67. *The Synthesis of Sugars from Simpler Substances. Part V.* Enzymic Synthesis of Sedoheptulose.*

By L. Hough and J. K. N. Jones.

An enzyme preparation from peas catalyses the reaction of D-erythrose (and its 2 : 4-ethylidene derivative) with triose phosphate to give sedoheptulose, identified as sedoheptulosan and its tetrabenzoate. In the presence of the enzyme preparation dihydroxymaleic acid and triose phosphate yielded D-xylulose which was recognised as its crystalline *iso*propylidene derivative. A preliminary account of these results has appeared elsewhere (Hough and Jones, *Chem. and Ind.*, 1952, 907).

In Parts III and IV * it has been shown that certain hydroxy-aldehydes combine with triose phosphate in the presence of an enzyme preparation from the pea (Stumpf, *J. Biol. Chem.*, 1948, **176**, 233), presumably by aldol condensation, to give monosaccharides containing the D-xylulose configuration on $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$. On this evidence reaction of D-erythrose with triose phosphate would be expected to give sedoheptulose (D-*altro*-2-ketoheptitol). We have now isolated this sugar from the condensation of D-erythrose and of its 2 : 4-ethylidene derivative with triose phosphate. The heptulose was isolated by partition chromatography on cellulose and was characterised by its colour reactions (Nordal and Klevstrand, *Acta Chem. Scand.*, 1950, **4**, 1320), and as the crystalline 2 : 7-anhydro-derivative, sedoheptulosan, and its crystalline tetrabenzoate. This identification was

* Part IV, L. Hough and J. K. N. Jones, *J.*, 1952, 4053.

facilitated by a generous gift of sedoheptulosan, for which we thank Dr. N. K. Richtmeyer (cf. Pratt, Richtmeyer, and Hudson, *J. Amer. Chem. Soc.*, 1952, **74**, 2200). The heptose was isolated as the free sugar and not as the phosphate because the enzyme preparation contained phosphatase (cf. Hough and Jones, *J.*, 1952, 4050). Horecker and Smyrniotis (*J. Amer. Chem. Soc.*, 1952, **74**, 2123) observed the formation of heptulose phosphate from D-erythrose, hexose diphosphate, and aldolase from yeast and rat liver. The synthesis of this heptulose is of interest because it occurs in the free state in plants of *Primula* and *Sedum* species, in the plant family *Saxifragaceae* (Nodal and Oiseth, *Acta Chem. Scand.*, 1952, **6**, 446), in yeast (Robison, Macfarlane, and Tagelaar, *Nature*, 1938, **142**, 114), and because it appears to be one of the primary intermediates involved in carbon dioxide fixation (Benson, Bassham, and Calvin, *J. Amer. Chem. Soc.*, 1951, **73**, 2970; Benson, Kawauchi, Hayes, and Calvin, *ibid.*, 1952, **74**, 4477). As triose phosphate will also combine with propaldehyde and acetaldehyde (Meyerhof, Lohman, and Schuster, *Biochem. Z.*, 1936, **286**, 301, 319) in the presence of the pea enzyme preparation (Hough and Jones, unpublished results), it is very probable that other aldehydes such as, for example, tartronic aldehyde, $CHO{\cdot}CH(OH){\cdot}CO_2H$ would also undergo condensation under these conditions. The last compound would give rise to 5-keto-D-gluconic acid, an epimer and possible precursor of D-glucuronic and D-mannuronic acid (cf. Fischer, Baer, and Nidecker, *Helv. Chim. Acta*, 1937, **20**, 1226). While these observations throw some light on the possible mode of formation in plants of sugars with the D-xylulose configuration, the origin of D-galactose, L-arabinose, D-galacturonic acid, chondrosamine, and D-mannoheptulose, sugars which may be derived from ketose precursors with the L-ribulose configuration, is unknown.

A further problem is raised by the synthesis of sedoheptulose, namely, the origin of D-erythrose. Glycollic aldehyde is known to dimerise in the presence of lime water to give tetrose (Jackson, *J.*, 1900, 129). We considered it possible that D-erythrose might arise by the enzymic dimerisation of glycollic aldehyde. Accordingly, we re-examined the mixture of sugars produced by condensing glycollic aldehyde with triose phosphate in the presence of a crude pea enzyme extract, but we could find no trace of heptulose. It has been suggested that dihydroxymaleic acid is a possible precursor of glycollic aldehyde and tartronic aldehyde in the plant (cf. Hough and Jones, *Nature*, 1951, **167**, 180) and therefore it was of interest to study the reaction of dihydroxymaleic acid (sodium salt) and triose phosphate in the presence of the enzyme preparation. From this reaction D-xylulose was isolated and identified as its crystalline *iso*propylidene derivative, but no heptulose or uronic acid could be detected in the mixture. Apparently the dihydroxymaleic acid is decarboxylated enzymically to glycollic aldehyde (cf. Neuberg and Kerb, *Biochem. Z.*, 1913, **53**, 406) which then undergoes condensation to ketopentose. Whilst the possibility that D-erythrose arises from glycollic aldehyde cannot yet be ruled out, it is conceivable that the tetrose may arise from a ketohexose by fission between $C_{(2)}$ and $C_{(3)}$ just as a ketopentose derivative has been shown to split into glycollic aldehyde and glyceraldehyde derivatives (Rappaport, Barker, and Hassid, *Acta Biophys.*, 1951, **31**, 326). It might arise by reduction of dihydroxyacetoacetic acid as suggested by Benson, Kawauchi, Hayes, and Calvin (*loc. cit.*). It is of interest to note that these authors have observed that during photosynthesis fructose and sedoheptulose phosphates are synthesised concurrently, followed by ribulose phosphate, and they suggest that pentose is formed from heptulose. The reverse process, the formation of sedoheptulose phosphate as a product of pentose phosphate metabolism has been observed by Horecker and Smyrniotis (*J. Amer. Chem. Soc.*, 1952, **74**, 2123). The formation of pentose was envisaged as arising from heptulose by fission between $C_{(6)}$ and $C_{(5)}$ (cf. the degradation of pentoses, Rappaport, Barker, and Hassid, *loc. cit.*).

Experimental

The following solvents were used in chromatographic separations on Whatman No. 1 paper: (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (*b*) *n*-butanol–pyridine–water (10 : 3 : 3) and (*c*) *n*-butanol–ethanol–water (40 : 11 : 19), all v/v. *p*-Anisidine hydrochloride solution was used as spray, to detect sugars, unless otherwise stated. Optical rotations were determined at 20° and in water. Microanalyses are by Mr. B. S. Noyes of Bristol. M. p.s are uncorrected. Evaporation of solutions was carried out under reduced pressure.

Synthesis of Sedoheptulose.—(2 : 4-Ethylidene D-erythrose and D-erythrose were prepared from D-glucose by the method of Rappaport and Hassid, *J. Amer. Chem. Soc.*, 1951, **73**, 5524.) (*a*) D-Erythrose (from 4 : 6-ethylidene D-glucose, 5·5 g.) in water (25 c.c.) was mixed with hexose diphosphate (sodium salt, 5 g.) in water (25 c.c.), and the pea enzyme (from peas, 50 g.; for preparation see Part III, *loc. cit.*) in water (125 c.c.) added. The pH of the solution was 7·2. After 72 hours at 30° the solution was heated to inactivate enzymes, then filtered, and the filtrate de-ionised with Amberlite resins IR-120 and IR-4B. Chromatographic examination of the concentrated solution disclosed erythrose, ribose, fructose, heptulose, glucose, and an unknown substance moving at a slower rate than any known hexose. The solution was concentrated to a syrup (1·31 g.) which was separated on a column of cellulose, with butanol half-saturated with water as mobile phase (Hough, Jones, and Wadman, *J.*, 1949, 2511). Portions of the effluent were heated on paper with the orcinol reagent (Nordal and Klevstrand, *loc. cit.*), which gives a pure blue colour with ketoheptoses and a green colour with ketohexoses, and the appropriate fractions selected and concentrated. The resulting syrup (101 mg.) was examined on paper chromatograms; it was observed to contain fructose, sedoheptulose, and a little glucose. In solvent (*c*), the heptulose moved slightly faster than glucose and a little more slowly than fructose. In order to destroy fructose and glucose and to convert the heptulose into heptulosan, the syrup was heated with N-sulphuric acid (10 c.c.) for 5 hours, excess of barium hydroxide added, and the solution heated for a further 2 hours. The solution was filtered before and after acidification with dilute sulphuric acid, de-ionised with Amberlite resin IR-4B, and concentrated to a syrup (42 mg.), which crystallised on nucleation with sedoheptulosan hydrate; it had m. p. 100—101°, not depressed on admixture with an authentic specimen, $[\alpha]_D$ −136° (*c*, 1·4). Its rate of movement in solvent (*c*) was 2·2 relative to galactose, in solvent (*a*), 1·12 relative to arabinose, and in phenol saturated with water, 1·14 relative to rhamnose. The positions of the sugars were detected with the ammoniacal silver nitrate reagent. It was indistinguishable from an authentic specimen on paper chromatograms or by *X*-ray crystallographic examination (Found : C, 40·2; H, 6·2. Calc. for $C_7H_{12}O_6,H_2O$: C, 40·0; H, 6·7%).

(*b*) 2 : 4-Ethylidene erythrose (from 5·5 g. of ethylidene glucose) was mixed with hexose diphosphate (sodium salt, 5 g.) in water (25 c.c.), and the enzyme solution (125 c.c.) from peas (50 g.) added. After 72 hours the solution was heated, filtered, cooled, and de-ionised with Amberlite resin IR-120. The acidic solution (pH 1—2) was heated at 100° for 3 hours to remove combined ethylidene groups, cooled, filtered, and de-ionised with Amberlite resin IR-4B. The neutral solution was evaporated to a syrup (2·1 g.) and examined on paper chromatograms. The presence of erythrose, ribose, sedoheptulosan, fructose, sedoheptulose, glucose, and a sugar moving more slowly than galactose was indicated. The syrup was fractionated on a column of cellulose, with butanol half-saturated with water as effluent, and the fractions giving positive tests for heptulosan and heptulose collected. The heptulosan moved at about the rate of xylose in solvent (*c*) and gave a blue colour with the orcinol spray. The combined fractions (60 mg.) were treated with hot N-sulphuric acid and then with barium hydroxide as described above. After filtration, the solution was de-ionised and concentrated to a syrup (31 mg.) which crystallised. It was recrystallised from methanol and then (24 mg.) had m. p. 100—101°, not depressed on admixture with an authentic specimen of sedoheptulosan hydrate, $[\alpha]_D$ −132° (*c*, 0·8). The tetrabenzoate had m. p. and mixed m. p. with an authentic specimen 165° (cf. Haskins, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1952, **74**, 2199).

(*c*) Glycollic aldehyde (8·0 g.) from dihydroxymaleic acid (20 g.) in water (100 c.c.) was mixed with hexose diphosphate (12·5 g.; sodium salt) in water (50 c.c.), and the pea enzyme (from 100 g. of peas) in water (300 c.c.) was kept at 25° for 72 hours (pH of solution 7·2). The sugars (4·0 g.) were isolated as described earlier (Hough and Jones, *J.*, 1952, 4047) and fractionated on a column of cellulose with *n*-butanol half-saturated with water as effluent. Neither sedoheptulose nor sedoheptulosan could be detected.

(*d*) Dihydroxymaleic acid (sodium salt, 5 g.) was suspended in water (100 c.c.), and hexose diphosphate (sodium salt, 5 g.) in water (100 c.c.) added, followed by enzyme solution (120 c.c.) from peas (50 g.). The solution (pH 8·0) was shaken continuously at 35° during 72 hours. At intervals samples were withdrawn and heated with 12% hydrochloric acid; furfuraldehyde-producing materials were rapidly formed. The solution was adjusted to pH 6 by acetic acid and filtered. The filtrate was heated, filtered, cooled, de-ionised with Amberlite resin IR-120, and concentrated. Chromatographic examination of the solution indicated the presence of xylulose, ribose, fructose, and hexose phosphates. Uronic acids were not detected by the naphtharesorcinol spray (Forsyth, *Nature*, 1948, **161**, 239). The solution was then heated at 100° for 3 hours to hydrolyse phosphate esters, cooled, filtered, de-ionised with Amberlite resin IR-4B, and

evaporated to a syrup (0·51 g.). This syrup was fractionated on a cellulose column with *n*-butanol half-saturated with water as developer. The fraction containing ketopentose (70 mg.) was collected, evaporated to a syrup, and examined chromatographically. It contained ketopentose and ribose. Accordingly, it was dissolved in acetone (25 c.c.) containing hydrogen chloride (1% w/v). After 3 hours, chromatographic examination (solvent *b*) showed that the ketopentose had been converted into an *iso*propylidene derivative which moved with the solvent front (R_F 0·84, solvent *a*). The solution was neutralised (Ag_2CO_3), filtered, and concentrated, and the syrup (66 mg.), n_D^{25} 1·4625, fractionally distilled in a micro-distillation apparatus. The distillate (48 mg.), b. p. 120°/0·1 mm., n_D^{26} 1·4625, crystallised on nucleation with an authentic specimen of 2 : 3-*iso*propylidene D-xylulose. When recrystallised from pentane it had m. p. 68—71°, not depressed on admixture with authentic specimens kindly supplied by Professor T. Reichstein and The Rockefeller Institute for Medical Research (Levene collection of chemicals) $[\alpha]_D$ +6° (*c*, 2·2) (Levene and Tipson, *J. Biol. Chem.*, 1936, **115**, 731; Prince and Reichstein. *Helv. Chim. Acta*, 1937, **20**, 107).

Heptulose was not detected in later fractions emerging from the cellulose column.

Control experiments, involving D-erythrose and the enzyme preparation in one case, and D-erythrose and hexose diphosphate in another, at pH 7·5, were treated as described above. In neither case was heptulose produced.

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