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SPECTROSCOPIC STUDY OF THE STRUCTURE OF SUCROSE IN THE AMORPHOUS STATE AND IN AQUEOUS SOLUTION

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ABSTRACT

The structure of noncrystalline sucrose in the amorphous, solid state and in aqueous solution was investigated. Differences of structure of amorphous solid samples, the quenched-melt, and freeze-dried sucrose, are revealed by differential thermal analysis (d.t.a.) and from the Fourier-transform infrared (F.t.-i.r.) spectra. Factor analysis of the F.t.-i.r. spectra of aqueous solutions of sucrose shows the existence of at least two forms of the sucrose molecule. Analysis of ^{13}C-n.m.r. spectra of amorphous and crystalline sucrose reveals a sensitivity of the fructosyl moiety to the morphology of the sample.

INTRODUCTION

X-Ray diffraction results permitted one of us[1] to propose that the sucrose molecule undergoes a folding in solution at high concentration, and a model for the structure of sucrose in aqueous solution was proposed. In this model, the sucrose molecule is not involved in any molecular hydrogen-bonding up to a concentration of 22.3% (w/w). Thereafter, one type of intramolecular hydrogen-bonding is formed between 22.3 and 65.3% (w/w), and two different types of intramolecular hydrogen-bonds (see Fig. 1) are formed in supersaturated solutions >65.3% (w/w). These results were inconsistent with n.m.r.-spectral studies[2] on dilute solutions of sucrose in D_2O and in deuterated Me_2SO, which suggested that, except for a slight flexing of the furanoid ring, sucrose is "basically a rather rigid molecule".

When the aim of an experiment study is the investigation of hydrogen bonding in a carbohydrate such as sucrose, it is difficult to find a solvent that does not interact with the solute molecule. Unfortunately, the only solvents available for

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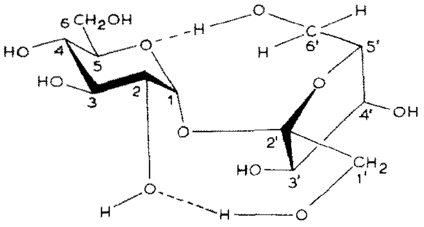

Fig. 1. Atom numbering in sucrose.

carbohydrates are such hydrophilic liquids as water, Me_2SO, and their deuterated derivatives. With these solvents, it is impossible to prevent the interference of hydrogen bonding between the solute and the solvent. An intramolecular bond via a water molecule may be envisaged, because hydrogen bonding in carbohydrates may be bifurcated[3]. It was concluded that it was necessary to find a solid form of sucrose, free from external hydrogen bonds, that could serve as a model of the sucrose molecule in dilute, aqueous solutions. A suitable sample may be the molten form, in which the effect of heat is to break inter- and intra-molecular hydrogen bonds. This structure is stabilized by rapid quenching. Moreover, classical theories of crystallization[4] find some comparisons between the molten state and dilute solutions. The structure of the quenched melt has been previously[5] compared to that of freeze-dried sucrose. It was found from an experimental study by X-ray and electron diffraction, and water-vapor sorption, that the quenched melt is completely amorphous, whereas the freeze-dried sample is microcrystalline. Although X-ray and conventional i.r. techniques have been used to study the crystallinity of amorphous sucrose[6] and lactose[7], these methods cannot differentiate such various types of amorphous carbohydrates as quenched melts and freeze-dried samples.

The solid-state i.r. spectra of casein–lactose mixtures were found[8a,b] difficult to interpret owing to the amorphous nature of the sample. Such difficulties may be removed by applying the F.t.-i.r. technique. Indeed, the F.t.-i.r. spectra show a better frequency accuracy and signal-to-noise ratio than conventional i.r. spectra. The direct interfacing of a computer to the spectrometer allows such arithmetical manipulations of the spectra as absorbance subtraction, which aid in resolving such problems as the identification of an interaction in a mixture spectrum. Moreover, the F.t.-i.r. spectrum of a mixture such as hydrogen-bonded and non-bonded sucrose molecules in aqueous solutions may be analyzed in order to determine spectroscopically the number of distinguishable components in the mixture. The method used for this purpose is called[9] factor major component analysis.

Many earlier studies[10–13] of the structures of carbohydrates used solution ^{13}C-n.m.r. spectroscopy. The advent of cross-polarization (c.p.), high-power proton-decoupling, and magic-angle sample spinning (m.a.s.) allows the study[14–17] of solids. Recently, Earl and Parrish[18] utilized this technique to study different crystalline forms of lactose.

The present work reports results of three different and distinct techniques: F.t.-i.r., c.p.-m.a.s. ^{13}C-n.m.r., and differential thermal analysis (d.t.a.) for analysis of the two noncrystalline forms of sucrose, the quenched melt and the freeze-dried. Likewise, factor analysis was applied to the F.t.-i.r. spectra of seven sucrose solutions of concentrations ranging from 10 to 70% (w/w). Our objective was to elucidate the structural differences between amorphous sucroses, with particular reference to intramolecular hydrogen-bonding and the role of the glucosyl and fructosyl moieties to these interactions.

EXPERIMENTAL

The quenched melt (QM) was obtained by melting at 186–190° fine crystals of sucrose (average dimension = 0.1 mm) and quenching the melt by immersion in liquid nitrogen. The freeze-dried (FD) sample was obtained by freezing at −40° at 10% (w/w) aqueous solution and drying the frozen material in a 1-cm thick layer under high vacuum (10^{-2} torr) in a Usifroid freeze-dryer for 24 h. The residual moisture after freeze-drying was 1.8%.

F.t.-i.r. spectra were recorded at 2-cm^{-1} resolution on a Digilab FTS 20 spectrometer. The solid samples were analyzed as KBr pellets and the aqueous solutions by use of a cell fitted with AgCl windows. Differential thermal analysis (d.t.a.) of amorphous sugars was described previously[19]. The ^{13}C-n.m.r. spectra were recorded at 37.7 MHz with a Nicolet NT-150 spectrometer equipped with cross-polarization accessory. Radiofrequency amplifiers delivering 550 W at 150 MHz and 1 kW at 37.7 MHz were adjusted to satisfy the Hartmann–Hahn[20] condition at roughly 50 kHz. Cross polarization and magic-angle spinning (c.p.–m.a.s.) spectra were recorded with a single, 1.0-ms contact time and a recycle time of 2.0 s. The magic angle of 54.7° was set by minimizing the intensity of the carbonyl resonance of glycine. Ten thousand transients were collected in the c.p.–m.a.s. experiment, and Kel-F spinners were used and spun at 2.1 kHz at the magic angle. For reference purposes, a Delrin polyoxymethylene rotor was used (89 p.p.m. from Me_4Si). Alteration of spin temperature was used to eliminate various artifacts[21]. The static field was not locked during accumulations.

RESULTS AND DISCUSSION

The d.t.a. thermograms of crystalline (C), freeze-dried (FD), and quenched-melt (QM) sucrose are shown in Fig. 2. The endothermic peak observed for C and FD at a temperature of 180° corresponds to the melting point of sucrose. Such an endotherm is not clearly observed for QM. The freeze-dried sample shows an exothermic peak at 110° corresponding to a recrystallization that is absent from the thermogram of the quenched melt. These results, and other data of a previous comparative study of the two non-crystalline solid forms of sucrose, permitted[5] us to conclude that the freeze-dried sample, although mostly amorphous, contains

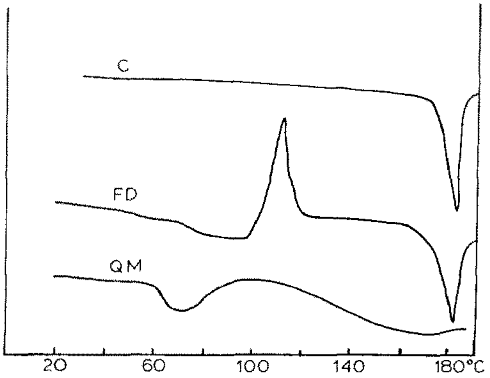

Fig. 2. D t.a. thermogram of crystalline (C), freeze-dried (FD), and quenched-melt (QM) sucrose

crystal nuclei that promote recrystallization at 110° during the heating. The quenched melt behaves as a glassy solid that shows a glass transition at 60° and endothermic behavior starting at a lower temperature of 135° rather than the melting point of sucrose. The observed thermal events are caused by a decrease in viscosity rather than by a phase transition.

F.t.-i.r. spectra of sucrose in the amorphous and crystalline-solid states and in dilute and concentrated aqueous solutions are shown in Fig. 3. Comparison of the most prominent i.r. bands in the frequency region between 1200 and 800 cm^{-1} shows similarities between supersaturated (70%) and crystalline samples on the one band, and the freeze-dried (FD) sample and saturated solution (66%) on the other. However, the spectrum of the quenched melt (QM), except for the background and the H_2O bending vibrations, is more comparable to the dilute (23%) solution than to the solid, freeze-dried (FD) sample. The better resolution of peaks in the 1200–800 cm^{-1} region for freeze-dried sucrose and concentrated solutions is a sign of a higher degree of order. The advantages of F.t.-i.r. spectroscopy over conventional i.r. as regards the differentiation of carbohydrates in solution or in the amorphous state have been reported[22] earlier. Moreover, it is possible to store the digitized F.t.-i.r. spectra and use them to obtain additional information.

Factor analysis[9] of the spectra of seven aqueous solutions of sucrose of 10, 23, 33, 40, 50, 66, and 70% weight concentrations was performed in the frequency region (1200–800 cm^{-1}) where the solvent (water) does not interfere. Factor analysis is a multivariate statistical technique performed on digitized absorbance spectra in order to determine the number of species contributing to a spectral region. This technique reveals spectroscopically discernible components in a mixture that give rise to reliably nonzero eigenvalues. The results are presented in the form of the minimum components fitting with independent functions (Fig. 4) or in the form of a graph giving the eigenvalues as a function of the different components of the mixture (Fig. 5). The lines in this graph serve only to group the data from a given analysis and emphasize the difference between the "nonzero" and the zero or error eigenvalues. In the "fingerprint" region, C–O stretchings and C–O–H bendings are found. They could display frequency shifts when they are engaged in intramolecular hydrogen-bonds. It was found from the factor analysis that the minimum number of components that fits with independent functions is three in the sucrose solutions analyzed (see Fig. 4). The eigenanalysis indicated that the spectra in the 1200–1800 cm^{-1} region (Fig. 5) were reliably expressed as linear combinations of at least two components, namely intramolecularly hydrogen-bonded and non-bonded sucrose molecules. The spectra calculated assuming three components are shown in Fig. 6. It may be seen from Fig. 6 that the upper spectrum exhibits more peaks than the two others, which are almost identical. It may be concluded from this observation that two species of sucrose molecules are present in aqueous solutions; one with relatively high order is comparable to crystalline sucrose and the other of lower order is comparable to the quenched melt. Factor analysis of F.t.-i.r. spectra of aqueous sucrose solutions leads to the same conclusion as the previous X-ray investigation: the number of species present in these solutions is more than one. The concentration dependence of the folding of sucrose has also been found[23] to fit with an interpretation of the rate of enzymic hydrolysis of concentrated sucrose solutions. It appears from the F.t.-i.r. results that the quenched melt could be taken as a model for the sucrose molecule in dilute solutions and the freeze-dried sample as a model for concentrated solutions.

A ^{13}C-n.m.r. study of structural differences between the two amorphous forms of sucrose was performed. Fig. 7 shows the solid-state ^{13}C-n.m.r. spectra of crystalline (C), freeze-dried (FD), and quenched melt (QM) sucrose. The resonance

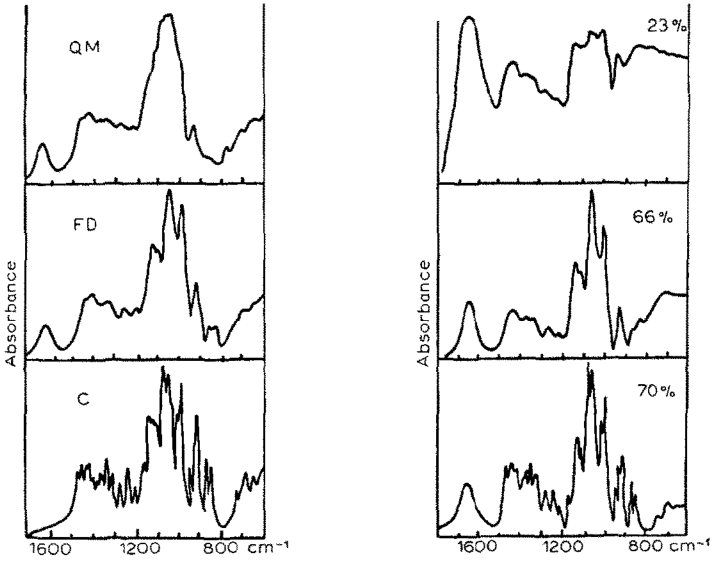

Fig. 3. F.t.-i.r. spectra of quenched-melt (QM), freeze-dried (FD), and crystalline (C) sucrose, and aqueous sucrose solutions of 23, 66, 70% (w/w) concentrations.

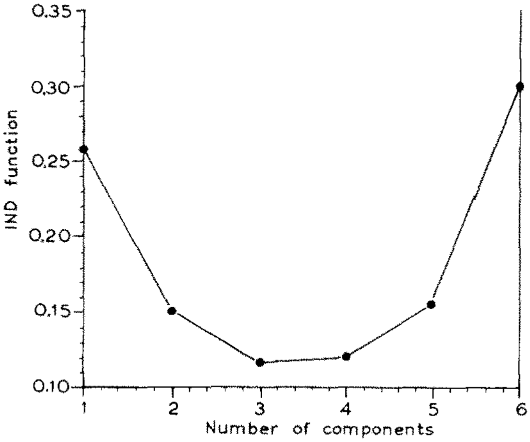

Fig. 4. IND function *vs* number of components for aqueous sucrose solutions.

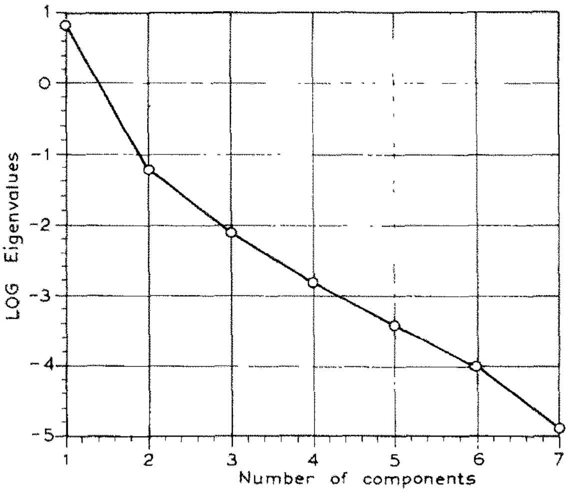

Fig. 5. Factor analysis over 1200–800 cm^{-1} for seven aqueous solutions of sucrose.

lines of the crystalline form are narrow as compared with the spectrum of amorphous sucrose. The chemical shifts of all crystalline resonances observed are listed in Table I from downfield to upfield; also listed are the chemical shifts for freeze-dried and quenched-melt samples. The ^{13}C-n.m.r. chemical shifts of sucrose in solution (resonances from downfield to upfield) and their assignments[12], are also listed in the last column of Table I. Comparison of the solid-state chemical shifts of the crystalline, freeze-dried, and quenched-melt samples with the chemical shifts in solution indicated that some resonances are shifted in the solid-state ^{13}C-n.m.r. spectra, making unambiguous assignment of the resonances difficult. However, the

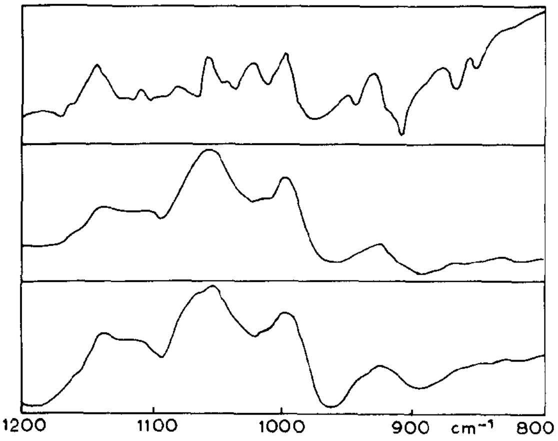

Fig. 6. Extracted F.t.-i.r. spectra between 1200 and 800 cm^{-1} from the factor analysis of seven solutions of sucrose.

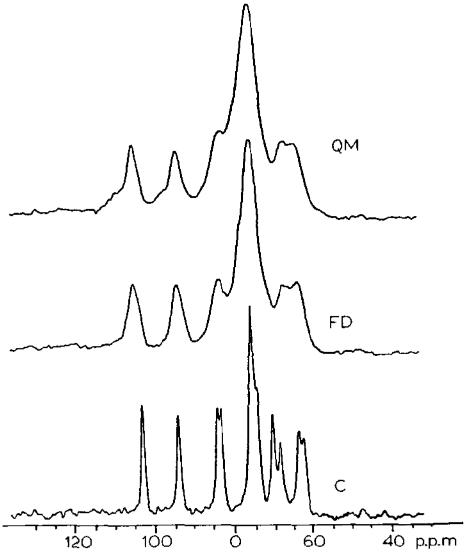

Fig. 7. Cross-polarization magic-angle sample spinning ^{13}C-n.m.r. spectra of quenched-melt (QM), freeze-dried (FD), and crystalline (C) sucrose.

chemical shifts indicate some interesting observations for the analysis, on the basis of physicochemical consideration of the sucrose molecule. The chemical shift of the C-2′ resonance in the crystalline form is δ 102.6, whereas it is δ 104.7 for the amorphous form (see Fig. 7 and Table I). The δ 2.1 change in the chemical shift for this resonance may be understood on the basis of the effect of intramolecular hydrogen-bonding in the crystalline and amorphous forms. It is known from other studies[24] that the most reactive site of the sucrose molecule is the D-fructosyl

TABLE I

TENTATIVE ASSIGNMENTS OF ^{13}C CHEMICAL SHIFTS (p.p.m. DOWNFIELD FROM Me$_4$Si) FOR SUCROSE IN VARIOUS PHYSICAL STATES

Freeze-dried	*Quenched melt*	*Crystalline*	*D$_2$O Solution*	*Assignments from ref. 12*
104.7	104.7	102.6	104.5	C-2′
93.4	93.4	93.5	92.9	C-1
		83.1	82.2	C-5′
82.4	81.35	82.0	77.3	C-3′
		73.9	74.8	C-4′
73.6	73.7	73.2	73.4	C-3
			73.3	C-5
		72.1	71.9	C-2
		68.2	70.0	C-4
65.3	65.0	66.3	63.2	C-6′
61.3	61.8	61.5	62.2	C-1′
		60.2	61.0	C-6

moiety. In other words, the breakage of intramolecular hydrogen-bonds in the amorphous form has an effect on the resonance of C-2 of D-fructose. In addition, the chemical shift of this C-2′ resonance for the amorphous form is similar to that of the solution ^{13}C-n.m.r. chemical shift for this resonance (see Table I). The chemical shift of C-1 of the D-glucosyl group is almost the same for the solution and in the three different solid forms.

The D-fructose moiety is more susceptible to morphological changes[24] than D-glucose. It is also of interest to compare the chemical shifts of the C-5′ and C-3′ resonances in the crystalline state and in solution. The difference in chemical shift for these two nuclei is δ 1.1 for the crystalline state whereas it is $\approx\delta$ 4.9 for the solution. For the model compound β-D-fructofuranose, the difference between the two resonances is[25] δ 5.2. The comparison between sucrose and β-D-fructofuranose clearly shows that the furanose puckering is the same for both molecules in solution. However, in the crystalline state, the D-fructose moiety is stabilized by intra- and inter-molecular hydrogen-bondings. This stability may be the reason for the observation of smaller chemical-shift differences between the C-5′ and C-3′ resonances in the crystalline sample in comparison with the solution of sucrose (see Table I). Because of excessive line-broadening in the spectra of freeze-dried and quenched-melt samples, it is difficult to distinguish them on the basis of solid-state n.m.r. spectra. The slight differences in the chemical shifts are listed in Table I.

CONCLUSION

Analysis of results of the three different techniques utilized in this study brings additional information for elucidating the structure of the sucrose molecule in the amorphous solid state and in aqueous solution. The ^{13}C-n.m.r. study shows that if breakage of the intramolecular hydrogen-bonding occurs in the solution, it involves mainly the fructosyl moiety. The F.t.-i.r. results are more explicit in determining the structural differences between the freeze-dried and the quenched-melt samples. Factor analysis of the F.t.-i.r. spectra of aqueous solutions helps in determining spectroscopically distinguishable forms of the sucrose molecule when the concentration is varied. The results of this work are helpful in interpreting such properties of sucrose in aqueous solutions as hydrolysis and the reactivity of the fructosyl moiety.

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