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

Ahmed M. Aman and R. S. Brown*

Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6

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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

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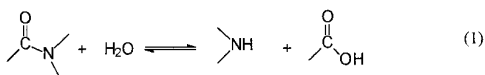
has long been known that hydrolytic enzymes are also capable of reforming peptides in solution.[2,3] The formation of amide bonds in aqueous media in the absence of enzymes is also of interest since this is implicated in the origin of life. Attempts[4−8] have been made to form peptides from amines and 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 $\lambda = 231$ nm.

D. Kinetics of Amide Formation and Hydrolysis by HPLC. For kinetics studies at 98 ± 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 ± 2 °C, pH 1.95 ± 0.03 and pH 3.72 ± 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 ± 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 ± 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 ± 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 ± 1 °C was determined in formate buffer ([buffer]$_{total}$ = 0.25−1.0 M) at pD 3.60 ± 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 ± 1 °C in the presence of phosphate (0.10−0.50 M) in 1.0 M formate buffer at pH 3.20 ± 0.03 and pH 3.60 ± 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 ± 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 ± 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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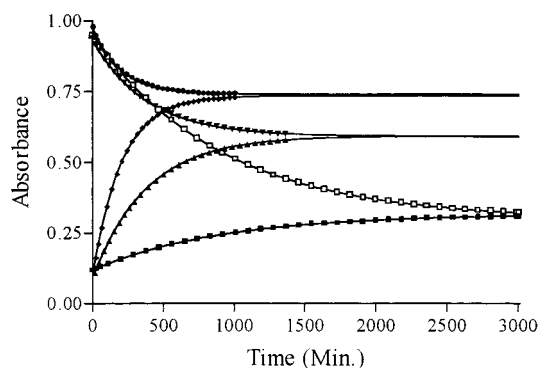
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Figure 1. Typical kinetic traces for formanilide equilibration at pH 3.60 at different [formate]$_{total}$, 0.10–1.00 M, μ = 1.0 (KCl), and T = 79 ± 1 °C. The lines are obtained from NLLSQ fitting of data to $A_t = A_\infty + (A_0 - A_\infty) \exp(-k_t)$. 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$_3$
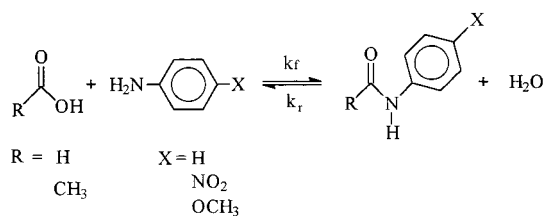
Results

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.

$$k_{obs} = k_f + k_r \qquad (2)$$

$$K' = k_f/k_r \qquad (3)$$

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

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

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 ± 0.03) × 10^{-5}	(0.23 ± 0.01) × 10^{-5}	12.3 ± 0.2
3.60 ± 0.03	0.10	(4.15 ± 0.02) × 10^{-5}	(1.13 ± 0.04) × 10^{-5}	13.1 ± 0.6
3.18 ± 0.03	0.10	(1.29 ± 0.06) × 10^{-4}	(2.56 ± 0.11) × 10^{-5}	12.9 ± 0.2
3.57 ± 0.03	0.50	(9.72 ± 0.30) × 10^{-5}	(1.20 ± 0.01) × 10^{-4}	11.9 ± 0.4
3.57 ± 0.03	1.00	(1.27 ± 0.04) × 10^{-4}	(3.36 ± 0.08) × 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] μ = 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 ± 0.04) × 10^{-5}	(0.19 ± 0.02) × 10^{-5}	3.6 ± 0.3
3.75 ± 0.05	acetate[d e]	(0.81 ± 0.02) × 10^{-6}	(1.07 ± 0.02) × 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

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 ± 0.05) × 10^{-5}	(0.77 ± 0.01) × 10^{-5}	20.6
2.79 ± 0.03	0.50	(7.60 ± 0.10) × 10^{-5}	(4.29 ± 0.05) × 10^{-5}	22.7
2.81 ± 0.03	0.90	(7.69 ± 0.02) × 10^{-5}	(8.22 ± 0.02) × 10^{-5}	20.7
3.18 ± 0.03	0.10	(3.32 ± 0.05) × 10^{-5}	(0.66 ± 0.02) × 10^{-5}	19.1
3.20 ± 0.03	0.50	(3.66 ± 0.05) × 10^{-5}	(3.60 ± 0.05) × 10^{-5}	18.2
3.20 ± 0.03	1.00	(4.09 ± 0.03) × 10^{-5}	(8.20 ± 0.02) × 10^{-5}	18.6
3.56 ± 0.03	0.001	(1.36 ± 0.02) × 10^{-5}		
3.60 ± 0.03	0.10	(1.42 ± 0.02) × 10^{-5}	(0.42 ± 0.01) × 10^{-5}	20.4
3.61 ± 0.03	0.50	(1.76 ± 0.03) × 10^{-5}	(2.64 ± 0.02) × 10^{-5}	20.9
3.59 ± 0.03	1.00	(2.15 ± 0.03) × 10^{-5}	(5.97 ± 0.04) × 10^{-5}	19.5
4.01 ± 0.03	0.10	(5.93 ± 0.02) × 10^{-6}	(1.83 ± 0.01) × 10^{-6}	20.9
4.02 ± 0.03	0.50	(7.90 ± 0.01) × 10^{-6}	(1.25 ± 0.01) × 10^{-5}	21.7
4.03 ± 0.03	1.00	(1.06 ± 0.01) × 10^{-5}	(2.94 ± 0.01) × 10^{-5}	19.0
3.60 ± 0.03[c]	0.001	(1.34 ± 0.03) × 10^{-5}		
3.58 ± 0.03[c]	0.50	(1.93 ± 0.09) × 10^{-5}	(1.77 ± 0.10) × 10^{-5}	22.3
3.61 ± 0.03[c]	0.75	(2.01 ± 0.12) × 10^{-5}	(2.76 ± 0.30) × 10^{-5}	21.9
3.60 ± 0.03[c]	1.00	(2.18 ± 0.03) × 10^{-5}	(3.96 ± 0.06) × 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 ± 0.05) × 10^{-4}	(5.34 ± 0.04) × 10^{-5}	0.26
3.18 ± 0.03	0.10	(9.86 ± 0.02) × 10^{-5}	(2.16 ± 0.20) × 10^{-6}	0.29
3.20 ± 0.03	0.50	(1.12 ± 0.03) × 10^{-4}	(1.17 ± 0.05) × 10^{-5}	0.27
3.20 ± 0.03	1.00	(1.28 ± 0.03) × 10^{-4}	(2.40 ± 0.06) × 10^{-5}	0.26
3.60 ± 0.03	1.00	(7.22 ± 0.07) × 10^{-5}	(9.33 ± 0.60) × 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 ± 0.05) × 10^{-5}	(4.01 ± 0.04) × 10^{-5}	41.0
3.18 ± 0.03	(2.13 ± 0.03) × 10^{-5}	(4.11 ± 0.06) × 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
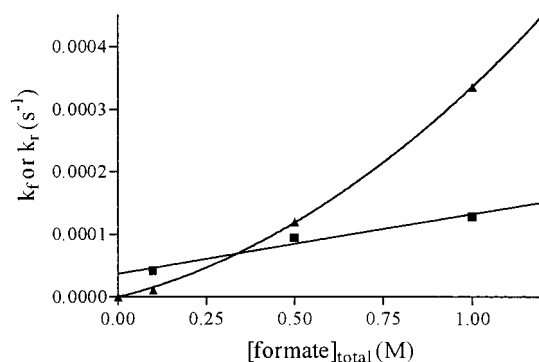
Figure 2. Pseudo-first-order rate constants of formation (k_f, ▲) and hydrolysis (k_r, ■) of formanilide vs [formate]$_{total}$ at 98 ± 2 °C (pH = 3.60 and $\mu = 1.0$ (KCl)). The lines were obtained from the fits to eqs 5 and 6.

can be fit to eqs 5 and 6,

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

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

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.

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

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

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