117. *The Hemicelluloses of Loblolly Pine* (Pinus taèda) *Wood. Part II.* The Constitution of Hexosan and Pentosan Components.*

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A copper-complexing hemicellulose fraction (I) from loblolly pine wood has been divided into a subfraction (II) containing residues of galactose, glucose, and mannose in the ratio 1 : 7 : 19, and a subfraction (III) containing residues of xylose and 4-*O*-methylglucuronic acid in the ratio 6 : 1.

Hydrolysis of methylated (II) yielded 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (1 part), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose (1 part), the 2 : 3 : 6-tri-*O*-methyl ethers of D-mannose and D-glucose (30 parts), and di-*O*-methyl hexoses (*ca.* 1 part).

Hydrolysis of methylated (III) gave 2 : 3 : 4-tri-*O*-methyl-D-xylose (3 parts), 2 : 3-di-*O*-methyl-D-xylose (60 parts), 3-mono-*O*-methyl-D-xylose (3 parts), 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid (1 part), and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-α-D-glucuronosyl)-D-xylose (10 parts). These and other results are discussed.

Part I of this series described the isolation from loblolly pine (*Pinus taèda* L.) wood of a hemicellulose fraction (I) containing residues of galactose, glucose, mannose, xylose, and 4-*O*-methylglucuronic acid. Structural features of its constituent polysaccharides were discussed in the light of partial acid-hydrolysis. A similar hemicellulose fraction has now been divided into a subfraction (II) containing residues of galactose, glucose, and mannose only, and a subfraction (III) containing residues of xylose and 4-*O*-methylglucuronic acid only.

Addition of Fehling's solution to an alkaline extract of loblolly pine sawdust yielded

* Part I, Jones and Painter, *J.*, 1957, 669.

an insoluble copper-complex, from which the hemicellulose fraction (I) was regenerated. Acetylation of this fraction with acetic anhydride and anhydrous zinc chloride yielded a mixture of polysaccharide acetates, which was dissolved in chloroform. Agitation of this solution with ice-cold, aqueous sodium carbonate caused part of it to form a stable emulsion, which was separated. From the remaining chloroform solution, a mixture of neutral polysaccharide acetates was recovered, and deacetylated to yield subfraction (II). Acidification of the emulsion, followed by extraction with chloroform, led to the recovery of a mixture of acidic polysaccharide acetates, which was deacetylated to yield subfraction (III).

Subfraction (II) had $[\alpha]_D$ — 20° in dilute alkali, and during hydrolysis with aqueous formic acid, the specific rotation (calculated as anhydrohexose) rose from —21° to a constant value of +21° in 10 hr. The hydrolysate was separated chromatographically into galactose, glucose, and mannose components; estimation of these by periodate oxidation [1] indicated that they were present, respectively, in the ratio 1 : 7 : 19.

On periodate oxidation, subfraction (II) consumed 1·02 moles of periodate per anhydrohexose unit, with the concomitant liberation of 0·133 mole of formic acid. Electrophoresis of the material on fibre-glass sheets [2] caused it to travel as a single, elongated spot, which was located by the method of Cifonelli and Smith.[3] The intrinsic viscosity of a solution of subfraction (II) in M-cupriethylenediamine was 0·12 dl./g.

Subfraction (III) had $[\alpha]_D$ —20° in dilute alkali, and yielded three sugars when hydrolysed with dilute sulphuric acid. These were chromatographically indistinguishable from xylose, 4-*O*-methylglucuronic acid, and 2-*O*-(4-*O*-methyl-α-D-glucuronosyl)-D-xylose. Iodometric titration of the three sugars indicated that subfraction (III) contained residues of xylose and 4-*O*-methylglucuronic acid respectively in the ratio 6 : 1.

The fully methylated derivatives of subfractions (II) and (III) were prepared as follows. Hemicellulose fraction (I) was methylated first by prolonged exposure to diazomethane, and then with methyl sulphate and alkali. The partly methylated product (OMe, 33%) was dissolved in aqueous alkali, and the solution was extracted with chloroform. The extract contained a small proportion of methylated pentosan (furfuraldehyde test), which was removed by the selective emulsification procedure described above, to furnish pure, partly methylated (II). The remaining alkaline solution was acidified and extracted again with chloroform, to furnish pure, partly methylated (III). Remethylation of the two products separately then gave the two fully methylated derivatives.

After hydrolysis with aqueous formic acid, the specific rotation of methylated (II) rose from —18·0° to a constant value of approximately +10°. The hydrolysate was resolved into four chromatographically distinct fractions (*a*, *b*, *c*, and *d* respectively) on filter-paper sheets. Fractions *a* and *b* contained the 2 : 3 : 4 : 6-tetra-*O*-methyl ethers of D-mannose and D-galactose respectively; these were characterised by conversion into their corresponding *N*-phenylglycosylamines. Fraction *c* could not be readily resolved on paper chromatograms, but application of selective methyl furanoside formation according to Reberr and Smith's directions [4] led to the isolation of 2 : 3 : 6-tri-*O*-methyl-D-mannose and 2 : 3 : 6-tri-*O*-methyl-D-glucose from this fraction; these sugars were characterised as their 1 : 4-di-*O*-*p*-nitrobenzoates. Fraction *d* consisted of a mixture of di-*O*-methylhexoses, none of which was positively identified. By weighing the respective fractions after isolation, it was estimated that *a*, *b*, *c*, and *d* were present in the approximate ratio 1 : 1 : 30 : 1.

The number-average molecular weight of methylated (III) was estimated, by osmometry, to be 13,550 ± 400. When hydrolysed with aqueous formic acid, the specific rotation of the material rose from —56·8° to an almost constant value of +34·4°. On chromatograms, the hydrolysis products travelled as three neutral spots (*a'*, *b'*, *c'* respectively) and two acidic spots (*d'* and *e'* respectively). After separation on filter paper sheets, *a'* was

[1] Hirst and Jones, *J.*, 1949, 1659.
[2] Bourne, Foster, and Grant, *J.*, 1956, 4311.
[3] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.
[4] Rebers and Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 6097.

identified as 2 : 3 : 4-tri-*O*-methyl-D-xylose by conversion into its corresponding *N*-phenylglycosylamine; *b'* was obtained as crystalline 2 : 3-di-*O*-methyl-D-xylose, and *c'* afforded crystalline 3-mono-*O*-methyl-D-xylose.

Component *d'* was chromatographically indistinguishable from 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid; owing to its low yield, it was characterised in admixture with component *e'* as follows. The two sugar acids were together converted into their methyl glycosides/methyl esters with methanolic hydrogen chloride, and then reduced with lithium aluminium hydride in ether. A portion of the products, on hydrolysis, yielded 2 : 3 : 4-tri-*O*-methyl-D-glucose, identified as its crystalline aniline derivative, and 3-mono-*O*-methyl-D-xylose, tentatively identified by its electrophoretic mobility and by comparison of its infrared absorption spectrum with that of an authentic specimen. A further portion of the products was fully methylated with Purdie's reagents, and then hydrolysed, to yield 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, characterised as its crystalline aniline derivative, and 3 : 4-di-*O*-methyl-D-xylose, characterised by conversion into crystalline 3 : 4-di-*O*-methyl-D-xylonolactone. It followed that component *d'* was 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, and that *e'* was 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose.

Iodometric titration of *a'*, *b'*, *c'*, *d'*, and *e'* indicated that they were present in the hydrolysate, respectively, in the approximate ratio 3 : 60 : 3 : 1 : 10.

These and previous results [5,6] show that the hemicelluloses of loblolly pine wood which form copper complexes contain two chemically distinct groups of polysaccharides. The hexosan group, represented by subfraction (II), is made up of essentially linear chains of D-mannose and D-glucose residues, linked through positions 1 and 4 in the β-configuration. In the particular hexosan fraction here studied (II), these chains bear, on an average, two non-reducing end-groups for every 27—33 anhydrohexose units; one of these is a D-mannopyranose residue, and the other a D-galactopyranose residue.

The isolation of about 3% of di-*O*-methylhexoses from the hydrolysate of methylated (II) probably arose from under-methylation of the polymer, or from demethylation during hydrolysis. However, the possibility that the chains contain a single branching point for every 27—33 anhydrohexose units cannot be excluded. The consumption by subfraction (II) of 1·02 moles of periodate per anhydrohexose residue is consistent with an essentially linear structure for the hexosans, but the liberation of 4 moles of formic acid for every 30 anhydrohexose residues is also consistent with slight branching of the chains. The average molecular weight of methylated (II) was too low to permit a satisfactory determination by osmometry, and viscosity data are very difficult to interpret. However, on the basis of experience with other mannoglucans, the intrinsic viscosity of subfraction (II) is believed to indicate an average D.P. of 20—30 for the hexosans.

The pentosan group of polymers is based on essentially linear chains of xylose residues, linked through position 1 and 4 in the β-configuration. In the particular fraction described here (III), all of these chains bear single terminal side-chains of 4-*O*-methyl-D-glucuronic acid residues, linked to position 2 of occasional xylose residues (1 in every 6—7), probably in the α-configuration. No neutral pentosan could be present in subfraction (III), since the acidity of the xylans formed the basis of their separation from (II) by the selective emulsification procedures.

If the xylans in subfraction (III) are considered as consisting of repeating units of 7 xylose residues and one 4-*O*-methylglucuronic acid residue, the number-average molecular weight of methylated (III) indicates an average of about 10 such repeating units per molecule, or a number-average D.P. of 85 ± 3. When considered together with the relative yields of the hydrolysis products of methylated (III), this figure indicates that the xylan skeletons contain, on an average, two branching points per molecule. From the identity of the major mono-*O*-methyl-D-xylose component (in *c'*) it appears that this branching takes place principally through position 2 of separate xylose residues.

[5] Ball, Jones, Nicholson, and Painter, *TAPPI*, 1956, **39**, 438.
[6] Jones and Painter, *J.*, 1957, 669.

It is improbable that either subfraction (II) or (III) is a homogeneous polysaccharide. The electrophoretic behaviour of the former suggests that it contains a mixture of different yet closely related polymers. Since partial hydrolysis of fraction (I) affords substantial quantities of glucosidomannose and mannosidoglucose,[6] it is evident that heteropolymer mannoglucans must represent a major part of subfraction (II): the manner in which the galactopyranosyl end-groups are distributed in it is not known; they may be attached to mannoglucan chains, or they may be present as separate mannogalactans. The apparent absence [6] of cellulose in the partial hydrolysate of fraction (I) indicates a paucity in subfraction (II) of contiguous glucose residues, and hence of separate glucan homopolymers. Subfraction (III) probably contains a mixture of closely related 4-*O*-methylglucuronoxylans, differing in their degree of branching and in the extent to which they bear uronic acid side-chains.

Hemicelluloses of the mannoglucan type have now been isolated from a number of other softwoods, including those of western hemlock,[7,8] white spruce,[9] Sitka spruce,[10,11] Norwegian spruce,[12,13] and western red cedar,[14] and important contributions in this field have been made by Aspinall,[10] Dutton,[11] Hamilton,[7,8,14] Lindberg,[12,13] Timell,[9] Wise,[15] and their co-workers. The pentosans of coniferous woods have received less attention, but detailed structural studies have been made of those in western hemlock,[16] Norwegian spruce,[17] and European larch [18] by Dutton and Smith,[16] and by Aspinall and his collaborators.[17,18]

In the case of loblolly pine, hexosans and pentosans of the types represented by subfractions (II) and (III) appear exclusively in the fraction of the hemicelluloses forming copper complexes. Although they constitute the main bulk of the non-cellulosic polysaccharides in the wood, smaller amounts of quite different polysaccharides are present and appear in fractions which do not form copper complexes. They include a group of xylans which bear unmethylated uronic acid residues, and polymers of the arabogalactan type.[5]

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper, with the following solvent systems: (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), (*b*) butan-1-ol–ethanol–water (40 : 11 : 19), (*c*) butan-1-ol–pyridine–water (10 : 3 : 3), and (*d*) butan-2-one–water (89 : 11) (all v/v). Sugars were located on the chromatograms by spraying with a solution of *p*-anisidine hydrochloride in butan-1-ol.[19]

Unless otherwise stated, optical rotations were determined for aqueous solution at 23° ± 3°, and the figures given are equilibrium values. Solutions were concentrated under diminished pressure.

X-Ray powder diffraction photographs were taken with Cu-K_α radiation (nickel filter). The powdered samples (*ca.* 1 mg.) were mounted in the camera on silica threads, to which they were attached with Vaseline petroleum jelly. Infrared absorption spectra were recorded on a Perkin-Elmer Model 21 instrument in conjunction with potassium bromide pellets, or with 10% w/v chloroform solutions in sodium chloride cells (0·1 mm.). Scanning was carried out between 4000 and 600 cm.$^{-1}$.

Preparation and Fractionation of Hemicelluloses forming Copper Complexes.—The hemicellulose fraction (I) forming copper complexes was prepared as previously described, but

[7] Hamilton, Kircher, and Thompson, *J. Amer. Chem. Soc.*, 1956, **78**, 2508.
[8] Hamilton and Kircher, *ibid.*, 1958, **80**, 4703.
[9] Timell and Tyminski, *TAPPI*, 1957, **40**, 519.
[10] Aspinall, Laidlaw, and Rashbrook, *J.*, 1957, 4444.
[11] Dutton and Hunt, *J. Amer. Chem. Soc.*, 1958, **80**, 5697.
[12] Lindberg and Meier, *Svensk Papperstidn.*, 1957, **60**, 785.
[13] Lindberg and Croon, *Acta Chem. Scand.*, 1958, **12**, 457.
[14] Hamilton and Partlow, *J. Amer. Chem. Soc.*, 1958, **80**, 4880.
[15] Merler and Wise, *TAPPI*, 1958, **41**, 80.
[16] Dutton and Smith, *J. Amer. Chem. Soc.*, 1956, **78**, 2505, 3744.
[17] Aspinall and Carter, *J.*, 1956, 3744.
[18] Aspinall and McKay, *J.*, 1958, 1059.
[19] Hough, Jones, and Wadman, *J.*, 1950, 1702.

without prior delignification of the sawdust.[6] The yield from 850 g. of wood was 9 g. Chromatography of a sample hydrolysed by 2N-sulphuric acid at 100° for 24 hr. indicated the presence of galactose, glucose, mannose, xylose, and 4-*O*-methylglucuronic acid.

The hemicellulose (I) (5·0 g.) was dried *in vacuo* at room temperature over silica gel (3 days), then mixed with finely ground anhydrous zinc chloride (1 g.). Acetic anhydride (100 ml.) was added, and the mixture heated at 80° for 4 hr. The resulting homogeneous solution was concentrated to 50 ml. and poured into ice-cold water (1 l.). The gelatinous precipitate was collected and washed by decantation, and then dissolved in chloroform (200 ml.). The solution was dried (Na_2SO_4) and filtered through decolourising charcoal (1 cm.), agitated with ice-cold N-sodium carbonate (1 l.) in a macerator (3 min.), poured into a separatory funnel, and set aside. The homogeneous chloroform layer which separated was removed and again agitated twice in the same way. It was finally washed with water, dried (Na_2SO_4), and poured into light petroleum (1 l.).

The cream-coloured precipitate was collected (centrifuge), washed with light petroleum, and air-dried. The product (1·5 g.) had $[\alpha]_D$ — 8·9° (*c* 0·9 in acetone) (Found: Ac, 44·2. Calc. for hexosan triacetate: Ac, 44·8%). It was deacetylated in acetone solution by the addition of methanolic sodium methoxide; the precipitated polysaccharides were collected by filtration, washed with methanol, then with acetone, and air dried. A portion of the product [subfraction (II)] was hydrolysed with 2N-sulphuric acid at 100° for 24 hr.; chromatography of the hydrolysate (solvent *b*) indicated the presence of galactose, glucose, and mannose only.

The upper layer in the separatory funnel consisted of a stable emulsion; it was washed by shaking it twice with chloroform, acidified, and shaken with chloroform (500 ml.), whereupon it collapsed to yield a homogeneous chloroform layer. The latter was separated, washed with water, dried (Na_2SO_4), concentrated to 100 ml., and poured into light petroleum (500 ml.). The precipitated material was collected, washed, and dried as before. The product (0·8 g.) had $[\alpha]_D$ — 45° (*c* 0·5 in acetone) (Found: Ac, 35·2. Calc. for pentosan diacetate: Ac, 39·8%). It was deacetylated as before to yield subfraction (III). Hydrolysis of a sample, followed by chromatography (solvent *a*), indicated the presence of xylose, 4-*O*-methylglucuronic acid, and 2-*O*-(4-*O*-methylglucuronosyl)xylose.

Preliminary Examination of Subfraction (II).—The unsubstituted material had $[\alpha]_D$ — 20° (*c* 1·0 in 0·1N-NaOH). A portion (76 mg.) was heated in 50% v/v formic acid (10 ml.) at 90°. The specific rotation (calc. as anhydrohexose) rose from −21° to +21° (const.) during 10 hr. The hydrolysate was concentrated to a syrup, which was then heated in N-sulphuric acid (10 ml.) at 90° for 30 min. The solution was cooled, neutralised with Amberlite resin IR-45, and concentrated to a syrup (60 mg.). The syrup (30 mg.) was resolved into galactose, glucose, and mannose components on a paper chromatogram (30 × 40 cm.) developed in solvent *c* during 3 days with intermediate drying. Their relative amounts were determined by the method of Hirst and Jones,[1] a correction being applied for carbohydrates extracted from the paper. A ratio for galactose : glucose : mannose of 1 : 7 : 19 was indicated.

Time (hr.)	3	6	12	36	108	300
IO_4^- (mole/162 g.)	0·452	0·536	0·732	0·748	1·016	1·016
$H{\cdot}CO_2H$ (mole/162 g.)	0·060	0·064	0·079	0·095	0·111	0·133

In the periodate oxidation of subfraction (II), 250 mg. samples were suspended in 0·025M-sodium metaperiodate (250 ml.), in which they largely dissolved. Aliquot parts were withdrawn periodically for the estimation of periodate uptake, by iodometric titration, and of formic acid liberated, by titration with base.

Electrophoresis of subfraction (II) was carried out on fibre-glass sheets [2] (10 × 40 cm.) with 0·2M-sodium borate as the electrolyte. A potential difference of 600 v was applied; an average current of 20—25 mA was observed. After 5 hr., the sheets were dried at 100° and treated with Cifonelli and Smith's reagents.[3] Subfraction (II) appeared as a single, elongated spot, 8 cm. distant from that of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose.

The intrinsic viscosity of subfraction (II) in M-cupriethylenediamine was determined by using a Craig–Henderson viscometer. A value of 0·119 dl./g. was obtained.

Preliminary Examination of Subfraction (III).—The deacetylated material had $[\alpha]_D$ — 20° (*c* 1·0 in 0·1N-NaOH). A portion (55 mg.) was hydrolysed with 2N-sulphuric acid (10 ml.) at 90° for 12 hr. The solution was neutralised with barium carbonate, filtered, de-ionised with Amberlite resin IR-120, and concentrated to a syrup (40 mg.). This was separated into xylose, 4-*O*-methylglucuronic acid, and aldobiuronic acid components on a paper chromatogram

(30 × 40 cm.) developed in solvent *a* for 2 days with intermediate drying. The relative proportions of the sugars were determined by iodometric titration; a blank chromatogram, developed under identical conditions, was used to correct the results for reducing material extracted from the paper. The ratio of the above components, respectively, was 14·80 : 1·00 : 1·83, corresponding to a ratio of 5·88 : 1·00 for the respective amounts of xylose and 4-*O*-methylglucuronic acid residues in the original polymer.

Preparation of Methylated (II) *and* (III).—Hemicellulose fraction (I) (8·0 g.) was dried thoroughly and then kept in ethereal diazomethane for 7 days. After evaporation of the latter, the material was methylated 8 times with methyl sulphate and aqueous sodium hydroxide (30% w/v) in the usual way. The product (OMe, 32·8%) was dissolved in 5N-sodium hydroxide (500 ml.), and the solution was extracted continuously with chloroform. Evaporation of the extract yielded a residue (2·1 g.), a sample of which gave traces of furfuraldehyde when boiled with 5N-hydrochloric acid (aniline acetate test). After further methylation, first with methyl sulphate and alkali, and then with Purdie's reagents, the residue (1·8 g.; OMe, 38·6%) was dissolved in chloroform (200 ml.) and shaken vigorously with N-sodium carbonate (500 ml.) (3 times). The lower layer was separated, washed with water, dried (Na_2SO_4), and evaporated to yield a residue (1·5 g.) which gave a negative furfuraldehyde test. After remethylation with Purdie's reagents, the product (1·39 g.; OMe, 42·2%) had $[\alpha]_D$ − 10·5° (*c* 2·5 in chloroform). A portion (10 mg.) was demethylated and hydrolysed with hydrobromic acid; [19] chromatography of the products indicated the presence of glucose and mannose, but not of xylose.

The above alkaline solution, after extraction of methylated (II), was acidified with sulphuric acid, and re-extracted continuously with chloroform. Evaporation of the extract gave a residue (4·0 g.), a sample of which gave a strong furfuraldehyde test. After remethylation with Purdie's reagents, the product (3·8 g.; OMe, 38·5%) had $[\alpha]_D$ − 66·8° (*c* 2·1 in acetone). A portion, demethylated and hydrolysed as before, indicated xylose and traces of uronic acids on chromatograms, but no hexoses.

Both methylated products showed slight infrared absorption in the hydroxyl region. They were purified by precipitation from chloroform solution by light petroleum, after which this absorption was negligible.

Examination of Methylated (II).—The material (1·1 g.) was dissolved in 90% w/w formic acid (50 ml.), and water (10 ml.) was added. The solution was heated at 95° {$[\alpha]_D$ −18·0° ⟶ +10° ± 5° (const.; 8 hr.)}. It was then concentrated to yield a syrup; water (20 ml.) was added, and the mixture was heated at 90° (30 min.). The water was then removed, and the product was heated twice more with water in the same way to hydrolyse formyl esters. The yield was 1·0 g. A portion (300 mg.) was separated, on two chromatograms (30 × 40 cm.) developed in solvent *b*, into four fractions (*a*, *b*, *c*, and *d* respectively).

Fraction *a* (8 mg.) travelled on chromatograms developed in solvent *b*, *c*, or *d* at a rate identical with that of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose. Its derived *N*-phenylglycosylamine had $[\alpha]_D$ − 80° (0·5 hr.) ⟶ −8° (const.; 10 hr.) (*c* 0·5 in MeOH), and m. p. 142° undepressed on admixture with 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine. A mixture with the corresponding D-glucosylamine derivative had a melting range 110—115°. An *X*-ray powder photograph of the material was identical with that of the authentic D-mannosylamine derivative.

Fraction b (6 mg.) {$[\alpha]_D$ +59° (*c* 0·4 in EtOH)} was indistinguishable, on chromatograms developed in solvent *b*, *c*, or *d*, from 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose. The derived *N*-phenylglycosylamine had m. p. 189°, undepressed on admixture with 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine. An *X*-ray powder photograph of the aniline derivative was identical with that of the last-named compound.

Fraction *c* (240 mg.) was inseparable chromatographically in solvents *b* and *c* from the 2 : 3 : 6-tri-*O*-methyl ethers of both D-mannose and D-glucose. In solvent *d* it was partially resolved into spots corresponding to these two sugars. A portion of the mixture (150 mg.) was dissolved in methanol (5 ml.) containing dry hydrogen chloride (1% w/w); the specific rotation of the solution changed as follows:

Time (hr.)	0·0	2·0	12·0	17·0	24·0
$[\alpha]_D^{23}$	+20·3°	+16·6°	0·0°	−1·0°	−0·4°

The solution was neutralised with silver carbonate, filtered and concentrated to a syrup (127 mg.), which was separated on a chromatogram (30 × 40 cm.) with solvent *c*. By spraying

of side-strips, a single reducing component was located and isolated in the usual way. From the area of paper immediately below the reducing component, a second, non-reducing component was isolated.

The reducing component (72 mg.) had $[\alpha]_D$ − 5° (*c* 3·6). When treated with *p*-nitrobenzoyl chloride in pyridine [4] it yielded crystals having $[\alpha]_D$ + 32° ± 1° (*c* 0·5 in chloroform) and m. p. 187—188°, undepressed on admixture with 2 : 3 : 6-tri-*O*-methyl-1 : 4-di-*O*-*p*-nitrobenzoyl-D-mannose (prepared from methylated lucerne seed mannogalactan). The non-reducing component (18 mg.) had $[\alpha]_D$ −4° (*c* 1·0). It was heated in 0·1N-sulphuric acid (5 ml.) at 100° (8 hr.), then the solution was neutralised with barium carbonate, filtered, and concentrated to a syrup (12 mg.), which was treated with *p*-nitrobenzoyl chloride and pyridine as above. The product had $[\alpha]_D$ −33° ± 1° (*c* 0·5 in chloroform) and m. p. 186°, undepressed on admixture with 1 : 4-di-*O*-*p*-nitrobenzoyl-2 : 3 : 6-tri-*O*-methyl-D-glucose (prepared from methylated amylopectin).

Fraction *d* (10 mg.) had $[\alpha]_D$ +20° ± 3° (*c* 0·6 in acetone). On chromatograms developed in solvent *d* for 24 hr. with intermediate drying, it travelled as three spots; the major component was chromatographically indistinguishable from 2 : 3-di-*O*-methyl-D-mannose. Demethylation of *d* with hydrobromic acid [19] gave a product travelling as mannose on chromatograms developed in solvents *b* and *c*.

Examination of Methylated (III).—The osmotic pressures of four different concentrations of the material in chloroform–ethanol (90 : 10 v/v) were determined, with a Zimm–Myerson–Stabin–Immergut osmometer. A number-average molecular weight of 13,550 ± 400 was indicated.

The methylated material (0·80 g.) was dissolved in 90% w/w formic acid, (25 ml.) and water (25 ml.) was added. The clear solution was heated at 90°; the specific rotation changed as follows:

Time (hr.)	0·00	0·33	0·67	1·00	2·00	3·00	4·50
$[\alpha]_D^{25}$	−56·8°	−26·3°	0·00°	+13·1°	+29·4°	+33·0°	+34·4°

The hydrolysate was concentrated to a syrup, which was heated with water as before to hydrolyse formyl esters. The product (0·75 g.) travelled, on chromatograms developed in solvents *a* and *c*, as three neutral spots (*a'*, *b'*, and *c'* respectively) and two acidic spots (*d'* and *e'* respectively). In solvent *d*, *d'* and *e'* travelled slowly as elongated yet separate spots. A portion (600 mg.) was separated on four chromatograms (30 × 40 cm.) with solvent *d*. A part of one chromatogram (10 × 40 cm.) was used to estimate the relative amounts of the five components by iodometric titration. A blank chromatogram was used to correct the results in the usual way. The ratio *a'* : *b'* : *c'* : *d'* : *e'* was 3·2 : 59·4 : 2·9 : 1·0 : 9·9.

Component a' (12 mg.) had $[\alpha]_D$ +18° (*c*. 2·4); it was indistinguishable on chromatograms developed in solvent *b*, *c*, or *d* from 2 : 3 : 4-tri-*O*-methyl-D-xylose, and crystallised in part on being seeded with this compound. The derived *N*-phenylglycosylamine had $[\alpha]_D$ −80° ± 5° (5 min.) ⟶ +40° ± 5° (24 hr.) (*c*, 0·8 in MeOH), and m. p. 100—102°, undepressed on admixture with 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine.

Component *b'* (350 mg.) was chromatographically indistinguishable (solvent *b*, *c*, or *d*) from 2 : 3-di-*O*-methyl-D-xylose, and completely crystallised on being seeded with this compound. The crystals had $[\alpha]_D$ +65° (5 min.) ⟶ +23° (12 hr.) (*c*, 2·0), m. p. and mixed m. p. 80°. The derived *N*-phenylglycosylamine had m. p. 126°

Component *c'* (15 mg.) had $[\alpha]_D$ +19° (*c* 3·0), and travelled on chromatograms (solvent *b*, *c*, or *d*) at a rate identical with that of both 2-*O*- and 3-*O*-methyl-D-xylose. On paper electrophoretograms, at least 90% of the material travelled at the same rate as 3-*O*-methyl-D-xylose, the remainder travelling as the 2-*O*-isomer. A further quantity of methylated subfraction (III) (1·5 g.) was hydrolysed as before, and the hydrolysate was passed, in aqueous solution, through a column (2 × 20 cm.) of Amberlite resin IR-45 (acetate form). Concentration of the effluent and washings yielded a syrup (1·1 g.) which was adsorbed on a column (2 × 20 cm.) of cocoa-bean shell charcoal.[20] Elution with aqueous ethanol (4% v/v; 1 l.) afforded an electrophoretically pure fraction (30 mg.) which crystallised when seeded with 3-*O*-methyl-D-xylose. The crystals had m. p. and mixed m. p. 96—98°.

Component *d'* (5 mg.) had $[\alpha]_D$ +45° ± 5° (*c* 1·0), and travelled on chromatograms developed in solvent *a* at a rate similar to that reported for 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid.

[20] Barth and Timell, *Canad. J. Chem.*, 1958, **36**, 1321.

Component *e'* (56 mg.) had $[\alpha]_D$ +49° (*c* 0·4) (Found: OMe, 32·1%; equiv., 374. Calc. for $C_{15}H_{26}O_{11}$: OMe, 32·5%; equiv., 382). The column of Amberlite resin IR-45 (above) was eluted with aqueous 10% v/v formic acid (300 ml.). Concentration of the effluent yielded a syrup (168 mg.), which was combined with the components *d'* and *e'* previously isolated, and then boiled with methanolic 2% w/w hydrogen chloride (50 ml.) for 6 hr. The solution was neutralised with silver carbonate, filtered, and concentrated to give a syrup; this was dissolved in ether (50 ml.) and reduced with lithium aluminium hydride (200 mg.). The product (135 mg.) was isolated in the usual way. A portion (60 mg.) was hydrolysed with aqueous 20% v/v formic acid (10 ml.) at 90° for 8 hr. Formyl esters were then hydrolysed as before, and the products were resolved on a chromatogram (30 × 40 cm.) (solvent *d*) into (i) 2 : 3 : 4-tri-*O*-methyl-D-glucose, identified by conversion into its *N*-phenylglycosylamine, $[\alpha]_D$ −125° ± 5° (5 min.) (*c* 0·54 in MeOH), m. p. 144—146°, and (ii) 3-*O*-methyl-D-xylose, tentatively identified by its electrophoretic mobility and its infrared absorption spectrum, $[\alpha]_D$ +18° (*c* 1·2).

A further portion of the reduced disaccharide was methylated with Purdie's reagents; the product (55 mg.) had $[\alpha]_D$ +104° (*c* 1·1 in chloroform) and showed negligible infrared absorption in the hydroxyl region. It was hydrolysed as before, and the products were separated into (iii) 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, identified by conversion into the corresponding *N*-phenylglucosylamine, $[\alpha]_D$ +240° (*c* 0·6 in acetone), m. p. and mixed m. p. 138°, and (iv) 3 : 4-di-*O*-methyl-D-xylose, identified by conversion into 3 : 4-di-*O*-methyl-D-xylonolactone, $[\alpha]_D$ −50° ⟶ −23° (3 days; *c* 1·1), m. p. and mixed m. p. 64—65°.

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