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THE EFFECT OF CATIONS ON THE ANOMERIC EQUILIBRIUM OF D-GLUCOSE IN AQUEOUS SOLUTIONS — A RAMAN-SPECTRAL STUDY

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ABSTRACT

Raman spectra of α- and β-D-glucose, each freshly dissolved in water, have been recorded. These conditions allow a more definite assignment of features to a particular anomer in the equilibrated mixture than is possible from a comparison with the spectra of the polycrystalline solids. A survey of the effect of metal cations on the α–β equilibrium of D-glucose has been carried out, using bands in the 950–800 cm^{-1} region to monitor the change. The Ca^{2+} ion has a marked effect, shifting the equilibrium to favor the α anomer. For other cations, the effect has been found to fall off in magnitude according to the series

$$Ca^{2+} > Sr^{2+} \approx La^{3+} > Na^{+} \approx Zn^{2+} > Cd^{2+} \approx Mg^{2+} \approx K^{+}.$$

INTRODUCTION

In solution, simple reducing sugars exist as equilibria of two or more isomeric species[1]. The observed composition of the equilibrium mixtures depends on the relative free-energies of the various isomers. Several attempts have been made to calculate the positions of anomeric equilibria (see, for instance, refs. 2 and 3). Such attempts have been only partially successful, because any solvation contributions are difficult to incorporate into the calculations.

That the solvent does indeed affect the anomeric (and other isomeric) equilibria is well established[4], and the mechanisms whereby the solvent, especially water, influences the relative free-energies of the conformers in solution have been studied in detail[5–8]. A summary conclusion of such thermodynamic and spectroscopic studies is that the various hydroxyl groups in a sugar molecule do not react equally with water, but that the dynamics, hydrogen bonding, and proton-transfer kinetics of the various hydrated hydroxyl groups depend sensitively on the detailed stereochemistry of the sugar molecule[9,10].

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The aqueous-solvent order can be perturbed by temperature or by solutes. It is particularly sensitive to the influence of ions. Thus, ions might be expected to cause shifts in the anomeric and conformational equilibria. On the other hand, some ions are also able to form stoichiometric complexes with sugars having some particular configurations. A primary objective of this work was to ascertain if particular cations could affect the point of balance of the anomeric equilibrium. The technique used to monitor the change was Raman spectroscopy, a technique that has proved useful in the study of complexes in electrolyte solutions[11]. This technique has been applied to aqueous solutions of D-glucose by Barrett[12], Mathlouthi and co-workers[13,14], Sivchik and Zhbankov[15], She *et al.*[16], Vasko *et al.*[17,18], and Spedding and Stamm[19]. The Raman spectrum of crystalline α-D-glucose has been reported by Vasko *et al.*[18], She *et al.*[16], Wells[20]. and Huvenne *et al.*[21]. The spectrum of crystalline β-D-glucose has been reported by Wells[20], Huvenne *et al.*[21], Cael *et al.*[22], and Hineno[23]. Normal-coordinate treatments have been reported by Vasko *et al.*[18], Huvenne *et al.*[21], Cael *et al.*[22], and Hineno[23].

Complexing between sugars and cations has been extensively studied by Angyal and co-workers, and has been reviewed[24]. For strong complexation to occur, Angyal has proposed that an axial–equatorial–axial arrangement of hydroxyl groups on three consecutive carbon atoms must exist. Complex-formation has been identified by n.m.r. spectroscopy, electrophoresis, ion-selective electrodes, and solubility measurements[24]. A number of complexes of inorganic salts with such sugars have been isolated[25] in the solid state (*e.g.*, the $CaCl_2$ complex of D-mannose). D-Glucose does not possess the required arrangement of hydroxyl groups, but this study suggests that Ca^{2+} does interact with D-glucose, causing a shift in the anomeric equilibrium, probably also through the mechanism of complex-formation.

EXPERIMENTAL

Aqueous solutions of D-glucose were prepared by dissolving α-D-glucose (Baker analyzed, anhydrous) in distilled water. Dissolution was aided by gentle heating for a very short time in a water bath at 80°. Solutions required about 2 h to reach anomeric equilibrium; to these solutions were added salts of analytical grade, and Raman spectra were recorded. When the spectrum of the virtually pure aquated α or β anomer was desired, the α-D-glucose or β-D-glucose (Sigma Chemical Company, St. Louis, MO) were rapidly dissolved in water to a specified concentration, and Raman spectra were recorded immediately. Anomerization was sufficiently slow as to allow bands of the α and β forms to be clearly differentiated. All samples were filtered through 0.6-μm Millipore filters prior to being flame-sealed in glass capillary tubes. Raman spectra were measured with a digitally driven Jarrell–Ash 25–100 spectrometer (1-m double Czerny–Turner monochromator), with an RCA 31034 photomultiplier and an SSR model 1105/1120 photon counting system. The 514.5-nm line of a Spectra Physics 165-03 Argon ion laser was used for excitation. Laser power at the sample was 1.5 W, and the spectral slit-width was 6 cm^{-1}. All were recorded at 23°.

RESULTS AND DISCUSSION

Spectra of D-*glucose solutions.* — Our primary objective was to ascertain the extent to which the anomeric equilibrium is affected, or to which complex species are formed, when selected cations are added to D-glucose in solution. To achieve this objective, the spectra of the α and β anomers in an aqueous medium must be understood; in particular, bands which can be clearly used to discriminate between the two anomers when in equilibrium need to be identified. To this end, spectra of solutions of the α anomer, the β anomer, and the equilibrated mixture were obtained.

Spectra of the α and β forms are presented in Figs. 1A and 1C, and 2A and 2C, respectively. Within 15 min, significant proportions of the other anomer were formed and, after ~2 h, the D-glucose solution had completely equilibrated (see Fig. 1B and 2B). Addition of the pure anomer to a saturated solution of sodium chloride further retards attainment of the anomeric equilibrium and allows the

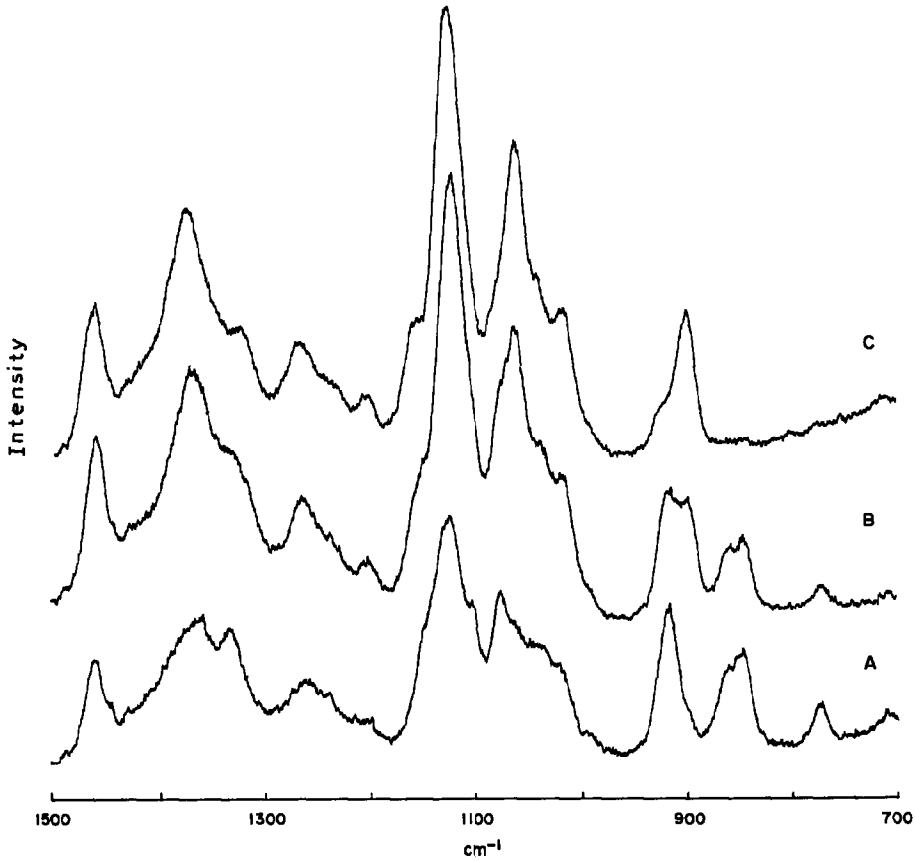

Fig. 1. Raman spectra of 2.0M D-glucose in the 1500 to 700 cm^{-1} region. A, Freshly prepared α-D-glucose. B, An equilibrated solution of α- and β-D-glucose. C, Freshly prepared β-D-glucose.

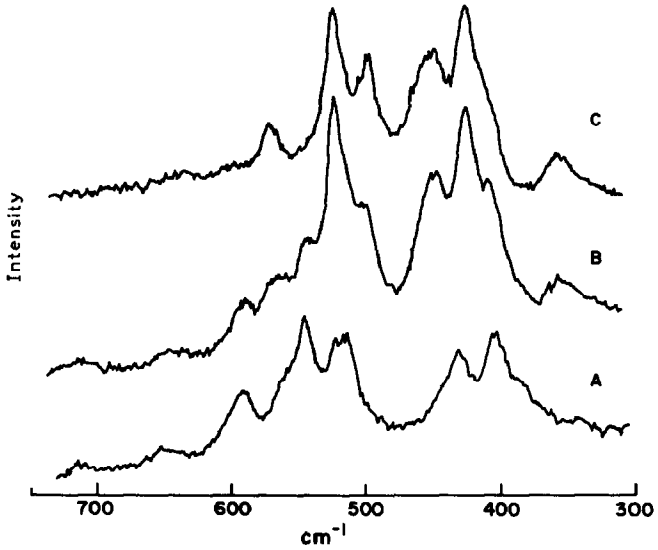

Fig. 2. Raman spectra of 2.0M D-glucose in the 700 to 300 cm^{-1} region. See Fig. 1 for explanation.

entire spectrum of the nearly pure anomer to be recorded. Consequently, a definite assignment of the bands of the equilibrated mixture to one, or both, of the anomers can be made. Table I contains frequencies of the spectra of 2M aqueous D-glucose. Our results for the equilibrated solution are in good agreement with those reported by Vasko *et al.*[17,18].

Having made a thorough study of the spectra of crystals and solutions, Koenig and collaborators remarked on the difficulty of achieving complete assignments for such a complex molecule; the normal modes are strongly coupled and thus, characteristic group vibrations frequently cannot be identified[22]. Also, spectral bands of solutions are broad. Although the α anomer, for example, may have a band with peak maximum shifted from that of the β anomer, intensity from the wings of the bands of the β anomer may underlie those of the α anomer, and the resulting line shape in the spectrum of the equilibrated mixture will be a composite, and *vice versa*. Thus, there are few regions of the spectrum that can be used to discriminate between the anomers.

Mathlouthi and Luu[13] assigned bands to group vibrations despite an admonition of Koenig and co-workers[18]. Their objective was to provide guidance for interpreting spectral changes resulting from modification of the molecular structure by such changes in the environment as temperature and concentration. They proposed some specific assignments for the α and β anomers on the basis of the expected intensity ratio ($\beta/\alpha = 1.78:1$).

This procedure for distinguishing between the anomers is not valid for two reasons. Firstly, in the equilibrated mixture, the bands of the two anomers overlap each other in many regions, and thus the ratio of intensities is not a ratio of populations of β to α anomers. A ratio of 1.78:1 can be fortuitous. Secondly, there is no

TABLE I

FREQUENCIES (cm^{-1}) OF α-D-GLUCOSE AND β-D-GLUCOSE IN AQUEOUS SOLUTIONS[a]

α-Anomer	*β-Anomer*	*Equilibrated*	*Attribution*[b]	*Equilibrated*[17]	*Glucose*[c]*+CaCl₂*
1464 m	1464 m	1464 m	α + β	1461 m	1464 m
F	F	F		1405 sh	F
F	1392 w,sh	F	α + β		F
1377 w,sh	1375 s,br	1374 s	α + β*	1373 vs	1378 m
1364 m,br	F	F	α + β	1349 m,sh	F
1336 m	F	1336 m,sh	α + β	1335 m,sh	1336 m,sh
F	1324 m,sh	F	α + β	1328 w,sh	F
				1298 w,sh	
1265 m,br	1265 m,br	1265 m,br	α + β	1278 m	1265 m,br
1240 w,sh	1240 w,br,sh	1240 w,sh	α + β	1222 w,sh	F
F	1202 w	1202 w	α + β*	1206 w	1210 w
1157 w,sh	1157 m,sh	1157 m,sh	α + β*	1152 w,shF	
1130 s	1128 vs	1128 vs	α + β*	1130 vs	1128 vs
1107 m,sh	F	F	α + β		1102 w,sh
1079 m	1080 w,sh	1077 m,sh	α* + β		1079 m
F	1063 s	1063 s	α + β*	1071 vs	F
1046 sh,br	F	1041 sh,br	α + β	1041 w,sh	1054 m,sh,br
F	1022 m,sh	1020 sh	α + β	1020 m,sh	1018 w,sh
920 s	924 w,sh	920 m	α* + β	913 s	918 s
	900 s	902 m	β	898 s	F w
868 m		862 m	α	859 sh	870 m
852 m		848 m	α	847 s	848 m
776 w		776 w	α	771 m	776 m
				747 w	
712 vw		710 vw	α	705 w	712 w
651 w		645 w	α	635 m	651 w
	634 vw		β		
591 m		588 w	α		591 m
F	571 w	F	α + β*		F w
562 sh		563 sh,br	α		560 m,sh
545 m		542 m,sh	α	541 w,sh	545 m
F	523 s	523 s	α + β*	514 vs	523 s
518 m	F	F	α + β		516 s,sh
F	502 w,sh	502 m,sh	α + β	498 m,sh	F w
F w	498 m	F	β		F w
F	451 m,br	450 m	α + β*	443 s	443 sh
431 m	F	F	α + β		430 m
F	425 s	425 s	α + β*	423 s	F
F	410 sh	410 m,sh	α + β*	409 m,sh	410 m
404 m	F	F	α		F
386 sh		F	α	381 w, sh	368 vw
	357 m	357 m	β		
342 w		F	α	341 m	
				294 w	
				274 w	

[a]Abbreviations: br, broad; m, medium; s, strong; sh, shoulder; v, very; w, weak. F, intensity present as the wing or foot of an adjacent band, but not a defined peak.

[b]The symbol * implies dominant intensity from the anomer so designated [c]Equilibrated 2M D-glucose with 4M $CaCl_2$.

a priori reason to believe that the specific or molar intensities of bands of the two anomers used in the ratio will be equal. Indeed, it is known that the integrated absorption intensity of the equatorial C–Cl stretching vibration of the *trans* isomer of 4-*tert*-butylcyclohexyl chloride is 47% greater than that of the axial C–Cl stretching vibrations of the *cis* isomer[26]. To be certain of the assignments, spectra of solutions of the pure anomers must be available. We now report these, and we find that several of the assignments of Mathlouthi and Luu[13] require qualification.

On the basis of the intensity ratio, Mathlouthi and Luu assigned a band at 1360 cm^{-1} (1374 cm^{-1} in our spectrum) to the β-anomer, and a band at 1327 cm^{-1} (1336 cm^{-1} in our spectrum) to the α anomer. Examination of Fig. 1B reveals that the ratio is about the expected 1.78:1, but, from Fig. 1A (α anomer) and Fig. 1C

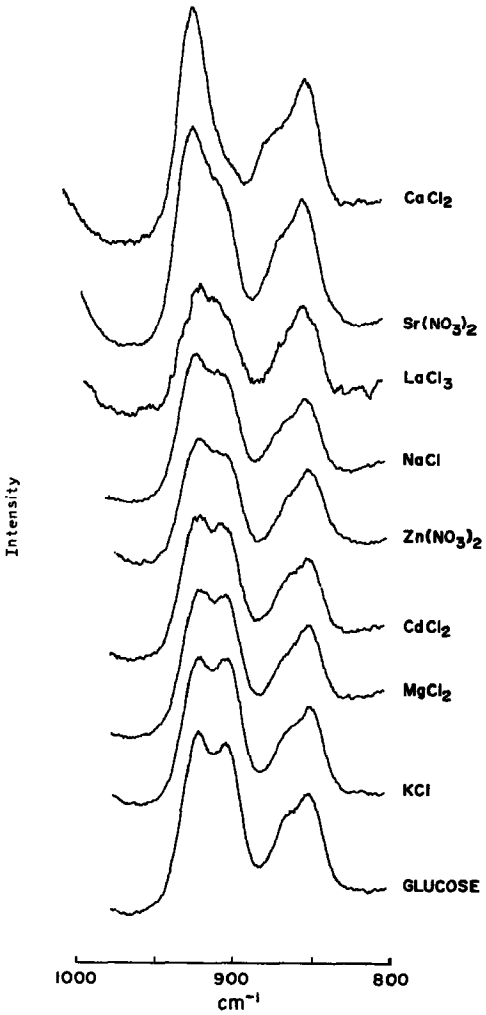

Fig. 3. Raman spectra of equilibrated 2.0M D-glucose solution (1000–800 cm^{-1}) containing the salts indicated. Salt concentrations are 4M, with the exception of $LaCl_3$, which is 3M.

(β anomer), it can be clearly seen that each anomer contributes to the intensity of both bands. The "1360-cm^{-1} band" is somewhat stronger and sharper for the β anomer, but it cannot be considered to be a discriminator. There is continuous, broad intensity from 1410 to 1310 cm^{-1}. Also the 1338-cm^{-1} band of the α anomer lies between the 1375-cm^{-1} band and its 1324-cm^{-1} shoulder of the β anomer, but is effectively masked by the superposition of bands of the α and β anomers in the equilibrated solution. Similarly, although the 1060-cm^{-1} band (1063 cm^{-1} in our spectrum) was correctly attributed to the β anomer[13], there is broad underlying intensity between 1090 and 1000 cm^{-1}, and thus, all bands in this range must be considered a superposition of bands from both anomers. Band overlap is also apparent in the infrared spectra of these solutions; difference spectra revealed which anomer contributes to the greatest extent to the intensity[27].

The region of the spectrum which shows easily distinguishable α- and β-anomeric bands is located between 930 and 830 cm^{-1}. This region has been called the "anomeric region" by Cael *et al.*[22], and will be referred to as such. The region includes four Raman bands (see Fig. 3, bottom trace). The band at 920 cm^{-1} is primarily due to α-D-glucose, with a small contribution at 924 cm^{-1} from β-D-glucose (*cf.*, Fig. 1A). The band at 902 cm^{-1} is designated as a "purely β" band (see Fig. 1C), and those at 862 and 848 cm^{-1} as "purely α" bands (see Fig. 1A). Mathlouthi and Luu[13] attributed a 910-cm^{-1} and an 893-cm^{-1} band to β-D-glucose. As is evident from Figs. 1A and 1C, the 910-cm^{-1} band (920 cm^{-1} in our spectrum) is quite prominent in the spectrum of the freshly prepared α anomer, and is nearly absent from the spectrum of the freshly prepared β anomer. Their assignment[13] of the 910-cm^{-1} band is, therefore, incorrect.

Below 700 cm^{-1}, there are four bands which clearly stand out, unmasked by bands of the alternative anomer. Thus, the 590- and 546-cm^{-1} bands arise from the α anomer; the 450- and 357-cm^{-1} bands arise from the β anomer. Other bands overlap to some degree. The differences can be gauged from Fig. 2.

In Table I, the letter F is used to indicate that intensity from the foot or wings of adjacent bands contributes intensity in a region where the peak of the other anomer occurs. For example, the 1364-cm^{-1} band of the α anomer and wings of the 1375- and 1324-cm^{-1}-bands of the β anomer contribute to give intensity at 1364 cm^{-1} in the equilibrated solution. The column labelled "anomer" indicates whether one (or both) anomer(s) is (are) contributing intensity at a particular frequency; if both are contributing, but one of the two is particularly prominent, that one is designated with either α^* or β^*.

The approximate potential-energy distribution for β-D-glucose and the calculated frequencies for the α and β anomers have been given by Cael *et al.*[22]. The modes for α-D-glucose were recalculated using a force field modified from that used by Vasko *et al.*[17]. Assignments for the solids were also given by Huvenne *et al.*[21] and Hineno[23]; these results are not reproduced here. The spectra of the solids consist of more and sharper lines, especially[20] at 100 K, than that of the solution. Frequently, correspondence between spectra of solid and solution is good. How-

ever, band intensities and positions can be markedly changed on going from the solid to the solution state, and, because of the broadness for the latter, a number of bands are encompassed under a single envelope. Thus, the normal modes of bands in the solution phase cannot be rigorously described.

Considering the spectra from 1500 to 300 cm^{-1}, the only region which provides clear distinction between bands of the α and the β anomer is the anomeric region between 930 and 830 cm^{-1}. For the solids, the 842-cm^{-1} band of the α anomer occurs in a clear region of the spectrum of the β anomer, and, for the solution, the corresponding 862–848-cm^{-1} doublet occurs in a region free from β anomer intensity. The reason for the doublet in the spectrum of the solution is not yet clear. The band is due to a coupling of CH_2, C-1-H, and C–O–H motions[22]. The 914-cm^{-1} band of solid α anomer has almost the same position as a weaker, 913-cm^{-1} band of the β anomer, beside which is a more-intense, 898-cm^{-1} band. These

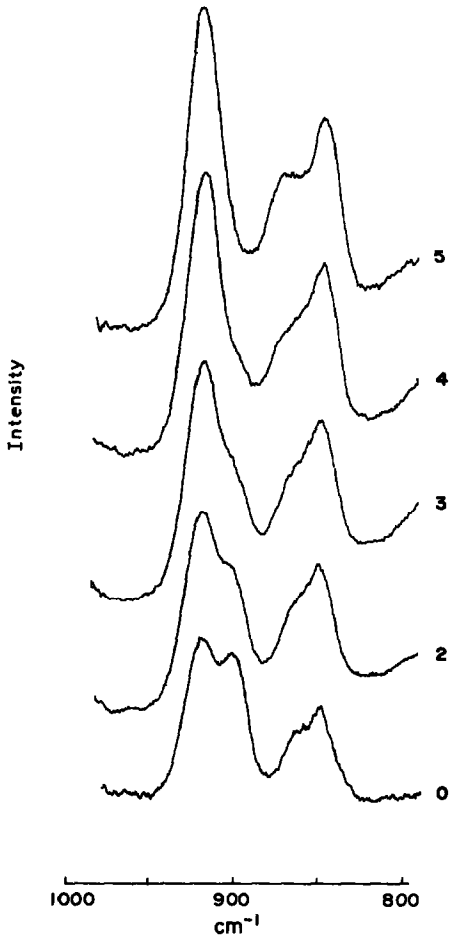

Fig. 4. Raman spectra of equilibrated 2.0M D-glucose solutions containing the specified concentrations (M) of $CaCl_2$.

correspond in solution to the 920-cm^{-1} line of the α anomer, under which is some intensity from the 924-cm^{-1} shoulder from the β anomer; the 900-cm^{-1} band of the β anomer overlaps, to give the doublet observed in the equilibrated mixture. The 924-cm^{-1} band arises from coupled CH_2 and C-1-H motions and C–O–H bending; the bending is lacking in the lower-frequency, 900-cm^{-1} mode. The atomic displacements have been illustrated by Cael *et al.*[22].

The addition of salts. — The anomeric regions of the Raman spectra of solutions of equilibrated D-glucose containing eight salts are presented in Fig. 3. For this series, the concentration of D-glucose is 2 mol.dm^{-3} and the salt concentrations are 4 mol.dm^{-3}. Considering first the sample containing $CaCl_2$, it can be seen that the 900-cm^{-1} band of the β anomer is greatly diminished, and the 920-cm^{-1} line of the α anomer stands out prominently. Assuming that the weaker, 924-cm^{-1} line of the β anomer is similarly lessened, the 920-cm^{-1} line can be attributed almost entirely to the α anomer. The 862–848-cm^{-1} lines of the α anomer are simultaneously clear, and altered very little. (Possibly the 862-cm^{-1} intensity is slightly diminished relative to the 848-cm^{-1} intensity.) Comparing this spectrum with the others, particularly at 900-cm^{-1}, the salt–glucose perturbation is seen to fall off in magnitude according to the series:

$$Ca^{2+} > Sr^{2+} \approx La^{3+} > Na^{+} \approx Zn^{2+} > Cd^{2+} \approx Mg^{2+} \approx K^{+}.$$

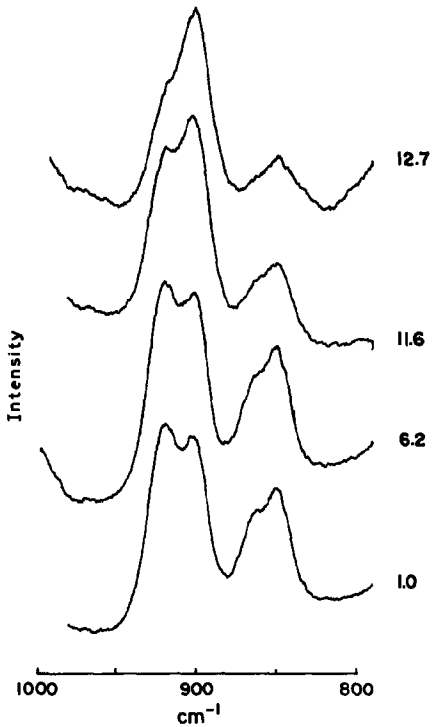

Fig. 5. Raman spectra of equilibrated 2.0M D-glucose solutions at the pH designated to the right of each trace.

The effect can be attributed to the cation, because, with two exceptions, the anion, chloride, is common to all. In order to avoid competition with extensive chlorozincate complexing, $ZnCl_2$ was not used.

The effect of increasing the concentration of $CaCl_2$ on the Raman spectrum of equilibrated, 2 mol.dm^{-3} D-glucose is shown in Fig. 4. As the concentration of $CaCl_2$ increases, the intensity of the 900-cm^{-1} band decreases, and ultimately disappears; the bands at 920, 862, and 848 cm^{-1} sharpen. The spectrum with 5 mol.dm^{-3} $CaCl_2$ is not significantly different from that of the aquated α anomer (see Fig. 1A). These spectra are consistent with the view that the point of balance of the α–β equilibrium is shifted by the Ca^{2+} to favor the α anomer.

In order to ensure that the observed effects were due to the presence of Ca^{2+} (and not the anion), Raman spectra of 4 mol.dm^{-3} solutions of $Ca(SCN)_2$, $Ca(NO_3)_2$, $CaBr_2$, and $CaCl_2$ were compared. All salts gave spectra similar to that shown in Fig. 4 for 4 mol.dm^{-3} $CaCl_2$.

To ascertain if a connection exists between the change in the spectrum and the acidity of the solutions, Raman spectra of samples of an equilibrated solution of D-glucose (alone) at differing pH values were recorded. The pH was adjusted by addition of small amounts of HCl or NaOH solution. The spectra are shown in Fig. 5. The acidic solutions showed no change in the spectrum of the anomeric region. The strongly basic samples showed a change: the intensities of the bands at 920, 862, and 848 cm^{-1} (α anomer) are diminished relative to that of the 900 cm^{-1} band (β anomer). This effect is the opposite of that of Ca^{2+}. A similar change in the ratio of the Raman spectral bands of D-fructose 1-phosphate to D-fructose 6-phosphate was observed by Barrett[12]. The equilibrium in basic medium is between anomers of the D-glucosate anion, for which the proportion of the β anomer is increased[28–30]. The change we report in the pattern of Raman intensity in Fig. 5 is consistent with the n.m.r.-spectral results. In the kinetics of the process, the catalytic activity of the D-glucosate ion was found to be 100 times that for hydronium[31].

Less attention has been given in this work to the C–H stretching region. However, it was noted that two overlapping bands, at ~2903 and 2951 cm^{-1}, changed in relative intensity on addition of $CaCl_2$, the latter increasing with respect to the former. When spectra of a freshly prepared solution of the β anomer were successively recorded as equilibrium was approached, the same effect was noted, and thus, the higher-frequency component can be assigned in large part to the α anomer. Thus, this region of the spectrum also reveals that Ca^{2+} causes the α–β distribution to shift to favor the α anomer.

Angyal observed no substantial change[32] in the n.m.r. spectra of dilute solutions of D-glucose, D-mannose, or D-arabinose on addition of $CaCl_2$. He noted that these sugars do not possess the *a–e–a* sequence of three oxygen atoms favorable for complex-formation[33]. Later, in studies of paper electrophoresis of polyols in solutions of calcium ion, he noted that practically all polyols and sugars (including the three just cited) show some mobility, even when other methods do not detect complex-formation[34]. Goulding[35] reported the separation of the α and β

anomers of D-glucose on a column of a Ca^{2+} or Sr^{2+} cation-exchange resin; β-D-glucose is eluted before α-D-glucose. He noted that the former has no *a–e* hydroxyl-group neighbors, whereas the latter has one such pair and can form weak complexes (compared to those sugars having the sequence *a–e–a*). We conclude that Raman spectroscopy is a technique which is sensitive to the presence of these weak complexes (in contrast to the view expressed by Back *et al.*[36]). However, the complexity of the vibrational spectrum is such that the task of elucidating the nature of these complexes is much more difficult than, for example, the study of small, ionic, complex ions[11].

It is interesting that the effect is largest for the Ca^{2+} ion, an important ion in biological systems. Unlike Zn^{2+}, Cd^{2+}, and Mg^{2+}, which have well defined hydration-spheres and which give a low frequency, polarized, Raman band from the $M^{2+}(H_2O)_6$ species[37], Ca^{2+} has less strongly bonded water molecules, with a less consistent coordination number[38], and gives no (or, at most, very weak) Raman intensity ascribable to $Ca^{2+}(H_2O)_x$. It may be asked if the shift in the anomeric equilibrium comes about because of changes induced in the solvation of the D-glucose molecules by the Ca^{2+} ion (an inductive effect) or whether the Ca^{2+} binds preferentially to the α-D-glucose, forming a complex which results in the shift of the equilibrium to favor the α form. In the latter case, signals from the bound sugar in the complex could be expected to be somewhat displaced from those of the solvated sugar, based on experience with the Raman spectra of inorganic complexes[11]. The changes in the spectrum of equilibrated D-glucose on addition of $CaCl_2$ are not great (see Table I, last column). Most of the changes result from a loss of intensity of bands attributed to β-D-glucose. There are a few exceptions: the shift of the 404-cm^{-1} band of the α anomer to 410 cm^{-1}, the enhanced intensity at 523 cm^{-1}, and the shift of the 1364-cm^{-1} band to 1378 cm^{-1} are examples, and there are other, more subtle changes. These give very little clue as to the structure of a complex other than that the C–O–H vibrations are perturbed. For a well-defined α-D-xylose complex in the solid state ($C_5H_{10}O_5 \cdot CaCl_2 \cdot 3\ H_2O$), many bands of D-xylose are markedly changed by the association[39] with Ca^{2+}. Similarly, the F.t.-i.r. spectra of Ca(α-L-arabinose)$X_2 \cdot 4\ H_2O$, where X = Cl^- or Br^-, have many differences from those of L-arabinose[40]. For the case of D-glucose, we must infer that the majority of the bands of the complex in solution and the bands of the α anomer are very similar. Despite this absence of distinctive bands, we favor an interpretation in terms of weak complex-formation, rather than the medium-induced shift, which would be expected to be nonspecific. Interaction with any small proportion of D-glucofuranose present[41] is probably undetectable.

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