842, and 950 cm^{-1}, while **10**-d_2 shows a strong band at 630 and medium intensity bands at 830 and 925 cm^{-1}. All of the cyclohexadienes, deuterated and undeuterated, show a medium intensity band at 1640 (C=C stretching), a strong band at 890 (=CH_2 hydrogen deformation), and a richly detailed series of eight or nine well-resolved bands in the 3000-cm^{-1} region (C–H stretching).

Compounds **7**-d_2 and **8**-d_2 isolated from pyrolysis were analyzed mass spectrometrically and showed the compositions 85.5 and 82.7% d_2, 11.2 and 13.4% d_1, and 3.4 and 4.0% d_0, respectively.

Pyrolyses of **7**-d_2 or **10**-d_2 regenerated **4**-d_2, isolated and identified by its nmr and infrared spectra. The other six pyrolysis products corresponded in gas chromatographic retention times to those obtained from **4**.

Conversion of 9 to *m*-cymene and 10 to *p*-cymene was effected as follows. A solution of 20 μl (18 mg) of **9** in 5 ml of dioxane containing a suspension of 15 mg of 5% palladium-on-calcium carbonate was stirred under hydrogen in a quantitative microhydrogenation apparatus until 1.2 molar equiv of gas had been absorbed. The catalyst was filtered off, and the solution was treated with 17 mg of tetracyanoethylene. After 6 hr vapor chromatographic analysis showed 50% conversion to *m*-cymene. Five milliliters of water was added, the mixture was extracted with pentane, and the pentane layer was dried and carefully concentrated to a volume of 400 μl with an efficient fractionating column. The residue was preparatively vapor chromatographed to give 4.5 μl of *m*-cymene, identified by its retention time and infrared spectrum.

Similarly, 25 μl of crude **10** (containing 20% of **7**) gave a 20% conversion to *o*-cymene, isolated (2 μl) and identified by comparison of its infrared and ultraviolet spectra with those of authentic materials (ref 27, no. 1640 and 534).

Ketimine Intermediates in Amine-Catalyzed Enolization of Acetone[1a]

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Abstract: Catalysis of the enolization of acetone by amines was followed by determining iodination or deuterium exchange of the ketone. Catalysis by free amine, ammonium ion, and the product of amine and ammonium ion was found. Enolizations catalyzed by a series of (free) amines fit the Brønsted relationship between log k and pK_a established for the base-catalyzed enolization of acetone by carboxylate anions and pyridine derivatives. The ammonium ion catalyzed enolizations do not fit the Brønsted relationship for catalysis by carboxylic acids, deviating by as much as 10^6. The conjugate acids of primary and secondary but not tertiary amines show this catalysis. It is suggested that the latter reactions proceed *via* the formation of a protonated ketimine (Schiff base) which then undergoes proton abstraction to give an enamine. This hypothesis is supported by the variation of the enolization rate constant with varying ratios of amine/ammonium ion. The hypothesis is further supported by the observation of kinetic terms involving an acid–base pair, the acid component of which is an ammonium ion. The ammonium ion catalyzed enolization by glycine is kinetically no better than that by methylamine, indicating that intramolecular catalysis by the internal carboxylate ion in the protonated Schiff base formed from glycine and acetone does not occur. The failure to observe such a reaction may be explained by the low value of the Brønsted β for the removal of a proton from protonated Schiff bases. The relative rates of enolization of protonated acetone, the protonated ketimine of acetone, and acetone are 10^{11}, 10^8, and 1, respectively. From the composite of two model systems, a calculation has been made indicating that an intramolecular catalysis of the enolization of a protonated ketimine intermediate will account for the enolization rate constant in catalyses by the enzyme aldolase.

The enzyme aldolase catalyzes the reaction between fructose 1-phosphate and glyceraldehyde plus dihydroxyacetone phosphate. Likewise, transaldolase catalyzes the transfer of a dihydroxyacetone molecule from one acceptor to another. A ketimine (Schiff base) formed from dihydroxyacetone and enzyme has been implicated in the mechanism of both these enzymatic catalyses, by experiments on the inactivation of the enzyme–substrate compound with sodium borohydride and isolation of the lysine derivative, $(CH_2OH)_2$-$CHNH^{\epsilon}$–lysine.[2,3] These enzymatic results have been interpreted to mean that the chemical steps in the aldolase reaction, starting with dihydroxyacetone phosphate, include: (1) ketimine formation, (2) enolization of the ketimine (enamine formation), and (3) condensation of enamine with glyceraldehyde 1-phosphate to give an enamine of the product. The isolation of a compound related to a ketimine does not, of course, prove that the ketimine is part of the catalytic pathway.

Aldimines and ketimines have been postulated as reaction intermediates in a number of reactions, including condensation and decarboxylation reactions. As a model for possible ketimine formation in the enzymatic catalytic pathway, we have investigated the enolization of acetone in the presence of amines. Some of these amines contain no other groups. Other amines such as glycine contain in addition to the amino group a secondary base, such as the carboxylate ion, which could serve as an intramolecular general basic catalyst of enolization after ketimine formation. Alternatively, the bifunctional amine could react with acetone to form a tetrahedral adduct such as a carbinolamine which then would undergo intramolecular general basic catalysis. Suggestions that the latter pathway is operative in the enolization of acetone in the presence of various amino acids has been made recently.[4] It is the purpose of this paper to determine

(1) (a) This research was supported by a grant from the National Science Foundation; (b) University of Oxford, Oxford, England.

(2) B. L. Horecker in "Comprehensive Biochemistry," Vol. 15, M. Florkin and E. H. Stotz, Ed., Elsevier Publishing Co., New York, N. Y., 1964, p 48.

(3) J. C. Speck, Jr., P. T. Rowley, and B. L. Horecker, *J. Am. Chem. Soc.*, **85**, 1012 (1963).

whether enolization of acetone can occur through prior ketimine formation, and further whether such enolization is enhanced by the formation of a ketimine containing an internal general base. The alternate formulation of intermediate carbinolamine formation will also be considered. With these model studies, we hope to shed some light on the mechanisms of catalysis of aldolase and transaldolase.

Experimental Section

Materials. The catalysts were recrystallized before use and wherever possible were of analytical reagent grade. Buffers were prepared by weighing the hydrochloride and neutralizing with standard alkali. The ionic strength of all solutions was kept at 0.4 with sodium chloride. Potassium iodide at 0.2 *M* accounted for half of the ionic strength. Acetone was of analytical reagent grade. N-Isopropylidene-*n*-butylamine was prepared according to Kosower and Sorenson[5] and was redistilled before each experiment.

Kinetics. A number of methods can be used to follow enolization reactions including halogenation, isotope exchange, racemization, and direct spectroscopy. In this work halogenation has been used primarily, but isotope exchange has also been used. The technique of halogenation depends on the fact that halogenation of enols (or enamines) is generally very fast.[6] This assumption breaks down when enolization becomes comparable to halogenation,[7] for example when very strong acid or base catalysts are used. The enolization of acetone was followed mainly through the use of the iodination reaction, by observing the decrease in triiodide ion absorption at 351 mμ of a solution containing buffer, ketone, and triiodide ion. Buffer (3.0 ml) was placed in a cuvette and equilibrated in the cell compartment of a Cary 14 recording spectrophotometer. Iodine solution in 0.2 *M* potassium iodide (to make *ca.* 3×10^{-5} *M* total iodine) was introduced with a lambda pipet and a blank rate was observed. Acetone was added as a solution in either water or methanol and the decrease in absorption at 351 mμ was observed. The rate of halogenation was calculated from the extinction coefficient at 351 mμ of the triiodide ion, 2.6×10^4.[8] Rates of iodination were zero order in iodine, except when the iodine was nearly all consumed. On occasion, a slight acceleration was observed initially; this phenomenon was also found previously[8,9] and is probably due to impurities oxidizing the triiodide ion. In a separate experiment triiodide ion was produced on adding potassium iodide to a solution of diisopropyl ketone, indicating the presence of an oxidizing impurity. In all the experiments, only the linear portion of the trace was used. The pH of the solution was checked after the reaction using a Radiometer 4C pH meter. The consistency of results obtained using different acetone concentrations indicates first-order dependence in acetone.

Although the reversible reactions of primary and secondary amines with iodine to produce the N-iodo derivative were successfully suppressed by using excess iodide ion which reverses the reaction, the rapid, irreversible reaction of trimethylamine with iodine[10] prevented the use of the iodination technique with this catalyst. Therefore, the enolization was followed by observing deuterium exchange between acetone and deuterium oxide at 1.41 μ, using the Cary spectrophotometer.[11] Trimethylamine buffer in deuterium oxide was placed in a 1-cm cuvette of 2-ml capacity. Neat acetone was added with a lambda pipet and the increase in absorption at 1.41 μ was observed. The trimethylamine–D_2O system was used to calculate the extinction coefficient of water at 1.41 μ (0.106). It was impossible to obtain first-order kinetics over the entire reaction because the rate constant became progressively smaller as the deuterium being substituted for hydrogen exerted its secondary deuterium isotope effect. Therefore zero-order rate constants were obtained from the beginning of the reaction. The infinity absorption from one run agreed with the absorption calculated from the extinction coefficient of water and three times the molarity of the acetone.

Results

Catalyses of the enolization of acetone by amines and other species were carried out by following the iodination or deuterium exchange of acetone. The zero-order rates of iodination of acetone were converted into first-order rate constants using the extinction coefficient of the triiodide ion and the molarity of acetone. The rates fitted the equation

$$\text{rate of enolization} = k_{obsd}[\text{acetone}] = (k_0 + k_a[A] + k_b[B] + k_{ab}[AB])[\text{acetone}] \quad (1)$$

where k_0 is the rate constant due to water, hydroxide ion, and hydronium ion catalysis, and where A and B represent acid and base catalysts, respectively.

When k_{obsd} is linear in [A] + [B] at constant buffer ratio (defining A and B as a conjugate acid–base pair) the product term, k_{ab}[A][B], must be equal to zero, and the system may be described as

$$k_{obsd} - k_0 = k_a[A] + k_b[B] \quad (2)$$

If we define r as the ratio [A]/[B], then eq 2 can be transformed to

$$k' = \frac{(k_{obsd} - k_0)}{[A] + [B]}\frac{(r+1)}{r} = k_a + k_b(1/r) \quad (3)$$

Thus, a plot of k' *vs.* $(1/r)$ yields k_a as intercept and k_b as the slope.

When k_{obsd} is concave in [A] + [B] at constant buffer ratio, then one must also consider the product term and the expression for the rate constant becomes

$$k_{obsd} - k_0 = k_a[A] + k_b[B] + k_{ab}[A][B] \quad (4)$$

This equation may be transformed to

$$k'' = (k_{obsd} - k_0)/[B] = k_a r + k_b + k_{ab}[A] \quad (5)$$

Thus, a plot of k'' *vs.* [A] gives a linear relation of slope k_{ab} and intercept $(k_a r + k_b)$. The intercept divided by r varies linearly with $1/r$, with a slope of k_b and an intercept k_a. An alternative procedure, used for ethylenediamine catalyses, was to plot $(k_{obsd} - k_0)/[A]$ *vs.* [B], and then plot intercepts *vs.* $1/r$. In either case, this procedure allows the calculation of k_a, k_b, and k_{ab}.

The results of these experiments are shown in Table I. The base-catalyzed terms, k_b, fit the Brønsted relationship between log k_b and pK_a for the base-catalyzed enolization of acetone by carboxylate anions[12] and pyridine derivatives.[13] The degree of fit is illustrated in Figure 1. It is suggested that k_b represents the simple proton abstraction from acetone by the amine in accordance with the mechanism already accepted for other basic catalysts.[14] The relatively large deviation of the borate ion term is a well-known phenomenon in general basic catalysis. It is due to the conjugate base existing largely as the hydrated anion $H_4BO_4^-$, a much weaker base than $H_2BO_3^-$.[14] There is no devia-

(4) I. V. Mel'nichenko, E. A. Shilov, and A. A. Yasnikov, *Ukr. Khim. Zh.*, **30**, 599 (1964); I. V. Mel'nichenko and A. A. Yasnikov, *ibid.*, **30**, 723 (1964).
(5) E. S. Kosower and T. S. Sorensen, *J. Org. Chem.*, **28**, 692 (1963).
(6) R. P. Bell and K. Yates, *J. Chem. Soc.*, 1927 (1962).
(7) G. Archer and R. P. Bell, *ibid.*, 3228 (1959); L. Zucker and L. P. Hammett, *J. Am. Chem. Soc.*, **61**, 2785 (1939).
(8) E. T. Harper and M. L. Bender, *ibid.*, **87**, 5625 (1965).
(9) J. R. Hulett, *J. Chem. Soc.*, 430 (1965).
(10) H. Yada, J. Tanaka, and S. Nagakura, *Bull Chem. Soc., Japan*, **33**, 1660 (1960).
(11) I. M. Klotz and B. H. Frank, *J. Am. Chem. Soc.*, **86**, 3889 (1964).
(12) R. P. Bell and O. M. Lidwell, *Proc. Royal Soc.* (London), **A176**, 88 (1940).
(13) J. A. Feather and V. Gold, *J. Chem. Soc.*, 1752 (1965).
(14) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, Chapter IX.

Table I. The Kinetics of Enolization of Acetone Catalyzed by Some Acids and Bases[a]

Catalyst[f]	pH range	pK_a	$K_a \times 10^6$ M^{-1} sec^{-1}	$k_b \times 10^6$ M^{-1} sec^{-1}	$k_{ab} \times 10^2$ M^{-2} sec^{-1}
Pyridine	4.5–5.5	5.2	-0.167 ± 0.167[d]	4.8 ± 0.15	
Phosphate	5.5–6.5	6.82	-0.228 ± 0.67[d]	16.7 ± 1.33	
Imidazole	6.5–7.5	6.95	5.78 ± 0.167	14.6 ± 0.2	
Glycine	8.5–9.5	9.6	79.0 ± 14.0	$(14.9 \pm 0.7) \times 10^2$	3.84 ± 0.26
Methylamine	8.6–10.6	10.6	$(3.04 \pm 0.34) \times 10^2$	$(19.1 \pm 0.3) \times 10^3$	
Ethanolamine	8.5–9.6	9.5	$(1.9 \pm 0.2) \times 10^2$	$(24.9 \pm 0.4) \times 10^2$	
Ethylenediamine	6–7	9.98	7.5 ± 4.5	$(39.2 \pm 2.3) \times 10^1$	3.64 ± 0.38
p-Toluidine	6–7	5.07	17.4[b]		2.61 ± 0.17
Tris(hydroxymethyl)-aminomethane	7.1–8.3	8.1	2.52 ± 0.45	36.0 ± 1.3	
Boric acid	8.5–9.5	9.23	$-3.17 \pm 13.4 \times 10^{-2}$[d]	19.7 ± 0.4	
Trimethylamine	8.5–9.5	9.76	$-0.92 \pm 1.0 \times 10^2$[d]	$26.1 \pm 0.5 \times 10^3$	
Acetate and glycine[c,e]	8.4–8.8				0.83×10^{-4}[b]

[a] At 25°, 0.2 *M* potassium iodide, 0.4 *M* ionic strength. [b] These values are in error by less than a factor of 2 and are suitable as estimates. [c] $k(CH_3CO_2^-)(NH_3^+CH_2CO_2^-)$. [d] These values are zero within experimental error. [e] Glycine concentration kept at 0.1 *M* and acetate ion concentration varied from 0.05 to 0.5 *M*. [f] Catalyst concentration 0.025–0.10 *M* throughout.

tion from the Brønsted relationship for the k_b term for glycine, and thus there is no evidence that a ketimine of acetone and glycine (anion) undergoes an intramolecular catalysis by carboxylate ion to form an enamine, or alternatively that a carbinolamine adduct undergoes intramolecular proton abstraction.

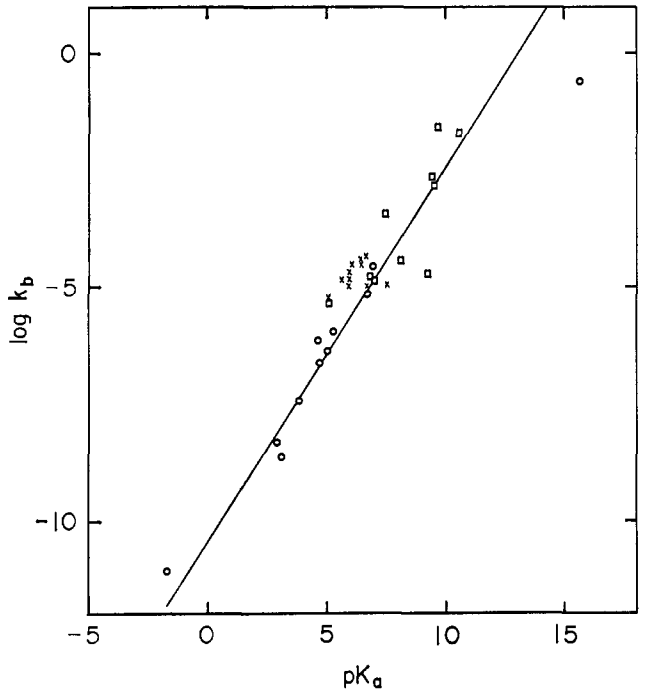


Figure 1. Catalysis of the enolization of acetone by pyridine, ×;[13] carboxylate anions and other general bases, O;[12] and amines, □ (present research). The line is an arbitrary line through all the points, giving $\beta = 0.8$.

In contrast to the basic rate constants the acidic rate constants for the ammonium ion catalyzed enolization of acetone do not fit the Brønsted relationship for catalysis by carboxylic acids,[11] as seen in Figure 2. In fact, some of the acidic rate constants of ammonium ions deviate by as much as 10^6 from the rate constants calculated on the basis of the pK_a's of the carboxylic acids. These deviations are too large to be explained by a difference in character between a positively charged ammonium ion and a neutral carboxylic acid. It must therefore be concluded that preequilibrium protonation followed by proton abstraction by the conjugate base, which is the mode of carboxylic acid catalysis,[15] cannot account for catalysis by ammonium ions.

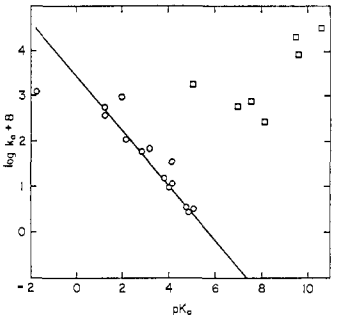
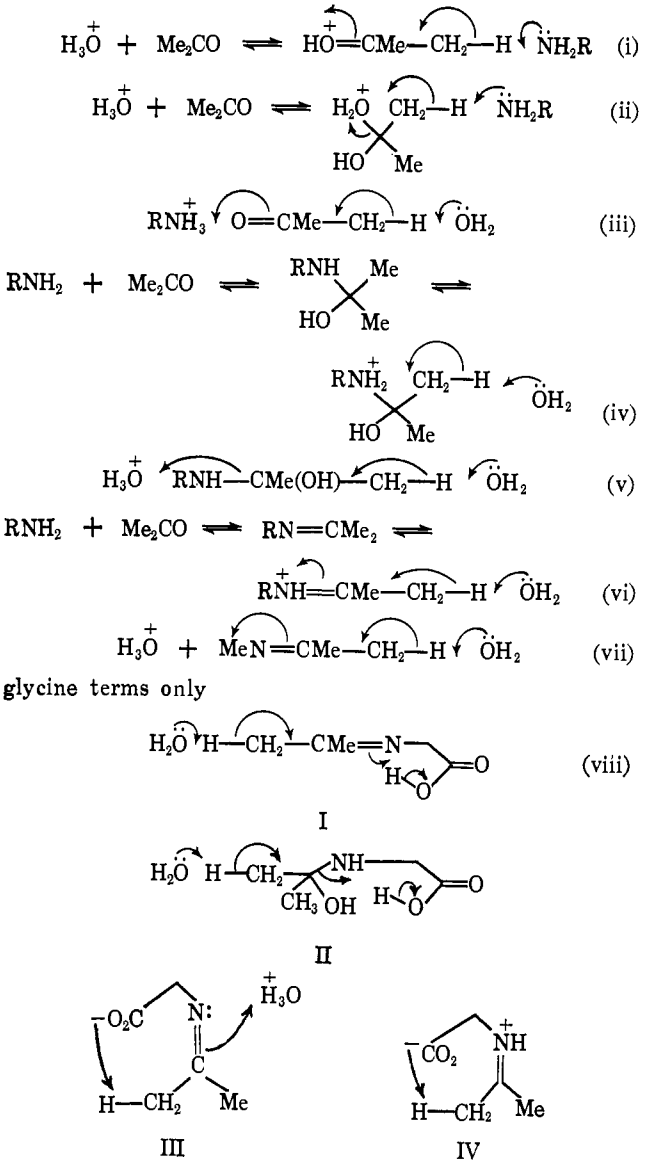


Figure 2. Catalysis of the enolization of acetone by carboxylic acids, O,[12] and ammonium ions, □ (present research). The line is an arbitrary line through the carboxylic acid points, giving $\alpha = 0.66$.

Discussion

Catalysis by Ammonium Ions. The significant finding of this research is that catalysis of the enolization of acetone by ammonium ions must be mechanistically different from the catalysis of the same reaction by carboxylic acids. Let us consider the various mechanisms that can be written for the ammonium ion catalyzed enolization reaction. The number of mechanistic possibilities is large. However, many of these possibilities can be eliminated. For example, mechanisms i and ii would require that the amine must react with the protonated acetone (or protonated hydrate) with a rate constant of 10^{14} M^{-1} sec^{-1}. This rate constant is faster than a diffusion-controlled process, and therefore mechanisms i and ii may be eliminated from consideration. Mechanism iii is unlikely since there is no reason why carboxylic acid catalysis should also proceed *via* this pathway but does not. The failure of the trimethylamine-catalyzed enolization of

(15) K. J. Pedersen, *J. Phys. Chem.*, **38**, 596 (1934); O. Reitz and J. Kopp, *Z. Physik. Chem.*, **A184**, 429 (1939); C. G. Swain, A. J. DiMilo, and J. P. Cordner, *J. Am. Chem. Soc.*, **80**, 5983 (1958).



acetone to show an acid term eliminates mechanisms iv and v as possibilities. Furthermore, these mechanisms are unlikely on the grounds that quaternary ammonium salts eliminate only at high temperatures and pH's. With the exception of glycine catalyses, all possible mechanisms of ammonium ion catalyses have therefore been eliminated except mechanisms vi and vii both of which are closely related to one another and both of which involve Schiff base intermediates.[16]

It was hoped to obtain direct evidence to support the hypothesis that a ketimine intermediate is formed in the ammonium ion catalyzed enolizations. Unfortunately, acetone and methylamine proved to be an intractable system; although the ketimine could be isolated, it was unstable under the experimental enolization conditions of pH 8–10 and aqueous solvent. Furthermore, at low pH's (3–4) where the ketimine was stable, the enolization of the ketimine was faster than the halogenation of the enamine and thus, the enolization could not be followed.[17]

Indirect evidence does, however, support the hypothesis of mechanism vi, involving a ketimine intermediate in the enolization reaction. This indirect evidence involves the variation of k' (see eq 3) with changing $1/r$ (r is the ratio [A]/[B]), as shown in Figure 3. At values of $1/r$ above 0.05, a linear curve is seen, the slope and the intercept of which give k_b and k_a, respectively. However, at values of $1/r$ below 0.05, the contribution from k_a decreases steadily and eventually disappears. This behavior suggests that a change in rate-determining step is taking place, and is very difficult to explain except in terms of a two-step process[18] involving (1) ketimine formation and (2) enolization.

The methylamine-catalyzed enolization above $1/r = 0.05$ follows the equation

$$\text{rate} = k_b[\text{methylamine}][\text{acetone}] + k_a[\text{methylammonium ion}][\text{acetone}] \quad (6)$$

as can be seen from the linear relationship with a finite intercept. At values of $1/r$ below $1/r = 0.05$, the k_a term changes into a term proportional to amine concentration, as can be seen from the fact that the intercept disappears below $1/r = 0.05$.

$$\text{rate} = k_b[\text{methylamine}][\text{acetone}] + k_b'[\text{methylamine}][\text{acetone}] \quad (7)$$

Equations 6 and 7 may be combined to a common expression, eq 8

$$\text{rate} = k_b[\text{methylamine}][\text{acetone}] + k_{\text{excess}}[\text{acetone}] \quad (8)$$

where k_{excess} is either k_b'[methylamine] or k_a[methylammonium ion]. The change of character of k_{excess} can be successfully rationalized if one assumes that k_{excess} is a composite quantity which is a measure of the rate of ketimine formation at acid pH and the rate of ketimine enolization at high pH. Thus eq 9 may be written for the over-all reaction.

$$\text{acetone} + CH_3NH_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (CH_3)_2C(OH)NHR \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (CH_3)_2C{=}NR \underset{K_2}{\overset{H^+}{\rightleftharpoons}} (CH_3)_2C{=}{}^+NHR \xrightarrow[H_2O]{k_e} \text{enamine} \quad (9)$$

The concentration of the carbinolamine, the ketimine, and the protonated ketimines are small compared with the acetone and amine concentrations, on the basis of the known ketimine equilibrium constant of 0.22 M^{-1}.[19] Furthermore, only the initial rate of enolization (to less than 5% completion) was measured. On these bases, a steady state in the three intermediates was assumed. It was further assumed that $k_{-1} > k_2$, as has been previously shown for this pH region.[19] With these two assumptions, the rate constant of enolization *via* eq 9 is

$$k_{\text{excess}} = \frac{k_1k_2k_e(\text{methylamine})}{k_{-1}(k_e + K_2k_{-2}/[H^+])} \quad (10)$$

If enolization is the rate-limiting step of the reaction, that is, if $k_e < K_2k_{-2}/[H^+]$, which would be the condi-

(16) Mechanisms viii, which apply to glycine catalyses, will be discussed later.

(17) These experiments were, in fact, carried out with N-isopropylidene-*n*-butylamine.

(18) *Cf.* T. C. Bruice and L. R. Fedor, *J. Am. Chem. Soc.*, **86**, 4886 (1964).

(19) A. Williams and M. L. Bender, *ibid.*, **88**, 2508 (1966).

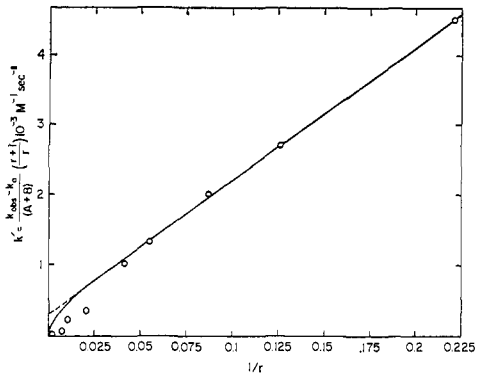


Figure 3. The methylamine-catalyzed enolization of acetone at 25° in aqueous solution. The line is a theoretical curve calculated according to the method given in the text.

tion at high pH (high values of $1/r$), then

$$k_{excess} = (K_a/K_2)(k_1k_2/k_{-1}k_{-2})k_e[CH_3NH_3^+] = (K_a/K_2)(K_1)k_e[CH_3NH_3^+] = k_a \quad (11)$$

where K_a is the ionization constant of methylamine and K_1 is the equilibrium constant of ketimine formation. On the other hand, if dehydration is rate limiting, that is, if $k_e > K_2k_{-2}/[H^+]$, which would be the condition at low pH (low values of $1/r$), then

$$k_{excess} = (k_1k_2/k_{-1})[CH_3NH_2] \quad (12)$$

Thus, it is seen that according to eq 9, k_{excess} should change character from dependence on methylammonium ion at high values of $1/r$ to dependence on methylamine at low values of $1/r$, as has been found experimentally.

The theoretical curve of Figure 3 for the methylamine-catalyzed enolization of acetone over the entire range of $1/r$ was calculated using eq 13. Equation 13 attributes a normal general basic catalytic function to methylamine (the k_b term) and in addition a contribution from catalysis through ketimine formation (the k_{excess} term). For this calculation, values of k_a and k_b were determined from five points at the higher

$$k_{calcd} = k_{obsd} - k_0 = \frac{[CH_3NH_2]k_1k_2k_e}{k_{-1}(k_e + K_2k_{-2}/[H^+])} + k_b[CH_3NH_2] \quad (13)$$

pH's (higher $1/r$) assuming that they fit a linear relationship. A value of k_e was calculated from eq 11 knowing k_a from the above determination, knowing K_1, the equilibrium constant of ketimine formation,[19] and estimating the value of K_2, the ionization constant of the protonated ketimine to be about 10^3K_a, namely $10^{7.6}$. Previously[19] we have calculated a value of k_1k_2/k_{-1} and shown that these constants are pH independent. It is then possible to calculate k_{-2} by dividing K_1 by k_1k_2/k_{-1}. Thus, from independent experiments we have the data to calculate a theoretical curve for Figure 3, shown as the solid line. Certainly the shape of the theoretical curve agrees with the experimental points in the region of curvature. Considering the difficulties involving accuracy at such high values of r (or low values of pH), it is gratifying that the falloff in linearity occurs in the same region of pH for both the theoretical and experimental values.[20]

Catalysis by Both Acids and Bases. A "product" term involving both an acid and a base is seen in some of the amine catalyses (Table I). It is of interest to consider the product terms which apply to the glycine–acetone system, collected in Table II. These product terms may be rationalized by mechanisms analogous to iii, iv, or vi. Mechanism iii is unlikely for the reasons already given. Thus, the only reasonable mechanisms are those involving carbinolamine (iv) or ketimine (vi) intermediates. The Brønsted relationship of the data of Table II helps to distinguish between mechanisms iv and vi for the product terms. The Brønsted relationship between log k and pK_a of the bases of Table II yields β = *ca.* 0.4. A relationship has been suggested between β and the pK_a of the enolizing proton for such proton transfer reactions.[21] Proton abstraction from a species involving weakly acidic protons should have a transition state resembling product and therefore a β close to unity. Alternatively, abstraction of a proton from a species having highly acidic protons should have a transition state resembling reactants and a β close to zero. On a quantitative basis, a β of 0.4 corresponds to a pK_a of the enolizing proton of about 8. In mechanisms iv and vi, a proton is removed from a protonated carbinolamine and a protonated ketimine, respectively. It is unlikely that the α hydrogen of the carbinolamine has a pK_a of 8, whereas in the protonated ketimine the α protons are probably acidic and could have a pK_a of 8. Interestingly, the Brønsted β calculated for the base-catalyzed abstraction of an α hydrogen from protonated acetone (the rate-determining step for the general acid catalyzed enolization of acetone by carboxylic acids) is *ca.* 0.5. Thus, the hypothesis that the product terms considered here proceed here *via* a protonated ketimine has a strong parallel in the general acid catalyzed enolization of acetone.

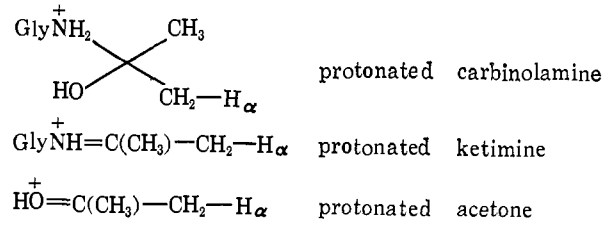


Table II. Catalysis of the Enolization of Acetone by Some Acid–Base Pairs Involving Glycine[a]

Acid	Base	pK_a of base	k_{ab} M^{-2} sec^{-1}	Ref
Glycine	Water	−1.74	0.42×10^{-5}	*b*
Glycine	Acetate	4.76	0.83×10^{-4}	*b*
Glycine	HPO_4^{2-}	6.82	1.1×10^{-2}	22
Glycine	Glycine	9.6	3.84×10^{-2}	*b*

[a] The terms $k_{glyH^+HPO_4^{2-}}$ and $k_{glyH^+Aco^-}$ are ambiguous and could refer to reaction of unprotonated glycine with the corresponding protonated species. This possibility, however, is not likely since reactions as listed in the table constitute a common Brønsted plot with the water and glycine product terms which are not ambiguous. [b] This research.

(20) Mechanism vii involving a general acid attack of oxonium ion on a ketimine could not account for the dependence of k's on $1/r$.

(21) R. P. Bell "The Proton in Chemistry," Methuen and Co., Ltd., London, 1959, p 172.

(22) E. A. Shilov and A. A. Yasnikov, *Ukr. Khim. Zh.*, **23**, 215 (1957).

Table III. Rates of Base-Catalyzed Enolization of Various Substrates

Substrate	$(CH_3)_2C{=}OH^+$	$(CH_3)_2C{=}N^+HGly$	$(CH_3)_2C{=}NH^+CH_3$	$(CH_3)_2C{=}O$
pK_a of substrate analog	-2^a	10^b	10^b	16^c
		k_2, M^{-1} sec^{-1}		
Water as a base	1.53[d]	2.04×10^{-3}	2.62×10^{-2}[f]	0.84×10^{11}[c]
Acetate ion as base	1.32×10^4[e]	7.2		2.5×10^{-7}[g]
Methylamine as base				1.87×10^{-2}
Glycine as base		5.5×10^2		1.49×10^{-3}

[a] Approximate pK_a of ROH_2^+. [b] Approximate pK_a of RNH_3^+. [c] Approximate pK_a of ROH. [d] $k_{H_2O} = k_{H_3O^+}K_S/[H_2O]$ where $k_{H_3O^+}$ is the rate constant of the hydronium ion catalyzed enolization of acetone[12] and K_S is the ionization constant of protonated acetone.[24] [e] $k_{acetate} = k_{acetic\ acid}K_S/K_a$, where $k_{acetic\ acid}$ is the rate constant of the acetic acid catalyzed enolization of acetone,[12] K_S is the ionization constant of protonated acetone,[24] and K_a is the ionization constant of acetic acid. [f] $k_{H_2O} = k_e$, which is defined in the text. [g] See ref 12.

The k_a of glycine (Table I) is of the same order of magnitude as the k_a of amines not containing a carboxylate ion. Thus, it appears that intramolecular catalysis of proton abstraction by internal carboxylate ion in the protonated glycine ketimine (mechanisms viii–IV) or in the ketimine assisted by an oxonium ion (mechanisms viii–III) (assuming that enolization is the rate-determining step of the reaction) does not occur.[23] The failure to observe such a reaction may be explained by the lower value of the Brønsted β for intramolecular proton abstraction from a protonated ketimine than for intermolecular proton abstraction. This explanation implies that the external general base, water, can compete with the internal general base, carboxylate ion, since 55 M water may be in excess over the apparent molarity of the carboxylate ion, and since the difference in basicities is not great. This hypothesis suggests that proton abstraction by an internal general base in a ketimine intermediate might be seen with a more favorable internal general base, such as could be found in a more rigid system with a general base of stronger basicity.

Reactivity of Substrates toward General Bases. The variation in enolization reactivity of different substrates toward a given general base is quite large. For example, protonated acetone and acetone differ by 10^{11} in their reaction with water as general base. Likewise, the difference in reactivity of these two substrates toward acetate ion as a general base is 10^{11}. The relative reactivity of the various substrates with water (or acetate ion) is related to the basicity of the heteroatom, as can be seen in Table III where an attempt has been made to estimate the relative basicities of the various heteroatoms.

Comparison of Model and Aldolase-Catalyzed Enolization. The comparison of the rate of enolization of the model systems considered here with the rate of enolization catalyzed by aldolase is of interest. Aldolase catalyzes a proton exchange which is presumably a measure of enolization.

The rate constant of this proton exchange is faster than the rate constant of condensation. Therefore, the rate constant of enzymatic enolization will be estimated from the rate constant of proton exchange rather than from the rate constant of condensation. Some interesting comparisons can be made. The ultimate comparison is yet to be achieved, but some conclusions can be drawn.

The rate constant of aldolase-catalyzed α-hydrogen exchange of dihydroxyacetone phosphate may be compared with the rate constants for various model systems, using the assumption that the aldolase-catalyzed enolization is a water-catalyzed reaction. On this basis, Table IV was constructed. This comparison is of limited validity because of the assumption that the enolization of the enzyme–substrate Schiff base is a water-catalyzed rather than an enzyme-catalyzed reaction. Furthermore, the enolization of dihydroxyacetone might be expected to be faster than that of acetone. However, it is clear that none of the models except protonated acetone approaches the enzyme–substrate ketimine base in efficiency. However, a protonated ketone is an unlikely reactant at the neutral pH's of the enzymatic reaction because the low concentration of protonated ketone at neutrality dictates that the rate of proton abstraction from the protonated ketone be faster than a diffusion controlled rate. On the other hand, a comparatively large concentration of protonated ketimine is present at neutral pH's and although the protonated ketimine reacts 10^3-fold slower than the protonated acetone, it is 10^8 faster than unprotonated acetone. Thus, of the three possible enolization models, the protonated ketimine model is by far the best one.

Table IV. Comparison of the Aldolase-Catalyzed α-Hydrogen Exchange of Dihydroxyacetone Phosphate with Various Enolizations of Acetone and Its Derivatives

System	k, M^{-1} sec^{-1}[c]	Ref
$(CH_3)_2C{=}OH^+ + H_2O$	1.53	25
$(CH_3)_2C{=}N\overset{+}{H}Gly + H_2O$	2.04×10^{-3}	*a*
$(CH_3)_2C{=}N\overset{+}{H}CH_3 + H_2O$	2.6×10^{-2}	*a*
$(CH_3)_2C{=}O + H_2O$	0.8×10^{-11}	12
Aldolase-N$=$C$R_2 + H_2O$[b]	12.5	26

[a] This work. [b] Corrected for titrium exchange. [c] The second-order rate constants refer to 55.5 M water.

In an intramolecular enolization of a ketone, the intramolecular general basic catalysis has been shown to be equivalent to about 50 M of the corresponding intermolecular general basic catalyst.[8] Presumably, in an aldolase-catalyzed enolization, intramolecular catalysis by an enzymatic group occurs, rather than cata-

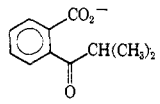
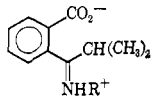
(23) Mechanisms viii (I and II) are unlikely because of their four-membered cyclic transition states.

(24) E. M. Arnett, *Progr. Phys. Org. Chem.*, 1, 325 (1963).

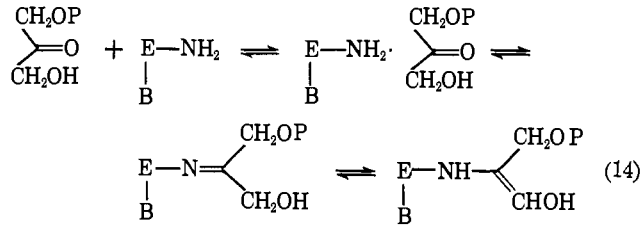
(25) W. J. Rutter, *et al.*, *J. Biol. Chem.*, **236**, 3185, 3193 (1961); I. A. Rose, E. L. O'Connell, and A. H. Mehler, *ibid.*, **240**, 1758 (1965).

(26) I. M. Rose and S. V. Rieder, *ibid.*, **231**, 315 (1958).

Table V. Comparison of Aldolase-Catalyzed α-Hydrogen Exchange of Dihydroxyacetone Phosphate with Some Intramolecular Enolizations

System	k, sec^{-1}	Ref
	50×10^{-7}	21
	500	See text
Aldolase-N=CR_2 + H_2O	690	38

lysis by external water, as assumed above. Therefore, it is of interest to compare the rate constant of this intramolecular catalysis of enolization with the rate constant of aldolase-catalyzed enolization. This comparison is shown in Table V. Since protonated ketimines enolize 10^8-fold faster than corresponding ketones (Table IV) the rate of the hypothetical intramolecular carboxylate ion catalyzed enolization of a protonated ketimine may be calculated from that of the corresponding ketone. From Table V it is seen that the rate of the hypothetical intramolecular carboxylate ion catalyzed enolization of a protonated ketimine compares very favorably with that of the aldolase-catalyzed enolization. Although the chemical analogy is far from complete it can be tentatively suggested that the aldolase-catalyzed enolization involves Schiff base formation, followed by proton abstraction to produce the corresponding enamine, as shown in eq 14.


(14)

Studies on the Mechanism of Oxime and Ketimine Formation[1]

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Abstract: The rate constant for ketimine formation from acetone and methylamine has been determined using two independent methods: by direct spectrophotometric observation and by determination of the rate of acetoxime formation from acetone and hydroxylamine in the presence of methylamine. The two methods yield equivalent rate constants. The pH dependence of ketimine formation indicates a reaction which is dependent only on free methylamine concentration. This result is in agreement with previous suggestions for the mechanism of this reaction. The rates of oximation of benzaldehyde and acetone were determined from approximately pH 6 to 12. With both substrates, a reaction dependent on oxonium ion and a reaction dependent on hydroxide ion were observed. In the acetone reaction, a reaction independent of pH was also observed. The oxonium ion catalysis and pH-independent reactions are interpreted as general acid catalyses by oxonium ion and water, respectively, on the basis of the fit of these data to a Brønsted plot, with $\alpha = 0.6$. The hydroxide ion reaction is interpreted as a specific hydroxide ion catalysis on the basis of its deuterium oxide isotope effect.

The rate of formation of the ketimine between methylamine and acetone was of interest in studies of enolization involving ketimine as possible intermediates.[3] Some of the kinetics of ketimine formation studied here were carried out by direct spectrophotometric measurements. However, since the equilibrium constant of ketimine formation from methylamine and acetone is very poor ($K_1 = 0.22\ M^{-1}$), the technique of using the oximation reaction to follow the formation of ketimine, developed by Cordes and Jencks, has also been used.[4] In this method hydroxylamine reacts very rapidly with the protonated ketimine and the rate of oximation is limited by the rate of formation of the ketimine. As background for our studies on ketimine intermediates in enolization reactions, studies on both ketimine formation and oxime formation are reported here.

Experimental Section

Materials. Acetone, benzaldehyde, hydroxylamine, and acetonitrile were of analytical reagent grade. Methylamine hydrochloride was an Eastman Kodak Co. product and was recrystallized from ethanol. Deuterium oxide (99.64%) was obtained from the Volk Radiochemical Co. The deuterium oxide buffers were prepared directly from heavy water and the protium buffer. For example, 0.1 M hydroxylamine hydrochloride will exchange with heavy water to give 0.2 M water, a dilution of the heavy water which is negligible for the purposes of our experiments. The ionic strength of the buffer solution was kept constant at 0.11 in the oximation of benzaldehyde and 0.2 in the other reactions. The pH of the buffers was adjusted in a Radiometer pH-Stat using 12 M sodium hydroxide. This treatment did not affect the ionic strength of the solutions and had a negligible effect on the concentrations. pD was calculated from the equation[5]

$$\text{pD} = \text{pH meter reading} + 0.4$$

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(3) A. Williams and M. L. Bender, *J. Am. Chem. Soc.*, **88**, 2502 (1966).

(4) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 826 (1962).

(5) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).