Transition State Characterization for Catalyzed Reactions

By Joseph L. Kurz

Equations are presented which allow the calculation, from experimental data, of the virtual equilibrium constant for dissociation of the catalyst from the transition state of a catalyzed reaction. These constants are shown to have wide applicability in the elucidation of transition state structures and of reaction mechanisms. Applications to acid–base catalysis are used as illustrations, and conclusions are drawn concerning the mechanisms of alkyl fluoride hydrolysis, β-diketone cleavage, proton transfer reactions and the enolization of ketones.

Introduction

A central objective in a study of the mechanism of any reaction is the determination of the structure of the transition state. There is much current activity being directed toward elucidation of the fine details of such structure. Recent examples are the work of Swain and Thornton[1] based on kinetic isotope effects and that of Robertson and co-workers[2] based on the determination of absolute thermodynamic parameters for transition states.

One fruitful approach to this problem appears to remain almost entirely unexploited. For many catalyzed reactions, it is possible to calculate the virtual equilibrium constant for the dissociation of the catalyst from the transition state, using experimental data. Since the possibility should have become obvious when the transition state theory was first formulated in terms of the thermodynamics of the activation process,[3] its long neglect is somewhat surprising. Some workers[4,5] have pointed out the utility of considering specific salt effects in terms of the formation constant of the complex between the transition state and one of the added ions, and Davies and Williams[6] have actually tabulated numbers which may be interpreted as being such constants. Quite recently, Hine has used a similar qualitative approach to discuss amide hydrolysis,[7,8] and has gone on to develop some quantitative relationships between the rates of corresponding acid-, base- and uncatalyzed reactions. The rate constant ratio discussed by Hine and Bayer[8] is identical with that which is shown to be equal to $K_a{}^{\ddagger}$ in the argument presented below.

Qualitatively, this approach to catalysis emphasizes the view that a catalyst accelerates a reaction by lowering the free energy of the transition state relative to that of the initial state as a result of its forming a more stable complex with the transition state. This approach becomes quantitatively useful whenever it is possible to measure the rates of two analogous reactions which differ only in whether a particular "extra" reactant (or catalyst) is present in the transition state. Although the most immediately apparent applications are to various types of catalysis, other reactions not usually grouped under this heading may also be treated (e.g., Sn2 attack of a nucleophile as compared to Sn1 solvolysis). Once the pertinent dissociation constant has been calculated, it may be used to characterize the structure of the transition state in a manner analogous to the use of similar data for stable compounds. Particularly for protonic equilibria does a large literature[9] exist concerning the correlation of such constants with molecular structure.
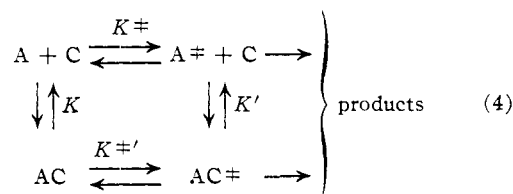
In this paper equations are derived and applied to examples of acid–base catalysis. Additional applications will be treated in future publications.

Theoretical Basis

Basic Equations.—Many catalyzed reactions appear to take place through a prior equilibrium association of the substrate, A, with a catalyst, C (eq. 1), followed by a rate-determining reaction of the catalyst–substrate complex (eq. 2). An analogous uncatalyzed reaction (eq. 3) is always possible in principle and often observable in fact.

$$A + C \xrightleftharpoons{K} AC \qquad (1)$$

$$AC \xrightarrow{k_c} \text{products} \qquad (2)$$

$$A \xrightarrow{k_u} \text{products} \qquad (3)$$
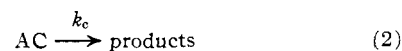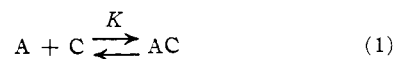
In terms of the equilibrium constants for the formation of the transition states and the complexes, this mechanism may be reformulated as in eq. 4. Here, $K^{\ddagger}$ and $K^{\ddagger\prime}$ are the equilibrium constants for the formation of the uncatalyzed and catalyzed transition

$$
\left.
\begin{array}{ccc}
A + C & \xrightleftharpoons{K^{\ddagger}} & A^{\ddagger} + C \longrightarrow \\[2pt]
\updownarrow K & & \updownarrow K' \\[2pt]
AC & \xrightleftharpoons{K^{\ddagger\prime}} & AC^{\ddagger} \longrightarrow
\end{array}
\right\} \text{products} \qquad (4)
$$

states from the corresponding reactants, while K and K' are the equilibrium constants for the association of the initial and transition states with the catalyst. Although true equilibrium exists between the transition and initial states only when the entire system is at equilibrium, the concentration of transition states which have been formed from reactants rather than products should always be at its equilibrium value with respect to the concentration of reactants[3] (at least in the liquid phase[10]). This, together with the assumption that A and AC are in equilibrium, requires that $AC^{\ddagger}$ be at its equilibrium concentration with respect to $A^{\ddagger}$. There is, of course, no direct dynamic equilibrium between these transition states.

From the transition state theory,[3] for reactions whose transmission coefficients are near unity, one may write for the catalyzed and uncatalyzed rate constants, respectively

$$k_u = (kT/h)K^{\ddagger}; \quad k_c = (kT/h)K^{\ddagger\prime}$$

(1) C. G. Swain and E. R. Thornton, *J. Am. Chem. Soc.*, **84**, 817 (1962); *Tetrahedron Letters*, **6**, 211 (1961).

(2) R. E. Robertson, R. L. Heppolette and J. M. W. Scott, *Can. J. Chem.*, **37**, 803 (1959).

(3) W. F. K. Wynn-Jones and H. Eyring, *J. Chem. Phys.*, **3**, 492 (1935).

(4) R. P. Bell, "The Proton in Chemistry," Cornell Univ. Press, Ithaca, N. Y., 1959, p. 127.

(5) J. L. Kurz and C. D. Gutsche, *J. Am. Chem. Soc.*, **82**, 2175 (1960).

(6) C. W. Davies and I. W. Williams, *Trans. Faraday Soc.*, **54**, 1547 (1958).

(7) J. Hine, "Physical Organic Chemistry," 2nd Ed., McGraw–Hill Book Co., Inc., New York, N. Y., 1962, pp. 111–112.

(8) J. Hine and R. P. Bayer, *J. Am. Chem. Soc.*, **84**, 1989 (1962).

(9) See, for example, H. C. Brown, D. H. McDaniel and O. Häfliger in E. A. Braude and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955, pp. 567–662.

(10) See, for example, S. W. Benson, "The Foundations of Chemical Kinetics," McGraw–Hill Book Co., Inc., New York, N. Y., 1960, p. 502.

But $K^{\ddagger\prime} = K^{\ddagger}K'/K$, so that

$$k_c = k_u K'/K \tag{5}$$

The mechanism under consideration would give rise to a linear empirical rate law

$$V = (k_1 + k_2[C])[A] \tag{6}$$

In terms of the mechanistic rate constants, this may be written as

$$V = (k_u + k_c K[C])[A] \tag{7}$$

which is transformed into eq. 8 when combined with eq. 5.

$$V = k_u(1 + K'[C])(A) \tag{8}$$

The negative logarithm of the virtual dissociation constant for the transition state–catalyst complex thus becomes, in terms of the empirical rate constants

$$pK_d^{\ddagger} = \log (k_2/k_1) \tag{9}$$

It is thus seen that the desired equilibrium constant is determined by two measured parameters instead of the three generally required for the indirect measurement of an equilibrium constant in a square array such as eq. 4. Since K appears in the rate equation only as a multiplier of k_c, the square array is equivalent to a triangular array which does not include the prior equilibrium. As a result, eq. 9 is also valid when applied to reactions in which no intermediate AC complex is formed and the rate-determining step involves reaction of catalyst with unbound substrate.

If the rate of the uncatalyzed reaction cannot be obtained, then the value of $pK_d^{\ddagger}$ cannot be calculated. However, if more than one catalyst is known, the equilibrium constant for replacement of one catalyst in the transition state by another (i.e., the relative K_d) is given by the ratio of the catalytic rate constants. This emphasizes the fact that under conditions which give rise to a linear rate law, differences in catalytic efficiency reflect differences in the transition state alone; ground state changes are irrelevant.

For the important special cases in which the catalyst is either hydrogen ion or hydroxide ion, the expressions for the $pK_a^{\ddagger}$ of the transition state become

for H$^+$ catalysis, $pK_a^{\ddagger} = \log (k_2/k_1)$ (10)

for OH$^-$ catalysis, $pK_a^{\ddagger} = \log (k_1/k_2) + pK_w$ (11)

where K_w is the dissociation constant for water. In both of these equations, $pK_a^{\ddagger}$ refers to the reaction

$$AH^{+\ddagger} + H_2O \longrightarrow A^{\ddagger} + H_3O^+ \tag{12}$$
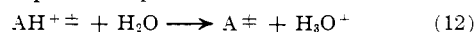
(For OH$^-$ catalysis, if strict conformity with eq. 4 is desired, AH$^{+\ddagger}$ in eq. 12 should be replaced by AOH$_2^{+\ddagger}$ or A‡, the corresponding change being made in the conjugate base.) The corresponding thermodynamic parameters for eq. 12 may be calculated from the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ determined for the catalyzed and uncatalyzed reactions. Thus for ΔH, one may write

for H$^+$ catalysis,

$$\Delta H = \Delta H^{\ddagger} \text{ (for } k_1) - \Delta H^{\ddagger} \text{ (for } k_2) \tag{13}$$

for OH$^-$ catalysis,

$$\Delta H = \Delta H^{\ddagger} \text{ (for } k_2) - \Delta H^{\ddagger} \text{ (for } k_1) + \Delta H \text{ (for } K_w) \tag{14}$$

Limitations on Applications.—A value may be calculated for $pK_d^{\ddagger}$ from any pair of reactions whose rate laws differ only in that one contains as a factor a reactant concentration not present in the other. However, this quantity will be readily interpretable only if the two reactions proceed by strictly analogous mechanisms. For example, in an application to ester hydrolysis it should be known that both catalyzed and uncatalyzed mechanisms involve the same position of cleavage (acyl or alkyl).

This restriction that both reactions must proceed by the same mechanism does not exclude those reactions whose uncatalyzed rate is attributed to catalysis by solvent. For example, when a hydrogen ion-catalyzed reaction occurs in aqueous solution, the corresponding uncatalyzed rate is usually considered to arise from general acid catalysis by water.[11] The transition states for these two mechanisms thus differ in that one is protonated while the other serves as an acceptor for a hydrogen bond from water. The water molecule in the latter transition state serves to solvate it in a manner analogous to the solvation of a ground state base.

Similarly, the differences in bond lengths and orders between the two transition states, although possibly large, are not different in kind from those between a stable acid and its conjugate base. The direction of these differences may be predicted from the Swain–Thornton rule.[1] Thus, structural parameters calculated from $pK_d^{\ddagger}$ should be interpreted as average values for the two transition states and the direction of deviation of each transition state from these averages predicted from a consideration of the structural effects of combination with the catalyst. This procedure is illustrated in the discussion of the mechanism of benzyl fluoride solvolysis. No such difficulty enters when comparisons are made solely between different $pK_d^{\ddagger}$'s, as in the last two examples discussed below.

Standard State for Water.—Some confusion is possible regarding the proper value to use for K_w and for the water activity when the transition state of a hydroxide ion-catalyzed reaction actually contains the OH$^-$ moiety. In this event, $pK_a^{\ddagger}$ refers to the ionization of a proton from a water molecule in the transition state of the corresponding uncatalyzed reaction. It is therefore desirable to be able to compare the value of $pK_a^{\ddagger}$ to that of pK_w. The value of pK_w commonly used in comparing the acidity of water with that of other acids is the one (15.7 at 25°) based on a standard state of unit molarity for water. The use of this value in this particular case would, however, be incorrect.

The correct value of pK_w for use in this comparison is that (14.0 at 25°) based on a standard state of pure liquid for water. This value is used in eq. 11 due to its having been introduced by the relation $pK_a + pK_b = pK_w$, which uses a standard state of pure water by convention. It may be shown that it is necessary to use this same standard state in formulating the rate equation in order to avoid the appearance in eq. 11 of a function of the water activity. For analogous reasons based on standard state consistency, when $pK_a^{\ddagger}$ is being estimated for a hydrogen ion-catalyzed reaction, the uncatalyzed rate term must be treated as a unit and not divided by the molarity of water before being used in eq. 10.

Examples

Specific Hydrogen Ion Catalysis.—An example of the use of transition state acidity as a criterion of mechanism for a specific hydrogen ion-catalyzed reaction may be based on the hydrolysis of benzyl fluoride

$$C_6H_5CH_2F + H_2O \longrightarrow C_6H_5CH_2OH + HF \tag{15}$$

Swain and Spalding[12] have published kinetic data for the hydrolysis of benzyl fluoride by both uncatalyzed and acid-catalyzed mechanisms. They found that the acid-catalyzed reaction followed h_o rather than $a_{H_3O^+}$ or h_R, and on this basis concluded that the transition state must have a structure close to $C_6H_5CH_2FH^+$, having neither tightly bound water nor carbonium ion

(11) R. P. Bell, "Acid–Base Catalysis," Oxford University Press, London, 1941, chap. 5.

(12) C. G. Swain and R. E. T. Spalding, J. Am. Chem. Soc., **82**, 6104 (1960).

character. The rate-determining step involving this transition state was considered to be

$$C_6H_5CH_2FH^+ \longrightarrow C_6H_5CH_2^+ + HF \qquad (16)$$

the conjugate acid of benzyl fluoride having been formed in a prior equilibrium protonation.

The values of $pK_a^{\ddagger}$, ΔH and ΔS which may be calculated from the data of Swain and Spalding for the acid-catalyzed transition state are given in Table I. The data refer to 10% acetone in water as solvent, so that these thermodynamic parameters will differ slightly from those valid in pure water. It is very unlikely, however, that any such deviation would be large enough to be significant in the argument based on those values. Also tabulated are the values of the corresponding parameters for HF and HFH$^+$. The value of pK_a for HF at 50° is extrapolated from the data of Broene and DeVries,[13] while the values of ΔH and ΔS are calculated from the data of the same authors. An approximate maximum value for the pK_a of H$_2$F$^+$ is estimated by assuming that ΔpK for the pair H$_2$F$^+$–HF is comparable to that for H$_2$O$^+$–H$_2$O ($\Delta pK = 17$) and for NH$_4^+$–NH$_3$ ($\Delta pK = 25$). Since the entropy of dissociation of an acid is primarily a function only of the solvation of the initial and final species,[14] it is assumed that its value for H$_2$F$^+$ is in the range characteristic of simple positively charged acids (e.g., NH$_4^+$).

If both transitions states had the structure which Swain deduced for the acid-catalyzed reaction from acidity function data (C–F bond nearly intact), the thermodynamic parameters for the transition state ionization would be expected to resemble those for HFH$^+$. Alternatively, if the fluorine moiety were completely free from the benzyl residue in both transition states, then the thermodynamic functions would coincide with those for HF. Intermediate degrees of bonding between carbon and fluorine would presumably give intermediate values.

TABLE I

THERMODYNAMICS OF IONIZATION AT 50°

	C$_6$H$_5$CH$_2$F solvolysis transition state	HF	H$_2$F$^+$
pK_a	1.8	3.4	<-10
ΔS, e.u.	-15.0	-17.7	~ 0
ΔH, kcal./mole	-2.1	-0.8	

It is clear from the data in Table I that the average structure of the two transition states which is measured by $pK_a^{\ddagger}$ has a carbon–fluorine bond which is nearly broken. Since the proton acts as an electron attracting substituent on fluorine, the Swain–Thornton rule[1] predicts that the carbon–fluorine bond will be shorter in the protonated transition state than in the nonprotonated one. On this basis it may be concluded that in the transition state for uncatalyzed solvolysis the C–F bond is almost completely broken, while in the acid-catalyzed solvolysis this bond is intermediate in strength between its condition in the protonated ground state and in the uncatalyzed transition state. The precise degree of bonding in the protonated transition state cannot be estimated from the semiquantitative estimates which are available for the thermodynamics of ionization of the various transition state models, and it is thus possible that it is little different from that in the ground state as proposed by Swain and Spalding. Their mechanism simply requires that a term for the stretching of the C–F bond in the acid-catalyzed transition state be added to the thermodynamic parameters estimated for the ionization of the weakly bonded

(13) H. H. Broene and T. DeVries, *J. Am. Chem. Soc.*, **69**, 1644 (1947).
(14) See, for example, J. T. Edsall and J. Wyman, "Biophysical Chemistry," Academic Press, Inc., New York, N. Y., 1958, chap. 8.

transition state using free HF as the model. Such corrections to $pK_a^{\ddagger}$ and ΔS might well be small.

If the transition state for the acid-catalyzed reaction does resemble benzyl carbonium ion, the failure of the rate to follow h_R might be due to the non-equivalence of OH and F in eq. 17 and 18. The former reaction

$$ROH + H^+ \rightleftharpoons R^+ + H_2O \qquad (17)$$

$$RF + H^+ \rightleftharpoons R^+ + HF \qquad (18)$$
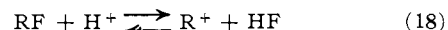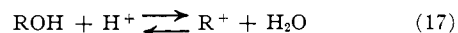
provides the basis for the definition of h_R,[15] while the latter corresponds to the solvolysis under consideration.

Specific Hydroxide Ion Catalysis.—Reactions commonly referred to as hydroxide ion-catalyzed may occur by two distinct mechanisms. Examples which illustrate the application of eq. 11 to both types of reaction are available. The substrate may be converted in a prior equilibrium to its conjugate base which then reacts (classical specific hydroxide ion catalysis[16]), or the substrate may combine with hydroxide ion, proceeding through a transition state which contains the hydroxyl moiety (often termed nucleophilic catalysis by hydroxide ion[17]). The analogous "uncatalyzed" reactions corresponding to these mechanisms would be, respectively, reaction of the un-ionized substrate or reaction with water.

As an example of classical specific hydroxide ion catalysis, the alkaline hydrolysis of β-diketones may be considered. This reaction appears to involve a prior equilibrium addition of hydroxide ion to a carbonyl, the adduct then either cleaving directly or losing a proton from the hydroxyl in a second equilibrium step to give a doubly charged anion which undergoes cleavage.[18] These reactions of the adduct may be formulated as shown in eq. 19 for 2,4-pentanedione, and the value of $pK_a^{\ddagger}$ may be calculated from the rate constants for the over-all cleavage.

$$\qquad (19a)$$

$$\qquad (19b)$$
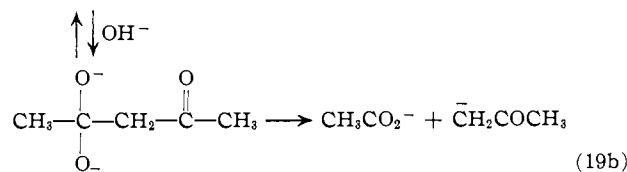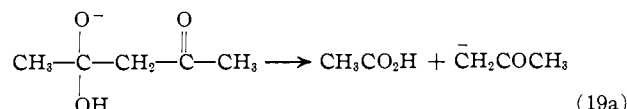
The transition state for reaction 19a would have the structure I, while that for 19b would be the corresponding
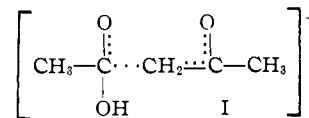
ing conjugate base arising from loss of the hydroxyl proton. The extent of cleavage present in I may be estimated from its pK_a relative to those of the reactant and product in 19a. If cleavage were complete and the fragments totally separated in the transition state, the value of $pK_a^{\ddagger}$ would be that for acetic acid (pK_a 4.8). Conversely, if no bond breaking were present in I, then the value of $pK_a^{\ddagger}$ would be that for the hydroxide adduct of pentanedione. This value may be estimated from the value of pK_a observed for acetaldehyde hydrate,[19] which is 13.6. The charge on the other oxygen is quite close to the proton and thus should have a

(15) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).
(16) Reference 11, chap. VII.
(17) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).
(18) R. G. Pearson and E. A. Mayerle, *J. Am. Chem. Soc.*, **73**, 926 (1951).
(19) R. P. Bell and D. P. Onwood, *Trans. Faraday Soc.*, **58**, 1557 (1962).
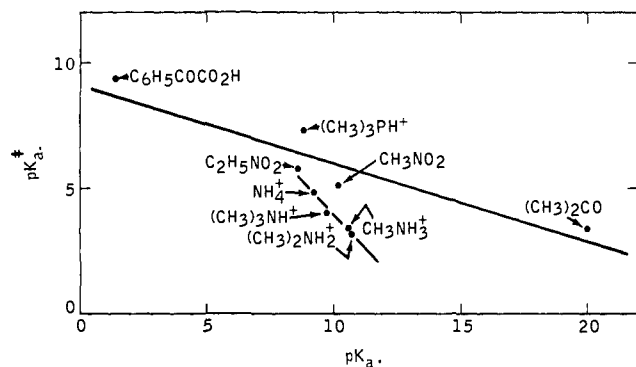
Fig. 1.—$pK^{\ddagger}$ vs. pK_a for donor acid. Lines are best fits for neutral acids and for substituted ammonium ions. No statistical corrections are included.

large effect which may be approximated by $pK_2 - pK_1$ for the dimensionally similar carbonic acid. Using the value of pK_1 corrected for H_2CO_3-CO_2 equilibrium,[20] ΔpK for H_2CO_3 is 6.4. Therefore, for the adduct in question, an approximate value of 20 may be taken for pK_a. The effect of the acetyl group on $pK_a^{\ddagger}$ has not been included, since an estimate of its magnitude (using $\rho^*(RCH_2OH) = 1.46$; $\sigma^*(CH_3COCH_2-) = 0.60/2.8$)[21] shows that it is within the uncertainty of the other estimates.

The data of Pearson and Mayerle[18] in combination with eq. 11 give $pK_a^{\ddagger}$'s of 12.4 and 13.0 for 2,4-pentanedione and 3-methyl-2,4-pentanedione, respectively. On the basis of the pK_a values estimated for the initial and final states, a $pK_a^{\ddagger}$ of 12.4 corresponds to a transition state which is 50% of the way to complete cleavage. Due to the approximations used in estimating the pK_a of the reactant and to the error in the rate constant ratio arising from the presence of barium ion in the experiments, the quantitative significance of this precise percentage is uncertain. However, it appears safe to draw the qualitative conclusion that the extent of bond cleavage in the average transition state corresponds to a position along the reaction coördinate which is closer to the mid-point between reactant and product than to either of these extremes. Again, however, a completely rigorous interpretation of this average value for the extent of bond cleavage must classify it as a lower limit for the dianionic transition state and an upper limit for the monoanionic transition state.

Proton transfer reactions comprise a particularly interesting class of those reactions which have hydroxide ion included in their transition states. Data are available for the rates of proton transfer from several acids to both water and hydroxide ion (eq. 20).

$$AH + H_2O \longrightarrow A^- + H_3O^+ \qquad (20a)$$

$$\updownarrow$$

$$AH + OH^- \longrightarrow A^- + H_2O \qquad (20b)$$
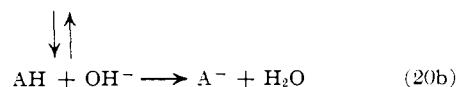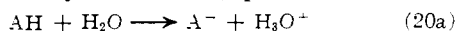
Such data can yield the $pK_a^{\ddagger}$ for the ionization of a proton from the water moiety in the transition state II.

$$A\cdots H\cdots O\text{—}H$$
$$\overset{\text{II}}{}\quad\overset{|}{H}$$
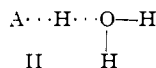
The acids for which the calculation has been made are listed in Table II and a plot of $pK_a^{\ddagger}$ as a function of the pK_a of the donor acid is given in Fig. 1.

The values of $pK_a^{\ddagger}$ may be taken as measures of the strengths in the transition states of the O–H bonds be-

(20) Reference 4, p. 179.
(21) R. W. Taft in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, chap. 9.

ing formed. If perturbations due to charges and other structural features in A were absent, then 100% transfer of the proton in both transition states would give a $pK_a^{\ddagger}$ equal to that of H_3O^+ (-1.7), and 0% transfer would give a $pK_a^{\ddagger}$ equal to that of H_2O (14.0). On this basis, the extent of proton transfer from the acids in Table II ranges approximately from 30% to 70% along the linear free energy scale defined by the pK_a's. The stronger acids show the lower percentages of transfer. Estimates of the extent of transfer could presumably be improved for the charged acids by the inclusion of corrections for the effect of the charges on $pK_a^{\ddagger}$.

TABLE II

ACIDITIES OF PROTON TRANSFER TRANSITION STATES AT 25°[aa]

Acid	pK_a	$pK_a^{\ddagger}$
NH_4^+	9.25[a]	4.80[g,h]
$CH_3NH_3^+$	10.62[a]	3.39[g]
$(CH_3)_2NH_2^+$	10.77[a]	3.16[g,h]
$(CH_3)_3NH^+$	9.80[a]	4.05[g,h]
$(CH_3)_3PH^+$	8.8[b]	7.3[b]
$C_6H_5COCO_2H$	1.4[c]	9.4[c]
$C_2H_5NO_2$	8.6[d]	5.8[d,e]
CH_3NO_2	10.2[d]	5.1[d,f]
CH_3COCH_3	20[d]	3.5[f]

[aa] Values for pK_a and for rate constants used in calculating $pK_a^{\ddagger}$ were taken from: [a] R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd Ed., Butterworths, London, 1959, pp. 517–522; [b] B. Silver and Z. Luz, J. Am. Chem. Soc., 83, 786 (1961); [c] M. S. Wheatley, Experientia, 12, 339 (1956); [d] R. G. Pearson and R. L. Dillon, J. Am. Chem. Soc., 75, 2439 (1953); [e] R. P. Bell and J. C. Clunie, Proc. Roy. Soc. (London), A212, 16 (1952); [f] K. F. Bonhoeffer, K. H. Geib and O. Reitz, J. Chem. Phys., 7, 664 (1939); [g] E. Grunwald, P. J. Karabatsos, R. A. Kroumhout and E. L. Purlee, J. Chem. Phys., 33, 556 (1960). [h] Rate constants for reaction with OH⁻ were calculated from equivalent conductances by the method of E. Grunwald, A. Loewenstein and S. Meiboom, ibid., 27, 630 (1957).

It is of interest to compare the observed correlation of $pK_a^{\ddagger}$ and pK_a to the prediction of the rule proposed by Swain and Thornton.[1] Since they define a more electron-supplying substituent as one which is more basic toward a proton, the observed decrease in $pK_a^{\ddagger}$ with increasing pK_a is in agreement with their rule. The complete series provided by the methylammonium ions indicates that the correlation is directly with the base strength of the amine in aqueous solution and not with the intrinsic electron-donating power of the substituents. The same factors (steric hindrance to solvation and/or back strain)[22] which alter the order of basicity of the methylamines from the simple order of the number of methyl groups retain most of their influence in the transition state. Since these factors would be expected to exert their influence primarily at the nitrogen rather than farther away at the abstracting water molecule, it is probable that the actual extent of proton transfer, and not merely the value of $pK_a^{\ddagger}$, correlates with the strength of the donor acid.

General Acid and Base Catalysis.—A reaction is conventionally considered to be general acid- or base-catalyzed if its empirical rate law consists of a sum of terms, one term for each acid or base present and each term proportional to the concentration of the corresponding acid or base.[23] If such a rate law contains terms for catalysis by both an acid and its conjugate base (eq. 21), then the formal requirements for the calculation of $pK_a^{\ddagger}$ are satisfied. Its value is given by

$$k_{obs} = k_{HA}[HA] + k_A[A^-] \qquad (21)$$

eq. 22, where pK_{HA} is the pK_a of the acid, HA.

$$pK_a^{\ddagger} = \log(k_{HA}/k_A) + pK_{HA} \qquad (22)$$

(22) Reference 9, p. 621.
(23) Reference 4, p. 130.

A classic example of a reaction whose rate follows eq. 21 is the enolization of acetone catalyzed by acetic acid and by acetate ion. Swain and co-workers[24-26] have presented convincing arguments that the acetic acid-catalyzed enolization proceeds through a prior equilibrium protonation of the ketonic oxygen followed by a rate-determining abstraction of a proton from carbon by acetate ion, and that the acetate ion-catalyzed enolization involves a similar proton abstraction by acetate ion assisted by hydrogen bonding of water to the oxygen. The hydrogen ion-catalyzed and uncatalyzed rates are attributable to analogous mechanisms with water taking the place of acetate as nucleophile.[24]

The transition states for the enolizations catalyzed by acetic acid or by hydrogen ion thus have the structure III, where B is, respectively, acetate ion or water.

$$\text{B}\cdots\text{H}\cdots\text{CH}_2\overset{\overset{\text{O—H}}{\vdots\vdots}}{=\!=}\text{CCH}_3 \quad \text{III}$$
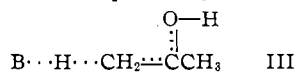
The rate data of Bell and Jones[27] may be used to obtain values of $pK_a{}^{\ddagger}$ for the ionization of the proton from

(24) C. G. Swain, J. Am. Chem. Soc., **72**, 4578 (1950).

(25) C. G. Swain, E. C. Stivers, J. F. Reuwer, Jr., and L. J. Schaad, ibid., **80**, 5885 (1958).

(26) C. G. Swain, A. J. DiMilo and J. P. Cordner, ibid., **80**, 5983 (1958).

(27) R. P. Bell and P. Jones, J. Chem. Soc., 88 (1953).

oxygen in these transition states. The values obtained are 4.8 when B is water and 4.0 when it is acetate ion.

According to the rule of Swain and Thornton,[1] changing B from water to acetate should lengthen the B$\cdots$H bond and shorten the carbon–oxygen bond in the acetone moiety. This latter shortening should decrease the basicity of the oxygen as is observed. This observation provides a verification of the Swain–Thornton rule which is independent of isotope effect arguments and measures the effect of changing the base on the orbital covering the enol proper instead of the effect on the orbital utilized in the B$\cdots$H bond (which is measured by the α-hydrogen isotope effect).

The 0.8 pK unit shift in the acidity of the proton which accompanies the change in nucleophile is of some interest as a quantitative measure of the deviation from the assumption[24] that the reactivity of the electrophile remains constant when the nucleophile is changed. This shift corresponds to a change of 1.1 kcal./mole in the free energy of the proton–oxygen bond in the transition state. This difference could produce a change in rate of a factor of six, which is small compared to the factor of more than 10^4 calculated[24] for the nucleophilicity of acetate relative to water using the assumption that the reactivity of any electrophile remains constant.

Glycine Analogs of Bradykinin[1]

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The synthesis of three analogs of the nonapeptide bradykinin is described: 6-glycine bradykinin, 7-glycine bradykinin and L-arginyl-heptaglycyl-L-arginine. The preparation of the decapeptide, 7-glycine kallidin, an analog of 1-L-lysyl bradykinin, is also reported. Biological activities of these peptides are tabulated.

For a study of the relationship between structure and activity of the nonapeptide bradykinin[2] some analogs were prepared, in which glycine replaced one or more of the amino acids in the sequence of the naturally occurring peptide.

The initial structure proposed[3] for bradykinin was that of a linear octapeptide in which the proline residue in position 7 of the nonapeptide structure was omitted. When this octapeptide was synthesized[4] it was found to be devoid of activity. The question whether the amino acid in position 7 had to be proline or whether the latter could be replaced by another amino acid, without loss in activity, still remained open. 7-Glycine bradykinin (XII) was prepared in an effort to explore this question. The second analog, 6-glycine bradykinin (VI), wherein serine is replaced by glycine, was synthesized in order to ascertain to what degree the serine side chain contributed to biological activity. This latter analog was also converted into 1-L-lysyl-6-glycine bradykinin (7-glycine kallidin) (XXII), the glycine analog of the biologically active decapeptide

(1) Some of the compounds described here were first discussed at The New York Academy of Sciences, Conference on Structure and Function of Biologically Active Peptides: Bradykinin, Kallidin and Congeners, March 22–24, 1962.

(2) (a) M. Rocha e Silva, W. T. Beraldo and G. Rosenfeld, Am. J. Physiol., **156**, 261 (1949); (b) E. Werle, Angew. Chem., **73**, 689 (1961).

(3) D. F. Elliott, G. P. Lewis and E. W. Horton, Biochem. J., **76**, 16 (1960).

(4) (a) R. A. Boissonas, St. Guttmann, P. A. Jaquenoud, H. Konzett and E. Stürmer, Experientia, **16**, 326 (1960); (b) R. Schwyzer, W. Rittel, P. Sieber, H. Kappeler and H. Zuber, Helv. Chim. Acta, **43**, 1130 (1960); (c) E. D. Nicolaides, H. A. DeWald, P. G. Shorley and H. O. J. Collier, Nature, **187**, 773 (1960).

kallidin,[5] by adding a lysine residue to the N-terminal arginine. Finally, a glycine analog embodying two characteristic features of bradykinin, namely, the terminal arginine residues separated by a peptide chain of seven amino acid residues, L-arginyl-heptaglycyl-L-arginine (XX) was prepared on the premise that these features might represent the minimum structural requirements for biological activity.

The schemes for the synthesis of 6-glycine- and 7-glycine bradykinin are shown in Fig. 1 and 2, respectively. These schemes differ, of course, in that they require two different protected C-terminal pentapeptide moieties III and IX, respectively, but follow parallel courses from there on. Thus the protected C-terminal pentapeptide ester, methyl benzyloxycarbonyl-L-phenylalanylglycyl-L-propyl-L-phenylalanylnitro-L-arginate (III), required for 6-glycine bradykinin was obtained in crystalline form and in good yield by allowing the p-nitrophenyl ester of the protected tripeptide benzyloxycarbonyl-L-phenylalanylglycyl-L-proline (II) to react with the dipeptide ester methyl-L-phenylalanyl-nitro-L-argininate. The p-nitrophenyl ester of II was prepared by coupling benzyloxycarbonyl-L-phenylalanine-p-nitrophenyl ester with glycyl-L-proline in aqueous pyridine followed by esterification with p-nitrophenol.

The protected C-terminal pentapeptide of 7-glycine bradykinin, methyl benzyloxycarbonyl-L-phenylalanyl-L-serylglycyl-L-phenylalanyl-nitro-L-arginine (IX), was secured by coupling the protected dipeptide benzyl-

(5) J. V. Pierce and M. E. Webster, Biochem. Biophys. Res. Commun., **5**, 353 (1961).