[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

# The Synthesis and Properties of D-Erythrose 4-Phosphate[1]

BY CLINTON E. BALLOU, HERMANN O. L. FISCHER AND D. L. MACDONALD

RECEIVED JULY 5, 1955

The cyclohexylammonium salt of 4-phosphoryl-D-erythrose dimethyl acetal has been synthesized by an unequivocal method from D-glucose and D-arabinose. This crystalline salt, which is relatively stable, can be converted readily into D-erythrose 4-phosphate, a compound implicated in many enzymatic transformations involving carbohydrates. Some of the chemical and enzymatic properties of this compound are described.

Recent evidence concerning the metabolic fate of D-glucose has emphasized that in many tissues the glycolytic pathway of Embden and Meyerhof is accompanied by an alternate pathway, commonly called the "hexose monophosphate shunt".[2] The details of the reaction sequence of this pathway are only now being worked out, but already considerable information has become available which implicates several sugars and sugar phosphates that heretofore had been considered of little metabolic significance. One of the postulated intermediates, which may play a central role in these and other enzymatic transformations of carbohydrates is D-erythrose 4-phosphate.

This sugar phosphate has been suggested as one product of the action of transaldolase on sedoheptulose 7-phosphate and D-glyceraldehyde 3-phosphate.[3] No tetrose phosphate could be isolated, but after enzymatic dephosphorylation, paper chromatographic examination revealed a component migrating at the same rate as D-erythrose. Further evidence for the transient existence of D-erythrose 4-phosphate was obtained by the isolation of a sedoheptulose diphosphate when aldolase and dihydroxyacetone phosphate were added to the reaction medium.[4] The phosphate groups of this sedoheptulose ester were found to be hydrolyzed by acid in a manner similar to those of D-fructose 1,6-diphosphate and this observation, coupled with the known properties of aldolase, led these workers to suggest that the dihydroxyacetone phosphate had condensed with D-erythrose 4-phosphate to produce sedoheptulose 1,7-diphosphate.

Similarly, D-erythrose 4-phosphate has been postulated as a product obtained by the action of transketolase on D-fructose 6-phosphate and D-glyceraldehyde 3-phosphate.[5] No direct proof of the presence of a four-carbon sugar phosphate was reported, but the demonstration of a five-carbon sugar phosphate as one product led to the inference that a tetrose phosphate was also present. Some evidence for the reverse reaction, the conversion by transketolase of tetrose phosphate and pentose phosphate into triose phosphate and hexose phosphate has also been presented.[4] Further investigation of the metabolic reactions of D-erythrose 4-phosphate has been hampered by the inability of these workers to isolate any of this material, although in recent work, Srere, Kornberg and Racker[6] appear to have obtained a mixture of the barium salts of D-fructose 6-phosphate and tetrose phosphate from the action of purified transketolase on D-fructose 6-phosphate and DL-glyceraldehyde.

The present paper is a report on the chemical synthesis of D-erythrose 4-phosphate by an unambiguous route, and some chemical and enzymatic studies on this synthetic material are also recorded. The procedure followed is similar to that which recently led to a successful synthesis of D-glyceraldehyde 3-phosphate.[7]

The D-erythrose (I) required for the preparation was obtained by two different methods. In the first, D-arabinose was converted into its diethyl mercaptal,[8] which was oxidized by perpropionic acid to a mixture of disulfones.[9] These were degraded, without separation of the individual components, by aqueous ammonia, to give mainly D-erythrose; paper chromatography revealed the presence in lesser amounts of two other components. The crude sirupy D-erythrose was treated with ethyl mercaptan and concentrated hydrochloric acid, and the resulting mercaptal was reacted, in pyridine solution with triphenylchloromethane, followed by acetic anhydride. The resulting crystalline 4-*O*-trityl-2,3-di-*O*-acetyl-D-erythrose diethyl mercaptal (II) was obtained in an over-all yield of 14% from D-arabinose. In the second method of preparing D-erythrose, 4,6-*O*-ethylidene-D-glucose[10] was oxidized by sodium metaperiodate, as has been reported for its enantiomorph,[11] to give 2,4-*O*-ethylidene-D-erythrose. Using the sequence of reactions just described, this was converted into the fully substituted mercaptal in an over-all yield of 45% based on ethylidene glucose.

The mercaptal was converted into the corresponding dimethyl acetal using mercuric chloride and mercuric oxide in methanol.[12] The reaction

(1) A preliminary report of this work has appeared: C. E. Ballou, H. O. L. Fischer and D. L. MacDonald, THIS JOURNAL, **77**, 2658 (1955). The communication should be corrected as follows: col. 1, line 27 for "4-*O*-trityl-2,3-di-*O*-benzyl-D-erythrose dimethyl acetal" read "4-*O*-trityl-2,3-di-*O*-benzoyl-D-erythrose dimethyl acetal."

(2) See, for instance, S. S. Cohen in "Chemical Pathways of Metabolism," Vol. I, edited by D. M. Greenberg, Academic Press, New York, N. Y., 1954, p. 173.

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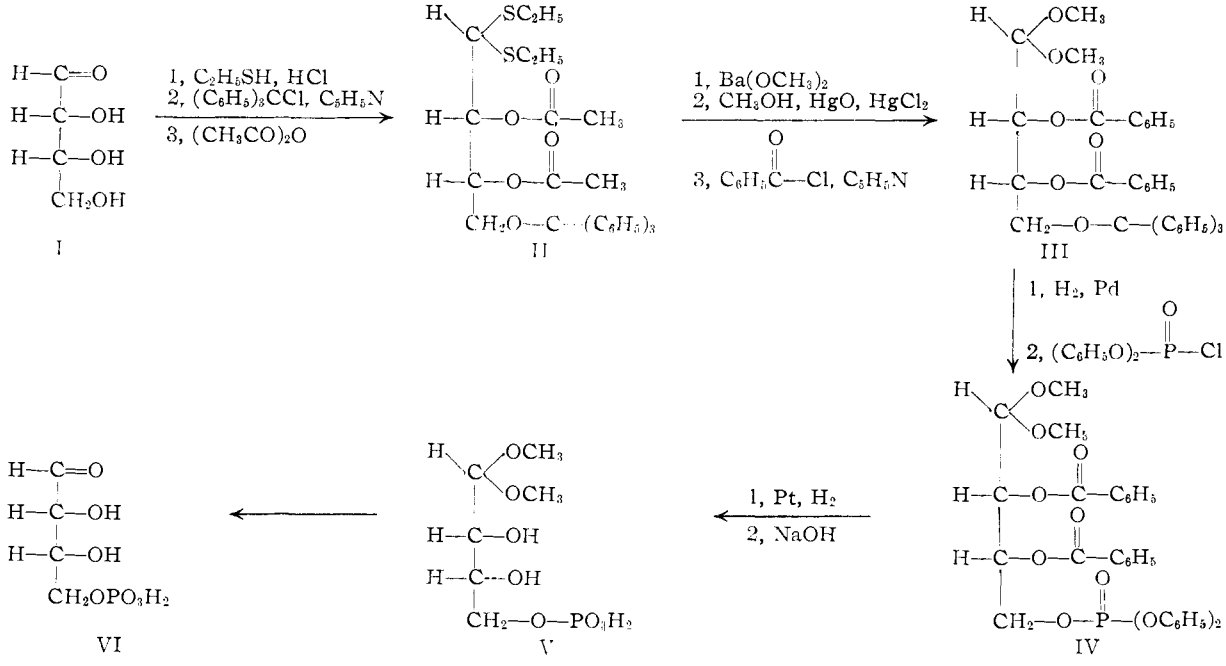


was sluggish with the fully substituted mercaptal, and better over-all results were obtained by prior deacetylation with barium methoxide. 4-*O*-Trityl-D-erythrose dimethyl acetal was obtained in the form of its crystalline diacetate or dibenzoate (III). After catalytic deacetylation of the diacetate, the material consumed one mole of periodate, consistent with the presence of a pair of vicinal hydroxyl groups.

The subsequent steps in the preparation were carried out without purification of the intermediates, since none of them was obtained in crystalline form. The trityl group in 4-*O*-trityl-2,3-di-*O*-benzoyl-D-erythrose dimethyl acetal was removed by catalytic hydrogenolysis using palladium and hydrogen, and the sirupy product was phosphorylated with diphenyl phosphorochloridate. The phenyl groups were removed by catalytic hydrogenolysis, using platinum and hydrogen, and this was followed by removal of the benzoyl groups by saponification. The resulting 4-phosphoryl-D-erythrose dimethyl acetal (V) was obtained in the form of its crystalline cyclohexylammonium salt. This material is stable at room temperature, and it can be converted readily into the free D-erythrose 4-phosphate (VI) as outlined below. The compound consumes one molar equivalent of periodate, consistent with its structure. Despite its asymmetry, the material did not possess any optical rotation, as contrasted with the considerable rotation of the corresponding derivative of D-glyceraldehyde 3-phosphate.[7]

After removal of the cyclohexylamine in aqueous solution, the acetal is readily hydrolyzed by the acidic phosphate group to produce the free D-erythrose 4-phosphate. The hydrolysis proceeds more rapidly than that of the dimethyl acetal of D-glyceraldehyde 3-phosphate,[7] and a yield of 90% is obtained after 18 hours at 40°. Like its acetal, D-erythrose 4-phosphate is optically inactive in acidic or neutral solution. In its behavior toward 1 *N* acid at 100°, the compound is very similar to D-glyceraldehyde 3-phosphate. With 1 *N* alkali at room temperature, inorganic phosphate is rapidly but incompletely liberated.

Treatment of a solution of equimolar amounts of D-erythrose 4-phosphate and dihydroxyacetone phosphate at *p*H 7 with crystalline rabbit muscle aldolase resulted in rapid disappearance of the triose and tetrose phosphates (alkali-labile phosphate) and the appearance of a new phosphate ester. This material, after dephosphorylation with potato phosphatase, was chromatographically indistinguishable from authentic sedoheptulose. The phosphate ester is probably the same as the sedoheptulose diphosphate already described.[4]

## Experimental[13]

**D-Erythrose.**—Eighty grams of D-arabinose diethyl mercaptal[8] was dissolved in 480 ml. of dioxane, and, while cooling the solution in ice, distilled perpropionic acid[14] (10% over the required 4 moles) was added during a period of ten minutes with mixing. After a further five minutes of cooling, the solution was set aside overnight. To the mixture of crystals and solution, 3 l. of ether was added portionwise, and after standing for 24 hours at −10°, the product was filtered off, washed with ether and dried *in vacuo* over potassium hydroxide. The product weighed 91.3 g. This material was a mixture of two disulfones, which exhibited $R_f$'s of 0.53 and 0.73 in butanol:acetic acid:water (4:1:5) using Whatman #1 paper. The starting mercaptal had $R_f$ 0.82.

Ninety grams of the sulfone mixture was mixed with 500 ml. of water, 4 ml. of concentrated ammonium hydroxide was added, and the mixture was left at room temperature for 48 hours. The *p*H of the mixture, initially 9.5, gradually fell, and was brought back to this figure three times during the 48-hr. interval by addition of more ammonium hydroxide. The precipitated bis-(ethanesulfonyl)-methane was filtered off (41.7 g.) and the filtrate was extracted several times with 100-ml. portions of chloroform to remove the residual bis-(ethanesulfonyl)-methane. The chloroform solution was dried (sodium sulfate) and concentrated *in vacuo* to give an additional 9.9 g. of crude bis-(ethanesulfonyl)-

(13) Analyses by The Microchemical Laboratory, University of California, and by Dr. A. Elek, Los Angeles.

(14) J. d'Ans and W. Frey, *Ber.*, **45**, 1845 (1912).

methane. The two crops were combined and recrystallized from absolute ethanol to give 49.2 g. (80% based on the mercaptal) of bis-(ethanesulfonyl)-methane, m.p. 101–102°, undepressed on admixture with authentic material.

The aqueous layer was passed successively through columns of Amberlite IR100-H and IR4B resins (3.2 cm. × 20 cm.) and after washing the columns with water, the combined percolate was decolorized with charcoal, to give about 3 l. of a colorless solution, containing 21.9 g. of D-erythrose as determined by the Willstätter–Schudel titration (58% based on the mercaptal). This solution was concentrated *in vacuo* (bath 45°) to give 25.0 g. of a viscous pale yellow sirup. Chromatography using Whatman #1 paper with butanol:acetic:water (4:1:5) revealed a main D-erythrose component and two minor, slower moving components of unknown composition.

**4-*O*-Trityl-2,3-di-*O*-acetyl-D-erythrose Diethyl Mercaptal.**—The above D-erythrose sirup (19.6 g.) was mixed with 15 ml. of methanol and warmed to give a homogeneous solution. To the cooled solution, 50 ml. of ethanethiol and 40 ml. of concentrated hydrochloric acid were added and the mixture was shaken while cooling in ice, for 15 minutes and then set aside at room temperature for 2.75 hours. The mixture was then poured into water (600 ml.) and the hydrochloric acid neutralized by the addition of freshly regenerated IR4B resin. The resin was separated by filtration, and washed alternately with water and methanol several times. The combined filtrate and washings were then concentrated *in vacuo*, and the resulting sirup was dried by distilling absolute ethanol from it several times. The weight of crude mercaptal was 23.4 g. (63%).

The mercaptal was dissolved in anhydrous pyridine (200 ml.), 29.4 g. of triphenylchloromethane was added and the homogeneous brown solution was set aside at room temperature. After 20 hours, the reaction mixture was cooled in ice, 100 ml. of acetic anhydride was added, and after a further 15 minutes at 0°, the mixture was set aside overnight. The solution was then cooled in ice, and the excess acetic anhydride was decomposed by the addition of water (20 ml.). After one-half hour, the solution was concentrated *in vacuo* and the residue was taken up in chloroform (250 ml.), washed with 1 *N* sulfuric acid, 1 *N* potassium carbonate and water and dried (sodium sulfate). The solvent was removed at reduced pressure and the residual sirup was taken up in hot methanol (500 ml.), treated with charcoal and filtered hot. 4-*O*-Trityl-2,3-di-*O*-acetyl-D-erythrose diethyl mercaptal (29.4 g., m.p. 103–105°) crystallized from the dark red solution. It was taken up in methanol, decolorized with charcoal, and from the cooled solution 26.9 g. of almost colorless material was obtained, m.p. 105–106°. From the combined mother liquors repeated recrystallization from methanol gave a further 1.9 g. of material of the same melting point, making the yield 32% based on the D-erythrose. For analysis, the product was recrystallized again from methanol to give m.p. 105–106°, $[\alpha]^{24}D$ +3.7 (*c* 4, chloroform).

*Anal.* Calcd. for $C_{31}H_{36}O_5S_2$ (552.7): C, 67.36; H, 6.57; S, 11.60. Found: C, 67.22; H, 6.67; S, 11.49.

**Alternate Preparation of 4-*O*-Trityl-2,3-di-*O*-acetyl-D-erythrose Diethyl Mercaptal.**—Sixty-four grams of 4,6-*O*-ethylidene-D-glucose (m.p. 175–180°)[10] was oxidized with sodium metaperiodate to 2,4-*O*-ethylidene-D-erythrose as has been reported for its enantiomorph.[11] The colorless sirup resulting from the oxidation was dissolved with swirling in 140 ml. of ethyl mercaptan, and to the ice-cold solution was added 50 ml. of concentrated hydrochloric acid. The mixture was shaken at 0° for 20 minutes, and then was made slightly basic by cautious addition of concentrated ammonium hydroxide. The reaction mixture was concentrated to dryness *in vacuo*, and the residue dried by distilling absolute ethanol from it two or three times. Absolute ethanol was then added and the insoluble ammonium chloride was removed by filtration. Removal of the alcohol at reduced pressure gave a mixture of mercaptal and a considerable amount of ammonium chloride; the product was then further dried by azeotropic distillation following the addition of benzene.

The resulting mixture was dissolved in anhydrous pyridine (400 ml.) and 88 g. of triphenylchloromethane was added. After standing for 22 hours, the solution was cooled in ice and 200 ml. of acetic anhydride was added. After one-half hour at 0° and 10 hours at room temperature, the product was worked up as described in the previous section. After three recrystallizations from methanol there was obtained 78 g. (45% based on the ethylidene glucose) of slightly yellow material, m.p. 105–106°. A further 2.5 g. of the same purity was isolated from the mother liquors.

**4-*O*-Trityl-D-erythrose Diethyl Mercaptal.**—One gram of 4-*O*-trityl-2,3-di-*O*-acetyl-D-erythrose diethyl mercaptal was dissolved in warm absolute methanol (50 ml.), and to the warm solution was added 0.2 ml. of 0.5 *N* barium methoxide. After two hours at room temperature, the solvent was removed at reduced pressure, the residue was taken up in methylene chloride and the solution was washed twice with water and dried with anhydrous sodium sulfate. The solvent was removed *in vacuo*, and the residual sirup, dissolved in 25 ml. of hot petroleum ether (b.p. 60–70°), was filtered from a trace of insoluble material, and seeded.[15] The product weighed 0.71 g. (83%), m.p. 74.5–75.5°. A second recrystallization from petroleum ether gave analytically pure material, m.p. 75–76°, $[\alpha]^{25}D$ −8.2 (*c* 1.7, methanol).

*Anal.* Calcd. for $C_{27}H_{32}O_3S_2$ (468.7): C, 69.20; H, 6.88; S, 13.69. Found: C, 69.09; H, 6.79; S, 13.69.

**4-*O*-Trityl-2,3-di-*O*-acetyl-D-erythrose Dimethyl Acetal.**—Five grams of the acetylated mercaptal was dissolved in 75 ml. of warm, dry methanol in a three-neck round-bottom flask. The solution was rapidly cooled to room temperature, and 2 ml. of 0.5 *N* barium methoxide solution was added. After one hour, when deacetylation was complete, the vessel was fitted with a mercury sealed glass stirrer and a reflux condenser. After the addition of 7.5 g. of mercuric oxide, the stirrer was adjusted to such a speed that the oxide was kept well suspended, and a solution of 7.5 g. of mercuric chloride in warm dry methanol was added.[12] The mixture was stirred at room temperature for 10 minutes and then under reflux in a water bath for 20 minutes.

The cooled solution was filtered, and the filtrate was concentrated *in vacuo* to dryness in the presence of a little mercuric oxide. The solid residue was extracted with two 50 ml. portions of chloroform, and the combined chloroform filtrate was washed three times with 100-ml. portions of water.[16] The organic layer, dried over anhydrous sodium sulfate, was concentrated *in vacuo* to a stiff sirup that weighed 3.75 g.

This sirup was acetylated in 20 ml. of dry pyridine with 5 ml. of acetic anhydride. After 18 hours at room temperature, a little water was added to destroy the excess acetic anhydride, and the solution was concentrated *in vacuo* to remove most of the pyridine. The residue was taken up in 100 ml. of chloroform, and the solution was washed with 100-ml. portions of water, cold 1 *N* hydrochloric acid, cold 1 *M* potassium bicarbonate and finally with water. The dry chloroform solution (sodium sulfate) was concentrated *in vacuo* to a sirup (5.0 g.), which crystallized on addition of 5 ml. of methanol. After several hours at 5° the crystals were filtered off and dried in air. The yield was 3.5 g. (79%). Recrystallization from a small volume of methanol gave 3.1 g. of granular crystals with m.p. 99–101°. The substance showed $[\alpha]^{25}D$ +10.8° (*c* 2.4, chloroform).

*Anal.* Calcd. for $C_{29}H_{32}O_7$ (492): C, 70.8; H, 6.5; $OCH_3$, 12.6. Found: C, 71.1; H, 6.6; $OCH_3$, 13.2.

Following deacetylation, 0.014 mM of the compound consumed 0.013 mM of periodate, consistent with the presence of two adjacent free hydroxyl groups.

**4-*O*-Trityl-2,3-di-*O*-benzoyl-D-erythrose Dimethyl Acetal.**—Three grams of the acetylated acetal was deacetylated in 50 ml. of dry methanol with 1 ml. of 0.5 *N* barium methoxide. After one hour the solution was concentrated *in vacuo* to a thick sirup and benzoylated in 15 ml. of dry pyridine with 3 ml. of benzoyl chloride. After 18 hours at room temperature, the reaction was worked up as described for the acetylation of the acetal above. The product crystallized from methanol in a yield of 3.5 g. (93%). After recrystallization from methanol, the dibenzoate melted at 122–124° and showed $[\alpha]^{25}D$ +18.3° (*c* 3, chloroform).

*Anal.* Calcd. for $C_{39}H_{36}O_7$ (616): C, 76.0; H, 5.9; $OCH_3$, 10.1. Found: C, 75.8; H, 5.9; $OCH_3$, 10.5.

(15) On working up an attempted acetonation (acetone and copper sulfate) of sirupy 4-*O*-trityl D-erythrose diethyl mercaptal, crystals were obtained, which proved to be the unacetonated starting material.

(16) Decolorization with charcoal and filtration through Celite was sometimes necessary to remove an orange color. Alternatively, one washing with 10% aqueous potassium iodide eliminated this difficulty.

This dibenzoate can be prepared directly from the demercaptalation reaction, but the over-all yield is better when the preparation is carried out *via* the diacetate.

**Detritylation and Phosphorylation.**—Three grams of 4-*O*-trityl-2,3-di-*O*-benzoyl-D-erythrose dimethyl acetal in 100 ml. of absolute ethanol was shaken with 3 g. of reduced and washed 5% palladium chloride-on-carbon catalyst[17] in the presence of hydrogen gas at atmospheric pressure for 16 hours. The hydrogen uptake (170 ml.) was in excess of the theoretical amount (110 ml.). The catalyst was centrifuged off and the ethanol solution was concentrated to dryness *in vacuo*. Crystals of triphenylmethane separated. Without separation, the mixture was dissolved in 10 ml. of dry pyridine, cooled in ice-water, and 2.5 g. of diphenyl phosphorochloridate was added dropwise. After 18 hours at 5°, the reaction was worked up as described for the acetylation of the acetal above. The yield of the phosphorylated product (contaminated with triphenylmethane) was 3.7 g.

**4-Phosphoryl-D-erythrose Dimethyl Acetal.**—The sirup from the above condensation was hydrogenated at atmospheric pressure in 250 ml. of absolute ethanol with 1 g. of platinum oxide catalyst. The hydrogen uptake was 1340 ml. in 10 hours. The catalyst was removed by centrifugation, and 30 ml. of 1 *N* sodium hydroxide was added to the ethanol solution. After 18 hours to allow saponification, the alcohol was removed by distillation *in vacuo*, and the residue, in 100 ml. of water, was extracted with ether to remove some water-insoluble material. The water layer was treated batchwise with 50 ml. of Dowex 50 (H+ form, 2 meq./ml.) to remove the cations, and was again extracted with ether to remove the cyclohexylcarboxylic acid. The water layer was immediately brought to about *p*H 9 (indicator paper) with cyclohexylamine, and the solution was concentrated *in vacuo* to dryness. The residue was dissolved in 5 ml. of absolute ethanol and ether was added to turbidity. Crystallization occurred, and after 18 hours at 5°, the mixture was filtered by suction on a hardened filter paper. The product was washed with ether on the funnel, then it was dried in air, and finally for an hour at room temperature in a high vacuum over phosphorus pentoxide. The yield was 0.6 g. (31%) of a product with m.p. 160–165°, $[\alpha]^{25}D$ 0 ± 0.2° (*c* 5, water or 1 *N* hydrochloric acid).

*Anal.* Calcd. for $C_6H_{15}O_8P \cdot 1.5C_6H_{11}NH_2$ (395): C, 45.5; H, 8.7; N, 5.3; P, 7.9; $OCH_3$, 15.7. Found: C, 45.4; H, 8.7; N, 5.6; P, 8.0; $OCH_3$, 16.1.

The substance was non-reducing, but became strongly reducing following mild acid hydrolysis. Periodate oxidation resulted in the consumption of 0.9 mole of periodate per mole of compound.

**D-Erythrose 4-Phosphate.**—A solution of 100 mg. of the cyclohexylamine salt of the acetal in 5 ml. of water was swirled for a minute with 2 ml. of Dowex 50 (H+ form, 2 meq./ml.). The resin was filtered off, and the filtrate was left in a tightly stoppered container at 40° for 18 hours. An aliquot (0.2 ml.) was removed and analyzed for aldehyde content by the Willstätter–Schudel titration and found to consume 0.018 meq. of oxidant, or 90% of the calculated requirement. No further increase in reducing power occurred. The above solution of the free acid of D-erythrose 4-phosphate (1%) showed no observable rotation in a 2-dm. tube, the $[\alpha]D$ being 0 ± 1°. Following neutralization with sodium hydroxide there was no change in optical activity.

**Acid and Alkaline Decomposition of D-Erythrose 4-Phosphate.**—A sample of D-erythrose 4-phosphate in 1 *N* sulfuric acid was heated in boiling water, and aliquots were withdrawn for inorganic phosphate analysis. Fifty per cent. decomposition occurred in about 20 minutes.

An aliquot of D-erythrose 4-phosphate was left in 1 *N* sodium hydroxide at room temperature for 20 minutes. Analysis for inorganic phosphate indicated that it was rapidly but incompletely liberated by this treatment.

**D-Erythritol 4-Phosphate.**—An aqueous solution of D-erythrose 4-phosphate prepared from 100 mg. of the dimethyl acetal as above was treated with an excess of sodium borohydride. After one hour, acetic acid was added to destroy the excess reducing agent, and the solution was concentrated to dryness. The residue was suspended in methanol and then reconcentrated, repeating several times to remove the borate. Finally, the residue was dissolved in water and the cations were removed by adding Dowex 50 (H+ form). After filtration to remove the resin, the solution was brought to *p*H 9 with cyclohexylamine and concentrated to a dry crystalline residue. This salt of D-erythritol 4-phosphate was recrystallized from hot absolute ethanol and dried for two hours at room temperature in a high vacuum.

*Anal.* Calcd. for $C_{16}H_{37}O_7PN_2$ (400): C, 48.0; H, 9.3; N, 7.0; P, 7.8. Found: C, 48.3; H, 8.9; N, 6.9; P, 7.5.

The substance consumed two moles of periodate per mole of compound consistent with the assigned structure.

**Aldolase-catalyzed Combination of D-Erythrose 4-Phosphate and Dihydroxyacetone Phosphate.**—A solution containing equimolar amounts of the two phosphates was adjusted to *p*H 7, then a small amount of crystalline rabbit muscle aldolase was added. After 5 minutes, the solution was essentially free of alkali labile phosphate (1 *N* sodium hydroxide at room temperature for 15 minutes). A chromatogram (butanol (74); acetic acid (19); water (50)) on acid-washed Whatman #1 paper showed a single spot migrating with fructose 1,6-diphosphate, but only traces of the triose and tetrose phosphates. When dihydroxyacetone phosphate alone was incubated with aldolase, no detectable amount of hexose diphosphate was formed.

Hydrolysis of an aliquot of the above solution with potato phosphatase, followed by chromatography of the solution (phenol saturated with water) gave only one spot migrating with authentic sedoheptulose and distinguished from fructose both in $R_f$ and color reaction with aniline oxalate spray.

The rate of hydrolysis of the phosphate groups in 1 *N* sulfuric acid at 100° was determined, and found to correspond roughly with that reported by Horecker and coworkers.[4]

NOTE ADDED IN PROOF.—Since submission of this paper for publication, additional studies of significance in characterizing this synthetic D-erythrose 4-phosphate have been completed. Kornberg and Racker have shown that it behaves qualitatively like the tetrose phosphate prepared enzymatically from D-fructose 6-phosphate in reactions catalyzed by transketolase.[18] Probably more significant is the finding of Srinivasan, Katagiri and Sprinson[19] that the substance is condensed with phosphoenolpyruvate by a cell free extract from an *E. coli* mutant 83–24 to give a 90% yield of dehydroshikimic acid. This is the first quantitative and specific biological test to be applied to D-erythrose 4-phosphate.

**Acknowledgments.**—The authors wish to thank the Eli Lilly Research Grants Committee and the Nutrition Foundation for support of this work.

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