STRUCTURAL INVESTIGATION OF WATER-SOLUBLE RAPESEED (*Brassica campestris*) POLYSACCHARIDES

PART II AN ACIDIC ARABINOGALACTAN*

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ABSTRACT

An acidic arabinogalactan isolated from rapeseed consists of L-arabinose, D-galactose, and D-glucuronic acid in molar proportions of 1 1 05 0 13 Electrophoretic and sedimentation data indicate that the acidic arabinogalactan is largely homogeneous Methylation studies show that the polysaccharide has a highly branched structure Hydrolysis of the methylated polysaccharide yielded 2,3,4,6-tetra-*O*-methyl-D-galactose (1 mol.), 2,3,5-tri-*O*-methyl-L-arabinose (14 mol), 2,3,4-tri-*O*-methyl-D-glucuronic acid (3 mol), 2,4,6-tri-*O*-methyl-D-galactose (4 mol), 2,3,4-tri-*O*-methyl-D-galactose (2 mol), 2,3-di-*O*-methyl-L-arabinose (5 mol), 2,4-di-*O*-methyl-D-galactose (13 mol), 3-*O*-methyl-L-arabinose (1 mol), and 2-*O*-methyl-D-galactose (2 mol) Periodate-oxidation data substantiate the methylation results The general structural features of the acidic arabinogalactan are discussed

INTRODUCTION

In a previous report[1], we described the isolation and characterization of an amyloid fraction from rapeseed Further work on the water-soluble fraction of the rapeseed cotyledon meal has resulted in the isolation of an acidic arabinogalactan for which the main structural features are now reported

RESULTS AND DISCUSSION

The crude, acidic arabinogalactan was isolated by fractionation of polysaccharide I[1] on DEAE-cellulose (borate form)[2] by elution with 50 mM sodium metaborate ($Na_2B_2O_4$ $8H_2O$) in a yield of 64 7% of fraction I or 0 086% of the alcohol-insoluble residue[1] Fractionation of the crude, acidic arabinogalactan on DEAE-cellulose (carbonate form)[3] yielded, *inter alia*, a major fraction (40%) Paper chromatography of a hydrolysate of this purified polysaccharide showed minute amounts of glucose, mannose, and xylose, in addition to the major components, namely

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arabinose, galactose, and uronic acid A final purification on DEAE-cellulose (phosphate form)[4] yielded the pure, acidic arabinogalactan (82 7%) and an impure fraction (1 3%) The neutral sugar components of the purified acidic arabinogalactan were D-galactose and L-arabinose (1 05 1) The uronic acid content, determined by decarboxylation[5], was 6 9%, giving an overall molar ratio of D-glucuronic acid–D-galactose–L-arabinose of 0 13 1 05 1. The rapeseed, acidic arabinogalactan showed a major, symmetrical, mobile peak both on boundary electrophoresis[6] and sedimentation analysis[7] The minor peak in each case amounted to *ca* 10% of the total, and thus the acidic polysaccharide appeared to be chemically homogeneous to the extent of *ca* 90% The detection of 1 01% of nitrogen in the polysaccharide suggested that the minor peak might have originated from a protein or a glycoprotein contaminant

After acid hydrolysis of the polysaccharide and fractionation of the sugars on an anion-exchange resin and subsequently on paper chromatograms, D-galactose and L-arabinose were obtained in crystalline form The major acidic components were identified as D-glucuronic acid and an aldobiouronic acid which was identified as a glucuronosyl-galactose, presumably the (1→6)-linked analogue

The polysaccharide was methylated, and the fully methylated product was subjected to methanolysis and hydrolysis After fractionation of the hydrolysis products on an anion-exchange column, the neutral *O*-methyl sugars were resolved into four fractions by paper chromatography The identities and proportion of the sugars in each fraction were determined by combined g l c–mass spectrometry[8 22] of the derived alditol acetates The major, acidic component was identified as 2,3,4-tri-*O*-methyl-D-glucuronic acid, following reduction with lithium aluminum hydride[9], hydrolysis, reduction with borohydride[10], acetylation, and combined g l c–mass spectrometry[8 22] The proportion of the uronic acid component was evaluated from the decarboxylation data

Analytical data, after conversion to the nearest whole numbers, are summarized as follows

Component	*Molar ratio*	*Mode of linkage*
2,3,4,6-Tetra-*O*-methyl-D-galactose	1	D-Gal*p*-(1→
2,3,5-Tri-*O*-methyl-L-arabinose	14	L-Ara*f*-(1→
2,3,4-Tri-*O*-methyl-D-glucuronic acid	3	D-Glc*p*A-(1→
2,3,4-Tri-*O*-methyl-D-galactose	2	→6)-D-Gal*p*-(1→
2,4,6-Tri-*O*-methyl-D-galactose	4	→3)-D-Gal*p*-(1→
2,3-Di-*O*-methyl-L-arabinose	5	→5)-L-Ara*f*-(1→
2,4-Di-*O*-methyl-D-galactose	13	→3,6)-D-Gal*p*-(1→
3-*O*-Methyl-L-arabinose	1	→2,5)-L-Ara*f*-(1→
2-*O*-Methyl-D-galactose	2	→3,4,6)-D-Gal*p*-(1→

Most of the above sugars were further characterized, either in the crystalline form or by the isolation of crystalline derivatives The molar ratio of arabinose–galactose

(1.1.1), calculated from the proportion of methylated sugars, was in excellent agreement with that (1·1 05) found for the original polysaccharide

Methylation analysis showed that the molecule has a highly branched structure Thus, of the 45 sugar residues that make up an average repeating-unit, there is 1 terminal, non-reducing end-group of D-galactose, 14 terminal, non-reducing end-groups of L-arabinose, and 3 terminal, non-reducing end-groups of D-glucuronic acid There are 13 residues of D-galactose, 1 of L-arabinose, and 2 of D-galactose at which branching through the 3 and 6, 2 and 5, and 3, 4, and 6 positions occurs in the polysaccharide The remaining 11 non-terminal residues consist of two (1→6)-linked D-galactose residues, four (1→3)-linked D-galactose residues, and five (1→5)-linked L-arabinose residues

The highly negative $[\alpha]_D$ values (−49 2° and −80 6°, respectively) for the unmethylated and methylated polysaccharide strongly indicated that the galactosidic bonds were predominantly of the β-D type and the arabinosidic were predominantly of the α-L type

Mild, acid hydrolyses (5mM sulphuric acid for 1, 2 5, and 5 h at 100°) of the polysaccharide showed that 3, 16, and 34% of the arabinose residues, respectively, were released without any release of galactose On further hydrolysis with 10mM sulphuric acid for 5 h at 100°, 100% recovery of arabinose and 10 7% of galactose was obtained This suggested that arabinose residues were entirely present in the furanoid form and were located in the exterior chains of the molecular structure

The repeating unit proposed above requires a periodate consumption of 0 70 mole, with the liberation of 0 13 mole of formic acid per "anhydro sugar" residue This is in reasonable agreement with the experimentally determined amounts of periodate consumed (0 76 mol) and formic acid produced (0 22 mol) The high titration value for formic acid is attributed to the presence of oxidized uronic acid fragments Smith degradation of the oxopolysaccharide yielded galactose, glycerol, arabinose, and threitol in the molar ratios 1.0 8 0 056 0 64, respectively The theoretical molar ratio of these components, based on the proposed repeating unit, would be 1 1 1 0 053 0 The small amount of threitol presumably arose from a pectin contaminant, and was not considered to be of any structural significance

Arabinogalactans of plant origin, which have been studied in some detail, fall into two classes Firstly, the highly branched arabinogalactans of the coniferous woods, characterized by the presence of (1→3) and (1→6)-linked β-D-galactopyranose residues, and containing terminal residues of L-arabinofuranose and D-glucuronic acid Occasionally, some doubly branched D-galactose residues have also been reported The second group consists of arabinogalactan associated with pectins and containing a basic framework of (1→4)-linked β-D-galactopyranose residues[12] The arabinogalactan from the oilseed soybean[13–18] belongs to the latter type The water-soluble arabinogalactan of rapeseed cotyledon meal belongs to the first group and appears to be closely related to the arabinogalactan from coffee beans[19] The arabinogalactans from lucerne (alfalfa), gum tragacanth, and lemon peel apparently contain all three inter-galactose linkages, but in none of these cases has conclusive evidence

been presented to show that the three types of linkages appear in a single, homogeneous molecular-species[20] The same argument applies to the arabinogalactan from centrosema seed[21].

EXPERIMENTAL

The general experimental methods have been reported previously[1]

Fractionation of crude, acidic arabinogalactan — The fraction[1] (3 53 g) eluted from DEAE-cellulose (borate form) with 50mM borate was fractionated on a column (3 5 × 11 in) of DEAE-cellulose (CO_3^{2-}), as described previously[3] Elution with water (1500 ml), followed by a continuous gradient of 0→0 5M ammonium carbonate (7 l) and collection of the effluent in 25-ml portions, yielded 7 fractions The water-eluted fraction (0 80 g), on hydrolysis, appeared to be similar to the amyloid fraction, characterized previously[1], except for traces of mannose in the acid hydrolysate The acid hydrolysate of the carbonate-eluted fraction *1* (0 35 g) showed mainly galactose and arabinose, small proportions of glucose and mannose, and traces of uronic acid Acid hydrolysis of fraction *2* (58 mg) showed the same sugar components with relatively more uronic acid Both fractions *3* and *4* (1 375 g and 62 mg, respectively), on hydrolysis, gave mainly galactose, arabinose, and uronic acid, with traces of glucose, mannose, and xylose Hydrolysates of fractions *5* and *6* (0 41 and 0 22 g, respectively) gave mainly galactose, arabinose, xylose, small amounts of uronic acid, and traces of glucose and mannose

The combined fraction *3* and *4* (1 25 g) was fractionated on a column (1 5 × 14 in) of DEAE-cellulose (phosphate form)[4] Elution with water (3 l), followed by a continuous, gradient elution with 0→0 5M sodium dihydrogen phosphate (4 l), yielded two fractions The fraction eluted with water was concentrated, dialysed for 20 h against running tap-water and 6 h against two changes of distilled water, and freeze-dried to yield 19 mg of material The phosphate fractions were combined and similarly recovered to yield 1 2 g of material

Acid hydrolysis of the water-eluted fraction gave mainly glucose, galactose, and arabinose, together with a small proportion of mannose, and traces of xylose and uronic acid The hydrolysate of the phosphate-eluted, acidic arabinogalactan gave galactose and arabinose, and a small proportion of uronic acid

Analysis of acidic arabinogalactan — The acidic arabinogalactan had $[\alpha]_D^{24}$ −49 2° (c 0 497, water); N, 1 01%, and uronic acid, 6 9%[5]. Electrophoretic examination[6] (Spinco Model H) of a 1% solution in 50mM borate buffer (pH 9 2) showed a minor and a major symmetrical peak Sedimentation analysis[7] of a 1% solution in 0 1M sodium tetraborate (pH 9 1) at 59,780 rev /min also showed a minor and a major symmetrical peak The areas under the two peaks, measured by triangulation, showed a ratio of *ca* 1.9 in each case A portion (5 0 mg) of the mixture of neutral sugars prepared by heating the polysaccharide with M sulphuric acid for 3 h at 100° was reduced with sodium borohydride, and the resulting mixture of alditols was

acetylated and examined by g l c with a 5-ft column* of ECNSS-M on Gas Chrom Q (100–120 mesh) at 165° and a nitrogen flow-rate of 45 ml/min The areas under the peaks gave a molar ratio of arabinose and galactose of 1 1 05 Partial and complete acid hydrolyses of the polysaccharide samples (5 0 mg) with 5mM sulphuric acid for 1,2 5, and 5 h, with 10mM sulphuric acid for 5 h, and with M sulphuric acid for 3 h, followed by reduction and acetylation of the products in the presence of erythritol (2 0 mg per sample) and by g l c analysis as above, but with programming at the rate of 2°/min from 143–185°, gave galactose–arabinose molar ratios of 0 0 0 043, 0 0 0 2, 0 0 0 42, 0 18 1 23, and 1 68 1 22, respectively

Acid hydrolysis of acidic arabinogalactan — Acidic polysaccharide (200 mg) was hydrolysed with M sulphuric acid (10 ml) for 3 h at 100° After neutralization ($BaCO_3$), filtration, deionization with Rexyn-101 (H^+) resin, and concentration, the mixture of acidic and neutral sugars was fractionated on a column of Rexyn-CGl (CO_3^{2-}) resin Washing with water (250 ml) removed the neutral sugars (176 mg), and gradient elution with 0–0 5M ammonium carbonate (collecting 3-ml fractions) yielded the uronic acid material in fractions 15–45 The uronic acid fraction (16 mg) was recovered by removing the bulk of ammonium carbonate by distillation *in vacuo* at 65–70° and the remainder with Rexyn-101 (H^+) resin

(*a*) *Neutral sugars* A portion (153 mg) of the neutral sugar fraction was separated on sheets of Whatman No 1 paper (Solvent *A*) Location and elution of appropriate strips gave galactose (84 mg) and arabinose (65 mg) The former crystallized from hot methanol to give D-galactose, m p and mixed m p 166–167°, $[\alpha]_D^{24}$ +114 2 (4 min)→+79° (equil) (*c* 1 2, water) The latter crystallized from 95% ethanol to give L-arabinose, m p and mixed m p 151–153°, $[\alpha]_D^{24}$ +147.5 (5 min)→ +104 3° (equil) (*c* 1 2, water)

(*b*) *Uronic acid fraction* The acidic sugars (16 mg) were separated on Whatman No. 1 paper by using 1-butanol–acetic acid–water (4 1 5) for 72 h The components recovered corresponded to glucurono-6,3-lactone (2 1 mg), a hexuronic acid (3 07 mg) tentatively identified as glucuronic acid, and an aldobiouronic acid (2 5 mg) On hydrolysis with M sulphuric acid for 3 h at 100°, with examination of the hydrolysate by paper chromatography and paper electrophoresis, the last component gave the unhydrolysed compound, glucuronic acid, and galactose

Methylation of acidic arabinogalactan — The polysaccharide (500 mg) was methylated, with cooling in an atmosphere of nitrogen, by dropwise addition of 30% aqueous sodium hydroxide (20 ml) and methyl sulphate (7 ml) The reagents were added at such a rate that the reaction mixture remained at a nearly neutral pH for the first few hours The addition lasted several hours, after which time the solution was stirred for 24 h After 10 such additions during 10 days, the reaction mixture was cooled, and acidified with 3M sulphuric acid in an atmosphere of nitrogen The solution was extracted with chloroform (4 × 240 ml), and the extract was dried with anhy-

*Prepared by soaking Gas Chrom Q (97 parts) with ECNSS-M (3 parts) in chloroform, followed by filtration and drying

drous sodium sulphate, filtered, and evaporated The solid residue (519 mg) was dissolved in methyl iodide (50 ml), and silver oxide (5 g) was added in 5 equal portions over 8 h. After 10 more such methylations, a product (430 mg) was recovered that had $[\alpha]_D^{23}$ −80 6° (*c* 3, chloroform) and showed no hydroxyl absorption in its infrared spectrum

Methanolysis and hydrolysis of the methylated, acidic arabinogalactan — The methylated polysaccharide (400 mg) was dissolved in 5% methanolic hydrogen chloride (250 ml), and the solution was refluxed for 28 h After neutralization (silver carbonate), filtration, and evaporation, the syrupy product was hydrolysed with 0 5M sulphuric acid (20 ml) for 35 h The hydrolysate was neutralized with barium carbonate, filtered, and evaporated to give a syrup (250 mg)

A solution of the mixture of neutral and acidic sugars (216 mg) was treated with Rexyn-101 (H^+) resin, filtered, and added to a column of Rexyn-CG1 (CO_3^{2-}) Elution of the column with water (250 ml) removed the neutral sugars (174 mg) Gradient elution with 0→0 5M ammonium carbonate (1 litre), with collection of effluent in 5-ml fractions, gave the uronic acid material in fractions 10–30 The acidic product (9 mg) was recovered by removing the bulk of the ammonium carbonate by distillation *in vacuo* at 65° and the remainder by passing the solution through a column of Rexyn-101 (H^+) resin

G l c –mass spectrometry of the neutral, methylated sugars — A portion (20 mg) of the neutral sugars was reduced with sodium borohydride (60 mg) in water (1 ml) for 24 h, the excess of borohydride was destroyed with glacial acetic acid, and the solution was deionized with Rexyn-101 (H^+) and 203 (OH^-) resins The resulting mixture of methylated alditols was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) for 1 h at 100° and overnight at room temperature Water was added to the reaction mixture which was then evaporated to dryness, and the residue was taken up in chloroform for g l c analysis G l c was carried out on a Pye 104 Gas Chromatograph, using dual 5-ft columns (see above) of ECNSS-M on Gas Chrom Q (100–120 mesh) at 180° and a nitrogen flow-rate of 45 ml/min or with temperature programming from 155→195° at a rate of 2°/min In view of the incomplete separation of 2,3,4,6-tetra-*O*-methylgalactitol diacetate from 2,3-di-*O*-methylarabinitol triacetate and to facilitate identification of individual components by g l c –mass spectrometry, a second portion (29 mg) of the neutral sugars was separated on a large sheet of Whatman No. 1 paper by using 1-butanol–ethanol–water (40 11 19) The yields of the four fractions were 1, 9 2 mg; 2, 8 7 mg; 3, 10 7 mg, and 4, 3 1 mg Portions (2–3 mg) of fractions 1, 2, 3 and 4 were reduced with sodium borohydride[10], acetylated, and examined by g l c as above G l c –mass spectrometry[8,22] was carried out with the above chromatographic column coupled to a Bell and Howell 21-490 mass spectrometer The mass spectra were recorded at an inlet temperature of 200°, ionising potential of 70 eV, and an ion-source temperature of 200° The identities and overall proportions of the methylated alditol acetates and their retention times with respect to 2,3,4,6-tetra-*O*-methylglucitol diacetate are summarized in the following Table

Fraction No	*Acetate of*	*Retention time (min)*	*Molar ratio*
1	2 3 4,6-Me_4-Galactitol	1 19	1 2
	2,3,5-Me_3-Arabinitol	0 54	13 9
2	2,3-Me_2-Arabinitol	1 27	5 3
	2,4,6-Me_3-Galactitol	2 09	4 3
	2,3,4-Me_3-Galactitol	3 02	2 1
3	2,4-Me_2-Galactitol	5 50	13 3
4	3-Me-Arabinitol	2 35	1
	2-Me-Galactitol	7 10	1 8

Although no standards for 3-*O*-methylarabinitol tetraacetate and 2-*O*-methyl galactitol pentaacetate were available, the presence of primary, fragment ions having *m/e* 189 and 117, respectively, left no doubt as to the identity of these components

Further characterization of methyl sugars — The remaining mixture of sugars was fractionated on sheets of Whatman No 1 paper by using 1-butanol–ethanol–water (40.11.19). Location and elution of appropriate strips gave the following fractions

Fraction 1. The syrupy fraction showed two components having R_F values (Solvent *B*) and colour reaction (*A*) identical with those of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,5-tri-*O*-methyl-L-arabinose It was separated on 3 sheets (7 × 22 in) of Whatman No 1 paper, giving (*a*) 2,3,5-tri-*O*-methyl-L-arabinose and (*b*) 2,3,4,6-tetra-*O*-methyl-D-galactose A solution of the former compound in water (1 ml) was oxidized with an excess of bromine in the presence of barium carbonate (40 mg) for 72 h Bromine was removed by aeration, and the acidified solution was extracted with chloroform for 24 h The chloroform extract was dried and concentrated, and the residual syrup was distilled [135–140°(bath)/0 01 mm Hg] to yield a product which was kept in methanol saturated with ammonia After 48 h in the cold, removal of methanol in a desiccator produced crystals Recrystallization from ethanol–ether in the cold gave 2,3,5-tri-*O*-methyl-L-arabinonamide, m p and mixed m p 138–139°, lit [23] m p 138°

A solution of fraction (*b*) (4 mg) in ethanol (0 5 ml) containing aniline (2 mg) was refluxed for 5 h Evaporation of the solvent, with crystallization from ethanol after seeding, gave 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m p and mixed m p 201°; lit [24] m p 197°.

Fraction 2 Paper chromatography (4 sheets, 7 × 22 inches, Solvent *B*) separated the fraction into (*a*) 2,3-di-*O*-methyl-L-arabinose, (*b*) 2,4,6-tri-*O*-methyl-D-galactose, and (*c*) 2,3,4-tri-*O*-methyl-D-galactose Each component (*a*, 13 mg; *b*, 10 mg, and *c*, 5 mg) was separately refluxed in ethanol (0 5 ml) containing aniline (7, 5, and 3 mg, respectively) for 5 h Crystallization of product (*a*) from ethanol–ether, with recrystallization from acetone in the cold, gave 2,3-di-*O*-methyl-*N*-phenyl-L-arabinosylamine, m p 133–136°; lit [25] m p 138° The products from fractions (*b*) and (*c*) crystallized from ethanol to give 2,4,6- and 2,3,4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m p 176–178° and 167–168°, respectively, lit [26,27] m p 179° and 167°, respectively

Fraction 3 This fraction was paper chromatographically and electrophoretically identical with an authentic sample of 2,4-di-*O*-methyl-D-galactose A portion (20 mg) was refluxed with aniline in the usual way to give 2,4-di-*O*-methyl-*N*-phenyl-D-galactosylamine, m p and mixed m p 216–127°, lit [28] value 214–217°

Fraction 4 Paper chromatography (1 sheet, 7 × 22 inches, Solvent *B*) separated fraction 4 into 3-*O*-methyl-L-arabinose and 2-*O*-methyl-D-galactose A solution of the former compound (3 mg) in 20% aqueous acetic acid (0 2 ml) containing sodium hydrogen sulphite (3 mg) and freshly distilled phenylhydrazine (11 mg) was heated for 3 h at 100° The precipitate obtained, after standing at room temperature for 5–6 h, was washed 3 times with 10% acetic acid and twice with water Crystallization from aqueous ethanol gave an osazone, m p 136–140°, lit [25] m p 163° for 3-*O*-methyl-L-arabinose phenylosazone Scarcity of material prevented further purification by recrystallization The latter component crystallized from ethanol to yield 2-*O*-methyl-D-galactose, m p 145–147°, lit [26] m p 145–148°

Examination of the methylated uronic acid fraction — The methylated uronic acid fraction (9 mg) was fractionated on a sheet (7 × 22 inches) of Whatman No 1 paper using 1-butanol–acetic acid–water (4 1 5) Three components (R_F 0 44, 0 34, and 0 18), recovered in trace amounts, could not be investigated further The major fraction (4 5 mg, R_F 0 61) was converted into the methyl ester methyl glycoside by refluxing with 2% methanolic hydrogen chloride for 10 h After reduction with lithium aluminum hydride[9] in dry ether, the product was recovered, and hydrolysed with 0 5M sulphuric acid for 12 h Paper chromatography (Solvent *B*) showed a single component having R_F and colour reaction (*A*) identical to that of 2,3,4-tri-*O*-methyl-D-glucose A solution of this product in water (1 ml) containing sodium borohydride (15 mg) was left at room temperature for 18 h The borohydride-reduced[10] product, recovered in the usual way, was acetylated with acetic anhydride and pyridine The resulting hexitol acetate, when examined by g l c –mass spectrometry[8,22] as described earlier, showed a single component having retention time and fragmentation pattern identical to that of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol

Periodate oxidation — Rapeseed acidic arabinogalactan (23 4 mg) was oxidized with 0 2M sodium metaperiodate (50 ml) at room temperature in the dark Aliquots (5 ml) were removed after 72 h, and the consumption of periodate and production of formic acid were determined[29,30] The results based on 149 g of polysaccharide (calculated on the arabinose–galactose–glucuronic acid ratios of 1 1 05 0 13) were as follows periodate consumed, 0 76 mole, formic acid produced, 0 22 mole

The solution remaining from periodate oxidation was treated with ethylene glycol (few drops) and dialysed for 18 h against running tap-water and 4 h against distilled water The concentrated dialysate (1 ml) was reduced with sodium borohydride (70 mg) After 18 h at room temperature, excess borohydride was destroyed with acetic acid, and the solution was deionized with Rexyn-101 (H^+) resin and concentrated Boric acid was removed by repeated distillation of methanol from the residue which was then hydrolysed with 0 5M sulphuric acid for 5 h at 100° The neutralised ($BaCO_3$) hydrolysate was examined by paper chromatography Large

amounts of galactose and glycerol and a small proportion of arabinose were detected, but no uronic acid fragments were found

The remaining reduced, hydrolysed polysaccharide was converted into the *O*-trimethylsilyl derivatives which were examined by g l c on a 9-ft column of 20% SE52 on Gas Chrom P The chromatograms were developed with temperature programming at a rate of 6°/min from 145→245°, using a nitrogen flow-rate of 45 ml/min The areas under the peaks showed galactose–glycerol–arabinose–threitol molar ratios of 1.0 8 0 053 0 04.

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