Equilibria and Kinetics of *N*-Hydroxymethylamine Formation from Aromatic Exocyclic Amines and Formaldehyde. Effects of Nucleophilicity and Catalyst Strength upon Mechanisms of Catalysis of Carbinolamine Formation[1]

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Abstract: The products of the reaction of exocyclic aromatic amines with formaldehyde are hydroxymethylamines and not imines; rate and equilibrium constants for carbinolamine formation have been determined for a series of substituted anilines and 1-methyl-4-aminopyridinium ion. The hydroxymethylamine formation constants (K_1) from hydrated plus unhydrated formaldehyde and aromatic amines of varying basicity (about pK_{a1} 6 to −6) are in the range 3–27 M^{-1} and show a relatively small dependence upon basicity (β_{nuc} values 0.04 and 0.16 for substituted anilines and *N*-methylanilines, respectively). The formation constants for cationic hydroxymethylamines from cationic amines and formaldehyde (K_2) are about two orders of magnitude less favorable than those for the neutral species and, reciprocally, the pK_{a2} values of *N*-hydroxymethylanilinium ions are about two pH units lower than their parent anilinium ions. Hydroxymethylamine formation occurs by general and specific acid and general and specific base catalyzed pathways, in addition to a pH-independent pathway. For the anilines, the Bronsted plots for general acid catalysts are nonlinear with the break occurring at a lower p*K* value than expected from the pK_0 value estimated for the alcoholic group of $>N^+HCH_2OH$ of ~9. A stepwise preassociation or spectator mechanism is suggested to account for these data and involves a preassociation of catalyzing acid, amine, and aldehyde in an encounter complex (K_E), the formation of (k_2) and proton transfer from the catalyst to an unstable zwitterionic tetrahedral intermediate (k_3), $T^{\pm}$, within the encounter complex, and dissociation or rotation (k_4) before product formation. With strongly acidic catalysts k_2 is the rate-determining step and with more weakly acidic catalysts k_3 is the rate-determining step. There appears to be a significant degree of stabilization of the transition state for C–N bond formation by general acids and hydronium ions. For the most weakly basic amine, 1-methyl-4-aminopyridinium ion, the hydronium ion and general acid catalyzed pathways appear to be more concerted based upon a *linear* Bronsted plot ($\alpha = 0.28$) as a consequence of the decreased stability of $T^{\pm}$. The rate constants for the uncatalyzed conversion of $T^{\pm}$ to a neutral tetrahedral intermediate are $1–5 \times 10^7$ s^{-1} and are consistent with a solvent-mediated intramolecular proton-transfer ("proton switch") mechanism of carbinolamine formation. For a more limited series of general base catalysts (p*K* = 9–11.5), the rate constants for the proton-transfer processes calculated from the observed rate constants and estimated equilibrium constants (K_n) for the formation of $T^{\pm}$ from amine and hydrated and unhydrated formaldehyde are in the range of $10^{7.4}$–10^9 M^{-1} s^{-1} for monofunctional general bases. These values approach those expected for diffusion-limited processes but the limited data with *linear* Bronsted plots (β values of 0.17 ± 0.05, 0.06 ± 0.02, and 0.13 ± 0.01 for the 4-cyano-, 4-nitro-, and 3-fluoro-4-nitroanilines, respectively) for general base catalysts do not unequivocally rule out a concerted mechanism of catalysis with proton removal by the base from the nucleophile as the N–C bond is formed.

Studies of the mechanism and catalysis of the reaction of formaldehyde with an isosteric series of exocyclic aromatic amines to form the *N*-hydroxymethyl adduct (eq 1) were undertaken in order to accomplish a quantitative test of the Cordes–Jencks equations.

$$>NH + HCHO \rightleftharpoons >NCH_2OH \qquad (1)$$

The group of equations derived by Cordes and Jencks[3,4a] represents quantitative statements of the Hammond postulate[5] and is arrived at through a correlation of the Bronsted relationship for general acid–base catalysis with relations of the Swain–Scott[6] type for nucleophilic reactivity. The previous quantitative tests of these equations that relate nucleophilicity with sensitivity to catalysis have employed neither an isosteric series of nucleophiles nor the same electrophile.[4] These equations are only applicable to a single-step of a reaction sequence and during the course of these studies it became apparent that carbinolamine formation in this and other similar systems[4e-g] can be a multistep process involving first the formation of a zwitterionic intermediate, $>N^+HCH_2O^-$, and second its further reaction to form $>N^+HCH_2OH$ and/or $>NCH_2OH$ depending upon the experimental pH value. The data in support of this conclusion and a discussion of the role of general catalysts in these reactions are presented herein.

The formation of *N*-hydroxymethyl adducts is related to the attack step to form the carbinolamine intermediate in Schiff base formation,[3,4] the second step (carbinolamine decomposition to amine and carbonyl compound) in Schiff base hydrolysis,[3,4] the attack step to form the tetrahedral intermediate in the aminolysis of esters,[3b] and the use of formaldehyde in studies of nucleic acids both as a structural probe and as a technique to maintain nucleic acid polymers as single-stranded species.[7,8] The present studies provide evidence that the formaldehyde reactions with aromatic exocyclic amino groups, including those of substituted purines and pyrimidines, involve the formation of hydroxymethyl adducts and not imines.[8c] Further, these studies relate to the nucleophilicity of amino groups of adenine, guanine, and cytosine toward electrophilic carbon atoms such as the methyl group of *S*-adenosylmethionine and the role of the latter compound in N-methylation reactions.[9]

Finally, this kinetic study of hydroxymethylation reactions has enabled empirically based estimates of the microscopic proton dissociation constants of exocyclic ammonium groups of biologically significant purines and pyrimidines[2] and these estimates are presented in the following paper.[10]

Experimental Section

Materials. Reagent grade formaldehyde, 37% containing 12–15% methanol (Fisher), was diluted to 1.0, 2.0, or 4.0 M stock solutions of formaldehyde, the concentrations of which were confirmed by titration with sodium sulfite.[11] Reagent grade inorganic and organic

salts were used without further purification. Deionized water of greater than 5×10^5 ohms cm specific resistance was used throughout. 4-Chloro-*N*-methylaniline [bp 118–119 °C (15 mmHg); n^{21}D 1.5799 (Bausch and Lomb Abbey refractometer), lit.[12] n^{21}D 1.5799] and 4-methoxy-*N*-methylaniline [bp 135–140 °C (0.2 mmHg); mp 31.5–33 °C (lit.[13] mp 40 °C, purified by sublimation). Anal. Calcd for $C_8H_{11}NO$: C, 70.04; H, 8.08; N, 10.21. Found: C, 69.93; H, 8.08; N, 10.46] were prepared by methylation of the acetanilides[13] with dimethyl sulfate, alkaline hydrolysis, ether extraction, and distillation. The trimethyl orthoformate method[12] was used to synthesize 4-methyl-*N*-methyl-*N*-formylaniline which was hydrolyzed with acid, neutralized, extracted into ether, distilled [bp 112 °C (24 mmHg), lit.[12] bp 120–121 °C (40 mmHg)], and converted to the hydrogen chloride salt (mp 100–102 °C). 4-Amino-1-methylpyridinium ion was synthesized as the iodide salt[14a] and recrystallized from hot ethanol [mp 188–190 °C (lit.[14a] mp 187–188 °C). Anal. Calcd for C_6H_9NI: C, 30.53; H, 3.84. Found: C, 30.32; H, 3.93]. 4-Chloro-, 4-methoxy-, and 4-methylaniline (Aldrich), and 4-nitro-, 4-nitro-*N*-methyl-, 4-cyano-, and 3,5-dinitroaniline (Pfaltz and Bauer) were purified prior to use by either recrystallization, distillation, or microsublimation and stored under vacuum protected from light and water. 4-Nitro-3-fluoroaniline was kindly supplied by Dr. B. M. Lynch. Examination of these compounds by thin-layer chromatography indicated homogeneity and the ^{1}H NMR, ir, and uv spectra were consistent with their structures. For kinetic and equilibrium studies, a solution of amines was prepared daily in water or aqueous ethanol and the concentration of the nucleophile determined gravimetrically. The reaction mixtures contained a final concentration of ethanol <1% by volume.

Preparation of Pivalideneaniline and Pivalidene-4-cyanoaniline. The Schiff bases of pivaldehyde[14b] (Chemical Procurement Labs) were prepared by 2–6-h azeotropic distillations of 0.05 mol of aldehyde and 0.025–0.05 mol of amine in 50 ml of benzene, in the presence of catalytic amounts of HCl (4.8×10^{-4} M) or glacial acetic acid. The reaction proceeded to about 100 and 67% completion with aniline and 4-cyanoaniline, respectively, as calculated from the water collected in the Dean-Stark tube. The product with aniline was distilled over crushed KOH under reduced pressure through a small Vigreux column [bp 60 °C (2 mm)] and the product with 4-cyanoaniline was passed through crushed NaOH and concentrated by a flash evaporation. Upon standing at room temperature the pivalidineaniline slowly crystallized from the caramel-colored solution (mp 45 °C). The structure of the pivalideneaniline was confirmed by the ir spectra (Perkin-Elmer Model 521 IR spectrophotometer, 1-mm NaCl cells) which showed neither primary or secondary amine (N–H) nor hydroxyl stretch bands but a band at 1645 cm^{-1} assigned as a C=N stretch.[15,16] A ^{1}H NMR spectrum (Jeolco C60H spectrometer) of the neat imine showed a singlet peak at 1.38 [$-C(CH_3)_3$] and 7.73 ppm (–CH–) and phenyl hydrogen signals at 7.21 and 7.30 ppm, downfield with respect to an external $(CH_3)_4Si$ standard, relative areas 9:1:5. A ^{1}H NMR spectrum of the pivalidene-4-cyanoaniline in $CDCl_3$ showed a singlet peak at 1.83 [$-C(CH_3)_3$] and 8.15 ppm (–CH–) and phenyl hydrogen doublets at 7.60 (J = 11 Hz) and 7.98 ppm (J = 13 Hz) downfield with respect to an external $(CH_3)_4Si$ standard, relative areas 9:1:4. The ir spectrum has a strong band at 1655 cm^{-1} (C=N stretch)[15,16] and was void of N–H or OH stretch bands. The percentage contamination was estimated to be less than 10% on the basis of minor peaks in the ^{1}H NMR and ir spectra.

Methods. Proton Dissociation Constants. The proton dissociation constant for various substituted anilinium ions was determined by either potentiometric or spectrophotometric titration[2,17,22a] at 25 °C, ionic strength 1.0 M. Spectra (uv and visible) were recorded with a Cary Model 14 or a Unicam-Pye SP 1800 spectrophotometer at 23–25 °C. Absorbance measurements were made with a Zeiss PMQ II or a Gilford Model 2000 spectrophotometer each equipped with a thermostated cell compartment maintained at 25 ± 0.1 °C. Measurements of pH were obtained with a Radiometer 25 or 26 SE pH meter with a GK 2301 B or C combined electrode standardized at pH 1, 4, 7, 10, and 12.88[18,19a] (B electrode). The concentration of hydronium and hydroxide ions was calculated from the observed pH values and the relationships C_{H^+} = antilog (−pH)/0.9 and C_{OH^-} = antilog (pH − 14)/0.67,[19b] respectively. The specific gravity of solutions of sulfuric acid was determined with a pycnometer and spectrophotometric titrations of 1-methyl-4-aminopyridinium ion and 3-fluoro-4-nitroaniline utilized H_2SO_4 and HCl solutions with H_+ and H_0 values, respectively.[20,25] Solutions of 1-methyl-4-aminopyridinium ion were stable >30 min at H_2SO_4 concentrations >95% and subsequent neutralization of solutions to pH 7 showed an unaltered pH 7 uv spectrum.

Equilibrium Constant Measurements. The methods for the determination of equilibrium constants from absorbance measurement have been previously described.[21a] The association constants for the formation of *N,N*-dihydroxymethylamine[21b] from *N*-monohydroxymethylamine and formaldehyde were determined for 1-methyl-4-aminopyridinium ion and 3,5-dinitroaniline by kinetic methods (see below).

Kinetic Methods. The rates of reaction of formaldehyde with aromatic amines were followed by measuring the changes in absorbance at suitable wavelengths (Table II). The chromophore concentration was approximately 1.0×10^{-4} M, and formaldehyde concentration was in tenfold or greater excess in order to yield pseudo-first-order kinetics. A stopped-flow mixing device adapted to the Gilford spectrophotometer was utilized for faster reactions. *N*-Hydroxymethylamine decomposition rates were measured spectrophotometrically following rapid and extensive dilution of equilibrium mixtures of formaldehyde and amine by conventional or stopped-flow techniques; this dilution caused the adduct to decompose to amine and formaldehyde. Ionic strength was maintained at 1.0 M with potassium chloride.

The methods for kinetic data analysis from chart recorder tracings were also applied to photographs of the storage oscilloscope traces from stopped-flow experiments and have been described.[2,22b] For the slower reactions, the absorbance was determined of aliquots removed from reaction mixtures maintained at 25 ± 0.1 °C in screw-top test tubes. For reactions which required more than 2 weeks to attain equilibrium, the data for the reactions up to 75–87% completion were analyzed by the Kézdy modification of the Guggenheim method.[2,23]

Non-buffer-catalyzed rates were obtained by extrapolation to zero buffer concentration (see below). Third-order catalytic constants were determined at a minimum of eight different formaldehyde and buffer concentrations and three different pH values. The standard errors of kinetic and equilibrium constants are ~10%.

Results

Equilibria. Proton Dissociation Constants. The pK'_{a1} values for the substituted anilinium ions and substituted *N*-methylanilinium ions are slightly greater than literature values[24] owing to a greater ionic strength employed in the present studies and are correlated with Hammett substituent constants[25] (σ^-) by slopes (ρ^-) of 2.86 ± 0.07 and 3.36 ± 0.04, respectively, in aqueous solution at 25 °C, ionic strength 1.0 M (not shown). These data may be compared with a ρ^- value of 2.89[26,27] for proton dissociation constants of meta- and/or para-substituted anilinium ions.

From the data of Table II and the equation $\Delta pK'_{a1} = 0.024 - 2.86\ \sigma^- = pK'_{a1_H} - pK'_{a1_X}$, where pK'_{a1_H} and pK'_{a1_X} apply to the conjugate acid of the unsubstituted and the substituted anilines, respectively, the effective substituent constants for 1-methyl-4-aminopyridinium ion, 3-fluoro-4-nitroaniline, and 3,5-dinitroaniline are 3.77, 1.77, and 1.50, respectively; the latter two values may be compared with 1.47 and 1.42, respectively, obtained by summation of the individual substituent constants.[28]

Basicity of Very Weak Nucleophiles. The H_0 and H_+ values at 50% protonation are measures of the tendency of solutions to donate a proton to neutral and cationic indicator bases,[25] respectively. In a study of three different types of weak bases by Brignell et al.,[41] the behavior of the second protonation of 4-aminopyridines to form the dication followed the H_0 scale with values of the activity coefficient ratio (n) in the range 0.86–1.08, in agreement with our values of n of 1.16 and 0.99 for the titration of 4-amino-1-methylpyridinium ion employing H_0 values from ref 20.[42] Thus, the protonation of 4-amino-1-methylpyridinium ion seems to follow H_0 quite closely[43] and the estimated pK'_{a1} values for this compound are −6.03 and −6.22, respectively.[20]

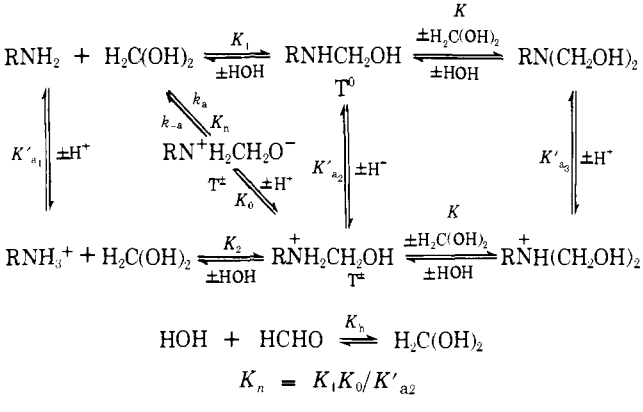
Equilibrium Constants for Formation of *N*-Hydroxymethylamines. The apparent equilibrium constants, K_{app} =

Table I. Ultraviolet Spectral Characteristics of Free Base, Protonated, and Hydroxymethylated Aromatic Exocyclic Amines

Compound	Wavelengths of maximum absorbance, nm (log ϵ)[a]					
	RNH_2	RNH_3^+	$RNHCH_3$	$RNHCH_2OH$[e]	$RN(CH_3)_2$	$RN(CH_3)CH_2OH$[e]
4-Amino-1-methylpyridinium ion[h]	268 (4.33)			273 (4.36) [81]		
	225 (4.12)					
Aniline	280 (3.12)	260	285 (3.18)	283 (3.14) [92]	283 (3.11)[b]	285 (3.18) [74]
	231 (3.89)	254	237 (3.99)	238 (4.01)	244 (3.94)	239 (3.97)
4-Chloroaniline	292 (3.11)		298 (3.10)	295 (3.18) [90]	311 (3.3)[c]	298 (3.1) [90]
	239 (4.02)		247 (4.04)	247 (4.08)	260 (4.2)	250 (4.07)
4-Cyanoaniline	270 (4.28)	278 (3.00)		275 (4.07) [73]		
	213 (4.16)	269 (3.00)		216		
		220 (4.01)				
3,5-Dinitroaniline	377 (3.30)	228		390		
	260 (4.10)			260		
	240 (4.08)			240		
3-Fluoro-4-nitroaniline	375 (4.20)			370 (4.20) [89]		
	226 (3.86)			225		
4-Methoxyaniline	295 (3.26)		300 (3.31)	298 (3.25) [86]	307 (3.3)[c]	295
	233 (3.88)		238 (4.02)	238 (3.97)	247 (4.0)	240
4-Methylaniline	287 (3.19)	259	290 (3.22)[f]	290		290 [53]
	232 (3.95)	268	238 (4.02)	240		244
		208 (3.90)	208 (4.08)			
4-Nitroaniline	382 (4.14)	375 (3.85)	407 (4.27)[g]	380 (4.30) [93]	392 (4.30)[d]	409 (4.23) [35]
	226 (3.83)	258 (3.87)	230 (3.90)	225	232 (3.96)	230

[a] ϵ = molar absorptivity (M^{-1} cm^{-1}), 25 °C. [b] References 33 and 51a. [c] L. Doub and J. M. Vandenbelt, *J. Am. Chem. Soc.*, **69**, 2714 (1947); P. Grammaticakis, *Bull. Soc. Chim. Fr., Ser. 5*, **18**, 220, 534 (1951), in ethanol. [d] B. M. Wepster, *Recl. Trav. Chim. Pays-Bas*, **76**, 357 (1957), in 96% ethanol. A. de Courville and L. Kerisit, *C. R. Acad. Sci., Paris, Ser. C*, **262**, 362 (1966). [e] Spectral data is for a solution containing % T^0 as denoted in brackets. [f] 4-Methyl-*N*-methylanilinium ion: 218 s (3.66), 210 (3.85). [g] 4-Nitro-*N*-methylanilinium ion: 255 nm. [h] Iodide ion absorbance: λ_{max} 226 nm (ϵ 13 500 M^{-1} cm^{-1}); E. Kosower, *J. Am. Chem. Soc.*, **77**, 3883 (1955).

Scheme I



$$HOH + HCHO \overset{K_h}{\rightleftharpoons} H_2C(OH)_2$$

$$K_h = K_1K_0/K'_{a2}$$

[*N*-hydroxymethylamine]$_t$/[hydrated + unhydrated formaldehyde][RNH_2]$_t$ (where t = sum of all ionization states present) for the addition of an aromatic amine to formaldehyde to form the *N*-hydroxymethylamine product, were determined from changes in absorbance (Tables I and II) at eight or more concentrations of formaldehyde at a single pH. The relatively low association constants for adduct formation from the aromatic amines and formaldehyde (K_1 = 3–27 M^{-1}, Table II) and the low concentrations of the chromophore (usually ~1.0 × 10^{-4} M) allow the approximation that the concentration of unbound formaldehyde at equilibrium is equal to the total concentration of formaldehyde.[21a] The values K_{app} are independent of the concentration of the chromophore under the experimental conditions used.[2] The pH dependence of the apparent equilibrium constant is presented in the form of a graph of K_{app} against the fraction of *N*-methylaniline as the free base (α_{AM}) and the association constants for neutral (T^0) and cationic (T^+) *N*-hydroxymethylamine formation, K_1 and K_2 (Scheme I), are determined from the right and left ordinate intercepts, respectively (eq 2, Table II, and Figure 1)

$$K_{app} = K_1\alpha_{AM} + (1.0 - \alpha_{AM})K_2 \quad (2)$$

where $K_1 = [T^0]/[RNH_2][F]$, $K_2 = [T^+]/[RNH_3^+][F]$, and F is total formaldehyde (hydrated plus unhydrated). In two cases, the determination of K_3 values for *N,N*-dihydroxymethylamine formation from *N*-monohydroxymethylamine and formaldehyde (Scheme I) by a kinetic method under pseudo-first-order conditions was possible because the rate of addition of the second molecule of formaldehyde to *N*-monohydroxymethylamine to form *N,N*-dihydroxymethylamine was sufficiently slower than the addition of the first formaldehyde molecule that the rate constants for both reactions could be obtained from the limiting slopes of the biphasic plots of log ($A_\infty - A_i$) against time, where A_∞ and A_i are the absorbance values at equilibrium and any given time, respectively. The K_3 values are more than tenfold lower than the values of K_1 (Table II).

Spectral Characteristics of *N*-Hydroxymethylamines and Schiff Bases. The spectra of the Schiff bases of pivaldehyde and 4-cyanoaniline and aniline are stable in dry dioxane and show marked shifts of λ_{max} for at least one absorption band (20–23 nm) with large changes in molar absorptivities when compared to the parent aniline in dioxane or water (Table III). Aliquots of a dioxane stock solution of pivalideneaniline and pivalidene-4-cyanoaniline, introduced into aqueous solution at pH 10.83, hydrated at sufficiently slow rates ($t_{1/2} \simeq 60$ min) that the spectra of the imines could be obtained in water (dotted line in Figure 2). The parent aniline can be identified spectrally as one of the breakdown products of pivalideneaniline (dashed line, Figure 2); the pivaldehyde present does not absorb significantly at these concentrations.[16] At pH 7, where the hydration of the imine is faster and the breakdown of the carbinolamine is the rate-determining step,[3b,4d] the spectrum of the carbinolamine intermediate (solid line, Figure 2) was obtained before the further decomposition to 4-cyanoaniline and pivaldehyde. The breakdown ($t_{1/2} \simeq 15$ min) of the carbinolamine of 4-cyanoaniline and pivaldehyde ex-

Table II. Proton Dissociation Constants, Equilibrium Constants for Formaldehyde Adduct Formation, and Substituent Constants for Aromatic Exocyclic Amines[a]

Compound (σ^-)[e]	pK'_{a1}[b]	pK'_{a2}[c]	K_1, M^{-1}	K_2, M^{-1}	pK_0	pK_n	pK_z	No. of expts	λ, nm[g]
4-Amino-1-methylpyridinium ion[f] (3.77)[d,l]	−6.03 (−6.0)[f]	[−7.90]	7.0		8.22	15.3	16.1	38	280, 285
3-Fluoro-4-nitroaniline (1.77)[d]	−0.30	[−2.20]	20.0		8.95	9.9	11.2	60	355
3,5-Dinitroaniline[m] (1.50)[d]	0.47 (0.37)[h]	[−1.40]	23.0		8.78	8.8	10.2	41	245
4-Nitro-*N*-methylaniline	0.90 (0.52)[i]		2.8					8	425
4-Nitroaniline (1.24)	1.18 (1.05)[j]	−0.33	27.3	0.85	9.13	8.0	10.4	50	370, 375, 378
4-Cyanoaniline (0.98)	2.00 (1.71)[k]	[0.1]	27.2		9.21	7.7	9.1	60	239, 270, 275 285
4-Chloroaniline (0.23)	4.16 (4.14)[j]	2.22	26.0	0.30				119	238, 250, 255 262, 290
4-Chloro-*N*-methylaniline	4.39 (4.21)[i]	3.02	10.6	0.45				50	252, 258
Aniline (0)	4.79 (4.64)[k]	2.44	22.3	0.10	9.58	5.8	7.2	38	245, 250
N-Methylaniline	5.10 (5.03)[i]	3.72	13.2	0.55				53	250
4-Methylaniline (−0.17)	5.30 (5.08)[k]	3.42	22.7	0.30	9.65	4.8	6.2	37	245
4-Methoxyaniline (−0.26)	5.61 (5.56)[j]	3.72	23.2	0.30	9.71	4.6	6.0	40	245, 255
4-Methyl-*N*-methylaniline	5.64 (5.54)[i]	3.73	16.2	0.20				48	245, 255
4-Methoxy-*N*-methylaniline	5.93 (5.78)[i]	4.59	18.6	0.85				37	240, 245, 250

[a] At 25 °C, ionic strength 1.0 M. [b] $K'_{a1} = [RNH_2]a_{H^+}/[RNH_3^+]$ determined spectrophotometrically (except 4-chloro-*N*-methylaniline, determined potentiometrically); values in parentheses are from the literature. $K_1 = [>NCH_2OH]/[>NH][F]$; $K_2 = [>NH^+(CH_2OH)]/[>NH_2^+][F]$; $K_3 = [-N(CH_2OH)_2]/[>NCH_2OH][F]$; $K_0 = [>N^+HCH_2O^-]a_{H^+}/[>N^+HCH_2OH]$; $K_z = K_0/K_{a2} = [T^\pm]/[T^0]$; $K_n = K_1K_z = [>N^+HCH_2O^-]/[>NH][F]$; where $F = HCHO + H_2C(OH)_2$. [c] $K'_{a2} = [RNHCH_2OH]a_{H^+}/[RNH_2^+CH_2OH]$; values in brackets are calculated from $pK'_{a2} = pK'_{a1} - 1.91$ based upon the data for the 4-methoxy-, 4-methyl-, 4-chloro-, and 4-nitroaniline and aniline which yield $\Delta pK = -1.91 \pm 0.29$. [d] Calculated from $\Delta pK'_{a1} = 0.024 - 2.86\sigma^-$, where $\Delta pK'_{a1} = (pK'_{a1_H} - pK'_{a1_X})$ (see text). [e] Values of σ^- taken from ref 26 were utilized to correlate the pK_{a1} values vs. σ^- and to calculate effective σ^- values for the upper three compounds of 3.77, 1.77, and 1.50, respectively (see footnote *d*). [f] For 4-aminopyridine: M. L. Bender and Y. L. Chow, *J. Am. Chem. Soc.*, **81**, 3929 (1959); see ref 41. [g] Wavelengths used for the determination of kinetic and equilibrium constants. [h] Reference 24b, $\mu = 0.72$ M. [i] Reference 51a, $\mu = 0.05$ M. [j] Reference 24a, $\mu = 0.06$–0.12 M. [k] Reference 27. [l] $K_3 = 0.08$ M^{-1}, determined kinetically at six formaldehyde concentrations, pH 0.08. [m] $K_3 = 1.4$ M^{-1}, determined kinetically at seven formaldehyde concentrations, pH 4.6 (Figure 3).

Table III. Ultraviolet Spectral Data of the Carbinolamine and Schiff Base of Pivaldehyde and Substituted Anilines

Compound	λ_{max}, nm: Dioxane	λ_{max}, nm: Water[a] (log ε)
Aniline	290	280 (3.12)
	240	230 (3.90)
Pivalideneaniline	280	266 (3.45)
	220	210 (4.04)
4-Cyanoaniline	272	270 (4.28)
	214	213 (4.16)
Carbinolamine[b]		278 (4.32)
		216 (4.03)
Pivalidene-4-cyanoaniline	275	271 (4.10)
	239	236 (4.24)

[a] 0.3% dioxane, free base species, ionic strength 1.0 M, ε = molar absorptivity (M^{-1} cm^{-1}), 25 °C. [b] Derived from 4-cyanoaniline and pivaldehyde.

hibits an isosbestic point at 240 nm, indicating that no other intermediates or products are involved. The uv spectral characteristics of anilines, carbinolamines, and imines formed from pivaldehyde are contained in Table III. The spectra of the carbinolamines of anilines and pivaldehyde (generated from the Schiff bases as described above) are *indistinguishable* from the spectra of solutions of the same substituted anilines and formaldehyde at a given pH (Table I, cf. Figure 2).

Kinetics of *N*-Hydroxymethylamine Formation. The rate of condensation of an aromatic amine with a large molar excess of formaldehyde, measured spectrophotometrically, is first order in respect to amine concentration. In the cases of 3,5-dinitroaniline and 1-methyl-4-aminopyridinium ion at concentrations of formaldehyde greater than 0.5 and 1.0 M, respectively, the absorbance changes are best described as the sum of two exponential curves with the rate constants for the second slower phase 25- to 80-fold smaller than the rate constants for the first phase. The first exponential is assigned to formation of *N*-hydroxymethylamine from amine and formaldehyde, and the second exponential is assigned to the formation of *N,N*-dihydroxymethylamine from *N*-monohydroxymethylamine and formaldehyde. The pseudo-first-order rate constants for both phases are linearly dependent upon the formaldehyde concentrations; i.e., both phases are first order in respect to formaldehyde concentration (Figure 3). However, the reverse reactions are significant as indicated by finite ordinate intercepts at zero formaldehyde concentrations (Figures 3 and 4) and enable kinetic estimates of K_1 and K_3 from the ratio of the slope to the ordinate intercept for each phase (Table II). Kinetic and equilibrium constant measurements for *N*-monohydroxymethylamine formation for these compounds utilized wavelengths near the isosbestic point for the *N*-hy-

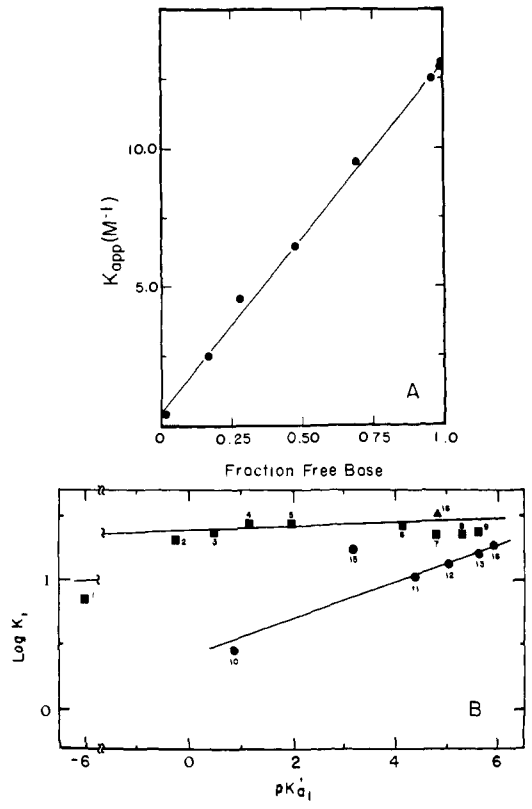


Figure 1. (A) Dependence of the apparent association constants for cationic and neutral *N*-methyl-*N*-hydroxymethylaniline formation from cationic and neutral *N*-methylaniline and hydrated plus unhydrated formaldehyde upon the fraction of free base amine at 25 °C, ionic strength 1.0 M, K_1 = 13.2 M^{-1} (right ordinate intercept), and K_2 = 0.55 M^{-1} (left ordinate intercept). The solid line was calculated from the parameters obtained by a least-squares method (Table II) and eq 2. (B) Dependence of association constants for hydroxymethylamine formation, K_1, from anilines and *N*-methylanilines and formaldehyde upon the pK'_{a1} or Hammett substituent constant, σ^-. Slopes for anilines (■), $\beta = 0.039 \pm 0.011$ and $\rho^- = -0.11 \pm 0.03$. Slopes for *N*-methylanilines (●), $\beta = 0.162 \pm 0.002$ and $\rho^- = -0.544 \pm 0.012$ (based on points 11-14). Compounds: (1) 4-amino-1-methylpyridinium; (2) 3-fluoro-4-nitroaniline; (3) 3,5-dinitroaniline; (4) 4-nitroaniline; (5) 4-cyanoaniline; (6) 4-chloroaniline; (7) aniline; (8) 4-methylaniline; (9) 4-methoxyaniline; (10), 4-nitro-*N*-methylaniline; (11) 4-chloro-*N*-methylaniline; (12) *N*-methylaniline; (13) 4-methyl-*N*-methylaniline; (14) 4-methoxy-*N*-methylaniline; (15, ●), sulfanilic acid (ref 44a); (16, ▲), tetrahydrofolic acid (ref 4q).

droxymethyl and *N,N*-dihydroxymethyl species and relatively low formaldehyde concentrations in order to minimize contributions due to *N,N*-dihydroxymethylamine formation.

The general expression for the observed pseudo-first-order rate constant for the approach to equilibrium for *N*-hydroxymethylamine formation according to the kinetic scheme of eq 3

$$RNH_2 + \left\{\begin{matrix} HCHO \\ + \\ H_2C(OH)_2 \end{matrix}\right\} \underset{k_{-s},\ k_{-H}a_{H^+},\ k_{-OH}(K_w/a_{H^+}),\ k_{-buf}}{\overset{k_s,\ k_H a_{H^+},\ k_{OH}(K_w/a_{H^+}),\ k_{buf}}{\rightleftharpoons}} RNHCH_2OH + HOH \quad (3)$$

is given by eq 4-8

$$k_{obsd} = (k_{nbc} + k_{buf}[B_T])\alpha_{AM}[F] + (k_{-nbc} + k_{-buf}[B_T])\alpha_{HM} \quad (4)$$

$$k_{nbc} = k_s + k_H a_{H^+} + k_{OH}(K_w/a_{H^+}) \quad (5)$$

$$k_{buf} = k_A(1 - \alpha_B) + k_B\alpha_B \quad (6)$$

$$k_{-nbc} = k_{-s} + k_{-H}a_{H^+} + k_{-OH}(K_w/a_{H^+}) \quad (7)$$

$$k_{-buf} = k_{-A}(1 - \alpha_B) + k_{-B}\alpha_B \quad (8)$$

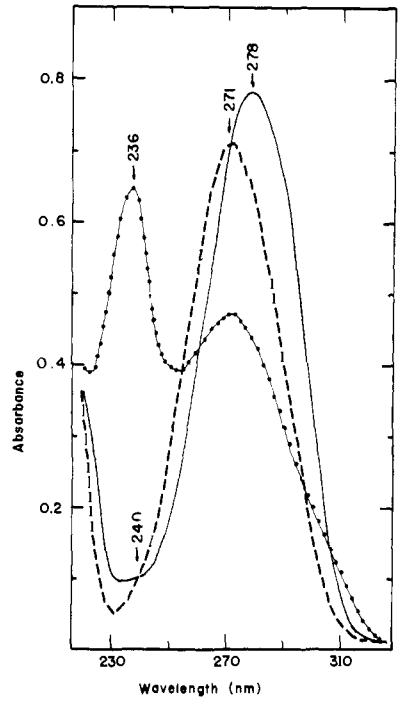


Figure 2. Spectrum of 4-cyanoaniline (1.0×10^{-4} M), pH 7.0 and 10.83 (dashed line); spectrum of carbinolamine of 4-cyanoaniline and pivaldehyde at pH 7.0 (solid line); spectrum of pivalidene-4-cyanoaniline at pH 10.83 (dotted line).

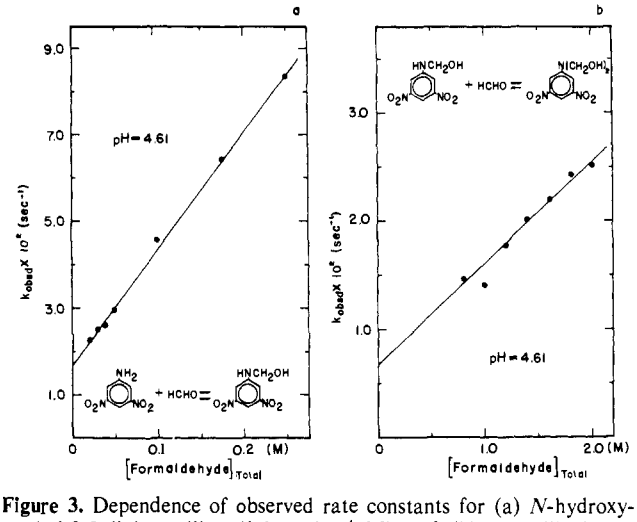


Figure 3. Dependence of observed rate constants for (a) *N*-hydroxymethyl-3,5-dinitroaniline (1.0×10^{-4} M) and (b) *N,N*-dihydroxymethyl-3,5-dinitroaniline formation upon total formaldehyde concentration at 25 °C, ionic strength 1.0 M, and pH 4.61. The slope is the second-order rate constant for adduct formation and the ordinate intercept is the first-order rate constant for adduct decomposition.

where F is the total (hydrated plus unhydrated) formaldehyde; B_T is total buffer; k_s, k_H, and k_{OH} are solvent, hydronium, and hydroxide ion catalyzed rate constants for *N*-hydroxymethylamine formation, respectively; k_A and k_B are the catalytic rate constants for the acidic and basic species of the buffer for *N*-hydroxymethylamine formation; and α_{AM}, α_{HM}, and α_B are the fraction of total amine, total hydroxymethylamine, and total buffer as the free base, respectively. The second-order observed rate constant for *N*-hydroxymethylamine formation (i.e., forward direction), $k_{obsd(f)}$, is obtained from the slope of a plot of k_{obsd} against total formaldehyde concentration (Figure 4). Since catalysis by the buffer makes a significant contribution to the observed rate, the forward rate constants

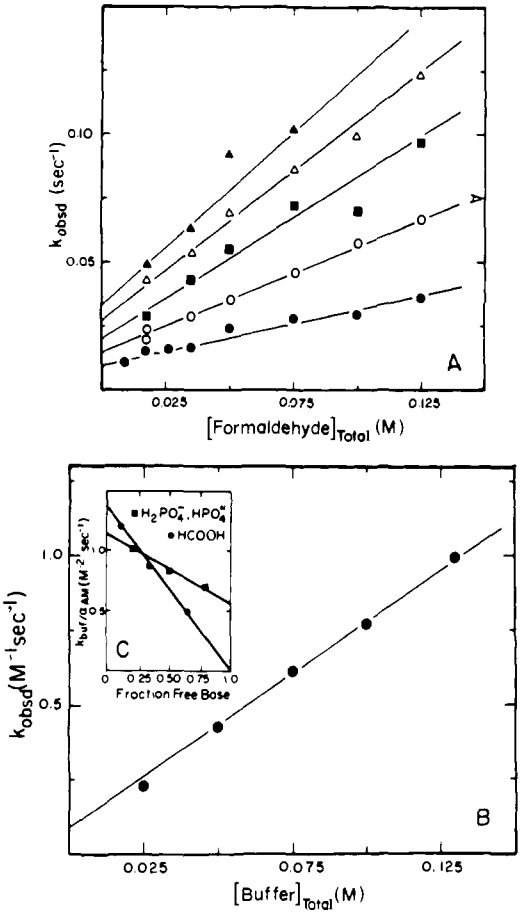


Figure 4. (A) Dependence of the observed rate constants for *N*-hydroxymethyl-4-nitroaniline (1.66×10^{-4} M) formation upon total formaldehyde concentration. Acetate buffer (20% free base) concentration (●, 0.025 M; ○, 0.05 M; ■, 0.075 M; △, 0.10 M; ▲, 0.125 M) at 25 °C, ionic strength 1.0 M, 397 nm, and pH 3.97. (B) Dependence of second-order rate constants (slopes from Figure 4 A) for *N*-hydroxymethylaniline formation upon total buffer concentration. The slope is k_{buf} (eq 6) and the ordinate intercept is k_{nbc} (eq 5). (C) Dependence of the third-order rate constants for buffer catalysis of *N*-hydroxymethyl-3-fluoro-4-nitroaniline formation with respect to the amine free base upon the fraction of the total buffer as the free base, α_B: formate (●), potassium phosphate (■), 25 °C, ionic strength 1.0 M. Right and left ordinate intercepts give k_B and k_A, respectively (eq 6).

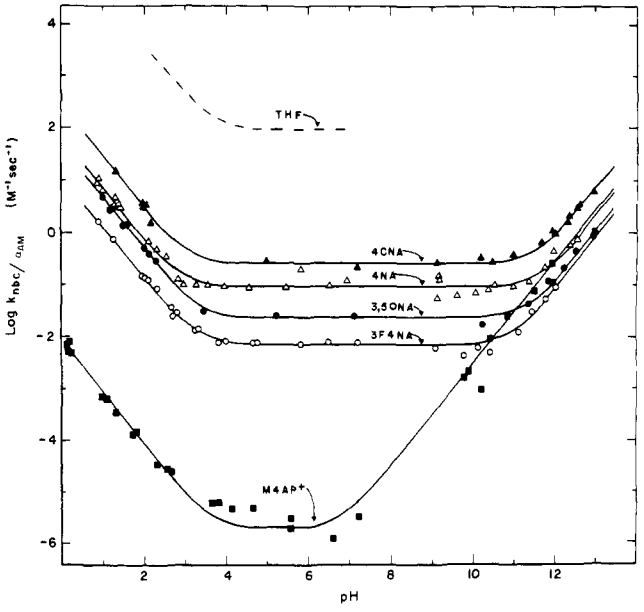


Figure 5. Dependence upon pH of the logarithm of the second-order rate constants for hydroxymethylamine formation from amine, as the free base, and total formaldehyde at zero buffer concentration (by extrapolation). Solid lines are calculated from the values of constants in Tables IV and V and eq 5; the dashed line represents tetrahydrofolic acid data from ref 4q. ▲, 4-cyanoaniline; △, 4-nitroaniline; ●, 3,5-dinitroaniline; ○, 3-fluoro-4-nitroaniline; ■, 4-amino-1-methylpyridinium ion.

for *N*-hydroxymethylamine formation for the non-buffer-catalyzed reaction, k_{nbc}, and for the buffer-catalyzed reaction, k_{buf}, were obtained from the ordinate intercept and slope, respectively, of plots of the second-order rate constants $k_{obsd(f)}$, the slopes of Figure 4A, against total buffer concentration (Figure 4B),[29] where $k_{obsd(f)} = [k_{nbc} + k_{buf}[B_T]]\alpha_{AM}$.

The pH dependence of the non-buffer-catalyzed apparent second-order rate constants for the reaction of formaldehyde with aromatic amines as the free base (k_{nbc}) to form the corresponding *N*-hydroxymethylamine indicates hydronium, solvent, and hydroxide ion terms in the rate law. The solid lines of Figure 5 are calculated from eq 5 and the constants in Tables IV and V. There is no downward deviation suggestive of a change in the rate-determining step (Figure 5) which is further evidence that significant imine formation is not observed.

The observed pseudo-first-order rate constants for the reaction of aromatic amines with formaldehyde are independent of the amine concentration and supports the formulation that the transition state involves a single amine molecule. There is less than a 10% change in the observed rate over the range of ionic strength 1.0–2.0 M which indicates that the rate enhancements for reactions of aromatic amines with formaldehyde at high buffer concentrations (>0.05 M) represent buffer catalysis rather than salt or activity coefficient effects.

As noted, the reaction of aromatic exocyclic amines with formaldehyde displays catalysis by buffers which is attributed to the conjugate acid or base of the buffer (or both in the cases of phosphate and 3-hydroxyquinuclidine buffers). The third-order catalytic constants for the basic and acidic species, k_B and k_A, are obtained from the right and left ordinate intercepts of graphs of the third-order rate constants for hydroxymethylamine formation (with respect to amine as the free base and total buffer concentration), k_{buf}, against the fraction of total buffer as the base, α_B (Figure 4 B, eq 6). The catalytic constants for general acids and bases are contained in Tables IV and V. The values for hydronium, solvent, and hydroxide ion were calculated from the data shown in Figure 5 and the rate law of eq 5 which describes the rate of the reaction exclusive of buffer catalysis with respect to the amine free base.

The third-order hydroxide ion catalyzed rate constant for *N*-hydroxymethyl-3,5-dinitroaniline formation was determined at pH >12.5 where the proton dissociation from formaldehyde hydrate ($pK_a = 13.3$)[30-32] to form the anion becomes significant. There are decreases in the observed rate constant because of a decrease in the fraction of the total formaldehyde as the neutral hydrated species and a correction was applied in the calculation of the third-order rate constant for hydroxide ion catalyzed hydroxymethylamine formation. No correction was necessary for those anilines whose rate constants could be determined by the dilution-decomposition technique of the corresponding hydroxymethylamine.

Discussion

Characterization of Aromatic Amine Formaldehyde Adducts. Although *N*-hydroxymethylamines formed from aromatic amines and formaldehyde have not been isolated,[4h,11,14c] the spectral, equilibrium, and kinetic data reported herein indicate that the major product of the interaction of formaldehyde and substituted anilines in aqueous solution is the *N*-hydroxymethylamine (eq 1).

Table IV. Logarithm of Third-Order Catalytic Constants[a] of General Acids for Attack of Aromatic Amines as the Free Base on Formaldehyde at 25 °C and Ionic Strength 1.0 M

		Logarithm									
		M4AP+		3F4NA		3,5DNA		4NA		4CNA	
Catalyst	pK^{HA}	k_A, M^{-2} s^{-1}	k_A/K_n, M^{-1} s^{-1}	k_A, M^{-2} s^{-1}	k_A/K_n, M^{-1} s^{-1}	k_A, M^{-2} s^{-1}	k_A/K_n, M^{-1} s^{-1}	k_A, M^{-2} s^{-1}	k_A/K_n, M^{-1} s^{-1}	k_A, M^{-2} s^{-1}	k_A/K_n, M^{-1} s^{-1}
H_3O^+	−1.7[b]	−2.15	13.1	1.16	10.9	1.70	10.5	1.85	9.9	2.51	10.2
Dichloroacetic acid	1.29	−2.74	12.6	0.70	10.6			1.45	9.5		
Cyanoacetic acid	2.33	−3.36	11.9	0.33	10.2			1.11	9.1	1.70	9.4
Chloroacetic acid	2.70	−3.33	12.0	0.42	10.3			1.11	9.1		
Formic acid	3.63	−3.82	11.5	0.13	10.0			0.93	8.9	1.52	9.2
Acetic acid	4.60	−3.96	11.3	0.04	9.9			0.90	8.9	1.43	9.1
Phosphate monoanion	6.50	−3.66	11.6	0.06	10.0			0.80	8.8		
3-Chloroquinuclidine	9.09			−1.52				−0.21	7.8	0.52	8.2
Hexafluoro-2-propanol	9.22							0.45	8.5		
3-Quinuclidinol	10.13			−1.92				−0.72	7.3	−0.41	7.3
Phosphate dianion	11.4	−3.80	15.4	−0.29				0.66		1.16	
HOH	15.7	−7.44	7.9	−3.90	6.0	−3.40	5.4	−2.79	5.2	−2.35	5.4

[a] $v_{cat.} = k_A[[HCHO] + [H_2C(OH)_2]][HA][RNH_2]$. [b] Catalytic constants for hydronium ion are expressed in terms of concentration (see Experimental Section).

Table V. Logarithm of Third-Order Catalytic Constants[a] of General Bases for Attack of Aromatic Amines as the Free Base on Formaldehyde at 25 °C and Ionic Strength 1.0 M

		Logarithm									
		M4AP+		3F4NA		3,5DNA		4NA		4CNA	
Catalyst	pK^{HA}	k_B, M^{-2} s^{-1}	k_B/K_n, M^{-1} s^{-1}	k_B, M^{-2} s^{-1}	k_B/K_n, M^{-1} s^{-1}	k_B, M^{-2} s^{-1}	k_B/K_n, M^{-1} s^{-1}	k_B, M^{-2} s^{-1}	k_B/K_n, M^{-1} s^{-1}	k_B, M^{-2} s^{-1}	k_B/K_n, M^{-1} s^{-1}
HOH	−1.7	−7.44	7.9	−3.90	6.0	−3.40	5.4	−2.79	5.2	−2.35	5.4
Phosphate dianion	6.50	−3.80	11.5	−0.28	9.6			0.66	8.7	1.16	8.9
Triethylenediamine	9.06			−1.22	8.7[c]						
3-Chloroquinuclidine	9.09			−1.19	*8.7*[c]			−0.17	*7.7*[c]	0.15	*7.9*[c]
3-Quinuclidinol	10.13			−1.07	*8.8*[c]			−0.11	*7.9*[c]	0.45	*8.2*[c]
Phosphate trianion	11.4	−0.82	14.5	−0.17	9.7			−0.57	7.4	1.27	9.0
Quinuclidine	11.50			−0.88	*9.0*[c]			−0.02	*8.0*[c]	0.58	*8.6*[c]
OH^-	15.7[b]	1.60	16.9	0.99	10.9	1.15	10.0	1.48	9.5	1.96	9.7
				β 0.13 ± 0.01				β 0.06 ± 0.02		β 0.17 ± 0.05	

[a] $v_{cat.} = k_B[[HCHO] + [H_2C(OH)_2]][B][RNH_2]$. [b] Catalytic constants for hydroxide ion are expressed in terms of concentration (see Experimental Section). [c] Slope (β) obtained from the italicized data. Data for triethylenediamine statistically corrected for comparison with quinuclidines.

The spectral characteristics of hydroxymethyl adducts of primary amines and formaldehyde in water are similar to the spectral characteristics of *N*-methylanilines (Table I). For example, *N*-hydroxymethylaniline [λ_{max} (log ϵ) 238 nm (4.01), 283 (3.14)] may be compared with *N*-methylaniline [239 nm (3.97), 285 (3.18)] and with *N,N*-dimethylaniline [244 (3.94), 284 (3.11)].[33] Other similar comparisons may be made from Table I when allowance is made for the fact that some of the spectral data were obtained in ethanol.

It was expected that imines would possess substantially different spectral properties on the basis of observations with aliphatic amines and aliphatic aldehydes[4i-n] and with aliphatic or aromatic amines and aromatic aldehydes.[4d] In fact, we have found that the major peaks of the spectrum of the Schiff bases of aniline or 4-cyanoaniline and pivaldehyde are quite *different* from both the parent amines (Table III, Figure 2) or their reaction products formed from formaldehyde.

The Schiff bases of aniline or 4-cyanoaniline and pivaldehyde are unstable in aqueous solution throughout the pH range 0–14. At neutral pH, carbinolamine breakdown to amine and aldehyde is slow with respect to the disappearance of the Schiff base spectrum,[4h] which enables the spectrum of the

carbinolamine to be obtained and indicates that this carbinolamine is substantially more stable thermodynamically than the Schiff base (Figure 2). The spectrum for the carbinolamine generated from the imine of pivaldehyde and 4-cyanoaniline, which shows a shift in wavelength of maximum absorbance to longer wavelengths with slightly increased molar absorptivity relative to 4-cyanoaniline, is virtually *identical* with the spectrum of *N*-hydroxymethyl-4-cyanoaniline (Table I, cf. Figure 2).

The spectral shifts observed upon formaldehyde adduct formation with *N*-methylanilines and the magnitudes of the association constant, K_1, are very similar to those for anilines and indicate that the formaldehyde adducts of aromatic amines are *N*-hydroxymethylamines (as previously shown for aliphatic amines[37a]) and *not* imines.[35] Note that for *N*-methyl aromatic amines, the resulting imine would necessarily be cationic and devoid of a lone pair of electrons on nitrogen to conjugate with the aromatic system and, hence, would be expected to exhibit both greater thermodynamic instability to hydration and significantly different spectral characteristics. Similar arguments indicate that the second formaldehyde molecule that adds to the aniline at high formaldehyde concentration leads to the formation of the *N,N*-dihydroxymethylaniline.

The reaction of substituted anilines and formaldehyde is readily reversible, since the spectrum of the aniline reappears at equilibrium following dilution. It would not be expected that the reactions of many of the possible aromatic amine formaldehyde adducts[11,14c,d,35] involving substitutions on the aromatic nucleus[2a] would be easily reversible upon simple dilution, since the reverse reactions involve carbon–carbon bond cleavage. Further, the determinations of the apparent association constants were independent of the concentration of the amine and the data could be well fit in terms of a 1:1 stoichiometric association of amine and formaldehyde.[36]

The rate constants for *N*-hydroxymethylamine formation were second order, being first order in respect to both amine and aldehyde, and were strictly first order for the decomposition of the *N*-hydroxymethylamine. Independent measurements of the apparent association constants for *N*-hydroxymethylamine formation, K_1, obtained from the ratio of the forward and reverse rate constants agree well with those obtained by equilibrium measurements. In virtually every sufficiently extensive kinetic study of Schiff base formation and hydrolysis, a change in rate-determining step with changing acidity occurs in the pH range 0–14.[3] The fact that there is no evidence from the pH–rate profile for a change in rate-determining step in the reaction of weakly basic exocyclic aromatic amines with formaldehyde (Figure 5) is support for the view that the kinetic observations refer to a single reaction, and from the several lines of evidence cited above, that reaction is simply *N*-hydroxymethylamine formation.

The calculated and experimentally determined values of the equilibrium constants for the reactions represented by K_3, K_4, and K'_{a3} of Scheme I indicate that the concentrations of formaldehyde adducts other than neutral and cationic *N*-hydroxymethylamine were minor components and could be neglected under the experimental conditions utilized for the kinetic studies and for the determination of the equilibrium constants K_1 and K_2.

(a) It was possible to measure kinetically the association constant for the addition of formaldehyde to *N*-hydroxymethylaniline to form the *N,N*-dihydroxymethylamine, K_3, because *N*-hydroxymethylamine forms more rapidly than the *N,N*-dihydroxymethylamine (Scheme I). The addition of formaldehyde to an *N*-hydroxymethylamine is 20- to 90-fold less favorable thermodynamically than the addition of formaldehyde to an amine and the equilibrium constants for the addition of formaldehyde to *N*-methyl derivatives (Table II) are of the same order of magnitude as to *N*-hydroxymethyl derivatives. The K_3 values are of the magnitude expected when consideration is given to steric, solvation, and orbital electronegativity factors.[37]

(b) The proton dissociation constants for cationic *N*-hydroxymethylamines, $K'_{a2} = (K'_{a1})(K_1/K_2)$, based on the cyclic nature of the equilibria (Scheme I), are 20–100 times greater than that for the parent amine, K'_{a1}, except for aniline where this ratio is 225.[38]

(c) The value of the proton dissociation constant for cationic *N,N*-dihydroxymethylamine, K'_{a3} (Scheme I), may be calculated from the equations developed by Condon[39] (eq 15 of ref 39 and for $-CH_2OH$,[40] $\Sigma\sigma^* = 0.555 \times 2 = 1.11$), quantitatively relating acidity of aromatic ammonium ions to polar, solvation, and statistical factors. The calculated value of K'_{a3} is about 10^4 larger than the proton dissociation constant of the parent amine, K'_{a1}, and permits estimates of the values of K_4 which are 100–300 times smaller than the values of K_1 from the relation, $K_4 = (K_3)(K'_{a2}/K'_{a3})$, based on the cyclic nature of the equilibria (Scheme I).

The nature of the spectral changes, the independence of the equilibrium constants on the concentration of the chromophore, the ready reversibility of the reaction upon simple dilution, the agreement between equilibrium constants determined spectrophotometrically and kinetically, and the pH–rate profile indicate that *only* the *N*-hydroxymethylamine need be considered as the principal product formed from aromatic amines under the experimental conditions employed.

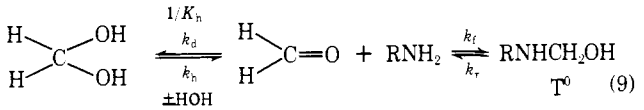
Equilibrium Constants. Equilibrium Constants for Hydroxymethylamine Formation. The differences in affinity between aromatic exocyclic and aliphatic amines for formaldehyde are not large[2] [$\beta_{nuc} = 0.15$ for a correlation for N-hydroxymethylation reactions of both aromatic exocyclic and aliphatic amines with amine basicity from Table II and the available data[37,44]]. These data may also be compared with the equilibrium constants for carbinolamine formation for aliphatic amines and isobutyraldehyde, which are correlated by $\beta_{nuc} = 0.227$.[44c]

Although polar and steric effects make contributions, the equilibrium constants for the formation of neutral and cationic *N*-hydroxymethylamines from formaldehyde are relatively insensitive to the polar character of the amine and this insensitivity has been attributed to the similar polar properties of hydrogen and the hydroxymethyl group[44b] (σ^* values are 0.49 and 0.56, respectively).[40]

When restricted to the aromatic exocyclic amines the dependence of the association constant for the neutral *N*-hydroxymethyl- and *N*-methyl-*N*-hydroxymethylaniline formation upon the basicity of the parent amine is small with β_{nuc} values for the two series of 0.04 ± 0.01 and 0.162 ± 0.002, respectively (Figure 1 B). The difference between the values of β_{nuc} (Figure 1 B) for *N*-hydroxymethylamine formation from anilines and *N*-methylanilines may be due to different sensitivities of the hydration energies for *N*-methyl-*N*-hydroxymethylanilines and *N*-hydroxymethylanilines[39] to field and resonance effects and is related to the difference in ρ^- values for the proton dissociation constants for the conjugate acids of anilines, *N*-methylanilines, and *N*-methyl-*N*-hydroxymethylanilines. Thus, for the aliphatic amines it has been suggested that the difference of more than an order of magnitude between the equilibrium constants (e.g., Table II) for formation of neutral and cationic hydroxymethylamines, K_1 and K_2, resides largely in a decrease in the solvation energy of the cation caused by replacement of a hydrogen by a hydroxymethyl group.[37] However, for aromatic exocyclic amines this effect may be partly offset by resonance augmented hydration of the *free base*, which is *decreased* upon *N*-hydroxymethylamine formation.[39] Thus the decrease in pK'_a value for the conjugate acids of aliphatic amines on hydroxymethylation is 2–3,[37a] whereas the decrease in pK' value for

the conjugate acids of aromatic amines upon hydroxymethylation is ~1.9 ($pK'_{a1} - pK'_{a2}$, Table II).

Formaldehyde Hydrate Dehydration Rate. Studies of nucleophilic reactions with carbonyl compounds of varying fractions of hydration have repeatedly shown that the unhydrated carbonyl compound is the reactive species (eq 9)[4h,22b,45]

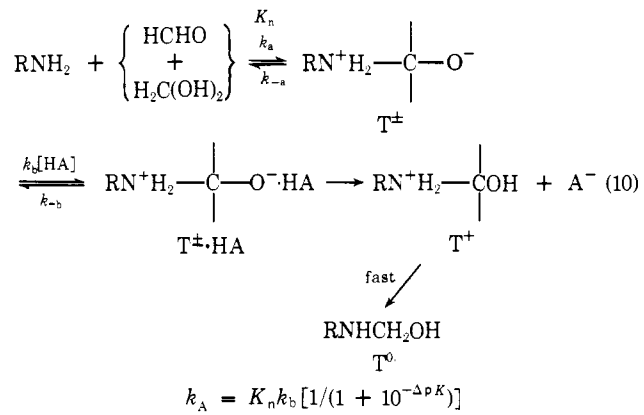


$$T^0 \quad (9)$$

where $K_1 = [T^0]/[RNH_2]([HCHO] + [H_2C(OH)_2]) = K_{ad}/(1 + K_h)$, $K_h = [H_2C(OH)_2]/[HCHO]$,[47] and $K_{ad} = [T^0]/[RNH_2][HCHO]$. The kinetic studies reported herein have been restricted to compounds for which attack rates were slow enough for formaldehyde dehydration to be adequate and, thus, not rate limiting.[46,47]

General Acid Catalysis of *N*-Hydroxymethylamine Formation. There are both buffer (Figure 4 B for 4-nitroaniline with acetic acid buffers) and solvated proton catalyzed pathways for *N*-hydroxymethylamine formation; the solvated proton catalyzed contribution to the non-buffer-catalyzed rates of *N*-hydroxymethylamine formation is shown at low pH in Figure 5. The fraction of the total buffer that is active catalytically as the fraction of ionization varies with changing pH is determined from plots (Figure 4 C) in which the third-order rate constants with respect to the free base amine and total buffer concentrations are plotted as a function of the fraction of buffer as the free base. In some cases *N*-hydroxymethylamine formation shows catalysis by both the acid and base components of the buffer (Figure 4 C). General acid catalysis has been observed for the addition of tetrahydrofolic acid,[4q] and model compounds thereof,[48] semicarbazide,[3a,4b] 2-methylsemicarbazide,[4g] thiosemicarbazide,[4c] 2-methyl-3-thiosemicarbazide,[4g] thiourea,[52] hydrazides,[4g] phenylhydrazine *p*-sulfonate,[4g] methoxyamine,[4f] to carbonyl compounds.

The nonlinear Bronsted plots for general acid catalysts for 4-cyano-, 4-nitro-, and 3-fluoro-4-nitroaniline (Figure 6) provide evidence for a change in the nature of the rate-determining step with changing pK^{HA} value of the catalyst and for a mechanism in which an intermediate is not at equilibrium with the medium with respect to transport processes.[4e,49] The change in rate-determining step means that the formation of the *N*-hydroxymethylamines must involve at least two kinetically significant steps, namely, the formation or breaking of bonds to the carbonyl carbon atom in the reaction *and* some process involving the acid that catalyzes proton transfer. The simplest explanation of this type of behavior is that the rate-determining step of the catalyzed reaction is a simple proton transfer step that is diffusion controlled in the direction that is thermodynamically strongly favorable[2,4e,49,50] (mechanism I, eq 10)



$$k_A = K_n k_b [1/(1 + 10^{-\Delta pK})]$$

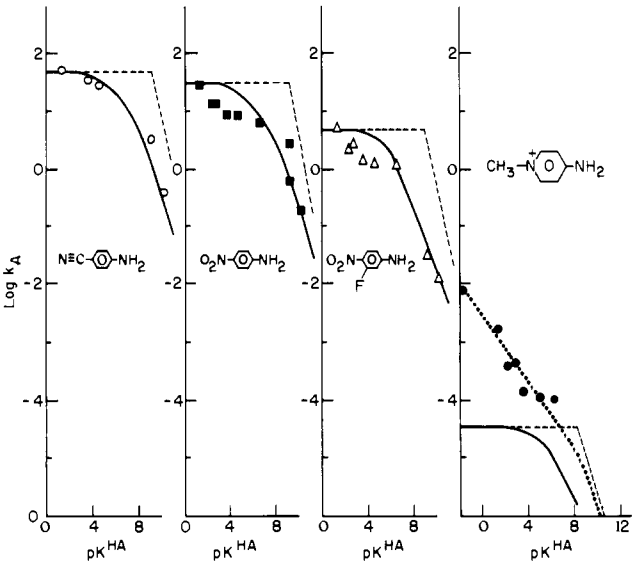


Figure 6. Bronsted plot for general acid catalysis of the addition of exocyclic aromatic amines to total formaldehyde (hydrated plus unhydrated) at 25 °C and ionic strength 1.0 M. The solid lines are theoretical curves for the preassociation mechanism of eq 11 and 12 with pK_0 values given in Table II and the individual rate and equilibrium constants given in the text. The dashed line shows the limiting slopes of 1.0 and 0 obtained from the fit of the data to the Bronsted curve for simple diffusion-controlled proton transfer (eq 10b). The dotted line is a theoretical curve for the concerted mechanism of eq 11 and 13 and the individual rate and equilibrium constants are given in the text with statistical corrections applied according to Bell and Evans.[45b]

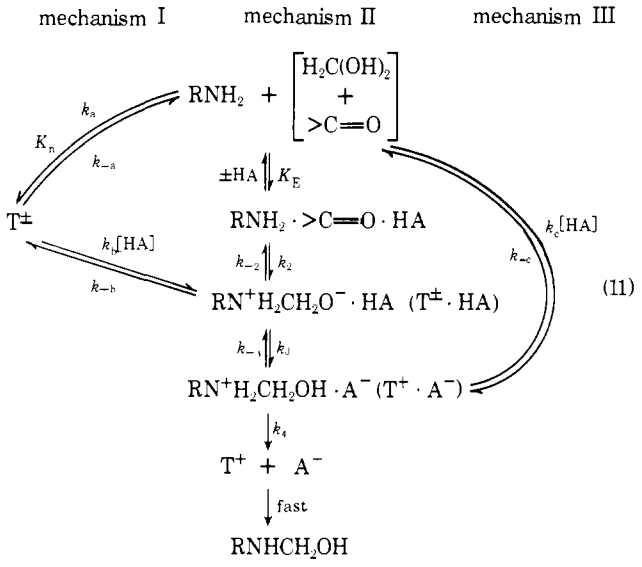
where $\Delta pK = pK_0 - pK^{HA}$, $K_n = [T^{\pm}]/[RNH_2][F]$, and $K_0 = [T^{\pm}]a_{H^+}/[T^+]$. According to this mechanism, the unstable intermediate $T^{\pm}$ that is formed initially by the attack of amine on the carbonyl group reverts rapidly to starting materials (k_{-a}) unless it is trapped by an encounter with an acid that protonates the alkoxide ion of $T^{\pm}$ so that amine expulsion is prevented. The rate of the proton-transfer step will be diffusion controlled and therefore independent of catalyst acidity for strong acids (slope = 0) and will follow a Bronsted slope of 1.0 for weak acids; for acids of intermediate strength the plot will be curved and the point of intersection of the lines of slope $\alpha = 0$ and $\alpha = 1.0$ will be close to $\Delta pK = 0$ between the catalyst and the intermediate.[50b] The dashed lines in Figure 6 show the limiting slopes of 0 and 1.0 obtained from the fit of the data to the Bronsted curve for a simple diffusion-controlled proton transfer. The observed nonlinear Bronsted plots for the more basic exocyclic amines in Figure 6 and the calculated rate constants $k'_A = k_A/K_n$ in the range of 10^9–10^{10} M^{-1} s^{-1} (Table IV) for the catalysts $pK^{HA} < 5$ are consistent with that expected for this mechanism, where K_n values are obtained from the relation $K_n = K_1K_0/K'_{a2} = [T^{\pm}]/[RNH_2][F]$ (Table II).

However, the positions of the breaks in the three left-most Bronsted plots in Figure 6 occur at lower pK^{HA} values by 3–4 units than expected from the estimated pK_0 values for the intermediates $T^{\pm}$ of 8–9 (see Appendix and Table II). Alternative mechanisms to account for the nonlinear Bronsted plots with break points at pK^{HA} values less than the pK_0 values, in which free $T^{\pm}$ does not occur as an intermediate, are the following: (i) preassociation or "spectator" and (ii) concerted mechanisms.[4e]

In the former, preassociation of the catalyzing acid, amine, and aldehyde in an encounter complex (K_E) is followed by the formation of $T^{\pm}$ within the encounter complex (k_2), proton transfer (k_3), and a dissociation or rotation (k_4) before product formation.

For very weak acids the rate-determining step is k_4 (the

same as for the expanded simple proton-transfer mechanism I, eq 11). However, for strong acids in the preassociation (or



(11)

"spectator")[51] mechanism, the rate-determining step is k_2 and the rate is higher than for simple proton-transfer mechanisms resulting in a break in the Bronsted plot at lower pK^{HA} value. For catalysts of intermediate acidity the rate-determining step is k_3. The steady-state rate law for the preassociation mechanism (mechanism II, eq 11) is given by eq 12.

$$k_A = K_E k_2 k_3 k_4/(k_{-2}k_{-3} + k_{-2}k_4 + k_3 k_4) \qquad (12)$$

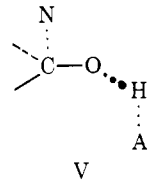
The solid lines in Figure 6 for the 4-cyano-, 4-nitro-, and 4-nitro-3-fluoroanilines have been calculated using the steady-state eq 12 with $K_E k_2$ values of 50 M^{-2} s^{-1}, 30 M^{-2} s^{-1}, and 5 M^{-2} s^{-1}, respectively (from k_A for strongly acidic carboxylic acids). The calculations employed the assumption that the pK_0 values are as in Table II, k_{-2} (for the breakdown of $T^{\pm}$ to amine and carbonyl compound within the encounter complex) is 5×10^{11} s^{-1}, and the following equations: $\log k_3 = 10 + 0.5\ \Delta pK$, $\log k_{-3} = 10 - 0.5\ \Delta pK$, $\Delta pK = pK_0 - pK^{HA}$, and $k_4 = 10^{11}$ s^{-1} (see ref 4e).

The calculated lines in Figure 6 for the three left-most compounds show satisfactory agreement with the observed rate constants with breaks at approximately the expected pK^{HA} value as a consequence of the change in rate-determining step from N-C bond formation within the encounter complex (k_2) with strong acids to predominantly rate-determining proton transfer (k_3) for the weaker acids examined. The dissociation step, k_4, with a Bronsted α value of 1.0 becomes rate determining only with acids that are so weak that they would *not* give detectable catalysis under the experimental conditions employed. The small deviations of the catalytic constants for some of the carboxylic acids on the Bronsted plots are similar to that observed in related reactions in which diffusion-limited proton-transfer processes occur[4e] and in simple proton-transfer reactions[50b] for which the transition to a slope zero apparently may not be complete even for large favorable ΔpK. If the assumption is made that $k_{-2} = 5 \times 10^{11}$ s^{-1}, the absolute magnitude of the observed constants, k_A, for strong acids may be calculated for the preassociation mechanism[4e] (eq 12) where $k_A = K_E k_2 = K_X k_{-2}$, $K_X = K_1 K_Z K_{assoc} = K_E K_2 = [T^{\pm}\cdot HA]/[F][HA][RNH_2]$, $K_Z = [T^{\pm}]/[T^0]$, and $K_{assoc} = [T^{\pm}\cdot HA]/[T^{\pm}][HA]$. Exact agreement with the observed k_A values (Table IV) for 4-cyano-, 4-nitro-, and 3-fluoro-4-nitroaniline is obtained with the constants in Table II and K_{assoc} values of 0.005, 0.008, and 0.07 M^{-1}, respectively. Such values are of the same order of magnitude as those proposed for encounter complex formation,[53] especially if significant orientation requirements exist within the encounter complex for the proton transfer to occur.[4e,53a]

As the lifetime of $T^{\pm}$ becomes shorter (related to progressively greater pK_n values as the basicity of the amine decreases, Table II), the $T^{\pm}$ species cannot exist as a discrete intermediate either free in solution or within an encounter complex and the reaction must proceed through mechanism III in which proton transfer and carbon-nitrogen bond formation to give T^+ occur more or less in a concerted manner with rate constant, k_c (eq 11). The steady-state rate law for this mechanism is given by eq 13.

$$k_A = k_c k_4/(k_{-c} + k_4) \qquad (13)$$

For weakly acidic catalysts, the relatively strong base A^- will catalyze a rapid breakdown of T^+ to reactants so that k_{-c} will exceed k_4 and k_4 will be rate determining. For strong acids the rate constants will be higher than for the previous nonconcerted mechanisms considered. The *linear* Bronsted plot for general acid catalysis of hydroxymethylamine formation from 1-methyl-4-aminopyridinium ion ($\alpha = 0.28 \pm 0.03$) and the calculated rate constants $k_b = k_A/K_n$ for mechanism I, eq 11 (Table IV), of $>10^{12}$ M^{-1} s^{-1} favor the hypothesis that there has been a shift of mechanism to a concerted mechanism of catalysis for this compound compared with that of the substituted anilines. Although a large free-energy advantage is expected to be gained by proton transfer from a catalyst (situated perpendicular to the normal reaction coordinate) to the carbonyl oxygen atom as the N-C bond is formed[34,40,49,51] (transition state V), a clear distinction between hydrogen bond



V

and concerted mechanisms (that is, whether there is a barrier for proton transfer between the acid and $T^{\pm}$) is not presently possible.

The stability and lifetime of $T^{\pm}$ is also decreased by lowering the dielectric constant of the medium and a concerted mechanism is apparently operative for even more strongly basic aniline derivatives in aqueous dioxane (50/50, v/v).[48a] Since the intermediate $T^{\pm}$ appears to be of borderline stability in aqueous solution, with a lifetime estimated to be on the order of 10^{-12} s, it is not unreasonable that even a relatively small additional destabilization (by decreasing solvent dielectric constant) of this extremely unstable intermediate would be sufficient to shift the reaction path to a concerted mechanism.

The dashed line for 1-methyl-4-aminopyridinium ion in Figure 6 shows expected behavior for a diffusion-limited proton-transfer reaction (eq 10) with $K_n = 10^{-15.3}$, $K_0 = 10^{-8.22}$, and a value of k_b of 10^{11} M^{-1} s^{-1}. The solid line for this compound for the preassociation mechanism was calculated as noted above (eq 12) with a limiting rate constant the same as that for the dashed lines. The dotted line which shows a satisfactory fit to the data (including the catalytic constant for hydronium ion) is calculated from eq 13 with $k_4 = 10^{11}$ s^{-1}, $\log k_c = -0.28\ pK^{HA} - 2.5$, and $\log k_{-c} = 0.72\ pK^{HA} + 4.4$. The dissociation step, k_4, becomes rate determining only with acids that are so weak that they would not give detectable catalysis under the experimental conditions employed.

The rate constants for catalysis by hydronium ion ($pK = -1.7$) are about two- to threefold larger than those for acidic carboxylic acids for the weakly basic anilines. It is possible that this enhanced rate reflects an additional degree of stabilization of the transition state for C-N bond formation by this much

stronger acid. Consistent with this suggestion is the fact that a plot of log k_H vs. pK_{a1} is linearly correlated by a β_{nuc} value of 0.57 ± 0.05 (Figure 7).

General Base Catalyzed *N*-Hydroxymethylamine Formation. The Bronsted β values, which reflect the dependencies of the third-order catalytic constants for general base catalyzed hydroxymethylamine formation upon the base strength or the catalyst, are 0.17 ± 0.05, 0.06 ± 0.02, and 0.13 ± 0.01 for 4-cyanoaniline, 4-nitroaniline, and 3-fluoro-4-nitroaniline, respectively, for monofunctional catalysts excluding data for water and hydroxide ion. General base catalysis has been observed in thiourea [52]and 2-methylthiosemicarbazide[4p] addition to carbonyl groups.

It is possible to consider detailed mechanisms involving simple diffusion-controlled proton transfers, preassociation, and concerted mechanisms for the general base catalyzed pathways of hydroxymethylamine formation.[49] The calculated rate constants $k'_B = k_B/K_n$ fall in the range of $10^{7.4}$–10^9 M^{-1} s^{-1} and approach those expected for a diffusion-controlled process. The Bronsted plots are neither clearly nonlinear (which might rule out a concerted mechanism) nor is a clear-cut trend to higher β values observed with decreasing nucleophilicity as the basicity of the amine decreases by $10^{2.3}$ (which might rule out diffusion-limited proton transfer or preassociation mechanisms). We conclude that the data are too few to definitely exclude any of the mechanistic possibilities for the base-catalyzed reactions of the substituted anilines with formaldehyde.

The increased instability and shorter lifetime of $T^{\pm}$ derived from 1-methyl-4-aminopyridinium ion and k_B/K_n values $>10^{11}$ M^{-1} s^{-1} suggest that the detailed mechanism of the general base catalyzed pathway for this compound is concerted.

The rate constants for the hydroxide ion catalyzed pathway for the most weakly basic amine very likely represents a specific base catalytic mechanism involving RNH^- attack on the carbonyl compound; the proton dissociation constant for $RNH_2 \rightleftharpoons RNH^- + H^+$ is $10^{-12.58}$ (ref 54) for 1-methyl-4-aminopyridinium ion. The fact that little unfavorable electrostatic interaction between cationic 1-methyl-4-aminopyridinium ion and hydronium ion is observed (see k_H correlation in Figure 7) supports a basis for the unexpected high rate for the hydroxide ion catalyzed reaction of 1-methyl-4-aminopyridinium ion and formaldehyde on grounds other than electrostatic and is consistent with pH-independent attack by the net neutral (zwitterionic) compound upon formaldehyde.

pH-Independent Reactions. The dependence upon amine basicity of the observed rate constant for uncatalyzed (or solvent catalyzed) carbinolamine formation from formaldehyde is nonlinear (Figure 7) with slopes of 0.87 and 0.21 for aromatic and aliphatic amines,[22b] respectively. A two-step mechanism has been proposed (eq 14)[4f,g] for similar reactions and appears consistent with the present data. Application of the steady-state assumption to the concentration of $T^{\pm}$ yields eq 15 for this mechanism.

$$RNH_2 + \left\{\begin{matrix} H_2C(OH)_2 \\ + \\ HCHO \end{matrix}\right\} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} T^{\pm} \xrightarrow{k_5} T^0 \qquad (14)$$

$$k_S = k_a k_5/(k_{-a} + k_5) \qquad (15)$$

For weakly basic amines the solvent mediated proton switch step k_5 is rate determining and the slope of a plot of log k_S against the pK_{a1} of the conjugate acid of the nucleophile is 0.87, the same as that for K_n, and is consistent with very little dependence of k_5 upon the basicity of the nucleophile. The dashed and dotted lines in Figure 7 represent the limiting behavior expected if the k_a and k_5 steps are solely rate determining for strongly and weakly basic amines, respectively. The solid line is calculated from eq 15 and log $k_{-a} = -0.66$ pK_{a1} + 11.2, log $k_a = 0.21$ pK_{a1} + 1.74, and $k_5 = 10^{7.7}$ s^{-1} for the water-mediated proton switch. The satisfactory agreement with the observed data supports the designations that C–N formation is the rate-determining step for strongly basic amines ($pK_{a1} > 6$) and that the solvent-mediated proton switch is the rate-determining step for the more weakly basic amines ($-2 < pK_{a1} < 6$).

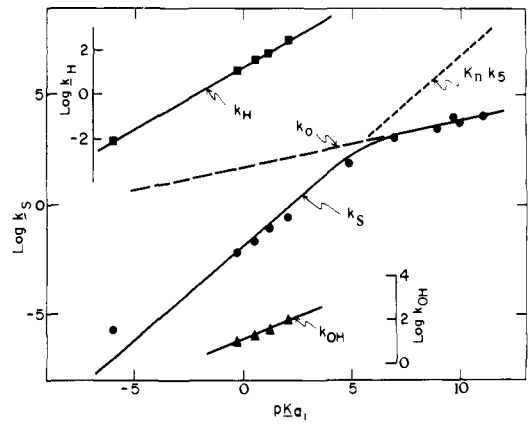


Figure 7. Dependence of the logarithms of the rate constants for hydronium ion (M^{-2} s^{-1}, ■), solvent (M^{-1} s^{-1}, ●), and hydroxide (M^{-2} s^{-1}, ▲) ion catalyzed formation of hydroxymethylamines from formaldehyde and amines upon pK_{a1} values for the nucleophiles (Tables IV and V and ref 22b). The parameters being correlated and the slopes are k_H, 0.57 ± 0.05; k_S ($pK_a < 5$), 0.87 ± 0.04; k_S ($pK_a > 5$), 0.21 ± 0.03; and k_{OH}, 0.41 ± 0.04. The solid line for k_S is a theoretical line for the stepwise mechanism of eq 14 and 15 and the rate and equilibrium constants are given in the text.

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Appendix

Estimates of $K_0 = [RN^+H_2CH_2O^-]a_{H^+}/[RN^+H_2CH_2OH] = [T^{\pm}]a_{H^+}/[T^0]$ are obtained following the considerations of Sayer and Jencks.[4e] Thus, from the estimated pK_a value of 9.98 for the alcoholic group of $CH_3N^+H_2CH_2OH$[4k] and correction for the phenyl substitution for methyl by $\Delta pK = -8.4\ (0.10)/2 = -0.42$ (employing an attenuation factor of 2 for the transmission of the substituent effect through an additional nitrogen atom[4e,55] that is present in carbinolamines) and the considerations of Fox and Jencks[50d] with a $\rho_I = +8.4$ 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 $\rho = +1.11$ value for the ionization of trifluoroacetophenone hydrates and σ values from the literature[28] (except that for $CH_3N^+C_4H_4$- of +2.42 based upon the pK values of benzoic and isonicotinic acids of 4.20 and 1.78,[56] respectively), the values of $\Delta pK = 1.11\ \sigma/2$ are −0.35, −0.43, −0.78, −0.61, and −1.34 are calculated for the exocyclic amines studied. The pK_0 values are then 9.21, 9.13, 8.78, 8.95, and 8.22 for the 4-cyano-, 4-nitro-, 3,5-dinitro-, and 3-fluoro-4-nitroanilines and 1-methyl-4-aminopyridinium ion derivatives, respectively. The correlations of pK_0, pK_z, pK_n, and log K_1 values with pK_{a1} provide the following slopes (β_{nuc}) and intercepts: 0.17, 8.97; −0.83, 10.9; −0.87, 9.5; and 0.04, 1.4, respectively, for the same series of amines.

References and Notes

(1) (a) This project was supported by the National Institutes of Health, U.S. Public Health Service, Grants GM 13 777 (R.G.K.), FR 5415 (University of Pennsylvania, School of Medicine), FR 15 (University of Pennsylvania, Medical School, Computer Facility), and Public Health Service Research Career Development Award No. KO4CA70487 from the National Cancer Institute. (b) Abbreviations: 4CNA, 4-cyanoaniline; 3,5DNA, 3,5-dinitroaniline; F, total formaldehyde (hydrated + unhydrated); 3F4NA, 3-fluoro-4-nitroaniline; THF, tetrahydrofolic acid; M4AP$^+$, 1-methyl-4-aminopyridinium iodide; 4NA, 4-nitroaniline; T^0, hydroxymethylamine or carbinolamine.

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(29) Alternative methods for data analysis are contained in ref 2. In several instances when the association constant for a particular amine was well determined, k'_{bul} and k'_{nbc} values first were determined from the slope and intercept, respectively, of a graph of k_{obsd} against total buffer concentration. Then by using equations $k_{nbc}\alpha_{AM} = k'_{nbc}/[[F] + 1/K_{app}]$ or $k_{bul}\alpha_{AM} = k'_{bul}/[[F] + 1/K_{app}]$ with the known apparent association constant for adduct formation, a correction was applied for the significant reverse reaction. The second-order ($k_{nbc}\alpha_{AM}$) or third-order ($k_{bul}\alpha_{AM}$) rate constants for hydroxymethylamine formation at a given pH were thus obtained. The calculated k_{nbc} values in all cases in which this technique was applied agreed to within 10% of the rate constants determined by other methods.

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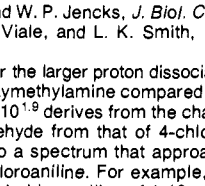
(36) Calculations using the equilibrium constant for *N,N'*-methylenedimorpholine[2,37] formation from morpholine and formaldehyde indicate that the percentage of the concentration of morpholine as the methylenediamine, IV, would be less than 2% under the experimental conditions used, where the concentration of amine is in the range of 5×10^{-5}–5×10^{-4} M (cf. Y. Ogata, M. Okano, and M. Sugawara, *J. Am. Chem. Soc.*, **73,** 1715 (1951), and ref 35).



IV

Morpholine (pK'_{a1} = 8.88)[37b] would be expected to form the methylenediamine at least as favorably as aniline (pK'_{a1} = 4.79) on the basis of the K_1 values for morpholine[37] and aniline of 925 and 22 M^{-1} (Table II), respectively.

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(38) Direct evidence for the larger proton dissociation constant of the conjugate acid of the hydroxymethylamine compared to the parent exocyclic amine of approximately $10^{1.9}$ derives from the change in the spectrum upon addition of formaldehyde from that of 4-chloroanilinium ion (similar to 4-chlorobenzene) to a spectrum that approaches that of free base *N*-hydroxymethyl-4-chloroaniline. For example, from the pK'_{a1} value for the conjugate acid of 4-chloroaniline of 4.16 and the pK'_{a2} value for the conjugate acid of *N*-hydroxymethyl-4-chloroaniline of 2.22, at pH 2.75 the fraction of 4-chloroaniline as the free base is 0.038 while the fraction of the hydroxymethyl derivative as the free base is 0.77. Thus, the bulk of spectral change results from the shift in the pK' value of the exocyclic nitrogen upon N-hydroxymethylation to produce *N*-hydroxymethyl-4-chloroaniline largely as the free base at pH 2.75.

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(42) In the spectrophotometric titrations of 4-amino-1-methylpyridinium ion and 3-fluoro-4-nitroaniline, the concentrations of the respective acid and base species in solution are small and may be represented by standard activities.[25] The dissociation constant obtained from the spectrophotometric H_0 titration is, therefore, a thermodynamic value, which may introduce a small (±0.3 pK unit) error when comparing these thermodynamic values with pK'_{a1} values obtained from aqueous solution at ionic strength 1.0 M.

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(46) Experiments with aniline or *N*-methylaniline with formaldehyde concentration in tenfold or greater excess than the amine concentration at pH 6 yielded biphasic stopped-flow traces of absorbance against time. While the time constant for the earliest phase did not fall clearly within the response time of the stopped-flow spectrophotometer, ($t_{1/2} \leq 6$ ms), the second slow phase absorbance change was readily resolvable and fit as a single exponential with regression coefficients >0.990 ($t_{1/2} \simeq 100$ ms). However, the pseudo-first-order observed rate constants *decreased* as a function of increasing nucleophile concentration and, thus, indicated that the dehydration of formaldehe hydrate was at least partially rate determining under these conditions.[2,45a] This conclusion was confirmed by simulation[2] studies with an analogue computer (Drs. W. R. Abrams and K. L. Brown) utilizing the scheme of eq 9, the values of k_l and k_r for tetrahydrofolic acid (pK' = 4.82),[4q] and the values of k_d and k_h for formaldehyde hydrate breakdown and formation, respectively.[45b,c] The simulation showed that the unhydrated formaldehyde concentration changes markedly during the course of the reaction[2] and that the dehydration rate of formaldehyde hydrate is at least partially rate determining.

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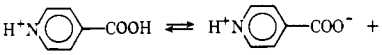
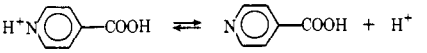
(56) The microscopic ionization constant for the proton dissociation reaction



is $10^{-1.78}$ based upon macroscopic constants of $10^{-1.77}$ and $10^{-4.84}$ for isonicotinic acid and an assumed microscopic ionization constant for



based upon that for the ethyl ester of $10^{3.45}$: "Handbook of Biochemistry: Selected Data for Molecular Biology", H. A. Sober and R. A. Harte, Ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1968, p J-179.

Estimates of Microscopic Ionization Constants for Heteroaromatic Exocyclic Amines Including Purine and Pyrimidine Nucleotides and Amides Based upon a Reactivity–Basicity Correlation for N-Hydroxymethylation Reactions with Formaldehyde[1a,2]

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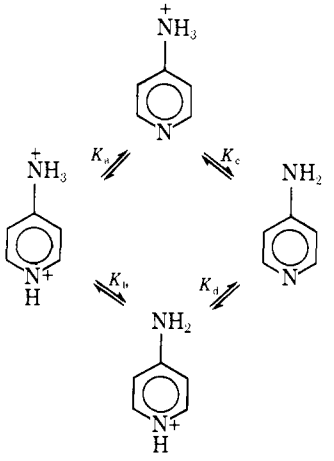
Abstract: The weaker basicity at the 2-amino and 4-amino sites of pyridines, pyrimidines, and purines, when compared to the endocyclic nitrogen atoms, precludes estimates of the pK values for the ionization of the conjugate acids of exocyclic amino groups by direct titration. A kinetic method is proposed for obtaining estimates of these microconstants for the aromatic exocyclic amino groups of heterocyclic compounds [$K_c = (ArNH_2)a_{H^+}/(ArNH_3^+)$], based upon the rates of reaction with formaldehyde to form the N-hydroxymethylamine. The Bronsted equation, $pK_c = (\log k_{0(u)} - 1.61)/0.87$, where $k_{0(u)}$ is the pH-independent rate constant for N-hydroxymethylation with respect to unhydrated formaldehyde, is based on aromatic exocyclic amines ($pK_c = -6.0$–2.0) and provides, with values of $k_{0(u)}$, ranges of values of pK_c at 25 °C for series of adenine, guanine, and cytidine derivatives of -2.8 to -2.2, -1.8 to -1.6, and -2.2 to -1.7, respectively. From the same Bronsted equation, the estimates of the microscopic proton dissociation constants of amides for N-protonation of benzamide and urea are estimated at -8.4 and -3.7, respectively.

The microscopic proton dissociation constants of exocyclic ammonium groups of pyrimidines and purines and the nitrogen atoms of cationic N-protonated amides are relevant to the interpretation of tritium exchange experiments designed to probe the secondary and tertiary structure of nucleic acids[3] and proteins,[4] respectively. However, determinations of the microscopic proton dissociation constants of exocyclic ammonium groups of 2- or 4-aminopyridines (e.g., K_c in eq 1, where the designations of macroconstants and microconstants are G and K, respectively)[5] and similar groups of pyrimidines and purines cannot be made by direct titration since the endocyclic nitrogen atoms are more basic[6-8] and the site with the highest proton affinity is dominant in the resulting macroconstant[5] (i.e., $1/G_2 \sim 1/K_d$ in eq 1).

Titration farther into regions of lower pH or H_0 provides a macroconstant for the proton dissociation of the dicationic species, G_1, which reflects, in the main, the basicity of the exocyclic amino group altered by protonation elsewhere on the molecule (i.e., K_b). The situation becomes more complex than that shown in eq 1 for heteroaromatic compounds containing more than a single endocyclic nitrogen atom, since the number of microscopic species is 2^n, where n is the number of ionizable groups.[5] Pyrimidines and purines are examples of such compounds.



$$G_1 = K_a + K_b$$

$$\frac{1}{G_2} = \frac{1}{K_c} + \frac{1}{K_d} \qquad (1)$$

Similar considerations are applicable to the direct determination of the microscopic ionization constant of the N-