intermediate to electronic effects on the acyl group or to the basicity of the thiol nucleophile. Thus, substitution of groups for hydrogen on the acyl carbon is expected to alter the constant term in eq 19 but have little effect on the coefficients of σ^* or pK_a. Thus, for a fixed acyl group the Brønsted coefficient for the formation of the anionic tetrahedral intermediate (β_{eq}) for acyl transfer from alcohols, thiols, or amines to thiols is expected to be ~0.8. Hupe and Jencks[27] have shown that the β_{nuc} value for acyl transfer from esters and thiol esters to thiolates is 0.3 for reactions in which formation of the anionic tetrahedral intermediate is the rate-limiting step. This suggests that the thiolate has lost ~37% (= 0.3/0.8) of its negative charge in going from the ground state to the transition state. Similarly, a comparison of the ρ^* for the rate of nucleophilic attack of thiolates on acyl compounds with the equilibrium ρ^* value (2.97) should provide a useful index of the structural similarity between the transition state and the anionic tetrahedral intermediate.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We also thank Dr. Jack Kirsch and the referees for several valuable comments.

References and Notes

(1) E. Baumann, *Chem. Ber.*, **18**, 258–263 (1885).
(2) C. A. Lewis, Jr., and R. Wolfenden, *Biochemistry*, **16**, 4890–4895 (1977).
(3) P. I. Clark, G. Lowe, and D. Nurse, *J. Chem. Soc., Chem. Commun.*, 451–453 (1977).
(4) J. P. Guthrie, *J. Am. Chem. Soc.*, **95**, 6999–7003 (1973).
(5) J. P. Guthrie, *J. Am. Chem. Soc.*, **100**, 5892–5904 (1978).
(6) J. Fastrez, *J. Am. Chem. Soc.*, **99**, 7004–7013 (1977).
(7) G. E. Lienhard and W. P. Jencks, *J. Am. Chem. Soc.*, **88**, 3982–3995 (1966).
(8) R. E. Barnett and W. P. Jencks, *J. Am. Chem. Soc.*, **91**, 6758–6765 (1969).
(9) C. A. Lewis and R. Wolfenden, *Biochemistry*, **16**, 4886–4890 (1977).
(10) J. F. Walder, "Formaldehyde", Reinhold, New York, 1964, pp 486–487.
(11) G. Ellman, *Arch. Biochem. Biophys.*, **82**, 70–77 (1959).
(12) S. S. Hall, A. M. Doweyko, and F. Jordan, *J. Am. Chem. Soc.*, **100**, 5934–5939 (1978).
(13) W. P. Jencks and K. Salvensen, *J. Am. Chem. Soc.*, **93**, 4433–4436 (1971).
(14) D. L. Rabenstein, *J. Am. Chem. Soc.*, **95**, 2797–2803 (1973).
(15) P. Greenzaid, Z. Luz, and D. Samuel, *J. Am. Chem. Soc.*, **89**, 749–756 (1967).
(16) R. P. Bell, *Adv. Phys. Org. Chem.*, **4**, 1–29 (1966).
(17) P. Greenzaid, *J. Org. Chem.*, **38**, 3164–3167 (1973).
(18) D. J. Hopper and R. A. Copper, *Biochem. J.*, **128**, 321–328 (1972).
(19) D. R. Trentham, C. H. McMurray, and C. I. Pogson, *Biochem. J.*, **114**, 19–24 (1969).
(20) K. Kirschner, *J. Mol. Biol.*, **58**, 51–68 (1971).
(21) H. F. Gilbert and W. P. Jencks, *J. Am. Chem. Soc.*, **99**, 7931–7947 (1977).
(22) E. G. Sander and W. P. Jencks, *J. Am. Chem. Soc.*, **90**, 6154–6162 (1968).
(23) D. J. Hupe, D. Wu, and P. Shepperd, *J. Am. Chem. Soc.*, **99**, 7659–7662 (1977).
(24) A. R. Fersht, *J. Am. Chem. Soc.*, **93**, 3504–3515 (1971).
(25) S. Takahashi, L. A. Cohen, H. K. Miller, and E. G. Peake, *J. Org. Chem.*, **36**, 1205–1209 (1971).
(26) W-M. Ching and R. G. Kallen, *J. Am. Chem. Soc.*, **100**, 6119–6124 (1978).
(27) D. J. Hupe and W. P. Jencks, *J. Am. Chem. Soc.*, **99**, 451–464 (1977).

The Timing of the Proton Transfer Process in Acid-Catalyzed Carbonyl Addition. Evidence for a Preassociation Mechanism for Catalysis of Carbinolamine Formation from Acethydrazide and *p*-Chlorobenzaldehyde[1]

J. M. Sayer* and Carl Edman

Contribution from the Department of Chemistry, University of Vermont, Burlington, Vermont 05405. *Received September 27, 1978*

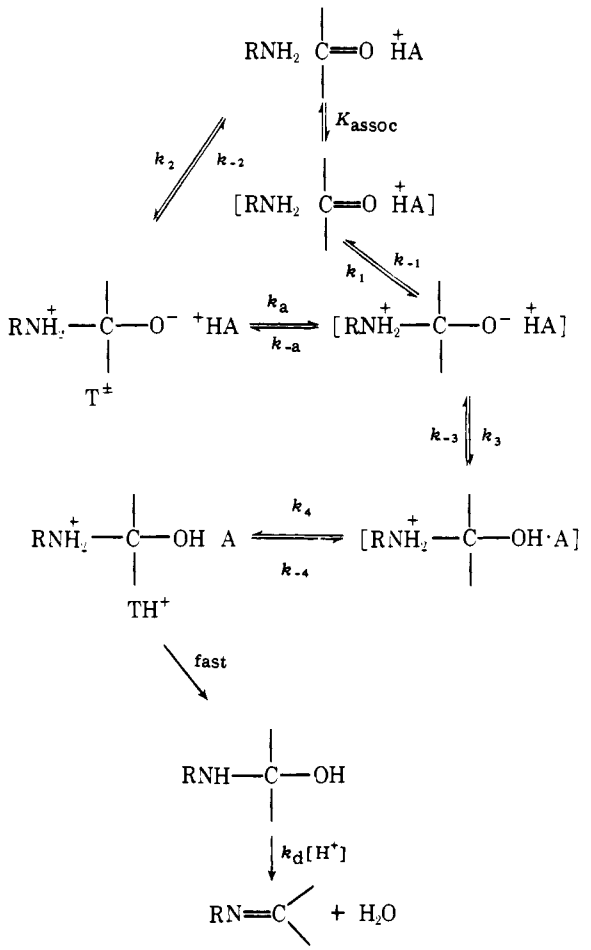
Abstract: General acid catalysis of carbinolamine formation from acethydrazide ($CH_3C(O)NHNH_2$) and *p*-chlorobenzaldehyde in aqueous solution probably occurs by a "preassociation" mechanism that involves rate-determining attack of the nucleophile on the aldehyde in the presence of the acid catalyst in a termolecular encounter complex, and rate-determining diffusion apart of the protonated carbinolamine and the conjugate base of the catalyst, in limiting cases of strongly and weakly acidic catalysts, respectively. Unlike amines of even slightly greater basicity, acethydrazide does not add to *p*-chlorobenzaldehyde by a mechanism involving a kinetically significant *free* zwitterionic carbinolamine ($T^{\pm}$). The data are most consistent with a mechanism in which there is a small amount of stabilization of the transition state for amine attack, and of the initial product of this attack, by hydrogen bonding of oxygen to strongly acidic catalysts. Evidence in support of the proposed mechanism includes (1) the absence in the pH–rate profile of a break at low pH corresponding to a change from rate-determining hydronium ion catalyzed protonation to uncatalyzed formation of $T^{\pm}$, (2) the absence of any detectable effect of increased solvent viscosity (50% aqueous glycerol) on the rate constants for catalysis by heterocyclic ammonium ions, and (3) a nonlinear Brønsted plot for general acid catalysis, with limiting slopes of ca. 0.11 for strongly acidic and ≥0.8 for weakly acidic catalysts. In contrast, the triazolium ion catalyzed reaction of methoxyamine (CH_3ONH_2) with *p*-chlorobenzaldehyde, which is known to involve rate-determining diffusion-controlled protonation of *free* $T^{\pm}$, is inhibited by a factor of approximately 12 in 50% aqueous glycerol.

The addition of weakly basic nitrogen nucleophiles to substituted benzaldehydes occurs in many cases by the initial formation of a highly unstable zwitterionic carbinolamine intermediate, $T^{\pm}$, that is trapped by rate-limiting diffusion-controlled proton transfer (k_a) from hydronium ion or a general acid catalyst at pH values greater than 1.0 (Scheme I, left-hand side).[2,3]

In order for a mechanism involving rate-determining protonation of free $T^{\pm}$ to be significant, $T^{\pm}$ must be sufficiently short lived that it reverts to starting materials faster than it

0002-7863/79/1501-3010$01.00/0

Scheme I



undergoes diffusion-controlled protonation (trapping) by hydronium ion or buffer acids; however, $T^{\pm}$ cannot be so unstable that it expels amine (k_{-1}) faster than the encounter complex ($T^{\pm}\cdot{}^{+}HA$) undergoes diffusional separation (k_{-a}). In the latter case, the lowest energy pathway from starting materials to ($T^{\pm}\cdot{}^{+}HA$) requires a "preassociation"[4] of amine, carbonyl compound, and catalyst (with or without hydrogen bonding to the catalyst) as shown on the right side (K_{assoc} and k_1) of Scheme I. An analogous preassociation mechanism has been suggested for the base-catalyzed addition of 2-methyl-3-thiosemicarbazide to *p*-chlorobenzaldehyde.[5]

A delicate balance exists between the relative energies of the two alternative pathways of Scheme I, and very small changes in the reactivity of the amine or the carbonyl compound are sufficient to cause a change in the detailed mechanism of the reaction.[3] We are interested in defining the effects of such small changes on the exact timing of the proton-transfer process in these reactions. Gilbert and Jencks[6] have shown that there is a variation in mechanism from simple trapping through preassociation mechanisms with increasing degrees of hydrogen bonding in the transition state, as thiol basicity is decreased, for general-acid-catalyzed addition of thiol anions to acetaldehyde. The present work provides further evidence for the same kind of mechanistic variation in general-acid-catalyzed carbinolamine formation, namely, the predominance of a preassociation mechanism for general-acid-catalyzed addition of acethydrazide ($CH_3C(O)NHNH_2$) to *p*-chlorobenzaldehyde. In contrast amines of only slightly greater basicity, such as methoxyamine, undergo general-acid-catalyzed addition predominantly by the trapping pathway.[2] Of particular interest is our demonstration that the reaction of acethydrazide is insensitive to a large variation in solvent viscosity effected by substitution of 50% glycerol for water as the solvent, whereas the corresponding reaction of methoxyamine, which involves rate-limiting diffusion-controlled trapping of $T^{\pm}$ by HA, is inhibited by a factor of approximately 12 in the more viscous solvent. Hence, the same diffusion process cannot be rate limiting for both reactions and the alternative, preassociation mechanism is indicated for catalysis of the reaction of acethydrazide.

Experimental Section

Materials. Organic reagents were recrystallized, sublimed, or distilled before use. Inorganic compounds were reagent grade and were used without further purification. Water was deionized and then glass distilled. *p*-Chlorobenzaldehyde acethydrazone was prepared from *p*-chlorobenzaldehyde (0.05 mol) and acethydrazide (0.045 mol) in 20 mL of ethanol containing 1 mL of glacial acetic acid, mp after recrystallization from ethanol–water, then ethanol, and drying in vacuo 151–153 °C.

5-Chloro-1-methylimidazole, 2,4-dimethylimidazole, and 2-methoxypropylamine were purified, stored, and used as the hydrochlorides, which were prepared from the commercially available free bases. 2,4,5-Trimethylimidazole hydrochloride was prepared by a modification of the method of von Pechmann[7] from equimolar quantities (0.3 mol) of diacetyl and acetaldehyde instead of from diacetyl alone. After several recrystallizations from ethanol the hydrochloride had mp 312–315 °C (lit.[7b] 316 °C); NMR (D_2O, DMPSA) δ 2.18 and 2.53 ppm (ratio 2:1).

Kinetics. Rates of acethydrazone formation and hydrolysis at 25 °C, ionic strength 1.0 M, were followed at 285 nm on a Cary 14, Perkin-Elmer 124, or Bausch and Lomb 710 spectrophotometer with recorder. The latter instrument was equipped with a Caltronics Linear Auto-Expander that was used for recorder scale expansion for reactions in which the total absorbance change was less than 0.2 unit. For hydrolysis experiments the concentration of acethydrazone in the final reaction mixtures was 5×10^{-5} M, introduced by addition of 50 μL of 3×10^{-3} M acethydrazone in 25% aqueous ethanol into 3.0 mL of aqueous reaction mixture.

For studies of catalysis of the forward reaction of acethydrazide with *p*-chlorobenzaldehyde a total acethydrazide concentration of $3\text{–}6 \times 10^{-3}$ M and a *p*-chlorobenzaldehyde concentration of 2×10^{-5} M were ordinarily used. The acethydrazide present in the reaction mixtures served as a buffer at pH values where the external catalysts used had inadequate buffering capacity; when necessary constancy of pH for a series of catalyst concentrations was maintained by adjusting the pH of the reaction mixtures, before initiation of reaction by aldehyde addition, with a small quantity of concentrated acid or base.

Second-order rate constants, k_{obsd}, for the reaction of acethydrazide free base were corrected[8] for partially rate-determining dehydration to give k_{ad}, the rate constant for the addition step, using eq 1 and a previously determined[9] value of $K_{ad}k_d$ of 3.88×10^4 M^{-2} s^{-1}

$$k_{ad} = k_{obsd}/(1 - k_{obsd}/K_{ad}k_d a_{H^+}) \qquad (1)$$

A single experiment in this laboratory at pH 6.47, 0.02–0.10 M phosphate buffer, gave $K_{ad}k_d = 3.7 \times 10^4$ M^{-2} s^{-1}, in agreement with the previously reported value.

For glycerol inhibition experiments stock solutions containing 83% glycerol by weight were prepared by mixing 42 g of glycerol with aqueous solutions of hydrochloric acid and 1.0 M acethydrazide or 1.07 M methoxyamine hydrochloride plus sufficient water to give a total volume of water of 8.3 mL. Reaction mixtures were prepared by making appropriate mixtures of either (A) 2.5 M aqueous triazole buffer, 50% hydrochloride, containing sufficient tetramethylammonium chloride to give an ionic strength of 2.5 M or (B) 2.5 M 5-chloro-1-methylimidazolium hydrochloride with (C) 2.5 M tetramethylammonium chloride, and mixing 2.0 mL of the resulting aqueous solution with 3.0 mL of the glycerol-containing stock solution. Since it was shown that no measurable volume change occurred on mixing, the resultant solutions contained 50% glycerol solvent and had an ionic strength of 1.0 M. After equilibration to temperature 3.0 mL of each reaction mixture was placed in a cuvette and reaction was initiated by addition with vigorous mixing of 30 μL of an acetonitrile solution of *p*-chlorobenzaldehyde. Final concentrations of *p*-chlorobenzaldehyde in the reaction mixtures were $2\text{–}4 \times 10^{-5}$ and 6×10^{-5} M for experiments using acethydrazide and methoxyamine as the nucleophile, respectively. Similar procedures were used for experi-

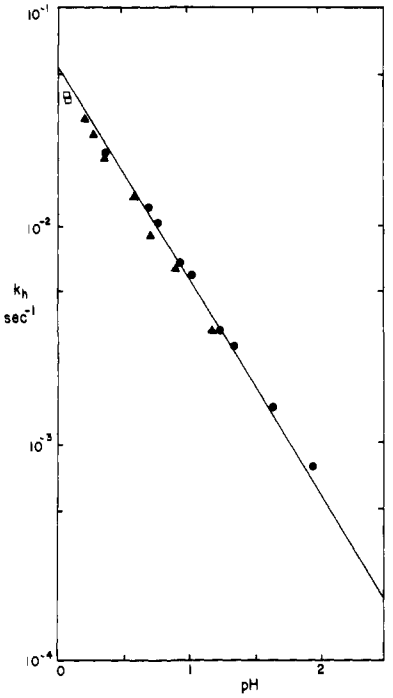


Figure 1. Observed rate constants, k_h, for hydrolysis of *p*-chlorobenzaldehyde acethydrazone at ionic strength 1.0 M, maintained with potassium chloride (●), lithium chloride (▲), or 1.0 M HCl (□), as a function of pH. The solid line of slope −1.0 is a theoretical line based on published values of the rate and equilibrium constants for acethydrazone formation (ref 3 and 9).

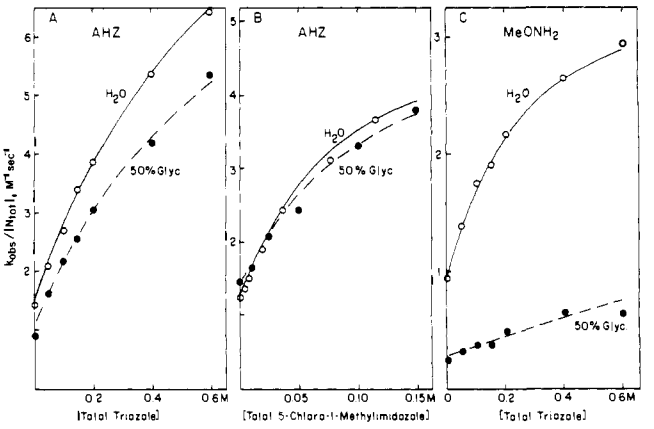


Figure 2. Effect of 50% aqueous glycerol solvent on catalysis by heterocyclic ammonium ions of acethydrazone (plots A and B) or *O*-methyloxime (plot C) formation from *p*-chlorobenzaldehyde at ionic strength 1.0 M (Me_4NCl). The quantity shown on the ordinate is an apparent second-order rate constant in terms of *total* acethydrazide or methoxyamine concentration. Apparent pH values were as follows: plot A (H_2O), 2.39; (50% glycerol), 2.43; plot B (H_2O), 3.56; (50% glycerol), 3.58; plot C (H_2O), 2.65; (50% glycerol), 2.59. The lines are theoretical curves based on the rate and equilibrium constants of Table II.

ments in phosphate buffer to determine the apparent rate constants, $K_{ad}k_d$, for the dehydration of the adducts in the presence of glycerol, except that in one case tetramethylammonium chloride was added to give an ionic strength of 1.65 M in the glycerol-containing stock solution. To 3.0 mL of this solution was added 0.1 M phosphate buffer, 50% dianion, ionic strength 0.15, and 0.15 M tetramethylammonium chloride to give a total volume of 5.0 mL and an ionic strength of 1.06. The rate constant for dehydration of the carbinolamine adduct of methoxyamine and *p*-chlorobenzaldehyde at ionic strength 1.0 (Me_4NCl) was determined from the ordinate intercept of a plot of $1/(k_{obsd} - k_0)$ against 1/[triazole] at pH 2.65 and agreed well with the value calculated from rate constants measured with potassium chloride as the compensating electrolyte.

For determinations of rate constants for catalysis in 50% glycerol and in aqueous tetramethylammonium chloride solutions, amine free base concentrations were calculated from pK_a values measured in 50% glycerol (see below) or aqueous trimethylammonium chloride, respectively, and apparent pH values of the reaction solutions measured with a glass electrode. Values of k_{ad} were determined from eq 1, again using the apparent pH and values of $K_{ad}k_d$ determined in the appropriate solvent-electrolyte system. Concentrations of the conjugate acid of the catalyst were calculated where necessary from the apparent pH and pK_a values determined in the solvent of interest. Since it was frequently necessary to adjust pH prior to a set of kinetic runs, using small amounts of acid or base, to assure constancy of pH for a series of catalyst concentrations when buffering capacity of the catalysts and/or acethydrazide was poor, acid/base ratios of the acid catalysts were calculated from the adjusted pH, rather than from the initial stoichiometry of the buffer solutions.

For pK_a determinations in 50% glycerol, compounds were dissolved in aqueous glycerol containing tetramethylammonium chloride, and titrations with aqueous KOH or HCl were carried out so that the mixtures being titrated had an ionic strength of 1.0 and contained 50% (by weight) glycerol at the *midpoint;* the volume of titrant added at the end point of titrations never exceeded 10% of the total of solution and generally was approximately 5% of the total volume. Apparent pH values were measured using a glass electrode standardized against aqueous buffer solutions.

Results

Previous work[3] has shown that the pH–rate profile for *p*-chlorobenzaldehyde acethydrazone formation exhibits a single break, between pH 3 and 4, indicative of the expected transition from rate-determining carbinolamine formation to dehydration as the pH is increased. No other break is observed above pH 1.5. The possibility that a second break,[2,3] indicative of a stepwise mechanism for proton transfer in carbinolamine formation, occurs at lower pH was most conveniently investigated by study of the *reverse* reaction, hydrolysis of the acethydrazone, since protonation of acethydrazide makes the equilibrium for acethydrazone formation unfavorable at low pH. Figure 1 shows the pH–rate profile for hydrolysis of the acethydrazone at pH values between 0 and 2.0. The solid line is calculated from published values of the rate constant for the forward reaction[3] and the equilibrium constant[9] measured under experimental conditions identical with those of this study, and is in good agreement with the data obtained here for the reverse reaction. The rate is clearly first order in hydronium ion throughout the pH range observed, and there is no break in the plot. In order to exclude the possibility that a small break is obscured by a salt effect from the substitution of hydrochloric acid for potassium chloride as the major electrolyte in solution at low pH values, lithium chloride was used instead of potassium chloride as the compensating electrolyte in one experiment. Under these conditions the pH–rate profile also exhibits no break. The apparent activity coefficient for hydronium ion (based on the measured pH) in this experiment varied from 0.81 (1.0 M HCl) to 1.3 (0.05 M HCl), but a plot of log k_h against the *measured* pH had a slope of −1.0 and was in good agreement with the pH–rate profile in the presence of potassium chloride. A plot of log k_h against −log c_{H^+} gave a slope of −0.88 and no apparent break.

Carbinolamine formation from *p*-chlorobenzaldehyde and acethydrazide is subject to general acid catalysis by buffers and other species according to a rate law $k_{ad} = k_{ad}^0 + k_{ad}^C$·[buffer], where k_{ad}^0, determined from the *y* intercepts of plots of k_{ad} against total buffer concentration, is an apparent rate constant for the addition step at zero buffer concentration, and k_{ad}^C, from the slopes of these plots, is the apparent rate constant for catalysis of the addition step by *total* buffer. This rate law is equivalent to $k_{ad} = k_{ad}^0 + k_{HA}[HA^+]$, where k_{HA} is the rate constant for catalysis by the conjugate acid and is given

Table I. General Acid Catalysis of Carbinolamine Formation from Acethydrazide and *p*-Chlorobenzaldehyde at 25 °C, Ionic Strength 1.0 M (KCl)

	catalyst conjugate acid of	pK_a	pH	concn range, M (total)	k_{ad}^C,[a] $M^{-2}\ s^{-1}$	k_{HA} (av),[b] $M^{-2}\ s^{-1}$
(1)	1,2,4-triazole	2.58[c]	2.42	0.05–0.50	115	
			3.06	0.05–0.50	56	210
(2)	acethydrazide	3.44[d]	3.25	0.004–0.012	182	
			3.38	0.002–0.015	98	250 ± 50
(3)	5-chloro-1-methylimidazole	5.40[e]	3.59	0.005–0.10	105	105
(4)	histamine	6.45[f,g]	2.80	0.02–0.15	113	113
(5)	imidazole	7.24[h]	2.32	0.05–0.25	110	
			2.48	0.05–0.25	99	
			2.55	0.025–0.60	63	
			3.41	0.05–0.25	66	
			3.48	0.05–0.50	61	80 ± 20
(6)	1-methylimidazole	7.20[i]	2.52	0.05–0.25	56	
			3.42	0.05–0.50	34	45 ± 10
(7)	glycinamide	8.30[g]	3.40	0.05–0.40	14	14
(8)	1,2-dimethylimidazole	8.35[e]	2.37	0.05–0.40	11	11
(9)	2,4-dimethylimidazole	8.76[g]	3.57	0.05–0.40	5.9	5.9
(10)	2,4,5-trimethylimidazole	9.41[g]	3.57	0.05–0.40	≤1.5	≤1.5
(11)	2-methoxyethylamine	9.72[j]	3.54	0.10–0.50	1.0 ± 0.6	1.0 ± 0.6
(12)	2-methoxypropylamine	10.46[k]	3.47	0.10–0.50	≤1.8	≤1.8

[a] In terms of total catalyst concentration. [b] In terms of the conjugate acid. [c] J. P. Fox and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 1436 (1974). [d] Reference 9. [e] A. C. Satterthwait and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 7031 (1974). [f] For the imidazole group. [g] Determined by titration (this work) at ionic strength 1.0 M (KCl). [h] Reference 8. [i] In 1.0 M tetramethylammonium chloride: D. G. Oakenfull and W. P. Jencks, *J. Am. Chem. Soc.*, **93**, 178 (1971). [j] W. P. Jencks and M. Gilchrist, *ibid.*, **90**, 2622 (1968). [k] Reference 2.

Table II. Effect of 50% Glycerol Solvent on Kinetic Constants for General Acid Catalysis of Adduct Formation from Nucleophiles and *p*-Chlorobenzaldehyde[a]

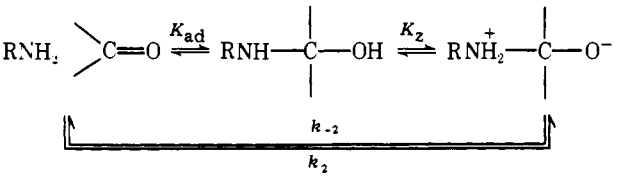
	50% glycerol	H_2O
	Acethydrazide	
pK_a	3.50	3.38
$K_{ad}k_d$, $M^{-2}\ s^{-1}$	3.7×10^4	3.5×10^4
k_{TrzH^+}, $M^{-2}\ s^{-1}$ [b-d]	400	390
k_{CMIH^+}, $M^{-2}\ s^{-1}$ [e]	130	120
	Methoxyamine	
pK_a	4.85	4.69
$K_{ad}k_d$, $M^{-2}\ s^{-1}$	2.82×10^5	1.8×10^5
k_{TrzH^+}, $M^{-2}\ s^{-1}$ [b-d]	5×10^2	6.1×10^3

[a] Ionic strength 1.0 M (Me_4NCl). [b] For general acid catalysis by 1,2,4-triazolium ion. [c] pK_a of triazolium ion in 50% glycerol, 2.36. [d] pK_a of triazolium ion in aqueous Me_4NCl, 2.43. [e] For general acid catalysis by 5-chloro-1-methylimidazolium ion.

by $k_{HA} = k_{ad}^C/f$ where f = the fraction of buffer present as the conjugate acid. Observed rate constants, k_{obsd}, increase nonlinearly with increasing buffer concentration because of the intervention of the dehydration step at high buffer concentration; rate constants (k_{ad}) for *carbinolamine formation* were calculated using eq 1. Rate constants for general acid catalysis by a series of primary and heterocyclic ammonium ions are summarized in Table I.

Rates of carbinolamine formation catalyzed by triazolium and 5-chloro-1-methylimidazolium ions were determined in water and in 50% aqueous glycerol at ionic strength 1.0 M maintained with tetramethylammonium chloride. Values of pK_a and of the dehydration rate constant, $K_{ad}k_d$, determined in aqueous tetramethylammonium chloride or 50% aqueous glycerol containing tetramethylammonium chloride were used for calculation of the catalytic constants for the addition step. Observed catalytic constants for the reaction of acethydrazide are remarkably insensitive to 50% glycerol (Figures 2A and 2B), whereas catalysis of methoxyamine addition by triazolium ion is greatly decreased in the glycerol-containing solvent (Figure 2C). Kinetic constants for the reactions of acethydrazide and of methoxyamine in 50% glycerol and in aqueous tetramethylammonium chloride are summarized in Table II.

Scheme II



Discussion

It appears well established[2,3] that general acid catalysis of addition reactions of moderately basic nitrogen nucleophiles to reactive carbonyl compounds generally occurs by a mechanism that involves formation of a zwitterionic intermediate, $T^{\pm}$ (Scheme I), which is then trapped by a kinetically significant proton-transfer process involving protonation of the oxygen of $T^{\pm}$ by general acid catalysts or the hydronium ion. Alternative mechanisms for catalysis should become significant as the stability and lifetime of $T^{\pm}$ are decreased, for example, by decreasing the basicity of the nucleophile. These include preassociation[10] and "concerted" mechanisms as well as protonation of the carbonyl compound in a prior equilibrium step giving rise to *specific* acid catalysis.

Our investigation of the detailed mechanism of acid-catalyzed carbinolamine formation from acethydrazide and *p*-chlorobenzaldehyde was prompted by the substantial positive deviation of the rate constant, k_H, for hydronium ion catalyzed addition of this nucleophile from a structure-reactivity correlation for other nucleophiles.[3] For the "trapping" mechanism shown on the left-hand side of Scheme I, k_a, a diffusion process is ordinarily rate determining for strongly acidic catalysts, and the observed rate constant for the reaction is $k_{HA} = k_a k_2/k_{-2}$. The effect of structure on k_{HA} is predictable in a straightforward manner since k_a is diffusion controlled and independent of the nucleophile, and the equilibrium constant k_2/k_{-2} is equal to $K_{ad}K_z$ where K_{ad} and K_z are the equilibrium constants defined in Scheme II. K_{ad} is an experimentally measured quantity and K_z should follow a relationship[3] $\Delta \log K_z = 0.8\Delta pK_{a_{nuc}}$. Hence, given the value of $k_{HA} = k_a k_2/k_{-2}$ for any nucleophile

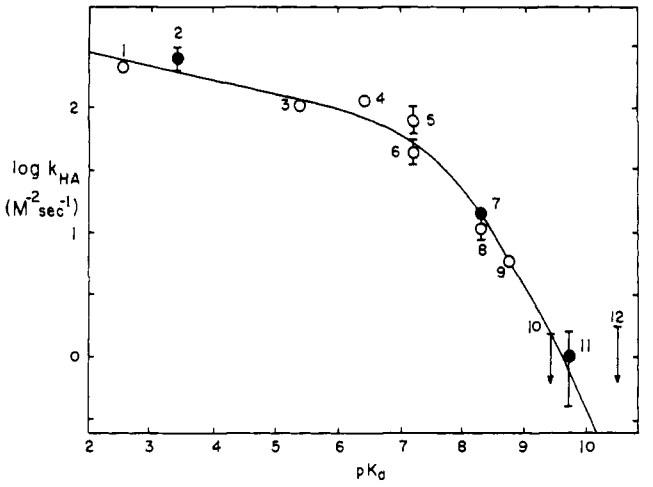


Figure 3. Brønsted plot for general acid catalysis of carbinolamine formation from acethydrazide and *p*-chlorobenzaldehyde by primary (●) and heterocyclic tertiary (○) ammonium ions. The line is a theoretical curve for a preassociation mechanism based on eq 4 and the individual rate and equilibrium constants (Table III) for the processes of Scheme I estimated as described in the text.

it is possible to predict quite accurately k_{HA} for a structurally similar nucleophile from the equation

$$\Delta \log k_{HA} = \Delta \log K_{ad} + 0.8\Delta pK_{a_{nuc}} \quad (2)$$

Calculation of log k_H for hydronium ion catalysis of the reaction of acethydrazide (pK_a = 3.44, K_{ad} = 0.7 M^{-1}) with *p*-chlorobenzaldehyde, via a diffusion-controlled trapping mechanism, from eq 2 and the appropriate rate and equilibrium constants for methoxyamine[2] (pK_a = 4.73, K_{ad} = 13.4 M^{-1}) gives $\Delta \log k_H = -2.3$ and k_H^{calcd} = 200 M^{-2} s^{-1}; the experimental value of this rate constant for acethydrazide is 3000 M^{-2} s^{-1}, suggestive of an alternative, faster mechanism for this reaction that does not involve a diffusion-controlled reaction of *free* T±. A similar calculation for catalysis by triazolium ion (in water, ionic strength 1.0 maintained with tetramethylammonium chloride; Table II) gives a calculated value of k_{TrzH^+} = 30 M^{-2} s^{-1} for the diffusion-controlled trapping mechanism for acethydrazide based on the observed value of 6100 M^{-2} s^{-1} for methoxyamine; the experimental value of 390 M^{-2} s^{-1} for acethydrazide is again slightly more than an order of magnitude larger than predicted.

We have obtained the following evidence that supports a preassociation mechanism (right-hand side of Scheme I) for acid catalysis of carbinolamine formation from acethydrazide and *p*-chlorobenzaldehyde. (1) There is no break in the pH–rate profile for the reverse of this reaction between pH 0 and 2. This means that, for *hydronium ion* catalysis of carbinolamine formation and its reverse, there is no change with decreasing pH from a rate-determining transition state that contains a hydronium ion to one that does not; for the trapping mechanism, such a change, from rate-determining encounter with the acid catalyst (k_a) to rate-determining attack and loss of the nucleophile (k_2 and k_{-2}), occurs as the pH is decreased. (2) An increase in solvent viscosity (50% aqueous glycerol) has no effect on the rate constants for catalysis of the reaction of acethydrazide by heterocyclic tertiary ammonium ions, whereas this solvent has a large (approximately 12-fold) retarding effect on the corresponding rate constant for the reaction of methoxyamine. (3) The Brønsted plot for general acid catalysis of carbinolamine formation from acethydrazide and *p*-chlorobenzaldehyde is nonlinear with limiting slopes, α, of 0.11 and ≥0.8 for strong and weak acids, respectively. This is consistent with a preassociation mechanism in which some stabilization of the transition state for amine attack is provided by hydrogen bonding to strongly acidic catalysts.

(1) Addition reactions of nitrogen nucleophiles to carbonyl compounds can exhibit one or two negative breaks[2,3] in their pH–rate profiles at pH values below neutrality corresponding to (a) a transition from hydronium ion catalyzed rate-determining carbinolamine dehydration to uncatalyzed (or water-catalyzed) conversion of T± to a neutral carbinolamine at pH ca. 4–6, and (b) a transition from diffusion-controlled protonation of T± (for the "trapping" mechanism of Scheme I) to uncatalyzed attack (k_2) of the nucleophile, at pH ca. 1–2. Previous results had shown that the reaction of acethydrazide with *p*-chlorobenzaldehyde exhibits a single break in the pH 5 region corresponding to (a) and no other break corresponding to (b) at pH values ≥1.5. Investigation of the pH dependence of this reaction was extended to pH ca. 0 in this work by following the rate of hydronium ion catalyzed acethydrazone hydrolysis. This reaction is strictly first order in hydronium ion between pH 0 and 2 as indicated by a pH–rate profile (Figure 1) with unit slope and no break at low pH. Calculation of the rate constants for acethydrazone formation (k_f) at pH values between 0 and 2 from experimental values of k_h and K_e = 5.5 × 10^4 M^{-1}, using the relationship $k_f = K_e k_h$, gave values in good agreement with a line of unit slope drawn through values of k_f determined previously for the reaction in the forward direction[3] between pH 1.4 and 2.2. We hence conclude that there is no experimental evidence for a second change in rate-determining step for acethydrazone formation, analogous to the transitions from rate-determining $k_a[H_3O^+]$ to k_2 at low pH that are observed for reactions of several other nucleophiles with aromatic aldehydes. For a preassociation mechanism the transition states for the kinetically significant steps k_1, k_3, and k_4 all contain the elements of amine, aldehyde, and acid catalyst, and hence no change in rate-determining step is possible as the concentration of the acid catalyst (in this case, hydronium ion) is varied. This absence of a change in rate-determining step for carbinolamine formation as a function of pH is a necessary, although not a sufficient, criterion for assignment of a preassociation mechanism for hydronium ion catalysis.

(2) The major piece of evidence that establishes the fundamental difference between the mechanism of carbinolamine formation from methoxyamine (which proceeds unambiguously by the stepwise trapping mechanism shown on the left of Scheme I) and acethydrazide is the effect of 50% glycerol solvent[11] on the rates of these two reactions catalyzed by heterocyclic ammonium ions. Rate constants for catalysis of carbinolamine formation from acethydrazide by triazolium and 5-chloro-1-methylimidazolium ions in water are identical, within experimental uncertainty, with those measured in 50% aqueous glycerol, a medium whose viscosity is approximately six times greater than that of pure water. In contrast, diffusion-controlled protonation by triazolium ion of the intermediate formed from methoxyamine and *p*-chlorobenzaldehyde is approximately 12-fold slower (Table II) in 50% glycerol than in aqueous solution. This is a larger retardation than expected from the relationship $k^{H_2O}/k^{glyc} = \eta_{glyc}/\eta_{H_2O}$ for diffusion[11] if η_{glyc}/η_{H_2O} is taken as 6.03[12] (for aqueous glycerol solutions containing no added salts); the effect of tetramethylammonium chloride on the viscosity of these solutions was not determined. This uncertainty does not affect the significant conclusion that there is a large *difference* in the effect of glycerol solvent on these two very similar reactions. This difference provides strong evidence that the reaction of acethydrazide, unlike that of methoxyamine, does not involve a rate-determining diffusion process, since it appears unreasonable that ordinary medium effects for these two similar reactions could differ by a factor of 12. For the reaction of acethydrazide, the absence of *any* detectable retardation in glycerol argues against "mixed" mechanisms in which preassociation and trapping pathways are of nearly equal energy,[6] and both contribute significantly

Table III. Estimated Values for Individual Rate and Equilibrium Constants of Scheme I as a Function of Catalyst pK_a

	pK_{HA} 2	4	6	8	10	α^a
$\log K_{assoc}k_1$	2.45	2.23	2.00	1.79	1.57	0.11
$\log k_{-1}$	←		10.34		→	0.0
$\log k_a$	←		9.7		→	0.0
$\log k_{-a}$	9.49	9.71	9.93	10.15	10.36	0.11
$\log k_3$	13.40	12.63	11.85	11.07	10.3	0.39
$\log K_3$	4.49	2.71	0.93	−0.85	−2.64	0.89
$\log k_4$	←		11.0		→	0.0

[a] $\alpha = -d(\log k)/d(pK_{HA})$ or $-d(\log K)/d(pK_{HA})$.

to the rate, for the acids studied. The Brønsted plot for general acid catalysis of the reaction in water (see below) can be fit by a theoretical curve using kinetic constants that correspond to relative rates for the preassociation and "trapping" mechanisms of ca. 6 and 3 for 1,2,4-triazolium and 5-chloro-1-methylimidazolium ions, respectively. The result of this calculation is thus consistent with, although it does not prove, the conclusion from the viscosity data that catalysis by these cations proceeds predominantly through a preassociation mechanism.

(3) **General Acid Catalysis.** The Brønsted plot for general acid catalysis of carbinolamine formation from acethydrazide (Figure 3) is nonlinear, with limiting slopes of ca. 0.11 for strong acids and ≥0.80 for weak acids. We believe that this is most consistent with a preassociation mechanism. In the limiting cases, this mechanism involves rate-determining attack of acethydrazide within a termolecular encounter complex of aldehyde, amine, and acid, with strongly acidic catalysts, and rate-determining diffusional separation of the encounter complex $[TH^+ \cdot A]$, with weakly acidic catalysts. Near the break in the Brønsted plot, a contribution of the proton-transfer process itself (k_3) may become kinetically significant.[13] The slope of 0.11 for strongly acidic catalysts suggests that some stabilization of the transition state for amine attack, and of the initial complex $[T^{\pm} \cdot HA^+]$, is provided by hydrogen bonding to the catalyst. The hydronium ion ($k_H = 3000\ M^{-1}\ s^{-1}$) shows an approximately fourfold positive deviation from the Brønsted plot of slope 0.11. Use of a statistical correction[14] ($p = 3, q = 1$) reduces this deviation to about twofold. This small deviation perhaps results from a slightly enhanced hydrogen-bonding capability of the hydronium ion, or from the ability of this species to hydrogen bond to the transition state through an intermediate water molecule.

An important consequence of hydrogen bonding in the transition state is that the added stabilization of $[T^{\pm} \cdot HA^+]$ makes its diffusional separation (k_{-a}) slow enough so that breakdown of $T^{\pm}$ within the complex (k_{-1}) is fast relative to k_{-a}. This is a necessary condition for a preassociation mechanism of catalysis. We believe that a hydrogen-bonding mechanism is probably most consistent with a reasonable estimate for the lifetime of $T^{\pm}$. If the reaction with acethydrazide involves a preassociation mechanism with *no* significant hydrogen bonding, then k_{-a} must be large (ca. $10^{11}\ s^{-1}$) and the requirement that k_{-1} be greater than k_{-a} means that k_{-1} for expulsion of acethydrazide must be about three orders of magnitude greater than the rate constant of $2.9 \times 10^8\ s^{-1}$ estimated for expulsion of methoxyamine from the corresponding adduct $T^{\pm}$. Since the equilibrium constant (k_{-2}/k_2) for breakdown of $T^{\pm}$ (Scheme II and eq 2) is predicted to be only 200 times greater for acethydrazide than for methoxyamine, it is unreasonable that the *rate* for breakdown of $T^{\pm}$ within the complex (k_{-1}) would be any more than 200 times larger for the weaker nucleophile. If, however, k_{-a} is decreased by hydrogen bonding, a preassociation mechanism becomes possible even if k_{-1} is 10^9–$10^{10}\ s^{-1}$, consistent with a somewhat longer lifetime for the zwitterionic acethydrazide adduct.

The Brønsted plot can be fit by a theoretical line for a preassociation mechanism with hydrogen bonding, calculated from eq 3 and estimated rate and equilibrium constants (Table III and Appendix) for the processes defined in Scheme I.

$$k_{HA} = K_{assoc}k_1K_3k_3k_4/(k_{-1}k_3 + K_3k_{-1}k_4 + K_3k_3k_4) \quad (3)$$

$$K_3 = k_3/k_{-3}$$

Although the parameters chosen to fit the experimental data are not unique, a line calculated from them gives a good fit to the experimental data of Figure 3, and absolute and relative values of the individual constants are reasonable. Stabilization of $[T^{\pm} \cdot HA^+]$ by hydrogen bonding causes k_{-a} to decrease, and the ratio k_{-1}/k_{-a}, which describes the partitioning of $[T^{\pm} \cdot HA^+]$ between the preassociation and trapping pathways, to increase, as the acidity of HA^+ is increased. Values of k_{-1}/k_{-a} significantly larger than unity for strong acids are consistent with our observation that 50% aqueous glycerol solvent has no detectable effect on k_{HA} for acids of $pK_a \leq 5.4$ and hence, with these acids, no experimentally significant fraction of the reaction is presumed to occur via the trapping pathway. According to Table III, k_{-1}/k_{-a} should be about 3 for 5-chloro-1-methylimidazolium ($pK_{HA} = 5.4$) and 6 for 1,2,4-triazolium ion ($pK_{HA} = 2.58$), and the trapping pathway, which is suppressed in 50% glycerol, should contribute, at most, only 25% to the observed rates. A variation in k_{HA} of 25% or less in 50% glycerol and water could conceivably be undetectable because of small compensating solvent effects or uncertainties in individual rate and equilibrium constants used for calculation of k_{HA}.

The value of $k_{-1} = 2.2 \times 10^{10}\ s^{-1}$ for expulsion of acethydrazide from $T^{\pm}$ within an encounter complex is two orders of magnitude larger than the estimated rate constant of $2.9 \times 10^8\ s^{-1}$ for breakdown of $T^{\pm}$ derived from *p*-chlorobenzaldehyde and methoxyamine.[2] This intermediate is expected to be less stable for acethydrazide both because of its lower basicity ($\Delta pK_a = 1.29$) and its less favorable equilibrium for neutral adduct formation ($\Delta \log K_{ad} = 1.3$). Because of the assumptions involved in its estimation, the value of k_{-1} is only approximate, but its magnitude suggests that this rate constant must be quite sensitive to the nature of the nucleophile. This provides the rationale for the observed change in the mechanism of catalysis by strong acids from a trapping mechanism for methoxyamine addition, for which $k_{-1} < k_{-a}$, to a preassociation mechanism for acethydrazide, for which $k_{-1} > k_{-a}$.

Although the present results indicate unambiguously that catalysis by strong acids of carbinolamine formation from acethydrazide and *p*-chlorobenzaldehyde does *not* involve kinetically significant trapping of free $T^{\pm}$, they do not enable a clear distinction to be made between a preassociation mechanism in which the hydrogen-bonded complex $[T^{\pm} \cdot HA^+]$ is a discrete intermediate and a "concerted" mechanism in which $[T^{\pm} \cdot HA^+]$ has no finite lifetime and the proton transfer occurs simultaneously with formation of the C–N bond. The

magnitude of calculated values for k_3 (based on a constant α value over a range of ΔpK from -2 to 6) suggests that, for strong acids, $[T^{\pm}\cdot HA^+]$ might be too unstable to exist ($k_3 >$ molecular vibrations). In this case the requirement for a concerted mechanism results not from the instability of $T^{\pm}$ with respect to amine expulsion but from the instability of the *complex* $[T^{\pm}\cdot HA^+]$ with respect to a proton jump. For weaker acids a concerted proton transfer is not required by the magnitude of k_3, and isotope effect data for a related reaction[13b] (the attack of methoxyamine on phenyl acetate) are most consistent with a discrete proton-transfer step within the encounter complex of $T^{\pm}$ and acid catalyst.

Acknowledgment. We thank Mr. Patrick Conlon for determination of pK_a values in aqueous tetramethylammonium chloride and 50% glycerol.

Appendix

Estimation of Rate Constants for the Individual Steps of Scheme I as a Function of pK_a of Acid Catalysts. The rate constants k_4 and k_{-4} of Scheme I were assumed to be invariant with pK_{HA}, i.e., there is no stabilization of $[TH^+\cdot A]$ by hydrogen bonding. The rate constant for diffusion together of $T^{\pm}$ and ^+HA was also assumed to be independent of pK_{HA}; this is consistent with results for the reaction of $T^{\pm}$ derived from methoxyamine and *p*-methoxybenzaldehyde,[2] and with limiting slopes of zero for Eigen plots for some simple proton-transfer reactions.[15] The value of log k_a was taken as 9.7 and log k_{-4} as 9.5. The difference corresponds to a slight difference in limiting rates for proton transfer between neutral and oppositely charged donor and acceptor molecules.[2,15] The value of log k_4 was taken as 11. Log k_{-1} was also assumed to be independent of pK_{HA}; this is equivalent to the assumption that the extents of stabilization by hydrogen bonding of $[T^{\pm}\cdot HA^+]$ at equilibrium and of the transition state are equal and is not unreasonable for a highly unstable intermediate.

We assume that pK-dependent hydrogen bonding of $T^{\pm}$ and HA^+ affects k_1, k_{-a}, k_3, and K_3, if such hydrogen bonding stabilizes the product $[T^{\pm}\cdot HA^+]$. The stabilization should be reflected in a negative α value for k_{-a}; i.e., as HA^+ becomes a stronger acid, stabilization of the complex is increased and k_{-a} decreases.[6] The experimental value of $\alpha = 0.11$ for $K_{assoc}k_1$ for strongly acidic catalysts and the assumed α value of zero for k_{-1} give an equilibrium α value of 0.11 for formation of $[T^{\pm}\cdot HA^+]$ from isolated reactants. This means that α for the equilibrium k_a/k_{-a} must also be 0.11, and, since α for k_a was taken as zero, α (i.e. $-d(\log k)/dpK_{HA}$) for k_{-a} must be -0.11. When HA is water, k_{-a} should be the same as for an encounter pair that is not stabilized, relative to isolated reactants in solution, by hydrogen bonding,[6] and log k_{-a} is set equal to 11 for water. The effect of decreasing pK_{HA} on k_{-a} is then described by the equation $\log k_{-a} = 11 - \{(15.74 - pK_{HA})\cdot(0.11)\}$.

A further consequence of hydrogen bonding is that the proton is in effect already "partly transferred" in the complex $[T^{\pm}\cdot HA^+]$ and therefore α for the equilibrium constant (K_3) for transfer of the proton *within the complex* to give $[TH^+\cdot A]$ should be less than unity. The *overall* equilibrium from free $T^{\pm}$ and HA^+ to TH^+ and A has a macroscopic equilibrium constant $K_{ov} = K_{HA}/K_{TH^+}$ and must have an α value of 1.0. The equilibrium constant $K_3 = k_3/k_{-3}$ is related to K_{ov} by the equation

$$K_3 = K_{ov}k_{-a}k_{-4}/k_ak_4 \qquad (4)$$

Values of log K_3 were calculated for $\log K_{ov} = pK_{TH^+} - pK_{AH}$ using $pK_{TH^+} = 8.2$,[16] estimated values of k_{-4}, k_a, and k_4 (see above), and $\log k_{-a} = 11 - \{(15.74 - pK_{HA})(0.11)\}$. Resultant values of log K_3 have $\alpha = 0.89$ as a consequence of the variation of log k_{-a} with pK_{HA}. If α for k_{-3} is taken as -0.5, α for k_3 must be 0.39, and log k_3 is taken as $11 + (0.39)(pK_{TH^+} - pK_{HA})$. The difference in α values in the forward and reverse directions results from the fact that α for the equilibrium $\neq 1$.

The rate constant $K_{assoc}k_1$ was determined from the experimental relationship $\log K_{assoc}k_1 = 2.67 - 0.11pK_{HA}$ observed for strongly acidic catalysts, and log k_{-1} was estimated to be 10.34 from the limiting experimental line for weakly acidic catalysts where $k_{HA} = K_{assoc}k_1K_3k_4/k_{-1}$, using the calculated or estimated values for $K_{assoc}k_1$, k_4, and K_3.

References and Notes

(1) Supported by a grant from the National Institute of General Medical Sciences of the National Institutes of Health (GM 22938). A portion of this work was presented at the Fifth Pharmacology-Toxicology Program Symposium of the National Institute of General Medical Sciences, Washington, D.C., Nov 1977.
(2) S. Rosenberg, S. M. Silver, J. M. Sayer, and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 7986 (1974).
(3) J. M. Sayer, B. Pinsky, A. Schonbrunn, and W. Washtien, *J. Am. Chem. Soc.*, **96**, 7998 (1974).
(4) W. P. Jencks and K. Salvesen, *J. Am. Chem. Soc.*, **93**, 1419 (1971); L. D. Kershner and R. L. Schowen, *ibid.*, **93**, 2014 (1971); M. I. Page and W. P. Jencks, *ibid.*, **94**, 8828 (1972).
(5) J. M. Sayer and W. P. Jencks, *J. Am. Chem. Soc.*, **95**, 5637 (1973).
(6) H. F. Gilbert and W. P. Jencks, *J. Am. Chem. Soc.*, **99**, 7931 (1977).
(7) (a) H. von Pechmann, *Ber.*, **21**, 1411 (1888); (b) R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, **115**, 217 (1919).
(8) J. M. Sayer and W. P. Jencks, *J. Am. Chem. Soc.*, **91**, 6353 (1969).
(9) J. M. Sayer, M. Peskin, and W. P. Jencks, *J. Am. Chem. Soc.*, **95**, 4277 (1973).
(10) W. R. Abrams and R. G. Kallen, *J. Am. Chem. Soc.*, **98**, 7777 (1976).
(11) C. Cerjan and R. E. Barnett, *J. Phys. Chem.*, **76**, 1192 (1972).
(12) "Handbook of Chemistry and Physics", 59th ed., CRC Press, Cleveland, Ohio, 1978, p D-278.
(13) (a) N.-Å. Bergman, Y. Chiang, and A. J. Kresge, *J. Am. Chem. Soc.*, **100**, 5954 (1978); (b) M. M. Cox and W. P. Jencks, *ibid.*, **100**, 5956 (1978).
(14) R. P. Bell and P. G. Evans, *Proc. R. Soc. London, Ser. A*, **291**, 297 (1966).
(15) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964).
(16) This pK value was estimated as follows: pK_{TH^+} for the methoxyamine adduct of *p*-chlorobenzaldehyde is estimated to be 8.5.[2] The effect of replacing the methoxy group by the $CH_3C(O)NH-$ group is to lower the pK of acethydrazide 1.29 units relative to methoxyamine; transmission of this substituent effect through one carbon and one nitrogen atom should reduce this effect[5] by a factor of 0.2 giving $pK_{TH^+} = 8.5 - (0.2)(1.29)$ or 8.24 for the acethydrazide adduct.