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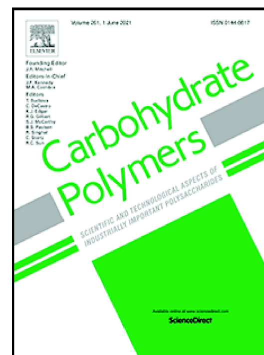

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A novel substitution pattern in glucuronoarabinoxylans from woody bamboos

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Abstract

(1→4)-β-D-Xylans are the second most abundant plant biopolymers on Earth after cellulose. Although their structures have been extensively studied, and industrial applications have been found for them and their derivatives, they are still investigated due to the diversity of their structures and uses. In this work, hemicellulose fractions obtained previously with 1M KOH from two species of woody bamboos, *Phyllostachys aurea* and *Guadua chacoensis*, were purified, and the structures of the glucuronoarabinoxylans (GAX) were studied by chemical and spectroscopic methods. In both cases, major amounts of α-L-arabinofuranose residues were linked to C3 of the xylose units of the backbone, and also α-D-glucuronic acid residues and their 4-*O*-methyl-derivatives were detected in minor quantities, linked to C2 of some xylose residues. Methylation analysis of the carboxyl-reduced derivative from GAX from *P. aurea* indicated the presence of terminal and 5-linked arabinofuranose units. NMR spectroscopy showed the presence of disaccharidic side chains of 5-*O*-α-L-arabinofuranosyl-L-arabinofuranose for the GAX from *P. aurea*, while for those of *G. chacoensis*, only single side chains were found. To the best of our knowledge, this disaccharide was not found before as side chain of xylans.

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Keywords: xylan, woody bamboo, *Phyllostachys aurea, Guadua chacoensis,* disaccharide side chain, Poaceae

1. Introduction

Xylans are plant cell wall polysaccharides widely used in many commercially relevant agricultural products, such as food, forage, and timber. They represent up to 50 % of the total mass of the cell walls of grasses, as woody bamboos, being the most important hemicellulose component of their primary and secondary cell walls. Moreover, they are considered the second most abundant plant biopolymers on Earth after cellulose (Scheller & Ulvskov, 2010).

They consist of linear chains of (1→4)-β-D-xylopyranose residues with different degree and pattern of substitution of their backbone depending on their origin. Based on the nature of glycosyl substitutions, xylans are generally categorized as glucuronoxylans that are mainly found in moss, seedless vascular plants, non-commelinid monocots and dicots (Kulkarni et al., 2012; Peña, Kulkarni, Backe, Boyd, O'Neill, & York, 2016; Teleman, Tenkanen, Jacobs & Dahlman, 2002); glucuronoarabinoxylans, found in grasses and gymnosperms except Gnetophyta (Verbruggen et al., 1998; Busse-Wicher et al., 2016; Jacobs, Larsson, & Dahlman, 2001); and arabinoxylans, present in cereal grains (McCleary et al., 2015).

Glucuronoarabinoxylans (GAX) from grasses have high amounts of α-L-arabinofuranose substituents on their backbone (Scheller & Ulvskov, 2010). They exhibit the greatest diversity of side chains and linkages, which have been shown to vary significantly between organisms and even between different tissues of the same plant (Peña et al., 2016). It has been hypothesized that the change in xylan chemical composition that occurred with GAX evolution led to the replacement of the functional role of pectins, extensins (Curry, Peña, & Urbanovich, 2023), and other hemicelluloses present in primary cell walls from dicots, with xylans in the case of commelinid primary cell walls (Carpita, 1996). The α-L-arabinofuranose residues are usually

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linked to C3 of the xylose units of the backbone, but also in minor amounts to both C2 and C3. In addition, these hemicelluloses show substitution with α-D-glucuronic acid residues and their 4-*O*-methyl-derivative in minor quantities, linked to C2 of some xylose residues. Most of these substituents are single chains. However, disaccharidic and even trisaccharidic side chains have been reported, which were obtained for example, from maize bran (Saulnier, Vigouroux, & Thibault 1995), by alkaline extraction of sorghum (Verbruggen et al., 1998), corn cobs (Ebringerová, Hromádková, Alfodia, & Hrıbalova, 1998), and barley husk (Höije, Sandström, Roubroeks, Andersson, Gohil, & Gatenholm, 2006), and also from *Festuca arundinacea* and *Panicum virgatum*, and many other species of grasses, generalizing their presence on these cell walls (Wende & Fry 1997, Bowman, Dien, Vermillion, & Mertens, 2014). They comprise 2-*O*-β-D-xylopyranosyl-L-arabinofuranose, 2-*O*-α-L-arabinofuranosyl-L-arabinofuranose, and 4-*O*-L-galactopyranosyl-2-*O*-β-D-xylopyranosyl-L-arabinofuranose. Besides, xylans have non-carbohydrate substituents linked through ester bonds, that are lost during the alkaline extraction usually performed to obtain them (Scheller & Ulvskov, 2010).

Bamboos (Bambusoideae) are among the fastest-growing plants; this characteristic makes them interesting sources of biomass. They comprise aerial and underground parts (culms, and rhizome and roots, respectively) (Shanmughavel & Francis, 1997). Bamboo shoots are the new culms that arise from the rhizomes, containing nodes and internodes in a vertically miniaturized form (Choudhury, Sahu, & Sharma, 2012). In previous papers, the cell wall material from two species of woody bamboos, *Phyllostachys aurea* and *Guadua chacoensis* were studied by a classical sequential extraction of their polysaccharides using different aqueous solvents, namely, 0.05 M CDTA at pH=6, 0.05 M Na_2CO_3, and 1M and 4M KOH solutions (Fry, 1988), and chemical and spectroscopic analyses of the extracts. In both cases, the highest yields were

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obtained for the first extract obtained with 1M KOH, which was constituted by major amounts of GAX and minor quantities of β-D-glucans, and possibly also pectins (Zelaya, Fernández, Vega, Mantese, Federico, & Ciancia, 2017, Fernández, Zelaya, Vega, Cobello, & Ciancia, 2019).

In this paper, purification of both extracts and fine structural determination of the purified GAX, by analysis of the original and carboxyl-reduced samples, allowed to detect differences in their substitution patterns. While the GAX from *G. chacoensis* showed the expected structural features, namely, single side chains of α-L-arabinofuranose and (4-*O*-methyl-)α-D-glucuronic acid, those isolated from *P. aurea* additionally presented important amounts of the disaccharide 5-*O*-α-L-arabinofuranosyl-L-arabinofuranose as side chains linked to C3 of some of the β-D-xylose units of its backbone. To the best of our knowledge, this disaccharide was not found before as side chain of xylans, so this paper contributes to the present understanding of the structural diversity of GAX from grasses.

2. Experimental

2.1. Extracts obtained from P. aurea and G. chacoensis with 1M KOH

The alcohol insoluble residues from shoots of both bamboo species were treated with α-amylase (type VI-B from bovine pancreas; Sigma), and the residues were sequentially extracted at room temperature with 0.05 M cyclohexane diamine tetraacetic acid (CDTA) at pH=6, 0.05 M Na_2CO_3, and 1M and 4M KOH solutions in the same way (twice with each solvent). The concentration of the sample subjected to each extraction procedure was 40g /L. The first extracts obtained with KOH 1M gave the highest yields for both bamboo species. They were dialyzed (MWCO 6,000-8,000) against water, and then freeze dried (Zelaya et al., 2017; Fernández et al., 2019). Here they are named PhyA and GuaC.

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2.2. Enzymatic treatment of PhyA and GuaC

Extracts PhyA and GuaC (100 mg) were incubated with cellulase (*Aspergillus niger*, Sigma) in sodium acetate buffer (50 mM, pH=5) at 50ºC for 48 h. The enzyme denaturalization was done by heating the reaction mixtures for 10 min in a boiling water bath and, when they reached room temperature, they were centrifuged. For each extract, the supernatant and the residue (residues PhyAp and GuaCp, for each woody bamboo species, respectively) were lyophilized for further analysis.

2.3. Reduction of carboxyl group

Reduction of the carboxyl groups of PhyAp and GuaCp was carried out according to Taylor & Conrad (1972). The GAX was dissolved in distilled water, and then 1 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDC) was added. The reaction mixture was agitated for 2 h at room temperature, keeping the pH at approximately 4.7 with addition of 0.1 M HCl when needed. Then, 20 mL 2 M $NaBH_4$ was added slowly to the mixture with a syringe within 60 min to reduce the carboxyl groups, keeping the pH at 7.0 with addition of 4 M HCl. Finally, the solution was dialyzed (MWCO 3,500 Da) against distilled water for 48 h and freeze-dried. This reduction process was repeated twice, and PhyAp-red and GuaCp-red were obtained.

2.4. General methods of analysis

The total carbohydrates content was analyzed by PhOH-H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The monosaccharide composition of the samples was determined by GC–MS of the alditol acetates, and N-propylaldonamide acetates, as described before (Zelaya et al., 2017).

2.5. Methylation analysis

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The samples (15 mg) were dissolved in DMSO (1 mL), and finely powdered NaOH (30 mg) was used as base (Ciucanu & Kerek, 1984), the mixture was agitated vigorously for 1 h, then cooled in an ice bath followed by addition of CH3I (0.5ml) drop by drop. The mixture was kept at room temperature for 1 h, and then it was poured into water (10 mL). Finally, it was dialyzed (MWCO 3,500) and freeze dried. The methylated samples were derivatized to the alditol acetates as indicated for the parent polysaccharides, and also to the aldononitrile acetates to determines the ratio 2MeXyl to 3MeXyl (Stortz, Matulewicz, & Cerezo, 1982).

2.6. Nuclear magnetic resonance spectroscopy

The samples (15 mg), previously exchanged by three repeated evaporations in D_2O, were dissolved in D_2O (1 mL) and placed in 5-mm tubes. Spectra were recorded at room temperature on a Bruker Avance II 500 spectrometer (Karlsruhe, Germany). For ^{1}H NMR experiments the parameters were: a spectral width of 6.25 kHz, a 76° pulse angle, an acquisition time of 3 s, a relaxation delay of 3 s, for 32 scans. For 125 MHz proton decoupled ^{13}C NMR experiments the parameters were: a spectral width of 29.4 kHz, a 51.4° pulse angle, an acquisition time of 0.56 s, a relaxation delay of 0.6 s, for 25,000 scans. Signals were referenced to internal acetone at 2.21 ppm for ^{1}H NMR and 31.1 ppm for ^{13}C NMR experiments. Pulse sequences for ^{1}H-^{13}C HSQC and HMBC techniques were supplied by the spectrometer manufacturer; spectra were recorded at room temperature, and they were obtained at a base frequency of 500 MHz for ^{1}H and 125 MHz for ^{13}C.

3. Results and Discussion

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Yields and analyses of the extracts obtained with 1M KOH during the sequential extraction of shoots from *Phyllostachys aurea* (PhyA) and *Guadua chacoensis* (GuaC) are shown in Table 1 (Zelaya et al., 2017; Fernández et al., 2019). It was clear that these extracts are constituted by major amounts of GAX and small amounts of mixed linkage glucans and/or xyloglucans. Besides, important differences in the molar ratio Xyl:Ara:uronic acids were found between GAX from both species (1: 0.67:0.07 and 1: 0.28:0.08, respectively). Two structural details are noteworthy, namely, the presence of terminal non-reducing galactose units in both extracts, and the important amount of 5-linked arabinofuranose residues detected only in GAX from *P. aurea*, which could arise from a small amount of pectin (Hosmer Caffall & Mohnen, 2009).

Table 1.

Yields and analyses of purified glucuronoarabinoxylans from shoots of *Phyllostachys aurea* (PhyAp) and *Guadua chacoensis* (GuaCp), and the carboxyl-reduced polysaccharides obtained from them (PhyAp-red and GuaCp-red)

Extract	Yield[a] %	Total Carbohydrate %	Total Protein %	Monosaccharide composition (mol %)						
				Ara	Xyl	Gal	Glc+4-*O*-Me Glc	GalA	GlcA	4-*O*-Me GlcA
PhyA[b]	12	49	19	28	41	5	21	3	1	2
PhyAp	85	88	-	34	57	-	-	-	6	3
PhyAp-red	72	92	-	34	57	-	9[d]	-	-	-
GuaC[c]	10	32	19	17	60	6	9	3	4	1
GuaCp	88	90	-	18	67	-	-	-	9	6
GuaCp-red	76	93	-	18	67	-	15[d]	-	-	-

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[a]Percentage of cell wall material for PhyA and GuaC; percentage of the total recovered after the treatment with cellulase, for PhyAp and GuaCp; percentage respect the non-reduced material for PhyAp-red and GuaCp-red. [b]PhyA is the first extract obtained with 1M KOH during the sequential extraction of *P. aurea* (Zelaya et al. 2017), included for comparison. [c]GuaC is the first extract obtained with 1M KOH during the sequential extraction of *G. chacoensis* (Fernández et al. 2019), included for comparison. [d]The percentages of glucuronic acids were the same as those of glucose plus 4-*O*-methylglucose in the reduced samples.

GAX were purified by treatment with cellulase, in order to eliminate the β-D-glucans present in the sample. After the enzyme treatment, the samples were centrifuged. The residues obtained (PhyAp and GuaCp) were analyzed, showing only the presence of xylose, arabinose, and (4-*O*-methyl)-glucuronic acid as monosaccharide constituents (Table 1). Comparison of the GAX before and after purification indicates that a small amount of pectic material, which comprised galacturonic acid, terminal galactose units, and possibly xylose and arabinose, were also lost during the purification step. It is also important to remark that, the total protein was lost after purification (for both the extracts). Besides, before purification, the solubility of the samples was very low, but after purification they were soluble in aqueous solutions.

Structural analysis of the GAX was carried out on the purified samples (PhyAp and GuaCp) and their uronic acids reduced derivatives (PhyAp-red and GuaCp-red). Table 2 shows results from methylation analyses of the latter polysaccharides. As glycosidic linkages of uronic acids are more difficult to hydrolyze than those from neutral monosaccharides, methylation analysis of the reduced derivatives is more reliable (Bemiller, 1967). This method was successfully applied for the study of other complex xylans comprising uronic acid units (Sznaider, Rojas, Stortz, & Navarro, 2020), as well as to many other polysaccharides (Chaves, Iacomini, & Cordeiro, 2019; Cui, Wang, Cao, Ismael, Wang, & Lü, 2023).

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Table 2

Methylation analyses of the uronic acids reduced derivatives of the purified samples obtained from shoots of *Phyllostachys aurea* (PhyAp-red) and *Guadua chacoensis* (GuaCp-red).

Monosaccharide[a,b]	Structural unit	PhyAp-red	GuaCp-red
2,3,5-Ara	Ara*f* (1→	24	18
2,3-Ara	→5) Ara*f* (1→	10	-
2,3,4-Xyl	Xyl*p* (1→	2	2
2,3-Xyl	→4) Xyl*p* (1→	22	32
2-Xyl[c]	→3,4) Xyl*p* (1→	21	16
3-Xyl[c]	→2,4) Xyl*p* (1→	12	16
2,3,4,6-Glc	Glc*p* (1→	9	15

[a]Monosaccharides having methyl groups at the positions indicated (for example, 2,3-Ara corresponds to 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylarabinitol, etc.). [b]In mol %. [c] Unresolved alditols, distinguished by aldononitriles.

Considering results from methylation analysis before (Zelaya et al., 2017; Fernández et al., 2019) and after purification, no evidence of degradation was found, and calculation of an approximate molecular weight gave values of ~7.0/7.5 KDa for both samples (Table 2 and see bellow Table 5).

The differences between the structures of both GAX are very clear. That from *P. aurea* has a higher degree of substitution (1:0.60 and 1:0.51, for PhyAp-red and GuaCp-red, respectively), calculated considering the substituted xylose units regards the total amount of 4-linked xylose. The substitution pattern is also different: the amount of arabinose side chains is higher for *P. aurea*, while that of glucuronic acid is higher for *G. chacoensis*. A common characteristic is the absence of disubstituted xylose units in the GAX backbones. Nevertheless, the most important

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difference is presence of significant amount of 5-linked arabinofuranose in PhyAp-red, while this unit was not found in GuaCp-red. Due to the high degree of homogeneity of PhyAp-red, the possibility that this structural unit were part of a contaminant polysaccharide, as a pectin arabinan moiety, is unlikely. Hence, it should be part of the GAX structure, this hypothesis was evaluated by NMR spectroscopy.

Fig. 1 shows the HSQC spectra of PhyAp and GuaCp and their reduced derivatives. In the anomeric region, the broad signal corresponding to the 4-linked β-D-xylopyranose units, partially substituted on C3 with α-L-arabinofuranose units was found at ~ 102.5/4.40 ppm. In addition, a signal at 102.2/4.61 ppm corresponds to the latter units substituted on C2. The signals at 98.4/5.30 and 98.5/5.33 ppm were assigned to C1/H1 of α-D-glucuronic acid and α-D-glucopyranose in the spectra of the original samples and their carboxyl-reduced derivatives, respectively. The presence of two signals corresponding to α-L-arabinofuranose units at 108.6/5.32 and 108.4/5.01 ppm are clear in PhyAp and PhyAp-red, while in the corresponding spectra of *G. chacoensis,* only the former signal was found, and it was assigned to terminal α-L-arabinofuranose units, while the second one, to 5-linked α-L-arabinofuranose units. Moreover, it was possible to assign the full spectra, which indicate that GAX from *P. area* have, not only single chains, but also disaccharidic arabinose chains, as shown in Table 3, while for the GAX of *G. chacoensis*, only single chains were detected. In particular, small signals at 83.3/4.17 and 67.7/3.72, 3.81 ppm corresponding to C4/H4 and C5/H5,5' are evident in the spectra of PhyAp and PhyAp-red, confirming the presence of 5-linked α-L-arabinofuranose units.

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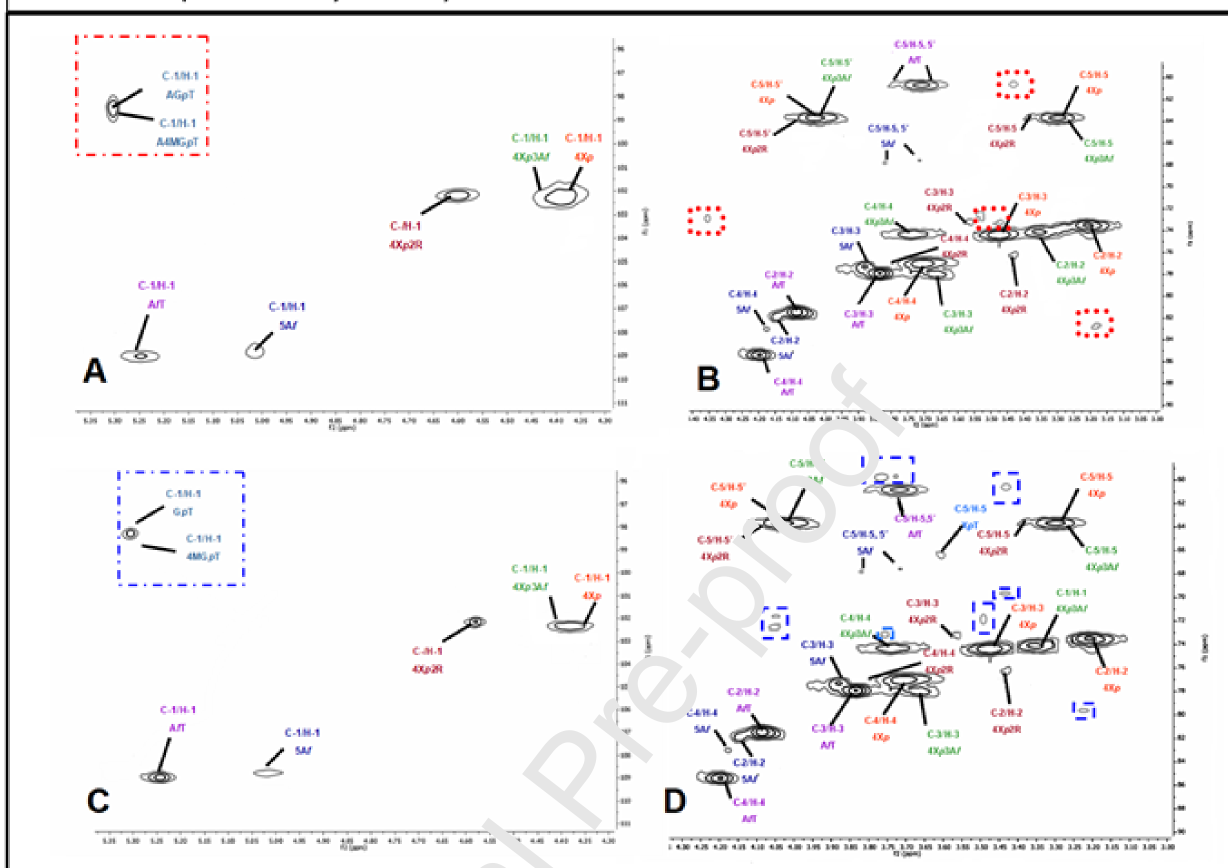

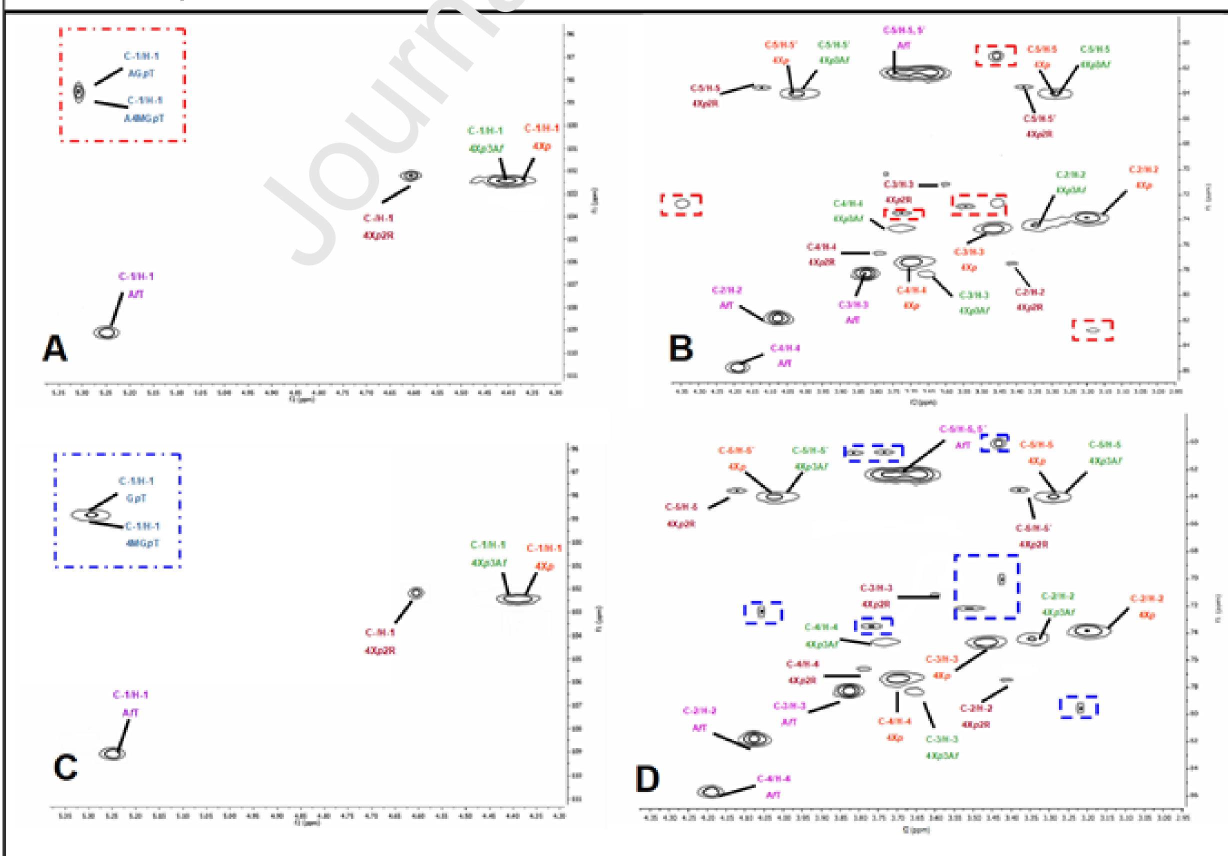

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Fig. 1. HSQC NMR spectra of PhyAp, PhyAp-red, GuaCp, and GuaCp-red Major signals present in (A) and (C) the anomeric region, and (B) and (D) the region corresponding to C2/H2-C6/H6 of the purified GAX and their carboxyl-reduced derivatives, respectively. Signals corresponding to glucuronic acid and glucose side chains are highlighted with red and blue boxes, respectively.

Table 3

NMR spectroscopic assignment of structures present in PhyAp and GuaCp and their reduced derivatives.

Unit[a]	Spectrum code	C1 / H1	C2 / H2	C3 / H3	C4 / H4	C5 / H5, 5´	C6 / H6, 6´	4- *O*-Me
→4)-β-D-Xyl*p*(1→	4X*p*	102.5 / 4.40	73.8 / 3.20	74.6 / 3.47	77.3 / 3.70	63.8 / 3.28, 4.00		
→3, 4)-β-D-Xyl*p*(1→	4X*p*3A*f*	102.5 / 4.40	74.5 / 3.35	78.2 / 3.66	74.6 / 3.74	63.7 / 3.28, 4.00		
→2, 4)-β-D-Xyl*p*(1→	4X*p*2R	102.2 / 4.61	77.2 / 3.41	73.2 / 3.59	76.4 / 3.78	63.5 / 3.37, 4.11		
α-L-Ara*f* (1 →	A*f*T	108.6 / 5.32	81.7 / 4.09	78.2 / 3.84	85.7 / 4.19	62.2 / 3.73, 3.80		
→5)-α-L-Ara*f* (1→	5A*f*	108.4 / 5.01	82.4 / 4.14	77.5 / 3.87	83.3 / 4.17	67.7 / 3.72, 3.81		
α-D-GlcA*p* (1→	GA*p*T	98.5 / 5.28	72.6 / 3.55	73.4 / 3.70	72.7/ 3.47	72.8 / 4.32		
4-*O*-Me-α-D-GlcA*p* (1→	4MGA*p*T	98.5 / 5.28	72.6 / 3.55	73.2/ 3.73	83.3 / 3.20	72.8 / 4.32		60.8 / 3.45
α-D-Glc*p* (1→	G*p*T	98.[illegible] / 5.30	72.1 / 3.53	73.4 / 3.75	70.1 / 3.42	72.5 / 4.05	60.9 / 3.72, 3.80	
4-*O*-Me-α-D-Glc*p* (1→	4MG*p*T	98.[illegible] / 5.30	72.2/ 3.51	73.4 / 3.75	79.5/ 3.23	71.4/ 4.05	60.9 / 3.72, 3.80	60.8 / 3.45

[a]Assignments were performed based on different NMR experiments, and previous reports (Ebringerová et al., 1998; Verbruggen et al., 1998; Mazumder & York, 2010; Cui, Wood, Blackwell, & Nikiforuk, 2000; Hromádková, Koštálová, Vrchotová, & Ebringerová, 2014; Liu et al. 2021)

To confirm the structure proposed for PhyAp, the HMBC spectrum of PhyAp-red was analyzed (Fig. 2). Table 4 gives the correlation values.

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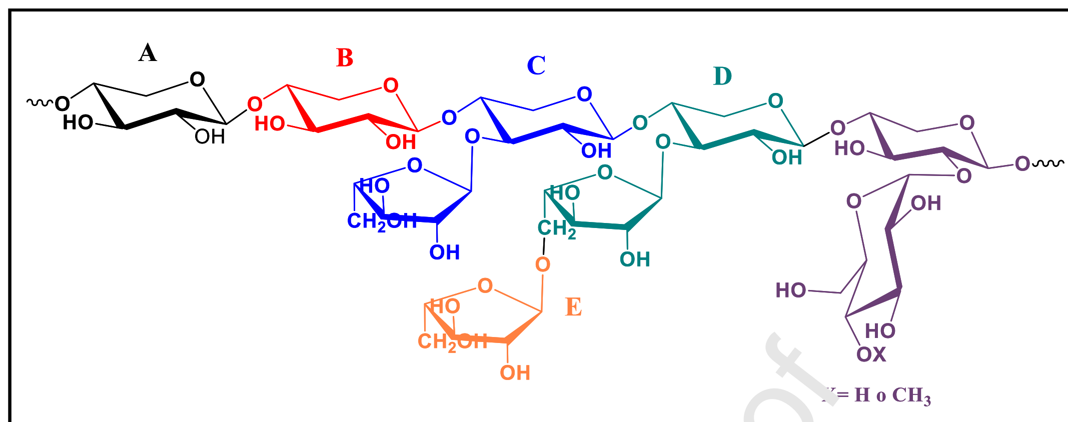
A



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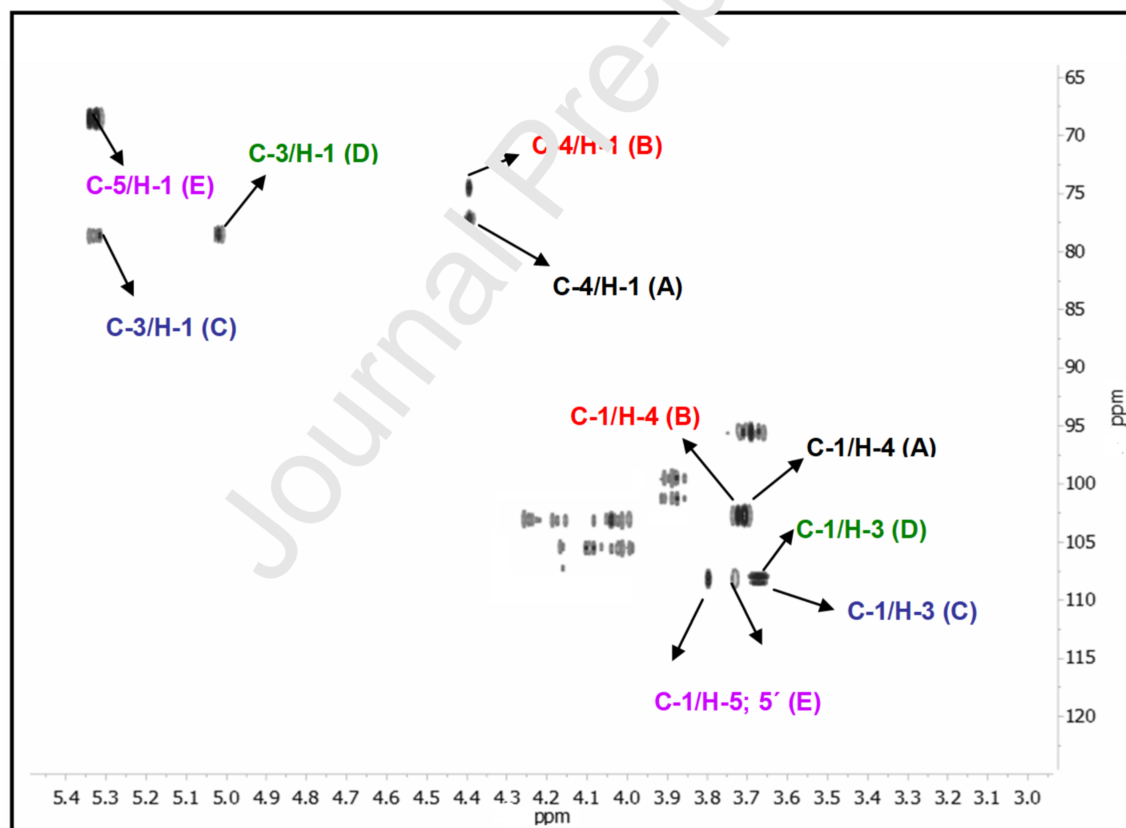

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Figure 2. (A) A possible fragment of PhyAp-red showing the linkages between the α-L-arabinofuranose and β-D-xylopyranose units. Capital letters refer to the different linkages. (B) HMBC spectrum with the corresponding assignments.

Table 4.

Correlation values of the HSQC spectrum of PhyAp.

Linkage	Assignment	Coupling	Signals (ppm)
A	β-D-Xyl*p* (1→4) β-D-Xyl*p*	C1/H4 - C4/H1	102.5/3.70 - 77.3/4.40
B	β-D-Xyl*p* (1→4) β-D-Xyl*p* (3-Ara*f*)	C1/H4 - C4/H1	102.5/ 3.74 - 74.6/ 4.40
C	α-L-Ara*f* (1→3) β-D-Xyl*p*	C1/H3 - C3/H1	108.6 / 3.66 - 78.2/5.32
D	5- α-L-Ara*f* (1→3) β-D-Xyl*p*	C1/H3 - C3/H1	108.4 / 3.66 - 78.2/ 5.01
E	α-L-Ara*f* (1→5) α-L-Ara*f*	C1/ H5, 5´ - C5/H1	108.6 / 3.81, 3.72 - 67.7/ 5.32

Correlation (D) indicates that a 5-linked α-L-arabinofuranose unit is linked to C3 of a 4-linked β-D-xylopyranose units of the backbone, and correlation (E) proves that the former unit has an α-L-arabinofuranose unit linked by C5.

In summary, although disaccharidic arabinose side chains in GAX from Poaceae have been characterized before, these units had (1→2)-linkages. In this work, it was shown that GAX from shoots of *Phyllostachys aurea* has 5-*O*-α-L-arabinofuranosyl-L-arabinofuranose as side chains, linked to C3 of some of the β-D-xylose units of its backbone in addition to the classical monosaccharidic side chains. Table 5 shows the composition in structural units for both GAX.

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Table 5.

Composition in structural units deduced by methylation analysis of purified GAX of the extracts obtained with 1M KOH from shoots of *Phyllostachys aurea* and *Guadua chacoensis*.

GAX	Xyl[a]	Xyl(Ara)$_2$[b]	Xyl(Ara)[c]	Xyl (GlcA)[d]	Xyl (MeGlcA)[e]	DP[f]
PhyA	12	5	7	3	2	51
GuaC	17	-	9	5	3	51

[a]Unsubstituted xylose units. [b]Xylose units substituted with 5-*O*-α-L-arabinofuranosyl-L-arabinofuranose. [c]Xylose units substituted with terminal arabinose. [d]Xylose units substituted with glucuronic acid. [e]Xylose units substituted with 4-*O*-methyl-α-glucuronic acid. [f]Degree of polymerization.

As this disaccharidic side chain was not found in shoots from *G. chacoensis*, it could be a characteristic of *Phyllostachys*, or at least some of its species. The genus *Phyllostachys* is worldwide distributed (Ibrahim et al., 2021), and it is one of the largest genera of woody bamboos (Hodkinson, Renvoize, Chonghaile, Stapleton, & Chase 2000), as well as being one of the most studied regarding its cell wall components (Bai et al., 2022).

In a detailed structural study of the oligosaccharides obtained by Driselase^{TM} treatment of shoots from *P. edulis* (Ishii & Hiroi, 1990; Ishii, 1991), the structure presented here was not found. On the other hand, a later work on the same material (Edashiige & Ishi, 1998) reported the presence of units of 5-linked arabinofuranose in the methylation analysis of the alkaline extracts, similarly to what was found for *P. aurea* (Zelaya et al., 2017). As, no purification of the alkaline extract was done, these units could correspond to pectin remains present in these samples and/or to the type of GAX side chains found in this work. Further investigations are needed to elucidate this issue.

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4. Conclusion

The use of cellulase as an enzyme for purification of GAX obtained by alkaline aqueous extraction proved to be a simple, inexpensive, and very efficient method. The purified GAXs and their reduced derivatives were studied in detail. In this way, it was possible to find the disccharide 5-*O*-α-L-arabinofuranosyl-L-arabinofuranose as side chain of the GAX from *P. aurea,* which was not present in that of *G. chacoensis*, being this an important structural difference. In addition, substitution with single side chains of α-L-arabinofuranose and (4-*O*-methyl)-α-D-glucuronic acid was found in both cases, but in different quantities. This method of purification could be extended to many other cases in which xylans are obtained with important amounts of other hemicelluloses, as mixed linkage glucans and/or xyloglucans, avoiding time consuming preparative chromatographic separations.

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Marina Ciancia: Investigation; Visualization; Roles/Writing - original draft; and Writing - Review & Editing; Funding acquisition

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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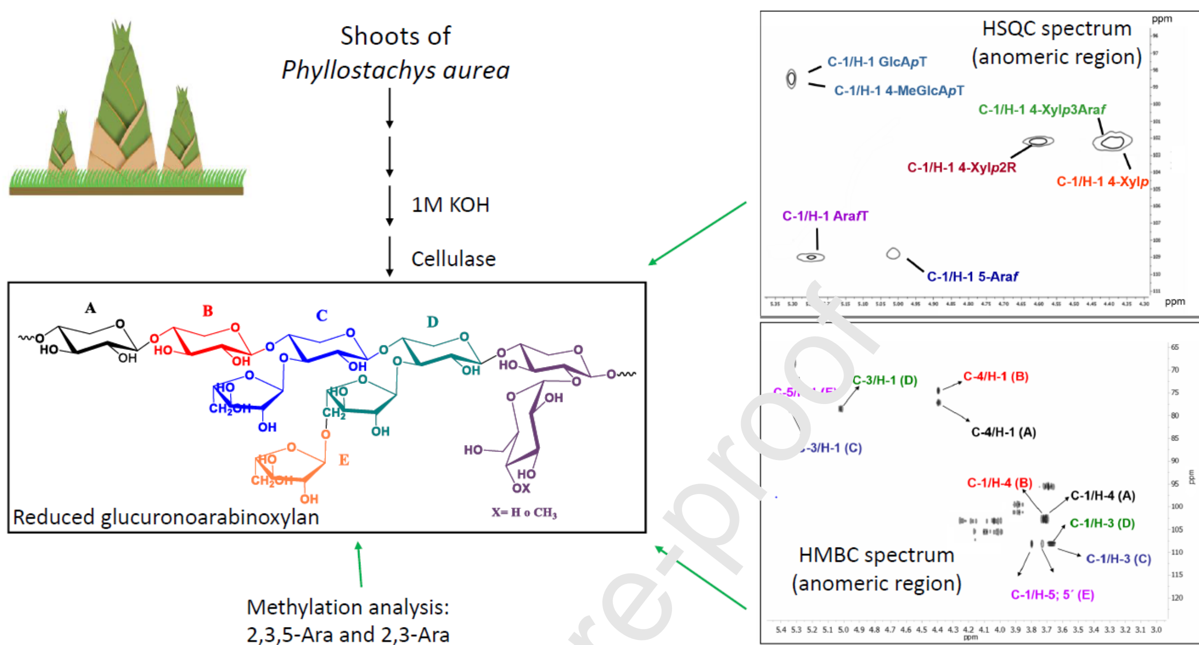

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