316. *The Structures of Two Water-soluble Polysaccharides from Scots Pine* (Pinus sylvestris).

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Aqueous extraction of Scots pine wood affords a mixture of a partly acylated galactoglucomannan and an arabinogalactan together with traces of an acidic xylan. The major polysaccharide components, after deacylation, have been fractionated *via* their copper complexes. The galactoglucomannan has been shown to contain essentially linear chains of 1,4-linked β-D-glucopyranose and β-D-mannopyranose residues, in the approximate ratio of 1 : 3, to which D-galactopyranose end-groups are attached probably as side-chains. The arabinogalactan has been shown to be a highly-branched polysaccharide containing main chains of 1,3-linked β-D-galactopyranose residues to which are attached side-chains carrying more D-galactopyranose residues present as end-groups or linked through positions 1 and 6, and L-arabinofuranose residues present mainly as end groups.

Coniferous woods contain varying proportions of arabinogalactans which can be extracted with water.[1] The polysaccharides from various species of larch wood are present in

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

greatest amount and have been studied in considerable detail. The less abundant arabinogalactans from spruces and pines have not been so fully characterised although it is clear that several of these polysaccharides contain highly branched structures. This paper reports the examination of an arabinogalactan from Scots pine wood (*Pinus sylvestris*) and of an accompanying galactoglucomannan.

Extraction of Scots pine sawdust with cold and hot water gave similar mixtures of polysaccharides, hydrolysis of which furnished glucose, mannose, galactose, and arabinose, together with smaller amounts of xylose and a trace of rhamnose. Since all preliminary attempts to fractionate the mixture of polysaccharides were unsuccessful, the corresponding mixture of methylated polysaccharides was prepared by simultaneous deacetylation and methylation of the acetylated polysaccharides. Hydrolysis of the mixture of methylated polysaccharides afforded the following methylated sugars, which were characterised by the formation of crystalline derivatives: 2,3,4,6-tetra-, 2,3,4- and 2,4,6-tri-, 2,4- and 2,6-di-, and 2-*O*-methyl-D-galactose, 2,3,6-tri- and 2,3-di-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,5-tri-, and 2,3- and 2,5-di-*O*-methyl-L-arabinose, and 2,3-di-*O*-methyl-D-xylose. In addition, the following sugars, which were present in only small amount, were characterised by paper chromatography of the sugars and their derivatives: 2,3,4-tri-*O*-methylrhamnose, 2,3,4,6-tetra-*O*-methylglucose, 2,3,4,6-tetra- and 2,3-di-*O*-methylmannose, 2,3,4-tri-, and 2- and 3-*O*-methylxylose, and the partially methylated aldobiouronic acid, *O*-(2,3,4-tri-*O*-methylglucopyranosyluronic acid)-(1→2)-3-*O*-methylxylose.

These results show that the majority of the D-mannose and D-glucose residues in the polysaccharides are present in 1→4 linked chains. Since most of the L-arabinose residues are found as end groups in the furanose form they must be present as constituents of a heteropolysaccharide, most probably an arabinogalactan. The D-galactopyranose residues occur in a variety of types of combination, but the majority are present as end groups or are involved in 1,3- and/or 1,6-linkages. Although these results, when taken in isolation, may be interpreted in a number of ways, the most probable conclusion that the major polysaccharide components of the mixture are a glucomannan and an arabinogalactan was confirmed in subsequent studies. The small proportion of D-xylose residues in the polysaccharide mixture is probably accounted for by the presence of an acidic xylan of the type commonly present in woods and normally isolated by extraction with alkali. Such a xylan has recently been isolated from Scots pine wood and has been shown to contain side-chains of L-arabinofuranose and 4-*O*-methyl-D-glucuronic acid residues attached to a basal 1→4-linked xylan chain.[2]

In order to obtain further information concerning the distribution of the D-galactopyranose residues involved in 1,3- and 1,6-linkages the mixture of polysaccharides was oxidised with periodate and the oxidised polysaccharides were degraded with phenylhydrazine by Barry's procedure.[3] The resulting degraded polysaccharides were subjected to partial acid hydrolysis and furnished galactose, glucose, mannose, traces of arabinose and xylose, and a mixture of disaccharides. Fractionation of the mixture of disaccharides afforded the crystalline 3-*O*-β-D-galactopyranosyl-D-galactose as the main component, together with mannobiose and two further galactobioses, probably the 1,4- and 1,6-linked isomers. Since partial hydrolysis of the polysaccharides afforded 3-*O*-β-D-galactopyranosyl-D-galactose as the main disaccharide with only small amounts of the 1,6-linked galactobiose, it follows that the main chains of the arabinogalactan are composed mainly of 1,3-linked β-D-galactopyranose residues and that the D-galactopyranose residues which are involved in 1,6-linkages are located mainly in side-chains.

The presence of periodate-resistant glucose and mannose residues in the mixture of water-soluble polysaccharides can be explained most simply if the 3·6% of acyl groups

[2] Garegg and Lindberg, *Acta Chem. Scand.*, 1960, **14**, 871.
[3] Barry, *Nature*, 1943, **152**, 537; Barry and Mitchell, *J.*, 1954, 4020.

which were shown to be present in the mixture were attached to positions 2 or 3 of some of the glucose and mannose residues of the glucomannan component. Such acyl groups would be readily removed with alkali, and no periodate-resistant glucose or mannose residues were indicated in the methylation study nor were any such residues detected when the glucomannan, which was isolated after fractionation of the polysaccharides regenerated from their acetates, was oxidised with periodate. Meier [4] has isolated an acetylated glucomannan from Scots pine wood by extraction of the chlorite holocellulose with water after swelling with dimethyl sulphoxide. Similar acetylated polysaccharides have been obtained from another species of pine (*P. densiflora*) [5] and from Norway spruce.[6] The acetyl groups in these pine polysaccharides were shown to be attached to secondary hydroxyl groups of mannose residues only.

When the mixture of polysaccharides obtained by deacetylation of the acetylated polysaccharides was treated with Fehling's solution the galactoglucomannan formed an insoluble copper complex from which the purified polysaccharide was isolated. The supernatant liquor afforded the arabinogalactan slightly contaminated with xylan. In studies on the separated polysaccharide fractions considerable use was made of gas–liquid partition chromatography for the analysis of complex mixtures of methylated and partially methylated methyl glycosides. By the general procedure of Bishop and Cooper [7] it is possible to separate highly complex mixtures of such methyl glycosides.[8] Indeed, the methyl glycosides of the majority of the methylated sugars, which were characterised as cleavage products from the mixture of methylated polysaccharides, were resolved sufficiently by gas chromatography to facilitate identification and semiquantitative estimation of their relative proportions.

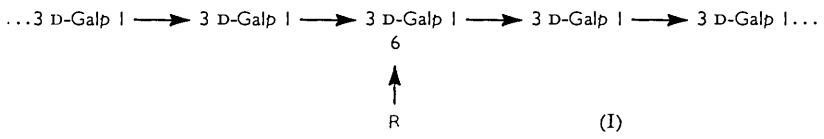
The results of the following examination of the glucomannan fraction confirmed the general conclusions drawn from the earlier methylation study and further indicated that galactose residues, present exclusively as end groups, were integral constituents of the polysaccharide. The cleavage products from the methylated polysaccharide were examined by paper chromatography of the reducing sugars and by gas–liquid chromatography of the derived methyl glycosides, and the presence of tetra-, 2,3,6-tri-, and 2,3-di-*O*-methylmannose, tetra-, 2,3,6-tri-, and 2,3-di-*O*-methylglucose and tetra-*O*-methylgalactose was indicated. All the hexose residues in the polysaccharide were cleaved by periodate, thus confirming that side-chains were attached only by 1,6-linkages. Partial hydrolysis of the polysaccharide was effected by acetolysis followed by deacetylation, the oligosaccharides were fractionated by chromatography on charcoal and cellulose, and chromatography and ionophoresis of the sugars and their hydrolysis products indicated the presence of 4-*O*-mannosylglucose, 4-*O*-glucosylmannose, 4-*O*-mannosylmannose, and *O*-mannosyl-(1→4)-*O*-mannosyl-(1→4)-mannose. From the proportions of methyl ethers of D-mannose and D-glucose isolated on hydrolysis of the mixture of methylated polysaccharides and from gas–liquid chromatography of the methanolysis products from the methylated glucomannan it can be estimated that the polysaccharide contained mannose, glucose, and galactose residues in the approximate ratios of 9 : 3 : 1.

These results, therefore, establish the presence, as a major component of the mixture of polysaccharides, of a partially acylated glucomannan containing linear chains of 1,4-linked β-D-mannopyranose and β-D-glucopyranose residues to which are attached D-galactopyranose end-groups, most probably linked to position 6 of glucose and mannose residues. This polysaccharide, which was isolated by aqueous extraction of the wood, is essentially similar in structure to the galactoglucomannans from other coniferous woods which are normally rather difficult to extract with alkali.[1]

[4] Meier, *Acta Chem. Scand.*, 1961, **15**, 1381.
[5] Koshijama, *J. Japan Wood Research Soc.*, 1960, **6**, 194.
[6] Annergren, Croon, Enström, and Rydholm, *Svensk Papperstidn.*, 1961, **64**, 386.
[7] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[8] Aspinall, *J.*, 1963, 1676.

The second polysaccharide fraction gave on hydrolysis galactose, arabinose, and xylose in the ratios of 24 : 2 : 1. Ultracentrifugal examination (by courtesy of Dr. C. T. Greenwood) gave a single peak in which a slight leading edge could be accounted for by the presence of a trace of xylan contaminant. Paper electrophoresis of the fraction in borate buffer showed a main polysaccharide component (M_G 0·5) with a trace of a second component, presumably the xylan. There was no indication of the presence of two arabinogalactans as found in several species of larch.[9] Hydrolysis of the derived methylated polysaccharide followed by paper chromatography showed 2,3,4,6-tetra-, tri-, and 2,4-di-*O*-methylgalactose, 2,3,5-tri-*O*-methylarabinose, and 2,3-di-*O*-methylxylose, together with traces of 2,3,4-tri-*O*-methylxylose, 2,3- and 2,5-di-*O*-methylarabinose, 2-*O*-methylgalactose, and methylated aldobiouronic acid. The presence of most of these cleavage products was further indicated by gas–liquid chromatography of the methyl glycosides formed on methanolysis of the methylated polysaccharide, and the presence of both 2,3,4- and 2,4,6-tri-*O*-methylgalactose was confirmed. The general conclusion concerning the distribution of 1,3- and 1,6-linked D-galactose residues in this highly branched polysaccharide, which was based on the Barry degradation of the original mixture of polysaccharides, was confirmed by the examination of a degraded galactan prepared by Smith's [10] procedure. The periodate-oxidised polysaccharide was reduced with potassium borohydride and subsequent mild acid hydrolysis removed the cleaved sugar units. The presence of a high proportion of 1,3-linked galactose residues in the degraded polysaccharide was established from the following experiments. Chromatographic examination of the products of partial acid hydrolysis showed the formation of 3-*O*-galactosylgalactose as the main disaccharide with only small amounts of the 1,6-linked isomer, whereas partial acid hydrolysis of the original polysaccharide gave similar amounts of both disaccharides. Oxidation of the degraded galactan resulted in the consumption of 0·22 mol. of periodate per sugar residue. Hydrolysis of the methylated polysaccharide gave tri-*O*-methylgalactose as the major product together with smaller amounts of tetra- and 2,4-di-*O*-methylgalactose and a trace of 2,3,5-tri-*O*-methylarabinose. Gas–liquid chromatography of the corresponding methyl glycosides provided further evidence for the identity of the cleavage products and indicated that the tri-*O*-methylgalactose fraction contained the 2,4,6-trimethyl ether with only small proportions of the 2,3,4-isomer.

On the basis of these results the partial structure (I) can be suggested for the degraded galactan. Since the 1,6-linked galactobiose could be detected as a minor partial hydrolysis



(I)

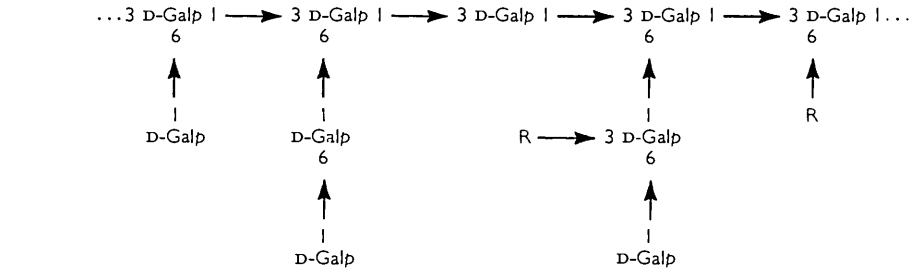
R = D-Galp 1... and less requently L-Araf 1....

product, it is probable that the degraded polysaccharide is still branched but to a much smaller extent than the original arabinogalactan. The small proportion of arabinofuranose end groups probably arise from the degradation of 3-*O*-L-arabinofuranosyl-L-arabinofuranose units which were present in the parent polysaccharide.

Although no unique structure can yet be suggested for the arabinogalactan from Scots pine the partial structure (II) indicates the known structural units, and other permissible variants can differ only in minor respects. Arabinogalactans from other species of pine,[1,11] although varying in the proportions of the component sugars, contain similar structural units but the distributions of the D-galactopyranose residues involved

[9] Bouveng and Lindberg, *Acta Chem. Scand.*, 1958, **12**, 1977; Bouveng, *ibid.*, 1959, **13**, 1869.
[10] Goldstein, Hay, Lewis, and Smith, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3D.
[11] Brasch and Jones, *Canad. J. Chem.*, 1959, **37**, 1538.

in 1,3- and 1,6-linkages have not been established. The Scots pine polysaccharide is similar to the arabinogalactans from European [1] and Western [12] larches in containing main chains of 1,3-linked β-D-galactopyranose residues to which other D-galactopyranose residues are attached as side-chains by 1,6-linkages. These polysaccharides all contain L-arabinose residues in the outer chains of the molecular structure, those in the Scots pine arabinogalactan are probably all present in the furanose form, whereas those in the larch polysaccharides are present in both furanose and pyranose forms.



(II)

R = D-Galp 1..., L-Araf 1..., or less frequently L-Araf 1 → 3 L-Araf 1... or L-Araf 1 → 5 L-Araf 1....

Experimental

Paper chromatography was carried out on Whatman Nos. 1 and 3MM papers with the following solvent system (v/v); (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (D) butan-2-one, half saturated with water; (E) benzene–ethanol–water (169 : 47 : 16, upper layer); (F) ethyl acetate–acetic acid–water (9 : 2 : 2); (G) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (H) pentan-2-one, 75% saturated with water. R_G Values of methylated sugars refer to rates of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent B. Demethylations of methylated sugars were performed by the method of Hough, Jones, and Wadman.[13] Paper chromatography of periodate-oxidised sugars was carried out by the method of Lemieux and Bauer; [14] the same procedure was used for the examination of the periodate-oxidised hexitols after their formation by reduction of the corresponding sugars with potassium borohydride. 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 by using a Pye Argon Chromatograph according to the procedure of Bishop and Cooper.[7] The following columns (120 × 0·5 cm. internal diam.) were used 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 [13] on acid-washed Celite at 175°; (*c*) 10% by weight of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)benzene] on acid-washed Celite at 200°. In all cases the methyl glycosides of reference compounds have been formed from sugars, which have been characterised independently, or from methylated polysaccharides known to yield particular sugars as cleavage products. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Isolation of Water-soluble Polysaccharides.—Scots pine sawdust was extracted twice with water at 60°, and the aqueous extracts were filtered, concentrated, and poured into ethanol (4 vol.) to give water-soluble polysaccharides (Found: acyl, 3·6%) as a pale brown powder in *ca.* 2% yield.

Methylation of Scots Pine Polysaccharides.—The water-soluble polysaccharides (10 g.) were acetylated in formamide by the addition of pyridine and acetic anhydride following Carson and Maclay's method.[15] Simultaneous methylation and deacetylation of the polysaccharide

[12] Bouveng, *Acta Chem. Scand.*, 1961, **15**, 78.
[13] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[14] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.
[15] Carson and Maclay, *J. Amer. Chem. Soc.*, 1946, **68**, 1015.

Analysis of hydrolysate of methylated polysaccharides.

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography * R_G	sugar	Sugars given on demethylation	Other evidence †
1	6		1·02	2,3,4-Me_3rhamnose (*t*)		
			1·00	Me_4mannose	mannose	H
				Me_4glucose (*t*)	glucose (*t*)	
2	52		1·00	Me_4mannose	mannose	
			0·98	2,3,5-Me_3arabinose	arabinose	D, E
				2,3,4-Me_3xylose (*t*)	xylose (*t*)	
3	56	−33°	0·98	2,3,5-Me_3arabinose	arabinose	D, E
				2,3,4-Me_3xylose (*t*)	xylose (*t*)	
4	7		0·98	2,3,5-Me_3arabinose (*t*)	arabinose (*t*)	D
			0·90	Me_4galactose	galactose	
5	22		0·90	Me_4galactose (*t*)	galactose (*t*)	
			0·86	2,3,6-Me_3glucose	glucose	D
				2,3,6-Me_3mannose	mannose	
6	228		1·04	Methyl glycosides	mannose, glucose	
			0·98	2,3,5-Me_3arabinose (*t*)	arabinose (*t*)	
7	397	+108°	0·90	Me_4galactose	galactose	D
8	60	+38°	0·86	2,3,6-Me_3glucose		
			0·85	2,5-Me_2arabinose		D, E, I
			0·83	3,5-Me_2arabinose (*t*)		
				2,3,4-Me_3arabinose (*t*)		
9	101	+60°	0·85	2,3,6-Me_3glucose	glucose	
				2,5-Me_2arabinose	arabinose	E, I
				2,3,6-Me_3mannose (*t*)	mannose (*t*)	
10	481	+1°	0·86	2,3,6-Me_3mannose	mannose	
				2,3,6-Me_3glucose (*t*)	glucose (*t*)	D
			0·77	2,3-Me_2xylose	xylose	
11	53	+5°	0·86	2,3,6-Me_3mannose	mannose	D
			0·66	2,3-Me_2arabinose (*t*)	arabinose (*t*)	
12	195	+104°	0·71	2,4,6-Me_3galactose	galactose	D
			0·66	2,3-Me_2arabinose (*t*)	arabinose (*t*)	
13	27	+110°	0·71	Me_3galactose (*t*)	galactose (*t*)	D
			0·66	2,3-Me_2arabinose	arabinose	
14	213	+119°	0·70	2,3,4-Me_3galactose	galactose	D
			0·66	2,3-Me_2arabinose (*t*)	arabinose (*t*)	
15	50		0·59	2,3-Me_2glucose	glucose	D, P, RP
			0·56	2,3-Me_2mannose (*t*)	mannose (*t*)	
16	57	−5°	0·59	2,3-Me_2glucose (*t*)	glucose (*t*)	
			0·56	2,3-Me_2mannose	mannose	D
				(?)Me arabinose (*t*)	arabinose (*t*)	
17	45	−12°	0·56	2,3-Me_2mannose		P, RP
18	18	−4°	0·56	2,3-Me_2mannose	mannose	D, I, P
			0·54	2,6-Me_2galactose (*t*)	galactose (*t*)	
19	20	+86°	0·54	2,6-Me_2galactose		D, I
20	27	+24°	0·54	2,6-Me_2galactose (*t*)		
			0·47	2,4-Me_2galactose (*t*)	galactose (*t*)	D, I
			0·38	2-Me xylose	xylose	
				3-Me xylose		
21	354		0·47	2,4-Me_2galactose		
22	19	+104°	0·47	2,4-Me_2galactose	galactose	I
			0·39	2-Me arabinose (*t*)	arabinose (*t*)	
23	24		0·47	2,4-Me_2galactose		
			0·33	(?) Me mannose	mannose	D, G
			streak	methylated acids	xylose	
24	63		0·29	2-Me galactose		
			0·20	galactose (*t*)		D, G
			streak	methylated acids		

* *t* = trace. † D, E, G, and H = paper chromatography in solvents D, E, G, and H, respectively. I = paper ionophoresis. P = paper chromatography of the periodate-oxidised sugar. RP = paper chromatography of the periodate-oxidised glycitol (from borohydride reduction of the sugar).

acetates (9 g.) was carried out in tetrahydrofuran by use of methyl sulphate and solid sodium hydroxide according to Hamilton and Kircher's method,[16] and the product was further methylated with methyl iodide and silver oxide to give methylated polysaccharides (3·3 g.), $[\alpha]_D$ −25° (*c* 2·5 in chloroform) (Found: OMe, 41·9%, not raised on further methylation).

[16] Hamilton and Kircher, *J. Amer. Chem. Soc.*, 1958, **80**, 4703.

Hydrolysis of Methylated Polysaccharides and Separation of Methylated Sugars.—The methylated polysaccharides (3·1 g.) were hydrolysed successively with boiling methanolic 4% hydrogen chloride (250 ml.) for 12 hr. and with 0·5N-hydrochloric acid (150 ml.) at 100° for 12 hr. An insoluble precipitate (108 mg., non-carbohydrate), which separated during the aqueous hydrolysis, was removed, and the resulting solution was neutralised with silver carbonate and concentrated to a syrup (3·1 g.). The syrup (3·1 g.) was separated on cellulose (80 × 3·8 cm.), (i) light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, later 1 : 1), saturated with water, and (ii) butan-1-ol, half saturated with water, being used as eluants to give 24 fractions. Some of the earlier fractions (240 mg.) contained incompletely hydrolysed methyl glycosides which were further hydrolysed and reseparated to give fractions 1—4. The Table summarises the results of preliminary examination of the various fractions.

Characterisation of Sugars from Hydrolysis of Methylated Polysaccharides.—Fraction 3. The major component was characterised 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. 135—136°.

Fraction 6. The syrup (228 mg.) contained unhydrolysed methyl glycosides and was heated with N-hydrochloric acid at 100° for 8 hr. The resulting mixture of sugars was separated on filter sheets in solvent D to give two main fractions and a trace of tri-*O*-methylarabinose. Fraction 5*a* (71 mg.), $[\alpha]_D$ +70° (*c* 1·5), was recrystallised from ether and had m. p. and mixed m. p. 119—120° (with 2,3,6-tri-*O*-methyl-D-glucose). Fraction 5*b* (133 mg.), $[\alpha]_D$ −9° (*c* 1·3), was characterised as 2,3,6-tri-*O*-methyl-D-mannose by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. 187—188°.

Fraction 7. The sugar was recrystallised from light petroleum (b. p. 60—80°) and had m p. and mixed m. p. 69—70° (with 2,3,4,6-tetra-*O*-methyl-D-galactose), and $[\alpha]_D$ +140° ⟶ +116° (equil.) (*c* 0·9). The aniline derivative had m. p. and mixed m. p. 198°.

Fraction 8. The mixture of sugars (60 mg.) was fractionated on charcoal–Celite (1 : 1; 50 g.) by gradient elution with water containing 0·0—5·5% of butane-2-one. The first fraction (17 mg.), $[\alpha]_D$ −2° (1·7), contained 2,5-di-*O*-methyl-L-arabinose as the major component and the sugar was identified by conversion into 2,5-di-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 126—128°.

Fraction 9. The major component was recrystallised from ether; it had m. p. and mixed m. p. 119—120° (with 2,3,6-tri-*O*-methyl-D-glucose), $[\alpha]_D$ +63° ⟶ −35° (*c* 0·8 in methanolic 1% hydrogen chloride), and furnished a 1,4-di-*p*-nitrobenzoate, m. p. and mixed m. p. 188—189°.

Fraction 10. After an unsuccessful attempt to separate the sugar components by chromatography on cellulose by using light petroleum–butan-1-ol mixtures, saturated with water, the syrup (275 mg.) was chromatographed on charocoal–Celite (1 : 1; 40 g.) by gradient elution with water containing 2·5—5·5% of butan-2-one. The major portion (240 mg.), $[\alpha]_D$ −4° (*c* 1·2), no longer contained 2,3,6-tri-*O*-methylglucose, and was further separated on filter sheets in solvent E to give chromatographically pure fractions 10*a* (181 mg.) and 10*b* (40 mg.). Fraction 10*a*, $[\alpha]_D$ −10° (*c* 1·8), was characterised as 2,3,6-tri-*O*-methyl-D-mannose by conversion into the 1,4-di-*p*-nitrobenzoate, m. p. 188—189°. Fraction 10*b*, $[\alpha]_D$ +22° (*c* 0·4), crystallised and had m. p. and mixed m. p. 79—80° (with 2,3-di-*O*-methyl-β-D-xylose), and afforded the aniline derivative, m. p. and mixed m. p. 122—123°.

Fraction 12. The main component was characterised as the crystalline sugar, m. p. and mixed m. p. 98—99° (with 2,4,6-tri-*O*-methyl-D-galactose), and as the aniline derivative, m. p. and mixed m. p. 165—166°.

Fraction 13. The main component was identified as 2,3-di-*O*-methyl-L-arabinose by conversion into 2,3-di-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 158—159°.

Fraction 14. The sugar was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 168—170°; the m. p. was depressed on admixture with the aniline derivative of 2,4,6-tri-*O*-methyl-D-galactose.

Fraction 15. The major component was purified by chromatography on filter sheets in solvent D, had $[\alpha]_D$ +46° (*c* 0·6 in acetone), and was identified as 2,3-di-*O*-methyl-D-glucose by conversion into 2,3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. and mixed m. p. 174—175°.

Fraction 17. Although attempts to prepare crystalline derivatives of 2,3-di-*O*-methyl-D-mannose failed, the syrup was chromatographically indistinguishable from the authentic sugar. Incomplete oxidation of the derived hexitol with periodate [14] gave *inter al.* a sugar chromatographically identical with 3,4-di-*O*-methyl-L-arabinose (R_F 0·55). Under similar conditions 2,3-di-*O*-methyl-D-glucitol afforded 3,4-di-*O*-methylxylose (R_F 0·50).

Fraction 19. Crystallisation of the syrup furnished 2,6-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 81—86°, which afforded the aniline derivative, m. p. and mixed m. p. 120—121°.

Fraction 21. The major component was recrystallised from acetone containing 1% of water; it had m. p. and mixed m. p. 101° (with 2,4-di-*O*-methyl-D-galactose monohydrate), $[\alpha]_D$ +123° ⟶ +85° (equil.) (*c* 1·1), and afforded the aniline derivative, m. p. and mixed m. p. 216—217°. Chromatographic examination of the mother liquors in solvent D showed 2,4- and a small amount of 2,3-di-*O*-methylgalactose. The presence of the latter sugar was also indicated by chromatography of the periodate oxidation products of the sugars and of the derived alditols.

Fraction 24. The two main components were separated by chromatography on filter sheets in solvent G. Fraction 25*a* (11 mg.) was recrystallised from acetic acid and had m. p. and mixed m. p. 147—148° (with 2-*O*-methyl-D-galactose). Fraction 25*b* (20 mg.) contained a methylated aldobiouronic acid (R_F 0·74 in solvent G, streaking in solvent B), which was converted into methyl ester methyl glycosides, reduced with lithium aluminium hydride in tetrahydrofuran, and hydrolysed to give two sugars chromatographically and ionophoretically indistinguishable from 2,3,4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose.

Degradation of Periodate-oxidised Polysaccharides with Phenylhydrazine.—The polysaccharides (10 g.) were oxidised with sodium metaperiodate solution (600 ml.; 0·27M) for 58 hr. (consumption of reagent was complete, corresponding to 1·11 mol. per hexose residue). The solution was treated with lead acetate to remove iodate and periodate, and then with dilute sulphuric acid to precipitate excess of lead. The resulting solution (700 ml.) was treated with phenylhydrazine (21 ml.) in 10% acetic acid (60 ml.), and the precipitate which separated was dried to a yellow powder (9·9 g.). The phenylhydrazine derivative (9·9 g.) was suspended in a mixture of ethanol (250 ml.), phenylhydrazine (30 ml.), glacial acetic acid (55 ml.), and water (100 ml.) and the suspension was refluxed for 4 hr. Removal of ethanol under reduced pressure yielded orange crystals (9·9 g.) which were separated and washed. The filtrate and washings were repeatedly extracted with ether, made 0·2N with respect to sulphuric acid and heated at 100° for 40 min. to cleave phenylosazone residue. The cooled solution was neutralised with barium carbonate, passed through Amberlite resin IR-120(H) to remove barium ions, extracted with ether, and concentrated (20 ml.). Since the addition of ethanol did not cause precipitation of the degraded polysaccharide the solution was concentrated to a syrup (590 mg.).

A portion (29 mg.) of the degraded polysaccharide was heated (boiling-water bath) in 0·5N-sulphuric acid (20 ml.) and samples (2 × 1 ml.) were withdrawn periodically for estimation of reducing power with Somogyi's reagent [17] and for paper chromatography. Hydrolysis was complete after 1·5 hr., and paper chromatography in solvent A after 45 min. showed the formation of galactose, glucose, mannose, traces of arabinose and xylose, and of oligosaccharides (maximum relative concentrations) with the same mobilities as 3-*O*-β-D-galactopyranosyl-D-galactose ($R_{galactose}$ 0·49) (major component), 6-*O*-β-D-galactopyranosyl-D-galactose ($R_{galactose}$ 0·30) (trace), and 4-*O*-β-D-mannopyranosyl-D-mannose ($R_{galactose}$ 0·60) (small amount). The remainder (570 mg.) of the degraded polysaccharide was heated (boiling-water bath) with 0·5N-sulphuric acid for 45 min., and the cooled solution was neutralised with Amberlite resin IR-45(OH) and concentrated to a syrup (274 mg.). The main disaccharide component (20 mg.) of the mixture was isolated after chromatography on filter sheets in sovent A and elution from charcoal (2 g.) with water containing 12·5% of ethanol. After recrystallisation from ethanol–water the disaccharide was identified as 3-*O*-β-D-galactopyranosyl-D-galactose by m. p. and mixed m. p. 146—147°, and by *X*-ray powder photograph. Paper ionophoresis of the mother liquor from the recrystallisation showed the presence also of traces of a disaccharide with the same mobility as 4-*O*-β-D-galactopyranosyl-D-galactose.

Fractionation of Scots Pine Polysaccharides.—0·5N-Barium methoxide in methanol (10 ml.) was added to the mixture of acetylated polysaccharides (7·2 g.) in chloroform (150 ml.) and methanol (50 ml.), a gelatinous precipitate was formed immediately, and the mixture was kept at 0° for 15 hr. The mixture was poured into water (400 ml.) and shaken vigorously. The aqueous layer was separated and the chloroform layer was extracted with water until free from carbohydrate. The combined aqueous extracts were neutralised carefully with 0·1N-sulphuric acid, and the filtered solution was concentrated (100 ml.) and poured into ethanol (400 ml.) to precipitate the mixture of polysaccharides (3·5 g.). Hydrolysis of a sample gave

[17] Somogyi, *J. Biol. Chem.*, 1952, **195**, 19.

galactose, glucose, mannose, arabinose, xylose, and traces of rhamnose and an aldobiouronic acid.

Fehling's solution (20 ml.) was added to the mixture (3·5 g.) of polysaccharides in water (100 ml.). The gelatinous precipitate which formed immediately was removed by centrifugation, suspended in water (50 ml.), and decomposed by the addition of ice-cold 0·5N-hydrochloric acid, and the regenerated polysaccharide was precipitated by the addition of ethanol (4 vol.). The polysaccharide was purified by reprecipitation as the insoluble copper complex and after regeneration gave galactoglucomannan (1·07 g.), $[\alpha]_D$ +4·5° (*c* 1·07 in water) and −9·2° (*c* 1·43 in N-sodium hydroxide).

The supernatant liquor from the above precipitation was cooled to 0°, carefully acidified with 0·5N-hydrochloric acid, and poured into ethanol (4 vol.) to give slightly impure arabinogalactan (1·8 g.), $[\alpha]_D$ +11° (*c* 1·8), hydrolysis of which followed by estimation of the sugars formed by Wilson's method [18] showed the presence of galactose, arabinose, and xylose in the proportions of 24 : 2 : 1, and traces of glucose, mannose, and an aldobiouronic acid. Examination of the polysaccharide by ultracentrifugation (by courtesy of Dr. C. T. Greenwood) showed a single peak with a slight leading edge probably due to the presence of a small amount of contaminating xylan. Paper electrophoresis of the arabinogalactan in borate buffer (kindly carried out by Dr. H. O. Bouveng) showed a main component (M_G 0·5) with a trace of a second component of lower mobility.

Periodate Oxidation of Galactoglucomannan.—The polysaccharide (11·9 mg.) was dissolved in 0·015M-sodium metaperiodate solution (10 ml.). The consumption of reagent, which was determined by the withdrawal of aliquot portions (1 ml.), dilution to 250 ml., and measurement of changes in light absorption at 223 mμ,[19] corresponded to 1·02 mol. per hexose residue (constant after 46 hr.). A further quantity (50 mg.) of polysaccharide was oxidised in 0·15M-sodium metaperiodate solution (100 ml.) for 48 hr. The solution was treated with lead acetate to remove iodate and periodate and with sulphuric acid to precipitate excess of lead. The resulting solution was treated with potassium borohydride (50 mg.) for 2 days, glacial acetic acid was added to destroy excess of hydride, and the mixture was concentrated to 10 ml., made N with respect to sulphuric acid and heated (boiling-water bath) for 6 hr. The cooled solution was neutralised with Amberlite resin IR-45(OH), passed through Amberlite resin IR-120(H) to complete deionisation, and concentrated to a syrup. Chromatography of the syrup showed erythritol and small amounts of glycerol but no reducing sugars.

Methylation of Galactoglucomannan.—The polysaccharide (819 mg.) was acetylated by Carson and Maclay's [15] method to give polysaccharide acetate (1·06 g.). A portion of the acetate was simultaneously methylated and deacetylated in tetrahydrofuran by Hamilton and Kircher's [16] method, and the product (201 mg.) was further methylated thrice with methyl iodide and silver oxide to give methylated galactoglucomannan (62 mg.) (Found: OMe, 42·9%, not raised on further methylation).

A portion (20 mg.) of methylated galactoglucomannan was hydrolysed successively with boiling methanolic 4% hydrogen chloride and with 0·5N-hydrochloric acid (boiling-water bath). Chromatography of the hydrolysate in solvents B, D, and E showed 2,3,4,6-tetra-*O*-methylgalactose (R_G 0·90), 2,3,6-tri-*O*-methylmannose (R_G 0·86), 2,3,6-tri-*O*-methylglucose (R_G 0·86), and 2,3-di-*O*-methylmannose (R_G 0·56) in large or moderate amounts, 2,3,4,6-tetra-*O*-methylmannose (R_G 1·00) and 2,3-di-*O*-methylglucose (R_G 0·59) in small amounts, and a trace of 2,3,4,6-tetra-*O*-methylglucose (R_G 1·00). A further quantity (20 mg.) of methylated polysaccharide was converted into a mixture of methylglycosides of methylated sugars by boiling with methanolic 4% hydrogen chloride for 18 hr. Gas chromatography on column *c* showed methyl glycosides of the following sugars: 2,3,6-tri-*O*-methylmannose (*T* 2·35) in large amount, 2,3,6-tri-*O*-methylglucose (*T* 1·71, 2·20), 2,3,4,6-tetra-*O*-methylgalactose (*T* 1·52, 1·61), and di-*O*-methylhexose (*T* 3·26) [probably an unresolved mixture of methyl glycosides of 2,3-di-*O*-methylmannose (*T* 3·37) and 2,3-di-*O*-methylglucose (*T* 3·20)] in moderate amounts, and small amounts of tetra-*O*-methylglucose (*T* 1·00, 1·31) and tetra-*O*-methylmannose (*T* 1·31). The methyl glycosides of tetra-*O*-methylmannose (*T* 1·17) were resolved from those of tetra-*O*-methylglucose (*T* 1·00, 1·27) by gas chromatography on column *a*.

Graded Acetolysis of Galactoglucomannan Acetate.—Galactoglucomannan acetate (0·7 g.) was dissolved in acetic anhydride–acetic acid–concentrated sulphuric acid (10 : 10 : 1; 8 ml.) at 0°

[18] Wilson, *Analyt. Chem.*, 1959, **31**, 1199.
[19] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.

and the solution was kept at room temperature for 92 hr. The mixture was poured into ice–water (50 ml.), neutralised with sodium hydrogen carbonate, and extracted with chloroform, and the extract was concentrated to a syrup (680 mg.). 0·5N-Barium methoxide in methanol (2 ml.) was added to the syrupy acetates (680 mg.) in chloroform (25 ml.) and methanol (25 ml.), and the mixture was kept at 0° for 24 hr. The mixture was poured into water (250 ml.), and the chlorform layer was separated and washed several times with water. The combined aqueous extracts were neutralised with sulphuric acid, filtered, and concentrated to a syrup (422 mg.). Chromatography of the syrup showed mannose, glucose, galactose, and a series of oligosaccharides.

The mixture of sugars (420 mg.) was separated on charcoal–Celite (1 : 1; 50 g.) by gradient elution with water containing 0·0—12·5% of ethanol to give six fractions. Fraction 1 (203 mg.) contained only monosaccharides. Fraction 2 (2 mg.) contained a single component which was chromatographically and ionophoretically indistinguishable from 4-*O*-β-D-mannopyranosyl-D-mannose and gave mannose only on hydrolysis. Fraction 3 (22 mg.) contained two components chromatographically and ionophoretically indistinguishable from the 1,4-linked mannobiose and the polymer-homologous mannotriose which were separated on filter sheets in solvent F. The faster-moving component (10 mg.) gave mannose only on hydrolysis. Partial hydrolysis of the slower-moving component gave mannose, one disaccharide with the chromatographic mobility of 4-*O*-β-D-mannopyranosyl-D-mnnose, and unchanged sugar. Fraction 4 (24 mg.) contained the same mannobiose as the major component together with mannotriose and a sugar with the chromatographic mobility of 4-*O*-β-D-mannopyranosyl-D-glucose. Fraction 6 (14 mg.) contained as the major component a sugar which was chromatographically and ionophoretically indistinguishable from 4-*O*-β-D-mannopyranosyl-D-glucose together with mannobiose. A sample of the major component was separated chromatographically; it gave mannose and glucose on hydrolysis, but after reduction with potassium borohydride hydrolysis yielded mannose as the only reducing sugar. Fraction 6 (8 mg.) contained a sugar which was chromatographically and ionophoretically indistinguishable from 4-*O*-β-D-glucopyranosyl-D-mannose with a trace of the above mannosylglucose. Hydrolysis of a sample gave mannose and glucose in equal amounts, whereas hydrolysis after reduction with potassium borohydride gave glucose and a trace of mannose.

Methylation of Arabinogalactan.—The polysaccharide (401 mg.) was acetylated by Carson and Maclay's [15] method to give polysaccharide acetate (434 mg.), which was then simultaneously methylated and deacetylated in tetrahydrofuran by Hamilton and Kircher's [16] method to yield a product (280 mg.) which was further methylated with methyl iodide and silver oxide to furnish methylated arabinogalactan (61 mg.) (Found: OMe, 44·0%, not raised on further methylation), $[\alpha]_D$ −31° (*c* 1·8 in chloroform).

A portion (20 mg.) of the methylated arabinogalactan was hydrolysed successively with boiling methanolic 4% hydrogen chloride and with 0·5N-hydrochloric acid (boiling-water bath). Chromatography of the hydrolysate in solvents B, D, and E showed 2,3,4,6-tetra- (R_G 0·90), 2,3,4- (R_G 0·70) and 2,4,6-tri- (R_G 0·72), and 2,4-di-*O*-methylgalactose (R_G 0·47) in large amount, 2,3,5-tri-*O*-methylarabinose (R_G 0·98) and 2,3-di-*O*-methylxylose (R_G 0·77) in small amount, and traces of 2,3,4-tri-*O*-methylxylose (R_G 0·98), 2,3- (R_G 0·66) and 2,5-di-*O*-methylarabinose (R_G 0·85), 2-*O*-methylgalactose (R_G 0·29), and methylated aldobiouronic acid (streaking in solvent B). A further quantity (20 mg.) of the methylated polysaccharide was converted into a mixture of methyl glycosides of methylated sugars by boiling with methanolic 4% hydrogen chloride for 18 hr. Gas chromatography on column *c* showed the presence of methyl glycosides of the following sugars 2,3,4,6-tetra- (*T* 1·53, 1·62), 2,3,4- (*T* 2·64, 2·94) and 2,4,6-tri- (*T* 2·10, 2·40) and 2,4-di-*O*-methylgalactose (*T* 3·68, 4·40) in large amount, and 2,3,5-tri- (*T* 0·47, 0·60), 2,3- (*T* 0·65, 0·97) and 2,5-di-*O*-methylarabinose (*T* 0·71, 1·05), and 2,3-di-*O*-methylxylose (*T* 0·77) in small amounts. Gas chromatography on column *b* also revealed traces of methyl glycosides of 2,3,4-tri-*O*-methylxylose (*T* 0·46) and 2,3,4-tri-*O*-methylarabinose (*T* 1·05).

Degradation of Periodate-oxidised Arabinogalactan.—Arabinogalactan (1·2 g.) was oxidised with 0·15M-sodium metaperiodate solution (200 ml.) for 80 hr. (consumption of reagent was complete, corresponding to 1·11 mol. per hexose residue). Ethylene glycol (4 ml.) was added to destroy excess of periodate and the solution was dialysed for 3 days. The solution was treated with potassium borohydride (1·2 g.) for 2 days, excess of borohydride was decomposed with acetic acid, and the solution was dialysed for 3 days and concentrated to 50 ml. 2N-Sulphuric acid (50 ml.) was added to the solution which was kept at room temperature for

3 hr., neutralised with barium hydroxide and barium carbonate, filtered, passed through Amberlite resin IR-120(H) to remove barium ions, concentrated, and poured into ethanol (4 vol.). The precipitated degraded galactan (160 mg.) was separated and the supernatant liquor was concentrated to a syrup (400 mg.). Chromatography of the syrup showed glycerol but no reducing sugars, and the absence of reducing sugars after hydrolysis showed that no glycosides were present. Hydrolysis of the degraded polysaccharide gave galactose and a trace of arabinose. Partial acid hydrolysis of the galactan with 0·5N-sulphuric acid (boiling-water bath) for 1 hr. also furnished a disaccharide with the chromatographic mobility of 3-*O*-β-D-galactopyranosyl-D-galactose ($R_{galactose}$ 0·49 in solvent A) with only traces of a second disaccharide with the mobility of 6-*O*-β-D-galactopyranosyl-D-galactose ($R_{galactose}$ 0·30). Partial acid hydrolysis of the original arabinogalactan under the same conditions yielded the two disaccharides in similar amounts. Oxidation of the degraded galactan with sodium metaperiodate resulted in the consumption of 0·22 mol. of reagent per hexose residue.

Methylation of Degraded Galactan.—Galactan (100 mg.) was methylated with methyl sulphate and sodium hydroxide, first in aqueous solution and then in tetrahydrofuran to give methylated galactan (64 mg.) (Found: OMe, 44·1%, not raised on further methylation with methyl iodide and silver oxide). A portion of the methylated galactan was hydrolysed and chromatography of the hydrolysate in solvents B, D, and E showed 2,4,6-tri-*O*-methylgalactose (R_G 0·72) as the main component with smaller amounts of 2,3,4,6-tetra- (R_G 0·90) and 2,4,-di-*O*-methylgalactose (R_G 0·47), and a trace of 2,3,5-tri-*O*-methylarabinose (R_G 0·98). The identity of these cleavage fragments was further indicated since gas chromatography of the methanolysis products from the methylated degraded galactan on column *c* showed methyl glycosides of 2,3,4,6-tetra- (*T* 1·53, 1·62), 2,4,6- (*T* 2·12, 2·40) and 2,3,4-tri- (*T* 2·64, 2·92, in small amount only), and 2,4-di-*O*-methylgalactose (*T* 3·68, 4·38), together with small amounts of unidentified products (*T* 0·72, 0·83) (possibly methyl ethers of alditols resulting from cleavage of galactose residues in the formation of the galactan). Gas chromatography on column *b* at 150° also indicated the presence of traces of methyl glycosides of 2,3,5-tri-*O*-methylarabinose (*T* 0·52, 0·69).

The authors thank Professor E. L. Hirst, C.B.E., F.R.S., for his interest and advice, Messrs. Alex Cowan and Sons Ltd. for financial assistance (to T. M. W.), and Imperial Chemical Industries Limited for a grant.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, August 30th*, 1962.]