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KINETICS OF ALKALI-CATALYZED ISOMERIZATION OF D-GLUCOSE AND D-FRUCTOSE IN ETHANOL–WATER SOLUTIONS

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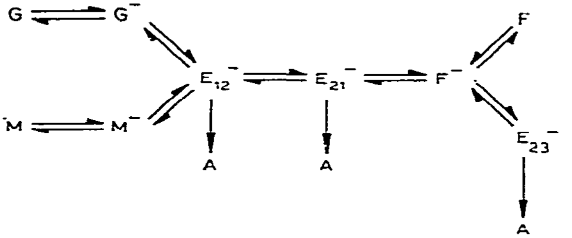
ABSTRACT

The kinetics of the alkaline isomerization of D-glucose and D-fructose in 0.1M ethanol–water solutions of sodium hydroxide were studied. To calculate the hydroxyl-ion-independent rate-constants, the ionization equilibria of the respective sugar were determined separately by potentiometric titration in the same solvent system at 21°. The sugars (D-glucose and D-fructose) in the reaction mixture were analyzed by g.l.c. as the trimethylsilylated oximes. By the micro-scale technique developed, reliable data could be obtained for reactions shorter than 10 seconds. The isomerization of D-glucose to D-fructose, and *vice versa*, accelerated markedly as a function of the alcohol concentration, and in 70% ethanol, their rates were 2.4 and 1.7 times the corresponding values in water solution. The activation energies of these isomerization reactions were found to be 122 kJ.mol^{-1} (D-glucose) and 112 kJ.mol^{-1} (D-fructose), and they were independent of the ethanol concentration.

INTRODUCTION

In an earlier paper, results from the oxidation of aldehyde end-groups of carbohydrates with oxygen in alkaline medium were reported[1]. It was shown that hydrocellulose and cellobiose were oxidized much faster in alcohol–water solutions than in pure water. The phenomenon observed was presumed to be the consequence of two separate factors: an increase in the solubility and mass transfer of oxygen, and an increase in the chemical reaction-rates. It was considered interesting to clarify, in more detail, the effect of solvent on the reactions of reducing sugars, both under oxidative and nonoxidative conditions. Results from the isomerization rates of D-glucose and D-fructose are now reported.



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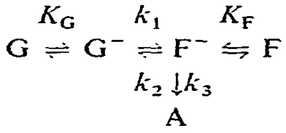
The alkali-catalyzed reactions of D-glucose, D-mannose, and D-fructose may be depicted according to the scheme in Eq. *1*, where G, M, and F denote D-glucose, D-mannose, and D-fructose; G^-, M^-, and F^-, the dissociated forms of the corresponding sugars; E^-_{mn} the enolate ion, where the negative charge is distributed over oxygen atom *m* and carbon atom *n*; and A denotes various degradation products. The ionization equilibria of the sugars are attained readily[2], and the negative charge is probably distributed over the anomeric and the ring-oxygen atom[3]. The ionization constants of the α and β anomers of D-glucose are known to be different[4]. In alkaline solutions, the mutarotation equilibrium is reached sufficiently rapidly[5] that the initial ratio between the anomers does not affect the isomerization rates. The isomerization between the ionized monosaccharides occurs *via* enolate ions. Although D-mannose can be formed from enolate ion E^-_{12}, this reaction is of minor importance, and can thus be excluded from the scheme[6].

In addition to isomerization, the enolate ions can be decomposed to various acids. Only small proportions of degradation products are formed from D-glucose, whereas the rate of degradation of D-fructose is relatively high[6], and it must occur mainly *via* enolate ion E^-_{23}, because no leaving group is present in enolate ion E^-_{21}. Therefore, a good approximation to the isomerization between D-glucose and D-fructose can be based on the simplified scheme in Eq. *2*, in which the enolate ions are excluded.



$$\begin{array}{c} G \overset{K_G}{\rightleftharpoons} G^- \overset{k_1}{\rightleftharpoons} F^- \overset{K_F}{\rightleftharpoons} F \\ k_2 \downarrow k_3 \\ A \end{array} \tag{2}$$

The following equations then give expressions for the formation of D-fructose and D-glucose, respectively.

$$d[F_{tot}]/dt = d([F] + [F^-])/dt = k_1[G^-] - (k_2 + k_3)[F^-] \tag{3}$$

$$d[G_{tot}]/dt = d([G] + [G^-])/dt = -k_1[G^-] + k_2[F^-] \tag{4}$$

For calculation of the concentrations of ionized sugars, ionization constants for D-glucose (K_G) and D-fructose (K_F) are required.

$$K_G = \frac{[G^-]}{[G][HO^-]} \tag{5}$$

$$K_F = \frac{[F^-]}{[F][HO^-]} \tag{6}$$

When the initial concentration of D-fructose is zero, the isomerization rate of D-glucose to D-fructose (k_1) (see Eq. *3*) after infinitesimal reaction-time can be calculated from the following expression.

$$\mathrm{d}[\mathrm{F}_{tot}]/\mathrm{d}t = k_1[\mathrm{G}^-] = k_1 \frac{K_G[\mathrm{HO}^-]}{1 + K_G[\mathrm{HO}^-]}[\mathrm{G}_{tot}], \tag{7}$$

where the term for $[\mathrm{G}^-]$ is derived from Eq. *5*. In analogy, if the D-glucose concentration is zero, the expression for the rate of isomerization of D-fructose to D-glucose (k_2), relating to Eq. *4*, is:

$$\mathrm{d}[\mathrm{G}_{tot}]/\mathrm{d}t = k_2[\mathrm{F}^-] = k_2 \frac{K_F[\mathrm{HO}^-]}{1 + K_F[\mathrm{HO}^-]}[\mathrm{F}_{tot}], \tag{8}$$

where the term for $[\mathrm{F}^-]$ can be calculated from Eq. *6*.

RESULTS AND DISCUSSION

Plots of the ionization constants of D-glucose and D-fructose at the range of concentration of ethanol studied are shown in Fig. 1. Comparative, literature data are available only for the water system[7,8], and they agree reasonably well with the present values. As may be seen, the ionization constants (and the acid strengths) of both sugars are increased with increase in the concentration of ethanol. Similarly, de Wit *et al.*[3] found that D-glucose has a lower $\mathrm{p}K_a$ value in $\mathrm{CD_3OD}$–$\mathrm{D_2O}$ than in $\mathrm{D_2O}$. The influence of the solvent can, at least partly, be attributed to the changes in anomeric equilibria[9,10], but no attempts were made to clarify this question.

A micro technique was developed for the determination of the isomerization rates. According to this method, the reaction was conducted in a capillary, glass

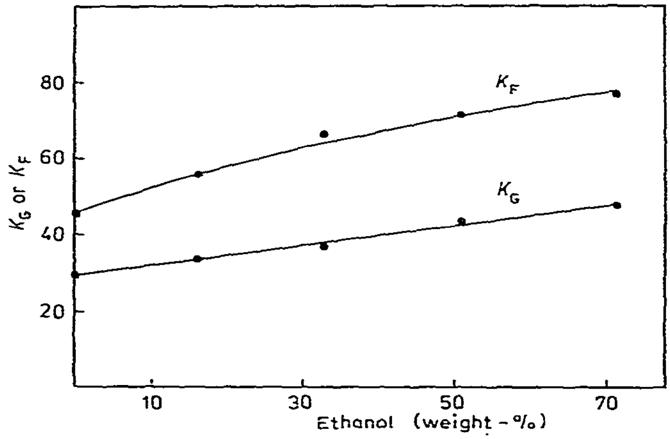


Fig. 1. Ionization constants of D-glucose (K_G) and D-fructose (K_F) as a function of the concentration of ethanol at 21°.

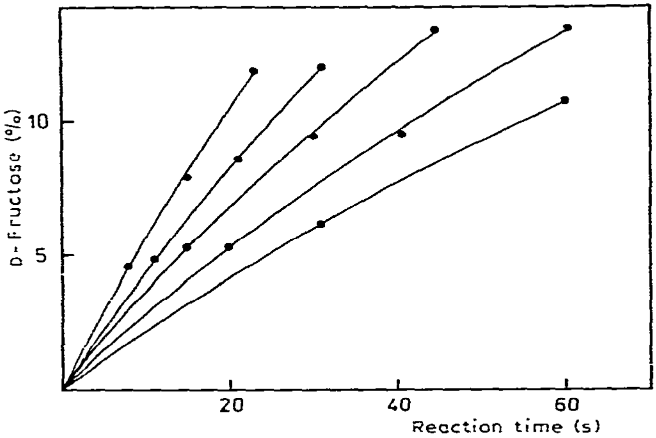


Fig. 2. Formation of D-fructose from D-glucose in 0.1M NaOH at 60°. (The ethanol concentrations corresponding to the curves, from left to right, are 72, 52, 33, 16, and 0 weight-%.)

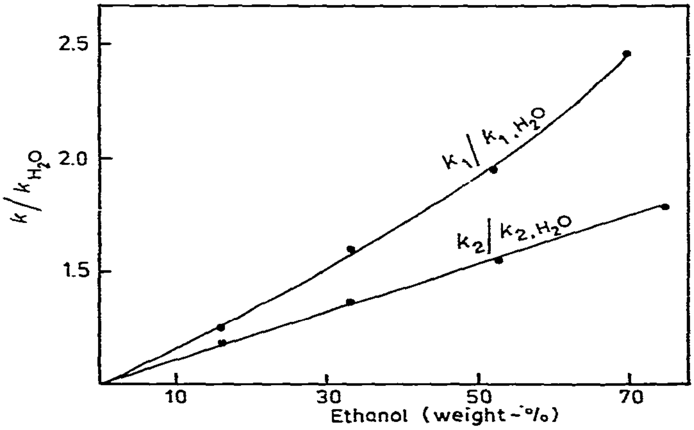


Fig. 3. The ratio of the isomerization rate-constants of D-glucose (k_1) and D-fructose (k_2) in ethanol–water solution to that in water solution. (The values are averages, from determinations conducted between 20 and 60°.)

coil, by which arrangement the heating and cooling occurred almost instantaneously, and it was possible to obtain accurate data for reactions requiring <10 s.

As an example of the analytical results, the amount of D-fructose formed from D-glucose at 60° is plotted against reaction time in Fig. 2, which shows that the isomerization is markedly accelerated when the concentration of ethanol in the solvent is increased. In Fig. 3, the isomerization-rate constants calculated for D-glucose and D-fructose in alcohol–water solutions are expressed as their ratio to the corresponding values in water solutions. For example, in 70% ethanol, the isomerization rates of D-glucose and D-fructose are 2.4 and 1.7 times those in water solution. The effect of alcohol on the degradation rate of D-fructose is of the same order. However, no

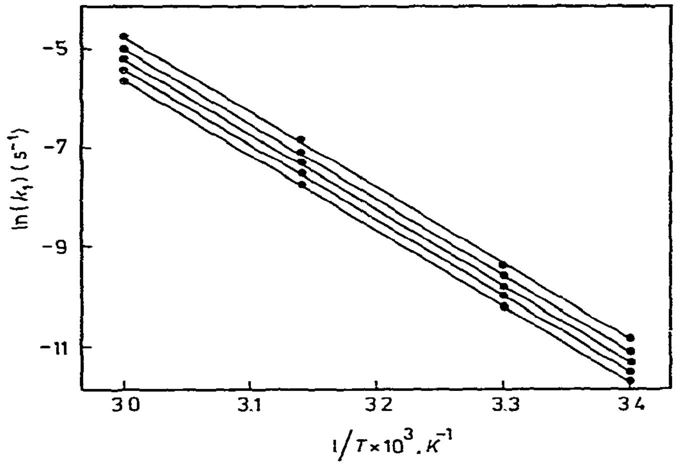


Fig. 4. The isomerization rate-constant of D-glucose as a function of temperature. (The concentrations of ethanol corresponding to the lines are 72, 52, 33, 16, and 0 weight-%, reading down.)

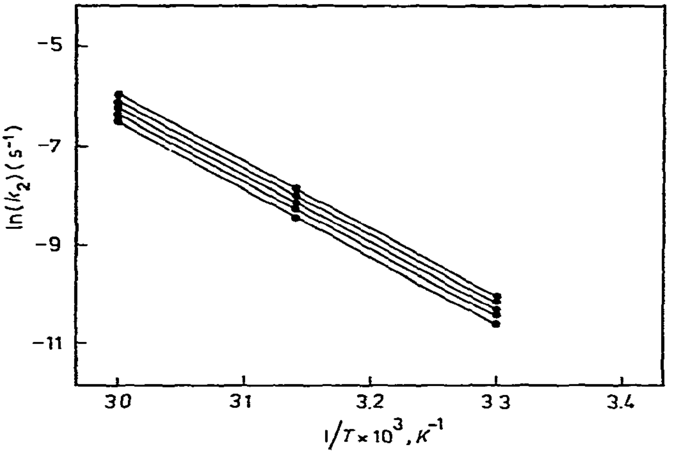


Fig. 5. The isomerization rate-constant of D-fructose as a function of temperature. (The concentrations of ethanol corresponding to the lines are 75, 52, 33, 16, and 0 weight-%, reading down.)

accurate kinetic data for this reaction could be obtained, as the relative change in the concentration of D-fructose was small under the present experimental conditions. As could be expected on the basis of literature data, the formation of D-mannose and the degradation of D-glucose were slow.

The absolute values of k_1 and k_2 are in good agreement with the literature data[11]. The present measurements, based on a large number of experimental data, gave activation energies of 122 and 112 kJ.mol^{-1} for the isomerization reactions of D-glucose and D-fructose, respectively (see Figs. 4 and 5). These differ somewhat from the findings of Kooyman *et al.*[11], who recently reported that the activation energies of the isomerization of D-glucose and D-fructose should be equal (121 kJ.mol^{-1}). However, a review of the work of Kooyman *et al.* discloses that the present value for the activation energy of D-fructose agrees better with the earlier experimental data than the 121 kJ.mol^{-1} reported.

EXPERIMENTAL

Determination of the ionization constants of monosaccharides. — A sample of ethanol–water solution (100 mL) was titrated with 0.1M sodium hydroxide under a nitrogen atmosphere to 1mM hydroxyl-ion concentration (not corrected for the ionized ethanol). The titration curve was recorded as a function of pH by using an automatic titrator (Metrohm Potentiograph E 436). The corresponding solutions containing 0.01M D-glucose and D-fructose were titrated to the same pH value, the measured pH values being corrected for the slightly lower ethanol concentrations compared with those in blank titrations. The concentrations of the ionized sugars were calculated from the differences in consumption of sodium hydroxide.

All of the sugar solutions were prepared at least 40 h before titration, and stored at the titration temperature (21°) in order to attain equilibrium between the anomeric and ring forms of the sugars.

The ionization constants at various temperatures were calculated by using ΔH values of -16.7 kJ.mol^{-1} and -22.2 kJ.mol^{-1} for K_G and K_F, respectively[12].

Determination of the isomerization rates of monosaccharides. — All of the solutions used for preparation of the reaction mixtures were kept, in vials sealed with septa, in an ice–water bath. Air was removed from the solutions by evacuation, and was replaced by nitrogen. The reaction mixture was prepared by introducing, under a nitrogen atmosphere, desired amounts of M sodium hydroxide, water, 99.5% ethanol, and, finally, 0.1M sugar solution containing the internal standard (0.1M xylitol) into a vial sealed with a septum. The total volume of the mixture was 10 mL, and the initial concentrations of sodium hydroxide and the sugar were 0.1 and 0.01M, respectively. The mixture was shaken, and a sample of it was transferred to a glass capillary coil (0.3 mm i.d. × 1.5 m) with a syringe. The coil and the needle of the syringe were connected with a suitable, Teflon tubing (i.d. 0.8 mm). The ends of the filled capillary were connected with Teflon tubing, and the coil was immersed in a thermostated, ultrasonic (80 kHz) water-bath. After the chosen reaction time, the coil was cooled in an ice–water bath, and the reaction mixture was blown out into a vial containing 0.02M acetic acid (1 mL), to neutralize the base. The sugars were analyzed by g.l.c. as their trimethylsilylated oximes, and the relative amount of the isomerization product was plotted against the reaction time. The isomerization rate-constant was calculated from the initial slope of the curve by using Eq. *7* or *8*.

Gas–liquid chromatographic analysis of monosaccharides. — The neutral, sugar solution was evaporated to dryness at 40° under diminished pressure. To prepare sugar oximes, hydroxylamine hydrochloride (10 mg) in pyridine (1 mL) was added, and the vial was shaken for 2 h at room temperature. The sugar oximes were per(tri-

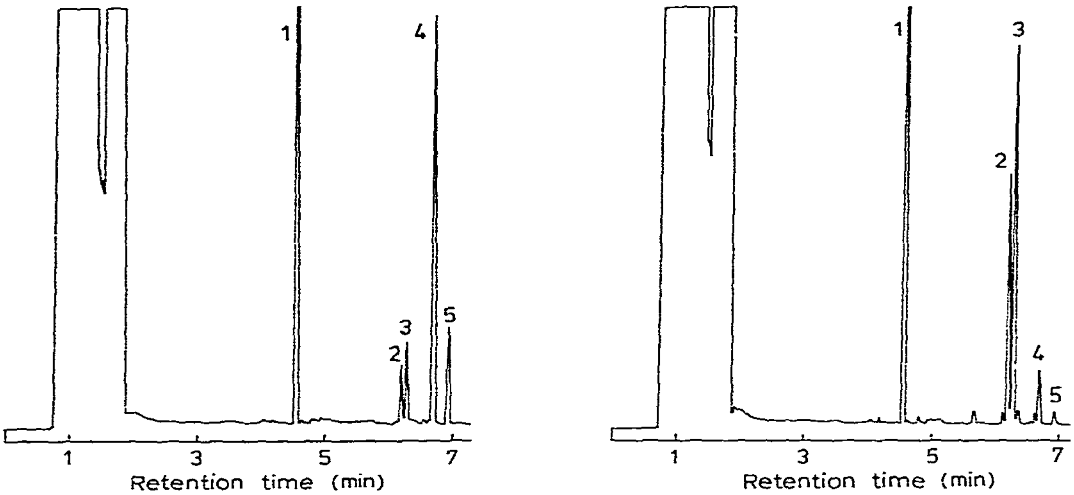


Fig. 6. Gas–liquid chromatograms from experiments on isomerization of D-glucose (left) and D-fructose (right). (Peaks: 1 = xylitol, 2 and 3 = D-fructose, 4 and 5 = D-glucose.)

methylsilyl)ated by adding trifluorobis(trimethylsilyl)acetamide (0.5 mL) containing 1% of chlorotrimethylsilane, and shaking the mixture for 20 min at room temperature. The (trimethylsilyl)ated samples were kept in a refrigerator if they were not to be analyzed immediately.

The (trimethylsilyl)ated compounds were analyzed with a Hewlett–Packard 5880 A gas chromatograph equipped with a flame-ionization detector and an OV-101 fused-silica capillary column (i.d. 0.20 mm × 10 m). Splitless injection was used, and the injection volume was 1 μL. The oven-temperature profile was 20°/min from 125 to 220°, and 3 min at 220°. The injection port and the manifold were kept at 260°. The flow rate of the hydrogen used as the carrier gas was 1 mL/min.

Typical chromatograms obtained for the reaction mixtures are shown in Fig. 6.

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