Ethyl Trichloroacetate Hydrolysis. III. Solvent Isotope Effects and Thermodynamic Activation Parameters for the Water-Catalyzed Hydrolysis

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Abstract: Values of the rate constant, $\Delta H^\ddagger$, $\Delta S^\ddagger$, and $\Delta C_p{}^\ddagger$ are reported for the hydrolysis of Cl_3CCO_2Et in H_2O and D_2O; the value of k_{H_2O}/k_{D_2O} passes through a minimum near 44°. These values are combined with data for the partition of the tetrahedral intermediate to give the following parameters for the first step (addition of water to the carbonyl group) in the hydrolysis mechanism at 25°: $k_{1,H_2O}/k_{1,D_2O} = 2.2$, and for H_2O solutions $\Delta H_1{}^\ddagger = 8.4$ kcal mol^{-1}, $\Delta S_1{}^\ddagger = -44$ cal deg^{-1}, and $\Delta C_{p,1}{}^\ddagger \approx -52$ cal deg^{-1} mol^{-1}. These values are quantitatively explainable on the basis of a transition state for the addition which includes a large extent of charge separation (e.g., one which is near in structure to a hydrogen-bonded dipolar intermediate, $L_2O\text{- - -}L_2O^+\text{-}C(CCl_3)(OEt)\text{-}O^-$). The presence of additional specifically bound water molecules in the transition state is not required by these explanations. The solvent isotope effect on the partition ratio for the tetrahedral intermediate suggests that the eliminations of water and of ethanol from that intermediate may involve different rate-determining processes.

Heat capacities[1-3] and entropies[4] of activation together with solvent isotope effects[1,5] are known to be useful probes of reaction mechanism and activated complex structure. The available evidence suggests that values of $\Delta C_p{}^\ddagger$ and k_{H_2O}/k_{D_2O} in particular are quite sensitive to changes in solvent configuration during the activation process.[1,5] In order to improve our understanding of the factors which contribute to observed values of these parameters, it is desirable to know such values for many different types of reactions. Unfortunately, few values of $\Delta C_p{}^\ddagger$ are known for reactions other than hydrolyses of alkyl halides or sulfonates.

This paper reports a study of k_{H_2O}/k_{D_2O}, $\Delta C_p{}^\ddagger$, and $\Delta S^\ddagger$ for the water-catalyzed hydrolysis of a carboxylic ester. The primary objective of this study was to obtain estimates of $\Delta C_p{}^\ddagger$ and $\Delta S^\ddagger$ for the carbonyl addition step in the hydrolysis mechanism. Such estimates were sought in order to extend our previous efforts[6] to separate the contributions to these parameters which are the results of covalent binding of individual solvent molecules and of cooperative structural reorganization in the surrounding solvent during the activation process.

Partition ratios for the tetrahedral intermediate in this hydrolysis are known,[7,8] and those ratios allow values of the activation parameters for the carbonyl addition step to be derived from the corresponding parameters which are observed for the hydrolysis. Although some values of $\Delta C_p{}^\ddagger$ for hydrolyses of carboxylic acid derivatives have been reported previously,[9-13] for none of these earlier examples have data been sufficiently complete to allow the estimation of contributions from separate steps in the mechanism; thus those $\Delta C_p{}^\ddagger$ values were not directly comparable to the values of $\Delta C_p{}^\ddagger$ for other classes of reactions.

Experimental Section

Ethyl trichloroacetate and other reagents were as described elsewhere.[7]

Hydrolysis rates were measured conductometrically using an Industrial Instruments RC-18 conductivity bridge. Conductance cells were designed in accord with the recommendations of Jones and Bollinger[14] and used bright platinum electrodes; lengths of capillary glass tubing (ca. 0.5-mm bore) connected the air spaces in the cell with the air above the bath to equalize pressure without allowing significant loss of solvent vapor. Runs using H_2O as solvent were usually made in cells of ca. 50-ml capacity, but rate constants measured using cells of ca. 10-ml and 200-ml capacities agreed with those from the 50-ml cell within experimental scatter; runs using D_2O as solvent were made in 10-ml cells.

Oil-bath temperatures were held constant to within ±0.003° and were measured with a Hewlett-Packard Dymec 2801 A quartz thermometer which was calibrated to within ±0.001° against a platinum resistance thermometer which in turn had been calibrated by the National Bureau of Standards.

Solutions of the ester (ca. 1×10^{-3} M) were degassed by boiling briefly in vacuo at room temperature before being transferred to the cell, which was then immersed in the oil bath. Conductances were measured during the second, third, and fourth half-times of the hydrolysis; the omitted first half-time was sufficiently long at all temperatures to allow the cell contents to approach sufficiently closely to thermal equilibrium with the bath. (Conductances of $NaClO_4$ solutions measured in the same cells were constant within ±0.027% after the same time interval.) The solvent in most runs contained 1.0×10^{-3} F $NaClO_4$ as backing electrolyte; within experimental scatter, no change in rate constant was observed when 1.0×10^{-3} F $HClO_4$ was substituted for the $NaClO_4$. (The common practice[1,14] of using the product of the hydrolysis as backing electrolyte could not be followed since it exacerbates the error in the rate constant which arises from the drift in conductance caused by decarboxylation of the trichloroacetate anion.) Rate measurements at $HClO_4$ concentrations up to 0.05 F were used to estimate the increase in the observed rate constant which resulted from acid catalysis by the product trichloroacetic acid and by $HClO_4$ backing electrolyte. This increase was found to be always less than 0.1%.

Results

The electrical conductance (C_t) at time t of a dilute aqueous solution of ethyl trichloroacetate increases as hydrolysis of the ester occurs and accurately obeys a first-order rate law (eq 1) for longer than 8 hydrolysis half-times.[15]

$$\ln (C_\infty - C_t) = -k_h t + \text{constant} \qquad (1)$$

The value of k_h, the first-order rate constant for hydrolysis, was usually calculated by the Guggenheim method[16,17] using a time interval between members of a pair of readings which was near 1.5 half-times. Drift-corrected values of k_h calculated for a single run by the Guggenheim method and from eq 1 agree within the experimental uncertainty (0.1-0.2%).

The temperature dependence of k_h was characterized by fitting the observed values to the Robertson equation[1] (eq 2) by least-squares. The observed values of k_h are given in

$$\log k_h = A/T + B \log T + C \qquad (2)$$

Table I. Rate Constants for the Water-Catalyzed Hydrolysis of Ethyl Trichloroacetate

Temp,[d] °C	$10^4 k_h$ in H_2O,[a] sec^{-1} Obsd[b]	$10^4 k_h$ in H_2O,[a] sec^{-1} Calcd[c]	$10^4 k_h$ in D_2O,[a] sec^{-1} Obsd[b]	$10^4 k_h$ in D_2O,[a] sec^{-1} Calcd[c]	k_H/k_D, calcd
5			0.1631	0.1631	
10	0.927	0.928	0.2387	0.2388	3.886
15	1.301	1.299	0.3413	0.3411	3.808
20	1.786	1.783	0.4762	0.4762	3.744
25	2.399	2.404	0.6504	0.6504	3.696
30	3.188	3.186	0.8716	0.8704	3.660
35	4.153	4.154	1.1402	1.1425	3.636
40	5.336	5.333	1.4739	1.4724	3.622
45	6.744	6.749			
50	8.426	8.425			
55	10.385	10.380			

[a] Solvents contain 1.0×10^{-3} F $NaClO_4$. [b] Average of between 2 and 14 observations. [c] From eq 2 with parameters from Table II. [d] ±0.003°.

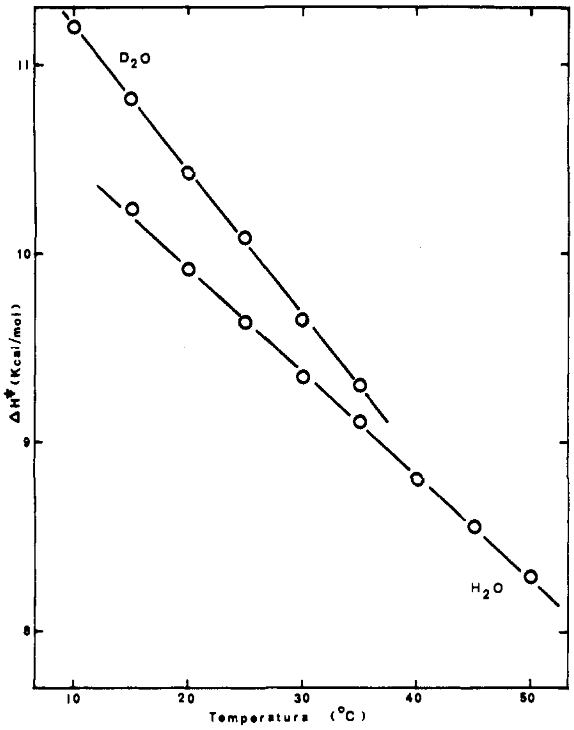


Figure 1. Temperature dependence of $\Delta H^\ddagger$. Values of $\Delta H^\ddagger$ are from eq 8 using 10° temperature intervals. The least-squares lines of best fit for H_2O and D_2O are shown.

$$\Delta H_h^{\ddagger} = R[-A \ln 10 + (B - 1)T] \quad (3)$$

$$\Delta S_h^{\ddagger} = R[C \ln 10 - \ln (k/h) + (B - 1)(\ln T + 1)] \quad (4)$$

$$\Delta C_{p,h}^{\ddagger} = R(B - 1) \quad (5)$$

Table 1 together with the corresponding values predicted from eq 2 using the A, B, and C parameters of best fit. Those parameters are given in Table II along with the values of $\Delta H_h^\ddagger$ and $\Delta S_h^\ddagger$ (for 25°) and the values of $\Delta C_{p,h}^\ddagger$ (averages over the experimental temperature ranges) which can be calculated from them via eq 3-5.[1] The standard deviations given in Table II for $\Delta H_h^\ddagger$, $\Delta S_h^\ddagger$, and $\Delta C_{p,h}^\ddagger$ are calculated from the standard deviations in A, B, and C, together with coefficients from the error correlation matrices given in eq 6 and 7 for A, B, and C taken in that order.[18]

$$\mathbf{R} \text{ (for } H_2O) = \begin{pmatrix} 1.0000 & 0.9998 & -0.9998 \\ 0.9998 & 1.0000 & -1.0000 \\ -0.9998 & -1.0000 & 1.0000 \end{pmatrix} \quad (6)$$

$$\mathbf{R} \text{ (for } D_2O) = \begin{pmatrix} 1.0000 & 0.9999 & -0.9999 \\ 0.9999 & 1.0000 & -1.0000 \\ -0.9999 & -1.0000 & 1.0000 \end{pmatrix} \quad (7)$$

As a check on the suitability of eq 2 and on the value of $\Delta C_p^\ddagger$ from eq 5, values of $\Delta H^\ddagger$ were calculated for each 10° temperature interval from eq 8 and plotted against the

$$\Delta H^{\ddagger} = [RT_1T_2/(T_2 - T_1)] \ln (k_2T_1/k_1T_2) \quad (8)$$

arithmetic mean temperatures of the intervals. The plots are shown in Figure 1; the slope of such a plot is $\Delta C_p^\ddagger$. Although the arithmetic mean temperatures are only approximations to the correctly defined temperatures for use in such a plot, this approximation should lead to no significant error in $\Delta C_p^\ddagger$.[2] The least-squares slopes of the lines in Figure 1 are included in Table II; for both solvents, these slopes are identical, within the experimental uncertainty, with the values of $\Delta C_p^\ddagger$ calculated from eq 5.

While these papers were in preparation, an independent report[11a] of values of $\Delta H_h^\ddagger$, $\Delta S_h^\ddagger$, and $\Delta C_{p,h}^\ddagger$ for the hydrolysis of ethyl trichloroacetate in light water appeared. Those values are included in Table II and are in satisfactory agreement with our values.

The solvent isotope effect on k_h, $k_{h,H_2O}/k_{h,D_2O}$, can be seen from Table I to decrease monotonically as temperature increases. However, a short extrapolation (via eq 2) to temperatures higher than those for which reliable values of k_{h,D_2O} could be measured predicts that $k_{h,H_2O}/k_{h,D_2O}$ will pass through a minimum value of 3.619 at 44.36°. This behavior is in qualitative agreement with that reported for solvent isotope effects on the hydrolysis rates of carboxylic anhydrides;[10] previous data for carboxylic ester hydrolyses[9] do not cover a sufficiently wide range of temperature to indicate whether the solvent isotope effect passes through an extremum. For ethyl trichloroacetate at 25°, $\delta_I \Delta H_h^{\ddagger}$[19] $= -402 \pm 10$ cal mol^{-1} and $\delta_I \Delta S_h^\ddagger = 1.25 \pm 0.04$ cal deg^{-1} mol^{-1}; also $\delta_I \Delta C_{p,h}^\ddagger = 20 \pm 2$ cal deg^{-1} mol^{-1}, although an additional error of a few cal deg^{-1} mol^{-1} may be present in this last quantity because of the 10° difference between the midpoints of the temperature ranges investigated for the two solvents.

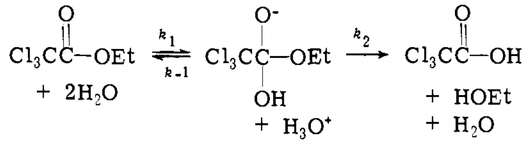
Table II. Derived Parameters for the Uncatalyzed Hydrolysis of Ethyl Trichloroacetate[a]

	In H_2O[b]	In D_2O[b]
A[c]	-5673.77 ± 56.075	-7115.19 ± 82.784
B[c]	-26.5256 ± 0.42382	-36.9788 ± 0.64616
C[c]	81.0467 ± 1.2369	111.179 ± 1.8767
$\Delta H_h^\ddagger(298.15)$[d]	9653 ± 7 cal mol^{-1} (9692 ± 28 cal mol^{-1})[h]	10055 ± 7 cal mol^{-1}
$\Delta S_h^\ddagger(298.15)$[e]	$-42.72 + 0.03$ cal deg^{-1} mol^{-1} (-42.63 ± 0.09 cal deg^{-1} mol^{-1})[h]	-43.97 ± 0.02 cal deg^{-1} mol^{-1}
$\Delta C_{p,h}^{\ddagger}$[f]	-55 ± 1 cal deg^{-1} mol^{-1} (-57 ± 4 cal deg^{-1} mol^{-1})[h]	-75 ± 1 cal deg^{-1} mol^{-1}
$\Delta C_{p,h}^{\ddagger}$[g]	-55 ± 1 cal deg^{-1} mol^{-1}	-76 ± 1 cal deg^{-1} mol^{-1}

[a] All uncertainties are standard deviations. [b] Solvents contain 1.0×10^{-3} F $NaClO_4$. [c] From eq 2. [d] From eq 3. [e] From eq 4. [f] From eq 5. [g] From the slopes of Figure 1. [h] From ref 11a.

Discussion

Parameters for Carbonyl Addition. As discussed in the preceding paper,[8] hydrolysis presumably takes place via the mechanism in eq 9. The first-order rate constants and acti-



$$Cl_3C\overset{\overset{O}{\|}}{C}{-}OEt + 2H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Cl_3C\overset{\overset{O^-}{|}}{\underset{\underset{OH}{|}}{C}}{-}OEt + H_3O^+ \xrightarrow{k_2} Cl_3C\overset{\overset{O}{\|}}{C}{-}OH + HOEt + H_2O \qquad (9)$$

vation parameters for the carbonyl addition (k_1) step are related to the corresponding quantities observed for the overall hydrolysis by eq 10-14,[10,11a] where $\alpha = k_{-1}/k_2$.

$$k_1 = k_h(1 + \alpha) \qquad (10)$$

$$\Delta H_1^{\neq} = \Delta H_h^{\neq} + \frac{\alpha}{1 + \alpha}(\Delta H_{-1}^{\neq} - \Delta H_2^{\neq}) \qquad (11)$$

$$\Delta S_1^{\neq} = \Delta S_h^{\neq} + \frac{\alpha}{1 + \alpha}(\Delta S_{-1}^{\neq} - \Delta S_2^{\neq} - R \ln \alpha) + R \ln (1 + \alpha) \qquad (12)$$

$$\Delta C_{p,1}^{\neq} = \Delta C_{p,h}^{\neq} + \frac{\alpha}{1 + \alpha}(\Delta C_{p,-1}^{\neq} - \Delta C_{p,2}^{\neq}) + \frac{\alpha}{(1 + \alpha)^2}\frac{(\Delta H_{-1}^{\neq} - \Delta H_2^{\neq})^2}{RT^2} \qquad (13)$$

$$\frac{k_{1,H_2O}}{k_{1,D_2O}} = \frac{k_{h,H_2O}}{k_{h,D_2O}}\frac{(1 + \alpha_{H_2O})}{(1 + \alpha_{D_2O})} \qquad (14)$$

Since values are known[7,8] for all of the quantities on the right-hand sides of eq 10-14 except $\Delta C_{p,-1}^{\ddagger} - \Delta C_{p,2}^{\ddagger}$, it is possible to evaluate k_1, $\Delta H_1^{\ddagger}$, $\Delta S_1^{\ddagger}$, and $k_{1,H_2O}/k_{1,D_2O}$; these values are given in Table III along with the value which $\Delta C_{p,1}^{\ddagger}$ would have if $\Delta C_{p,-1}^{\ddagger} = \Delta C_{p,2}^{\ddagger}$.[20]

Carbonyl Addition Activated Complex Structure. The kinetic parameters which are observed for water-catalyzed addition of water to carbonyl groups have often[21-25] been regarded as implying the relatively tight binding of several (greater than two) water molecules into the activated complex. One water molecule is presumably covalently bound to the carbonyl carbon in the transition state, a second is abstracting a proton from the first, and many others are present in the immediate neighborhood of the transition state. The question is: are any of those others sufficiently tightly bound to make it desirable to include them explicitly when writing the mechanism? For the carbonyl addition under study here, the values of $\Delta S_1^{\ddagger}$, $\Delta C_{p,1}^{\ddagger}$, and $k_{1,H_2O}/k_{1,D_2O}$ can be compared with the corresponding parameters for model equilibria; these comparisons show that no binding in excess of that present in the solvation of stable ground-state molecules is required in order to account for the observed values.

The k_1 step (eq 9) converts reactants which have no charge separation into products which contain fully charged H_3O^+ and $CCl_3C(OEt)(OH)O^-$ moieties. Attempts to estimate the extent of charge separation in the transition state which lies between those end points can be made in two ways; unfortunately, these two methods of estimation give quite different answers. First consider the Brϕnsted β, which should be an approximate indicator of the extent of proton binding to the catalyzing base molecule in the transition state. The value of β for the general-base catalyzed hydrolysis of ethyl trichloroacetate is uncertain since values for only two bases (water and acetate) are known. However, the β for ethyl dichloroacetate hydrolysis is 0.47, and the acetate/water rate constant ratios for these two esters are nearly identical;[26] thus β for ethyl trichloroacetate should be near 0.5. *If* the two processes of proton transfer and nucleophile-carbon bond formation have taken place to the same extent when the reactants reach the transition state, then this value would suggest that charge separation is about half complete. A second estimate of the extent of charge separation can be made from the free energies of activation for the forward (k_1) and reverse (k_{-1}) directions of the addition: $\Delta G_1^{\ddagger} = 21.5$ and $\Delta G_{-1}^{\ddagger} \sim 3.8$ kcal mol^{-1}.[7] These values suggest that structural reorganization is ca. 80-90% complete at the transition state.

Table III. Parameters for the k_1 Step[a]

	25°	45°[b]
$10^3 k_1$, sec^{-1}	1.01 ± 0.02[c]	2.48 ± 0.06[c]
$\Delta H_1^{\neq}$, kcal mol^{-1}	ca. $8._4$[d,e]	7.3 ± 0.2[d]
$\Delta S_1^{\neq}$, cal deg^{-1} mol^{-1}	ca. −44[f,g]	−47.6 ± 0.7[f]
$\Delta C_{p,1}^{\neq}$, cal deg^{-1} mol^{-1}	ca. −52[h,i]	−52 ± 1[h,i]
$k_{1,H_2O}/k_{1,D_2O}$	2.2 ± 0.2[j]	

[a] Quoted uncertainties are standard deviations propagated from the standard deviations in Table II and ref 8. [b] The midpoint of the temperature range covered by measurements of k_e/k_h is 45°.[8] [c] From eq 10 with k_h from Table I and α from ref 8. [d] From eq 11 with $\Delta H_h^{\neq}$ from Table II (via eq 3) and other parameters from ref 8. [e] Assuming $\Delta H_{-1}^{\neq} - \Delta H_2^{\neq}$ to have the same value at 25° as at 45°. [f] From eq 12 with $\Delta S_h^{\neq}$ from Table II (via eq 4) and other parameters from ref 8. [g] Assuming $\Delta S_{-1}^{\neq} - \Delta S_2^{\neq}$ to have the same value at 25° as at 45°. [h] From eq 13 with $\Delta C_{p,h}^{\neq}$ from Table II, assuming that $\Delta C_{p,-1}^{\neq} = \Delta C_{p,2}^{\neq}$ (see text). [i] If $\Delta C_{p,h}$ is significantly temperature dependent, then some additional small error in the estimated value of $\Delta C_{p,1}^{\neq}$ could arise from the difference between the midpoints of the temperature ranges used for measuring $\Delta C_{p,h}^{\neq}$ (32° from Table I) and $\Delta H_e^{\neq} - \Delta H_h^{\neq}$ (45°).[8] [j] From ref 7; ionic strength = 1.0. The value of α_{D_2O} is known only at 25° with ionic strength = 1.0.

It is probable that β and $\Delta G^{\ddagger}$ are most sensitive to different structural components of the activation process (e.g., β to the extent of proton transfer and $\Delta G_1^{\ddagger}$ to changes in carbon-oxygen covalent bonding); however, such speculation does not provide any sure basis for using either parameter to estimate the extent of charge separation. Further, the use of either as an indicator of activated complex structure carries the possibility of significant error; conclusions based on β can be wrong if interactions are present in the transition state which are absent in both the reactant and product states,[27,28] and the use of $\Delta G_1^{\ddagger}$ and $\Delta G_{-1}^{\ddagger}$ in this way assumes implicitly that the slope of the reaction profile (i.e., the slope of $\bar{G}°$ as a function of the reaction coordinate) has the same mean magnitude on both sides of the transition state.

In the following discussion it will be shown that if charge separation has proceeded to the extent of ca. 90% in the transition state (in agreement with the $\Delta G^{\ddagger}$ criterion), then *neither* enhanced hydrogen bonding *nor* additional orientation of solvent molecules is required by the values of $\Delta S_1^{\ddagger}$ and $\Delta C_{p,1}^{\ddagger}$ to be present in the transition state to an extent beyond that which is present in the equilibrium solvation of stable ground-state solutes. On the other hand, if charge separation has proceeded to the extent of only ca. 50% in the transition state (in agreement with the β criterion), then such hydrogen bonding or orientation *is* required to account for the values of $\Delta S_1^{\ddagger}$ and $\Delta C_{p,1}^{\ddagger}$. The value of $k_{1,H_2O}/k_{1,D_2O}$ could be consistent with either extent of charge separation but depends on how that charge is distributed.

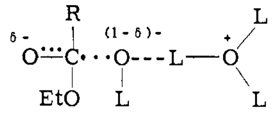
Entropy and Heat Capacity of Activation. In the k_1 step of eq 9, two processes occur which would be expected to contribute significantly to $\Delta S_1^{\ddagger}$; a covalent bond is formed between a water oxygen and the carbonyl carbon, and charge separation occurs to give a positive charge on a lyonium ion and a negative charge on the former carbonyl oxygen (or possibly on an OL^- in the transition state.)[29,30] The maximum contribution to $\Delta S_1^{\ddagger}$ from the covalent bond formation would correspond to formation of a full bond and should be close to that observed for the equilibrium addition of L_2O to CCl_3CHO in aqueous solution (about −26 cal deg^{-1} mol^{-1} for both H_2O and D_2O).[31] The maximum contribution from the charge separation would correspond to complete separation and should be close to that observed for the equilibrium ionization of $CCl_3CH(OH)_2$ to give $CCl_3CH(OH)O^- + H_3O^+$ (−22 cal deg^{-1} mol^{-1} in H_2O)[32] or to that observed for the autoprotolysis of water (about −27 cal deg^{-1} mol^{-1} for both H_2O and D_2O if L_2O, L_3O^+, and LO^- all three have molar standard states).[33] The sum of these two maximum contributions to $\Delta S_1{}^{\ddagger}$ is thus either −48 or −53 cal deg^{-1} mol^{-1} (depending on the location of the negative charge). The value of $\Delta S_1{}^{\ddagger}$ is −44 cal deg^{-1} mol^{-1} at 25° in H_2O (Table III); this is ca. 80-90% of that estimated to correspond to complete charge separation and bond formation and thus agrees with the $\Delta G^{\ddagger}$ criterion.

If charge separation were only about 50% complete in agreement with β, then the contribution to $\Delta S_1{}^{\ddagger}$ from that source would be reduced to near −11 or −14 cal deg^{-1} mol^{-1}. The contribution from covalent bond formation would remain near −26 cal deg^{-1} mol^{-1} if the extent of that formation were reduced to near 50%, because changes in bond force constants do not lead to significant changes in vibrational entropies unless the bond order is near zero. The sum of these two contributions falls short of the observed −44 cal deg^{-1} mol^{-1} by 4 or 7 eu. This deficit could be made up by specially oriented or hydrogen-bonded waters; the contribution of each such water molecule should be somewhat less negative than $-\Delta S°$(fusion) (ca. −5 cal deg^{-1} mol^{-1} for both H_2O and D_2O).[34]

The value, −52 cal deg^{-1} mol^{-1} (Table III), estimated for $\Delta C_{p,1}{}^{\ddagger}$ in H_2O is also ca. 90% of the value estimated for complete bond formation and charge separation. For equilibrium addition of H_2O to CH_3CHO, $\Delta C_p° \approx -10$ cal deg^{-1} mol^{-1}.[6] For water autoprotolysis, $\Delta C_p° = -47$ and −55 cal deg^{-1} mol^{-1} for H_2O and D_2O, respectively.[35] For ionization of $CCl_3CH(OH)_2$ into H^+ and $CCl_3CH(OH)O^-$, $\Delta C_p°$ is not known but is −37 cal deg^{-1} mol^{-1} for the closely related compound, $CF_3CH(OH)_2$.[32] The expected sum of the contributions to $\Delta C_{p,1}{}^{\ddagger}$ from complete bond formation and charge separation is thus −47 to −57 cal deg^{-1} mol^{-1}. Contributions to $\Delta C_{p,1}{}^{\ddagger}$ from additional hydrogen-bonded waters might be larger than the corresponding contributions to $\Delta S_1{}^{\ddagger}$ since $\Delta C_p°$(fusion) is significantly larger (ca. 9 cal deg^{-1} mol^{-1})[31] than $\Delta S°$(fusion) and since heat capacity changes which accompany changes in solvation are often quite large.[36]

Solvent Isotope Effect. In the above discussion of $\Delta S_1{}^{\ddagger}$ and $\Delta C_{p,1}{}^{\ddagger}$, explicit proposals of possible structures for the activated complex were deliberately avoided; those values can be explained by a wide range of structures; if any significant amounts of charge separation and covalent bonding are assumed to be present, inclusion of additional hydrogen-bonded waters can bring the "predicted" values of $\Delta S_1{}^{\ddagger}$ and $\Delta C_{p,1}{}^{\ddagger}$ into agreement with observation (or if the predictor prefers, a late transition state can be assumed to explain the observations without the invocation of additional waters).

The value of $k_{1,H_2O}/k_{1,D_2O}$ (2.2, Table III) can be explained easily only on the basis of a more restricted range of structures. For the assumed general-base catalyzed mechanism, an "alpha"[30] structure is more likely than a "beta" structure. Five such α-structures are shown as **1-5.** In these representations, a *dotted* line denotes a reacting bond, while a *dashed* line represents a stable hydrogen bond in which the proton lies in a potential minimum and participates in "solvation catalysis."[30] Structures **1** and **2** represent activated complexes for addition mechanisms in which carbon-oxygen bonding defines the reaction coordinate in the rate-determining step and lyon transfer either precedes (**1**) or follows (**2**) the rate-determining transition state. Similarly, **3** and **4** depict activated complexes in which lyon transfer defines the reaction coordinate in the rate-determining step, and **5** is meant to portray the activated complex in a concerted or "diagonal"[37] mechanism.



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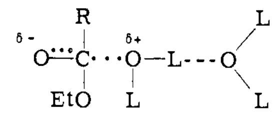

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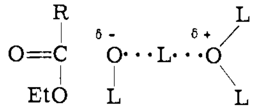

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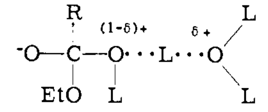

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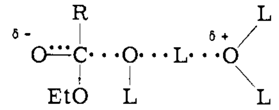

5

The ability of each of these structures to account for the observed solvent isotope effect can be assessed by Schowen's[30] approximate method. Structure **1** can be eliminated immediately since the L_3O^+ moiety which it contains would contribute a factor of $(1.5)^3$ which is by itself far larger than the total observed effect of 2.2.

Structure **4** can also be rejected. It implies a secondary contribution of ca. $(1.5)^{1+\delta}$ so that the primary contribution from the proton in transit could not exceed 2.2/1.5 = 1.5. Such a low primary effect would require either a very early ($\delta \approx 0$) or a very late ($\delta \approx 1$) transition state. A late transition state would lead to a secondary effect which was too large, and an early transition state could not account for the observed β, $\Delta S_1{}^{\ddagger}$ and $\Delta C_{p,1}{}^{\ddagger}$.

Structure **3** represents that class of transition states for which the dominant contribution to the activation process is similar to autoprotolysis. Such mechanisms have been proposed[29,30] to account for the very large solvent isotope effects sometimes observed for water-catalyzed additions of water since the secondary isotope effect by itself could be as large as 7. If **3** were correct for the addition under study here, then it would require $\delta \leq 0.5$. If $\delta = 0.5$, then the simple interpretation of β is correct, but no primary isotope effect from the lyon in transit could be present. While this is not impossible,[38] we consider it to be less likely than the explanation below based on structures **2** and **5.** If $\delta < 0.5$, a primary effect up to a maximum value of 2.2 could be present, but the small δ value which is implied by such a low primary effect is again in conflict with conclusions based on β, $\Delta S_1{}^{\ddagger}$ and $\Delta C_{p,1}{}^{\ddagger}$.

Structures **2** and **5** are those which appear to be most probable. Both predict secondary isotope effects of $(1.5)^{2\delta}$, and **5** predicts that a primary contribution will also be present. If the observed effect is entirely secondary, then $\delta \approx 1$; smaller values of δ are possible if a small primary effect is present. Thus a relatively late transition state with either structure could account for the observed isotope effect within the uncertainty of the Schowen approximation.

The inverse solvent isotope effect[7] on α is less easily explained.[39] If the k_2 transition state had the same general type of structure as the k_1 transition state (**2** or **5** with the EtO and OL interchanged and about the same value of δ), then the range of expected values would be $1 \leq \alpha_{H_2O}/\alpha_{D_2O} \leq 1.5$. If **3** were the structure of both transition states (that for k_2 again having EtO and OL interchanged), the value could be as high as 2. The inverse observed effect seems to require that the k_{-1} and k_2 steps have different mechanisms. For example, if the k_2 transition state were of the **1**, **3**, **4**, or **5** type, while the k_1 transition state had the **2** structure, then the solvent isotope effect on k_2 could be larger than that on k_{-1}, and the observed inverse effect on α could be explained. Perhaps such a dissymmetry in the mechanisms of the two paths open to the intermediate could also account for their observed[7] unsymmetric catalysis.

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(39) We believe that the probability of this value ($\alpha_{H_2O}/\alpha_{D_2O} = 0.56$)[7] being the result of experimental error is very small. The value of α_{H_2O} in ref 7 is corroborated by the oxygen-18 exchange data,[8] and the value of α_{O_2O} was independently remeasured and confirmed by one of the authors of ref 7 (J.L.K.) five years after the original measurement by the other author (J.M.F.).

Buffer Dependence of Carbonic Anhydrase Catalyzed Oxygen-18 Exchange at Equilibrium

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Abstract: The carbonic anhydrase (human C and bovine) catalyzed rate of exchange of oxygen-18 between bicarbonate and water was measured at chemical equilibrium with and without buffers. An increase of the buffer 1,2-dimethylimidazole ($pK_a = 8.3$) from 0 to 10 mM was accompanied by an enhancement of up to 80% in the rate of catalyzed oxygen-18 exchange near pH 8.3; this enhancement diminished as pH increased. However, the rate of exchange was not further enhanced by the addition of buffer beyond a 10 mM concentration. Similar effects were observed using tris sulfate. The addition of these buffers had no influence on the uncatalyzed rate of oxygen-18 exchange. Furthermore, 1,3-dimethylimidazolium sulfate and pyrrole, which are not buffers in the pH range studied, did not increase the carbonic anhydrase catalyzed exchange rate. Ionic strength was held constant in all experiments using Na_2SO_4; bicarbonate concentrations were as low as 0.5 mM, and enzyme was 5×10^{-9} M. We interpret these results as evidence for the involvement of a buffer-assisted proton transfer in the hydration–dehydration mechanism of carbonic anhydrase. In the absence of added buffers, the enzymatic rate is determined by alternating hydration–dehydration steps at equilibrium, which do not require proton transfer to the enzyme. In addition, bicarbonate itself may act as a buffer. As the buffer concentration is increased, the exchange data show a change in the rate-determining step. In the region of low buffer concentration, proton transfer is rate limiting and the catalytic exchange rate increases as buffer increases. Near 10 mM buffer, the maximum enzyme activity is reached and further buffer does not affect the catalytic rate.

Carbonic anhydrase (E.C. 4.2.1.1) catalyzes the reversible hydration of carbon dioxide with a very high turnover rate.[1,2] A likely mechanism for the carbonic anhydrase catalyzed hydration and dehydration of CO_2 is given in Figure 1. A similar scheme was thoroughly discussed by Lindskog and Coleman[3] and was found to be in agreement with the observed physicochemical properties of carbonic anhydrase. Of particular pertinence are the experimental observations that the activity of the enzyme depends on the state of ionization of a group in the active site with a pK_a near 7, that