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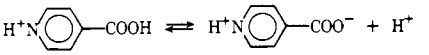
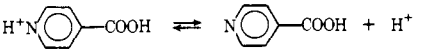
(56) The microscopic ionization constant for the proton dissociation reaction



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



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

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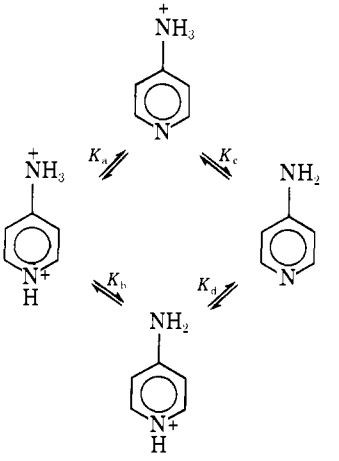
Abstract: The weaker basicity at the 2-amino and 4-amino sites of pyridines, pyrimidines, and purines, when compared to the endocyclic nitrogen atoms, precludes estimates of the pK values for the ionization of the conjugate acids of exocyclic amino groups by direct titration. A kinetic method is proposed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

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

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



$$G_1 = K_a + K_b$$

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

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

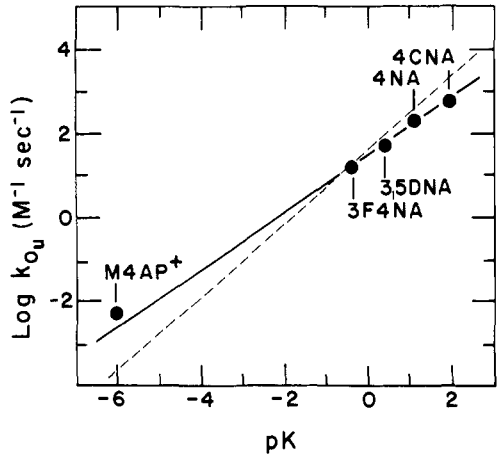
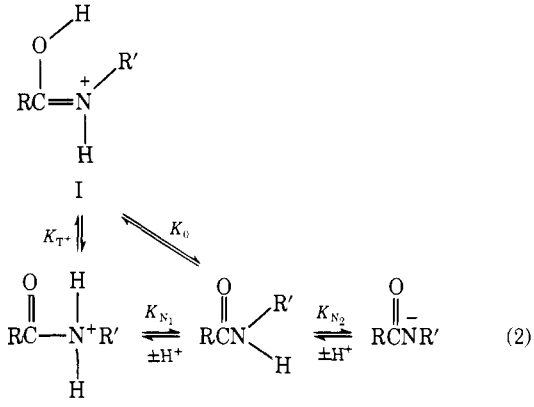


Figure 1. Dependence of pH-independent rate constants for the reaction of amines with unhydrated formaldehyde to form the hydroxymethylamine upon the pK of the conjugate acid of the nucleophile: 25 °C, ionic strength 1.0 M. The solid line is based on eq 5 and parameters obtained by least-squares methods; ordinate intercept (C) = 1.46; slope (β_{nuc}) = 0.63. The dashed line is a theoretical curve (ref 9, Figure 7) based on a stepwise mechanism for addition and has the following parameters which are used in eq 5: ordinate intercept (C) = 1.61; slope (β_{nuc}) = 0.87.

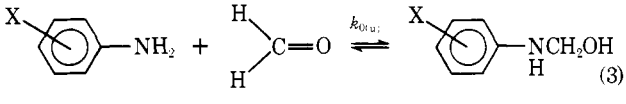
protonated cationic amide nitrogen, K_{N_1}, since protonation on oxygen is dominant (i.e., $1/K_0 \sim 1/G_{1(am)}$ in eq 2).



(2)

$$\frac{1}{G_{1(am)}} = \frac{1}{K_0} + \frac{1}{K_{N_1}}$$

The accompanying study of the catalysis of the reactions of formaldehyde with an isosteric series of weakly basic aromatic exocyclic amines (pK = −6.0–2.0) to form the hydroxymethylamine adducts[9] (eq 3) has resulted in a reactivity–



(3)

basicity correlation according to the Swain-Scott[10] and Bronsted equations[11-13a] (Figure 1).

We wish to propose the application of the reactivity–basicity correlation in order to provide empirical estimates of the pK_c values of the conjugate acid of the amino groups of 2- and 4-amino-substituted pyridines, pyrimidines, and purines.[13b] In addition, the use of the reactivity of the nitrogen atom containing functional groups with formaldehyde in order to provide estimates of the microscopic proton dissociation constants is extended to amides and ureas.

Experimental Section

Fresh solutions of adenosine 5′-monophosphate (Sigma, type V) were made daily. Other materials and the methods utilized in kinetic and equilibrium constant determinations have been described elsewhere.[9]

Table I. Estimated Microscopic Ionization Constants for the Exocyclic Amino Group of Nucleotides and Derivatives at 25 °C

Compound	$k_{0(u)}$, M^{-1} s^{-1} [a]	pK_c [b]	pK_c [f]
Adenosine	0.38 [d]	−3.0 ± 0.17	−2.3
5′-AMP	0.22, 0.37 [c]	−3.4 ± 0.18, −3.0 ± 0.17	−2.6, −2.3
5′-dAMP	0.46	−2.9 ± 0.17	−2.2
Poly A	0.15 [d]	−3.6 ± 0.19	−2.8
5′-CMP	1.2	−2.2 ± 0.15	−1.8
5′-dCMP	1.6	−2.0 ± 0.14	−1.6
5′-GMP	1.3 [e]	−2.1 ± 0.14	−1.7
5′-dGMP	0.49	−2.8 ± 0.16	−2.2

[a] Reference 15; $v = k_{0(u)}[ArNH_2][HCHO]$; ionic strength 0.2 M. [b] From least-squares fit of the data in Figure 1 and pK_c = (log $k_{0(u)}$ − 1.46)/0.63. [c] This work, pH 6.5; ionic strength 1.0 M; general buffer catalysis by phosphate (50% as free base) is observed with a catalytic constant of k_{buf} = 1.24 × 10^{-3} M^{-2} s^{-1} for the rate equation $v_{buf} = k_{buf}[ArNH_2][HCHO][B]$, where B is total buffer. [d] Ionic strength 0.08 M; pH 7.5, sodium phosphate buffer extrapolated from higher temperatures. Poly A exists largely as a single-stranded helix with stacked bases such that the exocyclic amino group is free to interact with formaldehyde: C. L. Stevens and A. Rosenfeld, *Biochemistry*, **5**, 2714 (1966). [e] A small perturbation of the kinetics for the reaction of 5′-GMP with formaldehyde, possibly attributed to a neighboring group effect (ref 15a), may create a small uncertainty in the estimate for the microconstant K_c for this compound. [f] From Figure 1 and eq 5, where $K_c = [ArNH_2]a_{H^+}/[ArNH_3^+]$, C = 1.61 and β_{nuc} = 0.87.

Results and Discussion

Microscopic Ionization of Nucleotides. For the reactions of weakly basic amines with formaldehyde, the similarity in spectral shifts, the magnitude of the equilibrium constants, and the pH dependence of the kinetics (notably the absence of evidence for a change in rate-determining step) indicate that the hydroxymethylamine is the principal product in the reactions with formaldehyde herein described.[9] The kinetic observations, therefore, are uncomplicated by Schiff base formation and pertain simply to hydroxymethylamine formation (eq 3).[9] The rate equation for the pH-independent and hydronium ion dependent N-hydroxymethylation reactions of aromatic amines with respect to the free base amine is given by eq 4.[9]

$$v = (k_0 + k_H a_{H^+})[>NH]([HCHO] + [H_2C(OH)_2]) \quad (4)$$

The pH-independent rate constant for hydroxymethylation with respect to unhydrated formaldehyde is $k_{0(u)}$, where $k_{0(u)} = k_0(1 + K_h)$ and K_h is the hydration constant for formaldehyde ($K_h = [H_2C(OH)_2]/[HCHO] = 2275$).[14] The fact that the rate constant, $k_{0(u)}$, for pH-independent hydroxymethylation reactions of 1-methyl-4-aminopyridinium ion, a heteraromatic exocyclic amine, falls on the line correlating reactivity with basicity for substituted anilines (Figure 1) supports the use of this correlation to estimate microscopic proton dissociation constants of exocyclic 2- and 4-ammonium groups of pyridines, pyrimidines, and purines (see below).

The pH-independent rate constants for hydroxymethylamine formation from unhydrated formaldehyde and nucleotides (calculated from ref 15) and eq 5 provide the estimates of the basicity of the compounds contained in Table I.

$$pK_c = (\log k_{0(u)} - C)/\beta_{nuc} = (\log k_{0(u)} - 1.61)/0.87 \quad (5)$$

Table II. Estimated Microscopic Ionization Constants for Amides and Urea at 25 °C[a]

Compound	k_H, $M^{-2}\ s^{-1}$	$k_{O(u)}$, $M^{-1}\ s^{-1}$	pK^b based upon k_H	pK^b based upon $k_{O(u)}$	pK^b based upon NMR
N-Methylacetamide[c]					−7.8
Propionamide[d]	1.8×10^{-4}		−8.9		
Benzamide[d]	1.35×10^{-4}	9.10×10^{-5}	−9.1	−8.4	
N-Methylurea[c]					−3.9
Urea	0.7×10^{-1}	1.37×10^{-1} [e,f]	−4.3	−3.7	
Methylolurea[e]	2.5×10^{-2}	5.0×10^{-2}	−5.1	−4.7	

[a] Rate constants corrected to 25 °C from the temperature dependence of the data of ref 17a-c. [b] $K_{N_1} = a_{H^+}[RCONH_2]/[RCONH_3^+]$, based upon eq 5 for $k_{O(u)}$ and eq 6 for k_H values. [c] Reference 18; note that the estimated pK_{N_1} value of −7.6 for acetamide (based upon a different technique) is contained in ref 19. [d] Reference 17a. [e] Reference 17c. [f] Reference 17b, $k_{O(u)} = 1.01 \times 10^{-1}\ M^{-1}\ s^{-1}$.

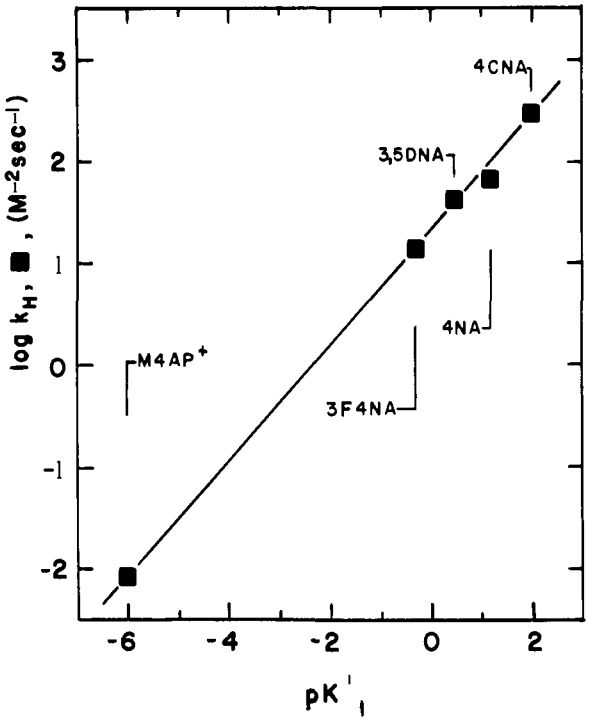


Figure 2. Dependence of hydronium ion catalyzed third-order rate constants for the reaction of aromatic exocyclic amines with formaldehyde upon the basicity of the amine: pK at 25 °C, ionic strength 1.0 M. The solid line is based on eq 6 and parameters obtained by a least-squares method; ordinate intercept (C) = 1.32 ± 0.06; slope (β_{nuc}) = 0.57 ± 0.05.

The constants C and β of eq 5 are the ordinate intercept and slope, respectively, of the data in Figure 1 obtained by a theoretical fit (Figure 1, dashed line).

There is some theoretical justification for the use of this kinetic method for estimates of microscopic proton dissociation constants which derives from the detailed mechanism of *N*-hydroxymethylamine formation. In the previous paper[9] we have presented evidence that the detailed mechanism of these pH-independent hydroxymethylation pathways is stepwise with first the formation of a zwitterionic tetrahedral intermediate $T^{\pm}$ ($>N^+HCH_2O^-$) and second trapping of the $T^{\pm}$ intermediate by a solvent-mediated proton switch. For the weakly basic sites being considered in the present communication, it is the second step, the trapping of the intermediate, that is the rate-determining step. The equilibrium constant for the prior rapid equilibrium step, the formation of $T^{\pm}$ from amine and formaldehyde, K_n, is strongly dependent upon the pK value of the conjugate acid of the amine ($\beta_{nuc} = 0.87$)[9] and therefore accounts directly for the ability to utilize eq 5 for the estimates of microscopic proton dissociation constants contained in Table I (pK_c).

This kinetic method for the estimates of basicity would be expected to be similarly applicable to the bases, nucleosides, or nucleotides, except when neighboring group effects (cf. 5′-GMP)[15] or unusual solvation[16] or steric influences[11] are operative. The data in Table I suggest that such influences, and also ionic strength effects, are not large within the series.

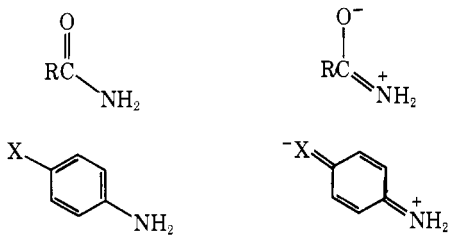
Basicity of Amides. The correlations of the rate constants for the solvent- and hydronium-catalyzed reactions of aromatic exocyclic amines with formaldehyde with the pK_c values of the conjugate acid of the nucleophiles (eq 5 and 6 and Figures 1 and 2) are used with the rate constants for hydroxymethylation of amides with formaldehyde to estimate *empirically* the microscopic proton dissociation constants of N-protonated cationic amides ($RCON^+H_2R'$) (Table II). Perhaps the relationship discussed above of the equilibrium constant, K_n, with the pK value of the N-protonated conjugate acid allows the use of a correlation based on aromatic amines to estimate pK_{N_1} values for amides. In addition, the following similarities between aromatic amines and amides may be noted, which are consistent with the use of the same method for the estimation of their respective basicities.

(1) Equilibrium constants for the addition of formaldehyde to an amide to form the hydroxymethyl derivative[17a] (eq 3) are of the same order of magnitude[12] [$K \sim 20\ M^{-1} = [RCONHCH_2OH]/([HCHO] + [H_2C(OH)_2])[RCOHN_2]$] as for aromatic amines.[9] Furthermore, the association constants for hydroxymethylamine formation from amides[17a] and aromatic amines are comparably independent of substituents and thus the basicity of the nitrogen atom.

(2) The kinetics of the reactions for hydroxymethylation of amides and aromatic amines similarly show (a) first-order dependence of rate in respect to both nucleophile and formaldehyde concentrations in the direction of association,[17] (b) buffer catalysis,[17a,c] and (c) rate-pH profiles, when correction is made for buffer contributions to the rate, with characteristic hydronium ion, hydroxide ion, and pH-independent terms in the rate law.[9,17] In contrast, the rate law for the reaction of aliphatic amines and formaldehyde fails to exhibit hydronium ion or hydroxide ion or buffer catalysis. From the limited data available for a Bronsted plot of the general acid catalysis of the reaction of benzamide and formaldehyde,[17a] the value of α is 0.39 ± 0.02, which is not unreasonable when this compound of p$K_{N_1} \approx -9$ (Table II) is compared with the Bronsted α value of 0.28 for 4-amino-1-methylpyridinium ion (p$K_c = -6$).[9]

(3) The weak basicity of the nitrogen atom of amides and exocyclic aromatic amines is due to contributions to the resonance hybrids of the similar valence bond structure of type I and II, respectively.

The rate equation for the pH-independent, k_0, and hydronium ion, k_H, catalyzed hydroxymethylamine formation from amides and ureas is given in eq 4. Estimates of microscopic proton dissociation constants of amides and ureas were



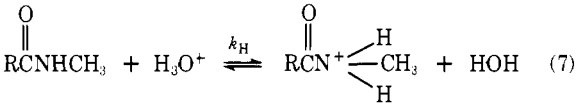
I II

obtained from the data in Figures 1 and 2 and eq 5 and 6 (Table II).

$$pK = (\log k_H - 1.32)/0.57 \quad (6)$$

The agreement of the present estimates with the following two additional independent methods is noteworthy.

(1) NMR measurements of the specific acid catalyzed rate of the amide proton[18] exchange (eq 7)

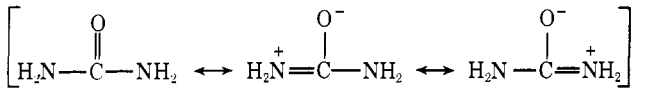


(7)

For example, the difference in pK_{N_1} values for *N*-methylacetamide and *N*-methylurea obtained from the NMR method is approximately 3.9, which compares favorably with a ΔpK_{N_1} for propionamide and urea of 4.6 (Table II).

(2) Kinetic studies of tertiary amine catalyzed ester hydrolysis which have been extended to provide an estimate of pK_{N_1} for *N*-methylacetamide of -7.6 [19] (Table II).

That the pK_{N_1} value for urea is about four units higher than acetamide may be attributed to a type of resonance, III, which



III

is less disrupted upon nitrogen protonation than is the resonance stabilization in an amide.[20]

Acknowledgment. We are grateful to Mrs. M. Frederick and Dr. P. Tobias for the data on the reaction of 5′-AMP and formaldehyde.

References and Notes

(1) (a) This project was supported by the National Institute of Health, U.S. Public Health Service, Grants GM 13 777 (R.G.K.), FR5414 (University of Pennsylvania, School of Medicine), and FR 115 (Computer Facility). (b) U.S. Public Health Service Predoctoral Trainee, 1966–1971.

(2) Abbreviations: p*K* is the negative logarithm of the proton dissociation constant; $ArNH_2$, aromatic exocyclic amine; $M4AP^+$, 4-amino-1-methylpyridinium ion; 4NA, 4-nitroaniline; 4CNA, 4-cyanoaniline (4-aminobenzonitrile); 3F4NA, 3-fluoro-4-nitroaniline; 3,5DNA, 3,5-dinitroaniline; Poly A, poly(adenylic acid); 5′-AMP, adenosine 5′-phosphate; 5′-dAMP, deoxyadenosine 5′-phosphate; 5′-CMP, cytidine 5′-phosphate; 5′-dGMP, deoxyguanosine 5′-phosphate.

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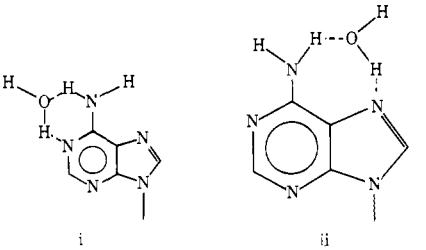
(16) (a) In this regard the similarity in K_1 values for anilines (Table II in ref 9) with that for adenine[16b] ($K_1 = 20$ M^{-1}) and evidence that the association constant for exocyclic aromatic *N*-hydroxymethylamines with formaldehyde to form the *N,N*-dihydroxymethylamines (K_3) is 20–90-fold smaller than K_1 (Table II in ref 9), indicate that an explanation put forth by Lewin[16b] for the predominance of *N*-hydroxymethylamine formation over *N,N*-dihydroxymethylamine based on specific solvation effects involving the N^6 and



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N^1 (i) or N^7 (ii) positions on adenine need not be invoked since these specific solvation effects are not possible for the substituted anilines. (b) S. Lewin, *J. Chem. Soc.*, 792 (1964).

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(20) (a) Applications of eq 5 and 6 provide estimates of microscopic proton dissociation constants of $10^{6.2}$ and $10^{7.5}$ from k_0 and k_H values,[20b] respectively, for $K_{N_1} = [-C(S)NH_2]a_{H^+}/[-C(S)NH_3^+]$ for thiourea. Since the macroscopic ionization constant for thiourea is $10^{0.96}$, the prototropic tautomerization constant $K_T = [H_2NC(SH)NH_2^+]/[H_2NC(=S)NH_3^+] \sim 10^{6.2}/10^{0.96} \sim 10^{5.3}$. (b) K. Dušek, *Collect. Czech. Chem. Commun.*, **25**, 108 (1960).