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THE CRYSTAL STRUCTURE OF NATIVE RAMIE CELLULOSE*

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ABSTRACT

Current computer-modeling information and rigid-residue, modeling techniques were used to analyze diffraction data from the literature for ramie cellulose. Chain models that gave the most satisfactory agreements of the observed and calculated intensity square roots were generally similar, including hydrogen-bonding schemes, to those obtained for *Valonia* cellulose by recent workers. The earlier conclusion that the ramie data could not be satisfied by a conventional cellulose model is therefore refuted, with R'' values of 0.158, 0.185, and 0.175 for the three possible packing-models, antiparallel, parallel up, and parallel down. A temperature factor of about 23 Å^2 was important in obtaining good overall fit. This value is appropriate for crystallinity that is much lower than that for *Valonia*. Small differences ($\sim$ 0.3 Å) in atomic positions from those in the early study also decreased R values. Besides slightly better R values when intensity square-roots calculated from antiparallel models were compared to the apparently high-quality observed data, a weak equatorial spot is best explained by antiparallel chains. Therefore, antiparallel chain-packing should continue to receive consideration, at least for cotton and ramie celluloses.

INTRODUCTION

Following a study in 1960, Jones concluded that no conventional model of cellulose I would provide a satisfactory fit of the calculated and observed diffraction-intensity data from ramie[1], a bast fiber composed of highly oriented and moderately crystalline cellulose. Since then, however, two simple proposed structures have been based on X-ray diffraction data from *Valonia ventricosa*, an algal cellulose having exceptionally high crystallinity[2,3]. In those reports, it was proposed that all structures of native cellulose are identical except for the extents of crystallinity. Those recent studies suggest, therefore, that satisfactory agreement of the diffraction intensities for ramie would be obtained if the cellulose model could be modified to compensate for the lower crystallinity of ramie. Besides learning whether lower crystallinity was the only important difference between ramie and *Valonia*, it was desired to gain

*Dedicated to my father on the occasion of his 60th birthday.

additional clarification of two points on which the recent proposed crystal-structures are in apparent disagreement with results from chemical treatments of cellulose.

Both proposed cellulose structures feature parallel-chain arrangements, with antiparallel arrangements ruled out by poorer fit of observed and calculated intensities[2,3]. This conclusion appears to rest on the diffraction data, because the choice of monomer geometry for the cellulose-chain model does not affect that conclusion[4]. However, from studies that furnished similar but stronger evidence for rayon (Fortisan)[5,6] and mercerized cotton[7], cellulose II is understood to have antiparallel chains. Some of those who have observed the mercerizing process that converts native cellulose I (cotton or ramie, but not *Valonia*) into cellulose II have been unable to envisage a practical scheme whereby the cellulose chains can accomplish a change from parallel to antiparallel chains while remaining in the solid state. One mechanism proposed for this conversion was epitaxial growth of folded chains around a cellulose I core. However, it is now thought that the epitaxial crystals will not form unless their molecules have chain lengths short enough to preclude chain-folding[8]. The other point of discrepancy concerns studies of availability and reactivity of hydroxyl groups[9]. Results from those studies seemed readily interpretable in terms of the older, antiparallel proposals that had O-6 in a *gt* disposition instead of the *tg* disposition of the newer proposals.

In addition to doubts based on experience with mercerization and chemical reactions, a third data-set, electron-diffraction intensities from *Valonia*, did not permit resolution of the question of chain polarity[10]. Further, a study of ramie cellulose III yielded identical R values for parallel and antiparallel chains[11]. Infrared and diffraction evidence suggest that the crystal structures of ramie and *Valonia* may differ. Infrared spectra categorize[12] *Valonia* and bacterial celluloses as I_A and cotton and ramie as I_B. Also, the diffraction spots that indicate a larger, 8-chain unit cell appear on both X-ray[2,13] and electron[14] diffraction patterns of I_A celluloses. However, these spots are not always found on electron-diffraction patterns of ramie and cotton[15,16], even though the patterns are of a quality comparable to X-ray diagrams from *Valonia*. If the two native forms, I_A and I_B, are different, it would not be without precedent. A similar natural polymer, chitin, apparently has parallel chains in the less-common, native β polymorph[17], whereas the widespread α-chitin has antiparallel chains[18]. In hopes of adding to knowledge on these points, it was decided to reexamine, with computing techniques and modeling information not available to Jones, the data carefully collected by his colleagues, Mann, Gonzalez, and Wellard[19].

DIFFRACTION DATA

Unit-cell dimensions and their standard deviations were those determined for a two-chain unit cell by Wellard[20], namely $a = 8.171$ (0.032) Å, $b = 7.846$ (0.019) Å, $c = 10.34$ (0.020) Å (fiber axis), and $\gamma = 96.38$ (0.13)°. These constants are similar to those for two-chain *Valonia* cells; ref. 3 reports values of 8.17, 7.86, and 10.38 Å, and 97.0°. Following some preliminary calculations, it was found necessary to modify

the literature data-set[19] in order to solve the structure. Although the data had been collected from five films with scanning densitometry, similar to current practice, many of the spots were reported to be composed of as many as seven reflections having widely varying *d*-spacings. The assignment of so many reflections to each spot decreased sensitivity of the diffraction error indicators to changes in the unit-cell model during the preliminary stage of analysis. Therefore, several of the reflections most distant from the observed peaks listed were omitted for 10 of the spots. After models that fit the remaining, trimmed data were developed, all reflections except 006 and 008 were used for final determinations of structure. The sixth- and eight-layer meridional reflections (006 and 008) were omitted because, due to their small *d*-spacings (1.72 and 1.29 Å), they are high-resolution reflections and are thus over-sensitive to small inacuracies in the model. Also, they are likely to be overlapped by other reflections and they are beyond the range considered by other workers, including Jones[1]. The 002 and 004 observed intensities were included after they had been divided by 2 for a crystallographic-multiplicity correction not mentioned by Mann *et al.*[19]. Jones[1] apparently doubled the calculated intensities instead. (Recent workers have not included meridional reflections because values of these intensities are not available from ordinary flat-plate X-ray photographs. A precession camera technique is available[21], however, in addition to the tilted-fiber and powder method used by Mann *et al.*).

The corrected intensity square-roots and the reflections assumed to contribute thereto are given in Table I, followed by groups of unobserved reflections having intensity square-roots for each group corresponding to the maximum possible value (or threshold of detection). Those groups were not trimmed at any time.

PROCESSING THE DIFFRACTION DATA

A local FORTRAN IV program, written for the CDC 1700* computer by Vincent Murphy, was used to construct models of cellulose unit-cells and calculate intensity square-roots and the resultant residual error. The modeling section uses a virtual-bond method, discussed in earlier papers[4,22]. Variables in the model of unit-cell structure are interchangeable monomeric geometry, ROT1 and ROT2 (rotation of corner and center chains about their axes), θ (rotation of the D-glucose residues about their virtual (O-4–O-1) bonds), SHIFT (translations along the chain axis of the center chain relative to the corner chain), and χ (rotational position of the primary alcohol group). In addition, flags are used for initial chain-direction and packing mode, resulting in the designations for the three possible packing models: parallel up, parallel down, and antiparallel[3]. Additional variability is allowed for θ and χ, permitting deviations from $P2_1$ symmetry and non-equivalence of the corner and center

*Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

TABLE I

X-RAY DATA FOR RAMIE CELLULOSE[a]

h	k	l	*Spot number*	$\sin^2\theta/\lambda^2$	d_{hkl}(Å)	I_{obs}‡	h	k	l	*Spot number*	$\sin^2\theta/\lambda^2$	d_{hkl}(Å)	I_{obs}‡
−1	1	0	1	.0070	5.97	59.0	−1	1	3	17	.0280	2.98	16.1
1	1	0	2	.0087	5.34	59.0	1	1	3	17	.0298	2.90	
2	0	0	3	.0151	4.06	158.0	−2	0	3	18	.0362	2.63	27.4
0	2	0	3	.0164	3.90		0	2	3	18	.0374	2.58	
−2	1	0	3	.0175	3.78		−2	1	3	19	.0385	2.55	11.4
−1	2	0	3	.0184	3.68		−1	2	3	19	.0395	2.51	
2	1	0	3	.0210	3.45		2	1	3	19	.0420	2.44	
1	2	0	3	.0219	3.37		1	2	3	19	.0430	2.41	
−2	2	0	4	.0281	2.98	9.5	−2	2	3	19	.0491	2.26	
3	0	0	5	.0341	2.71	12.3	−3	0	3	20	.0551	2.13	19.5
2	2	0	5	.0351	2.67		2	2	3	20	.0561	2.11	
−3	1	0	5	.0356	2.65		−3	1	3	20	.0566	2.10	
0	3	0	5	.0370	2.60		0	3	3	20	.0580	2.08	
−1	3	0	5	.0381	2.56		−1	3	3	21	.0592	2.05	13.8
3	2	0	6	.0558	2.12	7.1	3	1	3	21	.0619	2.01	
2	3	0	6	.0574	2.09		1	3	3	21	.0644	1.97	
4	0	0	7	.0606	2.03	26.9	0	0	4	22	.0374	2.58	33.6
−4	1	0	7	.0612	2.02		−1	1	4	23	.0444	2.37	8.6
−3	3	0	7	.0632	1.99		1	1	4	23	.0461	2.33	
0	4	0	7	.0657	1.95		−2	0	4	24	.0525	2.18	19.0
−1	4	0	7	.0660	1.95		0	2	4	24	.0538	2.15	
4	1	0	7	.0682	1.91		−2	1	4	24	.0549	2.13	
−4	2	0	7	.0700	1.89		−1	2	4	24	.0558	2.11	
−1	0	1	8	.0061	6.39	6.3	2	1	4	24	.0584	2.07	
0	1	1	8	.0064	6.23		1	2	4	24	.0594	2.05	
−1	1	1	9	.0093	5.17	3.1	3	1	0	25	.0408	2.47	(7.0)
1	1	1	9	.0111	4.74		1	3	0	25	.0434	2.40	
−3	0	1	10	.0364	2.62	23.2	−3	2	0	25	.0453	2.35	
2	2	1	10	.0374	2.58		−2	3	0	25	.0469	2.31	
−3	1	1	10	.0379	2.57		−2	0	1	26	.0175	3.78	(10.0)
0	3	1	10	.0393	2.52		0	2	1	26	.0187	3.65	
−1	3	1	10	.0405	2.48		−2	1	1	26	.0198	3.55	
3	1	1	11	.0432	2.41	16.7	−1	2	1	26	.0208	3.47	
1	3	1	11	.0457	2.34		2	1	1	27	.0233	3.27	(10.0)
−3	2	1	11	.0476	2.29		1	2	1	27	.0243	3.21	
−2	3	1	11	.0492	2.25		−2	2	1	28	.0304	2.87	(10.0)
0	0	2	12	.0093	5.17	7.9	3	2	1	29	.0581	2.07	(14.0)
−1	0	2	13	.0131	4.36	31.3	2	3	1	29	.0597	2.05	
0	1	2	13	.0134	4.31		−4	0	1	29	.0630	1.99	
−2	0	2	14	.0245	3.19	13.8	−4	1	1	29	.0636	1.98	
0	2	2	14	.0258	3.11		−3	3	1	29	.0655	1.95	
−2	1	2	14	.0268	3.05		0	4	1	29	.0681	1.92	
−1	2	2	14	.0278	3.00		−1	4	1	29	.0684	1.91	
2	1	2	15	.0303	2.87	13.4	−1	1	2	30	.0163	3.91	(10.0)
1	2	2	15	.0313	2.82		1	1	2	30	.0181	3.71	
−1	0	3	16	.0248	3.17	12.3	−2	2	2	31	.0374	2.58	(7.0)
0	1	3	16	.0251	3.15								

TABLE I *(continued)*

h	k	l	*Spot number*	$\sin^2\theta/\lambda^2$	d_{hkl}(Å)	$I_{obs}^{\frac{1}{2}}$	h	k	l	*Spot number*	$\sin^2\theta/\lambda^2$	d_{hkl}(Å)	$I_{obs}^{\frac{1}{2}}$
−3	0	2	32	.0434	2.40	(10.0)	3	2	2	34	.0651	1.96	(10.0)
2	2	2	32	.0444	2.37		2	3	2	34	.0667	1.93	
−3	1	2	32	.0449	2.36		−3	2	3	35	.0663	1.94	(10.0)
0	3	2	32	.0463	2.32		−2	3	3	35	.0679	1.92	
−1	3	2	32	.0475	2.29		−1	0	4	36	.0412	2.46	(7.0)
3	1	2	33	.0502	2.23	(10.0)	0	1	4	36	.0415	2.45	
1	3	2	33	.0527	2.18		−2	2	4	37	.0655	1.95	(10.0)
−3	2	2	33	.0546	2.14								
−2	3	2	33	.0562	2.11								

[a]Data computed from those given in ref. 19.

chains. However, this extra freedom was not found useful during the present study.

Instead of a minimization technique, this program examines variable space according to input ranges and increment-sizes of the foregoing continuous variables. Owing to the time-consuming nature of exhaustive-search, diffraction-intensity calculations, locations in variable space for fine-grid searches of minimal diffraction error were based to some extent on extensive experience with a variety of two-fold screw models in a similar study of *Valonia* cellulose[4].

Four different indicators of diffraction error were calculated. R_{obs} had the usual definition,

$$R_{obs} = \frac{\sum |I_{calc}^{\frac{1}{2}} - I_{obs}^{\frac{1}{2}}|}{\sum I_{obs}^{\frac{1}{2}}}.$$

The separate contribution to error of those unobserved reflections that had calculated intensities greater than the threshold of detection was assessed in a manner similar to Gardner and Blackwell's treatment of unobserved data[3],

$$R_{unobs} = \frac{\sum (I_{calc}^{\frac{1}{2}} - 0.67\, I_{thresh}^{\frac{1}{2}})}{\sum I_{obs}^{\frac{1}{2}} + \sum (0.67\, I_{thresh}^{\frac{1}{2}})}.$$

The total error of the determination was defined as

$$R_{tot} = R_{obs} + R_{unobs}.$$

The object of search in variable space was the minimum value of R'',

$$R'' = \left\{ \frac{\sum (I_{obs}^{\frac{1}{2}} - I_{calc}^{\frac{1}{2}})^2}{\sum I_{obs}} \right\}^{\frac{1}{2}},$$

where $I_{obs}^{\frac{1}{2}}$ for unobserved reflections is set equal to $0.67\, I_{thresh}^{\frac{1}{2}}$, if I_{calc} is greater than I_{thresh}. None of these R values incorporated any weighting scheme.

Before computing R values, the $I_{calc}^{\frac{1}{2}}$ values were corrected for isotropic thermal vibration and random lattice-disorder[23] by factor B and scaled by factor S. At each

grid-point in variable space, B and S were evaluated by using a regression analysis that found a minimum in U where

$$U = \sum_{i=1}^{N_{obs}} w_i \left(\ln \frac{(I_{obs})_i^{\frac{1}{2}}}{(I_{calc})_i^{\frac{1}{2}}} - S + B \sin^2\theta_i/\lambda^2 \right)^2.$$

Weights (w_i) used were the observed structure-factors. Although this technique does not, in general, yield values of B and S that would result in minimal values of R'', it had a substantial effect on the fit of the diffraction intensities. Before incorporating this algorithm, R'' had been reduced to 0.37, with an assumed temperature-factor of 4.0 and a resulting least-squares scale-factor of 0.68. With an unweighted regression otherwise similar to the one finally adopted to determine B and S, R'' for the same coordinates was 0.33 with $B = 8.5$ and $S = 0.62$. Addition of the weights dropped R'' to 0.246 and raised B to 18.5 and S to 0.94. For this particular data set, S is of interest because Mann *et al.* determined absolute intensities and S should therefore be about 1.0.

Initial calculations with the trimmed data used the coordinates of the non-reducing cellobiose residue[24] with θ at 68°, a combination found to be best suited to the data of Gardner and Blackwell[3] for *Valonia*[4]. The resulting minimum R'' value was, for a parallel-up model, 0.246. At $\theta = 68°$, the 0.04 Å shorter observed fiber-repeat spacing for ramie resulted in a glycosidic angle (τ) of 114.4° instead of 115.7° for *Valonia*. The value of θ was therefore decreased in 2° increments to increase τ to more-usual values. At each drop, the minimum R'' value decreased until use of this monomer was discontinued at $\theta = 62°$ because the concurrently changing O-3–O-5′ distance was rapidly approaching the minimally acceptable value of 2.6 Å. At that time, the residue geometry of Sarko and Muggli[2] was selected and refinement continued. Again, the data for ramie required a lower value of θ than did the data for *Valonia*. With the full data-set, the minimum in R'' was found when θ equaled 37°, instead of at 55° for the *Valonia* data. At 37°, the O-3–O-5′ distance is 2.58 Å and τ equaled 121.2°, and so θ was set equal to 39°, where the values are 2.59 Å and 120.5°, respectively. These changes in θ also result in changes in the linkage-conformation angles ϕ and ψ. At 55°, $\phi = 24.2°$ and $\psi = -27.3$, and at 39°, $\phi = 32.5°$ and $\psi = -36.3°$ (the conventions are the same as in ref. 3 and 4). The effect of the substitution of residue geometries and change of θ on diffraction error may be seen in the resulting minimum in R'' (trimmed data) for a parallel-up model, 0.164, a 33% reduction. Other than the rather short O-3–O-5′ distance and slightly high glycosidic angle[4], there are no intramolecular stereochemical defects at $\theta = 39°$.

At the end of the study of the trimmed data, a fruitless attempt was made to include the 006 and 008 reflections to help distinguish among the three packing modes. The R values increased and the temperature factor dramatically decreased, indicating that the reported observed values of 15.8 are too high for both $I^{\frac{1}{2}}_{006}$ and $I^{\frac{1}{2}}_{008}$. This could arise from overlapping reflections not included in calculation of the intensity, or be the result of a small absolute error in measurement, multiplied by large geometric and polarization factors. Upon reincorporation of the other omitted data, the con-

clusions obtained with the trimmed data-set were generally confirmed. Contrary to those earlier results, the parallel-chain models had equal values of ROT1 and ROT2 instead of differing from the final values by + and −3°. Values of SHIFT and χ changed slightly for all models (~0.01 and 2°, respectively). The R values changed slightly, providing some discrimination among the optimal models with the three packing types, and the temperature factors and scale factors increased slightly.

RESULTS AND DISCUSSION

Values of the chain rotation and translation parameters, O-6 position, and notable interoxygen distances for the antiparallel, parallel up, and parallel down models (in Gardner and Blackwell's notation, a_1, p_1, and $p_{\bar{2}}$) are presented in Table II. Also in Table II are the refined temperature-factors for all models (~23 Å^2) and the scale factors (all about 0.97), with resulting R values for each model. Jones wrote, "One might have expected that the limited X-ray data available for the celluloses would have been consistent with several potential crystal structures for each modification"[1]. Repeating his work with newer techniques and modeling information has confirmed that expectation for cellulose I. The R'' values of 0.158 (antiparallel), 0.185 (parallel up), and 0.175 (parallel down) are among the lowest values reported for polysaccharides, and may be compared to values of 0.215 given in ref. 3 and computed from the data in ref. 2 for the Meyer–Misch subcell. By current standards, then, a satisfactory match of observed and calculated intensities has been obtained for the ramie data with a conventional cellulose model.

Jones concluded that no conventional structure of cellulose I would suffice because calculated upper-level intensities, especially those on the first layer-line, were much stronger than the weak ones actually observed. As shown in Table III, the first-level intensity square-roots still show the poorest fit (spot numbers 8–11) but the calculated discrepancy for all three models composed of Sarko–Muggli residues is substantially lower than that obtained by Jones. This reduction is primarily due to improvements in the chain's modeling parameters. In fact, as the chain geometry was progressively changed (see next-to-last paragraph in Processing Diffraction Data), the major improvement was in the fit of the unobserved data (spots 25–37) and the first-level data. Table IV presents coordinates for the two chains of the ramie antiparallel models; the differences in atomic positions from those of Jones[25] average about 0.3 Å.

The high temperature-factor of about 23 Å^2, although important to the overall fit, actually worsens the fit of the first-level data. This value of B is similar to that reported by Kolpak and Blackwell for cellulose II (Fortisan)[5] and lower than the 32 Å^2 reported by the same research group for mercerized cotton[7]. These numbers are indications of lower crystallinity[23] than that of *Valonia* which has reported B values of 6.5 (ref. 2) and 2.5 (ref. 3). Effects of deficiencies in the rigid model that does not include hydrogen atoms are probably also included in B. As R'' was minimal when O-3 was closer to O-5′, and τ larger than might be thought most likely[4], the diffraction

TABLE II

COMPARISON AND DESCRIPTION OF CELLULOSE MODELS

Parameter	*Antiparallel* (a_1)	*Parallel up* (p_1)	*Parallel down* (p_2)
R_{obs}	0.177	0.191	0.200
R_{unobs}	0.051	0.064	0.046
R_{tot}	0.229	0.255	0.246
R''	0.158	0.185	0.175
ROT1[a]	61°	63°	63°
ROT2[a]	61°	63°	63°
χ[b]	−63°	−63°	−63°
SHIFT[c]	0.360	0.275	0.280
B	23.91	21.95	23.52
S	0.98	0.94	0.99
Interoxygen distances[d]			
O-6–*O*-3″	2.97 Å	2.92 Å	2.92 Å
	All models		
Residue geometry	Sarko–Muggli[e]		
τ	120.5°		
θ_f	39.0°		
ϕ(H-1–C-1–*O*–C-4′)[f]	32.5°		
ψ(C-1–*O*–C-4′–H-4′)[f]	−36.3°		
O-3–*O*-5′[d]	2.59 Å		
O-6–*O*-4	2.70 Å*		
O-6–*O*-2′	2.90 Å		

[a]As indicated in Fig. 1, the rotations are from a starting position where the plane passing through the linkage oxygen-atoms coincides with a plane perpendicular to the *a* axis of the unit cell. [b]The convention is the same as used in ref. 4 and opposite in sign to that used in ref. 3. Pure *gt* is 180°, pure *tg* is −60°, and pure *gg* is 60°. [c]The values are in fractions of the fiber repeat-distance. [d]Unprimed oxygen atoms are in the same monomer, singly primed ones are in the same molecule, and doubly primed atoms are on a different molecule. Those distances identified by asterisks are not considered to represent hydrogen bonds because O-4 is thought to be a poor hydrogen-bond acceptor. [e]Geometry used in ref. 2. [f]These conventions were used in ref. 4.

error could probably be decreased with a modeling technique that incorporates a flexible monomer. However, there are only 24 observed data in this set and the primary modeling variables number 8, and so it is questionable whether results based on a substantially increased number of variables would be more than cosmetic.

Each of the three final models has the same basic chain conformation; R'' dropped simultaneously for all three models as θ decreased. The hydrogen-bonding schemes for all are contained within the 020 planes as earlier discussed[3]. The rather long O-6–O-3″ (interchain) distances in Table II are probably artifacts due to rigid-residue modeling, rather than any particular characteristic, such as bifurcation of those proposed hydrogen bonds.

Instead of firmly concluding that the crystal structure of ramie cellulose is composed of antiparallel chains, I wish only to state that the current proposals for

TABLE III

COMPARISON OF OBSERVED AND CALCULATED INTENSITY SQUARE-ROOTS FOR DIFFERENT MODELS[a]

Spot number	$I_{obs}^{\frac{1}{2}}$	$I_{a_1}^{\frac{1}{2}b}$	$I_{p_1}^{\frac{1}{2}b}$	$I_{p_2}^{\frac{1}{2}b}$	$I_{a_1}^{\frac{1}{2}c}$
1	59.0	62.0	59.8	62.6	83.5
2	59.0	57.1	55.9	57.5	70.0
3	158.0	150.3	147.6	151.7	196.0
4	9.5	4.2	10.7	1.3	19.5
5	12.3	16.6	17.5	19.6	35.6
6	7.1	4.3	0.0	0.0	11.0
7	26.9	26.6	28.0	28.4	69.0
8	6.3	14.4	12.4	14.5	34.2
9	3.1	13.6	12.7	13.8	27.8
10	23.2	32.7	33.0	30.7	85.0
11	16.7	20.1	20.6	24.3	56.8
12	7.9	3.5	4.4	5.5	7.1
13	31.3	27.9	28.5	29.4	32.0
14	13.8	20.9	25.0	20.1	39.0
15	13.4	18.2	17.5	23.0	31.4
16	12.3	10.2	8.5	8.2	29.0
17	16.1	16.0	16.3	17.9	23.9
18	27.4	28.7	25.2	26.3	22.4
19	11.4	23.5	28.6	25.6	92.4
20	19.5	19.8	20.1	19.2	58.2
21	13.8	9.7	10.3	14.5	43.3
22	33.6	26.0	25.6	24.9	33.2
23	8.6	7.0	8.1	8.4	23.4
24	19.0	21.2	19.2	19.8	65.1
25	(7.0)	12.3	12.5	12.8	35.1
26	(10.0)	15.5	19.3	15.4	35.6
27	(10.0)	13.9	11.7	15.1	18.8
28	(10.0)	9.2	1.1	9.6	15.2
29	(14.0)	13.3	16.4	13.0	51.5
30	(10.0)	12.7	3.8	4.8	26.8
31	(7.0)	4.7	1.7	1.7	10.5
32	(10.0)	10.1	8.5	8.1	45.8
33	(10.0)	6.6	6.8	6.3	44.8
34	(10.0)	3.9	4.5	4.5	31.4
35	(10.0)	8.6	9.1	5.2	33.6
36	(7.0)	4.8	7.4	8.8	3.1
37	(10.0)	6.2	13.2	3.6	—

[a]Observed intensity square-roots ($I_{obs}^{\frac{1}{2}}$) are taken from groups of indexed reflections in Table I. Numbers in parentheses are maximum possible intensity square-roots for unobserved reflections. [b]The models a_1, p_1, and p_2 are further described in Table II. [c]These values were taken from ref. 1. Intensities of spots 12 and 22 were halved because of a multiplicity correction. The coordinates that produced these values are in ref. 24; the temperature factor is apparently 1.5 Å^2.

parallel cellulose chains were not confirmed by the ramie data. In favor of the parallel chains, the *trimmed* data-set did not support a conclusion based on R values and also produced unequal rotations of the chains for the parallel models, resulting in a possible explanation for spot 6 on the equator. This weak spot, also observed by Sarko and

TABLE IV

ATOMIC COORDINATES FOR BEST ANTIPARALLEL CELLULOSE MODEL (Å)

Atom	*Corner chain*[a]			*Center chain*[b]		
	x	y	z	x	y	z
C-1	−.440	−.128	3.929	3.210	4.027	2.689
C-2	−1.451	.342	2.915	2.199	3.557	3.703
C-3	−1.071	−.136	1.530	2.578	4.035	5.088
C-4	.326	.255	1.193	3.975	3.643	5.425
C-5	1.306	−.187	2.308	4.956	4.086	4.310
C-6	2.712	.316	2.188	6.361	3.583	4.500
O-1	−.737	.409	5.170	2.912	3.490	1.448
O-2	−2.726	−.125	3.262	.924	4.023	3.356
O-3	−1.986	.400	.583	1.664	3.499	6.035
O-4	.737	−.409	.000	4.387	4.307	6.618
O-5	.847	.349	3.553	4.497	3.550	3.065
O-6	3.296	−.187	.925	6.945	4.086	5.693

[a]The second residue in the corner chain is generated by the coordinates $-x$, $-y$, and $z + 5.171$.
[b]The second residue in the center chain is generated by $7.300 - x$, $7.798 - y$, and $z - 5.171$.

Muggli[2] but not by Gardner and Blackwell[3], requires some difference in the two chains other than pure translation along the z axis. Further, a p_1 model composed of a chain with a geometry best suited to *Valonia*[4] was tested against the trimmed data and a minimum in R'' was found when ROT1, ROT2, and SHIFT equaled those from the best model of the earlier study[4] and χ differed by only 5°. However, R'' for that model was substantially higher (0.246) than R'' for the three final models composed of Sarko–Muggli residues.

The antiparallel model does provide the best accounting for the ramie data on the basis of three of the four R values, a consideration that would normally enhance a small difference in any given R. The antiparallel model also provides the most satisfactory explanation of spot 6 on the equator. The explanation of the nonequivalence of parallel chains would be that they have unequal rotations, resulting in unequal hydrogen bonds, as reported by Sarko and Muggli[2]. Unequal intrasheet O-6–O-3″ hydrogen-bonds would seem to be an unnecessary complication. The antiparallel chains, on the other hand, can have equal rotation and still produce measurable intensity for spot 6. It may also be noted that the a_1 model is similar to the antiparallel model that best fits the diffraction data for *Valonia* (but was rejected at a high confidence level by Gardner and Blackwell[3]).

Because of these observations and a need for the p_1 and p_2 models to allow a parallel–antiparallel packing-mode transformation during the mercerization treatment, it appears that antiparallel chains must still be given serious consideration, at least for I_B celluloses. Fig. 1 contains such a packing model.

As analysis of the data collected by Mann *et al.* failed to confirm the recent findings of parallel cellulose chains, all three data sets[2,3,19] were compared. After

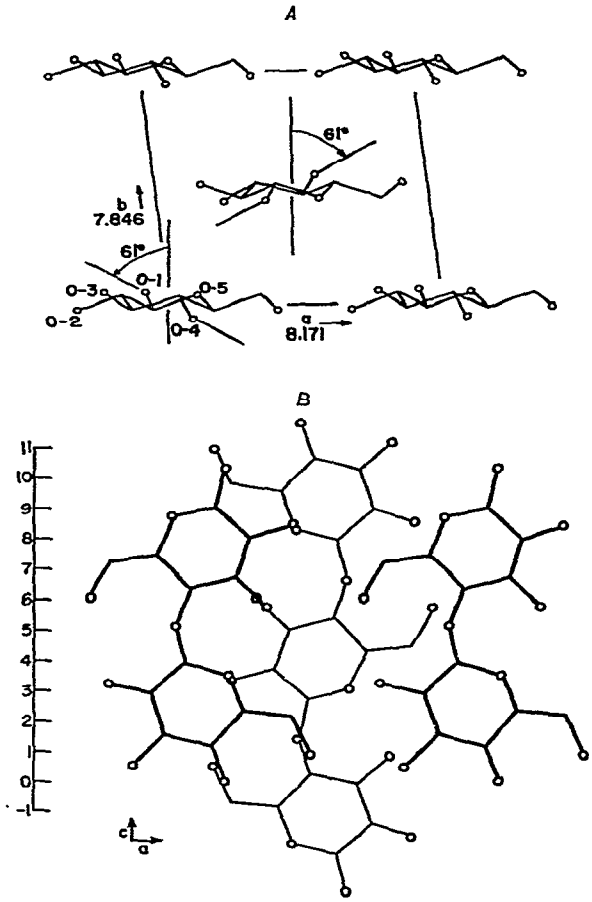

Fig. 1. Antiparallel cellulose model. *A*. Projection of atoms onto equatorial plane, showing labeling of oxygen atoms and unit-cell edges. The chain-rotation is also indicated. *B*. Projection of atoms onto fiber axis (*a*–*c* plane). This drawing appears similar to Gardner and Blackwell's model a_2 for *Valonia* but the center chain is shifted in the direction opposite to the model a_2 for Fortisan . *R* Values calculated using the *Valonia* data[3] suggest that if *Valonia* has antiparallel chains, the shift would be essentially the same as depicted here.

all were trimmed to eliminate noncomparable reflections, they were scaled by linear regression that embodied both determination of the slope (scale factor) and intercept (background). The $I^{\frac{1}{2}}$ values of Mann *et al.* agreed with those from ref. 2 to R_{obs} = 0.21 and with those of ref. 3 to R_{obs} of 0.27. A comparison of the two *Valonia* sets[2,3] yielded R_{obs} = 0.31. A similar comparison of data for Fortisan cellulose II produced R_{obs} values of 0.17 (refs. 5 and 19), 0.24 (refs. 6 and 19) and 0.49 (refs. 5 and 6). For both polymorphs, the data obtained by Mann *et al.* agreed better with those of current workers than those collected by current workers agreed. This observation, and the low values of *R* obtained herein, suggest that the early data were not demonstrably inferior. Despite the lower number of observed data for ramie (24 spots), it is actually

in a favorable position compared to I_A cellulose having roughly 35 X-ray spots. The I_A celluloses have eight-chain unit cells and therefore require either restrictive assumptions or four times as much data.

The continued finding of the *tg* disposition of O-6 in cellulose I lends support to the conclusion that this prevalent polysaccharide has an otherwise rare conformational feature. The *tg* conformation is not found in single crystals of model compounds (with one disordered exception) and its resulting "peri" interaction is theoretically not favored. However, newer studies have shown that a narrow range of *tg* positions is possible[26]. Another reason for doubt was that X-ray data for cellulose used previously lacked strong preference for the *tg* position over *gt*. Strong evidence against the other staggered position, *gg*, is readily found, but the *gt* position causes a special problem. Placing the O-6 atom in the *gt* position results in x and y (but not z) coordinates very similar to those for the *tg* position (see Table V). Structure factors of *hkO* reflections do not depend on z coordinates and therefore the calculated equatorial intensities of the *gt* and *tg* models are similar. Because the equatorial and normally unused meridional spots contain 85% of the observed intensity, differences in the indicated error are not large for refined models with O-6 in the two different positions. Even including the meridionals, which depend only on z coordinates, R_{obs} for the final a_1 model with O-6 *tg* was 0.177 *vs.* 0.208 for an optimized model with O-6 *gt*. For the *gt* model, however, $I^{\pm}_{002} = 30.9$ and $I^{\pm}_{004} = 21.4$, a strong disagreement with the observed data (spots 12 and 22, Table III). The *tg* models all provide reasonable agreement. Interestingly, the diffraction error for the first layer-line dramatically decreased for the *gt* model and all other layers were worse.

Equally strong evidence on the O-6 position is furnished by stereochemistry[3]. Calculations in conjunction with the present work show that the distance between O-6 *gt* and O-2″ is about 1.9 Å. Such a severe conflict is not likely to be resolved unless O-6 is moved from a *gt* position.

TABLE V

COORDINATES OF *O*-6 IN *gt* AND *tg* POSITIONS (Å)

	x	y	z
O-6_{gt}	3.520	−0.052	3.259
O-6_{tg}	3.296	−0.187	0.925

CONCLUSIONS

The O-6 position and hydrogen-bonding scheme result from the analysis of all three X-ray data-sets for native cellulose and from interpretation of interoxygen distances of 2.6 to 3.0 Å as hydrogen bonds. (Earlier studies[2,3] also examined the C-O–O angles for this arrangement.) This leaves unresolved the contradiction in O-6

position indicated by chemical methods[9] and the X-ray data. A possible explanation was offered by Kolpak *et al.*[7]. In some instances, the O-6 positions on the surfaces of cellulose crystallites may differ from those in the interior of the crystallites.

The large temperature-factor for ramie seems reasonable, contributing the expected modification to the cellulose model to compensate for the lower crystallinity of ramie. Whether there is a difference in packing mode associated with the differences in infrared spectra and diffraction patterns of I_A and I_B celluloses remains, to this writer, a mystery. It seems clear to me that the data will have to be more accurately collected and corrected before such decisions can be made with confidence. Further work to provide comparable treatment for observed and unobserved reflections would also be helpful.

The significance of the differences in chain conformational variables, observed with both the trimmed and full data-sets, is also unclear. The results suggest that either the chain conformations are different in I_A and I_B celluloses or that, because of current inadequacies in data acquisition, refinement of individual parameters for ring and pendant oxygen atoms should be regarded with some uncertainty.

It is clear that Jones underestimated the effect of small movements of atoms on the calculated diffraction-intensities. Also, the low glycosidic angle of his models (112° and less) gave them an important handicap in fitting these data, as did his low ($B = 1.5$ Å^2) temperature factor. The determination of absolute intensities by Mann *et al.* was helpful, but the grouping of reflections with such large differences in *d*-spacing seems undesirable in comparison with current work. Finally, the present work shows that it is feasible to include meridional intensities in a fiber-diffraction analysis. They not only add substantially to a small data-set, but can help to provide unique information such as that on the position of O-6.

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