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Anomerization of Furanose Sugars: Kinetics of Ring-Opening Reactions by ^1H and ^{13}C Saturation-Transfer NMR Spectroscopy

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Abstract: With the tetroses D-threose and D-erythrose, kinetic and thermodynamic parameters for the interconversion of α- and β-furanoses and the acyclic hydrate with the intermediate aldehyde form have been obtained from ^1H and ^{13}C NMR measurements. Unidirectional rate constants for the various equilibria involving the aldehyde have been determined, and from them the overall rate constants for interconversion of the abundant species. The approach can be applied to systems in which only solutions at tautomeric equilibrium are available or in which interconversions are too rapid to observe by other methods. Analyses by ^{13}C NMR were facilitated by the use of [1-^{13}C]tetroses. Aqueous solutions of the tetroses were examined at 17–81 °C by ^{13}C and ^1H NMR spectroscopy to evaluate tautomeric composition. Solutions of D-threose contain ~51% α-furanose and ~38% β-furanose at all temperatures, while the amount of aldehyde (a) increases from 0.7 to 4.7% and the amount of hydrate (h) decreases from 10.2 to 7.1%. $\Delta G°$ (kcal mol^{-1}), $\Delta H°$ (kcal mol^{-1}), and $\Delta S°$ (cal mol^{-1} K^{-1}) at 25 °C were estimated for the reaction a → α, −2.4, −6.0, and −12; a → β, −2.2, −6.0, −13; and a → h, −1.4, −7.3, −20, respectively. Rate constants for ring opening of the tetroses in ^2H_2O were obtained by ^1H and ^{13}C saturation-transfer NMR spectroscopy by saturating H-1 or C-1 of the aldehyde, respectively, and observing the transfer of saturation to the α- and β-furanose and hydrate resonances. Rate constants (±10%) for the ring-opening reactions of D-threose (unbuffered) are as follows: α-furanose, 0.034 s^{-1} (51 °C), 0.10 s^{-1} (66 °C); β-furanose, 0.083 s^{-1} (38 °C), 0.53 s^{-1} (66 °C). Ring-closing rate constants (±18%), determined from equilibrium constants, and ring-opening rate constants are as follows: α-furanose, 0.74 s^{-1} (51 °C), 1.5 s^{-1} (66 °C); β-furanose, 2.0 s^{-1} (38 °C), 5.9 s^{-1} (66 °C). Activation energies were estimated for β-threofuranose ring-opening (14 ± 3 kcal mol^{-1}) and ring-closing (8 ± 2 kcal mol^{-1}) reactions. From the unidirectional rate constants, overall rate constants at 66 °C for the conversion of α- and β-threofuranose were calculated as follows: $k_{\alpha\beta} = 0.08 \pm 0.01$ s^{-1}, $k_{\beta\alpha} = 0.11 \pm 0.01$ s^{-1}. Rate constants for ring-opening of D-threose and D-erythrose in 50 mM sodium acetate (p^{2}H 5.0) were compared. At 55 °C, α-erythrose (0.40 s^{-1}) > β-threose (0.36 s^{-1}) > β-erythrose (0.19 s^{-1}) > α-threose (0.11 s^{-1}). Rate constants obtained by ^{13}C and ^1H ST NMR spectroscopy were in good agreement.

Many biologically important compounds are capable of existing in solution in several tautomeric forms. The chemistry of these compounds is complicated by this structural diversity, since the rates of their reactions may be determined by the concentration of one or more reactive tautomers rather than the total concentration of the compound. In these cases knowledge of the tautomeric composition of the solution, of which tautomer(s) reacts, and of the rate(s) at which the reactive tautomer forms from other tautomers is essential to understanding the chemistry and biochemistry of the system. The monosaccharides, in aqueous solution, are such a system. Depending on the specific sugar, the interconverting tautomeric forms may include cyclic furanoses, pyranoses, and septanoses and linear hydrates (*gem*-diols), aldehydes, and/or ketones.[2] In some cases, dimers and/or oligomers may be present.[3] It has been assumed that the interconversion of aldose tautomers proceeds through the linear aldehyde, which for most simple pentoses and hexoses accounts for much less than 1% of the forms present. In most chemical and biochemical reactions, the sole or major pathway of reaction of monosaccharides involves only one of the tautomeric forms present in solution.[4] Thus, knowledge of the rate constants of interconversion of tautomers is key to understanding the chemical and biochemical

(1) (a) Section of Biochemistry, Molecules and Cell Biology, Cornell University. (b) Department of Chemistry, Cornell University. (c) Michigan State University.

(2) (a) Angyal, S. J. *Angew. Chem. Int. Ed. Engl.* **1969**, *8*, 157–166. (b) Grindley, T. B.; Vijeyalaksmi, G. *J. Chem. Soc., Chem. Commun.* **1978**, 1073–1074. (c) Angyal, S. J.; Pickles, V. A. *Aust. J. Chem.* **1972**, *25*, 1695–1710. (d) Angyal, S. J.; Bethell, G. S. *Ibid.* **1976**, *29*, 1249–1265. (e) Bell, R. P.; McDougal, A. O. *Trans. Faraday Soc.* **1960**, *56*, 1281–1285. Blackmore, P. F.; Williams, J. F.; MacLeod, J. K. *FEBS Lett.* **1976**, *64*, 222–226.

(3) (a) Collins, G.; George, W. O. *J. Chem. Soc. B* **1971**, 1352–1355. (b) Beck, W. S. *Methods Enzymol.* **1957**, *3*, 205. (c) Gardiner, D. *Carbohydr. Res.* **1966**, *2*, 234–239.

(4) Benkovic, S. J.; Schray, K. J. *Adv. Enzymol.* **1976**, *44*, 139–164.

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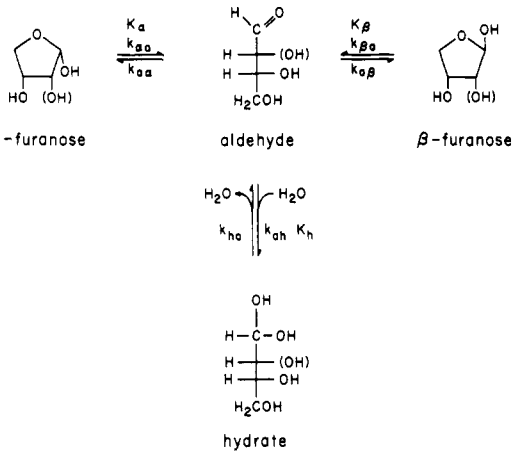
Scheme I



reactivities of monosaccharides, particularly those reactions that involve the free aldehyde and ketone forms present in very small amounts.

Although measurements of overall rate constants for the interconversion of cyclic forms have been numerous,[5] measurements of the rate constants for interconversion of linear carbonyl and cyclic forms have been limited to D-glucose[6a] and D-[UL-^{13}C]-fructose 1,6-bisphosphate (FDP).[6b] Polarographic techniques were used to estimate the unidirectional rate constants for D-glucose, and from these, the amount of free aldehyde (0.0026%) in solution was calculated. ^{13}C NMR line-broadening studies and the determination of equilibrium concentrations in solutions of D-[U-L-^{13}C]FDP allowed estimation of ring-opening and -closing rate constants. Ring-opening and -closing rate constants have been determined recently for thio sugars,[6c] and a computer simulation technique has been used to obtain similar rate constants for the furanose and pyranose forms of D-galactose.[6d]

During studies of ^{13}C NMR line widths in solutions of D-[1-^{13}C]tetroses and pentose 5-phosphates,[7] we found that solutions of these sugars contain, in addition to furanoses, measureable quantities of linear hydrates and aldehydes.[8] Although anomerization was too slow (<1 s^{-1}) at pH ~5 to obtain reliable ring-opening rate constants by ^{13}C line-broadening techniques, it appeared that saturation-transfer NMR (ST NMR) spectroscopy could be applied to these systems.

Continuous-wave ^{1}H ST NMR was first used by Forsén and Hoffman[9a-c] to study hydroxyl-proton exchange. FT NMR methods for measuring slow exchange rates have been evaluated by Campbell et al.[9d] Rate constants in the range 10^{-2}–10^{1} s^{-1} can be estimated by these methods.[10] The technique has been placed on firm theoretical ground,[9a] and both ^{1}H and ^{13}C ST NMR have been exploited in several chemical[11] and biochemical[12] studies.

In this report we describe the application of Fourier transform ^{1}H and ^{13}C ST NMR spectroscopy to the study of the tautomerization of the tetroses in ^2H_2O (Scheme I). These compounds are appropriate models for biologically important furanoses such as exist in pentoses and ketohexoses and their phosphate esters. The four interconverting forms in Scheme I can be observed by ^{1}H and/or ^{13}C NMR spectroscopy. The interconversion of these forms involves three, two-component equilibria, and the ring-opening ($k_{\alpha a}$, $k_{\beta a}$) and dehydration (k_{ha}) rate constants can be obtained by saturation-transfer NMR spectroscopy. From the equilibrium constants (K_α, K_β, K_h) and the rate constants ($k_{\alpha a}$, $k_{\beta a}$, k_{ha}), ring-closing ($k_{a\alpha}$, $k_{a\beta}$) and hydration (k_{ah}) rate constants can be calculated. Thus, unidirectional rate constants and thermodynamic parameters for the tautomerization of the tetroses can be obtained by NMR methods.

Experimental Section

Compounds. D-Erythrose and D-threose were prepared from 2,4-*O*-ethylidene-D-erythrose[13] and 2,4-*O*-ethylidene-D-threose,[14] respectively, and were greater than 95% pure by ^{1}H and ^{13}C NMR spectroscopy. D-[1-^{13}C]Erythrose and threose were prepared from K^{13}CN and D-glyceraldehyde as described previously.[8a] D-Glyceraldehyde was prepared from D-fructose by treatment with lead tetraacetate.[15]

^2H_2O (99.8 atom %) was purchased from Stohler Isotope Chemicals. $C^2H_3COO^2H$ (99.5 atom %) was purchased from Merck and Co., Inc., and NaO^2H (30%, 99 atom %) from Aldrich Chemical Co. Dowex 1-X8 (200–400 mesh) and Dowex 50-X8 (20–50 mesh) were purchased from Sigma Chemical Co. and converted to the acetate and hydrogen forms, respectively. Chelex 100 (100–200 mesh) was purchased from Sigma Chemical Co., activated,[16] and used in the hydrogen form.

Instrumentation. Proton-decoupled ^{13}C NMR spectra (75 MHz) used to evaluate equilibrium constants were obtained with a Bruker WM-300 FT superconducting spectrometer. ^{1}H NMR spectra (80 MHz) were obtained with a Varian CFT-20 FT spectrometer.

^{13}C ST NMR spectra at 100 MHz were obtained on a Bruker WH-400 superconducting FT spectrometer at the South Carolina Magnetic Resonance Laboratory of the University of South Carolina. Selective saturation was achieved by feeding the ^{13}C saturating radio frequency into the observe coils of the probe. ^{13}C NMR spectra were obtained with broad-band ^{1}H decoupling, and temperature was regulated to ±1 °C.

Double irradiation of ^{13}C resonances was achieved on a Bruker WM-300 superconducting FT spectrometer with an audio frequency to modulate the ^{13}C decoupler rf to produce two side bands with frequencies identical with the two resonances to be saturated. A Hewlett-Packard HP 4204A audio frequency synthesizer and a Programmed Test Sources PTS 160 radio frequency synthesizer were used. The modulated side bands were gated by the computer and amplified by an AV-4T power amplifier from Avantek to an output of ~0.5 W, sufficient for full saturation of the resonances.

^{1}H ST NMR spectra at 80 MHz were obtained on a Varian CFT-20 with modified software to vary saturation times (τ). Saturating power was measured with a Model 4370 RF wattmeter from Bird Electronic Corp. ^{1}H ST NMR spectra at 360 MHz were obtained with a Bruker WH 360/180 superconducting FT spectrometer at the Middle Atlantic NMR Research Facility at the University of Pennsylvania. Selective saturation was performed with standard Bruker instrumentation, and temperature for ^{1}H spectra was regulated to ±1 °C.

A microelectrode supplied by Microelectrodes, Inc., was used for p^{2}H measurements at 23 °C. Temperature was measured with a Fluke 2190A digital thermometer and a copper–constantan thermocouple.

Solution Preparation and Quantitation and Temperature Determination. Solutions of the tetroses were deionized by separate batchwise treatment with excess Dowex 1-X8 (200–400 mesh, acetate) and Dowex 50-X8 (20–50 mesh, hydrogen). Solutions were concentrated to ~0.1 mL at 30 °C in vacuo and evaporated several times from 1 to 2 mL of ^2H_2O. For unbuffered solutions, the residue was dissolved in 0.6 mL of

(5) (a) Pigman, W.; Isbell, H. S. *Adv. Carbohydr. Chem. Biochem.* **1968**, *23*, 11–57. (b) Isbell, H. S.; Pigman, W. *Ibid.* **1969**, *24*, 13–65. (c) Livingstone, G.; Franks, F.; Aspinall, L. J. *J. Solution Chem.* **1977**, *6*, 203–216. Capon, B.; Walker, R. B. *J. Chem. Soc., Perkins Trans.* **1974**, *2*, 1600–1610.

(6) (a) Los, J. M.; Simpson, L. B.; Wiesner, K. *J. Am. Chem. Soc.* **1956**, *78*, 1564–1568. (b) Midelfort, C. F.; Gupta, R. K.; Rose, I. A. *Biochemistry* **1976**, *15*, 2178–2185. (c) Grimshaw, C. E.; Whistler, R. L.; Cleland, W. W. *J. Am. Chem. Soc.* **1979**, *101*, 1521–1532. (d) Wertz, P. W.; Garver, J. C.; Anderson, L. *Ibid.* **1981**, *103*, 3916–3922.

(7) Pierce, J.; Serianni, A. S.; Barker, R., unpublished results.

(8) (a) Serianni, A. S.; Clark, E. L.; Barker, R. *Carbohydr. Res.* **1979**, *72*, 79–91. (b) Serianni, A. S.; Pierce, J.; Barker, R. *Biochemistry* **1979**, *18*, 1192–1199. (c) Serianni, A. S. Ph.D. Dissertation, 1980, Michigan State University, 195–198. (d) Serianni, A. S.; Nunez, H. A.; Pierce, J.; Rosevear, P. R.; Clark, E. L.; Barker, R. 28th Annual Conference on Mass Spectrometry and Allied Topics, American Society for Mass Spectrometry, New York, May 25–30, 1980; Abstr. RAMP10.

(9) (a) Forsén, S.; Hoffman, R. A. *J. Chem. Phys.* **1963**, *39*, 2892–2901. (b) Forsén, S.; Hoffman, R. A. *Acta Chem. Scand.* **1963**, *17*, 1787–1788. (c) Forsén, S.; Hoffman, R. A. *J. Chem. Phys.* **1964**, *40*, 1189–1196. (d) Campbell, I. D.; Dobson, C. M.; Ratcliffe, R. G.; Williams, R. J. P. *J. Magn. Reson.* **1978**, *29*, 397–417.

(10) Mann, B. E. *Prog. Nucl. Magn. Reson. Spectrosc.* **1977**, *11*, 95–114.

(11) (a) Mann, B. E. *J. Magn. Reson.* **1976**, *21*, 17–23. (b) *Ibid.* **1977**, *25*, 91–94.

(12) (a) Cheshnovsky, D.; Navon, G. *Biochemistry* **1980**, *19*, 1866–1873. (b) Clark, P. I.; Lowe, G.; Nurse, D. *J. Chem. Soc., Chem. Commun.* **1977**, 451–453. (c) Lowe, G.; Nurse, D. *Ibid.* **1977**, 815–817.

(13) Perlin, A. S. *Methods Carbohydr. Chem.* **1962**, *1*, 64–66.

(14) Ball, D. H. *J. Org. Chem.* **1966**, *31*, 220–223.

(15) Perlin, A. S. *Methods Carbohydr. Chem.* **1962**, *1*, 61–63.

(16) Willard, J. M.; Davis, J. J.; Wood, H. G. *Biochemistry* **1969**, *8*, 3137–3144.

^2H_2O, passed through a small column (0.5 cm × 1.5 cm) of Chelex 100 (H^+), and collected in a 5-mm NMR tube. The solution was flushed with N_2 for 3–5 min, and the tube was sealed with a plastic cap. For experiments at higher temperatures (>40 °C), a Teflon plug was inserted to minimize reflux in the tube.

For buffered solutions, ~4 mL of tetrose solution in H_2O was passed through Chelex 100 (H^+), the solution was concentrated as before to ~0.1 mL, and the residue was evaporated several times from 1 to 2 mL of ^2H_2O previously treated with Chelex 100 (H^+). The residue was dissolved in sodium acetate buffer (50 mM in ^2H_2O, p^{2}H 5.0) and transferred to either a 10-mm (^{13}C) or 5-mm (^1H) NMR tube. The p^{2}H of the solutions was measured, the solution was flushed with N_2 for 3–5 min, and the tube was sealed with a plastic cap. ^{13}C NMR tubes containing ~2 mL of solution were fitted with Teflon vortex plugs.

Concentrations of the tetroses were determined colorimetrically.[17] Temperature of the samples was measured through the use of standards containing unenriched tetroses. Standards in ^2H_2O or acetate buffer solution, as appropriate, were equilibrated in unsealed NMR tubes for 15–20 min in the probe, and the solution temperature was measured periodically in situ with a digital thermometer equipped with a thermocouple. Sample solutions, equilibrated and measured similarly, were found to have the same temperature as the standards.

Determination of Equilibrium Constants for D-Threose. Percentages of the four forms of D-threose in aqueous solution were determined by ^{13}C and ^1H NMR spectroscopy. Three spectra were obtained at each temperature, and peak areas were determined by integration. ^{13}C NMR spectra were obtained in ^2H_2O with D-[1-^{13}C]threose (0.1 M) in 50 mM sodium acetate, p^{2}H 5.0. Spectra of 1-^{13}C-enriched derivatives had excellent S/N ratios, permitting the accurate quantitation of amounts in solution.

Quantitative determination of amounts in solution by ^{13}C NMR spectroscopy can be complicated by differences in nuclear Overhauser enhancement (NOE), spin–lattice relaxation times (T_1), and insufficient digital resolution.[18] NOE effects were found to be negligible in experiments at 100 MHz using gated ^1H decoupling; that is, equilibrium constants measured in the presence and absence of NOE were statistically equivalent. Spin–lattice relaxation time differences between tautomeric forms were accounted for by using long delay times ($5T_1$) between pulses to permit full relaxation. The appropriate delay times were established from T_1 values for C-1 carbons measured by inversion–recovery at the temperatures at which equilibrium constants were to be determined. Digital resolution of all spectra was adequate for accurate quantitation.

As an additional check on the equilibrium constants determined by ^{13}C NMR, ^1H NMR spectra of D-threose (0.3 M) were obtained in ^2H_2O at 53 °C. Three spectra were obtained with 90° pulse angles and 50-s delay times. Peak areas were obtained by integration.

Rate Constant Determinations. 80-MHz ^1H ST NMR Experiments. Three spectra without aldehyde saturation (defined as $M_z(0)$ spectra) were obtained with either the saturating radio frequency off or off-resonance. Ten to twelve spectra with aldehyde saturation (defined as $M_z(\tau)$ spectra) were then obtained with τ values from 0.7 to 30 s. At least two spectra with $\tau > 20$ s (defined as $M_z(\infty)$ spectra) were obtained at the end of the experiment.

100-MHz ^{13}C and 360-MHz ^1H ST NMR Experiments. Three $M_z(0)$ spectra were obtained with $\tau = 0.005$ s. Spectra with different τ values (0.1–30 s for ^{13}C, 0.05–55 s for ^1H) were acquired automatically, and several τ values were repeated during each experiment to evaluate variation in instrument response. At least two $M_z(\infty)$ spectra were obtained with $\tau > 20$ s.

Two delay times were used for ST experiments: a hardware delay (0.005 s) between the termination of saturating power and acquisition and a relaxation delay (30 s for ^{13}C, 70 s for ^1H) between the end of acquisition and the initiation of saturating power.

Calculation of Rate Constants. Consider the partial reaction from Scheme I.

$$\underset{(a)}{\text{aldehyde}} \underset{k_{\beta a}}{\overset{k_{a\beta}}{\rightleftharpoons}} \underset{(\beta)}{\beta\text{-furanose}}$$

The Bloch equation, modified to account for chemical exchange and describing the change in intensity (M_z) of the H-1 or C-1 resonance of the β-furanose form as a function of time, t, after the application of a nonselective 90° pulse, is as follows:

$$\frac{dM_z^\beta(t)}{dt} = \frac{-[M_z^\beta(t) - M_z^\beta(0)]}{T_{1\beta}} - k_{\beta a}M_z^\beta(t) + k_{a\beta}M_z^a(t) \quad (1)$$

The saturation-transfer experiment is performed by saturating the aldehyde resonance (M_z^a), which causes $M_z^a \to 0$ and, thus, $k_{a\beta}M_z^a(t) \to 0$. The resulting equation, which contains terms for M_z^β, $T_{1\beta}$, and $k_{\beta a}$, can be integrated to the following form:

$$M_z^\beta(\tau) = M_z^\beta(0)(\tau_{1\beta}/\tau_\beta \exp(-\tau/\tau_{1\beta}) + \tau_{1\beta}/T_{1\beta}) \quad (2)$$

where $M_z^\beta(\tau)$ is the intensity of the β resonance at time τ after the onset of saturation of the aldehyde resonance and $M_z^\beta(0)$ is the intensity of the β resonance in the absence of this saturation. Now,

$$1/\tau_{1\beta} = 1/\tau_\beta + 1/T_{1\beta} \quad (3)$$

where $\tau_\beta = 1/k_{\beta a}$ and $T_{1\beta}$ = the spin–lattice relaxation time of the β resonance in the presence of aldehyde saturation. To obtain $k_{\beta a}$, a plot of ln $(M_z^\beta(\tau) - M_z^\beta(\infty))$, where $M_z^\beta(\infty)$ is the intensity of the β resonance a long time ($5(k_{\beta a} + T_{1\beta}^{-1})^{-1}$s) after applying the saturating irradiation, vs. saturation time (τ), gives a line with slope = $-1/\tau_{1\beta}$. The relaxation time $T_{1\beta}$ is determined from the relationship

$$M_z^\beta(\infty)/M_z^\beta(0) = \tau_{1\beta}/T_{1\beta} \quad (4)$$

The values of $M_z^\beta(0)$ and $M_z^\beta(\infty)$ can be determined with accuracy since they are obtained from the initial and final equilibrium intensities, respectively. Therefore both $\tau_{1\beta}$ and $T_{1\beta}$ can be computed with precision, and eq 3 can be applied to determine τ_β.

Similar computations give $k_{\alpha a}$, the rate constant for ring opening of the α-furanose, and k_{ha}, the rate constant for dehydration of the *gem*-diol.

Several conditions must be met for eq 2 to be valid. First, NOE and cross-relaxation effects in the system must be zero; both conditions are met by the present system. Second, saturation of the aldehyde must be complete and essentially instantaneous. This condition is achieved with the appropriate setting of saturation power, as discussed below. It should be noted that significant differences in the proportions of the aldehyde and cyclic forms in solution do not affect the calculation of rate constants as described above, nor is a knowledge of the T_1 of the aldehyde required.

For ^1H ST measurements, $1/T_1$ for the cyclic forms is <0.08 s^{-1}, and $0.16 < 1/\tau_1 < 0.58$ s^{-1}. In these cases, $1/\tau$ is determined primarily by the slope term ($1/\tau_1$) of eq 3, and small errors in T_1 do not affect significantly the calculated value of k. For ^{13}C ST measurements, $1/T_1$ is ~0.2 for all forms and $0.28 < 1/\tau_1 < 0.54$ s^{-1}, so that both terms of eq 3 contribute significantly to the value of k.

Results

^{13}C and ^1H NMR Spectra of the Tetroses and the Evaluation of Equilibrium Constants. ^{13}C and ^1H NMR spectra of the tetroses show the presence of four forms in aqueous solution. The 80-MHz ^1H NMR spectrum of D-threose is shown in Figure 1A. The chemical shifts of H-1 of the α- and β-furanoses and linear hydrate (h) of D-threose are found between 5.0 and 5.3 ppm, while the aldehyde H-1 (a) resonates at ~10.0 ppm. Resonances from H-2, H-3, H-4, and H4′ of the four forms occur between 3.5 and 4.2 ppm. The 80-MHz ^1H NMR spectrum of this region is complex, but can be interpreted at 180 MHz through the use of selective deuteration and computer simulation.[19] ^1H Chemical shifts and ^1H–^1H coupling constants for the tetroses have been verified at 600 MHz.[20]

The 20-MHz ^{13}C NMR spectrum of D-[1-^{13}C]threose in shown in Figure 1B. Only the resonances from the enriched carbons are shown. C-1 chemical shifts for the α- and β-furanoses and linear hydrate (h) are found between 90 and 104 ppm, while the aldehyde C-1 (a) resonates at 206 ppm.[8a,c,d] In this spectrum the signal to noise ratio for the aldehyde resonance exceeds 7:1. In spectra used to obtain equilibrium constants, the signal to noise ratio for the aldehyde resonance was always >12:1. Solutions of D-threose, over the temperature range 17–81 °C, contain the following percentages of α- and β-furanoses, hydrate, and aldehyde: 17 °C, 51.2 ± 0.5, 37.6 ± 0.4, 10.5 ± 0.4, and 0.67 ± 0.10; 20 °C, 50.9 ± 0.5, 37.7 ± 0.4, 10.5 ± 0.4, and 0.94 ± 0.10; 25 °C, 51.8 ± 0.5, 37.6 ± 0.4, 9.6 ± 0.4, and 0.96 ± 0.1; 37 °C, 51.2 ± 0.5, 37.6 ± 0.4, 9.7 ± 0.4, and 1.6 ± 0.1; 51 °C 51.4 ± 0.5, 37.8 ± 0.4, 8.3 ± 0.4, and 2.4 ± 0.2; 64 °C, 50.2 ± 0.5, 38.5 ± 0.4, 8.0 ± 0.3, and 3.3 ± 0.3; and 81 °C, 50.4 ± 0.5, 37.8 ± 0.4, 7.1 ± 0.3,

(17) Serianni, A. S.; Nunez, H. A.; Barker, R. *J. Org. Chem.* **1980**, *45*, 3329–3341.
(18) Shoolery, J. N. *Prog. Nucl. Magn. Reson. Spectrosc.* **1977**, *11*, 79–93.
(19) Serianni, A. S.; Barker, R. *Can. J. Chem.* **1979**, *57*, 3160–3167.
(20) Serianni, A. S.; Barker, R., unpublished results. Spectra were analyzed at 600 MHz at the NMR Facility for Biomedical Studies, Carnegie-Mellon Institute, Pittsburgh, PA.

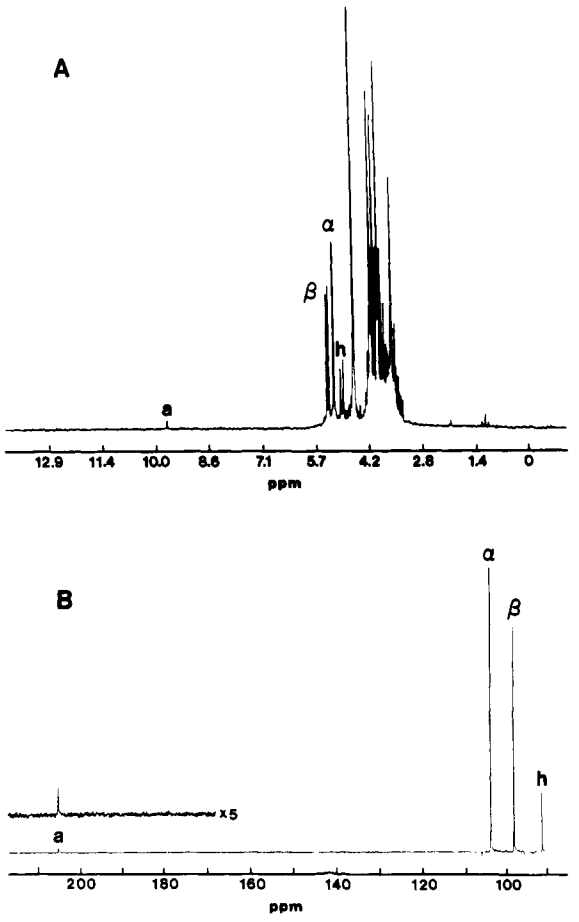


Figure 1. Tautomeric forms of D-threose in aqueous solution. (A) 80-MHz ^{1}H NMR spectrum of D-threose (0.3 M) at 31 °C. (B) 20-MHz ^{13}C NMR spectrum of D-[1-^{13}C]threose (0.2 M) at 29 °C showing resonances due only to the enriched carbons. Both spectra show the presence of α-furanose (α), β-furanose (β), hydrate (h), and aldehyde (a) resonances. H-1 and C-1 resonances for the furanoses and hydrate are found between 5.0–5.3 ppm and 90–104 ppm, respectively. H-1 and C-1 resonances for the aldehyde are found at 9.8 and 206.0 ppm, respectively. Assignments of H-1 and C-1 resonances to each form were made previously.[8,19]

and 4.7 ± 0.4. Free energies of conversion, $\Delta G°$ at 25 °C, were determined from equilibrium constants for the ring-closing and hydration reactions: for a → α, −2.4 ± 0.2 kcal mol^{-1}; for a → β, −2.2 ± 0.3 kcal mol^{-1}; and for a → h, −1.4 ± 0.2 kcal mol^{-1}.

A plot of equilibrium constants vs. $1/T$ for D-threose (Figure 2) was used to obtain values of $\Delta H°$ for the ring-closing and hydration reactions: for a → α, −6.0 ± 1.2; for a → β, −6.0 ± 1.2; and for a → h, −7.3 ± 1.5 kcal mol^{-1}. Entropies of conversion, $\Delta S°$ at 25 °C, calculated from $\Delta G°$ and $\Delta H°$, are as follows: for a → α, −13 ± 5; for a → β, −13 ± 5; and for a → h, −20 ± 5 cal mol^{-1} K^{-1}.

^{1}H and ^{13}C ST NMR Spectra. The large frequency separation between the site of irradiation (C-1 or H-1, aldehyde) and the observation sites (C-1 or H-1, α- and β-furanoses and hydrate) is ideal for both ^{1}H and ^{13}C ST NMR studies (Figure 1). A narrow rf bandwidth is, therefore, not essential for selective saturation, and no perturbation occurs in the region of observation.

The aldehyde resonance must be fully and essentially instantaneously saturated for eq 2 to be applicable to the calculation of ring-opening rate constants. Application of ^{1}H saturating rf power greater than 0.1 W at the D-threose aldehyde H-1 resonance frequency caused complete decay of $M_z^\beta(0)$ to $M_z^\beta(\infty)$. Increasing the power to 0.8 W did not decrease the value of $M_z^\beta(\infty)$ further, demonstrating that secondary effects on the intensity of the β-furanose H-1 resonance from the saturating rf are small. ^{1}H ST NMR spectra at 80 MHz were obtained with 0.6–0.8 W of saturating power to ensure both complete and instantaneous

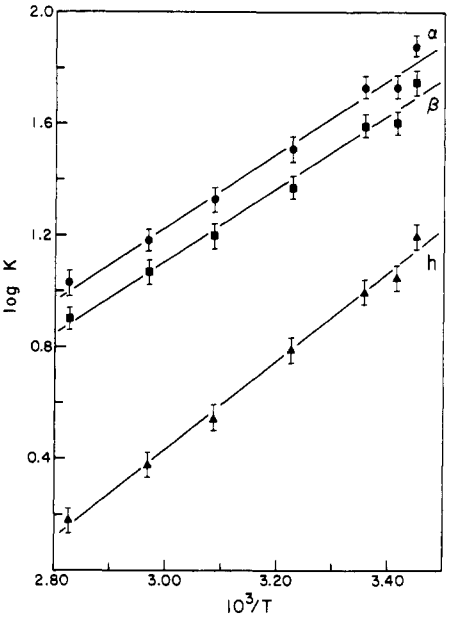


Figure 2. Temperature dependence of equilibrium constants for α-D-threofuranose (α), β-D-threofuranose (β) and D-threose hydrate (h). Equilibrium constants were determined by ^{13}C and ^{1}H NMR spectroscopy, as described in the text.

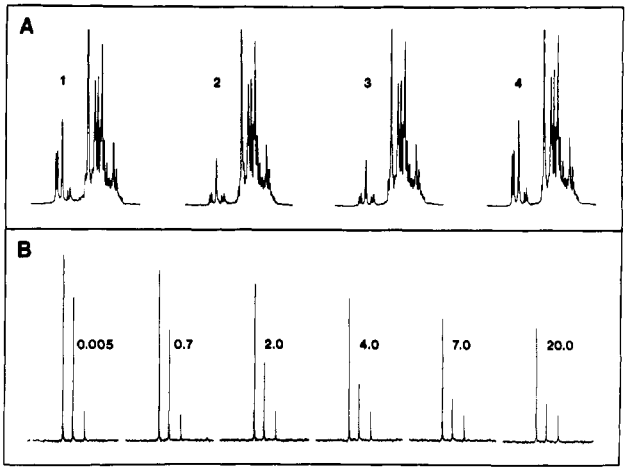


Figure 3. ^{1}H and ^{13}C ST NMR spectra. (A) ^{1}H ST NMR spectra (3.0–5.5 ppm) of D-threose (0.3 M, 57 °C). Spectrum 1 shows the H-1 resonance intensities of the α- and β-furanoses and hydrate in the absence of aldehyde saturation ($M_z(0)$). Spectra 2 and 3 are consecutive $M_z(\infty)$ spectra obtained with saturation of the aldehyde, showing the loss in intensity of H-1 for each form. Spectrum 4 shows the return of the initial intensities (spectrum 1) when saturation is removed. (B) ^{13}C ST NMR spectra of D-[1-^{13}C]threose at 55 °C showing only resonances of the enriched carbons. Spectra were obtained with different τ values, as indicated. The $M_z(\infty)$ spectrum is obtained after ~20 s of saturation.

saturation. A similar calibration was conducted for ^{13}C ST NMR measurements.

A $M_z(0)$ ^{1}H spectrum (no saturation) of the 3.0–5.5 ppm region for D-threose is shown in Figure 3A (spectrum 1). Spectra 2 and 3 are consecutive $M_z(\infty)$ spectra (with full saturation). In these spectra, the resonance intensities of H-1 of the α- and β-furanoses and hydrate have decreased by 48%, 79%, and 32%, respectively. Resonances of the other protons are essentially unaltered and are convenient indicators of spectrometer response during the experiment. Spectrum 4 in Figure 3A shows the return to the $M_z(0)$ intensities of spectrum 1 after terminating saturation or moving the saturating rf off-resonance. Slight changes in the intensities of the H-2, H-3, H-4, and H-4′ resonances between spectra 1 and 4 are caused by changes in instrument response and/or field homogeneity during the experimental period (13 h at 80 MHz, 1.5 h at 360 MHz).

Table I. Unidirectional Rate Constants[a] for D-Tetroses in ^2H_2O

T (±1 °C)	NMR[b]	form	k_{open} (±10%)	k_{close} (±18%)	conditions[c]
38	^1H	β-threo	0.083	2.0	A
48	^1H	β-threo	0.14	2.5	A
51	^1H	α-threo	0.034	0.74	B
		β-threo	0.21	3.5	
57	^1H	α-threo	0.052	0.97	A
		β-threo	0.25	3.5	
66	^1H	α-threo	0.10	1.5	A
		β-threo	0.53	5.9	
51	^1H	α-threo	0.057	1.2	C
		β-threo	0.19	3.2	
51	^1H	α-erythro	0.25		C
		β-erythro	0.14		
55	^{13}C	α-threo	0.11	2.2	C
		β-threo	0.36	5.3	
55	^{13}C	α-erythro	0.40		C
		β-erythro	0.19		

[a] Units of s^{-1}. [b] ^1H ST experiments were run at 80 MHz for determinations at 38, 48, 57, and 66 °C. Determinations at 51 °C were made at 360 MHz. ^{13}C ST experiments were run at 100 MHz. [c] Condition A, 0.3 M aldose in ^2H_2O, unbuffered; condition B 0.1 M aldose in ^2H_2O, unbuffered; condition C, 0.1 M aldose in 50 mM sodium acetate in ^2H_2O, p^{2}H 5.0.

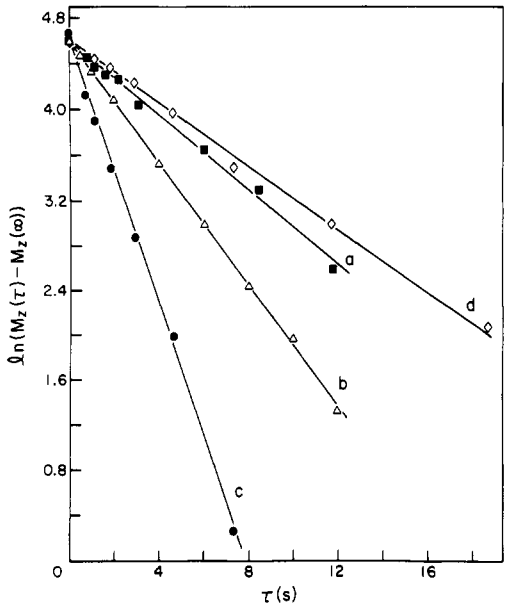


Figure 4. Decay of M_z as a function of saturation time, τ, using ^1H ST NMR. β-D-Threofuranose, unbuffered, at 38 °C (a), 51 °C (b), and 66 °C (c). α-D-Threofuranose at 66 °C (d). Determinations at 38 and 66 °C were made at 80 MHz with 0.3 M solutions; data at 51 °C were obtained at 360 MHz with 0.1 M solutions. Zero points are arbitrary.

Ring-opening and dehydration rate constants were determined by measuring the intensities of the α, β, and hydrate resonances as functions of saturation time, τ, as described in the Experimental Section. A sequence of ^{13}C ST NMR spectra with different τ values (0.005–20 s) is shown in Figure 3B. Above 20 s of saturation, spectra intensities were essentially the same ($M_z(\infty)$ had been reached).

Since the ^{13}C ST NMR data do not contain reference line(s) to evaluate changes in instrument response, similar τ values were repeated several times during the experimental period (1–2 h).

Unidirectional Rate Constants. Rate constants for ring opening in ^2H_2O for α- and β-threofuranose (0.3 M), measured at 80 MHz by ^1H ST NMR at several temperatures using the same unbuffered solution, are given in Table I. Rate constants were measured at 51 °C in a 0.1 M unbuffered solution at 360 MHz to examine the correlation between the data from different spectrometers. Semilogarithmic plots showing the decay of the β-threofuranose resonance intensities at 38, 51, and 66 °C are shown in Figure 4a–c. The decay of the α-threofuranose resonance intensities at 66 °C is also shown (Figure 4d) for comparison to that for β-threofuranose at the same temperature (Figure 4c). It is apparent that the β-furanose opens faster than the α-furanose to the linear aldehyde.

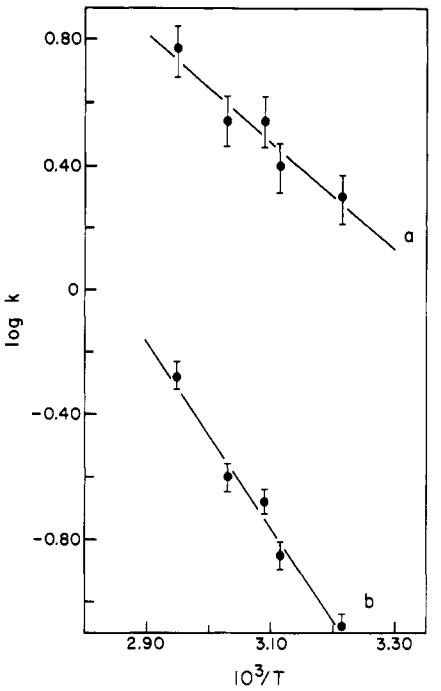


Figure 5. Temperature dependence of $k_{\alpha\beta}$ (a) and $k_{\beta\alpha}$ (b) for D-threose (0.3 M, unbuffered). Rate constants were determined by ^1H ST NMR spectroscopy. Energies of activation for $k_{\beta\alpha}$ and $k_{\alpha\beta}$ are 14 ± 3 and 8 ± 2 kcal/mol, respectively.

By use of ring-opening rate constants from ST measurements and equilibrium constants from van't Hoff plots (Figure 2), ring-closing rate constants for α- and β-threofuranose were calculated at each temperature (Table I). Energies of activation (E_a) for ring opening and closing were obtained from Arrhenius plots of the data (Figure 5).

Approximate values of $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ were calculated from transition-state theory.[21,22] $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ determined from NMR data are particularly subject to both statistical and systematic errors,[23] and these errors are compounded in the calculation of $\Delta S^{\ddagger}$. $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ for ring opening of α-threofuranose are ~21 kcal mol^{-1}, ~15 kcal mol^{-1}, ~−19 cal mol^{-1} K^{-1}; for ring closing they are ~19 kcal mol^{-1}, ~10 kcal mol^{-1}, and ~−29 cal mol^{-1} K^{-1}. $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for ring opening of β-threofuranose are ~20 kcal mol^{-1}, ~13 kcal mol^{-1}, and ~−23 cal mol^{-1} K^{-1}; for ring closing they are ~18 kcal mol^{-1}, ~7 kcal mol^{-1}, and ~−34 cal mol^{-1} K^{-1}.

A comparison of ring-opening rate constants obtained by ^1H and ^{13}C ST NMR and a study of the effect of configuration on these rate constants were simultaneously undertaken by examining acetate-buffered solutions (50 mM in ^2H_2O, p^{2}H 5.0) of unenriched and D-[1-^{13}C]threose and -erythrose. Unenriched tetroses and 1-^{13}C derivatives were analyzed by ^1H (360 MHz, 51 ± 1 °C) and ^{13}C (100 MHz, 55 ± 1 °C) ST NMR, respectively. Results are shown in Figures 6 and 7.

Discussion

Involvement of the Aldehyde in Anomerization. The anomerization of ring forms of aldoses in solution has been proposed to

(21) Daniels, F.; Alberty, R. A. "Physical Chemistry", 3rd ed.; Wiley: New York, 1966; pp 612–615.
(22) Hammes, G. G. "Principles of Chemical Kinetics", Academic Press: New York, 1978; pp 51–62.
(23) Martin, M. L.; Delpuech, J.-J.; Martin, G. J. "Practical NMR Spectroscopy", Heyden, Philadelphia, PA, 1980; 344–345.

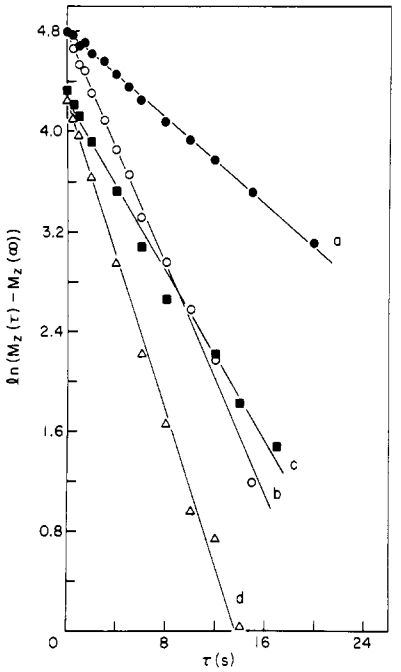
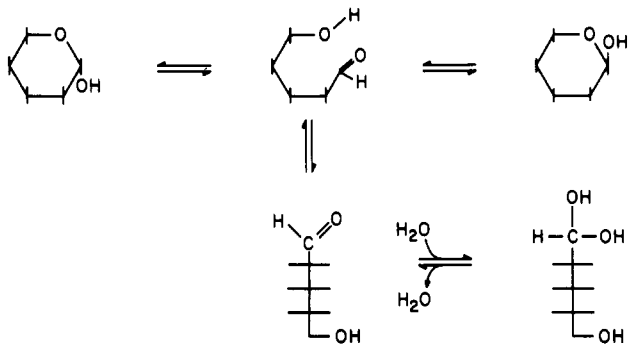


Figure 6. Decay of M_z as a function of ^1H saturation time, τ, for α- and β-D-threofuranose (a and b, respectively) and β- and α-D-erythrofuranose (c and d, respectively) at 51 ± 1 °C. ^1H ST NMR spectra were obtained at 360 MHz with 0.1 M solutions buffered with 50 mM sodium acetate (p^2H 5.0). Zero points are arbitrary.

Scheme II



involve the intermediate formation of an aldehyde, as shown in Scheme I. The transfer of saturation to furanose and hydrate forms when the aldehyde resonance is saturated provides direct, qualitative evidence that an aldehyde, or an aldehyde-like species, is an intermediate in the interconversion of aldose tautomers in solution. However, data demonstrating a slow rate of ^{18}O exchange with $H_2{}^{18}O$ relative to the rate of interconversion of ring forms in the hexoses have led to the proposal that the true intermediate is an aldehyde having a pseudoacyclic conformation,[5b] as shown in Scheme II.

The existence of a pseudoacyclic intermediate is of practical importance only if it and the acyclic aldehyde interconvert slowly relative to the rate of conversion of the pseudoacyclic aldehyde to the ring forms. In this case, the acyclic aldehyde, and the hydrate derived from it, stand in the same relationship to the true intermediate (pseudoacyclic aldehyde) as do the ring forms. In the case where the pseudoacyclic aldehyde and the acyclic aldehyde interconvert rapidly relative to the rate of conversion of the pseudoacyclic aldehyde to the ring forms, the distinction becomes moot. Certainly the acyclic aldehyde must assume a pseudoacyclic conformation for ring formation to occur.

Both ^1H and ^{13}C FT NMR spectroscopy (using 1-^{13}C-enriched aldoses) permits the tautomeric composition of aqueous (^2H_2O) solutions of the tetroses to be determined with an error of ±10% for species present in very small amounts. In spectra of pure monomeric tetroses, only one resonance attributable to aldehyde

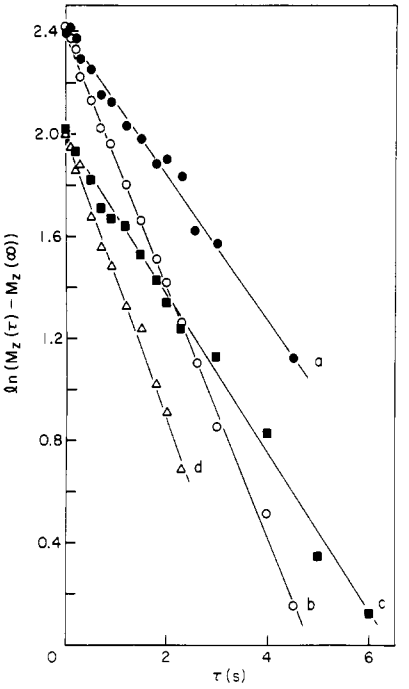


Figure 7. Decay of M_z as a function of ^{13}C saturation time, τ, for α- and β-D-[1-^{13}C]threofuranose (a and b, respectively) and β- and α-D-[1-^{13}C]erythrofuranose (c and d, respectively) at 55 ± 1 °C. ^{13}C ST NMR spectra were obtained at 100 MHz with 0.1 M solutions buffered with 50 mM sodium acetate (p^2H 5.0). Zero points are arbitrary.

forms is present. In fact, there is no reason to expect that the aldehydic ^1H and ^{13}C resonances of pseudoacyclic and acyclic forms would have different chemical shifts, so that the transfer of saturation from the "aldehyde" to the ring forms cannot reflect on the presence (or absence) of pseudoacyclic intermediates in tautomerization. However, the involvement of the "aldehyde" species in the interconversion can be assessed by analysis of the line width of the aldehyde resonance. Line broadening due to chemical exchange of the aldehyde resonance ($\pi\Delta\nu_a$) is predicted by the relationship

$$\pi\Delta\nu_a = k_{a\alpha} + k_{a\beta} + k_{ah}$$

Values of $k_{a\alpha}$ and $k_{a\beta}$ can be calculated from $k_{\alpha a}$, $k_{\beta a}$, and equilibrium constants, all of which can be measured ($k_{a\alpha} = K_\alpha k_{\alpha a}$, etc.). Since $k_{a\alpha}$ and $k_{a\beta} \gg k_{ah}$, the last term can be ignored. Now, if all of the "aldehyde" participates in the tautomerization, $k_{\alpha a}$ and $k_{a\beta}$ obtained by ST NMR will be accurate ring-closing rate constants and $\Delta\nu_a$ can be computed from them. If, however, only a small proportion of the "aldehyde" participates as an intermediate (a pseudoacyclic intermediate), the values assumed for the equilibrium constants will be in error. For example, if we assume that only 10% of the "aldehyde" is an intermediate, then the equilibrium constants would increase by a factor of 10, as would the values of $k_{a\alpha}$ and $k_{a\beta}$ and $\Delta\nu_a$ for the interconverting form. Thus, if a small proportion of the "aldehyde" form(s) is the true intermediate in tautomerization, and this species is in slow exchange with the remainder of the "aldehyde", then the observed line width of the "aldehyde" would be unaffected by the line broadening of the true intermediate due to chemical exchange. The proportion involved in exchange would be greatly broadened, while the larger proportion not involved would be broadened very little. Since we assume that both forms, if present, resonate at the same frequency, no line broadening would result from chemical exchange between them.

When the line width of the "aldehyde" ^{13}C resonance of D-erythrose is measured at 55 °C significant line broadening due to exchange is apparent (2.1 Hz). The line broadening calculated by using rate and equilibrium constants and assuming that all of the "aldehyde" serves as an intermediate in the reaction is 2.1 Hz. This agreement suggests that, regardless of the composition of

the "aldehyde", essentially all of it participates in the tautomerization, and the mechanism shown in Scheme I serves as an appropriate model for the tautomerization of the tetroses.

An additional method for examining the possibility that another pathway exists for the interconversion of the furanoses, other than through the aldehyde, involves the application of simultaneous saturating frequencies to the aldehyde and one of the furanose forms. If a separate pathway existed, the effect of the second saturating frequency would be to decrease the resonance intensity of the remaining furanose form to a greater extent than occurs with saturation of the aldehyde resonance alone. When such experiments were performed by ^{13}C ST NMR spectroscopy, the remaining resonance intensities were not significantly reduced, indicating that no significant second pathway exists.

Finally, k_{ah} and k_{ha} are small compared to rate constants for ring opening and closing so that ^{18}O exchange with $H_2{}^{18}O$ should proceed more slowly than mutarotation. Thus, such a finding need not imply that the two processes involve different intermediates.

On the basis of these considerations, it is appropriate to treat the system as consisting of the equilibria shown in Scheme I.

Equilibrium Constants and Thermodynamic Parameters. The effect of temperature on the relative proportions of pyranoses and furanoses in aqueous solutions of aldopentoses and hexoses has been studied previously, and increasing temperature favors furanoses.[2c] In solutions of D-threose, the percentage of aldehyde increases with temperature: 0.67 ± 0.1 (17 °C), 0.94 ± 0.1 (20 °C), 0.96 ± 0.1 (25 °C), 1.6 ± 0.1 (37 °C), 2.4 ± 0.2 (51 °C), 3.3 ± 0.3 (64 °C), and 4.7 ± 0.4 (81 °C). Although the small proportion of aldehyde leads to rather large errors in estimates of its concentration, these values give equilibrium constants suitable for the calculation of *approximate* thermodynamic parameters. Over the same temperature range, the amounts of α- and β-furanoses and hydrate remain nearly constant at ~51% and ~38%, respectively, while the amount of hydrate decreases from 10.5 ± 0.4% to 7.1 ± 0.3%.

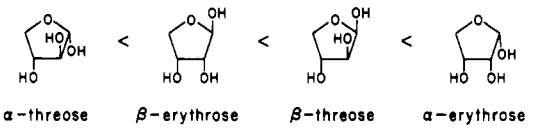
Free energies, enthalpies, and entropies of conversion of aldehyde to furanoses and hydrate at 25 °C (see Results) indicate that, whereas $\Delta G°$ and $\Delta H°$ favor ring formation and hydration, $\Delta S°$ favors the aldehyde form. $\Delta S°$ for hydration is approximately 1.7-fold greater than $\Delta S°$ for ring closure, as expected for a bimolecular reaction that immobilizes a water molecule, and is similar to that for the hydration of acetaldehyde at 25 °C (−16.4 cal mol^{-1} K^{-1}).[24]

In the overall conversion of β-furanose to α-furanose at 25 °C, $\Delta S° \simeq -1.0$ cal mol^{-1} K^{-1} and $\Delta H° \simeq 0$ kcal mol^{-1}. These values are similar to those reported for the interconversion of pyranoses.[5b]

Rate Constants and Activation Parameters. An unbuffered solution of D-threose was examined at various temperatures primarily to evaluate the saturation-transfer NMR method for determining rate constants in anomerizing systems. Studies in unbuffered solutions allow comparison of the rate constants for interconversion of the various forms of the same aldose. In addition, we find it possible with ion exchange and chelating resins to prepare such solutions in a highly reproducible fashion.

Ring-opening rate constants for β-threofuranose ($k_{\beta a}$) were found to increase from 0.083 to 0.53 s^{-1} between 38 and 66 °C (Table I). Rate constants for ring opening of the α-anomer ($k_{\alpha a}$) are about 5-fold smaller, indicating that configurational effects can be significant. Ring-closing rate constants for β-threofuranose ($k_{a\beta}$) in unbuffered solution are about 4-fold greater than those for the α anomer. It appears that, for the simple furanoses, the thermodynamically less favored ring (β-threofuranose) is kinetically favored, as in D-glucose,[6a] whereas, for FDP,[6b] the thermodynamically favored anomer (β-furanose) is kinetically favored. Activation energies (E_a) for ring opening and ring closing of β-threofuranose are 14 ± 3 and 8 ± 2 kcal mol^{-1}, respectively. In comparison, ring-opening activation energies calculated for the anomers of FDP[6b] were ~ 16 kcal mol^{-1}. Approximate values of $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ were calculated from transition-state theory.[21,22] Although interpretation of these parameters in terms of mechanism must be made with caution, the large, negative values of $\Delta S^{\ddagger}$ for both ring opening and closing may be partly due to ordering of solvent in the transition state.[5b,24]

Scheme III



	α-threose	β-erythrose	β-threose	α-erythrose
relative rate	0.26	0.52	0.83	1.0

For most monosaccharides, the overall rate constants for the interconversion of ring forms such as the α- and β-pyranoses are obtainable only if the anomers are available in crystalline form. The overall rate constants for interconversion are then measured by physical and/or chemical methods. These overall rate constants can be derived, however, from the unidirectional rate constants of interconversion of the cyclic and aldehyde forms. Thus, data of the kind obtained in this study can be used to assess the overall rate constants of interconversion of monosaccharides which are not amenable to study by more traditional techniques. This approach may be particularly useful in the study of pentose and ketohexose phosphates, which are frequently not known as crystalline anomers and whose rapid rates of interconversion prevent their study by other means.[7] For D-threose at 66 °C, the overall rate constants were calculated from

$$k_{\alpha\beta} = \frac{k_{\alpha a}k_{a\beta}}{k_{a\beta} + k_{a\alpha}} = 0.08 \pm 0.01 \text{ s}^{-1}$$

$$k_{\beta a} = \frac{k_{\beta a}k_{a\alpha}}{k_{a\beta} + k_{a\alpha}} = 0.11 \pm 0.01 \text{ s}^{-1}$$

Comparison of the rates of tautomerization of two or more compounds can be made only if the composition of the solutions is well-defined. For comparison of the behavior of D-erythrose and D-threose, ring-opening rate constants were determined with solutions of identical aldose concentration (0.1 M), ionic strength (50 mM, sodium acetate in ^2H_2O), and p^{2}H (5.0). As shown in Figures 6 and 7 and Table II, rate constants obtained by ^1H and ^{13}C ST NMR are in good agreement after correcting for temperature differences (4–6 °C). Ring-opening rate constants increase in the order shown in Scheme III. The thermodynamically favored rings having OH-1 and OH-2 trans (α-threo, β-erythro) open more slowly than rings having OH-1 and OH-2 cis (β-threo, α-erythro). Furthermore, ring opening is affected by the relative positions of OH-2 and OH-3; compounds with the cis configuration open more rapidly.

The effect of acid on the rates of anomerization was also examined. At 25 °C in 50 mM KCl/^{2}HCl (p^{2}H 1.6), $M_z(\infty)/M_z(0) \simeq 0.3$ for the anomeric protons of α- and β-erythrofuranose, indicating that the ring-opening rate constants are >0.15 s^{-1} as compared to those in acetate buffer at p^{2}H 5.0 (≪0.15 s^{-1}) and showing the expected acid-catalyzed rate enhancement. Clearly, the ST method can be applied to assess the effects of various catalysts and conditions on the anomerization of monosaccharides.

The rate constant for dehydration of D-threose hydrate cannot be estimated with precision at 55 °C in acetate buffer at p^{2}H 5.0, since aldehyde saturation causes only a 10% loss of intensity of the hydrate resonance. A measurement of the spin–lattice relaxation time for C-1 of the hydrate (~2.5 s) permits an estimation of the rate constant for dehydration of <0.05 s^{-1}.

This study has demonstrated that ^1H and ^{13}C ST NMR can be applied to the study of anomerization of furanose sugars. ^{13}C ST NMR studies require as little time as ^1H ST NMR analyses when 1-^{13}C-enriched derivatives are used to increase selectivity and sensitivity. ^{13}C ST NMR studies with 1-^{13}C-enriched compounds offer advantages of spectral simplicity and ease of quantitation. In addition, they can also be performed in either H_2O or ^2H_2O solutions. Ring-opening rate constants, heretofore unmeasureable, can be obtained from both methods and used with

(24) Schlager, L. L.; Long, F. A. *Adv. Phys. Org. Chem.* **1963**, *1*, 1–33.

equilibrium constants to determine ring-closing rate constants. These unidirectional rate constants can then be used to derive overall rate constants for the interconversion of cyclic forms, an approach that may be particularly useful in the study of biologically important sugars that may be available only as syrups or as solutions at anomeric equilibrium.

Thermodynamic and activation parameters can be estimated for each reaction, and the effects of temperature, pH, configuration, solvent, and catalysts can now be systematically examined. Although rate constants in the range 0.02–0.05 s^{-1} are difficult to estimate by ST NMR, various catalysts can be used to bring them into range, and intrinsic rate constants can be obtained by extrapolation. The extension of ST NMR methods to study pyranose or furanose anomerization of pentoses and hexoses may be feasible. D-Idose may be especially amenable to study since pyranose, furanose, hydrate, and aldehyde forms can be observed in the ^{13}C NMR spectrum of the 1-^{13}C-enriched derivative. Comparison of the unidirectional rate constants for α- and β-ribofuranose with those for α- and β-erythrofuranose would be particularly interesting, since in other systems, cyclizations to form furan rings involving secondary hydroxyls occur more readily than those involving primary hydroxyls.[17,25] Studies on the anomerization of the tetroses, aldose 5-phosphates, ketose phosphates, and their derivatives are in progress.

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Registry No. D-Erythrose, 583-50-6; D-threose, 95-43-2; 2,4-*O*-ethylidene-D-erythrose, 24871-55-4; α-threofuranose, 80877-72-1; β-threofuranose, 80877-73-2; α-erythrofuranose, 72599-80-5; β-erythrofuranose, 72599-81-6.

(25) Hudson, B. G.; Barker, R. *J. Org. Chem.* **1967**, *32*, 3650–3658.

The Concept of Lewis Acids and Bases Applied to Surfaces

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Abstract: The purpose of this paper is to apply the concept of Lewis acids and bases to provide an understanding of the influence of structure and composition on surface chemistry. The Lewis acid–base concept is briefly reviewed. The factors important for determining the strength of acid–base interactions, including orbital energies and orbital character, are illustrated with the aid of gas phase ion–molecule chemistry. The perturbational molecular orbital theory of acid–base interactions is adapted to the gas phase environment in order to show that changes in the ordering of base strength through a series of Lewis bases must be due to the criterion of maximum orbital overlap. The classification of metal surfaces as acid or base with respect to a molecular adsorbate is determined with the work-function change. The influence of surface composition modification by either electronegative or electropositive elements on CO adsorption is shown to follow an inductive effect analogous to that seen in molecules. On metal oxide surfaces and oxidized metal surfaces, the oxygen anions are assigned to be Lewis base sites and the electron-deficient metal atoms are assigned to be Lewis acid sites. For an oxygen monolayer on a metal surface, two types of structures are distinguished: the overlayer structure and the incorporation structure. Chemical and physical evidence is presented which indicates that the Lewis acid sites on a surface with an incorporation structure are localized. Finally, the chemical evidence from adsorption studies combined with the criterion of maximum orbital overlap indicates that it is the localized vs. delocalized character of the valence electronic states at the surface that determines adsorption selectivity changes.

I. Introduction: Lewis Acids and Bases

One of the ultimate goals for research into the chemical properties of solid surfaces is to establish an understanding of surface chemistry which is comparable to more traditional gas phase or solution phase organic and inorganic chemistry. Of particular importance in this endeavor is the need to establish the general principles or "rules of thumb" which can be used in practical situations to rationalize or predict the effects of structure and composition on surface chemistry. Such concepts as electronegativity, inductive effect, nucleophilicity, hard and soft, etc. which have proved useful for classifying and rationalizing traditional chemical phenomena will very likely be just as useful in surface chemistry. In fact, the same concepts of chemical bonding and chemical reactions currently used in traditional chemistry will most certainly be used in surface chemistry as well. The development of a chemical understanding of surfaces has been hampered by the generally heterogeneous nature of the solid surface. Experimentation on heterogeneous surfaces is analogous to performing solution phase chemistry on a complex mixture where any of a number of components may be responsible for observed chemical effects. In recent years the effort to describe and understand surface chemistry in molecular and atomic detail has been spurred (1) by the development of ultra-high-vacuum, clean-surface technology which allows a surface to be prepared and maintained in a well-defined structural and chemical state and (2) by the development of a variety of surface-sensitive spectroscopies which allow the structure and composition of the surface to be probed directly. With the accumulation of information on the chemical behavior of well-defined surfaces we can begin to apply the traditional chemical concepts to the solid surface.

The purpose of this paper is to apply the concept of Lewis acids and bases to the understanding of surface chemistry. The Lewis acid–base concept is one of the most generally useful classification schemes in traditional chemistry. It is a tool for systematizing reactive molecules and reactive sites on molecules that provides

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