APPLICATION OF INFRA-RED SPECTROSCOPY TO CELLULOSE AND WOOD POLYSACCHARIDES

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INTRODUCTION

The interpretation of the infra-red spectra of small molecules is one of the important triumphs of molecular physics. These spectroscopic studies have led to precise information concerning the rotational and vibrational properties of molecules and consequently their structure and shape. The spectra of polyatomic molecules, even symmetric ones, offer considerably greater difficulty due to the fact that there are *several* internuclear distances, *several* force constants and so on, which must be determined simultaneously. In the case of polymers, their very great asymmetry and polymorphism lead to complex selection rules and make it all but impossible to calculate the normal vibrations. As a result, the observation made over a decade ago[1] " . . . *the most that can be stated about a large organic molecule or polymer is that it does or does not contain certain chemical groups and even this statement has frequently to be hedged about with certain qualifications* " is still somewhat true.

As polymers go, polysaccharides are among the more difficult to study because the predominance of OH and CH groups with their similar absorption frequencies leads to ill-defined absorption maxima. The presence of several OH groups is in itself a sufficiently complicating factor but to this must be added the numerous hydrogen bonding possibilities which compound the number of different OH frequencies. However, recent reviews[2, 3] show clearly that the situation is far from hopeless. In the synthetic polymer field we are systematically approaching the ultimate goal of the polymer spectroscopist: to establish (i) band assignments, (ii) chain orientation, (iii) chain conformation, (iv) degree of crystallinity and (v) crystal structure, through the infra-red approach. The approach is mainly empirical basing itself on well-established properties of polymers and the known spectroscopic characteristics of the small molecules that constitute the polymer. Some of the foregoing goals are closely related to results which are generally derived from the X-ray diffraction method. There is ample evidence that the two techniques do indeed complement each other and examples of this in the polysaccharide field will be discussed below.

BAND ASSIGNMENT

Before structural or analytical information can be obtained from the infra-red spectrum of a polymer, accurate knowledge must be had of the relation between a given absorption band and the chemical group from

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which it arises. The achievement of this aim by theoretical analysis is out of the question for anything but the simplest polymer molecules. The spectra of the component crystalline carbohydrates are likewise of little help because the higher symmetry, greater crystallinity and different crystal structure of these small molecules often gives rise to much greater spectral complexity. There are times, however, when the spectra of low molecular weight water-soluble fragments (oligosaccharides), which crystallize with the same structure as the parent polymer, yield useful information. Reference will be made to this approach below.

The most straightforward approach to the problem of band assignments is through the familiar group frequencies. Empirically it has been found that, to a first approximation, a good part of the spectrum of a polymer

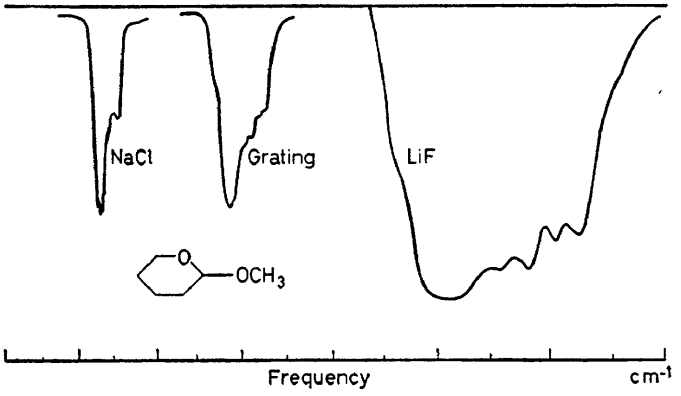


Figure 1. The spectrum of 2-methoxytetrahydropyran in the 2800 to 3000 cm^{-1} region with (*a*) NaCl prism, (*b*) grating of Perkin Elmer 221G instrument and (*c*) LiF prism

can be considered as a composite of the various bands which arise from the component groups within the monomer unit. Most simply this means that vibrational modes due to OH, NH, C=O stretching and CH_3 and CH_2 stretching and bending modes, *etc.*, have been found to be easily recognizable by their characteristic intensities and frequencies. This is not to say that there are never bands that do not admit of such a simple explanation. In particular, it is known that complex vibrational frequencies arise from combinations of the so-called internal group frequencies and the skeleton modes of polymers. The spectral characteristics which are related to the configurational and conformational properties of carbohydrates are likewise as yet uninterpreted. These are problems which remain to be solved. As the solutions are found we can expect a rapid application of the knowledge to the polymer field.

The group frequency approach can be illustrated by considering the spectra of model compounds based on tetrahydropyran. These spectra illustrate how confidence is acquired in the assignment of absorption bands to given chemical groups.

Pyran derivatives

The molecular skeleton of the most important carbohydrates of wood chemistry is the tetrahydropyran molecule. By examining the infra-red

spectrum of simple methyl and hydroxylated derivatives of this molecule it is possible to approach in stepwise fashion some of the characteristics of the spectrum of carbohydrates and polysaccharides in the 3 to 8μ region. The lack of crystallinity and liquid state of these molecular species also gives to their spectra characteristics which one associates with the spectra of very large molecules rather than those of well-crystallized small ones. The fact that these molecules readily undergo conformation changes makes them unsuitable, however, for identifying the ring vibrations and configurational characteristics of carbohydrate spectra which occur in the region beyond 8μ. (The whole field of conformational analysis of the simple pyran derivatives remains to be explored. The recent efforts[4, 5] of the Birmingham school along these lines as well as their pioneering efforts[6] in correlating configurational properties of carbohydrates with infra-red characteristics is recommended as first reference in this area. Some of the useful spectral differences between wood polysaccharides were first reported by them[6].)

By the use of a lithium fluoride prism, or a grating, much greater resolution is attainable in the C—H stretching region than one gets with simple rock-salt optics. The resulting spectra allow separation and assignment of specific bands to the various absorbing groups. An example of this increased resolution is shown in *Figure 1* where the spectrum of 2-methoxytetrahydropyran in the C—H stretching region is shown at three different degrees of resolution. It is evident that when more than one

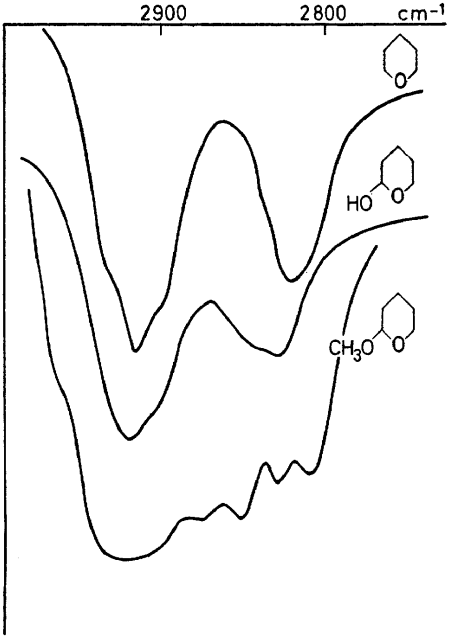


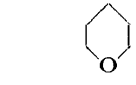
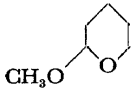
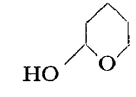
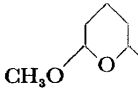
Figure 2. High resolution spectrum in the C—H region of 2-methoxytetrahydropyran, 2-hydroxytetrahydropyran and tetrahydropyran

alkane grouping is present in the molecule, sodium chloride optics are inadequate to resolve the various bands. The grating on the P.E. Model 221G is satisfactory for the spectrum in question but for highest accuracy the lithium fluoride prism is recommended for this purpose. When polarized beam techniques are used important conformational data can be derived from high resolution spectra in this region as we shall see further on.

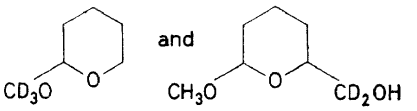
At least six separate absorption frequencies are detectable in the high resolution spectrum of 2-methoxytetrahydropyran. Four of them are at about the expected frequencies for the symmetric and antisymmetric modes of the methylene and *O*-methyl groups. Removal of the methyl group does indeed cause the disappearance of the 2835 and 2970 cm^{-1} bands, commonly associated with the *O*-methyl group, and perhaps of another band at 2893 cm^{-1} as may be judged from *Figure 2*. There is apparently a still further simplification of the spectrum on removal of the hydroxyl group. The two principal bands in tetrahydropyran are at the expected frequencies for the symmetric and antisymmetric stretching modes of a methylene group.

From spectra of the type shown in *Figure 2* the data of *Table 1* listing the frequencies assigned to the CH_2 modes (either on the ring or on the hydroxy methyl group) and the CH_3 modes of the methoxy group were compiled. It is worth noting as shown originally by Henbest and co-workers[20] that the low frequency absorption of the *O*-methyl is at 2835 or lower. This is quite a different frequency than the equivalent absorption band of the *C*-methyl group and important to know since the methoxyl grouping is a frequently occurring one in carbohydrates.

Table 1. Methylene and methyl group frequencies for tetrahydropyran and some of its derivatives

Compound	$\nu_s(CH_2)$	$\nu_a(CH_2)$	$\nu_s(CH_3)$	$\nu_a(CH_3)$
	2845	2935		
	2854	2935	2830	2980
	2853	2940		
	2850	2940	2835	2970

To confirm some of the assignments shown in *Table 1* we have made some deuterated analogues of the pyrans shown in the table, *viz.*



The benefit of such compounds in band assignment depends on the fact that the heavier deuterium atoms lead to lower stretching frequencies without affecting the remaining C–H group frequencies. The observed effect is shown in *Figure 3* where the two principal absorption frequencies of the CD_3 group are clearly visible. From the position of these bands it is possible to calculate the theoretical positions of the corresponding CH_3 modes. Generally it is more reliable to observe the high resolution spectra of the two compounds and observe the positions of the missing bands.

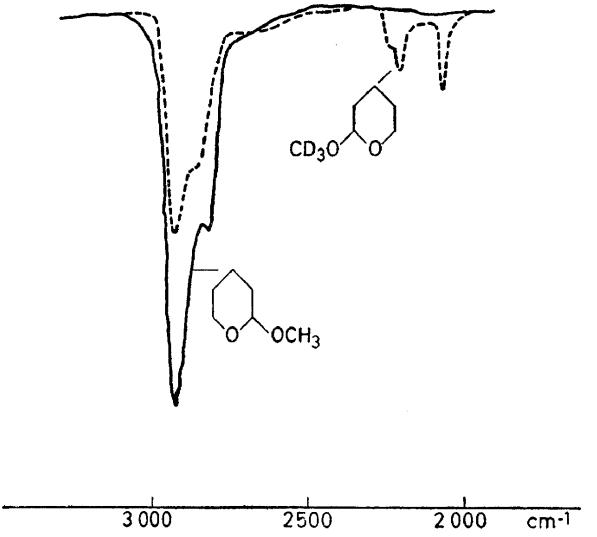


Figure 3. Spectrum of 2-methoxy-d_3-tetrahydropyran in the 2000 to 3000 cm^{-1} region, NaCl optics

One of the principal factors limiting resolution of polysaccharide spectra is the overlap of the CH and OH bending modes between 1500 and 1200 cm^{-1}. This problem is especially acute in the low crystallinity polysaccharides where multiple hydrogen bonds lead to multiplicity of OH modes. In such cases the deuteration technique, *i.e.* conversion of OH to OD, leads to a considerable sharpening of the spectrum in the region in question. As an example the problem is well illustrated by the spectrum of 2,3-dihydroxy-tetrahydropyran shown in *Figure 4*. Conversion of all OH to OD leads to the appearance of bands which are quite different from the ones in the original spectrum. It appears that the resolution of the latter was so poor

that little or no confidence can be placed in the recorded spectrum in this region. The absorption frequencies of the C—H bending modes can be determined from the spectrum of the deuterated material.

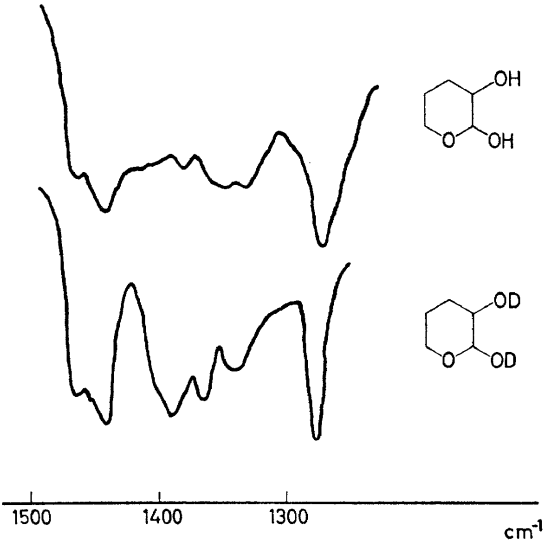


Figure 4. Spectrum of 2,3-dihydroxytetrahydropyran and the deuteroxy analogue in the 1200 to 1500 cm^{-1} region

Oligosaccharides

It is relatively easy to understand why the spectra of some neutral polysaccharides have been of little use for analytical or identification purposes. As was shown in the previous section, when one or more OH groups are attached to the pyran ring the C–H bending and O–H bending region suffers a considerable loss of resolution. The situation can be illustrated in the polysaccharide series by the spectrum of xylopentaose which is shown in the 1600 to 1200 cm^{-1} region in *Figure 5.* It is worth noting that the spectrum is identical to that of the " dry form " of the crystalline neutral xylan from esparto grass[7].

The spectrum of the original crystalline sample was recorded on a KBr disc. The band multiplicity and overlap is such as to make the spectrum useless in the 1500 to 1200 region. The situation is considerably improved by deuteration as shown in the same figure. The peak at 1465 is the expected CH_2 symmetric bending mode of the pyran ring and the blank in the spectrum from this region until the 1380 peaks confirms that this is indeed the region of strong O—H in-plane bending absorption. The shape of the band which fills in between the two peaks at 1465 and 1380 in the partially deuterated spectrum of *Figure 5* is probably characteristic of the O—H in-plane bending mode for low crystallinity polysaccharides in general, the breadth being due to the variety of environmental and hydrogen bonding possibilities.

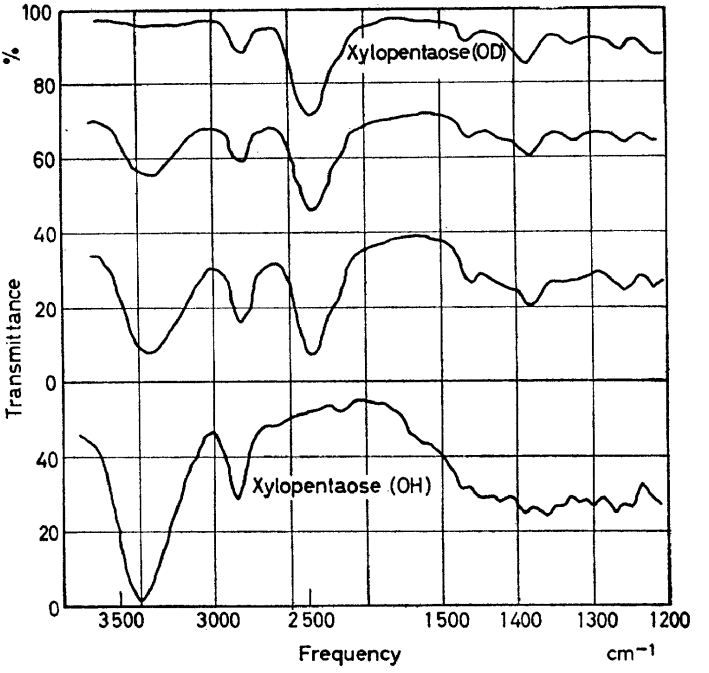
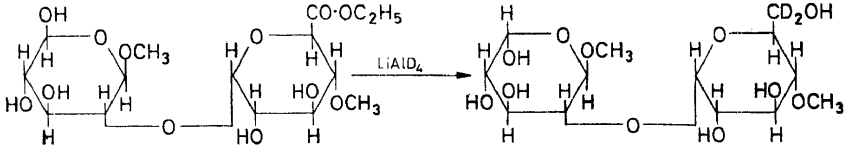


Figure 5. Spectrum of xylopentaose before and after treatment with deuterium oxide

The foregoing example illustrated one of the reasons why the oligosaccharides can be valuable aids in the interpretation of the polysaccharide spectra. The neutral xylan from esparto grass which is chemically equivalent to xylopentaose is considerably more difficult to deuterate. Another advantage of water-soluble oligosaccharides is that they are readily obtained in the completely amorphous form when freeze-dried from dilute aqueous solution.

The water solubility of oligosaccharides often allows chemical transformations which would be difficult or impossible with the parent polymer. For example, the transformations shown in equation (1) below:



could be readily performed on the aldobiuronic acid from xylan but only with difficulty and incompletely on the xylan itself. This transformation separated the methylene stretching frequencies due to the hydroxymethyl group of glucose from those of the xylopyranose ring. All these transformations are by way of establishing unequivocally the particular group responsible for a given absorption band.

Sometimes it is the property of high chemical purity combined with high crystallinity which makes a particular oligosaccharide valuable. The crystalline mannotetraose, for example, was particularly useful in establishing the exact positions of the absorption frequencies of the crystalline glucomannans which give poorly resolved spectra. Thus, only after deuteration (*cf. Figure 6*), does the infra-red spectrum of a crystalline mannan show the same resolution as that of the completely crystalline mannotetraose. Furthermore, deuteration of the mannan reveals an absorption band at 1600 indicating some impurity containing a carboxylic acid salt which is not present in the oligosaccharide. Finally, the contamination of the spectrum due to adsorbed water is almost negligible in the crystalline oligosaccharides. When using an oligosaccharide as a crystalline standard of a polysaccharide it is important that it should have the same unit cell as the parent polymer. This is the case for the regenerated mannan film and the crystalline mannotetraose used to obtain the spectrum shown in *Figure 6.*

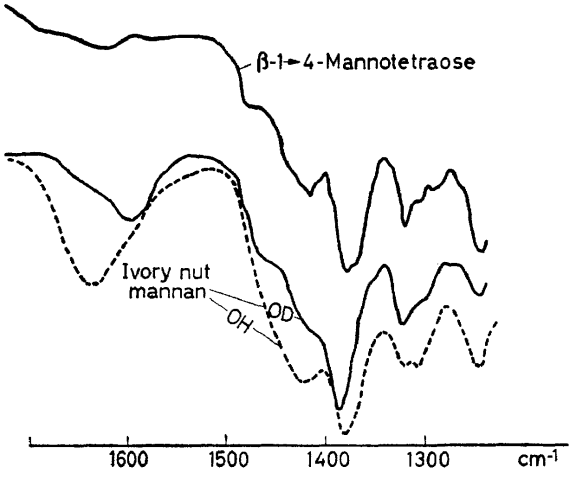


Figure 6. The spectrum of crystalline regenerated ivory nut mannan before and after deuteration compared to the spectrum of crystalline mannotetraose

Finally, if a particular oligosaccharide is not crystallizable and/or yields a rather ill-defined spectrum it is still sometimes possible to remedy the situation by conversion to the methyl derivative. *Figure 7* shows the spectra of methyl-2-*O*(4-*O*-methyl-α-D-glucopyranosyl)α,β-D-xylopyranoside and methyl-2-*O*(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)3-*O*-methyl-α,β-D-xylopyranoside in the 1500 to 1300 region. The effect of methylation is to remove the pronounced overlap of CH and OH bending modes. Since the CH_3 modes at 1450 and 1380 are easily identified, this treatment makes visible the shoulder at 1410 which most probably represents the methylene vibrations of the glucopyranosyl. The proper assignment of this band is important in the interpretation of the polarized spectrum of cellulose.

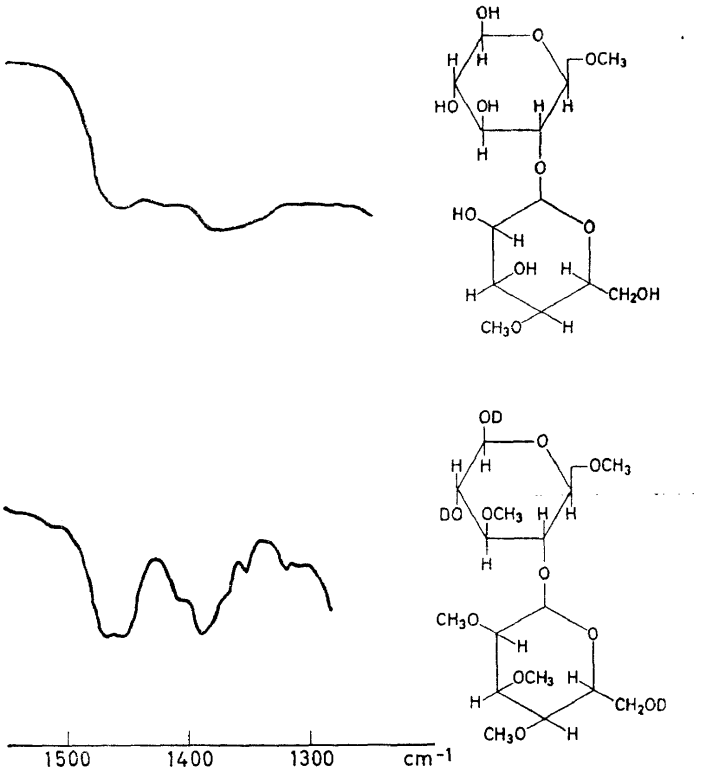


Figure 7. The spectra of methyl-2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)α,β-D-xylopyranoside and methyl-2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl) 3-*O*-methyl-α,β-D-xylopyranoside in the 1500 to 1300 cm^{-1}; the latter was treated with D_2O to convert residual OH to OD

Wood polysaccharides

We come now to the actual spectra of the polysaccharides which occur in wood. A list of the component sugars and the important polysaccharides which we may expect to encounter is shown in *Table 2.* From the table one deduces that a total of no less than six different sugars can be expected to contribute significantly to the spectrum of the holocellulose and, in the case of wood, a number of bands due to lignin must be added. A typical wood spectrum is shown in *Figure 8.* Obviously, if some use other than

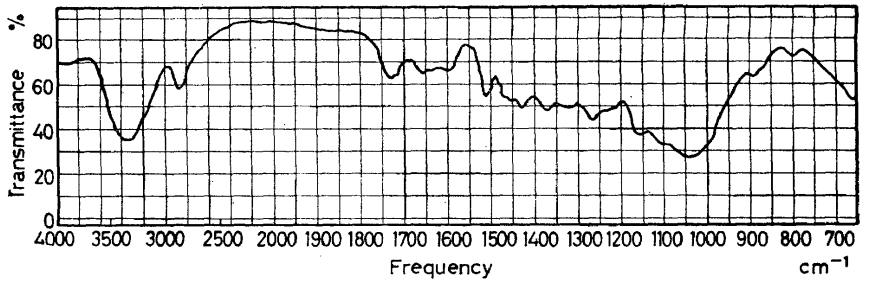


Figure 8. Infra-red spectrum of wood powder from white spruce in KBr disc

simple " fingerprinting " is to be derived from such a spectrum it will be necessary to study in detail the spectra of the component polysaccharide so that the absorption bands may be associated not only with a given component but with a given group in this component. Up to now two of the wood polysaccharides have been studied in detail: cellulose[8, 9] and xylan[10]. An outline of the semi-empirical approach which is used to make a full assignment of the absorption bands in a polymer has been given[3]. The following section will illustrate some aspects of the approach as it applies to wood polysaccharides.

Xylans—Xylans are one of the two most important hemicelluloses; they constitute about 25 to 33 per cent of the weight of hardwoods. To study

Table 2. Principal wood components and characteristic absorption bands in infra-red spectrum

Polymer	*Component monomer*	*Characteristic absorption* (cm^{-1})
Cellulose I	β-D-glucose	1425, 663
Xylan	β-D-xylose 4-*O*-methyl-α-D-glucuronic acid β-L-arabinose	890, 1735
Glucomannan	β-D-mannose β-D-glucose	805, 870
Water-soluble Polysaccharides	β-D-galactose β-L-arabinose	768
Lignin	Phenylpropane	1505, 1595

the spectrum of this material sample films were prepared by autoclaving aqueous slurries of alkali-extracted xylans to obtain solutions which were then air-dried on a glass plate. The chemical structure of a typical xylan is shown in *Figure 9.* The actual spectrum before and after deuteration is shown in *Figure 10.* The spectrum bears a good deal of similarity to that of cellulose as one might expect from the similarities between xylose and glucose. The 4-*O*-methyl glucuronic acid group leaves a characteristic

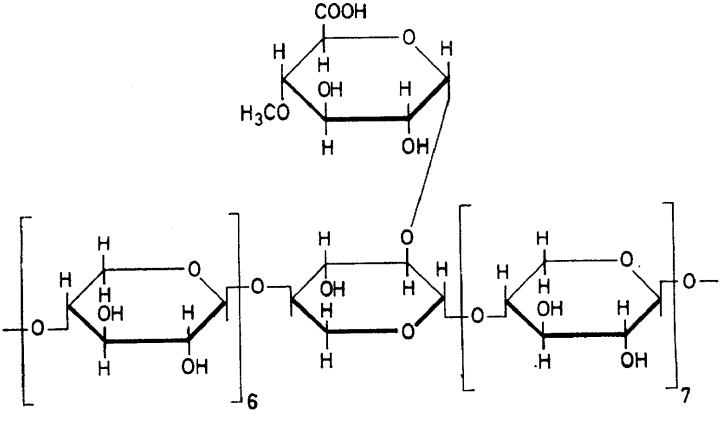


Figure 9. Chemical structure of 4-*O*-methylglucuronoxylan from milkweed

imprint on the spectrum contributing the strong absorption at 1600 and 1425. Since the moisture uptake of xylan is particularly large, the crystalline part itself being a hydrate[7], deuteration of all OH in the sample is both simple and valuable. From such a spectrum a fairly complete assignment of the CH and OH frequencies can be made[10]. Similar studies on other xylans from hard and softwoods have led to the same results despite small differences in chemical composition.

One may then ask what practical application can be made knowing the relation between a given absorption band and the chemical group from which it springs. The analytical applications are far from elegant at present. One can tell whether the carboxyl group is present as free acid or salt, depending on whether the absorption is at 1730 or 1600. In order to see the 1600 band clearly, deuteration is advisable. The intensity of these bands is related to the amount of carboxyl as may be judged from the relative intensities of the 1600 bands in *Figure 10.* Milkweed xylan is

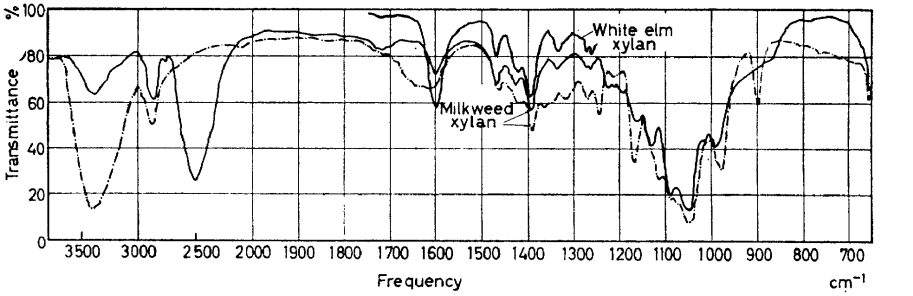


Figure 10. Infra-red spectra of 4-*O*-methylglucuronoxylan salts; deuterated — ; — · — · — rehydrogenated

known[11] to have about half as much glucuronic acid/xylose as white elm. Acetylation is easily detected when both the 1730 and the 1250 bands are strong. These applications are all fairly obvious and it is discouraging to think that there is no spectral characteristic whereby one can even recognize the presence of arabinose groups, for example, which occur to fairly high percentage in softwood xylans.

Cellulose—Band assignments are greatly facilitated when a polarized spectrum of the sample is available. For such a spectrum a uniaxially oriented polymer sample is required. The spectrum is then recorded with the incident infra-red beam polarized: (*A*) parallel to the fibre axis and (*B*) perpendicular to the fibre axis. Bands which show greater absorption in one case than in the other are said to be dichroic. If absorption is greater in *A* than in *B* then the band is parallel polarized (π band). If the reverse is true then the band is perpendicularly polarized (σ band). The ratio of the two optical densities is called the dichroic ratio.

A polarized spectrum of native cellulose crystallites[9] in the C—H stretching region is shown in *Figure 11.* Remembering that the C—H bonds of each glucose residue are approximately at right angles to the axis of the cellulose molecule it is evident that the absorption bands corresponding to

these vibrational modes must show maximum absorption when the incident beam is polarized perpendicular to the fibre axis assuming there is cylindrical symmetry about the molecular axis. With this in mind we readily assign the central σ bands at about 2900 to the C—H stretching modes. The

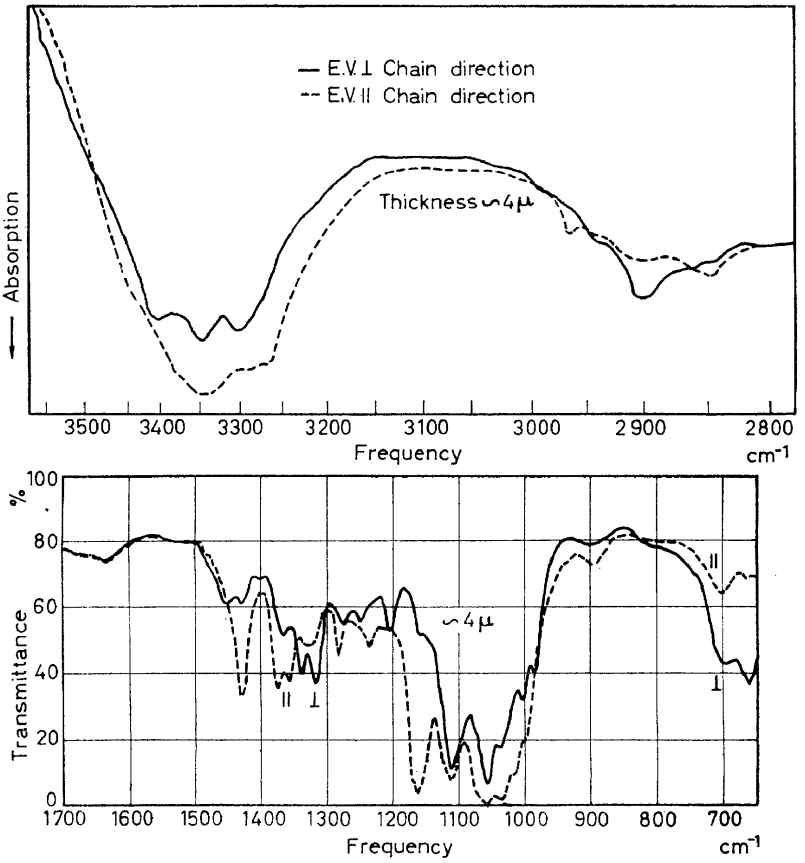


Figure 11. Polarized infra-red spectrum of ramie cellulose crystallites[9]; the spectrum was recorded with a LiF prism in the 3μ region

assignment is further strengthened by our experience with the pyrans whereby substitution on the ring tended to increase absorption in the central part of the C—H stretching region.

In *Figure 11* one also recognizes absorption at the frequencies characteristic of the CH_2 symmetric and antisymmetric stretching modes. Since these frequencies are so characteristic of this grouping one can usually rely on this assignment. Having made the assignment it is now the observed dichroism which assumes significance. The parallel polarization of the CH_2 symmetric stretching band at 2853 requires a similar polarization for the CH_2 symmetric bending mode which should be found somewhere

between 1460 and 1400. In the polarized spectrum of native cellulose in the 7 to 15μ region, shown in *Figure 11*, the only parallel band in the stipulated region is at 1430. Since its intensity is not greatly affected by deuteration the assignment to the CH_2 symmetric bending mode is reasonable and consistent.

Other polysaccharides in wood—With this illustration of how a detailed study is made of the spectrum of a given polysaccharide we may briefly consider the other important wood components and compare them with one another. Such a comparison is made in *Figure 12* which includes

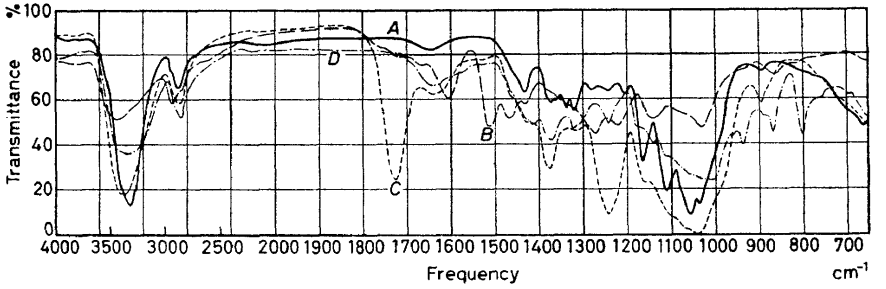


Figure 12. Infra-red spectra of (*A*) cellulose crystallites from prehydrolyzed cellulose wood pulp; (*B*) Klason lignin from red cedar; (*C*) *O*-acetyl-4-*O*-methylglucuronoxylan from white birch; (*D*) ivory nut mannan

spectra of an acidic *O*-acetyl xylan, wood cellulose crystallites, Klason lignin and ivory nut mannan. One notes the happy situation whereby the three non-cellulosic components have at least one strong band which occurs at a position corresponding to a " window " for all the others. This is fortunate for it means that acid xylan, mannan and lignin can be identified in the presence of the other wood components. The characteristic band for each polysaccharide is listed in *Table 2.*

Besides the ivory nut mannan spectrum, part of which is shown in *Figure 6*, the spectra of a series of glucomannan from hard and softwoods has been studied. These samples varied in their mannose glucose ratio from 20 to 3. However, except for the resolution of the spectra which decreased with increasing sugar heterogeneity there was no feature which was characteristic of the glucose component.

Included in *Table 2* is the arabogalactan polysaccharide which has been found characteristic of water-soluble wood polysaccharides from wood[13]. The particular sample examined was from Western Larch.

From a detailed study of the infra-red spectra of the various wood components as well as the spectra of both hard and softwoods it is possible to tabulate the principal absorption bands occurring in wood and make some proposals as to their origin. Such a compilation has been made in *Table 3.* It is very approximate, as it must be, since many of the absorption bands in the wood spectrum have contributions from several different components. The polarizations are the ones actually observed for wood sections[14].

Table 3. Infra-red spectrum of wood

Frequency (cm^{-1})	*Relative intensity*	*Polarization*	*Assignment*
3600–3200	s		H-bonded OH stretching
2970	sd		CH stretching
2945	sd	⊥	CH_2 antisymmetrical stretching
2914–2870	s	⊥	CH stretching
2850	m	∥	CH_2 stretching
1730–1725	s	⊥ or u	C=O stretching of acetyl or carboxylic acid
1670	w	u	Lignin
1635	m	u	Adsorbed water
1600	s	u	COO^- ion
1595	m	u	Lignin
1500	m	u	Lignin
1460	m	⊥	Lignin and CH_2 symmetrical bending on pyran ring
1455–1400	m		OH in-plane bending
1430	s	∥	CH_2 symmetrical bending mode of hydroxymethyl
1425	s		Carboxylic acid and COO^- vibration
1380	s	∥	CH bending
1335–1315	w	⊥	CH_2 wagging
1270	m or sd		Lignin
1240	m		C–O of acetyl
1162	s	∥	Antisymmetrical bridge oxygen stretching
1125–895	s		C–O stretching and ring vibrational modes
895	w	∥	Characteristic of β-link
875	sd	∥	Mode due to glucomannan
800	w	⊥	Mode due to glucomannan
768			Mode due to arabogalactan
700–650	m		OH out-of-plane bending

m: medium; s: strong; sd: shoulder; w: weak

SPECTRA OF WOOD SECTIONS

The composite nature of the infra-red spectrum of wood is shown by the series of spectra in *Figure 13.* These data were collected by recording the spectra of wood sections lightly impregnated with Nujol[14] after the section had been given the treatments shown in *Table 4.* As is well known, the gross morphology of the wood is maintained after the listed treatments and it was also shown that Nujol affected principally the intensity of the 1465 band.

The chemical treatments in *Table 4* were essentially of two kinds: (*a*) delignification, (*b*) removal of hemicellulose. Reference to *Figure 12* allows us to identify almost every band in the wood spectra with either cellulose, xylan, glucomannan or lignin. Delignification produces the very characteristic removal of the absorption bands at 1595 and 1505 and of a weaker band at 1270. In both hard and softwood one is left, after delignification, with a broad band at 1240 which is characteristic of an acetylated polysaccharide. Hardwood xylan is known to be acetylated but this is a good

clue that either the mannan or xylan in softwood also carry acetyl groups[21]. In both softwood and hardwood the C=O peak due to xylan is readily removed by treatment with alkali. It is not legitimate to conclude, how-

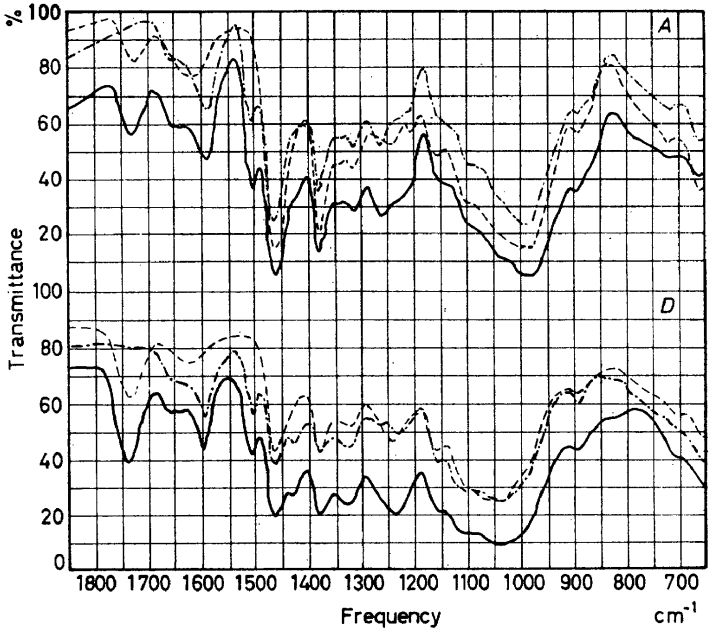


Figure 13. Infra-red spectra of wood cross-sections of western red cedar (*A*) and radial section of red maple (*D*). —: untreated; — — —: delignified; — · — · —: treated for removal of hemicellulose; all sections contain Nujol

ever, that all carboxyl-containing polysaccharides have been removed unless the samples are given an acid (ion exchanging) wash prior to recording the spectrum since the alkali treatment also causes a shift in the carbonyl frequency, due to salt formation.

The spectrum of the untreated Western Red Cedar (C–1) shows absorption shoulders at 875 and 800 which is characteristic of mannan. These

Table 4. Treatment of wood sections whose spectra are recorded in *Figure 13*

Wood	*No.*	*Section*	*Thickness* (μ)	*Treatment*
Western Red Cedar	C–1	Cross	30	Untreated
	C–2	Cross	30	Delignified*
	C–5	Cross	30	Treated with 8% NaOH, 20 min; H_2O
Red Maple	M–2	Radial	20	Untreated
	M–4	Radial	20	Delignified*
	M–8	Radial	20	Treated with 22% KOH, 20 min; 0·1N HCl, H_2O

* Cross and Bevan procedure repeated twice.

bands are somewhat weakened by the alkali treatment and are essentially absent in the spectra of hardwoods. They constitute, therefore, spectral characteristics for distinguishing between hard and softwoods. Another such characteristic is the shape of the absorption band from 1275 to 1200. Softwoods have their maxima at 1270 while hardwoods have theirs at 1240.

Obviously the foregoing data could have been recorded on powdered samples of wood mixed with potassium bromide although spectral resolution is superior with the wood sections. The real reason for working with wood sections was as a preliminary step to developing (hopefully) a micro-spectrophotometric method of studying the distribution of polysaccharides in the cell wall by direct spectroscopy. This goal is not yet achieved. (For a review of the situation with respect to infra-red microspectroscopy *cf.* ref. 15.) Wood radial sections have a special merit, independent of microspectroscopy, in that they provide an opportunity to answer the question of whether or not the wood hemicelluloses are oriented. *Figure 14* shows the polarized infra-red spectrum of a radial section from red maple and the same region recorded for an amorphous but oriented *O*-acetyl film of xylan from white birch. The similar polarizations for the two " xylan " bands, at 1725 and 1465, leaves little doubt that the xylan within the wood structure is oriented. The same conclusions can be made about the glucomannan fraction from the spectra of radial sections of Western Red Cedar and Douglas Fir[14]. This is an important piece of structural information for the wood chemist and is only obtainable *via* the infra-red approach.

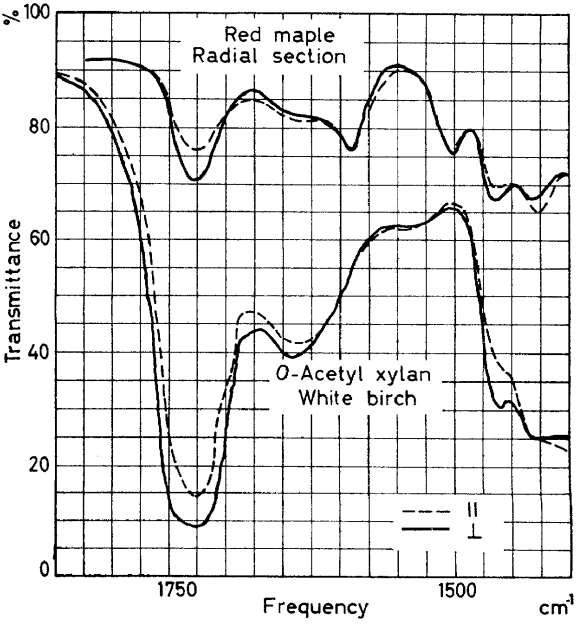


Figure 14. Polarized infra-red spectrum of radial wood section of Red Maple and oriented film of *O*-acetyl-4-*O*-methylglucuronoxylan from White Birch

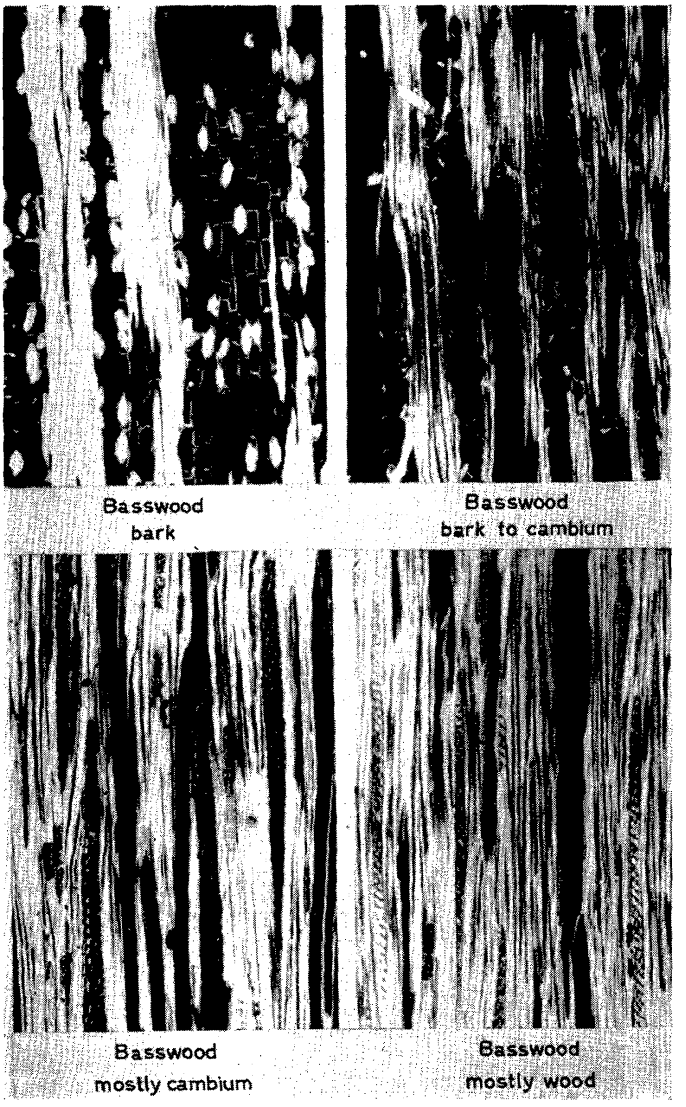


Figure 15. Photomicrographs of members of a series of tangential sections of basswood taken from bark → mature wood

The infra-red spectrum of a wood section can be a very valuable adjunct to microscopic examination of wood sections. For example, if the infra-red spectra of serial tangential sections of wood from the bark through the cambium are recorded, and at the same time photomicrographs are taken, an interesting correlation can be made between morphology and chemical composition. Examples of the type of data which might be obtained is illustrated for basswood sections (*tillia Americana*) and are shown in *Figures 15* and *16*.

The photomicrographs of *Figure 15* were taken between crossed Nicol prisms with the fibre axis at 45°. The interpretation of these photomicrographs depicting the transition from the bark area containing the hexagonal calcium oxalate crystals to the area of low birefringence constituting the cambial zone to, finally, the mature wood with maximum birefringence is well known. The transition represents the various stages of laying down carbohydrate and ligneous material for building up the cell and wood structure. Changes in the infra-red spectrum should reflect these additions to the primary cell wall and wood structure. Thus, the change in optical density of the carbonyl frequency at 1730, in *Figure 16*, can be taken as a

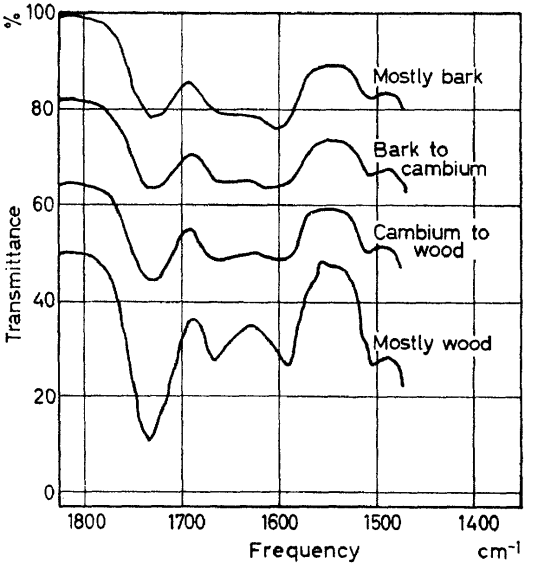


Figure 16. Infra-red spectra of representative spectra from a series of tangential sections of basswood

measure of the increasing density of uronide hemicellulose in the cell wall, since the thickness of each section was held constant. When the technique is perfected it will afford a convenient measure of wood constituents as a function of radial distance in the cambial zone which in turn can be correlated with the various cell wall layers visible in the microscope. It is doubtful if this instrumental technique can ever equal in precision the classical microchemical technique developed by Meier[16] but it does have the advantage of convenience.

MOLECULAR CONFORMATION AND HYDROGEN BONDING

So far we have touched only briefly on the more classical applications of infra-red spectroscopy to the polymer field. Most generally this approach has involved polarized spectra from which valuable information concerning the " backbone " conformation and orientation of pendant groups relative to the polymer " backbone " could be deduced. The latter application has been particularly fruitful in the case of cellulose where the classical X-ray approach has fixed the " backbone " conformation as that of a twofold screw but failed to provide the details of structure concerning hydrogen bonding and the arrangement of the hydroxymethyl group.

As stated above, the dichroism of the CH_2 symmetric stretching and bending modes observed[9] for doubly oriented films of cellulose I crystallites is parallel and that of the antisymmetric stretching is perpendicular. This means that the transition moment for the ν_S (CH_2), which is a vector bisecting the H—C—H bond angle, must make an angle less than 45° with the fibre axis. Examination of molecular models leads one to the conformation shown in *Figure 17* wherein the valence bond C_6–O_6 is *trans* to the C_5–C_4 bond. No other position is conformationally as stable and yields the correct dichroism. It is noteworthy that the conformation shown in the Newman diagram of *Figure 17* is that which would be predicted on the basis of simple conformational analysis assuming that hydrogen bonds would be of equal strength in any position. The position wherein C_6–O_6 is *trans* to C_5–O_5 is considered less stable than the one shown in *Figure 17* because of the additional conflict between O_6 and O_4 in this position analogous to a Peri conflict.

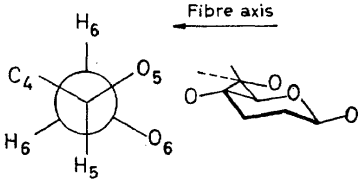


Figure 17. Schematic diagram showing orientation of CH_2OH in native cellulose relative to pyran ring as deduced from infra-red dichroism of methylene group

X-ray studies[17] lead to the conclusion that the latter position for O_6 best fits the data or possibly a combination of the two foregoing positions for O_6. There is, therefore, a net disagreement, at present, between the interpretation of the data from these two approaches. This is reflected in the various hydrogen bonding schemes which have been proposed for native cellulose[9, 17, 18] which will be discussed below.

The fixing of the hydroxymethyl group leads naturally to the question of hydrogen bonding in the cellulose I crystal. The polarized spectrum of ramie crystallites indicates the presence of five distinct hydrogen bonds, of which at least two show strong parallel dichroism *cf. Figure 11.* This means that any hydrogen bonding scheme for native cellulose must (*a*) be in agreement with the general tenets of the X-ray data, (*b*) have the correct CH_2 dichroism relative to the fibre axis and (*c*) have at least two hydrogen

bonds with a preferred orientation in the direction of the fibre axis (parallel hydrogen bonds). Two of the many proposed bonding schemes for cellulose I are shown in outline in *Figure 18.* The upper one is to be criticized on the grounds that it accounts for only a single parallel hydrogen bond. The lower scheme on the left does have two different parallel hydrogen bonds but would yield the incorrect dichroism for the CH_2

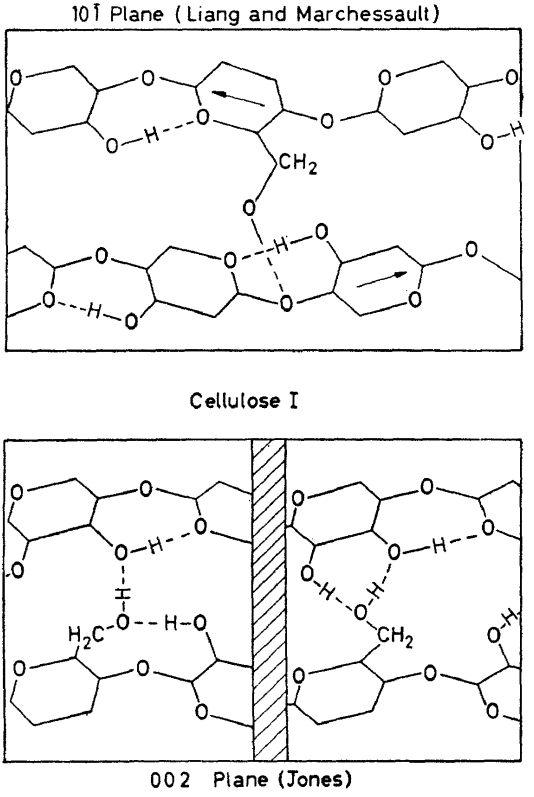


Figure 18. Three different hydrogen bonding schemes for native cellulose which encompass some of the requirements of the infra-red data and X-ray data

modes. The scheme on the right does have the required dichroism for the CH_2 modes and two different parallel hydrogen bonds (one of which is not included in the scheme as shown). It would appear, therefore, as the likeliest candidate to account for the hydrogen bonding in native cellulose. Unfortunately it gives very imperfect agreement with observed $0k0$ X-ray intensities[17].

Although certainty still eludes us as to the hydrogen bonding scheme in native cellulose it is clear that considerable progress has been achieved by the alliance of spectroscopy and crystallography if only that the very important hypothesis of intramolecular hydrogen bonding has been given some experimental backing.

NEAR AND FAR INFRA-RED SPECTRUM OF CELLULOSE

Two important spectroscopic regions of the infra-red spectrum of cellulose have not received attention as yet. These regions, the " near " and " far " infra-red, are generally considered to range from 1 to 3μ and 15 to 40μ respectively. The " near " infra-red is the easier part of the spectrum to investigate and interpret since absorption is usually due to overtones and combination modes. Another reason for interest in this region of the spectrum is the fact that much thicker samples can be used than in the infra-red. This is the result of the weaker absorption usually shown by overtone and combination bands compared to fundamentals.

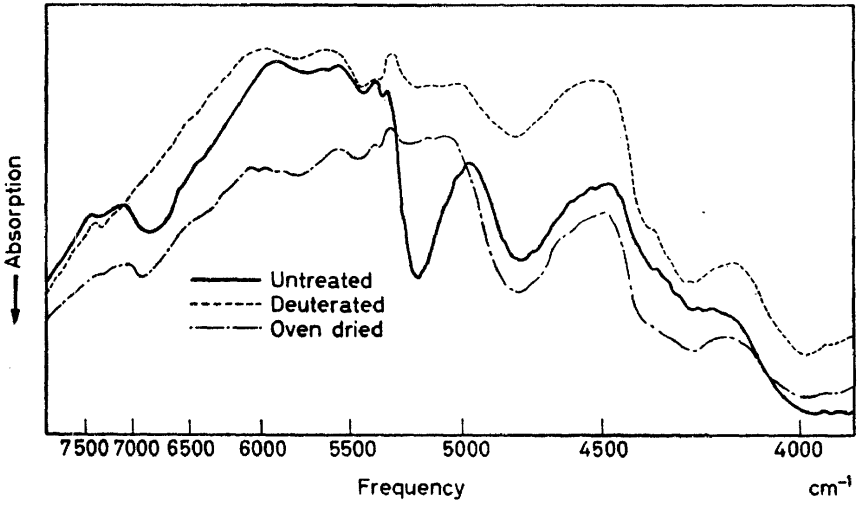


Figure 19. Near infra-red spectrum of air dry, oven dry and deuterated tyre cord rayon

Where 6 to 10 micron samples are required for the infra-red and the " far " infra-red it is possible to use $\frac{1}{4}$ inch fibre bundles in the " near " infra-red when a suitable immersion medium (CS_2 + CCl_4) is used.

Except for thep ioneering work on hydrogen bonding by Ellis and Bath[19], nothing has been published on the " near " infra-red spectrum of cellulose. *Figure 19* shows the spectrum in the " near " infra-red of conditioned tyre cord rayon, the dry material and finally the deuterated material[22]. It is evident that the 5190 band is exclusively due to bound water while the other bands appear to arise from the various combinations shown in *Table 5.* The dichroism was not pronounced for any of the bands when the polarized spectrum was recorded using a silver chloride plate polarizer. On the other hand, the 6770 band, which is the first overtone of the OH stretching fundamental, could be useful in hydrogen bonding[19] and derivative studies since this region of the spectrum is readily accessible on modern broad range ultra-violet spectrophotometers.

The " far " infra-red spectrum of polysaccharides contains a number of well defined bands with distinct dichroism. These bands probably arise

Table 5. Near infra-red spectrum of cellulose

Frequency (cm^{-1})	*Assignment*
3970, 3990	CO stretching + CH and CH_2 stretching
4235	OH and CH deformational modes + CH and CH_2 stretching
4365	CO stretching + OH stretching CH_2 bending + CH_2 stretching
4560	Cellophane only
4780	OH and CH deformation modes + OH stretching
5190	Absorbed H_2O
5310–5480	Background
6770	2 × OH stretching from cellulose and absorbed H_2O
7265	Background

from various modes of the pyran ring and may prove useful in conformation studies.

Acknowledgement is made to Dr T. E. Timell who kindly provided many of the samples used in our studies. Figure 18 was provided by Dr H. Marrinan. The writer is indebted to his colleague Dr C. Y. Liang who has collaborated in most of these studies and to Professor W. Cote, of the State College of Forestry, Syracuse, New York, for assistance in the preparation and examination of the wood sections. Technical assistance in these studies was received from Mrs J. Watson, Miss Joan Koch and Miss P. Fay.

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