Studies on Models for Tetrahydrofolic Acid. 8. Hydrolysis and Methoxyaminolysis of Amidines

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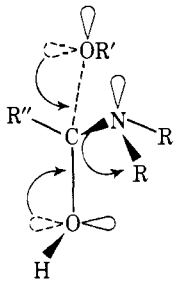
Abstract: The hydrolyses and methoxyaminolyses of a series of cyclic amidines and an acyclic analogue were examined in order to assess the possible importance of stereoelectronic control on the breakdown of the ensuing tetrahedral intermediates and to identify kinetically significant steps between these intermediates and the respective kinetic or thermodynamic isomers. All cyclic amidines react to form initially the product of kinetic control despite apparent conformational restrictions followed by decay to the thermodynamic isomers. The latter process unlike formation of the kinetic isomer is subject to general catalysis. Net formyl transfer to the attacking methoxyamine was also demonstrated in employing a cyclic amidine. The implication of these findings to the enzyme-catalyzed transfer of a one carbon unit from 5,10-methenyltetrahydrofolate to glycinamide ribonucleotide is discussed.

Previous studies related to the breakdown of orthoamide or tetrahedral intermediates formed in methoxyaminolysis or hydrolysis of formamidines have indicated initial formation of kinetic products based on prototropic control, i.e., preferential expulsion of the most basic amine and subsequent isomerization to the thermodynamically favored isomer.[1a] In relationship to the overall net formyl transfer from 5,10-methenyl-FH_4 to glycinamide ribonucleotide in the purine biosynthetic pathway, these observations lead one to invoke either an enzyme-mediated perturbation of the relative basicities of the involved amino groups (pK_a(N-5) = 4.8, pK_a(N-10) = −1.3)[1b] or equilibration before product release in order to account for net transfer. In their absence the *N*-10-formyl-FH_4 would be the immediate product of kinetic control.

Conceivably, another mechanism of action by the enzyme may involve a freezing of rotation in the tetrahedral intermediate to favor one conformation in which the lone pairs in the tetrahedral intermediate are aligned to expel preferentially one leaving group to give directly the anticipated (and observed) product(s). Lehn and Deslongschamps have recently demonstrated the importance of stereoelectronic control in directing the decomposition of tetrahedral intermediates formed in ester and amide hydrolyses.[2a,b] According to the principle of stereoelectronic control, the cleavage of a C–O or C–N heteroatom bond is allowed only if lone pairs on the other two heteroatoms (O or N) in the tetrahedral intermediate are oriented antiperiplanar to the bond undergoing fission; e.g., in amide hydrolysis,[2b] conformer **1** favors the expulsion of the



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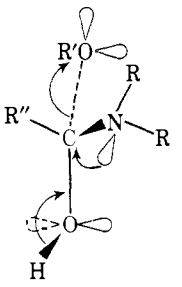

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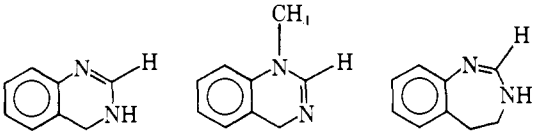
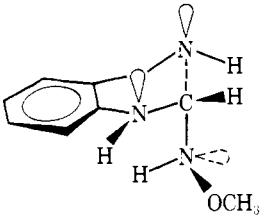
amino group to give the ester, whereas conformer **2** favors expulsion of alcohol. Supplemental to the above hypothesis are the postulates that (1) the energy barrier for the stereoelectronic cleavage of a tetrahedral intermediate is much lower than that for rotation to other conformers; (2) protonation and ejection of the amine leaving groups are synchronized; and (3) the O–H bond is equivalent to a lone orbital.

With this principle in mind, we have undertaken the synthesis and study of the hydrolysis and methoxyaminolysis of cyclic formamidines which incorporate restrictions on the number of possible conformers that may exist in the intermediate orthoamide species. For example, microscopic reversibility requires that the initial intermediates formed in the methoxyaminolysis of I or II have the lone pairs in the anilino



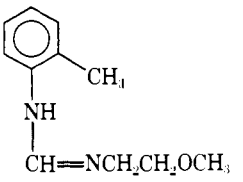
I II III

and benzylamine moieties in an antiperiplanar orientation relative to the methoxyamine nitrogen. However, in this conformer (Ii) the lone pair on the anilino nitrogen is prevented



Ii

from readily assuming an antiperiplanar relationship to the other carbon heteroatom bond due to a restriction of rotation imposed by the six-membered ring and conjugation with the aromatic ring. Expulsion of either the benzylamino moiety (the product of kinetic control) or the anilino nitrogen (the product of thermodynamic control) thus requires a conformational change. Comparison of the rates of hydrolysis and/or methoxyaminolysis and the nature of product formation for I, II, and III to the rotationally less restricted acyclic analogue IV should provide an assessment of the magnitude of the



IV

stereoelectronic factor in determining the mode of breakdown of orthoamide tetrahedral intermediates. The cyclic systems also furnish an opportunity to investigate a possible kinetic to thermodynamic product equilibration.

Results

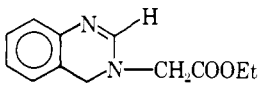
Hydrolysis Products. The hydrolyses of I–IV were allowed to proceed for periods of time equal to 10 half-lives, as measured spectrophotometrically. The products obtained by hydrolysis of I were isolated from buffers of pH 8.2 [tris(hydroxymethyl)aminomethane (Tris)] and pH 10.2 (carbonate) at 50 °C. Under both conditions, the product obtained was the thermodynamic product, *o*-amino-*N*-formylbenzylamine (Ia), as identified by nuclear magnetic resonance (NMR). Product identification by NMR in $CDCl_3$ was unambiguous owing to the doublet observed at δ 4.3 (J = 6 Hz) corresponding to the benzylic protons of the N-formylated benzylic side chain[3] compared with δ 3.8 (s) for the benzylic protons in the parent diamine.

Hydrolysis of the seven-membered formamidine III gave β-(*o*-aminophenyl)-*N*-formylethylamine as a brown oil: NMR ($CDCl_3$) δ 2.69 (t, J = 7 Hz), 3.40 (t, J = 7 Hz), 8.10 (s, -NHC*H*O). Structural assignment was possible through a comparison of the NMR spectra of the hydrolysis product with that of the parent diamine, β-(*o*-aminophenyl)ethylamine; the ethylamino side chain in the parent diamine appears at δ 2.4–3.1 ($CDCl_3$) as a complex multiplet indicative of the unsubstituted side chain. In addition, the onset of the aromatic region in the parent diamine occurs at approximately δ 6.5 due to the free amino group, as in the spectrum of the hydrolysis product of III. Due to these characteristic spectral patterns, the distinction between the kinetic and thermodynamic products is unambiguous. However, spectrophotometric evidence (see Kinetics) suggested that breakdown occurred initially to produce the kinetic isomer. Partial hydrolysis of III in 0.075 M carbonate buffer (μ = 1.0), pH 10.17 at 50 °C for 1 h (about 60% completion) gave a brown oil (65%). The NMR ($CDCl_3$) indicated a mixture of kinetic hydrolysis product (δ 2.4–3.0, multiplet; δ 8.42, s, -NHC*H*O), III, and the thermodynamic hydrolysis product in the ratio of approximately 1:1:1. Appearance of the kinetic hydrolysis product was further substantiated by thin-layer chromatography (TLC).

Attempts at intercepting a kinetic isomer in the hydrolysis of I or II that could arise from the protonation and expulsion of the more basic benzylic nitrogen proved to be unsuccessful. Hydrolysis of I or II at times corresponding to ≤20% of completion gave only the thermodynamic product Ia or IIa and unhydrolyzed amidine.

Hydrolysis of the acyclic formamidine IV at pH 10.2, 50 °C, gave the kinetic product anticipated on the basis of prototropy, *o*-methylformanilide, identical in all respects (NMR, IR, UV, MS) to an independently prepared sample.

Hydrolysis of the substituted 3,4-dihydroquinazoline V in Tris buffers, pH 8.3 (50 °C, μ = 1.0), for periods ranging from 20 to 50 h (~40 to 100% completion) led to the isolation of the thermodynamic hydrolysis product (by NMR). A downfield shift of the benzylic and the methylene protons of the ethylacetoxy moiety relative to the parent diamine (shift of 0.6–0.8 ppm) in addition to the appearance of the formyl proton of the hydrolysis product at δ 8.10 supports hydrolysis of V to the



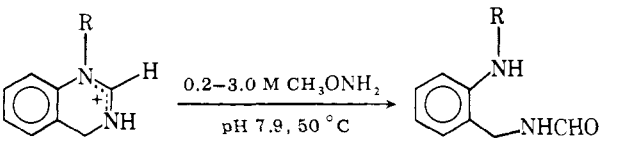
V

thermodynamic formyl isomer. The synthesis of V was undertaken in order to determine whether a diminution in the ΔpK_a between N_α and N_β (here $\Delta pK_a \sim 2$)[6] would allow for the interception of the kinetic hydrolysis product. Since attempts to observe formation of the hydrolysis product of kinetic control from V were unsuccessful, no detailed kinetic studies were performed on V.

In order to determine independently whether the kinetic product from hydrolysis of the 3,4-dihydroquinazoline (I), if formed, could have an appreciable lifetime under the conditions of hydrolysis, two precursors of the postulated kinetic isomer, *o*-(*N'*-formylamino)-*N*-trifluoroacetylbenzylamine (VI) and *o*-(*N'*-formylamino)-*N*-carbobenzyloxybenzylamine (VII), were synthesized. The trifluoroacetyl and carbobenzyloxy protecting groups were selected due to their ease of removal. A variety of conditions were chosen in which the removal of the trifluoroacetyl group was effected at pH values from 7.9 to 12.1 (50 °C). The products isolated at various time intervals, however, were only *o*-amino-*N*-formylbenzylamine and 3,4-dihydroquinazoline (I) (pH values ≤9) and solely *o*-amino-*N*-formylbenzylamine at pH values greater than 10. Estimates of the rate of loss of the trifluoroacetyl group and subsequent isomerization of the kinetic product obtained spectrophotometrically at 282 nm (pH 8–12, 50 °C, μ = 1.0) indicate that the rate of isomerization and consequently of removal of the trifluoroacetyl group exceeds that for the hydrolysis rate of I. Removal of the trifluoroacetyl group and formyl transfer to the benzylamino moiety was monitored by the increase in OD at 282 nm and compared directly with the rate of hydrolysis of I under identical conditions. For example, in 0.05 M carbonate buffer (μ = 1.0) at pH 10.16, 50 °C, VI loses the trifluoroacetyl group and isomerizes in a biphasic process to *o*-amino-*N*-formylbenzylamine ($\Delta\epsilon_{282\ nm}$ = 2640) with an initial rate constant of ca. 2.0×10^{-1} min^{-1} followed by a slower second phase. Under identical conditions, the rate of hydrolysis of I was measured to be 7.6×10^{-3} min^{-1}. Repetitive UV scans of the hydrolysis of VI in Tris buffers in pH 8 range reveal the formation of I and slower hydrolysis of the cyclic amidine. Similarly, attempts at removing the carbobenzyloxy-protecting group from VII by catalytic hydrogenation (room temperature) at pH 8–12 for periods from 15 min to 1 h led to the isolation of only mixtures of 3,4-dihydroquinazoline (I) and *o*-amino-*N*-formylbenzylamine (Ia).

Methoxyaminolysis Products. Preparative scale methoxyaminolyses of I–IV were performed in 0.10–0.20 M phosphate buffers at pH 7.9, 50 °C, with methoxyamine present in concentrations from 0.3 to 2.0 M. Again, the acyclic formamidine gave the anticipated product of kinetic control, *o*-[(*N*-methoxyformimidoyl)amino]toluene. Under these conditions III gave the thermodynamic methoxyaminolysis product, β-(*o*-aminophenyl)-*N*-methoxyformimidoylethylamine [NMR ($CDCl_3$) δ 2.42 (q, J = 7.5 Hz), 2.96 (t, J = 7 Hz), 3.7 (s, -$NHOCH_3$)] and a small amount (≤10%) of β-(*o*-aminophenyl)ethylamine resulting from hydrolysis of the methoxyaminolysis product. Again, product structural assignment was possible through a comparison of the NMR absorption of the ethylamino side chain in the product with that of the unsubstituted parent diamine as well as high-field aromatic absorption in the product (δ 6.5) indicative of the free anilino function.

Surprisingly, under the above conditions, the six-membered cyclic amidines I or II apparently did not undergo methoxy-



I, R = H
II, R = CH_3

Ia, R = H
IIa, R = CH_3

aminolysis after prolonged periods of time. The sole product isolation was that arising from hydrolysis *not* methoxyaminolysis of the six-membered amidines, in yields ranging from 60 to 80%. Once again, the structures assigned to the hydrolysis products are those of thermodynamic control, supported by NMR spectra.

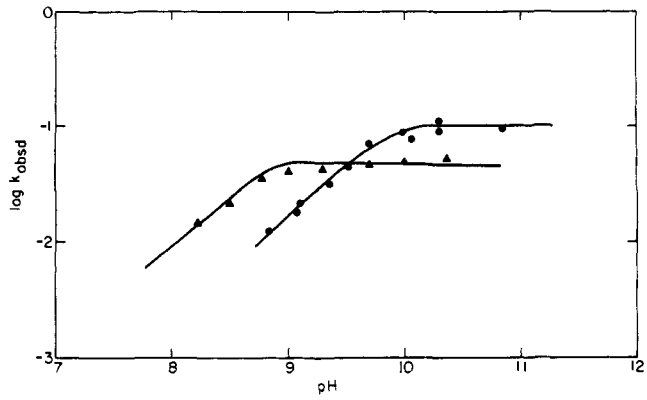


Figure 1. Log of observed first-order rate constants for hydrolysis of III (●) and IV (▲) as a function of pH, T = 50 °C, μ = 1.0 (KCl). Rate constants obtained in the Bruice–Maley cell in conjunction with the pH-stat apparatus.

Table I. Rate and Equilibrium Constants for the Hydrolysis of I, III, and IV as Described by Equations 1[a] and 2

	$k_{OH} \times 10^{-3}$, M^{-1} min^{-1}	k_B, M^{-1} min^{-1} [b]	$k_{B\text{-}OH}$, M^{-2} min^{-1} [b]	pK_a[c]
I		0.27 ± 0.02[d]	3.28 (±0.12) × 10^3 [d]	8.98 ± 0.02
III	3.52 ± 0.07[e]	0.10 ± 0.01[d]	1.01 (±0.03) × 10^3 [d]	9.83 ± 0.03
IV	2.10 ± 0.26[e]			8.70 ± 0.08

[a] Values obtained by Curfit[45] to eq 1. Maximum errors for parameters are computed by adopting a maximum tolerance of 10% in k_{obsd} values for III and 15% for IV. [b] Measured in carbonate buffer. [c] Spectrophotometric pK_a determined[6] (50 °C, μ = 1.0 (KCl)) for I = 8.98 ± 0.02 (lit.[9] pK_a = 9.19 ± 0.07 at 20 °C) and for IV = 8.51 ± 0.03. [d] Formation of the thermodynamic isomer. [e] Formation of the kinetic isomer.

NMR Methoxyaminolysis Experiments. The methoxyaminolyses were performed in an aprotic solvent in order to obviate hydrolysis and in an attempt to detect rapidly formed isomers. Amidine hydrochloride salts I, III, and IV were dissolved in ~0.5 mL of Me_2SO-d_6 in the sample tube and spectra recorded. Two equivalents of methoxyamine was added, and the tubes were shaken and quickly reinserted into the probe. In all cases, the decrease in the intensity of the formamidinium proton was in direct correspondence with the increase in intensity of the benzylic or methylene protons of the thermodynamic (i.e., N-substituted *o*-aminobenzylamine or β-(*o*-aminophenyl)ethylamine) products. Formation of the kinetic isomer from I would result in the appearance of the benzylic resonance at ca. δ 3.8 ($CDCl_3$) and lack of aromatic absorption in the region δ 6.5–7.0. In the case of III, the kinetic isomer would produce the ethylamino side chain absorption at ca. δ 2.4–3.1 ($CDCl_3$). No rapid appearance and disappearance of peaks attributable to the kinetic isomer was noted. The time required for the methoxyaminolyses reactions to achieved equilibrium between cyclic amidine and methoxyaminolysis products in Me_2SO varied greatly for the three cyclic amidines; in the case of the six-membered amidines, I and II, equilibrium was achieved only after a period of time exceeding 1 week (at room temperature). Equilibrium methoxyaminolysis of III, by contrast, was achieved within several minutes. Similarly, methoxyaminolysis of IV under the same conditions produced *o*-[(*N*-methoxyformimidoyl)amino]toluene (VIII) within a period of several minutes.

Kinetics. Hydrolysis. Hydrolyses of III and IV were examined in the absence of buffer in the pH range 8–11 employing a Bruice–Maley cell at 50 °C, μ = 1.0 with KCl. Buffer catalysis in the hydrolysis of the benzodiazepine III, monitored at 260 nm ($\Delta\epsilon_{260}$ = 6300), and the acyclic amidine IV, followed at 260 nm ($\Delta\epsilon_{260}$ = 4600), is negligible (≤5% over a 0.05–0.30 M range of carbonate buffer). The k_{obsd} vs. pH plot describes a titration curve (Figure 1) with k_{obsd} adequately defined[4,5] by eq 1:

$$k_{obsd} = \frac{a_H}{(K_a + a_H)} k_{OH} a_{OH} \quad (1)$$

Appropriate values of k_{OH} and K_a for III and IV at 50 °C as well as the spectrophotometrically determined pK_a for III and IV are tabulated in Table I.

In the latter stages of the hydrolysis of III ($t \geq 3t_{1/2}$, pH values ≥9.3) a concomitant increase of OD at 282 nm was noted. The final spectrum (λ_{max} 282 nm, ϵ = 2150) is coincident with that determined for the thermodynamic product. A representative spectrum for the hydrolysis of III in 0.12 M carbonate buffer, pH 9.35 at 50 °C, is presented in Figure 2. The rate of conversion of the kinetic product to the thermodynamic product was found to be dependent upon buffer (carbonate) concentration, according to eq 2

$$k_{obsd}' = k_{obsd}/f_{AM} = k_B[B] + k_{B\text{-}OH}[B]a_{OH} + k_0 \quad (2)$$

where B represents the free base form of the buffer, f_{AM} the reactive protonated form of the amidine, and $a_{OH} = 5.43 \times 10^{-14}/a_{H^+}$. Values of $k_B + k_{B\text{-}OH}a_{OH}$ were obtained as the slope of plots of k_{obsd}' against [B] and separated by replots against a_{OH} which yielded k_B as the intercept and $k_{B\text{-}OH}$ as the slope, respectively. A value of k_0, the rate constant for the apparent spontaneous isomerization, was found to be 0.04 ± 0.01 min^{-1} from the intercept of k_{obsd}' vs. [B] plots. Consequently, values for k_{OH} reported in Table I for III represent those for formation of the kinetic isomer, i.e., β-(*o*-*N*′-formylaminophenyl)ethylamine, which subsequently isomerizes to the thermodynamic product.

The hydrolysis of I was monitored by recording the decrease in OD_{290} or OD_{280} with time ($\Delta\epsilon_{290}$ = 3000). The spectrum corresponding to the hydrolysis of I was carefully scanned at selected pH values. In all cases, hydrolysis of I exhibited isosbestic behavior (252 nm) and no evidence for formation of a kinetic isomer preceding isomerization to the thermodynamic product was observed, contrary to the behavior of III. Difference UV spectra of I (pH 10.5 vs. pH 7.5) provided no evidence for the formation and existence of the tetrahedral hydroxide addition intermediate.[7,8] There appeared to be a strong dependency for the rate of hydrolysis of I upon increasing concentration of Tris or carbonate buffers. Hydrolysis of I in

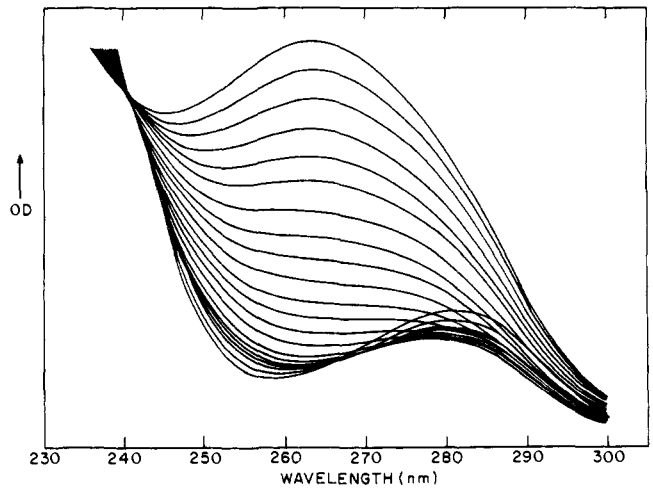


Figure 2. Repetitive UV scans of hydrolysis of III, 50 °C, pH 9.35, 0.12 M carbonate buffer (μ = 1.0).

Table II. Values of Rate Constants for Scheme I Obtained by Computer Simulation of OD_{260} vs. t[a]

$k_1 = 1.16 \times 10^{-2}\ M^{-1}\ min^{-1}$	$\epsilon_{III} = 8400$
$k_{-1} = 1.05 \times 10^{-2}\ min^{-1}$	$\epsilon_{KP} = 6700$
$k_2 = 1.25 \times 10^{-2}\ min^{-1}$	$\epsilon_{TP} = 5100$
$k_3 = 5.55 \times 10^{-4}\ min^{-1}$	

[a] The conditions are identical with those specified in Figure 3.

Table III. Observed Rate Constants for Methoxyaminolysis of III in the Presence of Varying Concentrations of Phosphate Buffer and Methoxyamine (T = 50 °C, μ = 1.0, KCl)

pH	$[CH_3ONH_2]$, M	$[B_T]$, M	k_{obsd} ($min^{-1} \times 10^3$)
	Phase II		
7.75–7.82	1		8.35
7.70–7.77	0.75		5.08
7.71–7.85	0.5		4.37
7.68–7.77	0.5		3.90
7.72–7.85	0.1		1.52
7.06–7.10	0.5	0.0148	13.2
7.07–7.10	0.5	0.030	12.5 ± 2.0
7.12–7.16	0.5	0.048	11.0
7.09–7.11	0.5	0.072	13.5 ± 0.2
7.11–7.14	0.5	0.096	13.2
7.11–7.14	0.5	0.144	21.5 ± 0.7
7.11–7.13	0.5	0.208	29.4 ± 1.8
	Phase III (Hydrolysis)		
7.12–7.16	0.5	0.048	0.550
7.11–7.14	0.5	0.096	0.613
7.11–7.14	0.5	0.144	0.615
7.11–7.13	0.5	0.208	0.575

carbonate buffers followed the rate law of eq 2. Values for k_B and $k_{B\text{-}OH}$ are listed in Table I; the value of k_0 for the spontaneous isomerization is 0.015 ± 0.002 min^{-1} and is associated with the formation of the thermodynamic isomer (see Discussion).

No extensive kinetic investigation of the hydrolysis of the methyl substituted analogue II was undertaken. However, in order to determine the effect of methyl substitution upon the hydrolytic stability of II relative to I, the rate of hydrolysis of II was measured under a set of conditions identical with that of I (0.05 M carbonate, pH 10.17, 50 °C, μ = 1.0). Under these conditions, the observed rate constant for hydrolysis of II was 0.0603 min^{-1}; the comparable value for I was 0.0076 min^{-1}.

Methoxyaminolysis. The methoxyaminolysis kinetics of 4,5-dihydro-3(*H*)-1,3-benzodiazepine (III) were examined in a series of phosphate buffers (pH 6.60–7.80) at 0.5 M methoxyamine, 50 °C, μ = 1.0 (KCl). The hydrolysis rate of III is negligible compared with methoxyaminolysis under these conditions.

Typically, plots of OD_{260} vs. time for methoxyaminolysis of III exhibited three distinct phases at pH 7.11 (Figure 3). At pH values outside the limits 7.1 ≤ pH ≤ 7.8, the three phases become less distinct due to apparent catalysis of phases I and II. Such triphasic behavior may be interpreted in terms of a rapid preequilibrium formation of the kinetic isomer (phase I), followed by its isomerization to give the thermodynamic product (phase II) and the latter's subsequent hydrolysis to β-(*o*-aminophenyl)ethylamine and formylmethoxyamine (phase III of Scheme I). Initial estimates of rate constants for phase II were obtained from OD_{260} vs. time plots adopted from the procedure of Bender.[10] Values of the rate constants associated with Scheme I are compiled in Table II. Values of extinction coefficients for II and TP of Scheme I at 260 nm, pH 7.11 (μ = 1.0), were determined experimentally. An estimate

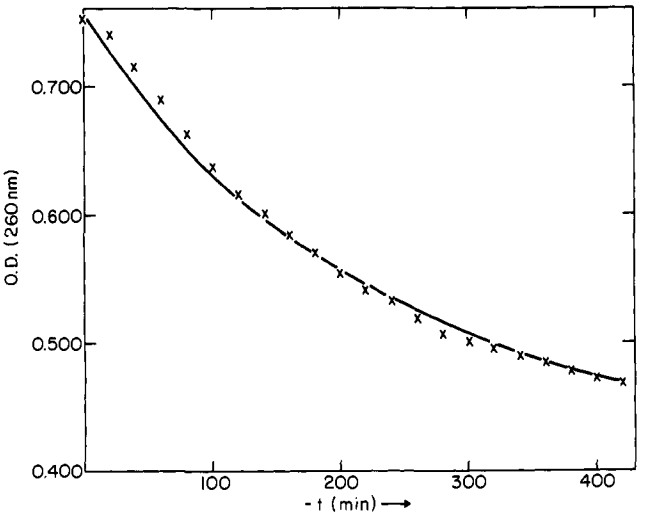
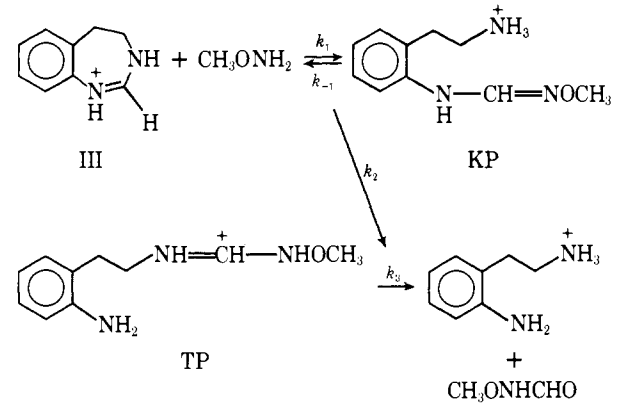


Figure 3. Plot of OD_{260} vs. time for the methoxyaminolysis of 4,5-dihydro-3(*H*)-1,3-benzodiazepine (III), pH 7.11, 0.03 M K·phos, $[CH_3ONH_2]$ = 0.5 M (T = 50 °C, μ = 1.0, KCl). Solid line represents computer simulation curve to Scheme I. Onset of phase II at ca. 30 min; onset of phase III at ca. 5 h.

Scheme I



of the extinction coefficient for the kinetic aminolysis product (KP) was available from VIII.

Attempts at extracting the dependence of phase II upon phosphate or *N*-methylmorpholine buffer concentration at pH 7.11 were unsuccessful due to the extreme experimental limitations imposed by the narrow pH range in which efforts are feasible. Although no quantitative data were obtained, it is apparent from k_{obsd} as a function of $[buffer]_t$ for the three separate phases that phase II of Scheme I is dependent upon buffer concentration (Table III) whereas the third phase shows no dependence upon buffer concentration (consistent with buffer-independent hydrolysis of the acyclic compound IV). Phase I (kinetic isomer formation) was also found to be accelerated by increasing phosphate concentration.

That the third phase represents hydrolysis of β-(*o*-aminophenyl)-*N*-methoxyformimidoylethylamine was independently confirmed by allowing a sample of III to undergo methoxyaminolysis at pH 7.17 (0.1 M phosphate–0.5 M NH_2OCH_3) equivalent to a time corresponding to 10 half-lives of phase III. Isolation and identification of the product provided 82% recovery of β-(*o*-aminophenyl)ethylamine.

Discussion

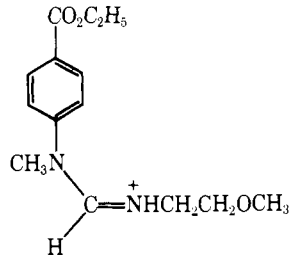
Previous studies have established the intermediacy of tetrahedral and orthoamide species in the course of hydrolysis[1,5,8] and aminolysis[4] of formamidinum salts. The results of this study are interpreted in terms of a step-wise mechanism, Scheme II, related to that proposed by Jencks and Satterthwait

for the aminolysis of acetate esters[11] and the hydrolysis of phenyl imidates.[12] The notations N_α and N_β designate the amino function of low and high pK_a, respectively.

According to this mechanism, the addition of hydroxide ion[5] to the protonated amidine to produce I_0 occurs in a rapid preequilibrium step and is followed by proton-transfer steps that generate the key product forming zwitterionic tetrahedral intermediates, $T^\pm$ and $K^\pm$. In the alkaline pH range studied, attack by hydroxide ion rather than water is the preferred route to the formation of the initial tetrahedral addition species, I_0. For diphenylimidazolinium chloride, the addition of hydroxide ion remains a preequilibrium process with respect to hydrolysis even under weakly acidic conditions.[5] Furthermore let us assume that orientation of the heteroatom lone pairs causes no perturbation of the relative pK_a values of N_α and N_β within the tetrahedral intermediates or the rate of the various proton transfer steps. Consistent with the postulates for stereoelectronic control,[13] this factor should be important only in determining the relative magnitudes of the breakdown steps (k_c, k_c') in which actual C–N bond cleavage occurs. Estimates of the pK_a values of the intermediates K^+ and T^+ for I were made by the method described by Fox and Jencks:[14]

$$pK_a(K_N^+) = 6.3, \quad pK_a(K_0^+) = 8.7,$$
$$pK_a(T_N^+) = 1.9, \quad pK_a(T_0^+) = 8.7^{15}$$

Hydrolysis of the acyclic amidine (IV) proceeds without detectable buffer catalysis and leads exclusively to the product expected for kinetic control. Hydrolysis of the related acyclic amidine (IX) likewise yields the *N*-formylaniline derivative



IX

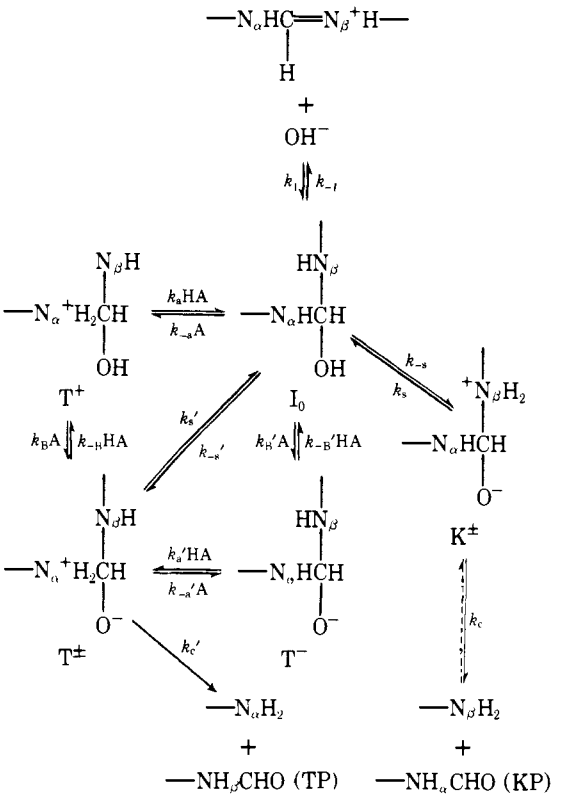
and occurs without buffer catalysis.[4] Consequently when N_β is a strongly basic amine, the intermediates I_0 and $K^\pm$ are in protonic equilibrium with each other and the solvent so that their relative concentrations are determined by the pH and the equilibrium constant for their interconversion. The absence of the thermodynamic isomer is explicable in terms of Scheme II. Given that its precursor $T^\pm$ likewise is in protonic equilibrium with the other intermediates, then the ratio of kinetic (KP) to thermodynamic product (TP) is given by eq 3.

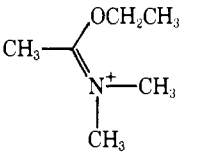
$$KP/TP = k_cK_{K_0^+}K_{T_N^+}/k_c'K_{K_N^+}K_{T_0^+} \qquad (3)$$

Adopting a value of $\alpha = 0.3$[16] ($\beta_{lg} = -0.7$) from the aminolysis of amides for the ratio k_c/k_c' and the pK_a values listed above permits evaluation of eq 3 with $KP/TP \geq 10$. It is plausible that the dependency of the decomposition of the tetrahedral intermediate is greater than 0.7 since the amine (N_β) of higher pK_a is being expelled by the amine (N_α) of lower pK_a and vice versa. However, k_{OH} for IV and IX differ by less than a factor of 2[17] despite a ΔpK_a[6] of approximately 2 units in the anilino nitrogens that participate in the expulsion of β-methoxyethylamine. Moreover, the formation of the kinetic product will be further enhanced if the proton transfer steps leading to $T^\pm$ become rate-determining as the basicity of N_α decreases (vide infra).

An estimation of the value of k_s may be obtained from related tetrahedral intermediates where the pK_a of K_N^+ is similar, namely, the hydrazinolysis of ethyl acetate[11] ($pK_a = 4.8$) and the hydrolysis of the *N,N*-dimethylimidate X[18] (pK_a = 5.8), where the value of k_s for both is reportedly in the range of 10^6 to 10^7 s^{-1}. If the proton switch pathway were solely responsible for maintaining prototropic equilibrium, then a maximal value for k_c is 10^5 to 10^6 s^{-1}. This estimate of k_c is about two orders of magnitude lower than values quoted for closely related zwitterionic intermediates,[11,12,19] suggesting that the $I_0 \rightleftarrows K^\pm$ equilibrium is maintained by buffer species (not shown).

Scheme II



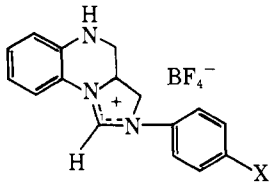


X

Similarly the initial formation of the kinetic hydrolysis product from III is not assisted by buffer catalysis, although subsequent isomerization of the kinetic to the thermodynamic isomer is dependent upon buffer concentration. Consequently the observed general base catalysis is not assigned to the hydration step. Isomerization may be viewed as rate-determining formation of the thermodynamic isomer from the relevant adduct I_0, the latter being in equilibrium with III and its kinetic isomer. In terms of Scheme II, the decreased basicity of the leaving amino function $-N_\alpha-$ (aniline rather than phenethylamine) now results in a proton-transfer step becoming rate limiting.[1,4] It appears that expulsion of weakly basic amines is generally sufficient to cause $k_c' > k_s'$, or $k_{-B}[HA]$ or $k_{-a}[A]$. Not only does the value of k_c' increase with decreasing amine basicity but the value of k_s' decreases as the basicity of the resident amine is significant reduced. The value of k_s' for the $T^\pm$ intermediate derived from the *N*-phenyl imidate[18] of X is 2×10^4 s^{-1} compared with 2×10^6 s^{-1} for X.

The proton-transfer steps to N_α (k_a or k_a'), which in the context of Scheme II give rise to the terms k_B and $k_{B\cdot OH}$, respectively, in eq 2, are thermodynamically unfavorable for buffer species with $pK_a \geq 6$ and probably constitute the rate-

limiting steps. The subsequent proton abstraction from T^+ to give $T^\pm$ will compete equally with reversion from T^+ to I_0 as long as the pH is sufficiently high so that the diffusion-controlled reaction with hydroxide ion or proton abstraction by other strong bases in the solution dominates. Similarly, the initial proton abstraction from I_0 to give T^- by strong bases or hydroxide ion should not be kinetically significant. Structure reactivity data for the general-base-catalyzed hydrolysis of XI

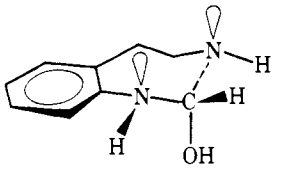


XI

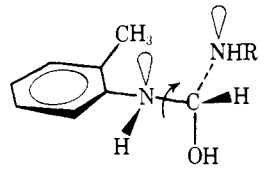
where X = COOEt correlate with $\alpha = 0.8$ for bases with $pK_a > 2$ and $\alpha \simeq 0$ for $pK_a < 2$.[20] Robinson and Jencks[5] have suggested that the general-acid-catalyzed hydrolysis of 1,3-diphenyl-2-imidazolinium chloride—the term [B][OH$^-$]—similarly represents a diffusion-limited process. Consequently the results are most easily explained by a stepwise rather than a concerted mechanism, although the latter is not excluded for the expulsion of very weakly basic amines by strongly basic groups with $T^\pm$ so that its stability becomes negligible.

The values of k_B reported for I and II are ca. 10^4-fold less than that for XI to its thermodynamic isomer.[1,20] According to Scheme II under these conditions, $k_B = (k_1/k_{-1})k_{HA}$. Since the pK_a of N_α is roughly comparable in the three systems, the difference in k_B must mainly reflect changes in the ratio k_1/k_{-1} and is consistent with the anticipated decrease in hydrolytic stability of the formamidinium salt with decreasing N_α and N_β basicity.

The discrepancy in the behavior of I imposed by simply a change in ring size is subject to two interpretations; namely, hydrolysis of I either proceeds directly to the thermodynamic isomer or proceeds through a low concentration of the kinetic product precluding its observation. Attack of hydroxide upon III and IV would lead to the respective conformers IIIi and IVi,[21] in which the aniline and phenethylamine (or β-

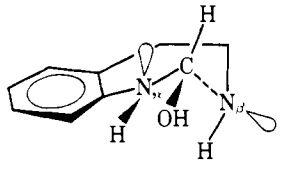


IIIi

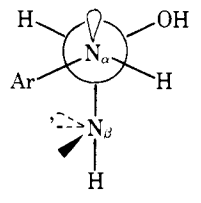


IVi

methoxyethylamine) nitrogen atoms are pyramidal[22] and antiperiplanar to the attacking nucleophile. Unrestricted rotation about the C–N_α bond as pictured in IVi provides access to a reactive conformer in which the lone pairs on N_α and N_β are suitably disposed to eject either the β-methoxyethylamine or aniline leaving group. Similarly, in IIIi, a facile interconversion of the chair to boat form of IIIi[23] produces a conformer (IIIii) in which either N_α or N_β may be expelled



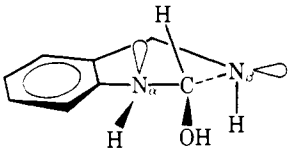
IIIii



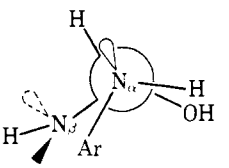
III'ii

according to the principle of stereoelectronic control. An alternative representation of the position of the lone pair orbital on N_α in relation to the C–N_β bond in IIIii is presented in terms of a Newman projection viewed along the N_α–C bond (III'ii). Thus, both IIIi and IVi breakdown according to relative basicities of the nitrogen atoms to produce initially a kinetic product, which, in the case of III, subsequently isomerizes to the more stable amide. One need only consider those conformers in which N_α retains a conjugation with the aromatic ring; rotation to give other nonresonating conformers would be a higher energy process.[24] The resonance energy provided by aniline conjugation is estimated to be on the order of 5 kcal/mol.[25] The possibility of pyramidal nitrogen inversion, which occurs freely in acyclic and seven-membered heterocycles,[26] likewise is not included in this discussion since inversions to "nonreactive" conformers are produced. It is also possible that the energy barriers are significantly higher for rotation and inversions within tetrahedral intermediates due to lone pair repulsion.[27]

On the other hand, attack of hydroxide upon the cyclic amidine I produces the intermediate Ii which, in adherence to microscopic reversibility, may expel the attacking nucleophile in a rapid preequilibrium process (k_{-1}). Although the barrier to ring flip to convert the half-chair conformer of Ii to the half-boat conformer (Ii → Iii) is slightly higher than that in the seven-membered ring system,[28] the barrier is presumably lower than that of a nonstereospecifically controlled cleavage of Ii[24] and the ring flip occurs preceding C–N bond cleavage.



Iii

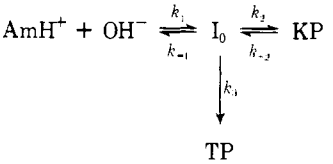


I'ii

The Newman projection corresponding to conformer Iii is represented by I'ii. However, unlike the conformer IIIii, stereoelectronic control then favors direct cleavage of the C–N_α bond since the orbitals of N_β and OH are now suitably disposed to eject N_α, whereas the cleavage of the C–N_β bond by participation of N_α would require a subsequent higher energy twisting mode in order to achieve the necessary antiperiplanar relationship to C–N_β. Due to this perturbation of activation energies, the intermediate Iii may break down to give the thermodynamic product directly (in opposition to the direction of breakdown favored by protonic equilibrium) with the expected kinetic parameters (k_0, k_B, $k_{B\cdot OH}$) associated with the $I_0 \rightarrow T^+$, $T^- \rightarrow T^\pm$ pathway.

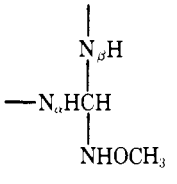
There are several difficulties with this interpretation that are more readily understood in terms of an abbreviated version of Scheme II given in Scheme III. Three experimental obser-

Scheme III



vations require a consistent rationale, namely: (i) the inability to detect the kinetic isomer in the hydrolysis of I contrasted with observation of related species in the hydrolysis and methoxyaminolysis of III; (ii) the description of both the hydrolysis of I and the isomerization of III in terms of the kinetic rate expression given by eq 2; and (iii) the apparent more rapid initial conversion of precursors of the kinetic product of I, namely, the trifluoroacetyl derivative VI, than I itself to the thermodynamic isomer. In the series I, III, and IV, cyclization to form I_0 from KP via k_{-2} is expected to favor formation of

the six- vs. seven-membered cyclic intermediate by a factor of ca. 10^2,[29] with $k_{-2} \simeq 0$ for IV. One can demonstrate by computer simulation[45] that observation i may result simply from a condition where the KP/Am equilibrium ≤ 1 for I relative to III caused by increases mainly in k_{-2}. In fact, the KP/Am equilibrium for III in the methoxyaminolysis reaction is ca. unity (k_1/k_{-1}, Table II). Two consequences of the instability of KP vs. the parent amidine in the case of I are observations ii and iii. Firstly in terms of Scheme III the KP/Am equilibrium would be rapidly established kinetically, so that the ensuing decay of amidine would be mainly through step k_3 and characterized by general-acid and -base catalysis. Secondly commencing with KP the initial partitioning of I_0 would result in a more rapid formation of TP since $k_{-2} > k_1$ followed by the latter's slower formation once equilibrium is established. Moreover the predicted kinetics are not truly first-order. Note that this rationale is consistent with the postulated preequilibrium formation of I_0 from amidine plus hydroxide ion, i.e., $k_{-1} > k_2$ and k_3. A more complete treatment of this basic scheme may be found in reference 1a. Stereoelectronic effects increasing the energy of the transition state lying between I_0 and KP should be manifest in an increased stability relative to cyclization of the kinetic isomer derived from I and should enhance the detection of that isomer which is contrary to our observation. In principle, however, it is possible that, in I, $k_2 < k_3$, owing to stereoelectronic factors which based on eq 3 require a decrease in k_2 of ca. 10^2 to favor direct formation of the thermodynamic isomer. This decrease then is not manifest in k_{-2} owing to compensation caused by the increased energy level of KP. Thus the magnitude of a stereoelectronic effect could fall within these limits and be masked experimentally in the present systems. However, there apparently are no stereoelectronic effects operating in formation of the TP, as discussed previously from the viewpoint of molecular models, from either I or III upon hydrolysis since the kinetic parameters are of nearly equivalent value. It is not clear exactly whether the methoxyaminolysis of I which does not compete favorably with hydrolysis in aqueous buffer and only proceeds slowly relative to III and IV in aprotic solvents is due to such effects. The slower partitioning of the key neutral orthoamide argues for a decrease in k_3 relative to either k_2 or k_{-1} vs. III or IV. It is possible, but not proved, that the proton transfer steps leading to and from XII become competitive with conformational interconversions and/or C–N bond cleavage so that XII returns mainly to starting amidine. From the above



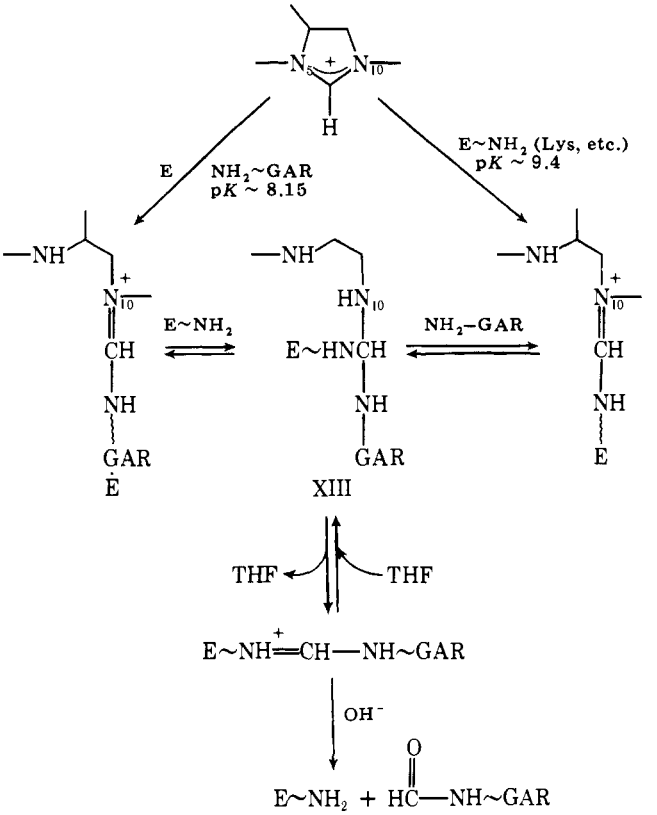
XII

it would not appear that such steps and C–N bond cleavage are always synchronized.

The methoxyaminolysis reactions of III which results in net formyl transfer represents our first demonstration of this pathway. The stepwise mechanism for this process is closely analogous to that proposed by Scheme II and has been described previously.[1,4] The formation/decomposition of XII is subject to general-acid–base catalysis when the attacking/leaving amine is weakly basic; in the case of strongly basic amines, the rate of C–N bond cleavage may become rate determining so that general-acid–base catalysis is not observed. This statement is subject to the proviso that the orthoamide has sufficient lifetime for either trapping by buffer species or equilibration with respect to proton transfer. The latter situation apparently is achieved if two relatively weakly basic amines act to expel an amine of higher pK_a to attain the amidine product.[30] In the present case, both attack by methoxyamine and presumably expulsion of the anilino nitrogen are subject to general-acid–base catalysis (Table III). The achievement of this transfer, of course, is dependent on the presence of a phenethylamine group intramolecularly disposed to intercept the initial amidine species (KP, Scheme I) which allows for rapid equilibration to the thermodynamic isomer due to a favorable entropy effect.[31] However, hydrolysis of the eventual amidine is still subject to kinetic control and leads to net transfer, owing to the lower pK_a of the methoxyamine residue in comparison with the phenethylamine. The implications of this model suggest the plausibility of a similar equilibration scheme being operative in the amidine–orthoamide equilibria accompanying enzymatic catalysis of formyl transfer.[32,33]

The initial condensation of either the amino moiety of GAR (glycinamide ribonucleotide) or a residual amino group of the enzyme gives rise to an N(10) amidinium species (Scheme IV).

Scheme IV



Attack of the complementary amino group upon the N(10) amidine produces the intermediate orthoamide species. According to the conclusions drawn above, the orthoamide species XIII mediates an equilibrium between the three enzyme-bound amidines leading to the accumulation of the thermodynamic favored product, the enzyme–glycinamide amidine (with loss of tetrahydrofolic acid). Selective hydrolysis of the enzyme-linked amidine under kinetic control with expulsion of the more basic amino group results in net formyl transfer to GAR and regeneration of the active amino group of the enzyme. Although in terms of stereoelectronic theory the breakdown of the orthoamide in a productive sense [expulsion of N(10)] may be aided through enzymatic manipulation[31,34] to favor a conformer in which the lone pair orbitals of NH_2–GAR and NH_2–E are disposed to favor cleavage of C–N(10) directly, direct proof of this point still is lacking.

Experimental Section

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Thin-layer chromatography were performed on Eastman

silica gel plates developed with 4:1 (v/v) ethyl acetate–cyclohexane. Compounds were visualized by UV fluorescence or treatment with iodine vapor. Ultraviolet spectra were taken on a Cary 118 or Cary 14 instrument. NMR spectra were recorded on a Varian A-60 instrument and chemical shifts (δ) are reported relative to Me_4Si.

Materials. *o*-Aminobenzylamine was prepared according to a modification of the procedure of Kornblum and Iffland[35] in which *o*-aminobenzonitrile (Aldrich) was hydrogenated over platinum oxide in acetic anhydride: bp 98–100 °C (1.5 mm) (lit.[35] bp 85–90 °C (1 mm)); mp 50–52 °C (lit.[35] 57–59 °C). 2-Methylaminobenzylamine was prepared according to the procedure outlined by Coyne and Cusic:[38] bp 113–115 °C (8 mm) (lit.[38] bp 88–96 °C (0.15 mm)).

3,4-Dihydroquinazolinium Acetate (I). A solution of 3.0 g (0.025 mol) of *o*-aminobenzylamine and 4.0 g (0.038 mol) of formamidine acetate[36] in 40 mL of absolute ethanol was brought to reflux for a period of 4 h. The resulting solution was cooled, the solvent removed in vacuo, and the precipitate recrystallized from benzene as the acetate salt (74%): mp 129–130 °C; NMR ($CDCl_3$) δ 2.05 (s, 3 H), 4.73 (s, 2 H), 6.8–7.3 (m, 4 H), 8.0–8.1 (s, 1 H); IR (Nujol) 1680, 1620 cm^{-1}.

The free base of the amidine salt was prepared by neutralizing a quantity of the acetate salt with saturated sodium bicarbonate. Extraction with methylene chloride, drying of the organic layer ($MgSO_4$), and distillation gave 3,4-dihydroquinazoline: bp 136–140° (0.35 mm), lit.[37] 134–136° (0.3–0.4 mm); mp 123–124° (lit.[37] 125–126°); NMR ($CDCl_3$) δ 4.62 (s, 2 H), 6.5–7.2 (m, 5 H); IR (Nujol) 1580 cm^{-1}; UV λ_{max} (pH 10.17) 291 nm (log ϵ = 3.80), lit.[37] λ_{max} (pH 11.5) 291 nm (log ϵ = 3.76) and λ_{max} (pH 7.0) 280 nm (log ϵ = 3.69).

1,4-Dihydro-1-methylquinazoline (II). A solution of 3 g (0.022 mol) of 2-methylaminobenzylamine and 25 g (0.024 mol) of formamidine acetate in 20 mL of absolute ethanol was refluxed for a period of 16 h. The solvent was removed in vacuo, and the residue was neutralized with an excess of aqueous 10% sodium bicarbonate, extracted into methylene chloride, dried ($MgSO_4$), and distilled (67%): bp 95.5–97.5 °C (2.5 mm) (lit.[37] 90–91 °C (2.5 mm)). The amidine was stored under argon at 0 °C to prevent decomposition: NMR ($CDCl_3$) δ 3.1 (s, 3 H), 4.62 (s, 2 H), 6.5–7.2 (m, 5 H); IR (neat) 1655, 1480, 740 cm^{-1}; UV λ_{max} (pH 7.9) 292 nm (log ϵ = 3.73) and 282 nm (log ϵ = 3.71) (lit.[37] UV λ_{max} (pH 7.0) 292 nm (log ϵ = 3.60) and 282 nm (log ϵ = 3.61)).

β-(*o*-Aminophenyl)ethylamine. The amine was prepared by either of two methods. The first method involved a modification of the Kornblum and Iffland procedure[35] in which a solution of 10 g (0.062 mol) of *o*-nitrophenylacetonitrile (Aldrich) in acetic anhydride was hydrogenated over platinum oxide. After hydrogen uptake was completed, the catalyst was filtered off and the solvent removed under vacuum to yield a dark oil. The oil was refluxed, under nitrogen, in a solution of 20 g of sodium hydroxide in 50 mL of water for a period of 22 h. The amine was extracted into methylene chloride, the organic layer dried, and the residue distilled (37%): bp 99–104 °C (1.25 mm) (lit.[35] bp 112–113 °C (2 mm)); NMR ($CDCl_3$) δ 2.4–3.1 (m, 4 H), 6.45–7.50 (m, 4 H).

An alternative procedure employed was based on that of Jen et al.[39] A solution of 28.5 g (0.19 mol) of *o*-nitrophenylacetamide (prepared in 65% yield from *o*-nitrophenylacetic acid) in 300 mL of dioxane was reduced by dropwise addition of 350 mL of 1 M diborane in tetrahydrofuran (Aldrich). The solution was stirred at room temperature for 20 h, refluxed for 50 h, cooled, and the excess diborane was decomposed by dropwise addition of methanol. The residue was stirred in water overnight and extracted into chloroform and the solvent removed to yield a red oil. Hydrogenation was effected over a 10% palladium catalyst. Distillation gave the diamine (43%): bp 87–90 °C (0.1 mm).

4,5-Dihydro-3(*H*)-1,3-benzodiazepinium Acetate (III). A solution of 1.1 g (0.008 mol) of β-(*o*-aminophenyl)ethylamine and 2 g (0.019 mol) of formamide acetate in 15 mL of absolute ethanol was refluxed under nitrogen for 16 h. The solvent was removed in vacuo and 30 mL of acetonitrile was added to the solid residue. Excess formamidine acetate was filtered off and the acetonitrile removed under vacuum from the filtrate to leave a residue. The latter was recrystallized from benzene to give the acetate salt (71%): mp 112–114 °C; NMR ($CDCl_3$) δ 2.07 (s, 3 H), 3.0–3.4 (m, 2 H), 3.6–3.9 (m, 2 H), 7.0–7.4 (m, 4 H), 8.3 (s, 1 H); IR (Nujol) 2940, 1665, 1390 cm^{-1}; UV λ_{max} (pH 10.2) 260 nm (log ϵ = 3.96) and λ_{max} (pH 7.11) 260 nm (log ϵ = 3.82). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.01; H, 6.66; N, 13.28.

The free base was generated by neutralization of the acetate salt by aqueous sodium bicarbonate. Extraction with methylene chloride, drying ($MgSO_4$), and removal of the solvent gave a solid: mp 114–116 °C; NMR ($CDCl_3$) δ 3.05 (t, J = 4 Hz, 2 H), 3.62 (t, J = 4 Hz, 2 H), 6.29 (m, 1 H), 6.90–7.3 (m, 5 H).

***o*-[[*N*-(2-Methoxyethyl)formimidoyl]amino]toluene (IV).** The procedure employed was that of Benkovic et al.[1a] To a solution of 5.0 g (0.047 mol) of freshly distilled *o*-toluidine (Eastman) and 4.75 g (0.047 mol) of *N*-formyl-β-methoxyethylamine (prepared from β-methoxyethylamine and 90% formic acid (bp 122–130 °C at 25–30 mm)) in 100 mL of chloroform was added 10.4 g (0.05 mol) of phosphorus pentachloride in several portions. After addition was completed, the mixture was brought to reflux for a period of 5 h. The reaction mixture was cooled and the solvent was removed under vacuum to leave a residue which was neutralized to pH ~10 with 1 N sodium hydroxide and the base extracted into methylene chloride and dried ($MgSO_4$). Distillation gave the amidine as a yellow-tinted oil (72%): bp 115–121 °C (0.05 mm); NMR ($CDCl_3$) δ 2.12, 2.25 (singlets, 3 H, 3:1 cis:trans[40]), 3.38 (m, 4 H), 3.56 (s, 3 H), 4.8–5.0 (bm, 1 H), 6.5–7.2 (m, 4 H), 7.48 (s, 1 H); IR (neat) 2900, 1640, 1110 cm^{-1}; UV λ_{max} (pH 9.9) 250 nm (shoulder, log ϵ = 3.87). Anal. Calcd for $C_{11}H_{16}N_2O$: C, 68.72; H, 8.39; N, 14.57. Found: C, 68.41; H, 8.00; N, 14.64.

3,4-Dihydro-3-ethylacetoxyquinazolinium Acetate (V). *o*-Nitrobenzyl bromide was prepared according to the procedure outlined by Kornblum and Iffland.[35] Glycine ethyl ester hydrochloride (Aldrich, 20 g, 0.14 mol) was dissolved in 150 mL of tetrahydrofuran–acetonitrile (2:1) and 35 g of anhydrous potassium carbonate was added. *o*-Nitrobenzyl bromide (15 g, 0.07 mol), dissolved in 25 mL of tetrahydrofuran, was added dropwise with stirring to the suspension for a period of 2 h. The reaction was worked up by addition of water, extraction with chloroform, drying ($MgSO_4$), and the removing of the solvent to give a red oil. The nitro group was reduced catalytically with 10% Pd/C in ethanol to give the diamine after removal of solvent (60% yield from *o*-nitrobenzyl bromide): NMR ($CDCl_3$) δ 1.27 (t, 3 H, J = 7 Hz), 3.4 (s, 2 H), 3.7 (s, 2 H), 3.8 (s, 2 H), 4.2 (q, 2 H, J = 7 Hz), 6.5–7.3 (m, 4 H).

The above diamine (2.4 g, 0.012 mol) was cyclized with 2.0 g (0.019 mol) of formamidine acetate in refluxing ethanol. The crude amidine salt was recrystallized from ether–acetonitrile to give the hygroscopic acetate salt of V as yellow needles (52%): mp 74–76 °C; NMR (Me_2SO-d_6) δ 1.22 (t, 3 H, J = 7 Hz), 1.9 (s, 3 H), 4.1 (s, 2 H), 4.2 (q, 2 H, J = 7 Hz), 4.5 (s, 2 H), 6.8–7.4 (m, 6 H); UV λ_{max} (pH 10.2) 300 nm (log ϵ = 3.90). Anal. Calcd for $C_{14}H_{20}N_2O_5$: C, 56.74; H, 6.82; N, 9.45. Found: C, 57.02; H, 6.35; N, 9.78.

***o*-(Amino)-*N*-trifluoroacetylbenzylamine.** Potassium carbonate (4 g) was suspended in a solution of 2 g (0.0167 mol) of *o*-aminobenzylamine in 50 mL of ether–chloroform (1:1). The flask was then immersed in a dry ice–acetone bath at −78 °C and 3.6 g (0.017 mol) of trifluoroacetic anhydride[41] (Aldrich) added dropwise, with stirring, over a period of 20 min. The reaction mixture was allowed to warm slowly to room temperature and stirred for an additional 24 h. The contents of the flask were then poured into ice–water and extracted quickly with chloroform. The chloroform extract was dried ($MgSO_4$) and the solvent removed to yield an oil that solidified upon standing (80%): NMR ($CDCl_3$) δ 3.5–4.5 (bm, 2 H, $-NH_2$), 4.2–4.4 (broadened doublet, 2 H), 6.5–7.3 (m, 5 H). The onset of the aromatic region at δ 6.5 indicated trifluoroacetylation at the benzylamine moiety.

***o*-(*N'*-Formylamino)-*N*-trifluoroacetylbenzylamine (VI).** Acetic–formic anhydride[42] was prepared by combining 2.6 mL (0.0276 mol) of acetic anhydride with 1.04 mL (0.0276 mol) of formic acid. The mixture was heated at 50 °C in an oil bath for 1.5 h. The oil bath was removed and the acetic–formic anhydride cooled to room temperature at which time 2 g (0.0092 mol) of *o*-(amino)-*N*-trifluoroacetylbenzylamine in 20 mL of chloroform was added dropwise with stirring to the anhydride. The mixture was stirred at room temperature for 14 h and then poured into 25 mL of water. The layers were separated, the aqueous layer was washed with 25 mL of chloroform, and the combined organic extracts were dried ($MgSO_4$). Removal of solvent gave an oil that solidified upon standing. Recrystallization from benzene/petroleum ether gave white crystals (62%): mp 96–98 °C; NMR ($CDCl_3$) δ 4.48 (d, J = 6 Hz, 2 H), 7.0–7.8 (m, 5 H), 8.4 (s, 1 H); IR (Nujol) 3300, 1670, 1180 cm^{-1}. Anal. Calcd for $C_{10}H_9N_2O_2F_3$: C, 48.79; H, 3.68; N, 11.38. Found: C, 47.40; H, 3.32; N, 10.68.

***o*-(Amino)-*N*-carbobenzyloxybenzylamine.** Potassium carbonate (2.75 g) was suspended in a solution of 2 g (0.0167 mol) of *o*-aminobenzylamine in 50 mL of chloroform. The contents of the flask were cooled by immersing the flask in an ice–water bath and 3.1 g (0.0182 mol) of benzyl chloroformate (Aldrich) was added dropwise with stirring. The contents of the flask were allowed to stand at room temperature for 24 h and poured into water, the layers were separated, and the organic layer was dried (K_2CO_3). Removal of the solvent gave an oil that solidified upon trituration with petroleum ether (75%): mp 108–111 °C; NMR ($CDCl_3$), δ 4.2 (d, J = 6 Hz, 2 H), 5.05 (s, 2 H), 6.5–7.5 (m, 9 H).

***o*-(*N'*-Formylamino)-*N*-carbobenzyloxybenzylamine (VII).** Acetic–formic anhydride (0.0294 mol) was prepared from acetic anhydride and formic acid. To the anhydride was added dropwise a solution of 2.5 g *o*-(amino)-*N*-carbobenzyloxybenzylamine in 40 mL of chloroform. After stirring for 20 h at room temperature, the reaction mixture was poured into water, the layers were separated, and the organic layer was washed once with 5% sodium bicarbonate and dried ($MgSO_4$). Removal of solvent in vacuo gave an oil. Trituration with ether resulted in solidification. Filtration and thorough washing with ether gave white crystals (58%): mp 113–116 °C; NMR ($CDCl_3$) δ 4.25 (d, J = 6 Hz, 2 H), 5.05 (s, 2 H), 5.5–5.9 (m, 1 H), 7.0–7.4 (m, 9 H), 8.0–8.3 (m, 2 H); IR (Nujol) 3300, 1690, 2150 cm^{-1}. Anal. Calcd for $C_{16}H_{16}N_2O_3$: C, 67.59; H, 5.67. Found: C, 67.76; H, 5.83.

***o*-[(*N*-Methoxylformimidoyl)amino]toluene (VIII).** To a solution of 10.0 g (0.075 mol) of *o*-methylformanilide (prepared from *o*-toluidine and 90% formic acid; bp 105–106 °C (2.76 mm)) and 3.5 g (0.075 mol) of methoxyamine in 200 mL of $CHCl_3$ was added 15.5 g (0.075 mol) of phosphorus pentachloride in portions. After stirring overnight, the solvent was removed in vacuo and the residue recrystallized from acetonitrile–ether (1:1) to give white hygroscopic needles: NMR (Me_2SO-d_6) δ 2.38 (s, 3 H), 3.78 (s, 3 H), 7.0–7.8 (m, 6 H); UV λ_{max} (pH 7.44) 248 nm (shoulder, log ϵ = 3.75). Anal. Calcd for $C_9H_{15}N_2O_2Cl$: C, 49.42; H, 5.95. Found: C, 49.06; H, 6.05.

Kinetics. The instruments employed have been described previously.[43] Kinetic runs and spectrophotometric pK_a determinations at constant pH in the absence of buffer were performed in the Cary 14 equipped with a 25-mL Bruice–Maley cell[44] in conjunction with a Radiometer TTTI titrator and SBUI syringe buret.

Kinetic runs were performed in either 2-mL cuvettes or the Bruice–Maley cell thermostated at 50 °C. Aliquots (10–60 μL) of stock solutions of I, III, and IV were added to either buffer solutions (μ = 1.0, KCl) or 1 M KCl (pH-stat) to bring the initial reactant concentration to approximately 10^{-4} M. Hydrolysis and/or methoxyaminolysis of I, III, and IV were monitored by the decrease in OD at 282 nm (I) or 260 nm (III and IV). Pseudo-first-order rates were obtained as slope × 2.303 of ln ($OD_t - OD_\infty$) vs. time plots. Plots of the logarithm of hydrolytic rate constants of III and IV vs. pH (Figure 1) and the plot of OD_t vs. time for methoxyaminolysis of III (Figure 3) were fit by the Curfit program provided by Dr. P. A. D. de Maine.[45]

Products. Preparative scale hydrolyses and methoxyaminolyses of I–V were performed by addition of 50–100 mg of the amidine (or amidine salt) into 25 mL of the appropriate buffer (containing 0.3 M methoxyamine as necessary). The reaction mixtures were allowed to stand at 50 °C for the desired period of time, extracted into methylene chloride or chloroform, and dried (K_2CO_3 or $MgSO_4$), and solvent was removed in vacuo. Product analyses and identification were made through UV, NMR, IR, MS, and TLC. Hydrolyses and methoxyaminolyses of I, II, IV, and V gave one component by the above criteria. Hydrolysis of III for periods up to 1 h (50 °C, pH 10.2, μ = 1.0) gave an oil (65 °C), NMR ($CDCl_3$) δ 2.4–3.0 (multiplet) and 8.42 (s, –NHC*H*O), in addition to spectra attributable to the thermodynamic hydrolysis product and III. TLC of the oil revealed the appearance of an additional spot to further support the intermediacy of a kinetic hydrolysis product. Hydrolysis of either III or this product mixture for longer periods of time (>10 half-lives) gave solely the thermodynamic hydrolysis product. The behavior of III under conditions of methoxyaminolysis was analogous.

Deprotection of VI was accomplished in a procedure similar to that described for the above hydrolyses. Generally, at pH values ≤9, the deprotection of VI gave mixtures of I and Ia. At higher pH values, Ia was the product isolated. Attempts at catalyzing the removal of the trifluoroacetyl group by addition of hydroxylamine to the buffers at pH values 8-9 resulted in no change in the product composition.

Deprotection of VII was effected by catalytic hydrogenation at room temperature on the Parr apparatus with Pd/C as the catalyst. Generally VII was dissolved in the buffer (or buffer–ethanol mixtures) and hydrogenation allowed to proceed until hydrogen uptake ceased (10–15 min). The catalyst was filtered, the aqueous phase extracted with chloroform, the organic layer dried, and solvent removed.

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(21) The amidine IV exists as a mixture of cis and trans isomers (Experimental Section). This isomeric distribution will have no effect upon the stereoelectronic discussion as it will affect only the diastereomeric distribution about N_β (IVi) and will not affect rotation about $C-N_\alpha$.
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Base-Catalyzed Ester Hydrolysis in a Toluene–Water System

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Abstract: Hydrolysis reactions of aromatic esters in a toluene phase with sodium hydroxide in an aqueous phase have been studied in a stationary system. Relative reactivities of various esters are remarkably different from those obtained in a homogeneous system. For example, the hydrolysis rates for three isomers of dimethyl phthalates are in the order of ortho > para > meta isomer, whereas in 56% aqueous acetone the order is para > meta > ortho isomer. The diffusion rate of esters through the bulk liquid was found to be the most important factor which controlled the heterogeneous reaction rate of the present system. The effects of the following variables were also determined: interfacial area, reaction volume, temperature, degree of stirring, and presence of surface active agent.

Because of slow reaction velocities, organic reactions between two substances located in different phases of a mixture have not been investigated extensively. Recently, however, two interesting developments have been made in this field. One of them is a micelle catalyzed reaction system,[1] and the other one involves reactions with so-called phase-transfer catalysts.[2] Except for these systems, only a few studies have been reported. Bell[3] investigated the oxidation of benzoyl-*o*-toluidine in benzene with a neutral aqueous solution of potassium permanganate. Several decades after this pioneer work, Menger[4] successfully established a methodology of interfacial reactions. He examined an imidazole-catalyzed hydrolysis of *p*-nitrophenyl laurate at a heptane–water boundary. He found that the reaction is an interfacial one and that the reaction profile is different from that of a homogeneous reaction. Judging from the very low activation energy (nearly 0), he concluded that the migration of reactants to the interface must be at least partially rate determining. Thus, in order to clarify the property of the binary phase reaction system, it is desirable to accumulate data with respect to the mass transfer phenomena. Although there are several studies on this subject from a chemical engineering point of view,[5-7] no diffusion study with simultaneous organic reaction has been reported. The present study was undertaken to clarify the contribution of diffusion process to the reaction rate in a binary phase system. Base-catalyzed hydrolysis reactions of various aromatic esters were utilized for this purpose. Corresponding homogeneous reactions have been already investigated intensively in this connection.[8-10] In addition, this system has the advantage that a variety of esters are readily available. In the present study, three structural isomers of dimethyl phthalates, three phthalates with different alkyl groups, and several benzoates with different substituents have been used as reactants in a toluene–water system.

Experimental Section

Materials. Most of the starting materials were guaranteed reagents and used without a further purification. Methyl *p*-anisate was not standardized, and it was used after recrystallizations. Guaranteed reagent toluene and distilled water were used as solvents for ester and NaOH, respectively.

Hydrolysis Reaction. The reaction was carried out under the following conditions unless otherwise stated. An aqueous solution of NaOH (0.186 N, 20 mL) was placed in a cylindrical vessel of 20 cm^2 in cross section and of 120 mL in capacity. Then a toluene solution of ester (0.050 M, 20 mL) was added carefully. The vessel was placed in a thermostated bath. Hydrolysis was usually carried out without stirring. After a required time, the content of remaining hydroxide ion in an aqueous phase was determined by titrating with a standard HCl solution. The ester content in toluene was determined by using GLPC with a 2-m PEGS column. The rate of ester disappearance agreed well with that of NaOH consumption in water. In cases of the hydrolysis of dialkyl phthalate, no half-ester and acid were found at all in toluene. The initial hydrolysis product, the half-ester, might be extracted into the basic water where fast homogeneous hydrolysis takes place.

Diffusion of Ester. The diffusion rate of ester from a toluene phase to an aqueous phase was determined in the absence of NaOH. A toluene solution of ester (0.050 M, 20 mL) was placed over pure water (20 mL). The concentration of ester transferred into water was determined spectrophotometrically after different intervals of time, ranging from 5 min to 90 h. Spectral measurements were made by using 1-cm silica cells in a Cary 14 spectrophotometer. The absorption coefficients for most esters have not been reported yet, and we determined them in 50% aqueous ethyl alcohol. The maximum wavelengths are listed below, together with log ϵ in parentheses: DMP, 283 (3.1), 276 (3.2), 228 (4.0); DMIP, 289 (3.0), 282 (3.0), 230 (4.1);