



AN ACIDIC XYLOGLUCAN FROM GRAPE SKINS

JOSÉ M. IGARTUBURU*, E. PANDO, F. RODRÍGUEZ LUIS and ANTONIO GIL-SERRANO†

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain; †Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, 41071 Sevilla, Spain

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Abstract—The structure of one hemicellulose A fraction (A-1) isolated from grape skins (*Vitis vinifera*) of the Palomino variety has been studied by methylation analysis, NMR spectroscopy and partial acid hydrolysis. Hemicellulose A-1 appeared to be homogeneous with a weight-average M_w of 35 544. It was shown to be a linear acidic xyloglucan constituted by a linear chain of xylopyranosyl and glucopyranosyl residues linked by β -(1 $\rightarrow$ 4) glycosidic bonds containing 4-*O*-methylglucuronopyranosyl acid, L-arabinofuranosyl and xyl-opyranosyl residues attached at position 2, in a ratio of one residue for every 10 units of xylose in the main chain, and L-arabinofuranosyl and glucopyranosyl residues attached at position 6 of the units of glucose, in a ratio of one residue for every four glucoses in the main chain. © 1997 Elsevier Science Ltd

INTRODUCTION

Grape pomace is the residue obtained after grapes have been pressed in order to produce must. The pomace from grapes of the *Palomino* variety is the main by-product of the sherry wine-making industry of the Jerez area. This by-product is currently under-exploited, its main application, after being fermented, is distillation into ethyl alcohol (vinic alcohol). However, this alcohol is produced more economically by distillation of surplus lower quality wine and currently only a small proportion of the total amount of pomace can be used in this way. Given this situation, we have opened a line of research dedicated to the study of this type of grape pomace. Our aim is to propose its transformation into other more economically valuable raw materials. A subordinate aim is to help solve the problem of the disposal of unused pomace which, at present, causes serious contamination of waterways.

Grape pomace consists of three different components, seeds (*granillas*), stalks (*raspones*) and skins (*hollejos*). Grape skin represents between 3 and 6% of the total dry weight of pomace. In previous research, we have analysed its lipid, protein and dietary fibre composition [1–8]. Dietary fibre is the main component of grape skin of the *Palomino* variety and represents ca 74% by the weight of this material; grape seeds contain a slightly higher proportion (76%) of dietary fibre. The major fraction of the poly-

saccharides from this dietary fibre is hemicellulose (30.7% by weight), followed by cellulose (ca 6%) [5]. The present paper describes the structural determination of a polysaccharide isolated from a hemicellulose A fraction of dietary fibre from skins of this grape cultivar.

RESULTS AND DISCUSSION

A study of dietary fibre from *Palomino* grape skin [5] by the Southgate method [9] has shown that hemicelluloses are the most important fraction of polysaccharides in this material (ca 31% by weight), followed by cellulose (ca 6%). Therefore, our study concentrates on the holocellulose fraction (hemicelluloses and cellulose) from this material. This fraction represents ca 26% of the total weight of grape skin.

Powdered grape skins were extracted sequentially with hexane, ethanol and chloroform-methanol, treated with 0.25 M methanolic sodium methoxide [10] and then extracted with water. Previous work by our group has shown that there is no starch in *Palomino* grape skins [5]; therefore it is not necessary to include a prior extraction stage with hot water or dimethylsulphoxide to solubilize this component. In addition, the fraction of mucilaginous, gums, and pectins is very small and is completely removed by extraction with water subsequent to sodium methoxide treatment. The residual solid was delignified with sodium chlorite–acetic acid [11] to yield the holocellulose. The hemicelluloses were recovered from this fraction by extraction with 10% aqueous sodium hydroxide [12], containing 10 mM sodium borohydride, under nitro-

* Author to whom correspondence should be addressed.

gen, and hemicellulose A was precipitated from the extract by acidification to pH 5 with 50% acetic acid. Hemicellulose B was then isolated from the supernatant solution by precipitation with ethanol, after dialysis against running water.

The hemicellulose A of grape skin was treated with Fehling's solution to fractionate it into its components [13]. Purified hemicellulose A-1 was isolated from the precipitate as a white powder and appeared to be homogeneous on gel-filtration, showing a weight-average M_w of 35 544 and had an $[\alpha]_D^{25} - 24.29^\circ$ (c 1.40, aqueous 1 M potassium hydroxide). This polysaccharide represents the major fraction of polysaccharides from grape skin dietary fibre. After acid hydrolysis [14, 15], the polysaccharide gave arabinose, xylose, glucose and 4-*O*-methylglucuronic acid in a molar ratio of 1:12.7:3.4:3.9.

Hakomori methylation [16] of A-1 gave a pale-yellow solid product with $[\alpha]_D^{25} - 17.39^\circ$ (c 1.38, chloroform), indicative of β -linkages, which was confirmed by the NMR spectra [17, 18] (δ 4.24 for H-1 and δ 102.2 for C-1). A portion of methylated polysaccharide was hydrolysed and the resulting sugars converted into their partially methylated alditol acetates and analysed by GC [19] and GC-mass spectrometry [20]; the results are summarized in Table 1. Another portion of methylated polysaccharide was reduced with lithium aluminium hydride [21] and then hydrolysed, and the resulting sugars analysed as their partially methylated alditol acetates by GC and GC-mass spectrometry; results are summarized in Table 1.

The methylation analysis revealed that hemicellulose A-1 is an acidic xyloglucan having a main chain of (1 $\rightarrow$ 4)-linked β -D-xylopyranosyl and β -D-glucopyranosyl residues in a molar ratio of 10:1. The presence and relative molar ratio of 2,4,6-Me₃-Glc (Table 1, columns A and B) indicates that the main chain contains one unit of glucose with a (1 $\rightarrow$ 3) gly-

cosidic linkage for every four units of glucose with a (1 $\rightarrow$ 4) glycosidic bond.

The points of branching in the backbone were indicated by the presence and molar ratio of 3-Me-Xyl and 2,3-Me₂-Glc expressed in columns A and B of Table 1. These data show that, for every 10 xylose residues in the main chain, there is one with a substituent at position 2, and for every two glucose residues in the main chain, there is one containing a substituent at position 6. These substituents are single L-arabinofuranosyl, xylopyranosyl, glucopyranosyl and 4-*O*-methylglucuronopyranosyl acid residues.

Partial acid hydrolysis [22] of the polysaccharide gave arabinose, xylose and glucose, in a relative molar ratio of 1:5.2:2.3 and six oligosaccharides (two neutral and four acidic oligosaccharides) that were isolated by preparative paper chromatography. Methylation analysis (Table 1) and spectroscopic data of the neutral oligosaccharides (δ 4.25 for H-1 and δ 102 for C-1) show them to be β -(1 $\rightarrow$ 4) linked xylobiose and xylotriose.

One of the acidic oligosaccharides (AOS-1) was shown to be 2-*O*-(4-*O*-methyl- α -D-glucopyranosyluronic acid)-D-xylopyranose (α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp), an aldobiouronic acid with $[\alpha]_D^{25} + 100^\circ$, indicative of an α -glycosidic linkage for the 4-*O*-methyl-D-glucuronic acid, confirmed by the NMR spectroscopic data (δ 5.18 for H-1 and δ 98.5 for C-1 of α -D-GlcpA). Its acetylated methyl ester methyl glycoside gave mass spectral peaks at m/z 417 (0.6), 403 (0.4), 345 (3.4), 289 (29.7), 229 (34.7) and 187 (base), consistent with the structure, methyl 3,4-di-*O*-acetyl-2- α -(methyl 3,4-di-*O*-acetyl-4-*O*-methyl-D-glucopyranosyluronate)-D-xylopyranose [23].

Spectroscopic data for the other three acidic oligosaccharides were similar to those of the above described aldobiouronic acid. Methylation analysis (Table 1) showed that their neutral residues were xylobiose, xylotriose and xylotetraose. Based on these

Table 1. Methylation analysis data for natural polysaccharide, reduced polysaccharide and oligosaccharides from hemicellulose A-1

Methylated sugars (as alditol acetates)					Molar ratios [§]				
	T*	T†	A	B	NOS-1	NOS-2	AOS-2	AOS-3	AOS-4
2,3,5-Me ₃ -Ara‡	0.48	0.66	0.1	0.1	—	—	—	—	—
2,3,4,6-Me ₄ -Glc	1.00	1.00	0.1	0.1	—	—	—	—	—
2,3,4-Me ₃ -Glc	2.49	1.13	—	0.4	—	—	—	—	—
2,3,6-Me ₃ -Glc	2.50	1.14	0.9	1.3	—	—	—	—	—
2,4,6-Me ₃ -Glc	1.95	1.11	0.1	0.2	—	—	—	—	—
2,3-Me ₂ -Glc	5.39	1.29	0.3	0.4	—	—	—	—	—
2,3,4-Me ₃ -Xyl	0.69	0.84	1.1	0.9	1.0	1.0	1.0	1.0	1.0
2,3-Me ₂ -Xyl	1.54	0.96	10.8	10.8	0.9	2.2	—	1.2	2.1
3-Me-Xyl	2.78	1.04	1.0	1.0	—	—	1.1	0.9	1.1

* R_f relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on a ECNSS-M column.

† R_f relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on a SPB-1 column.

‡ 2,3,5-Me₃-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, etc.

§ A, natural polysaccharide; B, carboxyl-reduced polysaccharide; NOS-1/2, neutral oligosaccharides 1 and 2, obtained on partial acid hydrolysis; AOS-2/4, acidic oligosaccharides 2 to 4 obtained on partial acid hydrolysis.

findings, these three acidic oligosaccharides are identified as β -D-Xylp(1 $\rightarrow$ 4)-[α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp], β -D-Xylp(1 $\rightarrow$ 4)- β -D-Xylp(1 $\rightarrow$ 4)-[α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp] and β -D-Xylp(1 $\rightarrow$ 4)-[β -D-Xylp(1 $\rightarrow$ 4)]₂-[α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp]. The presence of these oligosaccharides is consistent with the pattern of substitutions in the main chain previously described, based on methylation analysis.

The foregoing data indicate that hemicellulose A-1 is an acidic xyloglucan having a linear main chain of β -xylopyranosyl and β -glucopyranosyl residues (1 $\rightarrow$ 4)-linked; with a quarter of β -glucopyranose residues linked by (1 $\rightarrow$ 3) glycosidic bonds. The main chain carried units of 4-O-methyl-D-glucopyranosyluronic acid, β -D-xylopyranosyl, and L-arabinofuranosyl residues on the 2-position of xylose residues, in a ratio of 1:10. A quarter of the glucoses residues carried a glucose unit at their 6-position.

EXPERIMENTAL

General. Descending PC was performed on Whatman no. 3MM paper using EtOAc-HOAc-HCO₂H-H₂O (18:3:1:4) as eluent and diphenylamine-aniline as detection agent [24]. ¹H NMR and ¹³C NMR were recorded with Varian Unity-400 equipment. Spectra of the methylated polysaccharide was obtained in CDCl₃ and the spectrum referenced to the residual peak of the solvent. Spectra of the oligosaccharides were obtained in D₂O; in this case, the proton spectra were referenced to the residual peak of the solvent, while for the carbon spectra, MeOH was used as the int. standard reference.

GC-FID of alditol acetates were performed on a Supelcowax 10 M WCOT column (30 m $\times$ 0.53 mm id), using the temp. programme 220 $\rightarrow$ 230° at 3° min⁻¹. For partially methylated alditol acetates (PMAA), a SPB-1 WCOT column (30 m $\times$ 0.53 mm id), using the temp. programme 120 $\rightarrow$ 250° at 4° min⁻¹. GC-MS was carried out on a CP-SIL WCOT column (25 m $\times$ 0.32 mm id), using the temp. programme 100 $\rightarrow$ 250° at 5° min⁻¹. The ionization potential was 70 eV.

Gel permeation chromatography (GPC) was performed on a FPLC Superose 6HR 10/30 column (Pharmacia, V₀ 7.3 ml, V_i 21.9 ml), using 0.1 M aq. NaOH as eluent, at 0.5 ml min⁻¹. Solns (0.2 ml) containing 1 mg ml⁻¹ of polysaccharide were injected and the eluate monitored by differential refractometry. The column was calibrated with dextrans of known *M_r*.

HPLC-PAD of monosaccharides resulting from total acid hydrolysis was performed with a DIONEX DX-500 chromatograph equipped with a Dionex PA-1 column and pre-column. For analysis of neutral sugars, the following were used as eluents: A: NaOH 50 mM; B: NaOH 300 mM; C: de-ionized H₂O, using a programme which maintained a 1:1 isocratic mixt. of A and C for 15 min, and then passed to a 100% soln of B for 15 min, using a no. 8 curve gradient [25].

Uronic acids were analysed using the following as eluents: A: a soln of 100 mM NaOH and 600 mM NaOAc; B: de-ionized H₂O. Elution was performed under isocratic conditions using a mixt. of 25% A and 75% B [26].

Isolation and purification of polysaccharide. Grape skins (100 g) were triturated on a Braun AG-4050 miller and extracted successively with hexane, EtOH and CHCl₃-MeOH (1:1) for 18 hr in a Soxhlet. The residue (90.4 g) was stirred with methanolic 0.25 M NaOMe (1 l) for 24 hr at room temp. [10]. Insol. material was collected by centrifugation, washed with MeOH until neutral, and then treated with H₂O at room temp. for 24 hr. The remaining solid material (86.6 g) was delignified with NaClO₂ and HOAc [11]. The resulting holocellulose (46.6 g) was stirred with aq. 10% NaOH containing 10 mM NaBH₄ (1 l), for 24 hr at room temp. under N₂ [12]. The extract was filtered under red. pres. through cloth and hemicellulose A was pptd. by acidification to pH 5 with 50% HOAc. After storage for 3 hr at 5°, the ppt. was collected by centrifugation, washed with H₂O and EtOH, and then vacuum-dried to yield the crude polysaccharide (1.97 g). Hemicellulose B was subsequently isolated from the supernatant soln by pptn. with EtOH, after dialysis against running H₂O (2.09 g).

A soln of hemicellulose A (1.40 g) in aq. 5% KOH (300 ml) was treated with Fehling's soln [13] until pptn. was complete. Pptd. hemicellulose was collected by centrifugation, treated with 5% (v/v) HCl for 1 min at 0°, and then centrifuged. The residue was washed $\times$ 2 with EtOH and $\times$ 2 with Me₂CO and then dried over P₂O₅ *in vacuo*. This pptn. procedure was repeated $\times$ 2 to yield the purified polysaccharide (0.90 g) named hemicellulose A-1 (A-1) [α]_D²⁵ -24.29° (c 1.40, aq. 1 M KOH).

Methylation analysis. Polysaccharide A-1 (206.7 mg) was methylated by the Hakomori method [16]. A soln of methylated product in C₆H₆ was diluted with petrol (bp 30-60°) to ppt. the methylated polysaccharide (86.8 mg) [α]_D²⁵ -17.39° (c 1.38, CHCl₃). A portion of this material was hydrolysed and the resulting sugars converted into their partially methylated alditol acetates [14] and analysed by GC [19] and GC-MS [20].

To a soln of another portion (13.5 mg) of methylated polysaccharide in dry THF (10 ml), was added LiAlH₄ (400 mg) [21]. The reaction mixt. was refluxed under N₂ for 24 hr and then worked-up in the usual way. The reduced product was then extracted into CHCl₃ and dried for 48 hr *in vacuo* over P₂O₅. The product (11.5 mg) had IR absorption at 3600 cm⁻¹ (OH) but not at 1735 cm⁻¹ (ester C=O). The reaction product was hydrolysed and the resulting sugars were converted into their PMAA derivatives and analysed by GC [19] and GC-MS [20].

Methylation analysis of oligosaccharides was performed by the method of ref. [27].

Sugar analysis. (a) GC. Polysaccharide A-1 (10.3 mg) was treated with aq. 72% [14] H₂SO₄ (0.25 ml)

for 1 hr at 30° followed by dilution (2.75 ml H₂O) to 1 M H₂SO₄ and heating for 3 hr at 100°. When cool, the hydrolysate was neutralized with 0.6 ml of 15 M NH₃ soln, 50 µl of a soln of *myo*-inositol (20 mg ml⁻¹) added as int. standard and the soln centrifuged. The upper layer of the centrifuged soln (200 µl) was reduced using 1 ml of NaBH₄ soln in DMSO (20 mg ml⁻¹) at 40° for 90 min; 100 µl of 18 M HOAc were then added to decompose excess NaBH₄. 1-methylimidazole (200 µl) and Ac₂O (2 ml), were added to the reduced mixt. of monosaccharides and mixed. After 10 min at room temp., 10 ml of H₂O were added to decompose excess of Ac₂O. When cool, 1 ml CH₂Cl₂ was added and then mixed. After phase sepn, the lower phase was removed with a Pasteur pipette and the alditol acetates analysed by GC [15]. (b) HPAE-PAD. The polysaccharide (2 mg) was hydrolysed with 0.3 ml of 2M TFA at 121° for 2.5 hr. To the reaction mixt. was added 50 µl of *myo*-inositol soln as int. standard and the mixt. dried in a stream of N₂. The sample was then dissolved in 5 ml of de-ionized H₂O and analysed by HPAE-PAD to identify its neutral sugars [25] and uronic acids [26]. Uronic acids were also determined by the carbazole method using D-glucuronic acid as standard [28].

Partial hydrolysis. Hemicellulose A-1 (106 mg) was treated with 0.125 M H₂SO₄ [22] (12 ml) for 2 hr at 100°. The hydrolyzate was neutralized (BaCO₃), basified with 1 M KOH, then passed through a column of Amberlite IR-120 (H⁺) resin and concd. The syrupy residue was eluted from a column of Amberlite IRA-400 (AcO⁻) resin, first with H₂O to yield neutral oligosaccharides and then with aq. 10% HOAc to yield acidic ones. Prep. PC of the neutral sugars gave three monosaccharides, xylose (62.9%), glucose (25.8%) and arabinose (11.3%) and two oligosaccharides (NOS-1 and NOS-2) that were identified by methylation analysis [27] and from their ¹H NMR [17] and ¹³C NMR [18] spectroscopic data.

NOS-1. White crystalline solid, 15 µg. $[\alpha]_D^{25} - 30.0^\circ$ (c 1.3, EtOH-H₂O, 1:1). ¹H NMR (400 MHz, D₂O): δ 5.00 (d, $J_{1,2} = 4.0$ Hz; H-1 α-D-Xylp), 4.40 (d, $J_{1,2} = 8.0$ Hz; H-1 β-D-Xylp), 4.27 (d, $J_{1,2} = 8.0$ Hz; H-1 β-D-Xylp(1 → 4)-), 3.87 (dd, $J_{5\alpha,5\beta} = 12.0$ Hz, $J_{4,5\beta} = 5.0$ Hz; H-5β β-D-Xylp), 3.78 (dd, $J_{5\alpha,5\beta} = 12.0$ Hz, $J_{4,5\beta} = 5$ Hz; H-5β β-D-Xylp(1 → 4)-), 3.65–3.53 (group of signals corresponding to H-5α, H5β y H-4 of α-D-Xylp, H-4 de β-D-Xylp, y H-4 de β-D-Xylp(1 → 4)-), 3.47 (t, $J_{2,3} = J_{3,4} = 7.0$ Hz; H-3 α-D-Xylp), 3.43 (dd, $J_{1,2} = 5.0$ Hz, $J_{2,3} = 9.0$ Hz; H-2 α-D-Xylp), 3.36 (t, $J_{2,3} = J_{3,4} = 9.0$ Hz; H-3 β-D-Xylp), 3.36 (t, $J_{2,3} = J_{3,4} = 9.0$ Hz; H-3 β-D-Xylp(1 → 4)-), 3.19 (t, $J_{5\alpha,5\beta} = J_{4,5\alpha} = 11.0$ Hz; H-5α β-D-Xylp), 3.12 (t, $J_{5\alpha,5\beta} = J_{4,5\alpha} = 11.0$ Hz; H-5α β-D-Xylp(1 → 4)-), 3.10–3.02 (groups of signals corresponding to the protons H-2 of β-D-Xylp(1 → 4)-, -β-D-Xylp(1 → 4)- y β-D-Xylp). ¹³C NMR (100 MHz, D₂O): Table 2. This product (8 mg) was also submitted to methylation analysis (Table 1).

NOS-2. White crystalline solid, 13.6 mg. $[\alpha]_D^{25}$

–42.0° (c 1.05, EtOH-H₂O, 1:1). ¹H NMR (400 MHz, D₂O): δ 5.01 (d, $J_{1,2} = 4.0$ Hz; H-1 α-D-Xylp), 4.40 (d, $J_{1,2} = 8.0$ Hz; H-1 β-D-Xylp), 4.30 (d, $J_{1,2} = 8.0$ Hz; H-1 β-D-Xylp(1 → 4)-), 4.28 (d, $J_{1,2} = 8.0$ Hz; H-1 β-D-Xylp(1 → 4)-), 3.92 (dd, $J_{5\alpha,5\beta} = 12.0$ Hz, $J_{4,5\beta} = 5$ Hz; H-5β β-D-Xylp(1 → 4)-), 3.87 (dd, $J_{5\alpha,5\beta} = 12.0$ Hz, $J_{4,5\beta} = 5$ Hz; H-5β β-D-Xylp), 3.79 (dd, $J_{5\alpha,5\beta} = 12.0$ Hz, $J_{4,5\beta} = 5$ Hz; H-5β β-D-Xylp(1 → 4)-), 3.66–3.54 (group of signals corresponding to protons H-5α, H5β y H-4 of α-D-Xylp, H-4 of β-D-Xylp, H-4 of β-D-Xylp(1 → 4)-, and to H-4 of β-D-Xylp(1 → 4)-), 3.48 (t, $J_{2,3} = J_{3,4} = 10.0$ Hz; H-3 α-D-Xylp), 3.45 (dd, $J_{1,2} = 5.0$ Hz, $J_{2,3} = 10.0$ Hz; H-2 α-D-Xylp), 3.37 (t, $J_{2,3} = J_{3,4} = 10.0$ Hz; H-3 β-D-Xylp(1 → 4)-), 3.36 (t, $J_{2,3} = J_{3,4} = 10.0$ Hz; H-3 β-D-Xylp), 3.25 (t, $J_{5\alpha,5\beta} = J_{4,5\alpha} = 10.0$ Hz; H-5α β-D-Xylp(1 → 4)-), 3.20 (t, $J_{5\alpha,5\beta} = J_{4,5\alpha} = 10.0$ Hz; H-5α β-D-Xylp), 3.13 (t, $J_{5\alpha,5\beta} = J_{4,5\alpha} = 10.0$ Hz; H-5α β-D-Xylp(1 → 4)-), 3.10–3.03 (group of signals corresponding to protons H-2 of β-D-Xylp(1 → 4)-, -β-D-Xylp(1 → 4)- y β-D-Xylp). ¹³C NMR (100 MHz, D₂O): Table 2. This product (6.7 mg) was also submitted to methylation analysis (Table 1).

Prep. PC of the acidic sugars gave four oligosaccharides (AOS-1–4) that were identified by methylation analysis and NMR.

AOS-1. Yellowish-white-crystalline solid, 10.3 mg. $[\alpha]_D^{25} + 102^\circ$ (c 1.07, EtOH-H₂O, 1:1). ¹H NMR (400 MHz, D₂O): δ 5.18 (d, $J_{1,2} = 4.0$ Hz; H-1 α-D-GlcpA(1 → 2)-α-D-Xylp), 5.16 (d, $J_{1,2} = 4.1$ Hz; H-1 α-D-GlcpA(1 → 2)-β-D-Xylp), 4.87 (d, $J_{1,2} = 4$ Hz; H-1 α-D-Xylp), 4.51 (d, $J_{1,2} = 8$ Hz; H-1 β-D-Xylp), 4.09 (d, $J_{4,5} = 10.2$ Hz; H-5 α-D-GlcpA(1 → 2)-α-D-Xylp), 4.99 (d, $J_{4,5} = 10.1$ Hz; H-5 α-D-GlcpA(1 → 2)-β-D-Xylp), 3.73 (dd, $J_{5\alpha,5\beta} = 11.2$ Hz, $J_{4,5\beta} = 5.2$ Hz; H-5β β-D-Xylp), 3.28 (s, CH₃O-C-4, α-D-GlcpA(1 → 2)-D-Xylp), etc. ¹³C NMR (100 MHz, D₂O): δ 110.0 (C-1 β-D-Xylp), 98.5 (C-1 α-D-GlcpA(1 → 2)-α-D-Xylp), 97.9 (C-1 α-D-GlcpA(1 → 2)-β-D-Xylp), 97.2 (C-1 α-D-Xylp), 90.6 (C-1 α-D-Xylp), 83.5 (C-3 α-D-GlcpA(1 → 2)), 80.4 (C-2 α-D-Xylp), 79.3 (C-2 β-D-Xylp), 76.7 (C-4 α-D-GlcpA), 75.3 (C-3 β-D-Xylp), 73.1 (C-2 α-D-GlcpA(1 → 2) y C-3 α-D-Xylp), 72.3 (C-5 α-D-GlcpA(1 → 2)), 72.2 (C-4 α-D-Xylp), 70.4 (C-4 β-D-Xylp), 65.9 (C-5 β-D-Xylp), 61.8 (C-5 α-D-Xylp), 60.8 (CH₃O-C-4 α-D-GlcpA(1 → 2)). This oligosaccharide corresponds to an aldobiuronic acid. For identification, it was converted into its corresponding Me ester, Me glycoside [29], by treatment with 3% HCl in MeOH (5 ml) and boiling for 24 hr. The reaction product was acetylated with HOAc-pyridine (1:1) for 24 hr at room temp. EIMS (probe) 70 eV, *m/z* (rel. int.): 477 [abE₁] (0.25), 417 [abE₂] (0.59), 403 [baF₁] (0.40), 345 [abF₁] (3.37), 289 [aA₁] (29.66), 229 [aA₂] (34.72), 187 [aA₂-CH₂CO] (100), 155 [aA₃] (10.62), 127 [bC₁] (21.76), 85 [aK₂] (27.85), 43 [CH₃CO⁺] (76.17). These data correspond to methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-D-xylopyranose.

AOS-2. Pale yellow crystalline solid, 5.3 mg. $[\alpha]_D^{25}$

Table 2. ^{13}C NMR (δ) data of neutral oligosaccharides obtained by partial hydrolysis of hemicellulose A-1

Compound	Residue	C-1	C-2	C-3	C-4	C-5
NOS-1	β -Xylp(1 $\rightarrow$ 4)-	102.8	73.7	76.6	70.1	66.1
	β -Xylp	97.5	74.8	74.9	77.4	63.9
	α -Xylp	93.0	72.3	71.9	77.5	59.8
NOS-2	β -Xylp(1 $\rightarrow$ 4)-	102.8	73.7	76.5	70.1	66.2
	β -Xylp(1 $\rightarrow$ 4)-	102.6	73.6	74.6	77.3	63.9
	β -Xylp	97.5	74.8	74.9	77.3	64.4
	α -Xylp	92.9	72.3	71.8	77.5	59.8

+98.0° (*c* 1.04, EtOH-H₂O, 1:1). ^1H NMR (400 MHz, D₂O): δ 5.10 (*d*, $J_{1,2}$ = 5.0 Hz; H-1 α -D-GlcpA(1 $\rightarrow$ 2)- α -D-Xylp), 5.09 (*d*, $J_{1,2}$ = 4 Hz; H-1 α -D-GlcpA(1 $\rightarrow$ 2)- β -D-Xylp), 4.94 (*d*, $J_{1,2}$ = 4 Hz; H-1 α -D-Xylp), 4.37 (*d*, $J_{1,2}$ = 8 Hz; H-1 β -D-Xylp), 4.33 (*d*, $J_{1,2}$ = 8 Hz; H-1 β -D-Xylp(1 $\rightarrow$ 4)), 4.08 (*d*, $J_{4,5}$ = 10.0 Hz; H-5 α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp), 3.87 (*dd*, $J_{5\alpha,5\beta}$ = 11.0 Hz, $J_{4,5\beta}$ = 5.0 Hz; H-5 β β -D-Xylp), 3.75 (*dd*, $J_{5\alpha,5\beta}$ = 11.0 Hz, $J_{4,5\beta}$ = 5.0 Hz; H-5 β β -D-Xylp(1 $\rightarrow$ 4)), 3.23 (*s*, CH₃O-C-4, α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp), etc. ^{13}C NMR (100 MHz, D₂O): δ 102.1 (C-1 β -D-Xylp(1 $\rightarrow$ 4)), 97.9 (C-1 β -D-Xylp), 97.0 (C-1 α -D-GlcpA(1 $\rightarrow$ 2)), 92.5 (C-1 α -D-Xylp), 83.0 (C-3 α -D-GlcpA(1 $\rightarrow$ 2)), 77.0 (C-2 β -D-Xylp), 76.9 (C-4 β -D-Xylp; C-4 α -D-Xylp), 76.7 (C-3 β -D-Xylp(1 $\rightarrow$ 4); C-4 α -D-GlcpA(1 $\rightarrow$ 2)), 76.5 (C-2 α -D-Xylp), 74.9 (C-3 β -D-Xylp), 74.5 (C-2 β -D-Xylp(1 $\rightarrow$ 4)), 72.8 (C-2 α -D-GlcpA(1 $\rightarrow$ 2)), 71.9 (C-3 α -D-Xylp), 71.8 (C-5 α -D-GlcpA(1 $\rightarrow$ 2)), 70.4 (C-4 β -D-Xylp(1 $\rightarrow$ 4)), 65.5 (C-5 β -D-Xylp(1 $\rightarrow$ 4)), 63.5 (C-5 β -D-Xylp), 61.5 (CH₃O-C-4 α -D-GlcpA(1 $\rightarrow$ 2)), 59.2 (C-5 α -D-Xylp). This product (2.1 mg) was also submitted to methylation analysis (Table 1).

AOS-3. Pale yellow crystalline solid, 6.6 mg. $[\alpha]_D^{25}$ +90° (*c* 1.20, EtOH-H₂O, 1:1). ^1H NMR (400 MHz, D₂O): δ 5.11 (*d*, $J_{1,2}$ = 4.0 Hz; H-1 α -D-GlcpA(1 $\rightarrow$ 2)- α -D-Xylp), 5.09 (*d*, $J_{1,2}$ = 4 Hz; H-1 α -D-GlcpA(1 $\rightarrow$ 2)- β -D-Xylp), 4.98 (*d*, $J_{1,2}$ = 4 Hz; H-1 α -D-Xylp), 4.42 (*d*, $J_{1,2}$ = 8 Hz; H-1 β -D-Xylp), 4.41 (*d*, $J_{1,2}$ = 8 Hz; H-1 β -D-Xylp(1 $\rightarrow$ 4)), 4.38 (*d*, $J_{1,2}$ = 8 Hz; H-1 β -D-Xylp(1 $\rightarrow$ 4)), 4.26 (*d*, $J_{4,5}$ = 10.0 Hz; H-5 α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp), 3.78 (*dd*, $J_{5\alpha,5\beta}$ = 11.0 Hz, $J_{4,5\beta}$ = 5.0 Hz; H-5 β β -D-Xylp(1 $\rightarrow$ 4)), 3.27 (*s*, CH₃O-C-4, α -D-GlcpA(1 $\rightarrow$ 2)-D-Xylp), etc. ^{13}C NMR (100 MHz, D₂O): δ 102.5 (C-1 β -D-Xylp(1 $\rightarrow$ 4)), 102.2 (C-1 β -D-Xylp(1 $\rightarrow$ 4)), 98.0 (C-1 β -D-Xylp), 97.0 (C-1 α -D-GlcpA(1 $\rightarrow$ 2)), 92.5 (C-1 α -D-Xylp), 83.0 (C-3 α -D-GlcpA(1 $\rightarrow$ 2)), 77.3 (C-2 β -D-Xylp), 76.9 (C-4 α -D-Xylp), 76.6 (C-4 α -D-GlcpA(1 $\rightarrow$ 2); C-3 β -D-Xylp(1 $\rightarrow$ 4); C-4 β -D-Xylp(1 $\rightarrow$ 4); C-4 β -D-Xylp), 76.1 (C-2 α -D-Xylp), 74.9 (C-3 β -D-Xylp), 74.5 (C-3 β -D-Xylp(1 $\rightarrow$ 4)), 73.3 (C-2 β -D-Xylp(1 $\rightarrow$ 4)), 73.2 (C-2 β -D-Xylp(1 $\rightarrow$ 4)), 72.8 (C-2 α -D-GlcpA(1 $\rightarrow$ 2)), 71.8 (C-5 α -D-GlcpA(1 $\rightarrow$ 2)), 71.4 (C-3 α -D-Xylp), 69.9 (C-4 β -D-Xylp(1 $\rightarrow$ 4)), 65.7 (C-5 β -D-Xylp(1 $\rightarrow$ 4)), 65.3 (C-5 β -D-Xylp), 63.3 (C-5 β -D-Xylp(1 $\rightarrow$ 4)), 60.3 (CH₃O-

C-4 α -D-GlcpA(1 $\rightarrow$ 2)), 59.3 (C-5 α -D-Xylp). This product (2 mg) was also submitted to methylation analysis (Table 1).

AOS-4. White crystalline solid, 2.4 mg. $[\alpha]_D^{25}$ +85.0° (*c* 1.10, EtOH-H₂O, 1:1). ^1H NMR (400 MHz, D₂O): δ 5.13 (*d*, $J_{1,2}$ = 4.0 Hz; H-1 α -D-GlcpA(1 $\rightarrow$ 2)- α -D-Xilp), 5.11 (*d*, $J_{1,2}$ = 4 Hz; H-1 α -D-GlcpA(1 $\rightarrow$ 2)- β -D-Xilp), 4.99 (*d*, $J_{1,2}$ = 4 Hz; H-1 α -D-Xilp), 4.43 (*d*, $J_{1,2}$ = 8 Hz; H-1 β -D-Xilp), 4.41 (*d*, $J_{1,2}$ = 8 Hz; H-1 β -D-Xilp(1 $\rightarrow$ 4)), etc. ^{13}C NMR (100 MHz, D₂O): δ 102.6 (C-1 β -D-Xilp(1 $\rightarrow$ 4)), 97.5 (C-1 β -D-Xilp), 95.9 (C-1 α -D-GlcpA(1 $\rightarrow$ 2)), 83.0 (C-3 α -D-GlcpA(1 $\rightarrow$ 2)), etc. This product (2 mg) was also submitted to methylation analysis (Table 1).

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