KINETIC STUDY OF ALDOLIZATION REACTIONS OF TRIOSES

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Trioses can undergo in alkaline medium isomerizations, dehydrations, aldolizations and dealdolizations. The preferred course of the aldolization reactions and the composition of the reaction mixtures depends on the reaction conditions, especially on the starting triose concentration. D,L-Glyceraldehyde and dihydroxyacetone aldolize to give a mixture of D,L-fructose, D,L-sorbose, and D,L-dendroketose. This system of competitive and consecutive reactions of the first and second order was treated mathematically. Kinetic measurements were made at constant concentration of the base catalyst, NaOH or Na_2CO_3, maintained pH-statically. The results of the kinetic studies elucidate partly the mechanism of aldolization of both trioses.

Aldolization of trioses in alkaline medium was studied by many authors. Mixtures of hexoses resulting after aldolization of glyceraldehyde[1] were identified later as fructose and sorbose[2], and their ratio was determined after their separation in the form of phenylhydrazones[3]. Also the ratio of hexoses and trioses[4] was determined in solutions in which the aldolization apparently did not proceed quantitatively owing to a drop in pH. A branched ketohexose formed preferentially during aldolization of dihydroxyacetone was denoted as dendroketose[5]. Two teams of workers[6,7] carried out a kinetic study of the triose aldolizations and arrived at different results concerning the reaction order for dihydroxyacetone as well as the found constants and composition of the reaction mixtures after aldolization. They did not employ pH-stated media and determined the reaction components colorimetrically.

In connexion with our previous studies of the electrochemical and especially polarographic behaviour of trioses[8] and methylglyoxal[9] and the kinetics of their acid–base catalyzed conversions[10–12], with respect to the conditions under which the aldolization of trioses was followed[6,7] and differences in the published results, we continued to study thoroughly the aldolization kinetics of glyceraldehyde and dihydroxyacetone. To this purpose we employed a pH-stat to maintain the concentration of the base catalyst constant[12] and a polarographic method of the determination of trioses[13,14].

EXPERIMENTAL

Apparatus

The aldolizations were carried out in a universal titration vessel of the type EA 880 with an EA 109 U glass electrode and an EA 404 calomel electrode (Metrohm, Herisau). The indicator

electrode was connected to an automatic titrator of the type TTT 2 (Radiometer, Copenhagen) serving as a pH-stat in combination with a magnetic valve. Polarographic analyses were made on an OH 102 type polarograph (Radelkis, Budapest). The temperature of the titration and polarographic vessels was kept constant to within $\pm 0{\cdot}02°C$ by means of a U 10 thermostat (Prüfgeräte Medingen, Dresden). A gas chromatograph of the type 5754 G (Hewlett-Packard) was used.

Chemicals

D,L-Glyceraldehyde, *purum*, dihydroxyacetone, *puriss.*, in the dimeric form, isobutylamine, *purum*, and *o*-phenylenediamine for buffer solutions were products of Fluka A.-G. Sodium hydroxide, sodium carbonate and hydrochloric acid used to adjust the alkalinity of the studied solutions were in the form of "Titrisol" standard solutions (Merck). Paper chromatography was made on Whatman 1 paper, the developing system was an acetone–n-butyl alcohol–water mixture (7 : 2 : 1), and diphenylamine was used for detection[15]. Preparative chromatography was carried out on a powdered cellulose column (Whatman) with the same developing system using acetone and butyl alcohol of reagent grade or pure (Lachema, Brno) and distilled on columns. D-Fructose and L-sorbose (Lachema) of reagent grade recrystallized twice from an alcohol–ether mixture and served as chromatographic standards. D,L-Dendroketose was prepared by aldolization of dihydroxyacetone in a pH-stated aqueous hydroxide medium under optimum reaction conditions, and separated by column chromatography[16].

Method of Measurement

Kinetic measurements were made in nitrogen atmosphere in a pH-stated vessel by the method and in the apparatus described previously[12] together with the preparation of carbonateless solutions. The only difference was that solutions of NaOH and HCl used in titrations, NaOH and Na_2COl_3 used as basic catalysts in triose solutions were employed in the form of the mentioned standard solutions. The alkalinity of 0·0025—0·04M-NaOH and 0·025—0·5M-Na_2CO_3 was adjusted by adding stock solutions of 0·5 or 0·05M-NaOH (carbonate-free) or 1M-Na_2CO_3. The burette of the pH stat was filled with 1M-NaOH (carbonate-free). Solutions of 0·05—0·5M glyceraldehyde and 0·1—1M dihydroxyacetone were obtained from carbonate-free 0·2 or 1M glyceraldehyde and 0·5 or 2M dihydroxyacetone stock solutions. The triose concentration was always 0·5M. Concentration dependences at pH 12 were made in the presence of about 0·0a1M -NaOH. The temperature of measurement was 25°C; the dependences on temperature were m dewith 0·5M triose and 0·01M-NaOH, the temperature rising from 15 to 35°C at 5°C steps.

The ionic strength of the measured solutions was maintained by additions of KCl at 0·1 in the presence of NaOH and 0·3 in the presence of Na_2CO_3. However, it was not constant during the reactions owing to pH stating (additions of NaOH).

The triose concentration was measured during the reactions by an indirect polarographic method based on a different reactivity of the trioses against isobutylamine[13,14]. The determinations were made at 20°C in the presence of 0·01M *o*-phenylenediamine so that also methylglyoxal which could be formed from the trioses by isomerization and dehydration, could be determined as 2-methylquinoxaline. The composition of the reaction mixtures after completion of the aldolization and neutralization with 1·0 and 0·1M-HCl to pH 5—6 was determined by gas chromatography using the stationary phase OV-17 in an isothermal column. Sorbose was determined at a column temperature of 130°C, fructose at 190°C. Dendroketose and fructose were separated on the stationary phase XE-60 at 130°C after their conversion to polyalcohols by reduction

with $NaBH_4$. The reaction mixtures were in all cases silylated and chromatographed in the form of per-O-trimethylsilyl derivatives[16].

Dihydroxyacetone aldolized at optimum conditions was after finishing the reaction and neutralization with HCl separated chromatographically on a Whatman powdered cellulose column using the acetone–n-butyl alcohol–water system (7 : 2 : 1), and the obtained products were weighed. Glyceraldehyde was treated analogously.

Determination of Rate Constants

We assume that dealdolization does not proceed and the rate of aldolization in neutral solutions is practically zero, which was verified experimentally with both trioses. Their aldolization proceeds accordingly in two steps, formation of a carbanion and an addition:

$$A \xrightarrow{k_1} B, \quad A + B \xrightarrow{k_2} C, \qquad (A)$$

where A denotes a triose, B its carbanion intermediate and C aldolization product. The third (recombination-regeneration) step proceeds very rapidly and need not be therefore considered here. The ratio of the rate constants, k_1/k_2, determines the overall reaction order of the aldolization. Its kinetics is described by the following differential equations:

$$d[A]/dt = -k_1[A] - k_2[A][B], \qquad (1)$$

$$d[B]/dt = k_1[A] - k_2[A][B], \qquad (2)$$

where the brackets refer to molar concentrations. For $k_1 \gg k_2$ the total reaction is controlled by the rate of the addition; this is the case of dihydroxyacetone, for which the value of k_2 is found from a second-order kinetic equation. For $k_2 \gg k_1$ the aldolization attains a steady state for which according to the Bodenstein principle the concentration of the intermediate product, $[B]_s$, is constant, hence $d[B]_s/dt = 0$. This condition is introduced into Eq. (2) to obtain $[B]_s = k_1/k_2$, which is in turn introduced into (1); after integration we obtain

$$k_1 = (2{\cdot}3/2t) \log ([A]_0/[A]), \qquad (3)$$

where $[A]_0$ denotes concentration of the triose at $t = 0$ and [A] its concentration at a time t after attainment of the stationary state. This equation enables to calculate k_1 for glyceraldehyde whose aldolization is of the first order and the rate-determining step is the formation of a carbanion.

Benson[17] showed by analysis of the relationship derived by eliminating the time variable and integrating that [B] attains its limiting value $[B]_s$ only if $[A]_0\, k_2/k_1 \geqq 100$; the stationary state is then established practically during the whole reaction (for conversions from 0·1 to 1). This condition enables to estimate the value of k_2 for glyceraldehyde by determining its lowest initial concentration at which the aldolization is governed by a first-order kinetic equation, and the products of isomerization (dihydroxyacetone) and dehydration (methylglyoxal) are not observed.

From the kinetic study of mixtures of both trioses at an elevated aldolization rate it is possible to determine the constant k_1 for dihydroxyacetone. Its aldolization is then governed by a first-order kinetic equation and the constant k_1 refers to the first step, *i.e.*, formation of a carbanion.

RESULTS

The reaction order for the aldolization of both trioses and their mixtures was determined by the half-time method in 0·01M-NaOH at 25°C. The reaction half-time for 0·1–0·5M glyceraldehyde was 470 s. The half-time for the decomposition of dihydroxyacetone in 1 : 4, 1 : 1 and 4 : 1 triose mixtures (total concentration 0·5M) was also practically constant, $\tau_{1,2} = 280$ s. Aldolization of pure glyceraldehyde and of dihydroxyacetone in these mixtures is accordingly a reaction of the first order. For pure 0·025–0·5M dihydroxyacetone, the slope of the dependence of log $\tau_{1,2}$ on log $[A]_0$ shows that the aldolization is of the second order.

The kinetic measurements proper were carried out as described in the experimental part. The values obtained from the concentration dependences in 0·01M-NaOH (pH ≈ 12) at varying concentrations of the trioses are given in Table I; the mean values of the catalytic constants are $K_1 = 0{\cdot}074$ l mol^{-1} s^{-1} for glyceraldehyde

TABLE I

Rate Constants (k_1, k_2) and Catalytic Constants (K_1, K_2) for Catalysis with OH^- Ions of the Aldolization of D,L-Glyceraldehyde (k_1, K_1) and Dihydroxyacetone (k_2, K_2) 0·01M-NaOH, 25°C, $I \approx 0{\cdot}1$.

c, M	pH	$k_1 \cdot 10^4$ s^{-1}	$K_1 \cdot 10^2$ l mol^{-1} s^{-1}
		Glyceraldehyde	
0·1	11·95	6·8	7·6
0·2	12·0	7·4	7·4
0·3	11·95	6·8	7·6
0·4	12·0	7·5	7·5
0·5	12·0	7·0	7·0
c, M	pH	$k_2 \cdot 10^4$ l mol^{-1} s^{-1}	$K_2 \cdot 10^2$ l^2 mol^{-2} s^{-1}
		Dihydroxyacetone	
0·5	11·90	2·75	3·4
0·6	12·10	4·6	3·6
0·7	12·02	3·5	3·3
0·8	12·00	3·7	3·7
0·9	12·20	5·8	3·7
1·0	12·10	4·3	3·4

and $K_2 = 0{\cdot}035\ l^2\ mol^{-2}\ s^{-1}$ for dihydroxyacetone. The subscripts 1 and 2 are used to indicate the first or second order of the reaction.

The pH dependences at constant triose concentration, $[A]_0 = 0{\cdot}5$M, are given in Table II; the mean values of the catalytic constants are $K_1 = 0{\cdot}071\ l\ mol^{-1}\ s^{-1}$ for glyceraldehyde and $K_2 = 0{\cdot}032\ l^2\ mol^{-2}\ s^{-1}$ for dihydroxyacetone.

Kinetic data measured in the presence of varying amounts of sodium carbonate at pH $\approx$ 11 and constant triose concentration, $[A]_0 = 0{\cdot}5$M, are shown in Fig. 1. The mean values of the resulting catalytic constants corresponding to the catalysis of the aldolization with CO_3^{2-} ions are $K_1 = 4{\cdot}6 \cdot 10^{-4}\ l\ mol^{-1}\ s^{-1}$ for glyceraldehyde and $K_2 = 7{\cdot}5 \cdot 10^{-5}\ l^2\ mol^{-2}\ s^{-1}$ for dihydroxyacetone. From the dependences of the rate constants k_1 and k_2 on the concentration of Na_2CO_3 we found graphically (from the intersections of these dependences with the ordinate at constant pH) the catalytic constants corresponding to the catalysis with OH^- ions, $K_1 = 0{\cdot}085\ l\ mol^{-1}\ s^{-1}$ for glyceraldehyde and $K_2 = 0{\cdot}040\ l^2\ mol^{-2}\ s^{-1}$ for dihydroxyacetone.

The lowest concentration of glyceraldehyde at which in 0·01M-NaOH the aldolization is governed by a first-order kinetic equation and the presence of dihydroxyacetone in the reaction mixtures is not detectable is $[A]_0 = 0{\cdot}1$M. From the mentioned relation $[A]_0/K \geqq 100$ (Benson's condition) follows a rough estimate of the catalytic constant for the catalysis with OH^- ions corresponding to the second addition step of the aldolization of glyceraldehyde, $K_2 \approx 80\ l^2\ mol^{-2}\ s^{-1}$. Kinetic data for the aldolization of triose mixtures in 0·01M-NaOH (pH $\approx$ 12) at a total triose concentration 0·5M and their ratio 4 : 1, 1 : 1, or 1 : 4 are given in Table III. With a 1 : 4 glyceraldehyde–dihydroxyacetone mixture, the decomposition of glyceraldehyde

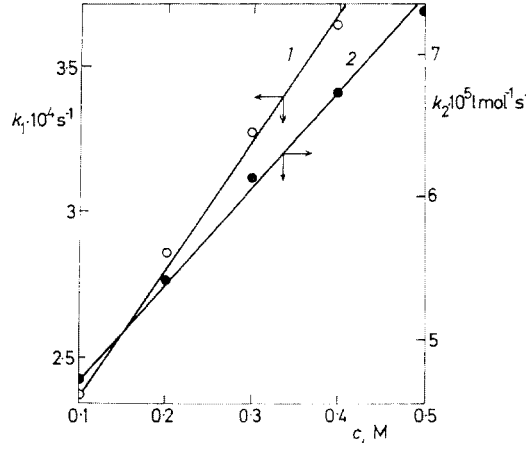

FIG. 1

Dependence of Aldolization Rate Constants for *1* D,L-Glyceraldehyde and *2* Dihydroxyacetone on Concentration of Na_2CO_3 at 25°C

corresponds to a second-order equation so that the value given in Table III is K_2 rather than K_1. The mean experimental error does not exceed 13%.

The temperature dependences in the interval 15–35°C are shown in Fig. 2 for $[A]_0 = 0{\cdot}5$M in the medium of 0·01M-NaOH. The activation energies were determined as $E_A = 19{\cdot}8$ kcal/mol for glyceraldehyde and 14·3 kcal/mol for dihydroxyacetone.

Analysis of the reaction mixtures after completing the aldolizations at suitably chosen reaction conditions revealed that in the triose concentration interval, pH and temperatures used the reaction products had a varying composition.

Paper chromatographic analyses showed that a considerable amount of fructose and sorbose was formed during aldolization of glyceraldehyde, the amount of fructose being markedly larger, and a small amount of dendroketose. During aldolization of dihydroxyacetone, mainly dendroketose is formed besides fructose and sorbose in small, approximately equal amounts. Under our conditions, the slowest motion on the chromatographic paper was observed with sorbose, then fructose (R_{sorb} = = 1·12) and dendroketose (R_{sorb} = 1·43). A temperature increase during aldolization was manifested by the formation of other two substances with R_{sorb} = 0·82 and 1·65. No aldohexoses were detected.

The preparative column chromatography of the reaction mixture after aldolization of 0·5M glyceraldehyde in 0·01M-NaOH yielded 80% of unbranched ketohexoses and 18% of dendroketose. The aldolization products of 1M dihydroxyacetone in the same medium contained up to 90% of dendroketose. Fructose and sorbose could not be separated in this way.

Gas chromatography showed the following content of ketohexoses in the reaction mixtures: After aldolization of glyceraldehyde 61–70% fructose, 20–24% sorbose,

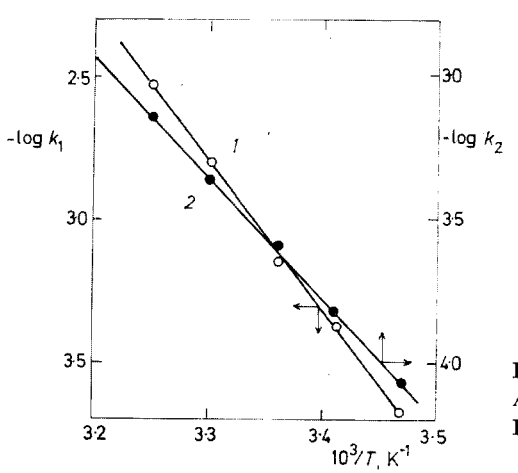

FIG. 2
Determination of Activation Energies for Aldolization of *1* D,L-Glyceraldehyde and *2* Dihydroxyacetone

TABLE II

Rate Constants (k_1, k_2) and Catalytic Constants (K_1, K_2) for Catalysis with OH^- Ions of the Aldolization of D,L-Glyceraldehyde (k_1, K_1) and Dihydroxyacetone (k_2, K_2)
0·5M Trioses, 25°C, $I \approx 0{\cdot}1$.

Glyceraldehyde			
M-NaOH	pH	$k_1 \cdot 10^4$ s^{-1}	$K_1 \cdot 10^2$ $l\,mol^{-1}\,s^{-1}$
0·003	11·47	2·2	7·1
0·007	11·80	4·8	6·9
0·01	12·00	7·0	7·0
0·022	12·35	15·0	6·7
0·035	12·54	23·0	6·7
Dihydroxyacetone			
M-NaOH	pH	$k_2 \cdot 10^4$ $l\,mol^{-1}\,s^{-1}$	$K_2 \cdot 10^2$ $l^2\,mol^{-2}\,s^{-1}$
0·0025	11·45	0·96	3·6
0·005	11·70	1·73	3·5
0·01	11·90	2·75	3·4
0·02	12·30	5·56	2·8
0·03	12·48	8·65	2·9

and 11–22% dendroketose; in the case of dihydroxyacetone 85–95% dendroketose, 2–8% sorbose, and 2–8% fructose. With decreasing initial concentrations of the trioses, the content of the main reaction products (fructose, sorbose, and dendroketose) becomes lower in favour of the complementary products in the range indicated. With the glyceraldehyde–dihydroxyacetone ratios 4 : 1, 1 : 1 and 1 : 4 the corresponding contents of the dendroketose are 8, 11 and 36%; the remainder is fructose and sorbose in the ratio approximately 3 : 1. The content of follow-up reaction products increases with temperature in accord with the results of paper chromatography and is 2–5% at 35°C after one hour from the commencement of the reaction.

Gas chromatography revealed not in a single case of the aldolization the presence of aldohexoses. The analysis of the triose aldolization products, the properties and behaviour of dendroketose, and the follow-up reaction products are described in another work[16].

TABLE III

Catalytic Constants (K_1, K_1') for Catalysis with OH^- Ions of the Aldolization of D,L-Glyceraldehyde (K_1) and Dihydroxyacetone (K_1') in their Mixtures

Total concentration of trioses 0·5M; 0·01M-NaOH (pH ≈ 12), 25°C, $I \approx 0{\cdot}1$.

Glyceraldehyde M	$K_1 \cdot 10$ l mol^{-1} s^{-1}	Dihydroxyacetone M	$K_1' \cdot 10$ l mol^{-1} s^{-1}
0·4	2·8	0·1	2·2
0·25	6·6	0·25	2·4
0·1	4·6—17·6	0·4	2·4

DISCUSSION

The aldolization of glyceraldehyde is a reaction of the first order with respect to glyceraldehyde. The rate-determining step is the formation of a carbanion of dihydroxyacetone through a glyceraldehyde and endiol carbanion. It cannot be decided whether the found rate constant k_1 corresponds to the formation of the glyceraldehyde carbanion or its conversion to the endiol carbanion. It certainly does not correspond to the conversion of the endiol to the dihydroxyacetone carbanion. The solution did not contain a significant amount of the endiol form. Not even traces of aldohexoses were detected in all the studied cases, an evidence for the formation of a carbanion of glyceraldehyde by splitting off a proton on the secondary alcoholic group by the action of the base catalyst. The second addition reaction step (k_2) corresponds to the formation of fructose and sorbose by the addition of a dihydroxyacetone carbanion to the neutral glyceraldehyde molecule. We have thus to deal with a crossed aldolization. The addition rate constant is by at least three orders of magnitude larger than that of the carbanion formation.

The aldolization of dihydroxyacetone is of the second order with respect to the triose. Its rate (k_2) is controlled by the addition of the dihydroxyacetone carbanion to the dihydroxyacetone molecule. The fact that this reaction is in mixtures of both trioses governed by a first-order kinetic equation enables to determine the rate constant of its first step (k_1), *i.e.*, the formation of the dihydroxyacetone carbanion from the triose by the addition of a base. The value of k_1 is by about an order of magnitude larger than that corresponding to the addition step. The insignificant influence of the ionic strength on the aldolization rate constants for both trioses is in accord with the assumed interaction of carbanions with neutral molecules.

The value of $K_1 = 0{\cdot}076$ l mol^{-1} s^{-1} for the aldolization of glyceraldehyde catalyzed by OH^- ions is in good agreement with the values obtained by other authors

using different analytical methods, namely $K_1 = 0{\cdot}058$ (ref.[6]) and $0{\cdot}111\ l\ mol^{-1}\ s^{-1}$ (ref.[7]). We recalculated their results for the temperature of 25°C by using the dependence of $-\log k_1$ on $1000/T$ (Fig. 2) measured in this work; the activation energy for this reaction, $E_A = 19{\cdot}8$ kcal/mol, not in good agreement with that found in the literature[6], 24·5 kcal/mol, which substantiates the use of our temperature dependence. The extrapolated mean values of the catalytic constants were divided by 2 as required by Eq. (3) for the stationary state. For the aldolization of glyceraldehyde catalyzed by carbonate ions, the mean value of $K_1 = 4{\cdot}2\ .\ 10^{-4}\ l\ mol^{-1}\ s^{-1}$ found by us is four times larger than that found by extrapolation of the literature data[7], namely $1\ .\ 10^{-4}\ l\ mol^{-1}\ s^{-1}$.

The dihydroxyacetone aldolization constants cannot be compared with those of other authors[6,7] since they found this reaction to be of the first order in contrast to the present work where a second-order reaction is assumed. This is the largest discrepancy between our and previous work. However, in agreement with others we found that in mixtures of both trioses the aldolization rate becomes much higher that with the pure compounds. If the aldolization of dihydroxyacetone were of the first order, its rate in the triose mixtures could not be higher than with the pure triose. Since this is not the case, we were able to determine the value of $K_1 = 0{\cdot}24$ $l\ mol^{-1}\ s^{-1}$ for the first aldolization step of dihydroxyacetone by following this reaction in mixtures of both trioses. This value is in good agreement with those found by extrapolation to 25°C of the data in the literature, namely, $K_1 = 0{\cdot}12$ (ref.[6]) and $0{\cdot}25\ l\ mol^{-1}\ s^{-1}$ (ref.[7]).

Since the aldolization of trioses is represented by a system of competitive-consecutive reactions of the first and second order, the type of the base-catalyzed reaction is determined by the initial triose concentration. It can be concluded from the participation of the addition step in each aldolization that higher concentrations of the trioses are favourable for the aldolization, and lower concentrations for isomerization or dehydration. This applies, of course, also when the triose concentration decreases during the aldolization. Lowering its initial value leads therefore to increasing amounts of the side products.

Increasing temperature of the aldolization results in increasing amounts of the side products formed at the expense of the little stable dendroketose, apparently products of dehydration and condensation follow-up reactions.

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