Intramolecular general base catalysis in the aminolysis of acetylimidazole and methyl formate by diamines

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Intramolecular General Base Catalysis in the Aminolysis of Acetylimidazole and Methyl Formate by Diamines[1]

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Abstract: Eleven diamines of varying structure show rate enhancements of 20- to 200-fold compared to simple aliphatic amines in their nucleophilic reactions with acetylimidazole; a smaller series shows a similar enhancement with methyl formate. In the reactions of monoamines the large dependence of the rate on amine basicity (β_{nuc} = 1.6) indicates that the transition state closely resembles products, with no significant proton removal from the attacking nitrogen atom. The rate enhancement in the diamine reactions is attributed to intramolecular general base catalysis of proton removal from the attacking nitrogen atom, which is important only for acyl compounds with relatively poor leaving groups that are highly susceptible to intermolecular general base catalysis. The low "effective molarity" of the catalyzing base of about 1.0 *M* and the small sensitivity to diamine structure suggest that the transition state for catalyzed proton transfer is loose, with minimal geometric requirements and a large internal entropy. A small rate enhancement in the reaction of 1,3-diaminopropane monocation is consistent with a mechanism involving general acid catalysis of the expulsion of imidazole anion.

There has been a recent renewal of interest in the question of how large a rate increase can be expected in a catalyzed reaction when the reactants and catalyst are brought together in an optimal alignment in the active site of an enzyme or in an intramolecular reaction.[2,3] Two aspects of the problem should be clearly distinguished.

(1) What is the factor by which the rate of a given reaction may be increased upon converting it from a bimolecular (or higher order) to a monomolecular reaction of an enzyme–substrate complex or chemically linked reacting groups? This factor has the units of *M* for conversion of a bimolecular to a monomolecular reaction and is often called the "effective molarity." The known entropies of gas-phase reactions, corrected for empirical entropies of solution, give rise to factors of up to about 10^8 *M* for the rate acceleration in reactions of typical molecules in solution, from the required loss of translational and (overall) rotational entropy of the reactants in the transition state. Several intramolecular reactions are known experimentally to give rate enhancements of up to 10^5 *M* when strain or other destabilizing influences in the reactants are certainly not present and factors of up to 10^8 *M* are known which probably can be accounted for entirely by the entropy effect.[3] The factor of 10^8 *M* is for a reaction proceeding through a tight transition state with relatively little freedom of internal motion (internal entropy) and this factor will be modified downward as the transition state becomes looser; the lower limit is expected for a diffusion-controlled reaction, in which the only restriction on the reactants in the transition state is that they be within a certain distance of each other.

(2) What is the susceptibility of the reaction to catalysis, either intermolecular or intramolecular? "Model" studies are frequently carried out with highly reactive compounds, such as *p*-nitrophenyl acetate, that are easy to examine, but compounds of biochemical sigficance are often much less reactive and more subject to catalysis. This susceptibility is usually expressed as the ratio of the rate constant for a given catalyzed reaction to the rate constant for the same reaction in the absence of catalyst. If the "uncatalyzed" reaction in fact represents catalysis by water, it may be converted to the same order as the intermolecular catalyzed reaction by dividing the observed rate constant by the concentration of water. For the usual general acid or base catalyzed reaction, the rate acceleration for a given catalyst is then determined by the Bronsted α or β value and the p*K* of the catalyst. However, in many cases the "solvent-catalyzed" reaction proceeds by a different mechanism than the catalyzed one and a number of reactions of biochemical interest are now being found to exhibit nonlinear Brønsted plots when transport processes involving the catalyst are kinetically significant.[4,5] For such reactions there is no predictable quantitative relationship between the rates of the catalyzed and uncatalyzed reactions and a meaningful comparison must include an analysis of the mechanisms in each case.

Ethylenediamine (1,2-diaminoethane) and related compounds provide a favorable system for the study of intramolecular general base catalysis of acyl aminolysis reactions by a catalytic group on the nucleophilic reagent. The mechanism of general base catalysis of ester aminolysis is known to involve the removal of a proton from the attacking nitrogen atom[6] and it is relatively easy to examine the effects of structural changes on the efficiency of catalysis, to provide some insight into the geometric requirements of the transition state. Initial attempts to study such intramolecular catalysis revealed little or no increase in the rate

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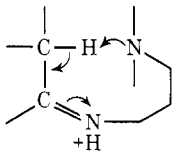
of reactions of diamines with acyl compounds that have good leaving groups, compared to aliphatic amines of comparable basicity.[7-10] In the aminolysis of phenyl acetate a series of diamines exhibits a small rate enhancement of up to 12-fold, but ethylenediamine itself is normal.[7,8] More recently, an enhanced reactivity of several orders of magnitude for ethylenediamine, compared to simple aliphatic amines, has been reported briefly in the aminolysis of an amide with a relatively poor leaving group, free acetylimidazole (pK_a of imidazole = 14.2);[11] no significant rate increase was found for the reaction of ethylenediamine with the more reactive acetylimidazolium ion, which has a much better leaving group (pK = 7).[12] Furthermore, the aminolysis of *p*-nitrophenyl acetate by ethylenediamine in chlorobenzene occurs to a significant extent through a reaction first order with respect to diamine concentration, whereas the reaction with *n*-butylamine occurs only through a reaction second order in butylamine, suggesting that intramolecular catalysis is significant for this ester in the absence of water.[13] A twofold enhancement of the rate of reaction of ethylenediamine monocation with isopropylmethyl phosphonofluoridate may represent intramolecular general acid catalysis.[14] A rate increase of sevenfold for *N*,*N*-dimethylamino-3-aminopropane and approximately 100-fold for *cis*- and *trans*-2-dimethylaminomethylcyclopentylamine compared to other, presumably inactive, diamines in catalyzing hydrogen exchange from the cationic imines of isobutyraldehyde and acetone provides evidence for intramolecular general base catalysis of proton abstraction from carbon that proceeds at an enhanced rate through an eight-membered cyclic transition state **1**.[15] We report here



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the results of a more extensive study of the reactions of acetylimidazole with ethylenediamine and other nucleophiles.

Experimental Section

Materials. Commercially available amines were generally purified by crystallization of the hydrochlorides or by distillation. However, dimethylamines (Aldrich Chemical Co.) which were examined in order to determine whether they exhibit a large rate enhancement compared to the corresponding unsubstituted amines, were used without further purification. *trans*-1,2-Cyclohexanediamine (Aldrich Chemical Co.) was converted to the sulfate.[16] The sulfate was carefully neutralized with methanolic sodium methoxide, the sodium sulfate filtered off, and the methanol removed on a rotary evaporator. The residual oil was taken up in ethanol and the hydrochloride salt of the amine was precipitated with hydrogen chloride gas and recrystallized three times from aqueous ethanol, mp ~325° dec.[17] Freshly boiled glass-distilled water was used throughout and the ionic strength was maintained at 1.0 *M* with potassium chloride except where otherwise indicated. The amines were used as buffers as well as reactants; the buffers were prepared by partial neutralization of the amine or amine hydrochloride just prior to the kinetic run. The pH values of the solutions were determined before and after a kinetic experiment and any run showing a pH drift greater than 0.03 unit was discarded.

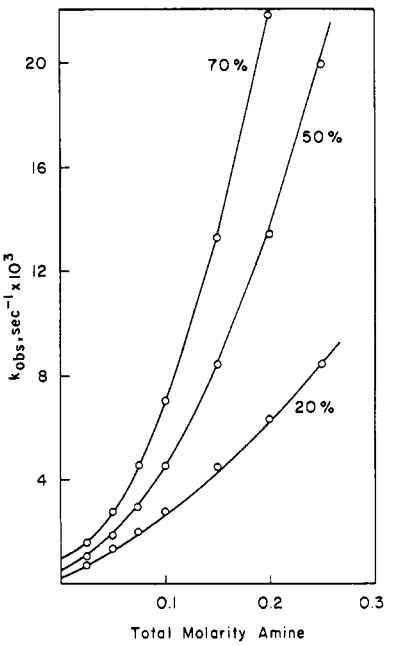

Figure 1. Observed pseudo-first-order rate constants for the reaction of acetylimidazole with cyanoethylamine at the indicated fractions of free base in the amine buffers at 25°, ionic strength 1.0. The lines are calculated from the rate constants in Table II.

Kinetics. The reactions were initiated by the addition of 25 μl of aqueous 0.01 *M* acetylimidazole, containing about 10^{-3} *M* imidazole, to 2.5 ml of the aqueous amine buffer solution preincubated at 25°, with thorough mixing. The disappearance of acetylimidazole was followed spectrophotometrically on a Zeiss PMQ II spectrophotometer fitted with a Beckman Model 1005 recorder at 245–265 nm. It was found that reactions with half-lives as short as 2 sec could be accurately measured by this technique. The values of the pseudo-first-order rate constants were calculated from linear plots of log $(A_t - A_\infty)$ against time or from the directly measured half-life on the recorder trace when this was shown to be constant over several half-lives. The aminolysis of methyl formate was measured as described previously.[6]

The products of aminolysis reactions were analyzed by conversion of amides to the hydroxamic acids.[6]

Results

The reactions of amines with acetylimidazole have been shown previously[18,19] to follow the rate law of eq 1, in which B represents a second molecule of amine, or the kinetically indistinguishable rate law of eq 2. The

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Table I. Observed Rate Constants for the Reactions of Acetylimidazole with Bases at 25°, Ionic Strength 1.0 M[a]

Base	Fraction free base or monocation	pH	No. of points	k_{int},[b] M^{-1} sec^{-1}	k_{cat},[b] M^{-2} sec^{-1}
2-Cyanoethylamine[c]	0.2	7.57	7	1.85×10^{-2}	0.060
	0.5	8.22	7	1.30×10^{-2}	0.26
	0.7	8.56	7	1.20×10^{-2}	0.47
Hydrazine[d]	0.1	7.24	10	1.53	11.0
	0.2	7.57	10	1.48	30.0
	0.3	7.84	10	1.48	64.0
	0.4	8.00	10	1.48	101.0
Ethanolamine	0.1	8.82	10	0.11	0.72
	0.2	9.14	10	0.27	1.0
	0.3	9.42	10	0.35	4.8
	0.4	9.57	10	0.63	6.2
	0.6	9.87	10	1.27	14.8
	0.7	10.10	10	1.49	23.9
2-Methoxyethylamine	2.05×10^{-4}	6.14	4	9.0×10^{-4} [e]	
			4	1.87×10^{-3} [e]	
	0.1[c]	8.70	10	0.032	0.248
	0.2[c]	9.06	10	0.050	1.34
	0.3	9.43	10	0.054	2.8
	0.5	9.82	10	0.11	10
Allylamine[f]	0.1	9.06	8	0.046	1.64
	0.3	9.65	10	0.18	14.4
	0.4	9.87	4	0.22	27.7
	0.5	10.05	10	0.30	57.6
n-Propylamine	0.1	9.77	10	0.95	2.5
	0.15	10.12	10	1.48	18.0
	0.20	10.25	10	2.15	45.0
Cyclohexylamine[g]	0.1	9.89	4	0.13	
	0.2	10.25	4	0.47	
	0.3	10.49	4	0.75	
tert-Butylamine	0.1	9.99	4	0.040	
	0.2	10.32	4	0.11	
3-Quinuclidinol[c]	0.1	9.24	4	0.118	
	0.2	9.61	6	0.242	
	0.3	9.87	4	0.36	
	0.5	10.22	8	0.60	
	0.7	10.58	8	0.82	
Quinuclidine[c]	0.07	10.40	10	0.17	
	0.1	10.61	10	0.31	
	0.15	10.82	10	0.50	
	0.2	10.97	8	0.62	
N-Methylmorpholine[c]	0.3	7.46	4	9.8×10^{-4}	
	0.5	7.83	4	1.8×10^{-3}	
	0.7	8.20	4	2.5×10^{-3}	
Malonate[c]	0.3	4.72	4	1.35×10^{-2}	
	0.5	5.04	4	9.8×10^{-3}	
	0.7	5.39	4	6.10×10^{-3}	
	0.9	5.98	4	2.45×10^{-3}	
Formate[h]	0.35	3.29	4	2.05×10^{-2}	
	0.5	3.51	4	2.29×10^{-2}	
	0.7	3.87	4	2.76×10^{-2}	
	0.9	4.34	4	1.98×10^{-2}	
Cacodylate[h]	0.7	6.51	3	0.109	
	0.9	7.10	3	0.044	
Hexafluoroisopropyl alcohol[c]	0.1	8.23	10	0.37	0.49
	0.3	8.84	10	0.33	1.29
	0.5	9.23	10	0.251	1.48
	0.7	9.66	10	0.215	1.23
1,3-Diaminopropane	1.59×10^{-3}	6.25	4	2.71×10^{-3} [i]	
			4	5.01×10^{-3} [i]	
	0.1	8.08	10	0.18	0.6
	0.2	8.50	10	0.73	0.36
	0.3	8.72	10	1.60	5.4
	0.4	8.90	10	3.10	8.0
	0.5	9.05	10	4.90	10.8
1,4-Diaminobutane	2.63×10^{-4}	6.25	4	2.23×10^{-3} [i]	
			4	4.06×10^{-3} [i]	
	0.1	8.86	10	0.21	1.5
	0.2	9.23	10	0.80	9.0
	0.3	9.50	10	1.80	20.0
	0.4	9.61	10	3.35	40.0
1,5-Diaminopentane	1.05×10^{-4}	6.25	4	1.70×10^{-3} [i]	
			4	3.20×10^{-3} [i]	
	0.07	9.13	10	0.185	2.5
	0.1	9.27	10	0.42	7.0
	0.15	9.49	10	0.78	17.0
	0.2	9.64	10	1.80	22.0
	0.25	9.76	10	3.00	32.0

Table I (*Continued*)

Base	Fraction free base or monocation	pH	No. of points	k_{int},[b] M^{-1} sec^{-1}	k_{cat},[b] M^{-2} sec^{-1}
N,*N*-Dimethyl-1,2-ethanediamine	0.1	5.94	2	0.028	
	0.3	6.54	2	0.041	
	0.6	7.09	2	0.11	
	0.9	7.73	2	0.47	
N,*N*-Dimethyl-1,3-propanediamine	0.1	7.82	2	0.052	
	0.3	8.37	2	0.31	
	0.5	8.72	2	0.87	
	0.6	8.86	2	1.36	
2-Dimethyl-1,2-ethanediamine	0.1	5.98	2	0.018	
	0.3	6.57	2	0.027	
	0.6	7.10	2	0.041	
	0.9	7.75	2	0.145	
N-2-Aminoethylpyrollidine	0.1	6.38	2	0.031	
	0.3	6.95	2	0.051	
	0.6	7.47	2	0.173	
	0.9	8.20	2	0.86	
N-2-Aminoethylpiperidine	0.1	6.22	2	0.031	
	0.3	6.85	2	0.053	
	0.6	7.41	2	0.19	
	0.9	8.15	2	0.96	
N-Ethyl-3-aminopiperidine	0.1	5.86	1	0.0091	
	0.3	6.45	1	0.0033	
	0.6	7.01	1	0.0069	
	0.9	7.75	1	0.017	
	0.1[i]	9.02	2	0.15	
	0.2[i]	9.35	2	0.32	
trans-1,2-Cyclohexanediamine	0.1	6.12	10	0.0075	
	0.3	6.70	10	0.0060	
	0.5	7.01	8	0.0060	
	0.1[i]	9.55	10	0.77	
	0.2[i]	9.73	10	1.25	
	0.3[i]	9.91	10	1.75	

[a] Ionic strength maintained with potassium chloride. Amines were examined in the range 0–0.05 *M* total amine concentration unless indicated otherwise. [b] k_{int} is the intercept and k_{cat} is the slope of a plot of $(k_{obsd} - k_0)$/[amine]$_{tot}$ against [amine]$_{tot}$; k_0 is the rate of acetylimidazole hydrolysis in the absence of amine. [c] Total buffer concentration in the range 0–0.25 *M*. [d] Ionic strength maintained with tetramethylammonium chloride. [e] k (sec^{-1}) extrapolated to zero imidazole concentration, from runs in 0.05–0.20 *M* buffer; 0.04 and 0.08 *M* total amine concentration. [f] Total buffer concentration in the range 0–0.10 *M*. [g] Total buffer concentration in the range 0–0.08 *M*. [h] Total buffer concentration in the range 0–0.40 *M*. [i] k (sec^{-1}), extrapolated to zero imidazole concentration, from runs in 0.05–0.20 *M* buffer, 0.05 and 0.10 total amine concentration. [j] Fraction of unprotonated amine using monocation–unprotonated base as buffer.

$$\text{rate} = k_1[RNH_2][AcIm] + k_2[RNH_3^+][AcIm] + k_3[RNH_2][B][AcIm] + k_4[RNH_2][BH^+][AcIm] \quad (1)$$

$$\text{rate} = k_1[RNH_2][AcIm] + k_2'[RNH_2][AcImH^+] + k_3[RNH_2][B][AcIm] + k_4'[RNH_2][B][AcImH^+] \quad (2)$$

experimental conditions for the rate measurements and the observed rate constants are given in Table I and the derived rate constants are summarized in Table II. Rate constants for the reactions of 1,2-diaminoethane and 1,2-diaminopropane with methyl formate are also given in Table II; these may be compared with previously reported rate constants for the reactions of simple primary amines with this ester.[6] Statistical corrections for structure–reactivity correlations were made as described by Bell and Evans.[20]

The procedure used for determining the rate constants in Table II has been described previously.[18,19] Typical experimental data are illustrated in Figures 1–4. The observed pseudo-first-order rate constants for simple primary amines such as cyanoethylamine (Figure 1) exhibit a sharp upward curvature in plots against amine concentration because of general acid–base catalysis of the aminolysis reaction by a second molecule of the amine buffer. The second- and third-order rate constants at each buffer ratio were obtained from the intercepts and slopes of plots of the observed second-order rate constants against total amine buffer concentration, as shown for the reaction of allylamine in Figure 2. The steep slopes and small intercepts in this figure illustrate the very large contribution to the observed rate of the catalyzed reaction, even at low amine concentrations. Consequently, the rate constants, k_1, for the uncatalyzed reactions of simple primary amines are not of high precision. Upper limits are given in Table II for a number of rate constants when no reaction was detected; these were usually estimated by assuming that a 20% increase in rate, caused by the term in question, was not detected.

In contrast, the rapid reaction of 1,3-diaminopropane with acetylimidazole exhibits very little curvature in plots of the observed first-order rate constants against amine concentration (Figure 3), indicating that catal-

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Table II. Summary of the Rate Constants for the Reactions of Acetylimidazole and Methyl Formate with Amines, Diamines, and Other Bases at 25°, Ionic Strength 1.0 M[a,b]

Amine or base	pK_a[c]	k_1(B, AcIm), M^{-1} sec^{-1}	k_2(BH$^+$, AcIm),[a] M^{-1} sec^{-1}	k_2'(B, AcImH$^+$), M^{-1} sec^{-1}	k_3(B,B, AcIm),[a] M^{-2} sec^{-1}	k_4(B,BH$^+$, AcIm), M^{-2} sec^{-1}	k_5(B^{2+}, AcIm), M^{-1} sec^{-1}
Trifluoroethylamine[d,e]	5.81	1.0×10^{-3}	2.4×10^{-2}	2.15	6.6×10^{-3}	0.048	
2-Cyanoethylamine	8.20	8.0×10^{-3}	2.0×10^{-2}	440	0.94	0.15	
Hydrazine[e]	8.20	1.5	1.5	3.3×10^{4}	550	64	
Methoxyethylamine	9.72[f]	0.16	2.3×10^{-2}	2.2×10^{4}	36		
Glycine	9.76[f]	0.08[g]	2.8×10^{-2}	2.2×10^{4}	25	3.3[d,e]	
Ethanolamine[h]	9.76	0.85	2.0×10^{-2}	1.7×10^{4}	52		
Allylamine	10.02	0.6	$\leq 2 \times 10^{-2}$	$\leq 3 \times 10^{4}$	170		
Cyclohexylamine	10.85	2.4	$<3 \times 10^{-2}$	$<3 \times 10^{5}$			
n-Propylamine	10.89[f]	10	9×10^{-3}	9.6×10^{4}	950		
tert-Butylamine	10.93	≤ 0.6					
Ethylamine	10.97	8.2	9×10^{-3}[d]	1.2×10^{5}	1370		
N-Methylmorpholine	7.83	3.56×10^{-3}	$<1.4 \times 10^{-4}$	<1.3			
3-Quinuclidinol	10.20	1.18	<0.3	$<6.6 \times 10^{5}$			
Quinuclidine	11.55	3.13	<0.07	$<3.5 \times 10^{6}$			
Formate	3.56[i]	$\leq 5 \times 10^{-3}$	0.143	7.2×10^{-2}			
Malonate	5.03	5×10^{-4}	2.1×10^{-2}	0.31			
Cacodylate	6.15	$\leq 1.2 \times 10^{-2}$	0.34	66			
Hexafluoroisopropoxide anion	9.22[i]	0.14	0.40	9.0×10^{4}		6.0	
1,2-Diaminoethane[e,j]	10.18 7.52	70	6.7×10^{-2}	1.4×10^{5}	1.7		
1,3-Diaminopropane	10.93 9.25	750	0.45	5.3×10^{5}	38[e]		4.6×10^{-2}
1,4-Diaminobutane	11.17 9.91	259	0.65	1.3×10^{7}	230		3.6×10^{-2}
1,5-Diaminopentane	11.20 10.34	310	<0.1	$<5.0 \times 10^{6}$	550[g]		2.6×10^{-2}
N,*N*-Dimethyl-1,2-ethanediamine	10.08 6.90	100	6.3×10^{-2}	1.1×10^{5}			2.0×10^{-2}
N,*N*-Dimethyl-1,3-propanediamine	10.80 8.80	140	3.2×10^{-1}	2.8×10^{6}			
2-Dimethyl-1,2-ethanediamine	10.25 6.93	36	4.5×10^{-2}	1.1×10^{5}			1.5×10^{-2}
trans-1,2-Cyclohexanediamine	10.33 7.08	6.0	1×10^{-3}	3×10^{3}			7.8×10^{-3}
N-2-Aminoethylpyrollidine	10.28 7.30	105	6.5×10^{-2}[g]	1.7×10^{5}[g]			2.5×10^{-2}
N-2-Aminoethylpiperidine	10.22 7.20	144	8.5×10^{-2}[g]	2.0×10^{5}[g]			2.5×10^{-2}
3-Amino-*N*-ethylpiperidine	9.96 6.81	1.53	1×10^{-2}[g]	1.3×10^{4}[g]			2×10^{-4}[g]
		Rate constants for methyl formate as substrate[k]					
1,2-Diaminoethane	10.28	0.48					
1,3-Diaminopropane	11.02	3.8					

[a] Ionic strength maintained with potassium chloride unless otherwise noted. For diamines k_3 is for $[H_2N(CH_2)_nNH_3^+]^2$ and k_2 is for the monocation. [b] k_5 is for the dication of diamines. [c] Determined from titration or the pH of buffer solutions. For diamines the highest pK_a is given which was calculated according to A. Albert and E. P. Sergeant, "Ionization Constants of Acids and Bases," Methuen and Company, London, 1962, pp 51–56. [d] Reference 19. [e] Ionic strength maintained at 1.0 M with tetramethylammonium chloride. [f] Reference 10. [g] Approximate value. [h] k for [AcIm][HOEtNH$_2$][OH$^-$] = 1×10^4 M^{-2} sec^{-1}. [i] J. M. Sayer and W. P. Jencks, *J. Amer. Chem. Soc.*, **91**, 6353 (1969). [j] Reference 12. [k] Ionic strength maintained at 1.5 M with tetramethylammonium chloride. Determined at 5 and 10% free base, 0.05–0.25 M total amine.

ysis by a second molecule of amine is relatively unimportant in this reaction. The predominant contribution of the term first order with respect to amine concentration is shown by the large intercepts and small slopes of the plots of observed second-order rate constants against amine concentration in Figure 4. As shown in Figure 5, the rate constants from the intercepts of Figure 4 increase much more rapidly than the fraction of amine monocation in the buffer, showing that most of the observed rapid reaction involves the free base form of the diamine. The terms contributing to the observed rate constants were analyzed according to eq 3 and 4, in which DA is the diamine, K_1 the dissociation constant of the monocation, and a the

$$k_{obsd} = k_0 + k_1[DA] + k_2[DAH^+] + k_3[DAH^+]^2 + k_4[DAH^+][DA] + k_5[DAH_2^{2+}] + k_6[DA]^2 \quad (3)$$

$$\frac{k_{obsd} - k_0 - k_5[DAH_2^{2+}]}{[DA]_{tot}a} = \frac{k_1K_1}{[H^+]} + k_2 + k_3[DAH^+] + k_4(K_1[DAH^+]/[H^+]) + k_6(K_1[DA]/[H^+]) \quad (4)$$

fraction of diamine monocation. The term k_5 for the contribution of DAH_2^{2+} was evaluated from measurements in imidazole buffers at low pH under conditions in which the other terms are small or negligible. The remaining terms first order in amine (k_1 and k_2) were separated utilizing the inverse dependence of the contribution of the k_1 term on hydrogen ion activity under the conditions of the experiments. The rate constants so obtained (Table II) were used to calculate the solid line in Figure 5, which shows satisfactory agreement with the data. The dashed line shows the small con-

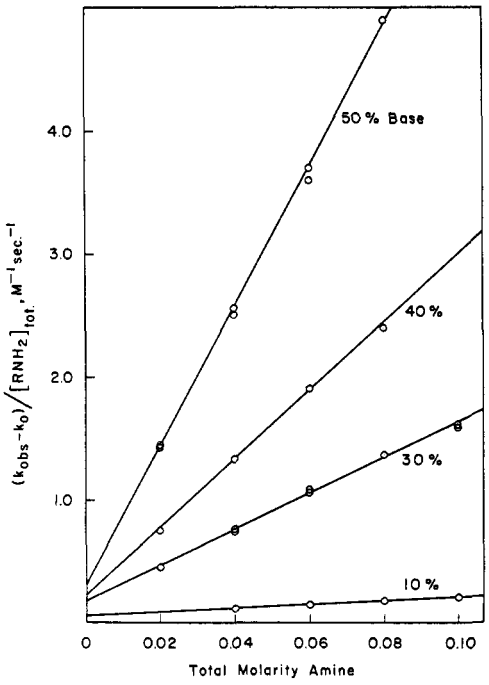


Figure 2. Observed second-order rate constants for the reaction of acetylimidazole with allylamine at the indicated fractions of free base as a function of total amine buffer concentration at 25°, ionic strength 1.0.

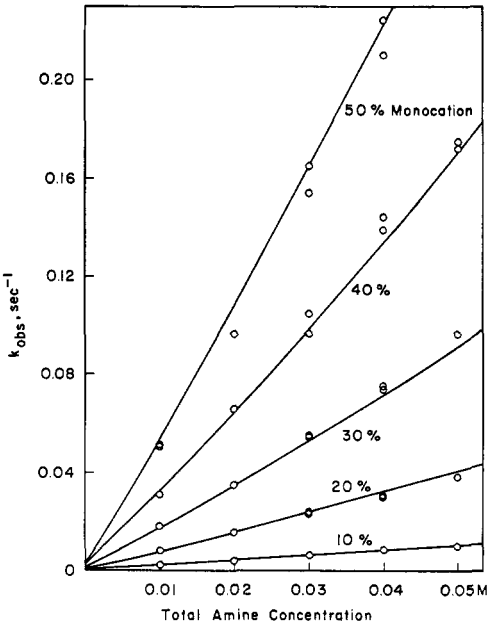


Figure 3. Observed pseudo-first-order rate constants for the reaction of acetylimidazole with 1,3-propanediamine in buffer solutions of the dication and monocation at 25°, ionic strength 1.0. The lines are calculated from the rate constants in Table II.

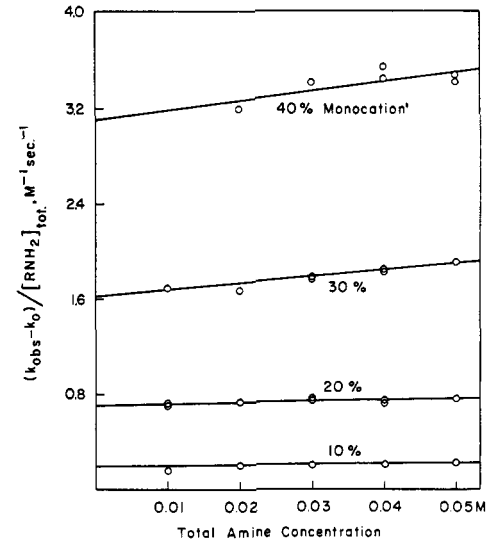


Figure 4. Observed second-order rate constants for the reaction of acetylimidazole with 1,3-propanediamine as a function of total amine concentration.

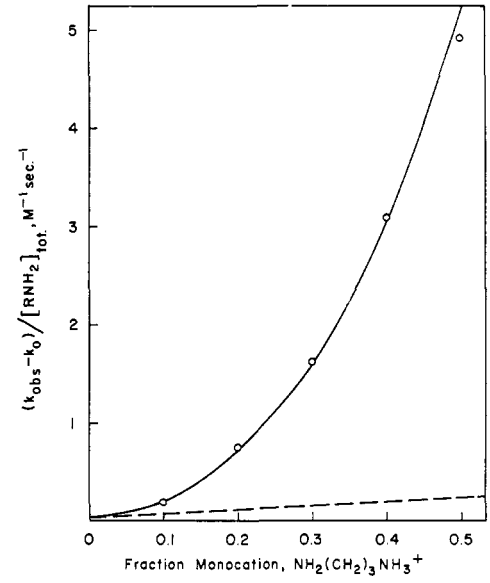


Figure 5. The dependence of the second-order rate constants for the 1,3-propanediamine reaction, from the intercepts of Figure 4, on the buffer composition. The solid line is calculated from the rate constants in Table II and the dashed line shows the contribution of the k_2 and k_5 terms, for the amine monocation and dication.

tribution of the k_2 and k_5 terms and emphasizes once again the importance of the free base term k_1. Because of the smaller rate constants for the reaction of *trans*-1,2-diaminocyclohexane with acetylimidazole it was possible to follow this reaction directly in buffers composed of the monocation and free base (Table I).

Rate constants for the reactions of several oxygen bases with acetylimidazole are included in Tables I and II. These reactions follow the rate law of eq 5 (or its kinetic equivalents).[18,19,22,23] Ethanolamine exhibits a hydroxide ion catalyzed reaction (Table II) that also presumably represents a reaction of the alcoholate anion (k_1, eq 5).

$$\text{rate} = k_1[RO^-][AcIm] + k_2[ROH][AcIm] + k_4[ROH][RO^-][AcIm] \quad (5)$$

The reaction of 0.05 and 0.10 *M* (total) *n*-propylamine with acetylimidazole at pH 10 gave a yield of amide product of >90%. Since 51–68% of the reaction under these conditions represents the uncatalyzed k_1 term, this indicates that this term represents aminolysis, rather than amine-catalyzed hydrolysis. At pH

(22) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **86**, 4651 (1964).

(23) J. Gerstein and W. P. Jencks, *ibid.*, **86**, 4655 (1964).

10.7 with 1.0 M (total) n-propylamine 97% of the observed rate represents the k_3 term and the yield of amide was also found to be $\geq$90%. The reaction of 0.1 M (total) 1,3-diaminopropane with acetylimidazole was found to give a >90% yield of amide at pH 9.2 and 9.6, where 90–95% of the observed rate represents the rapid k_1 term for reaction of the free base. A 78% yield was obtained at pH 8.0, where the reaction represents approximately two-thirds the k_5 term and one-third the k_2 term.

An attempt was made to demonstrate that the reaction of quinuclidine with acetylimidazole represents nucleophilic attack by searching for inhibition of the reaction by added imidazole, which might trap the intermediate acylated tertiary amine and regenerate starting materials (eq 6). No inhibition was found in

$$\mathrm{AcIm} + \mathrm{R_3N} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{Ac\overset{+}{N}R_3} + \mathrm{Im^-} \xrightarrow{\mathrm{OH^-}} \mathrm{AcOH} + \mathrm{NR_3} \qquad (6)$$

the presence of 0.2 M imidazole at pH 10.6, at which 86% of the reaction involves the free base form of both reactants. However, this negative result is not conclusive because the back reaction of imidazole anion may not be able to compete effectively with hydrolysis of the intermediate. Even with the much less reactive compound p-nitrophenyl acetate the imidazole anion reaction is not detectable under comparable conditions.[24]

Discussion

Free Diamines and Acetylimidazole. The rate constants k_1 for the reactions of a number of diamines of varying structure with free acetylimidazole are larger by some 20- to 200-fold compared to those for the reactions of simple primary amines of comparable basicity (Figure 6).[25,26] Correction for steric hindrance in the reaction with *trans*-1,2-cyclohexanediamine, based on the negative deviation in the reaction with acetylimidazolium ion (see below), brings the rate constant for this compound into the range observed with other diamines as shown by the dashed line in Figure 6; a smaller correction could be made for 3-amino-*N*-ethylpiperidine. This rate acceleration is interpreted as evidence for intramolecular general base catalysis of aminolysis by the second nitrogen atom in these diamines.

The reason that intramolecular general base catalysis is significant for reactions of acetylimidazole and not for some other acyl compounds is simply that general base catalysis is very important for the aminolysis of acetylimidazole (Figures 1 and 2), so that the intramolecular catalysis results in a significant rate enhancement compared to the uncatalyzed (water) reaction and is easily detectable. In searching for evidence in support of catalytic mechanisms of this and other kinds many investigators have made use of p-nitrophenyl acetate and related compounds, which are convenient to study because they react rapidly and give products that are easy to determine spectrophotometrically. However, reactive compounds with good leaving groups are poor candidates for such studies because they exhibit a fast uncatalyzed (water) reaction and little sensitivity to catalysis of any kind. Acyl compounds of physiological significance generally have poor leaving groups and are much more susceptible to catalysis. Free acetylimidazole, with a moderately poor leaving group of pK = 14.2,[11] provides a convenient model for the reactions of such physiological compounds because of its relatively high reactivity (as a consequence of its small resonance stabilization) and convenience for spectrophotometric assay. Methyl formate (pK of the leaving group = 15.5)[27] shows a similar rate acceleration in its reactions with diamines. These considerations support the hypothesis that appropriately located bases in the active sites of enzymes may provide significant rate increases through general base catalysis of the aminolysis of naturally occurring acyl substrates and (as a consequence of microscopic reversibility) will facilitate the expulsion of amines or ammonia from amides by general acid catalysis.

Although the absolute rate increase relative to the "water" reaction that is caused by intramolecular general base catalysis is considerable because of the importance of general base catalysis in these reactions, the contribution of the intramolecularity itself is small. The second-order rate constants for the reactions of diamines with acetylimidazole may be divided by the third-order constants k_3 for intermolecular catalysis of aminolysis by a second molecule of amine of comparable basicity to give an "effective molarity" of the adjacent amine in the intramolecular reaction; *i.e.*, the concentration of catalyzing amine that would give the same rate of reaction as the diamine. As shown in Table III, these "effective molarities" are only on the order of 1 M. Similar values are found for the methyl formate reaction. The small rate acceleration found earlier for the reaction of phenyl acetate with diamines also corresponds to an effective molarity of approximately 1 M,[7,28] confirming the conclusion that the ready detection of intramolecular catalysis in the acetylimidazole reaction is simply a reflection of the importance of the general base catalyzed compared to the uncatalyzed aminolysis of this compound. Intramolecular general base catalysis of the aminolysis of p-nitrophenyl acetate in chlorobenzene by diamines[13] is detectable because of the slow rate of the uncatalyzed reaction in the absence of water, and also corresponds to an effective concentration of the catalyzing amine close to 1 M.

The results shown in Figure 6 indicate that there is a

(24) J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 833, 837 (1964).

(25) The rate acceleration for ethylenediamine compared to glycine was stated previously to be a factor of approximately 10^3.[12] Although this number is within the range of error of the experimental measurements (Table II), the steep dependence of the rate upon basicity and the negative deviation of glycine from the correlation line of Figure 6 make the factor 20–200 a more general and conservative estimate.

(26) The rate constants for asymmetrical diamines and their monocations are plotted in Figures 6 and 7 as a function of their macroscopic pK_a values. Dissection into microscopic pK_a values is possible for the *N,N*-dimethyldiamines,[21] but does not clarify the correlations, in the absence of knowledge of the separate pK_a dependencies of the reactivities of the nucleophilic and catalyzing groups, and does not lead to any significant difference in the conclusions. The monocations of *N,N*-dimethylethanediamine and *N,N*-dimethylpropanediamine exist only about one-third (0.30–0.38), rather than one-half, in the form with a free primary amino group,[21] so that a small upward correction of the points for these compounds in Figure 7 is warranted if it is assumed that this is the reactive ionic form.

(27) P. Ballinger and F. A. Long, *J. Amer. Chem. Soc.*, **82**, 795 (1960).

(28) W. P. Jencks and J. Carrioulo, *ibid.*, **82**, 675 (1960); W. P. Jencks and M. Gilchrist, *ibid.*, **88**, 104 (1966).

Table III. Rate Enhancements and Effective Molarities in the Reactions of Diamines with Acetylimidazole and Methyl Formate

Amine	pK_a	Rate enhancement over RNH_2	Effective molarity
	Acetylimidazole		
1,2-Diaminoethane	10.18	186	0.55
1,3-Diaminopropane	10.93	118	0.94
1,4-Diaminobutane	11.17	18	0.20
1,5-Diaminopentane	11.20	19	0.25
	Methyl Formate[a]		
1,2-Diaminoethane	10.28	25	0.5
1,3-Diaminopropane	11.02	100	0.6

[a] The comparisons are based on rate constants for primary amines previously reported.[6]

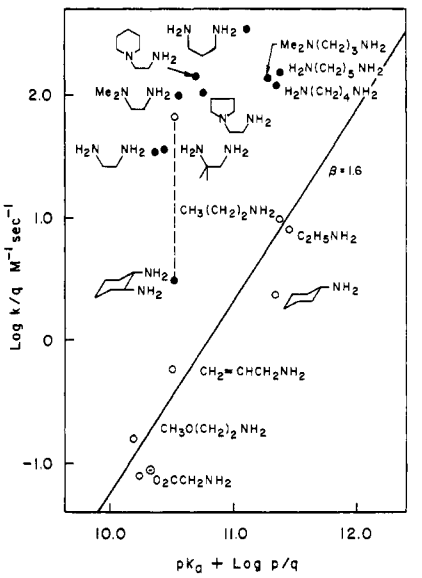
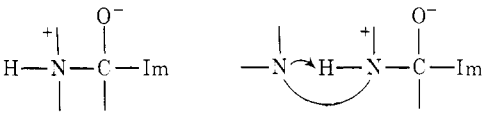


Figure 6. The dependence of the rate constants k_1 for the reactions of primary amines and diamines with free acetylimidazole on amine basicity at 25°, ionic strength 1.0. The data for monoamines and symmetrical diamines are statistically corrected.[20]

remarkably small sensitivity of the rate acceleration to variations in the structure of the diamine. There is no significant rate increase and little or no rate decrease upon the addition of geminal dimethyl groups to the alkyl chain or one nitrogen atom, upon the incorporation of one nitrogen atom into a five- or six-membered ring, upon the attachment of both nitrogen atoms to a cyclohexane ring, or upon an increase in the alkyl chain length from two to three carbon atoms, and there is still significant catalysis with diaminobutane and diaminopentane, which must form at least seven- or eight-membered rings in the transition state. The small effective molarity of the catalyzing amine group of a diamine could be caused by either (1) a potential energy or enthalpy effect, such as steric hindrance or ring strain in the transition state, or (2) an entropy effect that reflects a minimal requirement for order in a loose transition state. Although there may be a significant enthalpic barrier from eclipsing hydrogen atoms and ring strain in some cases, the small sensitivity to the structure of the diamine suggests that this effect is not predominant and that general base catalysis of aminolysis occurs through a transition state in which the proton transfer occurs either directly or through an intermediate water molecule with minimal structural requirements and a small loss of entropy.

The low effective molarity of about 1 *M* for the catalyzing group and the small sensitivity to structure suggest that general base catalysis of aminolysis approaches the lower limit of the range of significant rate accelerations brought about by intramolecularity or the binding of reactants to the active site of an enzyme. If a bimolecular reaction occurs through a tight transition state, the formation of this transition state requires the loss of most of the translational and rotational entropy of the reactants, amounting to some 40–50 eu for typical molecules in solution. If much of this entropy is removed beforehand by attaching the reactants to a chemical skeleton or to the active site of an enzyme in a proper orientation, little further entropy need be lost to reach the transition state and a large rate acceleration—10^8 *M* for 35 eu—will be observed. On the other hand, if the transition state has minimal structural requirements little entropy need be lost in its formation—the entropy of the reactants is retained in rotations and a variety of low frequency motions.[3] When there is a small entropy loss in the bimolecular reaction, there will be a correspondingly small rate acceleration from fixing and orienting the reactants in the corresponding monomolecular reaction. Evidently, the proton transfer portion of the aminolysis reaction falls in the latter category.

These considerations are consistent with what is known about the mechanism of general base catalysis of aminolysis from other criteria. It has recently been shown that the Brønsted plot for general base catalysis of the hydrazinolysis of acetylimidazole exhibits a curvature similar to that observed for simple proton transfer reactions.[5] This finding and a rule that states that concerted general base catalysis is expected only when an initially unfavorable proton transfer to the catalyst becomes favorable during the course of the reaction (*i.e.*, the p*K* of the catalyst is intermediate between those of the proton donor sites in the starting material and product)[29] suggest that the reaction occurs through a stepwise mechanism in which at least one limb of the Brønsted plot reflects an approach to a transport-limited process involving the catalyst and a tetrahedral addition intermediate. The simplest interpretation is that for strongly basic catalysts the rate-determining step is the diffusion-limited encounter of the base with the intermediate **2**, followed



2 **3**

by fast proton transfer and breakdown of the anionic tetrahedral addition intermediate. The results in the diamine series support a similar mechanism in which the reaction is made possible by rotation of the diamine chain to a position that permits proton transfer (**3**). The structural and entropic requirements for such a mechanism are small, similar to those for a simple

(29) W. P. Jencks, *J. Amer. Chem. Soc.*, **94**, 4731 (1972).

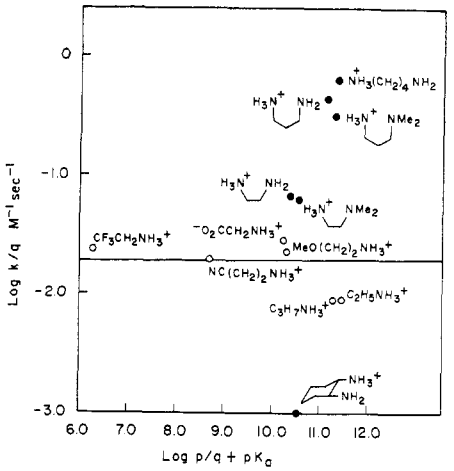
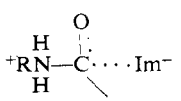


Figure 7. The dependence on amine basicity of the observed rate constants k_2 for the reaction dependent on the concentration of amine or diamine monocation and free acetylimidazole at 25°, ionic strength 1.0; statistical corrections as in Figure 6.

proton transfer reaction that is diffusion controlled in the thermodynamically favorable direction. We cannot specify in detail what part of the overall proton transfer process is rate determining in the diamine reactions. There is evidence that even the monocationic species of diamines exists in an extended, rather than a cyclic, hydrogen-bonded form in water,[21] so that movement of the catalyzing group into a position in which proton transfer may take place is probably required at some point in the reaction. However, the small sensitivity to structural variation is also consistent with the relatively small geometric and entropic requirements for formation of the hydrogen bond that is required in the transition state for the proton transfer process itself.[30] The tetrahedral addition intermediate is expected to break down very rapidly after proton removal, utilizing the driving force of the liberated electron pair on the nitrogen atom and the developing resonance stabilization of the amide product, as well as the electron pair on the oxygen anion.[5,6,29] In fact, it is possible that the lifetime of this anionic addition compound is so short that it does not exist as a discrete intermediate.[31]

There is strong evidence from the overall kinetics and the pH dependence of the breakdown of the tetrahedral addition intermediate that general base catalysis of the aminolysis of methyl formate involves proton removal from the attacking amine rather than some other kinetically equivalent mechanism; in this reaction the kinetics require that the proton removal take place in a step prior to the breakdown of the tetrahedral intermediate.[5,29] Furthermore, the high reactivity of 1,2-ethanediamine in the acetylimidazole reaction supports a mechanism involving proton removal from the attacking amine because this diamine is too short to permit a mechanism of catalysis involving donation to the distal nitrogen atom of the leaving imidazole[19] (although the reservation must always be made that the proton transfer could take place through an intermediate water molecule).

Although base catalysis plays an important mechanistic role in preventing the reversion of the tetrahedral addition intermediate **2** to starting materials and in liberating an electron pair on nitrogen to facilitate leaving group expulsion, the driving force for catalysis is best described as arising from the avoidance of the unstable products and transition states that would be required in its absence. The unusually large dependence on amine basicity of the rate of the uncatalyzed aminolysis of acetylimidazole, with a value of $\beta_{nuc} = 1.6$, means that the transition state of this reaction closely resembles products, with a large amount of charge development on the attacking nitrogen atom and no significant proton removal in the transition state **4**; the limiting interpretation is that the



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rate-determining step is the separation of the ion pair $CH_3CONH_2R \cdot {}^+Im^-$ (Figure 6[32,33]). The rate of this nucleophilic reaction rapidly becomes insignificant as the amine basicity is decreased, so that for amines of pK less than about 10 it is supplanted by general base catalysis of hydrolysis with a Bronsted β value of 0.5 ± 0.1.[19] General base catalysis of the nucleophilic reaction is important because it serves to avoid the unstable transition state **4** and the formation of the very similar immediate product $CH_3CONH_2R^+$, an unstable N-protonated amide with a pK on the order of −7.6.[34]

Other Reactions. The rate constants k_2 for the reactions of simple amine monocations with acetylimidazole are almost independent of amine pK, as shown by the horizontal line in Figure 7. This is because these reactions involve the free amine and acetylimidazolium ion (k_2', eq 2) and there is a cancellation of the effects of polar substituents on the concentration of free amine in solution and on its reaction rate with acetylimidazolium ion, for which $\beta_{nuc} = 1.0$.[19,35] The rate constant for 1,2-ethanediamine monocation shows a small positive deviation of sixfold from this line. Thus, there is only a small rate enhancement of questionable significance in the reaction of free 1,2-ethanediamine with acetylimidazolium ion, which has a good leaving group of pK = 7. For *trans*-1,2-cyclohexanediamine the effect of steric hindrance is larger than any rate enhancement and the rate constant shows a negative deviation.

Longer chain diamine monocations show a larger rate enhancement of about 30-fold (Figure 7). The rate constants for these compounds may also be compared to the rate constants k_1 for the reactions of free amines with free acetylimidazole. For the monocation of diaminopropane such a comparison shows a rate enhancement of 22-fold, but this is reduced to only eightfold after statistical correction of the pK

(30) J. Donohue in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968, p 443; J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, p 52; F. Covitz and F. H. Westheimer, *J. Amer. Chem. Soc.*, **85**, 1773 (1963).

(31) M. I. Page and W. P. Jencks, *ibid.*, **94**, 8828 (1972).

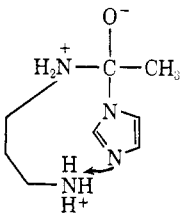
(32) M. I. Page and W. P. Jencks, *ibid.*, **94**, 3263 (1972).

(33) Although it is conceivable that the base-catalyzed reaction involves the removal of a proton from this ion pair, this interpretation is unlikely in view of the β value of 1.0[19] and because the rate of separation of the ion pair in water would probably be fast relative to the diffusion or rotation of a base into an appropriate position for proton abstraction.

(34) A. R. Fersht, *J. Amer. Chem. Soc.*, **93**, 3504 (1971).

(35) R. Wolfenden and W. P. Jencks, *ibid.*, **83**, 4390 (1961).

values; for diaminobutane monocation there is no rate enhancement after statistical correction. This relatively small enhancement may be accounted for by protonation of the leaving imidazole group by the RNH_3^+ group of the diamine **5**; inspection of molecular



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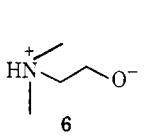
models suggests that such protonation is possible for 1,3-diaminopropane monocation. Although an exact comparison cannot be made because of the different pK values, the rate constant k_2 for diaminopropane monocation is smaller than the rate constant k_4 for general acid catalysis of the reaction of acetylimidazole with glycine by a second molecule of amine buffer[19] (Table II). This means that the "effective molarity" of the NH_3^+ group in this compound is less than 1 M, indicating once again that this catalysis, although probably real, is not very effective.

The diamine hydrazine also shows large positive deviations for the reactions of both the free base and the monocation, compared to simple amines of comparable basicity, but we do not believe that this involves intramolecular catalysis of proton transfer. Enhancement of the reaction rates of other diamines is observed only for terms first order with respect to amine concentration. The rate constants k_3 for general base catalyzed aminolysis of acetylimidazole by a second molecule of the primary amines examined here fall on a Brønsted line of slope 1.0 (not shown) similar to that reported previously for a more limited series of amines.[19] The comparable statistically corrected rate constants k_3 for diamine monocations fall on the same line, with the exception of that for 1,2-ethanediamine monocation which shows a threefold positive deviation that can probably be accounted for by general acid catalyzed aminolysis (k_4). In contrast, the rate enhancements for the hydrazine reaction are observed for the terms both first and second order in amine (Table II, compare cyanoethylamine). This means that, in contrast to other diamines, the rate enhancements with hydrazine occur regardless of whether or not the reaction is subject to intermolecular general acid–base catalysis and, therefore, are probably not caused by intramolecular general acid–base catalysis. Furthermore, a direct 1,2 proton shift through a four-electron three-center bond is probably unfavorable because of symmetry considerations.[36] The enhanced reactivity of hydrazine may be attributed to the "α effect,"[37] whatever that is.

Free ethanolamine and 3-quinuclidinol react with acetylimidazole some fivefold more rapidly than amines of comparable basicity that do not contain a hydroxyl group. This enhanced reactivity probably represents a kinetically indistinguishable (eq 7) reaction of the

$$k_1[HO\text{–}NH_2][AcIm] = k_1'[^-O\text{–}NH_3^+][AcIm] \quad (7)$$

oxygen atom of the dipolar ion **6**; intramolecular general base catalysis of alcoholysis (**7**) is not possible



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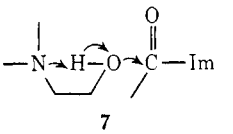

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in 3-quinuclidinol and the observed rate constants are much too large to be accounted for by reaction of the oxyanion with acetylimidazolium ion.[19] Werber and Shalitin have considered similar mechanisms to account for an enhanced nucleophilic reactivity of amino alcohols in other acyl transfer reactions.[38]

(36) R. Gleiter and R. Hoffmann, *Tetrahedron*, **24**, 5899 (1968).
(37) J. O. Edwards and R. G. Pearson, *J. Amer. Chem. Soc.*, **84**, 16 (1962).
(38) M. Werber and Y. Shalitin, personal communication.