

## Amine Catalysis in the Decarboxylation of Oxaloacetic Acid

KAI JULIUS PEDERSEN

*Chemical Laboratory, Royal Veterinary and Agricultural College, Copenhagen, Denmark*

It is known that the decarboxylation of  $\beta$ -keto carboxylic acids is catalysed by primary amines, but a satisfactory explanation of the reaction has not been given. The aim of this paper is to give a contribution to the solution of this problem by studying the amine catalysis in the decarboxylation of oxaloacetic acid. Three catalysts, ammonium ions, ethylammonium ions, and anilinium ions were examined in solvents of different acidity: solutions of hydrogen chloride and acetate buffer solutions. By an analysis of the results, separate rate constants were determined for each of the three simultaneous reactions: decomposition of undissociated oxaloacetic acid, of univalent hydrogenoxaloacetate ion, and of divalent oxaloacetate ion.

It was found that the uncatalysed decomposition of the univalent ion is accelerated by acetate ions. Aminium ions catalyse the decomposition of both the univalent and the divalent oxaloacetate ion. The former (and perhaps also the latter) catalysis is further accelerated by acetate ions. The anilinium ion is a much more effective catalyst than the two other ions, especially in the decarboxylation of the divalent oxaloacetate ion. It has not been possible to explain an apparent disagreement between the results on anilinium-ion catalysis in acetate buffers and in solutions of hydrogen chloride.

It is concluded from the experiments that aminium ions form reactive intermediates with the di- and uni-valent oxaloacetate ion (and possibly also with the undissociated acid), e. g., by reactions of the type  $>CO + NRH_3^+ \rightleftharpoons >CNRH^+ + H_2O$ . It has in all computations been assumed that the concentrations of such complexes are negligible. Results of glass-electrode measurements reported in the last part of the paper are in agreement with this assumption.

The decomposition of oxaloacetic acid in aqueous solution into pyruvic acid and carbon dioxide is catalysed by certain metal ions and by primary amines. The uncatalysed reaction and the metal-ion catalysis were examined in an earlier paper<sup>1</sup>, and a study of the amine catalysis is the object of the present paper. It is a general rule that the decarboxylation of  $\beta$ -keto carboxylic acids is catalysed, within certain pH-limits, by primary amines. This catalysis has been most thoroughly examined on acetoacetic acid (Widmark and Jeppson<sup>2</sup>, Ljunggren<sup>3</sup>, Beniya<sup>4</sup>) and on  $\alpha,\alpha$ -dimethyl acetoacetic acid (Pedersen<sup>5</sup>, Westheimer and Jones<sup>6</sup>). The catalysis of various amines and amino acids

in the decarboxylation of oxaloacetic acid has been studied by Kaneko <sup>7</sup> and by Bessman and Layne <sup>8</sup>. A satisfactory explanation of the amine catalysis has not been given. The author of the present paper has shown <sup>9</sup> that the decarboxylation is not preceded by an isomerisation of the keto-acid into an enol-form, and it has been suggested that a reactive intermediate is formed by a fast reaction of the amine with the keto-group, *e. g.*,



This suggestion has been examined and modified by Westheimer <sup>6,10</sup>.

In the present paper, three catalysts, ammonium ions, ethylammonium ions, and anilinium ions, were examined in solutions of various acidities. Since oxaloacetic acid is present in the solutions partly in the form of undissociated acid  $\text{H}_2\text{Ox}$  and partly in the form of the ions  $\text{HOx}^-$  and  $\text{Ox}^{2-}$  (degree of dissociation  $\alpha_1$  and  $\alpha_2$ , respectively), the rate of decarboxylation may be expressed as

$$-\frac{dx}{dt} = k_0 [\text{H}_2\text{Ox}] + k_1 [\text{HOx}^-] + k_2 [\text{Ox}^{2-}] = [k_0(1-\alpha_1-\alpha_2) + k_1\alpha_1 + k_2\alpha_2]x$$

where at least one of the coefficients depends on the nature and concentration of the catalyst. When the hydrogen ion concentration is constant, the reaction will be of first order, and the rate constant will be

$$k = k_0(1-\alpha_1-\alpha_2) + k_1\alpha_1 + k_2\alpha_2 \quad (1)$$

The solutions examined may be divided into two groups: highly acid solutions (where  $\alpha_2 < 0.013$ ) and weakly acid solutions (where  $\alpha_2 > 0.5$ ). The solvents to which oxaloacetic acid was added were in the former group water or solutions of hydrogen chloride, in the latter group buffer solutions of acetic acid and sodium acetate. The solutions contained, in addition, the chloride of the catalyst and potassium chloride up to an ionic strength of approximately 0.3. The temperature was 37.0° C, and the experimental method the same as in the earlier paper <sup>1</sup>. The first-order law was always found to hold for the highly acid solutions. In agreement with this, glass-electrode measurements <sup>11</sup> had shown that pH of a solution of oxaloacetic acid in pure water remains practically unchanged during the complete reaction. In the experiments on weakly acid solutions, small deviations from first-order law were observed. This is explained by a gradual change in pH, and consequently also in  $\alpha_1$  and  $\alpha_2$ , taking place when oxaloacetic acid decomposes. Here  $k_\infty$ , the rate constant corresponding to the final composition of the solution, was computed by means of a formula deduced in the earlier paper <sup>1</sup>. This formula could, however, not be applied to the experiments on anilinium ion catalysis since, here, not only  $\alpha_1$  and  $\alpha_2$  but, owing to displacement of the equilibrium:  $\text{AnH}^+ \rightleftharpoons \text{An} + \text{H}^+$ , also the coefficients  $k_0$ ,  $k_1$ , and  $k_2$  change during the reaction.

In accordance with practice in previous papers, the rate constants marked \* are defined in terms of decadic logarithms ( $k^* = 0.4343 k$ ), and the time unit is the minute. The degrees of dissociation  $\alpha_1$  and  $\alpha_2$  of oxaloacetic acid were calculated by means of the two dissociation constants of the acid obtained from

glass-electrode measurements<sup>11</sup>. In all calculations in this paper, it has been assumed that the concentrations of possible complexes of the amines with oxaloacetic or pyruvic acid are negligible. This assumption is supported by electrometric measurements on solutions of aniline and the acids (reported in the last part of this paper).

*Materials.* The oxaloacetic and pyruvic acid were of the same purity as the preparations used in earlier papers<sup>1,11</sup>. The other chemicals were purified either by distillation or by recrystallisation from redistilled water. Standard solutions of ethylammonium chloride and anilinium chloride were prepared from the pure bases and standard hydrochloric acid. The anilinium chloride solution was tested for excess or deficiency of hydrochloric acid by measurements with the glass electrode (see later). All solutions were made with water redistilled in an all-glass still.

#### EXPERIMENTAL RESULTS ON HIGHLY ACID SOLUTIONS

*Uncatalysed reaction* (Table 1, first 4 lines). When equation 1 is applied, and the last (small) term is calculated by means of  $k_2 \cdot 10^3 = 1.81$  (taken from experiments on weakly acid solutions), it is found that the following values of the two other coefficients

$$k_0 \cdot 10^3 = 0.176 \text{ and } k_1 \cdot 10^3 = 6.47 \quad (2)$$

give the best agreement with  $k^*$  found by experiment. When  $k^*$  is calculated from equation 1 and the values of the coefficients given here, the results shown in the next to the last column of Table 1 are obtained.

*Table 1. Decomposition of oxaloacetic acid (initially 0.0150 molar) at 37° C in the solvent: h molar hydrogen chloride, c molar ammonium chloride, (0.300 - h - c) molar potassium chloride. The data in the last column refer to solutions containing in addition 0.418 molar acetic acid.*

<i>h</i>	<i>c</i>	<i>a</i> <sub>1</sub>	<i>a</i> <sub>2</sub>	<i>k</i> ·10 <sup>3</sup>	<i>k</i> ·10 <sup>3</sup> calc.	<i>k</i> ·10 <sup>3</sup> acet. acid
0.0000	0.000	0.4841	0.0126	3.243	3.244	
0.01995	0.000	0.2352	0.0020	1.665	1.660	1.673
0.0499	0.000	0.1229	0.0005	0.942	0.950	0.963
0.0999	0.000	0.0670	0.0001	0.600	0.599	
0.0000	0.100	0.4841	0.0126	3.359	3.355	
0.0000	0.200	0.4841	0.0126	3.467	3.467	
0.01995	0.200	0.2352	0.0020	1.740	1.742	1.759
0.0499	0.200	0.1229	0.0005	0.987	0.990	1.009
0.0999	0.200	0.0670	0.0001	0.620	0.619	
0.0000	0.300	0.4841	0.0126	3.578	3.578	

*Ammonium-ion catalysis* (Table 1, last 6 lines). When the first term of equation 1 is calculated by means of  $k_0 \cdot 10^3 = 0.176$ , and the last term by means of the values of  $k_2^*$  obtained from experiments on ammonium-ion catalysis in acetate buffers (equation 10), we get agreement with the experimental results when we set

$$k_1 \cdot 10^3 = 6.47 + 1.47 [\text{NH}_4^+] \quad (3)$$

This is seen from the next to the last column where  $k^*$  calculated by means of the equation is given.

*Effect of acetic acid.* Addition of acetic acid has only a small effect on  $k^*$  as shown in the last column of Table 1.

*Ethylammonium-ion catalysis* (Table 2). When  $k_0 \cdot 10^3 = 0.176$  and values of  $k_2^*$  obtained from experiments on ethylammonium-ion catalysis in acetate

Table 2. Decomposition of oxaloacetic acid (initially 0.0150 molar) at 37° C in the solvent:  $h$  molar hydrogen chloride,  $c$  molar ethylammonium chloride,  $(0.300 - h - c)$  molar potassium chloride.

$h$	$c$	$\alpha_1$	$\alpha_2$	$k \cdot 10^3$	$k_1 \cdot 10^3$	$k_2 \cdot 10^3$ equ. 4
0.0000	0.100	0.4841	0.0126	3.605	7.10	7.10
0.01995	0.100	0.2352	0.0020	1.816	7.10	7.10
0.0000	0.200	0.4841	0.0126	3.994	7.79	7.73
0.01995	0.200	0.2352	0.0020	1.966	7.70	7.73

buffers (equation 11) are introduced into equation 1, we calculate the values of  $k_1^*$  given in the next to the last column of Table 2. They may be expressed by the following linear relation

$$k_1 \cdot 10^3 = 6.47 + 6.3 [\text{C}_2\text{H}_5\text{NH}_3^+] \quad (4)$$

Values calculated by means of this equation are given in the last column.

*Anilinium-ion catalysis.* The composition of the solutions examined and the rate constants found are given in Table 3. An analysis of the data shows that the catalytic effect of the anilinium ion on the decomposition of the divalent oxaloacetate ion is so enormous that its contribution to the total rate is large, although  $\alpha_2 < 0.013$ . The experiments fall in four groups. Within each group, the anilinium ion concentration is constant while the hydrogen ion concentration varies. For each group the coefficients  $k_0^*$ ,  $k_1^*$ , and  $k_2^*$  of equation 1 are determined by the method of least squares. The results are given in Table 3a. They may be expressed by the formulae

$$k_0 \cdot 10^3 = 0.176 + 1.19 [\text{C}_6\text{H}_5\text{NH}_3^+] \quad (5)$$

$$k_1 \cdot 10^3 = 6.47 + 404 [\text{C}_6\text{H}_5\text{NH}_3^+] + 260 [\text{C}_6\text{H}_5\text{NH}_3^+]^2 \quad (6)$$

$$k_2 \cdot 10^3 = 0.00181 + 38.4 [\text{C}_6\text{H}_5\text{NH}_3^+] + 55 [\text{C}_6\text{H}_5\text{NH}_3^+]^2 \quad (7)$$

The increase of  $k_0^*$  has, however, so small an influence on the total rate that it is uncertain whether it is significant. When the coefficients are calculated by means of equations 5, 6, and 7, the values given in the 3rd, 5th, and 7th column of Table 3a are obtained. In the last column of Table 3 are shown rate constants calculated by means of equation 1 and the values of the coefficients computed from equations 5, 6, and 7.

#### EXPERIMENTAL RESULTS ON WEAKLY ACID SOLUTIONS

In this section are reported results of measurements on solutions containing an acetate buffer system. Since, here, only a very small fraction (less than 1.2 %) of the oxaloacetic acid is undissociated and, further,  $k_0$  is always much smaller than  $k_1$  and  $k_2$ , we may neglect the first term of equation 1 and write

Table 3. Decomposition of oxaloacetic acid (initially 0.0150 molar) at 37° C in the solvent: *h* molar hydrogen chloride, *c* molar anilinium chloride, (0.300 - *h* - *c*) molar potassium chloride.

<i>h</i>	<i>c</i>	$\alpha_1$	$\alpha_2 10^3$	$k^* 10^3$	$k^* 10^3$ calc.
0.00000	0.0100	0.4808	12.41	10.06	10.05
0.00993	0.0100	0.3247	4.28	5.213	5.227
0.02043	0.0100	0.2316	1.90	3.329	3.329
0.02990	0.0100	0.1813	1.09	2.490	2.490
0.04985	0.0100	0.1228	0.47	1.640	1.637
0.09985	0.0100	0.0670	0.13	0.926	0.927
0.00988	0.0200	0.3250	4.29	8.242	8.265
0.02038	0.0200	0.2318	1.91	5.076	5.049
0.0498	0.0200	0.1228	0.47	2.334	2.342
0.0998	0.0200	0.0670	0.13	1.272	1.270
0.00474	0.0500	0.3940	7.05	25.36	25.47
0.00973	0.0500	0.3260	4.32	18.06	17.97
0.02023	0.0500	0.2325	1.92	10.49	10.48
0.04965	0.0500	0.1231	0.47	4.519	4.524
0.0996	0.0500	0.0671	0.13	2.314	2.316
0.1994	0.0500	0.0349	0.03	1.253	1.252
0.00948	0.1000	0.3276	4.37	35.35	35.55
0.01444	0.1000	0.2765	2.89	26.75	26.57
0.01998	0.1000	0.2336	1.94	20.40	20.34
0.02945	0.1000	0.1827	1.11	14.20	14.20
0.0494	0.1000	0.1236	0.47	8.48	8.50
0.0994	0.1000	0.0672	0.13	4.205	4.208
0.1490	0.1000	0.0461	0.06	2.851	2.851
0.1992	0.1000	0.0350	0.03	2.186	2.186

Table 3a.

<i>c</i>	$k_0^* 10^3$	$k_0^* 10^3$ calc.	$k_1^* 10^3$	$k_1^* 10^3$ calc.	$k_2^*$	$k_2^*$ calc.
0.0100	0.182	0.188	10.53	10.54	0.394	0.392
0.0200	0.198	0.200	14.67	14.65	0.785	0.792
0.0500	0.243	0.236	27.08	27.3	2.079	2.06
0.1000	0.295	0.295	50.0	49.5	4.34	4.39

Table 4. Decomposition of oxaloacetic acid (initially 0.0150 molar) at 37° C in the solvent: *a* molar acetic acid, *b* molar sodium acetate, (0.300 - *b*) molar potassium chloride.

<i>a</i>	<i>b</i>	$\alpha_{1\infty}$	$\alpha_{2\infty}$	$k^*_{\infty} 10^3$	$k^*_{\infty} 10^3$ calc.
0.1043	0.2991	0.064	0.936	2.16	2.16
0.2087	0.2991	0.113	0.887	2.42	2.43
0.3125	0.2991	0.157	0.842	2.70	2.68
0.4174	0.2991	0.198	0.801	2.90	2.90
0.1045	0.1993	0.095	0.905	2.37	2.35
0.2090	0.1993	0.164	0.835	2.70	2.73
0.4181	0.1993	0.275	0.722	3.36	3.34
0.6275	0.1993	0.359	0.636	3.79	3.79
0.0522	0.0997	0.114	0.886	2.42	2.40
0.1045	0.0997	0.186	0.813	2.77	2.79
0.2090	0.0997	0.298	0.698	3.36	3.38
0.4181	0.0997	0.448	0.542	4.20	4.16

$$k = k_1\alpha_1 + k_2\alpha_2 \quad (8)$$

Since  $\alpha_1$  and  $\alpha_2$  change a little during the reaction, we shall, where it is possible, compute  $k_\infty$ ,  $\alpha_{1\infty}$ , and  $\alpha_{2\infty}$ , the values for solutions where (practically) all oxaloacetic acid has been transformed into pyruvic acid. Inserting them into equation 8, we get

$$k_\infty = k_1\alpha_{1\infty} + k_2\alpha_{2\infty} \quad (9)$$

In order to calculate  $\alpha_1$  and  $\alpha_2$ , it is necessary to know with good accuracy the ratio  $K_{\text{HAc}}/K_2$ , where  $K_{\text{HAc}}$  is the dissociation constant of acetic acid and  $K_2$  the second dissociation constant of oxaloacetic acid. In the earlier paper<sup>1</sup>,  $k_2^*$  for the uncatalysed reaction was computed without using this ratio. Rate constants were determined for a series of solutions which differed only with respect to the concentration of free acetic acid. The reasonable assumption was made that  $k_1^*$  has the same value here as in solutions of hydrochloric acid. On the basis of equation 9, a formula was deduced which made it possible to determine  $k_2^*$  by extrapolation and, in addition, obtain a value of  $K_{\text{HAc}}/K_2$ . The latter did, however, not agree well with that found from electrometric measurements. For 0.3 molar sodium chloride at 37° C, it was estimated from measurements by Harned and Hickey<sup>12</sup> that  $K_{\text{HAc}} = 3.20 \times 10^{-5}$ , and glass-electrode measurements<sup>11</sup> have shown that  $K_2 = 1.97 \times 10^{-4}$ . Hence,  $K_{\text{HAc}}/K_2 = 0.162$ , while the much higher value 0.197 was found from the kinetic measurements for 0.3 molar sodium acetate. The discrepancy was confirmed in this paper, and experiments on amine catalysis have decisively shown that it is not permissible to assume that  $k_1^*$  has the same value for acetate buffer solutions as for solutions acidified with hydrochloric acid. In the following, we shall therefore, for the solutions examined, which have all an ionic strength of approximately 0.3, adopt the value 0.162 for the ratio  $K_{\text{HAc}}/K_2$ . By means of this value, we shall calculate  $\alpha_{1\infty}$  and  $\alpha_{2\infty}$ , and then, by means of equation 9, determine  $k_1^*$  and  $k_2^*$ . Fortunately, practically the same value of  $k_2^*$  for the uncatalysed reaction was obtained by the former and the latter method ( $1.80 \times 10^{-3}$  and  $1.81 \times 10^{-3}$ , respectively).

*Uncatalysed reaction.* In the three groups of solutions examined (Table 4), the concentration of sodium acetate (before addition of oxaloacetic acid) was 0.3, 0.2, and 0.1 molar, respectively. The rate constants found from the measurements are given in column 5. When equation 9 is applied to each group separately, the values of  $k_1^*$  and  $k_2^*$  shown in the last column are obtained. Introducing them into equation 9, we calculate the values of  $k_\infty$  presented in column 6. As seen from the last column,  $k_2^*$  is the same for all three groups (mean  $1.81 \times 10^{-3}$ ), while  $k_1^*$  increases with increasing acetate ion concentration and is considerably greater than the value  $6.47 \times 10^{-3}$  found for the highly acid solutions. If the latter value of  $k_1^*$  were adopted, a recalculation of the data in Table 4 by the old method<sup>1</sup> would give  $K_{\text{HAc}}/K_2 = 0.206$ , a value which is much higher than that derived from electrometric measurements (0.162).

*Ammonium-ion catalysis.* Within each of the four groups of experiments (see Table 5), both  $b$  and  $c$  are constant. When equation 9 is applied to each group separately, we find the values of  $k_1^*$  and  $k_2^*$  shown in the third and

Table 5. Decomposition of oxaloacetic acid (initial concentration 0.0150 molar, except in the experiments marked *x* and *y* where it was 0.0100 and 0.0200 molar, respectively) at 37° C in the solvent: *a* molar acetic acid, *b* molar sodium acetate, *c* molar ammonium chloride, (0.300 - *b* - *c*) molar potassium chloride.

	<i>a</i>	<i>b</i>	<i>c</i>	$\alpha_{1\infty}$	$\alpha_{2\infty}$	$k^*_{\infty} 10^3$	$k^*_{\infty} 10^3$ calc.
	0.1045	0.1993	0.050	0.095	0.905	3.86	3.83
	0.4181	0.1993	0.050	0.275	0.722	4.59	4.66
	0.6275	0.1993	0.050	0.359	0.636	5.07	5.04
	0.1045	0.1993	0.100	0.095	0.905	5.33	5.32
<i>x</i>	0.2090	0.1993	0.100	0.158	0.841	5.49	5.52
	0.2090	0.1993	0.100	0.164	0.835	5.62	5.54
<i>y</i>	0.2090	0.1993	0.100	0.171	0.828	5.65	5.57
	0.4181	0.1993	0.100	0.275	0.722	5.80	5.89
	0.6275	0.1993	0.100	0.359	0.636	6.15	6.15
	0.1045	0.0997	0.100	0.186	0.813	5.49	5.48
	0.2090	0.0997	0.100	0.298	0.698	5.73	5.75
	0.0522	0.0997	0.200	0.114	0.886	8.25	8.25
	0.1045	0.0997	0.200	0.186	0.813	8.21	8.21
	0.2090	0.0997	0.200	0.299	0.698	8.03	8.14
	0.4181	0.0997	0.200	0.448	0.542	8.14	8.03

Table 5a.

<i>b</i>	<i>c</i>	$k_1^* 10^3$	$k_2^* 10^3$	$k_1^* 10^3$ equ. 3	$k_2^* 10^3$ equ. 10
0.1993	0.050	8.03	3.39	6.54	3.42
0.1993	0.100	8.24	5.02	6.62	5.02
0.0997	0.100	7.52	5.02	6.62	5.02
0.0997	0.200	7.87	8.30	6.78	8.23

fourth column of Table 5a. Introducing them into equation 9, we calculate the values of  $k_2^*$  presented in the last column of Table 5. The coefficient  $k_2^*$  is independent of the acetate ion concentration and may be represented by the following linear function of the ammonium ion concentration

$$k_2^* 10^3 = 1.81 + 32.1 [\text{NH}_4^+] \quad (10)$$

$k_2^*$  calculated by means of this equation is given in the last column of Table 5a. Values of  $k_1^*$  calculated from equation 3, which was found to hold for acetate-free solutions, are shown in the next to the last column. They are much lower than those found for acetate buffers (column 3). The discrepancy cannot be explained away by an error in the value of the ratio  $K_{\text{HAC}}/K_2$ . If we, instead of 0.162, apply 0.206 (see above), the values computed for  $\alpha_{1\infty}$  and  $\alpha_{2\infty}$  will change considerably, but those for  $k_1^*$  only little, and they will still be much higher than those calculated from equation 3.

*Ethylammonium-ion catalysis.* When equation 9 is applied to each of the four groups of measurements presented in Table 6, the values of  $k_1^*$  and  $k_2^*$  shown in the fourth and fifth column of Table 6a are found. Introducing them into equation 9, we calculate the values of  $k^*_{\infty}$  given in the last column of Table 6. The coefficient  $k_2^*$  is nearly a linear function of the ethylammonium

Table 6. Decomposition of oxaloacetic acid (initially 0.0150 molar) at 37° C in the solvent: a molar acetic acid, b molar sodium acetate, c molar ethylammonium chloride, (0.300 - b - c) molar potassium chloride.

a	b	c	$\alpha_{1\infty}$	$\alpha_{2\infty}$	$k^*_{\infty} 10^3$	$k^*_{\infty} 10^3$ calc.
0.1043	0.1993	0.0500	0.095	0.905	4.67	4.68
0.2085	0.1993	0.0500	0.164	0.835	4.96	4.95
0.6260	0.1993	0.0500	0.358	0.636	5.72	5.72
0.1045	0.1993	0.1000	0.095	0.905	7.04	7.11
0.2090	0.1993	0.1000	0.164	0.835	7.36	7.30
0.4181	0.1993	0.1000	0.275	0.722	7.60	7.59
0.6275	0.1993	0.1000	0.359	0.636	7.79	7.81
0.1042	0.0997	0.1000	0.185	0.814	6.94	6.95
0.2087	0.0997	0.1000	0.298	0.698	7.14	7.14
0.4175	0.0997	0.1000	0.448	0.542	7.38	7.39
0.0421	0.0997	0.2002	0.098	0.901	11.13	11.17
0.1042	0.0997	0.2002	0.185	0.814	11.16	11.10
0.2089	0.0997	0.2002	0.298	0.698	11.04	10.98
0.4176	0.0997	0.2002	0.447	0.543	10.74	10.79

Table 6a.

b	c	$[\text{Ac}^-]_{\infty}$	$k_1 * 10^3$	$k_2 * 10^3$	$k_1 * 10^3$ equ. 4	$k_2 * 10^3$ equ. 11
0.1993	0.0500	0.184	8.35	4.29	6.78	4.32
0.1993	0.1000	0.184	9.61	6.85	7.10	6.83
0.0997	0.1000	0.085	8.51	6.60	7.10	6.56
0.0997	0.2002	0.085	10.47	11.26	7.72	11.32

ion concentration but it seems to increase a little also with increasing acetate ion concentration. The latter effect is, however, so small that it is uncertain whether it is significant. The values found for  $k_2^*$  may be expressed by the following equation

$$k_2^* 10^3 = 1.81 + (45.2 + 27 [\text{Ac}^-]) [\text{C}_2\text{H}_5\text{NH}_3^+] \quad (11)$$

$k_2^*$  calculated by means of this equation is given in the last column of Table 6a. The acetate buffer has a large influence on  $k_1^*$ . If  $k_1^*$  is calculated from equation 4, which holds for acetate-free solutions, the values given in the next to the last column are obtained. They are much lower than those found for acetate buffers (column 4). It has, however, not been possible to express the effect of acetate on  $k_1^*$  by means of a formula.

*Anilinium-ion catalysis.* As already mentioned, it was here too difficult to correct for the change in pH due to the reaction, but when the kinetic data were plotted in the usual way, only small deviations from the first-order law were found. The rate constants given in the next to the last column of Table 7, therefore, correspond to an average composition of the reacting solution. The values given for the concentrations of acetate ion  $[\text{Ac}^-]$  and anilinium ion  $[\text{AnH}^+]$ , and for  $\alpha_1$  and  $\alpha_2$ , are the averages of their initial and final values. For the dissociation constant of the anilinium ion we have used the value



Table 7. Decomposition of oxaloacetic acid (initially 0.0150 molar) at 37° C in the solvent: a molar acetic acid, b molar sodium acetate, (0.300 - b) molar potassium chloride,  $1.000 \times 10^{-3}$  molar anilinium chloride. In the experiments marked x and y, the concentration of anilinium chloride was  $0.500 \times 10^{-3}$  and  $2.000 \times 10^{-3}$ , respectively.

	a	b	[Ac <sup>-</sup> ]	[AnH <sup>+</sup> ] $10^3$	$\alpha_1$	$\alpha_2$	$k \cdot 10^3$	$k \cdot 10^3$ calc.
	0.1043	0.2991	0.276	0.294	0.069	0.931	7.08	7.07
x	0.2087	0.2991	0.277	0.215	0.119	0.881	5.80	5.83
	0.2087	0.2991	0.277	0.432	0.119	0.881	9.29	9.34
y	0.2087	0.2991	0.276	0.864	0.119	0.880	16.44	16.29
	0.3125	0.2991	0.277	0.523	0.163	0.836	10.65	10.70
	0.4174	0.2991	0.278	0.589	0.204	0.795	11.62	11.59
	0.1043	0.1998	0.178	0.393	0.104	0.896	8.54	8.55
	0.2087	0.1998	0.178	0.540	0.173	0.826	10.71	10.72
	0.4174	0.1998	0.179	0.690	0.283	0.714	12.54	12.49
	0.1043	0.0999	0.079	0.592	0.206	0.793	11.18	11.18
	0.2087	0.0999	0.080	0.723	0.317	0.679	12.45	12.53
	0.4174	0.0999	0.081	0.830	0.461	0.528	12.96	12.98

$3.52 \times 10^{-5}$  which, according to glass-electrode measurements<sup>13</sup>, holds for 0.3 molar sodium chloride at 37° C. An analysis of the data given in Table 7 has shown that the rate constants may be expressed by equation 8 when the coefficients have values given by the formulae

$$k_1 \cdot 10^3 = 6.47 + 4.4 \times 10^3 [\text{AnH}^+] \quad (12)$$

$$k_2 \cdot 10^3 = 1.81 + (16.4 + 4.8 [\text{Ac}^-]) 10^3 [\text{AnH}^+] \quad (13)$$

where  $k_1^*$  and  $k_2^*$  for the uncatalysed reaction have, in advance, been given values found earlier in this paper. Rate constants calculated by means of these equations are presented in the last column of Table 7.

There is a serious discrepancy between the results of the anilinium-ion catalysis obtained here and those found for solutions containing hydrochloric acid (compare equations 12 and 13 with equations 6 and 7). When rate constants for the solutions presented in Table 7 are calculated by means of equations 6 and 7, the values obtained are from 64 to 93 % too high. An attempt was made to explain this disagreement in the following way. It has hitherto in all the computations been assumed that the concentrations of reactive intermediates is negligible, but this may not be true in all cases. If a considerable fraction of the anilinium salt added to the acetate buffers were bound to oxaloacetate ions, the concentrations of free anilinium ion would be lower than shown in Table 7, and this might possibly explain the unexpectedly low rate found here. An examination showed, however, that it was not possible to explain the results of experiments on both acetate buffers and solutions of hydrochloric acid in this way. It has further been shown by electrometric measurements on solutions of oxaloacetic acid and aniline (reported in the last part of this paper) that the concentrations of possible complexes of this type are too small to explain the discrepancy.

## CONCLUSIONS OF KINETIC EXPERIMENTS

The rate constants for the uncatalysed decarboxylation of the undissociated oxaloacetic acid, the univalent ion, and the divalent ion were found to be  $k_0^* = 0.176 \times 10^{-3}$ ,  $k_1^* = 6.47 \times 10^{-3}$ , and  $k_2^* = 1.81 \times 10^{-3}$ , respectively. Higher values of  $k_1^*$  were found when the solvent contained an acetate buffer system. It seems as if acetate ions (or perhaps bases in general) catalyse the decomposition of the univalent oxaloacetate ion, but the experimental material is not large and accurate enough to draw a definite conclusion.

Table 8 gives a summary of the catalytic effects of the amines examined. It is seen that they have all a greater effect on the divalent ion than on the

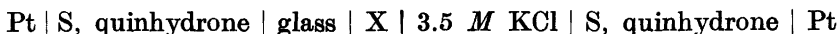
Table 8. Catalytic coefficients.

Catalyst, A <sup>+</sup>	Solvent	$\frac{(k_1^* - 6.47)10^3}{[A^+]}$	$\frac{(k_2^* - 1.81)10^3}{[A^+]}$
NH <sub>4</sub> <sup>+</sup>	Hydrochloric acid	1.47	(32.1)
	Acetate buffer	7.0 to 31.2	32.1
C <sub>2</sub> H <sub>5</sub> NH <sub>3</sub> <sup>+</sup>	Hydrochloric acid	6.3	(45.2)
	Acetate buffer	20.0 to 37.6	45.2 + 27 [Ac <sup>-</sup> ]
C <sub>6</sub> H <sub>5</sub> NH <sub>3</sub> <sup>+</sup>	Hydrochloric acid	404	38 400
	Acetate buffer	4 400	16 400 + 4 800 [Ac <sup>-</sup> ]

univalent ion, and that the anilinium ion is a much more effective catalyst than the ammonium and the ethylammonium ions. The reaction of the univalent oxaloacetate ion is undoubtedly catalysed more strongly by amines in acetate buffers than in solutions of hydrochloric acid. In the amine catalysis of the divalent ion, the effect of acetate ions is less certain, and, with the anilinium ion as catalyst, much lower values were found for acetate buffers than for solutions of hydrochloric acid, a result to which it has not been possible to give any explanation. It may be concluded from the experiments on amine catalysis that the aminium ions form reactive intermediates with both the univalent and the divalent oxaloacetate ion, and that the decarboxylation of (at least) the former is accelerated by acetate ions.

## GLASS-ELECTRODE MEASUREMENTS

The measurements were carried out at the temperature 25° C and by the method described in a previous paper<sup>11</sup>. The cells had the composition



where S denotes 0.00999 M HCl, 0.0900 M NaCl and X the solution under investigation. When  $E$  volts is the e.m.f. of the cell (after subtraction of a small asymmetry potential) and  $[H^+]$  the hydrogen ion concentration of solution X, we have

$$-\log [H^+] = A + 16.90 E \quad (14)$$

Table 9. Determination of  $A$  (equ. 14) for solutions of the composition:  $c$  molar anilinium chloride,  $(0.300 - c)$  molar sodium chloride,  $h$  molar hydrogen chloride at  $25^\circ C$ .

$c$	$h$	$-\log [H^+]$	$E$	$A$	$A$ (mean)
0.1000	0.01921	1.714	-0.0182	2.021	2.023
0.1000	0.00922	2.024	0.00005	2.023	
0.1000	0.004215	2.327	0.0179	2.024	
0.0500	0.00961	2.012	-0.00055	2.022	
0.0500	0.004600	2.316	0.0175	2.020	2.021

By means of this equation we may find  $[H^+]$  when  $A$  is known or  $A$  when  $[H^+]$  is known.

First  $A$  was determined for solutions containing anilinium chloride and small concentrations of hydrogen chloride (Table 9). In the calculation of  $[H^+]$  from the composition of the solutions, it is sufficient to know an approximate value of the dissociation constant of the anilinium ion. The values applied were  $2.33 \times 10^{-5}$  when  $c$  was 0.1 and  $2.26 \times 10^{-5}$  when  $c$  was 0.05.  $A$  calculated by means of equation 14 is shown in the two last columns of the table.

The measurements presented in Table 10 were carried out in order to determine the hydrochloric acid deficiency in a standard 1.000 molar anilinium

Table 10. Electrometric determination of the hydrochloric acid deficiency in a standard solution of anilinium chloride.

$h$ $10^3$	$E$	$[H^+]10^5$	$y$ $10^5$	$y$ $10^5$ (calc.)
-1.006	0.0617	86.0	1.648	1.648
0.000	0.0537	117.4	1.405	1.406
1.024	0.0449	165.3	1.054	1.037
2.047	0.03365	227.8	0.532	0.556

chloride prepared from pure aniline and hydrochloric acid. By dilution of the standard solution were prepared a 0.1000 molar "anilinium chloride", 0.200 molar sodium chloride and solutions containing in addition small concentrations of hydrogen chloride ( $h$  molar) or sodium hydroxide ( $-h$  molar). If a fraction  $\varepsilon$  of the aniline in the standard solution is not neutralised, we have for the diluted solutions, when  $K$  is the dissociation constant of the anilinium ion,

$$[H^+] ([H^+] + 0.1000\varepsilon - h) = K (0.1000(1-\varepsilon) - [H^+] + h)$$

which may also be written in the form

$$y = K - \varepsilon([H^+] + K) \quad (15)$$

where

$$y \equiv ([H^+] + K) ([H^+] - h) / 0.1000 \quad (16)$$

The hydrogen ion concentrations given in the third column of Table 10 are calculated from  $E$  by means of equation 14 and  $A = 2.023$  found in Table 9. Introducing them into equation 16, we calculate  $y$  given in the next to the last column. We get the best agreement with equation 15 when  $K = 2.33 \times 10^{-5}$  and  $\varepsilon = 0.0077$ . When  $y$  is calculated by means of equation 15 and these

Table 11. Determination of the dissociation constant  $K$  of the anilinium ion at 25° C in solutions of the composition:  $d$  molar aniline, 0.0500 molar hydrogen chloride or pyruvic acid, 0.250 molar sodium chloride.

$d$	Hydrogen chloride			Pyruvic acid		
	$E$	$-\log [H^+]$	$-\log K$	$E$	$-\log [H^+]$	$-\log K$
0.0750	0.1382	4.357	4.657	0.1391	4.372	4.663
0.1000	0.1557	4.652	4.652	0.15625	4.662	4.658
0.1500	0.17355	4.954	4.653	0.17385	4.959	4.657
		Mean:	4.654		Mean:	4.659

values of  $K$  and  $\epsilon$ , we obtain the results shown in the last column. As a result of the examination we have found that the standard solution had a hydrochloric acid deficiency of 0.77 % and that  $K = 2.33 \times 10^{-5}$  (compare  $K = 2.16 \times 10^{-5}$  found for 0.300 molar sodium chloride in a previous paper<sup>13</sup>).

In order to examine whether aniline or the anilinium ion form complexes with the pyruvate ion in dilute solution, measurements were carried out on solutions of aniline partly neutralised with either hydrogen chloride or pyruvic acid. The composition of the solutions and the results obtained are shown in Table 11. The dissociation constant of the anilinium ion was calculated from the measurements on the assumption that no complexes are formed. A correction has been made for the concentration of free pyruvic acid by means of the dissociation constant  $6.37 \times 10^{-3}$  for pyruvic acid<sup>11</sup>. Since practically the same values of  $K$  are found for solutions without and with pyruvate, we conclude that complexes are not formed to a measurable extent.

Measurements of the same kind with oxaloacetic acid instead of pyruvic acid were much more difficult and less accurate owing to the rapid decomposition of oxaloacetate in the presence of aniline. An attempt was, however, made (Table 12).  $E_0$  given in the third column of the table was found by

Table 12. Determination of the dissociation constant  $K$  of the anilinium ion at 25° C in solutions of the composition:  $d$  molar aniline,  $x_0$  molar oxaloacetic acid, 0.300 molar sodium chloride.

$d$	$x_0$	$E_0$	$\left(\frac{dE}{dt}\right)_0$	$-\log[H^+]_0$	$[HOx^-]_0$	$[Ox^{2-}]_0$	$-\log K$
0.00999	0.00297	0.1513	0.0014	4.571	0.00038	0.00259	4.67
0.01499	0.00299	0.1691	0.0012	4.872	0.00020	0.00279	4.67
0.01499	0.00600	0.1348	0.0031	4.293	0.00128	0.00471	4.68
0.02000	0.00600	0.1518	0.0026	4.580	0.00074	0.00526	4.69
						Mean:	4.68

extrapolation of the observed  $E$  values to the moment when the oxaloacetic acid was dissolved. The fourth column shows the increase of  $E$  in volts per minute at the beginning of the reaction. The next column gives  $-\log[H^+]_0$  calculated from  $E_0$  and values of  $A$  for 0.3 molar sodium chloride found earlier<sup>11</sup>. The initial concentrations of  $[HOx^-]$  and  $[Ox^{2-}]$  were computed by means of the values  $K_1 = 6.0 \times 10^{-3}$  and  $K_2 = 1.87 \times 10^{-4}$  for the

Table 13. Determination of the dissociation constants of pyruvic and oxaloacetic acid at 25° C from glass electrode measurements on solutions of the composition:  $x_0$  molar pyruvic or oxaloacetic acid,  $b$  molar sodium hydroxide,  $c$  molar anilinium chloride,  $(0.300 - c)$  molar sodium chloride.

$x_0$	$b$	-log $K$ for pyruvic acid			-log $K_1$ for oxaloacetic acid		
		$c = 0.0000$	$c = 0.0500$	$c = 0.1000$	$c = 0.0000$	$c = 0.0250$	$c = 0.0500$
0.0100	0.0000	2.174	2.193	2.191	2.225	2.230	2.234
0.0100	0.0050	2.186	2.199	2.187	2.217	2.224	
0.0200	0.0000	2.175	2.182	2.183			
0.0200	0.0100	2.180	2.191	2.192			
Mean:		2.179	2.191	2.188	2.221	2.227	2.234

first and second dissociation constant of oxaloacetic acid<sup>11</sup>, and the initial concentration of anilinium ion by means of the equation  $[\text{AnH}^+]_0 = [\text{HOx}^-]_0 + 2[\text{Ox}^-]_0 - [\text{H}^+]_0$ . Finally, the values of  $-\log K$  given in the last column of the table were found. They are so close to those found in Table 11 that we conclude, considering the lower accuracy, that the concentrations of the complexes are too small to be estimated by this method.

Table 13 gives a summary of a determination of the dissociation constant of pyruvic acid and the first dissociation constant of oxaloacetic acid in 0.300 molar sodium chloride and in solutions where a part of the sodium chloride had been replaced by anilinium chloride. In the computation it was assumed that the concentration of the complexes was negligible, and the measurements do not disprove this assumption, since the dissociation constants found are nearly independent of the concentration of anilinium ion.

As a result of the electrometric measurements we conclude that possible complexes of aniline or anilinium ion with either pyruvate or oxaloacetate ion are present in such small concentrations that they are undetectable by the method applied here. They were therefore neglected in all the computations in this paper.

#### REFERENCES

1. Pedersen, K. J. *Acta Chem. Scand.* **6** (1952) 285.
2. Widmark, E. and Jeppsson, C. A. *Skand. Arch. Physiol.* **42** (1922) 43.
3. Ljunggren, G. *Katalytisk kolsyreavspjälkning ur ketokarbonsyror* (Diss.) Lund 1925.
4. Beniya, K. J. *Biochem. (Japan)* **20** (1934) 451.
5. Pedersen, K. J. *J. Am. Chem. Soc.* **60** (1938) 595.
6. Westheimer, F. H. and Jones, W. A. *J. Am. Chem. Soc.* **63** (1941) 3283.
7. Kaneko, S. J. *Biochem. (Japan)* **28** (1938) 1.
8. Bessman, S. P. and Layne, E. C. *Arch. Biochem.* **26** (1950) 25.
9. Pedersen, K. J. *J. Phys. Chem.* **38** (1934) 559.
10. Westheimer, F. H. *Ann. N. Y. Acad. Sci.* **39** (1940) 401.
11. Pedersen, K. J. *Acta Chem. Scand.* **6** (1952) 243.
12. Harned, H. S. and Hickey, F. C. *J. Am. Chem. Soc.* **59** (1937) 1284.
13. Pedersen, K. J. *Kgl. Danske Videnskab. Selskab., Mat.-fys. Medd.* **14** (1937) No. 9.

Received February 4, 1954.