Anatomy of a Complex Mutarotation. Kinetics of Tautomerization of α-D-Galactopyranose and β-D-Galactopyranose in Water

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Abstract: The tautomerization (mutarotation) of α-D-galactopyranose and β-D-galactopyranose in dilute aqueous buffers was studied at 15 °C, pH 4.3, at 25 °C, pH 4.3, and at 25 °C, pH 6.2. In addition to optical rotatory measurements on the solutions, the proportions of the four cyclic tautomers (α-pyranose, β-pyranose, β-furanose, and α-furanose) were determined at intervals by gas–liquid chromatography. Plots of the GLC data show the simultaneous conversion of the starting pyranose to the anomeric pyranose and to furanoses during the course of the mutarotation. The thermodynamic parameters ($\Delta G°$, $\Delta H°$, $\Delta S°$) of the conversion reactions were calculated from data on the equilibrium proportions of the tautomers at the two temperatures. The kinetics of formation of each product tautomer were approximately first order. The rates of furanose formation were 8- to 13-fold greater than those of pyranose formation. Further analysis of the data, by a computer modeling approach and by a numerical computational approach, gave rate constants for the ring-opening and ring-closing reactions of each cyclic tautomer under the three conditions studied. The rate constants for the hydration–dehydration reactions of the open-chain forms of the sugar were also estimated from previous isotope exchange data. The rates of ring closure to furanose forms and to pyranose forms differ little from each other, but the ring-opening rates of the furanoses were 10 to 35 times faster than those of the pyranoses. When the calculated values of the rate constants were used in computer modeling, the models faithfully duplicated both the optical rotatory and the compositional changes observed in tautomerizing galactose solutions. Overall, the results provide the most complete description to date of a complex mutarotation and the first determination of the rate constants for the individual reactions underlying a mutarotation of this type.

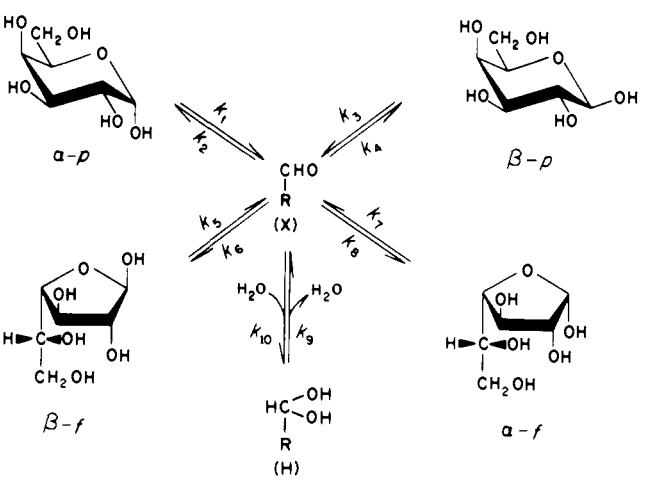
Introduction

The tautomerization (mutarotation) reaction that ensues when crystalline "reducing" sugars are dissolved in water or other suitable solvent is of considerable theoretical interest, and it is a major determinant of the chemical and biochemical behavior of the sugars. It is thus not surprising that the kinetics of mutarotation have been the subject of extensive studies over a period of many years.[1] From these studies it was concluded that the interconversion of the cyclic tautomers of a sugar proceeds (Scheme I) via a central intermediate, which is the aldehydo or keto form (X) of the sugar,[1a] in equilibrium with its hydrate (H). The open-chain forms were considered to be present at very low concentrations, and hence direct evidence for their presence was difficult to obtain. Recently, however, this lacuna has been filled, most notably by the work of Hayward and Angyal.[2,3]

It can be seen that, for a given sugar under given conditions (solvent, pH, temperature), a full description of the mutarotation/tautomerization process must include plots of the optical rotation *and* the tautomeric composition as a function of time until equilibrium is reached. In addition, one would like to have values of the rate constants (k_1 to k_8) for the individual steps of the tautomerization. Up to now, a complete, reliable set of data has not been reported for any sugar.

The first of the foregoing requirements is easily met for many sugars (e.g., glucose, mannose, xylose, and many common disaccharides) having "simple mutarotation", i.e., mutarotation strictly obeying the first-order rate law.[1a] In solutions of such sugars usually only two of the ring forms (normally the α- and β-pyranose) are significantly involved in the tautomeric equilibrium. The composition of the solutions can be calculated as a function of time from polarimetric measurements and a knowledge of the equilibrium ratio of the two major tautomers. The necessary data have been recorded for many sugars. The rate constants for the individual steps (k_1 to k_4) in this case cannot be extracted from

Scheme I



the optical rotatory/compositional data, but if the process is formulated as a simple, reversible interconversion of two molecular species (eq 1), the overall rate constants k_f and k_r can be calculated.

$$\alpha\text{-p} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \beta\text{-p} \qquad (1)$$

The mutarotations of a second class of sugars, of interest in the present work, show deviations from simple first-order kinetics and are designated "complex mutarotations".[1a] It is obvious that in such cases more than two of the tautomeric forms of the sugar are present in amounts sufficient to influence the optical rotation. It is also evident that the composition of a complexly mutarotating solution cannot readily be inferred from polarimetric readings. Direct measurements of the change, with time, of the proportion of each significant tautomer are required. Because suitable methods for making such measurements, namely, NMR spectroscopy and gas–liquid chromatography (GLC), are of relatively recent availability, only a few sugars of the second group have been examined in this way.

D-Galactose, in particular, has been investigated by Shallenberger et al.[4-6] and in the authors' laboratory,[7,8] in both cases by

(1) (a) Pigman, W.; Isbell, H. S. *Adv. Carbohydr. Chem.* **1968**, *23*, 11-57. (b) Isbell, H. S.; Pigman, W. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 13-65.

(2) Hayward, L. D.; Angyal, S. J. *Carbohydr. Res.* **1977**, *53*, 13-20.

(3) The keto forms of ketose sugars have been measured by NMR methods. Ketoses with special structural features may have substantial proportions (2 to >20%) of keto form in their aqueous solutions. See Wolff, G. J.; Breitmaier, E. *Chem.-Ztg.* **1979**, *103*, 232-233, and Angyal, S. J.; Bethell, G. S.; Cowley, D. E.; Pickles, V. A. *Aust. J. Chem.* **1976**, *29*, 1239-1247.

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GLC analysis. The Cornell workers were able to separate all four ring forms of the sugar, as the pentakis(trimethylsilyl) ethers, and determine the rates of change of the concentrations of the three major tautomers over the course of the mutarotation.[6] The α-furanose, present at equilibrium to the extent of ~1%, was not treated quantitatively. In our previous work the sugar was also treated as a three-component system, since the α-furanose was not resolved by our chromatographic procedure.[8] We concluded that the optical mutarotation could be correlated with the compositional changes only if a fourth component were reckoned in. Hence, a more definitive study was required.

In the present paper we describe the complete kinetics of tautomerization of α-D-galactopyranose and β-D-galactopyranose under three sets of conditions: 15 °C, pH 4.3; 25 °C, pH 4.3; and 25 °C, pH 6.2. By computer modeling techniques we have been able to show that the observed compositional changes quantitatively account for the observed complex optical mutarotation. In addition, we have been able to compute values of the rate constants k_1 to k_{10} for all of the interconversion reactions of the galactose tautomers according to Scheme I.

Experimental Section

α-D-Galactopyranose and β-D-Galactopyranose. The commercial D-galactose used was contaminated with a yellow impurity. This was removed by stirring a hot, concentrated solution of the sugar (~40%, w/v) with activated charcoal (Norite) for 1 h, filtering, and repeating the Norite treatment. Seven or more treatments were required to reduce the absorbance at 444 nm to a constant, minimal level. The pure galactose, crystallized from the final filtrate after the addition of methanol and ethanol,[9] had $[\alpha]^{20}_D$ +80.3° (*c* 5–20, equilibrium, water). The literature value[10] is $[\alpha]^{20}_D$ +80.2°.

Recrystallization of the purified sugar from water–methanol–ethanol and extraction of the dried, recrystallized product with 80% ethanol gave the pure α anomer in 70% yield. It had mp 169 °C (lit.[9] 165–167 °C) and an extrapolated, initial specific rotation, $[\alpha]^{20}_D$, of +150.6°, determined in 1 mM potassium hydrogen phthalate. The literature value[10] is $[\alpha]^{20}_D$ +150.7°. On analysis by gas–liquid chromatography[11] (GLC) only a faint trace of the β anomer could be detected.

The crystalline β anomer was prepared by a modification of the procedure of Hudson and Yanovsky.[12] A 50% (w/w) aqueous solution of the purified galactose was heated for 1 h on the steam bath, cooled to ~0 °C, diluted with 20 volumes of cold, absolute ethanol, and further cooled to −20 °C. The crystals deposited during 1 h at −20 °C were harvested and dried in vacuo. The dried product was then extracted with an equal weight of water for 5 min at ~0 °C, the undissolved sugar was filtered off and saved for reuse, and the filtrate was treated in the cold with ethanol as before. A second cycle of extraction and precipitation gave an 11% overall yield of material assaying 97.8% β-galactopyranose by GLC. The melting point was 144–146 °C (lit.[13] 143–145 °C), and the initial specific rotation, $[\alpha]^{20}_D$, was +55.0°; corrected for 2.2% of α-pyranose, +52.9° (lit.[10] $[\alpha]^{20}_D$ +52.8°).

Kinetic Measurements. A set of four clean, 19 × 36 mm vials was positioned in the bath compartment of a Lauda-Brinkmann Model K-2 circulator. The first vial held ~300 mg of dry sugar, while the second contained ~6 mL of buffer and supported a 5-mL syringe fitted with a needle carrying a Pyrex wool plug. The third vial held ~5 mL of buffer (heat transfer medium) and a supply of 10-μL pipets, and the fourth vial was empty. After thermal equilibration, the run was started by drawing ~5 mL of buffer into the syringe and squirting it onto the sugar (zero time). Sugar solution was quickly drawn back into the syringe, the needle was removed, and about half of the solution was deposited in the empty vial (no. 4). The remaining solution was injected into a 1.00-dm polarimeter cell, which was equipped with polyethylene inlet and overflow tubes, and thermostatted by water from the circulator. The cell was then placed in a Perkin-Elmer Model 141 polarimeter. Sampling and the recording of polarimeter readings were initiated by t = 1 min and continued, first at 1 min intervals (7–10 min), thereafter at longer intervals as appropriate. Equilibrium values were determined after 24 h.

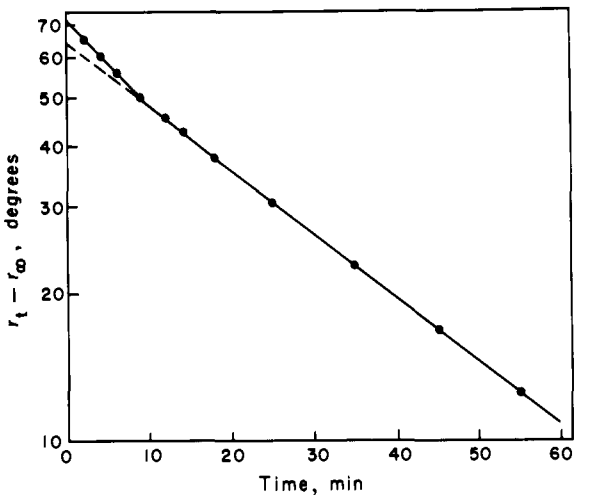

Figure 1. Semilogarithmic plot of the mutarotation of α-D-galactopyranose at 25 °C, pH 4.3. The points are experimental values from a single run. The line represents the best fit of these values to eq 2.

For experiments at pH 4.34, a single batch of 1.0 mM potassium hydrogen phthalate in triple distilled water was the buffer. Similarly, a 2.0 mM potassium maleate buffer was used for experiments at pH 6.24. Catalysis of the mutarotation by the buffer ions is negligible at these concentrations.[14]

Samples for compositional analysis by GLC were withdrawn from vial no. 4 by micropipet, discharged into *N,N*-dimethylformamide, frozen in liquid nitrogen, and trimethylsilylated as described by Bentley and Botlock.[15] The silylated samples were concentrated in a rotary evaporator at temperatures below 35 °C just prior to chromatography. Separation was accomplished on a 3 m × 2.2 mm (i.d.) aluminum column packed with 15% (w/w) silicone XF-1150 on 60–80 mesh Chromosorb A (system of Acree et al.[5]). The column was operated isothermally at 150 °C, with nitrogen as the carrier gas (25 mL/min), in instruments equipped with flame-ionization detectors. Each sample was run once at low attenuation and again at a higher attenuation to permit accurate determination of the relative areas of minor, intermediate, and major peaks. Areas were measured by cutting out and weighing the peaks. Control experiments indicated that the ratios of the galactose tautomers in a sample did not change during preparation for GLC. It was also shown that the response factors for the two pyranose anomers and the furanoses are identical.

Results

Optical Mutarotation. Semilogarithmic plots of the optical mutarotation of α-D-galactopyranose under all three of the conditions used had the classical biphasic form (Figure 1) associated with complex mutarotation.[16] The plots showed a short period of rapid optical rotatory change followed by a phase of slower change that strictly followed first-order kinetics until equilibrium was reached.

Equation 2 or its equivalent, eq 3, in which 10 is the base in

$$r_t = Ae^{-m_1't} + Be^{-m_2't} + C \quad (2)$$

$$r_t = A \times 10^{-m_1t} + B \times 10^{-m_2t} + C \quad (3)$$

the exponential terms, has been used to describe these mutarotations[1a,16b] and, indeed, our data were fitted by a least-squares method to eq 2. In the equations, r_t is the specific rotation (sodium D line) at time t, A is the value of $r_0 - r_\infty$ found by extrapolating the long segment of the semilogarithmic plot to t = 0, and B is the difference between A and the true value of $r_0 - r_\infty$. The

(4) Shallenberger, R. S.; Acree, T. E. *Carbohydr. Res.* **1966**, *1*, 495–497.
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(11) Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497–2507.
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(15) Bentley, R.; Botlock, N. *Anal. Biochem.* **1967**, *20*, 312–320.
(16) (a) Pigman, W. In "The Carbohydrates", 2nd ed.; Pigman, W.; Horton, D., Eds.; Academic Press: New York, 1972; Vol. IA, pp 165–175. (b) Bates, F. J. et al. *Natl. Bur. Stand. (U.S.), Circ.* **1942**, *C440*, 439–456.

Table I. Mutarotation Parameters for α-D-Galactopyranose[a,b]

temp, °C	pH	A, deg	B, deg	C, deg	m_1',[c] min^{-1}	m_2',[c] min^{-1}	ref
15	4.3	+65.2	+4.7	+81.2	0.012	0.137	this work
15	*d*			*+81.3*	*0.012*	*0.079*	*6, 17*
20	4.3				0.019[e]	0.172[e]	this work
20	*d*	*+64.9*	*+5.6*	*+80.2*	*0.018*	*0.182*	*10*
25	4.3	+64.0	+6.9	+79.4	0.030	0.216	this work
25	*d*			*+79.0*	*0.030*	*0.123*	*6, 17*
25	6.2	+63.7	+7.1	+79.4	0.032	0.344	this work

[a] Coefficients and exponential factors for the equation $r_t = Ae^{-m_1't} + Be^{-m_2't} + C$. See text for definitions of these quantities. [b] Each entry from the present work is the average of the values determined by least squaring the data from each of two runs (see text). [c] Literature values originally quoted as m_1 and m_2 (see text) were multiplied by 2.303. [d] Unbuffered water. [e] Calculated by the Arrhenius equation from the values determined at 15 and 25 °C.

equilibrium rotation, r_∞, is C and m_1' (or m_1) is the overall rate constant for the slow phase. The parameter m_2' (m_2) is a complex function of the slopes of the two segments of the plot.[1a,16b]

Values for A, B, C, m_1', and m_2' are assembled in Table I. It will be seen that the results of the present work are in excellent agreement with those of earlier investigators, except for m_2' at 15 °C, pH 4.3, and at 25 °C, pH 4.3. The reason for the discrepancy between our figures and those of Acree et al.[6] is not apparent. Since m_2' is at a minimum[14] at pH ~4.0, one should not obtain lower values by working in unbuffered water, as Acree et al. did. Indeed, if the pH of the water were ≥5, values higher than ours would be expected.

In our work with β-D-galactopyranose, the initial stages of the mutarotation were slightly affected by the presence in our sample of 2.2% of the α anomer. Hence, a comparison of our results with those in the literature could be made only for C and m_1'. For these the agreement was again excellent. Fortunately the contamination by α anomer did not interfere in other manipulations of data obtained with the β-pyranose.

Tautomeric Composition *vs.* Time. The course of the tautomerization of α-D-galactopyranose and β-D-galactopyranose at 25 °C, pH 4.3, as determined by GLC analysis, is plotted in Figure 2, and semilogarithmic plots for the α-anomer are given in Figure 3. Figure 3 also shows data collected at 25 °C, pH 6.2. For the *product* tautomers, the curves shown in the figures were obtained by fitting the data (plotted points) to the first-order rate equation by a least-squares method. First-order rate constants were calculated, and these, along with the corresponding half-times, are collected in Table II. The decay curves for the *starting* tautomers (Figures 2 and 3) were calculated by summing the best fit values for the other components and subtracting the sum from 100 mol %.

It could be shown (vide infra) that the progress to equilibrium of the product tautomers in the mutarotation of the galactopyranoses does not strictly follow first-order kinetics under the conditions studied. However, the deviations are small, as witnessed by the close fit of the calculated curves of Figures 2 and 3 to the experimental data. Hence, the "first-order treatment" is useful in characterizing the system.

In agreement with the results of our previous study of galactose tautomerization,[7,8] the curves of Figure 2 show no evidence of lag

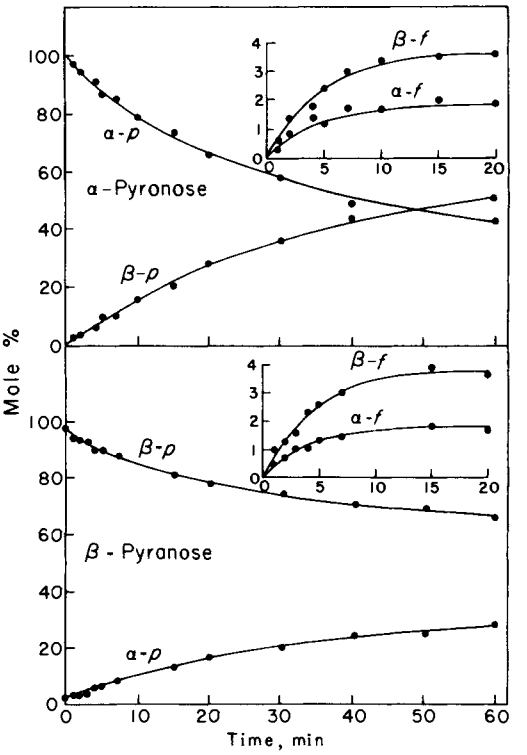

Figure 2. The change in composition, with time, of solutions of α-D-galactopyranose (top) and β-D-galactopyranose (bottom) at 25 °C, pH 4.3. In each case the points are experimental values from a single run, and the lines are the statistically fitted curves (see text).

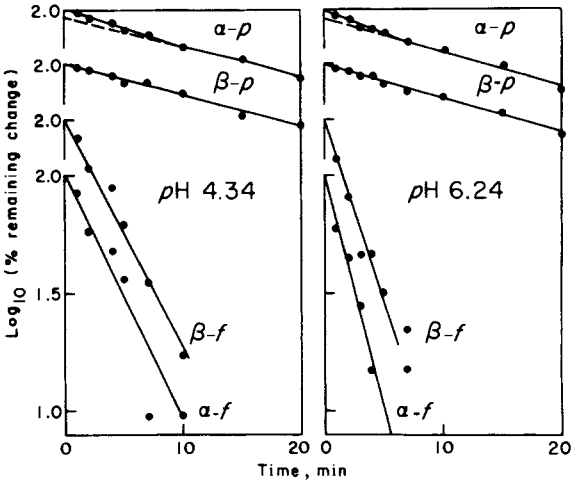

Figure 3. Logarithmic plots of the change in composition, with time, of solutions of α-D-galactopyranose at 25 °C, pH 4.3 (left), and at 25 °C, pH 6.2 (right). In each case the points are experimental values from a single run; the lines were statistically fitted (see text).

in the formation of any of the product tautomers and no maxima or minima. The biphasic nature of the decay curves (Figure 3) for the starting pyranose is to be expected, for this pyranose is

Table II. Kinetics of Approach to Tautomeric Equilibrium[a,b]

starting tautomer	temp, °C	pH	$10^2k \pm$ SE, min^{-1} ($t_{1/2}$, min) α-pyranose		β-pyranose		α-furanose		β-furanose	
α-p	15	4.3		(51.6)	1.20 ± 0.03	(57.8)	13.6 ± 1.9	(5.1)	13.3 ± 1.7	(5.2)
β-p	15	4.3	1.19 ± 0.04	(58.2)		(48.8)	13.0 ± 1.6	(5.3)	12.5 ± 1.0	(5.5)
α-p	25	4.3		(21)	3.0 ± 0.1	(23)	23.7 ± 2.6	(2.9)	19.9 ± 0.6	(3.5)
β-p	25	4.3	3.1 ± 0.2	(22)		(17)	26.5 ± 1.5	(2.6)	22.3 ± 1.0	(3.1)
α-p	25	6.2		(20)	3.2 ± 0.06	(22)	43.0 ± 2.3	(1.6)	35.6 ± 1.5	(2.0)
β-p	25	6.2	3.1 ± 0.1	(22)		(16.5)	42.2 ± 2.9	(1.6)	40.6 ± 2.1	(1.7)

[a] Each k is the weighted average of the values determined by least squaring the data from each of two runs (see text). [b] Half-lives: $t_{1/2} = 0.693/k$, except for the starting tautomer (see text).

Table III. Composition of Aqueous D-Galactose Solutions at Equilibrium

temp, °C	mol % α-pyranose	β-pyranose	α-furanose	β-furanose	ref and method used
15	32.5 ± 1.0[a]	63.8 ± 1.0	1.2 ± 0.1	2.5 ± 0.1	this work, GLC
15	*32.6*	*65.2*	*0.3*	*2.0*	*17, GLC*
25	32.0 ± 1.7	62.4 ± 1.7	1.8 ± 0.1	3.8 ± 0.1	this work, GLC
25	*32.0*	*63.9*	*1.0*	*3.1*	*6, GLC*
35	*29*	*64*	*4*	*3*	*18, ^{1}H NMR*[b]

[a] Standard error. [b] In D_2O.

Table IV. Thermodynamics of Equilibrium[a]

reaction	ΔG°_{298}, cal/mol	ΔH°, cal/mol	ΔS°_{298}, eu
α-p ⇌ β-p	−390	−110	+0.9
α-p ⇌ β-f	+1260	+7390	+20.6
α-p ⇌ α-f	+1700	+7160	+18.3

[a] The equilibrium constants (15 and 25 °C) for the reactions listed were calculated from the data of Table III.

necessarily the primary source of the rapidly formed furanose anomers.

The much greater rate of approach to equilibrium of the furanose anomers, as compared with the pyranoses, is clearly shown by the plots in Figure 3. These plots also demonstrate the greater sensitivity of the rates of furanose formation to increases in pH, which was expected from the studies of Isbell and Pigman[14] on the pH profiles of the parameters m_1' and m_2' in eq 3. The values of m_1' are minimal and nearly constant over the pH range 2.5 to 7. On the other hand, as already noted, m_2' shows a minimum at pH ~4.0 and increases sharply with either a decrease or increase in pH. Our values for the rates of formation of β-pyranose from α-pyranose (Table II) agree well with those of Acree et al.,[6] but our values for the rates of β-furanose formation are higher, in accord with our higher values for m_2'.

Equilibrium Composition and Thermodynamics. Determinations of the equilibrium proportions of the tautomeric forms of D-galactose in solution were a necessary part of the present work. The results are assembled in Table III, along with the values published by previous investigators. There is quite good agreement between our figures and those of Acree et al.,[6,17] also obtained by the GLC method. On the other hand, there is some discrepancy between our values for the α-pyranose and α-furanose at 25 °C and the ^{1}H NMR spectroscopic values of Angyal and Pickles.[18] These discrepancies are not entirely explained by the slightly higher temperature (35 °C) of the ^{1}H NMR measurements, which would cause an increase in the proportions of *both* furanoses. A more likely cause is the difficulty of accurately estimating the relative areas of the signals for the anomeric protons of the β-furanose, α-pyranose, and α-furanose. These signals are closely spaced even at 270 MHz.

Estimates of the thermodynamic parameters for the interconversion of the cyclic tautomers of D-galactose, derived from the data of Table III, are given in Table IV. Predictably, the figures relating to the α-p ⇌ β-p and the α-p ⇌ β-f reactions are similar to those quoted by Acree et al.,[6] who commented on the difference between the two types of equilibria. Each warrants a brief remark.

The enthalpy change for the α-p ⇌ β-p interconversion is small, in accord with the long known generalization that pyranose–pyranose equilibria are little affected by temperature.[16b] This generalization is supported by modern measurements on D-glucose,[19] D-mannose,[19] and 2-deoxy-D-ribose.[20]

Isbell and Pigman[14,21] noted that, in contrast to α-p ⇌ β-p equilibria, the equilibria between the pyranose forms and the "labile forms" (furanose anomers) of sugars are temperature sensitive. From extensive recent measurements on 2-deoxy-D-ribose[20] and D-fructose[22] and less complete data for other sugars,[18] it is evident that the furanose forms are the high-enthalpy forms, increasing in proportion with increasing temperature. Thus, we find $\Delta H^\circ \approx +7$ kcal/mol for the conversion of α-galactopyranose to the galactofuranoses (Table IV). Since these transformations involve only modest increases in Gibbs free energy, substantial entropy increases are involved. The structural bases for these thermodynamic differences between the pyranose and furanose forms of sugars have been discussed by Lemieux, Anderson, and Conner[20] and by Angyal and Pickles.[23]

Evaluation of the Rate Constants for the Individual Ring-Opening and Ring-Closing Steps. For an equilibrating, multicomponent system such as that of Scheme I, explicit calculation of the rate constants for the individual reaction steps is not possible from data on the changes in the composition of the system with time. Hence, to arrive at estimates of the rate constants k_1 to k_8 we further elaborated the computer modeling technique presented in our earlier paper.[8] As noted there, the modeling program was based on the analytical solution of the set of differential equations describing the system (four components plus central intermediate) of Scheme I. Given a value for the mole fraction of each cyclic tautomer at zero time, a statement of the equilibrium composition (mole fractions, including that of the central intermediate X), and stipulated values for the rate constants k_1, k_3, k_5, and k_7, it was possible using the program to model the progress of the system from any initial state to equilibrium. In the present work, we modified the program to provide for iterative adjustment of the initially supplied rate constants. Computation applied to the results of an experimental run then gave values for k_1, k_3, k_5, and k_7 consistent with the observed half-times for the approach of the individual components to equilibrium levels. For each run with a given starting anomer, this procedure furnished many sets of figures, covering a range of values for each rate constant. However, as described in the thesis of P.W.W.,[24] calculation for a particular pH and temperature gave only one set of figures satisfying the experimental findings for *both* starting anomers (α-pyranose and β-pyranose). Thus, we arrived at unique values for the rate constants k_1, k_3, k_5, and k_7 at each pH–temperature combination studied.

A more straightforward calculation of the rate constants of the galactose tautomerization became possible with the availability of a computer program devised by W. E. Stewart and J. P. Sørensen.[25] This program generates numerical solutions of the differential equations describing a reacting system and includes a least-squares routine for fitting the reaction parameters (rate constants and activation energies) to observed compositional changes. It is capable of dealing with systems of considerable complexity. Input, in the present case, was our complete set of kinetic data taken at 15 and at 25 °C, pH 4.3 (two runs with each starting tautomer at each temperature). Values of k_1, k_3, k_5, and k_7 calculated with the aid of the Stewart–Sørensen program are given in Table V, along with the values furnished by our original modeling approach. For the tautomerization at pH 4.3, identical results were obtained by the two methods. For pH 6.2, where data were collected at only one temperature, the values recorded are those provided by the modeling method.

Estimates of the rate constants (k_2, k_4, k_6, and k_8) for the ring-closure steps of the galactose tautomerization are also tabulated in Table V. For each tautomer under given conditions,

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Table V. Rate Constants for the Individual Steps of the Galactose Tautomerization

		ring-opening reactions,[a] min^{-1}				ring-closing reactions, min^{-1}		
tautomer	k_{op}	pH 4.3, 15 °C	pH 4.3, 25 °C	pH 6.2, 25 °C	k_{cl}	pH 4.3, 15 °C	pH 4.3, 25 °C	pH 6.2, 25 °C
α-pyranose	k_1	0.013 ± 0.0004	0.032 ± 0.001		k_2	38	50	52
		0.013	*0.033*	*0.034*				
β-pyranose	k_3	0.013 ± 0.0005	0.034 ± 0.001		k_4	70	100	100
		0.012	*0.034*	*0.035*				
β-furanose	k_5	0.20 ± 0.017	0.32 ± 0.027		k_6	45	58	180
		0.20	*0.32*	*0.98*				
α-furanose	k_7	0.21 ± 0.037	0.42 ± 0.072		k_8	23	36	100
		0.21	*0.42*	*1.20*				

[a] Values in roman type (with 95% confidence limits) were computed by the program of Stewart and Sørensen; values in italics were obtained by the authors' modeling approach (see text).

the ring-closing rate constant (k_{cl}) was calculated from the relevant ring-opening rate constant (k_{op}), and the relationship

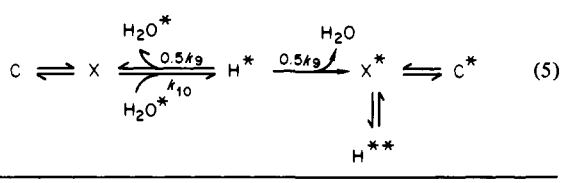
$$\frac{N_X}{N} = K = \frac{k_{op}}{k_{cl}} \tag{4}$$

where N_X is the mole fraction of the aldehydo intermediate and N is the mole fraction of the anomer concerned (Table III), both at equilibrium, and K is the equilibrium constant for the ring-opening reaction.

In assessing the figures presented in Table V it is necessary to consider certain features of the relationship between the k_{op}s, k_{cl}s, and N_X. Early in our work on the computer modeling of sugar tautomerization, we could show that the progress of the system to equilibrium is governed almost entirely by the ring-opening rate constants. In modeling calculations, the value assigned to N_X could be varied over a wide range with essentially no effect. From this it follows that our values for the ring-opening rate constants are firm, subject to the limitations of our experimental method. Reciprocally, the reliability of our estimates of the ring-closing constants is totally dependent on the reliability of the values chosen for N_X. As noted in the Introduction, the determination of this quantity has been a difficult problem. An earlier value of N_X for D-galactose at 25 °C based on polarographic data[26] has been shown to be unsound,[27] but acceptable estimates are now available from the circular dichroism measurements of Hayward and Angyal.[2] In calculating our ring-closing rate constants, we set $N_X = 0.00011$ at 15 °C and 0.00021 at 25 °C on the basis of Hayward and Angyal's results.

Kinetics of Hydration of the Aldehydo Form. In addition to the four cyclic tautomers and the aldehydo form, aqueous solutions of aldose sugars have long been presumed to contain a sixth molecular species, namely, the hydrated, open-chain or aldehydrol form (H, Scheme I). Recent NMR observations have verified this presumption for some rather special cases (vide infra), but in solutions of the more common sugars the amount of hydrated form is too small to measure directly. However, its presence is revealed by the finding that the anomeric oxygen of sugars exchanges with the oxygen of water.[28] From data on the rate of this exchange in the case of galactose,[8] it was possible to estimate the values of the rate constants (k_9 and k_{10}) associated with the hydration–dehydration equilibrium.

The central steps of the ^{18}O-exchange process are shown in eq 5, where C and C* represent, respectively, all unlabeled and all



(5)

labeled cyclic forms, X and X* the unlabeled and labeled aldehydo form, and H* and H** the singly and doubly labeled hydrate. The key intermediate is the *singly labeled* hydrate (H*), which is dehydrated to both X and X*. For purposes of calculation, we assume the rates of the two dehydrations are the same, although the reaction is probably subject to a small ^{18}O isotope effect. The important feature of the process is that when the exchange is carried out in a large molar excess of ^{18}O-labeled water, as was experimentally the case, the step H* → X* is effectively irreversible. Moreover, relative to the overall rate of isotope exchange, all other steps are in rapid equilibrium. This enables us to write eq 6, where k_{ex} is the rate constant for the overall exchange. From

$$\frac{d[C^*]}{dt} = -\frac{d[C]}{dt} = k_{ex}[C] = 0.5k_9[H^*] = 0.5k_{10}[X] \tag{6}$$

the relationships of eq 6, we obtain eq 7 and, since the ratio

$$k_{ex} = 0.5k_{10}\frac{[X]}{[C]} \tag{7}$$

[X]/[C] is effectively equal to N_X when N_X is small, eq 7 becomes eq 8. Knowing N_X, all we now require to complete the calculation

$$k_{ex} = 0.5k_{10}N_X \text{ or } k_{10} = \frac{2k_{ex}}{N_X} \tag{8}$$

are estimates of k_{ex}. Extrapolation from results previously obtained in this laboratory[8] at 50–70 °C gives k_{ex} for galactose in unbuffered water as 5.8×10^{-5} min^{-1} at 25 °C and 1.5×10^{-5} min^{-1} at 15 °C. Substitution of these figures, and the appropriate figures for N_X (see previous section), gives 0.55 min^{-1} at 25 °C and 0.27 min^{-1} at 15 °C as values for k_{10}, the pseudo-first-order rate constant for the hydration of *aldehydo*-galactose.

To obtain k_9 we note that

$$\frac{k_{10}}{k_9} = \frac{[H]}{[X]} \tag{9}$$

and seek an estimate of the hydrate/aldehyde ratio [H]/[X]. This ratio has been measured for glyceraldehyde[29] and its 3-phosphate,[30] for erythrose,[29] and for 3,6-anhydroallose.[31] These sugars exist to a substantial extent as open-chain forms in solution and are electronically similar to the common aldopentoses and -hexoses in that they bear a secondary hydroxy group at position 2. When normalized by assuming $\Delta H^\circ \simeq -7000$ cal/mol for the hydration reaction,[32] the values of [H]/[X] in these known cases cluster about 20 at 25 °C and 30 at 15 °C. Inserting these figures into eq 9 gives $k_9 \simeq 0.03$ min^{-1} at 25 °C and 0.01 min^{-1} at 15 °C.

Comparison of the Observed and Calculated Kinetics of Tautomerization. An obvious test of the correctness of the rate constants calculated by the Stewart–Sørensen method was to see

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whether the values, in conjunction with our modeling program, would yield accurate descriptions of galactose tautomerizations, over their full course from time zero to equilibrium. This test was in part accomplished by our iterative use of the modeling program for an independent evaluation of the rate constants. However, the comparison was incomplete, because the iterative procedure, in effect, fitted the progress curve to a single point ($t_{1/2}$) between the initial and equilibrium states. Hence, we did modeling computations for both starting anomers at each pH–temperature combination studied, using the values of k_1, k_3, k_5, and k_7 given in Table V and an ad hoc figure for N_X. The agreement between the computed progress curves (not shown) and the "best fit" experimental curves (Figure 2) was close. Divergences did not exceed 1.5 mol % for the pyranoses or 0.1 mol % for the furanoses.

When logarithmic plots (not shown) of the computed progress curves were examined, some of the lines were seen to have slight *s* curvature. From this it could be concluded that the kinetics of the formation of product tautomers in mutarotating galactose solutions are only approximately first order. Since the statistical fitting of our experimental data was based on a first-order equation, it could be further concluded that the discrepancies between the curves computed from the rate constants and the statistically fit curves were more apparent than real. The computed curves describe the experimental data as well as the "best fit" curves.

A more stringent test of the kinetic scheme presented here is to see whether the observed optical mutarotation curves for α- and β-D-galactopyranose are reproduced by modeling computations employing the proposed values of k_1, k_3, k_5, and k_7. This test was carried out by modifying the computer program so that, for successive time points, the specific rotation (r_t) of the tautomerizing galactose was obtained from its calculated composition according to eq 10, where $r_{\alpha\text{-p}}$, ..., $r_{\beta\text{-f}}$ are the specific rotations of the individual cyclic tautomers, and $N_{\alpha\text{-p}}$, ..., $N_{\beta\text{-f}}$ are the respective mole fractions at the time being considered.

$$r_t = r_{\alpha\text{-p}}N_{\alpha\text{-p}} + r_{\beta\text{-p}}N_{\beta\text{-p}} + r_{\alpha\text{-f}}N_{\alpha\text{-f}} + r_{\beta\text{-f}}N_{\beta\text{-f}} \quad (10)$$

As input for this calculation, in addition to the items listed in the previous section, we required figures for $r_{\alpha\text{-p}}$, ..., $r_{\beta\text{-f}}$ at both working temperatures. For the pyranose anomers the values were readily assembled from the data of the present work or from information in the literature, but for the α- and β-furanose the necessary data were lacking. Measurements of the specific rotations of the furanose forms of the common sugars have not been made, because it has not so far been possible to isolate and manipulate these anomers. We attempted a direct solution of this problem and devised a method[24] for obtaining a sample of D-galactose highly enriched in the β-furanose form. However, with the time and material available we were not able to accurately measure the specific rotation and anomeric composition of the sample.

Further consideration showed that accurate values for $r_{\alpha\text{-f}}$ and $r_{\beta\text{-f}}$ are not essential for the calculation of r_t from compositional data. Since the ratio $N_{\alpha\text{-f}}/N_{\beta\text{-f}}$ is nearly constant during the tautomerization, the net contribution of the furanose anomers to the optical rotation is proportional to the mole fraction of *total* furanose. The contribution is small even at $t = \infty$. Hence, the computation can be satisfactorily done with any pair of figures that satisfy eq 10. To obtain such figures for each of the two temperatures, we inserted our most accurately known sets of values of r_t and $N_{\alpha\text{-p}}$, ..., $N_{\beta\text{-f}}$ (those for $t = \infty$) into eq 10 and used the equation to adjust arbitrarily chosen trial values[33] of $r_{\alpha\text{-f}}$ and $r_{\beta\text{-f}}$.

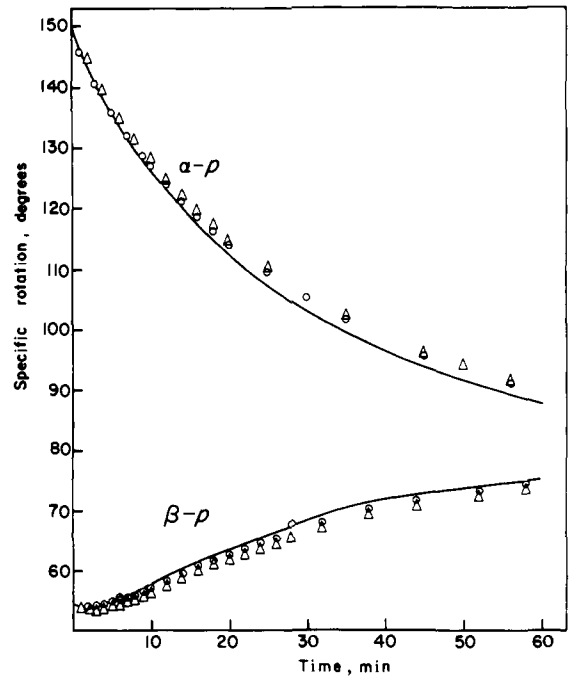

Figure 4. Computer simulation of the optical mutarotation of α- and β-D-galactopyranose at 25 °C, pH 4.3. The solid lines are the computed curves. The points are the experimental readings from the two runs made with each anomer at this temperature and pH.

Following this, execution of the simulations gave complete sets of mutarotation curves, which were compared with the experimental curves. The agreement was very good overall, as illustrated in Figure 4. In particular, the computed curve for the α-pyranose reproduces the rapid initial drop in rotation observed with this anomer. This drop was not accounted for in our earlier simulations,[8] which treated galactose as a system of three components plus a central intermediate. Also reproduced is the transient initial decrease in rotation characteristically observed with the β-pyranose.

Discussion

In 1938, Isbell and Pigman[14] analyzed the available data on fast and slow mutarotations and drew the inference that a complex mutarotation results from the initial rapid conversion of the starting pyranose to furanoses, superimposed on the slower and longer lasting, reversible conversion to the other pyranose. Evidence in support of this picture was furnished by a study of 2-deoxy-D-ribose, referred to earlier,[20] but that study was incomplete. Only the β-pyranose anomer could be examined, and the compositional data, obtained by NMR measurements, were not of the highest accuracy. Thus, an important result of the present work is the detailed confirmation of Isbell and Pigman's hypothesis for the case of D-galactose. This is provided in Figures 2 and 3, which document the concurrent and independent progress of the pyranose–furanose and pyranose–pyranose interconversions.

From a quantitative standpoint, the principal contribution of the work is the sets of rate constants, k_1 to k_8, derived for the ring-opening and ring-closing reactions of the individual cyclic tautomers of D-galactose. These constants, determined under conditions of minimal catalysis by acids and bases, are to the best of our knowledge the first obtained for the reactions underlying a complex mutarotation. There have been previous attempts, based on polarographic studies, to estimate the rate constants of the reactions of sugar tautomerization, but the sugars primarily investigated were D-glucose[27] and D-xylose,[38] which show simple mutarotation. Only four constants (k_1 to k_4) are involved.

(33) A reasonable estimate of the actual magnitudes of $r_{\alpha\text{-f}}$ and $r_{\beta\text{-f}}$ is obtained from the relationship M (methyl glycoside) − M (corresponding tautomer of sugar) ≃+115° (α anomer), or −115° (β anomer), where M is the molecular rotation at 589 nm (Brewster,[34] and Lemieux and Martin[35]). Calculation, based on literature values[36] of the specific rotations of methyl α- and β-D-galactofuranoside, gives $r_{\alpha\text{-f}}$ = +48° and $r_{\beta\text{-f}}$ = −57°. The Brewster–Lemieux approximation was developed from data for methyl aldopyranosides, but we believe it should apply to many aldofuranosides as well. The conformational basis for the optical rotatory powers of the methyl furanosides has recently been elucidated by Angyal.[37]

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Comparison of the polarographic values with our figures for the galactopyranoses is not appropriate, because the polarographic experiments were done in more concentrated buffers, at pHs >6.8. Moreover, the numerous assumptions required in calculating these rate constants from polarographic data leave the reliability of the constants open to serious question. The values reported here for the galactose system are subject to fewer reservations. They were obtained in a direct way and were shown to be consistent with experimental observations on both compositional and optical rotatory changes. Also supportive is the close correspondence between our values for the reactions of the galactofuranoses (k_5 to k_8) and Grimshaw, Whistler, and Cleland's estimate of the ring-opening and ring-closing rates of 6-thio-β-D-fructofuranose.[39]

The estimation of values for the rate constants (k_{10} and k_9) of the hydration and dehydration reactions of the open-chain forms completes the description of the kinetics of the galactose system. In addition, these calculations enable a correlation of the phenomena of mutarotation and the exchange of ^{18}O between water and the anomeric function of the sugar. Our figure for the hydration rate constant k_{10} at 25 °C (0.55 min^{-1}) is quite close to the known pseudo-first-order rate constant for the hydration of acetaldehyde at the same temperature (~2 min^{-1}).[40] In other words, the value of k_{10} computed from isotope exchange data is normal. It follows that there is no basis for arguing that the exchange reaction itself is anomalous (vide infra). Its slow rate is simply a consequence of the exceedingly low concentration of the open-chain forms of the sugar.

The methods used in the present work should be applicable to any sugar showing complex mutarotation, given the availability of samples, pure or highly enriched, of two of the cyclic tautomers of the sugar. Once the progress of each tautomer to equilibrium is charted, the program of Stewart and Sørensen would provide the most direct means of calculating the ring-opening and ring-closing rate constants. Computations with the present authors' modeling program would then be useful for testing the proposed rate constants and for visualizing the effects of changes in one or more of them. Studies of additional cases of complex mutarotation may uncover interesting features not found in the galactose and deoxyribose systems. Unfortunately, determination of the rate constants of the individual reactions associated with a simple mutarotation does not appear possible via either of our computational approaches.

In analyzing the results of our investigation, we have assumed that the reactions of galactose tautomerization are those shown in Scheme I. However, it must be noted that the success of our computations does not establish the validity of this scheme. It is unlikely that the tautomerization process can be correctly described by any simpler scheme, e.g., one without a central intermediate, even though such schemes have recently been used[41] to rationalize the data from early investigations on D-galactose. However, a more complex set of reactions, involving more than eight discrete steps, cannot be ruled out on the basis of present knowledge. Indeed, a serious proposal of this sort has been advanced by Isbell et al.,[42] who postulate a series of partially opened ("pseudoacyclic") species as intermediates in the interconversion of the cyclic tautomers of the sugars. In part, Isbell et al. were led to the idea of partially opened intermediates by the seeming contradiction between the rapidity of mutarotation and the slowness of the ^{18}O-exchange reaction. As pointed out above, this contradiction now seems to be resolved. It does appear, from recent work of Grimshaw, Whistler, and Cleland,[39] that partially opened intermediates participate in the α-β interconversion of sugars having sulfur as the ring heteroatom. However, Grimshaw et al. adduce arguments against the involvement of partially opened species in the tautomerization of normal sugars (oxygen the ring heteroatom), except possibly at high pH. Thus, the existence, in sugar solutions, of species of other than those shown in Scheme I remains to be established.

Examination of the values collected in Table V enables us to correlate the rate constants of galactose tautomerization with the major compositional feature of the system, namely, the preponderance of pyranose anomers at equilibrium. It is evident that the kinetic basis for this preponderance is to be found in the ring-opening reactions. The ring-opening rates of the pyranoses are slower, by an order of magnitude or more, than those of the furanoses. On the other hand, ring closure of the aldehydo form to furanoses is on the average no faster (pH 4.3), or only marginally faster (pH 6.2), than its closure to pyranoses.

This latter finding was unexpected, because quantitative studies of cyclization reactions have generally shown the formation of five-membered rings to be substantially faster than that of six-membered rings. Some of the studies have been done on simple aliphatic compounds that cyclize by an S_N2-type displacement on saturated carbon,[43] and it could be argued that such compounds are not appropriate models for an aldehydo sugar. However, open-chain derivatives of sugars (alditols, aldose dialkyl acetals, etc.) undergo a variety of reactions in which *either* a five-membered or a six-membered ring could form. In these cases, a substance having a five-membered ring is almost always the predominant or exclusive product.[44-46] (Lactone formation from aldonic acids may be an exception.[45b]) By comparison, ring formation from *aldehydo*-galactose appears anomalous. We can offer no rationalization of this "different" behavior, but if it proves to be characteristic of aldehydo sugars,[8] a search for the determining factors should be undertaken.

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