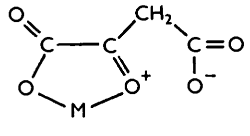
736. *The Interaction of Transition-metal Ions with Oxaloacetic Acid. Part I. The Rôle of Chelate Compounds in the Decarboxylation.*

By E. Gelles and R. W. Hay.

The nature of the chelate compounds formed by transition-metal ions with oxaloacetic acid in aqueous solution has been investigated spectrophotometrically and potentiometrically. The mechanism of the catalysed decarboxylation is discussed.

A variety of reactions in solution are catalysed by metal ions. The presence of metal–substrate complexes or chelate compounds has been demonstrated in many cases, but catalytic power and complex stability have been related quantitatively in only a few instances. The primary rôle of the metal ion in chelate compounds undergoing chemical change is to attract electrons. Withdrawal of electrons from the reaction centre in the substrate may facilitate reaction; however, the catalytic effect of the metal ion is governed not only by the degree of interaction with the substrate in the initial state of reaction, but also by the interaction in the transition state. Metal-ion catalysis of some oxidations involves electron transfer from the ligand;[1] withdrawal of electrons from the reaction centre in the substrate is exemplified by the catalysed hydrolysis of certain organic esters,[2] the halogenation of oxo-esters,[3] and the decarboxylation of β-oxo-carboxylic acids.[4]



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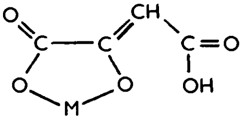

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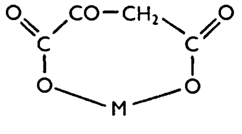

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One such reaction is the metal-ion catalysed decomposition of oxaloacetic acid into pyruvic acid and carbon dioxide. This reaction is of some biological importance.

Krampitz and Werkman [5] and Krebs [6] showed that decarboxylation was accelerated

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[1] Taube, *J. Amer. Chem. Soc.*, 1947, **69**, 1418; 1948, **70**, 1216; Duke, *ibid.*, 1947, **69**, 2885.

[2] Kroll, *ibid.*, 1952, **74**, 2034; Hoppé and Prue, *J.*, 1957, 1775.

[3] Pedersen, *Acta Chem. Scand.*, 1948, **2**, 252, 385.

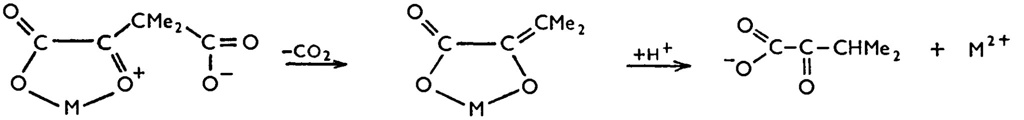
[4] *Idem, ibid.*, 1949, **3**, 676; 1952, **6**, 243, 285; Prue, *J.*, 1952, 2331; Gelles and Clayton, *Trans. Faraday Soc.*, 1956, **52**, 353.

[5] Krampitz and Werkman, *Biochem. J.*, 1941, **35**, 595.

[6] Krebs, *ibid.*, 1942, **36**, 303.

by various bi- and ter-valent cations. Kornberg, Ochoa, and Mehler [7] observed the appearance and decline of a strong absorption band at 260—300 mμ when metal ions were added to oxaloacetic acid under certain conditions. They attributed this to the slow formation of a strongly absorbing enolic complex B, which disappeared on decarboxylation.

Steinberger and Westheimer [8] showed that decarboxylation of αα-dimethylethoxaloacetic acid was not catalysed by metal ions, indicating that the catalytically active species formed by αα-dimethyloxaloacetic acid was a five-membered ring α-oxo-carboxylate chelate compound. They obtained chemical and spectroscopic evidence for a mechanism involving an enolic intermediate:



Dimethyloxaloacetic acid cannot form an enolic complex of type B, and so by analogy a ketonic complex A seemed likely to be the catalytically active species in the decarboxylation of oxaloacetic acid. Pedersen [9] interpreted the kinetics of the copper- and zinc-ion catalysed decomposition of oxaloacetic acid at low pH and metal-ion concentration in terms of the first-order decomposition of the acid, its two anions, and a species MA, where M^{2+} is the metal ion and A^{2-} the oxaloacetate anion. Williams [10] suggested that in addition to α-oxo-carboxylate complexes a dicarboxylate complex C might also be formed, and that this could retard decarboxylation under certain conditions.

Inadequate explanations have been advanced for the observed retardation of the reaction at high pH and metal-ion concentration.[10, 11] Previous work has established that at low pH and low concentration of metal ion the catalytically active species must be the complex A, but it has produced no clear evidence (i) on whether a catalytically inactive complex B is also formed, (ii) if this is formed slowly enough to account for the spectrophotometric observations,[7, 12] (iii) whether these observations in fact provide evidence for a strongly absorbing enolic pyruvate intermediate, (iv) on what the thermodynamic stabilities of the ketonic and enolic complexes, A and B, are, (v) how the proportion of these two complexes changes with pH, and whether increasing concentrations of the enolic complex at a high pH might account for the observed retardation in the rate of decarboxylation, (vi) whether other complexes, such as the dicarboxylate complex C, are also present under some conditions, and (vii) whether there is any correlation between the thermodynamic stability of the complexes and the catalytic power of the metal ions.

Our potentiometric and spectrophotometric studies indicate the rôle a number of chelate compounds play in the decarboxylation.

Thermodynamic information on the ketonic chelate compounds, which are the kinetically active species in decarboxylation, was obtained by comparing association constants for acids (I) and (II). Spectrophotometric studies demonstrated the presence of enolic chelate compounds which are not decarboxylated. Spectrophotometric studies on the chelate compounds of oxaloacetic acid (I) and its ethyl ester (III) have shown that oxaloacetate chelate compounds are formed very rapidly. The rise of optical density to a maximum, observed with some metal oxaloacetates under certain conditions, is due to formation of a strongly absorbing enolic pyruvate intermediate, which subsequently ketonises.

The measurement of association constants and rates of decarboxylation for metal oxaloacetates is described in detail in the following papers. In Part II, comparison of the

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[7] Kornberg, Ochoa, and Mehler, *J. Biol. Chem.*, 1948, **174**, 159; Speck, *ibid.*, 1949, **178**, 315.
[8] Steinberger and Westheimer, *J. Amer. Chem. Soc.*, 1951, **73**, 429.
[9] Pedersen, *Acta Chem. Scand.*, 1952, **6**, 243, 285.
[10] Williams, *Nature*, 1953, **171**, 304.
[11] *Idem, Biol. Rev.*, 1953, **28**, 381.
[12] Nossal, *Austral. J. Exp. Biol.*, 1949, **27**, 143, 313.

association constants for oxaloacetates with those for oxalates and succinates indicates the relative stabilities of keto-carboxylate and dicarboxylate chelate compounds of different ring size, and also shows that interaction of metal ion and substrate in the transition state of decarboxylation is likely to be much stronger than interaction in the initial state. In Part III the rate coefficients for catalysis by transition-metal ions are examined, and catalysis is related to the degree of interaction of metal ion and substrate in the transition state of decarboxylation.

RESULTS

The metal-ion catalysed decomposition of oxaloacetic acid to pyruvic acid and carbon dioxide involves the interaction of metal ions with oxaloacetate in the initial state, and with pyruvate in the final state. These states can be studied directly by thermodynamic methods, while interaction in the transition state is indicated by kinetic measurements.

For the initial state, experiments were made with oxaloacetic acid (I), αα-dimethyloxaloacetic acid (II), which cannot enolise, ethyl oxaloacetate (III), which cannot be decarboxylated or form a dicarboxylate complex, and ethyl αα-dimethyloxaloacetate (IV), which cannot enolise or be decarboxylated and can only form a ketonic α-oxo-carboxylate complex.

$HO_2C\cdot CO\cdot CH_2\cdot CO_2H$	$HO_2C\cdot CO\cdot CMe_2\cdot CO_2H$	$HO_2C\cdot CO\cdot CH_2\cdot CO_2Et$	$HO_2C\cdot CO\cdot CMe_2\cdot CO_2Et$
(I)	(II)	(III)	(IV)

Association constants at 25°.

Oxaloacetic Acid (I).—A detailed study of the association equilibria is described in the following paper. In the pH range investigated the significant association equilibria are

$$M^{2+} + A^{2-} \rightleftharpoons MA;\ K_{MA} = \{MA\}/\{M^{2+}\}\{A^{2-}\} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

and

$$M^{2+} + MA \rightleftharpoons M_2A^{2+};\ K_{M_2A^{2+}} = \{M_2A^{2+}\}/\{M^{2+}\}\{MA\} \quad . \quad . \quad . \quad (2)$$

where M^{2+} represents a metal ion, A^{2-} the oxaloacetate anion, and K_{MA} and $K_{M_2A^{2+}}$ are the thermodynamic association constants (Table 1).

TABLE 1. *Association constants of oxaloacetates.*

	Ca^{2+}	Mn^{2+}	Co^{2+}	Zn^{2+}	Ni^{2+}	Cu^{2+}
$10^{-3}K_{MA}$	0·4	0·7	1·4	1·7	3·2	75
$10^{-2}K_{M_2A^{2+}}$	<1	<1	2	2	1·5	—

αα-Dimethyloxaloacetic Acid (II).—The ionisation constants were calculated from the pH of mixtures of the acid with varying proportions of alkali.[13] The pH values were extrapolated to the time of addition of alkali (following paper).

If the total concentrations of the dicarboxylic acid and of sodium hydroxide are a and b respectively, and

$L = b + [H^+]$, $M = a - b - [H^+]$, $N = 2a - b - [H^+]$, $[OH^-] \ll [H^+]$, then

$$[\{H^+\}^2Lf_2/N] = [\{H^+\}Mf_2/Nf_1]K_1 + K_1K_2 \quad . \quad (3)$$

or

$$X = YK_1 + K_1K_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

where K_1 and K_2 are the two thermodynamic ionisation constants. Square brackets and braces are used to indicate concentrations and activities, respectively. f_1 and f_2 are ionic activity coefficients for univalent and bivalent ions, calculated from Davies's equation

$$-\log f_z = 0{\cdot}5z^2\left(\frac{I^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} - 0{\cdot}2I\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

The ionic strength I is calculated by successive approximations. K_1 and K_2 are obtained from the slope and intercept of the linear plot of X against Y. The results of some pH measurements and the quantities X and Y are given in Table 2.

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[13] Speakman, *J.*, 1940, 855.

The values obtained for the ionisation constants can be compared with approximate values of 3×10^{-2} and $1{\cdot}7 \times 10^{-5}$ obtained [8] by an indicator method at $I = 0{\cdot}5$, and corrected to zero ionic strength.

In the presence of copper the pH changed with time because of decarboxylation of the copper chelate compound; measurements could be made only over a limited range of pH and metal-ion concentration. The pH curve was extrapolated to the time of addition of metal ion. The two ionisation equilibria of the acid (II) are

TABLE 2. *Ionisation constants of αα-dimethyloxaloacetic acid.*

10^3a	5·76	5·67	5·76	5·59	5·52
10^3b	2·88	3·41	4·56	6·74	9·40
pH	2·66	2·77	$3{\cdot}31_5$	$4{\cdot}02_5$	4·99
10^8X	298	185	136	0·98	0·062
10^6Y	142	93·8	40·8	−20·7	−28·4

$K_1 = 1{\cdot}7 \times 10^{-2}$; $K_2 = 2{\cdot}4 \times 10^{-5}$

$$H_2A \rightleftharpoons HA^- + H^+ \quad \text{and } HA^- \rightleftharpoons A^{2-} + H^+ \quad . \quad . \quad . \quad . \quad (6)$$

If the only significant association equilibrium is

$$M^{2+} + A^{2-} \rightleftharpoons MA; \quad K_{MA} = \{MA\}/\{M^{2+}\}\{A^{2-}\} \quad . \quad . \quad . \quad . \quad (7)$$

we have for the total concentration of acid (II)

$$c_a = [H_2A] + [HA^-] + [A^{2-}] + [MA] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

for the total copper-ion concentration

$$c_m = [M^{2+}] + [MA] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

and for electroneutrality ($[OH^-] \ll [H^+]$)

$$2[M^{2+}] + [Na^+] + [H^+] = [Cl^-] + [HA^-] + 2[A^{2-}] \quad . \quad . \quad . \quad . \quad (10)$$

The ionic strength and the ionic activity coefficients are obtained by successive approximations:

$$I = \tfrac{1}{2}\left(4[M^{2+}] + [H^+] + [HA^-] + 4[A^{2-}] + [Na^+] + [Cl^-]\right) \quad . \quad . \quad . \quad (11)$$

From eqns. (6)—(10) we obtain

$$[H_2A]\left(2 + K_1/\{H^+\}f_1\right) = [Cl^-] + 2c_a - 2c_m - [Na^+] - [H^+] \quad . \quad . \quad . \quad (12)$$

The concentrations of all other species, and hence the association constant K_{MA}, can be calculated.

TABLE 3. *Association constant of copper αα-dimethyloxaloacetate.*

10^3c_a	10^3c_m	10^3[HCl]	pH	I	$10^{-3}\,K_{MA}$
5·61	1·96	5·10	2·07	0·0141	55
5·38	3·85	7·40	1·99	0·0215	51
5·54	3·85	2·53	$2{\cdot}16_5$	0·0169	45
5·26	7·41	7·12	2·00	0·0316	40

In view of errors due to rapid decarboxylation of the chelate compound the constancy of the values of K_{MA} given in Table 3 must be regarded as satisfactory, although the range of pH studied is too small to exclude the presence of other complexes. It is noted that the average value of K_{MA}, 48×10^3, is of the same order of magnitude as the association constant of copper oxaloacetate (cf. Table 1).

Ethyl Oxaloacetate (III).—The thermodynamic ionisation constant of this monobasic acid was determined in the usual way, by measurements with a glass electrode, to be $1{\cdot}8 \times 10^{-3}$, which is slightly smaller than the first ionisation constant of oxaloacetic acid ($2{\cdot}79 \times 10^{-3}$).

Measurements of pH on the copper chelate compound could not be interpreted in terms of the association equilibria:

$$M^{2+} + A^- \rightleftharpoons MA^+ \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (13)$$

$$M^{2+} + 2A^- \rightleftharpoons MA_2 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (14)$$

The data in Table 4 show that the hydrogen-ion concentration can exceed the initial ester concentration when cupric chloride is added. This implies that in addition to equilibria (13) and (14) there is appreciable formation of an enolic complex with the displacement of a proton,

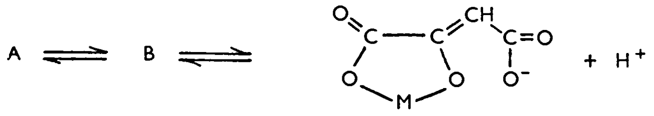
$$M^{2+} + A^- \rightleftharpoons MA_e + H^+ \quad . \; . \; . \; . \; . \; . \; . \; . \quad (15)$$

In contrast to ethyl oxaloacetate, oxaloacetic acid can form an enolic complex B from which a further proton will only be displaced at a higher pH.

TABLE 4. *Formation of an enolic copper complex from ethyl oxaloacetate.*

10^3[III]	2·00	1·96	1·92	1·88	1·85	1·82	1·78
10^3[CuCl$_2$]	—	1·96	3·85	5·66	7·40	9·09	10·71
pH	$2{\cdot}90_5$	2·84	$2{\cdot}80_5$	$2{\cdot}78_5$	2·77	$2{\cdot}75_5$	$2{\cdot}74_5$

Eqn. (1) represents the formation of A and B. Potentiometric measurements on oxaloacetates were made in a pH range in which the enolic complex B′ is not significant.



B′

Pyruvic Acid.—A value of $3{\cdot}24 \times 10^{-3}$ had been obtained [9] for the thermodynamic ionisation constant. If the monobasic acid is represented by HA

$$HA \rightleftharpoons A^- + H^+ \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (16)$$

and if the only significant association equilibrium is

$$M^{2+} + A^- \rightleftharpoons MA^+; \qquad K_{MA^+} = \{MA^+\}/\{M^{2+}\}\{A^-\} \quad . \; . \; . \; . \quad (17)$$

we have for the total concentration of metal

$$c_m = [M^{2+}] + [MA^+ \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (18)$$

for the total concentration of acid

$$c_a = [HA] + [A^-] + [MA^+] \quad . \; . \; . \; . \; . \; . \; . \; . \quad (19)$$

and for electroneutrality

$$[H^+] + 2[M^{2+}] + [Na^+] + [MA^+] = [Cl^-] + [A^-] \quad . \; . \; . \; . \; . \; . \quad (20)$$

If, on the other hand, the only significant association equilibrium is

$$M^{2+} + 2A^- \rightleftharpoons MA_2; \qquad K_{MA_2} = \{MA_2\}/\{M^{2+}\}\{A^-\}^2 \quad . \; . \; . \; . \quad (21)$$

then eqns. (18)—(20) become

$$c_m = [M^{2+}] + [MA_2] \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (22)$$

$$c_a = [HA] + [A^-] + 2[MA_2] \quad . \; . \; . \; . \; . \; . \; . \; . \quad (23)$$

$$[H^+] + 2[M^{2+}] + [Na^+] = [Cl^-] + [A^-] \quad . \; . \; . \; . \; . \; . \quad (24)$$

In either case

$$[HA] = [Cl^-] + c_a - 2c_m - [Na^+] - [H^+] \quad . \; . \; . \; . \; . \; . \quad (25)$$

the ionic strength and activity coefficients are obtained as before, and the concentrations of all other species can be calculated.

The results of some pH measurements on copper pyruvate are given in Table 5. Association constants were calculated on the assumption of there being only one complex, MA^+ or MA_2. It can be seen that the association constant for MA_2 remains approximately constant, while that for MA^+ changes as the pyruvate : copper ratio is increased; the predominant species is clearly MA_2.

TABLE 5. *Association constant of copper pyruvate.*

10^3c_a	10^3c_m	$10^4[Cl^-]$	$10^4[Na^+]$	pH	$\log_{10} K_{MA^+}$	$\log_{10} K_{MA_2}$
2·33	5·0	105	5·9	2·83	2·3	5·0
2·33	5·0	105	11·9	2·98	2·2	4·9
2·39	5·0	105	17·8	3·17	2·3	4·9
7·13	10·0	21	2·96	2·75	3·1	4·8
9·50	10·0	21	3·95	2·69	4·2	4·8

The monobasic acids, ethyl oxaloacetate and pyruvic acid, tend to form chelate compounds of the type MA_2 with no net charge. The compounds of ethyl oxaloacetate are more stable than those of pyruvic acid, which appear to be entirely ketonic. The dibasic acids oxaloacetic acid and αα-dimethyloxaloacetic acid tend to form chelate compounds of the type MA with no net charge, and these appear to have association constants of the same order of magnitude.

Chelate compounds from oxaloacetate.

The Ketonic Chelate Compound A.—Chelate compounds of oxaloacetic acid are not entirely ketonic. The ketonic compounds are decarboxylated to a strongly absorbing enolic pyruvate intermediate, as are those of αα-dimethyloxaloacetic acid (II). The monoester (IV), however, forms only stable ketonic chelate compounds. Fig. 1 shows the variation of extinction coefficient with wavelength for the anion of the ester (IV) and for its copper chelate compound. Experiments were carried out with anion concentrations of 1×10^{-3} and $3{\cdot}37 \times 10^{-3}$M and with copper concentrations of 1×10^{-2} and $3{\cdot}33 \times 10^{-2}$M. The association constants for the copper chelate compounds of esters (III) and (IV) are of the same order of magnitude. With the above concentrations the anion of ester (IV) should be completely chelated, and this is borne out by the constancy of the extinction coefficient as the copper concentration is increased. The α-oxo-acid peak occurs at 338 mμ ($\varepsilon = 43$). At 295 mμ, which is $\lambda_{max.}$ for the enolic chelate compounds, ε is 27 for the anion of ester (IV), and 68 for the copper chelate compound. Ketonic chelate compounds of α-oxo-carboxylates with other transition-metal ions are also expected to have extinction coefficients of around 70 at 295 mμ. This is borne out by further spectrophotometric measurements described below.

The Enolic Chelate Compound B.—Enols HO•CR⁚CH•CO•R′ have absorption maxima at about 260 mμ; on chelation with transition-metal ions the maximum absorption of the enolate ion is shifted to higher wavelength, and the extinction coefficient is increased two- or three-fold. Studies of enolic chelate compounds in chloroform show that $\lambda_{max.}$ and ε are not greatly affected by the nature of the bivalent ion.[14]

Fig. 2 shows that the peak at *ca.* 260 mμ is due to the enol of oxaloacetic acid. Fig. 3 shows the variation of extinction coefficient with wavelength for oxaloacetates (I) and (III) in ether and light petroleum (b. p. 40°). Both oxaloacetic acid and ethyl oxaloacetate appear to be completely enolised ($\lambda_{max.} = 260$ mμ, $\varepsilon = 8800$). (Oxaloacetic acid is not decarboxylated in these solvents.) Fig. 4 relates to the copper enolate of ethyl ethoxaloacetate, $Cu[EtO_2C(CO^-)\!:\!CH{\cdot}CO_2Et]_2$ (in chloroform; $\lambda_{max.} = 295$ mμ, $\varepsilon = 2 \times 10^4$). The enolic peak of the oxaloacetate (III) at 260 mμ and the shift of $\lambda_{max.}$ to 295 mμ on chelation of the oxaloacetate with Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} ions in aqueous solution is illustrated in Fig. 5. Enolic oxaloacetate complexes are expected to have $\lambda_{max.} = 295$ mμ ($\varepsilon \sim 2 \times 10^4$), a value independent of solvent or the nature of the transition-metal ion.

Speed of Formation of Chelates.—Addition of metal ions to oxaloacetic acid under certain conditions produces a rise of optical density with time, which has been attributed [7,12] to the slow formation of the enolic complex B. The speed of formation of the chelate compounds of ethyl oxaloacetate (III), which cannot be decarboxylated, has now been measured under comparable conditions and shown to be very fast. The optical density first measured 45 sec. after addition of metal ion changed very little with time (Table 7). The formation of a strongly absorbing intermediate on decarboxylation must therefore clearly play a part in determining

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[14] Belford, Martell, and Calvin, *J. Inorg. Nuclear Chem.*, 1956, **2**, 11.

the changes in absorption spectra of metal oxaloacetates. The copper compounds are formed at a measurable rate at a high pH, but for the other metal ions chelation appears to be complete within a fraction of a minute at pH <6.

Keto–Enol Equilibria.—Previous studies [15] of the enol content of oxaloacetic acid in aqueous solution have been conflicting. By use of a value of 8800 for $\varepsilon_{max.}$ of the enol, Nossal's spectrophotometric data [12] can be interpreted in terms of about 3% of enol at pH 2 increasing to 9% at pH 5·5. At this pH the acid is almost entirely in the form of the dianion. The enol content, as indicated by the optical density at 260 mμ, remains constant as the pH is increased, until an enolate ion is formed with the loss of a third proton at a much higher pH.

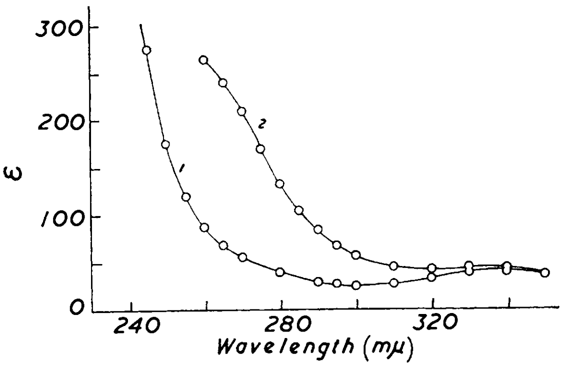

FIG. 1. *Variation of* ε *with* λ *for* (1) $^-O_2C{\cdot}CO{\cdot}CMe_2{\cdot}CO_2Et$, (2) *the copper chelate of compound* $HO_2C{\cdot}CO{\cdot}CMe_2{\cdot}CO_2Et$.

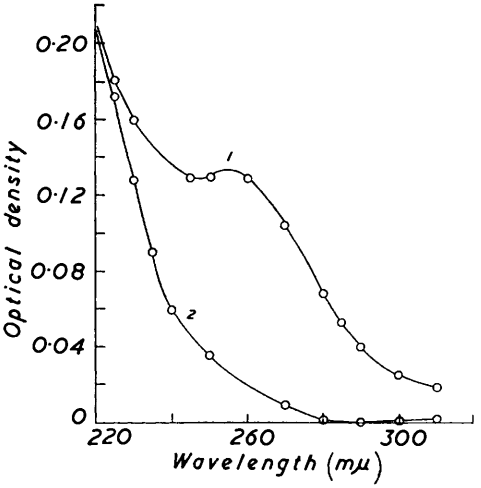

FIG. 2. *Variation of optical density with* λ *for* (1) $HO_2C{\cdot}CO{\cdot}CH_2{\cdot}CO_2H$, (2) $HO_2C{\cdot}CO{\cdot}CMe_2{\cdot}CO_2H$ (*both* $1{\cdot}25 \times 10^{-4}$M).

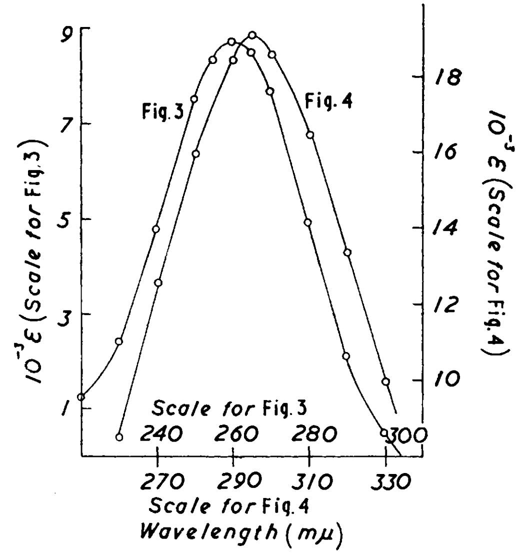

FIG. 3. *Variation of* ε *with* λ *for* $HO_2C{\cdot}CO{\cdot}CH_2{\cdot}CO_2H$ *in ether and in light petroleum* (*b. p.* 40°) *and for* $HO_2C{\cdot}CO{\cdot}CH_2{\cdot}CO_2Et$ *in ether.*

FIG. 4. *Variation of* ε *with* λ *for* $Cu[EtO_2C{\cdot}CO^-{\cdot}CH{\cdot}CO_2Et]_2$ *in chloroform.*

The enol content of oxaloacetic acid and its anions has been studied by comparing the

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[15] Meyer, *Ber.*, 1912, **45**, 2860; Hantzsch, *Ber.*, 1915, **48**, 1407.

extinction coefficients at 260 mμ of acids (I) and (II) at different values of pH. The values ε (I) = 420, ε (II) = 22, and ε (enol) = 8800 indicate an enol [$HO_2C{\cdot}C(OH){:}CH{\cdot}CO_2H$] content of 4—5% in undissociated oxaloacetic acid (in perchloric acid). At pH 6, ε (I) = 850, ε (II) = 92, and ε (enol) = 8800 indicate 9% of enol [$^-O_2C{\cdot}C(OH){:}CH{\cdot}CO_2^-$] for the dianion of oxaloacetic acid. In the above pH range optical densities changed with time owing to decarboxylation, and values were extrapolated to zero time. At pH 13 complete enolisation to the trianion $^-O_2C{\cdot}CO^-{:}CH{\cdot}CO_2^-$ is indicated by the stability of the solution. No evolution of carbon dioxide or change in optical density with time was detected with acid (I), in contrast to acid (II) which continues to decompose at this pH. The extinction coefficient of the enolate ion (ε_{260} = 3200) is lower than that for the enols.

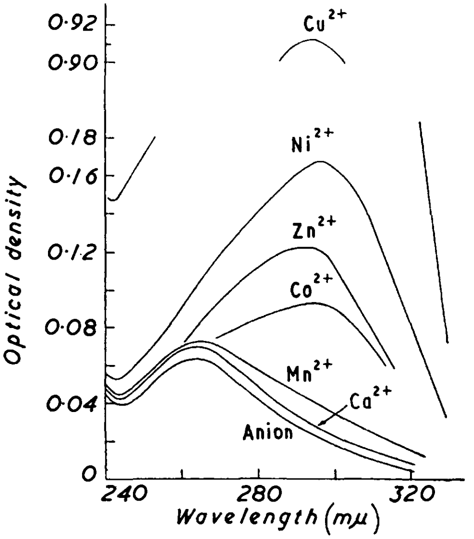


FIG. 5. *Variation of optical density with λ for* $NaO_2C{\cdot}CO{\cdot}CH_2{\cdot}CO_2Et$ ($1{\cdot}25 \times 10^{-4}$M) *with metal ions* ([$M^{2+}$] = $3{\cdot}3 \times 10^{-4}$M).

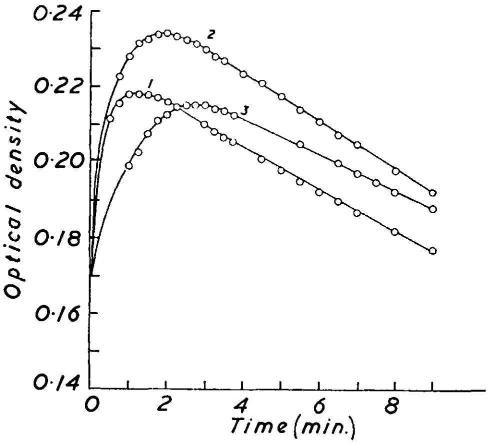


FIG. 6. *Variation of optical density with time* (λ = 290 mμ) *for* $HO_2C{\cdot}CO{\cdot}CH_2{\cdot}CO_2H$. [I] = $1{\cdot}25 \times 10^{-4}$M, [$Zn^{2+}$] = $3{\cdot}36 \times 10^{-4}$M, [Acetate] = 0·01M, (1) pH = 5·30, (2) pH = 5·66, (3) pH = 5·94.

Keto–enol equilibria in the metal oxaloacetates were investigated by measurement of optical densities at 295 mμ ($\lambda_{max.}$ for the enolic chelate compounds). The total concentration of chelate compound was calculated from the measured association constants and the initial concentrations, and the approximate proportions of ketonic and enolic forms, A and B, were estimated by using the values ε_{295} (A) = 70 and ε_{295} (B) = 20,000.

TABLE 7. *Speed of formation of ethyl oxaloacetate chelate compounds.*

[III] = $1{\cdot}14 \times 10^{-4}$, [$Zn^{2+}$] = $3{\cdot}20 \times 10^{-4}$M						[III] = $1{\cdot}20 \times 10^{-4}$, [$Cu^{2+}$] = $3{\cdot}23 \times 10^{-4}$M, pH = 5·34			
pH	time (sec.)	O.D.[a]	pH	time (sec.)	O.D.[a]	time (sec.)	O.D.[a]	time (sec.)	O.D.[a]
5·0	45	0·0725[b]	6·5	60	0·463	45	1·10	120	1·323
5·5	45	0·122[b]	,,	90	0·474	60	1·160	150	1·342
6·0	45	0·207[b]	,,	120	0·477	75	1·236	180	1·349
6·5	45	0·457	,,	480	0·480	90	1·274	240	1·350
			,,	600	0·480			300	1·350

[a] At 295 mμ. [b] Steady value.

The proportion of enolic complex increases with pH for ethyl oxaloacetate (III), since the formation of the enolic complex must involve the displacement of a proton (eqn. 15). The variation with pH is illustrated in Table 8. Under the conditions of these experiments the monoester is almost completely chelated. At low pH and sufficiently high concentration of zinc ion the optical density corresponds to a concentration of ketonic chelate compound ($\varepsilon = 70$) equal to the initial concentration of ester (III).

TABLE 8. *Keto-enol equilibria for zinc and copper ethyl oxaloacetates.*

10^4[III]	1·25	1·10
10^4[Zn^{2+}]	333	3·04
pH	2·75	6·50
O.D. at 295 mμ	0·009	0·48
Enolic complex (%)	0	22

10^4[III]	1·25	1·25	1·25	1·25
10^4[Cu^{2+}]	333	333	6·73	3·33
pH	2·24	2·54	4·34	5·34
O.D. at 295 mμ	0·20	0·51	1·13	1·36
Enolic complex (%)	8	20	45	55

With oxaloacetic acid the position of the keto-enol equilibrium between A and B is independent of pH. The loss of a proton from the enol B to give the complex B′ should occur at high pH, but the enol content of the metal oxaloacetates does not appear to increase below pH 6 or 6·5. With the known association constants and extinction coefficients Nossal's spectrophotometric data [12] on copper oxaloacetate can be interpreted. In the pH range 4—6 the optical densities extrapolated to zero time indicate a constant proportion (*ca.* 40%) of enolic complex. Experiments on copper oxaloacetate ([I] = $1·25 \times 10^{-4}$, [Cu^{2+}] = 10^{-1}M) at pH 2 and 3 gave extrapolated values of optical density between 0·9 and 1·0. The optical density falls very rapidly with time owing to decarboxylation. These values show that the same proportion of enolic copper complex is maintained over the pH range 2—6.

FIG. 7. *Variation of optical density with time* ($\lambda = 295$ mμ) *for* $HO_2C\cdot CO\cdot CH_2\cdot CO_2H$. [I] = $1·25 \times 10^{-4}$M, [M^{2+}] = $3·3 \times 10^{-4}$M, pH = 6·35.

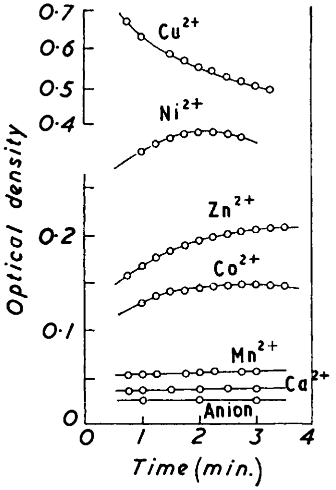
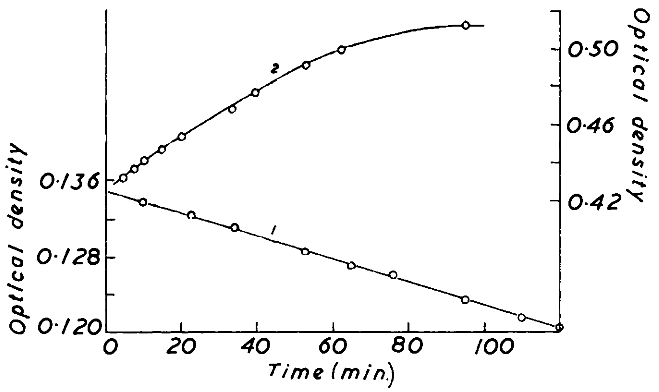


FIG. 8. *Variation of optical density with time* ($\lambda = 260$ mμ) *for* $HO_2C\cdot CO\cdot CH_2\cdot CO_2H$. (1) [I] = $1·25 \times 10^{-4}$M, pH = 2·0 (left ordinate), (2) [I] = $5·05 \times 10^{-4}$M, pH = 6·1 (right ordinate).

2 cm. cells.



Experiments with high concentrations of zinc ion at pH 2—3 indicate 15—20% of enol. This content appears to be maintained up to pH 6·5. Fig. 6 shows the optical density–time curves for several experiments in the pH range 5—6·5; the rise is due to the formation of a strongly absorbing enolic pyruvate intermediate. The initial optical density is independent of pH and with the known association constant and extinction coefficient gives a proportion of enolic zinc complex of about 15%.

Fig. 7 shows the optical density–time curves for a typical set of experiments with the series of transition-metal ions; the rise is again attributed to formation of an enolic pyruvate intermediate on decarboxylation. The results of these experiments are collected in Table 9. Optical densities are values extrapolated to 30 sec. after addition of metal ion. Owing to uncertainties in the extrapolation of optical densities, in the association constants, and in the various extinction coefficients, the proportions of enolic complex given are approximate.

TABLE 9. *Keto–enol equilibria for metal oxaloacetates.*

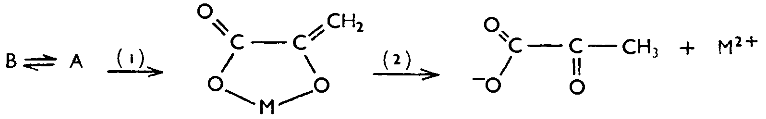
Oxaloacetate dianion: initial pH = 6·35.
[I] = 1·25 × 10^{-4}M, [M^{2+}] = 3·33 × 10^{-4}M.

	Dianion	Ca^{2+}	Mn^{2+}	Co^{2+}	Zn^{2+}	Ni^{2+}	Cu^{2+}
10^4[MA]	—	0·14	0·24	0·37	0·43	0·58	1·25
O.D. at 295 mμ	0·025	0·035	0·050	0·12	0·15	0·27	1
O.D. (B)	—	0·013	0·03	0·10	0·13	0·25	1
Enolic complex (%) ...	—	5	6	13	15	22	40

Pyruvate chelate compounds

The spectra of freshly prepared aqueous solutions of pyruvic acid and of metal pyruvates show no evidence of enolic species. Pyruvic acid and its anion give α-keto-acid peaks at 325 mμ (ε = 5) and 318 mμ (ε = 22). Copper pyruvate shows no absorption maximum and appears to be almost entirely ketonic ($\varepsilon_{295} \sim 200$).

A comparison of the optical density–time curves for the metal chelate compounds of ethyl oxaloacetate, which is not decarboxylated (Table 7), and for the metal oxaloacetates (Figs. 6 and 7) shows that the rise in optical density with time for the oxaloacetates (other than the copper complex) must be due to formation of a strongly absorbing species on decarboxylation. This is believed to be an enolic pyruvate intermediate, which subsequently ketonises:



The variation of optical density with wavelength suggests that the enolic pyruvate has an absorption maximum at a somewhat lower wavelength than B, and an extinction coefficient of several thousand. The disappearance of A and B on decarboxylation leads to a decrease in optical density, and a maximum will only be observed if ketonisation (2) is sufficiently slow to allow build-up of intermediate. It is found that the time of maximum optical density decreases with decrease of pH, and at low pH there is no maximum. The behaviour at two values of pH is illustrated for the unchelated acid in Fig. 8. At low pH the rate of fall of optical density (corrected for pyruvic acid) with time corresponds to the manometric rate of decarboxylation. Data for zinc oxaloacetate are illustrated in Fig. 6. If the rate of step (2) were simply proportional to hydrogen-ion concentration, then the maximum optical densities and the corresponding times should vary in an easily predictable manner with pH and with the ratio of the rate constants of steps (1) and (2). Measurements have been made in buffered and in unbuffered solutions of zinc oxaloacetate in the pH range 5—6·5 at 270 and 290 mμ. These measurements indicate that step (2) is a composite one, probably involving displacement of the metal ion by a proton and the catalysed ketonisation of the enol.

EXPERIMENTAL

Chemicals.—Oxaloacetic acid (Light & Co.) had m. p. 152° (decomp.) (Found, by titration against alkali: *M*, 132·6. Calc. for $C_4H_4O_5$: *M*, 132·1). Ethyl ethoxaloacetate was prepared by condensation of diethyl oxalate and ethyl acetate with sodium ethoxide.[16]

Ethyl oxaloacetate was prepared by alkaline hydrolysis of the copper enolate of ethyl ethoxaloacetate at room temperature.[17] Recrystallised from benzene it had m. p. 98° [Found: C, 44·8; H, 5·1%; *M* (by titration against alkali), 161·6. Calc. for $C_6H_8O_5$: C, 45·0; H, 5·0%; *M*, 160·1].

tert.-*Butyl αα-dimethylethoxaloacetate.* This was prepared by condensing diethyl oxalate and *tert.*-butyl *iso*butyrate with sodiotriphenylmethane.[8, 18, 19] Since the reaction times are critical, and have not been adequately reported, details are given: *tert.*-Butyl *iso*butyrate (0·19 mole) was added with shaking to sodiotriphenylmethane (0·19 mole) in ether (1300 ml.). After 25 min. at room temperature diethyl oxalate (0·19 mole) was added with shaking. After another 15 min. glacial acetic acid (15 ml.) was added and the mixture was extracted with

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[16] Rossi and Schinz, *Helv. Chim. Acta*, 1948, **31**, 473.
[17] Wislicenus and Endres, *Annalen*, 1902, **321**, 381.
[18] *Org. Synth.*, Coll. Vol. III, p. 142.
[19] Hudson and Hauser, *J. Amer. Chem. Soc.*, 1941, **63**, 3156.

water. The ether solution was washed with saturated sodium hydrogen carbonate solution and dried, the solvent distilled off, and the residue fractionated. *tert.*-Butyl αα-dimethylethoxaloacetate (23 g.; 46%) had b. p. 104—105°/4 mm., n_D^{20} 1·4252 (Found: C, 58·8; H, 7·9. Calc. for $C_{10}H_{20}O_5$: C, 59·0; H, 8·2%).

αα-Dimethyloxaloacetic acid was prepared by hydrolysis of the ester. The removal of the *tert.*-butyl group by hydrogen bromide in acetic acid and of the ethyl group by concentrated hydrochloric acid has been described.[8] The overall yield was 35%. The acid had m. p. 105° (decomp.) (from benzene) [Found: C, 44·8; H, 4·8%; *M* (by titration against alkali), 161·4. Calc. for $C_6H_8O_5$: C, 45·0; H, 5·0%; *M*, 160·1].

Ethyl αα-dimethylethoxaloacetate (yield 61%), b. p. 122—123°/15 mm., was prepared by condensing diethyl oxalate and ethyl *iso*butyrate with sodiotriphenylmethane [19] (Found: C, 55·8; H, 7·1. Calc. for $C_{10}H_{16}O_5$: C, 55·6; H, 7·4%). Its semicarbazone had m. p. 98°.

Ethyl αα-dimethyloxaloacetate was prepared by hydrolysis [20] of ethyl αα-dimethylethoxaloacetate (10 ml.) with concentrated hydrochloric acid (80 ml.) during 3 days. The oily product slowly deposited crystals (3 g.) (Found: C, 51·0; H, 6·5. Calc. for $C_8H_{12}O_5$: C, 51·0; H, 6·4%).

Pyruvic acid (B.D.H.) was distilled twice and then frozen (m. p. 13°) [Found (by titration against alkali): *M*, 88·2. Calc. for $C_3H_4O_3$: *M*, 88·1]. Only freshly prepared aqueous solutions were used.

Spectrophotometric Measurements.—A Hilger Uvispek spectrophotometer was used. Measurements on stable solutions were made in a cell compartment maintained by circulating water at 25°. Kinetic runs were made at room temperature (20°). Pairs of 1-cm. quartz cells were used, one of which contained the organic substrate, the concentration of the other components being identical in the two cells. In kinetic experiments optical-density measurements were begun 45 sec. after addition of the metal solution.

Potentiometric Measurements.—pH measurements were made with a Cambridge bench-type pH meter and a commercial glass electrode at 25°.

Microanalyses were carried out by Mr. J. M. L. Cameron.

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[20] Rassow and Bauer, *J. prakt. Chem.*, 1909, **80**, 87.