439. *The Synthesis of Sugars from Simpler Substances. Part VII.* Enzymic Synthesis of 5-Deoxy-D-Xylulose.*

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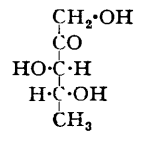
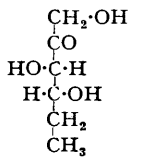
In the presence of an enzyme preparation from peas, acetaldehyde condenses with triose phosphate to give 5-deoxy-D-xylulose (I).

It has been observed that hydroxy-aldehydes will condense with triose phosphate in the presence of an enzyme preparation from peas containing aldolase (Stumpf, *J. Biol. Chem.*, 1948, **176**, 233) to give derivatives of D-xylulose (Hough and Jones, *J.*, 1952, 4047, 4052; 1953, 342). It was of interest to determine whether the absence of hydroxyl residues in the aldehyde affects the configuration of the hydroxyl groups in the final product. Therefore, we have investigated the enzymic reaction of acetaldehyde with triose phosphate. Previous work (Meyerhof, Lohmann, and Schuster, *Biochem. Z.*, 1936, **286**, 301) had demonstrated that triose phosphate will combine with acetaldehyde in the presence of muscle aldolase to give a 5-deoxypentose phosphate. The free sugar was converted into an unidentified phenylosazone, m. p. 165—167°, thus differentiating it from 2-deoxyribose. Racker (*J. Biol. Chem.*, 1952, **196**, 347) has observed that extracts of *Bacterium coli* contain an enzyme which produces 2-deoxy-D-ribose 5-phosphate on condensation of acetaldehyde and triose phosphate.

Acetaldehyde and triose phosphate in the presence of the pea enzymes gave several substances which were separated and detected on paper chromatograms. The major product moved to the same position and produced the characteristic colour reactions on being sprayed with *p*-anisidine hydrochloride or diphenylamine–trichloroacetic acid (Hough, Jones, and Wadman, *J.*, 1950, 1702) as did authentic 5-deoxy-D-xylulose (I).



(I)



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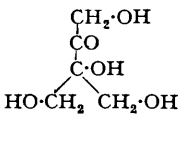

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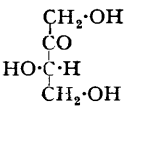

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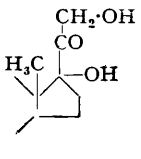

(V)

After removal of inorganic material on ion-exchange resins the solution was concentrated and the residual syrup chromatographed on a column of cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511). The fraction of effluent containing the deoxyxylulose was collected and concentrated to a syrup which had $[\alpha]_D$ −5° (authentic 5-deoxy-D-xylulose has $[\alpha]_D$ −4°). The derived phenylosazone was indistinguishable from an authentic specimen of 5-deoxy-D-xylose phenylosazone by m. p., mixed m. p., optical rotation, and by *X*-ray diffraction analysis. It differed from 5-deoxy-L-ribose phenylosazone (prepared from L-rhamnose; Ruff, *Ber.*, 1902, **35**, 2360).

5-Deoxy-D-xylulose was prepared by isomerisation of 5-deoxy-D-xylose in pyridine and separated from unchanged aldose by chromatography on sheets of filter paper. It yielded a phenylosazone which showed the same mutarotation, m. p. and mixed m. p., and had the

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same *X*-ray diffraction properties as did an authentic specimen of 5-deoxy-D-xylose phenylosazone (from 5-deoxy-D-xylose). The syrup was but slowly oxidised with sodium hypoiodite under conditions which resulted in the complete oxidation of D-xylose.

A faster-moving fraction eluted from the cellulose column contained a substance differing from 5-deoxyxylulose, but resembling 5-deoxyxylose on paper chromatograms. The optical rotation (+16°) of the syrup was similar to that of 5-deoxy-D-xylose (+13·3°) (Levene and Compton, *J. Biol. Chem.*, 1935, **111**, 325). It is possible that it was 5-deoxy-D-xylose arising from 5-deoxy-D-xylulose by isomerisation, since the enzymic reaction was carried out at pH 8.

The method of preparation of 5-deoxy-D-xylose and its phenylosazone from D-xylose (Levene and Compton, *loc. cit.*) was modified slightly in that the conversion of 1 : 2-*iso*propylidene 5-deoxy-5-iodo-D-xylose into 1 : 2-*iso*propylidene 5-deoxy-D-xylose was brought about by treatment with lithium aluminium hydride instead of by hydrogenation with Raney's nickel catalyst. This procedure, although giving the product in a lower yield, is a more convenient method.

We have observed that propaldehyde also will condense with triose phosphate in the presence of a crude enzyme preparation from peas and that the product has the properties of a 5 : 6-dideoxy-D-ketohexose. This material is very probably 5 : 6-dideoxy-D-fructose (II), but it is as yet incompletely identified. It will be of interest to see if formaldehyde will condense with triose phosphate under these conditions to give apioketose (III) and L-erythrulose (IV) (cf. Horecker and Smyrniotis, *J. Amer. Chem. Soc.*, 1953, **75**, 1009; Racker, de la Haba, and Leder, *ibid.*, p. 1110).

All the condensations of triose phosphate in the presence of the pea enzyme that we have examined have been with monofunctional and aliphatic aldehydes, and in every case have given a product with the D-xylulose configuration on $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$. It will be of interest to determine whether aromatic aldehydes such as phloroglucinaldehyde (cf. Geissmann and Heinreimer, *Botan. Review*, 1952, **18**, 145, 165), and bifunctional aldehydes such as glyoxal, malondialdehyde, and succindialdehyde will undergo condensation with triose phosphate in the presence of the pea enzymes, especially as the last three may lead to the formation of cyclic compounds [cf. the grouping on $C_{(17)}$ of 11 : 11-dihydro-17-hydroxycorticosterone (V)].

Experimental

For details of the solvents used in chromatographic separations on paper see Hough and Jones (*J.*, 1953, 342). Optical rotations were in H_2O, determined at 20° unless otherwise stated. Microanalyses are by Mr. B. S. Noyes of Bristol. Evaporation of solutions was carried out under reduced pressure.

1 : 2-iso*Propylidene* 5-*Deoxy*-D-*xylose*.—1 : 2-*iso*Propylidene 5-deoxy-5-iodo-D-xylose (Levene and Compton, *loc. cit.*) (9·2 g.) in dry ether (20 c.c.) was added slowly with stirring to an excess of lithium aluminium hydride (2 g.) suspended in dry ether (50 c.c.). After 30 min.' stirring, water (5 c.c.) was added dropwise to decompose excess of the reagent. Insoluble material was filtered off, and the filtrate evaporated to a small volume and a chloroform–water mixture (200 c.c.; 1 : 1) was then added. After separation of the chloroform layer, the aqueous layer was extracted with chloroform (2 × 100 c.c.). The combined extracts were dried ($MgSO_4$), filtered, and evaporated to a syrup. The syrup was crystallised from ether–light petroleum (b. p. 40—60°), to yield 1 : 2-*iso*propylidene 5-deoxy-D-xylose (3·7 g.), m. p. 68—69°, $[\alpha]_D$ −20° ± 2° (*c*, 3·0) (Found: C, 55·2; H, 7·7. Calc. for $C_8H_{14}O_4$: C, 55·2; H, 8·0%). This product was used for preparation of 5-deoxy-D-xylose and its phenylosazone.

5-*Deoxy*-D-*xylulose from* 5-*Deoxy*-D-*xylose*.—5-Deoxy-D-xylose (0·9 g.) was heated at 100° for 20 hr. in dry pyridine (20 c.c.). The solution was evaporated to a syrup. Chromatographic examination of the product showed that it contained, as well as 5-deoxy-D-xylose, a slower-moving substance which gave colour reactions (*p*-anisidine hydrochloride and diphenylamine–trichloroacetic acid sprays) which differed from those of 5-deoxy-D-xylose. This substance was separated from 5-deoxy-D-xylose by chromatography on several large sheets of filter paper (Flood, Hirst, and Jones, *Nature*, 1947, **160**, 86) with butanol–ethanol–water (40 : 11 : 19 v/v). The syrupy product had $[\alpha]_D$ −4° ± 1°.

Hypoiodite Oxidation of 5-*Deoxy*-D-*xylulose*.—Treatment of the syrup (2·19 mg.) with sodium hypoiodite (18 hr.) under conditions specified by Chanda, Hirst, Jones, and Percival (*J.*, 1950,

1289) resulted in 26% of it being oxidised. A similar determination on D-xylose resulted in 96% oxidation.

5-Deoxy-D-xylose Phenylosazone from 5-Deoxy-D-xylulose.—The syrup (113 mg.) was warmed at 50° for 18 hr. in aqueous phenylhydrazine acetate. On cooling, the yellow product was filtered off, washed with water, then with benzene, and finally recrystallised from aqueous methanol. The product (56 mg.) (Found: C, 65·2; H, 6·25; N, 18·1. Calc. for $C_{17}H_{20}O_2N_4$: C, 65·4; H, 6·4; N, 17·9%), $[\alpha]_D$ 67° → 0° (±7°) (*c*, 0·6 in pyridine–ethanol, 3 : 2 v/v) had m. p. 174—175°, undepressed on admixture with authentic 5-deoxy-D-xylose phenylosazone. It gave an *X*-ray diffraction pattern identical with authentic material prepared from 5-deoxy-D-xylose.

Condensation of Acetaldehyde with Triose Phosphate.—The sodium salt of hexose diphosphate (10 g.) was dissolved in water (500 c.c.) and the pH brought to 8 by the addition of 0·1N-sodium hydroxide. A solution of aldolase (200 c.c.) prepared from pea seeds (100 g.) by Stumpf's method (*loc. cit.*) was added. Redistilled acetaldehyde (4 c.c.) in water (100 c.c.) was then added drop-wise during an hour. The solution was incubated in a tightly stoppered flask at 37° for 4 days. Protein was then coagulated by heating the solution at 90° (1 hr.). The cooled solution was filtered, and the filtrate evaporated to 500 c.c. and deionised by successive passages through columns of Amberlite resins IR-120 and IR-4B. The effluent was concentrated to a syrup (5 c.c.) which was extracted with methanol. The methanolic solution was filtered and concentrated to a syrup, which was transferred to a column of cellulose (Hough, Jones, and Wadman, *loc. cit.*) and fractionated. Benzene–ethanol–water (30 : 10 : 3 v/v; top layer) (500 c.c.) was first passed down the column, followed by butanol. The effluent was collected on an automatic fraction cutter, and the fractions were examined chromatographically (solvent butanol–ethanol–water, 40 : 11 : 19 v/v). The sugars were detected after spraying the paper with ammoniacal silver nitrate and heating it. The presence of four substances which had rates of movement relative to rhamnose (1·0) of 2·4, 1·9, 1·73, and 1·53 was observed. One sugar moved at the rate of 5-deoxy-D-xylulose (1·53) and a second at the rate of 5-deoxy-D-xylose (1·73). The appropriate fractions which contained these sugars were collected and concentrated. The sugar which moved at the rate of 5-deoxy-D-xylulose also resembled it in that it gave a yellow colour with the *p*-anisidine spray and a pale green colour with the diphenylamine–trichloroacetic acid spray, while the sugar which resembled 5-deoxy-D-xylose gave an orange colour with the *p*-anisidine spray and a blue-green colour with the other reagent.

5-Deoxy-D-xylulose prepared by Enzymic Reaction.—This fraction, a syrup (A) (149 mg.), showed $[\alpha]_D$ −5° ± 1° (*c*, 1·49 in MeOH), whereas authentic 5-deoxy-D-xylulose has $[\alpha]_D$ −4° ± 1° (*c*, 1·1 in MeOH). When the sugar (A) (140 mg.) was dissolved in aqueous phenylhydrazine acetate and left for 18 hr. at 35°, a crystalline phenylosazone separated. It was collected on a filter, washed with water and with benzene, and dried. The product (61 mg.) was recrystallised from methanol–water and had $[\alpha]_D$ +74° → + 7° (±7°) (*c*, 0·6 in pyridine–ethanol, 3 : 2 v/v), m. p. 174—175°, not depressed on admixture with an authentic specimen (Found: C, 65·3; H, 6·3; N, 17·9. Calc. for $C_{17}H_{20}O_2N_4$: C, 65·4; H, 6·4; N, 17·9%). Meyerhof, Lohmann, and Schuster (*loc. cit.*) record m. p. 165—167°. The phenylosazone gave an *X*-ray diffraction pattern identical with that given by an authentic specimen (Levene and Compton, *loc. cit.*) and differed from that of 5-deoxy-L-ribose phenylosazone {$[\alpha]_D$ + 60° → 74° ± 7° (*c*, 0·6 in pyridine–ethanol, 3 : 2 v/v)} prepared from L-rhamnose (Ruff, *loc. cit.*).

5-Deoxy-D-xylose prepared by Enzymic Reaction.—This fraction (51 mg.) showed $[\alpha]_D$ +16°. 5-Deoxy-D-xylose has $[\alpha]_D^{24}$ +13·3° (Levene and Compton, *loc. cit.*). Neither a crystalline phenylosazone nor a crystalline *iso*propylidene derivative (Levene and Compton, *loc. cit.*) could be prepared from it. Although its behaviour on paper chromatograms was identical with that of 5-deoxy-D-xylose, conclusive evidence of its identity was not obtained.

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