J. Org. Chem. **1997,** *62,* 7351−7357 **7351**

Dissection of Nucleophilic and General Base Roles for the Reaction of Phosphate with *p*-Nitrophenyl Thiolacetate, *p*-Nitrophenyl Thiolformate, and Phenyl Thiolacetate

Manjinder S. Gill, Alexei A. Neverov, and R. S. Brown*

Department of Chemistry, Queen's University, Kingston, Ontario, Canada, K7L 3N6

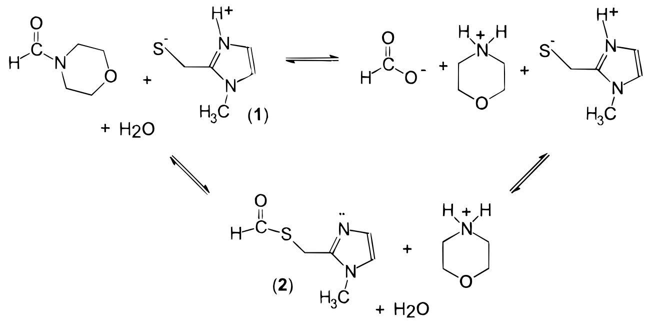
Received May 20, 1997⊗

Phosphate buffers are well-known to catalyze the decomposition of various active acyl compounds. This study was undertaken to determine the extent to which it acts as a nucleophile and general base toward some activated esters and thiolesters. Thus, the hydrolyses of *p*-nitrophenyl acetate (**3a**), *p*-nitrophenyl thiolacetate (**3b**), phenyl acetate (**4a**), phenyl thiolacetate (**4b**), and *p*-nitrophenyl thiolformate (**5**) have been studied in aqueous phosphate, $\mu = 1.0$ (K_2SO_4). Both phosphate monoanion and dianion are reactive toward the thiolesters **3b**, **4b**, and **5**. For **3b**, reaction of the dianion exhibits a solvent kinetic isotope effect (SKIE) of 1.00 ± 0.11 while that for the monoanion is 2.13 ± 1.1. For the reaction of phosphate dianion with **5**, the SKIE is 0.8 ± 0.2 and that for the monoanion at pH 3.05 is roughly 1.5. Phosphate dianion reacts with each thiolacetate and its oxygen analogue at comparable rates: the reactivity ratio of the formyl to acetyl thiolesters, **5**:**3b**, toward phosphate dianion is 685. ^1H NMR analysis of the **3b** hydrolysis mixtures in H_2O and D_2O containing phosphate shows the transient formation, and subsequent hydrolysis, of acetyl phosphate. Analysis of the kinetics of these processes indicates that in H_2O at pH = 8.5, phosphate dianion functions as both a nucleophile and general base toward **3b**, the nucleophilic role comprising 80−93% of the reaction. In D_2O, the process is entirely nucleophilic. For the reaction of phosphate dianion with **4b**, the ^1H NMR analysis indicates that the nucleophilic role comprises 40−50% of the reaction, the general base role being 50−60%. The reaction of phosphate dianion with **5** is entirely nucleophilic, while the monoanion reacts as a general base. The data are interpreted in terms of standard carbonyl addition/elimination mechanisms in which the ability of the attacking phosphate di- or monoanion to displace a given leaving group is tied to the pK_a of the conjugate acids of the nucleophile and leaving groups.

Introduction

In an earlier report we have described the catalysis of acyl transfer between certain formamides and water by an imidazole thiol.[1] This reaction proceeds as in Scheme 1 and was proposed as a mimic of the chemistry believed to occur in the active site of the cysteine proteases. As part of this program we were interested in the partitioning of the formyl thiolester intermediate (**2**) between attack of water and amine nucleophiles. Surprisingly, when **2** was placed in an aqueous phosphate buffer, an immediate quantitative formation of formyl phosphate[2,3] occurred (confirmed by ^1H and ^{31}P NMR). The apparent facility of the acyl transfer from a thiolester to phosphate seemed an unusual process and worthy of further study.

Scheme 1



Phosphate is a commonly used buffer for controlling the pH around neutrality, but it is often not a kinetically innocent species. Catalytic enhancement of the rate of hydrolysis of such species as activated esters,[4−6] phosphoimidazolide-activated nucleotides,[7] and acyl imidazoles[8] by phosphate has been reported numerous times. The mode of buffer catalysis has been discussed[4−8,9] in terms of general acid/base and nucleophilic mechanisms, but often the exact process is unclear unless one can actually observe an intermediate such as the one formed during nucleophilic catalysis.[10] To the best of our knowledge, the first unambiguous demonstration of a nucleophilic role for phosphate reacting with an activated ester was provided in 1984 by O'Connor and Wallace.[6] In that study, the hydrolysis of *p*-nitrophenyl acetate (pNPA) in the presence of phosphate was monitored by both UV/vis spectrophotometry and ^1H NMR, the latter showing the transient appearance of acetyl phosphate.

⊗ Abstract published in *Advance ACS Abstracts*, September 15, 1997.

(1) Keillor, J. W.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **1994**, *116*, 4669.

(2) Jahansouz, H.; Mertes, K. B.; Mertes, M.; P.; Himes, R. H. *Bioorg. Chem.* **1989**, *117*, 207.

(3) Neverov, A. A.; Brown. R. S., unpublished observations.

(4) (a) Bruice, T. C.; Lapinski, R. *J. Am. Chem. Soc.* **1958**, *80*, 2265. (b) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 1778. (c) Shames, S. L.; Byers, L. D. *J. Am. Chem. Soc.* **1981**, *103*, 6170 and references therein. (d) Koehler, K.; Skora, R.; Cordes, E. H. *J. Am. Chem. Soc.* **1966**, *88*, 3577. (e) Fedor, L. R.; Bruice, T. C. *J. Am. Chem. Soc.* **1965**, *87*, 4138. (f) Bruice, T. C.; Bruno, J. J.; Chou, W. *J. Am. Chem. Soc.* **1963**, *85*, 1659.

(5) Johnson, S. L. *Adv. Phys. Org. Chem.* **1967**, *5*, 237.

(6) O'Connor, C.; Wallace, R. G. *Aust. J. Chem.* **1984**, *37*, 2559.

(7) Kanavarioti, A.; Rosenbach, M. T. *J. Org. Chem.* **1991**, *56*, 1513.

(8) Jencks, W. P.; Carriuolo, J. *J. Biol. Chem.* **1959**, *234*, 1272, 1280.

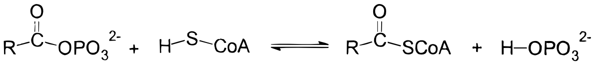
(9) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 24−242.

(10) Gold, V.; Oakenfull, D. G.; Riley, T. *J. Chem. Soc. B* **1968**, 515.

S0022-3263(97)00904-3 CCC: $14.00

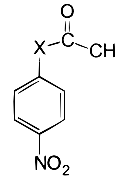
Subsequently, Byers has suggested, on the basis of detailed kinetic studies (including solvent kinetic isotope effects and activation parameters), that phosphonates[4c] and molybdate[11] are good nucleophiles toward both pNPA and *p*-nitrophenyl thiolacetate (pNPTA).

As pointed out by O'Connor and Wallace,[6] the reaction of activated esters with phosphate may serve as a model for a biological process wherein the enzyme phosphotransacetylase converts an acyl phosphate and CoA-SH into various acyl CoA esters,[12] eq 1. Given the analogy

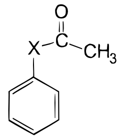


$$R\text{-}C(=O)\text{-}OPO_3^{2-} + H\text{-}S\text{-}CoA \rightleftharpoons R\text{-}C(=O)\text{-}SCoA + H\text{-}OPO_3^{2-} \quad (1)$$

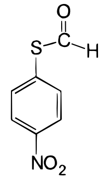
of the transfer from the formyl thiolester (**2**) to phosphate, and the rapidity of that reaction, we undertook a more complete study of this process to compare the reactivities of oxygen and thiol esters **3** and **4** toward phosphate at 37.5 °C, as well as the hydrolysis of *p*-nitrophenyl thiolformate (**5**) in aqueous phosphate buffer at 25 °C. The following indicates that (1) phosphate dianion is indeed a nucleophile toward all species with similar preference for oxygen and thiol esters; (2) phosphate monoanion acts as a general base; and (3) there is a competition between the nucleophilic and general base roles that depends upon the pK_a of the conjugate acids of the attacking nucleophile and leaving group.



3 a, X=O
b, X=S



4 a, X=O
b, X=S



5

Experimental Section

(a) Materials and General Methods. *p*-Nitrophenyl thiolacetate (**3b**),[13] phenyl thiolacetate (**4b**),[14] and *p*-nitrophenyl acetate (**3a**)[4b,15] were prepared according to the literature procedures. Phenyl acetate (**4a**) (Aldrich) was used as supplied. *p*-Nitrophenyl thiolformate (**5**) was prepared according to the procedure of Bax et al.[16] from the reaction of acetic formic anhydride[17] with *p*-nitrobenzenethiol and a catalytic amount of pyridine. Potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate were analytical grade (BDH) and were used as supplied. Acetonitrile was distilled from P_2O_5. Acetyl phosphate was prepared according to the procedure of Jencks et al.[18] Formyl phosphate was obtained as a solid mixture containing formic acid and acetic acid as impurities by the reaction of aqueous K_2HPO_4 with excess acetic formic anhydride at pH ~7 in an ice bath for 45 min, followed by precipitation by addition of cold acetone.

(B) Kinetics. The kinetics of the reactions were followed by UV/vis spectrophotometry and ^{1}H NMR using instruments and procedures previously described.[1] Buffer solutions (μ = 1 (K_2SO_4)) were made from purified, deoxygenated water and were further degassed by bubbling argon through them for 0.5−1 h before using. The rate of hydrolysis of thiol esters was followed by observing the change in absorbance at 284 nm (decrease) for **3b**; 263 nm (increase) for **4b**; 275 nm (decrease) for **3a**; 270 nm (increase) for **4a**; and 280 nm (decrease) or 412 nm (increase) for **5**. In a typical kinetic experiment, 3−3.5 mL of deoxygenated buffer solution in a quartz cell were equilibrated at 37.5 °C or 25 °C for 20−30 min in the spectrophotometer cell holder, after which the run was initiated by injection of 5−7 μL of a stock solution of thiol ester in dry CH_3CN. The concentration of substrates varied from 6 to 8×10^{-5} M. Reactions were followed to at least 5 half-times, and the pseudo-first-order rate constants (k_{obs}) were obtained for each run by nonlinear least-squares (NLLSQ) fitting of the abs vs time data to a standard exponential model ($A_t = A_\infty + (A_0 - A_\infty)\exp(-k_{obs}t)$). The final pH of the cell was measured after each run. For initial rates the reaction was followed up to 10% completion, and the final absorbance was obtained from the observed absorbance at that particular wavelength when equivalent amounts of the reaction products were added to the same buffer solution. For the reactions conducted in D_2O, the solution pD values were determined as pD = pH + 0.4.[19a]

(C) Product Studies. The products produced during the hydrolysis of the esters in the presence of phosphate were determined by ^{1}H NMR spectroscopy. A buffer solution of a particular pH or pD (0.5 mL) in an NMR tube was equilibrated to 37.5 °C or 25 °C for 0.5 h. The reaction was started by adding a stock solution of $(2-3) \times 10^{-4}$ M of **3b** or **4b** in 5−10 μL of CD_3CN or $(2-3.3) \times 10^{-3}$ M of thiolformate (**5**) in 12−20 μL of CD_3CN, and the spectra were recorded at various times. The rate constants for the hydrolysis of acetyl phosphate and formyl phosphate, $(0.3-4) \times 10^{-2}$ M, were also determined under similar conditions. The hydrolysis of formyl phosphate was conducted using two sample preparations. An authentic sample containing formic acid and acetic acid was used directly, or **5** was allowed to react with aqueous phosphate buffer (0.1 M) at pH ~8 for 1 min, after which the pH of the reaction mixture was quickly reduced to pH 3.05 with subsequent hydrolysis of the formyl phosphate transient being monitored by ^{1}HMR spectroscopy. In the trapping experiments, an aqueous pH 3.05 solution containing hydroxylamine (7.91×10^{-3} M) was allowed to react with **5** (3.73×10^{-3} M) at ambient temperature, and the spectrum was recorded after 30 min. This procedure was repeated with pH 3.05 aqueous solutions containing phosphate buffer (0.01 and 1.0 M). The fractions of formamide and formic acid were calculated from the integration of singlets for the respective formyl protons.

Results and Discussion

The following will be broken into two main parts. In the first we will describe the information that is gained simply by observing, using UV/vis spectroscopy, the rate of production of the hydrolysis products of the various esters in the presence of varying [phosphate]. These experiments will show the spontaneous rate of reaction, and the dependence of the rate on [phosphate], but they will not tell whether the phosphate acts as a nucleophile or a general base. This can be obtained using ^{1}H NMR, the analysis of which will comprise the second part of the discussion.

(i) UV/Vis Kinetics. (a) Water and Hydroxide Reactions. The pseudo-first-order rate constants (k_{obs}) for the hydrolysis of esters **3a,b** and **4a,b** were determined at a given pH (pD) at 37.5 °C as a function of [phosphate]$_{total}$. The primary data (Tables 1S−5S, Supporting Information), when plotted (not shown), give straight lines, the slopes and intercepts of which are the

---

[11] (a) Wikjord, B. R.; Byers, L. D. *J. Am. Chem. Soc.* **1992**, *114*, 5553. (b) Wikjord, B. R.; Byers, L. D. *J. Org. Chem.* **1992**, *57*, 6814.

[12] Lehninger, A. L.; Nelson, D. L.; Cox, M. M. *Principles of Biochemistry*; Worth Publishers: New York, 1972; p 100.

[13] (a) Douglas, K. T.; Yaggi, N. F.; Mervis, C. M. *J Chem. Soc., Perkin Trans. 2* **1981**, 171. (b) Dessoliw, M.; Lalio-Dirard, M.; Vilkas, M. *Bull. Soc. Chim. Fr.* **1970**, 2573.

[14] (a) Demaria, P.; Fini, A.; Hall, F. M. *J. Chem. Soc., Perkin Trans. 2.* **1973**, 196. (b) Street, J. P.; Brown, R. S. *J. Am. Chem. Soc.* **1985**, *107*, 6084.

[15] Chattaway, F. D. *J. Am. Chem. Soc.* **1931**, 2495.

[16] Bax, P. C.; Stevens, W. *Recl. Trav. Chim. Pays-Bas.* **1970**, *89* (3), 265.

[17] Krimen, L. I. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 8.

[18] Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 7938.

[19] (a) Covington, A. K.; Paabo, M.; Robinson, R. A.; Bates, R. G. *Anal. Chem.* **1968**, *40*, 700. (b) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 76th ed; CRC Press: Baco Raton, 1995; pp 8−57.

Table 1. Rate Constants for the Hydrolysis of *p*-Nitrophenyl Thiolacetate (3b) in Aqueous Phosphate Buffer at 37.5 °C[a]

pH	k_0 ($\mathrm{s^{-1}}$)[b]	k_{cat} ($\mathrm{M^{-1}\ s^{-1}}$)[c]
3.05[d]	$(5.75 \pm 0.46) \times 10^{-6}$	$(5.92 \pm 2.1) \times 10^{-6}$
4.05[d]	$(5.42 \pm 0.01) \times 10^{-6}$	$(9.72 \pm 0.01) \times 10^{-6}$
5.0[d]	$(5.26 \pm 0.45) \times 10^{-6}$	$(2.64 \pm 0.21) \times 10^{-5}$
5.7	$(6.74 \pm 0.1) \times 10^{-6}$	$(9.77 \pm 0.05) \times 10^{-5}$
6.1	$(9.58 \pm 1.79) \times 10^{-6}$	$(1.98 \pm 0.08) \times 10^{-4}$
6.5	$(1.08 \pm 0.73) \times 10^{-5}$	$(2.76 \pm 0.34) \times 10^{-4}$
7.0	$(1.88 \pm 0.46) \times 10^{-5}$	$(5.10 \pm 0.21) \times 10^{-4}$
7.5	$(2.95 \pm 0.68) \times 10^{-5}$	$(6.43 \pm 0.31) \times 10^{-4}$
8.5	$(1.65 \pm 0.001) \times 10^{-4}$	$(6.90 \pm 0.001) \times 10^{-4}$
9.0	$(4.00 \pm 0.25) \times 10^{-4}$	$(8.53 \pm 1.17) \times 10^{-4}$
9.5	$(1.07 \pm 0.09) \times 10^{-3}$	$(1.26 \pm 0.40) \times 10^{-3}$
10.0	$(3.84 \pm 0.20) \times 10^{-3}$	
10.5	$(1.18 \pm 0.06) \times 10^{-2}$	
11.0	$(3.38 \pm 0.05) \times 10^{-2}$	

[a] $\mu = 1$ (K_2SO_4). [b] From the intercept of linear regression of plots of pseudo-first-order rate constant for hydrolysis of **3b**; errors are from standard deviations of the plots. [c] Second-order rate constant for the buffer catalysis from the slope of plots of pseudo-first order rate constant for hydrolysis of **3b** vs [phosphate]$_{total}$; errors are from standard deviations of the plots. [d] Rate constants from initial rates of hydrolysis reactions.

Table 2. Rate Constants for the Hydrolysis of *p*-Nitrophenyl Thiolacetate (3b) in Phosphate Buffer in D_2O at 37.5 °C[a]

pD	k_0 ($\mathrm{s^{-1}}$)[b]	k_{cat} ($\mathrm{M^{-1}\ s^{-1}}$)[c]
2.70[d]	$(2.07 \pm 0.38) \times 10^{-6}$	$(4.05 \pm 1.73) \times 10^{-6}$
2.70[d]	$(2.71 \pm 0.25) \times 10^{-6}$	
4.55[d]	$(3.88 \pm 0.55) \times 10^{-6}$	$(3.02 \pm 2.50) \times 10^{-6}$
4.70[d]	4.27×10^{-6}	
6.0[d]		$(2.45 \pm 0.03) \times 10^{-5}$
6.7		$(1.77 \pm 0.61) \times 10^{-4}$
7.1		$(2.84 \pm 0.21) \times 10^{-4}$
7.50	$(7.75 \pm 3.9) \times 10^{-6}$	$(5.82 \pm 0.68) \times 10^{-4}$
8.50	$(7.47 \pm 0.62) \times 10^{-5}$	$(6.30 \pm 0.29) \times 10^{-4}$
9.60	$(2.58 \pm 0.16) \times 10^{-4}$	$(1.02 \pm 0.07) \times 10^{-3}$
10.6	$(2.20 \pm 0.10) \times 10^{-3}$	
11.0	$(7.90 \pm 0.20) \times 10^{-3}$	

[a] $\mu = 1$ (K_2SO_4). [b] From the intercept of linear regression of plots of pseudo-first-order rate constant for hydrolysis of **3b**; errors are from standard deviations of the plots. [c] Second-order rate constant for the buffer catalysis from the slope of plots of pseudo-first-order rate constant for hydrolysis of **3b** vs [phosphate]$_{total}$; errors are from standard deviations of the plots. [d] Rate constants from initial rates of hydrolysis reactions.

Table 3. Rate Constants for the Hydrolysis of Phenyl Thiolacetate (4b) in Phosphate Buffer (H_2O) at 37.5 °C[a]

pH	k_0 ($\mathrm{s^{-1}}$)[b]	k_{cat} ($\mathrm{M^{-1}\ s^{-1}}$)[c]
6.5	$(1.62 \pm 0.77) \times 10^{-6}$	
8.5	$(5.94 \pm 0.15) \times 10^{-5}$	
8.5[d]	$(5.31 \pm 0.31) \times 10^{-5}$	$(1.01 \pm 0.05) \times 10^{-4}$
8.5[e]	$(4.73 \pm 0.83) \times 10^{-5}$	$(8.29 \pm 1.1) \times 10^{-5}$
9.5	$(2.24 \pm 0.01) \times 10^{-4}$	
11.0	$(7.88 \pm 0.4) \times 10^{-3}$	

[a] $\mu = 1$ (K_2SO_4). [b] Fom the intercept of linear regression of plots of pseudo-first-order rate constant for hydrolysis of **4b**; errors are from standard deviations of the plots. [c] Second-order rate constant for the buffer catalysis from the slope of plots of pseudo-first-order rate constant for hydrolysis of **4b** vs [phosphate]$_{total}$; errors are from standard deviations of the plots. [d] $\mu = 2.5$ (K_2SO_4), duplicate runs, mean values of k_{obs} and standard deviations. [e] As in *d*, except [**4b**] = 1 mM.

Table 4. Rate Constants for the Hydrolysis of *p*-Nitrophenyl Acetate (3a) in Phosphate Buffer (H_2O) at 37.5 °C[a]

pH	k_0 ($\mathrm{s^{-1}}$)[b]	k_{cat} ($\mathrm{M^{-1}\ s^{-1}}$)[c]
6.5	$(2.00 \pm 0.06) \times 10^{-5}$	$(2.31 \pm 0.03) \times 10^{-4}$
7.5	$(2.84 \pm 0.76) \times 10^{-5}$	$(6.39 \pm 0.36) \times 10^{-4}$
8.5	$(2.17 \pm 0.14) \times 10^{-4}$	$(6.02 \pm 0.66) \times 10^{-4}$
9.0	$(4.91 \pm 0.52) \times 10^{-4}$	$(1.69 \pm 0.24) \times 10^{-3}$
9.5	$(1.20 \pm 0.15) \times 10^{-3}$	$(3.39 \pm 0.69) \times 10^{-3}$

[a] $\mu = 1$ (K_2SO_4). [b] From the intercept of linear regression of plots of pseudo-first-order rate constant for hydrolysis of **3a**; errors are from standard deviations of the plots. [c] Second-order rate constant for the buffer catalysis from the slope of plots of pseudo-first-order rate constant for hydrolysis of **3a** vs [phosphate]$_{total}$; errors are from standard deviations of the plots.

Table 5. Rate Constants for the Hydrolysis of Phenyl Acetate (4a) in Phosphate Buffer (H_2O) at 37.5 °C[a]

pH	k_0 ($\mathrm{s^{-1}}$)[b]	k_{cat} ($\mathrm{M^{-1}\ s^{-1}}$)[c]
7.80	$(1.76 \pm 0.09) \times 10^{-5}$	
8.75	$(7.24 \pm 0.29) \times 10^{-5}$	
9.60	$(4.01 \pm 0.09) \times 10^{-4}$	
11.0	$(9.21 \pm 0.21) \times 10^{-3}$	
8.5[d]	$(4.06 \pm 0.26) \times 10^{-5}$	$(3.11 \pm 0.38) \times 10^{-5}$
8.5[e]	$(3.87 \pm 0.19) \times 10^{-5}$	$(3.25 \pm 0.27) \times 10^{-5}$
8.5[f]	$(3.41 \pm 0.18) \times 10^{-5}$	$(3.70 \pm 0.25) \times 10^{-5}$

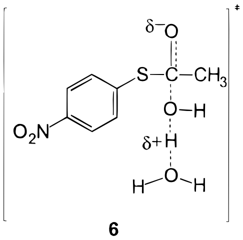
[a] $\mu = 1$ (K_2SO_4). [b] From the intercept of linear regression of plots of pseudo-first-order rate constant for hydrolysis of **4a**; errors are from standard deviations of the plots. [c] Second-order rate constant for the buffer catalysis from the slope of plots of pseudo-first order rate constant for hydrolysis of **4a** vs [phosphate]$_{total}$; errors are from standard deviations of the plots. [d] $\mu = 2.5$ (K_2SO_4). [e] As in *d*, except $\mu = 2.5$ (KCl). [f] As in *d*, except [**4a**] = 0.8 mM.

second-order rate constant for the phosphate reaction (k_{cat}) and spontaneous hydrolysis rate constant (k_o), respectively. These are given in Tables 1−5.

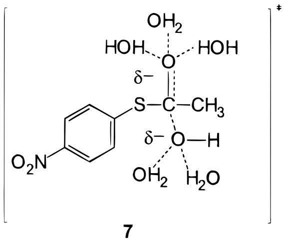
The spontaneous rate of hydrolysis of **3b** in H_2O and D_2O followed the rate expression given in eq 2. Using values for $[OH^-]$ and $[OD^-]$ based on $pK_w^{H_2O} = 13.614$ and

$$k_o = k_{L_2O} + k_{LO^-}[^-OL] \tag{2}$$

$pK_w^{D_2O} = 14.54$ at 37.5 °C,[19b] fits of the k_o data in Tables 1 and 2 to eq 2 gave rate constants k_{L_2O} and k_{LO^-} of $(7.13 \pm 0.79) \times 10^{-6}\ s^{-1}$ and $(17.1 \pm 1.83)\ M^{-1}\ s^{-1}$ in H_2O; in D_2O the values were $(3.29 \pm 0.75) \times 10^{-6}\ s^{-1}$ and $(29.6 \pm 7.0)\ M^{-1}\ s^{-1}$. Therefore, the water reaction has a computed solvent deuterium kinetic isotope effect (SKIE) of $k_{H_2O} / k_{D_2O} = 2.2 \pm 0.9$, while that for the lyoxide term is $k_{HO^-}/k_{DO^-} = 0.6 \pm 0.3$. These are in the typical range for hydrolyses of activated oxygen and thiol esters which show normal values for the water reaction[20] and unit or slightly inverse SKIE's for the acyl transfer to oxyanions including LO^- [21] consistent with transition states **6** and **7**, respectively.



6



7

The rate constants for the alkaline hydrolysis of **3a** and **4a,b** were calculated from fitting the data in Tables 3−5 to eq 2; the respective k_{HO^-} values at 37.5 °C are 23.9 ± 4.5, 5.6 ± 1.4, and 6.26 ± 2.8 $M^{-1}\ s^{-1}$. Although there

---

(20) (a) Alvarez, F. J.; Schowen, R. L. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1987, pp 1−60 and references therein. (b) Hegazi, M.; Mata-Segreda, J. F.; Schowen, R. L. *J. Org. Chem.* **1980**, *45*, 307.

(21) (a) Kellogg, B. A.; Tse, J. E.; Brown, R. S. *J. Am. Chem. Soc.*, **1995**, *117*, 1731. (b) Kellogg, B. A.; Brown, R. S.; McDonald, R. S. *J. Org. Chem.* **1994**, *59*, 4652. (c) Pohl, E. R.; Hupe, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 2763 and references therein.

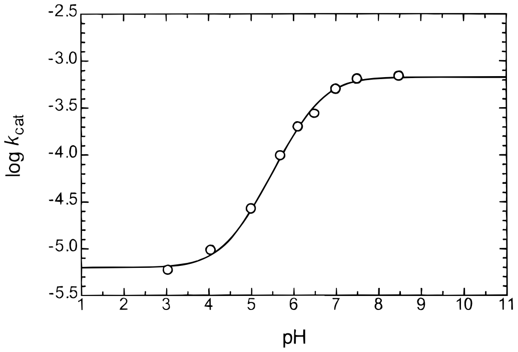


Figure 1. A plot of the second-order rate constants for the reaction of **3b** in H_2O, phosphate buffer, as a function of pH, $T = 37.5$ °C, $\mu = 1$ (K_2SO_4). The points are experimental, and the line is calculated from the NLLSQ fitting of the data to equation $k_{cat}^{obs} = k_M + k_D(1 + [H^+]/K_a^2)$.

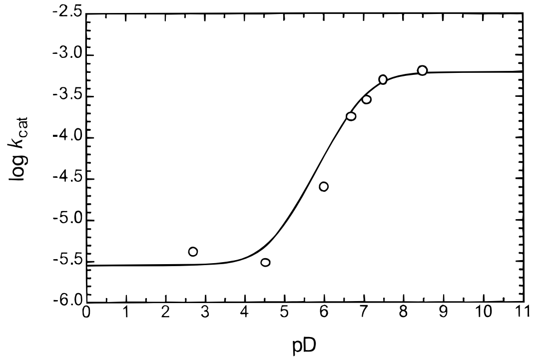


Figure 2. A plot of the second-order rate constants for the reaction of **3b** in D_2O, phosphate buffer, as a function of pD, $T = 37.5$ °C, $\mu = 1$ M (K_2SO_4). The points are experimental and the lines is calculated from the NLLSQ fitting of the data to equation $k_{cat}^{obs} = k_M + k_D(1 + [H^+]/K_a^2)$.

are substantial errors in the fit values, these data indicate that the analogous S- and O-esters are of comparable susceptibility to hydroxide attack; this can be compared with a reported reactivity ratio toward OH^- of about 1/2 reported for **3a**/**3b** at 25 °C.[22]

(b) Phosphate Reactions. Plots of the second order rate constants for the phosphate catalysis (log k_{cat} vs pH, data of Tables 1 and 2) for the reaction of **3b** in H_2O and D_2O are shown in Figures 1 and 2. The lines through the experimental points were calculated by NLLSQ fitting of the data to eq 3 where

$$k_{cat}^{obs} = k_M + k_D(1 + [H^+]/K_a^2) \tag{3}$$

k_M and k_D, are the rate constants for mono- and dianion attack on **3b**, and K_a^2 is the second dissociation constant of H_3PO_4. The so-derived constants are; $k_M = (6.31 \pm 0.56) \times 10^{-6}$ M^{-1} s^{-1}, $k_D = (6.68 \pm 0.47) \times 10^{-4}$ M^{-1} s^{-1}, and $K_a^2 = (3.04 \pm 0.38) \times 10^{-7}$ ($pK_a^2 = 6.52 \pm 0.05$) in H_2O. For the data in D_2O the fitting procedure consisted of two parts. The high pH domain was fit to eq 4 to determine k_D and K_a^2. These constants were then "fixed" to derive the value for k_M from a fit of all the data to eq 3. The computed rate and equilibrium constants

$$k_{cat}^{obs} = k_D(1 + [H^+]/K_a^2) \tag{4}$$

are $k_M = (2.95 \pm 0.83) \times 10^{-6}$ M^{-1} s^{-1}, $k_D = (6.69 \pm 0.23) \times 10^{-4}$ M^{-1} s^{-1}, and $K_a^2 = (6.84 \pm 0.74) \times 10^{-8}$ ($pK_a^2 = 7.16 \pm 0.05$) for the D_2O reaction. The reported value of the second pK_a of phosphoric acid in H_2O at 25 °C and $\mu = 1.0$ (NaCl) is 6.36,[23a] while the value at $\mu = 0$ is 7.2.[23b] The former value, assuming the same temperature dependence as at $\mu = 0$, should drop by only 0.01 unit[23b] at 37.5 °C and therefore agrees well with the best-fit value of 6.52 we determined here in H_2O. It is known that the second pK_a value for phosphate in D_2O is 0.64 unit higher than in H_2O, the reported value of 7.16[23c] agreeing exceptionally well with the best-fit value. The SKIE for the reaction of **3b** with the phosphate dianion, $k_D^{(H_2O)}/k_D^{(D_2O)}$, is 1.00 ± 0.11, while that for the reaction of the monoanion, is $k_M^{(H_2O)}/k_M^{(D_2O)}$, is slightly higher at 2.13 ± 1.1. The unit value for the reaction of the dianion can be compared with those for attack of molybdate^{2-} and the dianions of phosphonic acids on **3a,b** for which values in the range of 1.03 ± 0.1 [4c,11] are reported. Nucleophilic mechanisms have been proposed for the latter reactions. The values for the monoanion and dianion computed in our study appear different from each other and may suggest nucleophilic dianions, and general base monoanions, but the appreciable errors in the best-fit values for the monoanion rate constants limits our ability to draw meaningful mechanistic conclusions about this reaction.

Table 6. Rate Constants for the Hydrolysis of *p*-Nitrophenyl Thiolformate (5) in Phosphate Buffer (H_2O) at 25 °C[a]

pH	k_0 (s^{-1})[b]	k_{cat} (M^{-1} s^{-1})[c]
1.00	$(7.56 \pm 0.09) \times 10^{-4}$	$(2.67 \pm 0.40) \times 10^{-4}$
1.45	$(7.15 \pm 0.12) \times 10^{-4}$	$(2.80 \pm 0.57) \times 10^{-4}$
2.05	$(6.94 \pm 0.18) \times 10^{-4}$	$(6.04 \pm 0.83) \times 10^{-4}$
3.05	$(7.00 \pm 0.13) \times 10^{-4}$	$(1.21 \pm 0.06) \times 10^{-3}$
4.05	$(7.64 \pm 0.70) \times 10^{-4}$	$(3.29 \pm 0.32) \times 10^{-3}$
4.6		$(4.61 \pm 0.89) \times 10^{-3}$
5.7	$(6.02 \pm 0.47) \times 10^{-4}$	$(5.51 \pm 0.22) \times 10^{-2}$
		$(6.29 \pm 0.09) \times 10^{-2}$
6.1	$(6.26 \pm 0.14) \times 10^{-4}$	$(1.57 \pm 0.06) \times 10^{-1}$
		$(1.22 \pm 0.04) \times 10^{-1}$
6.5	$(1.36 \pm 0.14) \times 10^{-3}$	$(1.76 \pm 0.06) \times 10^{-1}$
7.1	$(1.96 \pm 0.33) \times 10^{-3}$	$(3.84 \pm 0.15) \times 10^{-1}$
7.5	$(2.48 \pm 0.34) \times 10^{-3}$	$(4.38 \pm 0.16) \times 10^{-1}$
8.1		$(5.16 \pm 0.20) \times 10^{-1}$

[a] $\mu = 1$ (K_2SO_4). [b] From the intercept of linear regression of plots of pseudo-first-order rate constant for hydrolysis of **5**; errors are from standard deviations of the plots. [c] Second-order rate constant for the buffer catalysis from the slope of plots of pseudo-first order rate constant for hydrolysis of **5** vs $[\text{phosphate}]_{total}$; errors are from standard deviations of the plots.

While we have not investigated the reaction of phosphate with pNPA (**3a**) in as much detail as with **3b**, **4a,b**, the data given in Table 4 indicate that in region between pH 7.5 and 8.5 where the dianion is the dominant species, $k_{cat} = (6.2 \pm 0.4) \times 10^{-4}$ M^{-1} s^{-1}. This value is very close to that obtained for dianion attack on **3b** (6.68×10^{-4} M^{-1} s^{-1}), which indicates that there is little difference in the attack of this anion on the *p*-nitro *S*- or *O*-ester. From the limited data for phenyl acetate (**4a**) and phenyl thiolacetate (**4b**) given in Tables 3 and 5, the ratio for their phosphate reaction at pH 8.5 is roughly $k_{cat}^{\mathbf{4a}}/k_{cat}^{\mathbf{4b}} = (3.3 \times 10^{-5}\ M^{-1}\ s^{-1}/9 \times 10^{-5}\ M^{-1}\ s^{-1}) = 1/3$.

(c) *p*-Nitrophenyl Thiolformate. A fit of the Table 6 data for the hydrolysis of *p*-nitrophenyl thiolformate (**5**) in H_2O at 25 °C to eq 2 gives $k_{H_2O} = (7.09 \pm 0.54) \times$

---

(22) Hupe, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 451.

(23) (a) Ilyasova, A. K.; Saulebekova, M. S. *Zh. Neorg. Chim.* **1983**, *28*, 553; *Chem. Abstr.* **1983**, *98*, 167945x. (b) Perrin, D. D. In *Dissociation Constants of Inorganic Acids and Bases in Aqueous Solution*; IUPAC, Pages Bros. Ltd.: Norwich, 1969. (c) Salomaa, P.; Hakala, R.; Vasala, S.; Aalto, T. *Acta Chem. Scand.* **1969**, *23*, 2116. (d) *CRC Handbook of Chemistry and Physics*, 50th ed; CRC Press: Boca Raton, 1969–70; p D117.

Table 7. Rate Constants for the Hydrolysis of *p*-Nitrophenyl Thiolformate (5) in Phosphate Buffer in D_2O at 25 °C[a]

pD	k_0 (s^{-1})[b]	chdk_{cat} (M^{-1} s^{-1})[c]
3.05	$(2.09 \pm 0.01) \times 10^{-4}$	$(4.29 \pm 0.03) \times 10^{-4}$
3.50	$(2.47 \pm 0.1) \times 10^{-4}$	$(6.0 \pm 0.46) \times 10^{-4}$
4.05	$(2.05 \pm 0.6) \times 10^{-4}$	$(7.30 \pm 2.8) \times 10^{-4}$
8.15		$(5.55 \pm 1.00) \times 10^{-1}$

[a] $\mu = 1$ (K_2SO_4). [b] From the intercept of linear regression of plots of pseudo-first-order rate constant for hydrolysis of **5**; errors are from standard deviations of the plots. [c] Second-order rate constant for the buffer catalysis from the slope of plots of pseudo-first-order rate constant for hydrolysis of **5** vs [phosphate]$_{total}$; errors are from standard deviations of the plots.

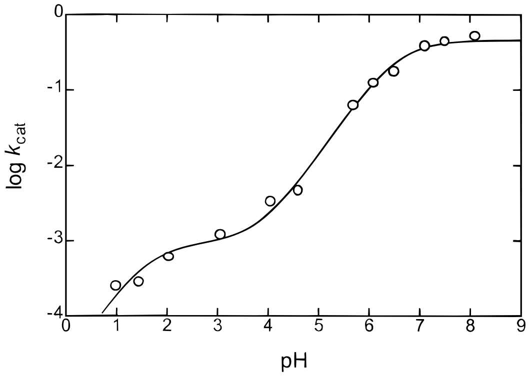


Figure 3. A plot of the second-order rate constants for the reaction of **5** in H_2O, phosphate buffer, as a function of pH, T = 25 °C, $\mu = 1$ (K_2SO_4). The points are experimental and the line is calculated from the NLLSQ fitting of the data to equation $k_{cat}{}^{obs} = k_M(1 + [H^+]/K_a{}^1) + k_D(1 + [H^+]/K_a{}^2)$.

10^{-4} s^{-1} and $k_{HO^-} = (7.35 \pm 1.6) \times 10^3$ M^{-1} s^{-1}. Water and hydroxide attack on the formyl ester are roughly 100-fold and 350-fold faster than on the corresponding acetyl ester **3b** as expected on the basis of steric grounds. These values can be compared with the findings of Pohl, Wu, and Hupe[24] who reported that **5** reacts roughly 1000-fold faster with anions than does **3b** regardless of whether the nucleophile is an oxyanion or thiol anion. A limited number of kinetic runs for the hydrolysis of **5** in the presence of phosphate in D_2O were conducted, the values for the rate constants being presented in Table 7. Between pD 3.05 and 4.05 (the pD independent region), the average value of the water reaction in D_2O is $k_{D_2O} = (2.2 \pm 0.2) \times 10^{-4}$ s^{-1}. The SKIE for this process is $k_{H_2O}/k_{D_2O} = 3.2 \pm 0.6$, consistent with the general process described above (transition state **6**) where two or more waters are involved in the attack on the carbonyl.[20]

Shown in Figure 3 is a plot of the log k_{cat} vs pH profile for the reaction of phosphate with **5** at 25 °C (data of Table 6). The line through the data is the best fit to eq 5 where k_M and k_D are rate constants for attack of the mono- and dianion, and $K_a{}^1$ and $K_a{}^2$ are the first and second dissociation constants for the ionization of H_3PO_4.

$$k_{cat}{}^{obs} = k_M(1 + [H^+]/K_a{}^1) + k_D(1 + [H^+]/K_a{}^2) \quad (5)$$

Using the previously computed dissociation constant ($pK_a{}^2 = 6.52$) as a fixed value gave $k_M = (9.27 \pm 2.01) \times 10^{-4}$ M^{-1} s^{-1}, $k_D = (4.58 \pm 0.41) \times 10^{-1}$ M^{-1} s^{-1} and $K_a{}^1 = (2.55 \pm 1.06) \times 10^{-2}$, $pK_a{}^1 = 1.60 \pm 0.15$. Comparing the k_{cat} value at pD 3.50 with the water value at pH = 3.05 gives a $k_{cat}{}^{H_2O}/k_{cat}{}^{D_2O}$ of (9.3×10^{-4} M^{-1} s^{-1}/6.0×10^{-4} M^{-1} s^{-1}) ≈ 1.5. Comparison of the best-fit value of the k_D value in water (($4.58 \pm 0.41) \times 10^{-1}$ M^{-1} s^{-1}), with the limiting value found for k_{cat} in D_2O (($5.5 \pm 1.0) \times 10^{-1}$ M^{-1} s^{-1}, Table 7) gives a computed value for $k_D{}^{H_2O}/k_D{}^{D_2O} = 0.8 \pm 0.2$ for the SKIE of the dianion attack on **5**. These values support a nucleophilic role for the dianion and either a general base or nucleophilic role for the monoanion in assisting the hydrolysis of **5**. Further delineation of these roles is possible using NMR analysis of the reaction media as described below.

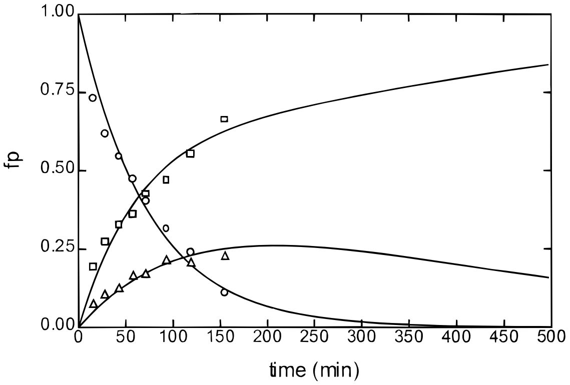


Figure 4. Plots of the fractions of **3b** (open circles), acetic acid (open squares), and acetyl phosphate (open triangles) as observed by ^1H NMR in H_2O in pH 8.5, 0.1 M phosphate buffer at 38 °C. The lines are calculated from NLLSQ fit to the data: for **3b** from first-order exponential decay; for acetyl phosphate from eq 7 and for acetic acid from eq 8.

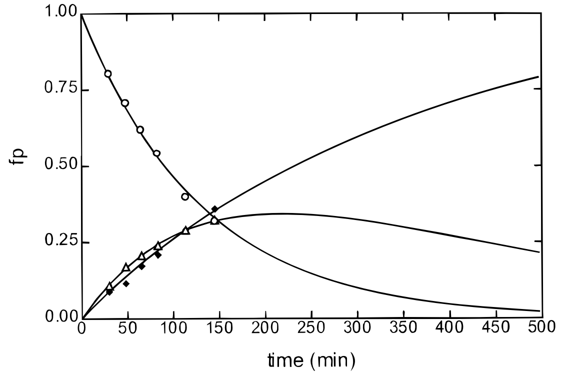


Figure 5. Plots of fractions of **3b** (open circles), acetic acid (filled squares), and acetyl phosphate (open triangles), as observed by ^1H NMR in D_2O, pD 8.5, 0.1 M phosphate buffer at 38 °C. The lines are calculated from NLLSQ fit to the data: for **3b** from first order exponential decay; for acetyl phosphate from eq 7 and for acetic acid from eq 8.

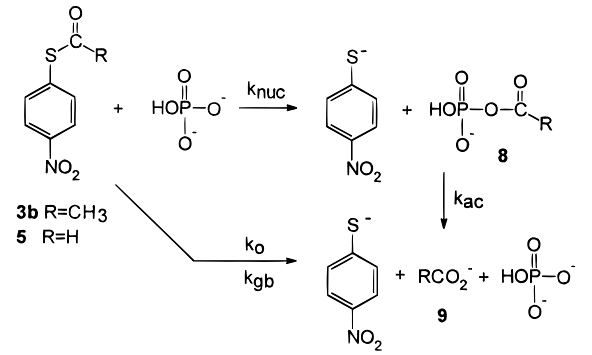
(ii) ^1H NMR kinetics. (a) Thiolesters 3b and 4b. The changes in the ^1H NMR intensities of the signals due to unreacted **3b** (singlet at δ 2.4), acetyl phosphate (doublet at δ 2.0), and the hydrolysis product, acetic acid (singlet at δ 1.83) were monitored in 38 °C H_2O, 0.1 M phosphate, pH 8.5 as a function of time. The same determination was conducted in D_2O solution at pD = 8.5. Plots of the fractions of the reactant and products vs time are shown in Figures 4 and 5, from which it can be seen that there is a substantial buildup of the transient intermediate, acetyl phosphate: this buildup is more prominent in D_2O than in H_2O.

Shown in Scheme 2 is the minimum process required to explain the data. In that scheme there are two roles for the phosphate, namely as a nucleophile (k_{nuc}) to give acetyl phosphate (**8**), or as a general base (k_{gb}) to assist in the direct formation of acetic acid. Spontaneous hydrolysis of the thiolester also can occur (k_o) to yield acetic acid directly.

---

[24] Pohl, E. R.; Wu, D.; Hupe, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 2759.

Scheme 2



The disappearance of **3b** follows an exponential first-order decay. Reversal of the acyl phosphate (**8**) is expected to be negligible as acetyl phosphate reacts with thiols very slowly if at all in aqueous solution.[25a,b] In fact this was confirmed under the present conditions by following the hydrolysis of authentic acetyl phosphate by ^{1}H NMR in phosphate buffer (1.0 M) of pH 8.5 in the presence of a large excess of thiophenol. Even though phenyl thiolate should be more nucleophilic than *p*-nitrophenyl thiolate, no signal assignable to **4b** was observed. The rate constant (k_{nuc}) representing the nucleophilic attack of phosphate on **3b** was calculated from standard equations for consecutive pseudo-first-order reactions.[26]

$$d[\mathbf{8}]/dt = k_{nuc}[\mathbf{3b}] - k_{ac}[\mathbf{8}] \qquad (6)$$

Integration of eq 6 using initial [**3b**] = 1 and [**8**] = 0 gives eq 7:

$$[\mathbf{8}] = k_{nuc}/(k_{ac} - k_{nuc}(e^{-k\ nuc\ t} - e^{-kt})) \qquad (7)$$

The fraction of acetic acid (**9**) was calculated from eq 8:

$$[\mathbf{9}] = 1 - [\mathbf{3b}] - [\mathbf{8}] \qquad (8)$$

The rate constant for the hydrolysis of acetyl phosphate (k_{ac}) was determined in a separate experiment under similar conditions by following the decomposition of authentic material using ^{1}H NMR. This k_{ac} was then used as a constant for the fitting of the data for the fraction of acetyl phosphate to eq 7 to evaluate the rate constant k_{nuc}. The computed rate constants are listed in Table 8. After supplying the rate constants for disappearance of **3b** and **8**, a reasonably good fit to the data for the fraction of acetic acid (**9**) was obtained from eq 8, the lines through the data in Figure 4 being those calculated on the basis of these constants. On the premise that the k_{cat} (entirely due to the dianion (k_D) at pH 8.5) determined for phosphate in the UV/vis kinetics consists of both a general base and nucleophilic processes, and the ^{1}H NMR experiment provides k_{nuc}, one can ultimately compute $k_{gb} = k_{cat} - k_{nuc}$. From the numbers given in Table 8, the nucleophilic process contributes 80−93% of the overall reaction, the general base process contributing 7−20%.

A similar experiment was conducted in D_2O, [phosphate]$_{total}$ = 0.1 M, pD = 8.5. NLLSQ fitting of the D_2O data to eq 7 gave $k_{nuc}^{D_2O} = (7.19 \pm 0.06) \times 10^{-4}$ M^{-1} s^{-1}, a value that is only slightly higher than the second-order rate constant $k_D = (6.69 \pm 0.23) \times 10^{-4}$ M^{-1} s^{-1}, obtained by UV/vis kinetics. Given the inherent differences in the methods and possibility of experimental error, we do not view these rate constants as being different, particularly at the 95% confidence level. Importantly, the ^{1}H NMR experiment indicates that in D_2O the phosphate reaction is entirely nucleophilic while, as above, in H_2O roughly 7−20% of the phosphate reaction goes via a general base route. This would be the expected effect since the general base route should have a significant SKIE while the nucleophilic route should show a much smaller one.

The reaction of phenyl thiolacetate (**4b**), monitored by ^{1}H NMR in a 38 °C, pH 8.5 aqueous solution of 1.0 M phosphate, also showed signals assignable to acetyl phosphate, but these were not as prominent as in the reaction of **3b**. Fitting of the data using eqs 6−8 and rate constants given in Table 8 shows that k_{nuc} contributes 40−50% to the reaction, k_{gb} contributing the rest. This suggests that the nucleophilic reaction depends upon the relative pK_a of the thiol leaving group (pK_a *p*-nitrothiophenol = 4.50, pK_a thiophenol = 6.43):[27] the higher the pK_a of the leaving group, the less prone it is to nucleophilic substitution by phosphate. This is a common finding for other carbonyl addition/elimination processes such as the reaction of acetate with aryl acetates where the nucleophilic reaction gives way to a general base one as the pK_a of the leaving aryloxy group increases.[10]

(b) Thiolester 5. The ^{1}H NMR spectrum of **5** (δ 8.22 (s)) in aqueous phosphate buffer (0.01 M) of pH 8.1 after 15 min showed signals due to formyl phosphate (**8**, >80%, δ 8.41 (d, J = 3.7 Hz)) and <20% of formic acid (**9**, δ 8.33 (s)): formyl phosphate further hydrolyses at a slower rate. Hydrolysis of **5** in pH 3.05 aqueous phosphate buffers (0.1 and 1.0 M), followed by recording the ^{1}H NMR spectrum after 15 min, showed no signal assignable to formyl phosphate. This does not necessarily rule out the formation of formyl phosphate as a transient since independent experiments showed that an authentic sample hydrolyzes quickly to formic acid under the pH 3 reaction conditions.

In an attempt to detect the formation of a transient intermediate of formyl phosphate at pH 3, a trapping experiment (Scheme 3) was conducted. Jahansouz *et al.*[2] have determined that the strong nucleophile, hydroxylamine, reacts rapidly with formyl phosphate and have used this reaction to quantify the amount of **8** (R = H) remaining in solution at various times during hydrolysis. Therefore, the hydrolysis of **5** was studied by ^{1}H NMR in a pH 3.05 aqueous solution of hydroxylamine (pK_a $HONH_3^+$ = 6.03)[23d] in the presence and absence of phosphate. Without phosphate, the observed products were formic acid (**9**) and formyl hydroxamic acid (HC(O)N-HOH, **10**) in a 1:2 ratio. In an aqueous phosphate buffer (0.1 M) of pH 3.05, the ratio was, within experimental error, the same. However, in a 1.0 M phosphate buffer, pH 3.05, formic acid and the hydroxamic acid were found to be produced in a 1.3:1 ratio. Using the rate constants given in Table 6, and assuming an exclusive nucleophilic role for the $H_2PO_4^-$, a computed ratio of 1:4 for formate:**10** at 1.0 M phosphate is determined. Conversely, if the exclusive role is general base, then the formate:**10** ratio should be 3:2, this value being experimentally indistinguishable from the 1.3:1 ratio observed.[28] These findings indicate that the dominant, and perhaps exclusive, role

---

[25] (a) Sabato, G. D.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4393. (b) Moore, S. A.; Jencks, W. P. *J. Biol. Chem.* **1982**, *257*, 10874.

[26] Frost, A. A.; Pearson, R. G. In *Kinetics and Mechanisms*; Wiley International: New York, 1961; pp 161−166.

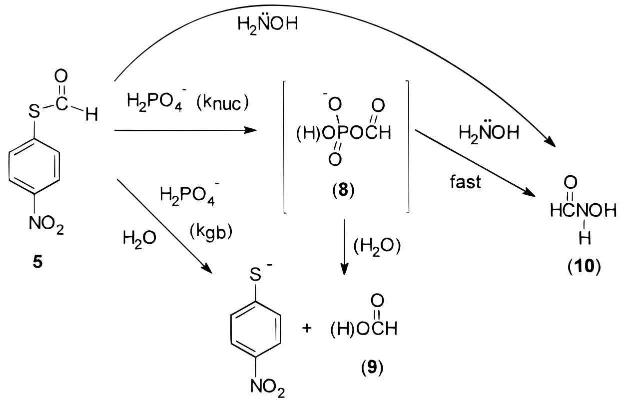
[27] Jencks, W. P.; Salvesen, K. *J. Am. Chem. Soc.* **1971**, *93*, 4433.

Table 8. Rate Constants for the Hydrolysis of *p*-Nitrophenyl Thiolacetate (3b)[a] and Phenyl Thiolacetate (4b)[b] in Phosphate Buffer at T = 37.5 °C

thiolacetatee	pH/pD	k_{obs} (s^{-1})[c]	k_{obs} (s^{-1})[d]	k_0 (s^{-1})[e]	k_{cat} (M^{-1} s^{-1})[f]	k_{ac} (s^{-1})[g]	k_{nuc} (M^{-1} s^{-1})[h]
3b	pH = 8.5	2.34×10^{-4}	$(2.26 \pm 0.11) \times 10^{-4}$	$(1.65 \pm 0.01) \times 10^{-4}$	$(6.68 \pm 0.47) \times 10^{-4}$	$(1.08 \pm 0.06) \times 10^{-4}$	$(5.79 \pm 0.03) \times 10^{-4}$
3b	pD = 8.5	1.36×10^{-4}	$(1.28 \pm 0.03) \times 10^{-4}$	$(7.47 \pm 0.62) \times 10^{-5}$	$(6.69 \pm 0.23) \times 10^{-4}$	$(8.26 \pm 1.05) \times 10^{-5}$	$(7.19 \pm 0.06) \times 10^{-4}$
4b	pH = 8.5	$(1.30 \pm 0.11) \times 10^{-4}$	$(1.10 \pm 0.09) \times 10^{-4}$	$(4.73 \pm 0.83) \times 10^{-5}$	$(9 \pm 1) \times 10^{-5}$	$(8.87 \pm 0.51) \times 10^{-5}$	$(4.05 \pm 0.06) \times 10^{-5}$

[a] $[Phosphate]_{total}$ = 0.1 M. [b] $[Phosphate]_{total}$ = 1.0 M. [c] From UV/vis kinetics, single run for **3b** and four runs for **4b**. [d] From ^1H NMR kinetics, single run. [e] From UV/vis kinetics, from the intercept of the linear regression of plot of k_{obs} vs $[phosphate]_{total}$, errors from standard deviations. [f] From UV/vis kinetics, from the fits of the k_{cat} data for **3b** to eq 3. Note that at pH 8.5, $k_{cat} = k_D$. Value of k_{cat} for **4b** average of two values in Table 3. [g] From ^1H NMR kinetics as the slope of the linear regression of the plot of ln of fraction of unhydrolyzed acetyl phosphate vs time, errors from standard deviations. [h] From NLLSQ fitting of the data for the fraction of acetyl phosphate (**8**) to eq 7, using [phosphate] = 0.1 M for **3b** and [phosphate] = 1 M for **4b**.

Scheme 3



for the phosphate monoanion at pH 3.05 is general base, while that for the dianion at pH 8.5 is nucleophilic.

Conclusions

Despite the fact that phosphate is a commonly used buffer and catalyzes numerous acyl transfer reactions, the number of cases where it has been unambiguously shown to be a nucleophile are surprisingly few. In the above study we have shown that *p*-nitrophenyl thiolacetate (**3b**), and *p*-nitrophenyl thiolformate (**5**) react with phosphate dianion in the neutral pH domain predominantly by a nucleophilic route. The study also shows that phosphate dianion behaves as both a nucleophile and general base toward phenyl thiolacetate (**4b**), the ratio of the two roles being roughly 1:1 at 37.5 °C. O'Connor and Wallace[6] have suggested that the nucleophilic to general base ratio for phosphate dianion reacting with *p*-nitrophenyl acetate (**3a**) at 37.5 °C is about 11:1 in D_2O, but could not refine the ratio further since the salt effect of phosphate on the reaction was uncertain. Under comparable conditions the reaction of phosphate dianion with the oxygen/thiolester pairs of **3a** or **3b** and **4a** or **4b** proceeds in much the same way in terms of reaction rate and mechanism. In two cases where we have data, phosphate monoanion behaves as a general base in promoting the hydrolyses of **3b** and **5**.

All the above observations are, after the fact, reconciled by well-established principles of carbonyl addition/elimination reactions, namely that nucleophiles cannot displace substantially poorer leaving groups from the addition intermediates.[29] The available data suggest that displacements of phosphate dianion on esters with leaving thiol anions or oxyanions having pK_a's for their conjugate acids of about 7 or less can occur readily. At lower pH, when the catalytic agent becomes the phosphate monoanion, the mode of catalysis for these esters changes to general base. This change in the mode of catalysis indicates that the nucleophilic addition to the carbonyl has a larger Brønsted coefficient than does the general base-promoted reaction, a finding that is also consistent with well-established principles for reactions at ester carbonyl groups. For example, nucleophilic addition of a variety of oxyanion and amine nucleophiles to activated esters, such as *p*-NPA, have Brønsted β-values of 0.66−0.92.[30] The reactions of a series of activated esters such as the ethyl chloroacetates and various anhydrides with neutral oxygen nucleophiles (water, alcohols) are subject to general base catalysis, but here the Brønsted β-values are in the range of 0.47.[31]

Acknowledgment. The authors gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada and Queen's University. In addition they are grateful to the reviewers of the manuscript for their helpful comments.

Supporting Information Available: Tables of rate constants for the hydrolysis of esters **3a,b** and **4a,b** in H_2O, T = 37.5 °C, at various pH values as a function of $[phosphate]_{total}$ (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO970904B

---

(28) The calculation is as follows: at the concentration of hydroxylamine and substrate **5** used (7.9×10^{-3} and 3.76×10^{-3} M), 1 part of formic acid and 2 parts of hydroxamic acid are produced at pH 3.05. From the rate constants given in Table 6, at pH 3.05, 1 M phosphate, no hydroxylamine, about 2/3 of the reaction goes via the phosphate process. If all the reaction of phosphate is general base, then the amount of formic acid is 3 parts, and the amount of hydroxamic acid is 2 parts. If all the reaction is nucleophilic and the formyl phosphate reacts quantitatively with hydroxyl amine, then the amount of formic acid is 1 part and the amount of hydroxamic acid is 4 parts.

(29) Jencks, W. P. In *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 463−554.

(30) Bruice, T. C.; Benkovic, S. J. In *Bioorganic Mechanisms*; Benjamin, Inc.: New York, 1966; Vol. I, p 43.

(31) Jencks, W. P.; Carrioulo, J. *J. Am. Chem. Soc.* **1961**, *83*, 1743.