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Kinetics of Oxygen Exchange at the Anomeric Carbon Atom of D-Glucose and D-Erythrose Using the Oxygen-18 Isotope Effect in Carbon-13 Nuclear Magnetic Resonance Spectroscopy†

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ABSTRACT: The ^{18}O isotope induced shift in ^{13}C nuclear magnetic resonance (NMR) spectroscopy affords a new and convenient method for the study of oxygen exchange at the anomeric carbon atom of simple sugars. The efficacy of the technique was confirmed by a study of the oxygen exchange reaction of D-[1-^{13}C]glucose. At pH 7.0 and 61 °C, the incorporation of ^{18}O from solvent $H_2{}^{18}O$ onto the C-1 carbon atom of the diastereomeric α- and β-pyranose sugars was followed by ^{13}C NMR spectroscopy in a continuous assay mode. The pseudo-first-order rate constant for exchange of both the α and the β anomers was 9.5×10^{-5} s^{-1}, which is in agreement with a rate constant obtained in a previous study by a chemical conversion–mass spectrometry technique. The new technique was applied to a study of the oxygen exchange at the anomeric carbon atom of D-[1-^{13}C]erythrose, a furanose sugar for which no experimental data were available. In unbuffered, aqueous solutions the incorporation of the ^{18}O label from the medium ($H_2{}^{18}O$) onto the C-1 carbon atom of the α- and β-D-[1-^{13}C]erythrose and the D-[1-^{13}C]erythrose hydrate forms was followed by ^{13}C NMR at 10, 23, and 36 °C. From analysis of the data for the α and β diastereomers, the pseudo-first-order rate constants for exchange were 1.4×10^{-4} s^{-1} at 10 °C, 4.8×10^{-4} s^{-1} at 23 °C, and 8×10^{-4} s^{-1} at 36 °C, and the apparent energy of activation for the exchange reaction was 12.1 kcal/mol. Particularly in conjunction with the use of specifically ^{13}C-enriched sugars, the new technique for studying oxygen exchange reactions of carbohydrates has many distinct advantages over earlier approaches, including the ability to follow simultaneously the exchange reactions of all of the sugar species for which a ^{13}C NMR signal can be detected, the continuity of the assay, the avoidance of possible artifacts due to incomplete or selective derivatization reactions, and the simplicity of the data analysis.

Mutarotation (tautomerization) of simple sugar molecules in aqueous solution is a fundamental interconversion process and one upon which many of the chemical and biological properties of the carbohydrates depend (Schray & Benkovic, 1978). The nonenzymatic conversion of simple sugars between their acyclic and diastereomeric α and β forms has been studied extensively. A sugar molecule may undergo a "simple" or a "complex" mutarotation, and the macroscopic rate constants for the mutarotation reaction have been measured by a variety of techniques (Pigman & Isbell, 1968; Isbell & Pigman, 1969). The experimental evidence supports a mechanism for anomerization in aqueous solution that involves an aldehydo or keto sugar intermediate. Moreover, as with other carbonyl derivatives in aqueous solution, the postulated aldehydo or keto sugar intermediate is expected to be in equilibrium with the hydrate (*gem*-diol), formed by nucleophilic addition of water to the carbonyl carbon. Thus, in the most simple system, three simultaneous equilibria involving four species exist: (1) the α sugar with the aldehydo or keto sugar, (2) the β sugar with the aldehydo or keto sugar, and (3) the aldehydo or keto sugar with its hydrate.

In recent years, more sophisticated experimental techniques have allowed a greater understanding of the mutarotation reaction, including estimates and measurements of the microscopic rate constants that govern the equilibria (Wertz et al., 1981; Serianni et al., 1982). The mutarotation reaction is typically studied by dissolving a diastereomerically homogeneous sugar in water and then following the resultant changes in optical rotation or other property until equilibrium is reached. Support for the presence of an aldehydo or keto sugar intermediate comes from the studies of oxygen exchange reactions at the anomeric carbon atom. The technique for studying the latter reaction involves incubating either an ^{18}O-labeled[1] sugar in normal water or an unlabeled sugar in [^{18}O]water and analyzing the ^{18}O content of the sugar as a function of time. Despite the importance of such experiments in providing data on the rate of formation and reactivity of

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[1] Abbreviations: ^{18}O, oxygen-18; ^{13}C, carbon-13; NMR, nuclear magnetic resonance; E_a, Arrhenius energy of activation; FID, free induction decay; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid.

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the aldehydo or keto sugar intermediate, surprisingly few results have been reported for the nonenzymatic oxygen exchange reaction that occurs at the anomeric carbon of simple sugars. Goto & Chitani (1941) reported that at 30 °C the rate of the nonenzymatic oxygen exchange reaction at the C-1 carbon atom of D-glucose was 5% of the rate of mutarotation. The position of oxygen exchange in D-glucose was later verified (Koshland & Stein, 1954). Rittenberg & Graff (1958) followed up these reports with the most extensive study of oxygen exchange in a sugar that has been reported. They studied the effect that pH and temperature have on the rate of oxygen exchange at the anomeric carbon atom in D-glucose. Brief studies were reported on oxygen exchange in maltose (Halpern & Leibowitz, 1959; Mayer & Larner, 1959), in fructose and its 1-phosphate and 1,6-diphosphate (Model et al., 1968), in D-galactose (Anderson & Garver, 1973), and in ribose and ribulose 5-phosphates (Johnson et al., 1973).

Quite possibly the paucity of experimental data on the rates of the oxygen exchange reactions of sugars is a result of the cumbersome nature of the analytical techniques that have been required for their measurement. Chemical conversion (involving combustion or derivatization) combined with mass spectrometry has been the only technique used to analyze the oxygen exchange reactions listed above. Analysis by such a method is severely limited because it is, of necessity, a discontinuous assay technique. Furthermore, the operations involved in sample preparation can be quite laborious, derivatization or combustion reactions may introduce artifacts, and data analysis can be tedious when correction factors (such as those involving isotopic abundance values) must be applied.

Because oxygen-18 causes an isotope-induced shift of the ^{13}C NMR signal of a directly bonded carbon nucleus (Risley & Van Etten, 1979), NMR spectroscopy affords an important alternative to chemical conversion–mass spectrometry techniques. It often permits a continuous and direct observation of oxygen exchange reactions (Risley & Van Etten, 1979, 1981) and can provide extensive information about biosynthetic pathways (Hill et al., 1982, and references cited therein). It appeared possible that this approach, together with the availability of specifically ^{13}C-enriched sugars, might provide a new approach to the study of oxygen exchange reactions of carbohydrates. The validity of this new approach is demonstrated here by a reexamination of the oxygen exchange reaction occurring at the anomeric carbon atom of D-glucose. The technique is then applied to a study of the temperature dependence of the oxygen exchange reaction at the anomeric carbon atom of D-erythrose, a sugar for which no exchange data had been reported.

Experimental Procedures

D-[1-^{13}C]Erythrose (90 atom % 1-^{13}C), a gift from Professor Robert Barker, was synthesized from K^{13}CN and D-glyceraldehyde (Serianni et al., 1979b). D-[1-^{13}C]Glucose (90 atom % 1-^{13}C, Merck) was a gift from Professor Klaus Hermann. [^{18}O]Water (97 atom % ^{18}O, normalized, KOR, Inc.), [^{2}H]-water (99.75 atom % ^{2}H, Baker Chemical Co.), glass-distilled deionized water, and analytical grade reagents were used in the preparation of the solutions. An NTC-200 NMR spectrometer fitted with a 12-mm probe and operating at 50.31 MHz was used to follow the oxygen exchange reactions. The solutions and instrument were equilibrated at the desired temperature (±1 °C) for a minimum of 30 min before the reaction was initiated. A ±400-Hz sweep width (quadrature phase detection), a 90° pulse angle, and a 16K data block were used; the acquisition time was 10.24 s, and the pulse delay was 5.00 s. A line-broadening factor was applied to the accumulated FID, and protons were broad-band decoupled. A vortex plug was used. A Corning Model 130 pH meter was used to measure solution pH.

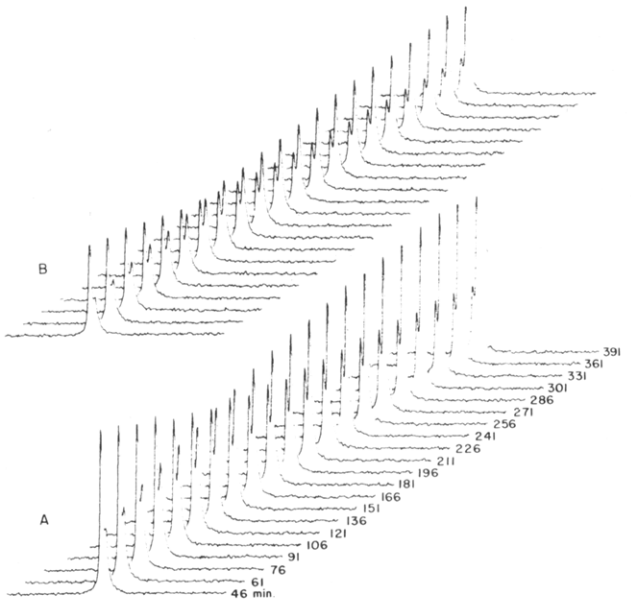


FIGURE 1: Nonenzymatic oxygen exchange at pH 7.0 and 61 °C at the anomeric carbon atom in D-[1-^{13}C]glucose as a function of time followed by ^{13}C NMR spectroscopy. The time after the addition of the sugar is given in (A). (A) β-D-[1-^{13}C]Glucopyranose. (B) α-D-[1-^{13}C]Glucopyranose. The *x* coordinate corresponds to magnetic field strength (increasing from left to right), with the separation between the two peaks of the β anomer being 0.016 ppm.

D-[*1-^{13}C*]*Glucose Oxygen Exchange at 61 °C.* A solution of 5 mM inorganic phosphate (pH ~7) was prepared in 1.0 mL of [^{2}H]water and 4.0 mL of [^{18}O]water. The oxygen exchange reaction at 61 °C was initiated when 29 mg of D-[1-^{13}C]glucose was added to the preequilibrated solution to give a final glucose concentration of 32 mM. The pH of the final solution was 7.02 (corrected for temperature). Spectra were taken every 15 min for 10 h.

D-[*1-^{13}C*]*Erythrose Oxygen Exchange.* Unbuffered solutions were prepared containing 3.0 mL of [^{18}O]water and 1.0 mL of [^{2}H]water. The oxygen exchange reaction was initiated by adding 1.0 mL of a 0.16 M solution of D-[1-^{13}C]erythrose (final concentration 32 mM) to the unbuffered solution at the preequilibrated temperatures. Spectra were taken every 10 min at 10 and 23 °C and every 5 min at 36 °C.

Data Analysis. Equations describing oxygen exchange reactions in systems such as carbohydrates have been detailed elsewhere (Model et al., 1968). For incorporation of [^{18}O]-water into an ^{16}O-labeled substrate, a plot of ln $[(F^* - F)/F^*]$ against time will give a slope equal to $-k$, where F^* is the atom percent excess ^{18}O in the substrate at equilibrium, F is the atom percent excess ^{18}O at time t, and k is the pseudo-first-order rate constant for the exchange reaction.

Results

The upfield ^{18}O isotope shifts exhibited by the anomeric carbon atoms of the sugars are as follows: β-D-glucose, 0.016 ppm; α-D-glucose, 0.018 ppm; β-D-erythrose, 0.019 ppm; α-D-erythrose, 0.017 ppm [all with respect to the unlabeled (^{16}O) sugar]. The experimental error is ±0.002 ppm. Figure 1 illustrates the time course of the oxygen exchange reaction for α- and β-D-glucose at 61 °C. Figure 2 illustrates the analogous reaction for α- and β-D-erythrose and D-erythrose hydrate at 10 °C. The exchange reactions of the α and β anomers were analyzed independently. The atom percent excess ^{18}O was

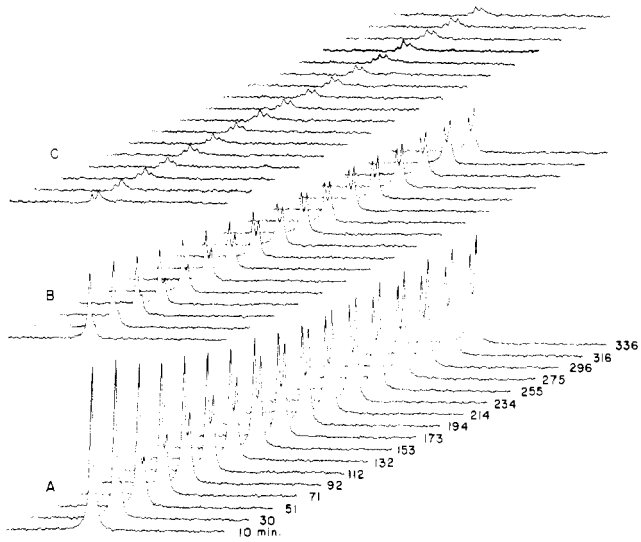


FIGURE 2: Nonenzymatic oxygen exchange at 10 °C in unbuffered water at the anomeric carbon atom in D-[1-^{13}C]erythrose as a function of time followed by ^{13}C NMR spectroscopy. The time after the addition of the substrate is given in (A). (A) β-D-[1-^{13}C]Erythrofuranose. (B) α-D-[1-^{13}C]Erythrofuranose. (C) D-[1-^{13}C]Erythrose hydrate. The x coordinate corresponds to magnetic field strength (increasing from left to right), with the separation between the two peaks of the β anomer being 0.019 ppm.

Table I: Rates of Oxygen Exchange at the Anomeric Carbon of D-Glucose and D-Erythrose

sugar	temp (°C)	pseudo-first-order rate constant × 10^6 (s^{-1}) α	β
D-glucose	61	95.0 (0.99_4)[a]	94.8 (0.99_5)
D-erythrose[b]	10	147 (0.99_8)	136 (0.99_6)
	23	486 (0.98_9)	481 (0.99_4)
	36	892 (0.99_7)	821 (0.99_6)

[a] Correlation coefficients are given in parentheses. [b] The energy of activation is 12.1 kcal/mol.

calculated either from direct peak-height measurements or by deconvolution of the spectra with a deconvolution routine available in the Nicolet software of the spectrometer. From these data, plots were made as described under Experimental Procedures. The resultant pseudo-first-order rate constants for the oxygen exchange reactions are listed in Table I. The standard deviation in k (from the linear least-squares analysis) is in no case greater than ±5%. The correlation coefficient for each plot is in parentheses.

Discussion

Oxygen exchange reactions at the anomeric carbon atom of simple sugars may be monitored either by the incorporation of the ^{18}O label from the medium into the sugar or by the loss of the ^{18}O label from the substrate into the medium. Both experimental procedures have been used previously, and Table II summarizes such data for the nonenzymatic oxygen exchange reactions that have been studied. Although the experimental results are limited, some observations may be made concerning the oxygen exchange reactions. In unsubstituted hexose and pentose sugars, the rate of oxygen exchange is quite slow at 25 °C. This is a consequence of the relative stability of the ring forms of the sugars. The substitution of one or more phosphate groups onto the sugar enhances the rate of oxygen exchange at the anomeric carbon atom, a result of the effect of the phosphate group(s) on the kind and extent of ring formation of the sugar. [This is also reflected in the greater mole fraction of hydrate present (Serianni et al., 1979a).]

In the present study, the nonenzymatic oxygen exchange reaction at the anomeric carbon atoms of D-glucose and of D-erythrose was monitored by measuring the incorporation of the ^{18}O label from the medium, $H_2{}^{18}O$, into the substrate. We also used 1-^{13}C-enriched sugars (90 atom %) in order to achieve greater accuracy and to permit us to follow relatively rapid exchange reactions.

The applicability of the ^{18}O isotope effect in ^{13}C NMR spectroscopy for following such oxygen exchange reactions was tested with D-[1-^{13}C]glucose. A concurrent objective was to provide independent confirmation of the oxygen exchange reaction for D-glucose, one of the few systems for which apparently reliable experimental data had been reported. A 5 mM phosphate buffer solution (pH 7) in 80% $H_2{}^{18}O$–20% ^2H_2O (v/v) was prepared and equilibrated at 61 °C. The oxygen exchange reaction was initiated by addition of D-[1-^{13}C]glucose to give a 32 mM sugar solution (pH 7.04 at room temperature). Two ^{13}C NMR signals were observed: the signal due to β-D-glucopyranose predominated and appeared downfield from that of its anomer, α-D-glucopyranose. Signals due to β- and α-D-glucofuranoses could not be observed. Upon incorporation of the ^{18}O label into the substrate, the ^{13}C NMR signal of the anomeric carbon atom is shifted upfield 0.016 ppm in β-D-glucose and 0.018 ppm in α-D-glucose. The isotope-induced shifts are slightly larger than those reported by Vederas (1980), who measured the isotope shifts using natural abundance ^{13}C with [$^2H_5$]pyridine as a solvent. Figure 1 illustrates the incorporation of the ^{18}O label from the medium onto the anomeric carbon atom of both the β and the α anomers as a function of time. The stacked plots have been prepared with a single scaling factor so that the observed distribution of the two diastereomers is shown in correct relative proportion. The data for the oxygen exchange reactions of β-D-glucose and α-D-glucose were quantitated independently by direct peak-height measurements. The measured peak heights were transformed into the atom percent excess ^{18}O in the substrate, and these values were subjected to analysis in order to obtain k. The resultant pseudo-first-order rate constants and correlation coefficients are listed in Table I. The rate constants calculated for oxygen exchange of α- and β-D-glucose are identical and are within experimental error of the reported value (Table II). This result demonstrates that the ^{18}O isotope effect in ^{13}C NMR can be successfully applied to the study of oxygen exchange reactions in simple sugars. Furthermore, this experiment clearly demonstrates the significant advantage of the present experimental technique over previous methods in that the rate of exchange can be followed continuously and independently for each diastereomer. These independent measurements of the rate of oxygen exchange not only complement one another but also allow one to determine if there is a significant difference between the rates of oxygen exchange in the diastereomers. D-Glucose shows no difference.

This method was then extended to a study of the oxygen exchange of D-erythrose, for which no data had been published. Whereas D-glucose exists primarily in a pyranose ring form, D-erythrose exists as a furanose ring form. In aqueous solution there are four ^{13}C NMR signals in the spectrum of D-[1-^{13}C]erythrose (Serianni et al., 1982), corresponding to the β-furanose, α-furanose, hydrate, and aldehydo sugars in a ratio of approximately 62:25:12:1 (but these ratios do vary with concentration, temperature, etc). Unbuffered solutions were prepared containing 60% $H_2{}^{18}O$ and 20% ^2H_2O. To the thermally equilibrated solution was added D-[1-^{13}C]erythrose to initiate the oxygen exchange reaction; the final concentration of the sugar was 32 mM.

Table II: Experimental Results from Earlier Studies of Nonenzymatic Oxygen Exchange Reactions

sugar	temp (°C)	exptl conditions	pseudo-first-order rate constant X 10^6 (s^{-1})	reference
glucose[a]	61	pH 1.5, oxalic acid	290	Rittenberg & Graff, 1958
	61	pH 2.15, HCl-KCl	100	
	61	pH 2.80, phthalate	25	
	61	pH 3.4, 5.0, acetate	19	
	61	pH 6.0-7.0, phosphate	45-100	
	61	pH 8.15, barbiturate	230	
	50	pH 7.0, phosphate	31	
	40	pH 7.0, phosphate	9.7	
fructose	25	pH 7.0, 0.2 M phosphate (or water) with 0.1% BSA and 10^{-3} M EDTA	<3.3	Model et al., 1968
fructose 1-phosphate	25	pH 7.0, 0.2 M phosphate (or water) with 0.1% BSA and 10^{-3} M EDTA	70	
fructose 1,6-diphosphate	25	pH 7.0, 0.2 M phosphate (or water) with 0.1% BSA and 10^{-3} M EDTA	400	
galactose[b]	70	unbuffered water	160	Anderson & Garver, 1973
	60	unbuffered water	58	
	50	unbuffered water	20	
	25 (extrapolated)	unbuffered water	0.97	Wertz et al., 1981
ribose 5-phosphate	25	unbuffered water	58	Johnson et al., 1973
ribulose 5-phosphate	25	unbuffered water	580	
ribulose 1,5-diphosphate	0	pH 7.8, triethanolamine	7700[c]	Sue & Knowles, 1978

[a] At pH 7.0, the energy of activation for the oxygen exchange reaction is 23.4 kcal/mol while for the mutarotation reaction it is 17.2 kcal/mol. [b] The energy of activation for the oxygen exchange reaction is 23.1 kcal/mol. [c] Estimated from the published experimental data.

Three of the D-[1-^{13}C]erythrose ^{13}C NMR signals were observed as a function of time: β-D-erythrose, α-D-erythrose, and the D-erythrose hydrate. Figure 2 illustrates the equilibration of ^{18}O label at 10 °C from the medium onto the anomeric carbon atom of the substrate for the α anomer, the β anomer, and the hydrate. The progress of the reaction to equilibrium is readily seen. As in Figure 1 for D-glucose, the stacked plots in Figure 2 have been generated with a single scaling factor so that the ratios of the sugar forms can be easily discerned. Upon incorporation of the ^{18}O label from the medium into the substrate, the ^{13}C NMR signal is shifted upfield 0.019 ppm in β-D-erythrose and 0.017 ppm in α-D-erythrose. The magnitude of the ^{18}O isotope shift in the β-furanose sugar is slightly larger than that in the α-furanose sugar, whereas the magnitudes of the isotope shift are reversed for the pyranose diastereomers in D-glucose. Under the experimental conditions employed here, quantitative data were not obtained from the spectral data for the exchange reaction of the erythrose hydrate,[2] but qualitative changes in the isotopic composition of the hydrate can be seen in Figure 2C. At intermediate times, three peaks can be discerned for the partially exchanged hydrate, corresponding to the presence of zero, one, or two atoms of ^{18}O.

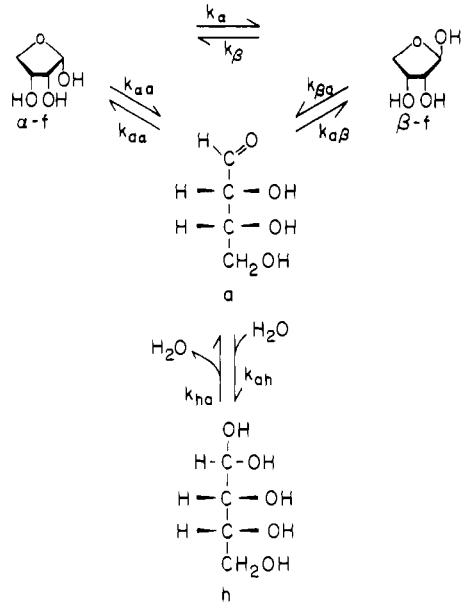
The data for the oxygen exchange reactions of the α and β diastereomers were analyzed independently at each temperature by direct peak-height measurements. The peak heights were transformed into values for the atom percent excess ^{18}O of the substrate, and these numbers were analyzed to obtain the exchange rate constants, k. The correlation coefficients for these plots and the resultant pseudo-first-order rate constants are listed in Table I. As a further check on the method of quantitation, the spectral data for the exchange reaction at 23 °C were deconvoluted and quantitated by electronic integration; the resultant rate constants were identical (within experimental error) with those calculated from peak-height measurements. From a plot of ln k vs. $1/T$, the energy of activation for the oxygen exchange reaction of D-erythrose was found to be 12.1 kcal/mol.

The pseudo-first-order rate constants calculated for the oxygen exchange reaction at the anomeric carbon atom of both the α and β anomers of D-erythrose are nearly equal, consistent with the greater rate of attainment of the anomerization equilibrium. Although k for α-D-erythrose appears slightly larger than k for β-D-erythrose at each temperature, the difference is probably not significant. Because of the ease with which the rapid exchange was measured, the experiments with D-erythrose demonstrate the advantages of using the ^{18}O isotope effect in ^{13}C NMR experiments over chemical conversion–mass spectrometry. The reaction was effectively followed in a continuous assay mode, and the oxygen exchange rates were obtained for each anomer of the sugar. Indeed, increases in concentration, magnetic field strength, or other experimental conditions might have permitted a more accurate measurement of the isotopically substituted hydrate or aldehydo forms, thus making available two additional measures of the exchange reaction.

The rate of the oxygen exchange reaction of D-erythrose is much faster than the rates of the other aldoses that have been measured (Table II), even those sugars that exist predominantly as furanoses. In fact, the rates in D-erythrose approach the rates observed for a phosphate-disubstituted keto furanose, where electrostatic effects may affect the extent of hemiacetal formation. Moreover, the energy of activation for the exchange reaction in D-erythrose is approximately half that obtained for other exchange reactions. These observations are consistent with an interpretation that the rate of exchange is dependent on the stability of the cyclic sugars—one measure of which is the amount of aldehydo (or keto) and hydrate sugars that is present—and whether one is investigating an aldose or ketose. This general problem is receiving much-deserved attention (Serianni et al., 1979a, and references cited therein). More extensive studies using the present techniques should provide complementary data on the rate of oxygen exchange as a function of the proportion of aldehydo, keto, or hydrate sugar forms.

[2] Although no attempt was made here to evaluate quantitatively the oxygen isotope content of the hydrate, a referee has emphasized that the two hydroxyl groups at C-1 of the hydrate are diastereotopic and exhibit different rates of reaction. The differences are expected to be small in systems such as the present one but may be substantial under some circumstances such as ones involving enzymatic reactions of the hydrate.

Scheme I



The tautomerization reaction of sugar molecules has received considerable research attention (Pigman & Isbell, 1968; Isbell & Pigman, 1969). Pentose and higher sugars have been the primary focus of such research, while the tetroses have apparently not been studied. These data show that sugars undergo either a simple or a complex mutarotation. The first-order rate law is strictly obeyed by sugars displaying simple mutarotation, and sugars that deviate from the first-order rate law are classified as exhibiting complex mutarotation. D-Glucose is a sugar of the former group and D-galactose of the latter group. The classification of the tetrose sugars with respect to mutarotation category has not been made experimentally; however, we shall assume a simple mutarotation.[3] The tautomerization of D-erythrose at equilibrium is illustrated in Scheme I. The α- and β-erythrofuranose (α-f, β-f) sugars are in equilibrium with the aldehydo (a) sugar, which is in equilibrium with its hydrate (h). Ring-opening rate constants ($k_{\alpha a}$ and $k_{\beta a}$) have been measured at 51 and 55 °C (Serianni et al., 1982), the rate of oxygen exchange has been measured here, and the ratios of the sugar species in solution at equilibrium are known. From these data it is possible to estimate the microscopic rate constants governing the tautomerization reaction and the macroscopic rate constant for mutarotation.

We calculated the microscopic rate constants for the tautomerization of D-erythrose at 23 °C assuming that the system was at equilibrium and the D-erythrose was undergoing simple mutarotation. The references that were used to make these calculations were Angyal & Wheen (1980), Serianni et al. (1979b, 1982), Wertz et al. (1981), and the present work. Equilibrium constants were defined as follows:

$$K_\alpha = \frac{k_{a\alpha}}{k_{\alpha a}} = \frac{[\alpha]}{[a]} \qquad K_\beta = \frac{k_{a\beta}}{k_{\beta a}} = \frac{[\beta]}{[a]} \qquad K_h = \frac{k_{ah}}{k_{ha}} = \frac{[h]}{[a]}$$

$$K_{\beta\alpha} = \frac{k_\alpha}{k_\beta} = \frac{[\beta]}{[\alpha]}$$

[3] Although only two cyclic forms of the aldotetroses are formed (the α and β furanose), the classification of tetrose sugars as displaying "simple mutarotation" is not obvious due to the considerable mole fraction of aldehydo and hydrate forms that is present at equilibrium. An initial classification as "simple" is reasonable until more extensive experimental determinations are made.

Table III: Microscopic Rate Constants and Associated Data for Mutarotation of D-Erythrose at 23 °C

$K_\alpha = 23$	$K_\beta = 57$ $K_h = 10$ $K_{\beta\alpha} = 2.5$
$k_{\alpha a} = 0.0065\ s^{-1}$	$k_{a\alpha} = 0.15\ s^{-1}$
$k_{\beta a} = 0.013\ s^{-1}$	$k_{a\beta} = 0.74\ s^{-1}$
$k_{ha} = 0.0088\ s^{-1}$	$k_{ah} = 0.088\ s^{-1}$
$k_\alpha = 0.039\ s^{-1}$	$k_\beta = 0.016\ s^{-1}$
$k_{ex} = 0.00048\ s^{-1}$	$k_m = 0.055\ s^{-1}$
E_a(α ring opening) $\simeq$ 24 kcal/mol	
E_a(β ring opening) $\simeq$ 16 kcal/mol	
E_a(oxygen exchange) = 12.1 kcal/mol	

The mole fractions of the four sugar species were as follows: α-f, 0.25; β-f, 0.63; h, 0.11; and a, 0.01. The oxygen exchange rate constant (k_{ex}) at 23 °C is $4.8 \times 10^{-4}\ s^{-1}$. The pseudo-first-order rate constant for hydration of the aldehydo sugar (k_{ah}) is $8.8 \times 10^{-2}\ s^{-1}$ and for dehydration of the hydrate (k_{ha}) is $8.8 \times 10^{-3}\ s^{-1}$. Extrapolation of plots of ln $k_{\alpha a}$ and ln $k_{\beta a}$ against $1/T$ to 23 °C gives $k_{\alpha a} = 6.5 \times 10^{-3}\ s^{-1}$ and $k_{\beta a} = 1.3 \times 10^{-2}\ s^{-1}$. From the equilibrium constants $K_\alpha = 23$ and $K_\beta = 57$ the ring-closing rate constants were calculated to be $k_{a\alpha} = 0.15\ s^{-1}$ and $k_{a\beta} = 0.74\ s^{-1}$. For calculation of the rate of mutarotation (k_m), the microscopic rate constants k_α and k_β were calculated from the cyclic system (top, Scheme I),[4] giving $k_\alpha = 0.039\ s^{-1}$ and $k_\beta = 0.016\ s^{-1}$. Thus, $k_m = k_\alpha + k_\beta = 0.055\ s^{-1}$ and $K_{\beta\alpha} = 2.5$ (in agreement with the experimental value of 2.5). The energies of activation for α ring opening, β ring opening, and oxygen exchange are approximately 24, 16, and 12.1 kcal/mol, respectively. The data are summarized in Table III. The rate of hydration of the aldehyde sugar ($0.088\ s^{-1}$) is similar in magnitude to the measured pseudo-first-order rate constant for the hydration of acetaldehyde at 25 °C ($\sim 0.033\ s^{-1}$) (Bell & Evans, 1966). The ring-opening rates for the α and β anomers are reversed at 23 °C compared to 55 °C (Serianni et al., 1982); i.e., the β anomer opens more rapidly at 23 °C than the α anomer while at 55 °C the converse situation is found. The rate of mutarotation is approximately 100 times the rate of oxygen exchange and supports the prediction by Serianni et al. (1982) that "... ^{18}O-exchange should proceed more slowly than mutarotation". Upon analysis of the calculated microscopic rate constants it is found that, as one might expect, the rates of dehydration and of ring opening are very similar in magnitude. On the other hand, the rates of hydration (a bimolecular reaction) and of ring closing (an intramolecular reaction) are not comparable; this is more clearly indicated by calculation of the "effective molarity" (Kirby, 1980).

At equilibrium the tautomerization of D-erythrose is significantly different from that observed for other simple sugars. First, the rate of oxygen exchange at the anomeric carbon atom of D-erythrose exceeds (by a factor of approximately 500) the rate observed for other simple sugars at similar temperatures (Table II), while E_a is approximately half. The E_a for mutarotation of D-erythrose has apparently not been measured, but there is no reason to expect that it should exceed the value of 16–18 kcal/mol observed for other sugars (Pigman & Isbell, 1968); indeed, the E_a may be as small as 9 kcal/mol if the ratios of the E_a's for mutarotation and oxygen exchange are the same as for D-glucose and D-galactose. The calculated rate of mutarotation of D-erythrose is approximately 100 times the

[4] Thus, $[\alpha]k_\alpha = [\alpha]k_{a\alpha} + [a]k_{a\beta}$. Dividing through by $[\alpha]$ and substituting K_α for $[\alpha]/[a]$ gives $k_\alpha = k_{\alpha a} + k_{a\beta}/K_\alpha$. Similarly, $k_\beta = k_{\beta a} + k_{a\alpha}/K_\beta$.

rate of oxygen exchange at 23 °C, whereas the corresponding ratio is approximately 1000 for D-glucose (Rittenberg & Graff, 1958) and D-galactose (Anderson & Garver, 1973). The rates of mutarotation of D-glucose and D-galactose are approximately 100 to 10000 times faster than that of D-erythrose. Because the reactions are at overall equilibrium, these comparisons reflect the relative differences in the rates at which these sugars tautomerize. The primary factor in the kinetic control appears to be the stability of the cyclic sugars. The aldotetrose (as well as the ketopentose) sugar can only cyclize with a primary alcohol to form a relatively unstable furanose ring, whereas aldopentoses and hexoses can cyclize with secondary alcohols to form relatively more stable furanose and/or pyranose rings. These stabilities are reflected in the proportions of acyclic sugar forms present at equilibrium—a significant proportion for D-erythrose but miniscule amounts for D-glucose and D-galactose—and the experimental conditions under which oxygen exchange can be conveniently measured for the three sugars—at and below room temperature for D-erythrose and at high temperature for D-glucose and D-galactose. These differences are also reflected in the microscopic rate constants for the reactions of D-erythrose and D-galactose (Wertz et al., 1981); within each tautomerizing system, the dehydration and ring-opening rate constants (of the most abundant and stable cyclic forms) are very similar in magnitude, but the rates of hydration are less by a factor of approximately 10 for D-erythrose and approximately 100 for D-galactose when compared to the rates of the ring-closing reactions.

Two points should be noted in the analysis. A solvent deuterium isotope effect k_H/k_D = 3.0–3.8 on the rate of mutarotation for sugars has been measured (Isbell & Pigman, 1969), and a kinetic ^{18}O isotope effect on the rate of oxygen exchange is expected.[5] Our oxygen exchange solutions did contain 20% ^2H_2O, and this quantity of ^2H could affect our experimental results by changing the rate of mutarotation and consequently the rate of oxygen exchange. At this time we cannot quantitate the ^2H isotope effect on the rate of oxygen exchange, but we believe that at a dilution of 20% the isotope effect should be relatively small. The primary kinetic ^{18}O isotope effect is expected to be negligible.

A direct comparison of the rates of nonenzymatic oxygen exchange of sugars with those of simpler carbonyl compounds has seldom been possible due to the significantly different experimental conditions under which the oxygen exchange reactions have been studied (Byrn & Calvin, 1966; Samuel & Silver, 1965)—principally with respect to solvent and catalyst. The present approach should facilitate such comparisons. Enzyme-catalyzed oxygen exchange reactions at the anomeric carbon atom of sugars can also be investigated by extensions of the present approach in order to gain insights into the mechanisms of the enzymatic reactions [see, for example, Heron & Caprioli (1975) and Schray & Benkovic (1978) and references cited therein]. The ^{18}O isotope effect in ^{13}C NMR spectroscopy provides a valuable new technique for studying these processes.

[5] A primary oxygen isotope effect, k_{16}/k_{18} of 1.03, has been reported for the acid-catalyzed hydrolysis of methyl α-D-glucopyranoside (Banks et al., 1961).

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