no starting material remained. The reaction mixture, 26 mg, was recrystallized from ethyl acetate to give **3α-acetamido-5β-androstane-4α,5,17β-triol 17-monoacetate** (**9a**) identical with that obtained from the *cis*-3α-acetamido-4α,5α oxide **4**.

3β-Acetamido-4β,5-oxido-5β-androstan-17β-ol Acetate (**7**).—A solution of 168 mg of 3β-acetamido-4β,5-oxido-5β-androstan-17β-ol acetate (**7**) in 25 ml of acetone and 2.5 ml of 0.2 *N* sulfuric acid at room temperature for 4 days, after which time no starting material remained. Recrystallization of the reaction product, 160 mg, from methanol–ethyl acetate gave 88 mg of **3β-acetamido-5α-androstane-4β,5,17β-triol 17-monoacetate** (**10a**), mp 290° dec, subl; $[\alpha]_D$ −15.6° (ethanol); tlc, R_f = 0.30 (methanol–ethyl acetate 1:9); ir 3430, 3355, 1730, 1716, 1648, 1632, 1523, 1250, 1038, 1023, 960, 945 cm^{-1}; nmr δ 0.75 (s), 1.08 (s), 1.87 (s), 1.98 (s) (deuteriochloroform and dimethyl sulfoxide-d_6).

Anal. Calcd for $C_{23}H_{37}NO_5$: C, 67.78; H, 9.53; N, 3.43. Found: C, 67.52; H, 9.22; N, 3.47.

Acetylation of 48 mg of 3β-acetamido-5α-androstane-4β,5,17β-triol 17-monoacetate (**10a**) with acetic anhydride and pyridine at room temperature for 3 days afforded 48 mg of **3β-acetamido-5α-androstane-4β,5,17β-triol 4,17-diacetate** (**10b**). Recrystallization from acetone–petroleum ether afforded **10b**, mp 312–313°; $[\alpha]_D$ −30.0° (ethanol); tlc, R_f = 0.58 (methanol–ethyl acetate 1:3); ir 3508, 3330, 1745, 1722, 1660, 1553, 1268, 1233, 1045, 1025 cm^{-1}; nmr δ 0.78 (s), 1.10 (s), 1.90 (s), 2.01 (s), 2.03 (s), 4.63 (br m), 5.02 (d, J = 4 cps).

Anal. Calcd for $C_{25}H_{39}NO_6$: C, 66.78; H, 8.74; N, 3.11. Found: C, 66.88; H, 8.89; N, 3.22.

3β-Acetamido-4α,5-oxido-5α-androstan-17β-ol acetate (**8**).—A solution of 11 mg of 3β-acetamido-4α,5-oxido-5α-androstan-17β-ol acetate (**8**) in 5 ml of acetone and 0.5 ml of 0.2 *N* sulfuric acid was kept at room temperature (22°) for 24 hr, after which time no starting material remained. Recrystallization from acetone–petroleum ether gave **3β-acetamido-5α-androstane-4β,5,17β-triol 17-monoacetate** (**10a**), mp 300° dec, subl. The product was identical with that obtained by the dilute sulfuric acid treatment of the corresponding *cis*-3β-acetamido-4β,5β oxide **7**.

Registry No.—**2**, 20429-62-3; **3**, 20429-63-4; **4**, 20445-48-1; **5**, 20429-64-5; **6**, 20588-73-5; **7**, 20429-66-7; **8**, 20429-67-8; **9a**, 20429-68-9; **9b**, 20429-69-0; **10a**, 20429-70-3; **10b**, 20429-71-4.

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Polar and Steric Effects in Acyl Phosphate Monoanion and Dianion Reactions

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The rate constants for hydrolysis of a series of aliphatic acyl phosphates have been determined at 60°. Complete pH–rate profiles for three of the derivatives, isobutyryl, trimethylacetyl, and 3,3-dimethylbutyryl phosphate, were obtained as well as the monoanion and dianion hydrolytic rate constants for isovaleryl phosphate. The values of ΔS^* were uniformly close to zero, consistent with the postulated unimolecular mechanism of hydrolysis of acyl phosphates. A decrease in the rate of hydrolysis was observed for the monoanion and dianion reactions as steric bulk and electron donation in the acyl group, as measured by the Taft σ^* constants, were increased. Second-order rate constants for reaction of pyridine with the monoanions and dianions were also correlated with σ^* constants. The ρ^* for k_{pyr} (monoanion) was +2.0 and for k_{pyr} (dianion) was +6.1. Imidazole and morpholine did not catalyze the hydrolysis of trimethylacetyl phosphate or 3,3-dimethylbutyryl phosphate.

Detailed mechanistic studies of acyl phosphate dianion,[2-4] monoanion,[2-4] and acid-catalyzed[5] hydrolysis reactions have been carried out. Acetyl phosphate monoanion and dianion hydrolysis takes place with unimolecular decomposition to metaphosphate,[4] but reaction with various nucleophiles can be observed.[3,4] Various tertiary amines and pyridine will attack at phosphorus, but imidazole and primary amines attack at the carbonyl group. There seems to be no relationship between the pK_a of the attacking amine and the position of attack.[6] As a consequence, it was thought that steric influences might be of extreme importance in these reactions. A study of the effects of increased steric size of the acyl group in acyl phosphate reactions was therefore undertaken.

Experimental Section

Materials.—Dilithium acetyl phosphate was purchased from CalBiochem Corp. and was used without further purification. All of the remaining aliphatic acyl phosphates were prepared by the method of Lipmann and Tuttle,[7] and isolated as the disodium salts as previously reported.[5] β-Chloropropionyl phosphate was analyzed as the disilver salt. *Anal.* Calcd for $C_3H_4ClO_5PAg_2$: C, 8.96; H, 1.00; P, 7.70. Found: C, 8.79; H, 0.89; P, 7.53. The acyl phosphates were stored in a desiccator at −4°, and fresh samples were prepared periodically.

Dioxane was purified by the method of Fieser[8] and was stored frozen. Deuterium oxide (99.8%) was obtained from Bio-Rad Laboratories. The remainder of the chemicals were reagent grade.

Kinetic Measurements.—The hydroxamic acid assay was used exclusively for the kinetic runs as described by Di Sabato and Jencks.[4] All rates were run in duplicate to at least 75% completion, with less than 5% deviation between the two rate constants in all cases. Each run was initiated by adding the acyl phosphate to the preequilibrated buffer solution making it approximately 2×10^{-3} *M* in acyl phosphate. Rate constants did not change when the acyl phosphate concentration was varied 50%. At appropriate time intervals, aliquots were removed and introduced into the hydroxylamine solution. The resulting mixture was then stoppered and shaken. Development time for complete formation of the hydroxamate was experimentally determined for each compound. At least nine points were employed for a rate determination, and infinity points were taken at ten half-lives. Temperature was controlled to ±0.1° by a Princo thermoregulator in a stirring-water bath. Pseudo-first-order rate constants (k_{obsd}) were calculated with an Oliuetti-Underwood Programma 101 using a computer program designed to calculate a least-

(1) This study represents part of the work to be submitted by D. R. Phillips in partial fulfillment of the requirements for the Ph.D. degree, University of Southern California.

(2) D. E. Koshland, Jr., *J. Amer. Chem. Soc.*, **74**, 2286 (1952).

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

(4) G. Di Sabato and W. P. Jencks, *J. Amer. Chem. Soc.*, **83**, 4393, 4400 (1961).

(5) D. R. Phillips and T. H. Fife, *ibid.*, **90**, 6803 (1968).

(6) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 2, W. A. Benjamin, Inc., New York, N. Y., 1966.

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

(8) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed, D. C. Heath and Co., Boston, Mass., 1955, p 284.

squares evaluation of the slope and intercept of a plot of ln $[(OD_0 - OD_\infty)/OD_t - OD_\infty)]$ *vs.* time.

Results

Rate constants for hydrolysis of the acyl phosphates at 60° and at various pH values are given in Table I. The pH–rate profiles for three branched acyl phosphates are presented in Figure 1. The lines are theoretical and were calculated from eq 1 employing the values of $k_{monoanion}$, $k_{dianion}$, and the second-order rate constant

$$k_{obsd} = k_{monoanion}\frac{(AcP^-)}{(AcP)_{total}} + k_{dianion}\frac{(AcP^{2-})}{(AcP)_{total}} + k_{OH}\frac{(AcP^{2-})(OH^-)}{(AcP)_{total}} \quad (1)$$

for hydroxide ion catalysis, k_{OH}, in Table II. The pK_a values were determined by measuring the pH of a half-neutralized solution of the disodium salt. The experimental pK_2 values at 25° are 4.86, 5.02, and 5.11 for isobutyryl phosphate, trimethylacetyl phosphate, and 3,3-dimethylbutyryl phosphate, respectively. These values were used as pK_2 of the compounds at 60°. pK_1 for the compounds could not be determined by this method due to rapid hydrolysis of all the compounds below pH 2. For this reason, theoretical lines were not continued below pH 3.

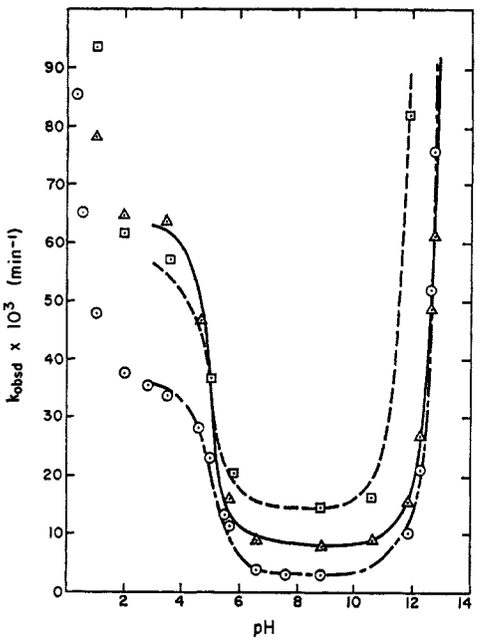


Figure 1.—Plot of k_{obsd} at 60° for hydrolysis of acyl phosphates *vs.* pH: ⊡, isobutyryl phosphate; ⊙, trimethylacetyl phosphate; △, 3,3-dimethylbutyryl phosphate.

TABLE I
OBSERVED RATE CONSTANTS FOR HYDROLYSIS OF ALIPHATIC ACYL PHOSPHATES AT VARIOUS pH VALUES (60° AND μ = 0.6 WITH KCl)

No.	Acyl group[b]	Buffer[a] (*M*)	pH 60°	$k_{obsd} \times 10^3$ min^{-1}
1	Isobutyryl	HCl (0.299)		159.4
		HCl (0.101)		93.5
		HCl	1.98	61.7
		Formate	3.57	56.9
		Acetate	5.02	36.3
		Imidazole	5.83	20.4
		Tris	8.83	14.5
		KOH	10.60	16.3
		KOH (0.0679)		82.2
2	Trimethylacetyl	HCl (0.497)		85.3
		HCl (0.299)		65.3
		HCl (0.101)		47.9
		HCl	1.98	37.7
		Formate	2.85	35.4
		Formate	3.48	33.7
		Acetate	4.61	28.1
		Acetate	5.02	22.8
		Acetate	5.51	13.7
		Imidazole	5.65	11.5
		Imidazole	6.61	3.97
		Imidazole	7.63	3.04
		Tris	8.83	3.14
		KOH (0.0679)		10.2
		KOH (0.170)		21.2
		KOH (0.424)		51.9
		KOH (0.501)		75.8
3	3,3-Dimethylbutyryl	HCl (0.299)		188.6
		HCl (0.101)		78.4
		HCl	1.98	64.6
		Formate	3.48	63.7
		Acetate	4.61	46.5
		Imidazole	5.65	15.7
		Imidazole	6.61	8.98
		Tris	8.83	8.78
		KOH	10.66	8.68
		KOH (0.0679)		15.6
		KOH (0.170)		26.7
		KOH (0.424)		48.8
		KOH (0.501)		61.0

[a] Buffer concentration was 0.05 *M* except where indicated. [b] Registry numbers are as follows: 1, 19926-78-4; 2, 19926-68-2; 3, 19926-69-3.

TABLE II
RATE CONSTANTS FOR SPONTANEOUS AND HYDROXIDE ION CATALYZED HYDROLYSIS OF ACYL PHOSPHATES AT 60° (μ = 0.6 WITH KCl)

No.	Acyl group[b]	$k_{monoanion} \times 10^3$ min^{-1}	$k_{dianion} \times 10^3$ min^{-1}	k_{OH}, l. mol^{-1} min^{-1}
1	Acetyl	128.0[a]	58.5[a]	15.7
2	Isobutyryl	56.9	14.5	1.0
3	Isovaleryl	57.6	16.9	
4	Trimethylacetyl	36.0	3.14	0.115
5	3,3-Dimethylbutyryl	63.7	8.78	0.10

[a] Value calculated employing the activation energy reported in ref 4. [b] Registry numbers are as follows (monoanion and dianion, respectively): 1, 19926-70-6, 19926-71-7; 2, 19926-72-8, 19926-73-9; 3, 19926-74-0, 19926-75-1; 4, 19926-76-2, 19926-77-3; 5, 19926-43-4, 19926-44-4.

The rate constants at various temperatures for trimethylacetyl phosphate and 3,3-dimethylbutyryl phosphate are reported in Table III. Activation parameters are also tabulated, calculated at 39.0°. For comparative purposes, the literature values for acetyl phosphate[4] are presented.

The effects of changing ionic strength and organic solvent can be seen in Table IV. For monoanion and dianion reactions of both trimethylacetyl phosphate and 3,3-dimethylbutyryl phosphate the rate is slightly increased by changing the ionic strength from 0.6 to 2.0 *M*. Similar effects are noted when the solvent is changed from water to 50% dioxane–water. The rate constant for hydrolysis of the dianion of trimethylacetyl phosphate is, however, doubled in 50% dioxane–H_2O and a rate decrease is observed for the dianion of 3,3-

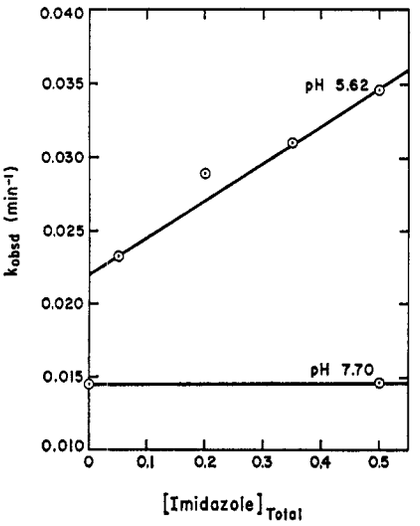


Figure 2.—Plot of k_{obsd} for hydrolysis of isobutyryl phosphate at 60° and μ = 0.6 *vs.* total imidazole concentration (moles per liter).

TABLE III

RATE CONSTANTS FOR THE HYDROLYSIS OF ACYL PHOSPHATES AT VARIOUS TEMPERATURES AND THE ACTIVATION PARAMETERS CALCULATED AT 39.0°

Acyl group	Temp, °C	k_{obsd}, min^{-1}	ΔH^*, kcal/mol	ΔS^*, eu
Monoanion				
Trimethylacetyl[a]	25	0.00055	22.9 ± 0.3	−4.4 ± 0.8
	60	0.0337		
	70	0.107		
	80	0.286		
3,3-Dimethylbutyryl[b]	40	0.00617	23.4 ± 0.1	−2.0 ± 0.4
	50	0.0214		
	60	0.0637		
	65	0.111		
	70	0.184		
Acetyl	39	0.0127		
Acetyl	60	0.128[c]	22.5[d]	−3.6[d]
Isobutyryl[b]	60	0.0569		
Isovaleryl[b]	60	0.0576		
Chloropropionyl[b]	39	0.00512		
Dianion				
Trimethylacetyl[e]	55	0.00205	27.7 ± 0.5	+5.4 ± 1.3
	60	0.00314		
	65	0.00736		
	70	0.0107		
	75	0.0222		
	80	0.0425		
3,3-Dimethylbutyryl[e]	50	0.00274	25.7 ± 0.1	+1.1 ± 0.5
	60	0.00878		
	65	0.0170		
	70	0.0304		
	75	0.0508		
Acetyl	39	0.0056		
Acetyl	60	0.0585[e]	25.4[d]	+3.7[d]
Isobutyryl[e]	60	0.0145		
Isovaleryl[e]	60	0.0169		
Chloropropionyl	39	0.00814		

[a] Rates were measured at pH 3.48, where the monanion is the predominant species, in 0.05 *M*, formate buffer, μ = 0.6 with KCl. [b] Rates were measured at pH 3.57, where the monoanion is the predominant species, in 0.05 *M* formate buffer, μ = 0.6 with KCl. [c] Value calculated from activation energy reported in ref 4. [d] Reference 4. [e] Rates were measured at pH 8.83 in 0.05 *M* Tris buffer, μ = 0.6 with KCl.

dimethylbutyryl phosphate.[9] The rate constants for hydrolysis of the monoanion and dianion of trimethylacetyl phosphate in deuterium oxide were also measured and are also reported in Table IV. Comparing these values to those determined in H_2O, D_2O solvent isotope effects (k_{D_2O}/k_{H_2O}) of 1.27 and 1.10 were obtained.

(9) It should be noted that the pK_2 values of the acyl phosphates may be considerably different in the mixed solvent than in water. Also, the effect of solvent on the rate constant includes its effect on the ratio of activity coefficients of the initial state and the transition state. It is probable that the observed differences between trimethylacetyl and 3,3-dimethylbutyryl phosphate indicate that these ratios are affected differently upon going from water to 50% dioxane–H_2O.

TABLE IV

HYDROLYSIS OF TRIMETHYLACETYL PHOSPHATE AND 3,3-DIMETHYLBUTYRYL PHOSPHATE WHEN SUBJECTED TO VARIOUS SOLVENT CONDITIONS AT 60° IN 0.05 *M* BUFFER AT IONIC STRENGTH 0.6 WITH KCl, EXCEPT WHERE INDICATED

Acyl group	μ	Buffer	Solvent	pH or pD[a]	k_{obsd}, min^{-1}
Trimethylacetyl	0.6	Formate	D_2O	3.30	0.0438
	2.0	Formate	H_2O	3.47	0.0390
	0.6	Formate	50% dioxane–H_2O	4.57	0.049
	0.6	Tris	D_2O	7.93	0.00345
	2.0	Tris	H_2O	8.82	0.00416
	0.6	Tris	50% dioxane–H_2O	8.96	0.00866
3,3-Dimethylbutyryl	2.0	Formate	H_2O	3.47	0.0655
	0.6	Formate	50% dioxane–H_2O	4.57	0.0814
	2.0	Tris	H_2O	8.82	0.0102
	0.6	Tris	50% dioxane–H_2O	8.96	0.00582

[a] pD values were determined from pH meter readings employing the glass electrode correction formula of T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961).

The second-order rate constants for pyridine catalysis are presented in Table V and show a decrease for the more highly branched compounds. No detectable reaction could be observed between imidazole or morpholine and the branched acyl phosphates. There was no catalysis at pH 5.65, 6.12, or 7.50 by 0.5 *M* imidazole nor at pH 8.10 by 0.5 *M* morpholine. However, a pronounced imidazole catalysis was observed in the case of isobutyryl phosphate at pH 5.62 where the monoanion would be at high concentration although no catalysis was observed at pH 7.70 where little monoanion would be present. A plot of k_{obsd} *vs.* total imidazole concentration is shown for isobutyryl phosphate in Figure 2.

TABLE V

SECOND-ORDER RATE CONSTANTS FOR PYRIDINE CATALYSIS OF THE HYDROLYSIS OF ACYL PHOSPHATES AT 60.0° AND μ = 0.60 WITH KCl

	k, l. mol^{-1} min^{-1}		
Acyl group	Pyridine[a]	Imidazole	Morpholine[b]
Monoanion			
Acetyl	0.163[c]		
Isobutyryl	0.114[c]	1.68	
Trimethylacetyl	0.0375[c]	*b*	
3,3-Dimethylbutyryl	0.0590[c]	*b*	
Dianion			
Acetyl	0.0412[d]		
Isobutyryl	0.0060[d]	*b*	*b*
Trimethylacetyl	0.00060[d]	*b*	*b*
3,3-Dimethylbutyryl	0.00134[d]	*b*	*b*

[a] Rate constants determined at four concentrations from 0.5 to 0.05 *M* amine. [b] No detectable reaction in 0.5 *M* amine. [c] In pyridine buffer, pH 5.22. [d] In 0.05 *M* Tris buffer, pH 8.89.

The pK_a of pyridine was determined at 60° and an ionic strength of 0.6 by half-neutralization and was found to be 4.75. The pK_a of imidazole at 60° was found to be 6.58 by extrapolation of values determined at a number of other temperatures.[10]

(10) J. B. Milstien and T. H. Fife, *J. Amer. Chem. Soc.*, **90**, 2164 (1968).

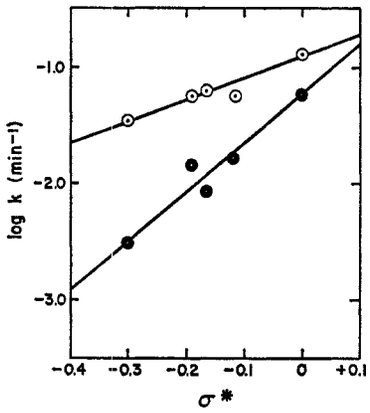
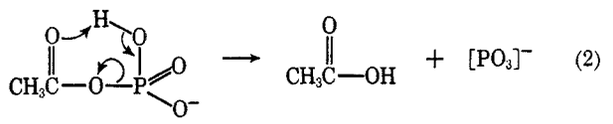


Figure 3.—Plot of log k at 60° for hydrolysis of acyl phosphate monoanions (⊙) and dianions (●) *vs.* σ^*.

Discussion

On the basis of present evidence it is likely that the monoanion and the dianion of acetyl phosphate hydrolyze by the unimolecular mechanisms 2 and 3, respectively.[4] The D_2O solvent isotope effect (k_{D_2O}/



k_{H_2O} = 0.94) for acetyl phosphate monoanion is in accord with an internal proton transfer that is either complete or partially rate determining.[11,12] Likewise, in the present study this ratio for trimethylacetyl phosphate monoanion was found to be 1.27. Salt effects and solvent effects are also similar for these compounds.

In general, increased branching decreases the rate of both monoanion and dianion hydrolysis. This rate decrease could be due to either steric or electronic factors, or to a combination of both. As seen in Table III, the entropies of activation for the branched compounds and acetyl phosphate are very similar, with ΔS^* for the monoanion of trimethylacetyl phosphate and the dianion of 3,3-dimethylbutyryl phosphate being only slightly more negative than the corresponding values for acetyl phosphate. Steric hindrance to solvation could lead to large rate reductions with increasing size of the acyl group, but it can be concluded from the similar entropies of activation that this is not an important factor.

Inductive effects in the aliphatic series could be quite important in producing the rate retardations observed for the highly branched compounds. Di Sabato and Jencks[4] found that the dianion rate of substituted benzoyl phosphates is facilitated by electron-withdrawing substituents (ρ = +1.2), while the hydrolysis of monoanions is relatively insensitive to electron withdrawal (ρ = +0.2). When the logarithms of the rate constants at 60° for the aliphatic series are plotted *vs.* the Taft σ^* constants,[13] a straight line relationship is obtained as shown in Figure 3. The value of ρ^* is +1.8 for the monoanion hydrolysis reaction and +4.3 for the dianion reaction. Thus, while the order of reactivity is in accord with inductive effects being of great importance, still the large magnitude of the ρ^* values might indicate that steric factors are indeed of some importance. Data obtained at 39° were also plotted *vs.* σ^* in order to incorporate more compounds.[14] This plot is shown in Figure 4. It can be seen that the same general relationship between the alkyl-substituted compounds is obtained at 39° as at 60°. However, inclusion of compounds having acyl groups with positive σ^* values, benzoyl, β-chloropropionyl, and chloroacetyl, makes it appear that the actual ρ^* values are approximately zero for the monoanion reaction and +2.2 for the dianion reaction. The large apparent ρ^* values when only alkyl-substituted derivatives are considered are due mainly to acetyl phosphate hydrolyzing considerably faster than expected in comparison with the other compounds. A possible explanation is that the $\rho^*\sigma^*$ plots are actually curved (dotted line in Figure 4) in which case the importance of steric factors in the hydrolytic reaction would be evident since linear $\sigma\rho$ plots are obtained with the benzoyl phosphates.[4]

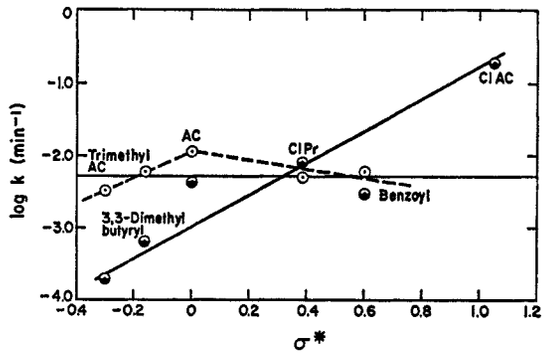


Figure 4.—Plot of log k at 39° for hydrolysis of acyl phosphate monoanions (⊙) and dianions (◒) *vs.* σ^*. Constants for benzoyl phosphate were from ref 4.

When the second-order rate constants, k_{pyr} (monoanion) and k_{pyr} (dianion) for the reaction of the acyl phosphates with pyridine at 60° are plotted against Taft's σ^* constants (Figure 5), reasonably straight lines are obtained with large ρ^* values of +2.0 and +6.1. Steric hindrance to approach of the nucleophile should only account for a part of the differences in the second-order rate constants since pyridine has been shown to attack predominantly at phosphorus when it reacts with either the monoanion or the dianion of acetyl phosphate.[3,4] Thus, two atoms, oxygen and the carbonyl carbon, separate the reaction center from the point of branching thereby reducing greatly the magnitude of any steric effect. This can be illustrated by the similar Taft E_s constants[13] for the groups—n-C_3H_7 (−0.36), i-C_5H_{11} (−0.35), t-$C_4H_9CH_2CH_2$ (−0.34)—although in this comparison replacement of the carbonyl carbon and the oxygen by two saturated carbon atoms may

(11) A. J. Kirby and A. G. Varvoglis, *J. Amer. Chem. Soc.*, **89**, 415 (1967).

(12) Solvent isotope effects of about unity might be expected if a zwitterionic species was involved as an intermediate since D_2O would have compensating effects on the equilibrium concentrations of monoanion and the zwitterion.

(13) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley & Sons, Inc., New York, N. Y., 1956, p 556.

(14) For chloroacetyl phosphate, $k_{dianion}$ is 0.190 min^{-1} at 39.0° and μ = 0.6. Elemental analysis on this compound was not possible due to its rapid partial hydrolysis in aqueous solutions during the isolation procedure.

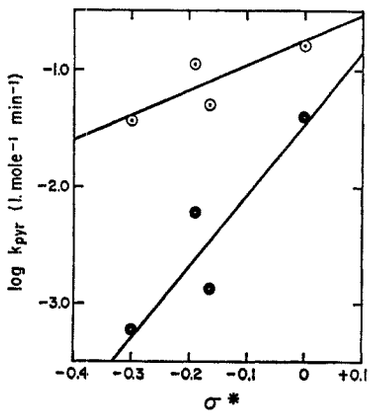
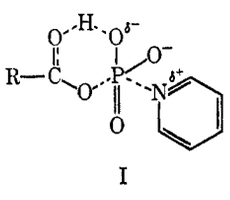


Figure 5.—Plots of log k_{pyr} at 60° for acyl phosphate monoanions (⊙) and dianions (●) *vs.* σ^*.

not be exact. In the case of β-glycerol phosphate[15] the C–O–P bond distance is similar to the C–C–C distance, but with acyl phosphates the C–O–P distance could be shorter because of resonance interaction between oxygen and the carbonyl group. It is therefore possible that steric and inductive effects are both affecting the nucleophilic attack of pyridine.

The value of ρ^* for the reaction of acyl phosphate dianions with pyridine is larger than for the dianion hydrolytic reaction. Inductively electron-donating groups would not only make it more difficult for the carboxylate anion to leave, as in the hydrolytic reaction, but would also impede the attack of pyridine. Also, ρ^* for the pyridine-catalyzed hydrolysis of acyl phosphate dianions is much larger than the ρ^* for the pyridine catalysis of acyl phosphate monoanion hydrolysis. This observation can be attributed to a proton-transfer step taking place in the monoanion reaction with pyridine, as in the hydrolytic reaction.[4] Thus the probable transition state for the pyridine reaction with the monoanion would appear as shown in I. A pentacovalent intermediate could also be forming, and the kinetic data do not eliminate this possibility.



I

Pyridine has been shown not to catalyze the hydrolysis of phenyl phosphate monoanions,[16] although weak catalysis was observed in the hydrolysis of *p*-nitrophenyl phosphate dianion.[17] The facile pyridine reaction with the monoanion of acyl phosphates may be due to the ease of proton transfer with these compounds, and in addition, the low pK_a of the carboxyl leaving group, compared with 9.9 for phenol and 7.1 for *p*-nitrophenol, should greatly facilitate the reaction. These differences in pK_a undoubtedly strongly influence the pyridine reaction with the dianion of acyl phosphates since proton transfer does not, of course, take place in the dianion reaction.

Reactions of acyl phosphates which occur at the carbonyl carbon center are subject to normal steric effects. Imidazole and morpholine, two amine bases which attack at the carbonyl of acetyl phosphate,[4] show no observable reaction with trimethylacetyl phosphate and 3,3-dimethylbutyryl phosphate. Hydroxide ion, a much smaller nucleophile, will catalyze the hydrolysis of the dianionic species of these two compounds, although at a reduced rate in comparison with acetyl phosphate, the relative rate ratios being acetyl 1.0, trimethylacetyl 0.0073, and 3,3-dimethylbutyryl phosphate 0.0064. The fact that imidazole is a good catalyst for the hydrolysis of isobutyryl phosphate at a pH value where the monoanion is at high concentration, but not at higher pH, indicates that the monoanion is the kinetically significant species with attack by the free base of imidazole taking place. A nucleophilic reaction involving the conjugate acid of imidazole and the dianion would not be expected on chemical grounds.[4] The monoanionic species should be considerably more reactive than the dianion in reactions involving attack of a nucleophile at the carbonyl since HPO_4^{2-} is a much better leaving group than PO_4^{3-}.

An important observation is that inductive and/or steric influences at the carbonyl carbon will not change the position of attack of amines. The relatively rapid acylation of imidazole by acetyl phosphate could possibly conceal a slower reaction at phosphorus, but the present data show that this is not the case. Imidazole will not preferentially attack at phosphorus even when relatively high electron density (trimethylacetyl phosphate) or large steric hindrance (3,3-dimethylbutyryl phosphate) occurs at the carbonyl carbon, no catalysis by imidazole being detected with those compounds. It is not clear why imidazole is such a poor nucleophile toward phosphorus in these compounds in comparison to pyridine.

Some enzymes which exhibit acyl phosphatase activity have histidine at their active sites. In the case of glyceraldehyde-3-phosphate dehydrogenase,[3] it has been suggested that histidine might attack at the carbonyl, as observed in the nonenzymatic reaction of imidazole with acetyl phosphate. However, when succinyl phosphate is utilized by succinic thiokinase, the acyl phosphate transfers the phosphoryl group to the enzyme to form a phosphoryl enzyme.[18] The phosphorylated group in the enzyme has been identified as 3-phosphohistidine.[18,19] The chemical data indicate that a phosphoryl group cannot be transferred directly from an acyl phosphate to histidine. Transfer must then be mediated through an intermediate carrier or else the enzyme, in an unknown manner, is inducing a change in mechanism from that normally seen in the nonenzymatic reactions.

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