320. *A Xylan from the Roots of Perennial Ryegrass* (Lolium perenne).

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Alkaline extraction of perennial ryegrass roots gave a polysaccharide of the xylan group. Hydrolysis of the methylated polysaccharide gave 2,3,5-tri- and 3,5-di-*O*-methyl-L-arabinose, 2,3,4-tri-, 2,3-di-, and 2-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose and 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methylglucopyranosyluronic acid)-xylose. Partial acid hydrolysis of the polysaccharide afforded a mixture of neutral oligosaccharides including 4-*O*-β-D-xylopyranosyl-D-xylose, 2-*O*-β-D-xylopyranosyl-L-arabinose, and *O*-D-galactopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 2)-L-arabinose, and an aldobiouronic acid. It is concluded that the polysaccharide is composed of chains of 1,4-linked β-D-xylopyranose residues to which are attached side-chains of single L-arabinofuranose units, and more complex *O*-D-galactopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 2)-L-arabinofuranose and possibly 2-*O*-β-D-xylopyranosyl-L-arabinofuranose units through position 3, and of 4-*O*-methylglucuronic acid units through position 2 of xylose residues.

The several polysaccharides of the xylan group whose detailed structures have received attention have been isolated from the aerial portions of land plants.[1] In continuation of our studies on the xylans from the *Gramineae* we report here an examination of a polysaccharide which has been isolated from the roots of perennial ryegrass (*Lolium perenne*).

Ryegrass roots were extracted successively with boiling ethanol–water (4 : 1) to remove colouring matter and soluble sugars, and with cold water to remove water-soluble polysaccharides, and the residue was delignified with acidified sodium chlorite solution. The hemicellulose component of the roots was isolated by alkaline extraction of the resulting holocellulose and, after reprecipitation, the isolated polysaccharide had 9·7% of uronic anhydride and gave on hydrolysis xylose (47%), arabinose (14%), glucose (8%), and galactose (2%). Attempts to fractionate the polysaccharide failed to effect a significant change in composition. However, since subsequent hydrolysis of the methylated polysaccharide afforded no glucose methyl ether, it is probable that the glucose was present as a constituent of a contaminating polysaccharide. The hexuronic acid residues were those of 4-*O*-methylglucuronic acid. After drastic hydrolysis an acidic component (probably an aldobiouronic acid) was isolated and separated chromatographically. By reduction of its methyl ester methyl glycosides with potassium borohydride and hydrolysis, the acidic component gave 4-*O*-methylglucose and xylose.

Hydrolysis of the derived methylated polysaccharide furnished 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-, 2,3-di-, and 2-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose and a partially methylated aldobiouronic acid. Small amounts of xylose and 3-*O*-methylxylose were also detected, the latter possibly arising from partial hydrolysis of the methylated aldobiouronic acid. Although insufficient of the partially methylated aldobiouronic

[1] Aspinall, *Adv. Carbohydrate Chem.*, 1959, **14**, 429.

acid was available for the complete characterisation of its cleavage products, the following experiments indicated that it was 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methylglucopyranosyluronic acid)-xylose. The methylated disaccharide was converted into the methyl ester methyl glycosides which were reduced with potassium borohydride. Hydrolysis of the partially methylated neutral disaccharide gave 2,3,4-tri-*O*-methylglucose and 3-*O*-methylxylose, identified by chromatography and ionophoresis. Complete methylation of the neutral disaccharide followed by hydrolysis furnished 2,3,4,6-tetra-*O*-methylglucose and 3,4-di-*O*-methylxylose. At a later stage of the investigation, after the partial acid hydrolysis products from the polysaccharide had been characterised and shown to contain 2-*O*-substituted L-arabinose residues, the methanolysis products from the methylated polysaccharide were examined by gas–liquid partition chromatography [2,3] and components having the retention times of the methyl glycosides of 3,5-di-*O*-methyl-L-arabinose, but not of 3,4-di-*O*-methyl-L-arabinose, were indicated in the mixture.

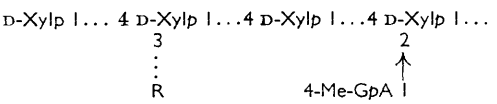
Partial acid hydrolysis of the polysaccharide furnished a mixture of neutral oligosaccharides which was fractionated by chromatography on charcoal–Celite, followed by partition chromatography on filter sheets to give four discrete components. 2-*O*-β-D-Xylopyranosyl-L-arabinose was characterised as the crystalline sugar and 4-*O*-β-D-xylopyranosyl-D-xylose by conversion into the crystalline phenylosazone. The third oligosaccharide gave xylose only on hydrolysis and was chromatographically indistinguishable from the polymer-homologous xylotriose. The fourth oligosaccharide was not isolated in sufficient quantity for complete characterisation by the formation of crystalline derivatives, but the following observations are consistent with its formulation as the trisaccharide *O*-β-D-galactopyranosyl-(1 ⟶ 4)-*O*-β-D-xylopyranosyl-(1 ⟶ 2)-L-arabinose. Hydrolysis of the sugar gave galactose, xylose, and arabinose, and hydrolysis of the derived glycitol (from borohydride reduction) gave galactose, xylose, and arabitol, showing arabinose to be the reducing residue. Partial acid hydrolysis gave a disaccharide with the chromatographic mobility of 2-*O*-β-D-xylopyranosyl-L-arabinose, but no second disaccharide could be detected. Periodate oxidation of the derived glycitol resulted in the consumption of 4·6 mol. of reagent with the release of 0·8 mol. of formaldehyde, pointing to the presence of a 2- or 4-*O*-substituted arabinose residue, whilst hydrolysis of the oxidised sugar showed the absence of unattacked sugar units. The sugar was methylated by the procedure of Kuhn *et al.*,[4] and the methanolysis products from the methylated derivative were examined by gas-chromatography on three stationary phases. Components having the retention times of the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3-di-*O*-methyl-D-xylose, and 3,4-di-*O*-methyl-L-arabinose were recognised. Since this technique permits the differentiation of the methyl glycosides of the various di-*O*-methyl-xyloses and -arabinoses,[2,3] there can be little doubt as to the identity of the cleavage products of the methylated trisaccharide. On the assumption that 2-*O*-β-D-xylopyranosyl-L-arabinose is formed on partial hydrolysis of the trisaccharide, the optical rotation ($[\alpha]_D$ +20°) of the sugar indicates that the D-galactopyranosyl residue has the β-configuration.

These results show that the polysaccharide contains main chains of 1,4-linked β-D-xylopyranose residues to which are attached a variety of units as side-chains. The isolation of the partially etherified aldobiouronic acid, 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methylglucopyranosyluronic acid)-xylose, from the methylated polysaccharide shows that the 4-*O*-methylglucuronic acid end-groups are present as single-unit side-chains attached by 1,2-linkages to the main chain. The isolation from the methylated polysaccharide of 2-*O*-methyl-D-xylose, which represents the main branching point in the molecule, shows that the arabinose-containing side-chains are joined to the basal chains by 1,3-linkages. Although no direct evidence is available in the case of this polysaccharide, it is probable that the L-arabinofuranose end groups in this as in other arabinoxylans [1] are present as

[2] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[3] Aspinall, *J.*, 1963, 1676.
[4] Kuhn, Trischmann, and Low, *Angew. Chem.*, 1955, **67**, 32.

single-unit side-chains. The polysaccharide also contains *O*-D-galactopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 2)-L-arabinofuranose units, and it is likely that these units are attached in a similar manner as side-chains. On the present evidence it is not certain whether the disaccharide, 2-*O*-β-xylopyranosyl-L-arabinose, arises from a further type of side-chain unit or whether it is formed only as a partial hydrolysis product from the trisaccharide unit. The mode of attachment of the more complex side-chain unit(s) to the basal xylan chain is not yet known, but it may be noted that evidence has now been obtained for the direct attachment of 2-*O*-β-D-xylopyranosyl-L-arabinofuranosyl side-chains to the backbone in barley-husk arabinoxylan.[5]

The annexed partial structure indicates the main features of this xylan from perennial ryegrass roots. The polysaccharide is similar to several other xylans from the *Gramineae* in respect of its main chain and single-unit side-chains. The xylan resembles those from barley husks,[6] maize cobs,[7] and esparto grass [8] in giving rise to 2-*O*-β-D-xylopyranosyl-L-arabinose as a partial hydrolysis product, although it is not yet known whether this disaccharide originates from identical sequences of sugar units in the parent polysaccharides. The presence of D-galactopyranose residues as integral constituents of a xylan is an unusual feature of this polysaccharide. In this respect this xylan resembles maize-fibre hemicellulose,[9–11] although the latter polysaccharide contains both D- and L-galactopyranose residues. It is of interest that maize-fibre xylan [9] affords, as a partial hydrolysis product, a similar trisaccharide to that isolated here, namely, *O*-L-galactopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 2)-L-arabinose, but that the D-galactopyranose end-group is replaced by that of the L-isomer. The xylan from the perennial



R = L-Araf ..., D-Galp I→4 D-Xylp I→2 L-Araf I..., or (possibly) D-Xylp I→2 L-Araf I....

ryegrass roots differs from those isolated from the leaves and stems of both perennial ryegrass (unpublished results) and cocksfoot grass [12] in containing more complex side-chains; these xylans contain only single-unit L-arabinofuranose and 4-*O*-methyl-D-glucuronic acid side-chains.

Experimental

Paper chromatography was carried out on Whatman nos. 1 and 3MM papers with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (D) benzene–ethanol–water (169 : 47 : 15, upper layer). R_G Values of methylated sugars refer to rates of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent C. Demethylations of methylated sugars were performed by the method of Hough, Jones, and Wadman.[13] Paper ionophoresis was in borate buffer at pH 10. Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas–liquid partition chromatography of the methyl glycosides of methylated sugars was carried out in a Pye argon chromatograph according to the procedure of Bishop and Cooper [2] (see also accompanying paper [3]). Separations were carried out on the following columns

[5] Aspinall and Ross, *J.*, 1963, 1681.
[6] Aspinall and Ferrier, *J.*, 1957, 4188.
[7] Whistler and McGilvray, *J. Amer. Chem. Soc.*, 1955, **77**, 2212; Whistler and Corbett, *ibid.*, p. 3822.
[8] Aspinall and Ferrier, *J.*, 1958, 1501.
[9] Whistler and Corbett, *J. Amer. Chem. Soc.*, 1955, **77**, 6328.
[10] Whistler and BeMiller, *J. Amer. Chem. Soc.*, 1956, **78**, 1163.
[11] Montgomery, Smith, and Srivastava, *J. Amer. Chem. Soc.*, 1957, **79**, 698; Srivastava and Smith, *ibid.*, p. 982.
[12] Aspinall and Cairncross, *J.*, 1960, 3877.
[13] Hough, Jones, and Wadman, *J.*, 1950, 1705.

(120 × 0·5 cm.) at gas flow rates of 80—100 ml./min.: (*a*) 20% by weight of Apiezon M on acid-washed Celite (80—100 mesh) at 150°; (*b*) 15% by weight of butane-1,4-diol succinate polyester [2] on acid-washed Celite at 150°; (*c*) 10% by weight of polyphenyl ether [*m*-di-(*m*-phenoxyphenoxy)benzene] on acid-washed Celite at 200°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Isolation of Perennial-ryegrass-roots Xylan.—Roots were shaken violently in acetone to dislodge adhering sand, cut into small pieces, and extracted with boiling ethanol–water (4 : 1) to remove soluble sugars and colouring matter, and then with cold water to remove water-soluble polysaccharides (mainly fructan). Batches (110 g.) of water-extracted roots were delignified with acidified sodium chlorite solution according to the procedure of Wise *et al.*[14] The resulting holocellulose (*ca.* 180 g.) was extracted twice with N-sodium hydroxide (2 l.) for 40 hr. in a ball-mill. The combined supernatant liquors were acidified to pH 5 with acetic acid, and the polysaccharide (20 g.) (Found: ash, 10·5%) was precipitated by the addition of acetone (1·5 vol.). The polysaccharide was reprecipitated from aqueous solution adjusted to pH 5 with acetic acid by addition of acetone (2 vol.), to give xylan (17·5 g.) (Found: ash, 3%) which was used in subsequent investigations. The xylan had $[\alpha]_D$ −100° (*c* 1·0 in 0·5N-sodium hydroxide) and uronic anhydride 9·7%, and hydrolysis, followed by paper-chromatographic separation in solvent A and estimation of the sugars in the hydrolysate,[15] gave xylose (47%), arabinose (14%), glucose (8%), and galactose (2%). Attempts to fractionate the polysaccharide by precipitation from aqueous solution with cupric acetate and ethanol, and by fractional precipitation of the acetylated polysaccharide from light petroleum–chloroform mixtures failed to give fractions differing significantly in composition.

Xylan (3 g.) was heated with N-sulphuric acid (150 ml.) on the boiling-water bath for 4 hr. The cooled solution was neutralised with barium carbonate, filtered, and concentrated to *ca.* 50 ml., treated with Amberlite resin IR-120(H) to remove barium ions, and poured on to a column of charcoal–Celite. Elution with water gave monosaccharides and only a trace of aldobiouronic acid, and elution with water containing 5% of butan-2-one gave aldobiouronic acid and small amounts of monosaccharides. Chromatographically pure aldobiouronic acid was obtained by separation on filter sheets in solvent A. The aldobiouronic acid was converted into methyl ester methyl glycosides, reduced with potassium borohydride, and hydrolysed to give xylose and 4-*O*-methylglucose (identified by chromatography of the sugar and of its periodate oxidation products [16]).

Preparation and Hydrolysis of Methylated Xylan.—The xylan (8 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide to give methylated xylan (2·84 g.), $[\alpha]_D$ −97° (*c* 1·0 in $CHCl_3$) (Found: OMe, 38·9%).

The methylated xylan (2·4 g.) was shaken overnight with methanolic 3% hydrogen chloride (300 ml.), and the resulting solution was refluxed for 6 hr. (to constant rotation). After removal of methanol under reduced pressure, water being added to maintain the volume of the solution, the solution was made N with respect to hydrochloric acid and heated on the boiling-water bath for 6 hr. (to constant rotation). The cooled solution was neutralised with silver carbonate, and the filtrate was treated with hydrogen sulphide to precipitate silver ions, filtered again, treated with barium carbonate into convert acids to barium salts, and concentrated to a syrup (2·09 g.). The syrup was absorbed on cellulose (70 × 3 cm.) and the column was eluted with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, and butan-1-ol, partly saturated with water, to give six fractions. A further fraction was obtained by elution with water.

Examination of Methylated Sugars.—Fraction 1. The syrup (194 mg.) had R_G 0·96 and $[\alpha]_D$ −18° (*c* 0·6) which corresponded to that of a mixture of 63% of 2,3,5-tri-*O*-methyl-L-arabinose ($[\alpha]_D$ −39·5°) and 37% of 2,3,4-tri-*O*-methyl-D-xylose ($[\alpha]_D$ +20°). The presence of these two sugars was indicated by paper chromatography in solvent D, and separation on filter sheets in this solvent gave fractions 1*a* and 1*b*. Demethylation of fraction 1*a* gave arabinose and the sugar was identified as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 133°. Demethylation of fraction 1*b* gave xylose and the sugar was identified as 2,3,4-tri-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. and mixed m. p. 100°.

[14] Wise, Murphy, and D'Addieco, *Paper Trade J.*, 1946, **122**, 35.
[15] Flood, Hirst, and Jones, *J.*, 1948, 1679.
[16] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

Fraction 2. The syrup (35 mg.), R_G 0·89 and $[\alpha]_D$ +116° (*c* 0·8), gave galactose on demethylation and was chromatographically indistinguishable from 2,3,4,6-tetra-*O*-methyl-D-galactose in solvents C and D. The sugar was characterised by conversion into the aniline derivative, m. p. and mixed m. p. 188—190°.

Fraction 3. Chromatography of the syrup (237 mg.) indicated tetra-*O*-methylgalactose, 2,3-di-*O*-methylxylose, and an unknown sugar, which was probably an ester of the partially methylated aldobiouronic acid (see fraction 7) since treatment with alkali resulted in the formation of the aldobiouronic acid. The syrup was heated in N-sodium hydroxide (10 ml.) at 50° for 30 min. and kept at room temperature for 3 hr. The solution was treated with Amberlite resin IR-120(H), and the isolated product was separated chromatographically on filter sheets by using solvent C, to give 2,3,4,6-tetra-*O*-methylgalactose, 2,3-di-*O*-methylxylose and partially methylated aldobiouronic acid in the proportion of 3 : 12 : 4. The sugars were added to the appropriate fractions for identification.

Fraction 4. The sugar (864 mg.), R_G 0·74, crystallised when seeded with 2,3-di-*O*-methyl-β-D-xylose, and had m. p. and mixed m. p. 80° and $[\alpha]_D$ +23·2° (equil.) (*c* 1·0). The sugar was further characterised by conversion into the aniline derivative, m. p. and mixed m. p. 121°, and into 2,3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 131—133°.

Fraction 5. The syrup (196 mg.) had R_G 0·38 and $[\alpha]_D$ +34·8° (*c* 1·0), which corresponded to that of a mixture of 94% of 2- and 6% of 3-*O*-methyl-D-xylose. The presence of these two sugars was indicated by paper ionophoresis. The main component was characterised as 2-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. and mixed m. p. 125—126°.

Fraction 6. The sugar (25 mg.) was chromatographically indistinguishable from D-xylose in solvents A and C, but an attempt to prepare the crystalline di-*O*-benzylidene dimethyl acetal was unsuccessful.

Fraction 7. The barium salt (101 mg.) was converted into the corresponding methylated aldobiouronic acid by treatment with Amberlite resin IR-120(H), and the resulting syrup was refluxed with boiling methanolic 5% hydrogen chloride for 4 hr. The product, after neutralisation with silver carbonate, was reduced with potassium borohydride (100 mg.) in water (10 ml.) for 3 hr. The excess of hydride was destroyed by the addition of dilute sulphuric acid, and extraction with chloroform furnished partly methylated disaccharide. A portion (5 mg.) of the syrup was hydrolysed with N-hydrochloric acid, and chromatography and ionophoresis showed 2,3,4-tri-*O*-methylglucose and 3-*O*-methylxylose. The remainder of the syrup was further methylated with methyl iodide and silver oxide to give a fully methylated disaccharide, hydrolysis of which gave two components which were chromatographically and ionophoretically indistinguishable from 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose.

Methanolysis of Methylated Xylan.—Methylated xylan (5 mg.) was heated in a sealed tube with methanolic 3% hydrogen chloride (2 ml.) for 12 hr. After neutralisation with silver carbonate, the resulting methyl glycosides were examined by gas chromatography on column *b*. The presence was indicated of methyl glycosides of the following sugars, 2,3,5-tri- (*T* 0·51, 0·69) and 3,5-di-*O*-methylarabinose (*T* 1·08, 2·76), 2,3,4-tri- (*T* 0·41, 0·55) and 2,3-di-*O*-methylxylose (*T* 1·50, 1·61, 1·86), and 2,3,4,6-tetra-*O*-methylgalactose (*T* 2·00).

Partial Acid Hydrolysis of the Xylan.—A suspension of the xylan (8 g.) was heated in 0·5N-sulphuric acid (1·8 l.) on the boiling-water bath for 30 min. Insoluble polysaccharide was separated at the centrifuge, and the cooled supernatant liquid neutralised with barium carbonate. The filtered solution was concentrated and degraded polysaccharide was precipitated by addition of ethanol (2 vol.). The combined precipitates were re-hydrolysed and the whole operation was repeated five times. The mother-liquors which remained after precipitations of degraded polysaccharides were combined, deionised, and concentrated to a syrup (3·6 g.). Chromatography of the syrup in solvent A showed xylose, arabinose, glucose, galactose, four discrete oligosaccharides (R_{xylose} 0·70, 0·64, 0·40, and 0·34), and traces of higher oligosaccharides. The syrup was adsorbed on charcoal–Celite (1 : 1; 45 × 2·5 cm.), and the column was eluted with water (1·5 l.) and with water containing 2% (1 l.), 5% (0·5 l. + 2 × 1 l.), 7% (1 l.), and 10% (2 l.) of ethanol. Fraction 1 (2·31 g.) contained monosaccharide only; fraction 2 (230 mg.) contained monosaccharides and traces of oligosaccharides 1 and 2; fraction 3 (84 mg.) contained oligosaccharides 1 and some oligosaccharides 2; fraction 4 (312 mg.) contained oligosaccharides 1 and 2; fraction 5 (94 mg.) contained oligosaccharides 1, 2, and 3 (traces); fraction 6 (56 mg.) contained oligosaccharides 2, 3, and 4; and fraction 7 (62 mg.) contained oligosaccharides 3 and

4, and higher oligosaccharides. Chromatographically pure samples of oligosaccharides 1—4 were obtained from fractions 3, 4, 6, and 7, respectively, by separation on filter sheets with solvents A and B.

Oligosaccharide 1. The sugar, R_{xylose} 0·70 in solvent A, $[\alpha]_D$ +28° (*c* 0·5), was chromatographically indistinguishable from 2-*O*-β-D-xylopyranosyl-L-arabinose in solvents A, B, and C, and crystallised. Although the sugar melted over a range (m. p. 84—92°), the m. p. was not depressed on admixture with 2-*O*-β-D-xylopyranosyl-L-arabinose trihydrate (m. p. 90—94°) and the identity of the sugar was also indicated by an *X*-ray powder photograph.

Oligosaccharide 2. The sugar, R_{xylose} 0·64 in solvent A, $[\alpha]_D$ −22° (*c* 0·5), gave only xylose on hydrolysis and was chromatographically indistinguishable from 4-*O*-β-D-xylopyranosyl-D-xylose. The sugar was characterised by conversion into the phenylosazone, m. p. and mixed m. p. 203—205° (dec.).

Oligosaccharide 3. The sugar, R_{xylose} 0·40 in solvent A, $[\alpha]_D$ +20° (*c* 0·2), gave galactose, xylose, and arabinose on hydrolysis, whereas the derived glycitol (from borohydride reduction) gave galactose, xylose, and arabitol. The sugar gave no colour reaction with triphenyltetrazolium hydroxide,[17] indicating the presence of a 2-*O*-substituted reducing residue. Partial hydrolysis of the sugar with 0·1N-sulphuric acid at 100° for 15 min. gave monosaccharide and 2-*O*-β-xylosylarabinose. The sugar (8 mg.) was reduced with potassium borohydride (15 mg.) in water (2 ml.) for 18 hr., and the solution was treated with Amberlite resin IR-120(H) to remove potassium ions and evaporated to dryness with methanol to remove boric acid as methyl borate. The resulting glycitol (6·5 mg.; quantity estimated by phenol–sulphuric acid reagent [18]) was oxidised with 0·15M-sodium metaperiodate (1 ml.). The consumption of reagent, which was measured spectrophotometrically,[19] after suitable dilution, was 4·6 moles per mole of glycitol. The formaldehyde present on completion of the oxidation was estimated by using the chromotropic acid reagent [20] and corresponded to 0·8 mole per mole of glycitol. Ethylene glycol (1 drop) was added to the remaining solution to destroy the excess of periodate, and sodium ions were removed with Amberlite resin IR-120(H). Iodic acid was neutralised with barium carbonate, and hydrolysis of the resulting solution with N-sulphuric acid gave on reducing sugars.

The sugar (10 mg.) was shaken in the dark with methyl iodide (1 ml.), *NN*-dimethylformamide (1 ml.), and silver oxide (2 g.) for 18 hr. The resulting methylated sugar was refluxed with methanolic 2% hydrogen chloride for 12 hr. and, after neutralisation with silver carbonate, the methyl glycosides were examined by gas-chromatography on columns *a*, *b*, and *c*. The methanolysis products, which were indicated, are shown in the Table.

	Relative retention times (*T*) of methyl glycosides					
	Column *a*		Column *b*		Column *c*	
Sugar	S	M	S	M	S	M
2,3,4,6-Tetra-*O*-methylgalactose ...	1·26m 1·38s	1·28 1·39	1·86sh 1·99	(1·87) 1·99	1·52sh 1·61	(1·53) 1·61
2,3-Di-*O*-methylxylose	0·45m	0·45	1·49m 1·60w	1·49 —	0·64m	0·64
	0·51s	0·51	1·86s	(1·87)	0·75s	0·75
3,4-Di-*O*-methylarabinose	0·64	0·65	2·26	2·26	0·99 1·52	1·00 (1·53)

S = Methyl glycosides from authentic sugars, M = methyl glycosides from methanolysis products from methylated trisaccharide, s = strong, m = medium, w = weak, sh = shoulder. Relative retention times shown in parentheses are those of components which were not resolved.

[17] Feingold, Avigad, and Hestrin, *Biochem. J.*, 1956, **64**, 351; Bailey, Barker, Bourne, Grant, and Stacey, *J.*, 1958, 1895.
[18] Dubois, Gillies, Hamilton, Rebers, and Smith, *Analyt. Chem.*, 1956, **28**, 350.
[19] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.
[20] McFadyen, *J. Biol. Chem.*, 1945, **158**, 107.

Oligosaccharide 4. The sugar, R_{xylose} 0·34 in solvent A, $[\alpha]_D$ −44° (*c* 0·2), gave xylose only on hydrolysis and was chromatographically indistinguishable from xylotriose.

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