the wave length shift by introduction of substituents. In infrared spectra the bands between 11~15 μ were analyzed as out-of-plane bending vibrations of the aromatic CH groups.

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## 17. **Tyunoshin Ukita, Kinzo Nagasawa, and Masachika Irie :** Organic Phosphates. I. Synthesis of 1,2-Diol Cyclic Phosphates.

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Isolation and identification of cyclic 2′,3′-nucleotides as intermediates in the mild alkaline hydrolysis of ribonucleic acid were reported by Markham and Smith.[1] Baer and Kates[2] proposed glycerol 1,2-cyclic phosphate (GCP) (I) as an intermediate in the acid hydrolysis of lecithin to glycerol-L-1, -DL-1 and -2 phosphates.

Recently, Ukita, Bates, and Carter,[3] assuming that GCP should also be an intermediate of the alkaline breakdown of lecithin analogs, tried to isolate this compound. Lecithin (II), L-α-glycerylphosphorylcholine (III), and benzyl-GP[4] (IV) were hydrolysed in various solvents under alkaline conditions. In no case, however, was GCP isolated from the hydrolysis product, although its presence in minor amount was qualitatively detected by paper chromatographic identification with synthetic GCP only in the case of the hydrolysis of (III) and (IV).

The instability of the synthetic GCP in alkaline solutions made it appear unlikely that the cyclic ester can be isolated from alkaline hydrolysates of lecithin.

Several researches on the chemical[5] and enzymatic[6] hydrolysis of cyclic nucleotides as well as on their properties as substrates for enzymatic synthesis of polynucleotides have been reported.[7] However, no investigations have been made on synthesis and properties of cyclic phosphates of simple aliphatic 1,2-diols other than that of glycerol, ones which are also important in biochemical respects.

This paper deals with the synthesis of 1,2-propanediol cyclic phosphate (PCP) (V), ethyleneglycol cyclic phosphate (ECP) (VI), and 2,3-butanediol cyclic phosphate (BCP) (VII), in order to have some evidence on the relationship between chemical or enzymatic properties of these products and their structural differences, which will be reported in subsequent papers.

Two methods have been developed for synthesizing 1,2-diol cyclic phosphates. In

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[1] R. Markham, J.D. Smith : Biochem. J. (London), **52**, 552(1952).

[2] E. Baer, M. Kates : J. Biol. Chem., **185**, 615(1950).

[3] T. Ukita, N.A. Bates, H.E. Carter : J. Biol. Chem., **216**, 867(1955).

[4] Following abbreviations are used : GCP=glycerol 1,2-cyclic phosphate, PCP=1,2-propanediol cyclic phosphate, ECP=ethyleneglycol cyclic phosphate, BCP=2,3-butanediol cyclic phosphate, EP=ethyleneglycol phosphate, BP=2,3-butanediol phosphate, GP=glycerol phosphate, PP=1,2-propanediol phosphate (preceded by alkyl or cation substituted or number of the position of hydroxyl group concerned with phosphoryl ester where necessary), and TFA=trifluoroacetic acid.

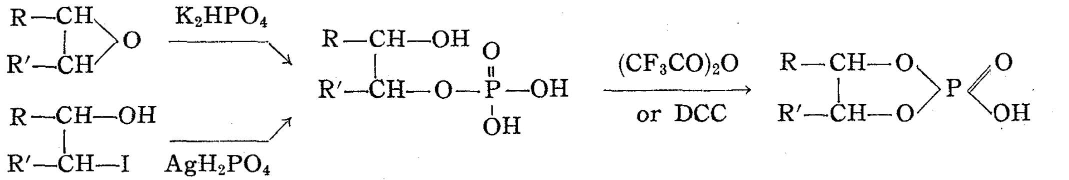
[5] L.A. Heppel, P.R. Whitfeld, R. Markham : Biochem. J. (London), **60**, 21(1955).

[6] C.A. Dekker : Fed. Proc., **13**, 197(1954); R. McDonald : Biochim. et Biophys. Acta, **18**, 139(1955); D.M. Brown, C.A. Dekker, A.R. Todd : J. Chem. Soc., **1952**, 2715; D.M. Brown, A.R. Todd : *Ibid.*, **1952**, 44.

[7] P.R. Whitfeld, L.A. Heppel, R. Markham : Biochem. J. (London), **60**, 15(1955); R. Markham, J.D. Smith : *Ibid.*, **52**, 558(1952).

the first method a monophosphate of 1,2-diol is dehydrated with a suitable dehydration reagent such as trifluoroacetic anhydride[3,8] or dicyclohexyl carbodiimide (DCC).[9] Trifluoroacetic anhydride was used in the previous experiment by Ukita, *et al.*[3] in the synthesis of GCP from β-GP. However, because of the instability of the reagent and the low yield of the product, the dehydration in the present work was preformed by DCC.

In the synthesis of GCP, a mixture of α- and β-GP was dehydrated with DCC in acetonitrile and after removal of unreacted, less-soluble barium-GP, the water-soluble barium-GCP was isolated in a yield of 33%. The trace of impurities detected on the paper chromatogram of the product was removed by subsequent column chromatography with Solka-Floc[10] and methanol.



(VIII) R=CH$_3$, R′=H
(IX) R=R′=H

(I) R=CH$_2$OH, R′=H (GCP)
(V) R=CH$_3$, R′=H (PCP)
(VI) R=R′=H (ECP)
(VII) R=R′=CH$_3$ (BCP)

In order to synthesize PCP (V) by this method, 1,2-propanediol-1 phosphate (α-PP) (VIII) had to be prepared. This compound had already been reportedly synthesized by both Todd[11] and Lardy[12] from 1,2-epoxypropane and dipotassium hydrogen phosphate without detailed descriptions on the properties of the product. α-PP obtained by this method showed Rf values of Rf$_1$ 0.27, Rf$_2$ 0.58, and Rf$_3$ 0.83[14] and gave the cyclohexylamine salt, $C_{15}H_{35}O_5N_2P\cdot{}^1/_2H_2O$, with m.p. 169~172°(decomp.). It was identified with the product synthesized from 1-iodo-2-propanol and monosilver phosphate.

The free α-PP thus obtained was dehydrated with DCC and the product, PCP, was isolated as the crystalline barium salt, $(C_3H_6O_4P)_2Ba$, in a yield of 54%. PCP showed Rf$_1$ 0.63 and Rf$_2$ 0.68.

Ethyleneglycol cyclic phosphate (ECP) (VI) was obtained also by the same type of reaction. The starting material, ethyleneglycol-1 phosphate (EP) (IX) was prepared from epoxyethane and dipotassium phosphate according to the report by Todd, *et al.*[11] (IX) showed Rf$_1$ 0.22 and gave the cyclohexylamine salt, $C_{14}H_{33}O_5N_2P\cdot{}^1/_2H_2O$, with m.p. 167~169°. The dehydration of (IX) with DCC was performed in a mixture of acetonitrile and aqueous pyridine and the product was purified as the barium salt by chromatography on Solka-Floc with aqueous isopropanol. Barium-ECP was obtained as needles, $(C_2H_4O_4P)_2Ba$, after reprecipitation with acetone from aqueous solution and it showed Rf$_1$ 0.55.

Another method of synthesis of cyclic phosphates consists of the direct phosphorylation of 1,2-diols with phosphoryl chloride. However, by this method usually the reaction product contains several by-products other than the desired cyclic phosphate, and sometimes no cyclic phosphate is found in the product even under the controlled reaction conditions. Thus, ECP was not obtained from ethyleneglycol and phosphoryl chloride.

PCP was synthesized by this method, however. When a pyridine solution of phos-

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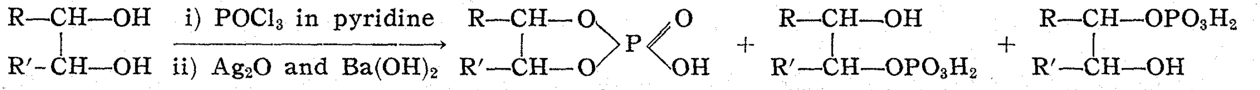
8) D.M. Brown, D.I. Magrath, A.R. Todd: J. Chem. Soc., **1952**, 2708.
9) H.G. Khorana: Chem. Revs., **53**, 145(1953).
10) The commercial cellulose powder manufactured by Brown Company, Boston, Mass., U.S.A.
11) F.R. Atherton, H.T. Openschow, A.R. Todd: J. Chem. Soc., **1945**, 385.
12) G.P. Lampson, H.A. Lardy: J. Biol. Chem., **181**, 693, 697(1949).

phoryl chloride was added to the pyridine solution of 1,2-propanediol the mixture of the reaction products showed five spots of phosphorus compounds with $Rf_1$ 0.63, 0.27, 0.36, 0.78, and 0.55 on a paper chromatogram. The first spot with $Rf_1$ 0.63 was that of the main product PCP, and the next two spots with $Rf_1$ 0.27 and 0.36 were proved to be those of (VIII) and (X), respectively.



(V) R=$CH_3$, R′=H
(VII) R=R′=$CH_3$

(VIII) R=$CH_3$, R′=H
(XI) R=R′=$CH_3$

(X) R=$CH_3$, R′=H

The last two faint spots with $Rf_1$ 0.78 and 0.55 were those of the by-products with minor yields, the structures of which need further study.

In order to separate PCP from the mixture, after hydrolysis and removal of chlorine with silver oxide, the phosphate mixture was converted to pyridinium salts and dissolved in anhydrous isopropanol. On saturation of this solution with ammonia, isopropanol-insoluble ammonium salts precipitated. The mixed ammonium salts of (VIII) and (X) thus precipitated were separated and converted into cyclohexylamine salts. By repeated recrystallization from hydrous acetone, the cyclohexylamine salt of (X) was isolated which showed $Rf_1$ 0.36, $Rf_2$ 0.65, and $Rf_3$ 0.93. The barium salt of (X) obtained from the latter was analysed to give the molecular formula of $C_3H_7O_5PBa$.

The isopropanol-soluble ammonium salts contained those of PCP and two side products with $Rf_1$ 0.78 and 0.55, but the amounts of the side products, in this case, were small enough not to interfere with further isolation of PCP. Thus after conversion into barium salts, the barium-PCP was purified by reprecipitation from ethanol with dry acetone.

To synthesize 2,3-butanediol cyclic phosphate (BCP) (VII), 2,3-butanediol was phosphorylated with phosphoryl chloride in pyridine. The isopropanol-soluble ammonium salt of (VII) was separated and converted into the barium salt, which showed $Rf_1$ 0.65 and $Rf_2$ 0.68.

The isopropanol-insoluble fraction of the ammonium salts was also converted to the barium salt and after removal of inorganic phosphate, butanediol phosphate (BP) (XI) was purified as the cyclohexylamine salt, $C_{16}H_{37}O_5N_2P\cdot{}^1/_2\,H_2O$, with m.p. 217~219° and $Rf_1$ 0.31 and $Rf_2$ 0.63.

As the barium salt of BCP obtained above did not give the desirable analytic data even after repeated purification, presumably because of the contamination with a trace of 2,3-butanediol, (XI) was dehydrated with DCC to BCP. The procedure for the dehydration was entirely the same as in the case of that type of reaction for the synthesis of PCP. The barium salt of BCP was purified as a white powder, which gave the molecular formula $(C_4H_8O_4P)_2Ba$ and showed $Rf_1$ 0.65 and $Rf_2$ 0.68.

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

## Experimental

**Glycerol 1,2-Cyclic Phosphate** (I) —A 5% aqueous solution of commercial Na-β-GP (containing ca. 23% of Na-α-GP) was passed through a column of $H^+$-type Amberlite IR-120 resin and the effluent was lyophilized. 4.5 g. of viscous GP was dissolved in a mixture of 50 cc. of $CH_3CN$ and 5 cc. of pyridine. To the solution, 5.5 g of DCC was added and dissolved with shaking. After 48 hrs., the mixture was diluted with water to 100 cc. and filtered to remove dicyclohexylurea. The filtrate was adjusted to pH 9 with $Ba(OH)_2$ and the precipitate which occurred was centrifuged off. After purification, 1.8 g. of Ba-GP was recovered from the precipitate. The supernatant was saturated with $CO_2$ to remove excess $Ba(OH)_2$ and centrifuged. After removal of $BaCO_3$ the solution was evaporated on a water bath *in vacuo* below 30° to remove organic solvents.

Dicyclohexylurea precipitated by this treatment was again removed by filtration and on lyophilization of the filtrate 1.45 g. of a white powder was obtained.[13] A solution of 0.9 g. of this powder in a small quantity of dil. MeOH was placed on top of a Solka-Floc column (2×33 cm.) prepared in MeOH. The column was eluted with MeOH at a flow rate of 5 cc. per 15 mins. and 5-cc. fractions were collected. Solvents were removed *in vacuo* from fraction Nos. 28~89 which gave positive P tests, and on trituration with acetone the residual viscous material turned into a white powder. After centrifugation and drying over $P_2O_5$ the powder weighed 500 mg. The product was recrystallized from dil. EtOH to give microscopic crystals. The sample was dried *in vacuo* for 3 hrs. over $P_2O_5$ at room temperature. *Anal.* Calcd. for $(C_3H_6O_5P)_2Ba$ : C, 16.23; H, 2.71; P, 13.95. Fouud : C, 16.35; H, 2.86; P, 13.67. $Rf_1$ 0.60.[14]

**1,2-Propanediol-1 Phosphate (VIII)** —1) A mixture of 2 g. of 1,2-epoxypropane, 12 g. of $K_2HPO_4$, and 50 cc. of water was heated at 110~120° for 10 hrs. in a sealed tube. After reaction, the mixture was added with $Ba(OH)_2$ solution to pH 8.0. The excess of $Ba(OH)_2$ was removed by saturation with $CO_2$, and centrifugation of the precipitate followed.

The supernatant was concentrated *in vacuo* at 50~60° on a water bath to a small bulk. The solution was filtered to remove a small amount of additional precipitate and the filtrate was added to its 5 volumes of EtOH to give a white powder. A mixture of 100 cc. of water and 20 cc. of $H^+$-type Dowex-50 resin was added to this powder and the mixture was heated at 100° for 75 mins. This treatment allowed the decomposition of bis(1,2-propanediol-1) phosphate ($Rf_1$ 0.74) into (Ⅷ). After removal of the resin by filtration, the filtrate was lyophilized to give (Ⅷ) as a viscous oil which revealed only one spot ($Rf_1$ 0.27, $Rf_2$ 0.58, $Rf_3$ 0.83) on paper chromatogram.

2) To a suspension of 2.27 g. of $Ag_3PO_4$ in 15 cc. of dehyd. ether, 1.0 cc. of 90% $H_3PO_4$ was added with stirring. The mixture was kept cooled at 5°, and added dropwise into a solution of 2 g. of 1-iodo-2-propanol in 20 cc. of dehyd. ether with vigorous stirring during 1 hr. After an additional stirring for 1 hr. at room temperature, the reaction mixture was concentrated into a small bulk and the residue was extracted with water. The extract was made alkaline by addition of $Ba(OH)_2$ solution. The excess of $Ba(OH)_2$ was precipitated with $CO_2$. $BaCO_3$ and Ba salt of inorganic phosphoric acid were removed by filtration. The filtrate was concentrated into a small volume and treated with $H^+$-type Amberlite IR-120 to remove cations. The acidic solution was added with cyclohexylamine to make pH 9~10 and lyophilized.

The residual 1.27 g. of cyclohexylamine salt was recrystallized from hydrated acetone to 0.86 g. of long needles, m.p. 169~172°(decomp.). The sample for analysis was dried to constant weight over $P_2O_5$ *in vacuo* at room temperature. *Anal.* Calcd. for $C_{15}H_{35}O_5N_2P \cdot \frac{1}{2}H_2O$ : C, 49.54; H, 9.99; N, 7.70; P, 8.53. Found : C, 49.33; H, 9.91; N, 7.71; P, 8.02. $Rf_1$ 0.26, $Rf_2$ 0.58, $Rf_3$ 0.83.

**1,2-Propanediol Cyclic Phosphate (V)** —1) To a mixture of 0.24 g. (1.0 mole) of (Ⅷ), 8 cc. of $CH_3CN$, 2.5 cc. of pyridine, and 0.1 cc. of water, a solution of 0.2 g. (1.1 moles) of DCC in 5 cc. of $CH_3CN$ was added and the mixture was kept at room temperature for 1 hr. After addition of 20 cc. of water, the precipitated dicyclohexylurea was removed from the reaction mixture by filtration. The solvent was removed by distillation *in vacuo* and the residue was dried, leaving a syrupy oil. This was dissolved in 25 cc. of dehyd. *iso*-PrOH and saturated with $NH_3$. The precipitate which occurred was removed by centrifugation and the supernatant was dried *in vacuo*. The aqueous solution of the *iso*-PrOH-soluble ammonium salt thus obtained was passed through a column of Amberlite IRC-50 ($Ba^{++}$-type) (50×1.5 cm.) to convert the product into the Ba-salt. On lypophilization of the effluent, 0.17 g. (54%) of Ba-PCP was obtained.

To the residue was added a few drops of water and the insoluble impurity was centrifuged off. 10 cc. of dehyd. acetone was added to the supernatant and the solvent was evaporated with a current of air. The syrupy residue was kept in a refrigerator to solidify into crystals. The sample for analysis was dried over $P_2O_5$ *in vacuo* at 100~110° to constant weight. *Anal.* Calcd. for $(C_3H_6O_4P)_2Ba$ : C, 17.50; H, 2.94; P, 15.07. Found : C, 17.93; H, 3.18; P, 15.76. $Rf_1$ 0.63, $Rf_2$ 0.68.

2) To a solution of 6.84 g. (1 mole) of 1,2-propanediol in 60 cc. of pyridine under chilling to −20°

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[13] On detection of phosphate by paper chromatography, this powder showed three spots with $Rf_1$ 0.23, 0.60 and 0.91. The first spot corresponded to GP, the second spot was that of the main product, GCP, and the third faint spot with $Rf_1$ 0.91 seemed to correspond to glycerophosphoryl derivatives of dicyclohexylurea (cf. C. A. Dekker : J. Am. Chem. Soc., **76**, 3522(1954) and G. M. Tener, H. G. Khorana : J. Am. Chem. Soc., **77**, 5349(1955)), because this spot appeared when excess of DCC was used or $CH_3CN$ lacked in reaction solvent; moreover the by-products was unstable in an acid or alkaline environment and this powder contained ca. 3.27% nitrogen.

[14] Paper chromatography : 10~40 $\gamma$ of P was applied to Toyo Roshi No. 3 filter paper and run ascendingly 15 hrs. with solvent systems of *iso*-PrOH : 5*N* $NH_4OH$ (2 : 1) (1), *iso*-PrOH : *tert*-BuOH : conc. $NH_4OH$ : $H_2O$ (40 : 20 : 1 : 39) (2), or *tert*-BuOH : $H_2O$ : picric acid (80 : 20 : 4 g.) (3). (Phosphorus was detected by the method of Bandurski and Axelrod (J. Biol. Chem., **193**, 405 (1951)). In this paper, the Rf values of P compounds found for each of these solvent systems are represented with the abbreviations $Rf_1$, $Rf_2$, and $Rf_3$, respectively.

and stirring, a solution of 13.8 g. (1 mole) of $POCl_3$ in 90 cc. of pyridine was added dropwise during 20 mins. After additional chilling and stirring for 1 hr., the mixture was set aside for 30 mins. at room temperature. Pyridine hydrochloride which precipitated was filtered off and the filtrate was concentrated to a small bulk *in vacuo* on a water bath at 50~60°.

To the residue was added a suspension of 30 g. of $Ag_2O$ in 200 cc. of ice water and the mixture was shaken to complete the hydrolysis. The precipitate (AgCl and $Ag_3PO_4$) was removed by filtration and the filtrate was extrated 3 times with ether. The water layer was treated with $H_2S$ to remove Ag ion and after removal of $H_2S$ by aeration, the silver-free aqueous solution was lyophilized.[15]

The phosphate pyridinium salt thus obtained was dissolved in 200 cc. of dehyd. *iso*-PrOH and the solution was saturated with $NH_3$ to separate isopropanol-soluble and -insoluble salts.

Isopropanol-soluble ammonium salt (V): Ammonia-saturated *iso*-PrOH solution was centrifuged and solvent was removed from the supernatant, *in vacuo* at room temperature. The residual crude ammonium PCP, a hygroscopic white powder (8.0 g, or 57%), was converted into the Ba-salt by passage of a 4% aqueous solution of the former salt through a column (80×2 cm) of Amberlite IRC-50 ($Ba^{++}$-type). This procedure was repeated once more and the effluent was lyophilized to give 8.53 g. of Ba-salt. 100 mg. of this salt was redissolved in a small quantity of water, insoluble impurity was centrifuged off, and the supernatant was again lyophilized.

The residue was dissolved in warm 95% EtOH to make a syrupy solution. On addition of dehyd. acetone to the syrup, Ba-PCP precipitated as a white powder. The sample for analysis was dried to constant weight over $P_2O_5$ *in vacuo* at 100~105°. *Anal.* Calcd. for $(C_3H_6O_4P)_2Ba \cdot H_2O$: C, 16.76; H, 3.28; P, 14.4. Found: C, 16.98; H, 3.04; P, 14.55. $Rf_1$ 0.63, $Rf_2$ 0.68. The electrometric titration of the product showed no secondary phosphate dissociation.

Isopropanol-insoluble ammonium salt: Mixture of $\alpha$- and $\beta$-PP—The *iso*-PrOH-insoluble portion (6 g.) was dissolved in 100 cc. of water and the solution was passed through an Amberlite IR-120 ($H^+$-type) column. To the acidic effluent was added $Ba(OH)_2$ solution to a weak alkaline reaction. After saturation with $CO_2$ and removal of excess $Ba(OH)_2$ as $BaCO_3$, the aqueous solution was concentrated *in vacuo* on a water bath. The dried residue was triturated with acetone to give a white powder (8.5 g.), 0.3 g. of this powder was dissolved in 3 cc. of water, and precipitated again with 5 cc. of 95% EtOH. The precipitation was repeated once more and the sample for analysis was dried over $P_2O_5$ *in vacuo* at 100~105° to constant weight. *Anal.* Calcd. for $C_3H_7O_5PBa$: C, 12.35; H, 2.40. Found: C, 12.37; H, 2.42.

On detection on a paper chromatogram this salt contains two phosphorus compounds, one with $Rf_1$ 0.27, $Rf_2$ 0.58, $Rf_3$ 0.83, and the other with $Rf_1$ 0.36, $Rf_2$ 0.65, $Rf_3$ 0.93. As the former spots are those of $\alpha$-PP the latter must be that of $\beta$-PP.

0.6 g. of mixed Ba-salt of (Ⅷ) and (X) thus obtained was converted to the cyclohexylamine salt in the same manner as was (Ⅷ)-2, and the mixture of cyclohexylamine salts was fractionally recrystallized from aqueous acetone to obtain the pure salt of $\beta$-PP with $Rf_1$ 0.36 which was sparingly soluble in the solvent used. This salt was again converted into the barium salt and dried over $P_2O_5$ *in vacuo* at 125° to constant weight. *Anal.* Calcd. for $C_3H_7O_5PBa$: C, 12.35; H, 2.40; P, 10.63. Found: C, 12.53; H, 2.24; P, 10.91. $Rf_1$ 0.36, $Rf_2$ 0.65.

**Ethyleneglycol-1 Phosphate (IX)**—The Ba-salt of this compound was synthesized according to the report by Todd, *et al.*[11] and the salt was converted into free acid by treatment with $H^+$-type Amberlite IR-120. To the aqueous solution of the acid was added cyclohexylamine to give a pH of 8.0. After concentration of the solution to a small bulk, cyclohexylamine salt was precipitated by addition of acetone. The produt was recrystallized from hydrated acetone to form needles, m.p. 167~169° *Anal.* Calcd. for $C_{14}H_{33}O_5N_2P \cdot \frac{1}{2}H_2O$ (dibasic cyclohexylamine salt): C, 48.15; H, 9.75;

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[15] At this stage when the product is detected on a paper chromatogram, two faint spots of by-products with $Rf_1$ 0.78 and 0.55 are revealed besides those of the cyclic phosphate, $\alpha$- and $\beta$-PP. As these by-products form *iso*-PrOH-soluble ammonium salts, they contaminate $NH_4$-CPP on further separation. Especially when 1,2-propanediol was added to pyridine solution of $POCl_3$ in the phosphorylation reaction, the ratio of the yields of PCP and these by-products amounts to 2 : 3 : 1. Thus the separation of PCP meets with difficulty. In this case, however, the two by-products can be isolated at the cost of the cyclic phosphate; the mixture of the Ba-salts of phosphates is converted to free acids by treating with Amberlite IR-120 ($H^+$ type). The acidic aqueous solution is heated at 65~70° to hydrolyse PCP into PP. As the by-products are stable in this reaction condition, only these products are contained in soluble fraction on further separation of ammonium salts with *iso*-PrOH. After removal of *iso*-PrOH, the mixed ammonium salts are dissolved in water and passed successively through columns of Amberlite IR-120 ($H^+$-type) and Amberlite IRC-50 ($Ba^{++}$-type). On lyophilization, the effluent gives mixed Ba-salts which on reprecipitation from aqueous solution by EtOH, are separated into sparingly soluble Ba-salt with $Rf_1$ 0.55 and soluble Ba-salt with $Rf_1$ 0.78.

N, 8.03; P, 8.88. Found (Sample dried over $P_2O_5$ *in vacuo* for 30 mins. at room temperature): C, 48.17; H, 10.14; N, 8.34; P, 8.79. Calcd. for $C_8H_{20}O_5NP$ (monobasic cyclohexylamine salt): C, 39.83; H, 8.30. Found (Sample dried over $P_2O_5$ *in vacuo* at 100° for 3 hrs.): C, 39.88; H, 8.47; $Rf_1$ 0.22.

**Ethyleneglycol Cyclic Phosphate (VI)**—To a solution of 2.20 g. of the pyridinium salt of (IX) in 35 cc. of $CH_3CN$ and 1 cc. of water, 1.6 g. of DCC dissolved in 5 cc. of $CH_3CN$ was added. After shaking for 30 mins. and standing for 2 hrs. at room temperature, the mixture was added to 20 cc. of water and the dicyclohexylurea which precipitated was removed by filtration. The filtrate was treated with $Ba(OH)_2$ in the same way as (I) and 1.7 g. of the water-insoluble Ba-salt of (IX) was recovered. After removal of excess $Ba(OH)_2$, the water soluble Ba-salt was obtained on lyophilization of the aqueous solution as a white powder. The powder was dissolved in a small quantity of water and put on the top of a Solka-Floc column (1.5×19 cm.) which was prepared in a mixture of water and *iso*-PrOH (1 : 2), the material was eluted with the same solvent at a flow rate of 0.3 cc. per minute and 2-cc. fractions were collected. Fraction Nos. 26~46, which gave positive phosphorus tests, were combined and the solvent was removed *in vacuo* at 30° to give a syrupy oil which on treatment with acetone, turned into crystals. After recrystallization from hydrated acetone, the sample for analysis was dried over $P_2O_5$ *in vacuo* at 130° for 6 hrs. Yield, 500 mg.. *Anal.* Calcd. for $(C_2H_4O_4P)_2Ba$: C, 12.52; H, 2.08; P, 16.17. Found: C, 12.89; H, 2.29; P, 16.48. $Rf_1$ 0.55. The electrometric titration of pure Ba-ECP showed no secondary phosphate dissociation.

**2,3-Butanediol Cyclic Phosphate (VII)**—To a solution of 9.01 g. (0.1 mole) of 2,3-butanediol in 60 cc. of pyridine, 15.4 g. (0.1 mole) of $POCl_3$ dissolved in 60 cc. of pyridine was added dropwise at −15° with stirring during 1 hr. After additional stirring for 1 hr. at room temperature, the precipitated pyridine hydrochloride was filtered off. The reaction mixture was treated the same as (V)-2) to obtain ammonium salts of the products.

The *iso*-PrOH-soluble ammonium salt was similarly converted to the Ba-salt which showed $Rf_1$ 0.65 and $Rf_2$ 0.68.

From the aqueous solution of the isopropanol-insoluble ammonium salt, cation was removed with $H^+$-type Amberlite IR-120 and the free acid was converted into Ba-salt by addition of $Ba(OH)_2$ solution. The mixture was saturated with $CO_2$ and the precipitated $BaCO_3$ was removed by centrifugation together with the insoluble Ba-salt of inorganic phosphoric acid. The aqueous solution which contained water-soluble Ba-salts was again converted into free acid by treatment with Amberlite IR-120 ($H^+$-type) and the acidic aqueous solution, after addition of cyclohexylamine, was lyophilized. The residual salt was recrystallized from hydrated acetone to needles, m.p. 191~193°. The crystals melted at this temperature solidified again and decomposed at 217~219° The sample for analysis was dried to constant weight over $P_2O_5$ *in vacuo* at room temperature. *Anal.* Calcd. for $C_{16}H_{37}O_5N_2P \cdot \frac{1}{2}H_2O$: C, 51.36; H, 10.07; N, 7.43; P, 8.23. Found: C, 50.95; H, 10.15; N, 7.60; P, 8.50. $Rf_1$ 0.31, $Rf_2$ 0.63.

The cyclohexylamine salt of BP thus obtained was again converted into the free acid by treatment with Amberlite IR-120 ($H^+$-type). 1.1 g. of the free acid was dehydrated with 1.46 g. of DCC in a mixed solvent of 10 cc. of pyridine, 30 cc. of $CH_3CN$, and 1 cc. of water. After setting aside for 3 hrs. at room temperature and removal of dicyclohexylurea, the solvent was distilled off from the reaction mixture. The residual mixture of the products was converted into ammonium salt in *iso*-PrOH and isopropanol-soluble ammonium salts was separated. An aqueous solution of the above salt was passed through a column of Amberlite IRC-50 ($Ba^{++}$-type) to convert the ammonium salt into the Ba-salt and the effluent was lyophilized. The yield of the crude Ba-salt was 0.44 g. To purify the salts, 0.1 g. of the salt was dissolved in 0.1 cc. of water and the solution was triturated with 10 cc. of dehyd. acetone. The precipitated white powder was washed twice with dehyd. acetone. 50 mg. of the pure salt thus obtained was dried to constant weight over $P_2O_5$ *in vacuo* at 105°. *Anal.* Calcd. for $(C_4H_8O_4P)_2Ba$: C, 21.87; H, 3.67; P, 14.11. Found: C, 21.97; H, 3.95; P, 14.38. $Rf_1$ 0.65, $Rf_2$ 0.68.

## Summary

Barium salts of glycerol 1,2-cyclic phosphate (GCP), 1,2-propanediol cyclic phosphate (PCP), ethyleneglycol cyclic phosphate (ECP), and 2,3-butanediol cyclic phosphate (BCP) were synthesized and Rf values of the pure products on paper chromatograms were given.

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