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Investigation of the enolization and carbonyl group migration in reducing sugars by FTIR spectroscopy

Varoujan A. Yaylayan *, Ashraf A. Ismail

Department of Food Science and Agricultural Chemistry, McGill University, 21111 Lakeshore, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada

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Abstract

The FTIR spectra of selected aldoses and ketoses were recorded in D_2O as a function of temperature between 30 and 80°C. The analysis of the spectra has revealed the presence of two sets of temperature-sensitive bands, one centered in the carbonyl region (1700–1750 cm^{-1}) and the other in the alkene absorption region (1630–1680 cm^{-1}). The latter was assigned to the enediol species formed as a result of enolization of acyclic *aldehydo* and *keto* forms of the sugars. Both absorption frequencies were confirmed by studies with selected ^{13}C-substituted sugars. The presence of α-hydroxyl groups in reducing sugars was found to shift the stretching frequencies of sugar carbonyl bands to higher values relative to simple alkyl-substituted carbonyl compounds. The relative concentration of enediol to that of carbonyl was also studied as a function of temperature and concentration. The ratio of intensities of enediol to carbonyl absorption bands was found to decrease with temperature and increase with concentration. Using D-[2-^{13}C]ribose and D-[1-^{13}C]ribose, further evidence for the occurrence of enolization was provided by observing the migration of carbonyl group from C-1 to C-2 in D-ribose and subsequent formation of D-*erythro*-pentulose (D-ribulose) as a result of reversal of enediol–carbonyl equilibrium through both hydroxyl groups. A mechanism is proposed for the enolization of sugars in neutral aqueous solutions involving hydrogen-bonded sugar dimers. These studies indicated that the relative concentrations of the enediols are as important as *aldehydo* or *keto* forms of reducing sugars in aqueous solutions.

Keywords: Sugars, reducing; Sugars, carbonyl; Sugars, enediol forms; Enolization; FTIR spectroscopy

* Corresponding author.

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1. Introduction

The physical and chemical properties of reducing sugars in solution depend on the relative concentrations of different tautomeric forms. Their biological properties can also have similar dependence [1]. In non-enzymatic glycation of proteins, 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. 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. In order to mutarotate, sugars must undergo a ring opening to the 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). 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 [2]. In a previous study [3] we demonstrated that FTIR spectroscopy can be employed in the detection of the carbonyl absorption band of the open form of D-fructose centered at 1728 cm^{-1}. Changes in the intensity of the band at 1728 cm^{-1} allowed the monitoring of the concentration of the open form of 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 with 30°C. The new equilibrium can be reversed with decreasing temperature. Data obtained [4] 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 and 80°C.

Under acid–base catalysis, reducing sugars are also known to undergo Lobry de Bruyn–Alberda van Ekenstein transformations [5]. The enediol mechanism of this transformation has been verified both in vivo [6] and in vitro [7]. It appears that the enediol forms of certain sugar derivatives in biological systems are the active forms with which enzymes react. The conversion of cytotoxic methylglyoxal into lactate, for example, by the enzyme glyoxylase II (thioester hydrolase glyoxylase II, EC 3.1.2.6), involves an enediolate intermediate of the methylglyoxal derivative [8]. Rubisco (ribulose-1,5-bisphosphate carboxylase, EC 4.1.1.39) adds carbon dioxide to the 2,3-enediolate form of ribulose-1,5-bisphosphate [9]. Enediol forms are known to play an important role in metal-catalyzed oxidative degradation of reducing sugars specially in biological systems [10]. Water is known to catalyze to a small extent enolizations of reducing sugars [11]; however, the contribution of enediols to the total concentration of acyclic forms of reducing sugars in neutral aqueous solutions is not known owing to a lack of analytical techniques for their detection. Taking advantage of the high absorptivity of enediols, FTIR spectroscopy was employed to estimate their relative concentration in aqueous solutions of reducing sugars.

2. Results and discussion

Carbonyl absorption frequencies of reducing sugars.—Generally, the position of the carbonyl stretching frequency is determined by the sample's physical state, conjugation,

hydrogen bonding, electronic effects of the substituents and the ring strain. In protic solvents, the intermolecular hydrogen bonding lowers the frequency of carbonyl absorption by 40–60 cm^{-1}, relative to that of the neat samples. In simple alkyl-substituted ketones, the two electron-donating alkyl groups lower the frequency of the carbonyl absorption relative to that of aldehydes, which have only one such electron-donating group. Consequently, simple ketones give rise to absorption bands at a lower frequency (1725–1710 cm^{-1}) than do aldehydes (1740–1720 cm^{-1}).

It is possible to extract from the position of the carbonyl absorption frequency a considerable amount of information, on the electronic and steric effects that arise from the nature of the substituents attached to the carbonyl group. Of special significance to reducing sugars are the substituents at the α-carbons, since the introduction of a halogen at these positions in simple ketones or aldehydes is known to lead to a shift to higher frequencies provided that the halogen can rotate to eclipse the carbonyl group [12]. The magnitude of this shift depends on the torsional angle. Most probably this shift arises from the field effect. This effect was demonstrated in carbohydrates by a shift to a higher frequency in the carbonyl absorption band of D-ribose (1722 cm^{-1}) when compared with the absorption of 2-deoxy-D-*erythro*-pentose (1717 cm^{-1}) due to the presence of a hydroxyl group at the α-carbon in D-ribose. The *keto* group of D-ribulose (D-*erythro*-pentulose), which has two such electron-withdrawing groups, absorbs at even higher frequency (1728 cm^{-1}) similar to D-fructose (see Table 1). Adding one hydroxyl group to the α-position of acetone increases the frequency of the carbonyl absorption from 1710 to 1720 cm^{-1}, whereas dihydroxyacetone absorbs at 1737 cm^{-1} (see Table 1). With the exception of D-ribose ($\upsilon_{C=O}$ 1722 cm^{-1}) and D-*ribo*-2-hexulose (D-psicose, $\upsilon_{C=O}$ 1724 cm^{-1}), all the pentoses and hexuloses studied absorb approximately at the same frequency (1728 cm^{-1}), which indicates the combined effect of the two α-hydroxyl groups on the absorption frequency of sugar carbonyls is very much dependent on their ability to attain eclipsed conformation with respect to the carbonyl group. Pentoses (except D-ribose) have more rotational and steric freedom to attain an eclipsed conformation, whereas in hexuloses (except psicose) the combined field effects of the two α-hydroxyl groups is equivalent to that of one α-hydroxyl group of pentoses. In D-*ribo*-2-hexulose (D-psicose) and D-ribose it seems the two α-hydroxyl groups cannot attain simultaneously an eclipse or near eclipse conformation. The common structural feature between the two sugars is the *ribo* configuration, which might impose rotational restriction through intramolecular H-bonding. Dihydroxyacetone, on the other hand, is able to assume a conformation in which the two α-hydroxyl groups can exert optimum field effect, thus shifting the absorption band to a much higher frequency of 1737 cm^{-1} compared with that of the hexuloses. In principle, the frequency of carbonyl absorption bands in a homologous series can be used to estimate relative torsional angles.

In aqueous solutions, ketoses in general show a more prominent carbonyl absorption band than the corresponding aldoses (see Fig. 1 for representative spectra). Hexoses, in contrast to hexuloses, do not show any detectable carbonyl absorption peak in D_2O owing to the hydration of the already very small concentrations of the acyclic *aldehydo* forms [1]. However, pentoses show a detectable *aldehydo* peak. D-Ribose 5-dihydrogen phosphate shows a more intense carbonyl absorption band at 1728 cm^{-1} compared with D-ribose, indicating a much higher concentration of the acyclic form in the phosphory-

Table 1
Carbonyl and enediol absorption bands of selected reducing sugars and sugar analogs in D_2O

Type	Compound	Carbonyl band (cm^{-1}) [a]	Enediol bands (cm^{-1}) [a]
Ketoses	Acetone	1710	n.o.
	2-Butanone	1695	n.o.
	Hydroxyacetone	1720,	1698
	Dihydroxyacetone	1737, 1724sh	1698
	D-Erythrulose	1728	n.o.
	D-Ribulose	1726	1650
	D-Fructose	1728	1647
	D-[2-^{13}C]fructose	1688	1643
	D-Tagatose	1728	1649
	D-Sorbose	1728	1649
	D-Psicose	1724	1649
Aldoses	D-Glyceraldehyde	1729, 1737sh, 1733sh,	n.o.
	D-Erythrose	n.o.	1636
	D-Lyxose	1727	1650
	D-Arabinose	1727	1648
	D-Xylose	1727	1648
	D-Ribose	1722	1646
	D-[2-^{13}C]ribose	1722	1646
	D-[1-^{13}C]ribose	1685	1646
	Ribose 5-phosphate (2 Na)	1728	1673, 1604
	2-Deoxy-*erythro*-pentose	1715	n.o.
	D-Glucose	n.o.	1647
	D-[1-^{13}C]glucose	n.o.	1645
	3-*O*-Methylglucose	n.o.	1646
	2-Deoxy-D-*arabino*-hexose	1722	1658
	D-Glucose 6-phosphate (Na)	1745	1647
Sugar analogs	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	1685	1606
	Ascorbic acid	1754	1686
	D-*arabino*-Hexose-2-ulose	1750	n.o.
	3-Deoxy-D-*arabino*-hexose-2-ulose	1765, 1735, 1719	1696, 1685

[a] n.o. = not observed; sh = shoulder.

lated derivative. Similarly, D-glucose 6-dihydrogen phosphate shows an absorption band (1745 cm^{-1}) in the carbonyl region that was not detected in glucose, indicating a much higher concentration of open-chain form. This observation is consistent with the literature data on the relative concentrations of acyclic forms [1]. In addition, the ability of the terminal phosphate group to exert an inductive effect to shift the carbonyl absorption bands to higher frequencies in both sugars indicates the close proximity of the phosphate groups to the carbonyl region. This observation gives further credence to the notion that acyclic forms of reducing sugars exist in a pseudo-cyclic conformation, at least in the phosphorylated derivatives.

The carbonyl absorption bands in reducing sugars can be assigned by observing a bathochromic effect or red shift when one of the atoms of the carbonyl group is replaced with a heavier isotope. This band should also be sensitive to temperature. The shift of the absorption band due to a specifically labeled carbonyl group in the FTIR spectrum of

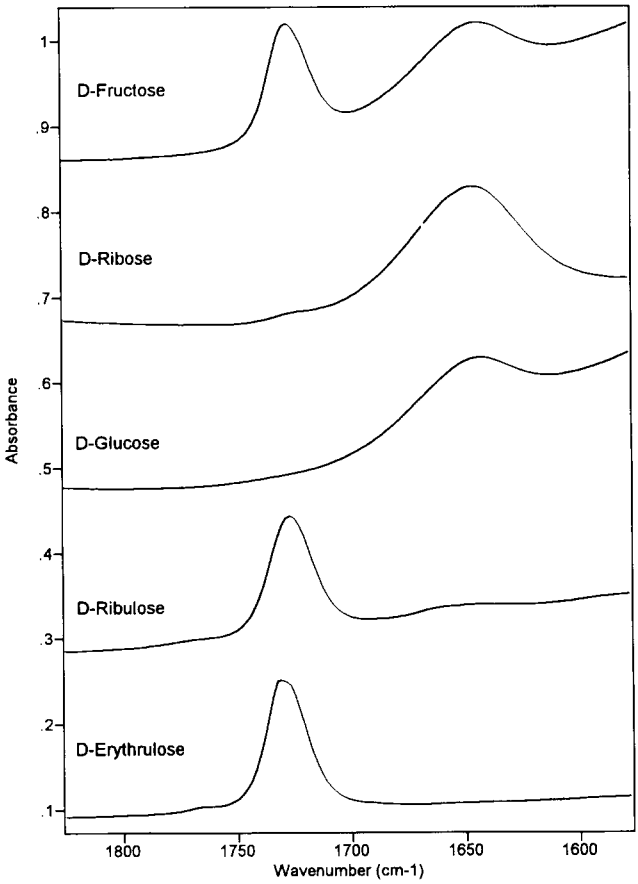

Fig. 1. Absorption of the carbonyl and enediol region (1800–1600 cm^{-1}) of selected reducing sugars in D_2O.

a sugar can also reveal the presence of overlapping bands in the carbonyl region due to the absorption of other carbonyl groups if present.

Water-catalyzed enolizations of reducing sugars.—Although enolization reactions require acid–base catalysis to proceed in appreciable amounts, FTIR analysis of different sugars has indicated that water can also catalyze enolizations to an extent that can be monitored by FTIR. Sugar enediols are reactive acyclic intermediates that are similar to *aldehydo* and *keto* forms, and knowledge of their relative concentrations may shed some light on their relative reactivities, especially during oxidative degradation, which is believed to proceed through enediol intermediates [10].

In general, enediols can be detected by FTIR spectroscopy owing to their absorption in the 1620–1690 cm^{-1} region. The FTIR analyses of different reducing sugars (Table 1) have indicated that in aqueous solutions, reducing sugars exhibit temperature-sensitive absorption bands in the 1630–1680 cm^{-1} region that can be ascribed to the presence of enediol species formed through water-catalyzed enolization. Aqueous solu-

tions of reducing sugars have different acidic pH values that might be dependent on the concentrations of enediol species. Theoretically, enediols can produce complex absorption patterns due to the presence of different stereoisomers and conformers. Aldoses, for example, can produce *cis*- and *trans*-1,2-enediols, and ketoses can produce *cis*- and *trans*-2,3-enediols in addition to *cis*- and *trans*-1,2-enediols. Enediols may exist in different conformations such as *syn–syn*, *syn–anti*, *anti–anti*, and *gauche–syn*. Intra- and inter-molecular H-bonding may stabilize one conformer relative to the other [13].

In order to verify the assignment of enediol bands, the spectra of D-[2-^{13}C]fructose and D-[1-^{13}C]glucose were compared with the spectra of unsubstituted derivatives. The enediol absorption bands were shifted to lower frequencies relative to the unsubstituted sugars (see Table 1), and the extent of these shifts was consistent with the theoretically expected values. In addition, the D-glucose enediol absorption band was compared with that of the 2-deoxy derivative. 2-Deoxy-D-glucose (2-deoxy-D-*arabino*-hexose) shows an absorption band in the enediol region centered at 1658 cm^{-1}, whereas glucose exhibits an absorption band centered at 1647 cm^{-1}. Deoxy sugars can generate only enols which are expected to absorb at a higher frequency than enediols. Oxidation of the C-2 hydroxyl group in glucose prevents enolization; accordingly, D-*arabino*-hexos-2-ulose (''glucosone'') shows no absorption in the enediol region. In addition, the effects of concentration, pH, temperature, and the type of sugar on the relative intensities of enediol absorption bands were studied in selected sugars. Increasing the concentration and the temperature of a fructose solution increases the intensity of both carbonyl and enediol absorption bands, as shown in Figs 2 and 3. Changing the pH of a fructose solution from 2 to 9 generates, in addition to the enediol peak at 1647 cm^{-1}, a new absorption peak centered at 1589 cm^{-1}. This band, which is also sensitive to temperature, could be assigned to the enediolate anion. When equimolar concentrations of tagatose, psicose, sorbose, and fructose were analyzed, fructose showed the highest concentration of carbonyl absorption, followed by sorbose, psicose, and tagatose. However, the relative intensities of enediol absorption bands were not in the same order. Psicose showed the highest relative concentration of enediol, followed by tagatose, fructose and sorbose (Fig. 4). Interestingly, the relative rates of enolizations under basic conditions show the same order [14]. If the catalytic activity of water to effect mutarotation of sugars is separated into acidic and basic functions, as shown below, then the contribution to the rate constant of the water molecule as a base catalyst was found to be 99% of its total catalytic activity [15]:

$$k_{H_2O} = k_{A(H_2O)}[H_2O] + k_{B(H_2O)}[H_2O]$$

where $k_{A(H_2O)}$ = rate constant of water as acid catalyst and $k_{B(H_2O)}$ = rate constant of water as base catalyst.

In neutral solutions, therefore, the basic catalytic function of water predominates for mutarotation reactions that are closely linked to enediol formation. One possible mechanism for water-catalyzed enolizations is the dimeric water as a bifunctional catalyst as shown in Fig. 5. This is based on the fact that two molecules of water are associated with each hydroxyl group of sugars as determined by calculation of hydration numbers of different sugars [16]. Alternatively, hydrogen-bonded dimeric acyclic sugar

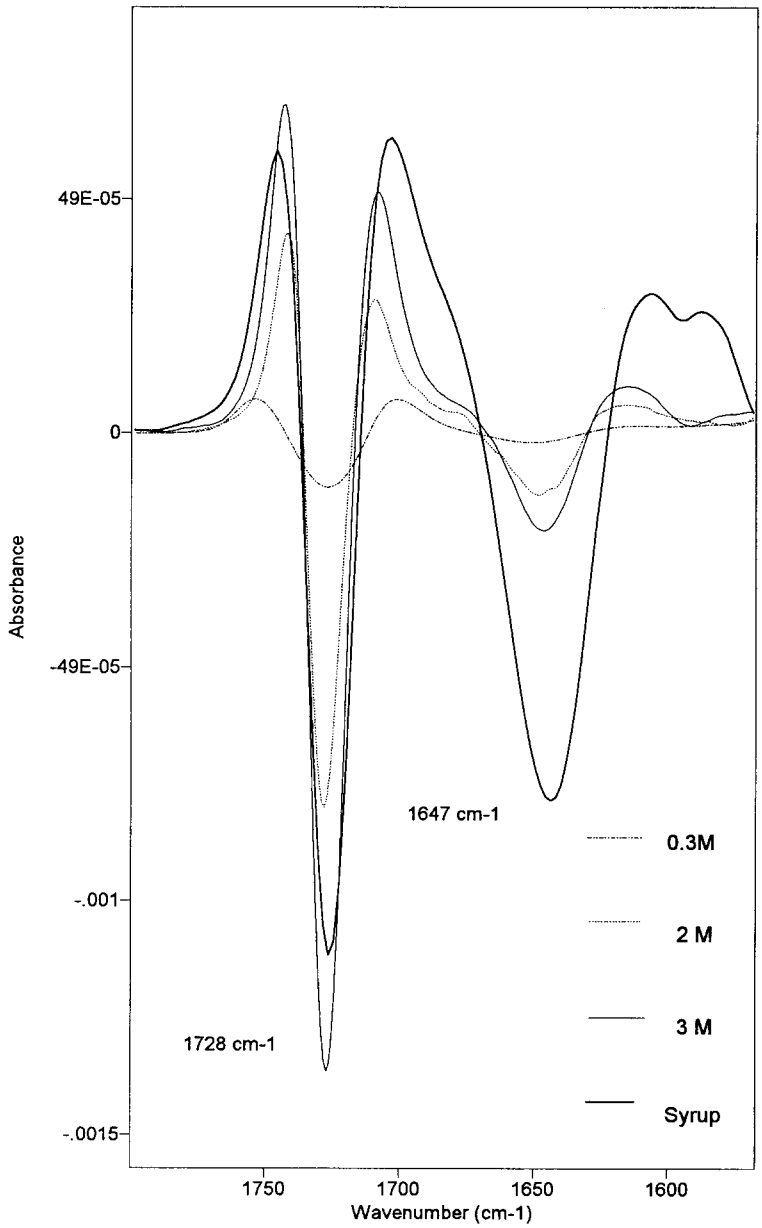

Fig. 2. Increase in the carbonyl and enediol absorption bands (second-derivative spectra) of D-fructose (syrup) as a function of increasing temperature (from 35 to 85°C).

molecules can also self-catalyze enolization reactions as shown in Fig. 5. The relative efficiency of reducing sugars to undergo water-catalyzed enolization could be related to their ability to induce modifications in the water structure to promote more efficient

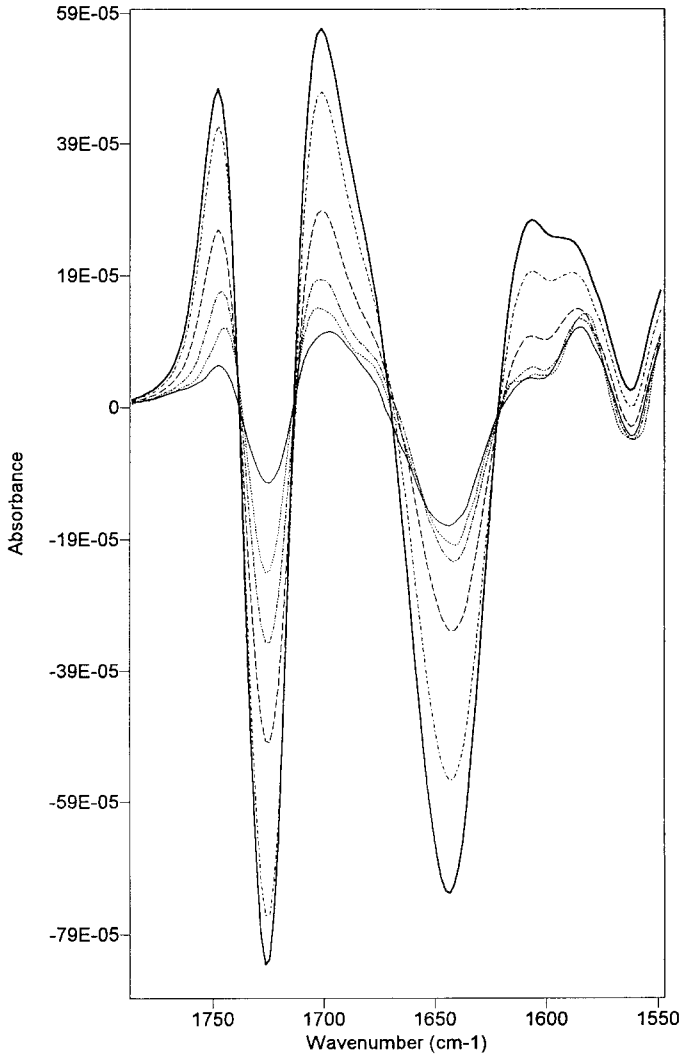

Fig. 3. Increase in the carbonyl and enediol absorption bands (second-derivative spectra) of D-fructose (at 85°C) as a function of increasing concentration (from 0.3 M to syrup).

catalysis or to the stability factors associated with the resulting enediol species through intra- and inter-molecular hydrogen bonding.

In order to estimate the relative concentration of enediol with respect to carbonyl forms, model compounds containing both enediol and a carbonyl group were studied by FTIR spectroscopy. In ascorbic acid the ratio of intensities of the permanent enediol to that of the lactone carbonyl was 2:1, and in 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) it was 2.8:1. These values indicate that the absorptivity of enediols is 2–3 times higher than that of carbonyls. Using a conservative estimate of a 3:1 ratio of

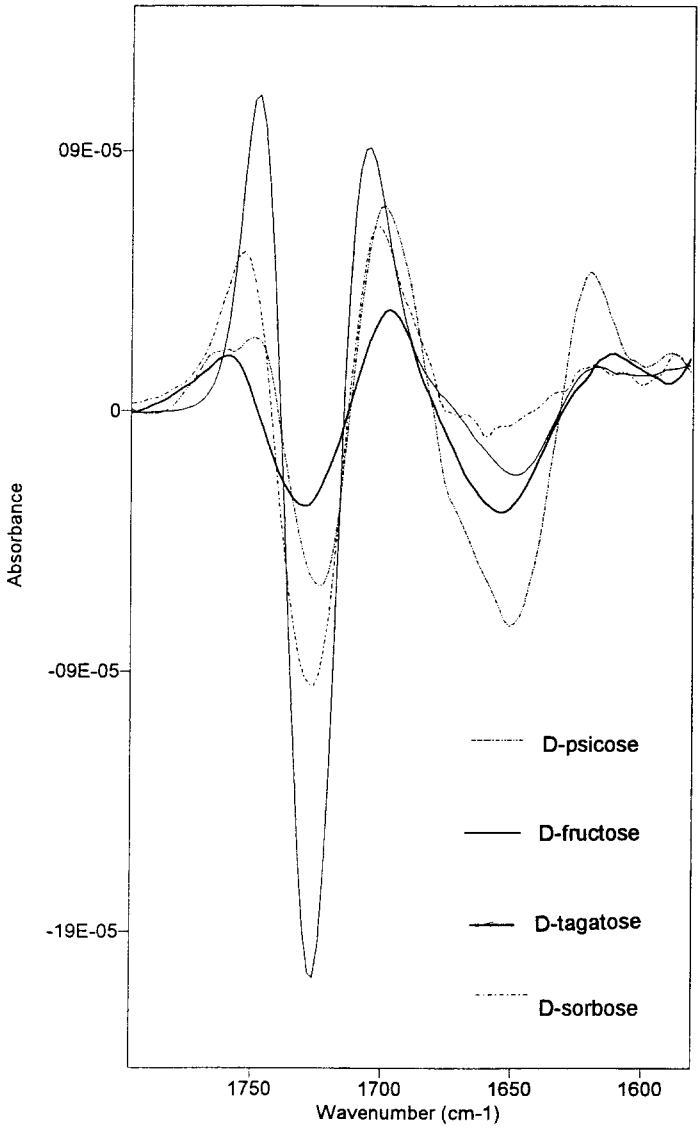

Fig. 4. Superposed second-derivative spectra (1750–1600 cm^{-1} region) of 0.3 M solutions of D-fructose, D-sorbose, D-tagatose, and D-psicose at the same temperature (65°C).

absorptivities, the relative enediol concentrations to that of carbonyl forms were calculated as a function of temperature (Table 2) and concentration (Table 3) for D-ribose and D-fructose as representative aldose and ketose sugars. The concentration of enediol species increases with increase in the concentration of carbonyl forms, as demonstrated earlier with fructose. However, the carbonyl band increases with temperature at a faster rate than the enediol absorption band, as shown in Table 2. At room

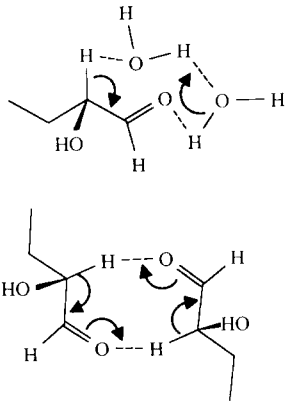

Fig. 5. Proposed dimeric-water catalysis and sugar dimer self-catalysis of enolization.

temperature both fructose and ribose have higher concentrations of enediol than carbonyl; however, as the temperature is increased, in the case of fructose the relative concentration of carbonyl forms becomes higher than that of the enediol. With ribose the enediol species remain in higher concentration, although the relative concentration drops. The effect of high temperature on the relative concentrations might be due to the breaking of hydrogen bonds that stabilize the enediols and/or promote dimerization necessary for self-catalysis of enolization as depicted in Fig. 5. With respect to ribose, the higher ratios of enediol forms might be partially attributed to the fact that most of the *aldehydo* forms are hydrated, and hence their observed concentrations are much lower. In addition, the dimer formation in aldoses is less sterically hindered relative to a

Table 2
Ratio of enediol to carbonyl absorption peaks as a function of temperature

Temperature (°C)	D-Ribose	D-Ribulose [a]	D-Fructose
30	28	0.20	2.3
65	18	0.12	0.5
85	16	0.09	0.3

[a] D-*erythro*-Pentulose.

Table 3
Ratio of enediol to carbonyl absorption peaks as a function of concentration

Concentration (M)	D-Ribose at 65°C	D-Fructose at 65°C
0.3	2	0.1
3.0	18	0.5
Syrup	23	1.5

keto sugar, hence self-catalysis is more efficient than in fructose. Unlike high temperature, high concentrations of sugars should promote hydrogen-bonded dimer formation and, in turn, increase the catalysis of enolizations. Table 3 lists the ratios of enediol to carbonyl absorption bands as a function of concentration at the same temperature. Both sugars show more than tenfold increase when the concentration is raised from 0.3 M to a syrup. Similar to ribose, other aldoses, in general, have more prominent enediol absorption bands than ketoses relative to the carbonyl.

Migration of the carbonyl absorption band as a consequence of enolization: a model study using D-*ribose,* D-*ribulose,* D-*[2-^{13}C]ribose and* D-*[1-^{13}C]ribose.*—If enediols are indeed formed in aqueous solutions of reducing sugars such as ribose, the equilibrium resulting from the reverse process can be initiated from both hydroxyl groups of enediol leading to the migration of the carbonyl group from C-1 to C-2 and formation of a mixture of ribose and ribulose through the common intermediate 1,2-enediol. It is possible to monitor by FTIR spectroscopy ribose–ribulose interconversion using properly substituted sugars.

D-Ribulose, similarly to D-fructose, exhibits two temperature-sensitive bands, the more intense band centered at 1726 cm^{-1} and the other at 1650 cm^{-1}. Previous studies [4] with D-[2-^{13}C]fructose and D-fructose have indicated that *keto*-fructose has an absorption band centered at 1728 cm^{-1}, as evidenced by a shift of the carbonyl band to 1688 cm^{-1} in the substituted fructose. The relative intensity of the carbonyl band of D-ribulose (D-*erythro*-pentulose) increased 2.5-fold during the heating cycle (30–80°C). A similar temperature rise in D-fructose affected a tenfold increase instead. The effect of temperature on the open-chain concentration of D-ribulose has also been investigated by ^{13}C NMR spectroscopy [17] in the temperature range 20–58°C. According to this study, increasing the temperature from 32 to 58°C increases the open-chain concentration 1.44-fold. FTIR measurements indicate that the integrated area of the carbonyl absorption band increases 1.57-fold when the temperature is raised from 30 to 60°C.

On the other hand, the prominent peak in the spectrum of D-ribose is centered at 1650 cm^{-1} and the less intense carbonyl peak at 1722 cm^{-1}. This is characteristic of *aldehydo* sugars in D_2O, which generally show a more prominent enediol absorption band relative to the carbonyl band due to the hydration of the *aldehydo* forms. If D-ribose-1,2-enediol can promote ketonization and formation of D-ribulose, then D-[1-^{13}C]ribose should exhibit two carbonyl bands, one at 1726 cm^{-1} (ribulose ketone band formed through enolization) and the other around 1680 cm^{-1} (ribose aldehyde band shifted from 1722 cm^{-1}). Similarly, D-[2-^{13}C]ribose should exhibit a band at 1722 cm^{-1} (D-ribose aldehyde band) and another around 1690 cm^{-1} (D-ribulose ketone band formed through enolization and shifted from 1726 cm^{-1}). To enhance the weak absorption of carbonyl bands in D-ribose, the second-derivative spectra were analyzed. Fig. 6 shows the superposed second-derivative spectra of D-[1-^{13}C]ribose, D-[2-^{13}C]ribose and D-ribose. The carbonyl peak centered at 1722 cm^{-1} in D-ribose is shifted to 1685 cm^{-1} in D-[1-^{13}C]ribose and the band centered at 1726 cm^{-1} in D-ribose is shifted to 1693 cm^{-1} in the spectrum of D-[2-^{13}C]ribose. The expected ratio $\upsilon_{^{12}C}/\upsilon_{^{13}C} = 1.025$, is very close to the experimentally observed ratio of $1722/1685 = 1.022$ for the *aldehydo* band and $1726/1691 = 1.021$ for the 2-*keto* band. This confirms the identity of the peaks and the occurrence of enolization.

3. Experimental

All the sugars were obtained from Aldrich Chemical. D_2O and D-[1-^{13}C]fructose were obtained from MSD Isotopes (Montreal, Canada), D-[2-^{13}C]ribose and D-[1-^{13}C]ribose were obtained from Sigma Chemical Co. Sugar solutions were prepared at concentrations ranging from 0.3 M to syrup in D_2O. The solutions were allowed to stand for a minimum of 24 h at room temperature prior to FTIR measurements. Processing of

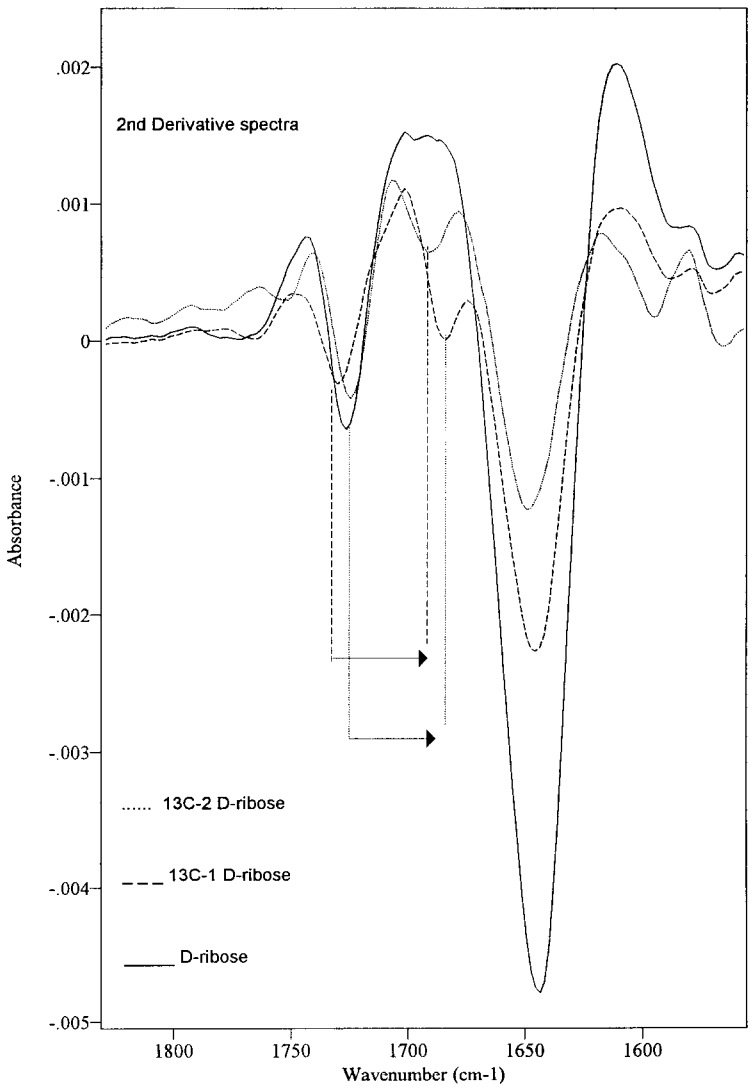

Fig. 6. Shift of the carbonyl absorption band in D-[1-^{13}C]ribose and D-[2-^{13}C]ribose.

the FTIR data was performed using the GRAMS/386 version 3.01 (Galactic Industries, 1994). Background D_2O and residual H_2O were subtracted from all spectra before derivatization. Second-order derivatization of the spectra was performed using the Savitsky–Golay function (second-order polynomial, 12 points).

Temperature studies.—Sugar solutions in D_2O were placed in a CaF_2 IR cell with a 50 μm 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/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 co-added.

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References

[1] S.J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 15–68.
[2] S.J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 49 (1992) 19–35.
[3] V.A. Yaylayan and A.A. Ismail, *J. Carbohydr. Chem.*, 11 (1992) 149–158.
[4] V.A. Yaylayan, A.A. Ismail, and S. Mandeville, *Carbohydr. Res.*, 248 (1993) 355–360.
[5] J.C. Speck, *Adv. Carbohydr. Chem.*, 13 (1958) 63–103.
[6] D. Cervantes-Laurean, D.E. Minter, E.L. Jacobson, and M.K. Jacobson, *Biochemistry*, 32 (1993) 1528–1534.
[7] M.J. King-Morris and A.S. Serianni, *Carbohydr. Res.*, 154 (1986) 29–36.
[8] D.S. Hamilton and D.J. Creighton, *J. Biol. Chem.*, 267 (1992) 24933–24936.
[9] G.H. Lorimer, Y.-R. Chen, and F.C. Haermena, *Biochemistry*, 32 (1993) 9018–9024.
[10] P. Thornally, S. Wolf, J. Crabe, and A. Stern, *Biochim. Biophys. Acta*, 797 (1984) 276–287.
[11] M.L. Wolfrom and W.L. Shilling, *J. Am. Chem. Soc.*, 73 (1951) 3557–3558.
[12] D.J. Pasto and C.R. Johnson, *Organic Structure Determination*, Prentice-Hall, Englewood Cliffs, NJ, 1969, pp 128–129.
[13] B. Floris, in Z. Rappoport (Ed.), *The Chemistry of Enols*, Wiley, Chichester, 1990, pp 147–305.
[14] H.S. Isbell, H.L. Frush, W.R. Wade, and C.E. Hunter, *Carbohydr. Res.*, 9 (1969) 163–175.
[15] H.S. Isbell and W. Pigman, *Adv. Carbohydr. Chem.*, 24 (1969) 22.
[16] J.L. Hollenberg and D.O. Hall, *J. Phys. Chem.*, 87 (1983) 695–696.
[17] J. Wu, A. Serianni, and T. Vuorinen, *Carbohydr. Res.*, 206 (1990) 1–12.