Reversible Formation of Intermediates during H_3O^+-Catalyzed Hydrolysis of Amides. Observation of Substantial ^{18}O Exchange Accompanying the Hydrolysis of Acetanilide and N-Cyclohexylacetamide

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Abstract: Careful mass spectrometric analysis of the ^{18}O content of $\sim 50\%$ enriched acetanilide (**2**) and N-cyclohexylacetamide (**3**) recovered from acidic media during the course of hydrolysis reveals that both species suffer ^{18}O loss. The percent of ^{18}O exchange per $t_{1/2}$ of hydrolysis increases as $[H_3O^+]$ decreases. For **2** at 72 °C the amount of exchange increases from 0.5 $\pm$ 0.5% (per $t_{1/2}$) in 1 M HCl to 9.4 $\pm$ 0.5% in glycine buffer, $[H_3O^+]$ = 0.003 M. For **3** at 100 °C the exchange is 1.05 $\pm$ 0.3% (per $t_{1/2}$) at 1 M HCl and 9.0 $\pm$ 0.4% in 0.01 M HCl. When these data are used to compute k_{ex} (the exchange rate constant), it shows a first-order dependence on $[H_3O^+]$ followed by a plateau at high $[H_3O^+]$ for both **2** and **3**.

Acid-catalyzed amide hydrolysis is generally considered to proceed by pathways involving irreversibly formed tetrahedral intermediates.[1] Numerous ^{18}O-exchange studies with labeled benzamides[2] and one with acetylimidazole[3] indicated that no detectable ^{18}O loss occurs in recovered starting material during the course of hydrolysis in strongly acidic media. However, McClelland observed that 90% ^{18}O-enriched benzamide exhibited 0.2% exchange per $t_{1/2}$ hydrolysis in 5.9% H_2SO_4 at 85 °C. Apparently, further increases in $[H_3O^+]$ do not lead to additional ^{18}O exchange, at least for benzamides.[2b,4b]

Recently we reported that substantial ^{18}O loss accompanies H_3O^+-catalyzed hydrolysis of labeled **1** between pH 4–5.[5] The major question is whether the exchange is a consequence of the unorthodox geometry of **1**[6] or is a fairly general phenomenon of
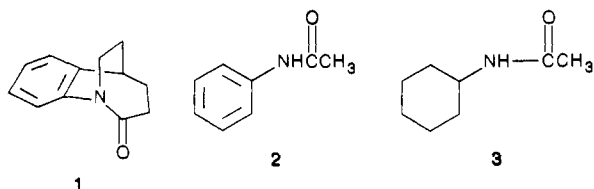
acid-catalyzed hydrolysis of amides when conducted at low $[H_3O^+]$.[7] Herein we report that two typical amides, acetanilide (**2**) and N-cyclohexylacetamide (**3**), indeed show increasing exchange when hydrolyzed at progressively lower $[H_3O^+]$. These unprecedented observations are not easily reconciled in terms of the currently accepted mechanism of $[H_3O^+]$-catalyzed amide hydrolysis.

Experimental Section

Materials. Acetanilide was commerical (Aldrich). N-Cyclohexylacetamide was prepared by acetylation of cyclohexylamine. Both materials were recrystallized from H_2O. Labeled materials were prepared as follows.

Acetyl chloride (0.785 g, 0.71 mL, 1.0 mm) was placed in a dried 5-mL flask equipped with a microdistillation apparatus protected from moisture and with the thermometer replaced by a serum stopper. The flask was then cooled to −20 °C and 0.19 mL of $H_2^{18}O$ (97.2 atom % ^{18}O, Cambridge Isotope Laboratories) added dropwise via syringe. The flask was warmed to 0 °C, and a slow but exothermic reaction occurred. After 30 min at room temperature, the flask was recooled to 0 °C and 0.45 g (0.29 mL) of freshly distilled PCl_3 added. The mixture was allowed to come to room temperature, and after 1 h, two layers had formed. The ^{18}O-labeled acetyl chloride was distilled directly into a flask held at 0 °C and containing 2.2 mmol of the desired freshly distilled precursor amine. After extraction with methylene chloride and washing the CH_2Cl_2 layer with dilute HCl and then saturated NaCl, the organic layer was dried

over $MgSO_4$. Following filtration, the volatiles were removed, yielding essentially a quantitative yield (based on 1 equiv of amine) of amide (50–55% ^{18}O enriched). The enriched amides were subsequently purified by recrystallization from H_2O.

Kinetics. (a) **Hydrolysis.** Pseudo-first-order rate constants (k_{hyd}) were obtained by observing the rate of decrease in absorbance of 1 × 10⁻⁴ M aqueous solutions of the acetanilide (**2**) at the wavelength of maximum change (240 nm) by using a Cary 210 UV–vis spectrophotometer interfaced as previously described.[8] Stock aqueous solutions of **2** (0.01 M) were prepared, and reactions were initiated by injecting 30 μL of the solution into a 1-cm quartz cuvette containing 3.0 mL of HCl or buffer solution (μ = 1.0 M, KCl) that had been equilibrated for 30 min at 72 °C in the cell holder. Rate constants were obtained by nonlinear least-squares fitting of the Abs vs. time curves to a standard exponential model ($A_t = A_{t\infty} + (A_{t0} - A_{t\infty})e^{-kt}$). The reactions were normally followed to completion except for the slowest, which were followed to two half-times. In all cases they exhibited clean first-order kinetics.

The rate of hydrolysis of N-cyclohexylacetamide was determined by an NMR technique[9] using a Bruker WH-200 spectrometer. A series of 0.05 M samples (0.02 M in the case of the lowest acid concentration) thermostated at 100 °C in HCl solutions (1.0, 0.5, and 0.05 N HCl; μ = 1.0 M KCl) were analyzed at various times to determine the solution composition. Immediately prior to NMR analysis, one drop of D_2O was added to ~ 300 μL of the solution. Careful integration of the O=C—CH₃ and >CH—N signals in both starting material (δ 1.88 and 3.4, respectively) and products (δ 1.84 and 2.9, respectively) provided the relative amounts of each material. The rate constants were calculated from the slope of the plot of ln [(*I* amide)/(*I* amide + *I* product)] vs. time, where *I* is the integrated intensity of the appropriate peak (methyl or methine).

(b) ^{18}O-**Exchange Kinetics.** A typical exchange experiment was conducted as follows. A 30-mL solution of amide **2** or **3** (0.005–0.001 M, μ = 1.0 M KCl, 50–55% ^{18}O enriched) was divided into three portions, each being sealed in an ampule. The ampules were thermostated at 72 °C (for **2**) or 100 °C (for **3**) for a time corresponding to the half-time ($t_{1/2}$) for hydrolysis. Samples were withdrawn from the temperature bath and plunged into an ice bath. These were then opened and extracted with 3 × 10 mL of purified CH_2Cl_2, and the combined extracts were washed

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(7) OH⁻-promoted hydrolysis of amides is well-known to promote large amounts of exchange.[1,2] The conditions herein include only the H⁺ domain: $[H_3O^+] > 10^{-3}$.
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Table I. Pseudo-First-Order Hydrolysis Rate Constants and ^{18}O-Exchange Data per $t_{1/2}$ Hydrolysis for Acetanilide at 72 °C, μ = 1.0 M KCl

[Buffer]^a (M)	k_{hyd} (s^{-1} × 10^6)^b	% ^{18}O found^c at $t_{1/2}$	mean (%)	% ^{18}O^e exchange/$t_{1/2}$	k_{ex} (s^{-1} × 10^7)^f
HCl, 1.0	262 ± 1	54.57 ± 0.07	54.54 ± 0.10	0.5 ± 0.5	17.9 ± 18.0
		54.50 ± 0.07			
HCl, 0.5	146 ± 1	54.45 ± 0.07	54.45 ± 0.07	0.6 ± 0.4	13.5 ± 9.1
		54.44 ± 0.03			
HCl, 0.1	34.1 ± 0.1	53.40 ± 0.10	53.32 ± 0.20	2.7 ± 0.7	13.5 ± 3.5
		53.25 ± 0.10			
HCl, 0.05	16.1 ± 0.1	51.70 ± 0.10	51.65 ± 0.15	5.7 ± 0.6	13.7 ± 1.5
		51.60 ± 0.10			
glycine, 0.4 [H⁺] = 0.032	10.4 ± 0.1	51.54 ± 0.10	51.43 ± 0.20	6.1 ± 0.7	9.5 ± 1.10
		51.32 ± 0.08			
glycine, 0.1 [H⁺] = 0.032	10.0 ± 0.1	51.42 ± 0.04	51.42 ± 0.10	6.1 ± 0.5	9.2 ± 0.84
		51.41 ± 0.09			
glycine,^{d} 0.4 [H⁺] = 0.01	3.51 ± 0.01	49.16 ± 0.06^d	49.22 ± 0.12	7.7 ± 0.5	4.1 ± 0.28
		49.29 ± 0.03^d			
glycine,^{d} 0.2 [H⁺] = 0.01	3.56 ± 0.01	49.22 ± 0.12^d	49.20 ± 0.14	7.7 ± 0.6	4.1 ± 0.34
		49.18 ± 0.08^d			
glycine, 0.4 [H⁺] = 0.003	1.15 ± 0.02	49.61 ± 0.05	49.65 ± 0.15	9.4 ± 0.6	1.64 ± 0.13
		49.69 ± 0.11			
glycine, 0.2 [H⁺] = 0.003	1.07 ± 0.05	49.66 ± 0.11	49.66 ± 0.11	9.4 ± 0.5	1.5 ± 0.36

^{a} [H₃O⁺] determined at 25 °C in the case of glycine buffers. ^{b} Determined by observing rate of change of absorbance at 240 nm in duplicate. Rate constants derived from nonlinear least-squares fitting of Abs vs. time data to standard exponential model. Error limits from least-squares standard deviations. ^{c} Initial ^{18}O content determined by isolation of labeled acetanilide at time 0 from 1 N HCl solution (54.73 ± 0.09; 54.87 ± 0.08; mean = 54.80 ± 0.15). ^{d} New sample labeled acetanilide used in this run; ^{18}O content by isolation from glycine at time 0 (53.37 ± 0.08; 53.30 ± 0.09; mean = 53.34 ± 0.14). ^{e} Normalized to 100% ^{18}O at time 0. Error limits calculated as sum of standard deviations of the mean plus that of time 0 sample normalized to 100%. ^{f} Calculated from percent ^{18}O content at $t_{1/2}$ hydrolysis as in text; error limits are cumulative sums of standard deviations in ^{18}O content and k_{hyd}.

Table II. Pseudo-First-Order Hydrolysis Rate Constants and ^{18}O-Exchange Data per $t_{1/2}$ Hydrolysis for *N*-Cyclohexylacetamide at 100 °C, μ = 1.0 M KCl

[HCl] (M)	k_{hyd} (s^{-1} × 10^6)^a	% ^{18}O found^b at $t_{1/2}$	mean (%)	% ^{18}O exchange^c/$t_{1/2}$	k_{ex} (s^{-1} × 10^8)^d
1.0	53 ± 2	50.11 ± 0.04	50.12 ± 0.05	1.05 ± 0.3	80.4 ± 27.0
		50.13 ± 0.02			
0.5	27.7 ± 1.0				
0.2		49.45 ± 0.05	49.37 ± 0.13	2.5 ± 0.5	42.1 ± 10.5
		49.30 ± 0.04			
0.1		48.19 ± 0.04	48.20 ± 0.17	4.8 ± 0.5	40.7 ± 6.3
		48.21 ± 0.16			
0.05	2.85 ± 0.04	47.80 ± 0.10	47.85 ± 0.20	5.5 ± 0.6	23.4 ± 3.7
		47.90 ± 0.15			
0.02		46.70 ± 0.10	46.80 ± 0.20	7.6 ± 0.6	13.0 ± 1.7
		46.90 ± 0.10			
0.01		46.08 ± 0.05	46.10 ± 0.10	9.0 ± 0.4	7.7 ± 0.7
		46.10 ± 0.10			

^{a} k_{hyd} determined by ^{1}H NMR analysis according to the method of Williams.^{9} ^{b} Sample separated at time 0 from 1 M HCl (50.72 ± 0.04; 50.59 ± 0.03; mean = 50.65 ± 0.11). ^{c} Normalized to 100% ^{18}O-enriched sample at time 0. Error limits calculated as sum of standard deviations of the mean plus that of time 0 sample normalized to 100%. ^{d} Calculated from percent ^{18}O content at $t_{1/2}$ hydrolysis; where k_{hyd} is not given it was calculated assuming a first-order dependence in [H₃O⁺] and a ±5% error, which is factored into k_{ex}.

with saturated NaCl until the aqueous layer was neutral. The organic layer was dried (MgSO₄) and stripped of solvent to yield a residue which was subjected to direct mass spectrometric analysis with an AEI MS-12 low-resolution mass spectrometer. The ^{18}O content of the reisolated material was calculated as $(I_{M^++2})/(I_{M^++2} + I_{M^+})$, where I is the peak intensity of the parent and enriched parent ions. Nine to sixteen separate determinations of the M⁺ and M⁺ + 2 intensities were recorded. Primary data are given in the supplementary material (Tables 1S and 2S). Values given in Tables I and II are the averages of two independent determinations along with the cumulative standard deviations. As a check to exclude anomalous exchange during the extraction and analysis procedure, the ^{18}O content of three independent samples removed at t = 0 was determined and compared with that of authentic material: in no case was the ^{18}O content different within the experimental accuracy.
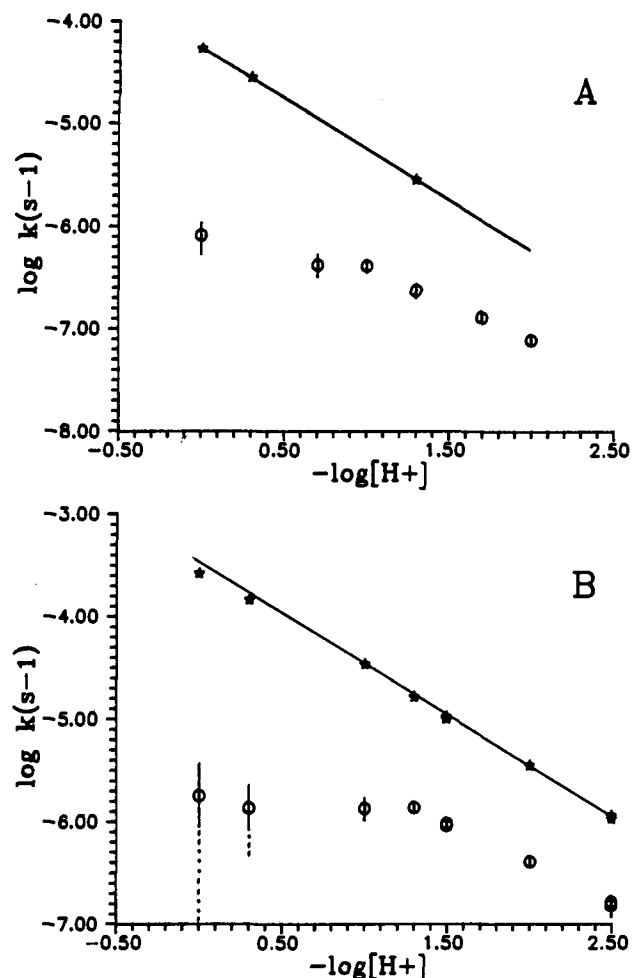
Results

Given in Tables I and II are hydrolytic rate constants and mass spectrometric ^{18}O-exchange data for ~50% labeled **2** and **3** reisolated from solution at the hydrolytic $t_{1/2}$ at various [H₃O⁺]. Duplicate isolation experiments were performed, and the error limits quoted in column three of Tables I and II are the standard deviations of 9-16 scans of the M⁺ and M⁺ + 2 peaks. From the mean values in column four it is readily seen that an increasing depletion of ^{18}O content occurs at lower [H₃O⁺] for both amides.

The depletion is expressed as percent of ^{18}O exchange per $t_{1/2}$ hydrolysis (normalized to 100% enrichment at zero time) in column five. Given in column six are the k_{ex} values, calculated according to $k_{ex}t_{1/2}$ = ln $(a/a - x)$, where a and $a - x$ are the ^{18}O contents at zero time and $t_{1/2}$, respectively. The error limits in k_{ex} are calculated based on the cumulative standard deviations in both ^{18}O contents and k_{hyd}. The errors are largest at low amounts of exchange and less so at high amounts, but their inclusion does not alter the conclusion that the amount of exchange increases as [H₃O⁺] decreases.

Finally, shown in Figure 1 is a plot of log k_{ex} and log k_{hyd} vs. $-$log [H₃O⁺] for both amides. The point of note is that the log k_{ex} values for both amides tend to plateau at high [H₃O⁺] but tend to a first-order dependence at low [H₃O⁺].^{10,11}

(10) As with other reported amides,^{1b,2d,11} the k_{hyd} values for **2** and **3** show significant deviations from linearity at high [H₃O⁺]. In the regions where significant increases in ^{18}O exchange occur, a first-order dependence of k_{hyd} on [H⁺] obtains.

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in base hydrolysis,[1,14] as well as in both acid and base hydrolysis of carboxylic esters,[1,15] and has been traditionally interpreted as implying the intermediacy of reversibly formed tetrahedral intermediates.

The generally accepted mechanism for amide hydrolysis in acid involves H_2O attack on an O-protonated amide[1,9] to produce a tetrahedral addition intermediate which undergoes rapid N-protonation and subsequent irreversible C–N cleavage (eq 1).
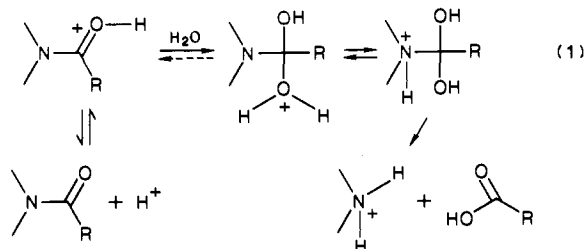
Our present results require that there be at least one intermediate (not necessarily given in eq 1) that is in equilibrium with starting amide and allows oxygen exchange. There are two major considerations in both quantitating the exchange data and relating it to the hydrolytic process. The first assumes that the intermediates are at equilibrium with respect to proton transfer. Thus, if there is a reversibly formed amide hydrate, both oxygens have an equivalent probability for loss (exclusive of $C-^{16}O/C-^{18}O$ kinetic isotope effects). The fact that changes in [glycine buffer] at low $[H_3O^+]$ affect neither k_{hyd} nor k_{ex} suggests the various intermediates *are* at equilibrium with respect to proton transfer, at least in the case of acetanilide.

A second and perhaps more serious assumption is that the intermediate leading to exchange is on the hydrolytic pathway.[1,4a] Inasmuch as microscopic adherance to eq 1 requires that the transition states leading to C–O or C–N cleavage each have the same molecular composition, (H^+, OH_2, amide), with that scheme it is difficult to explain why the k_{ex} and k_{hyd} rate constants diverge as a function of $[H_3O^+]$. Perhaps this indicates that there are two parallel processes, one leading to exchange and another to hydrolysis.

More work is clearly required to determine the scope, limitation, and structural constraints on the exchange process prior to proposing a scheme which explains these findings. Nevertheless, the observation of significant exchange accompanying acid-catalyzed hydrolysis of these two amides challenges our current understanding of this important process.

Figure 1. Plots of log k_{hyd} (*) and log k_{ex} (O) vs. –log $[H_3O^+]$ for N-cyclohexylacetamide (A) and acetanilide (B) determined at 100 and 72 °C, respectively, μ = 1.0 M KCl. The k_{ex} values are calculated from percent ^{18}O content at $t_{1/2}$ hydrolysis (see text). Dashed error bars in exchange data of B indicate there is no satisfactory lower limit due to error limits exceeding the value of k_{ex}. Straight lines through k_{hyd} data are unit slope first-order dependence on $[H_3O^+]$.

Discussion

Changes in C–O/C–N cleavage ratios as a function of pH have been noted in acid-catalyzed hydrolyses of certain imidate esters[12] and amide acetals[4b,13] and have been explained in terms of the involvement of tetrahedral intermediates differing in the site and state of protonation. In those cases, the C–O/C–N cleavage ratio increases at lower $[H_3O^+]$. Although phenomenologically a similar situation is observed with **2** and **3**, C–O cleavage regenerates amide (labeled or unlabeled), which ultimately hydrolyzes.

With the exception of McClelland's observations with benzamide (0.2% ^{18}O loss/$t_{1/2}$, 5.9% H_2SO_4, 85 °C),[4a] the occurrence of ^{18}O exchange accompanying acid-catalyzed amide hydrolysis has not been demonstrated. Such exchange is well documented

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