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REACTION OF N-ACYLPHOSPHORAMIDIC ACID WITH ALCOHOLS[1]

C. ZIOUDROU
Department of Biochemistry, Yale University,
New Haven, Connecticut, U.S.A.

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Abstract—The reactions of N-benzoylphosphoramidic acid and its *p*-methyl- and *p*-nitro-substituted derivatives with alcohols in the presence of organic bases result in the phosphorylation of the alcohol. The dependence of the phosphorylation upon base concentration and structure has been investigated. Several mono- and diesters of N-benzoylphosphoramidic acid have been prepared and some of their chemical properties are reported.

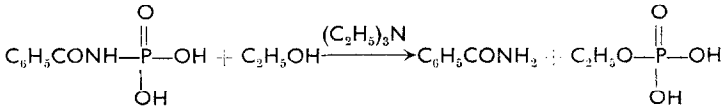
ALTHOUGH phosphoramidic acids have attracted considerable attention in recent years as reagents for the synthesis of pyrophosphates,[2] and as substrates for the enzyme phosphoramidase,[3] relatively little information is available about the properties of the N-acylderivatives of phosphoramidic acids. In the present communication we report studies on the phosphorylation of alcohols by N-acylphosphoramidic acids, as well as on other chemical aspects of these compounds.

N-Benzoylphosphoramidic acid was first prepared by Titherley and Worrall,[4] who treated benzamide with PCl_5 in benzene to obtain the dichloride $C_6H_5CONHP(O)Cl_2$, which was subjected to hydrolysis. More recently this synthesis has been extended[5] to a series of ring-substituted derivatives of benzamide. Because of the variety of melting points reported for N-benzoylphosphoramidic acid (see Experimental) and the unsatisfactory analyses of the different samples we isolated this acid and its *p*-methyl- and *p*-nitro-substituted derivatives in analytically pure form by converting them into their mono-cyclohexylammonium salts. Several mono-and diesters of N-benzoylphosphoramidic acid also have been prepared. As described in the experimental part of this communication, N-benzoylphosphoramidic acid and its *p*-substituted derivatives are suitable phosphorylating agents for alcohols on a preparative scale. The following compounds have been successfully phosphorylated and the products isolated in satisfactory yield (40–80 per cent) as the cyclohexylammonium salts: ethanol, benzyl alcohol, cyclohexanol, phenol. Ethanolamine was phosphorylated to yield free O-phosphorylethanolamine. Attempts to phosphorylate amines under the same conditions resulted only in the recovery of the amine salt of the N-acylphosphoramidic acids.

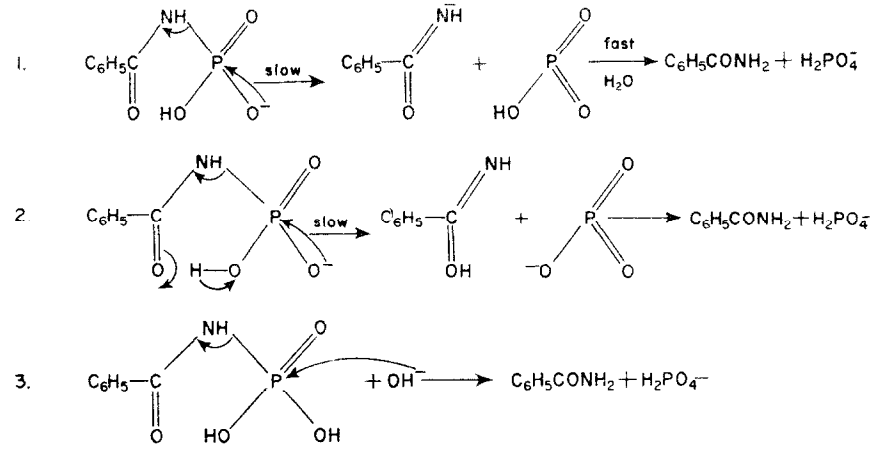
The rate of reaction of N-benzoylphosphoramidic acid with ethanol was studied at 56° as a function of the concentration of added base. The base triethylamine was

[1] This investigation was supported by grants from the National Science Foundation (C-7451) and the United States Public Health Service (RG-6452) to Professor Joseph S. Fruton.
[2] V. M. Clark, G. W. Kirby and A. Todd, *J. Chem. Soc.* 1497 (1957); R. W. Chambers and H. G. Khorana, *J. Amer. Chem. Soc.* **80**, 3749 (1958); J. G. Moffatt and H. G. Khorana, *Ibid*, **83**, 649 (1961).
[3] M. F. Singer and J. S. Fruton, *J. Biol. Chem.* **229**, 111 (1957); E. Boger and O. M. Friedman, *J. Amer. Chem. Soc.* **80**, 2583 (1958); R. A. Smith and D. J. Burrows, *Biochim. Biophys. Acta* **34**, 274 (1959).
[4] A. W. Titherley and E. Worrall, *J. Chem. Soc.* **95**, 1143 (1909).
[5] A. V. Kirsanov and R. G. Makitra, *J. Gen. Chem. U.S.S.R.* **26**, 905, 907 (1956); **27**, 450 (1957).

used because of the favorable solubility of the mono-and ditriethylammonium salts of the N-acylphosphoramidic acids. The observed rates follow first-order kinetics with respect to the phosphoramidic acid. As may be seen in Fig. 1 the rate of phos-



phorylation of ethanol is proportional to the concentration of triethylamine over the range zero to one mole and the maximum rate constant $9{\cdot}4 \times 10^{-3}$ min^{-1} is obtained at equimolar concentrations of N-benzoylphosphoramidic acid and triethylamine. At values of the molar ratio of base/acid above one the rate decreases slowly and the value of the maximal rate constant is approximately halved at a 10-fold ratio. The fact that the maximum rate is reached at equimolar concentrations of acid and base is concordant with the available data on the effect of pH on the hydrolysis of this compound. Halmann *et al.*[6] have reported that in the pH range 1–8 the rate of hydrolysis of N-benzoylphosphoramidic acid exhibits a maximal value at pH 4, where the predominant ionic form is the monoanion RCONHP(O)(OH)(O$^-$), (the first pK_a' for benzoylphosphoramidic acid is 1·99), and decreases rapidly with increasing hydroxide ion concentration. Experiments performed in this laboratory[7] are in agreement with this finding. Halmann *et al.*[6] on the basis of these results and the finding that the rate of solvolysis of N-benzoylphosphoramidic acid increases in D_2O suggest that the rate limiting step is controlled by the breakdown of a cyclic hydrate intermediate postulated by Westheimer for the hydrolysis of alkylphosphates.[8] In view of the uncertainties concerning the mechanisms involved in the hydrolysis of esters and amides of phosphoric acid,[8,9] the following mechanisms for the hydrolysis of the N-acylphosphoramidic acids should be considered:



The rate equation, rate $= k'$ [monoanion] which describes mechanism 1 and 2 is kinetically indistinguishable from the rate expression, rate $= k''$. [undissociated acid]

[6] M. Halmann, A. Lapidot and D. Samuel, *J. Chem. Soc.* **4672** (1960).
[7] L. T. Chylack and M. H. Knappenberger, unpublished work.
[8] C. A. Vernon, *Chem. Soc. Spec. Publ.* No. 8 (1957); W. W. Butcher and F. H. Westheimer, *J. Amer. Chem. Soc.* 77, 2423 (1955).
[9] M. Cohn, *J. Cellular and Comp. Physiol.* **54**, Supl. 17 (1959); M. Halmann and A. Lapidot, *J. Chem. Soc.* 419 (1960).

[OH^-] of mechanism 3 and the available data are insufficient to differentiate between the above equations.

It might be expected that at high concentrations of added triethylamine (where the predominant species is the dianion) the rate of ethanolysis would be negligible. The fact that this is not so (Fig. 1) suggests that ethoxide ion may be involved in a nucleophilic attack on the phosphorous atom (cf. equation 3).

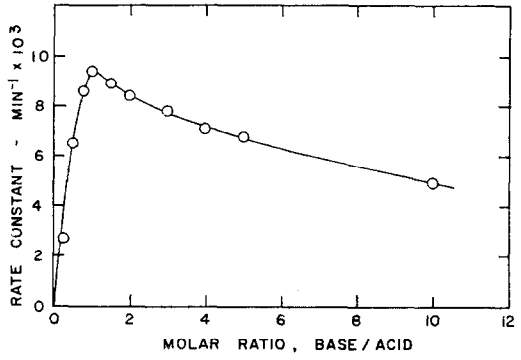


FIG. 1. The effect of concentration of triethylamine on the rate of ethanolysis of N-benzoylphosphoramidic acid at 56·4°.

The rate of ethanolysis of N-benzoylphosphoramidic acid was also studied at 56° in the presence of equimolar concentration of bases other than triethylamine. The salts of the N-benzoylphosphoramidic acid of the bases listed in Table 1 are soluble in ethanol at 56°. As may be seen from Table 1 variation of pK_a' or structure of the base did not change the rate of ethanolysis by a factor of more than 4.

TABLE 1. THE EFFECT OF pK' AND STRUCTURE OF ORGANIC BASES ON THE RATE OF ETHANOLYSIS OF N-BENZOYLPHOSPHORAMIDIC ACID AT EQUIMOLAR CONCENTRATIONS AT 56·4°

Base	pK'	First-order rate constant $\times 10^3$ min^{-1}
Piperidine	11·2[a]	10·8
Diethylamine	11·0[a]	10·0
Triethylamine	10·6[a]	9·4
t.-Butylamine	10·4[a]	8·8
Morpholine	8·3[a]	6·2
2,4,6-Trimethylpyridine	7·6[b]	7·3
Pyridine	5·2[b]	2·5

[a] H. K. Hall, *J. Amer. Chem. Soc.* **79**, 5441 (1957); pK_a' values in water at 25°.

[b] A. Gero and J. J. Markham, *J. Org. Chem.* **16**, 1835 (1951); pK_a' values in water at 25°.

Substitution in the *para*-position of the phenyl ring with methyl- or nitro-groups has a relatively small effect on the rate of ethanolysis or hydrolysis. At equimolar concentrations of acid and base the rate constant of ethanolysis for the *p*-methyl- and *p*-nitrobenzoylphosphoramidic acid at 56° are $8·6 \times 10^{-3}$ min^{-1} and $8·0 \times 10^{-3}$ min^{-1} respectively.

The maximal rate constant (at pH 4) of the hydrolysis of *p*-methyl- and *p*-nitro-derivatives at 37° are $14{\cdot}3 \times 10^{-3}$ min^{-1} and $10{\cdot}0 \times 10^{-3}$ min^{-1} respectively, the one of the parent compound being $12{\cdot}7 \times 10^{-3}$ min^{-1}.[7] The lack of sensitivity to electronic effects of the *p*-substituted derivatives of acylphosphoramidic acid supports mechanism 2 for hydrolysis and ethanolysis. Thus electron donating substituents increase the basicity of the carbonyl oxygen atom and its affinity for the proton of the hydroxyl group but at the same time lower the affinity for the electrons from the phosphorus-nitrogen bond. Electron withdrawing substituents produce the opposite effect.

TABLE 2. APPARENT IONIZATION CONSTANTS OF N-ACYLPHOSPHORAMIDIC ACIDS AND THEIR DERIVATIVES AT 30°

Compound	pK_a' values[a]
N-Benzoylphosphoramidic acid[b]	1·99, 6·42, ca. 14
p-methyl-N-benzoylphosphoramidic acid	2·04, 6·20
p-nitro-N-benzoylphosphoramidic acid	1·97, 6·24
Monophenyl-N-benzoylphosphoramidate	1·9, ca. 14 (13·4)
Diphenyl-N-benzoyl-phosphoramidate	7·7 (7·5)
Dibenzyl-N-benzoylphosphoramidate	8·9 (8·7)
Phosphoramidic acid[c]	(?), 8·2
Ethylphosphate[d]	<2, 6·9

[a] The values in parentheses were determined spectrophotometrically; the others were determined by titration. For details, see Experimental section.
[b] Halmann *et al.*[6] reported pK values of 2·67 and 5·67 for this compound at 27° in an aqueous medium of unstated ionic strength.
[c] T. Rathlev and T. Rosenberg, *Arch. Biochem. and Biophys.* **65**, 319 (1956).
[d] W. D. Kumler and J. J. Eiler, *J. Amer. Chem. Soc.* **65**, 2355 (1943) report pK' values 1·6 and 6·62 for 0·04 M solution of barium salt at 25°.

During the course of this study the apparent ionization constants of the N-acyl-phosphoramidic acids and some of their esters were determined. The data are summarized in Table 2.

It was reported by Halmann *et al.*[6] that, at high acidities, N-benzoylphosphoramidic acid undergoes acid-catalyzed hydrolysis to benzamide and phosphoric acid. In this connection it is of interest that the phosphorylation of ethanol by N-benzoyl-phosphoramidic acid has also been achieved in the present study by the addition of 2·5 N HCl. Under these acidic conditions of hydrolysis or ethanolysis, the reaction probably involves the protonation of the nitrogen to form the reactive species $C_6H_5CO\overset{+}{N}H_2$-P(O)(OH)(OH).

EXPERIMENTAL[10]

Preparation of acylphosphoramidic acids. N-Benzoylphosphoramidic acid was prepared in the manner described by Titherley and Worrall,[4] but successive preparations had m.p. between 136° and 156–157°. Yields varied between 40–60%, based on the amount of benzamide used. Although Titherley and Worrall reported the m.p. of benzoylphosphoramidic acid to be 157–158°, Kirsanov and Makitra[5] claimed that this value was too high, and that the correct m.p. was 136–138°. In a recent publication[6] it was reported that use of Titherley and Worrall's method gave a product melting

[10] All m.p. are uncorrected. Microanalyses were performed by Dr. S. M. Nagy, Massachusetts Institute of Technology to whom we wish to express our thanks.

at 157–158° without any indication of the difficulties in reproducing this value. Work in this laboratory yielded, in successive trials, preparations whose m.p. varied between 136° and 157°; for example, a sample melting at 146–147° gave satisfactory analytical data.

(Found: C, 41·4; H, 4·1; N ,7·0; P, 15·3; Calc. for $C_7H_8NO_4P$ (201·1): C, 41·8; H, 4·0; N, 7·0; P, 15·4%).

Because of the difficulty in obtaining samples of benzoylphosphoramidic acid having the same melting points, the monocyclohexylammonium salt was prepared. This proved to be a material having reproducible properties. The salt of benzoylphosphoramidic was prepared by the addition of distilled cyclohexylamine (4 ml, 0·033 mole) in 10 ml dry tetrahydrofuran to a solution of the acid (6 g, 0·03 mole) in 300 ml THF. The precipitate (8·5 g, 93%) was recrystallized from 94% methanol (water) and the addition of ether. Yield: 74% m.p. 150–151%.

(Found: C, 51·9; H, 7·2; N, 9·3; P, 10·2. Calc. for $C_{13}H_{21}N_2O_4P$ (300·3): C, 51·5; H, 7·1; N, 9·3; P, 10·24).

The infrared absorption band of the amide carbonyl of benzoylphosphoramidic acid appears at 6·05 μ (KBr disk) and is shifted to 6·12 μ in the case of the monocyclohexylammonium salt as expected from the increased electron-withdrawing capacity of the nitrogen in the ionized form. The monotriethylammonium salt of benzoylphosphoramidic acid melts at 132–134°.

(Found: N, 9·2; P, 10·5. Calc. for $C_{13}H_{23}NO_4P$ (302·3): N, 9.2; P, 10·3%).

p-Methyl-benzoylphosphoramidic acid was prepared from *p*-toluamide (10·3 g, 0·076 mole) and PCl_5 (16 g, 0·076 mole) in benzene as previously described. The crude product (13·1 g, 74%) was converted into the monocyclohexylammonium salt and recrystallized in the above described manner. Yield 54%, m.p. 158-159°.

Found: C, 52·9; H, 7·3; N, 8·9; P, 10·0. Calc. for $C_{14}H_{23}N_2O_4P$ (314·3): C, 53·4; H, 7·5; N, 8·9; P, 9·9.

p-*Nitrobenzoylphosphoramidic acid* was prepared from *p*-nitrobenzamide (16·6 g, 0·1 mole) and PCl_5 (21 g, 0·1 mole) with CCl_4 as the solvent for the preparation of the dichloride and the crude product (16 g, 65%) was converted to its monocyclohexylammonium salt and recrystallized from 60% acetone–water followed by addition of ether. Yield 42%, m.p. 194–196°.

Found: C, 45·3; H, 5·9; N, 11·9; P, 8·9. Calc. for $C_{13}H_{20}N_3O_6P$ (345·3): C, 45·3; H, 5·8; N, 12·2; P, 9·0%.)

Esters of N-*benzoylphosphoramidic acid.* The monophenyl ester was prepared by addition of 1·0 g (10·6 mmoles) phenol and 1·0 ml (12 mmoles) pyridine to a solution of 2·4 g (10 mmoles) N-benzoyldichlorophosphoramidate[4] (m.p. 110°). After the reaction mixture had been kept at 0° for 90 min and at room temp. for an additional hour, it was diluted with 20 ml $CHCl_3$ and washed with small volumes of 5% $NaCHO_3$, water and 30% NaCl. The chloroform solution was dried over $MgSO_4$ and evaporated to dryness. The residue was dissolved in 25 ml 4 N NaOH, and the solution heated for 10 min on a steam bath, chilled, and acidified with 5 N HCl to Congo red. The crystals (1·9 g) were collected, washed with cold water and ether, and recrystallized from methanol–ethyl acetate (2:3). Yield 70%; m.p. 170°. Molecular extinction coefficient in ethanol at 261 mμ, 1450.

(Found: C, 56·5; H, 4·4; N, 4·9; P, 11·5. Calc. for $C_{13}H_{12}NO_4P$ (277·22): C, 56·2; H, 4·3; N, 5·1; P, 11·2%).

The diphenyl ester was prepared by the addition of a suspension of 1·25 g (10·8 mmoles) sodium phenolate in 20 ml anhydrous methylene chloride to a solution of 1·2 g (5 mmoles) N-benzoyldichlorophosphoramidate. After the mixture had been stirred at room temp. under anhydrous conditions overnight, 0·65 g of insoluble material (NaCl) was removed by filtration. The filtrate was diluted with 30 ml CH_2Cl_2, washed with N H_2SO_4, 5% $NaHCO_3$, water, and dried over $MgSO_4$. Upon evaporation to dryness and the addition of ether, 1·1 g crystals were obtained. After recrystallization from chloroform-n-heptane the substance melted at 148°. Yield 63%. Molecular extinction coefficient in ethanol at 261 mμ, 1870.

Found: C, 64·3; H, 4·5; N, 4·0; P, 8·6. Calc. for $C_{19}H_{18}NO_4P$ (353·3): C, 64·5; H, 4·5; N, 3·9; P, 8·7%).

The dibenzyl ester was prepared by the addition of 3 ml (30 mmoles) benzyl alcohol and 2·4 ml (30 mmoles) anhydrous pyridine to a solution of 3·6 g (15 mmoles) N-benzoyldichlorophosphoramidate in 30 ml anhydrous methylene chloride. After being kept at 0° for 1 hr, and at room temp. overnight, the solution was diluted with 30 ml solvent, washed with N H_2SO_4, 5% $NaHCO_3$ and 30% NaCl, dried over $MgSO_4$, and evaporated to dryness. Upon treatment with ether, crystals (2·3 g)

were obtained. After recrystallization from benzene–n-heptane, the substance melted at 123–124°. Yield 50%. Molecular extinction coefficient in ethanol at 263 mμ, 1400, at 256 mμ, 1460.

Found: C, 65·8; H, 5·2; N, 3·6; P, 8·1. Calc. for $C_{21}H_{20}NO_4P$ (381·38): C, 66·0; H, 5·2; N, 3·6; P, 8·1%).

Phosphorylation of ethanol by cyclohexylammonium salt of benzoylphosphoramidic acid. The salt (1·5 g, 5 mmoles) was heated for 90 min under reflux with 60 ml anhydrous ethanol. The resulting solution was concentrated to dryness under red. press., and the residue was dissolved in 100 ml water. A 2 ml sample was withdrawn for the determination of residual phosphoramidic acid, by treatment with N H_2SO_4 at 100° for 10 min to release inorganic phosphate (estimated by the Fiske-Subbarow method[11]), and it was found that 97·4% of the initial benzoylphosphoramidic acid had undergone reaction with ethanol. The aqueous solution (98 ml) was concentrated to dryness under red. press., the residue was dissolved in 15 ml ethanol and addition of ether induced crystallization of the monocyclohexylammonium salt of ethylphosphate. Yield 0·82 g (74%). After recrystallization from ethanol–ether, the substance melted at 200–205° (decomp).

Found: C, 42·1; H, 8·7; N, 6·1; P, 13·7.[12] Calc. for $C_8H_{20}NO_4P$ (225·3): C, 42·7; H, 9·0; N, 6·2; P, 13·8%).

The ethanol–ether filtrate from the isolation of the salt of ethylphosphate was concentrated to dryness, and the residue was treated with 7 ml water. Benzamide (0·45 g, 75%) was isolated; it gave no m.p. depression with an authentic sample.

Phosphorylation of ethanol by benzoylphosphoramidic acid in presence of HCl. The free acid (0·25 g; 1·25 mmoles) was dissolved in 50 ml 2·5 N ethanolic HCl, and the solution was kept at 56° (glycerol bath) for 4 hr. At this time, analysis[11] of samples showed that no free inorganic phosphate was present and that 94% of phosphoramidic acid had disappeared.[13] The solution was concentrated to dryness under red. press., and the residue was treated several times with ethanol, which was removed by evaporation. The oily residue was dissolved in 3 ml ethanol, cyclohexylamine (0·13 ml) was added, followed by ether to induce crystallization. After 3 recrystallizations from ethanol–ether, 0·155 g (53%) of material melting at 200–205° (decomp) were isolated; it gave no m.p. depression with a sample of the cyclohexylammonium salt of ethylphosphate.

Phosphorylation of benzyl alcohol. N-Benzoylphosphoramidic acid (0·4 g, 2 mmoles) was dissolved in 2·5 ml (ca. 23 mmoles) of anhydrous benzylalcohol. After the addition of (0·28 ml, 2 mmoles) of triethylamine, the solution was heated for 2·5 hr at 78° in a glycerol bath. At this point phosphate analysis of a sample showed that the extent of phosphorylation was 80%. The solution was washed several times with ether, the residual syrup was dissolved in 12 ml 3 N H_2SO_4 and extracted with 20 ml portions of chloroform. The combined extracts were dried over $MgSO_4$, evaporated to dryness under red. press., and the residue was dissolved in 5 ml methanol. Cyclohexylamine (0·5 ml) was added, and the cyclohexylammonium salt precipitated by the addition of ether. After several recrystallizations and drying *in vacuo* over P_2O_5 at 56°, the resulting monocyclohexylammonium salt (0·36 g) melted at 238–240°.[14] Yield 63%. The dicyclohexylammonium salt initially obtained probably loses one mole of amine during recrystallizations and drying *in vacuo*.

Found: C, 54·0; H, 7·6; N, 4·9; P, 10·5. Calc. for $C_{13}H_{22}NO_4P$ (287·30): C, 54·3; H, 7·7; N, 4·9; P, 10·8%).

Molecular extinction coefficient at 256 mμ, 194 in 20% ethanol–water; authentic sample of dibenzylphosphate has a molecular extinction coefficient of 423 at 256 mμ in 20% ethanol–water.

Phosphorylation of cyclohexanol. The cyclohexylammonium salt of *p*-methylbenzoylphosphoramidic acid (0·4 g, 1·27 mmoles) was suspended in 5 ml anhydrous cyclohexanol and heated at 78° for 10 hr. Nearly all the material went into solution. At the end of the reaction, 85 mg undissolved starting material were removed by filtration and the filtrate was washed several times with ether. The residue was dissolved in 10 ml ethanol and crystallization of the monocyclohexylammonium salt of cyclohexylphosphate was induced by the addition of ether and a small amount of pet. ether. After recrystallization from methanol–ethyl acetate plus some pet. ether, the substance (0·16 g) melted at 208–210°. Yield 65%.

[11] C. H. Fiske and Y. Subbarow, *J. Biol. Chem.* **66**, 375 (1925).

[12] The low value for C is attributed to the occasional difficulties in C–H analyses of phosphorus containing compounds.

[13] At room temp, approximately 40 hr were needed to achieve this extent of reaction.

[14] J. Kumamoto and F. H. Westheimer, *J. Amer. Chem. Soc.* **77**, 2515 (1955), report m.p. 233° for the dicyclohexylammonium salt of benzylphosphate.

(Found: N, 5·1; P, 11·2. Calc. for $C_{12}H_{26}NO_4P$ (279·3): N, 5·0; P, 11·1%).

Phosphorylation of phenol. A mixture of the cyclohexylammonium salt of benzoylphosphoramidic acid (0·3 g, 1 mmole) and of phenol (0·4 g, 4 mmoles) was heated at 78° for 3 hr. The clear melt was cooled, dissolved in 3 ml methanol and crystallized by the addition of ether. The crystals (240 mg) were washed thoroughly with ether and after recrystallization from methanol–ether, the product melted at 202–205° and showed no m.p. depression when mixed with an authentic sample of the monocyclohexylammonium salt of phenylphosphate.

Found: C, 53·1; H, 7·9; N, 5·4; P, 11·5. Calc for. $C_{12}H_{20}NO_4P$ (273·28): C, 52·8; H, 7·4; N, 5·1; P, 11·4%).

An authentic sample of the salt of phenylphosphate had a molecular extinction coefficient of 460 at 261 mμ in water; the product gave a value of 435.

Phosphorylation of ethanolamine. Distilled ethanolamine (0·15 ml, 2·4 mmoles) was added to a solution of (500 mg, 2·3 mmoles) *p*-methylbenzoylphosphoramidic acid in 3 ml dimethylsulfoxide, and the resulting solution was heated at 56° for 6 hr. During the reaction, crystals and oil appeared. The mixture was diluted with 5 ml 50% ethanol and 40 ml ether were added to wash out *p*-toluamide and unreacted ethanolamine. The residual insoluble material was dissolved in 3 ml water. The pH was adjusted to 4·5 with HCl and addition of methanol followed by a few ml ether induced the crystallization of O-phosphorylethanolamine. Yield 150 mg (46%). After recrystallization from water–methanol–ether, the product melted at 235° (reported:[15] 242°), and showed no m.p. depression when mixed with a recrystallized sample of O-phosphorylethanolamine (California Biochemicals Corp.).

Found: C, 17·2; H, 5·8; N, 9·8; P, 22·1. Calc. for $C_2H_8NO_4P$ (141·1): C, 17·0; H, 5·7; N, 9·9; P, 22·0%).

Other experiments on phosphorylation of alcohols. The phosphorylation of benzylalcohol, cyclohexanol, and phenol has been studied at 78° with dimethylformanide as the solvent, and with 1 equivalent of the monotriethylammonium salt of the acylphosphoramidic acid and 1–2 equivalents of the hydroxy compound as reactants. The course of the reaction may be followed by analysis of samples for labile phosphate. The released benzamide, *p*-toluamide or *p*-nitrobenzamide can be removed by triturating the reaction mixture with ether and the products may be isolated by exhaustive extraction of acidified aqueous solutions with chloroform or ether, evaporation of the organic phase and precipitation of the mono- or dicyclohexylammonium salts from methanol–ether by the addition of cyclohexylamine. The yields of analytically pure compounds obtained in this manner are lower than those reported above.

Kinetics of ethanolysis of acylphosphoramidic acids. Solutions of 2–3 ml free acid (0·02 mole) in absolute ethanol, containing different concentrations (0·005 to 0·2 mole) of triethylamine were placed in sealed ampoules and kept at 56·4° (glycerol bath heated by acetone under reflux). At appropriate times (5 determinations per experiment), ampoules were withdrawn, and a 1 ml sample was hydrolyzed with 10 ml N H_2SO_4 for 10 min in a steam bath, in order to determine the amount of unreacted N-acylphosphoramidic acid by the Fiske-Subbarow method. Under these conditions of acid hydrolysis, the alkyl phosphates are stable, whereas the N-acylphosphoramidic acids are completely hydrolysed to inorganic phosphate. The rate of disappearance of acid-labile phosphate was taken as the rate of the phosphorylation of ethanol. All reactions were followed to 80–90% of completion, and the measured rates were found to follow first-order kinetics. The same procedure was used in the study of the rate of ethanolysis of N-benzoylphosphoramidic acid in the presence of equivalent concentration of bases other than triethylamine.

Determination of ionization constants. The apparent ionization constants of N-acylphosphoramidic acid were determined titrimetrically by means of a Radiometer TTTl automatic titrator. Solutions of 0·01 or 0·05 M monocyclohexylammonium salts in 0·1 M KCl were titrated with 0·1 N NaOH or N HCl at 30° ± 1°. The results were checked by back-titration. The pK' values were calculated by means of the Henderson-Hasselbach equation. For the determination of pK' values near pH 2, 0·05 M solutions were used and the appropriate corrections were made for changes in pH due to volume change and water titration. In the determination of the first ionization constant of the monophenyl ester of N-benzoylphosphoramidic acid, the solvent was 10% acetone containing 0·1 M KCl; the values to pK_1' and pK_2' of N-benzoylphosphoramidic acid are not altered in this solvent.

The ionization constants of the esters in the alkaline range of pH were determined by titration

[15] R. E. Plapinger and T. Wagner-Jauregg, *J. Amer. Chem. Soc.* **75**, 5757 (1953).

with alkali and also spectrophotometrically. In the titrimetric method, 0·05 M monoester or free acid solutions in 10% methanol containing 0·1 M KCl and 0·01 M of the diesters solutions in 40% methanol containing 0·1 M KCl were used. In the spectrophotometric determinations the concentration was 5×10^{-4} M in the same solvents and the increase in absorbance with pH was measured. For the mono- and dipheny! esters, the wavelength selected was 266 mμ; for the dibenzyl ester it was 262 mμ. The values of pK' obtained by the two methods were found to be in reasonable agreement and are shown in Table 2.

Acknowledgement—I wish to thank Professor Joseph S. Fruton for suggesting this problem and for helpful criticism and encouragement during the course of this work.