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75. Kinzo Nagasawa: Organic Phosphates. VI.[1] Alcoholysis of Catechol Cyclic Phosphates. (1).

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In the previous papers of this series, the author reported the syntheses and alcoholyses of 1,2-cyclic phosphates of several diols.[1~3] Among those the acid catalyzed alcoholysis of hydrobenzoin cyclic phosphate (HBCP[*2]) (I), with subsequent hydrolysis[4] or hydrogenolysis of the product was found to be available for the phosphorylation of simple alcohols according to the reactions represented in Chart 1.

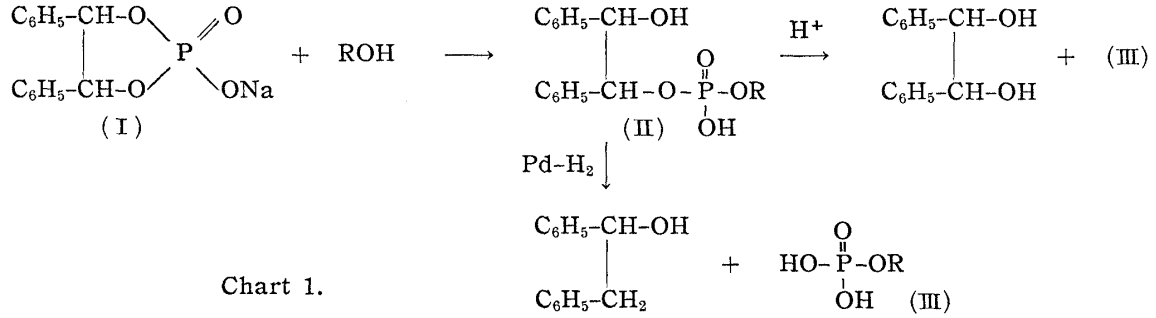

Chart 1.

This interesting property of HBCP led the author to test similar reactions for catechol cyclic phosphate (CCP) (IV), an *ortho*-phenolic cyclic phosphate. This paper deals with the results on these reactions which were found somewhat different from those observed for HBCP.

For the synthesis of CCP, a modified method of Cherbuliez and Schwarz[5] gave the desired compound in 70% yield.

The alcoholysis reactions of CCP were carried out using free acid CCP and alcohols instead of the sodium salt of (I) and acid catalyst used in the case of HBCP. Several mono-ols were mixed with CCP and reacted at room temperature or boiling point of the alcohols. In the cases of *tert*-butanol and phenol, dioxane was added as a solvent. The results are summarized in Table I.

The paper chromatographic detections of the phosphorus products showed that in all cases except for *tert*-butanol and phenol, products with larger Rf values corresponding to the alcohols used were found besides the spot of *o*-hydroxyphenyl phosphate (*o*-HPP)

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*2 Following abbreviations are used: HBCP, hydrobenzoin cyclic phosphate; CCP, catechol cyclic phosphate; *o*-HPP, *o*-hydroxyphenyl phosphate.

1) T. Ukita, K. Nagasawa, M. Irie: J. Am. Chem. Soc., **80**, 1373(1958).
2) T. Ukita, K. Nagasawa, M. Irie: This Bulletin, **5**, 121(1958).
3) T. Ukita, K. Nagasawa, M. Irie: *Ibid.*, **5**, 208(1958).
4) T. Ukita, H. Watanabe, M. Mikoshiba: Unpublished work.
5) E. Cherbuliez, H. Schwarz: Helv. Chim. Acta, **34**, 841(1951).

Table I. Alcoholysis Reaction of Catechol Cyclic Phosphate with Various Alcohols

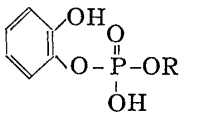
Solution spotted	Product of hydrolysis		Product of alcoholysis (Rf value)
	Inorg. P	*o*-HPP (Rf 0.32)	
CCP+dioxane	M	S	
o-HPP+dioxane	M	S	
CCP+MeOH	M	M	S (0.64)
CCP+EtOH	M	S	S (0.75)
CCP+*n*-PrOH	M	S	S (0.85)
CCP+*iso*-PrOH	M	S	S (0.85)
CCP+*n*-BuOH	M	S	S (0.86)
CCP+*tert*-BuOH	M	S	
CCP+cyclohexanol	M	M	S (0.87)
CCP+benzyl alcohol	M	M	S (0.87)
CCP+Phenol	M	S	

S: strong, M: medium.

(VI) (Rf 0.32). These results reveal that both primary and secondary alcohols are active to alcoholyse CCP. After above preliminary experiment for the detection of the products, reactions in preparative scales for ethanol, propanol, isopropanol, cyclohexanol, and benzyl alcohol were carried out and the main product was isolated by separating from by-products, *o*-HPP and inorganic phosphate, using the different solubility of their ammonium salts in isopropanol.

From the analysis and results of hydrolysis of these alcoholysis products, they were found to be alkyl *o*-hydroxyphenyl phosphate having corresponding alkyl group of the alcohol used. The reaction conditions, yield, and properties of these phosphodiesters are summarized in Table II.

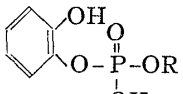
Table II.

ROH used	Reaction conditions			Alcoholysis product 	Yield (%)
	ROH/CCP mol. ratio	React. temp. (°C)	Time (hr.)		
EtOH	20	reflux	7	R=C_2H_5, m.p. 164~166°, Plates	56
n-PrOH	30	reflux	7	R=*n*-C_3H_7, m.p. 140°, Needles	47
iso-PrOH	20	reflux	8	R=*iso*-C_3H_7, m.p. 142, Needles	55
Cyclohexanol	20	reflux	10	R=C_6H_{11}, Ba-salt, Needles	65
Benzyl alcohol	20	25~30	24	R=C_7H_7, m.p. 156~158°, Plates	55

The type of reactions in acid and alkaline hydrolyses of these alkyl *o*-hydroxyphenyl phosphates was also different from that of 1,2-diphenyl-2-hydroxyethyl alkyl phosphates (II) previously reported.

In the case of (II), as shown in Chart 1, especially in acid reaction, the hydrolysis occurred at O-P linkage binding hydrobenzoin moiety to give monoalkyl phosphate (III) as a main product. On the contrary, all alkyl *o*-hydroxyphenyl phosphates (V) obtained this time were hydrolyzed both in acid and alkaline conditions at the O-P bond bearing alkyl groups to give *o*-HPP (VI) as a main product (Chart 2 and Table III).

Table III. Acid and Alkaline Hydrolyses of Alkyl *o*-Hydroxyphenyl Phosphate

Substrate 	Hydrolysis at 95~100°							Hydrolysis products containing phosphorus	
	with 1*N*HCl (min.)				with 1*N*NaOH (min.)				
	5	10	30	60	30	60	120		
R=C_2H_5	++	++	+++	+++	±	+	+	*o*-HPP,	orthophosphate
R=*n*-C_3H_7	++	++	+++	+++	−	−	−	〃	〃
R=*iso*-C_3H_7	+	++	++	+++	−	−	−	〃	〃
R=C_6H_{11}	+	+	++	+++	−	−	−	〃	〃
R=C_7H_7	+++	+++	+++	+++	+	+	++	〃	〃

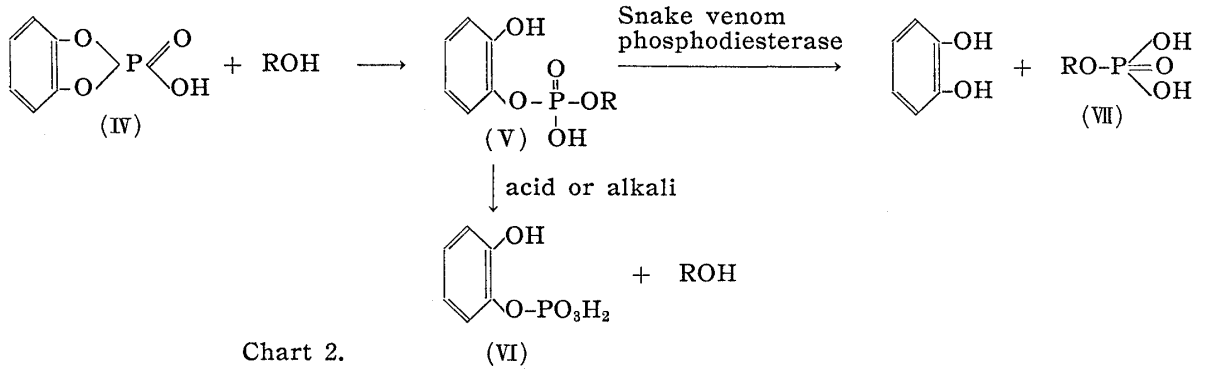


Chart 2.

Thus, the mode of hydrolysis of alkyl *o*-hydroxyphenyl phosphate is similar to those generally observed for the naturally occurring and synthetic phosphodiesters which have a C-hydroxyl group vicinal to one of the phosphate-bearing carbinol, such as glyceryl-phosphorylcholine[6,7] and ribonucleic acid.[8-11]

On catalytic hydrogenation of these alkyl *o*-hydroxyphenyl phosphates using platinum catalyst, no evidence for hydrogenolysis of O-P bond to give alkyl phosphates was observed. The indifference of this type of phosphodiesters against catalytic hydrogenolysis should be attributed to the free phenolic radical which interferes this reaction.

Besides the mode of hydrolysis and hydrogenolysis of these two types of phosphodiesters (II and V), enzymatic hydrolysis of these esters was investigated (Table IV). On

Table IV. Enzymatic Hydrolysis of Alkyl *o*-Hydroxyphenyl Phosphate

Substrate	Incubation at 37° (hr.) 5	10	15	24	48	Hydrolysis product containing P
R=C_2H_5	±	+	++	++	++	R=C_2H_5
R=*n*-C_3H_7	±	+	++	++	++	R=*n*-C_3H_7
R=*iso*-C_3H_7	−	±	+	++	++	R=*iso*-C_3H_7
R=C_6H_{11}	−	±	+	++	++	R=C_6H_{11}
R=C_7H_7	+	++	+++	+++	+++	R=C_7H_7
o-HPP						orthophosphate

Signs indicate the grades of hydrolysis observed on paper chromatograms, −, ±, +, ++, +++, +++, indicating nil, ca. 5%, ca. 25%, ca. 50%, and 100% hydrolysis.

incubation of these phosphodiesters with crude preparation of phosphodiesterase*[3] of snake venom (*Trimeresurus flavoviridis*), both alkyl *o*-hydroxyphenyl phosphate and alkyl 1,2-diphenyl-2-hydroxyethyl phosphate were found to be hydrolyzed by the enzyme giving monoalkyl phosphate (III or VIII), while alkyl hydroxyalkyl phosphodiesters or aliphatic cyclic phosphates, such as glycerol methyl phosphate, methyl 2-hydroxypropyl phosphate, or glycerol 1,2-cyclic phosphate, were found inert to the enzyme.[12]

The author is indebted to Mr. B. Kurihara and Miss R. Ohta for carrying out microanalyses.

6) A. Fono : Arkiv. Kemi Mineral. Geol., **24A**, No. 33, 14, 15(1947).
7) D.M. Brown, A.R. Todd : J. Chem. Soc., **1952**, 52.
8) R. Markham, J.D. Smith : Biochem. J., **52**, 552(1952).
9) D. Lipkin, P.T. Talbert : Chem. & Ind. (London), 143(1952).
10) D.M. Brown, A.R. Todd : J. Chem. Soc., **1952**, 44.
11) H.G. Khorana, G.M. Tener, R.S. Wright, J.G. Moffat : J. Am. Chem. Soc., **79**, 430(1957).
12) H. Ikezawa, A. Ohsaka, T. Ukita : Unpublished work.

*3 Kind gift of Dr. D. Mizuno, Department of Biochemistry, National Institute of Health, Tokyo.

Experimental

Catechol Cyclic Phosphate (IV)—A mixture of 13.2 g. of catechol (dried over P_2O_5 *in vacuo*) and 7.8 g. of P_2O_5 was heated at 120° in Claisen-type flask under a slightly reduced pressure. After 2 hr., the bath temperature reached 200° and pressure, 10 mm. Hg. After distillation of a small amount of unreacted catechol, a colorless vitreous distillate was obtained as a fraction of $b.p_3$ 218~220° in 14 g. (68%) yield. *Anal.* Calcd. for $C_6H_5O_4P$ (Catechol cyclic phosphate): C, 41.86; H, 2.93. Found: C, 41.51; H, 3.30.

(IV) thus obtained is very hygroscopic and on keeping in air gives *o*-HPP (VI) which further converts to catechol and inorganic phosphate. However, (IV) was stable for 6~12 months under exclusion of moisture.

Paper Chromatography—A sample containing 10~40 γ of P was applied on Toyo Roshi No. 53 filter paper and run ascendingly for 15 hr. with a solvent system of *iso*-PrOH : conc. NH_4OH : H_2O (7:1:2). Phosphorus was detected by the method of Bandurski and Axelrod,[14] and phosphates containing *o*-hydroxyphenyl group were detected also by spraying $FeCl_3$ solution.

Alcoholysis of Catechol Cyclic Phosphate with Several Monofunctional Alcohols—To 0.2 cc. each of MeOH, EtOH, *n*-PrOH, *iso*-PrOH, BuOH, *tert*-BuOH, cyclohexanol, benzyl alcohol, and phenol, 5~10 mg. of CCP was added. The mixture (contained 0.2 cc. of dioxane in the case of *tert*-BuOH and phenol) was kept at room temperature (15~20°) for 48 hr. and tested by paper chromatography. As a control a solution of CCP in dioxane was used. CCP was converted into *o*-HPP by hydrolysis during chromatography.

Isolation of Alcoholysis Product of CCP with Ethanol, Propanol, and Isopropanol—A mixture of 0.4 mole of each alcohol and 0.02 mole of CCP was refluxed under exclusion of moisture for 5~10 hr. From each mixture, excess of alcohol was removed by distillation and the residue was dissolved in 100 cc. of dehyd. *iso*-PrOH. The solution was saturated with NH_3 and the precipitated ammonium-*o*-HPP was removed by centrifugation. The supernatant was concentrated to a small volume and crystals were separated and recrystallized from dehyd. *iso*-PrOH. *Anal.* Calcd. for $C_8H_{14}O_5NP$ (Ammonium ethyl *o*-hydroxyphenyl phosphate): C, 40.85; H, 6.00; N, 5.95; P, 13.19. Found: C, 41.22; H, 6.16; N, 5.89; P, 13.09. *Anal.* Calcd. for $C_9H_{16}O_5NP$ (Ammonium propyl *o*-hydroxyphenyl phosphate and isopropyl *o*-hydroxyphenyl phosphate): C, 43.37; H, 6.48; N, 5.62; P, 12.45. Found (Ammonium propyl *o*-hydroxyphenyl phosphate): C, 43.38; H, 6.43; N, 5.62; P, 12.01. Found (Ammonium isopropyl *o*-hydroxyphenyl phosphate): C, 43.56; H, 6.55; N, 5.80; P, 12.30.

Isolation of Alcoholysis Product of CCP with Cyclohexanol—A mixture of 20 cc. of dehyd. cyclohexanol and 3.5 g. of CCP was refluxed for 10 hr. After removal of unreacted cyclohexanol by distillation, the residue was dissolved in 150 cc. of dehyd. *iso*-PrOH. The solution was saturated with NH_3 as above and the insoluble ammonium salts centrifuged. The supernatant, after concentration *in vacuo*, was extracted with ether to remove cyclohexanol and the aqueous layer was passed through a column of Amberlite IR-120 (H^+). The acid solution thus obtained was neutralized with satd. $Ba(OH)_2$ solution and evaporated to dryness *in vacuo*. The residue was extracted with 15 cc. of dehyd. MeOH and to the extract was added dry acetone. On keeping in a refrigerator overnight, microcrystals separated which were recrystallized from MeOH-acetone. *Anal.* Calcd. for $(C_{12}H_{16}O_5P)_2Ba$ (Barium cyclohexyl *o*-hydroxyphenyl phosphate): C, 42.35; H, 4.74; P, 9.62. Found: C, 42.03; H, 4.86; P, 9.18.

Isolation of Alcoholysis Product of CCP with Benzyl Alcohol—A mixture of 25 g. of benzyl alcohol (freshly distilled) and 2 g. of CCP was reacted at room temperature (25~30°) for 24 hr. After neutralization with 5% Na_2CO_3, unreacted benzyl alcohol was extracted with ether, and the aqueous layer was treated with Amberlite IR-120 (H^+) under cooling. The acid solution thus obtained was immediately lyophilized and residual syrup dissolved in 100 cc. of *iso*-PrOH was saturated with dry NH_3. After removal of insoluble ammonium salt the supernatant was evaporated to dryness. The residue obtained was dissolved in a minimum volume of dehyd. EtOH and added with dry acetone. The crystals separated were recrystallized from dehyd. EtOH-ether. *Anal.* Calcd. for $C_{13}H_{16}O_5NP$ (Ammonium benzyl *o*-hydroxyphenyl phosphate): C, 52.50; H. 5.42; P, 10.79. Found: C, 52.34; H, 5.53; P, 10.35.

Acid and Alkaline Hydrolysis of Alkyl *o*-Hydroxyphenyl Phosphate (V)—About 10 mg. of each *o*-hydroxyphenyl phosphates was dissolved in 0.5 cc. of 1*N* HCl and 1*N* NaOH, respectively. The solution was heated on a boiling water bath and 0.2-cc. aliquot was taken at intervals and examined by paper chromatography.

Enzymatic Hydrolysis of Alkyl *o*-Hydroxyphenyl Phosphate (V)—A mixture consisting of 5.4 cc. of NH_4Cl-ammonia buffer (0.1*M*, pH 9.0), 3.6 cc. of enzyme solution (0.2%), 1.8 cc. of $Mg(OAc)_2$

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

(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).