312. *The Synthesis of Sugars from Simpler Substances. Part VI.* Enzymic Synthesis of* D-*Idoheptulose.*

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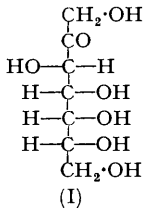
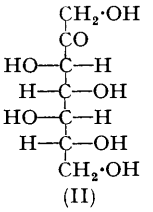
An enzymic preparation from peas catalyses the reaction of D-threose with triose phosphate to give D-idoheptulose, identified as D-idoheptulosan.

In Part V,* it was shown that D-erythrose combines with triose phosphate in the presence of an enzyme preparation from the pea (Stumpf, *J. Biol. Chem.*, 1948, **176**, 233) to give sedoheptulose (I). It was of interest to determine whether or not this is a general type of reaction, and particularly whether D-threose would undergo a similar condensation. Accordingly, D-threose was condensed with triose phosphate under the conditions specified by Hough and Jones (*J.*, 1953, 342) An examination at intervals, of portions of the reaction solution on paper chromatograms, indicated that material possessing the colour reactions of a heptulose (cf. Klevstrand and Nordal, *Acta Chem. Scand.*, 1950, **4**, 1320) was produced. After 90 hr. suitable manipulations, including heating with dilute acid, gave a mixture of sugar (20%) and anhydride (80%) (Pratt, Richtmyer, and Hudson, *J. Amer. Chem. Soc.*, 1952, **74**, 2210). The latter is stable when heated with alkalis, but the reducing sugars, such as idoheptulose (II), glucose, and fructose, which are present in the solution, are destroyed. The solution was therefore heated with barium hydroxide, filtered, freed from barium and other ions, and concentrated to a syrup. D-Idoheptulosan was isolated by chromatography on paper and identified by its analysis, its rate of movement on paper chromatograms, its colour reaction with the orcinol spray, its m. p. and mixed m. p., and its *X*-ray diffraction pattern (for determination of which we thank Mr. D. A. Brown) (cf. Pratt, Richtmyer, and Hudson, *loc. cit.*).



(I)



(II)

The synthesis of D-idoheptulose is a further example of the production of a sugar with the D-xylulose configuration on $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$, by the action of aldolase on a mixture of hexose diphosphate and an aldehyde. Aldolase is thus an enzyme which will transfer a triose fragment (very probably dihydroxyacetone phosphate) from D-fructose 1 : 6-diphosphate to an aliphatic aldehyde with the resultant formation of an optically active ketose, which possesses the D-xylulose configuration on the first four carbon atoms.

Experimental

The following solvents were used in chromatographic separations on Whatman No. 1 paper : (*a*) ethyl acetate–acetic acid–water (9 : 2 : 2); (*b*) *n*-butanol–ethanol–water (40 : 11 : 19), both by vol. *p*-Anisidine hydrochloride was used as a general spray to detect sugars; orcinol–trichloroacetic acid solution (Klevstrand and Nordal, *loc. cit.*) was used to detect heptulose and heptulosan. Evaporation of solutions was carried out under reduced pressure.

Synthesis of D-*Idoheptulose.*—D-Threose (8 g.) was prepared from D-xylose (30 g.) *via* calcium D-xylonate (Hudson and Isbell, *J. Amer. Chem. Soc.*, 1929, **51**, 2225) by the procedure used for the preparation of D-erythrose from D-arabinose (cf. Overend, Stacey, and Wiggins, *J.*, 1949, 1358). Ionic material was removed from the crude tetrose preparation by passage of the solution through columns of Amberlite resins IR120 and IR4B. A sample of the product, when separated on a paper chromatogram [solvent (*b*)] showed one main spot (R_F 0·40), which gave a yellowish-brown colour and moved faster than did D-erythrose (R_F 0·33), and a faint pink spot corresponding to xylose.

* Part V, *J.*, 1953, 342.

D-Threose (6 g.) and the sodium salt of hexose diphosphate (8 g.) were dissolved in water (300 c.c.) and the pH adjusted to 7·5 by 0·1N-sodium hydroxide. Crude aldolase solution (Hough and Jones, *J.*, 1952, 4053) (75 c.c.) from peas (250 g.) was added and the mixture kept at 20° for 90 hr. The solution was heated at 90° for 30 min., filtered, and concentrated to a syrup which was dissolved in water and filtered from a small amount of protein. Examination of a small portion of the solution on the paper chromatogram [solvent (*a*)] showed the presence of a heptulose, detected by spraying with orcinol and heating. It moved at the same rate as galactose. The filtrate was passed down a column of Amberlite IR120 resin, the acid effluent (50 c.c.) added to sulphuric acid (2N; 50 c.c.), and the mixture heated at 90° for 3½ hr. in order to convert the heptulose into the equilibrium mixture of heptulose (20%) and heptulosan (80%). The solution was then made alkaline by the addition of barium hydroxide and heated at 90° for 2 hr. The cooled solution was filtered, before and after acidification with cold dilute sulphuric acid, and then deionised by passage down a column of Amberlite resin IR4B. The effluent was concentrated to a syrup (170 mg.), which was examined chromatographically [solvent (*a*)]; it then contained a material which gave a positive test for heptulose (Nordal and Klevstrand, *loc. cit.*) and moved to the same position on the chromatogram as did an authentic specimen of idoheptulosan. Its rate of movement was 0·86 relative to ribose. As the syrup did not crystallise, it was fractionated on a sheet of paper [solvent (*a*)], and the appropriate section of the paper was eluted with methanol. The syrup (81 mg.) thus obtained was similarly refractionated [solvent (*b*)] and the purified product (32 mg.) recrystallised from ethanol or dioxan; it then had m. p. and mixed m. p. 172°, $[\alpha]_D^{20}$ −34° ±8° (*c*, 0·3) (Found: C, 43·9; H, 6·1. Calc. for $C_7H_{12}O_6$: C, 43·8; H, 6·3%).

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