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POLYSACCHARIDES FROM THE ROOTS OF THE MARSH MALLOW (*Althaea officinalis* L.): STRUCTURE OF AN ARABINAN

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ABSTRACT

A water-soluble L-arabinan isolated from the mucous material of the roots of the marsh mallow (*Althaea officinalis* L.) has a highly branched structure of ~90 α-L-arabinofuranosyl residues variously linked by (1→5), (1→3), and (1→2) bonds. Some of the L-arabinosyl groups are involved in branches through O-2, O-3, and O-5. There was good agreement between the results obtained by chemical and ^{13}C-n.m.r.-spectroscopic methods. The polymer has essentially the same structural features as those found for L-arabinans isolated from other plant sources.

INTRODUCTION

It has long been known[1] that a mucous material isolated from the roots of the marsh mallow (*Althaea officinalis* L.) may be used as a crude drug in treatment of catarrhs of the respiratory system, gastritis, ulcus ventriculi, and various inflammations of the nasal and oral cavities. Although polysaccharides constitute an appreciable proportion of this material, there have been few studies[2,3] on their detailed structures. It was thus of interest to broaden the present knowledge of the chemical structure of these polysaccharides and study simultaneously the possible relationship between their structures and therapeutic effects. In this paper, chemical data obtained for the L-arabinan from the roots of the marsh mallow (*Althaea officinalis* L.) are discussed and correlated with results obtained by ^{13}C-n.m.r. spectroscopy.

RESULTS AND DISCUSSION

Roots of the plant were crushed and twice macerated in cold water. Four water-soluble and four water-insoluble polysaccharide fractions were obtained by reprecipitations of the aqueous extracts with ethanol. The water-soluble fractions differed each from the other in the composition of the constituent sugars and in the uronic acid content (see Table I). In contrast, D-glucose was the only main sugar found in hydrolyzates of the water-insoluble fractions.

The first stage of the structural studies focused on fractionation of the

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TABLE I

WATER-SOLUBLE POLYSACCHARIDE FRACTIONS FROM THE ROOTS OF THE MARSH MALLOW (*Althaea officinalis* L.)

Fraction	*Yield*[a] (%)	*Molar ratios of monosaccharides*							
		Gal	*Glc*	*Man*	*Xyl*	*Ara*	*Rha*	*Fuc*	*Uronic acids*
A	3.2	1.0	1.3	trace	trace	0.3	0.1	trace	0.3
B	0.7	1.0	1.7	0.1	trace	0.6	0.3	trace	0.4
C	2.6	1.0	1.3	trace	0.1	1.5	0.7	trace	1.4
D	0.3	1.0	3.7	0.1	0.1	5.5	0.6	trace	2.0

[a] In percent of the total weight of roots

polysaccharide mixture C. The polymeric material, which had an L-arabinose content of ~21%, was suspended in 70% aqueous ethanol. The ethanol-soluble portion, having an increased content of L-arabinose (~45%), was then loaded onto a column of DEAE-Sephadex A-25 (carbonate form). Elution with water afforded a polysaccharide material (P_1) containing L-arabinose and D-glucose in the molar ratio 11:2, plus traces of D-galactose and L-rhamnose. Subsequent purification of P_1 on Sephadex G-75 yielded an L-arabinan that was homogeneous by electrophoresis and sedimentation analysis. The $\bar{P}_w/\bar{P}_n$ ratio revealed a low degree of polymolecularity, and the physicochemical properties are listed in Table II.

Chemical studies. — Partial hydrolysis of the L-arabinan with a water-soluble poly(styrenesulfonic) acid[4] in a dialysis bag under mild conditions gave L-arabinose and a mixture of oligosaccharides, which were separated by chromatography on a column of Sephadex G-15 and on paper. The saccharides thus obtained were successively methylated, hydrolyzed, and shown to contain 1→3 and 1→5 linkages by analysis of products as the corresponding alditol acetates[5] by g.l.c.–m.s.

Periodate oxidation of the polysaccharide showed consumption of 0.73 mol

TABLE II

PHYSICOCHEMICAL DATA FOR L-ARABINAN FROM THE *Althaea officinalis* L. ROOTS

$[\alpha]_D$	−147°
Electrophoretic mobility	4.1×10^{-8} m²V⁻¹s⁻¹
Consumption of IO_4^- per pentose residue	0.73 mole
Sedimentation constant S_{20}	3.86×10^{-13} s
Diffusion coefficient D_{20}	13.5×10^{-11} m²s⁻¹
Partial specific volume p_{20}	0.5265×10^{-3} m³kg⁻¹
Molecular weight M_w	14690
Molecular weight $\bar{M}_n$	11940
$\bar{P}_w/\bar{P}_n$	1.23

TABLE III

METHYLATED SUGARS FROM THE HYDROLYZATE OF THE METHYLATED L-ARABINAN

Sugar derivative	*Mole percent*
2,3,5-Me_3-Ara[a]	38
2,3,4-Me_3-Ara	1
2,5-Me_2-Ara	1
2,3-Me_2-Ara	31
2-Me-Ara	8
3-Me-Ara	8
L-Arabinose	13

[a]2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, etc

of periodate per pentose residue, and hydrolysis of the borohydride-reduced product gave mainly L-arabinose and glycerol. Permethylation of the L-arabinan was achieved by the Hakomori[6] method, followed by three Purdie[7] methylations. After hydrolysis of the methylated polymer, the partially methylated derivatives of L-arabinose were converted into the corresponding alditol acetates and identified[5] by g.l.c.–m.s. (see Table III). The methylation-analysis data indicate a theoretical periodate-consumption of 0.71 mol per pentose residue, in good agreement with the experimental value (0.73 mol).

The highly negative specific rotation ($-147°$) of the L-arabinan and its ^{13}C-n.m.r. spectrum (Fig. 1) suggest that most of the sugar residues are α-L.

^{13}C-N.m.r. studies. — The ^{13}C-n.m.r. shifts for the L-arabinan are given in Table IV. The tentative assignments were based on well known rules[8,9] valid for the ^{13}C chemical- shifts of *O*-alkylated saccharides and by comparison with the ^{13}C-spectral data of methyl *O*-methyl-α-D-arabinofuranosides[10]. Furthermore, the re-

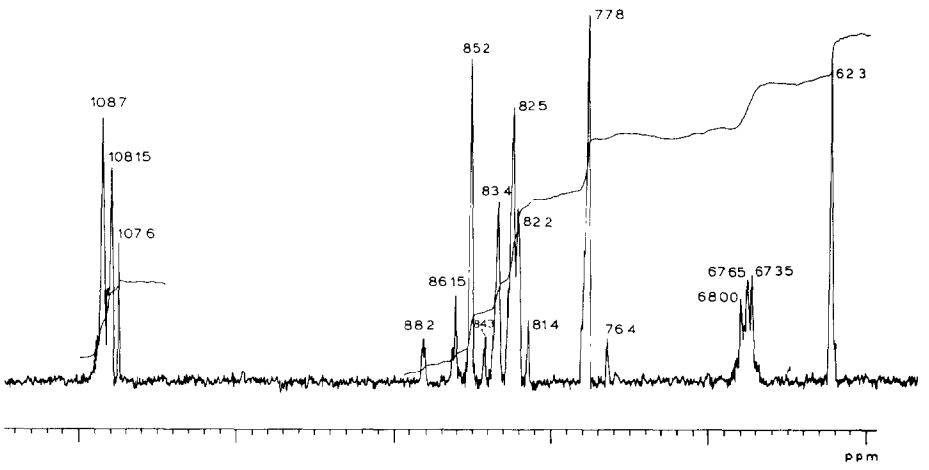


Fig. 1. The ^{13}C-n m r. spectrum of the L-arabinan (3% in D_2O)

TABLE IV

^{13}C-N.M.R. DATA FOR THE L-ARABINAN FROM THE ROOTS OF *Althaea officinalis* L

Sugar residues[a]	*Chemical shifts in δ(p.p.m.)*					*Relative proportions (%)*
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	
A	108.7	82.5	77.8	85.2	62.3	42
B	107.6	86.1	83.4	82.5	67.35	13
C	107.6	88.2	76.4	83.4	67.65	7
D	108.15	82.2	77.8	83.4	68.00	29
E	108.15	81.4	84.3	82.5		9

[a]For location of A, B, C, D, and E, see structure **I**. [b]These signals could not be assigned.

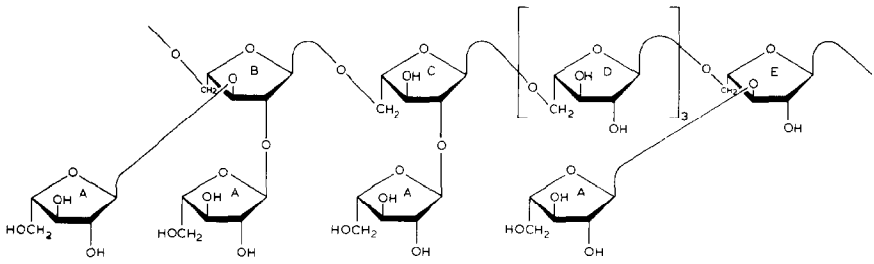
sults of methylation analysis of the L-arabinan were considered and also the fact that shifts produced by *O*-methylation and *O*-glycosylation, having the same direction, may differ[8] in the order of their magnitude. In this way, most signals of the spectrum could be attributed to the corresponding carbon atoms of the L-arabinan chain. A major ambiguity occurred as regards the correct assignment of the glycosidically bound C-5 atoms, which exhibited a group of 3 signals at 67.35, 67.65, and 68.00 p.p.m. The situation appeared rather complex in this instance, as four residues of this type may be found in the polysaccharide, and only three signals are seen. It is not possible to assign the signals without reference to model compounds, nor can it be stated whether the influence depends on the substitution of C-2 and/or C-3 of the residue, or on the neighboring units attached to C-5 and having several possible positions of substitution.

It has been shown earlier[8] that the integrated intensities of signals in the ^{13}C-n.m.r. spectrum of polysaccharides permit conclusions on the relative proportion of constituent sugars in the polymer investigated. Thus, for L-arabinan, integration of the spectrum gave the tentative values listed in Table IV, which were in good agreement with the results of methylation analysis.

Both chemical data and the ^{13}C-n.m.r. measurements reveal a highly branched structure for the L-arabinan, consisting of ~90 α-L-arabinofuranose residues, including an average of ~36 terminal and ~27 exclusively (1→5)-linked sugar residues. On the average, 7 and 8 of the L-arabinose residues are involved in branching through O-2, O-5 and O-3, O-5, respectively, whereas ~12 residues are the sites of branching at O-2, O-3, and O-5. Some of the terminal sugar residues (~1%) are present in the pyranoid form.

From the results presented, a structure (**I**) may be proposed consistent with the data obtained for this polysaccharide. Nevertheless, many other variations are also possible.

Genuine L-arabinans of plant origin, generally associated with pectin in the cell wall, have been investigated earlier[11–13]. The structural features of these L-arabinans were discussed mainly on the basis of methylation-analysis data and



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periodate-oxidation studies on the original polysaccharides. Recently, Joseleau *et al.*[14] have reported a partial interpretation of the ^{13}C-n.m.r. spectra of L-arabinans isolated from the bark of *Rosa glauca*. The results obtained were in accord with data determined by chemical methods. In our studies, we also extended assignments for ^{13}C signals appearing in the region 75–89 p.p.m., corresponding to the resonances of C-2, C-3, and C-4 of the L-arabinosyl residues. As both methods, methylation analysis and ^{13}C-n.m.r., gave similar information on the structure of the L-arabinan investigated, the approach presented appears to be very effective for elucidation of the structure of any type of polysaccharide, if suitable model compounds or literature data are available.

The isolated L-arabinan has essentially the same structural features as L-arabinans originating from other plant sources. Differences are apparent only in structural details, as in the degree of branching at O-2 and O-3, the molecular weights, and the ratio of furanoid and pyranoid forms of the terminal sugar residues. These findings may be considered as another demonstration of the heterogeneity of polysaccharides present in the plant tissues. Thus, the structural features of each polysaccharide type may be regarded as an average character of a family of related polymers.

EXPERIMENTAL

General methods. — Free-boundary electrophoresis was effected with a Zeiss 35 apparatus, using 0.05M sodium tetraborate buffer (pH 9.2), at 10 V/cm and 6 mA for 30 min, and a polysaccharide concentration of 10 mg/mL. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter for 1% aqueous solutions at 20°.

The polysaccharides were hydrolyzed with 72% sulfuric acid[15] or 2M trifluoroacetic acid for 2 h at 100°. The uronic acid content was determined by the carbazole method[16]. Preparative p.c. was performed by the descending method on Whatman No. 3MM paper with system *A*, 9:2:2 1-butanol–pyridine–water. Reducing sugars were detected with aniline hydrogenphthalate[17]. The mobilities (R_{Ara}) of oligosaccharides are expressed relative to that of L-arabinose.

G.l.c. was conducted with a Hewlett–Packard Model 5711A chromatograph and (*B*) a column (200 × 0.3 cm) of 3% of OV-225 on 80–100 mesh Chromosorb W (AW-DMCS), at a programmed temperature range of 120° (4 min) to 170° and 2°/min, on (*C*) a column (200 × 0.3 cm) of 20% of SE-96 on 80–100 mesh Chromosorb W (AW-DMCS), at 110° (2 min) to 210° and 4 °/min. Column *B* was applied for quantitation of the sugars as their alditol trifluoroacetates[18]. G.l.c – m.s. of alditol acetates[5] of methylated saccharides was performed with a JMS-D 100 (Jeol) spectrometer, using a column (200 × 0.3 cm) packed with 100–120 mesh Supelcoport coated with 3% of SP 2340. The inlet helium pressure was 101.3 kPa, temperature 160–240° at 6°/min, and the spectra were determined at 23 eV.

The ^{13}C-n.m.r. spectrum of the polysaccharide solution (3% in D_2O) was obtained in the Fourier-transform mode, by using a Bruker WM-250 spectrometer with complete proton-decoupling at 30°. The spectral width was 15 kHz; acquisition time, 0.5 s; data points, 8 k; and pulse width, 6 μs (45°). Chemical shifts were measured relative to internal dimethyl sulfoxide (39.45 p.p.m. from Me_4Si) as the standard.

The molecular weight ($\bar{M}_w$) of L-arabinan was estimated by ultracentrifugation (238,330 g) and extrapolation to zero concentration (MOM G 110 ultracentrifuge). Six photographs taken after 40 min at 6-min intervals showed that the polysaccharide sedimented as a single, symmetrical peak. The partial specific-volume was determined pycnometrically for a 1% solution of the polysaccharide. Osmotic pressure measurements with L-arabinan were performed in water at 35°, with a Knauer Membrane osmometer and Zweischicht-Membrane (Knauer) as the membrane.

Isolation of the L-*arabinan*. — Roots were obtained from plants cultivated at the Centre for Cultivation of Medicinal Plants, Faculty of Medicine of J. E. Purkyně University of Brno (Czechoslovakia) in November 1979. The material (9450 g) was crushed and then macerated in cold water (200 L) for 48 h. The extract was evaporated to lower volume (50 L) and poured into 96% ethanol (300 L) containing 1% (v/v) of acetic acid. The precipitate was treated with ethanol, suspended in water, and dialyzed against distilled water for 4 days. The water-insoluble portion, which separated, was removed by centrifugation and the solution was lyophilized to give a polysaccharide mixture *A* (see Table I). The water-insoluble fraction, which gave mainly D-glucose on acid hydrolysis, constituted 8.4% of the weight of starting material.

The residual, aqueous ethanolic solution was made neutral with calcium hydroxide, concentrated to 20 L, and poured into 96% ethanol (200 L) acidified with acetic acid (2 L). Following the procedure already described, a polysaccharide mixture B (Table I) and the water-insoluble, D-glucose-containing portion (1.8% of the roots by weight) were obtained.

A second maceration of the roots with cold water and subsequent isolation of the polysaccharide mixtures C and D (Table I) were accomplished similarly. Two water-insoluble polymer fractions (6.3 and 0.15% of the weight of roots, respec-

tively), separated during the isolation procedure, were again shown to be composed mainly of D-glucose.

A portion (15 g) of fraction C was suspended in aqueous ethanol (70%, 600 mL) and stirred for 3 h at 40°. The residue was separated by centrifugation, and the extraction was repeated three times. Hydrolysis of the ethanol-soluble portion (2.9 g, 19.3%) yielded L-arabinose, D-galactose, D-glucose, and L-rhamnose in the molar ratio 4.5:1:1:0.9 together with trace amounts of D-mannose, D-xylose, and L-fucose. The uronic acid content was ~17%. A solution of the ethanol-soluble portion (2.8 g) in water (150 mL) was added to the top of a column (100 × 3 cm) of DEAE-Sephadex A-25 (carbonate form). The column was eluted successively with water, ammonium carbonate (50–500mM) and 0.1, and 0.3M sodium hydroxide. The water-eluted fraction P_1 (650 mg, 23.2%) on acid hydrolysis gave L-arabinose, D-glucose (11:2), and traces of D-galactose and L-rhamnose. The material (600 mg) was purified by elution from a column (100 × 3 cm) of Sephadex G-75 with water to give a polysaccharide (225 mg, 37.5%), $[\alpha]_D$ −147° that afforded only L-arabinose on hydrolysis. Sedimentation analysis and free-boundary electrophoresis confirmed the homogeneity of the L-arabinan.

Partial hydrolysis of the L-*arabinan.* — The L-arabinan (100 mg) was dissolved in an aqueous solution of poly(styrenesulfonic) acid[4] (pH 2.1, 10 mL) and the solute was dialyzed at 65° in a dialysis bag against distilled water (50 mL). At hourly intervals, the dialyzate was replaced with fresh distilled water, previously heated to 65°. After 12 h, the combined dialyzates were evaporated to yield a syrupy product (72 mg) that was loaded onto a column (100 × 3 cm) of Sephadex G-15. Elution with water gave three fractions: (*1*) a mixture of higher oligosaccharides (10 mg); (*2*) a mixture containing di- and tri-saccharides (25 mg), and (*3*) L-arabinose (35 mg). Preparative p.c. of fraction *2* on Whatman No. 3 MM paper (solvent *A*), yielded components having R_{Ara} 0.81 (10 mg), 0.68 (8 mg), 0.41, and 0.15, the latter two being present only in traces. The oligosaccharides **2** (R_{Ara} 0.81) and **3** (R_{Ara} 0.68) were each (5 mg) methylated (methyl iodide and sodium hydride in *N*,*N*-dimethylformamide), subsequently hydrolyzed with 2M trifluoroacetic acid, and the products were analyzed as their alditol acetates[5] by g.l.c.–m.s. The identification of 2,3,5-tri-*O*-methylarabinose and 2,3-di-*O*-methylarabinose, or 2,3,5-tri-*O*-methylarabinose and 2,5-di-*O*-methylarabinose (in equimolar amounts) confirmed the presence of 1→5 and 1→3 linkages in **2** and **3**, respectively.

As the higher members of the homologous series of L-arabinose-containing oligosaccharides (R_{Ara} 0.41, 0.15) were only present in traces, they were not further investigated.

Methylation analysis of the L-*arabinan.* — The polysaccharide (40 mg) was solubilized in dry dimethyl sulfoxide (5 mL) and methylated[6] with methyl iodide in the presence of methylsulfinyl carbanion. The solution was then poured into water (30 mL), dialyzed for 48 h, and evaporated. The syrupy residue was dissolved in methyl iodide (5 mL), silver oxide (100 mg) was added[7], and the mixture was stirred and boiled under reflux for 24 h. The methylation procedure was repeated twice.

The methylated L-arabinan (30 mg) was hydrolyzed with 2M trifluoroacetic acid (5 mL) for 2 h at 105° and the partially methylated derivatives of L-arabinose were conventionally converted into the corresponding alditol acetates and quantitatively analyzed [5] by g.l.c.–m.s. The methylated sugars detected are listed in Table III.

Degradation of the L-arabinan. — The L-arabinan (10 mg), dissolved in aqueous sodium metaperiodate (15mM, 10 mL), was kept at room temperature in the dark, the consumption of periodate being spectrophotometrically monitored[19] on aliquots at 225 nm. After 70 h, the consumption of oxidant was 0.73 mol per pentose residue. Reduction of the resulting polyaldehyde by sodium borohydride (20 mg) followed by hydrolysis and g.l.c. analysis (column C), showed the presence of glycerol and L-arabinose detected as their *O*-trimethylsilyl derivatives[20].

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