The Synthesis of Sugars from Simpler Substances. Part IX. The Enzymic Synthesis of* 5 : 6-*Dideoxy*-D-threo*hexulose.*

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Propionaldehyde and D-fructose 1 : 6-diphosphate yield 5 : 6-dideoxy-D-*threo*hexulose in the presence of an enzyme preparation from peas which has aldolase activity. This result is in agreement with previous observations on the specificity of this enzyme system.

In Part VII (Gorin, Hough, and Jones, *J.*, 1953, 2140), the enzymatic preparation of 5 : 6-dideoxy*threo*hexulose from D-fructose 1 : 6-diphosphate and propionaldehyde was noted. This hexulose was identified on paper chromatograms. Since conclusive identification of the sugar was lacking, a new preparation of the dideoxyhexulose was made. A mixture in aqueous solution of disodium D-fructose 1 : 6-diphosphate, propionaldehyde, and an enzyme preparation from pea seeds (Stumpf, *J. Biol Chem.*, 1948, **176**, 233; Hough and Jones, *J.*, 1952, 4047) was kept at pH 6 for 22 hr. The solution was then heated to destroy enzyme activity and after precipitated protein had been removed by centrifugation, the resulting supernatant solution was evaporated. Extraction of the solid residue with hot acetone gave a syrup which, when examined on paper chromatograms, was shown to contain at least two components which moved at the rates corresponding to 5 : 6-dideoxy*threo*hexulose and dihydroxyacetone and gave with the *p*-anisidine hydrochloride and diphenylamine–trichloroacetic acid reagents (Hough, Jones, and Wadman, *J.*, 1950, 1702) corresponding colour reactions. The syrupy dideoxyhexulose was isolated by chromatography on sheets of filter paper (Flood, Hirst, and Jones, *J.*, 1948, 1679). Warming of the dideoxyhexulose with excess of phenylhydrazine acetate, gave in good yield a phenylosazone which had the same physical characteristics (m. p., mutarotation, and *X*-ray powder photograph) as authentic 5 : 6-dideoxy-D-*threo*hexose phenylosazone (Gorin, Hough, and Jones, *J.*, 1955, 2699). Under conditions whereby 1 mol. of D-xylose was oxidised by 0·94 mol. of sodium hypoiodite at pH 11·4 (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289), the dideoxyhexulose consumed only 0·35 mol. of the reagent; 1 mol. of 5-deoxy-D-*threo*pentulose consumed under the same conditions 0·26 mol. of sodium hypoiodite (Gorin, Hough, and Jones, 1953, *loc. cit.*).

The formation of a 5 : 6-dideoxyhexulose with the D-*threo*-configuration at $C_{(3)}$ and $C_{(4)}$ is in accordance with our previous observations concerning the specificity of the pea-seed enzyme preparation, which contains, amongst other enzymes, aldolase(s?), phosphatase, and triose phosphate isomerase (Stumpf, *loc. cit.*; Hough and Jones, *loc. cit.*). In all cases this enzyme preparation has catalysed the formation of ketoses with the D-*threo*-configuration at $C_{(3)}$ and $C_{(4)}$ (*i.e.*, D-*threo*pentulose derivatives) from a mixture of D-fructose 1 : 6-diphosphate and various aliphatic aldehydes, such as DL-lactaldehyde, glycolaldehyde, acetaldehyde, and aldotetroses. It was, however, found that L-glycerotetrulose was not formed by reaction with formaldehyde. On the other hand, Charalampous and Müeller (*J. Biol. Chem.*, 1953, **201**, 161; 1954, **211**, 249) have isolated from liver an enzyme preparation (1-phosphofructaldolase?) which is not identical with muscle aldolase (1 : 6-diphosphofructaldolase?), but which catalyses the reaction of formaldehyde and dihydroxyacetone

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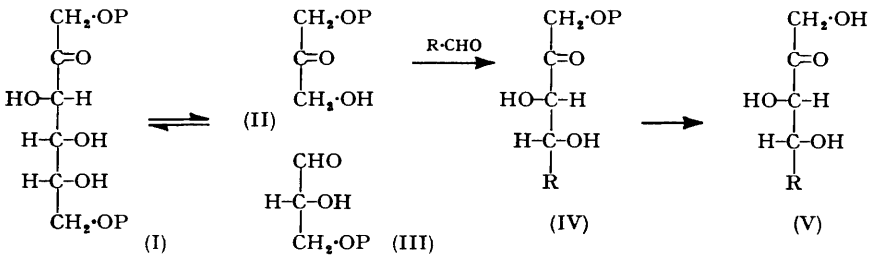
phosphate to give L-*glycero*tetrulose 1-phosphate. Our failure to obtain L-*glycero*tetrulose may, however, be due to an inhibition of phosphatase activity by the formaldehyde present in the reaction mixture.

We have attributed the syntheses by the pea-enzyme preparation to the action of either a single aldolase or mixture of aldolases which causes reversible dismutation of D-fructose 1 : 6-diphosphate (I ; P = PO_3Na_2) giving D-glyceraldehyde 3-phosphate (III) and dihydroxyacetone phosphate (II). In the presence of an introduced aldehyde, combin-



ation with dihydroxyacetone phosphate takes place to give a ketose 1-phosphate (IV). Although the phosphate esters have not been characterised in our products, we have assumed that hydrolysis of the ester takes place to give the free ketose (V), as a phosphatase has been shown to be present in the enzyme mixture.

It is significant that crystalline muscle aldolase (Byrne and Lardy, *Biochim. Biophys. Acta*, 1954, **14**, 495) and 1-phosphofructaldolase from liver (Leuthardt and Wolf, *Helv. Chim. Acta*, 1954, **37**, 1734) show the same specificity as the pea-enzyme preparation in giving ketose derivative with the D-*threo*-configuration at $C_{(3)}$ and $C_{(4)}$ from triose phosphate and aldehydes.

No evidence has been found to show that aldolase synthesises ketoses other than those having the D-*threo*-configuration at $C_{(3)}$ and $C_{(4)}$. However, D-tagatose 1 : 6-diphosphate, which has the L-*erythro*-configuration at $C_{(3)}$ and $C_{(4)}$, can be split by muscle aldolase between $C_{(3)}$ and $C_{(4)}$ (Tung, Ling, Byrne, and Lardy, *Biochim. Biophys. Acta*, 1954, **14**, 488).

Experimental

Evaporations were carried out under reduced pressure.

Condensation of Propionaldehyde with Triose Phosphate.—To disodium D-fructose 1 : 6-diphosphate (8 g.) in water (50 c.c.), freshly distilled propionaldehyde (5 c.c.) in water (100 c.c.) was added, and the resulting solution mixed with the enzyme preparation from pea seeds (100 g.) (Stumpf, *loc. cit.*; Hough and Jones, 1952, *loc. cit.*). The reaction mixture (pH 6) was incubated for 24 hr. at 22° and then adjusted to pH 5 by the addition of acetic acid. After 1 hr. at 90° to inactivate the enzymes, the solution was centrifuged to remove precipitated protein and the resulting supernatant liquor evaporated to dryness. Extraction of the residue with boiling acetone (500 c.c.), followed by evaporation of the extract, gave a golden syrup (1·07 g.). Examination on paper chromatograms (solvent : butan-1-ol–ethanol–water, 40 : 11 : 19 v/v) showed the presence of a sugar which was co-chromatographed with 5 : 6-dideoxy*threo*hexulose (rate of movement compared with rhamnose, 2·3; Gorin, Hough, and Jones, 1955, *loc. cit.*) and which gave a yellow colour with the *p*-anisidine hydrochloride and a pink colour with the diphenylamine–trichloroacetic acid spray reagents. Dihydroxyacetone was detected on the paper chromatogram by its brick-red colour with the *p*-anisidine hydrochloride reagent. It is probably an artefact from the breakdown of D-fructose 1 : 6-diphosphate.

The syrupy mixture was separated by chromatography on sheets of Whatman No. 1 filter paper (solvent : as above) and the appropriate sections of the paper chromatograms were cut out and eluted with water. After evaporation of the solvent, the fraction containing 5 : 6-dideoxy-D-*threo*hexulose (136 mg.) had $[\alpha]_D^{20}$ −13° (*c*, 1·2 in MeOH). Gorin, Hough, and Jones (1955, *loc. cit.*) quote $[\alpha]_D^{20}$ −8° (*c*, 2·5 in MeOH) for 5 : 6-dideoxy-D-*threo*hexulose, but this latter material may have been contaminated with a little 5 : 6-dideoxy-L-*erythro*hexulose. A portion of the hexulose was treated for 18 hr. with aqueous sodium hypoiodite at pH 11·4 under conditions specified by Chanda, Hirst, Jones, and Percival, (*loc. cit.*). It consumed

0·35 mol. of reagent per mol. of dideoxyhexulose, as compared with 0·94 mol. consumption when D-xylose was oxidised under the same conditions.

5 : 6-*Dideoxy*-D-threo*hexose Phenylosazone from* 5 : 6-*Dideoxy*-D-threo*hexulose*.—The dideoxyhexulose (91 mg.) in water was warmed at 40° for 16 hr. with 6 mol. of phenylhydrazine (0·40 c.c.) in acetic acid (0·6 c.c.). The precipitated phenylosazone was collected and washed with water. Two recrystallisations from benzene gave light yellow crystals (85 mg.) which had m. p. 160—171°, undepressed on admixture with authentic 5 : 6-dideoxy-D-*threo*hexose phenylosazone (Found : N, 16·9. Calc. for $C_{18}H_{22}O_2N_4$: N, 17·2%). The product gave an *X*-ray powder photograph indistinguishable from that given by the authentic material, and it showed $[\alpha]_D^{20}$ +32° (2 min.) ⟶ +8° ±8° (18 hr.; constant value; *c*, 0·17, in C_5H_5N–EtOH, 3 : 2 v/v). The phenylosazone prepared from 5 : 6-dideoxy-D-*xylo*hexose had $[\alpha]_D^{20°}$ +47° (5 min.) ⟶ +20° ± 7° [24 hr.; constant value; *c*, 0·60 in the same solvent (Gorin, Hough and Jones, 1955, *loc. cit.*)].

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