VOLUME 25 NUMBER 11

ACCOUNTS OF CHEMICAL RESEARCH®

NOVEMBER 1992

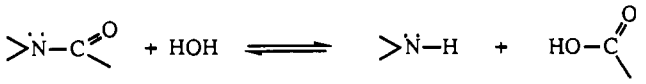
Recent Perspectives Concerning the Mechanism of H_3O^+- and OH^--Promoted Amide Hydrolysis

R. S. BROWN,* A. J. BENNET, AND H. ŚLEBOCKA-TILK

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Received February 25, 1992

The amidic unit is of fundamental importance in biological systems and to organic chemistry. Of primary concern to living systems are the acyl-transfer reactions of amides, not the least of which is the transfer to H_2O, or hydrolysis. In spite of the intense effort that has



been devoted to the enzyme-promoted hydrolysis of amides (peptides), it comes somewhat as a surprise that the nonenzymatic hydrolytic processes were, up until recently, rather ill-defined.[1] The historical reasons for this, no doubt, stem not from lack of interest nor importance, but from the slowness of the reactions for unactivated, or "normal", amides brought about by the inherent stability of the N–C(O) linkage that hampers the study under all but the most extreme conditions of pH and temperature.

It is the purpose of this Account to summarize the current state of understanding of the mechanisms of hydrolysis of some unactivated amides in acidic and basic media and to provide explanations about the factors that influence partitioning of the tetrahedral intermediates. The recent evidence upon which the present mechanistic analyses are made is based on the following three key series of experiments. First, the kinetics of hydrolysis as a function of $[H_3O^+]$ or $[OH^-]$ provide information about the stoichiometry of the rate-determining transition states (TS) for hydrolysis. Second, the kinetics of carbonyl ^{18}O exchange in unreacted amide recovered from the hydrolytic media as a function of $[H_3O^+]$ or $[OH^-]$ provide information about the reversible formation of tetrahedral intermediates. These are analyzed in terms of the processes depicted in Scheme I. In a typical experiment, an approximately 1/1 $^{18}O/^{16}O$ labeled amide is subjected to the hydrolysis conditions, recovered at various times, and then mass analyzed to determine the decrease in ^{18}O content as a function of time. The analysis requires the reasonable assumptions that the ^{16}O and ^{18}O oxygens are in rapid protonic equilibrium, that only half of the reversal from the tetrahedral intermediate leads to ^{18}O exchange, and lastly that the intermediates leading to hydrolysis and exchange are on the same pathway. Third, solvent kinetic isotope effects (SKIEs) on the hydrolysis and exchange processes in H_2O and D_2O are determined. These data provide information about the effects of H or D on the rate-determining TS for hydrolysis and exchange, and about whether protons

Stan Brown was born in High River, Alberta, Canada, and received a B.Sc. in honors chemistry from the University of Alberta in 1968. He received a Ph.D. at the University of California, San Diego, in 1972 and completed two years of postdoctoral research at Columbia University prior to obtaining an assistant professorship at the University of Alberta, Department of Chemistry, in 1974. Currently a professor of chemistry, his research interests include amide hydrolysis mechanisms, model enzyme studies, and electrophilic halogenation of olefins.

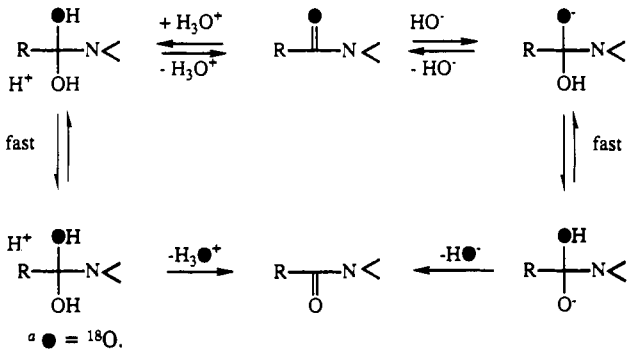
Andrew Bennet was born in Hanwell, England, and received both the B.Sc. and Ph.D. degrees from the University of Bristol. He undertook postdoctoral studies at the University of Kansas, followed by the University of Alberta. In 1991 he was appointed an assistant professor of chemistry at Simon Fraser University in Burnaby, British Columbia, Canada. His research interests include hydrolytic mechanisms of glycosidic and amidic units, as well as characterization of intermediates formed during electrophilic addition to olefins.

Henryka Ślebocka-Tilk was born in Lodz, Poland, and received the B.Sc., M.Sc., and Ph.D. degrees from the Technical University in Lodz. In 1980, she undertook postdoctoral studies at the Texas Technical University in Lubbock, TX, with John L. Kice. After a four-month period in Toronto in 1982, she joined the research group at the University of Alberta, where she is now a senior research associate.

(1) For contemporary summaries of the state of understanding of acid- and base-promoted amide hydrolysis, see: (a) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 2nd ed.; Harper and Row: New York, NY, 1987; pp 714–717. (b) Isaacs, N. S. *Physical Organic Chemistry*; Longman Scientific and Technical and J. Wiley and Sons: New York, NY, 1987; pp 484–486. (c) March, J. *Advanced Organic Chemistry*, 3rd ed.; J. Wiley and Sons: New York, NY, 1985; pp 338–341. (d) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: Oxford, 1983; pp 101–162. (e) O'Connor, C. J. *Q. Rev. Chem. Soc.* 1971, *24*, 553–564.

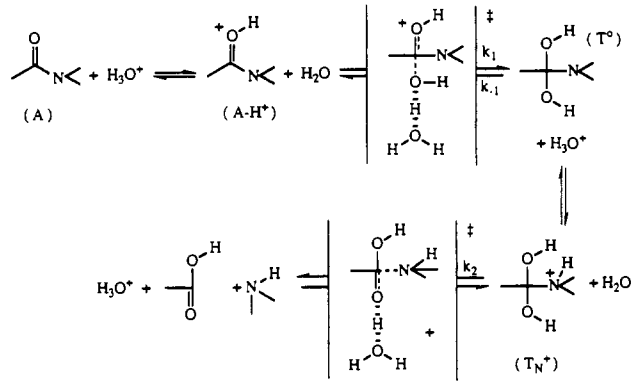
0001-4842/92/0125-0481$03.00/0

Scheme I[a]



[a] ● = ^{18}O.

Scheme II



are being transferred as part of these rate-limiting steps. The SKIE data are rigorously analyzed within the well-developed framework of isotopic fractionation factor analyses,[2] but for our purposes, the following three simplified considerations must be kept in mind.

First, for processes involving a proton being transferred or "in flight" between O and N, or O and O as part of the rate-limiting step, normal kinetic isotope effects of $k_H/k_D > 1$ are expected. Second, D_3O^+ is a stronger acid in D_2O than is H_3O^+ in H_2O, so amides would be expected to be more fully protonated and react faster in the former medium unless there are other compensating factors such as protons in flight as part of the rate-limiting step. Third, OD^- is a stronger nucleophile in D_2O than is OH^- in H_2O, so direct nucleophilic attack on the carbonyl would be expected to proceed faster in D_2O unless there are other compensating factors such as protons in flight as part of the rate-limiting step.

In what follows we will consider first the acid-catalyzed hydrolysis of certain anilides and toluamides, followed by the base hydrolysis of toluamides in which the amine portions contain an N of varying basicity.

H_3O^+-Catalyzed Amide Hydrolysis

Background. The currently favored mechanism for H_3O^+-catalyzed amide hydrolysis is depicted in Scheme II and involves a water-assisted H_2O attack on the O-protonated amide to yield T° and H_3O^+.[1] Plots of log k_{hyd} vs −log $[H_3O^+]$ generally show slopes of −1 and signs of a plateau at high $[H_3O^+]$ consistent with the onset of substantial equilibrium O protonation of the amide ($A\text{-}H^+$ $pK_a \sim 0$ to −2)[3] and reduction in the activity of H_2O. Early $^{18}O{=}C$ exchange studies of Bender,[4] Bunton,[5] and Yates[6] failed to detect loss of label in amide recovered from highly acidic media after partial hydrolysis: accordingly it was concluded that, in Scheme II, k_{-1} is negligible relative to the rate constants for product formation. However, in 1975 McClelland[7] reported that 90%-^{18}O-enriched benzamide suffered a 0.2% loss of label per hydrolytic $t_{1/2}$ when recovered from a 5.9% H_2SO_4 solution at 85 °C. This finding suggests a slight, but significant, reversal from T°.

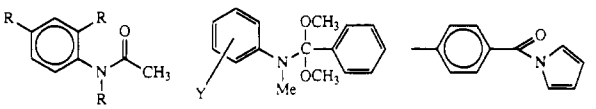
Recent Deductions Based on the ^{18}O-Exchange Kinetics and Solvent Kinetic Isotope Effects. For the mechanism presented in Scheme II, where k_1 is redefined to absorb the protonation step and k_2 is redefined to absorb T° ⇌ T_{N^+} equilibrium and breakdown of T_{N^+} to products, the $^{18}O{=}C$ exchange and hydrolysis rate constants (k_{ex} and k_{hyd}) are

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

$$k_{hyd} = k_1k_2/(k_{-1} + k_2) \quad (2)$$

$$k_{ex}/k_{hyd} = k_{-1}/2k_2 \quad (3)$$

The ratio of exchange to hydrolysis relates to the partitioning of the tetrahedral intermediate T°, the factor of 2 arising from the necessary assumptions that the oxygens are in protonic equilibrium and only half of the reversal by k_{-1} leads to ^{18}O loss. For acetanilide (**1a**) and *N*,2,4-trimethylacetanilide (**1b**) the exchange



1a R=H
b R=CH_3

2 3

and hydrolysis processes at 100 °C, μ = 1.0 (KCl), are both first order in $[H_3O^+]$, the k_{ex}/k_{hyd} ratios being 0.005–0.01 and 0.18–0.23, respectively.[8] The inherently small amount of exchange for **1a** complicates mechanistic evaluation. However, for **1b**, where the exchange is prominent, the solvent kinetic isotope effects are $(k_{ex})_{H/D} = 0.96 \pm 0.04$ and $(k_{hyd})_{H/D} = 0.98 \pm 0.04$. Certain mechanistic deductions can be made on the basis of these data. First, there must be an intermediate that partitions between exchange and hydrolysis: this is most likely the neutral tetrahedral addition intermediate, T°. Second, for these amides the predominantly rate limiting step must be the k_1 step since exchange is smaller than hydrolysis. Third, since both exchange and hydrolysis are first order in $[H_3O^+]$, each associated transition state must have one incorporated proton. This rules out the involvement of a neutral or zwit-

(2) (a) 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; pp 64–69. (b) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275–332. (c) Schowen, K. B. J. In *Transition States of Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1978; pp 225–283.

(3) (a) Guthrie, J. P. *J. Am. Chem. Soc.* **1974**, *96*, 3608–3615. (b) Arnett, E. M. *Prog. Phys. Org. Chem.* **1963**, *1*, 223–403.

(4) Bender, M. L.; Thomas, R. J. *J. Am. Chem. Soc.* **1961**, *83*, 4183–4189 and references therein.

(5) Bunton, C. A.; Farber, S. J.; Milbank, A. J. G.; O'Connor, C. J.; Turney, T. A. *J. Chem. Soc., Perkin Trans. 2* **1972**, 1869–1875 and references therein.

(6) Smith, C. R.; Yates, K. *J. Am. Chem. Soc.* **1972**, *94*, 8811–8817.

(7) McClelland, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 5281–5282.

(8) Bennet, A. J.; Slebocka-Tilk, H.; Brown, R. S.; Guthrie, J. P.; Jodhan, A. J. *J. Am. Chem. Soc.* **1990**, *112*, 8497–8506.

terionic TS as being responsible for any of the pathways leading away from T°.

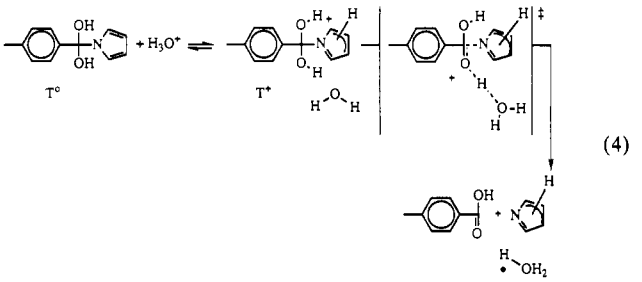
The SKIE data rule out most of the imaginable mechanisms. For **1b** the relevant analysis considers $(k_{ex}/k_{hyd})_{H/D}$, since this directly compares the SKIE on the two transition states involved for partitioning of the intermediate, e.g., $(k_{-1}/2k_2)_{H/D}$. Because the observed ratio is indistinguishable from unity, important constraints are placed on the k_{-1} and k_2 transition states with respect to the state of protonation and the numbers of protons in flight.[9] The preferred transition states are shown in Scheme II, from which it can be deduced that each involves water acting as a general base. It assists first in the delivery of another H_2O to the protonated amide (k_1 step), or in the microscopically reverse direction by the same transition state having H_3O^+ acting as a general acid to assist decomposition of T° (k_{-1} step), and then subsequently assists in deprotonation of the oxygen of T_{N^+} concurrent with C–N cleavage to form product (k_2 step). Of key importance in the analysis is that, for the breakdown of T_{N^+}, the required proton is fully installed on N so the only protons contributing to the SKIE are those associated with H_2O-mediated deprotonation of the O–H accompanying C–N cleavage. Thus the SKIE arises from an equilibrium isotope effect ($k_H/k_D < 1$) compensated by a normal effect attributable to a proton in flight.

The Effect of the Amine Leaving Group Basicity. Whether ^{18}O exchange is observed during H_3O^+-catalyzed amide hydrolysis depends upon the C–N/C–O cleavage ratio and on the ability to place the requisite proton on N. It is reasonable that the 20–40-fold-larger k_{ex}/k_{hyd} ratio observed for **1b** than **1a** stems from a difficulty in protonation of the nitrogen in T° which raises the energy of T_{N^+} for the former as well as for the subsequent C–N cleavage barrier. Formation of T_{N^+} encounters resistance because it introduces some steric compression between the developing sp^3 N and *o*-CH_3 in **1b** which is absent in the case of acetanilide. As a case in point, the same sort of steric effects make 2,6-dimethylanilinium ion more acidic than anilinium ion by 0.73 pK_a units.[10]

Additional evidence that the C–O/C–N cleavage ratio should be a function of the basicity of the amine N comes from studies of the H_3O^+-catalyzed cleavage of anilide amide acetals (**2**) by McClelland and Patel.[11] They observed that the rate constant for C–N cleavage dropped linearly when the pK_a of the corresponding anilinium ion of the leaving group was less than 4.5. Although these amide acetals might partition differently than the corresponding T° of a comparison amide, the behavior suggests some strong similarities in the requirements for protonation of N prior to C–N cleavage. In the limit, where the N cannot be protonated, the prediction would be that T° can only revert to starting material and the k_{ex}/k_{hyd} ratio should be very large.

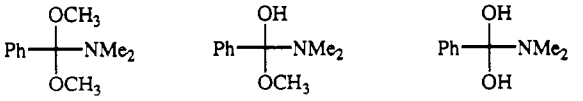
In an effort to test the above ideas, we have studied the H_3O^+-promoted hydrolysis and ^{18}O=C exchange of *N*-toluoylpyrrole (**3**).[12] Between pH 0 and 1 (72 °C, μ = 1.0 (KCl)), both processes are first order in $[H_3O^+]$ and $k_{ex}/k_{hyd} \simeq 50$. The corresponding SKIE data for **3** are $(k_{ex})_{H/D} = 0.81 \pm 0.08$, $(k_{hyd})_{H/D} = 0.91 \pm 0.18$, and $(k_{ex}/k_{hyd})_{H/D} = 0.87 \pm 0.20$. The exchange and hydrolysis of **3** can be accommodated by the mechanism shown in Scheme II and eqs 1–3, but given the large amount of exchange, $k_{-1} \gg k_2$ so that $k_{ex} = k_1/2$ and $k_{hyd} = k_1k_2/k_{-1}$. The exchange SKIE almost certainly rules out simultaneous H_2O attack and general acid catalyzed protonation of the C=O since this process should involve at least two protons in flight and an anticipated $k_H/k_D > 2$. The observed value of 0.81 is in the range typical for specific acid protonation followed by rate-limiting H_2O attack (with general base assistance by a second water). The slightly inverse SKIE for formation of T° from **3** when compared with the value for **1b** (1.0) probably indicates a slightly earlier transition state for the former that results from a destabilization of the starting amide caused by removal of amidic conjugation by the N lone pairs being occupied in an aromatic sextet.

The SKIE for breakdown of the tetrahedral intermediate formed from **3** ($(k_{hyd})_{H/D} = 0.91 \pm 0.18$) is, within experimental error, indistinguishable from unity. Since the slow step for hydrolysis is the k_2 step, the above SKIE refers principally to that step and suggests that the requisite proton is installed somewhere on the amine portion prior to the formation of product; otherwise, the k_H/k_D would be >1, reflecting the contribution of a proton in flight. The site of protonation, while uncertain, is probably on one of the ring carbons since the pyrrole N is very nonbasic (pK_a(pyrrole-H$^+$) < −3.8).[13] The breakdown mechanism, shown in eq 4, involves a preequilibrium formation of T$^+$, followed by rate-limiting, H_2O-assisted cleavage of the C–N bond to yield a carboxylic acid, a hydronium ion, and a tautomer of pyrrole.



(4)

As a final but important point in the discussion of acid-catalyzed amide hydrolysis, we consider the subtle differences in the partitioning of T° produced from the above amides and other tetrahedral species (**4** and **5**) that are taken as models for T°. Amide acetal **4** has



4 **5** **6**

been shown to exhibit an approximate 1/1 partitioning

(9) Detailed analysis of the SKIEs in terms of fractionation factors is given in ref 8.

(10) 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.

(11) McClelland, R.; Patel, G. *J. Am. Chem. Soc.* **1981**, *103*, 6908–6911.

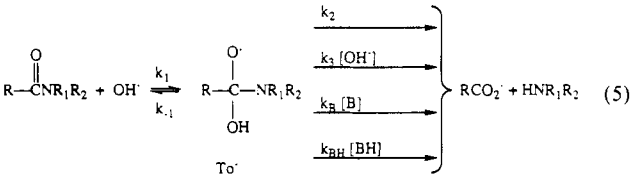
(12) Bennet, A. J.; Slebocka-Tilk, H.; Brown, R. S. *J. Am. Chem. Soc.* **1992**, *114*, 3088–3092.

(13) Chiang, Y.; Whipple, E. B. *J. Am. Chem. Soc.* **1963**, *85*, 2763–2767. The quoted value of −3.8 for the pK_a refers to a C-protonated form: the pK_a for the N-protonated form must therefore be substantially lower.

between C–O and C–N cleavage in acid.[14] That ratio must be greater than 100/1 in favor of C–N cleavage for the hydroxylated derivatives **5** and **6** (the T^{o} formed from *N,N*-dimethylbenzamide, which has been shown not to exhibit ^{18}O exchange under strongly acidic conditions[6]). McClelland[14] has advanced reasons for the partitioning difference, the most reasonable being H-bonding stabilization of the remaining OH groups in **5** or **6** with solvent (not possible with **4**) that develops in the C–N cleavage transition state. The observed SKIE near unity for C–N cleavage for the intermediates produced from amides **1** and **3** extends that and suggests that the hydroxyl groups of T^{o} are subject to a true general base process of solvent-mediated proton removal from the OH group concurrent with C–N cleavage.

Base-Catalyzed Amide Hydrolysis

Background. In base, the hydrolysis of amides in general follows the pathway shown in eq 5[1e,4,16] wherein the anionic tetrahedral intermediate To^- can either



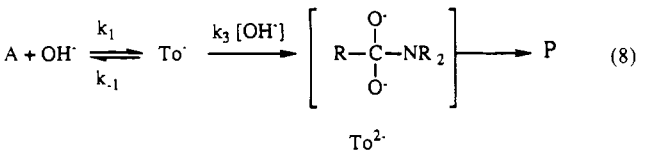
(5)

revert back to starting material or proceed to products by pathways that involve catalysis by various species in solution, including the buffer. In unbuffered media, steady-state treatment gives

$$k_{hyd} = \frac{k_1[OH^-](k_2 + k_3[OH^-])}{k_{-1} + k_2 + k_3[OH^-]} \quad (6)$$

$$k_{ex} = \frac{k_1 k_{-1}[OH^-]}{2(k_{-1} + k_2 + k_3[OH^-])} \quad (7)$$

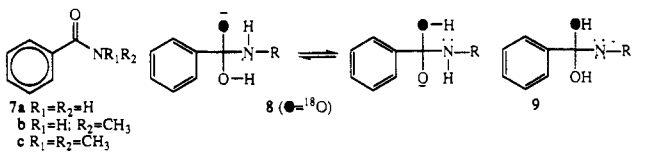
For the $^{18}O{=}C$ exchange process in base, the same assumptions concerning protonic equilibrium of the intermediates that were applied to the acidic conditions are necessary. Terms second order in $[OH^-]$ have been observed for the hydrolysis of acetanilides,[16b] formanilides,[15] trifluoroacetanilides,[16] and acetyl- or benzoylpyrroles.[17,18] This is interpreted in terms of a mechanism (eq 8) where a second OH^- removes a proton from To^- to yield the dianion To^{2-}, which expels amide ion, with or without general acid assistance by the solvent. At high $[OH^-]$, this trapping of To^- effectively halts reversal so that the rate-limiting step becomes the k_1 step, the formation of To^-. Many amides, such



(8)

as benzamides,[19,20] toluamides,[21] and simple aliphatic amides,[1e,22] do not exhibit terms second order in $[OH^-]$, so that the To^- formed from these is sufficiently reactive to break down to products without the assistance of a second hydroxide.

Early ^{18}O-exchange studies reported[19,20] for the series of substituted benzamides **7a–c** indicated that the k_{ex}/k_{hyd} ratios were independent of $[OH^-]$ with values of ~3.5 (**7a**), ~0.5 (**7b**), and 0 (**7c**). The inability of **7c** to undergo ^{18}O exchange was originally attributed[20] to the requirement for at least one N–H group in To^- to assist in the equilibration of the ^{16}O and ^{18}O oxygens (via **8** or **9**). Subsequently Deslongchamps and coworkers[1d,23] reinterpreted the original observations[20] of zero ^{18}O exchange for **7c** in terms of the theory of stereoelectronic control. In that interpretation, protonic equilibration of the oxygens was considered fast, but the lifetime of To^- was said to be too short to allow conformational changes at N (either by bond rotation or by N inversion) that would be required to assist in ejection of the $^{18}OH^-$.



7a $R_1{=}R_2{=}H$
b $R_1{=}H$; $R_2{=}CH_3$
c $R_1{=}R_2{=}CH_3$

8 (●=^{18}O)

9

Recent Deductions Based on ^{18}O Exchange. Despite the explanations given above, the reasons advanced for why **7c** exhibits no ^{18}O exchange (or only a small amount, at the limits of experimental detection) appear unsatisfactory since they take no cognizance of other factors, such as steric or leaving-group effects, that could seriously alter the C–O/C–N cleavage ratio. In an effort to assess the factors controlling this ratio, we embarked upon a controlled study of ^{18}O exchange and hydrolysis kinetics for secondary and tertiary toluamides **10a–c** and **11a–c**.[21] For each of these amides, both k_{ex} and k_{hyd} are linearly dependent upon $[OH^-]$ throughout the concentration ranges studied.[21] Given in Table I are the experimental k_{ex}/k_{hyd} ratios and the pK_a values for the corresponding ammonium ions. For the secondary toluamides **10a–c**, the k_{ex}/k_{hyd} ratios fall in the range ~0.4–0.7 despite the fact that

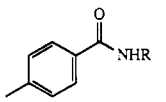
(14) McClelland, R. A. *J. Am. Chem. Soc.* **1978**, *100*, 1844–1849.
(15) DeWolfe, R. H.; Newcomb, R. C. *J. Org. Chem.* **1971**, *36*, 3870–3878.
(16) (a) Young, J. K.; Pazhanisamy, S.; Schowen, R. L. *J. Org. Chem.* **1984**, *49*, 4148–4152. (b) Eriksson, S. O. *Acta Chem. Scand.* **1968**, *22*, 892–906. (c) Pollack, R. M.; Dumsha, T. C. *J. Am. Chem. Soc.* **1973**, *95*, 4463–4465. (d) Biechler, S.; Taft, R. W. *J. Am. Chem. Soc.* **1957**, *79*, 4927–4935.
(17) Menger, F. M.; Donohue, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 432–437.
(18) Cipiciani, A.; Linda, P.; Savelli, G. *J. Heterocycl. Chem.* **1979**, *16*, 673–675.
(19) (a) Bender, M. L. *J. Am. Chem. Soc.* **1951**, *73*, 1626–1629. (b) Bender, M. L.; Ginger, R. D.; Kemp, K. C. *J. Am. Chem. Soc.* **1954**, *76*, 3350–3351. (c) Bender, M. L.; Ginger, R. D.; Unik, J. P. *J. Am. Chem. Soc.* **1958**, *80*, 1044–1048.
(20) (a) Bunton, C. A.; Nayak, B.; O'Connor, C. J. *J. Org. Chem.* **1968**, *33*, 572–575. (b) Bunton, C. A.; Lewis, T. A.; Llewellyn, D. R. *Chem. Ind. (London)* **1954**, 1154–1155.
(21) (a) Slebocka-Tilk, H.; Bennet, A. J.; Keillor, J. W.; Brown, R. S.; Guthrie, J. P.; Jodhan, A. J. *J. Am. Chem. Soc.* **1990**, *112*, 8507–8514. (b) Slebocka-Tilk, H.; Bennet, A. J.; Hogg, A. J.; Brown, R. S. *J. Am. Chem. Soc.* **1991**, *113*, 1288–1294 and references therein.
(22) (a) DeRoo, M.; Bruylants, A. *Bull. Soc. Chim. Belg.* **1954**, *63*, 140–157. (b) Calvet, E. *J. Chim. Phys.* **1933**, *30*, 140–166.
(23) (a) Deslongchamps, P.; Cheriyan, U. O.; Guida, A.; Taillefer, R. J. *Nouv. J. Chim.* **1977**, *1*, 235–241. (b) Deslongchamps, P.; Gerval, P.; Chiryan, U. O.; Guida, A.; Taillefer, R. J. *Nouv. J. Chim.* **1978**, *2*, 631–636. (c) Deslongchamps, P.; Bartlett, R.; Taillefer, R. J. *Can. J. Chem.* **1980**, *58*, 2167–2172.

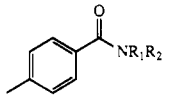
Table I. ^{18}O Exchange and Hydrolysis Data for Various Toluamides in Basic Media, μ = 1.0 (KCl), Along with pK_a Values for Corresponding Ammonium Ions[a]

amide	k_{hyd}(M^{-1} s^{-1})	k_{ex}/k_{hyd}	pK_a($H_2N^+R_1R_2$), 25 °C
10a[b]	1.15×10^{-4}	0.49 ± 0.03	10.8
	(1.0×10^{-4})[c]	(0.53 ± 0.04)[c]	
10b[b]	2.46×10^{-5}	0.64 ± 0.07	
10c[b]	2.48×10^{-6}	0.44 ± 0.04	10.8
11a[b,d]	1.15×10^{-3}	0.010 ± 0.002	10.64
	(1.28×10^{-3})[c]	(0.014 ± 0.002)[c]	
11b[e]	8.79×10^{-4}	0.13 ± 0.02	8.33
11c[f]	3.13×10^{-5}	32.2 ± 1.6	6.3
	(2.97×10^{-5})[c]	(35.6 ± 1.4)	
11d[g]	2.47×10^{-3}	9.0	4.05[h]
	(2.91×10^{-3})[c]		
11e[i]	1.29	0.24	<−3.8[j]
	(1.95)[c]		

[a] T = 100 °C unless otherwise noted; μ = 1.0 (KCl); error limits on k_{hyd} ±5% of quoted number. Unless otherwise noted, pK_a values are from the following: *CRC Handbook of Chemistry and Physics*, 48th ed.; CRC Press: Cleveland, 1967–1968. [b] From ref 21a. [c] In D_2O. [d] k_{ex}/k_{hyd} ratio determined from data at ~1.0 M NaOH. [e] T = 72 °C, μ = 1.0 (KCl); k_{ex} determined at 0.19 M NaOH and converted into second-order rate constant for determining k_{ex}/k_{hyd} ratio. [f] k_{ex}/k_{hyd} ratio determined at 1.08 M NaOH; in D_2O determined at 1.03 M NaOD; all values converted to second-order ones for comparison. [g] T = 73 °C; k_{ex}/k_{hyd} ratio determined in the low-[OH^-] range of [OL^-] = 0.035 M where both k_{ex} and k_{hyd} are approximately first order in [OH^-]; ref 25. [h] Reference 26. [i] T = 25 °C; k_{ex}/k_{hyd} ratio determined in the low-[OH^-] range where both k_{ex} and k_{hyd} are first order in [OH^-]; ref 25; $(k_{hyd})_{H/D}$ compared at pD = 9.43. [j] Reference 13.

the relative k_{hyd} drops from 1.0 to 0.2 to 0.02 in passing through the series. This would suggest that OH^- attack on the C=O is subject to steric hindrance by increasing bulk on the amine, but once To^- is formed, its partitioning ratio, k_{-1}/k_2, is relatively insensitive to steric effects. For the tertiary amides, **11a–c**, a series which should exhibit relatively constant steric effects, the k_{ex}/k_{hyd} ratio varies from ~0.01 to ~32, with the amide (**11c**) that has the least basic amine being the one most prone to reverse from To^- to re-form the amide. These data indicate that tertiary benzamides or toluamides can exhibit ^{18}O exchange, but the ability to do so in some way depends upon the amine basicity. Moreover, the data indicate that it is unnecessary to postulate that an NH group is absolutely required for equilibration of the ^{18}O and ^{16}O oxygens in To^- as in **8** or **9**.[20a] Also, each of **11b** or **11c** exhibits more ^{18}O exchange than **11a**, but the former two contain amines having steric bulks similar to or greater than the amine portion in the latter. Thus, if anything, conformational equil-





10a R=Et
b R=iPr
c R=tBu

11a R_1=R_2=CH_3
b R_1=R_2= —$(CH_2)_2O(CH_2)_2$
c R_1=Et; R_2=CH_2CF_3
d R_1=R_2=—$CH_2CF_2CF_2CH_2$—
e R_1=R_2=—CH=CH-CH=CH—

ibration at N in To^- is expected to be slower in the case of **11b,c** than in **11a**. This indicates that conformational equilibration at N and stereoelectronic requirements as proposed by Deslongchamps et al.[1d,23] are insufficient considerations for determining whether a given amide will or will not exhibit ^{18}O exchange accompanying its basic hydrolysis. Rather, the key element appears to be amine basicity.

Deductions Based on the SKIE. For the tertiary amides **11a–c** for which k_{ex} and k_{hyd} are both first order in [OH^-],

$$k_{ex} = \frac{k_1 k_{-1}[OH^-]}{2(k_{-1} + k_2)} \quad \text{and} \quad k_{hyd} = \frac{k_1 k_2 [OH^-]}{(k_{-1} + k_2)}$$

For **11a**, where exchange is always small relative to hydrolysis, $k_2 \gg k_{-1}$:

$$k_{ex} = \frac{k_1 k_{-1}[OH^-]}{2k_2}; \quad k_{hyd} = k_1[OH^-];$$

$$\text{and} \quad k_{ex}/k_{hyd} = \frac{k_{-1}}{2k_2}$$

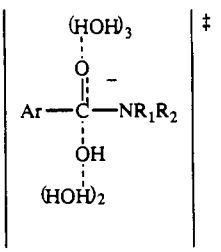
For **11c**, where hydrolysis is small relative to exchange, $k_{-1} \gg k_2$:

$$k_{ex} = \frac{k_1[OH^-]}{2}; \quad k_{hyd} = \frac{k_1 k_2 [OH^-]}{k_{-1}};$$

$$\text{and} \quad k_{ex}/k_{hyd} = \frac{k_{-1}}{2k_2}$$

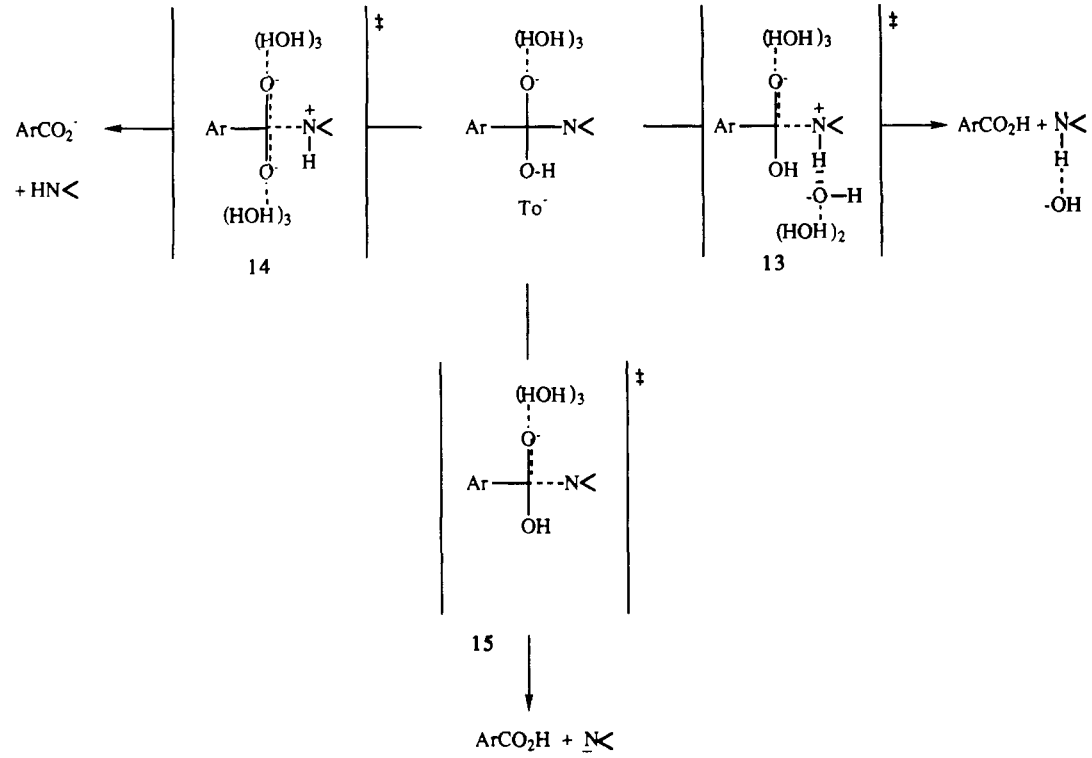
Thus, in these limiting cases it is possible to obtain simple expressions for all of the SKIE. The experimental observations[21] for **11a** and **11c** are that the SKIE is 0.91 ± 0.18 and 1.05 ± 0.04, respectively, for the k_{hyd} values, and that it ranges from 0.61 ± 0.12 (**11a**) to 0.90 ± 0.05 (**11c**) for k_{ex}. In the case of **11a**, which exhibits only a small amount of exchange relative to hydrolysis, the $(k_{ex})_{H/D}$ value contains a large uncertainty. The problem lies in the accuracy with which low amounts of ^{18}O exchange can be determined.[21a] However, since **11c** exhibits pronounced exchange, the $(k_{ex})_{H/D}$ is reliably given as 0.90 ± 0.05; this value, referring to the SKIE on the attack step, k_1, is expected and found to be similar to the $(k_{hyd})_{H/D}$ values for **11a**, since for this amide $k_{hyd} \sim k_1$.

The SKIE data $(k_{ex})_{H/D}$ and $(k_{hyd})_{H/D}$ for **11c** provide valuable information about the attack step (k_1) and the breakdown step (k_2).[21b] Because $k_{ex} \simeq 32k_{hyd}$, both the attack and breakdown are kinetically isolated and amenable to individual analysis. Importantly, since both k_{ex} and k_{hyd} are first order in [OH^-], each associated transition state must have one incorporated OH^- or its kinetic equivalent. By fractionation factor analysis,[24] the calculated k_H/k_D for the attack transition state (**12**) is 0.99 and agrees nicely with the observed $(k_{ex})_{H/D}$ of 0.90 ± 0.05. Here, the SKIE arises not from protons in flight, but from a reordering of the waters of solvation around the attacking OH^- and developing alkoxide anion in To^-.



12

Scheme III



The observed $(k_{hyd})_{H/D}$ of 1.05 ± 0.04 for 11c strongly suggests that in the breakdown of its To⁻ there are no protons in flight and the departing N has the requisite proton fully in place prior to CN cleavage. There are three possible monoanionic transition states shown in Scheme III, with two of them (13 and 14) being consistent with the observed SKIE.[21b] In 13, cleavage proceeds from a zwitterionic form with an associated OH^- that is H-bonded to the department protonated amine and leads to carboxylic acid and an amine:OH^- encounter complex; the calculated k_H/k_D is 1.27. For TS 14, where cleavage proceeds from a dianionic zwitterion leading to carboxylate and amine, the calculated k_H/k_D is 1.39. For any of the imaginable C–N cleavage transition states involving protons in flight to N as part of the rate-limiting step, large normal isotope effects of $k_H/k_D > 2$ are anticipated, contrary to the observed situation. TS 15, which involves expulsion of the amide anion, is predicted to show a SKIE of <1, clearly smaller than what is observed. As is shown in the next section, this path is favored as the amine basicity is reduced further.

Effect of Reduction in Amine Basicity on the k_{ex}/k_{hyd} Ratio. The data we have presented and analyzed so far for 11a–c indicate that (1) k_{ex}/k_{hyd} increases as the amine basicity decreases and (2) the amine N in To⁻ is fully protonated prior to rate-limiting C–N cleavage. Viewed together, the data lead to the prediction that drastic reduction in amine basicity should so inhibit N-protonation that the amine cannot be expelled from To⁻ since transition states 13 and 14 are too high in energy to be viable. Therefore, new pathways for breakdown will be required, and these may involve expulsion of the amide anion from To⁻ and/or the intervention of a second OH^- as in eq 8.

(24) The pertinent aspects of the fractionation factor analysis and assumptions pertaining to the positioning of the k_{ex} and k_{hyd} transition states are given in ref 21b.

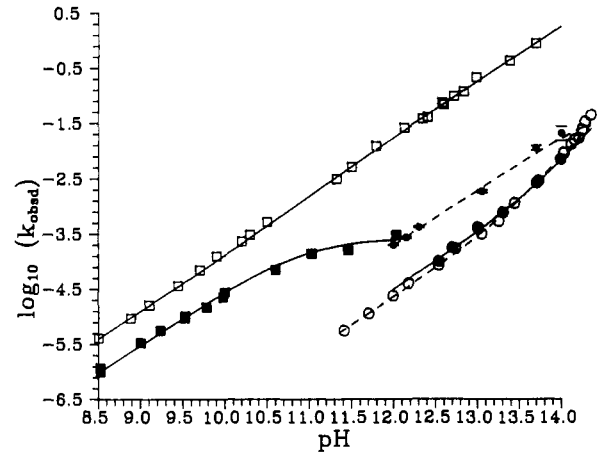


Figure 1. Plots of k_{hyd} (□) and k_{ex} (■) vs $[OH^-]$ for the base-promoted hydrolysis of 11e, T = 25 °C, μ = 1.0 (KCl). Plots of k_{hyd} (○) and k_{ex} (●) vs $[OL^-]$ for the base-promoted hydrolysis of 11d, T = 73 °C, μ = 1.0 (KCl); k_{hyd} in D_2O (●).

Amides 11d and 11e contain amine portions of low basicity, the pK_a's of the corresponding ammonium ions being 4.05[26] and <–3.8. Shown in Figure 1 are the hydrolysis and exchange pH vs log k_{obsd} profiles for 11d and 11e at 73 and 25 °C, respectively. For hydrolysis, both amides show changes in the kinetic order of the $[OH^-]$ dependence from first to second order with increasing $[OH^-]$. For 11e, this appears as a barely perceptible inflection in the profile that is flanked by two first-order domains. Of note is the leveling of k_{ex} of 11e depicted in Figure 1 that appears at the same $[OH^-]$ where the inflection in its k_{hyd} profile does. When analyzed in terms of eqs 5–8, the k_{ex} and k_{hyd} profiles are easily understood.

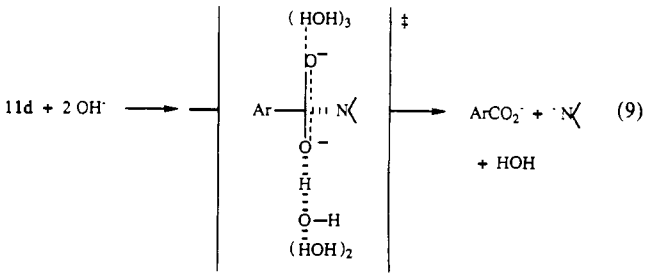
In the low-$[OH^-]$ domain where neither of the hydrolysis profiles shows $[OH^-]^2$ components, the k_{ex}/k_{hyd} ratios refer to the simple unimolecular partitioning of To⁻ (via k_{-1}, k_2) and so can be compared to those

values for **11a–c** (Table I). The most striking aspect is that the further reductions in amine basicity below that in **11c** lead not to increases in the C–O/C–N cleavage ratio, but to decreases. This signifies a change in mechanism with respect to the state of protonation of the amine as it departs. However, it must be remembered that since the k_{ex} and k_{hyd} processes are first order in [OH^-] in the domain in question, each TS must have one and only one incorporated hydroxide or its equivalent (e.g., To^-).

Since, for **11d**, $k_{ex}/k_{hyd} = 9.0$, the predominantly rate limiting step for exchange is k_1 while that for hydrolysis is k_2. As such, the SKIEs on k_{ex} and k_{hyd} are essentially relegated to single steps and can be compared with the effects noted for **11c** where $k_{ex}/k_{hyd} \simeq 32$. The observed $(k_{ex})_{H/D}$ of 0.89 (at [OL^-] = 0.01 M, μ = 1.0 (KCl)[25]) compares favorably with that seen for **11c** (0.90 ± 0.08) and suggests that both attack transition states are similar in terms of the waters associated with desolvation of the attacking OL^- and resolvation of the developing alkoxide (e.g., TS **12**). However, for **11d** the limiting $(k_{hyd})_{H/D}$ or $(k_1k_2/k_{-1})_{H/D}$[27] is 0.72 (probable error ±10%) and significantly lower than that seen for **11c** (1.05), which signifies a difference in the nature of their transition states for breakdown.

Three possible transition states for the breakdown of the To^- formed from OH^- attack on **11e** are shown in Scheme III. Two of these (**13** and **14**) are analogous to the breakdown transition states already considered for **11c** and yield predicted SKIE values >1. The third (**15**), which involves expulsion of the amide anion from To^-, is the only one that yields a predicted SKIE of <1 (actually 0.74, which agrees nicely with the experimental value of 0.72). It should be pointed out that, in the above case, the SKIE arises not from the protons in flight, but from reordering of solvent molecules during C–N cleavage. Furthermore, any strong general acid catalysis by solvent of the departure of the anionic N requires a proton in flight, which raises the expected SKIE to 1.6–2.7, clearly greater than the observed value.

Finally, we consider the mechanism involved in the [OH^-]^2 hydrolysis noted for **11d** and **11e**. The crucial observation is illustrated in Figure 1 as the closed circles that denote the hydrolytic profile for **11d** in D_2O. That the D_2O line crosses the H_2O line signifies that the SKIE changes from inverse (0.72) at low [OL^-] to normal at high values where the [OL^-]^2 term comes into play. The experimental value[28] of 1.54 signifies a significant contribution of a proton in flight during the OH^--promoted breakdown of To^-. For the likely transition state **16** (eq 9), the computed SKIE is >1 and involves removal of the O–H of To^- by hydroxide concurrent with C–N cleavage. For any process not involving a proton in flight, the SKIE would be inverse: this effectively rules out a complete removal of the



(9)

16

proton of To^- to yield To^{2-} (eq 8) prior to rate-limiting C–N cleavage.[25]

Conclusions

For the H_3O^+- or OH^--catalyzed hydrolysis of the amides investigated, we have presented mechanisms consistent with all of the presently available experimental observations. In both acid and base, the path chosen, as well as relative barrier heights for formation and partitioning of the intermediates, is in large part determined by the basicity of the amine portion. At the same time, the SKIE data provide important evidence that the oxygens in the tetrahedral intermediate(s) are in protonic equilibrium. If proton transfer were in some way limiting the equilibration of the ^{16}O and ^{18}O oxygens, then this would require a proton in flight as part of the rate-limiting equilibration, so far less ^{18}O exchange should be seen in D_2O relative to H_2O. Since the SKIE observed for all k_{ex} processes in either H_3O^+ or OH^- appears to be roughly unity, proton transfer cannot be limiting the exchange.

In acid, the general hydrolytic process depicted in Scheme II suffices to explain the existing ^{18}O-exchange and SKIE data. The simple, underlying theme involves specific acid protonation of the amide to activate it toward attack by water. That attack is assisted by a second H_2O to directly yield the neutral tetrahedral intermediate and H_3O^+, which avoids the formation of a highly unstable O-protonated tetrahedral intermediate. The breakdown of T^o, in all cases we have investigated, involves protonation by H_3O^+ to yield T_{N^+}, which then undergoes C–N cleavage. That process is assisted by simultaneous proton removal from an OH in T_{N^+} by solvent water, so that the immediately formed products are amine, carboxylic acid, and H_3O^+. ^{18}O exchange for normal amides containing a highly basic amine is understandably small because N protonation of To to form T_{N^+} and subsequent C–N cleavage are fast relative to reversal to re-form amide. However, ^{18}O exchange can be substantially increased by electronic or steric factors that decrease the N basicity (in T^o) thereby interfering with installation of the essential proton on the amine. For amides that are increasingly nonbasic so that any protonated forms (A–H^+ or T_{N^+}) are too high in energy to exist, the predicted pathways for hydrolysis in acid will involve concurrent general acid and general base mechanisms for both formation and breakdown of T^o.

The presently available evidence for the mechanism of hydrolysis of tertiary toluamides in base supports the ultrasimplified pathway given in eq 5. In all cases, the first step involves direct attack of OH^-, the SKIE arising from desolvation of the attacking hydroxide

(25) Brown, R. S.; Bennet, A. J.; Slebocka-Tilk, H.; Jodhan, A. *J. Am. Chem. Soc.* **1992**, *114*, 3092–3098.

(26) Roberts, R. D.; Ferran, H. E., Jr.; Gula, M. J.; Spencer, T. A. *J. Am. Chem. Soc.* **1980**, *102*, 7054–7058.

(27) From Table I the observed $(k_{hyd})_{H/D}$ at 0.035 M [OH^-] is 0.84. The limiting $(k_{hyd})_{H/D}$ was calculated by nonlinear least squares fitting of the separate k_{hyd} vs [OL^-] profiles to eq 6 from which can be obtained k_1, k_3/k_{-1} and k_2/k_{-1} in both OH^- and OL^- media. The computed limiting value of $(k_1k_2/k_{-1})_{H/D}$ of 0.72 was obtained from the NLLSQ fits; see ref 25.

(28) From the NLLSQ fits of the k_{hyd} vs [OL^-] data, the computed $(k_1k_3/k_{-1})_{H/D}$ is 1.54 (probable error ±10%).[25]

compensated by resolvation of the developing alkoxide as depicted for transition state **12**. The pertinent questions we have attempted to answer are, what factors control the k_1, k_2, and k_{-1} barrier heights, when can we anticipate large amounts of ^{18}O exchange, and when does the hydrolytic process require a second OH^-? The available evidence for the toluamides indicates that stereoelectronic effects as described by Deslongchamps et al.[1d,23] are of little consequence in determining the partitioning of the tetrahedral intermediate, To^-. Rather, the important factor in deciding whether a given amide will or will not exhibit ^{18}O exchange concurrent with hydrolysis in base is the basicity of the amine leaving group. It is also this factor that controls whether the amine leaves as its protonated form or as an anion. In the latter case, the hydrolytic process may be first or second order in $[OH^-]$, but this too is determined by the basicity of the amine, or more precisely by its willingness to depart as an anion. Both the ^{18}O-exchange and hydrolysis transition states that have relatively basic amines have one incorporated OH^-, and breakdown to product proceeds via a monoanionic form having a protonated N in place prior to C–N cleavage via transition states **13** or **14**. For amides (such as **11a**), where the amine basicity is high, conversion of To^- to the forms required for C–N cleavage is fast, so little exchange is observed. Reduction of the amine basicity (as in **11b,c**) destabilizes the N-protonated forms and concomitantly raises any of the transition states leading to products via these forms. ^{18}O exchange will be observed, but the k_{ex}/k_{hyd} ratio is independent of $[OH^-]$ since both k_{-1} and k_2 transition states have one incorporated OH^-. Further reductions in amine basicity destabilize the N-protonated forms so that **13** and **14** are no longer viable transition states. At the same time the electronic factors inhibiting N protonation also acidify the remaining OH of To^- and facilitate the departure of the N<, either as an anionic form H-bonded to solvent or with general acid catalysis but requiring no prior complete installation of the proton. The point of mechanistic transition appears to occur where the amine basicity lies between that in **11c** and **11d**. In the latter case, the hydrolysis kinetics will exhibit second-order terms in $[OH^-]$: ^{18}O exchange will be observed in the low-$[OH^-]$ domain with diminishing amounts as the $[OH^-]$ is raised. In the limit, at high $[OH^-]$, each time To^- is formed it will immediately react with a second $[OH^-]$ to form products, and ^{18}O exchange will cease to be observed.

The authors gratefully acknowledge the continuing financial support of the University of Alberta and NSERC of Canada. In particular, they are grateful to the staff of the Mass Spectrometry Laboratory of the Department of Chemistry, University of Alberta, without whose expertise the program described herein would not be possible.