

# MECHANISTIC STUDY OF THE ALDOL CONDENSATION OCCURRING IN THE ALKALINE SOLUTION OF 3-HYDROXY 2-OXO PROPRANOATE ( $\beta$ HYDROXYPYRUVATE)

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**Abstract**—With 3-bromo-2-oxopropanoate ( $\beta$  bromopyruvate) and its ethyl ester, the ionisation of the gemdiol of the hydrated form  $\text{BrCH}_2\text{-C}(\text{OH})_2\text{-COOR}$  **1** initiates the elimination of bromide anion yielding 3-hydroxy-2-oxopropanoate **2** ( $\beta$ -hydroxypyruvate). The mechanism of the reaction was investigated essentially by polarography in aqueous solution.

In neutral (and acid) media, the polarographic behaviour of **2** resembled that of other  $\alpha$ -ketoacids: reduction at the mercury electrode yielded glycerate.

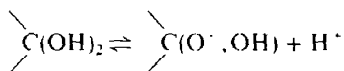
In alkaline media, there was evidence of the carbanion enolate  $\text{O}=\text{C}=\text{C}(\text{OH})\text{-COO}^-$ , **3'**. The overall rate constant was determined according to a kinetic law of the type:  $v = k_1[\text{2}][\text{OH}^-]$ ; found  $k = 1.56 \text{ min}^{-1}$  in NaOH 0.5N at 25°.

In the pH range 10.5 to 11.5, **3'** existed in minor amounts and initiated a slow aldol condensation with the tautomer 3-oxo-2-hydroxypropanoate **4** (tartronate semialdehyde) according to a kinetic law of the type  $v = \bar{k}[\text{2}]^2[\text{OH}^-]$  found  $\bar{k} = 211 \text{ mol}^{-1} \text{ min}^{-1}$  at 25° at pH 11.0.

The aldol product was isolated as a sodium salt and its structure established by  $^{13}\text{C}$  NMR.

The formation of stable hydrates is well known for ketones containing several electronegative groups. In previous papers we investigated two polyketones: leuconic acid<sup>2</sup> and ninhydrin.<sup>3</sup>

The pK values of their corresponding gemdiol-gemol-olate equilibria:



were determined by means of pH and found equal to 7.5 and 8.0 respectively. In these cases, ionisation of the OH group was followed by the opening of the polyoxo ring. The effect of this ring-opening reaction could be minimised by working for short periods and at low temperature.

This paper deals with the case of 3-bromo-2-oxopropanoic acid ( $\beta$ -bromopyruvic acid) and its ethyl ester for which there is polarographic evidence for the formation of the hydrate  $\text{BrCH}_2\text{-C}(\text{OH})_2\text{-CO}_2\text{R}$  in aqueous solvents.

The ionisation of the gemdiol group initiates two kinds of reactions: the elimination of bromide (leaving group) which predominates and which yields 3-hydroxy-2-oxopropanoic acid ( $\beta$ -hydroxypyruvic acid), in the pH range 7-10, and the formation of a carbanion enolate in more basic solutions: pH > 12. In the pH range 10.5-11.5, the carbanion enolate initiated a slow aldol condensation.

The reactivity of hydroxypyruvate may prove of biological interest: an important role was attributed to hydroxypyruvate in the enzymatic glycolysis in animal tissues. An attempt has been made in this paper to carry out the investigation of the asymmetric aldol condensation.

## EXPERIMENTAL

Measurements of concentrations of the studied compounds were essentially carried out by UV spectrometry and polarography: 3-bromo-2-oxopropanoate,  $\text{BrCH}_2\text{-CO-COO}^-$ , gives a cathodic wave corresponding to the hydrogenolysis of the C-Br bond.<sup>4</sup> Bromide ions give an anodic wave corresponding to the formation of HgBr at the dropping mercury electrode (d.m.e.). 3-hydroxy-2-oxopropanoate,  $\text{HOCH}_2\text{-CO-COO}^-$ , gives a cathodic wave yielding glycerate.

**Equipment.** The polarograms were recorded using a three electrode Tacussel polarograph type PRG 5. The characteristics of the capillary are as follows:  $m = 6.4 \text{ mg}$ ;  $t = 3.0 \text{ s}$ , mercury head  $h = 60 \text{ cm}$ ;  $m^2 \cdot s^{-1} \cdot \text{cm}^{-2} = 4.15$ .

Controlled potential electrolysis was carried out with a mercury pool electrode in a standard three electrode arrangement. The anodic and cathodic compartments were concentric and separated by sintered glass of porosity grade 4. The voltage source consisted of a Tacussel potentiostat type PRT 20/2. A Tacussel IG 5N integrating electronic milliammeter and voltameter were included into the electrolytic circuit. The electrolytic cell was thermostated and sealed.

pH values were determined by means of a Tacussel TS70N1 pH meter.

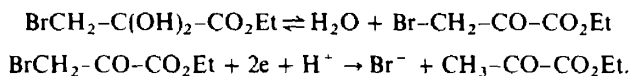
UV spectra were recorded using a Beckman Acta III spectrophotometer.

$^{13}\text{C}$ NMR spectra were recorded on a Varian XL 100 spectrometer operating at 25.2 MHz. The Na salt was dissolved in water or aqueous alkaline soln; a small amount

## RESULTS AND DISCUSSION

### I. Pathway for the elimination of bromide in slightly alkaline solutions of 1

In aqueous acid solution, ethyl  $\beta$ -bromopyruvate 1 is reduced<sup>4</sup> according to a  $2\text{F}\cdot\text{mol}^{-1}$  process:



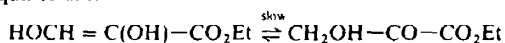
of  $\text{D}_2\text{O}$  was added to provide a lock signal. Proton broadband decoupled, proton off resonance decoupled and uncoupled spectra were used for assignment of resonances. Dioxane was used as an internal standard. Chemical shifts were referenced to TMS

$$\delta_{\text{TMS}} = \delta_{\text{dioxane}} + 67.4 \text{ ppm}$$

**Chemicals.** 3-bromo-2-oxopropanoic acid and its ethyl ester were Schuchart products and were used as starting materials.

The ethanolic soln of ethyl 2,3-dihydroxypropenoate 3 (ethyl dihydroxyacrylate  $\text{HOCH}=\text{C(OH)-CO}_2\text{Et}$ ) was synthesised according to the lit.<sup>5</sup>

In acid media the polarographic wave and the absorption bands of the enol form decreased with increasing time till the soln gave mostly the polarographic behaviour of the keto form ( $E_{1/2}$  mV.e.c.s. = -560-62 pH). This evolution corresponded to the displacement of the following equilibrium:



towards the right hand side. At 20' after 3 days, the height of the wave indicated that only 5% of the enol form was present in the bulk. Then the soln was used as a soln of ethylhydroxypyruvate.

Ethylhydroxypyruvate could also be prepared by dilution of ethyl  $\beta$ -bromopyruvate in slightly alkaline buffered soln as described in this paper (yield  $\approx 90\%$ ).

**Preparation of the sodium salt of the bicondensed product 6.** 3-Bromo-2-oxopropanoic acid (20 mmoles) were dissolved in 30 ml 2 M NaOH deaerated over 30 min by bubbling  $\text{N}_2$ . The soln showed a pH value of approximately 13.0 and was held 1 hr at 25'. (A more prolonged contact would cause a slow spontaneous decarboxylation accompanied by a drop in pH), and then acidified with glacial AcOH (2 ml). The pH of the acidified soln was roughly 4.0.

After addition of water (10 ml) the soln was made up to 200 ml with MeOH and was allowed to remain at 5 overnight. The white ppt was filtered off, washed with MeOH and dried *in vacuo* over  $\text{P}_2\text{O}_5$  for 48 hr. The product weighed 2.101 mg (yield = 73%).

**Procedures.** As supporting electrolytes, solns of 0.5 M  $\text{HSO}_4$  and sulfate were used at pH lower than 2. Mac Ilvaine buffers in the pH range 2-8, carbonate buffers 0.5 M in the pH range 9-11.

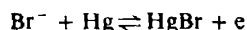
The 0.01 M stock solns of  $\alpha$  ketoester or  $\alpha$ -ketoacid studied were diluted at 20' in aqueous supporting electrolyte in the ratio 1/10 unless otherwise specified. Solns were deaerated with  $\text{N}_2$  and polarographic curves recorded within 10 min.

In the pH range 7.5-8.5, the cathodic current decreased with increasing time. However attempts to follow this decrease failed for two reasons:

(a) The height of the wave never reached more than 10% of the expected value for a two electron diffusion controlled process and did not provide suitable means of concentration measurement.

(b) The half-wave potential ( $E_{1/2}$  mV.e.c.s. = -100) was close to the reduction potential of oxygen. In order to get rid of the latter it would be necessary to deaerate the solution for more than 10 min.

We found it was easier to follow the course of the reaction (see the under-mechanism) by measuring the increase of the anodic current due to the reaction:



obtained by dilution of aliquots of the studied solution in an appropriate buffer (at pH 4.5 the oxidation wave was well defined).

Then the plot of  $\lg [(C_{\text{Br}^-})_x - (C_{\text{Br}^-})]$  vs time showed that the elimination of bromide proceeded according to a first order reaction with respect to bromide concentration. Values of the rate constant  $k$  at various pH are listed on Table 1.

Data given in Table 1 refer to solutions for which hydrolysis of the ester group was negligible. It appeared that  $k$  did not depend on the buffer concentration.

The obtained solution finally had the same characteristics as a solution in the same medium of  $\text{CH}_2\text{OH-CO-CO}_2\text{Et}$  synthesised as described in the Experimental.

These data indicated that the main reaction was the elimination of bromide according to the equation:



the independence of  $k$  upon the buffer concentration showed the occurrence of a basic catalysis related to the deprotonation of the gem-diol group.

In order to verify this assumption 20 ml of a  $5 \cdot 10^{-2}$  M solution of ethyl  $\beta$ -bromopyruvate were titrated against a solution of  $10^{-2}$  M NaOH. At 25° the pH value rapidly decreased with increasing time after

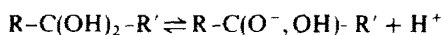
Table 1. Values of the rate constant  $k$  in neutral and mildly basic media at 20°  $v = d[\text{Br}^-]/dt = k \cdot [\text{BrCH}_2\text{-C(OH)}_2\text{-CO}_2\text{Et}]$

pH	7.30	7.45	7.65†	7.80	8.10	8.20
$k \text{ min}^{-1}$	0.017	0.024	0.052	0.068	0.11	0.12

†Buffer diluted in ratio 1:5

each addition of dilute NaOH. This phenomenon could be minimised by working for short periods and at 0°. Then the plot (extrapolated to zero time) of values of pH vs the amount of NaOH showed a pK value equal to 9.6.

For equilibria of the type:



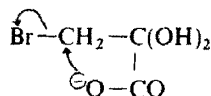
The correlation  $pK = 14.19 - 1.315 (\sigma_R^* + \sigma_{R'}^*)$  has been proposed<sup>6</sup> where  $\sigma_R^*$  and  $\sigma_{R'}^*$  are Taft's coefficients.  $\sigma_{CH_2, Br}^* = 1.03$  and  $\sigma_{CO_2Et}^* = 2.00$  gave  $pK = 10.2$ , value which is in rough agreement with the experimental result:  $pK = 9.6$ .

All the preceding results may be justified by the following reaction scheme:

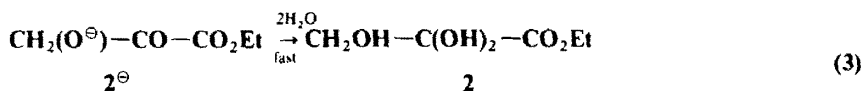
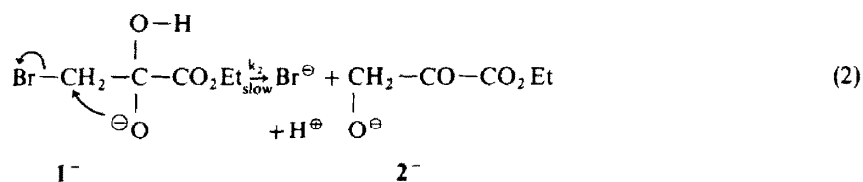
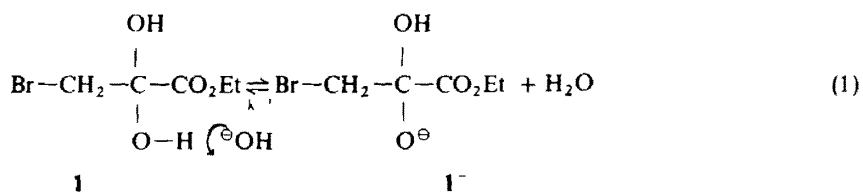
is likely to be higher than the one corresponding to the ester and therefore it could not be measured by means of pH. This observation results from the weakness of the electronegative effect of the carboxylate group compared with the ester group ( $\sigma_{COO}^* = 0.80$  gives  $pK = 11.8$ ).

Remarks

(1) Another scheme might be possible:



In this eventuality bromide would be eliminated in the whole range of pH greater than pK of the carboxylic



Applying to **1** the steady state approximation we found:

$$v = \frac{d[\text{Br}^-]}{dt} = \frac{k_1 \cdot k_2 \cdot [\mathbf{1}] \cdot [\text{OH}^-]}{k_{-1} + k_2}$$

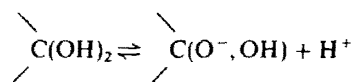
As reaction (2) is the rate determining step, we may assume that  $k_2$  is negligible compared with  $k_{-1}$  and then, as  $k_1/k_{-1} = K_1/K_e = 3.10^{-10}/10^{-14} = 3.10^4$ : at pH 8 and 25°,

$$v = 3.10^4 \cdot k_2 \cdot [\mathbf{1}] \cdot 10^{-6} = 3.10^{-2} \cdot k_2 \cdot [\mathbf{1}]$$

measured:  $v = 0.10 \cdot [\mathbf{1}]$ ; hence  $k_2 = 3.3 \text{ min}^{-1}$ .

Kinetic data obtained with the carboxylate species  $\text{BrCH}_2-\text{C}(\text{OH})_2-\text{CO}_2^-$  **2** under similar conditions are listed in Table 2.

The behaviour of  $\beta$ -bromopyruvate resembled its ester but the pK value of the equilibrium



group. This hypothesis is not in agreement with the observed results.

Finally when an aqueous solution of **1** is diluted in a slightly basic solution, the ionisation of the gem-diol group initiates the production of **2**. The yield is roughly 90%.

(2) Occurrence of a minor competitive reaction. After elimination of bromide, the solution showed an absorption band at 282 nm which cannot be attributed to **2** but to a product of a minor reaction.

The plot of  $\log(A_\infty - A_t)$  at 282 nm vs time was roughly a straight line whose slope gave a rate constant  $k'$ . However the kinetics were not exactly first order with respect to **1** since:

(a) The plot of  $\log t_{1/2}$  versus  $\log C$  at pH 7.9 is a straight line whose slope is equal to 0.3.

(b) The final apparent molar extinction at 282 nm increased with increasing concentration from  $\epsilon = 3001 \cdot \text{mol}^{-1} \text{ cm}^{-1}$  ( $c = 10^{-3} \text{ M}$ ) to  $\epsilon = 1.001 \cdot \text{mol}^{-1} \text{ cm}^{-1}$  ( $c = 10^{-2} \text{ M}$ ).

Table 2. Values of the rate constant  $k$  in neutral and mildly basic media at 20°C.  $v = d[\text{Br}^-]/dt = k \cdot [\text{BrCH}_2-\text{C}(\text{OH})_2-\text{CO}_2^-]$

pH	8.20	8.25	8.75	8.80	9.00†	9.10
$k \text{ min}^{-1}$	0.008	0.0135	0.025	0.037	0.048	0.08

† Buffer diluted in ratio 1:5

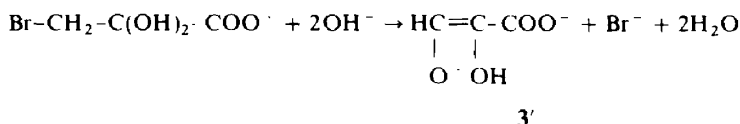
Data summarised in Table 3 agree with a kinetic law of the type:

$$v = \frac{dA_{282\text{nm}}}{dt} = k' \cdot [\text{BrCH}_2\text{-C(OH)}_2\text{-CO}_2\text{Et}]^{1.3} \cdot [\text{OH}^-]^{0.7}$$

and suggest the occurrence of a ketol condensation.

## II. Occurrence of an acid base/dehydration reaction in alkaline solution of hydroxypyruvate 2

*Polarographic behaviour of the  $\beta$ -hydroxypyruvate (and its ester).* At the mercury electrode in acid and

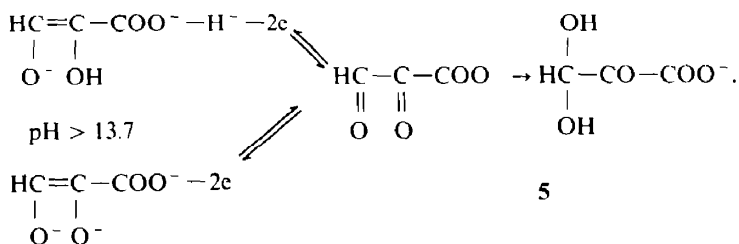


neutral media, the reduction of hydroxypyruvic acid resembles the enzyme catalysed reduction and yields glycerate by a  $2\text{F} \cdot \text{mol}^{-1}$  process; i.e. the cleavage of the C-OH bond, in  $\text{HOCH}_2\text{-CO-COOR}$  does not occur as in the case of  $\text{HOCH}_2\text{-CO-C}_6\text{H}_5$ <sup>7</sup> or in the case of  $\text{HOCH}_2\text{-CO-CH}_3$ .<sup>8</sup>

The plot of the polarographic intensity as a function of pH resembles those of other  $\alpha$ -ketoacids.<sup>9</sup>

However the polarographic cathodic current decreased with increasing time in slightly alkaline media in the pH range 10–12. This evolution was stopped by acidification of the solution. This observation may be explained by the occurrence of an aldol condensation whose rate increased with increasing pH. On the other hand, in alkaline media at pH greater than 12, an anodic wave appeared while the cathodic wave disappeared.

pH < 13.7



The value of the half-wave potential of the anodic wave ( $E_{1,2} = -550\text{ mV}$  in NaOH 0.5 N) indicates a highly oxidisable compound (with the same background, the half-wave potential of ascorbate would be roughly 200 mV more positive).

Simultaneously the alkaline solution exhibited an absorption band at 270 nm:  $\epsilon = 10,000\text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ .

At equilibrium a plot of the anodic current intensity on the one hand and of A 270 nm on the other hand, vs pH took the form of a dissociation curve the point of inflexion being situated at pH 11.6 in dilute NaOH (ionic strength  $I = 1$ ), pH 12.0 in phosphate buffered solution.

With  $\beta$ -bromopyruvate as starting material, the logarithmic analysis of the dissociation curve indicated that two ions  $\text{OH}^-$  were implied in the net reaction generating dihydroxyacrylate 3':

From these experiments, it can be deduced that the first  $\text{OH}^-$  ion was involved in the C-Br bond cleavage, and the second one in the carbanion enolate formation.

In agreement with usual assumptions, the carbanion enolate 3' was not reduced but oxidised at the dropping mercury electrode (d.m.e.). The oxidation constituted an irreversible  $2\text{F} \cdot \text{mol}^{-1}$  process owing to the follow-up hydration of the oxidised product, the mesoxalic semialdehyde 5 generated at the d.m.e.

In dilute NaOH (ionic strength  $I = 1$ ), the half-wave potential ( $E_{1,2}$ ) of oxidation of 3' was shifted to more negative potentials with increasing pH. The  $E_{1,2}$  pH plot indicated, in the pH range smaller than 13.7, a slope of 35 mV/pH, but no change with increasing pH at pH greater than 13.7: so it can be concluded that the oxidation was operating:

The results are in agreement with a pK value of 13.7 for the acid-base equilibrium:

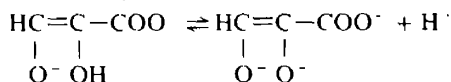


Table 3. Values of the rate constant  $k'$  in mildly basic media at 20°C.  $v = dA_{282\text{nm}}/dt = k' \cdot [\text{BrCH}_2\text{-C(OH)}_2\text{-CO}_2\text{Et}]$

pH	7.7	7.7†	7.9†	7.9	7.9‡	8.0
$k' \text{ min}^{-1}$	0.09	0.12	0.078	0.13	0.16	0.18
pH	8.1†	8.4				
$k' \text{ min}^{-1}$	0.11	0.26				

†  $[\text{CH}_2\text{Br-C(OH)}_2\text{-CO}_2\text{Et}] = 10^{-3}\text{ M}$ .  
 ‡  $[\text{CH}_2\text{Br-C(OH)}_2\text{-CO}_2\text{Et}] = 5 \cdot 10^{-3}\text{ M}$ .  
 §  $[\text{CH}_2\text{Br-C(OH)}_2\text{-CO}_2\text{Et}] = 10^{-2}\text{ M}$ .  
 in hydroethanolic (20%) solution.

he mesoxalate semialdehyde **5** was prepared by controlled potential electrolysis at the mercury pool electrode and isolated as disemicarbazone.

II. Kinetic investigations of the formation of the carbanion-enolate **3'**

Tables 4 and 5 summarise the kinetic data obtained in dilute aqueous NaOH (I = 0.5) and in Britton Robinson buffered solutions.

In dilute NaOH, the reaction was first order with respect to **1** and identical values were found for the rate constant *k* by measuring with increasing time:

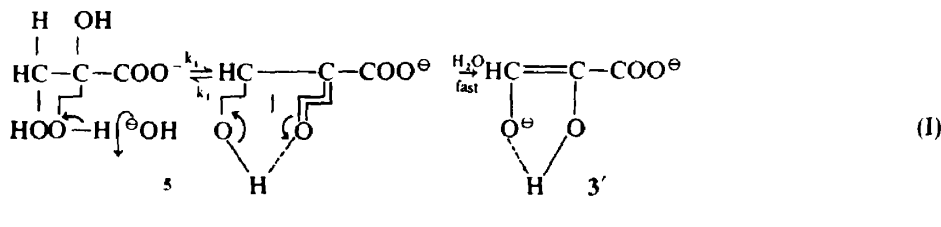
- (a) The decrease of the cathodic current *i<sub>c</sub>*;
- (b) The increase of the anodic current *i<sub>a</sub>*;
- (c) The increase of the UV extinction *A<sub>270nm</sub>*.

$$v = \frac{di_c}{dt} = \frac{di_a}{dt} = \frac{dA_{270}}{dt} = k \cdot [1]$$

Plot of log *k* vs pH showed kinetic of the type:

$$v = k \cdot [1] \cdot [OH^-]^{0.9}$$

in good agreement with the mechanisms (I) =



In phosphate buffered solutions, in the pH range 11.0–12.5, the experimental data are in agreement with the above kinetic law but the calculated values of *k* are greater than these obtained in dilute NaOH: in 0.01 N (I = 0.5) NaOH, log *k* = -1.05; in phosphate pH 12.0 buffered solution, log *k* = -0.60. It seems reasonable to consider this result as caused by the replacement of OH<sup>-</sup> by A<sup>-</sup> in the acid-base dehydration reaction (I). This hypothesis is supported by the dependence of the values of *k* on the nature and concentration of the buffer species.

IV. Occurrence of an oxo-enol equilibrium in acidic media

When a solution of carbanion enolate **3'** in 0.1 M NaOH is rapidly acidified, at 5°, with buffered solution at pH smaller than 2.0, a UV band appears at 250 nm ( $\epsilon_0 = 7,600 \text{ l. mol}^{-1} \text{ cm}^{-1}$  extrapolated value to *t* = 0). The absorbance decreases with increasing time until it reaches at the equilibrium  $\epsilon_e = 1,400 \text{ l. mol}^{-1} \text{ cm}^{-1}$  (value which corresponds to 22% of the endiol form).

Table 4. Values of the rate constant *k* in dilute NaOH solutions (I = 0.5)

$$v = \frac{dA_{270nm}}{dt} = \frac{di_a}{dt} = -\frac{di_c}{dt} = k \cdot [2] \cdot [OH^-]$$

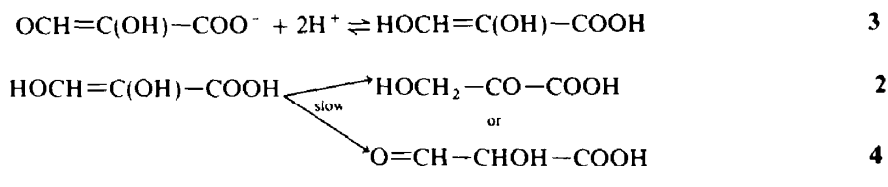
NaOH M: (I = 0.5)	0.5	0.2	0.1	0.05	0.042	0.02	0.008
	13.7	13.3	13.0	12.7	12.62	12.3	11.90
% $\text{HOCH}^{\ominus}\text{CO-COO}^{\ominus}$	100	100	100	100	80	75	50
$dA_{270nm}/dt, \text{k}^{\text{min}^{-1}}$	1.60		0.60	0.35			0.089
$di_a/dt, \text{k}^{\text{min}^{-1}}$	1.50	0.82	0.52	0.30	0.275	0.15	0.089
$-di_c/dt, \text{k}^{\text{min}^{-1}}$	1.56		0.50	0.32			0.087

Table 5. Values of the rate constant *k* in Britton Robinson Buffers

$$v = \frac{dA_{270nm}}{dt} = \frac{di_a}{dt} = k \cdot [2] \cdot [OH^-]$$

Britton/Robinson buffer pH	12.2	11.95	11.75	11.6	11.1
% $\text{HOCH}^{\ominus}\text{CO-COO}^{\ominus}$	56	40	30	20	10
$dA_{270nm}/dt, \text{k}^{\text{min}^{-1}}$	0.30	0.17		0.13	
$di_a/dt, \text{k}^{\text{min}^{-1}}$	0.40	0.14	0.225	0.11	0.056

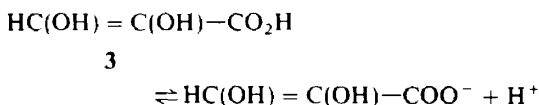
So it can be concluded that the acidification of the alkaline solution of enolate-carbanion **3'** yields first the enediol form which is later converted into an oxo form according to the reaction scheme:



When the acidification is limited to the pH range  $5 < \text{pH} < 8$ , the absorption decreases practically to zero. Then, if the latter solution is acidified to a pH value smaller than 2, the extinction increases until it reaches at the equilibrium the  $\epsilon_e$  value:  $1400 \text{ l. mol}^{-1} \text{ cm}^{-1}$ .

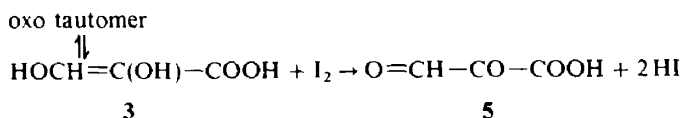
The rate of the enediol  $\rightleftharpoons$  oxo equilibrium was followed at pH 1.85 and 25°: the plot of  $(A_{250}^t - A_{250}^0)$  as a function of time is linear for the ascending and decreasing curves with the same slope and  $k = k_{\text{enediol}} + k_{\text{oxo}} = 0.065 \text{ min}^{-1}$ .

In the pH range 2.5 to 4.5, the plot of the percent of enediol as a function of pH has a shape of a dissociation curve whose point of inflexion (pH = 3.2) corresponds to the pK value of the acid-base equilibrium:



From several results, it is possible to deduce that tartronic semialdehyde **4** is the oxo form in equilibrium with the enediol form **3**:

(1) Contrary to an acid solution of hydroxypyruvic acid **1**, the acidified solution of **3'** was able to reduce iodine. In a first step corresponding to the formation of roughly 20% of **5**, the reaction was fast. Then the reduction of iodine occurred slowly depending on the oxo-enediol equilibrium:



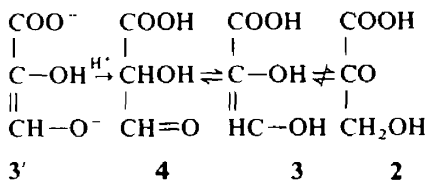
Finally **5** was obtained quantitatively.

(2) The acidified solution of **3'** showed spontaneous decarboxylation yielding quantitatively the glycolaldehyde  $\text{CH}_2\text{OH}-\text{CHO}$ . The kinetics were first order with respect to the starting material:  $k = 1.1 \times 10^{-2} \text{ min}^{-1}$  at pH 2.0 and 40°. In the same experimental conditions, the spontaneous decarboxylation of **1** would give  $k = 1.5 \times 10^{-4} \text{ mn}^{-1}$ .

(3) In agreement with the above results, the treatment of the acidified solution of **3'** with an excess of semicarbazide yielded quantitatively the semicarbazone of the glycolaldehyde whose the

polarographic and UV spectrophotometric behaviour are different from those of the semicarbazone of **2**.<sup>10</sup>

Finally the observed facts are in agreement with the sequence:



According to this scheme, a previous laboratory investigation<sup>11</sup> produced evidence that the  $\mathbf{3} \rightleftharpoons \mathbf{4}$  mixture constitutes an intermediary step in the spontaneous decarboxylation of dihydroxyfumaric acid into glycolaldehyde.

#### V. Kinetic investigations of the Aldol condensation

Whereas, in the pH range 11.0 to 12.5, the experimental data are in agreement with kinetics of the type:

$$v = \frac{di_a}{dt} = \frac{dA_{270}}{dt} = k \cdot |2| \cdot |\text{OH}^-|$$

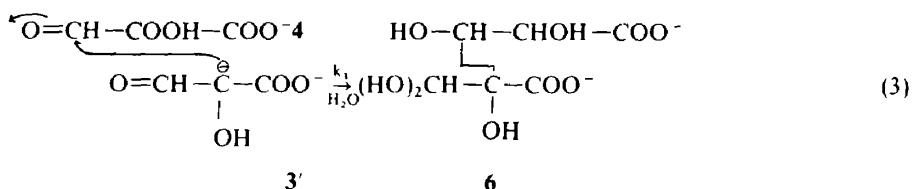
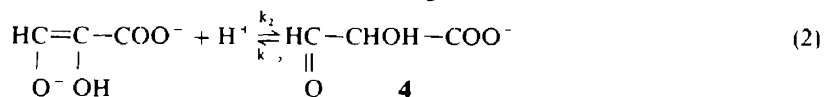
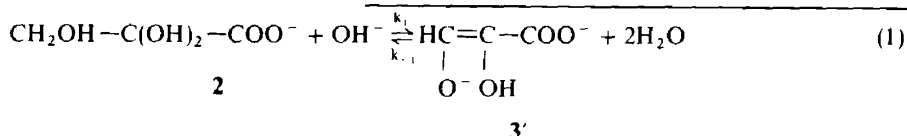
in the pH range 10.5–11.5, measurements of  $v = -di_c/dt$  did not agree with first order but with

second order (with respect to **|2|** kinetics:

$$v = \bar{k} \cdot |2|^2 \cdot |\text{OH}^-|^1$$

In this pH range, the carbanion enolate **3'** was present in low concentration as indicated by the graph of the plot of  $i_{\text{an}}$  or  $A_{270 \text{ nm}}$  vs pH. According to the results obtained in acidic media, the kinetic data may be justified by the occurrence of the acid-base dehydration reaction (1) followed by an aldol

condensation causing the decrease in the cathodic current:



Assuming that step (2) is fast and applying the stationary state approximation to the intermediate 3', we obtained:

$$|\text{3}'| = \frac{k_1 \cdot |\text{2}| \cdot |\text{OH}^-|}{k_{-1} \cdot |\text{H}_2\text{O}|^2 + k_3 \cdot |\text{4}|}$$

The experimental values determined for  $k$  in dilute NaOH ( $I=1$ ) and in phosphate buffered solutions (pH 11.5) by recording  $dI_3/dt$  or  $dA_{270\text{nm}}/dt$  are greater than those measured by plotting  $dI_3/dt = d|6|/dt$ . So we may assume that the step (3) is the limiting one and neglect the term  $k_3 \cdot |\text{4}|$  against  $k_{-1} \cdot |\text{H}_2\text{O}|^2$ .

$$|\text{3}'| = \frac{k_1 \cdot |\text{2}| \cdot |\text{OH}^-|}{k_{-1} \cdot |\text{H}_2\text{O}|^2}$$

On the other hand:

$$|\text{4}| = \frac{k_2}{k_{-2}} \cdot |\text{3}'| \cdot |\text{H}^+| = \frac{k_2}{k_{-2}} \cdot \frac{k_1 \cdot |\text{2}| \cdot |\text{OH}^-| \cdot |\text{H}^+|}{k_{-1} \cdot |\text{H}_2\text{O}|^2}$$

hence:

$$v = \frac{d|6|}{dt} = k_3 \cdot |\text{3}'| \cdot |\text{4}| = \frac{k_1 \cdot k_2 \cdot k_3 \cdot K_e \cdot |\text{2}|^2 \cdot |\text{OH}^-|}{(k_{-1} \cdot |\text{H}_2\text{O}|^2)^2 \cdot k_{-2}}$$

with  $K_e = |\text{H}^+| \cdot |\text{OH}^-|$

finally:  $v = \bar{k} \cdot |\text{2}|^2 \cdot |\text{OH}^-|$

This expression agrees with the experimental data: (Table 6) the reaction is second order with respect to |2| and first order with respect to |OH<sup>-</sup>| and the assumption of the occurrence of an aldol condensation between 3' and 4 in slightly basic media is confirmed.

Concerning the structure of the aldol 6, it was corroborated by the results of periodic oxidation and <sup>13</sup>C NMR.

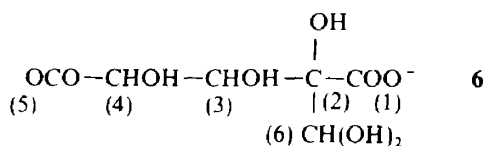
#### VI. Structure of the aldol 6

According to a previous work<sup>12</sup> the periodic oxidation of 2 consumed 1 mole of periodic acid and yielded 1 mole each of oxalic acid and formaldehyde. After aldol condensation at pH 12 under N<sub>2</sub>, the periodic oxidation yielded only 0.4 mole of oxalic acid and less than 0.1 mole of formaldehyde. At the same time carbon dioxide was produced in an amount corresponding to 0.4 mole.

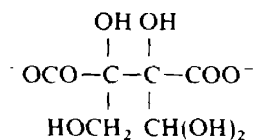
The disappearance of formaldehyde indicates the absence of a primary alcohol group. These results were interpreted by the formation of α ketotrihydroxyadipate,<sup>12</sup> but the results of <sup>13</sup>C NMR favour another interpretation.

The investigation of 6 as its sodium salt was carried out by <sup>13</sup>C NMR in water or dilute NaOH. Table 7 summarises the results obtained in aqueous solution and in 0.2 M NaOH, at 3', in order to avoid decomposition. (According to the ionisation of the CH(OH)<sub>2</sub> group in 6, or to the carbanion-enolate formation, one equivalent OH<sup>-</sup> was consumed per mole of 6. So, to reach a final (1M)/OH<sup>-</sup> concentration equal to 0.2 M, the sodium salt 6 was dissolved in 1.2 M NaOH).

The data obtained in neutral solution are in good agreement with the formula:



The alternative formula:



can be excluded owing to the lack of a specific signal due to the primary alcohol group.

In 0.2 M NaOH, the six signals corresponding to compound 6 are slightly displaced owing to the medium change and ionisation of one of the gemdiol OH groups. On the other hand, three new signals were observed whose intensities, in comparison with those of the signals due to compound 6, increase with increasing time, temperature and increasing pH (in 0.5 M NaOH).

Also it was established that the presence of electron donating substituent (OH) on the α-C atom and of electron acceptor substituent (COO<sup>-</sup>) on the β-C atom promotes cleavage of the C-C bond.<sup>13</sup> According to this concept and the above data, it seems reasonable

Table 6. Values of the rate constant  $\bar{k}$  in buffered solutions;  $c = 10^{-3}$  M

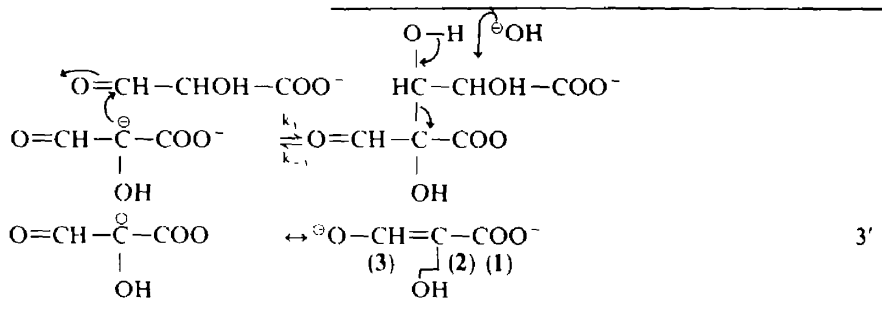
$$v = -\frac{d[\epsilon]}{dt} = \bar{k} \cdot [2]^2 \cdot [\text{OH}^-]$$

pH	11.4	11.0	10.6
$k \text{ l. mol}^{-1} \cdot \text{min}^{-1}$	24	21	7

Table 7.  $^{13}\text{C}$  NMR spectral data recorded at 3° q (quaternary), t (tertiary) carbons identified by off resonance decoupled and undecoupled spectra

Assignments 5	$\delta$ ppm neutral water	$\delta$ ppm NaOH 0.2 M	Assignments 3'
C <sub>1</sub> (q) C <sub>5</sub> (q)	178.9 and 177.2	181.5 and 180.4	
		176.4 149.1 127.5	C <sub>1</sub> (q) C <sub>3</sub> (t) C <sub>2</sub> (q)
C <sub>6</sub> (t)	100.2	105.7	
C <sub>2</sub> (q)	81.9	81.7	
C <sub>3</sub> (t)	81.2	80.1 and 79.7	
C <sub>4</sub> (t)	78.1		

to consider that, in alkaline media (pH > 13), the reverse reaction of aldolisation occurs, generating 3'



The reverse reaction was first order with respect to 6 and identical values were found for the rate constant  $k_{-3}$  by measuring with increasing time the increase of the anodic current ( $v = di_a/dt$ ) or, alternatively the increase of the molar absorptivity ( $v = d\epsilon/270 \text{ nm}/dt$ ). In dilute NaOH ( $10^{-2} \text{ M} < c < 1 \text{ M}$ ,  $I = 1$ ) the experimental values found for the rate constant  $k_{-3}$  did not vary with pH ( $K_{-3} = 0.25 \text{ min}^{-1}$  at 25 °C), according to a kinetic law of the type:

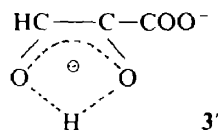
$$v = \frac{d[3']}{dt} = -\frac{d[6]}{dt} = k_{-3} \cdot [6]$$

From these data we can conclude that ionisation of the OH group precedes the rate limiting step: the cleavage of the C-C bond.

#### CONCLUSION

In slightly basic media, ionisation of the gemdiol group of  $\beta$ -bromopyruvate 1 initiates the bromine elimination yielding  $\beta$ -hydroxypyruvate 2. In basic media (pH > 13), the behaviour of  $\beta$ -hydroxypyruvate

may be explained by the presence in the bulk of the solution of a carbanion enolate 3'



the formation of which is initiated by an acid-base-dehydration equilibrium (half-evolution pH = 12). Owing to this  $pK'$  value, in the pH range 10.5 to 11.5, 3' existed in minor amounts but it initiated a slow aldol condensation with the tartronate semialdehyde 4. The mechanism and the value of the overall constant  $\bar{k}$  of the aldol condensation was determined in buffered solution according to a kinetic law of the type:  $v = \bar{k} \cdot [2]^2 \cdot [\text{OH}^-]$ .

The aldol product 6 was isolated as sodium salt and its structure established by  $^{13}\text{C}$  NMR.

The ability to produce a carbanion in slightly basic medium makes the hydroxypyruvate an interesting



product from a biological standpoint. Yet, among the reactions which are convenient in the elementary biochemical processes, there is the addition of a mobile C-H bond to an oxo bond, such as in the coenzyme A catalysed acetylation of the  $\alpha$ -ketoacids. Moreover, in rat liver, the synthesis of ascorbic acid proceeds by a similar pathway: via an asymmetric aldol condensation to 2-ketogulonate.

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- <sup>10</sup>D. Fleury and M. B. Fleury, *Ibid.* **36**, 331 (1971).
- <sup>11</sup>D. Fleury, *Bull. Soc. Chim. Fr.* 2533 (1972).
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