An Arabinogalactan Isolated from the Medicinal Plant *Maytenus ilicifolia*

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An arabinogalactan was obtained from the leaves of *Maytenus ilicifolia* by hot aqueous 2% KOH extraction, followed by a freezing−thawing process and anion-exchange chromatography. It consisted of arabinose, galactose, galacturonic acid, and rhamnose in a 69:20:6:5 molar ratio. Methylation analysis, partial acid hydrolysis, and ^{13}C NMR spectroscopy indicated that it was an arabinogalactan containing a (1→4)-linked *β*-Gal*p* main chain, substituted at O-6 with Ara units, which were in turn substituted at O-5 (Ara*f*) and/or O-4 (Ara*p*), O-3, O-3,5, and O-2,5. This arabinogalactan is probably linked to O-4 of some Rha*p* units of a type I rhamnogalacturonan, formed by repeating (1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p* groups.

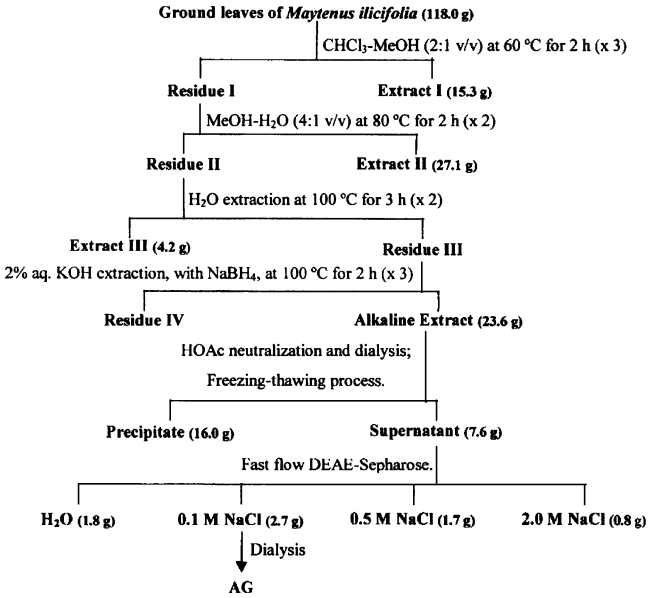
Maytenus ilicifolia Mart. ex Reissek (Celastraceae) is a medicinal plant popularly known as "espinheira-santa", found in the southern region of Brazil, Paraguay, Uruguay, and Argentina.[3] Its utilization is said to be mainly for treatment of stomach ulcers and gastritis, whose efficacy has been described.[1] Up to the present, only secondary metabolites of this plant have been chemically characterized, namely, triterpenes[2] and flavonoid glycosides.[3,4] There have been no reports on its primary metabolites, including polysaccharides. We report herein the isolation and structural analysis of an arabinogalactan which is probably linked to a type I rhamnogalacturonan (RGI) in the plant.

Arabinogalactans (AG) are essential polymers in the cell wall of plants and are found in all higher plants as structural polysaccharides.[5] They are also found as a main component of many gums and exudates,[5−8] affording highly viscous solutions, with a wide industrial application, and are often reported to be immunologically active.[9−11] Their chemical structures are very complex,[12,13] and members of this class of polysaccharides may exist as a pectin component[14] or linked to proteins.[5,15] The AG found in pectin fractions are type I arabinogalactans (AGI), which consist mainly of a (1→4)-linked *β*-Gal*p* main chain, with Ara*f* substituents at O-3, and can be linked to RGI.[14]

The water-soluble fraction (supernatant) obtained by a freeze−thawing process, according to Scheme 1, was heterogeneous (Figure 1A) when analyzed by HPSEC-MALLS; this fraction was thus submitted to chromatography using a fast-flow DEAE-Sepharose column, to give four fractions. HPSEC-MALLS analysis (Figure 1B) showed that the eluate obtained with 0.1 M NaCl (2.3 g %) contained a homogeneous polysaccharide (AG) with a molecular weight of 87 000 g/mol (d*n*/d*c* = 0.157). It contained arabinose, galactose, uronic acid, and rhamnose in a 69:20:6:5 molar ratio.

The ^{13}C NMR spectrum of AG (Figure 2A) showed a highly complex polysaccharide, with many signals in the C-1 region (*δ* 101.5−109.3). Although AG contained 6% of uronic acids, no signal was observed for the carboxyl groups, due to spectral conditions. The presence of *C*H$_3$-6 of rhamnosyl units was shown by a signal at *δ* 17.0.[16] Signals at *δ* 106.5, 107.1, 107.6, and 109.3 corresponded to C-1 of α-arabinofuranosyl units.[6,7,10,17,18] In turn signals at *δ* 103.2 and 104.4 were from C-1 of *β*-galactopyranosyl units[6,7,10,16−18] and at *δ* 101.5 and 101.7 from C-1 of α-rhamnopyranosyl and *β*-arabinopyranosyl units, respectively.[16,17]

Scheme 1. Extraction and Purification of the Arabinogalactan of Leaves of *Maytenus ilicifolia*



Methylation analysis (Table 1) showed that AG is a highly branched polysaccharide, containing nonreducing end-units of Ara*f* (2,3,5-Me$_3$-Ara) (25%) and Gal*p* (2,3,4,6-Me$_4$-Gal) (3%). The arabinosyl units were substituted at O-5 (Ara*f*) and/or O-4 (Ara*p*), O-3, O-3,5, and O-2,5, in accord with 2,3-Me$_2$-Ara (20%), 2,5-Me$_2$-Ara (10%), 2-Me-Ara (5%), and 3-Me-Ara (11%) derivatives, respectively. However, according to the predominant ^{13}C NMR signals of AG at *δ* 106.5 to 109.3 (Figure 2A), the great majority of the arabinosyl units are in the α-Ara*f* form. The galactopyranosyl units are, mainly, 4-*O*- and 4,6-di-*O*-substituted, in accord with 2,3,6-Me$_3$-Gal (10%) and 2,3-Me$_2$-Gal (3%) methylated derivatives, respectively. Also present were a 6-*O*- and 3,6-di-*O*-substituted galactopyranosyl units, as shown by the 2,3,4-Me$_3$-Gal (1%) and 2,4-Me$_2$-Gal (1%) derivatives, respectively. The rhamnosyl units were substituted at C-2 and C-2,4, as demonstrated by the presence of 3,4-Me$_2$-Rha (1%) and 3-Me-Rha (4%) derivatives, respectively.

The structure of the uronic acid found in AG, as well as its linkage types, was determined by carboxy reduction, followed by methylation analysis. The neutral product

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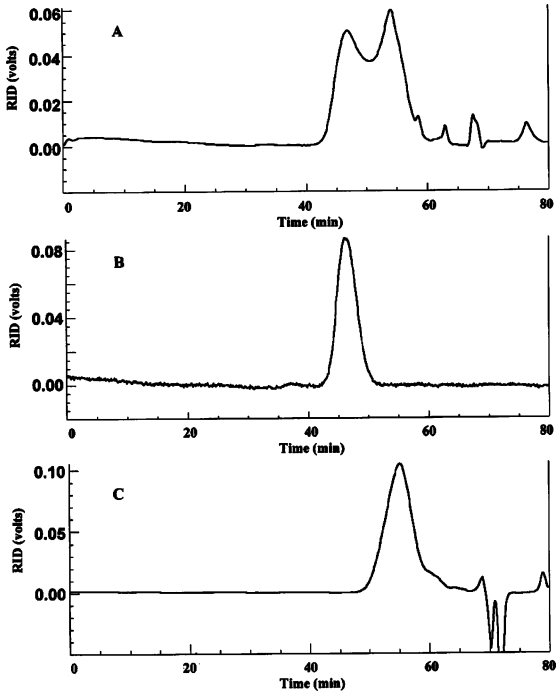


Figure 1. Elution profiles obtained on HPSEC-MALLS analysis of the supernatant of the freeze−thawing process (A), AG (B), and AG-HR.

showed an increase in the 2,3,6-Me_3-Gal derivative, indicating that (1→4)-linked galacturonic acid residues were present. The (1→4)-linked galacturonic acid, plus 2-*O*- and 2,4-di-*O*-substituted Rha*p* residues, strongly suggested a type I rhamnogalacturonan structure. These polysaccharides are formed by the repeating (1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p* group, often having C-4 of the rhamnosyl units substituted by an arabinogalactan sequence.[14]

Partial acid hydrolysis of AG (0.2 M TFA at 100 °C for 2 h) was carried out to elucidate the main chain. From 1 g of AG was obtained 120 mg of a polysaccharide (AG-HR),

Table 1. Profile of Partially *O*-Methylated Alditol Acetates Obtained from Methylation Analysis of AG[a]

O-Me-alditol acetate	%
2,3,5-Me_3-Ara	25
2,5-Me_2-Ara	10
2,3-Me_2-Ara	20
2-Me-Ara	5
3-Me-Ara	11
2,3,4,6-Me_4-Gal	3
2,4,6-Me_3-Gal	trace
2,3,6-Me_3-Gal	10
2,3,4-Me_3-Gal	1
2,3-Me_2-Gal	3
2,4-Me_2-Gal	1
3,4-Me_2-Rha	1
3-Me-Rha	4

[a] The content of galacturonic acid was 6%, according to the method of Filisetti-Cozzi and Carpita.[20]

which was homogeneous as shown by HPSEC-MALLS (Figure 1C). It contained arabinose, galactose, galacturonic acid, and rhamnose in a 3:39:31:27 molar ratio.

The ^{13}C NMR spectrum of AG-HR (Figure 2B) contained signals compatible with a rhamnogalacturonan, with signals at δ 99.1 and 97.8 of the C-1 of α-L-Rha*p* and α-D-Gal*p*A units, respectively, and at δ 77.6 and 76.6 of the C-4 and C-2 of α-D-Gal*p*A and α-L-Rha*p* substituted units, respectively.[19] The signal of the carboxyl groups of α-D-Gal*p*A units was observed at δ 172.0. It was also possible to see a signal intensification of C-1 of β-Gal*p* units at δ 103.4 and 104.3,[6,7,10,16−18] as well as the disappearance of the signals of arabinosyl units.

According to our results, AG is an arabinogalactan containing a (1→4)-linked β-Gal*p* main chain, with substituents of arabinose at O-6. Such a structure has now been described for the first time, since pectic arabinogalactans have been shown to contain a (1→4)-linked β-Gal*p* main chain, with Ara*f* substituents at O-3.[14] Our arabinosyl units are in turn substituted at O-5 (Ara*f*) and/or O-4 (Ara*p*), O-3, O-3,5, and O-2,5. This arabinogalactan is probably linked to a rhamnogalacturonan type I through

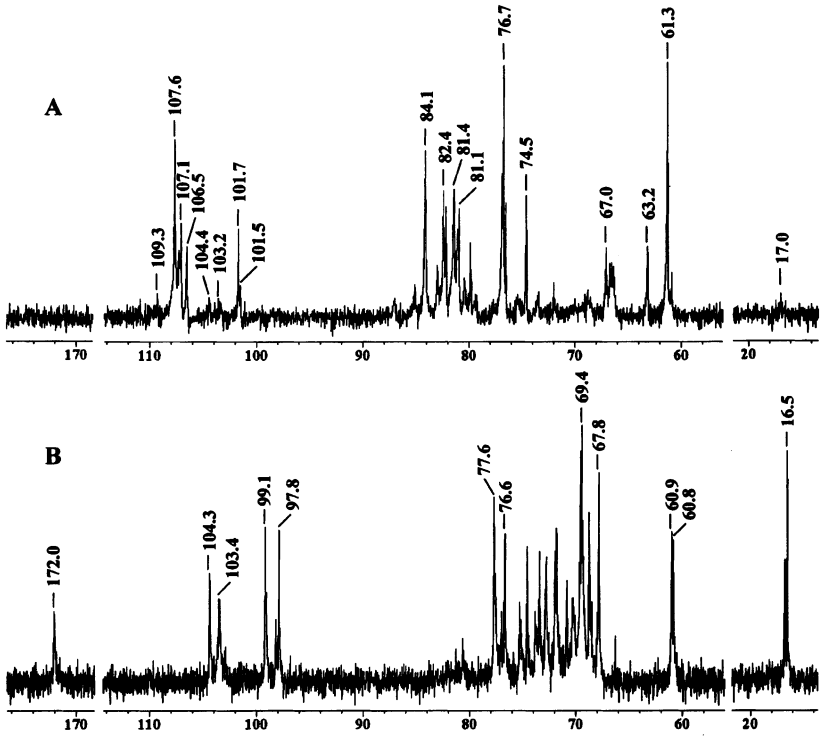
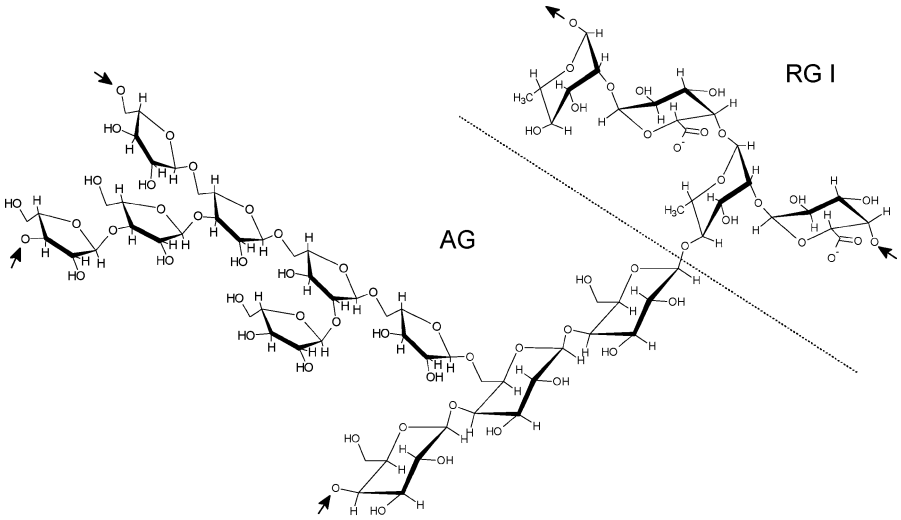


Figure 2. ^{13}C NMR spectra of AG (A) and AG-HR (B): solvent D_2O, at 50 °C, numerical values are in δ, ppm.

Chart 1



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C-4 of some of the rhamnosyl units, as shown in **1**.

M. ilicifolia is a popular medicinal plant cultivated in southern Brazil and neighboring countries, and its leaves are used widely as a tea infusion for treatment of stomach problems. On drinking such infusions, not only secondary metabolites, but also a great variety of primary metabolites, including polysaccharides, are ingested. On ^{13}C NMR examination of the infusion, many C-1 signals appeared arising from component polysaccharides, including those from α-Ara*f* and *β*-Gal*p* units, that should correspond to the arabinogalactan characterized.

The present investigation has led to the structural determination of a polysaccharide belonging to a class of polymers known for their biological activity.[9−11] We now intend to isolate and characterize other polysaccharides from plants, which are used locally as folk medicines.

Experimental Section

General Experimental Procedures. All extracts (see below) were evaporated at <40 °C under reduced pressure. The centrifugation conditions were 10 000 rpm for 15 min, at 25 °C. Uronic acid contents were determined according to the colorimetric method of Filisetti-Cozzi and Carpita, using *m*-hydroxydiphenyl.[20] The sample (100 mg) was carboxy-reduced via the carbodiimide ester reduced by sodium borohydride, as described by Taylor and Conrad.[21] Homogeneity and molar mass were determined by high-performance size-exclusion chromatography (HPSEC-MALLS), using a Waters 510 HPLC pump, at 0.6 mL/min, with four gel permeation Ultrahydrogel columns in series with exclusion sizes of 1×10^6, 4×10^5, 8×10^4, and 5×10^3 Da, using a refractive index detector. The eluent was 0.1 mol/L aqueous $NaNO_3$ with 200 ppm aqueous NaN_3. Samples, previously filtered through a membrane (0.22 μm; Millipore), were injected (250 μL loop) at a 2 mg/mL concentration. The specific refractive index increment (d*n*/d*c*) was determined for the fraction eluted with 0.1 M NaCl. The sample was dissolved in 50 mM $NaNO_3$ in five increasing concentrations, ranging from 0.2 to 1.0 mg/mL. Results were processed with software provided by the manufacturer (Wyatt Technologies). To analyze alditol acetates, obtained on successive hydrolysis, $NaBH_4$ reduction, and acetylation, the samples were examined by GC−MS, using a Varian model 3300 gas chromatograph linked to a Finnigan Ion-Trap, model 810 R-12, mass spectrometer on a DB-225 capillary column (30 m × 0.25 mm) and helium as carrier gas. The analyses were carried out from 50 to 220 °C at 40 °C/min, maintaining the temperature at 220 °C until the end of analysis (18 min). The partially *O*-methylated alditol acetate derivatives analyzed by GC−MS were carried out from 50 to 215 °C at 40 °C/min, maintaining at 215 °C for 31 min. ^{13}C NMR spectra were obtained using a 400 MHz Bruker model DRX Avance spectrometer with a 5 mm inverse probe at 50 °C in D_2O solution. Chemical shifts of the samples are expressed in ppm (δ) relative to acetone at δ 30.2.

Plant Material. Leaves of *M. ilicifolia* Mart. ex Reissek (Celastraceae) (118 g), collected in the region of Curitiba (Southern Brazil) in July 2001, were donated by Central de Produção e Comercialização de Plantas Medicinais, Aromáticas e Condimentares do Paraná Ltda. The plant was identified by Prof. O. Guimarães (Botany Department, Federal University of Paraná, Curitiba, Brazil) and is deposited in the Herbarium of UFPR, as voucher no. 30842.

Extraction and Purification of the Arabinogalactan (AG). The extraction and purification of arabinogalactan from the leaves of *M. ilicifolia* was processed according to Scheme 1. Ground leaves (118 g) were defatted with $CHCl_3$−MeOH (2:1 v/v; 0.5 L) at 60 °C for 2 h (× 3). Pigment was removed from the residue I with MeOH−H_2O (4:1 v/v; 0.5 L) at 80 °C for 2 h (× 2). The residue II was extracted with H_2O (0.5 L) at 100 °C for 3 h (× 2). Finally, residue III was extracted with 2% aqueous KOH (0.5 L) at 100 °C for 2 h (× 3) in the presence of a trace of $NaBH_4$. The alkaline extract (23.6 g, 20% yield) was neutralized with HOAc, dialyzed against water, and freeze-dried. This fraction was purified by a freeze−thawing process carried out on the supernatant and repeated until a precipitate no longer appeared. The precipitate (16.0 g, 13.6% yield) was isolated by centrifugation, and the supernatant (7.6 g, 6.4% yield) of this process was chromatographed using fast-flow DEAE-Sepharose, eluting successively with water (1.8 g, 1.5% yield) and 0.1 M (2.7 g, 2.3% yield), 0.5 M (1.7 g, 1.4% yield), and 2.0 M NaCl (0.8 g, 0.7% yield).

Monosaccharide Analysis. Monosaccharide components and their ratios were determined after hydrolysis of the polysaccharide (5 mg) with 2 M TFA (1.5 mL) at 100 °C for 8 h. The solution was evaporated to dryness and the residue dissolved in water (0.5 mL) to which $NaBH_4$ (2 mg) was added. After 18 h, HOAc was added, the solution evaporated to dryness, and the resulting boric acid removed as trimethyl borate by repeated evaporations from MeOH. Acetylation was carried out with Ac_2O−pyridine (1:1, v/v; 2 mL) at room temperature for 12 h, and the solution was added to excess ice−water, which was extracted with $CHCl_3$. This was evaporated to dryness at room temperature to give alditol acetates,[22,23] which were analyzed by GC−MS and identified by their typical retention times and electron impact profiles.

Methylation Analyses. The polysaccharides (5 mg) were methylated according to the method of Ciucanu and Kerek,

using powdered NaOH in Me_2SO−MeI.[24] The methylated derivatives were treated with 3% HCl−MeOH for 2 h at 80 °C, neutralized with Ag_2CO_3, and then hydrolyzed with 1 M H_2SO_4 at 100 °C for 8 h. The resulting mixture of *O*-methyl aldoses was neutralized with $BaCO_3$, filtered, reduced with $NaBH_4$, and acetylated as described above to give a mixture of partially *O*-methylated alditol acetates, which were analyzed by GC−MS. The resulting partially *O*-methylated alditol acetates were identified by their typical retention times and electron impact profiles.[25]

Partial Acid Hydrolysis. AG (1 g) was partially hydrolyzed with 0.2 M TFA (50 mL) at 100 °C for 2 h. The mixture was evaporated to dryness and redissolved in water (10 mL), and the resistant polysaccharide (AG-HR, 120 mg, 12.0% yield) was precipitated with excess acetone (30 mL), centrifuged, and freeze-dried.

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