101. *Studies of Seed Mucilages. Part V. Examination of a Polysaccharide extracted from the Seeds of* Plantago ovata *Forsk by Hot Water.*

By R. A. Laidlaw and E. G. V. Percival.

Extraction of these seeds with hot water, after exhaustive extraction with cold water, yielded a polysaccharide fraction (PII, equiv., *ca.* 4000; uronic anhydride, *ca.* 3%; pentosan, *ca.* 90%; methylpentosan, nil). Hydrolysis of PII gave D-xylose (80%), L-arabinose (14%), D-galactose (a trace), an aldobiuronic acid (*ca.* 0·3%), and an insoluble residue (*ca.* 3%). Acetylation, deacetylation and methylation, and fractional precipitation gave products *A* (63%), *B* (32%), and C (5%). Methanolysis and hydrolysis of *A* gave trimethyl D-xylopyranose (8 parts), trimethyl L-arabofuranose (3 parts), tetramethyl D-galactopyranose (*ca.* 0·3%), a 2 : 6-dimethyl hexose (1 part), 2 : 4-dimethyl D-xylopyranose (4 parts), 3-methyl D-xylopyranose (8 parts), and D-xylopyranose (1 part).

In Part III (Laidlaw and Percival, *J.*, 1949, 1600) the polysaccharide obtained from *Plantago ovata* Forsk seeds by extraction with cold water (PI) was shown to be highly complex. The main constituents liberated on hydrolysis were D-xylose (46%), L-arabinose (7%), and 2-D-galacturonosido-L-rhamnose (40%), but after acetylation and methylation no uronic acid residues remained. Although it was possible to indicate the mode of linkage of the xylose and arabinose residues in the methylated polysaccharide, it was thought that at least two associated polysaccharides were present in the mucilage, a polyuronide and a neutral fraction. This indeed agreed with Anderson and Fireman's observation (*J. Biol. Chem.*, 1935, **109**, 437) that products containing variable quantities of uronic acid residues could be isolated depending on purely mechanical differences in the mode of extraction. To throw further light on the matter, we

have studied the properties of the polysaccharide (PII) obtained by extraction of the seeds with hot water after exhaustive extraction with cold water. The proportion of uronic acid residues in PII is much less than in PI, the equivalent by titration being *ca.* 4000 (cf. 700 for PI), and PII contains only 3% of uronic anhydride; the pentosan content is correspondingly higher (90%) and L-rhamnose is absent. The uronic anhydride is considered to arise from an associated polyuronide. Hydrolysis of PII gave D-xylose (80%), L-arabinose (14%), a trace of galactose, and a small amount (0·3%) of a substance which appeared to be the barium salt of an aldobiuronic acid with properties different from that isolated from PI (*loc. cit.*).

Acetylation and methylation gave a methylated polysaccharide which was fractionated to give products *A*, *B*, and *C* (see table, p. 531). Products *A* and *B*, which together amounted to 95% of the methylated mucilage, appeared to be essentially similar, apart from the lower specific viscosity, and presumably, molecular weight of *B*.

Product *A*, on methanolysis afforded a mixture of methylated sugar glycosides which were separated by solvent extraction (Brown and Jones, *J.*, 1947, 1344) to give the four fractions discussed below, *viz.*, I (47·8%), II (15·7%), III (32·2%), and IV (4·4%). Fraction I was a mixture of trimethyl methyl-D-xylopyranosides and -L-arabofuranosides similar to that described previously (*loc. cit.*): crystalline trimethyl D-xylopyranose, its anilide, trimethyl D-xylopyranolactone, and 2 : 3 : 5-trimethyl L-arabonamide were isolated. From a consideration of the lactone equilibria it was estimated that trimethyl L-arabofuranose constituted about 14% of the products of hydrolysis of the methylated mucilage. Fraction II on hydrolysis and separation on a column of powdered cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511) gave six fractions. Of these, fraction II(1) was identical with I; II(2) was tetramethyl D-galactopyranose; II(3) has not been identified although it appears to be a 2 : 6-dimethyl aldohexose; II(4) proved to be a mixture of 2 : 3-dimethyl and 2 : 4-dimethyl D-xylose. Complete methylation gave trimethyl D-xylopyranose as the sole product, and the mixed lactone obtained on oxidation, the equivalent weight of which by titration corresponded to a dimethyl pentonolactone, showed $[\alpha]_D^{17}$ $+30·8° \longrightarrow 45·2°$ (96 hours) corresponding to a mixture of 2 : 3-dimethyl D-xylofuranolactone (40%) and 2 : 4-dimethyl D-xylopyranolactone (60%). Oxidation of the mixed sodium salts with periodic acid (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1473) gave formaldehyde in amount corresponding to the presence of 38% of sodium 2 : 3-dimethyl xylonate, and the syrupy amide gave a negative Weerman test (*Rec. Trav. chim.*, 1917, **37**, 16). A crystalline anilide, m. p. 158°, obtained from fraction II(4) was identified as slightly impure 2 : 4-dimethyl D-xylose anilide.

Fraction II(5) crystallised completely to give 2 : 4-dimethyl β-D-xylose which was identified by the properties of its lactone, by the facts that the derived amide gave a negative Weerman test and yielded no formaldehyde on periodate oxidation, and by comparison with a standard on the paper chromatogram.

Fraction II(6) proved to be 3-methyl xylose.

Fraction II(3) has defied identification; a syrup having $[\alpha]_D$ −19°, it was readily oxidised by sodium hypoiodite and was thus an aldose. Oxidation gave a furanolactone {$[\alpha]_D$ $-50° \longrightarrow -33°$ (18 days)}, showing the hydroxyl group on $C_{(4)}$ to be free. The derived crystalline *amide*, which analysis showed to be a dimethylhexonamide, gave a negative Weerman test showing the presence of a methoxyl group on $C_{(2)}$. The R_G value (Hirst and Jones, *J.*, 1949, 928) of fraction II(3), 0·80, is higher than that for any dimethyl hexose recorded so far, but the brown colour formed with aniline oxalate was easily distinguishable from the pink colour given by pentose derivatives. Oxidation with periodic acid (Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456) gave no acetaldehyde which, since the hydroxyl on $C_{(4)}$ is free, excludes all (except 5-substituted) 6-deoxyhexoses, in any event an unlikely possibility. No formaldehyde was released on oxidation with periodate (Reeves, *loc. cit.*) and this, coupled with the knowledge that the hydroxyl group on $C_{(4)}$ is free, makes it probable that a methoxyl group resides on $C_{(6)}$. That fraction II(3) was an individual substance appeared likely from the definition of the spot on the paper chromatogram and the isolation of a crystalline amide; complete methylation and hydrolysis gave a syrup which again gave a single spot on the chromatogram, although this observation would not exclude the presence of a mixture of D- and L-forms. The R_G value observed (0·96) for the last-mentioned product appears to exclude tetramethyl galactopyranose (R_G 0·88); this is confirmed by the failure to isolate a tetramethyl hexose anilide which also seems to remove the possibility of the parent sugar being mannose. The examination of the original hydrolysed PII on the paper chromatogram seems to exclude idose and talose as possibilities. Gulose on the other hand has the same R_G value as arabinose and would, therefore, escape detection, but gulose has not, so far, been isolated from a natural product.

Fraction III on hydrolysis and fractionation on a cellulose column gave four fractions as follows : III(*a*) having R_G 0·7; III(*b*), III(*c*), and III(*d*), having R_G 0·39.

Fraction III(*a*) gave a syrupy lactone {$[\alpha]_D^{18}$ $+12° \longrightarrow +27°$ (50 hours)}, and the derived amide gave a negative Weerman reaction and a 20% yield of formaldehyde on periodate oxidation. Examination on the paper chromatogram showed III(*a*) to contain a mixture of 2 : 3-dimethyl and 2 : 4-dimethyl xylose, and from the above evidence the relative proportions were taken as 1 : 4. Faint traces of II(3) and of a monomethyl xylose were also present. Fraction III(*b*) crystallised to give 3-methyl D-xylose (80%), together with a syrup which, on oxidation, gave crystalline 3-methyl D-xylofuranolactone as the only product. Fraction III(*d*) gave the same crystalline lactone on suitable treatment, and III(*c*) gave crystalline 3-methyl xylose anilide. No 2-methyl xylose derivatives were detected, although they occurred in the products of hydrolysis of methylated PI (Part III, *loc. cit.*).

Fraction IV consisted almost entirely of methyl D-xylosides, chromatographic analysis of the hydrolysed syrup giving one spot only, corresponding to xylose, and the identity of D-xylose was confirmed as the crystalline dibenzylidene dimethylacetal (Breddy and Jones, *J.*, 1945, 738).

On the methanolysis of product *B* and separation by solvent extraction, five fractions were obtained which on qualitative examination by paper chromatography appeared closely similar to the fractions obtained from *A*, and no other individual sugars were present.

Allowing for the demethylation known to occur during methanolysis (Part III, *loc. cit.*) it was calculated that product *A* on hydrolysis gave trimethyl D-xylopyranose (8 parts), trimethyl L-arabofuranose (3 parts), tetramethyl D-galactopyranose (*ca.* 0·3%), 2 : 6-dimethyl hexose (1 part), 2 : 3-dimethyl D-xylopyranose (1 part), 2 : 4-dimethyl D-xylose (4 parts), 3-methyl D-xylopyranose (8 parts), and D-xylopyranose (1 part). Thus, in the polysaccharide the following units must be present, linked as shown :

$$\text{X1; A1; G1; 3H4 (or 3H5); X3; X4; 4X2; 4X2}\ {}^{1}_{3}$$

{X = D-xylopyranose; G = D-galactopyranose;
A = L-arabofuranose; H = hexose.}

No information is available at present to show how these units are related in the mucilage molecule. Thus, no definite structural formula can be assigned to the PII repeating unit, but the formula indicated below, one of many possible variants, would explain the properties of the methylated polysaccharide.

$$\begin{array}{cccccccccccccc}
 & & X & & & & & & & & & & & \\
 & & 1 & & & & & & & & & & & \\
 & & 2 & & & & & & & & & & & \\
X1 & & 4X & X & & X & & X & & A & & A & A & X \\
 & & 1 & 1 & & 1 & & 1 & & 1 & & 1 & 1 & 1 \\
 & & 3 & 4 & & 4 & & 4 & & 4 & & 4 & 4 & 4 \\
\ldots & 1X2 & 1H3 & 1X3 & 1X2 & 1X3 & 1X2 & 1X4 & 1X2 & 1X3 & 1X2 & 1X3 & 1X2 & 1X2 & \ldots \\
 & & 4 & & & & & & & & & & & \\
 & & 1 & & & & & & & & & & & \\
X1 & & 2X & & & & & & & & & & & \\
 & & 4 & & & & & & & & & & & \\
 & & 1 & & & & & & & & & & & \\
 & & X & & & & & & & & & & &
\end{array}$$

X = D-xylopyranose; A = L-arabofuranose.

The galactose end-groups have been neglected in this formula, but it may be that in the mucilage molecule we have 13—14 such repeating units linked together and terminated by a D-galactopyranose residue.

Such a repeating unit should yield on oxidation with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427) 8 moles of formic acid, from the xylopyranose end-groups, *i.e.*, 0·00225 mol./g. The value found in practice for PII was 0·00234 mol./g., in good agreement with the theoretical figure. Further, a molecule of this constitution would use 20 moles of periodate or 0·00563 mol./g. The experimental figure (0·00535 mol./g. after 15 minutes when the initial rapid uptake appears to be complete) is in reasonable agreement. The higher figure (0·00674 mol./g.) obtained after 3 days may possibly be due to over-oxidation.

We may now compare the results from PII with those from PI. Methylated PI on hydrolysis yielded 2 : 3 : 4-trimethyl D-xylose (6 parts), 2 : 3 : 5-trimethyl L-arabinose (1 part), 2 : 4-dimethyl D-xylose (1 part), 3-methyl D-xylopyranose (4 parts), 2-methyl D-xylopyranose (1 part), and D-xylopyranose (2 parts). Thus, though we have in each case the same type of highly-branched structure considerable differences in detail are evident. Approximately the same

proportion of end-groups is present in each case, but in PII the amount of arabinose derivatives is doubled at the expense of the xylose derivatives. Again, PII appears to contain a much higher proportion of doubly-linked xylose units; 1 : 4-linked D-xylose, present in PII, could not be identified in PI, though it was found in the mucilage from *P. lanceolata* (Percival and Willox, Part IV, *J.*, 1949, 1607). Further, neither 2-methyl xylose could be obtained from methylated PII, and the proportion of D-xylose units linked through four positions was much less than in PI. Finally, no methylated derivatives of D-galactose, nor the unknown hexose, obtained from PII, were isolated from PI. Thus, PI and PII are to be regarded as distinct polysaccharides; it may well be that each of these is in itself a mixture of closely-related polysaccharides, but there is no evidence that they could be mixtures of relatively simple xylans and arabans constituted from single-sugar units. The relatively high yield of L-arabinose obtained on the hydrolysis of PII, its correspondence with the yield of trimethyl methylarabofuranosides obtained on methanolysis of the methylated polysaccharide, and the failure to detect any partly methylated L-arabinose derivatives in the hydrolysis of either methylated PI or methylated PII prove that the arabinose units occur as end groups as, for example, in gum arabic (Smith, *J.*, 1940, 1035) and are not derived from an associated araban.

Experimental.

Evaporations were conducted under diminished pressure unless otherwise stated. Temperatures are bath-temperatures. Fractions from the cellulose column were evaporated to dryness, dissolved in water, digested with charcoal, and filtered hot; the aqueous solution was then evaporated to dryness and exhaustively extracted with boiling acetone, and the extracts were evaporated to dryness.

Preparation of PII.—Product PI was removed from the seeds by extraction (Part III, *loc. cit.*), and the seeds were washed continuously with cold water until no more mucilage was removed. The residue was then extracted with hot water (90—95°), and the highly viscous solution separated by filtration through muslin. The filtrate, on cooling, set to a gel from which the mucilage was precipitated by the addition of acidified ethanol (4 volumes; 20 c.c. of concentrated hydrochloric acid per l.). The fibrous product was dried with ethanol and ether and in a vacuum over phosphoric oxide (Found: equiv., by titration, *ca.* 4000; uronic acid, *ca.* 3%; pentosan, 90%; methylpentosan, nil; ash, 1·41%, unchanged on treatment with sulphuric acid). Precipitation with ethanol alone yielded a product with 1·46% of ash (as sulphate, 1·83%).

Hydrolysis of PII with Oxalic Acid.—The dry mucilage (1·502 g.) was hydrolysed with oxalic acid (3%; 75 c.c.) at 100°. $[\alpha]_D^{16}$ was −11° (1 hour); +12·5° (2 hours); +25° (3 hours); +38° (6 hours); +40° (52 hours, constant). A larger quantity (46·92 g.) was hydrolysed as above for 6 hours. The insoluble residue (1·43 g.) was removed and the filtrate neutralised with barium carbonate, filtered, and evaporated to 200 c.c. This solution was filtered into ethanol (2 l.) to give a white barium salt (X) (0·19 g.). Evaporation of the alcoholic solution yielded a reducing syrup (S) (43·5 g.). The salt X showed Ba, 15·4% and $[\alpha]_D^{17}$ +39° in water (*c*, 1·0). Syrup S partly crystallised. Trituration of the mixture (22·47 g.) with glacial acetic acid gave D-xylose (7·02 g.) which, on recrystallisation from ethanol showed m. p. and mixed m. p. 140° and $[\alpha]_D^{17}$ +80° falling to +20° after 4 hours in water and yielded xylosazone, m. p. and mixed m. p. 157°. Evaporation of the acetic acid washings gave a syrup (S′) (15·46 g.), a portion of which was treated with phenylmethylhydrazine. No precipitate of galactose phenylmethylhydrazone was obtained.

Syrup S′, on treatment with diphenylhydrazine, yielded L-arabinose diphenylhydrazone, m. p. 194° not depressed on admixture with an authentic specimen, in an amount corresponding to the presence of 14·2% of anhydroarabinose in the mucilage.

Paper chromatographic investigations of S′ indicated the presence of xylose and arabinose along with a trace of galactose.

Acetylation.—The product PII (45 g.) was moistened with ethanol and dispersed with shaking in pyridine (750 c.c.), and acetic anhydride (500 c.c.) was added slowly with continuous stirring. The mixture was then heated at 100° for 3 hours and left at room temperature for a further 48 hours, whereafter the acetate was precipitated by pouring the mixture into water (10 l.) and washed thoroughly in running water till free from pyridine. Yield, 60 g. (Found: $CH_3{\cdot}CO$, 30·9%). The properties of this material were unchanged on reacetylation.

Methylation.—The acetate was methylated directly in three 20-g. portions with methyl sulphate and sodium hydroxide as described in Part III (*loc. cit.*), each portion receiving four methylations. The products were combined and dissolved in chloroform, the solution was dried (Na_2SO_4), and the methylated polysaccharide fractionally precipitated by the addition of light petroleum (b. p. 40—60°), to give the fractions listed in the table.

Fraction.	Yield, g.	OMe, %.	$[\alpha]_D^{16}$ ($CHCl_3$).	$\eta_{sp.}^{20}/c'$.*
A	20·0	35·8	−121°	55·5
B	10·0	35·8	−120	19·3
C	1·6	27·1	−112	very low

* c' is the concentration in g.-mols. of methylated anhydroxylose residues per l.

Fraction *C* was obtained by evaporation of the residual solution almost to dryness and subsequent trituration with light petroleum. The properties of *A* and *B* were unchanged on further methylation. Neither *A* nor *B*, nor *C* contained any appreciable amount of uronic acid.

Hydrolysis of the Methylated Polysaccharide.—Fraction *A* (18·7 g.) was hydrolysed with methanolic hydrogen chloride (3%; 350 c.c.) for 21 hours at 80°. After neutralisation with silver carbonate, filtration, and evaporation, a non-reducing syrup (21·1 g.) was obtained.

Fractionation by Solvent Extraction.—The above syrup (20·4 g.) was dissolved in water (50 c.c.) and extracted with light petroleum (b. p. 38—40°) for 16 hours to give fraction I (9·725 g.), η_D^{17} 1·4423. Extraction with the same solvent for a further 210 hours gave fraction II (3·207 g.), η_D^{15} 1·4577. The residual aqueous solution was then extracted with chloroform for 20 hours to yield fraction III (6·571 g.), η_D^{16} 1·4722. Evaporation of the residue gave fraction IV (0·888 g.), η_D^{17} 1·4840. Recovery was 100%.

Hydrolysis and Fractionation of Fraction B.—Fraction B (9·1 g.) on methanolysis as above yielded a syrup (10·1 g.). Separation of the glycosides was again effected by solvent extraction. Thus, the syrup (8·95 g.) yielded, with light petroleum, fractions V (14 hours) (3·944 g.), η_D^{19} 1·4413, VI (+ 7 hours) (0·201 g.), η_D^{16} 1·4545, and VII (+90 hours) (0·745 g.), η_D^{12} 1·4580. Chloroform gave fraction VIII (17 hours) (2·655 g.), η_D^{16} 1·4675, and evaporation of the aqueous residue furnished fraction IX (1·375 g.), η_D^{19} 1·4683. Recovery was 8·920 g. (99·7%).

Examination of Fraction I.—Fraction I (9·04 g.) was hydrolysed with nitric acid (2%; 150 c.c.) at 100°. $[\alpha]_D^{17}$ were +19° (1 hour); +7° (3 hours); +4° (6 hours, constant). Neutralisation with barium carbonate, filtration, and evaporation of the filtrate left a residue which was exhaustively extracted with boiling acetone. The acetone extracts were combined and evaporated to yield a reducing syrup (6·97 g.). Several crops (total, 2·83 g.) of crystalline material were removed during a prolonged period, which on recrystallisation from ether gave trimethyl α-D-xylopyranose, m. p. and mixed m. p. 92°, $[\alpha]_D^{17}$ +63° (2 minutes), +33° (30 minutes), +19° (2 hours, constant) (*c*, 1·43 in water) (Found: C, 50·2; H, 8·1; OMe, 47·9. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·4; OMe, 48·4%). With alcoholic aniline the characteristic trimethyl D-xylose anilide was obtained (Part III, *loc. cit.*), m. p. and mixed m. p. 98° and $[\alpha]_D^{17}$ −84° (4 minutes), −70° (45 minutes), −44° (90 minutes), +34° (345 minutes), +47° (24 hours, constant) (*c*, 0·32 in ethanol). The residual syrup (3·48 g.) was examined on the paper chromatogram which indicated the presence of trimethyl xylose and/or trimethyl arabinose, along with traces of other components.

Attempted Separation on a Column of Cellulose.—The residual syrup (cf. preceding paragraph) (3·40 g.) was fractionated on a column of cellulose (15″ × 1·2″) (Hough, Jones, and Wadman, *loc. cit.*), the eluent being light petroleum (b. p. 100—120°)–butanol (7 : 3) saturated with water. Four main fractions were collected: (i) 1·00 g., $[\alpha]_D^{17}$ −10° (*c* 2·67 in water); (ii) 1·13 g., $[\alpha]_D^{17}$ −11° (*c*, 3·25 in water); (iii) 0·55 g., $[\alpha]_D^{17}$ −5° (*c*, 1·57 in water) (these three gave spots on the paper chromatogram corresponding to trimethyl xylose and/or trimethyl arabinose); (iv) 0·27 g., obviously composed of less fully methylated materials. Recovery was 2·95 g. (87%). Syrups (i), (ii), and (iii) were recombined (2·30 g.), dissolved in water, and oxidised at room temperature with bromine till non-reducing (4 days). After treatment with silver carbonate and hydrogen sulphide, evaporation gave a syrupy product which was fractionally distilled to give (*a*) 1·06 g., b. p. 95—105°/0·01 mm., η_D^{18} 1·4496 (Found: OMe, 48·4. Calc. for $C_8H_{14}O_5$: OMe, 48·9%), and (*b*) 0·82 g., b. p. 105—125°, η_D^{18} 1·4551 (Found: OMe, 47·6%). Portion (*a*) showed $[\alpha]_D^{17}$ −25° (5 minutes), −24° (1 hour), −21° (31 hours), −14° (100 hours), −11° (200 hours), −10° (318 hours, constant) (*c*, 3·14 in water). Portion (*a*) (62·8 mg.) required 5·50 c.c. of 0·0565N-sodium hydroxide for neutralisation to phenolphthalein (Calc. for $C_8H_{14}O_5$: 5·84 c.c.) and on treatment with methanolic ammonia yielded crystalline 2 : 3 : 5-trimethyl L-arabonamide. On recrystallisation (ethyl acetate) this showed m. p. and mixed m. p. 136° and $[\alpha]_D^{17}$ +21° (*c*, 1·41 in water) (Found: C, 46·0; H, 8·05; N, 6·95; OMe, 43·7. Calc. for $C_8H_{17}O_5N$: C, 46·3; H, 8·3; N, 6·8; OMe, 44·9%). Portion (*b*) showed $[\alpha]_D^{17}$ −9° (5 minutes), −8° (4 hours), −4° (29 hours), +3° (100 hours), +6° (200 hours, constant) (*c*, 3·451 in water); 34·5 mg. required 2·95 c.c. of 0·0565N-sodium hydroxide for neutralisation (Calc. for $C_8H_{14}O_5$: 3·21 c.c.). It gave a syrupy amide, $[\alpha]_D^{16}$ +35° (*c*, 2·94 in water). Portion (*b*) partly crystallised when kept, to yield 2 : 3 : 4-trimethyl xylopyranolactone, m. p. and mixed m. p. 50° (Found: C, 50·6; H, 7·5; OMe, 48·1. Calc. for $C_8H_{14}O_5$: C, 50·6; H, 7·4; OMe, 48·9%).

Examination of Fraction II.—Fraction II (3·20 g.) was hydrolysed with nitric acid (2%; 100 c.c.) at 100°: $[\alpha]_D^{17}$ +28° (1 hour); +22° (2 hours); +16° (4 hours); +14° (6 hours, constant). Appropriate treatment gave a reducing syrup (2·65 g.). This syrup (2·5 g.) was combined with column-fraction (iv) from fraction I above, and fractionation effected on a column of cellulose (27″ × 1·2″) as before. This gave: (1) 0·11 g., R_G 0·94, (2) 0·065 g. (0·88), (3) 0·56 g. (0·80), (4) 0·69 g. (0·74), and (5) 0·59 g. (0·66); a further fraction, (6) 0·14 g. (0·39), was obtained by washing the column with water. Recovery was 2·16 g. (76·4%).

Fraction (1). This corresponded to fraction I.

Fraction (2). On treatment with alcoholic aniline this yielded 2 : 3 : 4 : 6-tetramethyl D-galactose anilide, m. p. 189°, not depressed on admixture with an authentic specimen (Found: OMe, 39·1. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%).

Fraction (3). The syrup showed OMe, 33·6% and $[\alpha]_D^{17}$ −19° (*c* 1·35 in water). On the paper chromatogram it gave a brown spot when sprayed with aqueous aniline oxalate and heated. Oxidation was easily effected with alkaline hypoiodite under standard conditions (Found: *M*, 180. Calc. for $C_8H_{16}O_6$: *M*, 208). A test oxidation on D-glucose gave *M*, 180 (Calc. for $C_6H_{12}O_6$: *M*, 180). Fraction (3) failed to form a crystalline anilide.

The syrup (3) (0·118) was kept at 80° with 1% methanolic hydrogen chloride. $[\alpha]_D^{17}$ was −75° (40 minutes) and −82° (230 minutes, constant).

Methylation.—Neutralisation with silver carbonate, filtration, and evaporation yielded a non-reducing syrup which was methylated **3** times with the Purdie reagents, and the highly mobile syrupy product distilled at 80°/0·01 mm. The distillate (43 mg.; OMe, 58·1%) was hydrolysed with nitric acid (2%; 10 c.c.) at 100°. $[\alpha]_D^{17}$ was −64° (zero time), −41° (50 minutes), −34° (2 hours, constant) (Calc. as methylglycosides). Appropriate treatment gave the free sugar as a syrup (20 mg.) which failed to crystallise. On investigation by means of the paper chromatogram this showed one spot only at R_G 0·96. No crystalline anilide could be obtained.

Lactone Formation.—Fraction (3) (0·11 g.) was converted into the lactone. The product (70 mg.)

did not crystallise (Found: OMe, 32·8%). $[\alpha]_D^{18}$ was −50° (10 minutes), −46° (95 hours), −36° (263 hours), −33° (432 hours, constant) (*c*, 0·606 in water). The lactone (9·1 mg.) required 2·08 c.c. of 0·0249N-sodium hydroxide for neutralisation (Calc. for $C_8H_{16}O_5$: 1·80 c.c.).

Amide Formation.—The above lactone was reclaimed from polarimetric solution by evaporation and redistillation. A portion of this material (50 mg.) was converted into the amide. A white crystalline solid was obtained, which on recrystallisation (ethyl acetate) had m. p. 131—132° and gave a negative Weerman test (Found: C, 42·9; H, 7·7; N, 6·8; OMe, 30·0. Calc. for $C_8H_{17}O_6N$: C, 43·0; H, 7·7; N, 6·3; OMe, 27·8%).

Periodate Oxidations.—The free sugar (15·2 mg.) was oxidised according to the method of Nicolet and Shinn (*loc. cit.*). No acetaldehyde was detected, whereas L-rhamnose (16·0 mg.) gave acetaldehyde in 90% yield.

Fraction (3) (19·0 mg.) was oxidised according to the method of Reeves (*loc. cit.*), but no formaldehyde was produced. A sample of D-xylose (17·0 mg.) under the same conditions yielded the formaldehyde–dimedon compound (12·5 mg.), m. p. and mixed m. p. 187°.

Fraction (4).—This was a syrup which showed $[\alpha]_D^{18}$ +16° (*c*, 3·85 in water) and OMe, 33·7%. On the paper chromatogram a pink colour was obtained on spraying of the spot with aniline oxalate and heating.

Anilide Formation.—Treatment of the syrupy fraction (4) (20 mg.) with alcoholic aniline gave a crystalline product, which, on recrystallisation (acetone–ether–light petroleum) had m. p. 158°, mixed m. p. with 2 : 3-dimethyl D-xylose anilide, 128° (Found: OMe, 28·0. Calc. for $C_{13}H_{19}O_4N$: OMe, 24·5%).

Lactone Formation.—Lactonisation of the syrup (0·3 g.) and distillation gave a syrupy product (0·21 g.) which showed $[\alpha]_D^{17}$ +30·8° (7 minutes); +38·4° (20 hours); +44·2° (48 hours); +45·2° (96 hours, constant) (*c*, 1·04 in water). A portion of the lactone was twice redistilled, but no significant difference in rotation figures was observed (Found: OMe, 33·6. Calc. for $C_7H_{12}O_5$: OMe, 35·3%). The lactone (20·8 mg.) required 4·80 c.c. of 0·0249N-sodium hydroxide for neutralisation (calc. for $C_7H_{12}O_5$: 4·75 c.c.). The lactone (41·0 mg.) was heated with sodium hydroxide (1N.; 0·5 c.c.) at 50° for 15 minutes. Water (1·5 c.c.) was added and the solution treated with periodic acid (Reeves, *loc. cit.*). This treatment gave the formaldehyde–dimedon compound (26·0 mg.), m. p. and mixed m. p. 188°. Treatment of the lactone with methanolic ammonia gave a syrupy amide, $[\alpha]_D^{17}$ +43° (*c*, 0·63 in water), which showed a negative Weerman test.

Methylation.—Fraction (4) (85 mg.) was treated with methanolic hydrogen chloride (3%; 20 c.c.) at 80° for 15 hours, to give a non-reducing syrup which was methylated 3 times with silver oxide and methyl iodide. The product on distillation at 80—100°/0·01 mm. was a highly mobile syrup (29 mg.) (Found: OMe, 58·4%). Hydrolysis was effected with nitric acid (2%; 7 c.c.) at 100°. $[\alpha]_D^{18}$ was +41° (zero time); +31° (1 hour); +15° (3 hours, constant). Appropriate treatment gave a syrup (17 mg.) identified on the paper chromatogram as trimethyl xylopyranose.

Fraction (5).—This crystallised completely. Trituration with acetone–light petroleum (1 : 3) gave 2 : 4-dimethyl β-D-xylose, m. p. 111°, not depressed on admixture with an authentic specimen. $[\alpha]_D^{19}$ was −13° (7 minutes); −1·5° (17 minutes); +9° (31 minutes); +19° (64 minutes); +21·5° (3 hours); +23° (24 hours, constant) (*c*, 2·1 in water) (Found: C, 47·3; H, 7·75; OMe, 34·7. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 34·8%). Lactonisation of this material gave syrupy 2 : 4-dimethyl xylonolactone, $[\alpha]_D^{17}$ −12° (15 minutes); −9° (2¼ hours); +15° (22 hours); +26° (46 hours); +27° (120 hours, constant) (*c*, 1·90 in water) (Barker, Hirst, and Jones, *loc. cit.*). Treatment with methanolic ammonia gave the syrupy amide, which showed a negative Weerman test, and on oxidation with periodic acid gave no formaldehyde.

Examination of Fraction (6).—Paper chromatography indicated the presence of 3-methyl xylose only, by direct comparison with a control. $[\alpha]_D^{19}$ +23° (*c*, 1·52 in water) was recorded.

Examination of Fraction III.—Fraction III (6·56 g.) was hydrolysed with nitric acid (2%; 150 c.c.) at 100°. $[\alpha]_D^{18}$ was +39° (1 hour); +24° (3 hours); +19° (6 hours, constant). This yielded a reducing syrup (4·92 g.) (Found: OMe, 18·2%). A fractionation was effected by means of a column of cellulose (27″ × 1·2″) with butanol (60%)–light petroleum (40%) saturated with water as eluent, to give four main fractions: (*a*) 0·95 g., (*b*) 2·26 g., (*c*) 0·44 g., and (*d*) 0·60 g. Recovery was 4·25 g. (92%).

Fraction (*a*), on examination by the paper chromatogram, was shown to consist mainly of 2 : 4-dimethyl xylose, with some 2 : 3-dimethyl xylose and a trace of the 2 : 6-dimethyl hexose component, while fractions (*b*), (*c*), and (*d*) showed the presence of monomethyl xyloses and/or arabinoses only.

Fraction (*a*) (0·80 g.) was oxidised, and distillation at 140—160°/0·05 mm. gave a syrupy lactone (0·53 g.) (Found: OMe, 33·4. Calc. for $C_7H_{12}O_5$: OMe, 34·8%), $[\alpha]_D^{18}$ +12° (5 minutes); +13·5° (1 hour); +15° (5 hours); +24° (24 hours); +27° (50 hours, constant) (*c*, 1·265 in water). The lactone (12·65 mg.) required 2·98 c.c. of 0·0249N-sodium hydroxide for neutralisation (Calc. for $C_7H_{12}O_5$: 2·89 c.c.). Treatment with methanolic ammonia yielded a syrupy amide which gave a negative Weerman test. Oxidation (of 41 mg.) with periodic acid gave formaldehyde as the dimedon compound (13 mg.), m. p. and mixed m. p. 187°.

Fraction (*b*) partly crystallised when kept. The crystalline material (1·50 g.) was separated by drainage on a tile and showed $[\alpha]_D^{17}$ +45° (5 minutes); +32° (17 minutes); +23° (50 minutes); +19° (19 minutes, constant) (*c*, 1·58 in water). Recrystallisation (absolute ethanol) gave 3-methyl D-xylopyranose as hygroscopic white needles, m. p. 95°, not depressed on admixture with an authentic specimen (Found: C, 42·7; H, 7·4; OMe, 21·0. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4; OMe, 18·9%). Exhaustive extraction, with boiling acetone, of the tile used in separating the crystalline component gave a syrup (0·40 g.), $[\alpha]_D^{17}$ +25° (*c*, 4·0 in water), which was oxidised to the lactone. Distillation at 160—170°/0·025 mm. gave 3-methyl γ-D-xylonolactone (0·20 g.) as a syrup which rapidly crystallised. On recrystallisation (ethyl acetate–light petroleum), this had m. p. 90°, not depressed on admixture with an authentic specimen; $[\alpha]_D^{17}$ +72° (zero time); +45° (245 hours); +40° (600 hours, constant) (*c*, 0·88 in water) (Found: OMe, 18·0. Calc. for $C_6H_{10}O_5$: OMe, 19·1%).

Fraction (*c*) (0·22 g.), on treatment with alcoholic aniline, gave crystalline 3-methyl D-xylose anilide as the sole product, which on recrystallisation from ethyl acetate had m. p. and mixed m. p. 137° (Found:

C, 59·9; H, 7·1; N, 5·85; OMe, 12·5. Calc. for $C_{12}H_{17}O_4N$: C, 60·2; H, 7·2; N, 5·9; OMe, 12·9%).

Fraction (*d*) (0·40 g.) was oxidised to the lactone. The product was crystalline 3-methyl γ-D-xylonolactone, m. p. and mixed m. p. 94°, $[\alpha]_D^{17}$ +73° (5 minutes); +67° (6 hours); +63° (27 hours); +54° (120 hours); +48° (246 hours); +39° (510 hours, constant) (*c*, 0·685 in water). The lactone (8·22 mg.) required 2·20 c.c. of 0·0249N-sodium hydroxide for neutralisation (Calc. for $C_6H_{10}O_5$: 2·04 c.c.) (Found: C, 44·0; H, 6·47; OMe, 17·5. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2; OMe, 19·1%).

Examination of Fraction IV.—Fraction IV (0·88 g.) was hydrolysed with nitric acid (2%; 50 c.c.) at 100°. $[\alpha]_D^{18}$ was +41·5° (zero time); +32° (1 hour); +18° (3 hours); +15° (5 hours, constant). The solution was neutralised with barium carbonate, filtered, evaporated to dryness, and exhaustively extracted with boiling absolute methanol. The extracts were combined, evaporated to small volume, refiltered, and evaporated to dryness, to give a reducing syrup (0·70 g.) (Found: OMe, nil). Examination by means of the paper chromatogram gave xylose only, and the presence of D-xylose was confirmed by the isolation of the crystalline dimethyl acetal, m. p. and mixed m. p. 206°, of dibenzylidene D-xylose.

Examination of Fraction V.—Fraction V (3·93 g.) was hydrolysed with nitric acid as before. $[\alpha]_D^{17}$ was +18° (70 minutes); +7° (3 hours); +5° (6 hours, constant). This gave a reducing syrup (3·00 g.) which partly crystallised to give trimethyl α-D-xylopyranose (1·50 g.), m. p. and mixed m. p. 89°. The residual syrup (1·08 g.) on the paper chromatogram indicated trimethyl xylose and/or trimethyl arabinose along with small amounts of components of fraction VI.

Examination of Fraction VI.—Fraction VI (0·20 g.) was hydrolysed with nitric acid. $[\alpha]_D^{17}$ was +24° (1 hour); +18·5° (3 hours); +16° (5 hours, constant). This gave a reducing syrup (0·15 g.), which on the paper chromatogram indicated presence of trimethyl xylose and/or trimethyl arabinose (trace), tetramethyl galactose (trace), 2 : 6-dimethyl hexose, 2 : 3-dimethyl xylose, 2 : 4-dimethyl xylose, and 3-methyl xylose (trace).

Examination of Fraction VII.—Fraction VII (0·74 g.) was hydrolysed with nitric acid. $[\alpha]_D^{17}$ was +24° (1 hour); +17° (2 hours); +14° (4 hours); +13° (6 hours, constant). The syrup (0·54 g.) obtained, on investigation by means of the paper chromatogram, was found to contain the same components as fraction II.

Examination of Fractions VIII and IX.—These syrups were combined and the product (4·01 g.) hydrolysed with nitric acid. $[\alpha]_D^{18}$ was +34° (1 hour); +22° (3 hours); +18° (6 hours, constant). Appropriate treatment gave a syrup (3·00 g.) which, on investigation by the paper chromatogram, disclosed xylose, 3-methyl xylose, and smaller amounts of 2 : 4-dimethyl xylose, 2 : 3-dimethyl xylose, and 2 : 6-dimethyl hexose.

Formic Acid from PII.—The polysaccharide was oxidised with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427), and the formic acid liberated was determined by titration with 0·01N-sodium hydroxide with the following results, expressed in mols. of formic acid/g.: 0·00129 (1 day); 0·00182 (3 days); 0·00217 (6 days); 0·00234 (10 days, constant).

Periodate Uptake of PII.—Product PII (*ca.* 0·4 g.) was dissolved in water (50 c.c.), and sodium metaperiodate (0·4M.; 25 c.c.) added. Portions (5 c.c.) were withdrawn at intervals, and the periodate content was determined by the arsenite method, with the following results, expressed in mols. of periodate consumed per g.: 0·00312 (10 minutes); 0·00535 (15 minutes); 0·00638 (1 day); 0·00674 (3 days, constant).

Thanks are expressed to the University of Edinburgh for a Post-graduate Fellowship (R. A. L.), and to Imperial Chemical Industries Limited and the Earl of Moray Endowment for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, November 9th*, 1949.]