One-Proton Solvation Bridge in Intramolecular Carboxylate Catalysis of Ester Hydrolysis[1,2]

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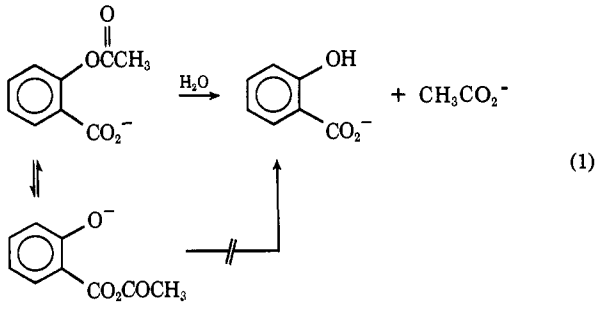
Abstract: The rate of hydrolysis of *O*-dichloroacetylsalicylic acid anion is independent of buffer strength and pH(D) in acetic acid–sodium acetate buffers in water, deuterium oxide, and their equimolar mixture at 25°. The rate constant k_n in solvents of mole fraction n deuterium oxide, mole fraction $1 - n$ of protium oxide, is given by $10^5k_n = (7432 \pm 33)[1 - n + (0.460 \pm 0.008)n]$. The solvent isotope effect of $k_{H_2O}/k_{D_2O} = 2.17$ thus arises from a single transition-state proton ("one-proton solvation bridge").

Many important hydrolysis reactions are *protolytically* catalyzed, *i.e.*, are accelerated through formation of a transition-state proton bridge from the reacting water molecule to a base catalyst.[4] An established example is the hydrolysis of aspirin anion (eq 1)



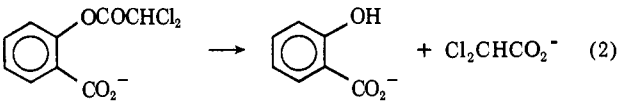
(1)

in which isotopic labeling studies show that, although the intramolecular acyl shift to generate the anhydride is rapid and reversible,[5] the main catalytic pathway is carboxylate-assisted attack of water on the ester function.[6] When the stability of the anhydride isomer is greatly increased (*e.g.*, in 3,5-dinitroaspirin), the mechanism shifts to the anhydride route. Fersht and Kirby believe the rate-determining step along this route to be phenoxy-assisted attack of water on the anhydride function and thus another example of protolytic catalysis.[6,7]

Protolytically catalyzed reactions generally proceed about twice as fast in protium oxide as in deuterium oxide ($k_{H_2O}/k_{D_2O} = 2.2$ and 2.1 for the ester route and the anhydride route, respectively, of aspirin compounds[7]). This isotope effect is doubtless a result of the interaction of water with the catalytic center in the transition state, but its detailed origin is a debatable matter.[8] As examples, it might be a primary isotope effect, a "solvation" effect from hydrogen-bond formation,[9] or a composite effect from many smaller changes in solvation character multiplying together to generate the observed effect. We have therefore made a "proton inventory" of such a transition state by examining the pH-independent hydrolysis of *O*-dichloroacetylsalicyclic acid (**1**) anion in mixtures of light and heavy water. This compound reacts more rapidly than aspirin and is thus more convenient for extensive kinetic study.

Results

The rate of hydrolysis of dichloroacetylsalicyclic acid (**1**) anion (eq 2) in acetic acid–sodium acetate



(2)

buffers is independent of buffer ratio and total buffer concentration in the ranges of $R \equiv [NaO_2CCH_3]/[HO_2CCH_3]$ from 1.87 to 10.51 (pH 4.88 to 5.56 in H_2O) and total buffer concentration from 0.10 to 0.50 M in the solvents protium oxide, deuterium oxide, and 50% H_2O–50% D_2O, as shown by data in Table I. The dependence of the first-order rate constant, k_n, on the atom fraction, n, of deuterium in mixtures of H_2O and D_2O is given in Table II. The data are described by eq 3 (illustrated in Figure 1), found by a linear least-squares fit.

$$10^5k_n = (7432 \pm 33) \times [1 - n + (0.460 \pm 0.008)n] \quad (3)$$

Discussion

pH and Buffer Independence of the Rates. It was expected by analogy with the pH–rate profile for aspirin hydrolysis (pH-independent rate from pH 4 to 8)[10] that **1** would hydrolyze at a rate independent of pH in the 4.9–5.6 region in protium oxide. This is confirmed. The further finding of pH(D) independence at corresponding buffer ratios in D_2O and in 50% H_2O–50% D_2O greatly simplified the experiments since it made unnecessary a detailed determination of the pH(D)–rate profile in each isotopic solvent. This observation

(1) Catalysis in Ester Cleavage. IV. For part III, see C. G. Mitton, M. Gresser, and R. L. Schowen, *J. Amer. Chem. Soc.*, 91, 2045 (1969).

(2) This research was supported by the National Science Foundation and the National Institutes of Health. Data reduction was carried out in the University of Kansas Computation Center. Further details may be found in S. S. Minor, M.S. Thesis, University of Kansas, 1971.

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

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(6) A. R. Fersht and A. J. Kirby, *ibid.*, 89, 4853, 4857 (1967); 90, 5826 (1968).

(7) A. J. Kirby and A. R. Fersht, *Progr. Bioorg. Chem.*, 1, 1 (1971).

(8) R. L. Schowen, *Progr. Phys. Org. Chem.*, 9, 275 (1972).

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

(10) L. J. Edwards, *Trans. Faraday Soc.*, 46, 723 (1950).

Table I. Invariance of the First-Order Rate Constant for Hydrolysis of 2×10^{-4} *M* *O*-Dichloroacetylsalicylate Anion to Buffer Composition and Strength in Protium and Deuterium Oxides and Their Equimolar Mixture at 25.0 ± 0.1°

$R = [NaO_2CCH_3]/[HO_2CCH_3]$	$[NaO_2CCH_3] + [HO_2CCH_3]$, *M*	$10^4 k_{obsd}$, sec^{-1}		
		H_2O	D_2O	$n \simeq 0.5$
1.874	0.5007	751, 744, 740	395, 357, 376	537, 533, 533, 549
(pH 4.88)[a]	0.4006	740, 774, 747	365, 380, 384	
	0.3004	732, 781, 732	376, 357	
	0.2003	735, 770, 739	388, 368	
	0.1001	747, 740, 766	380, 376, 372	
	Av for $R = 1.874$:	751 ± 12	375 ± 9	538 ± 6[b]
4.747	0.5011	759, 778, 751	361, 388	595, 572, 547, 568
(pH 5.21)[a]	0.4009	766, 747, 751	388, 395	
	0.3007	728, 781, 785	380	
	0.2004	774, 740, 774	384, 361, 372	
	0.1002	778, 766, 729	392, 384, 372	
	Av for $R = 4.747$:	759 ± 15	376 ± 11	571 ± 13[b]
10.506	0.5013	755, 788, 766	384, 365, 392	549, 584, 580
(pH 5.56)[a]	0.4010	736, 781, 766	376, 384	
	0.3008	751, 743, 751	388, 384	
	0.2005	751, 751, 755	384	
	0.1003	747, 785, 785	388, 384, 392	
	Av for $R = 10.506$:	760 ± 14	385 ± 5	571 ± 15[b]

[a] pH values are for H_2O. [b] Mixtures of H_2O and D_2O were prepared volumetrically in these three cases, rather than by weight as in the measurements of Table II. This may explain the lack of complete agreement in rate constants.

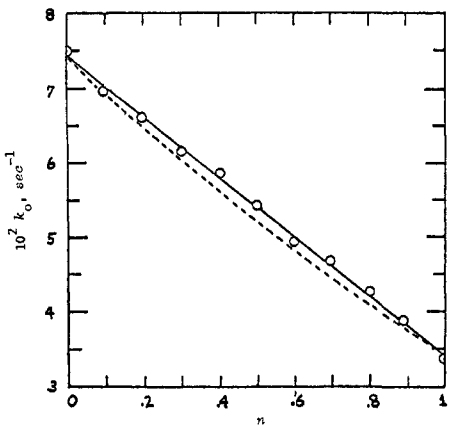


Figure 1. Observed first-order rate constants for hydrolysis of dichloroacetylsalicylate ion in sodium acetate–acetic acid buffers. The solid line is a plot of eq 3 with $k_0 = 0.0743$ sec^{-1} and $\phi^* = 0.46$. The dashed line is a plot of the expression for two protons each contributing a normal isotope effect of 1.48 (*i.e.*, eq 6 with $\nu = 2$, $\phi_i^* = 0.68$). The circles locating experimental points encompass the maximum error limits (average standard deviation 1%, range 0.3–1.7%).

Table II. Average First-Order Rate Constants for Hydrolysis of 2×10^{-4} *M* *O*-Dichloroacetylsalicylate Anion in Sodium Acetate–Acetic Acid Buffers (Total Concentration 0.5011 *M*, $R = 4.747$) in Mixtures of Deuterium Oxide (Mole Fraction *n*) and Protium Oxide (Mole Fraction $1 - n$) at 25.0 ± 0.1°

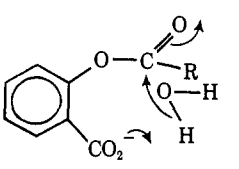
n, atom fraction D	$10^5 k_n$, sec^{-1}	No. of runs
0.0000	7510 ± 129[a]	5
0.0955	6994 ± 135	6
0.1984	6622 ± 61	7
0.2991	6173 ± 24	4
0.4012	5882 ± 38	6
0.5020	5438 ± 26	6
0.5981	4949 ± 29	5
0.6945	4699 ± 16	4
0.8009	4281 ± 54	6
0.8890	3886 ± 60	6
0.9964	3355 ± 26	6

[a] Error limits are standard deviations. Rate constants were obtained by a least-squares fit of absorbance–time data.

can be understood by noting that acetic acid and **1** differ little in acid strength (**1** should be slightly more acidic than aspirin which has a pK_a of 3.5) and that carboxylic acids of similar strength exhibit similar solvent isotope effects on acidity.[11] Since the pK_a of the buffer and the pK_a of the substrate should therefore change in nearly exactly the same way as deuterium is introduced into the solvent, a reaction conducted at the same buffer ratio in all solvents will remain at the same point on the pH(D)–rate profile. Thus, pH independence in protium oxide leads to pH(D) independence in all isotopic solvents.

In contrast to our finding that the strength of acetate buffers does not affect the rate with **1** as substrate at 25°, Fersht and Kirby[7] found a small amount of intermolecular catalysis by acetate with aspirin at 39°. This may indicate that the dichloroacetyl group, as opposed to the acetyl group, produces a preference for intramolecular over intermolecular catalysis or that the temperature dependences of the two processes differ. The question remains under study.

Mechanism. It is very unlikely that introduction of the dichloroacetyl group in **1** in place of the acetyl group of aspirin would lead to a change from the protolytic ("classical general base") mode of catalysis observed for aspirin to the nucleophilic mode by which 3,5-dinitroaspirin reacts. The rate-determining step in both the mechanisms appears to be intramolecularly base-catalyzed attack of water on a carbonyl group[6,7] (the ester function in aspirin and the anhydride function in the species derived from intramolecular migration of the acetyl group in dinitroaspirin) as shown in transition-state structures **2** and **3**. Both processes should there-



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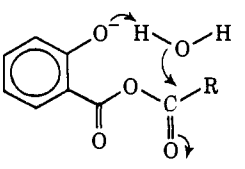

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(11) P. M. Laughton and R. E. Robertson in "Solute-Solvent Interactions," J. F. Coltzee and C. D. Ritchie, Ed., Marcel Dekker, New York, N. Y., 1969, Chapter 7.

fore be about equally affected by a change of R from CH_3 to Cl_2CH and the balance should remain in favor of **2**.

Proton Inventory of the Transition State. As Kresge has shown in a very lucid derivation,[12] reactions in mixtures of light and heavy water (atom fraction n of deuterium) yield rate constants k_n which are given in general by eq 4. Here the numerator contains con-

$$k_n = k_0 \prod_i^{TS}(1 - n + n\phi_i^*)/\prod_j^{RS}(1 - n + n\phi_j) \quad (4)$$

tributions from each exchangeable hydrogenic site of the transition state and the denominator from each such site of the reactant state. The ϕ_i^* and ϕ_j are *isotopic fractionation factors* for these individual sites and are defined by $\phi_k \equiv [(D/H)_k/(D/H)_{solv}]$, *i.e.*, they measure the deuterium preference for the site in question relative to the deuterium preference of a site in the solvent. When the isotopic reactants are all solvent molecules, as in this case, the denominator becomes unity. Then it is simple to see that ϕ_i^* is just the *inverse isotope effect contribution of the ith hydrogenic site of the transition state;* for example, if there is only one transition-state site which produces an isotope effect, the rate constant k_n will be the mole-fraction-weighted average of k_H (for pure H_2O) and k_D (for pure D_2O) as in eq 5. In the more general case

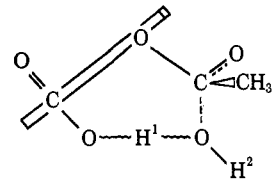
$$k_n = k_H(1 - n) + k_D(n) = k_H\left(1 - n + n\frac{k_D}{k_H}\right) = k_H(1 - n + n\phi) \quad (5)$$

of ν positions, each of which produces a particular isotope effect $(k_D/k_H)_i \equiv \phi_i^*$, k_n contains a factor for each position (eq 6). The rate constant k_n will be a

$$k_n = k_0 \prod_i^{\nu}(1 - n + n\phi_i^*) \quad (6)$$

nonlinear function of n (a polynomial of order ν) *except* in the special case when the solvent isotope effect arises from a single hydrogenic site in the transition state. Then and only then $k_n(n)$ will be linear.

The linear plot of Figure 1 and the linear fit of eq 3 show that intramolecular carboxylate catalysis of dichloroaspirin hydrolysis involves *one proton* which is isotopically distinct from water in the transition state and which produces an isotope effect $k_H/k_D = 2.17$. Adopting structure **2** for the transition state and ascribing the one-proton isotope effect to the bridging proton (H^1 in **4**), we are drawn to the conclusion that H^2 can yield no isotope effect. This is consistent with a smooth conversion of H^2 from a reactant water proton ($\phi = 1$) to a hydroxyl proton in the tetrahedral inter-



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mediate, also with $\phi = 1$.

(12) A. J. Kresge, *Pure Appl. Chem.*, **8**, 243 (1964).

One-Proton Solvation Bridges. A closely analogous reaction which has been studied in mixed isotopic solvents is the (intermolecularly) acetate ion catalyzed mutarotation of tetramethylglucose.[13] Although treated differently by Huang, Robinson, and Long, the data also fit a linear relationship quite closely and most strikingly give a solvent isotope effect $k_H/k_D = 2.23$, almost exactly equal to that for the dichloroaspirin reaction. Furthermore, in Johnson's tabulation of general-catalyzed ester hydrolysis reactions,[14] all cases of carboxylate catalysis ascribed to the protolytic category show solvent isotope effects in the range $k_H/k_D = 2.2 \pm 0.5$.

This suggests that the catalytic mechanism in all these reactions is the same, that it involves a single bridging proton between the catalytic function and the reorganizing substrate function (or *core*), and that the net binding of this bridging proton is reduced in the transition state sufficiently to produce an isotope effect around 2.2. On the assumption of rough cancellation of bending-frequency contributions between reactant and transition states, this corresponds to reduction of the OH stretching frequency to 2500 cm^{-1} in the transition state.

We conclude therefore that general-base catalysts in reactions of this type derive their catalytic power from formation of an unusually strong hydrogen bond to the reorganizing structure (reacting-bond region[15]) of the transition state. We call this a "one-proton solvation bridge."

Experimental Section

Materials. Potassium chloride (Matheson Coleman and Bell ACS Reagent), anhydrous sodium acetate (J. T. Baker Analyzed Reagent), glacial acetic acid (Fisher Reagent Chemical), salicylic acid (Baker AR), and dichloroacetic anhydride (Eastman Organic Chemical practical grade) were used as obtained. Acetonitrile (Fisher) was dried over molecular sieves before use. Deuterium oxide was from Diaprep, Inc., labeled as 99.8% minimum isotopic purity. Tap distilled water was glass distilled once from basic potassium permanganate solution, boiled to remove gases, and cooled with an Ascarite filter attached to prevent absorption of carbon dioxide. Solutions in light and heavy water and their mixtures were prepared by weight. The deuterium contents of mixtures were confirmed by Mr. Josef Nemeth, Urbana, Ill.

***O*-Dichloroacetylsalicylic acid (1)** was prepared from dichloroacetic anhydride and salicylic acid.[16] Two recrystallizations from benzene yielded colorless needles melting at 128° (lit.[17] 126–127°).

Kinetics. Rate measurements were performed with a Beckman DB-G ultraviolet–visible spectrophotometer equipped with a constant-temperature apparatus to maintin the cell temperature at 25.0 ± 0.1°. The release of salicylate ion was followed at 299 nm. A 3-ml quartz cuvette was filled with the desired buffer and allowed to attain temperature equilibrium. Into the cuvette was injected 25 μl of a solution of ~10 mg of dichloroaspirin substrate dissolved in 1 ml of dry acetonitrile. First-order rate constants were determined from the time–absorbance graph either by plotting $\log(A_\infty - A_0/A_\infty - A_t)$ *vs.* time or from a computer program which calculates first-order rate constants from given time and absorbance values.

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(14) S. L. Johnson, *Advan. Phys. Org. Chem.*, **5**, 275 (1960).
(15) C. G. Swain and E. R. Thornton, *J. Amer. Chem. Soc.*, **84**, 817 (1962); E. R. Thornton, *ibid.*, **89**, 2915 (1967).
(16) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Jap.*, **38**, 1979 (1965).
(17) F. Bergmann and L. Haskelberg, *J. Amer. Chem. Soc.*, **63**, 1437 (1941).