Mechanism of the Decarboxylation of Monoethyl Oxalacetate

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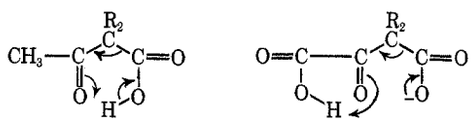
The monoethyl ester of oxalacetic acid was synthesized and its rate of decarboxylation studied. While the rate of decarboxylation of un-ionized monoethyl oxalacetate is not affected by a change in the solvent polarity, the rate of decarboxylation of its anion is facilitated by a lowering of the polarity of solvents. In contrast to the decarboxylation of the un-ionized monoester which exhibits the kinetic deuterium isotope effect, the decarboxylation of its anion is insensitive to the deuterium isotope. Reaction mechanisms consistent with experimental results are proposed for the decarboxylation of the un-ionized and anionic oxalacetates.

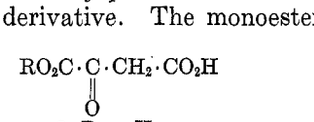
A great number of organic reaction mechanisms have been studied with a view that they may serve as chemical models for enzymic reactions.[1] One such system is the decarboxylation of β-keto acids.[2-5] Acetoacetic acid and α,α-dimethyl acetoacetic acid decarboxylate as free acids[6,7] by way of **1**, whereas oxalacetic acid and its α,α-dimethyl derivative decarboxylate mainly as their monoanions[2,8] by way of **2**. However, the intermediate **2** fails to explain why the monoester of α,α-dimethyl oxalacetic acid decarboxylates faster than its parent acid.[2] Furthermore, the pH–rate profile for the decarboxylation of oxalacetic acid (**3**) implicates the contribution of the un-ionized acid and its dianion to the rate.[8] In an attempt to resolve some of these uncertainties, the present work was undertaken to investigate the decarboxylation of monoethyl oxalacetate (**4**).



1 **2**

R = H or CH_3

Although a study of the decarboxylation of the monoethyl ester of α,α-dimethyloxalacetic acid has appeared,[2] a detailed kinetic study of the decarboxylation of **4**, which is the ester of the natural substrate of several enzymes,[9-11] may present a better model than its α,α-dimethyl derivative. The monoester **4** tautomerizes between keto and enol forms of which the keto tautomer is the active substrate for decarboxylase[9] and dehydrogenase[12] while the enol tautomer is responsible for the inhibitory effect of oxalacetate ion in an enzymic system.[13] The consideration of the keto–enol tautomerization in the decarboxylation study should lead to better understanding of the role of oxalacetates in enzymic reactions.



3, R = H
4, R = C_2H_5

Results and Discussion

To assess the structural requirement of carboxyl groups in decarboxylation, reactivities of oxalacetic acid and its esters to decarboxylate were examined. Within the pH range studied, oxalacetic acid (**3**) and 1-ethyl oxalacetate (**4**) decarboxylate in solutions. 4-Ethyl oxalacetate and diethyl oxalacetate, which are esterified at the 4-carboxyl group, do not undergo decarboxylation. The result is in agreement with the C_3–C_4 cleavage for the decarboxylation.[14,15] Although the decarboxylation of **3** has been studied in some detail,[8] a similar study of **4** is lacking. In discussing their results, Steinberger and Westheimer[2] assumed that the anion of the monoethyl ester of α,α-dimethyloxalacetic acid was the only species active in the decarboxylation. However, the sigmoidal pH–rate profile for the decarboxylation of **4** as shown in Figure 1 reveals that both un-ionized (**4a**) and anionic (**4b**) monoethyl oxalacetates decarboxylate.

At a given pH, **4** exists as **4a** and **4b**, which tautomerize between the enol and keto form. If keto tautomers are active in the decarboxylations,[2] according to Scheme I, the observed rate constant (k_{obsd}) can be expressed by

$$k_{obsd} = \frac{k_1[H^+] + k_2K_i}{(1 + K_e)([H^+] + K_i)}$$

SCHEME I

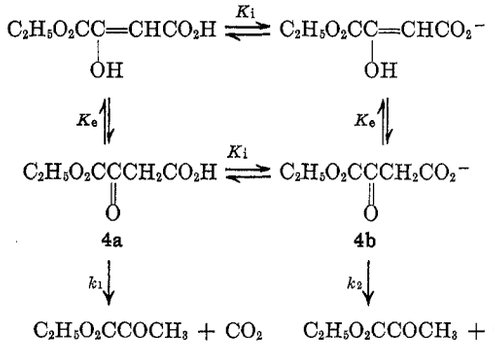


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TABLE I

KINETIC (30°, μ = 0.08 *M*) AND THERMODYNAMIC CONSTANTS OF OXALACETATES

Oxalacetate	$10^2 k_1$, min^{-1}	$10^2 k_2$, min^{-1}	K_e	K_i
$HO_2CCOCH_2CO_2H$		0.6[a]	0.09[b]	(3.16×10^{-3} and 4.27×10^{-5})[c]
$C_2H_5O_2CCOCH_2CO_2H$	0.4	4.5	0.19 ± 0.08	1.9×10^{-4}
$HO_2CCOCH_2CO_2C_2H_5$			0.32 ± 0.10	(1.8×10^{-3})[d]

[a] Reference 8. [b] Reference 17. [c] K. J. Pedersen, *Acta Chem. Scand.*, 6, 243 (1952). [d] E. Gelles and R. W. Hay, *J. Chem. Soc.*, 3673 (1958).

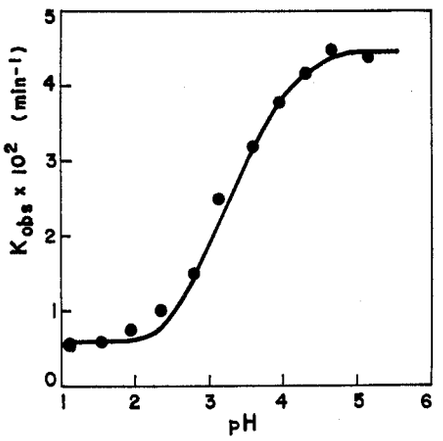


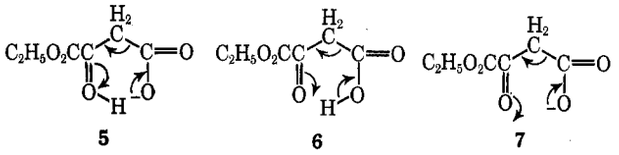
Figure 1.—pH–rate profile for the decarboxylation of 1-ethyl oxalacetate at 30°. KCl was added to maintain constant ionic strength at μ = 0.16 *M*.

where k_1 and k_2 are rate constants. K_i and K_e are the ionization constant and the enolization constant, respectively.[16] If $[H^+] \gg K_i$, and $k_1 \gg k_2K_i/[H^+]$,[17a] then $k_1 = k_{obsd}(1 + K_e)$, whereas if $[H^+] \ll K_i$[17b] and $k_2 \gg k_1[H^+]/K_i$, then $k_2 = k_{obsd}(1 + K_e)$. K_e is calculated from the percentage of keto tautomer by nmr spectrometry[18] and K_i is estimated from the pH–rate profile.[19] Results are summarized in Table I.

The faster rate of decarboxylation of **4** than that of **3** was attributed to a difference in ionization constants of the two carboxyl groups.[2] Indeed, the acidity of 1-carboxyl group is experimentally greater than that of the 4-carboxyl group by one pK unit. Thus, the monoanion of **3** is the mixture of monoanions, $^-O_2CCOCH_2COOH$ (**3a**) and $HO_2CCOCH_2COO^-$ (**3b**), consisting largely of the inert anion **3a**, whereas **4** can only exist in the active anion **4b**.

Table I shows that the per cent of enol tautomer in monoethyl esters of **3** is considerably higher (16 and 24% for 1- and 4-ethyl esters, respectively) than in the parent acid (8%). This result agrees with the previous report[18] that the esterification of carboxylic groups favors the enol tautomer.

Although the rate constant for the decarboxylation of un-ionized **4a** is only one-tenth that of monoanionic **4b**, both are active in the decarboxylation. While **4b** decarboxylates by way of **7**, the decarboxylation of **4a** may proceed by way of **5** or **6**, analogous to mechanisms proposed for acetoacetic acid.[7,20]



5 **6** **7**

According to the theory of Hughes and Ingold,[21] the rate of decarboxylation of **4b** *via* **7** is enhanced by solvents of low polarity, whereas that of **4a** *via* **6** is virtually unaffected by a change in the solvent polarity. The solvent effect on the decarboxylation of **4a** *via* **5** is less conclusive because the formation of **5** is favored by polar solvents but its decomposition is facilitated by nonpolar solvents. Table II shows that

TABLE II

SOLVENT EFFECT ON DECARBOXYLATION OF MONOETHYL OXALACETATE AT 30° AND μ (ACETATE ANION) = 0.08 *M*

Vol % ethanol	$10^2 k_{obsd}$, min^{-1} [a] pH 2.0[b]	pH 5.6[b]
0	0.40 ± 0.1	3.7 ± 0.5
16	0.34 ± 0.1	5.8 ± 0.8
36	0.42 ± 0.1	7.2 ± 0.4
50	0.40 ± 0.1	8.9 ± 0.8

[a] The observed rate constant (k_{obsd}) approximates k_1 and k_2 at pH 2.0 and pH 5.6, respectively. However, ethanol may also effect K_i (by suppressing the ionization) and K_e (by increasing the enol tautomer). Increased k_{obsd} values at pH 5.6 represent the lower limit as affected by ethanol; therefore, the conclusion that ethanol facilitates the rate of decarboxylation remains valid. [b] pH values quoted are direct meter readings.

the rate of the decarboxylation of **4b** is facilitated by a decrease in the polarity of solvents as expected, whereas that of **4a** is unaffected by the polarity of solvents.

The insensitivity of the decarboxylation of **4b** to the deuterium isotope (Table III) further substantiates

TABLE III

DEUTERIUM ISOTOPE EFFECT ON THE DECARBOXYLATION OF MONOETHYL OXALACETATE AT 30° AND μ (ACETATE ANION) = 0.08 *M*

	$10^2 k_1$, min^{-1}	$10^2 k_2$, min^{-1}
4 in H_2O[a]	0.46	4.0
Deuterated 4 in D_2O	0.15	3.9
k_H/k_D	3.07	1.03

[a] Monoethyl oxalacetate (4) used in this experiment has been treated by an identical procedure as deuterated **4**.

(16) Ionization constants for the enol and keto tautomers are assumed to be identical because **4** decarboxylates too rapidly to permit an experimental determination of an average K_i value. Enolization constants at pH 2.0 and 6.0 are identical within the experimental error (±5%).

(17) (a) This relationship holds only if $[H^+] \gg 1.14 \times 10^{-3}$. (b) This relationship is true as long as $[H^+] \ll K_i$.

(18) W. D. Kumler, E. Kun, and J. N. Shoolery, *J. Org. Chem.*, **27**, 1165 (1962).

(19) (a) The ionization constant obtained from the pH–rate profile corresponds to that of the keto tautomer. (b) If the average K_i[18a] can be determined potentiometrically, the ionization constant for the enol tautomer can be calculated.

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the intermediary formation of **7** in the decarboxylation of the anionic oxalacetate. The existence of a kinetic deuterium isotope effect (k_H/k_D = 3.1) implicates a proton transfer process leading to the decarboxylation of **4a**. However, the present experiment does not distinguish between the two mechanisms, namely **4a** → **6** → products and **4a** → **6** ⇌ **5** → products.

Experimental Section

Melting points were determined on a Fisher–Johns hot-stage apparatus. Infrared spectra were taken with a Perkin-Elmer Model 225 spectrophotometer. Ultraviolet spectra were taken with a Cary 14 spectrophotometer using 1.0-cm matched quartz cells. Nuclear magnetic resonance spectra were taken on JEOLCO JNM-C-60 and Varian Associates T-60 instruments. Chemical shifts are reported on the δ scale, parts per million downfield from tetramethylsilane or sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standard. Elementary analyses were performed by Organic Microanalyses, Montreal, Quebec, and Chemalytics, Inc., Tempe, Ariz. pH measurements were made with a Radiometer TTTlc. Trifluoroacetate (pH 1.5–3.0), acetate (pH 3.0–5.0), and phosphate (pH 5.0–6.5) buffers were prepared according to Gomori.[22] Ethanol was refluxed with magnesium turnings prior to the distillation.

Purification of Oxalacetic Acid (3) and Its Esters.—Oxalacetic acid and 4-ethyl oxalacetate were products of Nutritional Biochemical Corp. **3** was recrystallized twice from hexane as white crystals: mp 165–166°; uv (acetate buffer, pH 5.0) λ_{max} 260 nm (shoulder) (ϵ 1.3 × 10^3); nmr (CD_3COCD_3) δ 3.85 (s) and 5.95 (s). 4-Ethyl oxalacetate was recrystallized once each from chloroform and benzene as white crystals: mp 102–103°; uv (acetate buffer, pH 5.0) λ_{max} 260 nm (shoulder) (ϵ 0.6 × 10^3); ir (KBr) in the carbonyl stretching region, 1805, 1755, 1730, 1710, and 1664 cm^{-1}; nmr (CD_3COCD_3) δ 1.35 (t), 4.33 (q), 3.85 (s), and 6.00 (s). Diethyl oxalacetate was obtained from K & K Laboratories and purified by vacuum distillation: uv (acetate buffer, pH 5.0) λ_{max} 297 nm (ϵ 22.3 × 10^3); nmr (CD_3Cl) δ 1.25 (t), 1.36 (t), 4.08–4.50 (m), 3.80 (s), and 5.95 (s).

Synthesis of 1-Ethyl Oxalacetate (4).—Although the hydrolysis of the sodium enolate of diethyl oxalacetate was reported to yield **4**,[18] attempts by us and others[2] to prepare **4** or its α,α-dimethyl derivative by this procedure were unsuccessful. It gave white crystals, presumably 4-ethyl oxalacetate and a minor decarboxylating component. The following procedure was eventually employed to synthesize **4**. *tert*-Butyl acetate was prepared from *tert*-butyl alcohol and acetyl chloride in the presence of dimethylaniline.[23] The condensation of *tert*-butyl acetate with ethyl oxalate by means of isopropylmagnesium chloride[24] yielded 1-ethyl 4-*tert*-butyloxalacetate. The hydrolysis of 1-ethyl 4-*tert*-butyloxalacetate with HBr in glacial acetic acid[2] gave **4**, which was crystallized from hexane. Recrystallization of **4** from hexane twice gave white needles: mp 53–54°; uv (acetate buffer, pH 5.0) λ_{max} 262 nm (ϵ 1.4 × 10^3); ir (KBr) in the carbonyl stretching region, 1810, 1755, 1740, 1710, and 1640 cm^{-1}; nmr (CD_3COCD_3) δ 1.40 (t), 4.42 (q), 3.95 (s), and 6.13 (s).

Anal. Calcd for $C_6H_8O_5$: C, 45.00; H, 5.04. Found: C, 45.53; H, 5.27.

Preparation of Deuterated Compounds.—Deuterated trifluoroacetic acid was prepared by adding trifluoroacetic anhydride dropwise to D_2O. The distillation gave an azeotropic mixture (bp 91–92°). CH_3COOD was prepared by treating acetyl chloride with D_2O.[25] NaOD was obtained by dissolving sodium metal in D_2O. Deuterated **4** was prepared by an exchange reaction in D_2O followed by an immediate lyophilization. Deuterated buffers were prepared from CF_3COOD, CH_3COOD, and NaOD in D_2O. pH readings were corrected for pD values.[26]

Measurement of Enolization Constants.—Nuclear magnetic resonance spectra of freshly prepared solutions (in 0.1 *M* trifloroacetate buffer, pH 2.0 or phosphate buffer, pH 6.0) of monoethyl oxalacetates were taken. The ratio of ketonic CH_2 and esteric CH_3 (adjusted for the number of protons) was used to estimate the per cent of keto tautomer which was converted into enolization constant.

Rates of Decarboxylation.—The reactant–product relationship was established first by nmr spectrometry. A freshly prepared solution of **4** exhibits a triplet centered at δ 1.30 ppm (esteric CH_3) and a singlet at δ 3.20 ppm (ketonic CH_2). The esteric CH_2 was not detectable in H_2O. As the decarboxylation proceeded, the intensity of the ketonic CH_2 decreased with a concurrent appearance and a subsequent increase in the intensity of a singlet at δ 1.60 ppm corresponding to the CH_3 of ethyl pyruvate. For kinetic studies, rates of decarboxylation were determined manometrically in a Gilson differential respirometer as described previously.[8] The reaction mixture (2.5 ml) contained 200 μmol (based on the anionic species) buffer and 15 μmol of oxalacetate. The reaction was normally followed until 25% completion.

Registry No.—**3**, 328-42-7; **3** ethyl ester, 2401-96-9; **4**, 7597-72-0.

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