BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 2518—2523 (1970)

Syntheses of Organic Phosphates. II. 3-Hydroxy-2-pyridylmethyl, 6-Methyl-2-pyridylmethyl, 3-Pyridyl and 8-Quinolyl Phosphate*1

Yukito MURAKAMI, Junzo SUNAMOTO, Hiromi SADAMORI, Hiroki KONDO and Makoto TAKAGI

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Hakozaki, Fukuoka

(Received October 11, 1969)

The phosphorylation of 3-hydroxy-2-pyridylmethanol and 6-methyl-2-pyridylmethanol was carried out with pyrophosphoric acid. 3-Pyridinol and 8-quinolinol were phosphorylated in pyridine by using five- to ten-fold molar amount of phosphoryl chloride at room temperature or below. The chromatographic elution of the reaction mixtures on a column of cation-exchange resin, with water as an eluent, was found to be effective for separation and purification of the products in most cases, while 8-quinolyl phosphate was obtained directly from the concentrated reaction mixture. These phosphates, with the exception of 3-hydroxy-2-pyridylmethyl phosphate, follow acid dissociation processes similar to the case of pyridylmethyl phosphates. The effect of the 3-hydroxy group in 3-hydroxy-2-pyridymethyl phosphate on its acid dissociation processes was discussed. A symmetric pyrophosphate ester was also isolated in the course of phosphorylation of 3-pyridinol.

In an earlier paper[1] from our laboratories, synthesis and purification of 2-, 3- and 4-pyridylmethyl phosphate were reported in connection with the studies of biochemically interesting model compounds, such as vitamin B_6 phosphate. Hydrolysis of these pyridylmethyl phosphates was also investigated in the absence and presence of metal ions.[2-4] As a result, it became desirable to investigate the participation of a neighboring functional group

*1 Contribution No. 195 from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University.

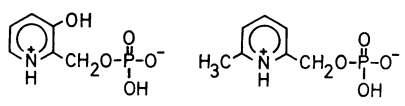
1) Y. Murakami, M. Takagi and H. Nishi, This Bulletin, **39**, 1197 (1966).

2) Y. Murakami and M. Takagi, *ibid.*, **40**, 2724 (1967).

3) Y. Murakami and M. Takagi, *J. Amer. Chem. Soc.*, **91**, 5130 (1969).

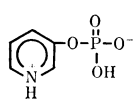
4) Y. Murakami and M. Takagi, This Bulletin, **42**, 3478 (1969).

in the hydrolysis of pyridylmethyl phosphate and the related compounds. The role of a hetero-aromatic nitrogen in such solvolysis reactions also needs to be clarified. In the present work, therefore, 3-hydroxy-2-pyridylmethyl phosphate (**1**), 6-methyl-2-pyridylmethyl phosphate (**2**), 3-pyridyl phosphate (**3**), and 8-quinolyl phosphate (**4**) have been synthesized. In addition, their ionization constants have been determined and discussed in terms of the structural effect.

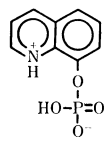


1

2



3



4

Syntheses and Purification. The phosphorylation of 3-hydroxy-2-pyridylmethanol and 6-methyl-2-pyridylmethanol, by using pyrophosphoric acid, was carried out according to the previous method for the syntheses of the unsubstituted pyridylmethyl phosphates.[1] Figure 1 shows the eluting behavior

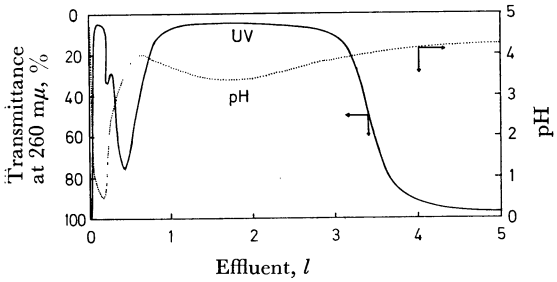


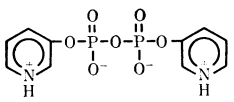
Fig. 1. Chromatographic separation of 3-hydroxy-2-pyridylmethyl phosphate from the phosphorylation mixture.
Mixture: 3.0 g of the alcohol underwent reaction with 23 g of pyrophosphoric acid
Column: Dowex 50W-X8 [H+] (312 meq.), 3×34 cm
Eluent: mineral-free water
Elution rate: 300 ml/hr

of the phosphorylation mixture of 3-hydroxy-2-pyridylmethanol on a chromatographic column of cation-exchange resin, Dowex 50W-X8, with water as an eluent. The front effluent (about 100—150 ml) was strongly acidic (pH=1.0). The ultraviolet measurement of this effluent showed the presence of aromatic species (λ_{max}=294 mμ), and the paper chromatogram indicated the presence of some phosphate compounds. However, no organic phosphates could be isolated from this portion because of their low yield. 3-Hydroxy-2-pyridylmethyl phosphate was obtained in a comparatively good yield from the following fractions, as described in the experimental section.

Since 3-hydroxy-2-pyridylmethanol has two phosphorylation sites, it was necessary to confirm which group, the 2-hydroxymethyl or the 3-hydroxy, was predominantly phosphorylated. Nuclear magnetic resonance spectra directly provide an evidence for the phosphorylation of the former group. The singlet resonance signal of the methylene group in 3-hydroxy-2-pyridylmethanol, the original alcohol, was found to shift downfield and to become the doublet signal through coupling with ^{31}P upon phosphorylation.

6-Methyl-2-pyridylmethanol was phosphorylated nearly quantitatively by a method similar to that used for 3-hydroxy-2-pyridylmethanol. The phosphorylation mixture showed an eluting behavior similar to that case 3-hydroxy-2-pyridylmethanol.

Phosphorylation of 3-pyridinol and 8-quinolinol was carried out by using five- to ten-fold molar amount of phosphoryl chloride at room temperature or below in pyridine. The phosphorochloridate of 3-pyridinol thus produced was hydrolyzed with a sufficient amount of water. After the reaction mixture was neutralized with ammonia, the products were separated chromatographically. Figure 2 shows the eluting behavior of the phosphorylation mixture of 3-pyridinol on a column of cation-exchange resin, Dowex 50W-X8, with water as an eluent. Effluent I was found to contain the corresponding symmetric pyrophosphate ester, which was not isolated because of its low yield. 3-Pyridyl phosphate was obtained from effluent II. When the reaction mixture was treated with just enough amount of water to hydrolyze all the phosphorochloridate in the presence of pyridine, the major product was identified to be the symmetric pyrophosphate ester, P^1,P^2-di(3-pyridyl) pyrophosphate (**5**), and no detectable amount of 3-pyridyl



5

phosphate was found in the reaction mixture as the eluting profile in Fig. 3 indicates. Meanwhile, 8-quinolyl phosphate was obtained exclusively as precipitates from the concentrated phosphorylation mixture even though the phosphorochloridate was hydrolyzed with a just enough amount of water in the presence of pyridine.

Acid Dissociation Constants. 3-Hydroxy-2-pyridylmethyl phosphate was found to be a tetrabasic acid, while the remaining orthophos-

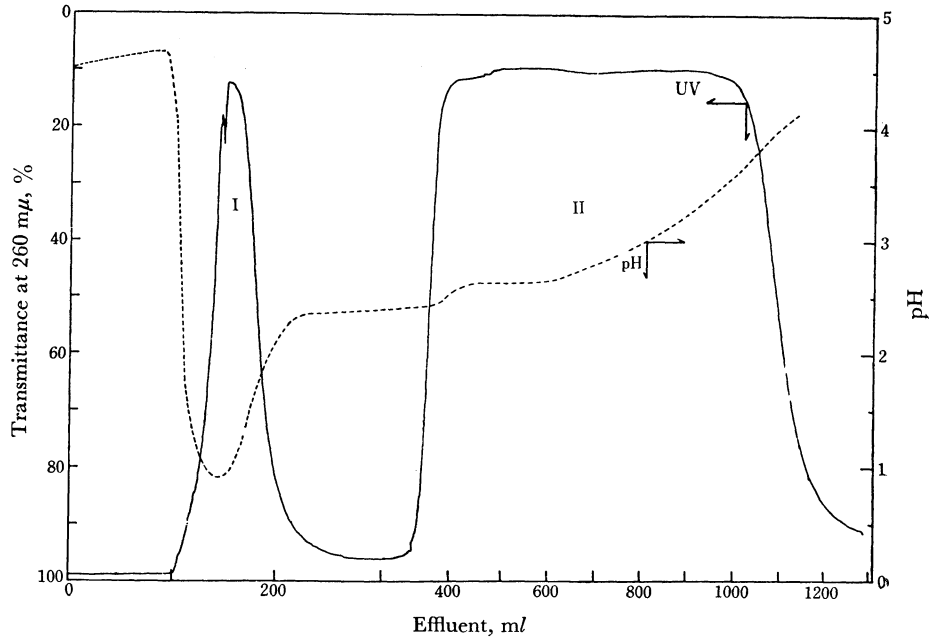


Fig. 2. Chromatographic separation of 3-pyridyl phosphate from the phosphorylation mixture.
Mixture: the phosphorylation mixture of 3-pyridinol (2.00 g) was hydrolyzed and neutralized with concd. ammonia; divided into two portions
Column: Dowex 50W-X8 [H^+] (50—100 mesh, 430 meq.), 2.8×48.5 cm
Eluent: distilled water
Elution rate: 2 ml/min for effluent I, 4 ml/min thereafter

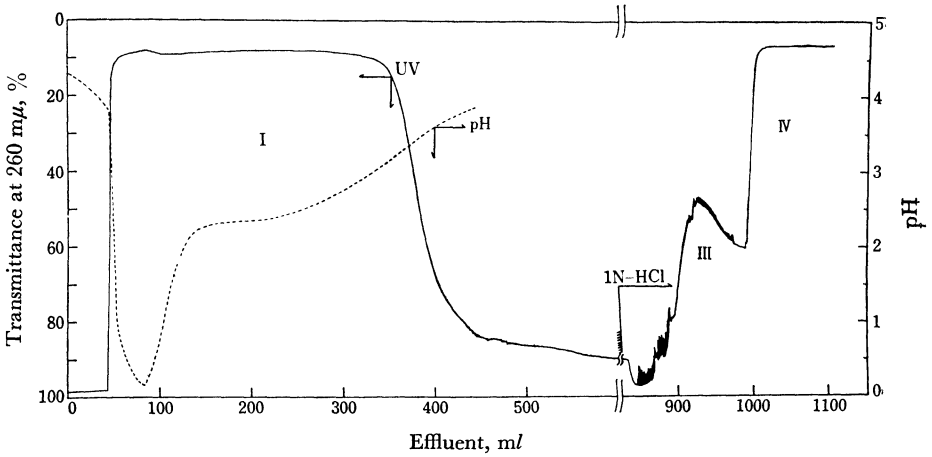


Fig. 3. Chromatographic separation of P^1,P^2-di(3-pyridyl) pyrophosphate from the phosphorylation mixture.
Mixture: the hydrolyzed phosphorylation mixture obtained from 1.4 g of 3-pyridinol
Column: Dowex 50W-X8 [H^+] (50—100 mesh, 240 meq.), 2.8×25 cm
Eluent: distilled water; 1N HCl for effluents III and IV
Elution rate: 2 ml/min
Effluent: I, the pyrophosphate ester; III, 3-pyridinol; IV, pyridine

phates were to be tribasic acids. Acid dissociation constants of these phosphates along with those of the related compounds are listed in Table 1.

Since pK_{H_2A} and pK_{HA} values for the tribasic phosphates are comparable in a qualitative sence with the corresponding values for pyridylmethyl

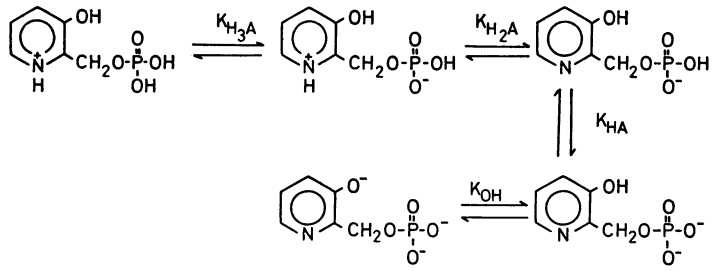


Chart 1. Acid dissociation processes for 3-hydroxy-2-pyridylmethyl phosphate.

Table 1. Acid dissociation constants

25.0±0.1°C, μ=0.10M (KNO_3)

Acid	pK_{H_2A}	pK_{HA}	pK_{OH}[a]
2-Pyridylmethyl phosphate[1]	4.42	6.29	—
3-Pyridylmethyl phosphate[1]	4.86	6.23	—
6-Methyl-2-pyridylmethyl phosphate	4.74	6.64	—
3-Hydroxy-2-pyridylmethyl phosphate	4.54	5.75	9.67
3-Pyridyl phosphate	3.86	5.64	—
8-Quinolyl phosphate	4.17	6.42	—
P^1,P^2-Di (3-pyridyl) pyrophosphate	3.58	4.51	—
3-Pyridinol	—	—	8.54
3-Hydroxy-2-pyridylmethanol	—	—	8.76

a) K_{OH} is the dissociation constant for the hydroxy proton.

phosphates, the present phosphates may undergo dissociation processes similar to those described previously.[1] Thus, K_{H_2A} is attributable to the dissociation constant of a pyridinium proton, while K_{HA} refers to that of the second phosphate proton. The most plausible proton-dissociation processes for 3-hydroxy-2-pyridylmethyl phosphate are shown in Chart 1 in the light of the acid dissociation steps for ordinary dibasic phosphates and hydroxypyridines.

3-Pyridyl phosphate demonstrated the lowest values of both pK_{H_2A} and pK_{HA} among the present six different phosphates listed in Table 1. This may be due to the phenolic character of the ester oxygen which may facilitate the electronic interaction between a pyridine ring and a phosphate group, while such an interaction would be minor in the remaining phosphates.

A comparatively low pK_{HA} value was observed for 3-hydroxy-2-pyridylmethyl phosphate. Through electron-withdrawing effect of the 3-hydroxy group and its participation in intramolecular hydrogen bonding with the phosphate group, the acidic nature of the phosphate proton seems to be greatly increased in this case. The above intramolecular hydrogen bonding is further confirmed by the fact that the dissociation of the 3-hydroxy proton is much suppressed as shown in Table 1.

The acid dissociation constants for pyrophosphoric acid have been evaluated at 20°C and the ionic strength of 0.1 in potassium chloride: pK_{H_3A} 2.22, pK_{H_2A} 6.08, pK_{HA} 8.45.[5] It is clear that the pK-values for P^1,P^2-di(3-pyridyl) pyrophosphate given in Table 1 are much higher than those of the first and the second protons of pyrophosphoric acid (pK_{H_4A} and pK_{H_3A}). Since these pK-values are comparable with those pK_{H_2A} values for other phosphate esters listed in Table 1, both K_{H_2A} and K_{HA} for the pyrophosphate ester may be attributed to the dissociation constants of pyridinium protons.

Experimental

3-Hydroxy-2-pyridylmethanol. To a mixture of sodium hydroxide (1.3 g), water (12.6 m*l*) and 3-pyridinol (3.0 g), 37% aqueous formaldehyde solution (2.5 m*l*) was added. After stirring for 3 hr at about 90°C, the mixture was neutralized with acetic acid, and evaporated to dryness. The yellow solid obtained was extracted with 200 m*l* of hot acetone. Through introduction of hydrogen chloride into the resulting extracts, the white hydrochloride was precipitated; yield 2.1 g (41%) upon recrystallization from ethanol; mp 213—216°C (dec.) (lit,[6] mp 209—211°C).

NMR (0.5M in D_2O, HOD as an internal standard): methylene, 0.31 ppm (singlet), ring protons, 3.22—3.57 ppm (multiplet).

This hydrochloride was converted to the corresponding free base with aqueous potassium hydroxide, and further purified by recrystallization from acetone followed by sublimation under reduced pressure; mp 145.4—146.5°C (lit,[6] mp 137—139°C) (Found: C, 57.62; H, 5.69; N, 11.12%).

3-Hydroxy-2-pyridylmethyl Phosphate. A mixture of 3-hydroxy-2-pyridylmethanol (3.0 g) and pyrophosphoric acid (23 g), which was freshly prepared by the brief heating of 85% orthophosphoric acid (13 g) and of phosphorus pentoxide (10 g), was placed in a 50 m*l* three-necked flask protected from moisture. This reaction mixture, after being heated at 80—85°C for

5) L. G. Sillén and A. E. Martell, "Stability Constants of Metal-Ion Complexes," Special Publication No. 17, the Chemical Society, London (1964).

6) N. Elming, *Acta Chem. Scand.*, **11**, 1496 (1957).

5 hr with sufficient stirring, was diluted with 16 m*l* of water and then reheated at 96°C for 30 min to hydrolyze any polyphosphates produced. After being cooled down to room temperature, an equal volume of water was added into the hydrolyzate. The resulting aqueous solution was applied to the top of a column (3×34 cm) of Dowex 50W-X8 [H^+] resin (312 meq.) and eluted with mineral-free water at 300 m*l* per hour. The ultraviolet absorption of the effluent was checked at 260 mμ with a ultraviolet detector, JEOL Model JLC-2B or Toyo UVICON-540, for the presence of aromatic species and the corresponding pH value was measured with a Hitachi-Horiba Model H-5 pH meter. After the front effluent (about 150 m*l*, pH=1.0) with absorption at 294 mμ came off, the acidity of the effluent gradually lowered, reaching pH 4.0 at a total effluent of 800 m*l*, in line with a decrease in the optical density. As the elution proceeded further, the effluent turned slightly acidic (pH=3.5), and the corresponding optical density at 295 mμ increased. After 3000 m*l* of the subsequent effluent was collected, the pH returned to 4.0 and the ultraviolet measurement did not show the presence of aromatic species. These fractions containing the phosphate ester (about 3000 m*l*, pH=3.5—4.0) were concentrated under reduced pressure below 30°C; white crystals precipitated; yield 2.53 g (66%) upon recrystallization from water, mp 201—203°C (dec.); neutralization equivalence 104.6 (calcd 102.5); $\lambda_{max}^{H_2O}$ (10^{-4}M) 252 mμ (ε 1270) and 288 mμ (ε 6910).

IR (KBr disc): 1250, 1175 cm^{-1} ($\nu_{P=O}$), and 1080 cm^{-1} ($\nu_{P-O-C(alk.)}$).

NMR (0.05M in D_2O, HOD as an internal standard): methylene, 0.64 ppm (doublet, J_{P-H}=7.40 Hz); ring protons, 3.33—3.62 ppm (multiplet); the accumulation (16 times) was carried out.

Found: C, 34.90; H, 4.02; N, 6.57; P*[2], 14.2%. Calcd for $C_6H_8NO_5P$: C, 35.14; H, 3.93; N, 6.83; P, 15.1%.

6-Methyl-2-pyridylmethanol. This was obtained from 2,6-lutidine *via* 2,6-lutidine *N*-oxide and 6-methyl-2-acetoxymethylpyridine in succession by a modification of the method in literature[7]; bp 86—92°C/0.2 mmHg; mp as the picrate 134.0—135.0°C (lit,[7] mp 129—132°C) (Found: C, 44.67; H, 3.38; N, 15.88%).

6-Methyl-2-pyridylmethyl Phosphate. A mixture of 44 g of phosphorus pentoxide and 56 g of 85% orthophosphoric acid were heated at 90—100°C with stirring. To the resulting viscous pyrophosphate, 5.4 g of 6-methyl-2-pyridylmethanol was added with caution at 67.5°C. Reaction temperature then immediately rose to about 110°C. After the exothermic reaction ceased, the reaction mixture was heated again up to 90—95°C and stirred for 9.5 hr. After being cooled down to room temperature, 100 m*l* of water was added and then reheated at 80—85°C for 1 hr in order to hydrolyze any polyphosphates produced. The hydrolyzate was subjected to the cation-exchange column chromatography by the use of Dowex 50W-X8 [H^+] resin (50—100 mesh, 425 meq.). Elution was carried out with mineral-free water at about 97 m*l* per hour. Eluting behavior was quite similar to that of 3-hydroxy-2-pyridylmethyl phosphate. From the later fractions of effluent (about 3840 m*l*), which were concentrated under reduced pressure at 40—45°C, white needle-like crystals were obtained; yield 7.5 g (92.7%) upon recrystallization from aqueous ethanol; neutralization equivalence 100.64 (calcd 101.57); $\lambda_{max}^{H_2O}$ (10^{-4}M) 269 mμ (ε 8350).

IR (KBr disc): ~3400 cm^{-1} (ν_{O-H}); 1268 cm^{-1} ($\nu_{P=O}$); 1182, 1164, 1102, and 1060 cm^{-1} ($\nu_{P-O-C(alk.)}$).

NMR (0.5M in D_2O, HOD as an internal standard): methyl, −1.87 ppm (singlet); methylene 0.44 ppm (doublet, J_{P-H}=8.29 Hz); 5H, 2.87 ppm (doublet); 3H, 2.93 ppm (doublet); 4H, 3.49 ppm (triplet, $J_{3-4}\simeq J_{4-5}$=7.80 Hz and J_{3-5}<1.0 Hz).

Found: C, 40.94; H, 5.04; N, 6.64; P, 14.6%. Calcd for $C_7H_{10}NO_4P$: C, 41.39; H, 4.96; N, 6.96; P, 15.3%.

3-Pyridyl Phosphate. A 2.00 g sample of 3-pyridinol dissolved in 25 m*l* of dry pyridine was added dropwise for 40 min into a mixture of 16.4 g of phosphoryl chloride and 25 m*l* of dry pyridine protected from moisture. The reaction flask was cooled with ice. The reaction mixture was then stirred for 3 hr at room temperature (10—15°C), followed by cooling with a freezing mixture (ice-sodium chloride). After the precipitated pyridine hydrochloride was removed immediately by filtration, pyridine and unreacted phosphoryl chloride were taken out by distillation *in vacuo* at 50°C from the filtrate. The residue was dissolved in 25 m*l* of acetonitrile, and then added in 80—90 m*l* of cold water in about 10 min with sufficient stirring. This aqueous mixture was neutralized with concd. ammonia, divided into two portions, each of which was concentrated to 10—15 m*l* *in vacuo* at 40°C and below. Each portion was then applied to the top of a column (2.8×48.5 cm) of Dowex 50W-X8 [H^+] resin (50—100 mesh, 430 meq.) and eluted with water at 2 m*l* per min until the front effluent (Fig. 2, I) came through; elution rate was doubled after then. The second part of eluted fractions (Fig. 2, II) was concentrated to about 10 m*l* *in vacuo* at 40°C and below; white needle-like crystals were precipitated by adding acetone; total yield 1.81 g (49%); mp 145.8—146.8°C (part. dec.); R_f 0.15—0.27 (Toyo filter paper No. 51A; developer, isopropanol : concd. ammonia : water=7 : 1 : 2 by volume); neutralization equivalence 87.4 (calcd 87.5); $\lambda_{max}^{H_2O}$ (2×10^{-4}M at pH 13) 268 mμ (ε 3340).

Found: C, 34.01; H, 3.62; N, 8.12; P, 17.5%. Calcd for $C_5H_6NO_4P$: C, 34.29; H, 3.46; N, 8.00; P, 17.7%.

***P*[1],*P*[2]-Di(3-pyridyl) Pyrophosphate.** The phosphorylation of 3-pyridinol (1.4 g) with phosphoryl chloride (11.4 g) was carried out in 80 m*l* of dry pyridine in a manner similar to that for 3-pyridyl phosphate. The unreacted phosphoryl chloride and pyridine were distilled off *in vacuo* from the reaction mixture without removing pyridine hydrochloride produced in the course of reaction. The mixture was stirred for 30 min at room temperature after addition of 40 m*l* of dry pyridine and 1 m*l* of water in succession, and was allowed to stand overnight. The solution was then concentrated to about 20 m*l* *in vacuo*, and charged to the top of a column (2.8×25 cm) of Dowex 50W-X8 [H^+] resin (50—100 mesh, 240 meq.). The elution was conducted with water at the flow rate of 2 m*l* per min, and with 1N hydrochloric acid subsequently as shown in Fig. 3.

*[2] A sample was decomposed by heating with concentrated nitric acid and 60% perchloric acid. The product was then analyzed for phosphorus by colorimetric method (R. J. L. Allen, *Biochem. J.*, **34**, 858 (1940)).

7) G. Kobayashi, S. Furukawa and Y. Kawada, *J. Pharm. Soc. Japan.*, **74**, 790 (1954).

The fractions of pH above 2 in the front effluent (Fig. 3, I) were collected, and concentrated *in vacuo* at 40°C and below; white precipitate (985 mg) was obtained upon addition of acetone. Repeated precipitation with water-acetone gave 100 mg of pure phosphate; mp 180—190°C (part. dec.); R_f 0.62; neutralization equivalence 165.3 (calcd 166.1)

Found: C, 35.74; H, 3.30; N, 8.84; P, 18.2%. Calcd for $C_{10}H_{10}N_2O_7P_2$: C, 36.16; H, 3.03; N, 8.43; P, 18.7%.

8-Quinolyl Phosphate. A 7.25 g sample of 8-quinolinol dissolved in 70 m*l* of dry pyridine was added dropwise in 2 hr into a mixture of 38.2 g of phosphoryl chloride and 70 m*l* of dry pyridine, while the reaction vessel was cooled with ice. Stirring was continued for another 5 hr. Pyridine hydrochloride formed was then recovered by filtration, and the filtrate was distilled *in vacuo* at 50°C to remove pyridine and the unreacted phosphoryl chloride. Into the residue, 100 m*l* of dry pyridine in one portion and subsequently 2.5 m*l* of water was added dropwise with shaking. Shaking was continued for another 30 min, and the reaction mixture was allowed to stand overnight at room temperature. The resulting yellowish brown mixture was concentrated *in vacuo* to 20—30 m*l*; filtered off to recover white precipitate (4.0 g). Repeated recrystallization from water-acetone (1 : 1) gave 1.95 g (17.5%) of needle-like crystals; mp 208—223°C (part. dec.); R_f 0.2—0.3 (developer, isopropanol : concd. ammonia : water=7 : 1 : 2), ~0.4 (developer, pyridine : concd. ammonia : water=6 : 3 : 1); neutralization equivalence 111.3 (calcd 112.6).

Found: C, 47.79; H, 3.66; N, 5.87; P, 13.0%. Calcd for $C_9H_8NO_4P$: C, 48.01; H, 3.58; N, 6.22; P, 13.8%.

The filtrate was applied to the top of column (3.0× 42.5 cm) of Dowex 50W-X8 [H^+] resin (50—100 mesh, 430 meq.) and eluted with water as described for 3-pyridyl phosphate; the eluting behavior was quite similar. Isolation of the pyrophosphate ester in its pure form from the front effluent was unsuccessful.

Acid Dissociation Constants. The acid dissociation constants of the phosphates prepared in this work were determined potentiometrically, with the use of a Hitachi-Horiba Model P pH meter with extension glass and calomel electrodes, at 25.0±0.1°C and the ionic strength of 0.10M in potassium nitrate.