211. *The Hemicelluloses of European Larch* (Larix decidua). *Part I. The Constitution of a Xylan.*

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Larch hemicellulose fractions are composed of residues of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose, and 4-*O*-methyl-D-glucuronic acid. From methylation and other experiments it is concluded that there is present a xylan containing unbranched chains of *ca.* 100 1 : 4-linked β-D-xylopyranose residues with every fifth or sixth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through position 2, and a smaller proportion of xylose residues carrying, on position 3, side-chains terminated by L-arabofuranose residues.

LARCHES are the only common deciduous conifers. They differ chemically from other coniferous woods in containing a much higher proportion of galactose-containing polysaccharides. The galactans are easily extracted with water and those from European [1] and Western [2] larches have been extensively studied. It was of interest to extend the investigations to the alkali-soluble hemicelluloses and this paper describes the structure of a xylan from European larch wood (*Larix decidua*).

Larch sawdust was extracted with hot water to remove ε-galactan and was then partially delignified with acidified sodium chlorite solution. The resulting holocellulose was extracted with 1% and 4% aqueous sodium hydroxide, to give hemicellulose fractions I and II, and with 10% sodium hydroxide to give fraction III (precipitated on acidification of the extract) and fraction IV (precipitated on subsequent addition of acetone). Hydrolysis and chromatography showed the various fractions to be composed of the same sugar residues but in different proportions. Fraction I contained mainly xylan and galactan, fraction II xylan together with appreciable amounts of glucomannan, and fractions III and IV mainly glucomannan with smaller amounts of xylan. Fractions I and II were fractionated by precipitation from water by ammonium sulphate,[3] to give fractions enriched with respect to xylan but still containing galactan or glucomannan.

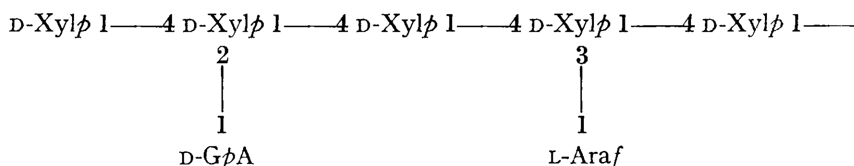
[1] Campbell, Hirst, and Jones, *J.*, 1948, 774; Jones, *J.*, 1953, 1692; Aspinall, Hirst, and Ramstad, *J.*, 1958, 593.
[2] White, *J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1507, 2838; Bouveng and Lindberg, *Acta Chem. Scand.*, 1956, **10**, 1515.
[3] Preece and Mackenzie, *J. Inst. Brewing*, 1952, **58**, 353, 457.

Since hydrolysis of the various hemicellulose fractions gave acidic components in addition to neutral sugars, a quantity of the unfractionated hemicellulose was hydrolysed and the acidic components were separated from the neutral sugars by absorption on an anion-exchange resin. The mixture of neutral sugars contained galactose, glucose, mannose, arabinose, and xylose, together with traces of rhamnose and two other sugars with greater chromatographic mobility. One of the last two sugars was tentatively identified by chromatography as 3-*O*-methylxylose. As far as we are aware this sugar has not previously been found to occur naturally, although the 2-methyl ether has recently been identified as a constituent of plum leaf hemicellulose.[4] The mixture of acidic components was eluted from the anion-exchange resin, and the three main components were separated by chromatography on filter sheets. The first fraction was identified by chromatography and optical rotation as 4-*O*-methyl-D-glucuronic acid. The second fraction was an *O*-(4-*O*-methyl-α-D-glucuronosyl)-D-xylose since the aldobiouronic acid had a high positive rotation ($[\alpha]_D$ +99°) and reduction of the derived methyl ester methyl glycoside with potassium borohydride followed by hydrolysis gave 4-*O*-methyl-D-glucose and D-xylose. The third fraction was probably an aldotriouronic acid since hydrolysis gave the aldobiouronic acid and xylose.

One of the xylan-rich fractions was methylated in the usual way and fractionation of the methylated polysaccharide gave a methylated xylan containing only traces of a methylated hexosan. Hydrolysis of the methylated xylan furnished 2 : 3-di-*O*-methyl-D-xylose and a methylated aldobiouronic acid, together with small amounts of 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methyl- and 2- and 3-*O*-methyl-xylose, which were only detected chromatographically. The structure of the methylated aldobiouronic acid was shown by the following experiments to be 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-α-D-glucuronosyl)-D-xylose. The acidic disaccharide was converted into the methyl ester methyl glycoside which was reduced with lithium aluminium hydride. Hydrolysis of part of the partially methylated disaccharide gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose. The remaining material was remethylated and hydrolysis of the fully methylated disaccharide gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-D-xylose. From the quantities of the major components isolated from the hydrolysis of the methylated polysaccharide it may be concluded that this xylan contains chains of 1 : 4-linked β-D-xylopyranose residues with approximately every fifth xylose residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue attached as a side-chain to position 2.

In order to obtain more detailed information regarding the fine structure of larch xylans a second series of methylation studies was carried out. The methylated xylan with uronic acid residues present as the sodium salts was readily separated from the methylated polysaccharides without uronic acid residues. The acidic groups were reduced with lithium aluminium hydride, and the reduced methylated xylan was remethylated. Hydrolysis of the methylated reduced xylan afforded 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4-tri-*O*-methyl-D-glucose, 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 2- and 3-*O*-methyl-D-xylose in the approximate molar ratio of 6 : 12 : 5 : 1 : 72 : 20 : 5. The tetra- and tri-*O*-methyl-D-glucose arise from the D-glucuronic acid residues in the xylan, not all tri-*O*-methyl-D-glucose residues being completely remethylated after the reduction. From the previous results it is clear that the 3-*O*-methyl-D-xylose arises from branching points in the main chain to which the uronic acid groups are attached. Since 3-*O*-methyl-D-xylose and the mixture of methyl ethers of D-glucose are present in approximately equimolecular amounts, and since 2-*O*-methyl-D-xylose and 2 : 3 : 5-tri-*O*-methyl-L-arabinose also occur in approximately the same molar proportions, it is probable that the 2-methyl ether arises from branch points to which terminal L-arabofuranose residues are attached. The accompanying partial structure for the xylan indicates the main features of the molecule.

[4] Andrews and Hough, *Chem. and Ind.*, 1956, 1278.



(D-Xyl*p* = D-xylopyranose, D-G*p*A = 4-*O*-methyl-D-glucuronic acid, and L-Ara*f* = L-arabofuranose)

It is not possible on the present evidence to indicate whether the L-arabofuranose residues are attached directly to the backbone of xylose residues as shown or whether 1 : 4-linked D-xylose residues are interposed with the arabinose residues terminating a longer side-chain. The former alternative is more probable as other xylans (from, *e.g.*, wheat straw [5] and barley husks [6]) are known to contain L-arabofuranose residues directly linked to the backbone of xylose residues.

A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood) gave a value of 18,000 $\pm$ 500 (degree of polymerisation, 107 $\pm$ 3) for the methylated xylan (as methyl ester). This value, taken together with the value of one non-reducing xylose end group per *ca.* 120 sugar residues, suggests that the backbone of xylose residues is unbranched. It is concluded, therefore, that this xylan fraction contains chains of 1 : 4-linked β-D-xylopyranose residues with, on the average, every fifth or sixth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through $C_{(2)}$. In addition, a small proportion (*ca.* 4%) of L-arabofuranose residues are present as integral parts of the xylan; these are present as non-reducing end-groups and are probably attached to the backbone through $C_{(3)}$ of xylose residues. In the first methylated xylan fraction, which we examined, the proportion of arabinose residues was very low ($<$1%) and it is probable that some of the xylan chains carried no arabinose residues. It is clear that in larch wood, as in monocotyledenous plants,[7] several closely related xylans occur side by side.

Several wood xylans have now been examined and all are characterised by the presence of 4-*O*-methyl-D-glucuronic acid residues attached as side-chains to D-xylose by 1 : 2-linkages. The proportions of uronic acid groups are in general somewhat higher in the xylans from soft woods (15—20%) (Western hemlock,[8] Norway spruce,[9] and larch) than in those from hard woods (8—15%) European [10] and North American [11] beech, birch,[12] and aspen [13]). Some of these xylans (Western hemlock, aspen, and larch) also contain a small proportion of L-arabofuranose residues. In contrast, the xylans from cereals [7] are, in general, characterised by a higher proportion of arabinose groups and a lower proportion of uronic acid groups. Despite considerable variations in detailed molecular architecture, however, it is not possible to draw a clear line of demarcation on structural grounds between the xylans from different lignified tissues.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 and 3MM filter papers with the following solvent systems (v/v): (A) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (B) ethyl acetate–pyridine–water (10 : 4 : 3); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (D) benzene–ethanol–water (169 : 47 : 15, upper layer); (E) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (F) butan-2-one, saturated with water containing

[5] Bishop, *J. Amer. Chem. Soc.*, 1956, **78**, 2840.
[6] Aspinall and Ferrier, *J.*, 1957, 4188.
[7] Hirst, *J.*, 1955, 2974.
[8] Dutton and Smith, *J. Amer. Chem. Soc.*, 1956, **78**, 2505.
[9] Aspinall and Carter, *J.*, 1956, 3744.
[10] Aspinall, Hirst, and Mahomed, *J.*, 1954, 1734.
[11] Adams, *Canad. J. Chem.*, 1957, **35**, 556.
[12] Saarnio, Wathén, and Gustafsson, *Acta Chem. Scand.*, 1954, **8**, 825.
[13] Jones, Merler, and Wise, *Canad. J. Chem.*, 1957, **35**, 634.

1% of ammonia; (G) pentan-2-one, 75% saturated with water; (H) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer). Methylated sugars were demethylated with hydrobromic acid.[14] Optical rotations were observed at 18° ± 2°. Extractions and reactions involving the use of alkali were performed, as far as possible, under nitrogen. In typical extractions larch holocellulose samples (*ca.* 80 g.) were extracted with alkali (*ca.* 1 l.) in a ball-mill for 12 hr. at room temperature.

Extraction and Fractionation of Larch Hemicellulose.—Larch sawdust was extracted successively with boiling benzene and boiling methanol to remove fats and colouring material, and then with water at 90° to remove ε-galactan. The residual sawdust was delignified with acidified sodium chlorite solution according to Wise's procedure.[15] The resulting holocellulose was extracted three times each with 1%, 4%, and 10% sodium hydroxide solutions to give fraction I (6% by weight of the air-dried sawdust), II (4·6%), III (4·0%), and IV (5·0%). Fractions I and II were isolated by acidification of the alkaline extracts with acetic acid (to pH 4—5) and precipitation with an equal volume of acetone. Fraction III was precipitated from the 10% sodium hydroxide extract on acidification, and fraction IV was precipitated on addition of an equal volume of acetone to the supernatant liquid after removal of fraction III. Fraction I, by precipitation from aqueous solution on the stepwise addition of ammonium sulphate, gave a xylan-rich fraction I*a* (precipitated with 50% ammonium sulphate) and galactan-rich fractions I*b* and I*c* (precipitated with 30% and 60% ammonium sulphate). Refractionation of fraction I*a* failed to yield xylan free from galactan. Fraction II was refractionated in a similar way, giving fractions II*a* and II*b* (precipitated with 30 and 50% ammonium sulphate). After five reprecipitations with ammonium sulphate fraction II*b* gave xylan A (1·6%), which was still contaminated with glucomannan. Fraction III was separated into fractions III*a* (1·0%) and III*b* (3·0%), soluble and insoluble in 2% aqueous sodium hydroxide. Fraction III*b* was further separated into fractions III*c* (0·3%), insoluble in 10% sodium hydroxide, III*d* (2·0%), soluble in 10% sodium hydroxide and precipitated on acidification, and III*e* (0·7%), soluble in 10% sodium hydroxide and precipitated, after acidification and removal of III*d*, by the addition of acetone. Fraction IV was separated into fractions IV*a* (4·3%) and IV*b* (0·7%), soluble and insoluble in water.

Chromatography [16] in solvent B of the hydrolysate from xylan A (Found: uronic anhydride, 11·7%) showed xylose (46·5%), mannose + arabinose (18%), glucose (9%), and galactose (3%).

Fraction III*c* (0·5 g.) in acetic anhydride (5 ml.), acetic acid (5 ml.), and concentrated sulphuric acid (0·28 ml.) was kept at room temperature for 7 days. The mixture was poured into ice-water (50 ml.), and the chloroform extract (2 × 25 ml.) was dried and concentrated. The resulting sugar acetates were deacetylated with barium methoxide in methanol and the sugars were examined by chromatography in solvent B. In addition to glucose and traces of mannose, xylose, and galactose, oligosaccharides having the same mobilities as cellobiose and cellotriose were detected. Fraction III*c* was probably a degraded form of cellulose which was extracted together with glucomannan (cf. Aspinall, Laidlaw, and Rashbrook [17]).

Samples of the various hemicellulose fractions were hydrolysed and the relative proportions of the sugars detected on paper chromatograms are indicated in the Table.

Hydrolysis of Larch Hemicellulose and Examination of the Acidic Components.—Hemicellulose (fractions I and II; 30 g.) in N-sulphuric acid (700 ml.) was heated on the boiling-water bath for 13·5 hr. (constant rotation). The solution was neutralised with barium hydroxide and barium carbonate, then filtered, and the barium salts were washed with water (3 × 30 ml.). The combined filtrate and washings were freed from barium ions by passage through a column of Amberlite resin IR-120(H) and the acids were adsorbed on a column of Amberlite resin IR-4B (OH). The eluate was concentrated to a syrup (20 g.), and chromatography showed galactose, glucose, mannose, arabinose, and xylose together with traces of rhamnose and two faster-moving sugars. One of the last two sugars was chromatographically similar to 3-*O*-methyl-D-xylose, but distinct from the 2-methyl ether, and gave xylose on demethylation.

The acids were displaced from the resin with N-sulphuric acid, and the eluate was neutralised with barium carbonate, filtered, freed from barium ions with Amberlite resin IR-120(H), and concentrated to a syrup (1·2 g.). Chromatography in solvent A showed three main components

[14] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[15] Wise, *Ind. Eng. Chem. Analyt.*, 1945, **17**, 63.
[16] Flood, Hirst, and Jones, *J.*, 1948, 1679.
[17] Aspinall, Laidlaw, and Rashbrook, *J.*, 1957, 4444.

having R_{xylose} 1·26, 0·69, and 0·17, and traces of sugars having R_{xylose} 1·60 (probably glucurone) and 0·30. Pure samples of the major acidic sugars were obtained by chromatography on filter sheets with solvent A. Fraction *a* (110 mg.) had $[\alpha]_D$ +83° (*c* 1·1 in H_2O) and was chromatographically indistinguishable from 4-*O*-methyl-D-glucuronic acid (R_{xylose} 1·26). Fraction *b* (231 mg.) had R_{xylose} 0·69 and $[\alpha]_D$ +99° (*c* 1·15 in H_2O). The acid was converted into the methyl ester methyl glycoside (185 mg.) which was treated with potassium borohydride (400 mg.) in water (10 ml.) for 18 hr. Excess of hydride was destroyed by the addition of acetic

Hemicellulose fraction	Method of isolation	Paper chromatography					
		Man	Glu	Gal	A	Xyl	UA
I	Extrn. with 1% NaOH	+	+	+++	++	+++	++
I*a*	Ammonium sulphate	+	+	+	+	+++	++
I*b* and I*c*	Pptn.	—	—	+++	++	+	tr
II	Extrn. with 4% NaOH	++	+	+	+	+++	++
II*a*	Ammonium sulphate	+++	++	+	tr	+++	++
II*b*	Pptn.	++	+	+	tr	+++	++
III	Extrn. with 10% NaOH	+++	++	+	—	++	+
III*a*	Sol. in 2% NaOH	+++	++	+	—	+++	++
III*b*	Insol. in 2% NaOH	+++	++	+	—	+	tr
III*c*	Insol. in 10% NaOH	tr	+++	tr	—	tr	—
III*d*	Sol. in 10% NaOH	+++	++	tr	—	tr	—
III*e*	Sol. in 10% NaOH	+++	++	+	—	+	tr
IV	Extrn. with 10% NaOH	+++	++	+	—	++	+
IV*a*	Sol. in H_2O	+++	++	+	—	+++	++
IV*b*	Insol. in H_2O	+++	++	+	—	+	tr

(Man = mannose, Glu = glucose, Gal = galactose, A = arabinose, Xyl = xylose, and UA = uronic acid. tr = trace.)

acid, and the solution was de-ionised by passage through columns of Amberlite resins IR-120(H) and IR-4B(OH) and concentrated to a syrup (140 mg.). The syrup (135 mg.) was hydrolysed with 0·8N-sulphuric acid (5 ml.) at 100° for 5 hr. After neutralisation with barium carbonate the resulting syrup (106 mg.) was separated on filter sheets with solvent H, to give fractions *d* (43 mg.) and *e* (35 mg.). Fraction *d* had $[\alpha]_D$ +54° (*c* 0·85 in H_2O) and was identified as 4-*O*-methyl-D-glucose by conversion into the phenylosazone, m. p. 149—152°, characterised by circular paper chromatography and by *X*-ray powder photography. Fraction *e* had $[\alpha]_D$ +20° (*c* 0·70 in H_2O) and was identified as D-xylose by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 210°. The acid fraction *c* (150 mg.) had R_{xylose} 0·17 and on hydrolysis gave xylose and the aldobiouronic acid having R_{xylose} 0·69.

Preparation of Methylated Xylan A, Hydrolysis, and Separation of Methylated Sugars.— Xylan A (10 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide to give methylated polysaccharide (3·5 g.), $[\alpha]_D$ −51·5° (*c* 0·34 in $CHCl_3$) (Found: OMe, 40·1%). A sample of this material was hydrolysed and chromatography of the hydrolysate showed methylated hexoses in addition to methylated pentoses and acidic components. The methylated polysaccharide was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—65°) mixtures and three main fractions were obtained, (1) [0·40 g., soluble in chloroform–light petroleum (1 : 4); OMe, 45·0%], (2) [0·95 g., soluble in chloroform–light petroleum (1 : 3); OMe, 38·5%], and (3) [1·0 g., soluble in chloroform–light petroleum (3 : 7); OMe, 37·9%]. Samples of these fractions were hydrolysed and the hydrolysates were examined chromatographically, fraction 1 giving methylated hexoses and traces of methylated pentoses, and fractions 2 and 3 giving methylated pentoses together with acidic components and traces of methylated hexosis. Fractions 2 and 3 were combined, dissolved in chloroform, and precipitated by light petroleum, to give methylated xylan A (1·8 g.), $[\alpha]_D$ −61° (*c* 0·23 in $CHCl_3$) (Found: OMe, 38·1%).

Methylated xylan A (1·5 g.) was hydrolysed successively with boiling methanolic 5% hydrogen chloride (170 ml.) for 8 hr. and with 0·5N-hydrochloric acid (100 ml.) at 100° for 12·5 hr. The cooled solution was neutralised with silver carbonate, hydrogen sulphide was passed through the filtrate to precipitate silver salts, and the solution was concentrated to a syrup. The syrup was dissolved in water, the acidic components were converted into barium salts by treatment with barium carbonate, and the solution was taken to dryness. The resulting syrup was extracted with chloroform to give syrup A (1·01 g.) and an insoluble residue, which was dissolved in water, passed through a column of Amberlite resin IR-100(H) to remove barium ions, and concentrated to give acidic fraction B (139 mg.).

Syrup A (1·01 g.) was fractionated on cellulose (40 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol half saturated with water as eluants, to give six fractions. Fractions 1—3 contained only neutral sugars. Fractions 4 and 6 contained only acidic components; these were combined and after removal of barium ions with Amberlite resin IR-100(H) gave acidic fraction C (116 mg.). Fraction 5 contained neutral and acidic sugars.

Examination of Neutral Sugars.—Chromatography of fraction 1 (26·5 mg.) in solvent C showed only one component (R_G 0·95). Since the syrup did not crystallise a small sample was rehydrolysed and a second component (R_G 0·73 in solvent C) was observed. The remainder of the syrup was rehydrolysed and chromatography in solvent D showed three sugars, 2 : 3 : 5-tri-*O*-methylarabinose, 2 : 3 : 4-tri-*O*-methylxylose, and 2 : 3-di-*O*-methylxylose. The proportions of the sugars were estimated by Pridham's method,[18] and the result indicated the presence in the original syrup of tri-*O*-methylxylose (10 mg.), tri-*O*-methylarabinose (5·5 mg.), and methyl di-*O*-methylxyloside (11 mg.). Fraction 2 (20 mg.) contained tri-*O*-methylhexose (R_G *ca.* 0·80 in solvent C) and on demethylation gave mannose, glucose, and a trace of galactose. Fraction 3 (404 mg.) crystallised on being seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. 78° and $[\alpha]_D$ +23° (equil.) (*c* 0·82 in H_2O) (Found: OMe, 35·1. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The identity of the sugar was confirmed by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 122—123°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. 132°. Fraction 5 (32 mg.) contained mono-*O*-methylxylose and a small amount of an acidic component and had $[\alpha]_D$ +44° (*c* 1·43 in H_2O). The neutral sugars (28 mg.) were separated from the acid on a filter sheet by using solvent C, and paper ionophoresis showed 2- and some 3-*O*-methylxylose. The syrupy mixture of methyl pyranosides, prepared by refluxing the sugars with dry methanolic hydrogen chloide, consumed 0·20 mol. of periodate (spectrophotometric determination [19] carried out by Dr. R. J. Ferrier), showing that the 2- and the 3-methyl ether were present in the ratio of 1 : 4.

Examination of the Acidic Fractions.—Chromatography of acidic fractions B and C in solvent E showed both to contain a main component and a second component moving slightly more slowly. On vigorous hydrolysis both fractions gave 2 : 3 : 4-tri-*O*-methylglucuronic acid, mono-*O*-methylxylose, and a trace of 2 : 3-di-*O*-methylxylose. Fraction C (95 mg.) was refluxed with methanolic 1% hydrogen chloride (15 ml.) for 6 hr. The product, after neutralisation with silver carbonate, was dissolved in methylal (15 ml.), lithium aluminium hydride (100 mg.) was added, and the solution was refluxed for 2 hr. Excess of hydride was destroyed by water, and the methylal layer was separated and concentrated. The aqueous layer was taken to dryness and the residue was extracted with acetone. The solid residue was suspended in water (25 ml.), shaken with Amberlite resin IR-100(H), taken to dryness, and extracted again with acetone. The combined extracts (70 mg.) were hydrolysed with 0·8N-hydrochloric acid (14 ml.) at 100° for 6 hr., and the hydrolysate (45 mg.) was separated on a filter sheet by using solvent E, to give fractions *a* (19 mg.) and *b* (7 mg.). Fraction *a* had $[\alpha]_D$ +50° (±3°) and was chromatographically identical with 2 : 3 : 4-tri-*O*-methyl-D-glucose. Fraction *b* was chromatographically and ionophoretically indistinguishable from 3-*O*-methyl-D-xylose and gave xylose on demethylation. Acidic fraction B (125 mg.), after conversion into methyl ester methyl glycoside, was reduced in a similar way, and the product was methylated with methyl iodide and silver oxide. The fully methylated disaccharide (95 mg.) was hydrolysed with 0·8N-hydrochloric acid (15 ml.) at 100° for 6 hr., and the hydrolysis products were separated on filter sheets with solvent E to give fractions *c* (53 mg.) and *d* (33 mg.). Fraction *c* had $[\alpha]_D$ +80° (±2°) and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 135—137°. Chromatography and ionophoresis of fraction *d* showed only 3 : 4-di-*O*-methylxylose, and the sugar was characterised by conversion into 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 65·5—66·5°.

Preparation of Methylated Reduced Xylan B.—Larch hemicellulose (fraction II; 20 g.) was methylated with methyl sulphate and sodium hydroxide, and the crude methylated polysaccharide which was isolated was separated into fractions C and D, respectively soluble and insoluble in acetone. The insoluble residue (D) was dispersed in acetone–water (1 : 1), further acetone (3 vol.) was added, inorganic salts, which separated, were filtered off, the filtrate was taken to dryness, and the residue was separated into fractions E and F, respectively soluble and

[18] Pridham, *Analyt. Chem.*, 1956, **28**, 1967.
[19] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.

insoluble in boiling chloroform. Fractions C and E were combined, dissolved in chloroform, and precipitated by the addition of light petroleum. This precipitate (7·5 g.) was probably a mixture of methylated glucomannan and methylated xylan (in the acid form) since chromatography of the hydrolysate showed methylated hexoses, methylated pentoses, and acidic components. The chloroform-insoluble residue (F) was dispersed in ethanol–water (9 : 1), and addition of ether precipitated methylated xylan B (12·0 g.; as sodium salt). Chromatography of the hydrolysate showed methylated pentoses together with acidic components, but no methylated hexoses. Methylated xylan B (11·9 g.; as sodium salt) in acetone–water (400 ml., 1 : 1) was shaken with Amberlite resin IR-120(H) for 30 min., further acetone (200 ml.) being added to maintain solution. Acetone was removed from the filtrate under reduced pressure at 15°, the aqueous dispersion was extracted with chloroform, and the dried extract was concentrated and poured into light petroleum to give methylated xylan B (7·9 g., as free acid). The methylated xylan acid (7·9 g.) was treated with methyl iodide and silver oxide, giving the methylated xylan methyl ester (7·3 g.), $[\alpha]_D$ −52° (*c* 0·89 in $CHCl_3$) (Found: OMe, 39·3%). The methylated polysaccharide (7·1 g.) in refluxing tetrahydrofuran (330 ml.) was reduced by lithium aluminium hydride (1·4 g.) for 3 hr. After destruction of excess of hydride by water the mixture was taken to dryness and the methylated polysaccharide was isolated by extraction with acetone. After a second reduction with lithium aluminium hydride, the product was remethylated with methyl iodide and silver oxide, to give methylated reduced xylan B (5·2 g.), $[\alpha]_D$ −52° (*c* 0·40 in $CHCl_3$) (Found: OMe, 37·2%).

Hydrolysis of Methylated Reduced Xylan B and Separation of Methylated Sugars.—0·5N-Hydrochloric acid was added slowly to methylated reduced xylan B (4·5 g.) in boiling methanol (300 ml.). The rates of addition of acid and of distillation of methanol were adjusted so that complete solution was maintained, acid (650 ml.) being added during 10 hr. while 630 ml. of distillate were collected. Water (100 ml.) was added and the solution (N with respect to hydrochloric acid) was heated at 100° for 4 hr. (constant rotation). The cooled solution was neutralised with silver carbonate and concentrated to a syrup (4·8 g.) which was fractionated on cellulose (70 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water, and butan-1-ol half saturated with water as eluants, to give four fractions.

Fraction 1. Chromatography of the syrup (500 mg.) in solvents D and G showed 2 : 3 : 4 : 6-tetra-*O*-methylglucose, 2 : 3 : 5-tri-*O*-methylarabinose, and 2 : 3 : 4-tri-*O*-methylxylose. Since hydrolysis of a sample gave small amounts of di- and mono-*O*-methylxylose (presumably derived from methyl glycosides) the syrup (*ca.* 480 mg.) was rehydrolysed with 0·5N-hydrochloric acid (50 ml.) at 100° for 5 hr., and the hydrolysate (433 mg.) was refractionated on cellulose (80 × 2 cm.) with the same solvents, to give six fractions. Fraction 1*a* (23 mg.) had m. p. 72—75° and $[\alpha]_D$ +81° (equil.) (*c* 0·45 in H_2O) and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 134—135°. Fraction 1*b* (178 mg.) contained 2 : 3 : 4 : 6-tetra-*O*-methylglucose and 2 : 3 : 5-tri-*O*-methylarabinose in the ratio of 0·73 : 1 (estimated by Pridham's method [18]). A sample (42 mg.) of 2 : 3 : 5-tri-*O*-methyl-L-arabinose, $[\alpha]_D$ −29° (*c* 0·42 in H_2O), was separated on filter sheets with solvent G and identified by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. 137—138°. Fraction 1*c* (27 mg.), $[\alpha]_D$ +19° (*c* 0·27 in H_2O), was chromatographically indistinguishable from 2 : 3 : 4-tri-*O*-methyl-D-xylose but attempts to prepare the aniline derivative failed. Fractions 1*d* (9 mg.), 1*e* (15 mg.), and 1*f* (9 mg.) were shown by chromatography to contain 2 : 3 : 4-tri-*O*-methylglucose, and 2 : 3-di- and mono-*O*-methylxylose respectively.

Fraction 2. The chromatographically pure syrup (492 mg.) (Found: OMe, 41·8. Calc. for $C_9H_{18}O_6$: OMe, 41·9%) had $[\alpha]_D$ +67° (*c* 0·82 in H_2O) and was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 144—145°.

Fraction 3. The sugar (2·325 g.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. 70—75° (Found: OMe, 34·5. Calc. for $C_7H_{14}O_5$: OMe, 34·9%). The sugar was identified by conversion into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 130—132°.

Fraction 4. Chromatography (solvent F) and ionophoresis of the syrup (730 mg.) disclosed 2- and 3-*O*-methylxylose. The optical rotation {$[\alpha]_D$ +23·0° (*c* 1·46 in H_2O)} of the syrup corresponded to that of a mixture of 2-*O*-methyl-D-xylose [20] ($[\alpha]_D$ +35·9°) and 3-*O*-methyl-D-xylose [10] ($[\alpha]_D$ +19·5°) in the ratio of 1 : 4. The periodate consumed (0·19 mol.) (spectrophotometric determination [19]) by the derived syrupy mixture of methyl pyranosides indicated that 19% of the 2-methyl ether was present in the mixture. The syrup (500 mg.) was

[20] Robertson and Speedie, *J.*, 1934, 824.

refractionated on cellulose (40 × 3 cm.) with solvent R, to give three fractions. Fraction 4*a* (312 mg.), $[\alpha]_D$ +19° (*c* 0·69 in H_2O), was chromatographically and ionophoretically pure, and was identified as 3-*O*-methyl-D-xylose by conversion into the phenylosazone, m. p. and mixed m. p. 170—171°. Fraction 4*b* (45 mg.) contained mainly 3-*O*-methylxylose with small amounts of the 2-methyl ether. Fraction 4*c* (89 mg.), $[\alpha]_D$ +33° (*c* 0·36 in H_2O), contained mainly the 2-methyl ether with small amounts of the 3-methyl ether. 2-*O*-Methyl-D-xylose was identified by conversion into the aniline derivative, m. p. and mixed m. p. 125—126°.

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