bead as the working electrode and an SCE electrode with a salt bridge as the reference.

Electron Spin Resonance Spectroscopy. The ESR apparatus consisted of a modified x-band Varian spectrometer employing 100-kHz field modulation.[30] ESR signal averaging and the spectral computer simulations were performed on an AT&T PC6300 microcomputer equipped with an 8087 numeric coprocessor.[31] Hyperfine splitting (hfs) constants are given in gauss (G). The radicals were generated by electrochemical or zinc metal reduction of acetonitrile or dimethylsulfoxide solutions of the appropriate parent compound. In all cases, the appearance potential for the ESR signal coincided closely with the first reduction wave observed in the cyclic voltammetry. The experimental and computer-simulated spectra obtained for **9** are included as supplementary material.

(30) Marshall, J. H. *J. Phys. Chem.* **1974**, *78*, 2225.
(31) Glarum, S. H., unpublished results.

Acknowledgment. We are grateful to W. D. Reents for exact mass measurements and S. H. Glarum for consultations on the ESR spectroscopy.

Registry No. **3**, 56496-29-8; **4**, 98304-01-9; **5**, 98304-02-0; **6**, 98330-18-8; **7**, 98330-19-9; **8**, 98304-03-1; $9^+BPh_4^-$, 98304-00-8; $9^+BF_4^-$, 98304-04-2; 10^+, 69461-26-3; 11^+, 98304-05-3; 12^+, 75961-51-2; 13^+, 68377-40-2; 14^+, 98304-06-4; hexachlorocyclotriphosphazene, 940-71-6.

Supplementary Material Available: Experimental and computer simulated spectra of **9** and details of the structure solution of 9^+, BPh_4^- (26 pages). Ordering information given on any current masthead page.

Solvation Effects on the Alkaline Hydrolysis of Some *p*-Nitrophenyl Esters

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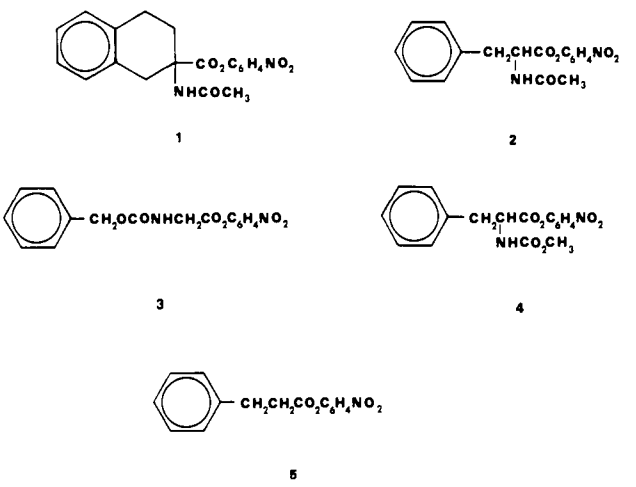
Abstract: The alkaline hydrolyses of six *p*-nitrophenyl esters were investigated. Two of the esters [2-acetamido-1,2,3,4-tetrahydro-2-naphthoate **1** and *N*-acetylphenylalaninate **2**] hydrolyze by a mechanism in which an oxazolinone is formed. The remaining esters [*N*-(benzyloxycarbonyl)glycinate **3**, *N*-(methoxycarbonyl)phenylalaninate **4**, 3-phenylpropanoate **5**, and acetate (NPA)] hydrolyze by a mechanism involving addition of hydroxide ion to the ester carbonyl group. Rather unexpectedly, esters **1–5** form an isokinetic series (estimated isokinetic temperature 87 K). Less unexpectedly, solvation changes upon activation appear to be responsible for the relationship: changes in ΔG^* for the series are linearly correlated with the logarithms of the inverse kinetic H_2O–D_2O solvent isotope effects (KSIEs). Small, nonspecific reorganizations of solvent molecules about similar R-group structures appear to be important in maintaining the regularity of change in ΔG^* and to contribute significantly to the KSIEs. The dissimilarity between the NPA R group and those of the other esters may explain the departure of this ester from the isokinetic relationship. Solvation models generated to rationalize the KSIEs (measured in 50:50 H_2O–D_2O mixtures and in the pure isotopic waters) imply that some specific as well as nonspecific solvent reorganizations accompany heavy-atom reorganization upon activation. Cooperativity among these solvation processes in turn produces the observed isokinetic behavior.

Many excellent studies have focused on the mechanism of alkaline hydrolysis of esters.[1] For ordinary esters with good leaving groups such as *p*-nitrophenoxide, the addition of hydroxide ion to the ester carbonyl group is generally thought to be rate determining, although partial rate limitation by a step preceding the addition and involving desolvation of the reactant hydroxide ion has been proposed.[2] In contrast, hydrolyses of substituted phenyl esters of carboxylic acids containing α-*N*-acylamino functions proceed in discrete steps leading to formation of oxazolinone intermediates (Scheme I). In the formation of 5-phenyloxazolin-2-one from *N*-benzoylglycine *p*-nitrophenyl ester, the only example of the oxazolinone mechanism which has been thoroughly investigated,[3-5] the expulsion of the leaving group from a quasi-tetrahedral intermediate is completely rate-controlling.[5]

Alkaline hydrolysis of esters **1–5** and *p*-nitrophenyl acetate (NPA) includes examples of both kinds of ester acyl group structure. Thus, alkaline hydrolysis of esters **1** and **2**, with α-*N*-acylamino functions, is expected to be dominated by the oxazolinone pathway. The nucleophilic addition mechanism should predominate alkaline hydrolysis of esters **3–5** and NPA.



During the course of studies of the alkaline hydrolysis of esters **1–5** and NPA, we have discovered a limited isokinetic series whose members include representatives of both hydrolytic mechanisms. As a rule, compounds forming an isokinetic series react by identical mechanisms, and even this is often an insufficient condition.[6] It was therefore unanticipated that esters **1–5**, which span 3.8×10^3 times in reactivity and represent at least two distinct hydrolytic

(1) For a historical perspective, see: (a) Johnson, S. L. *Adv. Phys. Org. Chem.* **1967**, *5*, 237–330. (b) Kirby, A. J. In "Comprehensive Chemical Kinetics"; Bamford, C. H., Tipper, C. F. H., Eds.; Elsevier: New York, 1972; Vol. 10, pp 57–207. (c) Euranto, E. K. In "Chemistry of Carboxylic Acids and Esters"; Patai, S., Ed.; Interscience: New York, 1969; pp 505–588.
(2) Kovach, I. M.; Elrod, J. P.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 7530–7534.
(3) (a) de Jersey, J.; Willadsen, P.; Zerner, B. *Biochemistry* **1966**, *8*, 1959–1966. (b) de Jersey, J.; Kortt, A. A.; Zerner, B. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 745–750.
(4) Williams, A. *J. Chem. Soc., Perkins Trans. 2*, **1976**, 947–953.
(5) Matta, M. S.; Andracki, M. E. *J. Am. Chem. Soc.* **1985**, *107*, 6036–6039.
(6) Exner, O. *Prog. Phys. Org. Chem.* **1973**, *10*, 411–482.

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Scheme I

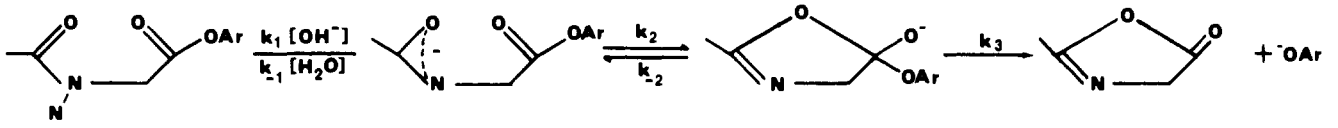

Table I. Apparent Second-Order Rate Constants and Kinetic Solvent Isotope Effects for the Alkaline Hydrolysis of Some *p*-Nitrophenyl Esters at 25 ± 0.1 °C.[a]

ester	k_0	$k_{0.5}$	k_1	$k_{0.5}/k_0$	k_1/k_0
1	(6.38 ± 0.10) × 10^4	(6.79 ± 0.13) × 10^4	(1.08 ± 0.04) × 10^5	1.06 ± 0.04	1.69 ± 0.06
2	(7.06 ± 0.11) × 10^3	(8.40 ± 0.11) × 10^3	(1.12 ± 0.03) × 10^4	1.18 ± 0.03	1.59 ± 0.06
3	(6.61 ± 0.11) × 10^2	(7.26 ± 0.00) × 10^2	(8.84 ± 0.07) × 10^2	1.10 ± 0.02	1.33 ± 0.03
4	(2.28, ± 0.06) × 10^2	(2.83 ± 0.00) × 10^2	(2.95 ± 0.04) × 10^2	1.24 ± 0.03	1.29 ± 0.05
5	(1.68 ± 0.03) × 10	(2.11 ± 0.03) × 10	(1.86 ± 0.04) × 10	1.26 ± 0.04	1.11 ± 0.05
NPA	(1.51 ± 0.02) × 10	(2.04 ± 0.08) × 10	(2.32 ± 0.03) × 10	1.35 ± 0.06	1.54 ± 0.03

[a] Apparent second-order rate constants in M^{-1} s^{-1}, obtained by dividing the pseudo-first-order rate constants by lyoxide ion activity. Buffered reaction mixtures contained 3.2% v/v acetonitrile. Ionic strength maintained at 1.0 with KCl.

mechanisms, would exhibit isokinetic behavior. In this paper, we explore the reasons for the isokinetic relationship, with emphasis on the role of solvent effects. The results of these studies allow us to infer the kinds of solvation processes that attend the hydrolyses.

Experimental Section

The *p*-nitrophenyl (*S*)-2-acetamido-1,2,3,4-tetrahydro-2-naphthoate (**1**) and *p*-nitrophenyl *N*-acetyl-L-phenylalaninate (**2**) were available from previous studies, as was *p*-nitrophenyl 3-phenylpropanoate (**5**).[7,8] The *p*-nitrophenyl *N*-(benzyloxycarbonyl)glycine (**3**) and NPA, used without further purification, were Sigma products.

Treatment of D-phenylalanine with methyl chloroformate[9] yielded *N*-(methoxycarbonyl)-D-phenylalanine, which has coupled with *p*-nitrophenol using dicyclohexyl carbodiimide in ethyl acetate to produce *p*-nitrophenyl *N*-(methoxycarbonyl)-D-phenylalaninate (**4**), mp 101–102 °C [lit.[9] mp 99 °C]; recrystallization was from chloroform–hexane. The stereochemical designations, irrelevant to the present studies, are not included in the text.

Alkaline hydrolyses of esters **1** and **2** were carried out in dilute (30–50 mM) phosphate buffers (KH_2PO_4/Na_2HPO_4) of pL 7.0–7.9 (L = H and D), while those of esters **3–5** and NPA employed dilute bicarbonate–carbonate buffers ($NaHCO_3/Na_2CO_3$) of pL 9.5–10.5. Ionic strength was maintained at 1.0 with KCl. The pL values employed were chosen to give convenient hydrolysis rates. Methods used to prepare solutions and determine pseudo-first-order rate constants and lyoxide ion activity have been fully described elsewhere.[5] The apparent second-order rate constants given in the text are the pseudo-first-order constants divided by lyoxide ion activity. Experiments at each set of conditions were carried out at least in triplicate.

Reversed-phase HPLC of esters **1–5** and NPA utilized a Beckman 112 solvent delivery module and a Beckman 160 UV absorbance detector at 260 nm. The recorder was a Hewlett-Packard 5880A Series GC terminal, level 4. The injector loop had a volume of 10 μL and the C-18 column was an Altex Ultrasphere-ODS, 4.6 mm × 15 cm, with column material of 5-μm particle size. The mobile phase, 70% acetonitrile and 30% 0.01 M sodium acetate–acetic acid buffer, adjusted to pH 4.7, was filtered through a 0.45-μm membrane filter (Metricel Alpha 450, Gelman) before use. A flow rate of 0.5 mL/min was maintained throughout. Capacity factor's k' were calculated from the relationship[10]

$$k' = (T_R - T_0)/T_0$$

where T_R is the elution time of a retained substance (the ester) and T_0 is the elution time of an unretained substance. The very hydrophilic compound formamide was used as the unretained substance.

Results

Apparent second-order rate constants k_n, where n is the mole fraction of D_2O in the aqueous solvent, were obtained at 25 °C for the alkaline hydrolases of esters 1–5 and NPA in H_2O (k_0), in D_2O (k_1), and in an isotopic water mixture containing equal mole fractions of H_2O and D_2O ($k_{0.5}$). The hydrolyses were invariably first-order in lyoxide ion and ester to at least 90% reaciton; catalysis by buffer species, lyonium ion, and water was negligible. These constants were used to calculate solvent isotope effects k_1/k_0 and $k_{0.5}/k_0$ for each ester. Table I lists the rate constants and the kinetic solvent isotope effects (KSIEs), which are all inverse. The higher reactivity of esters **1** and **2** compared with **3–5** and NPA is diagnostic of the oxazolinone mechanism for hydrolysis of the former esters.

Table II. Activation Parameters for the Alkaline Hydrolysis of Some *p*-Nitrophenyl Esters in Aqueous Solution[a,b]

ester	ΔG^*, kcal/mol	ΔH^*, kcal/mol	ΔS^*, eu	k'
1	10.9	14.5	12.2	1.43
2	12.2	14.8	8.6	0.99
3	13.6	15.3	6.0	1.38
4	14.2	12.2	−6.8	1.53
5	15.8	10.6	−17.3	3.10
NPA	15.8	14.3	−5.0	1.04

[a] ΔG^* and ΔS^* at 298 K. $\Delta G^* = RT \ln (K_bT/hk_0)$; ΔH^* was determined by multiplying −1.987 cal/(deg mol) by the slope of the line obtained from a graph of $\ln (k_0^T/T)$ vs. $1/T$. $\Delta S^* = (\Delta H^* - \Delta G^*)/T$. [b] The parameter k' is the reversed-phase HPLC capacity factor, as described in the text.

Additional k_0 were obtained at two or more additional temperatures betwen 15 and 33 °C. These, with k_0 acquired at 25 °C, gave linear Eyring plots from which the ΔH^* and ΔS^* values of Table II were derived. Table II also reports the reversed-phase HPLC capacity factor k' determined for each ester in aqueous acetonitrile.

Discussion

Isokinetic Relationship. The first five entries of Table II, listed in order of decreasing reactivity, exhibit a trend in ΔH^* and ΔS^* which is characteristic of enthalpy–entropy compensation.[6] Although this result was unanticipated, we were sufficiently impressed by the regularity of the trend to pursue a more rigorous test. Traditional graphs of ΔH^* vs. ΔS^* are inherently unreliable for assessing compensation because of the mutual dependence of the parameters.[6] We therefore graphed log k_0 at two temperatures ($T_2 > T_1$) for each ester (Figure 1).[6] A short interpolation or extrapolation occasionally was required to obtain the rate constant at a desired temperature, but this should not have a material effect on the graph.

Visual inspection of Figure 1 shows that NPA deviates from a straight line drawn between the other data points, and this ester was omitted from subsequent calculations. The regression line for esters **1–5** has slope b of 1.021 ± 0.028 (correlation coefficient $r = 0.998$, F ratio 1343, significant at the 99% level of confidence).

The isokinetic temperature, β, was estimated from eq 1.[6] The value of β from eq 1 is 87 K, but the uncertainty is from −48 to 145 K. Consequently, we are unable to say from these data that

$$\beta = T_1T_2(1 - b)/T_1 - bT_2 \quad (1)$$

temperature dependence reflects compensating enthalpy and en-

(7) Matta, M. S.; Rhode, M. F. *J. Am. Chem. Soc.* **1972**, *94*, 8573–8578.
(8) Matta, M. S.; Staley, D. D. *J. Biol. Chem.* **1974**, *249*, 732–737.
(9) Elmore, D. T.; Smith, J. J. *Biochem. J.* **1965**, *94*, 563–568.
(10) McCall, J. M. *J. Med. Chem.* **1975**, *18*, 549–552.

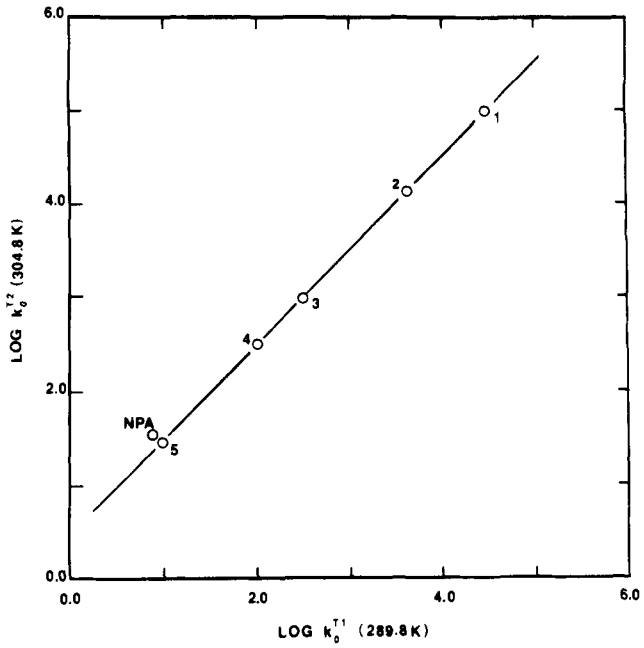

Figure 1. Graph of the logarithms of the apparent second-order rate constants k_0 for esters **1–5** and NPA at two temperatures. The data point for NPA was omitted in computing the regression line.

tropy ($\infty > \beta > 0$) or isoenthalpic behavior, which gives a slope of unity (β = 0 K) in the graph of log k_0^{T1} vs. log k_0^{T2}.[6] The uncertainty in β is in large part the result of the high sensitivity of this parameter to a slope b near unity. Nevertheless, the departure of NPA from the regression line for esters **1–5** suggests that modestly deviant data points can be detected. Experimental errors in k_0^{T1} and k_0^{T2} are liberally estimated to be ±5% of the mean values. If we assume that the error in the rate constants is confined to the higher temperature coordinate, the value of k_0^{T2} for NPA is too large by ~18%. The next largest deviation is by (*Z*)-glycinate **3**, for which the residual of k_0^{T2} is only slightly larger than the error estimate (~6%). Residuals of k_0^{T2} for the remaining four esters are well within the error limits (0.0–3.3%). Thus, we consider a limited isokinetic relationship to be established for esters **1–5**; the major uncertainty resides in the value of the isokinetic temperature, and not in whether such a temperature exists. This relationship is not shared by NPA.

Clearly β lies well below the range of experimental temperature, indicating that selectivity increases at higher temperatures. Isokinetic temperatures below the experimentally accessible range are characteristic of entropy control; i.e., the fastest reaction has the most positive ΔS^*.[11] Entropy control in turn suggests a strong influence of solvation in producing the observed isokinetic behavior. Because isokinetic behavior indicates regularity of the change in ΔS^* (and ΔH^* if such exists) throughout the series, further aspects of the relationships between this behavior and solvation effects can be probed by using ΔG^*, which is more accurately and precisely determined than either ΔH^* or ΔS^*.[6]

Relationship between ΔG^* and Solvent Isotope Effects. It is easily shown that the incremental change in ΔG^* for a reaction conducted in H_2O and D_2O is proportional to the logarithm of the kinetic solvent isotope effect (KSIE). A consequence of the solvation theory of isokinetic behavior is that values of ΔG^* and the logarithms of the KSIEs for the members of an isokinetic series should be linearly related. Figure 2 shows this relationship for esters **1–5** and NPA. The data points for esters **1–5** describe a straight line (r = 0.993, F ratio 222, significant at the 99% level of confidence). Figure 2 confirms the importance of solvation in producing the isokinetic relationship[12] and suggests that the

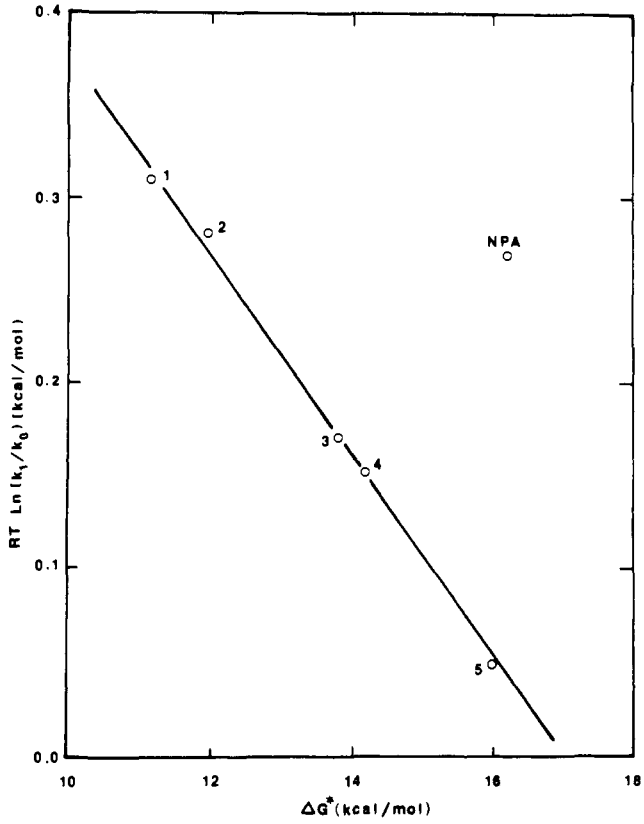

Figure 2. Logarithms of solvent isotope effects vs. the free energies of activation in protium oxide.

nature of the solvent reorganization (SR) processes, and how they produce isokinetic behavior, might be revealed by a detailed examination of the origins of the KSIEs.

R-Group Hydrophobicity and KSIEs. NPA, with its nearly minimal acyl R group, departs significantly from the isokinetic relationship shared by esters **1–5**. This hints that upon activation, solvation changes about the R-group aromatic rings and other structural features of esters **1–5** help to maintain linearity in the relationship. We therefore investigated the possibility of a correlation between ester reactivity and R-group hydrophobicity.

Hydrophobicities of organic compounds may be obtained from the distribution of the compound between an immiscible solvent, usually octanol, and water.[13] Because of the hydrolytic instability of *p*-nitrophenyl esters, however, the reveresed-phase HPLC capacity factors k' were determined. Values of k' are roughly proportional to octanol–water partition coefficients.[10] A compound need only be sufficiently stable to measure its elution time in order to obtain k'. The larger is k', the more hydrophobic is the ester, and the logarithm of k' is proportional to the free energy of transfer from the aqueous to the hydrophobic phase.

Figure 3 shows the relationship between log k' and ΔG^* for esters **1–5** and NPA. Although the data are necessarily sparse, lines drawn through the data points for esters **1** and **2** and esters **3–5** suggest that oxazolinone and nucleophilic mechanisms are influenced by acyl group hydrophobicity but in an opposite sense. For esters **1** and **2**, which hydrolyze by the oxazolinone pathway, ΔG^* decreases in proportion to acyl group hydrophobicity. The converse relationship is observed for esters **3–5**, which are subject to nucleophilic catalysis. An interesting and possibly significant feature of Figure 3 is that the slopes of both lines are identical except for the sign; the hydrophobic effects of nucleophilic catalysis

(11) Bunnett, J. F. In "Investigation of Rates and Mechanisms of Reactions", 2nd ed.; Friess, S. L., Lewis, E. S., Weissberger, A., Eds.; Interscience: New York, 1961; Part 1, pp 204–210 (Vol. 8 of series "Technique of Organic Chemistry"; Weissberger, A., Ed.).

(12) Attempts to correlate KSIEs with activation parameters are uncommon. In the one other instance of which we are aware, a much poorer correlation than described here was obtained in the basic methanolysis of aryl methyl carbonates and aryl acetates, although in a general sense the KSIEs become more inverse as ΔS^* becomes more positive. See: Mitton, C. G.; Gresser, M.; Schowen, R. L. *J. Am. Chem. Soc.* **1969**, *91*, 2045–2047.

(13) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175–5180.

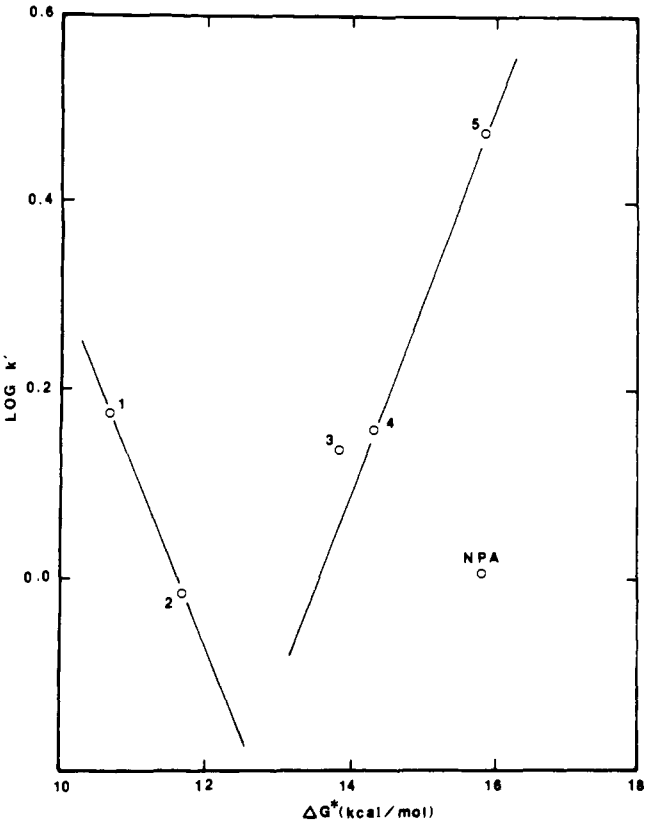

Figure 3. Logarithm of k', a measure of acyl group hydrophobicity, vs. free energy of activation, ΔG^*.

and of oxazolinone formation appear to be mirror images. The intersection of the lines in Figure 3 presumably represents the hydrophobicity of an ester for which the contribution of R-group-dependent SR is nil, regardless of mechanism.

If the magnitude of ΔG^* is related to ester hydrophobicity and the KSIE, it follows that SR associated with ester hydrophobicity contributes to the KSIE. With esters **1** and **2**, the direction of change in log k' is the same as the direction in change in the KSIE. That is, the most hydrophobic substrate has the most inverse KSIE. The converse is true for esters **3**–**5**. In terms of ester hydrophobicity, the isokinetic relationship is essentially pallindromic, with the most hydrophobic esters at the extremes. To achieve linearity in the isokinetic plot with esters **1** and **2**, the KSIEs for esters **3**–**5** *must* include one or more SR processes which are not the result of R-group hydrophobicity. Moreover, the hydrophobic and other SR processes *must* be mutually dependent for the isokinetic relationship to be maintained. Experimental support for these assertions comes from a more detailed analysis of the KSIEs, to which the remainder of this discussion is devoted.

Origins of KSIEs. Detailed information about SR processes in aqueous solution can sometimes be obtained from KSIEs determined in mixtures of H_2O and D_2O.[14] In this survey, the experimental determination of k_n/k_0 in the mixed solvents was limited to $k_{0.5}/k_0$, the midpoint solvent isotope effect.[14a] Three-point "proton inventory curves" of $k_{0.5}/k_0$ vs. n = 0.0, 0.5, and 1.0 (not shown) were constructed for each ester. The rudimentary proton inventory curves display a gradation from strongly "bulging down" or "bowl-shaped" for ester **1** to strongly "bulging up" or "dome-shaped" for ester **5**. In Figure 4, the size and direction of the midpoint curvature of the proton inventories is represented

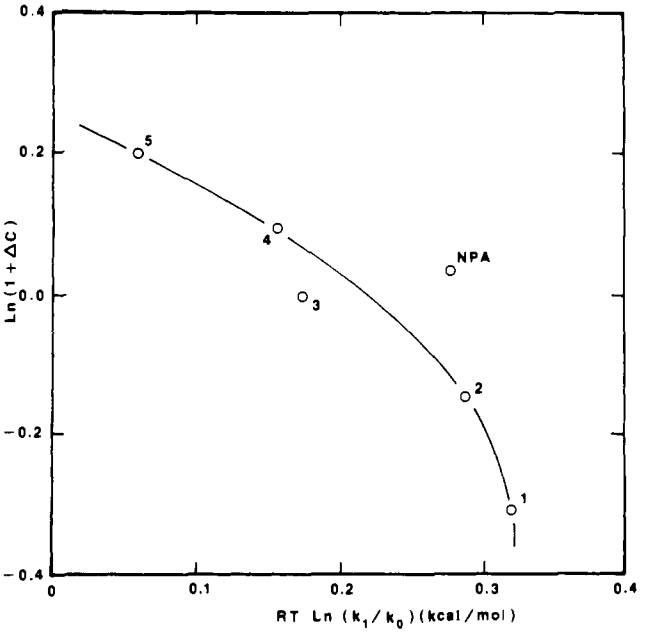

Figure 4. Logarithmic plot of midpoint curvature functions vs. solvent isotope effects.

by ΔC, which for each ester is the deviation at the midpoint from a straight line drawn between the KSIEs determined in the pure isotopic waters (eq 2). Dome-shaped proton inventories are

$$\Delta C = (k_{0.5}/k_0) - \tfrac{1}{2}[(k_1/k_0) + 1] \quad (2)$$

indicated in the figure by positive values of ln $(1 + \Delta C)$. Bowl-shaped and linear proton inventories, respectively, give negative and zero values. The graph of ln $(1 + \Delta C)$ vs. RT ln (k_1/k_0) for esters **1**–**5**, represented as curve linear, reveals a regular but complex relationship between $k_{0.5}/k_0$ and k_1/k_0.

Translation of proton inventories into detailed information about SR processes is usually attempted by using fractionation-factor theory. This technique of dissecting KSIEs holds that k_1/k_0 is the ratio of the product of the isotopic fractionation factors ϕ of all hydrogenic sites in the transition state (ϕ_i^T) to the product of those of the reactants (ϕ_j^R).[14] The ϕ_i^T and ϕ_j^R give the ratio of deuterium to protium at the ith and jth sites compared to the ratio of these isotopes in bulk water. The Gross–Butler equation (eq 3) permits the calculation of KSIEs in any mixture of isotopic waters containing a mole fraction of D_2O, when the appropriate transition-state (TS) and reactant-state (RS) ϕ's are known. In

$$k_n/k_0 = \overset{TS}{\prod}(1 - n + n\phi_i^T)/\overset{RS}{\prod}(1 - n + n\phi_j^R) \quad (3)$$

the more likely event that several values of k_n/k_0 and ϕ_j^R are known, eq 3 can be used to deduce ϕ_i^T and infer the kinds of SR that occur upon activation. From eq 3, $\phi_i^T > 1$ and $\phi_j^R < 1$ contribute to inverse KSIEs; $\phi_i^T < 1$ and $\phi_j^R > 1$ contribute to normal effects. A KSIE is produced by hydrogenic site only if the ϕ of the site changes upon activation to a kinetically significant TS. In reactions involving "generalized SR", with many sites each contributing a small isotope effect, it is convenient to count these contributions in the aggregate as the exponential Z^n (eq 4), where Z is the product of all normal and inverse effects of the generalized type.[14c]

$$k_n/k_0 = \overset{TS}{\prod}Z^n(1 - n + n\phi_i^T)/\overset{RS}{\prod}(1 - n + n\phi_j^R) \quad (4)$$

Equations 3 and 4 will in general give curved plots of k_n/k_0 vs. n. Information about solvation beyond that inferred from k_1/k_0 is furnished by analysis of this curvature in terms of RS and TS contributions to the KSIE.

The simplest physical origin of any KSIE is one in which only generalized SR occurring upon activation contributes to the effect. Equation 4 collapses to $k_n/k_0 = Z^n$, so that a midpoint effect equal to the square root of k_1/k_0 is predicted. This simple model fails to explain the curvature encountered in some of the proton in-

(14) (a) Albery, W. J. In "Proton Transfer Reactions"; Caldrin, E., Gold, V., Eds.; Chapman & Hall: London, 1975. (b) Schowen, K. B. In "Transition States of Biochemical Processes"; Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1978. (c) Schowen, K. B.; Schowen, R. L. *Methods Enzymol.* **1982**, *87*, 551–606. (d) Schowen, R. L. In "Isotope Effects on Enzyme-Catalyzed Reactions"; Cleland, W. W., O'Leary, M. H., Northrup, D. B., Eds.; University Park Press: Baltimore, 1977. (e) Gold, V. *Adv. Phys. Org. Chem.* **1969**, *7*, 259–331.

ventories. Exclusively generalized SR always produces bowl-shaped proton inventory curves, with the degree of curvature dependent on the magnitude of k_1/k_0. For example, the largest observed KSIE, 1.69 for ester **1**, gives a predicted midpoint effect of 1.30 by using the generalized SR model. This is scarcely distinguishable from the value of 1.35 for a straight line, and much different from the experimental value of 1.06. More complex models must be considered.

In the nucleophilic mechanism, hydrogenic sites undergoing changes in fractionation upon activation conceivably include Z sites and specific sites. We expect SR about the R groups to be of a generalized kind, making a Z-sites contribution to the KSIE. More specific solvent-related effects contributing to the KSIE may include any or all of the following. In the reactants, the lyoxide ion exhibits nonunitary and complex fractionation in L_2O (L = H or D).[14e] Possible TS contributions to the KSIEs may result from (a) residual fractionation of the lyoxide ion, (b) proton transfers of general catalysis by lyoxide of neutral hydrolysis, (c) specific solvation of an incipient tetrahedral-like oxyanion, analogous to the specific solvation of the RS lyoxide ion, and (d) transfer of a single proton to a developing oxyanion in formation of a full-fledged tetrahedral intermediate.

The SR conceivably associated with oxazolinone formation is similar but somewhat simplified, since possibilities (a) and (b) can be omitted. In the rapid proton abstraction that initiates the reaction, the lyoxide ion and its specifically solvating waters will have been transformed to bulk water ($\phi_w = 1.0$)[14] by the time the rate-controlling step is reached. Points (c) and (d) change to refer to the breakdown of a tetrahedral-like oxyanion and the transfer of a single proton from a full-fledged tetrahedral intermediate, respectively.

Solvation Models. Bulging proton inventory curves can be the result of contributions to the KSIE by multiple steps, each of which contribute to rate limitation.[14b,c] In attempting to fit various solvation models to the experimental proton inventory curves, however, we initially chose to consider that the proton inventory data for each ester are for an elementary rate-controlling step. Extant experimental evidence supports the choice for oxazolinone formation.[4,5] Our view is that for nucleophilic catalysis by hydroxide ion, the weight of experimental evidence does not support partial rate limitation by an uncoupled step involving desolvation of hydroxide ion and preceding addition of the nucleophile to the ester carbonyl.[15] Our objective then was to find a single solvation model, expressed in terms of the Gross–Butler equation, which could account for the various relationships already described for esters **1–5**. We tested and rejected many models in seeking this objective. Only our most satisfactory model is reported here.

We find that the best all-around fit to the overall and midpoint isotope effects is given by eq 5. The right-hand side of eq 5 was generated from known values of ϕ^R for the lyoxide ion, chemical intuition, and trial and error. NMR data[20] suggest that in aqueous

$$k_n/k_0 = Z^n(1 - n + n\phi^T)^3/(1 - n + 0.70n)^3 \qquad (5)$$

solution, the lyoxide ion exists as $[L_aOL_b]_3OL_c^-$, with $\phi_a = 1.0$, $\phi_b = 0.70$, and $\phi_c = 1.25$. The denominator of eq 5 includes only ϕ_b, since ϕ_a does not contribute to the KSIE. The effect of ϕ_c was ignored because ϕ's average 1.25 for the –OH protons of *gem*-diols and hemiacetals,[21] which may be considered as models for the TSs leading to tetrahedral intermediates in the hydroxide addition mechanism. Contributions to the KSIEs from ϕ_c and its TS counterpart should therefore cancel. Neglect of ϕ_c in the oxazolinone mechanism cannot be justified on the same grounds. As previously stated, the lyoxide ion and its solvating waters will have been transformed to bulk water at the rate-controlling step, so that ϕ_c is fully expressed in the KSIE. In fitting the proton inventories to eq 5, however, the inclusion of ϕ_c for compounds **1** and **2** makes little difference in the final results.

Table III. Calculated $k_{0.5}/k_0$ for Alkaline Hydrolysis of Some *p*-Nitrophenyl Esters[a]

ester	Z	ϕT	$k_{0.5}/k_0$
1	0.58	1.0	1.24 (0.18)
2	0.55	1.0	1.21 (0.03)
3	1.0	0.77	1.10 (0.00)
4	2.66	0.55	1.24 (0.00)
5	4.50	0.44	1.28 (0.02)
NPA	2.90	0.57	1.34 (–0.01)

[a] Calculated by using $Z^n(1 - n + n\phi^T)^3/(1 - n + 0.70n)^3$; the parenthesized numbers are the difference between the calculated and the mean experimental $k_{0.5}/k_0$.

An aggregate TS contribution to the KSIE was arrived at by calculating the arithmetic product of k_1/k_0 and RS contribution for a given ester. Using the TS contribution to k_1/k_0 as a constraint, and with $\phi_R = 0.70$, we calculated $k_{0.5}/k_0$ for various models of TS solvation. A Z-sites TS contribution seems to be required by the graph of log k' vs. ΔG^*. When Z is taken as the only TS contribution, however, exclusively bowl-shaped proton inventory curves are obtained. Therefore, an additional expression involving a hypothetical TS fractionation factor ϕ^T was incorporated into eq 5. This too seems required by the graph of log k' vs. ΔG^*. For each ester, again with the calculated aggregate TS contribution to k_1/k_0 as a constraint and $\phi^R = 0.70$, we iterated Z and ϕ^T to obtain the calculated value of $k_{0.5}/k_0$ that most closely matched the measured value. Early in these manipulations, we discovered that models similar to eq 5, but with linear or quadratic dependences on ϕ^T, are incapable of producing the strongly dome-shaped proton inventory curve for ester **5**. When the expression was expanded to cubic, the fit of the models to the experimental data significantly improved. Table III gives final values of Z and ϕ^T.

Except for ester 1, the correspondence between the experimental and calculated midpoint effects is good. Another step besides leaving group expulsion, possibly internal attack by oxygen of the α-amide anion (Scheme I), may partially limit the hydrolysis rate of **1**. Access of nucleophiles to the carbonyl group of this ester is known to be sterically hindered.[8]

The Z and ϕ^T values of Table III show in a general way how multiple solvation processes can conspire to produce isokinetic behavior in a complex reaction series. In going from ester **1** to ester **5**, the series switches from normal to inverse Z-sites TS contributions, creating a V-shaped dependence on ΔG^* reminiscent of the graph of log k' vs. ΔG^*. The value of k_1/k_0 declines along with Z in going from ester **1** to ester **2**. Although eq 5 does not succeed in simulating them, the solvation processes that accompany hydrolysis of **1** apparently act to maintain the isokinetic behavior of this ester. For esters hydrolyzing by rate-determining hydroxide ion addition, the incursion of another, mutually dependent TS

(15) Evidence for the influence of hydroxide ion desolvation on rates of alkaline ester hydrolysis comes from the observation that in comparison with other nucleophiles, hydroxide ion is less reactive than expected from its pK_a.[16] The basis for the argument that this desolvation may occur in a step completely decoupled from the addition of the nucleophile to the ester carbonyl group[2] is the small β-deuterium kinetic isotope effect (β-D-KIE) on alkaline hydrolysis of NPA and its trideuterated analogue. This has been taken as evidence for a heavy-atom TS with little progress toward a tetrahedral intermediate or two (or more) steps contributing to rate limitation, one of which manifests a β-D-KIE and another which does not. The latter step, if such exists, could be hydroxide ion desolvation. An alternative explanation of the small β-D-KIE for NPA, however, is that a single rate-determining step, carbonyl addition, is operative but that the effects of deuterium substitution in NPA are not additive.[17] Work in progress in our laboratory indicates that the β-D-KIE for ester **5**, which is identical in reactivity with NPA, is consonant with extensive rehybridization of the ester carbonyl group. Moreover, since the energy cost of the putative desolvation step should be constant, we would expect curvature in Hammett plots as the fractional contribution of the carbonyl addition step to rate limitation declines for good leaving groups. This is not observed. Hammett plots are linear ($\rho \simeq 1.0$) for substituted phenyl *N*-(methoxycarbonyl)phenylalaninates (unpublished results) and 3-phenylpropanoates,[18] as well as for acetates.[19]

(16) Hupe, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 541–464.
(17) Kovach, I. M.; Hogg, J. L.; Raben, T.; Halbert, K.; Rodgers, J.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *106*, 1991–1999.
(18) Matta, M. S.; Greene, C. M.; Stein, R. L.; Henderson, P. A. *J. Biol. Chem.* **1976**, *251*, 1006–1008.
(19) Fersht, A. R. *Ibid.* **1971**, *93*, 3504–3515.
(20) Gold, V.; Grist, S. *J. Chem. Soc., Perkin Trans. 2* **1972**, 89–95.
(21) Mata-Segreda, J. G.; Wint, S.; Schowen, R. L. *J. Am. Chem. Soc.* **1974**, *96*, 5608–5609.

solvation process produces a normal effect which more than compensates the inverse Z-sites effect. The total TS contribution is increasingly normal, so that k_1/k_0 continues to decline, and the isokinetic relationship continues to be maintained. The normal ϕ^T-sites effect is insufficient to generate a normal overall KSIE, but for ester **5**, it comes close.

Physical Implications of Solvation Models. The complexity of the systems makes us reluctant to state firm conclusions about the physical meaning of the Z and ϕ^T values. Reasonable speculative interpretation is possible, however. The Z values are of course presumed to be produced by generalized SR about R groups. Taken literally, the cubic dependence with $\phi^T < 1$ in carbonyl addition suggests three hydrogenic sites. A proton is bound considerably more loosely (has smaller force constants) in each of these sites than in bulk water and somewhat more loosely than in the specifically solvating waters of lyoxide. This is not the behavior expected of ϕ^T for residual TS fractionation of the waters solvating the RS lyoxide ion. As addition of hydroxide to the ester carbonyl proceeds, protons of the solvating waters of the nucleophile should more resemble bulk water. Equation 5 therefore gives no evidence for residual solvation of the hydroxide ion at the rate-determining TS for esters **3–5**. The specific TS solvation suggested by eq 5 could be the result of interaction of one proton of each of three water molecules with the three incipient unshared electron pairs of a developing tetrahedral-like oxyanion. Perhaps one of these protons is eventually transferred to the oxyanion, creating a full-fledged tetrahedral intermediate.

The mutual dependence of Z and ϕ^T coincides with traditional views of esterolytic reactions. The increasing polarization of the carbonyl group on its way to a tetrahedral-like oxyanion probably induces specific solvation about the carbonyl group and generalized SR[22] about the proximate R group. These heavy-atom and SR processes should be tightly coupled. Thus, the regular decrease in ϕ^T in going from ester **3** to ester **5** may indicate a trend toward more extensive rehybridization from sp^2 to sp^3 at the ester carbonyl group (a "later" TS); a later TS should have stronger interactions of the specifically interacting waters with the more extensively rehybridized carbonyl group, leading to smaller values of ϕ^T. Generalized SR, which should be most pronounced with hydrophobic R groups, may be the most important feature in determining the reactivity of the p-nitrophenyl esters, and therefore reaction progress toward a tetrahedral intermediate. Electronic and steric differences in the R groups cannot account for the 40-fold difference in reactivity between **3** and **5**. Activation of **5** requires extensive net generalized SR, however, at an apparent cost of ~1.0 kcal/mol and a later heavy-atom TS. Activation of **3** requires essentially no net generalized SR, and the heavy-atom TS is reached earlier. In this view, the reactivity difference between **3** and **5** is mainly a hydrophobic effect.

The data of Table III for NPA suggest that the ϕ^T for this ester is similar to that of esters **3–5**. Thus, our results do not support an independent hydroxide desolvation step which contributes to rate limitation.[2] NPA apparently deviates from isokinetic behavior with esters **1–5** because the Z-sites SR about the methyl group is more extensive than expected on the basis of this ester's overall hydrophobicity. The saponification of NPA represents an instance in which the combination of Z, ϕ^T, and ϕ^R produces an essentially linear proton inventory. In isolation, this result could be mistaken as emanating from unitary ϕ^R and a single TS hydrogenic site.

By analogy with N-benzoylglycine p-nitrophenyl ester,[5] leaving group expulsion is almost certainly the only step contributing to rate limitation for ester **2**. The fact that nonunitary values of ϕ^T are not required to fit eq 5 to the proton inventory suggests a TS which closely resembles the oxazolinone and p-nitrophenoxide,[5] and all SR is by water-like solvent molecules. The failure of specific solvation of the oxyanion of the developing p-nitrophenoxide to appear in the Gross–Butler equation may reflect very different requirements for solvation of the localized negative charge on alkoxide ions and delocalized negative charge on phenoxide ions.[2,16]

Summary. The alkaline hydrolysis esters **1–5** represent a limited isokinetic series, even though the hydrolysis of **1** and **2** is dominated by oxazolinone formation and that of **3–5** is dominated by nucleophilic addition of hydroxide ion to ester carbonyl. The regularity of change in ΔG^* is ascribed to mutually dependent, compensating solvation processes occurring upon activation to the rate-limiting step or steps. In nucleophilic addition, three kinds of processes are tentatively identified: (a) desolvation of reactant hydroxide ion; (b) reorganization of solvent about the R group of $RCO_2C_6H_5NO_2$; (c) another process or processes which may be specific solvation of a developing oxyanion on the path to a tetrahedral intermediate. In oxazolinone formation, desolvation of the reactant hydroxide ion and generalized reorganization of solvent about R may occur, but no specific solvation or other hydrogenic process about a collapsing tetrahedral intermediate can be distinguished.

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(22) The value of $Z = 4.5$ for ester **5** seems very large, but the reorganization of only 16 water molecules, each generating an inverse effect of 1.1, or of 150 water molecules, each generating an inverse effect of 1.01, is enough to produce it. Large Z values recently have been invoked by Stein[23] to explain the shapes of proton inventory curves in some enzymatic reactions.

(23) Stein, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 6039–6042.