INFRARED SPECTRA OF CARBOHYDRATES IN WATER AND A NEW MEASURE OF MUTAROTATION*

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SUMMARY

The infrared spectra of various carbohydrates in water have been recorded and found to be characteristic of each compound.

Infrared techniques have been used to determine mutarotation constants of α-D-glucose, β-D-glucose and β-D-mannose. Absorbance values at a constant characteristic wavelength were observed as they changed with time; and were applied in an equation previously used in polarimetric determinations of the mutarotation constants. The mutarotation constants determined by the infrared method were in good agreement with those determined polarimetrically.

INTRODUCTION

In the past thirty years, scattered reports have appeared in the literature concerning infrared investigations in which solutions of certain carbohydrates were studied. In none of these reports, however, was any indication given that the spectrum of a sugar might change in solution with time owing to changes in the configuration of the sugar molecule.

An investigation was therefore undertaken to obtain the infrared spectra of various carbohydrates in water and to determine whether any configurational changes could be detected by infrared spectrophotometry. Although in recent years many excellent studies have been made of carbohydrates in the solid state, it should be kept in mind that water solutions can give information which is more characteristic of biochemical systems.

MATERIAL AND METHODS

The experimental techniques involved were essentially those previously published from this laboratory in other studies of biochemical substances, such as compounds of the citric acid cycle[1] and amino acids and related compounds[2,3]. The instrument used was a Perkin-Elmer model 21 infrared spectrophotometer with a sodium chloride

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prism. The sample solution was contained in a barium fluoride cell of 0.025 mm thickness. In the reference beam a transmittance screen was used. This screen could be adjusted to allow varying amounts of light through from the source. It was based on a screen mentioned by POTTS AND WRIGHT[4]. The carbohydrate solutions observed were either 10 or 20 % (w/v) in water.

The materials studied were α-D-glucose (National Bureau of Standards, U.S.A.); β-D-glucose (Mann Research Laboratories (P), m.p. 149–150°, mutarotation: + 19.6 to 51.8° ($c = 10$); β-D-mannose (Mann, M.A., −16.0 to +14.3°); D-galactose (Pfanstiehl, anhydrous, C.P.); D-ribose (Nutritional Biochemicals Corp.); sucrose (J. T. Baker, analyzed); L-arabinose (Pfanstiehl, C.P.); D-levulose (Pfanstiehl, C.P.); glucose-1-phosphate, dipotassium salt (Schwarz Laboratories); and maltose hydrate (Pfanstiehl, C.P.).

RESULTS

The spectral change of α-D-glucose in water

Fig. 1 shows the spectrum of redistilled water in a 0.025 mm BaF_2 cell. The reference beam had only the transmittance screen. It can be seen that water has a transparent region from about 6.3 μ to 11 μ, which is the range used for these studies.

The observations from a set of experiments with α-D-glucose will now be described. It was considered possible at the outset that a band in the spectrum of α-D-glucose in water might be found which would change its intensity with time and thereby show up a structural change. Fig. 2 shows that a 20 % solution of α-D-glucose has such a band at 8.75 μ and that the transmittance of this band changes with time.

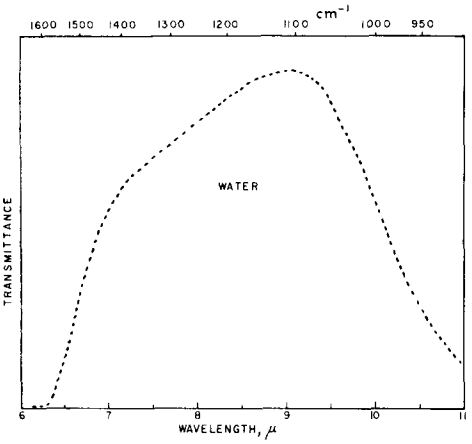

Fig. 1. Spectrum of water. Barium fluoride cell thickness 0.025 mm.

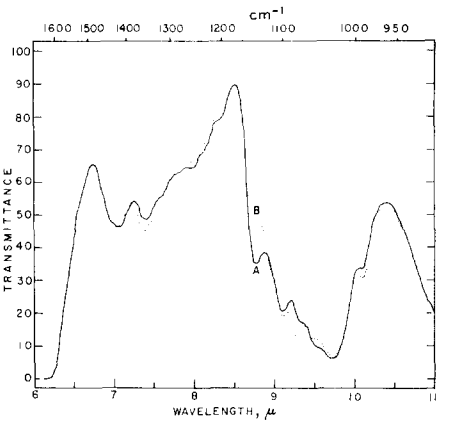

Fig. 2. —, the spectrum of α-D-glucose (20 % w/v) in water at time of dissolving; - - -, at end of mutarotation.

Other bands also exhibit changes in their intensities with time, but the changes are smaller.

The recording of the curve (solid line) was begun at 6.10 μ, 2.5 min after water had been added to the sugar. Point A represents the intensity of the band at 8.75 μ, 4.3 min after water had been added. Point B (dotted line) represents the intensity of the band at 8.75 μ at the end of the run, 169 min after dissolution.

It was reasoned that if the change in intensity of the band at 8.75 μ is a measure of the change from α-D-glucose to the well-known equilibrium mixture, then the same band would show a reverse change in percent transmittance if a sample of β-D-glucose were dissolved in water.

The spectral change of β-D-glucose in water

Fig. 3 demonstrates the anticipated reverse effect at 8.75 μ. Point A lies on the curve (solid line) representing the run at the beginning of the mutarotatin of β-D-glucose. Point B lies on the curve (dotted line) representing the equilibrium mixture at the end of the run. The change in percent transmittance here is downward from A to B.

Kinetics of mutarotation

Further corroboration that changes in the band intensity at 8.75 μ are indicative of mutarotation was found when an equilibrium mixture containing 36.4 % α-D-glucose and 63.6 % β-D-glucose was dissolved in water[5] and observed in the spectrophotometer. Fig. 4 shows that the percent transmittance of the 8.75 μ band remained at a constant value for the whole duration of the experiment (triangles). In this figure are plotted the relative changes in percent transmittance for the α-form, the β-form, and the equilibrium mixture, *i.e.*, in each case the difference between the percent transmittance at 8.75 μ and the percent transmittance at the highest part of the curve (8.50 μ) was plotted on the ordinate as Δ percent T, *versus* time in minutes on the abscissa.

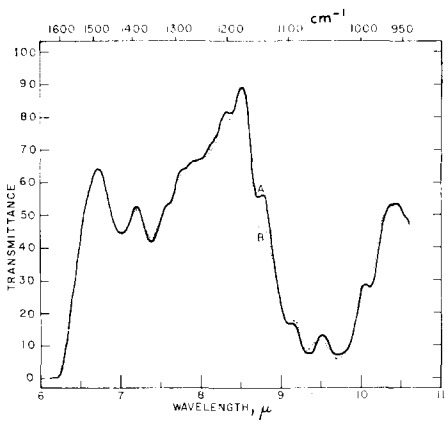

Fig. 3. —, the spectrum of β-D-glucose (20 % w/v) in water at time of dissolving; – – –, at end of mutarotation.

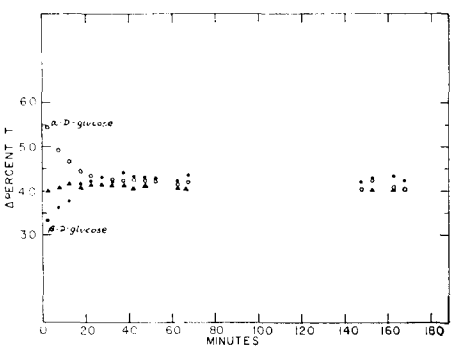

Fig. 4. ○, the relative changes in percent transmittance of the band at 8.75 μ for α-D-glucose; ●, β-D-glucose. ▲, for an equilibrium mixture. All in 20 % solutions.

It should be noted that the intensity changes of the α-D-glucose and of the β-D-glucose go in opposite directions and are essentially parallel to the effects seen in polarimetry.

The kinetics of such changes were calculated from absorbance values (as discussed below) for 10 % solutions observed in similar fashion. The reason for the use of 10 % solutions to calculate kinetic values was that previous workers[6,7], performing polari-

metric measurements, had used solutions of 10 % or less; and consequently their values could be used for comparison with the results obtained in the present study. The range of K values (mutarotation constants) are in good agreement with those found by HUDSON AND DALE[6] by polarimetric methods.

The formulation of the first-order constant for the change, $\alpha \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \beta$, was used for glucose in the following way:

$$K = k_1 + k_2 = \frac{1}{t} \log \frac{A_0 - A_\infty}{A_t - A_\infty}$$

where, t is the time in minutes after addition of water; A_0 is the initial absorbance of the band at 8.75 μ; A_∞ is the final absorbance of the band at 8.75 μ; and A_t is the absorbance at time t of the same band, all values being read from a continuous plot at constant wavelength of absorbance *versus* time. The equation given above is an adaptation of the one given by HUDSON AND DALE[6], where absorbance values have been substituted for optical rotation values. The speed of recording was 12.5 min/μ for the runs at constant wavelength.

Table I presents six representative experimental points of A_t taken from a group of over 50 experimental points from a plot of absorbance *versus* time at constant wavelength (8.75 μ). The points given in the table for A_t are chosen randomly, but with the intent of showing the progressive change in A_t with time and the method of calculation. The data presented in the table show an upward drift in the K value with time, which is attributable to the range of temperature to which the solutions were exposed in the BaF_2 cell. However, the range of K values agrees well with the range of values determined by HUDSON AND DALE[6] for α-D-glucose and β-D-glucose, as seen in Table II. The second column indicates polarimetric values of K determined at 25°, 30° and 40°. The third column indicates the range of values of K obtained by the infrared method, the average value of K, and the temperature range in the BaF_2 cell, which is within the range studied by HUDSON AND DALE.

TABLE I

REPRESENTATIVE VALUES FROM THE CALCULATION OF THE MUTAROTATION CONSTANT OF α-D-GLUCOSE

$K = \frac{1}{t} \log \frac{A_0 - A_\infty}{A_t - A_\infty}$ A_0 0.347; A_∞ 0.204; t *minutes after addition of water*

t (min)	A_t	K
3.25	0.327	0.0199
8.25	0.301	0.0204
20.75	0.257	0.0207
32.00	0.234	0.0214
47.00	0.213	0.0262
62.00	0.208	0.0260

The mutarotation constants obtained for a 10 % solution of β-D-mannose under the same conditions (27–35°) ranged from 0.042 to 0.080, which also is in satisfactory agreement with HUDSON AND SAWYER'S[7] reported values of 0.049 and 0.106 at 30° and 40°, respectively. Fig. 5 shows the changes undergone by β-D-mannose. The solid line represents the sugar shortly after dissolving. It can be seen that a band at 8.60 μ

TABLE II

A COMPARISON OF THE VALUES OF THE MUTAROTATION CONSTANT, K, OBTAINED BY HUDSON AND DALE[6] (POLARIMETRICALLY) WITH THE VALUES OBTAINED IN THE PRESENT STUDY (INFRARED)

Sugar	K	
	By polarimetry	*By infrared*
α-D-glucose	0.0105 at 25°	Temperature range, 27–35°
	0.0175 at 30°	0.0240 average
	0.0437 at 40°	(values range from 0.0186 to 0.0329)
β-D-glucose	0.01057 at 25°	Temperature range, 27–35°
	0.0168 at 30°	0.0200 average
	0.0395 at 40°	(values range from 0.015 to 0.0323)

has already begun to develop. The dotted line represents the sugar at the completion of mutarotation and indicates that the transmittance at 8.60 μ has decreased with time. Here, again, the mutarotation constant was calculated from absorbance readings at constant wavelength (8.60 μ), just as was done for glucose.

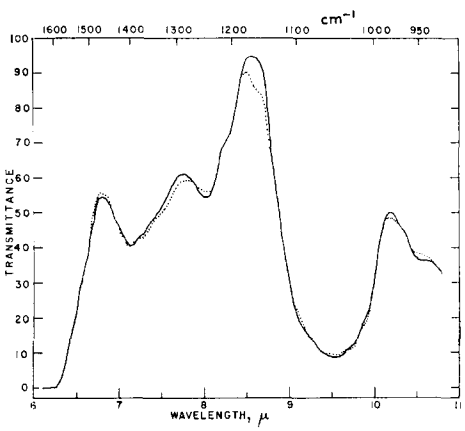

Fig. 5. —, the spectrum of β-D-mannose (20 % w/v) in water at time of dissolving; – – –, at end of mutarotation.

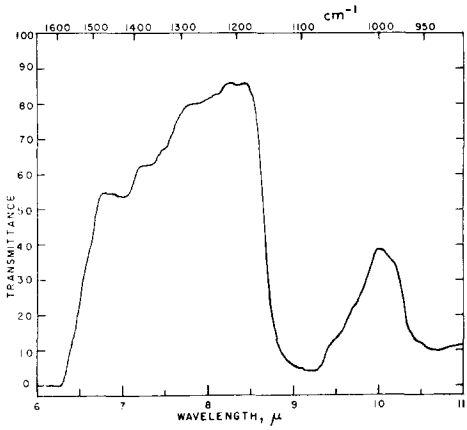

Fig. 6. The spectrum of glucose-1-phosphate, dipotassium salt (20 % w/v) in water.

Observations on sugars with no potential keto or aldehyde group

Fig. 6 shows the spectrum of glucose-1-phosphate (dipotassium salt). In this compound no mutarotation can take place because the hydroxyl group on carbon 1 has been esterified. No change in spectrum with time for this compound was observed. It is interesting, therefore, in this connection, that no absorption band is displayed at 8.75 μ, the band which undergoes the change in α-D-glucose and β-D-glucose.

Fig. 7 shows the spectrum of sucrose, which also cannot mutarotate. Again no change in spectrum with time was observed and there is no absorption band at 8.75 μ.

Spectra of some additional carbohydrates

Fig. 8 shows spectra of D-levulose (20 %), D-galactose (10 %), D-ribose (20 %), L-arabinose (20 %) and maltose hydrate (20 %) without regard for any possible

mutarotation changes. The transmittance scales have been displaced, in order to spread out the five spectra. It can be seen that in water solutions, carbohydrates show characteristic spectra just as they do in the solid state. Each spectrum shown in Fig. 8 is different from any of the others shown in this paper.

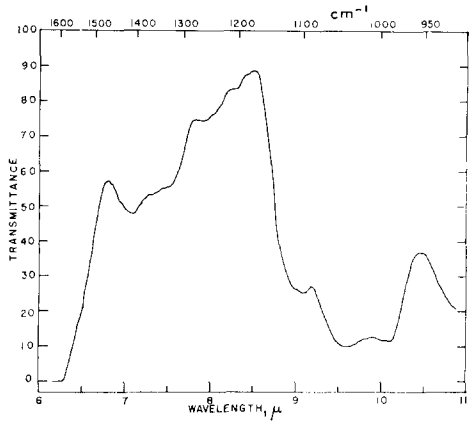

Fig. 7. The spectrum of sucrose (20% w/v) in water.

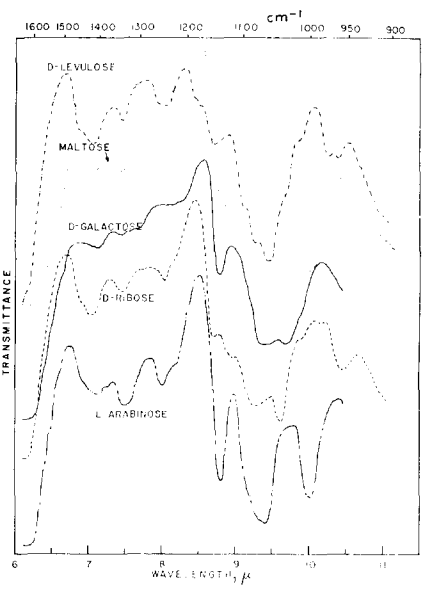

Fig. 8. The spectra of D-levulose (20%), D-galactose (10%), D-ribose (20%), L-arabinose (20%), and maltose hydrate (20%).

DISCUSSION

The data given in this paper show that infrared spectrophotometry can be applied in following structural changes which occur in carbohydrates in water solution. In particular, the process of mutarotation has been studied and the data relative to mutarotation constants compare favorably with those obtained polarimetrically by other workers.

It would be advantageous to be able to make an assignment of the absorption band at 8.75 μ (1143 cm^{-1}), but such an assignment cannot be made at this time. It has been stated[8] that, "beyond 960 cm^{-1}, C–O and C–C stretching, as well as C–H deformation and skeletal frequencies, make correlation between band positions and molecular structure difficult". Absorption due to the C–O–C antisymmetrical stretching has been observed[9,10] in the region 1150–1080 cm^{-1} in the case of cyclic ethers, such as tetrahydrofuran and 1,4-dioxane, but there is still not enough information available to decide what the effects of the α- or β-configurations might be in this case.

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THE BIOSYNTHESIS OF A MUSCLE-RELAXING SUBSTANCE

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SUMMARY

Under appropriate conditions it is possible to show that, during the incubation of muscle microsomes with ATP, a soluble dialyzable substance is found which is capable of inhibiting tension generation in glycerinated muscle fibers. A hypothesis has thus been made that the muscle-relaxing activity of MARSH extract is achieved through the formation of this substance. A number of conditions have been found, however, where the microsomes were active, but no soluble substance could be demonstrated. The conditions most favorable for forming the soluble muscle-relaxing substance have been investigated and some preliminary characterization of it has been achieved. Evidence is presented which suggests that ATP is a substrate for the formation of the substance.

INTRODUCTION

Although the muscle-relaxing activity of extracts of muscle[1] is associated with the microsomal fraction of the extract[2–5], some doubt exists as to whether the microsomes per se produce relaxation. Both LORAND[6] and WEBER[7] have noted that the size of the microsomes would preclude their penetration into a glycerinated muscle fiber and consequently an essential area of the fiber would be beyond the action of the microsome. It thus becomes necessary to postulate that the microsomes produce relaxation by forming or releasing some readily diffusible substance. The observations that inhibition of tension[8] and inhibition of myofibrillar ATP-ase[9,10] is preceded by a latency which is abolished by preincubation suggests that such a substance is indeed formed, or released, or both. Attempts by NAGAI *et al.*[10] to demonstrate the presence of such a substance, however, met with failure.

Abbreviations used: ATP, adenosinetriphosphate; CP, creatine phosphate; CPT, creatine phosphoryltransferase; CTP, cytidine triphosphate; SMRS, soluble musele-relaxing system.