INFRARED AND RAMAN SPECTROSCOPY OF CARBOHYDRATES

PART I: IDENTIFICATION OF O–H AND C–H-RELATED VIBRATIONAL MODES FOR D-GLUCOSE, MALTOSE, CELLOBIOSE, AND DEXTRAN BY DEUTERIUM-SUBSTITUTION METHODS

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ABSTRACT

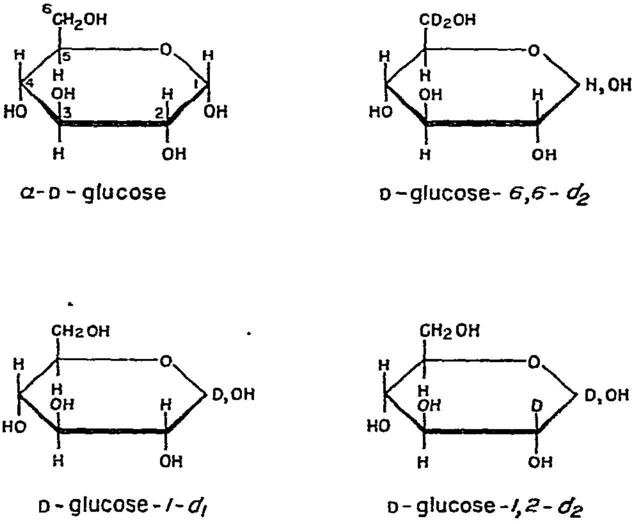
Laser-Raman spectra have been obtained from aqueous solutions of D-glucose, cellobiose, maltose, and dextran [(1 → 6)-α-D-glucan]. These four carbohydrates consist of one or more D-glucose residues, and their vibrational spectra are very similar. Comparison of the spectra of their solutions in water and deuterium oxide indicates that the Raman lines at approximately 1349, 1071, 1021, and 913 cm^{-1} (figures for D-glucose) for all four materials can be assigned modes related to the C–O–H group. In addition, a number of modes related to C–H have been identified by comparison of the infrared spectra of α-D-glucose and three *C*-deuterated D-glucoses in the solid state. Bands at 1457, 1337, 1219, and 1011 cm^{-1} for α-D-glucose are assigned to CH_2-related modes, and those at 1404, 1360, 1250, 1076, 1047, 911, and 836 cm^{-1} to C–H-related modes. The ratio of the frequency of an O–H or C–H deformation mode to that of the corresponding new band produced by deuteration is in the range 1.19 ± 0.02 in most cases.

INTRODUCTION

Effective use of i.r. and Raman spectroscopy to investigate the structure and conformation of carbohydrates requires an understanding of the vibrational origin of the observed frequencies. A complete assignment of the observed vibrations in carbohydrate molecules is a formidable task, especially for the region below 1500 cm^{-1}, and the work so far reported in the literature is limited and sometimes ambiguous. We have made a new study of the spectra of D-glucose, cellobiose, and maltose, which can serve as model compounds for polysaccharides of the glucan type, and have identified by deuteration methods some of the bands due to C–O–H and C–H deformations. (In addition, we have made a normal-coordinate analysis for the glucose molecule, details of which will be published as paper II of this series).

Examination of aqueous solutions of carbohydrates by i.r. spectroscopy is made difficult by the intense absorption of water in important regions of the spectrum and only limited spectroscopic work has been reported for solutions of such compounds (see, for example Refs. 1–3). However, water gives only one weak Raman line in the region 200–2000 cm^{-1}, at ~ 1640 cm^{-1}, and Raman spectroscopy is thus an ideal technique for the study of aqueous solutions. The Raman spectra of aqueous solutions of D-glucose, maltose, cellobiose, and dextran have now been recorded, and we have examined the effect of deuteration on the spectra of these carbohydrates, in order to make assignments for the C–O–H deformation frequencies. We have compared the Raman spectra of these materials in solutions of deuterium oxide with spectra of solutions in water. In D_2O solution, the OH groups of the carbohydrate are converted into OD, and spectral lines due to the vibration of the OH groups disappear and are replaced by the equivalent OD bands.

In the second phase of this work, frequencies related to CH_2 and CH vibrations were identified by use of three *C*-deuterated glucoses: D-glucose-*6,6*-d_2, D-glucose-*1*-d_1, and D-glucose-*1,2*-d_2. The chemical structures of these compounds are shown in Fig. 1. The solid-state i.r. spectra of these *C*-deuterated D-glucoses were compared with that of α-D-glucose. In the spectra of the deuterated compounds, the relevant frequencies of the CH_2 and C–H modes of undeuterated D-glucose should be absent, whereas new bands related to CD_2 and C–D vibrations would be expected to appear. Attempts to examine the Raman scattering of these compounds have so far been unsuccessful because of fluorescence of the specimens in the laser beam.



α-D-glucose D-glucose-6,6-d_2

D-glucose-1-d_1 D-glucose-1,2-d_2

Fig. 1. Derivatives studied by i.r. methods.

EXPERIMENTAL

The spectra of aqueous solutions of the carbohydrates were recorded by using a Raman spectrometer constructed in this laboratory. The essential features of the instrument are a Spex 1400 double monochromator, a Spex 1430 sample-illuminator, a RCA argon-ion laser having an average power of 0.5 W at both 488.0 or 514.5 nm, and a photon-counting system. The light emitted from the laser is linearly polarized, perpendicular to the scattering plane, when incident on the sample. The spectra were recorded at a scan speed of 45 cm^{-1}. min^{-1}, with a time constant of four seconds, and a slit width of 160 μm. The wavelength accuracy was ± 4 cm^{-1}.

α-D-Glucose and β-maltose were dissolved separately in distilled water or D_2O (Columbia Organic Chemicals Co.) to give 1:1 (w/w) solutions. Dextran was dissolved in H_2O or D_2O to give 1:3 (w/w) solutions. β-Cellobiose in H_2O or D_2O was supersaturated by application of heat and slight pressure, and the excess cellobiose was allowed to crystallize out on cooling to room temperature. The final concentrations of the cellobiose solutions were approximately 1:2 (w/w). Prior to use, all the solutions were kept for at least 24 h at room temperature. 1:4 (w/w) solutions of the carbohydrates in both H_2O and D_2O were also examined, and the same results were obtained. In addition solutions of α-D-glucose 1:1 (w/w) were prepared *in mixtures* of H_2O and D_2O, for which the H_2O:D_2O ratios were 1:1, 1:3, and 1:9 (v/v). The Raman spectra were recorded for all the above solutions in screw-top, glass vials sealed with Teflon tape to prevent evaporation (or exchange of hydrogen for the D_2O solutions). In the same way, Raman spectra were obtained for specimens of methanol (spectroscopic grade) prepared as 1:1 (v/v) solutions in both H_2O and D_2O.

Crystalline specimens of α-D-glucose were prepared from MeOH and MeOD solutions. The MeOH (or MeOD) was supersaturated with α-D-glucose by application of both heat and pressure. The α-D-glucose then was allowed to recrystallize out over a period of 48 h. The solid specimens were sealed in glass vials and the Raman spectra were recorded.

Samples of D-glucose-*6,6,d*$_2$ D-glucose-*1-d*$_1$, and D-glucose-*1,2-d*$_2$ were supplied by Merck, Sharp and Dohme of Canada, Ltd. Specimens for i.r. work were prepared as KBr discs, and their spectra were recorded by using a Perkin–Elmer 521 spectrophotometer.

RESULTS AND DISCUSSION

Raman spectra of solutions of carbohydrates in water and in deuterium oxide. — The Raman spectra of solutions of D-glucose, maltose, cellobiose, and dextran in H_2O and D_2O are given in Figs. 2–5 respectively, and the frequencies of the lines observed are listed in Table I. The solution spectra of these four carbohydrates in the region 700–1500 cm^{-1} are extremely similar, and it seems likely that assignments in this region will be relevant for studies of cellulose, amylose, and other polymers of D-glucose. In the region below 700 cm^{-1}, each carbohydrate examined has distinct features in its Raman spectrum and has a characteristic pattern of observed lines and intensities. Thus this region of the Raman spectrum is a potential identification for these molecules in solution, which is valuable in view of the difficulties encountered in i.r. work on these materials in aqueous solution[1–3].

COH Vibrational frequencies. — The lines at 1349, 1071, 1020, and 913 cm^{-1} (values for D-glucose) in the Raman spectra of the four carbohydrates are assigned to bending of the C–O–H groups. The line at 1349 cm^{-1} in the Raman spectrum of D-glucose in H_2O solution (see Fig. 2) is absent from the spectrum of this material

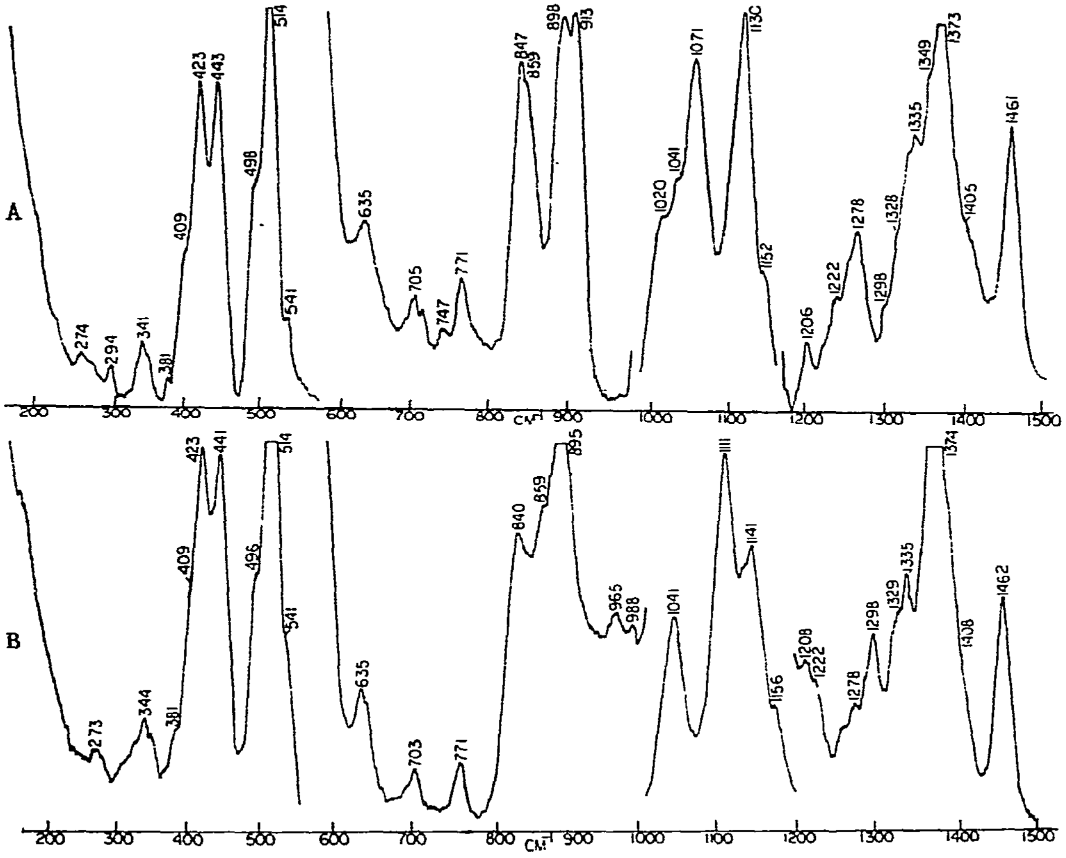


Fig. 2. Raman spectrum of D-glucose in solution: A, in H_2O; B, in D_2O.

in D_2O. Examination of Figs. 3, 4, and 5 indicates that a similar decrease in intensity of the shoulder at ~1349 cm^{-1} occurs in each case on changing the solvent from H_2O to D_2O. For maltose, this line decreases in intensity, although it does not disappear completely, perhaps due to overlap. Assignment of the 1349 cm^{-1} line to a C–O–H mode is further confirmed by comparison of the Raman spectra of the solid specimens of α-D-glucose that had been recrystallized separately from methanol (MeOH) and from deuterated methanol (MeOD); see Fig. 6. This Raman line was absent for α-D-glucose recrystallized from MeOD in which conversion of OH into OD had occurred.

Similar behavior is shown by the line at 1071 cm^{-1} for D-glucose in H_2O, which is absent for the D_2O solution. In the spectrum of maltose, there are two lines in this region, at 1079 and 1070 cm^{-1}. Both of these lines decrease in intensity upon deuteration and appear to be due to C–O–H vibrations. For cellobiose and dextran, the lines observed for the H_2O solution, at 1080 cm^{-1} and 1084 cm^{-1} respectively, are absent from the spectra of the D_2O solutions.

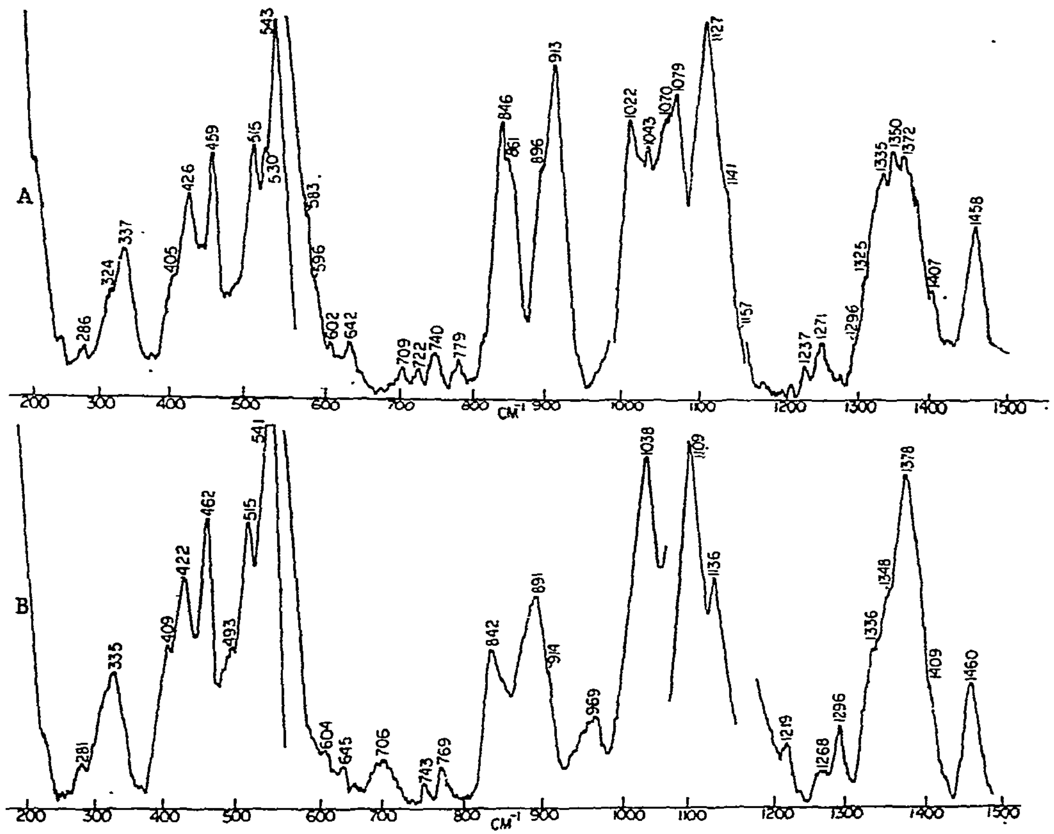


Fig. 3. Raman spectrum of maltose in solution: A, in H_2O; B, in D_2O.

The line at 1020 cm^{-1} in Raman spectrum of D-glucose is also assigned as a C–O–H frequency, as are the equivalent lines in the spectra of maltose at 1022 cm^{-1}, at 1020 cm^{-1} for cellobiose, and at 1024 cm^{-1} for dextran. In each case, this line is absent from the spectrum for the respective D_2O solution. Finally, the line at 913 cm^{-1} for D-glucose shows the same deuteration effects and is assigned to a C–O–H vibration. Similar lines occur for maltose at 913 cm^{-1}, for cellobiose at 915 cm^{-1}, and for dextran at 917 cm^{-1}, and all of these are very weak in the spectra of the respective solutions in D_2O.

For cellobiose, the intensity of the line at 890 cm^{-1} decreases considerably upon deuteration and is thought to be due, at least partially, to a C–O–H vibration. A summary of the assigned C–O–H frequencies is given in Table II.

Fig. 7 shows the 1300-cm^{-1} region of the Raman spectra of D-glucose in solution in mixtures of H_2O and D_2O. As the ratio of H_2O:D_2O changed from pure H_2O to pure D_2O, the intensity of the line at 1278 cm^{-1} decreased and that at 1298 cm^{-1} increased. Similar results were obtained for maltose and cellobiose (see Figs. 3 and 4). However, for dextran (also shown in Fig. 7), no such change occurs

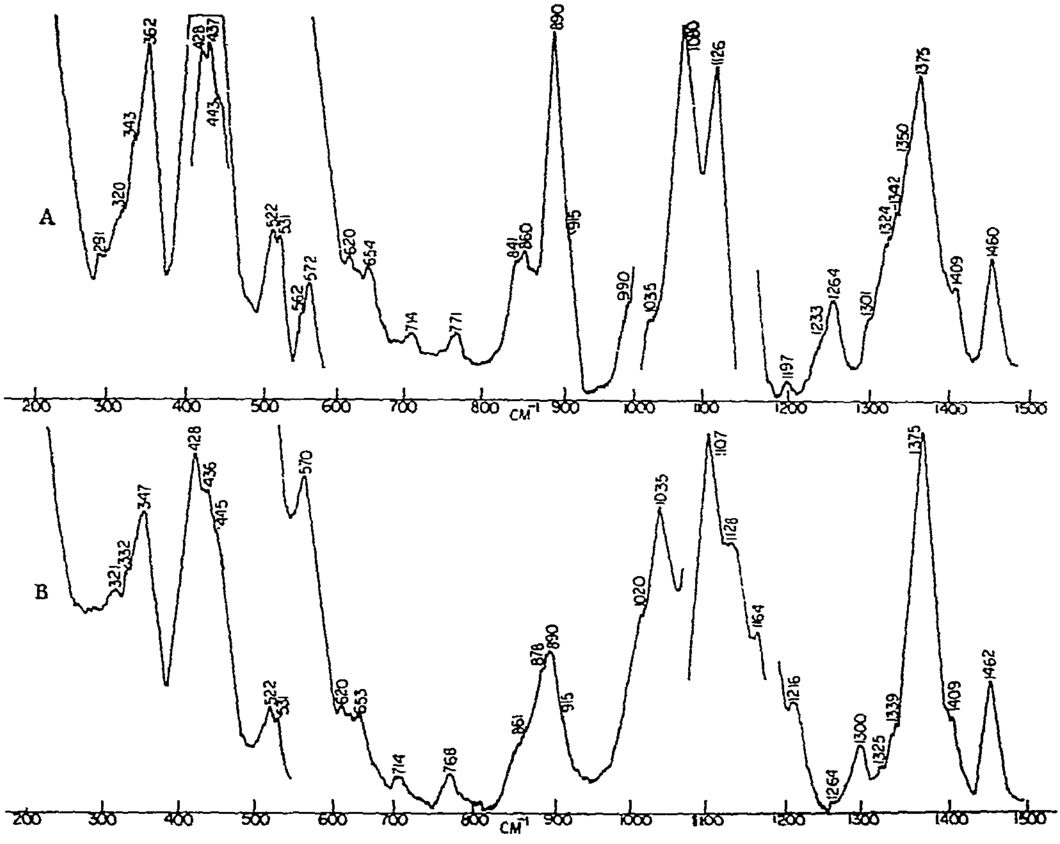


Fig. 4. Raman spectrum of cellobiose in solution: A, in H_2O; B, in D_2O.

for the line at 1274 cm^{-1}. These results suggest that these changes for D-glucose are related to *O*-deuteration of the CH_2OH side chain, or the 1–OH group, which in dextran are involved in the glycosidic linkage. However, an additional contribution from another structural source occurs in this frequency range, as is evidenced by the presence of the line at 1274 cm^{-1} in the Raman spectrum of dextran, and the fact that the line at 1278 cm^{-1} in that of D-glucose does not completely disappear with deuteration.

When deuteration occurs and a C–O–H line decreases in intensity, a new line due to a C–O–D mode should be observed at a lower frequency in the spectrum. A line at 1111 cm^{-1} appears upon deuteration of all four samples; it can be assigned to a C–O–D vibration, and this is the only such frequency that we have identified. The other C–O–D modes apparently either occur in regions of the spectra where they cannot be observed because of line overlap, or are inherently too weak to be detected, as was suggested by a study of deuteration effects for methanol and other aliphatic alcohols (see below).

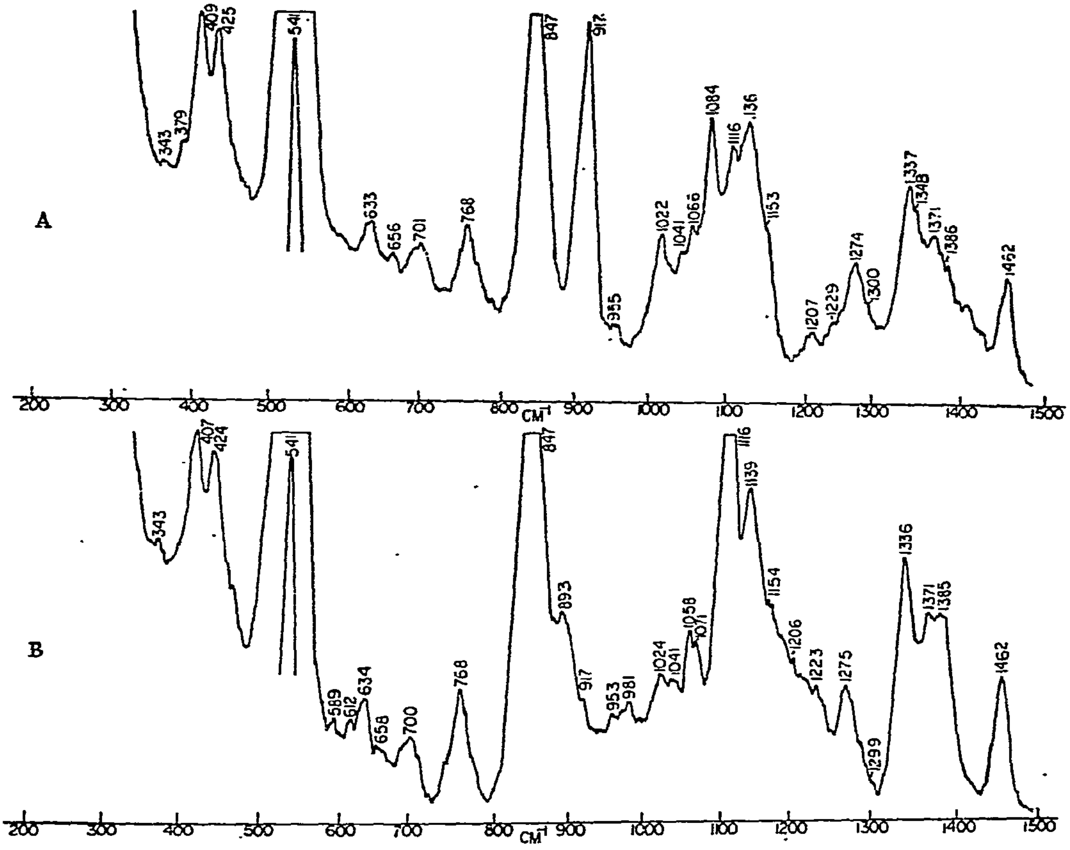


Fig. 5. Raman spectrum of dextran in solution: A, in H_2O; B, in D_2O.

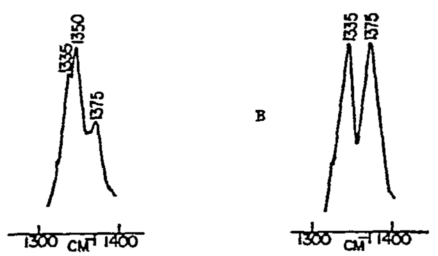


Fig. 6. 1350-cm^{-1} region of Raman spectrum of crystalline α-D-glucose: A, recrystallized from MeOH; B, recrystallized from MeOD.

Infrared studies of deuterated D-*glucoses in the solid state.* — The i.r. spectra for specimens of the *C*-deuterated D-glucoses in KBr discs are shown in Figs. 8–10. The observed frequencies are listed in Table III and compared with those for crystalline undeuterated α-D-glucose. Band assignments have been made on the basis of the differences between the spectra, as detailed below.

TABLE I

OBSERVED FREQUENCIES (cm^{-1}) AND INTENSITIES FOR THE RAMAN SPECTRA OF CARBOHYDRATES IN AQUEOUS (H_2O) SOLUTION*

D-*Glucose*	*Maltose*	*Cellobiose*	*Dextran*
1461 s	1458 s	1460 s	1462 m
1405 sh	1407 sh	1409 w	
			1386 sh
1373 s	1372 s	1375 s	1371 m
1349 m(sh)	1350 s	1350 s	1348 s
1335 m(sh)	1335 m(sh)	1342 w(sh)	1337 s
1328 sh	1325 sh	1324 sh	
1298 w	1296 w	1301 sh	1300 w
1278 m	1271 m	1264 m	1274 m
	1237 w	1233 sh	1229 w
1222 sh			
1206 w		1197 w	1207 w
1152 w	1157 sh		1153 sh
	1141 sh		
1130 vs	1127 vs	1126 w	1136 s
			1116 w
1071 vs	1079 vs	1080 vs	1084 s
	1071 sh(s)		1066 w
1041 w	1043 w	1035 w	1041 w
1020 s(sh)	1022 s	1022 m	1022 m
		990 sh	955 w
913 s	913 s	915 sh	917 s
898 s	896 sh	890 s	
859 sh	861 sh	860 m	
847 s	846 s	841 sh	847 vs
771 m	779 m	771 m	768 m
747 w	740 m		
	722 w	714 w	
705 m	709 w		701 m
635 m	642 w	654 w	656 w
		620 w	653 m
	602 w		
	596 sh		
	583 sh	572 s	
		562 sh	
541 w	543 vs		541 vs
	530 sh	531 s	
514 vs	515 s	522 s	525 s
498 sh			
	459 s		
443 s		443 sh	
		437 vs	
423 s	426 s	428 vs	
409 sh	405 sh		409 s
381 w			379 w
		362 s	
341 m	337 s	343 sh	343 w
	324 sh	320 sh	

*Key: vs, very strong; m, moderate; w, weak; sh, shoulder; br, broad.

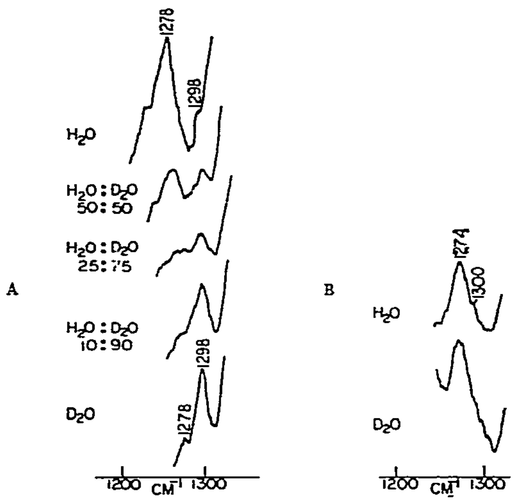


Fig. 7. A: 1300-cm^{-1} region of Raman spectra of D-glucose in solution in mixtures of H_2O and D_2O. B: 1300-cm^{-1} region of Raman spectra of dextran in H_2O and D_2O solutions.

TABLE II

FREQUENCIES (cm^{-1}) ASSIGNED TO C–O–H VIBRATIONS IN THE RAMAN SPECTRA OF CARBOHYDRATES IN AQUEOUS (H_2O) SOLUTION

Carbohydrate	*Frequencies (cm^{-1})*			
D-Glucose	1349	1071	1020	913
Maltose	1350	1070, 1079	1022	913
Cellobiose	1350	1080	1020	915, 890
Dextran	1348	1084	1024	917

Comparison of the i.r. spectrum of α-D-glucose[4] with that of D-glucose-*6,6-d*$_2$ shows the bands at 1457, 1337, 1219, and 1011 cm^{-1} for the undeuterated material are probably due to vibration of the CH_2 group. The 1460-cm^{-1} band is much decreased in intensity for D-glucose-*6,6-d*$_2$, and the bands at 1337, 1219, and 1011 cm^{-1} are completely absent from this spectrum. New bands appear at 1238, 967, 866, and 743 cm^{-1} in the i.r. spectrum of D-glucose-*6,6-d*$_2$, and are assigned as CD_2 motions. Six bands, at 1360, 1250, 1076, 1047, 911, and 863 cm^{-1} present in the spectrum of α-D-glucose, are not observed for D-glucose-*1-d*$_1$, and are assigned to C-1–H bending vibrational modes. These six bands are also absent from the spectrum of the *1,2-d*$_2$ derivative. In addition, the latter compound does not show the weak band at 1402 cm^{-1} given by α-D-glucose. This band is the only candidate to be assigned to a C-2–H deformation mode, and it must be assumed that such modes give rise to weak bands. In the spectrum of D-glucose-*1-d*$_1$, there are five bands that can apparently be assigned as vibrations related to the C-1–D group, based on the fact that they are present in the spectrum of this deuterated sample and are absent for undeuterated D-glucose. The new C-1–D bands are at 1158, 1063, 960, 874, and 810 cm^{-1}. Similar differences are found on comparison of the spectrum of α-D-glucose and its 1,2-*d*$_2$ derivative.

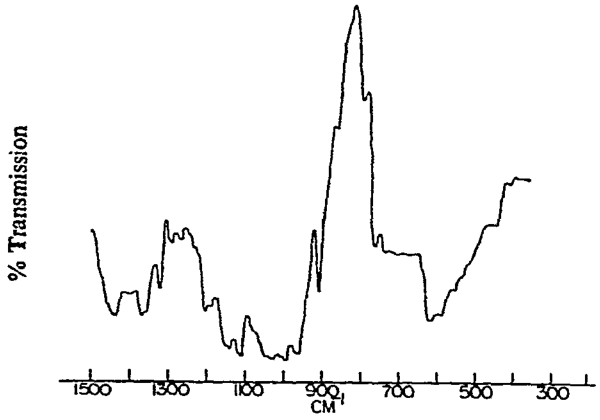


Fig. 8. I.r. spectrum of D-glucose-*6,6*-d_2 prepared as a KBr disc.

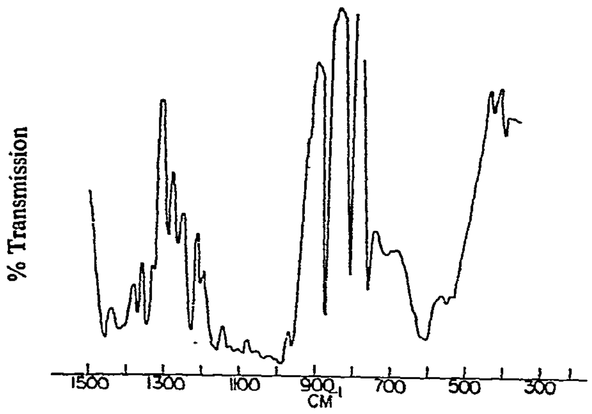


Fig. 9. I.r. spectrum of D-glucose-*1*-d_1 prepared as a KBr disc.

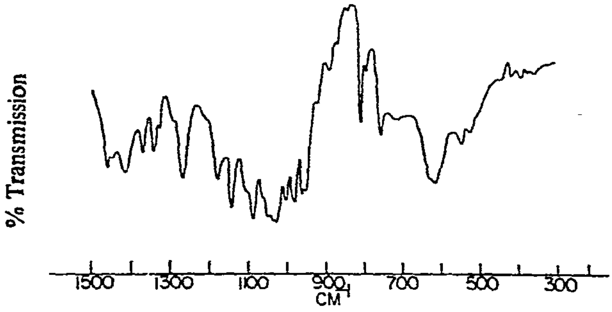


Fig. 10. I.r. spectrum of D-glucose-*1,2*-d_2 prepared as a KBr disc.

TABLE III

OBSERVED FREQUENCIES (cm^{-1}), INTENSITIES AND BAND ASSIGNMENTS FOR THE INFRARED SPECTRA OF *C*-DEUTERATED D-GLUCOSES IN THE SOLID STATE*

α-D-*Glucose*	*Assignment*	D-*Glucose-* 6,6-d_2	*Assignment*	D-*Glucose-* 1-d_1	*Assignment*	D-*Glucose-* 1,2-d_2	*Assignment*
1457 s	CH_2	1458 sh		1459 s		1457 s	
1442 sh		1440 s				1447 m	
1427 vw		1421 sh		1419 s-br		1421 s	
1402 w	C-2–H	1398 w		1404 sh			
1378 m							
1369 sh		1369 m		1369 m		1370 m	
1360 sh	C-1–H	1355 sh					
1337 m	CH_2			1346 s		1341 s	
1328 sh		1322 m		1323 w		1329 w	
1293 w		1296 m		1290 s		1294 s	
1270 m		1270 m		1264 m		1268 s	
1250 vw	C-1–H	1250 sh					
		1238 w	CD_2				
1219 m	CH_2			1228 vs		1220 s	
1197 m		1205 s		1205 m		1206 sh	
1189 w							
						1179 m	C-2–D
				1158 s	C-1–D	1163 w	
1142 m		1142 m				1143 s	
				1131 sh			
1116 sh				1115 sh			
1104 s		1112 s		1106 s		1106 s	
		1091 sh		1090 s		1088 s	
1076 m	C-1–H	1082 w					
				1063 s	C-1–D	1065 sh	
						1053 s	C-2–D
1047 m	C-1–H	1049 sh					
1026 s		1031 s		1035 s		1032 s	
1011 s	CH_2			1014 s		1003 m	
988 s		993 s		989 s		987 s	
		967 s	CD_2				
				960 m	C-1–D	961 m	
						954 m	C-2–D
						924 w	
911 m	C-1–H	908 s					
890 sh						896 w	
				874 vs	C-1–D		
		866 w	CD_2				
						849 w	C-2–D
836 s	C-1–H	837 w					
				810 vs	C-1–D	812 s	
		799 w				800 w	
768 m		768 m		761 m		761 m	
		743 m	CD_2				
721 w-br		717 w-br		716 m-br		724 m-br	
645 sh				640 sh		640 sh	
622 s-br		621 s-br		627 w		624 m-br	
603 s-br		594 s		612 s-br			
555 w-br		558 w		553 m		552 m	

TABLE III (*continued*)

α-D-Glucose	*Assignment*	*D-Glucose-6,6-d$_2$*	*Assignment*	*D-Glucose-1-d$_1$*	*Assignment*	*D-Glucose-1,2-d$_2$*	*Assignment*
		536 sh		534 w		533 w	
522 w							
		508 sh		497 vw			
		450 w				451 vw	
				431 w		427 w	
		418 w					
				401 w		403 w	

*See footnote to Table I.

In addition, new bands appear for the latter at 1179, 1053, 954, and 849 cm^{-1}, which are assigned to C-2–D-related modes. A variety of other differences between the spectra may be seen (see Table III), but the above are the only ones for which the changes across the series are sufficiently consistent to allow assignments to be made.

Frequency shifts produced by deuteration. — The effect of deuteration on the higher-frequency region of the i.r. spectrum has been extensively studied[5]. Conversion of the hydrogen-bonded OH groups into OD groups causes the replacement of bands at 3400 cm^{-1} with O–D stretching bands at ~2500 cm^{-1}. The stretching bands are due to pure vibrational modes, and the predicted ratio of equivalent O–H and O–D frequencies of $\sqrt{2}$ compares well with observed values for this ratio in the range of 1.3–1.4. However, the effect of deuteration on the O–H deformation bands has not been studied in such detail. In addition, most deuteration work on carbohydrates has been for i.r. techniques, and the changes in intensity on deuteration to be expected in the Raman spectra are largely unknown.

Assignments of the lines due to O–H and O–D related modes for methanol were made by comparison of the two spectra in Fig. 11. The line at 1112 cm^{-1} disappears on deuteration, and is assigned as an O–H mode; the two new lines at 1230 cm^{-1} and 945 cm^{-1} are assigned as O–D vibrations. It is unlikely that deuteration would produce a line at a higher frequency, and there is probably a weak O–H line beneath the strong lines due to CH_3 modes at 1453 and 1468 cm^{-1}. If this O–H band has a frequency of ~1460 cm^{-1}, then the ratio for the decline in frequency is $(1460/1230) \simeq (1112/945) = 1.18$. The intensity of the new O–D line at 945 cm^{-1} is very weak, and similar lines for the carbohydrates may well not be observed due to overlap. We have observed similar frequency-ratios for other aliphatic alcohols, although this behavior was not followed exactly for all frequency shifts. However, as the molecules get larger, the picture becomes complicated, probably as a result of complex modes and overtones.

The O–H and C–H deformation-frequencies for D-glucose that disappear on deuteration are listed in Table IV, together with the frequencies of the new O–D and

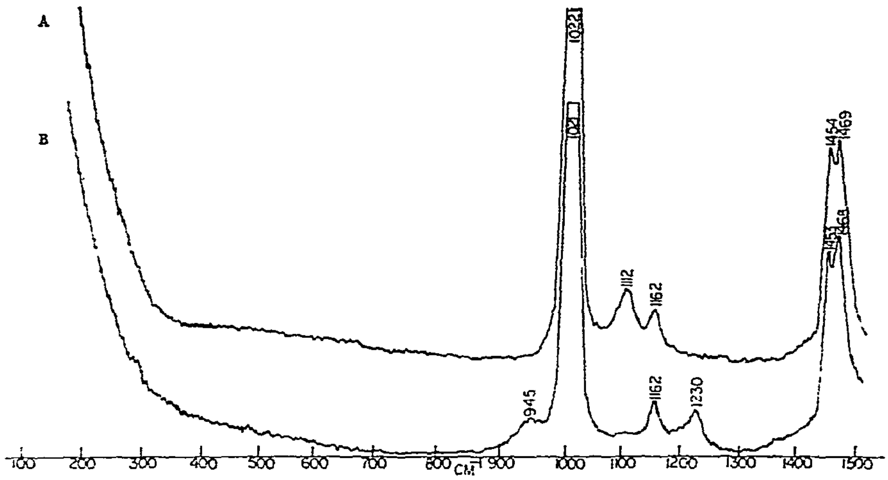


Fig. 11. Raman spectrum of methanol in solution: A, in H_2O; B, in D_2O.

C–D bands. Frequency ratios have been calculated for the bands in the order of decreasing frequency. It can be seen that the one value for the $\nu(OH)/\nu(OD)$ ratio is 1.21, which compares well with the observed figure for methanol, where the frequency correlation is unambiguous. For the C–H bands, six ratios are calculated and four of them lie in the range 1.19±0.02. Naturally, combinations can be chosen to give other ratios, although none show this measure of agreement, and some of the higher-frequency C–D bands must be left out. Some sort of correlation might also be expected between the band intensities, in that the O–D and C–D bands should have the same relative intensities, but lower absolute intensities, than the corresponding O–H and

TABLE IV

FREQUENCY SHIFTS FOR O–H AND C–H DEFORMATION MODES DUE TO DEUTERATION

Mode	*Frequency (cm^{-1})*	*Mode*	*Frequency (cm^{-1})*	*Frequency ratio*
O–H	1349	O–D	1111	1.21
	1071			
	1020			
	913			
C-1–H	1360	C-1–D	1158	1.17
	1250		1063	1.18
	1076		960	1.12
	1047		874	1.20
	911		810	1.12
	836			
C-2–H	1402	C-2–D	1179	1.19
			1053	
			953	
			849	

C–H bands. The intensity correlation in Table IV is only fair, since there is poor agreement in two examples. However, prediction of intensities is difficult, particularly if these bands are not due to vibrations that are completely pure. Further study is being made on this aspect of the work.

The band assignments reported in this paper have been made use of in experimental and theoretical studies of the spectra of other sugars and polysaccharides, details of which will be published shortly, as paper II of this series.

ACKNOWLEDGMENTS

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