Tetrahydrofolic Acid Model Studies. I. Equilibrium and Kinetic Studies of the Reactions of Symmetrically Substituted *N,N'*-Diphenylethylenediamines with Formaldehyde. Carbinolamine and Imidazolidine Formation[1,2]

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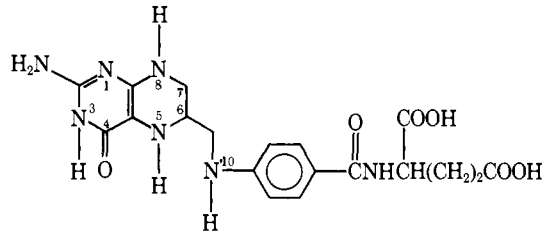
Abstract: The downward deviations in the pH-rate profiles in the pH region about 4-6 for the reactions of a series of symmetrically meta- or para-substituted *N,N'*-diphenylethylenediamines with formaldehyde in 50:50 (v/v) dioxane-water to form the imidazolidines have been shown to result from a change in rate-determining step with changing acidity. In alkaline solution, the rate-determining step is general-acid catalyzed dehydration of *N*-hydroxymethylamine adducts to form imines with Brϕnsted α values of about 0.60 which show little dependence upon the basicity of the nucleophile. In acid solution, the attack of weakly basic amines (pK'_a values of conjugate acids 1.69-4.51) upon carbonyl compound to form the *N*-hydroxymethyl adduct is rate determining with Brϕnsted α values for general-acid catalysis of 0.3. The rate constants increase with electron-donating substituents and are correlated by Hammett ρ^- values of −3.6, −2.4, and −4.2 (or β_{nuc} values of 0.77, 0.51, and 0.96) for the hydronium ion-catalyzed and pH-independent attack and the hydronium ion-catalyzed dehydration rate constants, respectively. The equilibrium constants for the formation of carbinolamines from amines and formaldehyde are about 3 M^{-1}. The equilibrium constants for the formation for free base and monocationic imidazolidines from free base and monocationic amines and formaldehyde exhibit ρ^- values of −1.08 and −0.64, respectively.

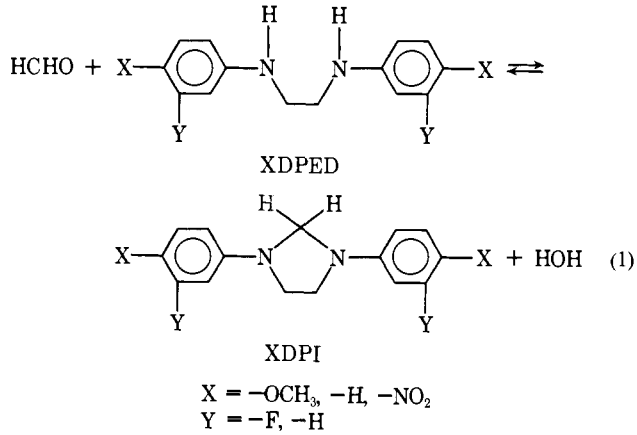
The following questions regarding structural features of tetrahydrofolic acid (THF) may be formulated with regard to its catalytic role in one-carbon unit transfers at the aldehyde oxidation level.[4,5] (1) What, if any, is the specific advantage of the pyrimidine aromatic system? (2) Is the difference in the basicity of the N_5(pK'_a = 4.82) and N_{10}(pK'_a = −1.25)[6] sites significant and, if so, how is it significant? (3) Does the N_5 site basicity in THF represent an optimum



tetrahydrofolic acid

in terms of reactivity and affinity toward carbonyl compounds which suit THF for its role with formaldehyde?

We have studied the reactions of formaldehyde and substituted *N,N'*-diphenylethylenediamines to form imidazolidines[7-9] (eq 1) in aqueous dioxane in an effort to provide



XDPED

XDPI

X = −OCH_3, −H, −NO_2
Y = −F, −H

answers to many of these questions. This report contains the results of these studies and includes the first data of which we are aware regarding substituent effects upon the rate constants for Schiff base formation via hydronium ion-catalyzed dehydration of carbinolamines formed from amines and formaldehyde. A recent study has been reported on the substituent effects upon the rate constants for hydronium ion-catalyzed dehydration of carbinolamines formed from hydrazines and *p*-chlorobenzaldehyde.[10] The present studies also provide the basis for further investigations of the reactions of imidazolidines including one-carbon transfer reactions to nucleophiles, especially carbanions, as models for THF-involved reactions which will be reported separately.

Experimental Section

Materials. Reagent grade 1,4-dioxane (Fisher) was purified according to the procedure of Fieser,[11] stored with metallic sodium, and distilled daily. DPED (Eastman Organic) was recrystallized from benzene, mp 63-66° (lit.[12] 65°). *p*-CH_3O-DPED was synthesized according to the procedure of Bennett et al.,[13] mp 104-105° (lit.[13] 104°). *p*-NO_2-DPED was synthesized according to the procedure of Linsker and Evans,[14] mp 216° (lit.[14] 216°). *m*-F-DPED was kindly supplied by Dr. D. R. Robinson and used without further purification, mp 69.5-74.0°. The ^1H NMR spectra (Jeolco C6OH) of the XDPED's were consistent with the assigned structures. The concentration of stock formaldehyde solutions (1.0 *M*), obtained from reagent grade formaldehyde (37% Fisher), was confirmed by titration with sulfite.[15]

Deionized water of greater than 5 × 10^5 ohms cm specific resistance was used throughout. The melting points are reported uncorrected.

***N*-Me-*p*-NO_2-DPED.** To a 100-ml round-bottomed flask fitted with a thermometer and reflux condenser were added 50 ml of DMSO, 0.25 g of $CuCl_2$, 21.3 g (0.134 mol) of *p*-chloronitrobenzene, and 4.95 g (0.067 mol) of *N*-methylethylenediamine. The solution was heated for 3 hr at 120°. After cooling, 150 ml of water was added, the resulting precipitate filtered, and the solid washed with 600 ml of water, dried over $CaCl_2$, and rewashed three times with 100 ml of ether (yield 5 g, mp 193-194°). Chromatography of the product by TLC on silica gel G with dioxane or chloroform revealed a single uv detectable component with R_f values of 0.56 and 0.28, respectively. The ^1H NMR spectrum in deuterated

Table I. Equilibrium Constants and Spectral Characteristics of Substituted *N,N'*-diphenylethylenediamines and Their Imidazolidine Products Formed from Formaldehyde[a]

Compd	σ^- [b]	pK'_{a_2} [c]	pK'_{a_1} [d]	pK'_{a_3} [e]	λ_{max} (log ϵ_D) [f]		K_D, M^{-1}	K_{DH}, M^{-1} [g]
					XDPED	XDPI		
p-CH_3O-DPED	−0.264	4.51	1.77	1.70	245 (4.29) 305 (3.50)	254 (4.53) 290 (3.70)	$2.52 \pm 0.37 \times 10^5$ [h]	3.90×10^2
DPED	0.0	3.64	1.82	0.51[i]	253 (4.39) 290 (3.63)	255 (4.49) 290 (3.69)	$1.33 \pm 0.13 \times 10^5$	0.99×10^2
m-F-DPED	+0.344	1.69	0	−1.14[j]	252 (4.40) 292 (3.65)	256 (4.55) 292 (4.73)	$5.50 \pm 0.25 \times 10^4$	8.14×10
p-NO_2-DPED	+1.239	−2.34[k]		−4.64[j]	400 (4.70)	400 (4.76)	$6.00 \pm 0.60 \times 10^3$	3.00×10

[a] All measurements in 50:50 (v/v) dioxane–water, ionic strength 0.10 *M*, 25°. [b] A. I. Biggs and R. A. Robinson, *J. Chem. Soc.*, 388 (1961). [c] $K'_{a_2} = a_{H^+}[XDPED]/[XDPEDH^+]$. [d] $K'_{a_1} = a_{H^+}[XDPEDH^+]/[XDPEDH_2^{2+}]$. [e] $K'_{a_3} = a_{H^+}[XDPI]/[XDPIH^+]$. [f] Wavelength (nm) used for spectrophotometric titration (log ϵ_D^+, ϵ_D) where ϵ_D^+ and ϵ_D are the molar absorptivity of monocation and free base (M^{-1} cm^{-1}), respectively (note that ϵ_D^{2+} is approximately zero at this wavelength); *p*-CH_3O-, 255 (3.78, 4.30), H-, 262 (3.58, 4.00), and *m*-F-, 250 (3.25, 4.51). [g] Calculated from $K_{DH} = K'_{a_2}K_D/K'_{a_3}$, see Scheme II. [h] $K_{D_{app}}$ (pH 3.91) = 4.92×10^3 M^{-1}. [i] Ionic strength 0.70 *M*. [j] Calcualted from $pK'_{a_3} = -4.20\sigma^- + 0.49$ based upon first two compounds. [k] Calculated from $pK'_{a_2} = -4.69\sigma^- + 3.41$ based upon first three compounds.

DMSO at 25° revealed a singlet at δ 3.1 (3 protons of $-CH_3$), a multiplet at δ 3.7 (4 protons of $-CH_2CH_2-$) and two pairs of overlapping doublets at δ 6.7, 6.8, 8.0, and 8.05 ($J \sim 9$ Hz) (8 aromatic protons) relative to Me_4Si. Anal. Calcd for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.06; N, 17.72. Found: C, 56.96; H, 5.06; N, 17.76.

Measurements of pH. Measurements of pH of aqueous solutions utilized a Radiometer Model 25 or 26SE pH meter with a combination electrode (GK 2021C), standardized with Beckman buffers at pH 4, 7, and 10. Measurements of pcH in 50:50 (v/v) dioxane–water utilized this meter and electrode with equilibration for at least 24 hr in this solvent[16] and standardization with 0.10, 0.05, and 0.01 *M* HCl in dioxane–water with ionic strength maintained at 0.1 *M* with KCl. The pH meter readings provide measurements of pcH, i.e., the negative logarithm of the hydrogen ion concentration rather than activity.[17] The difference between pH in water and pcH in dioxane–water is 0.26 calculated from the calomel electrode potential in 45% by (w/w) dioxane–water[18] (see Appendix). Therefore, pH = pcH (meter reading) + 0.26, and the data presented in this paper are expressed in terms of pH determined in this manner. The linearity of the pcH scale in this solvent was confirmed by titration of acetic acid; the slope was 1.0 for a plot of log [acetate]/[acetic acid] against pcH.

Kinetic Measurements. The rates of the reactions of aldehydes with amines were followed by measurements of absorbance at 400 nm for *p*-NO_2-DPED and 260 nm for the other XDPED's with a Coleman-Hitachi 124 recording spectrophotometer with a thermostated cell holder maintained at 25 ± 0.1°. The amine concentration was about $3\text{–}7 \times 10^{-5}$ *M*, and aldehyde was in tenfold or greater excess in order to yield pseudo-first-order kinetics. Reactions were initiated by addition of 10–20 μl of amine dissolved in ethanol or dioxane to a solution of carbonyl compound, buffer, and KCl (total volume 3.0 ml). The pseudo-first-order rate constants, k_{obsd}, were obtained by methods previously described.[19]

Proton Dissociation Constants. The ionization constant for acetic acid was determined by potentiometric titration, as described elsewhere,[20] and the pK'_a value for buffers from the pH of half neutralized solutions.

The proton dissociation constants for the mono- (D^+) and dicationic (D^{2+}) species of XDPED's (K'_{a_2} and K'_{a_1}, respectively) were determined from spectrophotometric titration at a single wavelength in the region 250–260 nm by a least-squares fit[1] to eq 2

$$\Delta A = A_D - A_X = C_1(1 + K'_{a_1}C_2/a_{H^+})/(1 + K'_{a_1}/a_{H^+} + K'_{a_1}K'_{a_2}/(a_{H^+})^2) \quad (2)$$

(Table I), where A_D and A_X are the absorbance of the same total amine concentration in alkali and at a given acidity, respectively, $C_1 = \Delta A_{max} = A_D - A_{D^{2+}}$, $A_{D^{2+}}$ is the absorbance of dication amine, $C_2 = (\epsilon_D - \epsilon_{D^+})/(\epsilon_D - \epsilon_{D^{2+}})$, and ϵ is the molar absorptivity at the given wavelength of the respective species denoted by the subscripting.

Proton dissociation constants for *p*-CH_3O-DPI and DPI, the imidazolidine products of *p*-CH_3O-DPED and DPED, respectively, were determined in dioxane–water 50:50 (v/v) by spectrophotometric titration at 255 and 260 nm, respectively, at the formaldehyde concentrations >0.5 *M*; at these carbonyl compound concentrations, the reactions to form XDPI's proceed to greater than 99% completion since, at different carbonyl compound concentrations, the final absorbance values were the same within experimental error at a given pH and total chromophore concentration. That no significant fraction of total chromophore was present as DPED under these conditions was confirmed by calculations utilizing the $K_{D_{app}}$ values or K_D and K_{DH} values in Table I and eq 3.

The equilibrium constants for the reactions of *N*-Me-*p*-NO_2-DPED and formaldehyde to form the carbinolamine, and formaldehyde and diamines to form imidazolidines were determined from absorbance measurements at 400 and 250 nm, respectively (Table I), as described elsewhere.[21]

Product Identification. The imidazolidine formed from formaldehyde and DPED was synthesized in methanol and recrystallized from aqueous ethanol, mp 123–124° (lit.[7] 124°). Anal. Calcd for $C_{15}H_{16}N_2$: C, 80.32; H, 7.19; N, 12.48. Found: C, 80.42, H, 7.17; N, 12.24. The uv spectrum of the product generated from DPED and formaldehyde in kinetic runs was that of synthetic *N,N'*-diphenylimidazolidine. Similarly, the imidazolidines from *p*-NO_2-DPED and *p*-CH_3O-DPED were synthesized, mp 240° dec and 132–134°, respectively. The ^1H NMR spectra of synthesized imidazolidines were consistent with assigned structures. For example, the ^1H NMR spectrum of DPI in deuterated chloroform at 25° and 60 MHz revealed a singlet at δ 3.5 (4 protons of $-CH_2CH_2-$), a singlet at δ 4.6 (2 protons of $>N-CH_2-N<$), and 10 aromatic protons δ 6.4–7.4. The imidazolidines are unstable at pH less than 2 as noted elsewhere[22] for related systems.

The values of $K_{hyd} = [>C(OH)_2]/[>C{=}O]$, for formaldehyde at 25°, utilized in this study are 2270[23] and 1135 ($K_{hyd}/2$) for water activity 1.0 and 50:50 (v/v) dioxane–water, respectively. Ionic strength in 50:50 (v/v) dioxane–water was maintained at 0.1 *M* with KCl.

Results

Proton Dissociation Constants. The proton dissociation constants for mono- and diprotonated amines and monoprotonated imidazolidine products and mixed solvents are contained in Table I; the proton dissociation constants for the general acid catalysts are contained in Tables III and IV. Ionizations which develop charge are in general hindered in the mixed solvent (e.g., the pK'_a value for acetic acid is 6.3 in aqueous dioxane compared with 4.6 in water), whereas isoelectric dissociations from conjugate acids of amines are more favorable in the mixed solvent (e.g., the pK'_a value for DPED is 3.64 in aqueous dioxane compared with 4.42 in water).

Equilibrium Constants. The spectrophotometrically determined pH-dependent and pH-independent constants for imidazolidine formation from the ethylenediamine derivatives

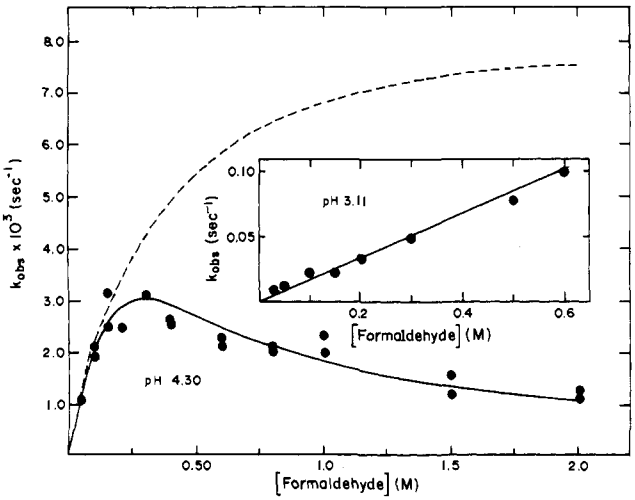


Figure 1. Dependence on total formaldehyde concentration of pseudo-first-order rate constants for the reaction of p-NO_2-DPED with formaldehyde in 50:50 (v/v) dioxane–water, 25°, ionic strength 0.10 M, pH 4.3 maintained with 0.20 M chloroacetate buffer. The *solid line* is calculated from eq 5, and the constants $K_1 = 3.21\ M^{-1}$ and $L_3 = 3.53\ M^{-1}$ are obtained from these data by a least-squares analysis, and the *dashed line* is calculated similarly except that $L_3 = 0$. Inset: dependence upon total formaldehyde concentration of pseudo-first-order rate constants for p-NO_2-DPI formation from p-NO_2-DPED and formaldehyde in 50:50 (v/v) dioxane–water, 25°, ionic strength 0.10 M, pH 3.1 maintained with 0.10 M chloroacetate buffer. The second-order constant, $k_{obsd}/[F]$, obtained from the slope is 0.17 M^{-1} sec^{-1}.

and formaldehyde ($K_{D_{app}}$, K_D, and K_{DH}) are contained in Table I. The $K_{D_{app}}$ values are related to the K_D and K_{DH} values through eq 3 and $K'_{a_3}K_{DH} = K'_{a_2}K_D$, where $K'_{a_3} = a_{H^+}[XDPI]/[XDPIH^+]$, $K_D = [XDPI]/[XDPED][F]$, $K_{DH} = [XDPIH^+]/[XDPEDH^+][F]$, $K'_{a_2} = [XDPED]a_{H^+}/[XDPEDH^+]$, $K_{D_{app}} = [XDPI_T]/[XDPED_T]/[F]$, F = formaldehyde (hydrated and unhydrated), $\alpha_{R_2NH} = 1/(1 + a_{H^+}/K'_{a_2})$, and T = total for the specified compound regardless of state of ionization. The second term on the right of the equality in eq 3 is insignificant under the conditions

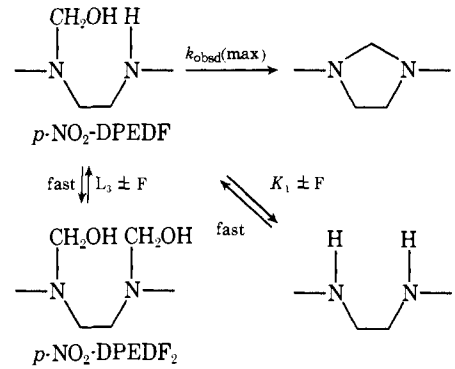
$$K_{D_{app}} = K_D\alpha_{R_2NH} + K_{DH}(1 - \alpha_{R_2NH}) \qquad (3)$$

of the experiments utilized to determine the values of $K_{D_{app}}$ (Table I).

Imidazolidine Formation from Formaldehyde. The pseudo-first-order rate constants for product formation, k_{obsd}, increase linearly with increasing formaldehyde (F) concentration below pH 4–6 (Figure 1, inset) and indicate that the rate is first order in respect to the carbonyl concentration.

Increases in the concentration of formaldehyde produced no change in the final absorbance readings and indicated that the reactions had proceeded to completion. This is consistent with the apparent equilibrium constant for imidazolidine formation and, as well, zero ordinate intercepts for plots of k_{obsd} vs. [F] (Figure 1, inset) which indicate that the reverse reactions under these conditions are insignificant.

At more alkaline pH values, the linear dependence of rate on formaldehyde concentration continues to obtain for the reaction of XDPED's with formaldehyde at low formaldehyde concentration. However, at high concentrations of formaldehyde as shown for p-NO_2-DPED, the rate of imidazolidine formation levels off as the formaldehyde concentration is increased and, at even higher formaldehyde concentration, the rates decrease (Figure 1). Spectra taken at high formaldehyde concentration at different times (Figure 2A) show that there is an initial decrease of absorbance (cf. an "initial burst" of absorbance)[6] followed by a slower formation of the imidazolidine product (manifested by a slower second-phase increase in absorbance at 400 nm). The leveling off and subsequent decrease of the rate of N,N'-di(p-nitrophenyl)imidazolidine formation (p-NO_2-DPI) as the formaldehyde concentration is increased (Figure 1) is therefore due to the conversion of p-NO_2-DPED to a monohydroxymethylamine (p-NO_2-DPEDF) adduct (the further reaction of which to form the imidazolidine product is rate determining) and to the accumulation of an unreactive or much less reactive N,N'-dihydroxymethylamine adduct (p-NO_2-$DPEDF_2$) (which acts to decrease the concentration of the p-NO_2-DPEDF causing the observed rates to decrease), respectively (eq 5).



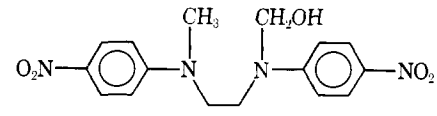
(4)

The data in Figure 1 were fitted to eq 5 by a least-squares method; eq 5 is derived from the mechanism in eq 4

$$k_{obsd} = k_{obsd}(max)K_1[F]/(1 + K_1[F] + K_1L_3[F]^2) \qquad (5)$$

by considering K_1 and L_3 rapid prior equilibria, and k_{obsd}(max) is the first-order rate constant for the formation of imidazolidine from p-NO_2-DPEDF, $K_1 = [p\text{-}NO_2\text{-}DPEDF]/[p\text{-}NO_2\text{-}DPED][F]$, and $L_3 = [p\text{-}NO_2\text{-}DPEDF_2]/[F][p\text{-}NO_2\text{-}DPEDF]$. In the absence of accumulation of p-NO_2-$DPEDF_2$, the observed rate constants for product formation vs. formaldehyde concentration would follow the dashed line in Figure 1. The behavior of the other DPED's is qualitatively similar, but insufficient number of data points at high formaldehyde precluded an accurate determination of L_3. However, it is estimated that L_3 is approximately equal to K_1 for the other DPED's.

N-Me-p-NO_2-DPED was synthesized, the formaldehyde adduct of which would serve as a model for the carbinolamines, p-NO_2-$DPEDF_2$ or p-NO_2-DPEDF.[24a] As can be seen from the spectra of Figure 2B, the spectrum of N-Me-p-NO_2-DPED in excess formaldehyde parallels the first-phase spectral changes observed with p-NO_2-DPED (Figure 2A). The value of the equilibrium constant of 0.96 M^{-1} measured for adduct formation (i) from N-Me-p-NO_2-



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DPED and formaldehyde is entirely consistent with hydroxymethylamine formation.[6,24a]

Catalysis of imidazolidine formation from formaldehyde and substituted amines by buffers in the pH range studied is proportional to the acid species of the buffer (Figure 3). Plots of k_{obsd} against buffer concentration (Table II) at all pH values where buffer catalysis was detectable show acceptable fits to eq 6, the complete steady-state rate equation for the mechanism depicted in eq 9 (see Discussion).

The general-acid catalyst term (HA) depends on the nature

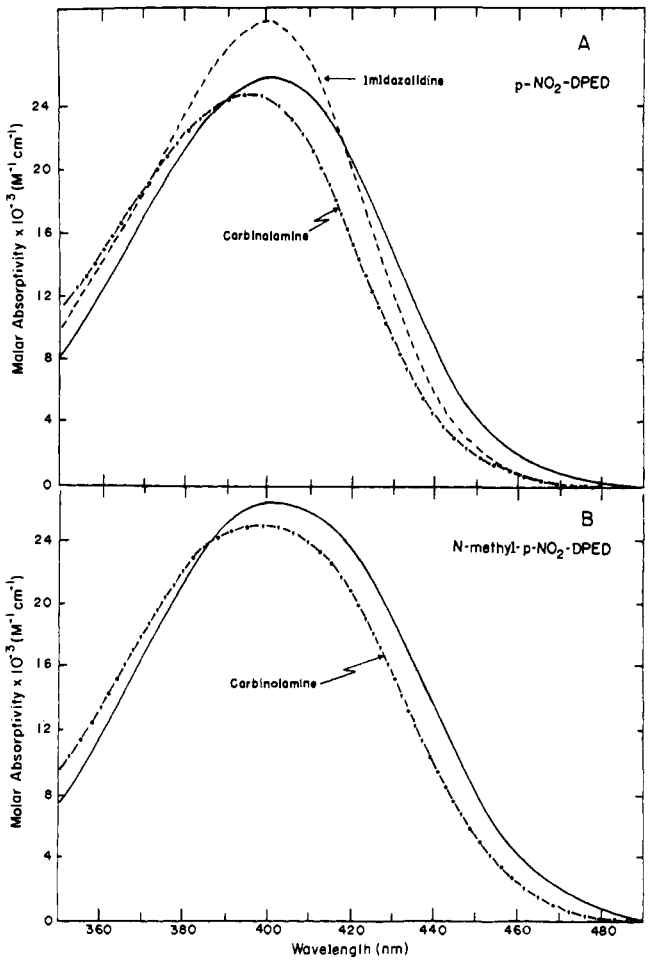


Figure 2. (A) Spectra of p-NO_2-DPED (——), mixture of carbinolamines of p-NO_2-DPED in 1.5 M formaldehyde (- · · · ·), and p-NO_2-DPI (- - -) in 50:50 (v/v) dioxane-water, 23-25°, ionic strength 0.10 M, pH 4.6 maintained with 0.10 M chloroacetate buffer. Estimated composition, 85% N,N'-dicarbinolamine and 15% N-carbinolamine. (B) N-Me-p-NO_2-DPED (——) and its formaldehyde adduct (- · - · - · -) in 1.5 M formaldehyde in 50:50 (v/v) dioxane-water, 23-25°, ionic strength 0.10 M, pH 4.6 maintained with 0.10 M chloroacetate buffer. Estimated 59% conversion to adduct.

$$k_{obsd} = A_1[F]\alpha_{R_2NH}/(Z_1 + 1) \quad (6)$$

where

$$A_1 = k_1 + k'_1a_{H^+} + k''_1[HA]$$

$$Z_1 = (k_1 + k'_1a_{H^+} + k''_1[HA])/K_1(k'_2a_{H^+} + k''_2[HA])$$

F = formaldehyde

α_{R_2NH} = fraction amine as the free base

HA = general-acid catalyst

K_1 = carbinolamine association constant

and state of ionization of the catalyst. For monobasic weak acids, monoprotonated weak dibasic acids and diprotonated dibasic weak acids [HA] = [buffer]/(1 + K'_{app}/a_{H^+}), [buffer]/(1 + a_{H^+}/K'_{app_1} + K'_{app_2}/a_{H^+}), and [buffer]/(1 + K'_{app_1}/a_{H^+} + $K'_{app_1}K'_{app_2}/a_{H^+}^2$), respectively. K'_{app}, K'_{app_1}, and K'_{app_2} are proton dissociation constants for monobasic weak acids, and diprotonated and monoprotonated forms of dibasic weak acids, respectively (referred to as K'_a in Tables II-IV). The other constants in eq 6 are defined in Tables III, IV, and V.

At low pH values, where attack of amine on formaldehyde is rate determining, eq 6 reduces to eq 7 (for more details, see ref 25a, p 590). The slopes of plots of $k_{obsd}/\alpha_{R_2NH}[F] - (k_1 + k'_1a_{H^+})$ against [HA] (eq 7) provide the

$$k_{obsd}/[F]\alpha_{R_2NH} = k_1 + k'_1a_{H^+} + k''_1[HA] \quad (7)$$

values for k''_1 (Table III). Error limits were obtained from a least-squares evaluation of the data.

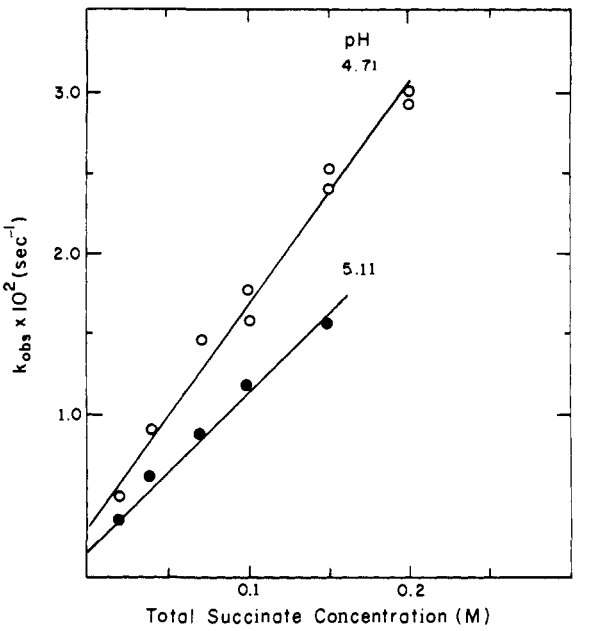


Figure 3. Dependence upon total succinate buffer concentration of the first-order rate constants for imidazolidine formation from m-F-DPED and formaldehyde (1.0×10^{-3} M) in 50:50 (v/v) dioxane-water, 25°, ionic strength 0.1 M. The *solid lines* are calculated from eq 7 and the constants in Table III.

At high pH values, where dehydration of the carbinolamine intermediate is rate determining, eq 6 reduces to eq 8.

$$k_{obsd}/[F]\alpha_{R_2NH} = K_1(k'_2a_{H^+} + k''_2[HA]) \quad (8)$$

The slopes of plots of $k_{obsd}/[F]\alpha_{R_2NH} - K_1k'_2a_{H^+}$ against [HA] equal $K_1k''_2$. Such plots were unsatisfactory for the determination of k''_2 since the buffers utilized in the study that were good catalysts (low pK'_a value) did not span the region of the pH-rate profile where k'_2 is completely rate determining. Consequently, plots of k_{obsd} against [buffer] in the region of the change in rate-determining step show deviations from linearity at higher buffer concentrations (Figure 4). The values of k''_2 (Table IV) were determined by iterative calculations employing eq 6 and values of k''_1 determined as described above. It must be stressed that values of k''_1, used in the analysis of k''_2, were calculated *independently* of k''_2, i.e., at a pH where k''_2 contributes insignificantly to the observed rate. Least-squares curve fitting procedures utilizing eq 6 were unsuccessful in the evaluation of k''_2 due to the relatively large contribution of k''_1 to the observed rate. Error limits for k''_2 were estimated and reflect the number of determinations and the closeness of the fit of the experimental data to eq 6.

Under no circumstances was general-base catalysis detectable by the conjugate base of the buffers (ref 25a, p 166).

Nonbuffer-catalyzed rate constants, k_{nbc}, were obtained by extrapolation of plots of k_{obsd} vs. [buffer] to zero buffer concentration. The pH dependence of the nonbuffer-catalyzed second-order rate constants for imidazolidine formation from p-CH_3O-DPED and DPED are bell-shaped (Figure 5, inset). The apparent inflection points for the reaction of formaldehyde with p-CH_3O-DPED are at pH 2.0 and 5.0 with a shoulder at about pH 6.5 and, for the reaction of

Table II. Pseudo-First-Order Constants, k_{obsd} (sec^{-1}), for General-Acid Catalysis of the Reaction of XDPED with Formaldehyde in 50:50 (v/v) Dioxane–Water, 25°, Ionic Strength 0.1 M[a]

XDPED	Catalyst	pK'_a	pH	[F] × 10^4	k_{nbc} × 10^2[b]	[Buffer] × 10^2	k_{obsd} × 10^2
p-CH_3O–	Dichloro-acetic acid	2.70	2.48	5.00	8.00	1.00	8.44
						2.00	8.88
						3.00	8.98
						4.00	9.76
						5.00	10.1
						7.00	11.7
						10.0	12.5
						15.0	13.8
			2.66	1.00	0.780	2.00	0.950
						4.00	1.10
						10.0	1.60
						20.0	2.30
p-CH_3O–	Malonic acid	3.92	3.89	2.00	1.20	2.00	1.46
						4.00	1.80
						8.00	2.45
						10.0	2.77
						15.0	3.28
						20.0	3.82
			4.38	2.00	0.940	1.00	1.10
						2.00	1.15
						4.00	1.60
						6.00	1.65
						8.00	1.80
						10.0	2.05
						15.0	2.70
						20.0	2.95
p-CH_3O–	Chloro-acetic acid	4.26	3.33	1.00	1.56	2.00	1.93
						4.00	2.04
						10.0	3.02
						20.0	3.98
			4.29	1.00	0.760	2.00	0.960
						4.00	1.00
						10.0	1.60
						20.0	2.32
			4.41	5.00	3.10	1.00	3.61
						2.00	3.84
						3.00	4.38
						4.00	4.89
						5.00	5.56
						7.00	6.27
						10.0	7.10
						15.0	9.25
p-CH_3O–	Formic acid	4.85	3.78	2.00	3.00	10.0	4.36
						20.0	5.79
						40.0	9.18
						80.0	13.3
			4.86	1.00	0.800	4.00	1.27
						10.0	1.83
						20.0	3.10
p-CH_3O–	Succinic acid	5.58	4.61	5.00	2.20	1.00	3.10
						2.00	4.50
						4.00	6.50
						6.00	8.90
						8.00	10.1
						10.0	12.0
						15.0	19.2
						20.0	16.7
			5.05	5.00	1.60	1.00	2.45
						2.00	4.15
						4.00	6.48
						6.00	8.12
						8.00	13.5
						10.0	15.2
						15.0	20.1
						20.0	24.5
p-CH_3O–	Acetic acid	6.22	5.28	1.00	0.650	10.0	1.78
						20.0	3.00
						40.0	5.45
						80.0	9.35
			5.71	1.00	0.310	4.00	0.620
						6.00	0.880
						8.00	0.960
						14.0	1.560
			6.28	1.00	0.250	5.00	0.600
						10.0	1.02
						20.0	1.72

Table II *(Continued)*

XDPED	Catalyst	pK'_a	pH	[F] × 10^4	$k_{nbc} \times 10^2$ [b]	[Buffer] × 10^2	$k_{obsd} \times 10^2$
			6.68	1.00	0.230	2.00	0.280
						4.00	0.350
						6.00	0.400
						10.0	0.500
p-CH_3O–	Succinic[c] acid	6.67	6.62	10.0	2.40	2.00	2.89
						3.00	3.55
						5.00	4.45
						7.00	5.00
						10.0	5.50
						15.0	5.70
						20.0	8.48
			7.06	10.0	1.80	2.00	2.09
						3.00	2.19
						4.00	2.30
						6.00	2.73
						8.00	2.76
						10.0	2.82
						15.0	3.20
p-CH_3O–	Malonic[c] acid	6.72	5.78	10.0	1.30	2.00	1.20
						4.00	1.75
						6.00	2.00
						8.00	2.30
						10.0	2.73
						15.0	3.23
						20.0	3.75
						30.0	4.84
			6.71	10.0	2.22	1.00	2.30
						2.00	2.16
						3.00	2.36
						5.00	2.67
						7.00	3.06
						10.0	3.27
						15.0	3.50
						20.0	3.86
			7.11	10.0	1.75	2.00	1.79
						4.00	1.82
						6.00	1.95
						8.00	2.05
						10.0	1.92
						15.0	2.55
						20.0	2.25
						30.0	2.50
H–	Dichloro-acetic acid	2.70	2.64	2.00	0.550	2.00	0.740
						3.00	0.850
						5.00	1.02
						10.0	1.32
						15.0	1.68
H–	Malonic acid	3.92	3.12	2.00	0.450	2.00	0.630
						4.00	0.840
						6.00	1.08
						8.00	1.33
						10.0	1.46
						15.0	1.92
						20.0	2.38
			3.94	2.00	0.260	1.00	0.290
						2.00	0.380
						4.00	0.630
						6.00	0.910
						6.00	0.840
						8.00	1.03
						8.00	1.18
						10.0	1.33
						15.0	1.90
						20.0	2.24
H–	Chloro-acetic acid	4.26	3.04	2.00	0.440	4.00	0.800
						10.0	1.33
						20.0	2.25
			4.26	2.00	0.130	2.00	0.330
						4.00	0.550
						10.0	1.16
						20.0	2.20
H–	Formic acid	4.85	3.86	2.00	0.500	10.0	1.50
						20.0	2.70
						40.0	5.20
						100	9.20
			4.81	2.00	0.170	5.00	0.550

Table II *(Continued)*

XDPED	Catalyst	pK'_a	pH	[F] × 10^4	k_{nbc} × 10^2 [b]	[Buffer] × 10^2	k_{obsd} × 10^2
						10.0	1.00
						15.0	1.30
						20.0	1.87
H–	Succinic acid	5.58	4.61	4.00	0.300	1.00	0.700
						2.00	1.06
						4.00	1.96
						6.00	2.80
						8.00	3.46
						10.0	4.35
						15.0	6.60
						20.0	7.50
H–			5.03	10.0	0.600	2.00	2.40
						4.00	4.30
						6.00	6.00
						8.00	7.80
						10.0	8.80
						15.0	13.0
H–	Acetic acid	6.22	5.26	2.00	0.300	10.0	0.920
						20.0	1.60
						40.0	2.72
						80.0	4.73
			6.27	2.00	0.110	5.00	0.232
						10.0	0.370
						15.0	0.405
						20.0	0.532
			6.22	10.0	0.350	1.00	0.500
						2.00	0.750
						4.00	1.14
						5.00	1.17
						5.00	1.28
						7.00	1.44
						10.0	1.95
						10.0	1.85
						15.0	2.30
						20.0	2.65
						20.0	2.70
			6.70	100	2.20	1.00	2.80
						2.00	3.68
						4.00	5.00
						5.00	5.70
						7.00	7.00
						10.0	7.95
						20.0	13.8
			5.74	50.0	2.00	1.00	3.00
						2.50	6.80
						5.00	10.9
						7.00	13.5
						10.0	17.4
						15.0	22.4
						20.0	29.3
						30.0	34.0
						40.0	37.5
H–	Succinic[c] acid	6.67	6.70	100	2.40	1.00	2.70
						2.00	3.65
						3.00	4.25
						5.00	6.00
						7.00	7.10
						10.0	7.70
						15.0	9.40
						20.0	11.2
			7.11	100	1.45	1.00	1.58
						2.00	1.88
						3.00	2.42
						4.00	2.60
						6.00	2.80
						10.0	3.53
H–	Malonic[c] acid	6.72	6.01	25.0	0.480	1.00	0.620
						2.00	0.770
						3.00	0.930
						4.00	1.09
						7.00	1.57
						10.0	2.00
						15.0	2.57
						20.0	3.18
						30.0	4.13
						40.0	5.10

Table II *(Continued)*

XDPED	Catalyst	pK'_a	pH	[F] × 10^4	$k_{nbc} \times 10^2$ [b]	[Buffer] × 10^2	$k_{obsd} \times 10^2$
			6.72	100	2.05	2.00	2.30
						3.00	2.65
						5.00	3.22
						7.00	3.70
						10.0	4.00
						15.0	4.80
						20.0	6.26
			6.84	100	1.50	1.00	1.65
						2.00	1.85
						4.00	2.20
						6.00	2.50
						8.00	2.80
						10.0	2.93
						15.0	3.43
						20.0	3.70
m-F–	Dichloro-acetic acid	2.70	2.59	2.00	0.600	2.00	2.00
						3.00	2.60
						5.00	4.00
						10.0	7.60
	Malonic acid	3.92	3.48	10.0	0.340	2.00	0.600
						4.00	1.20
						7.00	1.80
						10.0	2.50
						15.0	3.50
						20.0	4.80
			3.91	10.0	0.140	1.00	0.200
						2.00	0.350
						4.00	0.750
						7.00	1.20
						10.0	1.60
						10.0	1.80
						15.0	2.50
						15.0	2.75
						20.0	3.10
						20.0	3.75
m-F–	Chloro-acetic acid	4.26	3.30	10.0	0.350	2.00	0.600
						4.00	0.950
						6.00	1.30
						8.00	1.65
						10.0	2.20
						15.0	3.05
			3.75	10.0	0.250	5.00	0.85
						10.0	1.70
						20.0	3.20
						30.0	4.60
						40.0	5.90
			4.26	50.0	0.600	5.00	3.00
						7.00	4.00
						10.0	5.06
						15.0	7.10
						20.0	10.4
m-F–	Formic acid	4.85	3.91	10.0	0.150	5.00	0.900
						7.00	1.20
						10.0	1.60
						20.0	3.25
m-F–			4.33	100	1.40	5.00	5.80
						10.0	11.0
						20.0	22.0
						30.0	29.8
			4.86	100	1.00	5.00	3.40
						7.00	5.02
						15.0	9.00
						20.0	12.0
m-F–	Succinic acid	5.58	4.71	10.0	0.260	1.00	0.290
						2.00	0.435
						4.00	0.880
						8.00	1.44
						10.0	1.54
						10.0	1.63
						15.0	2.37
						15.0	2.50
						20.0	2.90
						20.0	2.96
			5.11	10.0	0.200	2.00	0.370
						4.00	0.630
						7.00	0.890

Table II *(Continued)*

XDPED	Catalyst	pK'_a	pH	[F] × 10^4	k_{nbc} × 10^2 [b]	[Buffer] × 10^2	k_{obsd} × 10^2
						10.0	1.19
						15.0	1.57
						20.0	1.84
m-F–	Acetic acid	6.22	5.80	100	1.60	2.50	1.50
						5.00	2.15
						7.00	2.50
						10.0	2.60
						15.0	3.45
						20.0	3.85
						30.0	4.60
						40.0	5.65
			6.21	100	0.600	5.00	1.00
						10.0	1.36
						15.0	1.53
						20.0	1.88
m-F–	Succinic[c] acid	6.67	6.32	500	0.530	1.50	0.680
						5.00	1.15
						6.00	1.36
						8.00	1.58
m-F–			6.71	100	0.320	2.00	4.12
						5.00	6.10
						7.00	6.30
						7.00	6.55
						10.0	8.00
						10.0	7.50
m-F–	Malonic[c] acid	6.72	5.71	10.0	0.400	2.00	0.350
						8.00	1.00
						10.0	1.27
						15.0	1.65
						20.0	2.06
						28.0	2.53
			5.89	10.0	0.123	1.00	0.200
						1.00	0.185
						2.00	0.264
						5.00	0.530
						5.00	0.550
						7.00	0.612
						10.0	0.963
						15.0	1.57
						15.0	1.63
			6.27	100	0.250	4.00	0.385
						6.00	0.472
						8.00	0.563
						10.0	0.600
						15.0	0.863
						20.0	1.18
			6.76	100	0.114	1.00	0.130
						2.00	0.162
						5.00	0.237
						5.00	0.257
						7.00	0.286
						7.00	0.298
						10.0	0.314
						15.0	0.400
						15.0	0.442
			7.01	100	0.105	2.00	0.130
						5.00	0.225
						7.00	0.250
						10.0	0.292
						15.0	0.310
						18.0	0.330
						20.0	0.345
p-NO_2–	Trichloro-acetic acid	1.82	1.82	605	0.850	5.00	1.40
						7.50	2.00
						10.0	2.70
						15.0	3.40
						20.0	4.00
						30.0	5.20
						40.0	7.50
						50.0	8.40
p-NO_2–	Dichloro-acetic acid	2.70	1.84	300	0.400	10.0	1.50
						20.0	2.70
						30.0	3.80
						40.0	4.50
						50.0	5.00
						100	9.50

Table II *(Continued)*

XDPED	Catalyst	pK'_a	pH	[F] × 10^4	k_{nbc} × 10^2 [b]	[Buffer] × 10^2	k_{obsd} × 10^2
			2.93	2000	0.650	2.50	1.00
						5.00	2.00
						10.0	3.20
p-NO_2–	Maleic acid	3.16	2.21	605	0.250	5.00	0.900
						10.0	1.30
						15.0	1.80
						20.0	2.45
						30.0	3.10
						50.0	4.40
p-NO_2–	Malonic acid	3.92	2.86	605	0.200	5.00	0.470
						7.00	0.600
						10.0	0.750
						15.0	1.12
						20.0	1.45
						30.0	1.98
						40.0	2.50
						50.0	3.09
p-NO_2–	Chloro-acetic acid	4.26	3.87	2000	0.250	2.50	0.350
						4.00	0.453
						4.00	0.475
						6.00	0.510
						8.00	0.575
						10.0	0.623
			3.32	1210	0.400	10.0	0.780
						20.0	1.18
						30.0	1.42
						40.0	1.93

[a] $v = k_{obsd}$[XDPED]. [b] Nonbuffer-catalyzed pseudo-first-order rate constant. [c] Ionic strength 0.4 *M*.

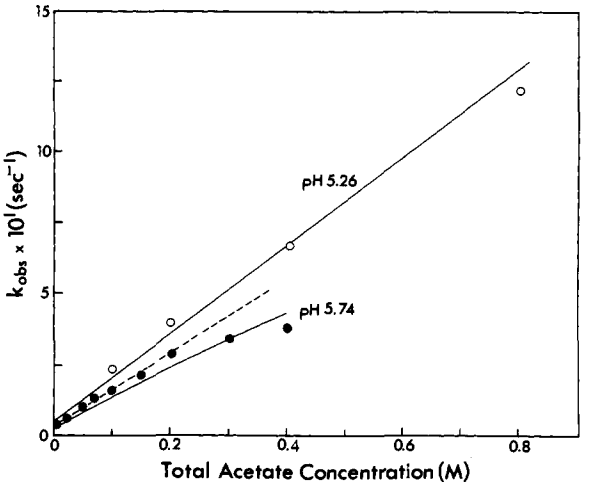


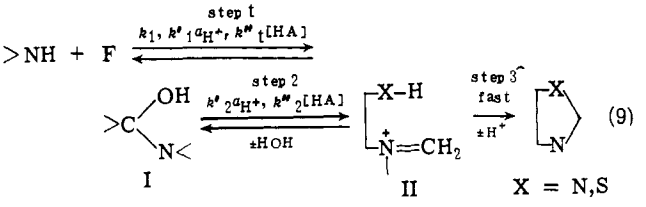
Figure 4. Dependence upon total acetic acid buffer concentration of the first-order rate constants for imidazolidine formation from DPED and formaldehyde (5.0×10^{-3} *M* in 50:50 (v/v) dioxane-water, 25°, ionic strength 0.10 *M*). The *solid lines* are calculated from eq 6, the constants in Tables III and IV and $k'_1 = 2.6 \times 10^5\ M^{-2}\ sec^{-1}$ for O—O. The *dashed line* is calculated assuming no change in rate-determining step.

formaldehyde with DPED, are at pH 2.0 and 3.8 with a shoulder at about pH 5.5. Logarithmic plots of rates against pH, with respect to amine as the free base, for these and related compounds show two hydronium ion-dependent limbs separated by pH-independent plateaus in the pH range 3.3-6.3 (Figure 5). The values for k'_1, k_1, and k'_2 (Table V) were calculated from the nonbuffer-catalyzed rate constants (Figure 5) by a least-squares analysis utilizing eq 10, which is identical with eq 6 but excludes the buffer terms. In Tables III and IV, for hydronium ion as a general acid catalyst $k''_1 = k'_1$ and $k''_2 = k'_2$ and for water as a general catalyst $k''_1 = k_1/[H_2O]$.

There was no effect on the rate of imidazolidine formation of variation in ionic strength from 0.10 to 0.70 *M* in 50:50 (v/v) dioxane-water in the pH range of 4.0-7.0.

Discussion

The explanation of the complex variation of the rate with pH (Figure 3) rests on the following principles which have been established for related reactions[6,10,19,24-30] involving Schiff base formation and are described in terms of eq 9.



(9)

(1) At alkaline pH, the rate-determining step of nitrogen derivative formation is the uncatalyzed,[6,10,19,25a,27,30] base-, or acid-catalyzed dehydration of the carbinolamine intermediate (I) (step 2, eq 9) to form the Schiff base (II).[31]

(2) At acid pH, the rate-determining step of nitrogen derivative formation is the uncatalyzed, acid-, or base-catalyzed attack of free base amine on the carbonyl group (step 1, eq 9).[6,10,19,24a,25a,27,30]

(3) The dehydration of carbinolamine addition intermediates formed from weakly basic amines is aided by acid and base catalysis.[6,10,19,25a,27,30]

(4) The attack of weakly basic amines upon carbonyl compounds is aided by general acid and base catalysis.[24a]

(5) For nitrogen derivatives formed from bifunctional amines and carbonyl compounds by nucleophilic attack of a neighboring atom (X in eq 9) upon the Schiff base (step 3, eq 9), e.g., thiazolidine[19] and imidazolidine[6] formation, step 1 or 2 remains rate determining. A previous interpretation[8,9] which specified that the cyclization step was rate determining for imidazolidine formation from amine and carbonyl compound is currently in question.[30]

(6) The change in rate-determining step from attack of amine upon formaldehyde to dehydration of the carbinolamine intermediate may be manifested at a given pH in the region of the change in rate-determining step by hyperbolic dependencies of the rate upon buffer concentration.[6,19,25a]

Table III. Third-Order Catalytic Constants, k''_1 (M^{-2} sec^{-1}), for General-Acid Catalysis of the Attack of XDPED on Formaldehyde in 50:50 (v/v) Dioxane–Water, 25°, Ionic Strength 0.1 M[a]

Catalyst	pK'_a	pH	Concn, M	p-CH_3O– × 10^{-3}	H– × 10^{-3}	m-F– × 10^{-3}	p-NO_2–
H_3O^+	−1.45[b]	1.3–5.0		3.3 ± 0.3 × 10^3 (28)	1.3 ± 0.1 × 10^2 (23)	6.6 ± 0.6 (28)	4.7 ± 0.6 (8)
Trichloroacetic acid	1.82	1.8	0.05–0.50				5.7 ± 0.2 (9)
Dichloroacetic acid	2.70	2.5–2.9	0.01–1.0	155 ± 8.6 (12)	10.5 ± 1.0 (5)	0.70 ± 0.3 (4)	3.5 ± 0.2 (6)
Maleic acid	3.16	2.2	0.05–0.40				1.9 ± 0.02 (8)
Malonic acid	3.92	2.9–4.4	0.05–0.50	6.3 ± 0.4 (14)	2.0 ± 0.2 (17)	0.31 ± 0.01 (16)	1.2 ± 0.2 (8)
Chloroacetic acid	4.26	3.3–4.3	0.01–0.40	4.5 ± 0.2 (16)	1.5 ± 0.9 (7)	0.25 ± 0.01 (16)	0.35 ± 0.06 (10)
Formic acid	4.85	3.8–4.9	0.01–0.80	4.1 ± 0.2 (7)	0.95 ± 0.06 (8)	0.18 ± 0.01 (12)	
Succinic acid	5.58	4.6–5.1	0.01–0.20	4.3 ± 0.4 (16)	1.5 ± 0.2 (14)	0.22 ± 0.002 (16)	
Acetic acid	6.22	5.3–6.7	0.01–0.78	1.6 ± 0.2 (15)	0.43 ± 0.04 (35)	0.02 ± 0.002 (15)	
Succinic acid	6.67[c]	6.3–7.1	0.01–0.20	0.70 ± 0.1 (14)	0.10 ± 0.01 (14)	0.05 ± 0.005 (10)	
Malonic acid	6.72[c]	5.7–7.2	0.01–0.30	0.30 ± 0.06 (16)	0.08 ± 0.01 (25)	0.15 ± 0.03 (37)	
HOH	17.2[d]	11.6–12.0		9.0 ± 0.8 × 10^{-4} (28)	1.0 ± 0.1 × 10^{-4} (23)	3.9 ± 0.3 × 10^{-5} (28)	8.3 ± 0.8 × 10^{-4} (14)

[a] $v = k''_1$[XDPED][F][HA], number of determinations is given in parentheses. [b] Based on water activity 0.5 in dioxane–water. [c] Ionic strength 0.4 M. [d] Calculated from $K_{WD} = 1.80 \times 10^{-16}$ (H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions", Reinhold, New York, N.Y., 1950).

Table IV. Second-Order Catalytic Constants, k''_2 (M^{-1} sec^{-1}), for General-Acid Catalysis of the Dehydration of N-Hydroxymethyl Intermediates of XDPED's and Formaldehyde in 50:50 (v/v) Dioxane–Water, 25°, Ionic Strength 0.1 M[a]

Catalyst	pK'_a	pH	Concn	p-CH_3O–	H–	m-F–	p-NO_2–
H_3O^+	−1.45[b]	4.0–8.5		2.2 ± 0.1 × 10^8 (28)	1.1 ± 0.09 × 10^7 (23)	1.8 ± 0.1 × 10^5 (24)	129 ± 8.0 (14)
Malonic acid	3.92	2.9–4.4	0.05–0.50				1.0 ± 0.3 (8)
Chloroacetic acid	4.26	3.3–4.3	0.01–0.40			190 ± 50 (16)	0.30 ± 0.09 (10)
Formic acid	4.85	3.8–4.9	0.01–0.80			140 ± 30 (12)	
Succinic acid	5.58	4.6–5.1	0.01–0.20	2000 ± 400 (16)	1000 ± 200 (14)	150 ± 30 (16)	
Acetic acid	6.22	5.3–6.7	0.01–0.78	630 ± 120 (15)	160 ± 36 (21)	14.0 ± 3.0 (15)	
Succinic acid	6.67[c]	6.3–7.1	0.01–0.20	400 ± 80 (14)	100 ± 20 (14)	5.0 ± 1.0 (10)	
Malonic acid	6.72[c]	5.7–7.2	0.01–0.30	320 ± 64 (16)	20 ± 4.0 (25)	2.5 ± 0.5 (37)	
HOH	17.2[d]	11.6–12.0		6.5 ± 1.2 × 10^{-6} (3)	4.5 ± 0.9 × 10^{-7} (3)	3.0 ± 0.6 × 10^{-10} (3)	

[a] $v = K_1 k''_2$[XDPED][F][HA] = k''_2[X][HA], number of determinations is given in parentheses; X, carbinolamine intermediate; K_1 values, see Table V. [b] Based on water activity of 0.5 in dioxane-water. [c] Ionic strength = 0.5 M. [d] Calculated from $K_{WD} = 1.80 \times 10^{-16}$, see footnote d, Table III.

Table V. Equilibrium and Rate Constants for the Reactions of Formaldehyde with Substituted Ethylenediamines[a]

Constants	Units	THF (W)[b]	p-CH_3O– (D/W)	H– (D/W)	m-F– (D/W)	p-NO_2– (D/W)
$k'_1v = k'_1[>NH][F]a_{H+}$	M^{-2} sec^{-1}	4.8×10^5	3.3×10^6	1.3×10^5	6.6×10^3	4.7
$k'_{-1}v = k'_{-1}[X]a_{H+}$	M^{-1} sec^{-1}	1.5×10^4	9.4×10^5	3.3×10^4	2.2×10^2	1.5
$k'_{1u}v = k'_{1u}[>NH][>C{=}O]a_{H+}$	M^{-2} sec^{-1}	1.1×10^9	3.6×10^9	1.4×10^8	7.3×10^6	5.2×10^3
$k_1v = k_1[>NH][F]$	M^{-1} sec^{-1}	90	2.5×10^1	2.9	1.0	2.3×10^{-2}
$k_{1u}v = k_{1u}[>NH][>C{=}O]$	M^{-1} sec^{-1}	2.1×10^5	2.8×10^4	3.2×10^3	1.1×10^3	2.1×10^1
$k_{-1}v = k_{-1}[X]$	sec^{-1}	2.8	7.2×10^{-1}	7.3×10^{-1}	3.3×10^{-1}	7.2×10^{-3}
$K_1k_2v = K_1k_2[>NH][F]$	M^{-1} sec^{-1}		6.3×10^{-4}	5.0×10^{-5}	2.5×10^{-8}	
$K_1k'_2v = K_1k'_2[>NH][F]a_{H+}$	M^{-2} sec^{-1}	8.6×10^8	7.4×10^8	4.4×10^7	5.5×10^5	4.2×10^2
$k'_2v = k'_2[X]a_{H+}$	M^{-1} sec^{-1}	2.7×10^7	2.2×10^8	1.1×10^7	1.8×10^5	1.3×10^2
$K_1 = [X]/[>NH][F]$	M^{-1}	32	3.5[d]	4.0[d]	3.0[d]	3.2
$K_1(1 + K_{hyd}{}^c) = [X]/[>NH][>C{=}O]$	M^{-1}	7.3×10^4	3.9×10^3	4.4×10^3	3.3×10^3	3.6×10^3

[a] $>C{=}O$, unhydrated formaldehyde; D, dioxane; D/W, 50:50 (v/v) dioxane–water, ionic strength 0.1 M; F, formaldehyde (hydrated and unhydrated); W, HOH; ionic strength 1.0 M; X, carbinolamine. [b] See ref 6. [c] $K_{hyd} = [>C(OH)_2]/[>C{=}O]$ and corrected by a factor of 2 for the difference in water activity in the mixed solvent (see ref 23). [d] Obtained from data similar to that in Figure 1.

Imidazolidine Formation from Substituted Diamines. The pH-rate profiles for the reaction of diamines with formaldehyde to form the imidazolidines in aqueous dioxane, with respect to total and free base diamines, are shown in Figure 5. Negative deviations, i.e., decrements in slope of −1 at pH 4–7, for the various compounds are observed in a pH range in which there is no significant change in the state of ionization of the nucleophile with (Figure 5) or without (Figure 5, inset) correction of the data to the free base species of nucleophile.[33,35] Therefore, the negative deviations clearly cannot be accounted for on the basis of ionizations of reactants. However, the results are consistent with the kinetic scheme of eq 9 in which there is a change in rate-determining step from step 2 to step 1 as the hydronium ion concentration increases. The *solid lines* in Figure 5 are calculated from the constants in Table V and eq 10 derived by the application of the steady-state assumption to the carbinolamine intermediate in the kinetic scheme of eq 9, where α_{R_2NH} is the fraction of total amine as the free base, and a_{H+} is the hydronium ion activity.

$$k_{obsd}/([F]\alpha_{R_2NH}) = (k_1 + k'_1a_{H+})k'_2a_{H+}/(k_1/K_1 + k'_1a_{H+}/K_1 + k'_2a_{H+}) \quad (10)$$

The assignment of the rate-determining step as attack (step 1, eq 9) for the hydronium ion-catalyzed and pH-independent limbs in the acid region and as the dehydration step (step 2, eq 9) for the hydronium ion-catalyzed limb in the alkaline region (Figure 5) is on the basis of previous data and interpretations for analogous reactions[6,10,19,24a,25a,27,30] and the following evidence.

(1) The rate constants k_1, k'_1, and $K_1k'_2$ fall within about sixfold for a comparison of the reactions of p-CH_3O-DPED (pK'_a = 4.51) and THF (pK'_a = 4.82) with formaldehyde based upon the above assignment of rate-determining steps (Table V) and despite solvent differences. There is no evidence that the cyclization step (step 3, eq 9) becomes rate determining in any of the diamine systems (including THF)[6] yet studied for their reactions with formaldehyde (cf. ref 8 and 9). It is noteworthy that p-NO_2-DPED undergoes no ionizations to form anilinium ions in the pH region studied, which might disfavor the accumulation of intermediates (especially the carbinolamine) that must be present at high formaldehyde concentration if cyclization were the rate-determining step. Despite this favorable situation for the accumulation of intermediates, no hyperbolic dependencies of rate upon formaldehyde concentration which might be evidence for the accumulation of intermediates could be demonstrated in the region assigned as the attack step (Figure 1, inset). Such nonlinear dependencies are readily demonstrable in the alkaline pH region (Figure 1).

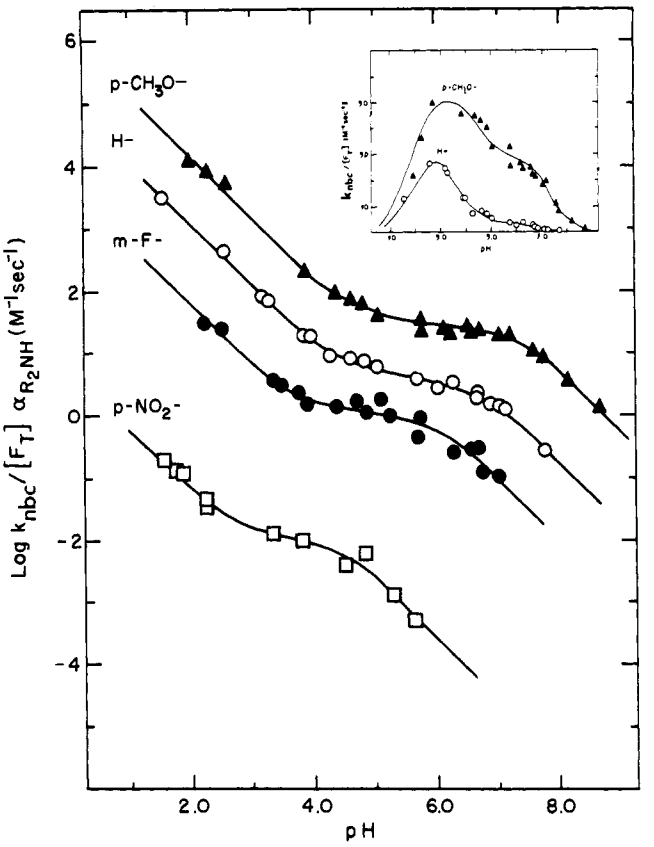


Figure 5. Dependence upon pH of the nonbuffer-catalyzed second-order rate constant, k_{nbc}, for imidazolidine formation from p-CH_3O-DPED (▲——▲), DPED (○——○), m-F-DPED (●——●), p-NO_2-DPED (□——□) as the free base forms and formaldehyde in 50:50 (v/v) dioxane-water, 25°, ionic strength 0.10 M. The *solid lines* were calculated from eq 10, and the constants contained in Table V were obtained from these data by a least-squares analysis. Inset: dependence upon pH of the nonbuffer-catalyzed second-order rate constants for imidazolidine formation from formaldehyde and p-CH_3O-DPED (▲——▲) or DPED (○——○) in 50:50 (v/v) dioxane-water, 25°, ionic strength 0.10 M. The *lines* were calculated from eq 10 and the constants contained in Table V.

The rate constant for formation of the carbinolamine from N-methyl-N,N'-di(p-nitrophenyl)ethylenediamine (N-Me-p-NO_2-DPED) and formaldehyde at pH 4.3 is within a factor of 2 of the value for the reaction of formaldehyde with p-NO_2-DPED and, with both amines, the rates of the reactions are first order in respect to formaldehyde concentration up to 1.5 M; no significant fraction of N-Me-p-NO_2-DPED is converted to an imidazolidine under these conditions.[36]

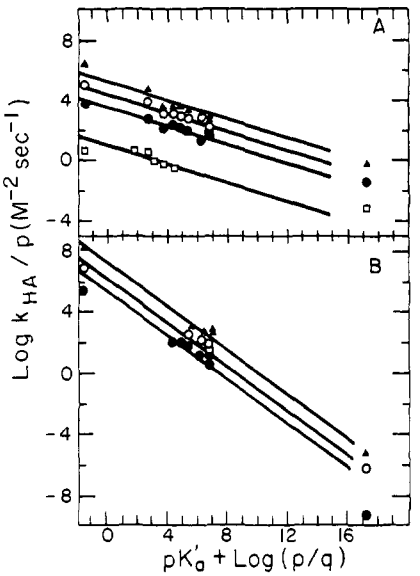


Figure 6. (A) Brønsted plots for general-acid catalysis of the attack of XDPED's on formaldehyde in 50:50 (v/v) dioxane-water, 25°, ionic strength 0.10 *M*: *p*-CH_3O- (▲——▲), H- (○——○), *m*-F- (●——●), *p*-NO_2- (□——□). The slopes of the lines are 0.30 ± 0.10. (B) Brønsted plots for general-acid catalysis of the dehydration of the carbinolamine intermediates of XDPED's and formaldehyde in 50:50 (v/v) dioxane-water, 25°, ionic strength 0.10 *M*: *p*-CH_3O- (▲——▲), H- (○——○), *m*-F- (●——●). The slopes of the lines are 0.62 ± 0.07.

(2) The attack of the amine on the carbonyl compound in terms of the above formulation is characterized from this study by general-acid catalysis with remarkably linear Brønsted plots (Figure 6A) associated with α values in the range of 0.3 in accord with that found for similar reactions.[6,19,24a,25b,27,30]

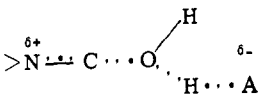
(3) In terms of the assignment of the rate-determining step in the alkaline region to the dehydration of the carbinolamine to form the Schiff base, this step is characterized in the present study also by linear Brønsted plots with α values in the range of 0.6 for general-acid catalysis[37] (Figure 5B), once again in accord with that found for similar reactions.[6,10,19,25a]

(4) The rates in the *alkaline* pH region rise, plateau, and decrease with increasing formaldehyde concentration (Figure 1). These changes are interpreted as the formation of the carbinolamine which is a competent intermediate and the *N,N'*-dicarbinolamine which is an unreactive intermediate off the productive pathway (eq 4). The equilibrium constants K_1 and L_3 for *p*-NO_2-DPED (eq 4), calculated from the rate data to be about 3 M^{-1}, fall in the range of such equilibria for *N*-hydroxymethylamine formation from *N*-Me-*p*-NO_2-DPED (see Results) and *N*-methylanilines,[24a] 1.0-18.6 M^{-1}.[38] It has previously been argued that imines derived from formaldehyde are less stable than their hydrates (carbinolamines)[19] and additionally the pH *independence* of these equilibrium constants, K_1, above pH 6 is incompatible with the assignment of the intermediate, which accumulates, as a cationic Schiff base.

(5) There is spectrophotometric evidence for the accumulation of an intermediate in the alkaline pH region at high formaldehyde concentrations, and the spectrum of the intermediate is consistent with that of a carbinolamine. For example, the spectral changes associated with the formation of the carbinolamine of *N*-Me-*p*-NO_2-DPED parallel the changes observed for the formation of the intermediate in the alkaline pH region from *p*-NO_2-DPED and formaldehyde (Figures 2A and 2B).

(6) At pH values in the region of the change in rate-determining step, the different sensitivities of the two steps to catalysis by general-acid catalysts (Figures 5 and 6) result in hyperbolic dependencies in plots of k_{obsd} vs. buffer concentration.

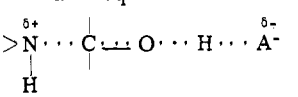
Carbinolamine Dehydration. The transition state III for step 2, eq 9, for Schiff base formation from carbinolamines formed from substituted *N,N'*-diphenylethylenediamines and formaldehyde involves proton donation to the leaving



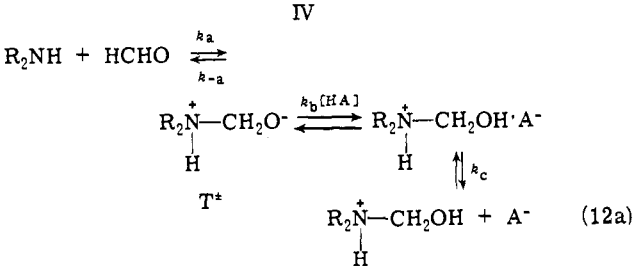
III

hydroxyl group as the C-N π bond is formed.[10,19,25a] The large Brønsted α values of 0.62 ± 0.07 (Figure 6B) for this step indicate that the proton of the general acid is close to the oxygen of the leaving hydroxide in the transition state. Since the Brønsted α value of 0.66 for the general-acid catalyzed dehydration step of the relatively basic *N*-hydroxymethylamines of cysteine (pK'_a = 8.90)[19] is similar to the Brønsted α values for the XDPED's (excluding *p*-NO_2-DPED, $pK'_a < 0$), there is only a very slight dependence of the Brønsted α values upon the basicity of the amine[19] or, in terms of the Cordes-Jencks equation,[24b,26] a $1/c_2$ value close to zero. Similarly, the Brønsted α values for general-acid catalyzed Schiff base formation by dehydration of a series of carbinolamines derived from substituted hydrazines and *p*-chlorobenzaldehyde are weakly dependent on the basicity of the amines employed.[10] The Brønsted α values for these latter reactions range from 0.63 for hydrazine (pK'_a = 8.26) to 0.73 for thiosemicarbazide (pK'_a = 1.88) and are correlated by a $1/c_2$ value from the Cordes-Jencks equation of 0.02 and are consistent with the interpretation that proton transfer is concerted.[24b]

Carbinolamine Formation. Brønsted α values of 0.30 ± 0.10 (Figure 6A) for the attack step (step 1, eq 9) have been determined for the reaction of a series of substituted *N,N'*-diphenylethylenediamines with formaldehyde. Two possible mechanisms for carbinolamine formation involve transition state IV, which involves in some sense concerted proton donation to the carbonyl group as the nucleophile attacks,[6,19,24a,b,25a,29,30] and eq 12a which involves a sequen-



IV

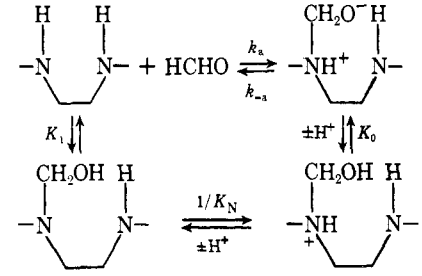


(12a)

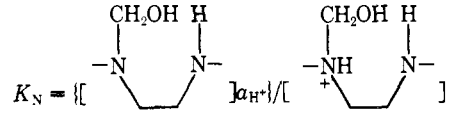
tial stepwise (rather than a concerted) mechanism within the encounter complexes.[24b,29]

Calculations[41,42a,b] based on eq 12a and the thermodynamic cycle (Scheme I) with order of magnitude guesses for pK_N and pK_0 of 2 and 10, respectively, and $k'_{lu} = 1.4 \times 10^8$ M^{-1} sec^{-1} (Table V), indicate that $k_b \sim 3 \times 10^{12}$ M^{-1} sec^{-1} for the reaction of neutral DPED with formaldehyde by a solvated proton-catalyzed pathway. This value of k_b which exceeds the rate constant for a diffusion-limited reaction suggests that the monocationic transition state for carbinolamine formation may in fact represent the reaction of

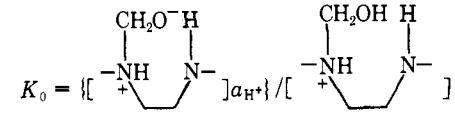
Scheme I



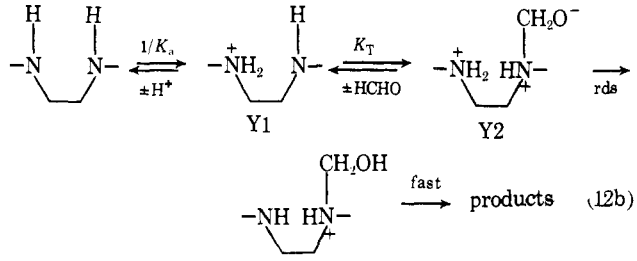
where



and



the monocationic DPED's by the mechanism of eq 12b in which the rate-determining step involves an intramolecular proton transfer from the adjacent $>NH_2^+$ to the dipolar intermediate.



where $K_T = [Y2]/[Y1]$

The magnitude of the rate constants for the buffer-catalyzed pathway[42b] according to eq 12a in the range $10^{10.7}$–$10^{15.1}$ M^{-1} sec^{-1} and the linearity of the Brønsted plots (the data for which is limited by the change in rate-determining step) both suggest that a concerted pathway through transition state IV may be operative. The occurrence of a concerted buffer-catalyzed pathway in the lower dielectric constant medium of the present study in contrast to a nonconcerted buffer-catalyzed pathway in water[24a] may be attributed to a destabilization of the dipolar intermediate in the mixed solvent.[24b]

Structure–Reactivity and Stability Correlations Regarding Reactions of Carbinolamines. The rate and equilibrium constants measured for the reaction of formaldehyde with a series of diamines to form imidazolidines obey linear free-energy relationships.[43,44] Thus, the rate constants for pH-independent attack, hydronium ion catalyzed attack, and dehydration (k_1, k'_1, and k'_2) for the present systems in aqueous dioxane and for the former pair of constants for substituted anilines in water[24a] are correlated by ρ^- values and β_{nuc} values as summarized in Table VI. The sensitivity of rate constants to the basicity of the amine is substantially altered in 50:50 (v/v) dioxane-water compared with those in water with β_{nuc} values for k_1 and k'_1 being inversely affected by the change to the mixed solvent (Table VI) and is consistent with different mechanisms as noted above.

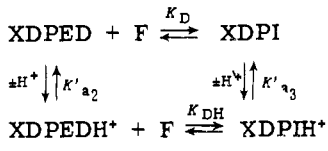
The equilibrium constants for free base carbinolamine formation from the free base amine reactants are relatively insensitive to the basicity of the reacting amine[10,19,24a,25a,45] (Table V). These results indicate that electron-donating groups on the phenyl ring substantially enhance to a similar extent the rates of both carbinolamine formation from and breakdown to reactants.

Table VI. Hammett σ^-–ρ^- Correlations in Rate Constants for Reactions of XDPED's with Formaldehyde in 50:50 (v/v) Dioxane–Water, 25°, Ionic Strength 0.1 M

Constants	Units	ρ^-	β_{nuc}
k'_1	M^{-2} sec^{-1}	−3.6 ± 0.12	0.77 ± 0.042 (0.57)[a]
k_1/H_2O	M^{-2} sec^{-1}	−2.4 ± 0.10	0.51 ± 0.043 (0.87)[a]
$K_1k'_2$	M^{-2} sec^{-1}	−4.2 ± 0.10	0.96 ± 0.025

[a] Value in parentheses for the reaction of ring-substituted anilines with formaldehyde in water, 25°, ionic strength 1.0 M (see ref 24a).

Scheme II



Structure–Stability Correlations of Neutral and Monocationic Cyclic Methylenediamines. The constants K_{DH} (Table I) were calculated from XDPI formation constants (K_D), the relation $K_{DH} = K'_{a_2}K_D/K'_{a_3}$ (from the cyclic relation of the reactions in Scheme II), and the K'_{a_2} and K'_{a_3} values and in some cases the Hammett[43] relationship (Table I).

The equilibrium constants for the formation of neutral and monocationic substituted imidazolidines from neutral and monocationic substituted DPED's and formaldehyde, K_D and K_{DH}, are correlated by ρ^- values of −1.08 ± 0.04 and −0.64 ± 0.02, respectively, on Hammett plots.

These data indicate that the pK'_{a_2} and pK'_{a_3} values for the monocationic XDPED's and XDPI's are markedly more sensitive to substituents in dioxane-water (ρ^- values of −4.69 ± 0.070 and −4.20 ± 0.14, respectively) than are the pK'_a values for the substituted anilinium ions in water, ρ^- = −2.88.[24a] These differences in relative stability for these substituted aromatic amines and anilinium ions in the mixed solvent dioxane-water with respect to similar equilibria in water may be attributed to important differences in the solvation[46] of the neutral and especially the monocationic aromatic amine reactants and products for the equilibria defined by K'_{a_2} and K'_{a_3}.

The decrease in basicity of symmetrical methylenediamines, pK'_{a_3} values, compared with the basicity of parent amines, pK'_{a_2} values (an effect which is directly related to the decrease in affinity of protonated XDPED's for formaldehyde of two to three orders of magnitude compared with the affinity of free base XDPED's for formaldehyde), may be attributed largely to different solvation effects on the secondary and tertiary ammonium cations. This argument is analogous to that applied to the difference in basicity of amines and *N*-hydroxymethylamines presented elsewhere.[20,45b]

The equilibria represented by K_D and K_{DH} show only a small difference in sensitivity to electronic effects, and this indicates that the destabilization by symmetrical electron withdrawal in methylenediamines is similar to that in monocationic methylenediamines. Within the range of these electronic effects then it does not appear that there is a substantial *net* greater sensitivity of $R_2NH^+CH_2$- than of R_2NCH_2- to destabilization by electron withdrawal.[20] However, the decreasing values of K_D in the present series

of compounds do suggest that NCN interactions depend upon electronic substituent effects, and that it may not be possible to define interaction values which are totally nitrogen atom substituent independent.[47]

Conclusions

The similarity in the rates, equilibria, and sensitivity to general-acid catalysis for the reactions of formaldehyde with *N,N'*-diphenylethylenediamines and THF leads to the following conclusions.

(1) The difference in basicity between the N_5 and N_{10} sites of THF is apparently not significant for imidazolidine formation and breakdown. The symmetrically substituted diamines have the same intrinsic basicity at both nitrogen atoms, and when symmetrical and asymmetrical compounds of comparable basicity at the most basic site are compared (e.g., *p*-CH_3O-DPED and THF), the reaction rates are similar. This follows from the fact that ring closure is not rate limiting in these systems. Whether an asymmetry of basicity in ethylenediamines is essential for the role of THF in one-carbon transfers at the aldehyde oxidation level (cf. the acyl level of oxidation)[48] cannot at present be answered.

(2) The increase in stability of formaldehyde adducts and reactivity of DPED's toward F with *increasing basicity* when taken together with the maximization of the fraction of free base amine at physiologic pH with *decreasing basicity* suggests that the THF pK'_a of about 5 represents an optimum in the design of the molecule to function as a facile but stable formaldehyde carrier.

(3) The pyrimidine moiety plays a crucial role in the determination of the weakly basic nature of the N_5 site in THF.[6]

Acknowledgment. We are grateful for the contributions of Dr. R. O. Viale to this work and thank Drs. W. P. Jencks and Jane Sayer for communication of results prior to publication and for their very helpful discussions.

Appendix

Calculation of a_{H^+} in Aqueous Dioxane. The mean ionic activity coefficient, $\gamma_\pm$, of a 0.10 *M* HCl solution in water and aqueous dioxane may be calculated from the observed electromotive force values (E_{obsd}) for the cell

$$[Pt,\ H_2(g)\,|\,HCl(m)\,|\,AgCl,\ Ag]$$

and the E° value for the AgCl|Ag electrode in these solvents utilizing the Nernst equation[49]

$$E_{obsd} + 0.1183 \log m = E^\circ - 0.1183 \log \gamma_\pm$$

where m = molality of HCl. The hydrogen ion activity, a_{H^+}, is given by $a_{H^+} = \gamma_\pm m$ (see Table VII).

Table VII

HCl, *M*	Solvent	E°	E_{obsd}	$\gamma\pm$	pH	Ref
0.10	H_2O	0.222	0.352	0.798	1.10	48
0.10	45%[a]	0.165	0.314	0.546	1.26	18

[a] Dioxane–water (w/w); approximately 50:50 (v/v) dioxane–water.

References and Notes

(1) This project was supported by the National Institutes of Health, United States Public Health Service, Grants No. 13,777 (R.G.K.), FR 15 (University of Pennsylvania Medical School Computer Facility), and RR00512 (Middle Atlantic NMR Research Facility). Further details on the work in this paper are available in the Doctoral Dissertation of G. P. Tuszynski, University of Pennsylvania, 1973. This investigation was supported in part by a Public Health Service Research Career Development Award No. 5 K04 CA 70487 from the National Cancer Institute.

(2) Abbreviations: D, dioxane; DMSO, dimethyl sulfoxide; DPED, *N,N'*-diphenylethylenediamine; DPI, *N,N'*-diphenylimidazolidine; F, formaldehyde (total hydrated and unhydrated); *m*-F-DPED, *N,N'*-di(*m*-fluorophenyl)ethylenediamine; *m*-F-DPI, *N,N'*-di(*m*-fluorophenyl)imidazolidine; *N*-Me-*p*-NO_2-DPED, *N*-methyl-*N,N'*-di(*p*-nitrophenyl)ethylenediamine; *p*-CH_3O-DPED, *N,N'*-di(*p*-methoxyphenyl)ethylenediamine; *p*-CH_3O-DPI, *N,N'*-di(*p*-methoxyphenyl)imidazolidine; *p*-NO_2-DPED, *N,N'*-di(*p*-nitrophenyl)-ethylenediamine; *p*-NO_2-DPI, *N,N'*-di(*p*-nitrophenyl)imidazolidine; THF, tetrahydrofolic acid; Me_4Si, tetramethylsilane; W, water; XDPED, disubstituted DPED where X = H, *p*-CH_3O, *m*-F, *p*-NO_2; XDPEDF, *N*-monocarbinolamine of XDPED; $XDPEDF_2$, *N,N'*-dicarbinolamine of XDPED; XDPI, disubstituted DPI, where X = H, *p*-NO_2, *p*-CH_3O, *m*-F.
(3) Predoctoral Fellow, National Institutes of Health, 1968–1973.
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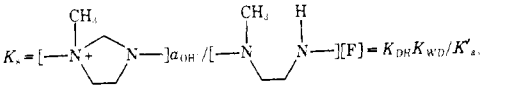

(37) A pH-independent term for the dehydration step (or, in reverse, hydroxide ion attack on the cationic imine)[26,39] has been observed for a number of carbinolamines,[10,25a,35,40] and indeed in the present systems involving formaldehyde in the pH region 11–12; these rate constants are designated k_2 in Table V and are correlated by $\log k_2 = -4.7\sigma^- - 6.9$.

(38) The pH-independent attack rate constant is 30-fold lower in dioxane-water than in water for the reaction of *p*-nitroaniline with formaldehyde to form the carbinolamine, while the equilibrium constant for this reaction is insensitive to solvent effects (K_1 = 14 M^{-1} in dioxane-water and 18 M^{-1} in water).[24a]
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(42) (a) J. Sayer, personal communication. (b) Estimates of K_0 = $[RN^+H_2CH_2O^-]a_{H^+}/[RNH_2CH_2OH]$ = $[T^-]a_{H^+}/[T^0]$ are obtained following the considerations of Sayer and Jencks.[35] Thus, from the estimated pK_a value of 9.98 for the alcoholic group of $CH_3N^+H_2CH_2OH$[42c] and correction for the phenyl substitution for methyl by ΔpK = −8.4 (0.10)/2 = −0.42 (employing an attenuation factor of 2 for the transmission of the substituent effect through an additional nitrogen atom[35,42d,42e] that is present in carbinolamines) and the considerations of Fox and Jencks[42l] with a ρ_I = +8.4 for the dissociation constants of substituted ammonium ions and alcohols, the calculated pK_0 value for $PhN^+H_2CH_2OH$ is 9.56. Based upon this same attenuation factor, a ρ = +1.11 value for the ionization of trifluoroacetophenone hydrates, and σ^- values from the literature,[42g] the values of ΔpK = $-1.11\sigma^-/2$ are 0.15, 0.0, −0.19, and −0.69 for *p*-CH_3O-, H-, *m*-F-, and *p*-NO_2-DPED, respectively (ignoring the effects of the $-CH_2CH_2NHPhX$ groups). The solvent effect on $-OH \rightarrow -O^- + H^+$ proton dissociations, based upon the pK'_a values for carboxylic acids (see Results) and K_{WD} (Table III) values in dioxane-water, is $10^{1.7}$. The pK_N values for K_N = $[>NCH_2O-H]a_{H^+}/[>^+NHCH_2OH]$ are obtained from the pK'_{a_2} value of the parent amine (Table I) and an acid strengthening effect of $10^{1.9}$ due to *N*-hydroxymethyl substitution.[24a] The pK_0, pK_N, and log K_n values are estimated from the following relationships: pK_0 = 9.56 + 1.7 + ΔpK, pK_N = pK'_{a_2} − 1.9, and log K_n = log K_1 − pK_0 + pK_N where K_n = $[T^{\pm}]/[R_2NH][F]$ = K_1K_0/K_N. The pK_0, pK_N, and log K_n values based upon the above and K_1 values from Table V for this series in dioxane-water are *p*-CH_3O-, 11.41, 2.61, −8.26; H-, 11.26, 1.74, −8.92; *m*-F-, 11.07, −0.21, −10.80; and *p*-NO_2-DPED, 10.57, −4.24, −14.31. The calculated rate constants, k_b, according to eq 12a are obtained from k_b = k''_1/K_n with k''_1 values from Table III and the K_n values from above. (c) J. Hine, J. C. Craig, J. G. Underwood, II, and F. A. Via, *J. Am. Chem. Soc.*, **92**, 5194 (1970); (d) A. Fisher, D. A. R. Happer, and J. Vaughan, *J. Chem. Soc.*, 4060 (1964); (e) R. Pollet and H. Vanden Eynde, *Bull. Soc. Chim. Belg.*, **77**, 341 (1968); (f) J. P. Fox and W. P. Jencks, *J. Am. Chem. Soc.*, **96**, 1436 (1974); (g) A. I. Biggs and R. A. Robinson, *J. Chem. Soc.*, 388 (1961).
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Mechanisms of Isoalloxazine (Flavine) Hydrolysis

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Abstract: The hydrolysis of 3-methyl-10-(2′,6′-dimethylphenyl)isoalloxazine (I) (30°, μ = 1.0, k = 3.16 × 10^2 M^{-2} sec^{-1}) exhibits no buffer catalysis, has a deuterium solvent kinetic isotope effect ($k_{obsd}^{H_2O}/k_{obsd}^{D_2O}$) of 0.6, and obeys the rate law $d[I]/dt = k[F][OH^-]^2$. The product, a ureido carboxylate (VII), arises from hydrolytic scission between positions 3 and 4 of the isoalloxazine ring. VII cyclizes in acid under anaerobic conditions to regenerate I. In the presence of oxygen, VII decarboxylates then cyclizes to form the ring contracted 3-methyl-9-(2′,6′-dimethylphenyl)isoimidazolone[4,5-*b*]quinoxaline (IX) (Scheme I). 3-Methyl-10-phenylisoalloxazine (III) hydrolyzes (k = 2.24 × 10^2 M^{-2} sec^{-1}) by the same mechanism as does I. The hydrolysis of 10-phenylisoalloxazine (VI) is first order in $[HO^-]$, k = 1.07 × 10^{-4} M^{-1} sec^{-1}. The change in kinetic dependence from $[HO^-]^2$ to $[HO^-]^1$ must be related to formation of the anion of VI (pK_a = 9.47) which is not susceptible to hydrolysis. 3,10-Dimethylisoalloxazine (IV) is hydrolytically reactive at the C-4 and C-10a positions. The kinetics of base hydrolysis of IV are biphasic as the result of two reaction paths each composed of two reactions with different pH dependence (Scheme II). 1,5-Dihydro-3-methyl-5-acetyl-10-phenylisoalloxazine (X) hydrolyzes in acid with a rate constant of 1.71 × 10^{-4} M^{-1} sec^{-1} giving 1,5-dihydro-3-methyl-10-phenylisoalloxazine which oxidizes to III in the presence of oxygen. The hydrolysis of 1,5-dihydro-3-methyl-5-acetyllumiflavine (XI) in acid has a rate constant of k = 8.67 × 10^{-5} M^{-1} sec^{-1}. In base, the hydrolysis of X is biphasic with the first step not involving removal of the 5-acetyl group. The largest rate constant of this biphasic reaction is only ca. 1 × 10^{-1} M^{-1} sec^{-1}. The rate constants for the acid and base hydrolysis of X and XI establish that neither the neutral nor anionic 1,5-dihydroisoalloxazine moieties are good leaving groups. For redox reactions which postulate facile expulsion of these species in dark (nonenzymatic) processes, this feature should be taken into account.

It is assumed that at least some flavine-catalyzed oxidation reactions proceed through covalent addition of the substrate to the isoalloxazine ring system.[1a,b] Many hypothetical but possible examples are provided in a recent review.[2] With this consideration in mind, it is of obvious importance to determine which positions of the isoalloxazine ring system are susceptible to nucleophilic (dark) addition reactions. Addition of SO_3^{2-} to the 5 position of flavine and flavinium compounds was established by Massey and Müller,[3a] and similar additions of phosphines have been established by Müller.[3b] The reaction of sulfite ion with 3-methyl-10-(2′,6′-dimethylphenyl)isoalloxazine (I) and 3-methyl-10-(2′,6′-dimethylphenyl)isoalloxazine-6,8-disulfonic acid (II) has been investigated in some detail.[4,5] Sulfite was found to add to the N(5) position of I and to the N(5) and C(4a) positions (by general-acid catalysis) of II to provide N-5 and **4a**, respectively. The objective of the present investigation has been to determine the mechanism of HO^--catalyzed hydrolysis of I and isoalloxazines III to VI in order to gain more insight into the mechanisms of nucleophilic additions to the isoalloxazine (flavine) nucleus. In addition, the hydrolyses of two N(5) acetyl-1,5-dihydroisoalloxazines have been examined in order to determine the susceptibility to displacement of substituents at the N(5) position of reduced flavine. The leaving abilities of the 4a and 5 positions are of some interest[6a] since metastable additions at these positions have been postulated to be involved in flavine catalysis.[1,6b,c]

The hydrolyses of isoalloxazine compounds were investigated as early as 1932.[7] Previous studies have been directed toward the hydrolysis of riboflavine,[7,8] lumiflavine,[9,10,12] and 10-methylisoalloxazine.[11a,b,c,12] The studies involving