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RELATION OF CERTAIN INFRARED BANDS TO CONFORMATIONAL CHANGES OF CELLULOSE AND CELLULOSE OLIGOSACCHARIDES

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ABSTRACT

The i r spectra of D-glucose and cellulose oligosaccharides up to cellopentaose have been compared with those of cellulose at various temperatures between that of liquid nitrogen and ~250° Significant changes in frequency and intensity of the bands at ~3400 cm^{-1} were observed The $a_{1372\,cm^{-1}}/a_{2900\,cm^{-1}}$ ratio for each carbohydrate studied decreased gradually as the temperature was increased above ambient The change of the band intensities at 1429 and 893 cm^{-1} with temperature was also investigated The observed spectral changes are assumed to be associated with changes of hydrogen bonding

INTRODUCTION

The preparation of oligosaccharides from cellulose has been studied by several groups of workers Wolfrom *et al* [1] obtained oligosaccharides, from cellotriose to celloheptaose, by chromatography of cellulose acetolysates followed by deacetylation Miller *et al* [2] prepared oligosaccharides from acid hydrolysates of cellulose by ethanol–water gradient elution from stearic acid-treated mixtures of charcoal and Celite We have used the latter method because it was more convenient for the preparation of large quantities of oligosaccharides

There have been several i r studies on cellulose, and significant information on crystal structure and hydrogen bonds has been obtained Nelson and O'Connor[3,4] investigated the i r spectra of cellulose I, II, and III, and amorphous cellulose in the region 850–1500 cm^{-1}, and proposed the ratio of peak intensities at 1372 and 2900 cm^{-1} as an index of crystallinity McCall *et al* [5] studied the changes in frequencies and intensities of the i r spectra of *Valonia ventricosa* cellulose and Lydrocellulose I and II at room and liquid-nitrogen temperatures, and suggested that the spectral changes occurring in the OH-stretching region on cooling were associated with hydrogen-bonding effects

Michell[6] obtained the i r spectra of cellobiose, cellotriose, and cellotetraose at 22° and −180°, and suggested that the higher oligosaccharides might have some disorder in their crystallinity, as there was little improvement in the spectrum of cellotetraose on cooling However, a systematic study of cellulose oligosaccharides of higher molecular weight has not been reported hitherto and we now record data on these compounds in relation to the effect of temperature on changes of spectral properties

EXPERIMENTAL

Materials — The acetates of cellulose oligosaccharides, prepared from cellulose by acetolysis[2], were deacetylated, and the resulting oligosaccharides were fractionated according to their molecular weights by ethanol–water gradient elution from a column of charcoal–Celite pretreated with 2 5% stearic acid The amounts of oligosaccharides in the eluates were determined by the orcinol–sulphuric acid reaction (absorbance at 550 nm) Each oligosaccharide was characterized by molecular weight and specific rotation, and purified by rechromatography

Whatman cellulose powder for chromatography, anhydrous D-glucose, and cellobiose were used as standards for i r spectra

Methods — I r spectra were obtained with a Perkin–Elmer Model 180 grating spectrophotometer by using the potassium bromide pellet technique A Hitachi IRH-3 vertical, heatable cell was used to obtain the high sample-temperatures Temperature was monitored with an Alumel-Chromel thermocouple in the sample holder, and controlled at various temperatures between room temperature and ~250° by a Hitachi IRC-2 temperature controller designed for operation with the IRH-3 variable-temperature unit Temperatures in the range ambient to liquid nitrogen were obtained by using a cryostat which consisted of an aluminium outer-jacket with KRS-5 (TlBr–TlI) windows and a heatable sample-holder with a refrigerant reservoir The system was evacuated to $\sim 1\,6 \times 10^{-6}$ mmHg Temperature was monitored by means of a platinum sensor and controlled by a Scientific Instrument Inc cryogenic temperature-controller Model No 3610A. A sub-heater was used and controlled by a slide transformer.

RESULTS AND DISCUSSION

Change of the absorptions at ~3400 cm^{-1}. — The absorption band at ~3400 cm^{-1} in cellulose, assigned to OH-stretching vibration by Higgins *et al.*[7], is associated with hydrogen bonding Zhbankov[8] suggested that intermolecular hydrogen bonds (3100–3450 cm^{-1}) would be more sensitive to change in temperature than intramolecular hydrogen bonds (3350–3560 cm^{-1})

Fig 1 shows i r spectra in the region 2500–4000 cm^{-1} for cellulose, D-glucose, and the oligosaccharides up to cellopentaose obtained at −178°, 28°, and 147°. The presence of adsorbed water in cellulose is clearly indicated by the band at 1640 cm^{-1};

this band is not overlapped by the spectrum of cellulose due to planar deformation vibrations of the water molecule[9], and its intensity is therefore related to moisture content The band at 1640 cm^{-1} vanished almost completely when KBr pellets containing cellulose and cellulose oligosaccharides were heated in a nitrogen atmosphere above 147° Therefore, i r spectra obtained at 147° are for anhydrous materials

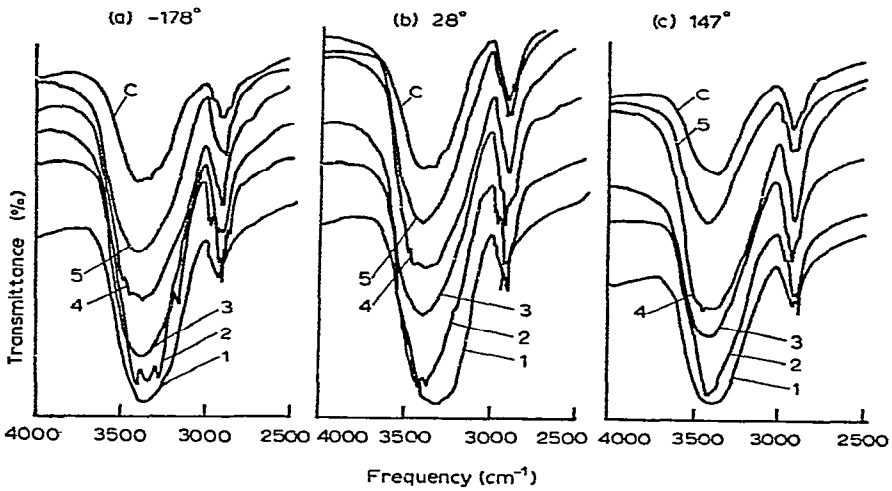

Fig 1 I r spectra (2500–4000 cm^{-1}) of oligosaccharides 1, D-glucose, 2, cellobiose, 3, cellotriose, 4, cellotetraose, 5, cellopentaose, C, cellulose

Fig 1 shows that change of temperature of the samples affects the spectra in the region investigated and may cause considerable overlapping of the bands due to the intra- and inter-molecular hydrogen bonds For D-glucose, a single, strong band centered at 3340 cm^{-1} at −178° shifted to 3390 cm^{-1} at 147° The bands for cellobiose that were prominent at 3150, 3270, 3365, and 3415 cm^{-1} at −178° had broadened and were observed at 3365 and 3420 cm^{-1} at 28° and shifted further to 3420 and 3480 cm^{-1} at 147°. The bands at 3150 and 3270 cm^{-1} observed at −178° disappeared with increasing temperature and appeared only as shoulders at 28° For cellotriose, a strong, single band centered at 3370 cm^{-1} at −178° shifted to 3400 cm^{-1} at 28° and to 3420 cm^{-1} at 147° Three bands observed in cellotetraose at 3360, 3445, and 3485 cm^{-1} at −178° did not shift much with increasing temperature However, only one band centered at 3420 cm^{-1} was observed at 205°. A single, strong band similar to that shown by cellotriose was observed for cellopentaose at 3400 cm^{-1} at −178° and shifted to 3410 cm^{-1} at 28° and to 3430 cm^{-1} at 147°. The bands at 3340 and 3400 cm^{-1} for cellulose at −178° shifted to 3410 and 3445 cm^{-1} at 28° and became one broad band centered at 3370 cm^{-1} at 147°

The bands at ~3400 cm^{-1} for cellobiose, cellotetraose, and cellulose were better resolved upon cooling, whereas the splitting of the bands for cellotriose and

cellopentaose was not prominent However, significant changes in frequency and intensity of the adsorptions for each of the compounds investigated occurred with change of temperature Figs 2 and 3 show the effect of temperature on the frequencies of the bands at 3400 cm^{-1}; these bands in cellulose oligosaccharides and cellulose shifted to higher frequencies on elevating the temperature This is a common tendency of OH-stretching bands associated with hydrogen bonding The shifts of these bands to lower frequencies on cooling may be caused by hindrance of the motion of their structural elements, indicating increased intermolecular bonding Relatively large shifts of the bands centered at 3340 cm^{-1} for D-glucose, 3370 cm^{-1} for cellotriose, and 3400 cm^{-1} for cellopentaose suggest that these bands are associated with intermolecular hydrogen bonds For cellobiose, the band at 3365 cm^{-1} is considered to be due to the intermolecular bonding because of its relatively large shift, the band at 3415 cm^{-1} shifted very slightly For cellotetraose, the band at 3360 cm^{-1} seems to be associated with intermolecular hydrogen bonding, judging from its relatively large shift with temperature On the other hand, the bands at 3445 and 3485 cm^{-1} were little affected by change in temperature, suggesting that they are due to intramolecularly hydrogen-bonded OH groups The bands for intermolecular hydrogen bonds for D-glucose, cellotriose, and cellopentaose shifted to a larger extent than those for cellobiose and cellotetraose This fact indicates a greater mobility of the structural elements of the former compounds, and a crystal structure of cellobiose and cellotetraose that is more stable to change in temperature A rotation about the glucosidic linkage, giving a "bent" or "bent and twisted" alignment of adjacent "anhydroglucose" units, may exist in cellotetraose because of the sharpness of the intramolecular-bonding bands at 3445 and 3485 cm^{-1} Thus, although a similar rotation about the glucosidic linkage would be expected for cellopentaose, intramolecular-bonding bands were not clearly observed The spectrum at ~3400 cm^{-1} for cellopentaose was similar to that for cellotriose, suggesting similarities in conformation and alignment of hydrogen bonds

Fig 4 shows the effect of changes in temperature on the relative intensities of the bands at ~3400 cm^{-1} The relative intensity, which is defined as the ratio of the intensity of this band to that of a suitable band in the same spectrum, is not significantly affected by concentration in the pellet and variation in scattered light The absorptivity of the band at ~2900 cm^{-1} was much less affected than that of other bands by change in temperature Therefore, the relative intensity is expressed as $a_{x\,cm^{-1}}/a_{2900\,cm^{-1}}$, where x refers to the bands at 3340 cm^{-1} for D-glucose, 3365 cm^{-1} for cellobiose, 3370 cm^{-1} for cellotriose, 3360 cm^{-1} for cellotetraose, 3400 cm^{-1} for cellopentaose, and 3340 and 3400 cm^{-1} for cellulose observed at $-178°$. The intensity of each band was calculated by the "base-line" method[10]

The relative intensity of the bands at ~3400 cm^{-1} decreases with increasing temperature This tendency is commonly observed for OH-stretching vibrations associated with hydrogen bonds and indicates a decrease in intermolecular hydrogen bonding Fig 4 shows that the decrease of relative intensity is considerably larger for cellotriose and cellopentaose than for cellobiose, cellotetraose, and cellulose, especially

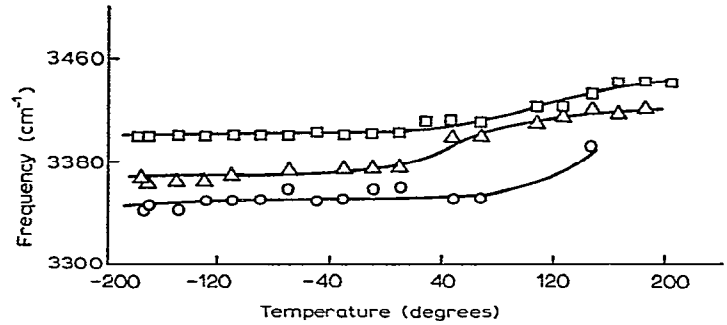

Fig 2 Effect of temperature on the frequencies of the bands at ~3400 cm^{-1} D-glucose (—○—), cellotriose (—△—), and cellopentaose (—□—)

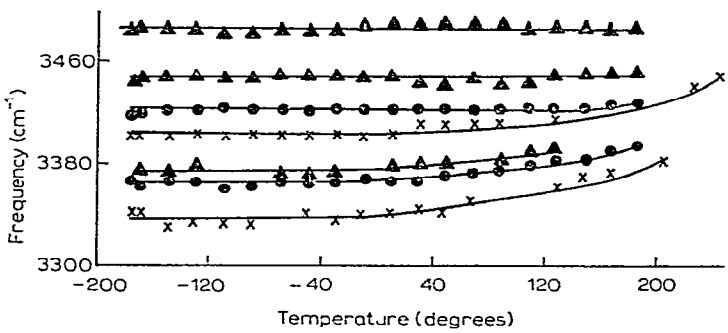

Fig 3 Effect of temperature on the frequencies of the bands at ~3400 cm^{-1} cellobiose (—●—) cellotetraose (—▲—), and cellulose (—×—)

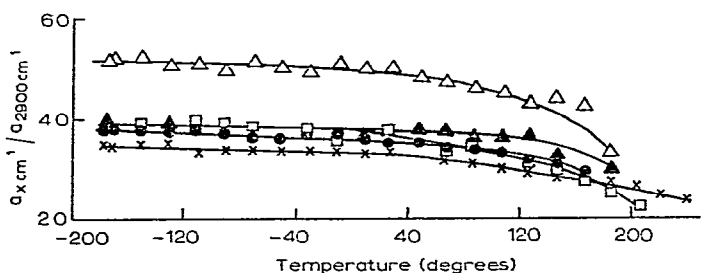

Fig 4 Effect of temperature on the relative intensities of the bands at ~3400 cm^{-1} cellobiose (—●—, x = 3365 cm^{-1}), cellotriose (—△—, x = 3370 cm^{-1}), cellotetraose (—▲—, x = 3360 cm^{-1}), cellopentaose (—□—, x = 3400 cm^{-1}), and cellulose (—×—, x = 3340 cm^{-1})

in the high-temperature region Increasing thermal vibrations and consequent rearrangement of the structural elements could be a factor contributing to the decrease in intensity with increasing temperature.

Fig 5 shows the relation between the relative intensity of the bands at $\sim 3400\ cm^{-1}$ and the number of D-glucose residues The relative intensity for D-glucose is extremely large compared with that for each of the oligosaccharides As the molecular weight increases, the relative intensity approaches the value for cellulose, although a study of oligosaccharides of moderately large d p will be necessary to clarify whether this change in spectral properties is continuous However, the foregoing data indicate that intermolecular hydrogen bonding in cellulose oligosaccharides tends to decrease with increasing number of "anhydrolucose" units

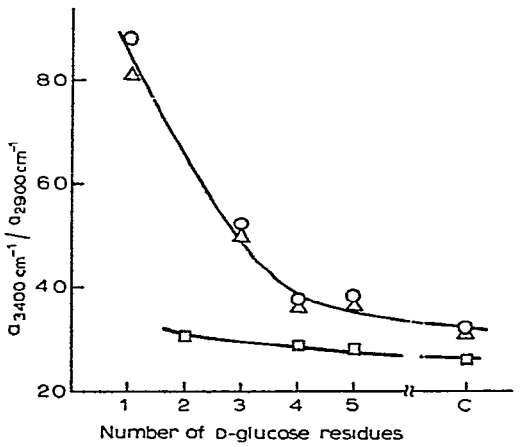

Fig 5 Relation between the relative intensities of the bands at $\sim 3400\ cm^{-1}$ and the number of D-glucose residues $-170°$ (—○—), 28° (—△—), 147° (—□—), C, cellulose

Changes in absorptions in the region 800–1500 cm^{-1} — The region 800–1500 cm^{-1} contains many characteristic absorption bands. The spectra of cellotriose and cellopentaose showed similarities in this region, chiefly in the resolution and intensity of the bands at 1372, 1335, 1315, and 1225 cm^{-1}, which are assigned as follows[7 11]. 1375 cm^{-1} C–H bending, 1335 cm^{-1} O–H in-plane bending; 1315 cm^{-1} CH_2 wagging, 1225 cm^{-1} possibly O–H in-plane bending The ratio of the intensity of the band at 1372 cm^{-1} to that of the band at 2900 cm^{-1} has been proposed as an index of crystallinity for cellulosic materials[4] Changes in the bands at 1429 and 893 cm^{-1} may also be related to the superstructure of cellulose and should not be neglected.

The band at 1375 cm^{-1} in α-D-glucose was assigned[12] to the CH-bending mode with some OH-bending contribution The ratio of the band intensities at 1372 and 2900 cm^{-1}, suggested[3,4] as an index of crystallinity of cellulose, has been applied to cellulose I and II and also to preparations containing a mixed lattice

The crystal lattice of a compound is deformed by heating, and completely disappears above the melting temperature Even below the melting temperature, increased thermal vibrations may cause rearrangement of the superstructure with consequent changes in the i r. spectrum Therefore, the band at 1372 cm^{-1} was investigated as an indicator of molecular motion within, or the deformation of, the crystal lattice of carbohydrates

The appropriate bands for D-glucose are usually observed[12] at 1360, 1369, and 1378 cm^{-1}. However, the band at 1360 cm^{-1} was not prominent at −178° The bands at 1359 cm^{-1} for cellobiose, 1370 and 1384 cm^{-1} for cellotriose, 1364 and 1376 cm^{-1} for cellotetraose, and 1375 cm^{-1} for cellopentaose were prominent at −178°. The band at 1372 cm^{-1} was well-resolved in the spectra of cellobiose, cellotetraose, and cellulose, but in those of cellotriose and cellopentaose it was much less intense and centered at ~1370 cm^{-1}.

The location of this band was scarcely affected by changes in temperature, and Fig 6 shows the effect of temperature on the relative intensity The $a_{1372\,cm^{-1}}/a_{2900\,cm^{-1}}$ ratio for each carbohydrate studied decreased gradually above ambient temperature Ramiah and Goring[13], from a study of the thermal behavior of glucose, cellobiose, cellulose, and other carbohydrates by dilatometry, proposed that the transition between 19 and 33° was caused by the breakage of weak hydrogen-bonds The band at 1372 cm^{-1}, assigned to the CH-bending mode with some OH-bending contributions[12], should reflect changes in conformation due to the greater freedom of movement of OH consequent on the breaking of hydrogen bonds

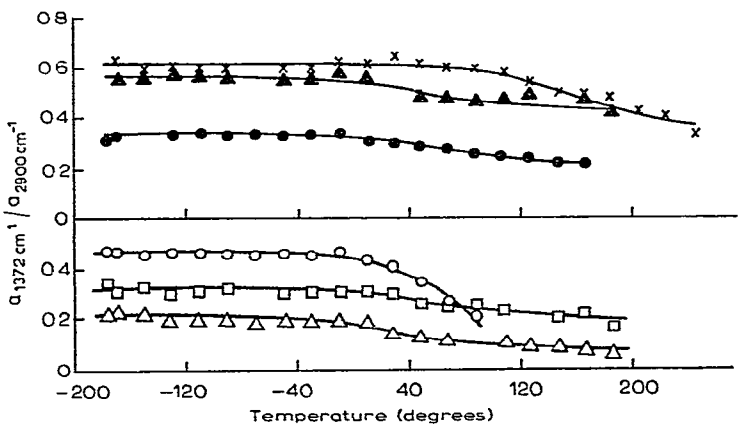

Fig 6 Effect of temperature on the relative intensities of the bands at ~1372 cm^{-1}. D-glucose (—○—), cellobiose (—●—), cellotriose (—△—), cellotetraose (—▲—), cellopentaose (—□—), and cellulose (—×—)

The band at 1429 cm^{-1} for cellulose is generally assigned to the CH_2-bending motion[7,14,15] and corresponds to the bands at 1415 cm^{-1} for D-glucose, 1426 cm^{-1} for cellobiose, 1410 cm^{-1} for cellotriose, 1417 cm^{-1} for cellotetraose, 1415 cm^{-1} for cellopentaose, and 1428 cm^{-1} for cellulose at −178°

O'Connor *et al* [16] proposed the ratio of absorptivities at 1429 and 893 cm^{-1} as an index of crystallinity for native cellulose Nelson and O'Connor[3], observing the similarity of the band at 1429 cm^{-1} for amorphous cellulose and for highly crystalline cellulose II, proposed the formation of the sterically most-favourable conformation, which would probably be that found in cellulose II and would be stabilized by intramolecular hydrogen bonds even in the amorphous state. They concluded that the band at 1429 cm^{-1} is associated with the environment of the C-6 group, *e g*, the formation (or breaking) of an intramolecular hydrogen bond involving O-6

As shown in Fig 7, the ratio $a_{1429\,cm^{-1}}/a_{893\,cm^{-1}}$ for cellulose decreased gradually with increasing temperature, changing abruptly at ~180° The latter temperature is in good agreement with that reported[17] for the glass transition Accordingly, the glass transition in cellulose may be dependent upon, or concurrent with, the rearrangement of intramolecular hydrogen bonds, at least in the amorphous parts

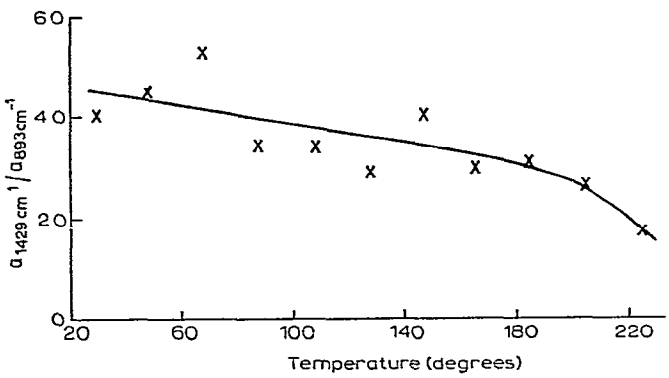

Fig 7 Effect of temperature on the relative intensity of the band at 1429 cm^{-1} for cellulose

The $a_{1429\,cm^{-1}}/a_{893\,cm^{-1}}$ ratio could not be applied to observe changes around position 6 of other oligosaccharides, since the absorptivity at 893 cm^{-1} for carbohydrates changes considerably with temperature

It has been concluded[7,14] that the band at 893 cm^{-1} for cellulose is probably due to a vibrational mode involving C-1 and the four atoms attached thereto. Nelson and O'Connor[3] suggested that this band should reflect changes in molecular conformation due to rotation about the interglycosidic linkages The corresponding bands for D-glucose (893 and 914 cm^{-1}) and cellobiose (888 and 894 cm^{-1}) were split into two parts, but appeared at 893 cm^{-1} for cellotriose, 892 cm^{-1} for cellotetraose, 894 cm^{-1} for cellopentaose, and 893 cm^{-1} for cellulose at −178°. Thus, the location of this band was not greatly dependent upon the number of "anhydroglucose" units If this band reflects the changes in conformation due to rotation about

the interglycosidic linkage, then, among the series of cellulose oligosaccharides, a pronounced difference in molecular conformation should not exist

Fig 8 shows the changes of the band intensity with temperature; the absorptivity in cellulose increases slightly with increase in temperature, but maintains an almost constant value up to 240°, the upper limit used in this experiment Therefore, it is

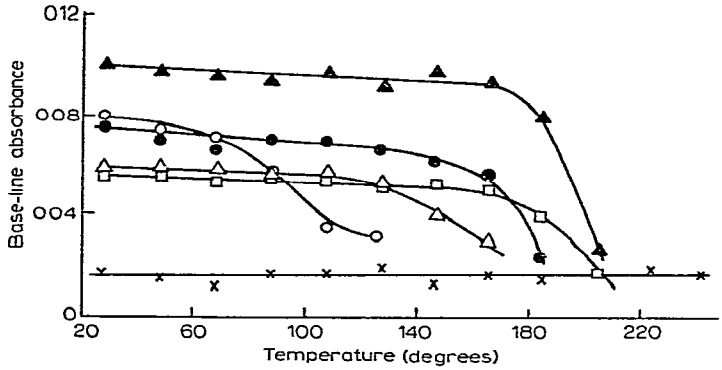

Fig 8 Effect of temperature on the intensities (base-line absorbances) of the bands at ~893 cm^{-1} for D-glucose (—○—), cellobiose (—●—), cellotriose (—△—), cellotetraose (—▲—), cellopentaose (—□—), and cellulose (—×—)

appropriate[16] to use this band intensity as the internal standard for the crystallinity index $a_{1429\,cm^{-1}}/a_{893\,cm^{-1}}$ However, the absorptivity of the band at ~893 cm^{-1} for glucose and cellulose oligosaccharides decreased rapidly above a certain temperature that is specific to each compound If this band is due to a vibrational mode involving C-1 and the attached four atoms and its intensity is affected by the changes in hydrogen bonding[3 7 14], then the abrupt decrease of this band intensity must reflect the changes in hydrogen bonding around position 1 It is also possible that rotation of the "anhydroglucose" units about the glucosidic linkage in cellulose oligosaccharides occurs more easily than in cellulose

It is concluded that the marked change in definition in the I R spectra on changing temperature reflects the effect of hydrogen bonding on the change of superstructure in cellulose oligosaccharides and cellulose The significant effect of change in temperature on the frequency and intensity of the bands at ~3400 cm^{-1} may be due to changes in intra- and inter-molecular hydrogen bonding The decrease in the ratio $a_{1372\,cm^{-1}}/a_{2900\,cm^{-1}}$ at temperatures above ambient for the carbohydrates studied may reflect changes in conformation due to greater freedom of movement of OH groups which might be produced by the breaking of a particular type of hydrogen bond The effect of temperature on the band intensities at 1429 and 893 cm^{-1} may be associated with hydrogen bonding involving positions 1 and 6

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