346. *Studies on Seed Mucilages. Part IV. The Seed Mucilage of* Plantago lanceolata.

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Methylated rib grass-seed mucilage prepared from the acetate (Part I, *J.*, 1940, 1501) gave, on methanolysis, trimethyl methyl-D-xylopyranosides (37%), tetramethyl methylgalactopyranosides (2·4%), 2 : 4-dimethyl methyl-D-xylosides (16%), 2 : 3-dimethyl methyl-D-xylosides (16%), 2 : 4 : 6-trimethyl methyl-D-galactosides (1·2%), 2-methyl methyl-D-xylosides (8%), 3-methyl methyl-D-xylosides (*ca.* 5%), and methyl-D-xylosides (7%).

The presence of 3 : 4-dimethyl xylose derivatives previously claimed (Part I, *loc. cit.*) could not be substantiated.

The aldobiuronic acid present in the portion of the mucilage resistant to hydrolysis appeared to be a mixture; the acidic component was identified as D-galacturonic acid.

In a preliminary examination of the mucilaginous polysaccharide from the seeds of the rib grass, *Plantago lanceolata* (Mullan and Percival, Part I, *J.*, 1940, 1501), among the products of hydrolysis of the methylated mucilage, 3 : 4-dimethyl xylose was thought to be present in a highly branched structure. James and Smith (*J.*, 1945, 744), however, isolated from methylated gum tragacanth a dimethyl xylose which gave a crystalline lactone of identical melting point with that reported in Part I, but showing $[\alpha]_D^{20°}$ −56° changing to −27° instead of $[\alpha]_D^{18°}$ +41° changing to +31° (in water). Evidence was presented which showed that the dimethyl xylonolactone isolated from the gum (James and Smith, *loc. cit.*) was probably 3 : 4-dimethyl D-xylonolactone. In order to clarify the position and to examine in more detail the constitution of rib grass-seed mucilage using improved methods of separating mixtures of methylated glycosides, the present investigation was undertaken.

The methoxyl content of the methylated mucilage obtained as in Part I (*loc. cit.*) could not be increased by using the thallium method. Fractional precipitation from chloroform solution by light petroleum gave two fractions *A* and *B*, and viscosity measurements indicated that the average molecular size of *A* was approximately twice that of *B*. Methanolysis of *A* was followed by distillation, to remove most of the fully methylated methylglycosides, the remainder being fractionated by extraction with light petroleum in a liquid extractor (Brown and Jones, *J.*, 1947, 1344), followed by chloroform. A fraction, insoluble in chloroform, remained. By these methods 2 : 3 : 4-trimethyl methyl-D-xylosides (37%) (identified as the crystalline trimethyl α-D-xylopyranose, the corresponding anilide, and by an examination of the lactone) and tetramethyl methyl-D-galactopyranosides (2·4%) (identified as the crystalline anilide) were separated. A search for fully methylated arabinose derivatives proved abortive.

After purification of the fractions extracted with light petroleum by adsorption on alumina (Jones, *J.*, 1944, 333) the following products were isolated: 2 : 4-dimethyl methyl-D-xylosides (16·1%), identified as the crystalline β-sugar and its anilide (Barker, Hirst, and Jones, *J.*, 1946, 783) and by the facts (*a*) that no formaldehyde was produced on the periodate oxidation of the free sugar (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476), proving substitution on $C_{(4)}$, and (*b*) that the derived amide gave a negative Weerman test, proving substitution on $C_{(2)}$; and 2 : 3-dimethyl methyl-D-xylosides (15·5%), identified as the crystalline amide (Hampton, Haworth, and Hirst, *J.*, 1929, 1739), the presence of a substituent on $C_{(2)}$ being confirmed by a negative Weerman test, and the absence of methoxyl residues on $C_{(4)}$ and $C_{(5)}$ by the formation of formaldehyde in almost theoretical yield (97%) on oxidation of the amide with periodate.

Early fractions extracted by chloroform were purified by chromatography on alumina and contained 2 : 4 : 6-trimethyl methyl-D-galactosides (1·2%), identified as the crystalline anilide (Hirst and Jones, *J.*, 1939, 1482), as well as the dimethyl methylxylosides already mentioned. A later chloroform fraction contained 2-methyl methyl-D-xylosides (8·4%), identified as the crystalline β-sugar (Robertson and Speedie, *J.*, 1934, 824), lactone, amide, and anilide, together with 3-methyl methylxylosides (*ca.* 5·0%). The evidence for the assignment of the methyl group to $C_{(3)}$ included the isolation of a monomethyl xylosazone, a positive Weerman test on the corresponding amide, the properties of the derived lactone, and the production of formaldehyde on oxidation with periodate (cf. Bell, *J.*, 1948, 992).

From the chloroform-insoluble residue crystalline β-methyl-D-xyloside was isolated. Methylxylosides (6·8%) were the main constituents of the residue, being identified as crystalline D-xylose, as the phenylosazone and by strip chromatography.

The following building units are present, therefore, in the portion of the mucilage isolated as the methylated derivative:

$$X1;\ 3X1;\ \overset{4}{\underset{2}{X}}1;\ \overset{4}{\underset{2}{X}}1;\ 3\overset{4}{X}1;\ 3\overset{4}{\underset{2}{X}}1;\ G1;\ 3G1$$

X = D-xylopyranose
G = D-galactopyranose

If the repeating unit is assumed to have one terminal D-galactopyranose residue, the inclusion of more than forty sugar residues would be necessary to explain the experimental results. If one neglects the galactose residues for the present and considers only xylose units, a structure such as that given below, of which there are many variants, could represent the general pattern.

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                X                 X
                1                 1
                2                 2
. . . 4X1 4X1 4X1 4X1 4X1 4X1 . . .
                3         3
                1         1
                X         X
                          3          X = D-xylopyranose
                          1
                          X
                          3
                          1
                          X
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The proportions of methylated methylxylosides which would be obtained on the methanolysis of a methylated polysaccharide constructed in the above fashion would be trimethyl 39% (37), 2 : 3-dimethyl and 2 : 4-dimethyl 18·2% (16), 2-methyl 8·4% (8·4), and 3-methyl methylxyloside 8·4% (5·3), and methylxylosides 7·8% (6·3); the experimental figures are given in parentheses, and have been corrected for demethylation found to occur during methanolysis (see Part III).

It will be noted that no 3 : 4-dimethyl xylose derivatives were recognised in the present series of experiments. Although the evidence that 3 : 4-dimethyl xylose was the principal constituent of the dimethyl pentose fraction appeared to be strong at the time (Part I, *loc. cit.*), it is now apparent that the dimethyl pentose fraction then examined was a complex mixture. The lactone thought to be 3 : 4-dimethyl D-xylonolactone, m. p. 67°, has now been found not to depress the melting point of 2-methyl D-xylonolactone (m. p. 66—68°). The constants quoted by James and Smith (*loc. cit.*) for 3 : 4-dimethyl xylonolactone are, therefore, inconsistent with the earlier views of one of us, and the claim that 3 : 4-dimethyl xylose is one of the principal products of the hydrolysis of methylated rib-grass-seed mucilage cannot be upheld. It should be pointed out, however, that, although the positive Weerman test (Part I, *loc. cit.*) might perhaps be explained by the presence of 3-methyl xylose in the mixture, the isolation of an optically active hydroxydimethoxyglutaric acid and D(−)-dimethoxysuccinamide remains unexplained.

No methylated derivatives of arabinose, rhamnose, or galacturonic acid were identified among the products of methanolysis. It is considered likely that the acidic nature of the polysaccharide as extracted from the seeds is due to an associated polyuronide which is eliminated on acetylation and methylation (cf. Part III, preceding paper), for the acetate was found to contain 7% of uronic anhydride and the methylated mucilage some 2·5%, falling to zero after four methylations.

A preliminary investigation of the barium salts obtained on hydrolysis of the mucilage with oxalic acid and neutralisation with barium carbonate was undertaken. The acidic portion was identified as D-galacturonic acid, but the original barium salt was certainly not a simple aldobiuronate. By hydrolysis using various conditions L-arabinose was obtained, and L-rhamnose, D-xylose, and D-galactose were also detected. A product approximating in composition to a barium aldobiuronate was isolated eventually and subjected to methylation and hydrolysis. The dimethyl ester of 2 : 3 : 4-trimethyl mucate was obtained on oxidation and esterification, but no conclusion has been reached as to the accompanying methylated pentose or methyl pentose residues.

Experimental.

All distillations were carried out under diminished pressure, and the temperatures quoted are bath-temperatures. The concentration of rotation solutions is 1% unless otherwise stated.

Preparation of the Mucilage.—The acid mucilage was extracted from the rib grass seeds as described in Part I (*loc. cit.*) (Found: uronic anhydride, 8·0%; equiv. by titration, 1025).

Autohydrolysis of the Mucilage.—The mucilage (0·5 g.) was heated with water (25 c.c.) at 95—100° for 20 hours, barium carbonate was added, the solution was filtered, concentrated to 10 c.c. at 40°, and poured

into methanol (300 c.c.) with stirring and the precipitate removed at the centrifuge. The alcoholic solution and washings were evaporated to dryness under reduced pressure, giving a syrup (0·09 g.) which was shown by strip chromatography to contain xylose and arabinose.

Oxidation with Periodate.—(*a*) The mucilage (0·553 g.) was oxidised with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427) and the formic acid liberated was determined by titration with 0·0103N-sodium hydroxide. A correction was applied for the acidity of the mucilage. The results expressed in mols. of $H{\cdot}CO_2H$ per g. were 0·00018 (½ hour), 0·00055 (18 hours), 0·0016 (66 hours), 0·0022 (163 hours), and 0·0020 (211 hours). (*b*) The mucilage (0·451 g.) was dissolved in water (100 c.c.) containing sodium metaperiodate (4·233 g.). The periodate remaining was determined at intervals on 5-c.c. portions by the arsenite method [Found (mols. of $NaIO_4$ per g.) : 0·0020 (10 minutes), 0·0026 (70 minutes), 0·0029 (21½ hours), 0·0038 (141 hours), and 0·0042 (168 hours, constant); the uptake was initially very rapid].

Methylation.—Acetylation and subsequent methylation were carried out as described in Part I (*loc. cit.*). A sample of the methylated product, $[\alpha]_D^{15°}$ −105° (in chloroform) (OMe, 35·0%), on a further methylation by the thallium method did not increase in methoxyl content (34·4%). The uronic anhydride content of the methylated mucilage was 2·5%, and in a sample methylated four times with sodium hydroxide and methyl sulphate the uronic anhydride content was nil.

Fractionation.—The methylated mucilage (55 g.) was fractionated into two portions *A* and *B* by dissolving it in chloroform (700 c.c.) and adding light petroleum (b. p. 40—60°) slowly with stirring. After addition of light petroleum (3 l.), a stable precipitate was obtained, fraction *A* (34 g.), $[\alpha]_D^{17°}$ −103° (in chloroform) (OMe, 33·4%). Further addition of light petroleum (total, 4 l.) gave a second fraction, *B* (21 g.), $[\alpha]_D^{17°}$ −111° (in chloroform) (OMe, 34·2%). *A* had η_{sp}/c' 125 and *B* η_{sp}/c' 69·5 (where η_{sp} is the specific viscosity and c' is the concentration in g.-mols. of methylated anhydroxylose residues per l., assuming the repeating unit to be $C_5H_8O_4$). It will be seen that the apparent molecular size of *A* is almost twice that of *B*.

Hydrolysis of the Methylated Mucilage.—Three series of preliminary experiments (on 10-g. samples of the methylated polysaccharide) were carried out to determine the best conditions for separation : by distillation, by a combination of distillation and chromatographic adsorption on alumina, and by solvent extraction. In all cases the proportion of trimethyl methylxylosides obtained was similar to that arrived at in the experiments described below. Tetramethyl galactopyranose, 2 : 4 : 6-trimethyl galactose, and 2 : 4-dimethyl xylose were also identified. It was found that the dimethyl methylpentoside fraction amounted to about 30% of the methylated methylglycosides obtained on hydrolysis and appeared to consist exclusively of dimethyl methylxylosides since complete methylation and hydrolysis gave trimethylxylopyranose in good yield.

Fraction *A* (31·0 g.) was then hydrolysed with 3% methanolic hydrogen chloride (600 c.c.) for 22 hours. The mixture of glycosides obtained was distilled to remove most of the fully methylated portion (*a*) (9·08 g.), b. p. 105—130°/0·06 mm., $n_D^{18°}$ 1·4414. The glycosides remaining were dissolved in water (30 c.c.), barium carbonate (1 g.) was added, and the aqueous solution extracted with light petroleum (200 c.c., b. p. 38—40°) for 4 hours in a liquid extractor. The solvent was removed to give fraction (*b*) (4·24 g.), $n_D^{19°}$ 1·4490. Fractions (*a*) and (*b*) were combined and redistilled to give (*c*) (8·56 g.), b. p. 83—97°/0·01 mm., $n_D^{17°}$ 1·4413, (*d*) (1·58 g.), b. p. 97—110°/0·01 mm., $n_D^{19°}$ 1·4420, and (*e*) (1·37 g.) (undistilled residue), $n_D^{17°}$ 1·4542 (OMe, 50·1%).

Extraction was continued with light petroleum, giving (*f*) (3·03 g.), $n_D^{15°}$ 1·4560 (OMe, 47·3%) (24 hours), and (*g*) (7·65 g.), $n_D^{18°}$ 1·4595 (OMe, 47·0%) (94 hours). Extraction was continued with chloroform to remove the less fully methylated glycosides, and yielded (*h*) (2·48 g.), $n_D^{15°}$ 1·4690 (OMe, 37·5%) (2 hours), and (*i*) (4·68 g.), $n_D^{19°}$ 1·4746 (OMe, 29·0%) (24 hours). Evaporation of the remaining solution gave a brown glass (*j*) (2·70 g.). Recovery was 98%.

Examination of the Distillation Fractions.—Fractions (*c*) and (*d*) were combined (10·14 g.) and hydrolysed with 2% nitric acid (150 c.c.) for 5 hours at 100°. $[\alpha]_D^{17°}$ were +18·8° (initial), +24·5° (1 hour), +18·2° (2 hours), and +15·0° (5 hours). The reducing syrup obtained crystallised almost completely in 12 hours, but left a syrupy portion (0·30 g.), which was passed through an alumina column (28 × 1·8 cm.) using a mixture of methanol and chloroform (1 : 5) for development. The resulting syrup (0·17 g.) had $[\alpha]_D^{17°}$ +3·7° (in water), and $n_D^{18°}$ 1·4560, and was converted into the lactone (0·13 g.), b. p. 100—110°/0·01 mm., $n_D^{17°}$ 1·4585, which crystallised partly on standing, the crystals having m. p. 47—48°, unchanged on admixture with 2 : 3 : 4-trimethyl D-xylonolactone (m. p. 53°). The remaining syrupy lactone (0·068 g.) had $[\alpha]_D^{18°}$ ±0° (3 hours), +9·5° (40 hours), +13° (64 hours). This rotational change is characteristic of trimethyl D-xylopyranolactone and it appeared that no fully methylated arabofuranose derivative was present in this fraction. The crystalline sugar (8·0 g.), on recrystallisation from dry ether, had m. p. 88—90° unchanged on admixture with 2 : 3 : 4-trimethyl α-D-xylose, and $[\alpha]_D^{21°}$ +54° (initial), +35° (15 minutes), +27° (30 minutes), +23·5° (45 minutes), and +20° (1 hour, constant) (*c*, 1·2 in water). The sugar (0·80 g.) was oxidised to the lactone, giving on distillation 0·65 g., b. p. 110—125°/0·04 mm., which crystallised immediately. Recrystallisation from dry ether gave needles, m. p. 53° [Found : C, 50·6; H, 7·4; OMe, 51·1. Calc. for $C_8H_{14}O_5$: C, 50·5; H, 7·4; OMe, 48·9%], $[\alpha]_D^{18°}$ +1·3° (initial), +4° (10½ hours), +10·5° (47½ hours), +16·5° (95½ hours), and +19° (120 hours, constant) (*c*, 1·6 in water). The anilide (0·16 g.), prepared from the crystalline 2 : 3 : 4-trimethyl xylose (0·48 g.), had m. p. 100—101° (Found : N, 5·3; OMe, 35·9. Calc. for $C_{14}H_{21}O_4N$: N, 5·2; OMe, 34·8%).

Fraction (*e*) (1·37 g.), dissolved in a mixture (25 c.c.) of light petroleum (b. p. 40—60°) and chloroform (4 : 1), was passed through an alumina column (25 × 3·5 cm.). Development was commenced using the original solvent and continued with a 2 : 1 mixture and finally with chloroform. Two main groups of fractions were collected : (i) $n_D^{18°}$ 1·4480—1·4489 (1·0 g.), and (ii) $n_D^{17°}$ 1·4540—1·4560 (0·3 g.). These were hydrolysed and, on conversion into the anilide, crystallised. The crystals (0·70 g.), removed on porous tile, had m. p. 192—193°, unchanged when mixed with an authentic sample of 2 : 3 : 4 : 6-tetramethyl galactose anilide (m. p. 192°), and $[\alpha]_D^{19°}$ −75° (*c*, 0·5 in acetone).

Examination of the Light-petroleum Fractions.—Fraction (*f*) (3·03 g.) was purified by an alumina column (30 × 1·8 cm.). Development was effected with a mixture of light petroleum and chloroform

(2 : 1) and then chloroform. One main group of fractions was collected, having $n_D^{19°}$ 1·4552—1·4565 (2·1 g.) and hydrolysed to a reducing syrup (1·9 g.) which crystallised partly. The crude product, removed on porous tile, crystallised from dry acetone by the addition of light petroleum (b. p. 80—100°) as needles (0·2 g.), m. p. 108—110°. The syrup (*g*) (7·65 g.), recovered from the tile by extraction with acetone, was purified by passage through alumina (20 × 3·5 cm.), and development effected by light petroleum–chloroform (1 : 1) and finally chloroform. Two main groups of fractions were collected : (i) $n_D^{18°}$ 1·4570—1·4582 (3·9 g.), and (ii) $n_D^{20°}$ 1·4552—1·4567 (1·5 g.). Fraction (i) was hydrolysed; the resulting reducing syrup (3·5 g.) crystallised partly; the crystals, recovered and recrystallised (1·1 g.) as described above, had m. p. 108—110°, not depressed on admixture with an authentic specimen of 2 : 4-dimethyl xylose (m. p. 108°).

Characterisation of 2 : 4-*dimethyl xylose*. The crystalline sugar (m. p. 108—110°) had $[\alpha]_D^{19°}$ +4° (17 minutes), +16° (67 minutes), +21° (2 hours), and +23° (3 hours constant) (*c*, 2·3 in water) (Found : C, 46·8; H, 8·0; OMe 33·8. Calc. for $C_7H_{14}O_5$: C, 47·1; H, 7·9; OMe, 34·8%). A portion of the crystalline sugar (0·13 g.) was oxidised to the lactone (0·07 g.), b. p. 130—135°/0·01 mm. $n_D^{14°}$ 1·4800 (last drop), $[\alpha]_D^{15°}$ −5° (initial), −1·5° (2 hours), +5° (5½ hours), +17° (24 hours), and +26° (72 hours, constant) (*c*, 0·6 in water). This rotational change is characteristic of a δ-xylonolactone. The syrupy amide (0·05 g.) was prepared from the lactone (0·05 g.) and gave a negative Weerman test, indicating the presence of a methoxyl group on $C_{(2)}$. The sugar (0·034 g.) was oxidised with periodate, but no formaldehyde was obtained (dimedon complex), indicating that $C_{(4)}$ was occupied by a methoxyl group.

The syrup recovered from the tile by acetone extraction, after removal of the crystalline 2 : 4-dimethyl xylose, was examined by strip chromatography; development of the chromatogram with ammoniacal silver nitrate produced spots corresponding to 2 : 4- (R_G value 0·66) and 2 : 3-dimethyl xylose (R_G value 0·74). The syrupy mixture (0·78 g.) was converted into the lactone (0·56 g.), b. p. 135—140°/0·05 mm., $n_D^{16°}$ 1·4750, $[\alpha]_D^{15°}$ +65·5° (initial), +50° (17½ hours), +55° (43 hours), +54·5° (113 hours), +51·5° (210 hours), +49° (600 hours) (*c*, 1·4 in water), a portion of which was converted into the amide; in the Weerman test this gave a small precipitate of hydrazodicarbonamide, corresponding to some 14% of a sugar not substituted on $C_{(2)}$, perhaps due to the presence of small amounts of 3 : 4-dimethyl xylose or 3-methyl xylose; a control experiment was run simultaneously on gluconamide.

Characterisation of 2 : 3-*dimethyl xylose*. Fraction *g* (ii) was hydrolysed, but the reducing syrup (1·4 g.) obtained did not crystallise. The derived lactone (0·91 g.) had b. p. 125—135°/0·05 mm., $n_D^{17°}$ 1·4665. $[\alpha]_D^{15°}$ +63° (initial), +63° (6 hours), +65° (24 hours), +64° (96 hours), +61° (192 hours), +57° (264 hours) and +54° (576 hours) (*c*, 0·8 in water), the rotational change being characteristic of a γ-lactone. The lactone (0·29 g.) was converted into the crystalline amide (0·15 g.), m. p. 132—133°, not depressed on admixture with an authentic sample of 2 : 3-dimethyl xylonamide (m. p. 133°), which gave a negative " Weerman " test indicating the presence of a methoxyl group on $C_{(2)}$ (Found : C, 43·5; H, 7·9; N, 7·0; OMe, 31·3. Calc. for $C_7H_{15}O_5N$: C, 43·5; H, 7·7; N, 7·25; OMe, 32·1%). A portion of the amide (0·027 g.) was oxidised with periodate; a quantitative yield of the formaldehyde–dimedon complex (0·040 g.) confirmed the absence of methoxyl groups on $C_{(4)}$ and $C_{(5)}$.

Investigation of the Chloroform Fractions.—Fraction (*h*) was distilled, giving 1·45 g., b. p. 110—120°/0·1 mm., $n_D^{22°}$ 1·4650 (OMe, 41·6%), and a waxy residue (0·9 g.) (not carbohydrate in nature). The distillate was purified by passage through alumina (28 × 1·8 cm.), and development effected by light petroleum–chloroform (2 : 1), then by chloroform, and finally by chloroform–methanol (4 : 1). One main group of fractions ($n_D^{18°}$ 1·4570—1·4580; 0·53 g.) was collected and then hydrolysed. The anilide of the resulting reducing syrup (0·48 g.) was fractionally crystallised; the first crop (0·07 g.) had m. p. 172°, unchanged on admixture with authentic 2 : 4 : 6-trimethyl galactose anilide (m. p. 172°), and $[\alpha]_D^{15°}$ −97° (initial), −87° (½ hour), −26° (5½ hours), +3° (11 hours), and +31° (23 hours, constant) (*c*, 0·3 in acetone); the second crop (0·1 g.) had m. p. 143° which was lowered by recrystallisation; the third crop (0·1 g.), m. p. 109—118°, gave a crystalline amide, m. p. 128—131°, unchanged on admixture with authentic 2 : 3-dimethyl xylonolactone (m. p. 133°), by way of the sugar and lactone. Fraction (*i*) (4·67 g.) was purified on an alumina column (17 × 3·5 cm.); light petroleum–chloroform (1 : 2), followed by chloroform and finally a chloroform–methanol mixture (2 : 1), were used for development. One main group of fractions, $n_D^{18°}$ 1·4700—1·4735 (3·4 g.), was collected and then hydrolysed to give a reducing syrup (3·1 g.) which was dissolved in the minimum quantity of ethanol; slow removal of the solvent gave crystals (1·1 g.), m. p. 135—137°.

Characterisation of 2-*methyl xylose*. The crystals, m. p. 135—137°, had $[\alpha]_D^{16°}$ −23° (initial), −1° (15 minutes), +20° (35 minutes), +34° (2 hours), and +35° (24 hours, constant) (*c*, 3·5 in water) (Found : C, 44·3; H, 7·7; OMe, 19·9. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4; OMe, 18·9%). Periodate oxidation and quantitative determination of the formaldehyde–dimedon complex indicated that 86% of the sugar was not substituted on $C_{(4)}$ and $C_{(5)}$. The crystalline sugar (0·1 g.) was converted into the osazone (0·04 g.) (OMe, 1·0%), m. p. 144°, mixed m. p. with an authentic sample of xylose phenylosazone (m. p. 156—158°) 154—155°. The sugar (0·35 g.) was oxidised to the lactone (0·23 g.), b. p. 155—160°/0·01 mm., $n_D^{14°}$ 1·4832. Crystallisation occurred after several days; m. p. 66—68°, unchanged on admixture with the supposed 3 : 4-dimethyl xylonolactone (m. p. 67°) (Part I, *loc. cit.*); $[\alpha]_D^{17°}$ +101° (initial), +98° (5 hours), +93° (71 hours), +81° (288 hours), and +74° (504 hours). The lactone (0·06 g.) gave a crystalline amide (0·04 g.), m. p. 96—98°, $[\alpha]_D^{18°}$ +52·5° (*c*, 2·0 in water) (Found : C, 40·0; H, 6·9, N, 8·3; OMe, 16·7. Calc. for $C_6H_{13}O_5N$: C, 40·2; H, 7·3; N, 7·8; OMe, 17·3%). The amide gave a negative Weerman test indicating the presence of a substituent on $C_{(2)}$. The anilide prepared from the sugar had m. p. 125—126°, $[\alpha]_D^{15°}$ +214° (*c*, 2·2 in ethyl acetate) (Found : C, 60·0; H, 7·1; N, 6·1; OMe, 12·8. Calc. for $C_{12}H_{17}O_4N$: C, 60·3; H, 7·1; N, 5·9; OMe, 12·9%).

Characterisation of 3-*methyl xylose*. After complete removal of the alcohol and crystalline sugar from fraction (i), a syrup was left which on periodate oxidation gave a 65% yield of formaldehyde (cf. Bell, *J.*, 1948, 992, for the oxidation of 3-methyl glucose). Examination of this syrupy fraction by strip chromatography showed, on development with ammoniacal silver nitrate, an elongated spot similar to that given by a mixture of 2- and 3-methyl xyloses. The osazone (0·10 g.), prepared from the syrup (0·18 g.),

was purified by passage through an alumina column (20 × 1·8 cm.), benzene–methanol (49 : 1) being used for development. Two bands were obtained; the less strongly adsorbed yielded an osazone (0·02 g.), m. p. 166—168°, unchanged on admixture with an authentic sample of 3-methyl xylose phenylosazone (m. p. 172°) (Found : OMe, 8·7. Calc. for $C_{18}H_{22}O_3N_4$: OMe, 9·1%). The syrup (0·30 g.) was converted into the lactone (0·11 g.), b. p. 160—170°/0·05 mm., $n_D^{18°}$ 1·4854, $[\alpha]_D^{17°}$ +67° (initial), +67° (5 hours), +63° (95 hours), +50° (287 hours), and +42° (528 hours, constant); the rotational change appeared to exclude the possibility of the presence of a δ-lactone such as would be given by 4-methyl xylose. The lactone was converted into the amide which gave hydrazodicarbonamide in the Weerman test, the yield, in comparison with gluconamide, indicating that 49% of the sugar was not substituted on $C_{(2)}$.

Isolation of β-Methylxyloside and D-*Xylose.*—A portion of the residue (*j*) (0·5 g.) was dissolved in the minimum quantity of ethanol and the solvent slowly removed. Crystals (0·1 g.), m. p. 154°, $[\alpha]_D$ −32° (*c*, 0·8 in water), were obtained. Hydrolysis and examination of the reducing syrup by strip chromatography showed only xylose to be present, confirming that the above crystalline product was β-methyl-D-xyloside. Hydrolysis of the remainder (2·2 g.) of the fraction and crystallisation from ethanol gave a crystalline sugar (1·2 g.), m. p. 141°, unchanged on admixture with authentic D-xylose (m. p. 141°). Examination, by strip chromatography, of the syrupy fraction remaining after removal of the solvent confirmed the presence of xylose. The development with ammoniacal silver nitrate also showed the presence, in this fraction, of small quantities of monomethyl xyloses, galactose, and an unidentified sugar (R_G value 0·25) which may be a monomethyl hexose.

Attempted Characterisation of the Barium Aldobiuronate.—(a) *Graded hydrolysis of the mucilage.* The acid mucilage (117 g.) was hydrolysed with 3% oxalic acid solution (2 l.) for 20 hours at 100°. The barium salt (10·3 g.) was isolated, as described in Part I (*loc. cit.*), as a pale yellow amorphous powder. This product (10·3 g.) was treated again with oxalic acid (200 c.c.; 3%) for 6 hours at 100°; the barium salt (4·0 g.) gave CO_2 7·3 and Ba 10·1%. The alcoholic solution of sugars (0·8 g.) obtained from the second hydrolysis was examined by strip chromatography, which showed the presence of xylose, rhamnose, and arabinose in approximately equal proportions, with a smaller quantity of galactose. The presence of L-arabinose was confirmed by the isolation of L-arabinose diphenylhydrazone (m. p. 204°) and L-rhamnose by the isolation of the L-rhamnose monohydrate (m. p. 90—94°) (0·03 g.). Hydrolysis was continued using 6% sulphuric acid (50 c.c.) for 6 hours at 100°; the barium salt (2·7 g.) obtained gave CO_2 9·4 and Ba 15·4%, whilst examination of the ethanolic solution of sugars (0·07 g.) by strip chromatography showed the presence of arabinose with traces of galactose and xylose.

(b) *Hydrolysis of the barium aldobiuronate.* The barium aldobiuronate (0·25 g.) was hydrolysed with 9·8% sulphuric acid (25 c.c.) for 25 hours at 100°. $[\alpha]_D^{17°}$ were +60° (initial), +54° (1 hour), +46° (5 hours), +24° (21½ hours) and +22° (25 hours). Samples were withdrawn, neutralised with barium carbonate, and run on a strip chromatogram against standards. A gradual increase in the intensity of the spot due to arabinose was observed from 1 to 25 hours, with an inverse change in intensity of the spot on the starting line due to the uronic and aldobiuronic acid. Traces of rhamnose and galactose were also present.

(c) *Oxidation with bromine and hydrobromic acid.* The barium aldobiuronate (0·5 g.) was dissolved in hydrobromic acid (6 c.c.; 6%) containing bromine (0·25 c.c.) and heated under reflux for 8 hours at 100°. During 14 days at 0° crystals separated, m. p. 208—209°, unchanged on admixture with an authentic sample of mucic acid (m. p. 213°). The filtrate yielded a small quantity of the benziminazole of L-arabonic acid; m. p. 231—233°; mixed m. p. with an authentic sample (m. p. 240°) 234—236°.

(d) *Methylation.* The barium aldobiuronate (1·8 g.) was methylated three times with sodium hydroxide and methyl sulphate, three times with silver oxide and methyl iodide, and once with diazomethane. Distillation gave fractions (i) (0·32 g.), b. p. 115—130°/0·05 mm., $n_D^{18°}$ 1·4503 (OMe, 54·1%), and (ii) (0·51 g.), b. p. 170—190°/0·05 mm., $n_D^{18°}$ 1·4687 (OMe, 49·1%). Fraction (ii) was hydrolysed with 7% hydrochloric acid (25 c.c.) for 10½ hours at 100°; $[\alpha]_D^{17°}$ were +121° (initial), +116° (1 hour), +95° (6 hours), and +82° (10½ hours). After the usual treatment with silver carbonate, hydrogen sulphide, and barium carbonate, a syrup was obtained which was exhaustively extracted with boiling ether, to give a syrup (*x*) (0·12 g.) and a residue (*y*) (0·40 g.) containing some inorganic material. (*x*) was examined by strip chromatography and on development with ammoniacal silver nitrate afforded two spots (R_G values 0·54 and 0·77, respectively). The mixed lactones (0·05 g.), prepared from the sugar, had b. p. 115—130°/0·01 mm., $n_D^{19°}$ 1·4635, and $[\alpha]_D^{16°}$ +20° (initial), +10° (8 hours), and +2° (constant value). They were apparently only δ-lactones. An amide (0·02 g.), prepared from the lactone (0·04 g.), had m. p. 147—148° (Found : C, 43·0; H, 7·85; N, 7·1; OMe, 28·2. Calc. for $C_7H_{15}O_5N$: C, 43·5; H, 7·7; N, 7·25; OMe, 32·1%). A mixed m. p. with an authentic sample of 3 : 4-dimethyl L-rhamnonamide (m. p. 144—145°) was 110—126°. The analytical figures suggested that the compound was a dimethyl pentonamide, but investigation of the unmethylated aldobiuronate suggested an arabinose structure. The dimethyl arabonolactones which could form δ-lactones are 2 : 3-, 2 : 4-, and 3 : 4-dimethyl arabinose. 2 : 3-Dimethyl arabonamide has m. p. 162°, and 2 : 4-dimethyl arabonamide m. p. 158°, neither figure being in agreement with the value found (147—148°). There remains the possibility that the 3 : 4-dimethyl arabopyranose linked through $C_{(2)}$ is involved in the structure of the methylated aldobiuronic acid.

The residue (*y*) was oxidised with bromine to the methylated mucic acid, esterified by boiling with methanolic hydrogen chloride (25 c.c.; 3%) for 23 hours, and neutralised by addition of silver carbonate; the filtrate was evaporated, giving a white residue which was exhaustively extracted with boiling ether. Concentration of these extracts gave a syrup (0·14 g.) which was distilled, having b. p. 120—135°/0·01 mm., $n_D^{19°}$ (1·4580 (OMe, 56·1%), and crystallising partly (m. p. 94—98°, unchanged on admixture with an authentic sample of the dimethyl ester of 2 : 3 : 4-trimethyl mucic acid).

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