Studies on Models for Tetrahydrofolic Acid. V. A Kinetically Significant Transport Process in General Base Catalyzed Aminolysis of a Formamidine[1]

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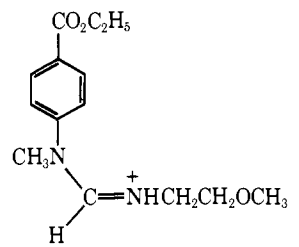
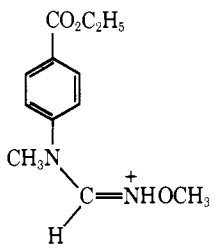
Abstract: The methoxyaminolysis of the unsymmetrically substituted formamidine I exhibits general base catalysis by amines, phosphate, and carboxylates. The catalytic constants are best correlated in the Bronsted relationship by two straight lines of $\beta \simeq 1$ and $\beta \simeq 0$ for $pK_{BH^+} < 1.5$ and $pK_{BH^+} > 1.5$, respectively, suggesting the existence of a short-lived covalent intermediate and kinetically significant transport processes. The data support a mechanism in which two sequential proton transfers occur, perhaps within a single encounter complex, the rate-limiting steps being (1) diffusion-controlled encounter of the intermediate and general base species for strong bases, and (2) either diffusion separation of or a "reorganization" within the encounter complex for weaker bases. General considerations for the aminolysis of amidines and certain enzymic reactions are discussed.

The chemistry of formamidines has been of interest in recent years principally because of their role in folate mediated biosynthesis of the purine nucleotides.[5,6] The relevant transformations involve formamidines as electrophilic substrates in hydrolytic or aminolysis reactions. Although the mechanisms for general acid–base catalysis of formamidine hydrolysis have been studied in detail,[7-14] relatively little is known about the mechanisms of aminolysis. To comment on these mechanisms, we have studied the general acid–base catalyzed methoxyaminolysis of ethyl *p*-{[*N*-(2-methoxyethyl)formimidoyl]methylamino}benzoate hydrochloride (I) to give the product amidine II. The choice of



I



II

methoxylamine as the nucleophilic reagent arises from the inability of non-α-effect amine nucleophiles to effectively compete with hydrolysis of the substrate. Design of the model formamidine I as a substrate for methoxyaminolysis is based on three considerations. (1) The basicities of the three participating amines, *N*-methylbenzocaine (NMB), β-methoxyethylamine (βMEA), and methoxylamine (MA), are sufficiently dissimilar to manifest divergent kinetic behavior. (2) None of the three parent amines is acidic enough to exist as an anion at kinetically significant concentrations in aqueous media,[14] and, as a consequence, the net transamidination is formally symmetrical with respect to the prototropy required in formation and decomposition of any covalent addition intermediates. (3) The basicities of the NMB and βMEA moieties approximate those of possible donor and acceptor sites in the biochemical reactions.[6]

The hydrolysis reactions of I and II are competitive side reactions under certain limiting conditions and are characterized in a brief study.

Results

The hydrolysis of I to give *N*-formyl-*N*-methylbenzocaine was examined in various buffers from pH 8 to 10.5. Buffer catalysis is not observed, and the pH dependence of k_{obsd} describes a simple titration curve. Since previous studies have established the protonated amidine as the reactive species,[9,13] the data are adequately described by a single term involving the hydrolytic reaction of hydroxide ion with the protonated formamidine AmH^+, as shown in eq 1. The

$$k_{obsd} = V\frac{1}{[Am]_T} = \frac{a_H}{K_a + a_H}k_{OH}a_{OH} \quad (1)$$

first-order dependence on hydroxide ion is apparent from the unit slope of the pH–rate profile (Figure 1) for reaction of the protonated formamidine. The best fit is achieved using $K_a = 1.48 \times 10^{-9}$ *M* ($pK_a = 8.83 \pm 0.1$) as the dissociation constant for monoprotonated I. The pK_a as determined spectrophotometrically is 8.98 ± 0.04.

The kinetics of aminolysis of I by methoxylamine in the presence of various buffers are described by the rate eq 2, in

$$k^{Am}_{obsd} = V\frac{1}{[Am]_T} = \frac{a_H}{K_a + a_H}\left\{[MA]_T\frac{K_{MA}}{K_{MA} + a_H}\left[k_0^{Am} + k_B^{Am}[B] + k_{MA}^{Am}[MA]_T\frac{K_{MA}}{K_{MA} + a_H}\right]\right\} \quad (2)$$

which $[MA]_T$ represents the total concentration of methoxylamine, K_{MA} is the dissociation constant for methoxylammonium ion, B is the base form of the buffer, and k_0^{Am}, k_B^{Am}, and k_{MA}^{Am} are apparent third-order rate constants associated with terms for the solvent, general base, and methoxylamine catalyzed aminolysis reactions, respectively. $[Am]_T$, $[AmH^+]$, and K_a are as defined above.

The observed rates of the aminolysis reactions are corrected for competing hydrolysis on the basis of uv assay of reaction products at $8\text{–}10t_{1/2}$. With the exception of triethanolamine (pH 7.5) and triethylenediamine buffers (pH 8.65), all runs were conducted between pH 6 and pH 7.4 with the formanilide product comprising less than 10% of the total product. At pH >7.5, hydrolysis becomes the predominant reaction. Hydrolysis of II also occasions a correction in k^{Am}_{obsd}, as discussed in the Experimental Section.

That the overall reaction represents methoxyaminolysis of I is shown by isolation and characterization of the product amidine II. The second-order dependence of k^{Am}_{obsd} on $[MA]_T$ is evident from the upward curvature of a plot of k^{Am}_{obsd} *vs.* $[MA]_T$ (Figure 2) and typically reflects aminolysis catalyzed by a second molecule of methoxylamine.[14] Data for the phosphate catalyzed reaction at various pH's (Figure 3) reveal the base form of the buffer as the catalytically active species. Accordingly, a plot of $k^{Am}_{obsd}/[MA]_B$

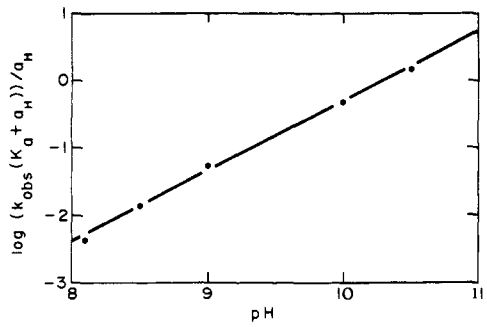


Figure 1. Log of observed first-order rate constants for hydrolysis of the protonated form of I as a function of pH: T = 25°; μ = 0.6, KCl.

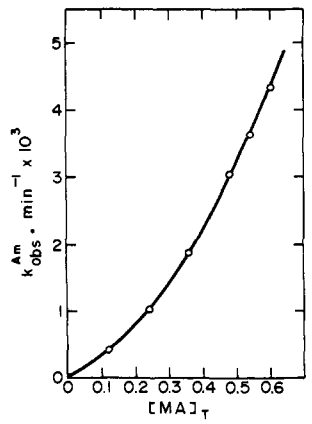


Figure 2. Observed first-order rate constants for methoxyaminolysis of I at various concentrations of methoxylamine: pH 6.7; T = 25°; μ = 0.6, KCl.

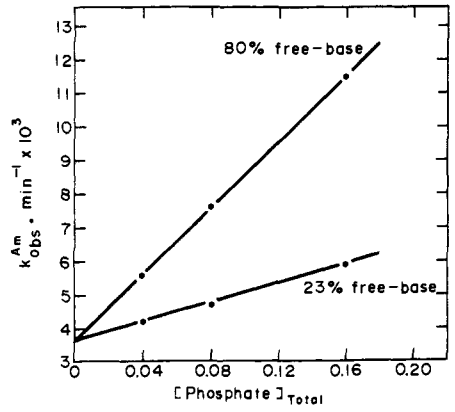


Figure 3. Observed first-order rate constants for methoxyaminolysis of I as a function of phosphate buffer concentration at 23% and 80% free-base form: T = 25°; μ = 0.6, KCl; $[MA]_T$ = 0.1 M. The solid lines are calculated from the rate constants in Table I according to eq 2.

vs. $[MA]_B$ (Figure 4) is linear with a slope of $k^{Am}{}_{MA}$ = 0.082 M^{-2} min^{-1} and intercept $k_0{}^{Am}$ = 2.25 × 10^{-2} M^{-1} min^{-1}, which are the apparent third-order and second-order rate constants for methoxylamine and spontaneous catalysis of aminolysis, respectively. Nucleophilic reaction of solution components other than methoxylamine is not evident in hydrolysis experiments and is assumed to be negligible in the aminolysis reactions. Catalytic constants for all buffers, except triethylenediamine, were determined in the pH range of 6 to 7.5. In this interval, $a_H \gg K_a$, $K_{MA} \gg a_H$, and eq 2 simplifies to eq 3. The values of $k_B{}^{Am}$ are obtained as the

$$k^{Am}{}_{obsd} = V\frac{1}{[Am]_T} = [MA]_T(k_0{}^{Am} + k_B{}^{Am}[B] + k_{MA}{}^{Am}[MA]_T) \quad (3)$$

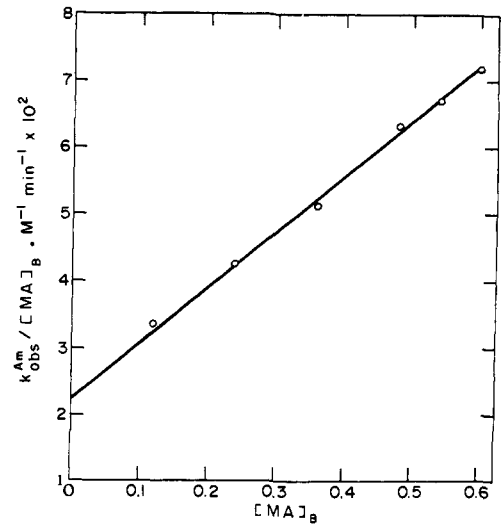


Figure 4. Apparent second-order rate constants for methoxyaminolysis of I as a function of free-base form of methoxylamine: pH 6.7; T = 25°; μ = 0.6, KCl.

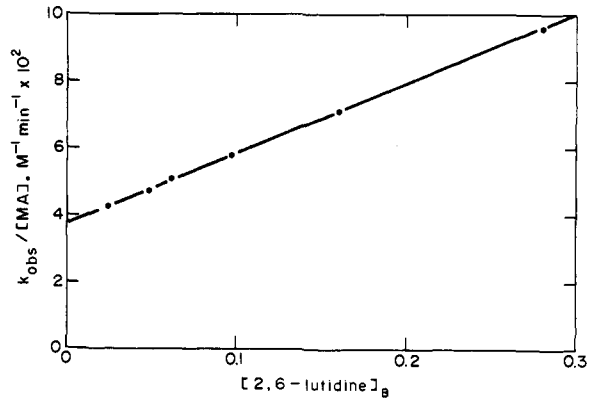


Figure 5. Apparent second-order rate constants for methoxyaminolysis of I as a function of concentration of free-base form of 2,6-lutidine: T = 25°; μ = 0.6, KCl; $[MA]_T$ = 0.2 M. The solid line is calculated from the rate constants in Table I according to eq 2.

slope of $k^{Am}{}_{obsd}/[MA]_T$ plotted as a function of [B]. Evaluation of the lutidine catalyzed reaction, as illustrated in Figure 5, is representative of the treatment. The apparent third-order rate constants, $k_B{}^{Am}$, are given in Table I. Note that the intercept of $k^{Am}{}_{obsd}/[MA]_T$ *vs.* [B] is the sum of the spontaneous and methoxylamine catalyzed aminolysis rates. A value for the spontaneous or solvent catalyzed reaction rate constant is obtained by subtracting $k_{MA}{}^{Am}[MA]$ (evaluated at the appropriate methoxylamine concentration) from the intercept. To allow comparison of the constants for solvent and buffer catalyzed reactions, the apparent second-order constant for solvent catalysis is divided by 55.5 M. The single value of $k_0{}^{Am}$ obtained alternatively as the intercept in Figure 4 is identical. The rate constants for general base catalyzed aminolysis are presented in the Bronsted relationship in Figure 6. Statistical corrections are not applied and, if applied, do not significantly alter the shape of the plot.[15]

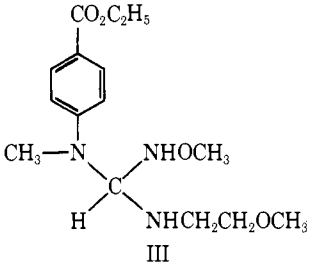
Discussion

Exclusive of the Bronsted plot, the aminolysis data do not demand a sequential, stepwise mechanism for conversion of I to the product amidine II since a change in rate-determining step is not evident. However, there is substantial indirect evidence that an orthoformamide such as III might be a discrete intermediate in the transamidination. In certain

Table I. Experimental Conditions and Rate Constants for General Base Catalyzed Methoxyaminolysis of I (T = 25°; μ = 0.6, KCl)

Buffer	pK_{BH^+}	$[B]_T$, M	% free base form	pH	No. of runs	k_B^{Am}, M^{-2} min^{-1} [d]
Hydroxide ion	15.7					$\leq 10^3$ [e]
Triethylenediamine (TED)	8.8[a]	0.05–0.15	41	8.65	3	0.232
Triethanolamine (TEA)	8.14[a]	0.05–0.15	17	7.43	3	0.256[e]
2,6-Lutidine (Lut)	6.88[a]	0.05–0.15	40	6.70	3	
		0.05–0.15	58	7.03	3	0.215
		0.05–0.15	80	7.50	3	
Phosphate (Phos)	6.62[a]	0.015–0.15	21	6.00	6	
		0.015–0.15	44	6.50	6	0.581
		0.05–0.15	66	6.90	3	
		0.015–0.15	72	7.00	6	
Pyridine (Pyr)	5.20[a]	0.10–0.20	98	6.99	3	0.19
Methoxylamine (MA)	4.70[a]	0.10–0.60	61	6.7	5	0.082
Phenylacetic acid (PhAc)	4.31[b]	0.15–0.25	>99	7.10	3	0.226
Methoxyacetic acid (MAc)	3.53[b]	0.10–0.25	>99	7.10	3	0.226
Chloroacetic acid (ClAc)	2.87[b]	0.10–0.20	>99	6.80	3	0.102
Cyanoacetic acid (CNAc)	2.47[b]	0.10–0.45	>99	7.23	3	0.076
Dichloroacetic acid (DClAc)	1.26[c]	0.10–0.50	>99	7.12	3	0.022
Trichloroacetic acid (TClAc)	0.66[c]	0.10–0.30	>99	7.10	3	0.021[e]
Trifluoroacetic acid (TFAc)	0.59[c]	0.10–0.50	>99	7.08	3	0.011[e]
H_2O	−1.74[b]	55.5				4.1×10^{-4} [f]

[a] Determined by half-neutralization of the buffer. [b] A. J. Kresge, H. L. Chen, Y. Chiang, E. Murrill, M. A. Payne, and D. S. Sagatys, *J. Amer. Chem. Soc.*, **93**, 413 (1971). [c] See ref 27. [d] As defined in eq 2; $[MA]_T$ = 0.1–0.2 M. [e] Upper limits as described in the Experimental Section. [f] Calculated as described in Results section.



III

favorable cases, substituted triaminomethanes have been isolated and characterized, but the instability of ortho amides with respect to conversion to amidines apparently precludes detectable accumulation in protic solvents.[16] Aminolysis of imidate esters and certain highly reactive acyl compounds[14] has been shown to proceed through metastable covalent addition compounds with discrete formation and decomposition steps.[17,18] Finally, formation and breakdown of III can be formulated without invoking intermediates which are sufficiently unstable to preclude a sequential and therefore demand a "concerted" mechanism.[17]

The rate law of eq 2 for general base catalyzed methoxyaminolysis of I demands a transition state involving the elements of neutral amidine, base form of methoxylamine, base catalyst, and a proton. Therefore, the rate law most likely represents general acid–base catalysis either of attack by MA on I or of the decomposition of protonated III. Rejection of rate-limiting catalyzed addition of MA is based on the following grounds. (1) The analogous addition of hydroxylamine or methoxylamine to ethylbenzimidate cation or of amines to a phthalimidium cation is not general base catalyzed.[18,19]

General base catalysis of MA attack on I would necessarily be a concerted process (i), in view of the instability of the amine anion ($pK_a \sim 30$) required in formulating the

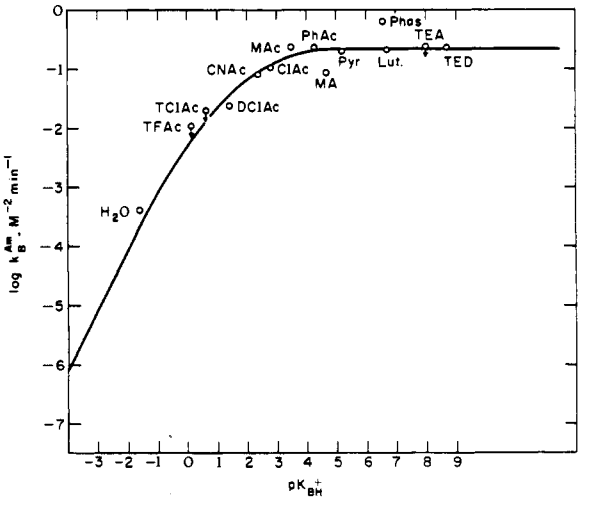
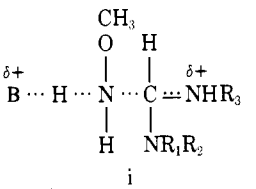


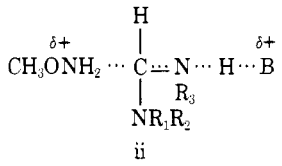
Figure 6. Bronsted plot of the apparent third-order rate constants for general base catalyzed methoxyaminolysis of I, T = 25°. Upper limits (see Experimental Section) are indicated by arrows. The catalytic constants and abbreviations are indicated in Table I. The solid curve is calculated from eq 6 using the parameters in Table II.



i

analogous stepwise process. For this mechanism, the limb of the Bronsted plot for catalysts $pK_{BH^+} > 2$ should have $\beta > 0$, since the catalysis would include both proton transfer and

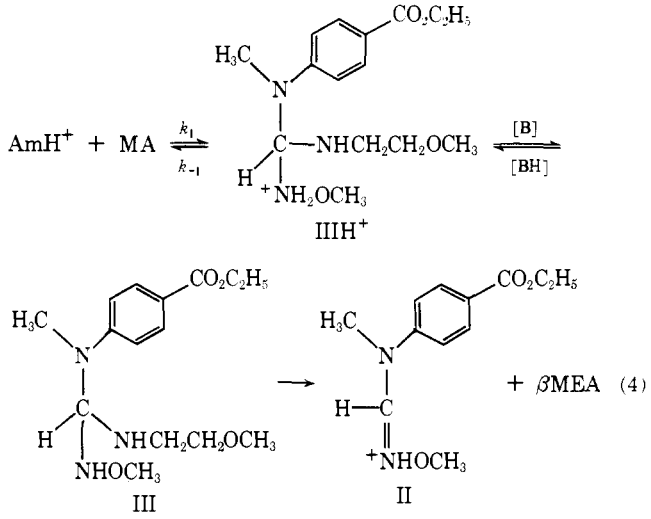
carbon–nitrogen bond formation. Moreover, the pK_a of the MA–AmH^+ adduct $IIIH^+$ is estimated as $\simeq 1.8$, indicating the protonated ortho amide to be sufficiently stable[20] so that concerted proton transfer and C–N bond cleavage which requires coupled multi-atom transfers and reorganization appear to offer no significant advantage over the stepwise process involving deprotonation of the adduct. (2) General acid donation of a proton to a distal nitrogen of the neutral amidine, concomitant with MA attack (ii), is discounted by the observation of general acid–base catalysis under experimental conditions where the protonated amidine ($pK_a \simeq 9$) comprises >90% of the total amidine. The rate law then is interpreted in terms of general base catalyzed reaction of protonated III or some subsequent, kinetically equivalent process.



ii

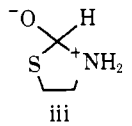
The Bronsted plot of the apparent third-order rate constants for general base catalysis of the aminolysis reaction cannot be adequately described by a single straight line but is best described as a curve contiguous with a well-defined limiting slope where $\beta \simeq 0$. Catalytic constants for amines limited by the experimental inaccessability of the appropriate pK_{BH^+} range nevertheless would lead to a β value significantly less than that for oxyanions if treated separately. Oxygen bases, moreover, are correlated only by a curve, in view of the fact that the upper limit of the rate constant for hydroxide catalysis, which is not observed, $k_{OH}^{Am} \leq 10^3\ M^{-2}\ min^{-1}$, falls below the line given by water and phenylacetic acid ($\beta = 0.64$) by at least three orders of magnitude. There is no basis from the present information for assigning a different mechanism to the water catalyzed reaction.

Similar Bronsted plots have been reported for catalysis of other nucleophilic displacement or condensation reactions which proceed through short-lived, covalent addition intermediates.[15,21,22] The apparent change in transition state with varying catalyst basicity and the occurrence of pK_{BH^+}-independent general acid–base catalysis are usually attributed to a mechanism featuring an intermediate of sufficiently short lifetime to preclude equilibration with respect to proton transfer from the components of bulk solvent, and, in the limit, imposition of a rate-limiting step involving transport and encounter of the intermediate and catalyst for at least one leg of the Bronsted plot.[17]



III II

The data for general base catalyzed methoxyaminolysis of I invite the above interpretation when considered in terms of a mechanism (eq 4) requiring general base catalyzed deprotonation of the initially formed ortho formamide $IIIH^+$ in the rate-limiting step. The feasibility of a transport process becoming kinetically significant is determined by the lifetime of $IIIH^+$ which, in turn, is determined by the magnitude of k_{-1}, the first-order rate constant for reversion of $IIIH^+$ to starting materials. Among supporting examples, the first-order rate constants for expulsion of the amine from iii ($pK_a = 9.1$) and the hydrazine group from iv



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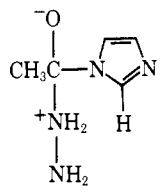


iv

($pK_a = 10$) are estimated to be $\geq 10^8\ sec^{-1}$.[21,15] Expulsion of methoxylamine from $IIIH^+$, assisted now by two adjacent nonbonding electron pairs rather than an oxyanion, should occur with a comparable rate constant. In contrast, the rate of bimolecular encounter of the intermediate and lyate species is estimated at *ca.* $10^3\ sec^{-1}$ at pH 7, thereby precluding equilibration of the intermediate with respect to proton transfer with solvent. Deprotonation of the methoxylamine nitrogen of $IIIH^+$ by both lyate and buffer species then becomes kinetically significant. At the limits of high and low pK_{BH^+} for an uncomplicated proton transfer mechanism, the rate-determining steps would be respectively diffusion-controlled encounter of $IIIH^+$ and a basic catalyst when the proton transfer is in the thermodynamically favorable direction ($\beta = 0$) and separation of the conjugate pair $\langle III \cdot BH^+ \rangle$ when proton transfer is unfavorable ($\beta = 1$). A Bronsted representation of the mechanism should undergo transition from $\beta = 1$ to $\beta = 0$ at a pK_{BH^+} which approximates that of the site of catalysis, *i.e.*, where $\Delta pK_a \simeq 0$ for the proton transfer.

The occurrence of the transition in the Bronsted plot at $pK_{BH^+} \simeq 2$ is consistent with the proposed mechanism and implicates the methoxylamine nitrogen ($pK_N = 1.8$ in the orthoformamide)[20] as the site of catalysis. A mechanism which is a kinetic equivalent of eq 4 features general acid catalyzed conversion of III to products with encounter-controlled (in the favorable direction) proton transfer to the βMEA nitrogen. This mechanism likewise is incompatible with the Bronsted data, however, in that it predicts a break at a much higher pK_{BH^+} ($pK_{N_2} > 4.8$ in the orthoformamide).[20] Rate-determining breakdown of III *via* a concerted mechanism involving general acid catalysis which becomes encounter limited with strong acids is compatible with the Bronsted data but is subject to the same criticism as the analogous mechanism for MA attack discussed above, *i.e.*, there is no apparent advantage for a concerted pathway since a stepwise route would be devoid of any prohibitively unstable intermediates. A mechanism involving preassociation of the catalyst with reactants is similarly discounted since such a mechanism predicts a substantial increase in the apparent pK_{N_1}.[22]

A more detailed view of the rate-limiting processes for catalysis by weak and strong bases is centered around the expanded mechanism of eq 5. The equation is formulated

$$IIIH^+ \underset{k_{-d}}{\overset{k_d[B]}{\rightleftharpoons}} \langle IIIH^+ \cdot B \rangle \underset{k_{-pt}}{\overset{k_{pt}}{\rightleftharpoons}} \langle III \cdot BH^+ \rangle \underset{k_{-x}}{\overset{k_x}{\rightleftharpoons}}$$

$$\langle III'H^+ \cdot B \rangle \overset{k_{pr}}{\rightleftharpoons} AmH^{+\prime} + \beta MEA \quad (5)$$

with the following definitions and assumptions. $IIIH^+$ and $III'H^+$ represent the ortho amide species protonated on the MA and βMEA nitrogens, respectively. The step designated k_x represents a composite of processes effecting conversion of the $\langle III \cdot BH^+ \rangle$ complex leading to $III'H^+$ *via* intracomplex reorganization or diffusional separation, reencounter, and protonation of the βMEA nitrogen of III. Protonation of the βMEA nitrogen is shown to be mediated by BH^+, as is supported below.

Let us first consider the possible behavior of catalysts within the limits $pK_{N_1} < pK_{BH^+} < pK_{N_2}$. Diffusion together of $IIIH^+$ and B will be rate-determining since the proton transfer step, k_{pt}, is strongly favorable thermodynamically and very likely exceeds the diffusion separation of $\langle IIIH^+ \cdot B \rangle$ estimated at 10^8–10^{10} sec^{-1}.[23] This line of reasoning is readily extended to include the case where $pK_{BH^+} > pK_{N_2}$, *i.e.*, aminolysis catalyzed by highly basic amines where proton transfer from BH^+ to the βMEA nitrogen in III becomes thermodynamically unfavorable. In order for $k_d[B]$ to remain rate-limiting, it is necessary that $k_{-d}\langle IIIH^+ \cdot B \rangle \ll k_x \langle III \cdot BH^+ \rangle$. Since the ratio of $\langle III \cdot BH^+ \rangle$ to $\langle IIIH^+ \cdot B \rangle$ is simply K_N/K_{BH^+}, whereas the proton transfer component of k_x is proportional to K_{BH^+}/K_N, the rate of the second proton transfer step remains invariant for bases of $pK_{BH^+} > 5$. On the other hand, the concentration of $\langle IIIH^+ \cdot B \rangle$ decreases so that the term $k_{-d}\langle IIIH^+ \cdot B \rangle$ decreases. Since diffusion together of $IIIH^+$ and B is already rate-determining for bases with limits $2 < pK_{BH^+} < 5$, no change in the rate step is anticipated, *i.e.*, the catalysis remains encounter limited.

The above scheme features general base mediation of the two sequential proton transfers in order to satisfy the inferred requirement for facile catalysis of the second proton transfer step. Allowing BH^+ to be replaced by water provides $\langle III \cdot H_2O \rangle$ whose rate of decomposition will become competitive with that for $\langle III \cdot BH^+ \rangle$ as the pK_{BH^+} approaches that for water. Since the ratio of $\langle IIIH^+ \cdot B \rangle / \langle III \cdot H_2O \rangle$ is *ca.* $2 \times 10^{-3} K_{BH^+}/K_{N_1}$, whereas the proton transfer component of k_x is not greater than $10^{12}K_{BH^+}/K_{N_2}$, it can be shown that the rate of diffusion separation, $k_{-d}\langle IIIH^+ \cdot B \rangle$, is *ca.* equal to $k_x \langle III \cdot H_2O \rangle$ for catalytic species of $pK_{BH^+} \simeq 8$. However, substitution of BH^+ by water will not become the dominant catalytic mode for decomposition of III until $pK_{BH^+} \simeq 13$, at which the higher concentration of water offsets the former's higher rate of proton transfer. Similarly substitution of H_3O^+ for BH^+ is unlikely since at pH 7, the concentration of catalyst generally exceeds that of H_3O^+ by at least a factor of 10^3. Although it is conceivable that the required proton transfers occur within a one encounter complex for those situations where the rate of proton transfer exceeds that for diffusional separation, the present data do not distinguish between these alternatives.

Let us now consider the nature of the rate-limiting step for catalysts of $pK_a < 2$, where diffusion is not rate limiting. It is unlikely that all three encounter complexes of eq 5 are at thermodynamic equilibrium, *i.e.*, k_{pr} is rate limiting, since the rate of proton transfer from the βMEA nitrogen of III to the catalyst for weakly basic catalysts is probably less than that for decomposition of the ortho amide species $III'H^+$. Moreover, this condition would lead to an observed $\beta = 0$ owing to the occurrence of two consecutive equilibria with pK_{BH^+} dependencies of +1 and −1 preceding k_{pr}. Assuming the diffusional component of k_x to be rate determining, the steady-state rate eq 6 can be derived that affords a satisfactory fit to the Bronsted plot. The best fit to the data is achieved using the constants of Table II and the expression $\log k_{pt} = 10 + 0.5\,(\Delta pK_a)$ to describe the rate of proton transfer. In the limit of $pK_{BH^+} \ll 2$, the denominator term $k_{-d}K_{BH^+}/K_{N_1}$ dominates, and the Bronsted slope of unity is manifest from equilibration of the $\langle IIIH^+ \cdot B \rangle$ and $\langle III \cdot BH^+ \rangle$ complexes, with the diffusion component of k_x rate limiting.

$$k_B^{Am} = \frac{k_1}{k_{-1}}\left[\frac{k_x k_d}{\frac{k_{-d}K_{BH^+}}{K_{N_1}} + \frac{k_{-d}k_x}{k_{pt}} + k_x}\right] \quad (6)$$

Table II. Rate and Dissociation Constants for Evaluation of Equation 6 and Free Energies

$k_1 = 10^{-2}\ M^{-1}\ sec^{-1}$	$k_x = 3.9 \times 10^{10}\ sec^{-1}$ [a]
$k_{-1} = 10^{11}\ sec^{-1}$	$K_{N_1} = 10^{-1.8}\ M$ [b]
$k_d = 10^{10}\ M^{-1}\ sec^{-1}$	$K_{N_2} = 10^{-4.8}\ M$
$k_{-d} = 3.9 \times 10^{10}\ sec^{-1}$ [a]	$K_{N_3} = 10^{-1.5}\ M$

$$\log k_{pt} = 10 + 0.5(\Delta pK)$$

$$\log k_{pt} = 12 - \log (K_{BH^+}/(K_N + K_B))$$ [c]

[a] "Best fit" value to data with the assumption of $k_{-d} = k_x$. [b] Calculated as in ref 20, a value of $K_{N_1} = 1.8$ was used in evaluation of eq 6. [c] This function was used in evaluation of free energies where ΔpK is large.

The degree of curvature for the theoretical curve in the vicinity of the break point is a consequence of the functions utilized to describe k_{pt}. The data obtained for acid–base proton transfer, although generally accommodated with $\beta = 0.5$ in the region $\Delta pK_a = 0$, can in some instances, particularly deprotonation of ammonium ion by oxyanions, be accurately approximated by a hyperbolic function.[24] The position of the curve generated by eq 6 relative to the abscissa depends in part on the ratio K_{BH^+}/K_{N_1}; values for pK_{N_1} of 1.8 and 2.5 (not shown) afford satisfactory description of the data; for the latter case, the point representing water catalysis deviates positively by *ca.* 1.3 log units. Since pK_{N_1} is estimated as 1.8, it appears, given the available data and uncertainty in the choice of a function for k_{pt}, that the agreement between the observed and calculated values for K_{N_1} are satisfactory.

Achievement of the diffusion limit in the overall reaction provides an absolute second-order rate constant which, in turn, permits evaluation of the preceding equilibrium for MA addition to I. Taking $k_B^{Am} = 0.22\ M^{-2}\ min^{-1}$ as an average of the catalytic constants where $\beta = 0$ and, again assuming that this represents diffusional encounter of $IIIH^+$ and B ($k_d \simeq 10^{10}\ M^{-1}\ sec^-$), one can evaluate the equilibrium of eq 7. The resulting value, $K_{eq} = 3.7 \times 10^{-13}$

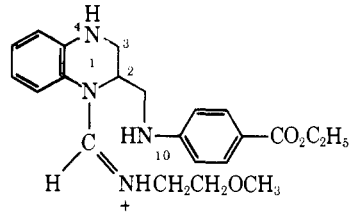
$$AmH^+ + MA_B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} IIIH^+ \quad (7)$$

$$K_{eq} = \frac{[IIIH^+]}{[AmH^+][MA]_B}$$

M^{-1}, intimates the instability of the protonated orthoformamide $IIIH^+$. For $IIIH^+$ to exist as a discrete intermediate, the first-order rate constant k_{-1} must not exceed $\sim 10^{12}\ sec^{-1}$;[17] and, in order that methoxylamine attack not become rate limiting overall, $k_{-1} > 10k_d[B]$, or k_{-1} must not be less than $\sim 10^{10}\ sec^{-1}$. With these limitations and K_{eq}, upper and lower bounds are estimated to be $k_1 = 10^{-1}$–$10^{-3}\ M^{-1}\ sec^{-1}$ and $k_{-1} = 10^{10}$–$10^{12}\ sec^{-1}$.

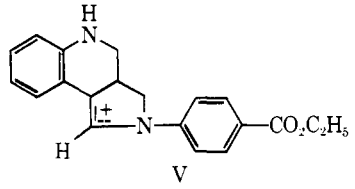
Since expulsion of *N*-methylbenzocaine (NMB) is not observed, a preference for protonation and expulsion of the most basic amino substituent in the $\langle$orthoformamide–$BH^+\rangle$ complex is indicated. The use of an α-effect amine nucleophile of low pK_a and a more basic (by 2–4 pK units) leaving group perhaps exaggerates the observed preference. However, a similar aminolysis reaction studied both in aqueous buffers and in DMSO using nuclear magnetic resonance affords corroborative data.[13] Hydrolysis of the ami-

dine IV in aqueous buffers proceeds through addition of hydroxide ion to the methenyl carbon and conversion of the

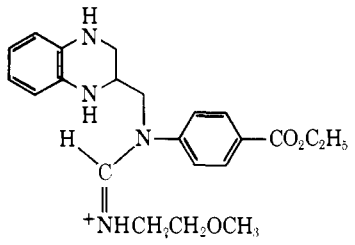


IV

resulting intermediate to the *N*-(1-formyl)tetrahydroquinoxaline derivative and β-methoxyethylamine. A pH ≤ 6.5, intramolecular aminolysis by the N-10 nitrogen competes with addition of hydroxide ion as evidenced by appearance of the amidine V. Since formation of the N-10 amidine VI

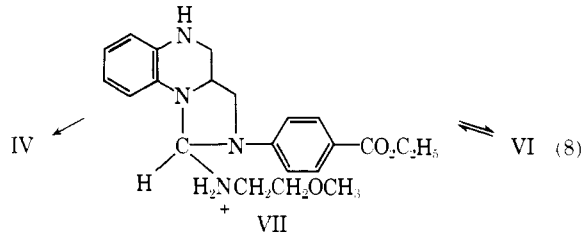


V



VI

is not observed, the more basic βMEA is implicated as the favored leaving group, although rapid, reversible expulsion of the N-1 nitrogen cannot be entirely discounted. The addition of 2 equiv of βMEA to a DMSO solution of V in the nmr probe reveals rapid formation of VI followed by slower isomerization of VI to IV. Under these conditions, covalent



(8)

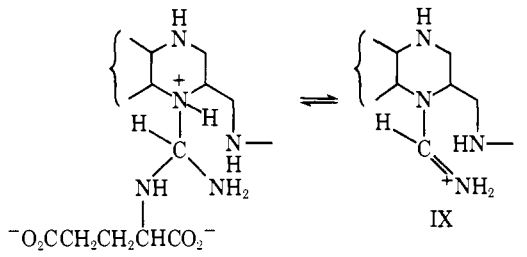
VII

addition of βMEA is unproductive, and product formation must reflect competition between expulsion of the N-1 and N-10 nitrogens from the respective tautomers of VII. Assuming that the relative pK_a's of the two potential leaving groups are not inverse in DMSO, the more basic amine leaves preferentially. Since formation of VI is reversible and, in analogy to the N-1 and N-10 formyl derivatives, the N-1 amidine is the more stable, expulsion of the less basic nitrogen (N-10) is eventually achieved.

These results, when considered together with the proposed mechanism for aminolysis of I, are most easily rationalized by assigning transport and proton transfer processes exclusively the role of determining the mode of decomposition for highly reactive orthoformamides. In short, within a complex of neutral orthoformamide and general acid catalyst, the site of C–N bond cleavage is dictated by the equilibrium or rate of protonation of each of the three possible sites. Two limiting cases were obtained. (1) Where the catalyst $pK_{BH^+} \ll pK_N$, in principle the rate of protonation of all three nitrogens will be identical, regardless of their respective basicities, so that the product amidines would form on a statistical basis. (2) Where the catalyst $pK_{BH^+} \gg pK_N$, the rate of protonation will reflect the intrinsic basicities of the various amines so that the more basic amine will be more rapidly protonated and expelled preferentially. Within the Eigen context, the first case corresponds to rate-limiting diffusion encounter of III and BH^+, whereas the second is equivalent to rate-determining diffusion separation of $\langle IIIH^+ \cdot B \rangle$, the degree of protonation of the amine being a function of their respective basicities relative to the catalyst. This analysis assumes the absence of steric effects or other molecular interactions that may direct protonation to a particular heteroatom. A consequence of this mechanism is observed in the kinetic product control found in the aminolysis and hydrolysis of the bridged methenyl salt V[12,13] as well as the hydrolysis of VI.

Construction of a free-energy reaction coordinate diagram (Figure 7) illustrates the decomposition mode of the intermediate ortho amide as a function of the pK_N of the leaving amine. Free energies are evaluated for catalysis by hypothetical buffers of $pK_B = 6$ and -2, *i.e.*, the limiting cases for strong and weak bases, and are subject to the assumptions that (1) the step designated k_x represents a reorganization diffusion within the $\langle III \cdot BH^+ \rangle$ complex; (2) each of the diffusion steps on a given pathway, including interconversion of complexes which differ only in orientation (k_d, k_{-d}, and k_x), are numerically equivalent to their counterparts on the alternate pathways; (3) the rate constants for C–N bond cleavage leading to the three possible amidines are equal, *i.e.*, $\beta_{lg} = 0$; and (4) the rate coefficient for proton transfer is calculated from $\log k_{pl} = 10 + 0.5(\Delta pK_a)$ as expressed above. Inspection of the resulting diagram for buffer of $pK_B = 6$ reveals preferential expulsion of the most basic leaving group. This corresponds to the experimentally observed result of <5% NMB expulsion for methoxylaminolysis in phosphate buffer. Furthermore, whenever expulsion of the most basic amine is reversible, the next most basic amine will be expelled. In terms of actual rate or equilibrium within the encounter complex, the outcome will be the same, *i.e.*, of the three possible catalyst–amine pairs, the one with more basic amine will dominate in the product distribution. It would be instructive to examine the reverse aminolysis, *i.e.*, aminolysis of II by βMEA. This reaction is not, however, experimentally realizable owing to competing hydrolysis. A system wherein the βMEA moiety is covalently linked to the product amidine is currently under investigation, in hopes of substantiating the proposed basis for leaving group abilities.

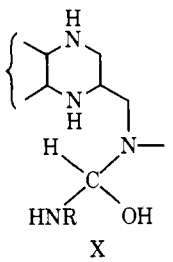
In the absence of data suggesting covalent participation of enzyme, the mechanisms for the enzymic reactions effecting (1) formimido group transfer from glutamic acid to H_4-folate, (2) cyclodeamination of 5-formimino-H_4-folate, and (3) 5,10-methenyl-H_4-folate donation of the methenyl carbon to glycinamideribonucleotide 5′-phosphate (GAR) to give the *N*-formylated product all formally demand intermediacy of orthoformamides related to VII and are subject to similar considerations.[5,6] Since aminolysis of amidines by non-α-effect amines is precluded by more rapid hydrolysis,[9,10] the reactions 1 and 3 demand enzymic mediation of the initial condensations. The net proton transfer required to transform VIII into 5-formimino-H_4-folate (IX) is thermodynamically favorable but must be enzymically directed to prevent expulsion of ammonia. Maximum catalytic efficiency would be achieved with one (or two) general acid–base site(s) of pK_a between ~1 and ~5 such that both transfer steps be thermodynamically favorable. Conversion of IX to 5,10-methenyl-H_4-folate, *via* the appropriate orthoformamide, also requires a favorable proton transfer.



VIII IX

The absence of strongly competing base sites in the intermediate is apparent from the quantitative nonenzymic conversion of IX to 5,10-methenyl-H_4-folate in aqueous acid.

The third enzymic reaction poses the problem of two similar mechanisms centered around either the 5- or 10-nitrogen of H_4-folate.[6] The results and conclusions above suggest that a single catalyzed proton transfer from the α-amino group of GAR to the 5-nitrogen represents the principal energy barrier for access of the 10-amidine analogous to VI. Presumably, the instability of the 10-amidine relative to the 5-isomer, as indicated in the model studies, would also be reflected in the relative rates of hydrolysis, making the former amidine the preferred intermediate for carbon transfer. Here, again, the intermediate (X) for hydrolysis



X

leading to carbon transfer requires protonation of the less basic nitrogen, which might render proton transfer to the 10-nitrogen the major barrier, thereby maximizing the efficiency of simple general acid catalysis. Involvement of the 10-nitrogen as the terminus for carbon transfer from H_4-folate is consistent with exclusive utilization of 10-formyl-H_4-folate in another enzymic formylation (5-formyl-H_4-folate is inactive) and the greater hydrolytic lability of the 10-isomer.[5]

Whether or not extrapolation to the enzymic reaction mechanisms is warranted, these data serve to illustrate further the critical balance between the basicities of the 5- and 10-nitrogens which is required in order that H_4-folate be, at once, a stable carrier and effective donor of the single carbon unit.

Experimental Section

Materials. Ethyl *p*-[(*N*-Formyl)methylamino]benzoate. This compound was prepared from ethyl *p*-(methylamino)benzoate and formic acid as previously described,[13] mp 56–57°: uv λ_{max} (pH 6.5–10.0) 265 nm (ϵ 17,300).

Ethyl *p*-{[*N*-(2-Methoxyethyl)formimidoyl]methylamino}benzoate (I). The procedure of Mandel and Hill[25] as modified by Benkovic, *et al.*,[13] was utilized to prepare this compound from ethyl *p*-[*N*-formylmethylamino]benzoate, 2-methoxyethylamine, and phosphorus pentachloride, mp 151–152° (hydrochloride salt): uv λ_{max} (pH 6.5) 260 nm (ϵ 20,000); λ_{max} (pH 10.5) 303 nm (ϵ 28,500).

Ethyl *p*-[(*N*-Methoxyformimidoyl)methylamino]benzoate (II). This compound was synthesized in a similar manner to I as previously described:[13] mp 68–69°; uv λ_{max} (pH 6.5–7.5) 305 nm (ϵ 26,000).

Products. Ethyl *p*-{[*N*-(2-methoxyethyl)formimidoyl]methylamino}benzoate (I) (200 mg, 0.67 mmol) was added to 15 ml of 0.2 *M* Tris, pH 8.00, containing 0.2 *M* methoxylamine and stirred for

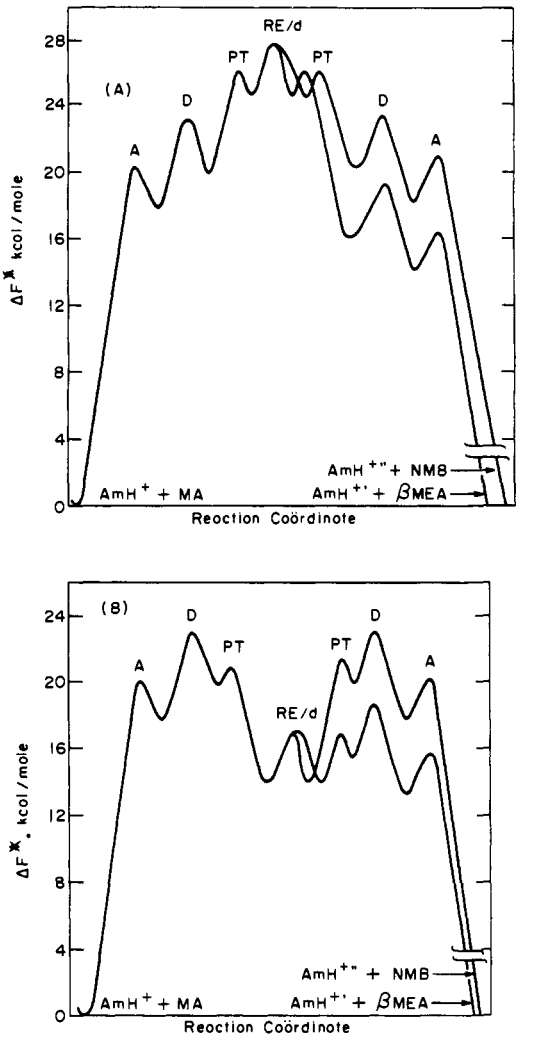


Figure 7. Free-energy reaction coordinate diagram based on the mechanism of eq 5 and 6 where k_x is a reorganization within or without the $\langle$III · BH$^+$$\rangle$ complex. The rate constants used are given in Table II. Values are calculated from $\Delta F^* = -1360 \log K + 17{,}400$ cal/mol, where k is a first-order rate constant (in reciprocal seconds) measured at 25°, and the maximum value of k ($\Delta F^* = 0$) is $10^{12.8}$ sec^{-1}. A catalyst concentration [B] = 0.1 *M* is assumed. (Top) catalysis by buffer of $pK_{BH^+} = -2$. (Bottom) catalysis by buffer of $pK_{BH^+} = 6$.

4 hr. The precipitate which formed was centrifuged, washed with water, and dried *in vacuo* to give a white solid (90 mg, 57%). This compound was identical (melting point, ir, nmr, uv) with ethyl *p*-[(*N*-methoxyformimidoyl)methylamino]benzoate (II).

To establish an upper limit for the amount of *N*-methylbenzocaine formed in the aminolysis reaction, the reaction was run in 25 ml of 0.12 *M* phosphate buffer, pH 6.75, 0.2 *M* methoxylamine, and 5×10^{-4} *M* I. After 5 half-lives, the reaction was extracted with diethyl ether (3×25 ml). The extract was dried over anhydrous potassium carbonate, and the solvent was removed on a rotary evaporator. The residue was dissolved in 1 ml of diethyl ether and gas chromatographed at 190° using a Barber Coleman Model 500 with a 6 ft × 0.25 in. glass U-column packed with 10% EGSS-X on 100/120 Gas Chrom Q. The instrument was equipped with a flame ionization detector. A control run employed an identical procedure in which 5×10^{-5} *M* *N*-methylbenzocaine was substituted for I. The chromatography indicated less than 5% of the aminolysis of I to proceed with expulsion of *N*-methylbenzocaine.

Kinetics. The instruments employed have been previously described.[26] All reagents used were reagent grade.

Kinetic runs (25°) were initiated by addition of 0.02 ml of a stock solution of the desired amidine hydrochloride salt in acetonitrile to a cuvette containing 0.1 *M* methoxyamine in 2 ml of aqueous buffer. The final reactant concentration was 5×10^{-5} *M*, μ = 0.6, KCl. The course of the aminolysis reaction was followed by

observing the change in OD at 305 nm with time through 8–10 half-lives. The pH of the initial and final solutions indicated a ΔpH < 0.02 unit. The reactions displayed pseudo-first-order kinetics, and k_{obsd} (aminolysis + hydrolysis) was taken as the slope from a plot of ln $(OD_\infty - OD_t)$ *vs.* time. The per cent aminolysis *vs.* hydrolysis was determined from the ultraviolet scan of the reaction after 8–10 half-lives by use of extinction coefficients at 260 and 305 nm of ethyl *p*-[(*N*-formyl)methylamino]benzoate and product amidine. The corrected k_{obsd} (aminolysis) was obtained from k_{obsd} × per cent aminolysis.

At pH <6.3, hydrolysis of the product amidine II proceeds at a rate which is significant relative to the aminolysis of I under these conditions. The hydrolysis is buffer catalyzed and gives rise to *N*-formyl-*N*-methylbenzocaine which exhibits no appreciable absorption at 305 nm. As a consequence, the observed value of OD_∞ at 305 nm is erroneously low by as much as 15% in some cases, and the observed first-order rates exhibit slight deviations from linearity after 2–3 half-lives. In such cases, the value of OD_∞ used in graphic determination of observed first-order rate constants is calculated from the known extinction coefficients of I and II at 260 and 305 nm, respectively, and the initial concentration of I, as determined by uv scan at $t \simeq 0$. The resulting plots of log $(OD_\infty - OD_t)$ *vs.* time are linear to at least 4 half-lives and afford a more reliable value of k^{Am}_{obsd}.

Upper limits on catalytic constants, $k_B{}^{Am}$, for buffer species exhibiting no observable catalysis are based on the assumption that a 20% increase in the observed rate relative to the spontaneous rate might not be detected.

The pK_a of amidine(I) was determined spectrophotometrically at 303 nm by the method of Albert and Serjeant[27] and was found to be 8.98 ± 0.04.

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(3) NIH Postdoctoral Fellow, 1972–1973.
(4) NIH Career Development Awardee.
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(20) The value of pK_{N_1} is estimated to be 1.8 ± 0.3. The pK_a' of *N*-methylmethoxylamine is calculated as 5.1 from ρ = 8.4 for the dissociation of substituted ammonium ions, σ = −0.05 for CH_3, and pK_a' = 4.7 for MA. Replacement of two hydrogens with two NH_2 (σ = 0.10) gives pK_{N_1} = 3.4. The effect of substituting two hydrogens with CH_3 and *p*-$COOC_2H_5C_6H_5$ is to lower the pK_a' of ammonia (10 − 2.7) units. Attenuation for transmission through N and C with fall off factors of 2 and 2.5 reduces this base weakening effect by 7.3/5.0 or 1.5 units. Substitutions at the hydrogen of the second NH_2 by $CH_3OCH_2CH_2$ lowers the pK_a' of ammonia (10 − 9.7) units; correction for transmission reduces this factor to 0.1. The total correction is ΔpK_a = 1.6 or pK_{N_1} = 1.8. The values of pK_{N_2} and pK_{N_3} are estimated on a similar basis to be 4.8 ± 0.3 and 1.5 ± 0.3, respectively.
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