ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 07, Issue, 08, pp.14844-14846, August, 2017

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

ANALYSIS OF BINDING IN AN OCTASACCHARIDE OF SIPARUNA GUIANENSIS AUBLET BY METHYLATION, AND NMR STUDIES

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ARTICLE INFO

Article History:
Received 20th May, 2017
Received in revised form
19th June, 2017
Accepted 18th July, 2017
Published online 30th August, 2017

Keywords:
D-galactose and D-Mannose,
Methylation, NMR,
Oxidation Periodate.

ABSTRACT

Siparuna guianensis Aublet is known by several popular names according to the country and / or region of distribution in Brazil. In the Cerrado regions in the states of Mato Grosso and Goiás, it is known by the name of negramina, is of the family Siparunaceae and consists of two genera, Glossocalyx and Siparuna, with approximately 65 species of shrubs and trees, except for 15 species that are trees of 20 to 40 meters high, with trunks with diameters greater than 120 cm, which usually occur in the Amazon. *S. guinanensis* is often cited as native to West Africa, where it is often found in areas of the types of neotropical vegetation at elevations between sea level and 3800 meters. With interest in its medicinal properties, a polysaccharide was isolated from the leaves of *S. guinanensis*, purified and partially hydrolyzed to obtain structural oligosaccharide. An oligosaccharide was isolated from the hydrolyzed product. At the conclusion the hydrolysis provided two monoaquats, galactose and mannose at a molar ratio of 3: 5. Methylation, Carbon-13 Nuclear Magnetic R (NMR)esonance and periodate oxidation studies indicated the presence of (1 → 4) and (1 → 6) between units of structural monosaccharides.

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Citation: Augustus Caeser Franke Portella, Aloisio Freitas Chagas Junior, Gessiel Newton Scheidt, AlexSander Rodrigues Cangussu and Raimundo Wagner de Sousa Aguiar. 2017. "Analysis of binding in an Octasaccharide of Siparuna guianensis Aublet by methylation, and NMR studies", *International Journal of Development Research*, 7, (08), 14844-14846.

INTRODUCTION

Siparuna guianensis also known in the north and northeast of Brazil as negraine, leaf-santa, sailor, capitiú, dog killer, catingoso, brave lemon and guianese cicatrizante, holy grass, scent mixture, dog killer , Jaqueira, catingueira-de-paca, fedegoso, among others (Valentini et al., 2010). It is a plant of the family Siparunaceae, which consists of two genera, Glossocalyx, a species from West Africa, and Siparuna, with approximately 65 phylogenetically basal species in the Neotropics, mostly in the Andes (Renner, 1997). The genus *Siparuna* includes shrub and tree species, whose height hardly exceeds 5 meters, except for 15 species that are trees of 20 to 40 meters of height, with trunks with diameters bigger than 120 cm, that usually occur in the Amazon and in the protected areas of the Guianas, suggesting that this group initially diversified after adaptation at high altitudes, as in the high Andes. (Renner and Hausner, 2005).

In several Brazilian states, the medicinal use of *S. guianensis* has been reported. According to Valentini et al., (2010) its main form of use is the decoction of its leaves for use in the form of baths, especially for influenza, fever and pain in the body. Portella et al. (2014) in previously reported studies report the use of essential oil extracted from the shoot of *S. guianensis* as a potential insecticide in the control of mosquitoes transmitting dengue, chicungunha and yellow fever. Numerous are the classes of chemical compounds that can be extracted from our plant species. One is represented by the polysaccharides which are natural polymers, which may consist of single or different types of monosaccharides. Cellulose, alginate and gum arabic are examples of homo-, co-, and heteropolysaccharides, respectively. Those with industrial applications are extracted from plants - including algae, animals and fungi or are obtained via microbiological fermentation. In the upper plants these can be obtained from exudates, seeds, fruits and tubers.

MATERIALS AND METHODS

Plant collection and extraction

The harvest of leaves and branches of *S. guianensis* Aublet was carried out in a fragment of Cerrado in the municipality of Formoso do Araguaia, in the north of Brazil, Latitude: 11 º 47' 48" S Longitude: 49 º 31' 44" W between the February and March 2014 and 2015 respectively. Exsicata of the botanical material was deposited at the Federal University of Tocantins (registry 10.298). The leaves (960 g) were dried and triturated by thorough maceration with EtOH: H_2O (7:3) at room temperature. The hydroalcohol extracts were evaporated under reduced pressure at 50 °C to ¼ of their initial volume and held for two days at 4 °C. Then these solutions and the filtrates were subjected to successive paroxysms with n-hexane, CH_2 C_{l2}, AcOEt and n-ButOH. The solvent from each organic phase was evaporated to obtain the respective extracts from the leaves. The extraction process followed the protocol described by Zhao et al. (2005), with modifications. For this, 55 g of the lyophilized product was subjected to two consecutive aqueous extractions, a cold aqueous extraction (AQF) and a hot aqueous extraction (AQQ). All analyzes were done in triplicates.

These extractions were conducted in three 500 ml beakers where distilled and deionized water (Milli-Q) was added to give a 1:20 solid / solute ratio. Under magnetic stirring, the cold extraction (AQF) was first carried out for 3 hours at room temperature (25 °C). At the end the sample was centrifuged at 3000xg for 15 minutes and at a temperature of 8 ºC. The supernatant was separated and stored and the moist solid part was weighed and the volume was completed, the initial weight being reached. Hot extraction (AQQ), which occurred on the same shaker, was continued for 3 hours at a temperature of 50 °C. This extract was also centrifuged and the supernatant collected. In the supernatants qualitative test for carbohydrates was done, according to Dubois et al. (1956), and qualitative test for starch, with lugol. Aqueous supernatants AQF and AQQ (from both batches and repeats) were named in order to identify batch and repeat. These had their volume reduced by rotating evaporation at 70 °C and vacuum in a rotating evaporator (60 rpm), until each sample reaches the volume of 200 ml.

Aiming at the separation of polysaccharides and oligosaccharides from the supernatants obtained in the first step, 3 volumes of 99.8% PA ethanol were added, promoting the precipitation of the polysaccharides. The samples were again centrifuged at 3000xg for 15 minutes at 8 °C and their portions were separated so that the low molecular weight carbohydrate (MBMM), oligosaccharide (Oligo) fraction was present in the supernatant, and the solids represented the fraction Polysaccharide (Polys).

Nuclear Magnetic Resonance Analysis

Nuclear magnetic resonance spectra were obtained using Bruker, Avance series, model DRX400 spectrometer. The lyophilized samples were diluted in D_2O, ratio 2: 1 (samples and water), and lyophilized, aiming the exchange of hydrogen by deuterium. After this initial procedure all samples were sent for analysis in a Bruker Advance DRX 400 Spectrometer equipped with a 5mm inverted detection probe.

The base frequency was 400.13 MHz for 1H and 100.63 MHz 13 C. The analyzes were done at 50 °C. 13C and 2D HSQC experiments were performed. The chemical signals were expressed relative to the internal standard of acetone set at 30.20 ppm (13C). The spectra were constructed and analyzed with the ACD / NMR Processor Academic Edition1, version 12.01 (March/ 2010).

RESULTS AND DISCUSSION

The 13C-NMR spectrum of the polysaccharide PPNA (Figure 1) shows approximately 4 signals in the anomeric carbon region (δ 110-90). The signal isolated at δ103.7 was assigned to the non-reducing β-Galp terminal (Goring and Mazurek, 1975; Tischer et al., 2002). The signals in δ 96.1, δ 96.0 and δ 92.2 were attributed to α-Manp, and the β- and α-Manp reducing units respectively. (Wagner et al., 2004). In the substituted carbon region, the signals in δ 81.4 and δ 80.5, refer to the C-3 of the substituted α-L-Araf and β-D-Galp units, respectively (11,12). From the literature data, the signals in δ 76.5, δ 75.8 and δ 69.3 could be attributed to the unsubstituted C-5, C-3 and C-4 respectively of β-D-Galp units (8,11). The signal at δ 61.5 can be attributed to unsubstituted C-6 of β-D-Galp units and to C-5 of α-L-Araf units (Delgobo et al., 1999).

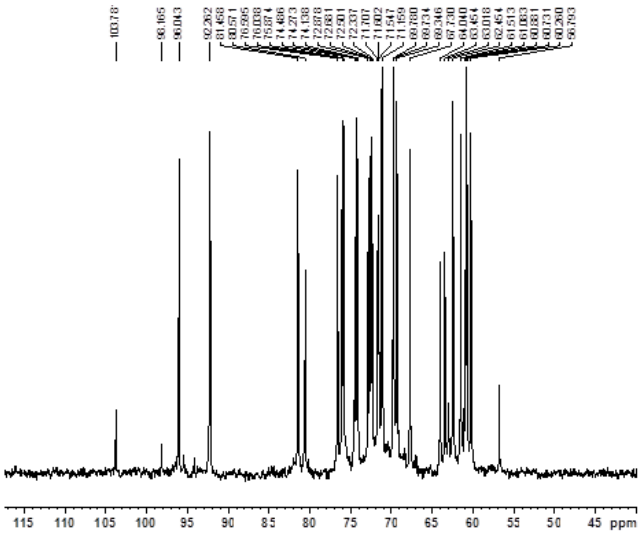

Figure 1 - 13C NMR Spectrum of the PPNAS2 Polysaccharide MeSO-d6 solvent at 60 ° C. Displacements are expressed in TM (ppm)

The C-5 and C-6 methoxyl positioning was verified by HSQC experiment with the correlation of the signal δ 76.5, δ75.8 and δ69.3 with respect to the hydrogen of the methyl group (Figure 2). By analyzing the ESI-MS, the monosaccharide composition and the 13C-NMR spectrum (Figure 3), oligo-0.3 was characterized as being predominantly disaccharide β-D-GlcpA- (1 → 6) -α- B-D-Gal.

The 13C-NMR spectrum of oligo-0.3 presents 2 signals in the anomeric carbon region. The signals at δ 81.4 and δ 67.7 were attributed to C-4 and the methyl group (Delgobo et al., 1999). The 4-Me-GlcpA units of oligosaccharide 4-Me-β-D-GlcpA (1 → 6) -α-β-D-Gal (oligo-0.34) are also present in lesser amounts in this fraction. Figure 4 also shows the analysis of the two-dimensional spectrum, showing the homonuclear correlations

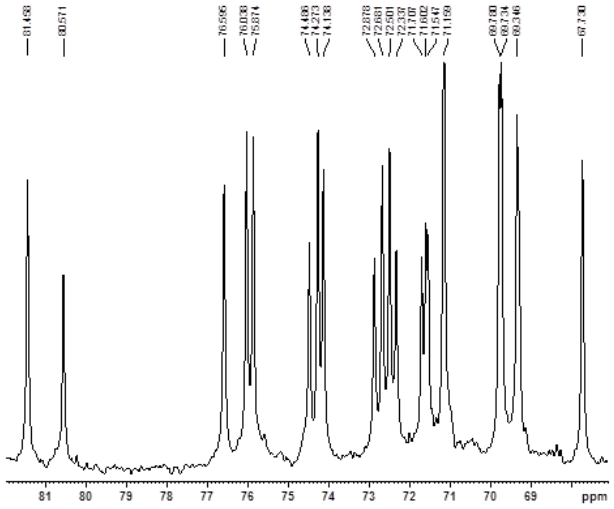

Figure 3 - 13C-NMR spectrum of Oligosaccharide 0.3. MeSO-d 6 solvent at 60 ° C. Displacements are expressed in TM (ppm)

Oligo-0.37 presented mannose as the main component and the results of ESI-MS and 13C NMR allowed its characterization as the oligosaccharide β-D-GlcpA- (1 → 4) –α-β-D-Manp. In the anomeric region of 13C-NMR Signals in δ 81.4 and δ 80.5 were attributed to β-Manp, α-Manp and α-Manp reductant β-GlcpA non-reducing terminals respectively. The signal at δ 76.5 was assigned to the substituted C-2 of the α-Manp units (Wagner et al., 2004).

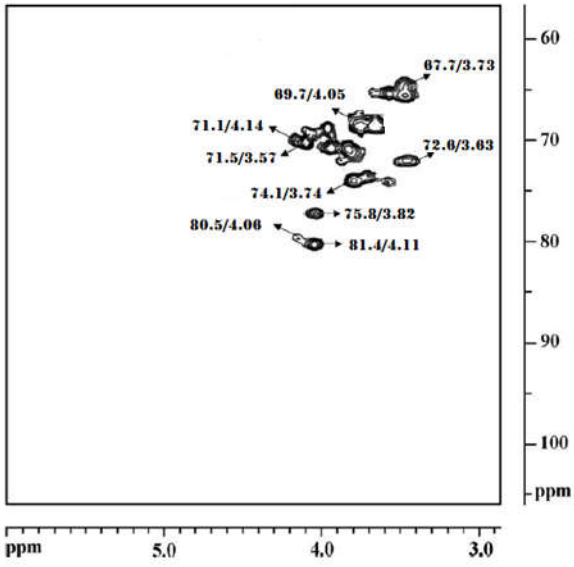

Figure 4: NMR Spectrum: 2D (HSQC) of the AQF portion of Oligosaccharide with internal standard of acetone and calibration 2.22 ppm (1H) and 30.25 ppm (13C)

Conclusion

Analysis of NMR data suggests that this polymer is probably composed mostly of galactose and has a (1 → 4) -β-Galp and (1 → 6) -β-D-galactan backbone where it can possible arabinose and rhamnose units are linked. It is possible to also notice the signal of great intensity in the region close to 70 ppm, which can be related even to the amino sugars.

Isolated carbohydrates present great complexity and it is necessary the continued study of these so that it is possible to clarify its structure and composition, with emphasis on the oligosaccharide fraction.

Therefore, because these polymers are widely studied as to their benefits when ingested, it is necessary to study the possible benefits that their use in food and feed may present. The analysis of NMR data suggests that this polymer is probably composed mostly of galactose and has a (1 → 4) -β-Galp and (1 → 6) major chain linkages between structural monosaccharide units.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Acknowledgement

The development of this research benefited from the New Researchers Program of the Federal University of Tocantins (PROPESQ / UFT).

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