[Chem. Pharm. Bull. 22(8)1743—1747(1974)] UDC 547.831.04 : 547.559.04 : 547.56.04

Studies of Phosphorylation. III.[1] Effect of Metallic Compound on the Phosphorylation of Alcohols, Phosphates, and Nucleosides by 8-Quinolyl Phosphates

Hiroshi Takaku and Yoshifusa Shimada

Laboratory of Organic Chemistry, Chiba Institute of Technology[2]

(Received October 24, 1973)

Influence of metallic compounds in phosphorylation of alcohols, phosphates, and nucleosides by means of 8-quinolyl phosphates was investigated. The mixed diesters of phosphoric acid (III) and pyrophosphates (V) were obtained in good yields when alcohols or phosphates were allowed to react with phenyl 8-quinolyl hydrogen phosphate (I) in the presence of a metallic compound. The effect of various metal components was examined and the copper[II] ion gave the best result. The reaction of tris(8-quinolyl) phosphate (VI) with nucleosides in the copper acetylacetonate resulted in the formation of corresponding nucleotides (VIII) in a high yield.

Our previous papers[1,3] reported that mixed diesters of phosphoric acid and nucleotides were synthesized by the reaction of alcohols and nucleosides with 8-quinolyl phosphates, an active phosphorylating reagent. It may be reasonable to assume that phenyl 8-quinolyl hydrogen phosphate (I) is protonated to give an active species (II), from which either of mixed diesters of phosphoric acid (III) can be obtained by further reaction with an alcohol (Chart 1).

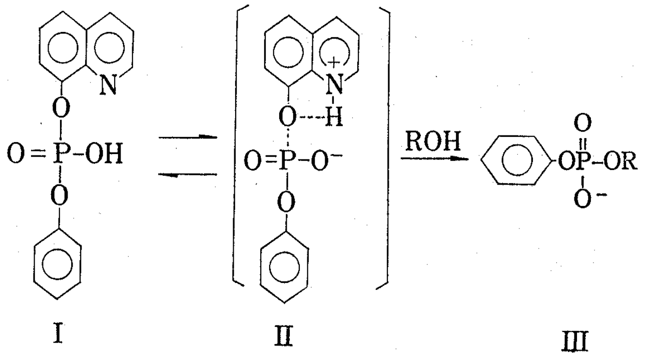

Chart 1

This reaction is not suitable for the preparation of mixed diesters of phosphoric acid (III) because of the low reactivity of I toward alcohols. Therefore, we expected that mixed diesters of phosphoric acid (III) would be synthesized in a fairly good yield when phenyl 8-quinolyl hydrogen phosphate (cyclohexylammonium salt) (I) is treated with alcohol in the presence of a metallic compound.

When I was treated with 1 equiv. of copper acetylacetonate and 3 equiv. of butanol in dry pyridine at 80° for 5 hr, butyl phenyl hydrogen phosphate (IIIc) was obtained in 73% yield along with a small amount of symmetrical P^1,P^2-bis (phenyl) pyrophosphate. In this reaction mixture, the three compounds, whose *Rf* were 0.79, 0.72, and 0.53, were detected by paper chromatography developed with solvent A. From these results, it was assumed that the three compounds were I (*Rf* 0.79), IIIc (*Rf* 0.72), and P^1,P^2-bis (pnenyl) pyrophosphate (*Rf* 0.53). Paper chromatogram of the reaction mixture showed the presence of a trace of P^1,P^2-bis (phenyl) pyrophosphate which could not be isolated.

By the use of this method, various mixed diesters of phosphoric acid (III) were obtained in high yields. These results are summarized in Table I.

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[1] Part II: H. Takaku, Y. Shimada, and K. Arai, *Bull. Chem. Soc. Japan*, **47**, 779 (1974).
[2] Location: *Narashino-shi, Chiba.*
[3] H. Takaku and Y. Shimada, *Chem. Pharm. Bull.* (Tokyo), **21**, 445 (1973).

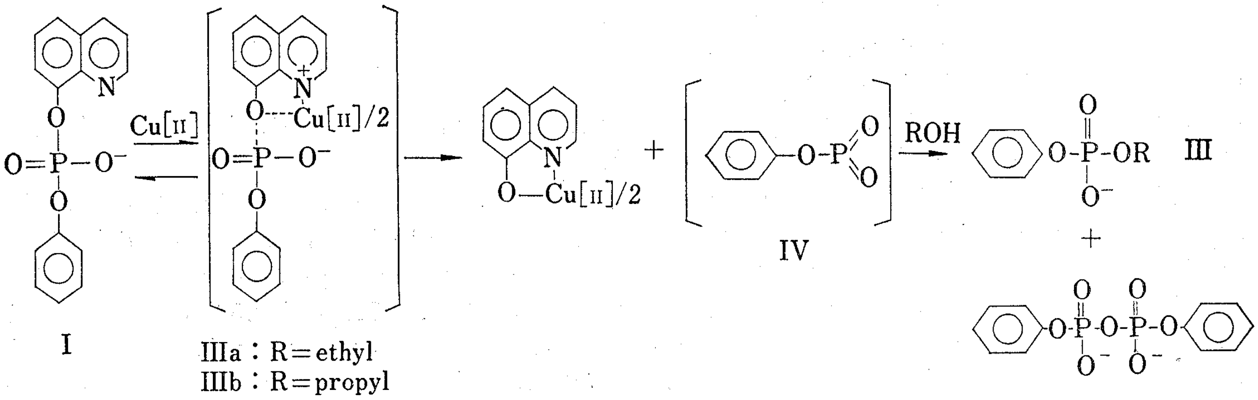
Furthermore, when I was treated with 10 equiv. of butanol, the result was almost the same as when 3 equiv. of butanol was used as shown in the above experiments. Thus, it can be said that the reagent (I) is effective for the phosphorylation of alcohols when treated with a metallic compound (see Table I).

TABLE I. Alkyl Phenyl Hydrogen Phosphates (III)[a)]

Compd. No.	R	Yield (%)	mp (°C)	*Rf*[b)]	Formula	Analysis (%) Calcd. (Found) C	H	N
IIIa	ethyl	73	109—112	0.67	$C_{14}H_{24}O_4NP$	55.86 (55.90)	8.04 (7.81)	4.65 (4.61)
IIIb	propyl	77	113—115	0.72	$C_{15}H_{26}O_4NP$	57.19 (57.02)	8.25 (8.38)	4.45 (4.51)
IIIc	butyl	73	108—110	0.74	$C_{16}H_{28}O_4NP$	58.41 (58.46)	8.51 (8.51)	4.25 (4.16)
IIIc	butyl[c)]	80	110—112	0.75	$C_{16}H_{28}O_4NP$	58.41 (58.65)	8.51 (8.87)	4.25 (4.09)
IIId	*sec*-butyl	70	179—180	0.72	$C_{16}H_{28}O_4NP$	58.41 (58.35)	8.51 (8.48)	4.25 (4.23)
IIIe	benzyl	68	142—143	0.71	$C_{19}H_{26}O_4NP$	62.86 (62.90)	7.22 (7.03)	3.86 (3.81)
IIIf	*p*-nitrobenzyl	69	187—190	0.70	$C_{19}H_{25}O_6N_2P$	55.93 (55.87)	6.18 (6.23)	6.86 (6.97)

a) monocyclohexylammonium salts
b) Paper chromatography was carried out by the descending technique using Toyo Roshi No. 51 paper. The solvent system used was isopropyl alcohol–concentrated ammonia–water (8:1:1, v/v).
c) Ten equiv. of butanol was used.

Of various metallic compounds examined, it was found that the yield of III increased when copper acetylacetonate was used as the metallic catalyst.[4)] The effect of metallic compounds in these reactions can be explained from the following mechanism (Chart 2). In the first stage of the reaction, the phosphate (I) reacts with cupper [II] ion to give, under the concurrent formation of 8-hydroxyquinoline–copper [II] complex, a reactive intermediate (IV) (metaphosphate). In the second stage, an intermediate (IV) is converted to diester of phosphoric acid (III) by its reaction with an alcohol.



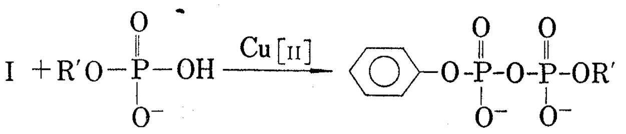
IIIa : R=ethyl
IIIb : R=propyl
IIIc : R=butyl
IIId : R=*sec*-butyl
IIIe : R=benzyl
IIIf : R=*p*-nitrobenzyl

Chart 2

[4)] In the case of nickel acetylacetonate, zinc acetylacetonate, and cobalt acetylacetonate, the yield of III decreased slightly.

Next, the synthesis of unsymmetrical or symmetrical pyrophosphates (V) was attempted by treatment of I with various phosphates in the presence of a metallic compound (Chart 3). When I was treated with 1.5 equiv. of benzyl dihydrogen phosphate in the presence of copper acetylaceonate in dry pyridine, the reaction progressed rapidly and the corresponding unsymmetrical P¹-benzyl, P²-phenyl pyrophosphate (Vc) was obtained in 75% yield.

In a similar manner, various unsymmetrical or symmetrical pyrophosphates (V) were obtained in a good yield. These results are summarized in Table II. In the above reaction, it was shown that the yield of V markedly decreased when triethylamine, tributylamine, or triethanolamine was used as the base.[5]



V

Va : R'=phenyl
Vb : R'=p-chlorophenyl
Vc : R'=benzyl
Vd : R = β-naphthyl

Chart 3

It was found that phenyl 8-quinolyl hydrogen phosphate (I) is the most effective reagent for the synthesis of pyrophosphates.

TABLE II. Preparation of Unsymm. and Symmetrical Pyrophosphates (V)[a]

Compd. No.	R'	Yield (%)	mp (°C)	Rf[b]	Formula	Analysis (%) Calcd. (Found) C	H	N
Va	phenyl	74	245—247	0.52	$C_{24}H_{38}O_7N_2P_2$	54.55 (54.42)	7.19 (7.16)	5.31 (5.48)
Vb	p-chlorophenyl	81	251—252	0.58	$C_{24}H_{37}ClO_7N_2P_2$	51.25 (52.03)	6.58 (6.47)	4.98 (4.63)
Vc	benzyl	75	220	0.58	$C_{25}H_{40}O_7N_2P_2$	55.15 (55.21)	7.33 (7.27)	5.15 (5.07)
Vd	β-naphthyl	85	218—220	0.59	$C_{28}H_{40}O_7N_2P_2$	58.13 (58.06)	6.92 (6.85)	4.84 (4.92)

a) biscylcohexylammonium salt
b) Paper chromatography was carried out by the descending technique using Toyo Roshi No. 51 paper. Solvent system used was isopropyl alcohol-concentrated ammonia-water (8: 1: 1, v/v).

Finally, the synthesis of nucleoside 5'-phosphates (VIII) was attempted by the use of tris (8-quinolyl) phosphate (VI) as the phosphorylating reagent in place of I (Chart 4). For example, when a solution of 1 equiv. of 2',3'-O-isopropylideneinosine, 1.5 equiv. of copper acetylacetonate, and 2 equiv. of VI was heated at 80° for 5 hr, 2',3'-O-isopropylideneinosine 5'-bis (8-quinolyl) phosphate (VIId) was formed. This phosphate (VIId) without isolation, was further treated with aqueous solution of cupric chloride at 100° for 1 hr. Purification by chromatography over DEAE-cellulose column, using a linear salt gradient, yielded pure 2',3'-O-isopropylideneinosine 5'-phosphate. After removal of isopropylidene group by its treatment with 70% acetic acid, inosine 5'-phosphate (VIIId) was obtained in 74% yield.

In the above reactions, addition of 0.75 equiv. of a metallic compound to 1 equiv. of VI gave inosine 5'-phosphate (VIIId) in 74% yield, but the same addition to 0.75 equiv. of VI gave VIIId and 5',5'-diinosine phosphate[6] in 57% and 22% yield, respectively. The Rf values of VIIId and 5',5'-diinosine phosphate were 0.65 and 0.38 (solvent B), and 0.25 and

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5) W. Kampe, *Chem. Ber.*, **98**, 1038 (1965).
6) N. Muramatsu and T. Takenishi, *J. Org. Chem.*, **30**, 3211 (1965).

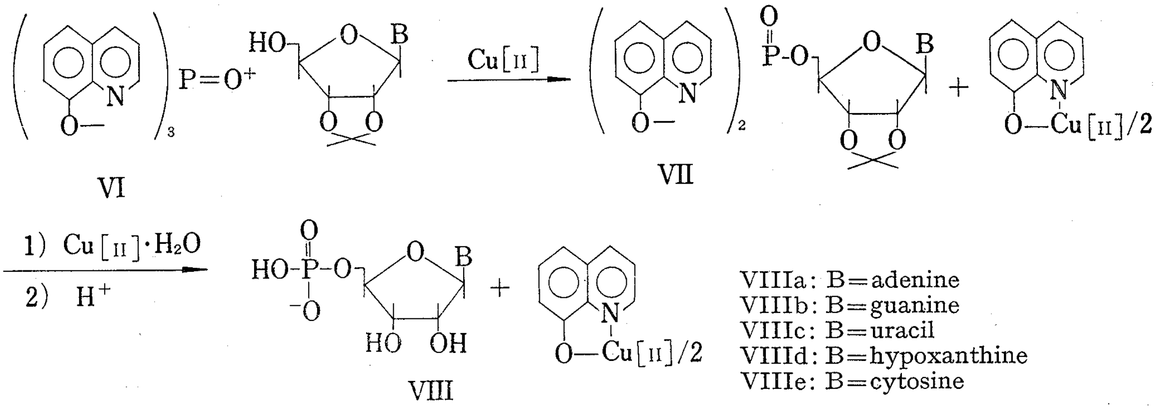

Chart 4

Table III. Preparation of Nucleotides (VIII)

Compd. No.	Nucleotide	Yield (%)	$Rf^{a)}$	UV Spectra (pH 2) $\lambda_{max}^{H_2O}$ (nm)	$\lambda_{min}^{H_2O}$ (nm)
VIIIa	adenosine 5′-phosphate	75	0.31	257	230
VIIIb	guanosine 5′-phosphate	70	0.49	256	228
VIIIc	uridine 5′-phosphate	71	0.68	262	230
VIIId	inosine 5′-phosphate	74	0.65	250	223
VIIIf	cytidine 5′-phosphate	63	0.70	279	242

a) Paper chromatography was performed by the descending technique using Toyo Roshi No. 51 paper. Solvent system used was isopropyl alcohol–saturated ammonia sulfate–water (2: 79: 19, v/v)

0.28 (solvent C), respectively, and ultraviolet (UV) spectrum of 5′,5′-diinosine phosphate in H_2O (pH 2) showed a maximum absorption band at 251 nm. The compounds (VIII) were detected by paper chromatography and their yield was determined by spectrophotometry.

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

From these facts, we may conclude that this metal-catalyzed reaction offers a unique mechanism and an effective method for the synthesis of mixed diesters of phosphoric acid, pyrophosphates, and nucleotides.

Experimental

General Methods and Materials——Reagent grade pyridine was distilled after treating with *p*-toluenesulfonyl chloride and dried over calcium hydride for several weeks. Alcohol was purified and dried by ordinary procedures. Paper chromatography was performed using the descending technique on Toyo Roshi No. 51 paper. The solvent systems used for paper chromatography were: isopropyl alcohol–concentrated ammonia–water (8: 1: 1) (solvent A); isopropyl alcohol–saturated aqueous ammonium sulfate–water (2: 79: 19) (solvent B); and *n*-propyl alcohol–concentrated ammonia–water (20: 12: 3) (solvent C). Ultraviolet lamp (254 nm) and Hanes Isherwood reagent[7] were used for the detection of spots.

Butyl Phenyl Hydrogen Phosphate (IIIc)——A solution of 390 mg (0.98 mmole) of phenyl 8-quinolyl hydrogen phosphate (cyclohexylammonium salt) (I) and 218 mg (2.94 mmole) of butanol in dry pyridine (5 ml) in the presence of 260 mg (0.98 mmole) of copper acetylacetonate was heated at 80° for 5 hr. After complete removal of the solvent under high vacuum, the residue was dissolved in water and the insoluble material (oxine-copper complex) was removed by filtration. The filtrate was evaporated to dryness to crystallize out. The crystals formed were collected and washed with cold acetonitril and ether and recrystallized from a mixture of acetone and chloroform. Monocyclohexylammonium salt of butyl phenyl hydrogen phosphate (IIIc) (249 mg, 73%) was obtained as white crystals; mp 108—110°. *Rf* 0.74 (solvent A). *Anal.* Calcd. for $C_{16}H_{28}O_4P$: C, 58.41; H, 8.51; N, 4.25. Found: C, 58.46; H, 8.51; N, 4.16.

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[7] C.S. Hanes and F.A. Isherwood, *Nature*, **164**, 1107 (1947).

Paper chromatogram of reaction mixture showed the existence of a trace of P¹,P²-bis(phenyl) pyrophoshate which could not be isolated.

In a similar manner, the diesters (III), such as IIIa, IIIb, IIId, IIIe, and IIIf were obtained in high yields as shown in Table I.

Preparation of Symmetrical and Unsymmetrical Pyrophosphate (V)——A Typical Procedure: A mixture of 390 mg (0.98 mmole) of phenyl 8-quinolyl hydrogen phosphate (cyclohexylammonium salt) (I) and 278 mg (1.5 mmole) of benzyl dihydrogen phosphate in dry pyridine (5 ml) was heated in the presence of 260 mg (0.98 mmole) of copper acetylacetonate at 80° for 1 hr. The solution was evaporated *in vacuo*, and the residue was dissolved in water (10 ml). After removal of the insoluble material (oxine-copper complex), the solution was concentrated to *ca.* 1 ml *in vacuo*, and then 270 mg of cyclohexylamine was added to the solution. The solution was kept standing overnight in a refrigerator. The precipitate was collected by filtration and washed with acetone and ether. Recrystallization of the crude product from a mixture of ethanol and water (1: 1 v/v) gave biscyclohexylammonium salt of P¹-benzyl,P²-phenyl pyrophosphate (Vc) (406 mg, 75%) as white crystals, mp 220°. *Rf* 0.58 (solvent A). *Anal.* Calcd. for $C_{25}H_{40}O_7N_2P$: C, 55.15; H, 7.33; N, 5.15. Found: C, 55.21; H, 7.27; N, 5.07.

P¹,P²-Bis(phenyl) (Va), P¹-(*p*)-chlorophenyl,P²-phenyl (Vb), and P¹-β-naphtyl,P²-phenyl (Vd) pyrophosphates were prepared in an analogous way. They are summarized in Table II.

Inosine 5′-Phosphate (VIIId)——A mixture of 286 mg (0.60 mmole) of tris(8-quinolyl) phosphate (VI) and 92.4 mg (0.30 mmole) of 2′,3′-O-isopropylidenosine in dry pyridine (3 ml) was heated in the presence of 117 mg (0.45 mmole) of copper acetylacetonate at 80° for 5 hr. The reaction mixture was treated with 0.48 ml of 43% aqueous cupric chloride at 100° for 1 hr, and concentrated to dryness. The residue was dissolved in water (5 ml) and the insoluble material (oxine-copper complex) was removed by filtration. The aqueous layer, containing the nucleotide, was chromatographed on a DEAE-cellulose (carbonate) column using a linear gradient of triethylammonium bicarbonate. The eluate was concentrated and treated with 70% actic acid at 70° for 1.5 hr. After separation of the product using chromatographic paper (Toyo Roshi No. 51, solvent B), spectrophotometric analysis of eluate showed the yield of inosine 5′-phosphate (VIIId) to be 74% [λ_{max} 250 nm (ε 12160)] at pH 2.0.

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

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