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# Determining the Crystal Structure of Cellulose III by Modeling

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# DETERMINING THE CRYSTAL STRUCTURE OF CELLULOSE $III_I$ BY MODELING

A Thesis

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# Dedication

My thesis is dedicated to my grandmother, Dorothy Moore (Momo), who passed away January 28, 2005 in her fight against cancer. She lived how she wanted, despite the pain, until the end. Momo loved me, her family, and everyone else who had the opportunity to know her. She was one of my biggest supporters throughout my graduate studies, always telling me how proud she was of me and giving me encouragement. It has been difficult thinking about her not being here to see my studies come to a close, but knowing that she watches over me gives me comfort.

# Acknowledgements

Dr. Alfred D. French gave me the opportunity to learn and appreciate carbohydrate chemistry and molecular modeling by doing this project. Glenn Johnson showed me how to perform the MM3 calculations and exposed me to UNIX and similar operating systems. Dr. Stevens always was helpful in answering challenging questions and providing support. Elena Graves and Dr. Ralph Berni prepared the cellulose III sample, and Dr. Paul Langan participated in helpful discussions. The cellulose III diffraction pattern was recorded at Center for Advanced Microstructure and Devices, Baton Rouge, Louisiana.

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# Abstract

Recently, a one-chain monoclinic unit cell for Cellulose $III_I$ having a single glucose in the asymmetric unit was proposed based on high-resolution diffraction patterns. The new work challenged a two-chain structure published 25 years earlier, although it did not provide new three-dimensional coordinates. Our goals were to solve the structure by modeling, find whether modeling would reject the previously determined two-chain unit cell, and compare the model with the anticipated experimental structure. Combinations of the O2, O3, and O6 hydroxyls produced 54 starting structures. Clusters of 13 cellotetraose chains terminated by methyl groups for each of the 54 starting structures were optimized with MM3(96). Hydroxyl groups on 16 of these 54 structures reoriented to give very similar hydrogen-bonding schemes in the interiors, along with the lowest energies. The one-chain cell models had much lower energy. The eight best “up” one-chain models agree well with the structure newly determined by experiment.

# Introduction

Cellulose was the first carbohydrate to be studied by computer modeling. In 1960, Jones[1] used standard bond lengths, angles and interatomic distances to construct models that were used as part of a mostly unsuccessful attempt to solve the crystal structure of ramie cellulose I from fiber diffraction data. The advantages of the method were clear, however, and since then, computer models have been an integral part of most fiber diffraction studies that seek to determine the atomic positions.[2] Augmentation of crystal structure determinations by modeling is often necessary because the small number of diffraction intensities from most fibers is inadequate to determine the x, y and z coordinates of all unique atoms in the structure. With a combined approach, diffraction data can provide some guidance and the modeling energy calculations supply the rest of the information. This approach has been taken to the logical extreme of attempting to solve structures of small organic molecules by modeling with no specific experimental data whatsoever.[3] Those efforts are as yet not sufficiently reliable for general use but are at the forefront of modeling development.

As modeling has become more sophisticated, methods for experimental study of crystalline fibers have also improved. New sources of highly crystalline cellulose have been identified, and the preparation of films of oriented crystallites allows the use of these crystallites regardless of their initial lack of orientation[4]. Neutron diffraction work has yielded the details of the hydrogen bond networks and very powerful synchrotron x-ray beams provide more diffraction data than laboratory generators. Together, the new techniques have resulted in sufficient data that high-resolution, model-free structure determinations of cellulose structures could, in principle, be carried out.

High-resolution structures are now available for cellulose Iα[5] and Iβ,[6] as well as cellulose II.[7] Most native cellulose is a mixture of the Iα and Iβ structures, with the Iα form being prevalent in cellulose that is produced by algae and bacteria, whereas Iβ is dominant in higher plants. The sample for the high-resolution study of cellulose II was produced by treating native cellulose I from flax with 23% NaOH, followed by rinsing and drying. Cellulose II can also be prepared by precipitation from solution, as in the manufacture of rayon, and by bacteria that are either mutants or at low temperature. A third major form, cellulose III, results from treatment with amines that are subsequently evaporated or rinsed off. Although their diffraction patterns are similar, subtle differences distinguish cellulose III that is made starting with cellulose I ($III_I$) from that starting with cellulose II ($III_{II}$). Finally, cellulose IV can be prepared by heating the other forms in glycerol at 260 C°. Recently, Wada et al. proposed that $IV_I$ is actually Iβ with lateral disorder.[8]

In 2001, Wada et al. proposed that cellulose $III_I$ has a single chain monoclinic unit cell with $P2_1$ symmetry and that the O-6 atoms were in the gt position.[9] Those results contradict a 1976 determination by Sarko et al., who had done a complete analysis based on limited X-ray diffraction data.[10] Their work was based on a two-chain unit cell and determined the O-6 groups to be in tg orientations. Although the pattern of Wada et al. has more than 100 intensities, they did not, in that work, attempt to solve the structure. Instead, the O-6 position was determined by accompanying NMR studies. Their results presented a unique opportunity. A modeling study could be independently carried out with an unknown that would inevitably be determined at high resolution. If successful, it was hoped that our project would encourage the incorporation of higher-

quality modeling methods in fiber diffraction studies. These combined methods would continue to be of use on less-crystalline samples. Of course, a successful prediction would lend credibility to modeling studies on other materials such as amorphous cellulose, for which experimental data are limited and more difficult to interpret.

The high-resolution experimental study of cellulose III has now been published,[11] and we can also compare those results with ours, which were presented at two meetings.[12]

## Methods

Given the results from Wada et al. regarding the O-6 position and unit cell dimensions and symmetry,[9] only the hydroxyl group orientations remain as explicit variables. Cellotetraose molecules were constructed with Chem-X with two-fold screw-axis symmetry and capped with methyl groups at the reducing and non-reducing ends to prevent the formation of unrealistic hydrogen bonds. The O-2, O-3 and O-6 hydroxyl groups on the tetraose models were placed in each of the three staggered orientations (Figure 1), so that they made torsion angles of –60°, 60° and 180° with the H2, H3 and C5 atoms. Thus, there were 27 combinations of hydroxyl orientations.

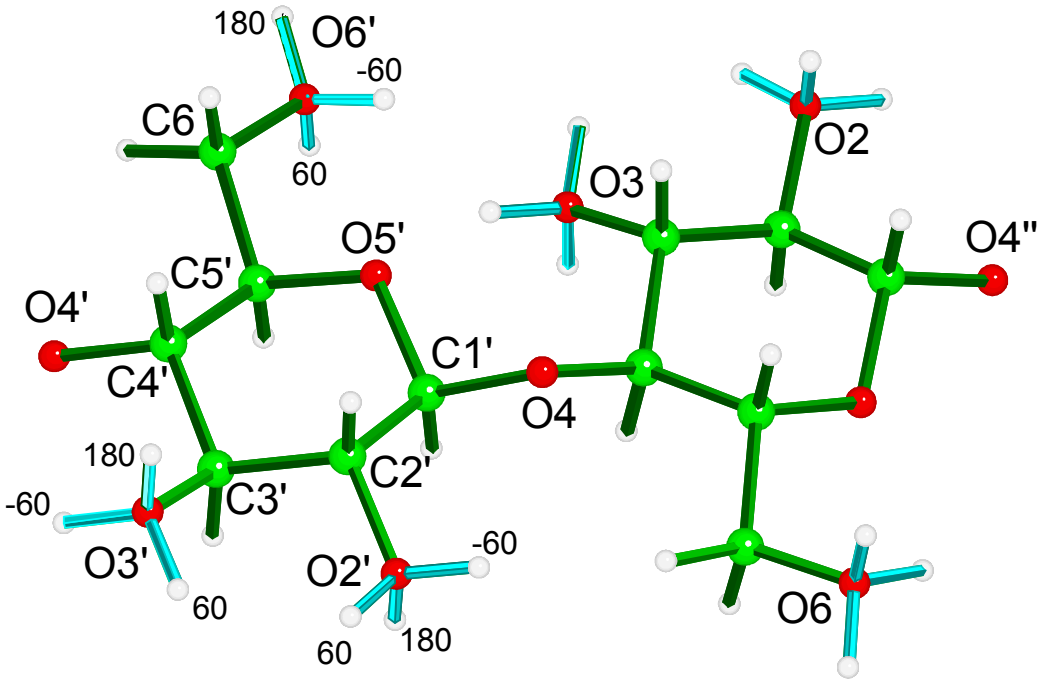


Figure 1. Cellobiose unit with the hydroxyl groups oriented in the 180, -60 and +60 orientations.

These models were placed visually in the unit cell according to Figure 5 in Wada et al., in both the “up” and “down” orientations,[13,14] for a total of 54 starting models. There was substantial confidence in the orientation presented by Wada since it was based on the report by Sarko et al.[10] That orientation would depend on the very strong hk0 reflections and is likely to be unaffected by other errors in the determination. Symmetry operators within Chem-X were used to generate clusters (minicrystals) with 13 chains, similar to previous designs[15], as shown in Figure 2.

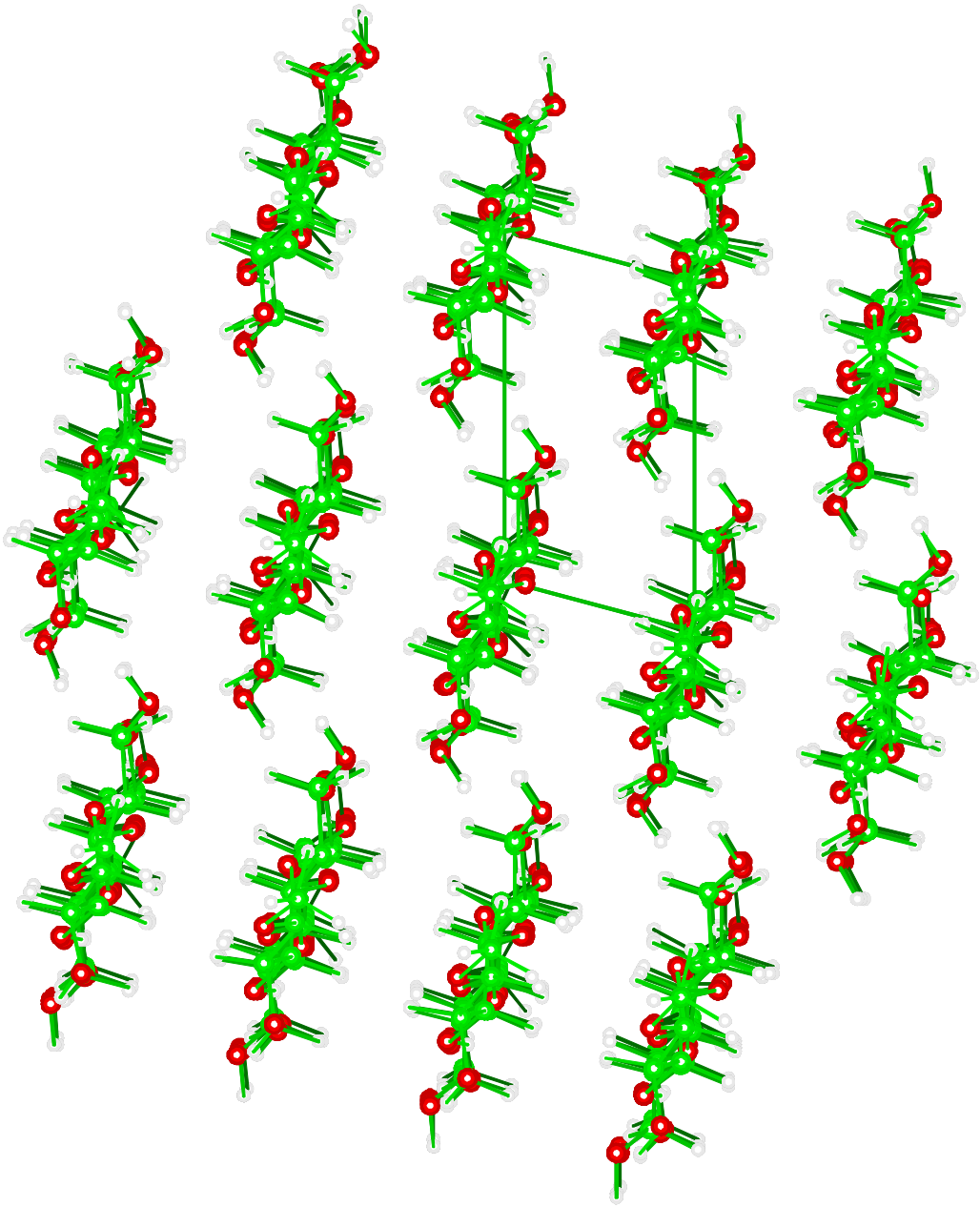

Figure 2. Minicrystal of cellulose III after energy minimization with MM3(96), viewed from above down the long molecular axes, which are parallel to the crystallographic c-axis. At the edges of the minicrystal, there is some visible variation in hydrogen position that resulted from different amounts of atom movement during minimization because the atoms have different environments than those in the interior of the minicrystal.

The 54 minicrystals were energy minimized with MM3(96), using a dielectric constant of 3.5 and the hydrogen bonding potential from MM3(92). We have found that those modifications result in better model crystal structures. No constraints, symmetry operators or periodic boundaries were placed on the structure during minimization. The plan was to observe the resulting energies and hydrogen bonding schemes and to select one or more likely structures for comparison with the proposed two-chain structure from Sarko et al.

The minicrystal method is subject to uncontrolled edge effects[16] regarding the positions of the external atoms. However, it has the advantage that it can provide energies that are based on a variety of different potential energy functions, including MM3, which is known to reproduce a number of phenomena related to carbohydrates. All energies are reported as kcal/mol of the structures in question. Thus, the energies reported for the tetraose-based minicrystals would be kcal for a mole of minicrystals. Other energies reported include kcal/mol of hexaose-based minicrystals and kcal/mol of a layer of cellobiose residues inside the hexaose-based minicrystal. These energies are reported below simply as kcal.

## Results and Discussion

Of the 54 models based on single-chain unit cells, 16 gave total minimized steric energies that were between 237 and 246 kcal. Eight of these were up models, and the other eight were down. A second group of 26 had energies between 318 kcal and 367 kcal, and the remaining structures had energies between 407 and 470 kcal. Only the group with energies of about 240 kcal is relatively homogeneous in energy and hydroxyl

orientation. That homogeneity is an additional confirmation that the lowest energy group represents the most likely structures. Table 1 shows that the best up model has an energy of 237.6 kcal, whereas the best down model has an energy of 236.7 kcal. These values can be compared to the energy of the minimized, tetramer-based model of Sarko et al., 340.3 kcal.

Table 1. Energies (kcal) and hydroxyl torsion angles (º) for two central glucose residues from the best tetraose-based models

| Model | Energy kcal | $\tau_2$ | $\tau_3$ | $\tau_6$ | $\tau_{2'}$ | $\tau_{3'}$ | $\tau_{6'}$ |
|---|---|---|---|---|---|---|---|
| Starting | ---------- | 60 | -60 | 180 | 60 | -60 | 180 |
| Best Up | 237.6 | 12.2 | -47.2 | -138.9 | 12.0 | -48.0 | -140.0 |
| Best Down | 236.7 | 12.0 | -48.0 | -139.5 | 12.2 | -47.2 | -140.0 |

Torsion angles were determined for the central cellobiose units in the minicrystals. Variations in the torsion angles for the hydroxyl groups on the minicrystal surfaces that result from the different environments than in the minicrystal interior, are among the edge effects. The different starting orientations lead different surface orientations and are the main factor responsible for the 9 kcal range of energies within the group that has the lowest energy. Because the energies are for all 26 cellobiose residues and 52 methyl groups in the minicrystal, the differences within the lowest-energy group are small per cellobiose unit. We were reluctant to choose between the up and down models in the lowest-energy group, given such small energy differences.

The interior hydroxyl groups of the 16 lowest energy structures rotated to nearly identical orientations during minimization even though they were in model crystal lattices. The H-C2-O2-H torsions were 12±5°, H-C3-O3-H values were –47±2°, and C5-

C6-O6-H torsions were –143±3° regardless of the up or down packing or initial hydroxyl orientation. For example, the hydroxyl groups on C2 and C2' rotated from initial values of 60° to final values near to 12°, a rotation of 48°. Hydroxyl groups on C2 of other structures in the low-energy group rotated to the same values near 12° starting from -60°, a rotation of 72°. The corresponding rotations at C3 and C6 of the lowest-energy structure were more than 107° and more than 40°, respectively. Hydroxyls on C6 atoms in other structures started at -60° and rotated about 72°. The extents of rotation of the hydroxyl groups were surprising since they were initially in staggered positions, normally considered to be energy minima, although nearly eclipsed conformations, such as the 12° torsion for O2H, are fairly common in carbohydrates and cyclitols. Such large rotations during minimization indicate that the attractiveness of the hydrogen bond system was so great that the hydroxyl groups overcame energy barriers. The similarity of the unprimed and primed torsion angles in Table 1 strongly supports the experimentally determined two-fold screw-axis symmetry.

Unit cell dimensions were assessed based on the interchain distances and angles. Those that were based on tetramer models were approximately a=4.5±0.09, b=8.0±0.1, c=10.35±0.03, $\alpha$=90.1±2, $\beta$=90.0±1.0, and $\gamma$=105.5±0.4 for the minimized models. Comparisons with the experimental values listed in Table 2 were satisfactory. Our minimized version of the model of Sarko et al.[10] gave a=10.44, b=7.95, c=10.36, $\alpha$=90.3, $\beta$=89.8, $\gamma$=122.85. Differences from the experimental values in Table 2 were also considered minor. The slight expansion of the unit cells, particularly along the a-axis, may be partly due to the lack of long-range packing forces in the minicrystals.

Table 2. Calculated energies and unit cell dimensions of hexamer models.

| Hexamer Model | Minicrystal Energy (kcal) | Cellobiose Layer Energy (kcal) | a(Å) | b(Å) | c(Å) | α(°) | β(°) | γ(°) |
|---|---|---|---|---|---|---|---|---|
| Best up one-chain | 322.2 | 84.5 | 4.58 | 7.95 | 10.33 | 90.3 | 90.1 | 107.9 |
| Best down one-chain | 323.2 | 86.5 | 4.58 | 8.00 | 10.31 | 90.2 | 90.1 | 107.9 |
| Wada et al.[9,a] | ---------- | ---------- | 4.45 | 7.85 | 10.31 | 90.0 | 90.0 | 105.1 |
| Two-chain | 477.5 | 137.2 | 10.45 | 7.92 | 10.33 | 90.2 | 89.8 | 122.8 |
| Sarko et al.[10,a] | ---------- | ---------- | 10.25 | 7.78 | 10.34 | 90.0 | 90.0 | 122.4 |

[a.] Experimentally determined.

Although our lowest-energy values for the tetramer-based models of 237 kcal for the Wada et al. structure[9] and 340 kcal for the Sarko et al.[10] structures strongly favored the single-chain unit cell of Wada et al., there was concern regarding chain-end effects of unknown magnitude. The central chain in the two-chain model is displaced 0.9 Å along the c-axis with respect to the corner chains. Therefore, its minicrystal energies would be susceptible to end effects. In the case of the minicrystals of the one-chain cell, all chain-ending methyl groups are in planes at the tops and bottoms of the minicrystals. Because of the shifting in the two-chain cell, its chain ends would not experience the same degree of stabilization from van der Waals attraction to their neighbors, as would the coplanar ends in the one-chain cell models. That problem was solved by comparing the energies of internal cellobiose layers in minicrystals that were built from methylated cellohexaose molecules. The energies for the cellobiose layer were based on subtraction of the energies of the best up and down methylated cellotetraose minicrystals from energies from analogous methylated cellohexaose minicrystals. Those

cellobiose layer energies, which do not have first-order end effects, are shown for the one- and two-chain cell structures in Table 2, along with the unit cell dimensions of the models based on the cellohexaose molecules. In this case, the energies of the “up” structure, both the full hexameric minicrystal and the cellobiose layer in the minicrystal, were slightly lower than those of its “down” counterpart but considerably lower than those of the two-chain cell structures.

Table 3 shows the geometries of the hydrogen bonds in which the central cellobiose unit in the minicrystal is involved, based on the hexameric models. Based on the criterion that the distance between the donated hydrogen and the acceptor oxygen atom is < 3.0 Å and the O—H…O angle is > 90°, there are three intramolecular and two intermolecular hydrogen bonds.

Table 3. Intra - and Intermolecular[a] hydrogen bonds in best "up" model.

| Type of bond | H-Bond | Length H…O (Å) | Length O…O (Å) | Angle (°) |
|---|---|---|---|---|
| Intramolecular | O3H…O5 | 1.92 | 2.73 | 142.4 |
| | O3H…O6 | 2.38 | 2.39 | 129.2 |
| | O3H…O4 | 2.77 | 3.00 | 94.6 |
| Intermolecular | | | | |
| Central chain donor | O2H…O6b | 1.82 | 2.76 | 169.3 |
| | O2H'…O6c' | 1.82 | 2.75 | 168.3 |
| | O6H…O2d | 1.79 | 2.72 | 163.1 |
| | O6H'…O2a' | 1.80 | 2.73 | 164.6 |
| Central chain acceptor | O6Ha…O2 | 1.79 | 2.71 | 163.2 |
| | O2Hc…O6 | 1.81 | 2.74 | 167.6 |
| | O2b'…O6' | 1.81 | 2.74 | 166.8 |
| | O6Hd'…O2' | 1.79 | 2.72 | 165.0 |

[a.] Letters a, b, c and d refer to neighboring glucose residues with the same z-coordinates as the central residue. See Figure 3.

The intramolecular hydrogen bonds, shown in Figure 3, are typical for β-1,4 linked carbohydrates.[17] The proton of the O-3 hydroxyl group is positioned to donate to the O-5' atom (see Figure 3) by virtue of the particular φ and ψ linkage torsion angles. The O-6' atom also accepts from O3-H. That frequently overlooked interaction stabilizes the gt position in many related molecules, despite H…O distances that are longer than are often considered to be hydrogen bonds.[18] The third intramolecular interaction in Table 3, O3-H…O-4 is indeed very weak, but its presence is noted.

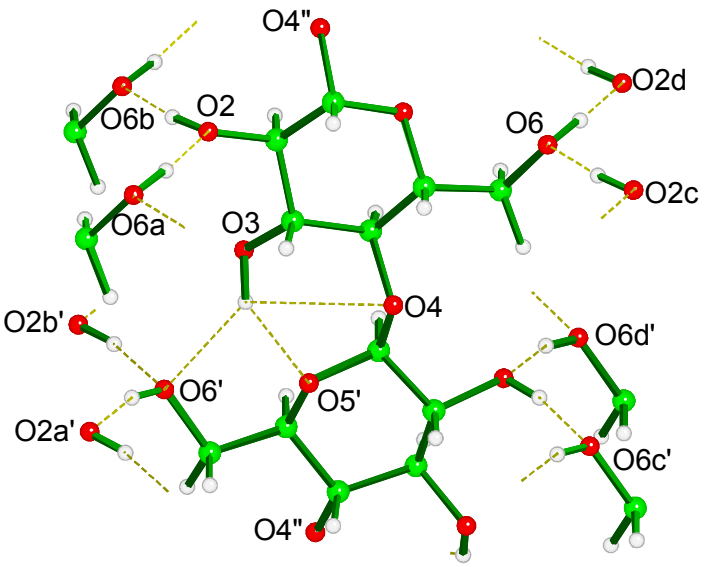


Figure 3. Hydrogen bonding in cellulose $III_I$. The central cellobiose unit of the minicrystal is shown along with hydroxyl and hydroxymethyl groups from the four neighboring cellobiose units, a—d. Groups from the a and c chains are in front of the central cellobiose, and those from the b and d chains are behind it. Hydrogen bonds are shown as dashed lines. The O6 and O2 atoms participate in infinite chains of donor…acceptor…donor hydrogen bonds, indicated by the dashed lines that would connect to cellobiose units in front of, or behind, the central cellobiose unit.

Although Table 3 shows four intermolecular hydrogen bonds in which the central cellobiose is the donor and four hydrogen bonds in which it is the acceptor, there is just one unique O6-H…O-2 hydrogen bond and one O2-H…O-6 hydrogen bond when there is actual two-fold symmetry. The near identity of these modeled geometries for the O6-H…O-2 hydrogen bonds confirms that the two-fold, single chain structure is consistent with the MM3 force field. The O2-H…O-6 geometries lead to a similar conclusion.

The intermolecular hydrogen bonds participate in “infinite” chains of donor-acceptor-donor linkages (Figure 3) that have excellent hydrogen bonding geometry. Such systems have increased strength and shortened interatomic distances because of the phenomenon of “cooperativity”.[19]

Van der Waals forces are also important, with stacking of the residues in the a-axis direction. Each of the methine hydrogen atoms is in van der Waals contact with one

or more methane hydrogen atoms on the neighboring molecules. Figure 4 illustrates the H…H distances > 3.2 Å for the best up model.

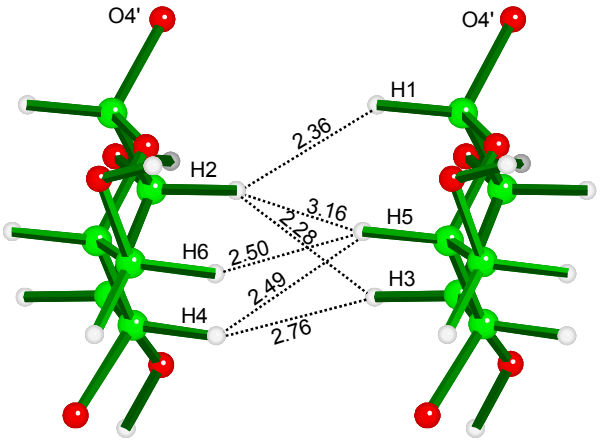


Figure 4. Two glucose residues from the center of the best up hexameric minicrystal, showing the H…H contacts < 3.2 Å.

Our best “up” model is similar in many respects to the high-resolution structure very recently published by Wada et al.[11] Interestingly, they were able to clearly rule out the down packing model, while our results were ambiguous on that point. The conformations of the primary alcohol groups in the experiment and model were 44° and 59°, respectively. Despite that difference, the resulting positions of the O-6 hydroxyl hydrogens are quite similar. The biggest difference is in the positions of the two protons attached to C-6. These relationships are shown in Figure 5, in which the central cellobiose unit from the hexaose-based up minicrystal is fitted to a cellobiose unit generated from the coordinates of Wada et al.[11] The root mean square difference between the positions of the 12 ring atoms and the linkage oxygen is only 0.1 Å.

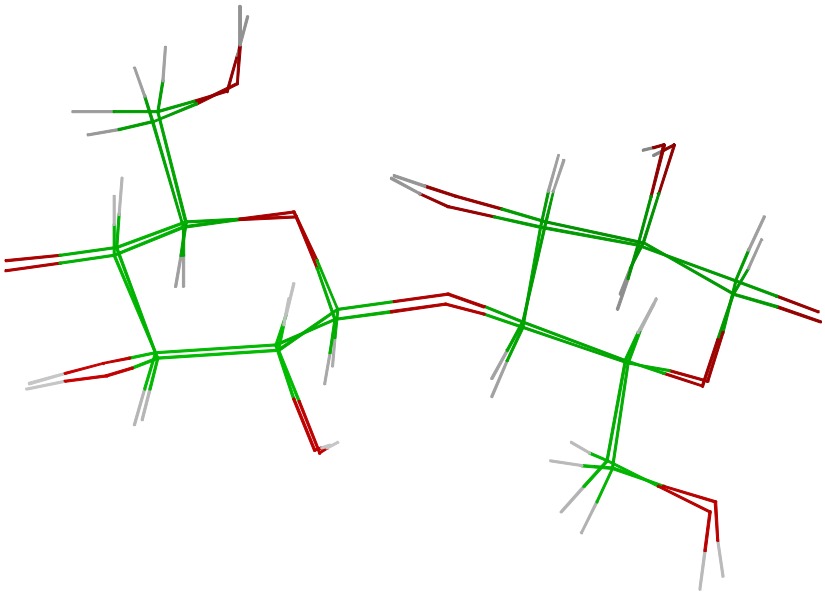

Figure 5. Superimposed cellobiose units from the experimental structure of Wada et al.11 and the best up model. The root mean square fit for the ring atoms and central linkage oxygen is 0.1 Å.

In the high-resolution structure of Wada et al.,[11] there was one slight ambiguity regarding the direction of the infinite cooperative hydrogen bonding network. Although their final result was quite similar to ours, they also considered an alternative that reversed the direction of the donor and acceptor hydroxyl groups. In the agreed upon network, our O-2 hydroxyls have 12° torsion angles, nearly eclipsing the C2-H hydrogen atoms. In the alternative network structure, the O2-H atoms are oriented anti to the C2-H hydrogens. Experimentally, this ambiguity arises because of the difficulty in precisely locating the proton between two oxygen atoms. If it is closer to O-2, then it is taken to be covalently bonded to O-2 and hydrogen-bonded to O-6, and vice versa. In a structural or modeling sense, direction of the hydrogen bonding in an infinite network is expressed by the rotational orientation of the hydroxyl groups. The modeling results were less ambiguous, because the various torsional and other steric terms in the force field resulted in the alternative systems being considerably higher in energy. Several

minicrystals having the alternative hydrogen-bonding scheme fell into the second lowest-energy group.

To understand why Sarko et al. proposed a two-chain structure, we reviewed their published (as supplementary data) crystallographic information and recorded a fiber diffraction pattern of ramie cellulose III prepared by the method of Calamari et al.[20] All of the diffraction spots on our low-resolution pattern could be indexed with the one-chain cell. All but two of their first-layer line spots (d-spacings = 2.78 Å and 2.55 Å could also be indexed with the one-chain cell. Those spots were not visible on our pattern. Their published pattern does not permit a close analysis, but one plausible explanation, that traces of cellulose I remained, is not likely because there is no 2.78 Å observed hk1 spacing from cellulose I.[21] It appears that Sarko et al. assumed that there were two chains in the cell. Ironically, Sarko and Muggli had earlier discussed a one-chain unit cell for cellulose I before the distinction between cellulose Iα and Iβ was understood.[22] In any case, the synchrotron fiber diffraction pattern by Wada et al. produced 114 reflections that were indexed by the proposed one-chain monoclinic unit cell, compared to Sarko's 23 reflections. The cell based on the larger number of reflections should overrule one based on so many fewer spots.

## Conclusions

Our molecular modeling study of cellulose $III_I$ concurs that the unit cell of Wada et al. is the more probable. However, our best up and down models show very small differences between them, either in the energies, the unit cell values, or the hydrogen

bond geometries. Therefore, either model could correspond to the structure of cellulose $III_I$. The final modeled coordinates are listed in Table 4.

Table 4. Atomic coordinates of modeled glucose monomer of up cellulose $III_I$.

| ATOM | X | Y | Z |
|---|---|---|---|
| O1 | -0.71816 | -0.47776 | 0 |
| C1 | 0.09394 | -0.31254 | 1.18442 |
| C2 | 0.02125 | 0.437675 | 3.95203 |
| C3 | 0.17484 | 1.163885 | 1.56558 |
| C4 | 0.78402 | 1.291385 | 2.95526 |
| C5 | -0.60447 | -1.11374 | 2.28449 |
| O2 | 0.11186 | -0.91633 | 3.51355 |
| O3 | 0.66243 | 2.659555 | 3.38147 |
| O4 | 1.05624 | 1.845775 | 0.66098 |
| O5 | 0.00193 | -0.88592 | 3.52615 |
| C6 | -0.55335 | -2.60868 | 1.99386 |
| O6 | -1.16506 | -3.30932 | 3.08837 |
| H1 | -1.03905 | 0.776125 | 4.0573 |
| H2 | 1.85235 | 0.975965 | 2.93495 |
| H3 | -0.84086 | 1.626185 | 1.54619 |
| H4 | 1.11733 | -0.72801 | 1.03337 |
| H5 | -1.66645 | -0.78826 | 2.39873 |
| H6A | 0.49943 | -2.94572 | 1.85343 |
| H6B | -1.10775 | -2.85088 | 1.05616 |
| HO2 | 1.52256 | 3.059395 | 3.31848 |
| HO3 | 0.60873 | 1.948075 | -0.17073 |
| HO6 | -0.64645 | -4.08777 | 3.26054 |

Table 5. Atomic coordinates of modeled glucose monomer of down cellulose $III_I$.

| ATOM | X | Y | Z |
|---|---|---|---|
| O1 | -0.26566 | 0.85319 | 0 |
| C1 | -0.99673 | -2.47271 | 3.11311 |
| C2 | -0.26665 | -0.22223 | 3.97863 |
| C3 | 0.25845 | 0.41033 | 1.22916 |
| C4 | 0.48834 | 1.05943 | 3.63036 |
| C5 | 0.07577 | 1.5262 | 2.24119 |
| O2 | -0.87046 | -3.36738 | 1.99746 |
| O3 | 0.94127 | 2.60653 | 1.84899 |
| O4 | 0.11194 | 2.10025 | 4.54465 |
| O5 | 0.94127 | 2.60653 | 1.84899 |
| C6 | -0.26665 | -0.22223 | 3.97863 |
| O6 | 1.33004 | 0.10133 | 1.14492 |
| H1 | -0.98977 | 1.85202 | 2.24954 |
| H2 | 1.59006 | 0.88423 | 3.66541 |
| H3 | -1.35054 | -0.00419 | 4.12287 |
| H4 | 0.94707 | -1.57249 | 2.75514 |
| H5 | -2.06017 | -2.1696 | 3.24951 |
| H6A | -0.68104 | -3.00519 | 4.04204 |
| H6B | 0.41797 | 3.39967 | 1.82384 |
| HO2 | 0.55395 | 1.94684 | 5.37151 |
| HO3 | -1.71577 | -3.78143 | 1.86147 |
| HO6 | 1.33004 | 0.10133 | 1.14492 |

## References


1. D.W. Jones. *J. Polym. Sci.*, **42** (1960) pp. 173-188.

2. P. J. C. Smith and S. Arnott. *Acta Crystallogr.*, A **34** (1978), pp. 3-11.

3. W. D. Motherwell, H. L. Ammon, J. D. Dunitz, A. Dzyabchenko, P. Erk, A. Gavezzotti, D. W. Hofmann, F. J. Leusen, J. P. Lommerse, W. T. Mooij, S. L. Price, H. Scheraga, B. Schweizer, M. U. Schmidt, B. P. van Eijck, P. Verwer and D. E. Williams. *Acta Crystallogr. B*. **58** (2002), pp. 647-661.

4. Y. Nishiyama, S. Kuga, M. Wada, T. Okano. *Macromolecules*, **56** (1997), pp. 6395-6397.

5. Y. Nishiyama, J. Sugiyama, H. Chanzy and P. Langan, *J. Amer. Chem. Soc.* **125** (2003), pp. 14300–14306.

6. Y. Nishiyama, P. Langan, and H. Chanzy. *J. Am. Chem. Soc*. **124** (2002), pp. 9074–9082.

7. P. Langan, Y. Nishiyama, and H. Chanzy. *Biomacromolecules* **2** (2001), pp. 410–416.

8. M. Wada, L. Heux and J. Sugiyama. *Biomacromolecules* **5** (2004) pp. 1385-1391.

9. M. Wada, L. Heux, A. Isogai, Y. Nishiyama, H. Chanzy, and J. Sugiyama, *Macromolecules* **34** (2001) pp. 1237–1243.

10. A. Sarko, J. Southwick and J. Hayashi. *Macromolecules* **9** (1976), pp. 857-863.

11. M. Wada, H. Chanzy, Y. Nishiyama and P. Langan. *Macromolecules* 2004, In Press.

12. Z. M. Ford, E. D. Stevens, G. P. Johnson and A. D. French, Southeast Regional Meeting, American Chemical Society (ACS), Atlanta, November 17, 2003; ACS National Meeting, Anaheim, CA, March 27, 2004, CARB028.

13. K. H. Gardner and J. Blackwell, *Biopolymers*, **13** (1974), pp. 1975-2001.

14. A. D. French and P. S. Howley. *Cellulose and Wood - Chemistry and Technology*, C. Scheurch, ed. Wiley and Sons. 1989. pp. 159-167.

15. A.D. French, D. P. Miller, and A. Aabloo, *Int. J. of Bio. Macromolecules* 1993, **15** 30--36.

16. K. Mazeau and L. Heux, *J. Phys. Chem. B.* **107**(2003) pp. 2394--2403.

17. A. D. French and G. P. Johnson. *Cellulose* **11** (2004), pp. 5—22.

18. Z. Peralta-Inga, G. P. Johnson, M. K. Dowd, J. A. Rendleman, E. D. Stevens and A. D. French. *Carbohydr. Res.* **237** (2002), pp. 851—861.

19. G. A. Jeffrey and W. Saenger, Hydrogen Bonding in Biological Structures, Springer-Verlag, Berlin 1991, p. 35 and others therein.

20. L. Y. Yatsu, T. A. Calamari, Jr. and R. R. Benerito. *Text. Res. J.*, 1986, **56**, 419—424.

21. J. Mann, L. Roldan-Gonzalez and H. J. Wellard, *J. Polym Sci.* **42** (1960), pp. 165–171.

22. A. Sarko and R. Muggli. *Macromolecules* 7 (1974), pp. 486–494.

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July 26, 2005

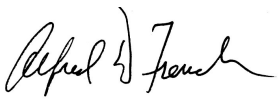
Robert C. Cashner, Ph.D.
Dean of the Graduate School
University of New Orleans
Lakefront Campus
New Orleans, Louisiana 70124

Dear Dean Cashner:

This letter is written on behalf of Ms. Zakhia Ford, a graduate student in Chemistry who is finishing her master's thesis. Part of her work has included a jointly authored paper, Determining the crystal structure of cellulose $III_I$ by modeling. It was published earlier this year in Carbohydrate Research, 340:827-833. 2005. The authors are Ford, Z. M., Stevens, E. D., Johnson, G. P. and French, A. D.

This paper was reviewed by Mr. Johnson, my technician, and myself, and was approved by the U. S. Department of Agriculture as an official publication. Ms. Ford made extensive contributions to the work and wrote the first draft of the paper. She retains the data used in the project. This letter transmits approval for the paper to be included in her thesis.

Sincerely,



Alfred D. French, Ph. D.
Research Chemist
Cotton Structure and Quality Research Unit

# Vita

Zakhia Moore Ford was born in New Orleans, Louisiana and was raised in Avondale, Louisiana. She graduated from Xavier Preparatory High School in 1996 and received her B.S. in Chemistry in 2000 from Xavier University of New Orleans. She entered the University of New Orleans' Chemistry Department in the fall of 2000 where she declared an Analytical Chemistry major with a minor in Physical Chemistry. Her advisor is Chairman and Distinguished Professor Dr. Edwin D. Stevens. Her research involves the characterization of materials using x-ray diffraction and molecular modeling of small carbohydrates. Zakhia has received research fellowships from government agencies such as the Department of Homeland Security and the Agricultural Research Service. In the fall of 2005, a Ph.D. in Analytical Chemistry is expected. She is married to Travis R. Ford, an Eleanor McMain graduate who received a B.A. in Communications in 2001 from the University of New Orleans. He is currently seeking a master's degree in education and teaches English at Marion Abramson High school in New Orleans.