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Studies of Phosphorylation. V.[1] 8-Quinolyl Dihydrogen Phosphate as a Phosphorylating Reagent in Nucleotide Synthesis

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Phosphorylation of nucleosides by means of 8-quinolyl dihydrogen phosphate has been described. Phosphorylation of nucleosides with 8-quinolyl dihydrogen phosphate could only be carried out in the presence of cupric chloride. By this method, nucleoside 5′-phosphates were obtained in good yields.

Keywords——nucleotide synthesis; phosphorylation of nucleoside; metal-catalized phosphorylation; phosphorylating reagent; monophosphate

In the synthesis of nucleotides, several methods for the phosphorylation of nucleosides have been offered and examined.[3] It is known that tri-substituted esters of phosphoric acid such as 2,2,2,-trichloroethyl phosphodiimidazolidate[4] can be advantageously used for the phosphorylation of nucleosides. However, mono-substituted phosphates are not suitable for the phosphorylation of nucleosides except when the reaction is carried out in the presence of an activator such as dicyclohexylcarbodiimide (DCC),[3d] trichloroacetonitrile,[5] 2,2′-dipyridyl disulfide and triphenylphosphine,[6] and 2,2′-dipyridyl diselenide and triphenyl phosphite.[7]

In this communication, we wish to report the synthesis of nucleoside 5′-phosphates (III) by the reaction of 8-quinolyl dihydrogen phosphate (I) with nucleosides in the presence of cupric chloride as an activating reagent of 8-quinolyl group.

For example, a mixture of I (1.5 mmol), 2′,3′-O-isopropylideneadenosine (1.0 mmol) and cupric chloride (1.5 mmol) in dry pyridine was treated at 80° for 5 hr. The mixture was concentrated to dryness under reduced pressure. The residue was washed with acetone and chloroform to remove unreacted adenosine and 8-hydroxyquinoline-copper complex, and the protecting group was removed by treatment with 80% acetic acid. After removal of acetic acid, the residue was dissolved in water. The solution was passed through a column of Dowex 50W-X8 (H^+ form) resin. Adenosine 5′-phosphate (IIIa) was isolated in 75% yield.

In a simialr manner, some nucleoside 5′-phosphates (III) were obtained in high yields as shown in Table I. In the above reaction, it was shown that the yields of III remarkably

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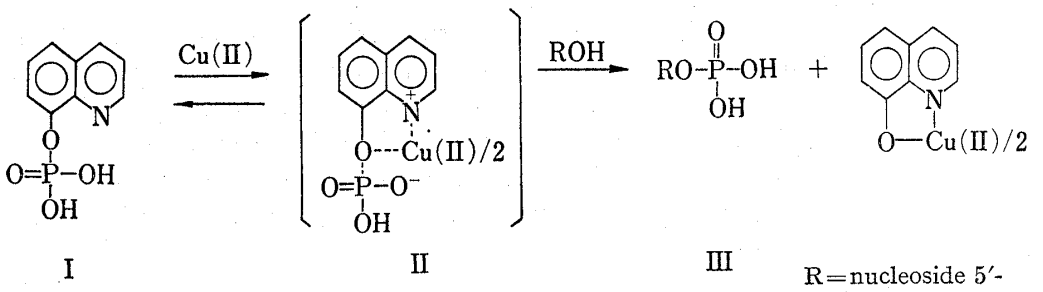
Table I. Preparation of Nucleotides (III)

Compd. No.	Nucleotide	Yield (%)	*Rf* value (A)	*Rf* value (B)	mp (°C)	UV spectra (pH 7) $\lambda_{max}^{H_2O}$ (nm) (ε)
IIIa	Adenosine 5′-phosphate	75	0.11	0.20	193—196[a]	259(15400)
IIIb	Uridine 5′-phosphate	76	0.10	0.29	[b]	262(10000)
IIIc	Cytidine 5′-phosphate	59	0.08	0.25	230—232[a]	271(9100)
IIId	Guanosine 5′-phosphate	72	0.05	0.15	188—196[a]	252(13000)

a) The mp corresponds to that reported in literature.[8]
b) The product was obtained as the barium salt.

decreased when 2 equivalents of cupric chloride was used. The products were identified with the authentic samples[8] on paper chromatography, melting point, and ultraviolet (UV).

This reaction seems to proceed through an intermediate (II), formed from I and cupric chloride. The intermediate (II) in turn reacts with nucleoside to give nucleotide (III) as shown in Chart 1.



I II III

Chart 1

Experimental

General Methods and Materials——Reagent grade pyridine was distilled after treatment with *p*-toluenesulfonyl chloride and dried over calcium hydride for 2 weeks. 8-Hydroxyquinoline and phosphoryl chloride were all commercial materials and purified by recrystallization or distillation before use. Paper chromatography was performed by the descending technique using Toyo Roshi No. 51 paper. The solvent systems used were: isopropyl alcohol–concentrated ammonia–water (7: 1: 2 v/v) (Solvent A) and ethyl alcohol–1 M ammonium acetate (pH 7.5) (5: 2 v/v) (Solvent B). Phosphorous-containing compounds were located on chromatograms with Hanes-Isherwood spray[9] followed by ultraviolet irradiation.[10]

8-Quinolyl Dihydrogen Phosphate (I)——To a solution of phosphoryl chloride (8.4 ml, 90 mmol) in a mixture of dioxane and pyridine (1: 1 v/v) (70 ml) was added dropwise 8-hydroxyquinoline (4.4 g, 30 mmol) dissolved in dioxane (30 ml) under cooling in an ice bath. The reaction mixture was kept standing at room temperature for 5 hr. After removal of pyridine hydrochloride, the solution was concentrated to dryness under reduced pressure at 35°. The residue was added slowly in portions into a mixture of pyridine and water (1: 1 v/v) (100 ml) with vigorous stirring. After 5 hr of stirring, the solution was evaporated under reduced pressure at a temperature below 40°. 8-Quinolyl dihydrogen phosphate (I) was obtained as white crystals. Repeated recrystallizations from a mixture of acetonitrile–water (1: 1 v/v) gave 4.7 g (70%) of needle-like crystals; mp 218—220°. *Rf*, 0.28 (solvent A). *Anal.* Cacd. for $C_9H_8NO_4P$: C, 48.01; H, 3.58; N, 6.22. Found: C, 47.58; H, 3.69; N, 5.93.

Nucleoside 5′-Phosphate (III)——General Procedure: 8-Quinolyl dihydrogen phosphate (I) (378 mg, 1.5 mmol) and 2′,3′-O-isopropylidenenucleoside (1.0 mmol) were rendered anhydrous by evaporation with pyridine several times. The residue was dissolved in dry pyridine (10 ml) and cupric chloride (196 mg, 1.5 mmol) was added. The mixture was kept at 80° for 5 hr, whereupon, water (1 ml) was added. After continuous stirring for 12 hr, the solution was concentrated to a gum. The residue was washed with acetone

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and chloroform and treated with 80% acetic acid at 100° for 90 min. It was concentrated under reduced pressure and the residue was dissolved in water (30 ml). The solution was applied to a column of Dowex 50W-X8 (H^+ form, 2.5 × 50 cm), which was eluted with water. The eluate was concentrated to dryness under reduced pressure and the crystalline residue was recrystallized from the appropriate solvent to give nucleoside 5′-phosphate (III). Characterization of the products was achieved by comparison on paper chromatography, melting point, and ultraviolet spectrum with authentic samples.[8] The results are summarized in Table I.

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Synthesis of the Hexadecapeptide corresponding to Positions 1 through 16 of Porcine Motilin, a Gastric Motor Activity Stimulating Polypeptide[1]

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The hexadecapeptide corresponding to positions 1 to 16 of porcine motilin was synthesized in a conventional manner. The synthetic peptide exhibited the activity of 3% of that of the synthetic motilin, when tested using rabbit duodenal muscle *in vitro*.

Keywords——motilin, a gastric motor activity stimulating polypeptide; motilin (1—16); motilin (5—22); contracting activity *in vitro* rabbit duodenal muscle; hydrogen fluoride deprotection

In 1975, we reported the first synthesis of the docosapeptide[3] corresponding to the revised amino acid sequence of a porcine gastric motor activity stimulating polypeptide, termed as motilin.[4] Shortly after our publication, Yamada, *et al.*[5] reported an alternative synthesis of this peptide, in which a new coupling reagent, diethyl phosphorocyanidate, was employed. In addition, two independent research groups, Mihara, *et al.*[6] and Fujino, *et al.*[7] have also synthesized this gut peptide using the liquid ammonia[8] and the methanesulphonic acid procedure[9] respectively.

1) Amino acids, peptides and their derivatives mentioned here are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=tosyl, OBzl=benzyl ester, ONP=*p*-nitrophenyl ester, DMF=dimethylformamide.
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