[CONTRIBUTION NO. 123 FROM THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

Acetyl Transfer Reactions of 1-Acetyl-3-methylimidazolium Chloride[1]

BY RICHARD WOLFENDEN AND WILLIAM P. JENCKS

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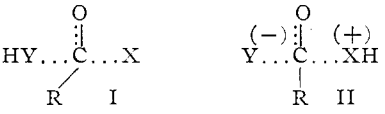
The reactions of 1-acetyl-3-methylimidazolium chloride ($AcMeIm^+$) with water, acetate monoanion, succinate and phosphate dianions and ammonia proceed with rate constants which, over a range of 10^6, do not differ significantly from those calculated for the corresponding reactions of N-acetylimidazolium ion.[2,3] This demonstrates that $AcMeIm^+$ is a satisfactory non-dissociating model for $AcImH^+$ and provides further evidence that many reactions of acetylimidazole with reagents of the form HY are, in fact, acid-catalyzed reactions of Y^-. The rapid neutral hydrolysis of $AcMeIm^+$ is subject to classical general base catalysis by N-methylimidazole; this reaction and the "water" hydrolysis are 2-4-fold decreased in D_2O. A mechanism for the neutral hydrolysis of acetylimidazole is discussed.

The mechanisms of acyl transfer and hydrolysis reactions which follow the rate law

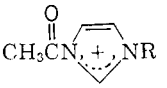
$$\text{rate} = k_{HY}[AcX][HY] \qquad (1)$$

are often ambiguous, since such reactions may involve either a direct attack of HY (transition state I) or a kinetically indistinguishable acid-catalyzed attack of Y^- (II).



(If a metastable tetrahedral addition intermediate[4] is formed, the Y–C bond may be fully formed in the transition state, but the same considerations apply.) A similar ambiguity exists for classical general acid–base catalyzed reactions. General acid catalysis, for example, can involve catalysis either by the undissociated acid or by a proton and the conjugate base of the acid.

Evidence has been presented previously[2,3] that a number of reactions of acetylimidazole which follow rate law 1 actually involve an acid-catalyzed reaction of Y^- (mechanism II). Since such reactions involve the acetylimidazolium cation ($AcImH^+$, IIIa), we have studied the reactions of 1-acetyl-3-methylimidazolium cation ($AcMeIm^+$, IIIb), which would be expected to react, unambiguously, according to mechanism II, except for the



IIIa, R = H
b, R = CH_3

replacement of $-CH_3$ for –H. This compound can also be regarded as a model for acid-catalyzed amide hydrolysis, which corresponds to the N- rather than the O-protonated form.

Experimental

1-Acetyl-3-methylimidazolium chloride ($AcMeIm^+$) was prepared by dropwise addition of 0.20 g. of redistilled acetyl chloride in 10 ml. of anhydrous ether to 0.21 g. of redistilled N-methylimidazole[5] in 15 ml. of anhydrous ether over a period of 15 minutes at room temperature with vigorous stirring. The white precipitate was filtered and washed with 200 ml. of anhydrous ether, with minimal exposure to the atmosphere. An ether slurry of the precipitate was poured into vials and the ether removed by evacuation in a desiccator over P_2O_5. The product, used without further purification, was an extremely hygroscopic white powder which melted at 110° and gave a 62% yield of acetohydroxamic acid on addition to neutral aqueous hydroxylamine solution.[6] The ultraviolet spectrum of the product in ethanol exhibits a shoulder at 240 mμ corresponding to the acetylimidazole peak at 245 mμ,[7] but differs from the acetylimidazole spectrum in that the extinction rises rapidly below 230 mμ.

Water, deuterium oxide, ethanol and dioxane were redistilled before use; water was made 10^{-4} M in ethylenediaminetetraacetic acid.

Kinetic Measurements.—The rate of disappearance of $AcMeIm^+$ was followed by measuring the decrease in absorbance of water solutions at 245 mμ with a Zeiss model PMQ II spectrophotometer, equipped with a thermostated cuvette compartment. Unless otherwise specified, the temperature was maintained at 25.0 ± 0.1° and the ionic strength was maintained at 0.20 by appropriate addition of NaCl. Reactions were initiated by the addition of approximately 0.1 mg. of the solid amide, with stirring, to 3 ml. of reaction mixture, to give a solution approximately 2×10^{-4} M in amide. Reactions were followed in duplicate for at least 4 half-times with at least ten optical density readings. Buffer concentrations were in large excess so that the pH, measured with the glass electrode of a Radiometer model 4 pH meter, remained constant throughout and good first-order kinetics were obtained. Pseudo-first-order rate constants were calculated from the equation $k = 0.693/t_{1/2}$. Sodium phosphate, sodium acetate, sodium succinate and N-methylimidazole buffers, neutralized with HCl, were used. Pseudo-first-order rate constants obtained at each of a number of concentrations of buffer at the same pH were plotted against total buffer concentration, yielding a line whose slope gave the second-order rate constant for reaction with the buffer at the pH of measurement and which could be extrapolated to give the rate constant for hydrolysis at zero buffer concentration at the pH of measurement. The reaction with ammonia was studied at several pH values in N-methylimidazole buffers, since the rates are too fast for study in ammonia buffers; the observed rates in the presence of 0.1 M ammonium chloride were corrected for uncatalyzed and buffer-catalyzed hydrolysis. In each case second-order rate constants, k_2', were determined at three or four pH values.

Results

The rate of hydrolysis of $AcMeIm^+$ remains constant from pH 1 to pH 6 and increases as neutrality is approached. In Fig. 1 the observed rate of hydrolysis at 25.0° and ionic strength 0.20, extrapolated to zero buffer concentration, is plotted as a function of pH. The dashed line represents the corresponding rate profile for acetylimidazole.[2] The increase in the rate of $AcMeIm^+$ hydrolysis near neutrality is proportional to (OH^-) and the hydrolysis of $AcMeIm^+$ follows the rate law

(1) Supported by grants from the National Science Foundation and from the National Cancer Institute of the National Institutes of Health (C-3975).

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

(3) W. P. Jencks and J. Carriuolo, *ibid.*, **234**, 1280 (1959).

(4) M. L. Bender, *J. Am. Chem. Soc.*, **73**, 1626 (1951).

(5) M. Häring, *Helv. Chim. Acta*, **42**, 1845 (1959).

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

(7) E. R. Stadtman in "The Mechanism of Enzyme Action," ed. W. D. McElroy and B. Glass, Johns Hopkins Press, Baltimore, Md., 1954, p. 581.

$$\text{rate} = 2.8\,[\text{AcMeIm}^+] + 9.0 \times 10^6[\text{AcMeIm}^+](\text{OH}^-) \quad (2)$$

The calculated rate from this equation is shown in Fig. 1 as the solid line. In 0.10 M DCl in D_2O at the same ionic strength and temperature k_{obs} = 1.08 min.$^{-1}$, giving $k_{H_2O}/k_{D_2O} = 2.6$.

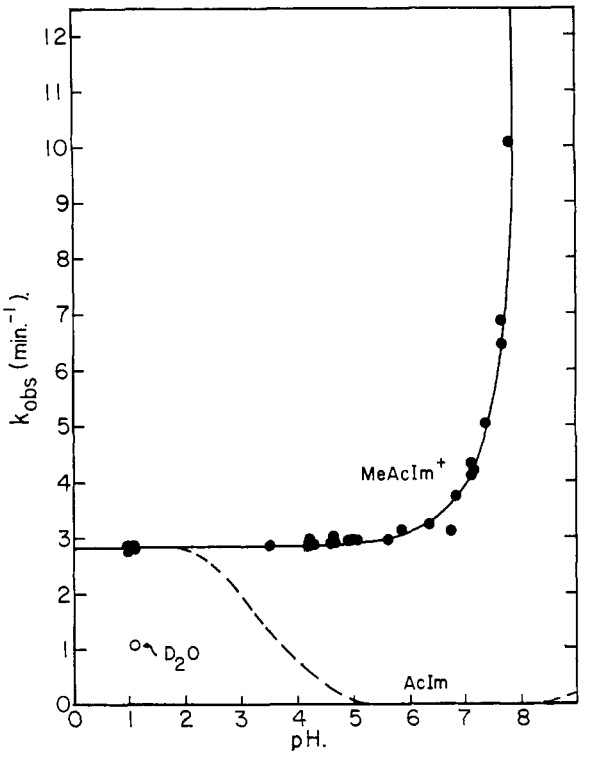


Fig. 1.—Rate of hydrolysis of AcMeIm$^+$, extrapolated to zero buffer concentration, as a function of pH, at 25° and ionic strength 0.2.

The rates of solvolysis of AcMeIm$^+$ in aqueous dioxane and ethanol are shown in Figs. 2 and 3. With increasing dioxane concentration the rates of AcMeIm$^+$ and AcImH$^+$ solvolysis decrease approximately proportionally to the water concentration, while the reaction of AcMeIm$^+$ with ethanol at two temperatures, which includes ethanolysis as well as hydrolysis, does not begin to decrease until high concentrations of ethanol are reached, and even exhibits a small maximum in the rate at intermediate ethanol concentrations. Such a rate maximum suggests that the reaction mechanism involves an interaction of the two components of this mixed solvent (*cf.* refs. 8, 9). The effect of increasing concentrations of NaCl on the rate of AcMeIm$^+$ solvolysis was also found to be identical with that obtained[2] with AcImH$^+$.

The data obtained for the nucleophilic reactions of various bases with AcMeIm$^+$ are given in Table I. In each case the reaction occurs mainly with a single ionic species, namely, the acetate monoanion, the succinate and phosphate dianions, and neutral ammonia. The possibility of a second-order reaction of ammonia due to general base-catalyzed ammonolysis, as previously described for the ammonolysis of acetylimidazole,[3] is not excluded by these data, since such a reaction would have become manifest only at rates too fast to measure by the present methods.

(8) J. Koskikallio, *Acta Chem. Scand.*, **13**, 665 (1959).
(9) J. B. Hyne, *J. Am. Chem. Soc.*, **82**, 5129 (1960).

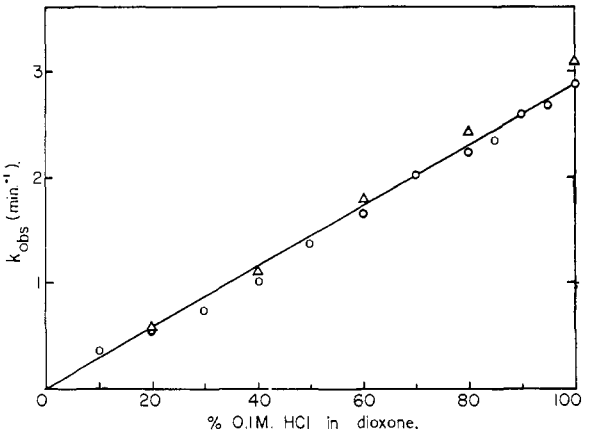


Fig. 2.—Rates of hydrolysis of AcMeIm$^+$ (Δ) and AcImH$^+$ (O) in water-dioxane mixtures at 25°.

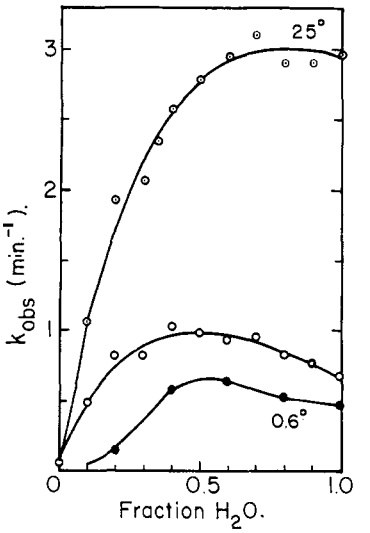


Fig. 3.—Rates of solvolysis of AcMeIm$^+$ in ethanol–water mixtures at 25°: ●, at 0.6° and 25.0°; O, in ethanol-water mixtures in dioxane with a total concentration of hydroxylic solvent of 16.7 M.

TABLE I

RATE CONSTANTS FOR REACTION OF AcMeIm$^+$ WITH NUCLEOPHILIC REAGENTS AT 25.0° AND IONIC STRENGTH 0.2

Reactant A$^-$ (pK)	Concn.[a] range	pH	A$^-$/total A	k_2',[b] l. mole^{-1} min.$^{-1}$	k_2,[c] l. mole^{-1} min.$^{-1}$
Acetate$^-$	0.05–0.20	5.56	0.86	14.5	16.8
(4.76)	.05- .20	5.04	.65	11.0	16.8
	.05- .20	4.64	.43	7.2	16.7
	.05- .20	4.24	.11	3.0	14.4
Succinate$^{=}$[d]	.02- .20	6.50	.94	39.5	42.2
(5.35)	.02- .20	5.77	.725	30.0	41.4
Phosphate$^{=}$	.005-0.02	6.09	.145	376	2590
(6.86)	.025- .10	5.78	.077	168	2180
	.04 - .01	5.63	.0555	136	2450
Ammonia	.10	6.72	3.24×10^{-3}	198	61,100
(9.21)	.10	6.27	1.15×10^{-3}	71.2	61,900
	.10	5.82	0.41×10^{-3}	27.2	64,100

[a] Average of six determinations at each pH. [b] $k_2' = (k_{obs} - k_{hydrol})/[\text{total A}]$. [c] $k_2 = (k_{obs} - k_{hydrol})/[\text{A}^-]$. [d] Ionic strength = 0.60 for succinate.

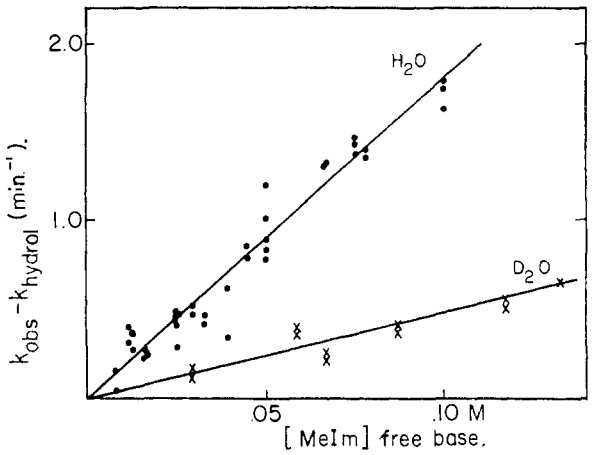


Fig. 4.—General base catalysis of AcMeIm$^+$ hydrolysis by N-methylimidazole at 25° and ionic strength 0.2. Rate constants determined in the *p*H range 6.36 and 7.67 and corrected for uncatalyzed hydrolysis, after extrapolation to zero buffer concentration.

At constant *p*H and ionic strength the rate of hydrolysis of AcMeIm$^+$ increases with the concentration of N-methylimidazole buffer. This increase is greater at more alkaline *p*H, and a plot of the increase in rate against the concentration of N-methylimidazole present as the free base at each of several *p*H values is linear (Fig. 4). This requires the addition of a term for general base catalysis to the rate law for AcMeIm$^+$ solvolysis

$$\text{rate} = k_1[\text{AcMeIm}^+] + k_2[\text{AcMeIm}^+](\text{OH}^-) + [\text{AcMeIm}^+]\Sigma_i k_{Bi}[B_i] \quad (3)$$

with $k_B = 18$ l. mole^{-1} min.$^{-1}$ for N-methylimidazole catalysis. The rate of the catalyzed reaction in D_2O solution is decreased to a value too small for accurate measurement (Fig. 4); an estimated value of 4.5 for the catalytic constant in D_2O gives a value of $k_{H_2O}/k_{D_2O} = 4$ for the catalyzed reaction.

Discussion

The rate of hydrolysis of AcMeIm$^+$ below *p*H 6 is identical with that of acetylimidazole in dilute acid solution, *i.e.*, to that of acetylimidazolium cation (AcImH$^+$).[2] The effect of dioxane and NaCl on the hydrolysis of AcMeIm$^+$ in 0.1 *M* HCl and the 2.6-fold rate reduction in D_2O correspond closely with the solvent and deuterium isotope effects for the hydrolysis of protonated acetylimidazole under the same conditions.[2] This constitutes strong evidence that protonation of acetylimidazole occurs on the free ring nitrogen and that AcMeIm$^+$ is a satisfactory model for reactions of the conjugate acid of acetylimidazole (AcImH$^+$).

The observed rate constants, expressed according to rate law 1, for the reactions of acetylimidazole with a series of reagents, HY, are summarized in column 2 of Table II. In column 3 are given the corresponding rate constants for the acid-catalyzed reaction of Y^- according to mechanism II and rate law 4

$$\text{rate} = k_{Y^-}[\text{AcXH}^+][Y^-] \quad (4)$$

calculated from the relation

$$k_{Y^-} = k_{HY}K_{\text{AcXH}^+}/K_{HY} \quad (5)$$

TABLE II

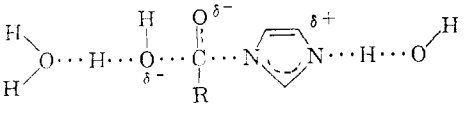
SECOND-ORDER RATE CONSTANTS FOR NUCLEOPHILIC REACTIONS WITH ACETYLIMIDAZOLE[2,3] AND AcMeIm$^+$ AT 25°

Reactant, Y^-	k_{HY}-[AcIm]-[HY], l. mole^{-1} min.$^{-1}$	k_{Y^-}-[AcImH$^+$]-[Y^-], l. mole^{-1} min.$^{-1}$	k_{Y^-}-[AcMeIm$^+$]-[Y^-], l. mole^{-1} min.$^{-1}$
Water		0.051[a]	0.051[a]
CH_3COO^-	1.3	19	17
Succinate$^=$	1.4	78	42
$HPO_4^=$	1.2	2100	2230
NH_3	0.20	81000	62000

[a] Second-order constant, calculated for a water concentration of 55 *M*.

in which K_{AcXH^+} and K_{HY} are the acid dissociation constants for AcImH$^+$ and HY, respectively. In column 4 are given the rate constants for the attack of these nucleophilic reagents on AcMeIm$^+$. The good agreement between columns 3 and 4, over a range of 10^6, confirms the conclusion that the reactions of these reagents with acetylimidazole follow mechanism II and rate law 4.

By analogy with the nucleophilic reactions just described, it is possible that the *p*H-independent, "water" hydrolysis of acetylimidazole may proceed wholly or in part, according to mechanism II, by a reaction of hydroxide ion with AcImH$^+$. From the observed neutral hydrolysis rate for acetylimidazole of 0.005 min.$^{-1}$, the second-order constant, k_{H_2O}, for the reaction of acetylimidazole with water is 9.0×10^{-5} l. mole^{-1} min.$^{-1}$, while the corresponding rate constant, k_{OH^-}, for the reaction of OH^- with AcImH$^+$, calculated from eq. 5, is 12.5×10^7 l. mole^{-1} min.$^{-1}$. This rate constant is 14 times higher than the observed rate constant for the reaction of hydroxide ion with AcMeIm$^+$, 0.9×10^7 l. mole^{-1} min.$^{-1}$. A significant fraction of the observed "water" reaction of acetylimidazole can, therefore, be accounted for as an acid-catalyzed reaction of hydroxide ion, but a different mechanism must be invoked to account for the remaining larger fraction of the observed reaction. Since it is most unlikely that water is a strong enough base to displace the imidazole anion and since the hydrolyses of both AcMeIm$^+$ and AcIm are subject to general base catalysis, we suggest that the major part of the reaction proceeds according to mechanism IV, which is similar to a proposed mechanism for the *p*H-independent hydrolysis of esters.[10]



IV

This transition state is similar to that of the OH^-–AcImH$^+$ mechanism, except that the necessary proton transfers have occurred only partly, rather than completely, in the transition state. Possible modifications of this mechanism might involve a fully formed O–C bond, corresponding to a metastable addition intermediate, or the transfer

$$\text{rate} = k_{H_2O}[\text{AcIm}][H_2O] = k_{OH^-}[\text{AcImH}^+][OH^-]$$

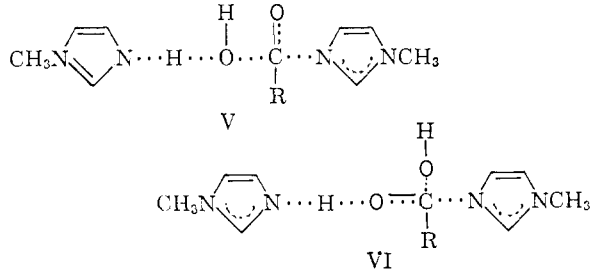
(10) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **83**, 1743 (1961).

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

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

are 9.0×10^{-5} and 0.14 l. mole^{-1} min.$^{-1}$, respectively. This gives a Brönsted slope, β, 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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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).

(6) D. E. Koshland, Jr., *ibid.*, **74**, 2286 (1952).

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).