# Amine Catalysis of β-Ketol Dehydration.   II.
Catalysis *via* Iminium Ion Formation.   General
Analysis of Nucleophilic Amine Catalysis

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Abstract: A kinetic study of the dehydration of 9-hydroxy-10-methyl-*cis*-decalone-2 (**1**) to form 10-methyl-$\Delta^{1,9}$-octalone (**2**) in aqueous solution has been made using a variety of primary and secondary amines as catalysts. Evidence, including a large primary kinetic isotope effect with appropriately deuterated **1**, has been found for catalysis involving rate-limiting α-proton abstraction by general bases from an iminium derived from **1**. When the general base is another molecule of the imine-forming amine, the bimolecular rate constant (k_{AB}) varies less than a factor of 2 over an amine basicity range of 10^5. This invariance results from a balancing of two effects: an increase in the effectiveness of the amine as a general base for α-proton abstraction with increasing pK_a ($\beta = 0.5$), and a decrease in the susceptibility of iminium ions to deprotonation with increasing pK_a. These findings lead to the conclusion that a nucleophilic amine catalyst for α deprotonation will be most effective when pH = $pK_a^N = pK_a^B$, where pK_a^N is the pK_a of the imine-forming amine and pK_a^B is the pK_a of the general base. The fact that this condition for maximum catalytic effectiveness can be met at neutral pH elucidates why many enzymes have evolved which operate *via* iminium ion formation. The maximum rate of catalysis for an enzyme constrained to operate with pH = $pK_a^N = pK_a^B$ is estimated and compared with experimental values.

As delineated in part I of this study,[1] the dehydration of β-hydroxy ketone **1** to form α,β-unsaturated ketone **2** is subject to complex general acid–base catalysis. Additional catalysis of this reaction by nontertiary amines *via* iminium ion formation was also expected, because such catalysis had been found previously in the conversion of β-acetoxy ketone **3** to **2**.[2] Similar, but more extensive, evidence of nucleophilic catalysis by primary and secondary amines of the dehydration of **1** is presented in this paper. These results are then used as the basis for a generalized discussion of this type of catalysis, in which conversion of a carbonyl group to an iminium ion facilitates removal, without its bonding electrons, of a group in the α position. Such processes are prevalent, both enzymically and in model systems. Examples include decarboxylations of β-keto acids,[3,4] aldol[5,6] and retroaldol[7] reactions, enolizations,[8–11] and dehydrations.[12,13] In this discussion, we offer an explanation why the iminium ion pathway is particularly well suited to biochemical systems.

1   1-C_1-d_2 2   3
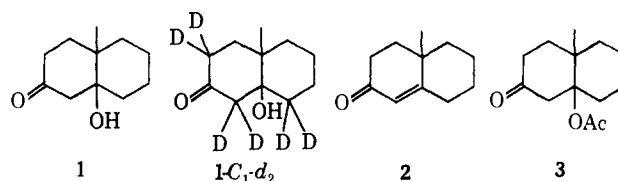
Results

The preparation of the substrates for this study, **1** and **1**-C_1-d_2,[14] was described in part I.[1] The reactions of dilute aqueous solutions of these β-hydroxy ketones were monitored by observing the increase in absorbance at 247 nm caused by formation of the product **2**. The reactions proceeded essentially quantitatively and afforded excellent pseudo-first-order kinetics.

The rate of formation of **2** from **1** is described by eq 1, where [H_3O^+] is a_H as measured by pH meter, [OH^-]

$$\frac{d[2]}{dt} = [f([B^n],[OH^-],[H_3O^+]) + k_A[RNH_3^+] + \sum_n k_{AB}^n[RNH_3^+][B^n]][1] \quad (1)$$

is K_w/a_H, [B^n] is the concentration of a given general base in solution, and [RNH_3^+] is the concentration of primary or secondary (but not tertiary) amine. The quantity $f([B^n],[OH^-],[H_3O^+])$ represents the complex dependence of the rate of dehydration of **1** on pH and general base and acid concentrations which was described in part I.[1]

Because primary and secondary amines exhibit large kinetic terms reflecting catalysis *via* iminium ion formation, as described below, it was not possible, as it was for tertiary amines, to measure all of the individual rate constants necessary to describe completely the quantity $f([B^n],[OH^-],[H_3O^+])$. However, in order to obtain values of the desired rate constants k_A and

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(11) M. A. Roseman, Ph.D. Thesis, Michigan State University, 1970.
(12) D. Portsmouth, A. C. Stoolmiller, and R. H. Abeles, *J. Biol. Chem.*, **242**, 2751 (1967).
(13) See W. A. Wood in "The Enzymes," Vol. V, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1971, p 582, for a consideration of "Schiff Base-Assisted Dehydrations," including the example cited in ref 12.

(14) The substance designated 1-C_1-d_2 is actually 1,1,3,3,8,8-hexadeuterio-9-hydroxy-10-methyl-*cis*-decalone-2, as its method of preparation (ref 1) makes clear.

Table I. Rate Constants for the Reactions of **1** and **3** with Primary and Secondary Amines in Aqueous Solution at 25°

Amine	$pK_a{}^a$	Substrate 1				Substrate 3^b		
		k_B', M^{-1} sec^{-1}	k_{AB}, M^{-2} sec^{-1}	k_A, M^{-1} sec^{-1}	No. of runs	k_B, M^{-1} sec^{-1}	k_{AB}, M^{-2} sec^{-1}	k_A, M^{-1} sec^{-1}
Cyanomethylamine	5.34	5.0×10^{-6}	2.45×10^{-3}	1.5×10^{-5}	104	3.8×10^{-5}	4.1×10^{-3}	1.0×10^{-4}
Trifluoroethylamine	5.70	2.5×10^{-6}	1.4×10^{-3}	6.0×10^{-6}	32	3.6×10^{-5}	1.8×10^{-3}	4.6×10^{-5}
Ethyl glycinate	7.73	7.0×10^{-5}	2.0×10^{-3}	6.0×10^{-7}	44	6.7×10^{-4}	4.0×10^{-3}	3×10^{-6}
Ethoxyethylamine	9.44	2.1×10^{-5}	2.9×10^{-3}		16	5.2×10^{-3}		
Allylamine	9.49	5.0×10^{-5}	1.4×10^{-3}		36	5.9×10^{-3}		
n-Butylamine	10.61	1.2×10^{-4}	1.2×10^{-3}		29	3.2×10^{-2}		
Morpholine	8.36	2.5×10^{-6}	$\sim 8 \times 10^{-5}$		56	3.5×10^{-3}		
Hexamethylenimine	11.10	2.0×10^{-4}			48	1.9×10^{-1}		
Piperidine	11.22	3.0×10^{-5}			24	1.3×10^{-1}		
Pyrrolidine	11.32	4.7×10^{-3}			24	3.1×10^{-1}		

^{a} The sources of all the pK_a values are given in ref 2. ^{b} Data from ref 2.
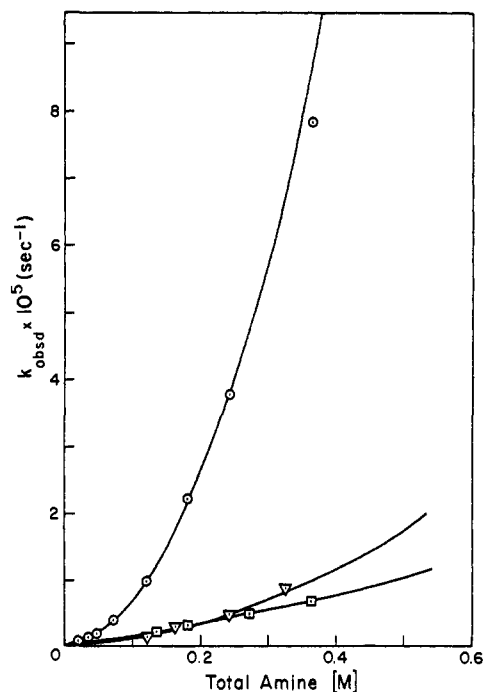

Figure 1. Plot of k_{obsd} values for the conversion of **1** to **2** vs. total cyanomethylamine buffer concentration at pH 5.25 (⊙), pH 6.94 (▽), and pH 3.02 (□). The lines were computed using eq 3 and the rate constants in Table I.

k_{AB}, which reflect nucleophilic catalysis, this quantity must at least be estimated. We have chosen the simplifying assumption shown in eq 2, where $k_H[H_3O^+]$ +

$$f([B^n],[OH^-],[H_3O^+]) \approx k_H[H_3O^+] + \sum_n k_B'^n[B^n] + f_{OH}[OH^-] \quad (2)$$

$f_{OH}[OH^-]$ at any specified pH is given by eq 3 in part I. This assumption of linear dependence on general base concentration ($k_B'[B]$) ignores the change in slope which occurs at 8.1×10^{-7} sec^{-1} for catalysis of **1** → **2**.[1] An error of approximately 8×10^{-7} sec^{-1} is therefore introduced, but this is $<1\%$ of the rate which k_{AB} terms exhibit ($\sim 10^{-4}$ sec^{-1}) in the pH–rate profiles shown below in Figure 4. Thus, for the purpose of evaluating k_A and k_{AB}, the rate of reaction may be adequately approximated by eq 3, where each $k_B'[B]$

$$\frac{d[2]}{dt} = \left\{ k_H[H_3O^+] + f_{OH}[OH^-] + \sum_n k_B'^n[B^n] + k_A[RNH_3^+] + \sum_n k_{AB}{}^n[RNH_3^+][B^n] \right\}[1] \quad (3)$$

term represents catalysis proportional to free amine. As discussed below, this term may include catalysis by either of two mechanisms.

In Table I are listed the rate constants, obtained as described below, for the various primary and secondary amines used in this study. For comparison, the corresponding values of k_B, k_A, and k_{AB} with keto acetate **3** as substrate[2] are also listed. The values of k_A in Table I were obtained from the slopes of plots of k_{obsd} vs. [RNH$_3^+$], using k_{obsd} values (corrected for $k_H[H_3O^+]$ if necessary) measured at pH's well below the catalyst pK_a, where k_{AB} and k_B' terms are negligible. These terms were found only for the low-pK_a primary amines in Table I. No such terms were detected for low-pK_a tertiary amines, such as N,N-dimethylcyanomethylamine (DCMA, $pK_a = 4.20$), or for any high-pK_a amines.

Similarly, the values of k_B' were determined from the slopes of plots of k_{obsd} (corrected for $f_{OH}[OH^-]$ if necessary) vs. [RNH$_2$] at pH's far enough above the pK_a so that k_A and k_{AB} terms were negligible. If this was not experimentally possible (as with n-butylamine, $pK_a = 10.61$), an approximate value of k_B' was obtained and used to calculate an approximate value of k_{AB}. By an iterative method, these two constants were refined to give the best fit with concentration dependence and pH–rate profile data.

Each of the primary amines used in this study exhibited $k_{AB}[RNH_3^+][RNH_2]$ terms, which caused upward curvature in plots of k_{obsd} vs. buffer concentration (Figure 1), and bell-shaped pH–rate profiles with a maximum at the amine pK_a, where the value of [RNH$_3^+$][RNH$_2$] is greatest (Figure 2). In Figure 1, nearly linear dependence of k_{obsd} on buffer concentration is found both when pH > pK_a, owing to dominance of $k_B'[RNH_2]$, and when pH < pK_a, owing to dominance of $k_A[RNH_3^+]$. When pH $\approx pK_a$, however, the $k_{AB}[RNH_3^+][RNH_2]$ term is the most important and second-order dependence on amine concentration produces upward curvature. The value of k_{AB} for each of the primary amines was obtained from the slope of a plot of ($k_{obsd} - k_B'[RNH_2] - k_A[RNH_3^+]$) vs. [RNH$_3^+$]·[RNH$_2$].

The points in Figure 1 are experimental; the curves were computed from the constants for CMA in Table I, using eq 3. Figure 2 shows the individual pH–rate profiles computed with the constants in Table I for the $k_B'[RNH_2]$, $k_A[RNH_3^+]$, and $k_{AB}[RNH_3^+][RNH_2]$ terms for 0.1 M CMA buffer. Their sum gives a good fit with the points determined experimentally under the

Figure 4. Six pH–rate profiles calculated for the conversion of 1 to 2 catalyzed by 0.5 M total buffer concentrations of primary amines, using eq 3 and the rate constants in Table I. The amines are (a) cyanomethylamine, (b) trifluoroethylamine, (c) ethyl glycinate, (d) ethoxyethylamine, (e) allylamine, (f) n-butylamine.
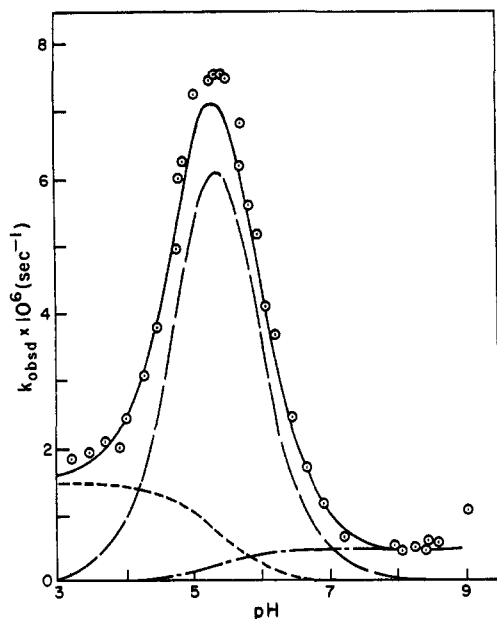
Figure 2. Plot of pseudo-first-order rate constants, k_{obsd}, for the conversion of 1 to 2 in the presence of 0.1 M cyanomethylamine buffer $vs.$ pH. The points are experimental and the lines were calculated for the $k_B'[RNH_2]$ term (— — —), the $k_A[RNH_3^+]$ term (- - - -), the $k_{AB}[RNH_3^+][RNH_2]$ term (— —), and the sum of these three terms (———), using eq 3 and the rate constants in Table I.

Figure 5. Three pH–rate profiles for the conversion of 1 to 2 catalyzed by 0.1 M CMA (○), 0.1 M CMA + 0.2 M DCMA (▽), and 0.1 M CMA + 0.4 M DCMA (□). The lines were calculated as the sum of the terms for CMA and DCMA plus 1.15 × 10⁻³· [HCMA⁺][DCMA].
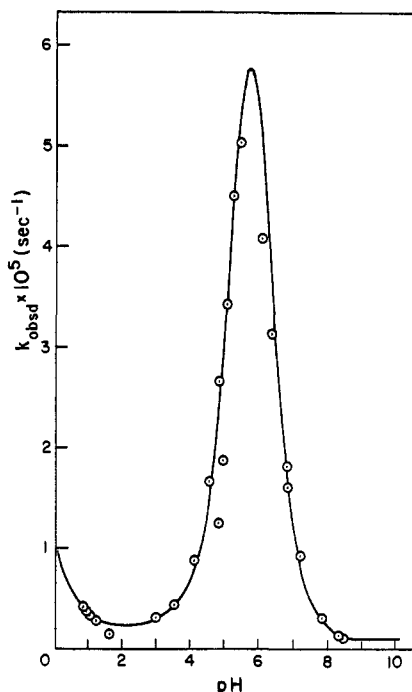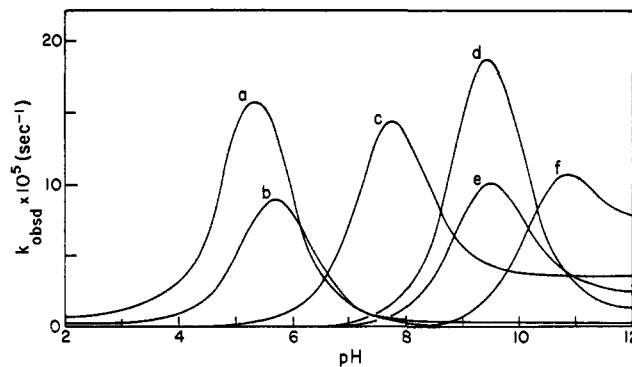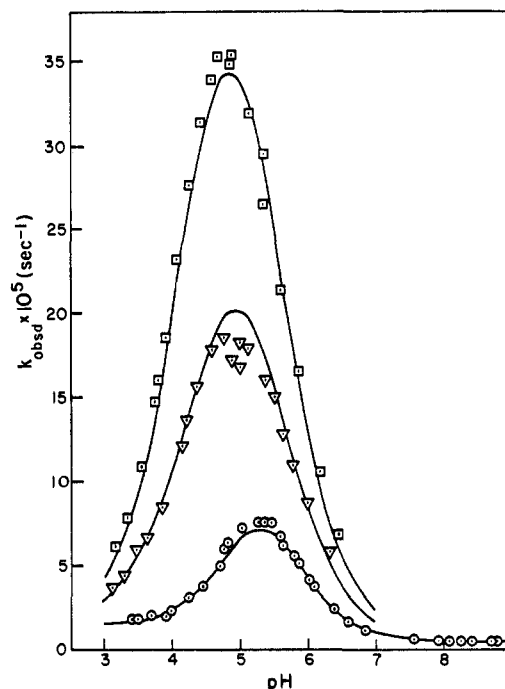
Figure 3. Plot of pseudo-first-order rate constants, k_{obsd}, for the conversion of 1 to 2 in the presence of 0.4 M trifluoroethylamine buffer $vs.$ pH. The points are experimental and the line was calculated using eq 3 and the rate constants in Table I.

same conditions, showing plateaus above and below the pK_a, caused by the k_B' and k_A terms, respectively, and a bell-shaped portion with its maximum at the pK_a caused by the $k_{AB}[RNH_3^+][RNH_2]$ term.

Using eq 3 and the rate constants in Table I, excellent fits were also obtained with pH–rate profiles measured for other primary amines, as shown in Figure 3 for 0.4 M trifluoroethylamine (TFE). In order to facilitate

visualization of the variation in k_A, k_{AB}, and k_B' terms which occur with changing amine pK_a, the pH–rate profiles for each of the primary amines used in this study have been computed for 0.5 M total amine concentration and are shown in Figure 4.

The addition of a general base B to a reaction mixture containing a primary amine produced a new third-order term $k_{AB}[RNH_3^+][B]$, proportional to protonated primary amine concentration times general base concentration. Figure 5 shows the experimental points in the pH–rate profiles for 0.1 M CMA (identical with those in Figure 2), for 0.1 M CMA + 0.2 M DCMA, and for 0.1 M CMA + 0.4 M DCMA. The curves shown in Figure 5 fitting the data points were obtained by adding to the CMA terms the $k_B'[B]$ term for DCMA and a new term, $k_{AB}[CMAH^+][DCMA]$, where $k_{AB} = 1.15 \times 10^{-3}$ M^{-2} sec⁻¹.
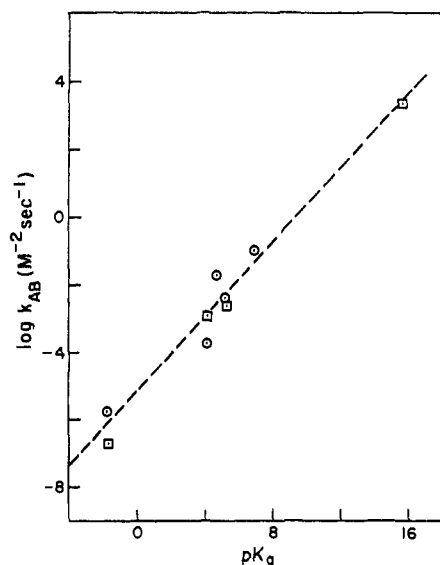
Figure 6. Plot of the logarithms of third-order rate constants, k_{AB}, obtained from $k_{AB}[HCMA^+][B]$ terms measured for the conversion of 1 to 2 (□) and 3 to 2 (○), $vs.$ the pK_a's of the general bases, B. The slope of the line is 0.5. The data for 3 were taken from ref 2.

Among the four secondary amines used, only morpholine showed upward curvature in a plot of k_{obsd} $vs.$ total amine concentration, indicating a significant k_{AB} term. However, the magnitude of this term was considerably smaller than those for primary amines, making the simplifying assumptions of eq 3 inapplicable and the value of k_{AB} for morpholine in Table I approximate. No catalysis proportional to protonated amine concentration was found for any of the secondary amines, but none with a very low pK_a was used.

When substrate $1\text{-}C_1\text{-}d_2$ was studied with CMA at its pK_a, the slope obtained from a plot of k_{obsd} $vs.$ [CMA]·[HCMA⁺] was about one-sixth the magnitude of a slope of a similar plot for 1. Since the k_{AB} term represents 96% of the catalysis of 1 → 2 by 0.4 M CMA at its pK_a, this is a good indication of a primary kinetic isotope effect in the k_{AB} term. As discussed in part I,[1] tertiary amines are capable of catalyzing isotopic exchange at C_1 and inducing thereby a diminution in the kinetic isotope effect observed as the reaction progresses. However, the pseudo-first-order plots for CMA with $1\text{-}C_1\text{-}d_2$ were linear to at least 1 half-life, showing that exchange at C_1 is not important. Such exchange, indicated by upward curvature in the pseudo-first-order plot, was observed for high-pK_a nontertiary amines such as pyrrolidine. However, the rate of exchange in such cases is of the same magnitude as that found for tertiary amines of comparable pK_a.

Discussion

Nucleophilic Amine Catalysis of the Conversion 1 to 2. As indicated in eq 3, the rate of formation of enone 2 from 1 is proportional to protonated primary (or secondary) amine concentration and protonated amine concentration times general base concentration. The analogous k_A and k_{AB} terms were shown in the case of keto acetate 3 to reflect catalysis via iminium ion formation.[2] The fact that the k_A and k_{AB} terms for CMA, TFE, and ethyl glycinate (EG) are very similar in magnitude for the elimination reactions of 1 and 3 suggests that the same mechanism is opera-

tive in both cases. A large, undiminishing, primary kinetic isotope effect found with $3\text{-}C_1\text{-}d_2$[2] was conclusive evidence that the rate-limiting step for the k_{AB} term in the elimination of acetic acid from 3 is amine catalyzed α-proton abstraction from an equilibrium concentration of iminium ion. The similar isotope effect found with $1\text{-}C_1\text{-}d_2$ establishes that in this case also the k_{AB} term is caused by the process shown in Scheme I.

Scheme I
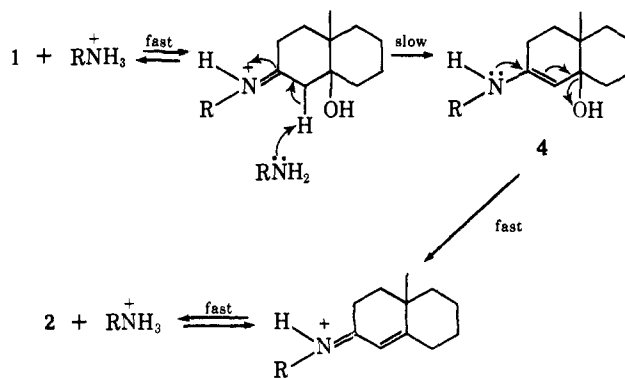
The fact that α-proton exchange occurs via general acid and base catalysis, as discussed in part I,[1] but not via the iminium ion mechanism shown in Scheme I, reflects the difference in the nature of the α-deprotonated intermediates enol 1e, enolate anion 1⁻, and enamine 4. It was shown[1] that enolate anion 1⁻, but not enol 1e, underwent general acid-catalyzed reprotonation at C_1 competitively with conversion to 2. Enamine 4, unlike 1e, is not in rapid equilibrium with an anionic species analogous to 1⁻, and it is not surprising that its kinetic behavior is more like that of 1e. Although it is impossible to determine whether there is general acid catalysis of the loss of hydroxide ion from 4, because this occurs after the rate-limiting step, analogy with the behavior[1] of 1e and 1⁻ suggests that such catalysis exists.

The k_A terms for the reaction of low-pK_a primary amines with 1 are ascribed, as they were for 3,[2] to the special case where water acts as the general base for abstraction of the α proton from the iminium ion, giving a rate $= k_A'[RNH_3^+][H_2O] = k_A[RNH_3^+]$. With neither substrate did low-pK_a tertiary amines such as DCMA exhibit k_A terms, ruling out a mechanism involving general acid catalysis.

The most general expression for this type of nucleophilic catalysis is that in which any general base, B^n, contributes a term $k_{AB}{}^n[RNH_3^+][B^n]$. As discussed above, a rate constant $k_{AB}{}^n$ of 1.15×10^{-3} M^{-2} sec⁻¹ is required to calculate the rates observed (Figure 5) after addition of DCMA buffer to a dehydration of 1 catalyzed by CMA. This behavior again is analogous to that found with 3 in the presence of CMA and added general bases.[2]

Figure 6 shows values of log k_{AB} obtained with either 1 or 3[2] from measured $k_{AB}[RNH_3^+][B]$ terms, where RNH_3^+ is protonated CMA, plotted $vs.$ the pK_a of the general base (B). The bases are either free amine, acetate ion, water, or hydroxide ion. The k_{AB} values for the case where B is water were obtained by dividing k_A by $[H_2O] = 55$ M. The k_{AB} values for both 1 and 3 are plotted together because the very different leaving groups of these two substances are significantly in-

volved only after the rate-determining proton abstraction. In Figure 6, the slope of the plot (0.5) is the Brønsted β for α-proton abstraction from a cyanomethyliminium ion. This value is consistent with the reported Brønsted β of 0.5 for the α-exchange reaction of the methyliminium ion derived from isobutyraldehyde-2-d[15] and is similar to the reported value of 0.4 for the α-proton abstraction from the iminium ion formed from glycine and acetone.[9] It is also consistent with the β values of 0.5 and 0.65 which can be derived from the observed[16,17] α values for general acid catalysis of the reverse reaction, C-protonation of enamines.

Should the base abstracting a proton from the iminium ion be hydroxide ion, the observed rate would be $k_{AB}[RNH_3^+][OH^-]$, which is kinetically equivalent to $k_B'[RNH_2]$. The measured free amine rate constants, k_B', thus may be combinations of the specific base-general acid catalysis found for tertiary amines[1] and catalysis involving iminium ion formation. Although these terms are both proportional to free amine, and therefore are not distinguishable kinetically, a given amine which has a large component of nucleophilic catalysis should produce a rate constant, k_B', larger than an amine of comparable pK_a which has none.

In Figure 7, the values of log k_B' are plotted vs. the pK_a's of the amines in Table I. The values of k_B' used for tertiary amines are assumed to be equivalent (eq 2) to the k_B^f values reported in part I of this study.[1] Large positive deviations from the line drawn through the tertiary amine points can be seen for certain primary and secondary amines. These rate accelerations are ascribed to nucleophilic catalysis in which hydroxide ion acts as the general base. This interpretation is confirmed by the fact that conversion of the measured $k_B'[RNH_2]$ term for CMA into the corresponding $k_{AB}[RNH_3^+][OH^-]$ term (by $k_{AB} = k_B'[RNH_2]/[RNH_3^+][OH^-] = k_B'[RNH_2][H_3O^+]/[RNH_3^+]K_w = k_B'K_a/K_w$) affords a value of log k_{AB} which fits nicely on the Brønsted plot for other general bases with CMA, as shown in Figure 6.

The substantial decrease in k_B' values upon changing the catalyst from pyrrolidine to hexamethylenimine to piperidine, despite their similar pK_a's, provides further evidence that nucleophilic catalysis involving hydroxide ion as the general base is occurring. This order of reactivity reflects the relative ease of iminium ion formation from these secondary amines. As noted previously,[18,19] the incorporation of an sp^2 atom, as in iminium ion formation, is more favorable in a five- or seven-membered ring system than in one with six members. Analogous positive deviations were not found with substrate 3, presumably because the k_B terms for 3 are much larger than the corresponding k_B' terms for 1. Similar masking by general base catalysis was probably responsible for Hine's failure to observe covalent catalysis by secondary amines of α-proton exchange in isobutyraldehyde.[20]

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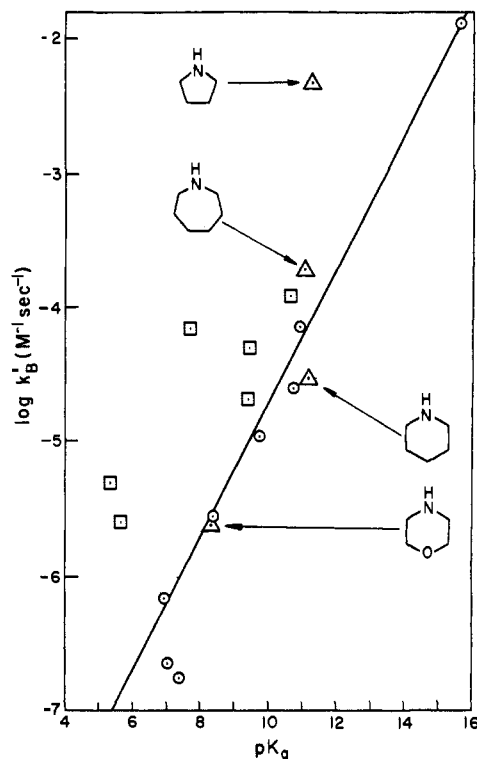(20) J. Hine and J. Mulders, *J. Org. Chem.*, **32**, 2200 (1967).

Figure 7. Plot of log k_B' values for the conversion of 1 to 2 catalyzed by primary (□), secondary (△), and tertiary amines and hydroxide ion (○) vs. amine pK_a.

The fact that the terms proportional to free amine are much smaller in the case of ketol 1 has another important consequence. With keto acetate 3, the k_B terms completely swamped the nucleophilic catalysis terms for any amine catalyst with $pK_a > 8$. With ketol 1, however, nucleophilic catalysts were readily studied up to $pK_a \cong 10.5$. Accordingly, it was possible to observe that the values of k_{AB} for the dehydration of 1 vary irregularly within a factor of only 2 over a range of amine catalyst basicities of greater than 10^5 (Table I; Figure 4). Our earlier conclusion[2] that k_{AB} is independent of catalyst pK_a, based on data from only three amines, was thereby confirmed.

As discussed above, these k_{AB} terms reflect rate-determining α-proton abstraction by free amine from an iminium ion formed by the same amine. Thus, we can say $k_{AB}[RNH_3^+][RNH_2][1] = k_{IB}[RN^+H=CR_2] \cdot [RNH_2]$ where k_{IB} is the second-order rate constant for this proton abstraction from the iminium ion. Results of Williams and Bender[21] indicate that the equilibrium concentration of imine depends only slightly on the amine pK_a, and Bender,[9] Hine,[22] and we[2] have assumed that $K_a^{imine} = 10^3 K_a^{amine}$ irrespective of amine pK_a, so that $[RN^+H=CR_2]/[RNH_3^+]$ should not depend upon the pK_a of RNH_3^+. Accordingly, k_{IB} will, like k_{AB}, be relatively independent of the basicity of the catalyst.

This important conclusion is the consequence of two compensating trends associated with a change in amine pK_a. Clearly, increasing the catalyst pK_a *increases* the efficiency of RNH_2 as an α-proton abstractor; a Brønsted β of 0.5 has in fact been determined for this

(21) A. Williams and M. L. Bender, *J. Amer. Chem. Soc.*, **88**, 2508 (1966).
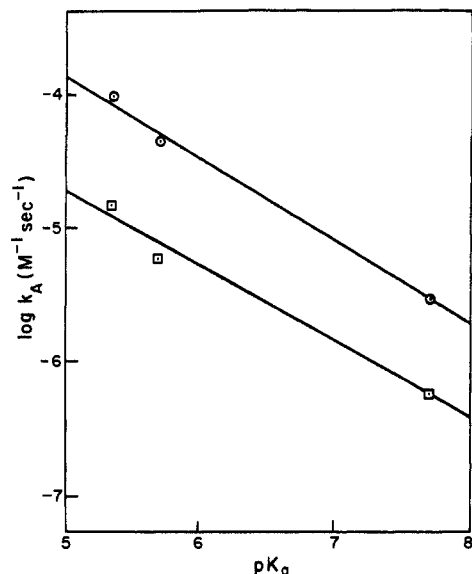(22) J. Hine, B. C. Menon, J. H. Jensen, and J. Mulders, *ibid.*, **88**, 3367 (1966).

Figure 8. Plots of log k_A values for the catalysis by protonated primary amines of the conversion of **1** to **2** (□) and of **3** to **2** (○), *vs.* the pK_a's of the primary amines. The slope of the lines is approximately -0.5. The data for **3** were taken from ref 2.

Figure 9. Plot of log k_{AB}' *vs.* $pK_a^N - pK_a^B$ (- - -), with a slope o -0.5; plot of α^{max} (see text) *vs.* $pK_a^N - pK_a^B$ (— — —); and plot of the logarithm of the product of k_{AB}' and α^{max} *vs.* $pK_a^N - pK_a^B$ (——).f

process (Figure 6). On the other hand, increasing catalyst pK_a *decreases* the susceptibility of the derived iminium ion to α-proton abstraction. Evidence that this is so may be seen in Figure 8 where values of log k_A are plotted against the pK_a's of RNH_3^+ for substrates **1** and **3**. In both cases the slope is roughly -0.5, indicating that for a given general base (in this case, water) an increase in the pK_a of the amine which forms the iminium ion results in a decrease in the rate of proton abstraction. The rates of abstraction of the α proton from a series of isobutyraldehyde iminium ions have been shown by Hine to exhibit similar behavior.[23]

General Analysis of Catalysis via Iminium Ion Formation. The factors discussed above place constraints on the design of a catalyst, such as an enzyme, which is to contain both a nucleophilic amine and a general base. It might initially be assumed that an ideal catalyst would contain a high-pK_a general base and a low-pK_a primary amine. However, at any pH low enough to generate an appreciable concentration of the protonated primary amine (and therefore produce the iminium ion in equilibrium with it), almost all of the high-pK_a general base would be protonated. While the rate constant k_{AB} would then indeed be large, the observed rate would be small, since only a negligible fraction of the catalyst would be in proper state of protonation. A balance must be struck between having a much lower pK_a primary amine than general base (with a small fraction of active catalyst but large k_{AB}) and having a much higher pK_a primary amine than general base (with a large fraction of active catalyst but small k_{AB}). It will be useful to put this argument into quantitative terms.

What one seeks to optimize is the rate of product formation per mol of catalyst (k_{obsd}/[cat]), where [cat] is the total concentration of catalyst regardless of state of protonation. For a catalyst containing both the

(23) J. Hine, B. C. Menon, J. Mulders, and J. P. Idoux, *J. Org. Chem.*, **32**, 3850 (1967). We unfortunately failed to refer to this precedent in our previous discussion of this topic (ref 2).
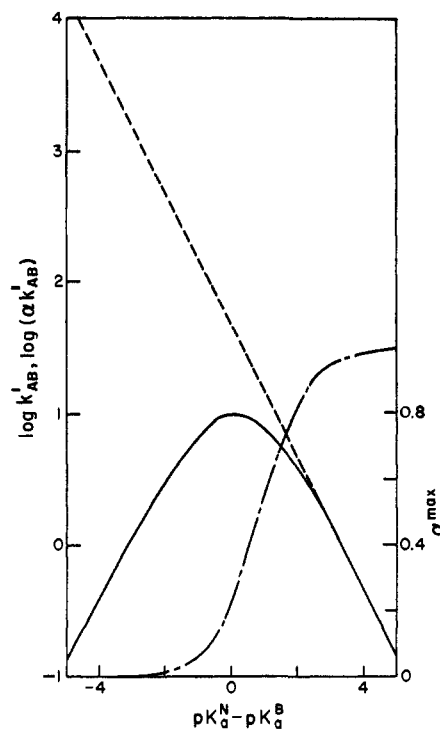
primary amine and the general base (H_2NEB), the rate is $k_{obsd} = k_{AB}'[H_3N^+EB]$. Since the total catalyst concentration is the sum of all the species in reasonable prototropic equilibria (*i.e.*, [cat] = $[H_3N^+EBH^+]$ + $[H_3N^+EB]$ + $[NH_2EB^+H]$ + $[NH_2EB]$), the quantity of interest, k_{obsd}/[cat], is equal to $k_{AB}'\alpha$, where α is the fraction of catalyst in the proper state of protonation (H_3N^+EB). Since α is a function of pH (*e.g.*, at very low pH's the catalyst is almost all $H_3N^+EB^+H$ and $\alpha \cong 0$), it is necessary to specify at what pH the catalyst is to operate. The product $[RNH_3^+][B]$ for any primary amine and general base will be at a maximum at a pH halfway between the pK_a of RNH_3^+ and that of BH^+. In order to maximize α, and hence k_{obsd}/[cat], it is desirable to operate at $pH_{max} \equiv (pK_a^N + pK_a^B)/2$, where pK_a^N is the pK_a of the imine-forming amine function and pK_a^B is the pK_a of the general base. The fraction of total catalyst in the proper state of protonation at pH_{max} is α^{max}, where α^{max} = $[H_3N^+EB]/$ $([H_3N^+EB^+H] + [H_3N^+EB] + [H_2NEB^+H] + [H_2NEB])$.

In Figure 9, log k_{AB}' is plotted against the difference in pK_a between the nucleophilic amine and the general base. The slope of this line is -0.5, consistent with either the Brønsted β for proton abstraction or the susceptibility of the iminium ion to proton abstraction, depending on which pK_a is changed in order to vary ($pK_a^N - pK_a^B$). Also plotted as a function of $pK_a^N - pK_a^B$ is α^{max}. The logarithm of the product of these two terms, log $k_{AB}\alpha^{max} = \log k_{obsd}$/[cat] is also shown. The value of k_{obsd}/[cat] is at a maximum when $pK_a^N - pK_a^B = 0$, meaning that the greatest catalytic efficiency would be obtained by having pH = $pK_a^N = pK_a^B$.

It should be reemphasized that the magnitude of k_{obsd}/[cat] is not altered by a change in pH^{max}, so long as the Brønsted β for α-proton abstraction is balanced by the changing iminium ion susceptibility to proton

abstraction. No inherent advantage would be gained by designing a catalyst to operate at high or low pH, since the largest k_{obsd}/[cat] would be obtained at any pH so long as $pK_a^N = pK_a^B = pH$. However, a nucleophilic amine enzyme, which necessarily functions at a physiological pH near neutrality, would be predicted to have both a primary amine and general base with pK_a's $\cong 7$ at the active site.

The available evidence is consistent with such a conclusion. First, it is clear from the above argument that hydroxide ion or water would be inefficient general bases in such enzymic processes. The imidazole moiety of a histidine residue, which has been suggested as a general base in reactions of this type[12] as well as in numerous other enzymic processes, has $pK_a = 6.0$ in the parent amino acid[24] and should therefore be very well suited for this role. The usual nucleophile, the primary ϵ-amino group of a lysine residue, on the other hand, has $pK_a = 10.53$ in the parent amino acid.[24] Such a pK_a obviously would be badly suited to furnish an optimum k_{obsd}/[cat]. Westheimer's recent finding[3,4] that the ϵ-amino group of the imine-forming[25,26] lysine residue in acetoacetate decarboxylase has a pK_a of 6.0 is accordingly of great significance to the present analysis. Evidence of such lowering of pK_a so that $pK_a^N \approx pH$ may be anticipated for other enzymic nucleophilic amines.

Implicit in this discussion is the reason why enzymes have evolved which function *via* a nucleophilic mechanism rather than *via* direct general base proton abstraction. As we have seen, with $pH = pK_a^N = pK_a^B$, k_{obsd}/[cat] is predicted to be relatively constant regardless of pH. A simple general base-catalyzed proton abstraction α to a carbonyl group, however, would have a rate strongly dependent upon the pK_a of the general base at the active site. If such a proton abstraction were to be effective at pH 6, it presumably would require a general base with $pK_a^B \cong 6$, in order to avoid essentially complete protonation of the base. We have shown previously[2] that conversion of a carbonyl group to an iminium ion, using an amine with $pK_a^N \cong 6$, increases the rate of proton abstraction by a given general base by a factor of 10^5. In other words, a nucleophilic enzyme which can bind substrate efficiently as iminium ion, and in which $pK_a^B = pK_a^N = pH = 6$ will have a k_{obsd}/[cat] 10^5 greater than that for direct proton abstraction by the general base. The more complex multifunctional pathway thus can provide a much more rapid rate-determining step.

It is also of interest to attempt to estimate the rate to be expected of an enzyme constrained to operate within the guidelines elaborated above, in order to compare this estimate with actual rates of enzymes which function *via* iminium ion formation. The rate constant for α-proton exchange for muscle aldolase is 690 sec^{-1},[9] and that for 2-keto-3-deoxy-6-phosphogluconic acid aldolase is 120 sec^{-1}.[11] Accordingly, an estimated rate of 10^2–10^3 sec^{-1} would be consistent with those found experimentally.

The rate of product formation according to the analysis above may be expressed as rate = k_{IB}[R_2C=N$^+$HE]·

[B*], where k_{IB} is the second-order rate constant for the reaction of the general base with the iminium ion, [R_2C=N$^+$HE] is the concentration of enzyme–substrate iminium ion, and [B*] is the "effective concentration"[27] of a proximate general base. For reactions in which $pK_a^N = pK_a^B$, k_{IB} is independent of pK_a and has a value of $\sim 10^1 M^{-1}$ sec^{-1}.[28] Therefore, the desired value of the rate per mol of enzyme is rate/[ENZ] (sec^{-1}) = $10 (M^{-1}$ sec^{-1})([R_2C=N$^+$HE]/[ENZ])· [B*](M). If we assume that all of the enzyme is able to bind substrate as protonated imine at neutral pH, then [R_2C=N$^+$HE]/[ENZ] ≈ 1, and only a relatively small effective concentration of B (10^1–$10^2 M$) would be necessary to explain the observed enzymic rate. However, it is more probable that most of the imine will be unprotonated at pH = pK_a^N, because $pK_a^N - pK_a^{Im} \approx 3$,[2,9] and, accordingly, the value of [R_2C=N$^+$HE]/[ENZ] will be $\sim 10^{-3}$. In order to produce an enzymic rate of 10^2–10^3 sec^{-1}, an effective concentration of general base on the order of 10^4–$10^5 M$ would then be required.

Attempts to evaluate effective concentrations of intramolecular general bases for α-proton abstraction directly from carbonyl compounds have not afforded values this large. Harper and Bender[29] and Bell and Fluendy[30] have found intramolecular α-proton abstraction by carboxylate anions with effective general base concentrations of 50 and 0.3 M, respectively. A similar study by Coward and Bruice,[31] in which the general base was a tertiary amine, revealed only a 0.05 M effective base concentration. These effective concentrations are undoubtedly lowered by the entropic barriers[32] to the formation of the required cyclic transition state. There is an additional constraint for α-deprotonation reactions in the requirement that the proton being abstracted should be situated so that continuous overlap is permitted between the orbital containing its bonding pair of electrons and the π orbital of the carbonyl group.[33] Inability to meet this criterion undoubtedly contributed to the low effective general base concentrations in the examples cited above. This view is supported by Hine's study[8] of intramolecular catalysis of enolization *via* iminium ions of acetone-d_6 with diamines. The maximum intramolecular catalytic term for diamines of the type $H_2N(C)_nNR_2$ occurred when $n = 3$, giving the eight-membered transition state shown in **5**.[8] The general base in **5** can adopt a position for abstraction of the α deuteron which allows effective orbital overlap.

Page and Jencks[32] have recently argued that effective concentrations up to $10^8 M$ are reasonable for intramolecular reactions, owing to the large loss of

(24) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution," Butterworths, London, 1965.

(25) G. A. Hamilton and F. H. Westheimer, *J. Amer. Chem. Soc.*, **81**, 6332 (1959).

(26) I. Fridovich and F. H. Westheimer, *ibid.*, **84**, 3208 (1962).

(27) The concept of "effective concentration" is required for a comparison of intramolecular reactions with analogous intermolecular reactions. For a discussion, see W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, Chapter 1.

(28) A value of $4 \times 10^1 M^{-1}$ sec^{-1} was calculated for CMA with keto acetate **3**.[2]

(29) E. T. Harper and M. L. Bender, *J. Amer. Chem. Soc.*, **87**, 5625 (1965).

(30) R. P. Bell and M. A. D. Fluendy, *Trans. Faraday Soc.*, **59**, 1623 (1963).

(31) J. K. Coward and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 5339 (1969).
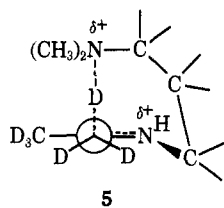
(32) M. I. Page and W. P. Jencks, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1678 (1971).

(33) See H. O. House, "Modern Synthetic Reactions," 2nd ed, W. A. Benjamin, New York, N. Y., 1972, pp 469–473, for a general discussion of this "stereoelectronic factor."

5

translational and rotational entropy which would be required to reach the transition states of the corresponding intermolecular reactions. Certain comparisons of inter- vs. intramolecular catalysis have suggested the existence of such large effective concentrations. One example is the factor of $\sim 10^7$ M found for intramolecular reaction of anions of dicarboxylic acid monophenyl esters.[34,35] Therefore, until better models are constructed to test the effective concentrations of intramolecular general bases for α-proton abstraction, it seems reasonable to conclude that values of 10^4–10^5 M are possible.

It is appropriate to mention here the several attempts which have already been made to observe intramolecular proton abstraction using an imine-forming amine which contains a general base. Bender and Williams[9] found no kinetic term reflecting intramolecular proton abstraction by carboxylate ion from the iminium ion in the case of glycine catalysis of acetone enolization. This result is not surprising because glycine has $pK_a^N - pK_a^B = 7.3$. In a study[36] of the enolization of isobutyraldehyde-2-d catalyzed by glycine and its higher homologs ($H_2N(CH_2)_nCOOH$), no such catalysis was detected. In a polarographic study of the aldol condensation of butyraldehyde in alcohol, Yasnikov[6] has claimed evidence for intramolecular proton abstraction from iminium ions by carboxylate anions with glycine and other amino acids, and by amines with lysine and ethylenediamine. However, this claim is based on observation of bell-shaped pH–rate profiles for these various catalysts at only one concentration. In the absence of concentration dependence data showing less than second-order dependence on catalyst for these terms, the most probable explanation for these curves is intermolecular catalysis analogous to that responsible for the curves displayed in Figures 2 and 3. Hine's recent studies of the α dedeuteration of isobutyraldehyde-2-d with polyethylenimines[37] and of acetone-d_6 with diamines[8] stand

out as the first investigations which have produced convincing evidence of intramolecular catalysis of this type. As noted above, the latter study has also contributed importantly to understanding the geometry of such processes.

The considerations above may be summarized in the following guidelines for the design of nucleophilic amine catalysts for α-deprotonation reactions. First, it is obviously desirable to incorporate features which will increase the stability of amine-carbonyl condensation product in the transition state. Second, the catalyst should possess an intramolecular general base with the correct geometry to abstract an α proton from the iminium ion. Third, it is unimportant what pH is chosen for maximum catalytic efficiency, so long as pH $\cong$ pK_a^N $\cong$ pK_a^B. Use of pH's near neutrality has the advantage of avoiding interference at high or low pH's from catalysis of the same or other reactions by general bases or acids, and it is appropriate for simulation of enzyme action.

Experimental Section

Materials. The substrates used in this study, 1 and 1-C_1-d_2, were prepared as previously described.[1,2] The amines used as catalysts were purified either by two recrystallizations of their hydrochloride salts from 1:1 methanol-1-propanol, or by distillation twice from barium oxide directly before use.

Apparatus. A Unicam SP 800B spectrophotometer equipped with an automatic cell changer and controlled by a Cary 1116100 program timer was used to obtain data on four runs simultaneously. The temperature within the cuvettes was maintained at 25.0 ± 0.1° by circulating water controlled by a P. V. Tamson bath through the cuvette housing.

Kinetics. The methods used to obtain k_{obsd} values were identical with those described previously.[1,2] Within a series of runs in which the buffer concentration was varied, all k_{obsd} values were corrected for minor variations in pH, as described in part I.[1] The pH–rate profiles were measured on solutions prepared by adjusting 5-ml aliquots of buffer solution (four times the desired final concentration) to the desired pH, followed by addition of enough solid KCl to make the solution 0.8 M in chloride ion after dilution to 10 ml. Such solutions were diluted 1:1 with aqueous solutions of 1 to initiate the reaction.

(34) E. Gaetjens and H. Morawetz, J. Amer. Chem. Soc., 82, 5328 (1960).

(35) T. C. Bruice and U. K. Pandit, Proc. Nat. Acad. Sci. U. S., 46, 402 (1960).

(36) J. Hine, B. C. Menon, J. Mulders, and R. L. Flachskam, Jr., J. Org. Chem., 34, 4083 (1969).

(37) J. Hine, F. E. Rogers, and R. E. Notari, J. Amer. Chem. Soc., 90, 3279 (1968).