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LASER-RAMAN SPECTRA OF D-GLUCOSE AND SUCROSE IN AQUEOUS SOLUTION

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ABSTRACT

Laser-Raman spectra of D-glucose in water at various concentrations were recorded below 1700 cm^{-1}. Assignments of the frequencies of D-glucose were proposed, based on earlier work on the vibrational spectra of this sugar, and determination by other techniques of the anomeric composition of aqueous solutions of D-glucose. The proportions of the anomers found from the ratio of the Raman intensities for the same modes of vibrations were similar to those found by other techniques. Assignments of the frequencies for sucrose were proposed, in the light of previous results on D-glucose and D-fructose, and the effect, on their Raman spectra, of the condensation of these two monosaccharides was pointed out.

INTRODUCTION

We recently discussed[1] the proportions of the furanoid and pyranoid forms of D-fructose in aqueous solutions as estimated from their laser-Raman spectra. Spectroscopic techniques, especially i.r. spectroscopy, have been used for a long time[2,3] to study the mutarotation of D-glucose in aqueous solutions, but, as noted by Tu *et al.*[4], the advantages of laser-Raman spectroscopy over i.r. spectroscopy for the study of aqueous solutions of sugars are very important, and recent studies[5–7] made by use of the Raman effect provide information permitting reliable characterization of the anomers of D-glucose.

Among the carbohydrates, D-glucose has been the most extensively studied by means of spectroscopic methods: i.r.[8,9] and the laser-Raman[5,7,10,11]; but, as Koenig *et al.*[7] remarked, the assignment of all of the observed vibrations of the Raman spectra below 1500 cm^{-1} is a formidable task. However, deuteration may help in assigning vibrations as C–H or O–H modes[7,9–11]. The calculation of the normal modes of vibration[5,10,12,13] could provide information on the contribution, to the vibration active in the Raman spectrum, of each of the groups in the molecule.

However, from the point of view of the biologist, it is less important to know the contribution of each of the modes of vibration, to the Raman line, than the assignment of the most probable vibration to that line. These assignments could be used as reference marks that should allow monitoring, by their shifts in frequencies and intensities, the modification of the structure by the environment when such physical factors as temperature and concentration are varied.

The results of techniques as different as oxidation methods[14], polarimetry[15], or n.m.r. spectroscopy[16] are in good agreement as regards the determination of the proportions of α and β anomers in aqueous solutions of D-glucose; these proportions are, respectively, 38 and 62%. The assignments of the frequencies below 1700 cm^{-1} of D-glucose in water should be based on these proportions and on earlier work on the Raman spectra of D-glucose and similar molecules[5,7,10,13]. Moreover, although glycosidic linkage has been actively studied by the Raman effect[4,6,11,13], no study of the Raman spectra of sucrose has, as far as we are aware, been made. We now interpret the Raman spectra of aqueous solutions of sucrose, with particular reference to the spectral differences observed between sucrose and the monosaccharide groups (α-D-glucopyranosyl and β-D-fructofuranosyl) that constitute it.

EXPERIMENTAL

Aqueous solutions of D-glucose were prepared by dissolving α-D-glucose (Merck) in distilled water. Concentrations were controlled, after filtration through a membrane, with an Abbé refractometer. The solutions of sucrose were obtained by dissolution of pure sucrose (Merck) in distilled water. All solutions were maintained, for at least 24 h, at the laboratory temperature before recording; the concentrations ranged from 5 to 50% (w/w) for D-glucose and from 10 to 66% for sucrose. The method of obtaining the Raman spectra was described previously[1]. The spectral range recorded was 1700 to 100 cm^{-1}.

RESULTS AND DISCUSSION

A. D-*Glucose solutions.* — In the region of frequencies below 1700 cm^{-1}, the spectral background shows two intensity maxima[17] (besides the D-glucose vibrations), namely, one at ~1640 cm^{-1} which is distinct, and another (more staggered and more diffuse) between 1000 and 400 cm^{-1} that had been observed by Walrafen[17]. The 1640-cm^{-1} maximum of the bending band of H_2O permits differentiation of D-fructose, in aqueous solutions, from D-glucose and sucrose[18]. Likewise, we noted that a band between 1000 and 400 cm^{-1}, which probably corresponds to the libration movement of H_2O, is almost absent from D-fructose spectra, whereas it is emphasized in the spectra of the solutions of D-glucose and sucrose (see Figs. 1 and 2).

In Table I are shown, as an example, the observed frequencies in the Raman spectra of a 22% (w/w) solution of D-glucose and their assignments based on the anomeric-equilibrium results[13-15] and on earlier, Raman-spectral studies of α-D-

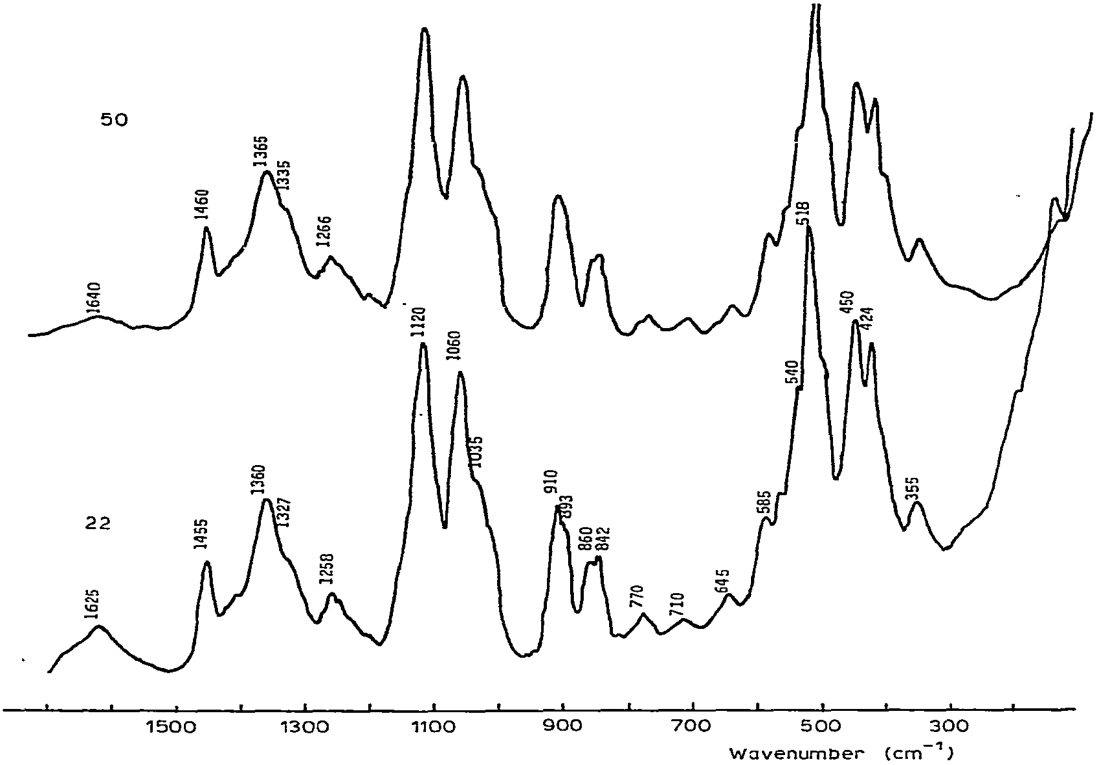


Fig. 1. Raman spectra of D-glucose solutions at 22 and 50% (w/w) concentration.

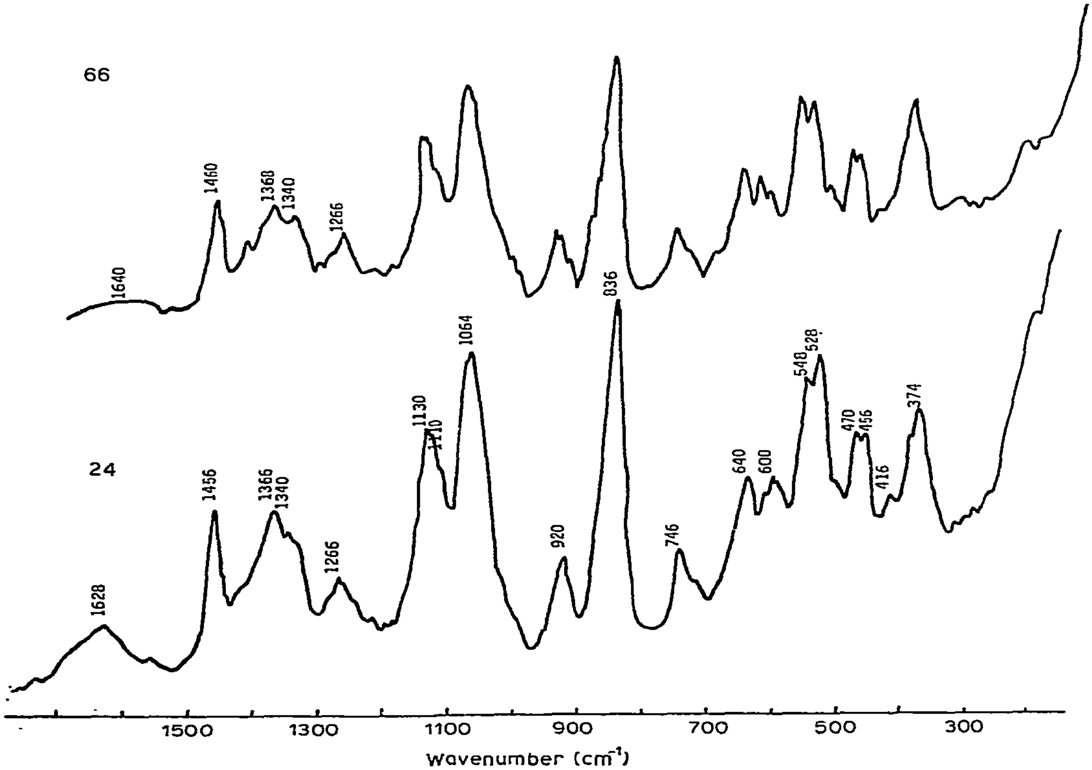


Fig. 2. Raman spectra of sucrose solutions at 24 and 66% (w/w) concentration.

TABLE I

ASSIGNMENTS OF THE FREQUENCIES OBSERVED[a] IN THE SPECTRA OF D-GLUCOSE IN AQUEOUS SOLUTION

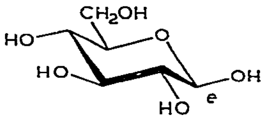
ν (cm^{-1})	*I*	ρ	*Assignments*
1625	13	0.62	δ (HOH)
1455	30	0.70	δ (CH$_2$)
1360	46	0.67	w (CH$_2$) β
1327	21	0.66	w (CH$_2$) α
1258	19.5	0.71	τ (CH$_2$)
1120	86	0.61	δ (COH)
1060	77	0.27	ν (C–O) β
1035	49.7	0.25	ν (C–O) α
910	37	0.20	δ (C–H) β
893			ν (C–C) β
860	23	0.21	ν (C–H) α
842			ν (C–C) α
770	6.6	0.38	δ (O-5–C-1–O-1) β
710	3	0.39	δ (O-5–C-1–O-1) α
645	7.2	0.10	lib. (H$_2$O)
585	16	0.09	δ (C-6–C-5–O-5)
540	54	0.13	δ (C-2–C-1–O-1) α
518	100[b]	0.14	δ (C-2–C-1–O-1) β
450	70	0.24	δ (CCO) *endo*
424	63.5	0.36	δ (CCC)
355	15	0.26	δ (COC)

[a] Key: I = relative intensity, ρ = depolarization ratio, δ = bending mode, w = wagging, τ = twisting, ν = stretching mode, α and β = D-glucose anomers, and *endo* = endocyclic. [b] Reference standard.

glucose[5-7], β-D-glucose[9,10], and D-glucose solutions[2,3,12,13]. The general aspect of these spectra seems to be independent of the concentration, but we have shown[18] that shifts of frequencies of such individual lines as the CH$_2$ bending-vibration at 1450 cm^{-1} occur when the concentration is varied. This vibration also differentiates between D-fructose (in water) and D-glucose and sucrose.

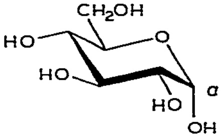
The frequencies at 1360 and 1327 cm^{-1} could be assigned to the wagging of CH$_2$ in α- and β-D-glucose, respectively. When recorded for the solid state, the spectra of these two anomers show an intense line at 1370 cm^{-1} for α-D-glucose, whereas the β-D-glucose spectrum shows only an intense line at 1340 cm^{-1}. Moreover, when the concentration was increased from 5 to 50%, there was observed a shift of these two frequencies between 1356 (to 1365 cm^{-1}) and 1325 cm^{-1} (to 1335 cm^{-1}). These shifts are comparable to those of the CH$_2$ bending-mode[18] between 1460 and 1450 cm^{-1}. This might be due to the formation of intermolecular hydrogen-bonds, including those of CH$_2$OH groups, creating constraints leading to increase in the frequencies of the CH$_2$ vibrations. The ratios of the intensities of these two vibrations, I(1360)/[I(1360) + I(1327)] = 0.68 and I(1327)/[I(1327) + I(1360)] = 0.32, are close to the proportions of β and α anomers in aqueous solution, thus supporting our assignments. The frequency at 1258 cm^{-1} could be assigned to the twisting mode of CH_2, which is in agreement with previous results[5,7,9]. This frequency also shifts, from 1256 to 1266 cm^{-1}, when the concentration of a D-glucose solution is increased from 5 to 50%.

The assignment of the frequencies in the 1360–1250-cm^{-1} region is, indeed, controversial, as noted by Koenig *et al.*[10], but our assignments, based on the ratio of anomers, and in good agreement with Raman results on polymers[19] containing CH_2OH groups, could constitute a good reference mark for the study of D-glucose solutions. The frequency at 1120 cm^{-1}, one of the most intense in the Raman spectrum of D-glucose, is present in the spectra of the solid forms of both α- and β-D-glucose.



β-D-Glucose

1



α-D-Glucose

2

It is found in the i.r. spectra of several sugars[3], and we assign it to the bending of C–O–H, in agreement with the Raman results of Koenig *et al.*[10] and the i.r. results of Hineno[9]. The frequencies at 1060 and 1035 cm^{-1} could be assigned to the C–O stretching-mode for β- and α-D-glucose, respectively. The most energetic vibration should correspond to the shorter, C–O bond-distance of the anomeric carbon atom. Crystallographic studies gave C-1–O-1 = 138.3 pm for β-D-glucose[20] and 140 pm for α-D-glucose[21] (see **1** and **2**). The ratio of the intensities of these frequencies, $I(1060)/[I(1060) + I(1035)] = 0.60$, permits finding proportions near to those found by other techniques for the α and β anomers; then, 1060 cm^{-1} may be assigned to C-1–O-1 stretching in β-D-glucose, and 1035 cm^{-1} to C-1–O-1 stretching in α-D-glucose.

The region of frequencies between 1000 and 800 cm^{-1} is stated by most investigators[5–13] to be characteristic of α and β anomeric differentiation, with this assignment differentiating the CH bending around the anomeric carbon atom. Moreover, the C–C stretching vibration is found[1,5,19] in this region. The Raman spectra of D-glucose solutions show two groups of unresolved lines, at 910 and 893 cm^{-1}, and 860 and 842 cm^{-1} (see Fig. 1); the first group is probably characteristic of the β anomer, and the second, of the α anomer. Then, the lines at 910 and 893 cm^{-1} could be assigned to ν(C–C) and δ(C-1–H-1) of β-D-glucose, and those at 860–842 cm^{-1} to ν(C–C) and δ(C-1–H-1) of α-D-glucose. These assignments are supported by the ratios of the intensities of the frequencies of the same modes of vibration that permitted finding 62% of β- and 38% of α-D-glucose, almost the same results as those found by other techniques[14–16]. The difference in the structures of the two anomers justifies our assignments: the β anomer has the H-1 substituent (on the anomeric carbon atom) in the axial position, which allows a more energetic vibration, and, therefore, a higher frequency than that of α-D-glucose, for which the CH bending takes place in the plane of the molecule. Furthermore, there is a difference in the

C–C bond-distances around the anomeric carbon atom that is, presumably, related to the differences in the frequencies of the stretching vibrations (C-1–C-2 = 152.5 pm for β-D-glucose[20], and 153.9 pm for α-D-glucose[21]).

At frequencies below 800 cm^{-1}, the vibrations of the deformations and the vibrations of the ring occur. Therefore, there should be found those vibrations of the ring that could differentiate α from β anomers; this is the case with the 770- and 710-cm^{-1} vibrations that we may respectively assign to O-5–C-1–O-1 in β- and α-D-glucose, and the distance between the two oxygen atoms linked to the anomeric carbon atom is also different in the two anomers (O-1–O-5 = 226.9 pm in β- and 232.6 pm in α-D-glucose)[22]. The equatorial arrangement of all of the OH groups in β-D-glucose allows water to have a long-range organization[23] in a tridymite form. The association, by hydrogen bonds, with water is more important in the case of β-D-glucose; this should explain the more important perturbation of the depolarization ratio of the 770-cm^{-1} vibration than of the 710-cm^{-1} as the concentration is changed. Moreover, the ratios of the intensities of these two frequencies permit estimation of the ratio of the anomers.

The 645-cm^{-1} band could be assigned[17] to the librational contour of H_2O; and the strongly polarized shoulder at 585 cm^{-1} could be assigned to the O-5–C-5–C-6 bending-mode for both anomers of D-glucose in water. The deformation of C–C–O

TABLE II

ASSIGNMENTS OF THE FREQUENCIES OBSERVED[a] IN THE SPECTRA OF SUCROSE IN AQUEOUS SOLUTIONS

ν (cm^{-1})	*I*	*ρ*	*Assignments*
1628	16.4	0.56	δ (HOH)
1456	33.6	0.93	δ (CH$_2$)
1366	45.7	0.67	w (CH$_2$)
1340	37.8	0.72	r (CH$_2$)
1266	25	0.71	τ (CH$_2$)
1130	68	0.53	δ (COH)
1110	60	0.20	ν (C–O) *endo*
1064	89	0.31	ν (C–O) *exo*
920	25	0.34	δ (C–H)
836	**100**[b]	0.10	ν (C–C)
746	21	0.38	δ (CCO) *endo* (Fru)
640	35.7	0.24	δ (CCO) *exo* (Fru)
600	31.4	0.45	δ (OCO$_1$)
548	60.7	0.25	δ (CCO) *endo* (Glc)
528	69	0.17	δ (CCO) *exo* (Glc)
470	38	0.10	δ (CCC) (Fru)
456	36.4	0.23	δ (CCC) (Glc)
416	17.8	0.84	δ (O—H–O)
374	40	0.25	δ (COC)

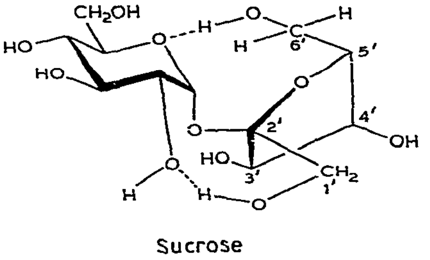
[a] Key: I = relative intensity, ρ = depolarization ratio, *endo* = endocyclic, *exo* = exocyclic, Fru = D-fructosyl moiety, Glc = D-glucosyl moiety, δ = bending mode, w = wagging, r = rocking, τ = twisting, and ν = stretching mode. [b] Reference standard.

around the anomeric carbon atom seems to be one of the most characteristic vibrations of the anomers. We have, indeed, observed that the most-intense frequencies in the spectra of solid samples of α- and β-D-glucose are respectively located at 540 and 518 cm^{-1}. The ratios $I(540)/[I(540) + I(518)] = 0.35$ and $I(518)/[I(518) + I(540)] = 0.65$ lead to assignment of the band at 540 cm^{-1} to C-2–C-1–O-1 in α-D-glucose, and 518 cm^{-1} to C-2–C-1–O-1 in β-D-glucose.

Frequencies below 500 cm^{-1} could be assigned to the endocyclic vibrations of the ring. Thus, that at 450 cm^{-1} could be assigned to the endocyclic C–C–O bending-mode, 424 cm^{-1} to the C–C–C bending, and 355 cm^{-1} to C-5–O-5–C-1. This C–O–C vibration is more intense in the spectra of sucrose solutions, because of the glycosidic linkage present.

B. Sucrose solutions. — The results of the study of the laser-Raman spectra of sucrose in water are summarized in Table II and Fig. 2. It must first be recalled that the 1628- and 1456-cm^{-1} frequencies, respectively assigned to H–O–H and H–C–H bending modes, show a displacement as the concentration is changed[18]. Walrafen[24] showed the structural effect of a 58% concentration of sucrose solution on water; that is what we[18] explained as due to the sucrose–sucrose association that occurs in such a solution, releasing water of hydration which is reorganized in clusters.

The 1500–1200 cm^{-1} region concerns, firstly, the vibrations of the groups that have a local symmetry; this is the case with the CH_2 groups (3 in the sucrose molecule 3). These groups, which can be individualized, should vibrate at the same frequencies in sucrose and the monosaccharides that constitute it, except for vibrations influenced by the environment[18].



Sucrose

3

Thus, we could assign, as in the spectra of D-fructose[1], the bands at 1366, 1340, and 1266 cm^{-1} to the wagging, the rocking, and the twisting modes, respectively, of CH_2 in the sucrose molecule. As the concentration is increased from 10 to 66%, shifts are observed in these frequencies, from 1360 to 1368 cm^{-1}, 1330 to 1340 cm^{-1}, and 1260 to 1268 cm^{-1}, respectively. These shifts are explainable if the two CH_2OH groupings of the D-fructofuranosyl moiety participate in intramolecular hydrogen-bonds[25] (see 3), while the CH_2OH group of the D-glucosyl moiety takes part in the intermolecular association that precedes crystallization.

The very sharp lines at 1130 and 1064 cm^{-1} found in the spectra of aqueous solutions of D-glucose and D-fructose[1] could be assigned respectively to the C–OH bending-mode and the C–O stretching-mode. The perturbation of the polarization ratio of 1130 cm^{-1} when the concentration is changed could probably be explained by the association, by hydrogen bonding, of the OH groups. The stretching vibration is more intense than the C–OH bending-vibration, which is opposite to that observed in the Raman spectra of D-fructose[1] and D-glucose (see Fig. 1). This could be due to the fact that the number of C–O bonds is more important in the sucrose molecule, because of the glycosidic bond C-1–O-1–C-2′ (see 3). In addition to the vibration at 1130 cm^{-1}, a shoulder at 1110 cm^{-1} is observed that could be assigned to the endocyclic, C–O stretching-modes (C-2′–O-2′ in the D-fructosyl moiety, and C-1–O-5 in the D-glucosyl moiety); these bonds are shorter than the exocyclic, C–O bonds that vibrate at 1064 cm^{-1}; their values are[22], respectively, 140.8 and 141.3 pm, whereas the mean value of the C–O bond-length is 143 pm.

These two bonds partake of the ketonic or the aldehydic character of the monosaccharides that constitute the sucrose molecule. They are more energetic, because of the more important energy[26] of the double bond (714.78 $kJ.mol^{-1}$) from which they derive, compared to a simple C–O bond (351.12 $kJ.mol^{-1}$). The 920-cm^{-1} frequency may be assigned to the bending of C–H; it is found in the spectra of both D-glucose and D-fructose. Deuteration permitted Koenig *et al.*[7] to ascribe this frequency to the deformation of C-1–H in D-glucose in solution.

The line at 836 cm^{-1} is the most intense in the spectrum of sucrose, and is greatly polarized. Tu *et al.*[4,6] situated at this frequency the deformation of CH around the anomeric carbon atom of D-glucose, but it is more probable that this frequency could be assigned to the C–C stretching mode in sucrose. On comparison of this assignment to that of D-fructose[1] and D-glucose (see Table I), it is seen that 836 cm^{-1} is lower than the frequencies of the C–C vibrations of D-fructose (874 cm^{-1}) and α-D-glucose (860 cm^{-1}), probably because of the "dimerization". The mass effect of sucrose (M.W. 342) compared to D-fructose or D-glucose (M.W. 180) should bring shifts in frequency, of the same modes of vibrations as in the monosaccharides, toward low frequencies. In the sucrose crystal, where the cohesion is greater than in solution, this vibration is found at 861 cm^{-1}.

In the region below 800 cm^{-1}, the vibrations of the skeleton of the sucrose molecule are found. This skeleton consists of a pyranoid (α-D-glucose) and a furanoid ring (β-D-fructose) linked by a C–O–C bridge (see 3). It would, therefore, be expected that the vibrations of these two rings should be present, with some shifts due to the "dimerization". Thus, we must separate the endocyclic vibrations of the D-fructofuranosyl group, which is more compact than the D-glucopyranosyl group. We could, then, assign 746 and 548 cm^{-1}, respectively, to the C–C–O bending mode in the furanoid and the pyranoid rings. These frequencies are higher than those of the equivalent modes of vibration in the monosaccharides[1]. The effect of the "dimerization" on the D-glucosyl group is that it is flattened in the sucrose molecule[27]; this confers more energy on the vibrations around the anomeric carbon atom. The exocyclic C–C–O vibrations are more intense than the endocyclic, and they might occur at 640 cm^{-1} for the D-fructosyl and 528 cm^{-1} for the D-glucosyl group; these frequencies are not very different from those of the exocyclic, C–C–O bands in the spectra of D-fructose[1] and D-glucose (see Table I). The vibration at 640 cm^{-1} is, indeed, the same as that of D-fructose[1], but a shift of 12 cm^{-1} is observed for the C–C–O of D-glucose. This frequency is intermediate between δ(CCO, exocyclic) in α-D-glucose (540 cm^{-1}) and δ(CCO, exocyclic) in β-D-glucose (518 cm^{-1}). Thus, the association of D-glucose with D-fructose that leads to the formation of the sucrose molecule has a most important effect on the structure of the D-glucosyl moiety, and this conclusion is in agreement with that of Jeffrey[27].

Moreover, it is around the glycosidic bridge, namely, C-1–O-1–C-2′, that the major differences between the spectra of sucrose and the monosaccharides that form it are found. Indeed, at 600 cm^{-1} is a vibration that does not exist in the spectra of D-glucose and D-fructose and that could be assigned to the bending of O–C–O about O-1 of the glycosidic bridge. Another characteristic vibration of this linkage is the C–O–C bending-mode, which occurs at 374 cm^{-1} and which is more intense than in the spectra of D-glucose and D-fructose. It was also noted that it is more energetic, as it occurs at a frequency 19 cm^{-1} higher than that of D-glucose (see Table I) and 30 cm^{-1} higher than that of D-fructose[1]. However, the double peak at 470–456 cm^{-1}, not resolved at all concentrations, is more representative of the two moieties of the sucrose molecule. The band at 470 cm^{-1} could be assigned to the C–C–C bending-modes of the D-fructosyl moiety, and that at 456 cm^{-1} could be assigned to the C–C–C mode of the D-glucosyl moiety. These frequencies, higher than the same modes of vibration in the monosaccharides, confirm the concept that the overall effect of the "dimerization" is an increase in the energy of the vibrations; it acts more by virtue of the higher cohesion in the disaccharide than by the mass effect (which normally leads to lower frequencies). The two intramolecular hydrogen-bonds, as well as the different intermolecular hydrogen-bonds, should be the origin of the vibration at 416 cm^{-1}. The particularly high depolarization-ratio ($\rho \sim 6/7$) of this frequency shows that it is an asymmetrical one, as is the case for the O---H–O bond; furthermore, the depolarization ratio is perturbed when the concentration is changed; this was foreseeable, as it is known[18] that the sucrose–water association is sensitive to the concentration.

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