266. *The Stability Constants of the Magnesium Complexes of the Keto and Enol Isomers of Oxaloacetic Acid at 25°.*

By S. S. Tate, A. K. Grzybowski, and S. P. Datta.

The stability constants for the 1 : 1 oxaloacetate di-anion : magnesium complexes have been determined, for both the keto and enol isomers of the ligand, by a combination of pH and optical-density measurements. The successive stability constants for the 1 : 1 and 2 : 1 oxaloacetate tri-anion : magnesium complexes have been determined by pH measurements.

In a previous paper[1] we showed that three protons are removed from oxaloacetic acid during titration with sodium hydroxide. These three titration dissociation constants have the following pK_a' values at 25° and $I \sim 0{\cdot}1$; $pK_{a1}' = 2{\cdot}22$; $pK_{a2}' = 3{\cdot}89$; $pK_{a3}' = 13{\cdot}06$. When oxaloacetic acid is titrated with sodium hydroxide in the presence of magnesium chloride a depression of the pH is observed; this is small in the region of the second dissociation but large in the region of the third dissociation. Examples of such titration curves are shown in Figs. 1 and 2. Because decarboxylation of oxaloacetate occurs in solution, particularly in the presence of Mg^{2+} ions, many of the points on the titration curve had to be obtained separately from fresh solutions.

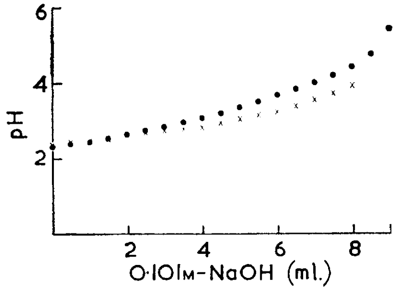

Fig. 1. Titration of the first two protons of oxaloacetic acid with NaOH in the presence of KCl and $MgCl_2$ at 25°, $I \sim 0{\cdot}1$.

Initially the solutions contained 0·5 mmole of oxaloacetic acid:

● in 40 ml. of 0·101M-KCl;

× with 1·0 mmole of $MgCl_2$ in 40 ml. of 0·025M-KCl.

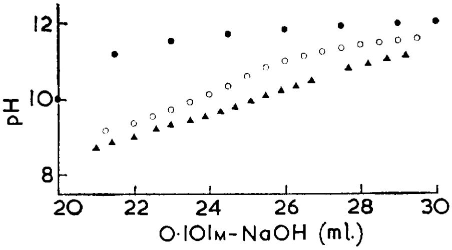

Fig. 2. Titration of the third proton of oxaloacetic acid with NaOH in the presence of KCl and $MgCl_2$ at 25°, $I \sim 0{\cdot}1$.

Initially all solutions contained 1·0 mmole of oxaloacetic acid:

● and 4·0 mmoles of KCl in 40 ml. of water;

○ and 0·33 mmoles of $MgCl_2$, 4·0 mmoles of KCl, in 50 ml. of water;

▲ and 0·5 mmoles of $MgCl_2$, 3·32 mmoles of KCl, in 48·3 ml. of water.

In the evaluation of the stability constants of the magnesium–oxaloacetate complexes the following acid dissociation equilibria have to be considered:

$$H_3A \rightleftharpoons H_2A^- + H^+,\ H_2A^- \rightleftharpoons HA^{2-} + H^+,\text{ and } HA^{2-} \rightleftharpoons A^{3-} + H^+,$$

for which the titration dissociation constants are defined as follows:

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

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

and

$$K_{a3}' = [H^+][A^{3-}]/[HA^{2-}]. \tag{3}$$

Complexes with Oxaloacetate Di-anion.—In the region below pH 5 (Fig. 1) it was

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[1] Tate, Grzybowski, and Datta, preceding paper.

assumed that the only magnesium complex formed was with the HA^{2-} ion, for which the equilibrium may be written:

$$Mg^{2+} + HA^{2-} \rightleftharpoons MgHA. \tag{4}$$

Since there is no depression of the pH in the presence of magnesium chloride in the region of the first dissociation of oxaloacetic acid, presumably no complex forms with the mono-anion. The stability constant for equilibrium (4), $K_{1,1}$, is defined by:

$$K_{1,1} = [MgHA]/[Mg^{2+}][HA^{2-}]. \tag{5}$$

Below pH 5 the conservation equations are: (6) for the total oxaloacetic acid, and (7) for the total metal.

$$A_T = [H_3A] + [H_2A^-] + [HA^{2-}] + [MgHA] \tag{6}$$

$$Mg_T = [Mg^{2+}] + [MgHA]. \tag{7}$$

The electroneutrality condition, ignoring $[OH^-]$, is

$$[Na^+] + [H^+] + 2[Mg^{2+}] = [H_2A^-] + 2[HA^{2-}] + [Cl^-]. \tag{8}$$

From equations (1), (2), (5), (6), (7), and (8), the concentrations $[H_3A]$, $[H_2A^-]$, $[HA^{2-}]$, $[MgHA]$, and $[Mg^{2+}]$ can be calculated and hence values for $K_{1,1}$ obtained. The values of log $K_{1,1}$ obtained from two titration curves are shown in Table 1, the average value being log $K_{1,1} = 1·96$.

TABLE 1.

The overall stability constant for the complex between Mg^{2+} ions and oxaloacetate di-anions; $I \sim 0·1$, 25°.

Each solution finally contained 1·0 mmole KCl, 1·0 mmole $MgCl_2$, 0·5 mmole oxaloacetic acid, and NaOH. The final volume was 40 ml. plus the volume of NaOH shown. The pH was measured as described in the text.

$$K_{1,1} = [MgHA]/[Mg^{2+}][HA^{2-}].$$

0·101M-NaOH (ml.)	pH	log $K_{1,1}$	0·101M-NaOH (ml.)	pH	log $K_{1,1}$	0·101M-NaOH (ml.)	pH	log $K_{1,1}$
5·75	3·19	2·05	7·0	3·55	1·95	8·0	3·93	1·78
6·0	3·24	2·09	7·0	3·56	1·94	8·0	3·87	1·92
6·5	3·39	2·02	7·25	3·64	1·89	Average log $K_{1,1}$ = 1·96 ± 0·09		
6·5	3·38	2·03	7·5	3·72	1·89			
			7·5	3·69	1·95			

The possibility remains of the formation of a 2 : 1 ligand : metal complex by the equilibrium,

$$MgHA + HA^{2-} \rightleftharpoons Mg(HA)_2^{2-}, \tag{9}$$

for which the stability constant, $K_{1,2}$, is defined as follows:

$$K_{1,2} = [Mg(HA)_2^{2-}]/[MgHA][HA^{2-}]. \tag{10}$$

To exclude this possibility, the results were inserted in Irving and Rossotti's equation,[2]

$$\bar{n}/(\bar{n} - 1)[HA^{2-}] = (2 - \bar{n})[HA^{2-}]K_{1,1}K_{1,2}/(\bar{n} - 1) - K_{1,1}, \tag{11}$$

where $\bar{n}$ is the mean number of ligand molecules bound per metal atom. The resulting plot is shown in Fig. 3. The slightly negative slope shows that $K_{1,2}$ is virtually zero; we are therefore correct in the assumption that only the 1 : 1 ligand–metal complex shown in equilibrium (4) need be considered, and that shown in equilibrium (9) is negligible.

Complexes with Oxaloacetate Tri-anion.—Preliminary inspection of the data at pH

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[2] Irving and Rossotti, *J.*, 1953, 3397.

values above 8 (Fig. 2) shows that at least two complexes must be formed between Mg^{2+} and A^{3-} ions according to the following equilibria:

$$Mg^{2+} + A^{3-} \rightleftharpoons MgA^{-}, \qquad (12)$$

and

$$MgA^{-} + A^{3-} \rightleftharpoons MgA_2^{4-}. \qquad (13)$$

The stability constants for equilibria (12) and (13) may be defined as follows:

$$K_{2,1} = [MgA^{-}]/[Mg^{2+}][A^{3-}], \qquad (14)$$

and

$$K_{2,2} = [MgA_2^{4-}]/[MgA^{-}][A^{3-}]. \qquad (15)$$

The evaluation of $K_{2,1}$ and $K_{2,2}$ presents considerable difficulties, since at the point on the titration curve corresponding to the complete removal of two protons from the oxaloacetic acid a large proportion of the magnesium in the solution is present as the complex MgHA.

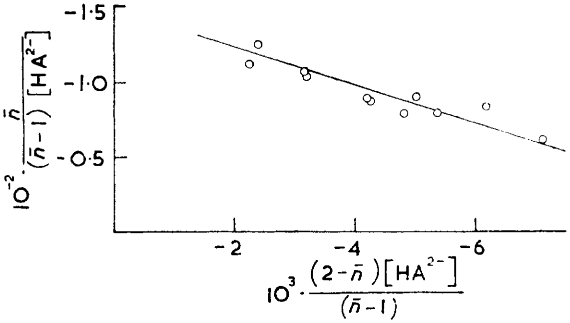

FIG. 3. Magnesium complex of oxaloacetate di-anion. Plot of the variables of equation (11). The points were calculated from the data in Table 1.

Therefore the formation of the complex MgA^{-} shown in equilibrium (12) must involve either the transfer of magnesium ions from the MgHA complex or the ionization of a proton from the latter species. Thus linear equations such as those of Irving and Rossotti[2] or Rossotti and Rossotti[3] for the resolution of successive stability constants cannot be applied.

At pH values above 8, $\bar{n}$ is defined by:

$$\bar{n} = ([MgHA] + [MgA^{-}] + 2[MgA_2^{4-}])/Mg_T$$

which can be combined with equations (5), (14), (15), and (18) to give:

$$\bar{n} = \frac{K_{1,1}[HA^{2-}] + K_{2,1}[A^{3-}] + 2K_{2,1}K_{2,2}[A^{3-}]^2}{1 + K_{1,1}[HA^{2-}] + K_{2,1}[A^{3-}] + K_{2,1}K_{2,2}[A^{3-}]^2}. \qquad (16)$$

The conservation equations for ligand and metal are:

$$A_T = [HA^{2-}] + [A^{3-}] + [MgHA] + [MgA^{-}] + 2[MgA_2^{4-}], \qquad (17)$$

and

$$Mg_T = [Mg^{2+}] + [MgHA] + [MgA^{-}] + [MgA_2^{4-}]. \qquad (18)$$

The electroneutrality condition, ignoring $[H^{+}]$, is:

$$[Na^{+}] + 2[Mg^{2+}] = 2[HA^{2-}] + 3[A^{3-}] + [MgA^{-}] + 4[MgA_2^{4-}] + [Cl^{-}] + [OH^{-}]. \qquad (19)$$

From equations (17) and (19), remembering that $2Mg_T = [Cl^{-}]$, we obtain:

$$[Na^{+}] + 2[Mg^{2+}] - 2A_T = [A^{3-}] - 2[MgHA] - [MgA^{-}] + 2Mg_T + [OH^{-}], \qquad (20)$$

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[3] Rossotti and Rossotti, *Acta Chem. Scand.*, 1955, **9**, 1166.

and from equations (18) and (20) we obtain:

$$[\mathrm{Na^+}] - 2\mathrm{A_T} - [\mathrm{OH^-}] = [\mathrm{A^{3-}}] + [\mathrm{MgA^-}] + 2[\mathrm{MgA_2^{4-}}]. \qquad (21)$$

From equations (17) and (21) we obtain:

$$3\mathrm{A_T} - [\mathrm{Na^+}] + [\mathrm{OH^-}] = [\mathrm{HA^{2-}}] + [\mathrm{MgHA}]. \qquad (22a)$$

Since all the terms on the left of equation (22a) are known, or can be calculated from the pH, we may write

$$3\mathrm{A_T} - [\mathrm{Na^+}] + [\mathrm{OH^-}] = a = [\mathrm{HA^{2-}}] + [\mathrm{MgHA}]. \qquad (22b)$$

Using the value of [MgHA] from equation (22b), equation (5) may be rewritten

$$[\mathrm{HA^{2-}}] = a/(1 + K_{1,1}[\mathrm{Mg^{2+}}]). \qquad (23)$$

From equations (3) and (23) we obtain

$$[\mathrm{A^{3-}}] = aK_{a3}'/[\mathrm{H^+}](1 + K_{1,1}[\mathrm{Mg^{2+}}]). \qquad (24)$$

Combining the conservation equations (17) and (18), we have

$$2\mathrm{Mg_T} - \mathrm{A_T} = 2[\mathrm{Mg^{2+}}] + [\mathrm{MgHA}] + [\mathrm{MgA^-}] - [\mathrm{HA^{2-}}] - [\mathrm{A^{3-}}]. \qquad (25)$$

Equations (14), (22b), (23), and (24) may be combined with equation (25) and rearranged to a quadratic in the $\mathrm{Mg^{2+}}$ ion concentration:

$$\begin{aligned}2K_{1,1}[\mathrm{Mg^{2+}}]^2 + \{2 + K_{1,1}(a + \mathrm{A_T} - 2\mathrm{Mg_T}) + aK_{a3}'K_{2,1}/[\mathrm{H^+}]\}[\mathrm{Mg^{2+}}]\\ + (\mathrm{A_T} - 2\mathrm{Mg_T} - aK_{a3}'/[\mathrm{H^+}] - a) = 0 \qquad (26)\end{aligned}$$

Equation (26) cannot be directly solved for $[\mathrm{Mg^{2+}}]$, since the value of $K_{2,1}$ is not known, and an iterative procedure has to be used.

Initially the presence of the complex MgHA is ignored, and equation (22a) becomes:

$$[\mathrm{HA^{2-}}] = 3\mathrm{A_T} - [\mathrm{Na^+}] + [\mathrm{OH^-}], \qquad (27)$$

and from equation (3) we have:

$$[\mathrm{A^{3-}}] = K_{a3}'[\mathrm{HA^{2-}}]/[\mathrm{H^+}]. \qquad (28)$$

The preliminary values of $[\mathrm{HA^{2-}}]$ and $[\mathrm{A^{3-}}]$ from equations (27) and (28) may be used to obtain a preliminary value of $\bar{n}$ from the relation

$$\bar{n} = (\mathrm{A_T} - [\mathrm{HA^{2-}}] - [\mathrm{A^{3-}}])/\mathrm{Mg_T}. \qquad (29)$$

Preliminary values of $K_{2,1}$ and $K_{2,2}$ can then be found from an equation like equation (11) where $[\mathrm{A^{3-}}]$ replaces $[\mathrm{HA^{2-}}]$, and $K_{2,1}$ and $K_{2,2}$ replace $K_{1,1}$ and $K_{1,2}$, respectively. The preliminary value of $K_{2,1}$ obtained in this way is inserted in equation (26) and a preliminary value of $[\mathrm{Mg^{2+}}]$ obtained. New values of the following terms can now be obtained: $[\mathrm{HA^{2-}}]$ from equation (23), $[\mathrm{A^{3-}}]$ from equation (28), and $\bar{n}$ from equation (29).

Using equation (3), equation (16) for $\bar{n}$ can be rearranged to give a modified form of the Irving and Rossotti equation:

$$\bar{n}/(\bar{n} - 1)[\mathrm{A^{3-}}] + K_{1,1}[\mathrm{H^+}]/K_{a3}' = (2 - \bar{n})[\mathrm{A^{3-}}]K_{2,1}K_{2,2}/(\bar{n} - 1) - K_{2,1}. \qquad (30)$$

The new values of $\bar{n}$ and $[\mathrm{A^{3-}}]$ are inserted in equation (30) and another value of $K_{2,1}$ obtained. This, in turn, is inserted in equation (26) and a new value of $[\mathrm{Mg^{2+}}]$ obtained. The iterative procedure is continued until constant values of $K_{2,1}$ and $K_{2,2}$ are obtained; about six cycles are necessary. Examples of plots of the Irving and Rossotti equation and its modification, equation (30), are shown in Fig. 4, and the values of $K_{2,1}$ and $K_{2,2}$ from two sets of titrations are shown in Table 2.

TABLE 2.

The first and second stability constants of the complexes between Mg^{2+} ions and oxaloacetate tri-anions; $I \sim 0{\cdot}1$, 25°.

The preliminary values were obtained from an equation similar to (11) without making allowance for the presence of the complex MgHA. The corrected values were obtained from equation (30) after allowing for MgHA as described in the text.

$$K_{2,1} = [MgA^-]/[Mg^{2+}][A^{3-}];\quad K_{2,2} = [MgA_2^{4-}]/[MgA^-][A^{3-}].$$

Total oxaloacetate (mmoles)	Total $MgCl_2$ (mmoles)	No. of points	Range of $\bar{n}$	Prelim. values log $K_{2,1}$	Prelim. values log $K_{2,2}$	Corr. values log $K_{2,1}$	Corr. values log $K_{2,2}$
1·0	0·5	11	0·86—1·50	5·98	4·82	6·32	4·78
1·0	0·33	9	0·75—1·54	5·93	4·87	6·22	4·86
			Average	5·95	4·85	6·27	4·82

At the start of each titration the solution contained KCl, $MgCl_2$, oxaloacetic acid, and approx. 2 mmoles of NaOH in a volume to make $I \sim 0{\cdot}1$. The titration was with approx. 0·1M-NaOH.

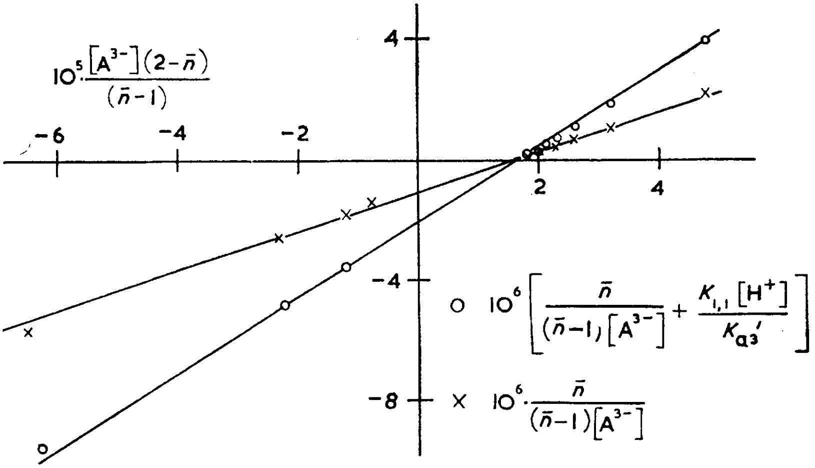

FIG. 4. Magnesium complexes of oxaloacetate tri-anion.

○ Plot of the variables of equation (30) after final iteration as described in the text.
× Plot of the variables of an equation similar to (11) for the uncorrected data.
The points were calculated from the first set of data in Table 2.

That this method of determining $K_{2,1}$ and $K_{2,2}$ is valid was checked in the following way. It is possible to derive, by another route, the following equation relating $[Mg^{2+}]$, $[A^{3-}]$, a of equation (22b), the constants $K_{1,1}$, $K_{2,1}$, and $K_{2,2}$, and Mg_T:

$$[Mg^{2+}]^2\{K_{1,1} + K_{1,1}K_{2,1}[A^{3-}] + K_{1,1}K_{2,1}K_{2,2}[A^{3-}]^2\} + \\ [Mg^{2+}]\{1 + aK_{1,1} + K_{2,1}[A^{3-}] + K_{2,1}K_{2,2}[A^{3-}]^2 - K_{1,1}Mg_T\} - Mg_T = 0 \quad (31)$$

which is a form of conservation equation for the metal. From equations (14), (15), and (21) we obtain:

$$[Na^+] - 2A_T - [OH^-] = [A^{3-}] + K_{2,1}[Mg^{2+}][A^{3-}] + 2K_{2,1}K_{2,2}[Mg^{2+}][A^{3-}]^2, \quad (32)$$

in which the left-hand side represents the extent of removal of protons from the acid in the third dissociation; in the absence of metal ions it would be $[A^{3-}]$. We now assume arbitrary values of $[Mg^{2+}]$, $K_{1,1}$, $K_{2,1}$, $K_{2,2}$, K_{a3}', Mg_T, A_T, and a, and calculate $[A^{3-}]$ from equation (32). This value is then inserted into equation (31) and a new value of $[Mg^{2+}]$ obtained. This is re-inserted into equation (32) and the process repeated until constant values of $[Mg^{2+}]$ and $[A^{3-}]$ are obtained. Then, using equation (24), for example, $[H^+]$ is obtained along a hypothetical titration curve. In order to simplify the calculations, the value of K_{a3}' is chosen such that $[OH^-]$ can be ignored. These calculated values of $[H^+]$ are then used in the procedure outlined above to recover $K_{2,1}$ and $K_{2,2}$. With $A_T = 0{\cdot}2$M and $Mg_T = 0{\cdot}1$M, $K_{a3}' = 10^{-9}$, $K_{1,1} = 10^3$, $K_{2,1} = 10^5$, and $K_{2,2} = 10^4$, the recovered values were $K_{2,1} = 0{\cdot}98 \times 10^5$ and $K_{2,2} = 1{\cdot}09 \times 10^4$, which may be considered satisfactory.

Keto–Enol Tautomerism.—As we have shown,[1] the di-anion of oxaloacetic acid exists in keto and enol forms; these arise by the dissociation of a proton from the corresponding mono-anions. The acid dissociation constants K_{a2K}', and K_{a2E}', which have been evaluated,[1] are defined as follows:

$$K_{a2K}' = [H^+][A_K^{2-}]/[HA_K^-] \qquad (33)$$

and

$$K_{a2E}' = [H^+][HA_E^{2-}]/[H_2A_E^-], \qquad (34)$$

where HA_K^- and $H_2A_E^-$ represent the keto and enol mono-anions, and A_K^{2-} and HA_E^{2-} the corresponding di-anions (the keto-species are shown with one less hydrogen because the keto-acid can only dissociate to give two hydrogen ions).

It therefore follows that two magnesium–oxaloacetic acid di-anion complexes (MgA_K and $MgHA_E$) can exist, with the stability constants:

$$K_{1,1K} = [MgA_K]/[Mg^{2+}][A_K^{2-}] \qquad (35)$$

and

$$K_{1,1E} = [MgHA_E]/[Mg^{2+}][HA_E^{2-}]. \qquad (36)$$

From equations (2), (33), and (34) we have

$$\begin{aligned} K_{a2}' &= [H^+]([A_K^{2-}] + [HA_E^{2-}])/([HA_K^-] + [H_2A_E^-]) \\ &= (K_{a2K}'K_{T2} + K_{a2E}')/(1 + K_{T2}), \qquad (37) \end{aligned}$$

where $K_{T2} = [HA_K^-]/[H_2A_E^-]$. Similarly, from equations (5), (35), and (36), we have

$$\begin{aligned} K_{1,1} &= ([MgA_K] + [MgHA_E])/[Mg^{2+}]([A_K^{2-}] + [HA_E^{2-}]) \\ &= (K_{1,1K}K_{T3} + K_{1,1E})/(1 + K_{T3}) \qquad (38) \end{aligned}$$

where $K_{T3} = [A_K^{2-}]/[HA_E^{2-}]$.

At pH values 5·2 and 5·6, where $[H_3A_E]$ and $[H_2A_K]$ are negligibly small, the conservation equations (6) and (7), suitably modified to include the concentrations of the keto and enol isomers separately, together with equations (37) and (38) may be combined to give the quadratic in the total concentration of di-anions:

$$\begin{aligned} ([A_K^{2-}] + [HA_E^{2-}])^2\{K_{1,1}(1 + [H^+]/K_{a2}')\} + ([A_K^{2-}] + [HA_E^{2-}])\{1 + [H^+]/K_{a2}' \\ + K_{1,1}(Mg_T - A_T)\} - A_T = 0, \qquad (39) \end{aligned}$$

from which we obtain $([A_K^{2-}] + [HA_E^{2-}])$. Since the value of K_{T3} is known,[1] the individual concentrations $[A_K^{2-}]$ and $[HA_E^{2-}]$ can be evaluated. Similarly, the individual concentrations $[HA_K^-]$ and $[H_2A_E^-]$ can be obtained from equation (37) and the known value [1] of K_{2a}' and K_{T2}. In the experiments at pH 7·0, $[HA_K^-]$ and $[H_2A_E^-]$ were negligible, and a simplified form of equation (39) was therefore used.

We have previously shown that the molar extinction coefficient at 280 mμ of the enol forms of oxaloacetic acid, $\varepsilon_{E280m\mu}$, is independent of the degree of dissociation of the protons and of the presence of Mg^{2+} ions and is very much greater than the extinction coefficient of the keto form.[1] It is therefore possible, from equilibrium optical density measurements at 280 mμ of oxaloacetic acid and magnesium chloride in various buffers, to compute the total concentrations of the keto and enol species, A_K and A_E, respectively, using the relationship,

$$A_K = (A_T\varepsilon_{E280m\mu} - D_e)/(\varepsilon_{E280m\mu} - \varepsilon_{K280m\mu}),$$

TABLE 3.

The stability constants and tautomeric equilibrium constants of complexes between Mg^{2+} ions and the keto and enol isomers of oxaloacetate di-anions from equilibrium optical density measurements at 25° and $I = 0{\cdot}1$. The pH values of the buffers were checked by glass-electrode measurements.

$$K_{1,1K} = [MgA_K]/[Mg^{2+}][A_K{}^{2-}];\ K_{1,1E} = [MgHA_E]/[Mg^{2+}][HA_E{}^{2-}];$$
$$K_{TM} = [MgA_K]/[MgHA_E].$$

Buffer system	pH	Oxaloacetic acid (M × 10^3)	$MgCl_2$ (M × 10^3)	10^3D_e (280 mμ)	log $K_{1,1K}$	log $K_{1,1E}$	K_{TM}
Acetic acid–sodium acetate	5·2	1·33	1·67	754	1·90	2·19	3·11
	5·2	1·33	3·33	802	1·91	2·20	3·09
,,	5·2	1·33	5·0	850	1·90	2·21	2·97
,,	5·6	1·33	1·67	757	1·91	2·19	3·24
,,	5·6	1·33	3·33	849	1·89	2·27	2·53
,,	5·6	1·33	5·0	885	1·89	2·25	2·69
,,	5·6	1·33	6·67	875	1·91	2·20	3·14
,,	5·6	2·67	1·67	1487	1·94	2·16	3·63
,,	5·6	2·67	3·33	1556	1·93	2·16	3·59
Triethanolamine–HCl	7·0	1·33	3·33	860	1·88	2·27	2·49
,, ,,	7·0	1·33	5·0	892	1·89	2·25	2·70
,, ,,	7·0	2·67	1·67	1493	1·93	2·14	2·70
,, ,,	7·0	2·67	3·33	1560	1·93	2·15	2·66
,, ,,	7·0	2·67	5·0	1648	1·92	2·17	3·40
				Averages	1·91	2·20	3·14
				± s.d.	0·02	0·04	0·42

where D_e is the equilibrium optical density. Since $A_K = [HA_K{}^-] + [A_K{}^{2-}] + [MgA_K]$ and $A_E = [H_2A_E{}^-] + [HA_E{}^{2-}] + [MgHA_E]$, we can obtain $[MgA_K]$ and $[MgHA_E]$. From these, together with $[Mg^{2+}]$ from the conservation equation, we can calculate $K_{1,1E}$ and $K_{1,1K}$. The results of such spectrophotometric experiments to determine these constants are shown in Table 3. Although acetate ions are said to form a weak complex with Mg^{2+} ions with log $K_1 = 0{\cdot}5$,[4] this would only alter the log $K_{1,1E}$ and log $K_{1,1K}$ values given here by about 0·02, which is within the experimental error.

From the concentrations of the keto and enol isomers of these complexes the tautomeric equilibrium constant $K_{TM} = [MgA_K]/[MgHA_E]$ can be calculated. The values of this constant are also given in Table 3.

With the complexes between Mg^{2+} ions and oxaloacetate tri-anions, A^{3-}, the situation is much simpler because the species A^{3-} is entirely enolic,[1] and therefore $K_{2,1}$ and $K_{2,2}$ apply only to the enolic forms of the ligand. No information is available about *cis–trans*-isomerism of enolic oxaloacetate in solution.

DISCUSSION

The values of the overall stability constant for the complex between Mg^{2+} ions and oxaloacetate di-anions found here, log $K_{1,1} = 1{\cdot}96$ at 25° and $I = 0{\cdot}1$, may be compared with values for similar complexes of other four-carbon dicarboxylic acids. Thus log $K_1 =$ 1·2, 1·36, and 1·55, for the succinate, tartrate, and malate complexes, respectively, with Mg^{2+}, at 25° and $I = 0{\cdot}2$.[4] The magnitudes of these constants are therefore very similar and there is no evidence for the formation of 2 : 1 ligand : magnesium complexes with any of these ligands. The value of log $K_{1,1} = 1{\cdot}6$ at 25° and $I = 0{\cdot}16$, found for the calcium oxaloacetate complex by Schubert and Lindenbaum,[5] is also very similar. These authors do not appear to have considered the possibility of complexes with oxaloacetate tri-anions.

It may be noted that the stability of the magnesium complexes of enolic oxaloacetate di-anions is greater than for the keto isomer, log $K_{1,1E} = 2{\cdot}20$ and log $K_{1,1K} = 1{\cdot}91$. This

[4] Cannan and Kibrick, *J. Amer. Chem. Soc.*, 1938, **60**, 2314.

[5] Schubert and Lindenbaum, *J. Amer. Chem. Soc.*, 1952, **74**, 3529.

is the reverse of what is found for the stabilities of the proton complexes. Thus $pK_{a1E}' = 1{\cdot}89$, $pK_{a1K}' = 2{\cdot}24$, and $pK_{a2E}' = 3{\cdot}72$, $pK_{a2K}' = 3{\cdot}90$.[1] This may be because in the magnesium complex the *cis*-enol form of the ligand is stabilized, but there is no information on the *cis*–*trans*-isomerism of oxaloacetic acid species in solution, and our stability and acid dissociation constants define the combined contributions from both these isomeric systems.

The most striking thing about the present results is the very great stability of the complexes between Mg^{2+} ions and oxaloacetate tri-anions. Both the 1 : 1 and 2 : 1 ligand : magnesium complexes are formed in solution, and their stability constants, $\log K_{2,1} = 6{\cdot}27$ and $\log K_{2,2} = 4{\cdot}82$, are much greater than any others reported for the complexes between Mg^{2+} ions and fairly simple organic acid ligands.[6] The stability of these oxaloacetate–magnesium complexes is such that they are present in solution even near pH 8 and may have to be considered in any detailed study of reactions such as the magnesium-activated enzymic cleavage of citrate to oxaloacetate and acetate.[7] The greater stability of the 1 : 1 ligand : magnesium complex as compared to the 2 : 1 complex, $\log K_{2,1} - \log K_{2,2} = 1{\cdot}45$, probably reflects the unfavourable electrostatic situation in the formation of the 2 : 1 complex.

Since the complexes between magnesium and oxaloacetate tri-anions are so much more stable than those with di-anions, the ionized enolate group is probably involved in complex formation. In both the *cis*- and *trans*-isomers, a five-membered ring could be formed with the metal, the enolate group, and the adjacent carboxylate group. A six-membered ring could be formed with the enolate and the more distant carboxylate groups but in the *cis*-isomer this would be sterically very difficult.

The formation of the complex MgA^- may also occur by the removal of a proton from the complex $MgHA_E$. The acid dissociation constant for this reaction $K_{ac}' = [H^+][MgA^-]/[MgHA_E]$ may be evaluated from the relation

$$pK_{ac}' = pK_{a3E}' - \log K_{2,1} + \log K_{1,1E}$$

where $pK_{a3E}' = 12{\cdot}18$ and refers to the dissociation of the third proton from enolic oxaloacetic acid.[1] The value found from the present data is $pK_{ac}' = 8{\cdot}11$. Therefore the presence of a magnesium ion complexed to enolic oxaloacetate increases the dissociation constant of the enolic hydrogen more than ten-thousand-fold. The enolic hydrogen in this magnesium complex is, however, a little less acidic than in the diethyl ester of oxaloacetic acid where its pK_a is reported to be 7·49 at 20°.[8]

The value of the keto–enol tautomeric equilibrium constant $K_{TM} = [MgA_K]/[MgHA_E] = 3{\cdot}1$ is considerably lower than $K_{T1} = 19$, $K_{T2} = 8{\cdot}2$, and $K_{T3} = 6$, the corresponding constants for fully protonated oxaloacetic acid, its mono-anion, and its di-anion, respectively.[1] Thus magnesium stabilizes the enol, which is consistent with the observed greater equilibrium optical density at 280 mμ of oxaloacetate solutions in the presence of increasing magnesium concentrations.[9]

EXPERIMENTAL

Magnesium chloride solutions were prepared from the AnalaR hexahydrate, and their magnesium content standardized by the gravimetric determination of magnesium as the pyrophosphate The oxaloacetic acid, sodium hydroxide solutions, and potassium chloride were the same as used previously.[1]

Titrations.—The general technique for the electrometric titrations was the same as previously described.[1] For the points shown in Table 1, potassium chloride and sodium hydroxide solutions were mixed and equilibrated in the titration vessel, then solid oxaloacetic acid and magnesium chloride solution were added and rapidly mixed. Readings of the pH were then made for about 8 min. and the resulting values extrapolated to zero time. This allowed for the decarboxylation of the oxaloacetic acid, the attainment of tautomeric equilibrium in the

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[6] " Stability Constants, Part I," *Chem. Soc., Special Publ.* No. 6, 1958.
[7] Dagley and Dawes, *Nature*, 1953, **172**, 345.
[8] Rumpf, d'Incan, and Schaal, *Bull. Soc. chim. France*, 1955, 122.
[9] Kornberg, Ochoa, and Mehler, *J. Biol. Chem.*, 1948, **174**, 159.

presence of Mg^{2+} ions being virtually instantaneous at 25°. The points shown in Fig. 2 were obtained from single solutions for each set; no changes in pH were observed with time in this region. The hydrogen and hydroxide-ion concentrations were calculated from the pH as in the determination of the acid dissociation constants.[1]

Optical-density Measurements.—The equilibrium optical density measurements at 25°, shown in Table 3, were made on a Hilger Uvispek spectrophotometer fitted with a thermostatically-controlled cell holder. Since the optical density decreased linearly with time, after an initial rapid decrease, readings were made during 5—6 min. and the linear parts of the plots were extrapolated to zero time.

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DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY COLLEGE,
GOWER STREET, LONDON W.C.1.

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