773. *The Synthesis of Sugars from Simpler Substances. Part IV.* Enzymic Synthesis of 6-Deoxy-D-fructose and 6-Deoxy-L-sorbose.*

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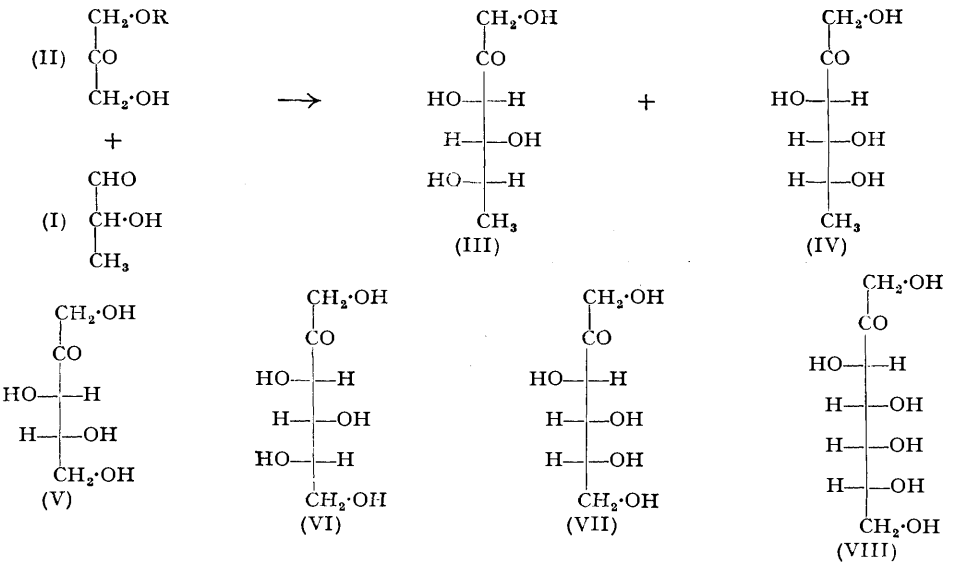
In the presence of an enzyme preparation from peas, DL-lactaldehyde combines with triose phosphate, to give optically active methyl pentose sugars. From the reaction mixture, 6-deoxy-D-fructose and 6-deoxy-L-sorbose have been isolated and characterised. The significance of these observations is discussed.

THE methyl pentose (6-deoxyhexose) sugars are of common occurrence in Nature, although they have always been found in the combined form linked glycosidically to some other fragment. Thus L-rhamnose (6-deoxy-L-mannose) occurs in a variety of plant materials combined either with other monosaccharides as in the gums and mucilages (Hirst and Jones, *Research*, 1951, **4**, 411) or with flavones, anthocyanins, or other aglycones. L-Fucose (6-deoxy-L-galactose) also is encountered in plant materials as, for example, in gum tragacanth (James and Smith, *J.*, 1945, 744) and occurs in polysaccharides of animal origin such as the blood-group substances (Aminoff, Morgan, and Watkins, *Biochem. J.*, 1950, **46**, 426); it is a common constituent of marine algæ. Other methyl pentoses are more rarely encountered; of these, D-fucose and D-quinovose (6-deoxy-D-glucose) occur in Jalep resin (Votoček, *Ber.*, 1910, **43**, 476), while several other methyl pentoses occur as methoxy- or deoxy-derivatives in cardiac glycosides (Elderfield, *Adv. Carbohydrate Chem.*, 1945, **1**, 147), the methoxy- and deoxy-group invariably being situated at $C_{(3)}$ and $C_{(2)}$ respectively.

Nothing is known concerning the origin of these methyl pentoses in Nature, although the most obvious suggestion is that they arise by reduction of the $C_{(6)}$-hydroxyl group of a hexose, but this is improbable since, amongst other objections, L-mannose, the precursor of L-rhamnose, is not known to occur in Nature. A further possibility envisages the combination by aldol-type condensation, either of two triose units, lactaldehyde (I) and dihydroxyacetone (II; R = H), or of acetaldehyde with two molecules of glycollic aldehyde or one molecule of tetrose (cf. Votoček, *Bull. Soc. chim.*, 1928, **43**, 1; Hough and Jones, *Nature*, 1951, **167**, 180). In order to test the second of these hypotheses, the condensation of DL-lactaldehyde with dihydroxyacetone in the presence of lime-water was attempted and it was observed that sugars moving on paper chromatograms at the rate of 6-deoxyketohexoses and showing the same colour reactions were produced (Hough and Jones, unpublished results) (DL-lactaldehyde was prepared from 1 : 1-dibutoxypropan-2-one by reduction with lithium aluminium hydride, followed by hydrolysis with dilute formic acid of the resultant 1 : 1-dibutoxypropan-2-ol). Accordingly, the enzymic synthesis of methyl

* Part III, preceding paper.

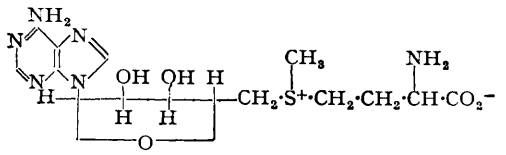
pentose was attempted by allowing a mixture of DL-lactaldehyde (I), the disodium salt of D-fructose-1 : 6 diphosphate and a crude aldolase preparation from peas to stand at pH 6·5. Under the influence of pea aldolase, fructose diphosphate is split into dihydroxyacetone phosphate (II; R = PO_3H_2) and D-glyceraldehyde-3 phosphate (Stumpf, *J. Biol. Chem.*, 1948, **176**, 233; Tewfik and Stumpf, *Amer. J. Bot.*, 1949, **36**, 567). This reaction is reversible : thus, D-frucose-1 : 6 diphosphate may be regenerated or the dihydroxyacetone phosphate may combine with lactaldehyde (I), to give a 6-deoxy-2-ketohexose-1 phosphate. It was observed chromatographically that considerable quantities of two 6-deoxyketohexoses were produced. After inactivation of the enzyme by heat, the solution was filtered, and the filtrate passed through a column of Amberlite resin IR-120 to remove cations. The resultant acidic solution was heated to hydrolyse phosphate esters, and then completely de-ionised by passage through a column of Amberlite resin IR-4B, and the effluent concentrated to a syrup. The sugar mixture was subjected to partition chromatography on a column of cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511), two optically active fractions being obtained with the properties of 6-deoxy-2-ketohexoses. The first fraction crystallised on nucleation with a specimen of 6-deoxy-L-sorbose (Müller and Reichstein, *Helv. Chim. Acta*, 1938, **21**, 263), from which it was indistinguishable chromatographically and by mixed melting point and optical rotation. Its osazone was identical with that prepared from authentic 6-deoxy-L-sorbose (III). The second fraction was obtained as a syrup showing properties in accordance with those of 6-deoxy-D-fructose (IV). Confirmation of this identity was obtained by conversion into crystalline 2 : 3-*iso*propylidene 6-deoxy-D-fructose (Morgan and Reichstein, *ibid.*, p. 1023), and by formation of the phenylosasazone which was indistinguishable by *X*-ray analysis from its enantiomorph, L-rhamnosazone. No trace of any other 6-deoxyketohexose or 6-deoxyaldohexose was detected.



It has already been observed (Meyerhof, Lohmann, and Schuster, *Biochem. Z.*, 1936, **286**, 301, 319) that D- and L-glyceraldehyde condense with dihydroxyacetone phosphate in the presence of aldolase to give the 1-phosphate of D-fructose (VII) and L-sorbose (VI) respectively, and it is clear that D- and L-lactaldehyde condense with dihydroxyacetone in an analogous manner. It is also significant that glycollic aldehyde condenses with triose phosphate under the influence of aldolase to give D-xylulose (Hough and Jones, preceding paper). In all these ketoses (III, IV, V, VI, VII) produced by condensation of an aldehyde with dihydroxyacetone phosphate in the presence of aldolase, the stereochemical configuration on $C_{(3)}$ and $C_{(4)}$ is the same, that is, they are all $C_{(5)}$ derivatives of D-xylulose (V).

One would expect, therefore, that, under these conditions, D-erythrose would condense with dihydroxyacetone phosphate to give D-altroheptulose (sedoheptulose), and it is precisely this sugar which was obtained by Horecker and Smyrniotis (*J. Amer. Chem. Soc.*, 1952, **74**, 2123; cf. Benson *et al.*, *J. Biol. Chem.*, 1952, **196**, 703).* It will be of interest to see if the 5-deoxy-2-ketopentose resulting from the condensation of acetaldehyde and dihydroxyacetone phosphate (Meyerhof, Lohmann, and Schuster, *loc. cit.*) has the D-xylulose configuration.

Although lactaldehyde can give rise to methyl pentoses, it is evident that this route will explain neither the presence in natural products of the common sugar L-rhamnose, nor the presence of L-fucose unless, in the latter case, isomerisation at $C_{(3)}$ of the 6-deoxy-L-sorbose is postulated. Clearly, other enzyme systems and other modes of origin of this class of sugar will require investigation. In this connection, the observation of Cartori (*J. Amer. Chem. Soc.*, 1952, **74**, 2942) that adenosine triphosphate participates in enzymic transmethylation reactions involving methionine as methyl donor, by formation of orthophosphate and *S*-adenosylmethionine (IX), is of interest. The latter compound is a possible precursor of 5-deoxy-5-methylthio-D-ribose, and this mechanism may also be operative in the biogenesis of other methyl pentoses and 2-deoxy-sugars.

(IX) 

Experimental

The following solvents were used in chromatographic separations: (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), (*b*) *n*-butanol–pyridine–water (10 : 3 : 3), (*c*) *n*-butanol–ethanol–water (40 : 11 : 19), and (*d*) the top layer of benzene–ethanol–water (169 : 47 : 15). R_G values quoted are relative to tetramethyl glucopyranose and are not intended as absolute values. Optical rotations were determined at 20° in aqueous solution unless otherwise stated. Microanalyses are by Mr. B. S. Noyes of Bristol. M. p.s are uncorrected. Evaporation of solutions was carried out under reduced pressure.

Pyruvaldehyde Dibutyl Acetal (1 : 1-*Dibutoxypropan*-2-*one*).—This was prepared from aqueous pyruvaldehyde (30%) by the method described in U.S.P. 2,421,559/1947 (cf. *Brit. Abs.*, 1950, B, II, 1033) except that toluene-*p*-sulphonic acid was used as catalyst in place of sulphuric acid. The yield was 60 g. of acetal, b. p. 100—105°/16 mm., n_D^{20} 1·4200, from 300 c.c. of pyruvaldehyde solution. Heating a portion with aqueous phenylhydrazine acetate gave methylglyoxal phenylosazone, m. p. 153° (Found: N, 21·5. Calc. for $C_{15}H_{16}N_4$: N, 22·2%).

DL-*Lactaldehyde Dibutyl Acetal*.—The foregoing acetal (55 g.) in ether (150 c.c.) was added to a cooled, well-stirred suspension of lithium aluminium hydride (14 g.) in ether (100 c.c.). Each addition caused a vigorous reaction and development of a yellow colour. Excess of the reagent was destroyed by water, and the crude DL-*lactaldehyde dibutyl acetal* isolated by ether-extraction and purified by distillation [yield 41 g.; b. p. 140°/16 mm. (bath-temp.), n_D^{20} 1·4262] (Found: C, 64·5; H, 12·1. $C_{11}H_{24}O_3$ requires C, 64·7; H, 11·8%).

DL-*Lactaldehyde*.—DL-Lactaldehyde dibutyl acetal (15 g.) was added to a mixture of glacial acetic acid (30 c.c.), water (40 c.c.), and formic acid (1 c.c.; 98%), and the mixture heated on the boiling-water bath for 30 minutes. The solvent mixture and butyl alcohol were removed by distillation at 40°, leaving a viscous oil with an odour resembling that of glyceraldehyde (4·8 g.). This crystallised in part. The crystals were filtered off and washed with ether and had m. p. 107° (Found: C, 48·8; H, 8·0. Calc. for $C_3H_6O_2$: C, 48·6; H, 8·1%). Heating with alcoholic *p*-nitrophenylhydrazine gave DL-lactaldehyde *p*-nitrophenylhydrazone, m. p. 112° (Found: N, 18·4. Calc. for $C_9H_{11}O_3N_3$: N, 20·2%). Lactaldehyde reduces hot Fehling's solution and yields acetaldehyde on oxidation with sodium metaperiodate solution. Its movement (R_G 0·60) in solvent (*b*) is faster than that of rhamnose (R_G 0·30 assumed), and it gives a yellow colour with the *p*-anisidine hydrochloride spray (Hough, Jones, and Wadman, *loc. cit.*).

* *Added in Proof.*—Sedoheptulose has now been isolated from the condensation of D-erythrose and hexose diphosphate in the presence of a crude pea aldolase preparation (Hough and Jones, unpublished results).

Synthesis of Methyl Pentose.—Hexose diphosphate (sodium salt; 9 g.) was dissolved in water (50 c.c.), and DL-lactaldehyde (4·8 g.) in water (25 c.c.) added. A solution (150 c.c.) of aldolase from peas (50 g.), prepared as described in Part III (*loc. cit.*), was then added and, after mixing, the surface of the solution was covered with a layer of toluene. The pH of the solution at this stage was 6·5. After 72 hours at 20°, examination of the solution on the paper chromatogram (solvent *a*) showed the presence of hexose, pentose, and 6-deoxyketohexose. The solution (pH 6·5) was heated at 90° for 30 minutes to inactivate enzymes, and filtered. The cooled solution was then passed through a column of Amberlite resin IR-120 to remove cations. The acidic effluent (pH *ca.* 1·0) was then heated at 90° for 8 hours to hydrolyse any phosphate esters present, cooled, and de-ionised by passage through a column of Amberlite resin IR-4B. The effluent (pH 7·5) was acidified with acetic acid (2 c.c.) and evaporated to a syrup which was dissolved in aqueous methanol. Excess of acetone was added to this solution, and the precipitate discarded. Concentration of the filtered solution gave a brown syrup [A; 2·63 g.; $[\alpha]_D$ −11° (*c*, 4·0)]. A portion of this material was examined on the paper chromatogram with solvent (*a*). After the paper had been sprayed with the *p*-anisidine hydrochloride spray and then heated, coloured spots indicating the presence of lactaldehyde (yellow, weak), two 6-deoxyketohexoses (yellow, strong), ribose (red, weak), fructose (yellow, medium), arabinose (red, weak), and galactose(?) (brown, weak) were detected. Solvent (*d*) gave the best separation of the two deoxyketohexoses, moving them 17 and 9·2 cm. respectively in 72 hours. They gave red colours with the resorcinol spray.

Separation and Identification of 6-*Deoxy*-L-*sorbose and* 6-*Deoxy*-D-*fructose.*—A portion (1 g.) of (A) (above) in acetone (2 c.c.) was placed on the top of a column of cellulose [Whatman cellulose powder, standard grade, previously washed with solvent (*d*)]. Solvent (*d*; 1·5 l.) was passed down the column, followed by pure *n*-butanol, the effluent being collected portionwise by means of an automatic fraction cutter (Hough, Jones, and Wadman, *loc. cit.*). Examination of the effluent on paper chromatograms showed that the two 6-deoxyketohexoses had been completely separated, and evaporation of the appropriate tubes of effluent gave fraction 1 (0·25 g.), $[\alpha]_D$ −21° (*c*, 0·43) which crystallised on nucleation with 6-deoxy-L-sorbose (Müller and Reichstein, *loc. cit.*). The crystals, recrystallised from acetone–light petroleum (b. p. 60—80°), had m. p. 88°, $[\alpha]_D^{22}$ −25°±2° (*c*, 0·72), and mixed m. p. with an authentic specimen 88—89° (Found: C, 43·7; H, 7·9. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·3%). Its rate of movement in solvents (*a*), (*b*), and (*c*), relative to that of rhamnose, was 1·24, 1·36, 1·34 respectively, values identical with those obtained with an authentic specimen. Phenylhydrazine acetate solution at 70° (3 hours) afforded 6-deoxy-L-gulosazone which was recrystallised from aqueous ethanol and dried at 60° *in vacuo* (Found: C, 43·7; H, 7·9. Calc. for $C_{18}H_{22}O_3N_4$: C, 43·9; H, 7·4; N, 16·4%).

Fraction 2 {0·7 g.; $[\alpha]_D$ −13°±2° (*c*, 1·8)} did not crystallise. Its rate of movement in solvents (*a*), (*b*), and (*c*) relative to that of rhamnose was 1·22, 1·39, and 1·20 respectively, and corresponded to 6-deoxyfructose. It gave a red colour with the resorcinol–hydrochloric acid spray. 6-Deoxy-D-glucosazone, formed as described above, had m. p. 176—178°, $[\alpha]_D$ −59° (1 hour) ⟶ −53°±3° (const.; *c*, 0·73 in ethanol) (Found: N, 16·7. Calc. for $C_{18}H_{22}O_3N_4$: N, 16·4%). The osazone on *X*-ray crystallographic examination was indistinguishable from 6-deoxy-L-glucosazone (L-rhamnosazone) which had m. p. 176—178°, $[\alpha]_D$ +80° (initial value) ⟶ +60°±3° (const.; *c*, 0·57 in ethanol). Analysis by paper chromatography of the products produced when the deoxyketohexose was heated in pyridine at 100° for 7 days in a sealed tube indicated that quinovose and rhamnose were produced.

2 : 3-iso*Propylidene* 6-*Deoxy*-D-*fructose.*—The sugar (0·38 g.) was dissolved in acetone (25 c.c.) containing gaseous hydrogen chloride (0·5 g.). After 1 hour, an examination of a portion of the solution on the paper chromatogram (solvent *b*) indicated that 6-deoxy-D-fructose was no longer present as such; in its place a faster-moving spot (R_G *ca.* 1·0) was observed. After 3 hours, the solution was neutralised with silver carbonate, then filtered, and the filtrate concentrated. On nucleation of the syrup (0·3 g.) with 2 : 3-*iso*propylidene 6-deoxy-D-fructose (Morgan and Reichstein, *loc. cit.*) crystallisation rapidly took place. The product had m. p. 112° and mixed m. p. (with an authentic specimen, m. p. 114°) 113°, after recrystallisation from ether–light petroleum (b. p. 60—80°), $[\alpha]_D$ +8° (*c*, 1·2 in ethanol) (Found: C, 52·9; H, 7·8. Calc. for $C_9H_{16}O_5$: C, 53·0; H, 7·8%).

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