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Glucuronoarabinoxylan from coconut palm gum exudate: Chemical structure and gastroprotective effect

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ABSTRACT

A glucuronoarabinoxylan (CNAL) was extracted with 1% aq. KOH (25 °C) from *Cocos nucifera* gum exudate. It had a homogeneous profile on HPSEC-MALLS-RI (M_w 4.6 × 10^4 g/mol) and was composed of Fuc, Ara, Xyl, Glc*p*A (and 4-*O*-Glc*p*A) in a 7:28:62:3 molar ratio. Methylation data showed a branched structure with 39% of non-reducing end units, 3-*O*-substituted Ara*f* (8%), 3,4-di-*O*- (15%), 2,4-di-*O*- (5%) and 2,3,4-tri-*O*-substituted Xyl*p* units (17%). The anomeric region of CNAL ^{13}C NMR spectrum contained 9 signals, indicating a complex structure. The main chain of CNAL was characterized by analysis of a Smith-degraded polysaccharide. Its ^{13}C NMR spectrum showed 5 main signals at δ 101.6, δ 75.5, δ 73.9, δ 72.5, and δ 63.1 that were attributed to C-1, C-4, C-3, C-2 and C-5 of (1 → 4)-linked β-Xyl*p*-main chain units, respectively. CNAL exhibited gastroprotective effect, by reducing gastric hemorrhagic lesions, when orally administered (1 and 3 mg/kg) to rats prior to ethanol administration.

1. Introduction

Cocos nucifera L. is a large palm belonging to the Arecaceae family. It is believed to have its origin in the Indo-Malayan region, from where it spread throughout the tropics (Bankar et al., 2011). The coconut palm is economically important because it provides food, drink, oil, folk medicine, among others. It can also be used for coastal stabilization as windbreaks and as a subsistence crop in many Pacific islands and other tropical regions (Renjith, Chikku, & Rajamohan, 2013; Rinaldi et al., 2009).

The coconut palm produces gum exudates, like other palms as *Livistona chinensis* (Maurer-Menestrina, Sassaki, Simas, Gorin, & Iacomini, 2003), *Scheelea phalerata* (Simas et al., 2004), and *Syagrus romanzoffiana* (Simas et al., 2006). The exudate process occurs mainly after some physical or microbiological injuries, and is found on the trunk of the palm. The coconut exudate is reddish-brown, clear, and vitreous. It can form an aqueous gel in water, although the gum has poor adhesive properties (Nussinovitch, 2010).

The wide industrial application of gum exudates is due to their water-retaining capacity to produce gels or highly viscous solutions, and for their ability to enhance the stability of emulsions and foams. It is known that these properties depend on the chemical structure of gum exudate polysaccharides and on their conformation in solution (Grein et al., 2013; Rinaudo, 2001; Rincón, Muñoz, Pinto, Alfaro, & Calero, 2009; Whistler, 1993).

Polysaccharides are the main components of gum exudates, having complex structures, consisting of a great variety of monosaccharides and glycosidic linkages, and a high number of branches as well (Aspinall, 1969). The most abundant polysaccharide gum exudates are arabinogalactans, such as arabic gum (from *Acacia senegal*), which is composed of Ara, Gal, Glc*p*A, and Rha as major monosaccharides. This polymer is composed of a main chain of (1 → 3)-linked β-D-Gal*p* residues, substituted at O-6 by complex side-chains composed of α-L-Ara*f*, β-D-Glc*p*A, α-L-Rha*p*, and β-D-Gal*p* (Anderson, Hirst, & Stoddart, 1966a, 1996b; Tischer, Gorin, & Iacomini, 2002). Other polysaccharides, such as glucuronoarabinoxylans (GAXs), were also isolated from gum exudates, although

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less common. These polymers have structure similarities with hemicellulosic glucuronoarabinoxylans from the primary plant cell wall, especially from species of the Poaceae family, such as sorghum (Verbruggen et al., 1998), maize (Allerdings, Ralph, Steinhart, & Bunzel, 2006), and wheat (Hromádková, Paulsen, Polovka, Kost'álová, & Ebringerová, 2013; Sun, Cui, Gu, & Zhang, 2011). Acetyl groups, ferulic acid and coumaric acid have also been found in GAXs from plant cell walls (Ishii, 1997). Glucuronoarabinoxylans from gum exudates, as those from palm species, are notably more highly branched than those of the hemicellulose type (Maurer-Menestrina et al., 2003; Simas et al., 2004, 2006).

Plant polysaccharides have showed a variety of biological activities, such as immunomodulatory (Moretão, Buchi, Gorin, Iacomini, & Oliveira, 2003; Schepetkin & Quinn, 2006; Simas-Tosin et al., 2012), anti-ulcer (Cipriani et al., 2008, 2009), antioxidant (Xie et al., 2012), antitumor (Xie et al., 2013), and as adjuvant in sepsis treatment (Dartora et al., 2013; Scoparo et al., 2013). Plant polysaccharides are good candidates as therapeutic biomacromolecules, considering that they are relatively nontoxic and have no significant side effects (Schepetkin & Quinn, 2006).

Glucuronoarabinoxylans from gum exudates are noteworthy molecules as candidates in industry or for therapeutic purposes, mainly because of its high yield, being around 80% of the gum weight (Maurer-Menestrina et al., 2003; Simas et al., 2006). These polymers may vary their chemical structure and conformation, which may be related to the different biological effects observed *in vitro* and *in vivo* (Moretão et al., 2003; Schepetkin & Quinn, 2006). Besides, the coconut palm is widely cultivated on the tropical regions of the planet, and despite of the great consumption of its fruit, the gum is discarded. Considering that there are no studies on coconut palm gum exudate, it was now chosen to evaluate the chemical and structural properties of its polysaccharides. The gastroprotective effects of the isolated glucuronoarabinoxylan were determined as well, using an *in vivo* model.

2. Materials and methods

2.1. Collection of the gum and isolation of polysaccharides

The coconut palm gum exudates were collected from the trunk of various tree specimens in Águas de Santa Bárbara (State of São Paulo, Brazil). The crude gum (9 g) was submitted to aqueous extraction (1.5%, w/v) at 25 °C (24 h). The remaining debris were removed by filtration and 3 volumes of ethanol (EtOH) were added to filtrate giving a precipitate, which was isolated by centrifugation (12,430 × g/20 min/10 °C). After dialysis (cut-off 12–14 kDa) and freeze-drying, the polysaccharide fraction CN was obtained (9% yield). The remaining gum residue was then submitted to aqueous extraction at 50 °C (24 h). The dispersion was filtered and the resulting soluble extract was added to 3 volumes of EtOH to give a precipitate, which was isolated as described above, giving rise to polysaccharide fraction CNH (12% yield). Finally, the remaining aqueous insoluble gum was treated with $NaBH_4$ in solution (pH 10.0), and then dissolved in 1% (w/v) aq. KOH (at 25 °C). After complete solubilization, the alkaline extract was neutralized with 50% (v/v) aq. acetic acid (HOAc) and was added to 3 volumes of EtOH, giving a polysaccharide fraction CNAL (50% yield), isolated as described above (Fig. 1).

2.2. Carboxy-reduction

Carboxy-reduction of polysaccharide CNAL (200 mg) was carried out using two successive cycles of the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide method (Simas-Tosin et al., 2013; Taylor & Conrad, 1972), to give a carboxy-reduced polysaccharide fraction (CR-CNAL). $NaBH_4$ being used as reducing agent.

2.3. Sodium periodate oxidation and controlled Smith degradation

In order to show the structure of the main chain of the CNAL it was submitted to controlled Smith degradation. CNAL was dissolved in H_2O (1 g in 100 mL) and 0.1 M $NaIO_4$ (100 mL) was then added. The solution was kept for 72 h in the dark, under magnetic stirring. After this time, 1 ml of oxidized solution was removed for determination of periodate consumption, according to the methodology described by Hay et al. (1965). Ethylene glycol (15 mL) was added to stop the reaction. The solution was dialyzed (cut-off 8 kDa/48 h) against tap water and treated with $NaBH_4$ (pH 10.0 for 16 h), neutralized with HOAc, dialysed (cut-off 8 kDa/48 h) and the volume was reduced to 50 mL. The last step of the procedure was a mild acid hydrolysis with TFA (0.1 M) until obtain pH 2.0, for 40 min

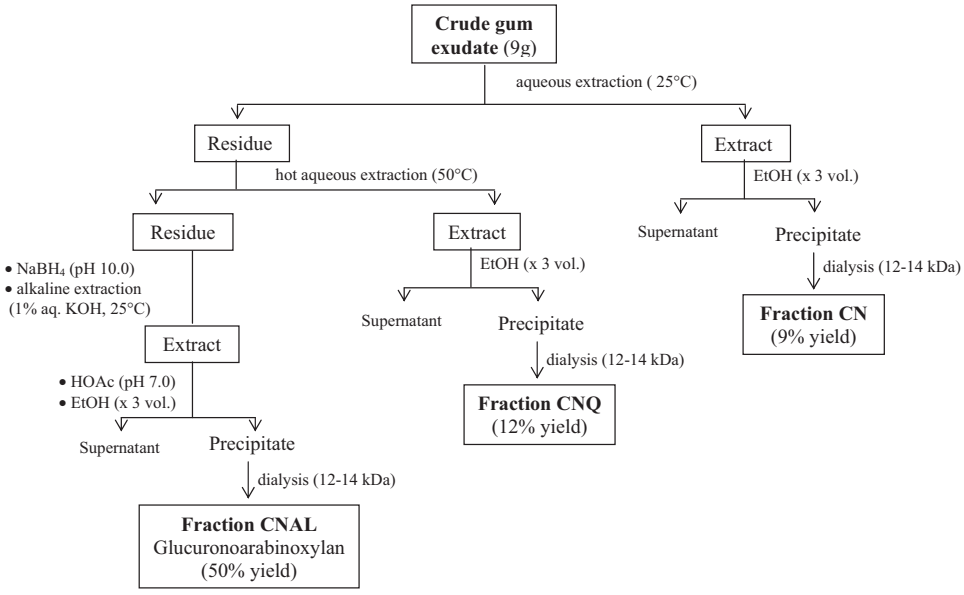

Fig. 1. Flow sheet diagram of isolation and the purification of polysaccharides from coconut gum exudate.