345. *Studies on Seed Mucilages. Part III. Examination of a Polysaccharide extracted from the Seeds of* Plantago ovata *Forsk.*

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Extraction of the seeds with cold water yielded a polysaccharide (PI) of equivalent *ca.* 700, giving on analysis uronic anhydride (20%), pentosan (52%), and methylpentosan (18%). Further extraction with hot water gave PII, equivalent *ca.* 4,000, containing *ca.* 3% of uronic anhydride.

Hydrolysis of PI gave D-xylose (*ca.* 46%), L-arabinose (*ca.* 7%), and an aldobiuronic acid (40%) shown to be 2-D-galacturonosido-L-rhamnose, and an insoluble residue (2%). From PII, L-arabinose (*ca.* 14%) and D-xylose (*ca.* 80%) were isolated, along with an insoluble residue (3%).

Acetylation, followed by deactylation and methylation of PI and subsequent fractional precipitation, gave materials *A* (82%) and *B* (18%). Methanolysis of *A* yielded trimethyl methyl-D-xylopyranosides (6 parts), trimethyl methyl-L-arabofuranosides (1 part), 2 : 4-dimethyl methyl-D-xylopyranosides (1 part), 3-methyl methyl-D-xylopyranosides (4 parts), 2-methyl methyl-D-xylopyranosides (1 part), and methyl-D-xylopyranosides (2 parts). *B* gave similar fractions, except that double the proportion of 2 : 4-dimethyl methyl-D-xylosides was present at the expense of monomethyl methyl-D-xylosides.

The aldobiuronic acid is probably a component of a polyuronide associated with a neutral polysaccharide rather than an integral part of a single acidic polysaccharide.

Anderson and Fireman (*J. Biol. Chem.*, 1935, **109**, 437) studied the mucilage extracted from the so-called light " Psyllium " seeds and suggested that it might be represented as a chain of D-xylose units, varying in number between 7 and 36, to which was attached an aldobiuronic acid formed by the union of L-arabinose and D-galacturonic acid.

In Part II of this series (*J.*, 1942, 58) the results of an investigation on the mucilage of dark " Psyllium " seeds (*Plantago arenaria*) were discussed. Hydrolysis of this mucilage gave D-xylose, L-arabinose, D-galactose, and D-galacturonic acid. It was evident that the polysaccharide isolated from *P. arenaria* differed in composition from that described by Anderson and Fireman (*loc. cit.*) and, since light " Psyllium " or ispaghula has been identified with *Plantago ovata* Forsk (Skryme, *Quart. J. Pharm.*, 1935, **8**, 161), it was decided to investigate the mucilages of this species, especially in view of the introduction of improved methods for the separation of mixtures of methylated sugars.

Anderson and Fireman (*loc. cit.*) pointed out that the composition of their extract varied with the conditions of extraction. Similar variations were found in our experiments; extraction with cold water gave a product (PI) having a higher uronic acid content and a lower pentosan content than the polysaccharide (PII) isolated by extracting the residue at 90—95°. The polysaccharide obtained by extraction with cold water and precipitation in acidified alcohol (PI) had an apparent equivalent weight of *ca.* 700, giving on analysis uronic anhydride (20%), pentosan (52%), and methyl pentosan (18%), whereas the fraction isolated from the residual seeds by water at 90—95° (PII) had an apparent equivalent weight of *ca.* 4000, and contained uronic anhydride (*ca.* 3%) and pentosan (*ca.* 90%).

The mucilages examined were thus very probably mixtures of at least two distinct polysaccharides : one, readily soluble in water, containing a high proportion of uronic acid units, and the other neutral. This may be compared with gum tragacanth (Jones and Smith, *J.*, 1945, 739, 749) which was shown to be a mixture of a polyuronide, a neutral polysaccharide, and another fraction, probably a sterol glucoside, and with pectin, which is a complex mixture of pectic acid, associated with an araban and a galactan.

Hydrolysis of PI with 3% oxalic acid gave, after neutralisation with barium carbonate, a complex barium salt *X* (44%) and a syrup containing D-xylose and L-arabinose. The arabinose, identified and estimated as the diphenylhydrazone, constituted some 6% of the original mucilage. More drastic hydrolysis of *X* gave a degraded barium salt *Y* (67·5% of *X*) and a reducing syrup (32·5%) which was chiefly D-xylose but contained L-arabinose (3% of *X* or 1·4% of the original

hydrolysed PI). Further hydrolysis of *Y* with sulphuric acid afforded rhamnose, along with traces of D-xylose and L-arabinose, and a barium salt *Z*, which, on drastic hydrolysis, yielded L-rhamnose and D-galacturonic acid. Anderson and Fireman (*loc. cit.*) did not report the presence of L-rhamnose among the hydrolysis products of their material, but the estimated proportions of L-rhamnose (18%) and D-galacturonic acid (20%) would agree with the assumption that a D-galacturonosido-L-rhamnose formed a building unit in PI.

As with the mucilage from *Plantago lanceolata* (Part I, *J.*, 1940, 1501), acetylation gave products which could be separated into fractions, soluble and insoluble in acetone–chloroform (1 : 1). The general properties of these acetates were unchanged on further acetylation, and methylation of the crude acetates and of the various fractions gave products which were essentially similar, apart from their specific viscosities, and, presumably, molecular sizes. Table I shows the results of fractional precipitations of a number of methylated products.

TABLE I.

Fraction.	Yield, %.	OMe, %.	$[\alpha]_D^{15°}$ ($CHCl_3$).	$\eta_{sp}^{20°}/c'$.
1	63	33·5	−121°	149
2	24	34·7	−123	97
3	13	34·5	−122	62
A	82	34·0	−121	145
B	18	35·0	−123	73

Where c' is the concentration in g.-mols. of methylated anhydro-xylose residues per l.

Methanolysis of A (32 g.) and B (7 g.) gave a mixture of methylated sugar glycosides, which were separated by solvent extraction with light petroleum (Brown and Jones, *J.*, 1947, 1344) and with chloroform, and by distillation, to yield the fractions discussed below.

The most highly methylated fractions were mainly trimethyl methylxylopyranosides, which gave on hydrolysis crystalline trimethyl α-D-xylopyranose which yielded a crystalline *anilide*. Polarimetric observations indicated, however, that this sugar was not the only component of the fraction, although no separation could be achieved by chromatography on alumina (Jones, *J.*, 1944, 333). After the removal of several crops of crystalline trimethyl xylose from the hydrolysed fraction over a prolonged period the remaining syrupy material was oxidised and the resulting lactones distilled. Crystalline 2 : 3 : 5-trimethyl L-arabonamide was prepared from the first fraction, and from the equilibrium rotations of the lactone fractions it was estimated that the fully methylated material consisted of a mixture of trimethyl methyl-D-xylopyranosides (85%) and trimethyl methyl-L-arabofuranosides (15%).

The fractions of methoxyl content corresponding to dimethyl methylpentosides gave on hydrolysis crystalline 2 : 4-dimethyl β-D-xylose, which was oxidised to the syrupy 2 : 4-dimethyl D-xylopyranolactone (Barker, Hirst, and Jones, *J.*, 1946, 783). The derived amide gave a negative Weerman test, and oxidation of the free sugar with periodic acid (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1473) gave no formaldehyde. Crystalline 2 : 4-dimethyl D-xylose anilide was also isolated. These findings were confirmed by mixed-m. p. determinations on the free sugar (kindly supplied by Dr. J. K. N. Jones) and on the anilide. Finally, by means of the paper chromatogram it was shown that 2 : 4-dimethyl D-xylose was the only sugar present. The fraction, therefore, consisted entirely of 2 : 4-dimethyl methyl-D-xylopyranosides.

The fraction corresponding to monomethyl methylpentosides, yielded, on methylation followed by hydrolysis, crystalline trimethyl xylopyranose as the sole product. Periodate oxidation of the free sugar gave formaldehyde in 27% yield, which seemed to indicate that some 4-methyl xylose was present. It may be recalled, however, that Bell (*J.*, 1948, 992) has shown that this is not necessarily a true estimate since several sugars, *e.g.*, 3-methyl glucose, 2 : 4-dimethyl galactose, 3 : 4-dimethyl mannose, etc., gave a yield of formaldehyde which was by no means quantitative. Oxidation of the free sugars gave a crystalline lactone admixed with syrup. The crystalline material, m. p. 93—94°, behaved as a γ-lactone and the sodium salt of the acid on periodate oxidation gave a high yield of formaldehyde, indicating the absence of a methoxyl residue on $C_{(4)}$. This was confirmed by the fact that the free sugar gave methyl-furanosides as well as methylpyranosides on treatment with cold methanolic hydrogen chloride. The amide derived from the crystalline lactone gave a positive Weerman test, showing that $C_{(2)}$ was unsubstituted; further, the free sugar yielded a monomethyl xylosazone. The sugar was therefore, 3-methyl xylose. In confirmation of this, the crystalline *lactone* and *anilide* were identical with products prepared from synthetic 3-methyl D-xylose (Levene and Raymond, *J. Biol. Chem.*, 1933, **102**, 331). The main constituent of the hydrolysed syrup was, therefore,

3-methyl D-xylopyranose which yielded 3-*methyl* D-*xylonolactone*, 3-*methyl xylonamide*, 3-*methyl xylose anilide*, and 3-*methyl xylosazone*.

The main bulk of the monomethyl xylose fraction was, therefore, 3-methyl methyl-D-xylopyranosides. There were, however, several indications that this was not the only constituent. The syrupy fraction of the lactone on hydrolysis and subsequent periodate oxidation yielded some formaldehyde, indicating the presence of a free hydroxyl group on $C_{(4)}$. The amide prepared from this syrupy lactone, however, gave a negative Weerman test. This would seem to indicate 2-methyl xylose as the parent sugar. Further, after treatment of the free sugars with aniline and removal of as much 3-methyl xylose anilide as possible, followed by hydrolysis and oxidation with nitric acid, the product appeared to be an optically active dihydroxymethoxyglutaric ester ($[\alpha]_D^{15^\circ}$ $+39^\circ$ in methanol) which must have been unsymmetrical and could have been derived from either 2- or 4-methyl xylose. That the former possibility was correct was confirmed by comparison, on a paper chromatogram, with an authentic specimen. The minor component (20%) was, therefore, 2-methyl methylxylopyranosides which yielded 2-methyl D-xylose, 2-methyl D-xylonolactone, 2-methyl D-xylonamide, and *dihydroxy*-2-*methoxy-xyloglutaric* ester.

The remaining glycosides contained a high proportion of free methylxylosides, with some 3-methyl methylxylosides. The presence of the former was established by the isolation of crystalline D-xylose and β-methylxyloside, and by the formation of dibenzylidene xylose dimethyl acetal (Breddy and Jones, *J.*, 1945, 738); presence of the 3-methyl methylxylosides was confirmed by isolation of the crystalline anilide. These findings were further confirmed by means of the paper chromatogram.

It was estimated that the methanolysis products of *A* were composed of trimethyl methyl-D-xylosides (6 parts), trimethyl methyl-L-arabinosides (1 part), 2 : 4-dimethyl methyl-D-xylosides (1 part), 3-methyl methyl-D-xylosides (4 parts), 2-methyl methyl-D-xylosides (1 part), and methyl-D-xylosides (2 parts).

In the polysaccharide, therefore, the following residues must be linked as shown,

$$\mathrm{X1;\quad A1;\quad \overset{1}{X}3;\quad 4\overset{1}{X}2;\quad 4\overset{1}{X}3;\quad 4\underset{3}{\overset{1}{X}}2}\qquad \begin{array}{l}(\mathrm{X} = \text{D-xylopyranose})\\ (\mathrm{A} = \text{L-arabofuranose})\end{array}$$

although no information is available at this stage to indicate how these residues are linked in the mucilage molecule.

The proportion of unsubstituted xylose units is too high to be accounted for by incomplete methylation of the mucilage, repeated attempts to raise the methoxyl content of the methylated polysaccharide having failed, even using the thallium method. A certain amount of demethylation has, however, been shown to occur during methanolysis. Thus, a sample of 3-methyl D-xylopyranose, on methanolysis followed by hydrolysis under the conditions used for the methylated polysaccharide, was shown to have been demethylated to the extent of *ca.* 5%, although trimethyl xylopyranose showed no appreciable demethylation when similarly treated. It is highly improbable therefore that the free xylose could have been formed in this way. In any event, the high proportion of end-groups found necessitates the presence of a relatively large proportion of free xylose units in the methylated molecule. Thus, a hypothetical repeating unit such as is indicated would give on hydrolysis a mixture of glycosides in the following

$$\begin{array}{cccccccc}
 & \mathrm{X} & & & & & & \\
 & 1 & & & & & & \\
 & 2 & & & & & & \\
\ldots & \mathrm{3X1} & \mathrm{3X1} & \mathrm{4X1} & \mathrm{4X1} & \mathrm{4X1} & \mathrm{3X1} & \ldots \\
 & 4 & & 2 & 2 & 2 & 4 & \\
 & 1 & & 1 & 1 & 1 & 1 & \\
 & \mathrm{X} & & \mathrm{X} & \mathrm{X} & \mathrm{X} & \mathrm{A} &
\end{array}$$

proportions: trimethyl methylxylosides, 44·7; trimethyl methylarabinosides, 8·9; dimethyl methylxylosides, 8·3; monomethyl methylxylosides, 30·9; methylxylosides, 7·1%, whereas the quantities estimated were 43·5, 7·5, 6·5, 32·5, and 10%, respectively. Such a structure, however, should yield on oxidation with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427) 5 moles of formic acid, *i.e.*, 0·00311 mol./g. The value found in practice for a specimen of the polysaccharide regenerated from the acetate was 0·00227 mol./g. Although this result is in agreement with the postulation of a highly branched structure, fewer terminal xylopyranose residues are indicated by the periodate method than by the methylation process, and further work will be necessary to explain this divergence.

The results obtained from methylated polysaccharide fraction B were closely similar, except that the proportion of 2 : 4-dimethyl methylxylosides appeared to be doubled at the expense of the monomethyl methylxylosides.

A comparison of the results reported here for *P. ovata* with those previously recorded for *P. arenaria* (Part II, *loc. cit.*) reveals that the anilide, m. p. 170°, $[\alpha]_D^{11°}$ −74°, isolated on that occasion was undoubtedly 2 : 4-dimethyl xylose anilide, and not a derivative of arabinose as suggested, and the L-arabinose in *P. arenaria* mucilage may possibly also occur as an end-group. No 3: 4-dimethyl xylose was detected in the present series of experiments, but this is not surprising, for the reasons outlined in Part IV. Apart from the confusion which has existed about the isolation of 3 : 4-dimethyl xylose from the methylated seed mucilages previously studied (Parts I and II, *loc. cit.*), the main points of difference between *P. ovata* and the others of this class are the absence of D-galactose in the present instance and the existence of a high proportion of xylose units with a free hydroxyl group on $C_{(3)}$. *P. arenaria* mucilage was shown to contain no L-rhamnose, although, on hydrolysis of the methylated mucilage, a high proportion (23%) of 2-methyl xylose was isolated.

The barium aldobiuronate *Z* was methylated and hydrolysed to yield 3 : 4-dimethyl L-rhamnose and 2 : 3 : 4-trimethyl D-galacturonic acid, identified as the crystalline methyl 2 : 3 : 4-trimethyl mucate. There is, as yet, no proof as to whether the linkage between these two units is α- or β- in form, but the highly positive rotation of the barium salt of the acid and of its methylated derivative would appear to indicate that it is α.

The aldobiuronic acid was, therefore, 2-D-galacturonosido-L-rhamnose, identical with that isolated from flax-seed mucilage (Tipson, Christman, and Levene, *J. Biol. Chem.*, 1939, **128**, 609) and slippery-elm mucilage (Gill, Hirst, and Jones, *J.*, 1939, 1469).

It will have been noted that no methylated uronic acid or methyl pentose derivatives were isolated from the hydrolysis products of the methylated mucilage. Analysis of the acetylated polysaccharide, however, showed that such residues were absent. Evidence was also obtained by direct methylation that PI could be separated into an insoluble, partly methylated derivative, devoid of uronic acid, and a soluble product still containing uronic acid residues. These observations lend support to the view already expressed that the aldobiuronic acid residue is a constituent of an associated polyuronide, although a direct separation has not so far been achieved.

Experimental.

All evaporations were conducted under diminished pressure unless otherwise stated; the temperatures recorded are bath-temperatures; the concentration of rotation solutions is 1% unless otherwise stated.

Preparation of PI.—*P. ovata* Seeds (150 g.) were steeped in water (3 l.) at 15° for 48 hours, with occasional stirring. The highly viscous solution was separated by filtration through muslin, and the residue washed with water. The solution was added to ethanol (3 volumes), and the fibrous product dried with ethanol and ether, and in a vacuum over phosphoric oxide. Yield, *ca.* 1·5% [Found: Ash, 5·2%; (as sulphate, 7·7%)]. Precipitation with acidified ethanol (20 c.c. of concentrated hydrochloric acid per l.) yielded an almost ash-free polysaccharide (1·5%) (Found: equiv., by titration, *ca.* 700; uronic anhydride, 20·3%; pentosan, 52%; methylpentosan, 18%).

Hydrolysis of PI *with* 3% *Oxalic Acid.*—The dry mucilage (2·001 g.) was hydrolysed with 3% oxalic acid (100 c.c.) at 100°. $[\alpha]_D^{16°}$ were +31° (1 hour), +47° (2 hours), +56° (3 hours), +60·5° (4 hours), +43·5° (52 hours, constant). A larger quantity (18·38 g.) was hydrolysed at 100° as above for 6 hours (final $[\alpha]_D^{16°}$ +65°). The insoluble residue (0·33 g.) was removed, and the filtrate neutralised with barium carbonate, filtered, and evaporated to 100 c.c. After removal of a trace of inorganic material, the solution was poured into ethanol (2 l.), to give a white barium salt (*X*; 7·00 g.). Removal of the ethanol yielded a reducing syrup (*S*; 8·71 g.).

S partly crystallised, to yield D-xylose (5·77 g.), which was separated by trituration with glacial acetic acid. The crystalline material had m. p. 139—141°, unchanged on admixture with an authentic specimen, $[\alpha]_D^{18°}$ +79° (initial value), +74° (10 minutes), +26° (80 minutes), +20° (4 hours, constant) in water, and yielded xylosazone, m. p. and mixed m. p. 157—159°.

The acetic acid washings were evaporated to give a reducing syrup (S_1) (2·90 g.), $[\alpha]_D^{18°}$ +34° in water (Found: methyl pentose, 2·4%). This syrup (0·353 g.) yielded L-arabinose diphenylhydrazone (0·231 g.), m. p. 190°, unchanged on admixture with an authentic specimen, corresponding to an arabinose content of 43·8%, or 6·1% of anhydro-arabinose in the mucilage. No galactose phenylmethylhydrazone could be obtained from S_1. After several days, S_1 crystallised to yield further quantities of D-xylose, identified as above.

Investigation of the Insoluble Residue.—The combined residues from several experiments (1·30 g.) with 72% sulphuric acid (Monier-Williams, *J.*, 1921, 803) yielded a reducing syrup (0·28 g.), with some apparently unchanged material (0·80 g.). Glucose was the only sugar identified on the paper chromatogram.

Acetylation. (*a*) PI (20 g.) was dispersed in pyridine (200 c.c.), and acetic anhydride (170 c.c.) added slowly with continuous shaking. The mixture was then heated at 100° for 3 hours and kept for a further 72 hours at room temperature. The acetate was precipitated by pouring into a large excess of water, filtered off, and washed in running water for 48 hours, to yield a white fibrous product [25 g.;

$\eta_{sp}^{20°}/c'$, 95·2 (*m*-cresol; *c'* as g.-mols. of acetylated anhydroxylose per l.); Ac, 35·0%], which was extracted with boiling acetone–chloroform (1 : 1) to give a soluble acetate (60%), $[\alpha]_D^{15°}$ −94° (in acetone) [Ac, 43·6%; $\eta_{sp}^{20°}/c'$, 88·6 (*m*-cresol)], and an insoluble acetate (40%) (Ac, 20·1%).

(*b*) PI (10 g.) with pyridine (150 c.c.) and acetic anhydride (100 c.c.) as above yielded a white fibrous product (13 g.; Ac, 29·1%). This gave a soluble acetate (40%), $[\alpha]_D^{12°}$ −65° (in chloroform) [Ac, 36·8%; $\eta_{sp}^{20°}/c'$, 188·1 (*m*-cresol)], and an insoluble fraction (60%) [Ac, 23·4%; $\eta_{sp}^{20°}/c'$, 161·6 (*m*-cresol)].

Methylation. Crude acetate from (*a*) above was methylated directly in three operations with dimethyl sulphate and sodium hydroxide as described by Mullan and Percival (Part I, *loc. cit.*) without extraction with solvents. This yielded a methylated polysaccharide (11·7 g.) {OMe, 35·3%; $[\alpha]_D^{14°}$ −126° (in chloroform); $\eta_{sp}^{20°}/c'$, 140 (*m*-cresol); these figures were unchanged on a further methylation}. Crude acetate from (*b*) above gave an almost identical product. The soluble acetate (3½ g.) from (*b*) yielded a methylated compound (1·1 g.) {OMe, 33·8%; $[\alpha]_D^{15°}$ −124° (in chloroform); $\eta_{sp}^{20°}/c'$, 152·0 (*m*-cresol)}. The insoluble acetate (5·5 g.) from (*b*) gave a methylated product (2·0 g.) {OMe, 36·4%; $[\alpha]_D^{15°}$ −127° (in chloroform); $\eta_{sp}^{20°}/c'$, 97·4 (*m*-cresol)}. To crude methylated product from (*b*) (1 g.) in chloroform (50 c.c.) was added thallous ethoxide (1 g.) in chloroform (10 c.c.); the mixture was evaporated to dryness, and the powdered residue heated with methyl iodide (50 c.c.) under reflux until a sample of the yellow solid gave no reaction with phenolphthalein (8 days); after filtration, the solvent was removed, and the residue exhaustively extracted with chloroform; the extracts were concentrated to small volume, and the methylated polysaccharide was precipitated by light petroleum {Found: OMe, 33·7%; $[\alpha]_D^{13°}$ −126° in chloroform; $\eta_{sp}^{20°}/c'$, 88·2 (*m*-cresol)}; this appeared to indicate that the polysaccharide was originally fully methylated.

The crude methylated polysaccharide from (*b*) was fractionated from chloroform solution to give the fractions listed in Table I.

The uronic acid contents of the various acetate fractions were *ca.* 2·5% (average), indicating considerable loss of uronic acid units during acetylation; the uronic acid contents of the various methylated materials were too small to determine accurately.

Direct Methylation of PI.—The polysaccharide (12 g.) was methylated three times with dimethyl sulphate and sodium hydroxide in the usual way. Two fractions were isolated, *viz.*, an insoluble fraction, purified by washing, drying in chloroform, and precipitating in light petroleum (4·5 g.) {OMe, 25·6%; $[\alpha]_D^{17°}$ −114° (in chloroform); uronic anhydride, nil}, and a soluble fraction (2 g.) [OMe, 15·1%; $[\alpha]_D^{17°}$ +78° (*c*, 3·692 in water); uronic anhydride, 14·7%].

Typical Hydrolysis of the Methylated Polysaccharide.—Fraction A (Table I) (30 g.) was boiled with methanolic hydrogen chloride (3%; 600 c.c.) till the rotation became constant ($[\alpha]_D^{15°}$ +55°, after 20 hours.) After neutralisation with silver carbonate and filtration, the solution was concentrated to give a non-reducing syrup (31·7 g.). Previous attempts had been made to fractionate a similar material entirely by high-vacuum distillation, but no clear-cut separation could be achieved.

Fractionation by Solvent Extraction.—The above syrup (31·7 g.) was first distilled in a Claisen flask, fitted with a vacuum-jacketed column, to give: (1) 11·511 g., b. p. 100—110°/0·08 mm., $n_D^{19°}$ 1·4411; (2) 3·310 g., b. p. 110—148°/0·04 mm., $n_D^{19°}$ 1·4517; and (3) 1·105 g., b. p. 148—155°/0·04 mm., $n_D^{19°}$ 1·4638. Fraction (2) was combined with the still-residue, dissolved in water (30 c.c.), and extracted for 9 hours with light petroleum (b. p. 38—40°) to give fraction (4), 3·714 g., $n_D^{17°}$ 1·4446. The residual aqueous solution was then extracted with chloroform to give (*a*) (½ hour) 1·344 g., $n_D^{17°}$ 1·4636, (*b*) (+1½ hours) 3·514 g., $n_D^{17°}$ 1·4690, (*c*) (+3 hours) 1·680 g., $n_D^{18°}$ 1·4727, (*d*) (+6 hours) 2·260 g., $n_D^{18°}$ 1·4747, (*e*) (+9 hours) 1·219 g., $n_D^{16°}$ 1·4777, and (*f*) (+15 hours) 1·059 g., $n_D^{18°}$ 1·4800. After treatment with charcoal, the residual aqueous solution was filtered hot and evaporated to a syrup (*g*), 2·547 g., $n_D^{18°}$ 1·4800. Fractions (3), (*a*), and (*b*) were combined, dissolved in water (50 c.c.), and extracted with light petroleum (b. p. 38—40°) for 96 hours, to give a syrup (*h*), 1·818 g., $n_D^{19°}$ 1·4550. The aqueous residue was evaporated to yield a syrup (*i*), 3·819 g., $n_D^{20°}$ 1·4702. Recovery was 29·627 g. (93%).

Hydrolysis and Fractionation of B.—Fraction B (Table I) (6·40 g.) was hydrolysed as above, the solution showing finally $[\alpha]_D^{15°}$ +58° after 28 hours. This yielded a non-reducing syrup (6·80 g.), which was dissolved in water (50 c.c.) and extracted continuously with light petroleum (b. p. 38—40°) to give fractions (*j*) (1½ hours), 1·526 g., $n_D^{22°}$ 1·4393, (*k*) (+2½ hours) 0·939 g., $n_D^{22°}$ 1·4398, and (*l*) (+12 hours) 0·854 g., $n_D^{22°}$ 1·4432. The aqueous residue was evaporated to 30 c.c. and extracted with chloroform to give fractions (*m*) (½ hour), 0·701 g., $n_D^{23°}$ 1·4595, (*n*) (+1½ hours) 0·610 g., $n_D^{20°}$ 1·4650, (*o*) (+5½ hours) 0·682 g., $n_D^{23°}$ 1·4713, (*p*) (+9 hours) 0·461 g., $n_D^{17°}$ 1·4713, and (*q*) (+12 hours) 0·252 g., $n_D^{16°}$ 1·4760. The aqueous solution was then evaporated to yield a syrup (*r*), 0·792 g., $n_D^{16°}$ 1·4866. Fractions (*n*), (*o*), and (*p*) were combined, dissolved in water (50 c.c.), and extracted with light petroleum for 10 hours to give fraction (*s*), 0·137 g., $n_D^{21°}$ 1·4520. The aqueous residue on evaporation yielded a syrup (*t*), 1·312 g., $n_D^{18°}$ 1·4724. Recovery was 6·513 g. (96%).

Examination of the Fully Methylated Fractions (1), (4), (j), (k), (l).—Preliminary attempts to separate these materials by chromatography on alumina were unsuccessful. Fraction (I) (11·3 g.) was hydrolysed by nitric acid (2%; 150 c.c.) at 100°. $[\alpha]_D^{15°}$ were +15° (zero time), +16° (1 hour), +9° (2 hours), +6° (3 hours), +4° (4 hours), and +3° (5 hours, constant). The solution was then neutralised with barium carbonate, filtered, and evaporated to a reducing syrup (9·7 g.). Crops of crystalline material, removed over a prolonged period (6·35 g.), showed m. p. 91°, alone or on admixture with an authentic specimen of trimethyl α-D-xylopyranose, and $[\alpha]_D^{13°}$ +50·8° (5 minutes), +31·1° (90 minutes), +30° (120 minutes, constant) (Found: OMe, 49·5. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). The residual syrup (1·83 g.) had $[\alpha]_D^{16°}$ −15° (*c*, 1·392 in water) (equilibrium value). This syrup (1·55 g.) was dissolved in water (10 c.c.), bromine (2 c.c.) was added, and the mixture kept at room temperature until non-reducing (4 days). After the usual treatment with silver carbonate, followed by hydrogen sulphide, and lactonisation, a syrup resulted which was distilled, giving fractions (i), 0·52 g., b. p. 90—105°/0·10 mm., $n_D^{16°}$ 1·4510 (Found: OMe, 47·9. Calc. for $C_8H_{14}O_5$: OMe, 48·9%), and (ii) 0·35 g., b. p. 105—125°/0·10 mm., $n_D^{16°}$ 1·4590 (Found: OMe, 49·8%). Lactone fraction (i) showed $[\alpha]_D^{15°}$ −35° (5 minutes), −31° (20 hours),

$-28°$ (50 hours), $-23°$ (120 hours), $-19°$ (170 hours), and $-16°$ (310 hours, constant) (*c*, 0·577 in water). 11·5 Mg. on titration with 0·0254N-sodium hydroxide to phenolphthalein required 2·41 c.c. (Calc. for $C_8H_{14}O_5$: 2·39 c.c.), and on treatment with methanolic ammonia yielded crystalline 2 : 3 : 5-trimethyl L-arabonamide which on recrystallisation (ethyl acetate) had m. p. 137—138°, alone or in admixture with an authentic specimen, and $[\alpha]_D^{16°}$ +21° (*c*, 1·3 in water) (Found: C, 46·3; H, 8·2; N, 6·6; OMe, 44·6. Calc. for $C_8H_{17}O_5N$: C, 46·3; H, 8·3; N, 6·8; OMe, 44·9%). Lactone fraction (ii) showed $[\alpha]_D^{15°}$ −11° (5 minutes), −9° (20 hours), −5° (50 hours), and +2° (170 hours, constant) (*c*, 0·546 in water); 27·3 mg. required 6·15 c.c. of 0·0254N-sodium hydroxide (Calc. for $C_8H_{14}O_5$: 5·65 c.c.); it gave a syrupy amide, $[\alpha]_D^{16°}$ +35° (in water).

Paper-chromatographic investigations on the hydrolysed, fully methylated fractions showed the presence in each of trimethyl xylopyranose with a small amount of 2 : 4-dimethyl xylose.

Preparation of 2 : 3 : 4-Trimethyl Xylopyranose Anilide.—Crystalline trimethyl xylose (0·21 g.), freshly-distilled aniline (0·11 g.), and absolute ethanol (2 c.c.) were heated under reflux for 1½ hours. The alcohol was removed in a vacuum-desiccator till only *ca.* 0·5 c.c. of solution remained, and nucleated with a crystal of tetramethyl galactopyranose anilide. Crystallisation overnight yielded 2 : 3 : 4-*trimethyl xylose anilide*, obtained on recrystallisation from ether as colourless crystals, very similar in appearance to trimethyl xylose, having m. p. 98—100° and $[\alpha]_D^{19°}$ −84° (4 minutes), −75° (20 minutes), ±0° (195 minutes), +34° (345 minutes), and +47° (24 hours, constant) (*c*, 1·84 in ethanol). When the ethanol was allowed to evaporate at room temperature, the anilide was again obtained in crystalline form. Redissolution in ethanol gave a solution with a negative specific rotation, rapidly changing to a positive value as above (Found: C, 63·2; H, 7·6; N, 5·3; OMe, 35·0. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·2; OMe, 34·8%).

Examination of Dimethyl Methylpentoside Fractions.—Fraction (*h*) (1·810 g.) was dissolved in light petroleum–chloroform (2 : 1), and fractionation effected on a column of alumina (20 × 2 cm.), to give the following fractions (using the solvent as eluent): (1) 0·225 g., $n_D^{18°}$ 1·4450, (2) 0·405 g., $n_D^{18°}$ 1·4520, (3) 0·294 g., $n_D^{18°}$ 1·4565, (4) 0·166 g., $n_D^{18°}$ 1·4581, (5) 0·297 g., $n_D^{18°}$ 1·4581 (using chloroform as eluent), (6) 0·258 g., $n_D^{18°}$ 1·4581, and (7) 0·120 g., $n_D^{18°}$ 1·4575. Recovery was 1·765 g. (98%).

Fractions (3) to (7) were combined and hydrolysed with 2% nitric acid to yield a reducing syrup (0·91 g.), which crystallised completely to give 2 : 4-dimethyl β-D-xylopyranose, which, on recrystallisation (acetone–light petroleum), had m. p. 108°, unchanged on admixture with an authentic specimen. Treatment with ethanolic aniline gave crystalline 2 : 4-dimethyl xylose anilide, m. p. 166—168°, not depressed on admixture with an authentic specimen.

Lactone Formation.—Oxidation of the free sugar (0·30 g.) with bromine in water yielded the syrupy 2 : 4-dimethyl xylopyranolactone (0·20 g.), $n_D^{20°}$ 1·4698, $[\alpha]_D^{19°}$ +25° (in chloroform), and $[\alpha]_D^{19°}$ +9° (½ hour), +10° (1 hour), +11° (3 hours), +23° (23 hours), +29° (50 hours), and +30° (100 hours, constant) (in water). The lactone (12·8 mg.) required 3·48 c.c. of 0·0202N-sodium hydroxide for complete neutralisation (Calc. for $C_7H_{12}O_5$: 3·61 c.c.), and yielded syrupy 2 : 4-dimethyl xylonamide, which gave a negative Weerman test.

Periodic Acid Oxidation of the Free Sugar.—This was carried out according to the method of Reeves (*loc. cit.*). No formaldehyde was detected, whilst a similar estimation on D-xylose (0·0177 g.) yielded formaldehyde, identified as the dimedon complex (0·0309 g.), m. p. 188—190°.

The above findings were confirmed when it was shown, by means of the paper chromatogram that fractions (*h*), (*m*), and (*s*) consisted almost entirely of 2 : 4-dimethyl methylxylosides, with traces of trimethyl and monomethyl methylxylosides.

Examination of the Monomethyl Methylpentoside Fractions.—Fraction (*e*) on complete methylation and hydrolysis gave trimethyl α-D-xylopyranose, m. p. and mixed m. p. 87°, as the only product.

Hydrolysis. Fraction (*c*) (1·65 g.) was hydrolysed with 2% nitric acid (50 c.c.) at 100°. $[\alpha]_D^{20°}$ were +74° (initial value), +45° (1 hour), +34° (2 hours), +29° (3 hours), +25° (4 hours), +23° (5 hours), and +21° (6 hours, constant). The reducing syrup (1·32 g.) obtained failed to crystallise.

Anilide formation. The syrup (0·23 g.) was converted into a crystalline anilide. Recrystallisation from ethyl acetate gave 3-*methyl xylose anilide* as white needles, m. p. 138°, unchanged on admixture with an authentic specimen, $[\alpha]_D^{18°}$ +77° (*c*, 0·8 in ethyl acetate) (Found: C, 60·0; H, 7·2; N, 5·9; OMe, 12·6. $C_{12}H_{17}O_4N$ requires C, 60·2; H, 7·2; N, 5·9; OMe, 12·9%).

Fractions (*d*), (*e*), (*i*), (*q*), and (*t*) were also hydrolysed as above and each yielded an identical anilide.

The anilide (0·12 g.) was heated with N-sulphuric acid (10 c.c.) at 100° for 3 hours. After neutralisation with barium carbonate, filtration, and washing with ether, the solution was concentrated to a syrup which did not crystallise. Periodic acid oxidation of the syrup (0·067 g.) yielded formaldehyde, identified as the dimedon complex (0·051 g.), m. p. and mixed m. p. 187°.

Periodic Acid Oxidation Experiments on the Free Sugar.—The hydrolysed fraction (*c*) was submitted to periodic acid oxidation by Reeves's method, and an average formaldehyde yield of 27% was obtained in three experiments. Similar results were obtained from fractions (*d*), (*e*), (*i*), (*q*), and (*t*).

Methylfuranoside Formation.—Fraction (*c*) (0·2435 g.) was dissolved in methanol. The calculated amount of concentrated methanolic hydrogen chloride was added, and the solution was diluted to 25 c.c. with methanol. The total acid content was 0·5%. The solution showed $[\alpha]_D^{16°}$ +34° (initial) +32° (4 hours), +27° (6 hours), +26° (8 hours), +22° (24 hours), and +21° (48 hours, constant) (*c*, 0·974). A sample of the pyranoside (prepared independently) in the same solvent showed $[\alpha]_D^{16°}$ +69° (*c*, 0·984). Portions (2 × 1 c.c.) of the solution were withdrawn at intervals and examined for the presence of furanosides (Levene, Raymond, and Dillon, *J. Biol. Chem.*, 1932, **95**, 699), with results given in Table II.

Osazone Formation.—Hydrolysed fraction (*d*) (0·1 g.) was converted into the osazone. After 1 hour at 95° the solution was cooled, and the osazone separated as yellow needles. Recrystallisation from aqueous ethanol yielded 3-*methyl xylosazone*, m. p. 148—150° (Found: OMe, 7·1. $C_{18}H_{22}O_3N_4$ requires OMe, 9·1%).

TABLE II.

Time (hr.).	Free sugar, %.	Furanoside, %.	Pyranoside, %.
3	54·5	21·3	24·2
$7\frac{1}{2}$	34·9	28·0	37·1
24	27·3	35·6	36·1
48	30·3	33·9	35·8
72	26·3	27·1	46·6

Lactone Formation.—The free sugar (*c*) was converted into the lactone as usual and distilled. The distillate partly crystallised (69%).

Investigation of the Crystalline Lactone Fraction.—This was shown to consist of 3-*methyl xylopyranolactone* and on recrystallisation from ethyl acetate–light petroleum had m. p. 93—94°, unchanged on admixture with a synthetic specimen, and $[\alpha]_D^{15°}$ +76° (5 minutes), +73° ($1\frac{1}{2}$ hours), +69° (24 hours), +62° (144 hours), +50° (359 hours), +42° (652 hours), and +41° (820 hours, constant) (*c*, 0·74 in water). 14·8 Mg. required 1·75 c.c. of 0·05165N-sodium hydroxide for neutralisation (Calc. for $C_6H_{10}O_5$: 1·77 c.c.) (Found : C, 44·0; H, 6·2; OMe, 17·2. $C_6H_{10}O_5$ requires C, 44·4; H, 6·2; OMe, 19·1%). A sample of the lactone prepared from synthetic 3-methyl xylopyranose (Levene and Raymond, *J. Biol. Chem.*, 1933, **102**, 331) had m. p. 94—95° and $[\alpha]_D^{17°}$ +72° (5 minutes), +65° (20 hours), +60° (100 hours), +50° (290 hours), +44° (405 hours), and +41° (620 hours, constant) (in water). The crystalline lactone gave a syrupy *amide* (Found : OMe, 18·0. $C_6H_{13}O_5N$ requires OMe, 17·3%). The amide (0·05 g.) yielded hydrazodicarbonamide (0·011 g.), m. p. and mixed m. p. 256° (Weerman reaction).

Periodic Acid Oxidation.—The lactone (20 mg.) was converted into the sodium salt of the acid by heating with N-sodium hydroxide (0·5 c.c.) at 50° for 15 minutes. Treatment with periodic acid (Reeves, *loc. cit.*) gave formaldehyde as the dimedon complex in 75% yield.

Investigation of the Syrupy Lactone Fraction.—This had $n_D^{17°}$ 1·4851 (Found : OMe, 17·4. Calc. for $C_6H_{10}O_5$: OMe, 19·1%) and was obtained by extraction of the tile used in separating the crystalline component. Evaporation of the extracts yielded a syrup which distilled at 170°/0·02 mm. The colourless distillate had $[\alpha]_D^{17°}$ +46° (*c*, 1·448 in water), unchanged after 100 hours. 14·5 Mg. required 3·00 c.c. of 0·0207N-sodium hydroxide for neutralisation (Calc. for $C_6H_{10}O_5$: 4·32 c.c.). On conversion into the amide a syrup was obtained which gave a negative Weerman test.

On oxidising the sodium salt of the acid from the syrupy lactone with periodic acid as above, formaldehyde was again obtained, identified by the port-wine colour obtained with acid–phenylhydrazine–ferricyanide.

Anilide Formation and Fractional Crystallisation.—Syrup (*i*) was hydrolysed with 2% nitric acid, and the product (2·798 g.) converted into the anilide. The ethanol was removed by stages in a vacuum-desiccator to yield various crops of crystals. Crop I (0·015 g.) had m. p. 146—148°. Crop II (0·422 g.) on recrystallisation from ethyl acetate had m. p. 147—149°, unchanged on admixture with crop I (Found : C, 60·4; H, 7·6; N, 6·3; OMe, 23·1. Calc. for $C_{13}H_{19}O_4N$: C, 61·6; H, 7·5; N, 5·5; OMe, 24·5%). Crop III (0·280 g.) and crop IV (0·065 g.) were 3-methyl xylose anilide, (III) m. p. and mixed m. p. 137°, and (IV) m. p. and mixed m. p. with crop III, 140° [Found : OMe, (III) 13·5, (IV) 13·0%]. Crop V (0·850 g.) was impure 3-methyl xylose anilide, m. p. 128—130° not depressed on admixture with an authentic specimen (Found : OMe, 13·6%). Crop VI was obtained as a syrup on the removal of the remaining ethanol. Crops I and II were combined and decomposed with sulphuric acid to yield 2 : 4-dimethyl xylose identified on the paper chromatogram. Crop VI was decomposed with sulphuric acid to yield a reducing syrup.

Nitric Acid Oxidation of the Syrup from Crop VI.—The syrup (0·4 g.) in concentrated nitric acid (5 c.c.) was heated at 50° for 2 hours. The temperature was raised slowly and kept at 90° for a further 4 hours, whereafter the nitric acid was removed by repeated distillation with water, and the water removed by distillation with methanol. The solution was then evaporated to a syrup which was esterified with boiling 3% methanolic hydrogen chloride. The product was distilled at 145—170°/0·05 mm. The clear yellow distillate (0·08 g.) had $n_D^{12°}$ 1·4588 (Found : OMe, 39·9. Calc. for $C_8H_{14}O_7$: OMe, 41·9%), $[\alpha]_D^{15°}$ +39° (*c*, 0·72 in methanol). No crystalline amide could be obtained.

Paper Chromatogram Studies.—Examination of hydrolysed fractions (*c*), (*d*), (*e*), (*i*), (*q*), and (*t*) by means of the paper chromatogram showed that the only sugars present in each case were 3- and 2-methyl xylose, with traces of 2 : 4-dimethyl xylose and xylose.

Examination of the Residual Fractions.—Fraction (*f*), $n_D^{18°}$ 1·4800 (Found : OMe, 31·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), with 2% nitric acid gave a reducing syrup, $n_D^{14°}$ 1·4886. This, on periodic acid oxidation, yielded formaldehyde (47%), identified as the dimedon complex, and with alcoholic aniline yielded 3-methyl xylose anilide (20%), m. p. 136°, unchanged on admixture with an authentic specimen.

Fraction (*g*), $n_D^{18°}$ 1·4800 (CO_2Me, nil), gave, by hydrolysis, a reducing syrup which yielded crystalline D-xylose, m. p. 138° not depressed on admixture with an authentic specimen, $[\alpha]_D^{17°}$ +80° (2 minutes), +33° (40 minutes), and +17·5° (24 hours, constant) (*c*, 0·511 in water) (Found : OMe, nil).

Fraction (*r*), $n_D^{16°}$ 1·4866 (Found : OMe, 21·6. Calc. for $C_6H_{12}O_5$: OMe, 18·9%), when kept for a long time, deposited some crystalline material which was separated by trituration with ethyl acetate as white needles, m. p. 145° (micro) not depressed on admixture with a sample of β-methylxyloside. Hydrolysis of (*r*) gave a reducing syrup which yielded dibenzylidene xylose dimethyl acetal (Breddy and Jones, *loc. cit.*).

Demethylation Studies.—3-Methyl xylose (0·5 g., OMe, 18·2%) was boiled with methanolic hydrogen chloride (3%, 25 c.c.) for 23 hours, to yield a non-reducing syrup which was hydrolysed with 2% nitric acid, to give a reducing syrup (0·4 g.; OMe, 18·6%) which crystallised almost completely. Examination by means of the paper chromatogram showed that *ca.* 5% demethylation had taken place [Found : xylose, 0·7% (starting product); 6·0% (end product)]. A sample of 2 : 3 : 4-trimethyl xylose, on the same treatment, showed no appreciable demethylation.

Examination of the Polysaccharide regenerated from PI *Acetate.*—Acetate (*b*) (2 g.) was deacetylated

by shaking it with sodium hydroxide (N /10 ; 250 c.c.) at 15° for 24 hours. The solution was neutralised with glacial acetic acid and evaporatedto *ca.* 50 c.c. and the polysaccharide was precipitated by pouring into an excess of ethanol.

Hydrolysis.—The material (0·20 g.) was hydrolysed with 3% oxalic acid solution (25 c.c.) at 100°. $[\alpha]_D^{17°}$ were +7·5° (1 hour), +20° (2 hours), and +32·5° (4 hours, constant). The solution was neutralised with barium carbonate, filtered, concentrated to *ca.* 5 c.c., and poured into 100 c.c. of ethanol. No appreciable precipitate was obtained. Evaporation gave a syrup which (paper chromatogram) contained xylose and arabinose only, rhamnose being absent.

Formic Acid from regenerated PI.—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. : 0·00034 (1 day) ; 0·00054 (3 days) ; 0·00111 (4 days) ; 0·00186 (7 days) ; 0·00227 (10 days, constant).

Periodate Uptake of regenerated PI.—PI (*ca.* 0·4 g.) was dissolved in water (50 c.c.), and sodium metaperiodate (*ca.* 2·14 g.) added. At intervals, 5-c.c. portions were withdrawn, and the periodate determined by the arsenite method, with the following results (expressed in mols. of periodate consumed per g.) : 0·0076 (½ hour) ; 0·0087 (1 day) ; 0·0090 (2 days).

Study of Barium Salt X *from* PI.—*X* had $[\alpha]_D^{18°}$ +78° (in water) and Ba content 14·5%. *X* (24·2 g.) was further hydrolysed with 5% oxalic acid solution (400 c.c.) for 12 hours at 100°. This yielded a barium salt *Y* (13·5 g.) and a syrup *T* (6·5 g.). *T*, on investigation by the paper chromatogram was shown to contain only xylose and arabinose. The arabinose, estimated as the diphenylhydrazone, constituted 9% of the syrup, equivalent to *ca.* 1·4% of the original mucilage. *Y* ($[\alpha]_D^{18°}$ +82·8° ; Ba, 17·3%) (10 g.) was hydrolysed with 5% sulphuric acid at 100° for 6 hours, neutralised, filtered, and treated as usual to give a barium salt *Z* (8·0 g.) and a reducing syrup (0·77 g.) which (paper chromatogram) consisted mainly of rhamnose, with traces of xylose and arabinose.

Z had $[\alpha]_D^{17°}$ +70° (*c*, 2·226 in water) (Found : Ba, 17·1 ; uronic anhydride, 37·3. Calc. for $C_{12}H_{19}O_{11}Ba_{\frac{1}{2}}$: Ba, 16·8 ; uronic anhydride, 43·2%). *Z* (2 g.) was oxidised by the method of Heidelberger and Goebel (*J. Biol. Chem.*, 1927, **74**, 616) to mucic acid (0·5 g.), m. p. 214° (decomp.), mixed m. p. with an authentic specimen, 215° (decomp.). *Z* (0·24 g.) was hydrolysed by 9% sulphuric acid (11 c.c.) at 100° for 18 hours, the solution showing finally $[\alpha]_D^{17°}$ +8·4°. The solution was then neutralised with barium carbonate and filtered, the filtrate concentrated to 5 c.c. and precipitated with ethanol, and the solvent removed to leave a reducing syrup. Examination on the paper chromatogram showed the presence of rhamnose with traces of xylose and arabinose.

Methylation of the Barium Salt Z.—*Z* (4·0 g.) was dissolved in water (70 c.c.), methyl sulphate (70 c.c.) added, and 30% sodium hydroxide solution (140 c.c.) run in during 4 hours, the mixture being stirred continuously (room temperature). After being kept overnight, the mixture was non-reducing. Solid sodium hydroxide (65 g.) was added, and methyl sulphate (110 c.c.) run in with stirring during 7 hours. The solution was again kept overnight, and was then heated at 100° for ½ hour, cooled, neutralised with concentrated sulphuric acid, and extracted with 6 × 300-c.c. portions of chloroform. Evaporation of the extracts yielded a syrup which was dissolved in water (50 c.c.) and remethylated with sodium hydroxide (150 c.c.) and methyl sulphate (75 c.c.) as above. After being kept overnight, the solution was worked up and extracted as before. The extracts were dried (Na_2SO_4), decanted, and concentrated to a syrup which was methylated four times with the Purdie reagents and twice with diazomethane. Distillation over barium carbonate at 170—190°/0·06 mm. yielded a yellow distillate (1·2 g.), $n_D^{16°}$ 1·4680 (Found : OMe, 48·7. Calc. for $C_{19}H_{34}O_{11}$: OMe, 49·5%).

The syrup (1·10 g.) was hydrolysed with aqueous hydrochloric acid (7% ; 50 c.c.) at 100° ; $[\alpha]_D^{17°}$ were +107° (zero time), +108° (1 hour), +96° (3 hours), +88° (5 hours), +81° (7 hours), +75° (9 hours), and +71° (12 hours, constant). The solution was cooled, neutralised with silver carbonate, and treated with hydrogen sulphide and barium carbonate. After evaporation, the residue was exhaustively extracted with anhydrous ether, leaving a white barium salt (0·6 g.). The ethereal extracts were combined and evaporated to yield a syrup (0·22 g.), $n_D^{18°}$ 1·4665, which, on drying at 40°/0·01 mm., crystallised completely. Recrystallisation from ether yielded 3 : 4-dimethyl α-L-rhamnose, m. p. 91—92° (Found ; OMe, 30·1. Calc. for $C_8H_{16}O_5$: OMe, 32·3%), $[\alpha]_D^{17°}$ +14° (12 minutes), +19° (35 minutes, constant) (*c*, 0·688 in water).

The crystalline sugar was converted *via* the lactone into the amide, which gave a positive Weerman test.

The above barium salt (0·6 g.) was oxidised to methyl 2 : 3 : 4-trimethyl mucate (0·3 g.) (Gill, Hirst, and Jones, *J.*, 1939, 1469), m. p. 101·5°, alone or mixed with an authentic specimen, $[\alpha]_D^{17°}$ +29·0 (*c*, 0·715 in water) (Found : C, 46·9 ; H, 6·8 ; OMe, 53·4. Calc. for $C_{11}H_{20}O_8$: C, 47·1 ; H, 7·2 ; OMe, 55·4%).

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