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The Structure of Cellulose by Conformational Analysis. 1. Cellobiose and Methyl-β-cellobioside

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ABSTRACT

Conformational analysis studies on the tertiary structure of cellobiose and methyl-β-cellobioside were carried out by using calculations of van der Waals, H-bond, electrostatic, and torsional energy interactions between the atoms and groups of the molecules. Energy maps as functions of the rotational angles Ψ° and Φ° of the glucosidic bond were obtained in increments of 20° and refined in increments of 1°. Two "primary" and one "secondary" conformations of minimum energy were obtained for both cellobiose and methyl-β-cellobioside, some of which are equivalent to results obtained by x-ray diffraction. The H-bond forces are shown to be, together with the van der Waals forces, the predominant factors in the fixation of the conformations of minimum energy. The position and energy contributions of the H-bonds patterns for the favored conformations are identified.

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INTRODUCTION

The molecular structure of cellulose was determined several decades ago. However, the tridimensional or "tertiary" structure of cellulose is still unknown, notwithstanding the considerable amount of time that has been dedicated to cellulose research in general. Today most researchers still favor the "bent-chain" conformation of cellulose rather than other types of conformation. This conformation was proposed more than 30 years ago [1, 2] on the basis of the observation of physical models representing the repetitive glucose residues of cellulose. It assumes that cellulose is not only a homogeneous polymer as regards gross chemical composition, but that it is also composed of monomer units of homogeneous configuration.

It is now accepted that cellobiose rather than glucose is the monomer unit of cellulose [3]. Up to now only one conformational analysis study has been carried out on the "tertiary" structure of cellulose and only a few on cellobiose [4, 5]. The first study [4], although important for the approximate definition of fully allowed and marginally allowed van der Waal conformational zones and suitable for the times in which it was carried out, is, however, inadequate to explain the structure of cellulose. It is limited to the calculation of energy minima of the skeleton of cellobiose only (eliminating completely the side chains and chemical groups attached to the skeleton) by using only the calculation of van der Waals interactions.

Other important interactions such as hydrogen bonds (H bonds), electrostatic, and bonds torsional stress forces were not taken into consideration. For both these reasons the assumption that the energy minima conformations which were found correspond to the more stable cellobiose structures is indeed very doubtful. Furthermore, only the energy of cellobiose in the "bent chain" or Hermans conformation [1] and Meyer and Misch conformation [5], thus in fixed positions, were calculated and compared. Only from this was it deduced that the "bent-chain" conformation is the more stable. Both cellulose conformations were quite far from the cellobiose minima. Again it was assumed that cellulose is composed of monomer units of homogeneous configuration.

The second, more recent, study by Melberg and Rasmussen [5] includes a simple force field calculation which allows internal distortion of the pyranose rings. This study, although of great interest, suffers from a definite conceptual disadvantage, namely that the authors have assumed that energy stabilization due to distortion of the pyranose rings is more marked than the energy stabilization obtainable by intra- and intermolecular H bonding. H bonding effects were not even considered. We will see in the discussion how important their contribution is in relation to pyranose rings distortion.

Atalla has recently proposed, from cellulose hydrolysis and Raman spectral studies, that the glycosidic linkages in the cellulose polymer are not the same [3], resulting in different degrees of sus-

ceptibility to hydrolytic attack. As the β-glucosidic nature of the bonds is not in question, we asked ourselves if this behavior was due to the presence of different conformations assumed by the cellobiose monomers and by the β-glucosidic bonds connecting such monomers. Our conformational analysis takes into consideration not only the total energy barrier to rotations around the β-glucosidic bonds of the cellulose chain, but also the individual energy contributions due to van der Waals, H-bond, electrostatic, and torsional forces. Furthermore, not only cellobiose-type structures were analyzed but also the configurations and energy minima of β-glucosidic bonds connecting two cellobiose residues. The investigation was not limited to the carbohydrate skeleton, but took into consideration all side chains and chemical groups in the glucose residues.

As the indications from Raman spectra of cellotetraose [3] were that nonequivalent glycosidic linkages occur in both mercerized and native cellulose, and considering that it now appears to be accepted that the structures of mercerized and native cellulose can be compared to the structures of cellobiose and methyl-β-cellobioside, respectively, our study was carried out on both monomer units. This first article is concerned with the conformations of cellobiose and methyl-β-cellobioside. The following articles are concerned first with the conformations of the β-glucosidic bonds linking the monomer units and the configuration of the cellulose chain, and second with the packing of cellulose chains in the crystallographic network which indicates the conformations constituting the crystalline and amorphous states of cellulose. Our main aim was to clarify the structure of "native" wood cellulose in wood, thus of Cellulose I.

EXPERIMENTAL

1. Computer Program

The computer program used is the same which was used by one of the authors [6] in clarifying the polypeptide sequence of (L)-proline 88 in the respiratory protein myoglobin. It was originally developed as program SZEN01 and 02 at the University of Rome, Italy, in 1968 to 1969 by the Liquori research group on conformational analysis of proteins and polypeptides. It can accommodate 30 bond rotational angles (15 Φ°s and 15 Ψ°s) maximum. It has been extensively modified by the authors of this article [7] for the calculation of conformational energies, allowed conformations, and atom coordinates of polymeric carbohydrates. This new BONDS program has several more capabilities beyond the original program.

2. Contributions to Conformational Energy

Conformational studies in the field of biological macromolecules have shown that, at least for polypeptide sequences, the conformational energy of a molecule can be represented with good accuracy by a sum of four types of contributions, namely

$$E_{(tot)} = E_{(vdW)} + E_{(HB)} + E_{(ele)} + E_{(tor)} \quad (1)$$

The same approach was used for the carbohydrates under analysis. $E_{(tot)}$ represents the total conformational energy of the molecule as a function of all the internal angles of rotation.

$E_{(vdW)}$ represents the contribution to the total energy due to van der Waal's interactions between all the couples of not-linked atoms whose relative position depends from one or more internal rotational angles ($\Phi°$,$\Psi°$). This contribution can be expressed by "Buckingham"-type functions

$$E_{(vdW)} = \Sigma_{ij}(a_{ij}e^{-b_{ij}r_{ij}} - c_{ij}r_{ij}^{-6}) \quad (2)$$

where the coefficients a, b, and c depend on the couple i,j of atoms or by "Lennard-Jones"-type functions

$$E_{(vdW)} = \Sigma_{ij}\left(\frac{d_{ij}}{r_{ij}^{12}} - \frac{c_{ij}}{r_{ij}^{6}}\right) \quad (3)$$

The more commonly used functions are those in the "Buckingham" form. Several sets of a, b, and c coefficients are available [9-11]. The work of Rees and Skerrett [4] has, however, shown that the final results, when using different sets of coefficients, are indeed similar. The coefficients used in our investigation were a set of coefficients from the Liquori research group (Table 1). These are more modern, refined, and effective than those of the Liquori set used by Rees and Skerrett in their investigation. The Liquori functions used by Rees and Skerrett were a mixture of Buckingham- and Lennard-Jones-type functions. The Liquori functions we used were only in the Buckingham form. The attraction coefficients c_{ij} in Eq. (2) were calculated with the formula of Slater [12] and Kirkwood:

$$c_{ij} = \frac{(3/2)e^{(h/m^{1/2})}\alpha_i\alpha_j}{(\alpha_i/N_i)^{1/2} + (\alpha_j/N_n)^{1/2}} \quad (4)$$

TABLE 1. Coefficients of "Buckingham"-Type Functions Used to Calculate van der Waals Interactions; Refined Liquori Set

Atoms involved in interaction	a ($\times 10^{-3}$)*	b	c
C–C	237.000	4.320	297.800
C–O	212.091	4.435	244.000
O–O	186.439	4.550	200.000
H–C	31.390	4.201	121.100
H–O	28.124	4.316	99.200
H–H	6.597	4.082	49.230
CH_3–C	291.300	1.665	981.200
CH_3–O	201.900	3.970	606.000
CH_3–H	41.090	3.705	380.200

*Values have been multiplied by 10^{-3}; thus C–C interaction = 237,000.

where α_i and α_j are the values of the polarizability of the atoms i and j, and N_i and N_j are the numbers of effective electrons (Table 2).

In Eq. (2), b_{ij} has been fixed to a constant value of 4.6 [13] and a_{ij} has been determined by imposing the condition of minimum at the distance which is the sum of the van der Waals radia of the atoms or groups considered. It must be pointed out that the van der Waals interactions of any chemical groupings were calculated as the sum of the single interactions between each couple of unlinked atoms. The only exception was the terminal $-CH_3$ group of methyl-β-cellobioside which was represented by means of a single function.

TABLE 2. Characteristics of Atoms Needed for the Calculation of van der Waals Interactions (c_{ij} term)

Atom type	Van der Waals radius	Polarization capability	Number of effective electrons
C	1.70	1.30	5.0
O	1.50	0.84	7.0
H	1.20	0.42	0.9
CH_3	2.00	2.17	8.0

$E_{(HB)}$ represents the hydrogen bond (H-bond) contribution between couples of atoms which do not belong to the same glucose residue. We have used a hydrogen bond function proposed by Stockmayer [14, 15] which has already been found to give representative results in polypeptide sequences [7, 9, 13]:

$$E_{(HB)} = 4\epsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] - \frac{\mu_a\mu_b}{r^3}\,(2\cos\theta_a\cos\theta_b - \operatorname{sen}\theta_a\operatorname{sen}\theta_b\cos(\gamma_a^\circ - \gamma_b^\circ)) \quad (5)$$

and which takes into consideration the angular dependence of the hydrogen bond. The first term in Eq. (5) describes the interaction between the hydrogen atom and the oxygen atom participating in the H bond. The second term describes the H bond as an electrostatic interaction between two pointlike dipoles of magnitudes μ_a and μ_b centered on the hydrogen and oxygen atoms. The directional character of the H bond is assured by the angular dependance of this function, and θ_a and θ_b are the angles that the bonds C–O and O–H form with the O--H segment linking the hydrogen and oxygen atoms. $(\gamma_a^\circ - \gamma_b^\circ)$ is the angle between the planes containing the H bond and the O–H and C–O bonds, ϵ, σ, and μ are obtained by minimizing the first term of Eq. (5) at the van der Waals distance between the hydrogen and oxygen atoms, and the whole function at a H-bond distance of 2.85 Å with aligned C–O and O–H bonds.

$E_{(ele)}$ describes the electrostatic contribution to the total energy. Dipolar momentums are generally expressed, in an approximation so-called "monopolar," by means of partial charges, the value of which is fixed in such a manner as to reproduce both bonds dipolar momenta and the total dipolar momentum. Using partial charges, the dipolar interactions can be calculated with a Coulomb-type law of form

$$E_{(ele)} = \Sigma i_{ij}\,\frac{q_i q_j}{\epsilon r_{ij}} \quad (6)$$

where q_i and q_j are the charges of atoms i and j, r_{ij} is the distance between them and ϵ is the dielectric constant. We used for q_i and q_j the fractional charges determined by Rao et al. [16] calculated by the MO-LCAO method [17], for aldohexopyranoses.

$E_{(tor)}$ describes the contribution to the total energy due to hin-

dered rotation around skeletal bonds. The formulas used for the torsional potentials of carbohydrate skeleton were those of Brant and Flory.

$$U(\Psi^\circ) = \tfrac{1}{2}U^\circ(\Psi^\circ)(1 - \cos 3\Psi^\circ) \quad \text{and} \quad U(\Phi^\circ) = \tfrac{1}{2}U^\circ(\Phi^\circ)(1 - \cos 3\gamma) \tag{7}$$

The values of the torsional barriers $U^\circ(\Psi)$ and $U^\circ(\Phi^\circ)$ used were, however slightly lower than that of 1.5 kcal/mol used by Flory and identical to those used by the Liquori research group [13]. It is necessary to point out, however, that in the case of polypeptides, this contribution to the total energy is indeed small and has very little importance in the determination of the allowed conformation of the polypeptide chain. We have found the same to apply in the case of cellobiose and cellulose.

3. Original Coordinates of the Atoms of Cellobiose and Methyl-β-cellobioside

The initial atomic coordinates were derived from the refined x-ray crystal structure coordinates of cellobiose of Chu and Jeffrey [18] and of Ham and Williams [19] for methyl-β-cellobioside. The valence bond angle at the glycosidic oxygen atom between the two glucopyranose rings was 116.1 and 115.8° for cellobiose and methyl-β-cellobioside, respectively [19]. It must be pointed out that these two sets of coordinates are indeed very different, which implies that the two molecules are conformationally much more different than what could be imagined by the simple difference in methyl group at C1'. The systematic rotations about bonds to the bridge oxygen were first carried out with increments of rotation of 20°. To pinpoint the actual minima and for energy calculations, the rotational increments for Φ° and Ψ° were 1°. The angles of rotation Φ° and Ψ° were the dihedral angles between C(1)-H(1) and O(4')-C(4') and between O(4')-C(1) and C(4')-H(4'), respectively (Fig. 1). The initial conformation $(\Phi^\circ,\Psi^\circ) = (0,0)$ is defined as having C(1), O(1), and O(4) of the first residue and C(4'), O(4'), and O(1') of the second residue in the same plane.

The rotation is considered to be positive when, viewing along C(1)O(1) (or C(4')O(4')) toward the bridge oxygen atom, the rotation is performed anticlockwise. This is different from the convention used from Rees and Skerrett [4] and equal to the convention used by Ramachandran [20], which is the most widely accepted one in conformational analysis. Thus, Rees and Skerrett rotated Ψ° anticlockwise about C(4')O(4') and Φ° clockwise about O(4')C(1).

For example, for this reason a (Φ°,Ψ°) set of angles expressed according to Rees and Skerrett convention as -25°,50° will be equal to a total angle between the mean plane of the two glucopyranose rings of 75°, while the same (Φ°,Ψ°) set of -25°,50° in the convention we

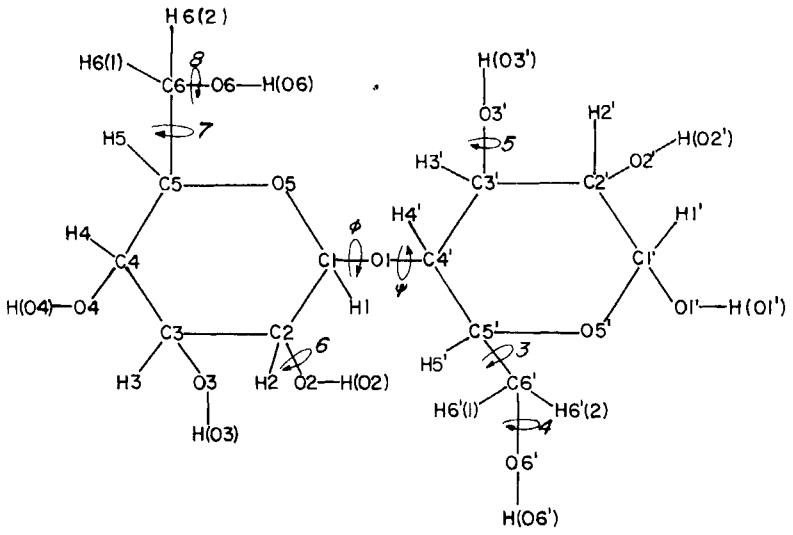


FIG. 1. Position of bonds rotated in cellobiose and methyl-β-cellobioside. H(01') = $-CH_3$ (methyl) in methyl-β-cellobioside.

have used will be equal to a total angle between the mean planes of the two glucopyranose rings of 25°. Thus, the minimum of the ring-to-ring conformation found by Rees and Skerrett using their convention is -25°, +142° (shift angle between ring planes = 167°) while expressed with the convention we have used, the same (Φ°,Ψ°) conformation would have been expressed as +25°, +142° (shift angle between ring planes = 167°).

The positions of 0° for the rotational angles of the side chains were assumed as the positions in which the atoms of the chains present themselves in the x-ray crystallographic data already reported [18, 19]. (This was done to facilitate calculation.) As such, their numerical values are of limited importance, but from these values the preferred atoms positions and coordinates can be retrieved at any time.

RESULTS

From the coordinates and positions of the atoms of cellobiose and methyl-β-cellobioside of Ham and Williams [19] and Chu and Jeffrey [18], it appears that fixing of the favorite conformations by H bonds, at least in the crystalline form of the molecules they have described, is mainly influenced only by the rotation of Angles Φ° and Ψ° and by the rotation of Angles 5, 7, and 8 (Fig. 1). In one case, Angles 3, 4, and 5 also have an influence.

First of all, Angles (Φ°,Ψ°) and 5 were each rotated 360° with 20° increments. It was found that rotation of the C(3')-O(3') (Angle 5) bond does considerably influence the values of Φ° and Ψ° of the conformations of minimum energy as well as the minimum energy values. Angle 7 presents two position ranges in which H-bond fixing of the conformation is possible. To cut down on the number of combinations, only one of these positions, the most likely one, was analyzed. This corresponded to the same position (of the group of atoms controlled by the rotation of Angle 7) found by x-ray crystallography [18, 19]. Angle 8 was instead rotated 360° with 20° increments simultaneously with the Φ°,Ψ°, and five angles. This means that 104,976 conformations were analyzed (namely, 324 energy maps in Φ°,Ψ° for each of the total, van der Waals, H-bond, electrostatic, and torsional contributions) for each monomer.

The results showed that rotation of Angle 8 around the C(6)–O(6) bond has little or no influence on the Φ°,Ψ° values of the conformations of minimum energy for both cellobiose and methyl-β-cellobioside. Thus Angle 8 is mostly 0° at the minimum energy of the conformation.

All this show that the position of the C(6)–O(6)–H(06) groups obtained by conformational analysis coincides with the x-ray analysis [18, 19] results. This is not the case with the position of the O(3')–H(03') bond as the rotation of Angle 5 influences considerably the value and position of the energy minimum in a Φ°,Ψ° map. In the case of the (Φ°,Ψ°) = (0°, -161°) conformation, the exact minimum was obtained also by rotation of Angle 6 and Angle 3 of 360°, at first with 20° increments and then with 1° increments. This was done because the O(6')H(06'). . .O(2)C(2) H bond was high. This conformation corresponds to that of Ham and Williams obtained by x-ray analysis. If Angle 6 is not rotated, the minimum is found at (Φ°,Ψ°) = (0°, -162°). The 20° increment map does not change, but the 1° increment map does change.

Several minima of energy were obtained for simultaneous 20° rotations over a 360° range of Φ°,Ψ° and 5 (Table 3). However, on refining all these approximate minima by simultaneous 1° rotations over a 60° range around the values of Φ°,Ψ° and 5 at the approximate minimum, all the apparent minima of energy melted into two main minimum energy conformations for cellobiose and two minimum energy conformations for methyl-β-cellobioside. These are shown in Table 3.

Furthermore, "secondary" minima are present, that is to say, most Φ°,Ψ° maps present two minima; a main "primary" energy minimum and another, less deep, minimum. These "secondary" minima were also refined by rotational increments of 1° of Φ°,Ψ° and 5 over the required ranges. Their values and the values of the proper primary energy minima allowed in the same "secondary" conformation are also shown in Table 3. The values of minimum energy of the six preferred conformations (three cellobiose, three cellobioside) give an indication of the relative stability of the con-

TABLE 3. (Φ°,Ψ°) Conformations and Values of Total energy Minima of the β-glucosidic Bond Connecting Two Glucose Residues within a Cellobiose and Methyl-β-cellobioside Monomer[a]

	Primary minimum			Secondary minimum		
	Angles conformation		Total energy at minimum	Angles conformation		Total energy at minimum
	Φ°,Ψ°	5	(kcal/mol)	Φ°,Ψ°	5	(kcal/mol)
Cellobiose: Primary minimum conformation	32°, 138°	104°	0.707	172°, 178°	104°	4.450
Cellobiose: Primary minimum conformation	56°, 178	5°	1.594	-	-	-
Methyl-β-cellobioside: Primary minimum conformation	-49°, -130°	-66	-0.605	171°, 170°	-66°	4.530
Methyl-β-cellobioside[b] Primary minimum conformation	0, -161°	-12°	0.055	0°, -22° 180°, -178°	-12° -12°	3.660 6.110
Cellobiose: Lowest energy conformation of secondary minimum	(20°, 159°)	(68°)	(2.380)	180°, 179°	69°	2.607
Methyl-β-cellobioside: Lowest energy conformation of secondary minimum	(-32°, -143°)	(-48°)	(0.830)	168°, 177°	-48°	3.019

[a]In the first four conformations the position of the secondary minima is the one obtained when the primary minimum is at its position of lowest possible energy. In the last two conformations the position of the primary minimum is the one obtained when the secondary minimum is at its position of lowest possible energy.

[b]Angle 3 = +7°; if angle 3 left = 0° energy = 0.431 kcal/mol.

formations. Figures 2, 3, and 4 show the (Φ°,Ψ°) total energy maps with 20°/20° and 1°/1° increments for the six minima. The molecular structures corresponding to these minima are shown in Figs. 5 and 6 (projections onto a plane of tridimensional structures).

All the hydrogen bonds formed and the values of their contributions to the total energy were calculated. For example, for the methyl-β-cellobioside minimum energy conformation (Φ°,Ψ°,5) = (-49°, -130°, -66°) in which the value of the total energy minimum is -0.605 kcal/mol, the contributions of the various energy components is

$$E_{(tot)} \ (=-0.605) = E_{(vdW)} \ (= +4.75) + E_{(HB)} \ (= -5.01) + E_{(ele)} \ (= -0.35) + E_{(tor)} \ (= 0.0)$$

and thus the contribution of the H bond to the fixing (stabilizing) of the conformation is indeed massive. It was of interest to determine which H bonds contribute to the total H-bond energy value. Different theories have been advanced in this respect, but no qualitative or quantitative calculation of the contributions, distribution, and location of the different H bonds has ever been presented. All the possible intramolecular H bonds in the conformations were taken into consideration. The breakdown in significant H-bond contributions for the six confirmations of minimum energy are shown in Table 4. What was found eliminates the hypothesis of Ham and Williams of a split H bond as well as the hypothesis of Chu and Jeffrey of a O(5)–H(03') H bond which is actually present in only very few of the conformations. This means that while the atomic positions obtained by x-ray analysis are obviously correct, they are not at all proof of the type, strength, and location of the H bonds. This situation may change once the molecule is packed in a crystalline network (see Parts 2 and 3 of this study). The energy minima shift position when comparing total energy with van der Waals energy maps. The shifts in minima positions are shown in Table 5. It is to be noticed that for most of the conformations calculated, it was assumed that the original coordinates of all C and O atoms, relative to their glucose ring, were exact, while the coordinates of the H were not considered as exact and thus the decision to rotate mainly Angle 5 together with Φ° and Ψ°.

DISCUSSION

The results and minimum total energy conformations obtained show that cellobiose and methyl-β-cellobioside can both exist in several stable conformations. The "primary" conformations are (32°,138°) and (56°,178°) for cellobiose and (-49°,130°) and (0°,-161°) for methyl-β-cellobioside. "Secondary" conformations most allowed are (180°, 179°) and (168°,177°) for cellobiose and methyl-β-cellobioside, respectively.

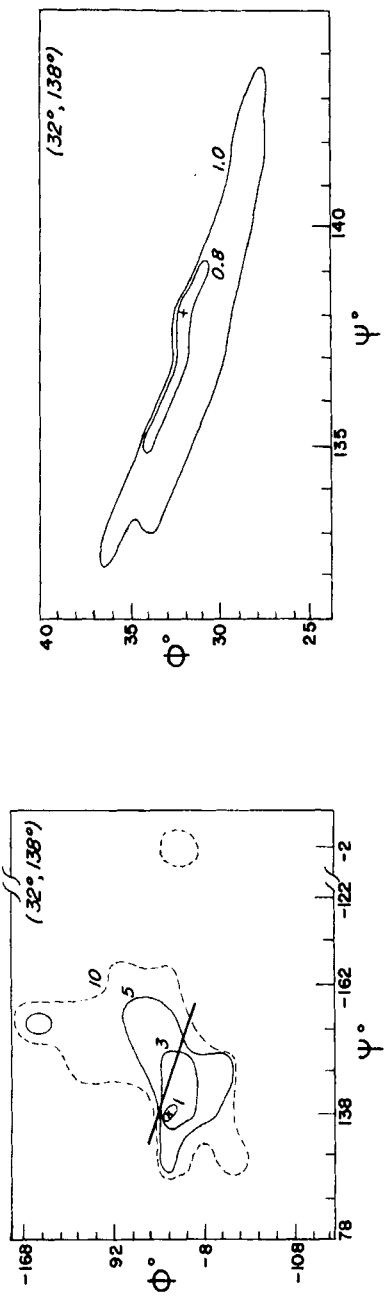


FIG. 2. 20° and 1° increments total energy maps of cellobiose conformations (32°, 138°) and (56°, 178°).

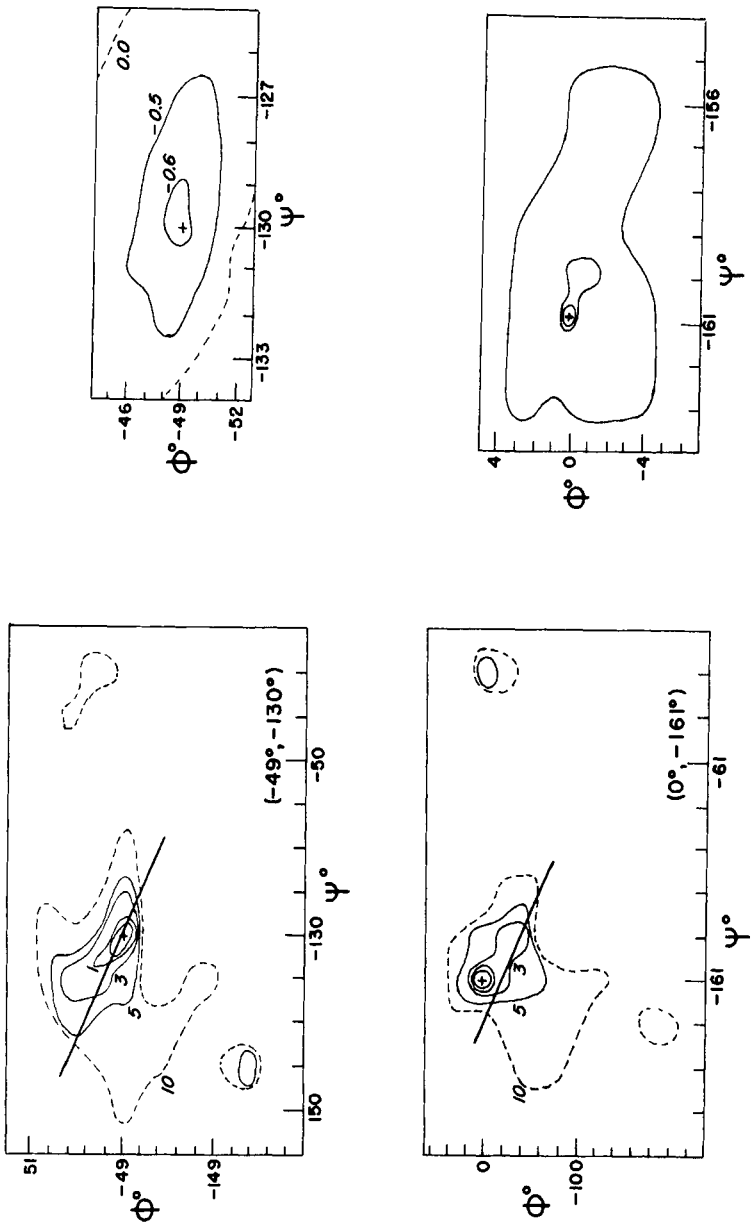


FIG. 3. 20° and 1° increments total energy maps of methyl-β-cellobioside conformations (-49°, -130°) and (0°, -161°).

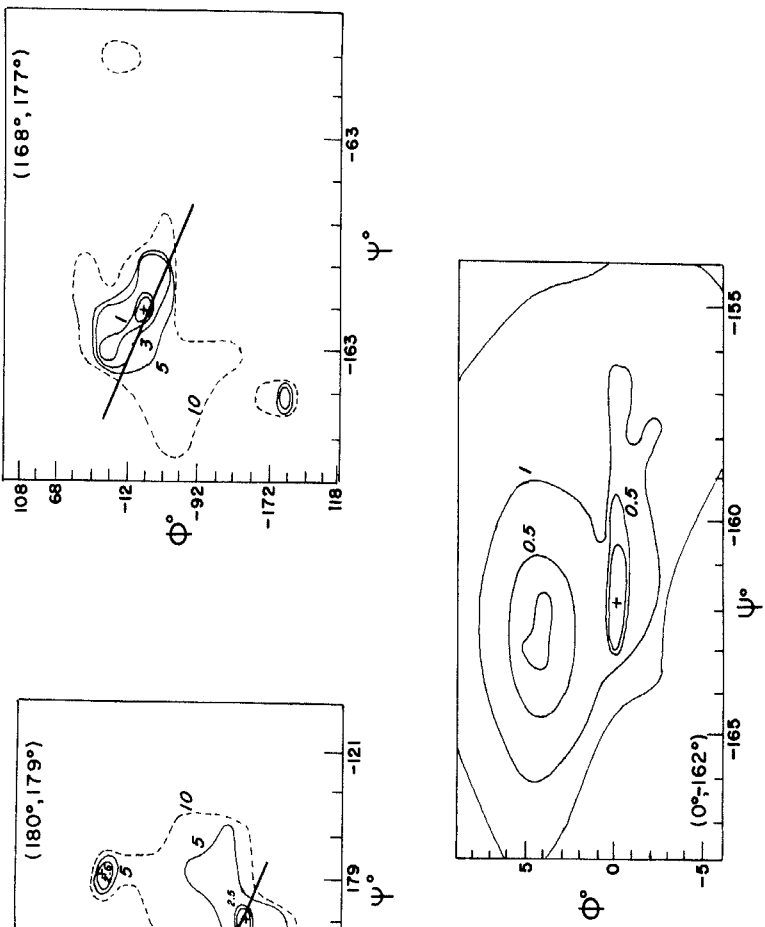


FIG. 4. Total energy maps of cellobiose (180°, 179°) and methyl-β-cellobioside (168°, 177°) conformations; 1° increments energy map of methyl-β-cellobioside (0°-162°) conformation (see Results).

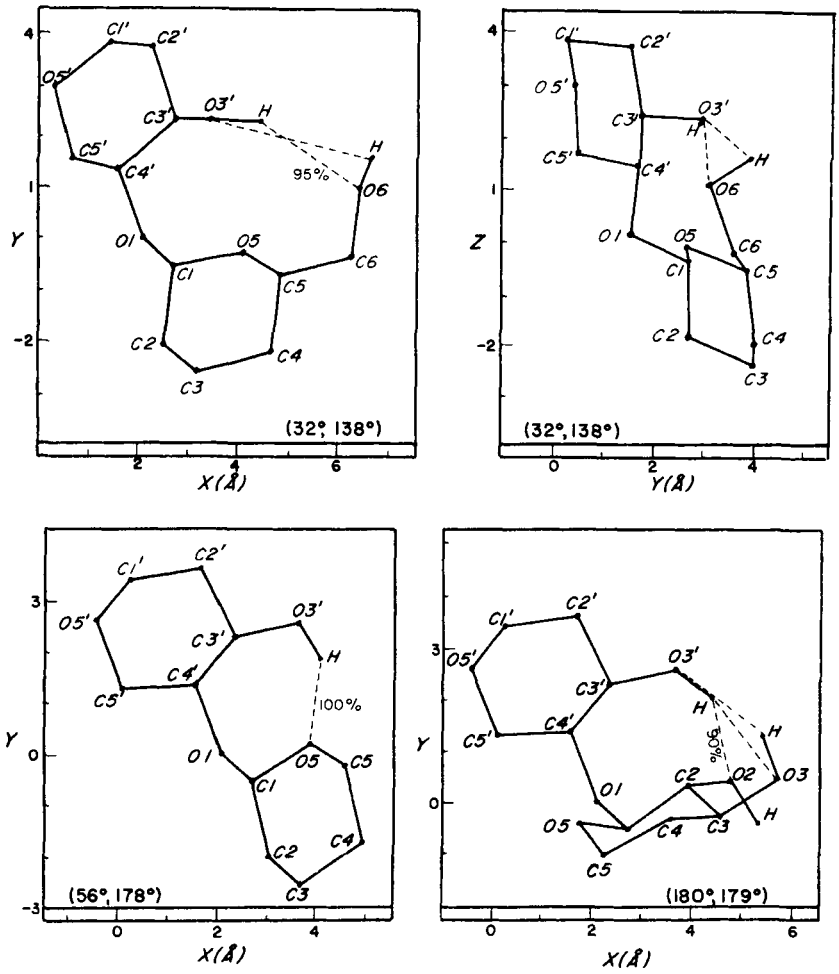


FIG. 5. Plane projections of cellobiose conformations (32°, 138°), (56°, 178°), and (180°, 179°) indicating atom positions and main H bonds formed (dashed lines).

In the 1° incremental maps, the total energy minima obtained are fairly shallow. They are, however, much steeper in the 20° incremental maps. This indicates that small shifts of the values of Φ° and Ψ°, thus small shifts in conformations from the minimum of total energy, are indeed allowed. However, greater variations from the Φ° and Ψ° values of minimum energy are not allowed and thus both the cellobiose and methyl-β-cellobioside molecules behave as fairly

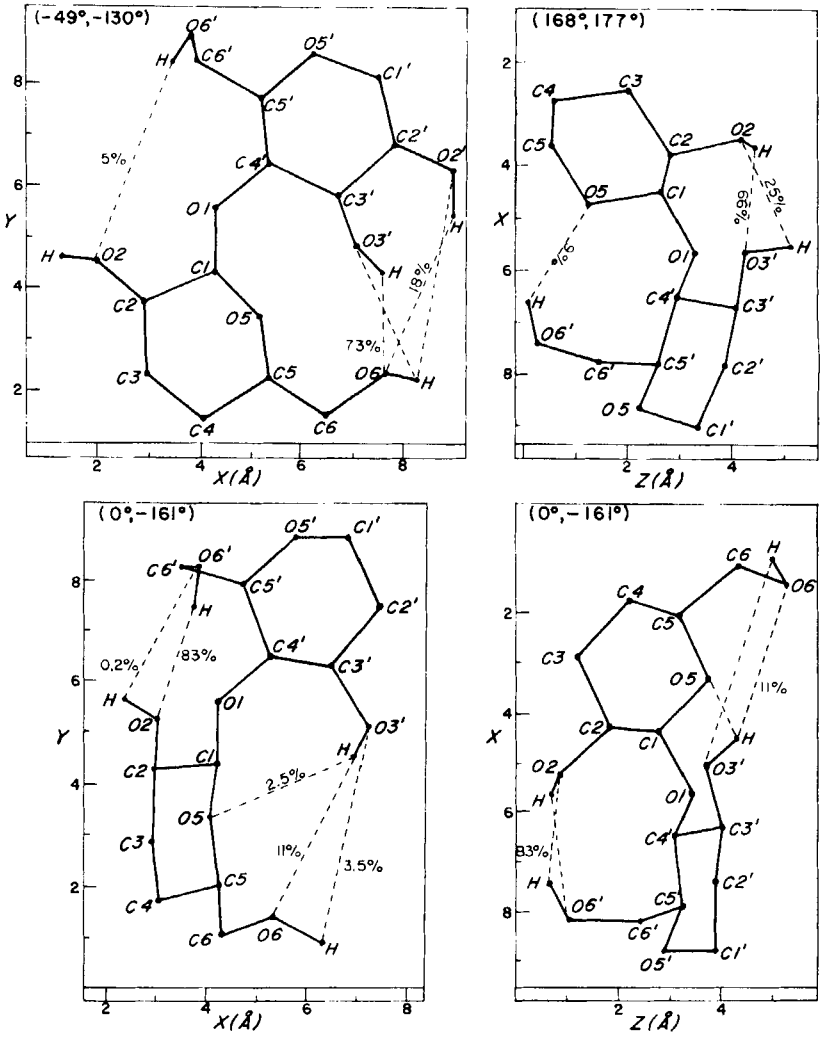


FIG. 6. Plane projections of methyl-β-cellobioside (-49°,-130°), (0°,-161°), and (168°,177°) indicating atom positions and H bonds formed (dashed lines).

rigid structures. The importance of the hydrogen bonds in the "fixing" of the most favored conformations is indeed predominant. For example, for the methyl-β-cellobioside minimum energy conformation (Φ°,Ψ°) = (-49°,130°) in which the value of the total energy minimum is -0.605 kcal/mol, the contributions of the various energy components are

$$E_{(tot)} (= -0.605) = E_{(vdW)} (= +4.75) E_{(HB)} (= -5.01) +$$

$$E_{(ele)} (= -0.35) + E_{(tor)} (= 0.0)$$

Similar relative proportions of the different energy contributions are also valid for the other favored conformations. This type of distribution of the contributing interactions is the cause of our criticism of the calculations and approach of Melberg and Rasmussen [5]. For instance, their minimum energy conformation, while it logically presents lower van der Waals energy minimum than ours as a consequence of the lower van der Waal hard spheres overlaps achieved by distortion of the pyranose ring, also presents a total energy minimum which is considerably higher than ours. The difference is even more marked than what would appear from the numerical values of energy if one considers that while they take into consideration only the skeletal bonds, we have also taken into consideration all the side chains.

All this indicate that when the stabilizing H bonds are taken into consideration, internal distortion of the pyranose rings will not occur more readily than the van der Waals hard spheres overlaps at the energy levels obtained. We think that Melberg and Rasmussen have inadvertently disregarded in their cellobiose study the stabilizing effect of the H bond and considered only ring distortion effects as a consequence of previous work on single pyranose rings conformations where the intermolecular H bond does not obviously apply. The discrepancies in total energy minima between Melberg and Rasmussen's [5] and our conformations become even more marked for the other conformations with higher minimum total energy. To consider the distortion of the pyranose ring as a stabilizing influence when instead is not, and to disregard the intramolecular H bond which instead does strongly stabilize the structure, is indeed a conceptual error. When the H bond is considered, it appears clearly that an H-bonded, undistorted (or very little distorted) pyranose ring is more stable than a distorted one. We have checked this, although this is not reported here, by using the same bond and valence angle-deformation functions used by Melberg and Rasmussen, and our idea proved correct. When the H bond is involved, the molecule prefers not to distort the pyranose ring.

Small variations of Φ° and Ψ° (of 1° or a few degrees maximum) will not alter the hydrogen bond pattern and, thus, shifts in conformation within these very narrow limits should occur very easily. Greater Φ° and Ψ° variations will instead alter considerably the predominant hydrogen-bond pattern of minimum energy and thus they are not easily allowed. It is interesting to note that one of the most favorite methyl-

TABLE 4. H-Bonds Distribution and Their Contributions to the Total Energy Minima of Favored Conformations

Cellobiose			Methyl-β-cellobioside		
Atoms groups	H bond (kcal/mol)	Contribution (%)	Atoms groups	H bond (kcal/mol)	Contribution (%)
Primary conformation (Φ°,Ψ°) = (32°,138°):			Primary conformation (Φ°,Ψ°) = (-49°,-130°):		
O(3')H(03')–O(6)C(6)	-2.1211	95	O(3')H(03')–O(6)C(6)[a]	-3.4935	73
O(6)H(06)–O(3')C(3')	-0.1129	5	O(2')H(02')–O(6)C(6)	-0.8722	18
Total contribution	-2.2340	100	O(6')H(06')–O(2)C(2)[b]	-0.2707	5.5
			O(6)H(06)–O(3')C(3')	-0.1256	2.5
			O(6)H(06)–O(2')C(2')	-0.0358	1
			Total contribution	-4.7978	100
Primary confirmation (Φ°,Ψ°) = (56°,178°):			Primary conformation (Φ°,Ψ°) = 0,-161°):		
O(3')H(03')–O(5)	-1.9751	100	O(6')H(06')–O(2)C(2)	-3.6805	83
			O(3')H(03')–O(6)C(6)	-0.4966	11
			O(6)H(06)–O(3')C(3')	-0.1566	3.5
			O(3')H(03')–O(5)	-0.1061	2.5
			O(2)H(02)–O(6')C(6')	-0.0106	0.2
			Total contribution	-4.4504	100

Secondary conformation (Φ°,Ψ°) = (180°,179°):			Secondary conformation (Φ°,Ψ°) = (168°,177°):		
O(3')H(03')–O(2)C(2)	-2.9916	90	O(2)H(02)–O(3')C(3')	-2.5780	66
O(3')H(03')–O(3)C(3)	-0.0510	2	O(3')H(03')–O(2)C(2)	-0.9728	25
O(3)H(03)–O(3')C(3')	-0.2456	7	O(6')H(06')–O(5)	-0.3336	9
Total contribution	-3.2882	100	Total contribution	-3.8844	100

[a]Note that the O(6) can form also a strong H bond (-4.53 kcal/mol) with the H(02)02 of the subsequent vicinal glucose residue of the following monomer in the chain. This is also valid for the (32°,138°) cellobiose. In an isolated monomer this cannot be formed. It is, however, important in "bent-chain" calculations and packing in the crystallite network (see Part 3 of this study). The presented set-up H-bond value = 4.63 kcal/mol, thus is still slightly favored.

[b]This is obtained without rotating the appropriate groups from their relative positions to their glucose ring. If the two side-chains are rotated, the O(6')H(06')–O(2)C(2) H-bonds value becomes -2.4 kcal/mol (see Part 3 of this study). However, both this and the previous value do not influence the relative positions of the isoenergetic curves in the energy maps and do not need to be considered in the balance of energy calculations of a chain.

TABLE 5. Shift in (Φ°,Ψ°) Values of Energy Minima Positions When Comparing Total Energy Maps with van der Waals Energy Maps

Total energy conformation			van der Waals energy conformation		
(Φ°,Ψ°)	Rings planes relative angular shift	Angular shift from planarity	(Φ°,Ψ°)	Rings planes relative angular shift	Angular shift from planarity
Primary minima:					
Cellobiose (32°,138°)	170°	10°	(29°,142°)	171°	9°
Cellobiose (56°,178°)	234°	54°	(56°,173°)	229°	49°
Cellobioside (-49°,-130°)	-179°	1°	(-29°,-150°)	-179°	1°
Cellobioside (0°,-161°)	-161°	19°	(-40°,-142°)	-182°	2°
Secondary minima:					
Cellobiose (180°,179°)	359°	1°	(175°,181°)	356°	4°
Cellobioside (168°,177°)	345°	15°	(178°,172°)	350°	10°

β-cellobioside conformations corresponds to $(\Phi^\circ,\Psi^\circ) = (0^\circ, -161^\circ)$ with a total angular shift of the mean rings planes of 161°, very similar indeed to the approximate value of 167° obtained by x-ray diffraction analysis by Ham and Williams [19]. It is also interesting that a 167° shift is also easily allowed (see Figs. 3 and 4) when the position, relative to its own glucosidic ring, of the groups controlled by the rotation of Angles 3, 4, and 6 (Fig. 1) is minimized or left unaltered from that obtained by x-ray analysis [19]. An equivalent total energy minimum conformation corresponding to the x-ray data of Chu and Jeffrey [18] for cellobiose was not obtained. The cellobiose conformation $(\Phi^\circ,\Psi^\circ) = 56^\circ, 178^\circ)$ with a mean rings planes angular shift of 234° is the nearest to the Chu and Jeffrey conformation (which has an angular shift of 204°).

This indicates that further stabilization of the cellobiose structure in its crystalline network is obtained by intermolecular secondary forces which allow the conformation of minimum total energy to be shifted from our calculated 234° to the experimentally obtained value of 204° of Chu and Jeffrey.

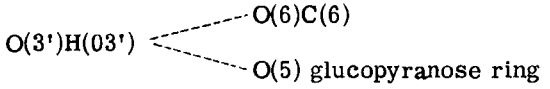
Most of the conformations of minimum energy obtained, namely the primary minima ones, are in the "fully allowed" van der Waals zone defined by Rees and Skerrett [4], while the rest are in the "marginally allowed" zone. As these "zones" were originally based on van der Waals energy calculations, and considering the predominant effect of the intramolecular H bonds, the use of total energy minima rather than van der Waals energy minima will have two effects on the zones limits: namely (1) most of the "marginally allowed" van der Waals only zone will become a "fully allowed" total energy zone, and (2) the passage from van der Waals to total energy will furthermore shift the position of the (Φ°,Ψ°) map of the allowed conformational zones. (As van der Waals only zones, the limits of Rees-Skerrett, however, still stand.)

It is also interesting to note that in methyl-β-cellobioside, one of the minimum energy conformations is only 1% different from the "twofold" helix, 180° rings-shift conformation. In cellobiose, the smallest difference is 10°. While it is interesting, this does not constitute as yet any proof of the feasibility and existence of a "twofold" helix conformation for cellulose. It only refers to the two glucose rings combination. The β-glucosidic bond between two glucose residues forming cellobiose-like structures is fixed in a near "twofold" helix conformation by a certain well-defined H-bonds pattern. The β-glucosidic bond before or after the cellobiose-like units will be fixed in a different position by a different H-bond pattern due to the different groups participating. This point will become clearer in the second part of this study.

It is important to note that, at least in the conformations of minimum energy, the β-glucosidic linkage in the primary conformations of methyl-β-cellobioside is considerably more shielded against water attack, hence hydrolysis, by the H-bond casing surrounding it than in cellobiose (see Figs. 5 and 6). Of the two primary conformations of methyl-β-cellobioside, the $(-49^\circ, -130^\circ)$ show better shielding on an

approximate single plane surrounding the glycoside linkage, thus it is a better conformation for packing in a crystalline network where the "above" and "under" positions would be shielded by cellobiosides with the mean planes of their glucose residues parallel to it. The (0°,-161°) instead presents a somewhat better "tridimensional" H bond shielding of the glucosidic linkage, indicating that while the (-49°,-130°) conformation may probably be the most resistant to hydrolytic attack when found in a crystalline network, the (0°,-161°) may be the most resistant conformation in an isolated chain.

In Table 4 the contributions of the various H bonds formed are quantified. The various H bonds are shown as dotted lines in Figs. 5 and 6. It is interesting to note that the stronger H-bond contributions are not those qualitatively foreseen by Chu and Jeffrey [18] and Ham and Williams [19]. Chu and Jeffrey foresaw, qualitatively, that in cellobiose the important H-bond contribution would have been the O(3')H(03')--O(5). This is true for the (56°,178°) cellobiose conformation which is the most similar to that obtained by them by x-ray analysis, and that is probably the one stabilized by secondary intermolecular forces in their crystalline network and not in the free molecule. It is obviously not true for the (32°,138°) cellobiose conformation. Ham and Williams instead foresaw, also qualitatively, that the strongest H-bond contribution would be given by a bifurcated H bond, namely



for methyl-β-cellobioside. Our results show that this bifurcated H bond does indeed occur (see Table 4 and Fig. 4 (0,-161°) conformation), but it is definitely not the strongest contribution to the H-bond energy: thus the O(3')H(03')--O(6)C(6) branch accounts for only 11% and the O(3')H(03')--O(5) branch for only 2.5% of the total H-bond energy of the conformation. Again this can be explained by crystal packing stabilization in their case, but is definitely not valid for the free molecule. A bifurcated H bond, between different groups is also present in the cellobiose (180°,170°) and methyl-β-cellobioside (-49°, -130°) conformations (see Table 4 and Figs. 5 and 6). However, with the exception of the (56°,178°) cellobiose conformation where the O(3')H(03')--O(5) H bonds constitute 100% of the H-bond contribution, the O(3')H(03')--O(6)C(6) H bond is probably the most common big contribution to the energy for most of the "primary" conformations. This H bond constitutes 95% of the total H-bond energy in the (32°, 138°) cellobiose conformation, 73% in the (-49°,-130°), and 11% in the (0°,-161°) methyl-β-cellobioside conformation, different from all the other cellobiose-like conformations, which is also unusual in presenting the strongest H-bond contributions between the groups O(6')H(06')--O(2)C(2). This interaction is also between similar types

of groups as the O(3')H(03')--O(6)C(6) of other conformations, but on the other side of the molecule. The H-bond patterns of the two most "favored secondary" conformations are somewhat different from those observed in the "primary" conformations. Here the atoms O(3'), H(03'), O(2) are the ones participating in the stronger H-bond contributions.

The "favored" conformations obtained by total energy minima, particularly in the case of cellobiose, and their similarity to the experimental results obtained by other authors [18, 19] by x-ray diffraction, confute the results obtained by Rees and Skerrett [4] using only van der Waals interactions, as already exposed in the Introduction of this article. In the case of two glucose rings structures such as cellobiose and methyl-β-cellobioside, it appears that not only their most likely conformations for two rings systems but also their conclusion on the probable structure of the cellulose polymer were incorrect. Their considerations on pure van der Waals skeletal interaction, when taken as isolated from the other interactions, are naturally still valid.

It is important, however, to note that in both structures the glycosidic linkage does not exist in a conformation consistent with twofold screw axis symmetry when the cellulose strand is taken by itself. Furthermore, the H-bonding system for methyl-β-cellobioside and native cellulose (Cellulose I) corresponds to what is foreseen by Atalla through Raman spectra investigations. The H-bonding system for cellobiose and mercerized cellulose (Cellulose II), which has much fewer H bonds, also correspond to the experimental results obtained by Raman spectroscopy. In the energy maps presented, the straight lines indicate where bent-chain ($0°, 180°$) conformations lie. They indicate what loss of energy is sustained by the molecule to arrange itself in a bent-chain conformation. If this energy gap is made up by a gain in energy stabilization due to interchain H bonds in the crystalline network packing, a bent-chain conformation will result. If this is not the case, the transformation of the conformation to a bent-chain one is not possible. The van der Waals energy minima obtained are higher than those obtained by Rees and Skerrett because all the atoms of the side chains and their interactions have been taken into account in our investigation.

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