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GALACTOMANNANS AND ARABINANS FROM SEEDS OF CAESALPINIACEAE

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Key Word Index—*Schizolobium parahybae*; *Schizolobium amazonicum*; *Cassia fastuosa*; Caesalpiniaceae; chemotyping; seed; polysaccharides; galactomannan; arabinan

Abstract—*Schizolobium parahybae* and *S. amazonicum* seeds yielded galactomannans with identical 3.0:1 Man:Gal ratios and with the same D-galactose distribution along the main chain. Although the galactomannan from seeds of *Cassia fastuosa* showed the same Man:Gal ratio, its fine structure differed significantly from that of the two *Schizolobium* species as shown by the analysis of oligosaccharides (DP 2 to 6) obtained by partial acid hydrolysis. Seed coats of *S. parahybae* and *S. amazonicum* furnished similar unusual neutral linear α-L-arabinofuranan (1→5) linked, as determined by methylation analysis, optical rotation and ^{13}C NMR spectroscopy. On the other hand, *C. fastuosa* seed coats furnished two acidic arabinans. These results in terms of using galactomannans and arabinans in chemotyping, support the suggestion of Rizzini that *S. parahybae* and *S. amazonicum* are not different species. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Endosperms of leguminous seeds have thickened cell walls with massive deposits of galactomannans. They are storage polysaccharides with a (1→4)-β-linked-D-mannan backbone, which carries single-unit (1→6)-α-linked-D-galactosyl branches, and have commercial importance [1]. It has been suggested that the yield and mannose:galactose ratio (Man:Gal) of such galactomannans could be used as markers in the taxonomy of the families of Fabales (Leguminosae) [1–4]. Man:Gal ratios between 1.0:1 (high Gal) and *ca* 5:1 (low Gal) can occur [1]. In general, low galactose galactomannans are obtained from the seeds of species belonging to the more primitive Caesalpiniaceae (Leguminosae Caesalpinoideae) and medium to high galactose galactomannans from more recent Fabaceae (Leguminosae Faboideae) [3]. However, galactomannans from seeds of different botanical sources can have identical degrees of galactose substitution but with differences in the statistical distribution of galactose residues along the mannan backbone [5].

Schizolobium parahybae (Vell.) Blake (guapuruvu) [6], previously called *Schizolobium parahybum*, and *Schizolobium amazonicum* Ducke (pinho cuiabano) are native Caesalpiniaceae species which grow in southern Brazil and the Amazon region, respectively [7]. They have been considered to be identical by Rizzini [8], although no synonym was found in the Index Kewensis [9]. Isolated endosperms from both species yielded *ca* 50% of galactomannan with identical 3.0:1 mannose to galactose ratios [10, 11]. The polysaccharides have closely related fine structures and D-galactose distribution along the main chain, as shown by the pattern of oligosaccharides obtained on partial acid hydrolysis [11].

On the other hand, in a preliminary investigation, we isolated a linear (1→5) linked α-L-arabinofuranan from the seeds of *S. parahybae* [12]. Its structure contrasts with the highly branched arabinans previously found in cell walls of roots [13, 14], seeds [15], pollen tubes [16], bark [17] and fruit [18, 19] of various species, which are generally associated with pectic material [20].

Arabinans have received less attention than galactomannans and could also be helpful in chemotyping. Recently, fat-mimetic properties were described for enzymatically debranched arabinans [21]. Chemical and NMR analysis suggest that it is linear, as is the polymer obtained from material precipitated from apple juice concentrate, and which may have been formed from a more complex polymer by partial enzymolyses [22]. The ability of the linear arabinan to match the rheological properties of high-fat products has resulted in a considerable number of opportunities for its use in fat replacement. It may also be employed in the food industry and has potential

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practical applications in cosmetics, pharmaceuticals and toiletries [21]. The fat-mimetic properties might be attributed to its polymorphism and tendency to form microcrystals in the solid state [23].

We now report the characterization of a neutral linear arabinan from *S. amazonicum* and two acidic arabinans from *Cassia fastuosa* Willd [24] which is also a member of the Caesalpiniaceae, in parallel with a comparison of the galactomannan of the latter and those of *Schizolobium* species.

RESULTS AND DISCUSSION

In previous investigations, we characterized the galactomannans from the seeds of *S. parahybae* and *S. amazonicum* which were obtained by water extraction at 4° [10, 11]. They had the same Man:Gal ratio (3.0:1). When the residues of cold aqueous extractions were treated sequentially with water at higher temperatures (25° and 60°), lower yields of less substituted galactomannans were obtained (4.0:1 and 4.3:1, respectively). The seeds of *C. fastuosa* also furnished, by cold aqueous extraction, a galactomannan with Man:Gal ratio 3.0:1, as determined by GC of derived alditol acetates. When water at 25° and 60° were used for extraction, galactomannans with Man:Gal ratios of 4.0:1 were obtained [25].

The ^{13}C NMR spectrum of the galactomannan from *C. fastuosa* obtained with cold water extraction confirmed a main chain of units of (1→4)-linked β-D-Man*p* substituted at O-6 by single-unit side-chains of α-D-Gal*p*. The Man:Gal ratio of 3.1:1 was also confirmed by the areas of the signals at δ 101.2 (C-1 of β-D-Man*p* residues) and that at δ 100.0 (C-1 of α-D-Gal*p* units). The CH_2 atoms (δ 61.7 and δ 62.3) from unbranched Man and Gal units respectively, are well documented [26]. The C-4 (Man) pattern of the lines showed clear splitting with different intensities of the *O*-substituted resonances depending on the substitution of the nearest neighbour (Fig. 1). The peak at the lowest field δ 78.0 can be assigned to two-substituted contiguous D-Man*p* units and the peak at high field (δ 77.5) originates from contiguous unsubstituted D-Man*p* residues. The intermediate peak (δ 77.8) represents superimposition of signals originating from diads in which only one of the two D-Man*p* units is substituted [11, 27].

Galactomannan from *C. fastuosa* (3.0:1) was submitted to partial acid hydrolysis under the same conditions used for galactomannans from *S. amazonicum* and *S. parahybae* (both 3.0:1) [11]. Table 1 shows the oligosaccharides obtained by mild acid hydrolysis from the three galactomannans. The yield of each DP determined by GPC was similar for the three polymers. The major fraction for each of the three galactomannans was the monosaccharide (*ca* 34%). Identification of the oligosaccharides with DP-2 to 6 was performed by correlation of their retention time on HPLC in the light of their ^1H and ^{13}C NMR analyses. As previously reported [11], on analysis of oli-

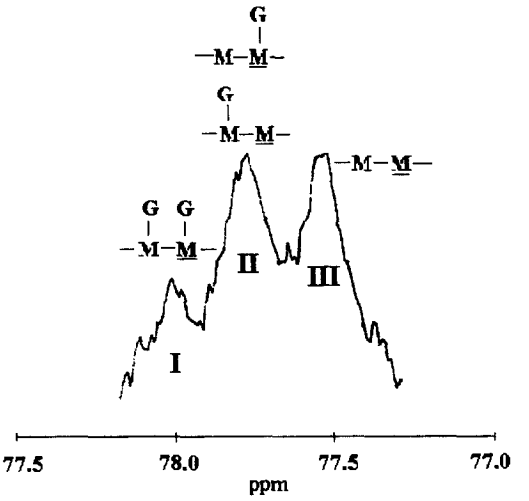

Fig. 1. ^{13}C NMR spectral region of C-4 of D-Man*p* units in *C. fastuosa* seed galactomannan. Chemical shifts given are for the underlined M (Man) residues.

Table 1. Oligosaccharides with DPs of 2-6 produced by partial acid hydrolysis of the galactomannan of *C. fastuosa* (*C.f.*) compared with those of *S. parahybae** (*S.p.*) and *S. amazonicum** (*S.a.*).

			Galactomannan source		
DP	Yield†	Compound‡	*C.f.*	*S.p.*	*S.a.*
DP 2	15	G-M	37.5	17.0	18.0
		M-M	62.5	83.0	82.0
DP 3	14	G^1M_2 §	39.0	14.5	15.1
		G^2M_2	24.2	30.8	32.5
		M_3	36.8	54.6	52.4
DP 4	14	$G^{1,2}M_2$	22.8	10.9	10.5
		G^1M_3	40.2	32.4	31.5
		G^2M_3	16.3	24.8	27.8
		M_4	20.7	31.8	30.1
DP 5	12	$G^{1,2}M_3$ ‖	20.9	9.4	7.6
		$G^{1,3}M_3$	19.3	17.9	15.7
		G^1M_4	39.3	45.0	42.0
		G^4M_4	8.7	10.9	16.4
		M_5	11.7	16.7	18.2
DP 6	11	1	31.8	17.7	23.2
		2	19.0	21.8	17.3
		3	36.7	43.5	39.9
		4	6.8	8.4	10.2
		5	5.7	8.4	9.3

*Ganter *et al.* [11].

†Yield (average value of each DP of *C.f.*, *S.p.* and *S.a.*) determined by GPC; DP 1 ~ 34%.

‡By HPLC.

§G^1M_2 compound correspond to M—M. The numerical subscript corresponds to the number of D-Man*p* units and the supercript corresponds to the position of the substituent.

‖This pentasaccharide may be $G^{1,2}M_3$ or $G^{2,3}M_3$.

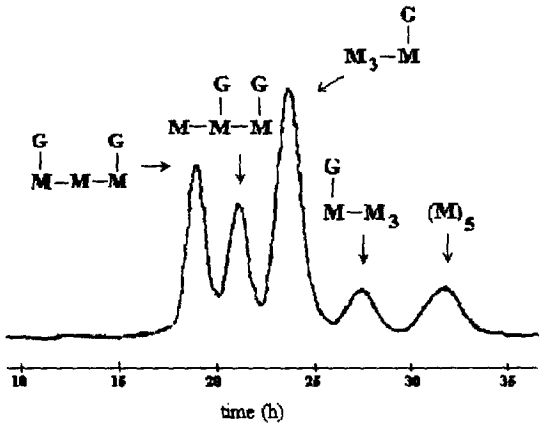

Fig. 2. HPLC chromatogram of the DP-5 series of galactomannan from *C. fastuosa*.

gosaccharide distribution, those from *S. amazonicum* and *S. parahybae*, with each isolated degree of polymerization (2 to 6) were similar. On the other hand, in spite of the same Man:Gal ratio, the distribution of the oligosaccharides in *C. fastuosa* galactomannan is quite different (Table 1); Fig. 2 shows the HPLC profile of the DP-5 series of the galactomannan from *C. fastuosa*. On comparison of the Man homo-oligosaccharides with DPs of 2–6 (Table 1), it can be seen that their relative proportion is lower than that found for *Schizolobium* species. This is in agreement with the resonances of C-4 of the D-Man*p* residues in their ^{13}C NMR spectra. For *S. amazonicum*, the proportion of the peak areas of I, II and III is 3%, 54% and 43% [11] and for *C. fastuosa* 6%, 57% and 37%, respectively (Fig. 1). Thus, peak III, corresponding to two contiguous unsubstituted D-Man*p* residues is less intense for *C. fastuosa* galactomannan. These results indicate that the unsubstituted regions along the main chain are less abundant in the galactomannan of *C. fastuosa*, when compared to that of the *Schizolobium* species. Consequently, the fine structure of galactomannan from *C. fastuosa* differs significantly from those of *S. amazonicum* and *S. parahybae*. Dea *et al.* [5] showed that galactomannans from distinct botanical sources with the same Man: Gal ratio, produced a different array of oligosaccharides when treated with β-endo-D-mannanase of *Bacillus subtilis*.

The seed coats of *S. amazonicum* and *C. fastuosa* were defatted and then extracted with water at 25° and at 80°. The residues arising from aqueous extractions were treated with 2 M NaOH by the procedure of Whistler and Feather [28], yielding HA and HB. The monosaccharide composition of the seed coat fractions for *S. amazonicum* and *C. fastuosa* are shown in Table 2 and Table 3, respectively. Although other monosaccharides were present, arabinose was the predominant component of all the isolated fractions. As xylose, mannose, galactose and glucose occurred in both the aqueous and alkaline extracts, they are probably components of different polysaccharides from the seed coat cell walls and not contaminants from the endosperm. For *S. amazonicum*, fraction S_3, extracted with 2 M NaOH, the only monosaccharide component present was arabinose (arabinan S_3).

However, for *C. fastuosa*, the hot water (C_2, Table 3) and 4 M alkaline extractions (C_5) afforded polymers in which arabinose was the only neutral sugar; colorimetric data showed the presence of 20% and 7% of uronic acid, respectively. The presence of uronic acid was confirmed by characteristic absorption at 1740 cm^{-1} for C=O group in their IR spectra as well as by PC. In contrast, uronic acid was not detected in polysaccharide S_3.

The three arabinans (S_3, C_2 and C_5) were homogeneous on Sephacryl S-300 column chromatography and were then characterized. The arabinan from *S. amazonicum* (S_3) had a M_r of 15100, $[\alpha]^{25}_D - 130°$; methylation products (Table 4), were 2,3-Me_2-Ara (99%) and 2,3,5-M_3 Ara (1%) corresponding to the non-reducing end. Its ^{13}C NMR spectrum was ident-

Table 2. Yields and neutral sugar composition of polysaccharides obtained from the seed coats of *Shizolobium amazonicum* by water and alkali treatments.

			Monosaccharide‡ (mol %)					
F*	Extractant	Yield† g %	Rha	Ara	Xyl	Man	Gal	Glc
S_1	H_2O, 25°	2.5	3.7	43.6	5.3	21.6	20.8	5.0
S_2	H_2O, 80°	0.6	1.4	61.7	5.9	12.4	14.1	4.5
S_3	2M NaOH (HA)	0.2	-	100.0	-	-	-	-
S_4	2M NaOH (HB)	0.9	1.9	25.2	17.8	22.4	21.5	11.2
S_5	4M NaOH (HA)	3.3	tr	57.7	7.7	16.7	5.1	12.8
S_6	4M NaOH (HB)	1.0	tr	13.8	11.3	36.1	14.2	24.5

*Fraction.
†Based on lipid free-seed coats.
‡Determined by GC of derived alditol acetates.
tr. trace

Table 3. Yields and neutral sugar composition of polysaccharides obtained from the seed coats of *C. fastuosa* by water and alkali treatments.

F*	Extractant	Yield† g %	Monosaccharide (mol %)‡					
			Rha	Ara	Xyl	Man	Gal	Glc
C_1	H_2O, 25°	0.8	3.0	38.0	36.4	10.3	11.2	1.1
C_2	H_2O, 80°	5.1	tr	99.0	tr	tr	tr	-
C_3	2M NaOH (HA)	1.5	1.1	56.8	28.8	2.4	7.3	3.5
C_4	2M NaOH (HB)	4.6	2.5	41.5	41.1	3.1	9.2	2.6
C_5	4M NaOH (HA)	2.8	tr	99.0	tr	tr	tr	tr
C_6	4M NaOH (HB)	4.6	7.9	50.3	11.3	6.9	15.6	8.0

*Fraction.
†Based on lipid-free seed coats.
‡Determined by GC of derived alditol acetates.
tr. trace.

Table 4. Methylation analysis of arabinans from seed coats of *S. amazonicum* (arabinan S_3) and *C. fastuosa* (arabinan C_2 and C_5).

Partially *O*-Methylated alditol acetate	mol %		
	Arabinan S_3	Arabinan C_2	Arabinan C_5
2,3,4-Me_3-Ara	-	1.0	-
2,3,5-Me_3-Ara	1.0	7.9	4.0
2,3-Me_2-Ara	99.0	83.2	96.0
3-Me-Ara	-	2.9	-
2-Me-Ara	-	4.2	-
Ara	-	0.7	-

ical to that of *S. parahybae* arabinan, confirming the same α-(1→5) linked arabinofuranan structure [12].

The occurrence of arabinans C_2 and C_5 (Table 3) and the predominance of arabinose in all the isolated polysaccharide fractions suggest for *C. fastuosa*, the presence of a family of arabinosyl polymers, as already described for other species [13, 17, 29].

The M_r for arabinan C_2 was 22900. This is similar to those found in the literature for other arabinans. In contrast, arabinan C_5 has a M_r of 4600; the possibility of degradation by a β-elimination process during alkali extraction was avoided by the presence of $NaBH_4$ during this step. Lower M_r values were also obtained for arabinans from *Sinapis alba* seeds [30] and from stem bark of *Rosa glauca* [15, 18, 29].

Arabinans C_2 and C_5 had negative specific rotations of $[\alpha]_D^{25} -62°$ and $[\alpha]_D^{25} -100°$ (0.5 M NaOH), respectively. Values of specific rotations for all arabinans range from $-178°$ to $-83°$. The lowest value found, $-62°$, for arabinanan C_2 is probably due to the presence of 20% of α-linked uronic acid units.

Methylation analysis of C_5 (Table 4) showed that the main chain is linear, as indicated by 2,3-Me_2-Ara (96%) and the relatively high yield of 2,3,5-Me_3-Ara (nonreducing ends) confirms the low M_r of the polymer.

Arabinan C_2 is branched at position O–2, O–3 and O–2,3 as indicated by the presence of 3-Me-Ara (2.9%), 2-Me-Ara (4.2%) and Ara (0.7%), respectively, by arabinosyl moieties (2,3,5-Me_3-Ara, 7,9%) and probably by uronic acid. Determination of branches by uronic acid was not reliable, probably due to the formation of acid-stable aldobiuronic moieties.

The ^{13}C NMR spectra of the arabinans are in agreement with the results of the methylation analysis. Several authors have reported ^{13}C assignments for branched arabinans [14, 17, 29]. Backinowsky *et al.* [31] obtained a spectrum for a synthetic linear (1→5)-α-arabinan with a M_r of 2000–3000. Based on these assignments and those of the linear α-L-arabinofuraran from *S. parahybae*, the spectra of arabinans C_2 and C_5 were interpreted. The ^{13}C NMR spectrum of arabinan C_5 is similar to that of S_3, with the exception of a small signal in the C-1 region (δ 100.8), which can be attributed to α-linked uronic acid. For arabinan C_2 a strong peak at δ 107.9 can be assigned to C-1 of unbranched (1→5) linked α-L-arabinofuranosyl units, that at 100.8 to the C-1 of 4-*O*-methyl α-D-glucopyranosyl (uronic acid), signals at δ 171.2 and δ 53.3 to C-6 and OCH_3 of 4-*O*-methyl substituted glucuronosyl acid residues, respectively [32]; signals at δ 81.3, δ 81.0, δ 77.2 and δ 67.5 are

Table 5. Characterization of arabinans from three species of the Caesalpiniaceae.

Source	Extractant	$[\alpha]_D^{25}$	M_r	Uronic acid* %
S. parahybae†	2M NaOH or DMSO	-133°	18.000	-
S. amazonicum (S_3)	2M NaOH	-130°	15.100	-
C. fastuosa (C_2)	H_2O 80°C	-62°	22.900	20
C. fastuosa (C_5)	4M NaOH	-100°	4.600	7

*Determined according to ref. [35].
†Zawadzki-Baggio *et al.* [12].

assigned to C-4, C-2, C-3 and C-5, respectively, of (1→5) linked α-L-arabinofuranosyl units. Small signals at δ 106.2 and δ 105.2 are due to C-1 of substituted α-L-arabinofuranosyl units, in agreement with methylation data. Table 5 compares the data for the arabinans.

The presence of an unusual neutral linear arabinan in *S. amazonicum* can be taxonomically important, since it has been found to date only in *S. parahybae* and not in *C. fastuosa*. These results, in terms of using the galactomannans and arabinans in chemotyping, support the suggestion of Rizzini [8] that *S. amazonicum* and *S. parahybae* are not different species. DNA homology tests should be used as final confirmation.

EXPERIMENTAL

General

Polysaccharides were hydrolysed with 1 M TFA (5 hr, 100°). After removal of acid, hydrolysates were reduced with $NaBH_4$ and then acetylated in Ac_2O-pyridine (1:1, 16 hr, at 25°). The resulting alditol acetates were analyzed by GC at 220° (FID and inj. temp. 250°), with a DB-210 capillary column (0.25 mm i.d. × 30 m), film thickness 0.25 μm and N_2 carrier gas. Methylated polysaccharides were hydrolysed with 72% w/w aq. H_2SO_4 (1 hr, 0–4°), following addition of H_2O to a final acid conc of 8% (5 hr, 100°). The soln was neutralized with $BaCO_3$ and the alditol acetates of partially methylated sugars analyzed by GC and by GC-MS on an OV-225 capillary column (0.25 mm i.d. × 30 m) linked to an ion trap, MS operated at 70 eV. The column was programmed from 50° to 220° at 40° min^{-1}. Whatman N° 1 paper and C_6H_6-*n*BuOH-pyridine-H_2O (1:5:3:3) were used for PC; sugars were detected using the alkaline $AgNO_3$ reagent [33]. ^{13}C NMR were recorded at 100 MHz, at 40°, with DDS (sodium 4,4-dimethyl-4-silapentane-1-sulphonate) as int. standard ($\delta = 0$). Samples were dissolved in 0.5 M NaOD in D_2O or D_2O, depending on their solubility in H_2O. Specific rotations were measured at 25° in 0.5 M NaOH. Total carbohydrate was estimated using the PhOH-H_2SO_4 method [34] and uronic acid according to ref. [35].

Plant material

Seeds of *S. parahybae* were collected on the campus of the Universidade do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, and seeds of *C. fastuosa* were collected on the Campus of Universidade Federal do Paraná, Curitiba, Paraná. These species were identified by Prof. Olavo Araujo Guimarães (Departamento de Botânica da Universidade Federal do Paraná). Vouchers specimens are deposited in the Herbarium (UPCB) of the Departamento de Botânica da Universidade Federal do Paraná, under the numbers 8749 for *S. parahybae* and 5904 for *C. fastuosa*. Seeds of *S. amazonicum* were collected at the National Forest of Tapajós, Pará, and supplied by EMBRAPA (Empresa Brasileira de Agropecuária)—CPATU—Belém, Pará, where a voucher specimen is deposited in the Herbarium (IAN), under the number 158478.

Polysaccharide isolation

Whole seeds were boiled in H_2O for 30 min and then kept at 4° for 48 hr until swelling took place. Thereafter, the endosperm, seed coat, and embryo were separated manually. The dry endosperm was milled and submitted to successive extractions at 4°, 25° and 60°, as previously described [10]. Seed coats were milled and treated with C_6H_6-EtOH (2:1) in a Soxhlet. Defatted and air-dried materials were submitted to aq. extraction at 25° and 80°, yielding S_1 and S_2 for *S. amazonicum* or C_1 and C_2 for *C. fastuosa*, respectively. The residues were extracted with 2 M NaOH and $NaBH_4$ (10 mg) at 25° and hemicellulose A (HA) then pptd by acidification to pH 5 with 50% (v/v) HOAc [28] (fr. S_3 for *S. amazonicum* and C_3 for *C. fastuosa*) and hemicellulose B (HB) isolated from the supernatant by pptn. with EtOH (fr. S_4 for *S. amazonicum* and C_4 for *C. fastuosa*). Residues were treated with 4 M NaOH and $NaBH_4$ (10 mg) at 25° and HA and HB then obtained as described above. These procedures furnished frs S_5 and S_6 for *S. amazonicum* and C_5 and C_6 for *C. fastuosa*.

Partial acid hydrolysis

Galactomanannan from *C. fastuosa* was solubilized in H_2O and H_2SO_4 added to a final conc of 25 mM.

The sol was refluxed for 5 hr, neutralized with $BaCO_3$ and filtered. The vol. of filtrate was reduced and EtOH (3 vols) added. The supernatant was conc and the resulting oligosaccharides analyzed by gel permeation chromatography (GPC) and HPLC. GPC of oligosaccharides was effected in a thermostated column (65°; 210 cm × 1.5 cm i.d.) filled with polyacrylamide gel (Biogel P2, 200–400 mesh; BioRad) using dist. H_2O as eluant (flow rate, 35 ml h^{-1}). Oligosaccharides were purified by ion-exchange HPLC on a "Phenomenex–Rezex–RDC–BR–Oligosaccharide" at 50° and/or a Nucleosil 5 μm C-18 column (Societe Françoise Chromato Colonne) with H_2O as eluant. Monosaccharide analyses were carried out on a CHO-682 column (Interchim) eluted with pure H_2O at 85°. Samples were desalted before injection using a mixed resin (Amberlite M 3). RI was used for detection and peak areas were determined by electronic integration.

Gel filtration of arabinan frs S_3, C_2 and C_5

The polysaccharides (1 mg in 0.5 ml of 50 mM NaOH) were applied to a column of Sephacryl S-300 (1.2 × 36 cm) and eluted with 50 mM NaOH. Elution of material was monitored by the PhOH-H_2SO_4 method at 480 nm and the M_rs estimated using a calibration curve prepared using standard dextrans with M_rs of 266000, 72000, 40000 and 17000 (Sigma).

Methylation analysis of fractions S_3, C_2 and C_5

Samples of polysaccharides (20 mg, dried over P_2O_5 in an Abderhalden apparatus) were dissolved in DMSO (2 ml) and powdered NaOH (80 mg) added. After vigorous stirring for 10 min at 25°, MeI (0.5 ml) was added and again stirred for 10 min. The mixts were neutralized with HOAc and extracted with $CHCl_3$ [36]. This procedure was repeated for each sample and the per-*O*-methylated polysaccharides shown to be free of OH groups by IR spectroscopy. Each methylated polysaccharide was hydrolysed with dil. H_2SO_4, as described above, and analyzed by GC and GC-MS.

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