701. *The Synthesis of Sugars from Simpler Substances. Part II.* The Synthesis of* DL-*Ribose* in vitro *from* D-*Glyceraldehyde and Glycollic Aldehyde.*

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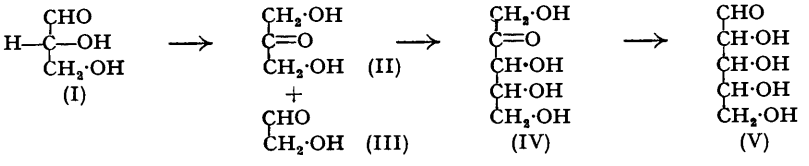
Investigation of the pentose sugars produced from the reaction of D-glyceraldehyde with glycollic aldehyde, in the presence of lime-water, has resulted in the isolation of DL-ribose (as its toluene-*p*-sulphonylhydrazone) and of other DL-pentoses. The origin of the pentoses in Nature is discussed.

IN Part I * it was observed that the products obtained from a solution of D-glyceraldehyde (I) and glycollic aldehyde (III) after 10 days at room temperature contained a complex mixture of pentose, hexose, and other sugars. A preliminary examination of this mixture on the paper chromatogram indicated that it contained all four pentose sugars (V), namely, arabinose, xylose, ribose, and lyxose. Conclusive evidence for the presence of DL-arabinose and DL-xylose was obtained by the separation of the mixture on a column of cellulose by partition chromatography and the formation, from the appropriate fractions, of crystalline DL-arabinose benzoylhydrazone and DL-xylose toluene-*p*-sulphonylhydrazone.

This reaction has now been repeated on a larger scale in the presence of lime-water, but allowed to proceed for one day only. The resultant mixture of sugars (*A*) was separated on a column of cellulose by partition chromatography at a temperature of 60° (Counsell, Hough, and Wadman, *Research*, 1951, **4**, 143). In this manner, an optically active fraction ($[\alpha]_D$ −10°), containing ribose as the only pentose sugar, was obtained. When this fraction was heated with toluene-*p*-sulphonhydrazide in methanol solution, crystalline DL-ribose toluene-*p*-sulphonylhydrazone was obtained. This derivative of D- and of DL-ribose is very sparingly soluble and is of value for both the characterisation and quantitative determination of ribose (*cf.* Easterby, Hough, and Jones, forthcoming publication). Two other fractions, possessing optical activity ($[\alpha]_D$ −10° and −12°, respectively) and containing pentose and other sugars, were also obtained.

The isolation of derivatives of the DL-pentose sugars, rather than of their optically active isomers, may be attributed to the predominant formation of the racemic sugars and also to the greater insolubility of their derivatives. Undoubtedly, some optically active sugars, some of which may be pentoses, are formed, but their identity is as yet unknown. The presence in the pentose fractions of such sugars as apiose and dendroketose, which because of their mode of synthesis will be optically inactive, and of optically active sugars such as hamamelose and psicose, is also to be expected (*cf.* Hough and Jones, *Nature*, 1951, **167**, 180). It would appear, therefore, that either the D-glyceraldehyde (I) concerned with pentose synthesis is first epimerised to



dihydroxyacetone (II), which may then react with the glycollic aldehyde (III) to yield DL-pentoses (V), or, alternatively, the D-glyceraldehyde is racemised to DL-glyceraldehyde before reaction with glycollic aldehyde. In this connection it is noteworthy that Fischer and Baer (*Helv. Chim. Acta*, 1936, **19**, 519) found that D-glyceraldehyde in alkaline solution is first converted into dihydroxyacetone, followed by reaction of the two to give D-ketohexoses. Dihydroxyacetone will, in fact, condense with glycollic aldehyde to give DL-pentose sugars (Hough and Jones, *loc. cit.*), and an examination of the condensation products (*A* : see above) from glycollic aldehyde and D-glyceraldehyde revealed that ketopentoses (IV) were present, since on the paper chromatogram they gave a characteristic blue colour with the resorcinol–hydrochloric acid spray reagent and moved at the same rate as riboketose and xyloketose. We consider, therefore, that in the above pentose synthesis, the main reaction involves the conversion of D-glyceraldehyde into dihydroxyacetone, which then reacts with glycollic aldehyde

* Part I, *J.*, 1951, 1122.

to give ketopentoses (IV), epimerisation of which affords the DL-pentose sugars. From this point of view, it is noteworthy that dihydroxyacetone phosphate will react with glycollic aldehyde in the presence of an aldolase of animal origin to give phosphorylated ketopentoses (Meyerhof and Kiessling, *Biochem. Z.*, 1935, **279**, 40; Lohmann and Schuster, *ibid.*, 1936, **286**, 301, 319; Schlenk and Waldvogel, *Fed. Proc.*, 1947, **6**, 288; Racher, *ibid.*, 1948, **7**, 180). The direct reaction of D-glyceraldehyde with glycollic aldehyde to give optically active pentose may also occur, but this reaction can occur in two different ways; by aldol-type condensation:

$$CH_2(OH)\cdot CH(OH)\cdot CHO + CH_2(OH)\cdot CHO \longrightarrow CH_2(OH)\cdot CH(OH)\cdot CH(OH)\cdot CH(OH)\cdot CHO$$

or by acyloin-type condensation:

$$CH_2(OH)\cdot CH(OH)\cdot CHO + OHC\cdot CH_2\cdot OH \longrightarrow CH_2(OH)\cdot CH(OH)\cdot CH(OH)\cdot CO\cdot CH_2\cdot OH.$$

Evidence for the latter type of reaction has been provided by Rappoport, Barker, and Hassid (*Arch. Biochem. Biophys.*, 1951, **31**, 326). Since Tewfik and Stumpf (*Amer. J. Bot.*, 1949, **36**, 567) have demonstrated the wide occurrence of aldolase in plants, it is conceivable that the pentoses are formed in Nature as their phosphorylated derivatives, *via* glycollic aldehyde and dihydroxyacetone derivatives (see Benson, *J. Amer. Chem. Soc.*, 1951, **73**, 2971), and that the reaction between derivatives of D-glyceraldehyde and glycollic aldehyde is of less common occurrence.

Experimental.

Reaction of D-*Glyceraldehyde with Glycollic Aldehyde.*—D-Glyceraldehyde (9·0 g.) (Baer and Fischer, *J. Biol. Chem.*, 1939, **128**, 463) was dissolved in water (75 c.c.) and glycollic aldehyde (6·0 g.) (prepared from 17 g. of dihydroxymaleic acid) in water (75 c.c.) was added. The solution was made alkaline by the addition of a slight excess of calcium hydroxide and the mixture set aside at 20° in a stoppered vessel. After 45 minutes a sample of the solution was examined on the paper chromatogram and observed to contain pentose sugars. After 20 hours the calcium was precipitated by addition of oxalic acid solution, and the solution after filtration de-ionised by passage through a column of Amberlite resin 1R4B. The aqueous solution was then concentrated, under reduced pressure, to a syrup (11·9 g.), which was fractionated by partition chromatography on a column of cellulose at 60°, butanol saturated with water at room temperature being used as the mobile phase (Counsell, Hough, and Wadman, *loc. cit.*). Concentration of the appropriate fractions of the effluent from the column (see Hough and Jones, *J.*, 1951, 1122, for details) gave five main fractions.

Fraction 1 (1·3 g.), consisted of glyceraldehyde, glycollic aldehyde, and ketopentoses. The ketopentoses move faster than ribose in the butanol–pyridine–water solvent (Hough and Jones, *loc. cit.*) and give a characteristic blue colour with the resorcinol–hydrochloric acid spray reagent. The positions of these sugars and their colour reactions were identical with those obtained from the ketopentose sugars obtained by epimerising arabinose and xylose, respectively. The glyceraldehyde and glycollic aldehyde moved faster than the ketopentose sugars and gave orange colours with the *p*-anisidine hydrochloride spray.

Fraction 2 (1·4 g.) ($[\alpha]_D$ −9°; *c*, 1·1 in water), contained ribose as the main component. The solution of sugars (1·3 g.) in methanol (25 c.c.) with toluene-*p*-sulphonhydrazide (1·8 g.) was boiled under reflux for 30 minutes. On cooling, DL-*ribose toluene*-p-*sulphonylhydrazone* (0·193 g.) crystallised. This material had m. p. 161—162° (decomp.) not altered on recrystallization of a sample from a large volume of methanol. The melting point was unaltered on admixture of the specimen with D-ribose toluene-*p*-sulphonylhydrazone, m. p. 164° (Found: C, 45·3; H, 5·7; N, 8·8. $C_{12}H_{18}O_6N_2S$ requires C, 45·3; H, 5·7; N, 8·8%). On dissolution in dilute hydrochloric acid, DL-ribose toluene-*p*-sulphonylhydrazone gave DL-ribose, which moved at the same rate on the chromatogram and gave the same colour reaction as D-ribose prepared in a similar manner from its toluene-*p*-sulphonylhydrazone. This fraction therefore contained at least 93 mg. of DL-ribose. A sample of the sugar, prepared from the toluene-*p*-sulphonylhydrazone by warming it with benzaldehyde in the presence of acetic acid, showed no optical activity.

Fraction 3 (1·8 g.) ($[\alpha]_D$ −10°; *c*, 0·11 in water), when examined on the paper chromatogram, showed ribose, xylose, and lyxose, together with other sugars and traces of arabinose. It was not further examined.

Fraction 4 (2·3 g.) ($[\alpha]_D$ −12°; *c*, 0·23 in water) contained arabinose, xylose, and other sugars. It was heated under reflux with benzhydrazide (2·1 g.) in methanol (25 c.c.) for 30 minutes. After the solution had cooled, DL-arabinose benzoylhydrazone (55 mg.), m. p. and mixed m. p. 195°, was collected.

Fractions 5 (1·7 g.) and 6 (1·7 g.) and the residue on the column did not contain pentose sugars and were not further examined.

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