Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 63 (2006) 30–39
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

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

F.F. Simas, J. Maurer-Menestrina, R.A. Reis, G.L. Sassaki, M. Iacomini, P.A.J. Gorin*

Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, CxP 19.046, CEP 81.531-990, Curitiba-PR, Brazil

Received 17 May 2005; accepted 16 June 2005
Available online 28 November 2005

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 1.4×10^5 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.

* Corresponding author. Tel.: +55 41 361 1670; fax: +55 41 266 2042.
E-mail address: cesarat@ufpr.br (P.A.J. Gorin).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.06.015

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.