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# Alkaline degradation of monosaccharides V\*: Kinetics of the alkaline isomerization and degradation of monosaccharides

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Abstract. A new kinetic model for the alkaline isomerization and degradation of monosaccharides is presented which includes the interconversion of D-glucose, D-fructose, D-mannose and D-psicose. Computer simulations using this model fit the experimental data and allow the determination of all relevant reaction rate constants. Additionally, it has been established that, for the alkaline degradation of either D-fructose, D-glucose or D-mannose, substantial amounts of acidic products, *i.e.* ~65% and ~20%, are formed via D-fructose and D-psicose, respectively.

The influence of some reaction parameters on the pseudo-first-order rate constants involved in the kinetic model has been investigated. The enolization of monosaccharides appears to be the rate-limiting step in both the isomerization and the degradation reactions. The enolization rate is markedly enhanced at higher HO<sup>-</sup> concentration or by the addition of calcium(II). Direct *retro*-aldolization of D-fructose and D-psicose occurs at  $[HO^-] > 10^{-2}$  M or in the presence of calcium(II), leading to an additional increase in the degradation rate towards lactic acid. The shift of the isomerization equilibrium by borate towards D-fructose and D-psicose is accompanied by a decrease of the degradation rate constants, due to stabilization of the monosaccharides as their borate esters.

## Introduction

In aqueous alkaline solutions, enolization of sugar anions causes the isomerization of (mono)saccharides, known as "the Lobry de Bruyn-Alberda van Ekenstein rearrangement"<sup>1</sup>. The intermediate enediol anion species have been generally accepted<sup>2-5</sup> as the starting intermediates in the alkaline degradation of monosaccharides. The first step in the degradation reaction is the elimination of a hydroxyl group in  $\beta$ -position to the ionized enol group. The resulting product of this so-called  $\beta$ -elimination reaction is an  $\alpha$ -dicarbonyl compound which may be considered<sup>6</sup> as the key intermediate in the subsequent alkaline degradation reactions, i.e. benzilicacid rearrangement, α-dicarbonyl cleavage and aldolization. The enediol anion and  $\alpha$ -dicarbonyl intermediates achieve only low concentrations. Enediol anion species, for instance, are detected in alkaline medium in amounts up to 0.6% of the monosaccharide concentration, as determined by UVspectroscopy<sup>2</sup>.

The kinetics of the isomerization and concomitant degradation of monosaccharides, e.g. D-fructose and D-glucose, in aqueous alkaline solutions have been studied by many investigators<sup>2,7-12</sup>. A survey of the relevant kinetic data has been given by *Kooyman* et al.<sup>13</sup>, which, together with some recent results<sup>2,14</sup>, represents the present-day knowledge of the kinetic behaviour of monosaccharides in alkaline medium.

It may be noted that formation of D-psicose has not been taken into consideration<sup>2,7-14</sup>, while D-mannose formation has often been omitted in the kinetic treatment of the alkaline degradation<sup>7,8,11-14</sup>.

Since we have established that substantial D-psicose and D-mannose formation occurs during the alkaline degradation of both D-glucose and D-fructose, we have reinvestigated the kinetics of the alkaline degradation reaction. With the aid of HPLC, accurate data of the reaction course as a function of time were obtained. The kinetic parameters thus derived require a kinetic model which differs from the models previously in the literature.

# Experimental

#### Materials

D-psicose was prepared<sup>15</sup> according to the literature. All other chemicals were obtained from Merck (analytical grade).

#### Apparatus

The alkaline degradation reactions were carried out in a 500-ml thermostatted vessel under nitrogen as described elsewhere<sup>6</sup>. During the experiments, both the temperature and the  $HO^-$  concentration

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were kept constant. Samples taken during the reaction were quenched by cooling in acetone-dry ice. The samples were neutralized by addition of a weak cation exchange resin (BioRex 70 H) at  $\sim 0^{\circ}$ C and filtered off.

#### Analysis method

Analysis of the monosaccharides present in the neutralized reaction mixtures was performed using HPLC<sup>15</sup> (carbohydrate column HPX 87 C from Bio-Rad, column temperature 60°C, eluent H<sub>2</sub>O, flow 0.4 ml/min, RI detection). The total amount of carboxylic acid degradation products was taken as 100% minus % total sugars. The carboxylic acid composition was also determined by HPLC analysis<sup>16</sup> (organic acid column HPX 87 H from Bio-Rad, column temperature 60°C, eluent 0.01 M trifluoroacetic acid aqueous solution, flow 0.6 ml/min, RI detection). Mutual interference of monosaccharides and acidic products allowed analysis of the monosaccharides, with an accuracy of 1%, up to a conversion of  $\sim 40\%$ . The latter acid products are stable compounds under alkaline conditions and do not react with the monosaccharides left in the reaction mixture. Thus, the kinetic data obtained by analysis of the reaction mixtures up to 40% will also be applicable to situations with a higher degree of conversion.

## **Results and discussion**

#### Analysis of the reaction course as a function of time

In preliminary experiments, the course of the alkaline degradation of D-fructose was followed in order to determine the applicability of HPLC as an analysis technique for kinetic measurements. For this purpose, the isomerization<sup>15</sup> and acidic degradation products<sup>16</sup> were analyzed on different columns as described in the experimental parts. Figs 1A and 1B present typical examples of liquid chromatograms obtained on the carbohydrate and the organic acid column, respectively. The reaction course as a function of time thus obtained is depicted in Fig. 2. Since the relative amounts of the acidic products did not change mutually during the



Fig. 1. HPLC analysis of products formed during the alkaline degradation of D-fructose (0.025 M D-fructose, 0.01 M KOH,  $H_2O, 78^{\circ}C, N_2$ ). Chromatographic conditions: see experimental. A. Separation of monosaccharides, sample taken after 10 min reaction time.

B. Separation of carboxylic acids, sample taken after 1 h reaction time. 6. glycolic acid

7. lactic acid

10. acetic acid

8. formic acid.

9. 2,4-dihydroxybutyric acid

1. D-glucose

- 2. D-mannose
- 3. D-fructose
- 4. D-psicose
- 5. saccharinic
- acids



Fig. 2. Reaction course of D-fructose in alkaline medium (0.025 M D-fructose, 0.01 M KOH, H<sub>2</sub>O, 78°C, N<sub>2</sub>); ▲, D--fructose; ▼, D-glucose; ●, D-mannose; ●, D-psicose; ●, saccharinic acids;  $\Delta$ , glycolic acid;  $\bigcirc$ , lactic acid;  $\nabla$ , formic acid;  $\Box$ , acetic acid;  $\blacksquare$ , 2,4-dihydroxybutyric acid;  $\times$ , > C<sub>6</sub> acids.



Fig. 3. Kinetic model for the alkaline isomerization and degradation of monosaccharides including enediol anions. A<sup>-</sup> denotes acidic products,  $k_{mn}$  denote the various reaction rate constants.

degradation reaction, use has been made of the total amount of acidic products, defined as 100% minus % sugars, as a measure of conversion. In consequence, the analysis of just the mono-saccharides as a function of time suffices for a kinetic picture of isomerization and degradation.

#### Kinetic model: pseudo-first-order rate constants

A kinetic model for the alkaline isomerization/degradation of D-glucose and D-fructose, which includes D-mannose and D-psicose as well as overall degradation rate constants, is given in Fig. 3. This kinetic model does not include sugar anions since the sugar anion concentrations at constant HO<sup>-</sup> concentration are proportional to the total sugar concentrations. The reactive enediol anion intermediates will have pseudo-steady-state concentrations  $[E_{1,2}^-]$  and  $[E_{2,3}^-]$ , *i.e.*:

$$\frac{d[E_{1,2}]}{dt} = 0 = k_{GE} * [G] + k_{FE} * [F] + k_{ME} * [M] - (k_{EG} + k_{EF} + k_{EM} + k_{EA}) * [E_{1,2}]$$
(1a)

and

$$\frac{d[E_{2,3}^{-}]}{dt} = 0 = k_{FE'} * [F] + k_{PE'} * [P] - (k_{F'F} + k_{E'P} + k_{E'A}) * [E_{2,3}^{-}]$$
(1b)

This implies that, with  $k_{EG} + k_{EF} + k_{EM} + k_{EA} = k_E$  and  $k_{E'F} + k_{E'P} + k_{E'A} = k_{E'}$ :

$$[E_{1,2}] = \frac{k_{GE}}{k_E} * [G] + \frac{k_{FE}}{k_E} * [F] + \frac{k_{ME}}{k_E} * [M]$$
(2a)

and

$$[E_{\overline{2,3}}] = \frac{k_{\rm FE'}}{k_{\rm E'}} * [F] + \frac{k_{\rm PE'}}{k_{\rm E'}} * [P]$$
(2b)



Fig. 4. Simplified kinetic model for the alkaline isomerization and degradation of D-glucose (G), D-mannose (M), D-fructose (F) and D-psicose (P) into acidic products (A). Pseudo-first-order rate constants  $(k_i)$  include different enolization and subsequent rearrangement steps.

Thus, the concentration of enediol anions is proportional to the monosaccharide concentrations and, therefore, can be eliminated in the kinetic model. This permits the use of the simplified kinetic model as presented in Fig. 4 which has been used throughout in our kinetic considerations. The various *pseudo-first-order rate constants*, which include HO<sup>-</sup>concentration-dependent enolization rate constants, have been determined (accuracy ~ 5%) by computer simulations of the experimental data on the basis of this simplified kinetic model. As an example, in Fig. 5 the computer simulations are given for the reaction course of D-fructose, Dglucose and D-mannose in alkaline medium.

It may be noted that the experimental data of the three degradations have been simulated by one and the same set of  $k_i$ . The various isomerization  $(k_1-k_8)$  and degradation  $(k_9-k_{12})$  pseudo-first-order rate constants obtained by the computer simulations are summarized in Table I. The data show that the isomerization of both D-glucose and D-fructose to D-mannose, the isomerization of D-fructose to D-psicose and the subsequent degradation of both D-mannose and



Fig. 5. Alkaline isomerization and degradation of D-fructose, D-glucose and D-mannose (0.025 M monosaccharide, 0.01 M KOH,  $H_2O$ , 78° C,  $N_2$ ). Experimental data:  $\bigcirc$  (F) D-fructose;  $\bigcirc$  (G) D-glucose;  $\triangle$  (M) D-mannose;  $\bigtriangledown$  (P) D-psicose;  $\times$  (A) acidic products. The curves were obtained by computer simulation using the kinetic model shown in Fig. 4.

D-psicose cannot be neglected in kinetic considerations. In particular, the degradation rate constant of D-psicose is noteworthy since it exceeds that of D-fructose.

Table I Pseudo-first-order rate constants for the reactions of monosaccharides in alkaline medium<sup>a</sup>.

Reaction pathway	Rate constant $(10^{-3} \text{ min}^{-1})$	
$G \rightarrow M$	$k_1$	7
$M \rightarrow G$	$k_2$	18
$M \rightarrow F$	$k_3$	49
$F \rightarrow M$	k4	15
$G \rightarrow F$	k <sub>5</sub>	115
F →G	k <sub>6</sub>	90
$F \rightarrow P$	k <sub>7</sub>	25
$P \rightarrow F$	$k_8$	45
$F \rightarrow A$	k <sub>o</sub>	50
$\mathbf{P} \rightarrow \mathbf{A}$	k <sub>10</sub>	70
$\mathbf{M} \rightarrow \mathbf{A}$	$k_{11}$	7
$G \rightarrow A$	k <sub>12</sub>	10
	1	

<sup>a</sup> 0.025 M monosaccharide, 0.01 M KOH, H<sub>2</sub>O, 78°C, N<sub>2</sub>.

The part of the acidic products which originates from D-fructose, D-psicose, D-mannose and D-glucose, respectively, has been obtained by computer calculations using the kinetic model of Fig. 4. The results given in Table II show that, whatever monosaccharide is degraded, about 20% of the products originate from D-psicose, whereas 63-68% of the products are formed via D-fructose. The high reactivity, *i.e.* enolization rate, of these ketoses is responsible for this phenomenon.

Table II The precursor role<sup>a</sup> of D-fructose (F), D-psicose (P), D-mannose (M) and D-glucose (G) in the formation of acidic products (A) upon alkaline degradation of monosaccharides.

Part (%) of A	Starting monosaccharide		
originating from;	D-fructose	D-glucose	D-mannose
F	68	63	63
Р	21	19	19
М	1	1	7
G	10	17	11

<sup>a</sup> Computer calculations using the kinetic model of Fig. 4 and the data of Table I.

In addition to the alkaline isomerization/degradation of D-fructose, D-glucose and D-mannose, that of D-psicose (Fig. 6) emphasizes that the isomerization (and consequently the degradation) of aldoses and 2-ketoses proceeds preferentially via 1,2- and 2,3-enediol anions as intermediates. Only the formation of D-sorbose, up to 3%, during the alkaline degradation of D-psicose, points to 3,4-enolization. However, the formation of D-sorbose as well as D-altrose and D-allose, as isomerization products of D-psicose, will be less than 1% in the alkaline degradation of D-fructose, D-glucose and D-mannose.

#### Influence of monosaccharide concentration

At a first approximation, the alkaline degradation rate of monosaccharides might be considered to be proportional to the total monosaccharide concentration, *i.e.*:

$$\frac{-\mathrm{d}\Sigma[\mathbf{S}_t]}{\mathrm{d}t} = k_{\mathrm{overall}} * \Sigma[\mathbf{S}_t]$$
(3)



Fig. 6. Reaction course of D-psicose in alkaline medium  $(0.025 \text{ M D-psicose}; 0.01 \text{ M KOH}, H_2O, 78^{\circ}C, N_2); \oplus, D-psicose; \bigcirc, D-fructose; \Box, D-glucose; \blacksquare, D-altrose; \nabla, D-allose; \triangle, D-sorbose; \times, acidic products.$ 

in which  $k_{overall}$  denotes the overall-pseudo-first-order degradation rate constant, thus including HO<sup>-</sup> dependence, and  $\Sigma[S_1]$  the total concentration of monosaccharides. Using the concentration profiles of the monosaccharides, as obtained by computer simulation (Fig. 5), equation (3) leads to the curves given in Fig. 7.

Clearly, overall-pseudo-first-order kinetics are only valid after a reasonable period of time, depending on the starting monosaccharide. The alkaline degradation started with D--glucose and D-fructose follows overall-pseudo-first-order kinetics after 15-20 min, whereas it takes more than 40 min for the degradation of D-mannose. This phenomenon can be explained by the fact that only overall-pseudo-first-order kinetics may be expected if the isomerization equilibrium has been attained<sup>7</sup>. As demonstrated by Fig. 5, the isomerization equilibrium is indeed achieved within 20 min for D-fructose and D-glucose, but not for D-mannose. From the rate constants given in Table I, the composition of the equilibrium mixture is calculated as 36% D-fructose, 35% D-glucose, 18% D-mannose and 11% D-psicose, which explains the overall-pseudo-first-order degradation rate constant  $k_{\text{overall}} = 0.030 \text{ min}^{-1}$ .

# Influence of hydroxyl ion concentration

As already mentioned, the reaction rate constants of the simplified kinetic model (Fig. 4) include the  $HO^-$  concentration. The influence of the  $HO^-$  concentration on the reaction rate constants is shown in Table III. The dependence observed can be understood using the general model shown in Fig. 8, including both the ionization and enediol anion formation from the different monosaccharides. Since the ionization of monosaccharides in alkaline medium is



Fig. 7. Logarithmic plot of the total concentration of monosaccharides versus time for the alkaline degradation of D-mannose (M), D-glucose (G) and D-fructose (F): deviations from overall-pseudo-first-order kinetics are due to isomerization reactions. Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH,  $H_2O$ , 78° C,  $N_2$ .

fast with respect to subsequent enediol anion formation, we may write:

$$\frac{-d[S^{-}]}{dt} = \frac{-d[S_{t}]}{dt} = k_{ts} * [S^{-}] - k_{2s} * [E^{-}]$$
(4)

with the total monosaccharide concentration  $[S_t] = [SH] + [S^-]$ . The conversion rate of the enediol anion can be expressed by:

$$\frac{-d[E^{-}]}{dt} = (\Sigma k_{2s} + k_d) * [E^{-}] - \Sigma k_{1s} * [S^{-}]$$
(5)

Considering E<sup>-</sup> as a reactive intermediate, we assume  $\frac{-d[E^-]}{dt} = 0$  so that  $[E^-]$  will be proportional to the various sugar concentrations:

$$[E^{-}] = \frac{\Sigma k_{1s}}{\Sigma k_{2s} + k_{d}} * [S^{-}]$$
(6)

Table III Influence of the  $HO^-$  concentration on the pseudo-first-order rate constants<sup>a</sup> according to the kinetic model shown in Fig. 4.

Rate constant	HO <sup>-</sup> concentration (M)				
$(10^{-3} \min^{-1})$	10-3	3.5 * 10 - 3	10-2	3.5 + 10 <sup>-2</sup>	10-1
k <sub>1</sub>	1.1	3.0	7	15	25
$k_2$	2.8	8.0	18	40	60
$k_3$	8.8	26.0	49	115	160
$k_4$	2.7	8.0	15	35	60
ks	20.0	55.0	115	260	360
$k_6$	15.7	45.0	90	220	330
k <sub>7</sub>	4.2	12.0	25	60	90
k <sub>8</sub>	8.0	23.0	45	100	140
ka	7.9	24.0	50	135	220
$k_{10}$	12.0	36.0	70	190	320
$k_{11}^{10}$	1.1	3.0	7	19	31
k <sub>12</sub>	1.6	5.0 ·	10	27	46

<sup>a</sup> Reaction conditions: 0.025 M monosaccharide, KOH, H<sub>2</sub>O, 78°C, N<sub>2</sub>.

Substitution of equation (6) in (4) gives:

$$\frac{-d[S_{t}]}{dt} = k_{1s} * [S^{-}] - \frac{k_{2s} \Sigma k_{1s}}{\Sigma k_{2s} + k_{s}} * [S^{-}]$$
(7)



Fig. 8. General model for the alkaline isomerization and degradation of monosaccharides.

$S_iH$ and $S_iH$	monosaccharides;
$S_i^-$ and $S_i^-$	monosaccharide anions;
E	enediol anion;
A -	acidic degradation products.

--G

**--** Δ

~ P

With:

$$K_{\rm as} = \frac{[S^{-}] * [H^{+}]}{[SH]} = \frac{[S^{-}] * K_{\rm w}}{[SH] * [HO^{-}]}$$
(8)

and

 $[SH] = [S_1] - [S^-]$ we get

$$[S^{-}] = \frac{[HO^{-}]}{K_{w}/K_{as} + [HO^{-}]} * [S_{t}]$$
(9)

Combination of (7) and (9) then results in:

$$\frac{-d[S_t]}{dt} = \frac{A * [HO^-]}{B + [HO^-]} * [S_t] = \Sigma k_i [S_t]$$
(10)

with  $A = k_{1s} - \frac{k_{2s}\Sigma k_{1s}}{\Sigma k_{2s} + k_d}$ ,  $B = K_w/K_{as}$  and  $\Sigma k_i$  the sum of the HO<sup>-</sup>-dependent pseudo-first-order rate constants  $k_i$  of

the simplified kinetic model (Fig. 4). For D-fructose, the kinetic data presented in Table III have been plotted versus

$$\begin{array}{c}
 \begin{bmatrix} HO^{-} \\ B + [HO^{-} ] \\ \hline B + [HO^{-} ] \\ \hline 0.3 \\ \hline 0.3 \\ \hline 0.4 \\ \hline 0.2 \\ \hline 0.1 \\ \hline 0 \\ \hline$$

Fig. 9. Pseudo-first-order rate constants  $k_i$ , according to the simplified kinetic model shown in Fig. 4, as a function of the  $HO^-$  concentration (pK<sub>w</sub> = 14.1 and pK<sub>as</sub> = 12.7). Reaction conditions: 0.025 M monosaccharide, KOH, H<sub>2</sub>O, 78°C, N<sub>2</sub>.

 $\frac{OH^{-}}{K_{W}/K_{QS}+OH^{-}}$ 

As would be expected from relation (10), a linear relationship is obtained for the pseudo-first-order isomerization rate constants  $k_4$ ,  $k_6$  and  $k_7$ . However, some deviation occurs for the degradation reaction  $(k_9)$  at  $[HO^-] > 10^{-2} M$ , which indicates the importance of a second ionization of D-fructose at high alkalinity in degradation product formation. Although some investigators<sup>8,12</sup> reported first-order kinetics with respect to the monosaccharide as well as the HO<sup>-</sup> concentration, Lai<sup>11,17</sup> and Bamford et al.<sup>7</sup> also found apparent deviations from simple first-order kinetics. These authors proposed that, at high HO- concentrations,  $[HO^{-}] > 10^{-2}$  M, dianionic sugar species will also be in-



Fig. 10. Direct retro-aldolization of the  $\beta$ -D-fructopyranose di-anion at high HO<sup>-</sup> concentration.

volved. Bamford et al.<sup>7</sup> assumed that ionization of a hydroxyl group, presumably on C4, occurs. If so, then an increased C3-C4 bond fission (retro-aldolization) into two trioses is to be expected as depicted in Fig. 10. This extra triose formation results in higher amounts of lactic acid, which is in accordance with the observed sharp increase of lactic acid production at higher HO<sup>-</sup> concentrations<sup>6</sup>.

# Influence of calcium(II) ion concentration

Vukov<sup>8</sup> and Mottard<sup>12</sup> observed that calcium(II) ions, in comparison with monovalent cations such as  $K^+$  and  $Na^+$ , accelerate the alkaline degradation of monosaccharides. In order to identify the role of calcium(II) in the alkaline isomerization/degradation pattern, we have performed kinetic experiments in the presence of different calcium(II) concentrations (0-0.06 M). The influence of calcium(II) on the overall-pseudo-first-order degradation rate constant  $k_{\text{overall}}$  [according to equation (3)], determined after the isomerization equilibrium has been attained, is depicted in Fig. 11.



Fig. 11. The overall-pseudo-first-order degradation rate constant as a function of the calcium(II) concentration. Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH,  $CaCl_2, H_2O, 78^{\circ}C, N_2.$ 

Clearly, the formation of Ca(II)-monosaccharide complexes will be responsible for the increase in the degradation rate. The influence of calcium(II) on the various rate constants of the simplified kinetic model (Fig. 4) demonstrates the role of calcium(II) in more detail as shown in Table IV. Comparison of the changes in the various isomerization-rate constants shows that calcium(II) slightly, but consistently, enhances the enolization rate, in particular in the case of D-fructose and of D-psicose. The complexation of calcium(II) is known to involve the anomeric hydroxyl which apparently favours enediol anion formation and thus isomerization of the monosaccharides. The average increase in the rate of isomerization of D-glucose  $(k_1, k_5)$ , D-mannose  $(k_2, k_3)$ , D-fructose  $(k_4, k_6, k_7)$  and D-psicose  $(k_8)$  is about 5 times lower than the increase of its degradation rate constants. Thus, in addition to a general increase in all reaction rates due to a higher rate of enolization, calcium(II) has an additionally favourable effect on the rate of formation of degradation products. In this respect, it may be noted that the enhanced production of lactic acid, observed upon addition of calcium(II)<sup>6</sup>, is also found at high HO<sup>-</sup> concentrations without calcium(II). Presumably, calcium(II) induces the second ionization of another hydroxyl group as depicted in Fig. 10, which leads to extra degradation product formation at relatively low  $HO^-$  concentrations.

Table IV Influence of calcium(II) on the rate constants<sup>a</sup> applying to the simplified kinetic model (Fig. 4).

Rate (10	e constant <sup>- 3</sup> min <sup>- 1</sup> )	Without Ca(II)	0.06 M CaCl <sub>2</sub>	
<i>k</i> <sub>1</sub>	$G \rightarrow M$	7	7	
k <sub>2</sub>	$M \rightarrow G$	18	19	
$  k_3$	M → F	49	52	
k4	$F \rightarrow M$	15	18	
k5	$G \rightarrow F$	115	120	
k <sub>6</sub>	F → G	90	100	
k <sub>7</sub>	$F \rightarrow P$	25	30	
k <sub>8</sub>	$P \rightarrow F$	45	50	
k.,	$F \rightarrow A$	50	90	
$k_{10}$	$P \rightarrow A$	70	110	
k <sub>11</sub>	M → A	7	9	
k <sub>12</sub>	$G \rightarrow A$	10	13	

<sup>a</sup> Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH, CaCl<sub>2</sub>, H<sub>2</sub>O, 78°C, N<sub>2</sub>.

# Influence of borate concentration

Alkali-catalyzed isomerization may be employed for the synthesis of ketoses from aldoses: *e.g.* D-fructose from D-glucose<sup>18-22</sup> and lactulose from lactose<sup>23</sup>. Fig. 5, for instance, shows that D-fructose is formed in concentrations of about 30%. Addition of borate<sup>24</sup> or boronates<sup>25,26</sup> to the reaction mixture is known to improve the equilibrium concentration of ketose. Thus, yields of  $\sim 90\%$  of cellobiulose and maltulose have been obtained by Hicks et al.27 by treating cellobiose and maltose, respectively, with triethylamine and a molar amount of boric acid at 70°C and pH 11. These authors also reported a very low rate of degradation under these circumstances. Since borate forms esters with polyhydroxy compounds<sup>28,29</sup> such as carbohydrates<sup>30</sup> in aqueous alkaline solutions, differences in the stability constants of the various monosaccharide borate esters involved are responsible for the observed shift in the isomerization equilibrium. The influence of borate on both the isomerization and degradation rate of the different monosaccharides can be determined by the use of the kinetic model shown in Fig. 4, which then gives the opportunity to establish which monosaccharides are preferentially esterified by borate.

The work of Makkee et al.<sup>30</sup> on the interaction of borate with D-glucose and D-fructose has revealed that, at molar borate/monosaccharide ratios >1, D-glucose and D-fructose are esterified with two borates in 5-membered rings (Fig. 12). At lower molar borate/monosaccharide ratios, esters of one borate with two monosaccharide units predominate (Fig. 13). In both monosaccharide diborate and bis(monosaccharide) borate esters, the anomeric hydroxyl is esterified by borate which is expected to retard enolization and the subsequent isomerization and degradation reactions. Kinetic experiments at low (0.4) and high (4) molar borate/monosaccharide ratios are shown in Fig. 14 for D--glucose and D-fructose, from which the kinetic data have been obtained by computer simulation (Table V). Since the signals of D-mannose and borate interfere in the liquid chromatogram, the kinetic data for the isomerization and degradation of D-mannose have been estimated assuming that the influence of borate on D-mannose conversion is

Table V Influence of borate on the pseudo-first-order rate constants<sup>a</sup> of the simplified kinetic model shown in Fig. 4.

Rate constant $(10^{-3} \text{ min}^{-1})$		Molar borate/monosaccharide ratio			
		0	0.4	4	
$k_1$	$G \rightarrow M$	7	7	3	
$k_2$ $k_3$	M → G M → F	49	48	17	
$k_4$	$F \rightarrow M$	15	10	1	
$k_6^{K_5}$	$F \rightarrow G$	90	53	8	
$k_7$ $k_8$	F → P P → F	25 45	16	2 4	
L	F → A	50	26	8	
$k_{10}$	$P \rightarrow A$	70	19	5	
$k_{11}$ $k_{12}$	$ \begin{array}{c} M \to A \\ G \to A \end{array} $	7 10	7 11	3 4	

<sup>a</sup> Reaction conditions: 0.025 M monosaccharide, 0.01 M KOH,  $Na_2B_4O_7 \cdot 10H_2O$ ,  $H_2O$ , 78°C,  $N_2$ .



Fig. 12. D-fructose (A) and D-glucose (B) diborate esters<sup>30</sup>.



Fig. 13. Bis(D-fructose) (A) and bis(D-glucose) (B) borate esters<sup>30</sup>.



Fig. 14. Alkaline isomerization and degradation of D-fructose and D-glucose in the presence of borate (0.025 M monosaccharide, 0.01 M KOH,  $Na_2B_4O_7 \cdot 10H_2O$ ,  $H_2O$ ,  $78^{\circ}C$ ,  $N_2$ ); A, borate/monosaccharide = 4; B, borate/monosaccharide = 0.4. Experimental data (see text):  $\bigcirc$  (F) D-fructose;  $\bigcirc$  (G) D-glucose; (M) D-mannose;  $\bigtriangledown$  (P) D-psicose; (A) acidic products. The curves were obtained by computer simulation (see text) using the kinetic model shown in Fig. 4.

comparable to that of D-glucose. On this basis, in Fig. 14 the D-mannose concentration and, consequently, the concentration of acidic products as a function of time have been calculated using the simplified kinetic model of Fig. 4. When the monosaccharide concentration exceeds that of borate, only the isomerization and degradation rate constants of D-fructose and D-psicose decrease substantially, whereas those of D-glucose and D-mannose are not influenced. On the other hand, if the borate concentration exceeds the monosaccharide concentration, all rate constants decrease substantially. From the kinetic data thus obtained, the following conclusions can be drawn:

- With an excess of monosaccharide, the borate esters of (i) D-fructose and D-psicose occur predominantly. As a consequence, only the isomerization and degradation rates of these monosaccharides decrease markedly and the isomerization equilibrium shifts towards these sugars.
- (ii) An excess of borate, on the other hand, causes the formation of (di)borate esters of all monosaccharides and thus largely reduces all isomerization and degradation rates. When the reaction is started with D-glucose, the formation of the relatively stable D-fructose diborate esters (A in Fig. 12) is responsible for the increase in the maximum D-fructose concentration to 53%.

# Conclusions

The kinetic model (Fig. 4) developed for the alkaline isomerization/degradation of monosaccharides gives a good description of the kinetic data. It is shown that both D-glucose and D-fructose give substantial isomerization to D-psicose and D-mannose. Subsequent degradation of, in particular, D-psicose is noteworthy since  $\sim 20\%$  of the acidic products formed by the alkaline degradation of either D-fructose, D-glucose or D-mannose originate from D-psicose. Additionally, the formation of  $\sim 65\%$  acidic products via D-fructose emphasizes that ketoses are the most important intermediates in the alkaline degradation reaction because of their higher reactivity, i.e. enolization rate, than aldoses. The influence of the HO<sup>-</sup> concentration on the various pseudofirst-order rate constants revealed that enolization of monosaccharides is the rate-determining step in both the isomerization and the degradation reactions. In addition, direct retro-aldolization of monosaccharides, in particular D-fructose, becomes of importance at HO<sup>-</sup> concentrations exceeding  $10^{-2}$  M. This is presumably due to the formation of di-anion species. Under these conditions, a second order dependence of the degradation rate constants on the HO<sup>-</sup> concentration becomes apparent.

Calcium(II) increases the enolization rate of monosaccharides, especially that of D-fructose. Furthermore, the influence of calcium(II) on the retro-aldolization reaction and thus on the degradation rates is comparable with the influence of HO<sup>-</sup> at high concentrations. In both cases, the increased retro-aldolization is reflected in the enhanced production of lactic acid.

In addition to the borate induced shift of the isomerization equilibrium towards D-fructose and D-psicose, due to the formation of various monosaccharide (di)borate esters and bismonosaccharide borate esters with different stability constants, there is a remarkable decrease in the degradation rate of the monosaccharides. Borate ester formation of monosaccharides involving the anomeric hydroxyl group,

therefore, has a stabilizing effect on monosaccharides in alkaline medium.

The rate-enhancing effect of Ca(II) versus the rate-retarding effect of borate finds its origin in the different character of the O-Ca and O-B bonds formed, *i.e.* ionic versus covalent oxygen bonding, respectively.

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