244. *The Synthesis of Sugars from Simpler Substances. Part I. The* in vitro *Synthesis of the Pentoses.*

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Aldopentose sugars have been synthesised from formaldehyde, from glycollic aldehyde and glyceraldehyde, and from dihydroxyacetone and glycollic aldehyde. The origin of the pentose sugars in Nature is discussed.

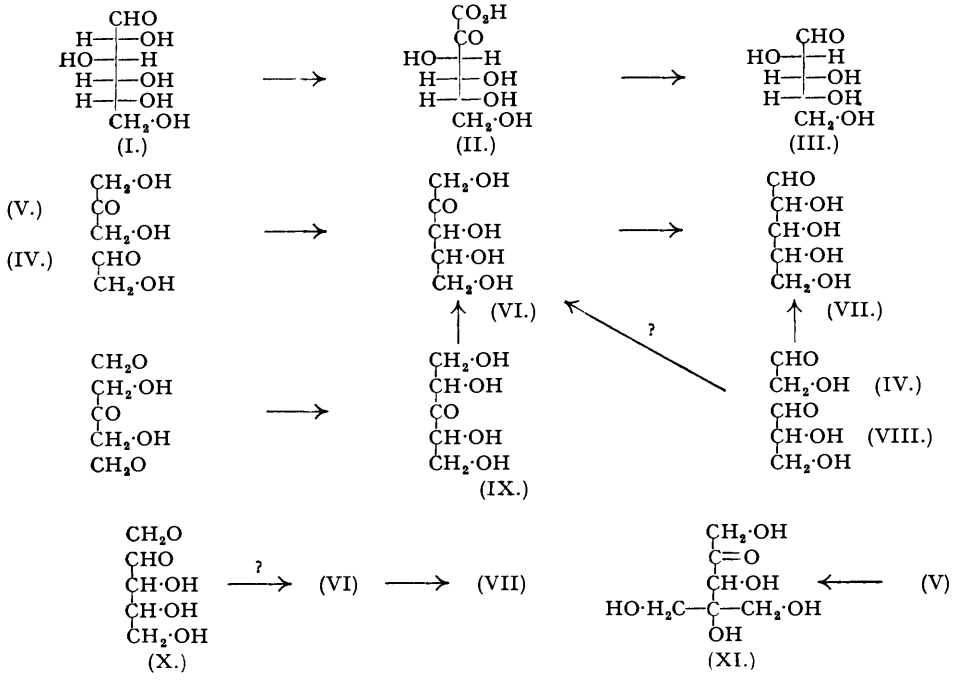
The biogenetical origin of the monosaccharides has long been the subject of scientific enquiry and, although it has been established that the hexoses can arise from the condensation of the two trioses, glyceraldehyde and dihydroxyacetone, as their phosphate derivatives, under the influence of enzymes, yet the origin of the pentose sugars remains obscure. The pentoses occur abundantly in plants, usually in combination in the form of a pentosan as, for example, in araban or xylan, or in combination with other sugars such as in the plant gums and mucilages. The frequent association of the pentoses with hexuronic acids and hexoses, such as the occurrence of araban, pectic acid, and galactan in pectic substances, has led to the suggestion that they are derived from hexoses by a process involving oxidation of the primary alcoholic group on $C_{(6)}$ of the hexose, yielding a hexuronic acid, subsequent decarboxylation of which affords a pentose. By this process, D-galactose would yield L-arabinose, and D-glucose would afford D-xylose, and it is precisely these hexose and pentose sugars which are associated one with another in plant materials. On the other hand, the isolation of D-mannuronic acid, and the failure to detect D-lyxose in plant products, has thrown some doubt on this hypothesis (see Hirst, *J.*, 1949, 522, for a detailed discussion of these problems).

It has also been suggested that the pentose sugars may arise from the 2-ketohexonic acids, *e.g.*, (II) ⟶ (III), again by decarboxylation. This suggestion receives support from the synthesis of D-arabinose (III) *in vitro*, from D-gluconic acid by use of Fenton's reagent (*J.*, 1899, **75**, 577), and of D-arabinose and D-ribose *in vivo* from D-glucose (I) *via* 2-keto-D-gluconic acid (II) (Dickens and Glock, *Nature*, 1950, **166**, 33; Cohen and Scott, *Science*, 1950, **111**, 543). D-Arabinose and D-ribose have been encountered as constituents of both plant and bacterial materials (Léger, *Compt. rend.*, 1912, **155**, 173; Corley, *J. Biol. Chem.*, 1929, **82**, 269). The absence of L-glucose, L-fructose, and L-mannose (all of which could yield L-arabinose) and of D-gulose and D-idose (the parent sugars from which D-xylose would be formed) in plant materials suggests that L-arabinose and D-xylose are not formed by this simple mechanism. On the other hand, the D-xylose might arise from glucose by a mechanism similar to the formation of D-ribose from this hexose.

The possibility remains that the pentoses are synthesised in a similar manner to the hexoses in the plant by aldol-type condensation of small fragments. Earlier work (Orthner and Gerisch, *Biochem. Z.*, 1933, **259**, 30) showed that glycollic aldehyde (IV) and dihydroxyacetone (V) combined in alkaline solution to form a mixture of reducing sugars, which, it was suggested, were mainly ketopentoses (VI) and from which an osazone with the characteristics of a pentosazone was isolated; Euler and Euler (*Ber.*, 1906, **39**, 45) detected arabinose amongst the condensation products of formaldehyde (cf. Karrer and Krauss, *Helv. Chim. Acta*, 1931, **14**, 820). Pentose sugars (VII) may also be envisaged as arising from the condensation of D- or L-glyceraldehyde (VIII) and glycollic aldehyde (IV), from dihydroxyacetone and two mols. of formaldehyde (XIV) and subsequent isomerisation of the ketose (IX), or from a tetrose (X) and formaldehyde.

We have examined the mixture of sugars produced on condensation of dihydroxyacetone and glycollic aldehyde in alkaline solution. The products were separated on the paper chromatogram and, by their rate of movement and by the colours produced on reaction with a variety of specific spray reagents (Hough, Jones, and Wadman, *J.*, 1950, 1702), all four pentose sugars were detected, arabinose and xylose predominating. The mixture of sugars was fractionated on a column of cellulose by partition chromatography, butanol–water being used as the mobile phase (Hough, Jones, and Wadman, *J.*, 1949, 2511). After separation, the fractions were shown to contain severally ribose, lyxose, xylose, and arabinose by paper chromatography. Efforts to crystallise these sugars directly were unsuccessful, but crystalline derivatives of DL-xylose and DL-arabinose were isolated, namely, DL-xylose toluene-*p*-sulphonylhydrazone and DL-arabinose benzoylhydrazone. After hydrolysis of the latter in the presence of benzaldehyde, crystalline DL-arabinose was obtained. Similarly, hydrolysis of the DL-xylose derivative afforded DL-xylose, a syrup. Since DL-arabinose may be resolved through its menthyl-

phenylhydrazone (Neuberg, *Ber.*, 1905, **38**, 868), and as glycollic aldehyde and dihydroxyacetone have been synthesised from bromoacetaldehyde and nitromethane, respectively (Piloty, *Ber.*, 1897, **30**, 3161), this constitutes a complete synthesis of D- and L-arabinose. The crystalline DL-derivatives were identical with authentic specimens prepared from D + L-xylose and D + L-arabinose respectively and, in each case, they possessed higher melting points than the corresponding D- or L-derivative. We are greatly indebted to Dr. T. Malkin for confirming the identity of these crystalline derivatives by *X*-ray analysis. The evidence indicated that ribose and lyxose were present in small quantity, but crystalline derivatives were not obtained.



D-Glyceraldehyde and glycollic aldehyde, on reaction in alkaline solution, gave a mixture of optically active sugars containing all four pentoses, xylose and arabinose again predominating. During this reaction some epimerisation of the D-glyceraldehyde to L-glyceraldehyde and dihydroxyacetone occurs, and consequently crystals of DL-xylose toluene-*p*-sulphonylhydrazone, D + DL-arabinose benzoylhydrazone and D + DL-sorbose were isolated and not the pure D-isomers. DL-Sorbose arises from the conversion of some of the D-glyceraldehyde into DL-glyceraldehyde and dihydroxyacetone and their subsequent condensation (cf. Fischer and Baer, *Helv. Chim. Acta*, 1936, **19**, 519).

One mode of condensation of dihydroxyacetone with formaldehyde might be envisaged as involving one molecule of ketose with two molecules of aldehyde with the formation of a symmetrical ketose (IX), with subsequent rearrangement first to a ketopentose (VI) and then to an aldopentose (VII). Aldopentoses were not detected as a result of this reaction at room temperature but, when the reaction mixture is heated in alkaline solution, aldopentoses are produced. This formation of pentose sugars may, however, result from the conversion of the formaldehyde into glycollic aldehyde and subsequent synthesis of the pentose sugars.

Utkin (*Chem. Abstr.*, 1950, **44**, 3910*a*) has isolated a ketohexose homologue of apiose termed dendroketose (XI) from the polymerisation of two molecules of dihydroxyacetone. We have observed that dihydroxyacetone alone in alkali gave a high yield of a ketose sugar which moves at the same rate as xylose on the paper chromatogram, butanol–ethanol–water being used as the mobile phase. Consequently, in reactions involving glyceraldehyde and dihydroxyacetone, the xylose fraction is likely to be contaminated with this sugar. Dendroketose gives a bright yellow colour with *p*-anisidine hydrochloride.

The polymerisation of formaldehyde in alkaline solution yields a complex mixture of sugars

("formose") in which pentose sugars can be detected. The syrup obtained from the polymerisation of paraformaldehyde with lime water was separated by partition chromatography on a column of cellulose, propanol–water being the mobile phase. A fraction rich in pentose sugars was collected and refractionated on a column of cellulose, with butanol–water as the mobile phase. This gave a portion of sugars which contained mainly xylose with small quantities of arabinose and ketose sugars. By utilising the fact that, when butanol–water is used as the mobile phase on the paper chromatogram xylose occupies a position between arabinose and lyxose, whereas when the phenol–water solvent system is employed, xylose moves more slowly and is completely separated from the other pentose sugars, a method for the separation of xylose from the other sugars is available. Accordingly, the mixture of sugars was separated on sheets of filter-paper, the phenol–water solvent being used. The majority of the impurities were separated from the DL-xylose, sufficient of which was extracted from the appropriate section of paper to give its characteristic toluene-*p*-sulphonylhydrazone derivative.

The preparation *in vitro* of pentose sugars from one-, two-, or three-carbon fragments by polymerisation under mild conditions suggests that the pentoses, D-xylose and L-arabinose, are formed in the plant from glycollic aldehyde and either dihydroxyacetone or, less likely, glyceraldehyde as such, or possibly through their phosphate derivatives. Their biosynthesis would then be analogous to that of the hexose sugars (cf. Hough and Jones, *Nature*, 1951, **167**, 180).

Experimental.

(Optical rotations were observed in aqueous solution at 20° unless otherwise stated. All R_G values quoted are those observed in *n*-butanol–ethanol–water mixture at *ca.* 20°.)

Unless otherwise stated, the products from each of the following reactions were separated on three separate sheet-paper chromatograms, using respectively as the mobile phase butanol–ethanol–water (40 : 11 : 19), butanol–pyridine–water (10 : 3 : 3), and phenol saturated with water containing 1% of acetic acid. After separation, the paper chromatograms were dried, sprayed with a solution of *p*-anisidine hydrochloride in butanol, and heated. In this manner, the four pentoses, namely, arabinose, xylose, lyxose, and ribose, are readily discerned, since they show up as characteristic red spots on a white background, the position of the spots being characteristic of each sugar (cf. Partridge, *Biochem. J.*, 1948, **42**, 238). Other chromatograms were sprayed with resorcinol–phosphoric acid mixture in aqueous ethanol, with which the ketohexoses give characteristic red colours (cf. Hirst and Jones, *Discuss. Faraday Soc.*, 1949, No. 7, 271)].

Reaction of D-*Glyceraldehyde with Glycollic Aldehyde.*—D-Glyceraldehyde (3·0 g.) (Baer and Fischer, *J. Biol. Chem.*, 1939, **128**, 463) was dissolved in water (10 c.c.), and glycollic aldehyde (2·0 g.) (prepared from 5·5 g. of dihydroxymaleic acid) in water (50 c.c.) was added. The solution was adjusted to pH 11 by addition of 0·3N-barium hydroxide, and the alkaline solution made up to 100 c.c. The quantity of barium hydroxide required to bring the solution to the required pH depends on the purity of the dihydroxymaleic acid, which usually contains traces of oxalic acid resulting in the formation of a colloidal precipitate of barium oxalate on the addition of the barium hydroxide. For this reason, it was not possible to follow any change in optical rotation of the solution. A portion of the yellow solution was withdrawn each day and examined on the paper chromatogram. After 10 days at 20° in a stoppered vessel, no further change was seen in the complex mixture of pentose and other sugars observable on the chromatogram. Sulphuric acid (2N.) was then added to the reaction mixture until no more barium sulphate was precipitated. The precipitate was removed by filtration, and the filtrate passed down a column of anion-exchange resin (Amberlite IR4B). The neutral solution was then concentrated under reduced pressure at 40° to an orange syrup (3·1 g.), which was separated on a column of cellulose, *n*-butanol half-saturated with water being used as eluent. Portions of the effluent were examined on the paper chromatogram, and the fractions so divided that, as far as possible, separation of the pentose sugars was achieved. Solvents were removed under reduced pressure yielding: fraction I (0·127 g.), $[\alpha]_D^{20}$ −6°; fraction II (0·091 g.), $[\alpha]_D$ −4°; fraction III (0·186 g.), $[\alpha]_D$ −2°; fraction IV (0·153 g.), $[\alpha]$ −22°; fraction V (0·145 g.); fraction VI (0·207 g.); fraction VII (0·315 g.); and residue, (0·290 g.). The total yield was 1·514 g. Examination of these fractions on the paper chromatogram showed that each consisted of two or more components and that fractions I—V inclusive contained aldopentoses. These fractions also contained ketohexoses since they gave, after separation on the paper chromatogram, characteristic red spots on spraying with resorcinol–phosphoric acid solution and heating; the positions of these spots corresponded with those of the four ketohexoses.

Fractions I and II contained pentose sugars, which moved to the same position as ribose (R_G 0·21) and lyxose (R_G 0·19) respectively on the paper chromatogram, gave a characteristic red colour with *p*-anisidine hydrochloride, and yielded furfuraldehyde on distillation with hydrochloric acid. Since no crystalline derivatives were isolated, this evidence is only presumptive and does not conclusively identity the sugars as ribose and lyxose.

Fraction III contained two pentose sugars, one of which was conclusively identified as xylose (R_G 0·17), and the other, present in small amount, showed properties identical with those of lyxose on the chromatogram. A portion of the syrup (90 mg.) was heated on the boiling-water bath with a solution of toluene-*p*-sulphonylhydrazine (90 mg.) in methanol (5 c.c.) for 30 minutes. The solution was then concentrated to *ca.* 2 c.c. and cooled and, after storage, the crystals (15 mg.) of DL-*xylose toluene*-p-

sulphonylhydrazone were collected on the filter, washed with methanol, and dried; they had m. p. 154° (decomp.) and $[\alpha]_D$ 0° ± 5° (*c*, 4·0 in pyridine) (Found: C, 45·2; H, 6·0; N, 8·8. $C_{13}H_{18}O_6N_2S$ requires C, 45·3; H, 5·7; N, 8·7%). This fraction therefore contains at least 15 mg. of DL-xylose.

An *X*-ray powder photograph of these crystals showed that they differed from an authentic specimen of D-xylose toluene-*p*-sulphonylhydrazone, m. p. 147° (decomp.), $[\alpha]_D$ −32° (*c*, 4·3 in pyridine), but were identical with DL-xylose toluene-*p*-sulphonylhydrazone, m. p. 154° (decomp.), prepared by dissolving equimolecular quantities of the corresponding hydrazones of D- and L-xylose in methanol and crystallising. [The L-xylose was prepared from D-sorbitol by oxidation with hydrogen peroxide in the presence of ferrous sulphate (Fenton's reagent)—details will be published later.] The DL-xylose derivative is more insoluble in methanol than the D-isomer.

Fraction IV contained only one pentose sugar, namely, arabinose. A portion of the syrup (0·07 g.) was heated on a boiling-water bath with benzoylhydrazine (0·05 g.) in alcohol (2 c.c.) for 1 hour. On concentration, the benzoylhydrazone (12 mg.), m. p. 194—195° (not depressed on admixture with DL-arabinose benzoylhydrazone) separated. This material showed $[\alpha]_D$ −25° [*c*, 3·6 (calc. on arabinose) in 2N-sulphuric acid] and evidently consisted of the D- and the DL-isomer. An *X*-ray powder photograph of the recrystallised material gave a picture identical with that of authentic DL-arabinose benzoylhydrazone, m. p. 194—195°; no D-isomer could be detected. All attempts to crystallise fraction IV by nucleation with D- and with DL-arabinose were unsuccessful. DL-Arabinose, prepared by recrystallisaton of equimolecular amounts of the D- and the L-sugar, has m. p. 163—164° (Wohl, *Ber.*, 1893, **26**, 742, gives m. p. 163·5—164·5° for this sugar). This fraction therefore contains at least 15 mg. of D + DL-arabinose.

Fraction V contained a low concentration of arabinose. A ketose sugar, which moved to the same position as fructose on the chromatogram and gave a red colour with the resorcinol spray, was also present. Glucosazone of characteristic crystalline shape was obtained on heating a portion of the syrup with a solution of phenylhydrazine acetate.

Fraction VI crystallised on storage. The crystals were separated after trituration with methanol and, after recrystallisation from this solvent, had m. p. 161—162° (mixed m. p. with L-sorbose, 161—162°). They were identified as D + DL-sorbose. The crystals gave a positive test for a ketose sugar and, with a variety of solvents, moved at the same rate as sorbose on the chromatogram, and had $[\alpha]_D$ +10° (*c*, 0·572) (D-sorbose has $[\alpha]_D$ +43·4°).

Fraction VI contained sugars which moved on the paper chromatogram at the same rate as galactose and gave colour reactions characteristic of aldohexoses; no derivative of this sugar, however, could be isolated.

Reaction of Dihydroxyacetone with Glycollic Aldehyde.—Dihydroxyacetone (0·9 g.) and glycollic aldehyde (0·6 g., from 1·48 g. of dihydroxymaleic acid) were dissolved in water (40 c.c.), and sufficient barium hydroxide (0·3N.) was added to bring the solution to pH 11. The solution was kept at this pH for 14 days. Barium ions were then precipitated by addition of 2N-sulphuric acid, and the filtered solution passed through a column of Amberlite IR4B resin to remove acids. The filtrate was concentrated under reduced pressure to a syrup (1·378 g.) which contained all four pentose sugars as well as other carbohydrate material. The syrup was fractionated on a column of cellulose, *n*-butanol half saturated with water being used as the mobile phase, and yielded the following fractions.

Fraction I (0·220 g.) contained a pentose sugar which moved at the same rate as ribose on the chromatogram; ketopentose sugars may also have been present; no crystalline derivative was isolated. Fraction II (0·092 g.) contained two pentose sugars, identified by their rate of movement on the chromatogram as ribose and xylose; lyxose may also have been present; ketose sugars were also detected; no crystalline derivative was isolated. Fraction III (0·050 g.) contained a small amount of xylose; the major component was an unknown sugar (dendroketose?), which moved to the same position on the chromatogram as did an authentic specimen of dendroketose and with *p*-anisidine hydrochloride gave a similar colour reaction.

Fraction IV (0·221 g.) contained the pentose, xylose, and the same unknown sugar (dendroketose?). A portion of this syrup (38 mg.) when heated with toluene-*p*-sulphonylhydrazine (40 mg.) in methanol gave DL-xylose toluene-*p*-sulphonylhydrazone (25 mg.), m. p. and mixed m. p. 154° (decomp.), the identity of which was confirmed by *X*-ray analysis. A little of the hydrazone was dissolved in warm 2N-hydrochloric acid, and a portion of the solution placed on the paper chromatogram. An authentic D-xylose derivative treated in this manner gave D-xylose which moved at the same rate and gave the same colour reactions as the sugar from the DL-xylose derivative. The toluene-*p*-sulphonylhydrazone derivative (110 mg. from 150 mg. of Fraction IV) was converted into the sugar by warming it for 1 hour with benzaldehyde (0·5 c.c.) dissolved in aqueous methanol (10 c.c.) containing one drop of acetic acid. The cooled solution was exhaustively extracted with ether, and the aqueous solution evaporated to a syrup of DL-xylose (48 mg.) which did not crystallise. Efforts to crystallise authentic DL-xylose were fruitless.* Fischer and Ruff (*Ber.*, 1900, **33**, 2145) give m. p. 129—131° for DL-xylose. Fraction IV contains at least 74 mg. of DL-xylose.

Fraction V (0·050 g.) contained ketohexose sugars, xylose, and arabinose. Fraction VI (0·211 g.) consisted of ketohexose sugars and arabinose. A portion of the syrup (100 mg.) was heated under reflux with a solution of benzoylhydrazine (100 mg.) in methanol (2 c.c.). On concentration of the solution, crystals of DL-arabinose benzoylhydrazone (40 mg.), m. p. and mixed m. p. 194°, separated. An *X*-ray powder photograph of the crystals was identical with that of an authentic specimen of DL-arabinose benzoylhydrazone. Conversion into the parent sugar was achieved by dissolving the derivative in a little 2N-hydrochloric acid. The resultant sugar moved at the same rate and gave the same colour

* *Added in Proof.*—This material has now been obtained crystalline.

reaction on the chromatogram as did L-arabinose. The benzoylhydrazone, m. p. 194° (40 mg. isolated from a second preparation of pentose sugars), was warmed with benzaldehyde (200 mg.), water (2 c.c.), glacial acetic acid (1 drop), and sufficient methanol to give complete dissolution. After 24 hours, the solution was diluted with water, and extracted with ether, and the aqueous solution then evaporated to a syrup, which crystallised. The DL-arabinose (8 mg.) was recrystallised from methanol; m. p. and mixed m. p. 163°. Fraction VI contained at least 44 mg. of DL-arabinose.

Preparation of Formose: Isolation of DL-*Xylose as its Toluene*-p-*sulphonylhydrazone.*—Paraformaldehyde (10 g.) was suspended in water (200 c.c.), and calcium hydroxide (2 g.) added. The solution was heated on the boiling-water bath for 45 minutes. During this time the paraformaldehyde dissolved, and the solution, initially colourless, became yellow; the odour of formaldehyde disappeared and was replaced by that of caramel. Karrer and Krauss (*loc. cit.*) found *ca.* 0·4% of pentose sugars to be present in a sample of formose prepared by a similar procedure. An examination of a portion of the reaction mixture on the paper chromatogram showed the presence of a complex mixture of ketohexose, aldohexose, and pentose sugars. The solution was cooled, and a solution of oxalic acid added to remove calcium salts, which were filtered off. The filtrate was passed down a column of Amberlite IR4B resin to remove acidic material, and the neutral solution concentrated to a syrup (8 g.) which was fractionated by chromatography on a column of cellulose, *iso*propyl alcohol and water (19 : 1) being used as the mobile phase. In this manner, a fraction (1·42 g.) containing all the four pentose sugars was obtained. This material was then further fractionated on a column of cellulose, butanol half saturated with water being the eluent. By this procedure, a fraction (0·600 g.) rich in xylose and containing a little arabinose (?) and ribose (?) was obtained. This fraction still contained sugars other than pentoses, since separation into three fraction on the paper chromatogram could be achieved by using phenol–water as mobile phase. Accordingly, a portion (0·4 g.) of the syrup was refractionated by separation on two sheets of Whatman No. 1 filter-paper (24″ × 18″), phenol–water being used as mobile phase. After separation, the paper was dried, and the appropriate section of filter-paper holding xylose was extracted (Flood, Hirst, and Jones, *J.*, 1948, 1679) with methanol (Soxhlet). The syrup (94 mg.) which remained on evaporation of the solvent did not crystallise. It was first extracted with ether to remove phenolic impurities and the insoluble residue was then boiled with a solution of toluene-*p*-sulphonylhydrazine (20 mg.) in methanol, and the solution evaporated to a small volume, whereupon DL-xylose toluene-*p*-sulphonylhydrazone (20 mg.), m. p. 154° (decomp.), separated. The product, recrystallised from methanol, had m. p. and mixed m. p. 154° (decomp.), and was identical with an authentic specimen in that on solution in dilute hydrochloric acid, it gave xylose which moved on the chromatogram at the same rate as an authentic specimen of sugar prepared from D-xylose toluene-*p*-sulphonylhydrazone.*

Condensation of Dihydroxyacetone and Paraformaldehyde.—Dihydroxyacetone (0·9 g.) and paraformaldehyde (0·9 g.) were dissolved in water (20 c.c.), and barium hydroxide (0·3N.; 1 c.c.) added. At intervals, portions of the solution were withdrawn; no pentose sugars could be detected even after 3 months. After this or the original solution was heated on the boiling-water bath for 30 minutes, the presence of pentose and other sugars could be detected on the paper chromatogram. When a portion of the solution was boiled with hydrochloric acid furfuraldehyde was formed.

Condensation of Glycollic Aldehyde and Paraformaldehyde.—Glycollic aldehyde (*ca.* 1·1 g.) in water (30 c.c.) and paraformaldehyde (0·6 g.) were mixed and the solution was adjusted to pH 11 by addition of 0·3N-barium hydroxide. After 6 days the pattern of sugar on the chromatogram was constant. The major component was an unknown sugar which gave an intense yellow colour with the *p*-anisidine spray and moved at the same rate as xylose in butanol–ethanol–water, and displayed the properties similar to those of dendroketose prepared from dihydroxyacetone (Utkin, *loc. cit.*). Small quantities of pentose sugars (mainly xylose) were also detected on the chromatogram.

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* *Added in Proof.*—We have since isolated DL-arabinose from formose in the following manner. The formose preparation was heated under reflux with methanolic hydrogen chloride (1%) and, after removal of the acid, the resulting glycosides were separated on a column of cellulose, butanol–water being used as the mobile phase. The faster-moving methylpentosides were separated and hydrolysed, and the reducing sugars refractionated on the column. This gave a fraction rich in arabinose, which, on reaction with benzhydrazide, gave DL-arabinose benzoylhydrazone, m. p. 194°, from which DL-arabinose was obtained.