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Synthetic Studies on Phosphorylating Reagent. III.[1] Selective Phosphorylation of Unprotected Nucleoside by Means of 2-(N,N-Dimethylamino)-4-nitrophenyl Phosphate

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Reaction of 2-(N,N-dimethylamino)-4-nitrophenyl phosphate (**1**), a new phosphorylating agent, with the alcohol involving primary and secondary hydroxyl groups led to selective phosphorylation of the primary hydroxyl group. Thus, 1,2-propanediol and glycerol were phosphorylated to give exclusively the corresponding 1-phosphates. When unprotected nucleosides were treated with this reagent **1**, nucleoside 5′-phosphates were obtained in satisfactory yield by simple work up.

In a previous paper,[1] we reported the preparation of a new phosphorylating reagent, 2-(N,N-dimethylamino)-4-nitrophenyl phosphate (**1**), and demonstrated that the reagent could be successfully utilized in the phosphorylation of simple alcohol and unprotected amino alcohol. It was shown that the electron attracting dimethylammonium group formed *in situ* by protonation enhanced the reactivity of the reagent.

Considering the bulkiness of the 2-(N,N-dimethylamino)-4-nitrophenyl group, one may expect the selective phosphorylation of the primary hydroxyl group of the poly hydroxy compounds, such as nucleoside and carbohydrate by using this reagent **1**. Although several reagents[3–9] have been employed thus far for direct phosphorylation of unprotected nucleoside, none has proved to be completely general in regard to the ease and efficiency with which they afford the nucleotides. Therefore, it is worthwhile to explore more convenient method for the phosphorylation of unprotected nucleoside.

The present paper describes a preliminary attempt for the selective phosphorylation of poly hydroxy compound by means of **1** and its application to unprotected nucleoside. Phosphorylation of 1,2-propanediol was first attempted. 1,2-Propanediol 1-phosphate (**3a**) has already been synthesized[10–12] from 1,2-epoxypropane and dipotassium hydrogen phosphate, however, direct phosphorylation of 1,2-propanediol has not been reported so far. Reaction of 1,2-propanediol (**2a**) with equimolar amount of **1** was carried out in absolute pyridine in the presence of acetic acid (3 mole) and triethylamine (1 mole) under reflux. The usual work up shown in the experimental afforded a phosphate in 81% yield as a biscyclohexylammonium salt, mp 169—170°, which was shown homogeneous by paper chromatography (PPC) in solvent

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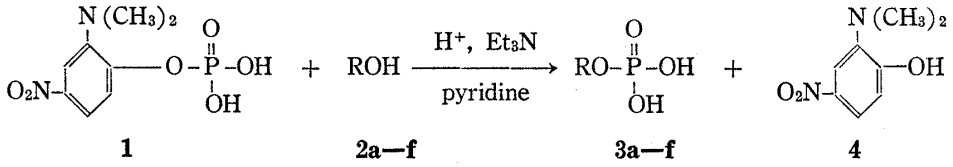
system A. The melting point and the *Rf* value (0.31) suggested occurrence of the selective formation of 1,2-propanediol 1-phosphate (**3a**). However, there was a little ambiguity on the position of phosphorylation site because of the close similarity of the *Rf* value with that (0.38 in the same solvent system) of 1,2-propanediol 2-phosphate. It has been reported by Ukita, *et al.*[12] that the hydrolytic cleavage of 1,2-propanediol 1,2-cyclic phosphate affords the 1-phosphate **3a** exclusively and this is confirmed by the direct comparison with the authentic 2-phosphate prepared by an independent route. In order to confirm the phosphorylation site, our product was subjected to the cyclization-cleavage experiment. Intramolecular phosphorylation of the product with dicyclohexylcarbodiimide (DCC) in pyridine gave a cyclic phosphate which was characterized to have cyclic phosphate linkage by PPC and paper electrophoresis. Acidic hydrolysis of this cyclic phosphate with 0.1 N hydrochloric acid afforded the original phosphate with the same *Rf* value and a small amount of another phosphate, judged to be the 2-phosphate from its *Rf* value (0.38), was also detected on PPC. These results clearly showed the validity of the structural assignment. Phosphorylation of glycerol (**2b**) under the same condition gave glycerol 1-phosphate (**3b**) in 88% yield as crystalline barium salt, and any other phosphate could not be detected on PPC of the reaction mixture. The structure was determined both by PPC and periodate titration according to Volis' method.[13]



1 **2a—f** **3a—f** **4**

R

a : $-CH_2-CH(OH)-CH_3$

b : $-CH_2-CH(OH)-CH_2OH$

c : 5′-adenosyl

R

d : 5′-guanosyl

e : 5′-uridyl

f : 5′-cytidyl

Chart 1

Table I. Phosphorylation Products

Products (**3a**—**f**)[a]	Yield (%)	*Rf*	mp (°C)	Recrystn. solv.	$\lambda_{max}^{H_2O}$ (pH2.0) mμ (ε)
1,2-Propanediol 1-phosphate (**3a**)	81	0.31[b]	169—172	acetone–H_2O	
Glycerol 1-phosphate (**3b**)	88	0.25[b]			
Adenosine 5′-phosphate (**3c**)	77	0.16[c]	195—199[d]	acetone–H_2O	257(15000)
Guanosine 5′-phosphate (**3d**)	46	0.09[c]	190—200[e]	acetone–H_2O	256(12200)
Uridine 5′-phosphate (**3e**)	65	0.17[c]	[f]	H_2O	262(10100)
Cytidine 5′-phosphate (**3f**)	57	0.13[c]	231—233[e]	EtOH–H_2O	280(13200)

a) The compounds were identified with authentic samples on PPC and paper electrophoresis (PEP).
b) solvent B
c) solvent A
d) Brown, *et al.* gave mp 192°;D.M. Brown, G.D. Fasman, D.I. Magrath, and A.R. Todd, *J. Chem. Soc.*, **1954**, 1448;
e) The mp corresponds to that reported in literature; A.M. Michelson and A.R. Todd, *J. Chem. Soc.*, **1949**, 2476
f) The product was obtained as the barium salt.

Next, we applied this method to the phosphorylation of unprotected nucleoside. Treatment of nucleoside (**2c**—**f**) with just one molar equivalent of the monotriethylammonium salt of **1** in the presence of acetic acid (3 mole) and triethylamine (1 mole) in absolute pyridine

13) L. Volis, G. Ellis, and L.A. Maynard, *J. Biol. Chem.*, **133**, 492 (1940).

under reflux led to a rapid and selective phosphorylation of the 5′-hydroxyl group, and no nucleoside 2′(3′)-phosphate was detected on PPC of the reaction mixture. However, significant amount of inorganic phosphate was always formed because of the susceptibility of the reagent toward moisture under the reaction condition. The isolation of the nucleotide could be achieved by simple procedure. The reaction mixture was concentrated to dryness under reduced pressure. The residue was washed with acetone to remove unchanged nucleoside and 2-dimethylamino-4-nitrophenol (**4**), and was dissolved in water. The solution was passed through a column of Amberlite IRC-50 (H^+ form) resin. Concentration of the eluate at the temperature below 40° under reduced pressure afforded the crude nucleotide, which was recrystallized from the appropriate solvent to give nucleoside 5′-phosphate **3c—f**. The products were identified with the authentic samples on PPC, melting point, and ultraviolet (UV) spectrum.

The successful application of this selective phosphorylation to a general synthesis of nucleoside 5′-phosphate encourages the hope that this type of reaction will find some utility in nucleotide chemistry.

Experimental

Reagents——Alcohols, amines and solvents were purified and dried by ordinary procedures. Paper chromatography was carried out by descending technique using Toyo Roshi No. 51A paper. Solvent systems used were: A, isopropyl alcohol–concentrated ammonium hydroxide–water (7: 1: 2, v/v); B, isopropyl alcohol-5N-ammonium hydroxide (2: 1, v/v).

UV lamp (254 mμ) and Hanes-Isherwood reagent[14] were used for the detection of spots on paper chromatogram. Melting points are uncorrected and were determined on a Yamato apparatus MP-21, and UV spectra were determined on a Hitachi EPS-3T spectrometer.

1,2-Propanediol 1-Phosphate (**3a**)——To a solution of 2-(N,N-dimethylamino)-4-nitrophenyl phosphate (**1**) monotriethylammonium salt (3.63 g, 0.01 mole) in absolute pyridine (30 ml) was added 1,2-propanediol (0.76 g, 0.01 mole), acetic acid (1.80 g, 0.03 mole) and triethylamine (1.01 g, 0.01 mole). The reaction mixture was refluxed for 3 hr and evaporated to dryness. The residue was dissolved in water (50 ml) and barium hydroxide (8.0 g, 0.025 mole) was added. The precipitate was removed by filtration and the solution was passed through a column of Dowex 50 W×8 (H^+ form, 1.0×100 cm). The product was eluted with water. The eluate was neutralized with cyclohexylamine and evaporated to dryness under reduced pressure. The residue was crystallized with acetone–ethyl ether (1: 1, v/v), and recrystallization from aqueous acetone gave 1,2-propanediol 1-phosphate (**3a**) biscyclohexylammonium salt (2.90 g, 81%) as colorless needles; mp 169—172° (decomp.) (lit.[4] mp 169—172°); *Rf* (solvent B) 0.31.

Glycerol 1-Phosphate (**3b**)——To a solution of **1** monotriethylammonium salt (3.63 g, 0.01 mole) in absolute pyridine (30 ml) was added glycerol (0.92 g, 0.01 mole), acetic acid (1.80 g, 0.03 mole) and triethylamine (1.01 g, 0.01 mole). The reaction mixture was refluxed for 3 hr and evaporated to dryness. The residue was dissolved in 60 ml of water and barium hydroxide (8.0 g, 0.025 mole) was added. The precipitate was removed by filtration. The solution was passed through a column of Dowex 50 W×8 (H^+ form, 1.0×100 cm), and the product was eluted with water. The eluate was neutralized with barium hydroxide and evaporated to dryness under reduced pressure. The residue was washed with ethanol to give glycerol 1-phosphate (**3b**) barium salt (2.71 g, 88%) as colorless powder; *Rf* (solvent B) 0.25. This product was determined on PPC and the titration with periodate acid showing 99% purity.

Nucleoside 5′-Phosphates (**3c—f**)——General Procedure: To a solution of **1** monotriethylammonium salt (3.63 g, 0.01 mole) and nucleoside (0.01 mole) in dry pyridine (30 ml) was added acetic acid (1.80 g, 0.03 mole) and triethylamine (1.01 g, 0.01 mole). The reaction mixture was refluxed for 3 hr and evaporated to dryness. The residue was washed with acetone (50 ml) and dissolved in water (50 ml). The solution was passed through a column of Amberlite IRC-50 (H^+ form, 1.0×30 cm) resin and the product was eluted with water. The eluate was evaporated to dryness under reduced pressure at the temperature below 40° and the crystalline residue was recrystallized from the appropriate solvent to give nucleoside 5′-phosphates (**3c—f**). The products correspond to the authentic samples on paper chromatography in solvent A, melting point, and UV spectrum.

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