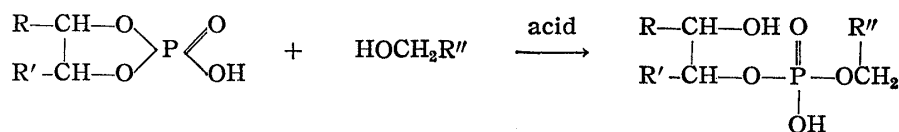


37. Tyunosin Ukita,* Kinzo Nagasawa,* and Masachika Irie*: Organic Phosphates. IV.¹⁾ Synthesis of Several Bis(2-hydroxyalkyl) and Methyl 2-Hydroxyalkyl Phosphates.

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The results of acid catalysed alcoholysis of GCP, PCP, ECP and BCP, were reported by the present authors in the preceding paper of this series.¹⁾ In that research, it was necessary to have several authentic phosphodiester which were used for the identification of the products of alcoholysis.



***GCP: R=CH₂OH, R'=H

PCP: R=CH₃, R'=H

ECP: R=R'=H

BCP: R=R'=CH₃

(I) R=R'=R''=H

(II) R=CH₃, R'=R''=H

(III) R=R'=CH₃, R''=H

(IV) R=R'=H, R''=CH₂OH

(V) R=CH₃, R'=H, R''=CH₃CH(OH)-

In this paper, the synthesis of methyl 2-hydroxyethyl phosphate (I), methyl 2-hydroxypropyl phosphate (II), methyl 1-methyl-2-hydroxypropyl phosphate (III), bis(2-hydroxyethyl) phosphate (IV), and bis(2-hydroxypropyl) phosphate (V) are reported.

Among these phosphates, (I), (IV), and (V) were respectively synthesized by Bailly *et al.*,²⁾ Plimmer, *et al.*,³⁾ and Davis, *et al.*⁴⁾ Thus, calcium salt of (I) was prepared by methylation of ethyleneglycol phosphate with dimethyl sulfate; barium salt of (IV) was obtained by phosphorylation of ethylene chlorohydrin, followed by hydrolysis of the product with lead dioxide, and barium salt of (V) was synthesized from propylene oxide by phosphorylation with orthophosphate. However, in each case the reports lack detailed properties of the products.

In the present series of experiments, all these compounds except (V) were synthesized by methods different from those by the authors cited and properties of the pure products on paper chromatograms were also examined.

Of these compounds (I) and (III) were prepared respectively by methylation of 2-hydroxyethyl phosphate and 1-methyl-2-hydroxypropyl phosphate with diazomethane, and the pure barium salt of (I) showed R_{f1} 0.63 and R_{f2} 0.65. In the synthesis of (III), the isopropanol-soluble ammonium salts separated from the reaction mixture gave two phosphate fractions when it was run through Solka-Floc⁵⁾ column with a solvent of 5N ammonia+isopropanol (1:2). The cyclohexylamine salt obtained from the faster moving phosphate fraction was purified as needles, m.p. 134~135.5°, C₁₂H₂₇O₅NP, and showed R_{f1} 0.84, R_{f2} 0.87. The analytical data of this product corresponded to methyl 1-methyl-2-methoxypropyl phosphate. From the slower moving phosphate fraction, cyclohexyl-

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*** The following abbreviations are used. GA: glycerophosphate, GCP: 1,2-glycerol cyclic phosphate, PCP: 1,2-propanediol cyclic phosphate, ECP: ethyleneglycol cyclic phosphate, BCP: 2,3-butanediol cyclic phosphate.

1) Part III, T. Ukita, K. Nagasawa, M. Irie: This Bulletin, 5, 208(1957).

2) O. Bailly, T. Gaume: Bull. soc. chim. France, [5], 3, 1396(1936).

3) R.H.A. Plimmer, W.J.N. Burch: J. Chem. Soc., 1929, 279, 292.

4) W. Davis, W.C.J. Ross: *Ibid.*, 1952, 4296.

5) Manufactured by Brown Company, Boston, Mass., U.S.A.

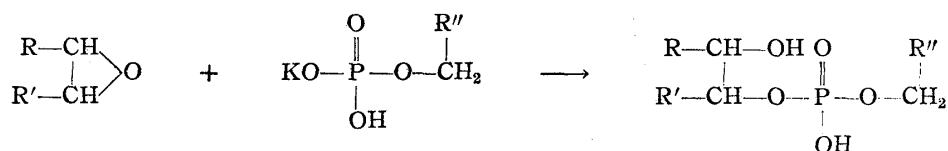
amine salt of (III) was purified as needles, $C_{11}H_{25}O_5NP$, which showed m.p. $108\sim 111^\circ$ and Rf_1 0.73, Rf_2 0.78.

To obtain (II), (IV), and (V), phosphorolysis of the corresponding epoxides was applied. The method was similar to that used in the synthesis of monophosphates of 1,2-diols in the first paper of this series.⁶⁾

Propylene oxide was heated with potassium methyl hydrogen phosphate under pressure and after removal of potassium ion with Amberlite IR-120 (H^+), the product was converted into barium salt with Amberlite IRC-50 (Ba^{++}). The purification of the salt by reprecipitation from water with acetone and subsequent recrystallization from alcoholic acetone gave pure barium salt of (II) as small needles, $(C_4H_{10}O_5P)_2Ba$, with Rf_1 0.70 and Rf_2 0.67.⁶⁾

A possibility that the phosphorolysis occurred to give methyl 1-methyl-2-hydroxypropyl phosphate might be excluded, because this type of reaction is well known to give phosphate of primary hydroxyl group.⁷⁾

(IV) was prepared similarly from 2-hydroxyethyl potassium hydrogen phosphate and ethylene oxide. The product, after purification by successive treatment with Amberlite IR-120 (H^+) and IR-4B (OH^-), was converted into barium salt and precipitated from methanol with acetone. The barium salt of (IV) was obtained as white powder, $(C_4H_{10}O_6P)_2Ba$, with Rf_1 0.55 and Rf_2 0.59.



(II) $R=CH_3$, $R'=R''=H$

(IV) $R=R'=H$, $R''=CH_2OH$

(V) $R=CH_3$, $R'=H$, $R''=CH_2CH(OH)-$

In the synthesis of (V), after the phosphorolysis of propylene oxide with 2-hydroxypropyl potassium hydrogen phosphate, the cation-free product was converted into ammonium salts in isopropanol. The separated isopropanol-soluble ammonium salt was derived to cyclohexylamine salt and recrystallized from acetone. The cyclohexylamine salt of (V) was obtained as needles, $C_{12}H_{25}O_6NP$, with m.p. $125\sim 128^\circ$ and Rf_1 0.74, Rf_2 0.81. The Rf values of the phosphoric diesters thus obtained are summarized in Table I.

TABLE I.

	Rf_1	Rf_2		Rf_1	Rf_2
(I)	0.63	0.65	(IV)	0.55	0.59
(II)	0.70	0.67	(V)	0.74	0.81
(III)	0.73	0.78			

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

Experimental

Methyl 2-Hydroxyethyl Phosphate (I)—To 2.05 g. of 2-hydroxyethyl phosphate were added 10 cc.

- 6) T. Ukita, K. Nagasawa, M. Irie; This Bulletin, 5, 121(1957).
- 7) Thus, as reported in the first paper of this series, 2-hydroxypropyl phosphate was obtained by the phosphorolysis of propylene oxide.
- 8) Techniques in paper chromatography: 10~40% of phosphorus was applied to Toyo Roshi No. 3 filter paper and run ascendingly for 15 hrs. with solvent systems of (1) *iso*-PrOH+5N NH_4OH (2:1), (2) *iso*-PrOH+*t*-BuOH+conc. NH_4OH +water (40:20:1:39), (3) *t*-BuOH+water+picric acid (80:20:4 g.), and (4) pyridine+*iso*-PrOH+water (5:70:30). Phosphorus was detected by the method of Bandurski and Axelrod (J. Biol. Chem., 193, 405(1951)). In this paper, the Rf values of phosphorus compounds found for each of these solvent systems are represented with the abbreviations Rf_1 , Rf_2 , Rf_3 , and Rf_4 , respectively.

of *iso*-PrOH and 100 cc. of dehyd. ether. The solution was added with 2.85 g. of CH_2N_2 (4.8 moles) dissolved in a small quantity of dehyd. ether under ice-cooling. After setting aside for 1 hr. at room temperature, the solvent was removed under a reduced pressure. The residue was dissolved in 50 cc. of distilled water and the solution was adjusted to pH 8.0~9.0 with $\text{Ba}(\text{OH})_2$. The excess of $\text{Ba}(\text{OH})_2$ was removed with CO_2 and the aqueous solution was lyophilized. The white powder (0.5 g.) thus obtained was dissolved in 10 cc. of water and added with EtOH, the precipitate formed was removed, and from the supernatant the Ba salt of (I) was precipitated with acetone. The salt was purified by three reprecipitations from hydr. EtOH (1:1) with acetone. The sample was dried on P_2O_5 *in vacuo* at 100° to constant weight. *Anal.* Calcd. for $(\text{C}_3\text{H}_5\text{O}_5\text{P})_2\text{Ba}$: C, 16.08; H, 3.58; P, 13.85. Found: C, 16.29; H, 3.60; P, 13.51. Rf_1 0.63 and Rf_2 0.65.⁸⁾

The electrometric titration of the pure barium salt showed no secondary phosphate dissociation.

Methyl 1-Methyl-2-methoxypropyl Phosphate and Methyl 1-Methyl-2-hydroxypropyl Phosphate (III)—To an ice-cooled solution of 2 g. (1 mole) of 1-methyl-2-hydroxypropyl phosphoric acid in 5 cc. of dehyd. MeOH 150 cc. of ether was added. The solution was added under stirring with 2.5 g. of CH_2N_2 (5 moles) dissolved in 100 cc. of ether and stirring was continued for 2 hrs. Solvents and excess of CH_2N_2 were removed and the residue was neutralised with dil. aq. ammonia. After 3 extractions with ether and CHCl_3 the aqueous layer was lyophilized. 1.7 g. of syrupy residue was dissolved in ca. 5 cc. of water, and placed on top of a Solka-Floc column (50×2 cm.), which was prepared in a mixed solvent consisting of 5N NH_4OH and *iso*-PrOH (1:2), and eluted with the same solvent system to collect 2-cc. fractions.

Methyl 1-methyl-2-methoxypropyl phosphate: Tubes 58~68, on evaporation of the solvent, gave 0.7 g. of the ammonium salt. An aqueous solution of this salt was passed through a column of Amberlite IR-120 (H^+). To the acid effluent was added cyclohexylamine to adjust to pH 9~10 and the mixture was lyophilized. On keeping in a refrigerator, the residue solidified into crystals which were recrystallized from dehyd. acetone to give needles, m.p. $134\sim 135.5^\circ$; yield, 0.17 g. *Anal.* Calcd. for $\text{C}_{12}\text{H}_{27}\text{O}_5\text{NP}$: C, 48.17; H, 9.18; N, 4.72; P, 10.45. Found: C, 48.04; H, 9.42; N, 4.57; P, 10.76. Rf_1 0.84, Rf_2 0.87.

Methyl 1-methyl-2-hydroxypropyl phosphate (III): Tubes 76~83 were combined and the solvent was removed to give 0.6 g. of the ammonium salt. The salt was treated as above and 0.75 g. of cyclohexylamine salt was obtained. The latter was precipitated from dehyd. acetone solution with ether and the oily precipitate crystallized after keeping in a refrigerator. Recrystallization from dehyd. acetone gave hygroscopic needles, m.p. $108\sim 111^\circ$. *Anal.* Calcd. for $\text{C}_{11}\text{H}_{25}\text{O}_5\text{NP}$: C, 46.77; H, 8.93; N, 4.96; P, 10.98. Found: C, 46.55; H, 9.26; N, 4.97; P, 11.15. Rf_1 0.73, Rf_2 0.78.

Methyl Phosphate—32 g. of MeOPOCl_2 ⁹⁾ (b.p.₁₅ $60\sim 64^\circ$) was dissolved in 400 cc. of water and to the solution was added equivalent amount of Ag_2CO_3 . After removal of AgCl by filtration, the filtrate was treated with H_2S to precipitate silver ion. The silver-free filtrate was neutralized with $\text{Ba}(\text{OH})_2$ to separate white crystals which were collected and washed several times with water. Dried Ba salt weighed 20 g. On concentration of the mother liquor additional 10.2 g. of Ba salt was obtained. *Anal.* Calcd. for $\text{CH}_3\text{O}_4\text{PBa}\cdot\text{H}_2\text{O}$: C, 4.53; H, 1.88; P, 11.70. Found: C, 4.54; H, 1.88; P, 11.95. Rf_1 0.16.

Methyl 2-Hydroxypropyl Phosphate (II)—A mixture of 2.34 g. (2 moles) of propylene oxide, 3.03 g. (1 mole) of MeKHPO_4 , and 50 cc. of water was heated in a sealed tube in an oil bath at $110\sim 120^\circ$ for 6 hrs. Water was removed at $40\sim 50^\circ$ under reduced pressure. From the residue propylene glycol, a by-product, was removed by distillation *in vacuo*. The vitreous residue was dissolved in 10 cc. of water and passed through a column of Amberlite IR-120 (H^+) to remove K ion. The acid effluent was again passed through Amberlite IRC-50 (Ba^{++}) and the aqueous solution of Ba salt was evaporated to dryness. On triturating the residue with acetone, 2.66 g. of white powder was obtained. To 0.5 g. of the powder a small quantity of water was added, and insoluble material that precipitated was centrifuged off. The supernatant was added with 95% EtOH to separate a small amount of insoluble matter which was again centrifuged off. After removal of the solvent from the supernatant, remaining Ba salt of (II) was dissolved in hydrated acetone ($\text{H}_2\text{O}:\text{Me}_2\text{CO}=1:10$) and precipitated with EtOH to give small needles. The sample for analysis was dried to constant weight over P_2O_5 *in vacuo* at room temperature. *Anal.* Calcd. for $(\text{C}_4\text{H}_{10}\text{O}_5\text{P})_2\text{Ba}$: C, 20.18; H, 4.24; P, 13.03. Found: C, 20.23; H, 4.40; P, 13.49. Rf_1 0.70, Rf_2 0.67.

Bis(2-hydroxyethyl) Phosphate (IV)—A suspension of 5 g. of Ba 2-hydroxyethyl phosphate in 50 cc. of water was treated with Amberlite IR-120 (H^+) to remove the cation. The aqueous acid solution was added with KOH solution to pH 5.0 and concentrated to 15 cc. To this solution 22 g. of ethylene oxide dissolved in 50 cc. of water was added and heated in a sealed tube at $80\sim 85^\circ$ for 4 hrs.

The orange-colored reaction mixture was diluted with water to 200 cc. and made free from cation

9) Prepared according to the method reported for the synthesis of EtOPOCl_2 (B. C. Saunders, G. J. Stacey, F. Wild, I. G. E. Wilding: J. Chem. Soc., 1948, 703.)

by treating with Amberlite IR-120 (H⁺). The aqueous acid solution thus obtained was passed through Amberlite IR-4B (OH⁻) column (1 × 26 cm.) and the column was washed with water. The phosphate was eluted from the column with 0.5N NaOH solution. From the fractions which showed a positive phosphorus test, Na ion was again removed with Amberlite IR-120 (H⁺). Ba(OH)₂ was added to the aqueous acid solution up to pH 10.0, and after removal of excess Ba(OH)₂ with CO₂, the aqueous solution was lyophilized to give Ba salt of (IV). The product was reprecipitated from MeOH three times with acetone. The sample for analysis was dried to constant weight over P₂O₅ *in vacuo* at 120°. *Anal.* Calcd. for (C₄H₁₀O₆P)₂Ba : C, 18.92; H, 3.74; P, 12.22. Found : C, 18.64; H, 3.53; P, 12.23. Rf₁ 0.55, Rf₂ 0.59.

Bis(2-hydroxypropyl) Phosphate (V)—1.7 g. (1 mole) of 2-hydroxypropyl potassium hydrogen phosphate, 1.2 cc. (2 moles) of propylene oxide, and 30 cc. of water were reacted similarly as in the case of (II). After removal of water and propylene glycol, the reaction mixture was converted into free acid by treatment with Amberlite IR-120 (H⁺) and lyophilized. The cation-free product thus obtained was dissolved in 50 cc. of dehyd. *iso*-PrOH and saturated with NH₃. The mixture was centrifuged to remove insoluble ammonium 2-hydroxypropyl phosphate (630 mg.). The residue obtained on evaporation of the supernatant was dissolved in 20 cc. of water and passed through a column of Amberlite IR-120 (H⁺) to remove cation. The effluent was added with cyclohexylamine to pH 9~10 and lyophilized. On keeping with trace of dry acetone, the residue solidified into crystals (0.9 g.). Recrystallization was performed with aqueous acetone (H₂O:Me₂CO=1:9). The sample for analysis was dried to constant weight over P₂O₅ *in vacuo* at 75°. *Anal.* Calcd. for C₁₂H₂₈O₆NP : C, 46.00; H, 9.01; N, 4.47; P, 9.89. Found : C, 45.63; H, 8.90; N, 4.69; P, 10.22. Rf₁ 0.74, Rf₂ 0.81.

Summary

Three methyl 2-hydroxyalkyl phosphates, methyl 2-hydroxyethyl phosphate (I), methyl 2-hydroxypropyl phosphate (II), and methyl 1-methyl-2-hydroxypropyl phosphate (III), and two bis(2-hydroxyalkyl) phosphates, bis(2-hydroxyethyl) phosphate (IV), and bis(2-hydroxypropyl) phosphate (V), were synthesized. The Rf values of the products were given.

(Received February 5, 1957)

U. D. C. 547.915 : 597.5

38. Takaaki Aoyagi* : Studies on the Lipid of *Pelteobagrus nudiceps*. I. The Isolation of a Substance Stimulating the Repair of Tuberculosis Foci.

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Shoyama, *et al.* found that *Pelteobagrus nudiceps*, heat-dried and powdered, could stimulate the repair of tuberculosis foci in various organs of guinea pig as well as in human lung. They¹⁾ identified the substance responsible for this activity as a lipid and also found that lipid of eel (*Anguilla japonica*) had no such activity. This paper is to report which fraction of this lipid is responsible for this activity. Results of pathological examinations carried out concurrently with this separation were reported elsewhere.^{2,3)} Very few data, so far, are available on the chemical constituents of *Pelteobagrus nudiceps*. Kamijo⁴⁾ described the general properties of ether-soluble substances of *Pelteobagrus fulvidraco* and *P. vacheli*.

* This work was carried out while the author maintained the scholarship given by Kamakura Institute of Tuberculosis.

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1) S. Shoyama *et al.* : Trans. Soc. Pathol. Japan, **40**, 370(1951).

2) S. Shoyama *et al.* : *Ibid.*, **41**, 401(1952).

3) S. Shoyama *et al.* : *Ibid.*, **42**, 535(1953).

4) N. Kamijo : Complete Chem. Abstr. Japan, **9**, 561(1935).