

THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

**IPC TECHNICAL PAPER SERIES
NUMBER 217**

**STUDIES ON THE STRUCTURE OF CELLULOSE USING
RAMAN SPECTROSCOPY AND SOLID STATE ^{13}C NMR**

RAJAI H. ATALLA AND DAVID L. VANDERHART

JANUARY, 1987

Studies on the Structure of Cellulose Using Raman Spectroscopy
and Solid State ^{13}C NMR

Rajai H. Atalla and David L. VanderHart

This manuscript is based on results obtained in IPC Project 3288 and is to be presented at the International Symposium on Wood and Pulping Chemistry in Paris, France on April 26-30, 1987

Copyright, 1987, by The Institute of Paper Chemistry

For Members Only

NOTICE & DISCLAIMER

The Institute of Paper Chemistry (IPC) has provided a high standard of professional service and has exerted its best efforts within the time and funds available for this project. The information and conclusions are advisory and are intended only for the internal use by any company who may receive this report. Each company must decide for itself the best approach to solving any problems it may have and how, or whether, this reported information should be considered in its approach.

IPC does not recommend particular products, procedures, materials, or services. These are included only in the interest of completeness within a laboratory context and budgetary constraint. Actual products, procedures, materials, and services used may differ and are peculiar to the operations of each company.

In no event shall IPC or its employees and agents have any obligation or liability for damages, including, but not limited to, consequential damages, arising out of or in connection with any company's use of, or inability to use, the reported information. IPC provides no warranty or guaranty of results.

STUDIES ON THE STRUCTURE OF CELLULOSE USING
RAMAN SPECTROSCOPY AND SOLID STATE ¹³C NMR

Rajai H. Atalla
The Institute of Paper Chemistry
Appleton, WI 54912 U.S.A.

and

David L. VanderHart
National Bureau of Standards
Gaithersburg, MD 20899 U.S.A.

ABSTRACT

Studies of cellulose using Raman and ¹³C NMR spectroscopy reveal new dimensions to the complexity of its structures and their relative stabilities. We present further evidence in support of our hypothesis that native celluloses are composites of two crystalline modifications, I_α and I_β, which coexist in most native forms, and we elaborate on our understanding of these structures and their relative stabilities. We also present studies of the transformation of algal cellulose to cellulose III, and its reversion to cellulose I, which are relevant to the broader question of stability of the native forms of cellulose.

Summary of Prior Results

In our past studies (1,2) the resonance multiplicities in the solid state ¹³C NMR spectra of native celluloses were examined at high resolution for a variety of native forms as well as samples of regenerated celluloses I and II. These spectra are an important source of new structural information that is complementary to that from other sources.

The spectra of pure samples of cellulose II can be rationalized in terms of nonequivalent sites within a unique unit cell. The spectra of the native celluloses, however, reveal multiplicities that cannot be ascribed to a unique unit cell. In particular, the sharper components of the C-4 and C-6 resonances, as well as the C-1 resonance, possess multiplicities that suggest that they arise from magnetically nonequivalent sites within crystalline domains. The narrow lines observed differ among the samples of native forms; the relative intensities are not constant and they are not in the ratios of small whole numbers as would be expected if they arose from different sites within a single unit cell.

We have found the proposal that native celluloses are composites of two distinct crystalline forms, identified as I_α and I_β, the most plausible basis for interpretation of the spectra. A decomposition of the spectra based

on such a model has been described in our earlier reports. The spectra were obtained by taking appropriate linear combinations of the spectra of the regenerated cellulose I and of the cellulose from *Acetobacter xylinum*; these two celluloses were judged to be the closest to the two extremes on the basis of a two component model.

In studies of the Raman spectra one of us (3) addressed the question of conformational states in the two forms of native cellulose. The question was whether the two forms I_α and I_β represent true solid state allomorphs, with molecules possessing identical conformations, or whether like celluloses I and II they contain molecules with different conformations as well as different lattices.

Comparisons of the Raman spectra in the conformation sensitive region revealed a great deal of similarity in the spectra of the native celluloses included in the previous studies using ¹³C NMR. In the OH stretching region, on the other hand, there were significant differences. These observations have been described in detail in recent reports by Wiley and Atalla (4,5), and lead to the conclusion that the I_α had I_β forms of cellulose represent lattices with nearly identical conformations of the heavy atom molecular skeletons, but with different hydrogen bonding patterns.

Recent Spectral Studies

The more recent studies undertaken in our laboratories have been directed at understanding the nature of the differences between the I_α and I_β forms. They have included additional Raman spectral studies, NMR relaxation studies, and studies on susceptibility to chemical reactions.

The Raman spectral studies have focused on the OH stretching region of the spectra. As noted earlier, Wiley and Atalla had found significant differences in this region between the spectra of celluloses in which the I_α and I_β forms were dominant. In an extension of this work, the question arose as to whether the different spectra could be resolved into those of the I_α and I_β components.

The spectra used in the resolution were those of the regenerated cellulose I which was known to be essentially of the pure I_β form, and that of *Cladophora glomerata*, an alga which produces celluloses which are very similar to those from *Valonia ventricosa*. Beginning with the proportions of the two forms determined from the Solid State ¹³C NMR spectra, it was possible to resolve the Raman spectra in the OH stretching region into two component spectra corresponding to the two pure forms.

The two component spectra are shown in Fig. 1, where it is seen that the I_α spectrum possesses bands which do not occur in the I_β spectrum and vice versa. Though in the studies by Wiley and Atalla it was not possible to distinguish the bands seen in Fig. 1, it was clear that the low frequency band in the spectrum of the I_α component does not occur in the spectrum of the I_β component. Similarly, the high frequency shoulder that is pronounced in the spectrum of the I_β component does not occur in the spectrum of the I_α component.

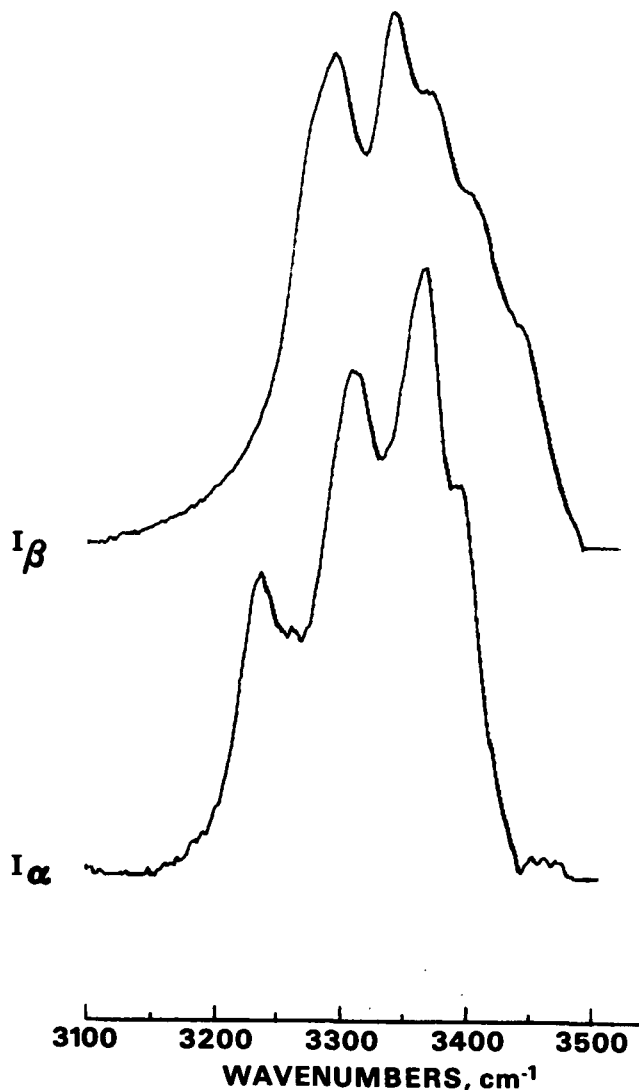


Figure 1. The resolved spectra of the I_α and I_β components of native celluloses in the OH stretching region of the Raman spectra.

In the investigations of Solid State ^{13}C NMR spectra, relaxation studies on both protons and carbon nuclei were used to confirm that unit cell inequivalences rather than crystal surface chain resonances determine the profiles of the sharp multiplets. It was also found that the higher plant celluloses contain a smaller fraction of

the I_α component than had previously been proposed.

Experiments based on weak ^{13}C - ^{13}C spin exchange were also conducted to probe the spatial environment, within a 0.7-1.0 nm radius, around carbons identified with individual multiplet components, which are assumed to belong exclusively to the I_α or I_β forms. The spectra of "nearest neighbors" were isolated for three different multiplet lines in an algal cellulose and for two lines in a higher plant cellulose. The results rule out the possibility that tertiary morphology can give rise to any multiplicity in these spectra. The results also provide strong support for the hypothesis of multiple crystalline forms in the algal celluloses; however, no clear evidence for multiple crystalline forms in the higher plant cellulose is found by this method.

In the studies of susceptibility to chemical reaction, samples of *Cladophora glomerata* cellulose were subjected to hydrolysis in boiling acid, and the resulting changes in the ^{13}C NMR spectra investigated. The hydrolysis was carried out in boiling 4N HCl for 44 hours. The mass yield was between 12 and 22%. The Solid State ^{13}C NMR spectra revealed a significant decline in the content of the I_α component. In one instance, shown in Fig. 2, the resulting spectrum suggested proportions of the two components similar to those in higher plants, though the resolution remained typical of the spectra of the algal celluloses.

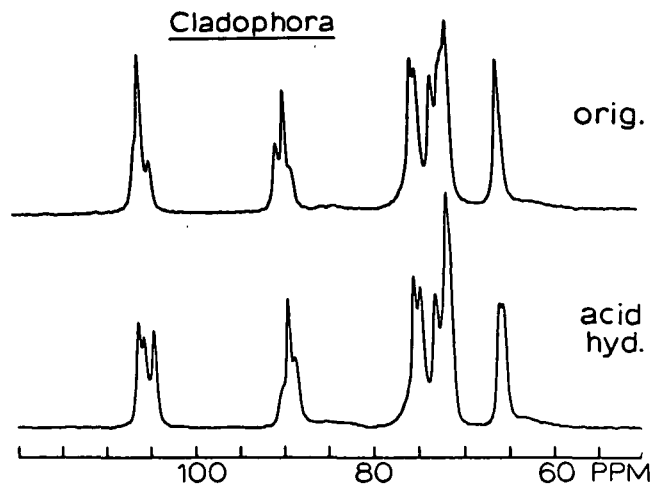


Figure 2. Solid state CP MAS ^{13}C NMR spectra of *Cladophora glomerata* cellulose before and after acid hydrolysis.

The results of the hydrolysis studies leave very little question that the susceptibility of the I_α form to acid hydrolysis is greater than that of the I_β form. It seems to us quite likely that differences in reactivity will be observed in other contexts as this matter becomes the subject of further studies.

Stability of Native Celluloses

In much of the early literature on polymorphism in cellulose it has been assumed that cellulose I is a less stable form of cellulose than cellulose II because the latter is the form most commonly recovered from solution. Although such an argument is not sound from a thermodynamic view, it has gained wide acceptance. One of us has questioned this on the basis of experimental results of regeneration studies (6,7). For example, it was found that celluloses regenerated at elevated temperatures into the cellulose IV lattice possessed Raman spectra indicating the presence of some cellulose in the conformation corresponding to cellulose I. That work also led to the preparation of regenerated cellulose I under more carefully controlled conditions (8).

In the context of this presentation it is interesting that the regenerated cellulose I has always been the pure I_{β} form as identified by ^{13}C NMR spectra. We believe this is not unrelated to recent observations of the effects of conversion of cellulose I to cellulose III, and its reversion to cellulose I upon boiling in water, which seem to alter the balance between the I_{α} and the I_{β} forms.

In this new series of observations samples of cellulose from Cladophora glomerata were converted to the cellulose III form according to a procedure suggested by Hayashi (9). The x-ray diffractogram of the converted form showed it to be of the highest crystallinity, approaching the same order of crystallinity as that of the native form. The solid state ^{13}C NMR spectra, shown in Fig. 3, differed from any that we had previously recorded for cellulose, in that none of the resonances of the carbons in the anhydroglucose units showed any multiplicities. Thus, the lattice and the molecular conformation were thought likely to be distinct.

Raman spectra confirmed our expectations on the basis of the NMR spectra. As shown in Fig. 4, the conformation sensitive region between 200 and 700 cm^{-1} showed some dramatic differences between the spectra of the cellulose III and that of the Cladophora cellulose from which it was prepared, as well as distinct differences between the cellulose III spectrum from that of cellulose II. These differences point to a change in the conformation of the cellulose skeleton, though not necessarily a very large one.

When the sample of cellulose III was boiled in water, it reverted to cellulose I. However, the Solid State ^{13}C NMR spectra, also shown in Fig. 3, revealed it to have been transformed from a cellulose in which the I_{α} form was dominant to

a cellulose in which the I_{β} form is dominant. Indeed, the spectra seemed to be quite similar to those expected from a highly crystalline higher plant cellulose. As seen in Fig. 5, the Raman spectrum also reverted, in the conformation sensitive region, to one characteristic of a higher plant crystalline cellulose similar, for example, to that of hydrocellulose from ramie. In the OH stretching region the spectrum of the recovered cellulose I was typical also of the celluloses in which the I_{β} form is dominant.

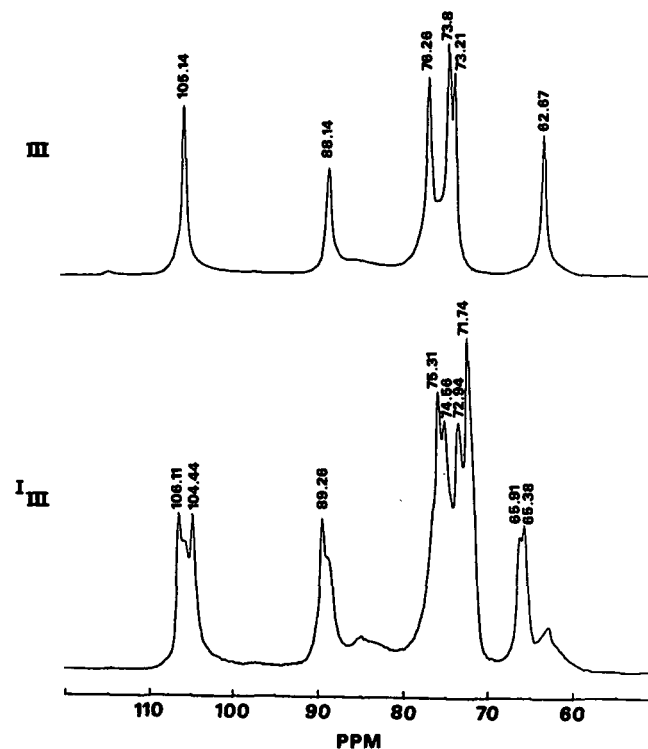


Figure 3. Solid state CP MAS ^{13}C NMR spectra of cellulose III prepared from Cladophora glomerata, and after recovery of cellulose I by boiling in water.

These observations suggest to us that the I_{β} form may be the more stable of the two native forms of cellulose. But they also suggest that the general assumption that cellulose I is a less stable lattice needs to be reexamined. If cellulose II were the more stable form, one would have expected the treatment in water to result in conversion of the cellulose III to cellulose II, particularly since both the Raman and ^{13}C NMR spectra leave little doubt that the original cellulose I lattice had been completely converted.

ACKNOWLEDGMENTS

The authors wish to acknowledge the help of Ms. Rebecca Whitmore and Mr. Clark Woitkovich in the preparation of some of the samples, and acquisition of the Raman spectra.

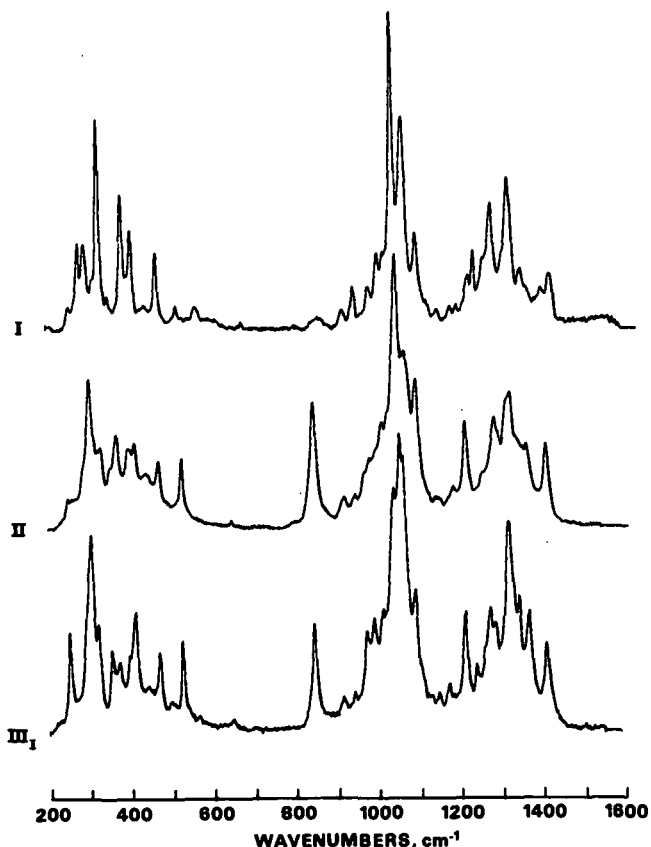


Figure 4. Raman spectra of celluloses I, II, and III, in the conformation sensitive region.

REFERENCES

1. ATALLA, R. H. AND VANDERHART, D. L. Native cellulose: a composite of two distinct crystalline forms. *Science* 223: 283-5 (Jan. 20, 1984).
2. VANDERHART, D. L. AND ATALLA, R. H. Studies of microstructure in native celluloses using solid state ^{13}C NMR. *Macromolecules* 17: 1465 (1984).
3. ATALLA, R. H. Polymorphism in native cellulose: recent developments. Structure, Function and Biosynthesis of Plant Cell Walls, W. M. Dugger and S. Bartnicki-Garcia, eds., American Society of Plant Physiologists, Rockville, MD, 1984. p. 381.
4. WILEY, J. H. AND ATALLA, R. H. Raman spectra of celluloses, in "Solid State Characterization of Cellulose." R. H. Atalla, ed., ACS Symposium Series, in press.
5. WILEY, J. H. AND ATALLA, R. H. Raman microprobe studies on cellulose fibers. Carbohydrate Research, special issue on physical chemical studies on macromolecular carbohydrates, in press.
6. ATALLA, R. H. AND NAGEL, S. C. Cellulose: its regeneration in the native lattice. *Science* 185: 522 (1974).
7. ATALLA, R. H., DIMICK, B. E., AND NAGEL, S. C. Studies of polymorphism in cellulose. Cellulose IV and some effects of temperature. ACS Symposium Series 48: 40-31 (1977). *Macromol.* 7: 182 (1985).
8. WHITMORE, R. E. AND ATALLA, R. H. Factors influencing regeneration of cellulose I from phosphoric acid solutions. *Int. J. Biol. Macromol.* 7: 182 (1985).
9. Hayashi, J., personal communication.

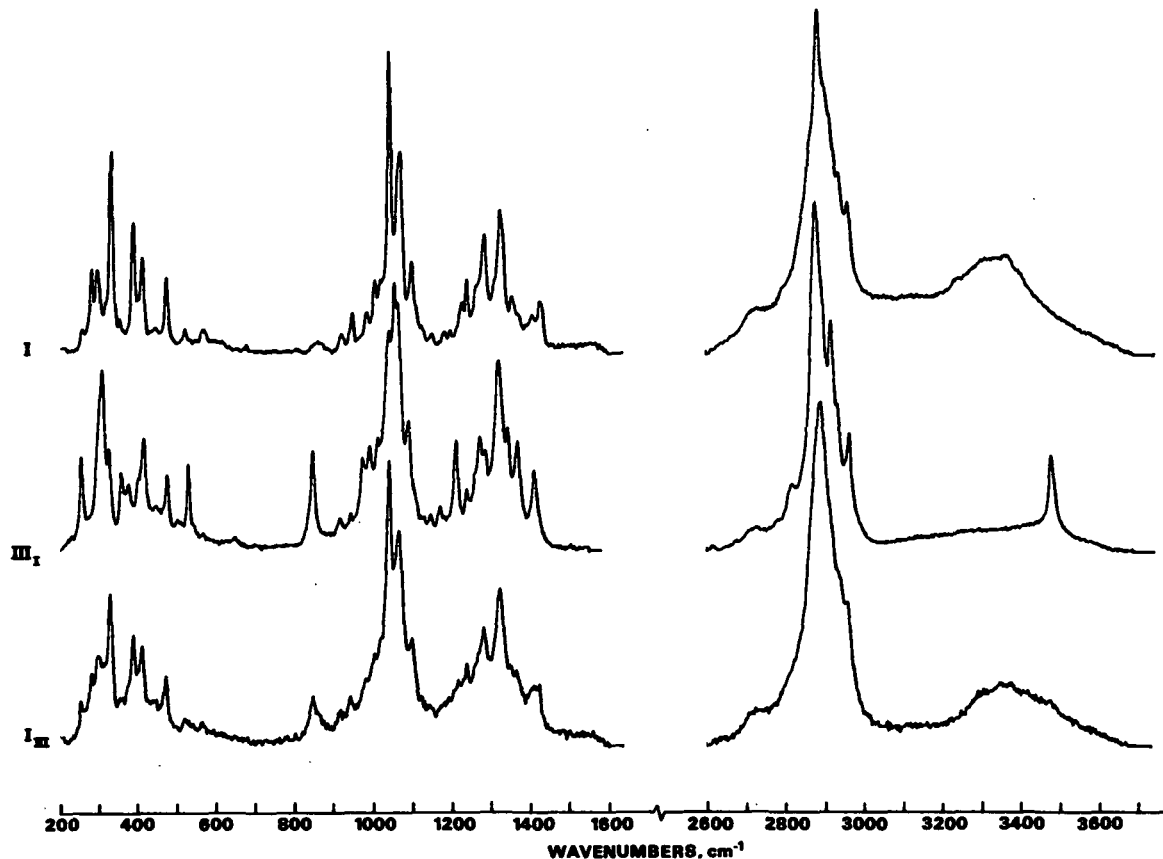


Figure 5. Raman spectra of *Cladophora* cellulose, in the native form, after conversion to cellulose III, and after reversion to the cellulose I lattice upon boiling in water.