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# Structure of the fucose-containing acidic heteroxylan from the gum exudate of *Syagrus romanzoffiana* (Queen palm)

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**Abstract**

The gum exudate of *S. romanzoffiana* contained a heteropolysaccharide (SRP), which was isolated in 80% yield, contained Fuc, Ara, Xyl, Gal, Glc and uronic acid in a molar ratio of 23:25:41:1:3:8, and had $[\alpha]_D^{25} - 72°$. It was homogeneous with $M_w$ $1.4 \times 10^5$ and $M_w/M_n \sim 1.0$ and formed high viscosity, aqueous solutions. Carboxy-reduction provided neutral material (SRP-CR), having glucose and its 4-*O*-methyl derivative in a molar ratio of ~3:1. Methylation analysis of SRP showed a highly branched structure with non-reducing end-units of Ara*f* (16%), Fuc*p* (10%) and Xyl*p* (12%), 3-*O*- (5%), 2-*O*- (17%), 3,4- (7%) and 2,4-di-*O*-substituted Ara*f* (10%), and 2,3,4-tri-*O*-susbtituted Xyl*p* units (21%). The $^{13}$C-NMR spectrum of SRP was complex and contained at least 8 C-1 signals. The $^{13}$C-NMR spectrum and methylation analysis of Smith-degraded polysaccharide (SRP-SM) were consistent with a (1→4)-linked β-Xyl*p* main chain. Partial hydrolysis of SRP gave rise to α-Glc*p*A-(1→2)-β-[Xyl*p*-(1→4)]$_{0\text{-}1}$-αβ-Xyl*p* and 4-Me-α-Glc*p*A-(1→2)-αβ-Xyl*p*. Milder hydrolysis conditions gave rise a mixture of oligosaccharides that were fractionated by charcoal–Celite column chromatography. The 10% aq. EtOH fraction (OL-10) gave a main GC spot with $R_{Gal}$ 1.2 and minor ones with $R_{Gal}$ 1.07 and 0.78, and was characterized as a mixture of β-Xylp-(1→2)-αβ-Xyl, Fuc*p*-(1→4)-αβ-Xyl*p*, and Fuc*p*-(1→3)-αβ-Ara. Small amounts of fucobiose were also present.


*Keywords:* Queen palm; *Syagrus romanzoffiana*; Gum exudate; Fucose-containing acidic heteroxylan

## 1. Introduction

The classification of complex polysaccharides of plant gum exudates can be based on the structure of their main chains, considering the frequent, high complexity of their side chains (Aspinall, 1969). Most of them contain a (1→3)-linked β-Gal*p* backbone substituted by (1→6)-linked β-Gal*p* side chains, among others (named arabino-galactans Type II) (Fincher, Stone, & Clarke, 1983; Whistler, 1993). Examples of this group are those of *Acacia* spp. (Mimosaceae), which may be used as an aid in chemotaxonomy (Anderson & Dea, 1969). However, relatively few examples of gum polysaccharides having D-Xyl*p* main-chains have been reported. These are from *Sapota achras* (Sapotaceae) (Dutton & Kabir, 1973), *Cercidium australe* (Cerezo, Stacey, & Webber, 1969) and *Cercidium praecox* (Léon de Pinto, Martínez, & Rivas, 1994), the last two species belonging to the family Mimosaceae. Recently, we reported the structural characterization of gum exudate polysaccharides of *Livistona chinensis* (Maurer-Menestrina, Sassaki, Simas, Gorin, & Iacomini, 2003) and *Scheelea phalerata* (Simas, Gorin, Guerrini, Naggi, Sassaki, & Delgobo, 2004), both species belonging to the Arecaceae family. It was shown that they are from a group having a main chain of (1→4)-linked β-Xyl*p* units, defined by Stephen (1983) as 'highly substituted acid β-xylans'. Both of these palm polysaccharides contained, among their complex side-chains, the unusual characteristic of fucosyl units as non-reducing end-units, not reported for gum polysaccharides of other families.

Another palm gum from *Syagrus romanzoffiana* (common name, Queen palm) is now shown to be of the same group. It contained a polysaccharide (SRP) with an identical main chain and 23% of fucosyl units. In order to give further chemotaxonomic weight to this and other chemical structures, SRP was subjected to a more detailed analysis.

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## 2. Materials and methods

### 2.1. Collection of gum exudate and isolation of its polysaccharide (SRP)

The gum exudate from *S. rommazofiana* was collected in Curitiba, State of Paraná, Brazil. This gum exudate formed gels in the presence of water at concentrations higher than 4% (w/v), and it was necessary to resort to a dilute solution (2% w/v) for aqueous extraction. A sample (2.0 g) was stirred overnight in $H_2O$ (100 mL), which gave a dispersion containing insoluble fragments, from which the larger ones were removed by passage through a fine cloth. The filtrate was added to excess EtOH (×3), to give a precipitate (SPR) in 80% yield. This was redispersed in $H_2O$, dialyzed against tap water (24 h), and then freeze-dried. The residue was dissolved in $H_2O$ to which excess EtOH was added, giving rise to a precipitate, which proved to be only sparingly soluble in water.

### 2.2. Investigation of low molecular weight material in the gum exudate

The exudate (30 g) was immersed in $H_2O$ (1.0 L) for 16 h and gum then triturated in a blender. The fine suspension was added to EtOH (3.0 L), the mixture filtered, and the filtrate evaporated to dryness. The residue (1.5% yield) was applied to a charcoal–Celite column (Whistler & Durso, 1950), which was eluted with $H_2O$ to give monosaccharides (127 mg), and successively with aq. EtOH at concentrations of 5% (36 mg), 10% (37 mg), 15% (114 mg), 30% (45 mg) and 50% (89 mg). PC examination of these fractions revealed complex mixtures of oligosaccharides that resisted further efforts to isolate pure samples.

### 2.3. General methods

Specific rotations of polysaccharides were measured at 25 °C in 0.5% (w/v) aqueous solutions, with a Rudolph Res polarimeter (model 589).

Polysaccharides and oligosaccharides were hydrolyzed with M TFA for 8 h at 100 °C to give monosaccharide mixtures. Part of the resulting solution was evaporated and examined using Whatman N° 1 filter paper (solvent: *n*-BuOH-Pyr-$H_2O$, 5:3:3, (v/v)) and the products were detected by the acetone-$AgNO_3$ dip reagent (Trevelyan, Procter, & Harrison, 1950). The remaining part of the hydrolyzed polysaccharide was reduced with $NaBH_4$, acetylated with $Ac_2O$-Pyr (1:1, v/v) for 18 h at 25 °C overnight and the derived alditol acetates were examined by gas liquid chromatography-mass spectrometry (GC-MS). This was performed using a Varian model 3300 gas chromatograph coupled to a Finnigan Ion-Trap (model 810 R-12) mass spectrometer using a DB-225 capillary column (30 m×0.25 mm i.d.) held at 50 °C during injection and then programmed at 40 °C/min to 220 °C (constant). He was the carrier gas.

The protein and uronic acid content of polysaccharide fractions were determined by the colorimetric methods described by Bradford (1976) and Blumenkrantz & Asboe-Hansen (1973), respectively. The 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide method (Taylor & Conrad, 1972) was used for carboxyreduction of SRP to SRP-CR.

### 2.4. Determination of molecular weight ($M_w$) and homogeneity of SRP using HPSEC-MALLS

A size exclusion chromatography (HPSEC) apparatus (Waters, MA, USA) coupled to a differential refractometer (RI) and a Wyatt Technology (Saint Barbara, CA, USA) Dawn-F Multi-Angle Laser Light Scattering detector (MALLS) was used for testing SRP (0.2% (w/v) in $H_2O$). Four Waters' columns, Ultrahydrogel 2000/500/250/120, were connected in series and coupled to refractive index and light scattering detectors. A 0.1 M $NaNO_3$ solution, containing $NaN_3$ (0.5 g $L^{-1}$) was used as solvent.

### 2.5. Partial acid hydrolyses of SRP

The polysaccharide (3.6 g) was dissolved in $H_2O$ (100 mL), and the solution was adjusted to pH 1.0 with M TFA and kept at 100 °C for 1 h. After precipitation with excess EtOH, a polysaccharide (SRP-PH-1; 42% yield) was obtained.

In order to obtain oligosaccharides containing uronic acid, SRP (2.0 g) was partially hydrolyzed in M TFA (40 mL) at 100 °C, for 2 h. The solution was evaporated to dryness and the residue applied to a charcoal–Celite column. This was eluted with $H_2O$ and then with 40% (v/v) aq. EtOH, which gave rise to a mixture (0.69 g) giving PC spots of components with $R_{Lact}$ 0.36, 0.47, 0.52, 0.58 and 0.59 (solvent: *n*-BuOH-Pyr-$H_2O$, 5:3:3, v/v). The mixture was fractionated on Whatman No. 3 filter paper with the same solvent to isolate oligosaccharides with $R_{Lact}$ 0.36 (142 mg), 0.47 (21 mg), 0.52 (18 mg), 0.58 (9 mg) and 0.59 (22 mg).

In order to obtain side-chain oligosaccharides less resistant to partial hydrolysis, SRP (2.0 g) was treated with 0.1 M TFA (100 mL) for 1 h at 96 °C. After EtOH precipitation and filtration, the supernatant was evaporated to a residue (470 mg), which was applied to a charcoal–Celite column, which was successively eluted with $H_2O$ and aqueous EtOH with concentrations of 2% (57 mg), 5% (85 mg), 10% (37 mg), 40% (70 mg) and 50% (16 mg). The 2% eluate contained a major component with $R_{Gal}$ 1.4, which was characterized by $^{13}$C-NMR as free fucose. The 10% fraction was deionized with ion-exchange resin (Amberlite IR-45, $-OH^-$ form) and the product (OL-10; 27 mg) had a major component with $R_{Gal}$ 1.2, with others having $R_{Gal}$ 1.07 and 0.78.

### 2.6. Methylation analysis

Polysaccharides (~10 mg) were methylated by the method of Ciucanu and Kerek (1984). The per-*O*-methylated products were refluxed with 3% MeOH–HCl for 3 h, neutralized with $Ag_2CO_3$ and then hydrolyzed with 0.5 M $H_2SO_4$ at 100 °C for 15 h. After $NaBD_4$ reduction and acetylation with $Ac_2O$-pyridine, the resulting mixture of partially O-methylated was examined by GC-MS using a battery of capillary columns. A DB-225 capillary column (30 m×0.25 mm i.d.), held at 50 °C during injection and then programmed at 40 °C/min to 215 °C (constant temp.), was used for overall identification and quantification. However, using 215 °C as a final temperature, it neither resolved acetates of 2,3,4-$Me_3$-Fuc, 2,3,4-$Me_3$-Ara and 2,3,4-$Me_3$-Xyl, nor those of 2- and 3-Me-Xyl. To resolve these acetates, it was necessary to employ a column of DB-23 (30 m×0.25 mm i.d.) and DB-210 of similar dimensions a final temperature of 215 °C. The 2,3,4-$Me_3$ group was resolved with a DB-23 column and its components had retention times of 7.05 (2,3,4-$Me_3$-Fuc), 7.11 (2,3,4-$Me_3$-Ara), and 7.16 min (2,3,4-$Me_3$-Xyl). The 2-/3-Me-Xyl group were resolved with a DB-210 column and had retention times of 12.37 (2-Me-Xyl) and 12.22 min (3-Me-Xyl).

Oligosaccharides ($R_{Lact}$ 0.52 and OL-10) (~2 mg) were methylated according to Tischer, Gorin, & Iacomini (2002), by dissolving them in a drop of water before addition of $Me_2SO$, MeI was then added followed by powdered NaOH. After vigorous agitation for 18 h, the suspension was acidified (dil. $H_2SO_4$) and then partitioned between $CHCl_3$ and $H_2O$ The organic layer, after washing twice with $H_2O$, contained per-*O*-methylated derivatives were treated with 3% HCl-MeOH for 2 h at 80 °C and neutralized with $Ag_2CO_3$. The product was hydrolyzed with M $H_2SO_4$ at 100 °C for 8 h, the resulting mixtures of partially *O*-methylated aldoses were reduced with $NaBD_4$, and then acetylated as described above to give a mixture of partially *O*-methylated alditol acetates, which were analyzed by GC-MS using DB-225 capillary column with final temperature of 180 °C.

In order to detect partially *O*-methylated fragments arising from glucuronic acid and/or 4-*O*-methylglucuronic acid residues in the above methanolysis step, the product was reduced with $NaBD_4$ in NaOMe in MeOH at 70 °C for 2 h. The product was then converted to a mixture of partially *O*-methylated alditol acetates as indicated above and submitted to GC-MS analysis. All mass spectra of partially *O*-methylated alditol acetates were characterized using selected mixtures, according to Sassaki, Gorin, Souza, Czelusniak, & Iacomini (2005).

### 2.7. Controlled smith degradation of SRP

The polysaccharide (2.0 g) was dissolved in 500 mL $H_2O$ and oxidized in 0.1 M $NaIO_4$ (500 mL) for 2 days in the dark. Ethylene glycol (30 mL) was added and the solution dialyzed and then reduced with $NaBH_4$ (pH 10.0) for 12 h. The solution was acidified with HOAc and redialyzed against tap (20 h) and then distilled $H_2O$ (20 h). The resulting solution was evaporated to 100 mL and partially hydrolyzed (Goldstein, Hay, Lewis, & Smith, 1965). In a modification of the hydrolysis conditions, the solution was adjusted to pH 2.0 with aqueous $H_2SO_4$, kept at 100 °C for 1 h (Gorin, Horitsu, & Spencer, 1965), neutralized with $BaCO_3$, and filtered. The filtrate was evaporated to 20 mL and added to EtOH (200 mL), to give a precipitate (SRP-SM), isolated in 14% yield.

### 2.8. Nuclear magnetic resonance spectroscopy

NMR spectra were obtained using a 400 MHz Bruker model DRX Avance spectrometer with a 5 mm inverse probe. $^{13}$C-NMR (100.6 MHz) analyses were performed at 30 °C on $D_2O$ solutions, except for the controlled Smith degraded polysaccharide, when 5% (w/v) NaOD in $D_2O$ was solvent. $^1$H-NMR and $^1$H (obs.) $^{13}$C HMQC of OL-10

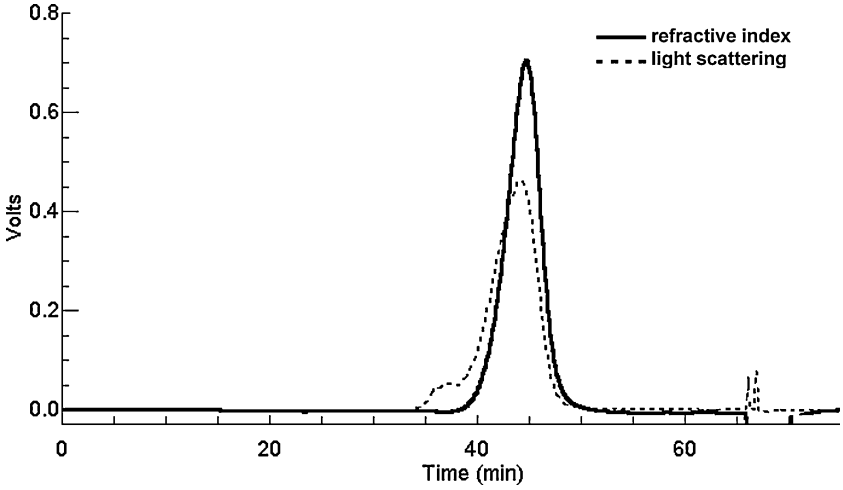


Fig. 1. Elution profile of SRP at HPSEC-MALLS using refractive index and light scattering detectors.

Table 1
Data on the native polysaccharide from *S. rommanzofiana* gum exudate (SRP), and those obtained after carboxy-reduction (SRP-CR), partial acid hydrolysis at pH 1.0 (SRP-PH-1), and on controlled Smith degradation (SRP-SM)

| | SRP | SRP-CR | SRP-PH-1 | SRP-SM |
|---|---|---|---|---|
| Yield (%)[a] | 80 | nd | 42 | 14 |
| Protein (%)[b] | 2.8 | nd | nd | nd |
| $M_w$ (g mol$^{-1}$)[c] | $1.4\times10^5$ | nd | $1.1\times10^4$ | nd |
| Specific rotation in $H_2O$, $[\alpha]_D^{25}$ | −72° | nd | nd | −53° |
| Uronic acid[d] | 8 | – | 8 | 3 |
| Neutral sugar (mol%)[e] | | | | |
| Fucose | 23 | 26 | 8 | – |
| Arabinose | 25 | 28 | 2 | 12 |
| Xylose | 41 | 37 | 82 | 85 |
| Galactose | 1 | – | – | – |
| Glucose | 2 | 7 | – | – |
| 4-Methyl-glucose | – | 2 | – | – |

nd: not determined.
[a] Obtained from the crude gum or SRP.
[b] According to method described by Bradford (1976).
[c] By HPSEC-MALLS.
[d] Percentage content determined colorimetrically (Blumenkrantz & Asboe-Hansen, 1973).
[e] By GC-MS of acetate alditol derivatives.

(in $D_2O$, at 70 °C) were carried out according to the Bruker Manual. Chemical shifts of the samples are expressed in $\delta$ PPM relative to external standard of acetone ($\delta$ 30.2 and $\delta$ 2.224 for $^{13}C$ and $^{1}H$ signals, respectively).

### 2.9. ESI-MS analyses

ESI-MS analysis of OL-10 was carried out using a Micromass Quattro Ultima spectrometer. The sample (~1 ηg/μL) was dissolved in $H_2O$ to which $CH_3CN$ was added to give a 1:1 mixture. The sample was applied using a manual loop injector (10 μL volume) on to a flow rate of 20 μL/min of the 1:1 solvent. The positive-ion mode was necessary, since the oligosaccharides with $R_{Gal}$ 0.78, 1.07, and 1.15 were neutral.

## 3. Results and discussion

SRP was isolated from the gum in 80% yield, had $[\alpha]_D^{25}$ − 72°, contained 2.8% protein, and Fuc, Ara, Xyl, Gal, Glc and uronic acid in a 23:25:41:1:2:8 molar ratio, and was sparingly soluble in water to form viscous solutions. HPSEC-MALLS analysis showed it to be homogeneous with $M_w$ $1.4\times10^5$ g.mol$^{-1}$ and a polydispersity ratio $M_w/M_n$ of ~1.0 (Fig. 1). Carboxy-reduction of SRP provided material (SRP-CR) with glucose and its 4-*O*-methyl derivative in a molar ratio of ~3:1. The chemical composition and other analytical data for SRP are shown in Table 1.

According to its monosaccharide composition, SRP contained neutral constituents similar to those of other polysaccharides obtained from the palms *Livistona chinensis* (LCP) (Maurer-Menestrina et al., 2003) and *Scheelea phalerata* (SPNa) (Simas et al., 2004). However, its Xyl:Ara ratio (2:1) was higher than that the 1:1 found for LCP and SPNa. In terms of the acidic carbohydrate components, all palm polysaccharides have a similar uronic acid content, although SPNa had a lower 4-Me-Glc*p*A ratio (Simas et al., 2004) when compared to the other polysaccharides. In addition, the main difference in chemical composition is the fucose content, which for SRP is 23%, much higher than those of LCP (6%) and SPNa (7%). However, each of the three polysaccharides were homogeneous (for SRP, see Fig. 1) and had similar $M_w$ values ($1.04\times10^5$ g mol$^{-1}$ for

Table 2
Neutral, partially *O*-methyalditol acetates formed on methylation analysis of *S. rommanzofiana* polysaccharide (SRP) and that obtained on controlled Smith degradation (SRP-SM)

| Partially *O*-methylated alditol acetate | % Area of fragment | | |
|---|---|---|---|
| | $t_r$[a] | SRP | SRP-SM |
| 2,3,5-$Me_3$-Ara | 7.12 | 16 | 16 |
| 2,3,4-$Me_3$-Fuc[b] | 7.61 | 10 | – |
| 2,3,4-$Me_3$-Xyl[b] | 7.61 | 11 | 4 |
| 3,5-$Me_2$-Ara | 8.34 | – | 4 |
| 2,5-$Me_2$-Ara | 8.56 | 4 | 2 |
| 2,4-$Me_2$-Fuc | 8.98 | 1 | – |
| 2,3-$Me_2$-Ara | 9.21 | – | 3 |
| 3,4- $Me_2$-Fuc | 9.28 | 1 | – |
| 2,3-$Me_2$-Xyl[c] | 9.48 | – | 45 |
| 3,4- $Me_2$-Xyl[c] | 9.48 | 20 | – |
| 2-Me-Xyl[d] | 11.87 | 7 | 1 |
| 3-Me-Xyl[d] | 11.87 | 10 | 25 |
| Xyl | 15.08 | 20 | – |

[a] Retention times in minutes, obtained with a DB-225 column.
[b] These were resolved and quantified by area comparison using a DB-23 column.
[c] The 3,4-isomer showed exclusively ions with *m/z* 117 and 130 and 2,3-isomer showed exclusively ions with *m/z* 118 and 129.
[d] These were resolved and quantified using a DB-210 column.

SPNa, $1.9\times10^5$ g mol$^{-1}$ for LCP, and $1.4\times10^5$ g mol$^{-1}$ for SRP).

Methylation analysis of SRP (Table 2) showed a high proportion of non-reducing end-units of Ara*f* (16%), Fuc*p* (10%), and Xyl*p* (11%) with 3-*O*-substituted Ara*f* (4%), 3-*O*- (1%) and 2-*O*-substituted Fuc*p* (1%), 2-*O*- (20%), 3,4-di-*O*- (7%), 2,4-di-*O*- (10%), and 2,3,4-tri-*O*-susbtituted Xyl*p* units (20%), showing a highly branched structure. The Glc*p*A and 4-Me-Glc*p*A units, present as 8% of total SRP, were detected as non-reducing end-units following carboxy-reduction of the methyl ester, methyl glycoside mixture, formed as an intermediate in the methylation analysis. This was carried out using $NaBD_4$ in MeOH containing NaOMe, which gave on further processing, the acetate of 2,3,4-$Me_3$-glucitol-1-D,6-$D_2$, which gave a typical GC-MS ion at *m*/*z* 191.

The C-1 NMR region of SRP (Fig. 2(A)) contained signals at $\delta$ 108.7, 107.8, and 106.7 from $\alpha$-Ara*f* units, and others at $\delta$ 103.4, 102.8, 102.1, 101.4, 100.0 and 98.5. That at $\delta$ 98.5 can be attributed to C-1 of $\alpha$-Glc*p*A and/or 4-Me-Glc*p*A (Swamy & Salimath, 1990; Cavagna, Deger, & Puls, 1984). Others at higher field at $\delta$ 82.0 and 60.8 correspond to 4-Me-Glc*p*A residues ($C$HOCH$_3$−4 and 4-O$C$H$_3$, respectively) (Maurer-Menestrina et al., 2003). Further signals at $\delta$ 65.8, 65.0, 63.8 and 62.2 arose from to C-5 of Xyl*p*, Ara*p* and/or Ara*f* (Delgobo, Gorin, Jones, & Iacomini, 1998), and as confirmation the same resonances appeared inverted in the $^{13}$C-NMR-DEPT (135) spectrum (data not shown). The $^{13}$C-NMR spectrum of SRP (Fig. 2(A)) showed at low field signal at $\delta$ 177.5, from –$C$O$_2$H-6 of uronic acid units. At high field there were two prominent signals at $\delta$ 16.6 and 16.2 from –$C$H$_3$-6 of fucosyl units. Considering the complexity of this spectrum, not all of the signals, especially in the C-2 to C-6 region, could be assigned.

As the NMR spectrum of SRP was poorly resolved, a mild partial hydrolysis was carried out to give a partly

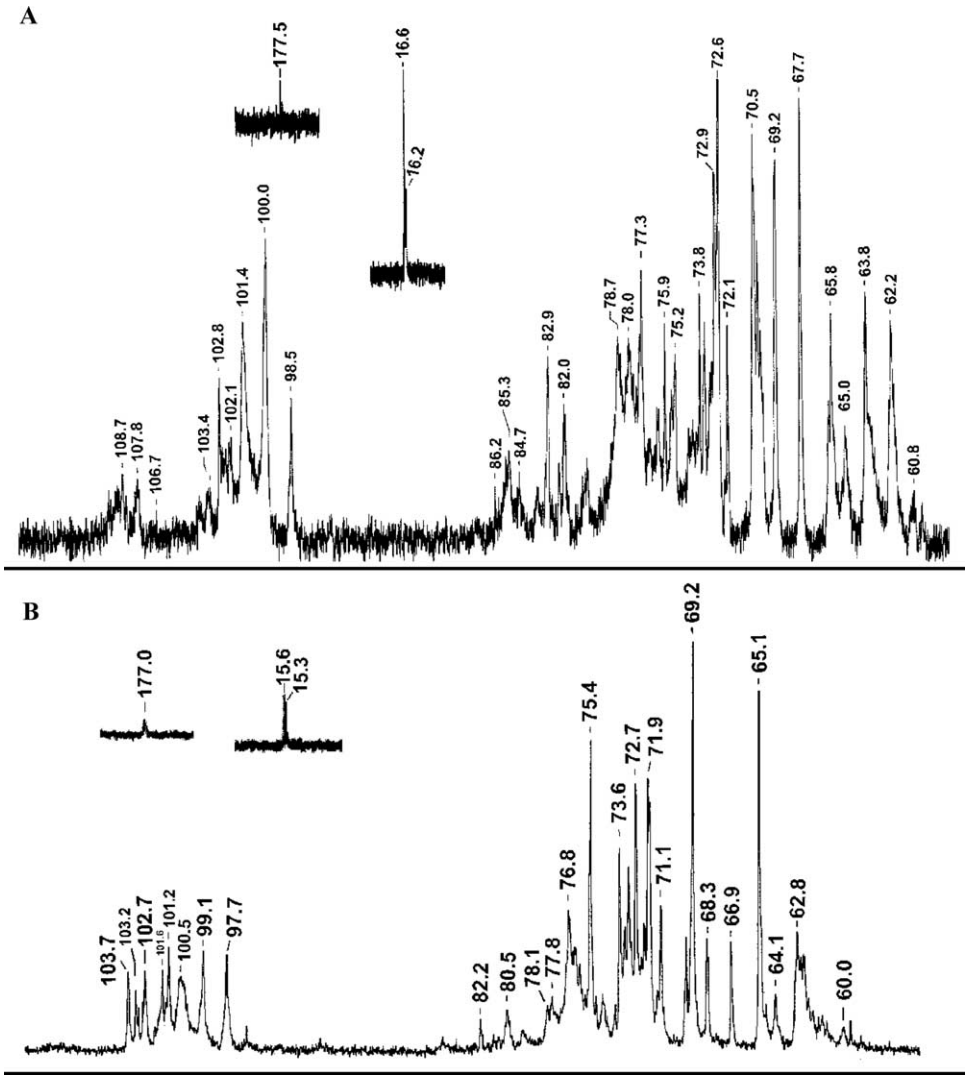


Fig. 2. $^{13}$C-NMR spectra of polysaccharide SRP from *S. romanzoffiana* (A) and polysaccharide SRP-PH-1 (B), obtained after partial acid hydrolysis at pH 1.0. Solvent $D_2O$ at 30 °C with numerical values in $\delta$ (PPM). Inserts: $C$O$_2$H and $C$H$_3$ regions in spectra A and B.

degraded polysaccharide (SRP-PH-1) in 42% yield and which contained Fuc, Ara, Xyl, and uronic acid in an 8:2:82:8 molar ratio. This showed that fucose and arabinose units are present in the side chains of SRP where they are main components. Its $^{13}$C NMR spectrum (Fig. 2(B)) was better defined than that of SRP. The main anomeric signals were at $\delta$ 103.7, 103.2, 102.7, 101.6, 101.2, 100.5, 99.1 and 97.7 and of α-Ara*f* were absent. The signal at $\delta$ 177.0 arose from -$CO_2$H-6 of uronic acid and C-6 signals of fucosyl units were of a lower intensity when compared with those of the $^{13}$C-NMR spectrum of SRP.

The structure of the main chain of SRP was identified by a controlled Smith degradation, which gave a product (SRP-SM), whose low yield (14%) showed a considerable removal of side chains. It contained Ara, Xyl, and uronic acid in a 12:85:3 molar ratio and had $[\alpha]_D -53°$. Methylation analysis (Table 2) confirmed that the length of the SRP side chains had diminished: non-reducing end-units Fuc*p* had disappeared, but those of 2,3,5-$Me_3$-Ara (16%) and 2,3,4-$Me_3$-Xyl (4%) remained. 2-*O*- (4%) and 3-*O*-substituted Ara*f* units (2%) were present as side-chain components, as were 4-*O*-substituted Ara*p* and/or 5-*O*-substituted Ara*f* units (3%). Internal Xyl*p* residues were 2,4-di-*O*- (25%), 3,4-di-*O*- (1%), and 4-*O*-substituted (45%). Uronic acid (3%) was found to be non-reducing end-units by the reduction procedure incorporating $NaBD_4$, which gave the acetate of 2,3,4-$Me_3$-glucitol-1-D,6-$D_2$, whose typical GC-MS ion was at *m*/*z* 191. The $^{13}$C NMR spectrum of SRP-SM (Fig. 3) contained five main signals, at $\delta$ 101.7; 72.7; 73.7; 76.4 and 63.0 corresponding, respectively, to C-1, C-2, C-3, C-4 and C-5, of the (1→4)-linked β-xylan main-chain (Ebringerová, Hromádková, Petráková, & Hricovíni, 1990; Léon de Pinto et al., 1994). The C-1 region also contained a signal at $\delta$ 103.8, which can be attributed to β-Glc*p*A (non-reducing end) (Gorin & Mazurek, 1975; Léon de Pinto et al., 1994). As with SRP-PH-1, C-1 signals of α-Ara*f* were absent, which also suggested the presence of β-Ara*f* units (non-reducing-ends). Thus, according to the monosaccharide composition, methylation data and $^{13}$C-NMR spectral data for SRP-SM, that it consisted of a (1→4)-linked β-Xyl*p* main chain, mainly substituted at O-2 with non-reducing end units of Ara*f*, Xyl*p*, and Glc*p*A.

An attempt was made to obtain more information on the structure the side chains of SRP by examination of the structurally related free, reducing oligosaccharides often present in plant gums (Menestrina, Iacomini, Jones, & Gorin, 1998; Delgobo, Gorin, Tischer, & Iacomini, 1999; Tischer et al., 2002). However, the complexity of the mixture and their closeness of the $R_F$ values of its components, made their isolation impossible.

More success was achieved on isolation of oligosaccharides formed by partial acid hydrolysis of SRP under two different conditions. Firstly, SRP was partially hydrolyzed with M TFA at 100 °C for 2 h to give uronic acid-containing oligosaccharides that on PC

α-Glc*p*A-(1 → 2)-β-Xyl*p*-(1 → 4)-αβ-Xyl

α-Glc*p*A-(1 → 2)-αβ-Xyl **1**

4-Me-α-Glc*p*A-(1 → 2)-αβ-Xyl **2**

β-Xyl*p* − (1 → 2)-αβ-Xyl **3**

α-Fuc*p*-(1 → 4)-αβ-Xyl **4**

α-Fuc*p*-(1 → 3)-αβ-Ara **5**

**6**

23

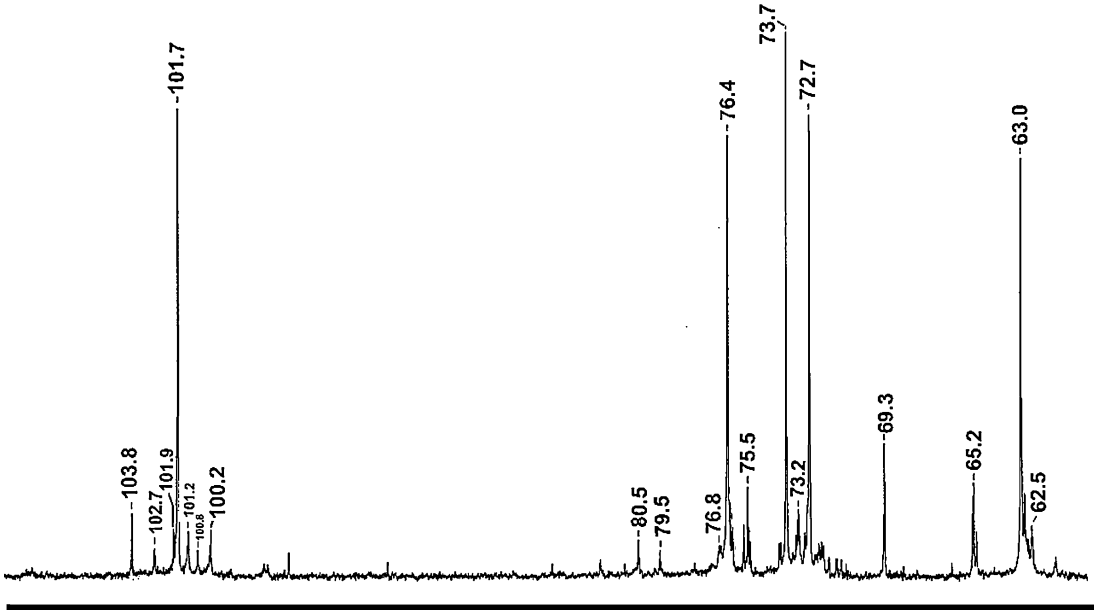


Fig. 3. $^{13}$C-NMR spectrum of SRP-SM obtained by controlled Smith degradation of SRP, solvent 5% NaOD in $D_2O$ at 30 °C. Numerical values in $\delta$ (PPM).

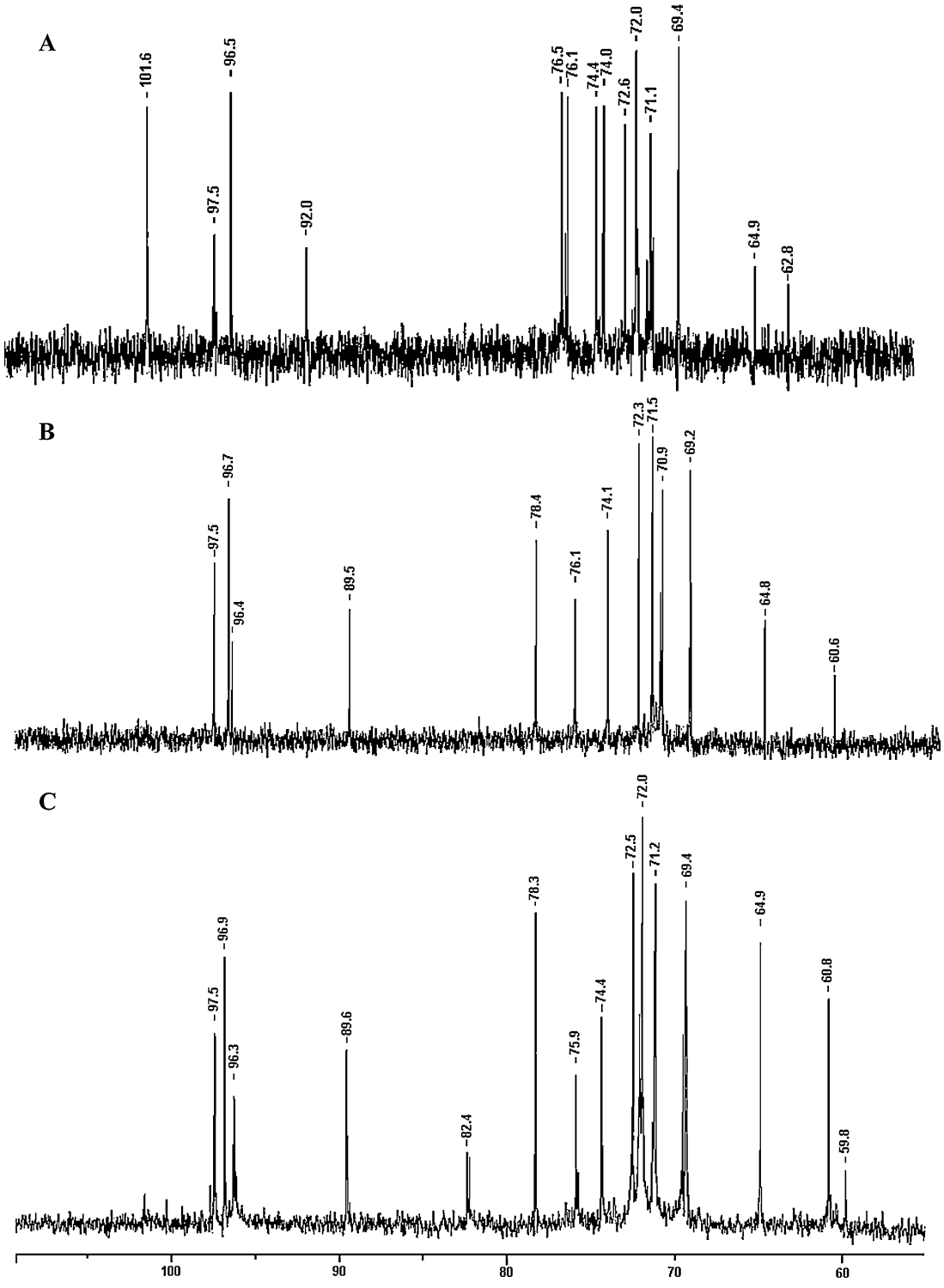


Fig. 4. $^{13}$C-NMR spectra of acidic oligosaccharides with $R_{Lact}$ 0.36 (A), 0.59 (B), and 0.52 (C). Solvent $D_20$ at 30 °C. Numerical values in $\delta$ (PPM).

had $R_{Lact}$ 0.36, 0.47, 0.52, 0.58 and 0.59. That with $R_{Lact}$ 0.36 was α-glucopyranosyl(uronic acid)-(1→2)-β-xylopyranosyl-(1→4)-αβ-xylose (**1**), according to its $^{13}$C-NMR spectrum (Fig. 4(A)), which had major C-1 signals at $\delta$ 101.6 (2-*O*-substituted β-Xyl*p*), 97.5 (non-reducing end units of α-Glc*p*A), 96.5 and 92.0 (4-*O*-substituted β- and α-Xyl reducing units, respectively). The signals at $\delta$ 76.5 and $\delta$ 76.1 were from *O*-substituted C-2 and *O*-substituted C-4 of Xyl*p* units, respectively (Maurer-Menestrina et al., 2003; Simas et al., 2004). The $R_{Lact}$ 0.59 component was 2-O-α-glucopyranosyl(uronic acid)-αβ-xylose (**2**), since its $^{13}$C-NMR spectrum (Fig. 4 (B)) contained C-1 signals at $\delta$

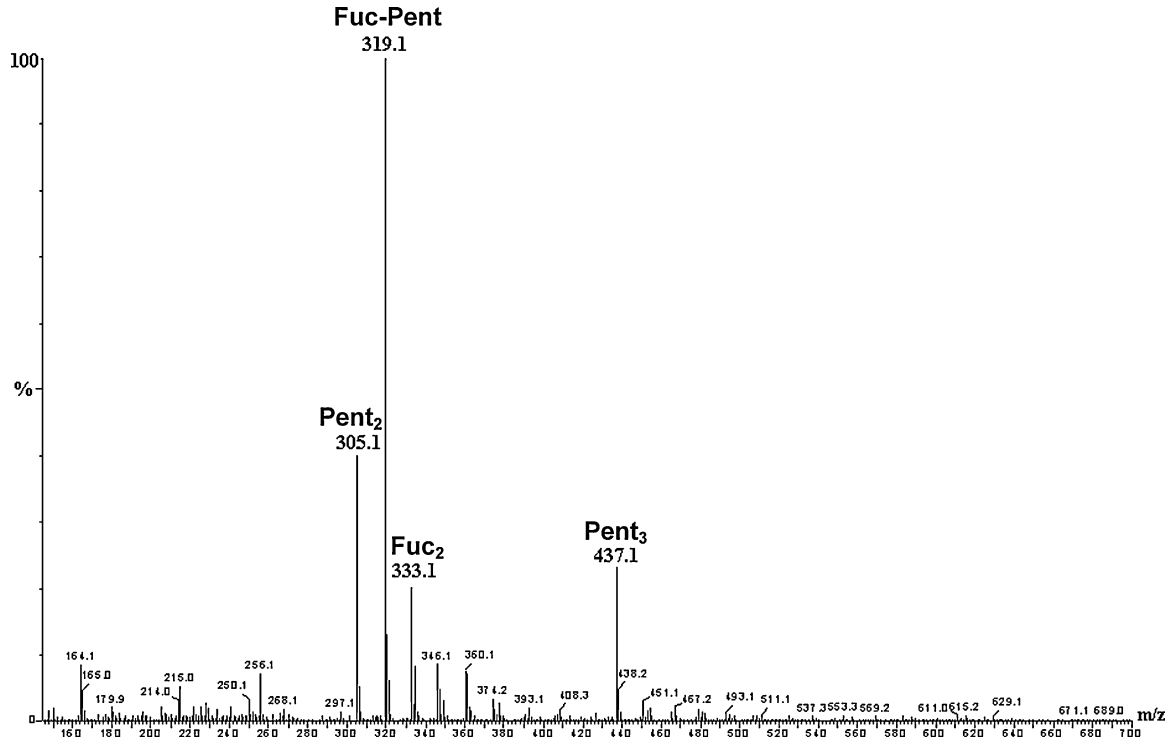


Fig. 5. ESI-MS (positive mode) of oligosaccharides of OL-10 fraction in Na+ forms.

97.5 and 96.4 (α-Glc*p*A linked to β- and α-Xyl reducing units, respectively), 96.7 and 89.5 (β- and α-Xyl*p* reducing units, respectively). The signals at $\delta$ 78.4 and $\delta$ 76.1 were from substituted C-2 of reducing β-Xyl*p* and reducing α-Xyl*p* units, respectively (Maurer-Menestrina et al., 2003; Simas et al., 2004). Other signals (C-2 to C-5 of Glc*p*A and Xyl units) showed in the spectra of $R_{Lact}$ 0.36 and $R_{Lact}$ 0.59 components corresponded to those found in the respective spectra of the aldotriouronic and aldobiouronic acid, obtained from the *S. phalerata* palm gum (Simas et al., 2004). The $R_{Lact}$ 0.52 fraction was characterized as 4-methyl-α-glucopyranosyl(uronic acid)-αβ-xylose (**3**), since methylation analysis involving a $NaBD_4$ reduction of carboxyl methyl ester groups gave rise to acetates of 3,4-$Me_2$-xylitol-1-D and 2,3,4-$Me_2$-glucitol-1-D,6-$D_2$. Its $^{13}$C-NMR spectrum (Fig. 4(C)) contained C-1 signals at $\delta$ 97.5 and 96.3 (4-Me-α-Glc*p*A linked to β- and α-Xyl*p* reducing units, respectively), 96.9 and 89.6 (β- and α-Xyl*p* reducing units, respectively). The signals appearing at $\delta$ 177.0 (not shown), $\delta$ 82.4, and $\delta$ 60.8 were from -*C*OOH, *C*HOCH$_3$−4, and CHO*C*H$_3$-4 of 4-Me-α-Glc*p*A units, respectively (Maurer-Menestrina et al., 2003). Cavagna et al. (1984) and Maurer-Menestrina et al. (2003) found similar signals and assigned an aldotriouronic acid structure to an oligosaccharide obtained from birch wood and an oligosaccharide obtained from *L. chinensis* gum exudate, respectively. The oligosaccharides with $R_{Lact}$ 0.47 and 0.58 could not be characterized.

The second hydrolysis of SRP under milder conditions, namely 0.1 M TFA for 1 h at 96 °C, liberated neutral oligosaccharides. These were isolated via charcoal–Celite column, which was eluted with water and then progressively with more concentrated aq. EtOH solutions. The 2% fraction contained fucose and the 10% aq. EtOH fraction was deionized to remove any possible uronic acid containing components to give fraction OL-10, which: on PC gave, a main spot, with $R_{Gal}$ 1.2 with minor ones at $R_{Gal}$ 1.07 and 0.78. Acid hydrolysis gave rise to Fuc, Ara, Xyl, Gal, and Glc in a 42:12:40:3:3 molar ratio. ESI-MS in the positive-ion mode (Fig. 5) detected molecular ions in the $Na^+$ form, the main one with *m/z* 319 (Fuc-Pent) > *m/z* 305 (Pent$_2$) > 437 (Pent$_3$) > 333 (Fuc$_2$). Methylation analysis showed the presence of non-reducing end-units of Ara*f* (4%), Xyl*p* (6%), and Fuc*p* (46%), 3-*O*- (2%) and 2-*O*-substituted Fuc*p* (4%), 3-*O*-substituted Ara*p* (17%), and 4-*O*- (∼9%) and 2-*O*-substituted Xyl*p* units (∼12%). In order to distinguish between (1→2)- and (1→4)-isomers of β-xylobiose, the $^{13}$C NMR data of Bock, Pederson, & Pederson (1984) were used to interpret an $^1$H(obs.)$^{13}$C HMQC, NMR spectrum of the mixture ($D_2O$ at 70 °C; Fig. 6). The presence of 2 low-field C-1 signals at $\delta$ 103.4 (5.045) > 103.5 (5.08) favored the (1→2)-isomer (**4**), since the (1→4)-isomer should only give one. In confirmation, examination of an authentic sample of β-Xyl*p*-(1→4)-αβ-Xyl now gave only one signal at $\delta$ 102.2. Fig. 6 contains other C-1′/H-1′ signals at $\delta$ 101.0 (5.49) and 101.7 (4.87), corresponding to predominant α-Fuc*p* non-reducing end-units. These, when the methylation data are considered, should be linked to remaining 4-*O*-substituted Xyl*p* reducing ends (∼12%), as in α-Fuc*p*-(1→4)-Xyl (**5**), and the 14% of 3-*O*-substituted Ara*p* units

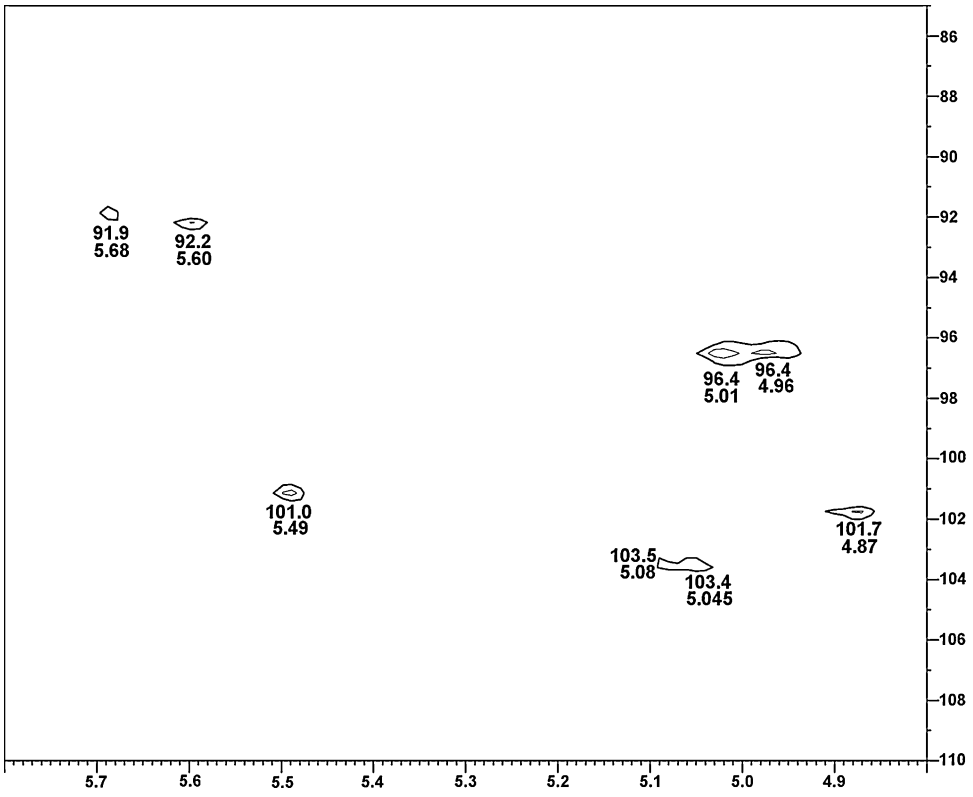


Fig. 6. C-1/H-1 region of $^{13}C$ HMQC, NMR spectrum of OL-10 fraction. Solvent $D_2O$ at 70 °C. Numerical values in $\delta$ (PPM).

were present as in α-Fuc*p*-(1→3)-Ara (**6**). This last assignment agrees with the methylation data obtained on the product (SRP-SM) of the controlled Smith degradation of SRP, whose 3-*O*-substituted Ara*f* units survived as 16% of Ara*f* non-reducing end-units.

## 4. Chemotaxonomic significance of SRP

The above analytical data show that the polysaccharide of *S. romanzoffiana* gum exudate can be classified as a 'heavily substituted β-xylan' (Stephen, 1983), a structure that is not common in plant gums, but similar to those of two previously investigated palm gums (Maurer-Menestrina et al., 2003; Simas et al., 2004). These, although more complex, are structurally related to hemicellulose xylans occurring universally in cell walls of higher plants. Plant gums containing β-xylan cores have been found in *Sapota achras* (Sapotaceae) (Dutton & Kabir, 1973), *Cercidium australe* (Cerezo et al., 1969), and *Cercidium praecox* (Léon de Pinto et al., 1994), the last two species belonging to the family Mimosaceae. Acidic heteroxylan gum exudates have, in common with those containing arabinogalactans, similar adhesive and water-retaining properties that are possibly related to their possible biological role in plant defense mechanisms (Fincher et al., 1983). Sandhu, Hudson, & Kennedy (1981) suggest that xylan main chains, which are substituted by hydrophilic (e.g. α-L-arabinofuranose) or ionic residues (e.g. uronic acids) similar to the polysaccharide from *S. romanzoffiana*, have: (a) hydrophilic character, (b) solubility in water, and (c) ability to form gels. However, linear non-substituted or lightly substituted (1→4)-linked β-xylans are insoluble in water, having a conformation with intermolecular hydrogen bonding, related to cellulose, defining their structural biological roles.

Exudate gums has been studied in detail directed to their potential in the chemical taxonomy of plants. A number of examples have been mentioned of structural similarities between polysaccharides from gums of botanically related species. The genus *Acacia* provides the most striking example of related species that give rise to gum polysaccharides of the same general type, having many structural features in common (Anderson et al., 1969; Aspinall, 1969).

In terms of our present study, touching on the classification of palms, Uhl & Dransfield (1987) consider that the genera *Syagrus* and *Scheelea* belong to the same subfamily (Arecoideae) and tribe (Cocoeae), but that of *Livistona* belongs to the Coryphoideae subfamily and the Corypheae tribe. Thus, the genera *Syagrus* and *Scheelea* are closer in this classification system, when compared with the genus *Livistona*. Considering the taxonomic position of these genera, we suggest that the type of gum exudate polysaccharide found for the palm gums could be a characteristic for the family Arecaceae. Further chemical studies of gum exudate polysaccharides

from other palm species palm will accordingly be investigated.

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## References

Anderson, D. M. W., & Dea, I. C. M. (1969). Chemotaxonomic aspects of the chemistry of *Acacia laeta* gum. *Phytochemistry*, *8*, 167–176.

Aspinall, G. O. (1969). Gums and mucilages. *Advances in Carbohydrates Chemistry and Biochemistry*, *24*, 333–379.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Bock, K., Pederson, C., & Pederson, H. (1984). Carbon-13 nuclear magnetic resonance data for oligosaccharides. *Advances in Carbohydrate Chemistry and Biochemistry*, *42*, 193–225.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.

Cavagna, F., Deger, H., & Puls, J. (1984). 2D-NMR analysis of the structure of an aldotriouronic acid obtained from birch wood. *Carbohydrate Research*, *129*, 1–8.

Cerezo, A. S., Stacey, M., & Webber, J. M. (1969). Some structural studies of brea gum (an exudate from *Cercidium australe* Jonhst.). *Carbohydrate Research*, *9*, 505–517.

Ciucanu, I., & Kerek, F. A. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Delgobo, C. L., Gorin, P. A. J., Jones, C., & Iacomini, M. (1998). Gum heteropolysaccharide and free reducing mono- and oligosaccharides of *Anadenanthera colubrina*. *Phytochemistry*, *47*, 1–8.

Delgobo, C. L., Gorin, P. A. J., Tischer, C. A., & Iacomini, M. (1999). The free reducing oligosaccharides of angico branco (*Anadenanthera colubrina*) gum exudate: an aid for structural assignments in the heteropolysaccharide. *Carbohydrate Research*, *320*, 167–175.

Dutton, G. G. S., & Kabir, S. (1973). Structural studies of sapote (*Sapota achras*) gum. *Carbohydrate Research*, *28*, 187–200.

Ebringerová, A., Hromádková, Z., Petráková, E., & Hricovíni, M. (1990). Structural features of a water-soluble L-arabino-D-xylan from rye bran. *Carbohydrate Research*, *198*, 57–66.

Fincher, G. B., Stone, B. A., & Clarke, A. E. (1983). Arabinogalactan-proteins: structure, biosynthesis, and function. *Annual Review of Plant Physiology*, *34*, 47–70.

Goldstein, I. J., Hay, G. W., Lewis, B. A., & Smith, F. (1965). Controlled degradation of polysaccharides by periodate oxidation, reduction, and hydrolysis. *Methods in Carbohydrate Chemistry*, *5*, 361–370.

Gorin, P. A. J., Horitsu, K., & Spencer, J. F. T. (1965). An exocellular mannan alternately linked 1,3-β and 1,4-β from *Rhodotorula glutinis*. *Canadian Journal of Chemistry*, *43*, 950–954.

Gorin, P. A. J., & Mazurek, M. (1975). Further studies on the assignment of signals in C-13 magnetic resonance spectra of aldoses and derived methyl glycosides. *Canadian Journal of Chemistry*, *53*, 1212–1223.

León de Pinto, G., Martínez, M., & Rivas, C. (1994). Chemical and spectroscopic studies of *Cercidium praecox* gum exudate. *Carbohydrate Research*, *260*, 17–25.

Maurer-Menestrina, J., Sassaki, G. L., Simas, F. F., Gorin, P. A. J., & Iacomini, M. (2003). Structure of a highly substituted β-xylan from the gum exudate of the palm *Livistona chinensis* (Chinese fan). *Carbohydrate Research*, *338*, 1843–1850.

Menestrina, J. M., Iacomini, M., Jones, C., & Gorin, P. A. J. (1998). Similarity of monosaccharide, oligosaccharide and polysaccharide structures in gum exudate of *Anacardium occidentale*. *Phytochemistry*, *47*, 715–721.

Sandhu, J. S., Hudson, G. J., & Kennedy, J. F. (1981). The gel nature and structure of the carbohydrate of ispaghula husk *ex Plantago ovata* Forsk. *Carbohydrate Research*, *93*, 247–259.

Sassaki, G. L., Gorin, P. A. J., Souza, L. M., Czelusniak, P. A., & Iacomini, M. (2005). Rapid synthesis of partially O-methylated alditol acetate standards for GC-MS: some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation. *Carbohydrate Research*, *340*, 731–739.

Simas, F. F., Gorin, P. A. J., Guerrini, M., Naggi, A., Sassaki, G. L., & Delgobo, C. L. (2004). Structure of a heteroxylan of the gum exudate of the palm *Scheelea phalerata* (uricuri). *Phytochemistry*, *65*, 2347–2355.

Stephen, A. M. (1983). Other plant polysaccharides. In G. O. Aspinall (Ed.), *The polysaccharides*. New York, NY: Academic Press.

Swamy, N. R., & Salimath, P. V. (1990). Structural features of acidic xylans isolated from red gram (*Cajanus cajan*) husk. *Carbohydrate Research*, *197*, 327–337.

Taylor, R. L., & Conrad, H. E. (1972). Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry*, *11*, 1383–1388.

Tischer, C. A., Gorin, P. A. J., & Iacomini, M. (2002). The free reducing oligosaccharides of gum arabic: aids for structural assignments in the polysaccharide. *Carbohydrate Polymers*, *47*, 151–158.

Trevelyan, W. E., Procter, D. P., & Harrison, J. S. (1950). Detection of sugars on paper chromatograms. *Nature*, *166*, 444.

Uhl, N. W., & Dransfield, J. (1987). Genera Palmarum: a classification of palms based on the work of H.E. Moore Jr. In L. H. Bailey (Ed.), *Hortorium and the International Palm Society* (pp. 610). Lawrence, Kansas, USA: Allen Press (ISBN 0-935868-30-5.).

Whistler, R. L. (1993). Exudate gums. In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial gums: Polysaccharides and their derivatives*. New York, NY: Academic Press.

Whistler, R. L., & Durso, D. F. (1950). Chromatographic separation of sugars on charcoal. *Journal of the American Chemical Society*, *72*, 677–679.