Studies on Seed Mucilages. Part VI. The Seed Mucilage of* Plantago arenaria.

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The mucilage from *P. arenaria* seeds is an acidic polysaccharide which gives on partial hydrolysis xylose (12 parts), arabinose (3 parts), galactose (1 part), and 2-*O*-α-D-galacturonosyl-L-rhamnose (1 part). Acetylation, deacetylation, and methylation gave a derivative which on hydrolysis yielded 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, 2 : 4-di-*O*-methyl-, 2-*O*-methyl-, and some unsubstituted D-xylose, 2 : 3 : 5-tri-*O*-methyl- and 2 : 5-di-*O*-methyl-L-arabinose, and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose. No methylated galacturonic acid or rhamnose derivatives were recovered.

SOME confusion has existed concerning the identity of seeds of the *Plantago* species (see Part III, *J.*, 1949, 1600), especially those classed as light and dark "psyllium," which have now been identified as *P. ovata* Forsk and *P. arenaria* respectively. The present investigations have been carried out on seeds which were identified botanically as *P. arenaria*.

A preliminary study of the mucilage from *P. arenaria* seeds (Nelson and Percival, Part II, *J.*, 1942, 58) showed that on hydrolysis it gave D-xylose, L-arabinose, D-galactose, and an aldobiuronic acid, thought to be composed of D-galacturonic acid and D-xylose. Hydrolysis of the methylated mucilage yielded 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2-*O*-methyl-D-xylose, and a mixture considered to be composed chiefly of 3 : 4-di-*O*-methylxylose, since the amide of the corresponding aldonic acid gave a positive Weerman test and the acid on further oxidation yielded an optically active hydroxydimethoxyglutaric acid. This dimethyl fraction also yielded a dimethylpentose aniline derivative which had m. p. 170°, $[\alpha]_D^{11}$ −74° in acetone; it was then thought that this was an arabinose derivative, but it is now highly probable that the substance was 2 : 4-di-*O*-methyl-*N*-phenylxylosylamine which has m. p. 170°, $[\alpha]_D^{20}$ −82° in dioxan (Barker, Hirst, and Jones, *J.*, 1946, 783).

* Part V, *J.*, 1950, 528.

In the present series of experiments, the mucilage was extracted from the seeds with cold water, after which no further material could be obtained by extraction of the seeds with hot water. The acidic polysaccharide was obtained by precipitation in acidified alcohol, and, although some of the arabinose residues are easily removed in acid conditions, quantitative hydrolysis of the polysaccharide in both its free acid and salt forms showed that this method of precipitation had left the molecule intact.

The polysaccharide contained 80% of anhydropentose and 7·2% of uronic anhydride. The equivalent was found by titration to be 1700, compared with a value of 2450 based on the uronic anhydride content, the latter being considered to be the more accurate. Hydrolysis with 3% oxalic acid yielded xylose (62%), arabinose (17%), galactose (6%), an aldobiuronic acid (13%), and an insoluble residue (2%), which appeared to be a mixture of cellulose and lignin. The aldobiuronic acid was shown to be 2-*O*-α-D-galacturonosyl-L-rhamnose by methylation, hydrolysis, and identification of equimolecular proportions of 2 : 3 : 4-tri-*O*-methyl-D-galacturonic acid and 3 : 4-di-*O*-methyl-L-rhamnose. The aldobiuronic acid previously isolated as the barium salt by Nelson and Percival (*loc. cit.*) has now been found by paper chromatography to give rhamnose on hydrolysis, after a preliminary mild hydrolysis to remove contaminating xylose. The above structure for the aldobiuronic acid is not in agreement with that published by Hostettler and Deuel (*Helv. Chim. Acta*, 1951, **34**, 2440) who isolated by acid hydrolysis of *P. arenaria* mucilage a product which on methylation and hydrolysis yielded 2 : 3 : 4-tri-*O*-methyl-D-galacturonic acid and 2 : 3-di-*O*-methyl-D-xylose. However these authors are now in agreement with our results (personal communication).

Acetylation of the mucilage gave a derivative which was fractionated with acetone–chloroform (1 : 1) to give a soluble portion X and an insoluble portion Y. The uronic anhydride contents of these products indicated that a partial loss of uronic acid had been suffered during the acetylation process. The acetates X and Y were deacetylated and methylated separately to give two main fractions, A and C respectively. Both of these products on hydrolysis yielded a complex mixture of the same methylated reducing sugars which were separated by partition chromatography on columns of cellulose with light petroleum–butanol saturated with water as mobile phase.

2 : 3 : 4-Tri-*O*-methyl-D-xylose, 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2 : 3-di-*O*-methyl-D-xylose, 2 : 4-di-*O*-methyl-D-xylose, and 2-*O*-methyl-D-xylose were identified by isolation of the sugars or derivatives in crystalline form. 2 : 5-Di-*O*-methyl-L-arabinose was isolated as a syrup and identified as the crystalline phenylhydrazide of the corresponding 2 : 5-di-*O*-methyl-L-arabonic acid. This sugar has been isolated from the hydrolysis products of methylated arabic acid (Smith, *J.*, 1940, 1035) and methylated cherry gum (Jones, *J.*, 1947, 1055) but has not been reported so far among the methylated products of the *Plantago* mucilages. However it now seems certain that a sugar, thought to be a 2 : 6-di-*O*-methylaldohexose, isolated from the hydrolysate of methylated *P. ovata* mucilage (Laidlaw and Percival, Part V, *J.*, 1950, 528) was in fact 2 : 5-di-*O*-methyl-L-arabinose. Among the similar properties possessed by the products isolated from *P. arenaria* and *P. ovata* are the specific rotations ($[\alpha]_D$ −18° and −19°), and the brownish-black colour produced on spraying with aniline oxalate on the paper chromatogram (as distinct from the usual pink colour given by pentose derivatives). The amides of the corresponding aldonic acids also had identical analytical data and melting points, and the course of hydrolysis of the syrupy lactones was the same ($[\alpha]_D$ −51° ⟶ −32°, −50° ⟶ −33°) (cf. Smith, *J.*, 1939, 744).

Contrary to earlier findings, no 3 : 4-di-*O*-methylxylose could be identified among the methylated products of the mucilage. However, there was present in the syrupy 2 : 3-di-*O*-methyl-D-xylose fractions [(*e*) and (*f*)] another di-*O*-methylpentose, thought to be an arabinose derivative, since fraction (*e*) on demethylation yielded arabinose as well as xylose. The syrupy amide derived from the aldonic acid of fraction (*e*) gave a positive Weerman test, suggesting that the sugar obtained by Nelson and Percival (see above) which yielded on oxidation an optically active hydroxydimethoxyglutaric acid was not in fact 3 : 4-di-*O*-methylxylose, but was probably 3 : 4-di-*O*-methylarabinose.

Small amounts of unsubstituted xylose were found among the methylated products

of the mucilage, but this may have arisen as the result of incomplete methylation or demethylation during acid hydrolysis and cannot be said with certainty to have any structural significance for the molecule. Three other methylated derivatives were present in small amounts but were not identified. Two of these gave a brown colour with aniline oxalate on the paper chromatogram, and from their positions (R_G 0·58 and 0·50) it is possible that they were either dimethylgalactose or monomethylpentose derivatives. The third sugar had R_G 0·24 and gave a pink colour with aniline oxalate; this was probably a monomethylpentose derivative. These residues must be triply-linked in the molecular structure of the mucilage and constitute some of the branch-points.

No trace of any methylated derivatives of either rhamnose or galacturonic acid was found among the hydrolysis products of the methylated mucilage. It is interesting that a similar disappearance of the aldobiuronic acid residues was also encountered during the study of the mucilages from both *P. ovata* and *P. lanceolata*. Since aldobiuronic acid residues are themselves stable during methylation it is possible that in the *Plantago* mucilages either (*a*) the aldobiuronic acid is attached to the rest of the molecule by an alkali-sensitive linkage, or (*b*) the mucilage consists of a neutral portion with an associated short-chain polyuronide. These possibilities were tested by a study of the alkali lability of the mucilage. The acetylated mucilage from *P. arenaria* was deacetylated with sodium methoxide and the resulting product was separated by sedimentation in alcohol into a fibrous material, which contained little or no uronic acid, and a gelatinous precipitate which gave a positive naphtharesorcinol test. Furthermore, the action of 2N-sodium hydroxide on the mucilage itself, followed by electrodialysis, was found to reduce the uronic anhydride content from 7·2% to 2·5%, removing an anion which gave a positive naphtharesorcinol test. On the other hand, electrodialysis of the untreated mucilage failed to remove any uronic acid. These results lend support to (*a*) and it seems possible that the galacturonic acid residue is attached to another residue in the molecule by a linkage of the ester type.

It was obvious that fraction A was incompletely methylated, since it yielded on hydrolysis a very high proportion of monomethyl pentose derivatives. However, on the basis of the quantitative evidence obtained from fraction C, it is possible to present the following figures for the sugar derivatives obtained from the methylated degraded mucilage: 2 : 3 : 4-tri-*O*-methyl-D-xylose (10 parts), 2 : 3 : 5-tri-*O*-methyl-L-arabinose (1 part), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (2 parts), 2 : 3-di-*O*-methyl-D-xylose (6 parts), 2 : 4-di-*O*-methyl-D-xylose (3 parts), 2 : 5-di-*O*-methyl-L-arabinose (2 parts), 2-*O*-methyl-D-xylose (8 parts), D-xylose (1 part), and unidentified derivatives (2 parts).

The monomethylpentose derivatives recovered from the methylated mucilage must have arisen from residues which are triply-linked in the molecule. From the high proportion of these it is clear that the mucilage possesses a very highly branched structure, for which it is at present impossible to put forward a unique molecular formula. Nevertheless, the presence of the following sugar residues in the molecule has definitely been established:

D-Xyl*p* 1 . . . .; . . . . 4 D-Xyl*p* 1 . . . .; . . . . 3 D-Xyl*p* 1 . . . .;

$\begin{matrix} \cdot\cdot\cdot\cdot 3 \\ \cdot\cdot\cdot\cdot 4 \end{matrix}$ D-Xyl*p* 1 . . . .; L-Ara*f* 1 . . . .; . . . . 3 L-Ara*f* 1 . . . .; D-Gal*p* 1 . . . .

The residue D-Gal*p*A–2 L-Rha*p* may also be a structural feature of the molecule, or may be part of an associated polyuronide, since there is still some doubt concerning the homogeneity of the mucilage.

Experimental

Evaporations were carried out at 40° under reduced pressure unless otherwise stated, and temperatures quoted are bath-temperatures.

In paper chromatographic studies the unsubstituted sugar mixtures were separated by elution with ethyl acetate–acetic acid–water (3 : 1 : 3 v/v) (solvent A) (Jermyn and Isherwood, *Biochem. J.*, 1949, **44**, 402) and methylated sugar mixtures with *n*-butanol–ethanol–water–ammonia (40 : 10 : 49 : 1 v/v) (solvent B) (Partridge, *Nature*, 1946, **158**, 270) unless otherwise

stated. The R_G values quoted are those observed in the latter solvent. All sugars were located by spraying the paper with an aqueous solution of aniline oxalate (Horrocks and Manning, *Lancet*, 1949, **256**, 1042).

The fractions obtained from the cellulose columns after removal of the solvent were dissolved in water and digested with charcoal, and the solutions were filtered and evaporated to dryness; the residues were exhaustively extracted with boiling acetone (unless otherwise stated), and the extracts filtered, evaporated, and dried *in vacuo* over phosphoric oxide.

Extraction of the Mucilage.—*P. arenaria* seeds (1 kg.) were soaked in water (16 l.) at room temperature for 48 hr. with occasional stirring. The viscous solution was separated from the seeds by filtration through muslin or by the use of a filter press and the seeds were re-extracted with 4—5 l. of water. The mucilage, precipitated by pouring the extracts into vigorously stirred alcohol (3 vols.) was dried by means of alcohol, and then ether, and finally *in vacuo* over phosphoric oxide, to give a white fibrous product in *ca.* 3% yield (Found: ash, 5·3; sulphated ash, 6·0%).

This material (1 g.) was hydrolysed with oxalic acid (3%; 50 c.c.) at 95—100° for 6 hr., and the solution neutralised with barium carbonate and filtered. After evaporation to *ca.* 10 c.c. the solution was poured into alcohol (250 c.c.), giving a white precipitate of a barium salt. Evaporation of the alcohol yielded a syrup (0·50 g.) which was dissolved in water, passed through two columns containing Amberlite resins IR-100 and IR-4B respectively, and evaporated to small volume. The sugars in this solution were separated by paper chromatography, eluted with cold water (Laidlaw and Reid, *Nature*, 1950, **166**, 476), and estimated by the Somogyi arsenomolybdate method (Nelson, *J. Biol. Chem.*, 1944, **153**, 375; Duff and Eastwood, *Nature*, 1950, **165**, 848) (Found: xylose, 72·3; arabinose, 20·5; galactose 7·2%).

Preparation of the Acid Mucilage.—Precipitation of the mucilage with 4% alcoholic hydrogen chloride gave an acid polysaccharide which was freed from chloride by trituration with alcohol (Found: ash, 1·2%; equiv., by titration, *ca.* 1700; uronic anhydride, 7·2; pentosan, 80%). This material was used in all the subsequent investigations.

Autohydrolysis of the Mucilage.—The mucilage (0·3 g.) in water (25 c.c.) was heated at 95—100° for 24 hr. The solution was neutralised with barium carbonate, filtered, evaporated to *ca.* 5 c.c., and poured into alcohol (400 c.c.). The white precipitate was removed by centrifugation, and the alcoholic solution evaporated to a syrup which was shown by paper chromatography to be arabinose.

Acid Hydrolysis.—The mucilage (10·00 g.) was heated at 95—100° with oxalic acid (3%; 250 c.c.), the following changes being observed: $[\alpha]_D^{17}$ +20° (2 hr.); +34° (3 hr.); +39° (4 hr.); +46° (5 hr., constant). The brown insoluble residue (0·1950 g.) was removed by filtration through a sintered-glass crucible (porosity 3), and the filtrate neutralised with barium carbonate, filtered, and evaporated to *ca.* 50 c.c. After removal of a trace of inorganic material, the solution was poured into ethanol (1500 c.c.), giving a white barium salt P (0·649 g.) which was removed by centrifugation. Evaporation of the alcoholic solution yielded a reducing syrup Q (3·686 g.). The syrup Q was dissolved in water and de-ionised, and the sugars present were estimated as before, after separation by paper chromatography (Found: xylose 72·6; arabinose 20·2; galactose 7·1%).

The insoluble residue (0·2 g.) with 72% sulphuric acid (Monier-Williams, *J.*, 1921, **119**, 803) yielded a reducing syrup, shown by paper chromatography to be glucose, and a brown residue of apparently unchanged material.

Isolation of a Barium Aldobiuronate.—The mucilage (150 g.) was hydrolysed with 3% oxalic acid for 12 hr. and treated as above to give the barium salt P. This was heated at 95—100° with 2% sulphuric acid for 7 hr. and the barium salt precipitated as before; the alcoholic washings were shown by paper chromatography to contain a little xylose, arabinose, and galactose. After a further treatment with 2% sulphuric acid for 5 hr. the washings contained only a trace of xylose and a little rhamnose, and the pure barium salt of an aldobiuronic acid (20 g.) was obtained as a white powder, $[\alpha]_D^{16}$ +65° (*c*, 0·3 in H_2O) (Found: Ba, 16·2; CO_2, 10·6. Calc. for $C_{12}H_{19}O_{11}Ba_{\frac{1}{2}}$: Ba, 16·8; CO_2, 10·8%).

Treatment of the Mucilage with Alkali.—The mucilage (*ca.* 2 g.) was heated with 2N-sodium hydroxide (120 c.c.) at 100° for 15 min. and the solution then left at room temperature for 16 hr. The solution was neutralised with acetic acid, diluted, and dialysed in a Cellophane bag against running water for 5 days. The solution was then evaporated to small volume and poured into methanol to give a fibrous product (uronic anhydride content, 4·3%).

A fresh portion of the mucilage was set aside in 2N-sodium hydroxide for 1 week. The solution was neutralised with dilute sulphuric acid, and removal of the uronic acid component

was attempted by electrodialysis. The membranes used were parchment (negative) and muslin impregnated with gelatin (positive). A potential difference of 130 v (D.C.) was applied and the initial current of 900 milliamp. dropped to 30 milliamp. in 1·5 hr. The solution in the anode compartment was evaporated to small volume and gave a positive naphtharesorcinol test. The electrodialysed mucilage was dialysed in a Cellophane bag for 1 week, evaporated to small volume, and precipitated in methanol to give a crisp brown solid (uronic anhydride content 2·5%). Electrodialysis of the mucilage itself effected no separation.

Periodate Uptake of the Mucilage.—(*a*) The mucilage (*ca.* 0·4 g.) was shaken with water (40 c.c.), and M-sodium metaperiodate (10 c.c.) was added. At intervals portions (5 c.c.) were withdrawn and the periodate contents determined by the arsenite method, with the following results [expressed as moles of periodate consumed/equivalent (2450)]: 13·4 (1 day); 13·9 (3 days).

(*b*) The mucilage (*ca.* 0·4 g.) was oxidised with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1948, 27), and the formic acid was estimated (0·01N-sodium hydroxide) with the following results (expressed as moles of formic acid produced/equivalent): 2·82 (1 day); 4·80 (5 days); 5·10 (7 days); 5·60 (12 days); 5·70 (14 days, constant).

Hydrolysis of the Periodate-oxidised Mucilage.—The mucilage (0·2836 g.) was oxidised with 0·2M-sodium metaperiodate (35 c.c.) for 5 days, and the solution neutralised with barium carbonate and filtered. Pure D-ribose (0·0964 g.) was added and the iodic acid removed by bubbling sulphur dioxide through the solution for 30 min. (Jayme and Sätre, *Ber.*, 1944, **77**, 242). The solution was then made 3% with oxalic acid and hydrolysed for 5 hr. at 100°, neutralised with barium carbonate, and filtered. Inorganic ions were removed by electrodialysis (Laidlaw and Reid, *J. Sci. Food and Agric.*, 1952, **3**, 19), and the solution was evaporated to a syrup (0·151 g.). The sugars in the syrup were separated on a paper chromatogram and estimated by the Somogyi arsenomolybdate method (Found: xylose 0·18 mg.; arabinose 0·03 mg.; ribose 0·35 mg.). This indicated that 25% of the xylose and 16% of the arabinose present in the mucilage had been unattacked by periodate.

Acetylation of the Mucilage.—The mucilage (60 g.) was moistened with ethanol and dispersed in pyridine (900 c.c.), and acetic anhydride (750 c.c.) was added slowly with stirring. The mixture was heated at 95—100° for 3 hr., left at room temperature for 48 hr. and poured into iced water. The semi-solid mass was triturated with ice and water, then washed with running water for 24 hours, and dried, giving a white fibrous product (75 g.). The acetyl content (34·6%) of this material was unchanged on re-acetylation. Extraction of the acetate with boiling acetone–chloroform (1 : 1) gave fraction X (20%) {(Ac, 37·4%; $[\alpha]_D^{17}$ −195° in $CHCl_3$; uronic anhydride, 2·3%); $\eta_{sp.}^{20}/c$ 8·5 (in *m*-cresol; *c* as g. per 100 c.c. of solution)}, leaving an insoluble material, fraction Y (80%) (Ac, 30·2; uronic anhydride, 3·9%).

The unfractionated acetate (1 g.) was dispersed in chloroform (15 c.c.). The mixture was cooled to 0° and a solution of 0·05 g. of sodium in 5 c.c. of methanol was added (Zemplén and Pacsu, *Ber.*, 1929, **62**, 1613). The mixture was shaken for 4 hr. at room temperature, and ice-water (5 c.c.) added, followed by acetic acid (10%; 1 c.c.) and water (10 c.c.). The mixture was poured into methanol (200 c.c.) from which two precipitates, G and H, were separated. The product G was a grey fibrous material which was hydrolysed with 3% oxalic acid solution to a syrup, shown by paper chromatography to contain xylose, arabinose and galactose. The syrup was completely soluble in alcohol and thus contained no uronic acid. Therefore, since the mucilage suffered only partial loss of uronic acid during the acetylation, the remaining uronic acid (*ca.* 3·5%) must have been removed under the alkaline conditions of the deacetylation. Investigation of material H, a fine gelatinous white precipitate, indicated that it was probably the sodium salt of the aldobiuronic acid. It gave a strongly positive naphtharesorcinol test and on treatment with Amberlite resin IR-100 yielded an acid (0·0177 g.) which was titrated with 0·005N-sodium hydroxide (Found: equiv., 282. Calc. for $C_{12}H_{20}O_{11}$: equiv., 340).

Identification of the Barium Aldobiuronate.—Hydrolysis of the barium aldobiuronate (0·5 g.) with sulphuric acid (9%; 20 c.c.) at 100° for 20 hr. gave a brown solution which was diluted, neutralised with barium carbonate, and filtered. The solution was digested with charcoal, filtered, and evaporated, giving a white residue which was extracted with hot methanol. The methanol extracts yielded a syrup which was shown by paper chromatography to be rhamnose. The residue was shaken in water with Amberlite resin IR-100, and the solution shown by paper chromatography to contain galacturonic acid.

A syrupy calcium aldobiuronate prepared from *P. arenaria* by Nelson and Percival (*loc. cit.*) was treated with Amberlite resin IR-4B. Examination on a paper chromatogram showed that

the calcium salt was contaminated with a large amount of xylose and some arabinose and galactose. When the calcium aldobiuronate was hydrolysed with 9% sulphuric acid and treated as the above barium aldobiuronate, the alcoholic extract on the paper chromatogram gave a dense spot of rhamnose with some xylose, arabinose, and galactose.

Oxidation of the barium salt (1 g.) by Heidelberger and Goebel's method (*J. Biol. Chem.*, 1927, **74**, 616) gave mucic acid, m. p. 215° (decomp.), mixed m. p. with an authentic specimen, 214° (decomp.). After removal of the mucic acid, the aqueous mother-liquor was neutralised with silver carbonate, silver removed as sulphide, and the solution evaporated to a partly crystalline syrup. This was heated with phenylhydrazine in ethanol at 80° for 2 hr. and, on cooling, crystals of rhamnonic phenylhydrazide separated. After recrystallisation from absolute alcohol these had m. p. 193°, $[\alpha]_D^{17}$ +17° (*c*, 1·1 in H_2O).

Methylation of the barium salt (12 g.) was carried out 4 times by the Haworth method at room temperature. After each methylation the solution was set aside overnight, heated at 100° for ½ hr., cooled, and then neutralised with concentrated sulphuric acid. The slightly acid solution was extracted with chloroform, and the extracts were dried (Na_2SO_4) and evaporated to a syrup. The product was methylated twice with the Purdie reagents and then with diazomethane in ether for 14 hr. at 0°. After evaporation of the diazomethane at room temperature the ether was evaporated to give a syrup which was distilled over barium carbonate (b. p. 180—200°/0·05 mm.). The pale yellow distillate (0·63 g.) had n_D^{16} 1·4684 (Found: OMe, 45·6. Calc. for $C_{19}H_{34}O_{11}$: OMe, 49·5%).

The syrup (0·57 g.) was hydrolysed at 100° with 2N-hydrochloric acid (25 c.c.), the following changes being observed: $[\alpha]_D^{16}$ +85° (15 min.); +77° (2 hr.); +76° 4 hr.); +72° (10 hr., constant). The solution was diluted, neutralised with silver carbonate, and filtered, and silver removed as sulphide. It was then aerated, warmed with barium carbonate, filtered, and evaporated to dryness. The residue was extracted with boiling anhydrous ether, leaving a white solid R (0·21 g.). Evaporation of the ethereal extracts yielded a syrup S (0·20 g.), $[\alpha]_D^{16}$ +19° (*c*, 2·6 in H_2O), n_D^{16} 1·4728 (Found: OMe, 31·8. Calc. for $C_6H_{12}O_5$: OMe, 32·3%). This travelled on the paper chromatogram at the same rate as 3 : 4-di-*O*-methylrhamnose and gave no acetaldehyde on oxidation with periodic acid (Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456). Oxidation of S (0·26 g.) with bromine in water and distillation of the product gave syrupy 3 : 4-di-*O*-methyl-L-rhamnonolactone (0·07 g.), b. p. 140/160°/0·01 mm., $[\alpha]_D^{16}$ −148° (10 min.), −142° (7 hr.), −126° (31 hr.), −120° (50 hr.), −117° (72 hr., constant) (*c*, 0·9 in H_2O). The lactone on treatment with methanolic ammonia gave a crystalline amide which gave a positive Weerman test.

The residue R was oxidised with bromine in water till non-reducing (3 days). The solution was aerated, neutralised with barium carbonate, filtered, and evaporated to a syrup which was esterified at 80° with methanolic hydrogen chloride (3%; 40 c.c.) for 14 hr. The solution was neutralised with silver carbonate, filtered, and evaporated, and the syrup distilled (b. p. 140°/0·05 mm.). The distillate, n_D^{17} 1·4545, crystallised overnight at 0°. After trituration with ether–light petroleum (1 : 3) and recrystallisation from ether–light petroleum (1 : 5) this had m. p. 100—101°, not depressed on admixture with authentic dimethyl 2 : 3 : 4-tri-*O*-methylmucate, and $[\alpha]_D^{17}$ +28° (*c*, 0·9 in H_2O).

Methylated Derivative of the Mucilage.—Fractions X and Y were treated separately in 12-g. portions with the Haworth reagents as described by Mullan and Percival (Part I, *J.*, 1940, 1501) without extraction with solvents. The products obtained after four treatments were dissolved in chloroform, dried (Na_2SO_4), and fractionally precipitated by addition of light petroleum (b. p. 60—80°). Fraction Y yielded fractions A and B and fraction X yielded fractions C and D (see Table). Further treatments with the Haworth reagents failed to increase the methoxyl content.

Fraction	Yield (g.)	OMe (%)	$[\alpha]_D^{14}$ ($CHCl_3$)	$\eta_{sp.}^{20}/c$	Fraction	Yield (g.)	OMe (%)	$[\alpha]_D^{14}$ ($CHCl_3$)	$\eta_{sp.}^{20}/c$
A	3·8	36·2	−102°	30·3	C	4·9	36·9	−105°	15·9
B	0·3	36·6	−103·5	8·9	D	0·4	36·2	−104	2·4

Fraction C after two methylations with the Purdie reagents gave a product E which had OMe 36·6%, $[\alpha]_D^{16}$ −104° in $CHCl_3$, $\eta_{sp.}^{20}/c$ 4·2 in *m*-cresol.

Hydrolysis of A and Fractionation by Solvent Extraction.—Fraction A (8 g.) was boiled with methanolic hydrogen chloride (3%; 160 c.c.) until the rotation was constant ($[\alpha]_D^{16}$ +54° after 20 hr.). The solution was neutralised with silver carbonate, filtered, and evaporated to dryness. The residue was extracted with boiling methanol, and the solution filtered and evaporated to a non-reducing syrup (9·1 g.), fractionation of which was attempted by solvent extraction (Brown

and Jones, *J.*, 1947, 1344). Extraction of an aqueous solution of the syrup (7·00 g.) with light petroleum (b. p. 30—40°) for 83 hr. gave fraction I (3·39 g.). Subsequent extraction with chloroform for 18 hr. gave fraction II (3·33 g.); evaporation of the aqueous solution yielded fraction III (0·25 g.).

Hydrolysis of Fraction I and Separation of the Methylated Sugars.—Fraction I (3·39 g.) was heated at 95—100° with nitric acid (2%; 150 c.c.). Changes found were: $[\alpha]_D^{15}$ +49° (3·5 hr.); +36° (9·5 hr.); +31° (12 hr., constant). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness, and the residue extracted with boiling methanol to yield a reducing syrup (3·05 g.). The syrup (3·02 g.) was fractionated on a column of cellulose (27 × 1·2") (Hough, Jones, and Wadman, *J.*, 1949, 2511) with, as eluant, light petroleum (b. p. 100—120°)–butanol (7 : 3) saturated with water to give fractions: (*a*) 1·01 g.; (*b*) 0·46 g.; (*c*) 0·21 g.; (*d*) 0·13 g.; (*e*) 0·42 g.; (*f*) 0·23 g.; and (*g*) 0·16 g. (eluted with water and extracted with 80% ethanol for purification). Recovery 88%.

Fraction (a). The syrup partly crystallised and the crystals (0·54 g.) were separated on a tile. After recrystallisation from anhydrous ether these had m. p. 90—92°, not depressed on admixture with authentic 2 : 3 : 4-tri-*O*-methyl-α-D-xylopyranose, $[\alpha]_D^{16}$ +67° (zero time), +51° (15 min.), +29° (100 min.), +19° (3 hr., constant) (*c*, 0·6 in H_2O) (Found: C, 50·2; H, 8·5; OMe, 47·7. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·4; OMe, 48·4%).

Extraction of the tile with acetone gave a syrup (0·43 g.), $[\alpha]_D^{19}$ +9·6° (*c*, 1·0 in H_2O). Oxidation with bromine in water at room temperature till non-reducing, and treatment with silver carbonate and hydrogen sulphide, gave a mixture of syrupy lactones which were fractionally distilled, to give fraction (1*a*), 0·10 g., b. p. 90—110°/0·15 mm., and (2*a*), 0·08 g., b. p. 110—120°/0·10 mm. Fraction (1*a*) had $[\alpha]_D^{16}$ +7° (5 min.), +2° (22 hr.), 0° (45 hr.), +13° (100 hr., constant) (*c*, 1·0 in H_2O); 2 c.c. of the polarimetric solution required 3·39 c.c. of 0·0322N-sodium hydroxide for neutralisation (Calc. for $C_8H_{14}O_5$: 3·40 c.c.). Fraction (1*a*) with methanolic ammonia gave after 10 months at 0° crystalline 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, recrystallised from ethyl acetate, m. p. and mixed m. p. 136°. Fraction (2*a*) had $[\alpha]_D^{16}$ −1° (½ hr.), +13° (4 hr.), +15° (72 hr.), +18° (200 hr., constant); 0·0163 g. required 2·54 c.c. of the above alkali for neutralisation (Calc. for $C_8H_{14}O_5$: 2·66 c.c.). The above data showed that fraction (*a*) was a mixture of tri-*O*-methyl-D-xylopyranose (0·94 g.) and tri-*O*-methyl-L-arabofuranose (0·06 g.).

Fraction (b). This crystallised. The crystals were separated on a tile and after recrystallisation (light petroleum) had m. p. 70—72° (not depressed on admixture with authentic 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose), $[\alpha]_D^{16}$ +142° (5 min.), +129° (30 min.), +117° (3 hr., constant) (*c*, 1·1 in H_2O) (Found: C, 50·9; H, 8·7; OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%). The residual syrup had $[\alpha]_D^{18}$ +114°, OMe, 51·7%. Treatment with alcoholic aniline gave 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine (in good yield), m. p. and mixed m. p. 193—194°, $[\alpha]_D^{17}$ +41·5° (equil., *c*, 0·6 in $COMe_2$) (Found: C, 61·8; H, 8·0; N, 4·3; OMe, 39·4. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·1; N, 4·5; OMe, 39·9%).

Fraction (c). This syrup was combined with fractions (*b*) and (ii) to give fraction H (see below).

Fraction (d). This was a mixture of 2 : 5-di-*O*-methyl-L-arabinose (cf. fraction H) and 2 : 3-di-*O*-methylxylose. After separation on a paper chromatogram and extraction with hot water these were estimated by oxidation with alkaline hypoiodite (Hirst, Hough, and Jones, *J.*, 1949, 928) and a phosphate buffer of pH 11·4 (Ingles and Israel, *J.*, 1947, 1344). It consisted of 44% of 2 : 5-di-*O*-methylarabinose (0·06 g.) and 56% of 2 : 3-di-*O*-methylxylose (0·07 g.).

Fraction (e). The syrup had n_D^{17} 1·4727, $[\alpha]_D^{16}$ +24° (15 min.), +26° (6 hr., constant) (Found: OMe, 34·1. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). When examined by paper chromatography with, as solvent, benzene–ethanol–water (167 : 47 : 16), two pink spots were obtained with aniline oxalate, one travelling at the same rate as 2 : 3-di-*O*-methylxylose and the other (in minor quantity) slightly faster than the latter. With solvent B only one spot was obtained. No indication was obtained of the presence of any 3 : 4-di-*O*-methylxylose, which travels more slowly than 2 : 3-di-*O*-methylxylose (Jones and Wise, *J.*, 1952, 3389).

Demethylation of the syrup (7 mg.) with hydrobromic acid (48%) (Hough, Jones, and Wadman, *J.*, 1950, 1702) and examination of the products on a paper chromatogram gave arabinose (R_G 0·12), xylose (R_G 0·15), mono-*O*-methylxylose (R_G 0·38), 2 unidentified pink spots (R_G 0·24 and 0·46), and unchanged material (R_G 0·64).

Oxidation of the syrup with bromine in water gave a syrup, b. p. 125—135°/0·03 mm., $[\alpha]_D^{16}$ +28° (initial). Treatment with methanolic ammonia yielded a syrup which gave a positive

Weerman test. A control test with 2 : 3-di-*O*-methylxylonamide was negative. Oxidation of a fresh portion of the syrup and fractional distillation of the lactones gave fractions: (1*b*) 0·03 g., b. p. 120—125°/0·04 mm.; (2*b*) 0·01 g., b. p. 150°/0·03 mm. Fraction (1*b*) had $[\alpha]_D^{16}$ +47° (4 hr.), +38° (25 hr.), +31° (120 hr.), and +27·5° (270 hr., constant) (*c*, 1·3 in H_2O). Fraction (2*b*) had n_D^{17} 1·4669, $[\alpha]_D^{16}$ +27° (½ hr.), +19° (5 hr.), +13° (18 hr.), 0° (47 hr.), +11° (121 hr.), +21° (143 hr.), +17° (166 hr.), +13° (212 hr.), and +8·5° (289 hr., constant) (*c*, 0·5 in H_2O).

Fraction (*e*) yielded a syrupy mixture of anilides.

Fraction (f). This crystallised. It was triturated with acetone–light petroleum (1 : 3) and recrystallised from acetone–light petroleum (1 : 10), giving long needles of 2 : 4-di-*O*-methyl-β-D-xylose, m. p. and mixed m. p. 111°, $[\alpha]_D^{17}$ −27·6° (5 min.), +10·6° (50 min.), and +21·5° (2 hr., constant) (*c*, 0·5 in H_2O) (Found: C, 47·1; H, 7·6; OMe, 34·3. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 34·8%).

Oxidation of the sugar (0·10 g.) with bromine in water yielded the syrupy lactone, b. p. 135—140°/0·01 mm., $[\alpha]_D^{20}$ −14° (8 min.), −7° (2 hr.), 0° (4·5 hr.), +14° (21 hr.), +23° (48 hr.), and +27° (118 hr., constant) (*c*, 0·3 in H_2O). The lactone yielded a syrupy amide which gave a negative Weerman test.

Fraction (g). Paper chromatography showed this to be a mixture of equal parts of 2-*O*-methylxylose and an unidentified sugar (R_G 0·52), which gave a brown colour with aniline oxalate.

Hydrolysis of Fraction II and Separation of the Methylated Sugars.—Fraction II (3·33 g.) was hydrolysed with nitric acid (2%; 150 c.c.); $[\alpha]_D^{16}$ were +60° (zero time), +36° (3 hr.), and +29° (6 hr., constant). The solution was neutralised with barium carbonate and yielded a reducing syrup (3·17 g.). The mixture (2·80 g.) was fractionated on a column of cellulose in the same way as the sugars from I to give fractions: (*h*) 0·09 g.; (i) 0·16 g.; (*j*) 0·18 g.; (*k*) 0·21 g.; (*l*) 0·08 g.; (*m*) 1·82 g. (extracted with ethanol for purification); and (*n*) 0·06 g. (eluted with water; extracted with 80% ethanol for purification). Recovery 93%.

Fraction (h) was combined with fractions (*c*) and (ii) to give fraction H (see below).

Fraction (i). This was a mixture of 2 : 5-di-*O*-methylarabinose (cf. fraction H) and 2 : 3-di-*O*-methylxylose. These were estimated to be present in the proportion 28 : 72 respectively, in the same way as fraction (*d*).

Fraction (j). Paper chromatography showed this to be a mixture of 2 : 3- and 2 : 4-di-*O*-methylxylose. Oxidation of the syrup (16·95 mg.) with periodic acid for 96 hr. gave formaldehyde (0·781 mg.) (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476). Pure 2 : 3-di-*O*-methylxylose (16·12 mg.) under the same conditions gave formaldehyde (1·902 mg., 70%). This indicates 39% of 2 : 3-di-*O*-methylxylose in (*j*).

Fractions (k) *and* (l). These were mixtures of 2 : 4-di-*O*-methylxylose, two sugars of R_G 0·58 and 0·50 respectively, and a trace of 2-*O*-methylxylose. Demethylation of fraction (*k*) with hydrobromic acid (48%) (*loc. cit.*) gave arabinose (R_G 0·12), xylose (R_G 0·15), a pink spot (R_G 0·24), 2-*O*-methylxylose (R_G 0·37), and some unchanged material.

Fraction (m). This crystallised and oxidation with alkaline hypoiodite indicated 94% of aldose. Recrystallisation from *n*-butanol gave 2-*O*-methyl-D-xylose, m. p. and mixed m. p. 135—137°, $[\alpha]_D^{17}$ −17° (3 min.), +12·5° (15 min.), +34° (75 min.), and +35° (100 min., constant) (*c*, 0·7 in H_2O) (Found: C, 43·7; H, 7·6; OMe, 18·6. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4; OMe, 18·9%). The syrup reclaimed from the mother-liquor had $[\alpha]_D^{17}$ +35° (*c*, 0·6 in H_2O).

Treatment of the crystals with aniline in ethanol gave 2-*O*-methyl-*N*-phenylxylosylamine; recrystallised from absolute alcohol, this had m. p. and mixed m. p. 124—125°, $[\alpha]_D^{16}$ +213° (*c*, 0·7 in ethyl acetate) (Found: C, 60·0; H, 7·0; OMe, 13·2. Calc. for $C_{12}H_{17}O_4N$: C, 60·3; H, 7·1; OMe, 12·9%).

Fraction (n). This was a mixture of 2-*O*-methylxylose, xylose, and a sugar of R_G 0·24.

Hydrolysis of Fraction III.—Fraction III (0·25 g.) was hydrolysed with nitric acid (2%; 30 c.c.); $[\alpha]_D^{17}$ were +47° (1 hr.), +25° (4 hr.), and +24° (5 hr., constant). This yielded a reducing syrup (0·22 g.) which was shown by paper chromatography to contain 2-*O*-methylxylose (*ca.* 0·1 g.), xylose (*ca.* 0·1 g.), arabinose (trace), and a sugar of R_G 0·24 (trace).

Hydrolysis of E and Separation of the Methylated Sugars.—Fraction E (4·5 g.) was boiled with methanolic hydrogen chloride (3%; 90 c.c.); $[\alpha]_D^{16}$ were +57° (3 hr.) and +64° (22 hr., constant). The solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (5 g.) which was hydrolysed with nitric acid (2%; 80 c.c.). $[\alpha]_D^{16}$ were +29° (4 hr.) and +27° (5 hr., constant). The solution was neutralised with barium carbonate and gave a reducing syrup (3·7 g.) which was fractionated on a column of cellulose as before to give

fractions: (o_1) 0·96 g.; (o_2) 0·16 g.; (o_3) 0·12 g.; (o_4) 0·28 g.; (*p*) 0·42 g.; (*q*) 0·29 g.; (*r*) 0·14 g.; (*s*) 0·02 g.; (*t*) 0·05 g.; (*u*) 0·15 g.; (*v*) 0·44 g.; (*w*) 0·02 g.; (*x*) 0·06 g. (eluted with water). For purification, fractions (*u*), (*v*), and (*w*) were extracted with ethanol, and (*x*) with 80% ethanol. Recovery was 85%.

Fraction (o_1). The syrup partly crystallised and was drained on a tile, to give 2 : 3 : 4-tri-*O*-methyl-D-xylose (0·53 g.) (recrystallised from ether), m. p. and mixed m. p. 89—91°. The tile yielded a syrup (0·26 g.) which was oxidised with bromine in water and fractionally distilled, to give fractions: (1*c*), 0·06 g., b. p. 80—100°/0·07 mm., n_D^{17} 1·4750, and (2*c*), 0·07 g., b. p. 100—125°/0·07 mm., n_D^{17} 1·4583. Fraction (1*c*) had $[\alpha]_D^{16}$ −5·7° (1 hr.), −0·6° (94 hr.), +3·2° (140 hr.), and +4·5° (284 hr., constant) (*c*, 1·57 in H_2O); 1 c.c. of the polarimetric solution required 4·29 c.c. 0·0196N-sodium hydroxide for neutralisation (Calc. for $C_8H_{14}O_5$: 4·42 c.c.). Treatment with methanolic ammonia gave an amide which failed to crystallise. Fraction (2*c*) had $[\alpha]_D^{17}$ −4·2° (10 min.), −1·2° (25 hr.), +9° (93 hr.), and +12° (200 hr., constant) (*c*, 1·7 in H_2O); 1 c.c. of the polarimetric solution required 4·69 c.c. of the above alkali for neutralisation (Calc. for $C_8H_{14}O_5$: 4·72 c.c.). The above data showed (o_1) to be a mixture of tri-*O*-methyl-D-xylopyranose (0·87 g.) and tri-*O*-methyl-L-arabofuranose (0·09 g.).

Fraction (o_2). This syrup was a mixture of tri-*O*-methylxylose and tetra-*O*-methylgalactose which were separated on a paper chromatogram and estimated with hypoiodite (*loc. cit.*) (Found: 0·09 g. and 0·08 g. respectively).

Fraction (o_3). The syrup had $[\alpha]_D^{17}$ +113·5° (*c*, 0·1 in H_2O). Treatment of the syrup (0·05 g.) with aniline (0·02 g.) in ethanol yielded 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine (0·06 g.), (recrystallised from absolute alcohol), m. p. and mixed m. p. 193—195°; (o_3) gave one discrete spot on the paper chromatogram travelling at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methylgalactose.

Fraction (o_4). This (0·28 g.) was a mixture of the two sugars found in fraction (*d*).

Fraction (p). Paper chromatography showed this to be a mixture of the same two sugars as were found in fraction (*e*), with 2 : 3-di-*O*-methylxylose as the major component. The syrup was inoculated with a crystal of 2 : 3-di-*O*-methylxylose (Chanda, Percival, and Percival, *J.*, 1952, 260) and after 7 months at 0° had partly crystallised. The crystals were removed, drained on a tile, and quickly washed with light petroleum, to give a white powder (0·024 g.), m. p. and mixed m. p. 79—80°, $[\alpha]_D^{16}$ +61° (7 min.), +31° (50 min.), +24° (3 hr.), and +22·6° (18 hr., constant) (*c*, 0·6 in H_2O).

Fraction (q). This crystallised and was treated as fraction (*f*), to give 2 : 4-di-*O*-methyl-β-D-xylose, m. p. and mixed m. p. 111°, $[\alpha]_D^{17}$ −26° (10 min.), +11° (50 min.), and +22° (2 hr., constant). It gave one discrete spot on the paper chromatogram, travelling at the same rate as 2 : 4-di-*O*-methylxylose.

Fraction (r). The syrup on a paper chromatogram gave a brown colour with aniline oxalate and had R_G *ca.* 0·58. It had $[\alpha]_D^{18}$ +78° (*c*, 0·4 in H_2O).

Fractions (s) *and* (t). These were syrupy mixtures of 2-*O*-methylxylose and two sugars of R_G 0·58 and 0·50, which both gave a brown colour with aniline oxalate.

Fraction (u). This syrup was obtained from the first 100 tubes of the monomethylxylose fraction eluted from the cellulose column. It had $[\alpha]_D^{16}$ +34° and no evidence of the presence of any 3-*O*-methylxylose was obtained.

Fraction (v). This crystallised and the crude material had $[\alpha]_D^{18}$ +9° (5 min.), and +31° (24 hr., constant). After recrystallisation from *n*-butanol the crystals had m. p. 136°, not depressed on admixture with 2-*O*-methyl-D-xylose, $[\alpha]_D^{17}$ −18° (2 min.), +14° (16 min.), +29° (1 hr.), and +36° (2 hr., constant) (*c*, 1·4 in H_2O).

Fraction (w). This syrup had R_G 0·24 and gave a pink colour with aniline oxalate [cf. demethylation products of (*e*) and (*k*)].

Fraction (x). This was shown to be xylose by paper chromatography.

Fractions (*d*), (*i*), and (o_4) were combined to give a syrup (0·50 g.) which was fractionated on a column of cellulose as before, to give fractions: (i) 0·02 g.; (ii) 0·24 g.; (iii) 0·09 g.; (iv) 0·09 g. Recovery was 88%.

Fractions (*c*), (*h*), and (ii) all gave the same spot on the paper chromatogram and were combined to give fraction H.

Fraction H. The syrup had OMe 31·6%, and $[\alpha]_D^{16}$ −18° (*c*, 1·1 in H_2O). On the paper chromatogram one discrete spot was obtained (R_G 0·80), which gave a grey-black colour with aniline oxalate. The material H failed to form a crystalline anilide. It (0·1279 g.) was dissolved in methanolic hydrogen chloride (1%; 15 c.c.), and rotational changes were observed at room temperature and at 80°. At room temperature $[\alpha]_D^{16}$ were −15° (5 min.), −9° (15

min.), 0° (30 min.), +21° (80 min.), +28° (150 min.), +28° (4 hr.), +18° (5 hr.), +11° (6 hr.), and −59° (26 hr.). At 80°, $[\alpha]_D^{16}$ were −67° (50 min.), −71° (100 min.), and −74° (220 hr., constant); 4 c.c. of the above solution ($[\alpha]_D$ −74°) were neutralised with silver carbonate and evaporated, to give a non-reducing syrup which was methylated twice with the Purdie reagents. Hydrolysis of the methyl glycoside with nitric acid (2%; 10 c.c.) at 100° for 6 hr. yielded a syrup which on the paper chromatogram gave one dark brown spot (aniline oxalate) (R_G 0·96).

The syrup (7 mg.) was demethylated with 48% hydrobromic acid (*loc. cit.*). Examination on a paper chromatogram gave the following spots (aniline oxalate): (1) pink, R_G 0·12, which travelled at the same rate as an arabinose control; (2) pink, R_G 0·34; (3) faint brown, R_G 0·45; (4) dark grey, R_G 0·80, unchanged material.

Oxidation of fraction H (0·1 g.) with bromine in water for 6 days gave a syrupy lactone (0·085 g.), b. p. 120—130°/0·06 mm., OMe, 28·6%, $[\alpha]_D^{17}$ −51° (zero time), −50° (24 hr.), −44° (192 hr.), −39° (336 hr.), −36° (384 hr.), and −32° (480 hr., constant) (*c*, 0·8 in H_2O). The lactone (0·030 g.) was recovered from the polarimetric solution by evaporation, and redistillation and was treated with methanolic ammonia for 2 days at 0°. Removal of the solvent gave crystalline 2 : 5-di-*O*-methyl-L-arabonamide (25 mg.) which, recrystallised from alcohol, had m. p. 131—132° (Found: C, 43·0; H, 7·7; N, 6·9. Calc. for $C_7H_{15}O_5N$: C, 43·5; H, 7·7; N, 7·25%). A Weerman test on the pure amide was negative.

To the lactone (30 mg.) was added phenylhydrazine (18 mg.) in anhydrous ether (6 c.c.). The solution was heated at 40° for 30 min., the ether removed, and the semicrystalline mass heated at 95° for 30 min. The solid was triturated with ether and recrystallised from alcohol–ether, to give crystals (30 mg.), m. p. 166—167°, not depressed on admixture with an authentic sample of 2 : 5-di-*O*-methyl-L-arabinose phenylhydrazine derivative kindly supplied by Dr. J. K. N. Jones. *X*-Ray powder photographs of the two samples by Dr. C. A. Beevers were identical.

Oxidation of fraction H with periodic acid (Reeves, *loc. cit.*) gave no formaldehyde. Glucose under the same conditions gave a precipitate of formaldehyde–dimedon compound.

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