Equilibrium Formation of Anilides from Carboxylic Acids and Anilinesin Aqueous Acidic Media

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Abstract: The formations of formanilide, *p*-methoxyformanilide, *p*-nitroformanilide, and acetanilide from their corresponding carboxylic acids and anilines in aqueous acidic media have been investigated at temperatures between 60 and 100 °C under a variety of conditions such as pH, D_2O, added phosphate, and added ethanol. In each case, the pseudo-first-order rate constants for the establishment of equilibrium (k_{obs}), from both the hydrolysis and formation directions, and the conditional equilibrium constant ($K' = $ [anilide]/[aniline]$_{total}$) were determined in excess formate. From K', and knowledge of how the pK_a values of RCOOH and anilinium ion depend on the various conditions, is derived a corrected equilibrium constant, K'_{eq}, defined as [anilide]/ ([aniline][RCOOH]). In the case of formanilide, the K' value is found to be invariant with temperature reductions, although the K'_{eq} value increases. In D_2O media, the K' value drops slightly, but after correcting for the medium induced changes in [aniline] and [RCOOH], the K'_{eq} value is the same as in water. In the presence of added KH_2PO_4, the rate of establishment of equilibrium increases but the K' and K'_{eq} values do not change relative to their values without phosphate. Added ethanol is found to increase both the rate of establishment of equilibrium and the K' equilibrium constants, but reduces K'_{eq}. The mechanism of formation of anilides in water under acidic conditions is discussed.

Introduction

The great bulk of studies concerning amide hydrolysis are conducted under acidic or basic conditions where the reaction proceeds essentially to hydrolyzed products.[1] Consequently, it is generally believed that amide bond formation in aqueous solution is unfavorable because the experimental conditions under which the reactions are conducted favor the hydrolysis process (eq 1). However, from the biochemical perspective, it

has long been known that hydrolytic enzymes are also capable of reforming peptides in solution.[2,3] The formation of amide

bonds in aqueous media in the absence of enzymes is also of interest since this is implicated in the origin of life. Attempts[4−8] have been made to form peptides from amines and acids or by coupling amino acids under conditions that resemble those of primitive earth. In studies simulating so-called prebiotic conditions in water, linear and cyclic polyphosphates,[5] cyanamide,[6] metal ions,[7] silica, alumina and clay,[8] and more recently iron sulfide plus H_2S[9] have been used for promoting amide bond formation from acids and amines.

Reformation of lactams from their amino acid hydrolysis products is well-documented and, in many cases, is very favorable due to the intramolecularity of the process.[10] Studies by Fersht and Requena,[11] Morawetz and Otaki,[12] Guthrie,[13] and

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us[14] have shown that it is possible, under certain conditions, to form substantial amounts of simple amides from intermolecular reaction of their constituent acids and amines in water either with or without catalysis. In view of the attractiveness of forming the amide bond under "green" conditions that avoid the use of acyl activating agents and dry, non-hydroxylic solvents, we have embarked upon a program to investigate the scope and limitations of the aqueous amide bond formation so as to define the potential applications. The following reveals our findings for the formation of simple anilides in aqueous acidic media under acidic conditions.

Experimental Section

A. Materials and General Methods. Aniline, formic acid, and *p*-nitroaniline were obtained from BDH. Acetanilide, formanilide, acetic anhydride, and *p*-methoxyaniline were purchased from Aldrich and used as supplied. Glacial acetic acid was obtained from Fisher Scientific. Methanol (HPLC grade) was obtained from EM Science. Aniline and acetic acid were distilled prior to use. All HPLC solvents were filtered through a 0.45 μm filter before use. All melting points were obtained using Fisher-Johns melting point apparatus and are uncorrected.

All buffers were prepared using purified deoxygenated water from an Osmonic Aries water purification system. The pH was measured at ambient temperature using a Radiometer Vit 90 video titrator equipped with a GK2321 C combination electrode, standardized by Fisher Certified pH 2, 4, 7, and 10 buffers.

B. Synthesis. *p*-Nitroformanilide and *p*-methoxyformanilide were synthesized from *p*-nitroaniline and *p*-methoxyaniline and formic acetic anhydride as described[15] by Krishnamurthy. *p*-Nitroformanilide was recrystalized 2 times from ethyl acetate before use and had a melting point of 196−197 °C (lit.[16] mp 196−198 °C). *p*-Methoxyformanilide was purified by recrystalizing 3 times from a chloroform−hexane mixture. The pure product had a melting point of 79−80 °C (lit.[17] mp 78−80 °C).

C. Analytical HPLC Conditions. For HPLC analysis a Hewlett-Packard 1050 series HPLC system, fitted with a variable-wavelength UV−vis detector and autoinjector, was used. For separation, an μ-Bonda-Pak C$_{18}$ (Waters) cartridge column was used. A gradient mixture of 0.005 M potassium phosphate buffer (pH 7.2) and methanol was used to separate aniline from formanilide. For each injection the initial solvent composition was 20% methanol:80% phosphate buffer, which after 15 min was modified to 30% methanol:70% phosphate buffer until 21 min, whereupon 100% methanol was used to wash the column for 9 min before the next injection. For separation of aniline and acetanilide, an isocratic mixture of 25% methanol:75% phosphate buffer (0.005 M, pH 7.2) was used. In both cases, the flow rate was 1.2 mL/min and the detector was set at λ = 231 nm.

D. Kinetics of Amide Formation and Hydrolysis by HPLC. For kinetics studies at 98 $\pm$ 2 °C, (5.0−5.3) × 10^{-3} M formanilide or aniline solutions were made in 10−20 mL of formate buffer (pH 3.2−4.2, [buffer]$_{total}$ = 0.1−1.0 M, μ = 1.0 M (KCl)) and were degassed by passing Ar through them for 30 min. These solutions were then divided into 10−20 autosampler vials that were then sealed with Teflon-lined septa. The vials were heated in a boiler containing boiling water, and at certain intervals, the vials were withdrawn, cooled immediately in ice water, and analyzed at ambient temperature by HPLC. The pseudo-first-order rate constants for appearance and disappearance of aniline and formanilide were obtained by NLLSQ fitting of peak area vs time data to a standard exponential model (vide supra). The response factors for formanilide and aniline under the experimental conditions were

obtained by injecting, and determining the peak areas of, a 1:1 formanilide and aniline mixture. For each run two rate constants could be obtained, for example, in the hydrolysis direction, the pseudo-first-order rate constant of disappearance of the formanilide as well as the rate constant for the appearance of aniline.

Kinetic data for formation and hydrolysis of acetanilide were obtained similarly at 98 $\pm$ 2 °C, pH 1.95 $\pm$ 0.03 and pH 3.72 $\pm$ 0.03 with (5.2−5.4) × 10^{-3} M solutions of acetanilide or aniline, μ = 1.0 (KCl). At lower pH, HCl comprised the buffer and 1.0 M acetic acid was added to the solutions. At higher pH, acetate buffer was used with the total [buffer] being 1.0 M.

E. Kinetics by UV−Vis Spectrophotometry. The rates of formation and hydrolysis of formanilide, *p*-nitroformanilide, and *p*-methoxyformanilide were observed at 79 $\pm$ 1 °C using a Cary-219 UV−vis spectrophotometer interfaced with an IBM 486 PC equipped with Olis software (Online Instrument Systems, Jefferson GA, 1992). Kinetic data were obtained by observing the rate of change in absorbance (increase for formation and decrease for hydrolysis) of formanilide at 246 nm and *p*-methoxyformanilide at 260 nm. For *p*-nitroformanilide, rates of formation and hydrolysis were obtained by following the rates of disappearance and appearance of *p*-nitroaniline, respectively, at 429 nm. Runs were initiated by injecting 5 μL of a stock solution in DME ((5.0−6.9) × 10^{-2} M) of either (*p*-H, *p*-NO$_2$, or *p*-OCH$_3$)-aniline or (*p*-H or *p*-NO$_2$ or *p*-OCH$_3$)-formanilide into 3 mL of formate buffer (pH 2.80−4.20, [buffer]$_{total}$ = 0.001−1.0 M, μ = 1.0 (KCl)), which had been thermally equilibrated at 79 $\pm$ 1 °C in the instrument cell holder for 30 min. The final pH of each of the runs was measured and shown to agree with the initial pH. The pseudo-first-order rate constants were obtained by NLLSQ fitting the absorbance vs time data to a standard exponential model ($A_t = A_\infty + (A_0 - A_\infty) \exp(-kt)$).

The acid-catalyzed hydrolysis of formanilide was followed at 79 $\pm$ 1 °C using 9.86 × 10^{-3} M HCl, μ = 1.0 (KCl), with runs being initiated as above. All the runs were followed for at least 5 half-lives.

F. D$_2$O Studies. The rate of formation and hydrolysis of formanilide at 79 $\pm$ 1 °C was determined in formate buffer ([buffer]$_{total}$ = 0.25−1.0 M) at pD 3.60 $\pm$ 0.03 where pD = pH$_{measured}$ + 0.40.[18] The rate of hydrolysis of formanilide was also obtained in 1.10 × 10^{-2} M DCl, μ = 1.0 (KCl). The kinetic data were obtained and analyzed as above.

G. Studies in the Presence of Phosphate. The rate of formation and hydrolysis of formanilide was determined similarly at 79 $\pm$ 1 °C in the presence of phosphate (0.10−0.50 M) in 1.0 M formate buffer at pH 3.20 $\pm$ 0.03 and pH 3.60 $\pm$ 0.03, with the ionic strength again being maintained at 1.0 (KCl).

H. Studies in Aqueous Ethanol. The rate of formation and hydrolysis of formanilide was obtained in 1.0 M formate buffer in 20% (v/v) ethanolic water, pH 3.59, μ = 1.0 (KCl). The kinetic data were obtained as above at 60 $\pm$ 0.3 °C. Kinetic data were also obtained in 80% (v/v) ethanolic water containing 1.0 M formate at pHs 3.60 and 4.92 at the same temperature. The pH was adjusted by adding a suitable amount of concentrated NaOH or HCl and measured before and after the reaction. The ionic strength in 80% ethanol−20%water was not corrected. The rate of formation and hydrolysis of formanilide was also obtained at 60 °C in 1.0 M aqueous formate buffer pH 3.60, μ = 1.0 (KCl).

I. Determination of pK_a. The pK_a's of aniline and formic acid were determined by titration at 24 $\pm$ 1 °C. For each determination, 0.048−0.051 mmol of aniline or formic acid was used in a 5 mL solution. In the case of aniline enough HCl was added to convert all of it to anilinium ion. The pH was measured using a Radiometer Vit 90 video titrator equipped with a GK2321C combination electrode and interfaced with an IBM PC. The pH was recorded as a function of added 0.0105 M NaOH, which was delivered by a Radiometer ABU 91 autoburet. The ionic strengths of all of the solutions were maintained at 1.0 using KCl. Data were analyzed by a computer version of Simms method.[19] The pK_a values reported are an average of three determinations. Reported in Table 10 (as calculated in the Appendix) are pK_a values corrected for the temperature and medium effects.

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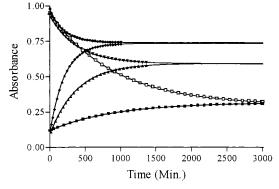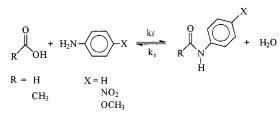(19) Huguet, J. Ph.D. Thesis, University of Alberta, 1980

Figure 1. Typical kinetic traces for formanilide equilibration at pH 3.60 at different [formate]$_{total}$, 0.10−1.00 M, $\mu = 1.0$ (KCl), and $T = 79 \pm 1$ °C. The lines are obtained from NLLSQ fitting of data to $A_t = A_\infty + (A_0 - A_\infty) \exp(-k_t)$. formation at 0.1 M (■), hydrolysis at 0.1 M (□), formation at 0.5 M (▲), hydrolysis at 0.5 M (▼), formation at 1.0 M (◆), and hydrolysis at 1.0 M (●).

Scheme 1

R = H   X = H
    CH$_3$ NO$_2$
                 OCH$_3$

Results

The approach to equilibrium for the anilides given in Scheme 1 was followed both from the direction of formation and hydrolysis under a variety of conditions such as temperature, pH, [RCOOH]$_{total}$, and the presence of the additives ethanol and phosphate. Shown in Figure 1 are typical absorbance vs time traces for the equilibration of formanilide with aniline and formate at pH 3.60 and 79 °C in the presence of three different [formate]$_{total}$. The pseudo-first-order rate constant for the approach to equilibrium (k_{obs}) can be obtained by NLLSQ fitting of the absorbance vs time data to a standard exponential model, $A_t = A_\infty + (A_0 - A_\infty) \exp(-kt)$. The k_{obs} can be expressed in terms of the forward and reverse rate constants, k_f and k_r, (Scheme 1) as in eq 2, while the conditional equilibrium

constant, K', is given in eq 3.

$$k_{obs} = k_f + k_r \tag{2}$$

$$K' = k_f/k_r \tag{3}$$

In practical terms, K' is the ratio of [anilide]/[aniline]$_{total}$ at equilibrium with a given excess of RCOOH. Given in Tables 1S−12S (Supporting Information) are the original k_{obs} data for appearance or disappearance of various the anilides as well as the conditional equilibrium constants, K', determined at equilibrium by HPLC methods or from the residual absorbance in the absorbance vs time UV kinetic plots. Given in Tables 1−8 are the pseudo-first-order rate constants for formation (k_f) and hydrolysis (k_r) of the anilides as well as the corrected equilibrium constant, K'_{eq}, which is based on the concentrations of nonionized aniline and formic acid with the concentration of water set as unity as in eq 4.[2b] The acid and amine concentrations can be

$$K'_{eq} = \frac{[\text{formanilide}]}{[\text{aniline}][\text{formic acid}]} \tag{4}$$

determined from the pK_a values for the various RCOOH and Ar−NH$_3^+$, corrected for the conditions of temperature and media as described in the Appendix, the corrected values being listed in Table 10. We deal with the specific conditions of temperature and structure below, followed by the effects of [formate] and additives.

A. Effect of Temperature and Structure on Anilide Equilibrium Constants. Given in Tables 1−3 are the experimental k_f, k_r, and K'_{eq} values for equilibration of formanilide and acetanilide at various pHs and [RCOOH]$_{total}$ at the indicated temperatures. The data show that the K'_{eq} for formanilide at 98 °C (12.6 M^{-1}) is larger than that for acetanilide (3.4 M^{-1}) by a factor of about 3.7. As expected, equilibrium is reached faster at higher temperature, but reducing the temperature from 98 to 79 °C leads to an increase in the K'_{eq} for formanilide of roughly 1.6-fold, (12.6 vs 20.1 M^{-1}). Given in Table 3 are data determined in D$_2$O at 79 °C that indicate that, at pH (pD) 3.60, the overall solvent deuterium kinetic isotope effect (DKIE) on

Table 1. Pseudo-First-Order Rate Constants[a] for Formation (k_f) and Hydrolysis (k_r) of Formanilide at 98 ± 2 °C and the Equilibrium Constants[b] (K'_{eq}) Obtained by HPLC at Various pHs and Buffer Concentrations in Aqueous Formate Buffer

pH	[formate]$_{total}$[c] (M)	k_r (s^{-1})	k_f (s^{-1})	K'_{eq} (M^{-1})
4.17 ± 0.03	0.10	$(1.22 \pm 0.03) \times 10^{-5}$	$(0.23 \pm 0.01) \times 10^{-5}$	12.3 ± 0.2
3.60 ± 0.03	0.10	$(4.15 \pm 0.02) \times 10^{-5}$	$(1.13 \pm 0.04) \times 10^{-5}$	13.1 ± 0.6
3.18 ± 0.03	0.10	$(1.29 \pm 0.06) \times 10^{-4}$	$(2.56 \pm 0.11) \times 10^{-5}$	12.9 ± 0.2
3.57 ± 0.03	0.50	$(9.72 \pm 0.30) \times 10^{-5}$	$(1.20 \pm 0.01) \times 10^{-4}$	11.9 ± 0.4
3.57 ± 0.03	1.00	$(1.27 \pm 0.04) \times 10^{-4}$	$(3.36 \pm 0.08) \times 10^{-4}$	12.7 ± 0.2

[a] Calculated from the pseudo-first-order rate constants (k_{obs}) for establishment of equilibrium, using $k_{obs} = k_r + k_f$ and $K' = k_f/k_r$. Errors are deviation from the mean for duplicate values and standard deviations for triplicate values. [b] Calculated using the nonionized concentrations at equilibrium (eq 4), taking concentration of water as unity and accounting for changes in the pK_a's due to temperature and ionic strength variations. [c] $\mu = 1.0$ (KCl).

Table 2. Pseudo-First-Order Rate Constants[a] for Formation (k_f) and Hydrolysis (k_r) of Acetanilide at 98 ± 2 °C and the Equilibrium Constants[b] (K'_{eq}) at Various pH Values in Aqueous HCl or Acetate Buffer

pH	buffer	k_r (s^{-1})	k_f (s^{-1})	K'_{eq} (M^{-1})
1.95 ± 0.05	HCl[c,e,]	$(3.47 \pm 0.04) \times 10^{-5}$	$(0.19 \pm 0.02) \times 10^{-5}$	3.6 ± 0.3
3.75 ± 0.05	acetate[d,e]	$(0.81 \pm 0.02) \times 10^{-6}$	$(1.07 \pm 0.02) \times 10^{-6}$	3.2 ± 0.3

[a] Calculated from the pseudo-first-order rate constants (k_{obs}) for establishment of equilibrium, using $k_{obs} = k_r + k_f$ and $K' = k_f/k_r$. Errors are deviation from the mean for duplicate values and standard deviations for triplicate values. [b] Calculated using the nonionized concentrations at equilibrium, taking concentration of water as unity and accounting for changes in the pK_as due to temperature and ionic strength variations. [c] 1.0 M acetate added. [d] [buffer]$_{total}$ = 1.0 M. [e] $\mu = 1.0$ (KCl).

Table 3. Pseudo-First-Order Rate Constants[a] for Formation (k_f) and Hydrolysis (k_r) of Formanilide at 79 ± 1 °C and the Equilibrium Constants[b] (K'_{eq}) at Various pHs or pDs and at Various Buffer Concentrations in Aqueous Formate Buffer

pH or pD	[formate]$_{total}$[d] (M)	K_r (s^{-1})	k_f (s^{-1})	K'_{eq} (M^{-1})
2.80 ± 0.03	0.10	$(7.02 \pm 0.05) \times 10^{-5}$	$(0.77 \pm 0.01) \times 10^{-5}$	20.6
2.79 ± 0.03	0.50	$(7.60 \pm 0.10) \times 10^{-5}$	$(4.29 \pm 0.05) \times 10^{-5}$	22.7
2.81 ± 0.03	0.90	$(7.69 \pm 0.02) \times 10^{-5}$	$(8.22 \pm 0.02) \times 10^{-5}$	20.7
3.18 ± 0.03	0.10	$(3.32 \pm 0.05) \times 10^{-5}$	$(0.66 \pm 0.02) \times 10^{-5}$	19.1
3.20 ± 0.03	0.50	$(3.66 \pm 0.05) \times 10^{-5}$	$(3.60 \pm 0.05) \times 10^{-5}$	18.2
3.20 ± 0.03	1.00	$(4.09 \pm 0.03) \times 10^{-5}$	$(8.20 \pm 0.02) \times 10^{-5}$	18.6
3.56 ± 0.03	0.001	$(1.36 \pm 0.02) \times 10^{-5}$		
3.60 ± 0.03	0.10	$(1.42 \pm 0.02) \times 10^{-5}$	$(0.42 \pm 0.01) \times 10^{-5}$	20.4
3.61 ± 0.03	0.50	$(1.76 \pm 0.03) \times 10^{-5}$	$(2.64 \pm 0.02) \times 10^{-5}$	20.9
3.59 ± 0.03	1.00	$(2.15 \pm 0.03) \times 10^{-5}$	$(5.97 \pm 0.04) \times 10^{-5}$	19.5
4.01 ± 0.03	0.10	$(5.93 \pm 0.02) \times 10^{-6}$	$(1.83 \pm 0.01) \times 10^{-6}$	20.9
4.02 ± 0.03	0.50	$(7.90 \pm 0.01) \times 10^{-6}$	$(1.25 \pm 0.01) \times 10^{-5}$	21.7
4.03 ± 0.03	1.00	$(1.06 \pm 0.01) \times 10^{-5}$	$(2.94 \pm 0.01) \times 10^{-5}$	19.0
3.60 ± 0.03[c]	0.001	$(1.34 \pm 0.03) \times 10^{-5}$		
3.58 ± 0.03[c]	0.50	$(1.93 \pm 0.09) \times 10^{-5}$	$(1.77 \pm 0.10) \times 10^{-5}$	22.3
3.61 ± 0.03[c]	0.75	$(2.01 \pm 0.12) \times 10^{-5}$	$(2.76 \pm 0.30) \times 10^{-5}$	21.9
3.60 ± 0.03[c]	1.00	$(2.18 \pm 0.03) \times 10^{-5}$	$(3.96 \pm 0.06) \times 10^{-5}$	21.6

[a] Calculated from the pseudo-first-order rate constants (k_{obs}) for establishment of equilibrium, using $k_{obs} = k_r + k_f$ and $K' = k_f/k_r$. Errors are deviation from the mean for duplicate values and standard deviations for triplicate values. [b] Calculated using the nonionized concentrations at equilibrium and taking concentration of water as unity. Changes in the pK_a's due to temperature, ionic strength, and solvent isotope effects have been considered. [c] pD. [d] $\mu = 1.0$ (KCl).

Table 4. Pseudo-First-Order Rate Constants[a] for Formation (k_f) and Hydrolysis (k_r) of *p*-Nitroformanilide at 79 ± 1 °C and the Equilibrium Constants[b] (K'_{eq}) at Various pHs and Buffer Concentrations in Aqueous Formate Buffer

pH	[formate]$_{total}$[c] (M)	k_r (s^{-1})	k_f (s^{-1})	K'_{eq} (M^{-1})
2.80 ± 0.03	1.00	$(2.33 \pm 0.05) \times 10^{-4}$	$(5.34 \pm 0.04) \times 10^{-5}$	0.26
3.18 ± 0.03	0.10	$(9.86 \pm 0.02) \times 10^{-5}$	$(2.16 \pm 0.20) \times 10^{-6}$	0.29
3.20 ± 0.03	0.50	$(1.12 \pm 0.03) \times 10^{-4}$	$(1.17 \pm 0.05) \times 10^{-5}$	0.27
3.20 ± 0.03	1.00	$(1.28 \pm 0.03) \times 10^{-4}$	$(2.40 \pm 0.06) \times 10^{-5}$	0.26
3.60 ± 0.03	1.00	$(7.22 \pm 0.07) \times 10^{-5}$	$(9.33 \pm 0.60) \times 10^{-6}$	0.25

[a] Calculated from the pseudo-first-order rate constants (k_{obs}) for establishment of equilibrium, using $k_{obs} = k_r + k_f$ and $K' = k_f/k_r$. Errors are deviation from the mean for duplicate values and standard deviations for triplicate values. [b] Calculated using the nonionized of concentrations at equilibrium and taking concentration of water as unity. Changes in the pK_a's due to temperature have been considered. For formic acid, the change in pK_a due to ionic strength variation has also been considered. However, the effect of ionic strength on the pK_a of *p*-nitroanilinium ion was not considered since at these pHs, a change of ±0.35 in pK_a unit will have less than 2% effect on the K'_{eq}. [c] $\mu = 1.0$ (KCl).

Table 5. Pseudo-First-Order Rate Constants[a] for Formation (k_f) and Hydrolysis (k_r) of *p*-Methoxyformanilide at 79 ± 1 °C and the Equilibrium Constants[b] (K'_{eq}) at pHs 2.80 and 3.20 in Aqueous Formate Buffer

pH[c]	k_r (s^{-1})	k_f (s^{-1})	K'_{eq} (M^{-1})
2.82 ± 0.03	$(4.08 \pm 0.05) \times 10^{-5}$	$(4.01 \pm 0.04) \times 10^{-5}$	41.0
3.18 ± 0.03	$(2.13 \pm 0.03) \times 10^{-5}$	$(4.11 \pm 0.06) \times 10^{-5}$	38.9

[a] Calculated from the pseudo-first-order rate constants (k_{obs}) for establishment of equilibrium, using $k_{obs} = k_r + k_f$ and $K' = k_f/k_r$. Errors are deviation from the mean for duplicate values and standard deviations for triplicate values. [b] Calculated using the nonionized of concentrations at equilibrium and taking concentration of water as unity and accounting for changes in the pK_a's due to temperature and ionic strength. [c] [buffer]$_{total} = 1.0$, $\mu = 1.0$ (KCl).

the K'_{eq} for formanilide is 1.1 ± 0.1 but that the DKIE on $K' = k_f/k_r$ is 1.5–1.6 ([formate]$_{total}$ = 0.5–1.0 M).

Given in Tables 4 and 5 are the k_f, k_r, and K'_{eq} values for the equilibration of *p*-nitroformanilide and *p*-methoxyformanilide at 79 °C at various pHs. The respective K'_{eq} values of 0.26 and 40 M^{-1}, coupled with the value of 20.1 M^{-1} for formanilide at 79 °C, indicate a strong dependence on the basicity of the aniline substituent.

B. Effect of [formate] on k_f and k_r. Shown in Figure 2 are typical plots of the pseudo-first-order rate constants for formation (k_f) and hydrolysis (k_r) of formanilide as a function of [formate]$_{total}$ at 98 °C, pH = 3.60, and $\mu = 1.0$ (KCl). Similar plots (not shown) can be made for the processes at 79 °C. The data indicate that the hydrolysis follows a linear dependency on the [formate], while the formation follows an exponential behavior. The kinetic/concentration data for the two processes
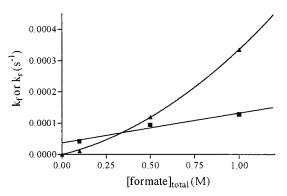

Figure 2. Pseudo-first-order rate constants of formation (k_f, ▲) and hydrolysis (k_r, ■) of formanilide vs [formate]$_{total}$ at 98 ± 2 °C (pH = 3.60 and $\mu = 1.0$ (KCl)). The lines were obtained from the fits to eqs 5 and 6.

can be fit to eqs 5 and 6,

$$k_r = k_{0r} + k_{1r}[\text{formate}]_{total} \tag{5}$$

$$k_f = [\text{formate}]_{total}(k_{1f} + k_{2f}[\text{formate}]_{total}) \tag{6}$$

where k_{0r}, the intercept for eq 5, is the spontaneous pseudo-first-order rate constant for hydrolysis at pH 3.60, k_{1r} is the second-order rate constant for formate-catalyzed hydrolysis, and k_{1f} and k_{2f} are the second- and third-order rate constants for formate-dependent hydrolysis and formation of formanilide. Fitting of the appropriate hydrolysis and formation data at 98 and 79 °C (from Tables 1 and 3) to eqs 5 and 6 gives the values

Table 6. Pseudo-First-Order Rate Constants for the Acid Catalyzed Hydrolysis, k_{0r} (s^{-1}),[a] of Formanilide at a Given pH or pD, Second-Order Rate Constants, k_{1r} (M^{-1} s^{-1}),[a] of Formate (buffer) Catalysis on Hydrolysis, and Second- and Third-Order Rate Constants, k_{1f} (M^{-1} s^{-1})[a] and k_{2f} (M^{-2} s^{-1}),[a] of Formate (buffer) Catalysis on Formation of Formanilide from Aniline and Formic Acid at Various Temperatures and pHs or pDs

temp (°C)	pH or pD	$k_{0r} \times 10^5$ (s^{-1})	$k_{1r} \times 10^6$ (M^{-1} s^{-1})	$K_{1f} \times 10^5$ (M^{-1} s^{-1})	$k_{2f} \times 10^5$ (M^{-2} s^{-1})
79 ± 1	2.80	7.02 ± 0.21	8.50 ± 3.50[b]	7.84 ± 0.09	1.45 ± 0.11
79 ± 1	3.20	3.24 ± 0.01	8.45 ± 0.01	6.24 ± 0.05	1.95 ± 0.06
79 ± 1	3.60	1.35 ± 0.01	8.01 ± 0.14	4.45 ± 0.14	1.47 ± 0.15
79 ± 1	4.00	0.54 ± 0.01	5.19 ± 0.13	2.02 ± 0.08	0.93 ± 0.08
79 ± 1	3.60[c,d]	1.38 ± 0.08	8.31 ± 1.10	3.04 ± 0.10	0.91 ± 0.10
79 ± 1	2.00[d]	43.8 ± 0.01			
79 ± 1	1.96[c]	53.2 ± 0.01			
98 ± 2	3.60	3.72 ± 0.56	95.0 ± 18.0	13.7 ± 0.10	19.9 ± 0.20

[a] The errors calculated from the standard deviation of the fit of k_r vs [formate]$_{total}$ and k_f vs [formate]$_{total}$ to eqs 5 and 6, respectively, at the given pH or pD. [b] At this pH the acid-catalyzed hydrolysis, k_{0r}, is much higher than the buffer catalyzed hydrolysis, k_{1r} (less than 11% at highest buffer concentration). [c] pD. [d] HCl or DCl was used as buffer; no formate added, $\mu = 1.0$ (KCl).

Table 7. Pseudo-First-Order Rate Constants of Formation (k_f) and Hydrolysis (k_r) of Formanilide and the Equilibrium Constants (K'_{eq}) in the Presence of Phosphate at 79 ± 1 °C in Aqueous Formate Buffer and the Second-Order Rate Constant for Phosphate Catalysis on Hydrolysis (k_r^{phos})[b] and Formation (k_f^{phos})[b]

pH[a]	[phosphate]$_{total}$ (M)	$k_r \times 10^5$ (s^{-1})	$k_f \times 10^5$ (s^{-1})	K'_{eq} (M^{-1})	$k_r^{phos} \times 10^5$ (M^{-1} s^{-1})	$k_f^{phos} \times 10^5$ (M^{-1} s^{-1})
3.21	0.10	(4.66 ± 0.02)	(9.50 ± 0.03)	18.5	6.14 ± 0.05	13.4 ± 0.10
3.21	0.30	(5.90 ± 0.10)	(12.2 ± 0.05)	18.6		
3.58	0.10	(2.47 ± 0.03)	(6.71 ± 0.02)	19.1	2.70 ± 0.09	6.90 ± 0.09
3.60	0.50	(3.52 ± 0.05)	(9.44 ± 0.01)	18.8		

[a] [buffer]$_{total} = 1.00$ M, $\mu = 1.0$ (KCl). [b] The errors are calculated from the standard deviation of the fit of k_r vs [phosphate]$_{total}$ and k_f vs [phosphate]$_{total}$ to a linear equation, $k_r = k_r^0 + k_r^{phos}$[phosphate]$_{total}$ or $k_f = k_f^0 + k_f^{phos}$[phosphate]$_{total}$, where k_r^0 and k_f^0 are the pseudo-first-order rate constants of hydrolysis and formation without added phosphate under the experimental conditions at the given pH. The data at zero phosphate concentration were obtained from Table 4.

Table 8. Pseudo-First-Order Rate Constants[a] of Formation (k_f) and Hydrolysis (k_r) of Formanilide and the Equilibrium Constants[b] (K'_{eq}) in Aqueous Ethanolic, Formate Buffer at 60.0 ± 0.3 °C

pH[c]	% (v/v) ethanol	k_r (s^{-1})	k_f (s^{-1})	K'_{eq} (M^{-1})[d]
3.59 ± 0.03	20	(5.32 ± 0.55) × 10^{-6}	(3.30 ± 0.02) × 10^{-5}	23.9
4.92 ± 0.05	80	(2.82 ± 0.03) × 10^{-6}	(1.85 ± 0.02) × 10^{-5}	14.4[e]
3.60 ± 0.05	80	(7.01 ± 0.02) × 10^{-6}	(7.14 ± 0.02) × 10^{-5}	9.25[e]
3.60 ± 0.05	0	(5.86 ± 0.03) × 10^{-6}	(1.65 ± 0.02) × 10^{-5}	32.7

[a] Calculated from the pseudo-first-order rate constants (k_{obs}) for establishment of equilibrium, using $k_{obs} = k_r + k_f$ and $K' = k_f/k_r$. Errors are deviations from the mean for duplicate values and standard deviations for triplicate values. [b] Calculated using the nonionized of concentrations at equilibrium and taking concentration of water as unity, accounting for the changes in the pK_a's due to the percent ethanol present in the solvent and variation of temperature. 20% v/v ethanol water corresponds to 16.4 wt %, and 80% v/v corresponds to 75.8 wt %. [c] [buffer]$_{total} = 1.0$ M. [d] Given the possibility that ethyl formate is produced during the reaction, that species could be involved in the formation of formanilide, and that the water concentration can no longer be taken as unity, the K'_{eq} should be written as K'_{eq}= ([amide][H$_2$O])/(([acid] + [ethyl formate])[amine]). [e] μ uncorrected.

for these rate constants compiled in Table 6. Two things are apparent from the data in Table 6. First, the k_{0r} constants increase linearly with increasing [H$_3$O$^+$], indicating that the hydrolysis in the absence of buffer is specific acid catalyzed. Second, from the D$_2$O data given in the table, the (k_{0r}^{H+}/k_{0r}^{D+}) ratio at pH (pD) 3.6 is 1.0 ± 0.1, while that at pH 2.0 is 0.9. The solvent DKIE on the buffer catalysis for hydrolysis at pH (pD) 3.6, ($k_{1r}^{H_2O}/k_{1r}^{D_2O}$), is 0.96 ± 0.13, while the DKIE for formation are ($k_{1f}^{H_2O}/k_{1f}^{D_2O}$) = 1.46 ± 0.10 and ($k_{2f}^{H_2O}/k_{2f}^{D_2O}$) = 1.61 ± 0.31.

C. Effect of Additives on the k_f, k_r, and K'_{eq} Constants. Given in Table 7 are the k_f, k_r, and K'_{eq} constants for the equilibration of formanilide at 79 °C at pHs 3.20 and 3.60 in a 1.0 M formate buffer in the presence of K$_2$PO$_4$. At these pH values, the phosphate exists as the monoanionic form and the ionic strength was held at 1.0 with added KCl. Although equilibrium is attained faster in the presence of phosphate, the K' and K'_{eq} values are unchanged relative to those obtained in the absence of phosphate. Plots (not shown) of the k_f and k_r values against [phosphate] give straight lines, the slopes of which give the phosphate catalysis on the formation (k_f^{phos}) and hydrolysis (k_r^{phos}) processes: these values are also listed in Table 7.

Given in Table 8 are the pseudo-first-order rate constants for formation and hydrolysis of formanilide in aqueous ethanolic solutions of [formate]$_{total}$ = 1.0 M, $T = 60$ °C, and ionic strength not held constant. In keeping with the trends of temperature on K'_{eq} noted in part A above, in the absence of alcohol, the corrected equilibrium constant increases at lower temperature although the rate of attainment of equilibrium decreases. Added ethanol has the effect of decreasing both k_r and k_f, but by different amounts, the net effect being to reduce K'_{eq} as the ethanol content increases.
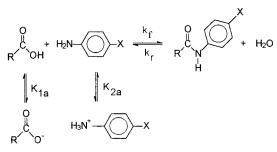
Discussion

A summary of the conditional equilibrium constants (K', defined as in eq 3) and corrected equilibrium constants (K'_{eq}, defined as in eq 4) for the anilides under all conditions investigated is presented in Table 9. The K' constant gives an indication of the expected [anilide]/[aniline]$_{total}$ at a given set of conditions when there is an excess of carboxylic acid present. It is known that the formation of the anilides depends on the concentrations of the nonionized acid and amine[2a,3,11,13,14] as depicted in Scheme 2. Therefore factors that alter the pK_a's of the amines and acids play an important role in controlling the extent of amide formation under a given set of conditions and

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Table 9. Conditional Equilibrium Constants (K') and Equilibrium Constants (K'_{eq})[a] of Formanilide, Acetanilide, *p*-Nitroformanilide, and *p*-Methoxyformanilide under Various Conditions
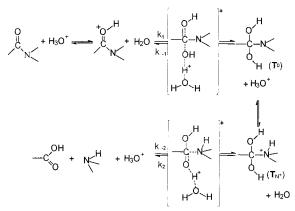
compound	pH	temp (°C)	[RCOOH]$_{total}$ (M)	K'	K'_{eq} (M^{-1})
formanilide	4.17	98	0.10	0.19	12.3
formanilide	3.60	98	0.10	0.27	13.1
formanilide	3.18	98	0.10	0.20	12.9
formanilide	3.60	98	0.50	1.23	11.9
formanilide	3.60	98	1.00	2.64	12.7
acetanilide	1.95	98	1.00[b]	0.06	3.60
acetanilide	3.75	98	1.00[b]	1.34	3.20
formanilide	2.80	79	0.10	0.11	20.6
formanilide	2.79	79	0.50	0.57	22.7
formanilide	2.81	79	0.90	1.07	20.7
formanilide	3.20	79	0.10	0.21	19.1
formanilide	3.20	79	0.50	1.00	18.2
formanilide	3.20	79	1.00	2.05	18.6
formanilide	3.60	79	0.10	0.29	20.4
formanilide	3.61	79	0.50	1.49	20.9
formanilide	3.59	79	1.00	2.77	19.5
formanilide	4.01	79	0.10	0.30	20.4
formanilide	3.20	79	0.50	1.58	21.7
formanilide	3.20	79	1.00	2.78	19.0
formanilide	3.58[c]	79	0.50	0.93	22.3
formanilide	3.61[c]	79	0.75	1.37	21.9
formanilide	3.60[c]	79	1.00	1.80	21.6
p-nitroformanilide	2.82	79	1.00	0.23	0.26
p-nitroformanilide	3.20	79	0.10	0.02	0.29
p-nitroformanilide	3.20	79	0.50	0.10	0.27
p-nitroformanilide	3.21	79	1.00	0.19	0.26
p-nitroformanilide	3.60	79	1.00	0.13	0.25
p-methoxyformanilide	2.82	79	1.00	0.98	41.0
p-methoxyformanilide	3.18	79	1.00	1.88	38.9
formanilide	3.21	79	1.00[d]	2.04	18.5
formanilide	3.21	79	1.00[e]	2.04	18.5
formanilide	3.58	79	1.00[d]	2.72	19.1
formanilide	3.58	79	1.00[f]	2.70	19.0
formanilide	3.60	60	1.00	2.82	32.7
formanilide	3.59	60	1.00[g]	6.21	23.9[i]
formanilide	3.60	60	1.00[h]	10.2	14.4[i]
formanilide	4.92	60	1.00[h]	6.54	9.3[i]

[a] Obtained using K'_{eq} = [formanilide]/[aniline][formic acid] and taking activity of water as unity. The concentrations of nonionized acid and nonprotonated amine were calculated using the corrected pK_a under the given conditions. [b] Acetate. [c] pD. [d] In presence of 0.10 M KH$_2$PO$_4$. [e] In presence of 0.30 M KH$_2$PO$_4$. [f] In presence of 0.50 M KH$_2$PO$_4$. [g] In 20% aqueous ethanol. [h] In 80% aqueous ethanol. [i] Given the possibility that ethyl formate is produced during the reaction, that species could be involved in the formation of formanilide, and that the water concentration can no longer be taken as unity, the K'_{eq} should be written as K'_{eq}= ([amide][H$_2$O])/(([acid] + [ethyl formate])[amine]).

Scheme 2

this is reflected in the corrected equilibrium constant, K'_{eq}. Several points of note are apparent from inspection of the data. (1) For a given amide, K' increases as the [formate]$_{total}$ increases, but K'_{eq} remains constant irrespective of the pH and [formate]$_{total}$. (2) In the set of formanilides, the *p*-substituent alters both K' and K'_{eq}. (3) Alteration of the acyl portion from formanilide to acetanilide decreases the equilibrium constant by a factor of 3.7 but decreases the rate of attainment of equilibrium by about

Scheme 3

240-fold. (4) For formanilide at a given pH and [HCOOH]$_{total}$, reduction in the temperature from 100 to 60 °C produces almost no change in K' but increases K'_{eq} by a factor of about 3. (5) Added phosphate increases the rate of attainment of equilibrium without affecting the K' or K'_{eq}. (6) Added ethanol increases the rate of attainment of equilibrium and the K' but reduces K'_{eq}. In the following we will deal with these observations in detail.

A. Mechanism of Specific Acid Catalyzed and General Catalyzed Hydrolysis and Formation. The hydrolysis of amides is specific acid catalyzed and adheres to the mechanism given in Scheme 3,[1,20,21] where preequilibrium protonation of the amide, followed by attack of H$_2$O assisted by one[20] or more[21] water molecules, generates a neutral tetrahedral intermediate (T_O) and an encounter complex with H$_3$O$^+$. Rapid protonation of the N to yield T_N^+ followed by water-assisted C$-$N cleavage generates the acid, amine, and H$_3$O$^+$. Consistent with such a mechanism is the fact that the solvent DKIE for H$_3$O$^+$-catalyzed hydrolysis is ~1.0 and little ^{18}O=C exchange is detected in labeled amides recovered from solution after partial hydrolysis,[20] indicating that the rate-limiting step is largely formation of T_O from the protonated amide. The data of Table 6 for formanilide indicate that the hydrolysis at zero added formate is linearly dependent on [H$_3$O$^+$] and that the solvent DKIE for that process is, as expected,[20] 1.0 ± 0.1.
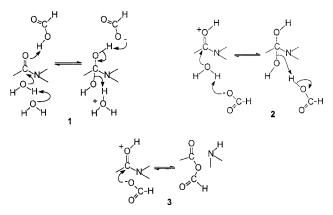
It is required that the reaction of acid-catalyzed amide formation follows the microscopic reverse of the hydrolysis, proceeding through the same intermediates with the same rate-limiting step(s). Thus the reaction must proceed through a reversible H$_3$O$^+$-catalyzed attack of the amine on RCOOH to yield T_N^+, which then deprotonates to give a T_O, which undergoes rate-limiting general acid catalyzed (by H$_3$O$^+$) C$-$OH cleavage to yield the protonated amide.

As previously mentioned in the Results, the observed rate constant for the attainment of equilibrium, k_{obs}, is the sum of the pseudo-first-order rate constants k_r and k_f. Figure 2 illustrates that both the latter constants are dependent on [RCOOH]$_{total}$, but in different ways. Fitting of the k_r and k_f vs [formate]$_{total}$ data in Tables 1 and 3 to eqs 5 and 6 gives the values for the formate-dependent rate constants presented in Table 6. The k_{0r} and k_{1f} terms relate to the H$_3$O$^+$-catalyzed hydrolysis of formanilide (discussed above) and its microscopic reverse, the

(20) (a) Bennet, A. J.; Slebocka-Tilk, H.; Brown, R. S.; Guthrie, J. P.; Jodhan, A. *J. Am. Chem. Soc.* **1990,** *112,* 8497. (b) Bennet, A. J.; Slebocka-Tilk, H.; Brown, R. S. *J. Am. Chem. Soc.* **1992,** *114,* 3088. (c) Brown, R. S.; Bennet, A. J.; Slebocka-Tilk, H. *Acc. Chem. Res.* **1992,** *25,* 481.
(21) (a) Cox, R. A.; Yates, K. *Can. J. Chem.* **1981,** *59,* 2853 and references therein. (b) Moodie, R. B.; Wale, P. D.; Whaite, T. J. *J. Chem. Soc.* **1963,** 4273.

H_3O^+-catalyzed formation of formanilide. Similarly, the k_{1r} and k_{2f} terms relate to a formate-catalyzed hydrolysis of formanilide and its microscopic reverse, the formate-catalyzed aminolysis of formic acid. At least three possible kinetically equivalent mechanisms can be presented for the formate-catalyzed hydrolysis as given in **1**−**3**. Mechanism **1** involves general acid

catalyzed attack of H_2O on formanilide or, in the reverse direction, general base plus H_3O^+-promoted decomposition of T_O. Mechanism **2** involves formate anion acting as a general base to deliver H_2O to a protonated amide and, in the reverse direction, formic acid acting as a general acid to assist in the decomposition of T_O. Finally, in mechanism **3**, formate acts as a nucleophile on the protonated amide to give a transient anhydride intermediate.

The solvent DKIE of $(k_{1r}^{H_2O}/k_{1r}^{D_2O}) = 0.96 \pm 0.13$ for the formate-promoted hydrolysis of formanilide at pH (pD) 3.60 and 79 °C permits us to favor mechanism **2** after consideration of the effect of D_2O on the concentrations of protonated amide, formic acid, and formate anion. Since it is generally observed that oxygen and nitrogen acids are stronger by a factor of ∼3 in H_2O relative to D_2O^{22} (since D_3O^+ is a stronger acid in D_2O than is H_3O^+ in H_2O), one expects that the amide would be more protonated by a factor of ∼3 in D_2O. Also, since the pK_a for formic acid under our conditions is 3.63 in water and 4.09 in $D_2O,^{23}$ for a given [formate]$_{total}$, there will be more HCOOD at pD 3.60 in D_2O than HCOOH at pH 3.60 in H_2O (70% vs 50%).[24] Mechanism **1** is disfavored since it should exhibit a large primary DKIE of $k_H/k_D = 2{-}5$ attributable to at least two protons in flight reduced by $^5/_7$ to account for the increase of [HCOOL] in D_2O. By contrast, mechanism **2** is similar to what is proposed in Scheme 3 for the specific acid-catalyzed hydrolysis of amides, but with formate replacing H_2O as the base in assisting the delivery of water to protonated amide. In this case, the DKIE should be ∼1 due to the competing effects of an inverse DKIE associated with preequilibrium protonation of the amide and a normal DKIE associated with the general base delivery of the attacking water,[20] modified by $^5/_3$ to account for the increased [formate anion] in H_2O. This same mechanism was found to be operative for the acetate-promoted hydrolysis of distorted anilide **4**.[25]

Mechanisms **2** and **3** are kinetically indistinguishable, but there is a preference for **2** based on the near unit value of the

(22) Gold, V. *Adv. Phys. Org. Chem.* **1969**, *7*, 259.
(23) Bell, R. P.; Kuhn, A. T. *Trans. Faraday Soc.* **1963**, *59*, 1789.

solvent DKIE for $(k_{1r}^{H_2O}/k_{1r}^{D_2O})$. Mechanism **3** is predicted to have a more inverse value than mechanism **2** which results from the pre-equilibrium protonation DKIE superimposed on a factor of $^5/_3$ to account for an increased [formate anion] in H_2O.

B. Effect of pH on the Conditional Equilibrium Constant, K'. As shown in Table 9, at constant [formate]$_{total}$, the conditional equilibrium constant maximizes at about pH 3.60 and then decreases at higher and lower pH. The conditional equilibrium constant includes the [formate]$_{total}$ so eq 3 becomes

$$K' = k_f/k_r = K''[\text{formate}]_{total} = [\text{anilide}]/[\text{aniline}]_{total}$$

where $K'' = [\text{anilide}]/([\text{HCOOH} + \text{HCOO}^-])([H_3N^+{-}Ar + H_2N{-}Ar])$. Because the formation reaction depends on the concentrations of RCOOH and amine, the optimum pH for synthesis of a given amide from its constituents occurs at values between the pK_a's of RCOOH and $H_3N^+{-}Ar$, expressed as $pH^{opt} = (pK_a^{acid} + pK_a^{ammonium})/2.^{2b,3}$ Using a pK_a value of 3.63 for HCOOH and 3.87 (100 °C) or 3.78 (80 °C) for anilinium ion (see Appendix), the calculated optimum pHs for formation of formanilide are calculated to be 3.83 and 3.71 at 80 and 100 °C, close to the experimentally observed optimum value of 3.60 given in Table 9.

There is an apparent solvent deuterium equilibrium isotope effect (EIE) on the formanilide $K'_H/K'_D = 1.56$ at pH (pD) 3.6 and $T = 80$ °C (Table 9). The data of Tables 3 and 6 clearly show that the source of the DKIE is in the k_f term since the k_r term has a unit kinetic isotope effect. Consideration of the effects of D_2O on the pK_a's of formic acid and anilinium[24] indicates that at pH (pD) 3.6 there is 50% less of the required neutral amine in D_2O than in H_2O (14% vs 29%) but $^7/_5$ more HCOOD. Thus the forward reaction, $k_f[\text{HCOOL}][L_2N{-}Ar]$ should show a solvent EIE of $^5/_7 \times ^2/_1 = 1.4$ due to simple concentration effects alone. It is notable that the solvent EIE on K'_{eq}, which depends on the true concentrations of the neutral acid and amine, is unity.

C. Effect of Structure on K' and K'_{eq}. Fersht and Requena[11] showed that, for a number of aliphatic amines, the corrected equilibrium constant for amide formation was related to the pK_a of the corresponding ammonium ion by eq 7. The experimental

$$\log K'_{eq} = 0.50 + 0.51 pK_a(\text{ammonium}) \qquad (7)$$

K'_{eq} from that study for formanilide is 8 times smaller than would be predicted using eq 7 probably due to the fact that aliphatic and aromatic amines are described by slightly different Brønsted relationships. In the present study of formanilides at 80 °C, the relationship between K'_{eq} and aniline basicity is

$$\log K'_{eq} = -0.95 + 0.57 pK_a(\text{anilinium}) \qquad (8)$$

It is also seen (Tables 1 and 2) that attainment of equilibrium is 240 times faster for formanilide than for acetamide under comparable conditions of temperature, pH, and reagent concentrations. The higher reactivity of $HC(=O)X$ can be attributed to steric factors since nucleophilic attack on the formate carbonyl

(24) Given that $pK_a = pH + \log[A{-}H]/[A]$, it can be calculated that for formic acid, $pK_a = 3.6$ in water, at pH 3.6 the ratio of [HCOOL]/[HCOO$^-$] is 1.0; in D_2O, where the pK_a of formic acid is 4.0, the ratio is 2.51. Similarly, for anilinium, $pK_a = 4.02$ in water at 79 °C, the ratio of [$L_3N^+{-}$Ar]/[$L_2N{-}$Ar] is 2.51 at pH 3.6, but in D_2O, where the $pK_a = 4.4$, the ratio is 6.3.
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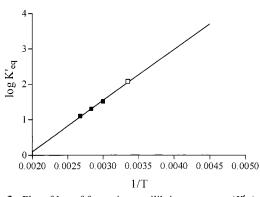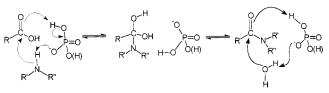
Figure 3. Plot of log of formation equilibrium constant (K'_{eq}) against $1/T$ for equilibrium formation of formanilide, $\mu = 1.0$ (KCl): ■, this work; □, at 25 °C (ref 11).

is less hindered than that on the $CH_3C(=O)X$.[26] However, the respective corrected equilibrium constants vary only by a factor of 3.7, indicating that the formation and hydrolysis processes are nearly equally sensitive to the steric effects. Morawetz and Otaki[12] came to the same conclusion after they observed that the amide equilibrium formation constants for a series of higher fatty acids under aqueous basic conditions were nearly the same but that CH_3NH_2 reacts 140 times faster with formate than with acetate.

D. Effect of Temperature on K' and K'_{eq}. The corrected equilibrium constant (K'_{eq}) for formanilide is inversely proportional to temperature, increasing 2.6 times from 100 to 60 °C (Table 9). A plot of log K'_{eq} vs $1/T$ (Figure 3) gives a straight line with positive slope from which can be computed a ΔH_{eq} of 6.60 ± 0.25 kcal/mol. This value is very close to the ΔH of ionization for anilinium (7.38 kcal/mol).[27] The pK_a of anilinium ion decreases from 3.78 to 4.30 from 100 to 60 °C, whereas the change in pK_a of formic acid over the same range is negligible because its ΔH of ionization is -0.04 kcal/mol[27] (see Appendix). The anticipated K'_{eq} from eq 8 for an aniline having a pK_a of 4.30 would be 31.6 M^{-1}, essentially the same as what is found experimentally (32.7 M^{-1}, Table 9). Thus the overwhelming result of the temperature effect is to alter the K'_{eq} due to changes in the amine basicity.

In practical terms, there is a near independence of the conditional equilibrium constant, K', on temperature, the values at pH 3.6 and [formate]$_{total}$ being 2.82, 2.77, and 2.64 at 60, 79, and 100 °C. This is due to an almost complete counterbalancing of the two competing effects of (a) increasing the amount of neutral amine as the temperature increases due to the drop in pK_a, which should serve to increase K', and (b) reducing the nucleophilicity of the amine which should serve to decrease the K' as in eq 8.

E. Effects of Phosphate and Ethanol Additives. The data in Table 7 show that phosphate catalyzes the formation and hydrolysis of formanilide, but as expected, the equilibrium position is not changed by the addition of KH_2PO_4. The accelerating effect is not great since, at pH 3.6, [formate]$_{total}$ = 1.0 M, and $\mu = 1.0$, the addition of 0.5 M phosphate only increases the rate of attainment of equilibrium by 50%. It is known that phosphate ions are able to mediate acyl transfer,[28,29,30] in this case to amine or water, through proton addition and removal during the formation and breakdown of tetrahedral

Scheme 4

intermediates. In some cases it is known that phosphate can act nucleophilically toward $RC(=O)X$ to give acyl phosphate intermediates. In a recent paper, we provided evidence that phosphate can act as a nucleophilic catalyst toward ester and thiolester hydrolysis.[31] This mechanism generally occurs when the conjugate acid of the departing group, X, has a low pK_a and can leave readily as an anion which is not the case for phosphate catalysis of amide equilibration where X would be OH or NHR. In an earlier study we suggested[14] that the catalytic mechanism of phosphate-assisted formation and hydrolysis of amides proceeds via either sequential general base/general acid process or a bifunctional concerted general base/general acid processes (Scheme 4).

To study the effect of the solvent polarity on the K' and K'_{eq}, the formation and hydrolysis of formanilide was carried out in both 20 and 80% (v/v) ethanol/water mixtures at 60 °C and pH 3.60 (Table 8). Addition of cosolvents complicates the definitions of pH and pK_a and also modifies the water concentration in the equilibrium process. Given the possibility that ethyl formate is produced during the reaction and that species could be involved in the formation of formanilide (vide infra) and that the water concentration can no longer be taken as unity, the K'_{eq} should be written as $K'_{eq} = ([amide][H_2O])/(([acid] + [ethyl\ formate])[amine])$. However, the values for K'_{eq} given in Tables 8 and 9 are computed in the same way as for the aqueous conditions to facilitate comparison. Under comparable conditions of pH and [formate]$_{total}$, both the rate constant for attainment of equilibrium (k_{obs}) and the conditional equilibrium constant (K') increased about 3.5 times in changing from 0 to 80% ethanol (see Table 9). The pK_a of formic acid increases with the addition of ethanol in aqueous media,[32] and under the experimental conditions, the pK_a of formic acid changes to ~5.33 in 80% ethanol from 3.63 in aqueous media, $T = 25$ °C. On the other hand the pK_a of anilinium ion decreases to ~3.78 in 80% ethanol from 4.30 in aqueous media.[33] The changes in pK_a's due to the addition of ethanol increase the concentration of nonionized forms of both aniline and formic acid at a given pH (here 3.6), and so the equilibrium should lie more toward anilide formation. However, it should be noted that, if the observed change in K' were only due to the increases in effective concentration of reactive species ([HCOOH] and [H$_2$NPh]), the change would be higher than 3.5-fold in 80% ethanol. The other competing factor which counterbalances the overall effect on K' is the drop in basicity of the amine.

Similar observations were made in studies of enzymatic formation of amide bonds,[2,3] and it is reported that, in the enzyme-catalyzed peptide formation, the addition of organic

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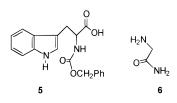
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Scheme 5

$$H_3O^+ + HCOOH + NH_2Ph \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H_3O^+ + HCONHPh + H_2O$$

$$H_3O^+ + HCOOH + EtOH \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} H_3O^+ + HCOOEt + H_2O$$

$$H_3O^+ + HCOOEt + NH_2Ph \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} HCONHPh + EtOH + H_3O^+$$

solvents shifted the equilibrium more toward amide bond formation. For example,[3b] in the chymotrypsin-catalyzed formation of the dipeptide benzyloxycarbonyl-L-tryptophanylglycinamide (from benzyloxycarbonyl-L-tryptophan (**5**) and glycinamide (**6**)), the addition of 85% 1,4-butanediol increased the

extent of amide formation by a factor of 84. The pK_a of the carboxylic acid group of **5** shifted from 3.6 in aqueous solution to 5.3 in 85% 1,4-butanediol, without a significant change in the pK_a of the amine group of **6** (from 8.2 to 8.1). In that study the enhancement in dipeptide formation in the above solvent system was attributed mostly to the change in pK_a of the carboxylic acid group of benzyloxycarbonyl-L-tryptophan. Presumably such effect could be operative in the nonenzymatic coupling of these two species as well.

Although the effects of the addition of ethanol can be explained in terms of the change in pK_a's, another possibility exists wherein ethyl formate is formed as a reactive intermediate and amide formation will occur through the aminolysis of ethyl formate (Scheme 5). Kistiakowsky studied[34] the equilibrium formation and hydrolysis of ethyl formate in acidic aqueous ethanol and showed that the acid-catalyzed rate of attainment of equilibrium (($k_2 + k_{-2}$) in Scheme 5) varies from 2.3×10^{-3} to 3.3×10^{-3} M^{-1} s^{-1} when the solvent composition changes from 21 to 80% ethanol at 25 °C. The equilibrium positions lay 17, 61, and 72% toward formation of ethyl formate in 21, 73, and 84% ethanol, respectively. The author also reported an 8−10% increase in the rate of attainment of equilibrium when the temperature was increased to 30 °C from 25 °C, with no change in the equilibrium position. From these data, it is clear that ethyl formate will be formed under our reaction conditions so the possibility of formanilide formation via aminolysis of ethyl formate cannot be ruled out at this point.

Conclusions

In this study we have shown that it is possible to form formanilide, acetanilide, *p*-nitroformanilide, and *p*-methoxyformanilide in reasonable amounts and in reasonable times from their amine and carboxylic acid constituents in aqueous acidic solution. The conditional equilibrium constant, K', gives an indication of the [anilide]/[aniline]$_{total}$ ratio expected once equilibrium is established. The equilibrium position for formanilides shifts more to formation with increasing basicity of the aniline, and at 1.0 M [formate]$_{total}$ and pH 3.60, the respective K' values for formanilide, *p*-methoxyformanilide, and *p*-nitroformanilide are 2.05, 1.88, and 0.19 at 80 °C. The half-time for

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Table 10. Computed pK_a's of Anilines and Acids at Various Temperatures and at $\mu = 1.0$

temp (°C)	pK_a in water	pK_a in 20% (v/v) ethanol	pK_a in 80% (v/v) ethanol
	Aniline		
25	4.89 ± 0.05^a	4.42^g	$3.78^{g,k}$
60	4.30^b	3.83^h	$3.19^{h,k}$
80	4.02^b		
100	3.78^b		
	Formic Acid		
25	3.63 ± 0.03^a	3.97^i	$5.33^{i,k}$
60	3.63^b	3.97^j	$5.33^{j,k}$
80	3.63^b		
100	3.64^b		
	Acetic Acid		
25	4.60^c		
100	4.62^b		
	p-Nitroaniline		
25	1.00^d		
80	0.65^d		
	p-Methoxyaniline		
25	5.34^e		
80	$4.35^{e,f}$		

^{a} Measured at $\mu = 1.0$ (KCl). ^{b} Calculated using $\Delta H_{ionization}$ and $\Delta S_{ionization}$ values from ref 27. ^{c} Available data[35] indicates that the pK_a of acetic acid drops from $\mu = 0$ to $\mu = 0.2$, but above $\mu = 0.2$, the pK_a is independent of ionic strength. The value used was that given at $\mu = 0.2$. ^{d} Reference 37. ^{e} Reference 38 at $\mu = 0.10$ M. ^{f} Extrapolated value using data from ref 36 (from 20 to 40 °C). ^{g} Calculated using data from ref 33. 20% v/v ethanol water corresponds to 16.4 wt % and 80% v/v corresponds to 75.8 wt %. pK_a values interpolated from data given between 0 and 20 wt %, and 65 and 80 wt % assuming a linear interpolation. ^{h} Assuming effect of temperature same as for aqueous media. ^{i} Calculated using data from ref 33. 20% v/v ethanol water corresponds to 16.4 wt % and 80% v/v corresponds to 75.8 wt %. pK_a values interpolated from data given between 0 and 20.3 wt %, and 65.1 and 79.9 wt % assuming a linear interpolation. ^{j} Assuming no effect of temperature change. ^{k} μ not corrected.

the attainment of equilibrium for the various formanilides varies from 1.6 to 8.6 h, and so for practical purposes, equilibrium can be established within 9−42 h ($5t_{1/2}$). The rate of attainment of equilibrium is subject to a large steric effect in the acyl portion such that acetanilide equilibrium is established 240 times slower than is formanilide equilibrium under comparable conditions of temperature and pH. Nevertheless, the corrected equilibrium constant, K'_{eq}, is only marginally affected by the steric factor, the value for acetanilide being 3.7 times smaller than formanilide.

Reducing the temperature slows the rate of establishing equilibrium but has virtually no effect on K', although K'_{eq} increases. This is due largely to the effect of increasing the basicity of the aniline at lower temperature which makes it more nucleophilic toward the acid although the RCOOH pK_a is essentially invariant with temperature. Added phosphate (0.5 M) increases the rate of establishing equilibrium for formanilide by 1.6 times at pH 3.6 and 80 °C but does not influence the equilibrium position. Both the rate of establishing equilibrium and the K' increase as the ethanol content increases in the medium. The effect results primarily from increasing the concentrations of the neutral forms of RCOOH and amine. From a practical point of view, an 80% ethanol solution at pH 3.6 containing 1.0 M [formate]$_{total}$ at 60 °C enhances the yield of formanilide to 91% from 73% in water, with equilibrium being attained in 12 h relative to 42 h.

All the experiments in this study were conducted using excess formate and concentrations of aniline or anilide which ensured homogeneous conditions for purposes of the kinetic work.

Synthetically, for amide formation in water, one can use much higher concentrations of acid and aniline since the forming anilide will quickly separate from the water layer and drive the equilibrium further to the side of amide formation.

Supporting Information Available: Tables of pseudo-first-order rate constants for the establishment of equilibrium and K' values for formanilide, acetanilide, *p*-methoxyformanilide, and *p*-nitroformanilide under various conditions (12 tables) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

Appendix: Calculation of pK_a's Listed in Table 10

1. Aniline. Isaacs has listed[27] the enthalpies and entropies for dissociation of various acids and ammonium ions: for anilinium ion $\Delta H_{\text{ionization}} = 7.38$ kcal/mol and $\Delta S_{\text{ionization}} = 3.7$ cal/(mol K) at $\mu = 0$. By titration in this study, the pK_a of anilinium ion was determined to be 4.89 ± 0.05 at 24 ± 1 °C and $\mu = 1.0$ (KCl). Using the expression $-\Delta G = RT \ln K_a$, ΔG of anilinium dissociation is calculated to be 6.67 kcal/mol ($K_a = 1.29 \times 10^{-5}$) at $\mu = 1.0$ and $T = 24 \pm 1$ °C.

Since $\Delta G = \Delta H - T\Delta S$, and assuming the ΔH is unaffected by ionic strength, ΔS can be calculated as 2.39 cal/(mol K) at $\mu = 1.0$ (KCl). Thus the ΔG at 100 °C and $\mu = 1.0$ becomes 6.49 kcal/mol. Hence, the pK_a of aniline under these conditions is 3.80.

Assuming that ΔS is insensitive to ionic strength, ΔH at $\mu = 1.0$ (KCl) becomes 7.77 kcal/mol. Therefore, the pK_a of anilinium ion at 100 °C becomes 3.75. An average pK_a of 3.78 was used for further calculations. Similar calculations were done at 80 °C ($pK_a = 4.02$) and at 60 °C ($pK_a = 4.30$).

2. Formic Acid. The pK_a of formic acid, 3.63 ± 0.03, was obtained by potentiometric titration at 24 ± 1 °C and $\mu = 1.0$ (KCl). It is reported[27] that $\Delta H_{\text{ionization}} = -0.04$ kcal/mol and $\Delta S_{\text{ionization}} = -17$ cal/(mol K) for formic acid at $\mu = 0$. From calculations similar to those above, the pK_a at 100 °C is 3.63. Therefore, although the change in ionic strength (from 0 to 1.0 M) alters the pK_a of formic acid by 0.12 unit, there is no effect due to the change in temperature.[35]

3. *p*-Methoxyanilinium Ion. Biggs has shown that the pK_a of *p*-methoxyanilinium ion decreases with increasing temperature[36] and has obtained pK_a's of *p*-methoxyanilinium ion at five different temperatures ranging from 20 to 40 °C and $\mu = 0.10$ M (KCl). The pK_a vs temperature plot follows a linear equation, $pK_a = a + bT$, where T is temperature in °C, $a = 0.018$, and $b = 5.79$. Using these data pK_a of *p*-methoxyanilinium was calculated to be 4.35 at 80 °C.

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