THE FORMATION OF SEDOHEPTULOSE PHOSPHATE FROM PENTOSE PHOSPHATE

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In 1932, Warburg and his collaborators (1) discovered an enzyme system in red blood cells which catalyzed the oxidation of glucose-6-phosphate with triphosphopyridine nucleotide (TPN) as the coenzyme. The product of the reaction, identified as 6-phosphogluconate (2), was later found to be further oxidized in the presence of the same coenzyme (3). This work, together with similar studies by Lipmann (4) and Dickens (5, 6), indicated the existence of a pathway for the direct oxidation of glucose-6-phosphate which was distinct from the Embden-Meyerhof glycolytic mechanism. The formation of pentose phosphate in the oxidation of 6-phosphogluconate, first demonstrated by Dickens (6), was confirmed and extended by Scott and Cohen (7) and by Horecker, Smyrniotis, and Seegmiller (8, 9). Ribulose-5-phosphate was identified as the product of the oxidative decarboxylation of 6-phosphogluconate in yeast and in animal tissues, and this ester was shown to be in equilibrium with ribose-5-phosphate (8).

In a preliminary communication (10), we have reported the identification of sedoheptulose-7-phosphate as a product of pentose phosphate metabolism. Evidence obtained with crude liver preparations (11) suggested that this substance was an intermediate in the conversion of pentose phosphate to hexose monophosphate. The present paper is concerned with the cleavage of pentose phosphate by purified enzymes from rat liver or spinach which results in the formation of sedoheptulose phosphate. The enzyme (or enzymes) which catalyzed this conversion has been shown to contain thiamine pyrophosphate as the prosthetic group (13, 14). On the basis of its ability to catalyze the formation and breakdown of ketol linkages, it is referred to as transketolase.

Methods

Materials—Barium ribulose-5-phosphate was prepared enzymatically by the oxidation of 6-phosphogluconate, as previously described (15). The

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1 The formation of sedoheptulose phosphate from pentose phosphate by spinach leaf extracts has recently been reported by Axelrod et al. (12).
specific rotation was $[\alpha]_D^{20} -28.2^\circ$ to $-31.9^\circ$, from which it was estimated that in these preparations about 15 to 20 per cent of the pentose was ribose-5-phosphate. Barium ribose-5-phosphate, barium fructose diphosphate, and thiamine pyrophosphate were commercial preparations. Reduced diphosphopyridine nucleotide (DPNH) was prepared by the method of Ohlmeyer (16) from DPN obtained by the method of Kornberg and Pricer (17). TPN was prepared according to Kornberg and Horecker (18). L-Erythulose o-nitrophenylhydrazone was prepared from erythritol with Acetobacter suboxydczns according to Müller, Montigel, and Reichstein (19). A sample was provided by Dr. G. C. Mueller of the University of Wisconsin. D-Erythrose was a gift of Dr. H. S. Isbell of the National Bureau of Standards. D-Glyceraldehyde-3-phosphate was supplied by Dr. H. A. Lardy of the University of Wisconsin. Sedoheptulosan monohydrate was provided by Dr. N. K. Richtmyer of this Institute. Fructose-1-phosphate synthesized by Pogell (20) was supplied by Dr. R. W. McGilvery of the University of Wisconsin. Ribose-1-C$^{14}$, obtained from Dr. H. S. Isbell, was esterified in the 5 position with a bacterial ribokinase. The preparation of this enzyme will be reported in a subsequent publication. Hydroxypyruvic acid was kindly provided by Dr. A. Meister of the National Cancer Institute.

Glycerophosphate dehydrogenase was a crude preparation from rabbit muscle (21) which also contained triose phosphate isomerase and aldolase. Crystalline aldolase was prepared according to Taylor et al. (22). Zwi- schenferment free of phosphogluconic dehydrogenase was prepared by the method of Kornberg (23). Hexose phosphate isomerase was prepared from rabbit muscle extract by precipitation with ammonium sulfate between 20 and 50 per cent saturation. The precipitate was stored as a paste at 5° and dissolved as needed. Acid phosphatase was purified from potato by the method of Kornberg.²

Dowex 1, 10 per cent cross-linked, was obtained from The Dow Chemical Company.

Analytical Methods—Spectrophotometric measurements were made at room temperature with a Beckman model DU spectrophotometer in 1.0 cm. cells. Manometric determinations were carried out with the conventional Warburg apparatus. Radioactivity was measured in a gas flow counter (24) with sufficiently thin samples to give negligible self-absorption (<10 per cent).

Thiamine pyrophosphate was determined by the method of Ochoa and Peters (25).

Mixtures of pentose and heptulose (or their phosphate esters) were analyzed in the orcinol test according to Mejbaum (26), except that heating

² Unpublished procedure.
was continued for 40 minutes (27). The solutions were read at 580 and 670 m\(\mu\) and the concentration of pentose and heptulose calculated by the solution of simultaneous equations. Recrystallized D-arabinose (\([\alpha]_{D}^{20} -104.5^\circ\)) and sedoheptulose monohydrate served as standards and were included in each set of determinations. The recovery of arabinose and sedoheptulose from known mixtures is shown in Table I. Occasionally other components of the reaction mixtures, such as fructose esters, interfered with the determinations; when large amounts of these were present suitable corrections were made. With sedoheptulose-7-phosphate the absorption at 580 m\(\mu\) is not significantly different from that with sedoheptulo-

### Table I

**Pentose and Heptulose Determination with Orcinol Test**

1.0 ml. of solution containing the quantities of pentose and heptulose indicated was treated with 0.1 ml. of 10 per cent orcinol in ethanol and 1.0 ml. of 0.1 per cent FeCl\(_3\) in concentrated HCl. The solutions were heated for 40 minutes at 100\(^\circ\) and cooled, and the densities determined with the Beckman spectrophotometer.

<table>
<thead>
<tr>
<th>Added</th>
<th>Optical density</th>
<th>Found*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedoheptulose</td>
<td>Arabinose</td>
</tr>
<tr>
<td>(\mu M \times 10^3)</td>
<td>(\mu M \times 10^3)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>40.0</td>
<td>0.121</td>
</tr>
<tr>
<td>10.0</td>
<td>30.0</td>
<td>0.175</td>
</tr>
<tr>
<td>20.0</td>
<td>20.0</td>
<td>0.235</td>
</tr>
<tr>
<td>30.0</td>
<td>10.0</td>
<td>0.286</td>
</tr>
<tr>
<td>40.0</td>
<td>0</td>
<td>0.336</td>
</tr>
</tbody>
</table>

* Calculated from equations derived from the first and last samples: heptulose \(= (3.8D_{580} - D_{670}) (0.034)\); pentose \(= (3.5D_{670} - D_{580}) (0.027)\); \(D\) refers to optical density.

Although the rate of color development is somewhat greater. However, since ribulose-5-phosphate yields a somewhat lower intensity at 670 m\(\mu\) than the aldopentose standards (15), accurate measurements of stochiometry could not be carried out with this substrate. For such measurements ribose-5-phosphate, which reacts quantitatively in the orcinol test, was employed. Either substrate could be used, since all of the transketo-lase preparations contained pentose phosphate isomerase. The amount of ribulose-5-phosphate formed from ribose-5-phosphate at equilibrium (about 25 per cent of the total pentose) did not appreciably alter the pentose values obtained in the orcinol test.

Glucose-6-phosphate was determined spectrophotometrically with TPN and Zwischenferment (28). Total hexose monophosphate was determined by the same procedure except that hexose phosphate isomerase was also
added. Total triose phosphate, together with hexose diphosphate, was determined spectrophotometrically with DPNH and the glycerophosphate dehydrogenase preparation (21). d-Glyceraldehyde-3-phosphate was determined with DPN and recrystallized glyceraldehyde-3-phosphate dehydrogenase (29). Protein was determined by the turbidimetric method of Büchler (30), standardized with protein samples of known nitrogen content.

**Enzyme Assays**—In crude preparations aldolase activity was determined by the method of Sibley and Lehninger (31). A unit of aldolase was defined as the amount of enzyme required to give a density of 1.0 at 540 mμ, measured in 16 mm. cuvettes with the Coleman junior spectrophotometer. The activity of crystalline aldolase was measured spectrophotometrically with hexose diphosphate, DPN, and crystalline glyceraldehyde-3-phosphate dehydrogenase.

Transketolase was assayed by the method developed by Racker for phosphofructokinase (21), which depends on the formation of triose phosphate. The reactions, which will be discussed more fully later, are in part represented by the following equations.

\[
\begin{align*}
(1) \text{Ribulose-5-phosphate} & \rightarrow \text{triose phosphate} + \text{“active glycolaldehyde”} \\
(2) \text{Triose phosphate} + \text{DPNH} + \text{H}^+ & \rightarrow \alpha\text{-glycerophosphate} + \text{DPN}^+
\end{align*}
\]

The test system contained 0.4 μM of ribulose-5-phosphate, 0.06 μM of DPNH, 0.14 mg. of glycerophosphate dehydrogenase preparation, 6.0 μM of cysteine, and 10.0 μM of glycylglycine buffer, pH 7.5. The total volume was 1.03 ml. In the presence of excess glycerophosphate dehydrogenase and triose phosphate isomerase the rate of DPNH oxidation (measured at 340 mμ) was proportional to the amount of transketolase present. Thus with 0.26, 0.53, and 0.79 γ of spinach transketolase the rate of density change was 0.013, 0.026, and 0.038 per minute, respectively. A unit of enzyme was defined as the amount required to give a decrease in optical density of 1.0 per minute under the conditions of the test. The specific activity is the number of units per mg. of protein.

**Purification from Liver (Table II)**

*Extraction*—Acetone powder of rat liver was prepared as previously described (32). The powder (24 gm.) was extracted for 15 minutes at 0° with 265 ml. of 0.02 m K2HPO4, centrifuged, and the residue discarded. (Acetone powder extract, 215 ml.)

*Ammonium Sulfate Fractionation*—The acetone powder extract was treated with 62.5 gm. of ammonium sulfate and centrifuged, and to the supernatant solution were added 26.8 gm. of ammonium sulfate. The precipitate was collected by centrifugation, dissolved in 75 ml. of 0.01 m phosphate buffer, pH 7.6, and adjusted to pH 7.7 with 2.5 ml. of 2 N ammonium hydroxide. (Ammonium Sulfate I, 74.0 ml.)
Methanol Fractionation—The methanol fractionation which followed was performed on a small scale to permit rapid cooling and centrifugation. To 37 ml. of the ammonium sulfate fraction, diluted to 120 ml. with cold water, were added 130 ml. of methanol, previously chilled to \(-12^\circ\). The addition was complete in about 30 seconds, following which the solution was cooled in an ice-ethanol freezing bath at \(-20^\circ\) for not longer than 2 minutes, until the temperature was \(-13^\circ\) to \(-16^\circ\). The precipitate was removed by centrifugation for 1 minute at \(-10^\circ\) in a Servall SS-1 centrifuge, and the slightly turbid supernatant solution was treated in the same manner with 95 ml. of cold methanol. The precipitate was suspended in 30 ml. of water and the insoluble residue centrifuged and discarded. The

<table>
<thead>
<tr>
<th>TABLE II</th>
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<tbody>
<tr>
<td>Purification of Transketolase from Rat Liver</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Fraction</th>
<th>With ribose-5-P</th>
<th>With ribulose-5-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total units</td>
<td>Total units</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone powder extract</td>
<td>388</td>
<td>409</td>
</tr>
<tr>
<td>Ammonium Sulfate I</td>
<td>170</td>
<td>207</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>87</td>
<td>138</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>42</td>
<td>77</td>
</tr>
<tr>
<td>Ammonium Sulfate II</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>&quot; III*</td>
<td>17</td>
<td>56</td>
</tr>
</tbody>
</table>

* Several preparations at the stage of Ammonium Sulfate II were pooled and refractionated with ammonium sulfate.

procedure was repeated and the solutions combined. (Methanol fraction, 58 ml.)

Acetone Fractionation—For the acetone fractionation the combined methanol fractions were diluted with 12 ml. of water and treated with 2 ml. of 4 M sodium acetate. The acetone additions required about 2 minutes, during which time the solution was cooled to \(-8^\circ\) in a freezing bath. After 3 minutes at this temperature the solutions were centrifuged for 2 minutes (International, size 2) and the precipitates dissolved in 8 ml. of cold water. The first fraction was collected after the addition of 22 ml. of acetone and two more fractions after the further addition of 11 ml. each time. The last two fractions were assayed separately; the bulk of the activity was usually, although not invariably, in the last fraction. (Acetone fraction, 9.2 ml.)

Calcium Phosphate Gel Adsorption and Ammonium Sulfate Fractionation II—The acetone fraction was treated with 7.5 ml. of calcium phosphate gel (33) which had been aged 3 to 6 months (dry weight 8 mg. per ml.). The gel was centrifuged and discarded. The solution (14.5 ml.) was treated
with 5.2 gm. of ammonium sulfate and centrifuged. The supernatant solution was treated with 0.5 gm. of ammonium sulfate and the precipitate collected and dissolved in 2.0 ml. of 0.02 M phosphate buffer, pH 8.0. (Ammonium Sulfate II, 2.5 ml.)

Properties of Purified Liver Preparation—With the crude extract the rate of pentose phosphate cleavage was as rapid with ribose-5-phosphate as with ribulose-5-phosphate. The purified preparations, however, were 2 to 3 times as active with ribulose-5-phosphate (Table II). The presence of pentose phosphate isomerase could be demonstrated in these preparations. Further efforts to remove this isomerase were not successful.

The liver preparations lost activity when stored overnight in the frozen state, but were fully reactivated by the addition of cysteine. With the assay system described, which contained cysteine, the purified liver enzyme preparations showed no decline in activity during storage for 10 months at −16°C.

Purification from Spinach (Table III)

Extraction and Ammonium Sulfate Fractionation I—60 gm. of spinach leaves, from which the stems were removed, were homogenized for 3 minutes in a Waring blender with 360 ml. of cold 50 per cent saturated ammonium sulfate which had been adjusted to pH 7.8 with concentrated ammonium hydroxide. The homogenates were filtered with Schleicher and Schuell No. 588 fluted filter paper. 16.9 liters of filtrate derived from 3.6 kilos of leaves were treated with 3.82 kilos of ammonium sulfate and filtered overnight in the cold room. The precipitate was dissolved in 500 ml. of water and adjusted to pH 7.1 with 2.2 ml. of 2 N ammonium hydroxide. (Ammonium Sulfate I, 600 ml.)

Ammonium Sulfate Fractionation II—The solution was diluted with water (180 ml.) to bring the ammonium sulfate saturation to 0.10, as determined by conductivity measurement with a model PM-2 Barnstead purity meter. The diluted solution was treated with 176 gm. of ammonium sulfate and centrifuged, and to the supernatant solution were added 78 gm. of ammonium sulfate. The second precipitate was collected by centrifugation, dissolved in 90 ml. of water, and neutralized with 0.3 ml. of 2 N ammonium hydroxide. (Ammonium Sulfate II, 108 ml.)

Calcium Phosphate Gel Adsorption and Ammonium Sulfate III—The solution was diluted with 1188 ml. of water to bring the protein content to 1.2 mg. per ml., treated with 1130 ml. of calcium phosphate gel (6.6 mg. dry weight per ml.), and centrifuged. The enzyme was eluted with 216 ml. of 0.01 M pyrophosphate buffer, pH 8.3. The eluate (262 ml.) was treated with 81.0 gm. of ammonium sulfate, centrifuged, and the supernatant solution treated with 30.0 gm. of ammonium sulfate. The precipitate obtained
was collected by centrifugation, dissolved in 25 ml. of water, and neutralized with 0.65 ml. of 0.2 N ammonium hydroxide. (Ammonium Sulfate III, 29.0 ml.)

**Acetone Fractionation**—Ammonium Sulfate III was dialyzed overnight against 0.1 M sodium acetate, adjusted to pH 7.2. The dialyzed solution was diluted to 77.4 ml. with 0.1 M sodium acetate to bring the protein content to 6.2 mg. per ml. and cooled in a freezing bath while 47.5 ml. of cold acetone were added. The solution was kept at -8° for 3 minutes, centrifuged rapidly, and the precipitate dissolved in 20 ml. of water. Three more fractions were collected in the same manner by the addition of 14, 15, and 25 ml. of acetone, respectively. These fractions were assayed separately and the most active ones (usually Fractions 3 and 4) were pooled.

(Acetone fraction, 42.0 ml.)

**Ammonium Sulfate IV**—The acetone fractions were diluted to 72.0 ml. and treated with 72.0 ml. of cold saturated ammonium sulfate, which had previously been adjusted to pH 7.8, and centrifuged. The supernatant solution was treated with 8.6 gm. of ammonium sulfate and centrifuged. The precipitate was dissolved in 3.0 ml. of 0.25 M glycylglycine buffer, pH 7.4. Two more fractions were collected by successive additions of 4.7 gm. of ammonium sulfate and the precipitates dissolved as before. Fractions 3 and 4, after assay, were combined. (Ammonium Sulfate IV, 6.6 ml.)

### Table III

**Purification of Transketolase from Spinach**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total units</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>24,500</td>
<td>0.4</td>
</tr>
<tr>
<td>Ammonium Sulfate I</td>
<td>17,100</td>
<td>1.6</td>
</tr>
<tr>
<td>&quot; II</td>
<td>14,000</td>
<td>9.2</td>
</tr>
<tr>
<td>&quot; III</td>
<td>9,440</td>
<td>18.2</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>7,270</td>
<td>37.0</td>
</tr>
<tr>
<td>Ammonium Sulfate IV</td>
<td>5,440</td>
<td>47.0*</td>
</tr>
</tbody>
</table>

*Assayed with ribulose-5-phosphate; the rate with ribose-5-phosphate was 50 to 60 per cent lower.

**Products of Pentose Phosphate Metabolism**

With Purified Liver Transketolase—Although the purified preparation was active in the assay system based on triose phosphate formation (Equations 1 and 2), by itself it catalyzed only a limited cleavage of ribose-5-phosphate or ribulose-5-phosphate. When the crude glycerophosphate
dehydrogenase preparation employed in the assay system was also present, a rapid and extensive disappearance of pentose phosphate occurred. The same result was obtained when crystalline muscle aldolase replaced the glycerophosphate dehydrogenase preparation. In the presence of both aldolase and liver transketolase the disappearance of pentose phosphate was accompanied by the formation of heptulose phosphate (Fig. 1). The spectrum obtained in the orcinol test with the products of the enzyme reaction represented that which would arise from a mixture of heptulose phosphate and pentose phosphate; the reaction therefore did not proceed to completion. The residual pentose phosphate was isolated by ion exchange chromatography and found to be an equilibrium mixture of ribulose-5-phosphate and ribose-5-phosphate. It was fully active in the transketolase assay system.

The stoichiometry of pentose phosphate conversion in the presence of aldolase is shown in Table IV. 1 mole of heptulose phosphate was formed for every 2 moles of pentose phosphate which reacted. Triose phosphate which accumulated, including that converted to hexose diphosphate, was only about one-half of the heptulose formed.

With Purified Spinach Transketolase—Purified spinach preparations with specific activity about 20 times greater than that of the best liver preparations catalyzed the conversion of pentose phosphate to heptulose phosphate in the absence of added aldolase (Table V). No aldolase activity could be detected in the spinach preparation; however, some increase in pentose utilization was observed when large amounts of aldolase were added.
In the presence of excess substrate the activity of the purified spinach preparation with ribose-5-phosphate was about 40 to 50 per cent of that obtained with ribulose-5-phosphate; however, pentose phosphate isomerase was still present. As in the case of the liver enzyme, the reaction did not go to completion and the pentose phosphate recovered from the reaction mixture by ion exchange chromatography had the properties of an equilibrium mixture of ribose-5-phosphate and ribulose-5-phosphate.

**Identification of Sedoheptulose**—The heptulose ester present after incubation of ribose-5-phosphate with the spinach enzyme (or with the liver preparation and aldolase) was hydrolyzed with acid phosphatase from potato and the sugar identified by the formation of sedoheptulosan tetra-benzoate.

Ribose-5-phosphate (457 µM) was incubated for 90 minutes at 25° with 88 mg. of purified liver transketolase and 38 mg. of aldolase in 92 ml. of 0.01 M glycylglycine buffer, pH 7.6, containing 0.006 M cysteine. The

<table>
<thead>
<tr>
<th>Table IV</th>
<th>Stoichiometry of Pentose Phosphate Conversion with Liver Transketolase</th>
</tr>
</thead>
<tbody>
<tr>
<td>The reaction mixture contained 6.26 µM of pentose phosphate, 0.36 mg. of liver transketolase, 0.37 mg. of aldolase, 11 µM of glycylglycine buffer, pH 7.5, and 7 µM of cysteine. The total volume was 1.1 ml.; the temperature was 23°.</td>
<td></td>
</tr>
<tr>
<td><strong>Min.</strong></td>
<td>30 min.</td>
</tr>
<tr>
<td>Pentose phosphate consumed</td>
<td>-3.59</td>
</tr>
<tr>
<td>Heptulose phosphate formed</td>
<td>+1.54</td>
</tr>
<tr>
<td>Triose phosphate formed</td>
<td>+0.74</td>
</tr>
<tr>
<td>Fructose diphosphate formed</td>
<td>+0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table V</th>
<th>Pentose Phosphate Conversion with Spinach Transketolase</th>
</tr>
</thead>
<tbody>
<tr>
<td>The reaction mixture (0.6 ml.) contained 3.10 µM of ribulose-5-phosphate, 0.01 mg. of spinach transketolase (28 units per mg.), 8 µM of glycylglycine, and 3 µM of cysteine. The pH was 7.4 and the temperature 25°. 0.08 mg. of aldolase was added as indicated. The pentose utilization values are approximate, since the precise value for ribulose phosphate in the orcinol reaction is not known.</td>
<td></td>
</tr>
<tr>
<td><strong>Min.</strong></td>
<td>Pentose phosphate utilized</td>
</tr>
<tr>
<td>No aldolase</td>
<td>Aldolase</td>
</tr>
<tr>
<td>µM</td>
<td>µM</td>
</tr>
<tr>
<td>30</td>
<td>1.16</td>
</tr>
<tr>
<td>60</td>
<td>1.59</td>
</tr>
<tr>
<td>90</td>
<td>1.97</td>
</tr>
</tbody>
</table>
reaction mixture, containing 170 μM of sedoheptulose phosphate and 56 μM of pentose phosphate, was brought to pH 5.2 by the addition of 100 ml. of 0.2 M sodium acetate buffer, pH 5.0, containing 0.01 M magnesium chloride, and incubated for 50 minutes with 3.7 mg. of potato phosphatase. The resulting solution, containing 157 μM of sedoheptulose, was heated for 5 minutes at 85° and deionized with a mixed bed deionizing column (Duo-lite A-4 and Amberlite IR-120(H+), 2 cm. × 50 cm.). The effluent and washings (337 ml.) were evaporated to dryness and the sedoheptulose converted to sedoheptulosan by heating at 100° for 1 hour with 1.0 ml. of 0.5 N HCl. This was converted to the tetrabenzoate by the procedure of Haskins, Hann, and Hudson (34). 29 mg. of product (48 μM) were obtained, which after two recrystallizations had the properties shown in

| TABLE VI
Sedoheptulosan Tetrabenzoate |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Derivative</td>
</tr>
<tr>
<td>(1) Authentic</td>
</tr>
<tr>
<td>(2) Liver enzyme product</td>
</tr>
<tr>
<td>(1) and (2) mixed</td>
</tr>
<tr>
<td>(3) Spinach enzyme product</td>
</tr>
<tr>
<td>(1) and (3) mixed</td>
</tr>
</tbody>
</table>

* Determined with the Fisher-Johns hot stage apparatus.
† C = 0.64 in CHCl₃.
‡ C = 0.39 in CHCl₃.

Table VI. The identity was further confirmed by examination of the x-ray diffraction patterns of the authentic and enzymatic products.

A similar preparation was carried out with spinach transketolase. The incubation mixture contained 690 μM of ribose-5-phosphate and 15.0 mg. of purified spinach preparation in 182 ml. of 0.01 M glycylglycine buffer, pH 7.6, containing 0.006 m cysteine. 268 μM of sedoheptulose phosphate were formed after 110 minutes at room temperature. This product was dephosphorylated, deionized, and converted to sedoheptulosan tetrabenzoate as before. The crude product weighed 16 mg. and after two recrystallizations had the properties shown in Table VI.

Thus the same reaction product was formed with the spinach transketolase preparation or the liver enzyme fortified with aldolase.

Isolation of Sedoheptulose Phosphate—The heptulose ester formed from pentose phosphate was isolated by ion exchange chromatography. The reaction mixture, 50 ml., contained 703 μM of ribose-5-phosphate, 15 units of spinach transketolase (1.0 mg. of protein), 170 μM of cysteine, and 280
μM of glycylglycine buffer, pH 7.7. After 165 minutes at room temperature the reaction mixture, containing 235 μM of sedoheptulose phosphate, was placed on a Dowex 1 (formate) column 13 cm. × 2.5 cm., washed with 50 ml. of water, and eluted with 0.2 N formic acid containing 0.03 N sodium formate (Fig. 2). The fractions containing heptulose phosphate (Fractions 24 to 29) were adjusted to pH 6.2 with 5.0 ml. of 4 N NaOH and 21.0 ml. of saturated barium hydroxide solution and precipitated with 4 volumes of ethanol. The precipitate was collected by centrifugation, washed with 10 ml. of 80 per cent ethanol, and dried in vacuo. The yield of dried barium salt was 117 mg.

50.0 mg. of product dissolved in 1.50 ml. of 0.02 N acetic acid were found to contain 85.5 μM of sedoheptulose and 86.5 μM of organic phosphate. This corresponds to a purity of 73 per cent and indicates the virtual absence of other phosphate esters. This was confirmed by enzymatic assay for triose phosphate and hexose diphosphate with α-glycerophosphate dehydrogenase and DPNH, and for glucose-6-phosphate with TPN and Zwi-
schenferment, which showed the absence of these compounds. The preparation contained 4.8 per cent of inorganic phosphate.

Reversibility of Sedoheptulose Phosphate Formation

The failure of pentose phosphate to undergo complete conversion to sedoheptulose phosphate suggested that the reaction is a reversible one. This was confirmed when a mixture of sedoheptulose phosphate and DL-glyceraldehyde-3-phosphate was incubated with spinach transketolase. Nearly the same amount of pentose phosphate was produced from these reactants as remained when pentose phosphate was the substrate (Fig. 3). The same was also roughly true for sedoheptulose phosphate. Under these conditions the reaction appears to favor the breakdown of pentose phosphate.

Evidence for the reversibility of sedoheptulose phosphate formation has also been obtained with the purified liver preparation. In the presence of 0.8 unit of liver transketolase, without added aldolase, 0.5 μM of pentose phosphate was formed in 30 minutes from 1.5 μM of sedoheptulose phosphate and 2.8 μM of DL-glyceraldehyde-3-phosphate. More extensive pentose synthesis by this preparation was prevented by the presence of enzymes which caused a rapid disappearance of the D-glyceraldehyde-3-phosphate added.

Coenzyme of Transketolase

The presence of thiamine pyrophosphate (ThPP) in the purified spinach preparation was first detected by paper chromatography of a boiled enzyme preparation by use of a modified thiochrome reaction. Quantitative assay of the enzyme preparation with yeast carboxylase (Table VII) showed it to contain nearly 1 equivalent of coenzyme per mole of protein, assuming the molecular weight to be 100,000 gm. per mole.

Transketolase was prepared free from coenzyme by precipitation at low pH in the presence of ammonium sulfate, with essentially the procedure which was introduced by Warburg and Christian (35) for the flavoproteins. 3.0 ml. of spinach transketolase containing 5.2 mg. of protein (19.7 units per mg.) were brought to 45 per cent saturation by the addition of 2.5 ml. of saturated ammonium sulfate solution and buffered with 0.07 ml. of 4 M sodium acetate. The solution was placed in an ice bath and 0.2 ml. of 2.2 M sulfuric acid were added until the solution was acid to methyl orange. After 5 minutes at 0° the precipitate was collected by centrifugation in the cold, dissolved in 1.0 ml. of 0.25 M glycylglycine buffer, pH 7.4, and neutralized with 2.0 N ammonium hydroxide. The resolved enzyme was stable

Unpublished procedure of Dr. S. Korkes of New York University.
when stored in the frozen state at \(-16^\circ\). Liver transketolase was prepared free of coenzyme in the same manner.

The yield of resolved enzyme by this procedure was 70 to 80 per cent, and no loss in specific activity was observed. In the presence of excess ThPP the specific activity of a resolved spinach enzyme preparation was 20.6 units per mg., compared with 19.7 units per mg. before acid precipitation. Under similar conditions the specific activity of the liver enzyme preparation before and after acid precipitation was 1.3 units per mg.

The cocarboxylase and Mg\(^{++}\) requirements are shown in Fig. 4. Neither of the resolved enzyme preparations showed appreciable activity in the absence of ThPP. While spinach transketolase required Mg\(^{++}\) for full activation, no such requirement was observed with liver transketolase. Spinach transketolase was fully reactivated at a ThPP concentration of about \(10^{-4}\) M, and half maximal rates were observed at \(1.5 \times 10^{-6}\) M. Comparable amounts of the active enzyme before resolution contained \(5 \times 10^{-7}\) M ThPP. The resolved enzyme thus required much higher ThPP concentrations for reactivation than were present in the intact enzyme. Furthermore, the activating effect of ThPP was not immediate, but required 4 to 6 minutes before linear rates were observed. Similar results have been reported for the activation of carboxylase by this coenzyme (36).

**Table VII**

**Thiamine Pyrophosphate Content of Spinach Transketolase**

<table>
<thead>
<tr>
<th>Addition</th>
<th>CO(_2)</th>
<th>ThPP ((\mu)M)</th>
<th>ThPP* (equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ThPP, (2 \times 10^{-4}) M</td>
<td>162</td>
<td>(1.5 \times 10^{-4})</td>
<td>0.8</td>
</tr>
<tr>
<td>“ 4 (\times 10^{-4}) ”</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled enzyme, 0.019 mg.</td>
<td>142</td>
<td>(2.8 \times 10^{-4})</td>
<td>0.7</td>
</tr>
<tr>
<td>“ 0.038 “</td>
<td>190</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Moles of ThPP per 100,000 gm. of protein.

Effect of Aldolase

With the purified liver transketolase preparations little heptulose phosphate was formed in the absence of added aldolase (Fig. 1). With crude
preparations, which contained aldolase, the conversion of pentose phosphate to heptulose phosphate occurred without further addition (Fig. 5, Experiment A). A synthetic mixture, made up with purified liver transketolase and crystalline aldolase to contain the same number of units of

![Fig. 4](http://www.jbc.org/)  
**Fig. 4.** The requirement of transketolase for ThPP and Mg++. Transketolase activity was followed by measurement of DPNH oxidation as described in "Methods." In A the absorption cells contained $3.9 \times 10^{-3}$ mg. of resolved spinach enzyme (8.8 units per mg.) and $1 \times 10^{-4}$ M ThPP and $2 \times 10^{-3}$ M MgCl$_2$ as indicated. In B the absorption cells contained 0.02 mg. of resolved liver enzyme (1.3 units per mg.) and $1 \times 10^{-3}$ M ThPP and $2 \times 10^{-3}$ M MgCl$_2$ as indicated. Mg$^{++}$ and ThPP were added last, after the other components of the reaction were mixed.

**Fig. 5.** Pentose phosphate metabolism with the crude liver preparation and with purified liver transketolase with added aldolase. The reaction mixture (0.8 ml.) contained 4.25 μM of ribulose-5-phosphate, 3.0 μM of glycyglycine buffer, pH 7.4, and 1.8 μM of cysteine. Enzyme additions, as indicated, were 0.12 ml. of crude liver preparation, containing 0.28 unit of transketolase and 11.4 units of aldolase (Sibley-Lehninger method); 0.01 ml. of purified transketolase, containing 0.28 unit of transketolase and 0.17 unit of aldolase; 0.014 ml. of crystalline aldolase, containing 11.2 units. Incubation was at 25°. Experiment A contained the crude liver preparation, and Experiment B purified liver transketolase. Experiment C and D contained purified liver transketolase; aldolase added at the start of the experiment in Experiment C and at the time indicated by the arrow in Experiment D. The crude preparation was a liver acetone powder extract precipitated with ammonium sulfate. The fraction precipitating between 40 and 70 per cent saturation was collected.

![Fig. 5](http://www.jbc.org/)  

each enzyme as were present in the crude liver preparation, was found to utilize pentose phosphate and form heptulose phosphate at the same rate (Experiment C). When aldolase was omitted from the purified system, the same initial rate was observed (Experiment B and D), but the reaction came to rest after only 26 per cent of the pentose phosphate had reacted. Addition of aldolase after the end-point had been reached resulted in a resumption of the reaction at essentially the initial rate. From these re-
sults it may be concluded that the transketolase equilibrium favors the formation of pentose phosphate. Heptulose phosphate accumulates only when a suitable mechanism for displacing the equilibrium is provided. This function can apparently be performed by aldolase as well as by some other component present in the purified spinach preparation; however, the mechanism is not yet clear.

Condensation Reactions with Tetrose and Triose Phosphate

Several new condensations have been observed, including the reaction of dihydroxyacetone phosphate with D-erythrose and of L-erythulose with glyceraldehyde-3-phosphate. While heptulose phosphate is formed in these reactions, evidence is available which indicates that these tetroses are not intermediates in the formation of the 7-carbon sugar from pentose phosphate.

D-Erythrose and Dihydroxyacetone Phosphate—Aldolase has been shown to catalyze the condensation of dihydroxyacetone phosphate with a number of aldehydes. On the basis of its known specificity (37), it would be expected that dihydroxyacetone phosphate and D-erythrose would react to form sedoheptulose-1-phosphate. This reaction was demonstrated with 47 μM of fructose diphosphate as a source of dihydroxyacetone phosphate, 100 μM of D-erythrose, and 20 mg. of aldolase (five times recrystallized) in 10 ml. of 0.01 M glycylglycine buffer, pH 7.4, containing 0.006 M cysteine. After incubation at room temperature for 24 hours the mixture was chromatographed on a Dowex 1 (formate) column, 14.5 cm. × 0.8 cm., as previously described. The fractions containing heptulose were adjusted to pH 6.2 with saturated barium hydroxide solution and precipitated with 4 volumes of ethanol. The precipitate was dried and dissolved in 0.01 N HCl, and barium was removed with a slight excess of potassium sulfate. 22 μM of heptulose phosphate were recovered. The acid lability of this ester is shown in Fig. 6. Although it was somewhat more stable than fructose 1-phosphate under comparable conditions (20), the rate of hydrolysis was consistent with esterification in the 1 position. The ester formed from pentose phosphate with transketolase was much more stable to acid hydrolysis and was presumably esterified in the 7 position (Fig. 6). Since this is the form which accumulated in the transketolase reaction, the labile ester, if it were an intermediate in the formation of sedoheptulose phosphate from pentose phosphate, should be converted to the stable form by the liver preparation. The aldolase reaction product (assumed to be sedoheptulose-1-phosphate) was incubated with purified liver transketolase under conditions identical with those employed for the formation of the stable ester from pentose phosphate. No decrease in acid lability was detected.
L-Erythrulose and Glyceraldehyde-3-phosphate—In the presence of purified spinach transketolase both pentose phosphate and heptulose phosphate were formed from L-erythrulose and DL-glyceraldehyde-3-phosphate (Table VIII). The presence of both of these substances was confirmed by an examination of the absorption spectrum obtained in the orcinol reaction, which accurately represented that of a mixture of pentose and heptulose. Since the total quantity of pentose phosphate plus heptulose phosphate formed (1.3 + 0.8 $\mu$M) was in substantial excess of the amount of d-glycer- aldehyde-3-phosphate utilized (1.2 $\mu$M), L-glyceraldehyde-3-phosphate must also have reacted. The difference (0.9 $\mu$M) was taken to represent the amount of L isomer consumed.

Table VIII

Condensation of L-Erythrulose with Dl-Glyceraldehyde-3-phosphate

Incubation was for 3 hours at 25° in 0.73 ml. of 0.01 M glycylglycine buffer containing 0.01 M cysteine. The pH was adjusted to 7.5 before the addition of 0.23 unit of purified spinach transketolase. d-Glyceraldehyde-3-phosphate was analyzed with the glycerophosphate dehydrogenase-DPNH assay system and thus includes any dihydroxyacetone phosphate or fructose diphosphate which might have been formed from d-glyceraldehyde-3-phosphate.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Initial $\mu$M</th>
<th>Final $\mu$M</th>
<th>Change $\mu$M</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Erythrulose</td>
<td>4.7</td>
<td>2.4</td>
<td>1.2</td>
</tr>
<tr>
<td>D-Glyceraldehyde-3-phosphate</td>
<td>2.4</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>L-Glyceraldehyde-3-phosphate</td>
<td>2.4</td>
<td>1.3</td>
<td>(0.9)*</td>
</tr>
<tr>
<td>Pentose phosphate</td>
<td>0</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Sedoheptulose phosphate</td>
<td>0</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Calculated on the assumption that 1 mole of triose phosphate is required for each mole of pentose phosphate or sedoheptulose phosphate formed.

Fig. 6. Acid lability of heptulose phosphate esters. Inorganic phosphate was determined after heating at 100° in 1.0 $N$ sulfuric acid for the time indicated. O represents heptulose phosphate formed from hexose diphosphate and d-erythrose with muscle aldolase; ●, the ester formed from pentose phosphate with the crude liver enzyme; ■, the product with purified liver transketolase and aldolase.
Condensation reactions have also been observed with d-glyceraldehyde as the aldehyde acceptor and with hydroxypyruvate as a source of "active glycolaldehyde" (Table IX). With d-glyceraldehyde, however, no heptulose could be detected. The pentose formed appeared to be ribulose on the basis of its behavior in paper chromatography and its reactivity in the

### Table IX

**Pentose Formation from Glyceraldehyde and Hydroxypyruvate**

The substrates were incubated for 4 hours at 25° with 0.15 unit of spinach transketolase (Q = 39.5 units per mg.) in 0.01 M glycylglycine buffer, pH 7.4, in a total volume of 0.32 ml. Ketopentose was determined with the cysteine-carbazole reaction (38).

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Additions</th>
<th>Ketopentose formed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d-Glyceraldehyde</td>
<td>L-Erythrulose</td>
</tr>
<tr>
<td>1</td>
<td>3.5</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>1.75</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table X

**Sedoheptulose Phosphate Formation with Ribose-5-phosphate-1-C¹⁴**

The substrates were incubated with 6.0 units of purified spinach transketolase in 31.5 ml. of 0.015 M glycylglycine buffer, pH 7.5. In Experiment 1, 98 µM of ribose-5-phosphate-1-C¹⁴ and 198 µM of L-erythrulose were present. In Experiment 2 no erythrulose was added. After 30 minutes at room temperature the solutions were chromatographed on Dowex 1 (formate) columns, 1 cm. X 15 cm., as described in the text. Erythrulose was determined in the effluent (Fractions 1 and 2) by the Nelson-Somogyi reducing procedure (40, 41) and the specific activity calculated from the reducing value. Sedoheptulose phosphate was precipitated as the barium salt from Fractions 35 to 41 (total volume, 37 ml.) in Experiment 1 and Fractions 36 to 44 (total volume, 44 ml.) in Experiment 2. Pentose phosphate was recovered as the barium salt from Fractions 49 to 57 (64 ml.) in Experiment 1 and Fractions 51 to 59 (67 ml.) in Experiment 2. The precipitates were dried, dissolved in 1.0 ml. of 0.02 M acetic acid, analyzed for sedoheptulose and pentose by the orcinol method, and counted.

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Pentose phosphate</td>
<td>2300</td>
</tr>
<tr>
<td>Sedoheptulose phosphate</td>
<td>4100</td>
</tr>
<tr>
<td>Erythrulose</td>
<td>0</td>
</tr>
</tbody>
</table>

* No reducing substance was present in the effluent in this experiment.
cysteine-carbazole reaction (39). Erythrulose, which was more reactive than hydroxypyruvate in this system, in no case furnished more than one 2-carbon unit for pentose synthesis. A similar reaction between dihydroxymaleic acid and triose phosphate has been described by Akabori, Uehara, and Muramatsu (38).

The formation of heptulose phosphate from erythrulose and triose phosphate is an indication that these substances might act as intermediates in the conversion of pentose phosphate to heptulose phosphate. However, experiments carried out to test this possibility indicated that L-erythrulose was not an intermediate in this reaction. With the ThPP-free spinach enzyme, in the absence of added ThPP, neither pentose phosphate nor heptulose phosphate was formed. On addition of the coenzyme both products appeared in the same proportion as with the unresolved enzyme (13). A direct aldol condensation between erythrulose and glyceraldehyde-3-phosphate would not be expected to involve ThPP; thus the heptulose phosphate which is formed must arise by way of pentose phosphate.

Further evidence against the participation of free erythrulose was obtained with ribose-5-phosphate-1-C14. This was converted to sedoheptulose phosphate in the presence of an excess of unlabeled erythrulose (Table X). Very little dilution of the heptulose was observed and the erythrulose recovered at the end of the reaction contained only a trace of radioactivity. From this result it is apparent that free erythrulose could not have been formed in the reaction, although the existence of an active form of erythrulose, perhaps bound to ThPP or as an enzyme complex, cannot be excluded.

**DISCUSSION**

Sedoheptulose phosphate has been identified as a product of pentose phosphate metabolism with transketolase preparations from rat liver and from spinach. While the position of the phosphate group has not been established, measurements of its lability in acid solution suggest that it is esterified in the 7 position.

The ThPP requirement for pentose phosphate cleavage and sedoheptulose phosphate synthesis is consistent with previous evidence (42) that glycolaldehyde is not formed in the cleavage of pentose phosphate, in spite of the fact that the other product has been identified as triose phosphate (43, 44). Glycolaldehyde may occur as a bound form, referred to as "active glycolaldehyde" (14). The formation of sedoheptulose phosphate is an indication that such an "active glycolaldehyde" molecule will participate in further condensation reactions, in addition to the condensation with triose phosphate to regenerate pentose phosphate.
The effect of aldolase on the formation of sedoheptulose phosphate with the purified liver enzyme preparation suggests a condensation between dihydroxyacetone phosphate and d-erythrose, and indeed the formation of sedoheptulose-1-phosphate from these substances in the presence of crystalline aldolase has been demonstrated. Hough and Jones (45) have identified sedoheptulose as the product formed from these substrates with a pea aldolase preparation. However, the absence of aldolase in the purified spinach enzyme preparation, together with the inactivity of sedoheptulose-1-phosphate in the liver system, makes it unlikely that this ester is an intermediate in sedoheptulose-7-phosphate formation. The effect of aldolase is apparently to displace the equilibrium in the direction of heptulose phosphate synthesis from pentose phosphate. Evidence has been presented for the reversibility of this reaction. However, the results described in Fig. 3 do not represent the true transketolase equilibrium, since the presence of other enzymes, such as pentose phosphate isomerase, will affect the ratio of pentose phosphate to heptulose phosphate. The results with the purified liver preparation (Fig. 5) suggest that the reaction favors the formation of pentose phosphate.

Heptulose phosphate is formed from L-erythrulose and glyceraldehyde-3-phosphate in the presence of the purified spinach enzyme preparation. However, the isotope experiment furnishes rather conclusive evidence that these substances are not intermediates in heptulose phosphate formation from pentose phosphate, since L-erythrulose added as a carrier does not become labeled when ribose-5-phosphate-1-C\textsuperscript{14} is the substrate, nor does it significantly dilute the isotope which appears in the sedoheptulose. The ThPP requirement for the formation of sedoheptulose phosphate from erythrulose and triose phosphate (13) is also taken as evidence against a direct aldol condensation of these substances. It is likely that in this case heptulose phosphate is formed by way of pentose phosphate.

The formation of sedoheptulose phosphate by 2-5 condensation remains to be considered. This would involve the accompanying reactions.

\[
\begin{align*}
\text{Ribulose-5-phosphate} & \quad \text{ThPP-enzyme} \quad \text{Glyceraldehyde-3-phosphate} \\
\text{H}_2\text{COH} & \quad \text{HCOH} \quad \text{H}_2\text{COH} \quad \text{H}_2\text{COPO}_4^- \\
\text{HCOH} & \quad \text{ThPP-enzyme} \quad \text{H}_2\text{COPO}_4^- \\
\text{HCOH} & \quad \text{H}_2\text{COPO}_4^- \\
\text{C-O} & \quad \text{H}_2\text{COH} \quad \text{H}_2\text{COH} \\
\end{align*}
\]
The "activated glycolaldehyde" complex can be formed either from ribulose-5-phosphate or sedoheptulose-7-phosphate or from other precursors such as L-erythrulose or hydroxypropyruvate. This scheme is consistent with the observation of Racker, de la Haba, and Leder (14) that with crystalline yeast transketolase no triose phosphate is formed from ribulose-5-phosphate unless ribose-5-phosphate or another aldehyde acceptor is present. Since our liver and spinach preparations contain pentose phosphate isomerase, no addition other than ribulose-5-phosphate is required. An explanation must be provided for the fact that the same enzyme which splits a sugar having the ribulose configuration will form the xylulose configuration found in sedoheptulose phosphate. This result may be due to a limited specificity on the part of transketolase, which may react with either configuration and thus give rise to the particular one which is thermodynamically more stable. Evidence that "active glycolaldehyde" can be formed from L-erythrulose has been presented, and xylose-5-phosphate and D-alloheptulose-7-phosphate might be found to react as well as their 3 epimers. Studies now in progress with C14-labeled pentose phosphate are intended to provide further information on the mechanism of sedoheptulose phosphate synthesis.

The transketolase-catalyzed equilibrium between ribulose phosphate and sedoheptulose phosphate is of interest in view of the evidence obtained by Calvin and his coworkers (46) that these substances appear in the early stages of CO2 fixation in photosynthesis. Their results indicate the formation of a C2 fragment which serves as a precursor of phosphoglyceric acid. "Active glycolaldehyde," which can be formed from either ribulose phosphate or sedoheptulose phosphate, may be identical with this C2 fragment.
The authors are indebted to Dr. Nelson K. Richtmyer for helpful suggestions and assistance in the identification of sedoheptulose, and to Mr. William C. White for the x-ray diffraction patterns.

SUMMARY

Procedures for the purification of transketolase from rat liver and from spinach are described. The activity of the enzyme was followed by measurement of triose phosphate formation from ribulose-5-phosphate. The reaction products are triose phosphate and an ester of sedoheptulose, which is presumably sedoheptulose-7-phosphate. The heptulose has been identified by the preparation of sedoheptulosan tetrabenzoate.

Evidence for the reversibility of the transketolase reaction has been obtained. In the presence of the purified spinach preparation pentose phosphate is formed from sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. The reaction appears to favor the synthesis of pentose phosphate.

Thiamine pyrophosphate is the coenzyme of transketolase. The spinach enzyme and the rat liver enzyme, after acid precipitation, show an absolute requirement for this substance. In the former case Mg$^{++}$ is also necessary for full reactivation.

Condensation reactions have also been observed with L-erythrulose and glyceraldehyde-3-phosphate, catalyzed by spinach transketolase, and with dihydroxyacetone phosphate and D-erythrose, catalyzed by crystalline aldolase. In the first case the products are pentose phosphate and heptulose phosphate; in the second case the product is an acid-labile ester, which is presumably sedoheptulose-1-phosphate. These reactions do not appear to participate in sedoheptulose-7-phosphate formation from pentose phosphate.

Several mechanisms for the transketolase reaction are considered. All of these involve the cleavage of ketol linkages and the formation of a 2-carbon intermediate which is designated as "active glycolaldehyde."

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