Structure of a Hemicellulose B Fraction in Dietary Fiber from the Seed of Grape Variety Palomino (*Vitis vinifera* cv. Palomino)

José M. Igartuburu,*,† E. Pando,† F. Rodríguez-Luis,† and Antonio Gil-Serrano‡

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apdo 40. 11510, Puerto Real, Cádiz, Spain, and Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, 41071 Sevilla, Spain

Received December 8, 1997

The structure of one of the hemicellulose B fractions (HB-1) extracted from the seeds of the grape variety Palomino (*Vitis vinifera* cv. Palomino) has been studied by means of methylation analysis, ^{1}H NMR, ^{13}C NMR, and partial acid hydrolysis. This hemicellulose seems to be a homogeneous polysaccharide with an apparent molecular weight of 35 000. Its structure is that of an acidic arabinoxylan, a linear chain of β-D-xylopyranosyl units, bonded together by (1→4) glycosidic links, containing a single L-arabinofuranosyl, α-D-xylopyranosyl and 4-*O*-methyl-α-D-glucopyranuronosyl residues joined by glycosidic links to position 2 of the xylose units of the main chain, in proportions of one branch to every seven units of xylose.

Andalucía is one of the main wine-making zones of Spain. In the Jerez/Xerez/Sherry zone, the main byproduct of the sherry wine-making industry is the pomace obtained when grapes of the Palomino variety are pressed. This byproduct consist of three different components, seeds, stalks, and skins. Grape seeds represents about 10−15% of the total dry weight of Pomace. The aim of our study of grape seed composition is to propose its transformation into more economically valuable raw materials that will allow an improved use of this resource, which is currently underexploited.

The hemicellulose group comprises the most abundant fraction of the polysaccharides that make up grape seed dietary fiber from Palomino grape[1] [*Vitis vinifera* cv. Palomino (Vitaceae)], the main grape variety cultivated in the Jerez Denomination of Origin wine area. In a previous paper,[2] we have described the isolation, purification, and structural determination of a fraction from the hemicellulose A of this grape seed. This paper describes the structural determination of a polysaccharide isolated from the hemicellulose B fraction of Palomino grape seed.

Results and Discussion

Analysis of Palomino grape seed dietary fiber by the Southgate method[1] has shown that hemicelluloses constitute the main fraction of polysaccharides present in this material (about 15%). The procedure to obtain the holocelluloses has been described in a previous paper.[2] The hemicelluloses were extracted from this material by treatment with 10% NaOH[3] under nitrogen, and hemicellulose A was precipitated from the extract by acidification to pH 5 with 50% acetic acid. Hemicellulose B was isolated from the supernatant solution by precipitation with ethanol after dialysis against running water.

The hemicellulose B of grape seeds was treated with Fehling's solution to fractionate it into its components.[4] Purified hemicellulose HB-1 was isolated from the precipitate and gave a single narrow band on size-exclusion chromatography, having an apparent molecular weight of 35 000 and a specific rotation of $[\alpha]^{25}_D$ −19.7° (*c* 1.22, 1 M sodium hydroxide). This compound represents the second most abundant fraction of the polysaccharides in grape seed dietary fiber. Acid hydrolysis[5,6] of the polysaccharide showed that it is composed of arabinose, xylose, and 4-*O*-methylglucuronic acid in a molar ratio of 1:134:21.

Hakomori methylation of HB-1[7] produced a yellow, solid product with a specific rotation of $[\alpha]^{25}_D$ −22.9° (*c* 1.18, chloroform), indicative of the existence of β-D-glycosidic linkages. This was confirmed by the NMR spectroscopic data (δ 4.24 ppm for H-1 and δ 102.3 ppm for C-1).[8,9] A portion of the methylated polysaccharide was hydrolyzed, and the resulting sugars were converted into their corresponding partially methylated alditol acetates and analyzed by GC[10] and GC−MS.[11] The results obtained are summarized in Table 1 and in Figure 1.

Another portion of the methylated polysaccharide was reduced with $LiAlH_4$[12] and then hydrolyzed, and the resulting sugars were converted into their corresponding partially methylated alditol acetates; these were analyzed by GC and GC−MS, and the results are summarized in Table 1 and in Figure 1. The results of methylation analysis of the polysaccharide revealed that hemicellulose HB-1 is a linear acidic xylan having a backbone of β-D-xylopyranosyl residues bonded together by (1→4) glycosidic links. This linear chain has single-unit branches of 4-*O*-methyl-D-glucopyranuronosyl, L-arabinofuranosyl, and D-xylopyranosyl residues attached at C-2. The relative proportions of 2,3,4-Me_3-Glc, 2,3,5-Me_3-Ara, and 2,3,4-Me_3-Xyl shown in columns A and B of Table 1 indicate that, approximately, there is one branch unit due to L-arabinofuranose and two due to D-xylopyranose for every four 4-*O*-methylglucopyranuronosyl residues, and the molar ratio of 3-Me-xyl in both columns indicates that there is one branch point for every seven units of xylose in the main chain.

Partial acid hydrolysis[13] of the natural polysaccharide enabled the isolation by preparative paper chromatog-

* To whom correspence should be addressed. Tel.: int. + 34/56/830217. Fax: int + 34/56/834924. E-mail: josemanuel.igartuburu@uca.es.
† Facultad de Ciencias, Universidad de Cádiz.
‡ Facultad de Química, Universidad de Sevilla.

S0163-3864(97)00551-X CCC: $15.00
Published on Web 06/12/1998

Table 1. Methylation Analysis Data for Natural Polysaccharide, Reduced Polysaccharide, and Oligosaccharides Obtained by Partial Hydrolysis from the Hemicellulose Fraction HB-1

			molar ratio[d]					
methylated sugars (as alditol acetates)	T^a	T^b	A	B	NOS-1	NOS-2	NOS-3	AOS-2
2,3,5-Me_3-Ara[c]	0.48	0.79	0.5	0.4				
2,3,4-Me_3-Glc	2.49	1.11		4.5				
2,3,4-Me_3-Xyl	0.68	0.85	1.0	1.0	1.0	1.0	1.0	1.0
2,3-Me_2-Xyl	1.54	0.96	47.9	40.8	1.1	2.2	2.9	
3-Me-Xyl	2.78	1.05	7.3	6.8				1.2

[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] 2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol; 2,3,4-Me_3-Glc = 1,5,6-tri-*O*-acetyl-2,3,4-tri-*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. [d] Key: A, native polysaccharide; B, carboxyl-reduced polysaccharide; NOS-1/3, neutral oligosaccharides 1−3, obtained on partial acid hydrolysis; AOS-2, acidic oligosaccharide 2 obtained on partial acid hydrolysis.

raphy of two monosaccharides, arabinose (5.5%) and xylose (94.5%), and five oligosaccharides (three neutral and two acidic oligosaccharides, see Figure 2). Methylation analysis (see Table 1) and the spectroscopic data (δ 4.25 ppm for H-1 and δ 102.5 ppm for C-1) of the neutral oligosaccharides NOS-1, NOS-2, and NOS-3 show that they belong to an homologous series of β-D-xylopyranose-containing oligosaccharides with (1→4) glycosidic links,[8,9,14] and we were able to identify them as xylobiose, xylotriose, and xylotetraose, respectively.

The smallest of the acidic oligosaccharides (AOS-1) is an aldobiouronic acid having $[\alpha]^{25}_D$ +100°, indicative of the existence of an α-D-glycosidic link; this was confirmed by the NMR spectroscopic data (δ 5.18 ppm for H-1 and δ 98.5 ppm for C-1 of the 4-*O*-methyl-D-glucopyranuronosyl unit).[8,9] A portion of this oligosaccharide was converted into its corresponding methyl ester, methyl glycoside, and then analyzed by MS.[15] The mass spectrum of this product showed a fragmentation pattern 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. Therefore, AOS-1 was identified as 2-*O*-(4-*O*-methyl-α-d-glucopyranosyluronic acid)-D-xylopyranose (α-D-Glc*p*A4Me-(1→2)-D-Xyl*p*).

Spectroscopic data of the other acidic oligosaccharide (AOS-2) were similar to those of the above-described aldobiouronic acid (δ 5.18 ppm for H-1 and δ 98.5 ppm for C-1 of 4-Me-α-D-Glc*p*A). Methylation analysis of this oligosaccharide showed that its neutral residue was xylobiose. Therefore, we have identified these two acidic oligosaccharides as α-D-Glc*p*A4Me-(1→2)-D-Xyl*p* and β-D-Xyl*p*-(1→4)[α-D-Glc*p*A4Me-(1→2)]-D-Xyl*p*. The presence of these oligosaccharides is consistent with the pattern of substitutions in the main chain that we have described above.

These findings indicate that hemicellulose HB-1 is an acidic arabinoxylan formed by a main chain of β-D-xylopyranosyl units joined by (1→4) glycosidic links, which show branch units of L-arabinofuranose, 4-*O*-methyl-α-d-glucopyranosyluronic acid, or D-xylopyranose joined at position 2, in a ratio of 1:7.

There are few precedents in the structural studies of polysaccharides isolated form grape or grape-related products.[16,17] One arabinogalactan−protein aggregate was isolated from must and might be involved on microfiltration membrane plugging and wine-color stabilization in wine-making technology. In a previous work[2] we have described an acidic xylan isolated from the hemicellulose A fraction of Palomino grape seed. This polisaccharide shown to have a linear backbone of β-D-xylopiranosyl residues (1→4)-linked with single 4-*O*-methyl-D- glucopyranosyluronic acid and β-D-xylopyranosyl residues attached at some positions 2, in a ratio of 1:20.

Experimental Section

General Experimental Procedures. Descending paper chromatography was performed using Whatman No. 3 MM paper, with ethyl acetate−acetic acid−formic acid−water (18:3:1:4) as eluent. Detection of the component sugars was performed with diphenylamine−aniline.[18] Optical rotations were recorded with a Perkin-Elmer 241 polarimeter, and the IR spectra were carried out with a Perkin-Elmer 257 spectrophotometer. ^{1}H NMR and ^{13}C NMR were performed with a Varian Unity-400 instrument. Spectra of the methylated polysaccharide were obtained in $CDCl_3$ and referenced by means of the residual peak of the solvent. Spectra of the oligosaccharides were obtained in D_2O; proton spectra were referenced by means of the residual peak of the solvent, whereas for the carbon spectra, MeOH was used as an internal reference standard. All of NMR spectra were acquired at 25 °C.

GC of the alditol acetates was performed with a Hewlett-Packard Model 5890A gas chromatograph, fitted with a flame-ionization detector and a Supelcowax 10 M WCOT column (30 m × 0.53 mm i.d.), using a program that maintained an isocratic temperature of 220 °C for 15 min and then progressed to 230 °C at a rate of 3 °C min^{-1}. For the partially methylated alditol acetates (PMAA), an SPB-1 WCOT column (30 m × 0.53 mm i.d.) was used, with a temperature program of 120−250 °C at a rate of 5 °C min^{-1}. GC−MS was performed with a Kratos MS-80 instrument fitted with a CP-SIL WCOT column (25 m × 0.32 mm i.d.), using a temperature program of 100−250 °C at a rate of 5 °C min^{-1}. The ionization potential was 70 eV.

Size-exclusion chromatography was performed with a FPLC Superose 10/30 column (Pharmacia, V_o 7.3 mL, V_i 21.9 mL), using 100 mM NaOH as eluent, at a rate of 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 differential refractometry. The column was calibrated using dextrans of known molecular weights (Dextrans T from Pharmacia with molecular weights of 500 000, 150 000, 70 000, and 40 000 u).

HPAE-PAD HPLC of the monosaccharides resulting from total acid hydrolysis was performed with a Dionex

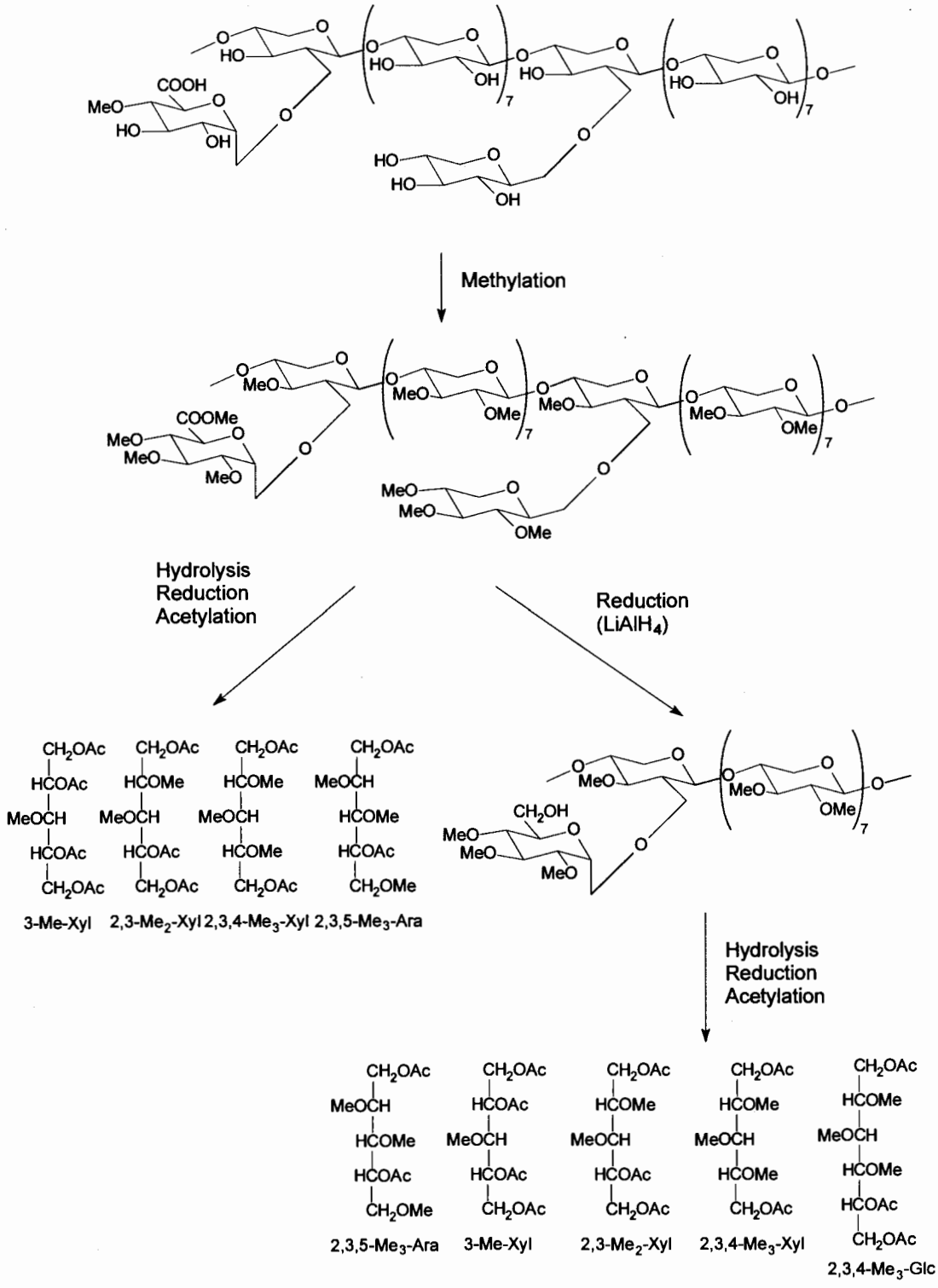


Figure 1. Methylation analysis of Hemicellulose HB-1.

DX-500 chromatograph equipped with a Dionex PA-1 column and precolumn. For 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. A program was used that 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.[19] Uronic acids were analyzed using the following as eluents: eluent A, a solution of 100 mM in NaOH and 600 mM in sodium acetate; eluent B, deionized water. The elution was performed under isocratic conditions using a mixture of 25% of A and 75% of B.[20]

Plant Material. Grape pomace was obtained from the grapes (*V. vinifera* cv. Palomino) collected in the Jerez/Xeres/Sherry Zone in September 1992 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.

Isolation and Purification of the Polysaccharide. Grape seeds (530 g) were triturated in a Braun AG-4050 mill and the meal extracted successively with

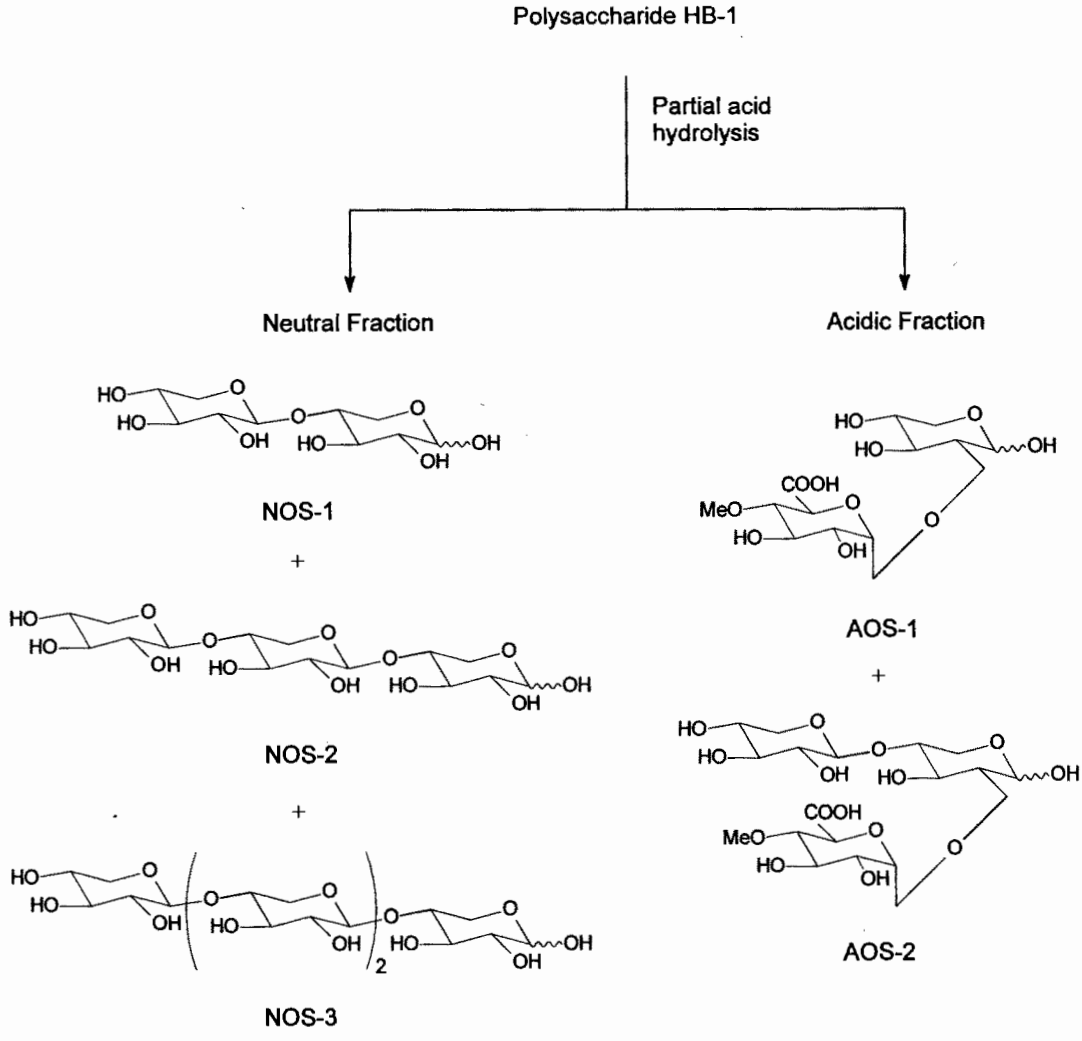


Figure 2. Oligosaccharides obtained by partial acid hydrolysis from hemicellulose HB-1.

hexane, ethanol, and chloroform–methanol (1:1) for 18 h in a Soxhlet extractor. The residue (479 g) was stirred for 24 h at room temperature with sodium methoxide 0.25 M in methanol (1 L).[21] The insoluble residue was collected by centrifugation and washed with methanol until neutral, and then it was extracted with deionized water at room temperature for 24 h. The remaining solid material (459 g) was delignified with sodium chlorite and acetic acid.[22] The resulting holocellulose (254 g) was stirred with aqueous 10% NaOH (5 L) containing 10 mM $NaBH_4$ for 24 h at room temperature under nitrogen. The resulting extract was vacuum-filtered through filter cloth. Hemicellulose A was precipitated from this extract 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 and then concentrated to small volume in a rotary evaporator. Hemicellulose B was precipitated by addition of 4 vol 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 (12.81 g).

A solution of the hemicellulose A (6.15 g) in 5% aqueous potassium hydroxide (600 mL) was treated with Fehling's solution[4] until precipitation was complete. The precipitated hemicellulose was collected by centrifugation, treated with 5% HCl in methanol, at 0 °C for 5 min, and then centrifuged. The residue was then washed four times with ethanol and twice with acetone and then vacuum-dried over phosphorus pentaoxide. This entire precipitation procedure was then repeated two more times; this preparation (1.15 g) gave designated hemicellulose B-1 (HB-1), $[\alpha]^{25}_D$ −19.67° (*c* 1.22, 1 M sodium hydroxide).

Methylation Analysis of the Polysaccharide. A quantity (139.5 mg) of HB-1 was methylated by the Hakomori method.[7] The methylated product was purified by precipitation from benzene with light petroleum (bp 30–60 °C) (86.5 mg): $[\alpha]^{25}_D$ −22.88° (*c* 1.18, $CHCl_3$).

A portion of the methylated polysaccharide was hydrolyzed, and the resulting sugars were converted into the corresponding partially methylated alditol acetates (PMAA),[5] which were analyzed by GC[10] and GC–MS.[11]

To a solution of another portion of the methylated polysaccharide (13.5 mg) in dry tetrahydrofuran (10 mL) was added $LiAlH_4$ (lithium aluminum hydride) (200 mg).[12] The mixture was refluxed in an atmosphere of N_2 for 24 h, after which time the reaction was stopped by addition of 2 mL of acetone and then filtered through Whatman No. 1 paper. The product of the reaction was then extracted in $CHCl_3$ and vacuum-dried for 48 h over

phosphorus pentaoxide. The resulting product showed an IR absorption at 3600 cm^{-1} (OH), but not at 1735 cm^{-1} (ester C=O). The reduction product was hydrolyzed, and the resulting sugars were converted into PMAA and analyzed by GC[10] and GC–MS.[11]

The methylation analysis of the oligosaccharides was performed by the method of Harris et al.[23]

Monosaccharide Composition of the Polysaccharides and Oligosaccharides. (a) By Gas–Liquid Chromatography. Polysaccharide HB-1 (12.0 mg) was treated with 0.25 mL of 72% (w/w) H_2SO_4 at room temperature for 1 h,[5] after which time the sample was diluted to a concentration of 1 M (2.75 mL) and then heated for 3 h at 100 °C. When cool, the hydrolyzate was neutralized with 0.6 mL of 15 M NH_3 solution; 50 μL of a solution of *myo*-inositol (20 mg mL^{-1}) were added as internal standard, and the hydrolyzate was clarified by centrifugation. A portion of 200 μL of the upper phase of centrifugate 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 excess sodium borohydride. Quantities of 1-methylimidazole (200 μL) and acetic anhydride (2 mL) were added to the mixture of reduced sugars 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 of dichloromethane was added, and the solution was mixed. After phase separation, the lower phase was removed with a Pasteur pipet, and the resulting alditol acetates were analyzed by GC.[6]

(b) By High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD HPLC). A quantity of 2 mg of the polysaccharide HB-1 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 (20 mg L^{-1}) solution as internal standard, and the mixture was completely dried in a current of N_2. The sample was then dissolved in 5 mL of deionized water and analyzed by HPAE-PAD HPLC.[19,20] Uronic acids were also determined by the carbazole method using D-glucuronic acid as the standard.[24]

Partial Acid Hydrolysis. Hemicellulose HB-1 (213 mg) was treated with 0.125 M sulfuric acid (20 mL) for 2 h at 100 °C.[13] The hydrolyzate was neutralized with $BaCO_3$, mixed with 1 M KOH, and then passed through a column of Amberlite resin IR-120 (H^+) and concentrated. The syrupy residue was eluted from a column of Amberlite resin IRA-400 (AcO^-), first with water to yield the neutral sugars and then with aqueous 10% acetic acid to yield the acidic oligosaccharides. Components of the neutral fraction were separated by preparative paper chromatography, which enabled the isolation of two monosaccharides (25 mg), arabinose (5.5%), and xylose (94.5%) (these monosaccharides were identified and quantified by GC), and three oligosaccharides (NOS-1, NOS-2, and NOS-3). These three oligosaccharides were crystallized from EtOH and were identified by methylation analysis and by their ^1H NMR and ^{13}C NMR data.

NOS-1. A white, crystalline oligosaccharide (10.1 mg) was isolated (EtOH) [$[\alpha]^{25}_D$ −33.0° (*c* 1.30, EtOH–H_2O, 1:1)], with the following spectroscopic data: ^1H NMR (D_2O, 400 MHz) δ 5.00 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 α-D-Xyl*p*), 4.40 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 β-D-Xyl*p*), 4.27 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 β-D-Xyl*p*-(1→4)-), 3.87 (1H, dd, $J_{5\alpha,5\beta}$ = 12.0 Hz, $J_{4,5\beta}$ = 5 Hz, H-5β β-D-Xyl*p*), 3.78 (1H, dd, $J_{5\alpha,5\beta}$ = 12.0 Hz, $J_{4,5\beta}$ = 5 Hz, H-5β β-D-Xyl*p*-(1→4)-), 3.65–3.53 (5H, group of signals corresponding to H-5α, H5β y H-4 of α-D-Xyl*p*, H-4 of β-D-Xyl*p*, and H-4 of β-D-Xyl*p*-(1→4)-), 3.47 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 7.0 Hz, H-3 α-D-Xyl*p*), 3.43 (1H, dd, $J_{1,2}$ = 5.0 Hz, $J_{2,3}$ = 9.0 Hz, H-2 α-D-Xyl*p*), 3.36 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3 β-D-Xyl*p*), 3.36 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3 β-D-Xyl*p*-(1→4)-), 3.19 (1H, t, $J_{5\alpha,5\beta}$ = $J_{4,5\alpha}$ = 11.0 Hz, H-5α β-D-Xyl*p*), 3.12 (t, $J_{5\alpha,5\beta}$ = $J_{4,5\alpha}$ = 11.0 Hz, H-5α β-D-Xyl*p*-(1→4)-), 3.10–3.02 (3H, group of signals corresponding to the protons H-2 of β-D-Xyl*p*-(1→4)-, -β-D-Xyl*p*-(1→4)- and β-D-Xyl*p*); ^{13}C NMR (D_2O, 100 MHz), see Table 2.

A quantity of 4 mg of this oligosaccharide was submitted to methylation analysis; the results are given in Table 1.

NOS-2. A white, crystalline oligosaccharide (5.7 mg) was isolated (EtOH) [$[\alpha]^{25}_D$ −45.3° (*c* 1.12, EtOH–H_2O, 1:1)] with the following spectroscopic data: ^1H NMR (D_2O, 400 MHz) δ 5.01 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 α-D-Xyl*p*), 4.40 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 β-D-Xyl*p*), 4.30 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1 -β-D-Xyl*p*-(1→4)–), 4.28 (1H, d, $J_{1,2}$ = 8.0 Hz H-1 β-D-Xyl*p*-(1→4)–), 3.92 (1H, dd, $J_{5\alpha,5\beta}$ = 12.0 Hz, $J_{4,5\beta}$ = 5 Hz, H-5β -β-D-Xyl*p*-(1→4)–), 3.87 (1H, dd, $J_{5\alpha,5\beta}$ = 12.0 Hz, $J_{4,5\beta}$ = 5 Hz, H-5β β-D-Xyl*p*), 3.79 (1H, dd, $J_{5\alpha,5\beta}$ = 12.0 Hz, $J_{4,5\beta}$ = 5 Hz, H-5β β-D-Xyl*p*-(1→4)–), 3.66–3.54 (6H, group of signals corresponding to the protons H-5α, H5β and H-4 of α-D-Xyl*p*, H-4 of β-D- Xyl*p*, H-4 of –β-D-Xyl*p*-(1→4)–, and to H-4 of β-D-Xyl*p*-(1→4)–), 3.48 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 10 Hz, H-3 α-D-Xyl*p*), 3.45 (1H, dd, $J_{1,2}$ = 5.0 Hz, $J_{2,3}$ = 10 Hz, H-2 α-D-Xyl*p*), 3.37 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 10 Hz, H-3 –β-D-Xyl*p*-(1→4)–), 3.36 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 10 Hz H-3 β-D-Xyl*p*), 3.25 (1H, t, $J_{5\alpha,5\beta}$ = $J_{4,5\alpha}$ = 10 Hz, H-5α –β-D-Xyl*p*-(1→4)–), 3.20 (1H, t, $J_{5\alpha,5\beta}$ = $J_{4,5\alpha}$ = 10 Hz, H-5α β-D-Xyl*p*), 3.13 (1H, t, $J_{5\alpha,5\beta}$ = $J_{4,5\alpha}$ = 10 Hz, H-5α β-D-Xyl*p*-(1→4)–), 3.10–3.03 (3H,group of signals corresponding to the protons H-2 of β-D-Xyl*p*(1→4)–, –β-d-Xyl*p*-(1→4)–, β-D-Xyl*p*); ^{13}C NMR (D_2O, 100 MHz), see Table 2.

A quantity of 2.6 mg of this product was submitted to methylation analysis; the results are given in Table 1.

NOS-3. A white, crystalline oligosaccharide (7.1 mg) was isolated (EtOH) [$[\alpha]^{25}_D$ −48.3° (*c* 1.01, EtOH–H_2O, 1:1)] with the following spectroscopic data: ^1H NMR (D_2O, 400 MHz) δ 4.91 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1 α-D-

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

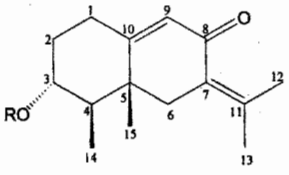
compd[a]	residue	C-1	C-2	C-3	C-4	C-5
NOS-1	β-Xyl*p*(1→4)–	102.8	73.7	76.6	70.1	66.1
	–β-Xyl*p*	97.5	74.8	74.9	77.4	63.9
	–α-Xyl*p*	93.0	72.3	71.9	77.5	59.8
NOS-2	β-Xyl*p*(1→4)–	102.8	73.7	76.5	70.1	66.2
	–β-Xyl*p*(1→4)–	102.6	73.6	74.6	77.3	63.9
	–β-Xyl*p*	97.5	74.8	74.9	77.3	64.4
	–α-Xyl*p*	92.9	72.3	71.8	77.5	59.8
NOS-3	β-Xyl*p*(1→4)–	102.9	73.8	76.6	70.2	66.2
	–β-Xyl*p*(1→4)–	102.7	73.7	75.0	77.4	64.1
	–β-Xyl*p*(1→4)–	102.7	73.7	75.0	77.4	64.1
	–β-Xyl*p*	97.5	74.7	74.7	77.4	63.9

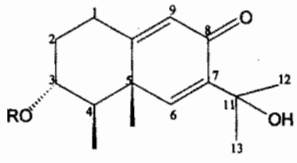
[a] NOS 1–3 denote neutral oligosaccharides 1–3, obtained on partial acid hydrolysis.

Table 1. ^1H and ^{13}C NMR Spectral Data of **1**–**4** (300 and 75 MHz, in $CDCl_3$)

	1		**2**		**3**		**4**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	NR[a]	29.8	NR	29.9	NR	30.1	NR	29.4
2	NR	31.4	NR	31.4	NR	32.7	NR	33.6
3	4.83 td (11.1,4.8)[b]	75.7	4.92 td (11.1,4.5)	75.1	4.97 td (11.1,4.8)	72.7	4.91 td (11.0,4.8)	76.8
4	NR	45.9	NR	45.9	NR	44.5	NR	42.6
5		42.0		42.1		43.0		39.0
6	2.91 d (13.5) 2.13 dq (13.5,1.5)	41.0	2.91 d (13.5) 2.18 dq (13.5,1.5)	41.1	6.88 s	147.8	NR	37.2
7		126.9		126.9		141.3		47.2
8		191.3		191.5		187.6	4.06 d (9.6)	68.8
9	5.73 d (1.5)	126.8	5.77 d (1.2)	126.9	6.07 d (1.5)	125.2	5.43 d (1.2)	123.9
10		164.0		164.4		166.2		145.7[c]
11		143.8		143.7		71.8		144.6[c]
12	2.04 d (1.5)	22.6[c]	2.08 d (1.8)	22.6[c]	1.44 s	28.9[c]	4.72 (br s) 4.87 (br s)	112.8
13	1.80 s	22.1[c]	1.84 s	22.1[c]	1.44 s	29.0[c]	1.71 s	19.8
14	0.93 d (6.9)	10.7	0.98 d (6.6)	10.7	0.94 d (6.6)	11.7	0.84 d (6.6)	11.8
15	1.04 s	17.0	1.03 s	17.1	1.08 s	18.5	1.00 s	22.1
1′		163.9		163.4		166.1		163.4
2′	6.24 d (10.2)	124.2	6.67 d (14.7)	126.0	5.79 d (10.5)	112.7	6.64 d (14.7)	126.3
3′	6.96 d (10.2)	159.5	7.62 d (14.7)	151.1	7.07 d (10.5)	152.8	7.57 d (14.7)	150.7
S–Me					2.41 s	19.2		
O ‖ S–Me	2.79 s	40.6	2.71 s	39.7			2.69 s	39.7
OH					4.75 br s			

[a] NR: not resolved. [b] Figures in the parentheses are coupling constants in Hz. [c] Assignment may be interchanged.





3 R: COCH=CHSMe (Z)

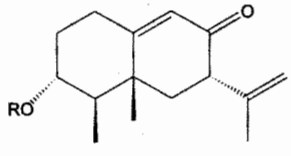
1 R: COCH=CHSMe (1′ 2′ Z 3′ O)

7 R: COCMe=CHMe (Z)

2 R: COCH=CHSMe (E)

6 R: COCH=CHSMe (Z)

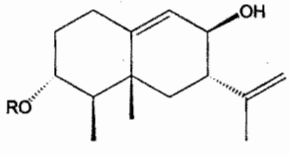
8 R: COCH=CHSMe (Z) (sulfoxide epimer of 1)



9 R: COCH=CHSMe (E)

5 R: COCH=CHSMe (Z)

10 R: COCH=CHSMe (Z)



4 R: COCH=CHSMe (E)

11 R: COCH=CHSMe (Z)

12 R: COCH=CHSMe (Z)

s)], and a (*Z*)-3-methylthioacrylate [δ_H 2.41 (3H, s), δ_C 19.2; δ_H 5.79 and 7.07 (each 1H, d, J = 10.5 Hz), δ_C 112.7 and 152.8] located at C-3 with α-equatorial orientation, and a methine proton with carbon bearing an ester group [δ_H 4.97 (1H, td, J = 11.1, 4.8 Hz)].

An HMBC experiment revealed the correlations as follows: H-12, 13 (δ 1.44) to C-11 (δ 71.8) and C-7 (δ 141.3); H-15 (δ 1.08) to C-6 (δ 147.8) and C-10 (δ 166.2); and H-6 (δ 6.88) to C-7 (δ 141.3) and C-8 (δ 187.6). This led to structure **3** with the same parent sesquiterpene as petasitin (**7**),[12] and the angeloyl ester moiety in **7** being substituted by (*Z*)-3-methylthioacrylate group in (*S*)-petasitin (**3**). Therefore, the structure of (*S*)-petasitin (**3**) was assigned as 3α-[(*Z*)-3-methylthioacryloxy]-11-hydroxyeremophila-6,9-dien-8-one.

Petasinol (**4**) was obtained as an amorphous solid. HRMS showed that **4** had the molecular formula $C_{19}H_{28}O_4S$. The IR spectrum indicated the presence of hydroxyl (3440 cm^{-1}) and an α,β-unsaturated carbonyl group (1710, 1625, 1220 cm^{-1}). Compound **4** had a (*E*)-3-methylsulfinylacryloxy side chain as in **2**: δ 2.69 (3H, s, $-SOCH_3$), 6.64, and 7.57 (1H each, d, J = 14.7 Hz). The ^1H NMR spectrum of **4** (Table 1) also revealed an isopropenyl group [δ 1.71 (3H, s), 4.72, and 4.87 (1H each, br s)], singlet and doublet methyl groups [δ 1.00 (3H, s) and 0.84 (3H, d, J = 6.6 Hz)], and a methine proton with triplet of doublets linked to ester [δ 4.91 (1H, td, J = 11.0, 4.8 Hz, H-3)]. An olefinic proton at higher field [δ 5.43 (1H, d, J = 1.2 Hz, allylic coupling with H-1 axial)] than the corresponding protons in **1**, **2**, and **3** showed that the C-9–C-10 olefin group was not conjugated. No ketone group was observed in its spectra, but a methine proton signal bearing a hydroxyl group was present at δ 4.06. This methine proton's coupling constant (J = 9.6 Hz) indicated that it was in quasi-axial orientation. The relatively lower field position of this proton at δ 4.06 showed that it was allylic. The structure of petasinol (**4**) was elucidated as 3α-[(*Z*)-3-methylsulfinylacryloxy]-8β-hydroxyeremophila-9,11-diene from HMBC correlations (Figure 1). The reduction of (*S*)-petasin (**5**) with $NaBH_4$ in MeOH yielded **11** [ν_{max} 3420, 1030 cm^{-1}; δ 4.05 (1H, d, J = 9.6 Hz, H-8)], which was subsequently oxidized with *m*-CPBA in CH_2Cl_2 at 0–5 °C and afforded compound **4** and a