Acyl Substituent Effects on Rates of Acyl Transfer to Thiolate, Hydroxide, and Oxy Dianions

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Abstract: The rates of transfer of a series of polar aliphatic acyl groups ($-0.3 \leq \sigma^* \leq 1.05$) from *p*-nitrophenol to hydroxide, to the thiol anion of *N*-acetyl-L-cysteine, and to a series of phosphonate dianions have been determined in aqueous solution. The rate constants for the alkaline hydrolysis of ten *p*-nitrophenyl esters can be correlated reasonably well ($r = 0.981$) to the single-parameter Hammett–Taft equation based on the polar substituent constant. The reaction constant for the alkaline hydrolysis is $\rho^* = 2.9$ with an interval estimator (90% confidence) of ± 0.3. The ρ^* value for the thiolysis reaction is only slightly larger ($16 \pm 13\%$) than the corresponding value for the alkaline hydrolysis reaction. These kinetic ρ^* values are essentially identical with the equilibrium ρ^* for hydroxide or thiolate addition to aldehydes (Kanchuger and Byers). To the extent that gem diolate and thiohemiacetalate formation are appropriate model reactions for the formation of the anionic tetrahedral intermediates in the corresponding acyl transfer reactions, the magnitude of the kinetic ρ^* values suggests that the transition states for the alkaline hydrolysis and for the thiolysis reactions are similar, in geometry and charge distribution, to the tetrahedral intermediates. Acyl transfer from *p*-nitrophenol to phosphonate dianions involves an uncatalyzed nucleophilic displacement by the oxy dianion. The ρ^* value for the reaction of (chloromethyl)phosphonate with the *p*-nitrophenyl esters is 2.4 (± 0.3). The second-order rate constants for the reactions between the phosphonates and *p*-nitrophenyl acetate, *p*-nitrophenyl chloroacetate, and *p*-nitrothiophenyl acetate show a small sensitivity to the basicity (pK_{a2}) of the nucleophile ($\beta_{nuc} \sim 0.3$). An explanation of the magnitudes of the ρ^* and β_{nuc} values, and of the anomalously low reaction rates (relative to oxy monoanions and amines of comparable basicities) for acyl transfer to the phosphonates, is that electrostatic interactions and desolvation of the oxy dianion make substantial contributions to the activation energy barrier for nucleophilic attack.

Structure–reactivity relationships have been used extensively in mechanistic investigations of carbonyl displacement reactions.[1,2] The dependence of the rates of acyl transfer reactions on such parameters as acceptor and leaving group basicities and on isotopic substitution (primary and secondary kinetic isotope effects) is a valuable probe of the mechanism of this prevalent class of reactions. Many factors influence the nature of any chemical reaction however, and, therefore, no single probe can yield an unambiguous picture of the transition state. Linear free-energy relationships, involving various nucleophiles and leaving groups, have been used extensively to provide a clearer characterization of acyl transfer reactions, but less use has been made of polar aliphatic acyl substituent effects in the study of these reactions.

Displacement reactions at the carbonyl carbon of *p*-nitrophenyl acetate have been investigated with an extensive series of nucleophiles.[3,4] The reactivity of oxy anions is dependent on the basicity of the nucleophile. Most of the oxygen nucleophiles examined have been monoanions. Three oxy dianions examined by Jencks and Carriuolo[4] (phosphate, arsenate, and carbonate) show negative deviations (by more than an order of magnitude) from the Brønsted correlation ($\beta = 0.8$) based on the reaction of over 45 nucleophiles with *p*-nitrophenyl acetate. Since many biochemical reactions involve nucleophilic attack by an oxy dianion (phosphate), we were interested in the comparison of the mechanistic aspects of acyl transfer to oxy dianions with those of reactions involving acyl transfer to other nucleophiles. Phosphonates provide a structurally homogeneous set of oxy dianions which covers a reasonably wide range of pK_a values. We report here a study of the effect of phosphonate basicity on the rate of some acyl transfer reactions. The effects of polar aliphatic acyl substituents on the phosphonate-catalyzed hydrolysis of *p*-nitrophenyl esters are compared with the effects on acyl transfer to hydroxide and to *N*-acetyl-L-cysteine. The utility of the Taft reaction constant, ρ^*, as an index of transition state structure is discussed.

Experimental Section

Materials. *p*-Nitrophenyl Esters. The *p*-nitrophenyl esters of acetic (PNPA) and trimethylacetic acid were obtained from Aldrich Chemical Co. The *p*-nitrophenyl esters of propionic acid, butyric acid, and *N*-carbobenzoxyglycine were obtained from Sigma Chemical Co. *p*-Nitrophenyl formate was from Alfa and *p*-nitrothiophenyl acetate (PNTPA) was obtained from Polysciences, Inc. The purity of the *p*-nitrophenyl esters was checked by monitoring the absorbance at 400 nm before and after basic hydrolysis in 0.1 M NaOH.

***p*-Nitrophenyl chloroacetate** was prepared from chloroacetyl chloride (Aldrich) and *p*-nitrophenol in ethyl acetate containing ~4% (v/v) triethylamine (0 °C). The amine hydrochloride was removed by filtration and the ethyl acetate was removed by rotory evaporation. The residue was dissolved in ether and acidified with HCl gas to precipitate remaining traces of the triethylamine. After filtration the diethyl ether solution was concentrated by rotory evaporation. Addition of petroleum ether resulted in the formation of white crystals: mp 96–98 °C (lit.[5] mp 95–97 °C); NMR[6] ($CDCl_3$) δ 4.4 (s, 2 H), 7.5 (d, 2 H), and 8.5 (d, 2 H).

The remaining *p*-nitrophenyl esters were prepared by coupling *p*-nitrophenol to the corresponding carboxylic acids in the presence of dicyclohexylcarbodiimide in ice-cold ethyl acetate by the general method of Bodanszky and duVigneaud.[7] The esters were crystallized from hexane–toluene (1:1). Yields varied between 51% (bromoacetate) and 86% (4-bromobutyrate). The properties of the crystalline esters follow. *p-Nitrophenyl iodoacetate*: mp 80–82 °C (lit.[8] 76–77 °C); NMR ($CDCl_3$) δ 4 (s, 2 H), 7.5 (d, 2 H), and 8.5 (d, 2 H). *p-Nitrophenyl bromoacetate*: mp 88–91 °C; NMR ($CDCl_3$) δ 4.2 (s, 2 H), 7.6 (d, 2 H), and 8.6 (d, 2 H). *p-Nitrophenyl 4-bromobutyrate*: mp 55–58 °C; NMR ($CDCl_3$) δ 2.3 (q, 2 H), 2.9 (t, 2 H), 3.6 (t, 2 H), 7.5 (d, 2 H), and 8.5 (d, 2 H).

***p*-Nitrophenyl 3-bromopropionate** was prepared by adding dicyclohexylcarbodiimide (41 g, 0.2 mol) to an ice-cold solution of 3-bromopropionic acid (26.2 g, 0.17 mol) and *p*-nitrophenol (18.1 g, .13 mol) in

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500 mL of ethyl acetate. The mixture was stirred for 2 h at 0 °C and then at room temperature for an additional 4 h. The mixture was filtered and washed with water (2×, 300 mL) and dried over $MgSO_4$. The solvent was evaporated and the residue was mixed with 250 mL of ether. The small amount of precipitate (dicyclohexylurea) was removed by filtration and the ether was evaporated to yield 30 g of a pale yellow viscous oil. TLC indicated the presence of a small amount of impurity. However, since basic hydrolysis of this material produced nearly (96%) a stoichiometric amount of *p*-nitrophenoxide, no attempts were made to further purify the material; NMR ($CDCl_3$) δ 3.3 (t, 2 H), 3.7 (t, 2 H), 7.5 (d, 2 H), and 8.5 (d, 2 H).

***p*-Nitrophenyl 3-(dimethylphosphono)propionate** was prepared from the 3-bromopropionate ester via an Arbuzov reaction. Freshly distilled trimethyl phosphite (25 mL, 0.21 mol) was added to 22 g of the *p*-nitrophenyl 3-bromopropionate (~0.08 mol). The mixture was stirred and heated to initiate the reaction. Once reflux temperature (111 °C) was reached heating was stopped and the mixture was allowed to cool to room temperature. Excess trimethyl phosphite was removed by distillation at 3 mm, yielding a light red viscous residue. The residue gave two spots on TLC (silica gel, toluene/ether, 1:1), one near the origin and one (corresponding to *p*-nitrophenyl 3-bromopropionate) with an R_f of 0.78. The two components were separated batchwise on silica gel: The residue from the Arbuzov reaction was dissolved in ether and mixed with silica gel. The gel was washed with ether and then washed with acetone. The acetone filtrates were collected and evaporated to yield a pale orange oil (14 g). This material showed a single spot on TLC (CH_3OH/benzene, 4:1; acetone/$CHCl_3$, 1:9) and was ~95% pure as judged by release of *p*-nitrophenoxide after alkaline hydrolysis; NMR (acetone-d_6) δ 2.2 (d of t, 2 H), 3.0 (d of t, 2 H), 3.8 (d, 6 H) 7.5 (d, 2 H), and 8.5 (d, 2 H). J_{H-P} = 20 Hz.

Phosphonates. The disodium salt of fluorophosphate was obtained from Alpha and purified by the method of Nowak and Mildvan;[9] chloromethylphosphonic acid was obtained from Chem Service, West Chester, PA. Phosphonoacetic acid was prepared from (triethylphosphono)acetate (Aldrich Chemical Co.) by the method of Swyryd et al.[10] (Trichloromethyl)phosphonic acid was prepared by acid hydrolysis of the diethyl ester of the phosphonate. The diethyl ester was prepared by the Arbuzov reaction[11] between carbon tetrachloride and triethyl phosphite (Aldrich Chemical Co.). (2-Bromoethyl)phosphonic acid was prepared by acid hydrolysis of diethyl (2-bromoethyl)phosphonate (Aldrich Chemical Co.). Acid hydrolysis of dimethyl methylphosphonate (Alfa) and of diethyl ethylphosphonate (Pfaltz and Bauer) resulted in the corresponding phosphonic acids.[12] The acid hydrolysis conditions and the subsequent purification of the phosphonic acids were essentially those employed by Kluger et al.[13] for the preparation of methylphosphonic acid. Allylphosphonic acid was synthesized by the Arbuzov reaction between allyl bromide and trimethyl phosphite followed by iodotrimethylsilane-catalyzed hydrolysis[14] of the dimethyl ester so obtained.

The phosphonic acids (and the sodium salts) are very hygroscopic. The phosphonates were dried by suspending the crystals in benzene or toluene and azeotroping off the water. The samples were stored in vacuo over P_2O_5. The purity of the phosphonates was checked by TLC (PEI-F cellulose plates, 1 M NaCl) and by ^{31}P NMR (JEOL FX 60). The concentration of the phosphonate solutions used for the kinetic measurements was determined by potentiometric titration (Brinkmann pH meter Model E512, Metrohm AH combination glass electrode). The thermodynamic pK_{a2} values used were those of Kresge and Tang[15] (XPO_3^{2-}, X = CH_3^-, $ClCH_2$–), those of Huebel and Popov[16] (phosphonoacetic acid), or those obtained by the method of Kresge and Tang.[15]

Spectrophotometric measurements were carried out with a Beckman 3600 spectrophotometer (with variable temperature control accessory). This instrument was used for most of the kinetic measurements but for the few very rapid reactions (with rate constants >1 min^{-1}) a Dionex Model D-110 stopped-flow spectrophotometer [interfaced with a Biomation 810 transient recorder[17] and a strip-chart recorder] was used.

Methods. General. The ester solutions (~0.05 M) were made up in either dioxane (dried by being passed through an alumina column and stored under N_2) or acetonitrile (distilled from P_2O_5). The ionic strength of the reaction mixture was maintained with NaCl. The reactions were initiated by the addition of 5–10 μL of the ester solution to a thermally equilibrated cuvette containing 1.1 mL of the other reactants. The final ester concentration in the reaction mixture was generally in the range of $2–7 \times 10^{-4}$ M with the *p*-nitrophenyl esters and $\sim 3 \times 10^{-5}$ M with *p*-nitrothiophenyl acetate. The final concentration of organic solvent was less than 1% v/v. The rates were measured by observing the absorbance due to *p*-nitrophenoxide (400 nm) or to *p*-nitrothiophenoxide (412 nm). The reactions were first order for at least 3 half-lives and were generally followed through 5 half-lives. End points were checked by the addition of 10 μL of 5 N NaOH to the reaction mixture. First-order rate constants were determined graphically and by a least-squares fit from plots of ln $(A - A_t)$ vs. time, using 15 points/run. The internal standard deviations of the rate constants (within one run) were consistently less than ±0.8%. Rate constants were determined at least in triplicate for each reaction. Plots of the pseudo-first-order rate constant vs. the concentration of the thiolate species of *N*-acetyl-L-cysteine or the concentration of the phosphonate dianion were found to be linear and independent of pH.

Solvent Isotope Effects. Solutions were prepared in deuterium oxide (99.8%, Norsk Hydro, Oslo, Norway). The reaction components (other than the esters) were prepared by dissolving them in D_2O and evaporating the solution prior to making up the final reaction mixture. The pD values were estimated from the formula: pD = pH (meter reading) + 0.41,[18] and adjusted with either NaOD (40% in D_2O) or DCl (38% in D_2O). The concentration of ^-OD (or of ^-OH) was calculated from the measured pD(H) values and the thermodynamic quantities for the ionization of D_2O (and H_2O) reported by Shoesmith and Lee.[19] pD(H) fluxuations during any kinetic run did not exceed 0.05 units.

Thiolysis and Thiol Ester Hydrolysis. *N*-Acetyl-L-cysteine (Sigma Chemical Co.) was used in the thiolysis reactions. Solutions were prepared (in degassed water) immediately before use. The concentration of free thiol was determined with Ellman's reagent[20] and was found to decrease by less than 5% during the course of the longest kinetic runs (~5 h). Similarly, the hydrolysis product of the thiol ester (*p*-nitrothiophenoxide) was stable under most of the reaction conditions. However, *p*-nitrothiophenoxide is not stable in the presence of hydrogen phosphonate (phosphite). For example, in the presence of 0.66 M HPO_3^{2-} (pH 7.1, 37 °C) a rapid decrease in the absorbance at 412 nm was observed following the addition of *p*-nitrothiophenol ($\sim 3 \times 10^{-5}$ M). The absorbance decreased from 0.4 to 0.2 in 1.4 min in a non-first-order process. This decrease was not observed in the presence of any of the other phosphonates [i.e., the absorbance decreased by less than 20% (pH 7, 37 °C) in 2 h when the solution was not protected from exposure to air]. The hydrogen phosphonate catalyzed hydrolysis of *p*-nitrothiophenyl acetate was followed by the decrease in the thiol ester absorbance (monitored at 280 nm). The rate constant obtained in this manner is identical with the value obtained by removing aliquots of the reaction mixture at various times, adding the sample to a solution of Ellman's reagent [1 mM 5,5′-dithiobis(2-nitrobenzoic acid), pH 7], and measuring the absorbance at 412 nm.[21]

Taft Polar Substituent Constants. The σ* values for most of the acyl substituents were obtained from the compilation of Hansch and Leo.[23] The acyl substituent constant for carbobenzyloxyglycine ($PhCH_2OC(=O)NHCH_2$–, σ* = 0.53) was obtained from the substituent constant for the carbobenzoxyamino substituent [σ* = 1.46, calculated from the experimentally determined pK_a of carbobenzoxyglycine (=3.80) and the

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(21) The stoichiometric reaction of Ellman's reagent indicates the presence of a product containing a free sulfhydryl group. Incubation of *p*-nitrothiophenoxide (50 μM) with hydrogen phosphonate (0.66 M) at pH 7.1 for 2 h at 37 °C resulted in a decrease in absorbance at 412 nm from 0.68 to <0.02. Addition of Ellman's reagent (1 mM) resulted in a rapid, and stoichiometric, formation of 2-nitro-5-thiobenzoate. The UV spectrum of the reaction mixture (prior to the addition of Ellman's reagent) gave no indication of the presence of *p*-nitrophenol, *p*-nitrophenylphosphonic acid, or nitrobenzene. The rate of the reaction is enhanced as the pH is lowered. A possible explanation of the hydrogen phosphonate induced decrease in absorbance is deoxygenation of the nitro group (to an amino group) by the phosphite species, $P(OH)_3$, in a manner analogous to the deoxygenation of nitro and nitroso compounds by alkylphosphites.[22]
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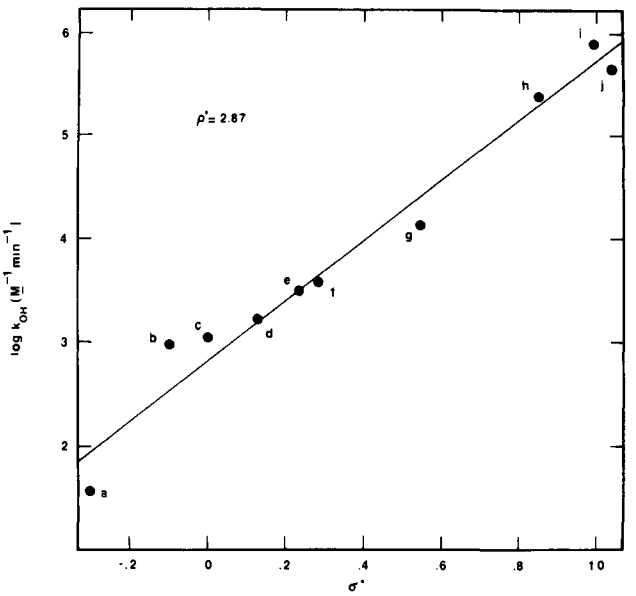

Figure 1. Taft plot for the specific base catalyzed hydrolysis of *p*-nitrophenyl esters (27 °C, μ = 0.5 M). The various acyl substituents are: (a) $(CH_3)_3C$–, (b) CH_3CH_2–, (c) CH_3–, (d) $Br(CH_2)_3$–, (e) $Br(CH_2)_2$–, (f) $(CH_3O)_2P(O)CH_2CH_2$–, (g) $C_6H_5CH_2OC(O)NHCH_2$–, (h) ICH_2–, (i) $BrCH_2$–, and (j) $ClCH_2$–. The slope of the line (least-squares fit) is 2.87 and the interval estimator of the slope (90% confidence) is 2.54–3.20.

equation of Fastrez[24] relating σ^* to the pK_a of substituted acetic acids] and the following relationship:[25]

$$\sigma^*_{XCH_2-} = 0.363\sigma^*_X - 0.003 \quad (1)$$

The σ^* value for $Br(CH_2)_3$– (=0.128 ± 0.006) was obtained by substitution of the value for $Br(CH_2)_2$– (σ^* = 0.36) into eq 1. Similarly, the σ^* value for the dimethyl phosphonoethyl substituent (=0.29 ± 0.02) was calculated based on the value of 0.80 for the $(CH_3O)_2P(=O)CH_2$– substituent.

Results

I. Polar Aliphatic Acyl Substituent Effects. A. Thiolysis and Specific-Base Catalysis. The rate of formation of *p*-nitrophenoxide from various *p*-nitrophenyl esters in the presence of hydroxide or of *N*-acetyl-L-cysteine (*N*-Ac-Cys) was examined as a function of pH (pH >6). Under these conditions the hydrolysis reaction is first order in [$^-$OH] and the pseudo-first-order rate constant is $k_{obsd} = k_{-OH}[^-OH]$, indicating no significant uncatalyzed hydrolysis. The pseudo-first-order rate constant in the presence of *N*-Ac-Cys is characterized by the following equation:

$$k_{obsd} = \frac{k_s[N\text{-Ac-Cys}]_{total}}{1 + 10^{(pK_a - pH)}} + k_{-OH}[^-OH] \quad (2)$$

The kinetically determined pK_a (9.2) is identical with the pK_a of *N*-Ac-Cys obtained by direct (spectrophotometric and potentiometric) titration under these reaction conditions (μ = 0.5 M, 27 °C). With the thiol concentrations employed (10^{-4}–10^{-3} *M*) the hydrolysis reaction was negligible relative to the thiolysis reaction.

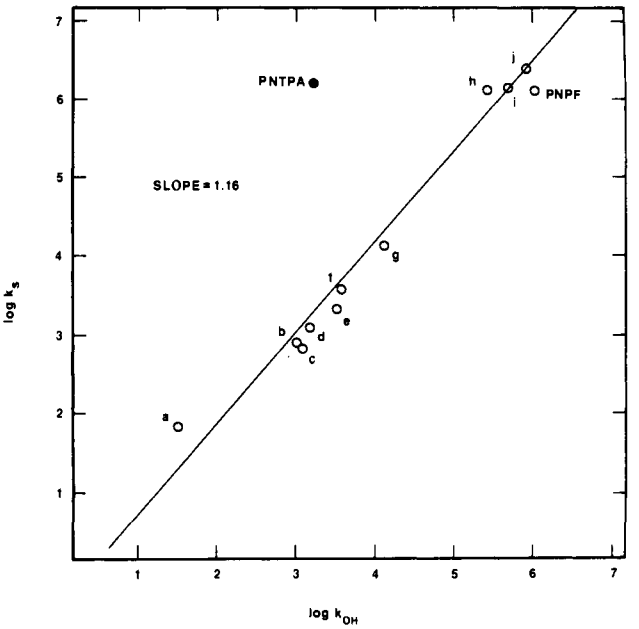

Figure 2. Correlation of the reactivity of *N*-acetyl-L-cysteine and of hydroxide with various acyl-substituted esters (27 °C, μ = 0.5 M). The ordinate is the logarithm of the second-order rate constant (M^{-1} min^{-1}) for the reaction of the thiolate anion with the esters. The substituents are the same as those in Figure 1 with the addition of the formyl ester (PNPF). The least-squares slope of the line is 1.16 and the interval estimator (90% confidence) is 1.03–1.29. The point for the thioester, PNTPA, is indicated for comparison and was not used in the correlation.

These results indicate that the thiolate anion, and not the protonated thiol, is the only species which reacts at a significant rate with these *p*-nitrophenyl esters.

The dependence of the second-order rate constant for the specific-base-catalyzed hydrolysis (log k_{-OH}) of various acyl-substituted *p*-nitrophenyl esters on the Taft polar substituent constant is illustrated in Figure 1. The data fit the linear free-energy relationship:

$$\log k_{-OH}\ (M^{-1}\ min^{-1}) = 2.87\ (\pm 0.32)\sigma^* + 2.83(\pm 0.20) \quad (3)$$
$$n = 10,\ r = 0.981$$

The figures in parentheses are the 90% confidence limits. The second-order rate constant for the reaction of the thiolate form of *N*-Ac-Cys shows a similar sensitivity to polar effects:

$$\log k_s\ (M^{-1}\ min^{-1}) = 3.43\ (\pm 0.42)\sigma^* + 2.76\ (\pm 0.20) \quad (4)$$
$$n = 10,\ r = 0.978$$

p-Nitrophenyl formate shows a positive deviation from each of these correlations (eq 3 and 4). This ester is ~50 times more reactive with hydroxide, and with *N*-Ac-Cys, than is predicted on the basis of the polar substituent constant for hydrogen (σ^*_H = 0.49). This deviant behavior of the hydrogen substituent is observed frequently in linear free-energy relationships. Hine[28] has pointed out the unreliability of σ^*_H as a measure of the polar character of the hydrogen substituent, and Hupe et al.[30] have recently analyzed the enhanced reactivity of formate esters, relative to substituted alkanoates. The formate ester, however, does not deviate from the correlation of log k_s with log k_{-OH} (Figure 2). This correlation is described by the equation

$$\log k_s = 1.16\ (\pm 0.13)\ \log k_{-OH} - 0.386\ (\pm 0.033) \quad (5)$$
$$n = 11,\ r = 0.9911$$

Equation 5 indicates that the specific-base-catalyzed hydrolysis

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(25) From a correlation of 20 well-characterized[23] polar substituent constants ($0 < \sigma^* < 3.56$): $\sigma^*_{XCH_2-} = 0.3625\ (\pm 0.019)\sigma^*_{X-} - 0.003\ (\pm 0.006)$; r = 0.9942. The figures in parentheses are the 95% confidence intervals. This equation is consistent with the customary value of 1/2.8 (=0.357) for attenuation by a methylene group.[26,27] However, there are limits to this generality.[28,29] For this reason, and in order to obtain a quantitative assessment of the uncertainties in the σ^* values, the decremental factor for an interposed methylene group was reevaluated with a large set of substituent pairs with properties similar to those of the substituents used in this study. The data used are given as supplementary material.
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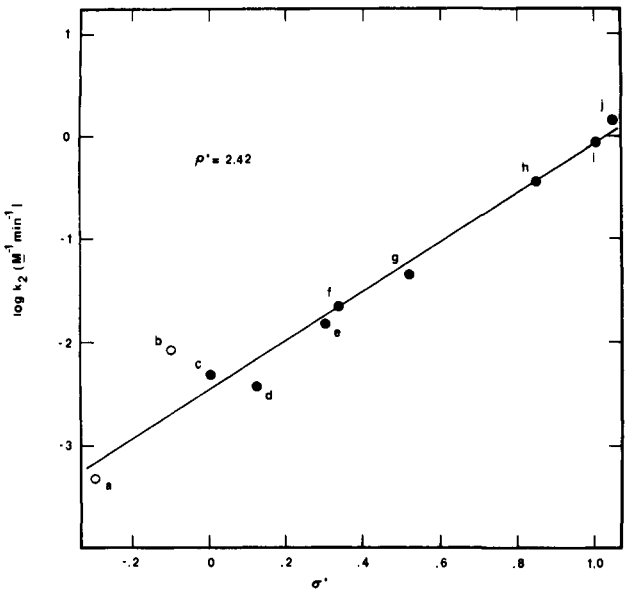

Figure 3. Taft plot for the reaction of (chloromethyl)phosphonate with various *p*-nitrophenyl esters (27 °C, μ = 2.8 M, pH 7.8). The letters indicate esters described in Figures 1 and 2.

and the thiolysis of *p*-nitrophenyl esters are nearly equally sensitive to the polar effects of the acyl substituent. The thiolester, *p*-nitrothiophenyl acetate (PNTPA), does not fit this correlation. The reaction of PNTPA with the anion of *N*-Ac-Cys is 700 times more rapid than is predicted on the basis of its reactivity with hydroxide. Similar deviations of thiolesters from linear free-energy relationships based on oxygen esters have been observed.[2,30,31]

B. Nucleophilic Attack by Phosphonates. The reaction of methyl phosphonate with *p*-nitrophenyl acetate (PNPA), with PNTPA, and with *p*-nitrophenyl 3-bromopropionate was examined in H_2O and in D_2O at various pL (pH or pD) values. There is no significant general-base catalysis in these reactions. For example, the pseudo-first-order rate constant for the reaction of methylphosphonate with PNPA is linearly dependent on the phosphonate buffer concentration (0.3 to 1 M) at pH 7.2. The pseudo-first-order rate constants for the reactions of methylphosphonate with these esters are characterized by the following relationship:

$$k_{obsd} = \frac{k_2^{lim}[P]_{TOT}}{1 + 10^{(pK_a - pL)}} + k_{-OL}[^-OL] \qquad (6)$$

where $[P]_{TOT}$ is the total concentration of the phosphonate. The pK_a (corresponding to the second ionization constant of methylphosphonic acid under the reaction conditions: 37 °C, μ = 2 *M*) is 7.62 (±0.08) in H_2O and 8.15 (±0.10) in D_2O. The solvent isotope effects for the specific-base-catalyzed hydrolysis of the three esters are identical, within the level of precision attained in these experiments, and equal to 0.6 (±0.1) = k_{OH}/k_{OD}. The second-order rate constants for the reaction of the phosphonate dianion with each of these esters (k_2^{lim}) in H_2O and in D_2O are identical: $k_{H_2O}^{lim}/k_{D_2O}^{lim}$ = 1.03 (±0.05).

The dependence of the rate of formation of *p*-nitrophenoxide from various *p*-nitrophenyl esters on the polar acyl substituent constant was examined in the presence of a phosphonate dianion. The reactions were carried out at pH 7.8 in the presence of 0.3–1.0 M (chloromethyl)phosphonate. Potentiometric titration of (chloromethyl)phosphonic acid, under the reaction conditions (27 °C and μ = 2.8 *M*), yields a pK_{a2} value of 5.9. Thus, at pH 7.8 the phosphonate is present in over 98% as the reactive dianionic species. Plots of the observed pseudo-first-order rate constant for *p*-nitrophenoxide formation vs. the (chloromethyl)phosphonate concentration are linear and the intercept (at $[ClCH_2PO_3^{2-}]$ = 0) yields a value of the rate constant for hydroxide attack. This value (at μ = 2.8 M) is approximately 15% larger than the value obtained at an ionic strength of 0.5 M for each of the esters examined. A Taft plot illustrating the dependence of the second-order rate constant for the (chloromethyl)phosphonate-catalyzed release of *p*-nitrophenoxide on the polar acyl substituent constant is shown in Figure 3. The equation of this line is

$$\log k_2\ (M^{-1}\ min^{-1}) = 2.42\ (\pm 0.29)\sigma^* - 2.49\ (\pm 0.24) \qquad (7)$$

$$n = 8,\ r = 0.9903$$

This equation was derived by using the data for all the alkyl-substituted esters with polar substituents (i.e., $\sigma^* \geq 0$). If the two nonpolar alkyl esters (*p*-nitrophenyl propionate and *p*-nitrophenyl pivalate) are included in the correlation analysis, the slope (=2.30) and the intercept (=2.40) are not significantly altered, but the correlation coefficient is reduced (r = 0.978). Although a poorer correlation is obtained when data with $\sigma^* < 0$ are included in the analysis, the single σ^* parameter accounts for over 95% (r^2 = 0.956) of the variance in the data.

The calculated rate constant (eq 7) for the reaction of (chloromethyl)phosphonate with *p*-nitrophenyl formate is 5×10^{-2} M^{-1} min^{-1} and the observed value is 1.8 (±0.4) M^{-1} min^{-1}. Thus, the positive deviation of the formate ester seen in the structure-reactivity correlations for the alkaline hydrolysis (eq 3) and thiolysis (eq 4) reactions is also apparent in the reaction of the (chloromethyl)phosphonate dianion with the *p*-nitrophenyl esters. The formate ester shows no significant deviation in the correlation between the rate constants for the (chloromethyl)phosphonate-catalyzed release of *p*-nitrophenoxide and those for the specific-base-catalyzed hydrolysis of the various acyl-substituted *p*-nitrophenyl esters:

$$\log k_2 = 0.80\ (\pm 0.07)\ \log k_{-OH} - 4.63\ (\pm 0.44) \qquad (8)$$

$$n = 11,\ r = 0.988$$

II. Effects of Phosphonate Basicity. The dependence of the second-order rate constant (k_2^{lim}) for the reaction of phosphonates

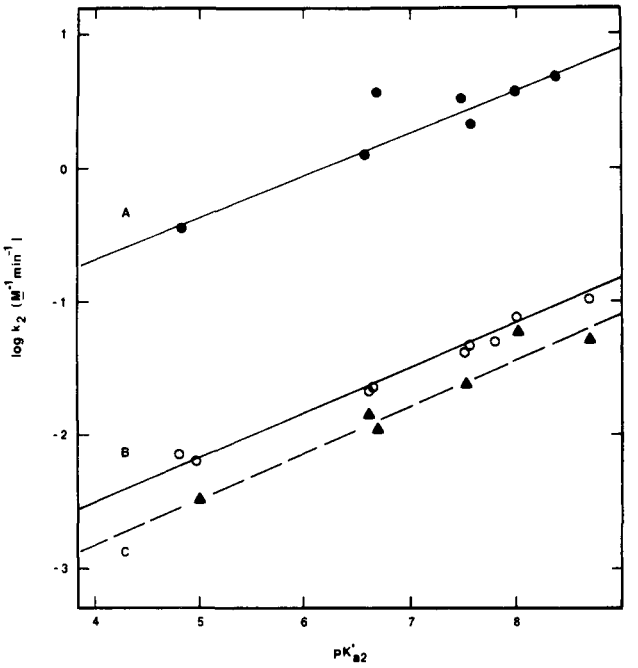

Figure 4. Dependence on basicity of the reactivity of phosphonates with: (A) *p*-nitrophenyl chloroacetate (27 °C, μ = 1.5 M), (B) *p*-nitrothiophenyl acetate (37 °C, μ = 2 M), and (C) *p*-nitrophenyl acetate (37 °C, μ = 2 M). The various phosphonates, XPO_3^{2-} (and thermodynamic pK_as) are: -F (4.8), $-CCl_3$ (4.93), -H (6.58), $-CH_2Cl$ (6.59), -OH (7.50, statistically normalized to correspond to the phosphonates, pK_{a2}^{corr} = 7.20 + log 2), $-CH_2CH_2Br$ (7.69), $-CH_2CH{=}CH_2$ (7.85), $-CH_3$ (8.00), $-CH_2CH_3$ (8.34), and $-CH_2CO_2^-$ (8.69). The Brønsted coefficient for each of these lines is ~0.3.

(31) Hupe, D. J.; Wu, D.; Shepperd, P. *J. Am. Chem. Soc.* **1977**, *99*, 7659–7662.
(32) (a) DeTar, D. F. *J. Org. Chem.* **1980**, *45*, 5166–5174. (b) DeTar, D. F. *J. Am. Chem. Soc.* **1980**, *102*, 7988–7990.

with PNTPA and with PNPA (μ = 2 M, 37°) on the pK_{a2} of the nucleophile is illustrated in Figure 4 (B and C). The logarithm of the rate constant for the PNTPA reaction is linearly dependent on the phosphonate pK_a:

$$\log k_2 = 0.31\ (\pm 0.03)\ \mathrm{p}K_{a2}^{*} - 3.69\ (\pm 0.08) \qquad (9)$$

$$n = 9,\ r = 0.992$$

In this series of reactions the pK_{a2} of each of the nucleophiles is greater than the pK_a of the thionitrophenol leaving group (pK_a = 4.5). A least-squares fit of the data with PNPA yields a slope of 0.34 (±0.05) (r = 0.982). Similarly, the reaction of the phosphonates with p-nitrophenyl chloroacetate (μ = 1.5 M, 27 °C) can be characterized by the Brønsted equation with β = 0.32 (±0.05) (r = 0.987). The pK_a of the p-nitrophenol leaving group is 7.14. Although the data for the reaction of the phosphonates with the p-nitrophenyl esters gives a reasonable fit to a linear dependence of log k_2 on pK_{a2} over the range of basicity examined, the data are not extensive enough to rule out the possibility of a break around pK_a = 7 (corresponding to ΔpK_a = 0).

Discussion

ρ_{acyl} as an Index of Transition-State Structure. The Taft reaction constant for the transfer of 10 acyl-substituted p-nitrophenyl esters to hydroxide is ρ^*_{acyl} = 2.9 (±0.3). Although the dependence of the specific-base-catalyzed hydrolysis of esters has been examined extensively as a function of the leaving group,[1] the effect of alterations in the structure of the acyl group on the hydrolysis rate has received only modest attention. Many of the investigations of ester hydrolysis have dealt with alkanoates containing alkyl group substitution at the acyl carbon. Basic hydrolysis of these esters shows a lack of a linear free-energy relationship based solely on the inductive substituent constant.[26,32,33] For example, Hancock et al.[34] have shown the importance of both steric and hyperconjugative effects, in addition to inductive effects, in the saponification of methyl esters (40% dioxane, 35 °C, ρ^* = 1.75). The nine compounds examined were methyl esters of aliphatic carboxylic acids. The contributions of factors such as steric and hyperconjugative effects, as well as solvation effects,[2,29-31,35] are expected to be more significant in those reactions involving compounds which cover a narrow range of σ^* values, especially if these values are near zero. Indeed, it has been cogently argued[29,36] that all alkyl groups should have identical inductive effects (i.e., σ^* = 0). It is necessary, therefore, to examine a series of compounds that cover a wide range of σ^* values in order to assess the importance of polar effects in a particular reaction. In this study the σ^* values for the various acyl substituents ranged from −0.3 to +1.05, corresponding to a span of reactivities (in the specific-base-catalyzed hydrolysis of p-nitrophenyl esters) of nearly four orders of magnitude. The largest deviations from the least-squares correlation to the Taft equation, based solely on the polar substituent constant (Figure 1), occur with the two esters containing acyl substituents [CH_3CH_2– and $(CH_3)_3C$–] with $\sigma^* < 0$. These esters also deviate from the Taft plot for the thiolysis reaction but in the correlation of the thiolysis rates with the hydrolysis rates (Figure 2), the deviations are not as significant. In this latter correlation (k_s vs. k_{OH}) the steric effects of the substituents are expected, at least partially, to cancel out.

There have been relatively few studies of transacylation reactions utilizing systematic variations in the electronic character of the acyl substituent, and most of these have dealt with aryl rather than with aliphatic groups. Kirsch et al.[37] have carried out a comprehensive examination of the rates of alkaline hydrolysis of 24 phenyl benzoates containing substituents in both the aryl group and the leaving group. The specific-base-catalyzed hydrolysis of the p-nitrophenyl benzoates (33% CH_3CN, μ = 0.3 M, 25 °C) is characterized by a Hammett reaction constant of ρ_{acyl} = 2.01 (±0.01). The Hammett reaction constant can be approximated in terms of the Taft reaction constant by the following relationship:[38]

$$\rho^* = 1.38\ (\pm 0.19)\rho \qquad (10)$$

Thus, the ρ_{acyl} value obtained by Kirsch et al.[37] corresponds to a ρ^* value of ~2.8, indicating essentially equal sensitivities to electron-withdrawing substituents in the acyl portion of the ester for the alkaline hydrolyses of p-nitrophenyl benzoates and alkanoates (ρ^* = 2.9).

The ρ^*_{acyl} value for the reaction of p-nitrophenyl esters with the thiolate species of N-acetyl-L-cysteine is very similar to the value for alkaline hydrolysis. The ρ^*_{acyl} values for these reactions can be compared with the ρ^* values for addition of hydroxide (ρ^*_{eq} = 3.00) or of thiolates (ρ^*_{eq} = 2.97) to aldehydes.[45] The value of ρ^*_{eq} for the equilibrium addition of the nucleophile to a series of aldehydes provides a useful reference for the interpretation of the kinetic ρ^*_{acyl} value. Equilibria additions to the carbonyl group can be considered as analogues of tetrahedral intermediate formation in the corresponding acyl transfer reactions. Sander and Jencks[47] have demonstrated a linear free-energy relationship between equilibrium constants for the addition of a series of nucleophiles to the carbonyl group of various aldehydes and the rate constants for the nucleophilic reactions with PNPA. The

Table I. Substituent Effects on Aldehyde Equilibria and p-Nitrophenyl Ester Reactivities

ArCHO + $^-$OH	$\rho = 2.24^a$	$ArCO_2NP + {}^-OH$	$\rho = 2.01^b$
RCHO + $^-$OH	$\rho^* = 3.00^c$	$RCO_2NP + {}^-OH$	$\rho^* = 2.9$ (±0.3)
RCHO + $^-$SR′	$\rho^* = 2.97^c$	$RCO_2NP + {}^-SR'$	$\rho^* = 3.4$ (±0.4)
RCHO + H_2O R = H–	$K^{obsd}/K^{calcd} = 324^d$	$RCO_2NP + {}^-OH$ R = H–	$k^{obsd}/k^{calcd} = 56$
R = Ph–	$K^{obsd}/K^{calcd} = 10^{-3}\,e$	R = Ph–	$k^{obsd}/k^{calcd} = 1.4 \times 10^{-3}\,f$
$CH_3CLO + {}^-OH$	$K_D/K_H = 1.30^g$	$LCO_2NP + {}^-OH$	$k_D/k_H = 1.24^h$
$CH_3CLO + {}^-SR$	$K_D/K_H = 1.18^g$	$LCO_2NP + {}^-SR$	$k_D/k_H = 1.03^h$

[a] Reference 46. [b] Reference 37. [c] Reference 45. [d] Greenzaid, P.; Luz, Z.; Samuel, D. *J. Am. Chem. Soc.* 1967, *89*, 749–756. K^{calcd} is based on $\rho^* = 1.68$[45] and $\sigma^*_H = 0.49$. [e] K^{calcd} is based on $\rho^* = 1.1$[46] and $\sigma^*_{Ph} = 0.60$. [f] k^{calcd} is based on eq 3 (text), k^{obsd} is from ref 37. [g] Based on the measured values[55] of K_D/K_H (=1.37 for D_2O addition; 1.25 for RSD addition) and corrected by a factor of 0.943 for the secondary deuterium isotope for ionization of the uncharged tetrahedral adduct [see: Okano, V.; DoAmaral, L.; Cordes, E. H. *J. Am. Chem. Soc.* 1976, *98*, 4201–4203]. RSH = β-mercaptoethanol. [h] Reference 35, RSH = β-mercaptoethanol.

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(35) Pohl, E. R.; Hupe, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 2763–2768.
(36) (a) Charton, M. *J. Am. Chem. Soc.* **1969**, *91*, 6649–6654. (b) Charton, M. *Ibid.* **1977**, *99*, 5678–5688.
(37) Kirsch, J. F.; Clewell, W.; Simon, A. *J. Org. Chem.* **1968**, *33*, 127–132.
(38) Equation 10 is an average of the ratios of five pairs of equilibria for which ρ and ρ^* have been measured: ionization of (a) XCO_2H [$\rho^* = 1.70$[39]] vs. $ArCO_2H$ [$\rho = 1.00$], (b) XCH_2CO_2H [$\rho^* = 0.663$[24]] vs. $ArCH_2CO_2H$ [$\rho = 0.49$[40]], (c) $XCH(OH)_2$ [$\rho^* = 1.32$[41]] vs. $ArCH(OH)_2$ [$\rho = 1.11$[42]], (d) XPO_3H^- [$\rho^* = 1.23$[43]] vs. $ArPO_3H^-$ [$\rho = 0.949$[44]], and (e) hydroxide addition to XCHO [$\rho^* = 3.00$[45]] vs. ArCHO [$\rho = 2.24$[46]]. The ratios (ρ^*/ρ) range from 1.19 (gem-diol ionization) to 1.70 (carboxylic acid ionization).
(39) Takahashi, S.; Cohen, L. A.; Miller, H. K.; Peake, E. G. *J. Org. Chem.* **1971**, *36*, 1205–1209.
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(47) Sander, E. G.; Jencks, W. P. *J. Am. Chem. Soc.* **1968**, *90*, 6154–6162.

correspondence of the activation volumes in the hydrolysis of *p*-nitrophenyl esters[48] and the volume changes accompanying hydration of the corresponding aldehydes[49] supports the appropriateness of aldehyde hydration as a model for the formation of the tetrahedral intermediate in ester hydrolysis. Additional parallels between equilibria involving aldehydes and rates involving the corresponding *p*-nitrophenyl esters are summarized in Table I.

Acyl transfer reactions in which the nucleophile (thiolate or oxy anion) is more basic than the leaving group involve rate-determining expulsion of the leaving group from the tetrahedral intermediate.[2,30] Formation of the tetrahedral intermediate is expected to be rate limiting in the alkaline hydrolysis and the thiolysis of *p*-nitrophenyl esters since the pK_a of water (=15.7), or of *N*-acetylcysteine (=9.2), is greater than that of *p*-nitrophenol (=7.1). The ratio of the kinetic and equilibrium reaction constants, $\rho^*_{acyl}/\rho^*_{eq}$, therefore, can be utilized as an index of the degree of advancement of the reactants in the transition state toward the anionic tetrahedral intermediate. For alkaline hydrolysis of *p*-nitrophenyl esters, $\rho^*_{acyl}/\rho^*_{eq}$ is ~ 1, indicating a nearly tetrahedral transition state structure. This is consistent with: (1) the high sensitivity ($\beta_{nuc} = 0.7$) of the rate constants to the basicity of various aryl oxide acyl acceptors ($pK_a > 7$) for acyl transfers from *p*-nitrophenol,[2] (2) the large secondary kinetic isotope effect for alkaline hydrolysis of *p*-nitrophenyl formate ($k_D/k_H = 1.24$),[35] and (3) the carbonyl oxygen kinetic isotope effects on the methanolysis of *p*-bromophenyl benzoate ($k^{16}/k^{18} = 1.018$) and phenyl benzoate ($k^{16}/k^{18} = 1.024$) which indicate extensive conversion of the carbonyl carbon–oxygen bond from a double bond to a single bond in the transition state.[50] Thus $\rho^*_{acyl}/\rho^*_{eq}$, as well as β_{nuc} and the ^2H- and ^{18}O-isotope effects, indicate a nearly tetrahedral transition-state structure for acyl transfer to oxygen nucleophiles (which are more basic than the leaving group). The nearly identical values of $\rho^*_{acyl}/\rho^*_{eq}$ for thiolysis (with *N*-acetylcysteine) and alkaline hydrolysis of *p*-nitrophenyl esters suggest a nearly tetrahedral transition-state structure for each of these reactions. Structure–reactivity studies with substituted phenyl acetates ($\beta_{lg} = 0.3$) indicate similar transition-state structures for thiolysis and hydrolysis[2] but other studies [e.g., dependence of rate constant on thiol basicity[2,30] and secondary kinetic isotope effects[35]] suggest a transition state for thiolysis which is more reactant-like than that for alkaline hydrolysis. The ambiguity in the transition-state structure for thiolysis of *p*-nitrophenyl esters can be resolved by a comparison of the transition state with the tetrahedral intermediate. The relationship between the transition-state structure and the tetrahedral intermediate can be assessed from an estimation of the free-energy content of these two species. The change in standard free energy for the addition of *N*-acetylcysteine to *p*-nitrophenyl acetate in forming the anionic tetrahedral intermediate can be estimated by the method of Guthrie.[51] The estimated free-energy change for formation of this intermediate is +15.2 kcal/mol.[52] If the "transition state" for thiolysis of *p*-nitrophenyl acetate is isoenergetic with the tetrahedral intermediate the predicted rate constant for the reaction is 2.7×10^3 M^{-1} min^{-1}.[53] The observed rate constant for the reaction of *N*-acetylcysteine with *p*-nitrophenyl acetate (27 °C, μ = 0.5 M) is 5.9×10^2 M^{-1} min^{-1}. Hupe and Jencks[2] determined the rate constant for the reaction of ethylthiolate (pK_a = 10.35) with *p*-nitrophenyl acetate to be 1.2×10^3 M^{-1} min^{-1} (25 °C, μ = 1 M). These results support the contention that the transition state for thiolysis of *p*-nitrophenyl esters is structurally similar to the anionic tetrahedral intermediate. The smaller secondary kinetic isotope effect for thiolysis of *p*-nitrophenyl formate than for alkaline hydrolysis (or for oxy anion attack) observed by Pohl and Hupe[35] is paralleled by a smaller secondary equilibrium isotope effect for thiol addition to acetaldehyde than for water addition.[55] One must, therefore, be cautious in attributing the smaller kinetic isotope effect in the thiolysis reaction to a transition state in which the acyl carbon is more sp^2-like in this reaction than it is in the specific-base-catalyzed reaction. Pohl and Hupe[35] present a detailed analysis of effects from sources other than hybridization changes which may contribute to a smaller kinetic isotope effect in the thiolysis reaction than in the hydrolysis reaction. Schowen et al.[56] also present a lucid analysis of the various factors which can contribute significantly to the magnitude of kinetic isotope effects in acyl transfer reactions.

The Taft reaction constant, ρ^*, is a measure of the sensitivity of a reaction to polar effects. The magnitude of ρ^* will be influenced by charge and bonding (bond moment) differences between the reactants in the ground state and transition state through electrostatic interactions (field effect) and electron withdrawing (inductive) effects. A reasonable model for the inductive component of ρ^*_{eq} for formation of the anionic tetrahedral intermediate is thiohemiacetal formation (ρ^* = 1.65) or aldehyde hydration (ρ^* 1.68).[45] The ionization of these addition adducts [ρ^*_{ion} = 1.32[41,45,51]] provides a measure of the electrostatic contribution to the ρ^*_{eq} value. Based on these model equilibria the experimental ρ^* value is expected to be influenced nearly equally by electrostatic ($\sim$44%) and inductive ($\sim$56%) effects. The magnitude of ρ^*_{acyl}, therefore, will be sensitive to the total charge around the acyl carbon atom in the transition state, relative to the ground state, but is expected to be relatively insensitive to the redistribution of this charge among the reacting atoms of the nucleophile and leaving group and the carbonyl oxygen. This is in contrast to the Brønsted coefficient which is sensitive to localized changes in effective charge on the nucleophile (β_{nuc}) or on the leaving group (β_{lg}).[2] Thus, ρ^*_{acyl} will be less sensitive than β to any imbalance in the extent to which a change in charge and a change in bond order have taken place in going from the ground state to the transition state.

Acyl Transfer to Phosphonates. The hydrolysis of *p*-nitrophenyl esters and *p*-nitrothiophenyl acetate is catalyzed by dianions of phosphonic acids (Figures 3 and 4). These reactions most likely involve an uncatalyzed nucleophilic attack by the phosphonate. This conclusion is based on: (1) the pH dependence of the reaction, (2) the magnitude of the Taft reaction constant (ρ^*_{acyl} = 2.4), (3) the absence of a kinetic solvent isotope effect ($k_{H_2O}/k_{D_2O} = 1.03 \pm 0.05$), (4) the requisite linear dependence of the first-order rate constant on phosphonate buffer concentration, (5) the 10^3- to 10^4-fold smaller rate constant for the (chloromethyl)-phosphonate (pK_a = 6.6) reaction than for the imidazole (pK_a = 7) reaction with the *p*-nitrophenyl esters, and (6) the magnitude of the entropy of activation for the reaction with methylphosphonate (ΔS^* = −14 eu[43]). Nucleophilic, rather than protolytic, catalysis by the phosphonates is consistent with the general observation of nucleophilic catalysis of the hydrolysis of esters with good leaving groups. The acetate-catalyzed hydrolysis of *p*-nitrophenyl acetate, for example, involves preferential (56%) nucleophilic catalysis[57] and the phosphate dianion catalyzed hydrolyses of *p*-nitrophenyl acetate,[4] 2,4-dinitrophenyl acetate,[5] and phenyl dichloroacetate[5] show no evidence of general acid/base catalysis.

The reaction of (chloromethyl)phosphonate with *p*-nitrophenyl esters is slightly less sensitive to polar acyl substituents (ρ^*_{acyl} =

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(49) Lewis, C. A., Jr.; Wolfenden, R. *J. Am. Chem. Soc.* **1973**, *95*, 6885–6688.
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(51) Guthrie, J. P. *J. Am. Chem. Soc.* **1978**, *100*, 5892–5904.
(52) This is based on the calculated equilibrium constant for the addition of ethyl thiolate to acetic acid [log $K = -13.1$[51]] to give the anionic tetrahedral intermediate. The σ^* values for RS– were assumed to be identical for R = CH_3CH_2– and R = $HC(CO_2^-)(NHAc)CH_2$–. The replacement of the hydroxyl moiety (σ^* = 1.55) by the *p*-nitrophenyl moiety (σ^* = 2.20) results in a stabilization of the tetrahedral intermediate by $\sim$2.6 kcal/mol [= $-2.3RT(2.97)(2.20 - 1.55)$, where the figure 2.97 is the ρ^* value for the formation of the thiohemiacetalate[45]].
(53) Calculated from the equation: $\ln k = \ln (k_bT/h) - \Delta G^*/RT$, where k_b is Boltzmann's constant and h is Planck's constant.[54]
(54) Eyring, H. *J. Chem. Phys.* **1935**, *3*, 107–115.
(55) Lewis, C. A., Jr.; Wolfenden, R. *Biochemistry* **1977**, *16*, 4886–4890.
(56) (a) Hogg, J. L.; Rodgers, J.; Kovach, I.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 79–85. (b) Kovach, I. M.; Hogg, J. L.; Raben, T.; Halbert, K.; Rodgers, J.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 1991–1999.
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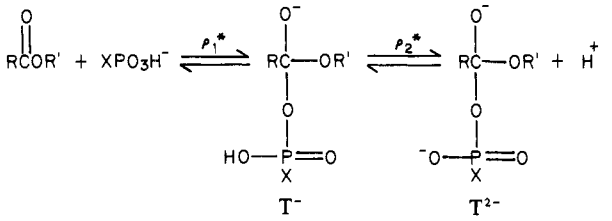
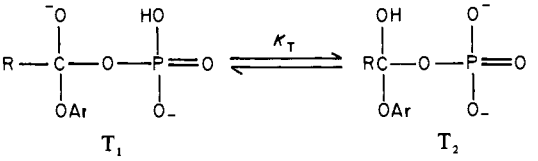
2.4) than are the reactions with either hydroxide or the *N*-acetylcysteine thiolate ($\rho^*_{acyl} = 3$). The equilibrium ρ^* value for formation of the tetrahedral adduct of the phosphonate dianion and the ester is expected to be somewhat larger than ρ^*_{eq} for hydroxide (or thiolate) addition of the carbonyl group. This larger ρ^*_{eq} value reflects an electrostatic component not present in the corresponding reactions of the monoanionic nucleophiles. This additional electrostatic component (ρ^*_2) corresponds to the ionization of the anionic tetrahedral intermediate (T^-):



The value of ρ^*_{eq} for formation of the tetrahedral intermediate, T^{-2}, is equal to $\rho^*_1 + \rho^*_2$, where ρ^*_1 (≈ 3) is the reaction constant for the step corresponding to formation of the tetrahedral intermediate with monoanionic nucleophiles (hydroxide and thiolate). The value of ρ^*_2 is estimated to be ~ 0.4. This is based on $\rho^* = 1.05$ for the first ionization of phosphonic acids[58] and an attenuation factor of 0.36 for the interposed carbon atom (eq 1) and of 1.07 for the interposed oxygen atom.[59] This yields a value of $\rho^*_{eq} \approx 3.4$ for the formation of the dianionic tetrahedral intermediate.

The ratio of the kinetic and equilibrium reaction constants, $\rho^*_{acyl}/\rho^*_{eq} = 0.7$, for acyl transfer to phosphonate dianions is 30% smaller than the corresponding value for reactions involving acyl transfer to the monoanions (hydroxide and thiolate). The smaller value may be the result of either (a) a more reactant-like transition state in the phosphonate reaction than in hydrolysis or thiolysis or (b) a more product-like transition state in the phosphonate reaction (i.e., rate-limiting breakdown of T^{2-}). Since the basicity of the nucleophile, (chloromethyl)phosphonate ($pK_{a2}^* = 6.6$), is similar to that of the *p*-nitrophenol leaving group ($pK_a = 7.1$), expulsion of the phosphonate and the phenol from the tetrahedral intermediate should occur with nearly equal ease if basicity is the major influence on the partitioning. Although basicity is undoubtedly important in determining the partitioning of the tetrahedral intermediate between reactants and products, the relative leaving ability of the phosphonate and *p*-nitrophenol is controlled to some extent by other effects. Polar acyl substituents, for example, are expected to destabilize the transition state for phosphonate expulsion more so than for phenoxide expulsion as a result of the greater electron demand for expulsion of the oxy dianion ($\rho^*_{eq} = -3.4$) than for expulsion of the monoanion ($\rho^*_{eq} = -3$). Thus, the relative basicities of the nucleophile and of the leaving group does not provide a quantitative answer to the question of whether formation or breakdown of the tetrahedral intermediate is rate limiting. Other considerations, however, indicate that the rate-limiting step in the reaction of phosphonates with the *p*-nitrophenyl esters is formation of the tetrahedral intermediate: (1) The Brønsted coefficient for the reaction of a series of phosphonates with *p*-nitrophenyl acetate (37 °C) or with *p*-nitrophenyl chloroacetate (27 °C) is 0.33 (±0.01). To the extent that the change in effective charge on the attacking nucleophile ($=\beta_{nuc}$) reflects changes in bond order, the magnitude of the Brønsted coefficient suggests an early transition in these two sets of reactions. Acyl transfer reactions involving rate-determining formation of the tetrahedral adduct with thiol anions or oxy anions are also characterized by a β_{nuc} value ~ 0.3.[2,30] The reaction of phosphonates with *p*-nitrothiophenyl acetate most likely involves rate-determining formation of the tetrahedral intermediate since each of the nucleophiles ($4.8 < pK_{a2} < 8.7$) is more basic than the leaving group ($pK_a = 4.5$). The β_{nuc} value for this series of reactions is 0.31 (Figure 4). (2) Imidazole is more reactive than phosphonates of comparable basicity by several orders of magnitude. At 27 °C the ratio of second-order rate constants for the reaction of imidazole ($pK_a = 7$) and (chloromethyl)phosphonate ($pK_a = 6.6$) is 50 M^{-1} $min^{-1}/4.9 \times 10^{-3}$ M^{-1} $min^{-1} = 10^4$ with *p*-nitrophenyl acetate ($\mu = 2.8$ M) and 1.8×10^3 M^{-1} $min^{-1}/1.4$ M^{-1} $min^{-1} = 1.3 \times 10^3$ with *p*-nitrophenyl chloroacetate ($\mu = 1.5$ M). This enhanced reactivity of imidazole over that of (chloromethyl)phosphonate is similar to the relative reactivities of imidazole and the phosphate dianion toward *p*-nitrophenyl acetate ($k_{IM}/k_{Pi} = 4.7 \times 10^3$)[4,60] or 2,4-dinitrophenyl acetate ($k_{IM}/k_{Pi} = 8.6 \times 10^2$).[5] The rate-limiting step in the nucleophilic imidazole-catalyzed hydrolysis of *p*-nitrophenyl acetate (or 2,4-dinitrophenyl acetate) is formation of the tetrahedral intermediate.[60,61] Imidazole is generally found to be $\sim 10^3$ times more reactive than phosphate in reactions involving rate-determining formation of the carbonyl-nucleophile adduct. The enhanced reactivity of imidazole, relative to phosphate, however, is not expected to be as pronounced in reactions involving rate-determining breakdown of the tetrahedral intermediate.[62] Nucleophilic catalysis of phenyl dichloroacetate hydrolysis by imidazole, for example, involves rate-determining breakdown of the tetrahedral intermediate [pK_a(leaving group) − pK_a(nucleophile) = 3]. The relative reactivity of imidazole and phosphate nucleophiles toward phenyl dichloroacetate is 10^2. (3) Formation of the tetrahedral intermediate involves a dynamic coupling of covalent bond formation and desolvation of the nucleophile.[2,30,35,56] It is expected that desolvation of the dianionic phosphonate makes a greater contribution to the activation energy barrier for formation of the tetrahedral intermediate than does desolvation of oxy monoanions in the corresponding reaction with these less solvated nucleophiles.[43] This more extensive solvent reorganization in acyl transfers to oxy dianions than in acyl transfers to monoanions will destabilize the transition state for phosphonate attack to a greater extent than the transition state for *p*-nitrophenoxide expulsion from the tetrahedral intermediate.

If the rate-limiting step in the reactions involving acyl transfer to the phosphonate dianions is indeed formation of the hypothetical tetrahedral intermediate (T^{2-}), the magnitude of the ρ^*_{acyl} value indicates that the transition state occurs some 25–35% earlier than in the more rapid reactions involving acyl transfer to hydroxide or to thiolates. This may reflect a domination of the rate-limiting step by desolvation of the oxy dianionic nucleophile. The solvation requirement of reactions involving dianionic acyl acceptors is likely to contribute to the anomalously low reactivity of the phosphate dianion. It is unlikely that the anomously low reactivity of phosphate is the result of a proton transfer which traps the tetrahedral intermediate (T_1) as its less reactive tautomer (T_2):



Since the alkoxide is more basic than the dianionic phosphate ester ($\Delta pK_a \sim 4.2$)[63] the equilibrium constant for this reaction is in favor of T_2 ($K_T \sim 10^4$). If T_1 was formed as a discrete intermediate, transfer of the proton from the acidic group ($pK_a \sim 3.8$) to the basic alkoxide ($pK_a \gtrsim 8$) will be very rapid ($k > 10^{12}$ min^{-1}) and the intermediate will be trapped as a species which is not on the normal reaction path. This can quantitatively account for the low reactivity of phosphate relative to other nucleophiles of comparable basicity. However, the phosphate dianion does not deviate from the Brønsted plots based on the reaction of *p*-nitrophenyl acetate, *p*-nitrothiophenyl acetate, or *p*-nitrophenyl

(58) Based on $\rho = 0.76$ for the first ionization constant or aryl phosphonic acids[44] and the conversion factor of 1.38 (eq 10).
(59) Based on a correlation of 13 pairs of substituent constants ($-0.2 < \sigma^*_X < 1.3$): $\sigma^*_{XO^-} = 1.068\sigma^*_X + 1.777$, $r = 0.9950$. The data used are given as supplementary material.
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chloroacetate with a series of phosphonates (Figure 4). Since the tetrahedral intermediate formed in the reaction with the phosphonates cannot tautomerize and the reactivity of phosphate is not abnormal relative to the reactivities of the phosphonates, the low reactivities of phosphate and phosphonates (compared to monoanionic or neutral acyl acceptors) most likely have a common origin. Electrostatic interactions between the incipient carbonyl oxy anion and the nonreacting oxy anion on phosphorous as well as solvent reorganization undoubtedly contribute to the destabilization of the transition state for acyl transfer to phosphonate dianions.[4,5,43] Thus, charge neutralization and desolvation of bound phosphate are methods by which enzymes can bring about the substantial rate enhancements in reactions involving nucleophilic attack by inorganic phosphate.

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Supplementary Material Available: A list of σ^* values used to obtain attenuation factors for the interposed methylene and oxygen and measured rate constants used in Figures 1–3 (5 pages). Ordering information is given on any current masthead page.

Effect of Phosphono Substituents on Acyl Transfer Reactions

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Abstract: The rate of release of *p*-nitrophenoxide from esters of phosphono-substituted carboxylic acids was examined as a function of pH(D), temperature, divalent metal ion (Mg^{2+} and Ca^{2+}) concentration, and acyl acceptor (^-OH and the thiolate of *N*-acetylcysteine). The hydrolysis of *p*-nitrophenyl 3-phosphonopropionate involves intramolecular nucleophilic catalysis by the dianionic phosphono substituent ($pK_{a2}^* = 7.5$) and is characterized by a first-order rate constant of 94 min^{-1} at 37 °C. A comparison of the rate constant of the unimolecular reaction with that of the corresponding bimolecular reaction (corrected for the inductive effect of the acyl substituent and for the phosphonate basicity) yields a rate constant ratio of $k_{uni}/k_{bi} = 7 (\pm 6) \times 10^3$ M. The magnitude of this rate enhancement is similar to those of analogous intramolecular reactions (e.g., hydrolysis of mono-*p*-nitrophenyl succinate or of *p*-nitrophenyl 4-(*N*,*N*-dimethylamino)butyrate but, unlike these reactions, the rate acceleration resulting from intramolecular nucleophilic catalysis by the dianionic phosphono group is enthalpic in origin ($\Delta\Delta H^* \approx 8$ kcal/mol). The entropy of activation for the intramolecular reaction is *less favorable* than that for the bimolecular reaction ($\Delta\Delta S^* \approx 9$ eu). The alkaline hydrolysis and the thiolysis rates of *p*-nitrophenyl phosphonoacetate are accelerated over 100-fold by the association of Mg^{2+} or Ca^{2+} with the ester. This rate acceleration is attributed to the formation of a six-membered bidentate coordination complex between the divalent cation and the incipient tetrahedral intermediate. The metal-promoted acyl transfer reactions of *p*-nitrophenyl phosphonoacetate provide a convenient system for the quantitative assessment of the role of metal ions in the catalysis of aqueous reactions.

Carbonyl displacement reactions have been extensively investigated. In particular, structure–reactivity relationships have provided a great deal of information on transition-state structures of acyl transfer reactions involving esters.[1,2] We have recently examined the effects of polar acyl substituents on the rates of reaction of *p*-nitrophenyl esters with hydroxide, with the thiol anion of *N*-acetylcysteine, and with a series of phosphonate dianions.[3] This provides a useful mechanistic framework for the quantitative assessment of the various factors which can influence the rate of a chemical reaction. Intramolecular reactions have received much attention because of their apparent similarity to many aspects of enzymatic reactions as well as their utility in the quantitative resolution of transition-state structures.[2] In this paper we describe the intramolecular catalysis of the hydrolysis of *p*-nitrophenyl 3-phosphonopropionate. This ester is similar in many respects to the monophenyl succinates examined by Gaetjens and Morawetz[4] in one of the earliest investigations of intramolecular nucleophilic catalysis of ester hydrolysis by carboxylate. We were interested in examining the hydrolysis of the 3-phosphonopropionate ester in order to evaluate the effect of an increase in basicity, an increase in negative charge density, and a change in geometry of the neighboring group on the intramolecular reaction. Also, in view of the fact that there are at least 58 enzyme-catalyzed reactions which involve a nucleophilic attack by inorganic phosphate,[5] the role of the phosphono substituent (a phosphate analogue) in the hydrolysis of *p*-nitrophenyl 3-phosphonopropionate is of particular interest.

Metal ion catalysis is important in many chemical reactions and is of wide prevalence in biochemistry.[6] For example, divalent metal ions are required as cofactors in enzyme-catalyzed phosphoryl transfer reactions.[7] Acyl transfer reactions of *p*-nitrophenyl phosphonoacetate seemed to provide an opportunity for exploring the effect of the interaction of divalent cations with the phosphono substituent on reactivity. We report here the effect of magnesium and calcium ions on the alkaline hydrolysis, and on the thiolysis, of the dianionic phosphonoacetate ester.

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