ACTA CHEMICA SCANDINAVICA 16 (1962) 2275–2283

Studies on a Galactan from Tension Wood of Beech (*Fagus silvatica* L.)

HANS MEIER*

Träkemiska avdelningen, Svenska Träforskningsinstitutet, Stockholm, Sweden

Chlorite holocellulose has been prepared from beech tension wood and has been extracted with dimethylsulphoxide, hot water and sodium hydroxide. The hemicellulose mixture extracted with the latter was fractionated with Fehling's solution and with cetyl-trimethylammonium hydroxide and a galactan was isolated with $\overline{DP}_n$ 380 and a uronic acid content of 28 %. On hydrolysis it yielded D-galactose, 69.3 %, L-arabinose, 6 %, D-xylose 4.2 % and L-rhamnose 20.5 %. From a partial hydrolysate of the galactan there were isolated — by carbon column chromatography and by chromatography on thick filter paper — the following neutral oligosaccharides: 6-*O*-β-D-galactopyranosyl-D-galactose, 4-*O*-β-D-galactopyranosyl-D-galactose, 6-*O*-β-D-galactopyranosyl-6-*O*-β-D-galactopyranosyl-D-galactose, 6-*O*-β-D-galactopyranosyl-4-*O*-β-D-galactopyranosyl-D-galactose, 6-*O*-β-D-galactopyranosyl-6-*O*-β-D-galactopyranosyl-6-*O*-β-D-galactopyranosyl-D-galactose, and the following acidic sugars: D-galacturonic acid, 4-*O*-methyl-D-glucuronic acid, 2-*O*-α-D-galacturonopyranosyl-L-rhamnose, 6-*O*-(4-*O*-methyl-β-D-glucuronopyranosyl)-D-galactose as well as a few others which were identified tentatively.

The galactan thus obviously contains both β-1,4- as well as β-1,6-linked galactose units, a combination which has not previously been demonstrated to occur in a natural polysaccharide.

Tension wood of several hardwood species has been shown to contain a higher percentage of galactose residues than normal wood [1,2]. In the present investigation the parent galactan was isolated from beech tension wood (*Fagus silvatica L.*) and characterised.

ISOLATION OF THE GALACTAN

The beech stem from which the galactan was isolated contained some 30 % typical tension wood which, in the fresh state in transverse sections through the stem, could be distinguished macroscopically from the normal wood by its light appearance. Microscopically it was found that the light area contained

* Present address: Botanisches Institut, Universität Fribourg, Fribourg, Switzerland.

Table 1. Carbohydrate analyses of polysaccharide fractions from beech tension wood.

	Yield g	Galactose %	Glucose %	Mannose %	Arabinose %	Xylose %
A: Beech tension wood, extracted with acetone (Klason lignin: 18.1 %)	961.7	4.9	73.3	trace	2.0	18.8
(Normal beech wood		1.3	57.6	4.3	1.0	35.8)
B: Polysaccharides from the delignification liquor						
B_1: Precipitated with 80 % ethanol	22.5	trace	31.8	4.6	21.8	41.8
B_2: Precipitated with 90 % ethanol	6.0	17.5	2.4	8.7	21.1	50.4
C: Holocellulose	833.5	4.6	75.8	trace	1.2	18.4
D: Polysaccharides from DMSO-extract	5.7	3.4	14.0	3.4	1.0	78.3
E: Polysaccharides from hot water extract	4.0	40.5	—	trace	11.9	47.7
F: Polysaccharides from 8 % sodium hydroxide extract	139.0	14.8	—	trace	6.5	78.7
G: Residual pulp	570.0	2.5	91.8	—	1.1	4.5

80 to 90 % tension wood fibres. The sugar analysis of a hydrolysate of the tension wood area showed only traces of mannose, a low xylose and a high glucose and galactose content (*A*, Table 1), which can be considered typical for tension wood. An analysis for normal beech wood is also given in Table 1.

Acetone-extracted, milled tension wood was delignified by the chlorite method and two fractions (B_1 and B_2, Table 1) were recovered from the delignification liquor. Both contained large amounts of materials other than polysaccharides. Only relatively small amounts of hemicelluloses had been dissolved during the chlorite treatment of the wood. The extraction of the holocellulose with DMSO (dimethylsulphoxide) and the subsequent extraction with hot water yielded two small fractions, *D* and *E*, respectively, of which the former was essentially a xylan and the latter a mixture of a xylan and a galactan. Large amounts of hemicellulose were then extracted with 8 % sodium hydroxide. This fraction, *F*, yielded on hydrolysis 14.8 % galactose which corresponded to about half of the galactose content of the wood. *F* was therefore subfractionated to separate the galactan from the large amounts of xylan also present in this fraction. Part of the xylan could be precipitated by adding Fehling's solution to an aqueous solution of *F*. The precipitate (F_1, Table 2) contained only a small amount of galactose residues. The polysaccharides (F_2)

Table 2. Carbohydrate analyses of the subfractions of *F*.

Fraction	Galactose %	Arabinose %	Xylose %	Rhamnose %
F_1	2.2	—	97.8	—
F_2	47.6	5.5	34.7	12.2
F_3	trace	—	100.0	—
F_4	69.3	6.0	4.2	20.5
F_5	16.5	2.5	81.0	trace

recovered from the soluble fraction contained 47.6 % galactose and 34.7 % xylose residues. When a sample of F_2 was redissolved in a small volume of water, no more xylan could be precipitated either by addition of Fehling's solution or of barium hydroxide. F_2 was then further fractionated with cetyltrimethyl ammonium hydroxide (CTA-OH).[3,4] CTA-OH was added to an aqueous solution of F_2 until precipitation was complete. The soluble fraction (F_3) was a pure xylan. The precipitate, which still was a mixture of a galactan and a xylan, was dispersed in water and sodium chloride solution was added until the precipitate was redissolved. On addition of water a new partial precipitation occurred. This new precipitate (F_4) was a galactan almost free from xylose residues whereas the soluble fraction (F_5) was essentially a xylan.

CHARACTERISATION OF THE GALACTAN (F_4)

Electrophoresis of F_4 on glass fibre sheets gave an elongated spot but no distinct components could be detected. F_4 contained 28 % uronic acids and had, in the acid form, $[\alpha]_D^{25}$ +11°. Its degree of polymerisation ($\overline{DP}_n$) was 380, determined osmometrically on the sodium salt in 0.1 M sodium chloride solution. The relative amounts of neutral sugars obtained by hydrolysis of F_4 (4 % sulphuric acid at 120° for 1 h) are given in Table 2. The xylose residues are most likely no integral part of the galactan but belong to a contaminating xylan.

Table 3. Carbon column fractionation of the neutral compounds from the partially hydrolysed galactan.

Fraction	Yield mg	Compound[a]		M_G[b]	R_{aG}[c]
25–49	620		galactose, arabinose, xylose rhamnose		
50–75	87	*a*	Gap $\xrightarrow[\beta]{1\ 6}$ Ga, monosaccharides	0.84	0.39
76–86	74	*b*	Gap $\xrightarrow[\beta]{1\ 4}$ Ga	0.45	0.59
		c	Gap $\xrightarrow[\beta]{1\ 6}$ Gap $\xrightarrow[\beta]{1\ 6}$ Ga	0.78	0.18
87–103	40	*d*	Gap $\xrightarrow[\beta]{1\ 6}$ Gap $\xrightarrow[\beta]{1\ 4}$ Ga	0.49	0.24
		e	Gap $\xrightarrow[\beta]{1\ 6}$ Gap $\xrightarrow[\beta]{1\ 6}$ Gap $\xrightarrow[\beta]{1\ 6}$ Ga	0.69	0.08
104–160 and 50 % ethanol eluate	180		higher oligosaccharides		

[a] Ga: D-galactose; *p*: pyranose form
[b] Paper electrophoretic mobility in 0.1 M borate buffer, pH 10.0 relative to glucose.
[c] Paper chromatographic mobility relative to galactose in solvent (i).

From a hydrolysate of F_4, after the acidic components had been removed by ion exchange, galactose crystallised directly. The mother liquor was fractionated by chromatography on thick filter paper, from which arabinose and rhamnose were recovered by elution with water. They were also obtained in the crystalline state and after recrystallisation of the three sugars their melting points and optical rotations were identical with those of D-galactose, L-arabinose and L-rhamnose, respectively.

A sample of F_4 was subjected to partial hydrolysis, the yield of lower oligosaccharides being improved by separation and further hydrolysis of the higher oligosaccharides. The hydrolysate was then separated into a neutral fraction (*I*) and an acidic fraction (*J*). *I* was fractionated by carbon column chromatography and by paper chromatography on thick filter paper. Table 3 gives the order in which the different compounds were eluted from the carbon column. All the oligosaccharides, on total hydrolysis, yielded galactose only. The two disaccharides (*a* and *b*) and the two trisaccharides (*c* and *d*) were obtained in the crystalline state. The structures of the disaccharides were determined by comparing them with authentic samples. The structures of the trisaccharides and of the tetrasaccharide (*e*) were elucidated by partial hydrolysis and chromatographic and electrophoretic identification of the products obtained. Substance *d*, a trisaccharide, previously unknown, was also reduced

Table 4. Carbon column fractionation of the acid compounds from the partially hydrolysed galactan.

Fraction	Yield mg	Compound[a]		R_{Gaa}[b]	M_{Gla}[c]	M_G[d]
17–35	130	*f*	Gaa	1.00	0.92	1.18
		g	MGla ca.	3.0	1.00	0.89
91–103	93	*h*	Gaa*p* $\xrightarrow[\alpha]{1\,2}$ Rh, minor amounts of *i*	0.74	0.65	0.67
104–131	116	*i*	MGla*p* $\xrightarrow[\beta]{1\,6}$ Ga, minor amounts of *j*	0.54	0.69	1.06
132–165	94	*j*	Gla*p* $\xrightarrow[\beta]{1\,6}$ Ga (?)	0.44	0.68	1.08
		k	MGla*p* $\xrightarrow[\beta]{1\,6}$ Ga*p* $\xrightarrow[\beta]{1\,6}$ (?)	0.38	0.54	0.92
166–209	156	*l*	MGla*p* $\xrightarrow[\beta]{1\,6}$ Ga*p* $\xrightarrow[\beta]{1\,6}$ Ga*p* $\xrightarrow[\beta]{1\,6}$ Ga (?)	0.19	0.46	0.88
		m	MGla*p* $\xrightarrow[\beta]{1\,6}$ Ga*p* $\xrightarrow[\beta]{1\,6}$ Ga*p* $\xrightarrow[\beta]{1\,4}$ Ga (?)	0.25	0.46	0.61
			Several other higher aldouronic acids			

[a] Gaa: D-galacturonic acid; MGla: 4-*O*-methyl-D-glucuronic acid; Gla: D-glucuronic acid; Rh: L-rhamnose; Ga: D-galactose; *p*: pyranose form.

[b] Paper chromatographic mobility relative to galacturonic acid in solvent *ii*.

[c] Paper electrophoretic mobility in 0.1 M acetate buffer, pH 4, relative to glucuronic acid.

[d] Paper electrophoretic mobility in 0.1 M borate buffer, pH 10, relative to glucose.

with sodium borohydride before it was subjected to partial hydrolysis, and yielded then *a* as the only reducing disaccharide.

The acidic fraction *J* from the partially hydrolysed galactan contained only small amounts of aldobiuronic acids but large amounts of higher aldouronic acids. It was therefore hydrolysed further and after the neutral sugars had been removed the acids were fractionated as the barium salts by carbon column chromatography. The fractions were then further fractionated by chromatography on thick filter paper. The compounds obtained are listed in Table 4. They were identified as described in the experimental part.

The uronic acids, the aldobi-, aldotri- and aldotetrauronic acids could easily be distinguished from each other by electrophoresis in acetate buffer (Table 4). Only two uronic acids, *i.e.* D-galacturonic acid (*f*) and 4-*O*-methyl-D-glucuronic acid (*g*) were detected, although a third, *viz.* glucuronic acid, was found in small amounts as a component of an aldobiuronic acid (*j*). Two aldobiuronic acids, *h* and *i* were found in relatively large amounts. An aldotriuronic acid, *k*, and two aldotetrauronic acids, *l* and *m*, all appeared to be derived from substance *i* which they yielded on hydrolysis together with galactose and 4-*O*-methyl-D-glucuronic acid.

DISCUSSION

That the galactan, F_4, is a homogeneous polysaccharide cannot be decided from the present study. F_4 could possibly consist of two acidic polysaccharides which are not separated by the fractionation technique used. The oligosaccharides obtained from the partial hydrolysate show, that both β-1,4- and β-1,6-galactopyranosidic linkages are present in the same molecular species which also contains 4-*O*-methyl-D-glucuronic acid residues. No aldotri- or aldotetrauronic acids were found containing 2-*O*-α-D-galacturonopyranosyl-β-rhamnose as a component. This can most probably be explained by the fact that rhamnosidic linkages are hydrolysed much faster than *e.g.* galactosidic linkages. No conclusions can be drawn from the present study as to whether the galacturonosyl-rhamnose is an integral part of the galactan or not. The same is true for the arabinose residues found in the hydrolysate of F_4. Since no oligosaccharides containing arabinose residues were found the latter are most likely to be present in the furanoside form as non-reducing end groups in the polysaccharide. The rhamnose residues in F_4 seem to a large extent to be present in aldobiuronic acid residues. However, relatively mild hydrolysis of the polysaccharide under conditions where aldobiuronic acids are not split also yielded rhamnose.

The 2-*O*-α-D-galacturonopyranosyl-L-rhamnose which in the present study was found to be a component of tension wood galactan has previously been found in a hydrolysate of pine wood by Roudier and Eberhard [5], but it has never been reported from a hardwood. The same authors have also isolated 6-*O*-β-D-glucuronopyranosyl-D-galactose from pine. The 6-*O*-(4-*O*-methyl-β-D-glucuronopyranosyl)-D-galactose units found in tension wood galactan represent a new aldobiuronic acid for wood and a polysaccharide containing both β-1,6-linked and β-1,4-linked galactose residues has not previously been demonstrated in nature. Gillham and Timell [6] have isolated from a hydrolysate

of white birch β-1,4-linked galactobiose which might have originated from a galactan similar in structure to tension wood galactan.

The galactan isolated from tension wood of beech differs markedly from compression wood galactan [7] but has a strong similarity to many gums. Both gum myrrh as well as mesquite gum [8] contain galactose, arabinose and 4-*O*-methyl-D-glucuronic acid as component sugars. In the former the uronic acid residues are partly linked to C_4 and partly to C_6 of galactose residues, which themselves are both β-1,3- and β-1,6-linked. 2-*O*-α-D-galacturonopyranosyl-β-rhamnose has been found in many different gums [8]. *Khaya grandifolia* gum [8,9] contains galactose, rhamnose, a trace of arabinose, galacturonic acid and 4-*O*-methyl-D-glucuronic acid, the same sugars which have been found in the tension wood galactan. Two aldobiuronic acids have been isolated from *Khaya grandifolia* gum, *viz.* 4-*O*-(4-*O*-methyl-D-glucuronopyranosyl)-D-galactose and 2-*O*-D-galactopyranosyl-L-rhamnose.

EXPERIMENTAL

Melting points are corrected. Concentration of solutions was effected under reduced pressure at a bath temperature of 40°.

Paper chromatography and paper electrophoresis. Whatman No. 1 and No. 3 MM papers were used. The chromatograms were run in the solvent systems (v/v):

(i) ethyl acetate-pyridine-water, 2:1:2 (upper layer).
(ii) ethyl acetate-acetic acid-formic acid-water, 19:3:1:4.
(iii) ethyl acetate-acetic acid-pyridine-water, 5:1:5:3.

The method of Saeman *et al.* [10] was used for quantitative determinations of monosaccharides. Paper electrophoreses were run in 0.1 M borate buffer of pH 10, in 0.1 M acetate buffer of pH 4, in germanate buffer [11] of pH 10.7 or in sulphonated phenylboronic acid buffer [12] of pH 7.

$\overline{DP}_n$-determination of F_4. The $\overline{DP}_n$ was determined osmometrically as described earlier for an arabinoglucuronoxylan [13]. For the calculation of the $\overline{DP}_n$ from the molecular weight of the polymer a molecular weight of 160 was assumed for the monomer units.

Isolation and fractionation of the polysaccharides. Tension wood areas from a 65 year old bent beech (*Fagus silvatica* L.) were cut out and wood meal was prepared in a Whiley mill. The fraction between 1 and 2 mm was extracted with acetone and chlorite holocellulose was prepared. The spent chlorite solution was dialysed against tap water for 8 days and concentrated to 3 l. After acidification with acetic acid hemicellulose fraction B_1 was precipitated with ethanol (12 l), centrifuged off, washed with ethanol and acetone, and dried. Further amounts of ethanol (15 l) were added to the centrifugate and the new precipitate (B_2) was centrifuged off and recovered in the usual way. The holocellulose (C) was extracted successively with dimethylsulphoxide, hot water and 8 % sodium hydroxide, the latter extraction being made under a nitrogen atmosphere. The polysaccharides from the different extracts were recovered by precipitation with ethanol (fractions D, E and F, respectively). A sample of F (87 g) was dissolved in water (2.5 l). Fehling's solution (1.5 l) was added, the precipitate was centrifuged, washed with dilute Fehling's solution and dissolved in 20 % acetic acid (2 l). The polysaccharides were then precipitated with ethanol (8 l) and washed free from copper with ethanol-water (4:1) containing small amounts of hydrochloric acid. Finally the precipitate was washed with ethanol and acetone and dried (F_1, yield 50 g). The centrifugate from the precipitation with Fehling's solution was treated with cation exchange resin (Dowex 50) and the hemicelluloses (F_2) were precipitated by the addition of ethanol and recovered in the usual way (yield 15.3 g).

A sample of F_2 (15 g) was dissolved in water (0.5 l) and neutralised with sodium hydroxide. A small insoluble fraction was discarded and a 5 % aqueous solution (150 ml) of cetyl-trimethylammonium hydroxide (CTA-OH) was added until precipitation was complete. The precipitate was centrifuged off and the centrifugate was treated with cation exchange resin (Dowex 50). By addition of ethanol a precipitate (F_3) was obtained

and recovered. The precipitate which had been obtained with CTA-OH was washed once with water by centrifugation and then dispersed in water (200 ml). On the addition of 10 % sodium chloride solution (50 ml) the precipitate dissolved. Water (1 l) was slowly added with stirring and the precipitate formed (F_4) was centrifuged off, dissolved in dilute acetic acid and recovered by precipitation with ethanol. The centrifugate from F_4 was neutralised with acetic acid, concentrated and the polysaccharides (F_5) were recovered by precipitation with ethanol. F_4, $[\alpha]_D^2$ +11.5° (*c* 2.0, H_2O) yielded sulphate ash, 2.4 %, and contained uronic acid, 28.2 %, calculated as galacturonic acid. Electrophoresis of F_4 in acetate buffer gave an elongated spot with $M_{\text{glucuronic acid}}$ *ca.* 0.3.

Identification of neutral monosaccharides from a hydrolysate of F_4. A sample of F_4 (1 g) was hydrolysed by heating for 1 h at 120° with 4 % sulphuric acid (200 ml). After neutralisation with barium hydroxide and centrifugation of the barium sulphate, the acidic sugars were removed by ion exchange using Dowex 1 resin in the acetate form. The solution containing the neutral sugars was concentrated to dryness and on trituration with moist methanol galactose crystallised. After two recrystallisations it had m.p. 162—165°, undepressed on admixture of D-galactose and $[\alpha]_D^{25}$ +79°(*c* 2.0, H_2O). The mother liquor was applied to thick filter paper which was irrigated with solvent (i) and rhamnose and arabinose were eluted from the paper. The rhamnose crystallised as the monohydrate and had m.p. 75° and 95° for hydrated and anhydrous forms, respectively. undepressed on admixture of L-rhamnose, and $[\alpha]_D^{25}$ +7.3° (*c* 1.0, H_2O). The arabinose also crystallised and had m.p. 150—154°, undepressed on admixture of L-arabinose, and $[\alpha]_D^{25}$ 103° (*c* 3.0, H_2O).

Partial hydrolysis of F_4 *and identification of the compounds obtained.* A sample of F_4 (3.9 g) dissolved in N sulphuric acid (400 ml) was heated on the steam bath for 30 min and then was cooled and neutralised with barium hydroxide. Barium sulphate was removed by centrifuging and washed twice with 25 % ethanol. Since the recovery of material was very incomplete, the barium sulphate precipitate was washed with dilute acetic acid whereby further material was obtained. Centrifugate and washings were combined and concentrated to 30 ml. By addition of ethanol (60 ml) the more highly polymeric material was precipitated and again partially hydrolysed as before. The whole procedure was repeated twice. All the low polymer fractions were combined (2.5 g) and the neutral sugars (*I*) were separated from the acidic (*J*) by adsorption of the latter on anion exchange resin (Dowex 1, acetate form), from which they then were eluted with 3 N acetic acid.

The neutral sugars (*I*) (1.2 g) were applied to a carbon/Celite column (4 × 40 cm). Gradient elution with aqueous ethanol (5 l, 0—25 %) effected a partial separation of the components (Table 3). All the fractions were fractionated further by chromatography on thick filter paper. All the oligosaccharides obtained yielded on hydrolysis galactose only. Partial hydrolysis of *c*, *d* and *e* was carried out on 3 mg samples in N sulphuric acid (0.5 ml) for 20 min at 100°.

a (22 mg), 6-*O*-β-D-galactopyranosyl-D-galactose crystallised from methanol with methanol as solvent of crystallisation, m.p. 97—100°, $[\alpha]_D^{25}$ +26° (c 2.5, H_2O)[14] and was chromatographically (solvent (i)) and electrophoretically (borate buffer) indistinguishable from an authentic sample.

b (39 mg), 4-*O*-β-D-galactopyranosyl-D-galactose crystallised from methanol and had m.p. 209—213°, undepressed on admixture of an authentic sample, $[\alpha]_D^{25}$ +67° (*c* 1.8, H_2O)[8,9] and was chromatographically (solvent (i)) and electrophoretically (borate buffer) indistinguishable from an authentic sample.

c (22 mg), 6-*O*-β-D-galactopyranosyl-6-*O*-β-D-galactopyranosyl-D-galactose crystallised from methanol and had m.p. 148—150° and $[\alpha]_D^{25}$ +5° (*c* 1.0, H_2O). A somewhat higher m.p. 157—160° and optical rotation $[\alpha]_D^{25}$ +16° have been reported by Aspinall *et al.*[15] Partial hydrolysis of *c* yielded substance *a* as the only disaccharide. On paper electrophoresis in sulphonated phenyl boronic acid *c* showed an M_G -value similar to that of substance *a*.

d (19 mg), 6-*O*-β-D-galactopyranosyl-4-*O*-β-D-galactopyranosyl-D-galactose crystallised from methanol and had m.p. 132—140° (explosive swelling) and 176—178° (melting) and

$[\alpha]_D^{25}$ +37° (*c* 2.3, H_2O). On partial hydrolysis *d* yielded the two disaccharides *a* and *b*. After reduction with sodium borohydride and partial hydrolysis *a* was obtained as the only reducing disaccharide. On paper electrophoresis in sulphonated phenyl boronic acid *d* showed an M_G-value similar to that of substance *b*.

e (6 mg), 6-*O*-β-D-galactopyranosyl-6-*O*-β-D-galactopyranosyl-6-*O*-β-D-galactopyranosyl-D-galactose yielded on partial hydrolysis galactose, *a* and *c*.

The acidic sugars (*J*, 1.3 g) from the partial hydrolysate of F_4 were further hydrolysed with 4 % sulfuric acid at 120° for 30 min and the neutral sugars were again separated as above from the acidic ones. The acidic sugars (0.7 g) were then applied as their barium salts to a carbon-Celite column (4 × 45 cm) which first was irrigated with water (2 l) and then with aqueous ethanol (5 l, 0—20 %). 30 ml fractions were collected and similar fractions were combined (Table 4). The main fractions were then further fractionated by chromatography on thick filter paper (solvent (ii)).

f (48 mg), D-galacturonic acid, was heated over night in an ampoule in 2 % methanolic hydrogen chloride in a boiling methanol bath. After neutralisation with silver carbonate the solution was centrifuged and concentrated. The methyl-(methyl-α-D-galactopyranosyl)-uronate crystallised from methanol-butanol and had m.p. 146—150°, undepressed on admixture with an authentic sample, and $[\alpha]_D^{55}$ +125°(*c* 1.0, H_2O).

g (38 mg), 4-*O*-methyl-D-glucuronic acid. From a sample of *g* (5 mg) the methyl ester methyl glucuronoside was prepared as decribed for substance *f*. The compound was dissolved in water (0.5 ml) and reduced by addition of sodium borohydride (20 mg). After 1 h the solution was neutralised with dilute acetic acid and deionised with a mixture of IR 4B and Dowex 50 resins. The sample was then hydrolysed with N sulphuric acid and neutralised. On paper electrophoresis in germanate buffer which separates the different monomethyl ethers of glucose very well, a strong spot with the same M_G-value as 4-*O*-methyl-D-glucose was obtained. Demethylation of the sample with boron trichloride [16] yielded glucose.

h (76 mg), 2-*O*-α-D-galacturonopyranosyl-L-rhamnose, had +76° (*c* 2.0, H_2O, Ba-salt) [7]. On hydrolysis with 8 % sulphuric acid (1 h at 120°) rhamnose and galacturonic acid were obtained. The paper chromatographic (solvents (ii) and (iii)) mobility of *h* was identical with that of an authentic sample of 2-*O*-α-D-galacturonpyranosyl-L-rhamnose (kindly supplied by Dr. A. Roudier) and when the chromatograms were developed with triphenyl tetrazolium chloride [7,17] *h* showed a negative formazan reaction.

i (117 mg), 6-*O*-(4-*O*-methyl-β-D-glucuronopyranosyl)-D-galactose, $[\alpha]_D^{25}$ +8° (*c* 1,1, H_2O, Ba-salt) [18] yielded on hydrolysis with 8 % sulphuric acid (1 h at 120°) 4-*O*-methyl glucuronic acid and galactose. The methyl glucuronoside was prepared from *i* (10 mg) and reduced with sodium borohydride as described above for *g*. The reduced compound was subjected to total hydrolysis and neutralised, and paper electrophoresis (germanate buffer) and paper chromatography (solvent (*i*)) indicated the presence of 4-*O*-methyl-glucose and galactose. The former, on demethylation with boron trichloride [16], yielded glucose. Partial hydrolysis of the reduced methyl ester methyl glucuronoside gave on paper electrophoresis (borate buffer, electrogram developed with anisidine hydrochloride) two spots with the same M_G-values as 4-*O*-methyl-glucose and galactose, respectively, and a third with M_G somewhat lower than that of 6-*O*-methyl-galactose but much higher than that of 4-*O*-methyl-galactose, indicating the presence of 6-*O*-(4-*O*-methyl-glucopyranosyl)-D-galactose. *i* showed a positive formazan reaction. A sample of *i* (26 mg) was converted into the methyl ester methyl glucuronoside and then fully methylated by the procedure of Kuhn *et al.* [19] The methylated product was dissolved in methanol and reduced by addition of sodium borohydride. The substance was recovered in the usual way and hydrolysed with N sulphuric acid. Chromatography of the reaction mixture on dimethylsulfoxide impregnated paper [20] with ethyl ether as irrigant and with the following reference substances: 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4-, 2,4,6- and 2,3,6-tri-*O*-methyl-D-galactose, revealed the presence of 2,3,4-tri-*O*-methyl-glucose and of 2,3,4-tri-*O*-methyl-galactose, giving further evidence for the structure proposed for *i*.

j (11 mg), yielded on hydrolysis galactose and an uronic acid together with its lactone which on paper chromatography in two solvents, (ii) and (iii), had the same R_F-values as glucuronic acid and its lactone, respectively. Although *j* could be easily distinguished chromatographically from substance *i* it had almost the same M_G-value as the latter on

paper electrophoresis in borate buffer, thus probably being 6-*O*-β-D-glucuronopyranosyl-D-galactose.

k (51 mg), *l* (7 mg) and *m* (11 mg) on hydrolysis all yielded, amongst other compounds, substance *i* and galactose. A comparison of the M_G-values of *k*, *l* and *m* (Table 4) with those of *a*, *c* and *d* (Table 3), respectively, gives some evidence for the structures proposed for *k*, *l*, and *m* in Table 4. Further evidence for the structures of *l* and *m* was obtained by partial hydrolysis, *l* yielding galactose, *i*, *k* and *a*, and *m* yielding galactose, *i*, *k* and *b* together with the unchanged compounds.

Acknowledgement. The author is much indebted to Professor Bengt Lindberg for his interest in this work and to Miss Anita Stridsberg and Mr. Nils-Håkan Wallin for skilful assistance.

REFERENCES

1. Gustafsson, C., Ollinmaa, P. J. and Saarnio, J. *Acta Chem. Scand.* **6** (1952) 1299.
2. Schwerin, G. *Holzforschung* **12** (1958) 43.
3. Scott, J. E. *Methods of Biochemical Analysis* **8** (1960) 145.
4. Bouveng, H. O. and Lindberg, B. *Acta Chem. Scand.* **12** (1958) 1977.
5. Roudier, A. J. and Eberhard, L. *Bull. soc. chim. France* **1960** 2074.
6. Gillham, J. K. and Timell, T. E. *Svensk Papperstidning* **61** (1958) 540.
7. Bouveng, H. O. and Meier, H. *Acta Chem. Scand.* **13** (1959) 1884.
8. Smith, F. and Montgomery, R. *The Chemistry of Plant Gums and Mucilages*, New York 1959.
9. Aspinall, G. O., Hirst, E. L. and Matheson, N. K. *J. Chem. Soc.* **1956** 989.
10. Saeman, J. F., Moore, W. E., Mitchell, R. L. and Millet, M. A. *Tappi* **38** (1954) 336.
11. Lindberg, B. and Swan, B. *Acta Chem. Scand.* **14** (1960) 1043.
12. Garegg, P. J. and Lindberg, B. *Acta Chem. Scand.* **15** (1961) 1913.
13. Meier, H. *Acta Chem. Scand.* **12** (1958) 1911.
14. Haq, S. and Adams, G. A. *Can. J. Chem.* **39** (1961) 1563.
15. Aspinall, G. O., Hirst, E. L. and Ramsted, E. *J. Chem. Soc.* **1958** 593.
16. Bonner, T. G., Bourne, E. J. and McNally, S. *J. Chem. Soc.* **1960** 2929.
17. Wallenfels, K. *Naturwiss.* **37** (1950) 491.
18. Jones, J. K. N. and Nunn, J. R. *J. Chem. Soc.* **1955** 3001.
19. Kuhn, R., Trischmann, H. and Löw, I. *Angew. Chem.* **67** (1955) 32.
20. Wickberg, B. *Acta Chem. Scand.* **12** (1958) 615.

Received May 25, 1962.