Carbohydrate Research, 173 (1988) 185–195
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SECOND DERIVATIVE F.T.-I.R. SPECTRA OF CELLULOSES I AND II AND RELATED MONO- AND OLIGO-SACCHARIDES

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(Received June 8th, 1987; accepted for publication, August 6th, 1987)

ABSTRACT

Second derivative F.t.-i.r. bands in the OH and CH stretching regions of the spectra of celluloses I and II and of related mono- and oligo-saccharides are much sharper than those in normal absorption spectra and the improved resolution enables more precise measurements of their frequencies. A multiplicity of bands was found in the OH stretching region, which indicated coupling. A much simpler pattern of bands was observed in the OD stretching region of the lightly deuterated compounds, indicating decoupling of the vibrations. There was a close correspondence between the peaks in the second derivative mode in the OH stretching regions of the spectra of cellotetraose and cellulose II, in agreement with postulates of a strong resemblance between their structures.

INTRODUCTION

Much of the useful information about the molecular structure and local environment of a chemical compound can be obtained from its i.r. spectrum. However, the information is often limited by the inability of the spectrophotometer to resolve overlapping bands in the spectra, especially of carbohydrates which are dominated[1] by vibrations of numerous OH and CH groups with similar absorption frequencies. One area of great interest is the OH stretching region where the bands reflect various types of hydrogen bonds which have a strong influence on the physical and chemical properties of the carbohydrates.

There has been interest in correlating OH stretching frequencies in carbohydrates with hydrogen-bond or O···O distances as determined by diffraction methods[2] and in relating OH and CH stretching frequencies to structural patterns, particularly those which might be related to sweetness[3,4]. In addition to the difficulties arising from overlapping bands, further complications exist as many of the OH stretching bands arise from coupled rather than from single vibrations[5].

The difficulties arising from broad overlapping bands may be lessened by measuring the spectra at reduced temperatures[5–8], and isotopic dilution has been used[5,8] to overcome problems resulting from coupling. The second derivative

mode[9,10] can provide more easily resolved spectra with sharper bands, but such spectra are much more sensitive to the noise level and are best combined with such techniques as smoothing and signal averaging which enhance the signal-to-noise level.

The improved resolution of bands in the F.t.-i.r. spectra of celluloses I and II and related mono- and oligo-saccharides, obtained in the second derivative mode, are now reported. Some of the compounds were also partly deuterated in order to show the usefulness of this combined approach for resolving band frequencies in the OH stretching region.

EXPERIMENTAL

The origins of the carbohydrates, the F.t.-i.r. spectra of which are reported, have been described[5,8,11]. The samples were dispersed in KBr discs and their spectra obtained by using a Mattson Alpha Centauri Fourier-Transform Infrared Spectrophotometer equipped with a water-cooled source, a computer-controlled iris, and a DTGS detector. The spectra were obtained at a resolution of 4 cm^{-1}, are averages of 64 scans, and have been smoothed[12] by three applications of 7-point smoothing followed by three applications of 9-point smoothing and derivativisation. Frequencies were obtained by using the "read cursor" facility.

RESULTS AND DISCUSSION

It follows from elementary calculus that the even derivatives of profiles of

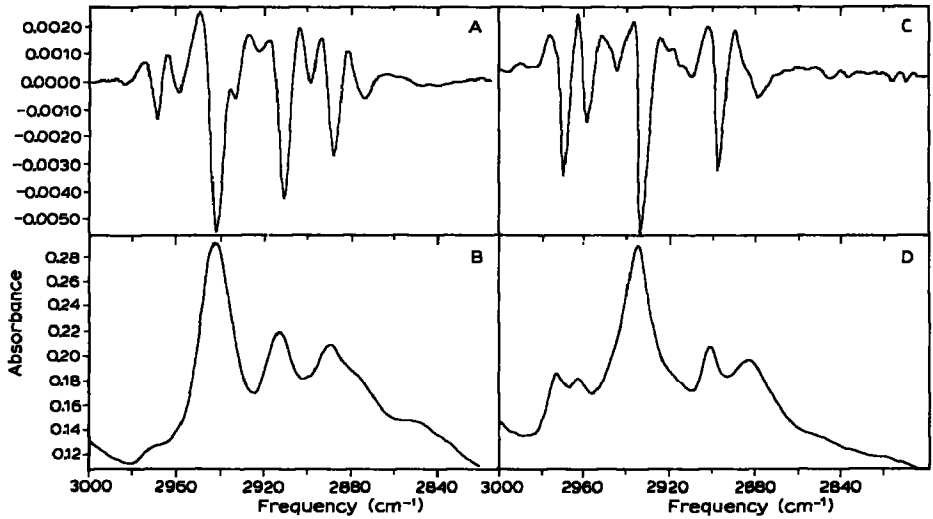

Fig. 1. F.t.-i.r. spectra in the CH stretching region: A, 2nd derivative spectrum of α-D-glucopyranose (peaks are shown as negative absorbance); B, normal spectrum of α-D-glucopyranose; C, 2nd derivative spectrum of β-D-glucopyranose; D, normal spectrum of β-D-glucopyranose.

functions describing the shapes of bands in i.r. spectra have the same abscissal value as the parent peak but are considerably sharper. This was first used[13] in i.r. spectroscopy in 1956, but its application to resolving complex bands in practical spectra has been restricted because of the need for the parent spectra to have a high signal-to-noise ratio. Spectra of this quality are now routinely available with the advent of computerised dispersive and F.t. spectrophotometers. The effects of various spectral parameters on measurements of derivative i.r. spectra have been determined[14–16]. Bands in the second derivative spectra have an appearance that is slightly different from those in normal spectra in that they have weak negative side-lobes as well as strong sharper central maxima.

D-*Glucopyranoses and methyl β-*D-*glucopyranoside.* — The spectra of α- and β-D-glucopyranose in the CH stretching region are shown in Fig. 1. In the second derivative spectra, the ordinate values of the peaks are negative. Clearly, the bands in the derivative spectra are much sharper and this enables the barely detectable broadening near 2938 cm^{-1} in the spectrum of α-D-glucopyranose to be resolved as

TABLE I

CH STRETCHING FREQUENCIES (CM^{-1}) OF α- AND β-D-GLUCOPYRANOSE

α-D-Glucopyranose

I.r.	*I.r. (2nd Deriv.)*	*Raman*[a]	*Calc.*[a]
2972	2970		2985
2961	2965	2961	
2944	2947	2947	2944
	2938		2939
			2937
	2928		2933
2913	2911	2911	2929
2903	2904		
2891	2893	2891	
2878	2880	2877	2883
2849		2850	

β-D-Glucopyranose

I.r.	*I.r. (2nd Deriv.)*	*I.r.*[b]	*Raman*[b]	*Calc.*[b]
2973	2975	2978	2976	2979
2963	2964			
2946	2952	2950	2945	2945
				2942
2936	2934	2934	2934	2937
			2932	
2922	2918			2930
2902	2904		2908	
			2898	
2883	2881		2880	2889

[a]Ref. 17. [b]Ref. 18.

a separate band and lends support to the reality of weak peaks near 2965, 2904, and 2880 cm^{-1}. However, the broad peak near 2849 cm^{-1} does not appear as a peak in the derivative spectrum. In the spectrum of β-D-glucopyranose, the broadenings near the base of the major band near 2934 cm^{-1} are shown in the derivative spectrum as separate bands near 2952 and 2918 cm^{-1}, respectively. In Table I, the peak frequencies in the parent and derivative spectra are compared with those in the Raman spectra and those calculated by normal co-ordinate analysis for α-[17] and β-D-glucopyranose[18].

The correspondence of frequencies between bands in the i.r. and Raman spectra is good but, in the i.r. derivative spectra, more bands are resolved and the number of major bands observed exceeds the theoretical number of seven. Possible reasons for this observation in the spectra of monosaccharides have been given[19].

Measurements of derivative spectra in the CH stretching region are easier to make than in the OH stretching region because, in the latter, there is background absorption arising from water vapour and there are greater apparent natural band-widths. The normal and derivative spectra of the OH stretching region for α- and β-D-glucopyranose are shown in Fig. 2. Four clear peaks can be identified[5] in the normal spectrum of α-D-glucopyranose at room temperature, and six peaks in the spectrum recorded at −180°. Depending on the applicable selection rules, up to 15 bands are possible[5]. In the derivative spectrum, there are about ten major peaks. The derivative spectrum shows the peak near 3405 cm^{-1} split into two peaks near 3402 and 3387 cm^{-1} in agreement with the splitting of ~20 cm^{-1} observed[5] for these bands in the spectrum of α-D-glucopyranose recorded at −180°. Little structure is discernible in the spectrum of β-D-glucopyranose and only changes in slope rather

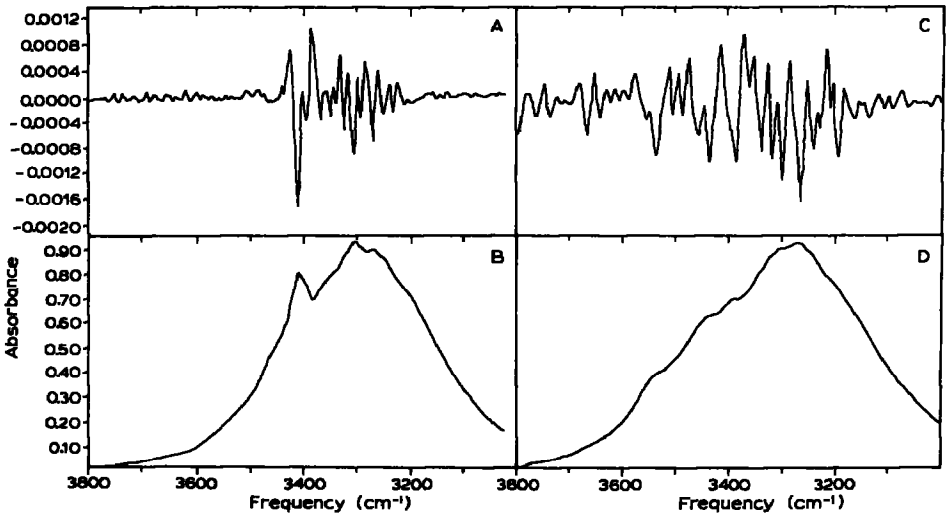

Fig. 2. F.t.-i.r. spectra in the OH stretching region: A, 2nd derivative spectrum of α-D-glucopyranose; B, normal spectrum of α-D-glucopyranose; C, 2nd derivative spectrum of β-D-glucopyranose; D, normal spectrum of β-D-glucopyranose.

than clear peaks are observed. The theoretical number of bands is the same as for α-D-glucopyranose, that is, 5–15 bands[5]. The derivative spectrum contains >15 peaks and some of the weaker peaks must be artifacts. The effects of coupling between OH stretching vibrations in creating a multiplicity of bands in this region can be ascertained by examining the spectra of compounds in which either the OH or OD groups are in low concentrations and there is a preponderance of the other[5]. However, these effects are more easily examined in the spectra of glycopyranosides which do not mutarotate when deuterated[8].

In Fig. 3 are shown the OH and OD stretching regions of the spectrum of lightly deuterated methyl β-D-glucopyranoside together with second derivative spectra in the same regions. The corresponding frequencies are collected in Table II. As might be expected, the decoupled spectrum in the OD stretching region is much simpler than that in the OH stretching region (4 peaks *versus* 6 peaks in the normal spectrum and 5 *versus* 10 peaks in the derivative spectrum). Methyl β-D-glucopyranoside crystallises from aqueous solution as a hemihydrate with chains of intermolecular hydrogen-bonds intersecting in pairs at the water molecules[20]. Thus, on a molecular basis, there are 5 distinguishable OH groups. In the OD stretching region, there is an intense central peak near 2496 cm^{-1}, somewhat less intense peaks near 2567 and 2433 cm^{-1}, and much weaker peaks near 2542 and 2461 cm^{-1}. The assignment of the bands to deuterated OH groups in the structure is not straightforward. According to the X-ray studies, the longest H···O contact is associated with the O-5···HO-2 bond and this would be expected to have the highest frequency (2567 cm^{-1}). There are two groups involved in ···O–H····O–H··· type bonding and this suggests their assignment to the most intense peak near 2496 cm^{-1}. However, the H···O distances involved are the shortest and thus assignment to the lowest frequency peak is indicated. For each of the major peaks, values of the ratio ν_{OH}/ν_{OD} are close to 1.35, as might be expected for decoupled vibrations.

Cellotetraose. — The OH stretching region of the spectrum of cellotetraose is of interest, since a strong correspondence was observed[21] between bands in this spectrum and those in the same region of the spectrum of a cellulose II film (viscose) from which absorption of OH groups in the amorphous region had been removed by deuteration.

In Fig. 4 are shown the OH and OD stretching regions of the spectrum of lightly deuterated cellotetraose together with the second derivative spectra in the same regions. The effect of deuteration of the cellotetraose is unlike that previously observed for cellulose II in that it resulted in sharper bands in the OD stretching region, whereas, for cellulose II, it caused a sharpening of the bands in the OH stretching region. For cellotetraose, only a small proportion of the groups were deuterated so that the OD stretching vibrations were not coupled, resulting in sharper bands, whereas, for cellulose II, the amorphous cellulose was deuterated, leaving sharper bands from the crystalline portion. In the OH stretching region, there are some 9 bands and 14 in the derivative spectrum. In the absence of symmetry in the four glucose residues of cellotetraose, there are 14 distinguishable

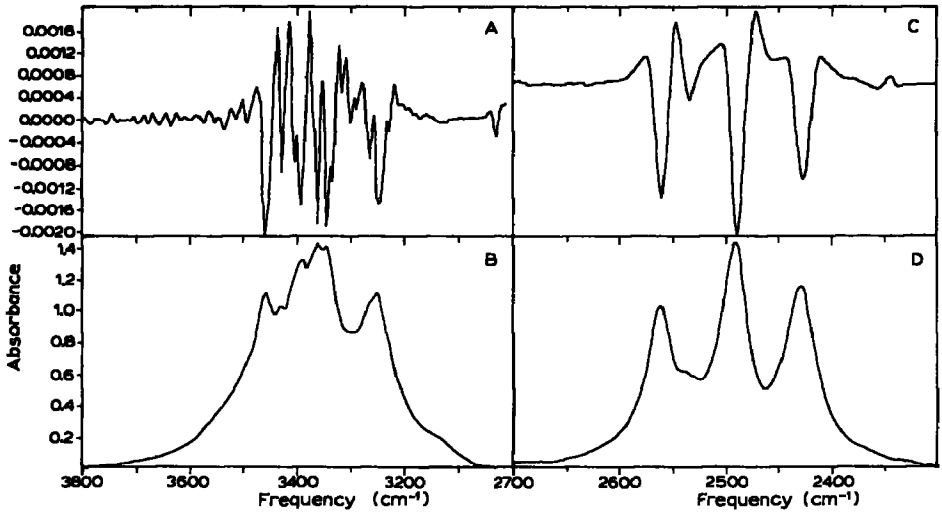

Fig. 3. F.t.-i.r. spectra of partly deuterated methyl β-D-glucopyranoside: A, 2nd derivative spectrum of OH stretching region; B, normal spectrum of OH stretching region; C, 2nd derivative spectrum of OD stretching region; D, normal spectrum of OD stretching region.

TABLE II

OH AND OD STRETCHING FREQUENCIES (cm^{-1}) OF PARTLY DEUTERATED METHYL β-D-GLUCOPYRANOSIDE

OH Stretching frequencies	
Normal spectrum	*2nd Derivative spectrum*
3460	3474
3432	3442
	3418
3392	3405
3366	3377
3351	3357
	3332
	3313
	3279
3254	3258
OD Stretching frequencies	
Normal spectrum	*2nd Derivative spectrum*
2561	2567
2539	2542
2491	2496
	2461
2429	2433

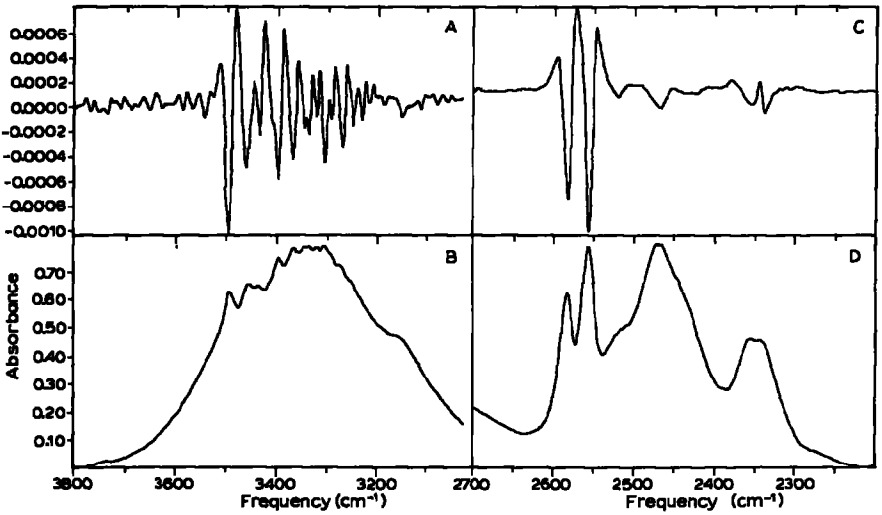

Fig. 4. F.t.-i.r. spectra of partly deuterated cellotetraose: A, 2nd derivative spectrum of OH stretching region; B, normal spectrum of OH stretching region; C, 2nd derivative spectrum of OD stretching region; D, normal spectrum of OD stretching region.

OH groups. The spectra in the OD stretching region are simpler with only 4 main peaks and some shoulders, and in the derivative spectra up to perhaps 7 peaks. Doubt exists about the region 2350–2400 cm^{-1} where there may be interference from remnant atmospheric CO_2 absorption.

Celluloses. — The OH stretching regions of the spectra of celluloses I and II together with the corresponding derivative spectra are shown in Fig. 5, and the frequencies are collected in Table III. These are compared with the i.r., Raman, and normal co-ordinate data[22] for cellulose I and the i.r. data[23] for cellulose II.

The second derivative spectrum of cellulose I contains more bands than could be detected from the envelope of the normal OH stretching absorption. It is reassuring that the frequencies match those observed in the Raman spectrum, since the vibrational species are expected to be active in both the i.r. and Raman[22]. In compiling Table III, the lower intensity peaks above 3500 cm^{-1} have been ignored, although they have been accepted as real in an earlier study[10], since they probably arise from water vapour which gives well delineated peaks that are imposed on the broad featureless tail of the cellulose spectrum. Support exists for this explanation in that the bands are more intense in the region 3800–3950 cm^{-1}, a region not known for absorption by the celluloses. Cael and co-workers[22] calculated six OH stretching modes for cellulose I, but noted that the nine resolvable lines found previously in the Raman spectrum necessitated that interchain coupling of the OH stretching modes be considered. The same conclusion applies to the eight peaks observed here in the i.r. derivative spectrum.

A similar situation exists with cellulose II, with 6 bands in the normal spectrum and 9 in the derivative spectrum. Assignments of the 5 bands resolved at

TABLE III

OH STRETCHING FREQUENCIES (CM^{-1}) OF CELLULOSES I AND II

Cellulose I

I.r.	*I.r. (2nd Deriv.)*	*I.r.*[a]	*Raman*[a]	*Calc.*[a]
	3460			
	3443			
3399		3408	3398	3398
		3376	3374	
3366	3362		3369	
3347	3352	3347	3354	
			3339	
3310	3312	3306	3307	
	3284		3295	
3286	3261	3271	3277	
3246	3222	3238	3235	

Cellulose II

I.r.	*I.r. (2nd Deriv.)*	*I.r.*[b]
	3553	
3520	3529	
	3505	
3490		3488
	3474	
3435	3442	3447
3398	3404	
3369	3361	3350
	3330	
	3313	3305
3161		3175

[a]Ref. 22. [b]Ref. 23.

the time have been made[20]. The predominance of intensity in both the normal and derivative spectra is at a higher frequency for cellulose II than for cellulose I, suggesting weaker hydrogen-bonding overall.

In Fig. 6, the OH stretching region of the second derivative spectrum of cellulose II is compared with that of cellotetraose. There is close correspondence between the peaks in the two spectra, with the greater number of peaks relative to the normal spectra giving increased confidence in the postulated strong resemblances of the underlying structures. Some differences are apparent, possibly due to the greater influence of end hydroxyl groups in the oligosaccharide and the presence of amorphous material in the sample of cellulose II.

In Fig. 7 are shown the normal absorption and derivative spectra of the CH stretching regions of celluloses I and II. The frequencies of the peaks are listed in

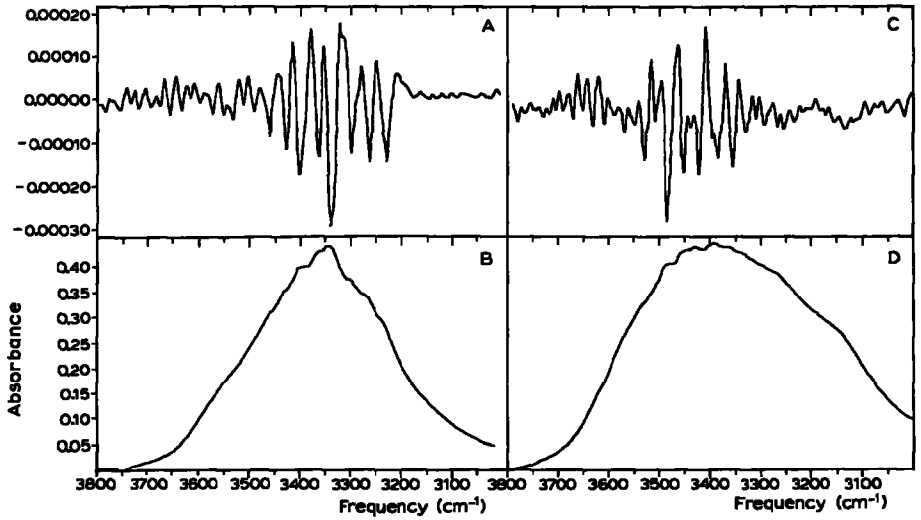

Fig. 5. F.t.-i.r. spectra in the OH stretching region: A, 2nd derivative spectrum of cellulose I; B, normal spectrum of cellulose I; C, 2nd derivative spectrum of cellulose II; D, normal spectrum of cellulose II.

Table IV together with those observed previously[22,23] in the i.r. and Raman spectra and, for cellulose I, calculated from a normal co-ordinate analysis[22].

Examination of Fig. 7 and Table IV shows that the use of the derivative technique has not led to the discovery of any major new bands. Nevertheless, those in the derivative spectra are much sharper and their peak frequencies are better defined.

Thus, the use of the second derivative technique as a means of elucidating bands in the spectra of carbohydrates has an advantage over the cooling technique, in that it is equally effective for bands arising from hydrogen-bonded and other groups and does not cause frequency shifts.

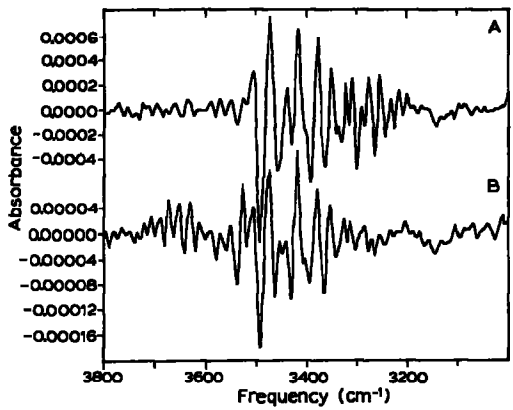

Fig. 6. Comparison of 2nd derivative F.t.-i.r. spectra in the OH stretching region of A, cellotetraose; B, cellulose II.

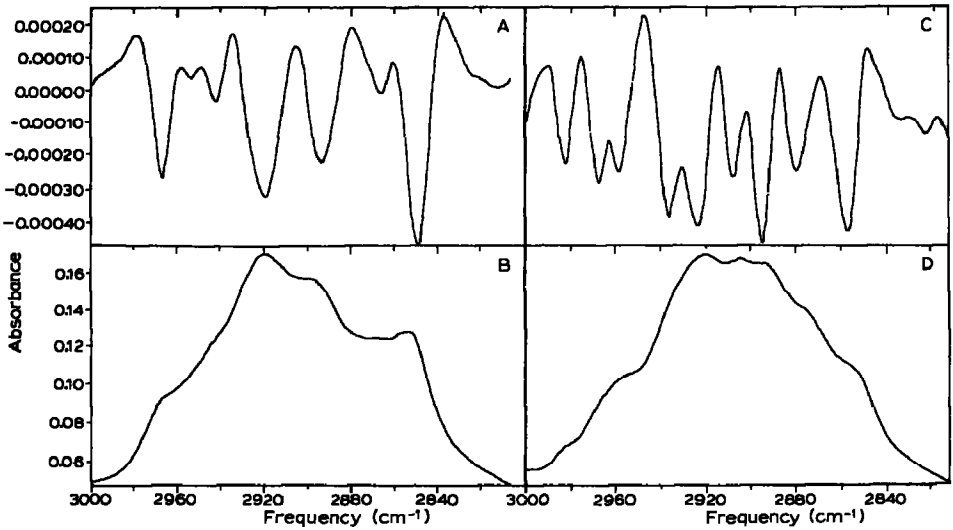

Fig. 7. F.t.-i.r. spectra in the CH stretching region: A, 2nd derivative spectrum of cellulose I; B, normal spectrum of cellulose I; C, 2nd derivative spectrum of cellulose II; D, normal spectrum of cellulose II.

TABLE IV

CH STRETCHING FREQUENCIES (cm^{-1}) OF CELLULOSES I AND II

Cellulose I				
I.r.	*I.r. (2nd Deriv.)*	*I.r.*[a]	*Raman*[a]	*Calc.*[a]
2966	2966	2966	2972	2961
	2958			
2941	2947	2942	2932	2946
		2942		
		2937		
		2933		
2920	2919	2919	2920	2929
	2911	2907		
2899	2894	2894	2889	
2870	2872	2866	2867	2868
2854	2850	2853	2850	

Cellulose II		
I.r.	*I.r. (2nd Deriv.)*	*I.r.*[b]
2980	2984	2981
2965	2965	2968
2954	2956	2955
	2935	2933
2919	2922	
2905	2903	2904
2893	2892	2891
2879	2875	2874
2855	2849	2850

[a]Ref. 22. [b]Ref. 23.

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