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A Study on Phosphorylation by Means of 8-Quinolyl Phosphates

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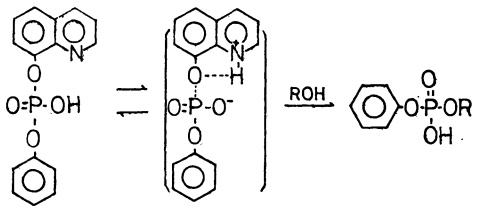
Synopsis. Phosphorylation of phosphates and nucleosides forming unsymmetrical pyrophosphates and nucleotides by the use of 8-quinolyl phosphates has been investigated. According to this method, various pyrophosphates and nucleotides were obtained in high yields.

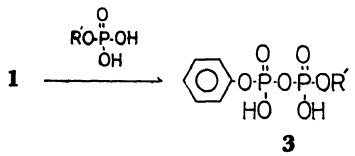
Recently, we reported[1] that mixed diesters of phosphoric acid (**2**) were prepared in a high yield by treating an active ester, phenyl 8-quinolyl phosphate (**1**), with alcohols as shown in the following scheme.



1 **1'** **2**

This paper deals with the reaction of 8-quinolyl phosphates[2] 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.

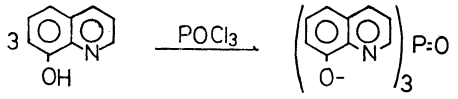
First, an attempt was made to synthesize unsymmetrical pyrophosphate (**3**) by the reaction of **1** with phosphates. When a mixture of one equiv. of **1** and 1.5 equiv. of *p*-chlorophenyl phosphate in dry pyridine was allowed to stand at 50 °C for 5 hr, the corresponding P^1-*p*-chlorophenyl P^2-phenyl 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]



3

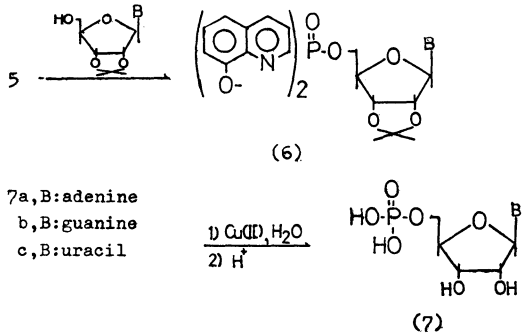
In a similar manner, various unsymmetrical pyrophosphates (**3**) were obtained in a high yield as shown in Table 1. When adenosine 5'-phosphate was used in the above experiments, P^1-adenosine-5' P^2-phenyl pyrophosphate (**3d**) was obtained in 54% yield. Thus it can be said that phenyl 8-quinolyl 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.



4 **5**

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.



(6)

7a,B:adenine
b,B:guanine
c,B:uracil

(7)

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 (%) C	Calcd (%) H	Calcd (%) N	Found (%) C	Found (%) H	Found (%) N
3a	*p*-Chlorophenyl	78	262—264	0.60	$C_{24}H_{37}ClN_2O_7P_2$	51.25	6.58	4.98	50.97	6.74	5.01
3b	Benzyl	73	218—220	0.58	$C_{25}H_{40}N_2O_7P_2$	55.15	7.33	5.15	55.53	8.01	5.24
3c	β-Naphthyl	82	226—227	0.59	$C_{28}H_{40}N_2O_7P_2$	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] Solv. C	Solv. D	UV spectra (pH 2) $\lambda_{max}^{H_2O}$(mμ)	$\lambda_{min}^{H_2O}$(mμ)
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 $C_{24}H_{37}ClN_2O_7P_2$: 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 $C_{16}H_{25}N_7O_{10}P_2 \cdot 5H_2O$: 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 ν_{max}^{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 $C_{27}H_{18}N_3O_4P$: 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′-phosphate[4] by paper chromatography and paper electophoresis. The R_f values are summarized in Table 2; ultraviolet absorption was $\lambda_{max}^{H_2O}$ 257 mμ.

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

References

1) H. Takaku and Y. Shimada, *Chem. Pharm. Bull.* (Tokyo), **21**, 445 (1973).

2) Hydrolysis of 8-quinolyl phosphate was studied recently by Murakami and his co-workers (Y. Murakami and J. Sunamoto, This Bulletin, **44**, 1827, 1939 (1971)).

3) W. Kampe, *Chem. Ber.*, **98**, 1038 (1965).

4) Purchased from Sigma Chemical Corp.

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

6) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 3756 (1958).