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A Hemicellulose B Fraction from Grape Skin (*Vitis vinifera*, Palomino Variety)

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The structure of a hemicellulose B fraction (B-1) isolated from grape skins (*Vitis vinifera*) of the Palomino variety has been studied by methylation analysis, ^{1}H NMR and ^{13}C NMR spectroscopy, and partial acid hydrolysis. Hemicellulose B-1 appeared to be homogeneous by gel filtration with a weight-average molecular weight of 22 600. This polysaccharide is a linear xyloglucan chain composed of xylopyranosyl and glucopyranosyl residues linked by β-(1→4) glycosidic bonds. Attached to this backbone, 4-*O*-methyl-D-glucuronopyranosyl acid, D-glucopyranosyl, and L-fucopyranosyl residues occur at position 2 in a ratio of one residue for every five units of xylose in the main chain, with D-xylopyranosyl residues attached at position 6 of glucose units in a ratio of one residue for every two glucose-derived moieties in the main chain.

In continuing our studies about the characterization of the pomace of Palomino grape, the main byproducts generated by the wine-making industry at Jerez/Xeres/Sherry zone, we have started the characterization of the polysaccharides from the skin of this grape variety. Grape pomace consists of three different components: seeds, stalks, and skins. Grape skins represent about 5% of the total dry weight of pomace. The aim of our study of Palomino grape skins is to propose its transformation into more economically valuable raw materials that will allow an improved use of this resource, which is currently underexploited. Dietary fiber is the main component of grape (*Vitis vinifera)* skin of the Palomino variety and represents about 74 wt % of this material. The major fraction of the polysaccharides from this dietary fiber are the hemicelluloses (30.7 wt % of the dietary fiber), followed by cellulose (about 6%).[1]

In previous reports, we have described the isolation and structural determination of an acidic xyloglucan from the so-called fraction A of the grape skin hemicelluloses[2] and two other hemicelluloses from the seeds of Palomino grapes.[3,4] This paper describes the structural determination of a polysaccharide isolated from the hemicellulose B fraction of dietary fiber from the grape skin under study.

Results and Discussion

The study of dietary fiber from *Palomino* grape skin[1] by the Southgate method[5] has shown that hemicelluloses are the most important fraction of polysaccharides in this material, followed by cellulose. Therefore our study concentrates on the holocellulose fraction (the total of the hemicelluloses and cellulose) from this material. This fraction represents about 37% of the total weight of grape skin. The procedures to obtain the holocellulose from grape skins have been described in a previous work.[2] The hemicelluloses were recovered from this fraction by extraction with 10% w/w aqueous sodium hydroxide,[6] containing 10 mM sodium borohydride, under nitrogen. The hemicellulose A fraction 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 B of grape skin was treated with Fehling's solution to fractionate it into its components.[7] Purified hemicellulose B-1 was isolated from the precipitate as a cream-colored powder and appeared to be homogeneous on gel filtration, showing a weight-average molecular weight of 22 600 and an $[\alpha]^{25}_D$ +11.21° (*c* 1.16, aqueous 1 M potassium hydroxide). This polysaccharide represents the second major fraction of polysaccharides from grape skin dietary fiber. After acid hydrolysis,[8,9] the polysaccharide gave fucose, xylose, glucose, and 4-*O*-methylglucuronic acid in the molar ratio of 1:7.9:7.5:2.

Hakomori methylation[10] of B-1 gave a pale yellow solid product with $[\alpha]^{25}_D$ +3.50° (*c* 2.86, chloroform), indicative of β-linkages for the xylose and glucose units, which was confirmed by the ^{1}H and ^{13}C NMR spectra[11,12] (δ 4.23 for H-1 and δ 102.2 ppm for C-1). An IR absorption band at 1741 cm^{-1} (methyl ester) confirmed the presence of uronic acid residues in the polysaccharide. A portion of the methylated polysaccharide was hydrolyzed, and the resulting sugars were converted into their partially methylated alditol acetates and analyzed by GC[13] and GC−MS;[14] another portion of methylated polysaccharide was reduced with lithium aluminum hydride[15] and then hydrolyzed, and the resulting sugars were analyzed as their partially methylated alditol acetates by GC and GC−MS. Data from these methylation analysis are summarized in Table 1.

Results of the methylation analysis revealed that hemicellulose B-1 is a xyloglucan. The presence and relative molar ratios of 2,3-Me$_2$-Xyl, 3-Me-Xyl, 2,3,6-Me$_3$-Glc, and 2,3-Me$_2$-Glc (Table 1, columns A and B) indicated that the main chain contains units of β-D-glucopyranose and β-D-xylopyranose with (1→4) glycosidic links in a molar ratio of 2:3. The points where the backbone is branched were indicated by the presence and molar ratio of 3-Me-Xyl and 2,3-Me$_2$-Glc expressed in columns A and B of Table 1. These data show that for every 14 xylose residues in the main chain there is one with a substituent at position 2, and for every glucose residue in the main chain, there is another one containing a substituent at position 6. These substituents are single L-fucopyranosyl, D-xylopyranosyl, D-glucopyranosyl, and 4-*O*-methyl-D-glucuronopyranosyl acid residues. The molar ratio of 2,3,4-Me$_3$-Xyl and 2,3-Me$_2$-Glc appears to suggest that branchings at position 6 of glucopyranose units are occupied by terminal β-D-xylopyranose residues, while the rest of the terminal residues are

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Table 1. Methylation Analysis Data for the Natural Polysaccharide, the Reduced Polysaccharide, and Oligosaccharides from Hemicellulose B-1

methylated sugars (as alditol acetates)	T^a	T^b	molar ratio[c] A	B	NOS-1	NOS-2	NOS-3	AOS-2	AOS-3
2,3,5-Me_3-Fuc[d]	0.48	0.66	0.3	0.4					
2,3,4,6-Me_4-Glc	1.00	1.00	0.1	0.1	1.0	1.0	1.0		
2,3,4-Me_3-Glc	2.49	1.13		0.6					
2,3,6-Me_3-Glc	2.50	1.14	1.0	1.0	1.1	2.1	2.9		
2,3-Me_2-Glc	5.39	1.29	1.5	1.5					
2,3,4-Me_3-Xyl	0.69	0.84	1.3	1.5				1.0	1.0
2,3-Me_2-Xyl	1.54	0.96	2.8	3.6					1.2
3-Me-Xyl	2.78	1.04	0.6	0.8				1.1	0.9

[a] Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on a ECNSS-M column. [b] Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on a SPB-1 column. [c] A, natural polysaccharide; B, carboxyl-reduced polysaccharide; NOS-1/3, neutral oligosaccharides 1, 2, and 3, obtained on partial acid hydrolysis; AOS-2/3, acidic oligosaccharides 2 and 3 obtained on partial acid hydrolysis. [d] 2,3,5-Me_3-Fuc = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylfucitol; 2,3,4,6-Me_4-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol; 2,3,4-Me_3-Glc = 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol; 2,3,6-Me_3-Glc = 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol; 2,3-Me_2-Glc = 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylglucitol; 2,3,4-Me_3-Xyl = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol; 2,3-Me_2-Xyl = 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylxylitol; 3-Me-Xyl = 1,2,4,5-tetra-*O*-acetyl-3-*O*-methylxylitol.

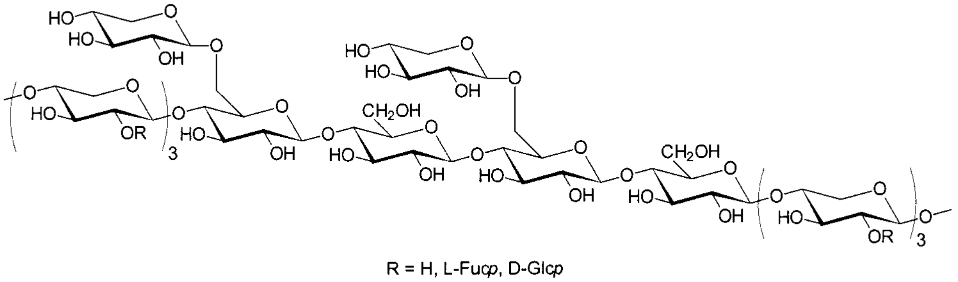

Figure 1. Average repetition unit of the polysaccharide B-1.

attached at some position 2 of the xylopyranose residues in the backbone.

The assignments of the D-,L-configuration to the different sugar moieties are based on the literature precedents for other hemicelluloses.[16]

Partial acid hydrolysis[17] of the polysaccharide gave fucose and xylose, in a relative molar ratio of 1:4, and six oligosaccharides (three neutral and three acidic oligosaccharides) that were isolated by preparative paper chromatography. Methylation analysis (Table 1) and spectroscopic data of the neutral oligosaccharides NOS-1, NOS-2, and NOS-3 (δ 4.25 ppm for H-1 and δ 102 ppm for C-1) showed them to be the (1→4)-linked β-D-glucopyranose oligomers cellobiose, cellotriose, and cellotetraose.

One of the acidic oligosaccharides (AOS-1) was shown to be 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose (α-D-Glc*p*A(1→2)-D-Xyl*p*), an aldobiouronic acid with $[\alpha]^{25}{}_{D}$ +100°, indicative of an α-glycosidic linkage for the 4-*O*-methyl-D-glucuronic acid, confirmed by the NMR spectroscopic data (δ 5.18 ppm for H-1 and δ 98.5 ppm for C-1 of α-D-Glc*p*A). Its acetylated methyl ester methyl glycoside gave mass spectral peaks at *m*/*z* ratios 417 (0.6), 403 (0.4), 345 (3), 289 (30), 229 (35), and 187 (100), consistent with the structure of methyl 3,4-di-*O*-acetyl-2-α-*O*-(methyl 3,4-di-*O*-acetyl-4-*O*-methyl-D-glucopyranosyluronate)-D-xylopyranose.[18]

Spectroscopic data for the other two acidic oligosaccharides (AOS-2 and AOS-3) were similar to those of the above-described aldobiouronic acid. Methylation analysis of these (Table 1) showed that their neutral residues were xylobiose and xylotriose. On the basis of these findings, these three acidic oligosaccharides were identified as β-D-Xyl*p*(1→4)-[α-D-Glc*p*A(1→2)-D-Xyl*p*], β-D-Xyl*p*(1→4)-β-D-Xyl*p*(1→4)-[α-D-Glc*p*A(1→2)-D-Xyl*p*], and β-D-Xyl*p*(1→4)-[β-D-Xyl*p*(1→4)]$_2$-[α-D-Glc*p*A(1→2)-D-Xyl*p*]. The presence of these oligosaccharides is consistent with the pattern of substitutions in the main chain previously described, based on the methylation analysis.

The foregoing data indicate that hemicellulose B-1 is an acidic xyloglucan having a linear main chain of β-xylopyranosyl and β-glucopyranosyl residues (1→4)-linked. The backbone carried units of 4-*O*-methyl-D-glucopyranosyluronic acid, β-D-xylopyranosyl, and D-glucopyranosyl residues on the 2-position of xylose residues, in a ratio of 1:5. A quarter of these branchings results from terminal fucose units, another quarter results from glucose units, and the rest have 4-*O*-methyl-D-glucopyranosyluronic acid residues. A half of the glucose units in the backbone carry a terminal β-D-xylopyranosyl residue. Figure 1 shows the repetition unit of the polysaccharide.

There are few precedents in structural studies of polysaccharides isolated from grape or grape-related products.[19,20] One arabinogalactan-protein aggregate was isolated from must and might be involved in microfiltration membrane plugging and wine-color stabilization in wine-making technology. In previous work, we have described an acidic xyloglucan isolated from the hemicellulose A fraction of Palomino grape skin. This polysaccharide was shown to have a linear backbone of β-D-xylopyranosyl and β-D-glucopyranosyl residues linked (1→4) with single 4-*O*-methyl-D-glucopyranosyluronic acid, β-D-xylopyranosyl, and L-arabinofuranosyl residues attached at some of the 2 positions, in a ratio of 1:10.[2]

Experimental Section

General Experimental Procedures. Descending paper chromatography was performed on Whatman No 3MM paper, using ethyl acetate−acetic acid−formic acid−water (18:3:1:4) as eluent and diphenylamine−aniline as detection agent.[21] Optical rotations were recorded with a Perkin-Elmer 241 polarimeter, and IR spectra with a Perkin-Elmer 257 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were performed with Varian Unity-400 equipment. The spectra of the methylated polysaccharide were obtained in deuterated chloroform and were referenced by means of the residual peak of the solvent. The NMR spectra of the oligosaccharides were obtained in deuterated water; in this case, the ^{1}H NMR spectra were referenced by means of the residual peak of the solvent, while for the ^{13}C NMR spectra, methanol was used as the internal standard for reference.

GC of the alditol acetates was performed with a Hewlett-Packard model 5890 A chromatograph, fitted with a flame-ionization detector, and a Supelcowax 10 M wall-coated open tubular column (30 m × 0.53 mm i.d.), using the temperature program 220 → 230 °C at 3 °C·min^{-1}. For partially methylated alditol acetates (PMAA) a SPB-1 wall-coated open tubular column (30 m × 0.53 mm i.d.) was employed, using the temperature program 120 → 250 °C at 4 °C·min^{-1}. GC–MS was performed with a Kratos MS-80 instrument fitted with a CP-SIL wall-coated open tubular column (25 m × 0.32 mm i.d.), using the temperature program 100 → 250 °C at 5 °C·min^{-1}. The ionization potential was 70 eV.

Gel permeation chromatography was performed on a FPLC Superose 6HR 10/30 column (Pharmacia, V_0 7.3 mL, V_i 21.9 mL), using 0.1 M aqueous NaOH as eluent, at 0.5 mL·min^{-1}. Solutions (0.2 mL) containing 1 mg·mL^{-1} of the polysaccharide were injected, and the eluate was monitored by measurement of its refractometic index. The elution volume of the polysaccharide was 16.4 mL, matching with a weight average molecular weight of 22 600. The column was calibrated with dextrans of known molecular weights (Dextrans-T from Pharmacia with molecular weights of 500 000, 150 000, 70 000, and 40 000). HPAE-PAD of the monosaccharides resulting from the total acid hydrolysis was performed with a Dionex DX-500 chromatograph equipped with a Dionex PA-1 column and precolumn. For the analysis of the neutral sugars, the following were used as eluents: eluent A, NaOH 50 mM; eluent B, NaOH 300 mM; eluent C, deionized water, using a program which maintained a 1:1 isocratic mixture of A and C for 15 min, and then passed to a 100% solution of B for 15 min, using a No. 8 curve gradient.[22] The uronic acids were analyzed using the following as eluents: eluent A, a solution of 100 mM NaOH and 600 mM sodium acetate; eluent B, deionized water. The elution was performed under isocratic conditions using a mixture of 25% A and 75% B.[23]

Plant Material. Grape pomace was obtained from the grapes (*V. Vinifera* cv. Palomino) collected in the Jerez/Xeres/Sherry zone in September 1996 and was provided by Domecq S.A. Grapes were pressed at Bodegas Domecq, and the pomace was washed with water and dried at room temperature, in the dark. Grape skins were separated from the rest of the pomace manually.

Isolation and Purification of the Polysaccharide. Grape skins (100 g) were triturated on a Braun AG-4050 mill and extracted successively with hexane, ethanol, and chloroform–methanol (1:1) for 18 h, using a Soxhlet. The residue (90.4 g) was stirred with methanolic 0.25 M sodium methoxide (1 L) for 24 h at room temperature.[24] Insoluble material was collected, by centrifugation, washed with methanol until neutral, and then treated with water at room temperature, for 24 h. The remaining solid material (86.6 g) was delignified with sodium chlorite and acetic acid.[25] The resulting holocellulose (46.6 g) was stirred with aqueous 10% sodium hydroxide containing 10 mM $NaBH_4$ (w/v, 1 L), for 24 h at room temperature under nitrogen.[6] The extract was filtered under reduced pressure through cloth, and hemicellulose A was precipitated by acidification to pH 5 with 50% acetic acid. After storage for 3 h at 5 °C, the precipitate was collected by centrifugation. The supernatant solution obtained in the previous step was dialyzed against running water for 24 h, then concentrated to a small volume in a rotary evaporator. Hemicellulose B was precipitated by addition of 4 volumes of EtOH. After storage for 16 h at 4 °C, the precipitate was collected by centrifugation, washed three times with EtOH, and vacuum-dried to yield the crude polysaccharide (2.09 g).

A solution of hemicellulose B (1.80 g) in aqueous 5% potassium hydroxide (350 mL) was treated with Fehling's solution[7] until the precipitation was complete. Precipitated hemicellulose was collected by centrifugation, treated with 5% (v/v) hydrogen chloride for 1 min at 0 °C, and then centrifuged. The residue was washed with ethanol (four times) and twice with acetone and dried over phosphorus pentaoxide in vacuo. This precipitation procedure was repeated twice to yield the purified polysaccharide (0.88 g) named hemicellulose B-1 (B-1): $[\alpha]^{25}_{D}$ +11.21° (*c* 1.16, aqueous 1 M potassium hydroxide).

Methylation Analysis of the Polysaccharide. Polysaccharide B-1 (202.4 mg) was methylated by the Hakomori method.[9] A solution of the methylated product in benzene was diluted with petroleum ether (bp 30–60 °C) to precipitate the methylated polysaccharide (156.3 mg), $[\alpha]^{25}_{D}$ +3.50° (*c* 2.86, chloroform); IR ν_{max} (KBr) 1741 (methyl ester), 1096 (ether, ν C–O–C at 2832), 755 (band characteristic of polysaccharides with xylose) cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 5.15 (1H, d, $J_{1,2}$ = 2.1 Hz, H-1 α-D-Glc*p*A), 4.23 (1H, d, $J_{1,2}$ = 7.0 Hz, H-1 β-D-Glc*p* and β-D-Xyl*p*), 3.54 (3H, s, MeO–C-3 β-D-Glc*p* and β-D-Xyl*p*), 3.48 (3H, s, MeO–C-2 β-D-Glc*p* and β-D-Xyl*p*), 3.41 (3H, s, MeO–C-6 β-D-Glc*p*); ^{13}C NMR (100 MHz, D_2O) δ 102.2 (C-1 β-D-Xyl*p* and β-D-Glc*p*), 101.0 (C-1 α-D-Glc*p*A(162)-α-D-Xyl*p*), 96.1 (C-1 β-D-Fuc*p*), 84.0 (C-4 β-D-Glc*p*), 83.1 (C-4 β-D-Xyl*p*), 82.3 (C-5 β-D-Glc*p*), 81.6 (C-3 β-D-Xyl*p*), 79.7 (C-3 β-D-Glc*p*), 76.2 (C-2 β-D-Xyl*p*), 75.3 (C-2 β-D-Glc*p*), 63.3 (C-5 β-D-Xyl*p*), 60.7 (C-6 β-D-Glc*p*), 59.2 (MeO–C-2 and MeO–C-3 β-D-Xyl*p*), 58.7 (MeO–C-2, MeO–C-3, and MeO–C-6 β-D-Glc*p*).

A portion of this material was hydrolyzed, and the resulting sugars were converted into partially methylated alditol acetates[8] and analyzed by GC[13] and GC–MS[14] (Table 1).

To a solution of another portion (27.8 mg) of the methylated polysaccharide, in dry tetrahydrofuran (10 mL), was added lithium aluminum hydride (500 mg);[15] the reaction mixture was refluxed under N_2 for 24 h and then worked up in the usual way. The reduced product was then extracted into chloroform and dried, for 48 h in vacuo, over phosphorus pentaoxide. The product (13.1 mg) had IR absorption at 3600 cm^{-1} (OH) but not at 1735 cm^{-1} (ester C=O). The reaction product was hydrolyzed, and resulting sugars were converted into PMAA and analyzed by GC[13] and GC–MS.[14]

The methylation analysis of the oligosaccharides was performed by the method of Harris et al.[26]

Sugar Analysis. (a) By Gas–Liquid Chromatography. Polysaccharide B-1 (9.5 mg) was treated with aqueous 72% (w/w)[8] sulfuric acid (0.25 mL) for 1 h at 30 °C followed by dilution (2.75 mL of water) to 1 M sulfuric acid and heating (3 h at 100 °C). When cool, the hydrolysate was neutralized with 0.6 mL of 15 M NH_3 solution, 50 μL of a solution of *myo*-inositol (20 mg·mL^{-1}) was added as internal standard, and the solution was centrifuged. An aliquot (200 μL) of the upper layer of the centrifuged solution was reduced using 1 mL of $NaBH_4$ solution in DMSO (20 mg·mL^{-1}) at 40 °C for 90 min. Then, 100 μL of 18 M acetic acid was added to decompose the excess of sodium borohydride. Next, 200 μL of 1-methylimidazole and 2 mL of acetic anhydride were added to the reduced mixture of monosaccharides and mixed. After 10 min at room temperature, 10 mL of water was added to decompose the excess of acetic anhydride. When cool, 1 mL dichloromethane was added and then mixed. After phase separation, the lower phase was removed with a Pasteur pipet, and the alditol acetates were analyzed by GC.[9]

(b) By HPAE-PAD. A quantity of 2 mg of the polysaccharide was hydrolyzed with 0.3 mL of 2 M trifluoroacetic acid at 121 °C for 2.5 h. To the reaction mixture was added 50 μL of *myo*-inositol solution as internal standard, and the mixture was dried completely in a current of N_2. The sample was then dissolved in 5 mL of deionized water and analyzed by HPAE-PAD to identify its composition of neutral sugars[22] and uronic acids.[23] The uronic acids were also determined by the carbazole method using D-glucuronic acid as the standard.[27]

Partial Hydrolysis. Hemicellulose B-1 (96 mg) was treated with 0.125 M sulfuric acid[17] (12 mL) for 2 h at 100 °C. The hydrolysate was neutralized ($BaCO_3$), basified with 1 M KOH, then passed through a column of Amberlite IR-120 (H^+) resin, and concentrated. The syrupy residue was eluted from a column of Amberlite IRA-400 (AcO^-) resin, first with water to yield the neutral oligosaccharides, and then with aqueous 10% acetic acid to yield the acidic ones. Preparative paper chromatography of the neutral sugars gave two monosaccha-

Table 2. ^{13}C NMR (δ, ppm) Data of Neutral Oligosaccharides Obtained by Partial Hydrolysis of B-1

compound[a]	residue	C-1	C-2	C-3	C-4	C-5	C-6
NOS-1	β-Glc*p*(1→4)-	102.7	73.4	75.6	69.7	76.1	60.8
	-β-Glc*p*	95.9	74.1	74.5	78.9	74.9	60.8
	-α-Glc*p*	92	71.4	71.5	79	70.3	60.4
NOS-2	β-Glc*p*(1→4)-	102.7	73.3	75.7	69.6	76.1	60.8
	-β-Glc*p*(1→4)-	102.5	73.1	74.2	78.6	75	60.1
	-β-Glc*p*	95.9	74.1	74.4	78.7	75	60.2
	-α-Glc*p*	92	71.4	71.5	78.9	70.3	60.1
NOS-3	β-Glc*p*(1→4)-	102.7	73.3	75.7	69.6	76.2	60.8
	-β-Glc*p*(1→4)-	102.5	73.1	74.2	78.5	75	60.1
	-β-Glc*p*(1→4)-	102.5	73.1	74.2	78.6	75	60.1
	-β-Glc*p*	95.9	74.1	74.4	78.7	75	60.1
	-α-Glc*p*	92.8	71.4	71.5	78.9	70.3	60.1

[a] NOS 1−3 denote neutral oligosaccharides 1−3, obtained by partial acid hydrolysis.

rides, xylose and fucose in proportions of 78.3%, and 21.7%, respectively, and three oligosaccharides (NOS-1, NOS-2, and NOS-3) that were identified by methylation analysis[24] and from their ^{1}H NMR[11] and ^{13}C NMR[12] spectroscopic data.

NOS-1. A quantity of 3.1 mg of a white-colored crystalline oligosaccharide was isolated: $[\alpha]^{25}_{D}$ +24.11° (*c* 1.12, EtOH/H_2O, 1:1); ^{13}C NMR (100 MHz, D_2O) spectroscopic data are given in Table 2.

A quantity of 2 mg of this product was submitted to methylation analysis; the results are given in Table 1.

NOS-2. A quantity of 3.5 mg of a white-colored crystalline oligosaccharide was isolated: $[\alpha]^{25}_{D}$ +27.3° (*c* 1.01, EtOH/H_2O, 1:1); ^{13}C NMR spectroscopic data are given in Table 2.

A quantity of 1.0 mg of this product was submitted to methylation analysis; the results are given in Table 1.

NOS-3. A quantity of 1.7 mg of a white-colored syrupy oligosaccharide was isolated: $[\alpha]^{25}_{D}$ +16.5° (*c* 0.85, EtOH/H_2O, 1:1); ^{13}C NMR spectroscopic data are given in Table 2.

A quantity of 1.0 mg of this product was submitted to methylation analysis; the results are given in Table 1.

Preparative paper chromatography of the acidic sugars gave three oligosaccharides (AOS-1, AOS-2, and AOS-3) that were identified by methylation analysis and ^{1}H NMR and ^{13}C NMR spectroscopy.

AOS-1. A quantity of 5.3 mg of a yellowish-white-colored crystalline oligosaccharide was isolated: $[\alpha]^{25}_{D}$ +100° (*c* 1.03, EtOH/H_2O, 1:1), with the following spectroscopic data: ^{1}H NMR (400 MHz, D_2O) δ 5.18 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 α-D-Glc*p*A(1→2)-α-D-Xyl*p*), 5.16 (1H, d, $J_{1,2}$ = 4.1 Hz, H-1 α-D-Glc*p*A(1→2)-β-D-Xyl*p*), 4.87 (1H, d, $J_{1,2}$ = 4 Hz, H-1 α-D-Xyl*p*), 4.51 (1H, d, $J_{1,2}$= 8 Hz, H-1 β-D-Xyl*p*), 4.09 (1H, d, $J_{4,5}$ = 10.2 Hz, H-5 α-D-Glc*p*A(1→2)-α-D-Xyl*p*), 4.99 (1H, d, $J_{4,5}$ = 10.1 Hz, H-5 α-D-Glc*p*A(1→2)-β-D-Xyl*p*), 3.73 (1H, dd, $J_{5\alpha,5\beta}$ = 11.2 Hz, $J_{4,5\beta}$ 5.2 Hz; H-5β β-D-Xyl*p*), 3.28 (3H, s, CH_3O−C-4, α-D-Glc*p*A-(1→2)-D-Xyl*p*), 3.64−2.96 (group of overlapped signals corresponding to the rest of the protons); ^{13}C NMR (100 MHz, D_2O) δ 110.0 (C-1 β-D-Xyl*p*), 98.5 (C-1 α-D-Glc*p*A(1→2)-α-D-Xyl*p*), 97.9 (C-1 α-D-Glc*p*A(1→2)-β-D-Xyl*p*), 97.2 (C-1 α-D-Xyl*p*), 90.6 (C-1 α-D-Xyl*p*), 83.5 (C-3 α-D-Glc*p*A(1→2)), 80.4 (C-2 α-D-Xyl), 79.3 (C-2 β-D-Xyl*p*), 76.7 (C-4 α-D-Glc*p*A), 75.3 (C-3 β-D-Xyl*p*), 73.1 (C-2 α-D-Glc*p*A(1→2) and C-3 α-D-Xyl*p*), 72.3 (C-5 α-D-Glc*p*A(1→2)), 72.2 (C-4 α-D-Xyl*p*), 70.4 (C-4 β-D-Xyl*p*), 65.9 (C-5 β-D-Xyl*p*), 61.8 (C-5 α-D-Xyl*p*), 60.8 (CH_3O−C-4 α-D-Glc*p*A-(1→2)).

The oligosaccharide AOS-1 corresponds to an aldobiuronic acid; for identification, 2.0 mg was converted into the corresponding methyl ester, methyl glycoside,[28] by treatment with HCl at 3% in methanol (5 mL) and boiling for 24 h. The product of this reaction was acetylated with acetic anhydride−pyridine (1:1) for 24 h at room temperature, after which the final reaction product was obtained, whose mass spectral analysis showed the following more relevant peaks: EIMS (probe) 70 eV, *m/z* 477 [abE_1] (0.3), 417 [abE_2] (0.6), 403 [baF_1] (0.4), 345 [abF_1] (3), 289 [aA_1] (30), 229 [aA_2] (35), 187 [aA_2-CH_2CO] (100), 155 [aA_3] (11), 127 [bC_3] (22), 85 [aK_2] (28), 43 [CH_3CO^+] (76). 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. A quantity of 4.7 mg of a pale yellow-colored crystalline oligosaccharide was isolated: $[\alpha]^{25}_{D}$ +96.0° (*c* 1.01, EtOH−H_2O, 1:1); ^{1}H NMR (400 MHz, D_2O) δ 5.10 (1H, d, $J_{1,2}$ = 5.0 Hz, H-1 α-D-Glc*p*A(1→2)-α-D-Xyl*p*), 5.09 (1H, d, $J_{1,2}$ = 4 Hz, H-1 α-D-Glc*p*A(1→2)-β-D-Xyl*p*), 4.94 (1H, d, $J_{1,2}$ = 4 Hz, H-1 α-D-Xyl*p*), 4.37 (1H, d, $J_{1,2}$ = 8 Hz, H-1 β-D-Xyl*p*), 4.33 (1H, d, $J_{1,2}$ = 8 Hz, H-1 β-D-Xyl*p*(1→4)), 4.08 (1H, d, $J_{4,5}$ = 10.0 Hz, H-5 α-D-Glc*p*A(1→2)-D-Xyl*p*), 3.87 (1H, dd, $J_{5\alpha,5\beta}$ = 11,0 Hz, $J_{4,5\beta}$ = 5.0 Hz, H-5β β-D-Xyl*p*), 3.75 (1H, dd, $J_{5a,5\beta}$ = 11.0 Hz, $J_{4,5\beta}$ = 5.0 Hz, H-5β β-D-Xyl*p*(1→4)), 3.23 (3H, s, CH_3O−C-4, α-D-Glc*p*A(1→2)-d-Xyl*p*), 3.60−2.90 (group of overlapped signals corresponding to the rest of the protons); ^{13}C NMR (100 MHz, D_2O) δ 102.1 (C-1 β-D-Xyl*p*(1→4)), 97.9 (C-1 β-D-Xyl*p*), 97.0 (C-1 α-D-Glc*p*A(1→2)), 92.5 (C-1 α-D-Xyl*p*), 83.0 (C-3 α-D-Glc*p*A(1→2)), 77.0 (C-2 β-D-Xyl*p*), 76.9 (C-4 β-D-Xyl*p*; C-4 α-D-Xyl*p*), 76.7 (C-3 β-D-Xyl*p*(1→4); C-4 α-D-Glc*p*A(1→2)), 76.5 (C-2 α-D-Xyl*p*), 74.9 (C-3 β-D-Xyl*p*), 74.5 (C-2 β-D-Xyl*p*(1→4)), 72.8 (C-2 α-D-Glc*p*A(1→2)), 71.9 (C-3 α-D-Xyl*p*), 71.8 (C-5 α-D-Glc*p*A(1→2)), 70.4 (C-4 β-D-Xyl*p*(1→4)), 65.5 (C-5 β-D-Xyl*p*(1→4)), 63.5 (C-5 β-D-Xyl*p*), 61.5 (CH_3O−C-4 α-D-Glc*p*-A(1→2)), 59.2 (C-5 α-D-Xyl*p*).

A quantity of 1.5 mg of this product was submitted to methylation analysis; the results are given in Table 1.

AOS-3. A quantity of 1.4 mg of a pale yellow-colored crystalline oligosaccharide was isolated: $[\alpha]^{25}_{D}$ +90.0° (*c* 1.02, EtOH−H_2O 1:1), with the following spectroscopic data: ^{1}H NMR (400 MHz, D_2O) δ 5.11 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 α-D-Glc*p*A(1→2)-α-D-Xyl*p*), 5.09 (1H, d, $J_{1,2}$ = 4 Hz, H-1 α-D-Glc*p*A-(1→2)-β-D-Xyl*p*), 4.98 (1H, d, $J_{1,2}$ = 4 Hz; H-1 α-D-Xyl*p*), 4.42 (1H, d, $J_{1,2}$ = 8 Hz, H-1 β-D-Xyl*p*), 4.41 (1H, d, $J_{1,2}$ = 8 Hz, H-1 -β-D-Xyl*p*(1→4)), 4.38 (1H, d, $J_{1,2}$ = 8 Hz; H-1 β-D-Xyl*p*(1→4)), 4.26 (1H, d, $J_{4,5}$ = 10.0 Hz, H-5 α-D-Glc*p*A(1→2)-d-Xyl*p*), 3.78 (1H, dd, $J_{5\alpha,5\beta}$ = 11.0 Hz, $J_{4,5\beta}$ = 5.0 Hz, H-5β β-D-Xyl*p*(1→4)), 3.27 (3H, s, CH_3O−C-4, α-D-Glc*p*A(1→2)-D-Xyl*p*), 3.64−2.93 (group of overlapped signals corresponding to the rest of the protons); ^{13}C NMR (100 MHz, D_2O) δ 102.5 (C-1 β-D-Xyl*p*(1→4)), 102.2 (C-1 -β-D-Xyl*p*(1→4)), 98.0 (C-1 β-D-Xyl*p*), 97.0 (C-1 α-D-Glc*p*A(1→2)), 92.5 (C-1 α-D-Xyl*p*), 83.0 (C-3 α-D-Glc*p*A(1→2)), 77.3 (C-2 β-D-Xyl*p*), 76.9 (C-4 α-D-Xyl*p*), 76.6 (C-4 α-D-Glc*p*A(1→2; C-3 β-D-Xyl*p*(1→4); C-4 -β-D-Xyl*p*(1→4); C-4 β-D-Xyl*p*), 76.1 (C-2 α-D-Xyl*p*), 74.9 (C-3 β-D-Xyl*p*), 74.5 (C-3 -β-D-Xyl*p*(1→4)), 73.3 (C-2 β-D-Xyl*p*(1→4)), 73.2 (C-2 -β-D-Xyl*p*(1→4)), 72.8 (C-2 α-D-Glc*p*A(1→2)), 71.8 (C-5 α-D-Glc*p*A-(1→2)), 71.4 (C-3 α-D-Xyl*p*), 69.9 (C-4 β-D-Xyl*p*(1→4)), 65.7 (C-5 β-D-Xyl*p*(1→4)), 65.3 (C-5 β-D-Xyl*p*), 63.3 (C-5 -β-D-Xyl*p*(1→4)), 60.3 (CH_3O−C-4 α-D-Glc*p*A(1→2)), 59.3 (C-5 α-D-Xyl*p*).

A quantity of 0.7 mg of this product was submitted to methylation analysis; the results are given in Table 1.

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