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Note

Quantitative determination of the effect of pH and temperature on the *keto* form of D-fructose by FT IR spectroscopy

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The scarce data available on the concentration of acyclic forms of reducing sugars is mainly due to the unavailability of fast and convenient analytical techniques for their detection and quantification. However, knowledge of the composition of reducing sugars in solution can have considerable practical and theoretical importance. The physical and chemical properties of these sugars in solution depend on the relative concentrations of different tautomeric forms. Their biological properties can also have similar dependence[1]. In the non-enzymatic glycation of proteins and amino acids (Maillard reaction), the concentrations of open-chain forms might be a crucial factor in determining the rate of the reaction if the mutarotation rate is slower than the reaction rate. The mutarotation of sugars in aqueous solutions is acid–base catalyzed. The reaction starts with an attack on the cyclic form of the sugar by either an acid or a base catalyst, followed by a slow opening of the ring. The acyclic intermediate thus formed plays a major role in the mutarotation reaction[2]. The low concentrations of this form detected in carbohydrate solutions prompted Isbell and Pigman to conclude that the rate of ring closure is faster than the rate of ring opening[3]. However, the equilibrium concentrations are determined by thermodynamic stability of the tautomers rather than by the rates of their formation.

Although most of the tautomeric forms of the reducing sugars in solution have never been isolated, they can be detected by different techniques, and their concentrations in the equilibrium mixture can be measured. Sugars that mainly show interconversions between pyranose anomers, such as glucose, are said to undergo "simple mutarotation"; on the other hand, sugars that also exhibit interconversions between furanose anomers, such as fructose, are said to undergo

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TABLE I

Equilibrium concentrations [a] of the *keto* form of D-fructose reported in the literature

Temperature (°C)	pH [b]	Method	Concentration	Solvent	Reference
20	5.2	CD	0.70	H_2O	7
21	7.0	^{13}C NMR	0.50	30% D_2O	8
21	4.9	^{13}C NMR	0.60	30% D_2O	8
25	4.4	GC/MS	0.36	H_2O	9
25		GC	0.60	H_2O	10
25		UV	2.0	H_2O	11
30		CD	0.70	D_2O	12
30		^{13}C NMR	3.00	$(CD_3)_2SO$	13
30	2.0	^{13}C NMR	0.80	10% D_2O	14
30	5.5	^{13}C NMR	0.55	10% D_2O	14
30	8.0	^{13}C NMR	0.50	10% D_2O	14
30	8.4	^{13}C NMR	0.58	10% D_2O	14
31		^{13}C NMR	0.80	D_2O	15
33		^{13}C NMR	2.00	C_5D_5N	16
40	7.0	^{13}C NMR	1.20	30% D_2O	8
50	7.0	^{13}C NMR	1.30	30% D_2O	8
50	4.9	^{13}C NMR	1.30	30% D_2O	8
80		^{13}C NMR	3.0	D_2O	17

[a] % of the total concentration. [b] Where pH is not reported, measurements were made in unbuffered solvent.

"complex mutarotation". In both cases, sugars must undergo a ring opening to the open-chain form (also known as *aldehydo* or *keto* forms) in order to mutarotate. The open-chain tautomer is usually present in very low concentrations, thus rendering its detection in the equilibrium mixture very difficult.

D-Fructose in aqueous solution is known to exist as a complex mixture of at least five equilibrating tautomeric forms: α- and β-pyranose, α- and β-furanose and the open-chain *keto* form. The latter has been stipulated[4] to undergo a rapid and reversible hydration reaction in aqueous solutions. However, the resulting hydrate has not yet been detected. α and β Anomerization reactions and furanose–pyranose interconversions proceed through a common, high-energy open-chain form that is usually stabilized by complexation with the solvent molecules. The proportions of the acyclic forms increase with temperature due to the entropy factor (acyclic forms have a greater degree of freedom) and the high enthalpy content (cyclizations are exothermic reactions and hence favored by lower temperatures). Table I summarizes the amounts of acyclic forms of D-fructose reported in the literature at different temperatures and pH values. Most recent studies have been done with ^{13}C NMR over a temperature range of 20–50°C. According to Table I, at room temperature D-fructose contains around 0.70% *keto* form, and this amount almost doubles when the temperature is raised to 50°C.

Evidence for the presence of the *keto* form of D-fructose in solution has been provided by methods such as polarography and ^{13}C NMR spectroscopy[5]. In a previous study[6] we demonstrated that FT IR spectroscopy can be employed in the detection of the carbonyl absorption band of the open form of D-fructose centered at 1728 cm^{-1} using D_2O or water as the solvent. Changes in the intensity of the band at 1728 cm^{-1} allowed the monitoring of the concentration of the open form of the *keto* sugar D-fructose at different temperatures and pH values. The concentration of the open form was observed to increase with increasing temperature and was an order of magnitude higher at 80°C compared to 30°C. The buildup of the open form was found to be extremely rapid. The new equilibrium can be reversed with decreasing temperature. The advantage of employing FT IR spectroscopy in the study of *keto* sugars is twofold. First, the *keto* form can be detected in low concentrations (> 0.5%) without the need to utilize ^{13}C-enriched compounds as is the case in ^{13}C NMR studies. Second, the time scale of IR is much faster and thus, in principle, rapidly exchanging conformers may be more easily detected by IR spectroscopy provided their concentrations fall within the sensitivity limits of the IR spectrometer. The potential of applying FT IR spectroscopy in studying the effect of environmental factors on the levels of the open-chain form of sugars has been demonstrated in the previous work. In this work we report the quantitative determination of the concentrations of acyclic forms of D-fructose as a function of temperature and pH.

Mathematical relationship between integrated intensities of the D-fructose carbonyl absorbance and temperature.—Solutions of D-fructose (after reaching mutarotational equilibrium) in D_2O showed an extremely weak absorption band in the carbonyl region centered at 1728 cm^{-1}. The intensity of this band was found to be sensitive to temperature. Fig. 1 shows the increase in the intensity of the carbonyl band as the temperature was increased gradually from 20 to 80°C. This process was found to be reversible. It should be noted that the carbonyl peak reaches its maximum height in less than 10 min, indicating that the new equilibrium concentration of the *keto* form at any given temperature is achieved very rapidly. Plotting the integrated intensities of the carbonyl band (1700–1750 cm^{-1}) versus temperature roughly defined a parabolic curve which could be fitted to a second-degree quadratic equation[6]. This equation relates the integrated intensity of the carbonyl band to the temperature. Using these equations and the molar aborptivity of the carbonyl band (see the section below), the percent composition of the open chain forms was calculated, and the results are given in Table II.

Confirmation of the carbonyl absorption peak by D-[2-^{13}C]fructose studies.—In order to confirm the identity of the peak centered at 1728 cm^{-1}, the spectrum of D-[2-^{13}C]fructose was compared to that of D-fructose. Infrared absorption wavelengths are shifted when one of the atoms is replaced with a different isotope. Increasing the mass of the atoms undergoing oscillations tends to decrease the frequency of the absorption. In unlabeled D-fructose the carbonyl peak was centered at 1728 cm^{-1} and in D-[2-^{13}C]fructose the absorption wavelength was

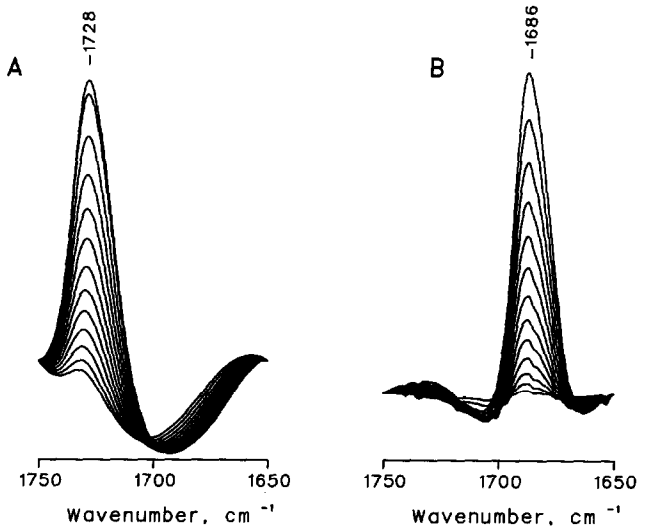

Fig. 1. (A) The increase in the carbonyl absorption band of D-fructose (in D_2O) as a function of temperature between 25 and 80°C. (B) The increase in the carbonyl absorption band of D-[2-^{13}C]fructose (in D_2O) as a function of temperature between 25 and 80°C

shifted to 1686 cm^{-1} (Fig. 1). The relationship that governs the frequency (ν) of an absorption in cm^{-1} units is given by eq. *1*, where, c is the speed of light, k the force constant, and μ the reduced mass.

$$\nu(cm^{-1}) = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}} \quad (1)$$

Increasing the atomic weight of the carbonyl carbon to 13 amu the expected ratio of $\nu_{C\text{-}12}/\nu_{C\text{-}13}$ is 1.025 which is very close to the experimentally observed ratio of 1728/1686 = 1.023, confirming the identity of the peak.

TABLE II

Percent composition of the open-chain form of D-fructose between 25 and 80°C at acidic, nuetral, and basic pH values

Temperature (°C)	pH 2	pH 3	pH 7	pH 8	pH 9
25	0.9	0.9	0.7	0.8	0.8
30	1.5	1.5	1.3	1.5	1.5
35	2.3	2.3	2.1	2.3	2.2
40	3.1	3.1	2.9	3.2	3.1
45	4.0	4.0	3.8	4.2	4.1
50	5.0	5.1	4.9	5.2	5.2
55	6.1	6.2	6.0	6.4	6.4
60	7.3	7.4	7.2	7.6	7.8
65	8.6	8.7	8.5	8.9	9.2
70	10.0	10.1	10.0	10.3	10.8
75	11.5	11.5	11.5	11.7	12.5
80	13.1	13.1	13.1	13.3	14.3

Quantitatition of the carbonyl absorption peaks.—To calculate the molar absorptivity of the carbonyl group of D-fructose, a model compound, 1,3-dihydroxyacetone (which has an absorption peak centered at 1737 cm^{-1}), was chosen since it approximates the electronic environment of the D-fructose carbonyl group. The error introduced by this model compound is negligible since the immediate substituents on the carbonyl carbon are the same as in the D-fructose. Hayward and Angyal[7] used the same approach to calculate the molar dichroic-extinction coefficients of reducing sugars. Calculations showed that the molar absorptivity of the carbonyl band of dihydroxyacetone in D_2O was 1286 L/mol/cm. The dependence of absorptivity of the carbonyl band on temperature was also studied and found to be negligible.

Effect of temperature and pH.—Data obtained by the digital integration of the intensities of open-chain carbonyl absorption bands were used to calculate the percent of open-chain forms of D-fructose between 25 to 80°C and at acidic and basic pH values (see Table II), using the molar absorptivity value of the carbonyl peak of 1,3-dihydroxyacetone (1286 L/mol/cm). The study was limited to a maximum temperature of 80°C and a pH of 9 since D-fructose remains intact under those conditions. Higher temperature and pH values will promote isomerizations, β-elimination, and decomposition reactions. Generally, the effect of acid or base catalysis on the ring-opening reactions was found to be minimal; however, basic catalysis was more effective than acid catalysis. Temperature, however, plays a crucial role in determining the equilibrium concentrations of the open-chain forms of D-fructose. ^{13}C NMR studies in D_2O indicated an approximate two-fold increase in the equilibrium concentration of the open-chain forms when the temperature was increased from 25 to 50°C (see Table I); however, in the same temperature range the FT IR measurements indicate a four-fold increase in the equilibrium concentrations, and at higher temperature ranges the increase is even larger. Further investigations are needed to explain the discrepancy of the calculated values between FT IR and NMR. However, one of the reasons for this difference might be the fact that the percent composition of open-chain form appears to vary linearly in the limited temperature range (20–50°C) of NMR studies[14], whereas, over the wider temperature range used in the present study, the variation was shown to be quadratic[6].

EXPERIMENTAL

Materials.—D-Fructose was obtained from Sigma Chemical Co. D_2O and D-[2-^{13}C]fructose were obtained from MSD Isotopes (Montreal, Canada). 1,3-Dihydroxyacetone was purchased from Aldrich Chemical Company and used without further purification. D-Fructose solutions were made up in concentrations ranging from 1.1 to 2.8 M in D_2O. The solutions were left to stand for a minimum of 48 h at room temperature prior to FT IR measurements. The pH of the solutions were adjusted by addition of DCl or NaOD.

Temperature studies.—A fructose solution in D_2O was placed in a CaF_2 IR cell with a 50-mm Teflon spacer. The temperature of the sample was regulated by placing the IR cell in a temperature-controlled cell holder. Infrared spectra were recorded on a Nicolet 8210 Fourier-transform spectrometer, purged with dry air, and equipped with a deuterated triglycine sulfate (DTGS) detector. The initial temperature of the cell was raised by 1°C per min, and every 5 min the temperature was kept constant for 15 min to record the spectra. A total of 128 scans at 4-cm^{-1} resolution were coadded.

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