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LASER-RAMAN SPECTRA OF D-FRUCTOSE IN AQUEOUS SOLUTION

MOHAMED MATHLOUTHI
Institut Universitaire de Technologie, Département "Biologie Appliquée", Université de Dijon, B.P. 510, 21014 Dijon Cédex (France)
AND DANG VINH LUU
Laboratoire de Spectroscopie Raman, Université des Sciences et Techniques du Languedoc, 34000 Montpellier (France)

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ABSTRACT

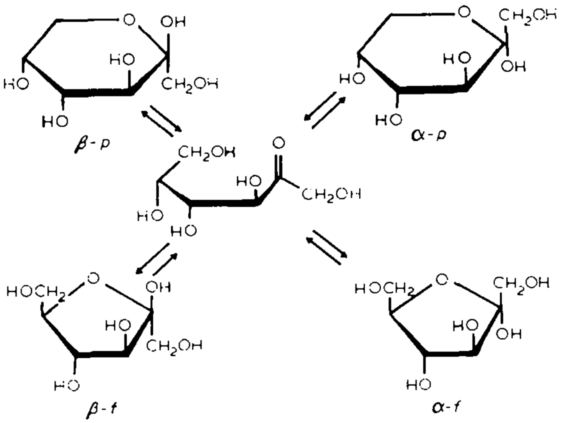
Laser-Raman spectra of D-fructose in water at different concentrations were recorded, and assignments of the frequencies were proposed, based on earlier work on the Raman spectra of other sugars, and determination by other techniques of the composition of aqueous solutions of D-fructose as regards different isomers. It was found that the frequencies of vibration of the furanoid are higher than those of the pyranoid ring. The proportions of the furanoses and pyranoses, found from the ratio of the Raman intensities for the same modes of vibration, were similar to those found by other techniques. Shifts of intensities and frequencies were observed in the region of O–H and C–H bands, and were assigned to probable association between molecules of D-fructose and water.

INTRODUCTION

D-Fructose is a natural sugar present in honey and in certain fruits. It is easier for liver to transform it than D-glucose[1] into glycogen. The recent tendency to use it as a substitute for sucrose, because of its nutritive qualities without risk[2], has led to a multiplicity of investigations of the physicochemical properties of its solution[3-5], but, as far as we are aware, no study of the Raman spectra of D-fructose solutions has been made.

It is known[6] that only β-D-fructopyranose occurs in the crystalline state, and the structure of the crystals has been determined by X-ray[7] and neutron diffraction[8] studies. When crystalline β-D-fructopyranose is dissolved in water, an equilibrium is established between its isomers[9]: β-D-fructopyranose, α-D-fructopyranose, β-D-fructofuranose, α-D-fructofuranose, and the acyclic form (see Scheme 1).

The composition of the aqueous solutions in regard to these different isomers depends on the temperature, the pH, and other factors[5]. Many attempts have been made to determine the proportions of these isomers. Thus, Perlin *et al.*[9] proposed the composition: β-pyranose, 60; α-furanose, 30; and β-furanose, 10%; and a trace



Scheme 1. Isomers present in an aqueous solution of D-fructose.

of α-pyranose, whereas Doddrell and Allerhand[10] deduced, from n.m.r. measurements, the proportions: β-pyranose, 57; α-pyranose, 3; β-furanose, 31; and α-furanose, 9%. On the other hand, Shallenberger[11], on studying the sweet taste of the different forms, gave the composition of the aqueous solution, in equilibrium at 20°, as β-pyranose, 76; α-furanose, 20; and undetermined components, 4%. More recently, Koerner *et al.*[12] proposed the composition: β-pyranose, 37; β-furanose, 34; α-pyranose, 18; and α-furanose, 11%. In all of these results, the proportion of β-D-fructopyranose is preponderant and this is true in solvents other than water[13]. Besides isomerism, solvation in aqueous solutions of sugars has attracted the attention of several authors[14-16], and its effect on the conformational equilibrium has been studied for some sugars other than D-fructose[14]. The complexity of the problem of the conformation of hexopyranoses when there is a solvation effect has been pointed out by Lemieux and Brewer[17].

Study of the Raman spectra of D-fructose may be conducted profitably by comparison with those of D-glucose[18-22], sucrose[23], lactose[24], hyaluronic acid[25], and 1-thiohexopyranosides[26]. In this paper, we propose assignments for the main frequencies of the Raman spectrum of D-fructose in aqueous solution, and specify the proportions of the furanoid and pyranoid isomers. I.r.-spectroscopic techniques have already been used to distinguish between five- and six-membered forms of sugar derivatives[27]. Verstraeten[28] identified the furanoid forms of ketoses from their i.r. spectra. Extensive information on the i.r. spectra of the more common sugars, including D-fructose, was provided by Tipson and Isbell[29].

EXPERIMENTAL

Samples were prepared by dissolving crystalline β-D-fructopyranose (Merck) in distilled water, and filtering through a Millipore membrane. Concentrations were controlled with an Abbé refractometer. The spectra of D-fructose solutions were recorded with a laser-Raman spectrometer having a double monochromator (a holographic grating, and a plane diffraction-grating etched with 1200 lines.mm^{-1}) and equipped with an Argon-ion laser (Spectra Physics 165). The ranges recorded were 3700 to 2700 cm^{-1} and 1700 to 100 cm^{-1}.

RESULTS AND DISCUSSION

A. Frequencies below 1700 cm^{-1}. — The laser-Raman spectra of solutions of D-fructose at 40, 60, and 80% (w/w) concentration are shown in Fig. 1. Experimental results and proposed assignments are summarized in Table I.

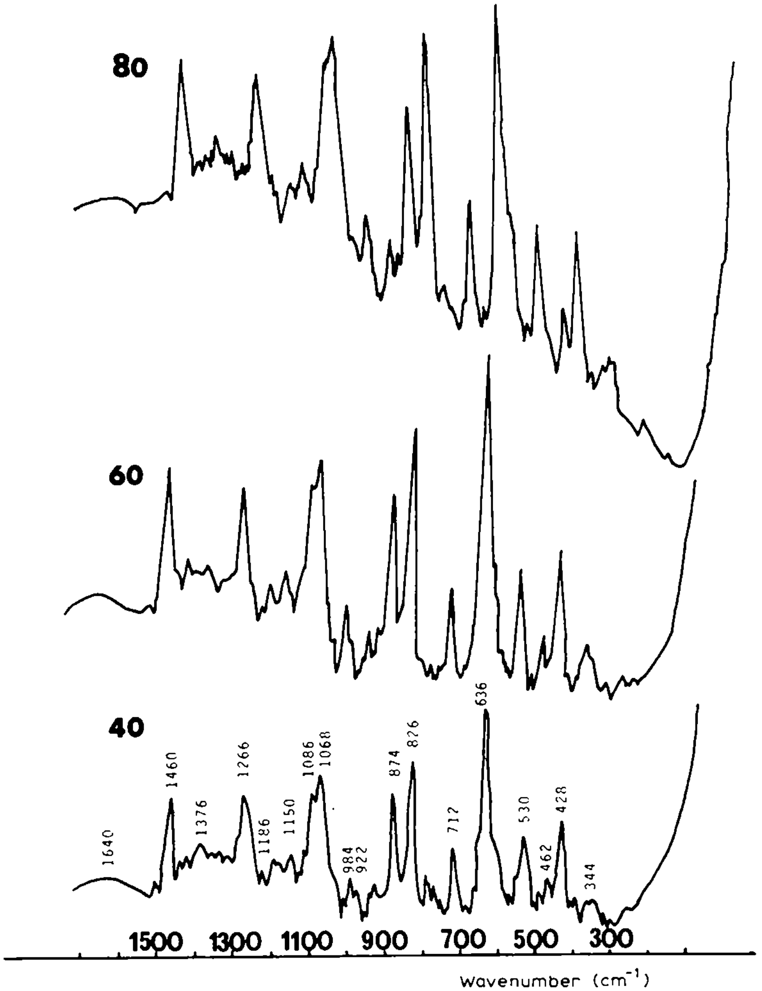


Fig. 1. Raman spectra of D-fructose in aqueous solutions at different concentrations: 40, 60, and 80% (w/w).

TABLE I

BANDS OBSERVED[a] IN THE LASER-RAMAN SPECTRUM OF D-FRUCTOSE IN AQUEOUS SOLUTION

ν (cm^{-1})	*I*	*ρ*	*Assignments*
1640	17	0.52	δ(H$_2$O)
1460	65	0.69	δ(CH$_2$)
1376	29	0.35	w(CH$_2$)
1266	59	0.67	τ(CH$_2$)
1186	19	0.67	ν(C–O)*f*
1150	27	0.66	ν(C–O)*p*
1086	76	0.47	δ(COH)
1068	84	0.36	ν(C–O)*exo*
986	24	0.57	δ(CCH)*p*
922	16	0.64	δ(CCH)*f*
874	54	0.10	ν(C–C)*f*
826	78	0.12	ν(C–C)*p*
712	33	0.12	δ(CCO)*f*
636	**100**[b]	0.20	δ(CCO)*exo*
530	41	0.46	δ(CCO)*p*
460	23	0.42	δ(CCC)*f*
428	41	0.43	δ(CCC)*p*
344	12	0.25	δ(COC)

[a]Key: *exo* = exocyclic; *f* = furanose; *p* = pyranose; I = relative intensity; *ρ* = depolarization ratio; δ = bending mode; w = wagging; τ = twisting; and ν = stretching mode. [b]Taken as reference.

The D-fructose molecule, either in its pyranose or furanose form, shows no element of symmetry (see Scheme 1). Consequently, assignments could not be based on the species of vibrations, which, as a rule, are all of the A species and are polarized.

The frequencies are functions of the bond distances and valence angles given by X-ray[7] and neutron diffraction[8] studies, and our assignments are based on analogy with molecules similar to those of D-fructose[18–26]. As there is no molecular symmetry, it was presumed that the oscillator depends on local symmetry; this is the case, for example, for the 1460-cm^{-1} vibration, which has the local symmetry of the C_{2v} group; this frequency remains constant when the concentration is varied between 5 and 80% (w/w), probably because molecules of D-fructose in aqueous solution are associated, and form "statistical balls", entities not influenced by the concentration[30]. The 1460-cm^{-1} band was assigned to the CH_2 bending mode; this assignment agrees with the Raman results of Koenig[18,19] and Tu[21] and their co-workers on D-glucose.

The bands at 1376 and 1266 cm^{-1} were respectively assigned to the wagging and twisting modes of CH_2. Snyder and Schatschneider[31] and Tadokoro *et al.*[32] assigned to these respective modes the values 1305 and 1257 cm^{-1}.

From earlier studies[9–12] of the composition of D-fructose solutions, summarized in Scheme 1, it was expected that a mixture of the vibrations of pyranose and furanose rings would be found, but the lack of the C=O stretching vibration obviously indicates the lack, or extremely small content, of the open-chain form.

In the 1200–1000-cm^{-1} region, the frequencies of C–O stretching and C–O–H bending modes might be found. We observed a double peak, at 1086 and 1068 cm^{-1}, not resolved at any concentration. The 1086-cm^{-1} vibration could be assigned to C–O–H bending, and that at 1068 cm^{-1} to the C–O stretching mode outside the ring, for both ring-forms. The relative perturbation of the depolarization ratio could be explained by the tendency of the O–H groups to form hydrogen bonds[33].

In contrast, the C–O grouping in the ring (C-2–O-6 and C-6–O-6 in the pyranose, and C-2–O-5 and C-5–O-5 in the furanose) should have a higher frequency. According to Sundaralingam[34], the oxygen atom in the ring is less disposed to engage in hydrogen bonds than the oxygen atom of OH; this could explain the polarization of the vibration at 1186 cm^{-1}, which may be assigned to the C–O stretching mode in the furanoid ring, and at 1150 cm^{-1}, which may be assigned to the C–O stretching mode in the pyranoid ring. The band at 922 cm^{-1} may be assigned to the deformation of C–H out of the furanoid ring, and at 986 cm^{-1}, to the deformation of C–H out of the pyranoid. It is in this region that Raman investigations[18–21] located the C–H deformation mode: Koenig *et al.*[18] assigned the frequency at 911 cm^{-1} to the C-1–H mode and Tu *et al.*[21] assigned the frequency at 1005 cm^{-1} to C-6–H-2.

We have also found that the C–C vibrations of the skeleton of the molecule lie in the region of 1000–860 cm^{-1}, a result comparable to those obtained with polymers[31,32]. Conformational studies of D-fructose in aqueous solution[9–12] gave proportions lying between 54 and 76% for the pyranoses, and 46 to 24% for the furanoses, which leads to our proposal that the frequencies at 874 and 826 cm^{-1} should be respectively assigned to C–C stretching modes in the furanoid and pyranoid rings (see Table I). The Raman intensity is, indeed, proportional to the mass of the scattering molecules; the proportions of the furanoses and the pyranoses were calculated from the ratio I(874)/I(826) = 0.69 : 1, which gives 59% of the pyranoses and 41% of the furanoses. These results agree well with those obtained by other techniques, and also seem to be in good agreement with the generally accepted conformations of the D-fructofuranoses[35] and the D-fructopyranoses[7] (see Table II). The more compact ring is the furanoid, for which the C–C distance remains almost constant; because of this, the external influence on the furanoid is lower than that exerted on the pyranoid ring. Thus, the furanoid ring should have a higher internal energy, and hence, higher frequencies for the modes of vibration of the bonds of the ring. Consequently,

TABLE II

C–C BOND DISTANCES (IN PICOMETERS) IN D-FRUCTOFURANOSE AND D-FRUCTOPYRANOSE

Bond	D-*Fructofuranose*[35] (*in sucrose*)	D-*Fructopyranose*[7]
C-1–C-2	153	152.0 (4)
C-2–C-3	153	154.0 (4)
C-3–C-4	153	151.8 (4)
C-4–C-5	152	152.4 (4)
C-5–C-6	151	149.4 (4)

assignment, by the ratio of the intensities, of the frequency at 874 cm^{-1} to the furanoid ring and at 826 cm^{-1} to the pyranoid ring is probably justified. Therefore, as a rule, for the same modes of vibration, the frequencies should be higher for the furanoid ring. This is true of the C–O stretching modes, for which I(1186)/I(1150) = 0.70 : 1 gives the same ratio of D-fructofuranoses (41%) to D-fructopyranoses (59%) as that found from the intensities of the C–C vibrations. As the out-of-plane deformation of C–H is situated outside the ring, it seems not to obey this rule; however, it permits verification, by the ratio I(986)/I(922), that the composition is 60% pyranoses and 40% furanoses.

The vibration at 712 cm^{-1}, observed in the spectrum of sucrose[23], and having a very weak intensity in the case of D-glucose[18-20], may be assigned to the bending of C–C–O in the furanoid ring. The frequency corresponding to the bending mode of C–C–O in the pyranoid ring should be the band at 530 cm^{-1}. The ratio I(712)/I(530) = 0.80 : 1 seems to be slightly high, but reasonable. The most intense peak, very polarized, in the spectrum of D-fructose occurs at 636 cm^{-1}, and may be assigned to the bending of C–C–O, with exocyclic O, because there are 12 C–C–O angles, 10 of which are exocyclic (for both rings).

Low-frequency bands at 530–300 cm^{-1} are probably due to ring vibration. We also assign the frequencies at 460 and 428 cm^{-1}, respectively, to the modes of C–C–C in furanoid and pyranoid rings. The ratio of intensities, I(460)/I(428) = 0.57 : 1, for these two vibrations is almost equal to those found in other fields of frequencies, and confirms the proportions of D-fructofuranoses and D-fructopyranoses existing in the solution. The 344-cm^{-1} band, which is very strong in the spectrum of sucrose because of the glycosidic linkage C–O–C, is assigned to the C–O–C bending-mode for both forms of the ring.

B. Frequencies between 3700 and 2700 cm^{-1}. — The bands observed in this region are shown in Table III and Fig. 2. The band (of weak intensity) at 2800 cm^{-1} could have as its origin either the superior harmonic of the deformation of CH_2,

TABLE III

BANDS OBSERVED IN THE LASER-RAMAN SPECTRUM OF A 20% AQUEOUS SOLUTION OF D-FRUCTOSE

ν (cm^{-1})	*I*[a]	*ρ*[a]	*Assignments*[a]
3449	187	0.26	ν_a(OH) in (H_2O)
3282	185	0.12	ν_s(OH) in (H_2O) ν_s(OH) in (CH_2OH) ν_a(OH) in (CH_2OH)
2988	54.5	0.31	ν(CH)
2946	**100**[b]	0.19	ν_a(CH) in (CH_2)
2908	55.4	0.27	ν_s(CH) in (CH_2)
2800	10	0	combination of $\delta(CH_2) + \tau(CH_2)$

[a] Key: I = relative intensity; ρ depolarization ratio; ν_s = symmetrical, stretching mode; ν_a = asymmetrical, stretching mode; δ = bending mode; and τ - twisting mode. [b] Taken as reference.

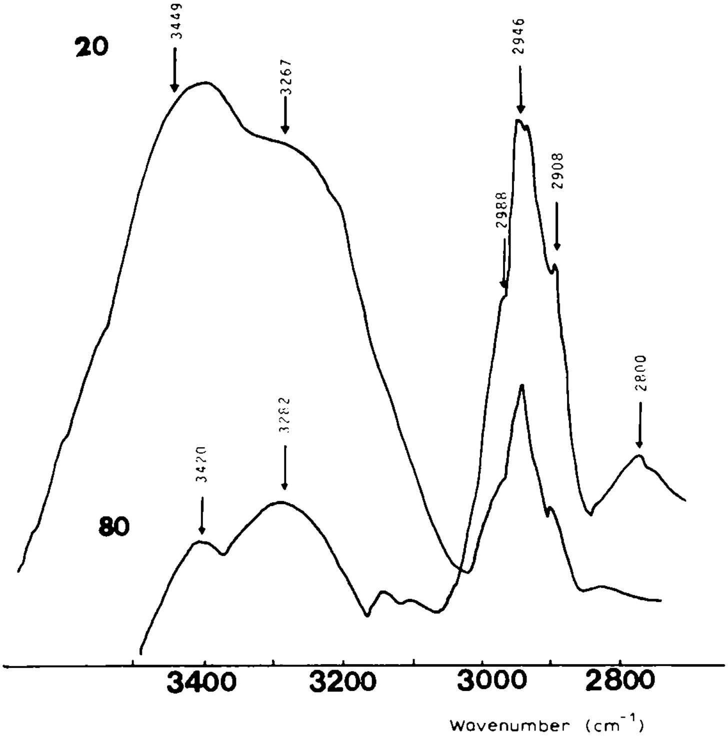


Fig. 2. Raman spectra of D-fructose in aqueous solutions at concentrations of 20 and 80% (w/w).

TABLE IV

SHIFT IN TWO MAXIMA IN THE LASER-RAMAN SPECTRA, WITH CONCENTRATION, OF AQUEOUS SOLUTIONS OF D-FRUCTOSE

Concentration (w/w)	$\nu_s(OH)$ *(cm^{-1})*	$\nu_a(OH)$ *(cm^{-1})*
0	3268	3459
5	3275	3463
10	3268	3453
20	3267	3449
30	3271	3443
40	3273	3433
55	3274	3427
80	3282	3420

or the combination of bending and twisting modes of CH_2, found for many such compounds as ethanol, ethylene glycol, poly(ethylene), and poly(propylene)[36]. The assignment of the 2908- and 2946-cm^{-1} frequencies, respectively, to symmetrical ν_s(C–H) and asymmetrical ν_a(C–H) modes of CH_2 seems to conform to the general rule. The frequency at 2988 cm^{-1} is assigned to ν(C–H); this is in good agreement with results on other sugars[19–21].

Comparative study of the intensities of the C–H vibrations permits differentiation of D-fructose from D-glucose and sucrose. We have, indeed, found[23] that the asymmetrical vibration ν_a(C–H) for CH_2 is stronger than the symmetrical vibration; the opposite is observed for D-glucose, and the spectrum of sucrose exhibits almost the same intensity for the two vibrations. This behavior is probably due to local orientations, which are different in the three sugars. The O–H band of D-fructose is confusable with that of water. For water, we observed two intensity-maxima, at 3459 and 3268 cm^{-1}. When the concentration of D-fructose was increased, there was a shift of these two maxima, particularly of that at 3459 cm^{-1} (see Table IV).

The shifts in frequency probably originate from the association (by hydrogen bonds) between water and the sugar. On the other hand, we observed an inversion of the intensity of the maxima at 80% concentration (see Fig. 2), the intensity of the 3420-cm^{-1} band being weaker than that of the 3282-cm^{-1} band.

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