Carbohydrate Research, 54 (1977) 33–44

THE ISOMERIZATION OF D-GLUCOSE INTO D-FRUCTOSE IN AQUEOUS ALKALINE SOLUTIONS

C. KOOYMAN, K. VELLENGA*, AND H. G. J. DE WILT
Chemical Engineering Laboratory, State University of Groningen, (The Netherlands)
(Received October 6th, 1975; accepted for publication, April 9th, 1976)

ABSTRACT

To relate kinetic data of previous publications about the isomerization of D-glucose into D-fructose in aqueous, alkaline media in the temperature range 22–71°, a simple kinetic model has been developed, which allows transformation of the rate constants published. The kinetic model has been based on the generally accepted enolate-ion isomerization mechanism, and comprises rate constants that are independent of the hydroxyl-ion concentration. The relationship between the hydroxyl ion-dependent and -independent rate constants is given. Given the reliability boundaries, it appears from the transformed literature data that the hydroxyl ion-independent rate constants of the forward and reverse reaction are about the same. The validity of the model at higher temperatures (67–104°) has been confirmed. From an Arrhenius plot, it has been deduced that the apparent activation energy, related to the hydroxyl ion-independent rate constants, of the forward and reverse reaction is ~ 121 kJ.mol^{-1}.

INTRODUCTION

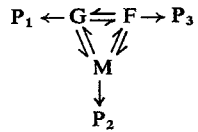
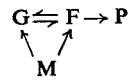
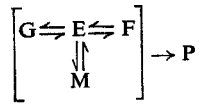
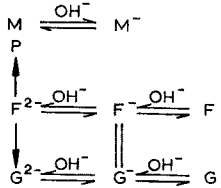
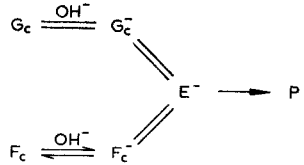
We have studied the kinetics of the isomerization of D-glucose into D-fructose in aqueous, alkaline media, because of the general importance of the reaction in the production of sucrochemicals.

Many authors[1–5] have reported on the kinetics of this reaction. However, comparison of the results is difficult because of differences in experimental conditions and the use of several kinetic models. A summary of relevant literature is given in Table I, including the range of process parameters studied and the kinetic models on which the calculations of the reported rate-constants have been based. From Table I, it appears that three different kinetic models have been used to describe the isomerization reactions, so that comparison of the various rate-constants is difficult. It is also difficult to compare the data of Bamford *et al.*[1], McLaurin and Green[2], and Garrett and Young[3], who all use the same kinetic model, because their experiments

*To whom correspondence should be sent.

TABLE I

A SUMMARY OF PUBLISHED STUDIES OF HEXOSE ISOMERIZATION

Authors	$[H]_0$ ($mmol.dm^{-3}$)	$[OH^-]_0$ ($mmol.dm^{-3}$)	*Temperature* (*degrees*)	*Kinetic model*[a]	*Remarks*
Bamford *et al.*[1]	440	1000–5000	25–45		
McLaurin and Green[2]	2	1000	22		
Garrett and Young[3]	100	10–660	25–50		The kinetic model is based on the theoretical model[3]: 
Lai[4 b]	100	10–660	25–50		As the mechanism has enolate ions as intermediates, the kinetic model must be based on negative ions[4].
De Wilt and Lindhout[5]	125	26	50–60		Part of an investigation into the oxidative degradation of hexoses in alkaline medium
Gottfried and Benjamin[6]	1000	30–180	70–130	No kinetic model presented.	Only experimental data available.
Kainuma and Suzuki[7]	1400–3500	62–162	56–97		

[a] G = glucose; G_c = cyclic glucose molecule; G^- and $G^=$ = glucose ion; G_c^- = cyclic glucose ion; K_G = dissociation constant for glucose [the same nomenclature holds for fructose (F) and mannose (M)]. E = enolate ion; P = by-product. [b] E has never been isolated because of the very low concentration, so the theoretical model is simplified to the kinetic model. [c] Used the results of Garrett and Young[3].

were carried out at different and/or varying hydroxyl-ion concentrations, and the kinetic model used to explain their data does not account for the hydroxyl-ion concentration, in spite of the fact that this is an important parameter in the isomerization reaction. This is the most-striking feature of the model used in Refs. 1–3. Some remarks above the model proposed by Lai[4] are given in the concluding paragraph.

Although De Wilt and Lindhout[5] used a kinetic model that is closely related to the reaction mechanism (see their kinetic model and the simplified mechanism given in Fig. 1), it involves the quantitative determination of the enolate-ion concentration, which is very difficult. This subject will be discussed below.

As a consequence of the above remarks, an attempt has been made to correlate the published kinetic models for the whole range of parameters to a kinetic model that is based on a chemical reaction mechanism. A generally accepted mechanism and the relevant rate-constants are given in Fig. 1.

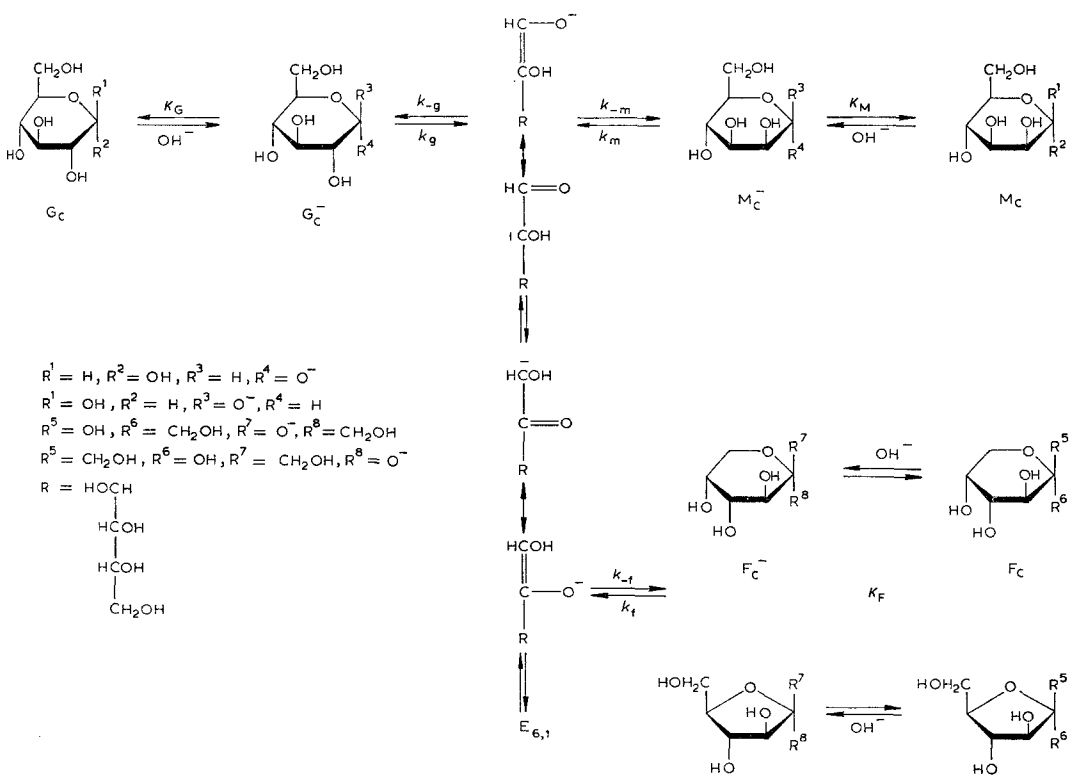


Fig. 1. A simplified, isomerization reaction-mechanism.

In considering Fig. 1, the following points are made. (*a*) The diagram has been restricted to the isomerization itself because, depending on the process conditions, by-products are formed by various mechanisms[9]. (*b*) The reaction mechanism also explains the formation of other monosaccharides *via* enolate ions ($E_{6,1}$) consisting

of six C atoms in a chain containing a C–C double-bond or a negatively charged C. However, the number of hexoses has been limited, for only the monosaccharides mentioned are present in any detectable quantities if D-glucose is the starting material[2]. (*c*) The hexoses each occur in different conformations. The rate of mutarotation between the conformations will be discussed in a separate paragraph. (*d*) At 25°, the rate constant of the de-protonation reaction of G_c is $>10^{10}$ dm^3.mol^{-1}.sec^{-1} (Ref. 10). As K_G is 31 dm^3.mol^{-1} (Refs. 5 and 11), the rate constant of the reverse reaction is $>3\times10^8$ sec^{-1}, so that the equilibrium G_c/G_c^- establishes itself momentarily. It is assumed that the same holds true for the F_c/F_c^- and M_c/M_c^- equilibria. (*e*) In the literature, it has been suggested that the rearrangement of one group of resonance structures of enolate ions to the other group proceeds either *via* an enediol mechanism[8,12] or *via* a hydride-shift mechanism[12]. (*f*) Two other isomerization mechanisms have been suggested in the literature: a hydride-shift mechanism[13] and a radical mechanism[14]. However, Isbell[14] reported that tracer studies exclude the hydride-shift mechanism and that, in the absence of free-radical initiators, the ionic enolate mechanism probably takes place. Lagercrantz[15] and Alekseeva *et al.*[16], however, have reported the formation of radicals under extreme alkaline conditions in the absence of initiators.

The rate equations, related to the reaction mechanism, include the enolate-ion concentration, which is very low with respect to the concentrations of hexoses and difficult to quantify[3,5]. It is therefore necessary to eliminate the enolate-ion concentration; if this is not done, the kinetic model will comprise rate constants that are related to, and are influenced by, a concentration that cannot be quantified, which would mean that the rate constants could not be determined.

If the isomerization reaction is carried out in a batch or plug-flow reactor, the enolate-ion concentration can be eliminated only if $d[E^-]/dt$ is very small with respect to d[Hexose]/dt. As the enolate-ion concentration is very small with respect to the hexose concentration[5], it can be assumed that $d[E^-]/dt \simeq 0$.

If a continuous, stirred tank-reactor is used in steady-state operation, integral mass-balances over the system can be composed for the enolate-ion concentration and the separate monosaccharides, in which the kinetic terms are related to the mechanism. After elimination of the enolate-ion concentration, the kinetic terms can be related to the following kinetic model:

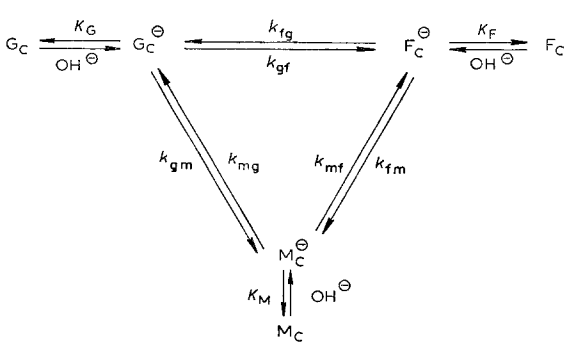


Fig. 2. A kinetic model, derived from the reaction mechanism (kinetic model I).

This model contains detectable concentrations only, so the rate constants can be determined. The relationships between the rate constants of the kinetic model, and their relations to the rate constants associated with the chemical mechanism of Fig. 1, are given in Table II (formulae I–VI).

Many rate constants reported for a reaction from a hexose I into a hexose J are based on the total hexose concentration, $[H_c]+[H_c^-]$. These rate constants are indicated in k_{IJ}. The relation between k_{ij} and k_{IJ} is given by formulae VII–XII (Table II).

TABLE II

RELATIONSHIP BETWEEN THE RATE CONSTANTS OF FIGS. 1 AND 2, AND THOSE WHICH ARE BASED ON THE TOTAL HEXOSE CONCENTRATION

$k_{gf} = k_g \frac{k_{-f}}{k_{-g}+k_{-f}+k_{-m}}$	I	$k_{GF} = k_{gf} \frac{K_G[OH^-]}{K_G[OH^-]+1}$	VII
$k_{gm} = k_g \frac{k_{-m}}{k_{-g}+k_{-f}+k_{-m}}$	II	$k_{GM} = k_{gm} \frac{K_G[OH^-]}{K_G[OH^-]+1}$	VIII
$k_{fg} = k_f \frac{k_{-g}}{k_{-g}+k_{-f}+k_{-m}}$	III	$k_{FG} = k_{fg} \frac{K_F[OH^-]}{K_F[OH^-]+1}$	IX
$k_{fm} = k_f \frac{k_{-m}}{k_{-g}+k_{-f}+k_{-m}}$	IV	$k_{FM} = k_{fm} \frac{K_F[OH^-]}{K_F[OH^-]+1}$	X
$k_{mg} = k_m \frac{k_{-g}}{k_{-g}+k_{-f}+k_{-m}}$	V	$k_{MG} = k_{mg} \frac{K_M[OH^-]}{K_M[OH^-]+1}$	XI
$k_{mf} = k_m \frac{k_{-f}}{k_{-g}+k_{-f}+k_{-m}}$	VI	$k_{MF} = k_{mf} \frac{K_M[OH^-]}{K_M[OH^-]+1}$	XII

TRANSFORMATIONS OF LITERATURE DATA

The published rate-constants, based on the kinetic models of Table I, were transformed to k_{ij} values by using equations I, III, and VII–XII (Table II).

The hexose dissociation constants which were used in the calculations are given by Lindhout and De Wilt[5] and Izatt *et al.*[11]: $K_G(25°) = 32\ dm^3.mol^{-1}$ ($\Delta H = -16.7\ kJ.mol^{-1}$); $K_F(25°) = 49\ dm^3.mol^{-1}$ ($\Delta H = -22.2\ kJ.mol^{-1}$); and $K_M(25°) = 83\ dm^3.mol^{-1}$ ($\Delta H = -18.0\ kJ.mol^{-1}$). The water dissociation constant was corrected for the temperature effect[17].

It is known that, with increasing alkalinity of an aqueous solution of a hexose, the relative concentration of double-negative hexose-ions increases. However, these ions were not included in our kinetic model. Bamford *et al.*[1] performed their experiments in strongly alkaline solutions (1–5M). We transformed only their kinetic data that had been measured in M alkaline media, because these conditions approximate

TABLE III

TRANSFORMED, LITERATURE RATE-CONSTANTS ($SEC^{-1} \times 10^{-4}$) AS A FUNCTION OF TEMPERATURE

Temp. (degrees)	*Ref.*	k_{gf}	k_{gm}	k_{gf}	k_{fm}	k_{mg}	k_{mf}
22	2	0.103	0.00515	0.114	0.0167	0.00515	0.0306
25	1	0.12		0.128			
	3	0.21		0.21			0.05
35	3	0.684–0.990		0.440–0.880		0.038–0.112	0.083–0.22
	4	0.75		0.80			
40	3	1.86		1.47		0.063	0.051
45	1	2.6		2.3			
50	3	5.6		5.0		0.600	2.2
	5	5.1		3.9			
56	7	15					
60	5	31		21			
71	7	105–120					

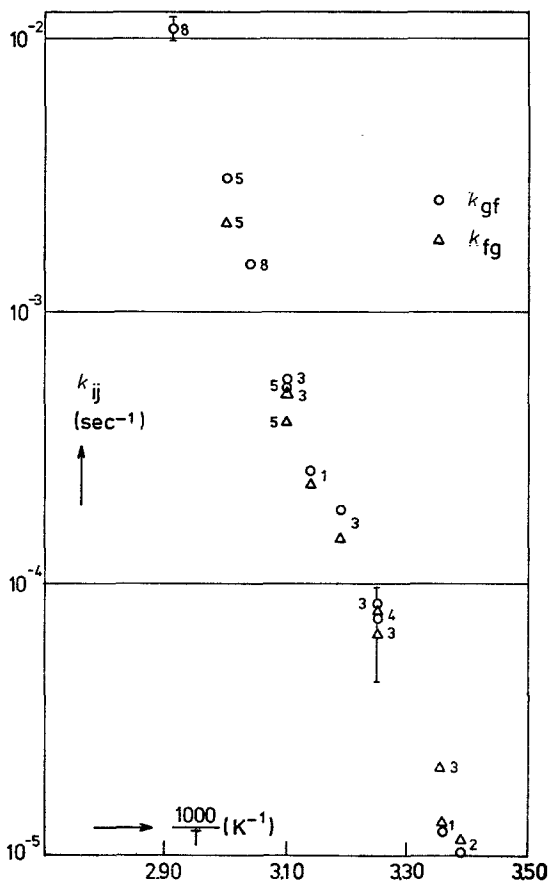


Fig. 3. Transformed, literature isomerization rate-constants.

most closely to those for which our model holds. Kainuma and Suzuki[7] did not present a kinetic interpretation of the experimental data in terms of rate constants. We estimated k_{GF} values from their experimental data at 56 and 71° only, because these data could be correlated to some extent with the initial conditions. The experimental data given by Gottfried and Benjamin[6] could not be used to estimate k_{GF} values, because of the lack of information about their experimental conditions.

The calculated k_{ij} values are given in Table III, and the k_{gf} and k_{fg} values are plotted against the reciprocal temperature in Fig. 3. The inaccuracies in some experiments, *e.g.*, change in the hydroxyl-ion concentration during the reaction, must be taken into account when interpreting Fig. 3. It appears that the kinetic model developed gives a fairly accurate description of the alkaline isomerization of D-glucose in the temperature range 22–71° at different hydroxyl-ion concentrations. Therefore, it has been checked experimentally to determine to what extent the simple kinetic model is also valid at higher temperatures.

EXPERIMENTAL

Reaction conditions. — Kinetic measurements were done on the D-glucose isomerization reaction in a continuous, stirred tank-reactor, for only in this reactor type is the hydroxyl-ion concentration essentially constant for an arbitrarily adjustable reaction-time. Parameters were: temperature, 67–104°; initial concentration of sodium hydroxide, 0.6–0.8M at 67–84°, 15mM at 84–104° (for explanation, see Results and Discussion); initial concentration of D-glucose, 0.08–0.4M; residence time, 25–150 sec. (The initial concentrations are equal to the concentrations in the reactor at infinitely short residence-time).

At 84–104°, the pH was measured with a Philips CAH 11 D combined electrode and a Metrohm E512 pH meter, which was calibrated at actual reaction temperatures. At 84°, the reaction was carried out at an initial concentration of sodium hydroxide of 0.7M and 15mM.

At each adjusted temperature, the concentrations of D-glucose, D-fructose, and D-mannose in the reactor were determined for three or four different residence-times. To avoid any oxidation, the reaction was performed under nitrogen.

Sampling. — Reaction samples were taken by quenching part of the reaction liquid in a solution of 4M HCl at −18°. The samples were also stored at this temperature.

Analysis. — The reaction samples were pre-treated as described by Verhaar and De Wilt[18]. The analysis was carried out with a Becker type 419 gas chromatograph. The coiled columns (2 m × 0.0625 in., inside diameter) consisted of AISI 321 stainless-steel packed with 10% of OV-17 on Chromosorb W-AW-DMCS (100–120 mesh). The carrier gas was helium, and a hydrogen flame-ionisation detector was used. The column temperature was 167° (isothermal). The sample volume injected was 2 μl, and a solution of pentadecane in hexane was used as internal standard. Peak areas were integrated with an Autolab System IV. The relative molecular responses of the sugars

were determined by injection of a standard sample of known composition before and after each reaction sample.

List of symbols. — H_c = cyclic hexose molecule; H_c^- = cyclic hexose ion; $[H]_a$ = analysed hexose concentration which is equal to $[H_c]+[H_c^-]$; $[H]_0$ = initial hexose concentration which is equal to $[H_c]_0+[H_c^-]_0$; P = by-product; K_H = dissociation constant of hexose H ($dm^3.mol^{-1}$); k_{+i} = rate constant for a reaction starting from a cyclic hexose ion i into an enolate ion; k_{-i} = rate constant for a reaction starting from an enolate ion into a cyclic ion i; k_{ij} = rate constant for a reaction starting from a cyclic hexose ion i into a cyclic hexose ion j; k_{IJ} = rate constant for a reaction from a hexose I into a hexose J; T = temperature (°C, °K); t = residence time (sec).

RESULTS AND DISCUSSION

Because of the relatively low concentration of mannose with respect of those of glucose and fructose, kinetic model I could be simplified. However, to calculate isomerization rate-constants from the experimental measurements, by-products had to be taken into account in the kinetic model. As these products are formed by various mechanisms[9], the side-reaction rate constants were based on the total hexose concentration. On account of the above considerations, kinetic model II was used to calculate the isomerization rate-constants.

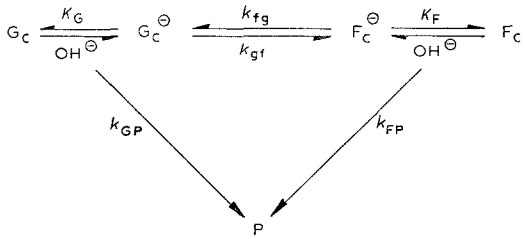


Fig. 4. The kinetic model used to determine the isomerization rate-constants (kinetic model II).

As a continuous, stirred tank-reactor was used, the following mass balances held for glucose and fructose:

$$[G]_0 = [G]_a + k_{GF}[G]_a t - k_{FG}[F]_a t + k_{GP}[G]_a t; \qquad \text{XIII}$$

$$0 = [F]_a + k_{FG}[F]_a t - k_{GF}[G]_a t + k_{FP}[F]_a t. \qquad \text{XIV}$$

As only the hexose concentrations and the residence time were known, rate constants could only be obtained if two more relations between the rate constants were known. These additional relations were derived from the data of Bamford *et al.*[1] and from Fig. 3.

Bamford *et al.*[1] determined side-reaction rate constants by extrapolating their

data, measured in a batch reactor under nitrogen, to initial conditions; this procedure cannot be applied to data obtained from a continuous, stirred tank-reactor. The measured ratio between k_{GP} and k_{FP} appeared to be 1/3 in M alkali at 45°. In the temperature range of 67–84°, we used the same value, as the experiments were carried out in solutions having the same initial concentration of sodium hydroxide. In the temperature range of 84–104°, no assumption had to be made, as the by-product formation was very small because of the low concentrations of sodium hydroxide. From Fig. 3, it can be deduced that the ratio between k_{gf} and k_{fg} approaches 1. Deviations from the ratio are included in the limits of accuracies of the rate constants, and in our calculations the ratio was taken to be 1. Because K_G and K_F values were obtained by extrapolating the results of De Wilt and Lindhout, and because the hydroxyl-ion concentrations were known, the ratio between k_{GF} and k_{FG} could be computed with the aid of equations VII and IX (Table II).

The values of k_{GF} and k_{FG} were calculated from the measured hexose concentrations with the aid of equations XIII and XIV, and the above relations between k_{GP}/k_{FP} and k_{GF}/k_{FG}. Values of k_{gf} and k_{fg} were determined from the k_{GF} and k_{FG} values calculated with equations VII and IX. Table IV presents the rate constants calculated and the reaction conditions used. In Fig. 5, both the isomerization rate-constants obtained from literature data and the rate constants obtained from the experiments reported herein are plotted against the reciprocal temperature.

The maximum spreading of the calculated values of k_{gf} for each adjusted temperature at three or four different residence times was 20%, due, for example, to errors in pre-treatment and analysis. The larger spreading in the calculated k_{IP} values appears to have had a relatively minor influence on the values of the isomerization rate-constants.

For most experiments in the temperature range of 67–84°, the initial glucose concentration was much smaller than the initial hydroxyl-ion concentration. Therefore, the formation of acidic by-products hardly diminished the hydroxyl-ion concentration, the actual hydroxyl-ion concentration being almost equal to the initial concentration.

TABLE IV

VALUES OF k_{gf} AND k_{GP} AND THE EXPERIMENTAL CONDITIONS AT WHICH THESE RATE CONSTANTS HAVE BEEN DETERMINED

Temp. (*degrees*)	$[G]_0$ ($mmol.dm^{-3}$)	$[OH^-]_0$ ($mmol.dm^{-3}$)	k_{GP} ($10^{-3}\ sec^{-1}$)	k_{gf} ($10^{-3}\ sec^{-1}$)
67	80	690	1.3	6.1
69	400	640	1.5	9.8
74	80	770	5.7	18
80	80; 400	700	4.6	43
84	80	780	6.4	69
94	80	15	12	180
104	80	15		390

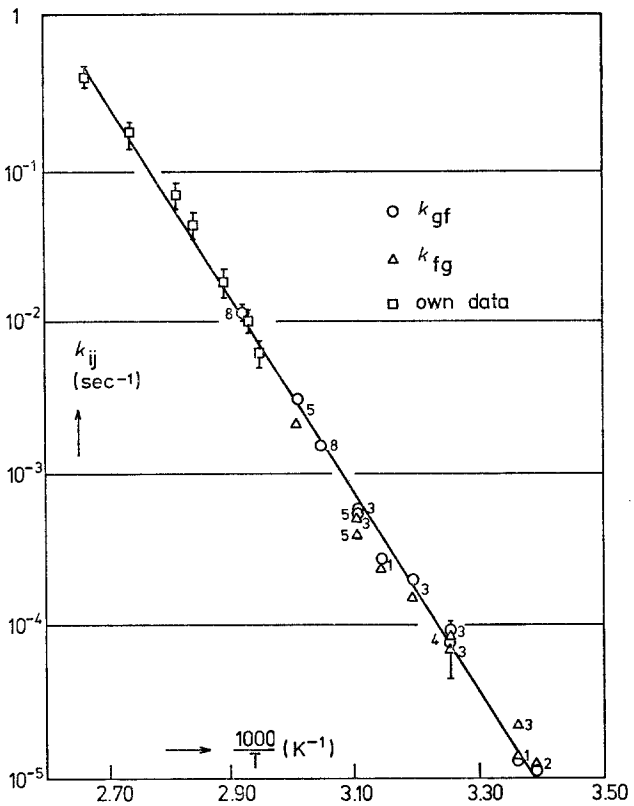


Fig. 5. Values of k_{gf} and k_{fg} as a function of temperature.

It might be expected that, at relatively high concentrations of glucose, the actual concentration of hydroxyl ion would be somewhat lower than the initial concentration. However, a small decrease in the hydroxyl-ion concentration has hardly any influence on the term $K_H[OH^-]/(1+K_H[OH^-])$, so that k_{gf} values could also be calculated under these conditions.

At temperatures above 84°, it was not possible to work at these concentrations of sodium hydroxide, as the isomerization rate-constants increased so rapidly with increasing temperature that the reaction could no longer be followed with the equipment used. Therefore, the experiments above 84° were performed at lower concentrations of sodium hydroxide. As any acidic by-products formed have a significant effect on the term $K_H.OH^-/(1+K_H.OH^-)$, the actual concentration of hydroxide ion had to be measured.

The kinetic model did not take into account the different conformations in which sugars may occur. Because the rate constants of the mutarotation of hexoses, given by Pigman and Anet[9], at 22° and pH 11 are: $k_{\alpha\text{-pyranose}\leftrightarrow\beta\text{-pyranose}} \simeq 10^{-2}$ sec^{-1} ($E_a = 66$–71 kJ.mol^{-1}) and $k_{\alpha\text{-furanose}\leftrightarrow\beta\text{-furanose}} \simeq k_{\text{furanose}\leftrightarrow\text{pyranose}} \simeq 10^{-1}$ sec^{-1} ($E =$ 54–58 kJ.mol^{-1}), it is clear that, under the above-reported reaction conditions, the

α/β pyranose and α/β furanose conformations were in equilibrium with each other, so that the rate constants measured were those of the isomerization reaction.

From Fig. 5, it has been calculated that the (apparent) activation energy of the isomerization reaction of glucose into fructose, and of the reverse reaction related to the hydroxyl ion-independent rate constants, k_{ij}, is 121 kJ.mol^{-1} for both the forward and reverse reaction.

The results of the literature study and the experimental data confirm that homogeneous, alkaline isomerization between the temperatures of 20 and 104° can be described kinetically with the kinetic model developed which includes the influence of the hydroxyl ions.

We agree with Lai's comment[4] on the conclusion of Garrett and Young[3] that the isomerization reaction is an S_N2 reaction in which glucose is not dissociated. Lai's criticism is that: (*a*) the difference in entropy between the activated complex and the initial components is positive, which would exclude an S_N2 mechanism; (*b*) the mechanism postulated by Garrett and Young is not in agreement with the generally accepted enolate-ion mechanism; (*c*) given the pK values of the hexoses and a high pH, the greater part of the hexoses in the solution must be ions and not molecules.

Bamford *et al.*[1] supposed that the mechanism on which the kinetic model is based contains double-negative hexose ions in the aldehyde form. However, they concluded that there is no relation whatever between the aldehyde glucose and fructose ions. The reaction mechanism, see Fig. 1, from which our kinetic model has been deduced would contradict the conclusion of Bamford *et al.*

Lai supposed that the isomerization reaction takes place *via* an enolate-ion mechanism and he used a kinetic model that looks like ours. But, unlike our model, Lai's kinetic model does contain double-negative hexose ions, although this assumption has hardly any influence on the k_{ij} values of the isomerization reaction. Moreover, Lai uses the 2nd dissociation constants, which have not been determined experimentally, and he does not give the relation between the mechanism and the kinetic model.

REFERENCES

1 C. H. Bamford, D. Bamford, and J. R. Collins, *Proc. Roy. Soc., Ser. A*, 204 (1950) 85–98.
2 D. J. McLaurin and J. W. Green, *Can. J. Chem.*, 47 (1969) 3947–3955.
3 E. R. Garrett and J. F. Young, *J. Org. Chem.*, 35 (1970) 3502–3509.
4 Y. Z. Lai, *Carbohydr. Res.*, 28 (1973) 154–157.
5 H. G. J. de Wilt and I. Lindhout, *Carbohydr. Res.*, 23 (1973) 333–341.
6 J. B. Gottfried and D. G. Benjamin, *Ind. Eng. Chem.*, 44 (1952) 141–145.
7 K. Kainuma and S. Suzuki, *Stärke*, 18 (1966) 135–140.
8 W. Pigman and D. Horton, *The Carbohydrates*, Vol. 1A, Academic Press, New York, 1972, pp. 175–178.
9 Ref. 8, pp. 170–172.
10 M. Eigen and G. Maass, *Angew. Chem.*, 75 (1963) 489–508.
11 R. M. Izatt, *J. Am. Chem. Soc.*, 88 (1966) 2641–2645.
12 J. Hine, *Physical Organic Chemistry*, McGraw-Hill, New York, pp. 271–272.
13 H. S. Isbell, K. Linek, and K. E. Hepner, *Carbohydr. Res.*, 19 (1971) 319–327.
14 H. S. Isbell, *Adv. Chem. Ser.*, 117 (1973) 70–87.

15 C. LAGERCRANTZ, *Acta Chem. Scand.*, 18 (1964) 1321–1324.
16 L. P. ALEKSEEVA, E. M. LEIBOVICH, E. I. CHUBKA, AND C. M. NIKITIN, *Khim. Drev.*, 14 (1973) 93–97.
17 C. LONG, *Biochemists Handbook*, Spon, London, 1961, pp. 19–20.
18 L. A. TH. VERHAAR AND H. G. J. DE WILT, *J. Chromatogr.*, 41 (1969) 168–179.
19 D. RITTENBERG AND C. GRAFF, *J. Am. Chem. Soc.*, 80 (1958) 3370–3372.