(6.3 M), and 9 cc. of substrate ($2\times10^{-3}M$) was incubated at 37°, 2.5-cc. aliquots were taken at intervals (5, 10, 15, 24, and 48 hr.). The aliquot was adjusted to pH 4.5 with AcOH and heated at 85° for 5 min. The solution thus treated was lyophilized and from the residue Mg ions were removed by adding 0.05 cc. of satd. $(NH_4)_2CO_3$ solution. The precipitate that formed was centrifuged and the supernatant was submitted to paper chromatography.

Summary

Catechol cyclic phosphate (CCP) (IV) was found to be alcoholyzed with several primary and secondary monofunctional alcohols to give the corresponding alkyl or aralkyl *o*-hydroxyphenyl phosphate (V) in a yield of 47~65%. These reactions proceeded by the catalytic function of the acid reagent, CCP, used.

The products (V) were further hydrolyzed by acid or alkali to give the corresponding alcohols and *o*-hydroxyphenyl phosphate. The catechol moiety of (V) was found inert against catalytic hydrogenolysis, while it was hydrolyzed by snake venom phosphodiesterase to alkyl or aralkyl phosphates.

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76. Tyunosin Ukita and Kinzo Nagasawa: Organic Phosphates. VII.[1] Alcoholyses of Catechol Cyclic Phosphate. (2).

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The results of investigations on alcoholyses of catechol cyclic phosphate (CCP)[*2] (I) were reported in the preceding paper of this series.[1] Thus, (I) is alcoholyzed with both primary and secondary alcohols to alkyl *o*-hydroxyphenyl phosphate, having the corresponding alkyl group of the alcohol used and these diesters, isolated from the reaction mixture, gave *o*-hydroxyphenyl phosphate (*o*-HPP) by further hydrolysis in acid or alkaline medium.

In further research, in the alcoholyses of catechol cyclic phosphate with polyols having two vicinal hydroxyl groups, the reactions proceeded differently from that observed for similar reactions of (I) with monofunctional alcohols and the results are reported in this paper.

(I) was mixed with each of ethylene glycol, 1,2-propanediol, glycerol, erythritol, or mannitol and warmed at 70~80° or kept at room temperature. Solvent such as pyridine or dioxane was used if necessary.

From the reaction mixture, an aliquot was taken to test the product on paper chromatogram and the results are summarized in Tables I and III.

As shown in Table I, in the cases of alcoholyses of (I) with ethylene glycol (A) and 1,2-propanediol (B), each reaction mixture revealed three phosphorus spots of the alcoholysis product with Rf values of 0.63, 0.55, 0.22, and 0.68, 0.63, 0.27, respectively, besides that of *o*-HPP (Rf_1 0.32).

Among these three new spots, in each case, only the ones (Rf_1 0.63 and 0.68) with the largest Rf values were found positive to ferric chloride coloration test. Further, after a mild acid treatment (0.1N HCl at 85° for 5 minutes), both reaction mixtures gave

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*2 The following abbreviations are used: CCP, catechol cyclic phosphate; *o*-HPP, *o*-hydroxyphenyl phosphate.

1) Part VI. K. Nagasawa: This Bulletin, **7**, 397(1959).

Table I. Alcoholysis Reaction of CCP with Polyols (1)

Solution spotted	Product of alcoholysis	Product of hydrolysis	Product of alcoholysis
	Before acid treatment		
	Rf_1	Rf_1	Rf_1
CCP+dioxane		0.32 S	
o-HPP+dioxane		0.32 S	
CCP+ethylene glycol	0.22 S	0.32M	0.55 S, 0.63M
CCP+1,2-propanediol	0.27 S	0.32W	0.63 S, 0.68 S
	After acid treatment		
CCP+ethylene glycol	0.22 SS	0.32M	
CCP+1,2-propanediol	0.27 SS	0.32W	

SS : very strong S : strong, M : medium, W : weak.

paper chromatograms on which two of above phosphate spots with larger Rf values than that of *o*-HPP disappeared with simultaneous increasing intensities of the phosphorus spots with the smallest Rf values (Rf_1 0.22 and 0.27).

From these properties of the product on paper chromatograms and from the relative positions generally observed for phosphodiesters, cyclic phosphates and phosphomonoesters on a similar paper chromatogram as reported in the previous papers,[2,3] the three new spots of alcoholysis products found for both reaction mixtures were assumed to be phosphodiester, cyclic phosphate, and phosphomonoester in the order of decreasing Rf values.

Thus the spots with Rf_1 0.63, 0.55, and 0.22 found for the case (A) and those with Rf_1 0.68, 0.63, and 0.27 for the case (B) should correspond to *o*-hydroxyphenyl 2-hydroxyethyl phosphate (IIa), ethylene glycol cyclic phosphate (IIIa), 2-hydroxyethyl phosphate (IVa), and *o*-hydroxyphenyl 2-hydroxypropyl phosphate (IIb), 1,2-propanediol cyclic phosphate (IIIb), 2-hydroxypropyl phosphate (IVb), respectively. Course of the reaction in these alcoholyses should be represented as in Chart 1.

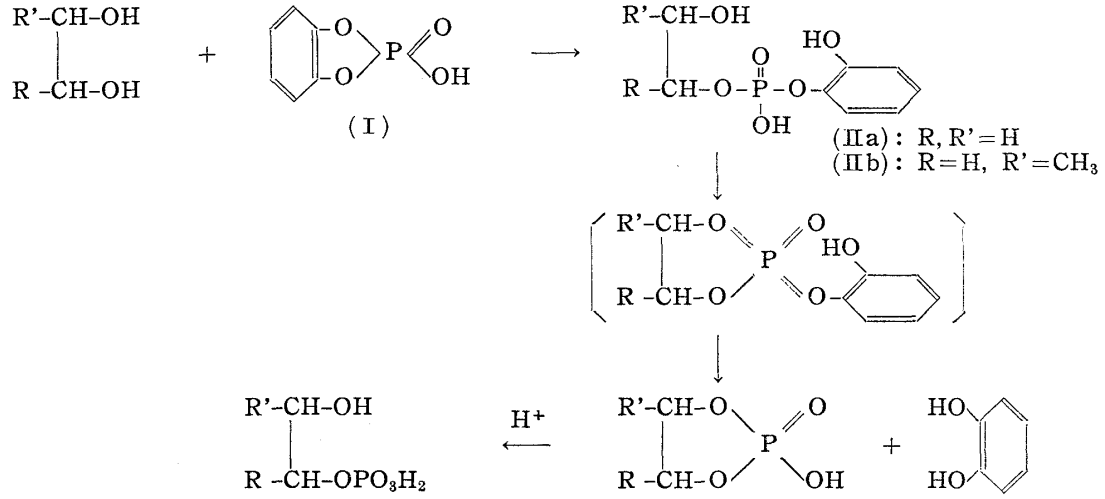

Chart 1.

To confirm these assumptions, the spots with Rf_1 0.55 and 0.22 in case of (A) and those with Rf_1 0.63 and 0.27 in case of (B) were compared and identified by paper chromatography

2) T. Ukita, K. Nagasawa, M. Irie : This Bulletin, **5**, 121(1957).
3) *Idem.* : *Ibid.*, **5**, 215(1957).

with authentic specimens[2] of (IIIa), (IVa) and (IIIb), (IVb), respectively.

Further, the intermediate compounds of type (II) was isolated from one of these reaction mixtures. CCP and 1,2-propanediol were reacted in a preparative scale at 37° for 24 hours. From the reaction mixture *o*-hydroxyphenyl 2-hydroxypropyl phosphate (IIb) was isolated as the barium salt, $(C_9H_{12}O_6P)_2Ba$, which gave reddish violet coloration with ferric chloride.

On hydrolysis of the ammonium salt of (IIb) with acid reagent such as *p*-toluenesulfonic acid or catechol cyclic phosphate in anhydrous pyridine at 90~100° for 2 hours, and detection of the product both by paper electrophoresis and paper chromatography, the only products observed in the reaction mixture were propanediol 1,2-cyclic phosphate, 2-hydroxypropyl phosphate, and catechol which were identified with authentic specimens (Table II). In another experiment, with 0.1*N* HCl at 37° after 4~5 hours, more than 50% of barium salt of (IIb) gave 2-hydroxypropyl phosphate and catechol.

Table II. Identification of Products in Transesterification Reaction of *o*-Hydroxyphenyl 2-Hydroxypropyl Phosphate with Propanediol 1,2-Cyclic Phosphate

	Products of transesterification reaction			Authentic sample		
	Rf_1	Rf_2	Mobility (mm.)	Rf_1	Rf_2	Mobility (mm.)
Propanediol 1,2-cyclic phosphate	0.63	0.57	100	0.63	0.56	98
2-Hydroxypropyl phosphate	0.27	0.74	83	0.27	0.74	83

This observation gave the evidence that in the case of ethylene glycol, the phosphorus spot with Rf value of 0.55 on the paper chromatogram of alcoholysis reaction mixture is that of ethylene glycol cyclic phosphate (IIIa).

Compared with the above results of alcoholysis with diols, in the similar reactions of CCP with polyols containing vicinally occurring three hydroxyl groups such as the cases with glycerol (C), erythritol (D), and mannitol (E), the paper chromatogram of the reaction mixture lacked the spots corresponding to phosphodiesters giving only one phosphorus spot with larger Rf values (Rf_1 0.60, 0.52, and 0.50) than that for *o*-HPP (Rf_1 0.32). After a mild acid treatment (0.1*N* HCl, at 85° for 5 minutes) of the reaction mixture none of these spots were observed on the paper chromatogram. Further, these phosphorus spots gave no coloration with ferric chloride and in the case of reactions with erythritol and mannitol, gave positive reactions for periodate-Schiff's reagent.[4,5]

Table III. Alcoholysis Reaction of CCP with Polyols (2)

Solution spotted	Product of alcoholysis	Product of hydrolysis	Product of alcoholysis
	Before acid treatment		
	Rf_1 (yield %)	Rf_1 (yield %)	Rf_1 (yield %)
CCP+dioxane		0.32	
o-HPP+dioxane		0.32	
CCP+glycerol	0.23 (12.2)	0.32 (23.0)	0.60 (60.5)
CCP+erythritol	0.20 (24.0)	0.32 (19.0)	0.52 (57.5)
CCP+mannitol	0.18 (5.5)	0.32 (29.5)	0.50 (64.0)
	After acid treatment		
CCP+glycerol	0.23	0.32	
CCP+erythritol	0.08, 0.20	0.32	
CCP+mannitol	0.05, 0.18	0.32	

Yield of each phosphate spot was determined by Allen's method after the spots were separated on paper chromatogram.[6]

4) J.G. Buchanan, C.A. Dekker, A.G. Long: J. Chem. Soc. **1950**, 3162.
5) J. Baddiley, J.G. Buchanan, R.E. Handschumacher, J.F. Prescott: *Ibid.*, **1956**, 2818.
6) G.W. Kenner, J. Mater: *Ibid.*, **1956**, 3524.

The corresponding product in the case of glycerol, glycerol 1,2-cyclic phosphate (IIIc), was isolated and identified with authentic specimen.

The phosphorus spots with the smallest Rf values (Rf_1 0.23, 0.20, and 0.18) found on the paper chromatogram prepared for the alcoholysis reaction mixture of CCP with these polyols were identified with monophosphate of the polyols used. Thus DL-erythritol 1-phosphate (Rf_1 0.20) (IVd) and D-mannitol 1-phosphate (Rf_1 0.18) (IVe) were isolated as cyclohexylammonium salt from the reaction mixture, and identified by analysis and periodate oxidation.

As shown in Table III, in the alcoholysis of CCP with erythritol and mannitol, the paper chromatogram prepared for acid-treated reaction mixture gave another faint phosphorus spot with smaller Rf values (Rf_1 0.08 and 0.05) than those of monophosphates. One of these products, in the case of the reaction with mannitol, was isolated and proved to be D-mannitol 1,6-diphosphate after analysis and periodate oxidation. The corresponding spot with Rf_1 0.08 for the case of erythritol should analogously be attributed to that of DL-erythritol 1,4-diphosphate. Because these spots of diphosphate were observed only after acid treatment of the alcoholysis reaction mixture, they should be secondarily formed from the corresponding dicyclic phosphate of polyols such as DL-erythritol 1,2 : 3,4- and D-mannitol 1,2 : 5,6-dicyclic phosphate, and the spots should overlap with each of DL-erythritol and D-mannitol 1,2-cyclic phosphates on the paper chromotogram prepared for the reaction mixture before acid treatment.

The results of observations described above give evidences that the phosphodiester-type intermediates formed during the alcoholysis of CCP with polyols were hydrolyzed to give the cyclic phosphate of the polyol used, contrary to the case of similar phosphodiester obtained in the alcoholysis of CCP with monoöls as reported in the preceding paper. Thus, for the former phosphodiesters the tendency to form a five-membered cyclic phosphate is larger for the aliphatic than for aromatic hydroxyl groups and the transition state thus formed is favorable for liberation of catechol moiety giving the cyclic phosphate of the polyol used.

Further these results showed that in the course of alcoholysis of CCP with polyols, when the polyol contains only one pair of vicinal hydroxyl groups, the intermediate compound of phosphodiester type is stable enough to be isolated, while in the case of polyols containing more than three vicinal hydroxyl groups, the corresponding intermediate is so unstable that no trace of this compound was observable on the paper chromatogram of the reaction mixture.

The authors are indebted to Mr. B. Kurihara and Miss R. Ohta for carrying out microanalyses.

Experimental

Paper Chromatography—Samples were applied on Toyo Roshi No. 53 filter paper and run ascendingly using following solvent systems : (1) *iso*-PrOH:conc. NH_4OH:H_2O (7:1:2); (2) PrOH: conc. NH_4OH:H_2O (6:3:1); (3) 80% EtOH containing 0.8% AcOH. The Rf values of phosphorus compounds found for each solvent system used are represented with abbreviations Rf_1, Rf_2, and Rf_3. For the detection of spots, Bandurski-Axelrod's method[7] for phosphate, periodate-Schiff's reagent[4,5] for polyols, and $FeCl_3$ solution for phenol group were employed.

For paper electrophoresis, strips of Toyo Roshi No. 53 filter paper were used. The strips moistened with buffer solution of pH 6.0 (BuOH:AcOH:pyridine:H_2O (15:2:10:500)) was subjected to a potential of 30 V/cm. for 60 min. Spots were detected on paper by the same techniques as those for paper chromatography.

Alcoholysis Reaction of Catechol Cyclic Phosphate with Several Polyols—To 0.2 cc. each of ethylene glycol, 1,2-propanediol, or glycerol ca. 10 mg. of CCP was added. In cases of erythritol and mannitol, a solution of 20 mg. of each polyol in a mixed solvent of 0.2 cc. of anhydr. dimethylformamide or dioxane and 0.2 cc. of pyridine was added with CCP. The mixture was heated at

7) R. S. Bandurski, B. Axelrod : J. Biol. Chem., **193**, 405(1951).

75~80° for 5 hr. An aliquot was withdrawn at intervals of 1, 2, and 5 hr. to apply to paper chromatography. The alcoholysis product on the paper chromatogram was detected by the reagent described above.

Isolation of the Alcoholysis Product of CCP with 1,2-Propanediol: (a) ***o*-Hydroxyphenyl 2-Hydroxypropyl Phosphate (IIb)**—A mixture of 17.6 g. (0.232 mole) of 1,2-propanediol and 2 g. (0.0116 mole) of CCP was shaken for 24 hr. at 37°. After neutralization with 10% Na_2CO_3 solution, the mixture was concentrated *in vacuo* to a syrup which was dissolved in 100 cc. of water.

The solution was treated with Amberlite IR-120 (H^+) in cold room and the acid solution obtained was kept at 37° for 5 min. to hydrolyze 1,2-propanediol cyclic phosphate to 2-hydroxypropyl phosphate. The resultant solution was immediately lyophilized, the residual syrup was dissolved in 150 cc. of *iso*-PrOH, and saturated with dry NH_3. The insoluble NH_4-salts (mainly consisting of ammonium 2-hydroxypropyl phosphate, *o*-hydroxyphenyl phosphate, and inorganic phosphate) were centrifuged and the supernatant was evaporated to dryness *in vacuo*. The residue was dissolved in 50 cc. of water and put on the top of a column (2.5×20 cm.) prepared with Amberlite IRA-400 (HO^-). After washing with ca. 1 L. of water, the column was eluted with satd. $Ba(OH)_2$ solution in a cold room. The alkaline effluent was immediately neutralized with CO_2, $BaCO_3$ that precipitated was removed, and the residue obtained on evaporation of the supernatant *in vacuo* was extracted with dehyd. MeOH (ca. 10 cc.). After filtration, the extract was added with dry acetone (40 cc.) and the solution was kept overnight in a refrigerator. The white powder that formed was collected by centrifugation and washed with dry acetone; yield, 1.2 g. (32.8%). The sample for analysis was dried to constant weight over P_2O_5 *in vacuo* at room temperature. *Anal.* Calcd. for $(C_9H_{12}O_6P)_2Ba$ (Barium *o*-hydroxyphenyl 2-hydroxypropyl phosphate): C, 34.19; H, 3.83; P, 9.81. Found: C, 33.92; H, 3.60; P, 9.90.

(b) **2-Hydroxypropyl Phosphate (IVb)**—A mixture of 4.4 g. (0.058 mole) of 1,2-propanediol and 1 g. (0.0058 mole) of CCP was heated at 100~110° in a Claisen flask for 7 hr. under a slightly reduced pressure. Excess of 1,2-propanediol was distilled off *in vacuo* and the residual syrup was dissolved in 50 cc. of water. The acid solution was set aside at 37° for 24 hr. to hydrolyze both 1,2-propanediol cyclic phosphate and *o*-hydroxyphenyl 2-hydroxypropyl phosphate to 2-hydroxypropyl phosphate. The hydrolysate was neutralized with satd. $Ba(OH)_2$ and the precipitate formed was centrifuged. The supernatant was concentrated to ca. 20 cc. and insoluble impurity was filtered off. To the filtrate was added 2 volumes of acetone, the white precipitate that appeared was redissolved in 10 cc. of water, and again precipitated with acetone; yield, 0.85 g. (50%). The sample for analysis was dried to constant weight at 100~110° over P_2O_5 *in vacuo*. *Anal.* Calcd. for $C_3H_7O_5PBa$ (Barium 2-hydroxypropyl phosphate): C, 12.35; H, 2.40; P, 10.63. Found: C, 12.52; H, 3.13; P, 10.88.

This sample was identified by paper chromatography with authentic sample of 2-hydroxypropyl phosphate. Rf_1 0.27, Rf_2 0.70.

1,2-Propanediol Cyclic Phosphate (IIIb) from *o*-Hydroxyphenyl 2-Hydroxypropyl Phosphate (IIb)—A mixture of 20 mg. of NH_4 salt of (IIb), 20 mg. of CCP (or anhydr. *p*-toluenesulfonic acid) and 0.2 cc. of anhydr. pyridine was heated in a sealed tube at 95~100° for 2 hr. The reaction mixture was applied as a line on 3 sheets of Toyo Roshi No. 53 filter paper (40×25 cm.), and run ascendingly at room temperature for 15 hr. using the solvent system (1). Each phosphate band separated on paper chromatogram was cut out and the cuttings were extracted with a small volume of water. The concentrated extracts were submitted to paper chromatography and paper electrophoresis for identification.

Isolation of Glycerol 1,2-Cyclic Phosphate (IIIc)—10.1 g. (0.11 mole) of freshly distilled glycerol was mixed with 20 cc. of anhydr. pyridine and 5.2 g. (0.03 mole) of CCP was added to the solution. The mixture was heated under stirring at 90~100° for 2 hr. With aliquot withdrawn from reaction mixture, the products and their yields were determined by paper chromatography.[6] Glycerol 1,2-cyclic phosphate (Rf_1 0.60; yield, 65.5%), and α- and β-glycerophosphate (Rf_1 0.23; yield, 7.0%) were found besides unreacted *o*-hydroxyphenyl phosphate (Rf_1 0.32; yield, 22.0%). From the reaction mixture the excess of glycerol and solvent were removed *in vacuo* and 40 cc. of water was added to the residual syrup. The aqueous solution was adjusted to pH 2.5~3.0 by addition of Amberlite IR-120 (H^+) and put on top of a column (1.6×20 cm.) of Amberlite IR-4B (HO^-). The column was eluted with 300 cc. of 10% NH_4OH after washing with ca. 500 cc. of water. The brownish effluent was evaporated to 50 cc. *in vacuo* and the insoluble coloring material was filtered with charcoal. The clear filtrate was passed through a column (2.7×30 cm.) of Amberlite IRC-50 (Ba^{++}) and the combined effluent and washings was concentrated to 20 cc. *in vacuo*. To the concentrate was added 5 cc. of 95% EtOH and the precipitate (mainly consisting of barium glycerophosphate, *o*-hydroxyphenyl phosphate, and inorganic P) was removed by centrifugation. The supernatant was evaporated to a syrup which was dissolved in 5 cc. of 50% MeOH-H_2O, this solution was placed on top of a column (2.4×25 cm.) of Toyo Roshi cellulose powder, prepared in

dehyd. MeOH, and eluted with the same solvent. The fractions containing glycerol 1,2-cyclic phosphate were collected, evaporated to a syrup which was dissolved in 5 cc. of water, and filtered. After removal of solvent from the filtrate, the residue was extracted with 10 cc. of hot dehyd. MeOH. On addition of 10 cc. of dry acetone the extract gave a powdery precipitate. The precipitate was washed twice with MeOH-acetone (1:2); yield, 1.86 g. (28.2%). The sample for analysis was dried at room temperature over P_2O_5 *in vacuo* for 6 hr. *Anal.* Calcd. for $(C_3H_6O_5P)_2Ba$ (Barium glycerol 1,2-cyclic phosphate): C, 16.23; H, 2.71; P, 13.95. Found: C, 16.63; H, 2.95; P, 14.16.

On detection on paper chromatogram, the sample obtained above was identified with authentic glycerol 1,2-cyclic phosphate. Rf_1 0.60, Rf_2 0.88.

Isolation of DL-Erythritol 1-Phosphate (IVd)—A mixture of 1.83 g. (0.015 mole) of anhydr. erythritol and 1.3 g. (0.0073 mole) of CCP in 50 cc. of dehyd. pyridine was warmed at 100~110° under vigorous stirring for 3 hr. Pyridine was removed by distillatoin *in vacuo* and the residual syrup was dissolved in 20 cc. of 1% H_2SO_4. The acid solution was refluxed for 1 hr. to hydrolyze both DL-erythritol 1,2-cyclic phosphate and CCP to DL-erythritol 1-phosphate and ohthophosphate, respectively. After neutrallization with satd. $Ba(OH)_2$, the hydrolysate gave a precipitate which was centrifuged and the supernatant was concentrated to 50 cc. The filtered condensate was added with same volume of 95% EtOH to precipitate barium DL-erythritol 1-phosphate. The white precipitate was washed with a small volume of dehyd. EtOH and dried in atmosphere; yield, 1.37 g. (55.7%).

0.6 g. of crude Ba salt obtained above was dissolved in 5 cc. of water and passed through a column (5×2 cm.) of Amberlite IR-120 (H^+). The combined acid effluent and washings was neutralized with aqueous cyclohexylamine and evaporated to dryness *in vacuo* below 35°. The brownish impurity was removed from the residue (0.5 g.) by extraction with 5 cc. of hot 95% EtOH to separate white precipitate. This was recrystallized from minimum volume of 95% EtOH to fine needles, m.p. 189°(decomp.); yield, 0.41 g. (31%). The sample for analysis was dried over P_2O_5 *in vacuo* for 2 hr. *Anal.* Calcd. for $C_{16}H_{37}O_7N_2P$ (Dicyclohexylammonium DL-erythritol 1-phosphate): C, 48.00; H, 9.25; N, 7.00; P, 7.75. Found: C, 48.02; H, 9.24; N, 7.20, P. 7.92. Rf_1 0.20, Rf_2 0.57.

On periodate oxidation, the sample obtained above consumed 1.99 molar equivalents of the reagent and the phosphate compound found in the oxidation product was identified with authentic glycol aldehyde phosphate[8,9] by paper chromatography.

Isolation of the Alcoholysis Product of CCP with D-Mannitol: (a) D-Mannitol 1-Phosphate (IVe)—A mixture of 9.1 g. (0.05 mole) of anhydr. D-mannitol in 200 cc. of anhydr. pyridine, and 1.72 g. (0.01 mole) of CCP was heated at 100~110° under vigorous stirring. After 30 and 90 min,. 1.72 g. each of CCP was added and the mixture was reacted for a total of 2.5 hr. The reaction mixture was concentrated *in vacuo* to a small volume and the concentrate was set aside in a refrigerator to separate unreacted D-mannitol. The syrupy filtrate freed from precipitate was evaporated to dryness and dissolved in 100 cc. of water. The solution was acidified by addition of Amberlite IR-120 (H^+) and refluxed for 2 hr. to hydrolyze both D-mannitol cyclic phosphate (Rf_1 0.50) and CCP. On analysis of the phosphorus compounds on paper chromatogram, this solution was detected to contain D-mannitol 1-phosphate (Rf_1 0.18, yield 70.4%), D-mannitol 1,6-diphosphate (Rf_1 0.04, yield, 9%), and orthophosphate (yield, 20.6%). After removal of the resin from the hydrolysate by filtration, the combined filtrate and washings was neutralized with satd. $Ba(OH)_2$ and the separated precipitate (largely $Ba_3(PO_4)_2$) was centrifuged. The supernatant was concentrated to ca. 150 cc. *in vacuo* and added with the same volume of 95% EtOH. The white precipitate (consisting of barium D-mannitol 1-phosphate with a small amount of D-mannitol 1,6-diphosphate) formed was collected and dried; yield, 7.15 g.

2.5 g. of crude Ba-salt was converted into NH_4-salt by treating with satd. $(NH_4)_2CO_3$. After removal of $BaCO_3$, the supernatant was concentrated to 5 cc. and placed on top of a column (30× 2.7 cm.) of Toyo Roshi cellulose powder which was prepared in a mixed solvent of *iso*-PrOH, water, and conc. NH_3 (7:2:1). The column was eluted with the same solvent mixture to collect 10-cc. fractions. The phosphorus-positive fractions giving a spot with Rf_1 0.18 were concentrated *in vacuo* to a small volume. The concentrate was decationized by passing through a column of Amberlite IR-120 (H^+) and the effluent was added with aqueous cyclohexylamine to pH 9. The mixture was evaporated to a syrup *in vacuo* below 35° and on addition of a small amount of 95% EtOH it solidified. The crystalline mass obtained was recrystallized from hot 95% EtOH to fine needles, m.p. 195°(decomp.); yield, 1.83 g. (37.8%). The sample for analysis was dried over P_2O_5 *in vacuo* at room temperature for 5 hr. *Anal.* Calcd. for $C_{18}H_{41}O_9N_2P$ (Dicyclohexylammonium D-mannitol 1-phosphate): C, 46.95; H, 8.98; N, 6.09; P, 6.74. Found: C, 46.35; H, 9.14; N, 6.00; P, 6.70. Rf_1 0.18, Rf_2 0.66.

8) J. Baddiley, *et al.*: Biochem. J., **64**, 601(1956).
9) H. S. Loring, *et al.*: J. Am. Chem. Soc., **78**, 3724(1956).

On periodate oxidation, this sample consumed 3.88 molar equivalents of the reagent and the phosphate compound found in the oxidation product was identified on paper chromatogram with authentic glycolaldehyde phosphate.

(b) **D-Mannitol 1,6-Diphosphate**—Among fractions obtained from cellulose column fractionation in above (a) the phosphate fractions giving a spot of Rf_1 0.04~0.05 were collected and evaporated to a small volume (20 cc.). The concentrate was passed through a column of Amberlite IR-120 (H^+) and the acid effluent was neutralized with cyclohexylamine. The mixture was evaporated to dryness *in vacuo* below 35° and 30 cc. of acetone was added to the syrupy residue dissolved in 10 cc. of dehyd. MeOH. The separated crystals were recrystallized from 50 cc. of dehyd. EtOH containing 0.5 cc. of water; needles (240 mg.), m.p. 206~207°(decomp.). The sample for analysis was dried over P_2O_5 *in vacuo* for 4 hr. *Anal.* Calcd. for $C_{24}H_{55}O_{12}N_3P_2$ (Tricyclohexylammonium D-mannitol 1,6-diphosphate): C, 45.04; H, 8.65; N, 6.56; P. 9.69. Found: C, 45.30; H, 8.91; N, 6.43; P, 9.96. Rf_1 0.05, Rf_2 0.17.

On periodate oxidation, the sample consumed 2.85 molar equivalents of the reagent and the phosphate found in the oxidation product was identified on paper chromatogram with authentic glycolaldehyde phosphate.

Summary

The alcoholysis of catechol cyclic phosphate (I) with several polyols was investigated. Ethylene glycol and 1,2-propanediol gave the corresponding hydroxyalkyl 1,2-cyclic phosphates (III) via intermediate hydroxyalkyl *o*-hydroxyphenyl phosphates (II). (III) were hydrolyzed by acid to the corresponding hydroxyalkyl phosphates (IV). In the case of the polyols containing more than three vicinal hydroxyl groups such as, glycerol, erythritol, and mannitol, the intermediates (II) were too labile to be detected on paper chromatogram of the reaction products, but converted into the corresponding cyclic phosphates (III) which were further hydrolyzed to (IV). Thus glycerol 1,2-cyclic phosphate, DL-erythritol 1-phosphate, and D-mannitol 1-phosphate were isolated and identified.

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77. Hideyo Shindo: Studies on the Infrared Spectra of Heterocyclic Compounds. VI.[1] Infrared Spectra of Substituted α-Pyridones and α-Quinolones. (1). The Region from 2000 to 4000 cm^{-1}.

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It is well known that α- and γ-hydroxypyridines and -quinolines are tautomeric with respect to the keto and the enol forms. Thus 2-hydroxypyridine (I) is tautomeric with 2-pyridone (II). Much study has been devoted to the structure of these compounds, and it has been established by infrared[2~5] and ultraviolet[6~8] spectroscopy, ionization constants,[9,10] dipole moment,[9] and X-ray analysis[11] that these compounds exist pre-

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1) Part V. H. Shindo: This Bulletin, **6**, 117(1958).
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5) S.F. Mason: J. Chem. Soc., **1957**, 4874.
6) G.W. Ewing, E.A. Steck: J. Am. Chem. Soc., **68**, 2181(1946).
7) H. Ley, H. Specker: Ber., **72**, 197(1939), H. Specker, H. Gawrasch: *Ibid.*, **75**, 1338(1942).
8) S.F. Mason: J. Chem. Soc., **1957**, 5010.
9) A. Albert, J.N. Philips: *Ibid.*, **1956**, 1294 (Approximate ratio of amide to enol tautomer was calculated as 340 and 3000 for 2-hydroxy-pyridine and -quinoline, respectively, in neutral aqueous solution).
10) S.F. Mason: J. Chem. Soc., **1958**, 674.
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