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POLYSACCHARIDES FROM THE BARK OF THE WHITE WILLOW (*Salix alba* L.): STRUCTURE OF AN ARABINAN

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ABSTRACT

A water-soluble arabinan $[\alpha]_D - 141°$, isolated from the bark of the white willow (*Salix alba* L.), has a highly branched structure and consists of approximately 90 α-L-arabinofuranose residues variously linked (1→5), (1→3), and (1→2). Some of the L-arabinose residues are involved in branches through positions 2, 3, and 5. The structure is essentially the same as that proposed for arabinans isolated from other plants.

INTRODUCTION

In previous reports[1,2], the isolation and structural characterization of a neutral D-galactan and an acidic heteropolysaccharide complex were described. We now report a structural study of a polysaccharide isolated from the bark of young twigs of the white willow (*Salix alba* L.)

RESULTS AND DISCUSSION

The cold- and hot-water extracts of delignified bark contained different fractions of pectic substances which, on acid hydrolysis, gave mainly D-galactose, L-arabinose, D-glucose, uronic acids, and lower proportions of D-xylose, L-rhamnose, and D-mannose (see Table I).

TABLE I

EXTRACTION OF THE POLYSACCHARIDES FROM THE BARK OF *Salix alba* L. HOLOCELLULOSE

Fraction	*Extractant*	*Yield* (%)	$[\alpha]_D$ (*degrees*)	*Molar ratios of saccharides*						
				Gal	*Glc*	*Man*	*Ara*	*Xyl*	*Rha*	*Uronic acid*
A	Cold water	11.2	+168	1.0	—	—	0.8	trace	trace	5.4
B	Hot water	1.5	+36	1.0	0.9	0.2	2.8	0.3	0.4	2.6

The hot-water extract, which had $[\alpha]_D$ +36° and an L-arabinose content of 34%, was treated with 70% aqueous ethanol. Fractionation of the ethanol-soluble polysaccharide on cross-linked microcrystalline DEAE-cellulose[3] (carbonate form) yielded a neutral L-arabinan which was homogeneous by electrophoresis and sedimentation analysis. The ratio $\bar{M}_w/\bar{M}_n$ revealed a low polydispersity, and the physicochemical constants are listed in Table II.

TABLE II

PHYSICO-CHEMICAL CONSTANTS OF ARABINAN

$[\alpha]_D$	−141°
Electrophoretic mobility	1.2×10^{-5} cm^2.V^{-1}.sec^{-1}
Consumption of IO_4^- per pentose residue	0.69 mol
Diffusion coefficient D_{20}	7.71×10^{-7} cm^2.sec^{-1}
Sedimentation constant S_{20}	2.04×10^{-13}
Partial specific volume ρ_{20}	0.5295
Molecular weight $\bar{M}_w$	13670
Molecular weight $\bar{M}_n$ (G.l.c.)	11620
$\bar{M}_w/\bar{M}_n$	1.17

Partial hydrolysis of the arabinan with acid under mild conditions gave crystalline L-arabinose, and a fraction containing a mixture of oligosaccharides was fractionated by chromatography on Sephadex G-10 and paper. The products obtained on methanolysis and hydrolysis of the methylated disaccharides were identified (g.l.c. of the glycosides or alditol acetates) and shown to contain α-(1→3) and α-(1→5) linkages.

TABLE III

METHYLATED SUGARS FROM THE HYDROLYSATE OF THE METHYLATED ARABINAN

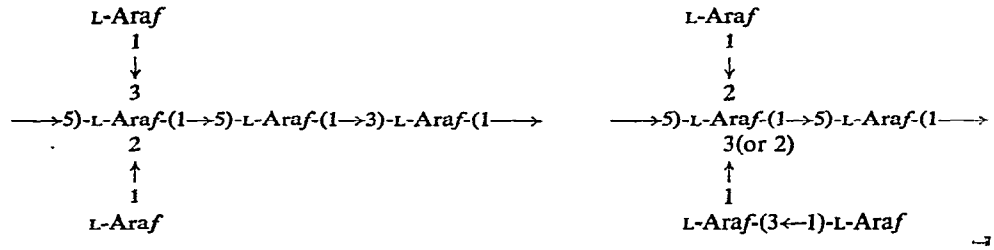
Sugar	*Molar ratio*	*Retention time* (T)[b]			R_A[c]
		Alditol acetates	*Glycosides*		
2,3,5-Me_3-Ara[a]	1.00	1.00	1.00 s	1.22 m	1.00
2,3,4-Me_3-Ara	0.06	1.47	1.61 s	1.67 w	0.61
2,3-Me_2-Ara	0.59	2.42	2.16 s	2.33 w	0.39
			2.40 m		
2,5-Me_2-Ara	0.11	2.55	2.69 s	2.96 w	0.72
3-Me-Ara	0.14	3.91	3.01 s	3.64 m	0.10
			3.17 w		
2-Me-Ara	0.22	3.30	3.45 m	4.10 s	0.21
L-Arabinose	0.34	4.93	—	—	—

[a]2,3,5-Tri-*O*-methyl-L-arabinose, etc. [b]G.l.c. on columns *B* and *C*; key: s, strong; m, moderate; w, weak. [c]T.l.c. (solvent *J*).

The polysaccharide consumed 0.69 mol of periodate per pentose residue, and hydrolysis of the reduced product gave mainly L-arabinose and glycerol. After hydrolysis of the methylated arabinan, the partially methylated sugars were converted into the corresponding glycosides and alditol acetates respectively and identified by g.l.c. (see Table III).

The identified disaccharides, as well as the methylation-analysis data, revealed a highly branched structure of the arabinan which consists of approximately 90 α-L-arabinofuranose residues, including an average of approximately 38 terminal sugar residues. Approximately 13 of the sugar units are involved in branching through positions 2 and 5 or 3 and 5, while 12 or 13 are linked through positions 2, 3, and 5. Some of the terminal sugar residues are present in the pyranoid form.

The identification of 2,5-di-*O*-methyl-L-arabinose and 3-*O*-α-L-arabinofuranosyl-L-arabinose suggests that the arabinan macromolecules have the structures shown below.



The methylation-analysis data predict a theoretical periodate-consumption of 0.72 mol per pentose residue, which is in good agreement with the experimentally determined value (0.69 mol).

The highly negative $[\alpha]_D$ value (−141°) of the polysaccharide suggests that the majority of the sugar residues are of the α-L type. The arabinan isolated from the willow bark, under mild conditions which avoided alkaline degradation of any acidic heteropolymer, appeared to be a native homoglycan, although it could be a fragment

TABLE IV

MOLAR COMPOSITION OF THE METHYLATED ARABINANS FROM DIFFERENT SOURCES

Sugar	*Maritime pine*[7] (Pinus pinaster)	*Aspen* (Populus tremuloides) *bark*[4]	*Willow* (Salix alba *L.*) *bark*
2,3,5-Me_3Ara[a]	1.00	1.00	1.00
2,3,4-Me_3-Ara	0.04	0.09	0.06
2,3-Me_2-Ara	2.44	1.26	0.59
2,5-Me_2-Ara	0.28	—	0.11
3-Me-Ara	0.13	0.21	0.14
2-Me-Ara	0.45	0.37	0.22
L-Arabinose	0.11	0.31	0.34

[a]2,3,5-Tri-*O*-methyl-L-arabinose, etc.

of a complex heteropolysaccharide if weak furanosidic linkages were present in the arabinan macromolecules. Arabinans from the bark of aspen (*Populus tremuloides*)[4], soybean cotyledon meal[5], and rapeseed (*Brassica campestris*)[6] have been isolated under conditions similar to those applied for the willow-bark arabinan. Arabinans occurring both in coniferous (maritime pine, *Pinus pinaster*[7]) and deciduous (aspen bark, *Populus tremuloides*[4]) species have essentially the same structural features. There are minor differences in the size and shape of the macromolecules which reflect differences of branch points at positions 2 and 3, and the ratio of furanoid and pyranoid forms of the terminal sugar residues, *etc.* (Table IV).

EXPERIMENTAL

General. — Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter for aqueous solutions at 20°. Free-boundary electrophoresis of polysaccharide solutions (10 mg/ml) was performed in 0.05M sodium tetraborate buffer (pH 9.2), using a Zeiss 35 apparatus at 150 V and 8 mamp for 30 min.

G.l.c. was performed with a Hewlett–Packard Model 5711A chromatograph; using *A* a column (305 × 0.3 cm) of 1% of XE-60 on 80–100 mesh Gas Chrom Z, at a programmed temperature range of 130–150° at 1°/min; *B* a column (200 × 0.3 cm) of 3% of ECNSS-M on 70–80 mesh, acid-washed Chromaton N-DMCS, at 164–180° and 2°/min; *C* a column (200 × 0.3 cm) of 10% of DEGS on 60–80 mesh Chromosorb W, at 150–205° and 4°/min; *D* a column (210 × 0.6 cm) of 20% of SF-96 on 60–80 mesh Chromosorb W-DMCS, at 180°; *E* a column (180 × 0.3 cm) of 10% of UC-W-98 on 80–100 mesh, acid-washed Chromosorb W, at 120–280° and 4°/min. Column *A* was used for quantitative analysis of the sugars as their alditol trifluoroacetates[8].

P.c. was performed by the descending method on Whatman Nos. 1 and 3MM papers, using *F* 8:2:1 ethyl acetate–pyridine–water, *G* 9:2:2 1-butanol–pyridine–water, and *H* 4:1:5 1-butanol–ethanol–water. Reducing sugars were detected with aniline hydrogen phthalate[9]. Thin-layer chromatography (t.l.c.) was performed on silica gel (Merck), using *I* chloroform–acetone (10:1) and *J* butanone saturated with water. The mobilities (R_A) of methylated sugars are expressed relative to that of 2,3,5-tri-*O*-methyl-L-arabinose and those (R_{ARA}) of oligosaccharides relative to that of L-arabinose. Uronic acid content was determined by the carbazole method[10].

Sedimentation analysis was performed with a Beckman Model E ultracentrifuge. The sample was run in an Al-centerpiece cell. Photographs taken at 32-min intervals (60,000 r.p.m.) showed that the polysaccharide sedimented as a single, symmetrical peak. The partial specific volume was estimated pycnometrically for a 1% solution of the polysaccharide.

Isolation of the arabinan. — Sawdust prepared from the bark of twigs of young white willow (*Salix alba* L.) was extracted with benzene–ethanol (3:1) and then delignified[11] using 80% ethanol. The resulting holocellulose (3540 g, 85% of the bark) was extracted with water, first at room temperature for 3 h and then at 80° for 3 h. The extraction of the residue was repeated twice. The combined hot-water extracts

were concentrated and freeze-dried to give fraction *B*, $[\alpha]_D$ +36° (*c* 1) (see Table I), a portion (57 g) of which was suspended in ethanol (70%, 2.5 l) and stirred at room temperature for 3 h. The residue was separated by centrifugation, and the extraction was repeated three times. Hydrolysis of the ethanol-soluble part (3.9 g) gave mainly L-arabinose together with small amounts of D-galactose, D-xylose, and D-galacturonic acid, and traces of L-rhamnose. The crude arabinan was purified by fractionation on cross-linked microcrystalline DEAE-cellulose[3] (15 g, carbonate form). Elution with water gave a product, $[\alpha]_D$ −141°, which afforded L-arabinose and traces of D-galactose and D-xylose on hydrolysis. Sedimentation analysis and electrophoretic mobility (μ 1.2×10^{-5} $cm^2.V^{-1}.sec^{-1}$) confirmed the homogeneity of the arabinan.

Partial hydrolysis of the arabinan. — The arabinan (1.14 g) was repeatedly treated at 100° with 12mM hydrochloric acid for 3 h. The hydrolysis mixture was neutralized after each treatment with Dowex-1x (HO^-) resin, and the part to be hydrolysed further was centrifuged. The fragments of low molecular weight (475 mg) were fractionated by elution from a column (100 × 3 cm) of Sephadex G-10 with water to give L-arabinose (270 mg), m.p. and mixture m.p. 154–156°, and a mixture containing oligosaccharides (200 mg). Preparative p.c. on Whatman No. 3MM paper yielded components with R_{ARA} 0.79, 0.66, 0.44, 0.19 (solvent *G*). The oligosaccharides **1**, R_{ARA} 0.66, $[\alpha]_D$ −15° (*c* 0.5); and **2**, R_{ARA} 0.79, $[\alpha]_D$ −79° (*c* 0.7); were each methylated as follows. Sodium hydride (25 mg) was added to a solution of **1** or **2** (5 mg) in *N*,*N*-dimethylformamide (3 ml), and the suspension was agitated ultrasonically for 30 min and then kept overnight at room temperature. Methyl iodide (3 ml) was added dropwise and the turbid solution was agitated ultrasonically for 30 min. Water (5 ml) was added and the methylated product was extracted with chloroform (3 × 10 ml). The combined extracts were washed with water and concentrated to dryness, and the residue was purified by t.l.c. (solvent *I*). The methylated disaccharides **1** and **2** were hydrolyzed with 0.1M hydrochloric acid and neutralized, and the products were analysed as glycosides and alditol acetates by g.l.c. (columns *B* and *C*). The identities of 2,3,5-tri-*O*-methyl-L-arabinose and 2,5-di-*O*-methyl-L-arabinose, or 2,3,5-tri-*O*-methyl-L-arabinose and 2,3-*O*-methyl-L-arabinose (in equimolar amounts), were confirmed by comparison of their relative retention times (*T*) with those of authentic standards.

The higher members of the homologous series of L-arabinose-containing oligosaccharides (R_{ARA} 0.44, 0.19) were not investigated.

Degradation of the arabinan. — The arabinan (11.5 mg) was oxidized with 15mM sodium metaperiodate (15 ml) in the dark at 5°. Periodate consumption was monitored spectrophotometrically[12]. After 65 h, the consumption of oxidant was 0.69 mol per pentose residue. After completion of the oxidation, sodium borohydride (20 mg) was added and the product was hydrolyzed with 0.25M sulphuric acid (3 ml). After deionization, only L-arabinose and glycerol could be detected by g.l.c. (column *E*) as their Me_3Si derivatives.

Methylation analysis of the arabinan. — A solution of the arabinan (46 mg) in dry methyl sulphoxide (5 ml) was added to a solution of sodium methylsulphinyl-

methanide in methyl sulphoxide (4 ml), prepared[13] from sodium hydride (250 mg) and methyl sulphoxide (5 ml). The suspension was kept at room temperature in a nitrogen atmosphere for 6 h. Methyl iodide (5 ml) was added dropwise with cooling, and the resulting solution was stirred overnight and then poured into water (30 ml). The solution was dialyzed for 2 days against water and concentrated. The syrupy residue was dissolved in methyl iodide (3 ml), barium oxide (200 mg) was added, and the mixture was stirred and boiled under reflux for 24 h. The methylation procedure was repeated twice.

The methylated arabinan (40 mg) was hydrolysed with 0.25M sulphuric acid (5 ml) at 100° for 8 h. P.c. of the neutralized hydrolysate indicated the presence of L-arabinose, mono-, di-, and tri-*O*-methyl-L-arabinoses with R_A values 0.12, 0.26, 0.31, 0.48, 0.55, and 1.00 (solvent *H*). The corresponding glycosides, prepared using 3% methanolic hydrogen chloride for 8 h at 100°, were identified by g.l.c. (column *C*) by comparing their relative retention times with those of standards.

A part (5 mg) of the mixture of methylated sugars was conventionally converted into the corresponding alditol acetates and quantitatively analysed by g.l.c. (column *B*). The methylated sugars detected are listed in Table III.

Determination of the molecular weight ($\bar{M}_n$) *of the arabinan.* — The arabinan (5 mg) was treated with a solution of sodium borohydride (15 mg) in water (2 ml) at room temperature for 24 h. The deionized product was hydrolyzed with 90% formic acid at 100° for 6 h, followed by hydrolysis at diluted concentration (5%) for 1 h. G.l.c. (column *D*) of the Me_3Si derivatives of the resulting mixture of L-arabinose and arabinitol revealed a molar ratio of 87:1 (average of two determinations) which corresponded to $\bar{M}_n$ 11620.

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