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.

Acknowledgment. This work was supported by a research grant from the U. S. Public Health Service (GM24765).

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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Experimental Section

The kinetic procedures were the same as those used previously.[3] Rates were determined by spectrophotometric measurement of *p*-nitrophenoxide (λ 400 nm) or of *p*-nitrophenol (λ 317.5 nm). For reactions carried out at temperatures below 27 °C or above 47 °C a Beckman DU spectrophotometer modified with an Update Model 122 digital display log converter amplifier was used. Temperature was controlled (±0.1 °C) with a Lauda Model K-2/R circulating constant-temperature bath. Temperature in the cell compartment was monitored with a Yellow Springs Tele-thermometer. The activation parameters, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, were determined from the following transformation[8] of the Eyring equation:

$$\ln (k/T) = -(\Delta H^{\ddagger}/R)(1/T) + [\Delta S^{\ddagger}/R + \ln (k_b/h)] \quad (1)$$

k is the rate constant (min^{-1} or M^{-1} min^{-1}), k_b is Boltzmann's constant, and h is Planck's constant ($k_b/h = 1.25 \times 10^{12}$ min^{-1} $molecule^{-1}$ K^{-1}). Rate constants were measured at five–seven temperatures covering a range of ~50 °C. The slope and the intercept of eq 1 were determined by an unweighted least-squares fit.

For the reactions in which the observed rate constant showed a hyperbolic dependence on the concentration of a reactant (e.g., pH dependence or divalent metal ion catalysis) the kinetic parameters were estimated by the median method of Cornish-Bowden and Eisenthal.[9] The nonparametric 95% confidence limits for the parameters were calculated by the method of Cornish-Bowden, Porter, and Trager.[10] The equations characterizing these reactions were expressed in the following form:

$$k_{obsd} = k^{lim}S/(K + S) \quad (2)$$

In the reactions showing an increase in rate with an increase in pH, S corresponds to $1/a_{H^+}$ and K corresponds to the reciprocal of the acid dissociation constant. In the metal ion catalyzed hydrolysis of *p*-nitrophenyl phosphonoacetate, k_{obsd} corresponds to the observed pseudo-first-order rate constant, k_{obsd}', corrected for the rate of hydrolysis in the absence of added divalent metal ions ($k_{obsd} = k_{obsd}' - k_0$). The limiting rate constant is $k^{lim} = k_{lim}' - k_0$ where k_{lim}' is the pseudo-first-order rate constant at saturating concentrations of the metal ion. K_d corresponds to the concentration of the metal ion, S, necessary for k_{obsd} to equal one-half k^{lim}.

***p*-Nitrophenyl (dimethylphosphono)acetate** was prepared from *p*-nitrophenyl bromoacetate by the method used in the preparation of *p*-nitrophenyl 3-(dimethylphosphono)propionate.[3] The product was isolated as a pale-red oil; $R_f = 0.55$ on TLC (silica gel, acetone/ether, 1:1), NMR[11] ($CDCl_3$) δ 3.3 (d, 2 H), 3.9 (d, 2 H), 7.6 (d, 2 H), and 8.6 (d, 2 H).

***p*-Nitrophenyl phosphonoacetate** was prepared by the trimethylsilyl iodide catalyzed hydrolysis[12] of *p*-nitrophenyl (dimethylphosphono)-acetate. The phosphonate dimethyl ester (8.7 g, 30 mmol) was dissolved in freshly distilled acetonitrile (30 mL). NaI (9 g, 60 mmol) was added and the mixture was placed under a nitrogen atmosphere. Trimethylsilyl chloride (7.6 mL, 60 mmol) was then added to the solution as it was being stirred. After being stirred for 20 min at room temperature the solution was filtered to remove the NaCl and evaporated under reduced pressure to remove the acetonitrile. Water (20 mL) was added to the silyl ester. The anilinium salt of the phosphonic acid was obtained by adding aniline (~6 mL) to the solution. The precipitate was removed by filtration and crystallized from ethanol/acetone (2:1) to yield 5 g of white crystals of the monoanilinium salt of *p*-nitrophenyl phosphonoacetate (47% yield), mp 183–185 °C. Anal. Calcd for $C_{14}H_{15}N_2O_7P$: C, 47.46; H, 4.27; N, 7.91; P, 8.74. Found:[11] C, 47.26; H, 4.44; N, 7.77; P, 8.97.

***p*-Nitrophenyl 3-phosphonopropionate** was prepared by hydrolysis of 30 mmol of *p*-nitrophenyl 3-(dimethylphosphono)propionate[3] by the procedure described above for the preparation of the phosphonoacetate ester. Crystallization of the monoanilium salt from ethanol/acetone (2:1) resulted in 4.8 g (43% yield) of white crystals with mp 160–161 °C. Anal. Calcd for $C_{15}H_{17}N_2O_7P$: C, 48.92; H, 4.65; N, 7.61; P, 8.41. Found:[11] C, 48.69; H, 4.59; N, 7.75; P, 8.76.

Preparation of the monoanilinium salts was found to be the most convenient and efficient means of purifying the *p*-nitrophenyl esters of phosphonoacetic and phosphonopropionic acid. Attempts at precipitation of the barium or calcium salts from an aqueous solution of the phosphonic acids were unsuccessful as were attempts at precipitation of the free acids by addition of acetone (20 volumes) to the aqueous solution. The monoanilinium salt could be converted to the free acid by mixing an aqueous solution with a cation exchange resin (Dowex 50X4). The small amount of aniline present in the kinetic experiments with the anilinium salt (~30 μM) had no detectable effect on the reaction rate when compared to the experiments using the free acid. The structural integrity of the phosphonoacetate moiety is maintained under the conditions used to measure the release of *p*-nitrophenoxide. After incubation of *p*-nitrophenyl phosphonoacete at 37 °C, pH 10.9 [0.01 M cyclohexylaminopropane sulfonate (CAPS), 1 M NaCl], for 6 h (corresponding to 9 half-lives for the hydrolysis reaction) the phosphonoacetate was recovered quantitatively. The reaction mixture was also analyzed for inorganic phosphate.[13] No phosphate was detected, indicating negligible fragmentation of the phosphonate (to phosphate, *p*-nitrophenoxide, and ketene) under these conditions.

Results

Taft Polar Substituent Constants. Although values of σ^* for charged groups are relatively unreliable[14] (e.g., medium dependent), it is desirable to have an estimate of the polar effect of the phosphono substituent in order to assess the factors, other than electronic, which are responsible for the reactivity of the *p*-nitrophenyl esters which contain this group. The σ^* values were estimated in the following manner. From the ionization of substituted benzoic acids (25 °C, $\mu \sim 0.1$–0.2 M) Jaffé et al.[15] determined the Hammett substituent constants of the dianionic phosphono group: $\sigma_p = -0.16$ and $\sigma_m = -0.02$. These values can be separated into inductive and resonance components by the method of Exner:[16] $\sigma_p = 1.14\sigma_I + \sigma_R$ and $\sigma_m = \sigma_I + 0.33\sigma_R$. This yields a value for the inductive substituent constant of the $-PO_3^{2-}$ group of $\sigma_I = 0.0538$. With use of the relationship $\sigma^* = 6.23\sigma_I$,[17] a value of $\sigma^* = 0.335$ for the dianionic phosphono substituent is obtained. Attenuation by a methylene group[18] yields values of $\sigma^* = 0.119$ for the $-CH_2PO_3^{2-}$ substituent and $\sigma^* = 0.040$ for the $-CH_2CH_2PO_3^{2-}$ substituent. The σ^* value of 0.12 for the dianionic phosphonomethyl substituent can be compared to the value of 0.52 for the monoanionic carboxymethyl substituent.[19] This is consistent with the larger value of σ_p for the $-CO_2^-$ group (+0.12) than for the $-PO_3^{2-}$ group (−0.16) obtained by Jaffé et al.[15] Similarly a σ^* value of 0.70 for the monoprotonated phosphonomethyl substituent can be estimated from the Hammett substituent constants of $\sigma_p = +0.17$ and $\sigma_m = +0.25$ for the $-PO_3H^-$ substituent.[15] From the acid dissociation constants of *p*- and *m*-phosphonobenzoic acids, the Hammett substituent constants for the ionized and un-ionized carboxylate group and for the monoprotonated phosphono group, and the relationship between pK_{a1} and σ for aryl phosphonic acids, all of which have been determined by Jaffé et al.,[15] a σ^* value of 1.11 is estimated for the un-ionized phosphonomethyl substituent. The substituent constant of the dimethylphosphonomethyl group is $\sigma^* = 0.80$.[20]

***p*-Nitrophenyl Phosphonoacetate.** At pH 10 (27 °C) the rate of release of *p*-nitrophenoxide from *p*-nitrophenyl phosphonoacetate is 250 times slower than the rate of release of *p*-nitrophenoxide from *p*-nitrophenyl acetate. Because of the low reactivity of *p*-nitrophenyl phosphonoacetate, most of the studies with this ester were carried out at elevated temperatures. The pH dependence of the rate of formation of *p*-nitrophenoxide from *p*-nitrophenyl phosphonoacetate was examined at 47 °C ($\mu = 1$ M, $8 < pH < 11$). The thermodynamic pK_{a2} of the phosphono

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Table I. Activation Parameters for *p*-Nitrophenoxide Release

reaction	μ, M[a]	k(37 °C)[b]	E_a,[c] kcal/mol	$\Delta H^{\ddagger}$,[d] kcal/mol	$\Delta S^{\ddagger}$,[d] eu
$^{2-}O_3PCH_2CH_2CO_2NP$	0.5	94.2 (±0.8)	10.9 (±0.2)	11.1 (±0.5)	−22.6 (±0.4)
$^{2-}O_3PCH_2CO_2NP + {}^{-}OH$	1.0	22.8 (±0.4)	12.7 (±0.9)	12.1 (±1.1)	−21.6 (±3.6)
$BrCH_2CH_2CO_2NP + {}^{-}OH$	1.0	1.20 (±0.7) × 10^3	11.1 (±0.3)	11.2 (±0.6)	−16.6 (±0.9)
$BrCH_2CH_2CO_2NP + CH_3PO_3^{2-}$	2.0	1.6 (±0.2) × 10^{-2}	21.8 (±0.3)	18.8 (±0.3)	−14.2 (±3.8)
$CH_3CO_2NP + CH_3PO_3^{2-}$	2.0	3.5 (±0.6) × 10^{-2}	20.5 (±0.4)	18.7 (±0.9)	−13.0 (±4.8)
$CH_3CO_2NP + CH_3CO_2^{-}$ [e]	0.2	2 × 10^{-3} [f]		15.7	−28.5
$^{-}O_2CCH_2CH_2CH_2CO_2NP$[e]	~0.15	6.5 × 10^{-1} [f]		19.1	−6.0[g]
$CH_3CO_2NP + Im$[h]	1.0	80[f]		8.7	−30
$CH_3CO_2NP + (CH_3)_3N$[i]	1.0	20[f]		12.3	−21.1
$(CH_3)_2NCH_2CH_2CH_2CO_2NP$[i]	1.0	6.5 × 10^4 [f]		11.9	−6.4

[a] Ionic strength maintained with NaCl. [b] Units are min^{-1} for the unimolecular reactions and M^{-1} min^{-1} for the bimolecular reactions. Error limits are standard deviations of 4–5 replicate determinations. [c] Arrhenius activation energy obtained by a least-squares fit to the equation: $\ln k = -(Ea/R)\,(1/T) + \ln Z$ ($r > 0.998$). The error limits are the 90% confidence intervals. [d] The enthalpy of activation was determined from the slope of the Eyring equation [eq 1] and the entropy of activation was determined from the intercept. The error limits are the 90% confidence intervals. [e] Reference 4. [f] Value calculated from $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. [g] The entropy of activation for *p*-nitrophenoxide release from mono-*p*-nitrophenyl succinate is expected to be somewhat more positive than this value. For the reaction of mono-*p*-methoxyphenyl and mono-*o*-methoxyphenyl succinates, $\Delta S^{\ddagger} = -10$ (±1) eu and for the reaction of mono-*p*-methylphenyl glutarate, $\Delta S^{\ddagger} = -18$ eu (ref 4). [h] Akiyama, M.; Hara, Y.; Tanabe, M. *J. Chem. Soc., Perkin Trans. 2* 1978, 288–292. [i] Reference 29.

substituent (determined by potentiometric titration) is 6.53 (±0.04) at 24 °C and the pK_{a2} at 47 °C (μ = 1 M) is 6.2. The rate of *p*-nitrophenoxide release is first order in hydroxide concentration. The first-order rate constant is described by the following equation:

$$k_{obsd}\ (min^{-1}) = 42\ (\pm 1)a_{-OH} + 0.001 \qquad (3)$$

No evidence for buffer catalysis was found. The rate constant (47 °C, μ = 1 M, pH 10.0) is independent of the buffer (CAPS, cyclohexylaminopropane sulfonic acid) concentration between 0.01 and 0.08 M. The second-order rate constant for the alkaline hydrolysis of *p*-nitrophenyl phosphonoacetate at 47 °C exhibits an inverse solvent deuterium isotope effect, k_{OH}/k_{OD} = 0.63 (±0.05).

In order to check for hydrogen exchange at C-2 in *p*-nitrophenyl phosphonoacetate, the hydrolysis was followed by ^1H NMR (0.15 M ester, 1 M NaCl, 0.01 M CAPS, pD = 10, $T \approx 25$ °C). The reaction was monitored over a 70-h period (~1 half-life) and no disappearance of the integrated NMR absorptions of the doublets (J = 21 Hz) corresponding to the C-2 hydrogens in either the unreacted ester or in the phosphonoacetate product was observed. This indicates that if exchange does occur the deuteration rate is less than one-tenth the hydrolysis rate.

The rate of alkaline hydrolysis of *p*-nitrophenyl phosphonoacetate was examined at four temperatures (27, 37, 47, and 54 °C) at pH 10 and pH 11 (0.01 M CAPS, 1 M NaCl). The activation parameters are summarized in Table I.

Divalent Metal Ion Catalysis. *p*-Nitrophenyl phosphonoacetate is unusually unreactive with respect to both alkaline hydrolysis and reaction with the thiol anion of *N*-acetyl-L-cysteine. The rates of these reactions are enhanced in the presence of $CaCl_2$ or $MgCl_2$. Figure 1 illustrates the dependence of the second-order thiolysis rate constant on the divalent metal ion concentration. The rate constants for both the thiolysis and the alkaline hydrolysis reaction show a hyperbolic dependence on divalent cation concentration (eq 2). At saturating concentrations of Ca^{2+} the rate of reaction with ^{-}OH or with ^{-}OD is enhanced by a factor of 110 (±2) and the rate of reaction with the thiol anion of *N*-acetyl-L-cysteine is enhanced by a factor of 99 (±2). At saturating levels of Mg^{2+} the thiolysis and hydrolysis rates are enhanced by a factor of 163 (±3). The hydrolysis of *p*-nitrophenyl (dimethylphosphono)acetate was followed at six pH values (pH 5.2–7.6, 27 °C, μ = 0.5 M). The pseudo-first-order constant is a linear function of hydroxide concentration:

$$k_{obsd}\ (min^{-1}) = 1.47\ (\pm 0.09) \times 10^7 a_{-OH} + 0.021 \qquad (4)$$

The alkaline hydrolysis rate is not altered in the presence of magnesium. Identical pseudo-first-order rate constants [k_{obsd} = 0.35 (±0.01) min^{-1}] were obtained for the reactions carried out at pH 6.3 in the presence of either 0.2 M $MgCl_2$ or 0.6 M NaCl. The rate constants for the hydrolysis and thiolysis of the *p*-nitrophenyl esters of the various phosphonocarboxylic acid derivatives are summarized in Table II.

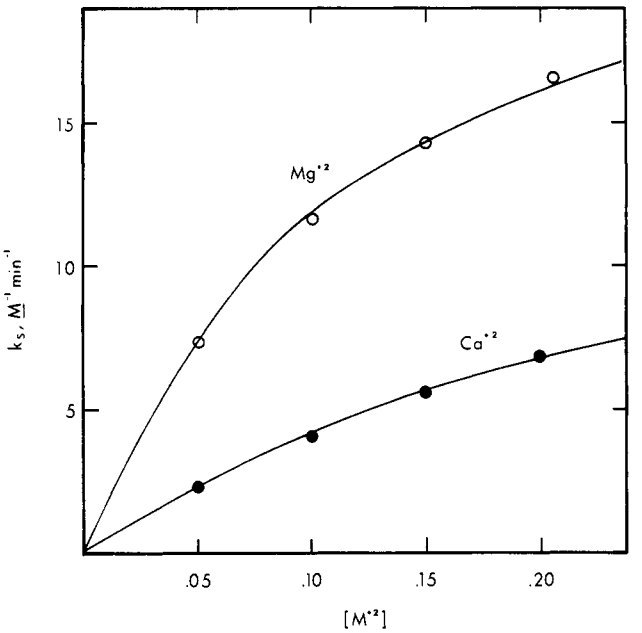


Figure 1. Effect of metal ions on the thiolysis of *p*-nitrophenyl phosphonoacetate. The abscissa is the molar concentration of $CaCl_2$ or $MgCl_2$. The ordinate is the corrected second-order thiolysis rate constant ($k_s = (k_{obsd} - k_0)/[RS^-]$, where k_{obsd} is the observed pseudo-first-order thiolysis rate constant in the presence of divalent cations and k_0 is the first-order thiolysis rate constant in the absence of added metal ions; $[RS^-]$ is the concentration of the thiolate of *N*-acetyl-L-cysteine). Reaction conditions: 27 °C, μ = 0.6 M (maintained with NaCl), pH 10–11 (0.01 M CAPS), $[RS^-]$ = 0.05–0.1 M, [ester] ~ 30 μM. The curves represent a theoretical fit to eq 2 with K_d = 0.11 M and k^{lim} = 25 M^{-1} min^{-1} for Mg^{2+} and K_d = 0.26 M and k^{lim} = 15 M^{-1} min^{-1} for Ca^{2+}.

***p*-Nitrophenyl 3-Phosphonopropionate.** The insertion of a methylene group into *p*-nitrophenyl phosphonoacetate yields a very reactive ester. The relative rate constants for *p*-nitrophenoxide release from the phosphonoacetate, acetate, and 3-phosphonopropionate esters at pH 6.3 (27 °C) are 4 × 10^{-3}:1.0:2 × 10^5. This enhanced reactivity of *p*-nitrophenyl 3-phosphonopropionate and the pH dependence of the reaction (dependent on a basic group with pK_a = 7.08 (±0.15) at μ = 0.13 M and pK_a = 6.88 (±0.09) at μ = 0.5 M) are indicative of intramolecular participation of the dianionic phosphono substituent. In order to distinguish between general-base catalysis and nucleophilic catalysis, we measured the solvent isotope effect on the first-order rate constant for *p*-nitrophenoxide release from the 3-phosphonopropionate ester. The results are illustrated in Figure 2. The pK_a characterizing the pH dependence of the reaction (23 °C, μ = 0.13 M) is 0.54 (±0.14) units higher in D_2O than in H_2O but the limiting first-

Table II. Kinetic Parameters for Reactions of *p*-Nitrophenyl Esters of Phosphonocarboxylic Acids[a]

reaction	k_{obsd}, M^{-1} min^{-1}	k_{calcd} or K_d
$^{2-}O_3PCH_2CH_2CO_2NP$	38 (±1)	k_{calcd}[b] = 8.8 × 10^2
$(CH_3O)_2P(O)CH_2CH_2CO_2NP + {}^-OH$	3.5 (±0.4) × 10^3	k_{calcd}[b] = 4.5 × 10^3
$^{2-}O_3PCH_2CO_2NP + {}^-OH$	4.7 (±0.4)	k_{calcd}[b] = 1.5 × 10^3
$^{2-}O_3PCH_2CO_2NP + {}^-SR$[c]	1.6 (±0.2) × 10^{-1}	k_{calcd}[b] = 1.5 × 10^3
$(CH_3O)_2P(O)CH_2CO_2NP + {}^-OH$	1.5 (±0.1) × 10^7	k_{calcd}[b] = 1.3 × 10^5
$CaO_3PCH_2CO_2NP + {}^-OH$	5.1 (±0.5) × 10^2	K_d[d] = 0.27 (±0.05) M
$CaO_3PCH_2CO_2NP + {}^-SR$[c]	1.5 (±0.1) × 10^1	K_d[d] = 0.26 (±0.03) M
$MgO_3PCH_2CO_2NP + {}^-OH$	7.7 (±0.8) × 10^2	K_d[d] = 0.10 (±0.02) M
$MgO_3PCH_2CO_2NP + {}^-SR$[c]	2.5 (±0.2) × 10^1	K_d[d] = 0.11 (±0.01) M

[a] Reaction conditions: T = 27 °C, ionic strength (maintained with NaCl) = 0.5 M (in absence of Ca^{2+} or Mg^{2+}) or = 0.6 M (in presence of Ca^{2+} or Mg^{2+}). Buffer concentration = 0.01 M (MES, PIPES, HEPES, or CAPS). [b] Second-order rate constant (M^{-1} min^{-1}) calculated from the Taft equation for the alkaline hydrolysis, or for the thiolysis, of ten *p*-nitrophenyl esters. [c] Thiolate of *N*-acetyl-L-cysteine. [d] Concentration of divalent cation required for half-maximal rate enhancement.

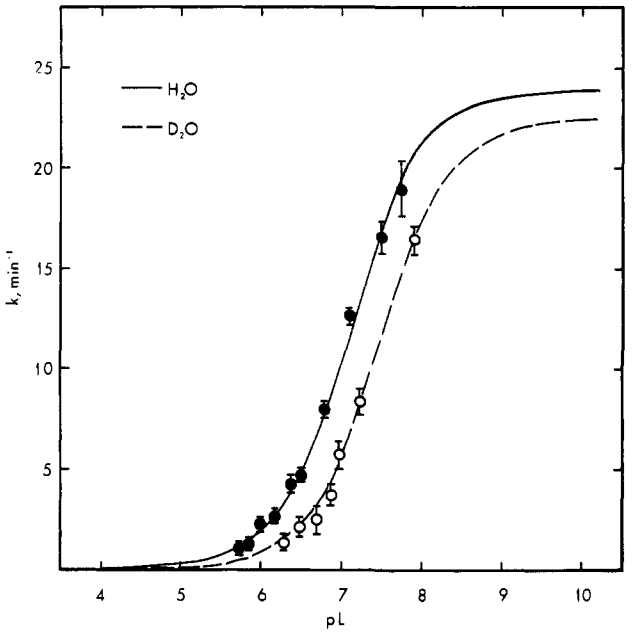
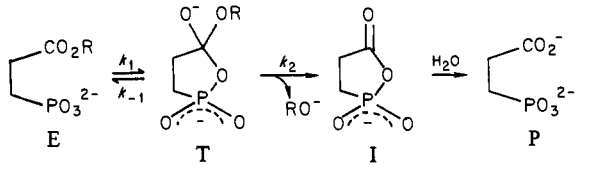


Figure 2. Dependence of the first-order rate constant for *p*-nitrophenoxide release from *p*-nitrophenyl 3-phosphonopropionate on pL = (= −log [lyonium ion]). Reaction conditions: T = 23 °C, μ = 0.13 M (NaCl), buffer concentration = 0.01 M (MES, 5.6 ≤ pL ≤ 6.6; PIPES, 6.0 ≤ pL ≤ 7.2; HEPES, 6.9 ≤ pL ≤ 7.8). The lines are theoretical for a pK_a = 7.08 and k^{lim} = 24 min^{-1} in H_2O and for a pK_a = 7.48 and k^{lim} = 22 min^{-1} in D_2O. The error bars are the standard deviations of three or more determinations. The relative differences between the observed and calculated rate constants for the hydrolysis reaction [i.e., $(k_{obsd} - k_{calcd}/k_{calcd})$] are independent of the magnitude of the rate constant. The mean value of the relative error is ±3.7% (−9.3%–+5.3%). If it is assumed that the hydrolysis reaction is first order in [$^-$OH], the correlation equation (based on a least-squares fit) is k = 3 × 10^7[$^-$OH] + 3.66 (r = 0.893). The fit of the data to this equation is not as good as the fit to eq 2 (text). The mean value of the relative errors is ±33% (−76%–+73%) and these relative errors show a systematic dependence on the magnitude of the rate constant.

order rate constants are indistinguishable in the two solvents (k^{lim} = 23 ± 1 min^{-1}). This suggests nucleophilic catalysis by the dianionic phosphono substituent.

The pH-independent rate constant is fairly insensitive to ionic strength. There is less than a 15% reduction in k^{lim} as the ionic strength is increased from 0.05 to 2.5 M (with either NaCl or Na_2SO_4). The effect of organic solvents on the reaction rate was examined by monitoring the release of *p*-nitrophenoxide following the addition of the ester to a mixture of the organic solvent and aqueous buffer solution (27 °C, final concentration of NaCl ~0.1 M). The apparent pH of the resulting solution was estimated by use of a glass electrode. *p*-Dioxane (dielectric constant = 2.21) inhibits the rate of release of *p*-nitrophenol from the 3-phosphonopropionate ester. Increasing the dioxane concentration to 50% v/v results in an increase in the apparent pK_a characterizing the reaction (by ~1 unit) and a 2–3-fold decrease in the limiting

Scheme I



first-order rate constant. Similar results were obtained with dimethylformamide (dielectric constant = 36.7) and dimethyl sulfoxide (dielectric constant = 45.7), although the apparent pK_a value increased by ~2 units at 50% v/v concentration of these organic solvents. Increasing the concentration of formamide (dielectric constant = 109) to 50% v/v results in an increase in the apparent pK_a by ~1 unit but has no effect (±10%) on the limiting first-order rate constant. The rate of release of *p*-nitrophenol was followed as a function of temperature in a 1:1 mixture of formamide and aqueous buffer solution (0.02 M MES, 0.2 M NaCl, pH 5.8). The observed first-order rate constants were reduced by 35 (±5)% in the presence of formamide at each temperature examined (15, 27, 37, and 47 °C).

The temperature dependence of the rate or release of *p*-nitrophenoxide from the 3-phosphonopropionate ester was determined over a pH range of 5.2–7.8 (μ = 0.5 M, 15–54 °C). The pK_a [=7.0 (±0.1)] characterizing the pH dependence of the rate of *p*-nitrophenoxide formation is insensitive to temperature (−1.4 kcal/mol < $\Delta H°_{ion}$ < 0.4 kcal/mol). A plot of the logarithm of the pH-independent first-order rate constant (k^{lim}) vs. $1/T$ was found to be linear (r = 0.9993), yielding a value for the Arrhenius activation energy of E_a = 10.9 (±0.2) kcal/mol. The temperature dependence of the free energy of activation, $\Delta G^‡$, for this intramolecular reaction, as well as that for the nucleophilic catalysis of *p*-nitrophenyl 3-bromopropionate hydrolysis by the dianion of methylphosphonic acid, is illustrated in Figure 3. The activation parameters are summarized in Table I.

Discussion

Intramolecular Nucleophilic Catalysis by the Phosphono Substituent. The rate of release of *p*-nitrophenoxide from the dianionic 3-phosphonopropionate ester is very rapid. The first-order rate constant for this reaction at pH 6.3 (27 °C), for example, is 2 × 10^5-times greater than that for hydrolysis of *p*-nitrophenyl acetate under the same conditions. A reasonable mechanism for the hydrolysis of *p*-nitrophenyl 3-phosphonopropionate, based on analogy with intramolecular nucleophilic catalysis of ester hydrolysis,[2,4,21,22] is presented in Scheme I. A catalytic role of the dianionic phosphonate group is apparent from the pH dependence of the reaction (Figure 2) and from the magnitude of the pH-independent rate constant, relative to the second-order rate constant for bimolecular nucleophilic catalysis of the *p*-nitrophenyl ester hydrolysis by dianions of phosphonic acids. For example, the pH-independent rate constant for *p*-nitrophenoxide release

(21) Bruice, T. C.; Pandit, U. K. *J. Am. Chem. Soc.* **1960**, *82*, 5858–5865.
(22) Bruice, T. C. In "The Enzymes", Boyer, P. D., Ed.; Academic Press: New York, 1970; Vol. 2, 3rd ed., pp 217–279.

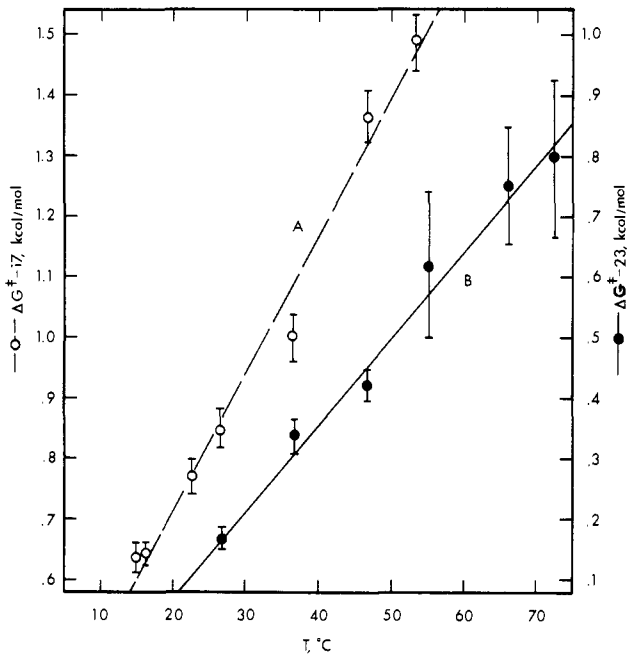


Figure 3. Eyring plot illustrating the activation energetics for *p*-nitrophenoxide release. A: Release of *p*-nitrophenol from *p*-nitrophenyl phosphonopropionate (μ = 0.5 M). The free energy of activation was calculated from the limiting first-order rate constant obtained from the pH dependence of k_{obsd} (5.6 ≤ pH ≤ 7.8). The thermodynamic parameters for the ionization of the phosphonate substituent (K_{a2}) are $\Delta H°_{ion}$ = −0.5 (±0.9) kcal/mol and $\Delta S°_{ion}$ = −33 (±2) eu. B: Reaction of methylphosphonate with *p*-nitrophenyl 3-bromopropionate (μ = 2.0 M). Reactions were carried out at pH 7.4–8.0. The thermodynamic parameters for the ionization of methylphosphonate (K_{a2}, μ = 2.0 M) are $\Delta H°_{ion}$ = +1.2 (±0.3) kcal/mol and $\Delta S°_{ion}$ = −30 (±1) eu.

from E is 6×10^3-times greater than the pseudo-first-order rate constant for *p*-nitrophenoxide release from *p*-nitrophenyl 3-bromopropionate in the presence of 1 M methylphosphonate dianion (37 °C, Table I). The absence of a significant solvent deuterium isotope effect in the hydrolysis of the dianionic 3-phosphonopropionate ester is indicative of a nucleophilic, rather than a protolytic, mechanism. The solvent isotope effect on the pH-independent rate of release of *p*-nitrophenoxide from E, k_{H_2O}/k_{D_2O} = 1.1 (±0.3), can be compared with the value of k_{H_2O}/k_{D_2O} = 1.03 (±0.05) for the nucleophilic reaction of the methylphosphonate dianion with *p*-nitrophenyl acetate, *p*-nitrophenyl 3-bromopropionate, or *p*-nitrothiophenyl acetate. The relative insensitivity of the pH-independent rate constant to the water concentration (e.g., no change in k^{lim} when the water molality is reduced 2.2-fold by the addition of 50% v/v formamide) is also more in keeping with a nucleophilic mechanism than with a mechanism involving intramolecular general-base catalysis by the phosphono substituent.

The pK_a which characterizes the pH profile for release of the phenol from *p*-nitrophenyl 3-phosphonopropionate is 7.1 at μ = 0.13 M and 6.9 at μ = 0.5 M. Based on the ionic strength dependence of pK_{a2} for other phosphonates, this extrapolates to a pK_a of 7.5 at μ = 0. This is reasonably close to the estimated[23] thermodynamic pK_{a2} of 7.6. Further evidence of the equivalence of the apparent (kinetically determined) pK_{a2} value and the true pK_{a2} of the phosphono substituent of *p*-nitrophenyl 3-phosphonopropionate is that the kinetic pK_a and the observed rate constant for the reaction are influenced differently by various changes in reaction conditions. A change in solvent from H_2O to D_2O has no effect on the pH-independent rate constant but results in an increase in the kinetic pK_a by 0.5 units. This shift in pK_a is of the same magnitude observed for the kinetic pK_{a2} of methylphosphonate in the bimolecular reaction with *p*-nitrophenyl esters[3] and for the thermodynamic pK_{as} of acids with pK_a values ~6–8.[24] Increasing the temperature from 15 to 54 °C results in an 11-fold increase in the limiting first-order rate constant for the intramolecular reaction but has no effect on the kinetic pK_a [$\Delta H°_{ion}$ = −0.5 (±0.9) kcal/mol]. Phosphate and phosphonate monoanions have acid dissociation constants which also show almost no temperature dependence (i.e., $\Delta H°_{ion} \sim 0$). The heat of ionization of the monoanion of methylphosphonic acid (pK_a* = 8.0) at μ = 2 M is +1.2 (±0.3) kcal/mol (Figure 3). $\Delta H°_{ion}$ = +0.8 kcal/mol for the monoanion of phosphoric acid[25] and $\Delta H°_{ion}$ = +1.3 kcal/mol for the dianion of phosphonoacetic acid.[26]

Knowledge of the basicity of the attacking group in the intramolecular nucleophilic phosphonate-catalyzed hydrolysis of *p*-nitrophenyl 3-phosphonopropionate provides a basis for the evaluation of the rate enhancement of the unimolecular reaction relative to its bimolecular counterpart. The dependence of the rate constant on the basicity of the phosphonate and on the acyl substituent have been determined for a series of reactions involving acyl transfer between *p*-nitrophenol and phosphonate dianions.[3] The second-order rate constant for the reaction of *p*-nitrophenyl acetate with dianions of phosphonic acids is characterized by the Brønsted equation (μ = 2 M, 37 °C):[3]

$$\log k_2 = 0.34 pK_{a2}* - 4.21 \quad (5)$$

This yields a value of $k_2 = 2 \times 10^{-2}$ M^{-1} min^{-1} for the reaction of *p*-nitrophenyl acetate with a phosphonate dianion of pK_{a2}* = 7.5 (= pK_{a2} of *p*-nitrophenyl 3-phosphonopropionate). Correcting this value for the different polar effects of the phosphonoethyl substituent (σ* = 0.04) and the methyl substituent (σ* = 0) yields a value of $k_{bi} = 10^{\rho*\Delta\sigma*} \times 2 \times 10^{-2} = 10^{2.4(0.04)} \times 2 \times 10^{-2} = 2.5 \times 10^{-2}$ M^{-1} min^{-1} for the bimolecular counterpart of the intramolecular reaction. A somewhat lower estimate of the corrected bimolecular rate constant is obtained from the observed second-order rate constant (=1.6×10^{-2} M^{-1} min^{-1}) for the reaction of methylphosphonate with *p*-nitrophenyl 3-bromopropionate (37 °C). The difference in σ* values (0.36–0.04) corresponds to a reduction in the rate constant by a factor of 5.86, and the difference in basicity of the phosphonate groups (ΔpK_{a2} = 8.0–7.5) corresponds to a rate reduction by a factor of 1.5 (based on a β_{nuc} value of 0.34). This yields a bimolecular rate constant, corrected for the acyl polar effect and the nucleophile basicity, of $k_{bi} = 1.8 \times 10^{-3}$ M^{-1} min^{-1}. The average value of these corrected bimolecular rate constants, $k_{bi} = 1.3$ (±1.1) $\times 10^{-2}$ M^{-1} min^{-1}, can be compared with the unimolecular rate constant for release of *p*-nitrophenoxide from the 3-phosphonopropionate ester. This value is 94 min^{-1} at 37 °C. This yields a value for the rate acceleration, brought about by reducing the reaction molecularity, of $k_{uni}/k_{bi} = 7$ (±6) $\times 10^3$ M. The magnitude of this rate enhancement is similar to those found for other acyl transfer reactions in which a five-membered cyclic intermediate is formed. For example, $k_{uni}/k_{bi} \approx 7 \times 10^4$ M (25 °C) for acyl transfer to a carboxylate[27]

(23) The thermodynamic pK_{a2} of *p*-nitrophenyl phosphonoacetate is 6.53. The σ* value for the $-CH_2CO_2NP$ substituent which corresponds to this pK_a is σ* = 1.3 (±0.1). This is obtained from a correlation of the pK_{a2} values of 17 phosphonic acids (XPO_3H^-) with standard σ* values: pK_{a2} = −1.23 (±0.07)σ* + 8.14 (±0.18); r = 0.9917, s = 0.0423. The data used are given as Supplementary Material. Attenuation of the electron-withdrawing influence of the $-CH_2CO_2NP$ group by a methylene[18] yields a σ* of 0.47 (±0.04) for the $-CH_2CH_2CO_2NP$ group. This corresponds to a value of pK_{a2} = 7.56 (±0.07) for *p*-nitrophenyl 3-phosphonopropionate.

(24) Laughton, P. M.; Robertson, R. E. In "Solvent Isotope Effects for Equilibria and Reactions in Solute–Solvent Interactions", Coetzee, J. G., Ritchie, C. D., Eds.; Marcel Dekker: New York, 1969; pp 407–412.

(25) Pitzer, K. S. *J. Am. Chem. Soc.* **1937**, *59*, 2365–2371.

(26) Heubel, P-H. C.; Popov, A. I. *J. Solution Chem.* **1979**, *8*, 615–625.

(27) The ratio of rate constants for the unimolecular reaction with mono-*p*-nitrophenyl glutarate and the acetate-catalyzed hydrolysis of *p*-nitrophenyl acetate is 462 M at 25 °C.[4] However, the acetate-catalyzed hydrolysis proceeds via two competing mechanisms–44% general base catalysis and 56% nucleophilic catalysis.[28] This brings the value of k_{uni}/k_{bi} to 825 M. Succinate monoesters form anhydrides 230 times faster than do the corresponding glutarate esters;[21] this yields a value of $k_{uni}/k_{bi} = 1.9 \times 10^5$ M. The σ* for the carboxyethyl substituent is ≈0.19 (based on the value of 0.52 for the $^-O_2CCH_2-$ group[19]). A ρ* value of 2.4 for the acetate reaction (assumed to be identical with that for the chloromethylphosphonate reaction) means that the rate constant ratio should be reduced by a factor of 2.9 ($=10^{2.4(0.19-0)}$) in order to correct for the inductive effect of the carboxylate group. This yields a corrected value of $k_{uni}/k_{bi} = 6.6 \times 10^4$ M.

and $k_{uni}/k_{bi} \approx 4 \times 10^3$ M (20 °C) for acyl transfer to a trialkylamino group.[29]

Although the magnitude of the rate enhancement for intramolecular nucleophilic catalysis by the phosphonate dianion is similar to that for analogous intramolecular reactions, the manifestation of the rate acceleration in the thermochemical activation parameters is notably different (Table I). The rate enhancement in the *p*-nitrophenyl 3-phosphonopropionate reaction, compared to its bimolecular counterpart, is enthalpic in origin. Conversion of the bimolecular reaction into the unimolecular reaction results in a decrease in $\Delta H^{\ddagger}$ by ~8 kcal/mol (corresponding to an increase in the rate constant by a factor of $e^{\Delta\Delta H^{\ddagger}/RT} = 5 \times 10^5$ at 37 °C). More striking, however, is the observation of a *less favorable* $\Delta S^{\ddagger}$ for the intramolecular reaction. The $\Delta S^{\ddagger}$ for the unimolecular reaction is more negative than that of the bimolecular reaction by ~9 eu (corresponding to a decrease in the rate constant by a factor of $e^{\Delta\Delta S^{\ddagger}/R} = 90$). A decrease in the entropy of activation resulting from the conversion of a bimolecular reaction into the corresponding unimolecular reaction is unusual. In displacement reactions involving acyl transfers to carboxylate or to tertiary amines, for example, $\Delta S^{\ddagger}$ is ~14 eu more positive for the intramolecular reaction compared with the intermolecular reaction (Table I). This difference in $\Delta S^{\ddagger}$ is typical of the entropic advantage usually found for intramolecular reactions over intermolecular reactions. Bruice[22] has compiled the activation entropies for 15 unimolecular acyl transfer reactions. The average value of $\Delta S^{\ddagger}$ (−14 ± 3 eu) for these reactions is more positive than the average value (−30 eu) for the corresponding bimolecular reactions.[22,31] Similar entropic enhancements in intramolecular reactions relative to the intermolecular counterparts are found in aliphatic nucleophilic substitution reactions. For example, cyclization reactions that yield five-membered rings have $\Delta S^{\ddagger}$ values which are typically 20 eu more positive than those for the corresponding bimolecular S_N2 reactions.[32]

The lack of an entropic contribution to rate enhancement of the *p*-nitrophenyl 3-phosphonopropionate reaction ($k_{uni}/k_{bi} \approx 7 \times 10^3$ M) reflects an unusually unfavorable $\Delta S^{\ddagger}$ for the intramolecular reaction and an unusually favorable $\Delta S^{\ddagger}$ for the intermolecular reaction. The intramolecular reaction has a $\Delta S^{\ddagger}$ (= −23 eu) which is ~9 eu more negative than that generally found in other intramolecular acyl transfer reactions.[22] The activation entropy for the intramolecular acyl transfer to the phosphonate dianion is also more negative (by ~12 eu) than the average $\Delta S^{\ddagger}$ of a diversity of reactions which proceed through a cyclic five-membered transition state.[33]

The entropy of activation for the bimolecular reaction of methylphosphonate with *p*-nitrophenyl acetate is 8–17 eu more positive than $\Delta S^{\ddagger}$ for the bimolecular reactions of trimethylamine, imidazole, or acetate with *p*-nitrophenyl acetate (Table I). The basicities of these nucleophiles span a range from pK_a 9.7 to 4.7. The unusually positive $\Delta S^{\ddagger}$ for the phosphonate reaction, therefore, cannot be attributed to differing rate-limiting steps in this reaction and the reaction with these other acyl acceptors.[34] The more positive values of $\Delta S^{\ddagger}$ (by ~14 eu) and $\Delta H^{\ddagger}$ (by ~6 kcal/mol) for acyl transfer to the dianionic phosphonate than for acyl transfer to monoanionic or neutral nucleophiles suggest that desolvation of the acyl acceptor makes a major contribution to the activation energy barrier for nucleophilic attack by methylphosphonate. A greater contribution from desolvation in the phosphonate reaction than in the reaction with the other nucleophiles is expected since the dianionic nucleophile is more extensively hydrated in the ground state than are the neutral or monoanionic nucleophiles.[35] The release of strongly interacting water molecules from the nucleophile, as bond formation proceeds, will make both $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ more positive.[36] A domination of the rate-determining step by solvent reorganization in reactions involving acyl transfer to phosphonate dianions is consistent with the results from structure–reactivity studies.[3]

In view of this putative role of solvent reorganization in displacement reactions by dianionic nucleophiles, an interpretation of the relative values of the activation parameters in the inter- and intramolecular reactions is now possible. The rate-determining step in bimolecular acyl transfer to phosphonates is formation of the tetrahedral intermediate.[3] If the rate-determining step in the intramolecular reaction is expulsion of *p*-nitrophenoxide from the tetrahedral intermediate (the k_2 step in Scheme I) the contribution of solvent reorganization to the free energy of activation will be less significant than it is in the intermolecular reaction. A smaller domination by solvation effects of the rate-determining step in the intramolecular reaction is also expected if steric interactions preclude the full development of the hydration shell around the phosphono substituent of *p*-nitrophenyl 3-phosphonopropionate. (The carbonyl C–P separation in the fully extended conformation is ~4.5 Å.)

Breakdown of the tetrahedral intermediate is, most likely, the rate-determining step in the *p*-nitrophenyl 3-phosphonopropionate reaction. Although the nucleophilic group (pK_{a2} = 7.5) and the leaving group (pK_a = 7.1) are of similar "leaving ability" in the bimolecular reaction which involves an acyclic tetrahedral intermediate, expulsion of the phosphonate from the cyclic tetrahedral intermediate in the unimolecular reaction is expected to be favored over expulsion of *p*-nitrophenoxide. The more rapid phosphonate expulsion, associated with ring opening, will favor the partitioning of the tetrahedral intermediate toward starting material, and the slower *p*-nitrophenoxide expulsion will be the rate-limiting step in the intramolecular reaction. This is analogous to other intramolecular reactions in which formation of a five-membered cyclic tetrahedral intermediate results in a greater enhancement of the partitioning step for nucleophile expulsion than for leaving group expulsion. For example, structure–reactivity studies on monophenyl succinates[2,4,22] indicate a greater degree of cleavage of the bond to the leaving group in the transition state of the unimolecular reaction than in the bimolecular counterpart. The relative magnitudes of the activation parameters for the *p*-nitrophenyl 3-phosphonopropionate reaction and the analogous intermolecular reactions are consistent with differing rate-determining steps in these two reactions. Rate-determining breakdown of the cyclic tetrahedral intermediate is also consistent with the low sensitivity of the pH-independent rate constant for the unimolecular reaction to solvent effects[37] (solvent polarity[37] and isotopic substitution[38–40]). A consequence of differing rate-determining steps in the inter- and intramolecular reactions in that the empirical rate constant ratio ($k_{uni}/k_{bi} \approx 7 \times 10^3$ M) is an underestimate of the rate enhancement for nucleophilic attack.

(28) Gold, V.; Oakenfull, D. G.; Riley, T. *J. Chem. Soc. B* **1958**, 515–519.
(29) Bruice, T. C.; Benkovic, S. J. *J. Am. Chem. Soc.* **1963**, *85*, 1–8. For the reactions with *p*-nitrophenyl benzoates[30] the ρ value for aminolysis (=1.43) is smaller than the ρ value for alkaline hydrolysis (=2.01). This corresponds to a ρ^* value of 2.04 [=(2.87)(1.43)/2.01]. The σ^* value of the *N,N*-dimethylaminoethyl substituent[20] is 0.13. Correcting for attenuation by a methylene group,[18] the calculated σ^* value of the 3-(*N,N*-dimethylamino)-propyl group is 0.044. This corresponds to a small effect on the rate constant for the unimolecular reaction resulting from the inductive effect on the acyl substituent ($10^{(2.04)(0.044)} = 1.23$).
(30) Kirsch, J. F.; Kline, A. *J. Am. Chem. Soc.* **1969**, *91*, 1841–1847.
(31) Bruice, T. C.; Benkovic, S. J. *J. Am. Chem. Soc.* **1964**, *86*, 418–426.
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(33) Mandolini, L. *J. Am. Chem. Soc.* **1978**, *100*, 550–554.
(34) Gresser, M. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 6970–6980.
(35) See, for example: Webb, J. L. "Enzyme and Metabolic Inhibitors"; Academic Press: New York, 1963; Vol. 1, pp 250–258.
(36) Kovach, I. M.; Hogg, J. L.; Raben, T.; Halbert, K.; Rodgers, J.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 1991–1999.
(37) See, for example: (a) Fagley, T. F.; Oglukain, R. L. *J. Phys. Chem.* **1969**, *73*, 1438–1447. (b) Wigfield, D. C.; Lem, B. *Tetrahedron* **1975**, *31*, 9–11. (c) Dafforn, G. A.; Koshland, D. E., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 7246–7257.
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(40) Schowen, K. B. J. In "Transition States of Biochemical Processes", Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1978; pp 225–283.

Electrophilic Catalysis of Phosphonoacetyl Transfer Reactions. The second-order rate constants for reactions of *p*-nitrophenyl phosphonoacetate with hydroxide and with a thiolate (*N*-acetylcysteine) are anomalously small compared to the values for the corresponding reactions of *p*-nitrophenyl esters having acyl substituents which are similar in polarity to the phosphonomethyl group ($\sigma^* = 0.12$). The alkaline hydrolysis rate constant of *p*-nitrophenyl 4-bromobutyrate ($\sigma^* = 0.13$), for example, is 320-times greater than that of *p*-nitrophenyl phosphonoacetate. The thiolysis rate constant of the phosphonoacetate deviates 9200-fold from the Taft correlation based on the rate constants for ten *p*-nitrophenyl esters.[3] It is unlikely that the low reactivity of *p*-nitrophenyl phosphonoacetate is due entirely to a steric effect. The alkaline hydrolysis and the thiolysis of *p*-nitrophenyl esters are fairly insensitive to steric effects of the acyl substituent. For example, the second-order rate constant for thiolysis of *p*-nitrophenyl trimethylacetate is only 6.8-times smaller than the rate constant for thiolysis of *p*-nitrophenyl acetate.[3] The bulky *tert*-butyl group ($\sigma^* = -0.30$) is less polar than the dianionic phosphonomethyl group ($\sigma^* = 0.12$), yet *p*-nitrophenyl trimethylacetate is *more reactive* than *p*-nitrophenyl phosphonoacetate (7-fold toward hydroxide, 500-fold toward the thiolate of *N*-acetylcysteine). The negative deviation of *p*-nitrophenyl phosphonoacetate from the correlation of the hydrolysis (or thiolysis) rate constants of a series of *p*-nitrophenyl esters with the Taft substituent constant (Table II) can be attributed to an electrostatic effect. The magnitude of the deviation of *p*-nitrophenyl phosphonoacetate (5.4 kcal/mol in the thiolysis reaction, 3.4 kcal/mol in the hydrolysis reaction) is reasonable for an electrostatic destabilization. The mutual potential energy of a negative charge (e.g., the oxyanion of the carboxyl group in the tetrahedral intermediate) and an oxygen atom of the phosphonate group will depend on the distance between these groups and on the "effective" local dielectric constant.[41] The maximum separation between the oxyanion (C–O oxygen) and an oxygen atom of the phosphonate moiety is ~5 Å. If the effective dielectric constant near these charged groups is between 25 and 39[42] the electrostatic interaction energy (3.4–5.4 kcal/mol) will correspond to the observed destabilization energy of the phosphono substituent (i.e., the difference between the observed $\Delta G^{\ddagger}$ and the theoretical value for the reaction of a nonionic ester).

The reactivity of *p*-nitrophenyl phosphonoacetate is enhanced in the presence of Mg^{2+} or Ca^{2+} (Table II). The dependence of the rate constant for the acyl transfer reactions on the divalent cation concentration (e.g., Figure 1) suggests the following kinetic mechanism:

$$M^{2+} + {}^{2-}O_3PCH_2CO_2NP \overset{K_d}{\rightleftharpoons} MO_3PCH_2CO_2NP \xrightarrow{k_c(X^-)} MO_3PCH_2C(O)X + NPO^- \quad (6)$$

It is likely that the kinetically determined "dissociation constant" (i.e., the concentration of divalent cation required for half-maximal rate enhancement) is a true thermodynamic dissociation constant. The kinetically determined dissociation constant is independent of the acyl acceptor (HO^- or RS^-) but the rate constant, k_c, for the reaction of the ester, saturated with the divalent cation, is 30-fold greater with hydroxide than with the thiolate (Table II). If the kinetically determined K_d value was a measure of the dissociation under steady-state (rather than equilibrium) conditions its value would be sensitive to changes in k_c. The value of the kinetically determined dissociation constant for Mg^{2+} (0.1 M) is in reasonable agreement with the calculated value of the thermodynamic dissociation constant (0.06 M), based on the linear relationship between log K_d and pK_{a2} for a series of magnesium–phosphonate complexes.[44]

The rate constant for the acyl transfer reaction in which *p*-nitrophenyl phosphonoacetate is saturated with the divalent cation (i.e., $[M^{2+}] >> K_d$) is two orders of magnitude greater than the rate constant for the corresponding reaction in the absence of divalent cations (Table II). The catalytic effect of the metal ions is dependent on the anionic phosphono substituent. The rate constant for the alkaline hydrolysis of either *p*-nitrophenyl acetate or *p*-nitrophenyl (dimethylphosphono)acetate ($\mu = 0.6$ M) is unaffected by increasing concentrations of $MgCl_2$ or $CaCl_2$ (up to at least 0.2 M). Divalent cations, in general, do not activate the carbonyl group of phenolic esters toward nucleophilic attack.[46] The catalytic effects of Ca^{2+} and Mg^{2+} on the acyl transfer reactions of *p*-nitrophenyl phosphonoacetate are comparable to the effects of these ions on the nucleophilic displacement reactions of dianionic acetyl phosphate.[47,48] Ionic interactions between the divalent cation and the dianionic phosphono group undoubtedly play a significant role in the metal ion promoted acyl transfer reactions of acetyl phosphate and of *p*-nitrophenyl phosphonoacetate. There are several possible mechanisms by which these electrostatic interactions can be utilized to enhance the reactivity of *p*-nitrophenyl phosphonoacetate.

One of the possible modes of metal ion catalysis is complexation with the dianionic phosphono substituent (ion pair formation). If the divalent cation remains coordinated to the phosphono moiety throughout the course of the reaction, the resulting enhancement of the polar effect of the acyl substituent will lead to an acceleration of the acyl transfer reaction. This enhanced polar effect is due to a field effect (charge neutralization) and to an inductive effect (increase in electron-withdrawing ability of the phosphono substituent). Based on the sensitivity to polar effects[3] ($\rho^* = 2.9$ for hydrolysis; $\rho^* = 3.4$ for thiolysis) the Ca^{2+}-induced rate enhancement of the *p*-nitrophenyl phosphonoacetate reactions ($k_c/k_0 = 102$) corresponds to an increase in σ^* by ~0.6 and the Mg^{2+}-induced rate enhancement ($k_c/k_0 = 160$) corresponds to an increase in σ^* by ~0.8. Although the magnitudes of these increases in the polar substituent constant (resulting from complexation of the divalent cation with the dianionic phosphono group) are reasonable (e.g., monoprotonation of the phosphonomethyl substituent corresponds to an increase in σ^* by 0.6, and complete protonation corresponds to an increase in σ^* by 1), it is unlikely that complexation of the metal ion exclusively with the phosphono ligand can result in the observed rate enhancements. The catalytic effect of the divalent cation can be described in terms of binding interactions. For example, the Mg^{2+}-induced rate enhancement of 160-fold indicates tighter binding of the cation to the ester in the transition state than to the ester in the ground state, by a factor of 160. This is an implausibly large change in the dissociation constant of the Mg^{2+}–phosphonate complex to be the result of the progression of the ester from the ground state to the transition state. In order for the dissociation constant to decrease by this factor the pK_{a2} of the conjugate acid of the phosphonate would have to increase 4 pK_a units (based on the linear free-energy relationship of Kluger et al.).[45] The pK_{a2}^* of *p*-nitrophenyl phosphonoacetate is 6.5 and the pK_a^* for the second ionization of the phosphono group in phosphonoacetic acid is 8.7.[26] The phosphono group in the tetrahedral intermediate (^-OH addition at the carbonyl group of the ester) has an estimated pK_a value of 7.7.[49] These increases in the pK_a values correspond to decreases in the dissociation constant for the Mg^{2+}–phosphonate complex by a factor of 3 in the tetrahedral intermediate and by a factor of 5.5 in the product. Since the rate-determining step

(41) (a) Kirkwood, J. G.; Westheimer, F. H. *J. Chem. Phys.* **1938**, *6*, 506–513. (b) Tanford, C.; Roxby, R. *Biochemistry* **1972**, *11*, 2192–2198.

(42) A dielectric constant of ~30 is expected at a distance of ~6 Å from a univalent ion. This is based on the empirical relationship of Conway et al.:[43] $D = 6d - 7$ ($3 \text{ Å} \leq d \leq 10 \text{ Å}$).

(43) Conway, B. E.; Bockrin, J. O'M.; Ammar, I. A. *Trans. Faraday Soc.* **1951**, *47*, 756–766.

(44) From the data of Kluger et al.[45] ($\mu = 0.3$ M, 30°), $\log K_d = -0.34pK_{a2} + 0.847$ ($n = 5$, $r = 0.996$). For *p*-nitrophenyl phosphonoacetate, at an ionic strength of 0.3 M, $pK_{a2} = 6.0$ ($pK_{a2}^* = 6.5$).

(45) Kluger, R.; Wasserstein, P.; Nakaoka, K. *J. Am. Chem. Soc.* **1975**, *97*, 4298–4303.

(46) See, for example: Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 7297–7300 and references cited therein.

(47) Koshland, D. E., Jr. *J. Am. Chem. Soc.* **1952**, *74*, 2286–2292.

(48) DiSabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4393–4400.

(49) This pK_{a2}^* value is based on the Taft relationship for ionization of phosphonic acids (see ref 23) and a substituent constant of $\sigma^* = 0.36$ for $-CH_2C(OH)(O^-)(ONP)$.[3,20]

in the alkaline hydrolysis (or thiolysis, with *N*-acetylcysteine) of *p*-nitrophenyl esters is formation of the tetrahedral intermediate[3] it is unlikely that the (virtual) dissociation constant of the Mg^{2+}–ester complex will decrease by a factor of 160 in the transition state if the metal ion remains coordinated exclusively to the phosphono substituent when the Mg^{2+}–ester complex proceeds to the transition state.

The divalent-cation-catalyzed hydrolysis of *p*-nitrophenyl phosphonoacetate can be explained if there is a change in the mode of metal ion chelation when the cation–ester complex progresses from the ground state to the transition state. The model studies of Kluger et al.[45,50] suggest a mode of chelation which can account for the divalent-cation-induced rate enhancement as one which involves a bidentate coordination of the metal ion by two oxygen atoms to form a six-membered cyclic complex. A chelate complex of Mg^{2+} or Ca^{2+} with an oxygen anion of the phosphono group and the incipient carbonyl oxy anion of *p*-nitrophenyl phosphonoacetate is expected to be a reasonably stable species. Heubel and Papov[51] have found that Mg^{2+} forms a tight complex with the trianionic form of phosphonoacetate ($K_d \sim 10^{-5}$ M). This most likely reflects the dissociation of Mg^{2+} from the bidentate coordination complex since the dissociation constant is three orders of magnitude smaller than the calculated value assuming complexation of the metal exclusively with the dianionic phosphono moiety of phosphonoacetate. The basicity of the carbonyl oxy anion in the tetrahedral intermediate is greater than that of the carboxylic acid group in the product and, therefore, the tetrahedral intermediate, or the structurally similar transition state, is expected to be a very good bidentate ligand for Mg^{2+}. The stable cyclic complex resulting from the bidentate coordination of the divalent cation will stabilize the transition state and, thus, accelerate the reaction.

Another possible role of the divalent cations which must be considered is the effect of the metal ions on the relative contribution of competing reaction pathways. The complexation of divalent cations with the phosphonoate moiety of *p*-nitrophenyl phosphonoacetate will increase the acidity of the methylene group and, therefore, potentially facilitate a β-elimination reaction. While we find no evidence of a β-elimination mechanism in the hydroxide-catalyzed release of *p*-nitrophenoxide from *p*-nitrophenyl phosphonoacetate it is possible that such a mechanism may account for the anomalously high reactivity of *p*-nitrophenyl (dimethylphosphono)acetate (Table II). *p*-Nitrophenyl 3-(dimethylphosphono)propionate shows no significant deviation from the Taft correlation between the second-order rate constant for alkaline hydrolysis and the polar substituent constant.[3] *p*-Nitrophenyl (dimethylphosphono)acetate, however, is over 100-times more reactive than is anticipated on the basis of the same Taft correlation. This may be a consequence of a mechanism involving an abstraction of hydrogen at the 2-position of the (dimethylphosphono)acetate ester by hydroxide followed by elimination of *p*-nitrophenoxide. It is interesting to note that a reaction which is analogous to the first step in this elimination mechanism, deuteroxide-catalyzed exchange at the 2-position of acetonyl phosphonate, is accelerated 2000-fold in the presence of saturating concentrations (0.2 M) of magnesium ion.[50] The rate of alkaline hydrolysis of *p*-nitrophenyl (dimethylphosphono)acetate is unaffected by the presence of 0.2 M Mg^{2+} reflecting the inefficiency of the nonionic phosphoryl oxygen in complexation with Mg^{2+}.

No incorporation of deuterium into either *p*-nitrophenyl phosphonoacetate or the phosphonoacetate product was observed when the ester was incubated in D_2O (±0.1 M magnesium) for a period of time corresponding to ~1 half-life for the hydrolysis reaction. The ratio of second-order rate constants for the alkaline hydrolysis and the thiolysis reactions are not altered by addition of either Mg^{2+} or Ca^{2+} [$k_{OH}/k_S = 31$ (±2)]. The magnitude of the solvent isotope effect for the alkaline hydrolysis of the calcium–*p*-nitrophenyl phosphonoacetate complex (k_{OH}/k_{OD} = 0.6–0.7) is the same as that for the alkaline hydrolysis of the free *p*-nitrophenyl phosphonoacetate or for the specific base-catalyzed hydrolysis of other esters.[40] These results indicate that the divalent-cation-induced rate enhancement of the alkaline hydrolysis of the phosphonoacetate ester reflects an acceleration of the normal carbonyl addition mechanism and not a change in mechanism.

The catalytic efficiency of the divalent-cation-catalyzed acyl transfer reactions of *p*-nitrophenyl phosphonoacetate reflects a geometric perturbation of the metal–ester complex. As the ester proceeds from the ground state to the transition state the developing bidentate coordination of the metal (with the carbonyl oxygen and a phosphono oxygen) will enhance the affinity of the cation for the ester and thus stabilize the transition state. There is a widespread occurrence of divalent metal ions as cofactors in phosphoryl and phosphate transfer reactions.[7] Although these metal ions play an important role in most biochemical reactions of phosphorylated substrates, the mode of association of the divalent cation with these reactants in the transition state remains uncertain.[45,48,50,52] Hydrolysis of *p*-nitrophenyl phosphonoacetate provides a convenient system for evaluating the contribution of the various modes of divalent cation–substrate interaction to a variety of metal ion catalyzed reactions. The 120-fold rate enhancement of the hydrolysis of dianionic acetyl phosphate in the presence of 0.1 M Mg^{2+},[47] for example, is similar to the 160-fold Mg^{2+}-induced rate enhancement of *p*-nitrophenyl phosphonoacetate hydrolysis. Complexation of Mg^{2+} with the phosphate group will facilitate the acetyl transfer reactions of the acyl phosphate (C–O cleavage) by making it a better leaving group. However, complexation with the phosphono substituent of *p*-nitrophenyl phosphonoacetate can contribute only ~3% of the observed rate enhancement of the phosphonoacetyl transfer reactions (i.e., ≤5-fold vs. the observed 160-fold acceleration). The divalent-cation-catalyzed acyl transfer reactions of dianionic *p*-nitrophenyl phosphonoacetate suggest that bidentate coordination of the metal ion with the incipient tetrahedral intermediate can account for the metal ion promoted reactions of dianionic acyl phosphates. A six-center chelate in the Mg^{2+}-catalyzed acyl transfer reactions of acetyl phosphate, proposed over 30 years ago by Koshland,[47] is also consistent with the results from a variety of model studies.[45,50,52]

The acyl transfer reactions of *p*-nitrophenyl phosphonoacetate can be conveniently monitored. Vast amounts of data exist on the mechanism of acyl transfer reactions. The dependence of the rate constant for alkaline hydrolysis of *p*-nitrophenyl esters on nucleophile basicity,[53] polar acyl substituents,[3] and the stereoelectronic structure of the tetrahedral intermediate[34,54] provides a useful framework for the interpretation, in terms of geometric and electronic effects, of the catalysis by divalent ions. The acyl transfer reactions of *p*-nitrophenyl phosphonoacetate can, therefore, serve as convenient and efficacious probes of transition state structures of metal ion facilitated processes in aqueous solution and in enzyme-catalyzed reaction.

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Supplementary Material Available: A list of the pK^*_{a2} values of 17 phosphonic acids (XPO_3H^-) and σ^*_X data for the pH dependence for hydrolysis of *p*-nitrophenyl 3-phosphonopropionate (2 pages). Ordering information is given on any current masthead page.

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