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CONFORMATION EFFECTS IN THE HYDROLYSIS OF CELLULOSE

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CONFORMATIONAL EFFECTS IN THE HYDROLYSIS OF CELLULOSE

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ABSTRACT

It is proposed that the different conformations of the glycosidic linkages, which are indicated by the Raman spectra of the various polymorphic forms of cellulose, result in differences in their degree of susceptibility to hydrolysis in acid or enzyme media. The basis for interpretation of structural data on cellulose has been reexamined and the view that anhydrocellobiose is the basic repeat unit, rather than anhydroglucose, has been developed. Crystallographic studies of cellobiose and β-methyl cellobioside suggest that both right and left handed departures from twofold helix conformations can be more stable than structures possessing a twofold screw axis of symmetry. Raman spectral studies further indicate that the glycosidic linkage conformation in β-methylcellobioside is representative of the form dominant in cellulose I, while that in cellobiose is representative of cellulose II. The conformation of the anhydrocellobiose unit represented by β-methylcellobioside and, in particular, participation of the primary hydroxyl group in a bifurcated intramolecular hydrogen bond, provide a basis for explaining the high resistance of native crystalline celluloses to hydrolytic agents.

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INTRODUCTION

Discussions of the hydrolysis of cellulose, in both acid media and enzyme preparations, have considered the influence of structure on the rates of hydrolytic degradation (1-6). In most instances the primary focus was on the variation in accessibility of the glycosidic linkage among the different types of cellulose. The differences between the responses of the various crystalline polymorphs were considered, but it was noted that the effect of crystalline polymorphic variation could not be separated from the effect of accessibility (4). In any event, the influence of polymorphic variation was assessed primarily in light of the prevailing notions concerning the structure of the different polymorphs, that is, in terms of cellulosic chains possessing twofold screw axis symmetry but packed differently in the crystalline lattice. This view of the structure of the different crystalline polymorphs has been based, in the main, on x-ray and electron diffractometric studies, with some elaboration based on infrared spectroscopic studies.

Recent studies of the Raman spectra of celluloses I, II, and IV have indicated that the different polymorphic forms involve two basically different molecular conformations, in addition to the differences in crystalline packing (7, 8, 9). The conformation variations suggested by the Raman spectra are such that they could play an important role in determining the susceptibility of glycosidic linkages to attack by hydrolytic agents. The questions raised by this possibility will be addressed in the present report.

A fundamental conceptual question, which will be central in the following discussion, is whether the structure of cellulose should be interpreted on the basis that the anhydroglucose unit is the repeating unit in the structure, or whether it is more appropriate to adopt the view that the anhydrocellobiose unit is the basic repeat unit. It will be held that the latter review can provide a more consistent interpretation of structural information. It has been recognized for some time in studies of the chemistry of cellulose that the repeat unit must be taken as the anhydrocellobiose unit. Development of the view that it should be regarded as the basic repeat unit in structural studies, as well, is rooted in the realization that two anhydroglucose units are necessary to define the nature of the linkage between them, and that the steric constraints associated with the β-1,4-linkage are as important in determining the structure of cellulose as they are in determining its chemical reactivity. Within the framework set forth, the conformational variations possible for the β-1,4-linkage in cellulose will be considered, and the potential influence of such variations on the response to hydrolytic attack will be examined.

In order to place the discussion in perspective, the next section will be devoted to a brief review of current ideas concerning the structure of cellulose, as well as to consideration of the implications of two crystallographic studies of cellobiose and β-methylcellobioside. The response of different celluloses to acid and enzymatic hydrolysis will then be examined in light of the structural considerations.

STRUCTURES OF CELLULOSE

The primary sources of information concerning the molecular structure of cellulose have been x-ray and electron diffractometric studies, conformational analyses, and vibrational spectroscopy. The work up to 1971 was very ably reviewed by Jones (10), and by Tonessen and Ellefsen (11,12). They generally concluded that although much evidence can be interpreted in terms of cellulose chains possessing a twofold axis of symmetry, in both celluloses I and II, none of the structures proposed for cellulose until that time could reconcile all of the known data derived from structural studies, primarily x-ray and electron diffractometric. Reese and Skerrett carried out a conformational energy analysis on cellobiose and xylobiose (13), focusing primarily on the contribution of nonbonded interactions while excluding the influence of hydrogen bonding. They found that, for the isolated molecules, potential minima occurred on either side of the twofold screw axis conformations. But the magnitudes of the differences in energy between the minima and conformations corresponding to twofold screw axis symmetry, were small enough for them to conclude that hydrogen bonding energy in the lattice might compensate enough to stabilize a chain structure with twofold screw axis symmetry. Until recently the more comprehensive studies in vibrational spectroscopy were those of Liang and Marchessault utilizing measurements of infrared dichroism on oriented samples of ramie (14). On the basis of these studies they proposed a unit cell, involving a particular pattern of hydrogen bonding between the chains, which has received popular acceptance as a representative structure of cellulose. Some remaining inconsistencies between the various structures proposed based on infrared data, conformational analyses, and diffractometric studies were the basis for the conclusions by Jones and by Tonessen and Ellefsen, that some significant questions remained concerning the structure of cellulose.

Since the reviews cited, two groups of investigations of the structure of cellulose have been reported. The first have been based on refinements of the x-ray and electron diffractometric analyses, in one instance in conjunction with analyses of molecular packing in the crystalline lattice. The second group have been based on analyses of the Raman spectra of the four major polymorphs of cellulose. The results of the two groups of studies are not consistent. Those based on diffractometry reaffirm the view that the molecular chains possess twofold axes of symmetry, and continue to interpret the differences between the polymorphic forms in terms of different modes of packing of the chains in the crystalline lattice. The Raman spectral observations, on the other hand, can only be interpreted in terms of significant differences between the conformations of the molecular chains in the different polymorphs. The two groups of studies will be reviewed in somewhat greater detail in the following subsections. The studies of the structure of cellobiose and methylcellobiocide will then be examined.

DIFFRACTOMETRIC STUDIES

The primary difficulty in diffractometric studies of polymeric materials in general is that the number of reflections recorded in a fiber diagram is usually less than 100 (15). This is in contrast with observation of 1000 to 2000 reflections in a typical study of the structure of a small molecule, where single crystals of optimum size can be grown. In the case of cellulose it is generally difficult to obtain even as many as 50 reflections. As a consequence, it is necessary to introduce assumptions concerning the structure of the monomeric entity and to utilize the limited scattering data to determine the orientation of the monomer groups with respect to each other (15). In all diffractometric studies of cellulose published so far the monomeric entity has been chosen as the anhydroglucose unit; the parameters of the glycosidic linkage

then are taken among those that are adjustable in search of an optimum fit to the crystallographic data. This is an assumption implicit in all of the analyses, but its validity needs to be questioned in light of the results on the dimeric species to be discussed below.

In addition to the question of the basic repeat unit to be selected in analyses of the diffractometric data, the studies of the structure of cellulose are complicated by the appearance of reflections which are disallowed by the symmetry generally ascribed to the cellulose chain. In one of the recent studies these reflections, which are rather weak relative to the other main reflections, have been assumed to be negligible (16). Thus, the refinement of the diffractometric data must be viewed as one constrained by the assumption of twofold screw axis symmetry. In the other recent study on the structure of cellulose, the inadequate informational content of the limited number of reflections from the cellulosic samples was complemented by conduct of an analysis of lattice packing energy. Thus the final structures were those constrained to produce minima in the energy of packing as well as optimum fits to the diffractometric data (17). One of the difficulties in this approach is that it is quite sensitive to statistical weighting in the energy computations. The calculation of packing energies utilized statistical weighting of hydrogen bonding potentials relative to Van der Waal's interactions that seem unrealistic; the weights were set at 20 to 1. These statistical weights had been initially derived in fitting the potential functions to crystallographic packing of monosaccharides, where the x-ray diffractometric data is adequate to establish the molecular structure and the relative position of the molecules in the unit cells. Serious questions must be raised, however, when these statistical weights are transferred to a polymeric species. The relative weights are particularly critical for the β-1,4-linkage, where structures possessing twofold screw axes of symmetry, of necessity, have

the two hydrogens on the 1 and 4 carbons significantly closer than the sum of their Van der Waals radii. Uncertainties concerning the distortions imposed by the statistical weighting, together with use of the anhydroglucose unit as the basic repeat unit in the cellulose molecule, raise serious doubts as to whether the remaining questions concerning the structure of the various polymorphs of cellulose have indeed been resolved.

RAMAN SPECTRAL STUDIES

The Raman spectral evidence concerning the structure of cellulose, which has been discussed in detail elsewhere (8,9), has suggested that the two most common polymorphs of cellulose, that is celluloses I and II, have basically different molecular conformations. Furthermore, celluloses III and IV may represent mixed lattices wherein the two basic conformations coexist; their spectra, in general, appear to be superimpositions of spectra of celluloses I and II. The basic difference between I and II is suggested by the spectra of highly crystalline samples shown in Fig. 1; the x-ray powder patterns of these samples are shown in Fig. 2. A number of arguments based on theoretical considerations, on comparisons with model compounds, and on comparisons with spectra of solutions, were developed (8) to support the conclusion that only different conformations of the molecular chains could account for Raman spectral differences of the magnitude indicated in Fig. 1. Analyses of the spectra of single fibers of native cellulose, before and after mercerization, suggested that the dominant conformation in native cellulose is a right-handed helical conformation, while that in mercerized cellulose is a left-handed helical conformation. Departures from the twofold helix conformation need not be very large ones to account for the spectral differences.

More recently calculations have shown that rotations about the glycosidic linkage, of the magnitude contemplated, can indeed result in shifts in characteristic bands of the types observed in Fig. 1 (18,19).

The findings summarized above, together with the information assembled by Reese and Skerrett on conformational analyses, led to a reassessment of available information concerning the conformation of the glycosidic linkage, its variability, and the assumptions about it implicit in past investigations of the structure of cellulose.

STRUCTURES OF CELLOBIOSE AND METHYLCELLOBIOSIDE

A search of the literature on relevant disaccharides revealed two crystallographic studies that proved to be of considerable importance. The study by Chu and Jeffrey of the structure of cellobiose (20) had been referred to in the discussions by Reese and Skerrett. Soon thereafter the structure of β-methylcellobioside had been worked out by Ham and Williams (21). While the contrast between the two structures is very significant and will be considered in detail, it is important to note that both structures have in common that the glycosidic linkage does not exist in a conformation consistent with twofold screw axis symmetry.

Comparison of the two structures, and their relationship to the proposed structures for cellulose is most conveniently discussed in terms of a diagram published by Reese and Skerrett (13). It defines conformations of the disaccharides as well as the structures of cellulose in terms of the dihedral angles about the bonds in the glycosidic linkage identified as ϕ and ψ. Though such maps were introduced by Ramachandran (22), we have found it convenient to adapt the map developed by Reese and Skerrett, Fig. 3. In addition to indicating the regions of minimum potential energy, it includes the locii of structures

possessing the same repeat distance for the anhydroglucose unit as well as the locii of structures possessing interoxygen distances of 2.5 and 2.8 Å for the intramolecular hydrogen bond between the C3 hydroxyl and the ring oxygen of the neighboring anhydroglucose unit. The locii of twofold and threefold helix structures are represented also. We have added the point corresponding to the structure of β-methylcellobioside indicated by W.

The first point immediately obvious from the diagram is that the structure of β-methylcellobioside corresponds to a right-handed departure from the two-fold helix line while the structure for cellobiose corresponds to a left-handed departure from the line. The other important contrast between the two structures is that the intramolecular hydrogen bond in cellobiose is rather isolated in the lattice, whereas the corresponding bond in the β-methylcello-bioside is bifurcated, with the oxygen on C6 of the nonreducing pyranose ring participating significantly in it. The structures are compared in Fig. 4 and the hydrogen bonding distances in Fig. 5. The distance of 3.1 Å between the oxygen on C6 and the hydroxyl oxygen on C3 in the case of the cellobiose structure is clearly too large to imply any significant degree of hydrogen bonding, whereas in the structure of β-methylcellobioside the distance is 2.91 Å, which is short enough to produce significant perturbation of the basic hydrogen bond between the C3 hydroxyl and the neighboring ring oxygen.

Observation of these differences suggested examination of the Raman spectra of the two compounds in the OH stretching region. The spectra are shown in Fig. 6 where it is clear that isolation of the intramolecular hydrogen bond in cellobiose permits a narrow rather intense band at 3440 cm^{-1}. In contrast the spectrum of the β-methylcellobioside shows only broad bands in this region. The significance of this comparison is much enhanced when it is noted that it

mimics comparisons of the spectra of celluloses I and II in the OH stretching region. It was first observed by Marchessault and Liang in their studies of infrared spectra of native and mercerized ramie (14,23) that the latter possessed sharp absorption bands above 3400 cm^{-1} in the OH stretching region, and that the dichroism of these bands was consistent with a transition moment parallel to the chain axis; absorption by native ramie fibers showed only broader bands at lower frequencies. Raman spectra of native and mercerized celluloses also reflect this effect (24).

The sharp narrow bands appearing in the OH region of cellobiose and mercerized celluloses imply isolated hydrogen bonds, unperturbed by the anharmonicities associated with participation in extended hydrogen bonding networks. The appearance of only broad bands in the spectrum of β-methylcellobioside reflects the anharmonicity introduced into the intramolecular hydrogen bond by the additional participation of the oxygen on C6. It may be inferred from the similar spectra of the native celluloses that the oxygens on C6 may indeed participate in bifurcated hydrogen bonds similar to the one observed in β-methylcellobioside.

The remaining questions in relation to the comparison made above is concerned with the appearance of two sharp bands in the infrared and Raman spectra of mercerized cellulose, while only one such band is seen in the spectrum of cellobiose. The spectrum of cellotetraose, recorded in the same region, also possesses two sharp bands almost identical to those in the spectrum of mercerized cellulose (18,19). There are indications that the broad band showing a high degree of dichroism in the infrared spectra of native cellulose, as well as the band that might correspond to the same region in the Raman spectrum of native celluloses, are both made up of two overlapping broad bands. The implication of all of these observations is that nonequivalent glycosidic linkages occur in both mercerized and native cellulose lattices.

The above considerations involving comparisons of the structures of cellobiose and β-methylcellobioside with the structures of mercerized and native cellulose, respectively, when taken together with the additional observation that the basic repeat unit derived from the diffractometric studies is 10.3 Å rather than 5.15 Å, require that data relating to the structure of cellulose be reexamined with the constraint that the anhydrocellobiose unit is the basic repeat unit rather than the anhydroglucose unit. To the authors knowledge no efforts have been made to interpret the diffractometric data in terms of a repeat unit based on the structure of cellobiose or on the structure of β-methylcellobioside. Experience with other polymers suggests that this avenue should be fully explored before the variety of recently published structures are reaffirmed as the ones most closely approximating the various crystalline polymorphs of cellulose.

The relevance of the question discussed above to the problem of hydrolytic degradation of cellulose arises from both stereochemical and electronic factors associated with the difference in conformation of the glycosidic linkage as well as with the participation of the oxygen on C6 in the bifurcated intramolecular hydrogen bond. The implications of these factors will now be considered.

CONFORMATIONAL EFFECTS IN HYDROLYTIC DEGRADATION

Among the patterns that emerge fairly early in any examination of the published literature on acid hydrolysis and on enzymatic degradation of cellulose, are the many similarities in the response to the two classes of hydrolytic agents. In both instances a rapid initial conversion to glucose and cellodextrins is followed by a period of relatively slower conversion, the rate of conversion in this second period depending on the prior history of the cellulosic substrate. In general the non-native polymorphic forms are degraded more rapidly during this second phase.

In addition it is found that the most crystalline of the native celluloses are particularly resistant to attack, with the most highly crystalline regions converted much more slowly than any of the other forms of cellulose.

The relationship of the patterns of hydrolytic susceptibility to the range of conformational variation discussed above can be interpreted in terms of the contrast between the states of the glycosidic linkage in cellobiose and β-methylcellobioside. The differences between the states which are likely to contribute to differences in observed reactivity are of two types. The first are differences in the steric environment of the glycosidic linkage, particularly with respect to activity of the C6 group as a steric hindrance to, or as a potential promoter of proton transfer reactions, depending on its orientation relative to the adjacent glycosidic linkage. The second type of difference is electronic in nature and involves readjustment of the hybridization of the bonding orbitals at the oxygen in the linkage. The potential contribution of each of these effects will be considered.

Steric Effects

Careful examination of scale models of the cellodextrins reveals that when C6 is positioned in a manner approximating the structure in β-methylcellobioside, the methylene protons are so disposed that they contribute significantly to creation of a hydrophobic protective environment for the adjacent glycosidic linkage. If, however, rotation about the C5 to C6 bond is allowed, the primary hydroxyl group can come into proximity with the linkage and provide a potential path for more rapid proton transfer.

Thus if, as suggested above on the basis of spectral data, the orientation of the C6 group in native cellulose is locked in by its participation in the bifurcated hydrogen bond to the C3 hydroxyl group, it may be responsible for the high degree of resistance to hydrolytic attack. Access to the linkage oxygen would be

through a relatively narrow solid angle, barely large enough to permit entry of the hydronium ions which are the primary carriers of protons in acidic media (25). If, on the other hand, the C6 group has greater freedom to rotate, as is likely the case in cellulose II, the hindrance due to the methylene protons can be reduced, and, in some orientations, the oxygen of the primary hydroxyl group may indeed provide a tunneling path, or a stepping stone, as it were, for transfer of protons from hydronium ions to the glycosidic linkages, hence greater susceptibility of non-native celluloses to hydrolytic attack.

The hypothesis put forth above concerning steric effects in acid hydrolysis would have as its corollary the proposal that the role of the C_1 component in cellulase enzyme system complexes is to disrupt the engagement of the C6 oxygen in the bifurcated intramolecular hydrogen bond and thus permit rotation of the C6 group into positions more favorable to hydrolytic attack.

The key role of C6 in stabilizing the native cellulose lattice is supported by recent findings concerning the mechanism of action of the dimethyl-sulfoxide-paraformaldehyde solvent system, which is quite effective in solubilizing even the most crystalline of celluloses. The crucial step in the mechanism proposed for action of this system is substitution of a methylol group on the primary hydroxyl at the C6 carbon (26, 27).

Electronic Effect

While the size of the system of bonds in the glycosidic linkage is clearly too large to permit computational investigation of the electronic structure by methods currently available, a number of qualitative observations related to the constraints of bond geometry are indicated. First it is clear that the hybrids of oxygen orbitals involved in the bonds to the carbons must be nonequivalent because the bond distances differ to a significant degree (20,21). The angle of approximately

116 degrees imposed on the linkage is likely to result in greater differences between the bonding orbitals and the lone pair orbitals than might be expected in a typical glycosidic linkage. Among themselves the lone pair orbitals are likely to be nonequivalent because of their different disposition with respect to the ring oxygen adjacent to C1 in the linkage; the differences may be small and subtle, but they are no less real. Given these many influences on the nature of hybridization at the oxygen in the linkage, it seems most unlikely that they would remain unaltered by changes in the dihedral angles of the magnitude of the difference between cellobiose and β-methylcellobioside. Hence a difference in electronic character must be expected.

The present state of the art does not permit estimation of the magnitude of the effects discussed, nor any speculation concerning the direction of changes in the relative reactivity of the glycosidic oxygen in the two different conformations. Yet it is clear that differences can be anticipated and they may be viewed, within limits of course, as altering the chemical identity of the glycosidic linkage as its conformation changes. It remains for future studies to define the differences more specifically.

A CONCLUDING PROPOSAL

In light of the observations discussed above relating to the structures of the two primary polymorphs of cellulose, as well as the variations in reactivity which have been rationalized in terms of their differing conformations, it is useful to consider an alternative conceptual framework for describing the structures of cellulose. As noted in the discussion of diffractometric studies, it has been the general practice to regard cellulose as a polymer of anhydroglucose units connected by β-1,4-linkages. An alternative view, that is perhaps more provocative of new insight into the nature of cellulosic matter, is that the cellulose chain is a polymer of glycosidic linkages held together by anhydroglucose units. Such a

view, when taken together with the possibility of different stable states of the linkage, corresponding to right-handed and left-handed conformations, elicits an interpretation of the phenomenology of cellulose in terms of a multistate model, not unlike the rotational isomeric model applied in the analysis of many other polymers with less constrained linkages. A detailed discussion of the implications of this proposal with respect to the physical and chemical properties of cellulose is beyond the scope of the present report. However, analyses of data on the hydrolysis of the glycosidic linkages need to be undertaken in light of the considerations which underly our proposal. In particular, variation of reactivity with conformation may require that the different polymorphs be viewed as differing in chemical character, as well as in physical structure.

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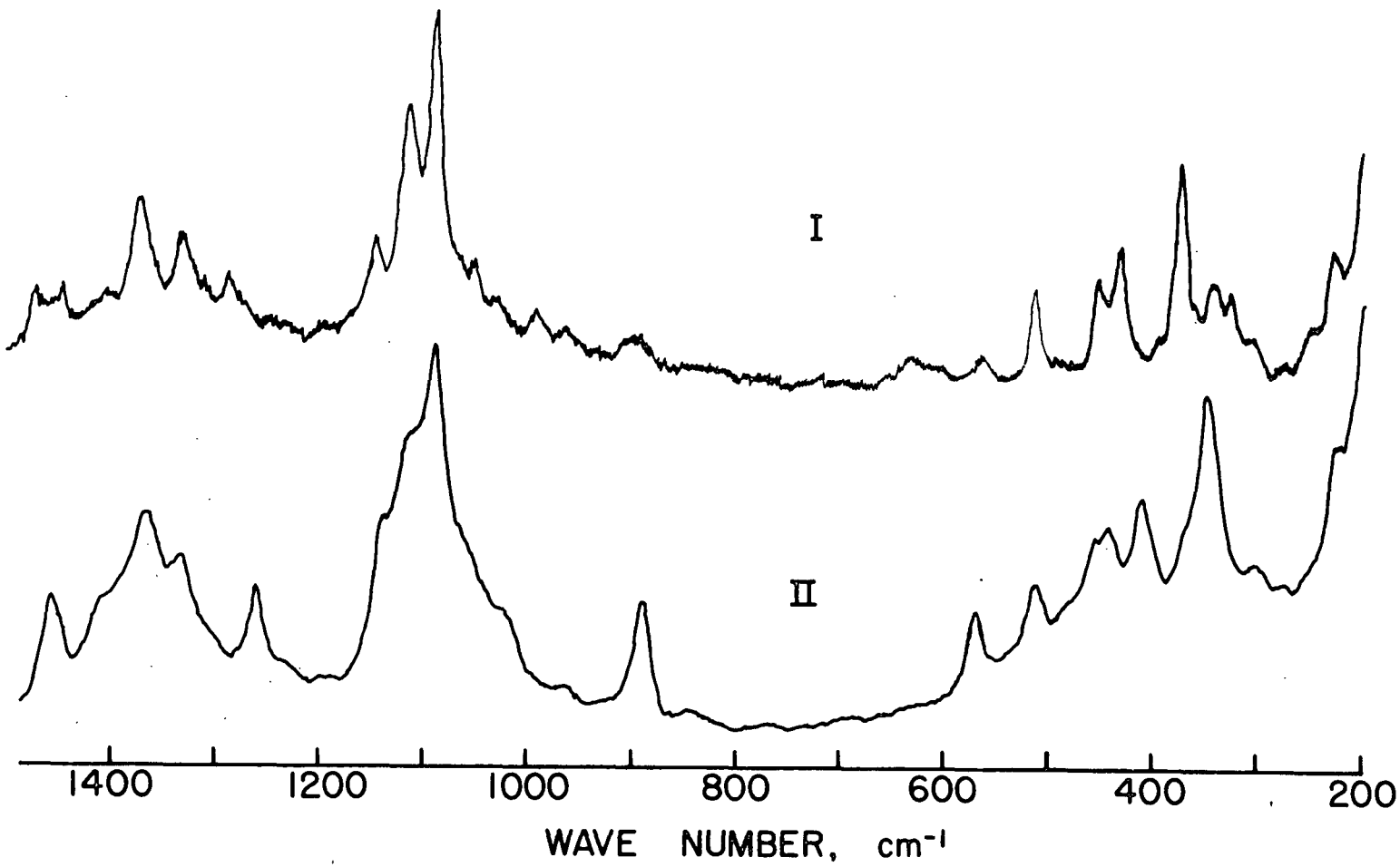


Figure 1. Raman spectra of highly crystalline samples of celluloses I and II.

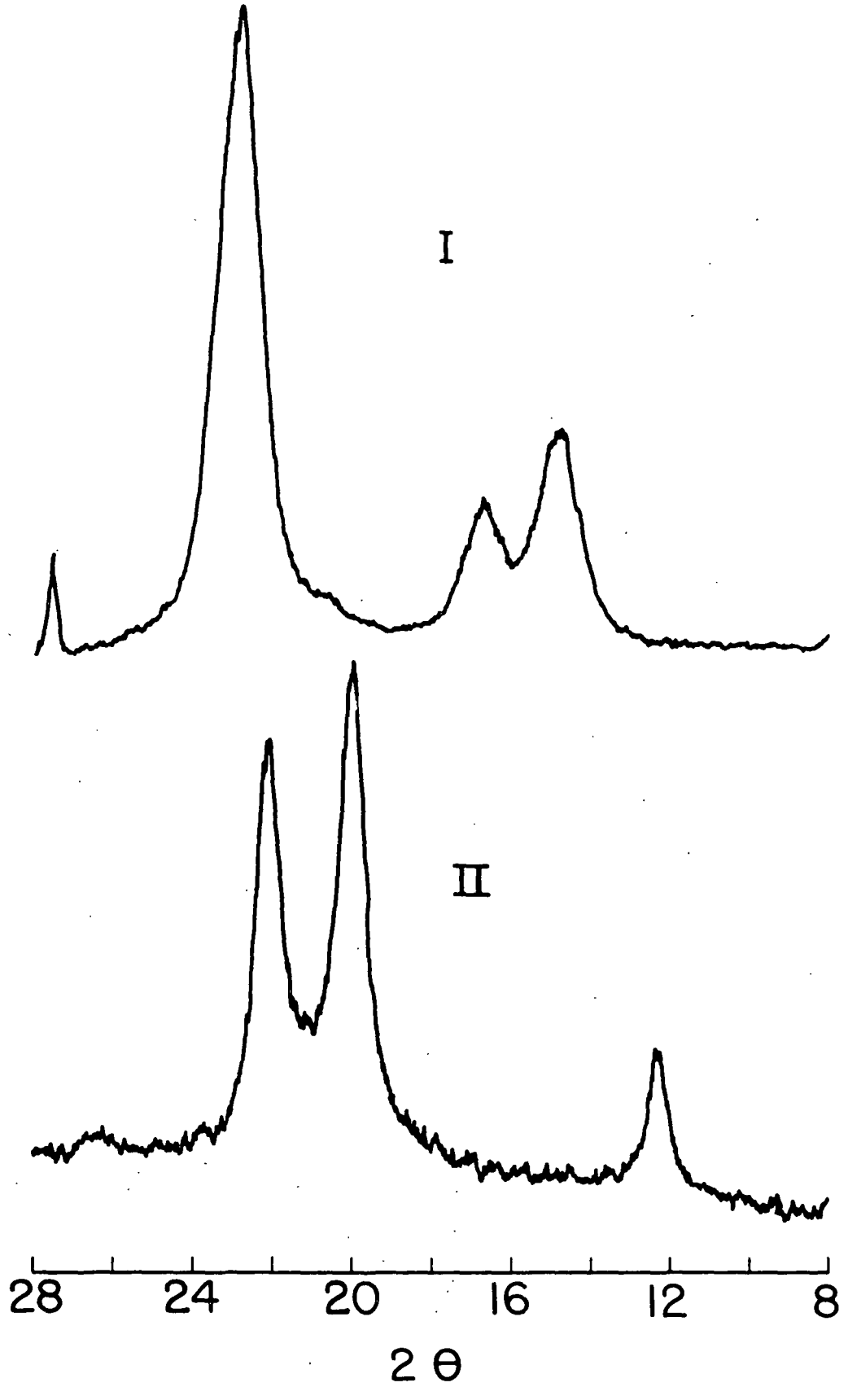


Figure 2. X-ray diffractograms for samples used for spectra in Fig. 1. (The reflection at 27.6° in diffractogram of cellulose I is due to TiO_2 used for calibration purposes.)

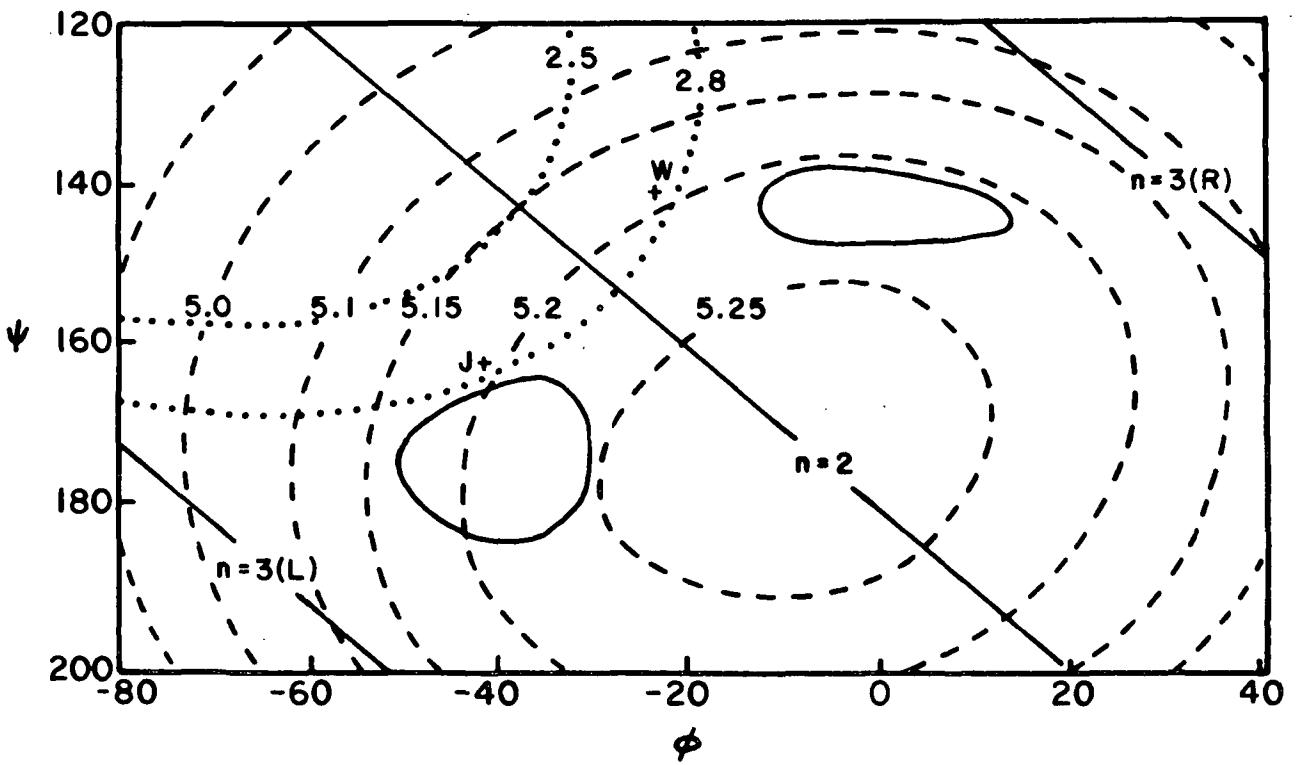
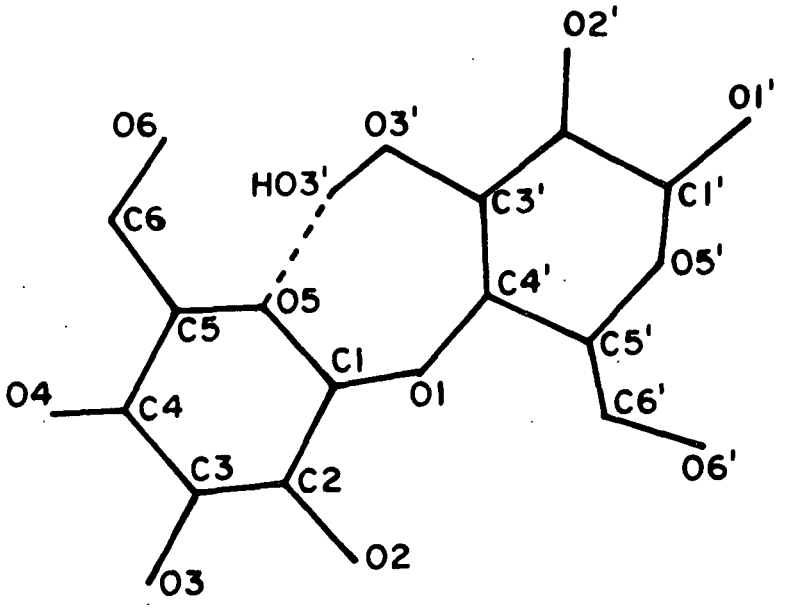
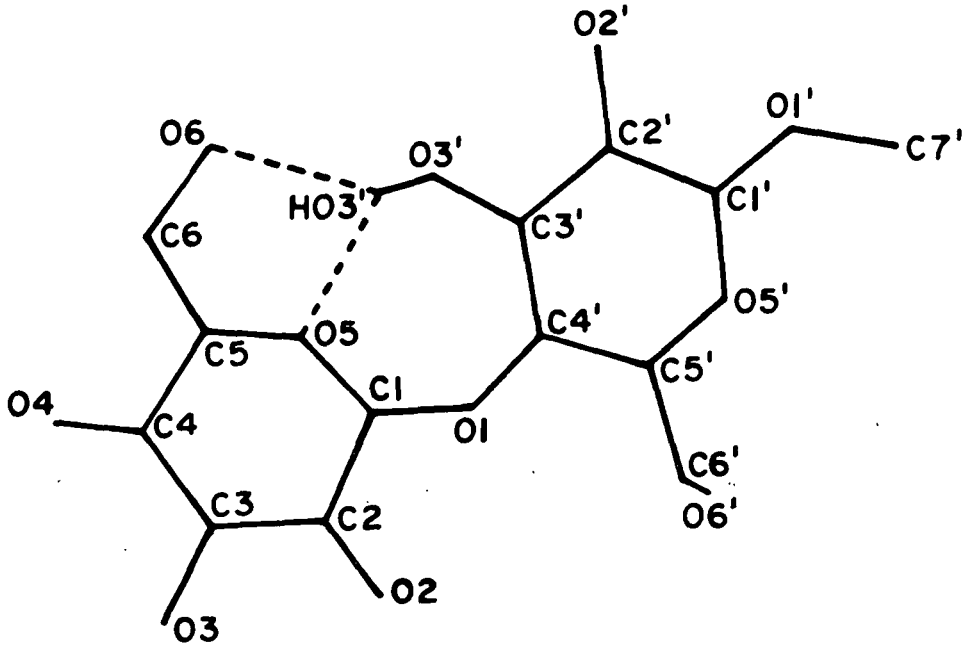


Figure 3. ψ/φ Map adapted from Ref. 13. (----) Locii of structures with constant anhydroglucose repeat periods. (....) Locii of structures of constant intramolecular hydrogen bond O-O distances. (——) contours of potential energy minima based on nonbonded interactions in cellobiose. J — cellobiose; W — β-methylcellobioside. n = 2, the twofold helix line; n = 3 the threefold helix lines, (R) right handed, (L) left handed. The Meyer-Migch structure is at ψ = 180, φ = 0.

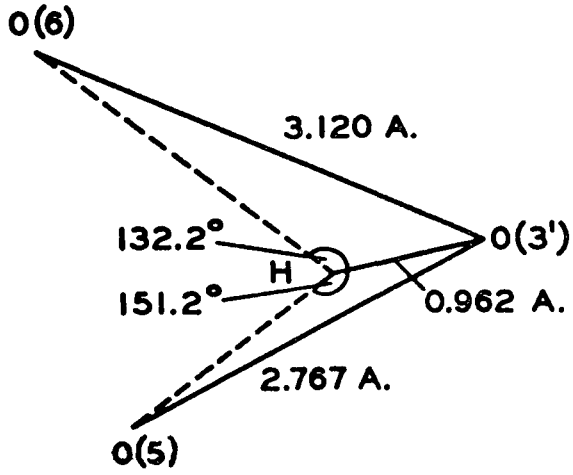


β CELLOBIOSE

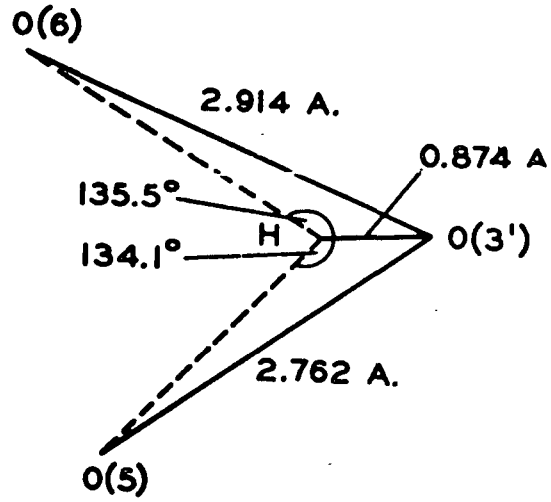


β METHYLCELLOBIOSIDE

Figure 4. Structures of β-cellobiose (20) and β-methylcellobioside (21).



β CELLOBIOSE



β METHYLCELLOBIOSIDE

Figure 5. Interatomic distances at the site the intramolecular hydrogen bond (21).

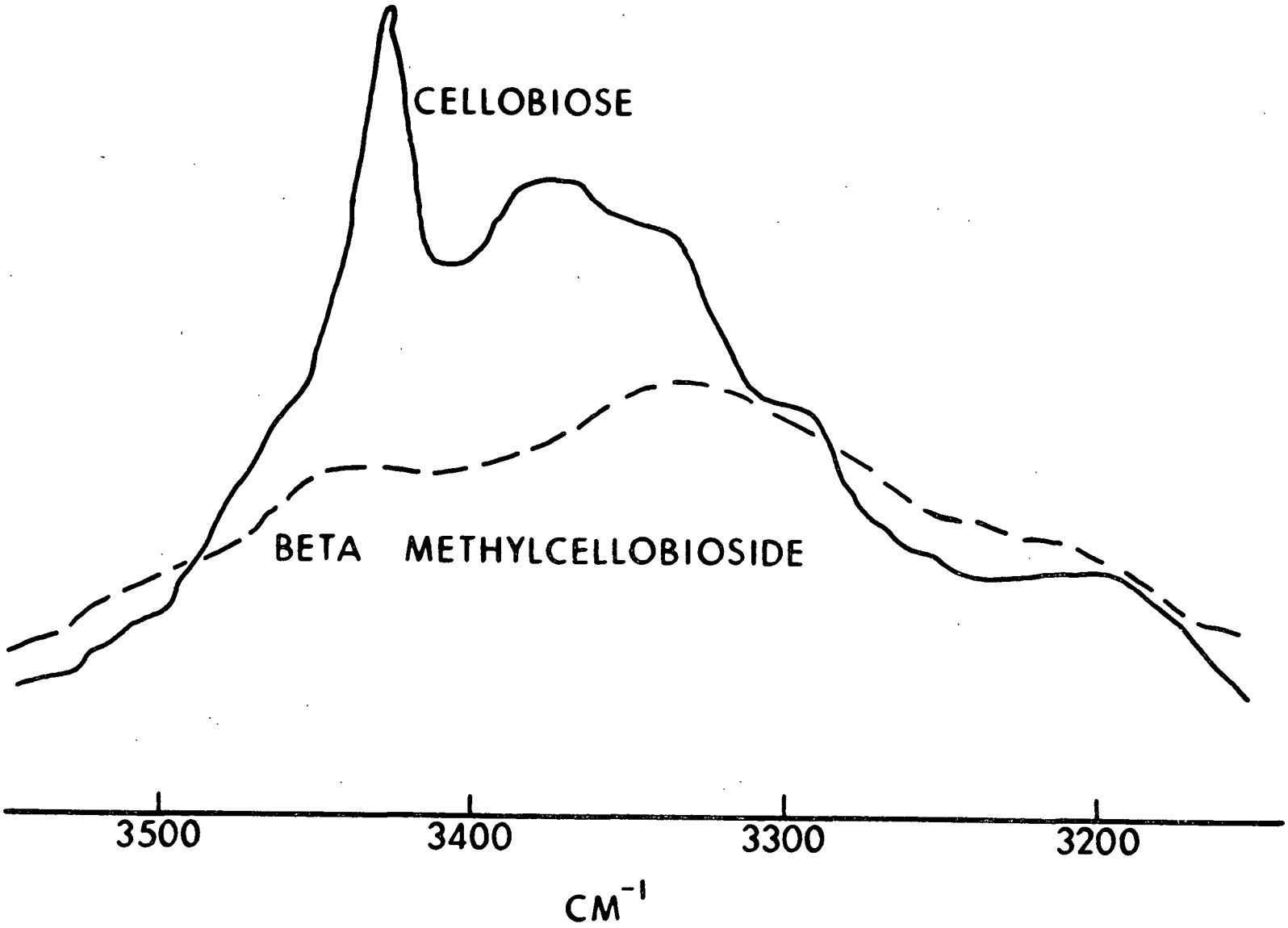


Figure 6. The OH stretching region in the Raman spectra of cellobiose and β-methylcellobioside.