STUDY OF REACTION KINETICS OF METHYLGLYOXAL IN ALKALINE MEDIUM

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The kinetics of aldolization and oxidation–reduction disproportionation of methylglyoxal as a system of two concurrent reactions was studied. In this system of base-catalyzed reactions, the disproportionation is of first and aldolization of second order with respect to methylglyoxal. The difference in reaction orders causes a dependence of the ratio of products on starting concentrations of methylglyoxal and base catalyst, *i.e.* NaOH or Na_2CO_3. A constant catalyst concentration, in spite of its consumption in neutralization of lactic acid formed by disproportionation of methylglyoxal, was maintained by a pH-stat. The rate constant and activation energies of the corresponding base-catalyzed reactions were determined. The reaction mechanism was partly elucidated by analysis of the reaction products and comparative measurements in D_2O.

The behaviour of methylglyoxal in alkaline medium was followed qualitatively by several authors[1–4]. Methylglyoxal is a frequent intermediate product in alkaline degradation of sugars, and the formation of "brown products" is attributed to its further chemical changes[5–7].

In our previous work we studied the kinetics of the formation of methylglyoxal by dehydration of trioses[8] as well as the kinetics and mechanism of its oxidation–reduction disproportionation to lactic acid[9] under conditions where other reactions practically do not proceed. Since it is known that besides the disproportionation also aldolization[1] of methylglyoxal can take place, it is of interest to determine quantitatively the mutual relation of both types of reactions in dependence on reaction conditions.

The polarographic determination of methylglyoxal[10–12] in combination with a pH-stat enables us to study experimentally the kinetics of the two concurrent reactions, aldolization and disproportionation of methylglyoxal.

EXPERIMENTAL

Apparatus and equipment. Kinetic measurements were performed in a closed thermostated titration vessel with magnetic mixing (Metrohm A.-G., Herisau), an accessory to an E 148c type compensator. Through openings with ground glass joints at the top of this vessel, the following parts were inserted: An alkali-resistant glass electrode, a calomel electrode (both from Metrohm), two-way valve with two inlet tubes for nitrogen, situated at different depths, enabling either bubbling of the solution or flow of nitrogen over the solution to maintain inert atmosphere, and finally an inlet from an automatic burette and a thermometer. The latter could be removed and the opening served to pour in a sample. The indicator electrodes were connected to a TTT 1 type titrator (Radiometer, Copenhagen), which controlled by means of a MNV 2 magnetic

valve a 2 ml automatic burette with a reservoir provided with a siphon filled with soda lime. The nitrogen was purified by bubbling through concentrated KOH solution to eliminate traces of CO_2, and a small overpressure of nitrogen was maintained in the measuring compartment. The titration vessel was thermostated at 25 ± 0·02°C (except for the temperature dependence) and the polarographic vessel (serving in the determination of quinoxaline) at 20 ± 0·02°C by a U 10 type thermostat (Prüfgeräte, Medingen/Dresden). Polarographic curves were recorded on an OH 102 Polarograph (Radelkis, Budapest) or on an LP 7 Polarograph (Laboratorní přístroje, Prague). Mass spectra were recorded on a MCH 1303 mass spectrometer (USSR), IR spectra on a Beckmann IR 5A apparatus, UV spectra on an ORD/UV-5 spectrophotometer (JASCO, Tokyo), and NMR spectra on a VS 487 B spectrometer (80 MHz) (Tesla, Prague). Preparative column chromatography was performed on a fraction separator of the type SF 62 (Mikrotechna, Prague).

Chemicals. Methylglyoxal was prepared by oxidation of acetone with selenium dioxide[13] or by dehydration of dihydroxyacetone by means of P_2O_5. The freshly prepared substance was immediately bound into the form of diisopropylacetal or was rapidly weighed and dissolved in D_2O. Stock solutions (mostly 0·2M) were prepared by hydrolysis of diisopropylacetal with diluted sulphuric acid. Their factor was adjusted by diluting to unity (for details see ref.[9]). 2-Methylquinoxaline was prepared by reaction of methylglyoxal with *o*-phenylenediamine in the medium of ethyl alcohol[14]. The *o*-phenylenediamine was redistilled and the chemicals for Britton–Welford phosphate buffers, for reaction media and analysis were of reagent grade (Lachema, Brno). Isobutylamine (reagent grade, Laborchemie, Apolda, GDR) was used in the preparation of amine buffers[10]. Chromatography was performed on a Whatman 1 paper with an acetone–butanol–water (8 : 1 : 1) system; a solution of KIO_4 and benzidine was used for detection[15]. Preparative column chromatography was carried out on a Whatman cellulose powder as well as on a microcrystalline cellulose[16] with the use of the same developing system. Thin-layer chromatography was performed on silica gel G (Merck, Darmstadt) with a butanol–ether–acetic acid–water developing system (4 : 5 : 2 : 1), detection was effected by 10% H_2SO_4 and heating[17]. All chromatographic solvents were redistilled on columns. Heavy water was furnished by the Institute for Research and Use of Radioisotopes, Prague.

Methods. In kinetic measurements, the air in the thermostated titration vessel was replaced by nitrogen, and a portion of the measured solution containing all components but methylglyoxal (*i.e.* base, KCl to adjust ionic strength, and water except for the portion contained in the methylglyoxal solution) was transferred in nitrogen atmosphere into the vessel. All solutions were prepared in nitrogen atmosphere to eliminate CO_2. Distilled water was boiled for a longer time and cooled under nitrogen. Carbonate-free alkali hydroxide was prepared by diluting its concentrated solution; 0·5 and 0·05 standard solutions were kept in automatic burettes with soda lime traps in nitrogen atmosphere.

The value of pH present on the pH-stat during titration was found from a preliminary experiment performed at equal conditions as the measurement proper. After inserting the indicator electrodes, alkali hydroxide inlet for pH-stating and a thermometer into the thermostated solution, the reaction was started by adding a neutralized methylglyoxal solution of the same temperature through the orifice for the thermometer and stopping the vessel by the thermometer. Samples for polarographic analysis were taken analogously at chosen time intervals and the consumption of 0·1M-NaOH for pH-stating was read off on the automatic burette. The sample volume was 0·1 – 5 ml according to the concentration of methylglyoxal. The reaction was stopped by lowering the value of pH to 7–8 by means of a buffer, diluting to $5 \cdot 10^{-4}$M and reacting with *o*-phenylenediamine (present in the buffer in a concentration of 0·01M). Methylglyoxal was determined polarographically after its condensation as 2-methylquinoxaline[10,11].

The behaviour of methylglyoxal was studied in carbonate-free NaOH, Na_2CO_3, and $NaHCO_3$.

The concentrations of the bases and methylglyoxal varied from 0·001 to 0·1M. The time courses of the reactions were followed at constant ionic strength $I = 0{\cdot}1$ in alkali hydroxide and $I = 0{\cdot}3$ in sodium carbonate; the concentration of one component was kept at 0·01M during a series of measurements while the other changed in the mentioned interval.

In the medium of heavy water (containing NaOH) the methylglyoxal was allowed to react to about one half and the remainder was after stopping the reaction (by neutralizing with HCl dissolved in D_2O) condensed with *o*-phenylenediamine to 2-methylquinoxaline. The latter was isolated by extracting into ether, dried over Na_2SO_4 overnight and distilled with water vapour, whereby it was separated from the remaining *o*-phenylenediamine. It was again isolated by extracting with ether, drying as above and distilling off the ether in vacuum without heating. A small volume was distilled in vacuum (b.p. 242°C at normal pressure[14]), whereby the 2-methylquinoxaline was isolated as a yellow liquid and stored in a sealed ampoule. Deuteration of its CH_3 group was followed by IR and mass spectroscopy, where the spectra of the deuterated compound were compared with those of the original one.

NMR spectra were recorded during the reaction of 0·5M methylglyoxal with 0·01M-NaOH in D_2O at 5°C; samples were cooled with liquid nitrogen. The reaction of 0·01M methylglyoxal and 0·01M-NaOH in water at 25°C was followed by UV spectroscopy.

The product of aldolization of methylglyoxal, isolated by preparative chromatography, was analyzed by mass spectroscopy, reduced with borohydride[18], and both the reduced and original compounds were analyzed by the method of oxidation with periodate[19]. Alternatively, a 0·01M solution of the aldolization product was dealdolized with 0·01M-NaOH (the relative molecular mass of HODA, hexane-4-ol-2,5-dione-1-al, served as reference) and the formation of lactic acid was followed from the decrease of the concentration of NaOH. The reactivity of the aldolization products toward *o*-phenylenediamine was also checked; the formation of quinoxaline derivatives was followed polarographically, they were isolated by the above-mentioned method, and we attempted to identify them by mass and IR spectroscopy.

Determination of rate constants. The conversions of methylglyoxal in alkaline medium can be due to disproportionation, aldolization, and dealdolization according to the schemes

$$\mathrm{A} \xrightarrow{k_1} \mathrm{B}; \quad 2\,\mathrm{A} \underset{k_2'}{\overset{k_2}{\rightleftharpoons}} \mathrm{C}.$$

These apply for the case of pH-stated reactions, where at constant base concentration the disproportionation and dealdolization are reactions of first order and aldolization of second order. Then A denotes methylglyoxal, B lactic acid, and C HODA. The rate of conversion of methylglyoxal is given by

$$\mathrm{d[A]}/\mathrm{d}t = -k_1[\mathrm{A}] - k_2[\mathrm{A}]^2 + k_2'[\mathrm{C}]\,. \qquad (1)$$

The solution of this equation would be very complicated[20], however it is considerably simplified if we assume that $\mathrm{d[C]}/\mathrm{d}t = k_2'[\mathrm{C}] = 0$, *i.e.* that the dealdolization can be neglected. Of several methods of solution of such a system of two concurrent reactions[21–23], we used that[23] which seems to be most convenient with respect to the possibility to follow the concentrations of methylglyoxal (polarographically) and acid (potentiometrically, from the consumption of NaOH during pH-stating). If we replace [A], [B] and [C] by $a - x$, y and z, where a denotes initial value of [A], x reacted amount of methylglyoxal at time t, then

$$y = \frac{k_1}{k_2} \ln \frac{k_1/k_2 + a}{k_1/k_2 + a - x}\,. \qquad (2)$$

The ratio k_1/k_2 can be determined from the slope of the tangent drawn to the curve $y = f(x)$ in the origin of coordinates:

$$k_2/k_1 = 1/a \operatorname{tg} \alpha - 1/a . \qquad (3)$$

By using the function $x = f(t)$ and integrating the expression for dx/dt we obtain

$$k_1 = \left[\frac{1}{t} \ln \frac{a}{a - x} - \ln \frac{k_1/k_2 + a}{k_1/k_2 + a - x}\right]. \qquad (4)$$

From the plot of the measured concentration of lactic acid against concentration drop of methylglyoxal the ratio k_2/k_1 can be found by using Eq. (3), and eventually the rate constants k_1 and k_2 can be evaluated by using Eqs (3) and (4).

In the case where, depending on reaction conditions ($y \approx x$, $y \gg z$), the disproportionation is preferred, the aldolization can be neglected against it. Then the value of k_1 can be calculated on the basis of the equation of first order from polarographic and potentiometric data. If $x \gg y$, then also $z \gg y$ and the disproportionation can be neglected; k_2 for aldolization can be calculated from polarographic data by the equation of second order for the case $a = b$, $2\,A \xrightarrow{k_2} C$. The consumption during potentiometric pH-stating was zero or negligible. When the values of x and y were comparable, the rate constants were evaluated by the mentioned method of solving the concurrent reaction system.

Besides using Eq. (3) it is possible to determine the ratio k_1/k_2 by comparing the corresponding average values of catalytic constants obtained from equations for first- and second-order reactions if only disproportionation or only aldolization proceeds. To this purpose the calculated rate constants are divided by the corresponding concentrations of OH^- ions. The mentioned ratio is then introduced into Eq. (4) and the rate constants for all other concentrations can be calculated.

RESULTS

The formation of aldolization products was followed by paper chromatography at 25°C at various concentrations of reactants. In 0·01M-NaOH with concentration of methylglyoxal $c = 0{\cdot}001 - 0{\cdot}5$M, the formation of aldolization products was observed at $c \geqq 0{\cdot}01$M. At constant $c = 0{\cdot}01$M, these products were detectable chromatographically if the concentration of NaOH did not exceed 0·05M; with equal concentrations of both reactants, at 0·01M and higher. In solutions of Na_2CO_3 at the same concentration conditions, the aldolization products were formed in all experiments. Similarly in carbonate buffers at the same concentration of Na_2CO_3. In solutions of $NaHCO_3$, both reactions under study were observed neither chromatographically not polarographically during three days. Two chromatographic spots were indentified, R_{F1} 0·617 and R_{F2} 0·805, the substance in the first spot being present in about a five fold excess against that in the second. The aldolization products of 0·05M methylglyoxal were kept for eight days in 0·05M-NaOH at ambient temperature without a measurable change in the hydroxide concentration.

The reaction order of the oxidation–reduction disproportionation was found[9] to be equal to one. The reaction order of aldolization was determined from the reac-

TABLE I

Rate Constants, k_1, k_2, and Catalytic Constants of OH^- Ions, K_1, K_2, for Oxidation–Reduction Disproportionation and Aldolization, Respectively, of Methylglyoxal in Solutions of Na_2CO_3 $I = 0{\cdot}3$, 25°C.

M-Na_2CO_3 resp. M-CH_3.CO.COH	pH	$k_1 \cdot 10^5$ s^{-1}	$k_2 \cdot 10^2$ $l\,mol^{-1}\,s^{-1}$	$K_1 \cdot 10$ $l\,mol^{-1}\,s^{-1}$	K_2 $l^2\,mol^{-2}\,s^{-1}$
0·1	11·15	15·6	5·9	1·09	42·0
0·05	11·1	11·8	4·54	0·94	36·0
0·01	10·6	4·2	1·6	1·05	40·3
0·002	10·8	7·2	2·7	1·14	43·7
0·001	10·65	4·9	2·18	1·16	42·1
		0·01M-Na_2CO_3			
0·1	10·68	4·54	1·74	0·95	36·4
0·05	10·45	9·43	3·61	1·06	40·5
0·01	10·92	9·52	3·64	1·15	43·9
0·002	10·96	9·77	3·74	1·08	41·1
0·001	10·9	7·43	2·86	0·94	35·9

TABLE II

Rate Constants, k_1, k_2, and Catalytic Constants of OH^- Ions, K_1, K_2, for Oxidation–Reduction Disproportionation and Aldolization, Respectively, of Methylglyoxal in Solutions of 0·01M-NaOH $I = 0{\cdot}1$, 25°C.

M-CH_3.CO.COH resp. M-NaOH	pH	$k_1 \cdot 10^4$ s^{-1}	$k_2 \cdot 10^2$ $l\,mol^{-1}\,s^{-1}$	$K_1 \cdot 10$ $l\,mol^{-1}\,s^{-1}$	K_2 $l^2\,mol^{-2}\,s^{-1}$
0·1	12·10	—	51·0	—	40·5
0·05	11·55	—	13·9	—	39·1
0·01	11·64	—	17·1	—	39·2
0·005	11·60	3·84	14·7	0·96	36·9
0·002	11·91	8·07	—	1·00	—
0·001	11·80	6·68	—	1·06	—
		0·01M-CH_3COCOH			
0·01	11·65	—	17·4	—	37·8
0·005	11·55	—	12·6	—	35·5
0·002	11·2	1·42	5·32	0·90	34·3
0·001	11·0	1·0	3·83	1·0	38·3

tion half times, $\tau_{1/2} = 180$ and 370 s for 0·1 and 0·05M methylglyoxal in 0·01M-NaOH, as $n = 1{\cdot}96 \approx 2$. The kinetic measurements in the media of Na_2CO_3 and NaOH are summarized in Tables I and II. The average values of the catalytic constants of OH^- ions in the medium of Na_2CO_3 are $K_1 = 0{\cdot}105\ l\ mol^{-1}\ s^{-1}$ for the disproportionation and $K_2 = 40{\cdot}2\ l^2\ mol^{-2}\ s^{-1}$ for the aldolization. The corresponding values for the medium of NaOH are $K_1 = 0{\cdot}098\ l\ mol^{-1}\ s^{-1}$ and $K_2 = 37{\cdot}7\ l^2\ .\ mol^{-2}\ s^{-1}$; the mean error does not exceed 11%.

The ratio of the rate constants, k_2/k_1, was calculated from Eq. (3) for every measured time dependence. These values were scattered in the interval 200–450 owing to inaccuracy in drawing a tangent in the origin to the experimental curve, and the constants k_1 and k_2 showed a large scatter as well. Therefore, our calculations are based on the ratio of the catalytic constants of OH^- ions, $K_2/K_1 = 383$, calculated from the first- and second-order kinetic equations under conditions where either disproportionation or aldolization is preferred. The dependences of the rate constants of both reactions on temperature are shown in Fig. 1. The values for aldolization were derived from the reaction of 0·01M methylglyoxal with 0·005M-NaOH, for disproportionation from the reaction of 0·001M methylglyoxal with 0·01M-NaOH; the corresponding activation energies, E_A, were calculated from the Arrhenius equation as 20·1 and 14·8 kcal/mol, respectively.

2-Methylquinoxaline, isolated after conversion of methylglyoxal in the medium of NaOH in D_2O, did not contain deuterium in its molecule. Its IR spectrum showed

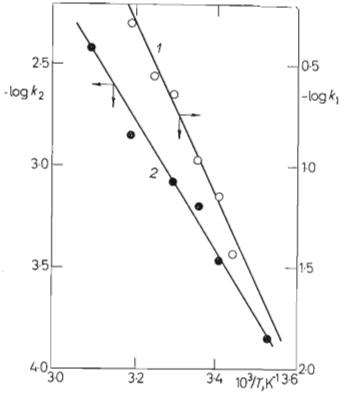

FIG. 1

Determination of Activation Energies for Oxidation–Reduction Disproportionation, *1*, and Aldolization, *2*, of Methylglyoxal

an absorption band, $\nu(CH_3—)$, at 2870 cm^{-1}, but no absorption band, $\nu(CD_3—)$, at 2250 cm^{-1}, and its mass spectrum confirmed the absence of deuterium by the value of molecular peak (m/e 144).

In the medium of D_2O, methylglyoxal gives a NMR spectrum with signals 5·48δ (H) and 1·58δ ($CH_3—$), the intensity of which decreases during conversion of this compound with NaOH (followed during three hours). Polarography showed that the decomposition was completed after 90 min. After 3 h the temperature was raised from 5 to 25°C and the changes in the spectrum were observed even after 21 h. — The UV spectrum during conversion of 0·01M methylglyoxal with 0·01M-NaOH (Fig. 2) showed a shift of absorption from λ_{max} 282 nm for the pure substance to 295 nm with a simultaneous 17-fold increase of absorption (after 4 h). Already after 15 min a new absorption band at 267 nm began to appear. After one day the absorption intensity reached the value of the preceding maximum and at the same time that at λ_{max} 295 nm became weaker until after three days it disappeared. Afterwards the spectrum did not change. Polarography showed that the methylglyoxal was completely decomposed under these conditions after an hour. During the aldolization in alkaline medium the solution first turns yellow, then brown, and on acidifying becomes almost clear. The colour change is reversible.

By following the reaction course of 0·01M methylglyoxal in a phosphate buffer (pH 12) polarographically, no anodic wave was obtained even with the use of a Kalousek commutator although the experimental conditions were favourable for aldolization. The same negative result was obtained in an acetate buffer (pH 4·6). When the reaction products after 15 min reaction were transferred into 0·5M-H_2SO_4, even after 90 min boiling under reflux no cathodic wave corresponding to fural derivative could be obtained.

The aldolization product obtained by preparative chromatography is a yellow-brown sirup, difficultly soluble in water but well in ethyl alcohol. Its mass spectrum shows the highest peak of $m/e = 279$, higher than the molecular mass of the monomer of HODA. Its IR spectrum does not contain the absorption band of the free CO group. A red-brown quinoxaline derivative is formed by its condensation reaction with an equivalent of *o*-phenylenediamine at 25°C for 3 h. This product gives three polarographic cathodic waves in the medium of 0·3M isobutylamine buffer with $E_{1/2} = -1·01$, $-1·53$ and $-1·74$ V (S.C.E.). The two more negative ones decrease with time until after three days they almost disappear. HODA gives in the same medium only two waves at $-1·53$ and $-1·74$ V attaining in total about 1/6 of the wave height of 2-methylquinoxaline corresponding to the original concentration of methylglyoxal. The more positive wave of the quinoxaline derivative of HODA attains one half of this value and its $E_{1/2}$ value is more negative than that of 2-methylquinoxaline ($-0·99$ V against S.C.E.). It does not change with time.

The preparative isolation of the quinoxaline derivative of HODA is difficult. The product is less extractable into ether than 2-methylquinoxaline and during

distillation with water vapour it decomposes to give *o*-phenylenediamine and an aliphatic residue. This was confirmed by thin-layer chromatography using 2-methylquinoxaline (R_F 0·74) and *o*-phenylenediamine (R_F 0·54) as standards. The organic residue formed a blue spot (R_F 0·96). During an analogous analysis of quinoxaline derivatives of the aldolization products, three spots were obtained (R_F 0·6, 0·78 and 0·86) whose colours, yellow, orange and orange, changed to light red, brown and brown on heating. The ratio of their intensities changed according to the way of treatment. From the isolated quinoxaline derivative of HODA, a precipitate separated after some time, soluble in alcohol but insoluble in water. The alcoholic solution yielded after evaporation a liquid, from which again a precipitate separated after some time. IR spectra showed characteristic quinoxaline bands. An absorption band of the free CO group, $\nu(CO) = 1715\ cm^{-1}$, was observed only in the spectrum of the precipitate of the quinoxaline product obtained by the KBr technique. The spectrum of the liquid portion was obtained as a liquid film. The mass spectrum showed the highest peak of $m/e = 352$ whereas the assumed molecular mass of the monomeric quinoxaline derivative is 216. A portion of the isolated aldolization product was reduced with borohydride and the original as well as the reduced samples were oxidized with periodate. The amount of formaldehyde determined by dimedone corresponded to one mole per two mol of HODA.

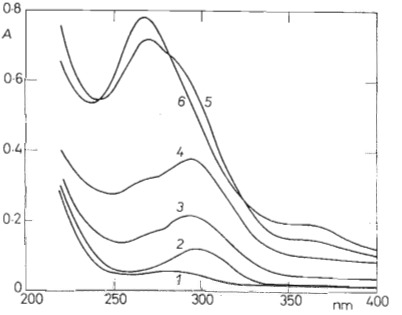

FIG. 2

Conversion of 0·01M Methylglyoxal in 0·01M-NaOH at Ambient Temperature Followed by UV Spectrophotometry

0·0025M Methylglyoxal; time: *1* 0 min; *2* 1 min; *3* 1 h; *4* 4 h; *5* 24 h; *6* 72 h.

DISCUSSION

The kinetic studies showed that aldolization of methylglyoxal at constant base concentration maintained by pH-stating is a reaction of second order with respect to methylglyoxal. In accord with the general aldolization mechanism[24-26] it can be assumed that the rate-determining step is interaction of an anion formed in the first reaction step with free methylglyoxal, which is in equilibrium with its hydrated form. The assumed enolic character of this anion[27] was not confirmed; the unreacted methylglyoxal isolated from the reaction mixture in D_2O + NaOH in the form of 2-methylquinoxaline contained no deuterium. Since small amounts of bound deuterium (under 5%) cannot be detected by the method used we assume that the rates of the first and second reaction step are comparable with each other. Lowering of the rate of the second step by decreasing concentration of methylglyoxal, which would perhaps enable the determination of a small amount of bound deuterium[28], is favourable for increasing the role of disproportionation. The independence of the rate of aldolization of Na_2CO_3 and $NaHCO_3$ concentrations and the good agreement between the average catalytic constants of OH^- ions in the media of NaOH and Na_2CO_3 are evidence for the dominant role of the specific acid–base catalysis. The small dependence of the rate constants on the ionic strength implies that the rate-determining step is an interaction between an ion and a dipolar molecule.

The method of automatic pH-stating proved very useful in the study of concurrent reactions in which one of the reaction products neutralizes the catalytically active acid or base. It enables to maintain a constant catalyst concentration and at the same time to determine analytically one reaction product. When it is possible to control the experimental conditions so that only the aldolization or only the oxidation–reduction disproportionation proceeds, it is more appropriate to calculate the ratio of the constants of the reactions from both extreme cases. In the method of the tangent drawn in the origin of the plot of the product concentration against the decrease in concentration of the starting substance, even small errors exert a large influence on the position of the tangent and hence on the mentioned ratio.

The oxidation–reduction disproportionation makes the existence of the free form of methylglyoxal in alkaline media impossible since the rate of its decomposition is greater than the rate of its formation by dehydration as follows by comparing the corresponding constants[8,9]. This applies even more for aldolization, for which the constant is about 400 times greater than for disproportionation. The higher activation energy for aldolization can be attributed to a greater role of space orientation of the reactants during bonding of the anion to the dipolar molecule[29].

It follows from the kinetic studies that increasing the concentration of methylglyoxal (other conditions being constant) causes aldolization in accord with the dependence of the half time of a second-order reaction on the concentration of the starting substance. Increasing the base concentration accelerates both reactions.

Rise of temperature favours aldolization in accord with its higher activation energy.

The study of the aldolization and its products by UV, IR, NMR and mass spectroscopy showed that the conversion of methylglyoxal in alkaline medium does not end by the formation of HODA. The HODA molecules react with one another. Their dimerization seems to be most probable as follows from the mass spectra and from the amount of formaldehyde evolved during oxidation of the reduced aldolization products with periodate. The formation of new maxima as well as the considerable increase of the intensity of UV absorption bands are an evidence for the replacement of the weakly absorbing CO groups most probably by a conjugated double-bond system. The reactions following after the aldolization require a further study.

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