base catalysis by the neighboring carboxyl group analogous to the hydrolysis of aspirin. However, in the presence of saturating concentrations of Cu^{2+}, Ni^{2+}, Co^{2+}, or Zn^{2+}, the plot of log k_{obsd} vs. pH is linear with a slope of 1.0 in the pH range 2–7, indicating that the carboxyl group is not participating in the presence of metal ion. Thus, any rate-enhancing effect of metal ion binding on intramolecular general base catalysis cannot compete with the metal ion ⁻OH reaction. Consequently, metal ion assisted general base catalysis in carboxypeptidase A enhanced hydrolysis of esters is unlikely. T. H. Fife and V. L. Squillacote, unpublished work.

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Lack of Concertedness in the Catalysis of the Enolization of Oxaloacetic Acid by General Acids and Bases. Formation of a Carbinolamine Intermediate in the Tertiary Amine Catalyzed Enolization Reaction
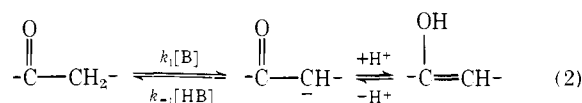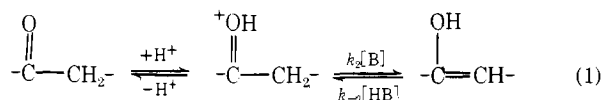
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Abstract: The interconversion of the keto–enol tautomers of oxaloacetic acid exhibits general-acid catalysis with acetate and pyridine buffers, is subject to general-base catalysis in the presence of carbonate and phosphate (HPO_4^{2-}/PO_4^{3-}) buffers, and exhibits both general-acid- and general-base-catalyzed pathways with imidazole and phosphate ($H_2PO_4^{-}/HPO_4^{2-}$) buffers. A Brønsted $-\alpha$ value of 0.43 and a β value of 0.35 were obtained for the general-acid and general-base catalytic rate constants. None of the buffer systems employed gave any evidence of the concerted general acid–general base catalyzed mechanism that had previously been reported to occur in the enolization of oxaloacetic acid. In the presence of tertiary amines of $pK_a > 8$, enolization occurs via the formation of a zwitterionic carbinolamine intermediate followed by amine-catalyzed elimination of a proton and tertiary amine from the protonated carbinolamine. Apparently, the quaternary ammonium group of the carbinolamine serves as an electron sink to enhance the rate of proton removal from the α-carbon of the ketone by the second molecule of tertiary amine. The elimination reaction apparently occurs only through protonated carbinolamine. The β value for the reaction of tertiary amines with oxaloacetic acid is 0.77.

Introduction

The interconversion of keto–enol tautomers may take place by two possible stepwise mechanisms, one subject to general-acid catalysis (eq 1) and the other to general-base catalysis (eq

$$-\overset{\overset{\displaystyle O}{\|}}{C}-CH_2- \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} -\overset{\overset{\displaystyle +OH}{\|}}{C}-CH_2- \underset{k_{-2}[HB]}{\overset{k_2[B]}{\rightleftharpoons}} -\overset{\overset{\displaystyle OH}{|}}{C}=CH- \quad (1)$$

$$-\overset{\overset{\displaystyle O}{\|}}{C}-CH_2- \underset{k_{-1}[HB]}{\overset{k_1[B]}{\rightleftharpoons}} -\overset{\overset{\displaystyle O}{\|}}{C}-\underset{-}{C}H- \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} -\overset{\overset{\displaystyle OH}{|}}{C}=CH- \quad (2)$$

2). In both of these reaction pathways, two consecutive steps are involved and the rate-limiting step in each is proton abstraction from the α-carbon atom. Alternatively, the reaction may take place through a concerted mechanism (eq 3) in which
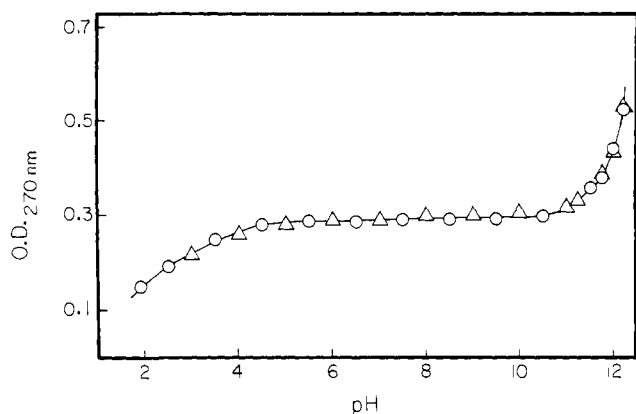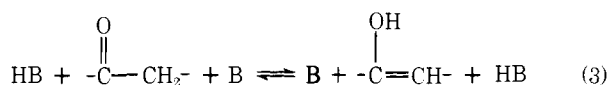
Figure 1. The absorbance at 270 nm of (O) an equilibrated aqueous solution of oxaloacetic acid (8.8×10^{-5} M, 30 °C, $\mu = 0.5$ with KCl) as a function of pH and ($\triangle$) an equilibrated solution of oxaloacetic acid (8.8×10^{-5} M, 30 °C, $\mu = 0.5$ with KCl) in 0.2 M 3-quinuclidinol minus the absorbance of 0.2 M 3-quinuclidinol as a function of pH.

$$HB + {}^{-}\overset{\overset{\displaystyle O}{\|}}{C}-CH_2{}^{-} + B \rightleftharpoons B + {}^{-}\overset{\overset{\displaystyle OH}{|}}{C}=CH{-} + HB \qquad (3)$$

proton donation to the carbonyl oxygen by the general acid and proton removal from the carbon atom by the general base take place concurrently. The "push–pull" catalysis of eq 3 has been of interest from the time of initiation of kinetic studies of keto–enol interconversion. Present interest continues due to questions concerning the feasibility of concerted movement of light and heavy atoms in a single transition state.[1] To date, however, except for the enolization reaction, other reported examples of push–pull catalysis have subsequently been shown to be incorrect.[2]

The possibility of the existence of a concerted mechanism for keto–enol interconversion is still open to controversy. Simultaneous catalysis by individual acid and base molecules will give rise to a third-order term (eq 4) in the rate law for enoli-

$$\nu = k_{ab}[HB][B][K] \qquad (4)$$

zation, unless a solvent molecule is functioning as a base or an acid in which case the mechanism of eq 3 will be kinetically indistinguishable from that of eq 1 or 2. Dawson and Spivey reported the existence of a third-order term in the acetic acid–sodium acetate catalyzed enolization of acetone.[3] Pederson analyzed Dawson and Spivey's results and concluded that the evidence for a concerted mechanism was unconvincing.[4] Swain, however, drew the opposite conclusion.[5] Upon repetition of the experimental work under more carefully controlled conditions, Bell and Jones[6] found that the product term was greater than originally reported by Dawson and Spivey, but they concluded that the concerted mechanism was not a "primary reaction pathway" of the enolization reaction. It has been suggested that the third-order term observed in the enolization of acetone may be due to a hydrogen-bonded acid–base pair rather than to concerted catalysis. When the present study was undertaken,[7] the most convincing experimental evidence, as judged by its frequency of citation, for the occurrence of the concerted mechanism was that of Banks[8] who reported that the enolization of oxaloacetic acid evidenced a third-order term in the presence of phosphate, imidazole, and triethanolamine buffers. Because of the low buffer concentrations (≤ 0.2 M) employed by Banks, a hydrogen-bonded acid–base pair is unlikely. We have reinvestigated the enolization of oxaloacetic acid employing low concentrations of buffer species (≤ 0.25 M) and have found no evidence for the concerted pathway that was reported by Banks. Recently, Hand and Jencks reported the occurrence of a third-order term in the acetate-catalyzed

enolization of cyclohexanone,[9] and Hegarty and Jencks found evidence for a concerted mechanism in the enolization of acetone with buffers of pK_a 2.8–5.[10] These authors were able to establish that catalysis does not involve a hydrogen-bonded acetate–acetic acid pair.

Experimental Section

Materials. Oxaloacetic acid (Aldrich) was used without further purification. Anal. Calcd for $C_4H_4O_5$: C, 36.38; H, 3.05. Found: C, 36.50; H, 3.14. The hydrochlorides of quinuclidine, 3-quinuclidinol, 3-chloroquinuclidine, and trimethylamine were recrystallized from water-ethanol, and imidazole was recrystallized from acetone-petroleum ether. Triethylamine and pyridine were distilled. All solids were dried in a vacuum dessicator over P_2O_5.

Buffer Solutions. The amine-amine hydrochloride buffer solutions were prepared just prior to use by the addition of standardized KOH or HCl to the amine hydrochloride or free amine. carbonate and phosphate buffer solutions were prepared using $KHCO_3$ and K_2CO_3, and KH_2PO_4, K_2HPO_4, and K_3PO_4. Generally, a minimum of five serially diluted buffer solutions were employed at each pH. The pHs of the serial dilutions agreed within 0.02 pH units. The pK_as of the buffers employed were determined by half-neutralization at 30 °C and $\mu = 0.5$ (KCl). Readings of pH were determined on a Radiometer Type PMH 26 pH meter.

Kinetic Measurements. All kinetic determinations were carried out in doubly glass-distilled water containing 10^{-4} M EDTA to sequester any extraneous metal ions, with $\mu = 0.5$ (KCl). Except where indicated, rate constants were determined at 30.0 ± 0.2 °C.

A stock solution of enolic oxaloacetic acid was prepared by dissolving the enolic salt in alcohol. Allowing the enolic salt to equilibrate in water (pH 3.7, 0.5 M KCl containing 10^{-4} M EDTA) provided a stock solution of predominately the keto tautomer. Thus, keto–enol interconversion could be monitored in the overall direction of ketonization using the ethanolic stock solution and in the direction of enolization upon addition of the aqueous stock solution to an aqueous solution of pH >3.7 (Figure 1). The concentration of the stock solutions was such that when added to the kinetic solution the oxaloacetate concentration was about 1.5×10^{-4} M. Enolization rates in the presence of lyate species were determined in a Radiometer pH–stat assembly specifically designed for a Cary 15 spectrophotometer;[11] at pHs >11 these rate constants were obtained with a Durrum-Gibson Model 13001 stopped-flow spectrophotometer. When buffer solutions were employed, the reactions were carried out under the pseudo-first-order conditions of $[buffer]_T \gg [oxaloacetic\ acid]$. Rate constants were determined on either a Cary 118, a Gilford Model 2000, or a Durrum-Gibson stopped-flow spectrophotometer. All spectrophotometers were thermostated. Rates were determined by following the change in absorption at 270 or 250 nm.

Calculation of the pseudo-first-order rate constants and least-squares slopes and intercepts and generation of the theoretical pH–rate profiles were done using a Hewlett-Packard Model 9820A computer.

Results

In Figure 1 is shown a plot of the equilibrium absorbance at 270 nm of a 8.8×10^{-5} M solution of oxaloacetic acid in water ($\mu = 0.5$, $T = 30$ °C) as a function of pH. This data is in good agreement with an earlier study of the pH-dependence of the keto-enol equilibrium of oxoaloacetic acid.[12] The pK_as of the carboxylic acid groups at 25 °C are 2.22 and 3.89.[13] The increase in absorption at low pH is due to increasing concentration of the enol tautomer upon ionization of the first carboxyl group. It has been reported that in aqueous solution at pH 7.4, 74.3% of oxaloacetic acid exists in the keto form, 17.8% in the enol form, and 7.8% is present as hydrated ketone.[14a] A more recent study has shown the composition of oxaloacetate at pH 6.89 to be 87.4% keto, 7.4% enol, and 5.2% hydrate.[14b] Loss of a proton from the enol tautomer ($pK_A = 13.03$ at 25 °C)[13] is responsible for the large increase in absorption in the basic pH region.

The pH–rate profile for keto-enol interconversion in the presence of lyate species is indicated by the solid line in Figure 2. At a given pH, the rate constant obtained using an equili-
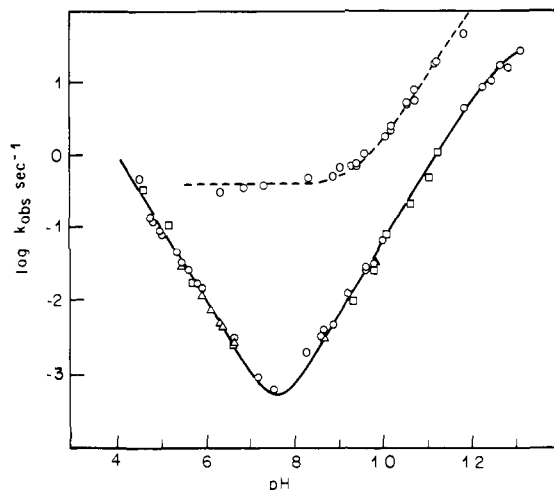
Figure 2. Solid line: pH–rate profile for the enolization of oxaloacetic acid (H_2O, 30 °C, $\mu = 0.5$). Rates determined with an autotitrator assembly employing primarily the keto tautomer as substrate (O) and the enol tautomer as substrate (△), and rates determined from intercepts of buffer dilution plots employing acetate, pyridine, imidazole, carbonate, and phosphate buffers (□). Dashed line: Intercepts obtained by extrapolation of the linear portions of amine buffer dilution plots at high $[N]_T$ to $[N]_T = 0$. The points are experimental and the lines are theoretical, having been generated from eq 5 and 9, respectively.
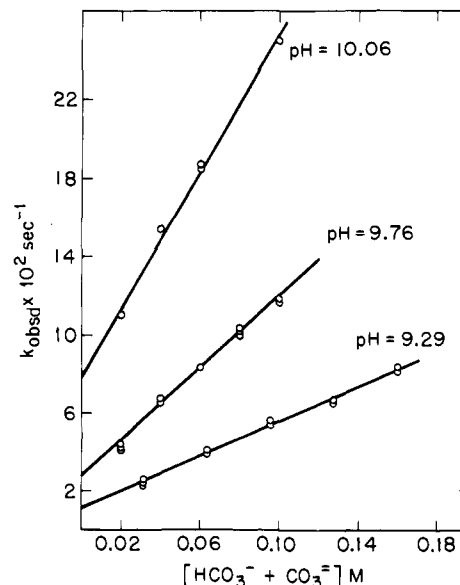

Figure 3. Plots of the observed first-order rate constants for the reaction of oxaloacetic acid in the presence of carbonate buffer vs. the total concentration of carbonate buffer at three hydrogen-ion concentrations.
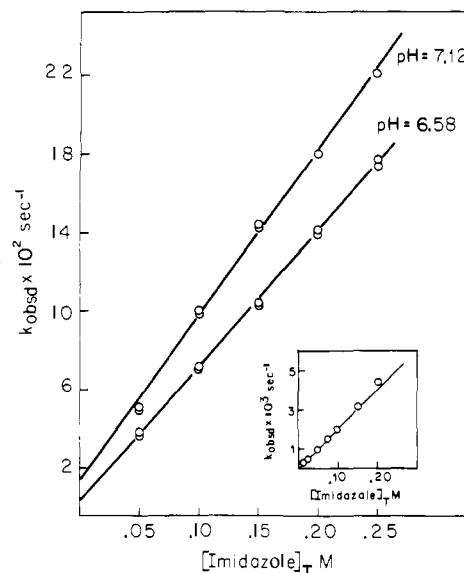

Figure 4. Plots of the observed first-order rate constants for the reaction of oxaloacetic acid in the presence of imidazole buffer (H_2O, $\mu = 0.5$, 30 °C) vs. the total concentration of imidazole at two hydrogen-ion concentrations. Inset: Data reported by Banks[8b] for the reaction of oxaloacetic acid in the presence of imidazole buffer (H_2O, $\mu = 0.1$, 1.5 °C, pH 7.12).

brated aqueous oxaloacetate stock solution and following the increase in absorption due to enol formation is identical to that obtained using an ethanolic stock solution of enolic oxaloacetate and monitoring the decrease in absorption due to ketonization; both techniques were employed in obtaining the points on the pH–rate profile. The points on the profile designated by squares were obtained from intercepts of buffer dilution plots employing acetate, pyridine, imidazole, phosphate, and carbonate as buffers. The line drawn through the experimental points was computer generated from the following empirical equation

$$k_{obsd} = k_H a_H + k_b \left(\frac{K_a}{K_a + a_H}\right) + k_{HO^-} \left(\frac{K_w}{a_H}\right) \quad (5)$$

where $k_H = 1.03 \times 10^4$ M^{-1} s^{-1}, $k_b = 38$ s^{-1}, $k_{HO^-} = 5$ M^{-1} s^{-1}, $pK_a = 12.75$, $pK_w = 13.83$, and a_H is the hydrogen-ion activity determined at the glass electrode. This study was not extended below about pH 4.5 because of the complications introduced by extensive formation of hydrated oxaloacetic acid when the carboxyl groups are undissociated.[14,15] Thus, the rate constants of Figure 2 pertain to the reaction of the dianion of oxaloacetic acid. For kinetic determinations requiring the use of a stopped-flow spectrophotometer, an equilibrated aqueous solution of oxaloacetate ($\mu = 0.5$ with KCl, 10^{-4} M EDTA, pH 3.7) was employed and the pH determined after mixing.

The buffer dilution plots obtained with phosphate (HPO_4^{-2}/PO_4^{-3}) and carbonate buffers (Figure 3) evidence general-base catalysis. Those obtained with acetate and pyridine buffers exhibit general-acid catalysis. The observed rate constants can, therefore, be expressed as in eq 6 and 7

$$k_{obsd} = k_{1y} + k_{gb} \left(\frac{K_a}{K_a + a_H}\right) [B]_T \quad (6)$$

$$k_{obsd} = k_{1y} + k_{ga} \left(\frac{a_H}{K_a + a_H}\right) [B]_T \quad (7)$$

where k_{1y} is the rate of the lyate species catalyzed reaction, k_{gb} and k_{ga} are the second-order rate constants for general-base and general-acid catalysis, K_a is the acid-dissociation constant of the buffer, and $[B]_T$ is the total concentration of buffer species present, i.e., $[HB] + [B]$. Plots of k_{obsd} vs. $[B]_T$ give slopes which, when divided by the mole fraction of buffer

present in the basic form $[K_a/(K_a + a_H)]$ or in the acid form $[a_H/(K_a + a_H)]$, give values of k_{gb} or k_{ga}, respectively. Imidazole and phosphate ($H_2PO_4^-/HPO_4^{-2}$) buffers evidence both general-base and general-acid catalytic terms with the observed rate constants for establishment of equilibrium correlated by the rate expression of eq 8.

$$k_{obsd} = k_{1y} + k_{ga} \left(\frac{a_H}{K_a + a_H}\right) [B]_T$$

$$+ k_{gb} \left(\frac{K_a}{K_a + a_H}\right) [B]_T \quad (8)$$

The buffer dilution plots are presented in Figures 4 and 5; the slopes are equal to $k_{ga}[a_H/(K_a + a_H)] + k_{gb}[K_a/(K_a + a_H)]$. Values of k_{gb} and k_{ga} are obtained from the slopes and intercepts, respectively, of secondary plots of [slope $(K_a + a_H)/a_H$]
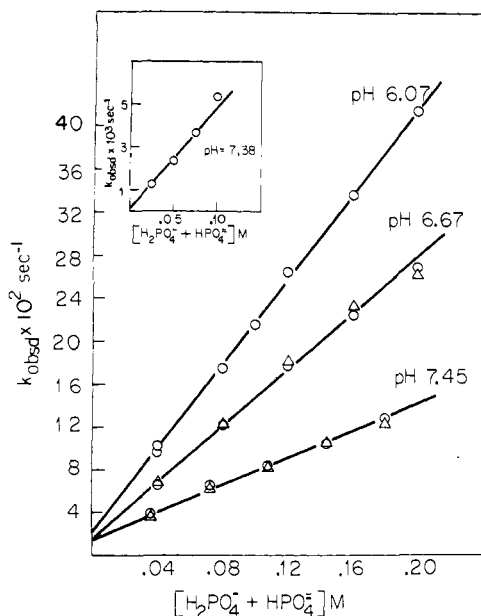
Figure 5. Plots of the observed first-order rate constants for the reaction of oxaloacetic acid in the presence of phosphate buffer (H_2O, $\mu = 0.5$, 30 °C) vs. the total concentration of phosphate buffer at three pH values. Rate constants determined employing primarily the keto tautomer as substrate (O) and the enol tautomer as substrate (Δ). Inset: Data reported by Banks[8b] for the reaction of oxaloacetic acid in the presence of phosphate buffer (H_2O, $\mu = 0.1$, 1.5 °C, pH 7.38).
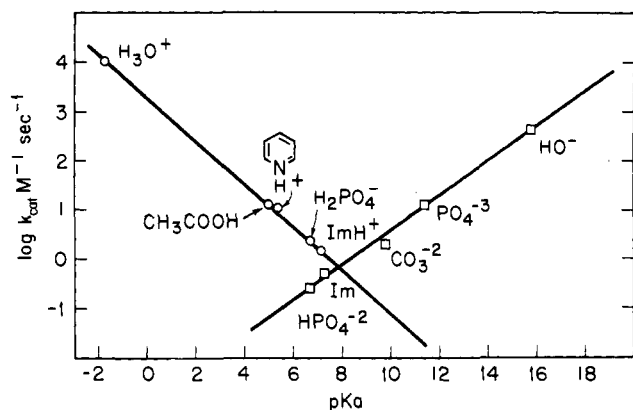
Figure 6. Plots of log k_{ga} (O) for the general-acid-catalyzed enolization of oxaloacetic acid vs. the pK_a of the general acid and log k_{gb} (□) for the general-base-catalyzed enolization of oxaloacetic acid vs. the pK_a of the general base.

vs. K_a/a_H. The logarithms of the k_{gb} and k_{ga} values thus obtained are plotted in Figure 6 vs. the pK_a of the catalyst. These values result in a Brønsted β value of 0.35 for general-base catalysis and a $-\alpha$ value of 0.43 for general-acid catalysis. The reaction of imidazole with oxaloacetic acid at 3.8 ± 0.2 °C results in the buffer dilution plot of Figure 7.

The enolization reaction was also investigated with tertiary amines as buffers. A keto-enol equilibrium mixture at pH 3.7 was stopped-flow mixed with amine at a more basic pH, resulting in the formation of a new equilibrium mixture as a result of increasing concentration of enolate anion and a consequently decreasing concentration of ketone (Figure 1). The amines employed are listed in Table I. Representative buffer dilution plots for the first-order reaction of oxaloacetic acid with tertiary amines are given in Figures 8-10. The intercepts of the linear plots of Figure 8 are plotted on the dashed line of Figure 2 as a function of the pH at which each was obtained. For the buffer dilution plots that evidence curvature (Figures 9 and 10), the points plotted on the dashed line of Figure 2 were
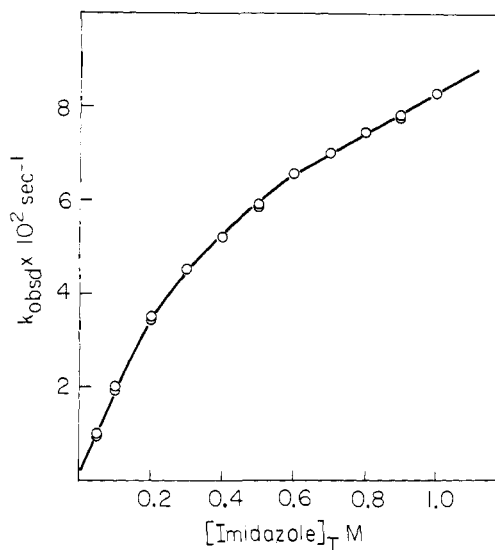

Figure 7. Plot of the observed first-order rate constants for the reaction of oxaloacetic acid in the presence of imidazole buffer at 3.8 °C vs. total imidazole buffer concentration ($\mu = 0.5$, pH 7.12).

Table I. Second-Order Rate Constants for Catalysis of Keto-Enol Interconversion of Oxaloacetic Acid by Tertiary Amines (Determined from Plots of k_{obsd} vs. [N]$_T$ at high [N]$_T$)

Amine	pK_a	k_N, M^{-1} s^{-1}
quinuclidine	11.11	155
triethylamine	10.63	7.7
3-quinuclidinol	10.11	15
trimethylamine	9.95	8.9
3-chloroquinuclidine	8.83	2.7

obtained by linear extrapolation of the rate constants at the higher buffer concentrations to zero buffer concentration. The reason for doing so will become evident (Discussion). The dashed line of Figure 2 was generated from eq 9

$$k_{obsd} = k_0 + k_{HO-}' \left(\frac{K_w}{a_H}\right) \qquad (9)$$

where $k_0 = 3.9 \times 10^{-1}$ s^{-1}, $k_{HO-}' = 8.8 \times 10^3$ M^{-1} s^{-1}, and p$K_w = 13.83$.

The slopes of the linear portions of the buffer dilution plots obtained with tertiary amines (i.e., Figure 8, and Figures 9 and 10 at high [N]$_T$) were divided by the fraction of total amine present as the free base. The resulting values of k_N are plotted in Figure 11 vs. the pK_a of the amine. The slope, β, is 0.77.

Discussion

General-Acid and General-Base Catalysis of Enolization. The pH-rate profile for keto-enol interconversion of oxaloacetic acid in the presence of lyate species (H_3O^+, H_2O, HO^-) is given by the solid line of Figure 2. At constant hydrogen-ion concentration, the pH-dependent rate constant (k_{obsd}) for approach to equilibrium is provided by the sum of the pH-dependent forward (k_f) and reverse (k_r) rate constants. Thus, identical k_{obsd} values are obtained, at any constant pH, when employing a stock solution of enol and monitoring the decrease in absorption due to ketonization, and when using an aqueous stock solution with oxaloacetic acid primarily in the keto form and following the increasing absorption associated with enolization.

The specific acid catalyzed pathway occurring below pH 7 is accounted for by preequilibrium protonation of the ketone followed by removal of an α proton by water (eq 1 with B = H_2O and HB = H_3O^+). In aqueous solution at constant pH,
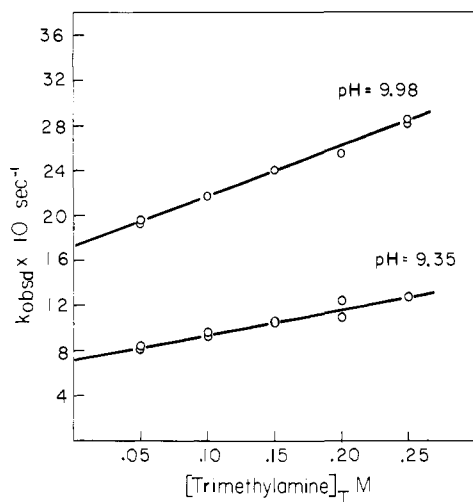
Figure 8. Plots of the observed first-order rate constants for the reaction of oxaloacetic acid with trimethylamine vs. the total concentration of trimethylamine at two pH values.
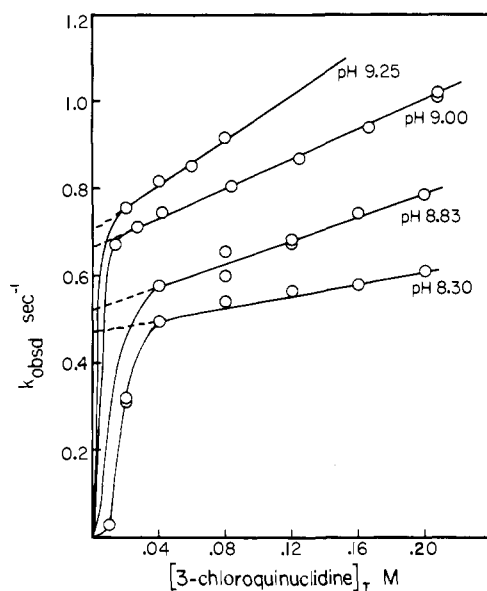

Figure 9. Plots of the observed first-order rate constants for the reaction of oxaloacetic acid with 3-chloroquinuclidine vs. the total concentration of 3-chloroquinuclidine at three pH values. Intercepts at $[N]_T = 0$ for the solid line buffer dilution plots were obtained from the pH–rate profile (solid line) of Figure 2. Intercepts obtained by extrapolating rates determined at high $[N]_T$ to $[N]_T = 0$ (dashed lines) are plotted on the dashed line of Figure 2.
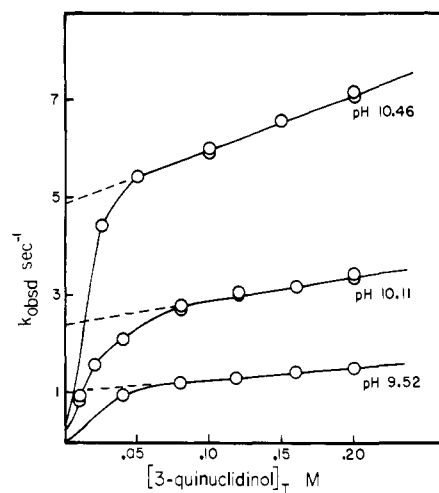

Figure 10. Plots of the observed first-order rate constants for the reaction of oxaloacetic acid with 3-quinuclidinol vs. the total concentration of 3-quinuclidinol at three pH values. Intercepts at $[N]_T = 0$ for the solid line buffer dilution plots were obtained from the pH–rate profile (solid line) of Figure 2. Intercepts obtained by extrapolating rates determined at high $[N]_T$ to $[N]_T = 0$ (dashed lines) are plotted on the dashed line of Figure 2.
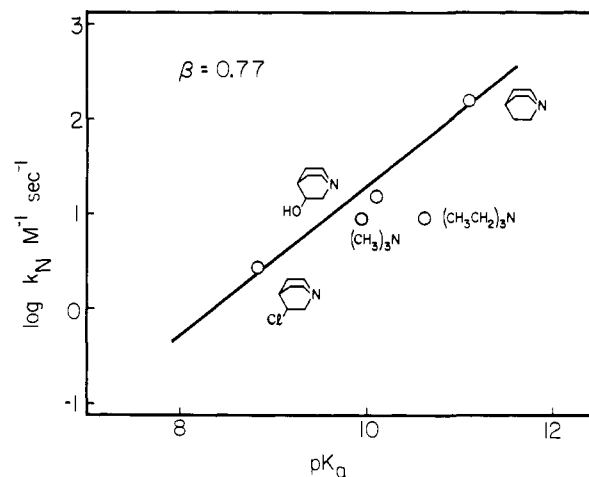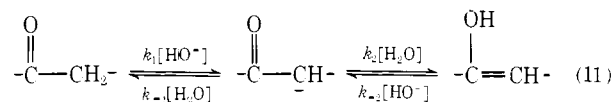

Figure 11. Brønsted plot for the reaction of tertiary amines with oxaloacetic acid. The second-order rate constants given as k_N pertain to values of k_1 for the mechanism of eq 17.

the rate-determining step of eq 1 may be assumed to be proton removal from carbon in the forward direction and proton donation to carbon in the reverse direction, since proton transfer to and from oxygen may be assumed to be rapid and to be controlled by the pH and the pK_a of protonated ketone. The kinetic expression for the mechanism of eq 1 is given by

$$k_{obsd} = k_f + k_r = k_2[H_2O] \left(\frac{a_H}{K_K + a_H}\right) + k_{-2}a_H \left(\frac{a_H}{K_E + a_H}\right) \quad (10)$$

where K_E and K_K are the acid dissociation constants of enol and protonated ketone, respectively. A deuterium solvent isotope effect (k_{H_2O}/k_{D_2O}) of 2.4 has been reported for the enolization of oxaloacetic acid under acidic conditions.[16]

Above pH 8, general-base catalysis is observed. Under basic

conditions in the presence of only lyate species, eq 2 may be written as eq 11.

As under acidic conditions, the rate-limiting step in the forward and reverse directions may be assumed to be proton removal from and proton donation to the α carbon of the ketone, respectively. The observed rate constants under basic conditions may be described by the rate law of eq 12

$$k_{obsd} = k_f + k_r = k_1[HO^-] + k_{-1}[H_2O] \left(\frac{K_{CH}}{K_{CH} + a_H}\right) \quad (12)$$

where K_{CH} is the dissociation constant of a proton from the α carbon of the ketone. Under basic conditions, the deuterium solvent isotope effect has been reported to be 4.5.[16] Since $K_K > a_H$ and $a_H > K_E$, summation of eq 10 and 12 results in the empirical expression of eq 5.

Table II. Theoretical Rate Constants for General-Acid- and General-Base-Catalyzed Keto–Enol Interconversion

| Mechanism	$k_f{}^a$ k_r	k_{obsd}
eq 1	k_f	$k_2 \left(\dfrac{K_A}{K_K + a_H}\right)$ [HB] $= k_2(K_A/K_K)$[HB] ^{b}
	k_r	k_{-2}[HB] $\left(\dfrac{a_H}{K_E + a_H}\right) = k_{-2}$[HB] ^{c}
eq 13	k_f	$K_1 k_2$[HB]
	k_r	k_{-2}[HB]
eq 2	k_f	k_1[B]
	k_r	$k_{-1} \left(\dfrac{K_{CH}}{K_{CH} + a_H}\right)$ [HB] $= k_{-1} \left(\dfrac{K_{CH}}{K_A}\right)$ [B] ^{d}
eq 14	k_f	k_1[B]
	k_r	$\dfrac{k_{-1}}{K_2}$ [B]

^{a} k_f = enolization; k_r = ketonization. ^{b} Since $K_K > a_H$. ^{c} Since $a_H > K_E$. ^{d} Since $a_H > K_{CH}$.

In the presence of buffers such as phosphate, carbonate, acetate, pyridine, and imidazole, keto–enol interconversion is subject to general catalysis. Linear buffer dilution plots are obtained and the logs of the intercepts of the plots (designated by squares) fall precisely on the pH-rate profile of Figure 2 established by use of a pH-stated spectrophotometer in the absence of buffer species. Some of the buffer dilution plots are shown in Figures 3–5. That the observed rate constants are the sum of the ketonization (forward) and enolization (reverse) reactions is evidenced in Figure 5 where superimposable buffer dilution plots were obtained using both pure enol as substrate and a keto–enol mixture with the keto form predominating.

There was no evidence of any upward curvature in any of the buffer dilution plots obtained during this study. Thus, the concerted mechanism of eq 3, which requires a second-order term in buffer species, can (at least in the concentration range of buffer employed) be ruled out for the enolization of oxaloacetic acid. In the insets to Figures 4 and 5 are plotted the data obtained by Banks which were responsible for her claim of the occurrence of a concerted mechanism for the ketonization of oxaloacetic acid in the presence of imidazole and phosphate buffers.[8b] The data are hardly conclusive for the establishment of a concerted mechanism, but, since the reactions were carried out at 1.5 °C, we repeated the experiment with imidazole at 3.8 °C to guarantee that our inability to detect a concerted mechanism was not the result of the higher temperature (30 °C) at which our study was done. We also extended the concentration range (0.015–0.20) employed by Banks to 1.0 M. These results are plotted in Figure 7. The possibility of a concerted mechanism under these conditions can obviously be put to rest. At this lower temperature, however, imidazole does not participate as a general acid–base catalyst as it does at 30 °C but apparently acts as a nucleophile toward oxaloacetic acid to form an intermediate carbinolamine, which then undergoes a general-base-catalyzed elimination reaction to give enol. This behavior is characteristic of tertiary amines of $pK_a > 8$ at 30 °C (see below).

At 30 °C, phosphate ($HPO_4{}^{-2}/PO_4{}^{-3}$) and carbonate buffers exhibit only general-base catalysis, imidazole and phosphate ($H_2PO_4{}^-/HPO_4{}^{-2}$) show both general-acid and general-base catalytic terms, while pyridine and acetate buffers appear to act only as general-acid catalysts. In previous enolization studies, it has occasionally been implied that with appropriate buffer systems the mechanisms given in eq 1 and 2 should each exhibit general-acid and general-base terms, since the rate of approach to equilibrium is given by the sum of the

forward and reverse rate constants. However, if one assumes either the preequilibrium proton transfer of eq 1 or a mechanism which is steady state in $-C(=OH^+)CH_2-$ (eq 13), both

(13)
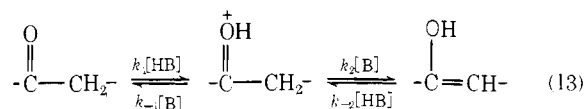
keto and enol formation are first order in HB. In like manner, the mechanisms of eq 2 and 14 are first order in B in both the

(14)
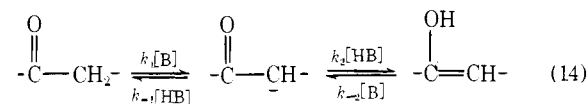
direction of ketonization and enolization. In Table II are given the results of the derivations employing eq 1, 2, 13, and 14, where K_A, K_K, K_{CH}, and K_E are the acid-dissociation constants of the general acid, protonated ketone, ketone carbon acid, and enol, respectively. From the table it is evident that either general-acid or general-base catalysis is seen in both the forward and reverse direction in the preequilibrium and steady-state mechanisms. The only condition under which a given reaction sequence could give rise to both general-acid and general-base catalysis is in the case of the mechanism of eq 2 when $K_{CH} > a_H$, i.e., pH >13. The rate-determining step may be assumed to be proton transfer to and from carbon, since proton transfer to and from oxygen is rapid in aqueous solution. Thus, when general-acid catalysis is observed, keto–enol interconversion proceeds by the mechanism given in eq 1 and the observed rate constant can be expressed as in eq 15

$$k_{obsd} = k_f + k_r = k_{1y} + \left(k_2 \left(\frac{K_A}{K_K}\right) + k_{-2}\right) [HB] \quad (15)$$

where k_{1y} is the contribution to catalysis in the forward and reverse directions by lyate species. Similarly, the observation of general-base catalysis signifies the mechanism of eq 2 for which the k_{obsd} value is expressed as

$$k_{obsd} = k_f + k_r = k_{1y} + \left(k_1 + k_{-1} \left(\frac{K_{CH}}{K_A}\right)\right) [B] \quad (16)$$

When both general-acid and general-base catalysis are observed, as with imidazole and phosphate buffers, the mechanisms of eq 1 and 2 are operating simultaneously. The k_{ga} and k_{gb} values plotted in Figure 6 do not refer to a single bond-making and -breaking process but are the composite terms found in parentheses in eq 15 and 16, respectively. These values result in a $-\alpha$ value of 0.43 and a β value of 0.35.

Catalysis of Enolization via Carbinolamine Formation. The enolization of oxaloacetic acid was investigated employing tertiary amines as buffers; primary and secondary amines were not used in order to avoid the possibility of imine formation. The reaction of tertiary amines, in the pH vicinity of their pK_as, with an equilibrated aqueous (pH 3.7) solution of oxaloacetic acid is characterized by a first-order increase in absorption as expected for the enolization reaction. Though the rate of change in absorbance is dependent upon amine concentration, the spectrum at t_∞ is identical to that obtained on identical pH jump in the absence of tertiary amine. However, tertiary amines of $pK_a > 8$ do not react with oxaloacetic acid by the general-base-catalyzed mechanism of eq 2. The observed first-order rate constants for the reaction of trimethylamine with oxaloacetic acid are plotted in Figure 8 vs. the concentration of trimethylamine. The plots are linear and by cursory examination appear to support general-base catalysis. However, the logs of the intercepts of these plots do not fall on the pH-rate profile for lyate species catalysis of keto–enol tautomerization (solid line) of Figure 2, as would be expected if

trimethylamine were acting as a general base to catalyze the enolization reaction via the mechanism of eq 2. Instead, they fall on the dashed line of Figure 2. The reaction of oxaloacetic acid with 3-chloroquinuclidine and 3-quinuclidinol results in the buffer dilution plots of Figures 9 and 10, respectively. In the case of these amines, rate constants were determined at very low buffer concentrations; these rate constants evidence a marked negative deviation from the linearity established with higher concentrations of amine. The intercepts at zero buffer concentration, to which the solid lines are drawn in Figures 9 and 10, are taken from the log k_{obsd} vs. pH profile for lyate species catalysis (Figure 2). The logs of the intercepts obtained by extrapolation of the linear portions of the plots (dashed line) fall, as did the intercepts of the plots in Figure 8, on the dashed line of Figure 2. Triethylamine buffer dilution plots evidence more marked departure from linearity, presumably due to the steric hindrance associated with this amine. Extrapolation of the linear portions of all the tertiary amine buffer dilution plots at high buffer concentrations to zero buffer concentration provides apparent first-order rate constants that fall on the dashed line of Figure 2. Minimum values of the second-order rate constants for reaction of oxaloacetate calculated from plots of k_{obsd} vs. $[N]_T$ at low amine concentration observable in Figures 9 and 10 are 121, 237, and 180 M^{-1} s^{-1} for 3-chloroquinuclidine, 3-quinuclidinol, and triethylamine, respectively. These may be compared to the predicted values of 1.6, 4.6, and 10.7 M^{-1} s^{-1} (determined from Figure 6) if the amines were reacting according to the mechanism of eq 2.

The addition–elimination mechanism of eq 17 is proposed
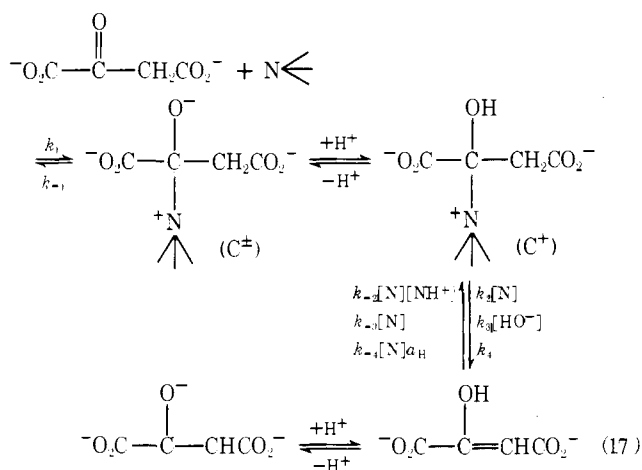
to account for the enhanced catalysis of the enolization of oxaloacetic acid by tertiary amines. The amine reacts with the ketone to form a zwitterionic carbinolamine ($C^{\pm}$) which is trapped by protonation. A second molecule of tertiary amine reacts in essentially an E_2-type mechanism with carbinolamine (C^+) to give enol by elimination of a proton and neutral amine. Thus, the quaternary ammonium moiety of C^+ serves as an electron sink to enhance the rate of proton removal from carbon, the slow step of the enolization reaction.

The assumption of a steady-state in $C^{\pm}$ and C^+ results in the expression

$$k_{obsd} = k_f + k_r =$$
$$\frac{k_1 k_2 a_H[N]^2 + k_1(k_3 K_w + k_4 a_H)[N] + [a_H/(K_E + a_H)]k_{-1}K_{C^+}(k_{-2}a_H[N]/K_A + k_{-3} + k_{-4}a_H)[N]}{k_2 a_H[N] + K_{-1}K_{C^+} + k_3 K_w + k_4 a_H}$$

(18)

where K_{C^+}, K_E, and K_A are the acid-dissociation constants of C^+, enol, and tertiary amine, respectively, and [N] is the concentration of amine present as the free base. Equation 18

simplifies to

$$k_{obsd} = k_f + k_r =$$
$$\frac{a_H(k_1 k_2 + k_{-1}k_{-2}K_{C^+}/K_A)[N]^2 + (k_1 k_4 a_H + k_{-1}k_{-3}K_{C^+} + k_1 k_3 K_w)[N]}{k_2 a_H[N] + k_{-1}K_{C^+} + k_3 K_w + k_4 a_H}$$

(19)

since $k_{-3} > k_{-4}a_H$ and in the pH range investigated, $a_H > K_E$. At any constant pH, eq 19 has the mathematical form of eq 20

$$k_{obsd} = \frac{K_I[N]^2 + K_{II}[N]}{K_{III}[N] + 1}$$ (20)

which predicts the observed first-order dependence on [N] at high concentrations of amine evidenced in Figures 8–10. At low amine concentration, the dependence on amine will vary from first to second order depending on the relative values of K_I and K_{II} and the amine concentration. At amine concentrations where the first-order dependence on amine concentration is observed, eq 19 reduced to eq 21.

$$k_{obsd} = k_f + k_r = \left(k_1 + \frac{k_{-1}k_{-2}K_{C^+}}{k_2 K_A}\right)[N]$$
$$+ \frac{k_1 k_4}{k_2} + \frac{k_1 k_3 K_w + k_{-1}k_{-3}K_{C^+}}{k_2 a_H}$$ (21)

Equation 21 predicts the observed dependence of the intercepts of the linear portions of the amine buffer dilution plots at zero amine concentration on a spontaneous term and a hydroxide ion dependent term (eq 9, see also the dashed line of Figure 2). That all of the intercepts do not fall precisely on the dashed line of Figure 2 is not unexpected, since the points refer to intercepts obtained with five different tertiary amines and the constants of the last two terms of eq 21 are not independent of amine pK_a. The mechanism of eq 17 requires that $k_2 a_H[N] > (k_{-1}K_{C^+} + k_3 K_w + k_4 a_H)$ for the k_{obsd} values to exhibit a first-order dependence on amine concentration, not an unrealistic requirement.[17] Amine-catalyzed elimination occurring through $C^{\pm}$ (k_2') requires that $k_2'[N] > k_{-1}$. Since the rate of collapse of the zwitterionic carbinolamine back to starting materials is expected to be greater than the rate of amine-catalyzed elimination, one can conclude that the elimination reaction occurs predominately through C^+ as indicated in eq 17.

If one assumes preequilibrium formation of $C^{\pm}$ and C^+, the mechanism of eq 17 prescribes the rate expression of eq 22

$$k_{obsd} = k_f + k_r = \frac{K_1 k_2 a_H[N]^2 + K_1(k_3 K_w + k_4 a_H)[N]}{K_1(K_{C^+} + a_H)[N] + K_{C^+}}$$
$$+ \frac{a_H}{K_E + a_H}(k_{-2}a_H[N]^2/K_A + (k_{-3} + k_{-4}a_H)[N]$$ (22)

where K_1 is the equilibrium constant for formation of $C^{\pm}$. Since a_H is greater than both K_{C^+} and K_E in the experimental pH range, eq 23 results.

$$k_{obsd} = k_f + k_r =$$
$$\frac{k_{-2}K_1 a_H^2[N]^3/K_a + a_H(k_2 K_1 + k_{-3}K_1 + k_{-4}K_1 a_H + k_{-2}K_{C^+}/K_A)[N]^2 + (k_3 K_1 K_w + k_4 K_1 a_H + k_{-3}K_{C^+} + k_{-4}K_{C^+}a_H)[N]}{K_1 a_H[N] + K_{C^+}}$$ (23)

Assuming that the third-order term in amine can be neglected, eq 23 also has the mathematical form of eq 20 at any constant pH. At amine concentrations where the first-order dependence on amine concentration is observed ($K_1 a_H[N] > K_{C^+}$), the preequilibrium assumption, like the steady-state assumption, fits the observed kinetic expression. The requirement that $K_1 a_H[N] > K_{C^+}$ for the first-order dependence on [N] to be

observed can alternately be expressed as in eq 24.

$$\frac{[C^{\pm}]a_H[N]}{[\text{ketone}][N]} > \frac{[C^{\pm}]a_H}{[C^+]} \tag{24}$$

Thus, at amine concentrations where linear buffer dilution plots are obtained, $[C^+] > [\text{ketone}]$. Conversion of ketone to carbinolamine will cause a shift in the keto–enol equilibrium which, when reestablished, will result in a decreased enol concentration. Enol and enolate ion in particular absorb light at 270 nm, while neither ketone nor carbinolamine has any appreciable UV absorbance. Therefore, at any given pH, the absorbance at 270 nm should be greater for an aqueous keto-enol equilibrium mixture than for the same concentration of tautomers in an aqueous solution of tertiary amine. The circles in Figure 1 indicate the equilibrium absorbance of a 8.8×10^{-5} M solution of oxaloacetic acid in 0.5 M KCl as a function of pH. The triangles in Figure 1 give the absorbance of the same concentration of oxaloacetic acid in 0.2 M 3-quinuclidinol minus the absorbance of 0.2 M 3-quinuclidinol at the given pH. The superimposability of the two plots shows that there is no appreciable buildup of any intermediate species. In addition, there is no discernible difference in the carbonyl infrared stretching frequency of oxaloacetic acid in D_2O in the presence and absence of triethylamine. Similar results have previously been reported for pyruvate and trimethylamine.[18] These observations argue against a preequilibrium assumption for the mechanism of eq 17.

Mechanisms in which the initial reaction of amine with oxaloacetic acid results in the formation of an intermediate which is off the reaction pathway can be ruled out. For example, the mechanism of eq 25, which involves the formation
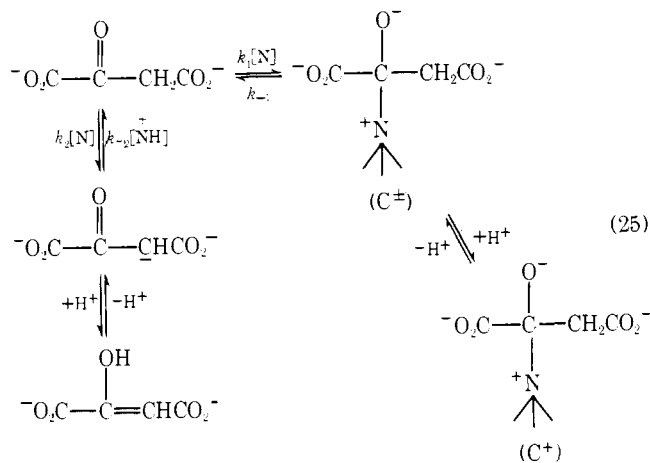
of an inactive carbinolamine in competition with the general-base-catalyzed mechanism of eq 2, results in a rate equation of the mathematical form given in eq 26.

$$k_{obsd} = \frac{K_I[N]}{K_{II}[N] + 1} \tag{26}$$

Equation 26 requires a zero-order dependence in amine at high amine concentration rather than the first-order dependence that is observed (Figures 8–10). If the addition intermediate is inactive as in eq 25, and catalysis of ketone to enol occurs with two molecules of amine, one in the acid form and one in base form (i.e., concerted general acid-general base catalysis), the required first-order dependence in amine at high amine concentration is obtained. However, at high amine concentration this mechanism also has the requirement that $[C^+] > [\text{ketone}]$, which is ruled out by the data of Figure 1.

Thus, tertiary amines of $pK_a > 8$ react with oxaloacetic acid by attacking the ketone carbonyl group to form a zwitterionic carbinolamine (eq 17) rather than by removal of a proton from the α-carbon atom (eq 2). Enolization of the intermediates ($C^{\pm}$

and C^+), present at only steady-state concentrations, then occurs by a subsequent general-base-catalyzed mechanism predominantly through C^+. Tertiary amine, hydroxide ion, and water function as general bases in this E_2-type reaction. Pyridine, imidazole, and oxyanion bases are apparently not sufficiently nucleophilic to form tetrahedral intermediates with oxaloacetic acid and thus they catalyze the enolization reaction via the mechanisms given in eq 1 and 2. At 3.8 °C, however, imidazole apparently catalyzes enolization via the addition–elimination mechanism (Figure 7), indicating a difference in the temperature coefficients of the two reactions.

It is well known that primary and secondary amines function as nucleophilic catalysts in a variety of reactions involving removal of an α substituent, without its pair of bonding electrons, from a carbonyl compound. Such reactions include enolization,[19] elimination from β-acetoxy ketones,[20] dehydration of β-ketols,[21] isomerization of β,γ-unsaturated ketones,[22] and decarboxylation of β-keto acids.[23] These all involve
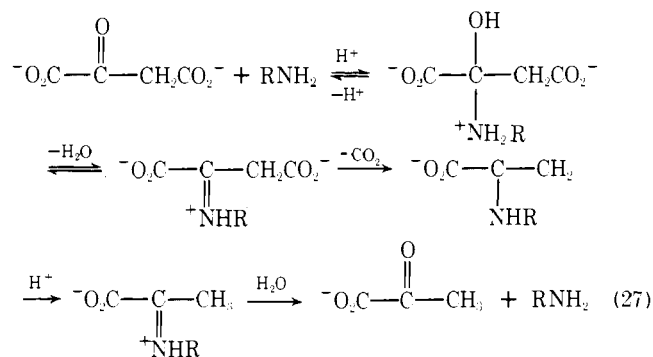
formation of an intermediate Schiff base (as shown in eq 27 for the amine-catalyzed decarboxylation of oxaloacetic acid) which functions as an electron sink for the elimination reaction. It has been reported, however, that the aniline-catalyzed decarboxylation of oxaloacetic acid in water occurs directly through the tetrahedral intermediate without the involvement of the Schiff base (eq 28).[24] The carbinolamine mechanism
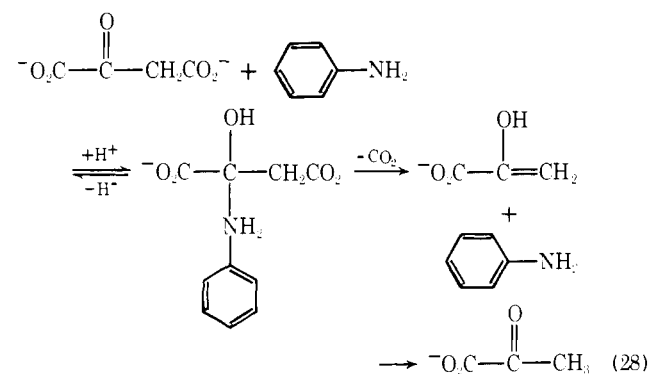
of eq 28 is not generally accepted because, unlike the mechanism of eq 27, it fails to account for the reported inability of tertiary amines to catalyze the decarboxylation reaction.[25] This lack of reactivity of tertiary amines as catalysts, however, is not a well-documented general phenomenon. Wohl and Oesterlin reported that tertiary amines did not catalyze the decarboxylation of oxaloacetic acid.[26] However, the only tertiary amine actually investigated was pyridine, and we have found that this amine is not sufficiently nucleophilic to form an addition compound with oxaloacetic acid. Primary, secondary, and tertiary amines have all been reported to exhibit catalytic activity in the decarboxylation of acetonedicarboxylic acid in water.[27] The experimental conditions, however, were not well controlled and the results, therefore, may be questionable. That aniline and oxaloacetic acid form a carbinolamine in water and a Schiff base in ethanol[24] suggests that the

aniline-catalyzed decarboxylation reaction in these solvents may involve the mechanisms of both eq 27 and 28 and that the choice of pathway will depend on the particular substrate and solvent employed. A carbinolamine has also been suggested to be the active species in the primary- and secondary-amine-catalyzed dealdolization of diacetone alcohol,[28] although a subsequent study[29] appears to support the previous suggestion of a Schiff base intermediate.[30] The results of the present study suggest that tertiary amines may catalyze the decarboxylation as well as the enolization of oxaloacetic acid. In our studies, a slow decrease in absorption is detected after the enolization reaction is complete, and this very well may be due to decarboxylation. The extent to which the decarboxylation reaction is increased by the presence of the tertiary amine has not been established.

Although the addition–elimination mechanism of eq 17 has not been previously recognized for enolization reactions, it may well be that this type of catalysis may find applicability in a variety of organic reaction mechanisms. For example, recently it has been reported that trimethylamine catalyzes the aromatization of 4-carbo-*tert*-butoxybenzene oxide. The addition–elimination mechanism of eq 29 has been established
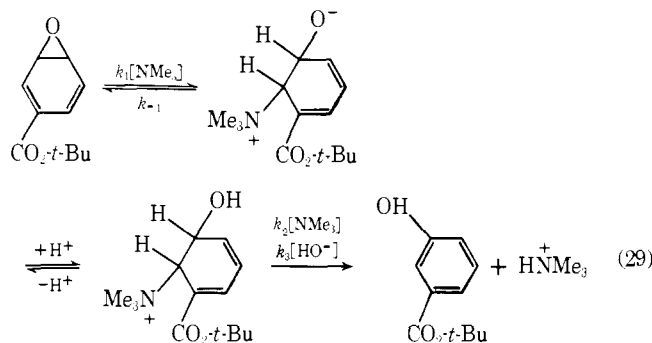
(kinetics plus NMR identification of intermediate).[31] The mechanism of eq 29 is of particular interest, since it provides a pathway other than the NIH shift for the aromatization of arene oxides. A mechanism other than the NIH shift has been reported to be prevalent in the hydroxylation of benzenoid hydrocarbons by hepatic monooxygenases.[32] Catalysis of·the dienone–phenol rearrangement by primary, secondary, and tertiary amines has been attributed to nucleophilic addition
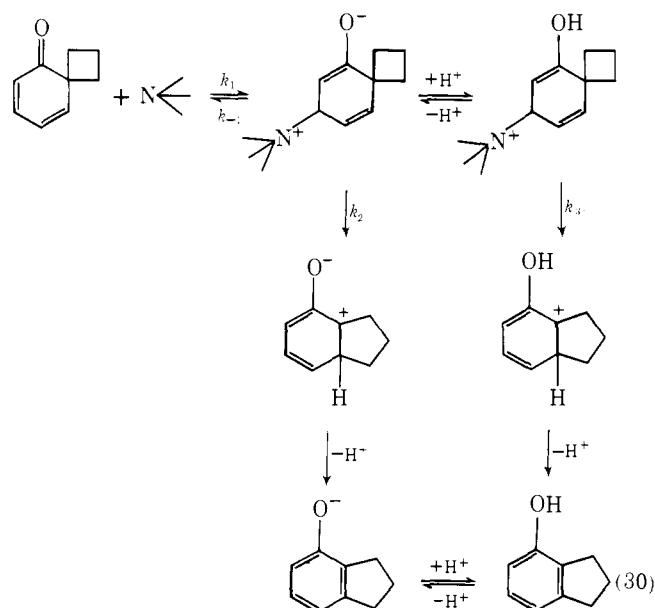
of amine followed by rearrangement and elimination of amine (eq 30).[33]

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