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Substituted galacturonan from starfruit: Chemical structure and antinociceptive and anti-inflammatory effects

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ABSTRACT

Starfruit (*Averrhoa carambola* L.) is an edible tropical fruit, which is usually consumed as a fresh table fruit or as fruit juice. It also exhibits various pharmacological activities. In this study, polysaccharides were extracted with boiling water and purified by freeze–thawing and Fehling treatments. After purification steps, a homogenous fraction was obtained. It was analyzed by sugar composition, gel permeation chromatography, methylation, and two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy analyses. It comprised arabinose (Ara), galactose (Gal), and galacturonic acid (GalA) in a molar ratio of 12.3:1.7:86.0. Methylation and NMR spectroscopy analyses showed that it contained a substituted galacturonan composed of (1→4)-linked α-D-Gal*p* A units branched at *O*-2 by (1→5)-linked α-L-Ara*f* and terminal α-L-Ara*f* and α-D-Gal*p* A units. The effect of PFSCW (10–300 mg/kg, i.p.) on nocifensive behavior induced by intraplantar injection of formalin in mice was evaluated. The fraction demonstrated antinociceptive and anti-inflammatory properties, suggesting that it may be useful in therapeutic intervention for the management of inflammatory pain.

1. Introduction

Inflammation has been recognized as a major risk factor for various progressive diseases in humans, including cancer, neurological disease, metabolic disorders, and cardiovascular disease, and targeting the reduction of chronic inflammation is a beneficial strategy to prevent these diseases [1]. Dietary fibers are plant cell wall polysaccharides, and the intake of these molecules is inversely associated with inflammatory markers [2]. Among these polysaccharides are pectins. Pectins are a family of complex polysaccharides that contain (1→4)-linked α-D-Gal*p* A residues [3]. Homogalacturonan (HG); rhamnogalacturonan I; and substituted galacturonans such as rhamnogalacturonan II, xylogalacturonan, apiogalacturonan, galactogalacturonan, arabinogalacturonan, and galacturonogalacturonan are the pectic polysaccharides that have been found in primary cell walls [4]. Anti-inflammatory activity has also been demonstrated by purified pectins, such as for those found in sweet pepper, celery stalks, *Comarum palustre*, and *Potamogeton natans* [5–8].

Averrhoa carambola L., also known as starfruit or carambola, is an edible tropical fruit of the Oxalidaceae family, usually consumed as fresh fruit or as fruit juice [9–11]. Several studies have reported that different parts of the *A. carambola* tree exhibit pharmacological activities [12]. In the fruits, antioxidant [13] and hypoglycemic effects [14,15] have been reported, while anti-inflammatory effects have been described for leaves [16] and stems [17]. Thus far, the biological activities of the polysaccharides of starfruit have not been reported. Recently, Leivas et al. [18] described a pectin from starfruit, formed by a type I rhamnogalacturonan containing arabinan and type I arabinogalactan as the neutral side chains. Herein, we describe the chemical structure of a substituted galacturonan extracted from *A. carambola* fruits and report the findings on its antinociceptive and anti-inflammatory effects.

2. Materials and methods

2.1. Plant material

Ripe starfruits of the cultivar B10 were purchased from the local market of Curitiba, State of Paraná, Brazil.

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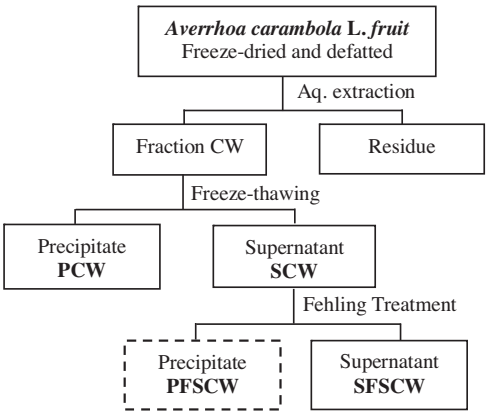

Fig. 1. Scheme of extraction and fractionation of a galacturonan from starfruit (*Averrhoa carambola* L.).

2.2. General analytical methods

All solutions were evaporated below 60 °C under reduced pressure. Centrifugation was performed at 12,000 × *g* for 20 min at 10 °C. Dialysis was performed using a 6–8 kDa molecular weight cut-off membrane (Spectra/Por®). Total lipid quantification was performed by extraction employing chloroform–methanol (1:1) as a solvent through the Soxhlet apparatus.

Uronic acid (UA) contents were determined using the *m*-hydroxybiphenyl colorimetric method [19] using galacturonic acid as the standard. Fraction was carboxy-reduced by the carbodiimide method [20] using $NaBH_4$ as the reducing agent, yielding products with the –COOH groups of its UA residues reduced to $–CH_2OH$.

The degree of methylesterification (DE) was determined by ^1H nuclear magnetic resonance (NMR) spectroscopy [21] using a Bruker AVANCE III 400 NMR spectrometer.

2.3. Extraction and purification of polysaccharides

Polysaccharides from starfruit were extracted with boiling water, yielding the aqueous fraction (CW) (Fig. 1) as previously described by Leivas et al. [18]. A freeze–thaw treatment was applied in this fraction to yield cold-water soluble (SCW) and insoluble (PCW) fractions. In this procedure, the sample was frozen and then thawed at room temperature, followed by centrifugation, until no more precipitate appeared.

Fraction SCW was dissolved in distilled water and then treated with Fehling's solutions [22], resulting in soluble (SFSCW) and precipitated (PFSCW) fractions, which were separated by centrifugation. Each fraction was neutralized with acetic acid (HOAc), dialyzed against tap water, and deionized with cation exchange resin.

Polysaccharide yields were expressed as percentages based on the weight of dried starfruit subjected to extraction (310.7 g).

2.4. Monosaccharide analysis

Neutral sugars were hydrolyzed with 2 M trifluoroacetic acid for 8 h at 100 °C, reduced with $NaBH_4$ [23], and acetylated with acetic anhydride-pyridine (1:1, v/v) for 18 h at 25 °C [24]. The resulting alditol acetates were analyzed by gas chromatography mass spectrometry (GC–MS) following the conditions employed by Leivas et al. [18].

2.5. Determination of homogeneity and molecular weight of polysaccharides

The homogeneity and molecular weight of water-soluble polysaccharides were determined by gel permeation chromatography (GPC). The procedure was carried out as previously reported by Leivas et al. [18].

2.6. Methylation analysis of polysaccharides

Fraction PFSCW was carboxy-reduced by the carbodiimide method [20] and methylated according to Ciucanu and Kerek [25] using powdered NaOH in DMSO-MeI. The per-*O*-methylated polysaccharide was then subjected to methanolysis in 3% HCl–MeOH (at 80 °C, 2 h), followed by hydrolysis with H_2SO_4 (0.5 M, 10 h, at 100 °C) and neutralization with $BaCO_3$. The resulting mixture of partially *O*-methylated monosaccharides was successively reduced with $NaBD_4$ and acetylated with acetic anhydride–pyridine. The products (partially *O*-methylated alditol acetates) were examined by capillary GC–MS. A capillary column (30 m × 0.25 mm i.d.) of DB-225, held at 50 °C during injection for 1 min, programmed at 40 °C/min to 210 °C, and held at this temperature for 31 min was used for separation. The partially *O*-methylated alditol acetates were identified by their typical electron impact breakdown profiles and retention times [26].

2.7. Nuclear magnetic resonance spectroscopy

^{13}C NMR and 2D $^1H/^{13}C$ heteronuclear single quantum coherence spectroscopy (HSQC) spectra were acquired at 50 °C on a Bruker AVANCE III 400 NMR spectrometer operating at 9.5 T, observing ^1H at 400.13 MHz and ^{13}C at 100.61 MHz, and equipped with a 5-mm multinuclear inverse detection probe with *z*-gradient. Samples were dissolved in D_2O, and chemical shifts were expressed as δ ppm relative to the CH_3 signal from acetone at δ 30.2 as the internal reference. ^1H NMR spectra were acquired at 70 °C, with 256 scans on a Bruker AVANCE III 400 NMR spectrometer. Samples were deuterium-exchanged 3 times by freeze–drying with D_2O solutions, finally dissolved in D_2O, and transferred into 5-mm NMR tubes. Chemical shifts were expressed as δ ppm relative to HDO at δ 4.22 as the internal reference.

2.8. Experimental animals

Experiments were conducted using adult female Swiss mice (25–35 g), obtained from the animal facility of Universidade Federal de Santa Catarina (UFSC, Florianópolis, SC, Brazil). Animals were housed in cages under a 12 h:12 h light:dark cycle (lights on at 6:00 am) at a controlled temperature (22 °C ± 2 °C) with ad libitum access to food and water. The animals were homogeneously distributed among groups and acclimatized in the experimental room for at least 1 h before testing and were used only once throughout the experiments. The experiments were performed after approval from the Ethics Committee for Animal Research of Universidade Federal de Santa Catarina (CEUA/UFSC protocol number PP00745) in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [27]. The number of animals used and the intensity of the noxious stimuli were at the necessary minimum to demonstrate the consistent effects of drug treatments.

2.9. Drugs

The following substances were used: formaldehyde, Tween 80 (Merck, Darmstadt, Germany); meloxicam (Movatec®, Boehringer Ingelheim, São Paulo, Brazil), indomethacin, diclofenac (Sigma