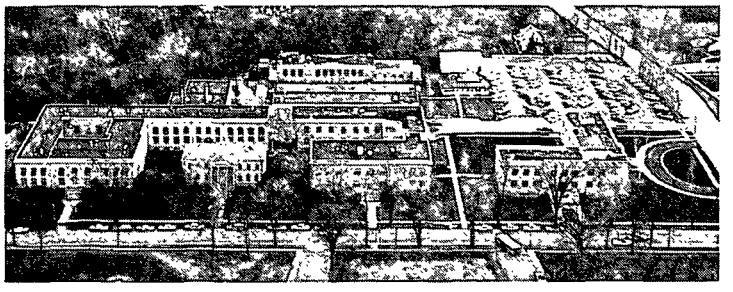

THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

IPC TECHNICAL PAPER SERIES
NUMBER 220

# BAND ASSIGNMENTS IN THE RAMAN SPECTRA OF CELLULOSES

JAMES H. WILEY AND RAJAI H. ATALLA

JANUARY, 1987

# Band Assignments in the Raman Spectra of Celluloses

James H. Wiley and Rajai H. Atalla

Portions of this work were used by JHW as partial fulfillment of the requirements for the Ph.D. degree at The Institute of Paper Chemistry

This paper has been submitted for consideration for publication in Carbohydrate Research

# BAND ASSIGNMENTS IN THE RAMAN SPECTRA OF CELLULOSES

James H. Wiley, Rajai H. Atalla

The Institute of Paper Chemistry, Appleton, Wisconsin 54912

## ABSTRACT

Our investigations of the vibrational spectra of celluloses have been extended by using the Raman microprobe to study the spectra of native celluloses. The microprobe allows spectra to be recorded from domains as small as 1 micron, so that, for fibers of simple morphology, the polarization of exciting and scattered radiation can be defined relative to molecular orientation. Series of spectra in which the polarization of the incident light was varied relative to the fiber axis were recorded from oriented fibers. Analysis of band intensities as a function of polarization revealed new information about the directional character of the vibrational displacements. In addition, a limited study of deuterated celluloses was conducted to identify the modes which involve hydrogen motions. The information from the studies of intensities and deuterated celluloses aided in the interpretation of the vibrational spectrum of cellulose. Although a complete assignment of the spectrum was not possible, this new information provides a more thorough characterization of the bands than has been possible in previous studies and establishes a foundation for future microprobe studies of native tissues.

## INTRODUCTION

A variety of spectroscopic and diffractometric techniques have been used to study the structure of cellulosic fibers. Vibrational spectroscopy has played a key role in this multifaceted approach. In order to derive structural information from the vibrational spectra of celluloses, the bands in the different spectral regions must be characterized. The large number of vibrational degrees

of freedom and the asymmetric structure of the cellulose molecule make interpretation of the spectrum difficult. The group frequency approach usually used in interpretation of vibrational spectra is not suited to interpretation of the molecular chain modes of cellulose. With the exception of a few modes associated with highly localized vibrations involving hydrogen atoms, most of the modes are highly coupled and delocalized. This is not surprising since the pyranose rings, and the linkages between them consist of systems of C-C and C-O bonds. These bonds have similar reduced masses and bond energies, so that their frequencies are sufficiently close for a high degree of coupling to occur between their vibrations.

In our laboratory, the problem of interpreting the spectrum of cellulose was approached by conducting a series of normal coordinate analyses of cellulose model compounds, and using the resulting information as the basis of the analysis. This report covers a closely related investigation utilizing the Raman microprobe[1-3] to study the dependence of band intensities on the polarization of the incident light relative to the orientation of the molecules. The study reveals the directional character of many of the vibrational modes and serves as a foundation for studies of morphologically complex aggregates of cellulose. In addition, a limited study of carbon-deuterated celluloses was undertaken to identify the bands involving OH and CH motions.

## BACKGROUND

Model Compound Studies — A series of normal coordinate analyses were undertaken in our laboratory to provide a basis for understanding the vibrational spectra of cellulose. The compounds chosen were the 1,5-anhydropentitols,[4-5] the straight chain pentitols,[6] the pentose sugars,[7] the inositols,[8-9] and the hexose

sugars.[10] For each group of compounds, the force constants were refined against the observed frequencies until a satisfactory fit was obtained. The force fields derived enabled successful prediction of the spectra of compounds not included in the refinements, and the calculated potential energy distributions were reasonable in comparison with the group frequency literature on the carbohydrates. The analyses were thus successful in developing a physically meaningful force field which is specifically tailored to the carbohydrates.

The hexose force field was used to extend the normal coordinate method to the cellodextrins[11] whose vibrational spectra more closely resemble the spectrum of cellulose. Because the number of vibrational degrees of freedom greatly exceeds the number of observed bands in the spectra of these compounds, it was neither possible nor meaningful to refine the force constants. Although the calculations predicted many more bands than are actually observed, the distribution of calculated frequencies was in qualitative agreement with the observed spectra. The force field derived appears to provide a good model for understanding the vibrational spectra of the cellodextrins and cellulose.

The potential energy distributions calculated for the model compounds were quite complex. Except for the internal vibrations of the methylene groups, the modes below 1500 $cm^{-1}$ are delocalized motions involving several internal coordinates. In earlier assignments of the cellulose vibrational spectrum, the modes below 1500 $cm^{-1}$ were assigned to localized group vibrations.[12-15] While the potential energy distributions generally agree qualitatively with the types of motions suggested in the earlier assignments, the calculations indicate that the motions are often more delocalized than was recognized in the early assignments. Above 1500 $cm^{-1}$, the CH and OH stretching modes do behave as relatively pure group modes.

Raman Spectroscopy — In cellulose all of the vibrational modes are potentially both infrared and Raman active. Raman spectroscopy, however, has some important advantages for recording spectra from cellulosic samples. Highly polar bond systems, which result in intense infrared bands, have relatively low polarizabilities and, hence, weak Raman intensities. Water, therefore, has very weak Raman bands and does not interfere with the spectrum of cellulose. The low frequency region, which is observed with difficulty in the infrared spectra, is easily observed in the Raman spectra. Finally, cellulosic materials are often optically heterogeneous substrates which scatter light intensely. In infrared spectroscopy, any processes other than absorption which cause attenuation of the incident beam are problematic. Since the refractive index of the sample will often go through large changes in the neighborhood of absorption bands, the scattering losses will vary with frequency over the infrared region. In Raman spectroscopy, refractive index variations are not a problem, since the excitation frequency is far removed from any absorption bands. Therefore, Raman spectra of samples such as cellulose, which scatter light strongly, are more accurate representations of the vibrational motions and the characteristic vibrational transitions.

Raman Microprobe — A recent innovation in Raman spectroscopy was the development of the Raman microprobe.[1-3] The microprobe is a specially designed optical microscope coupled with a conventional Raman spectrometer. The microscope performs two key functions. It focuses the exciting light on the sample down to a diameter of one micron; then it gathers the scattered light and transmits it to the entrance slit of the spectrometer. Since the microprobe acquires spectra from such small domains, the structural heterogeneity of the domains is greatly reduced relative to the domains examined in conventional Raman spectroscopy. The microprobe makes it possible to identify the morphological features from which spectra are recorded so that orientation, composition, and structure can be related to morphology.

The special attributes of the microprobe make new information available. In the present investigation, the microprobe was utilized to record spectra from morphologically homogeneous fibrillar domains. The polarization of the Raman scattered light was analyzed to aid in the assignment of the Raman spectrum of cellulose.

## EXPERIMENTAL

Sample Preparation — Valonia fibers were extracted from the purified cell walls of Valonia macrophysa that was grown in our laboratory. The alga was extracted with chloroform-methanol and boiled in a solution of 1% sodium hydroxide for 6 hours under nitrogen. Then it was bleached with sodium chlorite following the procedure in Browning.[16] Oriented fibrils were pulled from the cell wall with forceps and mounted on small washers for examination with the microprobe. Scanning electron micrographs of the fibrils showed that they had a high degree of parallel orientation.[17]

Deuterated cellulose fibers were prepared from purified filaments of Cladophora glomerata that had been grown in $D_2O$. The alga was adapted to growth in $D_2O$ following Crespi's methods.[18] The procedure used to purify Valonia was followed except that the bleaching step was omitted. Since the filaments were too small to be mounted on washers, they were stretched across copper specimen support grids normally used in electron microscopy.

Ramie (Boehmeria) fibers were purified by the same method used to purify Cladophora cellulose. The fibers were dried under tension and mounted on small washers. Scanning electron micrographs showed that the fibrils possessed a high level of parallel orientation.

A sample of deuterated bacterial cellulose was kindly provided by Dr. H. L. Crespi. It was prepared by growing Acetobacter xylinum in a deuterated growth medium.[18] The sample was purified as described for Valonia and then acid hydrolyzed.[17] The residue was made into the form of a pellet for examination with the conventional Raman system. A detailed description of the growth conditions used for the algae and the purification procedures is given elsewhere.[17]

Acquisition of Spectra — Spectra of the Valonia, Cladophora, and ramie fibers were recorded with a Raman microprobe developed by Instruments SA. The microprobe system consists of Jobin Yvon Ramanor HG2S coupled with a Nachet optical microscope. Since we wanted to compare the intensities in spectra recorded with different polarizations of the exciting light, special modifications were made to the microprobe in order to avoid problems arising from the dichroism inherent in the optical system of the microscope and the monochromator. First, a polarization scrambler was inserted at the coupling between the microscope and the monochromator. Second, a rotating mechanical stage was installed so that instead of changing the polarization of the incident light directly, we were able to rotate the sample relative to the plane of polarization of the incident light as shown in Fig. 1. The stage was aligned so that its axis of rotation coincided with the optical axis of the microscope. Therefore, it was possible to rotate the sample without changing the domain being examined.

[Fig. 1 here]

The exciting radiation was the 5145 Å line of an argon ion laser. The power incident on the sample was approximately 7 mW. A 40-X Nachet objective with a numerical aperture of 0.75 was employed. The spectral slit width was approximately 8 $cm^{-1}$. The acquisition time required for each spectrum was 8

hours. Multiple scans were recorded to reduce distortion of the relative intensities due to any drift in the laser power during a single scan.

The spectrum recorded from the deuterated bacterial cellulose pellet was acquired in the macro chamber of the same Raman spectrometer. The incident laser power was 150 mW. The spectral slit width was 3 $cm^{-1}$ and the acquisition time was 20 hours.

## RESULTS AND DISCUSSION

Analysis of Band Intensities — Sets of spectra in which the angle between the electric vector of the incident light and the fiber axis (see Fig. 1) was varied from 0 to 90° in 15° increments were recorded from Valonia and ramie fibers and are shown in Figures 2 and 3, respectively. Except for the band widths, the spectra of Valonia and ramie are very similar to each other below 3000 $cm^{-1}$, suggesting that the same vibrational modes occur in both celluloses. Above 3000 $cm^{-1}$, significant frequency differences are observed. The structural implications of these frequency differences will be discussed in a later section. Due to the similarity between the Valonia and ramie spectra, they can be compared to check the reproducibility of the band frequencies and the dependence of the band intensities on the polarization of the incident light.

[Fig. 2 and 3 here]

From Figures 2 and 3 it is clear that the band intensities are strongly dependent on the orientation of the incident electric vector relative to the fiber axis. The dependence of the band intensities on $\theta$, the angle between the incident electric vector and the fiber axis, was modeled by the following equation:

$$I = a + b(\cos\theta)^2 + c(\cos\theta)^4 \qquad [1]$$

where a, b, and c are constants related to the derivatives of the polarizability tensors with respect to the normal coordinates. The equation was derived according to Snyder's treatment of intensities for partially oriented polymers.[19] In the derivation, it was assumed that the cellulose chains are oriented parallel to the fiber axis and that the microfibrils are oriented randomly around their axes.

Equation [1] was fitted to the data in Figures 2 and 3 by a linear regression technique. The equation provided an adequate model for the dependence of the band intensities on θ for bands which were well resolved. Bands which were weak and/or poorly resolved could not be fitted as well. This approach to classification of the bands in the spectra has been adopted because it is useful as a basis for future applications of Raman microprobe spectroscopy in investigations of molecular organization in native plant tissue. The advantage of using ramie and algal cellulose spectra as reference spectra is that the organization of molecular chains within the fibrils is known to be simple, and parallel to the fibril axes.

Based on the relationships between the intensities and θ, the bands were divided into four groups. The classification of the bands is summarized in Table I. The first group of bands exhibits a single maximum and minimum in plots of intensity vs. θ as illustrated by curves a and c in Fig. 4. Such curves possess a single inflection point in the range of θ shown. This group of bands is designated as group A in Table I. The second group of bands exhibits two maxima, at 0 and 90°, and a single minimum between 0 and 90°C (see curves b and d in Fig. 4); these curves possess two inflection points. This group is designated as group B in Table I. The multiple maxima may arise from accidentally

degenerate modes which have maxima at 0 and 90° or from modes in which some elements of the polarizability tensor decrease during the vibration.

[Table I and Fig. 4 here]

The bands were further categorized by whether they were most intense when θ was 0 or 90°. The bands that are most intense when θ equals 0° are designated by a 0 subscript in Table I. Example plots of intensity vs. θ for $A_0$ and $B_0$ bands are given by curves a and b, respectively, in Fig. 4. These bands result from vibrations in which the maximum change in polarizability is parallel to the chain axis. Those bands that are most intense when θ equals 90° are designated by a 90 subscript. Example plots are given by curves c and d in Fig. 4. For these modes, the maximum change in polarizability is perpendicular to the chain axis. The direction in which the maximum change in polarizability occurs is related to the direction of the vibrational displacements. The intensity study can, therefore, reveal information about the directions of the vibrations. In addition, it provides a more thorough characterization of the bands in the spectrum of cellulose than has been possible in previous studies.

Spectra of Deuterated Celluloses — A limited study of deuterated celluloses was conducted to identify the modes which involve hydrogen motions. Figure 5 shows a spectrum from a pellet of deuterated bacterial cellulose recorded in the conventional Raman mode. Figure 6 includes spectra from an oriented sample of deuterated Cladophora cellulose recorded with the incident electric vector parallel and perpendicular to the fiber axis. The residual intensities in the CH (2800-3000 $cm^{-1}$) and OH stretching (3200-3500 $cm^{-1}$) regions indicate that the samples are not fully deuterated. Due to the residual hydrogen present in the samples, the full effect of deuteration on the spectrum of cellulose could

not be determined. For most of the bands, however, we were able to determine whether the effect of deuteration was small or large. This information is listed in Table I.

[Fig. 5 and 6 here]

Band Assignments — In previous assignments of the vibrational spectrum of cellulose it has generally been assumed that the vibrational motions could be described in terms of simple group motions. The normal coordinate analyses demonstrated that assignments in the traditional sense are not meaningful. In the region below 1500 $cm^{-1}$, only the internal motions of the methylene groups can be adequately approximated as group motions. The rest of the modes are delocalized motions involving more than one group or site in the molecule. Furthermore, cellulose possesses many more vibrational degrees of freedom than the number of bands observed in the infrared and Raman spectra. Therefore, it is possible that some modes are accidentally degenerate. The observed bands may actually arise from a composite of several vibrational motions all having approximately the same frequency.

The assignments described below are not assignments in the traditional sense but are rather descriptions of the types of motions occurring in each region of the spectrum. The types of motions were identified from the potential energy distributions for the hexoses[10] and the cellodextrins[11] and the study of deuterated celluloses. The assignments also involve a description of the directional character of the vibrations based on the intensity study. The spectrum has been divided into six regions for convenience.

250-550 $cm^{-1}$ Region — In the region between 250 and 550 $cm^{-1}$, several closely spaced medium intensity bands were observed in the Raman spectra of Valonia and ramie celluloses (see Fig. 2 and 3). A similar pattern has been observed in the infrared spectra of native celluloses.[11-15] The potential energy distributions are to a large extent delocalized, indicating that the vibrational modes are quite complex. Displacement drawings based on the normal coordinate calculations for the disaccharides[11] show that almost every atom in the molecule participates in these modes. The predominant motions are skeletal bending modes involving the CCC, COC, OCC, and OCO internal coordinates. Small amounts of methine bending (CCH and OCH) and skeletal stretching (CC and CO) contribute in the region. Torsional motions, which are out-of-plane bending about the CO and CC bonds, become significant below 300 $cm^{-1}$. The small sensitivity of the bands to deuteration is consistent with the small contribution of CH coordinates in the potential energy distributions.

According to the classifications in Table I, the bands at 331, 459, and 520 $cm^{-1}$ are types $A_0$, $B_0$, and $A_{90}$, respectively. The 344, 381, and 437 $cm^{-1}$ modes all belong to the B category, but their distribution in groups $B_0$ and $B_{90}$ is uncertain due to divergences between the Valonia and ramie data. Since the 331 and 459 $cm^{-1}$ modes are most intense when the incident electric vector is parallel to the chain axis, these modes are skeletal bending modes where the major change in polarizability is parallel to the chain axis. An accordion-like bending motion of the pyranose rings in the chain is a plausible description of this mode. Since the 459 $cm^{-1}$ band falls in the B category, it may actually be a composite of a motion where the change in polarizability is parallel to the chain axis and a motion where the change in polarizability is perpendicular to the chain axis. Alternatively, some of the polarizabilities

may decrease during the vibration. The 520 $cm^{-1}$ mode is most intense when the incident electric vector is perpendicular to the chain axis.

The normal coordinate calculations for cellotetraose[11] showed that the frequency distribution below 700 $cm^{-1}$ is sensitive to the dihedral angles at the glycosidic linkages. Raman spectra of various types of cellulose have demonstrated that the Raman spectra are very sensitive to the polymorphic form of the cellulose.[20-21] The observed spectral differences are very similar to the differences observed in the frequency distributions for the alternate structures of cellotetraose. This has reinforced the conclusion that celluloses I and II possess different conformations of the glycosidic linkages.[20-21]

550-950 $cm^{-1}$ Region — In the region between 550 and 950 $cm^{-1}$ of the Raman spectra of Valonia and ramie celluloses (see Fig. 2-3), the bands are weak and widely spaced. The region between 750 and 800 $cm^{-1}$ is devoid of any significant features. Infrared spectra of native celluloses differ from the Raman spectra between 550 and 750 $cm^{-1}$ in that several medium intensity peaks are observed.[11-15] Between 750 and 850 $cm^{-1}$, the IR spectra are also devoid of any significant features. Both the Raman and IR spectra possess a weak, poorly resolved cluster of bands at approximately 900 $cm^{-1}$.

The potential energy distributions[10-11] indicate that between 550 and 750 $cm^{-1}$ the predominant internal coordinates are CCC, COC, OCO, CCO, and OH out-of-plane bending. The OH bending modes are observed in infrared spectra but are absent from Raman spectra because of the large dipole moment and low polarizability associated with the OH bond. No bands are calculated between 750 and 800 $cm^{-1}$, which is consistent with the observed frequency pattern. A cluster of

peaks is calculated around 900 $cm^{-1}$ that involves HCC and HCO bending localized at the C6 positions.

The deuteration sensitivities of the bands in the Raman spectrum between 550 and 850 $cm^{-1}$ were small. This observation is consistent with the dominance of the CCC, COC, OCO, and CCO internal coordinates in this region. Since the peaks around 900 $cm^{-1}$ involve primarily methine bending coordinates, these bands should be strongly deuteration sensitive. We were unable to identify a peak in the appropriate region of deuterated cellulose spectra which could correspond to the 900 $cm^{-1}$ bands. New peaks are shifted into the region around 900 $cm^{-1}$ by deuteration so that it is difficult to tell if the 900 $cm^{-1}$ band is still present. It appears, however, that the 900 $cm^{-1}$ band is shifted by much less than would be the case if this was a pure CH bending mode.[22] Therefore, it is likely that the bands around 900 $cm^{-1}$ are more delocalized than predicted by the cellodextrin calculations.

In assigning the modes around 900 $cm^{-1}$, several empirical observations are useful. The band is significantly more intense in the spectra of ramie than in Valonia (see Fig. 2-3). Below 3000 $cm^{-1}$, this is the most significant difference between the ramie and Valonia spectra. A comparison of the spectra of ramie, cotton, bacterial, algal, and amorphous celluloses suggested that the intensity of the 900 $cm^{-1}$ band is related to the lateral size of the cellulose crystallites. The intensity of the 900 $cm^{-1}$ band was also found to correlate in some instances with the intensity of the broad upfield shoulders for the C4 and C6 carbons in the solid state $^{13}C$ NMR spectra of native celluloses.[23] The broad shoulders arise from cellulose chains on the crystallite surfaces and in the amorphous regions. These results suggest that the intensity of the 900 $cm^{-1}$ peak is proportional to the amount of disorder in the cellulose. Since the likely sites of

disorder in the cellulose molecules are the glycosidic linkages, the C6 positions, and the hydroxyl groups, the 900 $cm^{-1}$ band is likely to involve one or more of these sites. Further investigations are underway to test this hypothesis.

950-1180 $cm^{-1}$ Region — In the region between 950 and 1180 $cm^{-1}$, several closely spaced intense bands are observed in both the Raman spectra of Valonia and ramie celluloses (see Fig. 2-3) and in the infrared spectra of native celluloses reported in the literature.[11-15] The normal coordinate calculations[11] show that the band density in the 950 to 1180 $cm^{-1}$ region is very high. The potential energy distributions are dominated by CC and CO stretching motions. Small amounts of HCC, HCO, and skeletal atom bending also contribute to the bands. The motions are highly coupled, often involving coupling between the glucose rings. The high Raman and infrared intensities of the bands are consistent with the large band density and dominance of CC and CO stretching motions predicted by the normal coordinate calculations. It is difficult to determine the deuteration sensitivities because many new bands appear in the region due to deuteration. The 1071, 1095, 1118, and 1123 $cm^{-1}$ bands exhibit very little sensitivity to deuteration, which is consistent with the negligible contribution of CH, $CH_2$, and OH coordinates to the modes responsible.

The 997, 1034, 1057, 1095, and 1123 $cm^{-1}$ modes all fall in the $A_0$ category. The band at 1118 $cm^{-1}$ is a $B_0$ mode. Since these bands are most intense when the electric vector of the incident light is parallel to the fiber axis, they must result from CC and CO stretching motions which are parallel to the chain axis. The 968 $cm^{-1}$ band is a $B_{90}$ mode. It must result from skeletal stretching motions that are predominantly perpendicular to the chain axis. In addition to 1118 and 968 $cm^{-1}$ bands, the band at 1152 $cm^{-1}$ also belongs in the B category. The ramie and Valonia data are not consistent, however, as to

whether the 1152 $cm^{-1}$ band is a $B_0$ or a $B_{90}$ mode. The directionality of the 1152 $cm^{-1}$ mode is, therefore, uncertain.

1180-1500 $cm^{-1}$ Region — Between 1180 and 1270 $cm^{-1}$ the Raman and infrared spectra of Valonia and ramie celluloses exhibit only weak and widely spaced bands. The potential energy distributions for the cellodextrins indicate that the 1180 to 1270 $cm^{-1}$ region is a transition region.[11] Below 1180 $cm^{-1}$, CC and CO stretching coordinates dominate the potential energy distributions, while above 1270 $cm^{-1}$ HCC, HCO, HCH, and COH bending coordinates are most significant. Between 1180 and 1270 $cm^{-1}$, the modes involve significant amounts of skeletal stretching as well as methine bending.

The cellodextrin calculations showed that the frequency distribution is sensitive to the conformation of the glycosidic linkages in the 1200-1300 $cm^{-1}$ region.[11] The differences in the frequency distributions correspond closely with the differences observed between the spectra of celluloses I and II. A medium intensity band is observed in the Raman spectrum of cellulose II at 1261 $cm^{-1}$ that is not observed in the spectrum of cellulose I. This observation lends support to the proposal that celluloses I and II possess different molecular conformations. The sensitivity of the bands to conformation may arise from the nature of the potential energy distributions. Since the 1180 to 1270 $cm^{-1}$ region is a transition region, many different types of internal coordinates contribute to the modes, thereby increasing the amount of delocalization.

In the 1270 to 1500 $cm^{-1}$ region several closely spaced medium intensity bands are observed in both the Raman and infrared spectra of native celluloses. The bands are strongly sensitive to deuteration. The normal coordinate calculations also predict a high density of bands in the region.[11] The predominant

internal coordinates in the potential energy distributions are CCH, OCH, COH, and HCH bending. Between 1430 and 1500 $cm^{-1}$ the major internal coordinate is HCH bending; from 1430 to 1350 $cm^{-1}$ it is COH bending; and from 1350 to 1270 $cm^{-1}$ it is HCC and HCO bending. Except for the internal modes of the $CH_2OH$ groups, the motions are quite delocalized. The dominance of CH and OH bending coordinates is consistent with the strong deuteration sensitivity of the bands.

The Raman bands at 1279, 1334, 1337, and 1406 $cm^{-1}$ are all $A_0$ modes. Since these modes are most intense when the electric vector of the incident light is parallel to the chain axis, they must result primarily from HCC and HCO bending motions where the change in polarizability is parallel to the chain axis. Although COH bending coordinates also contribute to the potential energy distributions above 1300 $cm^{-1}$, OH bending is very weak in Raman spectra so that the intensities will be dominated by the motions of CH groups.

The Raman bands at 1455 and 1479 $cm^{-1}$ fall in the $B_{90}$ and $A_{90}$ categories, respectively. The potential energy distributions show that these bands are HCH bending modes which contain a very small proportion of COH bending.[11] The bands are most intense when the electric vector of the incident light is perpendicular to the fiber axis. Therefore, the vibrations must be oriented so that the change in polarizability accompanying the vibration is also perpendicular to the chain axis. This can only occur in the so called gt and tg rotational conformations for the methylene groups.[24] In the gt conformation, the C6-O6 bond is gauche to the C5-O5 bond and trans to the C4-C5 bond, whereas in the tg conformation it is trans to the C5-O5 bond and gauche to the C4-C5 bond. The Raman spectra do not provide a basis for discriminating between the two forms. Since the 1455 $cm^{-1}$ band is a B mode, it might also be a degenerate

vibration or result from a vibration where elements of the polarizability decrease during the motion.

2800-3000 $cm^{-1}$ Region — Between 2800 and 3000 $cm^{-1}$, the Raman spectra of Valonia and ramie contain several closely spaced very intense bands. In the infrared spectra, the band structure is very similar but the bands are not as intense.[12,14-15,25] The bands are strongly deuteration sensitive. The normal coordinate calculations predict that the CH and $CH_2$ stretching vibrations occur in this region. These modes are isolated from the other motions in the molecule and, therefore, behave as group vibrations.

The 2868 and 2885 $cm^{-1}$ bands fall into the $B_{90}$ category in both the Raman spectra of ramie and Valonia. The 2965 $cm^{-1}$ band is a $B_0$ band in both sets of spectra. Although it is difficult to assign the bands in this region due to the overlapping of the bands and the possibility of Fermi resonance, the most intense band at 2885 $cm^{-1}$ is most likely due to the methine protons. The methine CH bonds are perpendicular to the chain axis and hence would result in stretching bands that are most intense when the electric vector of the incident light is perpendicular to the chain axis. Also, there are more methine protons than $CH_2$ protons so that the methine stretch should be the most intense CH band.

The $CH_2$ group should exhibit both symmetric and antisymmetric stretching bands. The antisymmetric stretching band will be at higher frequency than the symmetric stretching band. Since the symmetric methylene bending mode is most intense with the incident electric vector perpendicular to the fiber axis, the symmetric methylene stretch is also expected to be most intense in the perpendicular mode, while the antisymmetric stretch is expected to be most intense in the parallel mode. The 2965 $cm^{-1}$ band is most intense with incident electric

vector parallel to the fiber axis and is a plausible frequency for a $CH_2$ antisymmetric stretching mode.

Since there are several modes other than the methine stretching band that are most intense when the incident electric vector is perpendicular to the chain axis, the symmetric $CH_2$ stretching mode is more difficult to identify. As mentioned above, the 2868 $cm^{-1}$ band is a $B_{90}$ mode. The 2848 and 2904 $cm^{-1}$ bands were classified as $B_{90}$ bands based on the Valonia data, but they were not resolved in the ramie spectra. The 2941 $cm^{-1}$ band was classified as a $B_0$ band based on the Valonia data and as a $B_{90}$ band based on the ramie data. Since the symmetric $CH_2$ stretching frequency is usually at least 100 $cm^{-1}$ lower than the antisymmetric stretching frequency,[26] either the 2848 or the 2868 $cm^{-1}$ band is most likely the $CH_2$ symmetric stretching mode.

3200-3500 $cm^{-1}$ Region — Between 3200 and 3500 $cm^{-1}$, the Raman spectra of Valonia and ramie contain several closely spaced medium intensity bands. In the infrared spectra of native celluloses, the band frequencies are the same as in the Raman spectra, but the bands are much more intense. The bands are strongly deuteration sensitive. The normal coordinate calculations[10-11] predict that the OH stretching vibrations occur in this region. As was the case with the CH motions, the OH motions are isolated from the other internal motions of the cellulose molecule. Hydroxyl stretching motions, however, can couple with lattice modes due to their involvement in intermolecular hydrogen bonds. Since the normal coordinate calculations are based on an isolated molecule approximation, they cannot predict the coupling of lattice modes with internal modes in this region.

All of the bands were found to be most intense when the electric vector of the incident light was parallel to the fiber axis, suggesting that the OH

groups are oriented predominantly parallel to the chain axis. The bands are not clearly resolved, however, so it is possible that some of the bands might be more intense with the electric vector perpendicular to the chain axis.

Although the OH bands in both the Valonia and ramie spectra appear to be most intense with the incident electric vector parallel to the fiber axis, the band frequencies differ significantly. The spectra of Valonia have a peak at 3231 $cm^{-1}$ that is not observed in the spectra of ramie. The spectra of ramie, on the other hand, have a peak at 3429 $cm^{-1}$ that is not observed in Valonia spectra. The frequency differences suggest that the hydrogen bonding pattern in Valonia cellulose differs from the hydrogen bonding pattern in ramie cellulose. These differences in the hydrogen bonding patterns are related to the structural differences between the $I_\alpha$ and $I_\beta$ forms of native cellulose which are discussed in more detail elsewhere.[17,27]

## CONCLUSIONS

Based on the relationships between band intensities and the polarization of the incident light, the bands in the Raman spectrum of cellulose were classified into four groups. The classification of the bands in this manner revealed information about the direction of the vibrational motions in cellulose. The directions of the vibrations are such that the major change in polarizability associated with the motions is either parallel or perpendicular to the chain axis. Raman spectra recorded from deuterated celluloses allowed the vibrational modes involving CH and OH motions to be identified. These spectra demonstrated that most of the modes are complex coupled vibrations. Results from normal coordinate analyses of cellulose model compounds were used to determine the types of motion most likely to occur in each region of the

spectrum. These calculations also suggested that the vibrational motions are very complex. The information from the normal coordinate calculations, intensity studies, and spectra of deuterated celluloses aided in the interpretation of the vibrational spectrum of cellulose. The importance of these results, even though they are not complete assignments, lies in the foundation they establish for microprobe studies of native tissues by providing a thorough characterization of the bands in the vibrational spectrum of cellulose.

ACKNOWLEDGMENT

The authors wish to thank Dr. H. L. Crespi for furnishing the sample of deuterated bacterial cellulose and for his advice concerning the growth of algae in heavy water, and Drs. U. P. Agarwal and N. S. Thompson for the many helpful discussions they contributed to this work. Portions of this work were used by J. H. Wiley as partial fulfillment of the requirements for the Ph.D. degree at The Institute of Paper Chemistry.

REFERENCES

1. P. Dhamelincourt, F. Wallart, M. Leclercq, A. T. N'Guyen, D. O. Landon, Anal. Chem. 51 (1979) 414A-421A.

2. G. J. Rosasco Advances in Infrared and Raman Spectroscopy. R. J. H. Clark and R. E. Hester, Eds. Heyden, London, 3 (1980).

3. M. E. Andersen, R. Z. Muggli, Anal. Chem. 53 (1981) 1772-1777.

4. L. J. Pitzner An investigation of the vibrational spectra of the 1,5-anhydropentitols. Doctoral Dissertation. Appleton, WI, The Institute of Paper Chemistry (Jan. 1973) 402 p.

5. L. J. Pitzner, R. H. Atalla, Spectrochimica Acta 31A (1975) 911-929.

6. G. M. Watson An investigation of the vibrational spectra of the pentitols and erythritol. Doctoral Dissertation. Appleton, WI, The Institute of Paper Chemistry (June 1974) 178 p.

7. S. L. Edwards An investigation of the vibrational spectra of the pentose sugars. Doctoral Dissertation. Appleton, WI, The Institute of Paper Chemistry (Jan. 1976) 245 p.

8. R. M. Williams An investigation of the vibrational spectra of the inositols. Doctoral Dissertation. Appleton, WI, The Institute of Paper Chemistry (June 1977) 377 p.

9. R. M. Williams, R. H. Atalla, J. Phys. Chem. 88 (1984) 508-519.

10. H. A. Wells An investigation of the vibrational spectra of glucose, galactose, and mannose. Doctoral Dissertation. Appleton, WI, The Institute of Paper Chemistry (Jan. 1977) 431 p.

11. K. P. Carlson An investigation of the vibrational spectra of the cellodextrins. Doctoral Dissertation. Appleton, WI, The Institute of Paper Chemistry (Jan. 1979) 153 p.

12. M. Tsuboi, J. Polym. Sci. 25 (1957) 159-171.

13. C. Y. Liang, R. H. Marchessault, J. Polym. Sci. 39 (1959) 269-278.

14. H. G. Higgins, C. M. Stewart, K. J. Harrington, J. Polym. Sci. 51(1961) 59-84.

15. J. Blackwell, P. D. Vasko, J. L. Koenig, J. Appl. Phys. 41 (1970) 4375-4379.

16. B. L. Browning Methods in Wood Chemistry. Wiley Interscience, New York, 2 (1967) p.295.

17. J. H. Wiley Raman Spectra of Plant Celluloses. Doctoral Dissertation. Appleton, WI, The Institute of Paper Chemistry, 1986.

18. H. L. Crespi Biosynthesis with deuterated microorganisms. Technical Committee Meeting on Modern Trends in the Biological Applications of Stable Isotopes., Leipzig, Germany. (Dec. 6-10, 1976) p. 1-15.

19. R. G. Snyder, J. Mol. Spect. 37 (1971) 353-365.

20. R. H. Atalla, Appl. Polym. Symp. 28 (1976) 659-669.

21. R. H. Atalla Spectroscopic studies of polymorphy in cellulose: a new structural model. Proceedings of the International Symposium on Wood and Pulping Chemistry, Stockholm, June, 1981. SPCI Report No. 38, 1 (1981) 57.

22. S. Krimm, J. Chem. Phys. 32 (1960) 1780-1783.

23. D. L. VanderHart, R. H. Atalla, Macromolecules 17 (1984) 1465-1472.

24. M. Sundaralingam, Biopolymers 6 (1968) 189-213.

25. C. Y. Liang, R. H. Marchessault, J. Polym. Sci. 37 (1959) 385-395.

26. R. Zbinden Infrared Spectroscopy of High Polymers, Academic Press, New York, 1964.

27. J. H. Wiley, R. H. Atalla, ACS Symposium Series, in press.

Table I. Summary of intensity maxima, deuteration sensitivities, and band assignments for the Raman spectra of Valonia and ramie.

| Band Frequency[a] ($cm^{-1}$) Valonia | ramie | Intensity Classification | Deuteration Sensitivity | Assignment |
|---|---|---|---|---|
| 331 | 331 | $A_0$ | weak | heavy atom bending, |
| 344 | 344 | $B_?$ | " | some heavy atom stretching |
| 381 | 380 | $B_?$ | weak | " |
| 437 | 437 | $B_?$ | " | " |
| 459 | 458 | $B_0$ | " | " |
| 520 | 519 | $A_{90}$ | " | " |
| 913 | 910 | $B_0$ | ? | HCC and HCO bending at C6 |
| 968 | 969 | $B_{90}$ | ? | heavy atom (CC and CO) |
| 997 | 995 | $A_0$ | ? | stretching |
| 1034 | 1037 | $A_0$ | ? | " |
| 1057 | 1057 | $A_0$ | ? | " |
| 1095 | 1095 | $A_0$ | weak | " |
| 1118 | 1117 | $B_0$ | " | " |
| 1123 | 1121 | $A_0$ | " | " |
| 1152 | 1151 | $B_?$ | ? | heavy atom stretching plus HCC and HCO bending |
| 1279 | 1275 | $A_0$ | ? | HCC and HCO bending |
| 1292 | 1291 | ? | ? | " |
| 1334 | 1331 | $A_0$ | strong | " |
| 1337 | 1337 | $A_0$ | " | HCC, HCO, and HOC bending |
| 1378 | 1378 | $B_?$ | " | " |
| 1406 | 1407 | A0 | " | " |
| 1455 | 1456 | $B_{90}$ | " | HCH and HOC bending |
| 1477 | 1475 | $A_{90}$ | " | " |
| 2868 | 2866 | $B_{90}$ | " | CH and $CH_2$ stretching |
| 2885 | 2889 | $B_{90}$ | " | " |
| 2941 | 2943 | $B_?$ | " | " |
| 2965 | 2963 | $B_0$ | " | " |
| 3291 | 3286 | $B_0$ | " | OH stretching |
| 3334 | 3335 | $?_0$ | " | " |
| 3361 | 3363 | $?_0$ | " | " |
| 3395 | 3402 | $B_0$ | " | " |

a Only the bands resolved in both the Valonia and ramie are included in the table.

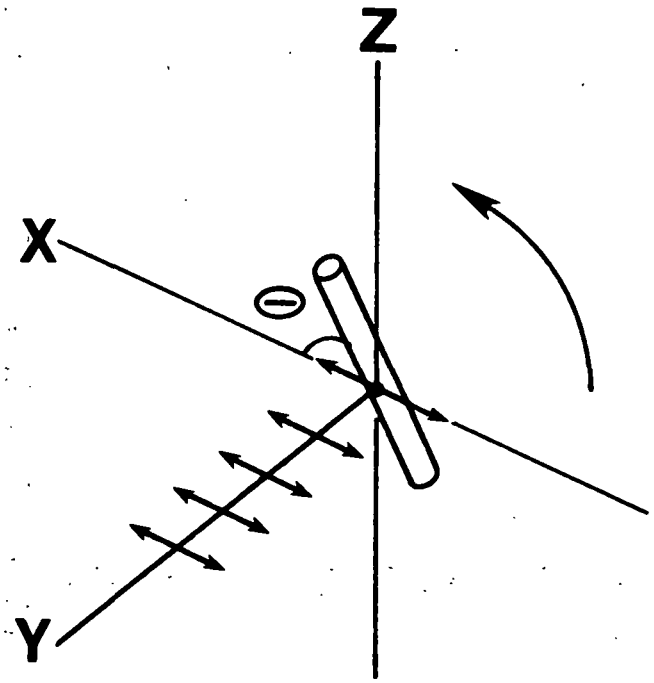


Fig. 1. Representation of the experiment in which the angle between the electric vector of the incident light and the fiber axis was varied from 0 to 90° by rotating the fiber relative to the plane of polarization of the incident light.

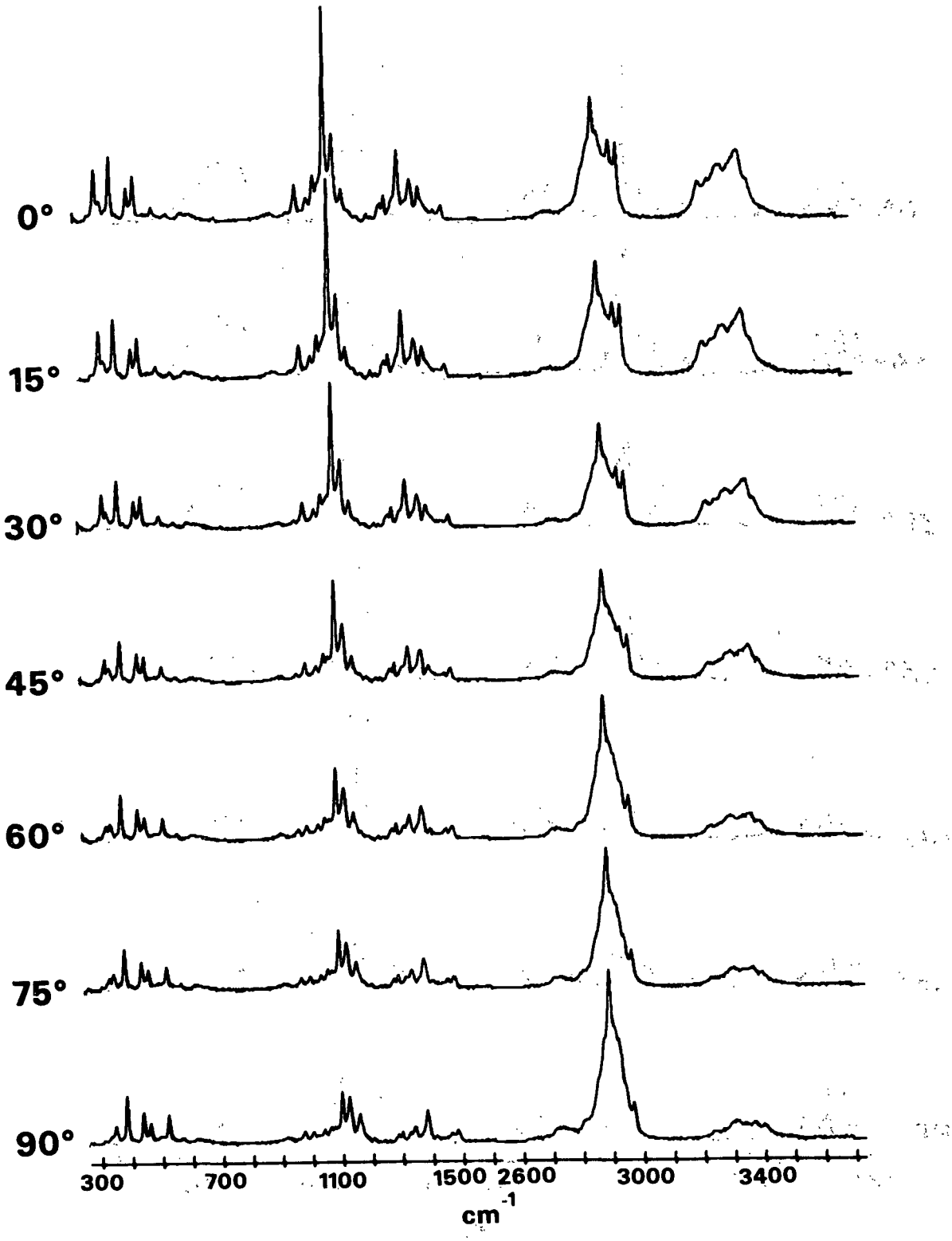


Fig. 2. Polarized Raman spectra of a Valonia fiber. The angle between the electric vector of the incident light and the fiber axis was varied from 0 to 90° in 15° increments.

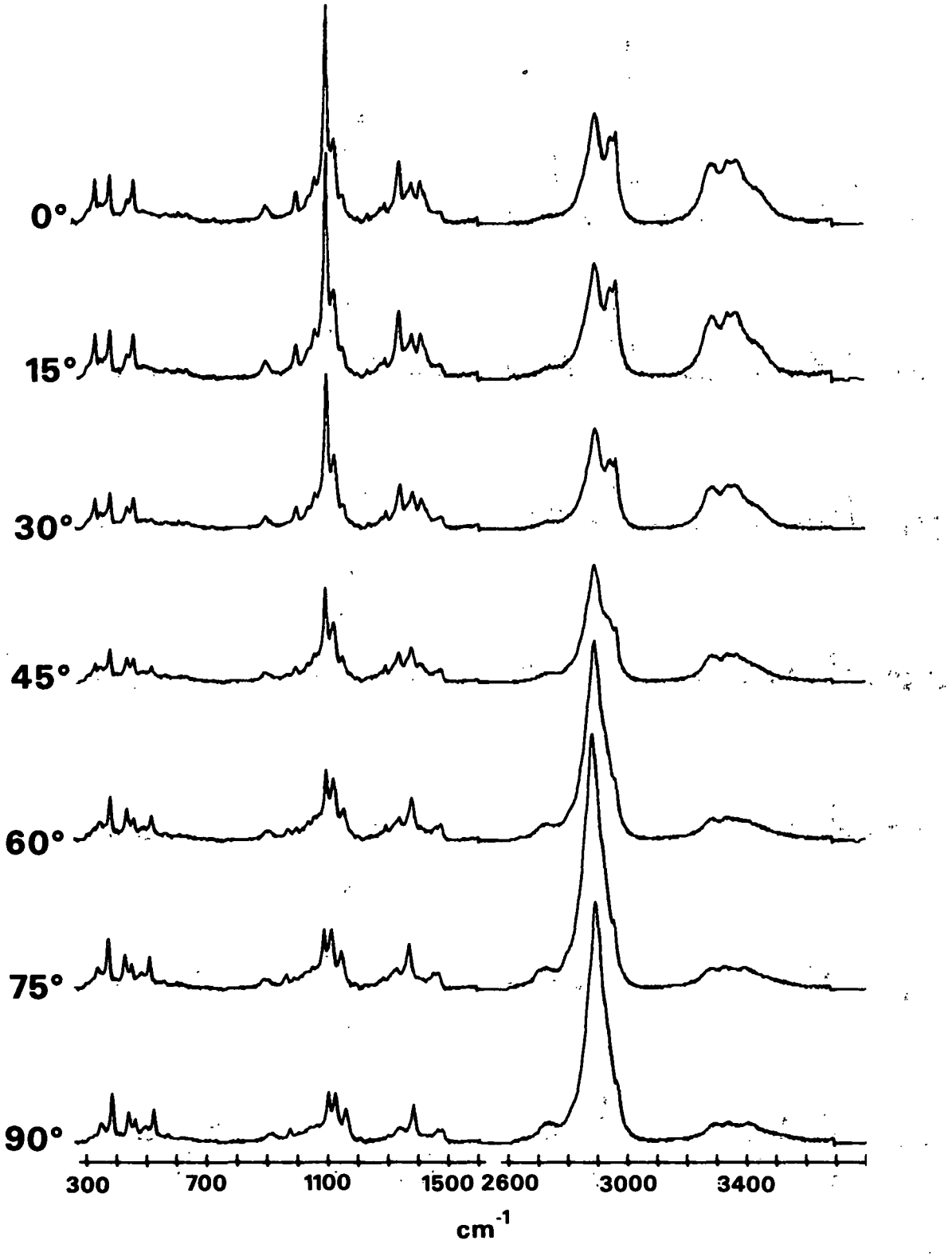


Fig. 3. Polarized Raman spectra of a ramie fiber. The angle between the electric vector of the incident light and the fiber axis was varied from 0 to 90° in 15° increments.

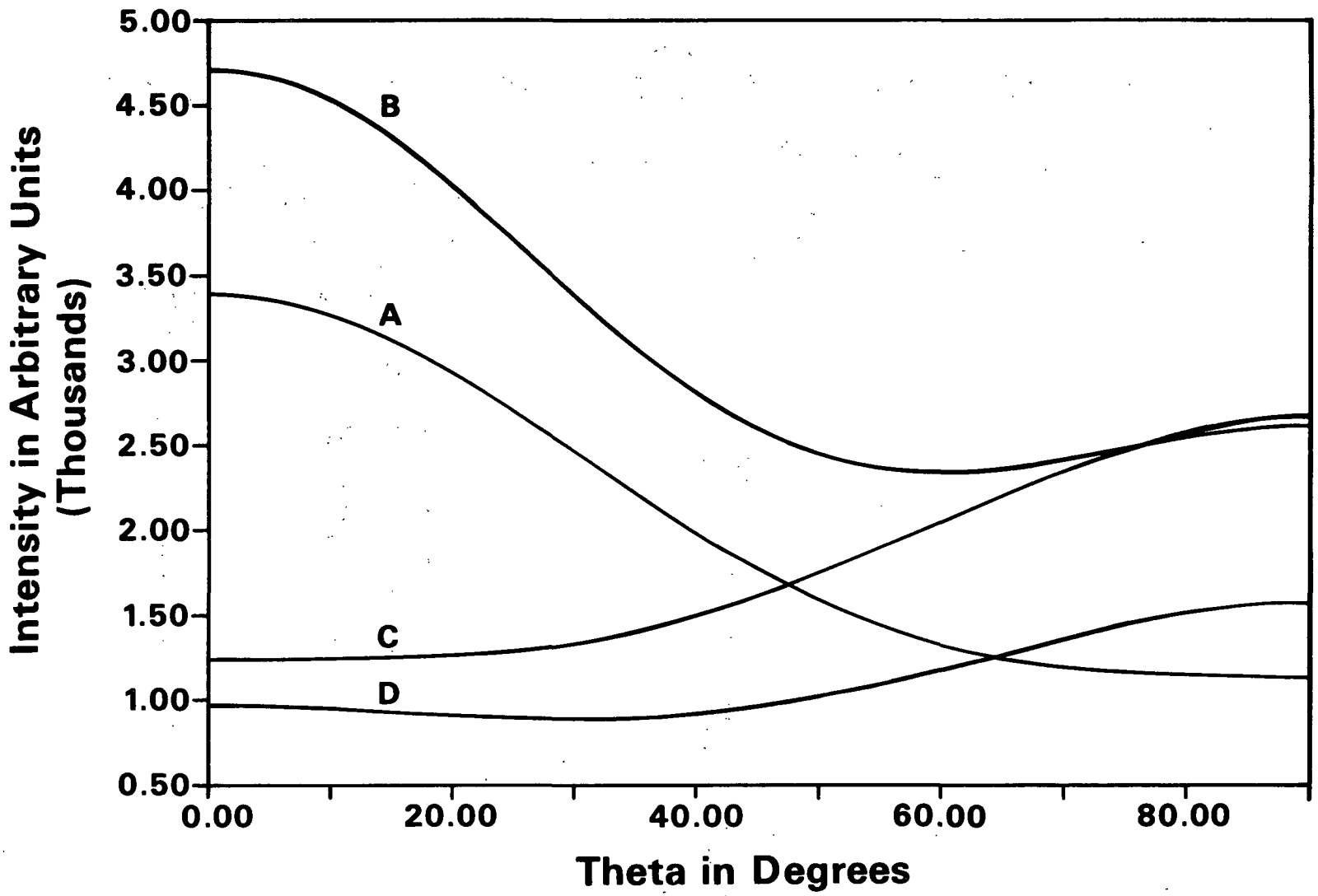


Fig. 4. Plots of intensity vs. the angle between the incident electric vector and the fiber axis. a) $A_0$ band, b) $B_0$ band, c) $A_{90}$ band, and d) $B_{90}$ band.

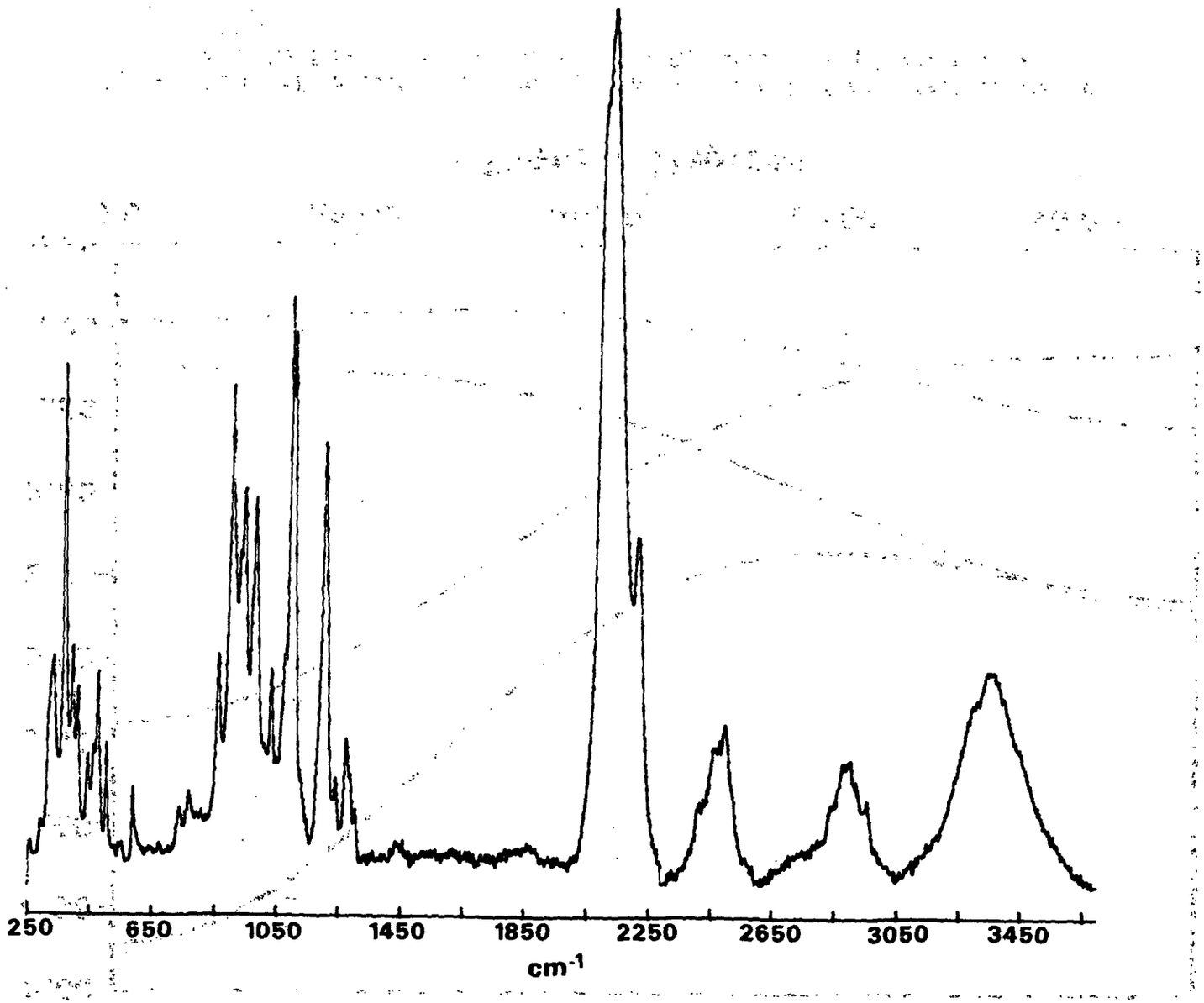


Fig. 5. Raman spectra of a pellet of deuterated bacterial cellulose.

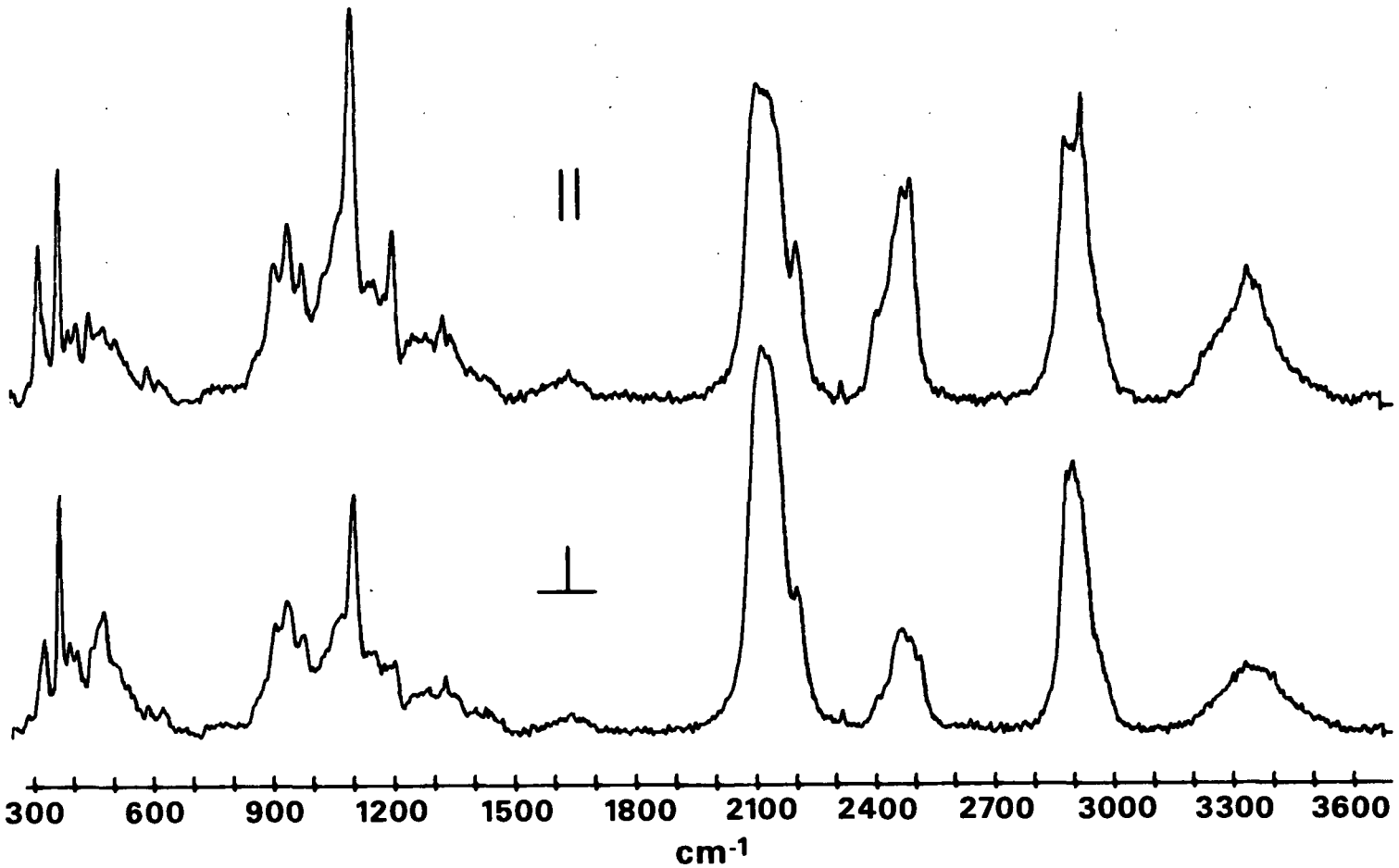


Fig. 6. Raman microprobe spectra of a deuterated Cladophora fiber. a) electric vector parallel to fiber axis, b) electric vector perpendicular to the fiber axis.