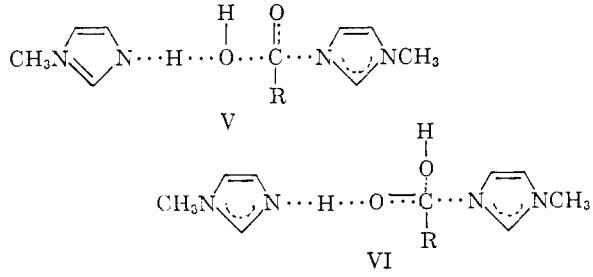
of a proton from water to the carbonyl oxygen atom. The rate constants for general base catalysis of acetylimidazole hydrolysis by water and by imidazole according to eq. 6

$$\text{rate} = [\text{AcIm}]\Sigma_i k_{B_i}[B_i] \qquad (6)$$

are $9.0 \times 10^{-5}$ and 0.14 l. mole$^{-1}$ min.$^{-1}$, respectively. This gives a Brönsted slope, $\beta$, of 0.37, which is similar to the value of 0.45 observed for general base catalysis of ester hydrolysis.

The hydrolysis of $AcMeIm^+$ is also subject to general base catalysis by N-methylimidazole. This catalysis must be classical general base catalysis, because nucleophilic displacement would only regenerate starting material and the rate of N-methylimidazole-catalyzed hydrolysis is decreased in solvent $D_2O$. The existence of general base catalysis of this reaction is somewhat unexpected, since the relative importance of general base catalysis decreases as the leaving group becomes better in the aminolysis of substituted phenyl acetates, and the reactions of *p*-nitrophenyl acetate, which has a leaving group with a $pK$ of 7, are generally not detectably subject to classical general base catalysis.[11,12] The $pK$ of N-methylimidazole, the leaving group of $AcMeIm^+$, is also 7 and the rates of its reactions are several orders of magnitude greater than those of *p*-nitrophenyl acetate, yet general base catalysis makes an important contribution to the observed rates of reaction of this compound. The methyl group on the imidazole moiety of $AcMeIm^+$ reduces the ambiguity in respect to possible mechanisms for this general base catalysis,[10] as discussed above, and leaves V and VI as reasonable transition states, in which, again, the O–C bond may be partly or fully formed.



V



VI

(11) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 675 (1960).

(12) T. C. Bruice and M. F. Mayahi, *ibid.*, **82**, 3067 (1960).

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[CONTRIBUTION NO. 124 FROM THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

# Mechanism and Catalysis of Reactions of Acyl Phosphates. I. Nucleophilic Reactions[1]

BY GIOVANNI DI SABATO AND WILLIAM P. JENCKS

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Acyl transfer reactions from acetyl phosphate to *n*-butylamine, ammonia, glycine and hydroxylamine are subject to general base catalysis, as shown by a greater than first-order dependence of the rate on the concentration of amine buffers. The reactions of acetyl phosphate at neutral *p*H with hydroxylamine, aniline, morpholine, N-methylimidazole, glycine and glycylglycine give C–O bond breaking and proceed predominantly through an acid-catalyzed pathway; *i.e.*, they involve a reaction of amine base with the acetyl phosphate monoanion. The existence of these catalyzed reaction paths partially accounts for the relatively high reactivity of acetyl phosphate. The reaction with glycine and the neutral and base-catalyzed hydrolysis of acetyl phosphate are catalyzed by calcium ion. The reactions with pyridine, 4-methylpyridine, triethylenediamine and probably trimethylamine give P–O bond breaking; in the presence of fluoride, fluorophosphate is formed, suggesting that these reactions represent nucleophilic catalysis of phosphoryl transfer. Fluoride also reacts directly with the acetyl phosphate monoanion to give fluorophosphate, but does not react appreciably with the acetyl phosphate dianion.

The high reactivity of acetylimidazole at neutral *p*H is due not so much to a high reactivity of the compound itself as to the availability of facile acid- and general base-catalyzed paths for its reactions.[2] The present study was undertaken to determine the extent to which the reactions of acetyl phosphate, another "energy-rich" compound of some biochemical importance,[3–6] proceed by similar catalytic pathways in aqueous solution near neutrality. In the course of the study several unexpected characteristics of acyl phosphate reactions were encountered.

(1) Supported in part by the National Cancer Institute of the National Institutes of Health (Grant C-3975 and Training Grant CRT-5033) and the National Science Foundation. For a preliminary report, see Abstracts, 137th Meeting, American Chemical Society, Cleveland, Ohio, 1960, p. 77-O.

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

(3) F. Lynen, *Ber.*, **73**, 367 (1940).

(4) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **153**, 571 (1944).

(5) D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **73**, 4103 (1951).

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## Experimental

Dilithium acetyl phosphate was prepared by the procedure of Avison.[7] Acetyl phenyl phosphate was prepared by a modification of this method.[2] Other reagents, except for reagent grade inorganic salts, were recrystallized or redistilled before use. Water and deuterium oxide were glass-distilled. The reactions were started by adding a freshly prepared solution of acyl phosphate to a reaction mixture at 39.0° which contained a large excess of the other reactant under investigation. Aliquots were withdrawn at appropriate time intervals and analyzed for remaining acyl phosphate by conversion to hydroxamic acid.[8] The extent of the reaction, $x_\infty - x_t$, was plotted on semi-logarithmic graph paper and a pseudo-first-order rate constant obtained from the half-time, using the formula $k = 0.693/t_{1/2}$. Five to ten points were used for the determination of each first-order constant. The observed rate constants were corrected for rates of hydrolysis, determined separately at the same *p*H and ionic strength. Second-order rate constants were obtained from the slopes of plots of the observed first-order constants against the concentration of the second reactant. Catalytic constants were obtained from the slope of plots of the appropriate observed first- or second-order con-

(7) A. W. D. Avison, *J. Chem. Soc.*, 732 (1955).

(8) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

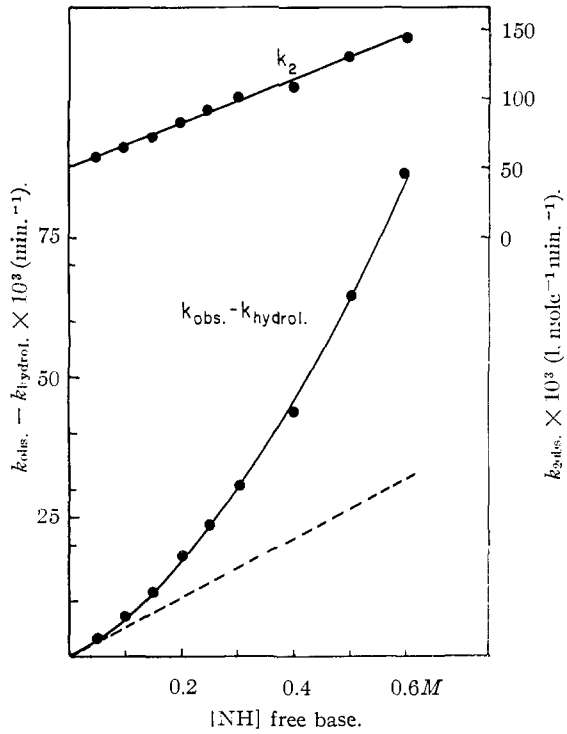



Fig. 1.—The effect of ammonia concentration on the ammonolysis of acetyl phosphate at $p$H 9.5, 39.0° and ionic strength 0.6: solid line, rate for the catalyzed plus uncatalyzed reaction, calculated from the rate constants of Table I; dashed line, calculated rate for the uncatalyzed reaction; upper line, observed second-order rate constants as a function of ammonia concentration.

stants against the concentration of the catalyst. Reactions leading to the disappearance of thiols were carried out with $10^{-3}$ $M$ ethylenediaminetetraacetic acid in the reaction mixtures and were followed by measuring the disappearance of the free thiol group by the nitroprusside method.[9] For calcium-catalyzed reactions, excess ethylenediaminetetraacetic acid was added to the assay mixtures to prevent calcium carbonate precipitation. Since the thiol was not present in excess, complex kinetics, between zero and first order, were obtained in these experiments and no attempt was made to calculate rate constants.

Inorganic phosphate release on the reaction of acetyl phosphate with tertiary amines in methanol was determined by the Fiske and SubbaRow method[10] after precipitation as the calcium salt.[4] Fluorophosphate was determined as the difference in inorganic phosphate before and after hydrolysis in 1 $M$ HCl for 10 minutes at 100°. Reaction mixtures containing fluoride were incubated in polyethylene tubes. Fluoride and triethylenediamine were shown not to interfere with the phosphate analyses after dilution, although more concentrated tertiary amines do cause interference. Fluorophosphate was shown to be stable under the conditions of incubation of the reaction mixtures, except at acid $p$H. Fluorophosphate was identified by paper electrophoresis on Whatman #31 double thickness paper in 0.02 $M$ sodium citrate–0.003 $M$ citric acid and in 0.08 $M$ sodium acetate–0.02 $M$ acetic acid buffers with a potential of 10 v./cm. for 4 hours at 3–5°. For these experiments, the reactions were run with 0.4 $M$ acetyl phosphate and 2 $M$ fluoride, so that the ionic strength would not be excessive after dilution to give 0.2–0.3 μmole/0.02-ml. aliquot. The dried sheets were sprayed with molybdate mixture,[11] dried at 65° for 1 hour, sprayed with 1% $SnCl_2$ in 1 $M$ HCl, and exposed to ammonia vapors; migration in cm.: fluorophosphate, pyrophosphate and phosphate, 30, 25.5, 20 (citrate buffer) and 24, 21.5, 18 (acetate buffer), respectively. The reaction product moved at the same rate as known fluorophosphate and did not separate from fluorophosphate added to the reaction mixtures.

The reaction of hydroxylamine with acetyl phosphate, as with other activated acyl groups, gives both N-acylation (hydroxamic acid) and O-acylation (O-acetylhydroxylamine) in the initial reaction, which is followed by a slower, hydroxylamine-dependent, isomerization of the O-acetylhydroxylamine to hydroxamic acid.[12] At $p$H 4.1 and 6.4, hydroxamic acid formation accounts for 74–77% of the initial reaction; this is followed by a slower formation of hydroxamic acid at a rate equal to the rate of isomerization of O-acetylhydroxylamine. The ratio of O to N acylation was not affected by variation of the hydroxylamine concentration from 0.1 to 0.5 $M$ at $p$H 6.4. The rate constants reported here were obtained from the initial first-order formation of hydroxamic acid and were multiplied by 0.75 to give the rates of reaction of the hydroxylamine nitrogen atom.

## Results

The rate of the reaction of acetyl phosphate with ammonia at $p$H 9.5 increases more rapidly than the first power of the ammonia concentration at constant $p$H and ionic strength (Fig. 1). This is evidence for general base catalysis of the reaction by a second molecule of ammonia, as previously demonstrated for the reaction of phenyl acetate with a number of amines and of ethyl formate with $n$-butylamine.[13,14] The rate constants for the catalyzed and uncatalyzed reactions (Table I) were calculated as previously described[13] according to the rate law

$$v = k_2[AcP^-][NH_3] + k_3[AcP^-][NH_3]^2 \quad (1)$$

and the solid line in Fig. 1 was drawn from the rate constants so obtained. The rate of the reaction with 0.1 and 0.2 $M$ ammonia at $p$H 9.4 was found to be unchanged on variation of the ionic strength from 0.6 to 1.0 by addition of KCl.

The reactions of acetyl phosphate with $n$-butylamine and glycine were studied in the same manner and also were found to be subject to general base catalysis (Table I). As in the case of phenyl acetate, and presumably for the same reasons,[13] no such catalysis was found of the reactions with piperidine, trimethylamine, morpholine and triethylenediamine (diazobicycloöctane).

The reaction with hydroxylamine shows a $p$H–rate maximum as well as a term second-order in respect to hydroxylamine; the rates at $p$H 6.5 and $p$H 7.0 are entirely accounted for by the rate law

$$v = k_2'[AcP^-][NH_2OH] + k_3'[AcP^-][NH_2OH]^2 \quad (1a)$$

This is the only compound for which such a catalyzed reaction of the acetyl phosphate monoanion was detected.

The rate of the reaction of aniline with acetyl phosphate shows a maximum at $p$H 4.7 and is very small at $p$H 7 (Fig. 2). Such a $p$H–rate maximum is consistent with a reaction of the acetyl phosphate dianion ($AcP^=$) with the conjugate acid of aniline

$$v = k_2''[AcP^=][RNH_3^+] \quad (2)$$

or a kinetically indistinguishable reaction of the acetyl phosphate monoanion ($AcP^-$) with free aniline

$$v = k_2'[AcP^-][RNH_2] \quad (3)$$

(9) H. R. Mahler, S. J. Wakil and R. M. Bock, *J. Biol. Chem.*, **204**, 453 (1953).
(10) C. H. Fiske and Y. SubbaRow, *ibid.*, **66**, 375 (1925.)
(11) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).
(12) W. P. Jencks, *J. Am. Chem. Soc.*, **80**, 4581, 4585 (1958).
(13) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).
(14) J. F. Bunnett and G. T. Davis, *ibid.*, **82**, 665 (1960).

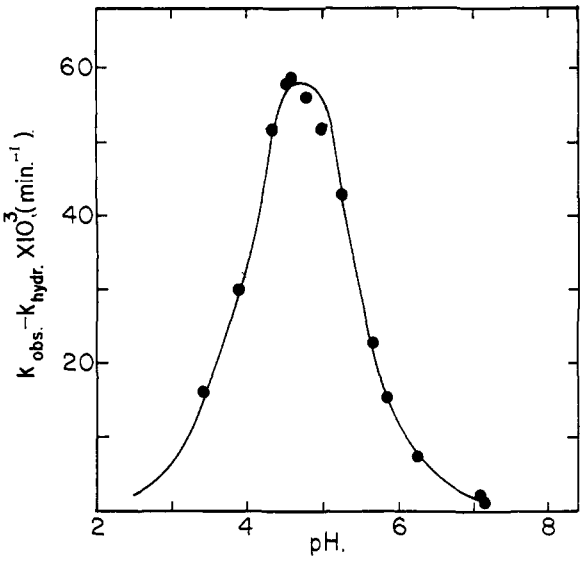



Fig. 2.—Effect of $p$H on the rate of the reaction of aniline (total aniline concentration 0.3 $M$) with acetyl phosphate at 39.0° and 0.6 ionic strength: solid line, rate calculated from the rate constants of Table I.

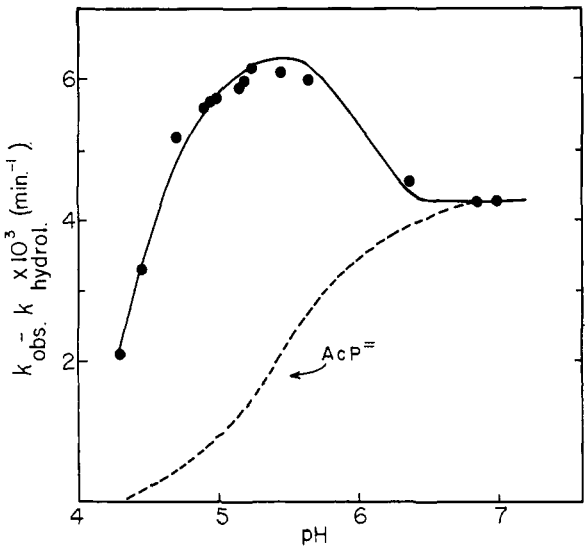



Fig. 3.—Effect of $p$H on the rate of the reaction of pyridine (total pyridine concentration 0.5 $M$) with acetyl phosphate at 39.0° and 0.6 ionic strength: solid line, calculated for the sum of the $AcP^-$ and $AcP^=$ reactions; dashed line, calculated for the $AcP^=$ reaction alone.

The rate constant, calculated according to (3), is given in Table I and was used to calculate the solid line in Fig. 2, which agrees satisfactorily with the experimental data. The rate of the reaction was found to be linear in respect to aniline buffer concentration from 0.075 to 0.45 $M$ at $p$ 4.5, which shows that there is no significant general base catalysis of this reaction.

The reactions of acetyl phosphate with hydroxylamine, aniline, morpholine, N-methylimidazole, glycine and glycylglycine proceed at least in part according to rate law 2 or 3. For nucleophilic reagents with a $p$K near to that of acetyl phosphate (4.95[15]), this results in a $p$H–rate maximum, as in Fig. 2, while for more basic reagents it results in a broad plateau between the two $pK$ values. The reaction with pyridine shows a rate maximum, corresponding to an $AcP^-$ reaction according to rate law 3, but also shows a $p$H-independent rate at higher $p$H, corresponding to a reaction of free pyridine with $AcP^=$ (Fig. 3). These reactions and certain other reactions of tertiary amines with acetyl phosphate occur by a different mechanism from those of primary and secondary amines, as discussed below. The rate constants for these and for some related reactions with acetyl phosphate and acetyl phenyl phosphate are summarized in Table I.

Both the hydrolysis and the aminolysis of acetyl phosphate are catalyzed by calcium ion. The $p$H dependence of the calcium-catalyzed hydrolysis (Table II) indicates that two reactions are involved: a $p$H-independent reaction, which presumably involves attack by water, and a reaction which increases with increasing $p$H. Although the increase in rate is not strictly proportional to the concentration of hydroxide ion, it is tentatively concluded that the latter reaction represents a calcium-catalyzed attack of hydroxide ion. The aminolysis of acetyl phosphate by glycine at $p$H 9.8 is catalyzed by calcium and the rate of this reaction increases linearly with the concentration of glycine (Table II). The product of this reaction is acetylglycine (Table III). The acetylation of the sulfhydryl group of mercaptoacetate also is catalyzed by calcium. The free sulfhydryl group of 0.0005 $M$ mercaptoacetate was found to disappear with a half-time of 9 minutes at $p$H 8.4 in the presence of 0.004 $M$ acetyl phosphate and 0.12 $M$ $CaCl_2$, although a control without calcium showed no reaction in this period of time.

The products of the reactions of acetyl phosphate with ammonia, butylamine, piperidine, morpholine and glycine were examined by assay of the reaction mixtures for amides by a slight modification of the method of Katz, Liebermann and Barker.[2] The reactions were carried out under conditions in which the predominant reaction was with the acetyl phosphate dianion and the general base-catalyzed reaction, if present, accounted for a large fraction of the observed reaction (Table III). The expected amide was formed in each instance, indicating that the reaction proceeds with C–O bond breaking. It has been shown previously, by isolation of the products, that the reactions of acetyl phosphate with aniline[16] and with glycine,[5] under conditions in which the reaction with $AcP^-$ is predominant, also give the expected acylated amines, and that acetamide is formed in the reaction of bis-phenylammonium acetyl phosphate with aqueous ammonia.[17] It is well known that acetyl phenyl phosphate and related compounds quite generally undergo nucleophilic attack principally at the carbonyl group, although attack at phosphorus can be detected under special conditions.[2,18]

(15) F. Lipmann and L. C. Tuttle, *Arch. Biochem. Biophys.*, **13**, 373 (1947).

(16) F. Lipmann, *J. Biol. Chem.*, **160**, 173 (1945).

(17) R. Bentley, *J. Am. Chem. Soc.*, **70**, 2183 (1948).

(18) (a) H. Chantrenne, *Biochim. Biophys. Acta*, **2**, 286 (1948); (b) T. Wieland and F. Jaenicke, *Ann.*, **613**, 95 (1958); (c) H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **81**, 4660 (1959); (d) N. S. Corby, G. W. Kenner and A. R. Todd, *J. Chem. Soc.*, 1234 (1952).

TABLE I

RATE CONSTANTS FOR REACTIONS OF 0.004 *M* ACETYL PHOSPHATE AND ACETYL PHENYL PHOSPHATE WITH AMINES AT 39° AND IONIC STRENGTH 0.6

| | AcP$^{-a}$ $k_2'$, M.$^{-1}$ min.$^{-1}$ | AcP$^{-a}$ $k_2$, M.$^{-1}$ min.$^{-1}$ | AcP$^{=a}$ $k_3$, M.$^{-2}$ min.$^{-1}$ | AcPhP$^{-b}$ $k_2$, M.$^{-1}$ min.$^{-1}$ |
|---|---|---|---|---|
| Piperidine | | 2.1[c] | [d] | |
| *n*-Butylamine | | 0.91[e] | 0.85[e] | |
| Trimethylamine | | .008[c] | [d] | 3.6 |
| Glycine | 876[g] | .08 | 0.19 | 2440[h] |
| Ammonia | <1[i] | .052 | 0.15 | 82[i] |
| Morpholine | 165[c] | .15[c] | [d] | 2470[k] |
| Triethylenediamine | 55[c] | .028[c] | [d] | 2.6[l] |
| Glycylglycine | 51.9[m] | .047[m] | [d] | 460[n] |
| N-Methylimidazole[o] | 1.8[p] | .0034[p] | [d] | 58[q] |
| Imidazole[o] | | | | 20[r] |
| 2,4,6-Trimethylpyridine | [s] | [s] | [s] | [t] |
| 2,6-Dimethylpyridine | [s] | [s] | [s] | [u] |
| 2-Methylpyridine | [s] | [s] | [s] | |
| 4-Methylpyridine | 0.1[c] | .013[c] | | 5.3[f] |
| Pyridine | .046[v] | .0087[v] | | 1.2[w] |
| Aniline | .54[x] | | | 0.31[f] |
| Hydroxylamine[y] | 41.8 | | 810[z] | |

[a] AcP rate constants are from the rate law $v = k_2'[AcP^-][amine] + k_2[AcP^-][amine] + k_3[AcP^-][amine]^2$. [b] Rate constants calculated for reaction with amine free base. [c] Determined from 3–5 runs, generally in 0.1–1.0 *M* solutions of 10%, 50% and 90% neutralized amine buffers. [d] No detectable reaction. [e] Calculated from 8 runs in 0.05–0.8 *M* solutions of 50% and 10% neutralized *n*-butylamine buffers. [f] Calculated from 3–4 runs in 0.015–0.1 *M* solutions of 50% neutralized amine buffers. [g] $k_{obs} - k_{hydr} = 0.0064$ min.$^{-1}$ at *p*H 5.8, 6.5, 7.0 and 7.4 in 0.1 *M* potassium phosphate buffer and 0.5 *M* glycine; $k_2 = (k_{obs} - k_{hydr}) \cdot K_{AcP^-}/K_{glyc^-}$. [h] Calculated from 9 runs in 0.1 *M* potassium phosphate buffer between *p*H 5.8 and *p*H 7.3. [i] No detectable reaction in 3.0 *M* $NH_4Cl$ at *p*H 6.1. [j] Calculated from 5 runs in 0.005–0.025 *M* ammonia in 0.1 *M* tris-(hydroxymethyl)-aminomethane–HCl buffer, *p*H 8.5–8.6. [k] Calculated from 3 runs in 0.005 *M* and 0.01 *M* morpholine in 0.1 *M* potassium phosphate buffer, *p*H 6.2 and 6.6. [l] Calculated from 4 runs in 0.01–0.02 *M* solutions of 50% neutralized amine buffers in the presence of 0.002 *M* AcPhP. [m] $k_{obs} - k_{hydr}$ at *p*H 7.3 = 0.0093 to 0.0095 min.$^{-1}$; $k_2$ determined from 10 runs in 0.1–0.8 *M* glycylglycine at *p*H 8.6–8.7. [n] Calculated from 3 runs in 0.005–0.02 *M* glycylglycine in 0.1 *M* potassium phosphate buffer *p*H 6.8 and 6.4. [o] In 0.1 *M* sodium arsenate to prevent reverse reaction. [p] Calculated from 9 runs in 0.4 and 0.8 *M* solutions of 10, 20, 50, 70 and 90% neutralized amine buffers. [q] Calculated from 3 runs in 0.001–0.005 *M* N-methylimidazole in 0.1 *M* tris-(hydroxymethyl)-aminomethane–HCl buffer, *p*H 7.2. Calculated from 3 runs in 0.005 and 0.015 *M* imidazole in 0.1 *M* tris-(hydroxymethyl)-aminomethane–HCl buffer, *p*H 7.2. [s] No detectable reaction in 0.05–0.5 *M* solutions of 50% neutralized amine buffer. [t] $k_{obs} - k_{hydr}$ reaches a maximum value of 0.003 min.$^{-1}$; half-maximal rate at 0.07 *M* half-neutralized 2,4,6-trimethylpyridine buffer. [u] $k_{obs} - k_{hydr}$ reaches a maximum value of 0.006 min.$^{-1}$; half-maximal rate at 0.24 *M* half-neutralized 2,6-dimethylpyridine buffer. [v] Calculated from 20 runs in 0.5 *M* pyridine between *p*H 4.3 and *p*H 7.3 and a linear plot of rate against pyridine concentration, 0.125–0.5 *M* at *p*H 5.7. [w] Calculated from 4 runs in 0.025–0.18 *M* pyridine in 0.1 *M* potassium phosphate buffer, *p*H 6.8, and 4 runs in 0.05–0.5 *M* solutions of 50% neutralized amine buffers. [x] From data of Fig. 2 and a linear plot of rate against aniline concentration, 0.075–0.45 *M*, at *p*H 4.5. [y] At 25° in the presence of EDTA, $2 \times 10^{-4}$ *M*; calculated from 5–7 runs in 0.025–0.20 *M* solutions of 75% and 90% neutralized $NH_2OH.HCl$ buffers. [z] Value calculated for the reaction with $AcP^-$.

The reactions with tertiary amines cannot be examined in this manner since the products are not stable. The position of bond-breaking was investigated by carrying out these reactions in 85–90% methanol, in which C–O bond breaking would give predominantly methyl acetate and inorganic phos-

TABLE II

CATALYSIS BY 0.12 *M* $CaCl_2$ OF THE HYDROLYSIS AND REACTION WITH GLYCINE OF ACETYL PHOSPHATE AT 39° AND IONIC STRENGTH 0.6

| *M* | *p*H | $k_{obs} - k_{control} \times 10^3$ min.$^{-1}$ | *M* | *p*H | $k_{obs} - k_{control} \times 10^3$ min.$^{-1}$ |
|---|---|---|---|---|---|
| 0.2 collidine | 5.72 | 3.4 | 0.4 TRIS | 8.63 | 18.0 |
| .2 collidine | 6.69 | 3.6 | .2 TRIS | 8.94 | 23.4 |
| .4 collidine | 6.57 | 3.6 | .18 glycine | 9.80 | 34[b] |
| .2 TRIS[a] | 7.29 | 4.0 | .24 glycine | 9.80 | 44[b] |
| .2 TRIS | 7.69 | 6.6 | .30 glycine | 9.80 | 56[b] |
| .2 TRIS | 8.13 | 11.0 | .60 glycine | 9.80 | 112[b] |
| .2 TRIS | 8.65 | 17.0 | | | |

[a] Tris-(hydroxymethlyl)-aminomethane. [b] Corrected for hydrolysis and uncatalyzed reaction of glycine under identical conditions.

TABLE III

ANALYSIS OF PRODUCTS

Acetyl phosphate and primary and secondary amines: amide analysis

| | *M* | *p*H | | Yield of amide,[a] % |
|---|---|---|---|---|
| Ammonia | 1.2 | 9.8 | | 99 |
| Butylamine | 0.8 | 10.9 | | 100 |
| Piperidine | .8 | 11.5 | | 100 |
| Morpholine | .8 | 9.0 | | 100 |
| Aniline | | | | 79[b] |
| Hydroxylamine | 1.0 | 6.5 | | 84[c] |
| Glycine | 0.5 | 9.5 | | 100[d] |
| Glycine | 0.6 | 9.9 | 0.12 *M* $CaCl_2$ | 100 |

Acetyl phosphate and tertiary amines: reaction in 85–90% methanol[e]

| | *M* | Yield of methyl acetate, % | Yield of inorganic phosphate, % | Buffer-catalyzed reacn.,[f] % |
|---|---|---|---|---|
| Solvolysis[g] | | 13 | 15 | 0 |
| Triethylenediamine | 0.8 | 8 | 4 | 55 |
| Imidazole | .4 | 42 | 46 | 42 |
| N-Methylimidazole | .8 | 44 | 50 | 55 |
| Pyridine | .8 | 11 | 4 | 78 |
| 4-Methylpyridine | .8 | 16 | 20 | 73 |
| Trimethylamine | .4 | 42 | 46 | 15 |
| | 1.2 | 28 | 42 | 36 |
| | 2.4 | 14 | 46 | 57 |

Acetyl phenyl phosphates and tertiary amines: reaction in 90–95% methanol

| | *M* | Yield of methyl acetate,[e] % |
|---|---|---|
| Solvolysis[h] | | 91 |
| Imidazole | 0.5 | 98 |
| N-Methylimidazole | .5 | 100 |
| Pyridine | .5 | 95 |
| Triethylenediamine | .5 | 98 |

[a] Hydroxamic assay of amides, using the appropriate amide as standard.[12] [b] By isolation of acetanilide.[16] [c] Acetohydroxamic acid formed by neutral hydroxylamine method, using acetohydroxamic acid as standard; total acetyl phosphate measured by phosphate determination after precipitation of inorganic phosphate as the calcium salt.[4] [d] 100% yield of acetylglycine reported at *p*H 8.5–9.0.[5] [e] Buffers are half-neutralized except pyridine, which is 10% pyridine hydrochloride; methyl acetate determined by alkaline hydroxylamine assay; inorganic phosphate determined after precipitation as the calcium salt. [f] Fraction of the total reaction proceeding by reaction with amine, determined by measurement of reaction rates. [g] Identical rates and products in 0.4 and 0.8 *M* half-neutralized collidine buffers. [h] Sodium phenyl phosphate buffer, 0.1 *M*.

phate, while P–O bond breaking would give acetate and methyl phosphate. As shown in Table III, the hydrolysis of $AcPhP^-$ and its reactions with pyridine, imidazole, N-methylimidazole and triethylenediamine give methyl acetate as the product, indicating that these reactions proceed with C–O bond breaking. On the other hand, the hydrolysis of acetyl phosphate and its reactions with triethylenediamine, pyridine and 4-methylpyridine proceed predominantly with P–O bond breaking, while the reactions with imidazole and N-methylimidazole give C–O bond breaking. The results with trimethylamine are anomalous in that the methyl acetate and phosphate analyses do not give comparable results, presumably because one of the reaction intermediates reacts preferentially with water in the mixed solvent; the results strongly suggest, however, that in the case of this tertiary amine also, at least part of the reaction occurs with P–O bond breaking.

That P–O bond breaking occurs in the reaction of pyridine with acetyl phosphate, but not acetyl phenyl phosphate, was confirmed by a study of catalysis of acyl transfer from these compounds to the sulfhydryl group of mercaptoacetic acid[2] (Table IV). Pyridine catalyzes a rapid acetylation

TABLE IV

PYRIDINE CATALYSIS OF ACYL TRANSFER TO MERCAPTOACETIC ACID FROM ACETYL PHOSPHATE AND ACETYL PHENYL PHOSPHATE, MEASURED BY SULFHYDRYL DISAPPEARANCE[a]

| | Free sulfhydryl by nitroprusside method Klett reading of 0.2-ml. aliquot | | | | | | |
|---|---|---|---|---|---|---|---|
| | Acetyl phosphate | | | Acetyl phenyl phosphate | | | |
| | Control | 0.5 *M* pyridine | | Control | | 0.5 *M* pyridine | |
| Time | *p*H 7.3 | *p*H 7.3 | *p*H 5.3 | *p*H 7.3 | *p*H 5.4 | *p*H 7.3 | *p*H 5.3 |
| 10 sec. | 116 | 111 | 112 | 115 | 120 | 37 | 98 |
| 1.5 min. | | | | | | 0 | |
| 4 min. | | | | | | | 44 |
| 120 min. | 102 | 108 | 108 | 108 | 115 | | |

[a] At 39°, ionic strength maintained at 0.6 with KCl; 0.004 *M* acetyl phosphate or acetyl phenyl phosphate, $5 \times 10^{-4}$ *M* mercapto acetic acid and 0.001 *M* ethylenediaminetetraacetic acid; controls in 0.05 *M* potassium phosphate buffer.

of mercaptoacetate by $AcPhP^-$, presumably *via* the intermediate formation of the acetylpyridinium ion (eq. 4)

$$CH_3COPO_3^-R + N\!\!\swarrow\!\!\searrow \longrightarrow CH_3C(=O)\overset{+}{N}\!\!\swarrow\!\!\searrow \xrightarrow{RSH} CH_3C(=O)SR \qquad (4)$$

but there is no catalysis of acyl transfer from acetyl phosphate at *p*H 5.3 and 7.3. Thus, under conditions in which over half of the observed acetyl phosphate disappearance is *via* pyridine-catalyzed attack on $AcP^-$ and $AcP^=$, and attack on carbon may be detected by the sulfhydryl trapping reagent, no such attack on carbon occurs.

The rates of acetyl phosphate disappearance in 0.8 *M* half-neutralized pyridine buffers and in 0.5 *M* pyridine at *p*H 7 (where the pyridine-catalyzed reaction is independent of *p*H) were found to be identical in water and in deuterium oxide solutions. There is, thus, no solvent deuterium isotope effect which might suggest stretching of a solvent O–H bond in the transition state[19] of the pyridine-catalyzed reaction of either $AcP^-$ or $AcP^=$.

The solvolysis of $AcP^-$ at *p*H 3.5 in the presence of fluoride ion gives fluorophosphate as one product, measured as acid-labile phosphate, which does not react as inorganic phosphate under the conditions of the Fiske and SubbaRow analysis.[10] This appears to represent a reaction of fluoride with $AcP^-$, rather than the trapping of a reactive intermediate, since the rate of $AcP^-$ disappearance is increased in the presence of fluoride (Table V).

TABLE V

REACTIONS OF $AcP^-$ WITH FLUORIDE AND OF $AcP^=$ AND TRIETHYLENEDIAMINE WITH FLUORIDE AT 39°

| | $k_{obs} \times 10^3$, min.$^{-1}$ | Final fluorophosphate $M \times 10^3$ ($AcP^- = 0.015$ *M*) |
|---|---|---|
| Control, *p*H 3.5[a] | 10.8 | 0 |
| 0.2 *M* fluoride | 12.1 | 0.9 |
| .4 *M* fluoride | 13.9 | 2.2 |
| .6 *M* fluoride | 15.1 | 2.8 |
| .8 *M* fluoride | 15.0 | 3.4 |
| | | ($AcP^= = 0.077$ *M*) |
| Control, *p*H 9.5[b,c] | 6.4 | 0 |
| Same, 1.6 *M* KF | 6.4 | 0 |
| 0.4 *M* triethylenediamine, *p*H 9.3[c,d] | 13.1 | 0 |
| Same, + 0.4 *M* KF | 14.4 | 9.0 |
| Same, + 0.8 *M* KF | 16.5 | 13.0 |
| Same, + 1.2 *M* KF | 16.5 | 17.5 |
| Same, + 1.6 *M* KF | 16.9 | 26.5 |

[a] Potassium formate buffer, 2.0 *M*; 0.5 equivalent of HCl added to KF, ionic strength 1.8 maintained with KCl. [b] Potassium carbonate buffer, 0.2 *M*. [c] Ionic strength 2.0 maintained with KCl. [d] Triethylenediamine half-neutralized with HCl.

The correspondence between increase in rate and fluorophosphate formation is not exact, but this is probably to be expected in view of the sensitivity of the rate to *p*H in this region and the difficulty of maintaining exact *p*H control in the presence of concentrated fluoride. The yield of fluorophosphate decreases with increasing *p*H in the region of $AcP^-$ ionization (Table VI) and drops to an insignificant level at neutral or alkaline *p*H, in which AcP is present as the dianion. This indicates that $AcP^-$, and not $AcP^=$, reacts with $F^-$ and means that $F^-$ cannot be reacting with any common intermediate (such as $PO_3^-$) formed from $AcP^-$ and $AcP^=$. Although quantitative studies in acid solution are complicated by the rapid acid-catalyzed hydrolysis of fluorophosphate and the presence of HF and $HF_2^-$, the high yield of fluorophosphate in acid solution suggests that the uncharged free acid of AcP also may react with fluoride.

Although fluoride does not react directly with $AcP^=$, if fluoride is included as a trapping reagent in a reaction mixture containing $AcP^=$ and triethylenediamine, fluorophosphate is formed in a yield which increases with increasing $F^-$ concentration (Table V). This is accompanied by a small rate increase, which levels off as the $F^-$ concentration is increased. Fluorophosphate also is formed if fluoride is added to solutions of $AcP^-$ and $AcP^=$

(19) G. O. Dudek and F. H. Westheimer, *J. Am. Chem. Soc.*, **81**, 2641 (1959).

TABLE VI

FLUOROPHOSPHATE FORMATION IN AcP REACTIONS IN THE PRESENCE OF 2.0 *M* KF AT 39°

| Buffer, *M* | Approx. *p*H | Fluorophosphate[a] formed, % |
|---|---|---|
| **Solvolysis** | | |
| HCl, 0.1[b] | 1.0 | 44 |
| Potassium formate, 1.6 | 2.5[c] | 48, 42 |
| Potassium formate, 1.6 | 3.8[d] | 42, 49 |
| Potassium formate, 1.6 | 4.7[e] | 36, 38 |
| Potassium acetate, 1.6 | 5.8 | 15, 18 |
| Tris-(hydroxy- 0.2 | 7.2 | 5 |
| methyl)- .4 | 7.2 | 3 |
| amino- .2 | 8.1 | 4 |
| methane .4 | 8.2 | 2 |
| **Reactions with amines** | | |
| Pyridine, 0.5 | 7.2[f] | 28 |
| Pyridine, 1.25 | 5.3 | 29, 31 |
| 4-Methylpyridine, 0.9 | 6.0 | 23 |
| Trimethylamine, 2.0 | 9.7 | 13 |

[a] 0.04 *M* AcP incubated 20–24 hours, except in 0.1 *M* HCl, which was incubated 42 minutes. [b] 2.0 *M* KF, 2.1 *M* HCl. [c] KF neutralized 90% with HCl. [d] KF neutralized 50% with HCl. [e] KF neutralized 10% with HCl. [f] In tris-(hydroxymethyl)-aminomethane buffer, 0.35 *M*.

undergoing pyridine-, 4-methylpyridine-, and trimethylamine-catalyzed hydrolysis (Table VI).

Although fluoride reacts with $AcPhP^-$ with a rate constant of $4.9 \times 10^{-3}$ l. mole $min.^{-1}$, the reaction in this case appears to be with the carbonyl group, rather than phosphate, since the reaction of $AcPhP^-$ with 0.5 *M* KF in 50% methanol gave a 67% yield of methyl acetate. The reaction with fluoride accounts for approximately 70% of the $AcPhP^-$ disappearance under those conditions.

The rate of hydrolysis of the phosphorus-containing product from the reaction of $AcP^-$ with $F^-$ was found to be 0.038 $min.^{-1}$ at 39° in 0.4 *M* HCl, compared to a fluorophosphate control of 0.037 $min.^{-1}$; the product from the reaction with triethylenediamine and fluoride disappeared with a rate constant of 0.035 $min.^{-1}$ under the same conditions, compared to a control of 0.036 $min.^{-1}$. Flavin, *et al.*,[20] report a rate constant of 0.040 $min.^{-1}$ for the hydrolysis of fluorophosphate in 0.5 *M* HCl at 40°. Fluorophosphate was further identified as a reaction product by paper electrophoresis at *p*H 5.2 and 6.0.

## Discussion

**Catalysis of Acyl Transfer Reactions of Acetyl Phosphate.**—These results demonstrate that the reactions of acetyl phosphate near neutrality, like those of acetylimidazole,[2] are subject to both acid[21] and general base catalysis. The aminolysis of phenyl acetate in aqueous solution is also subject to general base catalysis and the relative importance of the catalyzed reaction is similar for the two compounds. Thus, in a 0.4 *M* glycine –0.6 *M* glycine anion buffer, 73% of the observed reaction with phenyl acetate[13] and 59% of the observed reaction with acetyl phosphate proceed *via* general base catalysis. With a better leaving group, general base catalysis is generally less important.[13,22] Thus, general base catalysis is not observed in the aminolysis of *p*-nitrophenyl acetate, acetyl phenyl phosphate and the acetyl phosphate monoanion, with the single exception of the reaction of $AcP^-$ with hydroxylamine.

Near neutral *p*H the observed reactions of most nucleophilic reagents with acetyl phosphate occur principally through the pathway described by eq. 2 or 3. It is evident from the rate constants given by Koshland that the reactions of acetyl phosphate with amines cannot be entirely accounted for by reactions of the free amines with the acetyl phosphate dianion,[5] and Kurz and Gutsche, in a careful study of specific cation effects on the reaction of glycine with acetyl phosphate,[23] have independently described a reaction which follows eq. 2. These reactions almost certainly occur by attack of a free base on $AcP^-$ (eq. 3) rather than by reaction of the conjugate acid of the nucleophilic reagent with $AcP^=$ (eq. 2) because: (i) on chemical grounds, it is much more likely that the free electron pair of a nucleophilic reagent should attack a carbonyl group than that its conjugate acid, which has no free electron pair, should do so; (ii) $AcP^-$ should be several orders of magnitude more reactive as an acylating agent than $AcP^=$, because $HPO_4^=$ is a better leaving group than $PO_4^{\equiv}$; and (iii) the rates of reaction of $AcPhP^-$ (which is similar in structure to $AcP^-$, with the substitution of phenyl for H, but cannot react according to eq. 2) with glycine, glycylglycine, morpholine and aniline are either approximately equal to, or are larger than, the rates of reaction of $AcP^-$, calculated according to eq. 3 (Table I). Except for the reaction with aniline, the rates are faster for $AcPhP^-$, as would be expected from the electron-withdrawing properties of the phenyl group, which facilitates the expulsion of phenyl phosphate ($pK_2$ 5.88[24]). Since the observed rate can reasonably be accounted for by a simple reaction of free amine with $AcP^-$, it does not appear to be necessary to postulate a cyclic mechanism[23] for this reaction path.

It is well known that reactions of acetyl phosphate are catalyzed by cations such as $Mg^{++}$,[6] $Ca^{++}$[4] and even $Li^+$.[23] The results reported here show that $Ca^{++}$ catalyzes a neutral, as well as a base-catalyzed hydrolysis and that it catalyzes the attack of glycine and of mercaptoacetate on the carbonyl group. No indication of concerted general base catalysis of the $Ca^{++}$-catalyzed reaction with glycine could be detected. It is likely that the function of the calcium is to facilitate reaction of the acyl group with water, $OH^-$, $RS^-$ or glycine by decreasing electrostatic repulsion by neutralization of negative charge and by aiding the expulsion of the phosphate group through general acid catalysis.

**Reactions Giving P–O Bond Breaking.**—The reactions of acetyl phosphate with several tertiary amines, including triethylenediamine, pyridine, 4-methylpyridine and probably trimethylamine, re-

(20) M. Flavin, H. Castro-Mendoza and S. Ochoa, *Biochim. Biophys. Acta*, **20**, 591 (1956).

(21) Kinetically, these are general acid-catalyzed reactions, according to rate law 2; mechanistically, they involve specific acid catalysis of the acylation of a nucleophilic reagent (eq. 3). We feel that they are best described in the latter terms.

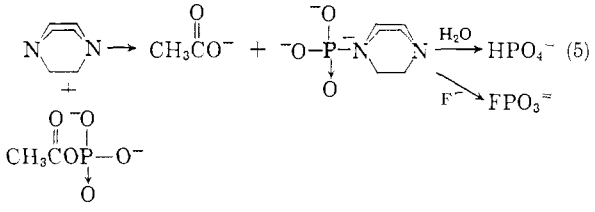
(22) T. C. Bruice and M. F. Mayahi, *J. Am. Chem. Soc.*, **82**, 3067 (1960).

(23) J. L. Kurz and C. D. Gutsche, *ibid.*, **82**, 2175 (1960).

(24) J. D. Chanley and E. Feageson, *ibid.*, **77**, 4002 (1955).

sult in P–O bond breaking, as first demonstrated for the reaction with pyridine by Park and Koshland[25] by experiments in $H_2{}^{18}O$. Although nucleophilic reactions with monosubstituted phosphates in aqueous solution are not common, phosphoramidate reacts with heterocyclic tertiary amines, but not with primary amines and amino acids.[26] No explanation is readily apparent for the specificity of reaction of nucleophilic reagents with acetyl phosphate: aniline, with a basicity similar to that of pyridine, and imidazole and N-methylimidazole, which react as heterocyclic tertiary amines, all attack the acyl group, while the more basic triethylenediamine gives P–O cleavage.

These reactions could occur: (a) by direct attack of amine on phosphorus to give an unstable phosphoramidate intermediate (eq. 5)



(b) by general base catalysis of the attack of solvent on phosphorus, as was demonstrated by Dudek and Westheimer for the solvolysis of tetrabenzyl pyrophosphate in propanol[19]; or (c) by attack of the amine on the carbonyl group to form a tetrahedral addition intermediate, followed by an elimination to give $PO_3^-$, acetate and amine. Mechanism b is made less likely, but not ruled out, by the absence of a solvent deuterium isotope effect in the pyridine–catalyzed hydrolysis. Mechanism a is strongly supported by the finding that fluorophosphate is formed if fluoride is added as a trapping reagent during the tertiary amine-catalyzed hydrolysis of acetyl phosphate, under conditions in which there is no direct reaction of fluoride with acetyl phosphate (eq. 5, Tables V and VI). The small increase in the rate of the triethylenediamine-catalyzed reaction in the presence of fluoride probably represents a salt effect, since it levels off at intermediate fluoride concentrations while the yield of fluorophosphate continues to increase; this suggests that the attack of the nitrogen base on phosphorus is the rate-determining step of the reaction. The rate of the reaction with triethylenediamine at *p*H 10 was found not to be affected by 1 *M* acetate, indicating that any such phosphoramidate intermediate does not react readily with acetate to regenerate acetyl phosphate. These reactions in the presence of fluoride appear to be examples of nucleophilic catalysis of phosphoryl transfer in aqueous solution.

The *uncatalyzed* attack of fluoride on the phosphorus atom of $AcP^-$ (but not $AcP^=$) is surprising in view of the generally very low reactivity of anions with phosphate anions and the fact that fluoride is notably deficient in those qualities, such as polarizability and basicity, which generally accompany high nucleophilic reactivity. Fuoride does not appear to be acting as a trapping reagent for a reactive intermediate, such as $PO_3^-$, since the rate of $AcP^-$ disappearance is increased by fluoride, no significant formation of fluorophosphate occurs during the solvolysis of $AcP^=$, and no evidence for a common ion effect of acetate could be obtained. Fluoride is also an extraordinarily reactive nucleophilic reagent, better than ethoxide, phenoxide, thiophenoxide and acetate anions, toward diisopropyl phosphorochloridate.[27]

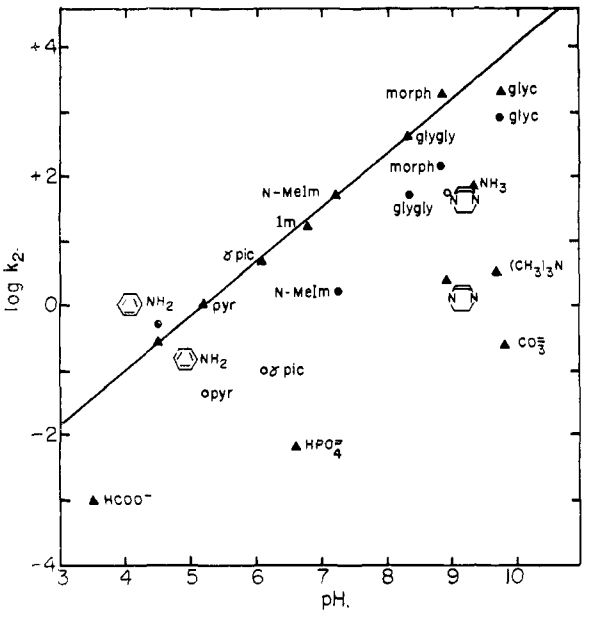



Fig. 4.—Rates of reactions with acetyl phenyl phosphate and acetyl phosphate monoanions at 39° plotted against the basicity of the attacking reagent: ▲, acetyl phenyl phosphate; ●, acetyl phosphate, C–O bond cleavage; ○, acetyl phosphate, P–O bond cleavage.

**Reactivity of Nucleophilic Reagents with Acyl Phosphates.**—The rate of reaction of acetyl phenyl phosphate with a series of amines increases with increasing basicity of the amine, and a logarithmic plot of the rate constants against the basicity of the amine has a slope of 0.85 (Fig. 4) similar to slopes of 0.7–0.8 for phenyl acetates.[13,28,29] Negatively charged attacking reagents, particularly oxygen anions, are considerably less reactive and sulfhydryl reagents, which react as the anion, react only very slowly with acyl phosphates; this is one reason why it is possible to demonstrate nucleophilic catalysis of acyl transfer between these compounds. Although amines which attack carbon follow a similar pattern in their reactivity with $AcP^-$ and $AcPhP^-$ there is less regularity for the former compound (Fig. 4) and still less for reactions of $AcP^=$ (Table I). It is especially difficult to understand why ammonia reacts slowly with acetyl phenyl phosphate and not at a detectable rate with the acetyl phosphate monoanion and why certain tertiary amines, which readily attack the carbonyl group of acetyl phenyl phosphate, will only catalyze a slow

(25) J. H. Park and D. E. Koshland, Jr., *J. Biol. Chem.*, **233**, 986 (1958).

(26) (a) T. Rathlev and T. Rosenberg, *Arch. Biochem. Biophys.*, **65**, 319 (1956); (b) Th. Müller, T. Rathlev and Th. Rosenberg, *Biochim. Biophys. Acta*, **19**, 563 (1956).

(27) I. Dostrovsky and M. Halmann, *J. Chem. Soc.*, 508 (1953).

(28) T. C. Bruice and R. Lapinski, *J. Am. Chem. Soc.*, **80**, 2265 (1958).

(29) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960).

P–O splitting with the acetyl phosphate monoanion. Pyridine does not form an unreactive complex with acetyl phosphate, since the rate of reaction of 0.22 *M* aniline with acetyl phosphate at *p*H 5.2 was found to be unchanged on the addition of 0.5 *M* pyridine. No reaction of acetyl phosphate with acetate, arsenate, tris-(hydroxymethyl)-aminomethane or phosphate could be detected.[30]

(30) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, **83**, 4400 (1961).

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[CONTRIBUTION NO. 125 FROM THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

# Mechanism and Catalysis of Reactions of Acyl Phosphates. II. Hydrolysis[1]

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The following evidence supports the hypothesis that the hydrolysis of simple acyl phosphates near neutrality proceeds through monomeric metaphosphate ion, the dianion ($AcP^{=}$) by direct elimination of carboxylate ion and the monoanion ($AcP^{-}$) by proton transfer and elimination of carboxylic acid, while the hydrolysis of the acetyl phenyl phosphate monoanion ($AcPhP^{-}$) proceeds through a nucleophilic reaction of water with the acyl group: (1) Values of $\Delta S^{\ddagger}$ for hydrolysis are 3.8 e.u. for $AcP^{=}$, −3.6 e.u. for $AcP^{-}$ and −28.8 e.u. for $AcPhP^{-}$. (2) There is no significant solvent deuterium isotope effect on the hydrolysis of $AcP^{=}$ and $AcP^{-}$, while the hydrolysis of $AcPhP^{-}$ is decreased 2.5-fold in deuterium oxide. (3) The hydrolysis of simple acyl phosphates is much faster than that of other phosphate esters and $AcPhP^{-}$. (4) The rate of hydrolysis of substituted benzoyl phosphate dianions is increased by electron-withdrawing substituents ($\rho$ = 1.2), while hydrolysis of the corresponding monoanions is much less sensitive to structure. (5) Solvolysis of $AcP^{=}$ and $AcP^{-}$ is known to proceed with P–O bond cleavage, while the solvolysis of $AcPhP^{-}$ in 90% methanol proceeds with C–O cleavage. (6) The rates of hydrolysis of $AcP^{=}$ and $AcP^{-}$ are slightly increased by concentrated salt solutions, while that of $AcPhP^{-}$ is decreased. (7) The addition of acetonitrile to the reaction medium has little or no effect on the rates of hydrolysis of $AcP^{=}$ and $AcP^{-}$, while it considerably decreases that of $AcPhP^{-}$. (8) Values of $\Delta V^{\ddagger}$ for hydrolysis are −1.0 cm.³/mole for $AcP^{=}$, −0.6 cm.³/mole for $AcP^{-}$ and −19 cm.³/mole for $AcPhP^{-}$.[2] (9) Solvolysis of $AcP^{=}$ in 7 *M* $NaClO_4$ gives pyrophosphate.

There is a considerable body of evidence which is consistent with the hypothesis that the solvolysis of phosphate monoester monoanions and related compounds proceeds through protonation and elimination of the leaving group to give the unstable monomeric metaphosphate anion as the initial reaction product.[3-10] The experiments reported here provide support for such a mechanism in the solvolysis of simple acyl phosphate monoanions and suggest that the corresponding dianions eliminate carboxylate ion to give metaphosphate ion directly; substituted acyl phosphates, such as acetyl phenyl phosphate, undergo normal acyl–oxygen cleavage.

## Experimental

Rate determinations were carried out as described in the preceding paper.[11] Substituted benzoyl phosphates were prepared by the procedure of Avison.[12] The desired anhydride was prepared from the acid and acid chloride in dry pyridine and added, without isolation, to 0.5 to 1.0 molar equivalent of $K_2HPO_4$ in water at 0°, to give a final pyridine concentration of 30–40%. The mixture was stirred at 0° for 30 minutes (at room temperature for the anisoyl compound) and extracted three times with ether. The benzoyl and toluyl compounds were prepared directly from the anhydrides. The monoacyl phosphates were separated from contaminating diacyl phosphate by precipitation as the barium salts at *p*H 7.2–7.4, and were redissolved by stirring with $Na_2SO_4$ just before use. The compounds were obtained in approximately 50% yield and were used without further purification. Reactions of the substituted benzoyl phosphates were followed by analyzing aliquots of the reaction mixtures for remaining acyl phosphate with the alkaline hydroxylamine method of Hestrin,[13] except for 3,5-dinitrobenzoyl phosphate, which was assayed by the neutral hydroxylamine method.

## Results

The rates of acetyl phosphate monoanion ($AcP^{-}$) and dianion ($AcP^{=}$) hydrolysis are summarized in Table I; the rates in 0.6 *M* KCl at 39° agree satisfactorily with those previously obtained by Lynen[14] and by Koshland.[15,16] The rates of monoanion and dianion hydrolysis are not significantly altered in solvent deuterium oxide and are not changed or are slightly increased by concentrated KCl, $NaClO_4$ and NaCl. The rates are also insensitive to the concentration of tris-(hydroxymethyl)-aminomethane (TRIS) buffer and do not show a significant reaction or common ion effect with concentrated phosphate, acetate or arsenate buffers. In 30 and 50% acetonitrile the rate of $AcP^{=}$ hydrolysis is unchanged and that of $AcP^{-}$ is slightly decreased. The rates of hydrolysis at 0°, 25° and 50° and the derived $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values also are recorded.

The rate of hydrolysis of acetyl phenyl phosphate increases with increasing concentrations of phosphate buffer in $H_2O$ and $D_2O$ (Fig. 1). The de-

(1) Supported in part by grants from the National Cancer Institute of the National Institutes of Health (C-3975 and CRT-5033) and the National Science Foundation. For a preliminary report, see Abstracts, 137th Meeting, American Chemical Society, Cleveland, Ohio, 1960, p. 77-O.

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