

**ELECTRON DENSITY STUDIES OF METHYL CELLOBIOSIDE**

**Edwin D. Stevens**  
**University of New Orleans**  
**New Orleans, LA**  
**Michael K. Dowd**  
**Glenn P. Johnson**  
**Alfred D. French**  
**USDA-ARS, SRRC**  
**New Orleans, LA**

**Abstract**

Experimental X-ray diffraction crystallography measures the variations in electron density that result from the periodic array of atoms in a crystal. Normally, the positions and types of atoms are determined from the diffracted intensity data based on an approximation that the electron density about an atom is spherical. However, the diffraction data can also be refined based on a multipole approximation that permits the structure's electron density to show a more resolved picture. For example, lone pairs of electrons become visible and increases in the density between atoms indicate when bonding is occurring. Alternatively, Bader's Atoms-In-Molecules topological theory can be applied to analyze the electron density that is obtained either from experiment or from theoretical electronic structure theory calculations. Even rather weak interactions, such as hydrogen bonds, can be resolved with this technique. In the present effort, both experiment and theory worked together to gain added insight into the structure of cellulose III<sub>1</sub>. Methyl  $\beta$ -cellobioside has structural similarities to cellulose III, which results from ammonia mercerization of cotton. Unlike the cellulose polymorph, methyl  $\beta$ -cellobioside provides sufficiently high quality crystals for these electron density studies.

**Introduction**

Many properties of cotton fibers can be rationalized only at the molecular and microfibrillar or nanocrystalline levels. There are several approaches that can furnish information, among them X-ray diffraction and molecular modeling. These studies can now be carried out with a useful degree of accuracy directly on some cellulosic materials, but cotton fibers themselves are quite complex. This complexity limits the amount of experimental data that can be recorded by using cotton fibers to diffract X-rays. From the molecular modeling side, only the molecules and nanocrystals that make up the microfibrils can be modeled at the atomic level. Bigger structures exceed the limits of modeling systems that explicitly represent each atom. Still, there are interesting problems at a smaller scale where we do not really understand the forces that hold the cellulose molecules to each other in nanocrystals. For example, are the direct and relatively strong hydrogen bonds in cellulose crystals the main organizing force, or are non-polar van der Waals forces more important (Bergenstrahle et al., 2010)? This understanding has implications for biosynthesis as well as numerous chemical treatments of cellulosic materials.

Often, smaller carbohydrate systems can furnish more precise information than large polysaccharides such as cellulose. Over the years one of our focuses has been the study of small fragments of cellulose, such as the title molecule, methyl  $\beta$ -cellobioside. As a fragment of cellulose, its importance to cellulose knowledge has long been recognized. Its methanol adduct crystal structure was solved more than 40 years ago (Ham and Williams, 1970). Since then, a greater importance has become more apparent because of its close relationship to the crystal structure of cellulose III<sub>1</sub> (Wada et al., 2004; Ford et al., 2005), a polymorph that results from treatment of cellulose I with amines or liquid ammonia. The latter treatment is a commercial process called ammonia mercerization.

Besides the increased relevance of methyl  $\beta$ -cellobioside, new methods of studying and interpreting molecular interactions have become available. For example, the concept of C-H...O hydrogen bonds has become widely, although not universally, accepted. Last year French and Johnson (2010) presented a paper on the interactions between two methanol molecules done entirely with electronic structure theory. It analyzed hydrogen bonding based on the topological theory of Atoms-In-Molecules (Bader, 1990). Our paper showed that Bader's "Bond Paths" (BPs) and "Bond Critical Points" (BCPs) that identify bonds can be detected in the electron density of the methanol dimer for a wide range of geometries. The methodology applies to both ordinary covalent bonds and to weaker interactions such as C-H...O hydrogen bonds (Koch and Popelier, 1995). Analysis of the properties of the electron density at the BCPs provides useful information on the strength of the hydrogen bonds. The Atoms-In-Molecules approach has

been extensively used to study hydrogen bonds (Grabowski, 2006). Our work with the methanol dimer showed that BCPs occurred for longer distances between donated protons and acceptor oxygen atoms than commonly thought.

Another development since the first crystal structure of methyl  $\beta$ -cellobioside is the study of electron density with X-ray diffraction. From a practical standpoint, electron density analysis to crystals of molecules as large as methyl cellobioside depends on the better data that can now be obtained with highly-sensitive, digital intensity detectors that simultaneously collect data for many reflections as well as many repeated measurements. Two approaches have been taken. In one approach an atom's electron density is described as a collection of monopole, dipole, quadrupole, octapole and hexadecapole functions. In normal single crystal diffraction studies, the density is considered to be spherical, but the multipolar model allows the density to have bumps and other distortions. The difference that results between the electron density interpreted as multipolar atoms and as spherical atoms is referred to as a "difference density plot" that reveals lone pairs of electrons on oxygen atoms and increased electron density in the interatomic bonds. The second approach to analyzing electron density is to use the Atoms-In-Molecules approach. This approach permits ready comparison of the experimental and theoretical results. Recently we published a paper on an analysis of the structure of trehalose dihydrate using these methods (Stevens et al., 2010). Among other things, it showed how the water of crystallization was held in by several O-H...O and C-H...O hydrogen bonds. Our paper followed the first such carbohydrate paper on this theme, on sucrose (Jaradat et al., 2007).

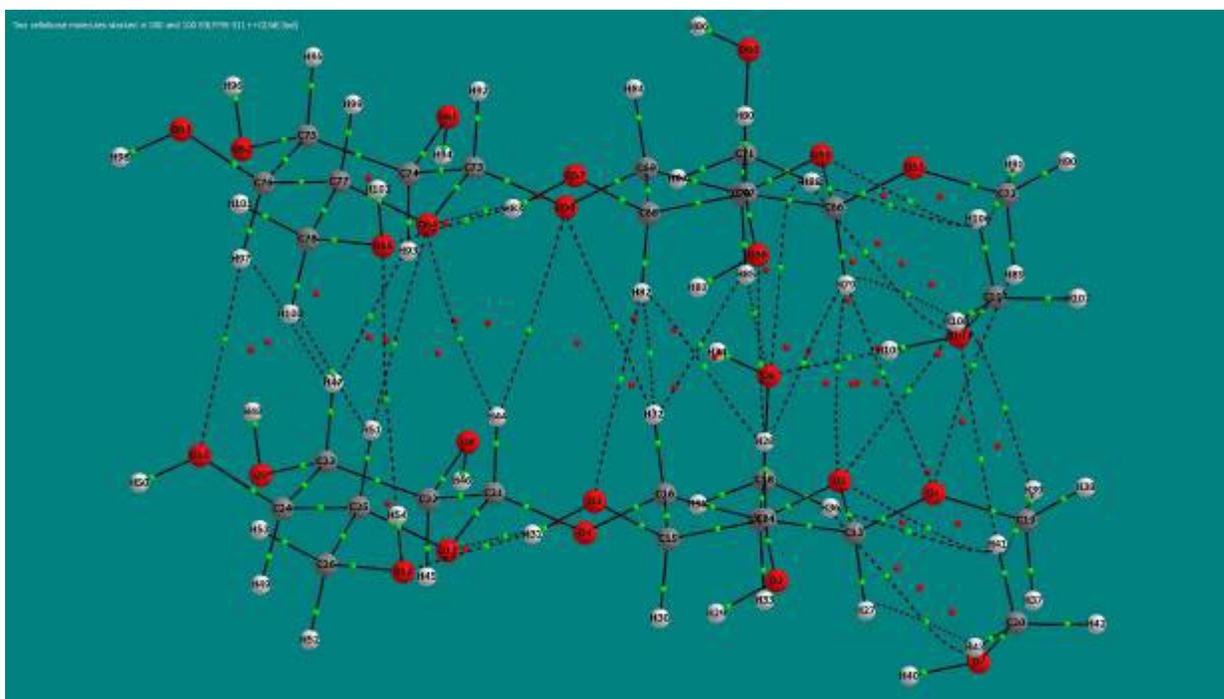


Figure 1. Atoms-In-Molecules molecular graph showing BPs, BCPs, and the atoms for the methyl  $\beta$ -cellobioside methanol adduct. Two complete cellobiose molecules are shown, one above the other, with the methyl groups at the right side (C19 and C72). The methanol group with C104 and O103 is also shown. The white spheres are hydrogen atoms, the grey spheres are carbon, and the comparably sized red spheres are oxygen atoms. Covalent BPs are solid black lines, and weak interactions, including O-H...O and C-H...O hydrogen bonds are shown as dashed lines. Interactions between hydrogen atoms are also shown. Green beads are the BCPs and the small red beads are Ring Critical Points (RCPs). (Many RCPs are for rings formed in part with non-covalent BPs.) This drawing was produced by AIMAll (Keith, 2010) from B3LYP/6-311++G(3df,3pd) quantum calculations. Similar information is provided by the diffraction study. (When viewing an electronic version, zoom in to see the detail better.)

## Results

Several interactions are observed between cellobiose molecules in the methyl  $\beta$ -cellobioside methanol adduct. Figure 1 shows a stacked pair of methyl cellobioside molecules. On the left side, the chair form of the glucose ring is

most easily seen. Also on that side there is an intermolecular O-H...O linkage from O12-H54...O65 (both are O6 groups.) In the lower middle, the bifurcated hydrogen bond from O3-H31...O11 and O3-H31...O12 is shown. In this projection, however, the other weak interactions between the two cellobioside molecules are far more extensive than the O-H...O hydrogen bonds. This stacking is nearly identical to the stacking seen in cellulose III<sub>I</sub>, but it is not the same as the stacking observed in cellulose I (Nishiyama et al., 2002), which lacks the strong O6...O6 hydrogen bonding between the layers, and has its chains offset. Another interesting point is that the density of cellulose Iβ is slightly higher than that of III<sub>I</sub>, even though it looks like the chains could not get closer together than this.

Between the theoretical calculations and the experimental data from this complex structure, there are many details that can be exploited to gain a better understanding of the forces that govern the shape of the cellobiose molecule and the interactions among cellobiose molecules. This new information can either be applied to cellulose when there is a direct analogy, such as in the case of the relationship here between methyl β-cellobioside/methanol adduct and cellulose III<sub>I</sub>, or it can be put in the library of information used to better understand cellulose as well as other carbohydrate materials.

### Acknowledgment

This project was aided by funding from Cotton Incorporated.

### References

- Bader, R. F. W. (1990) Atoms in Molecules: A Quantum Theory (International Series of Monographs on Chemistry). Oxford University Press Inc., New York
- Bergensträhle, M., Wohler, J., Himmel, M. E., Brady, J. W. (2010) Simulation studies of the insolubility of cellulose. *Carbohydr. Res.* 345:2060-2066
- Ford, Z. M., Stevens, E. D., Johnson, G. P., French, A. D. (2005) Determination of the crystal structure of cellulose III<sub>I</sub> by molecular mechanics modeling. *Carbohydr. Res.* 340:827-833
- French, A. D., Johnson, G. P. (2010) Hydrogen bonding in the methanol dimer. Proceedings, Beltwide Cotton Conferences, 1581-1584.
- Grabowski, S. J. (2006) Hydrogen bonding – new insights. Springer, Dordrecht The Netherlands
- Ham J. T., Williams D. G. (1970) Single-crystal x-ray structure determination of methyl-β-cellobioside methanol solvate. *Acta Cryst B*26:1373-1383
- Jaradat, D. M. M., Mebs, S., Chęcińska, L., Luger, P. (2007) Experimental charge density of sucrose at 20 K: bond topological, atomic, and intermolecular quantitative properties. *Carbohydr. Res.* 342:1480-1489
- Keith, T. (2010) AIMAll (Version 10.05.04) ([aim.tkgristmill.com](http://aim.tkgristmill.com))
- Koch, U., Popelier, P. L. A. (1995) Characterization of C-H...O Hydrogen Bonds on the Basis of the Charge Density. *J Phys Chem* 99:9747-9754
- Nishiyama, Y., Langan, P., Chanzy, H. (2002) Crystal structure and hydrogen-bonding system in cellulose Iβ from synchrotron X-ray and neutron fiber diffraction. *J. Am. Chem. Soc.* 124:9074-9082
- Stevens E. D., Dowd M. K., Johnson, G. P., French, A. D. (2010) Experimental and theoretical electron density distribution of α,α-trehalose dihydrate. *Carbohydr. Res.* 345:1469-1481
- Wada, M., Chanzy, H., Nishiyama, Y., Langan, P. (2004) Structure determination of cellulose III<sub>I</sub> by Synchrotron X-ray and Neutron Fiber Diffraction *Macromolecules* 37:8548-8555