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# THE STUDY OF THE MUTAROTATION OF D-GLUCOSE, D-FRUCTOSE, AND D-RIBOSE BY USE OF THEIR CIRCULAR DICHROISM*

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## ABSTRACT

After mutarotation, D-fructose, D-ribose and, to a much smaller extent, D-glucose have c.d. maxima in the region 270–290 nm as well as below 210 nm. The ellipticities at the former wavelengths can be attributed to an acyclic form since compounds having no carbonyl group, such as sucrose, D-glucitol, methyl α- and β-D-glucosides, lack a maximum there whereas D-glyceraldehyde has a maximum at 290 nm. The ellipticities at the short wavelengths are due, at least in part, to ring structures, since all the model compounds absorb there. Sucrose has an especially strong ellipticity Evidence is presented to indicate that both D-fructose and D-ribose have the acyclic form present in the original dry crystals before mutarotation. Ellipticity changes of D-fructose show a single first-order step. The first-order kinetics at the acyclic maximum indicates a rate of formation considerably lower than the rate of mutarotation. The increase of D-ribose in ellipticity at this maximum indicates two simultaneous first-order reactions, one of them being much slower than the other. The mutarotation of α-D-glucose can be followed easily only at a short wavelength and gives a single first-order plot, the rate constant of which agrees with the polarimetric mutarotation constant, and in which the accumulation of acyclic intermediates is not observed to any significant extent. These observations support the suggestion that mutarotation of a sugar proceeds through *pseudo*-acyclic intermediates which, thereafter, give rise to either the acyclic form or another ring.

## INTRODUCTION

The mutarotation of simple sugars is often assumed to include transformations of ring forms *via* an acyclic carbonyl structure, the concentration of which rapidly

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attains a steady state. In the case of some monosaccharides, for instance D-fructose or D-ribose, this form has been reported to attain a relatively large concentration: $\sim$1-2% as estimated by u.v. and circular dichroism (c.d.) spectra, for the former,[1] and 8.5% determined by the dropping mercury electrode for the latter[2]. In other cases, most notably that of D-glucose, the concentration of the carbonyl form is barely measurable, although in the biologically important phosphate esters of glucose it may be considerably increased[3]. Furthermore, the techniques that have been used with D-glucose disturb the steady-state concentration by the act of measurement. Using polarographic methods, Cantor and Peniston[2] have estimated a concentration of 0.024% of carbonyl groups, and Los *et al.*[4] 0.0026%.

The circular dichrometer is able to examine events in mutarotation without disturbing equilibria. The region near 275 nm has been especially of interest with sugars containing a free carbonyl group. Listowsky *et al.*[1] have described the ellipticity, in this region of equilibrium, of D-fructose and several of its phosphate derivatives, D-glyceraldehyde, as well as several other mono- and disaccharides. Totty *et al.*[5] observed an ellipticity near 290 nm for L-arabinose and reported it to be constant for at least 24 h.

In addition to their intrinsic significance to understanding the mechanism of spontaneous mutarotation, our studies were initiated to provide a basis for further investigations into the mechanism of the biological catalysis of mutarotation by the enzyme mutarotase[6,7].

## RESULTS AND DISCUSSION

The ellipticities of D-fructose and D-ribose have been measured at their wavelength maxima (Table I). In addition, we have found a weak signal for D-glucose at 290 nm (Table I) in 3M solution.

TABLE I

C.D. MAXIMA OF D-RIBOSE, D-FRUCTOSE, AND D-GLUCOSE[a]

| *Compound* | $\lambda_{max}$ *(nm)* | $[\theta]_{app}^{b}$ (°) |
|---|---|---|
| D-Ribose | 283 | −2.4 |
| | 221 | −2.9 |
| | 195[c] | −240.2 |
| D-Fructose | 272 | +25.2 |
| | 205 | −28.0 |
| | 195[c] | +81.9 |
| D-Glucose | 290 | −0.038 |
| | 195[c] | +134.8 |

[a] All extreme values for ellipticities, whether positive or negative, are reported as maxima. [b] See Experimental section for definition. [c] The ellipticity continues to increase below this wavelength but the DV is > 0.7 kV.

That the 270–290-nm ellipticity represents the acyclic form of these sugars has been suggested by theoretical considerations for a carbonyl n → π* transition[1]. These bands are absent in the c.d. spectra of compounds that lack an acyclic carbonyl form, such as sucrose, D-glucitol, and methyl α- and β-D-glucopyranoside, as observed by ourselves (Table II) and, earlier, by Listowsky and Englard[6] with the methyl glucosides.

TABLE II

C.D MAXIMA OF SUCROSE, D-GLUCITOL, METHYL α-D-GLUCOPYRANOSIDE, AND METHYL β-D-GLUCOPYRANOSIDE[a]

| *Compound* | $\lambda_{max}$[b] *(nm)* | $[\theta]$ (°) |
|---|---|---|
| Sucrose | 195[c] | +300 |
| D-Glucitol | 193.5 | +51.3 |
| Methyl α-D-glucopyranoside | 195[c] | +21.2 |
| Methyl β-D-glucopyranoside | 210 | −3.0 |
| | 195[c] | +50.8 |

[a]All extreme values for ellipticities, whether positive or negative, are reported as maxima. [b]The ellipticity for all of these compounds is ≃ 0 at wavelengths > 230 nm. [c]The positive ellipticity continues to increase below this wavelength, but the DV is > 0.7 kV.

Additional evidence that the acyclic form absorbs at 270–290 nm is the presence of these bands in the spectrum of D-glyceraldehyde. At 290 nm, we have observed glyceraldehyde to have a $[\theta]_{app}$ −76.9° at 1 h after addition to water. Feeley *et al.*[9] have reported temperature (25° to 78°) enhancement of the ellipticity of glyceraldehyde from $\Delta\varepsilon \times 10^3$ −42.8° to −291° at this wavelength and also a small, somewhat delayed, positive increase at 330 nm, neither of which completely reverts upon cooling. These authors also noted that the apparent mol. wt. decreases by almost half during several days at 37° until it nears that of $C_3H_6O_3$. Although they have not included ellipticity measurements of solutions aged at 37°, they attribute the dichroic changes to increased amounts of the two products of the slowly equilibrating system: Glyceraldehyde dimer ⇄ hydrated acyclic monomer (presumably aldehydrol) ⇄ acyclic carbonyl group. This scheme would require a diol at C-1 to absorb rather strongly at long wavelengths where other hydroxyl groups do not. Swenson and Barker[10] found for D-glyceraldehyde a $[\theta]_{app}$ −46° to −47° before and after purification by charcoal, but did not mention the age of the solutions. They also presented i.r. data indicating the absence of an acyclic carbonyl form in these solutions. By contrast, n.m.r. studies have shown[11] that the acyclic carbonyl form is predominant in some ketose phosphates. The form of glyceraldehyde monomer remains an open question but, at least at present, the ellipticity at 290 nm can be attributed to some kind of acyclic form.

Tables I and II also show ellipticities near 200 nm. Bands have previously been reported in this region for D-galactose[8,12], D-glucose[8,12], and D-xylose[12]. The spectra of the methyl D-glucosides, especially when evaluated in conjunction with that of sucrose, implicate ring structures as the absorbing species. Pickett *et al.*[13] report u.v. absorbance at this wavelength for oxygen-containing 5- and 6-membered heterocyclic compounds. However, the c.d. spectrum of acyclic D-glucitol, as well as those of other compounds containing hydroxyl groups, implies a role for the latter functional group. Harrison *et al.*[14] support this point with their study of the u.v. absorbance of simple alcohols. Possibly both ring structures and hydroxyl groups contribute to the c.d. spectra.

We have also studied the c.d. spectra of some of the sugars as a function of time at a fixed wavelength. The extremely low signal of D-glucose at 290 nm makes kinetic measurements impossible at this wavelength, but mutarotation of this sugar can be observed at the low wavelengths. The change at 195 nm as a function of time, after the sample is dissolved in water, is a continuous increase in positive ellipticity. This change cannot be extrapolated back to zero ellipticity at zero time but rather to $[\theta]_{app}$ +69.5°, which indicates an ellipticity attributable to the $\alpha$-pyranose form of the sugar present in the original crystalline state. The absorption of the pyranose ring in the c.d. range suggests that other ring forms absorb in this range. At 195 nm, the ellipticity becomes more positive until a limiting value of $[\theta]_{app}$ +134.3° is reached. These large changes, coupled with the negligible ellipticity at 290 nm, imply the opening of the original ring form as a slow kinetic step, followed by the rapid closing to a second ring form without the accumulation of an intermediate acyclic species. The overall positive ellipticity of D-glucose at 195 nm approximately doubles during the course of the entire reaction. If one assumes (a) a transformation from an initial ring form to a second ring form, (b) a very minor accumulation of a carbonyl form, and (c) the second ring form present in twice the concentration of the first at equilibrium, the molar ellipticity of the second ring form may be estimated at ~2.5 times greater than that of the first form, resulting in a doubling of the initial to the final ellipticity value. Table II indicates that, at 195 nm, methyl $\beta$-D-glucopyranoside has a positive $[\theta]$ 2.5 times as great as the $[\theta]$ of methyl $\alpha$-D-glucopyranoside, and the known concentrations of $\beta$- and $\alpha$-pyranose forms in a solution of D-glucose at equilibrium indicate that the $\beta$ form is present at about twice the concentration of the $\alpha$ form. It is, of course, possible that the acyclic form may be present and shows considerably more ellipticity at 195 nm than it has at 290 nm, but the model compound, D-glyceraldehyde, has at its 290 nm maximum at least 2.3 times the molar ellipticity that it has at 195 nm. In addition, dimeric D-glyceraldehyde may absorb at 195 nm, thereby increasing this ratio for the acyclic D-glyceraldehyde present.

The relationship between log $\Delta$ (see Experimental section) at 195 nm *vs.* time after dissolution for D-glucose is an integrated first-order kinetic plot (Fig. 1). The slope between 0 and 40 min is 0.0143 min$^{-1}$ and the regression coefficient ($r$) of the points is 0.999. The linearity of the plot supports the contention that the rate-limiting step in the mutarotation of D-glucose is the opening of the initial ring form with any carbonyl form in rapid equilibrium with the second ring form. The slope, when multiplied by the ln-log conversion factor 0.4343, gives a value (0.0062 min$^{-1}$) that is in accord with the mutarotation constant (0.0063 $\text{min}^{-1}$) for α-D-glucose reported earlier[15].

The behavior of D-fructose is more striking than that of D-glucose. The increase in positive ellipticity of the ketose form at 272 nm as a function of time may be easily observed. In contrast, at 200 nm, after a sharp decrease in positive ellipticity, a passage through zero ellipticity is followed by a slow increase in negative ellipticity. In this case again, the observation that the ellipticity at 200 nm does not proceed from a null value toward another positive or negative value, or *vice versa*, suggests that at least two forms of the sugar absorb, one of them being a ring form. The overall change in ellipticity at 200 nm throughout the experiment is not great. At both 195 nm

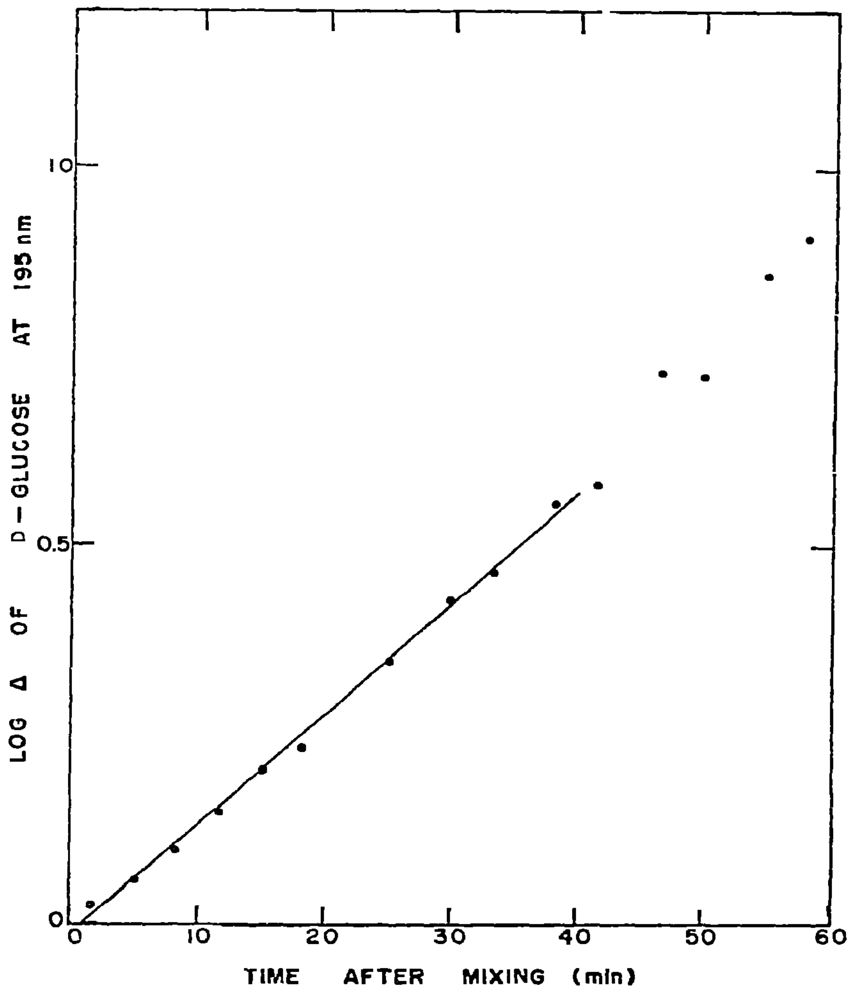

Fig. 1. Log Δ (see Experimental section) *vs.* time after dissolution for a solution of α-D-glucose at 195 nm.

and 205 nm, no detectable change in the c.d. signals with change in time took place, even at the greatest scale-expansion. The significant ellipticity at 272 nm indicates a measurable increase of the acyclic form of D-fructose, which in turn may contribute some ellipticity, less positive than that of the original, crystalline ring-form, to the ellipticity and its changes at 200 nm. At 272 nm, extrapolation to zero time gives an ellipticity greater than zero, which would indicate that the original, dry crystals already contained the acyclic form of D-fructose to an extent of roughly 54% of the total amount that is present in solution at equilibrium. Preliminary observations in this laboratory have shown that D-galactose and L-arabinose have ellipticities that increase with time in this region; upon extrapolation to zero time, they also imply the

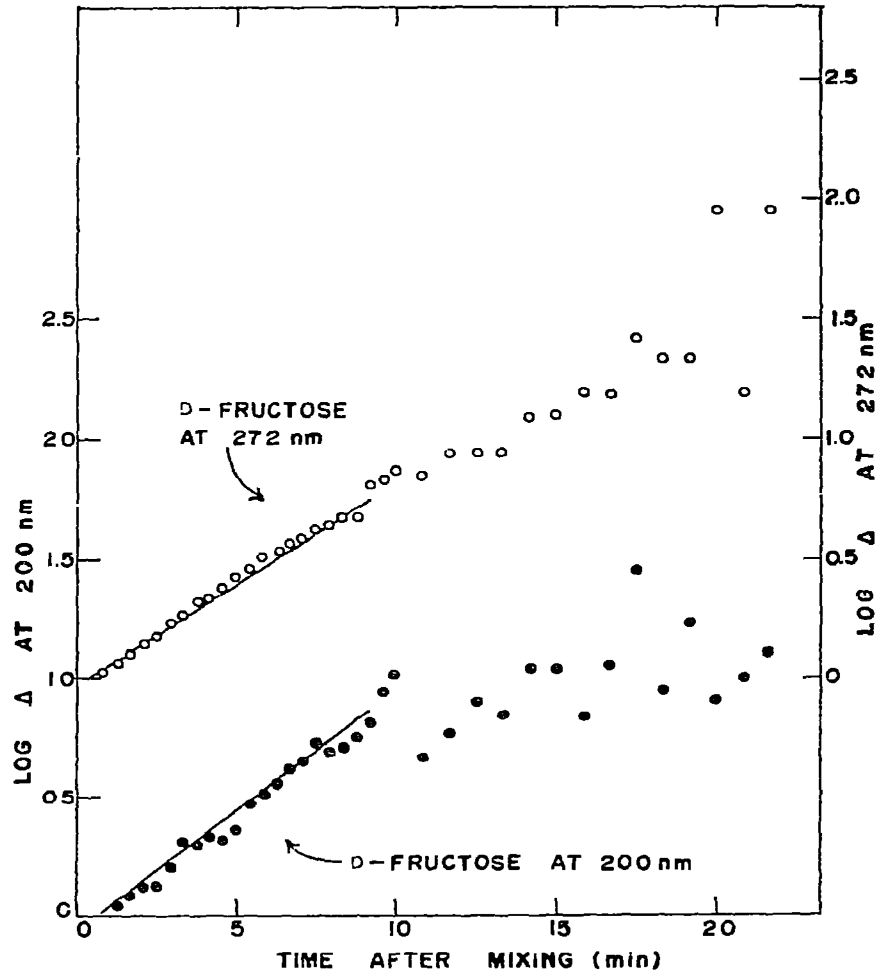

Fig. 2. Log $\Delta$ vs. time after dissolution for a solution of D-fructose at 272 (—○—○—) and 200 nm (—●—●—).

presence of acyclic structures in the original crystalline material. Isbell and Pigman have reported[16] sugar transformations in the solid state, but their brief statement discussed only D-glucose and D-lyxose.

In the plot of log $\Delta$ *vs.* time for a D-fructose solution (Fig. 2), first-order kinetics are observed for the first 9 min at both 272 nm (slope = 0.084 min$^{-1}$; $r = 0.996$) and 200 nm (slope = 0.096 min$^{-1}$; $r = 0.990$). After this initial period, the data scatter and fall away somewhat from the initial, straight line. Even up to 20 min, linear regression curves with $r$-values $>0.9$ can be found at both wavelengths with similar, although not identical slopes. The behaviors at both wavelengths are related and imply the appearance of a measurable acyclic form, from a prior ring, by a first-order

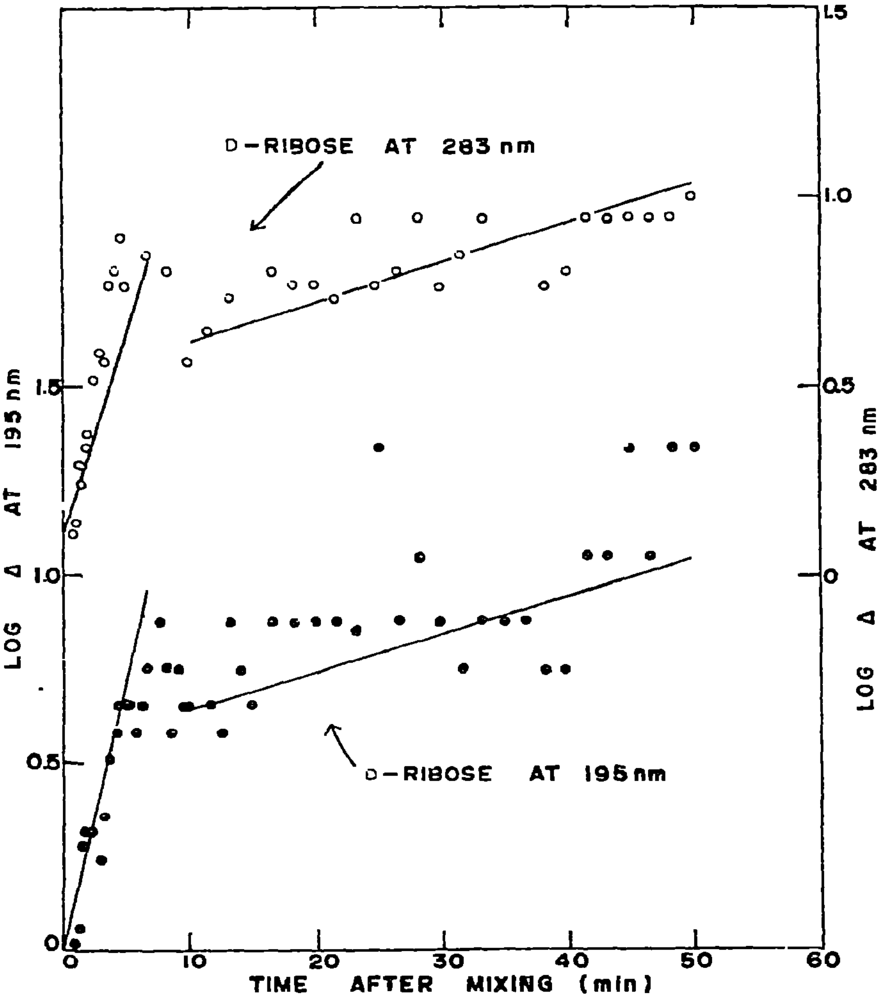

Fig. 3. Log $\Delta$ *vs.* time after dissolution, for a solution of D-ribose at 283 (—○—○—) and at 195 nm (—●—●—).

reaction. The slopes (Fig. 2) multiplied by 0.4343 generate values (0.0365 $\text{min}^{-1}$ and 0.0417 $\text{min}^{-1}$) that are lower than the value obtained[16] from the mutarotation constant (~0.115 $\text{min}^{-1}$) for a solution of D-fructose of pH 6.5 at 20°. The formation of a true acyclic structure, by a collateral reaction, from a transient *pseudo*-acyclic intermediate of the type described by Isbell and Pigman[16] is suggested by these results. In this model, a *pseudo*-acyclic form results from the slow-step disruption of the anomeric semi-acetal bond of one ring form, without considerable conformational change, and then alters to another transient form which resembles and rapidly closes to give a second ring form: A completely open-chain, acyclic conformation results from either *pseudo*-acyclic intermediate by a reaction that is the slowest of all. Such an interpretation is consistent with the presence of a $\beta$-D-furanose ring[17].

D-Ribose progressively exhibits at 283 nm a more negative ellipticity with time. This change is only very small overall. As in the case of D-fructose, the zero time value of $[\theta]_{\text{app}}$ is above zero ellipticity and indicates the existence of an acyclic form in the dry crystals to the extent of about 70% of the amount that is present in the solution at equilibrium. At the greatest scale-expansion of the circular dichrometer, some mutarotation may be detected at 220 nm, 215 nm, and 205 nm, but the changes are too small to be measured. At 200 nm and 195 nm, small but measurable changes in $[\theta]_{\text{app}}$ are observed with time, those at 195 nm being the larger; at both wavelengths, the initial negative ellipticity becomes less negative but not zero, indicating at least two forms of the sugar. At 195 nm as well as at 283 nm, an abrupt, apparently linear initial change takes place.

For solutions of D-ribose, the plots of log $\Delta$ *vs.* time (Fig. 3) give linear fits at both 283 nm and 915 nm for the first 7 min (at 283 nm, slope = 0.124 $\text{min}^{-1}$, $r = 0.881$; at 195 nm, slope = 0.118 $\text{min}^{-1}$, $r = 0.952$). These slopes, corrected by a factor of 0.4343, give 0.0539 $\text{min}^{-1}$ and 0.0512 $\text{min}^{-1}$, respectively. After the first 7 min, the scatter of the data makes the fitting of large segments with linear curves difficult and, after 50 min, impossible. Between 10 and 50 min, however, the plots are linear with rather low regression-coefficients at 283 nm ($r = 0.617$) and 195 nm ($r = 0.631$). The slopes × 0.4343 are 0.00352 $\text{min}^{-1}$ and 0.00430 $\text{min}^{-1}$, respectively. The mutarotation constants of L-ribose for the fast and slow reaction were reported[15] as 0.321 $\text{min}^{-1}$ and 0.0492 $\text{min}^{-1}$, respectively.

The well-known variation of the optical rotation of D-ribose shows the presence of at least three forms[18]. Our data indicate that during the first 50 min a minimum of two (and more likely three) forms of D-ribose are present, at least one of which is acyclic. On the basis of the previously described spectrum of D-glyceraldehyde[9], we assume that only one acyclic form is absorbing near 283 nm.

The initial fast reaction is followed by a slow reaction that increases the concentration of acyclic D-ribose (Fig. 3). Both apparent rate constants are smaller than the comparable mutarotation constant reported earlier[18], although the fast-reaction rate constant illustrated in Fig. 3 is similar in magnitude to the slow-reaction mutarotation constant. (Because of the low $r$-value for the slow reaction illustrated in Fig. 3, we have not corrected the apparent rate-constant of the fast reaction for the presence of the slow one).

The magnitude of the rate constants illustrated in Fig. 3 renders it unlikely that the acyclic form is an obligatory intermediate in the conversion of the original crystalline ring form into a rapidly-appearing, second ring form. The observation that the concentration of acyclic D-ribose (Fig. 3) increases after the initial reaction also rules against the role of an intermediate for this form since such role would require this form either to increase to a steady state, or to increase and then decrease. It is more likely that the acyclic form arises from the complete opening (at different rates) from *pseudo*-acyclic forms that are intermediates to various ring transformations. Thus, the fast reaction illustrated in Fig. 3 may be significant for the interpretation of the polarimetric mutarotation in that it generates a terminal product rather than an intermediate.

## EXPERIMENTAL

*Materials*. — Sucrose (lot 712447) and α-D-glucose were obtained from Fisher Scientific Co., Pittsburgh, PA 15219; D-fructose (control 2059) and D-glucitol (lot 6436) from ICN Life Sciences Group, Cleveland, Ohio 44128; D-ribose and methyl α-D-glucopyranoside (lot 93c-1460) from Sigma Chemical Co., St. Louis, MO 63178; and methyl β-D-glucopyranoside (lot 45352) from Calbiochem, La Jolla, CA 92037. All these compounds had $[\alpha]_D^{20}$ in agreement with literature values. D-Glyceraldehyde (lot x4004, $[\alpha]_D^{25}$ +16.06°), obtained from Schwarz/Mann, Orangeburg, NY 10962, was an oil containing about 10% of $H_2O$.

*Circular dichroism determinations*. — All measurements were made in $H_2O$ (all pH values in the range 6.4–6.8) at 21 ± 1° with a Cary Model 61 circular dichrometer, with a dynode voltage (DV) not $>0.7$ kV. A higher DV resulted in unmanageably small signal-to-noise ratios. The instrument had been calibrated at 290 nm with (*d*) 10-camphorsulfonic acid in $H_2O$. Spectra of solutions of D-ribose (100mM, 350–210 nm; 25mM, 210–195 nm), D-fructose (100mM, 350–205 nm; 10mM, 205–195 nm), D-glucose (3M, 350–260 nm; 50mM, 255 nm and below), D-glyceraldehyde (25mM), sucrose (100mM, 300–205 nm; 10mM, 200 nm and below), methyl α-D-glucopyranoside (100mM, 350 nm–200 nm; 20mM, 195 nm and below), methyl β-D-glucopyranoside (50mM), and D-glucitol hydrate (100mM, 350–200 nm; 10mM, 195 nm and below) were recorded 1 h after mixing, except for the first three compounds observed at equilibrium.

*Mutarotational kinetic measurements*. — These measurements were made with the dichrometer for solutions of α-D-glucose (at 195 nm, scale expansion 0.10, DV = 0.46 kV, 50mM), D-fructose (272 nm, 0.10, 0.32 kV, 100mM; 200 nm, 0.02, 0.69 kV, 50mM), and D-ribose (283 nm, 0.01, 0.35 kV, 100mM; 195 nm, 0.2, 0.67 kV, 10mM). The solutions were prepared from finely ground, dry crystalline material that was stirred rapidly with a magnetic mixer as water (10.0 ml), kept at 21 ± 1°, was rapidly added. Zero time, at which point the chart timing-device on the instrument was started, was the time at which the solvent entered into contact with the dry material. Rapid stirring for additional mixing was continued for several sec (for D-glucose, a minimum of 50 sec; D-fructose, 20 sec; and D-ribose, 5 sec), after which the solution was transferred to the cell. The pen recording was begun after a period of time equivalent to the pen period of the experiment. A blank experiment with water was performed later in the same cell under the same conditions. The effect of pen noise was minimized (after the completion of the measurement) by treating the primary tracing on the chart in the following manner: Within sequential intervals, the highest pen fluctuations were connected by hand-drawn lines as well as the lowest fluctuations, and at timed spacings, printed on the chart, the means of the two lines were taken. To a large extent, the scatter of data to the right in Figs. 2 and 3 is a consequence of a small increase in signal within moderately noisy tracing.

*Calculations* — The molar ellipticity $[\theta]$ is defined by the equation: $[\theta] = \theta_{obs} \times$ mol. wt./$(10 \times l \times c)$, where $\theta_{obs}$ is the experimental ellipticity (in degrees), $l$ the length of path (1 cm), and $c$ the concentration in g/ml. The apparent molar ellipticity $[\theta]_{app}$ is defined similarly, except that in expressing the concentration we have used the overall concentration of the sugar, with no effort to correct for the amounts of the various isomeric forms that may be present.

To evaluate the kinetic data, preliminary plots (not included in this report) of log $[\theta]_{app}$ *vs.* time were made for each compound at a given wavelength. These plots could be fitted in their early parts with linear curves that, in turn, could be extrapolated to zero time to retrieve a value of $[\theta]_{app,t=0}$; ultimately the plots approached limiting values from which $[\theta]_{app,t=\infty}$ could be derived. Subsequently, the actual kinetic parameters were derived from plots of an alternate form of the integrated first-order rate equation, log $\Delta$ *vs.* time, where:

$$\Delta = ([\theta]_{app,t=0} - [\theta]_{app,t=\infty})/([\theta]_{app,t=t} - [\theta]_{app,t=\infty}).$$

The values for $[\theta]_{app,t=0}$ and $[\theta]_{app,t=\infty}$, respectively, are: D-glucose (195 nm) +69.5° and +134.3°; D-fructose (272 nm) +13.7° and +25.2°; D-fructose (200 nm) +15.8° and −2.9°; D-ribose (283 nm) −1.6° and −2.3°; and D-ribose (195 nm) −272.9° and −227.1°. D-Ribose did not approach, at 195 nm, a fixed limiting value, and so an extreme value at about 50 min was used for $[\theta]_{app,t=\infty}$; after 50 min, the $[\theta]_{app}$ at this wavelength began gradually to become slightly more negative again.

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