vapor pressures of the corresponding ethers. The melting points of the tetrabromide compounds are approximately 60° lower than those of the tetrachloride products. The study of systems involving tin tetraiodide revealed little or no tendency for the tetraiodide to form addition compounds with tetrahydrofuran and tetrahydropyran.

The replacement of one of the chlorine atoms in silicon tetrachloride by a hydrogen atom does not cause addition compound formation with diethyl ether. With tetrahydrofuran a 1:1 compound of low stability is obtained. This compound, however, may be the result of weak hydrogen bonding between the silicon and oxygen or some other process, rather than coördination between the silicon and oxygen.

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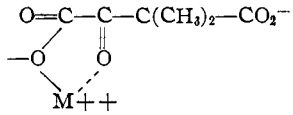
[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY OF THE UNIVERSITY OF CHICAGO]

Metal Ion-catalyzed Decarboxylation: A Model for an Enzyme System[1]

BY RUDOLPH STEINBERGER[2] AND F. H. WESTHEIMER

A study of the decarboxylation of dimethyloxaloacetic acid, A, $RO_2C—CO—C(CH_3)_2—CO_2H$ (R = H) has shown that the first product of decarboxylation is the enol form of α-ketoisovaleric acid. Similarly, the monoethyl ester of A, (R = C_2H_5) yields the enol form of ethyl α-ketoisovalerate. The enols have been identified spectroscopically and by bromine titration. The decarboxylation of dimethyloxaloacetic acid is catalyzed by heavy metal-ions, whereas that of the mono ester is not. The *p*H–rate profiles for these decarboxylation reactions show that the anion of the ester, the mono and dianions of the diacid, and a complex of the diion with metal ions, undergo decarboxylation. The effect of metal ions, M, on the decarboxylation reaction has been interpreted in terms of a complex with the structure



The results obtained with dimethyloxaloacetic acid have been compared with those for the enzymatic and non-enzymatic decarboxylation of oxaloacetic acid itself, and a tentative hypothesis has been formulated to account for the role of the protein in the enzymatic process.

Introduction

In a recent communication, the authors[3] outlined a synthesis of dimethyloxaloacetic acid, A, $HO_2CCOC(CH_3)_2CO_2H$ and of its monoethyl ester, B, $C_2H_5O_2CCOC(CH_3)_2CO_2H$; they further showed that the decarboxylation of the acid is catalyzed by heavy metal ions, whereas the monoethyl ester is essentially unaffected by these ions. From these facts it was concluded that the metal ion which catalyzes the decarboxylation of dimethyloxaloacetic acid (and by analogy of oxaloacetic acid) must be coördinated with that carboxyl group of the diacid which is not lost during the reaction.

The present paper gives in detail a proposed mechanism by which metal ions catalyze certain decarboxylations. The proposed mechanism for the decarboxylation of A^-, the monoanion of dimethyloxaloacetic acid, is shown in equations (1) and (2)

$$\underset{(A^-)}{HO_2C—CO—C(CH_3)_2—CO_2^-} \longrightarrow HO_2C—\underset{\displaystyle O^-}{\underset{|}{C}}=C(CH_3)_2 + CO_2 \quad (1)$$

$$\underset{\displaystyle O^-(C^-)}{HO_2C—\underset{|}{C}=C(CH_3)_2} + H^+ \longrightarrow HO_2C—CO—CH(CH_3)_2 \quad (D) \quad (2)$$

The intermediate first formed is the enol, C, or enolate ion, C^-, of α-ketoisovaleric acid, D; this ion is stable enough to be identified both spectroscopically and by bromine titration. During the catalyzed decarboxylation, a metal ion complex of the diion is probably formed. The effect of various complex-forming agents upon the rate of the catalyzed decarboxylation is interpreted, and a possible role for the enzyme in an enzyme-catalyzed decarboxylation of oxaloacetic acid is discussed.

Experimental

Kinetic Method

Apparatus.—Most of the kinetic determinations were made in a manometric apparatus of a familiar type.[4] The reaction rate was followed by measuring the amount of carbon dioxide evolved at various stages of the reaction. The sample of β-keto acid was introduced into the reaction mixture by means of a platinum bucket, which was suspended from the ground glass stopper of the reaction vessel; the bucket was dropped into the reaction mixture by rotating the stopper. Both reaction flask and manometer were immersed in a thermostat held at 25.00 ± 0.02°. Readings were taken through a window in the bath.

In most of the reactions here reported the reaction flask was shaken at a rate of 240 to 310 oscillations per minute. Slower rates were sometimes satisfactory; but in concentrated buffer solutions the slower shaking rate failed to bring about the prompt evolution of CO_2, even from creased flasks, and apparent induction periods, as long as an hour, were observed.

Retention of CO_2.—The manometric method just mentioned was used with solutions having a *p*H < 6. There is undoubtedly some retention of CO_2 by solutions where the *p*H > 5; this effect, however, is quite small and the amount of CO_2 evolved corresponds almost quantitatively to that shown in equation (1). In general, the evolution of CO_2 is somewhat slow at the beginning of an experiment, but reaches the first-order rate before the reaction has proceeded 5% to completion.

(1) Presented at the 118th Meeting of the American Chemical Society, at Chicago, September, 1950.

(2) Atomic Energy Commission Predoctoral Fellow, 1949–1950.

(3) R. Steinberger and F. Westheimer, THIS JOURNAL, **71**, 4158 (1949).

(4) J. Brönsted and C. King, *ibid.*, **47**, 2523 (1925).

Spectrophotometric Method.—To determine the rate of decarboxylation in basic solution, the decrease in optical density at 3100 to 3500 Å. was used. As is explained below, this method is satisfactory only when the concentration of enol, C, is very small. The experimental data indicate that this condition is met in moderately concentrated buffers. The decrease in the concentration (determined spectrophotometrically) of the diacid gives the same first-order rate constant as that obtained from the evolution of CO_2 in the manometric experiments.

Spectrophotometer.—All optical measurements were made on a Beckman model DU quartz spectrophotometer. Measurements at both 1-cm. and 10-cm. path lengths were made in cell compartments kept at 25.00° by circulating water from the thermostat.

Synthesis.—A general outline of the synthesis of dimethyloxaloacetic acid and of its monoethyl ester, was given in the previous communication.[3]

The condensation of ethyl oxalate and *t*-butyl isobutyrate by means of sodium triphenylmethide gives the ethyl-*t*-butyl ester of dimethyloxaloacetic acid, $C_2H_5O_2CCOC(CH_3)_2CO_2C(CH_3)_3$. The *t*-butyl isobutyrate reacts with sodium triphenylmethide more slowly than does ethyl isobutyrate. With this exception, the synthesis parallels that reported by Hauser[6]; yield of ester 40%; b.p. 107–111° (7 mm.); d^{25} 1.0124; n^{20} 1.4257.

In order to hydrolyze the ethyl-*t*-butyl ester to the monoethyl ester, $C_2H_5O_2CCOC(CH_3)_2CO_2H$, 10 g. of the former was mixed with an equal volume of a saturated (approximately 5 *M*) solution of HBr in glacial acetic acid; after 5 minutes at room temperature the mixture was placed in a vacuum desiccator. The acetic acid, hydrogen bromide and *t*-butyl bromide were removed by pumping off the gases (mechanical pump, Dry Ice and liquid nitrogen traps) for several hours, and then allowing the mixture to stand in vacuum over soda lime overnight. The crude liquid monoester was dissolved in ligroin (90–110°) and cooled in an acetone–Dry Ice-bath. When the walls of the vessel were scratched, the ester crystallized. The crystals were filtered, washed with cold petroleum ether and dried. The hygroscopic solid melted at 38–39°; yield 55%.

Five grams of the crude monoester was hydrolyzed to the diacid by adding it to 50 ml. of concd. hydrochloric acid and allowing the mixture to stand at room temperature for three days. The hydrochloric acid and ethyl alcohol were evaporated off in a stream of dry air and the acid was recrystallized twice from dry benzene; yield 60%; m.p. 105.5–106.5° with decomposition.

The syntheses outlined above were adopted because hydrolysis of the diethylester of dimethyloxaloacetic acid leads to the undesired monoethyl ester,[6] $HO_2C—CO—C(CH_3)_2CO_2C_2H_5$; no means could be found to complete the hydrolysis of this ester, to form the diacid, A. Presumably, the carboxyl group adjacent to the quaternary carbon atom is sterically protected. The synthetic method here adopted takes advantage of the fact that *t*-butyl esters can be cleaved by a mechanism[7] (presumably through a solvated carbonium ion) unavailable to the corresponding ethyl ester.

Isolation of Products.—The collected products from a set of completed kinetic experiments were acidified with concd. HCl and treated with a solution of 2,4-dinitrophenylhydrazine in 6 *M* HCl. The yellow precipitate was digested on the steam-bath for about an hour, filtered, washed and dried. The derivative from the monoester experiments was recrystallized from ethanol, whereas that from diacid runs was recrystallized from acetone or acetone–ligroin mixtures. The 2,4-dinitrophenylhydrazone[8] of α-ketoisovaleric acid melted at 194–194.5°. Calcd. for $C_{11}H_{12}O_6N_4$: C, 44.6; H, 4.08; N, 18.9. Found: C, 44.8; H, 4.21; N, 19.1. The analysis of the derivative of ethyl α-ketoisovalerate has already been reported.[3]

The reaction mixtures from the completed reactions catalyzed by metal ions were freed from these ions by treatment with H_2S; the derivatives of the organic products were isolated as already described. They proved to be identical (melting point method) with the products of the uncatalyzed experiments.

(5) B. Hudson, Jr., and C. Hauser, THIS JOURNAL, **63**, 3156 (1941).

(6) B. Rassow and R. Bauer, *J. prakt. Chem.*, [2] **80**, 87 (1909).

(7) B. Tronov and N. Ssibgatullin, *Ber.*, **62B**, 2850 (1929); S. Cohen and A. Schneider, THIS JOURNAL, **63**, 3382 (1941).

(8) G. Ramage and J. Simonsen, *J. Chem. Soc.*, 532 (1935).

Determination of Ionization Constants.—The first and second ionization constants of dimethyloxaloacetic acid were determined by an indicator method. The optical density due to one colored form of an indicator in a buffer of the diacid, A, was compared with the optical density of the same concentration of the same indicator in a corresponding oxalic acid buffer. Since the ionization constants of dimethyloxaloacetic acid are of the same order of magnitude as those of oxalic acid, this method permitted the determinations to be almost as precise as those for oxalic acid itself. Since all the kinetic measurements here reported were made at an ionic strength of 0.5 mole/liter, the ionization constants desired are approximate values of the "concentration" equilibrium constants, K'. The constant K_1' for oxalic acid was estimated from the value of the thermodynamic constant[9] and activity coefficients estimated by extrapolating Kielland's formula[10] to 0.5 m./l. The constant K_2' was similarly calculated; the value found agrees approximately with that of McComas and Rieman.[11] The "*p*H" of each buffer was measured with a Beckman glass electrode *p*H meter; on the assumption that the meter measures hydrogen ion concentration, the values so obtained are in agreement with those calculated by the method outlined above, and with standard values for 0.1 molar $HClO_4$ and for a dilute phthalate buffer. Clearly, none of the measurements of acidity here reported is accurate enough to distinguish unambiguously between activity and concentration. All the measurements, however, were made with the same instrument and with solutions of the same ionic strength and are self-consistent.

Thymol blue and methyl red, respectively, were used to determine K_1' and K_2' of dimethyloxaloacetic acid; both indicators were used at a concentration of 10^{-5} molar. The values here used for K_1' and K_2' of oxalic acid are 0.11 and 1.7×10^{-4}, respectively. The values here found for K_1' and K_2' of dimethyloxaloacetic acid are 6.0×10^{-2} and 7.0×10^{-5}, respectively. The colorimetric data are given in Table I.

TABLE I

IONIZATION CONSTANTS OF DIMETHYLOXALOACETIC ACID

Solution	Composition	Optical density
HIn (Thymol blue)		1.448
		(λ = 5400 Å.)
In^-		0.060
Oxalate buffer	0.05 *M* H_2Ox, 0.05 *M* HOx^-	.787
Diacid buffer	.05 *M* H_2A, 0.05 *M* HA^-	.700
HIn (Methyl red)		0.108
		(λ = 4000 Å.)
In^-		1.65
Oxalate buffer	$(HOx^-) = (Ox^=) = 5 \times 10^{-4}$ *M*	0.232
Diacid buffer	$(HA^-) = (A^=) = 5 \times 10^{-4}$ *M*	.333

All solutions brought to an ionic strength of 0.5 with $NaClO_4$.

Results

The enol, C (or possibly the enolate ion, C^-), was identified spectroscopically. The spectrum of an aqueous solution of 0.001 mole per liter of the pure diacid in distilled water was observed. It showed at 3350 Å. the low absorption peak (ϵ = 38) characteristic of α-ketoacids.[12] (Here $\epsilon = D/cl$, where D is the optical density recorded on the Beckman spectrophotometer, c is the concentration of the diacid in moles per liter, and l is the cell length in centimeters.) The decarboxylation was allowed to proceed at room temperature and the spectrum of the solution was observed at intervals. The intensity of the absorption maximum at 3350 Å. diminished, until after 6 hours it had fallen to half its original value. At the same time,

(9) H. Harned and B. Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publishing Corp., New York, N. Y., 1943, pp. 210, 580.

(10) J. Kielland, THIS JOURNAL, **59**, 1675 (1937).

(11) W. McComas, Jr., and W. Rieman III, *ibid.*, **64**, 2948 (1942).

(12) C. Fromageot, M. Pelletier and P. Ehrenstein, *Bull. soc. chim.*, **51**, 1283 (1932).

the absorption below 2900 Å. greatly increased. After 6 hours, however, the new, very intense maximum around 2400 Å. began to decrease, whereas the absorption at wave lengths greater than 3000 Å. increased. The final absorption is not identical with the original one; the maximum is at 3100 Å. rather than at 3350 Å. But the facts cited clearly indicate that the carbonyl absorption, which was almost absent after 6 hours, had reappeared as the reaction approached completion. The absorption curves are presented in Fig. 1.

Evidently an intermediate, which absorbs strongly at wave lengths of 2300–2700 Å. first forms and then slowly disappears, whereas the concentration of dimethyloxaloacetic acid (which absorbs at 3350 Å.) continuously decreases, and the product, α-ketoisovaleric acid, which absorbs at 3100 Å., appears rather late in the reaction. The intermediate, which has its absorption maximum at 2400 Å., is almost certainly the enol. On the basis of the quantitative estimate (see below) of the quantity of enol present, the compound has an extinction coefficient of 7500; for comparison, α-ethoxycrotonic acid[13] has its absorption maximum at 2200 Å. and an extinction coefficient of 8,700. Although the two compounds are not strictly similar, the intense absorption at 2400 Å. suggests the α,β-unsaturated acid structure.

When the decarboxylation is carried out in buffered solution, the maximum amount of enol present at any time is much less. Presumably the enol is still formed during the decarboxylation, but the buffer catalyzes its ketonization.[14] Thus when the decarboxylation is carried out in an 0.25 molar acetate buffer, the maximum absorption at 2600 Å. is only 7% of that in the absence of any buffer. When the decarboxylation is catalyzed by copper (0.001 *M*) the maximum is 3.4 times as great as it is in the uncatalyzed experiment; presumably this increase is due to a copper enolate. The ketonization is essentially complete after one hour.

It is possible to estimate the amount of enol formed in the decarboxylation reaction. Since the extinction coefficient of dimethyloxaloacetic acid is known, and since the rate of decarboxylation has been measured manometrically, the part of optical density at 3350 Å. due to the diacid can be calculated for any instant. The difference between the observed optical density at 3350 Å. and that due to dimethyloxaloacetic acid can then be determined. This optical density may then be assigned to α-ketoisovaleric acid; and since the extinction coefficient of this compound is known, its concentration can be calculated. The sum of the concentrations of dimethyloxaloacetic acid and of α-ketoisovaleric acid gives the total concentration of keto acids; the difference between this sum and the initial concentration of dimethyloxaloacetic acid gives the concentration of the enol. This method of computation assumes that the enolic acid does not absorb at 3350 Å. The known absorption spectrum of olefinic acids and the absorption spectrum of the reaction mixture at the end of 6 hours both show that this assumption is reasonable. If, however, part of the optical density at 3350 Å. is due to the enol, then the true concentration of ketoisovaleric acid is somewhat less than that calculated, and the concentration of enol somewhat greater. In other words, the calculation here outlined gives the lower limit for the percentages of enol present. The calculated percentages of enol under various experimental conditions are shown in Table II.

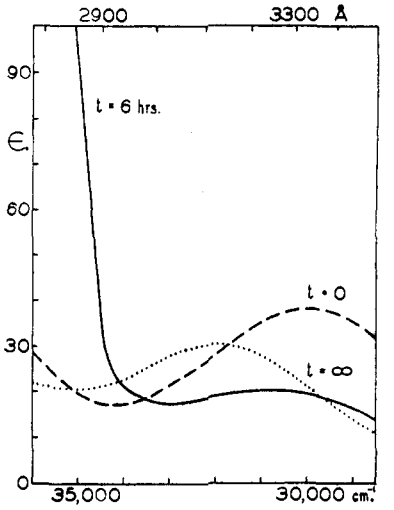


Fig. 1.—Ultraviolet absorption spectrum, determined at several times, of the solution obtained on decarboxylation of the diacid.

TABLE II

GREATEST CALCULATED ENOL CONTENT DURING DECARBOXYLATION

$(H_2A)_0$	$[Cu^{++}]$	Buffer	Enol, %	$t_{max.}$	λ, Å.
0.001*	...	.....	41	6 hr.	3300
.001	0.001	.....	48	5.5 min.	3400
.001	...	0.25 *M* OAc^-: 0.25 *M* HOAc	0.6	1 hr.	3500
.001	...	0.25 *M* OAc^-: 0.25 *M* HOAc	1.9	1 hr.	3300

The enol content of the solution marked by the asterisk was also determined by bromine titration, as follows: 5 ml. of standardized aqueous bromine solution in water was added to a sample of the solution undergoing decarboxylation. After a few seconds, solid potassium iodide was added and the amount of iodine liberated was determined by titration with sodium thiosulfate. The amount of bromine consumed was assumed equal to the amount of enol present in the reaction mixture. The value found was 35%.

It is at first surprising that the enols here formed ketonize slowly enough to permit their identification in solution. Presumably the conjugation of the olefinic double bond with the carboxyl group makes them somewhat more stable than simple enols; perhaps the enol here formed is also stabilized by steric effects.[15]

Kinetics of the Uncatalyzed Decarboxylation of the Monoester.—The monoester is decarboxy-

(13) L. Owen, *J. Chem. Soc.*, 385 (1945).

(14) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 229.

(15) R. Fuson, L. Armstrong, D. Chadwick, J. Kneisley, S. Rowland, W. Shenk, Jr., and Q. Soper, THIS JOURNAL, 67, 386 (1945).

lated according to a first order rate law. A sample kinetic experiment is shown in Table III.

TABLE III

SAMPLE KINETIC EXPERIMENT FOR THE MONOESTER

Concn. monoester approximately 0.015 M,[a] 20 ml. 0.05 M citrate buffer, pH = 2.7, μ = 0.5, T = 25.00 ± 0.02°

t, min.	P	$P_\infty - P$	log $(P_\infty - P)$	k, min.$^{-1}$
0	27.0	120.0	2.079	
4	38.0	109.0	0.037	0.0241
8	49.0	98.0	1.991	.0253
12	59.5	87.5	0.942	.0263
16	68.0	79.0	.898	.0260
20	75.8	71.2	.853	.0260
24	82.8	64.2	.808	.0260
28	89.0	58.0	.763	.0260
32	94.5	52.5	.720	.0258
38	101.7	45.3	.656	.0256
40	104.5	42.5	.628	.0259
44	108.2	38.8	.589	.0256
48	112.0	35.0	.544	.0256
52	115.2	31.8	.502	.0255
56	117.0	30.0	.477	.0248
60	121.1	25.9	.413	.0256
	147.0		Av.	.0256

[a] Due to the hygroscopic nature of the ester, it was usually not weighed out. The diacid was generally weighed out accurately.

The first order rate constants for the decarboxylation depend upon pH; the data are shown in Fig. 2. Here the open circles represent experimental points. The solid line through these points was

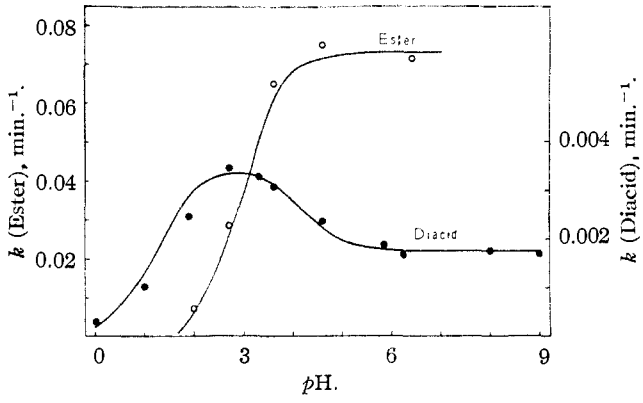


Fig. 2.—Rates of decarboxylation *vs.* pH: monoester, open circles, rate scale at left, solid line calculated for pK 3.0, k_{A^-} 0.073; diacid, filled circles, rate scale at right; solid line calculated for pK_1 1.22, pK_2 4.16, k_{HA^-} 0.00350, $k_{A^{--}}$ 0.00175.

calculated from the constants summarized in Table V, on the basis of the assumption that only the anion, B^-, of the monoethyl ester B can be decarboxylated. The fraction of the monoester which is ionized was calculated for each pH from the assumed ionization constant; then the rate was calculated from the equation

$$d(CO_2)/dt = k_{B^-}(B^-) \qquad (3)$$

Thus the solid line (marked "Ester") in Fig. 2 essentially represents a "titration curve" for B; the rate reaches a plateau where the ester is almost fully ionized. The ester is decarboxylated too rapidly to permit an experimental determination of its pK at 25°; the value (1×10^{-3}) here chosen corresponds reasonably with the other constants in Table V.

Kinetics of the Uncatalyzed Decarboxylation of the Diacid.—The diacid, in the absence of metals, undergoes first order decarboxylation. The individual rate constants show about the same average deviation as those in Table III; the fact that the rate constant is independent of the initial diacid concentration and of the buffer concentration is shown in Table IV.

TABLE IV

RATE OF DECARBOXYLATION OF DIMETHYLOXALOACETIC ACID

[Diacid]$_0$ M	Buffer	pH	$k \times 10^3$, min.$^{-1}$	Dev., %
1.0×10^{-3}	0.25 M acetate	4.6	2.35[a]	5.7
2.5×10^{-3}	.05 M acetate	4.6	2.33	1.3
6.25×10^{-3}	.05 M acetate	4.6	2.38	3.5
1.0×10^{-2}	.05 M acetate	4.6	2.42	5.2
1.25×10^{-2}	.25 M acetate	4.6	2.20	4.3
		Av.	2.30	

[a] Determined spectrophotometrically.

The dependence of the rate of decarboxylation of the diacid on pH is also shown in Fig. 2. The filled circles are experimental points. The solid line through these points was calculated, from the constants summarized in Table V, on the basis of the assumption that both the monoion, A^-, and the diion, $A^=$, of the diacid can be decarboxylated. The fraction of the diacid which is present as the monoion and the fraction which is present as the diion were calculated for each pH from the experimentally determined ionization constants. Then the rate was calculated from the equation

$$d(CO_2)/dt = k_{A^-}(A^-) + k_{A^=}(A^=) \qquad (4)$$

Note that different rate scales have been employed in Fig. 2 for the diacid and for its monoester.

Comparison with Decarboxylation of Dimethylacetoacetic Acid.—Table V shows that dimethyloxaloacetic acid and its ethyl ester are decarboxylated most rapidly by way of their monoanions. By contrast, dimethylacetoacetic acid is decarboxylated largely by way of the free acid.[16] Presumably, the electrostatic effect of the carboxyl group stabilizes the enolate ion, C^-, $HO_2C{-}C{=}C(CH_3)_2$ (O$^-$), more than it does the corresponding enol. This stabilization should lower the energy (and presumably the free energy) of activation because C^- is one of the structures contributing to the resonance hybrid of the activated complex for decarboxylation.

TABLE V

RATE AND EQUILIBRIUM CONSTANTS

Compound	pK_1	pK_2	Rate constants, min.$^{-1}$ Mono-anion	Di-anion
$C_2H_5O_2C{-}CO{-}C(CH_3)_2{-}CO_2H$, B	3.0	..	0.073	....
$HO_2C{-}CO{-}C(CH_3)_2{-}CO_2H$, A	1.22	4.16	.0035	0.00175

(16) K. Pedersen, THIS JOURNAL, 51, 2098 (1929).

The rate constant for the decarboxylation of the monoanion of dimethyloxaloacetic acid is only 1/20 that of the anion of the monoester. This difference is reasonable in the light of the fact that there are really two monoanions, A^- and A'^-, in equilibrium with each other. Whereas the ion A^- should probably be decarboxylated at the same rate as is the anion of the ester, the ion A'^- is analogous to the diacid itself, and should be rela-

$HO_2C—CO—C(CH_3)_2—CO_2^-$ $^-O_2C—CO—C(CH_3)_2—CO_2H$
A⁻ A'⁻

tively stable. Since pyruvic acid is about 20 times as strong an acid as is acetoacetic,[17] the equilibrium between the ions may well favor A'^- by a factor of 20; the ion A^-, then, is decarboxylated at a rate not far different from that of the ion of the monoester.

Similarly, it is at first surprising that the diion is decarboxylated half as fast as is the monoion, since the negative charge on the carboxylate ion groups should interfere with the stabilization of the activated complex and lower the rate by much more than a factor of two. However, the monoion, A^-, is probably decarboxylated 40 times as fast as is the diion, $A^=$; the apparent similarity in their rates of decarboxylation results from the fact that the mixture of monoions consists largely of the inert anion, A'^-.

Kinetics of the Metal-Ion Catalyzed Decarboxylation.—In the presence of low concentrations of metal ions, the decarboxylation usually still approximates a first order reaction. Table VI shows the effect of various metal ions on the rate.

TABLE VI

EFFECT OF METAL IONS ON THE RATE OF DECARBOXYLATION OF DIMETHYLOXALOACETIC ACID

Metal ion	Concn.	*p*H	*k*, min.$^{-1}$
None	...	4.6	0.0024
Cu^{++}	0.001	4.6	.143
Al^{+++}	.001	4.6	.128
Ni^{++}	.01	4.6	.0216
Mn^{++}	.01	4.6	.0058
None	...	2.4[a]	.0032
Fe^{++}[b]	.002	2.4[a]	.0102
Fe^{+++}	.002	2.3[a]	.301
None	...	0	.00032
Pd^{++}	.01	0	.00061

[a] Solution unbuffered. [b] Experiment conducted under nitrogen.

The effect of *p*H on the rate of a metal-catalyzed decarboxylation is shown in Fig. 3. The points of Fig. 3 cannot be connected by a single smooth curve; they show a discontinuity at *p*H 3.5, presumably due to the fact that solutions more acid than *p*H 3.5 were made up with chloroacetate buffers, whereas those more alkaline were made up with acetate buffers. All the buffers used had an anion concentration of 0.25 mole per liter. The rate of the uncatalyzed decarboxylation, which is controlled by the *p*H but not by the buffer composition, contrasts with the rate of decarboxylation catalyzed by metal ions, which depends upon the composition of the buffer solution. Despite the discontinuity in the *p*H–rate curve, evidently it is the diion of dimethyloxaloacetic acid which forms a complex with Cu^{++}, and which undergoes decarboxylation. No explanation is offered here for the slight decrease in rate at high *p*H.

(17) Landolt-Börnstein, "Physikalisch-chemische Tabellen," Ew. III, Springer Verlag, Berlin, 1936, p. 2108; A. Hantzsch and A. Miolati, *Z. physik. Chem.*, **10**, 1 (1892).

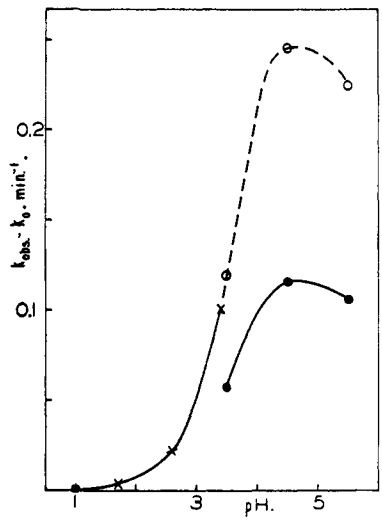


Fig. 3.—Rate of Cu^{++}-catalyzed decarboxylation of diacid *vs.* *p*H: ■, rate in 0.1 *M* $HClO_4$; ×, rates in chloroacetate buffers; ●, rates in acetate buffers; O, 2.12 times rates in acetate buffers; —, curve through experimental points; - - - -, chloroacetate curve extended through "corrected" acetate points.

Copper Complexes.—The efficiencies of copper ion as a decarboxylation catalyst in the presence of various complex-forming agents is shown in Table VII. Citrate greatly reduces the catalytic activity of Cu^{++}; on the other hand, the rate is greater in the presence of pyridine than it is in the presence of acetate. These observations will later be discussed in more detail.

TABLE VII

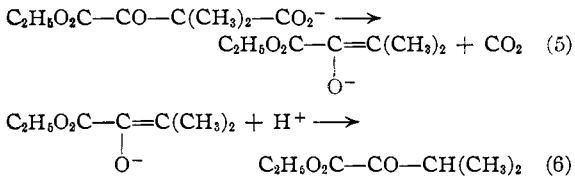
RATES OF Cu^{++} CATALYZED DECARBOXYLATION

10^3 conc. in m./l. Cu^{++}	A	*p*H	Buffer	Conc., m./l. Base	Acid	*k*, min.$^{-1}$
0.5	2.5	4.6	Acetate	0.25	0.25	0.049
.5	5.0	4.6	Acetate	.25	.25	.049
.5	12.5	4.6	Acetate	.25	.25	.042
.5	25	4.6	Acetate	.25	.25	.039
.5	5	5.4	Pyridine	.25	.25	.084
.5	5	*ca.* 5	Acetate	.22	.22	.069
			Pyridine	.03	.03	
1.0	5	4.6	Acetate	.05	.05	.229
1.0	5	5.4	Pyridine	.05	.05	.385
1.0	5	3.5	Acetate	.25	2.5	.060
1.0	5	3.4	Chloroacetate	.25	.25	.108
1.0	5	3.3	Citrate	.25[a]	.25	*ca.* .005

[a] Monosodium citrate.

Discussion of Results

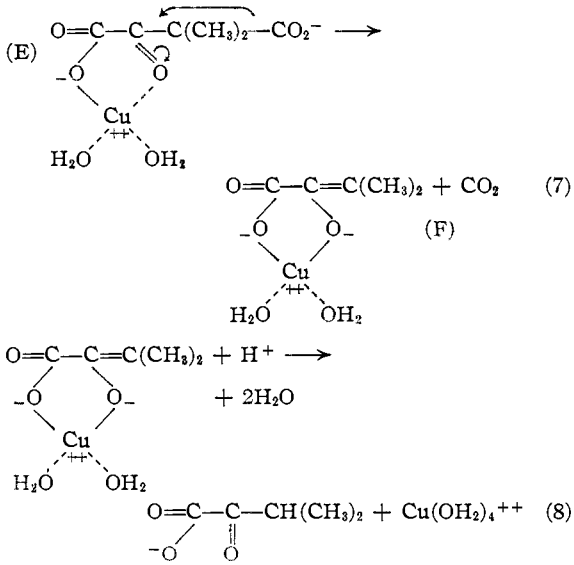
Mechanism of the Uncatalyzed Decarboxylations.—The decarboxylation of the monoester probably occurs according to equations (5) and (6)



The evidence that the proposed mechanism is correct consists of the facts that (a) a derivative of the ester of α-ketoisovaleric acid has been isolated, (b) the presence of the enol as an intermediate has been demonstrated spectrophotometrically, and (c) the *p*H–rate profile (Fig. 2) identifies the ion as the reactive species.

The monoanion of the diacid is probably decarboxylated according to equations (1) and (2). The evidence that the proposed mechanism is correct, consists of the facts that (a) a derivative of α-ketoisovaleric acid has been isolated, (b) the presence of the enol as an intermediate has been demonstrated spectrophotometrically, and by bromine titration, and (c) the *p*H–rate profile indicates that the diacid is decarboxylated only slowly, compared to either the mono- or the dianion. The dianion, $A^{=}$, presumably decarboxylates by a path similar to that for the monoanion.

Mechanism of the Metal-Ion Catalyzed Decarboxylation.—The mechanism proposed for the decarboxylation catalyzed by metal ions is illustrated (for copper ion) by equations (7) and (8).

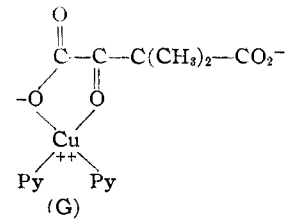


Reaction (8) is presumably general base catalyzed, and is analogous to the reverse of the metal ion catalyzed enolization of acetoacetic ester.[18]

The evidence that the proposed mechanism is correct consists of the facts that (a) a derivative of α-ketoisovaleric acid has been isolated (b) the enolic intermediate has been identified spectroscopically (c) the *p*H–rate profile (Fig. 3) shows that a complex of the diion is decarboxylated (d) the ester of dimethyloxaloacetic acid is decarboxylated without metal ion catalysis,[3] which shows that the copper ion must be associated with the carboxylate ion gamma to the one lost (e) since copper is usually, and palladium always,[19] planar in coördination compounds, the metal atom is probably not coordinated with the carbonyl oxygen and with both of the carboxylate ion groups. Furthermore, since Al^{+++} is a strong catalyst, no valence change of the metal ion is involved in the reaction. In addition to all this evidence, the observations made on decarboxylations catalyzed by ferric ion strongly support the proposed mechanism. When dimethyloxaloacetic acid is added to a colorless solution of ferric ammonium sulfate (a good catalyst; see Table VI), the solution turns bright yellow. The shade deepens rapidly as the decarboxylation proceeds, and the color is progressively green, blue and deep blue. When the decarboxylation is complete, the color gradually fades and the solution finally becomes colorless. Since dimethyloxaloacetic acid cannot enolize, the yellow complex is probably a ferric-dimethyloxaloacetate complex (perhaps analogous to E), and the blue color must be identified with a transitory ferric enolate (analogous to F) of α-ketoisovaleric acid. Incidentally, the fact that no color appeared during decarboxylations catalyzed by ferrous ions shows that the solutions used were free from ferric ion.

The mechanism here outlined assigns a reasonable function to the metal ion. During decarboxylation, an electron pair initially associated with the carboxylate ion group is transferred to the rest of the molecule (see arrows on E in equation 7). A metal ion, because of its positive electric charge, should assist this transfer; further, the higher the charge and the more readily the metal coordinates with the carbonyl group, the greater should be the catalytic activity of that ion. This general rule is amply borne out by Table VI.

Furthermore, the effect of various coördinating agents upon copper ion catalysis is in agreement with the rule here suggested. Negative ions, *e.g.*, citrate and acetate, diminish the catalytic activity of Cu^{++}; the amount of diminution is much greater for citrate than it is for acetate. These facts are in agreement with the assumption that, in buffer solutions containing these ions, only the complex E (containing Cu^{++}, dimethyloxaloacetate and two water molecules) loses carbon dioxide. Negative ions compete with dimethyloxaloacetate for the cupric ion, and thus reduce the concentration of E. Those negative ions which form the most stable complexes[20] with Cu^{++} must also effect the largest diminution of the rate. However, a complex-forming agent which does not destroy the charge on the copper ion does not destroy its catalytic activity. Thus pyridine,



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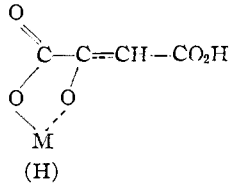
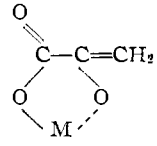
which readily forms complexes with Cu^{++}, promotes the decarboxylation. Probably the complex, G, can also undergo decarboxylation. Only an intricate series of quantitative measurements can determine the relative rates of decarboxylation of the aquo complex, E, and of the pyridinium complex, G.

Comparison with the Decarboxylation of Oxaloacetic Acid.—The decarboxylation of oxaloacetic acid is also catalyzed[21,22] by metal ions, and the presence of an enol or metal enolate in the decarboxylation mixture has been established. The structure H has generally been ascribed[23] to the enolate of the metal and it has been assumed that



(H)

H is the active intermediate in the decarboxylation. Of course, such an enolate may be formed. But the strict analogy with dimethyloxaloacetic acid suggests that H is not an active intermediate in the decarboxylation. On the other hand, the enolate, I, is probably formed during decarboxyla-



(I)

tion and would have about the same ultraviolet spectrum as H.

The Function of the Enzyme.—It is interesting to consider the function of the enzyme in the metal-ion catalyzed decarboxylation of oxaloacetic acid. The fact that neither the enzymatic[24] nor the non-enzymatic[21,23] decarboxylation of acetoacetic acid is promoted by cations, whereas both the enzymatic[22,25,26] and the non-enzymatic[21,22,23] decarboxylation of oxaloacetic acid is thus catalyzed, suggests that the essential feature of the enzyme is as a complexing agent for the metal. To be more precise, it is here proposed that the protein part of the enzyme has two quite different functions (a) to impart specificity with respect to substrate to the enzyme system, and (b) to complex the metal in such a way as to enhance its activity.

The effect of the enzyme on most metal ions is rather small. Thus the enzyme at a concentration of 7.5 mg./ml. enhances[22] the activity of 0.001 molar Zn^{++} only by a factor of 1.3. The effect is no greater than that which can be obtained, in acetate buffers, by complexing copper with pyridine. Of course, the concentration of enzyme is small. On the basis of the assumption that the molecular weight of the enzyme is 40,000, the enzyme may be a hundred times as effective as pyridine in promoting metal ion catalysis.

The enzyme causes a somewhat larger increase in rate for catalysis by the Mn^{++} and Cd^{++} than it does for that by other ions. For both these ions (at a concentration of 0.001 mole per liter) the enzyme (at a concentration of 7.5 mg. per ml.) causes a fivefold increase in rate. The effect with Cd^{++} is difficult to explain; but under no conditions is Cd^{++} a particularly good catalyst. However, the best catalyst for the decarboxylation, and the one which is actually present in biochemical systems, is the complex of enzyme and manganous ion. Although the special activity of manganous ion may represent some unknown and specific enzymatic effect, it is interesting to speculate upon the possibility that, when complexed with the apoenzyme, manganous ion is oxidized to manganic ion. A similar oxidation of manganous to manganic ion has been observed with another enzyme system.[27] And it is clear from the effect of ferrous and ferric ions on the decarboxylation of dimethyloxaloacetic acid (see Table VI), that a metal may be a much more effective catalyst when it is in its higher valence state.

There are several enzymes which are effective in the decarboxylation of oxaloacetic acid, or the oxidative decarboxylation of malic acid.[26] Clearly, the model system here described can only correspond more or less exactly to one of these systems. Perhaps some of these systems are essentially different from the one here described; perhaps they are merely variations on the same mechanism.

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