	9354 (9)	8770 (10)	5100 (3)	83
H6B(2u)	9256 (9)	4642 (10)	5337 (3)	90	H6B(2d)	8567 (9)	7057 (10)	4749 (3)	83
HO2(2u)	1747 (26)	706 (58)	6213 (34)	101	HO2(2d)	1561 (15)	7760 (15)	3962 (31)	89
HO3(2u)	2940 (70)	3552 (7)	4838 (33)	109	HO3(2d)	2151 (97)	5979 (26)	5281 (33)	112
HO6(2u)	10457 (120)	2717 (81)	5890 (23)	118	HO6(2d)	9537 (69)	8395 (54)	3947 (11)	102
H1(3u)	5904 (9)	3923 (8)	4044 (3)	70	H1(3d)	5899 (9)	6593 (9)	6029 (3)	76
H2(3u)	6681 (8)	1461 (8)	3514 (3)	57	H2(3d)	8229 (7)	9681 (8)	6636 (2)	62
H3(3u)	6883 (8)	4313 (9)	2907 (3)	61	H3(3d)	7150 (9)	6414 (9)	7057 (3)	77
H4(3u)	3964 (9)	1115 (9)	2658 (3)	66	H4(3d)	6005 (8)	8820 (8)	7441 (2)	64
H5(3u)	3571 (10)	3654 (10)	3294 (3)	78	H5(3d)	3878 (8)	5792 (8)	6805 (3)	66
H6A(3u)	891 (9)	398 (9)	2988 (3)	77	H6A(3d)	2061 (8)	6513 (9)	7418 (3)	71
H6B(3u)	772 (9)	2002 (9)	2760 (3)	77	H6B(3d)	2632 (8)	8178 (9)	7085 (3)	71
HO2(3u)	9367 (21)	3884 (88)	3452 (5)	100	HO2(3d)	10107 (55)	9210 (26)	6195 (30)	92
HO3(3u)	6692 (30)	1743 (64)	2340 (33)	120	HO3(3d)	9454 (96)	9305 (7)	7550 (38)	131
HO6(3u)	240 (126)	2469 (44)	3628 (27)	112	HO6(3d)	1191 (63)	6744 (71)	6291 (13)	108
H1(4u)	3591 (11)	964 (11)	1724 (3)	82	H1(4d)	6015 (9)	8718 (8)	8388 (3)	66
H2(4u)	2564 (9)	3350 (10)	1153 (3)	78	H2(4d)	4066 (9)	5625 (9)	8898 (3)	78
H3(4u)	2206 (10)	274 (9)	700 (3)	75	H3(4d)	5310 (10)	8731 (10)	9450 (3)	85
H4(4u)	4598 (12)	3487 (12)	315 (3)	102	H4(4d)	6790 (11)	6444 (12)	9753 (3)	105
H5(4u)	5574 (11)	1266 (12)	975 (3)	94	H5(4d)	8590 (9)	9517 (9)	9164 (3)	78
H6A(4u)	7936 (13)	2917 (14)	381 (4)	122	H6A(4d)	10409 (9)	8312 (9)	9776 (3)	88
H6B(4u)	7899 (13)	4519 (14)	635 (4)	122	H6B(4d)	9782 (9)	6971 (9)	9256 (3)	88
HO2(4u)	132 (63)	297 (21)	1320 (40)	122	HO2(4d)	2958 (23)	7758 (77)	8460 (36)	122
HO3(4u)	759 (118)	2146 (92)	257 (28)	124	HO3(4d)	4278 (104)	7390 (127)	10230 (19)	183
HO4(4u)	5533 (80)	2083 (78)	−239 (35)	152	HO4(4d)	7366 (136)	9207 (117)	10252 (34)	150
HO6(4u)	10070 (53)	4295 (121)	1146 (37)	163	HO6(4d)	11717 (108)	10154 (8)	9108 (38)	136
					H1(wat)	1455 (53)	3759 (45)	9545 (15)	10 (9)
					H2(wat)	304 (71)	3563 (58)	10067 (21)	34 (12)

[a] Standard deviations are given in parentheses. [b] U_{iso} is defined as 1.2 times the equivalent isotropic value (U_{eq}) of their connecting atom. [c] As shown in Figure 2B, the sugar residues of each tetrasaccharide are numbered 1−4 starting from the reducing end; u and d correspond respectively to the tetrasaccharide that is "up" and the one which is "down".

hydroxymethyl conformation that is *gt* for the glucose moieties of cellulose II as well as for those of the β-D-cellotetraose molecules.

Our results[17] indicate also that there is a slight difference between the geometry of the glucose rings in the β-D-cellotetraose molecules that are up versus that that are down. A similar observation was also found in the structure of methyl β-D-cellotrioside.[14] It would be important to see whether this situation occurs also in cellulose II. So far, the crystalline structure of cellulose II has been refined with glucose rings of fixed geometry. Our results show that some modification in the glucose ring conformation may be expected for cellulose II, with differences between the rings of the chains located at the center of the unit cell of the cellulose II cell versus those of the corner chains. Such a difference in ring conformation is presently being tested in a refinement of the crystal structure of cellulose II where, in particular, all the hydroxymethyl groups would be in the *gt* position.

Acknowledgment. The authors acknowledge the technical help of P. Colin Morel for the preparation and purification of β-D-cellotetraose and T. Eriksson for his help with the programs FIT2D and DENZO.

References and Notes

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(17) Since the submission of our manuscript, a structure very similar to ours and determined in part with the help of synchrotron radiation was published by: Gessler, K.; Krauss, N.; Steiner, T.; Betzel, C.; Sandmann, C.; Saenger, W. *Science* **1994**, *266*, 1027–1029. Our results and conclusions are essentially the same as theirs. We could collect a thousand more reflections than them. Our final R factor of 4.8% is slightly better than their 8.7%. The precision of our data allows one to localize the position of the hydrogen atoms of the sugar hydroxyl groups as well as those of the water molecule.

MA9450694