BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 44, 2741—2744 (1971)

p-Nitrophenyl Phosphates as Phosphorylating Reagents of Alcohols

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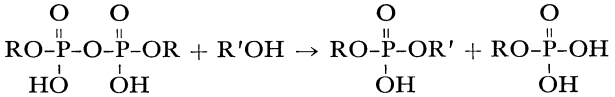
(Received December 21, 1970)

Phosphorylation of alcohols by means of P^1,P^2-di-*p*-nitrophenyl pyrophosphate and *p*-nitrophenyl phosphate was investigated. Alcohols were phosphorylated in almost quantitative yields by use of P^1,P^2-di-*p*-nitrophenyl pyrophosphate. It was found that *p*-nitrophenyl phosphate acts as an effective phosphorylating reagent for alcohols in dry pyridine to give the corresponding alkyl phosphate.

It is known that tetra-substituted[1] and tri-substituted[2] pyrophosphates can be advantageously used for the syntheses of esters of phosphoric acid and nucleotides. However, P^1,P^2-di-substituted pyrophosphates are not suitable for the phosphorylation of alcohols except when the reaction is carried out in the presence of an activator such as trichloroacetonitrile[3] or dicyclohexylcarbodiimide.[4]

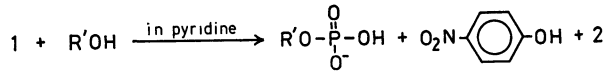
In the present experiment, we tested the phosphorylation of alcohol with various P^1,P^2-di-aryl substituted pyrophosphates with the expection that the usefulness of the method would considerably increase if a generally applicable reagent, P^1,P^2-di-substituted pyrophosphate, could be found. We found that P^1,P^2-di-*p*-nitrophenyl pyrophosphate (**1**) is effctive for the phosphorylation of alcohols without using any activating reagent. As an example, when **1** was treated with excess alcohol at 80°C for three hours, alkyl *p*-nitrophenyl phosphate (**2**) and *p*-nitrophenyl phosphate (**3**) were obtained in almost quantitative yields(Table 1).



(**1**) (**2**) (**3**)

R = *p*-nitrophenyl, R' = alkyl

In the course of the experiment, it was found that alkyl phosphate (**4**) and *p*-nitrophenol were unexpectedly obtained in high yields along with **2** when the reaction was carried out in dry pyridine.



(**4**)

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2) a) F. Cramer and K. G. Gärtner, *Chem. Ber.*, **91**, 704 (1958). b) F. Cramer and R. Wittmann, *ibid.*, **94**, 328 (1961). c) F. Cramer and D. Voges, *ibid.*, **92**, 752 (1959). d) A. M. Michelson, *Biochim. Biophys. Acta*, **91**, 1 (1964).

3) a) F. Cramer and H. J. Baldauf, *Angew. Chem.*, **72**, 627 (1960). b) F. Cramer, K. H. Scheit, and H. J. Baldauf, *Chem. Ber.*, **95**, 1657 (1962).

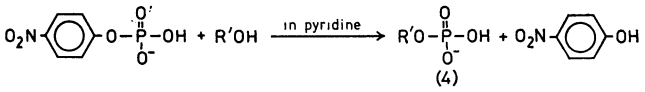
4) a) M. Smith, G. I. Drummond, and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 698 (1961). b) G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, *ibid.*, **80**, 6223 (1958). c) H. Schaller and H. G. Khorana, *ibid.*, **85**, 3828 (1963).

TABLE 2. PREPARATION OF ALKYL PHOSPHATES (**4**)

Alkyl	Yield (%)	Mp[a] (°C)	R_f value[b]	Formula	Calcd			Found		
					C	H	N	C	H	N
Ethyl	87	162—163	0.26	$C_8H_{14}NO_4P$	43.85	6.44	6.39	44.03	6.44	6.39
n-Propyl	90	140—142	0.28	$C_9H_{16}NO_4P$	46.34	6.92	6.01	45.82	6.94	5.82
β-Cyanoethyl	73	154.5—155	0.34	$C_9H_{13}N_2O_4P$	44.27	5.36	11.47	45.97	5.76	11.67
n-Butyl	92	141—142	0.40	$C_{10}H_{18}NO_4P$	48.58	7.34	5.66	47.82	7.39	5.51
n-Pentyl	81	136—137	0.52	$C_{11}H_{20}NO_4P$	50.56	7.72	5.36	51.98	7.97	5.67
i-Pentyl	89	140—140.5	0.55	$C_{11}H_{20}NO_4P$	50.56	7.72	5.36	50.95	7.87	5.07
n-Octyl	62	129—130	0.72	$C_{14}H_{26}NO_4P$	55.44	8.58	4.62	55.60	8.75	4.63
n-Hexadecyl	41	108—108.5[c]	0.05	$C_{16}H_{38}NO_4P$	56.64	11.21	4.13	57.84	11.32	3.80
Bornyl	53	178—180	0.64	$C_{16}H_{26}NO_4P$	58.72	8.02	4.28	58.46	8.04	4.08

a) Monoanilinium salt
b) Solvent system used: *i*-propyl alcohol, concentrated ammonium hydroxide, water (7:1:2 v/v).
c) Monoammonium salt

It seems that the formation of **4** and *p*-nitrophenol was caused by the subsequent reaction of alcohol and **3** which was initially formed. The reaction of **3** with alcohol in dry pyridine was tried separately in order to elucidate the mechanism of the formation of **4** and *p*-nitrophenol. When a mixture of **3** and excess ethanol in dry pyridine was heated at 110—120°C for two hours, ethyl phosphate and *p*-nitrophenol were obtained in high yields.

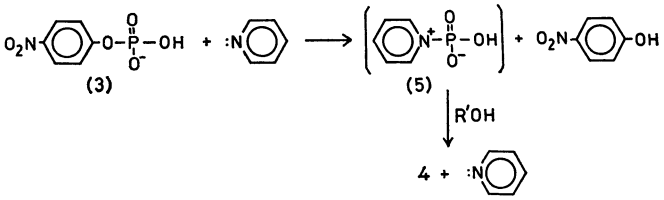


(4)

This indicates that *p*-nitrophenyl phosphate (**3**) could be successfully employed as an effective phosphorylating reagent for the synthesis of various alkyl phosphates (**4**). It was established that several alkyl phosphates (**4**) can be prepared in high yields by a simple procedure from **3** and alcohols.[5] The results are summarized in Table 2. In the above reactions, it was shown that the yields of **4** depend on the amount of alcohols. As an example, the yields of **4** remarkably decreased when one equivalent of alcohols was used.

Of various tertiary amines examined, it was shown that pyridine is superior to other amines[6] such as triethylamine, tri-*n*-butylamine and *N*-methylmorpholine. It was also found that *p*-nitrophenyl phosphate (**3**) is the most suitable reagent in comparison with other aryl phosphates such as phenyl-, *p*-tolyl-, and *o*-hydroxyphenyl phosphate.

The reaction seems to proceed through an intermediate, inner salt of *N*-phosphopyridinium hydroxide (**5**), formed from **3** and pyridine. The inner salt (**5**) in turn reacts with alcohol to give alkyl phosphate **4** as shown in the following scheme.



On the other hand, when *p*-nitrophenyl phosphate **3** was heated alone in pyridine, several *p*-nitrophenyl polyphosphates were obtained. These polyphosphates can be detected and determined by UV spectra and phosphorus analysis after being chromatographed on paper (Table 3).

TABLE 1. REACTION OF P^1,P^2-DI-*p*-NITROPHENYL PYROPHOSPHATE (**1**) WITH ALCOHOLS

Alcohol	Yield (%) of **2**	R_f value[a]	Yield (%) of **3**
Ethanol	60	0.83	68
n-Propanol	83	0.84	77
n-Butanol	96	0.86	94
s-Butyl alcohol	99	0.90	98
i-Pentanol	86	0.87	83
n-Octanol	96	0.89	70

a) Solvent system used: *i*-propyl alcohol, concentrated ammonium hydroxide, water (7:1:2 v/v).

5) The reason why 2 can not be used for this type of phosphorylation could not be explained.
6) In the cases of triethylamine and tri-*n*-butylamine, the reactions proceeded very sluggishly.

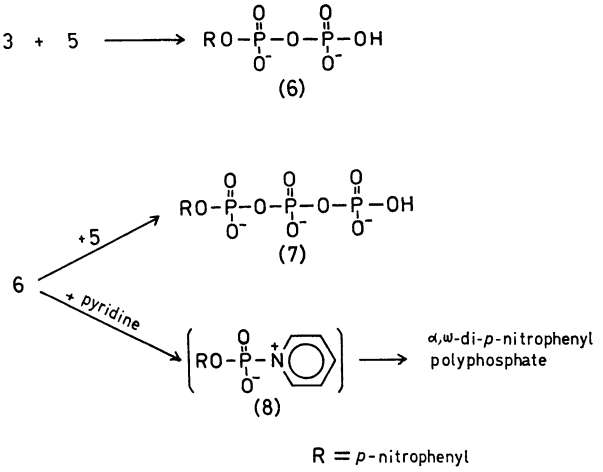
TABLE 3. REACTION OF *p*-NITROPHENYL PHOSPHATE (**3**) WITH PYRIDINE

(R = *p*-nitrophenyl)

Product	R_f value[a]	Yield[b] (%)	Phosphorus content[c]	$\lambda_{max}^{H_2O}$ (mμ)
Rp	0.36	15	1.00	309
Rp_2	0.24	7	2.01	292
Rp_3	0.12	0.8	3.21	289
Rp_4	0.08	0.7	4.97[d]	288
Rp_2R	0.73	4	1.00	288
Rp_3R	0.58	2	1.49	290
Rp_4R	0.47	0.6	1.90	288

a) Solvent system used: *i*-propyl alcohol, concentrated ammonium hydroxide, water, (7:1:2 v/v).
b) A remaining part was proved to be *p*-nitrophenol and inorganic phosphates.
c) Values are relative to *p*-nitrophenyl group.
d) The compound is contaminated with inorganic phosphate.

In this reaction, intermediate **5** would react with **3** to give *p*-nitrophenyl diphosphate (**6**). Compound **6** would further react with **5** to form *p*-nitrophenyl triphosphate (**7**), whereas inner salt of *p*-nitrophenylphosphopyridinium hydroxide (**8**) and inorganic phosphate would be produced by the immutability reaction of polyphosphates, reported by Moffatt.[7] Compound **8** thus successively reacts with **3**, **6**, or **7** to afford α,ω-di-*p*-nitrophenyl polyphosphates.



Experimental

General Procedure. Paper chromatography was performed by the descending technique using Toyo Roshi No.51 paper. Solvent system used was: isopropyl alcohol, concentrated ammonium hydroxide, water (7 : 1 : 2 v/v) (Solvent *A*). The phosphorus containing compounds were dected by means of a spray of Hanes-Isherwood reagent[8] on paper. The R_f values of all compounds described are given in Tables 1, 2, and 3. In all reactions, dried materials were employed. Pyridine was purified and dried by distillation over *p*-toluenesulfonyl chloride and stored over calcium hydride for several days. Evaporation was carried out using a rotatory evaporator under reduced pressure. *p*-Nitrophenyl phosphate was prepared by a modified procedure of Cramer[9] as described below. P^1,P^2-Di-*p*-nitrophenyl pyrophosphate was prepared according to a previous paper.[10]

p-*Nitrophenyl Phosphate (3).* *p*-Nitrophenyl phosphorodichloridate[11] (83.5 g) was added slowly in portions into 400 m*l* of water with vigorous stirring. The temperature was kept below 15°C during the reaction. Water was evaporated under reduced pressure at a temperature below 60°C. After addition of benzene (150 m*l*), the solution was concentrated under reduced pressure until some white crystals appeared and was allowed to stand in a refrigerator overnight. *p*-Nitrophenyl phosphate (40.0 g, 56%) was obtained as pale yellowish crystals. Recrystallization from benzene containing a trace of ether afforded white needles, mp 156—158°C. By recrystallization from the mother liquid, an additional product (14.3 g, 20%) was obtained.

Reaction of P¹,P²-*Di*-p-*nitrophenyl Pyrophosphate (1) with Alcohols.* A mixture of P^1,P^2-di-*p*-nitrophenyl pyrophosphate (**1**) (11.56 mg, 0.02 mmol) and alcohol (2.0 m*l*) was heated at 100°C for 3 hr. The mixture was then concentrated to dryness and the residue was dissolved in water (2.25 m*l*). Chromatography was performed on paper using Solvent *A* for development. Yields of the compounds, **2** and **3**, were determined spectrophotometrically using $\varepsilon_{291m\mu}=1\times10^4$ (pH 7) for alkyl *p*-nitrophenyl phosphate (**2**) and $\varepsilon_{307m\mu}=1\times10^4$ (pH 7) for *p*-nitrophenyl phosphate (**3**). The results are summarized in Table 1.

Reaction of P¹,P²-*Di*-p-*nitrophenyl Pyrophosphate (1) with Alcohols in pyridine. A Typical Procedure*: A solution of di-pyridinium salt of P^1, P^2-di-*p*-nitrophenyl pyrophosphate (**1**) (324 mg, 0.5 mmol) and *s*-butyl alcohol (3 m*l*) in dry pyridine (4 m*l*) was concentrated by heating at 100°C for 4 hr. Chromatography was performed on paper using Solvent *A*. Three products were detected: *s*-butyl *p*-nitrophenyl phosphate (R_f 0.85, 92%), *s*-butyl phosphate (R_f 0.43, 96%) and *p*-nitrophenol (R_f 0.77, 96%). The yields of the compounds were determined spectrophotometrically.

Isolation of s-*Butyl Phosphate.* The oily residue was dissolved in water (10 m*l*) and the solution was washed with three portions of ether (3×20 m*l*) for removal of *p*-nitrophenol. The aqueous layer was concentrated to dryness. The residue was dissolved in dry pyridine (5 m*l*) and pyridine was evaporated for complete removal of moisture. White precipitate was stored in desiccator over phosphorus pentoxide. The percipitate was poured into a solution of freshly distilled aniline (0.5 m*l*) in dry benzene (20 m*l*). The suspension was heated until it became clear. After cooling, the solution separated white crystals which were collected by filtration. Monoanilinium salt of *s*-butyl phosphate (102 mg) was obtained: mp 155.5—156°C. Found: C, 48.84; H, 7.91; N, 5.10%. Calcd for $C_{10}H_{18}O_4NP$: C, 48.55; H, 7.29; N, 5.66%. From the mother liquid, the salt (10 mg) was obtained. Total yield was 112 mg (92%).

Preparation of Alkyl Phosphates (3). To a solution of an anhydrous alcohol (4 m*l*) in dry pyridine (2 m*l*), *p*-nitrophenyl phosphate (438 mg, 2 mmol) was added. The mixture was heated at 120°C for 1.5 hr and then was concentrated under reduced pressure. The gummy residue was dissolved in water (50 m*l*) and washed with three portions of ether (3×10 m*l*). Ethyl, β-cyanoethyl, *n*-propyl, *n*-butyl and *i*-butyl phosphates were obtained according to method A and the other derivatives listed in Table 2 were isolated by method B.

Method A. The aqueous solution was concentrated to dryness under reduced pressure at a temperature below 15°C. The residue was dissolved in 10% of aqueous pyridine (10 m*l*) and 30% aqueous barium acetate solution was added with vigorous stirring. After 2 hr, the precipitate was collected by filtration and washed with ethyl alcohol. Amorphous barium salt of alkyl phosphate was isolated by centrifuging. The barium salt was further suspended in water and then Dowex 50W-X8 (pyridinium form) was added with stirring over a period of 2 hr. After filtration, the resin was washed with water. The filtrate and washings were combined and concentrated to dryness. The syrup was dissolved in dry pyridine and further concentrated to dryness. This was repeated three times for complete removal of moisture. After addition of freshly distilled aniline (0.4 m*l*), the precipitate was collected by filtration and recrystallization from benzene containing a small amount of ethyl alcohol gave a monoanilinium salt of alkyl phosphate (**4**). The yields and the physical properties are shown in Table 2.

7) a) W. E. Wehrli and J. G. Moffatt, *J. Amer. Chem. Soc.*, **87**, 3760 (1965). b) D. L. M. Verheyden, W. E. Wehrli, and J. G. Moffatt, *ibid.*, **87**, 2257 (1965).
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9) F. Cramer and M. Winter, *Chem. Ber.*, **92**, 2761 (1959).
10) K. J. Chong, S. S. Pong, and T. Hata, This Bulletin, **43**, 2571 (1970).
11) M. Y. Kraft and V. V. Katyshkina, *Dokl. Akad. Nauk. S.S.S.R.*, **86**, 726 (1952); *Chem. Abstr.*, **47**, 8032 (1953).

Method B. The aqueous solution was concentrated to dryness. Pyridine (10 m*l*) was added and removed by evaporation. This was repeated three times for complete removal of moisture. The gummy residue was dissolved in dry chloroform (50 m*l*) and allowed to stand at room temperature for 6 hr. The pyridinium salt of inorganic phosphate was removed by filtration. After evaporation of chloroform, this procedure was repeated three times for complete removal of inorganic phosphate. The resulting residue was dissolved in chloroform and freshly distilled aniline (0.4 m*l*) was added. The precipitate was collected by filtration and recrystallization from benzene. A mono-anilinium salt of alkyl phosphate (**4**) was obtained as shown in Table 2.

Reaction of p-*Nitrophenyl Phosphate (4) with Pyridine in the Absence of Alcohol.* *p*-Nitrophenyl phosphate (65.7 mg, 0.3 mmol) was dissolved in dry pyridine (0.1 m*l*). The solution was sealed and heated at 110°C for 1.5 hr. After cooling, water (3 m*l*) was added and the aqueous solution was washed with three portions of ether (3×10 m*l*). The aqueous solution was then concentrated to a small volume. Chromatography was performed on paper (Toyo Roshi No. 51) using Solvent *A* for development. The results are summarized in Table 3.

The authors heartily thank Professor Teruaki Mukaiyama for his encouragement and discussions throughout the investigation and also Mr. Masaru Koezuka for his help with elemental analysis.