265. *The Acid Dissociations of the Keto and Enol Isomers of Oxaloacetic Acid at 25°.*

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The three titration dissociation constants of oxaloacetic acid have been determined from pH measurements with glass electrodes. The first two dissociations are presumably from the carboxyl groups, and the third from the enolic hydroxyl group. The molar extinction coefficients at 280 mμ of the enol forms of this acid have been measured over a range of pH. From a combination of pH and optical-density measurements, the first and second acid dissociation constants of the keto and enol isomers have been resolved, and the third dissociation has been shown to arise only from the enol forms.

Oxaloacetate occupies an important position in the metabolism of several classes of compound, and it is a substrate in many enzyme reactions which are the subject of detailed study. In such studies it is important to have information about the affinity of oxaloacetate both for protons and for metal ions such as Mg^{2+}. In this Paper we report the results of a detailed investigation of the acid dissociation constants of the keto and enol isomers of oxaloacetic acid by a combination of glass-electrode and spectrophotometric measurements. Studies of the magnesium complexes are described in the following Paper.

Oxaloacetic acid can exist in keto and enol forms. The solid is probably the *trans*-enolic isomer whilst its aqueous solutions contain both enolic forms.[1,2] There appears to be no information about the relative concentrations of the *cis*- and the *trans*-isomer in solution. The keto-isomer has not been prepared as a solid. Experiments with oxaloacetic acid are further complicated by its spontaneous decarboxylation in solution to pyruvic acid, especially in the pH region corresponding to its second dissociation.

Titration Constants.—When an oxaloacetic acid solution is titrated with sodium hydroxide, the change of pH observed (Fig. 1) indicates that three protons are liberated from each molecule of oxaloacetic acid. These dissociations may be depicted as follows:

$$K_{a1}' = [H^+][H_2A^-]/[H_3A], \quad (1)$$

$$K_{a2}' = [H^+][HA^{2-}]/[H_2A^-], \quad (2)$$

and

$$K_{a3}' = [H^+][A^{3-}]/[HA^{2-}], \quad (3)$$

where K_{a1}', K_{a2}', and K_{a3}' are the successive titration constants, and $[H_3A]$, $[H_2A^-]$, $[HA^{2-}]$, and $[A^{3-}]$ represent the sums of the concentrations of the undissociated acids and the mono-, di-, and tri-anions, respectively, of all isomeric species of oxaloacetic acid.

[1] Banks, *J.*, 1961, 5043.
[2] Gruber, Pfleiderer, and Wieland, *Biochem. Z.*, 1956, **328**, 245.

The first and second titration constants overlap considerably but the third is well separated from the others. Values of K_{a1}' and K_{a2}' can be obtained by using a modification of Speakman's equation [3] for overlapping dissociations:

$$\frac{\bar{h}[H^+]}{(\bar{h}-1)} = K_{a1}'K_{a2}'\frac{(2-\bar{h})}{[H^+](\bar{h}-1)} - K_{a1}', \quad (4)$$

where $\bar{h}$ is the average number of protons removed per molecule of acid, summed over all isomers. The value of $\bar{h}$ was determined from a knowledge of the stoicheiometry of the

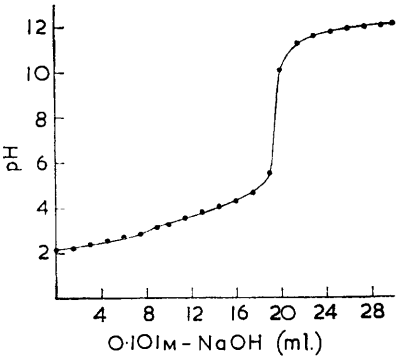


FIG. 1. Titration curve of oxaloacetic acid with NaOH at 25°. Solutions contained 0·132 g. (1 mmole) of oxaloacetic acid in 40 ml. of 0·1M-KCl. The first 7 points were obtained from one solution and the last 8 were obtained from another solution. The 7 points in the middle of the curve were each obtained from a separate solution.

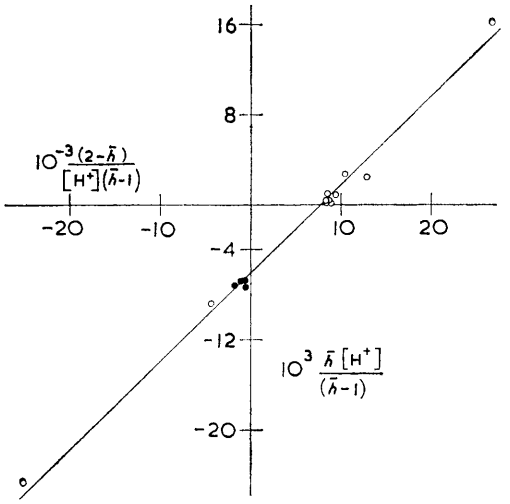


FIG. 2. Speakman plot for resolution of the first and second titration constants of oxaloacetic acid at 25 [equation (4)]. ○ Points from experiment (*a*) of Table 1. The points ● denote two indistinguishable observations.

solutions, and the hydrogen-ion concentration was calculated from the experimentally determined pH values by using the relation $[H^+] = \text{antilog}\,[-\text{pH} - \log\gamma_{H^+}]$, where γ_{H^+} was assumed equal to $\gamma_{\pm}(HCl)$ at the relevant ionic strength. Values of $\gamma_{\pm}(HCl)$ were calculated from the data of Bates and Bower.[4] An example of a plot of the variables of equation (4) is shown in Fig. 2. The third titration constant, K_{a3}', was evaluated from the relationship:

$$pK_{a3}' = (\text{pH} + \log\gamma_{H^+}) - \log([Na^+] - 2A_T - [OH^-])/(3A_T - [Na^+] + [OH^-]), \quad (5)$$

where A_T is the total concentration of oxaloacetic acid of all species. The hydroxide-ion concentration was found from the relationship,

$$[OH^-] = \text{antilog}\,(\text{pH} - pK_w - \log\gamma_{\pm}),$$

[3] Speakman, *J.*, 1940, 855.
[4] Bates and Bower, *J. Res. Nat. Bur. Stand.*, 1954, **53**, 283.

where pK_w is the negative logarithm of the ionic product of water calculated from Robinson and Stokes's equation.[5] The values of the titration dissociation constants found are given in Table 1.

TABLE 1.

Titration dissociation constants of oxaloacetic acid at 25° and $I \sim 0{\cdot}1$.

All solutions were initially 40 ml. 0·1M-KCl to which weighed amounts of solid oxaloacetic acid were added. Titration was with 0·1M-NaOH. K_{a1}' and K_{a2}' were obtained using equation (4).

	Total oxaloacetic acid (mmoles)	No. of points	Range of $\bar{h}$	$10^3K_{a1}'$	$10^4K_{a2}'$
(*a*)	1·0	21	0·48—1·62	6·04	1·29
				$pK_{a1}' = 2{\cdot}22$	$pK_{a2}' = 3{\cdot}89$

K_{a3}' was obtained using equation (5).

	Total oxaloacetic acid (mmoles)	No. of points	$10^{14}K_{a3}'$	pK_{a3}'
(*b*)	1·0	7	Av. 8·69	13·06 ± 0·13
(*c*)	—	—	—	13·03 ± 0·11

In (*a*) the first 11 points were from two different solutions, the others were all from individual solutions. In (*b*) all the points were from one solution. (*c*) Values from optical density measurements, see Table 4.

Keto-Enol Tautomerism.—Since, in aqueous solution, oxaloacetic acid and its anions can exist as both keto and enol isomers,[1,2] separate dissociation constants can be defined for each isomer. For the keto isomer:

$$K_{a1K}' = [H^+][HA_K^-]/[H_2A_K], \text{ and } K_{a2K}' = [H^+][A_K^{2-}]/[HA_K^-];$$

and for the enol isomers,

$$K_{a1E}' = [H^+][H_2A_E^-]/[H_3A_E], \quad K_{a2E}' = [H^+][HA_E^{2-}]/[H_2A_E^-],$$

and

$$K_{a3E}' = [H^+][A_E^{3-}]/[HA_E^{2-}],$$

where the subscripts K and E denote the keto and enol isomers, respectively. The assumption is made here that only the enol isomer is able to dissociate to give a third proton, and therefore the keto species are shown with one less hydrogen atom.

Further, the isomers of the various protonated species are related by the tautomeric equilibrium constants:

$$K_{T1} = [H_2A_K]/[H_3A_E], \quad (6)$$

$$K_{T2} = [HA_K^-]/[H_2A_E^-], \quad (7)$$

and

$$K_{T3} = [A_K^{2-}]/[HA_E^{2-}]. \quad (8)$$

From these definitions of the keto and enol dissociation constants and the tautomeric equilibrium constants, the titration constants may be defined as follows:

$$K_{a1}' = \frac{[H^+]([HA_K^-] + [H_2A_E^-])}{[H_2A_K] + [H_3A_E]} = \frac{K_{a1K}'K_{T1} + K_{a1E}'}{1 + K_{T1}}, \quad (9)$$

$$K_{a2}' = \frac{[H^+]([A_K^{2-}] + [HA_E^{2-}])}{[HA_K^-] + [H_2A_E^-]} = \frac{K_{a2K}'K_{T2} + K_{a2E}'}{1 + K_{T2}}, \quad (10)$$

and

$$K_{a3}' = \frac{[H^+][A_E^{3-}]}{[A_K^{2-}] + [HA_E^{2-}]} = \frac{K_{a3E}'}{1 + K_{T3}}. \quad (11)$$

From equations (9) and (10) it is evident that the first two titration constants, K_{a1}' and K_{2a}', combine the corresponding dissociation constants for the keto and enol isomers and the appropriate tautomeric equilibrium constants. Equation (11) shows that the third titration constant, K_{a3}', combines only the third dissociation constant of the enol and the

[5] Robinson and Stokes, "Electrolyte Solutions," Butterworths, London, 1955, p. 496.

tautomeric equilibrium constant, K_{T3}, for the di-anions; this is a consequence of the assumption that only the enol isomers can dissociate to give a third proton.

The ultraviolet absorption spectra of the keto and the enol forms of oxaloacetic acid in water are very different. Thus, at 280 mμ the enol forms have a molar extinction coefficient about 140 times greater than for the keto form. Therefore, if the relevant molar extinction coefficients are known, the equilibrium optical densities of oxaloacetate solutions at different pH values, together with the values of the titration dissociation constants, will give sufficient information to allow the calculation of the individual dissociation constants of the keto and enol isomers.

To determine the molar extinction coefficient of the enol forms ($\varepsilon_{E\,280\,m\mu}$), small volumes of fresh oxaloacetic acid in anhydrous methanol at −15° were added to various aqueous buffer solutions in the cuvette of a recording spectrophotometer at 2°, and the change of optical density at 280 mμ with time was recorded. The solid oxaloacetic acid used to make the methanolic solution was enolic [1,6] and the rate of isomerisation at −15° is negligible. Therefore extrapolation of the optical density readings to zero time should

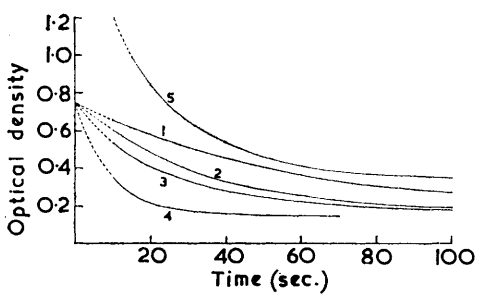


FIG. 3.

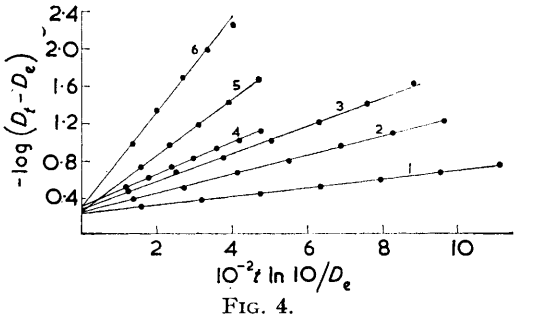


FIG. 4.

FIG. 3. Changes, with time, in optical density at 280 mμ of oxaloacetic acid solutions at 2°.

Curves 1–3: $2{\cdot}0 \times 10^{-4}$M-oxaloacetic acid in triethanolamine–HCl buffer (pH 7·4) with 0, $1{\cdot}67 \times 10^{-3}$, and $5{\cdot}0 \times 10^{-3}$M-$MgCl_2$, respectively. Curve 4: $2{\cdot}0 \times 10^{-4}$M-oxaloacetic acid in triethanolamine–HCl buffer (pH 8·4). Curve 5: $5{\cdot}0 \times 10^{-4}$M-oxaloacetic acid in $5{\cdot}0 \times 10^{-3}$M-NaOH.

FIG. 4. Determination of the molar extinction coefficient of enolic oxaloacetic acid at 2°. Plots of the variables of equation (12).

Plots 1–4: $2{\cdot}0 \times 10^{-4}$M-oxaloacetic acid in triethanolamine–HCl buffer (pH 7·4) with 0, $1{\cdot}67 \times 10^{-3}$, $3{\cdot}33 \times 10^{-3}$, and $5{\cdot}0 \times 10^{-3}$M-$MgCl_2$, respectively. Plots 5 and 6: $2{\cdot}0 \times 10^{-4}$M-oxaloacetic acid in triethanolamine–HCl buffer (pH 8·4) with 0 and $8{\cdot}3 \times 10^{-4}$M-$MgCl_2$, respectively.

give the value for the enol forms. The measurements were made at 2° because at higher temperatures the rate of isomerisation was so great as to make the results inaccurate. Some measurements were also made in buffer solutions containing magnesium chloride, to determine the effect of Mg^{2+} ions on ε_E. Examples of these optical density records are shown in Fig. 3, from which it is apparent that accurate extrapolation to zero time is difficult. To overcome this difficulty, these plots were converted into straight lines by application of the equation describing first-order kinetics of reversible reactions. From this we obtain the relation:

$$\log (D_t - D_e) = -kD_0 t \ln 10/D_e + \log (D_0 - D_e), \qquad (12)$$

where D_t and D_e are the observed optical densities at time t sec. and at equilibrium, respectively, D_0 is the optical density at time zero, which is equal to the optical density of the solution of the enol forms, and k is the first-order rate constant for enolisation. Plots of $\log (D_t - D_e)$ against $t \ln 10/D_e$ gave straight lines, as shown in Fig. 4. Since D_e

[6] Meyer, *Ber.*, 1912, **45**, 2860.

TABLE 2.

Molar extinction coefficient at 280 mμ of the enol form of oxaloacetic acid and oxaloacetate at 2°. $\varepsilon_{E\ 280\ m\mu}$ was obtained from plots of the variables of equation (12).

Buffer system $I \sim 0{\cdot}1$	$MgCl_2$ (M × 10^3)	Oxaloacetic acid (M × 10^4)	pH *	$\varepsilon_{E\ 280\ m\mu}$
HCl	—	2·0	1·1	2463
,,	—	5·0	1·1	2855
HCl–KCl	—	2·0	1·5	2315
,,	—	2·0	1·8	2622
Triethanolamine–HCl	—	2·0	7·4	3551
,, ,,	—	2·0	7·4	3769
,, ,,	1·67	2·0	7·4	3836
,, ,,	3·33	2·0	7·4	3684
,, ,,	5·0	2·0	7·4	3476
,, ,,	—	2·0	8·4	3590
,, ,,	0·83	2·0	8·4	3452
NaOH–KCl	—	5·0	~11·5	3410
,,	—	2·0	11·94	3438
,,	—	5·0	11·94	3866
	Av. excluding values in HCl and in HCl–KCl			3607
Diethyl ether	—	2·0	—	3550
	α-Oxoglutarate			
Triethanolamine–HCl	—	40·0	8·4	26

* The pH values of the buffers were measured at 25°.

TABLE 3.

First and second dissociation constants of the keto and enol isomers of oxaloacetic acid and the second tautomeric equilibrium constant from equilibrium optical density measurements at 25° and $I = 0{\cdot}1$. The pH values of the buffers were checked by glass-electrode measurements.

Buffer system	pH	Oxaloacetic acid (M × 10^3)	$10^3 D_c$ (280 mμ)	$10^3 K_{a1K}'$	$10^4 K_{a2K}'$	$10^2 K_{a1E}'$	$10^4 K_{a2E}'$	K_{T2}
HCl	1·1	0·56	115	—	—	—	—	—
,,	1·1	1·12	227	—	—	—	—	—
,,	1·1	2·24	460	—	—	—	—	—
HCl–KCl	1·2	0·56	114	—	—	—	—	—
,,	1·2	1·12	225	—	—	—	—	—
,,	1·2	2·24	463	—	—	—	—	—
HCl–KCl	1·5	0·56	116	6·03	1·35	0·68	3·76	(16·96)
,,	1·5	1·12	235	5·98	1·36	0·75	3·43	(15·31)
,,	1·5	2·24	483	5·88	1·39	0·89	2·86	(12·56)
HCl–KCl	1·8	0·56	133	5·79	1·22	1·09	2·04	10·20
,,	1·8	1·12	258	5·84	1·21	0·98	2·26	(11·40)
,,	1·8	2·24	517	5·84	1·21	0·98	2·25	(11·33)
Glycine–HCl	2·2	0·56	152	5·78	1·22	1·12	1·98	9·88
,,	2·2	1·12	302	5·78	1·12	1·10	2·01	10·01
,,	2·2	2·24	617	5·76	1·23	1·15	1·93	9·58
Glycine–HCl	2·6	0·56	195	5·67	1·25	1·32	1·68	8·19
,,	2·6	1·12	380	5·69	1·24	1·27	1·74	8·53
,,	2·6	2·24	743	5·71	1·24	1·23	1·80	9·58
Glycine–HCl	3·0	0·56	263	5·48	1·30	1·67	1·33	8·19
,,	3·0	1·12	501	5·53	1·28	1·57	1·41	8·53
,,	3·0	2·24	998	5·54	1·28	1·56	1·42	8·84
AcOH–NaAc	4·0	1·33	655	5·59	1·26	1·47	1·51	7·29
,,	4·0	2·66	1310	5·59	1·26	1·47	1·51	7·29
AcOH–NaAc	4·4	0·66	343	5·55	1·27	1·53	1·44	6·91
,,	4·4	1·33	688	5·53	1·28	1·55	1·43	6·80
AcOH–NaAc	4·8	0·66	351	5·52	1·28	1·63	1·36	6·45
,,	4·8	1·33	701	5·52	1·28	1·61	1·37	6·53
AcOH–NaAc	5·2	0·66	351	5·59	1·26	1·52	1·46	7·01
,,	5·2	1·33	701	5·59	1·26	1·48	1·50	7·23
AcOH–NaAc	5·6	0·66	351	—	—	—	—	—
,,	5·6	1·33	702	—	—	—	—	—
$N(CH_2{\cdot}CH_2{\cdot}OH)_3$–HCl	7·4	0·66	352	—	—	—	—	—
,,	7·4	1·33	702	—	—	—	—	—
$N(CH_2{\cdot}CH_2{\cdot}OH)_3$–HCl	8·4	0·66	358	—	—	—	—	—
,,	8·4	1·33	715	—	—	—	—	—
		Averages		5·69	1·26	1·29	1·89	8·17
		± s.d.		0·16	0·04	0·30	0·66	1·28

is known, D_0 can be obtained from the intercept on the log $(D_t - D_e)$ axis. The values of $\varepsilon_{E\,280\,m\mu}$ obtained under different conditions are shown in Table 2, from which it can be seen that the value of $\varepsilon_{E\,280\,m\mu}$ is unaffected by the presence of Mg^{2+} ions and is fairly constant except in very acid solutions. The latter results are probably inaccurate because of the very high ketonisation rates in these solutions. The average value, $\varepsilon_{E\,280\,m\mu} = 3607$ at 2°, did not differ significantly from the value in ether, 3550, in which solvent oxaloacetic acid is enolic [7] and presumably fully protonated.

The molar extinction coefficient of the keto form of oxaloacetic acid and oxaloacetate at 280 mμ ($\varepsilon_{K\,280\,m\mu}$) was assumed to be equal to that (26) of the next higher homologue α-oxogluratic acid.

Measurements were then made at 25° of the equilibrium optical densities at 280 mμ of oxaloacetate at different pH values, using the same technique. In the pH region corresponding to the dissociation of the second proton, the equilibrium optical densities showed a tendency to decrease linearly with time, after the initial rapid decrease. This was presumably due to decarboxylation to pyruvate. For these solutions, the linear parts of the optical-density records were extrapolated to zero time to give the true D_e. The values of D_e found at different pH values are shown in Table 3.

From these data the values of the three tautomeric equilibrium constants were calculated as follows. An overall tautomeric equilibrium constant, K_T, is defined, $K_T = A_K/A_E$.

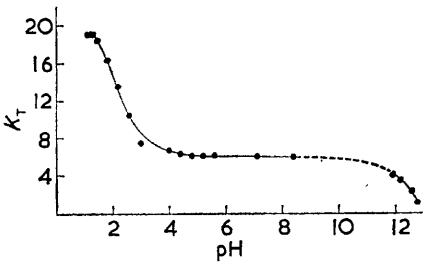


FIG. 5. The dependence on pH of the overall keto–enol tautomeric equilibrium constant (K_T = [total keto]/[total enol]) of oxaloacetic acid at 25°.

The total concentrations of the keto and enol species were calculated as described in the text from the data given in Table 3.

where A_K and A_E represent the sums of the concentrations of all keto species and all enol species of oxaloacetic acid, respectively. In any given solution, $A_K = (A_T\varepsilon_{E\,280\,m\mu} - D_e)/(\varepsilon_{E\,280\,m\mu} - \varepsilon_{K\,280\,m\mu})$ and $A_E = A_T - A_K$, where A_T is the total concentration of oxaloacetic acid of all species. The variation of K_T with pH at 25° is shown in Fig. 5. There are two regions where K_T is invariant with pH, first below pH 1·2 where K_T may be identified with K_{T1}, and secondly in the range pH 5·2—8·4 where K_T may be identified with K_{T3}.

From the values of the titration dissociation constants, K_{a1}' and K_{a2}', and the tautomeric equilibrium constants, K_{T1} and K_{T3}, the values of the second tautomeric equilibrium constant, K_{T2}, and the separate dissociation constants for the keto and enol isomers, K_{a1K}', K_{a2K}', K_{a1E}', and K_{a2E}' may be calculated as follows. At pH values below 8·4, the conservation equations are

$$A_T = [H_2A_K] + [HA_K^-] + [A_K^{2-}] + [H_3A_E] + [H_2A_E^-] + [HA_E^{2-}]; \quad (13)$$

$$A_K = [H_2A_K] + [HA_K^-] + [A_K^{2-}], \quad (14)$$

and

$$A_E = [H_3A_E] + [H_2A_E^-] + [HA_E^{2-}]. \quad (15)$$

From equations (9), (10), and (13), the total concentration of undissociated acid can be obtained:

$$([H_2A_K] + [H_3A_E]) = A_T/(1 + K_{a1}'/[H^+] + K_{a1}'K_{a2}'/[H^+]^2). \quad (16)$$

The total concentration of mono-anions $([HA_K^-] + [H_2A_E^-])$ can be obtained from

[7] Hantzsch, *Ber.*, 1915, **48**, 1407.

equations (9) and (16), and then that of the di-anions ($[A_K^{2-}] + [HA_E^{2-}]$) using equation (10). Rearrangement of equation (6) gives

$$[H_3A_E] = ([H_2A_K] + [H_3A_E])/(1 + K_{T1}),$$

which together with equation (16) gives the individual concentrations of the two undissociated isomeric forms $[H_2A_K]$ and $[H_3A_E]$. Similarly, equation (8) gives

$$[HA_E^{2-}] = ([A_K^{2-}] + [HA_E^{2-}])/(1 + K_{T3}),$$

from which the individual concentrations of the di-anions $[A_K^{2-}]$ and $[HA_E^{2-}]$ can be obtained. The individual concentrations of the mono-anions ($[HA_K^-]$ and $[H_2A_E^-]$) are now obtainable from the conservation equations (14) and (15).

The second tautomeric equilibrium constant, K_{T2}, can be calculated from equation (7), and the individual dissociation constants K_{a1K}', K_{a2K}', K_{a1E}', and K_{a2E}' from the concentrations of the relevant species. Values for the second tautomeric equilibrium constant and the first and second dissociation constants of the keto and enol isomers of oxaloacetic acid are shown in Table 3.

It has been assumed above that the oxaloacetate tri-anion is entirely in the enolic form. To test this, the third titration dissociation constant K_{a3}' was determined from a combination of pH and optical-density (at 280 mμ) measurements on equilibrium solutions of oxaloacetic acid in excess sodium hydroxide at ionic strength 0·1 at 25°. In these solutions the only oxaloacetate species assumed to be present were A_K^{2-}, HA_E^{2-}, and A_E^{3-}. From D_e and A_T the concentration of the keto isomer $[A_K^{2-}]$ was found and inserted into equation (8) together with $K_{T3} = 6·09$, this enabled $[HA_E^{2-}]$ to be calculated. Then $[A_E^{3-}]$ could be found from the conservation equation. These concentrations of oxaloacetate species were combined with $[H^+]$ in equation (3) to give K_{a3}'. The values found are shown in Table 4; they are in excellent agreement with those found from potentiometric titration using equation (5). The individual third dissociation constant of the enolic oxaloacetic acid, K_{a3E}', can be seen from equation (11) to equal $K_{a3}'(1 + K_{T3})$; its value is given in Table 4.

TABLE 4.

Third dissociation constant of oxaloacetic acid from equilibrium optical density measurements at 280 mμ, at 25°.

Buffer system: NaOH–KCl; $I = 0·1$.

K_{a3}' is the third " overall " dissociation constant and K_{a3E}' is the third dissociation constant of the enol [equation (11)].

pH *	Oxaloacetic acid (M × 10^3)	10^3D_e	$10^{14}K_{a3}'$	$10^{13}K_{a3E}'$
11·90	0·66	532	(15·2)	(10·8)
11·90	1·33	975	11·2	7·91
12·17	0·66	571	10·02	7·10
12·17	1·33	1083	8·50	6·03
12·17	2·66	1971	6·14	4·35
12·57	0·27	353	11·8	8·40
12·57	0·66	760	8·42	5·97
12·57	1·33	1482	7·94	5·63
12·81	0·27	542	(18·7)	(13·3)
12·81	0·66	1182	13·3	9·43

Average of 8 results: $K_{a3}' = 9·67 \times 10^{-14}$; $K_{a3E}' = 6·85 \times 10^{-13}$.

* Calculated from Bates, Pinching, and Smith, *J. Res. Nat. Bur. Stand.*, 1950, **45**, 418.

The values of the acid dissociation and tautomeric equilibrium constants, together with their standard errors, are summarised in Table 5.

TABLE 5.

Summary of the acid dissociation constants and tautomeric equilibrium constants of the keto and enol isomers of oxaloacetic acid at 25° and $I \sim 0{\cdot}1$.

	pK_{a1K}'	pK_{a2K}'	pK_{a1E}'	pK_{a2E}'	pK_{a3E}'	K_{T1}	K_{T2}	K_{T3}
	2·24	3·90	1·89	3·72	12·18	19·10	8·17	6·09
± s.d.	—	—	—	—	0·11	—	1·28	—

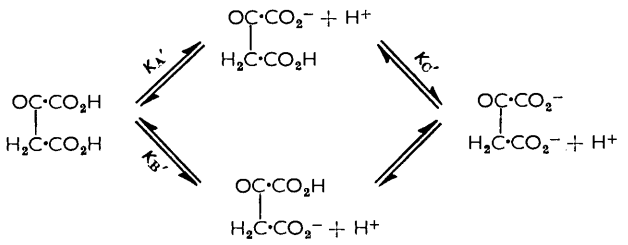
DISCUSSION

The first and second titration dissociation constants at 25° and $I \sim 0{\cdot}1$ found in this work, $pK_{a1}' = 2{\cdot}22$ and $pK_{a2}' = 3{\cdot}89$, may be compared with the values for the same constants found by Pedersen [8]: $pK_{a1}' = 2{\cdot}31$ and $pK_{a2}' = 3{\cdot}89$, these last values being calculated from Pedersen's equations for the variation of the dissociation constants with ionic strength. The agreement between these two sets of values is satisfactory. Pedersen's values were obtained from cells containing glass electrodes and 3·5M-potassium chloride salt bridges to quinhydrone–hydrochloric acid reference electrodes. For the first dissociation, measurements were mostly made with solutions containing oxaloacetic acid and sodium chloride only and corrections were applied for the dissociation of the second proton. In the present work all the measurements used in evaluating these constants were on solutions containing oxaloacetic acid, sodium hydroxide, and potassium chloride, and a graphical method was used to resolve K_{a1}' and K_{a2}'. Pederson did not consider the third dissociation of oxaloacetic acid or the difference between the keto and enol isomers.

The molar extinction coefficient of the enol isomer appears to be unaffected by the degree of dissociation of the protons. Thus we find $\varepsilon_{E\,280\,m\mu} = 3550$ in ether, and an average value of 3607 in water at pH 7·4—11·9. These values are somewhat higher than $\varepsilon_{E\,280\,m\mu} = 3320$ in ether reported by Banks.[1] The value of the ratio of the keto : enol di-anions, $K_{T3} = 6{\cdot}09$, gives 14·1% enol at pH 5·2—8·4. This is slightly lower than Banks's value of 15·5—16%, the difference being due to the higher $\varepsilon_{E\,280\,m\mu}$ found here.

Evidence for the correctness of our value of $\varepsilon_{E\,280\,m\mu}$ and the assumption that it does not change between 2 and 25° is afforded by the excellent agreement between the values of pK_{a3}' obtained independently from glass-electrode and optical-density measurements, 13·06 and 13·03, respectively.

The results given in Table 5 show that the first acid dissociation is stronger in the enol than in the keto isomers ($pK_{a1K}' - pK_{a1E}' = 0{\cdot}35$). For the second dissociation the enol is again stronger but the difference is less ($pK_{a2K}' - pK_{a2E}' = 0{\cdot}18$). It should, however, be remembered that even these constants for the keto and enol isomers are composite ones. Thus, for example, there are two routes by which the di-anion of the keto isomer can be formed:



The intrinsic dissociation constants K_A', K_B', K_C', and K_D' are related to the measured dissociation constants K_{a1K}' and K_{a2K}' as follows:

$$K_{a1K}' = K_A' + K_B' \quad \text{and} \quad 1/K_{a2K}' = 1/K_C' + 1/K_D'.$$

[8] Pedersen, *Acta Chem. Scand.*, 1962, **6**, 243.

Similar sets of intrinsic dissociation constants exist for the *cis*- and the *trans*-enol isomers. Therefore, the measured dissociation constants K_{a1E}' and K_{a2E}' are complex constants combining the intrinsic dissociation constants of both the enol isomers and the *cis–trans*-tautomeric equilibrium constants. The present work cannot, of course, give any information about these intrinsic constants.

The greater error in the values of the first and second enolic dissociation constants than in those for the keto isomer is presumably due to the smaller amount of enolic isomers present in the solutions. As mentioned above, the amount of enolic oxaloacetic acid below pH 8·4 is less than 15% of the total.

Experimental

Oxaloacetic Acid.—Some of the oxaloacetic acid was prepared by the method of Wohl and Oesterlin [9] and some was purchased from L. Light & Co.* The two samples were combined and recrystallised from 1 : 4 (v/v) acetone : chloroform and dried (P_2O_5). The m. p. was approximately 151°, which is in the range reported by others.[1,2] The pH titration curve shows a marked inflexion at a point corresponding to the expected equivalent weight (Fig. 1). Detailed analysis by this method is difficult because of the decarboxylation occurring at these pH values.

Carbonate-free Sodium Hydroxide Solution.—This was made by diluting the carbonate-free clear supernatant from a saturated sodium hydroxide solution, followed by standardisation against constant-boiling hydrochloric acid.

Potassium Chloride.—This was prepared as described previously.[10]

Titrations.—The electrometric titrations were carried out in a cell comprising a saturated calomel electrode and a glass electrode (EIL type GHS 23). The measurements were made at 25° using a precision laboratory-built pH meter. The cell was standardised with 0·05M-potassium hydrogen phthalate (pH 4·01) and 0·01M-borax (pH 9·18). Only part of the total dissociation range was covered in each titration. The potassium chloride solution (0·1M) with or without added sodium hydroxide solution, depending on the pH range to be covered, was equilibrated at 25° in a thermostatically-controlled liquid paraffin bath. Solid oxaloacetic acid, in an open glass container, was then dropped in, rapidly dissolved, and pH measurements were commenced. Carbon dioxide-free nitrogen was bubbled through the solution during the titration, to achieve the required stirring and to keep out atmospheric carbon dioxide. The titration vessel was closed with a stopper carrying the two electrodes, the tube from the burette, and the nitrogen bubbler. To improve the mixing of the solution during the titration, a small glass stirrer operated by a battery-driven motor was also used. In the pH range corresponding to the first dissociation (pH 2—3), the decarboxylation was so slight that, if it occurred at all, no change in pH due to it could be observed. This also largely applies to the third dissociation range (pH 10—12). However, in the range corresponding to the second dissociation (pH 3—~5) the decarboxylation was fairly rapid, the rate increasing with increase in the pH. To allow for this, the pH measurements corresponding to each point on the titration curve were carried out over a period of time and extrapolated to zero time, *i.e.*, the moment when oxaloacetic acid was dissolved. The concentration of oxaloacetic acid in each titration was ~0·02M (*i.e.*, 1 mmole added to ~50 ml. of solution).

Optical Density Measurements.—The measurements at 2° were made on an Optica CF4 recording spectrophotometer fitted with a thermostatically-controlled cell holder. Dry nitrogen was blown over the cells to prevent condensation. The equilibrium optical density measurements at 25° were made on a Hilger Uvispek spectrophotometer also fitted with a thermostatically-controlled cell holder.

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[9] Wohl and Oesterlin, *Ber.*, 1901, **34**, 1139.
[10] Datta and Grzybowski, *J.*, 1962, 3068.