Seed Gum of *Stryphnodendron barbatiman* (Barbatimão)

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ABSTRACT

Stryphnodendron barbatiman (barbatimão) is a native tree that is found throughout the "Cerrados," a region of Central Brazil. Plant seeds, on water extraction, furnished 28 g% galactomannan (dry-weight basis), the monosaccharide composition of which (galactose to mannose ratio, 1.0:1.5) fits in the legume heteromannan group. This seed gum, after Sevag deproteinization, still retained 6 g% of associated protein and had a molecular weight of about 1.8 MD on gel filtration. A high intrinsic viscosity (1300 cP) was observed for the polysaccharide sample obtained after reflux of the crushed seeds in 80% aqueous methanol.

Index Entries: *Stryphnodendron barbatiman;* galactomannan; seed gum; viscosity.

INTRODUCTION

Galactomannans are the energy-reserve polysaccharides in seeds of endospermic leguminous plants. They are notably used as additives in food, cosmetics, oil-well drilling muds, mining, and explosives. These polymers generally consist of a main chain of (1→4)-linked β-D-mannopyranosyl residues, most of which are substituted at 0-6 with single-unit

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α-D-galactopyranosyl side-chain residues. The ratio of galactose to mannose residues depends on the plant source and can range from 1.0/1.0 to 1.0/5.6. Two galactomannans are in large-scale commercial production: guar gum from *Cyamoposis tetragonolobus* and locust bean gum from *Ceratonia siligua*. Their respective monosaccharide ratios are 1.0/1.6 and 1.0/3.3 (*1,2*).

Polysaccharide dimensions and molecular shape are important in relation to the field of polymer science. In the case of heteromannans, an understanding of two kinds of molecular associations that are present is of paramount importance. These are (a) the interaction of similar chains (tertiary structure) caused by the relatively rigid (1→4)-β-D-mannopyranan backbone that leads to high viscosities of dilute solutions and (b) the interaction between dissimilar chains (quaternary structure) that results in the formation of elastic gel combinations with such polysaccharides as carragenan, agar, and xanthan gum. This occurs when a low galactose content is present in the galactomannan. Consideration of these properties and parameters can elucidate that biological function of the polysaccharide as well as the increase of their applications in industry (*3,4*). Rheological properties characterize the behavior of polymeric systems facing stress or deformation. They determine the relationships among stress, deformation, and deformation rate (*5*). Data obtained at various temperatures and different deformation regimens give valuable information on the properties of polysaccharide molecules, such as basic structure and structural transitions (*6*), and on, their importance for the selection of processing and operational alternatives. Presently, only locust bean and guar gum, among the viscous polysaccharides of the heteromannan group, are extensively used. However, many other galactomannans offer interesting physiochemical properties and, since there is a constantly increasing demand for plant gums, new sources are under continuing investigation (*7*). This report describes some properties of the galactomannan obtained from the seeds of *Stryphnodendron barbatiman,* which is a native leguminous tree found throughout one of the largest areas of Central Brazil, the "Cerrados."

MATERIALS AND METHODS

Polysaccharide Source

Seeds of *S. barbatiman* were collected at the Estaçao Experimental de Paraguaçu Paulista of the Instituto Florestal de São Paulo.

Polysaccharide Isolation (*see* Flow Diagram, Fig. 1)

Crushed seeds (100 g) were extracted with 9/1 (v/v) benzene-ethanol in a Soxhlet apparatus for 16 h, the residual mass being twice refluxed (4 h cycle) with 4/1 (v/v) methanol-water. Extracted lipophilic and low-mol-wt

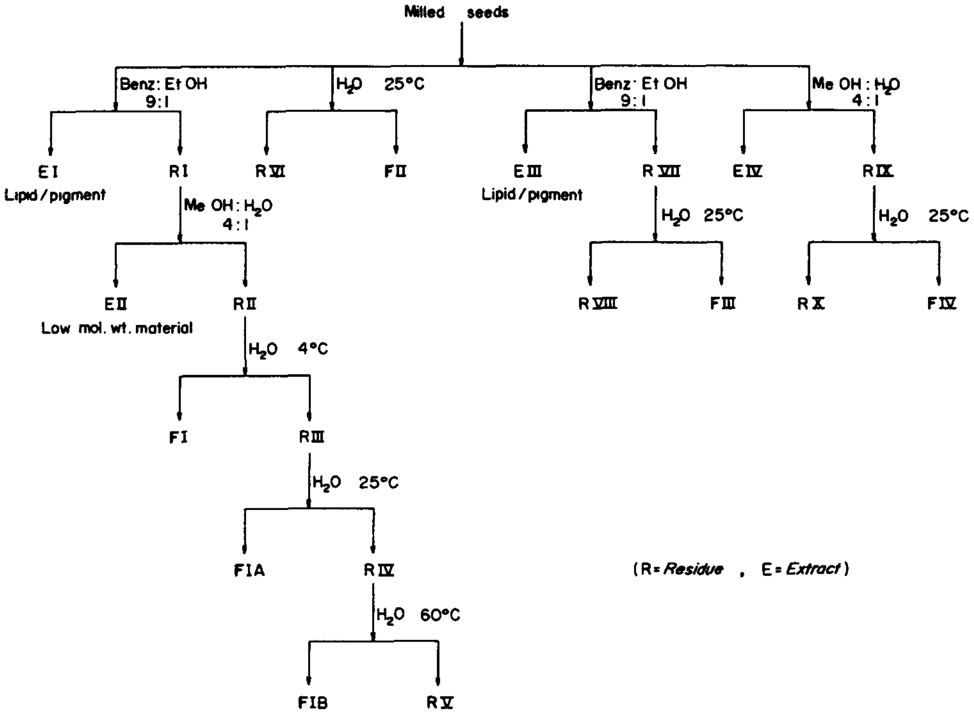

Fig. 1. Flow diagram of the isolation of galactomannans fractions F-I to F-IV from seeds of *S. barbatiman.*

material was discarded, and extraction was continued with water (2 L; 4°C) for 24 h with constant mechanical stirring. The insoluble residue was submitted to two additional aqueous extractions, raising the temperature to 25 and 60°C, respectively. Separate extracts were concentrated at 40°C under reduced pressure, 2 vol of ethanol were added, the precipitated polysaccharide was filtered and washed with ethanol, and the collected fractions designated F-I, F-IA, and F-IB, respectively. Since rheological properties depend in part on the extraction procedure, some variations were carried out, namely

1. Direct aqueous extraction of the crushed seeds at 25°C, omitting the organic solvent pretreatments (F-II),
2. The same procedure preceded only by the benzene-ethanol step for lipid/pigment removal (F-III), and
3. The same procedure with a methanol-water step instead of pretreatment with the organic-solvent mixture (F-IV).

Polysaccharide Analyses

Total carbohydrate and protein were assayed by the phenol-sulfuric acid and the modified Lowry methods (*8,9*). Total acid hydrolyses were performed with 1*M* trifluoroacetic acid at 100°C for 5 h and the released monosaccharides converted into alditol acetates (*10*). Gas-liquid chromatography (GLC) was carried out isothermically in a model 2440 Varian chromatograph at 190°C using a 3% OV-225 column (0.15 id×200 cm; gas Chrom Q support), with nitrogen as the carrier gas (40 mL/min) and a FID* detector. Paper chromatography of mono- and oligosaccharides was performed on Whatman no. 1 filter paper with benzene-butanol-pyridine-water (1/5/3/3; v/v; upper phase). Sugars were detected using the alkaline silver nitrate reagent (*11*). ^{13}C-Nuclear magnetic resonance (NMR) spectroscopy was performed with a Brucker AC 300 instrument.

Gel Filtration of Polysaccharide Fractions

A Sepharose CL-4B chromatographic column (45×1.8 cm) was calibrated with standard dextrans (mol wt range 0.15, 0.26, 0.5 and 2.0 MD; Sigma Chemical). The void volume was determined with the 40-MD dextran. Polysaccharide samples (0.5 mL; 3.0 mg/mL solution) were applied and eluted with water or 7*M* urea. One-milliliter fractions were collected and their absorbance monitored at 280 nm (directly for protein) and 490 nm (after the phenol-sulfuric reaction) for carbohydrate.

Polysaccharide Purification

Polysaccharide "deproteinization," with the removal of non-covalently associated material, was carried out on polysaccharide fractions F-I and F-II (aqueous, 3 g% solutions) using three sequential shaking cycles with a 5/1 (v/v) mixture of chloroform-butanol as proposed by Sevag and described by Staub (*12*). Galactomannan fraction F-II was further purified by means of copper-complex formation as follows: A 25 mg/mL solution of a deproteinized sample was poured into an excess of Fehling solution (*13*). The resulting insoluble complex was removed by centrifuging, decomposed with the required amount of 1*M* hydrochloric acid, and precipitated with an excess of ethanol.

Viscosimetry Measurements

Determinations were carried out in a Ostwald viscosimeter at 25°C. Intrinsic viscosity $[\eta]$ was obtained by plotting the reduced viscosities $[\eta_{red}]$ against polysaccharide concentrations. The influence of pH and additives was evaluated by repeating the viscosimetric measurements after the inclusion of citric, fumaric, or lactic acid (final pH range, 2.5–4.0) or sodium citrate (final pH range, 6.0–8.0).

RESULTS AND DISCUSSION

Crushed, defatted seeds of *S. barbatiman,* free of low-mol-wt compounds, were found to be a good galactomannan source. Successive aque-

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*Flame Ionization Detector.

Table 1
Monosaccharide Composition of the Polysaccharide Fractions
Obtained from the Seeds of *Stryphnodendron barbatiman* (bartatimão)

Fraction	°C[a]	Yield, g%[b]	Ara	Xyl	Gal	Man
			Monosaccharide composition			
F-1[c]	4	16	1.5	0.9	38.7	58.9
F-1A	25	8	3.9	1.5	37.5	57.1
F-1B	60	4 (28)[d]	8.4	3.5	34.5	52.6
F-II[e]	25	26	—	—	40.0	60

[a] Extraction temperature.

[b] Dry-wt basis.

[c] Further analyses were performed using F-I with a protein content reduced from 12 to 6 g% by partial deproteinization using the Sevag method. The partially purified galactomannan had an optical rotation $[\alpha]^{25}{}_D = +93.7$ (0.32 g%, water).

[d] Overall yield.

[e] After Sevag and Fehling treatments.

ous extractions at 4, 25, and 60°C gave rise to an overall recovery of 28 g% of polysaccharide (dry-wt basis; Table 1). The largest polysaccharide yield occurred in the cold extraction (F-I; 57%), and this fraction also had the lowest content of minor monosaccharide components (arabinose, xylose). One-half and one-quarter of this yield was obtained in the further cycles, respectively. The further fractions (F-IA and F-IB) had higher contents of arabinose and xylose (5.4 and 11.9%, respectively). In this way, the combined mannose and galactose content dropped from 97.2 to 87%, but, more important, the ratio between the main monosaccharide components remained unchanged (1.0/1.5). A polysaccharide preparation was then obtained at 25°C, without the organic-solvent pretreatments (F-II). Its yield (26 g%) and protein content after Sevag deproteinization (6 g%) were almost equivalent to that obtained using the overall-temperature-gradient procedure. After repurification by copper-complex formation, this fraction was homogeneous (galactose-mannose, 40/60; Table 1), clearly indicating that the minor monosaccharides, previously seen upon total hydrolysis, arose from a contamination of cell-wall hemicellulosic material, most probably an arabinoxylan. Purified F-II was analyzed by ^{13}C-NMR, giving rise to a spectrum typical for a galactomannan structure (*14–16*), and confirmed the monosaccharide ratio as calculated from C-i signal areas of the expected α-D-galactopyranose and β-D-mannopyranose units.

Galactomannan fractions F-I and F-II were analyzed by gel filtration on a calibrated column of Sepharose CL-4B. A partially deproteinized F-I sample (Sevag treatment only) was eluted as a single peak with water, and a mol-wt determination plotting K_{av}* against the reference dextrans' mol-wt log) indicated about 1.8 MD (Fig. 2). The eluted material was monitored for carbohydrate and protein. When 7*M* urea was used as the eluant, part of the protein was dissociated, indicating that complete removal of

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*K_{av} Distribution Coefficient.

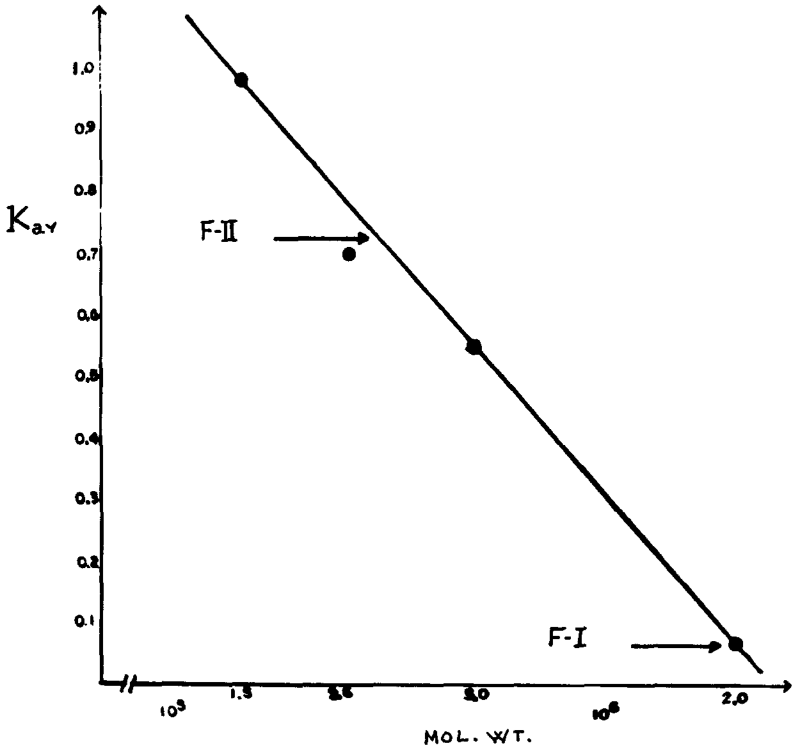

Fig. 2. Molecular-weight determination for *S. barbatiman* seed galactomannan fractions. (Column calibration and conditions for F-I and F-II fraction extractions and treatments are described in the text. The standards used were dextrans (Sigma Chemical Co.) with mol wt 0.15, 0.26, 0.5, and 2.0 MD).

protein was not achieved using the Sevag method. However, the carbohydrate component was eluted as a single peak coincident with the residual protein, suggesting a covalent linkage with the galactomannan. Such covalent linkages have been reported as controversial by other authors (*17*). On the other hand, for the extensively purified fraction F-II, under the same column operational conditions, a mol wt of 0.28 MD was obtained, thus indicating that alkaline processing (Fehling reagent) may cause molecular dissociation or partial degradation of the native polysaccharide. The alkaline degradation of neutral branched polysaccharides has been reported. Degradation of (1]4) linked and 0-6 single-unit-substituted hexopyranans proceeds with the formation of 0-glycosylsaccharinic acids from the branched residues. Such degradations, for instance, were seen in guar gum (*18*), the structure of which is similar to the barbatiman-seed galactomannan.

The viscosity values of galactomannan fractions F-I–F-IV were quite different. Partially deproteinized fraction F-I (the polysaccharide extracted at 4°C after double-cycling of organic solvents under reflux) had an intrinsic viscosity of 1300 cP (Fig. 3), which is comparable to that of guar

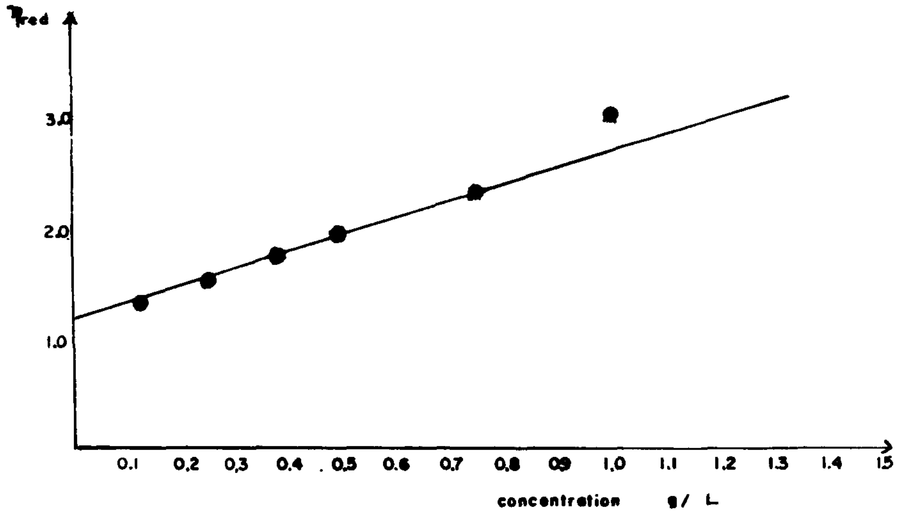

Fig. 3. Viscosimetry on *S. barbatiman* seed galactomannan fraction F-I. [Experimental conditions as explained in the text; cP = (L/g)·10^{-3}].

gum. The influence of pH and temperature variation on viscosities of the same sample were also assessed. In the tested pH ranges (*see* Materials and Methods section), no influence on the viscosity was observed as a result of the inclusion of each of the four additives (organic acids and the sodium salt of citric acid). This agrees with the lack of charged groups in galactomannan structure and with a diminished contribution of the minor protein content to the overall rheological behavior, at least in terms of pH. Concerning the temperature effect, assays were carried out from 10 to 55°C. The polysaccharide sample underwent some reduction (ca. 20%) in the relative viscosity with the temperature increase in this range (results not shown). The intrinsic viscosity was also determined on purified F-II, and, unexpectedly, it dropped to 27 cP. This may be a result of the two different conditions employed to isolate this fraction (*see* Fig. 1): (a) the omission of both organic-solvent pretreatments and (b) the additional strong alkaline medium used in the copper complex formation for complete protein and/or hemicellulose removal. In order to determine the origin of this dramatically negative effect, crude F-II (that is, galactomannan obtained without pretreatment) was investigated without the Sevag and Fehling treatments. Again the same very low intrinsic-viscosity value was obtained. Since the extraction at 25°C did not cause any significant viscosity loss, it is clear that organic-solvent pretreatment plays a major role in the rheological properties of the final product. This hypothesis was confirmed when analyzing fractions III (isolated by hot benzene-ethanol pretreatment) and IV (isolated via hot aqueous-methanol pretreatment): their respective intrinsic viscosity values were 122 and 1270 cP, thus correlating with fractions II (no organic solvent pretreatment)

Table 2
Viscosimetric Data for *S. barbatiman* Seed Galactomannan Fractions

	Fractions (extraction temperature, °C)			
	I (4°C)	II (25°C)	III (25°C)	IV (25°C)
Pretreatments, reflux				
Benzene–ethanol	+	–	+	–
Methanol–water	+	–	–	+
Posttreatments				
Sevag	+	+	–	–
Fehling	–	+	–	–
Intrinsic viscosity, cP[a]	1300	27	122	1270

[a] For experimental details, *see text*

and I (both organic-solvent pretreatments). A summary of the treatments and the resulting viscosimetric data are compiled in Table 2. Reflux with the polar methanol–water mixture is thus the significant step for modifying the native galactomannan behavior to a high-viscosity state. Whether this effect results from the removal of some active low-mol-wt component(s) or, more probably, provokes changes in the galactomannan intra- and/or interchain interactions (secondary and/or tertiary structures) is a matter requiring further clarification. Work in progress is dealing with this point. The experimental approach conducted herein revealed a valid option for viscous polysaccharide processing, taking into account that most, if not all, studies on rheological properties of galactomannans are based on commercial products, because of their availability (*19,20*).

CONCLUSIONS

Stryphnodendron barbatiman seeds can be considered as a potential source for production of viscous polysaccharide, since the plant is ubiquitous in one of the largest tropical areas of Brazil. Galactomannan with useful rheological properties can be obtained when the appropriate extraction process is used. In terms of the highest viscosity presently observed, pretreatment of seeds with hot aqueous methanol followed by aqueous extraction at 25°C or higher temperatures is a valid procedure.

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