1524 BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48 (5), 1524—1527 (1975) [Vol. 48, No. 5

Synthetic Studies on Phosphorylating Reagent. I. The Phosphorylation of Alcohols by Means of *N*-[2-(Dihydrogen phosphoroxy)-5-nitrobenzyl]pyridinium Hydroxide

Yoshihiko TAGUCHI, Yoshitaka MUSHIKA, and Naoto YONEDA

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., 16–89, Kashima-3-chome, Yodogawa-ku, Osaka 532

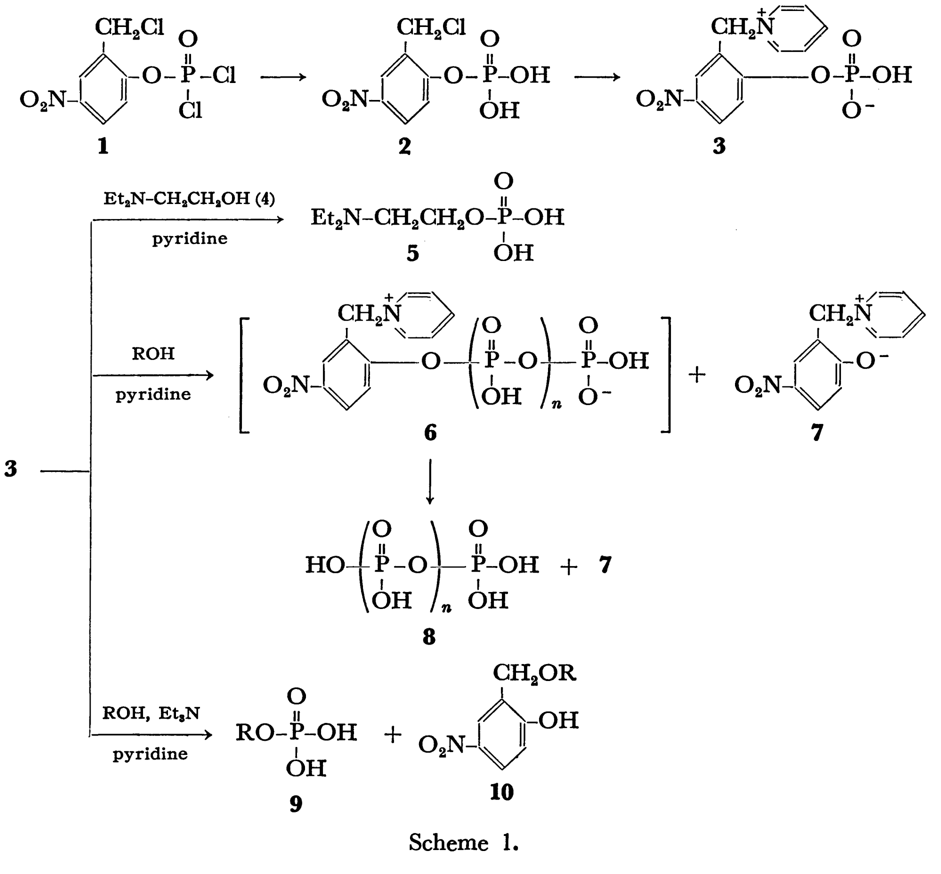
(Received December 28, 1974)

The phosphorylation of alcohols by means of *N*-[2-(dihydrogen phosphoroxy)-5-nitrobenzyl]pyridinium hydroxide (**3**), which was prepared from 2-(chloromethyl)-4-nitrophenyl phosphorodichloridate (**1**) through two steps, was demonstrated. Alcohol with a tertiary amino group in its alkyl moiety was readily phosphorylated with **3** to afford the aminoalkyl dihydrogen phosphate in good yield. On the other hand, the phosphorylation of the usual neutral alcohol proceeded only in the presence of a strong base such as triethylamine to give the corresponding alkyl dihydrogen phosphate (**9**). Thus, several alkyl dihydrogen phosphates **9** were prepared. In this phosphorylation, the formation of alkyl 2-hydroxy-5-nitrobenzyl ether (**10**) was also observed.

In our previous papers,[1–5] we reported on the preparation of various phosphoric esters by means of 2-(chloromethyl)-4-nitrophenyl phosphorodichloridate (**1**), a phosphorylating reagent with an activatable protecting group, and demonstrated that the reagent is very useful for the preparation of alkyl dihydrogen phosphate and dialkyl hydrogen phosphate. However, the reagent could not be utilized in the phosphorylation of amino alcohol because of the complicated reaction with the basic part of the reactant.

The present study was undertaken with an aim to explore a more versatile reagent which could be used in the phosphorylation of amino alcohol. An inner salt of *N*-[2-(dihydrogen phosphoroxy)-5-nitrobenzyl]-pyridinium hydroxide (**3**), which has no active chlorine atom, was chosen as a candidate for this purpose, since, in our earlier study,[4] the inner salt of *N*-[2-(alkyl hydrogen phosphoroxy)-5-nitrobenzyl]pyridinium hydroxide was shown to retain its reactivity toward alcohol.

The inner salt **3** was obtained quantitatively by the reaction of pyridine with 2-(chloromethyl)-4-nitrophenyl dihydrogen phosphate (**2**) which was prepared by the hydrolysis of **1**. The isolation of pure crystals of **3** was somewhat difficult because of the instability upon recrystallization from aqueous ethanol. The reaction of a stoichiometric amount of 2-(*N*,*N*-diethylamino)ethanol (**4**) with **3** in pyridine at 90—95 °C afforded a mixture of the resinous compounds, which were characterized on paper chromatography (PPC) as a phenolic polymer and an inorganic polyphosphoric acid. However, when a 10-equivalent amount of **4** was used, 2-(*N*,*N*-diethyl-



Scheme 1.

Table 1. Reaction of alcohol with **3** in the presence of triethylamine

R	Alkyl dihydrogen phosphate (**9**)[a]				Ethereal derivative (**10**)			
	Compd No.	Yield[b] (%)	Mp (°C)	R_f[c]	Compd No.	Yield[b] (%)	Mp (°C)	R_f[c]
Ethyl	**9a**	46	163—164	0.24	**10a**	36	83—85	0.85
n-Butyl	**9b**	40	138—140	0.36	**10b**	31	54—55	0.85
s-Butyl	**9c**	39	159—160	0.36	**10c**	44	54—55	0.85
n-Amyl	**9d**	31	137—139	0.49	**10d**	46	51—52	0.82
n-Dodecyl	**9e**	45	131—133	0.67	**10e**	30	49—50	0.85
Cyclohexyl	**9f**	55	169—170	0.40	**10f**	56	62—63	0.82
Benzyl	**9g**	53	168—169	0.35	**10g**	58	89—91	0.77

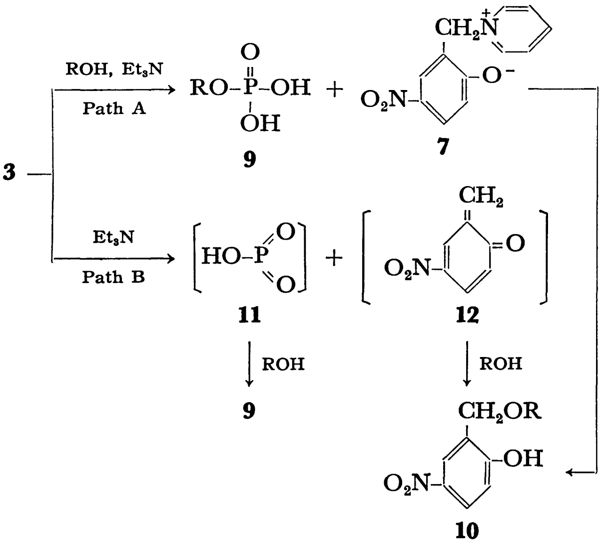
a) Alkyl dihydrogen phosphates were isolated as monoanilinium salt. b) Yields of **9** and **10** were based on 2-(chloromethyl)-4-nitrophenyl dihydrogen phosphate (**2**). c) Solvent A was used.

amino)ethyl dihydrogen phosphate (**5**) was obtained in 71% yield.

The elemental analysis and the downfield shift of the methylene protons adjacent to the phosphate group[6] in the nuclear magnetic resonance (NMR) spectrum supported the structure of the product. On the other hand, the phosphorylation of 2-aminoethanol resulted in the multiformation of intractable compounds. The reaction of an alcohol which does not contain a *tert*-amino residue, such as ethanol and benzyl alcohol, did not proceed at all under similar conditions, however, inorganic polyphosphoric acid and *N*-(2-hydroxy-5-nitrobenzyl)pyridinium hydroxide (**7**) were produced as the main products. This unexpected result could be explained by the self-condensation reaction of **3**, since the nucleophilicity of the second dissociable hydroxyl group in the phosphate group is larger than that of alcohol. An inner salt of *N*-[2-(polyhydrogen polyphosphoroxy)-5-nitrobenzyl]pyridinium hydroxide (**6**) formed would then be hydrolysed to give inorganic polyphosphoric acid (**8**) and **7** during work-up. Therefore, triethylamine was added to the reaction system in order to prevent the self-condensation.

First, the reaction with two equivalent amounts of ethanol was carried out in pyridine in the presence of three equivalents of triethylamine at 90—95 °C, however, ethyl dihydrogen phosphate (**9a**) was obtained in only 11% yield, followed by a considerable amount of the resinous phenolic polymer and inorganic polyphosphoric acid. Therefore, the reaction with ten equivalents of ethanol and three equivalents of triethylamine was attempted under the same conditions. The phosphorylation of ethanol occurred readily; ethyl dihydrogen phosphate (**9a**) was isolated in an improved yield as its monoanilinium salt, which was identified with an authentic sample on infrared (IR) spectrum. Similar results (Table 1) were obtained in the cases of other primary and secondary alcohols, such as *n*-butyl, *s*-butyl, *n*-dodecyl, cyclohexyl, and benzyl alcohol.

In these reactions, nearly equal amounts of alkyl 2-hydroxy-5-nitrobenzyl ethers (**10a-g**) to **9a-g** were isolated. The products were identified by a direct comparison with each authentic specimen, which was prepared by an alternate method.[2,7–8] It was considered that the formation of **10** is closely related to the mechanism of the phosphorylation. One conceivable mechanism (Path A) is the S_N2-type phosphorylation with alcohol, followed by the formation of alkyl 2-hydroxy-5-nitrobenzyl ether (**10**) *via* pyridinium compound **7** formed.



Scheme 2.

However, this seemed not to be the case in view of the following results: the phosphorylation with **3** required a large excess of alcohol in order to improve the yield of the phosphate **9a-g**, whereas the same type of phosphorylation[1–3] with *N*-[2-(alkyl hydrogen phosphoroxy)-5-nitrobenzyl]pyridinium hydroxide, which probably proceeded through S_N2 mechanism, has been shown to proceed satisfactorily with a stoichiometric amount of alcohol; the formations of large amounts of inorganic polyphosphoric acid and the phenolic polymer were observed when the reaction was run with a stoichiometric amount of alcohol. Another mechanism (Path B) involves the decomposition of the reagent **3** to the quinomethide (**12**) and methaphosphoric acid (**11**) under the present reaction conditions and subsequent condensations with alcohol giving alkyl ether **10** and alkyl dihydrogen phosphate **9**, respectively. This mechanism is supported not only by the above results, but also by the formation of **9** and **10** in nearly equal amounts in all cases. The quinomethide **12** has been proposed by Horton and Koshland[9] as an active species in the reaction of 2-(bromomethyl)-4-nitrophenol with tryptophan. In view of the equivalence of the requisite structure, it seems likely that the same intermediate is generated also in the present reaction.

From these considerations, Path B is a plausible mechanism of this phosphorylation.

In conclusion, it has been shown that a new compound, *N*-[2-(dihydrogen phosphoroxy)-5-nitrobenzyl]-pyridinium hydroxide **3** has phosphorylating ability toward the alcohol in the presence of triethylamine and toward the *tert*-amino alcohol in the absence of the base. Further modification of the reagent would be necessary to improve its efficiency as a phosphorylating reagent.

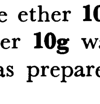
Experimental

Paper chromatography was carried out by ascending technique using Toyo Roshi No. 51 paper. Solvent systems used were: isopropyl alcohol–concd NH_4OH–H_2O (7: 1: 2 v/v) (Solvent A) and *n*-butanol–AcOH–H_2O (5: 1: 4 v/v, upper layer) (Solvent B). UV lamp (254 nm) and Hanes–Isherwood reagent[10] were used for the detection of spots on paper chromatogram. Melting points are uncorrected and were determined on a Yamato apparatus MP-21. NMR spectra were determined on Hitachi Perkin-Elmer R-20A instrument (Me_4Si). IR spectra were determined on a Shimadzu IR-27G spectrometer.

Materials. Alcohols, amines, and solvents were purified and dried by the ordinary procedures.

2-(Chloromethyl)-4-nitrophenyl phosphorodichloridate (**1**) was prepared as described in the previous paper.[2]

2-(Chloromethyl)-4-nitrophenyl Dihydrogen Phosphate (2) (Improved Procedure).[2] To a solution of 3.04 g, (0.01 mol) of 2-(chloromethyl)-4-nitrophenyl phosphorodichloridate (**1**) in 15 ml of acetone was added 15 ml of cold water in one portion with stirring under ice-cooling and the stirring was continued at a temperature below 15 °C for 30 min. After concentration of the reaction mixture to dryness under reduced pressure, the residue was crystallized with $CHCl_3$ to afford 2.28 g (85%) of the phosphate **2** as a white powder; mp 137—139 °C. The product was identified with an authentic sample by the criteria of PPC and NMR spectroscopy.

Inner Salt of N-*[2-(Dihydrogen phosphoroxy)-5-nitrobenzyl]pyridinium Hydroxide (3).* To 16 ml (0.20 mol) of anhydrous pyridine was added 5.35 g (0.02 mol) of 2-chloromethyl-4-nitrophenyl dihydrogen phosphate (**2**) with stirring at room temperature. After being allowed to stand at room temperature overnight, the reaction mixture was diluted with 30 ml of acetone, the precipitate was then collected by filtration. The precipitate was recrystallized from 50% ethanol to afford 3.94 g (64%) of analytically pure **3** as white needles; mp 204—205 °C (decomp.); R_f 0.28 (Solvent A) and 0.61 (Solvent B); UV (H_2O, nm): 261.5 (log ε 3.90), 267.5 (3.90) and 303 (3.96); IR (Nujol, cm^{-1}): 2700—2300 ($\rangle\overset{+}{N}\langle$), 1630 (), 1515, 1348 (NO_2), 1280 (P=O), and 1040 (P–O–C). Found: C, 46.39; H, 3.86; N, 8.97%. Calcd for $C_{12}H_{11}N_2O_6P$: C, 46.46; H, 3.57; N, 9.03%.

*2-(*N,N*-Diethylamino)ethyl Dihydrogen Phosphate (5).*[11] To a reaction mixture containing 0.01 mol of **3**, which was prepared from 2.67 g (0.01 mol) of **2** and 20 ml of anhydrous pyridine, was added 11.7 g (0.10 mol) of 2-(*N,N*-diethylamino)ethanol (**4**), and then the mixture was heated at 90—100 °C for 7 hr with stirring. After concentration of the reaction mixture to dryness under reduced pressure, water (60 ml) was added to the residue. A water-insoluble, gummy compound was removed by filtration, and the filtrate was passed through a column (1.5 cm × 60 cm) of Amberlite IR 120 resin (H^+ form). The column was washed with water until the washings became neutral to litmus. The column was eluted with 5% ammonia. The eluent was concentrated to dryness under reduced pressure, and an aqueous solution of the residue was applied to a column (1.5 cm × 50 cm) of Amberlite IRC 50 resin (H^+ form). The column was sufficiently washed with water. The combined original eluent and washings were evaporated to dryness under reduced pressure, and the colorless viscous residue was crystallized with acetone to afford 1.40 g (71%) of 2-(*N,N*-diethylamino)ethyl dihydrogen phosphate (**5**) as white powder; mp 155—159 °C. Recrystallization from ethanol afforded analytically pure **5** as hygroscopic white powder; mp 159—160 °C; R_f 0.23 (Sovlent A); IR (Nujol, cm^{-1}): 2600—2330 ($=\overset{+}{N}H-$), 1270 (P=O), 1180 and 1085 (P–O–C alkyl and organic phosphoric salt); NMR (D_2O, ppm): 1.54 (t, 6H, $CH_3 \times 2$), 3.36—3.73 [m, 6H, $(CH_3\text{-}C\underline{H}_2)_2N\text{-}C\underline{H}_2\text{-}$], 4.42 (m, 2H, $-CH_2O-$). Found: C, 36.20; H, 8.32; N, 7.05%. Calcd for $C_6H_{16}NO_4P$: C, 36.55; H, 8.18; N, 7.10%.

Reaction of Alcohol with 3. Typical Procedure; Reaction with Benzyl Alcohol: To a reaction mixture containing 0.01 mol of **3**, which was prepared from 2.67 g (0.01 mol) of **2** and 20 ml of anhydrous pyridine, was added 10.81 g (0.10 mol) of benzyl alcohol and 2.8 ml (0.02 mol) of triethylamine, then the reaction mixture was heated at 90—100 °C for 10 hr. After concentration of the reaction mixture to dryness under reduced pressure, an aqueous solution of the residue (H_2O, 50 ml) was extracted with three 20 ml portions of ether. The aqueous layer was passed through a column (1.0cm × 50cm) of Amberlite IR 120 resin (pyridinium form), and the column was washed with water. The combined original eluent and washings were neutralized with a small amount of pyridine and were evaporated to dryness under reduced pressure. To the acetone solution of the residue was added 1.6 ml of aniline, and the precipitate was collected by filtration. The precipitate was washed with a small amount of water to remove the salt of inorganic phosphoric acid, and then the insoluble precipitate was recrystallized from 95% ethanol to afford 1.49 g (53%) of monoanilinium salt of **9 g** as white needles; mp 168—169 °C. The product was identified with an authentic specimen by a comparison of its R_f-value of PPC and the IR spectrum and by the mixed melting point test.

The combined ethereal extracts were again extracted with three 20 ml portions of 2M-NaOH. After acidification of the alkaline layer with 10% HCl, the aqueous layer was extracted with three 20 ml portions of ether. The combined ethereal extracts was evaporated to dryness, and the residue was extracted with 100 ml of a boiling mixture of benzene and *n*-hexane (1: 3 v/v). After removal of an insoluble gummy substance by decantation, the solution was decolorized with charcoal and concentrated to dryness. The residue was crystallized from boiling *n*-hexane which contained a small amount of benzene to afford 1.58 g (58%) of the ether **10g** as pale yellow prisms; mp 89—91 °C. The ether **10g** was identified with an authentic specimen which was prepared by the established procedure.[8]

In a similar manner, alcohols, such as ethyl, *n*-butyl, *s*-butyl, *n*-amyl, *n*-dodecyl, and cyclohexyl alcohol, were treated with **3** in the presence of triethylamine to afford the corresponding alkyl dihydrogen phosphates **9a**—**f** and alkyl 2-hydroxy-5-nitrobenzyl ethers **10a**—**f**.

We wish to express our thanks to Professors Teruaki Mukaiyama and Tsujiaki Hata, and Drs. Takashi Takayanagi, Ichiro Chibata, Muneji Miyoshi and Ichizo Inoue for their valuable discussions and suggestions.

References

1) Y. Mushika and N. Yoneda, *Chem. Pharm. Bull.* (Tokyo), **19**, 696 (1971).

2) T. Hata, Y. Mushika, and T. Mukaiyama, *J. Amer. Chem. Soc.*, **91**, 4532 (1969).

3) T. Hata, Y. Mushika, and T. Mukaiyama, *Tetrahedron Lett.*, **1970**, 3505.

4) Y. Mushika, T. Hata, and T. Mukaiyama, This Bulletin, **44**, 232 (1971).

5) Y. Mushika and N. Yoneda, *Chem. Pharm. Bull.* (Tokyo), **19**, 687 (1971).

6) I. Feldman and R. P. Agarwal, *J. Amer. Chem. Soc.*, **90**, 7329 (1968).

7) T. Obata and T. Mukaiyama, *J. Org. Chem.*, **32**, 1063 (1967).

8) V. I. Starrovskaya, *Zh. Obshch. Khim.*, **24**, 2068 (1954). We obtained **10** by the reaction of 2-(chloromethyl)-4-nitrophenol with alcohols in the presence of triethylamine, and its details will be reported elsewhere.

9) H. T. Horton and D. E. Koshland, Jr., *J. Amer. Chem. Soc.*, **87**, 1126 (1966).

10) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

11) E. Cherbuliez and J. Robinowitz, *Helv. Chim. Acta*, **41**, 1168 (1958). They have described that the phosphate **5** was a white crystal having a melting point of 118—123 °C and its crystallization was difficult. However, by improving this purification, authers obtained a hygroscopic white crystal, mp 159—160 °C, as an authentic sample.