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A Convenient Method for the Preparation of Mixed Diesters of Phosphoric Acid and Pyrophosphates

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It was found that mixed diesters of phosphoric acid and pyrophosphates were obtained in good yields when alcohols and arylphosphates, respectively, were allowed to react with 4-methoxyphenyl N-(2-aminophenyl) phosphoramidate in the presence of cupric chloride.

Keywords——phosphorylation; phosphorylating reagent; mixed diester of phosphoric acid; pyrophosphate; metal-catalyzed phosphorylation

Several methods for the preparation of mixed diesters of phosphoric acid and pyrophosphates have been suggested and examined.[2] It was recently demonstrated in our laboratory that phenyl 8-quinolyl phosphate reacts with cupric chloride to give an intermediate complex, from which mixed diesters of phosphoric acid can be obtained by further reaction with alcohols.[3] More recently, Koizumi *et al.* reported[4] the preparation of alkyl phenyl phosphates by the used of phenyl N-(2-aminophenyl) phosphoramidate, but this method, employing an excess of alcohols under reflux, was not wholly satisfactory.

We have found that 4-methoxyphenyl N-(2-aminophenyl) phosphoramidate (**1**) reacts with alcohols in the presence of cupric chloride to give the corresponding mixed diesters of phosphoric acid (**2**).

We describe here the catalytic reaction of **1** and its application for the preparation of mixed diesters of phosphoric acid (**2**) and pyrophosphates (**3**).

Table I. Effect of $CuCl_2$ in the Phosphorylation of *n*-Butyl Alcohol with 4-Methoxyphenyl N-(2-Aminophenyl)phosphoramidate (**1**)

$CuCl_2$ (0.075 mmol)	Temp. (°C)	Time (hr)	Yield of **2d** (%)
	22	24	0[b]
$CuCl_2$	22	24	15
	50	12	0[b]
$CuCl_2$	50	5	82

a) In the above reactions, **1** (0.1 mmol) and *n*-butyl aclohol (1.0 mmol) were used.
b) The starting material was recovered quantitatively.

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4-Methoxyphenyl N-(2-aminophenyl) phosphoramidate (**1**) was prepared as a phosphorylating agent in 72% yield from *o*-phenylenediamine and 4-methoxyphenyl phosphorodichloridate⁽⁵⁾ on reflux in dry benzene for 4 hr, using a modification of Koizumi's procedure.⁽⁴⁾

We carried out the phosphorylation of *n*-butyl alcohol with **1** in the presence and absence of metal catalysts in pyridine. The results are summarized in Table I. As shown in Table I, no reaction was observed when **1** (0.1 mmol) was treated with *n*-butyl alcohol (1.0 mmol) in dry pyridine (1 ml) for 12 hr at 50°, and the starting material (**1**) was recovered. On the other hand, **1** (0.1 mmol) and *n*-butyl alcohol (1.0 mmol reacted with each other in the presence of cupric chloride (0.075 mmol) in dry pyridine (1 ml) for 5 hr at 50°, yielding the corresponding *n*-butyl 4-methoxyphenyl phosphate (**2d**) in 82% yield together with a trace of P^1,P^2-bis(4-methoxyphenyl) pyrophosphate (**3a**). Paper chromatography of the reaction mixture showed the presence of a small amount of **3a**, which could not be isolated, The yield of **3** depended on the amount of cupric chloride, and better results were obtained in the presence of 0.75 molar equivalent.

Table II. Preparation of Alkyl 4-Methoxyphenyl Phosphates (**2**)

Compound No.	Alkyl	Yield of 2 (%)	*Rf* value[a)]	PE[b)]
2a	Methyl	69	0.80	0.59
2b	Ethyl	79	0.82	0.60
2c	*n*-Propyl	79	0.86	0.63
2d	*n*-Butyl	82	0.86	0.61
2d	*n*-Butyl[c)]	75		
2e	*sec*-Butyl	75	0.88	0.55
2f	*n*-Octyl	81	0.82	0.66

a) Paper chromatography was perfotmed by the descending technique using Toyo Roshi No. 51A paper. The solvent system used was isopropanol–conc. ammonia–water (7: 1: 2, v/v).
b) P.E. refers to paper electrophoretic mobility relative to 4-methoxyphenyl phosphate. The buffer used was potassium phosphate (0.2 M, pH 8).
c) This reaction was carried out with three molar equiv. of *n*-butyl alcohol.

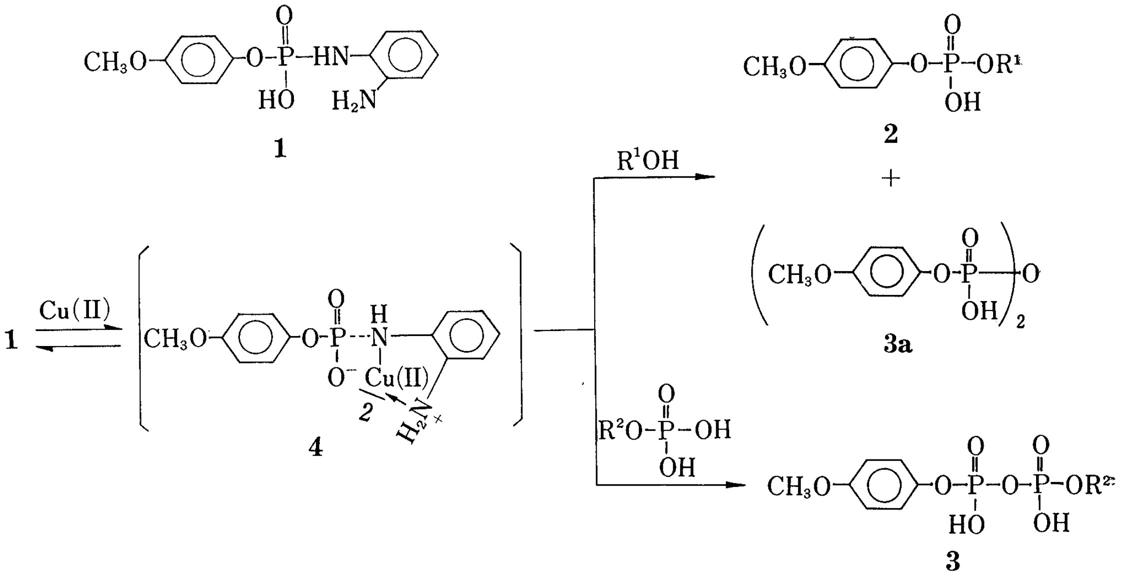

Chart 1

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5) V.V. Katyskina and M.Y. Kraft, *Zh. Obshch. Khim.*, **26**, 3060 (1956).

In a similar manner, variuos mixed diesters of phosphoric acid (**2**) were obtained in high yields as shown in Table II.

In the above reactions, when **1** was treated with 3 molar equiv. of *n*-butyl alcohol, the yield of **2d** decreased (see Table II). Of various metallic compounds examined, it was found that the yield of **3** increased when cupric chloride was used as the metallic catalyst.[6]

The reaction seems to proceed through an intermediate (**4**) formed from **1** and cupric chloride. The intermediate (**4**) in turn reacts with alcohol to give alkyl 4-methoxyphenyl phosphate (**2**) [Chart 1].

Next, the reaction of **1** and aryl phosphate in the presence of cupric chloride was attempted. For example, when **1** (0.1 mmol) was reacted with 4-nitrophenyl phosphate (0.13 mmol) in the presence of cupric chloride (0.075 mmol) in dry pyridine (1 ml) for 5 hr at 22°, the corresponding P^1-4-methoxypheny;, P^2-4-nitrophenylpyrophosphate (**3b**) was obtained in 88% yield.

TABLE III. Preparation of Unsymmetrical and Symmetrical Pyrophosphates (3)

Compound No.	Aryl	Time (hr)	Yield of 3 (%)	*Rf* value[a]	P.E.[b]
3a	4-Methoxyphenyl	5	51	0.57	0.89
			85[c]	0.60	0.89
3b	4-Nitrophenyl	5	44	0.72	0.87
			88[c]	0.70	0.83

a) Paper chromatography was performed by the descending technique using Toyo Roshi No. 51 paper. The solvent system used was isopropanol–conc. ammonia–water (7: 1: 2, v/v).
b) P.E. refers to paper electrophoretic mobility relative to 4-methoxyphenyl phosphate. The buffer used was potassium phosphate (0.2 M, pH 8).
c) The reactions were carried out in the presence of $CuCl_2$.

P^1,P^2-bis(4-methoxyphenyl) pyrophoshate (**3a**) was similarly obtained as shown in Table III. In the absence of cupric chloride, the rate of the reaction was very slow (see Table III).

In conclusion, it appears that **1** is a useful phosphorylating agent for alcohols and phosphates in the presence of a metallic catalyst.

Experimental

Reagent-grade pyridine was distilled after treatment with *p*-toluenesulfonyl chloride and dried over calcium hydride for 2 weeks. Paper chromatography was performed by the descending techique using Toyo Roshi No. 51A paper. The solvent system used was: isopropanol–conc. ammonia–water (7: 1: 2, v/v). Paper electrophoresis was carried out in an apparatus similar to that described by Markham and Smith[7] with the following buffer solution: 0.2 M potassium phosphate (pH 8). Phosphorus-containing compounds were located on chromatograms by the method of Hanes–Isherwood,[8] followed by ultraviolet irradiation.

4-Methoxyphenyl N-(2-Aminophenyl) phosphoramidate (1)——A solution of *o*-phenylenediamine (10.8 g, 0.1 mol) in dry benzene (150 ml) was treated with 4-methoxyphenyl phosphorodichloridate[5] (12.3 g, 0.05 mol) at 0°. The reaction mixture was stirred for 1 hr at room temperature then refuxed for 4 hr. The solvent was evaporated off *in vacuo* and the residue was added to ice-water (1000 ml). The precipitate was collected by filtration and washed with ice-water. Recrystallization of the crude product from a mixture of ethanol and water gave **1** (10.4 g, 72%) as white crystals, mp 177—178°. *Anal.* Calcd for $C_{13}H_{15}N_2O_4P \cdot H_2O$: C, 50.00; H, 5.49; N, 8.97. Found: C, 49.54; H, 5.52; N, 8.72. The compound lost water after drying under reduced pressure at 50° to give the anhydrous compound, mp 154—156° C. *Rf*=0.71 (isopropanol–conc.ammonia–water, 7: 1: 2 v/v). PE=0.61 [to 4-methoxyphenyl phosphate, phosphate buffer (pH 8)]. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1} 3400, 3240, 1620, 1500, and 1210. *Anal.* Calcd for $C_{13}H_{15}N_2O_4P$: C, 53.07; H, 5.14; N, 9.52. Found: C, 52.72; H, 5.10; N, 9.51.

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6) With nickel chloride, zinc chloride, and cobalt chloride, the yield of **2d** decreased slightly.
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Alkyl 4-Methoxyphenyl Phosphates (2); General Procedure——Cupric chloride (10.1 mg, 0.075 mmol) was added to a solution of 4-methoxyphenyl N-(2-aminophenyl) phosphoramidate (1) (29.4 mg, 0.1 mmol) and alcohol (1.0 mmol) in dry pyridine (1 ml) with vigorous stirring for 5 hr at 50°. After complete removal of the solvent under high vacuum, the residue was dissolved in water and the insoluble materal (*o*-phenylenediamine-copper) was removed by filtration. The solution was treated with Dowex 50W-X2 (pyridinium form) and the resin was removed by filtration. The solution was concentrated *in vacuo* and the residue was dissolved in water (10 ml). The yield of **2** were determined spectrophotometrically it terms of λ max (H_2O, pH 7) 280 nm (ε=1,830) for alkyl 4-methoxyphenyl phosphates (**2**) after separation by paper electrophoresis. The results are summarized in Table I and Table II.

Unsymmetrical and Symmetrical Pyrophosphates (3); General Procedure:——Cupric chloride (10.1 mg, 0.075 mmol) was added to a solution of 4-methoxyphenyl N-(2-aminophenyl) phosphoramidate (**1**) (29.4 mg, 0.1 mmol) and aryl phosphate (0.13 mmol) in dry pyridine (1 ml) with stirring for 5 hr at 22°. The yields of **3** were determined by the method descrived above. In this solvent, P^1,P^2-*bis*(4-methoxyphenyl) pyrophosphate (**3a**) has λ_{max} (H_2O, pH 7) 274 nm (ε=2,660), and P^1-4-methoxyphenyl P^2-4-nitrophenyl pyrophosphate (**3b**) has λ_{max} (H_2O, pH 7) 290 nm (ε=10,000). The results are summarized in Table III.

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Characterization of an Acidic Tripeptide in Neurotoxic Dialysate[1]

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An acidic tripeptide was isolated from neurotoxic dialysate by ultrafiltration with an Amicon Centriflo DM-5 membrane, followed by gel filtration on Sephadex G-15 and Sephadex G-10, and ion-exchange chromatography on DEAE-Sephadex A-25. The tripeptide thus obtained was identified as H-Glu-Asp-Gly-OH by amino acid analysis, Edman degradation, and measurement of physical constants and analytical data in comparison with those of the authentic tripeptide. This tripeptide inhibited LDH activity.

Keywords——uremic toxins: uremic neuropathy; acidic tripeptide; middle molecular hypothesis; lactate dehydrogenase

Research on uremic toxins has now been going on for more than 150 years, but despite these eforets the true nature of uremic toxicity has not been fully elucidated yet. Many substances are known to accumulate in the body fluids of patients with uremic symptoms.[3,4] However, none of the peptides have been identified chemically, and their biological roles are quite unknown.[5,6] The middle molecular hypothesis formulated by Babb *et al.*[5] on the basis of manipulation of clinical hemodialysis strategies suggested that uremic neuropathy was due

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1) Amino acids, peptides and their derivatives mentioned in this paper are the L-configuration. abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochem. Biophys. Acta*, **263**, 205 (1972). Other abbreviations: DMF=dimethylformamide, Et_3N=triethylamine, TFA=trifluoroacetic acid, WSCI=water-soluble carbodiimide, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, Tos=*p*-toluenesulfonic acid, LDH=lactate dehydrogenase (EC 1.1.1.27), AP-M=aminopeptidase-M.

2) Location: *Tsutsumimachi 3-16-1, Sendai, 980, Japan.*

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