2-Keto-3-deoxy-D-arabo-heptonic Acid 7-Phosphate Synthetase*

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In the preceding paper (1) it was demonstrated that cell-free extracts of *Escherichia coli* converted erythrose-4-phosphate and phosphoenolpyruvate almost quantitatively to 5-dehydroquinic acid. It was postulated that the initial reaction in this conversion is a condensation of phosphoenolpyruvate and erythrose-4-phosphate to yield inorganic phosphate and 2-keto-3-deoxy-D-arabo-heptonic acid 7-phosphate. The present paper describes the purification and properties of the enzyme carrying out this reaction, and the identification of the product as KDHP by comparison with the chemically synthesized compound. The name KDHP synthetase is suggested for this enzyme. The later stages of the enzyme purification were greatly aided by the discovery of Weissbach and Hurwitz (3) (communicated to us before publication) that P-formylpyruvic acid, derived from ribose-5-phosphate, is a gift of Professor C. E. Ballou. Ammonium salts of dihydroxyacetone phosphate and n-glycerovvaleric acid were gifts of Professor Z. Dische. The cyclohexylammonium diacetamide was prepared according to an unpublished procedure.2 The name KDHP synthetase is suggested for this enzyme. The discovery of Weissbach and Hurwitz (3) was also estimated spectrophotometrically (8). Inorganic phosphate was determined by the method of Fiske and SubbaRow (9). P-enolpyruvate was measured enzymatically according to the method of Ficko and SubbaRow (9). P-enolpyruvate was measured enzymatically according to Kornberg and Price (10). Light absorption measurements were carried out on a Cary Recording Spectrophotometer or a Beckman model B Spectrophotometer.

**Experimental**

Matteral-—The dimethylacetal of cyclohexylammonium erythrose-4-P was prepared according to the method of Ballou et al. (5), and used as sodium erythrose-4-P (5). Barium P-enolpyruvate was a gift of Mr. W. E. Price, Jr., and was converted to the potassium salt. KDHP and 3-deoxy-D-arabo-heptonic acid 7-phosphate (configuration of α-hydroxyl unknown) were prepared according to an unpublished procedure.2 2-Keto-3-deoxyglucuronic acid 6-phosphate and 2-keto-3-deoxygalactonic acid were gifts of Professor E. Racker. Sedoheptulose 1,7-diphosphate, pure by enzymatic assay (6), was a gift of Professor E. Racker. Sedoheptulose 7-phosphate was a gift of Professor B. L. Horecker. n-Glucosamine 6-phosphate and N-acetyl-D-glucosamine 6-phosphate were gifts of Professor S. Roseman. n-Erythrose (from the diacetamide) and 2,3-diketovaleric acid were gifts of Professor Z. Dische. The cyclohexylammonium salt of dihydroxyacetone phosphate and n-glycerol aldehyde 3-phosphate were gifts of Professor C. E. Ballou.

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† Career Investigator of the American Heart Association.

† The following abbreviations are used: erythrose-4-P, n-erythrose-4-phosphate; KDHP, 2-keto-3-deoxy-D-arabo-heptonic acid 7-phosphate; EDTA, sodium ethylenediaminetetraacetate; Tris, tris(hydroxymethyl)aminomethane.

KDHP is correctly named as abover ather than, as in previous publication, 2-keto-3-deoxy-7-phosphoglucoheptonic acid.

The mutant strains used in this work were kindly furnished by Professor B. D. Davis. Yeast hexokinase (30 per cent pure) was a gift of Professor E. Racker. Neurospora DPNase (60 per cent acetone fraction) was a gift of Professor N. O. Kaplan.

The following materials were obtained from commercial sources: 3-phosphoglyceric acid, fructose 1,6-diphosphate, and ribose-5-phosphate, Sigma Chemical Company; phosphoenolpyruvate (containing P-enolpyruvate-ATP transphosphorylase), Worthington Biochemical Corporation; β-ketobutyraldehyde dimethylacetal (a gift), Henley Laboratories; 2-thiobarbituric acid, Eastman-Kodak; crystalline bovine serum albumin, Schwarz Laboratories, Inc.; 2-keto-3-deoxy-D-arabo-heptonic acid, Eastman-Kodak; crystalline bovine serum albumin, Maurice Position Laboratories; diethylaminoethyl cellulose, Brown and Company; AT, ADP, and DPNH, Sigma Chemical Company; crystalline lactic dehydrogenase (containing P-enolpyruvate-ATP transphosphorylase), Worthington Biochemical Corporation.

**Analytical Procedures**—Protein was determined by the method of Lowry et al. (7); however, before the addition of potassium iodide, protein was also estimated spectrophotometrically (8). Inorganic phosphate was determined by the method of Fiske and SubbaRow (9). P-enolpyruvate was measured enzymatically according to Kornberg and Price (10). Light absorption measurements were carried out on a Cary Recording Spectrophotometer or a Beckman model B Spectrophotometer.

**Enzyme Purification**—*E. coli* mutant 83-24 (5) was grown for 18 hours with aeration at 37°C in medium A (11) supplemented with 0.2 per cent yeast extract (Difco) and 0.2 per cent casein hydrolysate. The cells were harvested by centrifugation at 2°C, washed with cold water, suspended in 0.1 M potassium phosphate buffer, pH 7.4 (20 ml./5.0 gm. of wet bacteria), and disrupted by means of sonic oscillation for 30 minutes in a 1-kW Raytheon oscillator cooled with circulating ice water. Centrifugation at 13,000 X g yielded a clear greenish yellow supernatant solution containing 16 mg. of protein per ml. All subsequent operations were carried out at 2°C.

Two hundred ml. of the cell free extract was treated with 28 ml. of 2 per cent proteinate sulfate solution,4 and the precipitate was removed by centrifugation. To 218 ml. of the supernatant solution were added slowly with stirring 54 gm. of (NH₄)₂SO₄ and, after stirring for another 20 minutes, the precipitate was removed by centrifugation and discarded. The procedure was repeated with 21 gm. of (NH₄)₂SO₄ on the supernatant solution. The precipitate was removed by centrifugation, dissolved in 45 ml. of a/30 potassium phosphate buffer pH 7.4 and dialysed against the same buffer (4 changes of 2 liters each at 2-hour intervals).

4 0.5 ml. of 2 per cent proteinate solution for every 70 mg. of protein as estimated spectrophotometrically (8).
The rest of the fractionation procedure was best carried out on a smaller scale. Twenty ml. of the dialysate was adjusted to pH 5.4 with 1 N acetic acid and the precipitate was removed by centrifugation. The supernatant solution (cooled by an ice bath) was treated with 17 ml. of acetone (precooled to −15°) slowly with stirring and the precipitate was removed by centrifugation and discarded. The supernatant solution was treated similarly with 12 ml. of acetone, and the precipitate was removed by centrifugation, dissolved in 5 ml. of M/30 potassium phosphate buffer, pH 7.4, and dialyzed against 2 liters of 0.01 M phosphate buffer, pH 6.8, as described previously.

The diethylaminoethyl cellulose column used in the next step was prepared as follows. To 5 gm. of diethylaminoethyl cellulose suspended in 100 ml. of H₂O at room temperature, 1.5 ml. of 1.0 M H₂SO₄ was added with stirring. (The pH of the solution was 0.8.) The cellulose was removed by filtration on a Buchner funnel, washed several times with 0.01 M potassium phosphate buffer, pH 6.8, and suspended in 70 ml. of the same buffer. Ten ml. of this suspension was placed on a chromatographic column (13 mm. diameter) and packed by mild suction (length, 30 mm.). The column was transferred to a cold room at 2° and equilibrated with cold buffer by allowing approximately 50 ml. to pass through the column (2 hours).

One ml. of the dialyzed acetone fraction was placed on the column and eluted successively with 5.0 ml. each of phosphate buffer, pH 6.8, of the following molar concentrations (flow rate 5 ml. in 20 minutes): 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.10, 0.16, and 0.20. The desired enzyme activity was present in the two 0.1 M fractions. Four such fractions (from two column operations) were combined (3.8 mg. of protein), and immediately concentrated by precipitation with (NH₄)₂SO₄ (80 per cent saturation). The precipitate was dissolved in 0.8 ml. M/30 potassium phosphate buffer pH 7.4, dialyzed against the same buffer, and clarified by centrifugation. Recovery of protein was 1.8 mg.

A summary of the enzyme purification is given in Table I. The (NH₄)₂SO₄ and acetone fractions were stable for at least several weeks at −15°. The cellulose fractions were stored at 2°.

**Assay**—The activity of the enzyme was determined by measuring the amount of KDHP formed from erythrose-4-P and P-enolpyruvate. A unit of enzyme was defined as that amount of enzyme which will form 0.1 µmole of KDHP in 5 minutes under the following conditions.

The incubation mixture contained 100 µmoles of potassium phosphate buffer (pH 6.4), 0.5 µmole each of erythrose-4-P and P-enolpyruvate, and enzyme fraction in a total volume of 1.0 ml. The reaction was started by the addition of enzyme after preincubation of the other constituents at 37° for 10 minutes. After 5 minutes at 37° the reaction was stopped by the addition of 0.4 ml. of 10 per cent trichloroacetic acid and the mixture was centrifuged. Aliquots were removed for the estimation of KDHP by cleavage with periodic acid to β-formylpyruvic acid (butyric acid-2,4-dione), and estimation of the latter by the color produced with thiobarbituric acid.

**Estimation of KDHP**—0.01 to 0.05 µmoles of compound in 0.25 ml. of solution was treated with 0.25 ml. of 0.025 M periodic acid in 0.125 N H₂SO₄. After 45 minutes at room temperature, 0.5 ml. of 2 per cent sodium arsenite in 0.5 N HCl was added to destroy the excess periodate (2 minutes at room temperature). Two ml. of 0.3 per cent thiobarbituric acid solution were added and the tubes were placed in a boiling water bath for 5 minutes.

(To prepare the thiobarbituric acid solution, 300 mg. of thiobarbituric acid were dissolved in about 70 ml. of H₂O with the aid of 3 ml. of 1 N NaOH; 2.5 ml. of 1 N HCl was added, and the pH adjusted to 2 in a final volume of 100 ml.) After cooling in a water bath at 40° the pink color was measured immediately at 549 µm in a spectrophotometer against a blank run with water under the same conditions. The rate of production of chromogenic material from KDHP by periodic acid is shown in Fig. 1.

**Paper-chromatographic Identification**—A solution equivalent to 2 to 5 µg. of KDHP was spotted on acid washed Whatman No. 1 paper and developed for 24 hours by descending chromatography with a mixture of tertiary amyl alcohol-formic acid (95 per cent)-water (3:3:1). The paper was dried at room temperature and sprayed with 0.1 M periodic acid in 0.125 N H₂SO₄. After 20 minutes at room temperature the paper was sprayed with 10 per cent sodium arsenite in 0.5 N HCl. The paper became colored by liberated iodine which was removed by further reaction with

![Fig. 1. Development of color with KDHP in the periodate-thiobarbiturate reaction.](https://example.com/f1.png)
TABLE II
Behavior of various compounds in periodate-thiobarbiturate reaction

<table>
<thead>
<tr>
<th>Compound</th>
<th>λmax</th>
<th>εmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDHP*</td>
<td>549</td>
<td>7.2 × 10⁴</td>
</tr>
<tr>
<td>2-Keto-3-deoxygalactonic acid†</td>
<td>549</td>
<td>7.2 × 10⁴</td>
</tr>
<tr>
<td>2-Keto-3-deoxygluconic acid 6-P*</td>
<td>549</td>
<td>7.2 × 10⁴</td>
</tr>
<tr>
<td>1,3,4,5,6-Pentahydroxycyclohexene-carboxylic acid‡‡</td>
<td>549</td>
<td>7.2 × 10⁴</td>
</tr>
<tr>
<td>Malondialdehyde (1,1,3,3-tetramethoxypropane)‡‡‡</td>
<td>532</td>
<td>13.0 × 10⁴</td>
</tr>
<tr>
<td>3-Deoxy-n-arabino-heptonic acid 7-P*</td>
<td>(none)</td>
<td></td>
</tr>
<tr>
<td>β-Ketobutyraldehyde dimethy acetate‡ ‡</td>
<td>542</td>
<td>3000</td>
</tr>
<tr>
<td>Glyoxal‡‡</td>
<td>546</td>
<td>2560</td>
</tr>
<tr>
<td>Glycaldehyde‡ ‡</td>
<td>517</td>
<td>136</td>
</tr>
<tr>
<td>Acetoacetic acid, aceto pyruvic acid (2,4-diketovaleric), dimedon, acetoxyaceton</td>
<td>(none)</td>
<td></td>
</tr>
</tbody>
</table>

* Treated with periodate and thiobarbiturate.
† Shikimic acid was hydroxylated with OsO₄ to give the pentahydroxycarboxylic acid and then treated with periodate to give β-formylpyruvic acid (13).
‡ Treated with thiobarbiturate only.

TABLE III
Conversion of P-enolpyruvate-erythrose-4-P condensation product and of synthetic KDHP to 5-dehydroquinate*

<table>
<thead>
<tr>
<th>Substrate†</th>
<th>Amount incubated</th>
<th>Dehydroquinate formed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>amoles</td>
<td>amoles</td>
</tr>
<tr>
<td>Synthetic KDHP.</td>
<td>0.20</td>
<td>0.17</td>
</tr>
<tr>
<td>Synthetic KDHP + EDTA ‡</td>
<td>0.20</td>
<td>0</td>
</tr>
<tr>
<td>Enzymatic product§ + EDTA ‡</td>
<td>0.27</td>
<td>0</td>
</tr>
</tbody>
</table>

* The enzyme preparation used was a fraction from E. coli strain 83-24 capable of converting KDHP to 5-dehydroquinate, and the latter product and for synthetic KDHP.
† Treated with periodate and thiobarbiturate.
‡ Treated with thiobarbiturate only.
§ 3 amoles each of erythrose-4-P and P-enolpyruvate were incubated with 300 amoles of potassium phosphate buffer, pH 7.4 and 0.5 ml of acetone fraction (1.3 mg of protein) in a total volume of 3.0 ml at 37° for 15 minutes. Unchanged P-enolpyruvate was destroyed by incubation with 0.04 ml of lactic dehydrogenase and 3 amoles each of ADP and DPNH (final volume 3.44 ml). After 30 minutes the pH was adjusted to 2.0 with 6 N HCl and then brought to 6.0 with 1 N NaOH. The precipitated proteins were removed by centrifugation. Aliquots of the supernatant solution were then used for the determination of KDHP and for conversion to dehydroquinate.

RESULTS
Scope and Sensitivity of Periodate-Thiobarbiturate Reaction

Several compounds structurally related to β-formylpyruvic acid, or expected to give rise to it on periodate oxidation, were tested in the procedure described under "Estimation of KDHP." The results are summarized in Table II. Simple aliphatic aldehydes, α, β-diketones, β-keto acids, and β-formylacetic acid (derived from 3-deoxy-n-arabino-heptonic acid 7-phosphate) did not yield colored products under these conditions. Glycaldehyde and glyoxal gave brown solutions with very small absorptions at 517 and 546 μm, respectively. In addition to the four compounds in Table II, which would be expected to yield β-formylpyruvic acid with periodate, only 3-keto-butyraldehyde dimethy acetate gave a pink color, but it was quite unstable and weak.

With KDHP, the optimal reaction time with periodate was 45 minutes (Fig. 1). However, this could be reduced to 20 minutes by the addition of 10⁻² molar concentrations of glycine or glycylglycine. The function of these compounds is unknown, and is under further investigation.

Identification of Product of Erythrose-4-P and P-enolpyruvate Condensation as KDHP

Chromatographic and Chemical Comparison with Synthetic KDHP—Erythrose-4-P and P-enolpyruvate were incubated with enzyme and aliquots were spotted on acid-washed Whatman No. 1 filter paper along with synthetic KDHP, erythrose-4-P, and P-enolpyruvate. The papers were developed with tertiary amyl alcohol-formic acid-water. KDHP was detected by the periodate-thiobarbituric acid reaction as indicated earlier, and also by spraying with semicarbazide (14, 15) (0.1 per cent semicarbazide hydrochloride in 0.15 per cent sodium acetate). The semicarbazide-treated paper was dried in an oven at 95° for 10 minutes. Under ultraviolet irradiation the semicarbazones were revealed as dark spots. By either method, a strong spot corresponding to KDHP (Rₚ = 0.49) was present in the enzymatic reaction mixture. Identical Rₚ values of 0.28 were also found for synthetic KDHP and enzymatic product with ethanol-water-acetic acid on Whatman No. 50 paper.

Biological Activity of Enzymatic and Synthetic Product—Synthetic KDHP and the product of the P-enolpyruvate-erythrose-4-P condensation were incubated with an enzyme preparation capable of converting KDHP to 5-dehydroquinate, and the latter was estimated microbiologically (16). The results, presented in Table III, showed identical behavior for the enzymatically formed product and for synthetic KDHP.

* P. R. Srinivasan and D. B. Sprinson, unpublished results (cf. (12)).
Properties of KDHP Synthetase

Phosphate, glucosamine B-phosphate and N-acetylglucosamine 6-phosphate, prepared in the same way. The pH of the reaction mixtures was determined on duplicate solutions prepared in the same way.

Fig. 3. Rate dependence of P-enolpyruvate-erythrose-4-P condensation on the erythrose-4-P concentration. In addition to erythrose-4-P, the reaction mixture contained 1.0 μmole of P-enolpyruvate, 50 μmoles of potassium phosphate buffer (pH 6.4), and 0.01 ml. of acetone fraction (51 μg. of protein) in a final volume of 0.5 ml. See “Assay” for procedure.

Fig. 4. Rate dependence of the P-enolpyruvate-erythrose-4-P condensation on the P-enolpyruvate concentration. The procedure was the same as in Fig. 3 using 1.0 μmole of erythrose-4-P and varying amounts of P-enolpyruvate.

Table IV

Stoichiometry of KDHP synthetase reaction

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>P-enolpyruvate disappeared</th>
<th>Orthophosphate formed</th>
<th>KDHP formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.53</td>
<td>1.62</td>
<td>1.56</td>
</tr>
<tr>
<td>2</td>
<td>1.68</td>
<td>1.68</td>
<td>1.68</td>
</tr>
</tbody>
</table>

The reaction mixture (3 ml.) contained 3 μmoles each of erythrose-4-P and P-enolpyruvate, 240 μmoles of Tris-maleate buffer, pH 7.0, and 0.25 ml. of acetone fraction (1.1 mg. of protein) which was dialyzed against 0.2 M Tris-maleate buffer, pH 7.0. The reaction was started by the addition of enzyme after preincubation of the other constituents at 37° for 10 minutes. Before and after an incubation period of 15 minutes aliquots were withdrawn for the determination of P-enolpyruvate, KDHP, and orthophosphate.

Inhibitors—With the acetone fraction, EDTA (4 x 10⁻³ M) did not inhibit the reaction. The addition of EDTA (12), does not inhibit KDHP formation in the acetone and cellulose fractions. Nevertheless, the addition of DPNase results in an 81 and 50 per cent inhibition of KDHP formation in the crude and (NH₄)₂SO₄ fractions, respectively, while the addition of DPNase results in a 97 and 44 per cent inhibition. These unexpected inhibitions are being further investigated.
0.2 ml. aliquots were used for KDHP analysis. The reaction mixture (0.5 ml.) was incubated at 37° for 15 minutes, and deproteinized by the addition of 0.2 ml. of 10 per cent trichloroacetic acid. 

None of the compounds tested inhibited the reaction. The values reported in the table were corrected accordingly.

* 0.25 µmole of erythrose-4-P, 0.30 µmole of P-enolpyruvate, 50 µmoles of potassium phosphate buffer pH 7.0, and inhibitor were preincubated at 37° for 10 minutes. After the addition of 0.01 ml. of acetone fraction (73 µg. of protein) the reaction mixture (0.5 ml.) was incubated at 37° for 15 minutes, and deproteinized by the addition of 0.2 ml. of 10 per cent trichloroacetic acid. 0.2 ml. aliquots were used for KDHP analysis. 

† The incubation mixture (2 ml.) contained 2.0 µmoles of erythrose-4-P, 2.5 µmoles of P-enolpyruvate, 200 µmoles of potassium phosphate buffer pH 6.4, 0.08 ml. of acetone fraction (0.6 mg. of protein), and, when added, 4.0 mg. of serum albumin, or 4 µmoles of 2-mercaptoethanol. The latter was preincubated with the enzyme for 10 minutes at 37° before addition of the other components.

‡ At this concentration of 2-mercaptoethanol (2 X 10⁻⁴ M) the thiobarbiturate assay for KDHP was inhibited 27 to 30 per cent. The values reported in the table were corrected accordingly.

**TABLE V**

**Inhibition of KDHP synthetase by phosphorylated compounds**

<table>
<thead>
<tr>
<th>Compound†</th>
<th>Amount added</th>
<th>Yield of KDHP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles</td>
<td>µmoles</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td>Sedoheptulose 1,7-diphosphate</td>
<td>0.25</td>
<td>0.12</td>
</tr>
<tr>
<td>Sedoheptulose 1,7-diphosphate</td>
<td>0.50</td>
<td>0.08</td>
</tr>
<tr>
<td>Sedoheptulose 1,7-diphosphate</td>
<td>1.0</td>
<td>0.06</td>
</tr>
<tr>
<td>Sedoheptulose 1,7-diphosphate</td>
<td>1.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Sedoheptulose 7-phosphate</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Fructose 1,6-diphosphate</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>3-Deoxy-d-arabo-heptonic acid 7-P.</td>
<td>1.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**TABLE VI**

**Extent of reaction in P-enolpyruvate-erythrose-4-P condensation**

<table>
<thead>
<tr>
<th>Additions</th>
<th>KDHP formed at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 minutes</td>
</tr>
<tr>
<td>None</td>
<td>1.2</td>
</tr>
<tr>
<td>Crystalline bovine serum albumin</td>
<td>1.2</td>
</tr>
<tr>
<td>2-Mercaptoethanol†</td>
<td>1.6</td>
</tr>
</tbody>
</table>

As far as is known at present only erythrose-4-P and P-enolpyruvate are condensed by this enzyme. That the product of the condensation is KDHP is shown by comparison of the enzymatic product with synthetic KDHP in chemical and chromatographic behavior, and in enzymatic conversion to dehydroquinase.

**DISCUSSION**

**Thiobarbiturate Reaction with β-Keto Aldehydes**—It was first observed by Weissbuch and Hurwitz (3) that a compound resulting from the action of periodate on 2-keto-3-deoxyaldehydic acids reacts with thiobarbiturate to yield an intense pink color with an absorption maximum at 549 mμ. From the structure of several compounds giving this test they concluded that β-formylpyruvic acid is responsible for the color reaction. Further support for their conclusion is shown in Table II. Although β-formylpyruvic acid has not been obtained in pure form, it has previously been isolated as its bis-2,4-dinitrophenylhydrazine from 1,3,4,5,6-pentahydroxyxylocellosecarboxylic acid (13). When the periodate-thiobarbiturate reaction was applied to this compound, an absorption curve identical with that given by KDHP and related compounds was obtained. Since β-ketobutyraldehyde, unlike several other dicarbonyl compounds, also gave a pink color† with thiobarbiturate (λmax = 542 mμ, μ), it would appear that the structure of R·CO·CH2·CHO is essential for color formation. The previous application of this test to malondialdehyde (λmax = 532 mμ (21-23)) would exemplify the case where R = H. Since β-formylpyruvic acid reacts slowly with periodate (3), the molar absorptivity index of KDHP recorded in Table II may represent a minimal value.

**Properties of KDHP Synthetase**—It may be seen from Table I that the fraction obtained from the diethylaminoethyl cellulose column represented a 60-fold purification of the KDHP synthetase. In a preliminary investigation this fraction appeared to be homogeneous in the Spinco analytical centrifuge. Most of the work reported in this paper was done with the acetone fraction, which was shown to be free of aldolase (by its inability to cleave sedoheptulose 1,7-diphosphate), P-enolpyruvate-ATP transphosphorylase, the enzyme system for the conversion of KDHP to 5-dehydroquinate, dehydroquinic-dehydrase (26), and DPNH oxidase.

As far as is known at present only erythrose-4-P and P-enolpyruvate are condensed by this enzyme. That the product of the condensation is KDHP is shown by comparison of the enzymatic product with synthetic KDHP in chemical and chromatographic behavior, and in enzymatic conversion to dehydroquinase. The enzyme preparation used in the latter test is inactive with 3-deoxy-d-arabo-heptonic acid 7-phosphate, 2-keto-3-deoxy-d-arabo-heptonic acid (diphosphorylated KDHP), 2-keto-3-deoxygluconic acid 6-phosphate, or any related compounds. Furthermore, although erythrose-4-P disappearance was not detected when incubated with enzyme in the presence of inorganic phosphate under the following conditions: (a) with ADP, Mg++ and P-enolpyruvate-ATP transphosphorylase; (b) with ADP, Mg++ and P-enolpyruvate-ATP transphosphorylase, lactic dehydrogenase and DPNH; (c) with the substrates and enzymes of (b) plus hexokinase and glucose. 

* We are indebted to Dr. H. C. Lawler for aid with this determination.
was not measured, the stoichiometry of the reaction (Table IV) is in accord with the concept that erythrose-4-P and P-enolpyruvate are condensed to form KDHP and inorganic phosphate. The specificity of the enzyme for its substrates suggests its use for the assay of either erythrose-4-P or P-enolpyruvate.

KDHP synthetase is apparently a sulfhydryl enzyme, since its inhibition by p-chloromercuribenzoate is reversed by cysteine. Although it is stable during preparation, the purer fractions (starting with the acetone fraction) showed a sharp halt in activity after 10 to 15 minutes, when the yield of KDHP was 50 to 60 per cent of the theoretical. However, when 2-mercaptoethanol was added, complete conversion of the substrates to KDHP occurred. This behavior may be due to a greater tendency of the enzyme to oxidation in the presence of its substrates.

**Mechanism of Condensation Reaction**—The condensation of erythrose-4-P and P-enolpyruvate may be tentatively regarded (as shown in Scheme I) as a nucleophilic attack on P-enolpyruvate, by a reagent here symbolized as OH\(^{-}\), resulting in the release of phosphate and the formation of the open chain form of KDHP. It is analogous to the CO\(_2\) fixation reaction (27, 28): P-enolpyruvate\(^{-}\) + HCO\(_3\)^{-} → oxaloacetate\(^{-}\) + HPO\(_4\)^{2-}. Since the pyranose form of KDHP is highly favored both in the solid state and in solution\(^2\), cyclization to this form would presumably occur spontaneously. The effect on KDHP formation of 6- and 7-carboxylate forms of KDHP is highly favored both in the solid state and in the corresponding aldehydes (glyceraldehyde 3-phosphate, and N-acetyl-n-mannosamine, respectively).

**Scheme I**

An enzyme which catalyzes the condensation of d-erythrose 4-phosphate and phosphoenolpyruvate to form 2-keto 3-deoxy-D-arabo-heptonic acid 7-phosphate and inorganic phosphate was purified from extracts of *Escherichia coli*. After a 60-fold purification it appeared homogeneous in the ultracentrifuge. The name 2-keto-3-deoxy-D-arabo-heptonic acid 7-phosphate synthetase is suggested for this enzyme.

The yields of 2-keto-3-deoxy-D-arabo-heptonic acid 7-phosphate were quantitative, and attempts to reverse the reaction were unsuccessful. Only phosphoenolpyruvate and d-erythrose 4-phosphate were activated by the enzyme. It had the properties of a sulfhydryl protein and showed maximal activity in the presence of 2-mercaptoethanol. No cofactor requirements could be demonstrated. The reaction was inhibited by fructose 1,6-diphosphate, sedoheptulose 1,7-diphosphate, and 3-deoxy-D-arabo-heptonic acid 7-phosphate.

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10 A \(\Delta F^\circ\) value of approximately -1000 calories can be calculated from the published data (32) for the reaction: pyruvate\(^{-}\) + N-acetyl-D-mannosamine → N-acetylneuraminate\(^{-}\).
2-Keto-3-deoxy-d-arabo-heptonic Acid 7-Phosphate Synthetase

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