obtain a relative response of 6-methylchrysene to the internal standard, *o*-dichlorobenzene The solution was then treated at 70.0 ± 0.1° in a sealed ampoule under a reduced pressure of nitrogen for 106 min. The reaction mixture was then analyzed by glc. It was found that 0.4390 ± 0.002 mmol of 6-methylchrysene had reacted. The solvent and residual *tert*-butyl hypochlorite were then removed and the residue was dried in a vacuum desiccator at 0.05 mm for 24 hr. The mixture was then dissolved in benzene and stirred and refluxed with 1 *N* aqueous silver nitrate overnight. The resulting silver chloride was filtered, dried, and found to amount to 0.4731 mmol. An nmr spectrum of the resulting organic mixture was taken. Based on the integration of the aldehyde proton peak at 10.2 and the methylene peak at 5.84, it was found the product consisted of 24% 6-chrysenyl aldehyde and 76% 6-chrysenylmethanol. Therefore 0.5444 mmol of silver chloride should have been obtained. Hence 87% of the 6-methylchrysene consumed was accounted for by silver chloride formed. A product study using the above method was run on 1-methylnaphthalene to determine the accuracy of the method. Only 90% of the 1-methylnaphthalene consumed was accounted for by silver chloride formed. This probably defines the accuracy of the method.

Reaction of 9,10-Dimethylanthracene with *tert*-Butyl Hypochlorite. Addition of *tert*-butyl hypochlorite to a benzene solution of 9,10-dimethylanthracene and trichloroethylene at room temperature showed rapid reaction even without an initiator. About 60% of the dimethylanthracene was consumed within 85 min. After removal of the solvent and residual *tert*-butyl hypochlorite, the reaction mixture was treated with silver nitrate solution. Precipitation of silver chloride indicated that substitution had taken place at the methyl groups.

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Proton Transfer and Heavy-Atom Reorganization in Amide Hydrolysis. Valence-Isomeric Transition States[1,2]

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Contribution from the Department of Chemistry, University of Kansas, Lawrence, Kansas 66044. *Received June 18, 1970*

Abstract: The hydrolysis in protium and deuterium oxides of ten ring-substituted trifluoro-*N*-methylacetanilides, $XC_6H_4N(CH_3)COCF_3$, in basic solution proceeds by rate-determining breakdown of tetrahedral intermediate to products with water catalysis (k_1 process) at low base concentrations, with hydroxide catalysis (k_2 process) at intermediate base concentrations, and by rate-determining formation of the tetrahedral intermediate (k_a process) at high base concentrations. The k_a process yields a linear free-energy relation (slope 0.35 in H_2O) with pK_b of the leaving-group aniline for all substituents (X = *p*-OCH_3 to *m*-NO_2) and occurs 1.2–1.7 times faster in deuterium oxide than in protium oxide. The k_1 and k_2 processes, on the other hand, exhibit biphasic free-energy relations with pK_b of the leaving group, with very small slopes (0.02 and 0.09 in H_2O, respectively) for $pK_b < 9$ and large slopes (0.3 and 0.7 in H_2O, respectively) for $pK_b > 9$. Solvent isotope effects for both processes decrease with increasing pK_b, in the case of the k_2 process dropping abruptly from normal to inverse values at $pK_b \sim 9$. Breakdown of the tetrahedral intermediate proceeds along two parallel pathways with valence-isomeric transition states, one involving mainly proton transfer from catalyst to nitrogen with generation of an ammonium intermediate and the other involving mainly C–N bond cleavage to give a hydrogen-bonded anilide ion intermediate. The former is favored by "poor" leaving groups (electron donors, $pK_b < 9$) and the latter by "good" leaving groups (electron acceptors, $pK_b > 9$).

When more than one chemical bond is made or broken in the course of a molecular transformation, the individual bonding changes may be either microscopically simultaneous (*concerted* or sequential in time (*stepwise*, a *step* being passage through a single activated complex). A *concerted reaction* in solution is one for which the reaction path consists of (a) an assembly of reactants into a common region ("diffusion together") which may or may not be a "solvent cage," within which the reactants may or may not be separated by or linked together by one or more solvent molecules; (b) passage through a single activated complex, the reaction coordinate motion transforming reactant molecules into product molecules; (c) departure of the product molecules from the common region ("diffusion apart"). A *stepwise reaction* will involve (a) diffusion together; (b) passage through a series of activated complexes, separated by stable structures (*i.e.*, collections of molecules with net restoring forces for all vibrational degrees of freedom); component molecules of these intermediate structures will diffuse apart and rediffuse together before the next step in the sequence if the rate of diffusion apart exceeds the rate of the next step—otherwise, the next step will occur without diffusion apart; the last step in the sequence will produce the product molecules; (c) diffusion apart of product molecules.

The class of reactions in which proton transfer is combined in the overall transformation with the reor-

(1) This research was supported by the National Science Foundation and the National Institutes of Health and was carried out in part at the Computation Center of the University of Kansas. Part of these results have appeared in preliminary form in L. Kershner and R. L. Schowen, Abstracts of the 153rd National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, paper no. S174. Further details are available in L. D. Kershner, Ph.D. Thesis in Chemistry, University of Kansas, 1969.

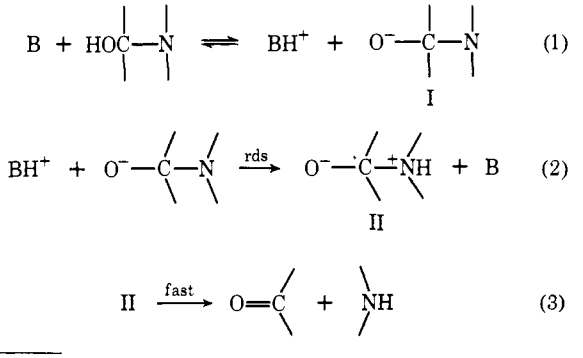
(2) Amide Hydrolysis. IV. For part III, see R. L. Schowen, H. Jayaraman, L. Kershner, and G. W. Zuorick, *J. Amer. Chem. Soc.*, **88**, 4008 (1966).

(3) National Defense Education Act Fellow.

(4) Holder of a Research Career Development Award of the National Institute of General Medical Sciences.

ganization of a heavy-atom framework includes the largest part of acid–base-catalyzed processes and very likely a considerable fraction of enzymic catalyses.[5] In some protolytically catalyzed reactions, the base catalyst or the conjugate base of the acid catalyst can be shown on kinetic grounds not to be present in the rate-determining activated complex (*specific catalysis*), which necessitates a stepwise mechanism. Those reactions in which the entire catalyst is present in the rate-determining activated complex (*general catalysis*) are not necessarily concerted, however, because this is only one of several alternatives which may hold. These are (i) rate-determining simple proton transfer; (ii) heavy-atom reorganization with solvating catalyst; (iii) heavy-atom reorganization with spectator present; (iv) heavy-atom reorganization concerted with proton transfer; (v) rate-determining diffusion.

Rate-Determining Simple Proton Transfer. Proton transfer alone may be rate determining, with heavy-atom reorganization either preceding or succeeding it. When the proton is being transferred among O, N, or S, it has been customary to imagine it alone as an unlikely rate-determining process in reactions which exhibit Brønsted coefficients not equal to zero or one and which exhibit primary isotope effects.[6] It is known that such proton exchanges are usually diffusion controlled in the thermodynamically favored direction[7] (*i.e.*, diffusion together of reactants is rate determining for "downhill" reactions and diffusion apart of products for "uphill" reactions) although the overall reaction might very well have a quite small rate constant if diffusion of low-concentration intermediates is rate determining or if the reverse proton exchange is the one which is diffusion controlled. Nevertheless, the molecular structure of the molecules in the diffusion activated complex will be that of the reactants (diffusion together) or products (diffusion apart) of the proton exchange and therefore will give unit or zero Brønsted coefficients and, at most, secondary isotope effects. This argument is valid so long as consideration is limited to reaction sequences in which all species are in equilibrium with their "diffusion conjugates," *i.e.*, species among which they may be interconverted by diffusion alone. If this condition is not met, as may frequently be the case, the argument that proton transfer alone among O, N, and S will not give rise to nonzero, non-unit Brønsted coefficient and to primary isotope effects



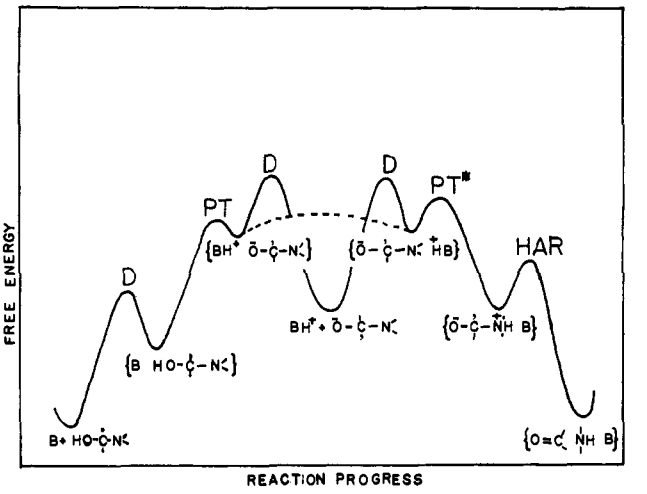
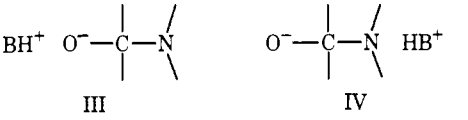


Figure 1. Conceivable free-energy diagram for general-base-catalyzed breakdown of the tetrahedral intermediate in amide hydrolysis. The dashed line shows the low (or zero) free energy of activation for the "conducted tour" of BH^+ from O to N (if the two ion pairs are, in fact, distinct). Diffusion activated complexes are labeled D, those for proton transfer PT, and those for heavy-atom reorganization HAR. The dashed line gives the true reaction pathway, with PT* being the observed activated complex.

becomes invalid. For example, consider the mechanism shown in eq 1–3 for the general-base-catalyzed decomposition of the tetrahedral intermediate in amide hydrolysis.[2,8] It is unlikely that nitrogen protonation (eq 2) will be rate determining as shown, and yet give $\beta \neq 0$ or 1, and primary isotope effects, *if* the first step (eq 1) represents conversion to diffusion-separated BH^+ and I because, as shown in the solid line of Figure 1, the activated complex for the overall process would then be that for diffusion apart or together of BH^+ and I. However, reconversion of I and BH^+ to reactants, on the one hand, and their conversion to II and B, on the other hand, proceed *via* two different conjugates (III and IV) which differ only in that BH^+ is nearer O in



III and nearer N in IV. The implicit assumption in the argument given above is that the most rapid pathway for interconversion of these two is diffusion apart to their common diffusion conjugate, $BH^+ + I$, and diffusion back together. This is not necessarily the case.[9] Cram and coworkers[10] have elegantly demonstrated that the component members of caged pairs may experience an extensive "conducted tour" among various isomeric species more rapidly than they diffuse apart, even in solvents which permit the latter process with greater facility than does water. Indeed, in water, another attractive route to interconversion of III and IV for the particular case of $B = H_2O$ exists in the form of Grotthus chain conduction of the proton from one side to the other[9,11]

(5) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.
(6) R. E. Barnett and W. P. Jencks, *J. Amer. Chem. Soc.*, **91**, 2358, 6758 (1969); *cf.* G. M. Blackburn, *Chem. Commun.*, 249 (1970).
(7) (a) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964); (b) M. Eigen in "Fast Reactions and Primary Processes in Chemical Kinetics: Nobel Symposium 5," S. Claesson, Ed., Interscience, 1967.
(8) M. L. Bender and R. J. Thomas, *J. Amer. Chem. Soc.*, **83**, 4183 (1961).
(9) M. Eigen, *Discuss. Faraday Soc.*, **39**, 1 (1965); *cf.* W. J. Albery, *Progr. Reaction Kinetics*, **4**, 355 (1967), and R. P. Bell, J. P. Millington, and J. M. Pink, *Proc. Roy. Soc., Ser. A*, **303**, 1 (1968).
(10) D. J. Cram, F. Willey, H. M. Relles, and D. A. Scott, *J. Amer. Chem. Soc.*, **88**, 2759 (1966).

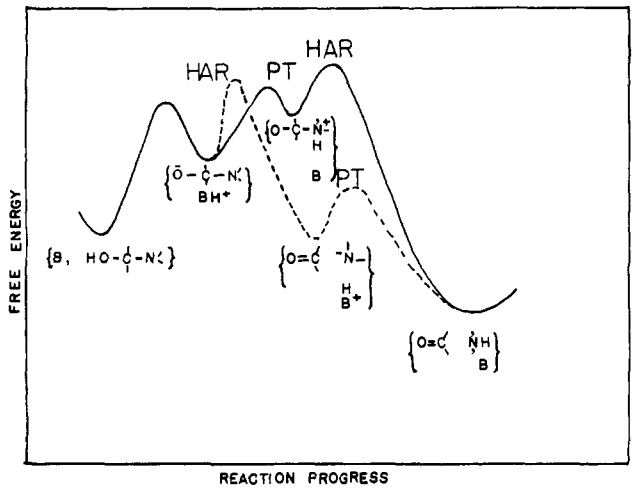
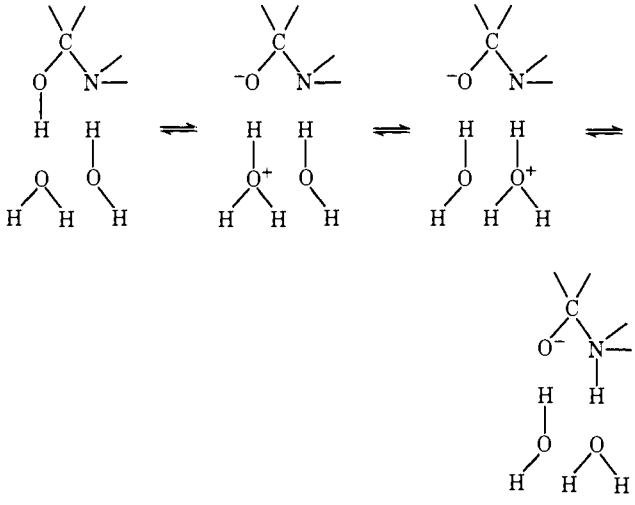
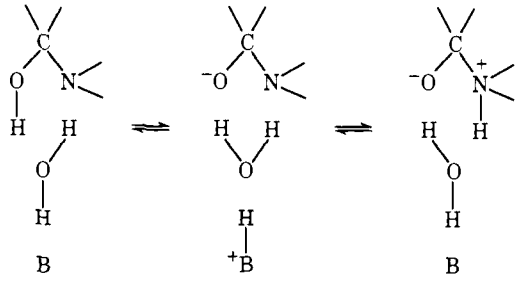


Figure 2. Two conceivable temporal sequences for proton transfer (PT) and heavy-atom reorganization (HAR) in amide hydrolysis with the latter rate determining in both cases. The full line shows rapid formation of a zwitterion and its rate-determining decomposition, while the dashed line represents rate-determining C–N cleavage, followed by a rapid proton switch from one hydrogen-bond minimum to the other. Diffusion is regarded as a slow and unimportant side reaction throughout.



Finally, the two ion pairs may not even be structurally distinct; proton removal from either O or N may lead to exactly the same ion pair



Thus the *possibility* (and the probability) shown in the dashed line of Figure 1 must be included, that the main route of reaction does not pass through the diffusion-separated ions. Then there is no difficulty in picturing a rate-determining proton transfer among O, N, and S in the simplest sense of the word (activated complex PT*). The diffusion steps are merely slow, unimportant side reactions.

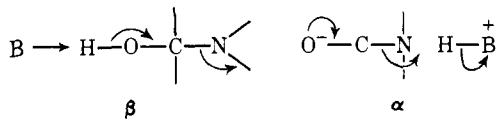
Heavy-Atom Reorganization with Solvating Catalyst. Just as the proton-transfer (PT*) step in Figure 1 can be rate determining, so can the heavy-atom-reorganization (HAR) step, if its activated complex has a higher free energy than any other along the reaction path (Figure 2). In principle the reorganization could precede (dashed line) or succeed (solid line) the proton transfer. In this mechanism, the BH^+ and B moieties, respectively, may be envisioned as specifically solvating the rate-determining activated complex.[12] If the conducted tour of BH^+ among basic sites (or B among acidic sites) is rapid compared to the reorganization process, then the activated complex with the catalyst bound at the most basic (toward BH^+) or acidic (toward B) position will be that which is observed ("solvation rule").[12] On the other hand, if the conducted tour is relatively slow, the catalyst may be trapped at a less favorable position. In such a case, the "anthropomorphic rule" of Reimann and Jencks,[11] which holds that the catalyst will solvate that position which has most *increased* in binding power (acidity or basicity) on going from reactants to transition state, might emerge (coincidentally) as correct. Since this rule demands that the activated complex, anthropomorphically speaking, remember the acidities or basicities of its various binding sites in their previous condition (or incarnation) in the reactant molecule—in order to compare these with their binding powers in the activated complex and thereby direct the catalyst to that position whose attractive power has most considerably increased—it cannot describe a situation in which open, equilibrating paths are open to all activated complexes. However, if some were closed off by a relatively slow rearrangement pathway along the conducted tour, then the rate-determining process might occur before the activated complex could forget what it knew about the reactants. In fact, if diffusional relaxation is, as considered here, relatively slow compared to processes along the main reaction path, it is easy to imagine cases in which the catalyst, far from binding at the most favorable site, will not be interacting favorably at all with the remainder of the activated complex but will be present merely as a trapped spectator, or captive audience at the heavy-atom reorganization. Indeed its presence might be a *destabilizing* factor.

Heavy-Atom Reorganization with Spectator Present. Figure 3 illustrates the way in which B may cause the observed activated complex (for the HAR* step) to be of higher free energy than an unobserved one with B absent. The reaction will not follow the HAR route because it is blocked by the relatively slow diffusion step required to expel B. It is, of course, still correct to think of B as a catalyst because its presence was originally required to tautomerize the tetrahedral intermediate to a reactive form. Interestingly enough, however, B is not required by the observation of a kinetic order in B (and thus the presence of B in the rate-determining activated complex) to be exerting a stabilizing influence (relative to a state from which it is absent) in that activated complex.

(11) J. E. Reimann and W. P. Jencks [*J. Amer. Chem. Soc.*, **88**, 3973 (1966)] have argued that the water-catalyzed (or uncatalyzed) N–O proton transfer in nitrone formation occurs by a "one-encounter" mechanism of this type (involving solvent) or by direct 1,3-migration of the proton.

(12) C. G. Swain, D. A. Kuhn, and R. L. Schowen, *J. Amer. Chem. Soc.*, **87**, 1553 (1965).

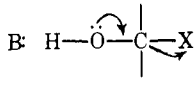
Heavy-Atom Reorganization Concerted with Proton Transfer. Although, as just demonstrated, protolytically catalyzed reactions may involve as a rate-determining process either proton transfer alone, or heavy-atom reorganization with the catalyst as solvator or spectator, it is certainly true that they may also involve concerted proton transfer and heavy-atom reorganization. It is well known that for carbonyl-forming eliminations, two distinct modes of elimination, which might be called β and α, are possible. The carbon–



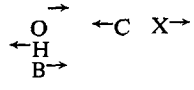
carbon analog of β-carbonyl elimination, in which olefins rather than carbonyl compounds are formed (the E2 reaction), has been the subject of very extensive mechanistic study, particularly with respect to the question of how nearly synchronous are proton transfer and leaving-group departure. Thornton[13] has summarized the evidence and concluded that in fact the two processes are quite closely concerted (for example, primary kinetic isotope effects are observed for both hydrogen and nitrogen or sulfur in alkylammonium and -sulfonium salt eliminations). The complete selectivity–reactivity picture, as well as the isotope-effect data, are rationalized by the reaction coordinate diagram

$$\vec{B}\ \vec{H}\ \vec{C}\ \vec{C}\ \vec{X}$$

The reaction-coordinate motion thus leads to simultaneous C–X cleavage and proton transfer. Thornton, in the same paper,[13] has given a clue to why the same reaction coordinate should not describe the β elimination of carbonyl adducts. The fact that orbital overlap between the incipient p orbitals of the nascent olefin greatly stabilizes the E2 transition state leads to a strong demand for coplanarity of the HCCX chain in these activated complexes. In carbonyl elimination, however, it is likely that greater stabilization can be obtained by overlap with one of the *unbound* lone pairs of the oxygen atom



Translated into geometrical terms, this means that the BH moiety should here be located in a plane perpendicular to that of the OCX chain, and the reaction coordinate would become approximately



so that a bending motion of the proton (rather than a stretch as in the E2 transition state) is now coupled to the CX stretch to form the reaction coordinate.[14] As has been shown elsewhere,[14] such a reaction coordinate for carbonyl elimination will have several observable sequelae: small hydrogen isotope effects (no matter how symmetrically disposed between its two binding partners the proton may be in the activated complex, the isotopic zero-point energy difference associated with

(13) E. R. Thornton, *J. Amer. Chem. Soc.*, **89**, 2915 (1967).
(14) R. L. Schowen, *Progr. Phys. Org. Chem.*, in press.

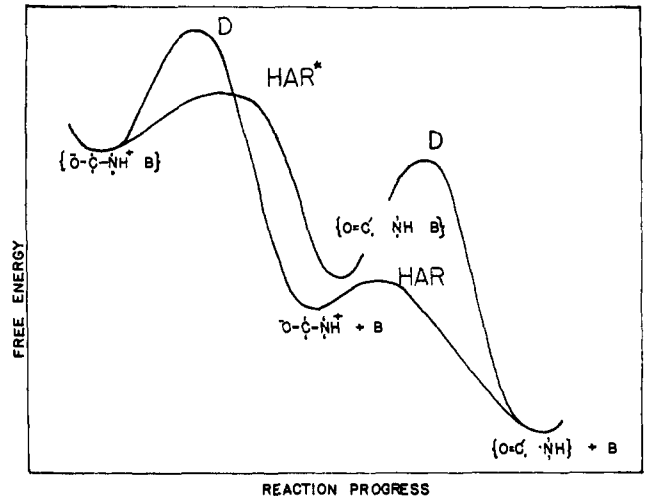
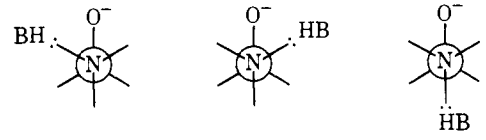


Figure 3. Rate-determining heavy-atom reorganization with B present and *destabilizing* the activated complex (since HAR* → HAR + B is exergonic); the departure of B is shut off by the high diffusion barrier. If the HAR* step is to be rate determining for the overall reaction then the reactant at the left of this figure must be generated in an uphill proton transfer reaction; *i.e.*, the PT* step of Figure 1 must be uphill, rather than downhill as shown there, sufficiently for HAR* to be the highest activated complex along the main reaction route.

its stretching motions can never be entirely lost); relative insensitivity of position of the proton to other structural features of the activated complex and a tendency of the proton to move, with changes in other structural features of the activated complex, in an "anti-Hammond" direction (domination of the usually small "perpendicular" effects[13] on its position between the two ends of the transition state). The importance of this hypothesis for our present discussion is that it seems to us to make truly concerted proton transfer and heavy-atom reorganization in β elimination to form carbonyl groups quite unlikely. As for α elimination, the driving force for coplanarity of any sort is quite absent, as is any constraint on the geometrical relation of the HB to the reorganizing heavy atoms. If a staggered conformation for the activated complex is assumed, the choice among the three possibilities will presumably depend on factors



such as steric or dipolar interactions. In case the trans coplanar arrangement is favored, it is possible but not necessary that an E2 reaction coordinate might result. The other two conformers should have an uncoupled reaction coordinate. Thus one might expect occasionally to find somewhat larger isotope effects for α than for β carbonyl eliminations.[14] It is worth noting that if the three transition-state conformers were of equal stability in light water (thus $k_H = k_1^H + k_2^H + k_3^H = 3k_1^H$) with the gauche conformers giving no isotope effect ($k_1^D = k_1^H$, $k_2^D = k_2^H = k_1^H$) and the anti conformer a very large isotope effect ($k_3^D \sim 0$) for BH *vs.* BD, the reaction would exhibit a solvent isotope effect, k_{H_2O}/k_{D_2O}, of only 1.5 (*i.e.*, $k_H/k_D = 3k_1^H/2k_1^H = 3/2$).

Rate-Determining Diffusion. Of course, if the highest free-energy species along the actual reaction path *is* a diffusion activated complex, then the process will

exhibit the characteristics (β = 0 or 1, no primary isotope effects) outlined above for such reactions. Barnett and Jencks[6] have summarized some cases which appear to fit in this category.

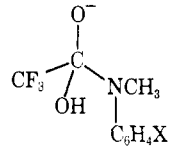
Amide Hydrolysis. The preceding arguments set the stage for an experimental attack on the problem, how synchronous are proton transfer and heavy-atom reorganization in carbonyl elimination? Previous work[2,15,16] on the basic hydrolysis of 2,2,2-trifluoro-*N*-methylacetanilides (eq 4) indicates this reaction to have

$$CF_3CON(CH_3)C_6H_4X + H_2O \xrightarrow{HO^-} CF_3CO_2^- + CH_3NHC_6H_4X \quad (4)$$

some advantages as a field for such an investigation. The reaction in sodium hydroxide solutions is first order in substrate amide and follows the kinetic law of eq 5,

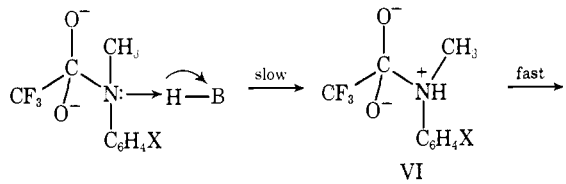
$$k_0 = \frac{k_a(k_1 + k_2[HO^-])[HO^-]}{k_a + k_1 + k_2[HO^-]} \quad (5)$$

where k_0 is the observed first-order rate constant, k_a is the rate constant for addition of hydroxide ion to the carbonyl group to generate the tetrahedral intermediate V, k_1 is the rate constant for conversion of reactants into the activated complex for water-catalyzed decomposition of V to products (*i.e.*, it is equal to the equilibrium constant k_a/k_{-a}, multiplied by k_1', the rate constant for water-catalyzed decomposition of V), and k_2 is the similar



V

rate constant for conversion of reactants into the activated complex for hydroxide-ion-catalyzed decomposition of V. In buffered solutions, general catalysis of the decomposition of V is observed. On the basis of reasonably large solvent isotope effects on k_1 and k_2, we postulated[2] that the rate-determining process for decomposition of the tetrahedral intermediate from the unsubstituted anilide (V, X = H) was simple proton transfer (eq 6) to generate the intermediate VI pre-



VI

products (6)

viously proposed by Bender and Thomas.[8] We also noted that, in the general-catalyzed decomposition of hemiacetals (*e.g.*, the mutarotation of glucose), heavy-atom reorganization with solvating catalyst, rather than proton transfer, appears to be rate determining.[12] The difference may be rationalized[2] on the grounds that ammonium ions (as in a transition state resembling VI) are more stable than amide ions (partial negative charge on nitrogen might arise in a transition state for C–N bond cleavage with solvating BH), while alkoxonium ions (as in the hemiacetal analog of VI) are less stable than alkoxide ions (as may be partially formed in the activated complex for heavy-atom reorganization). The prediction was then made that if sufficiently electron-withdrawing substituents could be introduced into the anilide ring of V (at X), the mechanism should change from proton transfer to heavy-atom reorganization because the stability of an activated complex with partial *negative* charge on N would then exceed that of one with partial positive charge on N. We report here experiments which test this prediction.

(15) S. S. Biechler and R. W. Taft, *J. Amer. Chem. Soc.*, 79, 4927 (1957).

(16) S. O. Eriksson, *Acta Pharm. Suecica*, 6, 139 (a valuable review), 121, 63 (1969), and preceding papers.

Results

Kinetics. The kinetics of the hydrolysis of all *N*-methyltrifluoroacetanilides investigated in this study were found to describe within experimental error the same two-step mechanistic scheme reported earlier for the *N*-methyl-2,2,2-trifluoroacetanilide.[2] The observed first-order rate constant is given by eq 5 in H_2O or its equivalent containing DO^- in D_2O.

The observed rate constants for the hydrolysis of *p*-bromo- and *p*-methyl-*N*-methyltrifluoroacetanilide as a function of lyoxide concentration are presented in Figures 4 and 5. In the high pH region, the observed rate constant approaches a simple first-order dependence on base concentration ($k_0/[LO^-]$ independent of $[LO^-]$) whereas a more complex dependence on lyoxide concentration is found in the lower pH region.

Data Treatment. The rate constants for the indi-

Table I. Rate Constants for the Base-Catalyzed Hydrolysis of *N*-Methyl-2,2,2-trifluoroacetanilides, $XC_6H_4NCH_3COCF_3$, in Protium and Deuterium Oxides at 25.0 ± 0.1° (μ = 0.01 *M*, Added KCl)[a,b]

X	k_a, M^{-1} sec^{-1}	k_1, M^{-1} sec^{-1}	k_2, M^{-2} sec^{-2}
	Protium Oxide Solvent		
m-NO_2	140.0 ± 4.0	25.7 ± 3.30	78100 ± 1130
m-Br	31.3 ± 0.3	6.66 ± 1.89	21700 ± 340
m-Cl	30.2 ± 0.4	6.41 ± 1.39	19400 ± 290
p-Br	25.7 ± 0.3	4.08 ± 0.21	9090 ± 70
p-Cl	22.5 ± 0.5	4.01 ± 1.17	8790 ± 230
m-OCH_3	10.9 ± 0.3	3.33 ± 0.27	4050 ± 39
H	10.9 ± 0.2	2.23 ± 0.32	2500 ± 49
m-CH_3	6.93 ± 0.10	2.21 ± 0.20	2460 ± 43
p-CH_3	5.74 ± 0.06	2.20 ± 0.15	2460 ± 27
p-OCH_3	5.71 ± 0.08	1.90 ± 0.31	2190 ± 32
	Deuterium Oxide Solvent		
m-NO_2	172.0 ± 6.0	14.8 ± 5.56	85200 ± 2800
m-Br	42.7 ± 0.8	3.27 ± 1.82	27100 ± 700
m-Cl	40.9 ± 0.6	2.97 ± 1.73	27000 ± 490
p-Br	32.7 ± 0.5	1.88 ± 1.08	11800 ± 220
p-Cl	28.1 ± 0.5	1.73 ± 1.35	11100 ± 290
m-OCH_3	18.6 ± 0.4	1.23 ± 0.49	3240 ± 67
H	13.7 ± 0.4	0.993 ± 0.574	2060 ± 65
m-CH_3	9.75 ± 0.20	0.785 ± 0.081	1810 ± 19
p-CH_3	7.90 ± 0.10	0.680 ± 0.076	1760 ± 18
p-OCH_3	7.72 ± 0.05	0.605 ± 0.054	1500 ± 6.3

[a] Error limits are standard deviations. [b] These rate constants were determined from 1063 kinetic runs under first-order conditions, of which 903, for lyoxide concentrations from 10^{-3} to 9 × 10^{-3} *M*, were at μ = 0.01 *M* and 160, at higher lyoxide concentrations up to 45 × 10^{-3} *M*, were at μ = 0.05 *M*. Biechler and Taft[15] demonstrated the complete independence of observed rate constants ($[HO^-] = 10^{-3}$–10^{-2} *M*) and ionic strength in this reaction from μ = 0.01 to μ = 0.5 *M*, so that ionic strength differences of 0.01–0.05 *M* will not affect the values tabulated here. A listing of our first-order rate constants is available on request.

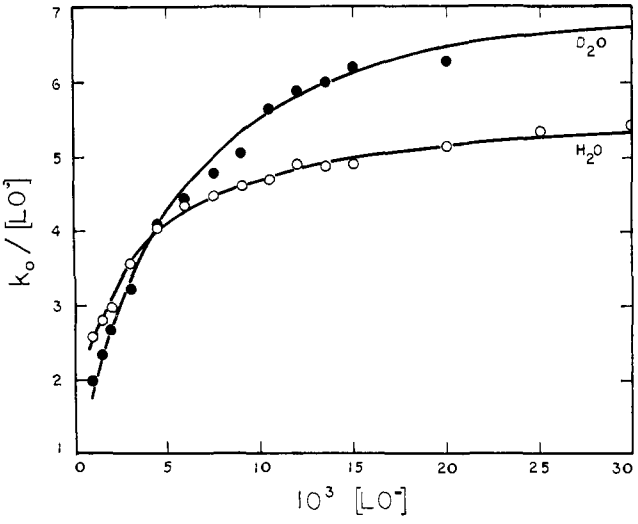


Figure 4. Observed first-order rate constants *vs.* lyoxide concentration for hydrolysis in light and heavy water of a typical electron-donor-substituted compound, p-$CH_3C_6H_4N(CH_3)COCF_3$. The data are plotted to show explicitly the change from first order, to second order, and back to first order in lyoxide ion, as its concentration increases. The solid lines are calculated from eq 5 (or its equivalent for D_2O) and the rate constants of Table I.

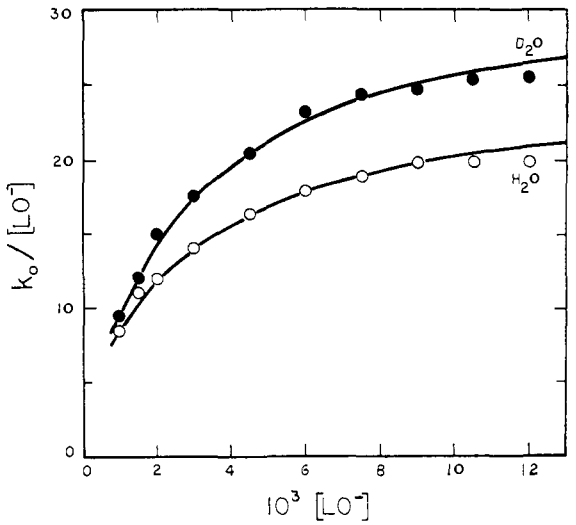


Figure 5. Observed first-order rate constants *vs.* lyoxide concentration for hydrolysis in light and heavy water of a typical electron-acceptor-substituted compound, p-$BrC_6H_4N(CH_3)COCF_3$. The data are plotted to show explicitly the change from first order, to second order, and back to first order in lyoxide ion, as its concentration increases. The solid lines are calculated from eq 5 (or its equivalent for D_2O) and the rate constants of Table I.

vidual processes in the hydrolysis reaction were evaluated in the following way. Equation 5 may be re-

$$\frac{k_0 k_a}{k_a(LO^-) - k_0} = k_1 + k_2(LO^-) \qquad (7)$$

arranged to give eq 7 which with the proper value for k_a and the experimentally determined values for k_0 and (LO^-) generates a straight line of slope k_2 and intercept k_1. An initial approximate value of k_a was obtained in each case from the plot of $k_0/(LO^-)$ *vs.* (LO^-), which gives k_a as a limiting asymptotic value at high base concentration. By using this value of k_a, the corresponding values of k_1 and k_2 and their respective standard deviations were calculated by a linear least squares treatment using an Olivetti Underwood Programma 101 desk computer. Successive approximations were made as to the value of k_a, by addition to or subtraction from the original value in small increments. The best value for k_a was taken as the one which gave the smallest standard deviations in both k_1 and k_2 for the experimental data employed. The rate constants thus obtained are given in Table I.

The kinetic data for each compound were also treated using a nonlinear least squares computer program developed by Mr. Dennis Drake in this laboratory. This program involves the iterative solution of a set of simultaneous equations (normal equations of curve fitting) following Deming,[17] as reviewed by Murr[18] and Buddenbaum,[19] for a case of two variables, k_0 and (LO^-), and three parameters, k_a, k_1, and k_2 (eq 5). It was found that the normal equations of curve fitting, when applied to these data and this function, constituted a system of ill-conditioned simultaneous equations.[20] Ill-conditioned simultaneous equations are ones for which the solutions change drastically with small variations in the coefficients. Such variations can arise in least squares fitting, as here, from the errors present in the physical measurements. Because of this difficulty none of the rate constants given in Table I was determined by this method. It would seem advisable to test for ill-conditioning whenever nonlinear least-squares methods are used for data fitting.

Solvent Isotope Effects. The hydrolysis of trifluoro-*N*-methylacetanilides in protium and deuterium oxide yields the isotope effects given in Table II.

Table II. Solvent Isotope Effects[a] for the Reaction of *N*-Methyltrifluoroacetanilides, $XC_6H_4NCH_3COCF_3$, at 25.0 ± 0.1°

X	$k_a^{D_2O}/k_a^{H_2O}$	$k_1^{H_2O}/k_1^{D_2O}$	$k_2^{H_2O}/k_2^{D_2O}$
m-NO_2	1.23 ± 0.08	1.7 ± 0.4	$(1.09 \pm 0.05)^{-1}$
m-Br	1.36 ± 0.04	2.0 ± 1.7	$(1.25 \pm 0.05)^{-1}$
m-Cl	1.35 ± 0.04	2.2 ± 1.8	$(1.39 \pm 0.05)^{-1}$
p-Br	1.27 ± 0.04	2.2 ± 1.5	$(1.30 \pm 0.03)^{-1}$
p-Cl	1.25 ± 0.05	2.3 ± 1.9	$(1.27 \pm 0.07)^{-1}$
m-OCH_3	1.71 ± 0.09	2.7 ± 1.3	1.25 ± 0.04
H	1.26 ± 0.05	2.3 ± 1.7	1.22 ± 0.06
m-CH_3	1.41 ± 0.06	2.8 ± 0.5	1.36 ± 0.02
p-CH_3	1.38 ± 0.06	3.2 ± 0.6	1.40 ± 0.02
p-OCH_3	1.35 ± 0.03	3.1 ± 0.8	1.46 ± 0.02

[a] Error limits are standard deviations.

Acid–Base Equilibria for *N*-Methylanilines. The ionization constants of the product *N*-methylanilines, determined for use in interpretive structure–reactivity correlations, are presented in Table III. The *N*-methylaniline K_a's fit the σ^- version of the Hammett relationship[21] quite well with a ρ^- value of +3.47 ± 0.04. This compares with ρ = +3.54 for *N*,*N*-dimethyl-

(17) W. E. Deming, "Statistical Adjustment of Data," Wiley, New York, N. Y., 1943.
(18) B. L. Murr, Ph.D. Thesis in Chemistry, Indiana University, 1961.
(19) W. E. Buddenbaum, Ph.D. Thesis in Chemistry, Indiana University, 1965.
(20) S. D. Conte, "Elementary Numerical Analysis," McGraw-Hill, New York, N. Y., 1965, pp 163–168.
(21) H. H. Jaffé, *Chem. Rev.*, 53, 191 (1953).

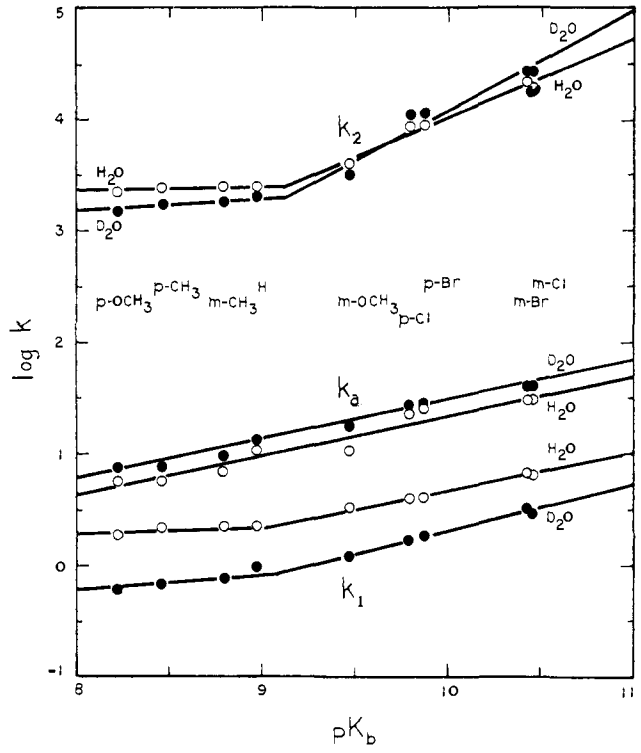


Figure 6. Free energy relations (log k *vs.* pK_b of the leaving-group amine) for the three activation processes of amide hydrolysis.

aniline[22] and $\rho = +2.77$ for aniline[21] K_a's in aqueous solution.

Table III. Basicity of *N*-Methylanilines, $XC_6H_4NCH_3$, in Water[a,b]

X	10^5K_a,[c] M
p-NO_2	29,350 ± 1710
m-NO_2	327 ± 27
m-Br	26.6 ± 1.4
m-Cl	28.9 ± 0.9
p-Br	7.38 ± 0.28
p-Cl	6.17 ± 0.23
m-OCH_3	2.94 ± 0.11
H	0.924 ± 0.036
m-CH_3	0.619 ± 0.019
p-CH_3	0.287 ± 0.010
p-OCH_3	0.167 ± 0.012

[a] K_a is defined as the equilibrium constant for the reaction $ArNH_2CH_3^+ \rightleftarrows ArNHCH_3 + H^+$. Molarity ($M$) units were used to calculate K_a. [b] All acidity constants were determined at 25.0 ± 0.10° and at a constant ionic strength of 0.05 M (added KCl). [c] The acidity constants are the arithmetic mean of from four to six individual determinations of the value. The error limits are average deviations from the mean.

Discussion

The data just presented consist of substituent effects and isotope effects (as a function of substituent) for the rate processes of eq 8–10. Thus we are able to proceed

$$CF_3CON(CH_3)Ar + HO^- \xrightarrow{k_a} \text{TSA} \quad (8)$$

$$CF_3CON(CH_3)Ar + HO^- + H_2O \xrightarrow{k_1} TS1 \quad (9)$$

$$CF_3CON(CH_3)Ar + 2HO^- \xrightarrow{k_2} TS2 \quad (10)$$

toward characterization of three transition states, TSA (for addition to form the tetrahedral intermediate V), TS1 (for water-catalyzed conversion of V to products), and TS2 (for hydroxide ion catalyzed conversion of V to products.

Substituent and Isotope Effects on Addition. In Figure 6 are plotted, along with other data, log k_a for $CF_3CON(CH_3)C_6H_4X$ in light and heavy water *vs.* pK_b of $CH_3NHC_6H_5X$ (in light water). The lines are straight with slopes both equal to 0.34; this corresponds to a ρ value (the plots *vs.* σ are equally straight) of 1.18 (1.0 was obtained for addition of hydroxide ion to para-substituted acetanilides by Bender and Thomas[8]). This is as expected for the rate process of eq 8 and shows that the increase of electron density in the anilide portion of the molecule on formation of TSA is about 35% of that when the *N*-methylanilinium ion is neutralized (eq 11).[23]

$$CH_3NH_2C_6H_4X^+ + HO^- \longrightarrow CH_3NHC_6H_4X + H_2O \quad (11)$$

From Figure 6 and Table II we note that all the additions occur more rapidly in deuterium oxide than in protium oxide by factors of 1.2–1.7. This also is expected;[14] processes in which hydroxide ion is bound to a substrate will generally proceed more rapidly in heavy water by a factor of 2 if binding is complete, and by a factor of 2^x if the new bond to hydroxide has order x. The variation in $k_a^{D_2O}/k_a^{H_2O}$ with substituent does not present a clear picture and may simply reflect the variety of factors contributing to the detailed values of solvent isotope effects for carbonyl addition.[24]

Substituent Effects on Formation of TS1 and TS2. Change in Mechanism. Far from showing the linearity of the free energy relation for addition, both plots for formation of the elimination transition states TS1 and TS2 show dramatic breaks at a leaving group p$K_b \sim 9$, with practically no substituent effect for electron donors and a large acceleration by electron acceptors. The slopes and equivalent Hammett ρ values are collected in Table IV. Such a sharp break

Table IV. Slopes of Plots of Log k_1 and Log k_2 *vs.* pK_b of Leaving Group with Equivalent Hammett ρ Values in Parentheses

	pK_b < 9	pK_b > 9
$k_1^{H_2O}$	0.02 (0.07)	0.3 (1.1)
$k_1^{D_2O}$	0.08 (0.3)	0.4 (1.4)
$k_2^{H_2O}$	0.09 (0.3)	0.7 (2.4)
$k_2^{D_2O}$	0.09 (0.3)	0.9 (3.1)

in the free energy relation, with a larger slope for the more reactive substrates, is an excellent indication of a change in mechanism.[25] Furthermore the change must be from an activated complex having a charge density near the substituent little different from that in the reactant (pK_b < 9, slopes near zero) to one in which the net negative charge density has increased by

(22) A. V. Willi, *Helv. Chim. Acta*, **40**, 2019 (1957).

(23) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1962, p 156 ff.

(24) S. L. Johnson, *Advan. Phys. Org. Chem.*, **5**, 237 (1967).

(25) See ref 23, p 187 ff.

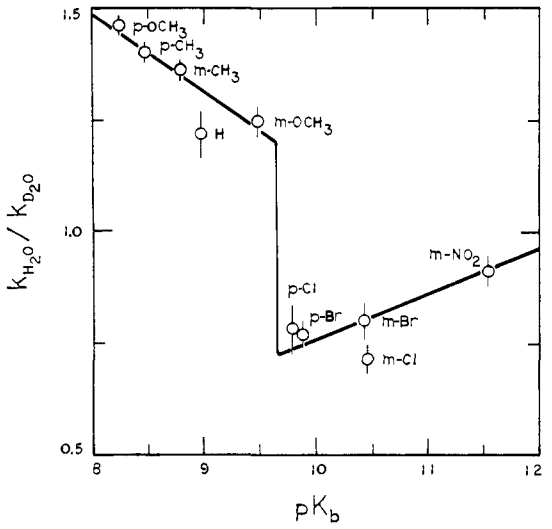


Figure 7. Solvent isotope effects on the base-catalyzed breakdown of the tetrahedral intermediate, as a function of leaving group pK_b.

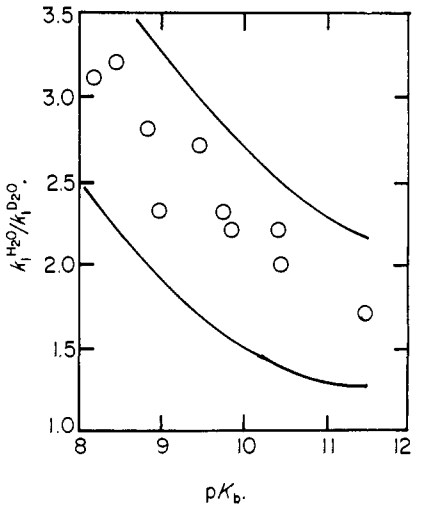
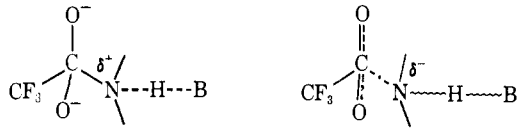


Figure 8. Solvent isotope effects on the water-catalyzed breakdown of the tetrahedral intermediate, as a function of leaving group pK_b. The circles show "best" values, while the lines give the approximate loci of maximum and minimum values.

30% (k_1) to 70% (k_2) of the increase on neutralization of *N*-methylanilinium ions (slopes of 0.3 and 0.7 in H_2O). Our current hypothesis is that the two activated complexes are those (i) for simple proton transfer to nitrogen from a general base (VII), which is of lower energy with electron donors in the leaving group ($pK_b < 9$), and (ii) for heavy-atom reorganization with solvating catalyst (VIII), with proton transfer being completed in a subsequent step, which is of lower energy with electron acceptors in the leaving group ($pK_b > 9$). Further support of this view is given below, but we can



first rule out two alternative hypotheses on the basis of data not included here. First, the reaction continues to show superimposed general base catalysis[26] with electron acceptors in the leaving group,[27] which shows that the larger slope at $pK_b > 9$ does not arise from an uncatalyzed expulsion of anilide ion from the oxydianion shown in eq 6. Second the basic methanolysis of these substrates exhibits the same break in the structure–reactivity plot,[28] ruling out the possibility that N protonation of the oxydianion ($pK_b < 9$) is giving way to O–H proton abstraction from the tetrahedral intermediate ($pK_b > 9$) with concerted C–N bond cleavage in both cases. In the basic methanolysis reaction, the OH group of the tetrahedral intermediate is replaced by OCH_3.

Isotope Effects on the Formation of TS2. The isotope effect results for k_2 are far more precise than are those for k_1 and are therefore taken up first. Figure 7 shows the dependence of $k_2^{H_2O}/k_2^{D_2O}$ on pK_b of the leaving group. It can be seen at a glance (*cf.* Table II) that the isotope effect plummets from normal values (1.2–1.5 times faster in H_2O) to inverse values (1.1–1.4 times faster in D_2O) at the same point (pK_b of 9–10) where the break occurs in the linear free energy relation of Figure 6. Clearly the transition state with relative positive charge on nitrogen ($pK < 9$) gives normal isotope effects, while that with negative charge on nitrogen ($pK > 9$) gives inverse effects. This is fully consistent with structures VII (proton undergoing transfer, CN bond intact) and VIII (solvating catalyst, CN bond breaking) with B = HO^- for TS2.

The magnitude of the effects involved becomes still more impressive when the fact is considered that two hydroxide ions are being bound in the formation of TS2 from reactants. Complete binding of each would lead to an inverse secondary isotope effect of 2, giving 4 for the maximum effect if both hydroxides were fully bound.[14] In fact, one *is* fully bound at the tetrahedral intermediate stage, while another is presumably partially bound in continuing on to TS2. Thus the secondary contribution to the observed solvent isotope effect on k_2 will be between 2 and 4 (k_D/k_H). The observed effect will presumably be the product of this secondary contribution and a possible primary effect for proton transfer (eq 12). Thus the primary effects in TS2 are

$$(k_{H_2O}/k_{D_2O})_{obsd} = (k_H/k_D)_{sec}(k_H/k_D)_{prl} \qquad (12)$$

$(k_H/k_D)_{prl}$ = 2.4–6 ($pK_b < 9$) and 1.4–3.6 ($pK_b > 9$). The former are quite consistent with rate-determining proton transfer and the latter with solvation catalysis. A complete numerical analysis of these effects will be available elsewhere.[14]

Isotope Effects on the Formation of TS1. Figure 8 indicates that the same falloff from larger to smaller isotope effects on going to less basic leaving groups is observed on the k_1 process. All isotope effects here are normal, as might be expected from the fact that one hydroxide ion and (presumably) one water molecule are bound in the formation of TS1. The limiting structures in terms of secondary contributions to the observed solvent isotope effects are IX and X, which would produce[14] $(k_H/k_D)_{sec}$ of 1/2 and 3.4/2 = 1.7, respectively. Thus the observed effects of k_{H_2O}/k_{D_2O} about 3 ($pK_b < 9$) and about 2 ($pK_b > 9$) correspond to

(26) R. L. Schowen and G. W. Zuorick, *J. Amer. Chem. Soc.*, **88**, 1223 (1966).
(27) D. Drake, Ph.D. Thesis in Chemistry, University of Kansas, 1970.
(28) C. R. Hopper and C. Bazikian, unpublished work.

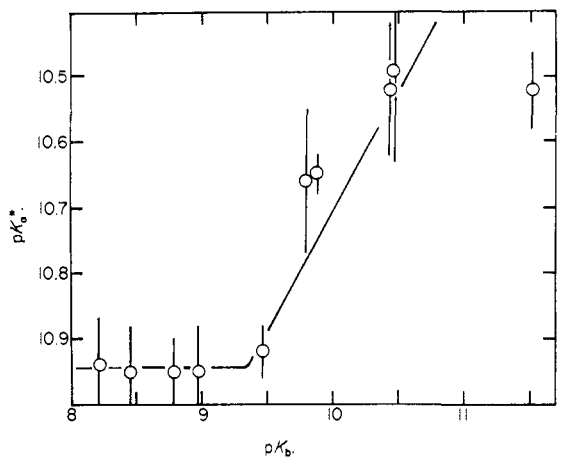
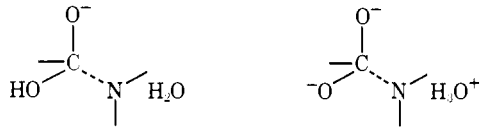


Figure 9. Acidity of the TS1 transition state as a function of leaving group pK_b.

primary contributions in TS1 of 1.8–6 ($pK_b < 9$) and 1.2–4 ($pK_b > 9$). Neither of these results raises strong questions about structures VII and VIII with B = H_2O for TS1.



IX X

Acid Dissociation of TS1 to TS2. According to Kurz's excellent theory of transition-state acidities,[29] we can envision the dissociation of TS1 (VII or VIII, B = H_2O) to give a proton and TS2 (VII or VIII, B = HO^-) as in eq 13. The K_a*'s are given by eq 14 (K_w is

$$(TS1)^- \rightleftharpoons (TS2)^{2-} + H^+ \quad (13)$$

$$K_a^* = (k_2/k_1)K_w \quad (14)$$

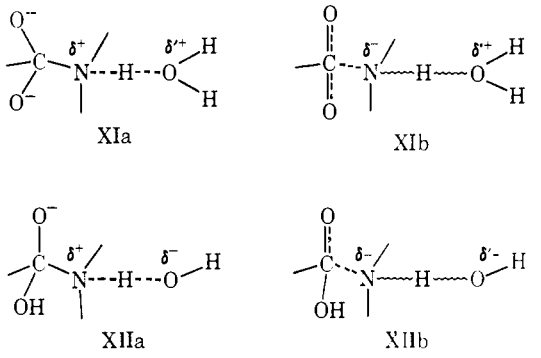
the autoprotolysis constant of water) and are shown in Table V and Figure 9 as a function of leaving group

Table V.[a] Acid Dissociation Constants in Protium Oxide and Acidity Isotope Effects for Transition States TS1, Derived from $XC_6H_4N(CH_3)COCF_3$, at 25°

X	pK_a^* (H_2O)	$K_b^*(D_2O)/K_b^*(H_2O)$
m-NO_2	10.52 ± 0.06	1.9 ± 0.5
m-Br	10.49 ± 0.14	2.5 ± 2.3
m-Cl	10.52 ± 0.10	3.1 ± 2.7
p-Br	10.65 ± 0.03	2.9 ± 2.1
p-Cl	10.66 ± 0.11	2.9 ± 2.6
m-OCH_3	10.92 ± 0.04	2.2 ± 1.1
H	10.95 ± 0.07	1.9 ± 1.5
m-CH_3	10.95 ± 0.05	2.0 ± 0.4
p-CH_3	10.95 ± 0.07	2.3 ± 0.5
p-OCH_3	10.94 ± 0.07	2.1 ± 0.6

[a] K_a^* is defined by eq 13; K_b^* is the equilibrium constant for the reaction $LO^- + (TS1)^- \rightarrow L_2O + (TS2)^{2-}$ in L_2O.

pK_b. TS1 is obviously an acid of about the strength of a phenol; but its acidity exhibits a remarkable dependence on its structure. For $pK_b < 9$, pK_a^* is essentially independent of structure in the aniline ring, while for $pK_b > 9$, the acidity of TS1 rises sharply (the slope of pK_a^* *vs.* pK_b is about 0.4; since ρ^- for pK_b is 3.47, as shown above, ρ^- for pK_a^* is 1.3). These results may be used to differentiate between two possible structures for TS1, XI, and XII, the a structures corresponding to $pK_b < 9$, the b structures to $pK_b > 9$.



XIa XIb XIIa XIIb

Structures XI nicely explain the phenomena of Figure 9. In XIa, electron withdrawal at N has two opposing effects: (1) it increases the acidity of the OH_2 protons in the usual way, and (2) it changes the geometry of the transition state by a "parallel effect,"[13] bringing the transferring proton closer to the more weakly basic N, thus decreasing the positive charge δ' on O and decreasing the acidity of the OH_2 protons. A rough balance of the two effects explains the observed $\rho = 0$. In XIb, on the other hand, the "parallel effect" shortens the CN bond, making the N again less basic. But now the NH bond is a "solvation bond"[12] and will become longer as *N* becomes less basic. Thus δ'^+ is increased and the acidity increased by the geometric changes as well as by the normal effect of electron withdrawal. The result of the positive superposition is $\rho = 1.3$.

For structure XII, the predictions are not in accord with the results. For XIIa, electron withdrawal should shorten NH, increasing δ^+ and thus the acidity of OH, the effect of geometric change thus adding to the normal electronic effect, although both should be attenuated by the insulating carbon. In XIIb, the CN distance should again be decreased by electron withdrawal, making OH more alcohol-like and less carboxylic acid-like and thus less acidic, in opposition to the usual effect. Thus the results of Figure 9 argue strongly for structure XI. It must, however, be kept in mind that the O–N proton transfer may be mediated by a chain of several molecules of water or by a single bridging water molecule,[11] in which case XI and XII are merely limiting forms of the same structural type.

Table V also shows the isotope effects on the dissociation of TS1, although the poor precision of k_1 in both isotope solvents makes the values quite uncertain. If we make the (probably bad) assumption that the primary contributions to TS1 and to TS2 are about equal, we can interpret these effects as simple secondary ones. The limiting structures for the dissociating portion of structures XI are H_3O^+, for a reactant-like transition state, and H_2O, for a product-like transition state (*i.e.*, with the proton transferred not at all, and completely, respectively). The isotope effect for neutralization of H_3O^+ by HO^- should equal the inverse of that for autoprotolysis of water,[30] or k_D/k_H about 7.

(29) J. L. Kurz, *J. Amer. Chem. Soc.*, **85**, 987 (1963).

(30) (a) A. K. Covington, R. A. Robinson, and R. G. Bates, *J. Phys. Chem.*, **70**, 3820 (1966); (b) V. Gold and B. M. Lowe, *J. Chem. Soc. A*, 936 (1967); (c) L. Pentz and E. R. Thornton, *J. Amer. Chem. Soc.*, **89**,

No isotope effect (k_H/k_D = 1) should result from reaction of HO^- with H_2O to give H_2O and HO^-. If we call the charge on the water oxygen of TS1 x, then the observed acidity isotope effect should be about 7*. The observed values are in the range 2–3, yielding x about 0.4–0.6, consistent with a proton "40–60% transferred." This term is to be understood in the sense of the solvation rule[12] for structure XIb: that is, the bond is loosened to this extent by hydrogen bonding, although the proton is not actually participating in the reaction coordinate and will presumably not be fully bound to nitrogen in the first stable structure following the transition state. Extent of proton transfer in stable hydrogen-bonded complexes has been defined and measured by the Arnett–Schleyer–Taft consortium.[31a]

The value of pK_a* may be used to find an independent value of x. The pK_a of an H_3O^+-containing transition state (x = 1) should be −1.74, while the pK_a of an H_2O-containing transition state[31b] (x = 0) should be 15.74. The observed values of 10.5–11 thus correspond to x = 0.25–0.30, or to about 70–75% proton transfer. In view of the errors involved in the isotope effects, this value is to be preferred.

Valence-Isomeric Transition States. The net picture that emerges for the catalytic breakdown to products of the tetrahedral intermediate in amide hydrolysis is that two competitive pathways exist, *via* transition states VII and VIII. These structures are *valence isomers* in the sense that their rough or gross geometrical characters are probably similar, but their electron distribution (or the relative strengths of various bonds) differ. "Good" leaving groups, which favor the development of electron density, lead to a lower energy for structure VIII than for VII and thus this pathway, with heavy-atom reorganization dominating the reaction coordinate and the catalyst playing a solvating role, is observed. The class of good leaving groups will usually include oxygen functions. "Poor" leaving groups, which prefer positive charge development, favor VII, with proton transfer as the dominant reaction-coordinate contributor.

Experimental Section

Materials. *m*-Bromoaniline (J. T. Baker Chem. Co., Baker Grade), *p*-bromoaniline (Eastman White Label), *m*-chloroaniline (Matheson Coleman and Bell), *p*-chloroaniline (Matheson Coleman and Bell, Practical Grade), *m*-methoxyaniline (Aldrich Chemical Co.), *p*-methoxyaniline (Eastman White Label), and *m*-nitroaniline (unknown source, mp 112.5–113.5°, lit.[32] mp 111.8°) were used in the procedure described below for the preparation of *N*-methylanilines.

***N*-Methylanilines.** The anilines were converted to the acetanilides by the Lumiere-Barbier method.[33] The appropriate aniline was transformed into the aniline hydrochloride by reaction with hydrochloric acid in dilute aqueous solution. The amine hydrochloride was purified by heating with decolorizing charcoal (Nuchar-C190-N, Fisher Scientific Co.) and the solution was filtered. To the resulting clear aniline hydrochloride solution acetic anhydride (J. T. Baker Chemical Co.) was added followed immediately by an aqueous sodium acetate (J. T. Baker Chemical Co.) solution. After cooling, the mixture was filtered and the resulting solid acetanilide was recrystallized from an appropriate solvent.

The *N*-methylacetanilides were obtained from the corresponding acetanilides by the method of Patcher and Kloetzel.[34] An excess of powdered potassium hydroxide (J. T. Baker Chemical Co., Analyzed Reagent Grade) was added to a solution of the acetanilide in warm acetone (J. T. Baker Chemical Co.). Methyl iodide (Aldrich Chemical Co.) in an equal volume of acetone was added dropwise to the refluxing mixture. The hot solution was filtered, the solvent was removed under reduced pressure, and the resulting aryl-substituted *N*-methylacetanilide was purified by recrystallization from a suitable solvent or by vacuum distillation.

The *N*-methylacetanilides were hydrolyzed to the desired *N*-methylanilines by refluxing for a period of 2–6 hr in a 15% aqueous ethanol solution to which an excess of potassium hydroxide was added. After hydrolysis, the solution was diluted with water and extracted several times with ether. The ether was removed under vacuum and the amine obtained was purified by vacuum distillation or by crystallization. For $XC_6H_4NHCH_3$ (X, mp or bp, $n^{20}D$): *m*-NO_2, mp 66–67° (lit.[35] mp 67.5°); *m*-Br, bp 82° (0.3 mm), 1.6070; *m*-Cl, bp 82° (0.5 mm) (lit.[36] bp 234.5–235.5° (764 mm)), 1.5832); *p*-Br, bp 150° (20 mm), 1.6119; *p*-Cl, bp 68° (0.3 mm) (lit.[37] bp 242° (768 mm)), 1.5823; *m*-CH_3O, bp 94° (0.6 mm) (lit.[38] bp 131° (17 mm)), 1.5673; *p*-CH_3O, bp 85° (1.0 mm) (lit.[39] bp 111–113° (9 mm)), mp 34–36° (lit.[40] mp 37°).

***N*-Methyl-2,2,2-trifluoroacetanilides.** The *N*-methylanilines obtained above and *N*-methyl-*p*-nitroaniline (Eastman White Label), *N*-methylaniline (Matheson Coleman and Bell), *m*-methyl-*N*-methylaniline (Eastman White Label), and *p*-methyl-*N*-methylaniline (Eastman White Label) were treated with trifluoroacetic anhydride according to the procedure of Bourne, Henry, Tatlow, and Tatlow.[41] The appropriate *N*-methylaniline was dissolved in dry ether and cooled to 0°. A slight molar excess of trifluoroacetic anhydride (Pierce Chemical Co.) was added slowly. The rate of addition was such that the temperature of the reacting solution remained below 5°. After the solution had warmed to room temperature, the solvent was removed under diminished pressure and ice–water was added to the residue. The precipitated *N*-methyl-2,2,2-trifluoroacetanilide was collected and recrystallized or distilled under vacuum. For $XC_6H_4NCH_3COCF_3$ (X, mp): *p*-NO_2, 126.5–128°; *m*-NO_2, 70.5–72.5°; *m*-Br, 23.5–25°; *m*-Cl, 28.5–30°; *p*-Br, 63.5–65°; *p*-Cl, 66–68°; *m*-CH_3O, 47–48°; H, 26–28° (lit.[15] mp 25–26°); *m*-CH_3, 20.5–22.5°; *p*-CH_3, 30.5–32°; *p*-CH_3O, 42–43.8°.

Kinetic Determinations. Hydrolysis reaction rates were determined spectrophotometrically using a Cary Model 14 or Cary Model 16 spectrophotometer. All base and substrate solutions

Table VI. Ultraviolet Spectral Data for *N*-Methylanilines in Aqueous Solution[a]

X	λ_{max}, nm	ϵ^b	λ_{max}, nm	ϵ^b
p-NO_2	230.5	14,300	408.0	18,200
m-NO_2	239.0	15,600		
m-Br	245.0	9,700	293.0	2,250
m-Cl	243.5	9,600	292.0	1,830
p-Br	247.0	13,100	294.0	1,550
p-Cl	246.0	12,600	295.0	1,620
m-OCH_3	241.0	11,200	286.0	2,320
p-H	238.0	10,000	285.0	1,590
m-CH_3	239.0	9,990	286.0	1,620
p-CH_3	240.0	10,800	290.5	1,690
p-OCH_3	237.0	10,400	299.0	2,040

[a] Determined in 0.05 *M* aqueous sodium hydroxide solution at 25.0 ± 0.1°. [b] Molar absorptivity, cm^{-1} M^{-1}.

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were thermostated, prior to use, in a constant-temperature bath. A 1.50-ml aliquot of the appropriate base solution was transferred to the reaction cell (Pyrocell Manufacturing Co.) in the thermostated cell jacket of the spectrophotometer and allowed to equilibrate thermally for at least 3 min. The reaction was then initiated by rapid injection of 1.00 ml of the substrate solution into the cell from a thermostated syringe. The increase of ultraviolet absorption due to formation of the *N*-methylaniline product ($XC_6H_4NHCH_3$) *vs.* time was monitored at these wavelengths (X, λ in mμ): *m*-NO_2, 240; *m*-Br, 245; *m*-Cl, 243.5; *p*-Br, 247; *p*-Cl, 246.5; *m*-CH_3O, 239; H, 238; *m*-CH_3, 242; *p*-CH_3, 238; *p*-CH_3O, 300. For all cases except X = *p*-OCH_3, the substrates have no absorption maxima in the 200–320-mμ range and show only a small absorption at the wavelength used for kinetic studies. For X = *p*-OCH_3, there was an interfering substrate absorption maximum at 224 mμ, and therefore the much weaker absorption maximum of the product at 300 mμ was used. The base concentration was in large excess over the substrate concentration so that pseudo-first-order kinetics was observed in all cases. The observed first-order rate constants were calculated from the kinetic data by a linear least-squares treatment of the integrated first-order rate law using a GE 625 computer. Reactions in deuterium oxide were carried out with use of a drybox and serum-capped vessels.

***N*-Methylaniline Acidities.** The pK_a values were determined in aqueous solution by the spectrophotometric method of Willi[22] using a Cary Model 16 spectrophotometer. All measurements were carried out at 25.0 ± 0.1° and at a constant ionic strength of 0.05 *M* maintained with added potassium chloride. Sodium acetate–acetic acid (Fisher Scientific Co., Reagent Grade) buffers and dilute hydrochloric acid solutions were used for the control of pH. Spectral data obtained during the work are given in Table VI.

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Studies of the Chymotrypsinogen Family of Proteins. XI. Heat-Capacity Changes Accompanying Reversible Thermal Unfolding of Proteins[1,2a]

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Abstract: The reversible thermal unfolding processes (transition I) of chymotrypsinogen A, dimethionine sulfoxide chymotrypsinogen A, diphenylcarbamyl-α-chymotrypsin, and ribonuclease A have been quantitatively characterized in the pH interval 2–3.5 using a spectrophotometric van't Hoff method. An attempt is made to evaluate baseline errors and the form of the relationship between heat-capacity change and temperature. Comparisons of results with those obtained using calorimetric data indicate that baseline errors have been minimized. It is found that although the heat-capacity change within error is independent of temperature, the errors are so large as to exclude the use of van't Hoff data in establishing the form of the temperature dependence. Nevertheless an analysis of the data based on temperature-independent heat-capacity changes provides values for the enthalpy change and the average heat-capacity change in good agreement with those obtained calorimetrically. The results are qualitatively consistent with the analysis given by Brandts[3,4] but demonstrate the need for a more accurate heat-capacity expression.

The first reversible thermal unfolding transition, transition I, of globular proteins takes them from the most completely folded state, state A, to the first fully or partially unfolded state, state B.[5] Large, positive, heat-capacity changes accompanying transition I were first reported by Brandts[6,7] in studies of chymotrypsinogen A (CGN) and ribonuclease A (RNase). Similar observations have been reported for α-chymotrypsin and its mono- and dimethionine sulfoxide derivatives by Biltonen and Lumry[8,9] and for myoglobin by Hermans and Acampora.[10] Danforth, *et al.*,[11] and Tsong, *et al.*,[12] measured ΔC_p° for ribonuclease calorimetrically and found it to be about 2.0 kcal/mol deg in agreement with Brandts' van't Hoff value.[7] Pace and Tanford[13] investigated β-lactoglobulin A in aqueous urea solutions and found ΔC_p° = 2.1 kcal/mol deg. More recently Schwartz, Wadsö, and Biltonen[14] have measured ΔC_p° calorimetrically at several temperatures using proteins of the chymotrypsinogen A family. Large positive ΔC_p° values were also observed. Such ΔC_p° values appear to be a general characteristic of unfolding transitions and have been attributed by Brandts to changes in the interaction of nonpolar polypeptide side chains with water. As a result of the magnitude of ΔC_p° van't Hoff plots at lower temperatures are very curved. In order to analyze these plots Brandts gave a provisional expres-

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