

Aspects of the hydrolysis of formamide: revisitation of the water reaction and determination of the solvent deuterium kinetic isotope effect in base

H. Slebocka-Tilk, F. Sauriol, Martine Monette, and R.S. Brown

Abstract: A study of the hydrolysis of formamide is reported with the aims of isolating the water reaction for hydrolysis from the acid and base hydrolysis terms and determining the solvent deuterium kinetic isotope effect (dkie) on base-catalyzed hydrolysis. Respective activation parameters (ΔH^\ddagger and ΔS^\ddagger) of (17.0 ± 0.4) kcal mol⁻¹ and (-18.8 ± 1.3) cal mol⁻¹ K⁻¹ for the acid reaction and (17.9 ± 0.2) kcal mol⁻¹ and (-11.1 ± 0.5) cal mol⁻¹ K⁻¹ for the base reaction were determined from Eyring plots of the second-order rate constants over the range of 27–120°C. Kinetic studies at the minima of the pH/rate profiles in the pH range from 5.6 to 6.2 in MES buffers at 56°C, and in the pH range of 4.25–6.87 in acetate and phosphate buffers at 120°C are reported. At 56°C the available data fit the expression $k_{\text{obs}}^{56} = 0.00303[\text{H}_3\text{O}^+] + 0.032[\text{HO}^-] + (3.6 \pm 0.1) \times 10^{-9}$, while at 120°C the data fit $k_{\text{obs}}^{120} = (0.15 \pm 0.02)[\text{H}_3\text{O}^+] + (3.20 \pm 0.24)[\text{HO}^-] + (1.09 \pm 0.29) \times 10^{-6}$. Preliminary experimental estimates of E_a (ln A) of 22.5 kcal mol⁻¹ (15.03) for the water rate constant (k_w) are calculated from an Arrhenius plot of the 56 and 120°C data giving an estimated k_w of 1.1×10^{-10} s⁻¹ ($t_{1/2} = 199$ years) at 25°C. Solvent dkie values of $k_{\text{OH}}/k_{\text{OD}} = 1.15$ and 0.77 ± 0.06 were determined at $[\text{OL}^-] = 0.075$ and 1.47 M, respectively. The inverse value is determined under conditions where the first step of the reaction dominates and is analyzed in terms of a rate-limiting attack of OL^- .

Key words: formamide, activation parameters, water reaction, acid and base hydrolysis, solvent kinetic isotope effect.

Résumé : On a effectué une étude de l'hydrolyse du formamide dans le but d'isoler la réaction d'hydrolyse par l'eau des termes d'hydrolyses acide et basique et de déterminer l'effet isotopique cinétique du deutérium du solvant (eicd) sur l'hydrolyse catalysée par la base. En se basant sur les courbes de Eyring des constantes de vitesse du deuxième ordre entre 27 et 120°C, on a déterminé les paramètres d'activation respectifs (ΔH^\ddagger et ΔS^\ddagger) de $(17,0 \pm 0,4)$ kcal mol⁻¹ et $(-18,8 \pm 1,3)$ cal mol⁻¹ K⁻¹ pour la réaction acide et de $(17,9 \pm 0,2)$ kcal mol⁻¹ et $(-11,1 \pm 0,5)$ cal mol⁻¹ K⁻¹ pour la réaction basique. On rapporte aussi les études cinétiques aux minima des profils pH/vitesse, à des pH allant de 5,6 à 6,2, dans des tampons de MES, à 56°C et à des pH allant de 4,25 à 6,87, dans des tampons d'acétate et de phosphate, à 120°C. À 56°C, les données peuvent être accommodées par l'expression $k_{\text{obs}}^{56} = 0,00303[\text{H}_3\text{O}^+] + 0,032[\text{HO}^-] + (3,6 \pm 0,1) \times 10^{-9}$ alors que, à 120°C, les données peuvent être accommodées par l'expression $k_{\text{obs}}^{120} = (0,15 \pm 0,02)[\text{H}_3\text{O}^+] + (3,20 \pm 0,24)[\text{HO}^-] + (1,09 \pm 0,29) \times 10^{-6}$. Sur la base d'évaluations expérimentales préliminaires de E_a (ln A) de 22,5 kcal mol⁻¹ (15,03) pour la constante de vitesse de l'eau (k_w) faites à l'aide d'une courbe d'Arrhenius des données de 56 à 120°C, on a évalué que, à 25°C, $k_w = 1,1 \times 10^{-10}$ s⁻¹ ($t_{1/2} = 199$ ans). Des valeurs de l'eicd du solvant de $k_{\text{OH}}/k_{\text{OD}} = 1,15$ et $0,77 \pm 0,06$ ont été déterminées pour des valeurs respectives de $[\text{OL}^-] = 0,075$ et 1,47 M. La valeur inverse a été déterminée pour des conditions dans lesquelles la première étape de la réaction est dominante et on l'a analysée en fonction d'une attaque cinétiquement limitante de OL^- .

Mots clés : formamide, paramètres d'activation, réaction de l'eau, hydrolyses acide et basique, effet isotopique cinétique du solvant.

[Traduit par la Rédaction]

Introduction

Due to the obvious relevance to biological processes, considerable attention has been devoted to understanding the mechanism of hydrolysis of amides under both acidic and

basic conditions (1, 2). Far less is understood about the mechanism by which water alone promotes the hydrolysis of amides. Water reactions or their kinetic equivalent, HO^-

Received 2 April 2002. Published on the NRC Research Press Web site at <http://canjchem.nrc.ca> on 22 October 2002.

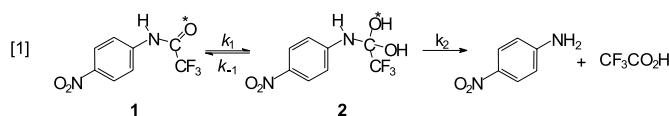
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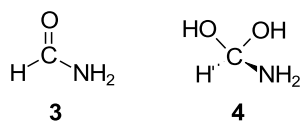
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attack on the protonated amide, have been observed when the amide is activated in some way (3). Recently there has been interest in determining the water hydrolysis rate constants for peptides so that these values could be used as a background for comparison with enzyme-catalyzed rates (4). The latter water reactions are very much slower than those of activated amides so new, highly sensitive assay techniques using ^{14}C radioisotopic detection (4a) or post-hydrolytic conversion of products to fluorescent tracers (4b, 4c) were developed to follow reactions to a few percent completion at 25°C. Radzicka and Wolfenden (4d) determined activation parameters for water hydrolysis of some simple dipeptides by monitoring the hydrolyses in buffered media between pH 4.2 and 7.8 at several temperatures between 120 and 200°C, extrapolating the water rate constant to 25°C. In the latter studies, the $t_{1/2}$ for the water reactions at 25°C varied from 7 (4a) to 600 (4d) years.

Despite the above, information about the mechanism of the water reaction is sparse, particularly with respect to the partitioning of neutral tetrahedral addition intermediates. Earlier, we reported (5) $^{18}\text{O}=\text{C}$ exchange and solvent deuterium kinetic isotope (dkie) studies of the water reaction observed for trifluoromethyl-*p*-nitroacetanilide (1) (eq. [1]) an activated amide previously studied by Komiyama and Bender (3d).



In that study we observed no $^{18}\text{O}=\text{C}$ exchange accompanying the hydrolysis in the water region, so we could not provide evidence that the reaction proceeded through a putative reversibly formed *gem*-diol intermediate (2). That intermediate, if formed, must break down to products faster than it reforms ^{18}O -exchanged starting material ($k_2 \gg k_{-1}$) but how relevant those findings are to the water reaction of normal, and unactivated, amides remains to be established.



The water reaction of the simplest amide, formamide (3) has been investigated computationally to determine the overall reaction enthalpy for the neutral hydrolysis (6) and the free energy of activation for a hypothetical reaction involving one or more water molecules (7). The activation free energy for reaction with a single H_2O in the gas phase at 25°C is calculated to be $\sim 55 \text{ kcal mol}^{-1}$ (7a, 7b) but for the reaction in aqueous solvent involving three waters the value falls to $\sim 48 \text{ kcal mol}^{-1}$ (7c). The rate-limiting step for the three-water reaction is the formation of the tetrahedral addition product (4) with the great bulk of the free energy of activation ($\sim 33 \text{ kcal mol}^{-1}$) attributed to the entropy of restriction of freedom of the reaction partners in the transition state. More recently, Guthrie and Pitchko (8) introduced a valuable method termed the No Barrier Theory for calculating the activation free energies for hydration of carbonyl compounds, and computed a ΔG^\ddagger value of $31.9 \text{ kcal mol}^{-1}$ for hydration

of dimethyl formamide (DMF) via a three-water cyclic mechanism.

The high computed activation free energy for the water reaction of formamide and DMF can be compared to the activation parameters commonly found for the acid- and base-catalyzed hydrolysis of amides. Guthrie (9) reports that the free energies of activation for the acid hydrolysis of DMF and dimethyl acetamide at 25°C are 26.3 and 25.6 kcal mol^{-1} , respectively, while the corresponding base hydrolyses have values of 22.6 and 24.1 kcal mol^{-1} , respectively. Bolton and co-workers (10, 11) gave E_a (ΔS_{75}^\ddagger) values of 18.8–21.5 kcal mol^{-1} (-17.4 to $-22.0 \text{ cal K}^{-1} \text{ mol}^{-1}$) for the acid hydrolysis of some primary amides and for *N*-methyl and *N,N*-dimethyl acetamide as well as ΔH^\ddagger and ΔS^\ddagger values of 11.7–17.4 kcal mol^{-1} (-29 to $-39 \text{ cal K}^{-1} \text{ mol}^{-1}$) for the base hydrolysis of a series of primary amides. Langlois and Broche (12) gave E_a (ΔS_{70}^\ddagger) values of 18.4 kcal mol^{-1} ($-24.7 \text{ cal K}^{-1} \text{ mol}^{-1}$) for the acid hydrolysis of DMF, and 14.9 kcal mol^{-1} ($-28.2 \text{ cal K}^{-1} \text{ mol}^{-1}$) for its base hydrolysis. Although no experimental activation parameters for any of the reactions of 3 are available, it is striking that the computed barrier for its water reaction (7c), and that of DMF (8) is much larger than any experimental values for the acid- or base-catalyzed pathways of simple amides. An experimental search for the water reaction of some esters and simple amides like benzamide, butyramide, and acetamide was undertaken at high temperature in NaHCO_3 – HOAc buffered media from pH 5–8 (13) but only the hydroxide process was claimed to be observed.

Our interest in amide water reactions was piqued by a report (14) stating that the pH/rate profile for hydrolysis of formamide between pH 1 and 9 at 80°C contains a slight plateau region at pH 6. The observed kinetics were fit to a general expression ($k_{\text{obs}} = 0.0178[\text{H}^+] + 0.211[\text{OH}^-] + k_w$) with the water reaction having a rate constant (k_w) of $8.4 \times 10^{-8} \text{ s}^{-1}$, ($t_{1/2} = 95$ days). In the authors' words: "the k_w term is probably real, even though it is never large enough to contribute more than about 50% to the total reaction." Given the possibility that one might separate the water reaction from the H_3O^+ or HO^- reactions at some other temperature, we undertook a study of the hydrolysis of formamide at 56 and 120°C. Assessment of both the acid- and base-catalyzed hydrolyses at various temperatures was required to ascertain their importance in the neutral pH region where the water term is expected and so we obtained the activation parameters for both these processes. As part of the latter investigation we performed solvent deuterium kinetic isotope effect studies at 27°C in the base domain.

Experimental

Materials

MES buffer (morpholinoethanesulphonic acid) was reagent grade (Sigma). Acetic acid was distilled prior to use; NaH_2PO_4 and Na_2HPO_4 were used as supplied (Fisher). H_2O was made free from dissolved CO_2 and stored under Ar. D_2O (CDN Isotopes, 99.9 atom % D) was used as supplied as was formamide (99.5+%, A.C.S. reagent grade, Aldrich).

Kinetics

The rates of hydrolysis of formamide were determined by ^1H NMR analyses (Bruker DMX-Avance 500 spectrometer

Table 1. Second-order rate constants for formamide hydrolysis in base ($I = 0.1$ M KCl).^a

T (K) (°C)	[OH ⁻] (M)	k_{HO^-} (M ⁻¹ s ⁻¹)
393 (120)	9.33×10^{-6} ^b	3.32
	3.98×10^{-6} ^b	3.76
353 (80) ^c		0.211
329 (56)	0.075	$(3.0 \pm 0.1) \times 10^{-2}$
	0.015	$(3.05 \pm 0.07) \times 10^{-2}$
319 (46)	0.075	$(1.37 \pm 0.08) \times 10^{-2}$
300 (27)	0.075	$(2.3 \pm 0.1) \times 10^{-3}$
	0.015	$(2.2 \pm 0.1) \times 10^{-3}$
	1.47	$(3.07 \pm 0.1) \times 10^{-3}$ ^d
	0.075 (D ₂ O)	$(1.98 \pm 0.01) \times 10^{-3}$
	1.47 (D ₂ O)	$(4.03 \pm 0.1) \times 10^{-3}$ ^d

^a $\Delta H^\ddagger = 17.9 \pm 0.2$ kcal mol⁻¹; $\Delta S^\ddagger = -11.1 \pm 0.5$ cal K⁻¹ mol⁻¹.^bCalculated from pH 6.87 and 6.55 buffer results in Table 4 assuming rates at those pH values are controlled by the HO⁻ process. See Results for calculation of [HO⁻] at 120°C.^cData from ref. (14).^dIonic strength not controlled. Average of duplicate runs.**Table 2.** Second-order rate constants for formamide hydrolysis in acid ($I = 0.1$ M KCl).^a

T (K) (°C)	[H ₃ O ⁺] (M)	$k_{\text{H}_3\text{O}^+}$ (M ⁻¹ s ⁻¹)
393 (120)	5.6×10^{-5}	0.184 ^b
353 (80) ^c		0.0178
329 (56)	0.1	$(3.3 \pm 0.1) \times 10^{-3}$
	0.01	$(3.1 \pm 0.1) \times 10^{-3}$
300 (27)	0.1	$(1.83 \pm 0.08) \times 10^{-4}$
	0.01	$(1.57 \pm 0.11) \times 10^{-4}$

^a $\Delta H^\ddagger = 17.0 \pm 0.4$ kcal mol⁻¹; $\Delta S^\ddagger = -18.8 \pm 1.3$ cal K⁻¹ mol⁻¹.^bCalculated from the pH 5.6 data in buffer given in Table 4.^cData from ref. (14).

equipped with a broadband inverse probe). The ¹H NMR spectra in water were accumulated using a standard presaturation water suppression technique. Solutions of base (0.015 and 0.075 M, ($I = 0.1$ M (KCl))) in either H₂O or D₂O were prepared under CO₂-free conditions and stored under Ar. Base concentrations were determined by titration with standardized 0.1 N HCl, phenolphthalein indicator. Hydrolysis in acid was carried out in 0.01 M and 0.1 M HCl, $I = 0.1$ M (KCl). For reasons of solubility of the formamide, ionic strength was not kept constant for the kinetic experiments in MES buffer (pH 5.6, 5.9, 6.2; [buffer]_t = 0.01, 0.02, and 0.04 M for each pH). Each NMR tube contained 0.5 mL of the acid, base, or buffer solution and 5×10^{-4} – 1×10^{-3} M of formamide along with 10 μL of D₂O as a lock. For slower reactions, sealed NMR tubes were thermostated at the desired temperature and removed at various times to collect the NMR spectra. For faster reactions, the NMR data were acquired continuously from the tube placed in the probe held at the desired temperature, and every 32 scans were summed separately (the time being recorded as the midpoint of the number of scans utilized). This process was repeated up to at least two hydrolysis half-times. For

Table 3. Pseudo-first-order rate constants for formamide hydrolysis in buffered media at 56°C.^{a,b}

pH (Uncorrected) at ambient temperature	pH (Corrected) measured at 56 °C	k (s ⁻¹) (Extrapolated to [buffer] = 0)
5.6	5.5	$(1.48 \pm 0.02) \times 10^{-8}$
5.9	5.8	$(9.85 \pm 0.16) \times 10^{-9}$
6.2	6.1	$(9.50 \pm 0.39) \times 10^{-9}$

^aMES buffers, ionic strength not controlled.^bSee Table 1S, Supplementary material³ for original data.**Table 4.** Pseudo-first order rate constants for hydrolysis of formamide in buffered media at 120°C.

pH (Measured at 25°C)	k (s ⁻¹) ^a
4.25 ^b	1.035×10^{-5}
4.75 ^b	3.75×10^{-6}
5.33 ^b	2.8×10^{-6}
5.75 ^b	3.8×10^{-6}
6.15 ^c	5.9×10^{-6}
6.50 ^c	1.5×10^{-5}
6.87 ^c	3.1×10^{-5}

^aAcetate buffer, ionic strength not controlled.^bPhosphate buffer, ionic strength not controlled.^cSee Table 2S, Supplementary material³ for original data.

very slow reactions under buffered conditions (pH 5.6, 5.9, and 6.2), sealed NMR tubes with solutions of formamide in 0.5 mL of MES buffer were placed in a thermostated bath held at 56°C and the NMR spectra were collected at various time intervals over a period of 6 months (Tables 1–4).

The specific kinetic experiments for the 120°C conditions are as follows: a 10 mL solution of amide in acetate or phosphate buffer of known concentration and pH, measured at 25°C, buffered at various values between pH 6.15 and 6.87 for phosphate, and 4.25–5.75 for acetate, was divided into 10 portions, each being sealed in an ampoule. The ampoules were thermostated at 120°C (silicon oil, Hi-temp bath 160A, Fisher Scientific) for a maximum time corresponding approximately to one half-time of hydrolysis. Samples were withdrawn from the bath at various times and plunged into an ice bath. These were opened and, after the addition of 10 μL of D₂O to 0.5 mL of the sample solution, analyzed by NMR. The rate constants quoted in Tables 3 and 4 were obtained from the intercepts of the plots of the observed rate constant vs. [buffer]. All the individual rate constants were determined in duplicate (original data in Tables 1S and 2S).³

Solvent deuterium kinetic isotope experiments were undertaken in D₂O at [NaOD] = 0.075 and 1.47 M, respectively, at 27°C. For the latter concentration, two independent runs were undertaken, while a single run was performed at the former concentration.

Pseudo-first-order rate constants (k_{obs}) for formamide hydrolysis under the various conditions were obtained by observing the rate of increase of the intensity of the signal at δ 8.46 attributable to the O=C-H proton of the formic acid

³Supplementary material may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc.ca/cisti/irm/unpub_e.shtml for information on ordering electronically).

formed (A) and the decrease of the intensity of the signal at δ 7.55 for that of formamide (B). The formamide signal at δ 7.55 appears as a doublet ($J = 14.9$ Hz) coupled to one of the NH protons while the two NH protons appear as two broad triplets coupled to ^{14}N . The one coupled to the formamide proton appears at δ 7.17 ($J_{\text{N-H}} \sim 60$ Hz), while the other NH appear as a sharper triplet at δ 7.55 ($J_{\text{N-H}} \sim 62$ Hz). Rate constants were evaluated from the slopes of the $\ln(A/(A+B))$ vs. time plots with the errors in k_{obs} being determined as the standard deviation of the linear regression lines. Between 15 and 20 experimental points were used for each plot.

Activation parameters for the acid- and base-promoted hydrolyses were determined from plots of the log of the second-order rate constant ($k_{\text{H}_3\text{O}^+}$ or k_{HO^-}) vs. $1/T$ at four and five different temperatures including the reported data of Hine et. al (14) at 80°C with a total of seven and eight independent kinetic runs.

pH measurements of the reaction mixtures at ambient temperature and at 56°C were made with a Radiometer TTT2 unit and a Radiometer pHC4406 combined pH electrode. In the case of the reactions at 120°C , the pH was measured at ambient temperature before the reaction.

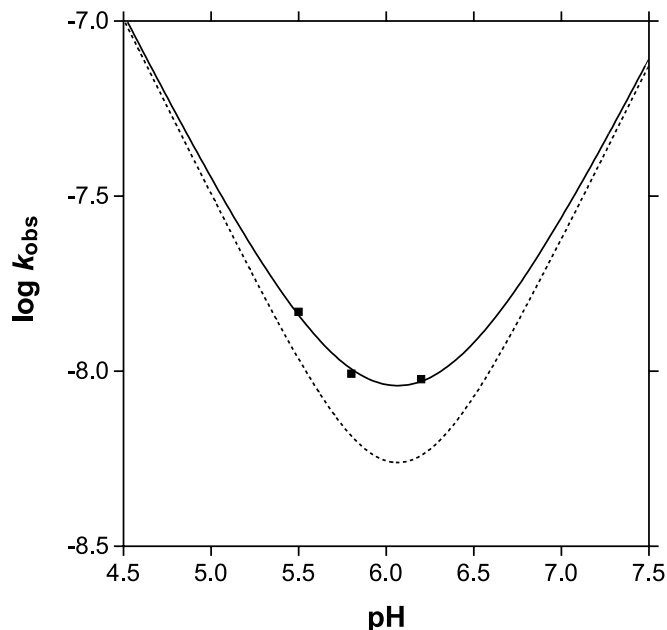
Results

Given in Tables 1 and 2 are the second-order rate constants for the HO^- and H_3O^+ catalyzed hydrolysis of formamide in water at various temperatures (including the 80°C data (14)). The 120°C acid and base values were derived under buffered conditions (Table 4, vide infra) at the lowest and highest pH values, where the respective reactions are first order in $[\text{H}_3\text{O}^+]$ and $[\text{HO}^-]$. Also included in Table 1 are the k_{OD^-} values at two $[\text{OD}^-]$ that give $k_{\text{OH}^-}/k_{\text{OD}^-}$ values of 1.15 at $[\text{OL}^-] = 0.075$ M, and 0.77 ± 0.06 at $[\text{OL}^-] = 1.47$ M, $T = 27^\circ\text{C}$.

Given in Table 3 are the rate constants determined under buffered conditions (MES, extrapolated to $[\text{buffer}] = 0$) at 56°C , while in Table 4 are the data at 120°C (acetate, phosphate extrapolated to $[\text{buffer}] = 0$). The original data in buffers used to construct these tables are given as Tables 1S and 2S, supplementary material.³ The contribution of the acid plus base terms in the neutral pH region can be computed from the second-order rate constants provided in Tables 1 and 2. The hydroxide contribution at $5 < \text{pH} < 7$ requires a correction of the $[\text{HO}^-]$ (13) attributable to the change in the K_w of water from $1 \times 10^{-13.997}$ at 25°C to $1 \times 10^{-13.11}$ at 56°C , and $1 \times 10^{-11.90}$ at 120°C (determined from the linear interpolation of the reported data (15) for K_w at 55 and 60°C , or the data at higher temperatures (16)).

Shown in Figs. 1 and 2 as dotted lines are the computed pH/rate profiles for hydrolysis only considering the H_3O^+ and HO^- terms at 56°C and 120°C , along with the experimental pseudo-first-order rate constants (■) in buffered conditions. The solid lines through the data in Figs. 1 and 2 come from NLLSQ fits of the 56 and 120°C data to the expression given in eq. [2], modeled for a process involving acid, base, and water terms. For the 56°C computation, values for the H_3O^+ and HO^- terms (Tables 1 and 2) were set as constants.

Fig. 1. Solid line: NLLSQ fit of the 56°C hydrolysis data in Table 3 to eq. [2] where $k_{\text{H}_3\text{O}^+} = 0.0032 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{OH}^-} = 0.0303 \text{ M}^{-1} \text{ s}^{-1}$ are set constants and the autoprotolysis constant of water is $\text{p}K_w = 13.11$. Experimental data (■) for pseudo-first-order rate constants for hydrolysis from Table 3. Dotted line: computed pH/rate profile at 56°C for the hydrolysis of formamide assuming only the acid and base terms are operative.



$$[2] \quad k_{\text{obs}}^t = k_{\text{H}_3\text{O}^+}[\text{H}_3\text{O}^+] + k_{\text{OH}^-}[\text{OH}^-] + k_w$$

The best fit lines are: $k_{\text{obs}}^{56} = 0.00303[\text{H}_3\text{O}^+] + 0.032[\text{HO}^-] + (3.6 \pm 0.1) \times 10^{-9}$, and $k_{\text{obs}}^{120} = (0.15 \pm 0.02)[\text{H}_3\text{O}^+] + (3.20 \pm 0.24)[\text{HO}^-] + (1.09 \pm 0.29) \times 10^{-6}$.

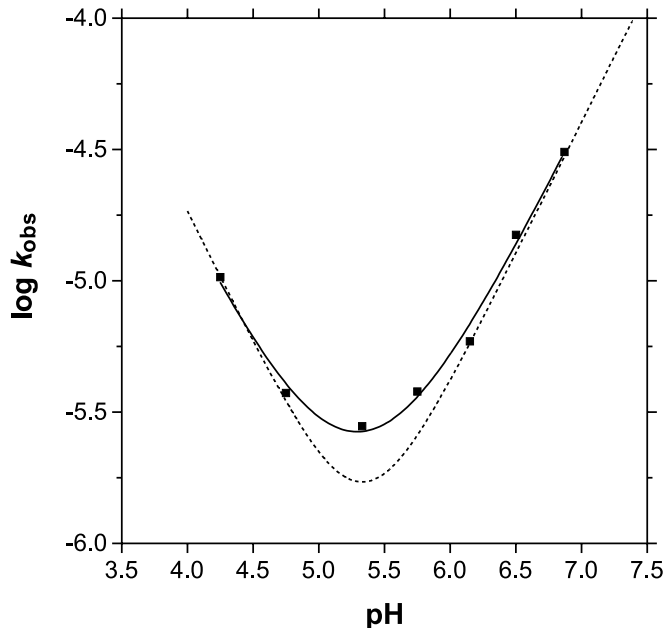
Discussion

Water reaction?

Activation parameters (ΔH^\ddagger and ΔS^\ddagger) of (17.0 ± 0.4) kcal mol^{-1} and (-18.8 ± 1.3) cal $\text{mol}^{-1} \text{ K}^{-1}$ for the H_3O^+ catalyzed reaction and (17.9 ± 0.2) kcal mol^{-1} and (-11.1 ± 0.5) cal $\text{mol}^{-1} \text{ K}^{-1}$ for the HO^- reaction were determined from standard Eyring plots of the second-order rate constants given in Tables 1 and 2. These experimental values for formamide compare favorably with those reported for other amides (9–12). On the other hand, the activation parameters for the OH^- catalyzed hydrolysis gave a ΔG_{25}^\ddagger of 21.2 kcal mol^{-1} which is about 25% lower than the recently reported computational value of 27.3 kcal mol^{-1} (17).

We envision that the acid, base, and water pathways must have different activation parameters so that one might isolate the water process by changing the reaction temperature. However it is not easily predicted whether one should move to higher or lower temperatures than the 80°C used by Hine et al. (14) because the literature gives little guidance concerning the activation parameters of the three processes in any given case. Computations can sometimes be of assistance but for formamide the calculated free energy of activation for the water reaction of 48 kcal mol^{-1} at 25°C (7c) seems too high relative to the experimental activation

Fig. 2. Solid line: unconstrained NLLSQ fit of the 120°C hydrolysis data in Table 4 to eq. [2] with the autoprotolysis constant of water being set at $pK_w = 11.90$. Experimental data (■) for pseudo-first-order rate constants for hydrolysis under buffered conditions from Table 4. Dotted line: computed pH/rate profile for the hydrolysis of formamide at 120°C assuming only the acid and base terms are operative.



parameters we determined here for the acid and base processes for it to play any significant role at easily accessible temperatures. Guthrie and Pitchko's (8) recent computed value of $\Delta G_{25}^\ddagger = 31.9 \text{ kcal mol}^{-1}$ for DMF hydrating via a three-water cyclic mechanism seems more reasonable as an upper limit for that amide, this value being some 5 to 6 kcal mol^{-1} larger than the acid and base values (9). Nevertheless, taken at face value the extant experimental rate data for formamide (14) suggest that the free energies for these processes must be similar if the water, acid, and base processes are competitive at 80°C. Esters, which are generally more reactive than their amide counterparts, might provide a starting point for comparison, but apparently the water reactions are exceedingly slow and little kinetic data are available. The water reaction for hydrolysis of methyl acetate has been estimated (18) to have a rate constant at 25°C of $3 \times 10^{-10} \text{ s}^{-1}$ corresponding to a half-time of 70 years, however, a plateau is not observed at intermediate pH in the hydrolysis of ethyl acetate (19). A more recent study involving six nonactivated esters at higher temperatures (13) concluded that only the hydroxide reaction is important down to pH values of about 5, except for *tert*-butyl acetate where a pH-independent reaction is said to occur. Kirby (20) has noted that activated esters such as alkyl and aryl haloacetates and substituted phenyl acetates do have water reactions with low activation enthalpies (7–14 kcal mol^{-1}) and large negative activation entropies (–40 to –50 cal $\text{K}^{-1} \text{ mol}^{-1}$). This would be expected for highly ordered transition states in which charge buildup is avoided by several proton transfers accompanying the formation of a possible *gem*-diol intermediate. In our earlier study (5), we determined that the respective activa-

tion parameters of $\Delta H^\ddagger = 14.4 \pm 0.6$ and $11.7 \pm 0.3 \text{ kcal mol}^{-1}$ and $\Delta S^\ddagger = -36.1 \pm 1.6$ and $-52.3 \pm 1.3 \text{ cal K}^{-1} \text{ mol}^{-1}$ for the water-promoted hydrolysis of trifluoromethyl-*p*-nitroacetanilide and trifluoromethylacetanilide. Although the activation parameters were not determined for the acid and base processes, the above values are quite close to what was observed for the water reactions of activated esters.

How relevant these numbers might be to the hydrolysis of more normal, less-activated amides is unclear. As far as we know, the only other experimental activation parameters for water hydrolysis of amides were determined by Radzicka and Wolfenden (4d) who reported E_a values of 23–25 kcal mol^{-1} for the hydrolysis of three dipeptides, including glycyl-glycine, at pH 6.8 with temperatures between 120 and 200°C. The experimental $\Delta G_{25}^\ddagger = 31.71 \text{ kcal mol}^{-1}$ for the hydrolysis of *N*-acetyl glycyl-glycine (4d) compares very well with the computed 31.9 kcal mol^{-1} value for DMF (8). These values can be compared with the reported (21) activation parameters for HCl- or NaOH-promoted hydrolysis for glycyl-glycine (ΔH^\ddagger (ΔS^\ddagger), 20.3 kcal mol^{-1} (–24 cal $\text{K}^{-1} \text{ mol}^{-1}$) and 16.9 kcal mol^{-1} (–26.8 cal $\text{K}^{-1} \text{ mol}^{-1}$), respectively). Of course one must be mindful that acid, base, and neutral hydrolyses of dipeptides may involve different states of ionization of the substrates which may affect the various activation parameters in unpredictable ways.

Given the above, we set out to isolate the water reaction by obtaining the hydrolysis kinetics of formamide from initial rates at 56°C in the pH range of 5.6 to 6.2. The Table 3 data and Fig. 1 show a plateau at the pH minimum that we attribute to an observable water reaction having a computed k_{obs} value of $(3.6 \pm 0.1) \times 10^{-9} \text{ s}^{-1}$, $t_{1/2} = 6.1$ years! Concomitantly, we investigated the hydrolysis at 120°C in acetate and phosphate buffers. The enthalpies of ionization of these are small so that the dissociation constants do not vary much with temperature (15, 22). The kinetic data in Table 4, illustrated in Fig. 2, also plateau around the pH minimum. An unrestricted fit of these experimental buffer data to eq. [2] gives the values $k_{H_3O^+} = (0.15 \pm 0.02) \text{ M}^{-1} \text{ s}^{-1}$, $k_{OH^-} = (3.20 \pm 0.24) \text{ M}^{-1} \text{ s}^{-1}$, and $k_w = (1.09 \pm 0.29) \times 10^{-6} \text{ s}^{-1}$. For the latter term, $t_{1/2} = 7.3$ days.

One can, through use of the best-fit rate constants above, determine that at the pH minima of 6.1 (56°C) and 5.3 (120°C), the water reaction contributes about 40% of the overall rate at either temperature. This, coupled with Hine's careful statements that the k_w term is never large enough to contribute more than about 50% to the total reaction at 80°C, is at first glance surprising. However, further analysis indicates this is a consequence of the similarity of the activation parameters for all three hydrolysis processes, and an interesting artifact of the drop in the autoprotolysis constant of water with increasing temperature which raises the $[\text{HO}^-]$ and makes the base term more prominent at any given pH at high temperatures.

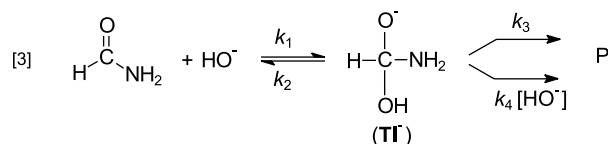
While it is now clear that there must be a water term in the hydrolysis of formamide, it is unfortunate that our best-fit water rate constants must have a 10–30% error which limits our ability to make conclusions and predictions. Nevertheless, since this is the only non-peptidic, nonactivated amide that has an experimentally demonstrated water reaction, we feel justified in putting forth a two-point experimental estimate of the Arrhenius parameters E_a (ln A) of 22.5 kcal

mol⁻¹ (15.03) based on our 56 and 120°C data. Inclusion of Hine's 80°C k_w (14), which lies slightly above the two-point plot, does not change these values greatly. The estimated k_w at 25°C is thus $1.1 \times 10^{-10} \text{ s}^{-1}$ ($t_{1/2} = 199 \text{ years}$).⁴

Solvent deuterium kinetic isotope effect in base

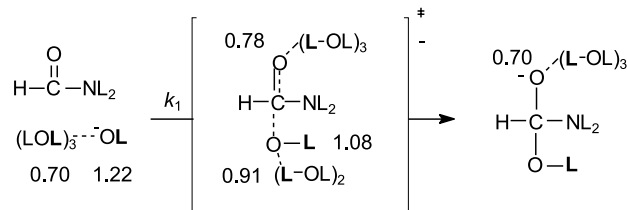
The HO⁻ promoted hydrolysis of formamide has recently been investigated by Marlier et al. (23) through the application of heavy atom kinetic isotope effects. They point out that the experimental data concerning the ¹⁸O content of the formate product arising from alkaline hydrolysis in a solvent enriched with ¹⁸OH₂ could be accommodated by either of two scenarios. With water as the attacking nucleophile, the ¹⁸O solvent kinetic isotope effect (¹⁸ k_{obs}) would be 1.022 while with hydroxide as the attacking nucleophile, ¹⁸ k_{obs} would be 0.982. Based on the experimental precedence that almost all the known cases of heavy atom isotope effects for attacking nucleophiles are normal, they proposed that a water molecule solvating the hydroxide was the actual nucleophile (through a general base pathway) and not hydroxide itself. A general base mechanism was also proposed in their earlier report of the heavy atom isotope effects for the alkaline hydrolysis of methyl formate (24), and supported by a more recent analysis of a proton inventory study of the base-promoted hydrolysis of ethyl acetate (25). Given our expectation that the general base process is expected to exhibit a substantial primary solvent deuterium kinetic isotope effect (skie) due to a proton in flight, we undertook studies on the alkaline hydrolysis of formamide in D₂O. All previous solvent dkie studies of the base-catalyzed hydrolysis of amides such as benzamides, toluamides, and anilides (1d, 2) yield values of about 1.0 or slightly inverse which are consistent with a direct nucleophilic mechanism.

Analysis of the dkie requires consideration of the hydrolytic mechanism with respect to rate-limiting steps and partitioning of any anionic tetrahedral intermediates. Marlier et al. (23) and earlier, Kirsch (26), demonstrated that the rate law for alkaline hydrolysis of formamide contains both first- and second-order terms in hydroxide which is analyzed in terms of the mechanism presented in eq. [3] for which steady-state analysis gives the expression in eq. [4].

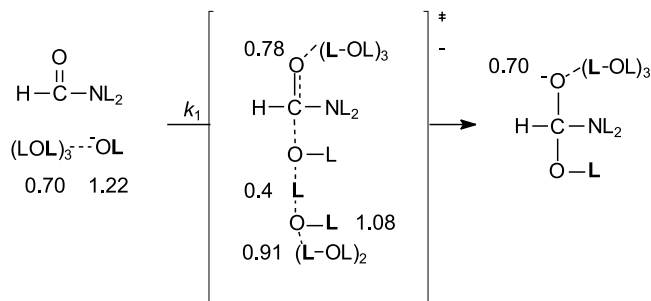


$$[4] \quad k_{\text{hyd}} = \frac{k_1[\text{HO}^-](k_3 + k_4[\text{HO}^-])}{k_2 + k_3 + k_4[\text{HO}^-]}$$

Scheme 1.



Scheme 2.



Graphical analysis (23, 26) of the partitioning of the tetrahedral intermediate (**TI**⁻, eq. [3]) indicates that the k_3/k_2 ratio is 1.05, while the k_4/k_2 ratio is 2.15 M⁻¹. At low [HO⁻] (<0.1 M), the second-order in [HO⁻] pathway is relatively unimportant and **TI**⁻ undergoes a substantial reversal, so k_{hyd} can be approximated as $k_1[\text{HO}^-]k_3/(k_2 + k_3)$. Analysis of the dkie at low [OH⁻] is complicated since no step is entirely or substantially rate-limiting. On the other hand, at high [OH⁻] the tetrahedral intermediate is driven forward via rapid capture by the second hydroxide so reversal becomes less important and attack becomes the rate-limiting step with k_{hyd} being approximated as $k_1[\text{HO}^-]$.⁵ The skie data reported in Table 1 indicate that at [LO⁻] = 0.075 M, where about 90% of the reaction proceeds by the first-order pathway ($k_{\text{OH}}/k_{\text{OD}} = 1.15$). At [LO⁻] = 1.47 M, where about 75% of the reaction proceeds via the second-order pathway ($k_{\text{OH}}/k_{\text{OD}} = 0.77 \pm 0.06$) which can thus be taken as the skie on k_1 .

We analyze the latter data through the use of fractionation factors using the same general methodology we applied successfully to the base-promoted hydrolysis of some secondary and tertiary toluamides (27, 2a) and some esters (28). The solvent dkie can be predicted (29) as $k_{\text{OD}}/k_{\text{OH}} = \Pi\phi^{\text{TS}}/\Pi\phi^{\text{GS}}$, where $\Pi\phi^{\text{TS}}$ and $\Pi\phi^{\text{GS}}$ are the products of the various fractionation factors in the transition and ground states. Two possible cases that are considered are shown in Schemes 1 and 2 which involve, respectively, direct hydroxide attack and hydroxide acting as a general base for delivery of one of its solvating waters. In analyzing the dkie, we adopt Gold and Grist's (30) suggestion that HO⁻ has a fractionation factor of 1.22 while its three solvating waters each have fractionation factors of 0.7. During the direct attack of

⁴J.P. Guthrie (University of Western Ontario, private communication) informed us that the his No Barrier Theory calculations for the water reaction of dimethyl formamide (DMF, (ΔG_{25}^\ddagger 31.9 kcal mol⁻¹)) lead to a computed water rate constant of $2.6 \times 10^{-11} \text{ s}^{-1}$.

⁵This analysis is identical to that proposed by Marlier (ref. 23) and is substantially correct. However, at [HO⁻] = 1.47 M the partitioning of the tetrahedral intermediate is ~4.1 in favour of product formation relative to reversal. Of that ratio, some 3.1 parts are attributed to the second-order pathway (k_4) and one part is attributed to the spontaneous pathway (k_3). Strictly speaking, while the dkie is substantially attributable to the attack step (k_1), there is a small additional component attributable to k_4 which should be inverse given OD⁻ in D₂O is a stronger base than is HO⁻ in H₂O.

hydroxide we assume in Scheme 1 that one of the solvating waters has been lost to liberate a lone pair required for attack on C=O, and that the developing O⁻ in **TI**⁻ is associated with three solvating waters with fractionation factors similar to those of hydroxide. We assume that the transition state for attack (k_i) is late, being ~70% along the reaction coordinate to the tetrahedral species.

In the case of the general base mechanism given in Scheme 2, we assume that the fractionation factor for the proton in flight between the hydroxide and water is 0.4 (corresponding to a normal primary k_{H} of 2.5). All the assumed values are shown in bold type in Schemes 1 and 2: these give predicted solvent k_{H} 's of ~1.0 for the direct attack of hydroxide and 2.2 for the general base mechanism.

The only substantial difference between the processes shown in Schemes 1 and 2 concerns the presence of the proton in flight which is solely responsible for the predicted normal k_{H} in the latter. Making the transition state in Scheme 1 later gives only a small increase in the predicted k_{H} because of the compensating effects of the loosening of waters solvating the attacking HO⁻, and tightening of the waters solvating the developing O⁻ in **TI**⁻. By converse, an earlier transition state for the direct attack of hydroxide, say 50% along the way to **TI**⁻, gives a predicted k_{H} of 0.85, close to the experimentally observed value of 0.77 ± 0.06 . That value seems to rule out the pathway given in Scheme 2, or any other pathway where there is much contribution from one or more protons in flight. In the limit of that mechanism, where the proton is nearly fully transferred from the attacking water to the general base HO⁻, its associated fractionation factor would be close to unity leading to a drop in the predicted k_{H} toward the observed value. In the end this simply becomes a mechanism of direct hydroxide attack and cannot be distinguished from the process in Scheme 1.⁶

Conclusions

The main goals of the above study were to provide information about the water reaction of formamide, and provide the solvent k_{H} for the base reaction. Several important conclusions can be advanced.

We have determined the activation parameters for the H₃O⁺ and HO⁻ promoted hydrolyses of formamide. The experimental ΔH^\ddagger and ΔS^\ddagger values are (17.0 ± 0.4) kcal mol⁻¹ and (-18.8 ± 1.3) cal mol⁻¹ K⁻¹ for the H₃O⁺ catalyzed reaction and (17.9 ± 0.2) kcal mol⁻¹ and (-11.1 ± 0.5) cal mol⁻¹ K⁻¹ for the HO⁻ process. The values fit within the general range determined for acid- and base-catalyzed hydrolysis of other simple amides. However, the ΔH^\ddagger and ΔS^\ddagger values for the HO⁻ process give an experimental ΔG_{25}^\ddagger of 21.2 kcal mol⁻¹ which is substantially lower than the recently computed value of 27.3 kcal mol⁻¹ (17) which should prompt further efforts to refine the computational approaches.

We have determined k_{w} values of $(1.09 \pm 0.29) \times 10^{-6}$ s⁻¹ at 120°C and $(3.6 \pm 0.1) \times 10^{-9}$ s⁻¹ at 56°C, from which we

estimated activation parameters of $E_{\text{a}} = 22.5$ kcal mol⁻¹, $\ln A = 15.03$ from a two-point Arrhenius plot.

While it is apparent that a water reaction does exist at 56°C and at 120°C, in neither case does it contribute more than about 40% to the observed rate at the respective pH minima of 6.1 and 5.3. Hine et al. (14) also concluded that at 80°C, no more than 50% of the rate at the pH minimum can be attributed to the water reaction. Taken together, these studies indicate that it is improbable that conditions will be found where the water term for hydrolysis of formamide can be isolated (meaning contributing more than 95% at any given pH) from the corresponding acid and base reactions. This is an interesting consequence of two major factors: (1) the relative activation parameters of all three processes for this specific amide; and (2) the increase in the autoprotolysis constant of water as a function of temperature that increases the [HO⁻] at any given pH, the net effect being to drive the pH minimum for the reaction to lower values at higher temperatures.

We have presented solvent deuterium kinetic isotope data for the base-catalyzed reaction at high [HO⁻] where the kinetics are largely determined by the first step leading to the tetrahedral intermediate. The observed inverse value of $k_{\text{OH}}/k_{\text{OD}} = 0.77$ gives little evidence for a significant contribution of a proton in flight and strongly supports the mechanism involving the direct attack of hydroxide, similar to that believed operative for other amides, and at variance with a suggested water attack mediated by HO⁻.

Acknowledgements

The authors gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada (NSERC) and Queen's University. In addition they acknowledge the generous donation of NMR time and expertise by Bruker Canada. In addition they are grateful for the helpful comments of the referees and for insightful personal communications from Professor J. P. Guthrie, University of Western Ontario.

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⁶This analysis differs from the one used by Mata-Segreda in assessing the mechanism for hydroxide attack on ethyl acetate (25). In that analysis the assumed transition structure involved HO⁻...H-(OH)...C=O with no solvating waters and a proton in flight between the attacking water and the general base HO⁻. A future publication from these laboratories will present our analysis of the proton inventory study of the base hydrolysis of formamide.

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