with hexanes containing 20% ether) and distillation (120 °C: 2.5 mm) (yield 43%): ^{1}H NMR ($CDCl_3$) δ 3.40 (s, $(OCH_3)_2$), 5.0 (d, J = 4.5 Hz, H-1), 6.30 (dd, J = 17, 4.5 Hz, H-2), 6.87 (d, J = 17 Hz, H-3), 7.85 (m, ArH); IR (thin film) 2940, 2835, 1785, 1640, 1470, 1455, 1380, 1275, 1175, 1125, 1105, 1050, 965, 890, 840, 690, 670 cm^{-1}; MS, m/e (M^+) calcd for $C_{13}H_{12}F_6O_2$ 314.0741, found 314.0719.

4-Nitro-*trans*-cinnamaldehyde dimethyl ketal, 1f, was prepared from 4-nitro-*trans*-cinnamaldehyde and was chromatographed over alumina, eluting with ether containing 40% hexanes. The main band afforded **1f** as colorless needles after recrystallization from hexanes (60%): mp 60–61 °C (lit.[24] 66–67 °C); ^{1}H NMR ($CDCl_3$) δ 3.39 (s, $(OCH_3)_2$), 4.99 (d, J = 4.5 Hz, H-1), 6.29 (dd, J = 16.5, 4.5 Hz, H-2), 6.84 (d, J = 16.5 Hz, H-3), 7.57 (d, J = 9 Hz, ArH), 8.22 (d, J = 9 Hz, ArH); MS, m/e (M^+) calcd for $C_{11}H_{13}NO_4$ 223.0884, found 223.0885.

Protonations. The ketal (15–20 mg) was weighed into an NMR tube, and the tube was cooled in dry ice/acetone. FSO_3H (0.3 mL) was added slowly down the wall of the tube and solution was effected by stirring with a thin glass rod.

Irradiations were carried out directly on FSO_3H solutions (**2c** and **2f**) in NMR tubes. The samples were placed in a partially silvered quartz Dewar with methanol as the heat-transfer medium and irradiated with light of wavelength 350 nm at ca. −70 °C.

NMR Spectral Simulations. Calculated spectra were obtained by using the program DNMR 3 by G. Binsch and D. A. Kleier, Quantum Chemistry Program Exchange.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council of Canada, the Deutsche Forschungsgemeinschaft, and the Fonds der Chemischen Industrie. All calculations have been carried out at the Kölner Rechenzentrum.

Registry No. 1a, 63511-97-7; **1a** (aldehyde), 24680-50-0; **1b**, 95123-63-0; **1b** (aldehyde), 56578-35-9; **1c**, 63511-93-3; **1c** (aldehyde), 14371-10-9; **1d**, 95123-64-1; **1d** (aldehyde), 49678-02-6; **1e**, 95123-65-2; **1e** (aldehyde), 95123-61-8; **1f**, 95123-67-4; **1f** (aldehyde), 49678-08-2; **1g**, 95123-66-3; **2g**·FSO_3^-, 95123-69-6; **2b**·FSO_3^-, 95123-71-0; **2c**·FSO_3^-, 95123-73-2; **2d**·FSO_3^-, 95123-75-4; **2e**·FSO_3^-, 95123-77-6; **2f**·FSO_3^-, 95123-79-8; **2g**·FSO_3^-, 95123-81-2; **4c**, 95123-83-4; **5a**, 95123-84-5; **5b**, 95123-85-6; **5c**, 77406-43-0; **5d**, 95123-86-7; **5e**, 95123-87-8; **5f**, 95123-88-9; **6**, 57344-16-8; **20**, 95123-62-9; 3,5-bis(trifluoromethyl)benzoic acid, 725-89-3; 3,5-bis(trifluoromethyl)benzyl alcohol, 32707-89-4; 3,5-bis(trifluoromethyl)benzaldehyde, 401-95-6.

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Anomerization of Furanose Sugars and Sugar Phosphates

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Abstract: Thermodynamic and kinetic parameters for the ring-opening and -closing reactions of several aldo- and ketofuranoses and their phosphate esters have been determined by NMR line-width and saturation-transfer methods. Cyclic forms interconvert via a single, acyclic carbonyl form under either acid or base catalysis. Ring-opening rates do not correlate with thermodynamic stability of the rings. For aldofuranose phosphates, α anomers open faster than β anomers; for ketofuranose phosphates the converse is observed. Intramolecular catalysis of anomerization by the phosphate group of sugar phosphates is documented. Biological and mechanistic implications of the observed kinetics are discussed.

Aqueous solutions of monosaccharides and their phosphate esters often contain several interconverting, tautomeric forms. Furanoses, pyranoses, septanoses, acyclic hydrates, aldehydes, ketones, and oligomers have been detected.[2] The chemistry of the monosaccharides is complicated by this structural diversity as the rates of their chemical and biochemical reactions may be determined by the concentration of a minor form and its rate of production from other forms, rather than by the total sugar concentration. Knowledge of the rates of interconversion of tautomeric forms is therefore required to interpret the observed chemical and biochemical reactivities of monosaccharides.

Studies of anomerization (the interconversion of cyclic anomers) of simple sugars began in the mid-19th century. Considerable kinetic data are available,[3] and overall rate constants for the interconversion of predominant forms have been determined for a variety of sugars. The reaction is subject to general acid and base catalysis, and the ring structure influences reaction rates.

The simplest mechanism for interconversion of tautomers (tautomerization)[4] involves the acyclic carbonyl form as the obligatory and sole intermediate (Scheme I). This mechanism has been proposed to interpret anomerization kinetic data,[3] including that which yields unimolecular ring-opening rate constants.[5] The role of the acyclic carbonyl form as the key intermediate, however, has been questioned, and pseudocyclic intermediates have been postulated.[3b,6] Recent kinetic experiments on sugars containing sulfur as the ring heteroatom have suggested that, in these compounds, interconversions of cyclic forms occur faster than formation of the acyclic carbonyl from cyclic forms.[7] Since the interpretation of most experiments on anomerization has depended on assumptions regarding the nature of the reaction intermediate, methods are needed to examine this intermediate and to test the validity of these assumptions.

Interest in the anomerization of sugar phosphates has been stimulated by the advancing studies of enzyme mechanisms and metabolic regulation. While the molecular dynamics of proteins have received considerable attention, the solution behavior of their substrates has often been neglected and investigators are confronted with the difficulties of studying the properties of sensitive and selective catalysts with reagents of undefined and changing nature. Unfortunately, measurements of the anomerization of

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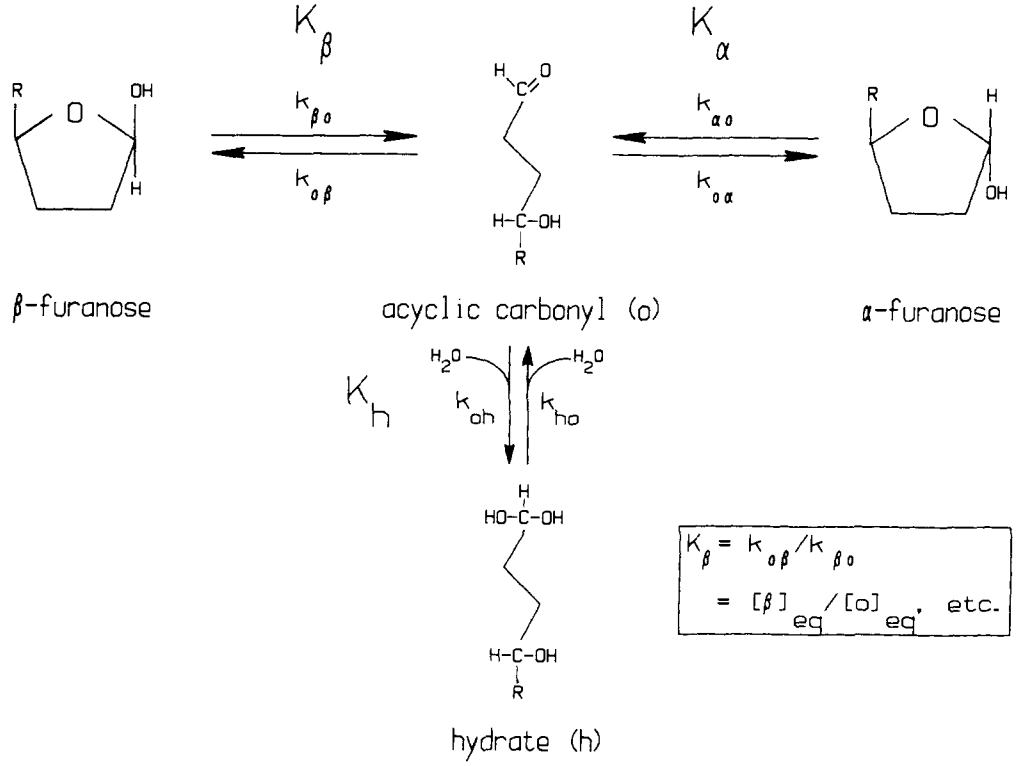
Scheme I



sugar phosphates are hampered by the unavailability of pure, crystalline anomers and by the rapid interconversion of sugar phosphate tautomers. One approach to this problem utilizes rapid reaction techniques in which the sugar phosphate is mixed with an enzyme that specifically converts one tautomer to product under conditions where the rate of product formation approximates the rate of tautomerization. By this method, glucose-*6*-P[8] was shown to anomerize 240 times faster than glucose under similar conditions, and the importance of intramolecular catalytic participation of the substituent phosphate group in this rate acceleration was demonstrated.[9] Similar studies with fructose-*6*-P[10] and fructose-*1,6*-P_2[11] yielded rate constants for interconversion of α- and β-furanose forms that are similarly much larger than those for simple, nonphosphorylated sugars.

Although anomerization can be studied by measurement of overall rate constants, the unimolecular rate constants of ring opening and closing more completely describe the kinetics. Unimolecular rate constants have been reported for only one sugar phosphate (fructose-*1,6*-P_2) by measurement of ^{13}C NMR line widths.[12] From these rate constants, the form of fructose-*1,6*-P_2 (namely, the acyclic carbonyl form) acted upon by aldolase was inferred.

Previous NMR studies of the tetroses and pentose *5*-phosphates[13] have revealed that aqueous solutions of these sugars contain, in addition to furanose forms, small but measurable quantities of acyclic hydrate and carbonyl forms. In addition, unimolecular rate constants for ring opening and closing of the tetroses have been measured recently by saturation-transfer (ST) NMR methods.[14] The present study extends these earlier observations to the biologically important pentose 5-phosphates and ketohexose phosphates (Chart I). Direct evidence for the role of the carbonyl form as the obligatory and sole intermediate in anomerization is presented. The effects of ring configuration and intramolecular phosphate catalysis on anomerization are discussed and consideration is given to their implications for enzyme catalysis and metabolic regulation.

Experimental Section

Materials. Unenriched sugars and sugar phosphates, ion-exchange resins, and enzymes were obtained from Sigma Chemical Co. Deuterium oxide (99.8 atom % ^2H) from Stohler Isotope Chemicals was distilled prior to use. [^{13}C]Methanol (90 atom %) was purchased from Merck. ^2HCl (20%, 99+ atom %), NaO^2H (30%, 99+ atom %) and acetic-$^2H_4$ acid (99.5 atom %) were obtained from Aldrich Chemical Co. Chemicals were reagent grade or better and used without further purification.

Compounds. Monosaccharides used in this study had the D configuration. Erythrose and threose were prepared from their respective 2,4-*O*-ethylidene derivatives.[15]

5-*O*-Methylxylose was prepared by acid hydrolysis (0.1 N H_2SO_4, 85 °C for 2 h) of 1,2-*O*-isopropylidene-5-*O*-methyl xylofuranoside[16] and purified by chromatography on Dowex 50-X8 (200–400 mesh) in the barium form.[17]

Methyl 5-*O*-methyl ribofuranosides were prepared from ribose[18] and purified by chromatography on Dowex 1-X2 (200–400 mesh) in the hydroxide form.[19] The glycosides were hydrolyzed (0.04 N HCl, 100 °C for 2.5 h) and 5-*O*-methylribose was purified by chromatography on Dowex 50-X8 (200–40 mesh)(Ba^{2+}).

Aldoses and aldose phosphate enriched with ^{13}C were prepared as described previously.[13a,13b,20] Methyl [1-^{13}C]arabinofuranoside-*5*-P was prepared by treating the free acid with 0.1 N H_2SO_4 in anhydrous methanol for 28 h at room temperature. [2-^{13}C]Fructose-*1,6*-P_2 was obtained by the action of hexokinase, glucose-*6*-P isomerase, fructose-*6*-P kinase, and myokinase on Mg^{2+}–ATP and [2-^{13}C]glucose.[21]

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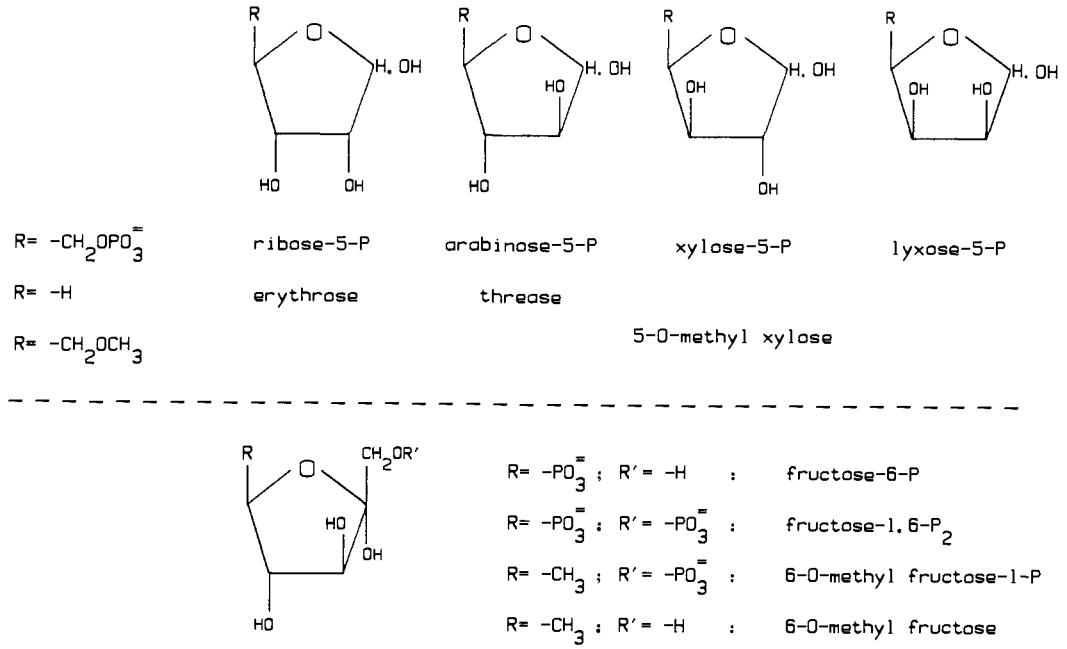
Chart I



[2-^{13}C]Fructose-*6-P* was obtained from [2-^{13}C]fructose-*1,6-*P_2 by the sequential action of acid phosphatase and hexokinase.[12] Fructose-*6-P* (0.5 mmol) was purified by chromatography on a DEAE-Sephadex A-25 column (2 × 50 cm) in the bicarbonate form using a linear bicarbonate gradient (3 L, 0–0.4 M $NaHCO_3$, pH 7.5). Fractions containing the ketose phosphate were pooled, treated batchwise with excess Dowex 50-X8 (20–50 mesh)(H^+), filtered, and concentrated in vacuo at 30 °C.

6-*O*-Methyl[2-^{13}C]fructose-*1-P* was prepared by the action of fructose-*1,6-*P_2 aldolase and triosephosphate isomerase on 3-*O*-methylglyceraldehyde and [2-^{13}C]fructose-*1,6-*P_2. 5-*O*-Methylribose was treated with lead tetraacetate (in the manner described for the preparation of glyceraldehyde-*3-P* from fructose-*6-P*)[13b] to yield 2-*O*-formoyl-3-*O*-methylglyceraldehyde, which was incubated with 0.1 N H_2SO_4 for 4 h at 35 °C to give 3-*O*-methylglyceraldehyde. The solution was deionized by successive treatments with Dowex 1-X8 (OAc^-) and Dowex 50-X8 (H^+). [2-^{13}C]Fructose-*1,6-*P_2 (0.6 mmol) and 3-*O*-methylglyceraldehyde (5 mmol) were incubated with 500 units (1 unit = 1 μmol·min^{-1}) of aldolase and 1000 units of triosephosphate isomerase at pH 7.2 in a total volume of 120 mL. The reaction was complete when 25 μL of the reaction solution no longer lowered the absorbance at 340 nm when added to a solution (975 μL) containing aldolase, glycerol phosphate dehydrogenase, and NADH. (Aldolase apparently has a high Michaelis constant for 6-*O*-methylfructose-*1-P*, and under the assay conditions, fructose-*1,6-*P_2 and the triosephosphates promote rapid oxidation of NADH whereas 6-*O*-methylfructose-*1-P* does not.) The ketose phosphate was purified by anion-exchange chromatography as described for fructose-*6-P*. The presence of triosephosphate isomerase and aldolase in the reaction mixture caused the ^{13}C label of 6-*O*-methyl[2-^{13}C]-fructose-*1-P* to be diluted to 45 atom % ^{13}C due to isotopic equilibration at C-2 and C-5 of [2-^{13}C]fructose-*1,6-*P_2.

6-*O*-Methyl[2-^{13}C]fructose was obtained by treating 6-*O*-methyl[2-^{13}C]fructose-*1-P* with acid phosphatase, deproteinizing with ethanol, and deionizing with Dowex 50-X8 (H^+) and Dowex 1-X8 (OAc^-).

All compounds were greater than 95% pure as determined by ^{1}H NMR, ^{13}C NMR, enzymatic, and/or phosphate analysis. Tetrose concentrations were determined colorimetrically,[22a] sugar phosphate concentrations were determined by phosphate analysis,[22b] and other reducing sugar concentrations were determined by reacting the sugar with a slight excess of lead tetraacetate, quenching with potassium iodide, and titrating the liberated iodine with sodium thiosulfate.

Instrumentation. Fourier transform NMR spectra were obtained by using 5-mm (^{1}H) or 10-mm (^{13}C) samples containing 15–100% ^2H_2O for field frequency locking. ^{1}H NMR spectra were obtained at 300 MHz on a Bruker WM-300 spectrometer. ^{1}H-Decoupled ^{13}C NMR spectra were obtained at 15, 20, 75, and 100 MHz on Bruker WP-60, Varian CFT-20, Bruker WM-300, and Bruker WH-400 spectrometers.

^{1}H ST-NMR spectra were obtained by using standard Bruker hardware and software. Selective saturation of ^{13}C resonances (at 75 and 100 MHz) was achieved by feeding the output of a Programmed Test Sources-160 radio frequency synthesizer into the ^{13}C observation coils. Double irradiation of ^{13}C resonances was achieved on the Bruker WM-300 by using an audio frequency (Hewlett Packard HP4204A) synthesizer to modulate the ^{13}C decoupling frequency so as to produce sidebands with frequencies identical with those of the two resonances to be saturated. Decoupling frequencies were gated by the computer and amplified (Avantek AV-4T) to a maximum power of approximately 0.5 W.

A microelectrode (Microelectrodes Inc.) was used for pH measurements of samples in the NMR tube. Temperature was measured with a Fluke 2190A digital thermometer and a copper–constantan thermocouple.

Solution Preparation and Temperature Measurement. Solutions of nonphosphorylated sugars were treated batchwise with excess Dowex 1-X8 (OAc^-) and Dowex 50-X8 (H^+), concentrated at 30 °C in vacuo, and concentrated several times from ~3 mL of ^2H_2O. Distilled ^2H_2O was added to achieve the desired sugar concentration, the solution was passed through a small (0.5 × 2 cm) column of Chelex 100 resin (H^+), and the eluant was collected in an NMR tube. Solutions of KCl, ^{2}HCl, or appropriate buffer in ^2H_2O were treated in a similar fashion with Chelex resin and added to the desired concentration.

Solutions of phosphorylated sugars (pH ~4) were treated with Chelex resin and concentrated to ~0.5 mL. Solutions were made 15% in ^2H_2O and 2 mM in ethylenediaminetetraacetic acid, and the pH was adjusted with 2 N NaOH. All NMR tubes were filled to a height slightly greater than the coil length of the probe, fitted with Teflon vortex plugs (10-mm tubes), and sealed with plastic caps.

Temperature measurements were obtained with standard solutions of KCl (for neutral sugars) or glycerol-*1-P* (for phosphorylated sugars) at the same pH and concentration as the sample being analyzed. A thermocouple was securely positioned in the standard solution, the sample was lowered into the probe, and the temperature was recorded after ~25 min of equilibration. Actual sample solutions, occasionally equilibrated and measured similarly, had the same temperature as the standards.

Nonuniform sample heating due to broad-band ^{1}H decoupling at high fields was minimized by using chirp frequency modulation[22c] of the decoupling radio frequencies for ^{13}C ST-NMR measurements at 100 MHz and two-level decoupling protocols for ^{13}C equilibrium measurements at 75 MHz. Temperatures reported are accurate to within 1 °C.

Equilibrium Measurements. Equilibrium constants were determined from ^{13}C NMR spectra of monosaccharides enriched with ^{13}C at the anomeric carbon. A rapid-pulse technique[23a] was applied with pulse

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widths and recycle times calculated from the Ernst relationship. Conservative estimates of ^{13}C spin–lattice relaxation times of 3 and 40 s were used for the anomeric carbons of aldoses[23b] and ketoses, respectively. The accuracy of this method was verified by measuring equilibrium proportions by using the more reliable but time-consuming 90° pulse–$7T_1$ delay method for [1-^{13}C]erythrose. Solution proportions obtained by both procedures and determined from spectra obtained with or without nuclear Overhauser enhancement were identical within experimental error. Line-broadening factors of 2–3 Hz were used to produce ideal line shapes, and measurements were made in triplicate at each temperature.

^{13}C NMR Line-Broadening Measurements. ^{13}C NMR spectra were obtained at 15 MHz with a digital resolution of 0.12–0.24 Hz/point, and the line widths were determined by measuring the width at half-height of plotted spectra. The line width of internal $^{13}CH_3OH$ (0.1% v/v) was used to determine ^{13}C resonance line widths in the absence of chemical exchange. The observed line widths of the C-1 resonances of the aldose phosphates were found to depend on the position of the carrier frequency used for the ^1H decoupling. The magnitude of this effect was determined by setting the ^1H carrier frequency near the resonance frequency of the methanolic protons and comparing the line widths of $^{13}CH_3OH$ and methyl [1-^{13}C]arabinofuranoside-*5-P* at various temperatures. At all temperatures the line widths of the glycosides were 0.32 Hz greater than the methanol line width. Therefore, 0.32 Hz was added to the observed line width of the internal $^{13}CH_3OH$ in line-broadening experiments, and this corrected value was subtracted from the observed line widths of the anomeric carbons to determine line broadening due to chemical exchange. It should be noted that this correction is only important when obtaining rate constants < 10 s^{-1} since with faster exchange rates a difference of 0.32 Hz causes little error (<10%) in the calculated rate constant.

Spectra were obtained by using 90° pulses and long relaxation delays. Measurements were performed in triplicate at each temperature and pH.

Saturation-Transfer NMR Measurements. Single-resonance saturation experiments were conducted at 300 MHz (^1H) and 75 and 100 MHz (^{13}C). The carbonyl resonance was saturated for defined times before applying the 90° observe pulse.[14] Care was taken to minimize systematic errors associated with instrumental instability by randomly varying the order and magnitude of the presaturation times.

Double-resonance saturation ^{13}C NMR experiments were performed by continuously saturating the desired resonances (except during data acquisition) and performing a standard, nonselective inversion–recovery pulse sequence.[23c] Single irradiation experiments with [1-^{13}C]erythrose showed that both the presaturation and inversion–recovery techniques gave equivalent exponential time constants for intensity loss and recovery, respectively.

Calculation of Rate Constants. Rate constants from line-broadening measurements were calculated from the observed line widths according to the expression[24a]

$$k_i = \pi(\Delta\nu_i - \Delta\nu^\circ) \quad (1)$$

where $\Delta\nu_i$ and $\Delta\nu^\circ$ are the observed line widths (in hertz) for an exchanging nucleus and for a nonexchanging nucleus, respectively. The validity of this approximation was checked for those cases in which exchange rates were extremely large and the proportion of the acyclic carbonyl form was very small (e.g., ribose-*5-P* and arabinose-*5-P*), by obtaining measurements at 75 MHz and by modeling of the two-site NMR-exchange equations.[24b]

ST-NMR data obtained by the presaturation technique were adequately described by the equation[14,25]

$$dI_t/dt = \rho I_0 - (\rho + k)I_t \quad (2)$$

where I_t is the intensity of the observed resonance at time t after the onset of saturation of the carbonyl resonance, I_0 is the intensity at $t = 0$ (i.e., without application of the saturation frequency), ρ is the nuclear relaxation rate (T_1^{-1}), and k is the first-order rate constant for the chemical conversion of the observed nucleus into the saturated nucleus. The data were fitted by the method of least squares to the linearized solution of eq 2

$$\ln (I_t - I_\infty) = A - Bt \quad (3)$$

where I_∞ is the intensity of the observed resonance at a time $t > 10[\ln 2/(\rho + k)]$ after the onset of saturation, $A = \ln (I_0 - I_\infty)$, and $B = p + k$. Note that

$$I_\infty/I_0 = \rho/(\rho + k) \quad (4)$$

The values of ρ and k were determined from the slope and intercept of semilogarithmic plots. An independent measurement of I_∞/I_0 was used to check the derived value of k [$=(1 - I_\infty/I_0)B$].

Inversion–recovery ST-NMR data were fitted to the equation

$$\ln (I_\infty - I_t) = \text{constant} - Bt \quad (5)$$

where I_∞ and B have their former meanings but now t is the time between inversion of the observed nucleus with a 180° pulse and its observation with a 90° pulse.

A minimum of 10 useable data points (usually 15) always gave excellent fits to the above equations over a range of times > $2[\ln 2/(\rho + k)]$.

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Table I. Equilibrium Compositions of Selected Furanose Phosphates[a]

sugar	percent in solution[b]			
	β	α	hydrate	free carbonyl
ribose-*5-P*	63.9	35.6	0.5[d]	0.1[c]
arabinose-*5-P*	40.4	57.3	2.2	0.2[c]
xylose-*5-P*	42.4	52.6	4.7	0.3[d]
lyxose-*5-P*	24.8	70.5	4.3	0.4[d]
fructose-*6-P*	81.8	16.1	*e*	2.2
fructose-*1,6-*P_2	86.0	13.1	*e*	0.9

[a] Determined by ^{13}C NMR analysis of the ^{13}C-enriched compounds (0.4 M in 15% ^2H_2O) at 15 MHz; 6 °C, pH 4.5. [b] The error in the measurements is <8% of the value given, unless otherwise noted. [c] These values may be in error by as much as 50% due to the very low amounts in solution. [d] The error in these values is <15%. [e] Resonances for these forms were not observed.

Results and Discussion

Solution Composition of Furanoses and Furanose Phosphates. In this study we wished to determine the rate constants for ring opening and ring closing in a number of biologically important aldose and ketose phosphates. These values are of interest in evaluating enzyme–substrate interactions and the rates of chemical transformations and in establishing the relationship of configuration to reactivity. Ring-opening and -closing rates can be obtained by NMR methods provided that the various forms present in solution can be identified and quantified. The stereochemical relationships of the aldoses and ketoses included in this study are shown in Chart I.

Resonances attributable to α-furanose, β-furanose, hydrate, and free carbonyl forms are obervable in the low-temperature (6 °C) ^{13}C NMR spectra of the [1-^{13}C]aldofuranose phosphates (Table I). At 6 °C it can be seen that cyclic forms account for 95–99% of the total sugar present; the remainder comprises hydrate and carbonyl forms at a ratio of ~10. At higher temperatures, the percentage of free carbonyl form is expected to increase as it does for the simple sugars,[2d,14] and indeed for solutions of xylose-*5-P*, heats of reaction for ring opening ($\Delta H^\circ_{\text{cyclic}\rightarrow\text{open}} \sim 5$ kcal/mol) indicate that this is so. However, at higher temperatures line broadening of the carbonyl resonance severely degrades the signal to noise in the spectra, precluding a more thorough analysis of the thermodynamics of tautomerization of the aldofuranose phosphates.

A study of the effect of temperature on the tautomeric equilibria of fructose-*6-P* was aided by the large proportion of carbonyl form present in aqueous solutions of this compound (Figure 1). The heat of reaction for ring opening of the β anomer ($\Delta H^\circ_{\beta\rightarrow o} = 4.8 \pm 0.5$ kcal·mol^{-1}) is significantly greater than that for the α anomer ($\Delta H^\circ_{\alpha\rightarrow o} = 3.8 \pm 0.4$ kcal·mol^{-1}). The overall heat of reaction for the conversion of β- to α-furanose ($\Delta H^\circ_{\beta\rightarrow\alpha} \simeq 1$ kcal·mol^{-1}) indicates that the ratio of β-to-α forms of fructose-*6-P* will decrease with increasing temperature. Entropy of ring opening for both anomers is ~10 cal·K^{-1}·mol^{-1}. ΔH° and ΔS° for the anomerization of fructose-*6-P* were ~20% less than those for threose, its simplest structural analogue.[14] Results from similar studies on fructose-*1,6-*P_2 over a more limited temperature range are similar to those obtained for fructose-*6-P*.

Aqueous solutions of the aldopentose phosphates contain less carbonyl form (~0.1–0.4%) than those of ketose phosphates (~1–2%). In addition, the aldehydes are appreciably hydrated whereas the ketones are not. This behavior is also observed for acylic aldose and ketose phosphates.[13b] For example, erythrose-*4-P* is greater than 90% hydrated in aqueous solution, whereas ribu-

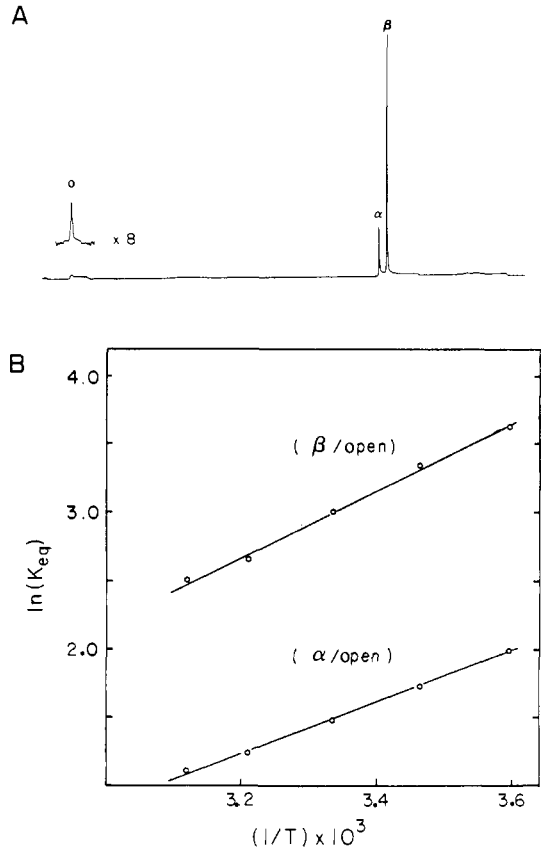

Figure 1. Temperature dependence of the tautomeric equilibria of [2-^{13}C]fructose-*6-P* (0.4 M sugar at pH 4.5 in 15% ^2H_2O). (A) The 15-MHz ^{13}C NMR spectrum at 42 °C. (B) Temperature dependence of the ring-opening equilibria. $\Delta H°_{\beta\to o}$ = 4.8 ± 0.5 kcal/mol; $\Delta H°_{\alpha\to o}$ = 3.8 ± 0.4 kcal/mol.

lose-*1,5-P*$_2$ is only 12% hydrated despite the electron-withdrawing properties of the phosphate group which should render the carbonyl carbon of ribulose-*1,5-P*$_2$ more electrophilic.[26]

The proportions of the acyclic forms in the aldopentose phosphates are influenced by configuration; the proportion is higher when, in the furanose forms, OH-3 and C-5 are cis (*xylo* and *lyxo*) than when they are trans (*ribo* and *arabino*) (Table I, Chart I). These observations are consistent with the observation that xylose-*5-P* and lyxose-*5-P* isomerize more readily to xylulose-*5-P* at pH 6 than arabinose-*5-P* and ribose-*5-P* isomerize to ribulose-*5-P*. Stored solutions of xylose-*5-P* and lyxose-*5-P* have been found to contain up to 20% keto sugar, behavior consistent with isomerization occurring by enolization of the carbonyl form.

aldo-Pentofuranose phosphates with OH-1 trans to OH-2 are generally more stable (Table I) than those having the cis arrangement. *xylo*-Pentofuranose phosphate is an exception, indicating that destabilization arising from two cis-1,3 interactions in the β anomer is equivalent to that arising from a single cis-1,2 interaction in the α anomer. Nonphosphorylated furanoses behave similarly,[2a] as do 5-*O*-methylribose, 5-*O*-methylarabinose, 5-deoxyxylose, and 5-deoxylyxose (data not shown).

Knowledge of the tautomeric compositions of pentose phosphates may permit predictions of the solution behavior of hexoses and their derivatives. As the equilibrium compositions of 5-*O*-methyl- and 5-deoxypentose solutions are very similar to those of the pentose phosphates of the same configuration, it appears that variations in substituents at C-5 have little effect on the tautomeric equilibria. This being the case, furanose forms of hexoses and hexose phosphates should have stabilities similar to those of the configurationally related pentose derivatives. In addition, similar equilibria should be established between the furanose and acyclic forms in the hexoses as are observed in the

(26) Bell, R. P. *Adv. Phys. Org. Chem.* **1966**, *4*, 1.

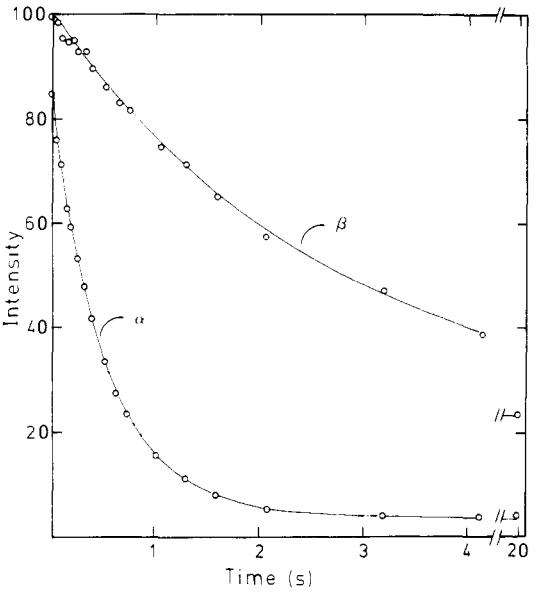

Figure 2. ^{1}H ST-NMR experiment with 5-*O*-methylxylose in ^2H_2O at 40 °C, [$^2H^+$] = 0.004 M. The curves were drawn from the exponential form of eq 3 with, for the β anomer, I_0 = 100, I_∞ = 23.4 ρ = 0.091 s^{-1}, k = 0.28 s^{-1} and for the α anomer, I_0 = 85, I_∞ = 3.9, ρ = 0.11 s^{-1}, k = 1.8 s^{-1}.

Table II. Acid-Catalyzed Ring-Opening Rate Constants for Selected Furanoses at 40 °C[a]

sugar	$K_\alpha(\alpha/o)$	$K_\beta(\beta/o)$	$k_{2_{H^+}}(\alpha\to o)$,[b] M^{-1} s^{-1}	$k_{2_{H^+}}(\beta\to o)$,[b] M^{-1} s^{-1}
erythrose	14	35	550	150
threose	34	25	91	970
5-*O*-methylxylose	73	57	410	62
6-*O*-methylfructose	5.5	21	8.5	6.0

[a] Obtained by ^{1}H (threose and 5-*O*-methylxylose) or ^{13}C (erythrose and 6-*O*-methylfructose) ST-NMR measurements over an 8- to 10-fold range of $^2H^+$ concentrations in ^2H_2O. The ionic strength was adjusted to 0.20 M with KCL. Values are calculated by linear regression against eq 6 (see text) and are accurate to within 10%. (Note that −log [$^2H^+$] = pH_{obsd} + 0.4.) [b] Rate constant for ring closure are obtained as $k_{o\alpha} = K_\alpha k_{\alpha o}$; $k_{o\beta} = K_\beta k_{\beta o}$.

pentoses, and the concentration of furanose forms can be used to estimate the amount of the acyclic carbonyl present. For instance, altrose contains approximately 20% α-furanose and 13% β-furanose forms at 40 °C.[2b] Altrofuranoses have the *arabino* configuration, and the data for arabinose-*5-P* at 6 °C (Table I) can be used to predict that solutions of altrose at 40 °C should contain approximately 0.2% of the acyclic aldehyde form. (It is assumed that the temperature dependence of the tautomeric equilibria for arabinose-*5-P* is similar to that for the tetroses and fructose-*6-P*.) Hayward and Angyal[2d] predict that 0.07% of the aldehyde is present from circular dichroism studies. The amount of furanose forms in solutions of hexoses is easily measured, and the amount of carbonyl form can be approximated from the data in Table I. Values obtained in this way may be useful in the interpretation of kinetic and physical data for reactions of carbohydrates and their derivatives that are known to involve carbonyl forms.

Carbonyl Form and Anomerization of Simple Furanoses. A single-resonance saturation NMR experiment with 5-*O*-methylxylose is representative of the experiments to be described. This compound exists in solution in four forms (54% α-furanose, 42% β-furanose, 4% hydrate, 0.7% carbonyl at 40 °C). The application of a saturating radio frequency to the resonance of the aldehydic proton causes the α and β resonance intensities to decay in a first-order fashion predicted by eq 2. The more rapidly descending curve for the α-furanose anomer reveals that α-xylofuranose opens more rapidly to the aldehyde form than does β-xylofuranose (Figure 2).[27] It is apparent from Figure 2 that the thermodynamic

Table III. Double Irradiation Experiment with [1-^{13}C]Erythrose at 40 °C[a]

conditions	signals irradiated	signal obsd	$\rho + k$, s^{-1}	k, s^{-1}
$pH_{obsd} = 2.24$[b]	o	α	1.14	0.98
	o + β	α	1.16	0.99
	o + α	β	0.49	0.31
	o	β	0.50	0.33
$pH_{obsd} = 6.50$[c]	o	α	1.26	1.1
	o + β	α	1.31	1.1
	o + α	β	0.57	0.37
	o	β	0.56	0.37

[a] [1-^{13}C]Erythrose was 0.15 M in ^2H_2O containing 3 mM, 2,6-lutidine with ^{2}HCl and KCl, I = 0.20 M. [b] [$^2H^+$] = 2.3 mM. [c] [$^2H^+$] = 0.13 μM.

stabilities of the cyclic forms do not correlate with kinetic stabilities, since $K_{\alpha/\beta} = 1.3$ whereas $k_{\alpha o}/k_{\beta o} = 6.4$.

A more detailed analysis of four furanoses (Table II) confirms the expectation that the ring-opening reactions are acid-catalyzed. At [$^2H^+$] > 10^{-4} M, the ring-opening reactions obey the relationship

$$k_{obsd} = k_o + k_{2_H^+}[^2H^+] \quad (6)$$

Values for k_o, the ^2H_2O-catalyzed rate constant, varied between 0.02 and 0.06 s^{-1} for the aldoses and were <0.02 s^{-1} for 6-*O*-methylfructose. (These values were determined by rather large extrapolations of the data and are not known with precision.) Again, thermodynamic stabilities do not correlate with kinetic stabilities.

A general scheme for interconversion between tautomers is shown in Scheme I where the carbonyl form is depicted as the sole and obligatory intermediate. The possible existence of additional pathways for exchange between cyclic forms other than via the carbonyl form was examined by double-resonance saturation experiments with [1-^{13}C]erythrose.

The only observed ^{13}C-enriched resonances in solutions of [1-^{13}C]erythrose correspond to the C-1 carbons of α-furanose, β-furanose, hydrate, and carbonyl forms. Only two conditions allow for the existence of another, more enigmatic intermediate; either the undetected intermediate has a ^{13}C resonance coincident with that of the carbonyl resonance or the resonance of the intermediate cannot be detected. In the first case, the single-resonance saturation experiment still gives ring-opening rate constants. In the second case, the proposed intermediate may be (a) present either at a very low level or (b) may exchange so rapidly with the cyclic forms that the associated line broadening renders it undetectable. If either of these conditions exist, then the single-resonance saturation experiment will not yield ring-opening rate constants but will yield a rate constant that is composed of the rate constant for ring opening and the exchange rate between the carbonyl form and the proposed intermediate. A double-resonance saturation experiment, where the carbonyl resonance and one cyclic resonance are saturated simultaneously, can discriminate between these possibilities. If single- and double-resonance saturation experiments yield the same result, then anomerization involves only forms with coincident carbonyl resonances. If they do not, the presence of an undetected intermediate is implied.

When both the aldehyde and α-furanose resonances of [1-^{13}C]erythrose are simultaneously saturated (Figure 3, Table III), equilibrium intensities of the β-furanose and hydrate resonances at t = 20 s (right spectrum) are virtually the same as when the aldehyde resonance alone is saturated (center spectrum). Under conditions where the reaction proceeds predominantly (>95%) by either $^2H^+$ or O^2H^- catalysis, rates of saturation transfer and calculated ring-opening rate constants were unaffected by the additional saturation of a cyclic carbon resonance (Table III). These results are consistent with a mechanism of exchange between cyclic forms where the form (or forms) that produces the free carbonyl resonance is the kinetically important intermediate. Equally important, single-resonance saturation experiments will yield accurate ring-opening rate constants.

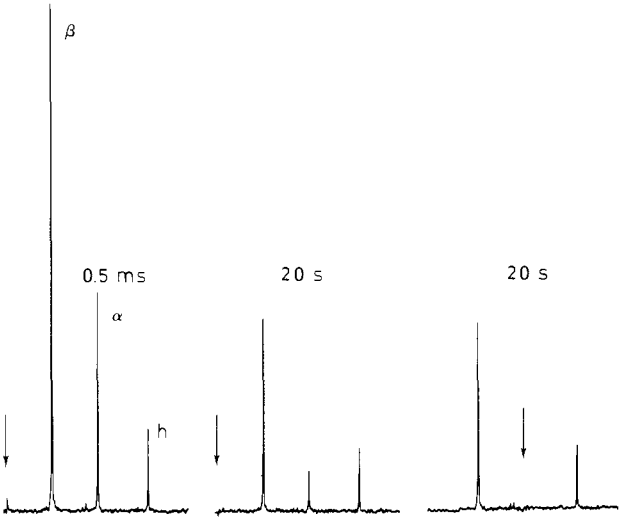

Figure 3. Double-resonance saturation experiment with [1-^{13}C]erythrose at 40 °C, [$^2H^+$] = 0.002 M. Only α-furanose, β-furanose, and hydrate resonances are shown. Two frequencies were applied for the indicated times prior to data acquisition; one frequency was applied at the aldehyde resonance frequency, and the other was set to the position indicated by the arrow. Note the similar β-furanose and hydrate resonance intensities in the center and right spectra.

As discussed previously[14], the presence of more than one form contributing to the observed carbonyl resonance can be determined from line-width analysis. If only one carbonyl form is present, then it should experience line broadening due to exchange with the other forms predicted by

$$\Delta\nu_{exchange} = \frac{1}{\pi}(k_{o\alpha} + k_{o\beta} + k_{oh}) \quad (7)$$

where $\Delta\nu_{exchange}$ = line width due to chemical exchange. When more than one carbonyl form is present, only a fraction (e.g., 20%) of the species giving rise to the free carbonyl resonance is in rapid exchange with the cyclic forms, and the equilibrium constants used in the above calculation would be in error (by a factor of 5). The resonance of the exchanging fraction would be severely broadened while that of the nonexchanging fraction (80% of the signal) would be unaffected, and the observed carbonyl resonance would not be broadened to the extent predicted by eq 7. When the experimentally determined equilibrium and rate constants at [$^2H^+$] = 2.3×10^{-3} M are used, the predicted line broadening of 26 ± 3 Hz can be compared with the experimentally observed line broadening of 25 ± 1 Hz. The corresponding values when [$^2H^+$] = 1.3×10^{-7} M are 28 ± 3 and 26 ± 1 Hz, respectively. The agreement between predicted and experimental values for erythrose suggests that, regardless of its structure (linear, pseudocyclic, etc.), essentially all the carbonyl form participates in the anomerization reaction; that is, there is only one kinetically significant carbonyl form.

Pseudocyclic intermediates[2] were invoked to explain both anomalous anomerizations and the slow rate of oxygen exchange from water into the hemiacetal oxygen of sugars relative to the rate of interconversion between cyclic forms. Our results demonstrate that only one kinetically significant carbonyl form in the anomerization of erythrose is important, regardless of the conditions of catalysis. The observed differences in oxygen exchange vs. anomerization rates may be explained by noting that exchange of water oxygen with a hemiacetal oxygen requires both hydration and dehydration of the carbonyl form prior to ring closure. Since hydration and dehydration rates are slower than ring-opening and -closing rates (Figure 2 and ref 14), the appearance of oxygen

(27) The ratio of α-to-β peak heights at t = 0 is <1, even though the equilibrium ratio of α-to-β forms is 1.3. This difference is caused by the higher rate of exchange of the α anomer, which broadens its resonance more than for the β anomer. The signal *height* of the α anomer at t = 0 is smaller than that of the β anomer, but its *area* is greater.

Table IV. Tautomerization of Various Sugars at 25 °C

sugar	pH 6.9 $10^3(k_{\alpha o} + k_{\beta o})$, s^{-1}	$(k_{o\alpha} + k_{o\beta})$, s^{-1}	$k_{mut}(H^+)$,[e] $M^{-1}\,s^{-1}$	$10^{-3} \times k_{mut}(OH^-)$,[e] $M^{-1}\,s^{-1}$
glucopyranose[a]	1.5	20	0.01	0.08
galactopyranose[b]	1.3	3		
galactofuranose[b]	130	17		800[d]
erythrofuranose[c]	480	15	100[d]	4000[d]
6-*O*-methylfructo-furanose[c]	200	5	3[d]	900[d]

[a] Calculated from data in ref 5a. [b] Calculated from data in ref 5b. [c] Values for erythrose and 6-*O*-methylfructose were obtained at 40 °C and adjusted by using activation energies of 14 kcal·mol^{-1} (ring closing) and 8 kcal·mol^{-1} (ring opening) previously determined for threose.[14] These values are for catalysis by ^2H_2O and are expected to be smaller than the corresponding values for H_2O by a factor of ~3.[2b] [d] These values are approximations and should only be taken to indicate the order of magnitude of the reported rate constant. The values for erythrose and 6-*O*-methylfructose are for catalysis by $^2H^+$ and O^2H^-, which are expected to be smaller than the corresponding values for H^+ and OH^- by a factor of ~1.4.[2b] [e] $k_{mut} = k_{\alpha\beta} + k_{\beta\alpha} = (k_{\alpha o}k_{o\beta} + k_{\beta o}k_{o\alpha})/(k_{o\alpha} + k_{o\beta})$.

label at the hemiacetal oxygen is slower than the rate of interconversion of ring forms. The slow exchange of water oxygen with carbonyl oxygen is not inconsistent with the presence of only one kinetically significant carbonyl form in anomerization.

This conclusion is based on observations with erythrose. The rates of hydration of various aldehydes affect the extension of this finding to anomerization of other sugars. The rate constant for hydration of acetaldehyde in neutral aqueous solution at 25 °C is ~0.03 s^{-1}.[28] Those for galactose (0.01 s^{-1}) and erythrose (0.09 s^{-1}) are similar,[5b,29] indicating similar reactivity of the carbonyl carbon, contrary to the decreased reactivity one would expect in pseudocyclic forms. We conclude that anomerization is best represented by the mechanism presented in Scheme I.

Comparison between Overall and Unimolecular Rate Constants of Anomerization. The fact that kinetic and thermodynamic stabilities of furanose rings are not correlated serves to emphasize the importance of obtaining unimolecular rate constants for mechanistic studies. The traditional method of studying the kinetics of anomerization involves measuring the rate of equilibration of one cyclic form with another (i.e., mutarotation). By this method, the observed complex rate constant, k_{mut}, is related to the unimolecular rate constants of Scheme I by the expression

$$k_{mut} = k_{\alpha\beta} + k_{\beta\alpha} = \frac{k_{\alpha o}k_{o\beta} + k_{\beta o}k_{o\alpha}}{k_{o\alpha} + k_{o\beta}} \quad (8)$$

Using the data in Table II for the $^2H^+$-catalyzed reaction of 5-*O*-methylxylose, we find $k_{mut}(^2H^+) = 99\ M^{-1}\,s^{-1}$, which implies $k_{\alpha\beta}(^2H^+) = 43\ M^{-1}\,s^{-1}$ and $k_{\beta\alpha}(^2H^+) = 56\ M^{-1}\,s^{-1}$. However, $k_{\alpha o}(^2H^+) = 410\ M^{-1}\,s^{-1}$, whereas $k_{\beta o}(^2H^+) = 62\ M^{-1}\,s^{-1}$. In other words, even though the rate constant for α-furanose ring opening ($k_{\alpha o}$) is much larger than that for β-furanose ring opening ($k_{\beta o}$), the constant for conversion of α-furanose to β-furanose ($k_{\alpha\beta}$) is smaller than that for conversion of β-furanose to α-furanose ($k_{\beta\alpha}$). This is due to the fact that the carbonyl form partitions asymmetrically between the two cyclic forms. This assymetry is dramatically illustrated by the finding that for the $^2H^+$-catalyzed reaction of 5-*O*-methylxylose, we find $k_{o\alpha}/k_{o\beta} = 8.5$, whereas for the configurationally similar tetrose, threose, $k_{o\alpha}/k_{o\beta} = 0.13$. Clearly, caution must be used in interpreting k_{mut} in mechanistic terms.

Furanose and Pyranose Interconversions. Anomerization data for selected sugars (Table IV) show that, at neutral pH where catalysis by H_2O predominates, rate constants for ring closure of the aldopyranoses, aldofuranoses, and the ketofuranose differ by factors of ~2–7, whereas the ring-opening rate constants differ by factors of up to 370. These results also indicate that both H^+ and OH^- catalysis of anomerization are 10^4-fold more effective in aldofuranoses than in aldopyranoses.[30] Since the solution composition of these compounds is not strongly affected by pH, it follows that both ring-opening *and* ring-closing rates are increased substantially by H^+ and OH^- ions. Ring structure affects the rate constants for H^+ and OH^- catalysis more than those for H_2O catalysis, suggesting that these species catalyze mechanistically distinct reactions.[3c]

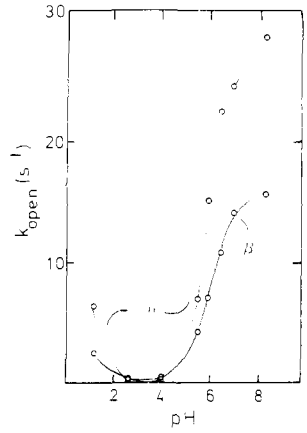

Figure 4. pH dependence of the ring-opening rate constants for [1-^{13}C]ribose-5-*P* (0.3 M sugar in 15% ^2H_2O at 24 °C). Rate constants were obtained from line-broadening experiments except for the values at pH 2.3 and 4.0 which were obtained by ^{13}C ST-NMR experiments.

Table V. Ring-Opening Rate Constants for Sugar Phosphates at 40 °C[a]

sugar	pH 4.2[c] $K(\alpha/\beta)$[b]	$k_{\alpha o}$, s^{-1}	$k_{\beta o}$, s^{-1}	pH 7.5[d] $k_{\alpha o}$, s^{-1}	$k_{\beta o}$, s^{-1}
ribose-5-*P*	0.60	0.86	0.44	100	40
arabinose-5-*P*	1.47	0.64	0.49	50	33
xylose-5-*P*	1.19	0.42	0.17	33	9.6
lyxose-5-*P*	2.21	0.24	0.22	13	13
fructose-6-*P*	0.28	0.20	0.22	18	21
6-*O*-methylfructose-1-*P*	0.25	0.68	1.2	18[e]	130[e]
fructose-1,6-P_2	0.25	1.2	1.9	28[e]	140[e]

[a] Conditions: 0.15 M sugar phosphate in 15% ^2H_2O. [b] Values are the average of three determinations and are accurate to within 6%. [c] From ^{13}C ST-NMR measurements at 75 or 100 MHz. Values are accurate to within 12% except for those of fructose-1,6-P_2 and 6-*O*-methylfructose-1-*P* which may be in error by as much as 50% due to high exchange rates and long relaxation times. [d] From ^{13}C line-width measurements at 15 and 75 MHz. Values are averages of three determinations and are accurate to within 10%. [e] The apparent line widths contained contributions from ^{13}C–^{31}P coupling. Intrinsic line widths were calculated from computer simulation by varying the values of the coupling constant and the intrinsic line width until the best fit to the observed spectrum was obtained.

Phosphate-Catalyzed Tautomerization. The ring-opening rate constants of aldose and ketose phosphates are higher than those of their nonphosphorylated analogues. These rate constants are unaffected by the concentration of aldose phosphate over a 6-fold range, demonstrating that intramolecular catalysis by phosphate predominates over intermolecular phosphate catalysis. The observed linear relationship at intermediate pH values between rate constants and the ionization state of the phosphate group (pK = 6.1) supports this contention. Below pH 2 and above pH 7, H^+ and OH^- catalysis is observed (Figure 4).

The effect of phosphate ionization on catalysis was examined by measuring the ring-opening rate constants at pH 4.2 (mo-

(28) Bell, R. P.; Evans, P. G. *Proc. R. Soc. London, Ser. A.* **1966**, *291*, 297.
(29) Risley, J. M.; Van Etten, R. L. *Biochemistry* **1982**, *21*, 6360.

(30) (a) Although not directly comparable, ring-opening rates for 2-(hydroxymethyl)benzaldehyde (I) and 2-(hydroxyethyl)benzaldehyde (II)[30b] indicate that OH^- ion catalysis of ring opening in I is ~20-fold greater than that in II, while H^+ catalysis of ring opening in I is ~0.8 times that in II. (b) Harron, J.; McLelland, R. A.; Thankachan, C.; Tidwell, T. T. *J. Org. Chem.* **1981**, *46*, 903.

Table VI. Comparison of Ring-Opening Rate Constants for Fructose Analogues at pH 7.5 and 40 °C[a]

sugar	$k_{\beta o}$, s^{-1}	$k_{\alpha o}$, s^{-1}	$k_{\beta o}/k_{\alpha o}$
6-*O*-methylfructose[b]	1.3	0.9	1.4
6-*O*-methylfructose + 0.15 M glycerol-*1*-*P*	5.3	4.5	1.2
fructose-*6*-*P*	21	18	1.2
6-*O*-methylfructose-*1*-*P*	130	18	7.2
fructose-*1*,*6*-P_2	140	28	5.0

[a] Conditions: 0.15 M sugar in 15% ^2H_2O. Values are accurate to within 12%. [b] These values were obtained from the ordinal intercept of a plot of k_{obsd} vs. [2,6-lutidine] at pH 7.5; I = 0.30 M with KCl.

noanion) and 7.5 (dianion). Ring-opening rate constants at pH 4.2 were generally 60–100 times smaller than those at pH 7.5 (Table V). All the sugar phosphates studied showed similar behavior, although the catalytic efficiency of the phosphate group depends on ring configuration. For aldofuranose phosphates, $k_{\alpha o} > k_{\beta o}$ at both pH values, whereas for ketofuranose phosphates, $k_{\beta o} > k_{\alpha o}$. Phosphorylation of the primary hydroxyl at C-1 of ketoses greatly increases ring-opening rates (compare values for fructose-*6*-*P* with those for fructose-*1*,*6*-P_2 and 6-*O*-methylfructose-*1*-*P* in Table V). It appears that the phosphate dianion is a better catalyst than the monoanion based on the rate constants obtained at pH 4.2 and 7.5, although differential contributions to these numbers through catalysis by H_2O, H^+, and OH^- are also present. The relative contributions of these species to catalysis can be estimated by examining rates of anomerization of several fructofuranose analogues.

Ring opening of 6-*O*-methylfructose at pH 7.5 (Table VI) is catalyzed by both H_2O and OH^-. Rate constants are 20–30 times greater than those obtained at pH 4.2 ($k_{\alpha o} \simeq k_{\beta o} \simeq 0.04$ s^{-1}) where H_2O catalysis predominates. Rates of anomerization at pH 7.5 increase 4–5-fold in the presence of equimolar glycerol-*P* acting as an *intermolecular* general base catalyst. Replacement of the methoxyl group in 6-*O*-methylfructose with a phosphate group (to form fructose-*6*-*P*) greatly enhances ring-opening rates without affecting the relative rates of ring opening of the two cyclic anomers, while phosphorylation at C-1 dramatically increases ring-opening rates and alters the relative rates of ring opening of the two anomers. It appears that intramolecular participation by phosphate is the predominant catalytic factor for ring opening in furanose phosphates. Therefore, the ratio of ring-opening rate constants at pH 4.2 and 7.5 (Table V) provides an adequate approximation of the relative catalytic contributions by the phosphate mono- and dianion, respectively.

Differences in the relative ring-opening rates of the α- and β-furanose phosphates are interesting since these differences may reflect on the mechanisms of ring opening. Plausible mechanisms include catalysis by removal of a proton from O-1 and/or catalysis by protonation of O-4. Regardless of whether the aldose phosphates are in the mono- or dianionic form (Table IV), the α anomer always opens faster than the β anomer. Since O-1 and the phosphate group are trans in the α anomer, direct participation of the phosphate group in the deprotonation of O-1 seems unlikely. Therefore, if phosphate acts directly, it must do so by facilitating the protonation of the ring oxygen. The phosphate group may, however, play an indirect role by hydrogen bonding with one or more H_2O molecules, thereby positioning the catalyst (H_2O) to more effectively donate H^+ to O-4 and receive H^+ from O-1.[31] The slower rate of reaction of the β anomers may be due to steric interference of O-1 with the cis phosphate group at C-5.

In ketose phosphates, the α anomer opens more slowly than the β anomer. Here, the C-1 hydroxymethyl group is cis to the C-5 phosphohydroxymethyl group and may interfere with the required orientation of the phosphate group over the ring.

6-*O*-Methylfructose-*1*-*P* anomerizes nearly as rapidly as fructose-*1*,*6*-P_2, demonstrating that the C-1 phosphate is catalytically competent. Accordingly, the slowest observed rate of anomerization for the ketose phosphates is for fructose-*6*-*P*, which must overcome the unfavorable interaction between C-6 and either C-1 or *O*-1 to allow catalysis by phosphate. It is appropriate here to reemphasize that the relative ring-opening rates of α- and β-6-*O*-methylfructose and fructose-*6*-*P* are similar (Table VI), even though their absolute rates are quite different. When viewed in isolation, this result can be interpreted to imply that inductive effects are important. However, when coupled with observations on the ketose 1-phosphates, the argument for inductive effects weakens, and all these results can be accommodated within the mechanistic scheme outlined above.

The relative enhancement of ring-opening rates by the phosphate mono- and dianion depends on the configuration of the furanose (Table V). The ring-opening rate constants for a given anomer in the monoanionic forms differ by less than a factor of 4, whereas the dianionic forms reveal large differences (>10-fold). The relative catalytic efficiency of the phosphate group, therefore, depends in a complicated way on the stereochemistry of the substituents at C-2 and C-3. The origin of this dependency may lie in steric effects mentioned previously.

Table VII. Relative Rates[a] of Ring Closure Reactions

	pentose 5-phosphate		1,4-anhydro-alditol	pento-furanoside[b]
	α	β		
ribo	10	37	50	12.4 (β)
arabino	9.3	8.6	10	1.9 ($\alpha + \beta$)
xylo	2	4	29	2.5 ($\alpha + \beta$)
lyxo	2.6	1	1	1 (α)

[a] Rates are relative within each class of compound. [b] Letters in parentheses indicate which anomers are formed in the initial phase of reaction.

Ring Closure Rates. The pentose 5-phosphates are a full set of stereoisomers, and it is interesting to determine whether they display any pattern of ring closure rates that can be explained on the basis of known or suspected interactions in the transition state. Earlier studies of 1,4-anhydropentitol and pentofuranoside formation showed that ring closure followed the sequence *ribo*, *xylo*, *arabino*, *lyxo*. This ordering was explained as arising from intramolecular steric effects and by interference with the protonated leaving group by OH-2 when they were syn to each other.[32] In the pentose 5-phosphates, ring closure involves attack by OH-4 on one face of the C-1 carbonyl group. The effects of configuration here are quite different, as might be expected (Table VII). Although the uncertainty in the computed values for ring closure rates is higher due to uncertainty in the concentration of the aldehyde form, it is unlikely that the errors would alter the general order of the rates. Certainly, differences between α and β forms are real.

Interaction between C-5 and the newly formed OH-1 does not inhibit ring closure since only in lyxose does the β anomer form less readily than the α anomer. As in all other systems, ribofuranosyl rings form much more readily than the other pentofuranosyl rings, indicating the importance of cis interactions between C-5 and ring hydroxyl groups. It is interesting to speculate whether this enhanced reactivity had any role in establishing the biological importance of this isomer.

Biological Effects of Anomerization. A number of enzymes of carbohydrate metabolism are specific for a given tautomer of their substrate. Fructose-*1*,*6*-P_2 phosphatase appears to be specific for α-fructose-*1*,*6*-P_2[33] whereas phosphofructokinase is specific for β-fructose-*6*-*P*.[34] It is possible that, in a metabolic pathway, an

(31) We have measured a temperature-independent solvent isotope effect of 2.1 for ring opening of arabinose-*5*-*P* at p^{2}H 7.5. This value is intermediate between those found for H_2O and H^+-catalyzed anomerization of glucose and related compounds[3c] and may be due to hydrogen bonding of water by phosphate which would increase the bifunctional catalytic behavior of H_2O. In effect, the H_2O molecule is rendered more acidic at one end and more basic at the other.

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enzyme could produce a tautomer that cannot be acted on by the next enzyme in the sequence. In this situation, the rate of anomerization may affect metabolic flux through the pathway.[35] For example, fructose-*1,6-P*$_2$, fructose-*6-P*, and ribose-*5-P* are involved in the photosynthetic carbon reduction cycle and are substrates for fructose-*1,6-P*$_2$ aldolase, fructose-*1,6-P*$_2$ phosphatase, transketolase, and ribose-5-P isomerase. Steady-state rates of photosynthesis in whole spinach leaves have been reported to be 20–60 nmol of CO_2 fixed·s^{-1}·mg^{-1} of chlorophyll at room temperature.[36] Ribose-*5-P* and fructose-*1,6-P*$_2$ are present in chloroplasts at levels of approximately 3.2 and 4 nmol·mg^{-1} of chlorophyll, respectively, under steady-state conditions.[37] From the measured values of the ring-opening rates, tautomeric proportions, and activation energies for tautomerization, one may use eq 8 to calculate rates of anomerization for these two compounds at pH 7.5 and 28 °C [$k_{\alpha\beta}$(ribose-*5-P*) ~20 s^{-1}; $k_{\alpha\beta}$(fructose-*1,6-P*$_2$) ~9.3 s^{-1}]. These rate constants correspond to in vivo rates of 23 and 4.8 nmol of β anomer produced·s^{-1}·mg^{-1} of chlorophyll, respectively, and are similar in magnitude to the observed metabolic rate. Therefore, under appropriate conditions, the anomerization reaction may affect the partitioning of substrates between different metabolic pathways. This conclusion is in accord with the analyses of possible effects of anomerization on the glycolytic and gluconeogenic pathways.[35] The presence of enzymes with anomerase activity could change the partitioning ratio, and if these anomerases were allosteric, they could represent important controls in the regulation of metabolic activity. Enzymes with anomerase activity have been characterized[38] and may be important in maintaining high metabolic fluxes, even for those pathways where rapidly anomerizing sugar phosphates are utilized.

Even without enzymes with anomerase activity, the phosphorylation of simple sugars provides a mechanism for increasing the rates of interconversion between different forms of the sugar. There are many cases where multiple forms of the sugar (or sugar phosphate) are present and the biologically active form is only a minor component (e.g., fructose-*1,6-P*$_2$, erythrose-*4-P*, glyceraldehyde-*3-P*). In these cases, rapid interconversion of the sugar species allows the particular enzymes to work at a faster rate than they would if they had to wait for a slow tautomerization to provide the catalytically active form of their substrate. For the pentoses, phosphorylation of the primary hydroxyl group has two functions: it restricts the resultant ring to an inherently less stable furanose configuration *and* catalyzes the rates of interconversion between the different forms of the sugar phosphates. For the tetroses, phosphorylation completely interferes with ring formation and immediately provides for an increased amount of the biologically active, free carbonyl form. For the trioses, the solution proportions of the various forms are not substantially altered upon phosphorylation, but rates of hydration and dehydration are substantially increased, thereby allowing for rapid production of the minor but biologically active free carbonyl form. In all these cases, sugar phosphorylation not only couples intermediary metabolism and oxidative phosphorylation (by way of ATP) but allows for immediately increased rates of metabolism.

Acknowledgment. This work was supported, in part, by a grant (GM-21731) from the Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD. We acknowledge the use of the South Carolina Regional NMR Facility, Columbia, SC, supported by NSF Grant CHE 70-04825, and the Cornell NMR Facility, supported by NSF Grant CHE 70-04825. We thank Dr. Ruth R. Inners for her assistance in obtaining the 100-MHz ^{13}C spectra and S. G. Huang for making instrumental modifications for obtaining ^{13}C saturation measurements at 75 MHz and for useful discussions.

Registry No. α-D-Erythrofuranose, 72599-80-5; β-D-erythrofuranose, 72599-81-6; α-D-threofuranose, 80877-72-1; β-D-threofuranose, 80877-73-2; α-D-5-*o*-methylxylofuranose, 94707-49-0; β-D-5-*o*-methylxylofuranose, 94707-50-3; α-D-6-*o*-methylfructofuranose, 94707-51-4; β-D-6-*o*-methylfructofuranose, 94707-52-5; glucopyranose, 2280-44-6; galactopyranose, 10257-28-0; galactofuranose, 19217-07-3; α-D-ribofuranose 5-phosphate, 34980-65-9; β-D-ribofuranose 5-phosphate, 34980-66-0; α-D-arabinofuranose 5-phosphate, 69926-02-9; β-D-arabinofuranose 5-phosphate, 69881-35-2; α-D-xylofuranose 5-phosphate, 94798-99-9; β-D-xylofuranose 5-phosphate, 94799-00-5; α-D-lyxofuranose 5-phosphate, 94799-01-6; β-D-lyxofuranose 5-phosphate, 94799-02-7; α-D-fructofuranose 5-phosphate, 41452-28-2; β-D-fructofuranose 6-phosphate, 41452-29-3; α-D-6-*o*-methylfructofuranose 1-phosphate, 94707-53-6; β-D-6-*o*-methylfructofuranose 1-phosphate, 94707-54-7; α-D-fructofuranose 1,6-diphosphate, 34693-23-7; β-D-fructofuranose 1,6-diphosphate, 34693-15-7.

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Conformational Enantiomerism in Bilirubin. Selection by Cyclodextrins

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Abstract: Intramolecularly hydrogen-bonded, bichromophoric (4*Z*,15*Z*)-bilirubin IXα adopts either of two enantiomeric conformations that are in dynamic equilibrium in solution. Added α-, β-, or γ-cyclodextrin binds preferentially to one conformational enantiomer, and the complex exhibits a bisignate circular dichroism Cotton effect in the vicinity of the bilirubin long wavelength electronic transition. Analysis of these data, within the framework of exciton coupling models, indicates a preference for complexation of the left-handed (or negative) chirality enantiomer of bilirubin with cyclodextrin.

The constitutional structure of bichromophoric (4*Z*,15*Z*)-bilirubin IXα (**1**), the yellow-orange lipophilic and cytotoxic pigment of jaundice, was proved over 40 years ago;[2] however, its conformational structure has only recently been characterized,[3] and that

0002-7863/85/1507-2456$01.50/0