

A Study on Phosphorylation by Means of 8-Quinolyl Phosphates

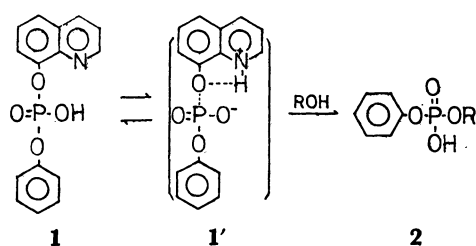
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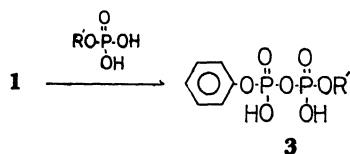
Synopsis. Phosphorylation of phosphates and nucleosides forming unsymmetrical pyrophosphates and nucleotides by the use of 8-quinolyl phosphates has been investigated. According to this method, various pyrophosphates and nucleotides were obtained in high yields.

Recently, we reported¹⁾ that mixed diesters of phosphoric acid (2) were prepared in a high yield by treating an active ester, phenyl 8-quinolyl phosphate (1), with alcohols as shown in the following scheme.



This paper deals with the reaction of 8-quinolyl phosphates²⁾ such as 1 or tris-(8-quinolyl) phosphate (5), with phosphates and nucleosides which give unsymmetrical pyrophosphates and nucleotides that are expected from the above reaction.

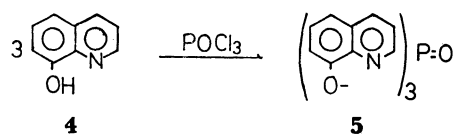
First, an attempt was made to synthesize unsymmetrical pyrophosphate (3) by the reaction of 1 with phosphates. When a mixture of one equiv. of 1 and 1.5 equiv. of *p*-chlorophenyl phosphate in dry pyridine was allowed to stand at 50 °C for 5 hr, the corresponding *P*¹-*p*-chlorophenyl *P*²-phenyl pyrophosphate (3a) was obtained in 78% yield. However, when a small quantity of triethylamine was added to the pyridine solvent, the yield of 3a decreased markedly.³⁾



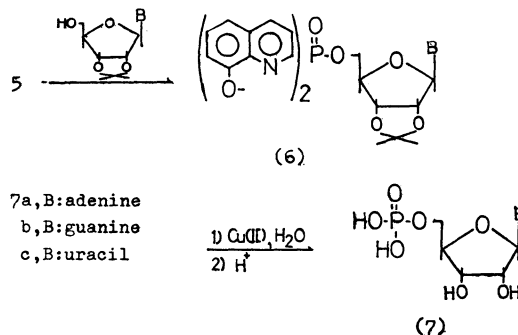
In a similar manner, various unsymmetrical pyrophosphates (3) were obtained in a high yield as shown in Table 1. When adenosine 5'-phosphate was used

in the above experiments, *P*¹-adenosine-5' *P*²-phenyl pyrophosphate (3d) was obtained in 54% yield. Thus it can be said that phenyl 8-quinolyl phosphate (1) is effective for the synthesis of pyrophosphates.

Next, the synthesis of nucleoside 5'-phosphate was tried by the use of a phosphorylating reagent, tris-(8-quinolyl) phosphate (5). The reagent (5) was prepared in 75% yield by treating six equiv. of 8-quinolinol (4) with one equiv. of phosphoryl chloride.



When a mixture of one equiv. of 2',3'-*O*-isopropylideneadenosine and two equiv. of 5 in dry pyridine was heated at 80 °C for 8 hr, 2',3'-*O*-isopropylideneadenosine 5'-bis-(8-quinolyl) phosphate (6a) was formed. The phosphate (6a) without isolation, was further treated with aqueous solution of cupric chloride and 2',3'-*O*-isopropylideneadenosine 5'-phosphate was obtained. After removal of the protecting group, adenosine 5'-phosphate (7a) was obtained in 70% yield, which was determined by UV absorption after separation by paper chromatography. Nucleoside 5'-phosphates were characterized by comparison of ultraviolet absorption properties with those of authentic samples,⁴⁾ *R*_f values in paper chromatography, and paper electrophoresis.



Similarly, other nucleoside 5'-phosphates were ob-

TABLE 1. PREPARATION OF UNSYMMETRICAL PYROPHOSPHATES (3)

Compd No.	R'	Yield (%)	Mp ^{a)} (°C)	R _f ^{b)}	Formula	Calcd (%)			Found (%)		
						C	H	N	C	H	N
3a	<i>p</i> -Chlorophenyl	78	262—264	0.60	C ₂₄ H ₃₇ ClN ₂ O ₇ P ₂	51.25	6.58	4.98	50.97	6.74	5.01
3b	Benzyl	73	218—220	0.58	C ₂₅ H ₄₀ N ₂ O ₇ P ₂	55.15	7.33	5.15	55.53	8.01	5.24
3c	β -Naphthyl	82	226—227	0.59	C ₂₈ H ₄₀ N ₂ O ₇ P ₂	58.13	6.92	4.84	58.45	7.03	4.97

a) Bis-cyclohexylammonium 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).

TABLE 2. PREPARATION OF NUCLEOTIDES (7)

Compd No.	Nucleotide	Yield (%)	R_f Value ^{a)}		UV spectra (pH 2)	
			Solv. C	Solv. D	$\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (m μ)	$\lambda_{\text{min}}^{\text{H}_2\text{O}}$ (m μ)
7a	Adenosine 5'-phosphate	70	0.30	0.29	257	230
7b	Guanosine 5'-phosphate	68	0.49	0.23	256	228
7c	Uridine 5'-phosphate	65	0.68	0.31	262	230

a) Paper chromatography was carried out by the descending technique using Toyo Roshi No. 51 paper. Solvent systems used were: 2-propyl alcohol-saturated ammonium sulfate-water (2:79:19 v/v) (Solvent C) and *n*-propyl alcohol-concentrated ammonia-water (20:20:3 v/v) (Solvent D).

tained in a high yield as shown in Table 2.

Experimental

Paper chromatography was carried out the descending technique using Toyo Roshi No. 51 paper. The solvents used were isopropyl alcohol-concentrated ammonia-water (8:1:1 v/v) (Solvent A), or (7:1:2 v/v) (Solvent B), 2-propyl alcohol-saturated ammonium sulfate-water (2:79:19 v/v) (Solvent C), and *n*-propyl alcohol-concentrated ammonia-water (20:20:3 v/v) (Solvent D). Paper electrophoresis was carried out on Toyo Roshi No. 51A paper at 80 V/cm in system E (0.05 M potassium phosphate (pH 7.5)). Nucleotides were confirmed by Hanes and Isherwood spray,⁵⁾ followed by ultraviolet irradiation.

Phenyl 8-quinolyl phosphate (**1**) was prepared from the phosphorylation of 8-quinolinol (**4**) with phenyl phosphorodichloridate according to the previous paper.¹⁾

*P*¹-*p*-Chlorophenyl *P*²-Phenyl Pyrophosphate (**3a**). A mixture of 602 mg (2 mmol) of phenyl 8-quinolyl phosphate (**1**) and 624 mg (3 mmol) of *p*-chlorophenyl phosphate in dry pyridine (10 ml) was allowed to stand at 50 °C for 5 hr. To the reaction mixture, 991 mg (10 ml) of cyclohexylamine and water (10 ml) was added, and the mixture was kept overnight in a refrigerator. The precipitate was collected by filtration and washed with water and acetone. The precipitate was recrystallized from a mixture of ethanol and water (1:1 v/v). Bis-cyclohexylammonium salt of *P*¹-*p*-chlorophenyl *P*²-phenyl pyrophosphate (**3a**) (876 mg, 78%) was obtained as white prisms; mp 262–264 °C; R_f 0.60 (Solvent A).

Found: C, 50.97; H, 6.74; N, 5.01%. Calcd for $\text{C}_{24}\text{H}_{37}\text{ClN}_3\text{O}_7\text{P}_2$: C, 51.25; H, 6.58; N, 4.98%.

*P*¹-Adenosine-5' *P*²-Phenyl Pyrophosphate (**3d**).⁶⁾ A solution of 452 mg (1.5 mmol) of phenyl 8-quinolyl phosphate (**1**) and 347 mg (1 mmol) of adenosine 5'-phosphate in dry

pyridine (10 ml) was allowed to stand at 50 °C for 8 hr. The pyridine solution was evaporated *in vacuo* and the residue mixed with water (10 ml). The solution was adjusted to pH 8 and passed through a column of Amberlite IR-120 (H⁺) (50–100 mesh) ion-exchange resin. The eluate was neutralized with 6 M ammonium hydroxide and evaporated to dryness. The residue was dissolved in methanol (10 ml). Bis-ammonium salt of *P*¹-adenosine-5' *P*²-phenyl pyrophosphate (**3d**) (339 mg, 54%) was separated by the addition of ether; R_f 0.40 (Solvent B).

Found: C, 30.17; H, 5.41; N, 15.49%. Calcd for $\text{C}_{16}\text{H}_{25}\text{N}_7\text{O}_{10}\text{P}_2 \cdot 5\text{H}_2\text{O}$: C, 30.60; H, 5.62; N, 15.56%.

Tris-(8-Quinolyl) Phosphate (**5**). To a solution of 8.70 g (60 mmol) of 8-quinolinol (**4**) in dry pyridine (20 ml) was added dropwise a solution of 1.53 g (10 mmol) of phosphoryl chloride in dry pyridine (10 ml) with stirring at 0 °C. The mixture was stirred for 2 hr and further at room temperature for 3 days. After removal of pyridinium chloride, the filtrate was concentrated *in vacuo*. *Tris*-(8-quinolyl) phosphate (**5**) was separated and collected 3.59 g (75%). It was recrystallized from dimethylformamide; mp 202–203 °C; R_f 0.90 (Solvent B). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1620–1570. (C=C and C=N) and 1253 (P=O).

Found: C, 67.39; H, 3.75; N, 8.97%. Calcd for $\text{C}_{27}\text{H}_{18}\text{N}_3\text{O}_4\text{P}$: C, 67.64; H, 3.75; N, 8.76%.

Adenosine 5'-Phosphate (**7a**). A mixture of 286 mg (0.60 mmol) of *tris*-(8-quinolyl) phosphate (**5**) and 92 mg (0.30 mmol) of 2',3'-*O*-isopropylideneadenosine in dry pyridine (3 ml) was heated at 80 °C for 8 hr. The mixture was treated 0.48 ml of 43% aqueous cupric chloride at 100 °C for 1.5 hr and concentrated. The residue was treated with 80% acetic acid at 70 °C for 1.5 hr. Adenosine 5'-phosphate (**7a**) was obtained in an over-all yield of 70%, as determined by means of the ultraviolet absorption after separation with paper chromatography. This sample is identical with an authentic specimen of adenosine 5'-phosphate⁴⁾ by paper chromatography and paper electrophoresis. The R_f values are summarized in Table 2; ultraviolet absorption was $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 257 m μ .

Similarly, guanosine and uridine 5'-phosphate were obtained in high yields as shown in Table 2.

References

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