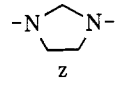
Tetrahydrofolic Acid Model Studies. II. Equilibrium and Kinetic Studies of the Reaction of Tetrahydroquinoline and Tetrahydroquinoxaline Derivatives with Formaldehyde. Carbinolamine, Imidazolidine, and Hexahydropyrimidine Formation[1,2]

George P. Tuszynski,[3] M. Frederick, and Roland G. Kallen*

Contribution from the Department of Biochemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174. *Received March 3, 1975*

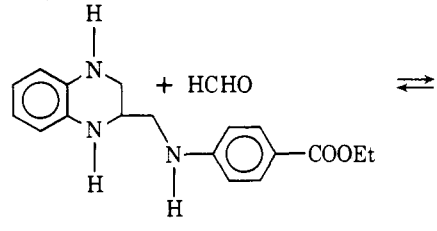
Abstract: The reactions of the free base forms of ethyl *p*-[*N*-(tetrahydro-2-quinolinylmethylene)amino]benzoate (**2**) and ethyl *p*-[*N*-(tetrahydro-3-quinolinylmethylene)amino]benzoate (**3**) with formaldehyde to form the imidazolidine **5** and the hexahydropyrimidine **6**, respectively, exhibit pH–rate profiles containing two hydronium ion catalyzed limbs separated by a pH-independent plateau. These data are interpreted in terms of a carbinolamine intermediate and a change in rate-determining step with changing acidity. In alkaline solution, the rate-determining step is the general-acid-catalyzed dehydration of the carbinolamine intermediate. In acidic solution, the rate-determining step is the attack of amine upon formaldehyde, which exhibits catalysis by general acids and the solvated proton, and, as well, a pH-independent pathway. In contrast to an earlier formulation by others, the reaction of ethyl *p*-[*N*-(1,2,3,4-tetrahydro-2-quinoxalinylmethylene)amino]benzoate (**1**) with formaldehyde to form the imidazolidine **4** appears to proceed with the accumulation of a hexahydropyrimidine intermediate **4a**, the *formation* of which shows kinetic characteristics comparable to those described above for the formation of **5** and **6**. These pH–rate data for the formation of **4a** reveal the occurrence of a change in rate-determining step and provide evidence for the existence of a carbinolamine intermediate, which also is observable spectrophotometrically under appropriate experimental conditions. The further reaction of the hexahydropyrimidine intermediate to form the imidazolidine product is many-fold slower than the rate of **1** → **4a** formation at all pH values and the **4a** → **4** isomerization exhibits a pH–rate profile which also contains two hydronium ion catalyzed limbs separated by a pH-independent plateau. There is, therefore, a change in rate-determining step with changing acidity and the presence of an intermediate (iminium cation) in the isomerization of **4a** to **4**. The intermediate, **4a**, has been isolated and partially characterized. The equilibrium constants for the formation of neutral hexahydropyrimidines and imidazolidines from neutral diamines and formaldehyde fall in the range 3.7×10^2–$7.3 \times 10^3\ M^{-1}$ and 8.0×10^4–$6.3 \times 10^5\ M^{-1}$, respectively. The existence and accumulation of hexahydropyrimidines in the reactions of the tetrahydroquinoxaline derivatives with formaldehyde and *not* in the reactions of tetrahydrofolic acid (THF) with formaldehyde suggest that one role of the adjacent pyrimidine moiety is to inactivate the N_8 site in order to diminish this type of spurious reaction in THF involved reactions.

From a cursory examination of the structure of tetrahydrofolic acid (THF), the possible chemical basis for the multiplicity of functional groups in that molecule in relation to the roles of THF in one-carbon metabolism is inapparent. Nevertheless, secure in the assumption that the complex structure of THF does not merely reflect requirements for binding processes,[4] a number of studies have emerged designed to provide structure–function correlative information. In most cases, model compounds have been employed and studies have involved in one manner or another imidazolidines (z) which are "active formaldehyde" analogs.[5-8]

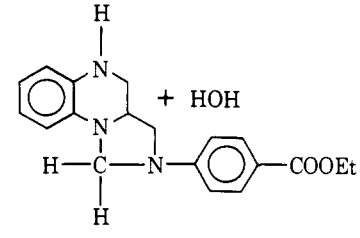


z

Extensive kinetic studies by Benkovic and his associates[6,7] of imidazolidine formation from tetrahydroquinoxaline derivatives (**1**) and formaldehyde (eq 1) indicated a change in rate-determining step with changing pH. The data were interpreted in terms of Scheme I and used to provide preliminary structure–function hypotheses regarding THF. In the formulation of the mechanism in Scheme I, the central intermediate that accumulated was assigned as a *carbinolamine* with (i) carbinolamine formation occurring in a rapid prior equilibrium step; (ii) hydronium ion catalyzed dehydration (k_2') of the carbinolamine to form an iminium cation intermediate as the rate-determining step in alkali; and (iii) hydroxide and solvent-catalyzed ring closure (k_3' and k_3) of the iminium cation intermediate as the rate-determining step in the more acid pH region. On the basis of our



1



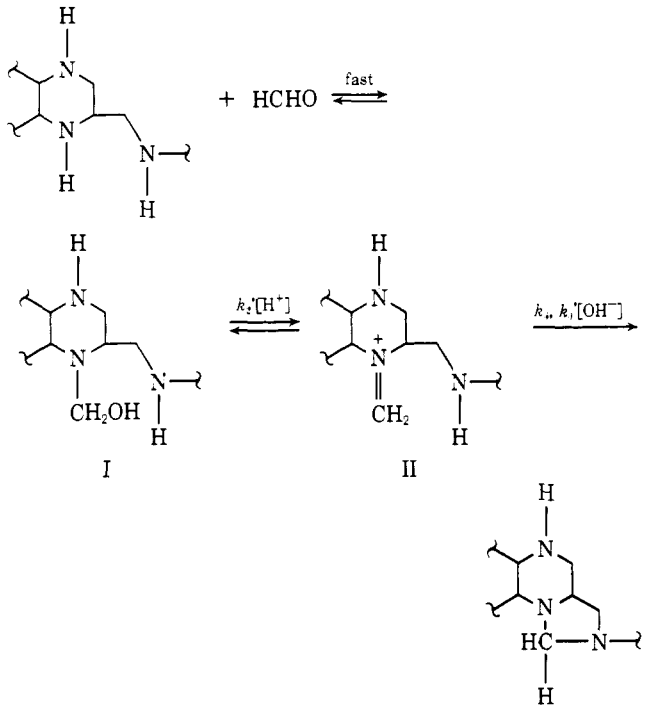
4

(1)

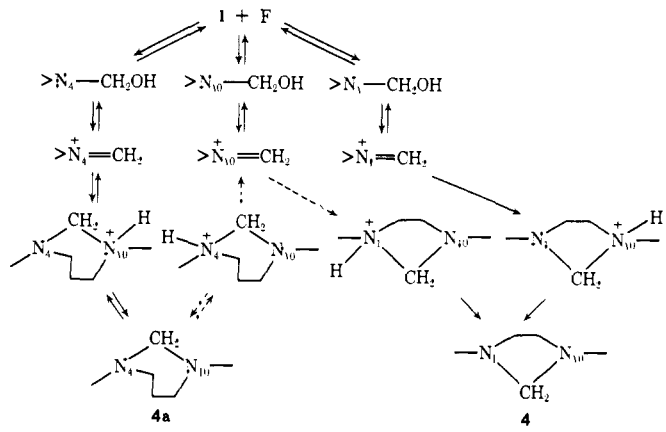
previous studies of formaldehyde reactions with amines (including THF itself)[8,9] a number of serious discrepancies became apparent regarding the interpretation of the experimental data for the tetrahydroquinoxaline derivatives in terms of Scheme I.

(i) The identity of the intermediate formed from amine and formaldehyde which was revealed by the rectangular hyperbolic progression from first-order to zero-order dependence of the rate of product formation upon formaldehyde concentration (characterized by an equilibrium constant for adduct formation of $5 \times 10^3\ M^{-1}$) appears to be incompati-

Scheme I



Scheme II



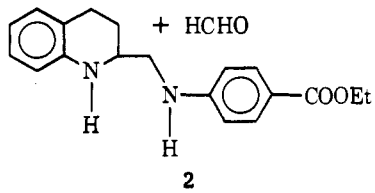
ble with simple carbinolamine formation on the basis of the magnitude of the equilibrium constant[8,9,11] and the "lag"[10b] in absorbance measurements attributed to the formation of that intermediate in a one-step reaction.

(ii) Cyclization has *not* been demonstrated to be rate determining in the formation of any cyclic systems by a direct pathway *from bifunctional amine derivatives*[10a] *and carbonyl compounds* (e.g., in the formation of thiazolidines or imidazolidines including N_5,N_{10}-methylene tetrahydrofolic acid).[8,9,12]

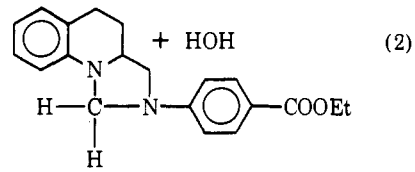
(iii) The rates in the alkaline region attributed to dehydration of the hydroxymethylamine intermediate (I in Scheme I) show a several-fold greater dependence upon the effects of para substituents of the aminobenzoyl moiety[6,7] in **1** than expected for the transmission of inductive effects through the insulating ethylene groups.

(iv) The rate constants for product formation, when allowance is made for basicity and possible reactivity differences at the nucleophilic center, are more than an order of magnitude less than expected in the alkaline region for the dehydration of the carbinolamine, I, to its Schiff base, II (Scheme I).[8-10,12]

In order to clarify these discrepancies of interpretation and to contribute further to an understanding of the relationship of the structure to the function of THF, we have extended our earlier studies of formaldehyde reactions to a consideration of this particular system involving the same tetrahydroquinoxaline derivative (eq. 1, **1**). We now present uv, ^{1}H NMR and ir spectroscopic, thermodynamic, and kinetic evidence that the intermediate observed kinetically by Benkovic et al.[6,7] *is not a carbinolamine but rather represents the intervention and accumulation of hexahydropyrimidine* (**4a**), a possibility considered and rejected previously by those workers[6,7] in the kinetic pathway to the formation of the more stable imidazolidine product (Scheme II). This conclusion is based in large part upon kinetic and equilibrium studies of compounds **2** and **3** with formaldehyde to form compounds **5** and **6** (eq 2 and 3), respectively, which serve as models for the formation of the cyclic compounds **4** and **4a**, which involve the N_1 and N_4 sites of **1**, respectively. Further, with the isolation and demonstration of the kinetic competency of the hexahydropyrimidine intermediate **4a**, the mechanism of imidazolidine formation from **1** *must* be revised and the previous conclusions[6,7] relevant to THF

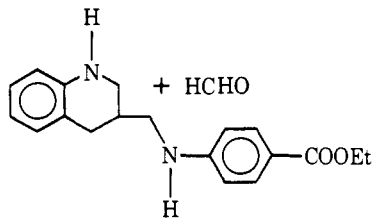


2

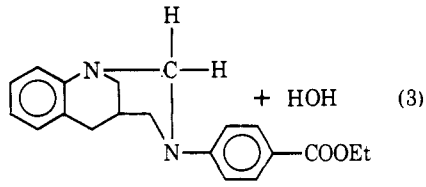


(2)

5



3



(3)

6

based upon the mechanism of imidazolidine formation in the model system require substantial modification.

Experimental Section

Materials. Reagent grade 1,4-dioxane (Fisher) was purified according to the procedure of Fieser,[13] stored with metallic sodium, and distilled daily. Deionized water of greater than 5×10^5 ohm cm specific resistance was used throughout. Kinetic and equilibrium studies were conducted at 0.1 *M* maintained with KCl, unless noted otherwise. Melting points are recorded uncorrected and the ^{1}H NMR standard was tetramethylsilane.

Synthesis of Diamines and Imidazolidines. In Table I, the characterization of the synthetic products (Figure 1, A and B) is summarized. Products were subjected to TLC on silica gel G and examined under ultraviolet light after development in the following solvents (ratios expressed as v/v): A, 10:1 cyclohexane–ethanol; B,

Table I. Characteristics of Tetrahydroquinoline and Tetrahydroquinoxaline Derivatives and Their Formaldehyde Adducts

Compd	Synthesis ref	% yield	Mp, °C[h]	C[a]	H[a]	N[a]	Ir	^{1}H NMR	R_f, TLC[e] A	B	C	D	E	F
1	6	80	131–134	*69.42*	*6.89*	*13.58*	*b*	*d*			0.085	0.65	0.60	0.71
			(131–134)[f]	69.43	6.79	13.49								
1a	6	95	138–139				*c*				0.20			
			(138–139)[f]											
2	7	10	93–94	*73.39*	*7.34*	*8.87*	*b*	*d*	0.25					
			(93–94)[f]	73.50	7.10	9.05								
2a	7	80	138–139				*c*				0.32			
			(138–139)[f]											
3	*e*	5	122–123	*73.19*	*7.06*	*8.92*	*b*	*d*		0.62				
				73.50	7.10	9.05								
3a	*e*	>98	83–85				*c*				0.11			
4	6	>98	168–169	*70.58*	*6.47*	*12.51*		*d*			0.20	0.65	0.87	0.71
			(168–169)[f]	70.56	6.54	12.98								
4a	*e*	>98	100 dec	*70.50*	*6.85*	*12.68*	*b*	*d*			0.24	0.62	0.58	0.68
				70.56	6.54	12.98								
5	7	~70	149–150	*74.23*	*6.88*	*8.51*		*d*	0.71					
			(148–149)[f]	74.60	6.80	8.70								
4[g]	*e*										1.0		0.42	0.72
4a[g]	*e*										1.0		0.60	

[a] Found, in italics (Huffman Laboratories). [b] N–H stretching at 3300–3400 cm^{-1} and bending at 1510 cm^{-1}. [c] C=N stretching at 1630 cm^{-1}. [d] See Figure 1. [e] See Experimental Section. [f] Literature values. [g] Borohydride reduction products. [h] Values uncorrected.

5:2 cyclohexane–ethanol; C, chloroform; D, ethanol; E, tetrahydrofuran; F, 1,4-dioxane. Additional details regarding the syntheses and modifications of published procedures are given below.

Ethyl *p*-[*N*-(2-Quinoxaldylidene)amino]benzoate (1a). The preparation of quinoxaline-2-carboxaldehyde was by the method of Borche and Doeller:[14] mp 107–108° (lit.[14] 107–108°). The preparation of the Schiff base was by the method of Benkovic et al.[6]

Ethyl *p*-[*N*-(1,2,3,4-Tetrahydro-2-quinoxalinylmethylene)amino]benzoate (1). The preparation of this amine was by the method of Benkovic et al.[6]

Ethyl *p*-[*N*-(2-Quinaldylidene)amino]benzoate (2a). The preparation of quinoline-2-aldehyde was by the method of Kaplan:[15] mp 68–70° (lit.[15] 68–70°). The preparation of the Schiff base was by the method of Benkovic et al.[6] with the following modifications. (1) Following 5 hr of reflux, the reaction mixture was permitted to stand overnight and refluxed for an additional hour the following day. This aided the removal of all of the water and improved the yield of Schiff base. (2) The Schiff base–benzene solution was evaporated to dryness in vacuo to give a yellow oil which was triturated with cold ethanol to induce crystallization. (3) The Schiff base crystals were washed with a small amount of cold ethanol and then recrystallized from toluene–petroleum ether.

Ethyl *p*-[*N*-(Tetrahydro-2-quinolinylmethylene)amino]benzoate (2). The Schiff base (1.5 g, 0.005 mol) was dissolved in 100 ml of absolute ethanol and catalytically reduced with hydrogen at atmospheric pressure for 48 hr with 0.3 g of 10% Pd on carbon. The hydrogenation apparatus was built according to the description given by Vogel and the precautions prescribed were employed in its operation.[16] After 24 hr the uptake of H_2 had slowed (probably due to catalyst poisoning), and following filtration, a new aliquot of catalyst was added and the hydrogenation allowed to continue for an additional 24 hr. The theoretical amount of H_2 was taken up, and the reduced Schiff base was evaporated to dryness in vacuo giving a yellow oily residue. Examination of the TLC of the oil in solvent A under ultraviolet light revealed a single major component of R_f value 0.25. The oil was triturated with a small amount of petroleum ether and placed in the freezer until crystallization occurred (1 week). The yellow, sticky crystals were washed with cold ether yielding 0.15 g of white crystals. The uncrystallized oil was used for the synthesis of the imidazolidine adduct.

Ethyl *p*-[*N*-(3-Quinaldylidene)amino]benzoate (3a). Quinoline-3-aldehyde was prepared by the reaction of 3-quinolyllithium and *N,N*-dimethylformamide. To a flame-dried 250-ml flask equipped with a magnetic stirring bar and serum cap was added ca. 100 ml of dry ether and 10.4 g (50 mmol) of freshly distilled 3-bromoquinoline. The solution was cooled to −60° using a dry ice–isopropyl alcohol bath, and 22 ml (53 mmol, 2.4 *M*) of *n*-butyllithium in hexane was added slowly over a period of 15 min. The dark red mixture of 3-quinolyllithium was allowed to stir at −60° for 30 min and then 4.1 ml (53 mmol) of freshly distilled, dry *N,N*-dimethylformamide in 10 ml of dry ether was added. The resulting red mixture was allowed to stir under nitrogen for 3 hr at −60° and was then slowly warmed to −10° and hydrolyzed with 10 ml of aqueous methanol. After the solution was brought to room temperature, another 50 ml of water was added and the product salted out with sodium chloride. The ether layer was washed with water and saturated sodium chloride solution, dried with $MgSO_4$, and reduced in volume to give a yellow oil. Hot water was added, filtered away from the residual oil, and cooled. The resulting colorless needles were filtered to give 1.2 g of quinoline-3-aldehyde: mp 67–68° (lit.[17] mp 70°). The preparation of the Schiff base was by the method of Benkovic et al.[7] with the exception of the modifications listed above for the 2′ Schiff base (**2a**). Evaporation of the benzene resulted in the precipitation of yellow crystals.

Ethyl *p*-[*N*-(3-Tetrahydroquinolinylmethylene)amino]benzoate (3). The Schiff base (2.0 g, 0.0065 mol) was reduced according to the procedure given for the 2′ Schiff base (**2a**). Trituration of the oil with petroleum ether at −5° resulted in crystallization of 0.1 g of white crystals which was washed with a small amount of cold ether. A single uv-active spot (R_f 0.62) was observed upon TLC of the material in solvent B.

Imidazolidine Adducts. The imidazolidine adducts **4** and **5** were prepared from **1** and **2**, respectively, and formaldehyde by the method of Benkovic et al.[6,7] We were unsuccessful in isolating **6**, the formaldehyde adduct of **3**, by the aforementioned method, presumably as a result of the relatively unfavorable equilibrium constant for its formation. The limited amount of **3** available precluded further efforts to isolate **6**. However, the kinetics of **6** formation and the uv spectral characteristics are consistent with the assigned structure (see Discussion).

The intermediate **4a**, which is formed in the conversion of **1** to **4**, was isolated as follows. To a 1000-ml round-bottom flask 31.4 mg of **1**, 50 ml of purified dioxane, and 48.8 ml of distilled water were added and the mixture was stirred until dissolution was effected. The reaction was initiated by addition of 1.2 ml of a formaldehyde solution (0.084 *M*) (final concentration 1×10^{-3} *M*). The formation of the intermediate **4a**, which required about 5 min, was monitored by repeatedly scanning the uv spectrum of an aliquot of the reaction mixture in a 0.05-cm path-length quartz cell. The reaction mixture was rapidly frozen on the surface of a rotating flask in a dry ice–isopropyl alcohol bath and lyophilized.

Tetrahydroquinoxaline and Tetrahydroquinoline. The preparation of tetrahydroquinoxaline was by the method of Bohlmann:[18] mp 96–97° (lit.[18] 96–97°). Reagent grade tetrahydroquinoline (Aldrich) was used without further purification.

Ethyl *p*-Aminobenzoate (EPAB). Reagent grade EPAB (Baker) was used without further purification.

Kinetic Measurements. The rates of the reactions of aldehydes

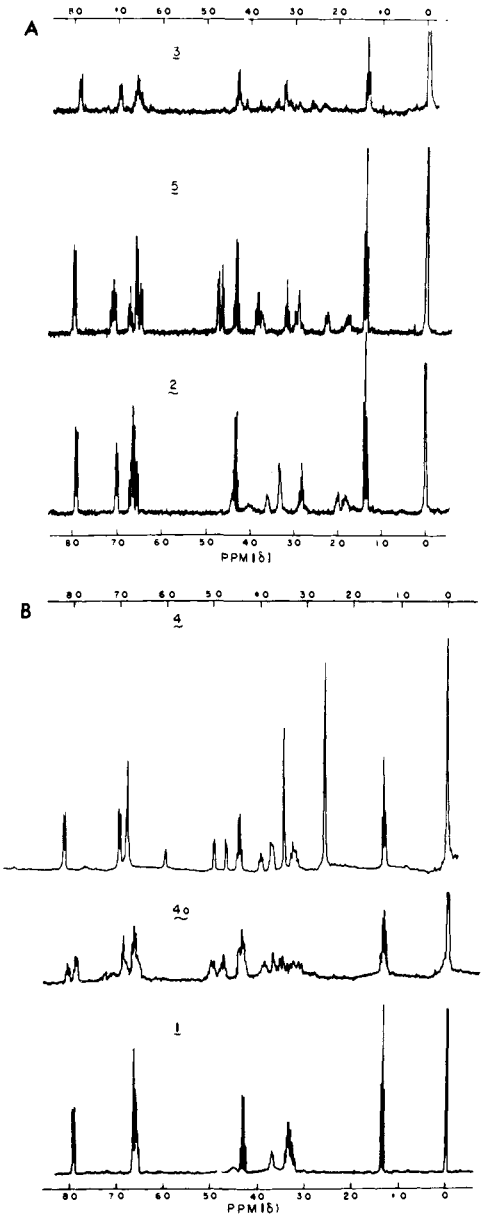


Figure 1. (A) The ^{1}H NMR spectra of compounds **2**, 3, and **5** in $CDCl_3$, 20°. The spectra of compounds **2** and **5** represent ten Fourier transforms and that of 3 is a single scan. (B) The ^{1}H NMR spectra of compounds 1 and **4a** in $CDCl_3$ and **4** in Me_2SO-d_6, 20°. The spectra of compounds 1, **4a**, and **4** represent ten Fourier transforms.

with aromatic amines were followed by measurements of absorbance with Coleman-Hitachi 124 and Gilford 2000 recording spectrophotometers with thermostated cell holders maintained at 25 ± 0.1°. The aldehyde concentration was in tenfold or greater excess over the amine concentration of about $3\text{–}7 \times 10^{-5}$ *M* in order to yield pseudo-first-order kinetics. Non-buffer-catalyzed rates were obtained by extrapolation to zero buffer concentration. Reactions were initiated by addition of 10–20 μl of amine dissolved in 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.[9,12] Analysis of pH–rate profiles was accomplished with a computer by least-squares methods.[19a]

Proton Dissociation and Equilibrium Constants. The proton dissociation constants for amine mono- and dications were determined from spectrophotometric titrations and analyzed with a computer by least-squares methods.[8,19a] Compound **3**, tetrahydroquinoline, and tetrahydroquinoxaline were titrated at 250, 300, and 315 nm, respectively. The equilibrium constants for carbinolamine and imidazolidine formation were determined from formaldehyde concentration dependence of absorbance measurements as described elsewhere.[12,19b] Kinetic titrations were analyzed with a computer by least-squares methods.[8,19a] The equilibrium constants for formation of **4** and **5** were calculated from the rate constants obtained independently for the formation and hydrolysis of the imidazolidines. The imidazolidine hydrolysis rates, monitored at 310 nm, were measured at imidazolidine concentrations of $3.0 \times 10^{-5}\text{–}5.0 \times 10^{-5}$ *M* at pH 5 in the presence of 0.02, 0.05, and 0.10 *M* mercaptoethanol. The formation of the hemithioacetal from formaldehyde and the thiol[12] as the carbonyl compound is released during the hydrolysis was utilized to pull the hydrolysis reactions to completion; the hydrolysis rates showed no dependence upon the thiol concentration indicating the adequacy of the formaldehyde trap. The final spectra were those of **1** and **2** and indicated the absence of linear thiol adducts of the type $>NCH_2SR$, which are known to be unstable under these conditions for these systems.[20a]

Evidence against Amine–Formaldehyde Polymerization Reactions. The following parameters, measured under pseudo-first-order conditions in respect to formaldehyde, showed *no* dependence upon amine concentration in the range $1 \times 10^{-5}\text{–}5 \times 10^{-5}$ *M* (higher amine concentrations were precluded by large extinction coefficients and solubility considerations, Table III) in the pH range studied indicating that the present kinetic and equilibrium studies are not perturbed by amine–formaldehyde polymer formation: (1) rates of imidazolidine formation from amine and formaldehyde; (2) equilibrium constants for imidazolidine formation from amine and formaldehyde; (3) equilibrium constant for formation of H**4** from **4** and formaldehyde, where H**4** is the N_4-hydroxymethyl adduct of **4**; (4) rates of **4a** formation from **1** and formaldehyde; (5) equilibrium constant for formation of H**4a** from **4a** and formaldehyde, where H**4a** is the N_1-hydroxymethyl adduct of **4a**.

Instruments. The following instruments were used for recording spectra: Varian HR 220 equipped with a Fourier transform system for ^{1}H NMR, Hitachi RMH-2 for mass spectra, Perkin-Elmer 521 grating infrared spectrophotometer for infrared, and a Pye Unicam SP 1800 and Cary 14 for ultraviolet.

Results

The designation of structures of **1**, **2**, **3**, **4a**, **4**, and **5** is supported by the following evidence. (i) Elemental analyses (Table I). (ii) The ir spectra of **1**, **2**, **3**, and **4a** in $CHCl_3$.[20b] (iii) The ^{1}H NMR spectra of **1**, **2**, and **3** show aromatic resonances (δ 7.5–8.0) and ethyl group resonances (J = 4.0 Hz, δ 1.3 and 4.3) in the ratio of 8:5 with respect to the ethyl group protons and methylene and amine hydrogen resonances (δ 2.2–4.6) in the ratio of 9:5 (for **2** and **3**) and 8:5 (for **1**) with respect to the ethyl group protons (Figure 1, A and B).

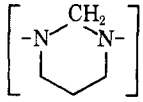
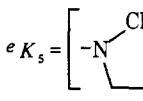
The ^{1}H NMR spectra of formaldehyde adducts, **4** and **5**, display the same characteristic resonances as those of the parent amines with the addition of a new band which appears as an AB quartet at about δ 4.7. This resonance is attributed to the methylene protons of formaldehyde in the imidazolidine ring system (ratio of 2:5 with respect to the ethyl group protons for **5** and approximately 1:1 with respect to the methylene protons of the ethyl group for **4**).

The ^{1}H NMR spectrum of **4a** is complicated by the occurrence of duplicate resonances associated with each of the resonance groups observed for the other amines and imidazolidines (note especially compound **1**). These duplicate resonances are most noticeable in the aromatic region at about δ 8.0 and 6.8 (total of eight protons) and in the region of the methylene proton absorbances where the two poorly resolved AB quartets of the formaldehyde methylene protons are observed. An increased complexity of the ethyl group resonance (total of five protons at δ 1.3 and 4.3) is also seen. Slowly at room temperature or rapidly at elevated temperatures (in the aprotic solvent, $DCCl_3$) the spectrum resolves itself to single aromatic resonances (δ 8.3 and 6.8) and methylene resonances (δ 4.7) with the disappearance of the complexity of the ethyl group resonances and, subsequently, a precipitate (presumably of the more stable isomer) ap-

Table II. Equilibrium Constants for the Reactions of Compounds 1–3, Tetrahydroquinoline, and Tetrahydroquinoxaline with Formaldehyde and Protons[a]

Compd	pK_a'	K_1, M^{-1} [b]	K_6, M^{-1} [d]	K_5, M^{-1} [e]
Tetrahydroquinoline	4.76	89.0 (S)[j]		
Tetrahydroquinoxaline	4.74, ~0.26	60.0 (S)[j]		
1	3.78,[l] −0.33	44.8 (S),[f] 41.6 (K)[c]	7.3 0.3×10^3[k]	6.3×10^5[g]
2	2.58,[l] −1.1	2.1 (K)[c]		8.0×10^4[g]
3	3.49	55.1 (K)[c]	3.7 0.6×10^2[k]	
4a	(2.3)[m]	30.2 (S),[h] 51.0 (K)[c]		
4		80.3 (S)[i]		

[a] 50:50 (v/v) dioxane–HOH, $\mu = 0.1$ *M*, 25°; S = spectrophotometric, K = kinetic. The values of the equilibrium constants for tetrahydroquinoxaline and its derivatives have *not* been corrected for statistical factors which would be necessary for a direct comparison with tetrahydroquinoline and its derivatives. For constants determined kinetically (K) see Figure 4, A, and Results section for explanation. [b] $K_1 = [R_2NCH_2OH]/[R_2NH][F]$. [c] Determined from dependence of observed pseudo-first-order rate constants for product formation upon formaldehyde concentration in the alkaline region as in Figure 4, A, and eq 4.

[d] $K_6 =$  / [amine][F]. [e] $K_5 =$  / [amine][F].

[f] Determined at pH 10.1 where the further reaction of hydroxymethylamine adduct to cyclic compounds is very slow ($t_{1/2}$ = 100 min); whether one or two equivalent and independent sites for adduct formation with formaldehyde are involved was not determined; however, it seems likely that the value of K_1 reflects carbinolamine formation at the N_4 site based upon the steric effects observed for carbinolamine formation from formaldehyde and compounds 2 and 3. [g] Determined kinetically from forward and reverse rate constants measured independently. [h] S at 255 nm, pH 8.49, and K at 310 nm, pH 6.71. [i] At 260 nm, pH 8.16. [j] At 250 nm, pH 7.00. [k] Average of kinetic and spectrophotometric determinations. [l] Reference 7, for compound 1 referred to as k_{a_1}' in text. [m] Estimated in footnote 32c and referred to as k_{a_2}' in text.

pears. The spectrophotometric monitoring of the formation of **4a**, the chromatographic homogeneity and distinguishability of **1** from **4a** on TLC (Table I), and the ratio of formaldehydic methylene to ethyl methylene protons (1:1) in the ^1H NMR spectrum of **4a** are evidence against the possibility that the double sets of resonances are due to a mixture of **1** and **4a**. The chromatographic homogeneity and specific and distinguishing derivatizations of **4a** and **4** by borohydride (Table I) indicate that the double sets of ^1H NMR resonances in the spectrum of **4a** cannot be attributed to a mixture of **4** and **4a**. These observations are consistent with the existence of two conformeric hexahydropyrimidines, one of which is the more stable (see Discussion).

(iv) Mass spectra of **4a** and **4** reveal different fragmentation patterns having the same parent ion peaks at *m/e* 323 (theoretical *m/e* 323) for the two compounds.

Imidazolidine (5) and Hexahydropyrimidine (6) Formation from Formaldehyde and 2 and 3. Equilibria. The proton dissociation constants for compounds **2** and **3** were obtained by spectrophotometric titration and the equilibrium constants for the formation of the cyclic derivatives **5** and **6** from formaldehyde and **2** and **3**, respectively, were obtained from the formaldehyde concentration dependence of the absorbance changes at equilibrium or independent measurements of forward and reverse rate constants (Table II). These data indicate that the formation of the cyclic five-membered imidazolidine is more than 200-fold more favorable than the formation of the six-membered hexahydropyrimidine from their respective reactants **2** and **3** and formaldehyde. The equilibrium constants for *N*-hydroxymethylamine formation from the *least* sterically hindered secondary amines, tetrahydroquinoline and tetrahydroquinoxaline, and formaldehyde were determined spectrophotometrically (Table II) and are fourfold smaller than the *least* favorable equilibrium constant for the formation of a cyclic product, K_6 (Table II).

Kinetics. The addition of formaldehyde to compounds **2** and **3** results in spectral changes (Table III and Figure 2, A and B) due to the formation, at equilibrium, of the cyclic methylene derivatives **5** and **6**, respectively. The kinetics of the approach to equilibrium are first order in chromophore concentration and are further characterized by (i) pH–rate profiles for the non-buffer-catalyzed rate of product formation with respect to the free base amine which reveal two hydronium ion catalyzed limbs separated by a pH independent region (Figure 3) indicative of a change in rate-determining step with changing pH; and (*ii*) rates of product formation which exhibit a first-order dependence upon formaldehyde concentration at pH values below 7.0 and which exhibit a dependence upon formaldehyde concentration which

Table III. Spectral Characteristics for Various Formaldehyde Adducts of Aromatic Amines Including 1–3, Tetrahydroquinoline, and Tetrahydroquinoxaline

Compd	λ_{max}, nm	Molar absorptivity $\times 10^{-3}$ M^{-1} cm^{-1}
1	309	24.0
4a	250 (s),[a] 312	6.00, 23.3
H4a	255 (s), 310	7.15, 25.3
H1	260 (s), 310	7.50, 24.2
4	312	26.9
H4	260 (s), 312	6.94, 30.3
2	253, 308	5.60, 18.4
Carbinolamine of 2	255, 308	5.72, 18.4
5	260 (s), 310	6.00, 22.0
3	257, 308	3.46, 16.0
Carbinolamine of 3	257, 310	4.96, 16.2
6	310	15.1
Tetrahydroquinoline	249, 298	4.95, 1.23
Carbinolamine of tetrahydroquinoline	255, 298	7.23, 1.52
Tetrahydroquinoxaline	253, 310	3.68, 2.56
Carbinolamine of tetrahydroquinoxaline	263, 310	5.67, 3.56
Ethyl *p*-aminobenzoate	293	18.1
Carbinolamine of ethyl *p*-aminobenzoate	296	22.4

[a] s = shoulder.

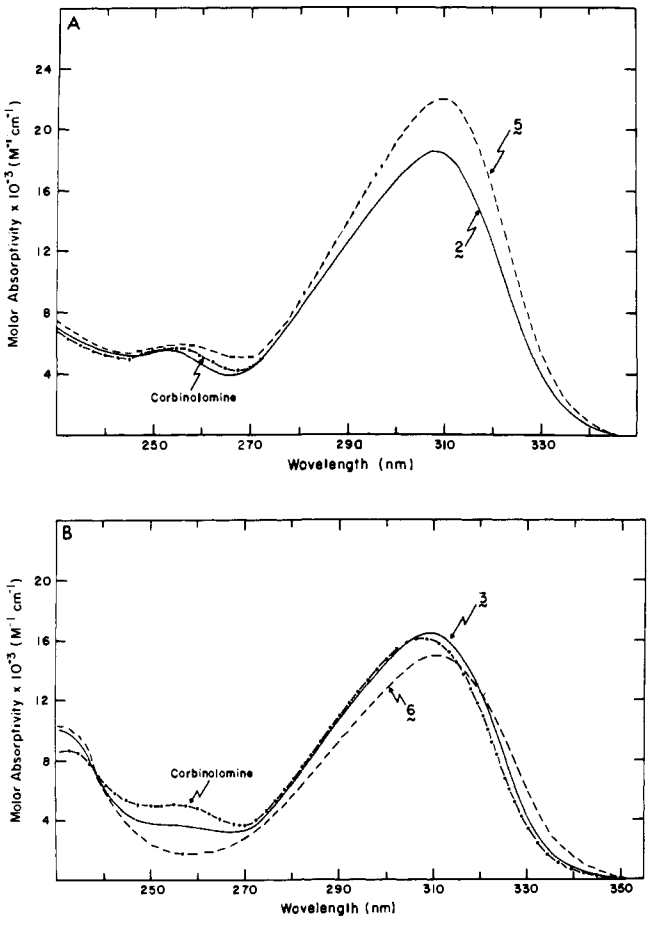


Figure 2. (A) Spectra of **2** in the absence (—) and presence of 0.50 *M* formaldehyde in 50:50 (v/v) dioxane–water, 23–25°, ionic strength 0.10 *M*, pH 9.46 maintained with 1×10^{-3} *M* DABCO buffer. Carbinolamine of **2** (- · · -). Spectrum of **5** (- - - -). (B) Spectra of 3 in the absence (—) and presence of 0.50 *M* formaldehyde in 50:50 (v/v) dioxane–water, 23–25°, ionic strength 0.10 *M*, pH 9.46 maintained with 1×10^{-3} *M* DABCO buffer. Carbinolamine of 3 (- · · -). Spectrum of 6 (- - - -).

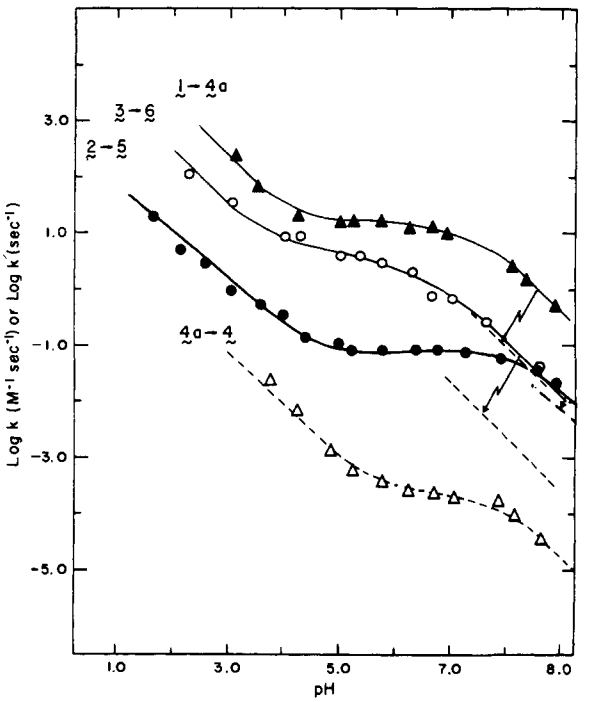


Figure 3. Dependence on pH of the second-order rate constants corrected to the fraction present as the free base amine and measured at 0.01–0.05 *M* formaldehyde for **4a** formation (▲—▲) from **1** and formaldehyde, **6** formation (○—○) from 3 and formaldehyde, **5** formation (●—●) from **2** and formaldehyde, and the dependence upon pH of the first-order rate constants for **4** formation (△ · - - △) from **4a** at 10^{-3} *M* formaldehyde. The respective first-order dehydration rate constants, $k_2'[H^+]$, are shown as the arrow directed dashed lines. Solid lines and dashed lines for the upper three curves are calculated based upon the values contained in Table IV obtained by a least-squares method and eq 5. The dashed line (△ - - - △) for **4** formation from **4a** is calculated from eq 8b and the constants contained in Table V which were obtained from the data by a least-squares analysis.

progresses from first order to zero order as the formaldehyde concentration is increased at pH values greater than 7.0 (not shown). The latter observation is consistent with the rapid accumulation in the alkaline pH region of the respective carbinolamines with formation constants determined kinetically (from eq 4 and data *similar* to that shown in Figure 4, A, for **4a** formation from **1** and formaldehyde)

$$k_{obsd} = k_{obsd\,(max)}/(1 + 1/K_1[F]) \quad (4)$$

of 2 and 55 M^{-1} for **2** and **3**, respectively, where K_1 is the equilibrium constant for carbinolamine formation from compounds **2** or **3** and formaldehyde, and $k_{obsd\,(max)}$ is the first-order rate constant for the rate-determining step in the further conversion of the intermediate to product, i.e., the dehydration of the carbinolamine.

Unaccountably, previous observations of the reaction of **2** with formaldehyde to form **5** failed to detect both the break in the pH–rate profile at about pH 7.0 and the occurrence of the rectangular hyperbolic dependence of the observed rate constants for **5** formation upon the formaldehyde concentration in the alkaline region (Figure 4, A).[7]

The pH–rate profiles for **5** and **6** formation were analyzed in terms of eq 5 derived from the mechanism of eq 6 by the application of the steady-state assumption to the carbinolamine intermediate, where α_{R_2NH} is the fraction free base form of the amine, and the rate constants are contained in Table IV.

$$k_{obsd}/([F]\alpha_{R_2NH}) = (k_1 + k_1'a_{H^+})k_2'a_{H^+}/(k_1/K_1 + k_1'a_{H^+}/K_1 + k_2'a_{H^+}) \quad (5)$$

$$>NH + F \underset{k_{-1},\ k_{-1}'[H^+]}{\overset{k_1,\ k_1'[H^+]}{\rightleftharpoons}} >NCH_2OH \xrightarrow{k_2'[H^+]} \text{product} \quad (6)$$

This mechanism has previously been applied to many reactions[21] involving Schiff base formation from weakly basic amines including the reactions of substituted *N,N'*-diphenylethylenediamines[8] and of tetrahydrofolic acid[9] with formaldehyde.

The spectra of the carbinolamines of **2** and **3** (Figure 2, A and B) reveal the most significant absorbance changes in the wavelength region of 255–265 nm and are therefore assigned as the N_1-hydroxymethylamine derivatives; carbinolamine formation at the N_{10} site would be expected to be associated with absorbance changes in the wavelength region of 310 nm as a result of the major contribution to the absorbance in this region by the ethyl *p*-aminobenzoate moiety.

The structural assignment of the product of the reaction of **3** with formaldehyde as **6** is based upon the similarity of uv spectral changes (Figure 2, A and B) and equilibrium (Table II) and kinetic constants (Table IV) for the formation of both **5** and **6** including the above cited change in rate-determining step with pH. The latter observations are *not* consistent with the formulation of the reaction of **3** with formaldehyde as simple carbinolamine formation.

Formation of 4a and 4 from Formaldehyde and 1. Equilibria. The equilibrium constants were determined spectrophotometrically for the formation of **4a** and **4** from **1** and form-

Table IV. Rate and Equilibrium Constants for the Reactions of Formaldehyde with Tetrahydroquinoline- and and Tetrahydroquinoxalinediamines[a]

		2 → 5	3 → 6	1 → 4a	DPED → DPI[c]
k_1'	$v = k_1'[>NH][F]a_{H^+}, M^{-2}\ sec^{-1}$	8.7×10^2	1.3×10^4	1.0×10^5	1.3×10^5
k_{-1}'	$v = k_{-1}'[C]a_{H^+}, M^{-1}\ sec^{-1}$	4.2×10^2	2.4×10^2	2.4×10^3	3.3×10^4
k_{1u}'[b]	$v = k_{1u}'[>NH][>C=O]a_{H^+}, M^{-2}\ sec^{-1}$	9.9×10^5	1.5×10^7	1.1×10^8	1.4×10^8
k_1	$v = k_1[>NH][F], M^{-1}\ sec^{-1}$	9.3×10^{-2}	3.9	1.6×10^1	2.9
k_{1u}[b]	$v = k_{1u}[>NH][>C=O], M^{-1}\ sec^{-1}$	1.0×10^2	4.4×10^3	1.8×10^4	2.2×10^3
k_{-1}	$v = k_{-1}[C], sec^{-1}$	4.5×10^{-2}	7.1×10^{-2}	3.8×10^{-1}	7.3×10^{-1}
$K_1'k_2'$	$v = K_1'k_2'[>NH][F]a_{H^+}, M^{-2}\ sec^{-1}$	2.0×10^7	6.9×10^6	2.4×10^8	4.4×10^7
k_2'	$v = k_2'[C]a_{H^+}, M^{-1}\ sec^{-1}$	9.5×10^5	1.2×10^5	5.8×10^6	1.1×10^7
K_1	$K_1 = [C]/[>NH][F], M^{-1}$	2.06	55.1	41.6	4.0
	$K_1(1 + K_{hyd})$[b] $= [C]/[>NH][>C=O], M^{-1}$	2.3×10^3	6.3×10^4	4.7×10^4	4.4×10^3
	$K_{hyd} = [>C(OH)_2]/[>C=O], M^{-1}$	1.14×10^3	1.14×10^3	1.14×10^3	1.14×10^3

[a] >C=O, unhydrated formaldehyde; F, formaldehyde (hydrated and unhydrated); C, carbinolamine intermediate; DPED, *N,N'*-diphenylethylenediamine; DPI, *N,N'*-diphenylimidazolidine. [b] Corrected utilizing K_{hyd} for F which has been corrected for the decreased activity of H_2O in dioxane–water [P. Valenta, *Collect. Czech. Chem. Commun.*, 25, 853 (1960)]. [c] Reference 8.

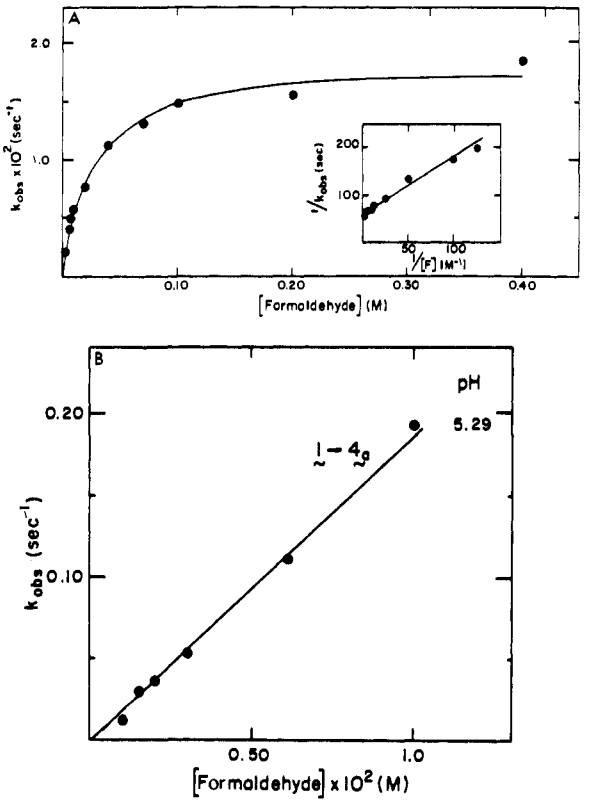


Figure 4. (A) Dependence of k_{obsd} (sec^{-1}) for formation of **4a** and the N_1-hydroxymethylamine adduct thereof upon formaldehyde in 50:50 (v/v) dioxane-water, 23–25°, ionic strength 0.10 *M*, pH 8.46 maintained with 1×10^{-3} *M* DABCO buffer. The solid line is calculated from eq 4 and the constants $K_1 = 41.6\ M^{-1}$ and $k_{obsd\ (max)} = 1.82 \times 10^{-2}\ sec^{-1}$ which were obtained from these data by a least-squares analysis. (B) Dependence of k_{obsd} (sec^{-1}) for formation of **4a** and the N_1-hydroxymethylamine adduct thereof upon formaldehyde concentration in 50:50 (v/v) dioxane-water, ionic strength 0.10 *M*, 25°, pH 5.29 maintained with 1×10^{-1} *M* acetate buffer. The solid line is calculated based upon the constant $k_1 = 1.6 \times 10^1\ M^{-1}\ sec^{-1}$ obtained by a least-squares method and the equation $k_{obsd}\ (sec^{-1}) = k_1[F]$.

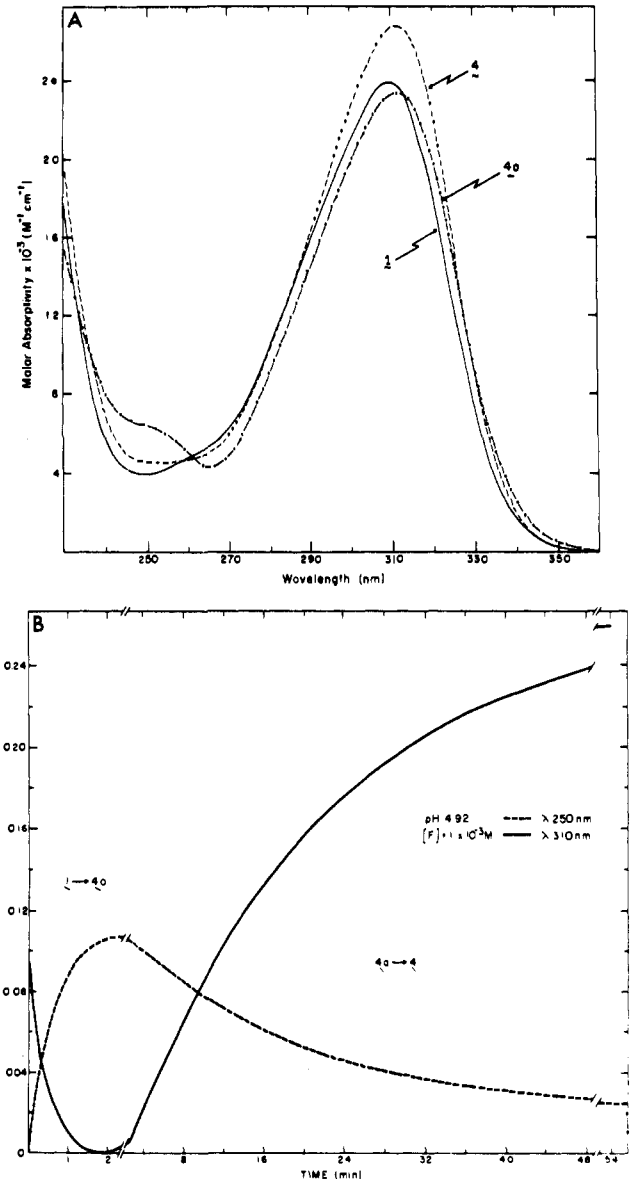


Figure 5. (A) Spectra of **1** in the absence (—) and presence of 1×10^{-3} *M* formaldehyde in 50:50 (v/v) dioxane-water, 23–25°, ionic strength 0.10 *M*, pH 8.46 maintained with 1×10^{-3} *M* DABCO buffer. **4a** (- · - · -); **4** (- - -). (B) Time dependence of the Δ absorbance change at 310 (—) and 250 nm (- - - -) in the reaction of 3.3×10^{-5} *M* **1** with 1×10^{-3} *M* formaldehyde in 50:50 (v/v) dioxane-water, 25°, ionic strength 0.10 *M*, pH 4.92 maintained with 1×10^{-3} *M* formate buffer.

aldehyde under appropriate conditions (see below) based on the spectra in Figure 5, A. The proton dissociation constants for mono- and dicationic **1** were obtained from previous studies[6,7] (Table II).

Kinetics. At formaldehyde concentrations of 10^{-4}–10^{-3} *M* and at suitable wavelengths, the time dependence of the absorbance changes for the reaction of **1** with formaldehyde appears *biphasic* over the entire pH range studied (Figure 5, B). These observations indicate the accumulation of an

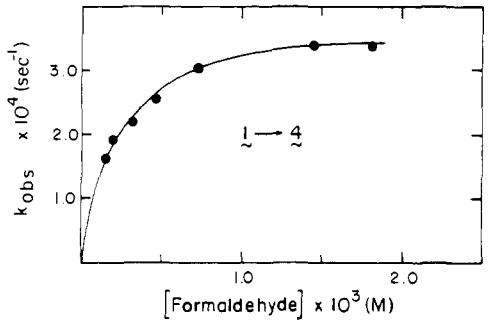


Figure 6. Dependence of k_{obsd} (sec^{-1}) for formation of **4** upon formaldehyde concentration in 50:50 (v/v) dioxane–water, ionic strength 0.10 M, pH 6.71 maintained with 1×10^{-3} M acetate buffer. The solid line is calculated from eq 7 and the constants $K_6 = 4.9 \times 10^3$ M^{-1} and $k_{obsd\,(max)} = 3.83 \times 10^{-4}$ sec^{-1} obtained from these data by a least-squares analysis.

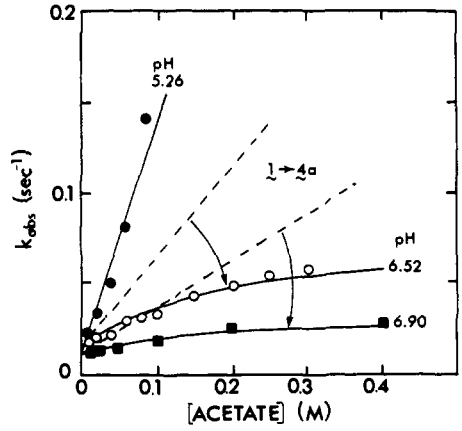


Figure 7. General-acid catalysis by acetic acid (HA) of the reaction of **1** and 1×10^{-3} M formaldehyde to form **4a** at pH 5.26 (●), 6.52 (○), and 6.90 (■) in 50:50 (v/v) dioxane–water, ionic strength 0.40 M. The solid lines are calculated from the steady-state rate equation, $k_{obsd}/\alpha_{R_2NH} = [(k_1 + k_1'[H^+] + k_1''[HA])(k_2'[H^+])[F]]/(k_{-1} + k_{-1}'[H^+] + k_{-1}''[HA] + k_2'[H^+])$, and the constants contained in Table IV, $k_1'' = 1500$ M^{-2} sec^{-1}, $k_{-1}'' = 360$ M^{-1} sec^{-1}, where k_1'' and k_{-1}'' are the general-acid-catalyzed rate constants for acetic acid for the formation of the carbinolamine intermediate and its breakdown to reactants respectively, where $[HA] = [buffer]/(1 + K_a'/[H^+])$, and K_a' is the proton dissociation for the general acid. The general-acid-catalyzed dehydration rate constant of the carbinolamine for acetic acid makes an insignificant contribution to the rate under these conditions, based on the Bronsted α value of 0.75 (ref 8). The dashed lines are calculated assuming no change in rate-determining step with buffer concentration.

intermediate in the early "a" phase absorbance changes and a slower further reaction of this intermediate to form the product **4** in the subsequent "b" phase absorbance changes. The spectrum of the intermediate generated in situ agrees precisely with synthetic **4a** and is shown in Figure 5, A. The equilibrium constant for the formation of **4a** from **1** and formaldehyde was determined by the formaldehyde concentration dependence of the absorbance changes at the completion of the "a" phase absorbance changes and is about 1×10^4 M^{-1}.

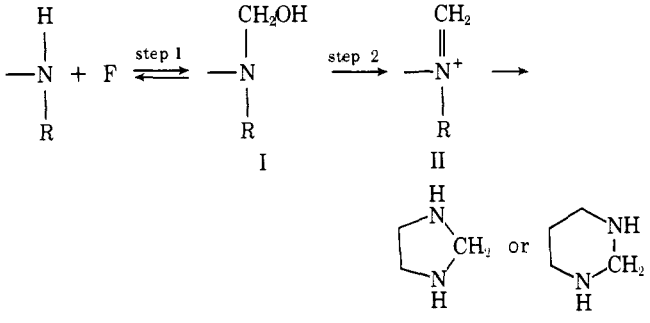
The rate constants obtained from absorbance changes which are associated with product (**4**) formation show a rectangular hyperbolic dependence upon formaldehyde concentration which is described by eq 7 (Figure 6) where K_6 is the equilibrium constant for **4a** formation from **1** and formaldehyde and $k_{obsd\,(max)}$ is the first-order rate constant for the conversion of **4a** to **4**. The "b" phase absorbance changes represent the reaction studied by Benkovic et al.[6] (at formaldehyde concentrations of 10^{-3} M) and the magnitude of the rate constants, the pH dependence of the rate constants, and the values of K_6[22] reported by those workers are in satisfactory agreement with the present data.

$$k_{obsd} = k_{obsd\,(max)}/(1 + 1/K_6[F]) \qquad (7)$$

Further study of the "a" phase absorbance changes leading to the *formation* of the intermediate **4a** has revealed the following: (i) a pH–rate profile which indicates that there is a change in rate-determining step with pH (Figure 3, labeled **1** → **4a**); (ii) a progression from a linear to a rectangular hyperbolic dependence of the rate of **4a** formation upon buffer concentration as the pH values proceed from 5.26 through 6.52 to 6.90, which is evidence for a change in rate-determining step with increasing buffer concentration (Figure 7); (iii) a linear dependence of the observed rate constant upon formaldehyde concentration in the acid region which is in accord with rate-determining attack (Figure 4, B) of **1** upon formaldehyde to form a carbinolamine intermediate; (iv) a rectangular hyperbolic dependence of the observed rate constant upon the formaldehyde concentration in the alkaline pH region where the formation of **4a** from the carbinolamine is rate determining and which is described by eq 4 (Figure 4, A) with a K_1 value of 41.6 M^{-1}: this value agrees with the value of 44.8 M^{-1} determined from absorbance measurements.

These observations upon the complicated "a" phase absorbance changes for the formation of **4a** from **1** and formaldehyde are analogous to the observations of the reactions of **2** and **3** with formaldehyde to form **5** and **6** and are consistent with the formation of an intermediate *prior* to the formation of **4a** and that intermediate is most reasonably the carbinolamine of **1**.

Scheme III



The macroscopic constant for K_1 of 41.6 and 44.8 M^{-1} probably refers to the formation of the hydroxymethylamine derivative at the N_4 site of **1** based upon the wavelength region of the absorbance changes (Figure 8) and the expectation that the N_1 and N_{10} sites, by the virtue of their rather greater steric hindrance, would have lower affinities, i.e., K_1 values of 2–3 M^{-1} [8] (Table II).

Discussion

Imidazolidine 5 and Hexahydropyrimidine 6 Formation from Formaldehyde and 2 and 3. The change in rate-determining step revealed by the pH–rate profile and the rectangular hyperbolic dependence of rate upon formaldehyde concentration in the alkaline region are evidence for the existence of at least one intermediate in each of the pathways to the formation of the products **5** and **6** from formaldehyde and **2** and **3**, respectively. Similar reactions of bifunctional amines with carbonyl compounds which display similar kinetics have been interpreted in terms of Scheme III[8,9,12,21] in which there are two intermediates, a carbinolamine (I) and a Schiff base (II) and the rate-determining step changes from hydronium ion catalyzed dehydration (step 2)

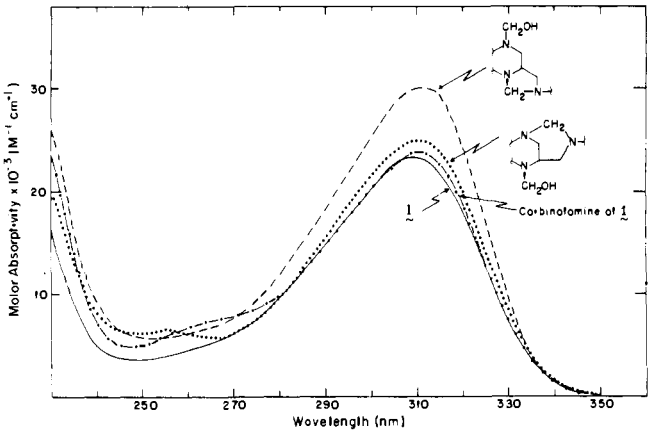


Figure 8. Spectra of **1** in the absence (—) and presence of 0.50 *M* formaldehyde in 50:50 (v/v) dioxane-water, 23–25°, ionic strength 0.10 *M*, pH 9.46 maintained with 1×10^{-3} *M* DABCO buffer. Spectra of carbinolamine of 1 (· · · ·); carbinolamine of **4a** (. . . . .); carbinolamine of **4** (- - - - -).

of the carbinolamine to form the Schiff base in alkali to the attack of the amine upon the carbonyl compound (step 1) as the pH decreases. The following evidence argues for applicability of this Scheme III to the present data for the formation of **5** and **6** as well.

(1) The uv spectral properties of both the carbinolamines and products (Table III) are consistent with such an assignment.[8,9,11]

(2) The magnitude of the equilibrium constants for the formation of the carbinolamines and the products from both **2** and **3** and formaldehyde are also plausible for Scheme III.[8,9] Thus the equilibrium constants for the formation of *N*-hydroxymethylamines derivatives of **2** and **3** when allowance is made for the increased steric hindrance in **2**[9,23] are similar to those for tetrahydroquinoline and tetrahydroquinoxaline[24a] (Table II) and DPED[8] (Table IV).

(3) The equilibrium constants for imidazolidine formation from various ethylenediamines and formaldehyde[8] or glyoxalate[24b,c] are in the range 10^3–10^6 M^{-1} and are similar to that for the formation of **5** from **2** and formaldehyde ($K_5 = 8.0 \times 10^4$ M^{-1}).

(4) The greater than 200-fold more favorable formation constant for the five-membered ring in **5** compared with the six-membered ring system **6** from formaldehyde and reactants expressed by ratio of K_5/K_6 is probably as expected.[25]

(5) The kinetic constants for the hydronium ion catalyzed and pH independent attack of the amines on formaldehyde and for the hydronium ion catalyzed dehydration of the hydroxymethylamine intermediates are similar to those for compounds of comparable basicity (compare **2** and **3** with DPED in Table IV).

Formation of 4 from Formaldehyde and 1. The identity of the intermediate **4a,** characterized by an association constant of about 7×10^3 M^{-1} from **1** and formaldehyde, is designated to be the *six-membered cyclic hexahydropyrimidine and not the carbinolamine of* **1** (cf. ref 6) on the basis of the following *independent* kinetic, thermodynamic, and structural considerations.

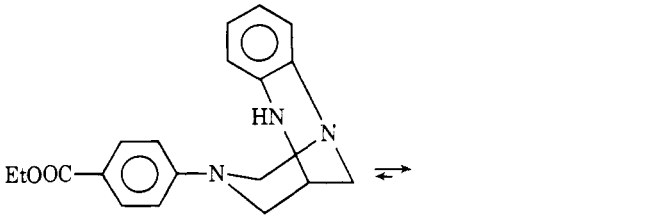
(1) The elemental and mass spectral analysis (*m/e* of parent ion 323) for **4a** isolated directly from a reaction mixture is consistent with the structure of **4a** and clearly distinguishable from that of the isomeric product, **4.**

(2) The ir spectrum of compound **4a** is similar to that of **1** and retains secondary amine NH stretching and bending frequencies[20b] at 3300–3400 and 1510 cm^{-1}, respectively.

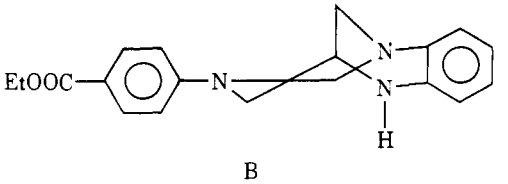
(3) The uv spectra of **6** and **4a** reveal decreases in the intensity of the 310-nm band compared with the parent compounds [Figures 2 (B) and 5 (A)] that are consistent with cyclic hexahydropyrimidine structures in which the lone pair of electrons is constrained at the N_1 and N_4 sites, respectively, in an orientation orthogonal to the electrons of the adjacent phenyl rings thus decreasing the orbital overlaps.[26]

(4) Both the pH-rate profile, which indicates a change in rate-determining step with pH (Figure 3), and the rectangular hyperbolic dependence of the rate of **4a** formation upon formaldehyde concentrations in the alkaline pH region ($K_1 = 41.6$ M^{-1}, Figure 4, A) *require* that an intermediate occur on the pathway *to* the formation of **4a** and these data are consistent with the designation of the prior intermediate as a carbinolamine[8,9,11] (cf. K_1 values for tetrahydroquinoline and tetrahydroquinoxaline in Table II). The observations and formulations of mechanism in this system are analogous to those for **2** and **3** for their reactions with formaldehyde and, indeed, the values for K_1 and the kinetic constants for the formation of **4a** based upon this formulation are similar to those for imidazolidine and hexahydropyrimidine formation from similar reactants including **2** and **3** (Table IV). It does *not* appear that the rate-determining step for formation of **4a** from formaldehyde involves *cyclization* either by an SN2 displacement upon the N_4-hydroxymethylamine or by addition to the cationic imine to form the cyclic product since the rates of formation of imidazolidines and thiazolidines in alkali from formaldehyde and *o*-phenylenediamine or *o*-aminobenzenethiol, respectively, are very similar [10a,27] These possible transitions states would involve attack by a thiolate anion and an amine for *o*-aminobenzenethiol and *o*-phenylenediamine, respectively, and the nucleophilicities of thiolate anions[28] are considerably greater than amines of comparable basicity.[12]

(5) The ^1H NMR spectrum in deuterated chloroform of the isolated **4a,** while apparently complicated by the occurrence of conformational isomers, is consistent with a bicyclic hexahydropyrimidine derivative since such compounds have been shown to exhibit such isomerism[29,30] (Figure 1). The observation of two quartets in the ^1H NMR spectrum of **4a** centered at about δ 4.7 attributed to the formaldehyde methylene protons and the double sets of aromatic and ethyl group resonances is consistent with the postulation of two conformeric isomers, the chair form A and the boat form B. Studies at 5, 25, and 40° show slow increases in the ^1H NMR resonance assigned to the B conformation and indicate that in $DCCl_3$ the preferred conformation is B in



A



B

agreement with the work of Bohlmann et al.[30] The latter workers showed that the boat form was the preferred conformation for quinolizidines, which are bicyclic compounds similar in structure to **4a.**

(6) The magnitude of the equilibrium constant for the formation of **4a** from **1** is more than tenfold greater than for

the formation of **6** from **3**. The latter reaction might be expected to be less favorable than the formation of **4a** from **1** as a consequence of greater strain in **6** than that in **4a** due to the presence of a methylene group rather than a nitrogen atom at the 4 position[31] in **6**.

(7) The hydronium ion catalyzed rate constant for product formation from **4a** in the alkaline region is tenfold lower than those unequivocally assigned to the hydronium ion catalyzed dehydration of carbinolamines of compounds of similar basicity[8] but may not be unreasonable for the isomerization of the hexahydropyrimidine to imidazolidine. This isomerization reaction of **4a** to **4** will be discussed in further detail in the next to last section.

(8) The ratio of the equilibrium constants K_5/K_6 for the reactions of **1** with formaldehyde is within threefold of the ratio K_5/K_6 for the reactions of **2** and **3**, respectively, with formaldehyde (Table II).

The rates of cyclization by attack of N_{10} on the cationic imines at N_4 and N_1 to form **4a** and **4**, respectively, are expected to be faster for the formation of the five-membered as opposed to the six-membered ring on entropic grounds. Thus, the greater rates of **4a** than **4** formation directly from **1** must be related to the greater rate of cationic imine formation at the N_4 than the N_1 site. It would appear that steric effects of the neighboring side chain are significant in this respect since at all pH values examined in which the rates of product formation are first order in respect to formaldehyde concentration, the rates of **6** formation exceed or equal those of **5** formation (Figure 3).

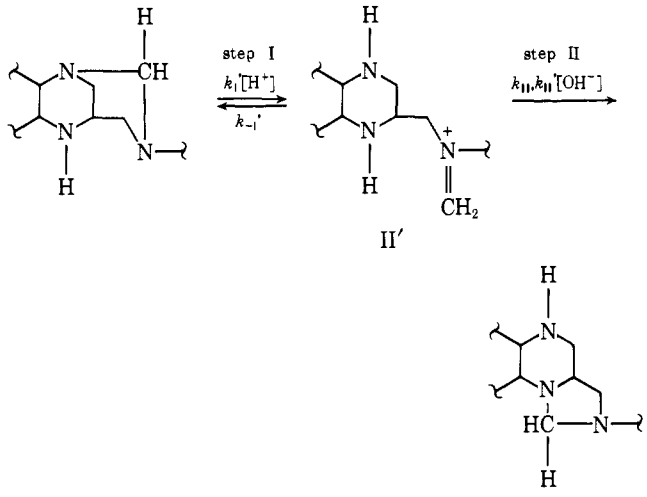
We conclude from the kinetic and thermodynamic data, which are supported by the structural data, that Scheme II applies, with the existence of the **4a** intermediate, to the mechanism of **4** formation from **1** and formaldehyde at relatively low formaldehyde concentration (the conditions employed by Benkovic et al.[6]).

Imidazolidine (4) Formation from Hexahydropyrimidine (4a). Some of the many species in solutions of **1** and formaldehyde including the critical hexahydropyrimidine intermediate (**4a**) and the final imidazolidine product (**4**) are depicted in Scheme II.[32a] The possible pathways from **1** to **4**, under the condition in which **4a** accumulates, in reality become a question of possible pathways from **4a** to **4** and can be divided into two groups, *direct* and *indirect* pathways both of which differ significantly from the earlier mechanistic formulations for **4** formation.[6,7] One direct pathway is illustrated by the dashed arrows proceeding through the N_{10}-iminium cation directly to **4** (Scheme II). One indirect pathway that can be formulated depicts the formation of **4** by the breakdown of **4a** to reactants, **1** and F, and then the successive formation of $>N_1CH_2OH$, $>^+N_1{=}CH_2$, and **4** (Scheme II).

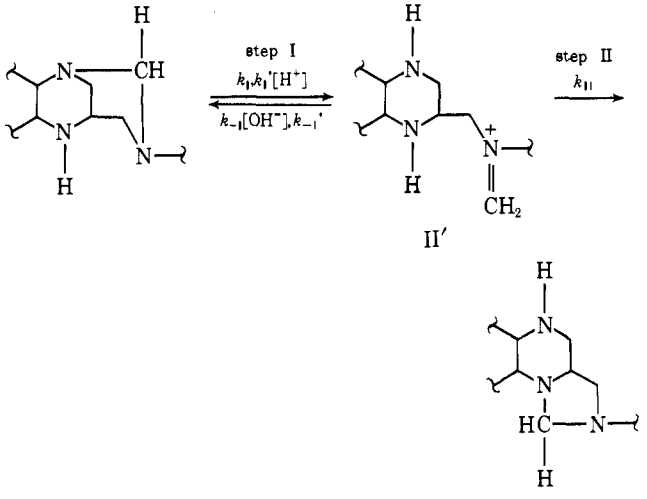
In the discussion as to whether an indirect or a direct pathway is followed in proceeding from **4a** to **4**, it must be noted that there are few data available regarding the following: (i) the stabilities and reactivities of the N_1- and N_{10}-carbinolamines to form carbonyl compounds or Schiff bases, (ii) the stabilities and reactivities of the N_1-, N_4-, or N_{10}-iminium cations to attack by nucleophiles (water, hydroxide ion, and amines), (iii) rate constants (including those for possible general-acid catalysts) for cyclization and ring-opening reactions for hexahydropyrimidines and imidazolidines, and (iv) macroscopic and microscopic proton dissociation constants for many conjugate acids of the species depicted in Scheme II.[32b]

In terms of the mechanism of the direct pathway for **4a** → **4** (dashed arrows Scheme II), the rate-determining step may either be ring opening, step I, to form the N_{10}-iminium cation (II′) or cyclization to effect ring closure, operating upon the N_{10}-iminium cation, step II (Scheme IV or V). In

Scheme IV



Scheme V



the specific acid-general base catalysis mechanism of Scheme IV, the hydronium ion catalyzed ring opening to form the N_{10}-iminium cation is followed by solvent- or base-catalyzed ring closure whereas in the general-acid-catalyzed mechanism of Scheme V, the ring opening to form the iminium cation is acid (H^+) and solvent catalyzed, while the cyclization to form **4** is solvent catalyzed.[34] The pH–rate profile for **4a** to form **4** (Figure 3) may be explained in terms of a transition of rate-determining steps from water- and base-catalyzed ring closure to acid-catalyzed ring opening with increasing pH (Scheme IV) or a transition of rate-determining steps from acid- and water-catalyzed ring opening to water-catalyzed ring closure with increasing pH (Scheme V). Both mechanisms account for a change in rate-determining step as a function of pH and, at selected pH values, a change in rate-determining step as a function of buffer concentration.[6] For Schemes IV and V, k_{obsd} can be quantitatively accounted for in terms of eq 8a and 8b, respectively, which reduce at low and high pH values to eq 9a,b and 10a,b, respectively, where K_{WD}[33] is the autoprotolysis constant of water in this solvent (Table V) and $K_I' = k_I'/k_{-I}'$ (Scheme IV) or $K_I = (k_I + k_I'a_{H^+})/(k_{-I} + k_{-I}'a_{H^+}/K_{WD})$ (Scheme V).

$$k_{obsd} = [k_I'a_{H^+}(k_{II} + k_{II}'a_{OH^-})]/(k_{-I}' + k_{II} + k_{II}'a_{OH^-}) \quad (8a)$$

$$k_{obsd} = [(k_I + k_I'a_{H^+})k_{II}]/(k_{-I}a_{OH^-} + k_{-I}' + k_{II}) \quad (8b)$$

$$k_{obsd} = K_1'(k_{II}a_{H^+} + k_{II}'K_{WD}) \quad (9a)$$

$$k_{obsd} = k_I'a_{H^+} \quad (9b)$$

$$k_{obsd} = k_I + k_I'a_{H^+} \quad (10a)$$

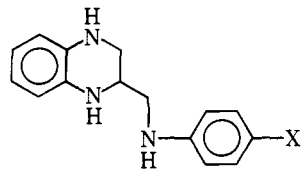
$$k_{obsd} = K_Ik_{II}a_{H^+}/K_{WD} \quad (10b)$$

The ring opening step in Schemes IV and V is expected to be influenced by the basicity at the N_4 site (which is expected to be little affected by substituents on the phenyl ring)[32b] and the electron-donating ability of the N_{10} site with respect to π-bond formation in the generation of the N_{10}-iminium cation (which is expected to be very sensitive to substituents). The net effect anticipated then is a substantial substituent effect upon ring opening of **4a** to form the N_{10} Schiff base. In contrast, the ring closure step to form the imidazolidine will be influenced by the stability and, hence, the concentration of the N_{10}-iminium cation *and* its electrophilicity; for example, electron-withdrawing substituents would decrease the former but increase the latter. Substituents, therefore, may be expected to have more or less *opposite* effects upon these two factors for step II and the *net* effect might well be small on the ring closure reaction to form **4**. However, the ratios of constants k_{-1}'/k_{II}' (k_{-1}/k_{II}) in Scheme IV (V) represent the partitioning ratio of N_{10}-iminium cation to hexahydropyrimidine–imidazolidine and in terms of these mechanisms are 360, 7.7, and <1, for *p*-COOEt, *p*-Cl, and *p*-CH_3, respectively (Table V).[7] It is not readily expected that the relative rates of cyclization by attack of the N_1 and N_4 atoms on the N_{10}-iminium ion to form the five- and six-membered rings should be as para-substituent dependent on the aforementioned partitioning ratios indicate (for either the non-buffer-catalyzed or buffer-catalyzed pathways). These data argue against the direct mechanisms of Schemes IV and V.

Despite the many-fold uncertainties pointed out earlier in this section of the Discussion which make tenuous a clear-cut choice between the indirect and direct mechanisms, it is of interest that the indirect route to **4** via **1** and formaldehyde (at 10^{-3} *M*) via rate-determining attack of free **1** on formaldehyde in the acid region and rate-determining dehydration of the N_1-carbinolamine to form the iminium ion in the alkaline region (eq 6) can be fit to eq 5 in which the fraction of total tetrahydroquinoxaline derivative, T, as free base **1**, α_{R_2NH}, is given by $\alpha_{R_2NH} = [\mathbf{1}]/[T] = 1/[1 + a_{H^+}/K_{a_1}' + K_6[F](1 + a_{H^+}/K_{a_2}')]$ where $K_{a1} = [\mathbf{1}]a_{H^+}/[$cationic **1**$]$ and $K_{a2} = [\mathbf{4a}]a_{H^+}/[$cationic **4a**$]$ and takes into account the rapid accumulation of **4a** prior to significant formation of **4**. The following rate constants are obtained by this treatment with the value of K_6 from Table II, $K_{a_1}' = 10^{-3.78}$, and $K_{a_2}' = 10^{-2.3}$ (ref 32c): $K_1k_2 = 1.3 \times 10^8\ M^{-2}$ sec^{-1}, $k_I = 1.7\ M^{-1}$ sec^{-1} and $k_I' = 3.8 \times 10^5\ M^{-2}$ sec^{-1}. These calculated rate constants are sufficiently close to those for DPED ($pK_a' = 3.64$, ref 8) of $4.4 \times 10^7\ M^{-2}$ sec^{-1}, $2.9\ M^{-1}$ sec^{-1}, and $1.3 \times 10^5\ M^{-2}$ sec^{-1}, respectively, that the indirect pathway appears available for the formation of **4**. The previously observed buffer catalysis for **4** formation with a Bronsted α value of 0.35 for the attack of the N_1 nitrogen of **1** upon formaldehyde is consistent with this formulation [and comparable to the Bronsted α value (0.30 ± 0.10) for the similar reaction of DPED[8]].

The several-fold more rapid rate of **4a** → **4** at higher formaldehyde concentration has previously been interpreted in terms of an analogous indirect route.[1] However, it does not appear possible to readily accommodate the following previous observations[7] on the *p*-CH_3 and *p*-Cl tetrahydroquinoxaline derivatives with the mechanism of the indirect pathway: (i) the marked substituent dependence of the rates of product formation for the data below pH 7, and (ii) the lack of detectable general-acid catalysis of product formation below pH 7. The failure to detect a change in the rate-determining step with changing pH with the *p*-CH_3 derivative makes it difficult to draw conclusions about the substituent dependence of the process(es) occurring at pH greater than 8. Indeed this failure is surprising based on our studies with THF[9] and especially symmetrically substituted *N,N'*-diphenylethylenediamines[8] and formaldehyde, since in no case of reactions of formaldehyde with amine derivatives that proceed through Schiff base formation has there been a failure to see a change in the rate-determining step with changing pH (spanning a range of pK_a values of about 9[12] through 4–5[10a,27] to 2.3[8]). It must be noted, however, that the pH range reported was restricted, 6.4–7.7, and electron-donating substituents shift the pH of the change in the rate-determining step to progressively more alkaline pH.[8] In summary, the complexity of the tetrahydroquinoxaline derivative system, in contrast to THF itself, leads directly to the major conclusions contained in the next section.

Table V. Rate Constants for the Isomerization of Substituted Hexahydropyrimidines to Substituted Imidazolidines[a]



Substituent X	k_I',[b] M^{-1} sec^{-1}	k_I,[c] sec^{-1}	K_Ik_{II}/K_{WD},[d] M^{-1} sec^{-1}
CH_3[f]	$>5.3 \times 10^4$	*e*	5.3×10^4
Cl[f]	1.7×10^3	1.5×10^{-4}	1.3×10^4
EtOOC[f]	3.8×10^1	2.5×10^{-4}	6.7×10^3
EtOOC[g]	5.0×10^1	2.4×10^{-4}	1.8×10^4

[a] Rate constant designation for Scheme V; **4a** generated in situ at $1 \times 10^{-3}\ M$ formaldehyde. [b] $k_I' = K_1k_3$, where $K_1 = k_1/k_{-1}$ in ref 7. [c] $k_I = K_1K_{WD}k_3'$ in ref 7. [d] $K_Ik_{II}/K_{WD} = k_I$ in ref 7. [e] Not determined. [f] Taken from ref 7. [g] This work.

Considerations Relevant to the Structure of Tetrahydrofolic Acid (THF). The pyrimidine portion of THF clearly contributes to the determination of the redox potential of the tetrahydropteridine moiety[5] in order to poise this potential such that it is suitable for the roles of THF and tetrahydropteridine derivatives in the reactions catalyzed by thymidylate synthetase and various hydroxylases,[36] respectively. Whether the existence of the N_8 atom is the result of the common utilization of THF in various redox and 1-carbon transfer reactions or is an evolutionary remnant cannot readily be answered presently. But since the K_1 values and K_5 values are about 20- and 8-fold more favorable for **1** than **2**, the presence of a nitrogen atom in place of a carbon atom at the 8 position may be necessary to aid the stability of THF adducts of formaldehyde. In order to maximize the affinity toward F by the presence of an N_8 atom but at the same time to avoid spurious reactions such as hexahydropyrimidine formation, the adjacent pyrimidine ring of the tetrahydropteridine moiety serves to make the N_8 site in THF substantially less basic and less reactive than either the N_5 site in THF or the analogous N_4 site tetrahydroquinoxaline derivatives. Thus, the tendencies for carbinolamine and hexahydropyrimidine formation, which should decrease with the decreased basicity at the N_8 site in THF as a result of the adjacent pyrimidine ring, may be expected to be substantially lower than those tendencies involving the N_4 site in **1**; the nucleophilic reactivity at the N_8 site in THF should be drastically reduced as well.[8,11] On balance it appears that tetrahydroquinoxaline derivatives are *not* especially good model compounds for THF, at least for reac-

tions involving compounds at the aldehyde level of oxidation, despite repeated implications to the contrary.[6,7] Nevertheless, these studies with tetrahydroquinoxaline models for THF have opened new possibilities regarding the structural determinants which suit THF to its diverse roles, and the model system presents opportunities for the study of the mechanisms of methylenediamine reactions and interconversions thereof which have not been studied and which are relevant to bioorganic mechanisms. In addition, the structure–reactivity correlative information presented in this study can be of importance in the design of new folic acid antagonists which in the past have proven to have some effectiveness in the chemotherapy of cancer.[37]

Acknowledgments. We gratefully acknowledge the technical assistance of Ms. Linda Nussbaum and Ms. Connie Williams. We also wish to thank Dr. Terry Newirth for accomplishing the essential synthesis of quinoline-3-aldehyde.

References and Notes

(1) This project was supported by the National Institutes of Health, U.S. Public Health Service, Grant No. 13777 (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.

(2) Abbreviations: DABCO, triethylenediamine (1,4-diazabicyclo[2.2.2]octane); DPED, *N,N'*-diphenylethylenediamine; DPI, *N,N'*-diphenylimidazolidine; F, formaldehyde (hydrated and unhydrated); THF, tetrahydrofolic acid; **1**, ethyl *p*-[*N*-(1,2,3,4-tetrahydro-2-quinoxalinylmethylene)amino]-benzoate; **2**, ethyl *p*-[*N*-(tetrahydro-2-quinolinylmethylene)amino]benzoate; **3**, ethyl *p*-[*N*-(tetrahydro-3-quinolinylmethylene)amino]benzoate; **4** and **5**, imidazolidine adducts of **1** and **2**, respectively; **6**, hexahydropyrimidine derivative of **3**; H4 and H4a, *N*-hydroxymethyl adduct of **4** and N_1-hydroxymethyl adduct of **4a**, respectively; and H1, *N*-hydroxymethylamine of **1**.

(3) Predoctoral Fellow, National Institutes of Health, 1968–1973.

(4) M. Friedkin, *Annu. Rev. Biochem.*, **32**, 185 (1963).

(5) L. V. Jaenicke and E. Brode, *Justus Liebigs Ann. Chem.*, **624**, 120 (1959).

(6) S. J. Benkovic, P. A. Benkovic, and D. R. Comfort, *J. Am. Chem. Soc.*, **91**, 5270 (1969).

(7) S. J. Benkovic and R. Chrzanowski, *J. Am. Chem. Soc.*, **92**, 523 (1970).

(8) G. P. Tuszynski and R. G. Kallen, *J. Am. Chem. Soc.*, **97**, 2860 (1975), and references cited therein.

(9) R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, **241**, 5845, 5851, 5864 (1966).

(10) (a) For a series of unsymmetrical diamines one might anticipate that the rate of cyclization of the progressively more weakly basic site upon the iminium ion at the more strongly basic site (when sufficiently different basicity obtains) might become sufficiently slow that cyclization per se becomes the rate-determining step. However, the progression of substituents from *p*-$COOCH_2CH_3$, *p*-Cl, and *p*-CH_3 in the series studied[7] tends in the opposite direction, that is, to make the diamine sites more equivalently basic. (b) This "lag" is due to the preequilibrium formation of **4a**, the rate of formation of which is first order in amine and formaldehyde under these conditions. The "lag" cannot be due to rate-determining dehydration of formaldehyde, since the rate of dehydration of formaldehyde hydrate to free formaldehyde is orders of magnitude faster than any rates of product formation measured in this study [P. Le Henaff, *C. R. Hebd. Seances Acad. Sci.*, **256**, 1752 (1963)].

(11) W. R. Abrams and R. G. Kallen, manuscript in preparation.

(12) R. G. Kallen, *J. Am. Chem. Soc.*, **93**, 6227, 6236 (1971).

(13) L. F. Fieser, "Experiments in Organic Chemistry", 3rd ed, D. C. Heath, Boston, Mass., 1955, p 284.

(14) W. Borsche and W. Doeller, *Justus Liebigs Ann. Chem.*, **537**, 39 (1938).

(15) H. Kaplan, *J. Am. Chem. Soc.*, **63**, 2654 (1941).

(16) A. Vogel, "A Text Book of Practical Organic Chemistry", Spottiswoode, Ballantyne and Co., London, England, 1961, p 471.

(17) A. H. Cook, I. M. Heilbron, and L. Steger, *J. Chem. Soc.*, 413 (1943).

(18) F. Bohlmann, *Chem. Ber.*, **85**, 390 (1952).

(19) (a) W. E. Wentworth, *J. Chem. Educ.*, **42**, 96 (1965); (b) R. O. Viale and R. G. Kallen, *Arch. Biochem. Biophys.*, **146**, 271 (1971), and references cited therein.

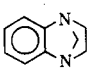
(20) (a) J. Sayer, personal communication; G. P. Tuszynski and R. G. Kallen, unpublished results. (b) The ir spectra of **1**, **2**, **3**, and **4a** in $CHCl_3$ reveal secondary amine NH stretching and bending frequencies at 3300–3400 and 1510 cm^{-1}, respectively, carbonyl stretching of an aromatic ester at 1725 cm^{-1}, and aromatic absorbances at 1600, 1480, 1180, 1100, 1020, and 830 cm^{-1},[20c] (c) J. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds", Prentice-Hall, Englewood Cliffs, N.J., 1965, p 22.

(21) W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969, and references cited therein.

(22) Referred to as K_1 in ref 6 and 7.

(23) J. De Luis, Ph.D. Thesis, Pennsylvania State University, 1964.

(24) (a) The spectral changes observed in the formaldehyde titration of tetrahydroquinoxaline to form the formaldehyde adduct occurred within 1–2 sec and final spectra were stable for several hours at pH values of 5.0 and 7.0. These observations are consistent with the formation of the hydroxymethylamine of tetrahydroquinoxaline and *not* the N_1,N_4-methylene adduct thereof. Furthermore, examination of molecular models of the methylene adduct indicates the occurrence of considerable strain in placing a methylene group across the N_1 and N_4 sites of the rigid quinoxaline ring system in order to form the bicyclic derivative, n.



n

(b) B. E. Leach and D. L. Leussing, *J. Am. Chem. Soc.*, **93**, 3377 (1971); (c) G. P. Tuszynski, K. L. Brown, and R. G. Kallen, manuscript in preparation.

(25) Experiments are underway to measure the entropic and enthalpic differences for formation of these ring systems, since there are no such data available to date.

(26) W. R. Remington, *J. Am. Chem. Soc.*, **67**, 1838 (1945).

(27) G. P. Tuszynski and R. G. Kallen, unpublished results.

(28) (a) G. E. Lienhard and W. P. Jencks, *J. Am. Chem. Soc.*, **88**, 3982 (1966); (b) R. G. Kallen and M. Frederick, submitted for publication; (c) J. Hine, "Physical Organic Chemistry", McGraw-Hill, New York, N.Y., 1962, p 186.

(29) A. S. Lindsey, *J. Chem. Soc.*, 1685 (1965).

(30) F. Bohlmann, D. Schuman, and C. Arnt, *Tetrahedron Lett.*, 2705 (1965).

(31) E. S. Gould, "Mechanism and Structure in Organic Chemistry", Holt, Rinehart and Winston, New York, N.Y., 1960, p 207.

(32) (a) The complex composition of such solutions is due to the presence of three reactive nitrogen sites, N_1, N_4, and N_{10}. Thus, there are many other species present, which for simplicity have been omitted from Scheme II, such as di- and tricarbinolamines, di- and triimines, compounds with various combinations of hydroxymethyl and imine functions on the same molecule, carbinolamines and imines of the cyclic compounds, etc. Furthermore, there are in most instances several states of protonation of these species. (b) Unfortunately, data were not reported on even the macroscopic titration constants of the *p*-Cl and *p*-CH_3 derivatives in the solvent of study[7] which might have permitted estimates of the substituent effects on N_4 basicity in the series of tetrahydroquinoxaline derivatives. (c) The K_{a_2} value of $10^{-2.3}$ is estimated from that of the parent amine ($pK = 3.78$) and a ΔpK of -1.5 for the difference in pK values between aliphatic or aromatic cationic methylenediamines and the pK value of the parent amine: R. G. Kallen, "Methods in Enzymology: Vitamins and Coenzymes", Vol. 18, Academic Press, New York, N.Y., 1971, p 705; R. G. Kallen, R. O. Viale, and L. K. Smith, *J. Am. Chem. Soc.*, **94**, 576 (1972).

(33) K_{WD} is defined as the autoprotolysis constant of H_2O in 50% dioxane–water, 25°, and is 1.8×10^{-16} (H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions", Reinhold, New York, N.Y., 1950, p 581).

(34) (a) General-base catalysis of the attack of weakly basic amines upon carbonyl compounds via proton removal from the amine has been observed.[11,21,35] General-base catalysis of transimination reactions has also been reported[34b,c,35a] which is closely related to the reaction steps in Schemes IV and V (see ref 21, pp 505–506). (b) K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **86**, 2413 (1964); (c) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 826 (1962).

(35) (a) L. do Amaral, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **88**, 2225 (1966); (b) J. M. Sayer and W. P. Jencks, *ibid.*, **94**, 3262 (1972).

(36) R. L. Blakley, "The Biochemistry of Folic Acid and Related Pteridines", Wiley, New York, N.Y., 1969, p 241.

(37) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors", Wiley, New York, N.Y., 1967, p 267.