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STRUCTURAL INVESTIGATION OF AN ACIDIC XYLAN FROM RAPESEED*†

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ABSTRACT

An acidic xylan isolated from rapeseed was ~80% pure, as indicated by sedimentation and sugar-analysis data Methylation analysis showed that, on average, for every 7 sugar units, the xylan has 1 non-reducing terminal residue of 4-*O*-methyl-D-glucuronic acid attached to position 2 of a D-xylose residue, with the remaining 5 non-terminal D-xylose residues linked (1→4)

INTRODUCTION

In previous reports[1–5] we described the isolation from rapeseed and characterization of an amyloid, an acidic arabinogalactan, an arabinan, two pectic polysaccharides, and a fucoamyloid Further work on the sodium hydroxide-soluble fraction of the rapeseed Cotyledon meal gave an acidic xylan, for which the main structural features are now reported

RESULTS AND DISCUSSION

The acidic xylan-containing fractions[5] (4 and 5) from DEAE-cellulose (CO_3^{2-}) were combined to yield a polysaccharide An attempt to fractionate this material with barium hydroxide was largely unsuccessful, as the complexing and non-complexing fractions contained essentially the same sugars, there was relatively less arabinose and more mannose in the former fraction However, prior to precipitation with barium hydroxide, the sodium hydroxide solution yielded a small amount of insoluble material which, on the basis of the monosaccharide constituents, appeared to be an insoluble fucoamyloid The presence of soluble and insoluble fucoamyloids has been reported in mustard seed; they are structurally similar, and may be bonded to cellulose by hydrogen bonds[6,7]

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Fractionation of the combined material with Cetavlon[8] gave a soluble fraction which, on the basis of its rotation ($[\alpha]_D$ +39°) and monosaccharide constituents appeared to be similar to the pectic polysaccharides reported earlier Acid hydrolysis of the insoluble fraction ($[\alpha]_D$ −16 5°) gave mainly xylose and uronic acids, together with small proportions of galactose, glucose, and arabinose, and traces of fucose and mannose Further purification of this fraction on DEAE-cellulose[9] (CO_3^{2-}) yielded a minor fraction similar to the fucoamyloid and a major fraction which, on final purification with Febling's solution, yielded an acidic xylan ($[\alpha]_D$ −49°) that gave mainly xylose and uronic acid components, together with small proportions of glucose and arabinose, on hydrolysis

The rapeseed acidic xylan showed a major, symmetrical peak and two minor peaks on sedimentation analysis[10]. The major peak was ~79% of the total, and the two minor peaks, suspected to be due to the contaminating pectic polysaccharide and fucoamyloid, corresponded to 14 and 7%, respectively The neutral sugar components of the acidic xylan were glucose, arabinose, and xylose (1:2·24); on the basis of a glucose–xylose ratio of 2·1 for fucoamyloid[5], and an arabinose–xylose ratio of 2.1 for pectic polysaccharide[4], it is concluded that the acidic xylan was contaminated to the extent of ~19%, a figure in excellent agreement with that determined by sedimentation The $[\alpha]_D$ value (−49°) for the acidic xylan, when corrected for a 7% contamination with fucoamyloid ($[\alpha]_D$ +56°) and a 14% contamination with pectic polysaccharide ($[\alpha]_D$ +65°), was −77°, in good agreement with the values reported for xylans

The polysaccharide was methylated[11] and the fully methylated product was subjected to methanolysis and hydrolysis, with fractionation of the hydrolysis products on an anion-exchange resin The neutral fraction contained two major sugars (p c) which were identified, on the basis of R_F and M_G values, demethylation analysis[12], and g l c –m s of the derived glycitol acetates, as 2,3-di-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose G l c analysis of the respective glycitol acetates showed a molar ratio of 7.1 The acidic fraction, when reduced with lithium aluminium hydride[14], followed by sequential hydrolysis, reduction with sodium borohydride[15], acetylation, g l c , and combined g l.c.–m s [13], showed mainly two glycitols, corresponding to 2,3,4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose (molar ratio, 3.1) The overall molar proportions (converted into the nearest whole number) for the three major components were 2,3,4-tri-*O*-methyl-D-glucuronic acid, 1 3-*O*-methyl-D-xylose, 1, and 2,3-di-*O*-methyl-D-xylose, 5

The methylation analysis showed that, for every 7 sugar units, there was 1 non-reducing, terminal 4-*O*-methylglucuronic acid residue attached to position 2 of a D-xylose residue, with the remaining 5 non-terminal residues of D-xylose being linked (1→4)

The high, negative optical rotation for the methylated and unmethylated polysaccharide showed that the glycosidic linkages were mainly β-D

EXPERIMENTAL

The general experimental methods have been reported previously[1]

Fractionation of the crude, acidic polysaccharide — The fractions 4 and 5 (2.9 g) eluted[5] from DEAE-cellulose (CO_3^{2-}) with 0.5M sodium hydroxide were dissolved in 10% sodium hydroxide (900 ml). The suspension was centrifuged, and the residue was treated with another portion (150 ml) of sodium hydroxide and centrifuged to yield a residue (0.68 g), which was washed with 50% acetic acid, and then with 80% ethanol, ethanol, and ether. A saturated solution of barium hydroxide (200 ml) was added dropwise to the combined extract (1000 ml). The precipitate was collected by centrifugation, and washed with dilute barium hydroxide and water, and the complex was decomposed with cold 50% acetic acid. The material was recovered by addition of ethanol (4 vol), suspended in water, dialysed, concentrated, and freeze-dried to yield the barium hydroxide-complexing fraction (0.23 g). The barium hydroxide-soluble supernatant was neutralized with 50% acetic acid, and the material was similarly recovered by ethanol precipitation, dialysis, and freeze-drying to yield the barium hydroxide-noncomplexing fraction (1.3 g). Acid hydrolysis (M sulphuric acid, 100°, 3 h) of the initial sodium hydroxide-insoluble residue produced the same sugars, in the same proportion, as the fucoamyloid[2]. The hydrolysates of the barium hydroxide-complexing and non-complexing fractions contained galactose, glucose, mannose, arabinose, xylose, fucose, and uronic acid.

A solution of the combined barium hydroxide-complexing and non-complexing fraction (1.4 g) in 0.5M sodium hydroxide (100 ml) was centrifuged at 3,000 r.p.m. for 0.5 h. A small amount of insoluble material was discarded, the solution was neutralized with 50% acetic acid and centrifuged again, a small amount of precipitate was discarded, and the clear supernatant was poured into ethanol (4 vol). The precipitate was collected, suspended in water, dialyzed, concentrated, and freeze-dried to yield a solid (1.33 g).

The polysaccharide (1.3 g) was dissolved in water (200 ml), and the solution was clarified by centrifugation, followed by filtration through a thick pad of filter-paper pulp. The precipitate was discarded, and Cetavlon[8] (cetyltrimethylammonium bromide) (750 mg) was added to the clear solution. Centrifugation at 15,000 r.p.m. for 20 min gave a precipitate which was washed with water, and dissolved in M sodium chloride. The solution was diluted to 0.25M with respect to sodium chloride and mixed with ethanol (4 vol). A solution of the resulting precipitate in water was dialyzed for 24 h against running tap-water and for 4 h against distilled water. The dialysate was centrifuged to remove a small amount of insoluble material, and freeze-dried to yield the Cetavlon-insoluble fraction (0.35 g). The Cetavlon-soluble fraction (0.61 g), obtained by similar treatment of the supernatant, had $[\alpha]_D^{20}$ +39° (*c* 0.25, water), and gave galactose, glucose, mannose, xylose, and fucose on hydrolysis. The Cetavlon-insoluble fraction had $[\alpha]_D^{20}$ −16.5° (*c* 0.25, water), and gave mainly xylose on hydrolysis, together with small proportions of uronic acid, galactose, glucose, and arabinose, and traces of fucose and mannose.

Purification of the Cetavlon-insoluble fraction — The Cetavlon-insoluble fraction (330 mg) was fractionated on a column (1 × 12 in.) of DEAE-cellulose[9] (CO_3^{2-}). Elution with water (1 litre) gave a neutral fraction (10 mg) which, from hydrolysis results, was similar to the fucoamyloid[5] isolated earlier. Elution with 0.5M ammonium carbonate (500 ml) gave a major, acidic fraction (192 mg), $[\alpha]_D^{24}$ −47° (*c* 0.5, M sodium hydroxide), and final elution with 0.5M sodium hydroxide (500 ml) gave a second fraction (96 mg), $[\alpha]_D^{24}$ −43° (*c* 0.22, 0.5M sodium hydroxide). Paper chromatography and electrophoresis of the hydrolysates of the two acidic fractions showed the same components, namely, xylose and uronic acids, together with small proportions of arabinose and glucose, and traces of galactose. The combined fraction showed N, 0.7; ash, 5.62; moisture, 6.64%.

A solution of the combined acidic fractions (120 mg) in 0.5M sodium hydroxide (10 ml) was centrifuged at 15,000 r.p.m. for 10 min. A minute amount of precipitate was discarded, and the clear supernatant was mixed with a freshly prepared Fehling's solution (2 ml). The gelatinous precipitate was removed, washed with water, suspended in ice-cold water (10 ml), and acidified with M hydrochloric acid. The green solution was mixed with ethanol (4 vol.) and centrifuged, and the slightly green precipitate was reprecipitated from an acidic solution. The final precipitate was suspended in water, dialyzed for 24 h against running tap-water and for 4 h against distilled water, concentrated, and freeze-dried to yield an acidic xylan (63 mg).

Analysis of acidic xylan — The acidic xylan had $[\alpha]_D^{22}$ −49° (*c* 0.3, water) after correction for a moisture content of 7.1%. Sedimentation analysis[10] of a 0.5% solution in acetate buffer (pH 5.03) at 52,000 r.p.m. showed two minor peaks and a major, symmetrical peak; the ratios of the three peaks were ~1:2.11.3. A portion (2 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[11], and the resulting mixture of glycitols was acetylated, and examined on a Pye-104 Gas Chromatograph with dual columns (5 ft) of 3% of OV-225 on Chromosorb W-Hp (80–100 mesh), temperature programming at 2°/min from 185 → 250°, and a nitrogen flow-rate of 45 ml/min. Peak areas evaluated by a CSI digital integrator (model 208), after correction for response factors, gave molar ratios of glucose, arabinose, and xylose of 1:2:24.

Acid hydrolysis of acidic xylan — Acidic xylan (5 mg) was hydrolysed with M sulphuric acid (0.5 ml) for 3 h at 100°. After neutralization ($BaCO_3$) and filtration, the mixture of acidic and neutral sugars was stirred with Dowex 1 X2 (CO_3^{2-}) resin for 42 h. Washing with water (15 ml) removed the neutral sugars, and elution with 0.25M ammonium carbonate (20 ml), followed by removal of ammonium carbonate with Rexyn-101 (H^+) resin, yielded the uronic acid material.

Paper chromatography of neutral sugars in solvent *A* showed mainly xylose with traces of arabinose and glucose.

Paper chromatography of the acidic sugars in 1-butanol–acetic acid–water (4:1:5) showed two major components having R_{RHA} 0.78 (4-*O*-methylglucuronic acid) and 0.30. Paper electrophoresis (acetate buffer, pH 5) showed two components, and

paper electrophoresis (borate buffer, pH 10) also showed two components (M_{GLC} 0.85 and 0.77). Glucuronic and galacturonic acids, under the same conditions, showed M_{GLC} 1.09 and 1.05, respectively. The two components were separated by electrophoresis in borate buffer. Location and elution of the strips gave the two fractions, which were recovered by removal of sodium ions with Rexyn-101 (H^+) resin and boric acid by repeated co-distillation with methanol. Hydrolysis of each fraction with M sulphuric acid for 3 h at 100° showed (p.c. and electrophoresis) the unchanged uronic acid and xylose.

Methylation analysis of acidic xylan — Acidic xylan (28 mg) was dissolved in dry, warm methyl sulphoxide (5 ml) by stirring for 10 h. The solution was flushed with nitrogen, a 2M solution (1 ml) of methylsulphinyl carbanion[11] was added dropwise, and the resulting solution was stirred for 12 h at room temperature. Methyl iodide (1 ml) was then added dropwise, with external cooling (ice–water), the reaction mixture was stirred at room temperature for 5 h, then poured into water (50 ml), dialysed for 24 h against running tap-water, concentrated, and extracted continuously with chloroform. The extract was dried (Na_2SO_4) and concentrated to give a syrup (29 mg), $[\alpha]_D^{25}$ −24° (*c* 2.9, chloroform), which showed only weak i.r. absorption for hydroxyl.

A solution of the methylated xylan (29 mg) in 2% methanolic hydrogen chloride (20 ml) was boiled for 18 h, then neutralized (Ag_2CO_3), and concentrated. The syrupy product was hydrolysed with M sulphuric acid (1 ml) for 32 h at 100°. The hydrolysate was neutralized ($BaCO_3$), concentrated to 10 ml, and stirred with Dowex 1 x2 (CO_3^{2-}) resin (10 ml) for 24 h. The slurry was added to a column containing more (5 ml) resin, and elution with water (75 ml) removed the neutral sugars (15 mg). The acidic sugars (5 mg) were removed by elution with 0.25M ammonium carbonate (50 ml) and recovered after removal of ammonium carbonate with Rexyn-101 (H^+) resin.

(*a*) *Neutral sugars*. A portion (5 mg) of the neutral, methylated sugars was fractionated on Whatman No. 1 paper with solvent *B*, to give two major components **1** (R_F 0.49, M_G 0.00) and **2** (R_F 0.18 and M_G 0.78). Traces of at least five other components (R_F 0.38, 0.66, 0.36, and 0.47) were shown (p.c., electrophoresis, and g.l.c.) to be contaminants from pectin[4] and fucoamyloid. Portions (1–2 mg) of fractions **1** and **2**, when demethylated with boron trichloride[12], gave (p.c.) xylose in each case. Portions (1–2 mg) were reduced with sodium borohydride[5], and the products were acetylated, and examined by g.l.c. as described above (temperature programming, 200→250° at 1°/min). Component **1** gave 2,3-di-*O*-methylxylitol triacetate, and component **2** gave 3-*O*-methylxylitol tetra-acetate. The identity of the components was confirmed on a combined Finnigan 3100 D GC/MS[13], with a U-shaped column (5 ft × 0.25 in.) of 3% of OV-225 on Chromosorb W-HP (80–100 mesh) and temperature programming from 200→225° at 2°/min. The separator temperature was 250°, analyser temperature 90°, ionizing electron energy 70 eV; the spectra were recorded as bar graphs by means of the Finnigan 600 MS Data System.

A second portion (2–3 mg) of the neutral sugar mixture was similarly reduced,

acetylated, and examined by g l c The molar ratio (uncorrected for response factors and converted into the nearest whole number) of the methylated glycitol acetates corresponding to fractions **1** and **2** was 1·7.

(*b*) *Acidic sugars* Paper chromatography [1-butanol–acetic acid–water (4:1 5)] of the methylated uronic fraction showed major (R_F 0.59) and minor components (R_F 0.44) together with a trace of a component having R_F 0 30 The fraction (5 mg) was treated with boiling, 2% methanolic hydrogen chloride for 10 h The resulting methyl ester methyl glycoside was reduced with lithium aluminum hydride[14] (30 mg) in a (1:1) mixture of dry ether–tetrahydrofuran (5 ml) The product was recovered in the usual way and hydrolysed with 0 5M sulphuric acid for 3 h P c (solvent *B*) and electrophoresis (borate buffer, pH 10) showed two components having R_F 0 56 and 0.18 (M_G 0 00 and 0.77). Traces of a third component (M_G 0 34) were also detected The remainder of the reduced uronic acid fraction was reduced, acetylated, and examined by g l c and g l c–m s The molar ratio (uncorrected for response factors and converted into the nearest whole number) of the methylated glycitol acetates, identified as 2,3,4-tri-*O*-methylglucitol triacetate and 3-*O*-methylxylitol tetra-acetate, was 3:1

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