Proximate Charge Effects. 3.[1] Enthalpies of Solvent Transfer of Reactants and Transition States in the Saponification of Acetylcholine

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Abstract: The rates and activation enthalpies of the alkaline hydrolysis of acetylcholine were measured in water, in 60 mol % ethanol-water, and in 85 mol % ethanol–water. Unlike the alkaline hydrolysis of ethyl acetate, which exhibited a large increase in ΔH^* on going from water solvent to 60 mol % ethanol–water solvent, no such solvent effect was observed in the case of acetylcholine. Calorimetric determination of the relative enthalpies of the reactants in water and in aqueous ethanol revealed that whereas there was a large desolvation of the ethyl acetate saponification transition state in aqueous ethanol relative to water, no such desolvation of the acetylcholine saponification transition state took place.

Interionic attraction energies are well-known as a potent factor influencing interactions between molecules and inducing conformational changes within molecules. In particular, forces of interionic attraction should introduce modifications in the conformation of the transition state of a reactant in which a permanent charge is proximate to a reaction center which itself becomes charged in the course of the reaction. A reaction that lends itself to the study of this possibility is the alkaline hydrolysis of an ester. The mechanism of this reaction is well established,[2] the rate-determining step being the formation of the negatively charged tetrahedral intermediate. There is considerable charge localization on the acyl oxygen of the transition state lending to this intermediate as has been demonstrated[3] by the large exothermic enthalpy of transfer of this transition state into a hydrogen-bonding medium. This reaction should, therefore, lend itself to the above-mentioned modification of its transition state by the introduction of a proximate positively charged group. Thus, the hydrolysis of acetylcholine might well be facilitated if the transition state were coiled, I, due to an electrostatic interaction between the proximate positive charge and the carbonyl oxygen rather than linear, II (Figure 1). In a study of the alkaline hydrolysis of meta- and para-substituted ethyl β-phenylpropionates, Fuchs and Caputo[4] found an unusually high rate for the m-$(CH_3)_3N^+$ ester. This they attributed to a cyclic transition state in which there was a strong ion–ion attraction between the $^+(CH_3)_3N$ group and the negatively charged carbonyl oxygen in the transition state. The salt effect on the rate of the acid as well as the basic hydrolysis of acetylcholine has been examined.[5] Calculation of the distance between the quaternary ammonium group and the ester carbonyl showed that this distance was slightly less for the alkaline hydrolysis than for the acid hydrolysis, leading the authors[5] to suggest that there may be some coiling of the chain when it has oppositely charged ends.

The acid-catalyzed hydrolysis of acetylcholine is somewhat slower than that of a comparable ester lacking a proximate positive charge such as ethyl acetate.[6] The rate of the basic hydrolysis, on the other hand, is considerably faster than that of an uncharged model.[6] This might be caused by the inductive effect of the positively charged quaternary ammonium group on the negatively charged tetrahedral transition state or by the coiled transition state suggested above.

Table I. Rate Constants[a] for the Alkaline Hydrolysis of Acetylcholine

solvent[b]	temp, K	k_2, L mol^{-1} s^{-1}
H_2O	291.15	1.689 ± 0.071
H_2O	322.90	15.210 ± 0.55
0.60 aq ethanol	273.16	$1.456 \times 10^{-3} \pm 0.246 \times 10^{-3}$
0.60 aq ethanol	291.15	$7.00 \times 10^{-3} \pm 2.87 \times 10^{-3}$
0.60 aq ethanol	322.90	$55.05 \times 10^{-3} \pm 5.62 \times 10^{-3}$
0.85 aq ethanol	291.15	$0.948 \times 10^{-3} \pm 0.157 \times 10^{-3}$
0.85 aq ethanol	322.90	$11.02 \times 10^{-3} \pm 1.78 \times 10^{-3}$

[a] Extrapolated to zero ionic strength (log k_2 vs. $I^{1/2}$), using 4–7 kinetic runs at concentrations giving ionic strengths from 8×10^{-5} to 2×10^{-1}. Uncertainty given as the standard deviation of the extrapolated value. [b] Solvent composition indicated as the mole fraction of the organic solvent.

Table II. Activation Parameters for the Alkaline Hydrolysis of Acetylcholine and Ethyl Acetate

ester	solvent[a]	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal deg^{-1} mol^{-1}
acetylcholine chloride	H_2O	12.23[b]	−15.4
acetylcholine chloride	0.60 aq ethanol	12.14	−26.8
acetylcholine chloride	0.85 aq ethanol	13.72	−25.2
ethyl acetate[c,d]	H_2O	11.1	−25.8
ethyl acetate[d]	0.60 aq ethanol	14.9	−20.0

[a] Solvent composition indicated as mole fraction of ethanol. [b] Literature values for the alkaline hydrolysis in water: $\Delta H^{\ddagger}$ = 12.8 kcal/mol (Buterworth, J.; Eley, D. D.; Stone, G. S. *Biochem. J.* 1953, *53*, 30) and $\Delta H^{\ddagger}$ = 12.2 kcal/mol (Wright, M. R. *J. Chem. Soc. B* 1968, 545). [c] Tommila, E.; Koivisto, A.; Lyyra, J. P.; Antell, K.; Heims, S. *Acad. Sci. Fennicae, Ser. A.* 1952, *47*, 3. [d] Roberts, D. D. *J. Org. Chem.* 1964, *29*, 2714.

Saponification Rates

The rate of the alkaline hydrolysis of acetylcholine was measured conductometrically at several temperatures in three solvents: water, 60 mol % aqueous ethanol, and 85 mol % aqueous ethanol. The results are shown in Table I. Activation enthalpies and activation entropies calculated from these data are listed in Table II. Also shown in Table II, for comparison, are literature values of the activation parameters of the alkaline hydrolysis of ethyl acetate.

The greater rate of hydrolysis of acetylcholine over its uncharged model in water is seen to be entirely an entropic effect, the activation enthalpy being actually a little higher for acetylcholine. In the less polar medium[7] 60 mol % aqueous ethanol on the other

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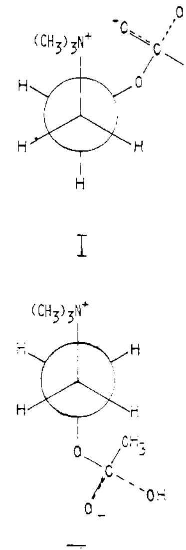

Figure 1. I, Coiled transition state, having an electrostatic interaction between the proximate positive charge and the negative reaction center; and II, linear transition state, with both charges optimally solvated.

hand, there is a substantial decrease in the activation enthalpy (3.8 kcal/mol) for the saponification of acetylcholine over that of ethyl acetate. As the kind of proximate charge catalysis suggested above would become more manifest in a less polar medium, this result does suggest the existence of such electrostatic facilitation of acetylcholine hydrolysis. This is so because the ethyl acetate hydrolysis transition state would experience increased solvation on transfer from the less polar medium to water, whereas the cyclic acetylcholine hydrolysis transition state would not be expected to have such a corresponding increase in solvation energy on transfer from aqueous ethanol to water.

Enthalpies of Solvent Transfer

The above expectation can be tested by measuring the enthalpies of transfer of the reactants in the saponification of ethyl acetate and acetylcholine from water to aqueous ethanol. The enthalpy of transfer of a compound from one solvent to another, $\delta\Delta H_{s1\rightarrow s2}$, is the difference between the heats of solution, ΔH_s, of the compound in the two solvents of interest:

$$\delta\Delta H_{s1\rightarrow s2} = \Delta H_{s2} - \Delta H_{s1}$$

The enthalpies of transfer of ethyl acetate and acetylcholine chloride were obtained by this method in a straightforward manner, using the appropriate calorimetric measurements. The enthalpies of transfer of OH^- and the $^+(CH_3)_3NCHCH_2OCOCH_3$ ion were determined by calorimetry, using the $(n\text{-}C_4H_9)_4N^+(n\text{-}C_4H_9)_4B^-$ extrathermodynamic assumption used in our earlier work.[3] The enthalpies of transfer of these compounds and ions are shown in Table III.

If one assumes that the enthalpy of activation for the saponification reaction is approximately equal to the activation enthalpy for the first step of this reaction, that is the formation of the tetrahedral anion intermediate,[8] then one can calculate the enthalpy of transfer of the transition state for this step from one solvent to another. For a single step, for which the activation enthalpies in two solvents are available, one can calculate the enthalpy of solvent transfer for the transition state in this step from the equation

$$\delta H^{\ddagger}_{s1\rightarrow s2} = \delta\Delta H_{s1\rightarrow s2} + \delta\Delta H^{\ddagger}$$

Table III. Enthalpies of Solvent Transfer, $\delta\Delta H_{s1\rightarrow s2}$, of Compounds and Ions, 298.16 K (kcal/mol)

	solvent transfer[a]	$\delta\Delta H_{s1\rightarrow s2}$
acetylcholine chloride	$H_2O \rightarrow$ 0.60 aq ethanol	2.22
acetylcholine chloride	$H_2O \rightarrow$ 0.85 aq ethanol	1.73
Cl^-	$H_2O \rightarrow$ 0.60 aq ethanol	1.6[b]
Cl^-	$H_2O \rightarrow$ 0.85 aq ethanol	0.6[b]
$CH_3CO_2CH_2CH_2N(CH_3)_3^+$	$H_2O \rightarrow$ 0.60 aq ethanol	0.6
$CH_3CO_2CH_2CH_2N(CH_3)_3^+$	$H_2O \rightarrow$ 0.85 aq ethanol	1.1
ethyl acetate	$H_2O \rightarrow$ 0.60 aq ethanol	3.33
OH^-	$H_2O \rightarrow$ 0.60 aq ethanol	-0.5[b]
OH^-	$H_2O \rightarrow$ 0.85 aq ethanol	-2.9[b]

[a] Composition of mixed solvents indicated as mole fraction of ethanol. [b] From ref 1.

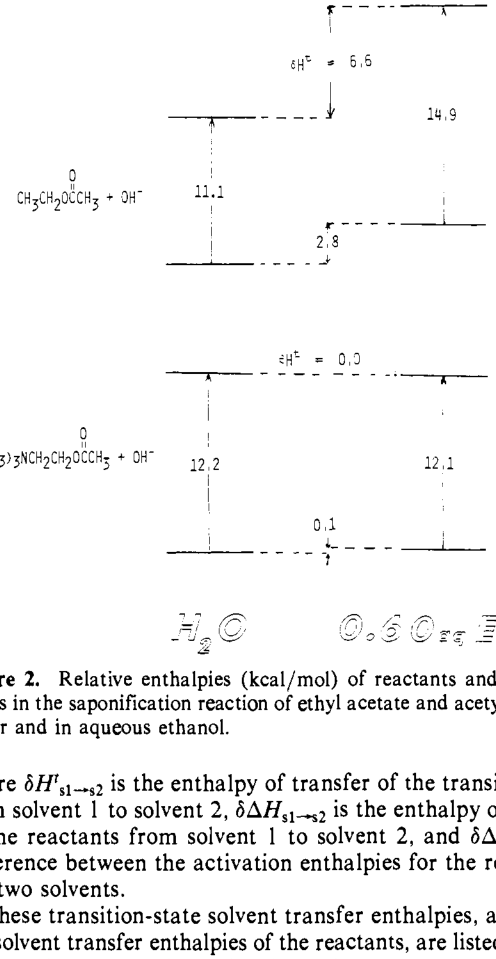

Figure 2. Relative enthalpies (kcal/mol) of reactants and transition states in the saponification reaction of ethyl acetate and acetylcholine in water and in aqueous ethanol.

where $\delta H^{\ddagger}_{s1\rightarrow s2}$ is the enthalpy of transfer of the transition state from solvent 1 to solvent 2, $\delta\Delta H_{s1\rightarrow s2}$ is the enthalpy of transfer of the reactants from solvent 1 to solvent 2, and $\delta\Delta H^{\ddagger}$ is the difference between the activation enthalpies for the reaction in the two solvents.

These transition-state solvent transfer enthalpies, along with the solvent transfer enthalpies of the reactants, are listed in Table IV and shown graphically in Figure 2.

Before discussing these solvent transfer enthalpies, it is necessary to consider to what extent they reflect solute–solvent interactions. An enthalpy of transfer of a solute from one solvent to another consists of two contributions: (1) the difference between the solute–solvent interactions in the two solvents and (2) the difference between the cavity formation energies in the two solvents. Since only (1) is of the interest here, it is necessary to show to what extent (2) contributes to the value of Table IV. To estimate the differences between the cavity formation energies in our solvents, we use as a model the enthalpy of transfer of benzene. The enthalpy of transfer of benzene from water to 60 mol % aqueous ethanol was found to be 0.49 kcal/mol.[1] Though not

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(7) Dielectric constants: H_2O, 78; 0.60 aqueous ethanol, 33; 0.85 aqueous ethanol, 27 (Akerlof, G. *J. Am. Chem. Soc.* **1932**, *54*, 4125).

(8) This is a reasonable assumption. It was found by Bender et al. (Bender, M. L.; Ginger, R. D., Unik, J. P. *J. Am. Chem. Soc.* **1958**, *80*, 1044) that in the basic hydrolysis of ethyl benzoate in 33% dioxane–water the energy of activation of the hydrolysis reaction was $E_h^{\ddagger}$ = 12.9 kcal/mol and the energy of activation of the first step in the reaction was $E_1^{\ddagger}$ = 13.2 kcal/mol.

Table IV. Enthalpies (kcal/mol) of Solvent Transfer of Reactants and Transition States in the Reaction $CH_3COOR + OH^- \rightarrow CH_3CO_2^- + ROH$ at 25 °C

	reactants		
	$CH_3COOC_2H_5$ + OH^-	$CH_3COOCH_2CH_2N(CH_3)_3^+$ + OH^-	transition state
$H_2O \rightarrow$ 0.60 aq ethanol	2.8		6.6
$H_2O \rightarrow$ 0.60 aq ethanol		0.1	0.0
$H_2O \rightarrow$ 0.85 aq ethanol		-1.8	-0.3

Table V. Heats of Solution[a]

solute	solvent[b]	ΔH_S, kcal/mol
acetylcholine chloride	H_2O	2.26 ± 0.03
acetylcholine chloride	0.60 aq ethanol	4.48 ± 0.11
acetylcholine chloride	0.85 aq ethanol	3.99 ± 0.14
ethyl acetate	H_2O	-2.23[c]
ethyl acetate	0.60 aq ethanol	1.10[d]

[a] Integral heats of solution, 25.0 °C, measured at concentrations of 0.001-0.01 M, uncertainties given as the standard deviation of the mean of 4 measurements. [b] Solvent composition given as mole fraction of ethanol. [c] Stern, J. H.; Hermann, A. *J. Phys. Chem.* 1967, *71*, 306. [d] Reference 3.

negligible, this value is an order of magnitude smaller than the enthalpy of transfer of the ethyl acetate saponification transition state (6.6 kcal/mol, Table IV), and hence it is reasonable to discuss this latter transition-state transfer enthalpy in terms of solute-solvent interactions.

Examination of Figure 2 shows that the substantial increase in the ethyl acetate hydrolysis activation energy on going from water to aqueous ethanol is due to a large increase in the desolvation of the transition state on going to the less polar medium. This desolvation is not observed in the case of the acetylcholine hydrolysis transition state. A possible reason for this is that the acetylcholine transition state is internally solvated as shown in Figure 1.

Experimental Section

Solution Preparation. The mixed solvents were prepared from weighed amounts of distilled water and ethanol (Commercial Solvents Corp.). Dissolved carbon dioxide was removed from the solvent by passing purified nitrogen gas through a sintered glass bubbler immersed in the solvent. All subsequent operations were carried out under a nitrogen atmosphere. Acetylcholine chloride (Aldrich) and NaOH (Matheson, Coleman and Bell, Analyzed Reagent, A.C.S.) solutions were standardized by titration with $AgNO_3$ and HCl, respectively.

Rate Measurements. A sodium hydroxide solution was placed into an all-glass conductance cell and allowed to come to thermal equilibrium in a constant-temperature bath. To this was added an equal volume of acetylcholine solution which had also been equilibrated in the same constant-temperature bath. These operations were carried out under an atmosphere of pure nitrogen. The cell was shaken and conductance measurements were taken at appropriate intervals with a Wayne-Kerr conductivity bridge (Model B224) which operates at 1592 Hz.

The fraction conversion, f, was obtained from

$$f = \frac{L_0 - L_t}{L_0 - L_\infty}$$

where L_0 = the initial conductance, L_∞ = the final conductance, and L_t = the conductance at time t.

Thirty to fifty points per kinetic run were taken, ranging from at least 20% to 70% conversion. Initial concentrations ranged from 4×10^{-5} M to 3×10^{-4} M in water, 0.007-0.1 M in 0.60 aqueous ethanol, and 0.008-0.1 M in 0.85 aqueous ethanol. The conductance change ranged from 5% to 15% of the total conductance value. L_0 values were checked by adding the (separately measured) conductances of acetylcholine chloride and sodium hydroxide. L_∞ values were checked by adding the (separately measured) conductances of sodium acetate, choline chloride, and sodium hydroxide and correcting for the choline-anhydrocholine equilibrium.[1] In both cases, fair agreement was found between these conductances and the ones observed at the beginning and end of the kinetic runs.

To calculate the second order rate constant, k_2, for the process $^+(CH_3)_3NCH_2CH_2OAc + OH^- \rightarrow {}^+(CH_3)_3NCH_2CHOH + AcO^-$, it is not possible to use the standard form of the integrated second-order rate equation because OH^- is also consumed by a second process, namely the equilibrium:

$$^+(CH_3)_3NCH_2CH_2OH + OH^- \rightleftarrows {}^+(CH_3)_3NCH_2CH_2O^- + H_2O \quad (1)$$

The instantaneous OH^- concentration at every kinetic point, t, was calculated with the equation

$$(OH^-)_t = B_0 - A_0f - ((KB_0 + 1) - [(KB_0 + 1)^2 - 4K^2A_0f(B_0 - A_0f)]^{1/2})/2K$$

where B_0 = the initial OH^- concentration, A_0 = the initial acetylcholine concentration, f = the fraction conversion, K = the equilibrium constant for the choline-anhydrocholine equilibrium (eq 1) taken from ref 1 (B_0 was always either equal to or greater than A_0).

The second-order rate constant, k_2, was then calculated at each of the 30-50 points from the expression

$$k_2 = \frac{\Delta f/\Delta t}{(OH^-)(1 - f)}$$

The mean of these k_2 values yielded the second-order rate constant at a particular ionic strength (I). In each solvent, rate measurements were done at four to seven different initial concentrations. The above four to seven k_2 values were extrapolated to zero ionic strength by plotting ln k_2 vs. $I^{1/2}$ to obtain a rate constant for the pure solvent, e.g., at zero ionic strength. This gave a linear plot, the intercept being the k_2 at zero ionic strength and the standard deviation of this intercept being taken as the error of the k_2 value (both tabulated in Table I).

Calorimetry. The molar heat of solution (integral) of acetylcholine chloride was measured in the three solvents of interest with a LKB 8700 calorimeter.

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Registry No. Acetylcholine, 51-84-3; ethyl acetate, 141-78-6; acetylcholine chloride, 60-31-1.