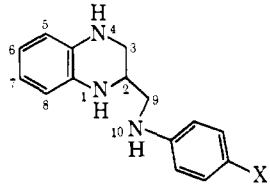
Studies on Models for Tetrahydrofolic Acid. IV. Reactions of Amines with Formamidinium Tetrahydroquinoxaline Analogs

S. J. Benkovic,*[1a] T. H. Barrows, and P. R. Farina[1b]

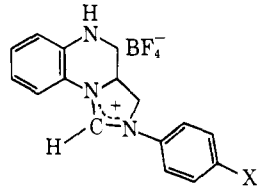
Contribution from the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received June 2, 1973*

Abstract: The condensation of amines with formamidinium model compounds for 5,10-methenyltetrahydrofolic acid leads to *N*-formimidoyl products. The reaction involves decomposition of a presumed orthoamide intermediate to yield the formamidine product of kinetic control; the latter then isomerizes to the thermodynamically more stable *N*-formimidoyl derivative. In the absence of intramolecular effects, efficient transfer of the one-carbon unit to yield the *N*-formyl amine generally requires the pK_a of the condensing amine to be less than the pK_a of the formamidine nitrogens owing to the need for amine protonation prior to expulsion. The implications of kinetic *vs.* thermodynamic control for the mechanisms of folate catalyzed transfer reactions are discussed.

The biosynthesis of inosinic acid, a precursor for adenine and guanine nucleotides, requires two transformylation reactions involving 5,6,7,8-tetrahydrofolic acid (FH_4).[2] Our previous work on tetrahydroquinoxaline models for FH_4 (Ia,b and IIa,b) has shown



Ia, X = CO_2Et
b, X = Cl

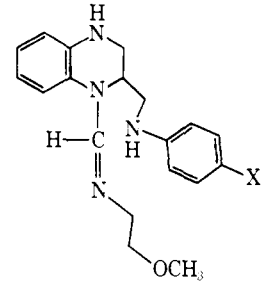


IIa, X = CO_2Et
b, X = Cl

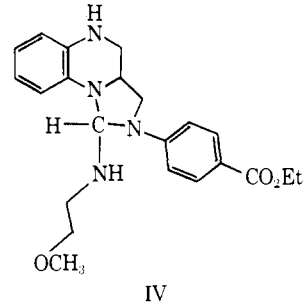
that they encompass many salient characteristics of the natural coenzyme but possess fewer dissociable acid–base groups and greater stability toward redox processes.[3] These latter features coupled with systematic variation of the para substitution should yield a degree of insight into the formyl transfer process. We report herein experiments employing model compounds (IIa,b) in a study related to the transfer of the formamidine carbon of 5,10-methenyltetrahydrofolic acid (5,10-methenyl FH_4) to glycinamide ribonucleotide.

Results

Structural Assignments. Condensation of IIa with excess 2-methoxyethylamine yields an adduct which was isolated as a white crystalline solid. Four possible structures were considered for this adduct; formimidoyl substitution at N-4, N-1 (IIIa), or N-10 and orthoamide (IV).[4,5] The orthoamide structure was ruled out by an ir absorption at 1640 cm^{-1} (CH=N)[6] and an nmr signal at δ 8.1 (s, 1 H), assigned to the formamidine hydrogen.[7]



IIIa, X = CO_2Et
b, X = Cl



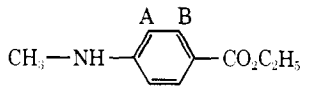
IV

Elemental analysis confirms that the adduct is in the free base form, as expected from the method of synthesis.

Assignment of the formimidoyl substituent to the N-1 and/or N-4 position rather than N-10 is based upon the following data. The nmr spectrum attributed to the aromatic protons of the tetrahydroquinoxaline moiety resembles the N-1 rather than N-10 formyl derivative of Ia.[3b] In the N-10 formyl case the nmr spectrum reveals a near singlet corresponding to the four aromatic protons of the tetrahydroquinoxaline moiety and, in the N-1 formyl case, a wider multiplet arising from the same protons. The spectra of the three compounds are displayed in Figure 1. It is unlikely, however, that this spectral region can be utilized to distinguish between N-1 or N-4 substitution. A comparison of the aromatic proton chemical shifts of the benzocaine moiety for the methoxyethylamine adduct with a series of structurally similar compounds (Table I) likewise is informative. Compounds V and VI were synthesized according to a modification of the procedure of Mandel and Hill.[9] The assigned structures of V and VI are consistent with spectral and elemental data (*cf.* Experimental Section). It is apparent that formimidoyl substitution at the N-10 position would shift the A protons of Ia approximately 0.5 ppm downfield. Since the A protons of the adduct and Ia have nearly identical chemical shift values, it can be concluded that the compound is not substituted at the N-10 position.

(1) (a) National Institutes of Health Career Development Awardee; (b) National Institutes of Health Postdoctoral Fellow, 1972–1973.
(2) For an extensive review see R. L. Blakley, "The Biochemistry of Folic Acid and Related Pteridines," North-Holland Publishing Co., Amsterdam, 1969.
(3) (a) S. J. Benkovic, P. A. Benkovic, and D. R. Comfort, *J. Amer. Chem. Soc.*, **91**, 5270 (1969); (b) S. J. Benkovic, W. P. Bullard, and P. A. Benkovic, *ibid.*, **94**, 7542 (1972).
(4) J. Hocker and R. Merten, *Chem. Ber.*, **105**, 1651 (1972).
(5) P. A. S. Smith, "Open-chain Nitrogen Compounds," Vol. 1, W. A. Benjamin, New York, N. Y., 1965, Chapter 4.
(6) K. Uyeda and J. C. Rabinowitz, *J. Biol. Chem.*, **240**, 1701 (1965).
(7) An orthoamide proton would give rise to an nmr signal at δ 3.0–5.8.[4,8]
(8) H. Bredereck, G. Simchen, and H. U. Schenck, *Chem. Ber.*, **101**, 3058 (1968).
(9) H. G. Mandel and A. J. Hill, *J. Amer. Chem. Soc.*, **76**, 3978 (1954).

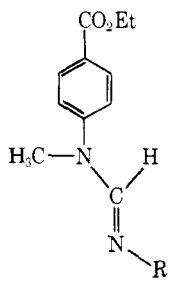
Table I. Chemical Shift Values for Benzocaine Derivatives[a]



1

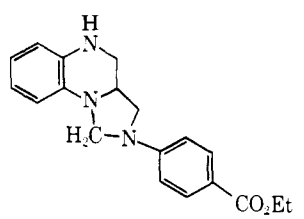
Compd	δ_A	δ_B
IIIa	6.57	7.78
Ia	6.55	7.86
1	6.54	7.89
V[b]	7.07	7.98
VI	7.00	7.98

[a] All spectra were obtained in deuteriochloroform and an AB coupling constant of 9 Hz was observed in each case. [b] Free base form.



V, R = $CH_2CH_2OCH_3$
VI, R = OCH_3

The designation of formimidoyl substitution at N-1 rather than N-4 was based on the following evidence: (a) acidification of IIIa at pH 2 gives quantitatively the formamidinium salt IIa as measured by changes in uv absorption; (b) treatment of IIIa in DMSO with sodium borohydride yields VII which is identical with



VII

an authentic sample;[3a] and (c) hydrolysis of IIIa at pH 10 forms the N-1 formyl product exclusively.

The adduct containing trifluoroethylamine (VIII) was prepared in the same manner as IIIa. The structure assigned to VIII is consistent with both elemental analysis and spectral data, *e.g.*, ir absorption at 1635 cm^{-1} (CH=N) and the fact that the nmr region containing the aromatic protons is superimposable for IIIa and VIII. Hydrolysis at pH 10 yields the corresponding N-1 formyl derivative exclusively. At pH 4 quantitative production of IIa was observed.

Compound IIIb was prepared from the *p*-chloroformamidinium fluoroborate model (IIb) according to the above procedure. Evidence for the proposed structure is furnished by the presence of ir absorption at 1630 cm^{-1} (CH=N) and an nmr signal at δ 8.2 (s, 1 H) characteristic of the formamidine proton at N-1. Proof for this designation is furnished by the nmr spectrum of IX which exhibits a formamidine proton signal at δ 7.9. Compound IX was prepared in the same manner as V.

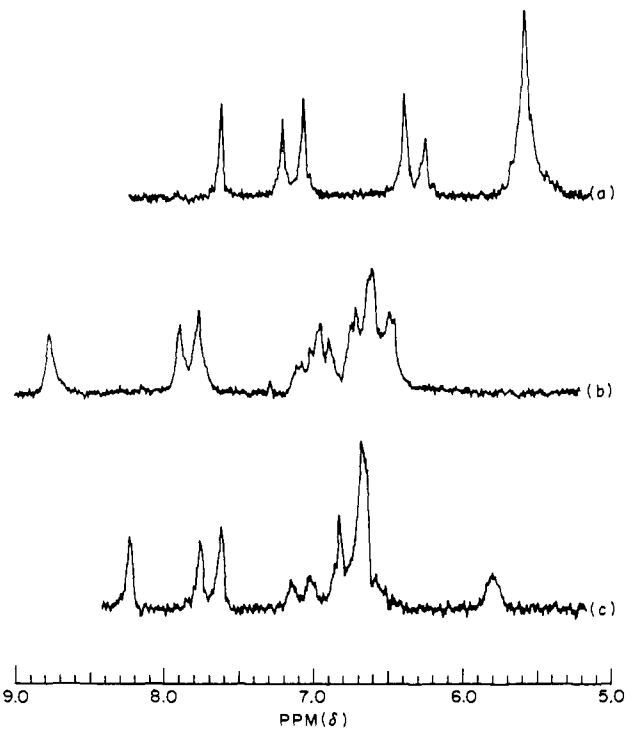
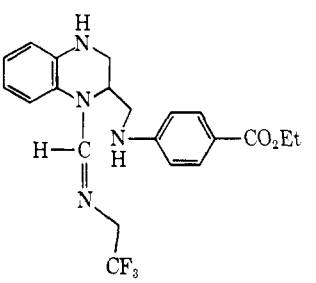
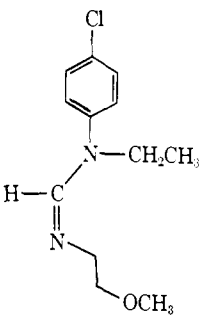


Figure 1. Nmr spectra of (a) the N-10 formyl derivative of Ia in $CDCl_3$; (b) the N-1 formyl derivative of Ia in $CDCl_3$; and (c) IIIa in DMSO-d_6.



VIII



IX

Isomerization. Hydrolysis of IIa has been shown to yield the N-10 formyl derivative of the *p*-carbethoxytetrahydroquinoxaline model which can isomerize to the more stable N-1 isomer.[3b] The possibility of a similar rapid isomerization (N-10 → N-1 product) after addition of 2-methoxyethylamine to IIa was investigated by nmr.

The formamidinium proton of IIa at δ 9.96 in DMSO-d_6 disappeared upon addition of ~2 equiv of methoxyethylamine and was replaced by a new peak at δ 8.2. This signal which appeared as two partially overlapped singlets showed a rapid decrease in the high-field signal and a corresponding increase in the low-field one. After several minutes the spectrum featured a sharp singlet, as well as aromatic absorptions, identical with the formamidine singlet and aromatic protons of IIIa, respectively, in DMSO-d_6. The product which precipitated after addition of water to the DMSO-d_6 solution was lyophilized and recrystallized from acetonitrile to give a white solid identical (ir, uv, mass spectrum) with an authentic sample of IIIa.

The experiment was repeated using IIb. After addition of methoxyethylamine to IIb in DMSO-d_6 the

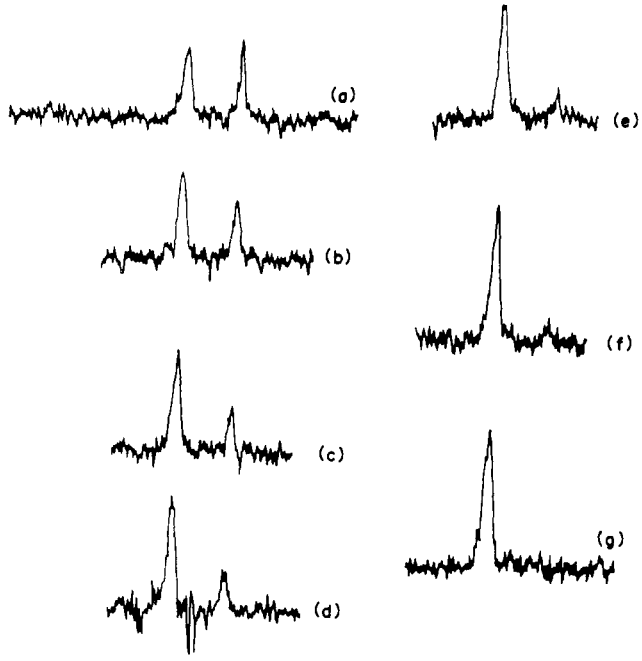


Figure 2. Nmr spectrum of IIb in DMSO-d_6 after addition of ~2 equiv of 2-methoxyethylamine at 36°: t = 40 sec (a); 81 (b); 111 (c); 167 (d); 236 (e); 292 (f); 792 (g).

formamidinium peak at δ 9.84 disappeared and two new peaks appeared at δ 7.99 and 8.23 (Figure 2). The peak at δ 8.23 increased as the peak at δ 7.99 decreased. After several minutes the peak at δ 7.99 was no longer detectable ($t_{1/2} \simeq$ 40 sec, 36°). The peak at δ 8.23 was identical with the formamidine signal for an original sample of IIIb in DMSO-d_6 which features substitution at N-1. The transitory peak at δ 7.99 is consistent with the formamidine signal of IX (δ 7.97) and therefore corresponds to the N-10 isomer of IIIb. Note that both N-1 substituted IIIa and IIIb give the same formamidine proton resonance in DMSO-d_6 (δ 8.2).

Kinetics and Product Distribution. Hydrolysis of IIIa in aqueous buffers from pH 7 to 10 gives mixtures of the N-10 and N-1 formyl derivatives of Ia. At pH 7, the product composition is *ca.* 90% N-10 formyl whereas at pH 10 the product is exclusively N-1 formyl. The reported hydrolysis constants were extrapolated to zero buffer concentration, although the effects of varying buffer were generally insignificant (less than 30% of k_{obsd}). The influence of varying buffer on product distribution was negligible. After 10 half-lives, pH 7 and 10 solutions of IIIa were extracted with chloroform to give, after crystallization, pure N-10 and N-1 formyl compounds, respectively. The products thus obtained were identical with authentic samples.[3b] Below pH 6, appearance of IIa was detected by an increase at 365 nm.[10]

Scheme I has been fitted to the rate data (Figure 3) using eq 1, where a_H is the activity of hydrogen ion as

$$k_{obsd} = \frac{a_H}{K_a + a_H}\left[\frac{k_1 K_w}{a_H} + k_2\right] \qquad (1a)$$

measured by the glass electrode, and K_w is the autoprotolysis constant of water, 10^{-14}. The values employed are collected in Table II. A satisfactory fit

(10) The hydrolysis of IIa in this pH region has been previously described.[3b]

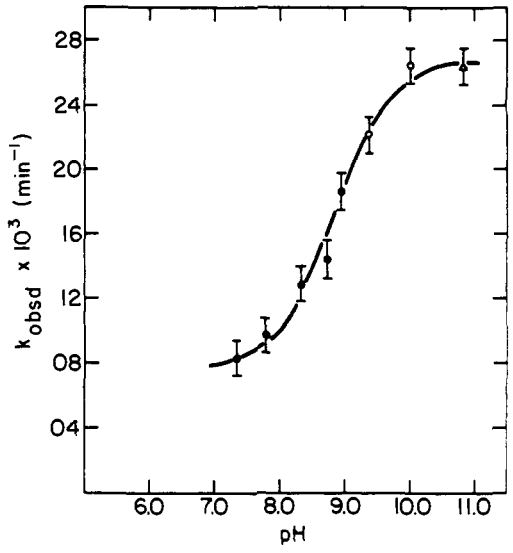


Figure 3. Plot of k_{obsd} *vs.* pH for hydrolysis of IIIa in aqueous buffers at 25° and μ = 0.2, KCl. Points are experimental and the line is calculated from eq 1a. The buffers used were tris(hydroxymethyl)aminomethane (●), triethylamine (△), and carbonate (○).

Scheme I

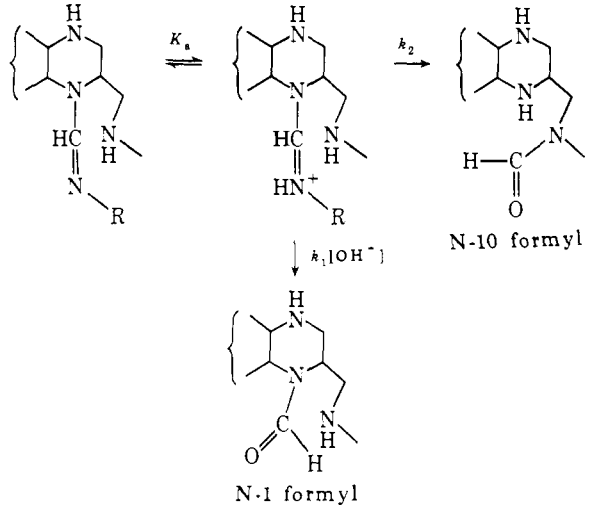


Table II. Rate Constants for the Hydrolysis of the Formamidine Models[a]

Compd	k_1, min^{-1}	k_2, min^{-1}	pK_a
IIIa	$3.8 \pm 0.4 \times 10^3$	$7.7 \pm 0.7 \times 10^{-3}$	8.85 ± 0.1[b]
V	$3.4 \pm 0.2 \times 10^3$		8.98 ± 0.04[c]

[a] T = 25°, μ = 0.2, KCl. [b] Obtained from kinetic data. A pK_a = 8.95 ± 0.1 was determined spectrophotometrically at 271 nm. [c] Determined spectrophotometrically at 303 nm.

to the observed product distribution (Figure 4) can be calculated from eq 1a where

$$\%\text{N-10} = 100\frac{k_2 a_H}{k_1 K_w + k_2 a_H} \qquad (1b)$$

and

$$\%\text{N-1} = 100\frac{k_1 K_w}{k_1 K_w + k_2 a_H} \qquad (1c)[11]$$

(11) The formation of N-10 formyl presumably occurs *via* IIa which in tris(hydroxymethyl)aminomethane buffers, pH 7–9, partitions to *ca.* 7% N-1 formyl.[3b] The calculated per cent of N-1 formyl has been adjusted accordingly.

Ethyl *p*-[[*N*-(2-methoxyethyl)formimidoyl]methylamino]benzoate (V) was prepared as a model for the unstable N-10 isomer of IIIa. Hydrolysis of V in aqueous buffers from pH 7 to 10 gave exclusive formation of ethyl *p*-[(*N*-formyl)methylamino]benzoate with negligible buffer catalysis. Hydrolysis of V follows the rate law $k_{obsd} = k_1K_w/(K_a + a_H)$. The values for the desired kinetic constants are listed in Table II.

Hydrolysis of IIIb, the *p*-chloro analog, in aqueous buffers from pH 8 to 10 gives mixtures of the N-10 and N-1 formyl derivatives of Ib. Due to similarities in the uv spectra of the two formyl isomers, which were overlooked in a previous study,[3b] the product distribution was determined by preparative tlc (Table III).

Table III. Distribution of Formyl Isomers upon Hydrolysis[a]

Compd	pH	%N-1	%N-10
IIa[3b]	7–9	93 ± 2	97 ± 2
IIb	8.2	47 ± 5	63 ± 5
	9.3	45 ± 5	55 ± 5
	10.7	40 ± 5	60 ± 5
IIIa	7.5	24 ± 4	76 ± 4
	8.5	63 ± 4	37 ± 4
	10.0	100 ± 4	
IIIb	8.2	47 ± 5	63 ± 5
	9.3	62 ± 5	38 ± 5
	10.7	100 ± 5	
VIII	7.3	23 ± 4	77 ± 4
	10.0	100 ± 4	

[a] $T = 25°$, $\mu = 0.2$, KCl.

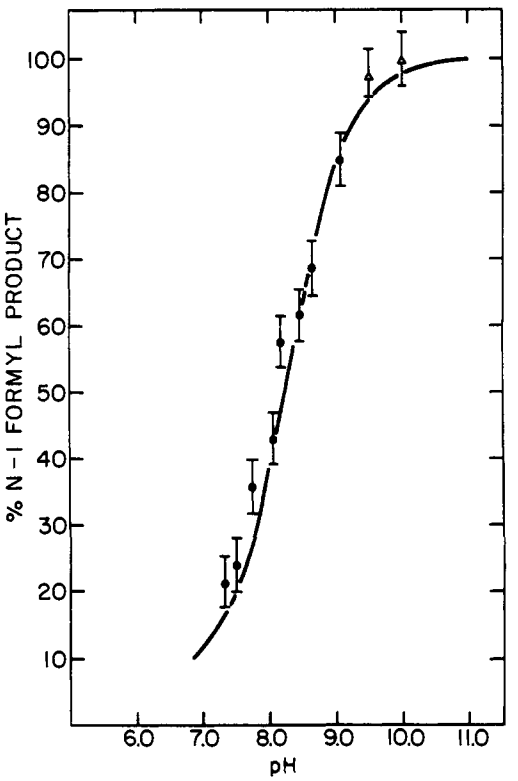


Figure 4. Plot of %N-1 formyl product obtained after 8 to 10 half-lives *vs.* pH. Solid line represents the product distribution calculated from eq 1b and 1c.

Below pH 8 the hydrolysis of IIIb resulted in the initial accumulation of IIb. Hydrolysis of IIb over the pH region of 8 to 11 gave a relatively constant ratio of the two isomeric formyl products (45% N-1 formyl, 55% N-10 formyl) as determined by the tlc method.

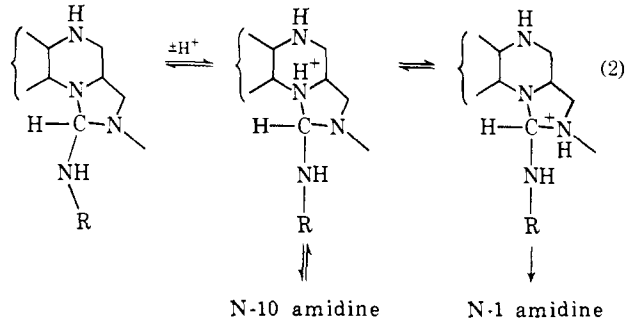
The hydrolysis of VIII in which trifluoroethylamine replaces methoxyethylamine from pH 6.8 to 7.8 also gave the two formyl isomers (Table III). Above pH 8 the solubility of VIII in aqueous buffers decreases markedly but apparently yields 100% *N*-formyl derivatives of Ia. Buffer solutions made with 25% dioxane effected greater solubility but dramatically slowed the rate of hydrolysis. Below pH 6 the initial accumulation of IIa again was noted.

Discussion

The addition of a primary amine to models for 5,10-methenyl FH_4 (IIa and IIb) yields the N-10 amidine as the product of kinetic control which isomerizes to the isolable N-1 amidine. This is manifested by the rapid disappearance of a formamidine proton (δ 8.2 IIIa, δ 8.0 IIIb) which is replaced by the lower field formamidine proton of the N-1 amidine. The fact that IIIa and IIIb in aqueous acid (pH 2) regenerate their respective formamidinium salts strongly argues for formimidoyl substitution at N-1. Atomic CPK models reveal that a six-membered ring formamidinium salt formed between N-4 and N-10 rather than a five-membered ring between N-1 and N-10 would suffer interruption of conjugation between the two rings and should not give rise to the uv absorption at 365 nm (*e.g.*, protonated V gives rise to λ_{max} at 261 nm). Furthermore, reduction of IIa by sodium borohydride in tetrahydrofuran yields VII quantitatively.[3b] Likewise in the case of IIIa, treatment with sodium borohydride gives VII, presumably arising from trapping of the five-membered formamidinium salt. Although formation of the initial N-10 amidine probably proceeds through an orthoamide intermediate which undergoes ring opening, no evidence for accumulation of such a species was found. Orthoamides derived from secondary amines, however, have been isolated and characterized.[4,5]

The accumulation of an N-10 amidine from ring cleavage of the presumed orthoamide and its isomerization to N-1 is consistent with the kinetic and thermodynamic disposition of the *p*-carbethoxyformamidinium salt (IIa)[3b] and the corresponding derivative of FH_4. Hydrolysis of 5,10-methenyl FH_4 under basic conditions yields mostly N-10 formyl FH_4. This species undergoes slow isomerization to N-5 formyl FH_4 under certain conditions, *e.g.*, autoclaving or prolonged incubation in weakly basic medium. The formation of an N-10 product is apparently controlled by the inherent basicities of the N-1 and N-10 nitrogens. Protonation of the more basic tetrahydroquinoxaline nitrogens of IV (macroscopic pK_a 4.2) can result in ring opening to the N-10 amidine followed by isomerization to the thermodynamically more stable N-1 isomer (reaction 2). All of the cases in (2) emphasize the fact that the expulsion of nitrogen almost always requires addition of a proton, so that the leaving group is the free amine rather than the unstable amine anion.[12] Moreover, these data lead to the tentative proposal that the preferred mode of decomposition for a monoprotonated orthoamide is dictated entirely by the site

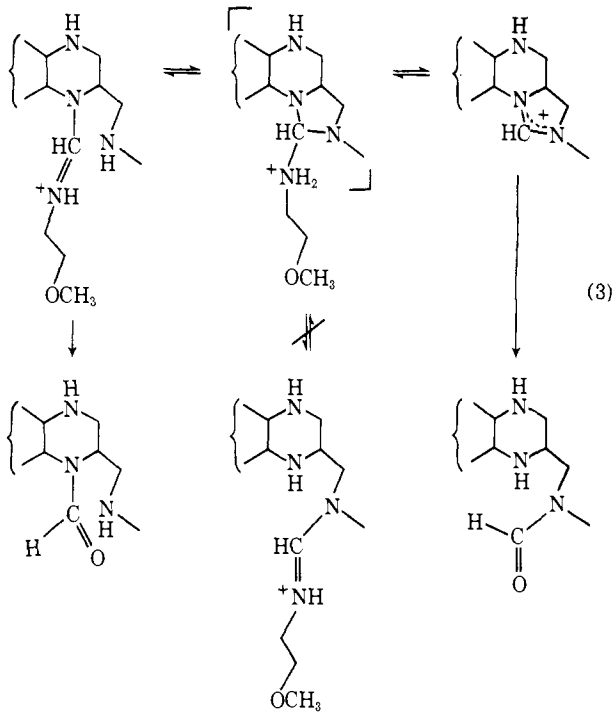
(12) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, p 542.



of protonation, with expulsion of the neutral amine occurring at a rate which might be competitive with proton transport processes. The net transamidination requires *catalyzed* tautomerization of the initially formed adduct to that leading to product. In the case of competing reversible expulsion of a higher pK_a amine as in (2) leading to the less stable amidine, protonation and expulsion of the less basic amine (N-10) proceeds, albeit at a slower rate, due to an unfavorable ΔpK_a for proton transfer, even though this process gives a more stable amidine. The dominant role of prototropy in these and related reactions perhaps underlies the *apparent* opposition of kinetic and thermodynamically controlled product formation which typifies the reactions of FH_4.

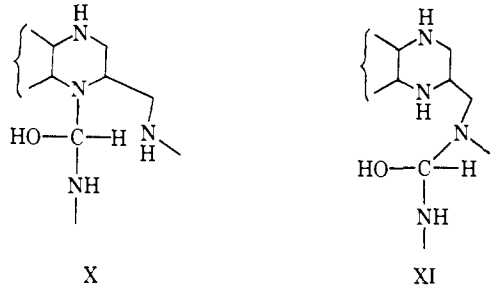
The hydrolysis of IIIa and IIIb is described by the rate law of eq 1 which is first order in protonated IIIa or IIIb and hydroxide ion. This description is preferred based on analogy with the aminolysis of imidate esters[13] and hydrolysis of formamidines which proceed through cationic species.[14] An alternate postulation involving attack by water on the amidine free base would require initial formation of a nitrogen anion ($pK_a \simeq 30$) which is generally considered to be an unlikely species in aqueous media. The absence of significant buffer catalysis is attributed to two factors: (a) structure-reactivity considerations for the reverse reaction, aminolysis of amides, suggest that the water-catalyzed reaction will become more significant with increasing pK_a, *i.e.*, decreasing β; and (b) the total change in k_{obsd} over the pH range investigated is only threefold.

Examination of the effect of pH on product distribution bears directly on the question of a potential equilibrium between formimidoyl substitution at quinoxaline nitrogens and the exocyclic nitrogen. The hydrolysis of the bridged formamidinium salts IIa and IIb yields nearly 100% and 60% N-10 formyl, respectively, at pH 7–10, the remainder being N-1 formyl. Since the presence of IIa and IIb was detected at the lower pH, only those distributions at pH 9–10 can be used to probe the potential equilibrium. However, both IIIa and IIIb give exclusively N-1 formyl at the more alkaline pH values. These observations, therefore, are in accord with hydrolysis solely of the initial amidine species at high pH with increasing contribution from IIa and IIb as the pH is lowered. This analysis which is depicted below includes the presumed orthoamide species, a likely precursor for formamidine formation (reaction 3).



An attempt was made to determine the equilibrium distribution of the two formimidoyl isomers for the *p*-carbethoxy model by measuring the rate of hydrolysis of V, an analog of the N-10 isomer. At pH 10 the hydrolysis of V is *ca.* 1.2-fold faster than IIIa. Assuming that 5% N-10 formyl could have been detected, one can estimate that the maximal concentration of the N-10 formimidoyl isomer is less than *ca.* 4%. The inability to observe a detectable equilibrium between the N-1 and N-10 formimidoyl isomers is not entirely unexpected since a previous study showed that the probably analogous formyl isomers were at an equilibrium ratio of *ca.* 100:1 (N-1 : N-10).[3b] Although increasing the pK_a of the N-10 nitrogen might be expected to increase the equilibrium concentration of the N-10 isomer, the rate of hydrolysis of this species should be decreased. This compensation rationalizes the failure to detect isomerization *via* product distribution with IIIb as well.

The absence of any transfer of a formyl group from IIIa (N-1) or V (N-10) to yield *N*-formyl-2-methoxyethylamine or from VIII to yield *N*-formyl-2,2,2-trifluoroethylamine suggests that such a transfer is governed as noted above by the basicities of the participating nitrogens. Thus, the higher pK_a nitrogen of 2-methoxyethylamine is preferentially protonated and expelled from the tetrahedral intermediate X or XI if



X XI

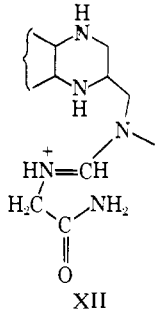
the latter were accessible. Formyl transfer only appears possible if either (a) the steps involving proton transfer to N-1 or N-10 become competitive with pro-

(13) E. Hand and W. P. Jencks, *J. Amer. Chem. Soc.*, **84**, 3505 (1962).

(14) D. R. Robinson and W. P. Jencks, *J. Amer. Chem. Soc.*, **89**, 7088, 7098 (1967).

ton transfer to the exocyclic amine or (b) the pK_a of N-10 or N-1 is perturbed so that it is greater than that of the attacking amine. As will be discussed in a forthcoming paper, catalysis of transfer reactions involving N-10 apparently are diffusion limited in terms of the approach and departure of the catalytic species.

Assuming that a pathway similar to the model reactions exists for condensation of glycinamide ribonucleotide (GAR) with 5,10-methenyl FH_4, several biological implications become apparent. Since the pK_a of the natural substrate GAR (pK_a = 8.15)[15] is between the pK_a's of 2-methoxyethylamine (pK_a = 9.72)[16] and 2,2,2-trifluoroethylamine (pK_a = 5.75),[17] it appears that an enzyme mediated counter-thermodynamic protonation would be necessary for transformylation. This might be effected by either a precisely positioned proton-donating residue on the enzyme directed toward a specific nitrogen, assuming that transport processes between the various basic sites are inhibited, *e.g.*, lack of solvent,[3b,18] or by reversibility of all steps preceding carbon–nitrogen bond cleavage at N-10. The difference in the rate of formyl transfer from the N-10 relative to the N-1 position, provided that the proton transfer and associated diffusion steps are no longer rate determining within the enzyme–substrate complex, ultimately would reflect the difference in leaving group tendencies between protonated N-1 and N-10 nitrogens and probably be a factor of 10^2 to 10^3. Thus, enzymic transformylations proceeding through orthoamide and amidine intermediates, the latter at N-10 of the cofactor, are an attractive plausibility.[19] Finally, it is noteworthy that condensation of IIa with glycinamide followed by hydrolysis does not lead to *N*-formylglycinamide (>5%). Evidently the presence of an amide function (XII) capable



XII

of intercepting the intermediate formimidoyl species (N-1 or N-10) still is insufficient to redirect the reaction toward net formyl transfer.[20]

Experimental Section

Materials. Synthesis of ethyl *p*-[*N*-2′-(1,2,3,4)tetrahydroquinoxalinylmethylene]aminobenzoate and the related *p*-Cl derivative (Ia and Ib) has been previously described.[3a,21] The corresponding formamidinium fluoroborate salts (IIa and IIb) were prepared according to the published procedure by condensation of Ia and Ib with triethyl orthoformate in ethanol and acidification with aqueous fluoroboric acid.[3b]

The N-1 and N-10 formyl derivatives of Ia and the N-1 formyl derivative of Ib are known compounds.[3b] The preparation of the unknown N-10 formyl derivative of Ib is described below.

The purity of tetrahydroquinoxaline derivatives was analyzed on silica gel tlc (Brinkmann PF-254) by migration with ethyl acetate:cyclohexane (80%:20%, v/v) and visualization with uv light or by spraying with 5% $FeCl_3$ in 0.1 *N* HCl. The ferric chloride spray produced characteristic colors which were stable for about 0.5 hr.

Melting points were taken with a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer Model 257 and were calibrated with polystyrene film. Nuclear magnetic resonance spectra were taken on a Varian Associates A-60 instrument, and chemical shifts are reported in δ units relative to tetramethylsilane. Ultraviolet spectra were taken on a Cary 14 instrument. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind. Solvents, unless otherwise stated, were reagent grade and used as received.

Ethyl *p*-[[[1,2,3,4-Tetrahydro-1-[*N*-(2-methoxyethyl)formimidoyl]-2-quinoxalinyl]methyl]amino]benzoate (IIIa). 2-Methoxyethylamine (Aldrich) (0.10 ml, 1.4 mmol) was added to a solution of *p*-carbethoxyformamidine fluoroborate (IIa) (200 mg, 0.49 mmol) in acetonitrile (5 ml). White thread-like crystals formed upon cooling to −45° (Dry Ice–acetonitrile) and were filtered and washed with cold ether (172 mg, 91%). Recrystallization from absolute ethanol and drying *in vacuo* gave a white powder: mp 124–126°; ir (KBr) 1685, 1640, and 1610 cm^{-1}; uv λ_{max}^{EtOH} 308 nm (ϵ 30,500) and 275 (18,000); nmr ($CDCl_3$) δ 8.10 (s, 1 H) [7.78 and 6.57 (qAB, J = 9 Hz), 7.2 to 6.4 (m)] (8 H), 4.3 to 3.3 (m, 14 H), and 1.33 (t, 3 H, J = 7 Hz); *m/e* 396 (M^+).

Anal. Calcd for $C_{22}H_{28}N_4O_3$: C, 66.67; H, 7.07; N, 14.14. Found: C, 66.79; H, 7.44; N, 14.05.

2-[(*p*-Chloroanilino)methyl]-1,2,3,4-tetrahydro-1-[*N*-(2-methoxyethyl)formimidoyl]quinoxaline (IIIb). 2-Methoxyethylamine (0.12 ml, 1.7 mmol) was added to a solution of *p*-chloroformamidinium fluoroborate (IIb) (300 mg, 0.8 mmol) in acetonitrile (15 ml) and the resulting solution stored in the cold for several hours. Crystallization was induced by scratching and cooling to −45°. After recrystallization from acetonitrile the product was dried *in vacuo* at 56° to yield a white powder (75 mg, 26%): mp 105–107°; ir (KBr) 1630 and 1590 cm^{-1}; uv λ_{max}^{EtOH} 318 nm (ϵ 8700) and 250 (25,600); nmr (acetone-d_6) δ 8.26 (s, 1 H) [7.08 and 6.72 (qAB, J = 9 Hz), 7.2 to 6.5 (m)] (8 H), 3.58 (s, 5 H), 3.35 (s, 6 H), and 2.69 (s, 3 H); *m/e* 358 (M^+).

Anal. Calcd for $C_{19}H_{23}N_4OCl$: C, 63.60; H, 6.42; N, 15.62. Found: C, 63.40; H, 6.27; N, 15.74.

Ethyl *p*-(Methylamino)benzoate (I). Sulfuric acid (11 ml) was added slowly to a suspension of *p*-(methylamino)benzoic acid (Eastman) (15.1 g, 0.1 mol) in ethanol (100 ml). The reaction was refluxed with stirring for 2.5 hr to give a clear solution. The solution was concentrated under reduced pressure to approximately half its volume and neutralized with saturated aqueous sodium bicarbonate. Extraction with methylene chloride and drying ($MgSO_4$) gave a white crystalline product (13.4 g, 75%). Recrystallization from heptane gave ethyl *p*-(methylamino)]benzoate: mp 66–67° [lit.[22] 65–67°].

Ethyl *p*-[(*N*-Formyl)methylamino]benzoate. Ethyl *p*-(methylamino)benzoate (21 g, 0.120 mol) was added to a flask containing 75 ml of benzene and 75 ml of formic acid (90%) fitted with a Dean-Stark trap. The reaction mixture was refluxed with constant stirring for 3 hr and the water which collected in the separator was removed periodically. Evaporation of the solution gave a syrup which was dissolved in ether, washed several times with aqueous saturated sodium bicarbonate, washed with water, and dried ($MgSO_4$). The solvent was evaporated to dryness to yield ethyl *p*-[(*N*-formyl)-methylamino]benzoate (22.1 g, 89%), mp 56–57°. Recrystallizations from 1:1 diethyl ether:petroleum ether (low boiling) gave an analytical sample: mp 57–58.5°; ir (Nujol) 1718, 1680, 1610, 780, and 715 cm^{-1}; nmr ($CDCl_3$) δ 8.63 (s, 1 H), 8.05 and 7.24 (qAB, 4 H, J_{AB} = 8.5 Hz), 4.36 (q, 2 H, J = 7 Hz), 3.33 (s, 3 H), and 1.38 (t, 3 H, J = 7 Hz); uv λ_{max} (pH 7.5) 265 nm (ϵ 17,300); *m/e* 207 (M^+).

Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.96; H, 6.39; N, 6.76.

Ethyl *p*-[[*N*-(2-Methoxyethyl)formimidoyl]methylamino]benzoate Hydrochloride (V). A flask containing ethyl *p*-[(*N*-formyl)methyl-

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amino]benzoate (5.5 g, 0.026 mol), 2-methoxyethylamine (2.0 g, 0.027 mol), and phosphorus pentachloride (8.0 g, 0.038 mol) in 100 ml of chloroform was fitted with a condenser and drying tube and the solution refluxed for 3 hr. The solution was transferred to a 1 l. flask and cooled in Dry Ice–acetone, followed by the addition of 500 ml of anhydrous ether to give a white semisolid. The precipitate was allowed to warm to room temperature, filtered, and dried *in vacuo* (4.4 g, 57%). Recrystallization from ethyl acetate: acetonitrile gave white needles: mp 151–152°; ir (Nujol) 1719, 1698, and 1610 cm^{-1}; uv λ_{max} (pH 7.5) 261 nm (ϵ 18,800) and λ_{max} (pH 10.5) 303 nm (ϵ 28,500); nmr ($CDCl_3$) δ 8.67 (broad d, 1 H), 8.23 and 7.67 (qAB, 4 H, J_{AB} = 8.5 Hz), 5.17 (broad s, 1 H), 4.46 (q, 2 H, J = 7 Hz), 3.87 (broad s, 7 H), 3.45 (s, 3 H), and 1.42 (t, 3 H, J = 7 Hz); *m/e* 263 (M^+ − HCl).

Anal. Calcd for $C_{14}H_{21}O_3N_2Cl$: C, 55.90; H, 7.04; N, 9.43. Found: C, 55.98; H, 6.92; N, 9.24.

The free base form of V was prepared by rapid neutralization with aqueous sodium bicarbonate, extraction with chloroform, and evaporation to give a colorless oil: nmr ($CDCl_3$) [δ 8.11 (s), 7.98 and 7.07 (qAB, J = 9 Hz)] (5 H); 4.33 (q, 2 H, J = 7 Hz) [3.58 (s) and 3.37 (m)] (10 H); and 1.35 (t, 3 H, J = 7 Hz).

Ethyl *p*-[(*N*-Methoxyformimidoyl)methylamino]benzoate (VI). This compound was synthesized in a similar manner to V. Methoxyamine was prepared, from methoxyamine hydrochloride (Eastman), by dissolving the salt in a small amount of water and making it alkaline with sodium hydroxide pellets. It was distilled (bp 50°), weighed, diluted with chloroform, and dried ($MgSO_4$). The addition of phosphorus pentachloride to the solution of methoxyamine and ethyl *p*-[(*N*-formyl)methylamino]benzoate produced vigorous bubbling which subsided in several minutes, after which the solution was raised to reflux temperature. The fine, white solid collected after ether precipitation was briefly suspended in aqueous sodium bicarbonate with stirring and filtered (37% yield), mp 66–68°. Recrystallization from petroleum ether (low boiling) gave white crystals which were dried *in vacuo:* mp 68–69°; ir (Nujol) 1699, 1605, and 1570 cm^{-1}; uv λ_{max} (pH 7.5) 305 nm (ϵ 26,000); nmr ($CDCl_3$) δ 8.36 (s, 1 H), 7.98 and 7.00 (qAB, 4 H, J_{AB} = 9 Hz), 4.35 (q, 2 H, J = 7 Hz), 3.79 (s, 3 H), 3.30 (s, 3 H), and 1.40 (t, 3 H, J = 7 Hz); *m/e* 236 (M^+).

Anal. Calcd for $C_{12}H_{16}O_3N_2$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.77; H, 6.58; N, 11.63.

Ethyl *p*-(3,4,4a,5-Tetrahydroimidazo[1,5-*a*]quinoxalin-2(1*H*)-yl)-benzoate (VII). Compound IIIa (4.4 mg, 0.011 mmol) was dissolved in DMSO (1 ml) and $NaBH_4$ added (0.25 ml of a 0.05 *M* solution in DMSO, 0.012 mmol). The solution exhibited a single $FeCl_3$ positive spot on tlc (R_f 0.59). The product was isolated from preparative tlc and recrystallized from ethanol, mp 168–169°. The mp, ir, uv, mass spectrum, and tlc are identical with an authentic sample of VII.[3a]

Ethyl *p*-[[[1,2,3,4-Tetrahydro-1-[*N*-(2,2,2-trifluoroethyl)formimidoyl]-2-quinoxalinyl]methyl]amino]benzoate (VIII). 2,2,2-Trifluoroethylamine (Columbia Organic Chemical Co.) (3 ml) was added to *p*-carbethoxyformamidinium fluoroborate (IIa) (200 mg, 0.49 mmol) and the mixture allowed to stand for several hours, whereupon the solid dissolved and white thread-like crystals slowly formed. The product was collected and washed with ether (180 mg, 88%), mp 161–163°. Recrystallization several times from acetonitrile and drying *in vacuo* at 56° yielded a white powder: mp 162–163°, ir (KBr) 1690, 1635, and 1590 cm^{-1}; uv λ_{max}^{EtOH} 306 nm (ϵ 30,900) and 274 (17,300); nmr (DMSO-d_6), δ 8.46 (s, 1 H) [7.64 and 6.75 (qAB, J = 9 Hz), 7.2 to 6.4 (m)] (8 H), 4.13 (q, 2 H, J = 7 Hz), 3.5 to 2.9 (m, 7 H), 1.25 (t, 3 H, J = 7 Hz); *m/e* 419 (M^+).

Anal. Calcd for $C_{21}H_{22}N_4F_3$: C, 60.14; H, 5.25; N, 13.36. Found: C, 59.90; H, 5.46; N, 13.43.

***N*-Ethyl[*N*-(2-methoxyethyl)formimidoyl]-*p*-chloroaniline (IX).** *N*-Ethyl-*p*-chloroformanilide was prepared from *p*-chloroaniline, triethyl orthoformate, and sulfuric acid according to the procedure of Roberts and Vogt.[23]

N-Ethyl-*p*-chloroformanilide (9.5 g, 0.052 mol), 2-methoxyethylamine (4.0 g, 0.054 mol), and phosphorus pentachloride (16.0 g, 0.076 mol) were dissolved in 200 ml of chloroform and the solution was refluxed for 3 hr. The solvent was removed under vacuum, and 250 ml of 1 *N* NaOH was added to the resulting oil. The mixture was extracted with ether (3 × 100 ml), dried ($MgSO_4$), and evaporated under vacuum. The product was obtained as a colorless liquid by fractional distillation (8.0 g, 64%): bp 114° (0.02 mm); ir (neat) 1640 and 1590 cm^{-1}; uv λ_{max}^{EtOH} 267 nm (ϵ 28,200); nmr (DMSO-d_6) δ 7.97 (s, 1 H), 7.27 (qAB, 4 H, J = 9 Hz), 3.87 (q, 2 H, J = 7 Hz), 3.49 (s, 4 H), 3.28 (s, 3 H), 1.10 (t, 3 H, J = 7 Hz); *m/e* 240 (M^+).

Anal. Calcd for $C_{12}H_{17}N_2OCl$: C, 59.87; H, 7.07; N, 11.64. Found: C, 59.86; H, 6.94; N, 11.53.

The N-10 Formyl Derivative of Ib. Attempted recrystallization of crude IIIb (200 mg, 0.56 mmol) from hot 2-propanol (20 ml, Fisher purified grade) gave the N-10 formyl product as colorless flakes (74 mg, 42%): mp 115–117°; ir (KBr) 1655 and 1590 cm^{-1}; uv λ_{max}^{EtOH} 310 nm (ϵ 4400); ($CDCl_3$) δ 8.36 (s, 1 H), 7.33 and 7.07 (qAB, 4 H, J = 9 Hz), 6.48 (broad s, 4 H), and 4.0 to 3.0 (m, 7 H); *m/e* 301 (M^+).

Anal. Calcd for $C_{16}H_{16}N_3OCl$: C, 63.68; H, 5.31; N, 13.93. Found: C, 63.82; H, 5.47; N, 13.99.

An authentic sample of the N-1 formyl derivative of Ib (mp 167–170°)[3b] was found to have an R_f of 0.52 (light yellow to $FeCl_3$) whereas the above compound exhibited an R_f of 0.48 (bright blue to $FeCl_3$). The presence of N-1 formyl as well as some additional N-10 formyl product in the mother liquor was detected by tlc.

Kinetics. The instruments employed have been previously described.[24] All reagents and solvents used were reagent grade.

Kinetic runs (25°) were initiated by addition of 0.02 ml of a stock solution of the desired compound to a cuvette containing 2 ml of the aqueous buffer. The final reactant concentration was 5 × 10^{-5} *M*, μ = 0.2, KCl. The stock solutions were freshly prepared with either tetrahydrofuran (tetrahydroquinoxaline derivatives) or acetonitrile (benzocaine derivatives). The course of the reactions was monitored by observing the decrease in OD at 305 (IIIa), 308 (IIIb), 300 (V), or 310 nm (VIII), corresponding to the disappearance of reactant. k_{obsd} (±5%) was taken as slope × 2.303 from a plot of log [(OD_0 − OD_∞)/(OD_t − OD_∞)] *vs.* time. Product distributions for IIIa were determined from ultraviolet spectra after 8–10 half-lives by the previously described method.[3b] The pH of reaction solutions taken before and after runs agreed within 0.02 unit. Values of a_{OH^-} were taken as $10^{-14}/a_{H^+}$.

Product distributions for the hydrolysis of IIb and IIIb were determined as follows. After 10 half-lives, 2-ml buffered solutions containing 5 × 10^{-5} *M* products were concentrated to a small volume, extracted with absolute ethanol, and separated by preparative tlc. The concentration of product in each band was obtained by extraction with absolute ethanol (3 × 2 ml) and summation of the uv spectrum for each extract. Comparison of the spectra of the two products with spectra of original mixtures showed recovery of >90%.

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