p-Nitrophenyl Phosphate as a Phosphorylating Reagent in Nucleotide Synthesis

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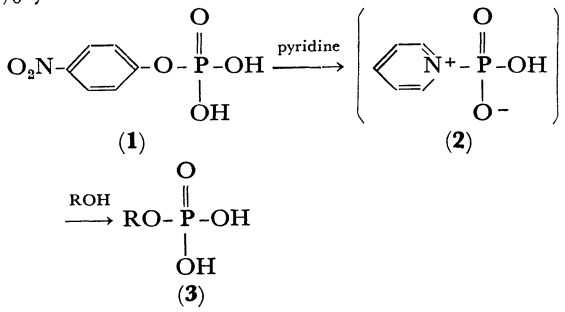
It is known that tri-substituted esters of phosphoric acid such as triethyl phosphate are transesterified into the corresponding trialkyl phosphate by treatment with alcohol or sodium alkoxide.[1] In the case of mono-substituted phosphates, however, the interchange reaction proceeds very sluggishly and therefore can not be applied to the syntheses of desired monoalkyl phosphates. It was found that pyridine was a remarkably effective solvent for the ester-interchange reaction of *p*-nitrophenyl phosphate (**1**) with alcohols.[2] The reaction is of particular interest since it is an example of ester-interchange reaction, probably proceeding through a reactive *N*-phosphopyridinium intermediate (**2**) towards alcohols.

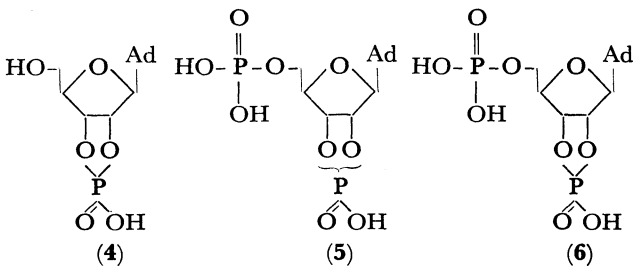
Phosphorylation of nucleosides by the use of **1** was investigated. When 2′,3′-*O*-isopropylidene adenosine (0.3 mmole) was allowed to react with **1** (0.6 mmole) in dry pyridine (0.3 m*l*) at 110°C for two hours, 2′,3′-*O*-isopropylidene adenosine 5′-phosphate was isolated by using DEAE cellulose column chromatography. After removal of the protecting group by treating 30% formic acid, adenosine 5′-phosphate was obtained in 85% yield.



Similarly, other nucleotides were obtained in high yields as shown in Table 1.

It is noted that the yields of nucleotides depend on the concentrations and the molar ratios of **1** to nucleosides. When a large excess of pyridine was employed, nucleoside polyphosphates were formed. 2′,3-*O*-Isopropylidene adenosine (0.05 mmol) was treated with **1** (0.25 mmol) in 1.0 m*l* of dry pyridine at 110°C for four hours. After removal of the protecting group, adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP) were obtained in 32% and 33% yields, respectively, along with adenosine 5′-phosphate (35%). These polyphosphates were isolated by means of DEAE cellulose column chromatography.

Phosphorylation of unprotected free nucleoside by the present method was attempted. When adenosine (0.1 mmol) was treated with **1** (0.7 mmol) in dry pyridine (3.0 m*l*) in the presence of tri-*n*-butylamine, adenosine 2′,3′-cyclic phosphate (**4**) and adenosine 2′(3′),5′-diphosphate (**5**) were obtained in 43% and 39% yields, respectively. The cyclic phosphate (**4**) was converted with 0.1 N hydrochloric acid into adenosine 2′(3′)-phosphate in quantitative yield. When **4** (0.1 mmol) was further treated with **1** (0.7 mmol) in dry pyridine (3.0 m*l*), adenosine 2′,3′-cyclic phosphate 5′-phosphate (**6**) was obtained in 57% yield. Data on the chromatographic and spectral properties of these compounds (**4**, **5** and **6**) agree with those of authentic samples.[3,4]



Ad = adenine residue

TABLE 1. PREPARATION OF NUCLEOTIDES

Nucleotide[a]	Yield (%)	R_f value[c]	UV Spectra (pH 2) $\lambda_{max}^{H_2O}$ (mμ)	$\lambda_{min}^{H_2O}$ (mμ)
Adenosine 5′-phosphate	85	0.14	257	231
Guanosine 5′-phosphate	80	0.09	256	228
Uridine 5′-phosphate	82	0.16	262	231
Cytidine 5′-phosphate	70	0.16	279	242
4-*S*-Thiouridine 5′-phosphate	52	0.17	331	285
Thymidine 3′-phosphate[b]	43	0.15	267	235

a) 2′,3′-*O*-Isopropylidene nucleoside was used as a starting material except in the case of thymidine derivative.
b) 5′-*O*-Trityl thymidine was used.
c) Paper chromatography was performed by descending technique using Toyo Roshi No. 51 paper. Solvent system used: isopropyl alcohol, concentrated ammonium hydroxide, water (7 : 1 : 2 v/v).

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3) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 6204 (1958).
4) J. G. Moffatt and H. G. Khorana, *ibid.*, 663 (1961).