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Registry No. 1, 89771-75-5; **2**, 617-12-9; ESP synthase, 9068-73-9; chorismate mutase, 9068-30-8; anthranilate synthase, 9031-59-8; (1*R*,2*R*)-[1-^{2}H,^{3}H]glycerol, 90195-13-4; methyl 3-[(1-carbomethoxyvinyl)oxy]-4-methoxy benzoate, 81776-92-3; methyl 3-[(1-carbomethoxyethyl)oxy]-4-methoxybenzoate, 96454-31-8; tritiated D,L-lactic acid, 96553-57-0.

Complex Isomerization of Ketoses: A ^{13}C NMR Study of the Base-Catalyzed Ring-Opening and Ring-Closing Rates of D-Fructose Isomers in Aqueous Solution

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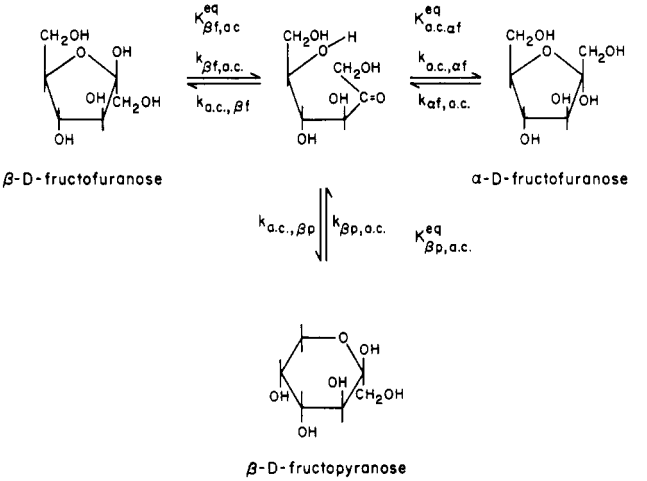
Abstract: In the present study, the isomerization of D-[2-^{13}C]fructose has been characterized under alkaline conditions with ^{13}C NMR spectroscopy. No detectible resonances arising from the open-chain hydrate or α-D-fructopyranose could be observed in ^{13}C NMR spectra acquired above pH 7 and between 17 and 50 °C. Resonances in spectra arising from the ^{13}C-enriched carbon of the α-furanose and β-furanose and the acyclic keto form of D-fructose were observed to broaden drastically with increasing pH or temperature while, in the same spectra, the line width of the anomeric carbon resonance of the β-pyranose form remained less than 2 Hz. Apparent first-order rate constants for ring opening of the α- and β-furanose forms of the sugar ($k_{\alpha f,a.c.}$, $k_{\beta f,a.c.}$) were determined by fitting measured line widths to a model for three-site exchange. At all pH values and temperatures studied, it was found that $k_{\beta f,a.c.}$ was nearly twice as large as $k_{\alpha f,a.c.}$ Second-order rate constants for furanose ring opening were determined from the pH dependence of the apparent first-order rate constants and were found to be (2.88 ± 0.5) × 10^6 and (3.6 ± 0.6) × 10^6 M^{-1} s^{-1} for ring opening of the α-furanose and β-furanose forms, respectively. Thermodynamic activation parameters, $\Delta H^{\ddagger}$, $\Delta G^{\ddagger}_{298}$, and $\Delta S^{\ddagger}_{298}$, were determined from the temperature dependence of $k_{\alpha f,a.c.}$ and $k_{\beta f,a.c.}$ at pH 8.4. Under these conditions, $\Delta S^{\ddagger}_{298}$ was found to be positive for both furanose ring-opening reactions, in marked contrast to the large negative $\Delta S^{\ddagger}_{298}$ determined from furanose ring-opening rates of D-galactose, D-threose, D-erythrose, and 2-deoxy-D-ribose under acidic conditions. The positive $\Delta S^{\ddagger}_{298}$ values have been rationalized in terms of a model involving the organization of polar solvent around the stabilized D-fructofuranose anions. Values of $\Delta H°$, $\Delta G°_{298}$, and $\Delta S°_{298}$ characterizing interconversions between cyclic structures and between cyclic and acylic forms were determined from the temperature dependence of equilibrium constants at pH 8.4. In all cases, $\Delta G°_{298}$ is determined both in sign and magnitude by $\Delta H°$. Apparent first-order ring-closing rates to D-fructofuranoses were determined from ring-opening rate constants, $k_{\alpha f,a.c.}$ and $k_{\beta f,a.c.}$, and corresponding equilibrium constants, $K^{eq}_{\alpha f,a.c.}$ and $K^{eq}_{\alpha f,a.c.}$ At all temperatures studied, the ring-closing rate to β-fructofuranose was found to be roughly five times that of the corresponding rate to the α-furanose form. Inversion-transfer ^{13}C NMR experiments were carried out on D-[2-^{13}C]fructose at pH 8.4 and 27 °C in order to further characterize the relatively slow β-pyranose ring-opening and ring-closing rates ($k_{\beta p,a.c.}$ and $k_{a.c.,\beta p}$). From inversion-transfer data and equilbrium intensities, an upper limit of 0.1 s^{-1} was determined for $k_{\beta p,a.c.}$ and 20 s^{-1} for $k_{a.c.,\beta p}$. Under identical conditions, ring-closing rate constants to the α- and β-furanose forms were found to be about 80 and 500 s^{-1}.

Reducing sugars are known to exist in aqueous solution as complex equilibrium mixtures of isomeric forms, including pyranoses, furanose, and septanoses.[1-5] Interconversions between these forms is thought to proceed through a series of steps involving an initial base or acid-catalyzed ring opening to an acyclic keto or aldehyde intermediate in equilibrium with an open-chain hydrate (*gem*-diol), followed by a conformational rearrangement in acyclic structure and final ring-closing (Scheme I).[6,7] In the case of most simple pentoses and hexoses, ring-closing reaction rates are typically 10-1000 times faster than corresponding ring-opening rates,[8-12] making the amount of acyclic and open-chain forms present at equilbrium quite small (usually less than 1%). Many chemical and biochemical reactions involving monosaccharides involve only one of the isomeric forms present in

Scheme I



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solution.[13-15] Ultimately, the rates of such reactions may be limited by the unidirectional rates of conversion of isomers not involved in the reaction to the acyclic intermediate. Hence, detailed knowledge of unidirectional rate constants defining isomeric interconversions is requisite to our understanding of rates of chemical and biochemical reactions involving monosaccharides.

Since greatly enhanced ring-opening rates in alkaline solution make kinetic measurements difficult,[6] measurements of unidirectional rates from monosacchardie isomers have typically been carried out in neutral or slightly acidic solution. This is particularly true when the acquisition of kinetic data involves the measurement of rapidly changing spectroscopic properties after dissolving a pure crystalline cyclic form of the sugar in aqueous media. It is likely that because of these difficulties, ring-opening and ring-closing rates of cyclic D-glucose, D-galactose, and 2-deoxyribose isomers were determined only below neutral pH.[8-10] On the other hand, steady-state kinetic measurements have proved to be effective in the determination of base-catalyzed isomerization rates of thiosugars where the overall steady-state ring-opening rate could be controlled by varying the concentration of a sulfohydryl chromophore able to react with the acyclic form of the sugar.[16] Nevertheless, in all of the kinetic studies, unidirectional rate constants could only be obtained by computer simulation of kinetic data based on an assumed scheme for complex interconversions between isomers.

Many of the problems associated with using kinetic velocity measurements to obtain unidirectional rate constants on rapidly interconverting sugar forms can be avoided by directly determining rates from an equilibrium mixture of isomers with NMR resonance line widths. Ultimately, rate constants which can be determined by using the line widths are limited by the chemical shift difference between resonances of the two nonexchanging In ^{13}C NMR spectra, where the frequency difference between cyclic and acyclic keto resonance is on the order of kilohertz, rates greater than ~ 1 s^{-1} can be measured. Unidirectional rates slower than this lower limit can be measured with NMR saturation or inversion–transfer techniques.[18,19] Recently, these magnetization–transfer techniques have been used to determine furanose ring-opening rates for D-threose and D-erythrose at pH 5.[12]

In the present study, unidirectional rate constants describing isomer interconversions have been determined for D-fructose in alkaline solution with ^{13}C NMR. Under all pH and temperature conditions studied, ^{13}C resonances arising from the β-pyranose, β-furanose, α-furanose, and acyclic keto forms of D-fructose were observed, while signals arising from α-pyranose and open-chain hydrate forms were absent. Therefore, it is assumed that α-pyranose and open-chain hydrate forms of D-fructose are present at negligible concentrations and that conversions between isomers may be represented according to Scheme I. Rate constants for the ring-opening of furanose anomers have been determined under alkaline conditions by fitting resonance line widths to NMR exchange models. Ring-closing rates $k_{a.c.,\beta f}$ and $k_{a.c.,\alpha f}$ were calculated from ring-opening rates and measured equilibrium constants, $K^{eq}_{\beta f,a.c.}$ and $K^{eq}_{\alpha f,a.c.}$. An upper limit to rate constants for ring-opening and ring-closing of the β-pyranose form ($k_{\beta p,a.c.}$ and $k_{a.c.,\beta p}$) could be estimated from measured line widths of the acyclic carbonyl resonance and cyclic isomer interconversion rates, as determined by ^{13}C NMR inversion–transfer methods. From a temperature dependence of rate data, thermodynamic activation parameters could be calculated. It has been found that rates and corresponding thermodynamic activation parameters for base-catalyzed D-fructose isomer interconversions differ significantly from similar data acquired under neutral or acidic conditions.

Experimental Section

Materials. Sodium [^{13}C]cyanide and initial samples of D-[2-^{13}C]-fructose (90% enriched in ^{13}C) were supplied by the Los Alamos Scientific Laboratory, University of California, Los Alamos, NM. "Sweetzyme Q" (an immobilized form of glucose isomerase) was purchased from Novo Laboratories, Inc. (Wilton, CT); Dowex-1 and Dowex-50 ion exchange resins, D_2O (99%), D-arabinose, palladium–barium sulfate (5%), and molybdic acid (85%) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade and were used without further purification. D-[1-^{13}C]Mannose was prepared from sodium [^{13}C]cyanide and D-arabinose according to the procedure of Serianni et al.[20] The product (60% yield) was separated from D-[1-^{13}C]glucose by fractionation on a 2 × 90 cm column of Dowex 50-X8 (barium form, 200–400 mesh) with H_2O as eluent.

D-[2-^{13}C]Glucose was prepared from D-[1-^{13}C]mannose according to the procedure of Hayes et al.[21] Accordingly, an aqueous mixture of 0.5 M D-[1-^{13}C]mannose and 10 mM molybdic acid (85%) was incubated at pH 4.5 and 90 °C for 6 h. The pH of the reaction mixture was readjusted to 8.5 and 10 g of "Sweetzyme Q" was added. Further incubation at 70 °C for 12 h converted 30% of the D-[2-^{13}C]glucose in the reaction mixture to D-[2-^{13}C]fructose, as was determined by peak integration in the ^{13}C NMR spectra of the reaction mixture. D-[2-^{13}C]-fructose was purified by column chromatography on Dowex 50-X8, as described previously.

NMR Spectral Conditions. Normal Fourier transformed ^{13}C NMR spectra were acquired at 75.2 MHz (70.4 kG) on a Bruker WM-300 at Los Alamos Scientific Laboratory, University of California. Spectra were acquired with use of a 10° pulse angle and a delay time between pulses of 1 s. In order to minimize differential intensity effects arising from nuclear Overhauser enhancements, the proton decoupler was gated off during the delay time. Spectra acquired under these conditions were identical with spectra acquired by using delay times greater than 60 s. Samples were run in 10-mm NMR sample tubes. The probe temperature was regulated to ±1 °C with a flow of dry nitrogen. Chemical shifts were measured digitally with the anomeric carbon resonance of β-D-[2-^{13}C]-fructopyranose (99.1 ppm) as an internal standard. Peak intensities were determined by digital integration or by cutting out and weighing peaks from horizontally expanded spectra.

Inversion–transfer Fourier transform ^{13}C NMR spectra were run at 50.1 MHz (46.9 kG) on a JEOL FX-200. Selective inversion was achieved with use of the "Dante" pulse sequence, as described by Morris and Freeman.[22] Spectra were acquired under conditions of complete proton decoupling, with a 45-s delay time between successive pulses. The probe temperature was maintained at 27 °C ± 1 °C with a flow of dry air.

NMR Sample Preparation, Quantitation, and Temperature Determination. Following chromatographic separation, dilute solutoins of D-[2-^{13}C]fructose were concentrated by evaporation under reduced pressure to less than 0.5 mL and dissolved in aqueous, unbuffered solutions containing 10% D_2O. In a few cases, samples were prepared under a nitrogen atmosphere with degassed solutions, in order to prevent carbonate from entering the system. The pH of the samples was adjusted with 0.001–0.1 N HCl and degassed NaOH. D-Fructose concentrations were measured colorimetrically with phenol–sulfuric acid.[23] The sample temperature in the NMR probe was determined by equilibrating standard unenriched D-fructose samples in the NMR probe and measuring the temperature with a digital thermometer equipped with a thermocouple.

Computations. Exchange rates for ring-opening of D-fructofuranose anomers were determined by reading digitized NMR data for the furanose anomeric carbon and the acyclic keto carbonyl carbon resonances into an iterative version of DNMR3, a computer program written for the calculation of multiple-site exchange-broadened NMR spectra.[24,25] Populations of each of the exchanging populations were held at fixed values, in proportion to intensities determined by peak integrations. Line widths for the furanose and acyclic keto carbonyl resonances in the absence of exchange were estimated from line widths of resonances arising from β-D-fructopyranose (about 2 Hz), cyclic isomer known to undergo much slower exchange to the acyclic keto form.[26] Ring-closing

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exchange rates were calculated from ring-opening exhange rates and measured furanose–acyclic keto equilibrium constants.

The inversion–transfer experiments were performed by selectively inverting the magnetization of the anomeric carbon resonance of β-D-[2-^{13}C]fructopyranose, β-D-[2-^{13}C]fructofuranose, or α-D-[2-^{13}C]fructofuranose. Recovery of magnetization following inversion can be described by the set of coupled first-order differential equations:[18]

$$d[M_{\alpha f}]/dt = -R_{\alpha f}(M^{\circ}_{\alpha f} - M_{\alpha f}) + k_{\beta f,\alpha f}(M^{\circ}_{\beta f} - M_{\beta f}) + k_{\beta p,\alpha f}(M^{\circ}_{\beta p} - M_{\beta p}) \quad (1)$$

$$d[M_{\beta f}]/dt = k_{\alpha f,\beta f}(M^{\circ}_{\alpha f} - M_{\alpha f}) - R_{\beta f}(M^{\circ}_{\beta f} - M_{\beta f}) + k_{\beta p,\beta f}(M^{\circ}_{\beta p} - M_{\beta p}) \quad (2)$$

$$d[M_{\beta p}]/dt = k_{\alpha f,\beta p}(M^{\circ}_{\alpha f} - M_{\alpha f}) + k_{\beta f,\beta p}(M^{\circ}_{\beta f} - M_{\beta f}) - R_{\beta p}(M^{\circ}_{\beta p} - M_{\beta p}) \quad (3)$$

Where, for example, $M^{\circ}_{\alpha f}$ and $M_{\alpha f}$ represent the magnitudes of the z components of magnetization of the α-furanose anomer at equilibrium and time τ following the inverting pulse, respectively. As described by the above equations, the intensities of any two of the three anomeric carbon resonances is influenced by the inversion of z magnetization of the third anomeric carbon resonance through the rate constants describing the overall exchange rate between isomers ($k_{\beta f,\alpha f}$,$k_{\beta p,\alpha f}$, etc., where, for example, $k_{\beta f,\alpha f}$ is the rate constant for exchange from the β-furanose anomer to the α-furanose anomer) and by the relaxation rate for each of these resonances in the presence of exchange ($R_{\alpha f}$, $R_{\beta f}$, and $R_{\beta p}$, where, for example, $R_{\alpha f} = k_{\alpha f,\beta f} + k_{\alpha f,\beta p} + 1/T_{1\alpha f}$, and $T_{1\alpha f}$ is the spin–lattice relaxation time for the α-furanose anomeric carbon resonance in the absence of exchange).

Since it is difficult to solve these coupled expressions in closed form, values of magnetization were calculated digitally for any given set of unknown parameters by incrementing the delay time following the inverting pulse. As written, eq 1–3 contain nine unknowns. However, three rate constants, $k_{\alpha f,\beta f}$, $k_{\alpha f,\beta p}$, and $k_{\beta f,\beta p}$, may be expressed in terms of the rate constants $k_{\beta f,\alpha f}$, $k_{\beta p,\alpha f}$, and $k_{\beta p,\beta f}$ and the three measured equilibrium contants $K^{eq}_{\alpha f,\beta f}$, $K^{eq}_{\alpha f,\beta p}$, and $K^{eq}_{\beta f,\beta p}$. The remaining six rate constants and relaxation rates were determined by fitting experimental inversion–recovery data to eq 1–3, using a multivariable nonlinear least-squares computer fitting subroutine ZXSSQ in the IMSL statistical package (International Mathematical and Statistical Libraries, Inc., Houston, TX).

All calculations involving multivariable fits were carried out on an IBM 4341 at the University of Texas Regional Computer Center. As a check to the validity of the fit, calculated curves based on output parameters were displayed graphically along with experimental points on the high resolution graphics screen of an IBM Personal Computer.

Results and Discussion

pH Dependence of D-Fructose Isomerization Reactions. The 75.1-MHz proton-decoupled ^{13}C NMR spectrum of 1.5 M D-[2-^{13}C]fructose in H_2O (10% D_2O) at 30 °C and pH 2.0 is shown in Figure 1α. The intense resonances at 99.1, 102.6, andd 105.5 ppm arise from the ^{13}C-enriched carbon of the β-pyranose, β-furanose, and α-furanose isomers while the resonance at 214.2 ppm has previously been assigned to the carbonyl carbon of the acyclic keto form of the sugar.[27] Resonances visible between about 60 and 80 ppm arise from unenriched carbons of the cyclic forms of D-fructose. Notice that even at the elevated concentrations used in acquiring the pH 2.0 spectrum (a ^{13}C concentration equivalent to 150 M of naturally enriched sample), there is an apparent absence of resonances arising from the enriched carbon of the α-pyranose anomer or the open-chain hydrate form of D-fructose. The anomeric carbon resonance of α-fructopyranose has previously been observed at elevated temperatures (99.0 ppm at 80 °C and neutral pH),[27,28] while the corresponding resonance of the open-chain hydrate is expected from ^{13}C NMR data of other monosaccharides to lie between 90 and 104 ppm.[12,29] Hence, if trace amounts of these two forms of D-fructose are present at 30 °C, resonances arising from them must be obscured by more intense signals in the spectrum.

Parts B–D of Figure 1 show that upon raising the pH of the D-[2-^{13}C]fructose solution to pH 9.5, there is an apparent broadening of resonances arising from the α-furanose, β-furanose, and acyclic keto forms of the sugar. This broadening is so pronounced that above pH 9.6, only resonances arising from β-D-fructopyranose can be clearly identified. Such broadening of resonances is consistent with slow exchange of nuclei between cyclic and acyclic keto forms of D-fructose[11,12] where the resonance line widths at half-height ($\Delta\nu$) are given by

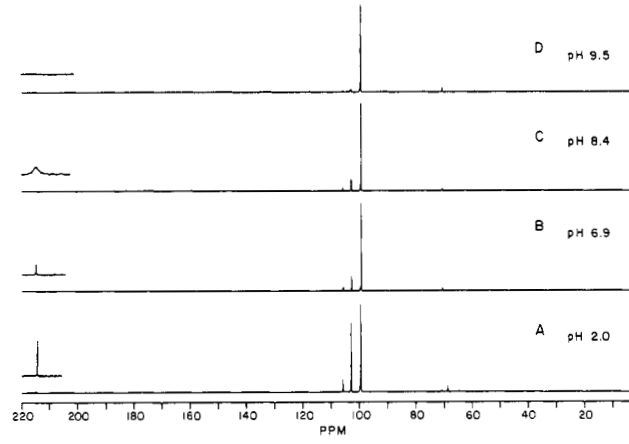

Figure 1. Proton-decoupled ^{13}C NMR spectra of 1.5 M D-[2-^{13}C]fructose at 30 °C. Fourier-transform time-domain spectra were acquired with a 10° flip angle and a delay between acquisitions of 1 s. The resonance at 214.2 ppm is also shown on a vertically expanded scale 32 times that used in plotting the entire spectrum. Two-hertz line broadening was applied prior to transformation of all spectra shown spanning the chemical shift range from 0 to 220 ppm. Ten hertz (spectra A and B) or twenty hertz (spectra C and D) was used in transforming vertically expanded peaks.

$$\pi\Delta\nu_{\beta p} = \pi\Delta\nu^{0}_{\beta p} + k_{\beta p,a.c.} \qquad \pi\Delta\nu_{\beta f} = \pi\Delta\nu^{0}_{\beta f} + k_{\beta f,a.c.}$$
$$\pi\Delta\nu_{\alpha f} = \pi\Delta\nu^{0}_{\alpha f} + k_{\alpha f,a.c.} \quad (4)$$
$$\pi\Delta\nu_{a.c.} = \pi\Delta\nu^{0}_{a.c.} + (k_{a.c.,\beta p} + k_{a.c.,\beta f} + k_{a.c.,\alpha f} + k_{a.c.,e})$$

Here $\Delta\nu^0$ and $\Delta\nu$ are resonance line widths (in Hz) in the absence and presence of chemical exchange. As shown in Scheme I, $k_{\beta p,a.c.}$, $k_{\beta f,a.c.}$, and $k_{\alpha f,a.c.}$ represent pseudo-first-order rate constants from the β-pyranose, β-furanose, and α-furanose forms to an acyclic conformation while $k_{a.c.,e}$ is the corresponding rate constant to the fully extended keto form of the sugar. Taken along with the pH-dependent line-broadening effects shown in Figure 1, the above relationships imply that ring-opening rates from the furanose anomers are much more rapid than the corresponding rate from the β-pyranose form. As previously pointed out by Lemieux et al.[10], this is most likely a result of ring strain in the five-membered-ring forms.

Since spectra of D-fructose were acquired under spectral conditions so as to minimize differential effects of nuclear Overhauser enhancement (NOE) and relaxation, integrated intensities of assigned resonances may be used to estimate pH-dependent changes in isomeric compositions.[30] At 30 °C, the acyclic keto composition was significantly higher at pH 2.0 (0.80 ± 0.4%) than at pH 5.5, 8.0, or 8.4 (0.55 ± 0.03%, 0.50 ± 0.03%, and 0.58 ± 0.08%, respectively). Corresponding compositions of the β-pyranose, β-furanose, and α-furanose forms of D-fructose were the following: 65.2 ± 3.3%, 28.2 ± 1.4%, and 5.8 ± 0.3% at pH 2.0; 67.2 ± 3.4%, 25.5 ± 1.3%, and 6.7 ± 0.3% at pH 5.5; 68.0 ± 3.4%, 25.8 ± 1.3%, and 6.2 ± 0.3% at pH 8.0; 67.7 ± 3.4%, 25.7 ± 1.3%, and 6.6 ± 0.3% at pH 8.4; 68.7 ± 3.4%, 24.6 ± 1.2%, and 5.7 ± 0.3% at pH 9.5; 69.7 ± 3.5%, 24.2 ± 1.2%, and 6.1 ± 0.3% at pH 9.6. Within experimental error, there appears to be no pH dependence in the fraction of D-fructose cyclic forms present.

Ring-closing rates from the acyclic keto intermediate can be calculated from the line broadening observed in furanose anomeric carbon resonances and acyclic keto–furanose equilibrium constants measured from integrated peak intensities. It is not possible to

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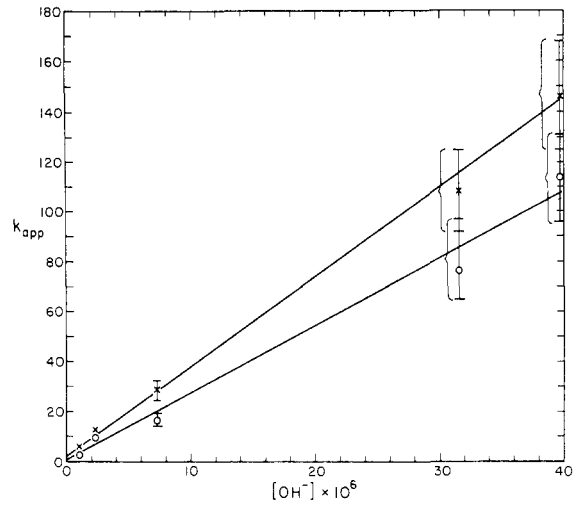


Figure 2. Apparent first-order rate constants for β-D-[2-^{13}C]fructofuranse (×) and α-D-[2-^{13}C]fructofuranose (○) as a function of increasing hydroxide ion concentration. Rate constants were calculated by simultaneously fitting the anomeric and carbonyl resonance line widths to eq 4. Lines in the figure represnt linear least-squares fits to experimental data.

estimate the ring-closing rate to the β-pyranose isomer, since there is no measurable line broadening seen in the β-pyranose anomeric carbon resonance, even at pH 9.5 (Figure 1D). However, if $k_{a.c.,e}$, $k_{a.c.,\beta p} \ll k_{a.c.,\beta f}$, $k_{a.c.,\alpha f}$, then the line broadening measured from the acyclic keto carbonyl resonance in high pH spectra will be the same as the line broadening calculated from eq 4, neglecting $k_{a.c.,\beta p}$ and $k_{a.c.,e}$. When such a comparison is carried out with data taken from the pH 8.4 spectrum (Figure 1C), the calculated line width of the carbonyl resonance (189 Hz) is in good agreement with the measured line width (180 Hz). This result implies that ring-closing rates to the furanose anomers are much faster than the ring-closing rates to the β-pyranose anomer, a result consistent with D-fructose mutorotation data.[26]

There is some discrepancy in the literature as to the exact nature of keto intermediates involved in the exchange process between isomers. On one hand, ^{18}O exchange studies have demonstrated that the rates of isotope incorporation into hexoses from $H_2{}^{18}O$ are much slower than the respective mutorotation rates, leading to the proposal that the true intermediate is an aldehyde having a pseudo-acyclic conformation.[7] Such a pseudo-acyclic form in slow exchange with the fully extended form was also found to be consistent with kinetic data taken from the base-catalyzed anomerization of thiosugars.[16] On the other hand, saturation transfer NMR studies on tetroses suggest that if such a pseudo-acyclic intermediate exists, it must be in rapid equilibrium with the fully extended aldehydo form.[12] There is no reason to believe the carbonyl resonance of the fully extended keto form of D-fructose should have a chemical shift different from the corresponding resonance of the acyclic keto form. However, since there is no detectible narrow component superimposed on the broadened resonance at 214.2 ppm (Figure 1C), we can conclude that either the fully extended keto form is in fast exchange with the acyclic keto form or that the fully extended keto form is in slow exchange with the acyclic form but is present at a low enough abundance so as to be undetectible (about 10% or less based on the signal-to-noise ratio of the carbonyl resonance).

In order to further characterize the base-catalyzed exchange behavior between furanose anomers and the acyclic form of D-fructose, apparent first-order rate constants were determined as a function of pH by fitting digitized spectra to eq 4, using a nonlinear least-squares fitting routine.[24,25] Rate constants determined by this method may be expressed as[7]

$$k_{app} = k_{H_2O} + k_{S^-}(S^-) + [k_{CO_3^{2-}}(CO_3^{2-}) + k_{HCO_3^-}(HCO_3^-)] + k_B(OH^-) \quad (5)$$

Here k_B, k_{S^-}, $k_{CO_3^{2-}}$, and $k_{HCO_3^-}$ are second-order rate constants

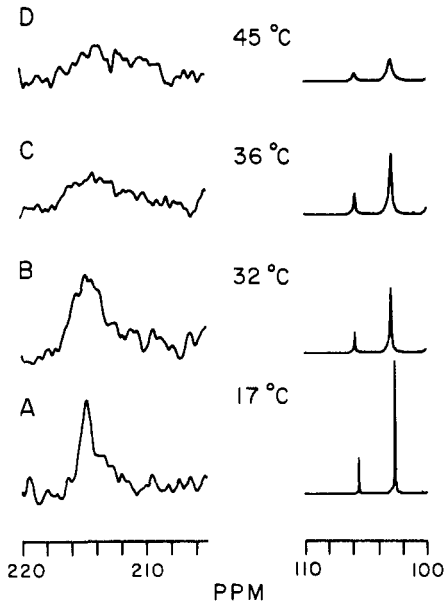


Figure 3. Resonances appearing in the carbonyl and anomeric carbon regions of ^{13}C NMR spectra of 1.5 M D-[2-^{13}C]fructose at pH 8.4. The resonance assigned to the anomeric carbon of β-D-[2-^{13}C]fructopyranose (99.1 ppm) is not shown in the figure but has a line width less than 2 Hz at all indicated temperatures. Anomeric carbon resonances were Fourier transformed from the time domain with 2 Hz broadening while 20 Hz line broadening was used in transforming the carbonyl resonance at 214.2 ppm. The carbonyl region has a vertical scale 32 times that shown for the anomeric carbon region.

arising from basic catalysis by hydroxide, the fructosyl anion, and any carbonate and bicarbonate anions which may be present in unbuffered aqueous D-fructose solutions. Figure 2 shows a plot of $k_{\beta f,a.c.}$ and $k_{\alpha f,a.c.}$ as a function of hydroxide concentration. The second-order rate constants for hydroxide-catalyzed ring-opening for furanose anomers determined from the slopes of the best-fit lines are $k_B^{\alpha f,a.c.} = (2.88 \pm 0.5) \times 10^6$ M^{-1} s^{-1} and $k_B^{\beta f,a.c.} = (3.6 \pm 0.6) \times 10^6$ M^{-1} s^{-1}. These values are roughly two orders of magnitude larger than rate constants determined from the base-catalyzed mutorotation of the β-pyranose form.[31] Slopes determined from a log–log plot of the same data shown in Figure 2 confirm the first-order dependence of k_{app} on hydroxide ions (slopes = 1.00 ± 0.1 and 0.90 ± 0.1 from the pH dependence of log $k_{\alpha f,a.c.}$ and log $k_{\beta f,a.c.}$, respectively). The intercepts of the best fit lines in Figure 2 yielded upper limits for the sum of apparent first-order rate constants, $k_{H_2O} + k_{S^-}(S^-) + k_{HCO_3^-}(HCO_3^-) + k_{CO_3^{2-}}(CO_3^{2-})$, of 0.5 and 3.5 s^{-1} for ring-opening of the α-furanose and β-furanose anomers. However, neither a threefold variation in D-fructose concentration nor measures taken to scrupulously exclude carbonate had any observable effect on anomeric or carbonyl carbon resonance line widths in spectra recorded at pH 8.4 or 9.0. Hence, these values probably represent upper limits for k_{H_2O} and, as such, are consistent with values of k_{H_2O} previously reported for ring-opening of furanose anomers of other aldoses and thiosugars in aqueous solution.[10,12,16]

Temperature Dependence of D-Fructose Isomerization Reactions. Figure 3 shows the temperature dependence of furanose anomeric carbon and acyclic carbonyl carbon resonances between 17° and 45 °C at pH 8.4. Although not shown in the figure, the β-pyranose resonances have line widths less than 2 Hz at all temperatures examined. The resonance broadening effects observed with increasing temperature at pH 8.4 are similar to temperature-induced changes observed in spectra of D-[U-^{13}C]fructose-1,6-biphosphate at pH 7.2.[11] Apparently the intramolecular catalytic effect resulting from phosphate abstraction of the anomeric proton on D-fructose-1,6-biphosphate is large enough at neutral pH so as to produce ring-opening rates nearly equivalent to those seen in

(31) Isbell, H. S.; Wade, C. W. R. *J. Res. Natl. Bur. Stand.* **1967**, *71A*, 137–148.

Table I. Thermodynamic Parameters for Isomerization Equilibria of D-Fructose, D-Threose, and D-Galactose[a]

equilibria	$\Delta H°$, kcal	$\Delta G°_{298}$, kcal	$\Delta S°_{298}$, cal/(mol·K)	$\Delta H°/\Delta G°_{298}$
β-fructopyranose ⇌ acyclic keto	10.4 ± 0.5	3.0 ± 0.2	24.8 ± 2.4	3.5 ± 0.4
α-fructofuranose ⇌ acyclic keto	4.2 ± 1.0	1.4 ± 0.2	9.5 ± 4.5	3.5 ± 1.7
β-fructofuranose ⇌ acyclic keto	6.3 ± 1.1	2.3 ± 0.3	13.5 ± 4.5	2.9 ± 0.8
β-fructofuranose ⇌ α-fructofuranose	2.0 ± 0.9	0.8 ± 0.1	4.1 ± 3.3	2.7 ± 1.4
α-fructofuranose ⇌ β-fructopyranose	−4.6 ± 0.5	−1.5 ± 0.2	−10.4 ± 2.4	3.1 ± 0.8
β-fructofuranose ⇌ β-fructopyranose	−3.2 ± 0.9	−0.7 ± 0.1	−8.4 ± 3.4	4.8 ± 2.0
α-threofuranose ⇌ acyclic keto	6.0 ± 1.2	2.4 ± 0.2	13 ± 5	2.6 ± 0.7
β-threofuranose ⇌ acyclic keto	6.0 ± 1.2	2.2 ± 0.3	13 ± 5	2.9 ± 0.9
α-galactopyranose ⇌ β-galactopyranose	−0.11	−0.39	0.9	0.3
β-galactofuranose ⇌ α-galactopyranose	−7.39	−1.26	−20.6	5.9
α-galactofuranose ⇌ α-galactopyranose	−7.16	−1.70	−18.3	4.2
α-2-deoxy-ribofuranose ⇌ β-2-deoxy-ribopyranose	−1.87	−0.73	−3.9	2.6
β-2-deoxyribofuranose ⇌ β-2-deoxyribopyranose	−4.30	−0.86	−11.7	5.0
β-2-deoxyribofuranose ⇌ α-2-deoxyribofuranose	2.30	0.13	8.0	17.7

[a] Parameters for D-fructose interconversions were calculated from the temperature dependence of equilibrium constants (Figures 5 and 6). Parameters for D-galactose, 2-deoxy-D-ribose, and D-threose were taken from ref 9, 10, and 12, respectively.

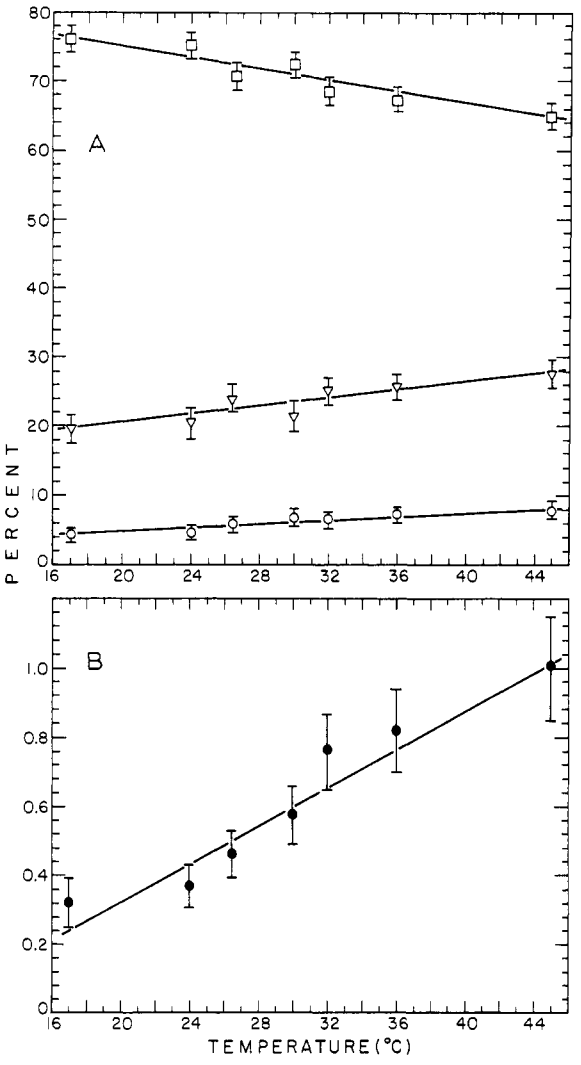


Figure 4. Percent composition of β-D-fructopyranose (□), β-D-fructofuranose (▽), α-D-fructofuranose (○), and the acyclic keto form of D-fructose (●) as a function of temperature at pH 8.4. Composition was determined by integration of anomeric and carbonyl carbon resonances in ^{13}C NMR spectra of 1.5 M D-[2-^{13}C]fructose. Conditions for spectral acquisition are described in the text.

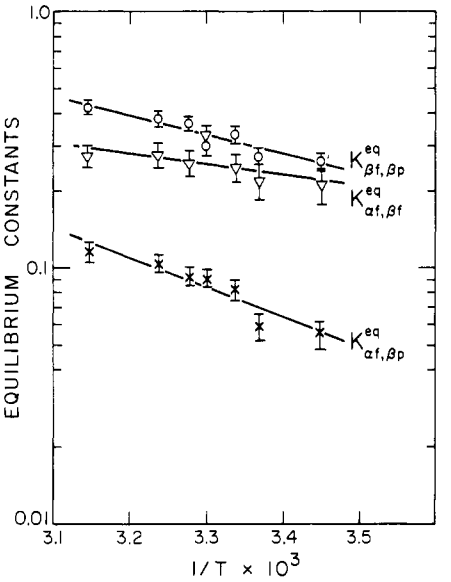


Figure 5. Equilibrium constants describing isomerization of cyclic forms of D-fructose at pH 8.4 as a function of inverse temperature (K). Equilibrium constants were determined from compositions shown in Figure 4. As defined in the figure, $K^{eq}_{\beta f,\beta p} = (\beta f)/(\beta p)$, $K^{eq}_{\alpha f,\beta f} = (\alpha f)/(\beta f)$, and $K^{eq}_{\alpha f,\beta p} = (\alpha f)/(\beta p)$, where αf, βf, and βp represent the α-furanose, β-furanose, and β-pyranose forms of D-fructose. Lines in the figure represent linear least-squares fits to experimental data points.

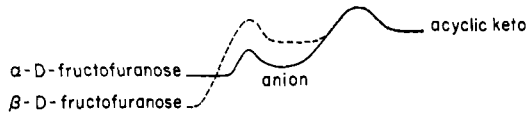
D-fructose only under alkaline conditions.[11,12b,14]

In addition to the broadening of some of the D-fructose resonances observed with increasing temperature, there are also progressive changes in resonance intensities. Data obtained by digital integration of anomeric carbon and acyclic keto carbonyl resonances at pH 8 are displayed in Figure 4. Over the limited range of temperatures shown, the percent composition of D-fructose forms appears to vary linearly. Such linear variation of isomeric composition with temperature was also found for D-fructose and 2-deoxy-D-ribose at neutral pH.[10,26] A calculated linear least-squares fit to the experimental data yields slopes of −0.43%/°C, 0.30%/°C, 0.13%/°C, and 0.03%/°C for the β-pyranose, β-furanose, α-furanose, and the acyclic keto forms of D-fructose. The fractions of D-fructose ring forms predicted from Figure 4A are in good agreement with corresponding values previously reported for the sugar in neutral aqueous solution at temperatures between 20 and 36 °C.[26,27,32] However, the percent of acyclic keto form extrapolated from Figure 4B at 20 °C (0.3%) is only half that estimated by fitting far-UV circular dichroism spectra of β-fructose to carbonyl dichroic extinction coefficients derived from model compounds.[33] Although not specified, it is possible that the larger fraction of fully extended form estimated by this latter method was obtained from slightly acidic D-fructose solutions where the fraction of acyclic keto form estimated from ^{13}C NMR spectra is nearly 1.5 times that reported at pH 8.4 (0.8 ± 0.03% at pH 2.0, 30 °C, compared to 0.58 ± 0.08% at pH 8.4, 30 °C).

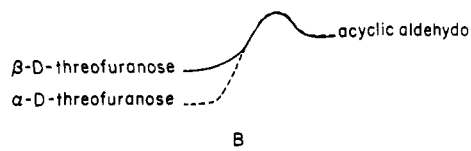
Figures 5 and 6 show plots of the inverse temperature dependence of equilibrium constants calculated from isomeric compo-

(32) Hyvonen, L.; Varo, P.; Kowistoinen, P. *J. Food Sci.* **1979**, *42*, 657–659.
(33) Hayard, L. D.; Angyal, S. J. *Carbohydr. Res.* **1977**, *53*, 13–20.

Scheme II



A



B

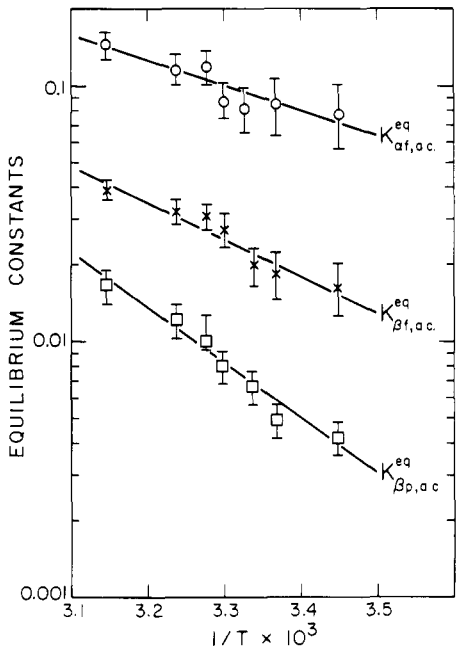


Figure 6. Equilibrium constants describing isomerization of D-fructose at pH 8.4 as a function of inverse temperature (K). Equilibrium constants were determined from compositions shown in Figure 4. Equilibrium constants are $K^{eq}_{\alpha f,a.c.}$ = (a.c.)/(αf), $K^{eq}_{\beta f,a.c.}$ = (a.c.)/(βf), and $K^{eq}_{\beta p,a.c.}$ = (a.c.)/(βp), where a.c., βf, and βp represent the acyclic keto form, α-furanose, β-furanose, and β-pyranose forms of D-fructose. Lines in the figures represent linear least-squares fits to experimental data points.

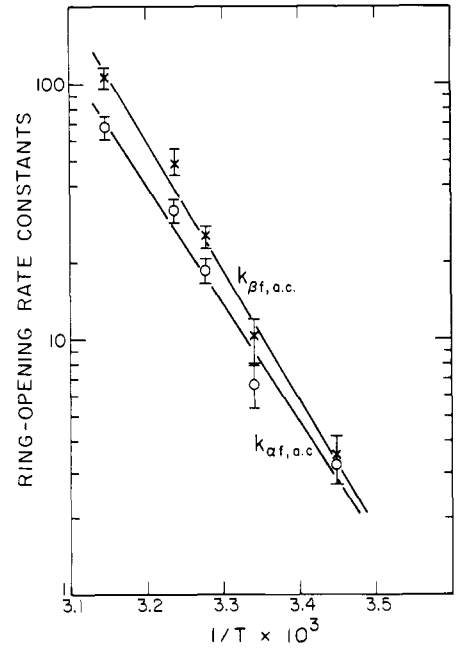


Figure 7. Inverse temperature (K) dependence of apparent first-order ring-opening rate constants determined by simultaneously fitting anomeric and carbonyl carbon NMR resonance line widths to eq 4. Experimental data were taken from ^{13}C NMR spectra of 1.5 M D-[2-^{13}C]-fructose at pH 8.4. Lines drawn through the data represent linear least-squares fits.

sitions at pH 8.4. Thermodynamic parameters calculated from these plots for are summarized in Table I along with similar data taken from the literature for D-threose, 2-deoxy-D-ribose, and D-galactose. Noteworthy is the fact that the overall free energy change at 25 °C is determined both in sign and magnitude by the enthalpy change for all examples shown. This trend also turns out to hold true for nearly all sugars for which isomeric equilibria have been studied.[7] The ratios $\Delta H°/\Delta G°_{298}$ (column 5) further illustrate the correlation between free energy and enthalpy. Particularly in the case of equilibria shown for D-fructose and D-threose, the $\Delta H°/\Delta G°_{298}$ ratio is nearly constant, having a mean value of 3.2 ± 1.2. As illustrated by the data listed for D-galactose and 2-deoxy-D-ribose, there is a greater deviation of the $\Delta H°/\Delta G°_{298}$ ratio away from this mean for isomeric equilibria of other sugars, particularly if those equilibria involve furanose–furanose or pyranose–pyranose interconversions.

It is tempting to interpret the entropy changes listed in Table I in terms of changes in conformational flexibility, ignoring effects of the solvent. For example, $\Delta S°_{298}$ terms for pyranose or furanose–acyclic keto equilibria are typically large and positive, in agreement with the greater flexibility expected for the acyclic or fully extended form of the sugar, while $\Delta S°_{298}$ terms for furanose–pyranose interconversions are large and negative, probably as a result of the loss in flexibility of the C5 or C6 methoxy group upon pyranose ring formation. Conversely, $\Delta S°_{298}$ terms for equilibria involving only pyranoses or furanoses are relatively small in magnitude, in agreement with structural similarities between the ring forms undergoing conversion.

Figure 7 shows the inverse temperature dependence of D-fructofuranose ring-opening rate constants at pH 8.4 derived by fitting anomeric carbon resonance line widths to eq 4. At all temperatures, ring-opening from the β-furanose form is kinetically more favorable than ring-opening from the α-furanose form of the sugar. It is interesting to note that for all sugars for which unidirectional rate data are availble, ring-opening rates for furanose forms having an anomeric hydroxyl cis to the hydroxyl on the neighboring carbon are greater than corresponding rates for furanose forms having the same two hydroxyls trans to one another, irrespective of whether the study was carried out in acid or alkaline aqueous media.[9-12,16] Base-catalyzed ring-opening involves an initial rapid equilibrium between the sugar and the monovalent anion found by deprotonation of the anomeric hydroxyl, followed by a rate-determining step involving the protonation of the ring oxygen and electron redistribution.[7,34] A plausible explanation as to why furanose forms having cis hydroxyls are kinetically unstable might be attributed to the unfavorable electrostatic interaction between the anomeric hydroxyl anion and the neighboring electronegative hydroxyl. This added electrostatic instability would then result in a lower activation energy relative to that predicted for the furanose anion having trans hydroxyls (Scheme II). A similar explanation is less definitive in the case of acid-catalyzed furanose ring-opening reactions. However, in the case of the sugars studied under acidic conditions (D-galactose, 2-deoxy-D-ribose, and D-threose), the cis hydroxyl furanose form is thermodynamically least stable.[9,10,12] It is quite possible that this form remains relatively unstable up to a time just prior to the activated complex formation, resulting in a lower activation energy.

Thermodynamic activation parameters were calculated from the data of Figure 7 with transition-state theory.[35] $\Delta H^{\ddagger}$, $\Delta G^{\ddagger}_{298}$, and $\Delta S^{\ddagger}_{298}$ for ring-opening of α-fructofuranose were 21.0 ± 2.0

(34) DeWitt, G.; Kieboom, A. P. G.; Van Bekkum, H. *Carbohydr. Res.* **1984**, *127*, 193–200.

(35) Moore, W. J. "Physical Chemistry", 3rd ed.; Prentice-Hall, Inc.; Englewood Cliffs, NJ, 1962; Chapter 8.

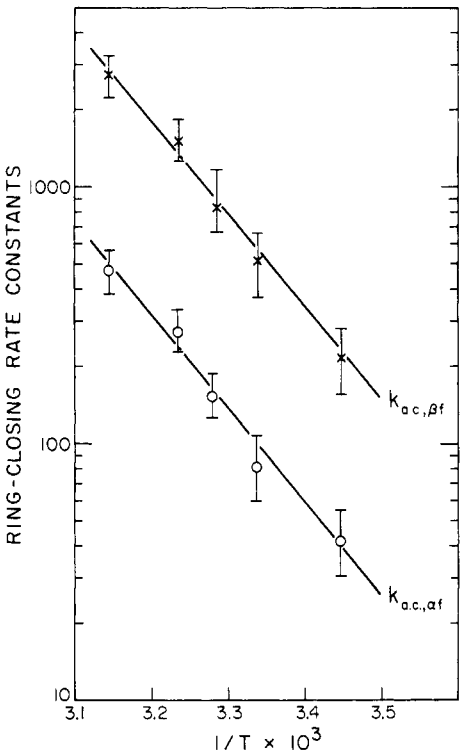


Figure 8. The inverse temperature (K) dependence of apparent first-order ring-closing rate constants determined from ring-opening rate constants (Figure 7) and anomer–acyclic keto equilibrium constants (Figure 6). Lines through the data represent linear least-squares fits.

kcal/mol, 16.1 ± 0.2 kal/mol, and 16.4 ± 7.4 cal/(mol·K). Corresponding values for ring-opening of β-fructofuranose were 23.0 ± 2.5 kcal/mol, 16.0 ± 0.2 kcal/mol, and 23.5 ± 9.0 cal/(mol·K). Because ring-opening is the rate-limiting step, values of activation parameters calculated from D-fructose ring-opening rates may be compared to similar data for other sugars calculated from cyclic isomer interconversion rates. Such a comparison shows that base-catalyzed D-fructofuranose ring-opening is one of the few examples for which $\Delta S^{\ddagger}_{298}$ is positive.[7] This difference may arise as a result of a much lower entropy of the furanose forms of D-fructose or as a result of the much greater entropy of the activated complex. The negative $\Delta S^{\ddagger}_{298}$ calculated from rate data on other sugars under neutral or acidic conditions has been attributed to organization of solvent around the protonated form of the activated complex.[7,12] If this is indeed the case, then it is equally likely that in alkaline solution, the stable anionic forms of D-fructofuranose organize solvent to the extent of making the contribution of the solvent to $\Delta S^{\ddagger}_{298}$ negligible. The positive $\Delta S^{\ddagger}_{298}$ calculated for the base-catalyzed ring-opening of D-fructofuranoses would then support increased flexibility of the activated complex. The fact that ΔS°_{298} values found for converting D-fructofuranose anomers to the acyclic keto form (Table I) are within experimental error of respective $\Delta S^{\ddagger}_{298}$ values, supports the resemblance of activated complex to the stable acyclic form.

From the rate data for ring-opening and the inverse temperature dependence of equilibrium constants, rate data for ring-closing can be calculated. These data are shown in the form of an Arrenhius plot in Figure 8. Activation parameters determined from NMR data are particularly subject to both statistical and systematic errors,[36] and these errors are compounded in the calculation of ring-closing rates due to the error associated with the measured equilibrium constants. From the data of Figure 8, $\Delta H^{\ddagger} \simeq \Delta G^{\ddagger}_{298} \simeq 16$ kcal/mol and $\Delta S^{\ddagger}_{298} \simeq 0$ for ring-closing to either of the two furanose anomers. At all temperatures studied, the ring-closing rate to β-fructofuranose is roughly five times that of the corresponding rate to the α-furanose anomer. Ring-closing rates to either of the furanose forms are roughly ten times the respective ring-opening rates.

(36) Martin, M. L.; Delpulch, J.-J.; Martin, G.-J. "Practical NMR Spectroscopy"; Heyden: Philadelphia, PA, 1980; pp 344–345.

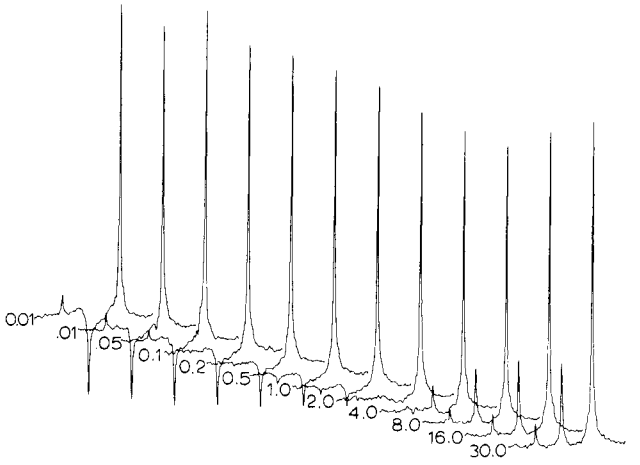


Figure 9. Inversion–transfer ^{13}C NMR spectra of the anomeric carbon region of 0.688 M D-[2-^{13}C]fructose at pH 8.4 and 27 °C. From the left side of the spectra, anomeric carbon resonances have been assigned to the α-furanose, β-furanose, and β-pyranose forms of the sugar. τ values to the left of each spectrum are equivalent to the wait time (in seconds) between the end of the pulse sequence used in inverting the β-furanose anomeric carbon resonance and the start of spectral acquisition.

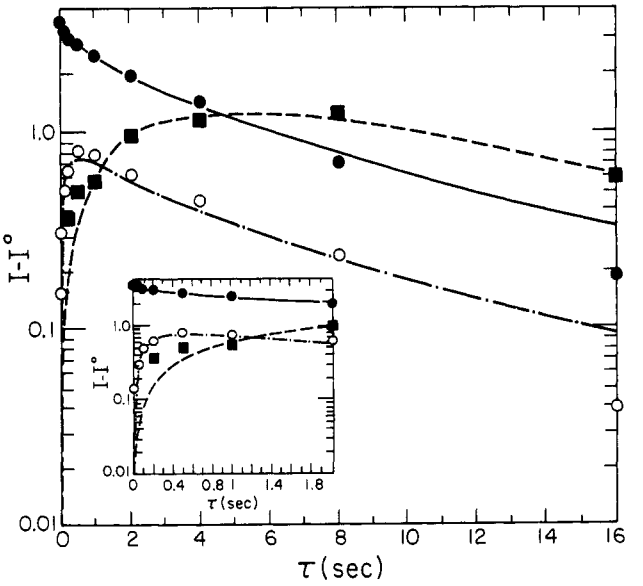


Figure 10. The difference between momentary and equilibrium peak intensities as a function of τ for the inversion–transfer experiment (Figure 9). Data points were taken from peak heights of anomeric carbon resonances of the α-furanose (○), β-furanose (●), and β-pyranose (■) forms of D-fructose at pH 8.4 and 27 °C. Lines drawn through the data points are non-linear least-squares best fits to eq 1. Parameters obtained from the fitting are summarized in Table II.

Cyclic Isomerization Rates Determined by NMR Inversion Transfer. In order to more fully describe the base-catalyzed isomerizations of D-fructose in terms of microscopic rate constants, an estimate of ring-opening and ring-closing rates from the β-pyranose form is needed. Since no detectible line broadening was observed in the β-pyranose anomeric carbon resonances even near pH 10, alternative methods must be utilized to estimate ring-opening rates. Figure 9 shows a sequence of ^{13}C NMR spectra generated by selectively inverting the magnetization of the β-furanose anomeric resonance. Spectra were acquired at 27 °C and pH 8.4. Values for each spectra correspond to wait times in seconds between the inverting pulse sequence and data acquisition. Inverted magnetization is transferred to the α-furanose and β-pyranose forms of D-fructose at rates equal to respective isomeric interconversion rates.[18] The difference between equilibrium intensities and intensities following inversion are shown

Table II. Rate and Relaxation Parameters Obtained Fitting Inversion–Transfer Data on Anomeric Resonances of D-Fructose at pH 8.4 (27 °C)

experiment[a]	$k_{\beta f,\alpha f}$	$k_{\beta p,\beta f}$	$k_{\beta p,\alpha f}$	$R_{\alpha f}$	$R_{\beta f}$	$R_{\beta p}$
invert $M_{\alpha f}$	1.9	$<10^{-2}$	<0.1	5.2	2.8	0.3
invert $M_{\beta f}$	1.7	<0.1	$<10^{-2}$	6.1	2.1	0.2
invert $M_{\beta p}$	1.8	$<10^{-2}$	<0.1	4.7	3.2	0.2

[a] $M_{\alpha f}$, $M_{\beta f}$, $M_{\beta p}$ are magnetizations associated with the anomeric carbon of the α-furanose, β-furanose, and β-pyranose forms of D-fructose.

in Figure 10. Rate parameters describing variations of resonance intensities were determined by fitting experimental data to eq 1–3 (as described in the Experimental Section) and are summarized in Table II. Considering the complexity of the fitting procedure, values for $k_{\beta f,\alpha f}$, $R_{\alpha f}$, $R_{\beta f}$, and $R_{\beta p}$ are remarkably consistent, irrespective of which type of inversion experiment is carried out. The mean value of 1.8 for $k_{\beta f,\alpha f}$ is also consistent with rate constants determined by resonance line broadenings. For example, using the measured equilibrium constant $K^{eq}_{\beta f,\alpha f}$ at pH 8.4 and 27 °C and $k_{\beta f,\alpha f}$ determined by the inversion–transfer method, one calculates a $k_{\alpha f,\beta f}$ of 7.3 s^{-1}. This value compares favorably with the rate constant for ring-opening of the α-furanose isomer determined from line-broadening measurements ($k_{\alpha f,a.c.}$ = 6.6 ± 1.3 s^{-1} at pH 8.4 and 26.5 °C). The close agreement between $k_{\alpha f,\beta f}$ and $k_{\alpha f,a.c.}$ also indicates that the ring-closing rate to the β-furanose form must be much faster than ring-closing to either the α-furanose or β-pyranose forms, a finding consistent with ring-closing rate constants calculated from line-broadening data and measured equilibrium constants (Figure 7). On the other hand, the rate measured by the inversion–transfer method for conversion of the β-furanose form to the α-furanose form ($k_{\beta f,\alpha f}$) is only 18% of the ring-opening rate from β-furanose to the acyclic form, as determined by line-width measurement ($k_{\beta f,a.c.}$ = 10.1 s^{-1} at pH 8.4 and 26.5 °C). However, this lower value is consistent with the ratio of rates determined from line widths for the conversion of acyclic intermediate to α-furanose and β-furanose forms (80 s^{-1}/500 s^{-1} × 100 = 16%). This result implies that once the β-furanose is converted to the acyclic form, roughly 20% of the acyclic form is converted to α-furanose while 80% returns to β-furanose. A negligible amount is converted to β-pyranose.

Interconversion rate constants listed in Table II from the β-pyranose form ($k_{\beta p,\beta f}$, $k_{\beta p,\alpha f}$) are certainly of less significance than $k_{\beta f,\alpha f}$ values, and they may arise as artifacts of the complex fitting routine. However, the magnitude of the values is certainly consistent with very slow ring-opening rates from the β-pyranose form and somewhat larger ring-closing rates to the β-pyranose form. For example, if 0.1 s^{-1} is taken as an upper limit for the β-pyranose–β-furanose conversion rate, then $k_{\beta p,a.c.}$ is also on the order of 0.1 s^{-1} and $k_{a.c.,\beta p}$ is less than 20 s^{-1}, a rate much slower than ring-closing to either the β-furanose or α-furanose forms (500 and 80 s^{-1}, respectively). Hence, dynamically only about 3% of the acyclic keto form is converted to the β-pyranose form. However, this form dominates the isomeric equilibrium due to its much smaller ring-opening rate.

Acknowledgment. The author acknowledges support of this research by the Robert A. Welch Grant AT-885 and by Associated Western Universities, Inc. Initial samples of D-[2-^{13}C]fructose were provided by the National Stable Isotope Resource facility at Los Alamos National Laboratories. This facility is supported by National Institutes of Health Grant 1P41 RR02231-01. The author also thanks Drs. Robert London and Clifford J. Unkefer for their helpful suggestions.

Registry No. D-Fructose, 57-48-7; β-fractopyranose, 7660-25-5; α-fructofuranose, 10489-79-9; β-fructofuranose, 470-23-5.

Single Transition State for Sulfuryl Group ($-SO_3^-$) Transfer between Pyridine Nucleophiles

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Contribution from the University Chemical Laboratories, Canterbury, England CT2 7NH.
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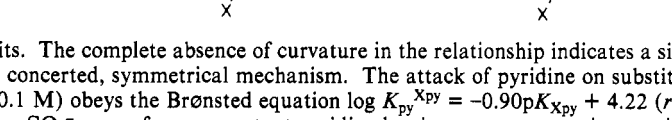
Abstract: The second order rate constants for the nucleophilic attack of pyridines on isoquinoline-*N*-sulfonate (25 °C, 0.1 M ionic strength) obey an excellent linear relation, log $k_{X_{py}}^{isq}$ = 0.23p$K_{X_{py}}$ − 1.91 (r = 0.995), with the pK of the attacking



pyridine over eight pK units. The complete absence of curvature in the relationship indicates a single transition state for the reaction consistent with a concerted, symmetrical mechanism. The attack of pyridine on substituted pyridine-*N*-sulfonates (25 °C, ionic strength at 0.1 M) obeys the Brønsted equation log $K_{py}^{X_{py}}$ = −0.90p$K_{X_{py}}$ + 4.22 (r = 0.998). The β_{EQ} for the equilibrium transfer of the $-SO_3^-$ group from a constant pyridine leaving group to a variant pyridine nucleophile (+1.13) is close to that predicted from a previous study. The electronic structure of the transition state possesses considerable sulfur trioxide character as deduced from the changes in effective charge on the entering and leaving pyridine nitrogen atoms.

There is much interest in the existence of preassociation stepwise mechanisms where an intermediate is not stable enough to diffuse outside an encounter complex containing a product-yielding reagent molecule.[1] Such processes have been considered in carbonyl addition,[2] $-PO_3^{2-}$ group transfer,[3,4] ligand exchange, and aromatic electrophilic substitution;[4] the following scheme (eq 1) summarizes the possibilities for a nucleophilic substitution reaction

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