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AN ARABINOGALACTAN FROM THE FIBRES OF COTTON (*Gossypium arboreum* L.)

ANTONY J. BUCHALA AND HANS MEIER
Institut de Biologie végétale et de Phytochimie, Université de Fribourg CH-1700 Fribourg (Switzerland)
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ABSTRACT

An acidic arabinogalactan has been isolated from fibres of the cotton plant (*Gossypium arboreum* L.) at the stage of intensive secondary-wall formation. The polysaccharide contains arabinose, galactose, rhamnose, and glucuronic acid residues in the molar ratios 1.1:2.0:1.0:0.2. Periodate oxidation and methylation studies showed that there is a main chain of (1→3)-linked galactopyranosyl residues to which side chains are attached at O-6. The side chains consist of (1→6)-linked galactopyranosyl residues substituted at O-3 by (1→5)-linked arabinofuranosyl chains. Terminal galactopyranosyl, rhamnopyranosyl, and glucopyranuronosyl groups are also present. Enzymic hydrolysis showed that the configurations of the galactose and arabinose residues are D and L, respectively.

INTRODUCTION

Arabinogalactans and glycoproteins containing significant amounts of arabinose and galactose are common constituents of plant tissues. The form and function of such macromolecules has been reviewed[1]. Arabinogalactans *per se* have been subdivided[2] into two main groups: the arabino-4-galactans (Aspinall Type I) and the arabino-3,6-galactans (Aspinall Type II). The former are constituents of pectic complexes in seeds and bulbs and of coniferous compression wood; the latter frequently occur in most plant organs of angiosperms, coniferous woods, and angiosperm exudates. They are also found in the extracellular media of various tissues in suspension culture. We now report on structural studies of a Type II acidic arabinogalactan.

EXPERIMENTAL

General — Paper chromatography (p.c.) was performed on Schleicher and Schuell paper No. 2043b and t.l.c. on Kieselgel G (Merck) with *A*, ethyl acetate–pyridine–water (8:2:1), *B*, ethyl acetate–acetic acid–formic acid–water (18:8:3:9), *C*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4), *D*, acetone–1-butanol–acetic acid–water (12:8:1:5:8), *E*, 1-butanol–ethanol–water–ammonia (4:1:5 trace), *F*, butanone–water–ammonia (10:1 trace), *G*, acetone–water (88:12). Chromatographic detection reagents were alkaline silver nitrate, 3% *p*-anisidine hydrochloride,

and 5% 1-naphthol–conc sulphuric acid G l c was performed on a Packard 428 chromatograph, using columns (2 m × 2 mm i d) containing (*a*) 3% of ECNSS-M on Gas Chrom Q (100–200 mesh), (*b*) 8% of *m*-bis(phenoxyphenoxy)benzene on AW HMDS Chromosorb W (100–120 mesh), (*c*) 2 5% of OV-225 on AW DMCS Chromosorb G (100–120 mesh) or (*d*) a capillary column (25 m × 0 5 mm) of WCOT with OV-225 Polysaccharides were hydrolysed according to Saeman *et al* [3], hydrolysates were neutralised with barium carbonate and the neutral sugars were examined by p c (irrigant *A*) Quantitative analyses of the sugars were effected by g l c of their derived glycitol acetates (column *a*) Uronic acids were determined by the method of Blumenkrantz and Asboe-Hansen[4] and hydrolysates examined by t l c (irrigant *D*)

Isolation of the arabinogalactan — Cotton fibres harvested during the stage of secondary-wall formation (30–40 days *post anthesis*), were homogenised as described by Huwyler *et al* [5] The wet material (corresponding to 28 g of dry fibres) was extracted with water (1 L 4 × 24 h) at 4° The water-soluble material was dialysed using a hollow fibre system with a nominal, molecular retention limit of 5000 D (Amicon Corp Lexington, Mass , U S A), and lyophilised (yield 830 mg) A solution of the material in water was clarified by centrifugation, and fractionated on a column of DEAE-cellulose (DEAE-Sephacel, chloride form Pharmacia) Fractions were eluted with water and then step-wise with increasingly concentrated solutions of NaCl (300 mL of each) Elution was monitored by the phenol–sulphuric acid method[6] The principal fraction eluted with 0 1M NaCl gave, on acid hydrolysis arabinose, galactose, and rhamnose (molar ratios 1 1 2 0 1) glucuronic acid, and traces of glucose, xylose, and other acidic material This fraction was retained for study No further fractionation could be achieved on columns of Bio-Gel P-100 or DEAE-Sepharose CL 6B (Pharmacia) Electrophoresis of the polysaccharide dyed with Procion MGS Red was performed on cellulose acetate[7]

Periodate oxidation of the arabinogalactan — A sample (20 mg) of the arabinogalactan was oxidised in the dark at 4° with 0 05M sodium metaperiodate The periodate consumed after 28 days was 0 8 mol per 'anhydro sugar' residue The oxopolysaccharide was dialysed, and reduced with sodium borohydride The resulting polyalcohol (5 mg) was hydrolysed with 4% sulphuric acid, and the products were examined by paper chromatography (irrigant *A*), galactose, glycerol, and traces of arabinose were detected The hydrolysate was reduced with sodium borohydride, acetylated, and examined by g l c (column *a*) Components having retention times identical to those of the galactitol and glycerol acetates were detected in the molar ratio 1 1 2

Another sample (10 mg) of the polyalcohol was partially hydrolysed with M trifluoroacetic acid at room temperature for 4 days The hydrolysate was evaporated to dryness and applied to columns of Sephadex G-25 (where the material of high molecular weight was excluded from the gel) and Sephadex G-75 (where the material of high molecular weight was eluted as a single, broad peak over the entire included volume) The material was recovered, and methylated by the method of Hakomori[8] as

described below for the undegraded arabinogalactan On hydrolysis[9] 2 3 4 6-tetra-*O*-methylgalactose, 2 3,4-tri-*O*-methylgalactose, 2 4 6-tri-*O*-methylgalactose and 2 4-di-*O*-methylgalactose were detected and identified by g l c (column *d*) of their derived glycitol acetates (see below)

Methylation of the arabinogalactan — A sample (20 mg) of the polysaccharide was methylated by the method of Hakomori[8] The material was recovered by dialysis and extracted with light petroleum and the extract was evaporated to dryness The residue (25 mg) was soluble in chloroform and showed no hydroxyl absorption in its i r spectrum The methylated polysaccharide was then reduced with lithium aluminium deuteride and hydrolysed[9] by the formic acid–sulphuric acid method T l c of the hydrolysate (irrigants *E* and *F*) revealed 2 3,4 6-tetra-*O*-methylgalactose, 2 3 4-tri-*O*-methylgalactose 2 4,6-tri-*O*-methylgalactose 2,4-di-*O*-methylgalactose 2 3 5-tri-*O*-methylarabinose 2 3-di-*O*-methylarabinose, and 2 3 4-tri-*O*-methylglucose A sample of the hydrolysate (~5 mg) in aqueous methanol was reduced with an excess of sodium borohydride After 12 h, the remaining borohydride was decomposed with acetic acid, and the borate produced was removed by repeated co-distillation with methanol at room temperature The residue was acetylated with acetic anhydride (1 mL) and pyridine (1 mL) in a sealed tube for 30 min at 120° The excess of acetic anhydride was hydrolysed with water, the solution was evaporated to dryness, and the products were extracted into chloroform and examined by g l c (columns *a*, *c* and *d*) The methylated glycitol peracetates of the following compounds were identified by comparison with authentic compounds and by comparison of their mass spectra (Hewlett–Packard HP 5992B) with those recorded in the literature[10] 2 3 4 6-tetra-*O*-methylgalactose 2,3,4-tri-*O*-methylgalactose, 2 4 6-tri-*O*-methylgalactose 2,4-di-*O*-methylgalactose 2 3 5-tri-*O*-methylarabinose 2,3-di-*O*-methylarabinose 2 3 4-tri-*O*-methylglucose, and 2 3,4-tri-*O*-methylrhamnose A number of other peaks were observed in trace quantities corresponding to the contaminant β-D-glucan and arabinoxylan degradation products

Partial acid hydrolysis of the arabinogalactan — A sample (50 mg) of the arabinogalactan was treated with 0 05M trifluoroacetic acid at 100° for 2 h, arabinose and only traces of galactose were released The hydrolysate was evaporated to dryness and the residue was dissolved in water and fractionated on a column of Bio-Gel P-2 (200–400 mesh) The material of high molecular weight was recovered (20 mg) Total acid hydrolysis of a sample of this material gave mainly galactose rhamnose, and glucuronic acid, with traces of arabinose and other acidic material The remaining material was treated with 0 4M trifluoroacetic acid at 100° for 1 h the hydrolysate was evaporated to dryness, and the residue was chromatographed on the same column of Bio-Gel P-2 very little material of high molecular weight remained and no distinct peaks corresponding to oligosaccharides were obtained The fractions were examined by p c (irrigants *A*, *B*, and *C*), and similar fractions were combined and then purified by p c (irrigant *B*) on Whatman 3MM paper Six components were obtained (irrigant *B* R_{Gal} 0 78, 0 66, 0 59, 0 40 0 27, and 0 16), each giving, on acid hydrolysis, only galactose (t l c , irrigants *D* and *G*) Samples of the first four of the

components were methylated[10], and the products heated in sealed tubes at 90° with anhydrous, 3% methanolic HCl for 12 h The methanolysates were taken to dryness and examined by g l c (columns *b* and *d*) Each sample gave a peak chromatographically identical to that of the methyl glycosides of 2,3,4 6-tetra-*O*-methylgalactose, and components 1 3 and 4 gave peaks chromatographically identical to those of the methyl glycosides of 2,4,6-tri-*O*-methylgalactose, whilst component 2 gave peaks corresponding to methyl 2 3,4-tri-*O*-methylgalactosides Quantitatively, the peak-area ratios were approximately those to be expected for a homologous series of β-(1→3)-linked galactose-containing oligosaccharides (components 1, 3 and 4) and 6-*O*-galactopyranosylgalactose (component 2) In addition the R_{Gal} values (irrigant *B*) correspond well with those previously recorded[11] and a plot of the negative logarithm of the R_{Gal} values (components 1 3 4 5, and 6) as a function of the assumed d p is linear

Hydrolysis of the arabinogalactan with 4% sulphuric acid gave, besides the monosaccharides an acidic component (R_{Gal} 0 78, irrigant *B*) that was retained on an anion-exchange resin (Dowex 1, acetate form) eluted with water, but was desorbed with 40% aqueous acetic acid The component was further purified by p c (irrigants *C* and *A*) and then treated with 4% sulphuric acid at 100° for 12 h in a sealed tube P c (irrigants *A*, *B* and *C*) revealed the presence of galactose and glucuronic acid (and glucuronolactone) along with unhydrolysed material These data suggest that the acidic component was probably 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose

Enzymic hydrolysis of the arabinogalactan and determination of the configuration of the galactose and arabinose residues — A sample of an acid hydrolysate of the arabinogalactan containing ~0 5 mg of galactose was dissolved in 0 1M phosphate buffer (2 mL, pH 7 0) containing 30 units of D-galactose oxidase (U S Biochemical Corp) and incubated at 25° for 2 days The solution was evaporated to dryness, and p c of the residue (irrigant *A*) did not detect galactose

A sample of the arabinogalactan (2 mg) was dissolved in 0 2M acetate buffer (2 mL pH 5 0) containing pectinase (2 mg) from *Aspergillus niger* (Sigma) and incubated at 30° for 1 day The solution was taken to dryness, and t l c (irrigant *D*) of the residue revealed arabinose and galactose, but no uronic acids

A sample (10 mg) of the arabinogalactan dissolved in 0 1M phosphate–citrate buffer (5 mL, pH 4 0) containing α-L-arabinofuranosidase[12] was incubated at 30° for 1 day The enzyme was deactivated by heating at 100° and the hydrolysate applied to a column of Bio-Gel P-2 (see above) The material of low molecular weight consisted almost entirely of arabinose On acid hydrolysis, the material of high molecular weight released mainly galactose, but some arabinose was still present A second treatment was carried out as above, the material of high molecular weight recovered and a sample hydrolysed The material still contained ~10% of arabinose residues, and it was evident that the enzyme preparation also contained sugar residues in the form of material of high molecular weight that complicated the analysis of the results The remainder of the material was methylated by the method of Hakomori[10]

and examined as described above All of the methyl sugars derived from the original arabinogalactan were present, but in different proportions

RESULTS AND DISCUSSION

The material of high molecular weight solubilised with water at 4° from maturing, homogenised cotton fibres contained arabinose, galactose, glucose, mannose, rhamnose, and xylose residues in the molar ratios 1 0 9 1 2 0 0 5 0 1 0 1 and acidic sugars The material gave a negative iodine-reaction for starch Its protein content was ~15% and that of hydroxyproline was ~1 3% Chromatography of this material on DEAE-cellulose (chloride form) gave a fraction, eluted with water, containing polysaccharides composed of all of the above-mentioned sugars, but enriched in glucose A major fraction (~60%) eluted by 0 1M NaCl gave, on acid hydrolysis, arabinose, galactose, and rhamnose in the molar ratios 1 1 2 0 1, and traces of xylose and glucose The uronic acid content[4] was ~8% and that of protein[13] ~2 5% This material had $[\alpha]_D^{20}$ −18° (c 0 5, water), and electrophoresis of the polysaccharide dyed with Procion Red[7] gave a single band Under the same conditions, an arabinogalactan from *Larix occidentalis* also gave only one band, but is recognised to contain two components of similar overall structure which differ in their degree of branching and molecular weight[14 15] The polysaccharide from cotton fibres could not be further fractionated by chromatography on DEAE-Sepharose CL 6B or Bio-Gel P-100 and was considered to be sufficiently homogeneous for structural studies.

The arabinogalactan was methylated by the method of Hakomori[8 10], to yield a product (~90%) that displayed no i r peak attributable to hydroxyl groups The methylated polysaccharide was completely reduced with lithium aluminium deuteride and hydrolysed[9] The hydrolysate was examined by t l c, and a sample was reduced with sodium borohydride, acetylated, and examined by g l c–m s[10] The following sugars were identified 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4-tri-*O*-methylgalactose, 2,4,6-tri-*O*-methylgalactose, 2,4-di-*O*-methylgalactose, 2 3 5-tri-*O*-methylarabinose, 2,3-di-*O*-methylarabinose, 2,3,4-tri-*O*-methylglucose, and 2,3,4-tri-*O*-methylrhamnose, which were present in the peak-area ratios of 0 35 0 27 0 23 1 0 55 1 31 0 16 0 05 The ratio for arabinose to galactose derivatives in the methylated polysaccharide (~1 1) is in fair agreement with that found for the original arabinogalactan (1 1 2), but it is evident that the methylation, or the subsequent work-up, resulted in the loss of rhamnose and glucuronic acid residues However, the method of Blumenkrantz and Asboe-Hansen[4] for the determination of uronic acid residues gives erroneously high values when certain plant phenolic substances are present[16], so that the apparent loss due to the methylation is probably overestimated Deuteration was detected only in 2,3,4-tri-*O*-methylglucose, showing that this sugar alone was derived from uronic acid residues

The arabinogalactan reduced 0 8 mol of periodate per mol of "anhydro sugar and acid hydrolysis of the reduced oxopolysaccharide released galactose and glycerol in the molar ratio 1 1 2 Smith degradation of the reduced oxopolysaccharide[15],

methylation[10] of the resulting material of high molecular weight, followed by hydrolysis gave (g l c of their glycitol acetate derivatives) 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4-tri-*O*-methylgalactose. 2 4,6-tri-*O*-methylgalactose, and 2,4-di-*O*-methylgalactose in the peak-area ratios 3 3 1 1 9 These results indicate that the arabinogalactan contains a branched core of (1→3)- and (1→6)-linked galactopyranosyl residues

Mild, acid hydrolysis of the arabinogalactan removed almost all of the arabinose residues before galactose residues were released, indicating that the former were linked in the furanoid form. Further acid hydrolysis released a series of (1→3)-linked galactose-containing oligosaccharides and a (1→6)-linked galactobiose Acid hydrolysates also contained an acidic oligosaccharide tentatively identified as a 6-*O*-(glucopyranosyluronic acid)galactose

The fact that D-galactose oxidase completely oxidised the galactose in acid hydrolysates showed that this had the D configuration Treatment of the arabinogalactan with an α-L-arabinofuranosidase removed ~90% of the arabinose residues, showing that they were indeed linked in the furanoid form and that they had the L configuration However, repeated treatment did not remove the remaining arabinose residues The methylation analysis indicates that arabinose is only (1→5)-linked, so most probably the resistance to hydrolysis of the residual arabinose is due to steric reasons A similar enzyme preparation did not completely remove the arabinose residues from an arabinoxylan isolated from wheat flour[17] nor from an arabinogalactan also isolated from wheat flour[18] The material of high molecular weight remaining after treatment with the arabinofuranosidase was methylated although the material was evidently contaminated with methyl sugars derived from the enzyme preparation, it could be seen that the removal of the arabinose residues gave rise to an increase in the proportion of 2,3 4-tri-*O*-methylgalactose and a corresponding decrease in the proportion of 2,4-di-*O*-methylgalactose in a hydrolysate

The results are consistent with a highly branched molecule containing a (1→3)-linked β-D-galactopyranosyl backbone where most of the galactosyl residues carry side chains at O-6 These side chains consist of (1→6)-linked D-galactopyranosyl residues (average length, 3 residues) substituted at O-3 by short chains of (1→5)-linked L-arabinofuranosyl residues also about three residues long There are also terminal rhamnopyranosyl, glucopyranosyluronic acid, and D-galactopyranosyl residues No estimation was made of the molecular weight of the polysaccharide

The presence of (1→5)-linked L-arabinofuranosyl side-chains has been reported for several purified Type II arabinogalactans[19–21], and their presence can be inferred from methylation evidence for other polysaccharides if the possibility of pectic arabinan can be excluded[22,23] Such arabinans are invariably branched, and the lack of mono-*O*-methylarabinose derivatives in the hydrolysate of the methylated arabinogalactan from cotton fibres precludes their presence

The arabinogalactan from cotton fibres has a similar chemical composition to the Type II arabinogalactans isolated from maple sap[24], rapeseed[20], bamboo shoots[25], and tobacco[11], and has some but not all, of the structural features already

known for such arabinogalactans[1] All of the Type II arabinogalactans have the same essential structure, but the variations in the side chains are numerous[1]

Because of their high water-solubility, it is difficult to locate arabinogalactans in plant tissue, but most work to date seems to indicate that such polysaccharides are not contained within the cell wall[1] The work of Albersheim and co-workers can be interpreted as indicating the presence of Type II arabinogalactans in the primary cell-wall of dicotyledonous plants[23,26], and the results described here and those of Maltby *et al* [22] are consistent with localisation either in the primary cell-wall or the cytoplasm

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