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87. Tyunosin Ukita and Kinzo Nagasawa : Organic Phosphates. XIV.*1 Reaction of Catechol Cyclic Phosphate with Monoacetoneglucose; A Novel Synthesis of D-Glucose 6-Phosphate.

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In the previous works on organic phosphates,[1,2] it was reported that catechol cyclic phosphate (CCP)*3 is alcoholyzed with appropriate hydroxylic compounds to the corresponding *o*-hydroxyphenyl phosphodiester-type compounds and that hydrolysis of the phosphodiesters resulted in the cleavage of phosphoryl ester bonds in a different direction according to the structure of the hydroxylic compounds used for alcoholysis, as shown in Chart 1.

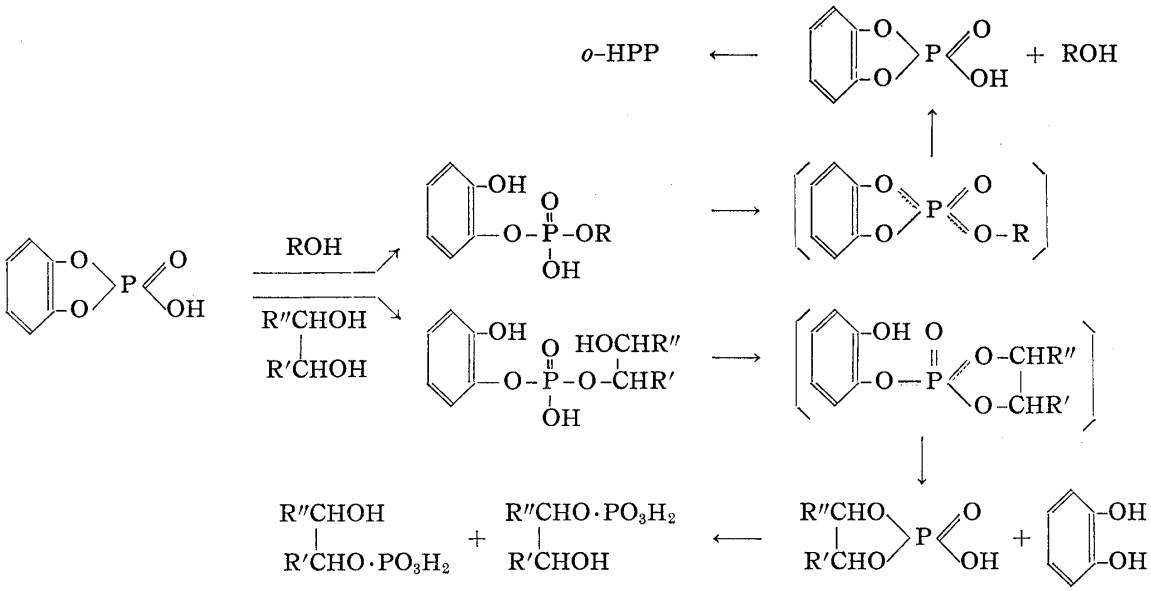

Chart 1.

The phosphodiester formed from CCP with 1,2-diol is hydrolyzed to give a final product of 1,2-diol phosphomonoester via an intermediate cyclic phosphate of the latter, while the *o*-hydroxyphenyl phosphodiester composed of a monofunctional hydroxylic compound gives *o*-hydroxyphenyl phosphate (*o*-HPP).

The different mode of these reactions can be explained by the formation of a different type of transitional intermediates of cyclic phosphotriester given in Chart 1, in brackets, which furnish the final product by their further preferential cleavage of P-O-C linkage not involved in the cyclic phosphate moiety.

As the applications of this type of reaction, CCP was alcoholyzed with DL-erythritol, D-mannitol, or riboflavin, and the product was hydrolyzed by which DL-erythritol 1-phosphate, D-mannitol 1-phosphate, D-mannitol 1,6-diphosphate, or riboflavin 5′-phosphate was obtained in a good yield.[2,3]

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*1 Part XIII : This Bulletin, **9**, 369 (1961).

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

1) K. Nagasawa : This Bulletin, **7**, 397 (1959).
2) T. Ukita, K. Nagasawa : *Ibid.*, **7**, 401 (1959).
3) *Idem* : *Ibid.*, **7**, 465 (1959).

In the present series of work, further research on the reaction of CCP with 1,2-*o*-isopropylidene-D-glucofuranose, which has a glycolic hydroxyl group in its 5- and 6-positions, was attempted to provide a new route to D-glucose 6-phosphate and the results obtained are reported herein.

The monoacetoneglucose was reacted with CCP in pyridine at 85~90° to afford an alcoholysis product (I) giving a spot of Rf_1 0.73*[4] on paper chromatogram. The barium salt of (I), isolated in a pure state by a preparative scale experiment, was analyzed to have a molecular formula of $(C_{15}H_{20}O_{10}P)_2Ba$, colored reddish violet with $FeCl_3$, and did not reduce the Fehling reagent. (I) did not consume periodate and the electrometric titration curve obtained for this product showed a monobasic property. These properties of (I) are in good agreement with the structure of phosphodiester composed of *o*-hydroxyphenyl- and 1,2-*o*-isopropylidene-D-glucofuranose group for this compound. On alkaline hydrolysis (*N* sodium hydroxide, 37°, 30 minutes), (I) gave, besides catechol, two phosphoryl esters (II and III) which gave the respective Rf_1 value of 0.45 and 0.32 on paper chromatogram, while in acid condition (0.1*N* hydrochloric acid, 37°, 6 hours), (I) was hydrolyzed to additional two other phosphates (IV), Rf_1 0.15, and (V), Rf_1 0.08, besides (II) and (III). Among these four products, (V) was identified with D-glucose 6-phosphate by paper chromatography.

When (II) was further hydrolyzed in an acid medium, it gave (IV) and (V), while (III) was decomposed to (V) and orthophosphate by a similar treatment. From these results, it is evident that acid hydrolysis of (I) gives two final products, (IV) and (V).

(II) and (III) were isolated as their crystalline ammonium salts from both acid and alkaline hydrolysates of (I) by chromatography on a cellulose powder column. Ammonium salt of (III) had a sharp melting point of 166~167° and it gave a spot positive to phosphorus and phenolic coloration on paper chromatogram. However, when (III) was submitted to paper electrophoresis, it was separated into two spots, which were respectively identified with *o*-HPP and monoacetoneglucose monophosphate (III'). From the phosphorus analyses of the two crystalline (III) and its two components (*o*-HPP and III'), isolated from the separated spots on the paper electropherogram, it was revealed that (III) is a double salt consisting of one mole each of *o*-HPP and (III'). (III') thus isolated as crystalline ammonium salt melted at 124~126° and its analytical data agreed with those of monoacetoneglucose monophosphate. This phosphate was identified with authentic ammonium salt of 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate by mixed melting point and paper chromatography. Furthermore, (III') was hydrolyzed with 0.1*N* hydrochloric acid at 37° for 10 hours and formed D-glucose 6-phosphate.

The ammonium salt of (II), m.p. 94~96°, was negative to both ferric chloride coloration and the Fehling reagent, and was proved to be an isomer of the ammonium salt of (III') from its analytical data. Because (II) did not consume periodate to form formaldehyde and differed from 1,2-*o*-isopropylidene-D-glucofuranose 3-phosphate (VI) in both melting points and behavior on paper chromatogram, the structure of 1,2-*o*-isopropylidene-D-glucofuranose 5-phosphate was assumed for this compound (II). This assumption proved true by further examination on the structure of (IV), which was obtained from the hydrolysate of (II) besides (V). After separation of the hydrolysate on repeated paper chromatography in preparative scale and subsequent reprecipitation, (IV) was obtained as a powdery barium salt and its analytical values agreed with that for glucose monophosphate. (IV) reduced the Fehling reagent strongly and consumed ca. 3 molar equivalents of periodate but didn ot form formaldehyde. In an acid condition (*N* hydrochloric acid, 100°), (IV) was hydrolyzed to D-glucose and orthophosphate 2.5 times more rapidly than D-glucose 6-phosphate.

From these results, the phosphoryl substituted in glucose monophosphate (IV) must be at positions other than C-1 and C-6. Furthermore, a glucose monophosphate which has

*[4] See experimental part.

the phosphoryl substituted in hydroxyl groups other than at C-5 or C-6 position must form formaldehyde as its oxidation product with periodate. Because (IV) gave no trace of formaldehyde on consumption of that reagent, the structure of (IV), which was obtained besides (V) by acid hydrolysis of (II), must be represented as D-glucofuranose 5-phosphate.*5

The above-described observations revealed an interesting difference in the products from acid hydrolysis of two isomeric monoacetoneglucose monophosphates (II and III'). Hydrolysis of (II) gave both (IV) and (V), while that of (III') gave only (V). Furthermore, in a more detailed examination of the hydrolysis product of (II) on paper chromatogram, a small amount of (III') was detected, while no trace of (II) was detected on a similar treatment of (III'). This finding evidently indicates that, in the acid condition used, a migration of phosphoryl group between C-5 and C-6 positions occurred for the phosphate (II) but not for (III') prior to the hydrolysis of isopropylidene group. Since a similar treatment of (IV) with acid gave no evidence of phosphoryl migration, D-glucose 6-phosphate (V) obtained by acid treatment of (II) must have resulted from (III') derived from (II) by migration of phosphoryl group via the cyclic phosphate (VIII).

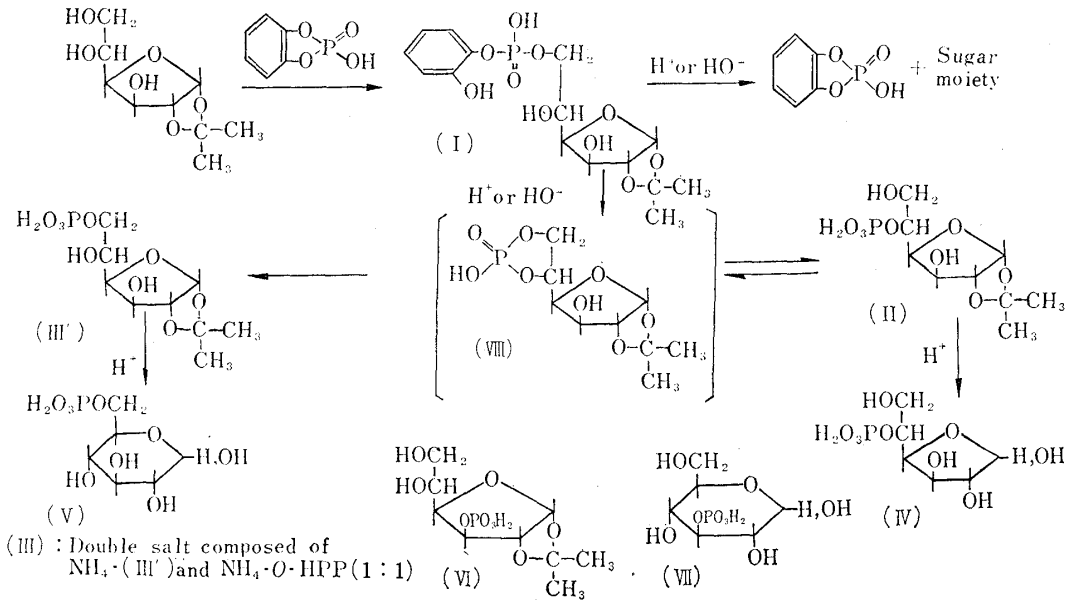

Chart 2.

The overall reactions in the hydrolysis of (I) are schematically given in Chart 2. The phosphodiester (I), which contains vicinal free hydroxyl group on each of the two phosphoryl-bearing carbon atoms of hydroxylic compounds, as has been reported by Brown, *et al.*[4,5] in the case of 2-hydroxycyclohexyl 1-glycerophosphate or glycerol esters of myo-inositol phosphate, is converted in acid or alkaline condition to both CCP*6 and 1,2-*o*-

*5 (IV) was compared with authentic D-glucopyranose 3-phosphate (VII) by paper chromatography and they were found to be not identical. (VII) was oxidized with periodate and produced ca. 1 mole of HCHO.

*6 CCP formed is so labile that it was instantly hydrolyzed to *o*-HPP. In the course of this alcoholysis as well as hydrolysis of (I), paper chromatogram revealed a faint spot with Rf_1 0.63, presumably the spot of 1,2-*o*-isopropylidene-D-glucofuranose 5,6-cyclic phosphate (VIII), but no identification of the spot could be made because of its small quantity.

4) D.M. Brown, G.E. Hall, H.M. Higson : J. Chem. Soc., **1958**, 1360.
5) D.M. Brown, G.E. Hall, R. Letters : *Ibid.*, **1959**, 3547.

isopropylidene-D-glucofuranose 5,6-cyclic phosphate*6 (VIII), and the latter is further hydrolyzed to a mixture of (II) and (III'). By further hydrolysis, (II) gave both (IV) and (V), the former by simple hydrolytic removal of isopropylidene group in (II) and the latter via (VIII) and (III') by a reversible conversion between (VIII) and (II). In a similar reaction of (III'), however, (V) was the only final hydrolysis product, because of the irreversible conversion of (VIII) to (III').

Thus, the overall reaction between monoacetoneglucose and CCP showed a fairly complex feature, especially in the course of the hydrolytic decomposition of the phosphodiester-type intermediate (I). However, the final reaction products are two glucose monophosphates, (IV) and (V), the latter of which was easily separated from the former as a sparingly soluble heptahydrated barium salt. The overall yield of (V) from monoacetoneglucose was 55~60%.

Experimental

Paper Chromatography and Paper Electrophoresis——Samples were applied on Toyo Roshi No. 53 filter paper and run ascendingly, using the 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) *tert*-BuOH-H_2O-picric acid (80 cc.:20 cc.:4 g.). The Rf values found with these solvent systems are designated as Rf_1, Rf_2, and Rf_3, respectively. For the detection of spots, Bandurski-Axelrod method[6] for P, aniline-hydrogenphthalate reagent[7] or periodate-Schiff reagent[8] for sugars, and 5% $FeCl_3$ solution for phenolic 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=20:2:10:500) were subjected to a potential of ca. 30 v./cm. for 1 hr. Spots were detected on paper by the same techniques as those in paper chromatography. For paper electrophoresis in preparative scale, samples were streaked on Toyo Roshi No. 27, thick filter paper. The paper was subjected to electrophoresis as described above, except for using buffer solution of pH 6.0 (BuOH-AcOH-pyridine-H_2O=20:1:5:500).

The mobility (M) for each P spot was represented by the ratio of the distance of the spot from start line to that for DNP-glutamic acid used as a standard.

Synthesis of D-Glucose 6-Phosphate through the Alcoholysis of CCP with 1,2-*o*-Isopropylidene-D-glucofuranose——A mixture of 1 g. (4.5 mmoles) of 1,2-*o*-isopropylidene-D-glucofuranose and 20 cc. of dehyd. pyridine was added with 0.85 g. (5 mmoles) of CCP and heated for 3 hr. at 85~90° with vigorous stirring. After the reaction, the solvent was repeatedly evaporated with addition of a small volume of H_2O. The resultant vitreous residue was dissolved in 10 cc. of H_2O and decationized with Dowex-50 (H^+), and the filtrate and washings were combined (12.5 cc., 0.4*M* solution). The acid solution was heated at 95° for 5 hr., diluted with 80 cc. of H_2O, and neutralized with saturated $Ba(OH)_2$ solution. After centrifugation of the hydrolysate with added charcoal, the supernatant (ca. 120 cc.) was concentrated to ca. 10 cc. in a reduced pressure and set aside overnight in a refrigerator. The plate crystals of barium D-glucose 6-phosphate containing 7 moles of crystal water were separated and washed with 50% EtOH, and dried in air (1.41 g.).

The crystalline Ba salt was dissolved in 12 cc. of H_2O containing Dowex-50 (H^+), the acidic solution was collected by filtration, and adjusted to pH 6.5 with *N* NaOH. To the neutral solution, an equivalent volume of 5% $(AcO)_2Ba$ was added and the insoluble impurity was centrifuged off. To the clear supernatant 4 volumes of dehyd. EtOH was added to precipitate barium D-glucose 6-phosphate. The precipitate was collected by centrifugation, washed successively with 80% EtOH and dehyd. EtOH, and dried at 70° over P_2O_5 *in vacuo* (0.98 g., 55%); $[\alpha]_D^{16}$ +19.6° (c=1.63, 0.1*N* HCl).*7 *Anal.* Calcd. for $C_6H_{11}O_9BaP$ (Barium D-glucopyranose 6-phosphate) : C, 18.22; H, 2.81; P, 7.84. Found: C, 18.40; H, 2.88; P, 7.51.

This sample was identified by paper chromatography with authentic D-glucose 6-phosphate; Rf_1 0.08, Rf_2 0.26, and Rf_3 0.35.

*7 Authentic barium D-glucopyranose 6-phosphate was synthesized through the procedure reported by Lardy and Fischer,[9] and showed a rotatory value of $[\alpha]_D^{16}$ +19.9° (c=1.63, 0.1*N* HCl).

6) R.S. Bandurski, B. Axelrod : J. Biol. Chem., **193**, 405 (1951).
7) R.J. Block, E.L. Durrum, G. Zweig : "Paper Chromatography and Paper Electrophoresis," 2nd Ed., 181 (1958).
8) *a*) J. Baddiley, J.G. Buchanan, R.E. Handschumacher, J.F. Prescott : J. Chem. Soc., **1956**, 2818; *b*) J.G. Buchanan, C.A. Dekker, A.G. Long : *Ibid.*, **1959**, 3162.
9) H.A. Lardy, H.O.L. Fischer : J. Biol. Chem., **164**, 513 (1946).

On periodate oxidation, this sample consumed the same amount of periodate as the authentic sample, as shown in the following table.

IO_4^- consumed (mol. equiv.) at 15~18°

Reaction time	60 min.	300 min.	24 hr.
Sample prepared by CCP reaction	2.83	3.06	3.09
Authentic D-glucose 6-phosphate	3.05	3.18	3.18

Isolation of the Alcoholysis Intermediate of CCP with 1,2-*o*-Isopropylidene-D-glucofuranose: *o*-Hydroxyphenyl 1,2-*o*-Isopropylidene-D-glucofuranose 6-Phosphate (I)——A mixture of 2 g.(9 mmoles) of 1,2-*o*-isopropylidene-D-glucofuranose, 2.3 g. (13.5 mmoles) of CCP, and 40 cc. of dehyd. pyridine was heated at 85~90° for 2 hr. with vigorous stirring. The solvent was evaporated *in vacuo* and the resultant syrup was dissolved in 30 cc. of cold MeOH. To this solution 150 cc. of iso-PrOH was added and the mixture was saturated with NH_3 gas. The precipitate that appeared was centrifuged off and the supernatant was evaporated *in vacuo* to a pale reddish syrup (3.1 g.), 2 g. of which was dissolved in 10 cc. of H_2O and adjusted to pH 3 with Dowex-50 (H^+) and adsorbed on a column (20×2.5 cm.) of Amberlite IR-4B (HO^-). After washing with 500 cc. of H_2O, the column was treated with 300 cc. of 10% NH_4OH to elute the phosphates. Ammoniac eluate was concentrated to a small volume at a room temperature, filtered to remove coloring material, and further concentrated to dryness (1.22 g.). A solution of 0.4 g. of the material thus obtained dissolved in 4 cc. of H_2O was applied to preparative paper electrophoresis.

The phosphate separated on filter paper was extracted with H_2O and converted to its Ba salt by passing through a column (30×1.9 cm.) of Amberlite IRC-50 (Ba^{2+}). The effluent was concentrated to ca. 20 cc., filtered with added charcoal to remove insoluble impurity, and the clear filtrate was lyophilized. The hygroscopic white powder (0.28 g.) thus obtained was dissolved in a minimum volume of dehyd. MeOH and two volumes of dehyd. Me_2CO was added to produce a precipitate. The white precipitate formed was collected, washed with dehyd. Me_2CO, and dried over P_2O_5 at room temperature *in vacuo* (0.11 g.); $[\alpha]_D^{14}$ −5.0°(c=1.3, H_2O). Rf_1 0.73. *Anal.* Calcd. for $(C_{15}H_{20}O_{10}P)_2Ba$ (Barium *o*-hydroxyphenyl 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate) : C, 39.13; H, 4.38; P, 6.74. Found: C, 39.06; H, 4.46; P, 6.89.

This sample colored reddish violet to $FeCl_3$ and no reaction was observed with the Fehling reagent or periodate. However, it was degraded easily with 0.1*N* HCl at 37° for 6 hr. to give (II), (III′), and *o*-HPP.

Isolation of the Hydrolysis Products of (I) : (a) 1,2-*o*-Isopropylidene-D-glucofuranose 5-Phosphate (II)——A solution of 1 g. of the crude sirupy (I) dissolved in 8.5 cc. of H_2O was acidified with Dowex-50 (H^+) (0.2*M* solution) and kept at 37° for 1 hr.[*8] After filtration, the combined filtrate and washings were neutralized with 10% NH_3, concentrated to a small volume in a reduced pressure, and again filtered with added charcoal to remove colored impurity. The filtrate was evaporated to dryness and the syrupy residue was chromatographed on a cellulose column (35×2.6 cm.) prepared from a suspension of cellulose in iso-PrOH-conc. NH_4OH-H_2O (7:1:2). The fraction Nos. 40~51 and 56~70 (each 5 cc.) were collected separately and the combined former fractions were concentrated to a small volume, filtered with added charcoal. After evaporation to dryness, the residual syrup was dissolved in a minimum volume of warm 80% EtOH and filtered. The same volume of dehyd. EtOH was added to give crystals. Recrystallization from hydr. EtOH gave needles, m.p. 94~96°, which was dried at room temperature over P_2O_5 *in vacuo* for analysis; yield, 0.15 g. Rf_1 0.45, Rf_2 0.56. *Anal.* Calcd. for $C_9H_{23}O_9N_2P$ (Ammonium 1,2-*o*-isopropylidene-D-glucofuranose 5-phosphate) : C, 32.32; H, 6.93; N, 8.37; P, 9.27. Found : C, 32.40; H, 6.62; N, 8.28; P, 9.14.

This sample reacted with neither the Fehling reagent nor periodate. On treating with 0.1*N* HCl at 37°, it was completely hydrolyzed to (IV) and (V) within 10 hr.

(b) Double Salt (III) of Ammonium 1,2-*o*-Isopropylidene-D-glucofuranose 6-Phosphate and *o*-Hydroxyphenyl Phosphate——The fraction Nos. 56~70 from the foregoing chromatography (a) was concentrated to ca. 20 cc., treated with charcoal and filtered. The filtrate was evaporated to dryness and left a vitreous residue which was triturated with dehyd. EtOH. Recrystallization of the solidified residue from warm 80% EtOH gave fine needles, m.p. 165.5~166.5°(decomp.); yield, 0.19 g. The sample for analysis was dried at room temperature over P_2O_5 *in vacuo*. *Anal.* Calcd. for $C_9H_{23}O_9N_2P$-$C_6H_{13}O_5N_2P$ (Ammonium 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate·ammonium *o*-hydroxyphenyl phosphate) : P, 11.10. Found : P, 11.25.

(III) obtained as above colored reddish violet to $FeCl_3$ and did not reduce the Fehling reagent. On paper electrophoresis, this crystalline sample gave two phosphorus-positive spots which were

[*8] That the conditions described here are sufficient to hydrolyze (I) to (II) and (III) was checked by paper chromatography.

identified with *o*-HPP (M 1.05) and (III′) (M 0.88). P analysis of these two spots separated on filter paper proved that (III) is composed of equimolar amounts of *o*-HPP and (III′) as shown in the following table.

	Optical Density at 760 mμ
o-HPP (M 1.05)	0.059
(III′) (M 0.88)	0.058

(c) **1,2-*o*-Isopropylidene-D-glucofuranose 6-Phosphate (III′)**——A solution of 0.1 g. of NH_4-salt of (III) dissolved in 2 cc. of H_2O was applied to preparative paper electrophoresis. The band corresponding to (III′) was extracted with H_2O and passed through Amberlite IRC-50 (Ba^{2+}) column to convert it into Ba salt. The combined eluate and washings were evaporated to ca. 5 cc. *in vacuo* and filtered to remove a small amount of $Ba_3(PO_4)_2$. To the clear filtrate, saturated $(NH_4)_2CO_3$ was added to precipitate $BaCO_3$ which was centrifuged off. The supernatant was evaporated to dryness, the vitreous residue was dissolved in warm 80% EtOH, and added with the same volume of dehyd. EtOH to precipitate crystals. Recrystallization by the same procedure as above gave fine needles, m.p. 124~126°; yield, 38 mg. Rf_1 0.32, Rf_2 0.49. For analysis, the sample was dried over P_2O_5 at room temperature *in vacuo*. *Anal.* Calcd. for $C_9H_{23}O_9N_2P$ (Ammonium 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate) : C, 32.32; H, 6.93; N, 8.37; P, 9.27. Found : C, 33.32; H, 7.20; N, 8.12; P, 9.10.

On periodate oxidation, this sample did not consume the reagent and it was easily hydrolyzed with 0.1*N* HCl at 37° for 10 hr. into D-glucose 6-phosphate as the only product. This sample was identified with authentic ammonium 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate by paper chromatography and mixed fusion.

(d) **D-Glucofuranose 5-Phosphate (IV)**——To the mother liquor obtained after separation of crystalline heptahydrated Ba salt of D-glucose 6-phosphate as described above, 4 volumes of dehyd, EtOH was added, the precipitate formed was collected, washed with 80% EtOH, and dried in air (0.27 g.). The powder thus obtained*9 was dissolved in 2 cc. of aqueous suspension of Dowex-50 (H^+) giving an acid solution which was applied on preparative paper chromatography using solvent I. The band corresponding to (IV) was extracted with H_2O, the extract was evaporated to ca. 5 cc., and filtered with added charcoal to remove insoluble material. The clear filtrate was decationized with Dowex-50 (H^+), neutralized with saturated $Ba(OH)_2$ solution, and evaporated to ca. 0.5 cc. The insoluble material that appeared was removed by centrifugation. To the supernatant, 4 volumes of dehyd. EtOH was added, the precipitates was collected, washed successively with 80% EtOH and dehyd. EtOH, and dried in air (42 mg.). The powder thus obtained was reprecipitated by the same procecure to give an amorphous white powder which was dried over P_2O_5 at 60° for 2 hr. *in vacuo* (30.5 mg.). Rf_1 0.15, Rf_2 0.31, Rf_3 0.36. *Anal.* Calcd. for $C_6H_{11}O_9BaP$ (Barium D-glucofuranose 5-phosphate) : C, 18.22; H, 2.81; P, 7.84. Found : C, 18.20; H, 3.28; P, 7.66.

On periodate oxidation, (IV) consumed 2.02 and 2.97 molar equivalents of the reagent respectively after 30 min. and 70 hr., but no HCHO was detected during oxidation. On hydrolysis of (IV) with *N* HCl at 100° for 2 hr., 54% of (IV) was degraded to orthophosphate and D-glucose contrary to the case of D-glucose 6-phosphate in which 22% of it was decomposed under the same condition. During the treatment of (IV) with 0.25*N* HCl at 100° for 2 hr. or with *N* HCl at 100° for 2 hr., no evidence for phosphoryl group migration was observed.

1,2-*o*-Isopropylidene-D-glucofuranose 6-Phosphate (III′) through Phosphorolysis Reaction of 1,2-*o*-Isopropylidene-5,6-anhydro-D-glucofuranose with K_2HPO_4[11]——A mixture of 1 g. (5 mmoles) of 1,2-*o*-isopropylidene-5,6-anhydro-D-glucofuranose,[12] 1.55 g. (9 mmoles) of K_2HPO_4, and 25 cc. of H_2O was refluxed for 24 hr. The reaction mixture was passed through a column of Amberlite IRC-50(Ba^{2+}), the combined eluate and washings were concentrated to ca. 15 cc., and filtered with added charcoal. To the filtrate, 60 cc. of dehyd. EtOH was added, the precipitate formed was collected, washed with 80% EtOH, and dried in a desiccator over $CaCl_2$ (0.8 g.). The powdery Ba salt thus obtained was

*9 On analysis of phosphorus compounds on paper chromatogram, this powder proved to contain *o*-HPP (55%), D-glucose 6-phosphate (31%), and (IV) (14%). In order to separate (IV) from mixed D-glucose 6-phosphate, cellulose column chromatography was not effective, and the separation was successful by preparative paper chromatography technique.

10) G.W. Kenner, J. Mater : J. Chem. Soc., **1956**, 3524.
11) G.P. Lampen, H.A. Lardy : J. Biol. Chem. **181**, 693 (1949). This literature reported the synthesis of D-glucose 6-phosphate through the phosphorolysis of 1,2-*o*-isopropylidene-5,6-anhydro-D-glucofuranose with K_2HPO_4, but the intermediate, 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate involved in the course of the reaction, was not isolated.
12) H. Ohle, L.V. Vargha : Ber., **62B**, 2435 (1929).

dissolved in 5 cc. of H_2O and added with satd. $(NH_4)_2CO_3$ to precipitate $BaCO_3$ which was removed by centrifugation. The supernatant was evaporated to leave a vitreous residue which was dissolved in warm 80% EtOH and precipitated in crystalline form by addition of the same volume of dehyd. EtOH. Recrystallization by the procedure as above gave fine needles, m.p. 125~126° (0.31 g.). Rf_1 0.32, Rf_2 0.49. *Anal.* Calcd. for $C_9H_{23}O_9N_2P$ (Ammonium 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate) : C, 32.32; H, 6.93; P, 9.27. Found : C, 31.93; H, 6.40; P, 9.13.

1,2-*o*-Isopropylidene-D-glucofuranose 3-Phosphate[13] **(VI)**——To an ice-cold mixture of 2.6 g. (10 mmoles) of 1,2:5,6-*o*-diisopropylidene-D-glucofuranose and 9 cc. of dehyd. pyridine, 2.95 g. (11 mmoles) of diphenyl phosphorochloridate was added dropwise with vigorous stirring during 1 hr. and the mixture was kept standing at room temperature for 30 min. The reaction mixture was poured into 200 cc. of ice-water with stirring and the separated oily substance was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with cold dil. HCl and H_2O, and dried over anhyd. Na_2SO_4. After evaporation of the solvent, the syrupy residue solidified on keeping in a desiccator (4.2 g.).

One g. of crude 1,2:5,6-*o*-diisopropylidene-D-glucofuranose 3-diphenylphosphate obtained as above was dissolved in 20 cc. of dehyd. MeOH and catalytically hydrogenated with 0.2 g. of Adams PtO_2 at room temperature in H_2 atomosphere for 5 hr. The catalyst was removed from the reaction mixture, the filtrate was neutralized with saturated $Ba(OH)_2$, and evaporated to dryness. The residual powder, which is composed of 1,2:5,6-*o*-di-isopropylidene-D-glucofuranose 3-phosphate with contamination of a small amount of 1,2-*o*-isopropylidene-D-glucofuranose 3-phosphate, was dissolved in 15 cc. of 0.3*N* H_2SO_4 and kept at room temperature for 2 hr. The hydrolysate was neutralized with saturated $Ba(OH)_2$, $BaSO_4$ that seperated was removed by centrifugation, and saturated $(NH_4)_2CO_3$ was added to the filtrate. After centrifugation of $BaCO_3$ formed, the supernatant was evaporated in a reduced pressure and separated crystals weighed 0.45 g. The crystals were dissolved in 3.5 cc. of warm 80% EtOH, 7 cc. of dehyd. EtOH was added, and set aside in a refrigerator overnight. The plate crystals were collected, washed with cold dehyd. EtOH, and dried over P_2O_5 at 60° *in vacuo* (0.36 g.), m.p. 147~148° (decomp. 199°). *Anal.* Calcd. for $C_9H_{23}O_9N_2P$ (Ammonium 1,2-*o*-isopropylidene-D-glucofuranose 3-phosphate) : C, 32.32; H, 6.93; N, 8.37; P, 9.27. Found : C, 32.28; H, 6.86; N, 8.58; P, 9.40. Rf_1 0.41, Rf_2 0.53.

On periodate oxidation, this substance consumed 1.05 mol. equiv. of the reagent within 75 min. and liberated 1.0 mol. equiv. of HCHO.

D-Glucopyranose 3-Phosphate (VII)——Dowex-50 (H^+) was added to a solution of 0.1 g. of NH_4-salt of (VI) dissolved in H_2O to prepare 0.5*M* solution of free (VI). The acid solution thus obtained was heated at 80~85° for 1 hr. and neutralized with saturated $Ba(OH)_2$. After evaporation, the concentrate (ca. 2 cc.) was filtered and 4 volumes of dehyd. EtOH was added to the clear filtrate, which was washed successively with 80% EtOH and dehyd. EtOH, and dried in the air. To purify this material, the reprecipitation procedure was repeated once more and the product was dried *in vacuo* over P_2O_5 at 60° for 2 hr. (90 mg.). *Anal.* Calcd. for $C_6H_{11}O_9BaP$ (Barium D-glucopyranose 3-phosphate): C, 18.22; H, 2.81; P, 7.84. Found : C, 18.14; H, 3.14; P, 7.77. Rf_1 0.16; Rf_2 0.31; Rf_3 0.45.

On periodate oxidation, (VII) consumed 1.0 and 2.9 mol. equiv. of the reagent, respectively, after 30 min. and 40 hr., and liberated 0.90 mol. equiv. of HCHO.

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Summary

The alcoholysis of catechol cyclic phosphate with 1,2-*o*-isopropylidene-D-glucofuranose was examined. A primary alcoholysis product (I) was formed in a good yield and its structure was determined as *o*-hydroxyphenyl 1,2-*o*-isopropylidene-D-glucofuranose 6-phosphate. (I) was hydrolyzed with acid or alkali into two isomeric monoacetoneglucose monophosphates which were identified with 1,2-*o*-isopropylidene-D-glucofuranose 5-phosphate (II) and 6-phosphate (III'). On further acid hydrolysis, (II) gave a mixture of D-glucofuranose 5-phosphate (IV) and D-glucopyranose 6-phosphate (V), while (III') only gave (V).

The sequences in the hydrolysis reaction of (I) to produce these several products and the availability of this alcoholysis reaction for the preparation of D-glucose 6-phosphate were discussed.

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13) This compound has been isolated as amorphous barium salt by phosphorylation of 1,2:5,6-*o*-diisopropylidene-D-glucofuranose using $POCl_3$, followed by partial hydrolysis (cf. E.E. Percival, E.G.V. Percival : J. Chem. Soc., **1945**, 874).