Concurrent ^{18}O Exchange Accompanying the Acid-Catalyzed Hydrolysis of Anilides. Implications for the Lifetimes of Reversibly Formed Intermediates

A. J. Bennet,[†] H. Slebocka-Tilk,[†] R. S. Brown,*[,†] J. Peter Guthrie,*[,‡] and A. Jodhan[§]

Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2, Department of Chemistry, University of Western Ontario, London, Ontario, Canada N6A 5B7, and Mass Spectrometry Laboratory, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2. *Received February 22, 1990*

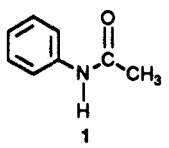
Abstract: Hydrolysis of acetanilide and *N*,2,4-trimethylacetanilide in acid is accompanied by oxygen exchange. The log k_{hyd} and log k_{ex} vs -log [H^+] profiles are parallel and show no significant divergence. The deuterium solvent kinetic isotope effects on exchange and hydrolysis are unity. A possible alternative mechanism for exchange in acetanilides involving enolization and elimination to give a transient keteniminium ion has been ruled out by the absence of concurrent deuterium incorporation into the acetyl group and shown to be thermodynamically prohibited. The deuterium solvent kinetic isotope effects (KIE) allow two mechanisms for hydration: the most probable is an attack of water with concerted loss of a proton to a second water; less likely is an attack of water with concerted transfer of a proton by way of a second water to the nitrogen. The solvent KIE allows only one mechanism for amine expulsion, concerted loss of amine from T_{N^+} and loss of a proton to solvent water. A decision on the mechanism of hydration can be made by the use of estimated energies of the intermediates and a two-dimensional extension of Marcus theory. These calculations rule out the mechanism leading directly to T_{N^+} and favor one leading to T^o.

I. Introduction

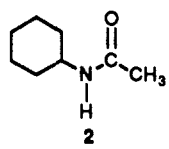
The generally accepted mechanism for H_3O^+-catalyzed amide hydrolysis (depicted in Scheme I) involves the initial attack of H_2O on the O-protonated amide to produce T^o.[1,2] Under conditions where the [H_3O^+] is large, subsequent proton transfer to N is thermodynamically favorable and should be fast,[2] as should the breakdown of T_{N^+} to products. In general, the rate-limiting step at high [acid] and in the absence of buffers is considered to be attack of H_2O on A-H^+. Plots of log k_{hyd}^{obsd} vs -log [H_3O^+] generally show a slope of -1 and signs of a plateau at high [H_3O^+] are consistent with the onset of substantial equilibrium O protonation of the amide (A-H^+, $pK_a \approx -2$ to 0)[3] and reduction in the activity of H_2O.

Of fundamental importance to the understanding of the process were early carbonyl ^{18}O exchange studies of Bender,[4] Bunton,[5] and Yates[6] which were designed to cast light on the importance of reversibly formed tetrahedral intermediates. In those studies, no loss of label was detected in amide recovered from highly acidic media after partial hydrolysis, and it was concluded that in Scheme I, k_{-1} is negligible relative to the rate constants for product formation. However, in 1975 McClelland reported that 90% ^{18}O-enriched benzamide suffered a 0.2% loss of label per hydrolytic half-time ($t_{1/2}$) when recovered from a 5.9% H_2SO_4 solution at 85 °C.[7] This finding is important in that it suggested that H_3O^+-catalyzed amide hydrolysis adheres to a mechanistic category like that of other carboxylic acid derivatives that also proceed via the formation of tetrahedral addition intermediates.

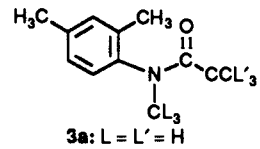
Recently we reported that acetanilide (**1**) and *N*-cyclohexylacetamide (**2**) suffer ^{18}O exchange concurrent with acid hydrolysis.[8] Unexpectedly, the amount of exchange apparently increased with decreasing [H_3O^+], an observation that cannot be easily reconciled within the mechanistic context of Scheme I. In view of the potential ramifications of the latter anomaly, we have now undertaken an investigation of the hydrolysis and exchange rate constants to expand the study with **1** and another anilide (**3**) which was hoped to show larger amounts of exchange than **1**. The



1



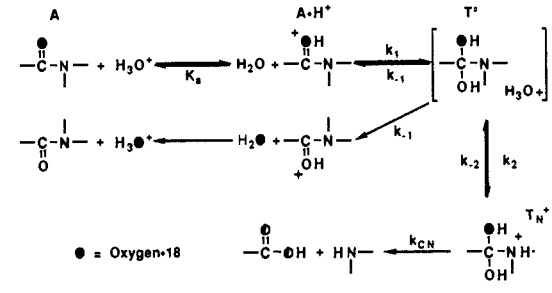
2



3a: L = L' = H
b: L = D; L' = H
c: L = H; L' = D

[†] University of Alberta.
[‡] University of Western Ontario.
[§] Mass Spectrometry Laboratory.

Scheme I. Generalized Pathway for Acid-Catalyzed Amide Hydrolysis



results of this study indicate that (1) the order of exchange in [H_3O^+] is **3a** > **1**, (2) the log k_{hyd} and log k_{ex} vs -log [H_3O^+] profiles for both **1** and **3a** are parallel and show no signs of significant divergence in contrast to our previous report[8] on **1**, and (3) the k_{ex}/k_{hyd} ratio in H_2O and D_2O is the same.

II. Experimental Section

Materials. Acetanilide (**1**) was commercial (Aldrich). *N*,2,4-Trimethylacetanilide (**3a**) was prepared from 2,4-dimethylaniline which was

(1) For leading references to generalized mechanisms, see: (a) Jencks, W. P. In *Catalysis in Chemistry and Enzymology*; McGraw-Hill Inc.: New York, 1969; pp 523–527. (b) Lowry, T. H.; Richardson, K. S. In *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper and Row Inc.: New York, 1987; pp 714–717. (c) Deslongchamps, P. In *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: Oxford, 1983; pp 101–162. (d) O'Connor, C. J. *Quart. Rev. Chem. Soc.* **1971**, *24*, 553. (e) Bender, M. L. *Chem. Rev.* **1963**, *1*, 53.

(2) Satterthwait and Jencks (Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7031) have presented studies that indicate that attack of H_2O on a cationic imidate ester directly generates H_3O^+ and a OR analogue of T_O. In the thermodynamically favorable direction, proton transfer from the nascent H_3O^+ to T_O should occur prior to diffusional separation of the two.

(3) (a) Arnett, E. M. *Prog. Phys. Org. Chem.* **1963**, *1*, 223. (b) Guthrie, J. P. *J. Am. Chem. Soc.* **1974**, *96*, 3608. (c) Arnett, E. M.; Quirk, R. P.; Larsen, J. W. *Ibid.* **1970**, *92*, 3977. (d) Fersht, A. *Ibid.* **1971**, *93*, 3504.

(4) (a) Bender, M. L.; Thomas, R. J. *J. Am. Chem. Soc.* **1961**, *83*, 4183. (b) Bender, M. L.; Ginger, R. D.; Kemp, K. C. *Ibid.* **1954**, *76*, 3350. (c) Bender, M. L.; Ginger, R. D. *Ibid.* **1955**, *77*, 348.

(5) (a) Bunton, C. A.; Farber, S. J.; Milbank, A. J. G.; O'Connor, C. J.; Turney, T. A. *J. Chem. Soc., Perkin Trans. 12* **1972**, 1869. (b) Bunton, C. A. *J. Chem. Soc.* **1963**, 6045. (c) Bunton, C. A.; O'Connor, C. J.; Turney, T. A. *Chem. Ind. (London)* **1967**, 1835.

(6) Smith, C. R.; Yates, K. *J. Am. Chem. Soc.* **1972**, *94*, 8811.

(7) McClelland, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 5281.

(8) Slebocka-Tilk, H.; Brown, R. S.; Olekszyk, J. *J. Am. Chem. Soc.* **1987**, *109*, 4620.

0002-7863/90/1512-8497$02.50/0

N-acetylated (acetic anhydride/pyridine), and the product (2,4-dimethylacetanilide) was recrystallized from H_2O, mp 129.5–130.5 °C (lit.[9] mp 129 °C). This product was N-methylated by a modification of a procedure of Deslongchamps et al.[10]

To a mixture of 0.52 g of 80% NaH in mineral oil (which was previously washed with 3 × 30 mL of dry benzene) in 50 mL of dry THF and cooled to 0 °C in an ice bath was added dropwise a solution of 2,4-dimethylacetanilide (2.58 g, 15.8 mmol) in 50 mL of dry THF. After the addition, the mixture was stirred at room temperature for 2 h, and then recooled to 0 °C. To this was added dropwise 2.2 mL (35.3 mmol) of CH_3I in 50 mL of dry THF, and the resulting solution was stirred at room temperature overnight. The volatiles were then removed under reduced pressure, and the residue was taken up in CH_2Cl_2. Following filtration and removal of the volatiles in vacuum, the residue was recrystallized from heptane to give *N*,2,4-trimethylacetanilide (**3a**) in 58% yield (1.62 g), mp 58.0–59.0 °C. Anal. C, H, N. Mass spectrum, *m/z* (intensity): 177 (94.9), 162 (16.6), 135 (86.6).

2,4-Dimethylacet-d_3-anilide was prepared from 1.2 mL (16.9 mmol) of acetyl-d_3 chloride and 4.5 mL (36.4 mmol) of freshly distilled 2,4-dimethylaniline in 20 mL of CH_2Cl_2. The mixture was stirred at room temperature overnight and then poured into dilute HCl. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried and then evaporated, and the residue was recrystallized from H_2O to give 1.36 g (49%) of the d_3-acetanilide. HRMS *m/z*: 166.1186 (M^+ calcd for $C_{10}H_{10}D_3NO$ 166.1185).

The above d_3-anilide (1.36 g, 8.2 mmol) was N-methylated as described for **3a**: yield 0.957 g (65%); mp 58.0–59.0 °C. HRMS *m/z*: 180.1339 (M^+ calcd for $C_{11}H_{12}D_3NO$ 180.1342). ^2H NMR (CCl_4): δ 1.842 (s). IR ($CHCl_3$ cast, ν cm^{-1}): 1622.9.

2,4-Dimethyl-*N*-methyl-d_3-acetanilide (**3b**) was prepared by *N*-CD_3 methylation of 2,4-dimethylacetanilide (2.58 g, 15.8 g) in 50 mL treated with 0.52 g of 80% NaH in mineral oil for 2 h. The mixture was added dropwise to a solution containing NaI (3.098 g, 20.7 mmol) and methyl-d_3 tosylate[11] (2.5 mL) in 50 mL of dry THF and the resulting solution was treated and worked up as for **3a**: yield 1.04 g (37%); mp 56.5–58.0 °C. HRMS *m/z*: 180.1346 (calcd for $C_{11}H_{12}D_3NO$ M^+ 180.1342). ^2H NMR (CCl_4): δ 3.189 (s). IR ($CHCl_3$ cast ν cm^{-1}): 1649.4.

Preparations of ^{18}O-labeled **1**, **3a**, and **3c** were effected according to the methodology described earlier.[8]

Product Analysis. *N*,2,4-Trimethylacetanilide (**3a**) (0.115 g, 0.649 mmol) was heated in 10 mL of 1.0 M HCl for 100 h at 100 °C. After cooling, the solution was extracted with 2 × 10 mL CH_2Cl_2 and then made basic with solid K_2CO_3. The aqueous phase was then extracted with 2 × 10 mL of CH_2Cl_2, and the organic extracts were dried. After removal of the volatiles, 72 mg (82% crude) of a slightly brown oil were recovered. This was distilled (Kügelrohr, 100–110 °C, 18 Torr) to give a colorless liquid which proved to be the expected trimethylanilide. HRMS: 135.1046 (M^+ calcd for $C_9H_{13}N$ (135.1048). ^1H NMR ($CDCl_3$, 80 MHz): δ 6.8–7.0 (m, 2 H), 6.46 (d, 1 H, ortho H, J = 6.5 Hz), 3.24 (br s, 1 H), 2.84 (s, 3 H), 2.22 (s, 3 H), 2.08 (s, 3 H). On heating this product in 1 M DCl/D_2O, the peak at δ 6.46 disappears. Anal. C, H, N.

^1H NMR Experiment on Hydrolysis of 3a in D_2O. Into 300 μL of 1.0 M DCl/D_2O was placed 7.8 mg of **3a**. At time 0, the 400-MHz ^1H NMR spectrum (ref. N^+Me_4) gave resonances at δ 6.6–6.9 (m, 3 H) and four major singlets at δ 1.41, 1.76, 1.91, and 2.76 along with four minor singlets at δ 1.70, 1.89, 1.90, and 2.92. (These arise from the syn/anti conformers of the amide and indicate a major/minor conformer ratio of 4:1). After the mixture was heated for 22 h at 100 °C, the ^1H NMR spectrum showed a mixture of starting material and products. After 106 h, only peaks attributable to product were formed. The ortho H of *N*,2,4-trimethylaniline (δ 6.46) exchanges for D under these conditions as do the protons of acetic acid. However, those same protons in the starting amide do not exchange.

^2H NMR Experiments To Rule out Exchange via Enolization. A weighed quantity of 2,4-dimethyl-*N*-methyl-d_3-acetanilide (**3b**) was dissolved in DCl solution (10 mL) with a constant ionic strength (μ = 1.0, KCl). The samples were sealed in ampules which were subsequently heated at 100 °C for a specified time. After the ampule was cooled, its contents were extracted with 3 × 10 mL of CH_2Cl_2. The combined organics were washed with brine and H_2O and then dried. After removal of the volatiles, the residue was dissolved in CCl_4 (0.3 mL) and analyzed by ^2H NMR using comparison integration of the *N*-CD_3 region (δ 3.19) and C(O)CD_3 region (δ 1.84). The data are as follows

initial amide, mg	[DCl]	time, h	maximal % D in $C(O)CH_3$	$t_{1/2}$ hydrolysis, h (approximate)
46.7	1.00	6.5	3.17	6.5
44.5	0.25	15.0	2.14	30
32.9	0.25	30.0	4.20	30

The maximal % D in the $C(O)CH_3$ peak is calculated as the ratio of the intensities of the δ 1.84/δ 3.19 multiplied by a factor of 3 to account for single deuterium in the acetyl peak relative to the *N*-CD_3 integration. No incorporation of D was seen in the aromatic ring of the amide.

Kinetics. (a) Hydrolysis. The hydrolysis kinetics of **1** and **3a** were monitored by UV-vis spectrophotometry by using the methodology and equipment previously described.[8] In the present series of experiments, the slow hydrolysis of the trimethylacetanilide necessitated the use of degassed (refluxed or distilled and stored under argon) acidic solutions. If degassed solutions were not used, the solutions of amide, on prolonged heating, showed UV bands at 260–270 nm arising from decomposition of the amine product. The hydrolysis of **3a** was followed by observing the rate of decrease of a 2.5 × 10^{-4} M solution of amide at 230 nm. The amide (~2.4 mg) was placed into 100 mL of the degassed solution (μ = 1.0, KCl except for [HCl] > 1.0 M) and 4 mL of this solution was placed into each of 12 ampules. The ampules were sealed under argon and then placed into a "boiler" so constructed as to allow the ampules to be immersed in the vapors of refluxing water (100 °C). After an equilibration period of 10 min, an ampule was removed, and the others were removed at various time intervals up to roughly 4 $t_{1/2}$ hydrolysis. The contents of each ampule were analyzed by using an HP 8451A diode-array UV-vis spectrophotometer, and the absorbance at 230 nm was recorded. Observed rate constants (k_{obsd}) were evaluated by fitting the absorbance vs time data to a standard exponential model. After the kinetic data were obtained, the acid solutions were titrated with standardized NaOH solutions to ensure than the acid concentration did not change during a kinetic run.

Additional hydrolysis experiments for acetanilide at 72 °C were conducted in the present work to both check the original data[8] and extend the [HCl] range to 5 M. The experimental protocol was the same as already described.[8]

(b) ^{18}O exchange. The ^{18}O content of initially ~40% ^{18}O-labeled acetanilide recovered form acidic solutions was monitored by using mass spectrometric equipment and methodologies analogous to those described[8] with the exception that more amide (0.01 M) was used and the period of time of residence in the hydrolysis medium was extended to between 3–5 $t_{1/2}$ hydrolysis.

(i) **Acetanilide.** Fifteen 20-mL ampules were each charged with ~25–30 mg of 40% ^{18}O-enriched acetanilide. Four 10-mL ampules were charged with 1–2 mg each of the same material. Three of the 20-mL ampules were then each charged with 20 mL of the appropriate HCl solution (0.25, 0.50, 1.0, 2.0, and 5.0 M) and sealed. They were then placed in a boiler containing 54/46 ethyl acetate/cyclohexane mixture, the azeotrope of which boils at 72.8 °C.[12] The ampules were kept in the vapor for times corresponding to ~3–5 $t_{1/2}$ hydrolysis in that same medium. They were then removed and cooled in ice, and each was extracted and worked up as previously described.[8] The ^{18}O content at t = 0 was determined by heating the contents of the four 10-mL ampules (0.5 M HCl, μ = 1.0 (KCl)) in the ethyl acetate/cyclohexane azeotrope vapor for 10 min, and then cooling the ampules, and extracting the contents. Control experiments to determine the ^{18}O content at t = 0 were done with different batches of ^{18}O-labeled **1** at acid concentrations of 0.1, 0.02 (glycine buffer), 0.05, 0.25, and 1.0 M. In the latter four cases, the initial amount of labeled **1** was 10 mg with ~70% ^{18}O label. The recovered material was recrystallized from H_2O and its mass analysis indicated that it had exactly the same ^{18}O content of the same batch of labeled **1** that had not been subjected to the hydrolytic medium. In the case of the latter four acid concentrations, 80 mg of 71% labeled **1** was placed in 100 mL of the aqueous acid (250 mL for 0.05 M HCl) (previously equilibrated at 72 °C), and the solutions were thermostated at 72 °C in a water bath for periods of time corresponding to ~3 $t_{1/2}$ in that medium. The workup on these samples consisted of extraction with CH_2Cl_2 (3 × 40 mL), and the combined organic phases were washed with 40 mL each of saturated $NaHCO_3$, brine, and then H_2O. The organic phase was dried and filtered, and the volatiles were removed. The residue (~10 mg) was recrystallized from 0.5–1.0 mL of H_2O. The crystals were filtered, dried, and then submitted for mass analysis with an AEI

(9) *Dictionary of Organic Compounds*, 5th ed.; Chapman and Hall Inc.: New York, 1982; pp 2067.

(10) Deslongchamps, P.; Gerval, P.; Cheriyan, U. O.; Guida, A.; Taillefer, R. J. *Nouv. J. Chim.* **1978**, *2*, 631.

(11) Murray, A.; Williams, D. L. In *Organic Synthesis With Isotopes*; Interscience: New York, 1958, 1499.

(12) *CRC Handbook of Chemistry and Physics*, 48th ed.; CRC: Cleveland, Ohio, 1967–68.

Table I. k_{hyd} and k_{ex} Data for Acetanilide (1) in H_2O as a Function of $[H_3O^+]$, T = 72 °C, μ = 1.0 (KCl)

$[H_3O^+]$	k_{hyd}, 10^6 s^{-1} [a]	k_{ex}, 10^8 s^{-1} [b]
5.0	714 ± 16	870 ± 106
4.0	802 ± 8	
3.0	701 ± 19	
2.0	537 ± 4	394 ± 56
1.0	279 ± 2 (261 ± 1)[c]	202 ± 46, 258 ± 30 (194 ± 42)[d]
0.5	(146 ± 1)[c]	91 ± 23
0.3	101 ± 2[e]	
0.25		75 ± 7, 46 ± 12, (50 ± 7)[d]
0.175	59.6 ± 1.0	
0.10	(34.1 ± 0.1)[c]	27 ± 5
0.07	24.4 ± 0.2	
0.05	(16.1 ± 0.1)[c]	(10.4 ± 1.5)[d]
0.032[f]	(10.2 ± 0.2)[c]	
0.02[f]		(4.2 ± 0.6)[d]
0.01[f]	(3.53 ± 0.2)[c]	
0.003[f]	(1.11 ± 0.06)[c]	

[a] Mean and SD of mean from at least three separate kinetic runs unless otherwise stated. [b] k_{ex} and SD calculated as in text and Appendix I (supplementary material). [c] Previous work.[8] [d] Recrystallized recovered acetanilide. [e] Duplicate kinetic runs. [f] Glycine buffers.[8]

MS-12 low-resolution mass spectrometer. When **1** was recovered and recrystallized, duplicate exchange experiments were performed, while in cases where it was recovered but not recrystallized, triplicate runs were done. For mass analysis, 21 scans of the M^+ and $M^+ + 2$ peaks were done for each sample and the ^{18}O content was evaluated as

$$\% \ ^{18}O = I_{M^+ + 2}/(I_{M^+} + I_{M^+ + 2})$$

where I = peak intensity. Primary data are given in Table S1, supplementary material.

(ii) ***N*,2,4-Trimethylacetanilide (3a).** To evaluate the ^{18}O content of this anilide at t = 0, 1.0 mg of amide was placed in each of three ampules containing 10 mL of the acid solution (0.05, 0.1, 0.25, 0.5, 1.0, 1.98, 3.0, 4.0, and 5.0 M). For $[H^+]$ of < 1.0 M, the ionic strength was kept constant, (μ = 1.0, KCl). The ampules were sealed, placed in the boiler which contained refluxing H_2O (100 °C) for 10 min, removed and then plunged into ice/H_2O, and the contents were worked up as described for acetanilide. The residue was then subjected directly to low-resolution mass analysis. As before, 21 scans of the M^+ and $M^+ + 2$ peaks were made, and the ^{18}O content of this amide was recovered from the hydrolytic medium after incomplete hydrolysis. Amide (10 mg) was placed into ampules containing 10 mL of the appropriate acid (1.97, 3.0, 4.0, and 5.0 M). Triplicate determinations were performed at each $[H^+]$. The ampules were then sealed and immersed in the 100 °C boiler for periods of time between 2 and 3 $t_{1/2}$ hydrolysis. For $[H^+]$ of 1.0 M and less, 1–8 mg of amide was used, and the ampules were heated at 100 °C for 1 $t_{1/2}$ hydrolysis before workup. The reactions in 0.1 and 0.5 M HCl were repeated with use of μ = 1.0 ($N^+Me_4Cl^-$) to check for an anomalous specific salt effect. In these two cases, the amount of ^{18}O exchange was identical (within experimental error) with the ones where KCl was added to maintain the ionic strength. In separate experiments, 2–8 mg of **3a** were subjected to hydrolysis in 1.0 and 0.25 M D_3O^+ for ~1 and 2 $t_{1/2}$ hydrolysis in each medium. Isolation and mass analysis indicated ^{18}O depletion in D_2O media was (within experimental uncertainty) the same as in H_2O. HRMS of the $M^+/M^+ + 2$ region of these samples indicated no extraneous co-contaminants nor peaks attributable to D incorporation in recovered **3a**. Primary data concerning the M^+ and $M^+ + 2$ intensities are given in Table S2, supplementary material. The k_{hyd} values for **3a** in the above D_2O solutions were determined from 10 samples of amide in D_2O (~3 mg/mL) which were heated at 100 °C for various times and then directly analyzed by ^1H NMR.

(iii) ***N*,2,4-Trimethylacet-d_3-anilide (3c).** To check whether enolization was responsible for exchange, 45% ^{18}O-labeled amide with $C(O)CD_3$ was subjected to hydrolysis in D_3O^+ media of 1.0 M and 0.25 M at 100 °C for a period of time corresponding to 1 $t_{1/2}$ hydrolysis in those media. (The k_{hyd} data at 100 °C were obtained by UV-vis spectrophotometric analysis of the contents of 10–12 ampules charged with D_3O^+ solution and removed at various time intervals up to ~4 $t_{1/2}$). For the exchange, duplicate runs of 1 mg of labeled amide in 10 mL of D_3O^+ (μ = 1.0, KCl) were used for both the t = 0 and $t_{1/2}$ extractions which were performed as above. In these two cases, ^{18}O was lost from the recovered anilide in amounts comparable to those observed for ^{18}O loss from non-deuterated *N*,2,4-trimethylacetanilide in H_2O media. This indicates there is not a large solvent or CD_3 isotope effect on the exchange. Primary data are given in Table S3, supplementary material.

Table II. k_{hyd} and k_{ex} Data for *N*,2,4-Trimethylacetanilide (3a) as a function of $[H_3O^+]$, T = 100 °C, μ = 1.0 (KCl)

$[H_3O^+]$	k_{hyd}, 10^6 s^{-1} [a]	k_{ex}, 10^7 s^{-1} [b]
5.00		29.9 ± 0.5
4.19	21.3 ± 0.3	
4.00		35.1 ± 0.3
3.00		38.4 ± 2.2
2.95	28.3 ± 0.2	
1.98		41.8 ± 0.7
1.97	29.4 ± 0.05	
1.02	18.2 ± 0.6	
1.00		32.4 ± 1.2 (30.5 ± 1.8)[c]
0.50		21.9 ± 0.04, (22.3 ± 2.5)[d]
0.49	11.0 ± 0.2	
0.25		12.8 ± 0.2
0.20	4.80 ± 0.06	
0.11	2.71 ± 0.04	
0.10		5.85 ± 0.06, (5.42 ± 0.47)[d]
0.06	1.68 ± 0.02	
0.05		3.20 ± 0.02

[a] Single runs using 12 ampules see text; SD from nonlinear least-squares fitting of abs (230 nm) vs t up to 4 $t_{1/2}$. [b] k_{ex} from triplicate or duplicate samples recovered from hydrolytic solution after 2–3 $t_{1/2}$ hydrolysis at $[H_3O^+]$ > 1.0 and ~$t_{1/2}$ for $[H_3O^+]$ < 1 M. [c] Value obtained from separate experiment where amide was recovered at t = 0, 10, and 20 h and fitted to $k_{ex} = \ln (a/a - x)/t$ by linear least squares. [d] Ionic strength held at 1.0 with $(CH_3)_4N^+Cl^-$.

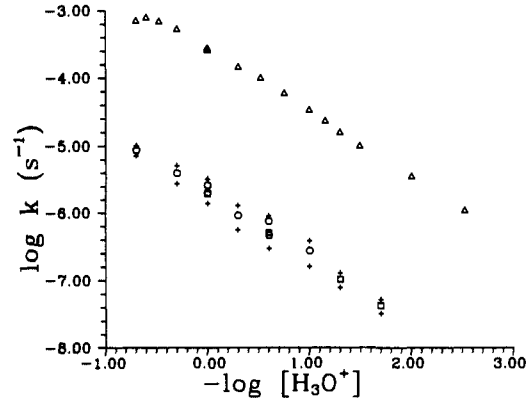


Figure 1. Plots of log k_{hyd} and log k_{ex} vs −log $[H_3O^+]$ for acetanilide (**1**) in H_2O, T = 72 °C, μ = 1.0 (KCl): ○, k_{ex} from amide reisolated from medium after partial hydrolysis and subjected directly to mass analysis; □, amide recovered and purified by recrystallization prior to mass analysis; +, error limits in k_{ex} (±2 × sd); △, k_{hyd}.

III. Results and Discussion

(i) Acetanilide (1) and *N*,2,4-Trimethylacetanilide (3a). Given in Table I are the k_{hyd} and k_{ex} values observed for **1** at 72 °C, μ = 1.0 (KCl) while Table II contains the same constants for **3a** at 100 °C, μ = 1.0 (KCl). At $[H_3O^+]$ > 1.0 M, the ionic strength was not kept constant. In all cases, duplicate or triplicate exchange experiments were done. Some of the k_{hyd} values for **1** were taken from previous work, with those for the extended [acid] being newly acquired. The k_{ex} values are newly acquired and were calculated according to $k_{ex}t = \ln (a/a - x)$, where a and $a - x$ are the ^{18}O contents at zero time and time t, respectively. The error limits in k_{ex} given in Tables I and II are calculated on the basis of the cumulative standard deviation in both ^{18}O contents as indicated in Appendix I (supplementary material). Given in Figures 1 and 2 are the plots of log k vs −log $[H_3O^+]$ for both processes for **1** and **3a**, respectively. The plots for the exchange and hydrolysis processes for both amides are parallel as a function of $[H_3O^+]$ and show no evidence of convergence at $[H_3O^+]$ < 1 M.

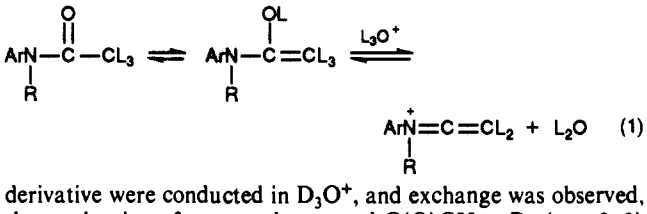
One must consider the reasons for the apparently increasing ^{18}O exchange in **1** in the previous work[8] and why the data now presented are more reliable. In the previous work, the amide solutions used for exchange were quite dilute (0.005–0.001 M) which led to the isolation of small amounts of recovered acetanilide

(<1 mg) at times corresponding to $t = 0$, and $t_{1/2}$ hydrolysis. This residue was then directly subjected to low-resolution (AE IMS12) mass analysis to analyze the relative intensities of the M^+ and $M^+ + 2$ peaks at m/z 135, 137. However, on analysis of small amounts of material, co-contamination by small amounts of adventitious phthalates (m/z 135, 149, 163)[13] can produce an artificial enhancement of what was assumed to be the M^+ of the recovered amide. This is particularly a problem in cases where small amounts of true exchange are in evidence.[14]

In the present series of exchange experiments with **1**, the above problems were addressed in two ways. Firstly, larger amounts of **1** were used which allowed the recovery of larger amounts of (~10 mg) amide. In four cases having $[H_3O^+]$ at 0.02 M (glycine buffer), 0.05, 0.25, and 1.0 M HCl 80 mg of 71% ^{18}O-labeled **1** in 100 mL of solution (250 mL for 0.05 M HCl) were used and the 10 mg of amide recovered after ~3 $t_{1/2}$ hydrolysis was recrystallized from H_2O to yield purified **1** that was then subjected to mass analysis. The second general protocol for maximizing the observable differences in intensity between the M^+ and $M^+ + 2$ peaks due to true exchange was to increase residence time of the amide in the hydrolytic medium (3–5 $t_{1/2}$ of hydrolysis).

Anilide **3a** was investigated for exchange accompanying the H_3O^+-catalyzed hydrolysis by using the same protocol of large [**3a**] and increased residence time in the hydrolytic medium. Our present experience with **3a** indicates it is much easier to obtain reliable results for its ^{18}O exchange than is the case for acetanilide. This is because its inherent exchange is greater than with **1** which leads to larger intensity changes in the $M^+ + 2$ peaks and because in our experiments there appears to be little if any co-contaminants having m/z = 177, 179. As a point of note supporting the latter, the mass analysis of recovered **3a** ranging from submilligram to ~10 mg amounts gives the same percentage of ^{18}O after identical isolation experiments. Also, HRMS analysis of recovered **3a** indicated no co-contaminants having peaks close to $M^+/M^+ + 2$ which, at low-resolution analysis, would add intensity to one or the other peak.[13] As can be judged from Tables I and II and Figures 1 and 2, the ratio of k_{ex}/k_{hyd} is greater for **3a** (~0.18–0.23) than for **1** (0.005–0.01).

(ii) Control Experiments for Exchange in 3. A possibility exists that the exchange in the acetanilides occurs by a parallel process that produces intermediates other than those along the hydrolytic pathway. One such process, given in eq 1, involves an enolization of the $C(O)CL_3$ group. In this case, if the reaction of a $C(O)CH_3$



(1)

derivative were conducted in D_3O^+, and exchange was observed, the production of some α-deuterated $C(O)CH_{3-x}D_x$ (x = 0–3) should proceed in amounts at least as large as the ^{18}O exchange. To address this question, 33–47 mg of 2,4-dimethyl-*N*-methyl-d_3-acetanilide (**3b**) was subjected to hydrolysis in 1.0 and 0.25 M DCl solutions for times up to $t_{1/2}$ hydrolysis in those media. The recovered amide was analyzed by ^2H NMR in the *N*-CD_3 (δ 3.19) and $C(O)CD_3$ (δ 1.84) regions with the *N*-CD_3 resonance as a calibrant. Comparison of the integrated intensities of those regions (corrected by a factor of three to normalize the *N*-CD_3 to one deuterium) showed that at most, 2–4% D was incorporated into the $C(O)CH_3$ group.[15]

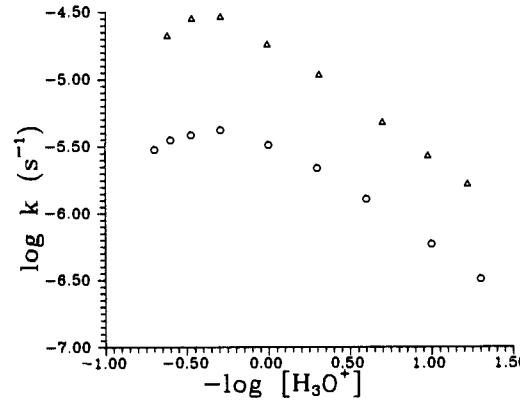


Figure 2. Plots of log k_{hyd} and log k_{ex} vs $-\log [H_3O^+]$ for *N*,2,4-trimethylacetanilide (**5a**), T = 100 °C, μ = 1.0 (KCl): ○, k_{ex}; △, k_{hyd}. Error limits are encompassed within symbol diameter.

Table III. ^{18}O Contents and k_{ex} Values for **3a** and **3c** recovered from L_3O^+ Hydrolysis Media, T = 100 °C, μ = 1.0 (KCl)

amide	$[L_3O^+]$ (L)	recovery time, h	% ^{18}O found[a]	k_{ex}, 10^6 s^{-1}[b]
3a	1.0 (H)	0.0	53.21 ± 0.05	
			53.26 ± 0.03	
			53.24 ± 0.03	
	1.0 (H)	8.1[c]	48.51 ± 0.12	3.24 ± 0.12
			48.36 ± 0.17	
	0.25 (H)	32.6[c]	45.78 ± 0.08	1.28 ± 0.02
			45.81 ± 0.06	
3a	0.25 (D)	0.0	56.54 ± 0.11	
			56.23 ± 0.07	
			56.45 ± 0.09	
	1.00 (D)	6.5	52.16 ± 0.07	3.40 ± 0.06
			52.03 ± 0.05	
	1.00 (D)	13.0	48.17 ± 0.09	3.35 ± 0.11[e]
			48.26 ± 0.22	
	0.25 (D)	24.0	50.53 ± 0.11	1.32 ± 0.06
			50.13 ± 0.17	
	0.25 (D)	50.6	44.37 ± 0.10	1.34 ± 0.02[e]
			44.09 ± 0.11	
3c	1.0 (D)	0.0	45.40 ± 0.08	-
			45.44 ± 0.10	
	1.0 (D)	6.5[c]	41.81 ± 0.07	3.55 ± 0.12
			41.79 ± 0.07	
	0.25 (D)	0.0	45.23 ± 0.11	-
			45.23 ± 0.10	
	0.25 (D)	30.0[c]	38.44 ± 0.06[d]	1.45 ± 0.16
			38.77 ± 0.10	
			38.86 ± 0.07	

[a] Errors in ^{18}O content evaluated as standard deviations in 16–21 scans of relative intensities of M^+ and $M^+ + 2$ peaks. [b] Errors in k_{ex} evaluated from square root of sum of squares of standard deviations in ^{18}O content at $t = 0$ and time t. [c] These times correspond roughly to $t_{1/2}$ hydrolysis. [d] Error limit used in calculating k_{ex} was based on the mean of ^{18}O content ± maximum excursion from the mean. [e] k_{hyd} in D_2O are 18.6 × 10^{-6} s^{-1} and 6.02 × 10^{-6} s^{-1} at 1.0 and 0.25 M D_3O^+, respectively.

In a second set of experiments, ^{18}O-labeled **3a** and its $C(O)CD_3$ derivative (**3c**) were each subjected to hydrolysis in 1.0 and 0.25 M DCl solutions and the material recovered after ~$t_{1/2}$ hydrolysis in those media was subjected to MS analysis. The ^{18}O content and k_{ex} values are given in Table III along with those for **3a** hydrolyzed in H_3O^+ media. Two points are of note. First, when normalized to 100% ^{18}O content at $t = 0$, the data indicate that the amount of ^{18}O exchange is between 8 and 14% at the times of isolation. Since the amount of $C(O)CH_{3-x}D_x$ (x = 0–3) is at most 2–4% for **4b** under similar conditions, the ^{18}O exchange cannot principally result from some enolization process. Second, from the values of k_{ex} for **3a**,**c** in D_3O^+ and H_3O^+, there is no apparent large solvent or $C(O)CD_3$ isotope effect on the exchange process.

As another test of the idea that ^{18}O exchange might represent a side reaction by way of a keteniminium ion, we have estimated

(13) Hites, R. A. In *CRC Handbook of Mass Spectra of Environmental Contaminants*; CRC Press: Boca Raton, FL, 1985.

(14) In the previous work[8] care was taken to purify by distillation all solvents used for extraction. It should be pointed out that the experimental protocol in terms of number of extractions, volume of solvent, concentrations of amide employed etc. lead to reproducible results in duplicate experiments and in experiments repeated on different occasions.

(15) In fact the δ 1.84 region in the ^2H NMR spectrum of recovered **3b** showed no discrete peaks. However, amplified integration of the baseline between δ 2.0 and 1.5 indicated a maximum of 2–4% (in different $[D_3O^+]$) could have occurred.

Table IV. Deuterium Solvent Kinetic Isotope Effects for *N*,2,4-Trimethylacetanilide[a]

process	[L$^+$] M	k_{H_2O}, 10^6 s^{-1}	k_{D_2O}, 10^6 s^{-1}	k_{H_2O}/k_{D_2O}	(k_{ex}/k_{hyd}) H_2O/D_2O
exchange	1.0	3.24 ± 0.12	3.37 ± 0.09[b]	0.96 ± 0.04	
	0.25	1.28 ± 0.02	1.33 ± 0.04[b]	0.96 ± 0.03	
hydrolysis	1.0	17.8 ± 0.6[c]	18.6 ± 0.1	0.96 ± 0.03	1.00 ± 0.05
	0.25	5.9 ± 0.15[d]	6.02 ± 0.1	1.00 ± 0.03	0.98 ± 0.04

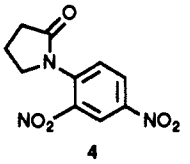
[a] Data from Tables II and III. [c] Average of two values in Table III. [c] Calculated from a rate constant at 1.02 M HCl, assuming linearity in [HCl]. [d] Calculated from a rate constant at 0.20 M HCl, assuming linearity in [HCl].

the free energy change for dehydration of acetanilide to *N*-phenyl ketenimine. The calculations, described in Appendix 2, (supplementary material) lead to the conclusion that for the reaction

$$CH_3—C(OH)=NH^+—Ar \rightleftharpoons CH_2=C=NH^+—Ar + H_2O$$

ΔG = 51 kcal/mol (at 25 °C). This is much higher than the observed free energies of activation, 25.8 kcal/mol at 72 °C for acetanilide and 29.9 kcal/mol at 100 °C for *N*,2,4-trimethylacetanilide. Even given the imprecision of the estimations, the thermodynamic barrier is too high for this to be a viable mechanism.

(iii) Mechanistic Implications. The concurrence of ^{18}O exchange accompanying base hydrolysis of amides[1,4a,4b,16] as well as during both acid and base hydrolysis of carboxylic esters[1,17] is well documented. This has traditionally been interpreted as implying the intermediacy of reversibly formed tetrahedral intermediates along the reaction pathway. There is a large body of data for the reactions of other species (imidates,[2,18] amide acetals,[19] and aminolysis of esters[20]) which proceed by way of analogous tetrahedral intermediates. Similar evidence for ^{18}O exchange in amides under acidic conditions was not found in early studies[4c,5,6] undoubtedly because of the low levels of ^{18}O incorporation in the starting amide and inherently small amount of exchange. Nevertheless, McClelland's observation with 90% ^{18}O-labeled benzamide (0.2% exchange/$t_{1/2}$, 5.9% H_2SO_4, 85 °C)[7] as well as Abdallah and Moodie's findings with the hydrolysis of **4**[21] and our recent ones with **1** indicate that in acid exchange may be more prevalent than was originally believed.[1]



4

(a) Deductions from the Observed Exchange and Its pH Dependence. For both **1** and **3a**, exchange and hydrolysis are both acid catalyzed with an essentially constant ratio k_{ex}/k_{hyd} over the accessible range of acid concentrations. There may be small changes at the highest acid concentrations used, but these are not dramatic, and at these acidities activity coefficient effects become quite important yet are poorly understood. Even in the best case discovered to date, i.e., **3a**, exchange is slow relative to hydrolysis, with $k_{ex}/k_{hyd} \leq 0.23$.

Certain deductions can be made from these results. First, there must be an intermediate which can partition between exchange and hydrolysis; this is assumed to be the tetrahedral intermediate in the generally accepted addition–elimination mechanism for hydrolysis. Second, the predominantly rate-determining step for hydrolysis must be addition of water to form the tetrahedral intermediate, because exchange is always slow relative to hydrolysis. Third, whatever is the rate-determining step for exchange, the rate-determining step for cleavage of the intermediate to products must also be acid catalyzed, because k_{ex}/k_{hyd} is independent of [H$^+$]. This means that cleavage of the zwitterion is not kinetically significant at any of the acidities used in our studies. McClelland has suggested that for tetrahedral intermediates derived from benzimiditonium ions the zwitterion is important until significant concentrations of sulfuric acid have been added to the medium.[22] However we are led to the conclusion that the zwitterion, as a discrete species in an energy well, is not kinetically significant for acetanilide at any of the acidities involved.

(b) Deductions from the Deuterium Solvent Kinetic Isotope Effects. The experimental observations, for **3a**, are that the solvent kinetic isotope effect is experimentally indistinguishable from 1.0 for both the hydrolysis and exchange reactions and is independent of [H$^+$] between 0.25 and 1.00 M HCl. It can be seen that where comparison can be made for a given **3** in H_2O or D_2O (Table II, III), k_{ex} or k_{hyd} remains almost the same within experimental error. The isotope effects are summarized in Table IV.

For the mechanism of Scheme I, with k_1 redefined to absorb the protonation step, and k_2 redefined to absorb the $T^o \rightleftharpoons T_{N^+}$ equilibrium and breakdown of T_{N^+} to products, the observable rate constants are given by

$$k_{hyd} = k_1k_2/(k_{-1} + k_2)$$

$$k_{ex} = k_1k_{-1}/2(k_{-1} + k_2)$$

$$k_{ex}/k_{hyd} = k_{-1}/2k_2$$

When $k_2 \gg k_{-1}$ we may write

$$k_1 \approx k_{hyd}$$

In the present case (**3a**), $k_{ex}/k_{hyd} \approx 0.2$, $k_2 = 2.5k_{-1}$, and so this approximation is not strictly applicable. The exact expression is

$$k_1 = k_{hyd}(1 + 2(k_{ex}/k_{hyd}))$$

but in fact the same KIE are calculated for either the exact or approximate expressions. For ease of interpretation it is desirable to express the KIE in terms of products or quotients of microscopic rate constants, with no sums or differences.

For the mechanistic analysis which follows we will assume that the qualitative conclusions from the data for **3a** will apply to **1** as well.

The solvent deuterium kinetic isotope effect data permit the elimination of most of the imaginable mechanisms. We analyze the situation by calculating the isotope effect expected for a mechanism using the procedures outlined by Schowen[23,24] and Schowen[25] and making the following assumptions.

(16) (a) Bunton, C. A.; Nyak, B.; O'Connor, C. J. *J. Org. Chem.* **1968**, *33*, 572. (b) Slebocka-Tilk, H.; Brown, R. S. *Ibid.* **1988**, *53*, 1153. (c) DeWolfe, R. H.; Newcomb, R. C. *Ibid.* **1971**, *36*, 3870.

(17) (a) Shain, S. A.; Kirsch, J. F. *J. Am. Chem. Soc.* **1968**, *90*, 5848. (b) Lane, C. A.; Cheung, M. F.; Dorsey, G. F. *Ibid.* **1968**, *90*, 6492. (c) For a review of early oxygen isotopic exchange reactions of organic compounds, see: Samuel, D.; Silver, B. L. *Adv. Phys. Org. Chem.* **1965**, *3*, 123–186.

(18) (a) Smith, V. F.; Schmir, G. L. *J. Am. Chem. Soc.* **1975**, *97*, 3171. (b) Caswell, M.; Schmir, G. L. *Ibid.* **1979**, *101*, 7323. (c) Lee, Y. N.; Schmir, G. L. *Ibid.* **1979**, *101*, 6277. (d) Chaturvedi, R. K.; Schmir, G. L. *Ibid.* **1968**, *90*, 4413.

(19) (a) McClelland, R. A. *J. Am. Chem. Soc.* **1978**, *100*, 1844. (b) McClelland, R. A.; Patel, G. *Ibid.* **1981**, *103*, 6908. (c) McClelland, R. A. *Ibid.* **1984**, *106*, 7579. (d) Brown, R. S.; Ulan, J. G. *Ibid.* **1983**, *105*, 2382.

(20) (a) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7018. (b) Blackburn, G. M.; Jencks, W. P. *Ibid.* **1968**, *90*, 2638. (c) Fife, T. H.; De Mark, B. R. *Ibid.* **1976**, *98*, 6978. (d) Jencks, W. P. In *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; p 526. (e) Camilleri, P.; Ellul, R.; Kirby, A. J.; Mujahid, T. G. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1617.

(21) Abdallah, J. M.; Moodie, R. B. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1243.

(22) McClelland, R. A.; Potter, J. P. *Can. J. Chem.* **1980**, *58*, 2318.

(23) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275.

(24) Schowen, R. L. In *Isotope Effects on Enzyme Catalyzed Reactions*; Cleland, W. W., O'Leary, M. H., Northrup, D. B., Eds.; University Park Press: Baltimore, 1977.

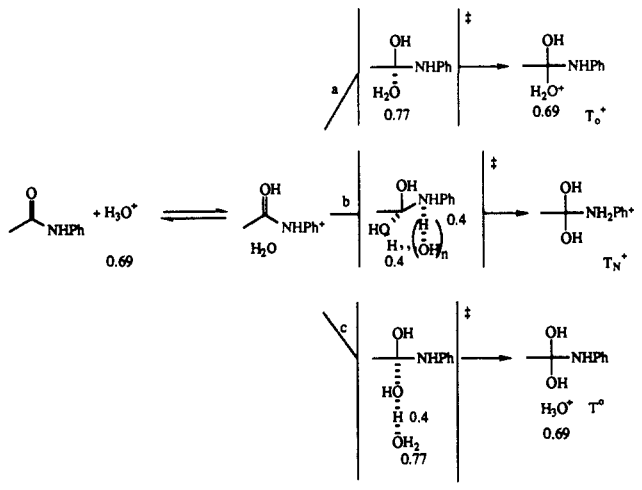


Figure 3. Fractionation factors for the alternative paths for the addition of water to protonated acetanilide.

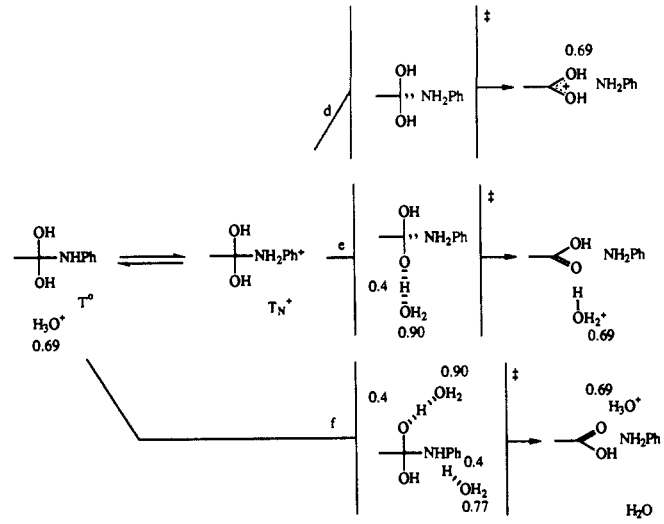


Figure 4. Fractionation factors for the alternative paths for the expulsion of aniline from T_{N^+}.

(1) Transition states are close to tetrahedral species, with the position of the transition state along the reaction coordinate being 0.7 for formation and 0.3 for breakdown. (Approximately this transition-state position is suggested by the energetics calculations given below.)

(2) The primary kinetic isotope effect for a proton in flight between oxygens or oxygen and nitrogen is based on a transition-state fractionation factor of 0.4 (with values of 0.3–0.5 used as limits).[24]

(3) The O-protonated amide has the same fractionation factor as a carboxylic acid, because the charge resides mainly no nitrogen. Thus it is expected to behave, in terms of its fractionation factors, as a moderately acidic OH rather than as an oxonium ion.

(4) Protons transferred between waters or water and substrate will show relatively normal primary KIE's. There has been a long-standing debate over whether the proton transferred by a general catalyst would show a primary isotope effect of 1 (solvation rule[26,27]) or a more normal effect with a potential maximum value based in the usual way on the vibrational frequency of the bond from hydrogen to catalyst.[25] Schowen has discussed these problems.[23,24] Kresge[28] and Jencks[29] have demonstrated very narrow isotope effect maxima for general-base-catalyzed reactions, but these were for reactions which involved trapping by a catalyst, with the actual proton transfer process being rate limiting only for a narrow range around $\Delta pK_a = 0$. Outside this range the primary isotope effect would be small. For the reactions considered here, there would be no such problem of change in the nature of the rate-determining process and one does not expect unusually small primary isotope effects. Schowen has shown that a plausible explanation of the observed solvent isotope for hydration of a carbonyl compound is possible assuming normal primary isotope effects.[24]

(5) A cyclic concerted mechanism would have a large primary kinetic isotope effect because there are two or more protons in flight: this assumption was made by Bell et al. in their analysis of ketone hydration.[30]

(6) The fractionation factor for the OH group in T°, T_{O^+}, or T_{N^+} is similar to that of a gem diol (1.0).[31]

We will now examine three possible transition states (TS) for the acid-catalyzed addition of water to acetanilide: (a) Fully stepwise addition leading to T_{O^+}. (b) Fully concerted addition of water with solvent-mediated transfer of a proton to nitrogen, leading to T_{N^+}. (c) Concerted proton transfer from the water adding to the carbonyl to another water molecule, leading to T°. The three transition states and the associated fractionation factors are shown in Figure 3. (In Figures 3 and 4, only nonunit fractionation factors are shown beside the appropriate H; unit fraction factors are omitted for clarity.)

Path a leads to a predicted KIE of 0.55. We conclude that path a can be excluded.

Path b with three water molecules leads to a predicted KIE of 5.16. No plausible fractionation factors predict a KIE less than 2.64. If the number of protons in flight were 2 rather than 3, and the associated fractionation factor was 0.5, the predicted KIE is 1.32. This seems too high, but we must ask whether a TS with only two water molecules is feasible. The closest models are proton-exchange processes, for which it has been shown[32,33] that the preferred TS for benzoic acid in methanol has two solvent molecules in an eight-membered H-bonded ring. The bond lengths are not the same for the TS for H_2O addition, but there are some strong similarities. Thus, we conclude that a TS with two bridging waters is more probable and that the observed KIE probably rules out path b, although not rigorously so at the present state of knowledge.

Path c leads to predicted KIE's of 1.39 and 1.11 if the fractionation factors for the proton in flight are 0.4 or 0.5, respectively, the latter being close to the experimental value. Path c is thus the preferred mechanism and this suggests that the addition leads directly to T° and hydronium ion.

It is important to remember that the observed solvent isotope effects on k_{ex}/k_{hyd} refer specifically to the transition states leading to the amide or product irrespective of the nature or number of the tetrahedral intermediate(s). Since the observed solvent KIE on k_{ex}/k_{hyd} is unity the isotope effects on k_{-1} and k_2 must be identical as must the products of fractionation factors for these two transition states. Deslongchamps has suggested that for exchange to occur during hydrolysis of carboxylic acid derivatives a conformational isomerization is required.[34] If conformational isomerization were rate limiting for our observed exchange then it must involve a protonated adduct, because the exchange process is acid catalyzed. The above solvent KIE on k_{ex} does not point to which of a heavy-atom bond cleavage or a conformational change is rate limiting for k_{-1}. However, it is difficult to envision how the putatively required[34] conformational changes of any tetrahedral intermediate from **3** would be more rapid than those

(25) Schowen, K. B. J. In *Transition States of Biochemical Processes*; Gandour, R. D.; Schowen, R. L., Eds.; Plenum Press: New York, 1978.
(26) Swain, C. G.; Thornton, E. R. *J. Am. Chem. Soc.* **1962**, *84*, 817.
(27) Thornton, E. R. *J. Am. Chem. Soc.* **1967**, *89*, 2915.
(28) Bergman, N.-A.; Chiang, Y.; Kresge, A. J. *J. Am. Chem. Soc.* **1978**, *100*, 5954.
(29) Cox, M. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1981**, *103*, 572.
(30) Bell, R. P.; Millington, J. P.; Pink, J. M. *Proc. R. Soc. London A* **1968**, *303*, 1.
(31) (a) Keeffe, J. R.; Kresge, A. J. *Can. J. Chem.* **1989**, *67*, 792. (b) Bone, R.; Wolfenden, R. *J. Am. Chem. Soc.* **1985**, *107*, 4772.
(32) Grunwald, E. *Prog. Phys. Org. Chem.* **1966**, *3*, 317.
(33) Grunwald, E.; Jumper, C. F.; Meiboom, S. *J. Am. Chem. Soc.* **1963**, *85*, 522.
(34) Deslongchamps, P. In *Stereoelectronic Effects in Organic Chemistry*; Pergamon: London, 1983.

in the analogous but far less sterically encumbered intermediates from **1**. Nevertheless, the k_{ex}/k_{hyd} ratio for **3** is a minimum of 18 times larger than for **1**, which suggests that the exchange process is not conformationally restricted. The energetics calculations (vide infra) also bear this out.

Since the observed solvent KIE on k_{ex} or k_{hyd} is close to unity, then the product of fractionation factors for the reverse and forward transition states must equal the product of fractionation factors for the initial state, i.e., $0.69^3 = 0.33$. This places some important constraints on the possible modes of decomposition leading to products.

We consider three possibilities as in Figure 4: (d) a stepwise cleavage of T_{N^+} leading to carboxylic acidinium ion and amine; (e) a concerted process where loss of a proton to water occurs at the same time as C–N cleavage of T_{N^+}; (f) a fully concerted process in which transfer of a proton from hydronium ion to nitrogen is concerted with C–N cleavage and with transfer of a proton from the developing acidinium ion to water. The corresponding transition states and fractionation factors are shown in Figure 4.

For these processes (d, e, and f) the products of fractionation factors for the k_2 transition state are 1.0, 0.32, and 0.08, respectively. Mechanism f is almost certainly ruled out because it requires a very large isotope effect on k_{ex}/k_{hyd} with more exchange occurring in D_2O. Mechanisms d and e are really variations of a common pathway that differ in the degree of solvent participation as an acceptor base. The product of fractionation factors in d is too large and can only be reduced toward the required value by loosening the association of a proton with the tetrahedral intermediate and increasing its association with a water, thus logically merging into the favored mechanism, e.

We conclude that of the various mechanisms possible for formation and breakdown of the intermediates (T^0, T_{N^+}), the KIE data are most consistent with paths b (Figure 3) and e (Figure 4).

(c) Deductions from the Energies of the Intermediates. We can specify somewhat more closely the nature of the reaction coordinate diagram for this hydrolysis, and choose between the alternative mechanisms for water addition, by estimating the free energy levels of the tetrahedral intermediates. These calculations, described in Appendix 3 (supplementary material), lead to the free energies in Table V.

We have described a procedure,[35] based on Marcus theory,[36-38] for analyzing whether a reaction will be concerted or not, by drawing three-dimensional reaction coordinate diagrams (two dimensions corresponding to different bond changing processes, and one dimension of energy). We can apply this procedure to anilide hydrolysis, and both gather insights into the nature of the process and impose further constraints upon the energies of the intermediates. Our procedure uses eq 2

$$G = \alpha x + \beta x^2 + \gamma y + \delta y^2 + \epsilon xy \qquad (2)$$

Where $\alpha = \Delta G^\circ_x + 4\tilde{G}_x$, $\beta = -4\tilde{G}_x$, $\gamma = \Delta G^\circ_y + 4\tilde{G}_y$, $\delta = -4\tilde{G}_y$, and $\epsilon = \Delta G_{reaction} - \Delta G_x - \Delta G_y$, and where ΔG_x and ΔG_y are the free energies of the "corner" intermediates, $\tilde{G}_x$ and $\tilde{G}_y$ are the intrinsic barriers for the "edge" reactions, and $\Delta G_{reaction}$ is the overall free energy change from starting material to immediate product of the (potentially) concerted reaction. If the surface described by eq 2 has a lowest activation energy path which avoids the barriers of the edge reactions, then the reaction is concerted; if the lowest activation energy path proceeds along the edges, then the reaction is stepwise. We illustrate the behaviors possible with Figure 5. When the two possible corner intermediates are high energy and the intrinsic barriers are both small, then the concerted reaction is observed, Figure 5a; when the two possible corner intermediates are high energy and the intrinsic barriers are both large, then a stepwise reaction is observed, Figure 5b; when one corner intermediate is high in energy and the other is only slightly above the starting point, then the reaction is stepwise for high or low intrinsic barriers, Figure 5 parts c and d, respectively.

In order to apply the procedure in a truly predictive fashion, the intrinsic barriers must be known. Although we have some

Table V. Calculated Free Energies of Formation for Various Intermediates and Transition States in Aqueous Solution at 72 °C Relative to Acetanilide, with $[H_3O^+] = 10^a$

compound	ΔG°_f (aq) calculated	by fitting
MeC(OH)(OH)(NHPh)	20.17 ± 3[b]	17.2
MeC(OH)(OH_2^+)(NHPh)	28.90 ± 5[c]	27.4
MeC(OH)(OH)(NH_2Ph^+)	17.66 ± 5[c]	13.1
MeC(O^-)(OH)(NH_2Ph^+)	30.85 ± 5[c]	26.3
C–O ts	25.81[d]	
C–N ts	22.74[e]	
protonation ts	24.23[f]	
$CH_3C(OH)=NHPh^+$	2.37 ± 1[g]	2.37
H_3O^+, HO^-, $CH_3C(OH)=NHPh^+$	28.99 ± 7[h]	27.0
H_3O^+, $CH_3C(OH)_2NHPh$	24.7 ± 5[i]	20.7
$CH_3C(OH)_2^+$, $PhNH_2$	20.41 ± 7[j]	22.8
H_3O^+, $CH_3C(OH)(O^-)NH_2Ph^+$	30.90 ± 7[k]	24.3
H_3O^+, CH_3COOH, $PhNH_2$	14.01 ± 8[l]	10.8

[a] Calculated values were obtained as described in Appendix 3 (supplementary material); values by fitting were obtained by varying within the uncertainty limits to obtain transition states and free energies of activation consistent with the kinetic isotope effects and the observed rates. [b] Estimated from the rates of alkaline hydrolysis at 25 °C as described in Appendix 3 (supplementary material). [c] Based on the estimated p*K*a values in Table A2 (supplementary material). The estimated uncertainties are ±3 for the tetrahedral intermediate energy and ±2 for the p*K*a estimation. [d] Free energy of activation for the hydrolysis reaction, calculated from second-order rate constant derived from a linear least-squares fit to the data for $[H_3O^+] \leq 0.3$ M. [e] Free energy of activation for the C–N cleavage process calculated from the free energy of activation for the hydrolysis process and the free energy difference derived from $k_2/k_{-1} = k_{hyd}/2k_{ex}$; k_{ex} was calculated from a second-order rate constant derived from a linear least-squares fit to the data for $[H_3O^+] \leq 0.3$ M. [f] Free energy of activation for N-protonation of T^0, assumed to be a diffusion-controlled process. At 72 °C the rate constant for a diffusion-controlled reaction was calculated to be 1.94×10^{10} M^{-1} s^{-1} by using the Stokes–Einstein equation.[50] [g] The pK_{BH^+} is −1.2 at 25 °C;[51] the temperature effect is taken to be $dpK/dt = (pKa - 0.9)/T = -0.007$.[52] This leads to a p*K* of −1.5 at 72 °C. [h] Based on the following: the pK_{BH^+} of acetanilide, the ion product for water at 72 °C, 12.74,[53] the equilibrium constant for hydrogen-bonded ion pair formation, the equilibrium constant for formation of an encounter complex of protonated acetanilide and this hydronium hydroxide ion pair, estimated as described in Appendix 3 (supplementary material). The estimated uncertainties are ±2 for the hydrogen-bond energy in solvated hydroxide, ±2 for the electrostatics, ±1 for encounter complex formation, and ±2 for hydrogen-bond formation between hydroxide and hydronium ion, for a total possible error of ±7. [i] Hydronium ion hydrogen bonded to a hydroxyl of the tetrahedral intermediate. The estimated uncertainties are ±3 for tetrahedral intermediate formation and ±2 for hydrogen-bond formation, for a total possible error of ±5 kcal/mol. [j] The pK_{BH^+} for acetic acid is taken as −6.56 at 25 °C; this is the value of H_0 where acetic acid is half protonated;[54] correcting for the effect of temperature after Perrin[52] this becomes −7.74 at 72 °C. Based on the equilibrium constant for amide hydrolysis and the equilibrium constant for protonation of acetic acid, $\Delta G = 12.23$; and the equilibrium constant for encounter complex formation taken as $K_{encounter} = 0.017$, ignoring loss of hydrogen bond (presumed weak) and any special interactions of amine + cation, $\Delta G = 2.80$. The estimated uncertainties are ±2 for amide hydrolysis, ±3 for pK_{BH^+} of acetic acid, and ±2 for encounter complex (more uncertainty than normal). The total possible error is ±7 kcal/mol. [k] Hydronium ion hydrogen bonded to the O^- of a zwitterionic tetrahedral intermediate. The estimated uncertainties are ±2 for the p*K*a; ±2 for the hydrogen-bond formation. The total possible error is ±4 kcal/mol on top of the uncertainty (±3) in the energy of the tetrahedral intermediate. [l] Hydronium ion hydrogen bonded to acetic acid, which in turn is in contact with aniline. The estimated uncertainties are ±2 for amide hydrolysis, ±3 for pK_{BH^+} of acetic acid, ±2 for hydrogen-bonded formation, ±1 for encounter complex formation. The total possible error is ±8 kcal/mol.

(35) Guthrie, J. P.; Pike, D. C. *Can. J. Chem.* **1987**, *65*, 1951.
(36) Cohen, A. O.; Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 4249.
(37) Marcus, R. A. *J. Am. Chem. Soc.* **1969**, *91*, 7224.
(38) Marcus, R. A. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155 and references cited therein.

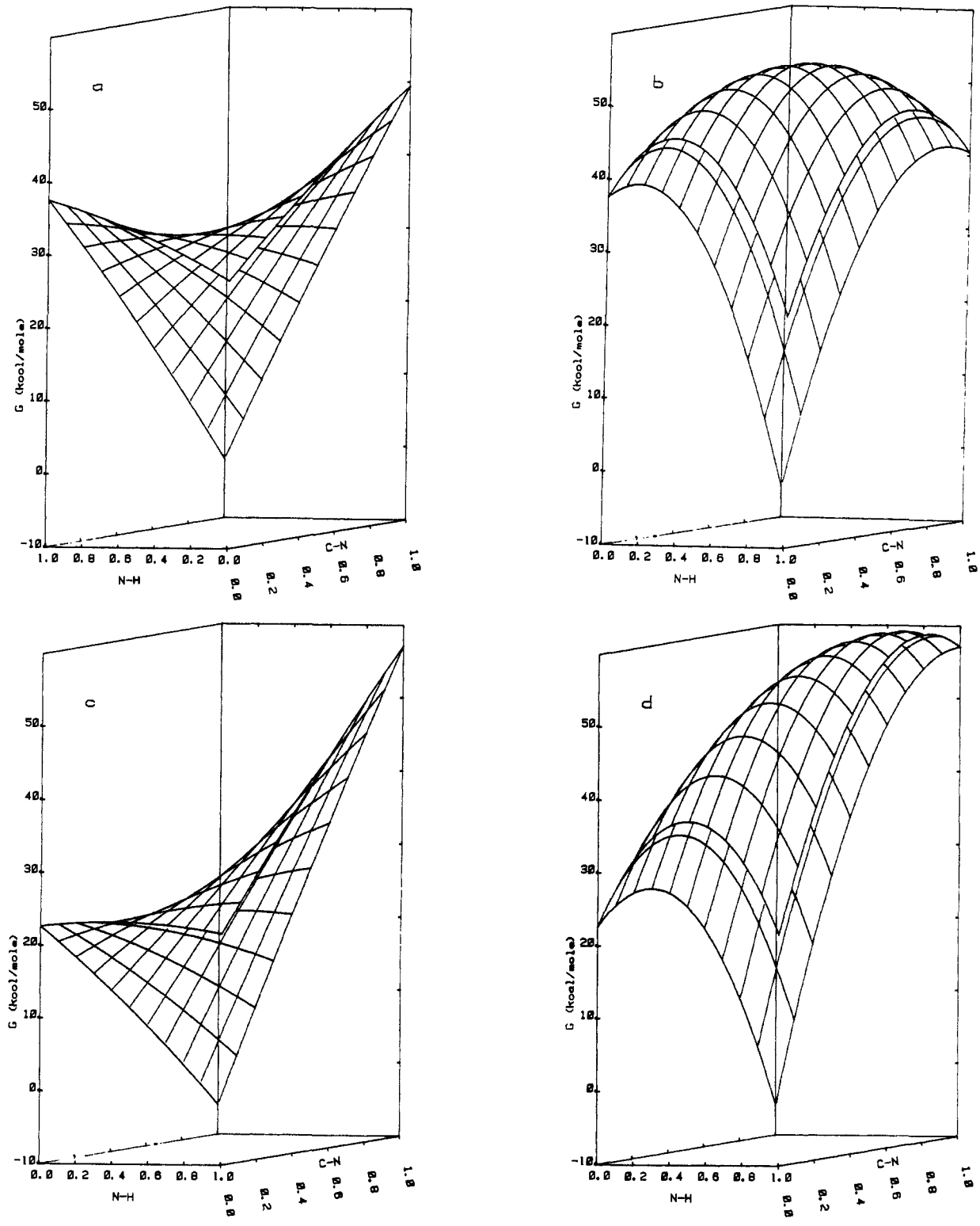


Figure 5. Effects of corner energies and intrinsic barriers on the shape of the surface calculated from eq 2. (a) The two possible corner intermediates are high energy, and the intrinsic barriers are both small, leading to concerted reaction. (b) The two possible corner intermediates are high energy and the intrinsic barriers are both large, leading to stepwise reaction. (c) One corner intermediate is high in energy, the other is only slightly above the starting point, and the intrinsic barriers are high, leading to stepwise reaction. (d) The same but with low intrinsic barriers also leads to stepwise reaction, because the rate-limiting activation barrier is along the edge coordinate leading to the low energy intermediate, although after this barrier is crossed there is no energetic reason to proceed to the intermediate and reaction may follow a range of paths to the product.

confidence that the intrinsic barriers for proton transfers between oxygen and oxygen or nitrogen should be small,[39] i.e., 1 kcal/mol, we have little basis for estimating a value for the intrinsic barriers for C–O or C–N bond formation, except that they are expected to be less than 14 and more than 4 kcal/mol. These estimates are based on a survey of reactions of bases with electrophilic carbon by Hine;[40] although larger intrinsic barriers were found, they applied to reactions of delocalized bases or electrophiles, where

(39) Kresge, A. J. *Chem. Soc. Rev.* **1973**, *2*, 475.

(40) Hine, J. *J. Am. Chem. Soc.* **1971**, *93*, 3701.
(41) Cox, R. A.; Yates, K. *Can. J. Chem.* **1981**, *59*, 2853.
(42) Satterwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7031.

one might expect that more reorganization, hence more energy, was needed to reach the transition state. We have used intrinsic barriers derived from work at 25 °C. Examination of the expected temperature dependence suggests that these barriers, expressed in terms of free energies, will be quite insensitive to temperature in the range of interest here, particularly in the light of the other uncertainties in the values used.

For the hydration process, we will first consider the mechanism we consider less likely, namely a fully concerted reaction leading to T_{N^+}. The two stepwise components are simple addition leading to the O-protonated tetrahedral species and a water-mediated proton switch transferring a proton from this water to the nitrogen. The available data (Appendix 4, supplementary material) suggest that the intrinsic barrier for water-mediated proton switch reactions is relatively large, ranging from 7 to 13 kcal/mol. We explored the implications of the lowest plausible values, namely 4 for C–O bond formation and 7 for the water-mediated proton switch. Equation 2 clearly predicts a stepwise reaction and in fact only predicts a concerted path with the observed activation energy if both intrinsic barriers are lowered to 2 kcal/mol, and then only if energies of some species are at the low limit of the uncertainty bounds. These intrinsic barriers seem too low to be credible, and we conclude that this mechanism is not viable.

The alternative mechanism leading to T° involves a simple proton transfer and C–O bond formation as the edge reactions. For the proton transfer edge reaction the corner intermediate is an encounter complex of a hydronium–hydroxide ion pair and protonated anilide. For the simple proton transfer an intrinsic barrier of 1 kcal/mol appears plausible. Equation 2 predicts a concerted reaction, with the observed free energy of activation if the energies of various corner species are near the limits of their uncertainties and the intrinsic barrier for C–O cleavage is 5 or even 4 kcal/mol, again at or near the lower limit of what seems plausible. Since a concerted path is possible and consistent with what we know about the thermodynamic and kinetic barriers, we conclude that this is the preferred mechanism.

Now we turn to the product-forming step, where the solvent isotope effect data led to a clear choice of mechanism. The arguments given above demand that a concerted mechanism for the k_2 step be predicted by eq 2 with the calculated energies of the corner species (within the estimated uncertainties) and an intrinsic barrier for C–N cleavage between 4 and 5 kcal/mol. This in fact is what is found.

Figure 6 shows contour diagrams for the addition and elimination steps of the reaction, with the transition state positions calculated by eq 2.

Figure 7 shows the free energies for a two-dimensional projection of the reaction-coordinate diagrams which are consistent with all of the facts presently known. We cannot say whether the immediate product of the addition reaction, an encounter complex of hydronium ion and T°, is converted to T_{N^+} faster than hydronium ion diffuses away or whether diffusional separation and diffusion-limited protonation occur with T° as an obligatory intermediate. The rates expected for diffusional separation and diffusional motion within the encounter complex are both about 10^{11} s^{-1} and would not be detectable. There appears to be no problem with rotation to allow exchange because this rotation is expected to have a rate constant of 10^8 s^{-1} and an activation energy of 8 kcal/mol.[43,44] The tetrahedral species involved in this hydrolysis have very short lifetimes, both because they are interconverted by proton transfers which are inherently fast, and because the breakdown processes with either C–O or C–N cleavage are very rapid from the appropriate intermediate. The lifetimes so calculated for the mechanism shown in Figure 7 are as follows: encounter complex of $T°/H^+$, 1×10^{-11} s; T°, 5×10^{-11} s; T_{N^+}, 3×10^{-8} s. For T_{N^+}, the thermodynamically preferred form of the tetrahedral intermediate, one can also calculate a lifetime based on the rates of conversion to starting material or products: this lifetime is 10^{-7} s. Thus, the intermediate has a long enough lifetime for rotational isomerization, although just barely.

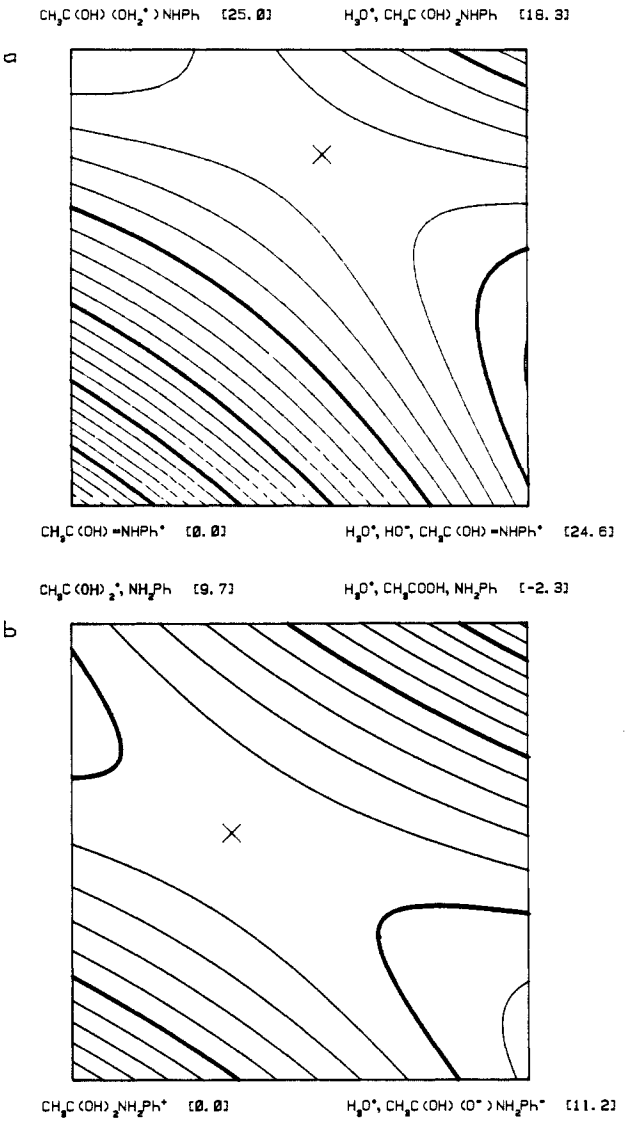


Figure 6. Contour diagrams for the addition of water and expulsion of aniline, calculated by using equation 2. The relative energies of the corner species (in kcal/mol) are shown. The intrinsic barriers used were 1 kcal/mol for proton transfer and 4 kcal/mol for C–O and C–N bond making or breaking. The position of the transition state is indicated. Contours are drawn every kilocalorie, with heavier lines every 5 kcal.

(d) Remaining Complexities. Mechanisms where proton transfer to nitrogen is either concerted[41] or occurs faster than diffusional separation of the newly formed hydronium ion[42] have both been proposed for related reactions. These proposals have very important implications for the idea of stereoelectronic control of carbonyl reactions.[34] If, as proposed by Deslongchamps,[34] the addition of water to an O-protonated amide leads directly to the tetrahedral species with the nitrogen lone pair antiperiplanar to the newly formed C–O bond, then it is very difficult to see how transfer of a proton from the newly generated hydronium ion to this nitrogen lone pair can be faster than diffusional separation. It would require a long chain of hydrogen-bonded water molecules to span the distance between the hydronium ion and nitrogen. There is unlikely to be any preformed solvation shell suitable for this process, because the nitrogen was very weakly basic and unlikely to accept a hydrogen bond until the new C–O bond was extensively formed. Rotation about the C–N bond is likely to be too slow to compete with diffusional separation, although this is not as firmly settled as one would hope. The available data, for *tert*-butylmethylbenzylamine[43] and *tert*-butyldimethylamine,[44] indicate that the rate of rotation about the C–N bond is about

(43) Bushweller, C. H.; O'Neil, J. W.; Bilofsky, H. S. *J. Am. Chem. Soc.* **1971**, *93*, 542.
(44) Bushweller, C. H.; O'Neill, J. W.; Bilofsky, H. S. *J. Am. Chem. Soc.* **1970**, *92*, 6349.

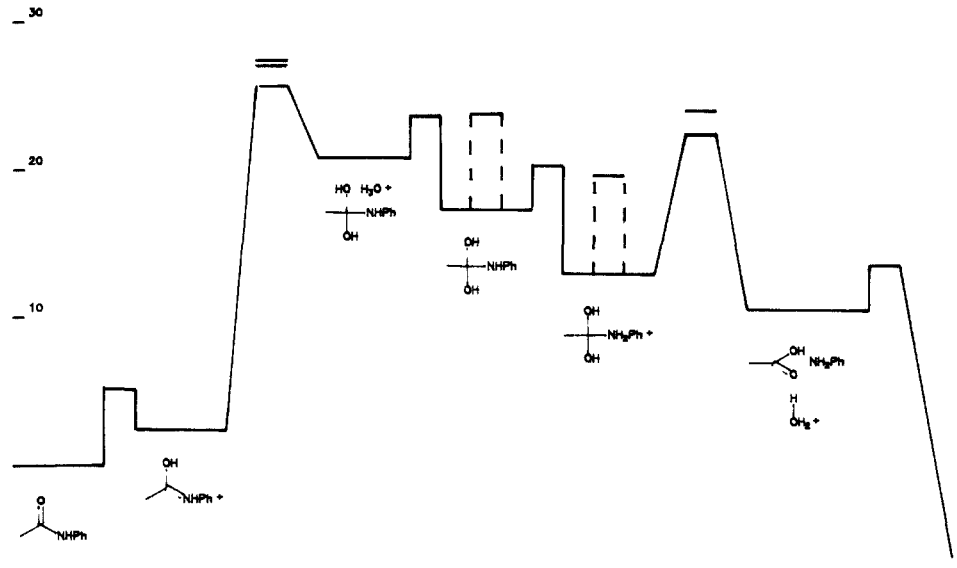


Figure 7. Two-dimensional projection of the reaction coordinate diagram for acetanilide hydrolysis at 72 °C, using energies for the intermediates consistent with all the known constraints. This set of values assumes intrinsic barriers of 1 kcal/mol for proton transfer and 4 kcal/mol for C–O or C–N bond cleavage. For the C–O and C–N transition states the energies of the "corner intermediates" are also shown. Activation energies for bond rotation (with an assumed rate constant of 10^8 s^{-1}) are shown for both T° and T_{N^+} (dotted lines).

10^8 s^{-1}, which is much less than the rate of diffusional separation, i.e., 10^{11} s^{-1}.[45] What this means is that if the attack of water upon the protonated amide leads to T_{N^+}, then the reaction must be a syn addition. Satterthwait and Jencks have presented compelling arguments that the mechanism of hydrolysis of *N*,*N*-dimethyl-*O*-arylacetimiditonium ions must involve addition of water to give the T_{N^+}, and not T°.[42] Perrin has shown that there is no strong preference for anti addition to amidinium ions.[46,47] Sinnott has argued that least motion rather than stereoelectronic control governs the preference for syn vs anti addition.[48] Syn addition would be expected to lead to an eclipsed adduct, but collapse to one or the other staggered adducts should occur without activation barrier, so that the effect is simply of a more complicated reaction coordinate, which becomes one of bond rotation at the end. As was shown above, the energetics of the intermediates favor direct formation of T°, as does the kinetic solvent isotope effect. Our data do not permit us to state whether addition is syn or anti.

If addition of water to the protonated amide leads directly to T° for *N*,2,4-trimethylacetanilide, then one expects increased crowding upon N protonation, because of the greater solvation needed by the cation. This would produce a modest shift in pK_a; 2,6-dimethylanilinium ion is more acidic than anilinium ion by 0.73 pK_a units.[49] Such a pK_a change would not affect the rate of protonation unless T_{N^+} became less stable than T°, making the protonation reaction less diffusion controlled. The shift in relative energy levels of T° and T_{N^+} is the most plausible explanation for the shift in k_{ex}/k_{hyd} from **1** to **3a**. It should be noted that if the major effect of the additional methyl groups is to raise the energy of the tetrahedral intermediate side of the reaction coordinate diagram for C–N cleavage, then acetanilide is likely to be more concerted than **3a**.

IV. Conclusions

In Figure 7 we have presented an internally consistent picture of the detailed mechanism of the reaction which is in accord with all the available data. The acid-catalyzed hydrolysis of acetanilides proceeds by way of T°, which is formed by attack of water on the protonated amide with accompanying loss of a proton to a second water molecule. Product formation occurs by preequilibrium protonation at nitrogen, followed by C–N bond cleavage with concerted loss of a proton to give carboxylic acid amine and hydronium ion in an encounter complex. There are uncertainties remaining, but new kinds of experimental evidence will be required to remove them.

Acknowledgment. The authors gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada and the Universities of Alberta and Western Ontario.

Supplementary Material Available: Tables of original MS data for acetanilide (S1), *N*,2,4-trimethylacetanilide (S2), *N*,2,4-trimethylacet-d_3-anilide (S3), Appendix 1, used for calculating the standard deviations of k_{ex} rate constants, Appendices 2–4, used in calculating the energetics of the various intermediates, and associated references 50–80 (70 pages). Ordering information is given on any current masthead pages.

(45) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1.
(46) Perrin, C. L.; Nunez, O. *J. Am. Chem. Soc.* **1986**, *108*, 5997.
(47) Perrin, C. L.; Nunez, O. *J. Am. Chem. Soc.* **1987**, *109*, 522.
(48) Sinnott, M. L. *Adv. Phys. Org. Chem.* **1988**, *24*, 113.
(49) Jencks, W. P.; Regenstein, J. *CRC Handbook of Biochemistry*, 1st ed.; Sober, H. A., Ed.; The Chemical Rubber Co.: Cleveland, Ohio, 1968; pp J150–J189.
(50) Ridd, J. H. *Adv. Phys. Org. Chem.* **1978**, *16*, 1.
(51) Barnett, J. W.; O'Connor, C. *J. Chem. Soc., Perkin Trans. 2* **1973**, 220.
(52) Perrin, D. D.; Dempsey, B.; Serjeant, *pK_a Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981.
(53) Dean, J. A., Ed. *Lange's Handbook of Chemistry*, 12th ed.; McGraw-Hill: New York, 1979.
(54) Deno, N. C.; Puttman, C. U., Jr.; Wisotsky, M. J. *J. Am. Chem. Soc.* **1964**, *86*, 4370.