POLYSACCHARIDES FROM THE FLOWERS OF *Malva mauritiana* L.: STRUCTURE OF AN ARABINOGALACTAN

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A water-soluble arabinogalactan composed of D-galactose and L-arabinose in the mole ratio 1 : 1.4 has been isolated from the flowers of *Malva mauritiana* L. Partial acid hydrolysis, methylation analysis, periodate oxidation, and ^{13}C NMR spectroscopy were employed in structure elucidation. The arabinogalactan was shown to have a highly branched structure. The core consisted of 1,6-linked β-D-galactopyranose units, about 65% of which were substituted in position C-3 by side-chains of mainly 1,5-linked α-L-arabinofuranosyl residues.

In our previous work we described the isolation of the mucilage from the flowers of *Malva mauritiana* L., and the structure of an α-D-glucan component of its neutral portion[1]. Now, we report on the purification and structure determination of another neutral polysaccharide component, an arabinogalactan.

EXPERIMENTAL

Material and Methods

The flowers of *Malva mauritiana* L. were purchased from Slovakofarma, Medicinal plants, Malacky. Solutions were concentrated under diminished pressure below 40 °C. Free-boundary electrophoresis of 1% solutions of polysaccharides was effected with a Zeiss 35 apparatus, using 0.05 M sodium tetraborate buffer (pH 9.2) at 150 V/cm and 6 mA for 30 min. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter for 1% aqueous solutions at 20 °C. The number average molecular mass ($\overline{M}_n$) was determined osmometrically at 30 °C, using a Knauer Vapour-pressure osmometer. Infrared spectra of the methylated products were recorded with a Perkin–Elmer 9836 spectrometer. The polysaccharides were hydrolyzed with 2 M trifluoroacetic acid for 1 h at 120 °C. Paper chromatography was performed by the descending method on Whatman No. 1 and 3MM papers with system S, ethyl acetate–pyridine–water (8 : 2 : 1). The saccharides were detected with anilinium hydrogen phthalate[2]. The mobilities (R_{Ara}) of oligosaccharides are expressed relative to that of L-arabinose.

Gas chromatography (GL) was carried out on Hewlett–Packard Model 5711A instrument on column (0.3 × 200 cm) with stationary phase 3% OV-225 on Chromosorb W (AW-DMCS, 80-100 mesh) at 120 °C (4 min) to 170 °C (2 °C/min), and a column (0.3 × 200 cm) with the stationary phase 3% SP 2340 on Chromosorb W (AW-DMCS, 80–100 mesh) at 180–220 °C (4 °C/min). Gas chromato-

graphy-mass spectrometry (GL-MS) of alditol acetates[3] of methylated saccharides was performed with a JMS-D 100 (JEOL) spectrometer, and a column (0.3 × 200 cm) with 3% SP-2340 on Supelcoport (100–200 mesh) as stationary phase. The temperature was 160–240 °C (6 °C/min), and the spectra were measured at 23 eV.

^{13}C NMR spectra of polysaccharides in D_2O were recorded at 35 °C on a Bruker AM-300 spectrometer at an operating frequency of 75 MHz with inverse-gated decoupling. Chemical shifts were measured relative to methanol as internal reference (50.15 ppm from tetramethylsilane). ^{13}C NMR DEPT experiment[4] was carried out using Bruker software.

Isolation of Arabinogalactan

The crude mucilage isolated from flowers of *M. mauritiana* (SLM) was resolved on a DEAE-Sephadex A-50 column (5 × 100 cm) to neutral (NP) and acid (AP) portions[1]. The neutral portion (1 g in 10 ml water) was loaded on a column (4 × 100 cm) of Sephadex G-75 and eluted with water. The eluate, assayed with phenolsulfuric acid, contained four distinct fractions (Table I). The first fraction (NP-1, 120 mg) was a practically homogeneous α-D-glucan[1], while the other ones (NP-2, 250 mg; NP-3, 260 mg; NP-4, 140 mg) contained all 7 sugar components found in the starting NP, though in different ratios. Rechromatography of NP-3 (250 mg in 2 ml water) on a column (2.5 × 120 cm) of Sephadex G-50 gave a polysaccharide (180 mg) homogeneous upon free-boundary electrophoresis and size exclusion chromatography. It was composed of D-galactose and L-arabinose in the mole ratio 1 : 1.4 and trace amounts of D-xylose.

Methylation Analysis

The dry samples of oligo- and polysaccharides (1–3 mg) were solubilized in dry dimethyl sulfoxide (1 ml) and methylated by the Hakomori method[5]. The methylated products were isolated by partition with chloroform, hydrolyzed with 2 M trifluoroacetic acid for 1 h at 120 °C, then converted into partially methylated alditol acetates and analyzed[3] by GL-MS (Table II).

TABLE I
Sugar composition of Sephadex G-75 fractions of the neutral polysaccharides (NP)

Fraction	Mole ratios of monosaccharides							
	D-Gal	D-Glc	D-Man	L-Ara	D-Xyl	L-Fuc	L-Rha	UA[a]
SLM[b]	1.00	0.22	0.22	0.62	0.30	0.03	0.42	0.68
NP[c]	1.00	1.41	0.40	1.03	0.34	0.10	0.29	–
NP-1[d]	–[e]	1.00	–[e]	–[e]	–	–	–	–
NP-2	1.00	1.47	0.30	1.89	–[e]	–[e]	1.00	–
NP-3	1.00	0.34	0.17	1.55	–[e]	–[e]	–[e]	–
NP-4	1.00	1.52	0.69	0.73	1.08	0.14	–[e]	–

[a] Uronic acids. [b] Crude mucilage. [c] Neutral polysaccharides. [d] Sephadex G-75 fractions of NP. [e] Traces.

Partial Hydrolysis

The arabinogalactan (100 mg) was hydrolyzed with 1 M trifluoroacetic acid (20 ml) for 30 min at 100 °C. The hydrolysis product was separated on a column (2.5 × 150 cm) of Sephadex G-25 by water elution to three fractions: polymeric residue (1F, 33 mg), mixture of oligomers (2F, 11 mg), and arabinose (3F). The polymeric portion was homogeneous upon gel chromatography on Sephadex G-50. On total hydrolysis it gave D-Gal as the only sugar. Part of the polysaccharide (2 mg) was dissolved in dry dimethyl sulfoxide and methylated by the Hakomori method[5]. The results of methylation analysis are given in Table II.

The mixture of oligosaccharides was separated by preparative paper chromatography on Whatman 3MM paper in system S to three distinct components of R_{Ara} 0.80 (*1*), 0.51 (*2*), and 0.33 (*3*). The compounds *1* and *2* on acid hydrolysis afforded L-Ara only. Chromatographic mobility of *1* was in good agreement with that of arabinofuranosyl-1,5-α-L-arabinofuranose, isolated from the arabinan[6] of *Althaea officinalis* L. and rhamnoarabinogalactan[7] of *Plantago lanceolata* L. Compound *3* was composed of L-Ara and D-Xyl in the mole ratio 2.9 : 1. The oligosaccharides were methylated and the products of methylation analysis of *1* and *2* were identified by GL-MS as 2,3,5-tri-*O*-methylarabinose and 2,3-di-*O*-methylarabinose, pointing to 1,5 linkage between the sugar units. Their equimolar ratio in *1* and 1 : 1.9 in *2* confirmed that *1* was a disaccharide and *2* a trisaccharide. Compound *3* provided 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*-methylarabinose, and 2,3-di-*O*-methylxylose in the ratio 1 : 1.9 : 0.9, evidencing that the bond between Araf units was 1,5 similarly as in *1* and *2*, and Xyl was an interunit bound by 1,4 linkage.

TABLE II
Sugar linkage analysis of native (A), acid-degraded (B), and oxidized (C) arabinogalactan

Sugar derivative	Mole %			Linkage indicated
	A	B	C	
2,3,5-Me_3-Ara[a]	29.6			Araf-(1-
2,3,4-Me_3-Ara	1.8			Arap-(1-
3,5-Me_2-Ara	0.9		1.5	-2)-Araf-(1-
2,5-Me_2-Ara	3.0		7.3	-3)-Araf-(1-
2,3-Me_2-Ara	17.1		–	-5)-Araf-(1-
2-Me-Ara	1.3		3.1	-3,5)-Araf-(1-
3-Me-Ara	5.8		12.1	-2,5)-Araf-(1-
2,3,4,6-Me_4-Gal	–[b]	2		Galp-(1-
2,3,6-Me_3-Gal	2.2	–[b]		-4)-Galp-(1-
2,3,4-Me_3-Gal	12.0	98		-6)-Galp-(1-
2,4-Me_2-Gal	26.3	–	76.0	-3,6)-Galp-(1-

[a] 2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, etc. [b] Traces.

Periodate Oxidation

The arabinogalactan (10 mg) was oxidized with 15 mM sodium periodate (10 ml) at 5 °C in the dark. The consumption of periodate was monitored spectrophotometrically[8] at 225 nm. The reaction was complete after three days. The excess periodate was destroyed by ethylene glycol (0.2 ml) and the resulting polyaldehyde was reduced with sodium borohydride (50 mg) overnight. After removing the excess borohydride with acetic acid, the solution was concentrated and separated on a column of Sephadex G-25. The yield of the modified polymeric residue was 3.1 mg. Part of it (1.5 mg) was methylated[5] and the products were analyzed as in the case of the native polysaccharide (Table II).

RESULTS AND DISCUSSION

The mucous material was resolved by ion-exchange chromatography to neutral (NP) and acid (AP) portions[1]. The mixture of neutral polysaccharides (NP), composed of D-Gal, D-Glc, L-Ara, D-Xyl, L-Fuc, and L-Rha, was separated by gel permeation chromatography on Sephadex G-75 to four fractions (NP 1-4), differing in composition of the constituent sugars (Table I).

The fraction eluted first (NP-1) contained an α-D-glucan, the structure of which has already been described[1]. Though sugar analysis of NP-2 revealed a high content of L-Ara, the presence of D-Glc, D-Gal, and L-Rha pointed to heterogeneity of this fraction. Fraction NP-3 was remarkable by dominance of two saccharide components, namely D-Gal and L-Ara. The last fraction contained all the constitutive saccharides of the starting NP, except of L-Rha.

As the composition of NP-3 suggested the presence of one or two dominant polymers, it was further purified by size-exclusion chromatography to give a homogeneous polysaccharide composed of D-Gal and L-Ara in 1 : 1.4 mole proportion and trace amounts of D-Xyl. Its $\overline{M}_n$ was 18 900 and optical rotation –61°. Partial acid hydrolysis of arabinogalactan under mild conditions gave, besides the degraded polymer, a mixture of arabinooligomers and arabinose.

Compositional analysis of the degraded polysaccharide revealed galactose as the only constituent sugar. Consequently, all arabinose units were located in side-chains and were removed on partial acid hydrolysis. The polymer had $[\alpha]_D$ +10° and $\overline{M}_n$ 8 033 (degree of polymerization 50). The type of linkages in the degraded polymer was proved by the products of methylation analysis. 2,3,4,6-Tetra-*O*-methylgalactose and 2,3,4-tri-*O*-methylgalactose (Table II) evidenced a linear chain of 1,6-linked sugar units.

The ^{13}C NMR spectrum of the degraded polymer (Fig. 1) confirmed the above mentioned result. It showed only one anomeric signal at 104.5 ppm, assigned to C-1 of galactopyranose units involved in β-glycosidic linkage[9]. The signal at 70.65 ppm (negative amplitude in DEPT experiment) indicated the substitution of the C-6 atom. The other signals at 74.91, 73.78, 71.90, and 69.81 ppm arised from resonances of C-5, C-3, C-2, and C-4, respectively[7,10].

Methylation analysis of arabinogalactan showed a variety of linkages and pointed to highly branched structure of the polymer (Table II). About 33% sugar units were involved in branches. The main derivatives of D-Gal residues were 2,3,4-tri-*O*-methyl- and 2,4-di-*O*-methylgalactose, indicating 1,6 (12%) and 1,3,6 linkages (26.3%). Such glycosidic linkages are common[11] for many plant arabinogalactans (Type II). Besides these two main derivatives also a low amount of 2,3,6-tri-*O*-methylgalactose (2.2%) was detected, showing the presence of 1,4 linkages, which are common[11] for arabinogalactans isolated from plant sources (Type I). The dominant derivatives of L-Ara, i.e. 2,3,5-tri-*O*-methylarabinose and 2,3-di-*O*-methylarabinose, proved the terminal- (29.6%) and 1,5-linked (17.1%) position of arabinofuranose units (Table II). The high content of 1,5-linked Araf units confirms the existence of side pentose chains, the length of which may be 1 to 4 units, as proved by the products of partial acid hydrolysis. Side units (7.1%) are branched at position C-2 and C-3, as indicated by the 2- and 3-*O*-methylarabinose derivatives. Some of the arabinose residues (1.8%) are present in

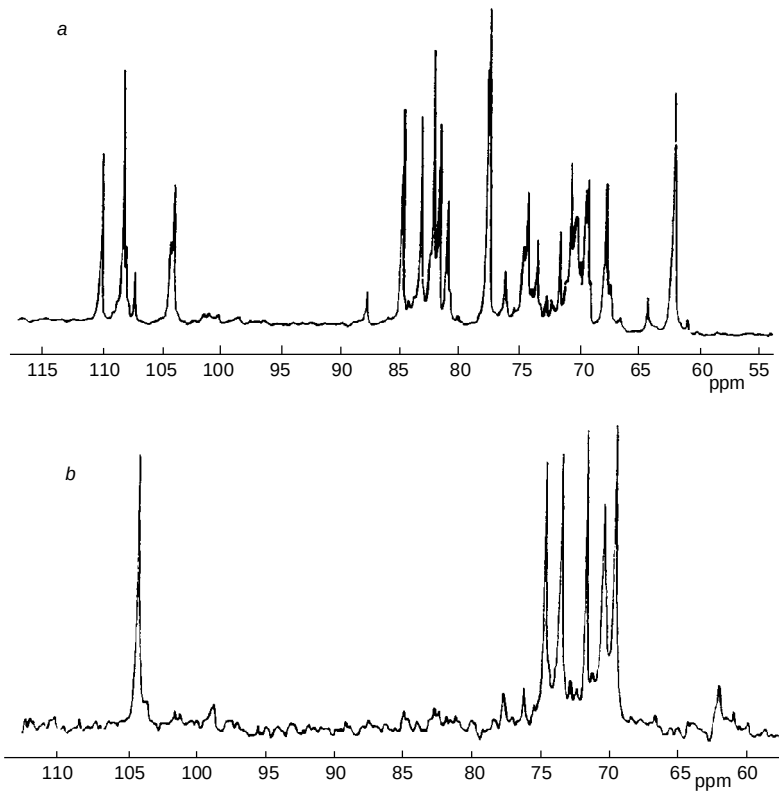

FIG. 1
^{13}C NMR spectra of *a* native and *b* degraded arabinogalactan

the pyranoid form (Table II). The occurrence of D-Xyl unit in side-chains, though in very low amount, is noticeable. The relative mole proportion of D-Gal and L-Ara resulting from sugar linkage analysis (1 : 1.5) was in agreement with the results of GLC (1 : 1.4). Periodate oxidation of arabinogalactan supported the results of methylation analysis. The amount of the polymeric residue (31%) after oxidation of the intact polysaccharide reflected the high degree of substitution of the polymer. Sugar linkage analysis indicated 1,2- (1.5%), 1,3- (7.3%), 1,3,5- (3.1%), and 1,2,5-linked (12.1%) arabinose and 1,3,6-linked galactose (76%) as periodate resistant units.

The signals in the ^{13}C NMR spectrum of arabinogalactan were interpreted on the basis of the spectral data for respective monosaccharides[9,12], related polysaccharides[6,10], and the degraded polymer[7]. The spectrum of arabinogalactan is a complex one (Fig. 1). The 6 main signals in the anomeric region indicated a branched structure of the polymer. The four main signals observed in the lowest magnetic field reflected the resonances of C-1 of nonreducing terminal and internal α-L-arabinofuranosyl residues. Absence of these signals in the ^{13}C NMR (Fig. 1) spectrum of the degraded polysaccharide confirmed the complete loss of side-chains during mild acid hydrolysis. The other anomeric signals at 104.63 ppm and 104.39 ppm arose from C-1 resonances of 1,6- and 1,3,6-linked β-D-galactopyranose residues, respectively. In accordance, the signal at 104.39 ppm was missing on the spectrum of the degraded linear galactan. The resonances of the substituted carbons were observed at 88.17 ppm (C-2 of 1,2-and 1,2,5-linked Araf), 83.59 ppm (C-3 of 1,3- and 1,3,5-linked Araf), 81.40 ppm (C-3 of 1,3,6-linked Galp), 70.57 ppm (C-6 of 1,6- and 1,3,6-linked Galp), and 68.16–67.74 ppm (C-5 of 1,5-, 1,2,5-, and 1,3,5-linked Araf). Due to complexity of the spectrum, unambiguous assignment of all resonances was not possible.

It can be concluded that the structure elucidation by chemical and spectral analysis revealed, accordingly, a highly branched structure of the arabinogalactan, the main chain of which consists of 1,6-linked β-D-Galp residues substituted (65%) in position C-3 by side-chains of 1,5-linked α-L-Araf units. It is known from the literature[11] that arabinogalactans with 1,3-linked β-D-Galp units in the backbone are widely distributed in plants. Arabinogalactans with the core composed of 1,6-linked β-D-Galp units have been already isolated from the roots of *Angelica acutiloba* L. (ref.[13]), seeds of *Coix lacryma* L. (ref.[14]), berries of *Viscum album* L. (ref.[15]), leaves of *Plantago lanceolata* L. (ref.[7]) and *Tridax procumbens* L. (ref.[16]). Though the relative composition of constitutive sugars as well as the occurrence and length of side-chains differ each from the other in these polysaccharides, the common type of the glycosidic linkage in the core may serve as a new feature for classification of related polysaccharides.

REFERENCES

1. Capek P.: Collect. Czech. Chem. Commun. *57*, 2400 (1992).
2. Partridge S. M.: Nature *164*, 443 (1949).

3. Jansson P. E., Kenne L., Liedgren H., Linberg B., Lönngren J.: Chem. Commun., Univ. Stockholm *8*, 1 (1976).
4. Rutar V.: J. Magn. Reson. *48*, 155 (1982).
5. Hakomori S.: J. Biochem. (Tokyo) *55*, 205 (1964).
6. Capek P., Toman R., Kardosova A., Rosik J.: Carbohydr. Res. *117*, 133 (1983).
7. Kardosova A., Capek P.: Collect. Czech. Chem. Commun. *59*, 2714 (1994).
8. Aspinall G. O., Ferrier R. J.: Chem. Ind. (London) *1957*, 1216.
9. Bradbury J. H., Jenkins G. A.: Carbohydr. Res. *126*, 125 (1984).
10. Cartier N., Chambat G., Joseleau J. P.: Carbohydr. Res. *168*, 275 (1987).
11. Aspinall G. O.: *The Polysaccharides*, Vol. 2, p. 122. Academic Press, New York 1983.
12. Pfeffer P. E., Valentine K. M., Parrish F. W.: J. Am. Chem. Soc. *101*, 1265 (1979).
13. Yamada H., Kiyohara H., Cyong J. C., Otsuka Y.: Mol. Immunol. *22*, 295 (1985).
14. Yamada H., Yanahira S., Kiyohara H., Cyong J. C., Otsuka Y.: Phytochemistry *26*, 3269 (1987).
15. Wagner H., Jordan E.: Phytochemistry *27*, 2511 (1988).
16. Raju T. S., Davidson E. A.: Carbohydr. Res. *258*, 243 (1994).