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Determination of the neutral and acidic glycosyl-residue compositions of plant polysaccharides by GC-EI-MS analysis of the trimethylsilyl methyl glycoside derivatives

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Abstract

A method, using GC-EI-MS analysis of the trimethylsilyl methyl glycoside derivatives, has been developed to identify the neutral and acidic glycosyl-residue compositions of plant polysaccharides. Monosaccharides (15) including four pentoses (arabinose, xylose, 2-*O*-methylxylose, and apiose), three hexoses (galactose, glucose, and mannose), three 6-deoxy hexoses (rhamnose, fucose, and 2-*O*-methylfucose), two hexuronic acids (galacturonic and glucuronic acids), two 2-keto-3-deoxy sugars (Kdo and Dha), and aceric acid were quantified. The monosaccharide derivatives were identified by their retention times and by their characteristic GC-MS fragmentation patterns. These monosaccharides are components of plant cell wall polysaccharides and red wine polysaccharides. Thus, GC-MS analysis of trimethylsilyl methyl glycoside derivatives is suitable for determining the neutral and acidic glycosyl-residue compositions of plant cell wall polysaccharides and of polysaccharides present in plant-derived foods and beverages.

Keywords: GC-EI-MS; Methanolysis; Trimethylsilylation; Cell walls; Pectic polysaccharides; Rhamnogalacturonans; Apiose; 2-*O*-methyl fucose; 2-*O*-methyl-xylose; Aceric acid; Kdo; Dha

1. Introduction

Fruit-derived beverages such as wine contain complex mixtures of plant- and microbial-derived polysaccharides including arabinans (Belleville, Williams & Brillouet, 1993; Villettaz, Amado & Neukom, 1981), arabinogalactans (Brillouet, Bosso & Moutounet, 1990; Pellerin, Vidal, Williams & Brillouet, 1995; Pellerin, Waters & Brillouet, 1993), rhamnogalacturonans (Doco & Brillouet, 1993; Pellerin, Doco, Vidal, Williams, Brillouet & O'Neill, 1996), mannans (Llaubères, Dubourdieu & Villettaz, 1987; Villettaz, Amado & Neukom, 1980; Waters, Pellerin & Brillouet, 1994) and glucans (Dubourdieu, Ribereau-Gayon & Fournet, 1981). Rhamnogalacturonans are structurally complex pectic polysaccharides that contain several different glycosyl residues (O'Neill, Albersheim & Darvill, 1990). The predominant glycosyl residues of rhamnogalacturonan I are galacturonic acid, arabinose, galactose, and rhamnose (O'Neill et al., 1990). Rhamnogalacturonan II (RG-II), which is a major polysaccharide component of red wine (Doco & Brillouet, 1993; Pellerin et al., 1996), contains 11 different glycosyl residues including the unusual monosaccharides apiose, 2-*O*-methylfucose, 2-*O*-methylxylose, aceric acid [3-*C*-carboxy-5-deoxy-L-xylose] (Spellman, McNeil, Darvill, Albersheim & Henrik, 1983), Kdo [3-deoxy-D-*manno*-2-octulosonic acid] (York, Darvill, McNeil & Albersheim, 1985) and Dha [3-deoxy-D-*lyxo*-heptulosaric acid] (Stevenson, Darvill & Albersheim, 1988).

The neutral glycosyl-residue compositions of plant polysaccharides are typically determined, after acid hydrolysis, by GC and GC-MS analyses of the alditol acetate derivatives (Albersheim, Nevins, English & Karr, 1967; Fox, Morgan & Gilbart, 1990). However, the acidic glycosyl-residue compositions cannot be determined directly by this procedure. Thus, colorimetric assays for hexuronic acids (Blumenkrantz & Asboe-Hansen, 1973), and for Kdo and Dha (York et al., 1985) are often used in

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Abbreviations: GC, Gas Chromatography; EI, Electron Impact; MS, Mass Spectrometry; RG-II, rhamnogalacturonan II; Aceric acid, 3-*C*-carboxy-5-deoxy-L-xylose; Kdo, 3-deoxy-D-*manno*-octulosonic acid; Dha, 3-deoxy-D-*lyxo*-heptulosaric acid; Apiose, 3-*C*-hydroxymethyl-D-*glycero*-aldotetrose; TMS, per-*O*-trimethylsilylated methyl glycosides; HPAEC-PAD, High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

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combination with the alditol acetate procedure to estimate the neutral and acidic glycosyl-residue content of a polysaccharide (Brillouet et al., 1990; Doco & Brillouet, 1993; Segarra, Lao, López-Tamames & de la Torre-Boronat, 1995). In contrast, both the neutral and acidic monosaccharides released by acid hydrolysis of a polysaccharide can be quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Hardy, Townsend & Lee, 1988). However, standards are required, since the monosaccharides are identified on the basis of their retention times alone.

Neutral and acidic monosaccharides are liberated, as their corresponding methyl glycosides, by treating polysaccharides with anhydrous methanol containing HCl (Chambers & Clamp, 1971). The conversion of the methyl glycosides to their trimethylsilyl (TMS) methyl glycoside derivatives typically results in the formation of several components (derivatives of the α- and β-, and pyranose and furanose forms) for each sugar. The relative proportions of each component and their retention times on high-resolution gas chromatography capillary columns are characteristic of each monosaccharide. However, only those glycosyl residues for which standards are available can be identified on the basis of their retention time alone.

GC-MS is an established procedure for identifying the TMS methyl glycoside derivatives of neutral and acidic monosaccharides (Bleton, Mejanelle, Sansoulet, Goursaud & Tchapla, 1996). The EI mass spectra of hexose, pentose, 6-deoxyhexose, and uronic acid derivatives are sufficiently different to allow their unambiguous identification (DeJongh et al., 1969; Kakehi & Honda, 1990; Kotchetkov & Chizhov, 1966; Petrzika & Linow, 1982). However, the EI mass spectra of monosaccharides within a class (e.g. glucose, galactose, and mannose) are similar and thus are of limited value for identification purposes. Nevertheless, the ability of EI-MS analysis to distinguish different classes of monosaccharide derivatives when combined with the high resolution of capillary GC provides unambiguous identification of complex mixtures of monosaccharides.

We now report that all the glycosyl residues, including the unusual monosaccharides apiose, 2-*O*-methylfucose, 2-*O*-methylxylose, aceric acid, Kdo, and Dha, that are present in plant cell walls and in wine polysaccharides are identified and quantified by GC-MS analysis of their TMS methyl glycoside derivatives. Each glycosyl residue was identified by its characteristic pattern of multiple peaks and their retention times and by specific fragment ions in its EI mass spectra. The EI mass spectra and fragmentation mechanism of the unusual monosaccharides are discussed.

2. Materials and methods

2.1. Materials

Materials and reagents were analytical-reagent grade. L-Fucose, L-rhamnose, 2-*O*-methyl D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, Kdo, D-galacturonic and D-glucuronic acid were obtained from Sigma (St Louis, USA). The trimethylsilylation reagent (TriSil®) was purchased from Pierce (Rockford, USA). Red wine polysaccharides were precipitated by the addition of four volumes of 95% ethanol (Doco, Quellec, Moutounet & Pellerin, 1999) to a wine produced from mature grapes of the Carignan noir cultivar in 1996 at the Unité Experimentale d'Œnologie INRA Pech-Rouge (Gruissan, France). Apples (*Malus domestica*) and carrots (*Daucus carota*) (100 g) were peeled, sliced, and then homogenized in presence of 3 mM ascorbic acid using a Waring blendor (2 min) (Doco, Williams, Vidal & Pellerin, 1997). The homogenates were filtered through Nylon mesh (40 μ). The filtrates were then treated with four volumes of 95% ethanol and kept at −20°C for 24 h. The precipitates that formed were isolated by centrifugation at 4°C for 25 min (8500*g*) and the pellets washed with 95% ethanol. The precipitates were dissolved in water and then freeze dried.

2.2. Sample preparation

Methanolysis and trimethylsilylation were performed as previously described (Chambers & Clamp, 1971; York, Darvill, McNeil, Stevenson & Albersheim, 1986). Methanol containing 0.5 M HCl was prepared by adding acetyl chloride (140 μl) to anhydrous methanol (1 ml). Monosaccharides and polysaccharides (100–500 μg) were suspended in MeOH/HCl (0.5 ml) and kept for 16 h at 80°C. The cooled solutions were concentrated to dryness at 40°C under a stream of nitrogen gas. An excess of TriSil® reagent (0.3 ml) was added and the solutions kept for 20 min at 80°C. The reagents were removed at 40°C with a stream of nitrogen gas. The residue was then extracted with hexane (1 ml). The hexane was concentrated to 50 and 2 μl used for GC-EI-MS analysis. All analyses were performed in triplicate.

2.3. Gas chromatography-mass spectrometry

GC-MS was performed with a Hewlett Packard HP-6890 GC coupled to a HP 5973 mass selective detector operated in the EI mode and under the control of a HP Productivity ChemStation. The GC was equipped with a capillary split/splitless inlet and a flame ionization detector (FID). Two DB-1 fused-silica capillary columns (30 m × 0.25 μm i.d., 0.25 μm film thickness, J&W Scientific) were coupled to a single injector inlet using a two-holed ferrule. The outlet of one column was connected directly to the FID detector. The outlet of the second column was connected to the mass detector by a deactivated fused-silica column (0.25 m × 0.11 μm i.d.). Hydrogen (flow rate of 2 ml/min, average velocity 58 cm/s) was the carrier gas. Samples were injected in the pulsed split mode with a split ratio of 20:1. The injector and the FID were operated at 250°C. The

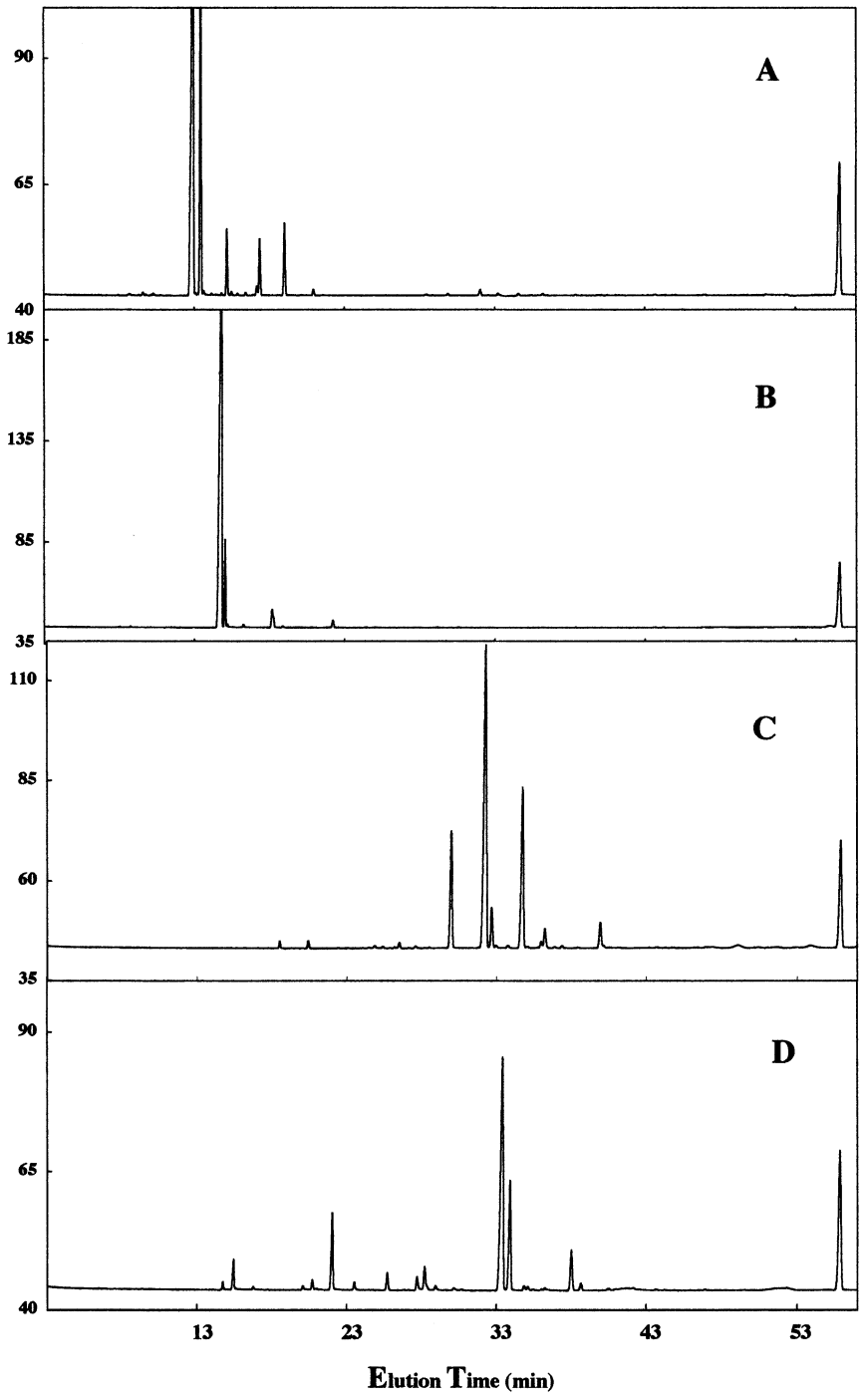

Fig. 1. The GC-FID profiles of the TMS methyl glycoside derivatives of standard monosaccharides: (a) L-arabinose; (b) L-rhamnose; (c) D-galactose; and (d) D-galacturonic acid.

transfer line to the MSD was set at 280°C. The GC was operated with temperature programming (120–145°C at 1°C/min, 145–180°C at 0.9°C/min, and 180–230°C at 50°C/min). EI mass spectra were obtained from *m/z* 50–650 every 2.48 s in the total ion-monitoring mode using a source temperature of 230°C, a quadrupole temperature of 106°C, a filament emission current of 34.6 μA, and an ionization voltage of 70 eV.

3. Results

3.1. General

Arabinose, galactose, rhamnose, fucose, xylose, galacturonic acid, and glucuronic acid are the predominant glycosyl residues present in pectic polysaccharides and are also representative of pentoses, hexoses, 6-deoxy

Table 1
Retention times on OV-1 column of sugars found in pectic polysaccharides

Compound	Retention time[a] (min)	% of maximal peak[b]
Arabinose	12.98 ± 0.02	100.0
Arabinose	13.48 ± 0.02	42.0 (±1.1)
Arabinose	15.19 ± 0.02	7.2 (±0.5)
Arabinose	17.38 ± 0.02	7.7 (±1.2)
Rhamnose	14.77 ± 0.06	100.0
Rhamnose	15.00 ± 0.06	9.2 (±0.4)
Rhamnose	18.14 ± 0.05	3.8 (±0.3)
Rhamnose	22.19 ± 0.05	1.2 (±0.3)
Fucose	14.89 ± 0.03	24.3 (±3.7)
Fucose	15.95 ± 0.04	100.0
Fucose	17.17 ± 0.03	52.2 (±2.1)
Fucose	17.36 ± 0.03	9.5 (±2.2)
Xylose	18.62 ± 0.02	100.0
Xylose	19.90 ± 0.01	52.0 (±2.7)
Xylose	22.99 ± 0.02	4.8 (±0.2)
Xylose	27.27 ± 0.02	5.3 (±0.1)
Galactose	29.99 ± 0.02	25.0 (±3.6)
Galactose	32.31 ± 0.03	100.0
Galactose	32.68 ± 0.02	7.2 (±1.3)
Galactose	34.76 ± 0.02	43.2 (±0.5)
Galacturonic acid	22.03 ± 0.02	17.4 (±3.1)
Galacturonic acid	25.77 ± 0.05	37.0 (±1.5)
Galacturonic acid	28.24 ± 0.01	16.9 (±0.4)
Galacturonic acid	33.44 ± 0.01	100.0
Galacturonic acid	33.93 ± 0.01	34.6 (±0.2)
Galacturonic acid	38.01 ± 0.01	11.8 (±1.2)
Galacturonic acid	38.64 ± 0.02	4.4 (±0.8)
Glucuronic acid	23.69 ± 0.15	9.4 (±3.1)
Glucuronic acid	37.23 ± 0.04	31.4 (±4.1)
Glucuronic acid	37.81 ± 0.06	100.0
Glucuronic acid	40.43 ± 0.01	5.0 (±0.5)

[a] Average ± standard deviation.
[b] % Average.

hexoses, and hexuronic acids. Authentic standards of these monosaccharides were converted to their corresponding TMS methyl glycoside derivatives and analyzed by GC. Between four and seven peaks are obtained for each monosaccharide (Fig. 1 and Table 1). The multiple peaks correspond to the TMS methyl glycoside derivatives of the α and β-anomers and the pyranose and furanose ring forms of the monosaccharide. The relative proportions of the peaks are characteristic of each monosaccharide and their retention times are reproducible (Table 1). Thus, GC separates all of the TMS methyl glycoside derivatives that are likely to originate from the predominant glycosyl residues present in pectins. The GC profiles obtained after methanolysis and trimethylsilylation of plant polysaccharides are complex. Indeed, GC analysis of the TMS methyl glycoside derivatives generated from red wine polysaccharides detected at least 50 peaks (Fig. 2). The major peaks were identified on the basis of their retention times. However, GC-MS analysis was required to identify the peaks corresponding to those glycosyl residues that are minor components of pectic and hemicellulosic polysaccharides.

3.2. GC-MS characterization of the TMS methyl glycoside derivatives of pentoses, hexoses, 6-deoxy hexoses, and uronic acids

The EI mass of the TMS methyl glycoside derivatives of pentoses, hexoses, 6-deoxy hexoses, and hexuronic acids spectra obtained in this study are similar to those previously reported (Bleton et al., 1996; DeJongh et al., 1969; Petrzika & Linow, 1982). In all cases ionization occurs with the removal of an electron from the ring-oxygen atom (Kotchetkov & Chizhov, 1966). Molecular ions and fragment ions in the high-mass range (>200 amu) were typically of low intensity. Nonetheless, these ions provide information on characteristic fragmentation pathways. The fragment ions and their structures are classified according to the nomenclature of Kotchetkov and Chizhov (1966) as modified by Petrzika and Linow (1982).

The EI mass spectra of the pentoses (arabinose and xylose), hexoses (galactose, glucose, and mannose), 6-deoxy hexoses (fucose and rhamnose), and hexuronic acids (galacturonic and glucuronic acids) contain similar types of fragment ions (Table 2) whose origins have been discussed (Bleton et al., 1996; DeJongh et al., 1969; Petrzika & Linow, 1982). The masses of these ions are diagnostic since they are formed by fragmentation of the molecular ion which itself has a different mass for each monosaccharide class (Table 2). For example, the TMS methyl glycoside derivatives of hexuronic acids, which contain a methyl-esterified carboxylic acid group, give fragment ions that are 44 amu lower than the corresponding fragments ions of a hexose derivative (Table 2). The fragments ions of the pentose and 6-deoxy hexose derivatives are 102 and 88 amu lower, respectively, than the corresponding fragments ions of a hexose derivative (Table 2).

3.3. GC-MS characterization of the TMS methyl glycoside derivatives of Dha, aceric acid, 2-O-methylxylose, 2-O-methylfucose, Kdo and apiose

The pectic polysaccharide RG-II contains several unusual sugars including apiose, 2-*O*-methylfucose, 2-*O*-methylxylose, aceric acid, Kdo and Dha. There are, as far as we are aware, no published reports of the EI mass spectra of the TMS methyl glycoside derivatives of these monosaccharides. Red wine RG-II was used as a source of Dha, aceric acid, 2-*O*-methyl-fucose, and apiose since they are not commercially available (Pellerin et al., 1996). Apiose is a major constituent of *Lemna* (Duck Weed) cell walls (Hart & Kindel, 1970) and these walls were also used as a source of this sugar.

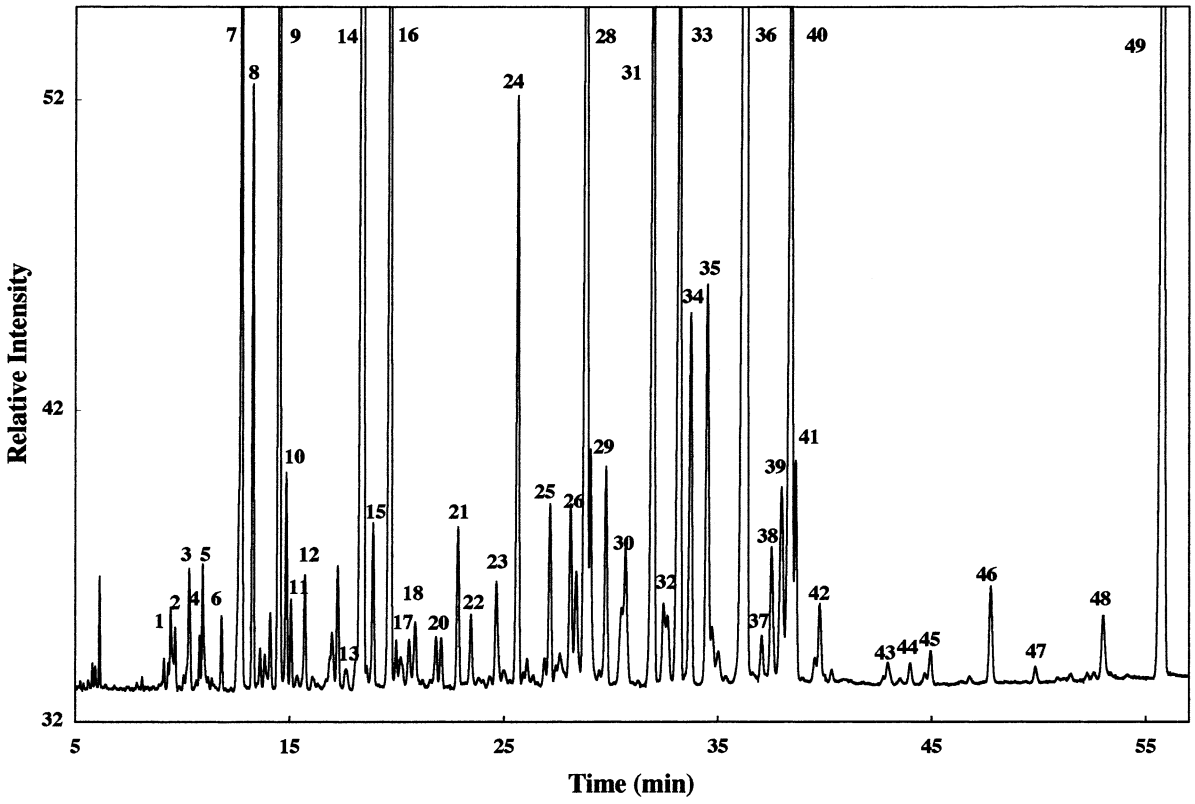

Fig. 2. The GC-EI-MS total ion current profile of the TMS methyl glycoside derivatives generated after methanolic-HCl treatment of red wine polysaccharides. The number above each peak corresponds to the monosaccharide identified in Table 3. Peaks 18, 19, and 43 are contaminants that were present in the reagents used to prepare the derivatives. Peak 49 corresponds to meso-inositol (internal standard).

The EI mass spectra of the Dha, aceric acid, 2-*O*-methylxylose, 2-*O*-methylfucose, Kdo, and apiose TMS methyl glycoside derivatives contain fragment ions that are consistent with previously published fragmentation pathways for TMS methyl glycoside derivatives (Kotchetkov & Chizhov, 1966; Petrzika & Linow, 1982). However, the EI mass spectrum of each monosaccharide derivative also contained characteristic fragment ions that allowed their unambiguous identification (Table 2).

Table 2
Ions obtained for TMS derivatives produced after methanolysis and trimethylsilylation of all the sugars present in plant polysaccharides

	$M^{\cdot+}$	$M^{\cdot+}-CH_3^{\cdot}$	$M^{\cdot+}-CH_3O^{\cdot}$	$M^{\cdot+}-CH_3^{\cdot}-CH_3OH$	$M^{\cdot+}-TMSiOH$	$M^{\cdot+}-COOCH_3^{\cdot}$	$M^{\cdot+}-TMSiO$	$M^{\cdot+}-CH_3^{\cdot}-TMSiOH$
Usual sugars								
Pentoses	380	365	349	333	290	–[a]	–	275
Hexoses	–	467	451	435	392	–	–	377
6-deoxy-Hexoses	394	379	363	347	–	–	305	289
Uronic acids	–	423	407	391	–	379	349	333
Unusual sugars								
DHA[b]	–	393	–	361	–	349	–	303
Aceric acid[c]	–	335	–	–	–	–	–	–
2-Me-Xyl[d]	322	307	291	275	232	–	–	217
2-Me-Fuc[e]	336	–	305	289	–	–	–	231
KDO[f]	554	539	–	507	464	495	–	–

[a] Ions not found.
[b] Dha = 3-deoxy-D-*lyxo*-heptulosaric acid.
[c] Aceric acid = 3-*C*-carboxy-5-deoxy-L-xylose.
[d] 2-Me-Xyl = 2-*O*-methylxylose.
[e] 2-Me-Fuc = 2-*O*-methylfucose.
[f] Kdo = 3-deoxy-D-*manno*-octulosonic acid.

Table 3
Retention time (min), relative retention times (RRT) with inositol as internal standard and monosaccharide identification from GC analysis on OV-1 column for TMS derivatives obtained for a crude wine polysaccharide preparation

Peak number	Retention time	RRT	Monosaccharides
1	9.26	0.166	Aceric acid
2	9.45	0.169	Aceric acid
3	10.34	0.185	2-*O*-Me-Fucose
4	10.81	0.193	2-*O*-Me-Xylose
5	10.96	0.197	2-*O*-Me-Xylose
6	11.84	0.212	Apiose
7	12.40	0.225	Apiose
	12.78	0.229	Arabinose
8	13.30	0.238	Arabinose
8′	13.82	0.247	Apiose
9	14.54	0.260	Rhamnose
10	14.89	0.266	Fucose
	15.01	0.268	Rhamnose
11	15.19	0.272	Arabinose
12	15.95	0.285	Fucose
13	17.26	0.309	Fucose
14	18.47	0.330	Xylose
15	19.03	0.339	Arabinose
16	18.92	0.354	Xylose
17	21.87	0.391	Galacturonic acid
20	22.11	0.396	Rhamnose
21	22.88	0.409	Xylose
22	23.49	0.420	Glucuronic acid
24	25.68	0.459	Galacturonic acid
25	27.18	0.486	Xylose
26	28.14	0.503	Galacturonic acid
27	28.40	0.508	Dha
28	28.89	0.517	Mannose
29	29.79	0.533	Galactose
30	30.52	0.546	Dha
	30.71	0.549	Mannose
31	32.03	0.573	Galactose
32	32.49	0.581	Galactose
	32.67	0.585	Dha
33	33.44	0.595	Galacturonic acid
34	33.76	0.604	Galacturonic acid
			Kdo
35	34.53	0.618	Galactose
36	36.36	0.651	Glucose
37	37.06	0.663	Glucuronic acid
38	37.51	0.671	Glucuronic acid
39	37.98	0.680	Galacturonic acid
40	38.42	0.687	Glucose
41	38.64	0.691	Galacturonic acid
42	40.33	0.722	Glucuronic acid
44	44.01	0.787	Glucose
45	45.18	0.808	Kdo
46	47.75	0.854	Glucose
47	50.04	0.895	Kdo
48	53.31	0.954	Kdo
49	55.89	1	Inositol

3.4. The EI mass spectra of the TMS methyl glycoside derivatives of Dha

Three peaks (R_t 28.40, 30.52 and 32.67 min, Fig. 2 and Table 3) with mass spectra that are consistent with the expected fragmentation pathway of TMS derivatives of Dha (Fig. 4a) were detected by GC-EI-MS. The EI mass spectra of the Dha derivatives (Fig. 3a and Table 2) contain ions that result from the fragmentation of the molecular ion, although they do not contain a molecular ion ($M^{\cdot+}$, *m/z* 408). The ion at *m/z* 393 (1.8%, relative to the ion at *m/z* 204) most likely forms by the loss of $CH_3^{\cdot}$ from $M^{\cdot+}$. This ion undergoes further fragmentation to give ions at *m/z* 361 ($M^{\cdot+}$–$CH_3^{\cdot}$–CH_3OH 6.3%), 333 ($M^{\cdot+}$–$CH_3^{\cdot}$–$HCOOCH_3$, 2.5%), and 303 ($M^{\cdot+}$–$CH_3TMSiOH$, 2.7%). The ion at *m/z* 349 (18.0%) is formed from $M^{\cdot+}$ by the loss of the $COOCH_3^{\cdot}$ group present on C-2 (glycosidic carbon) or on C-6. The elimination of a TMSiOH group from this fragment gives an ion at *m/z* 259 ($M^{\cdot+}$–$COOCH_3^{\cdot}$–TMSiOH, 9.9%) which itself then loses a TMSiOH group to give the ion at *m/z* 169 ($M^{\cdot+}$–$COOCH_3^{\cdot}$–$[TMSiOH]_2$, 18.5%). The ion at *m/z* 287 (2.0%) is most likely formed from $M^{\cdot+}$ by the loss of $CH_3O^{\cdot}$ and TMSiOH. The two-carbon fragment C-3–C-4 (*m/z* 204) is characteristic of TMS ethers of monosaccharides (Bleton et al., 1996; Petrzika & Linow, 1982). The loss of radicals from the first ion of the B series (*m/z* 320) may result in the formation of fragment ions at *m/z* 305 ($B_1 - CH_3^{\cdot}$), *m/z* 217 ($B_1 - CH_2OSiTM^{\cdot}$), *m/z* 129 ($B_1 - [TMSiO]_2CH^{\cdot}$), and *m/z* 217 ($B_1 - CH(COOCH_3)OCH_3^{\cdot}$). The absence of the C series of ions (Kotchetkov & Chizhov, 1966) is characteristic for a 3-deoxy-sugar since the cleavage between C-2 and C-3 is impeded.

3.5. The EI mass spectra of the TMS methyl glycoside derivatives of aceric acid

Aceric acid is, as far as we are aware, the only naturally occurring branched, acidic, deoxy-sugar (O'Neill et al., 1990; Spellman et al., 1983). Two minor peaks (R_t 9.17 and 9.37 min, see Fig. 2 and Table 3) with mass spectra consistent with the expected fragmentation pathway of TMS derivatives of aceric acid (Fig. 4b) were detected by GC-MS. No molecular ion was present in the mass spectra of these derivatives, although an ion at *m/z* 335 (18.5%) that corresponds to ($M^{\cdot+} - CH_3^{\cdot}$) was present (Fig. 3b). The elimination of radicals from the first ion of the B series (*m/z* 306; $M^{\cdot+} - CH_3CHO$, 28.5%) may give fragment ions at *m/z* 291 ($B_1 - CH_3^{\cdot}$, 15%), *m/z* 275 ($B_1 - CH_3O^{\cdot}$, 74.8%), *m/z* 247 ($B_1 - COOCH_3^{\cdot}$, 19.8%), *m/z* 217 ($B_1 - TMSiO^{\cdot}$, 20.2%), and *m/z* 133 ($B_1 - (CH_3)_3SiOC(COOCH_3)CH^{\cdot}$, 23.5%). The first ion of the C series (*m/z* 290 $M^{\cdot+} - CH_3OCHO$, 21.8%), which is initiated by a cleavage between C-1–C-2, is unstable, and loses $CH_3^{\cdot}$, $COOCH_3^{\cdot}$ or $TMSiO^{\cdot}$ to give ions at *m/z* 275 (74.8%), 231 (49.5%), and 201 (12%), respectively. The ion at *m/z* 262 that results from cleavage of the C-1–C-2 and C-3–C-4 bonds, is also unstable and immediately loses a silanyl radical ($TMSiO^{\cdot} = 89$ mass units) to give the ion at *m/z* 173. As expected the mass spectrum of the aceric acid derivatives did not contain an ion at *m/z* 204 corresponding to the C-3–C-4 two-carbon fragment

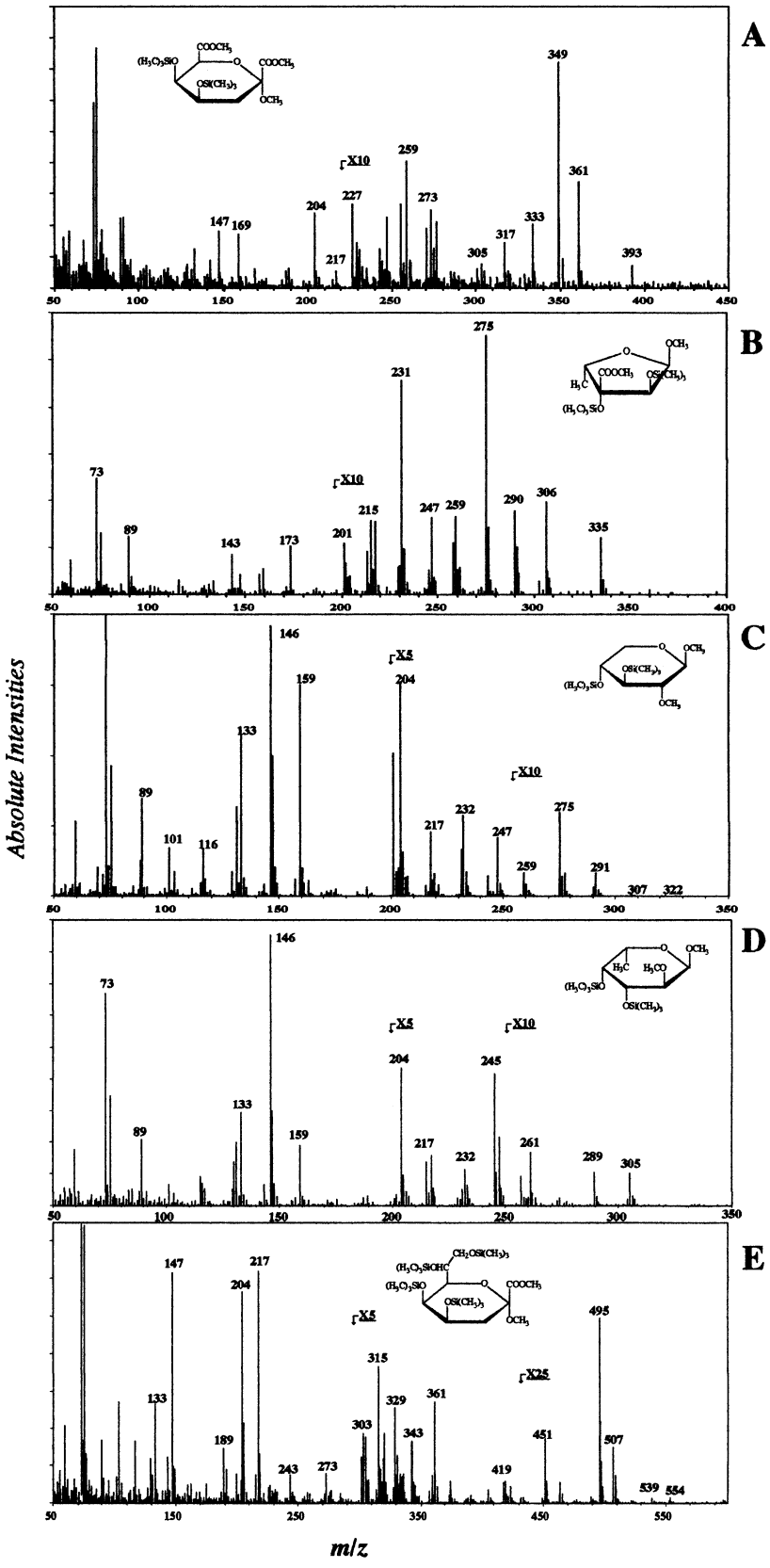

Fig. 3. The EI mass spectra of the TMS methyl glycoside derivatives of selected monosaccharides present in RG-II: (a) Dha [3-deoxy-D-*lyxo*-heptulosaric acid]; (b) aceric acid [3-*C*-carboxy-5-deoxy-L-xylose]; (c) 2-*O*-methylxylose; (d) 2-*O*-methylfucose; (e) Kdo [3-deoxy-D-*manno*-octulosonic acid]. The multiplication factors used to expand selected regions of each mass spectrum are shown.

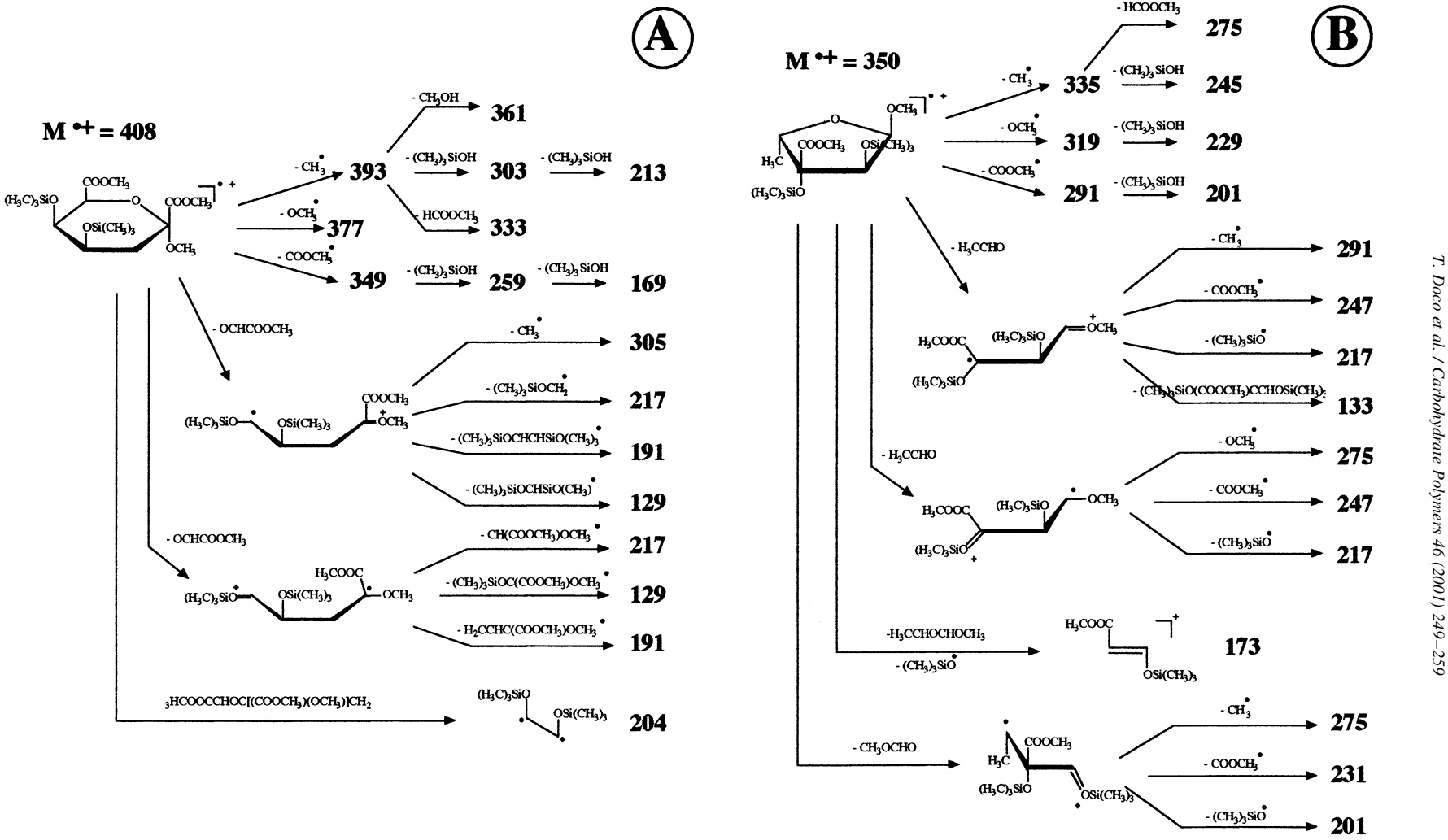

Fig. 4. Proposed structures and origins of the fragment ions in the EI mass spectra of the TMS methyl glycoside derivatives of (a) Dha [3-deoxy-D-*lyxo*-heptulosaric acid] and (b) aceric acid [3-*C*-carboxy-5-deoxy-L-xylose].

Table 4
Carbohydrate composition of total wine polysaccharides, soluble polysaccharides from apple and carrot, and grape cell wall polysaccharides calculated from the *O*-trimethylsilylated methyl glycosides produced by methanolysis–trimethylsilylation and analyzed by GC-EIMS

Monosaccharide	Wine[a]	Apple[b]	Carrot[b]	Grape[c]
Apiose	13 ± 2	–	–	8
Arabinose	45 ± 3	16 ± 2	76 ± 9	105
Rhamnose	31 ± 2	3 ± 0	6 ± 1	36
Fucose	6 ± 1	2 ± 0	2 ± 1	15
2-*O*-Me-Xylose	4 ± 1	–	–	4
2-*O*-Me-Fucose	3 ± 1	–	–	2
Aceric acid	< 1	–	–	2
Galactose	125 ± 9	36 ± 1	366 ± 20	113
Glucose	49 ± 2	16 ± 2	145 ± 6	254
Mannose	165 ± 1	2 ± 0	24 ± 1	55
Xylose	5 ± 1	4 ± 0	8 ± 1	115
Galacturonic acid	211 ± 10	71 ± 5	33 ± 3	543
Glucuronic acid	30 ± 8	8 ± 4	19 ± 3	10
DHA	11 ± 4	–	–	10
KDO	13 ± 2	–	–	13

[a] mg/l of red wine (average ± standard deviation $n = 3$).
[b] μg/g of fresh weight (average ± standard deviation $n = 4$).
[c] μg/g of fresh weight, performed one time for this experiment.

(Bleton et al., 1996; Petrzika & Linow, 1982), since this sugar is branched at C-3.

3.6. The EI mass spectra of the TMS methyl glycoside derivatives of 2-O-methylxylose

Four peaks (R_t 10.95, 11.14, 14.64, and 16.80 min) with mass spectra (Fig. 3c) consistent with the expected fragmentation pathways of the TMS methyl glycoside derivatives of 2-*O*-methylxylose were detected by GC-EI-MS analysis. The molecular ion (*m/z* 322) was present albeit at low intensity (Fig. 3c). The fragment ions at *m/z* 307, 291, and 232 result from the elimination of $CH_3^{\cdot}$, $CH_3O^{\cdot}$, and TMSiOH, respectively, from $M^{\cdot+}$ and provide additional evidence that the molecular weight of the derivative is 322. The ion at *m/z* 307 can lose CH_3OH and TMSiOH to give ions at *m/z* 275 and 217, respectively. The first ion (*m/z* 292) of the B series is present, albeit at low intensity, and may fragment to give the more abundant ions at *m/z* 277, 247, 189, 159 and 133. The most abundant ion in the mass spectrum (*m/z* 146) most likely correspond to the C-2–C-3 fragment that contains one silanol and one *O*-methyl group. A C-2–C-3 fragment that contains two silanol groups gives an ion at *m/z* 204. The difference of 58 mass units is equal to the difference in the masses of CH_3O– and TMSiO–. Thus, the high relative abundance of the ion at *m/z* 146 (100% for the major peak) and the low intensity of the ion at *m/z* 204 are likely to be characteristic of 2-*O*-methylxylose.

3.7. The EI mass spectra of the TMS methyl glycoside derivatives of 2-O-methylfucose

Only one peak (R_t 10.34 min, see Table 3) with mass spectra (Fig. 3d) consistent with the expected fragmentation pathways of the TMS methyl glycoside derivatives of 2-*O*-methylfucose was detected by GC-EI-MS analysis. The molecular ion (*m/z* 336, 0.4%) was present albeit at low intensity (Fig. 3d). Fragmentation of the molecular ion may give the fragments C-2–C-3 (*m/z* 146, 100%) and C-3–C-4 (*m/z* 204 16.6%). The C-3–C-4 fragment contains two silanol groups, whereas the C-2–C-3 fragment contains one CH_3O– and one TMSiO– and thus its mass is decreased by 58 mass units. Ions of the B series at *m/z* 277, 247, 189, 159 and 133 may be produced by the loss of C-5 and oxygen as acetaldehyde, with subsequent stepwise elimination of other substituents. The C series of ions (*m/z* 276, 187 and 97) may be generated by the cleavage of the C-1–C-2 bond, although the intensities of these ions are low.

3.8. The EI mass spectra of the TMS methyl glycoside derivatives of Kdo

There is little information concerning the EI mass spectral fragmentation patterns of the TMS methyl glycoside derivatives of Kdo, even though this monosaccharide is a component of bacterial lipopolysaccharides (Sutherland, 1985) and of RG-II (York et al., 1985).

The TMS methyl glycoside of authentic Kdo gave six peaks (R_t 45.25, 50.03, 51.43, 52.77, 53.32, and 53.58). However, only four peaks corresponding to Kdo were detected by GC analysis of the TMS methyl glycoside derivatives generated from RG-II (Table 3). A molecular ion (*m/z* 554, 0.1%) was present in the EI-mass spectra of the Kdo derivatives. The ions at *m/z* 539, 495, and 464 correspond to the elimination from $M^{\cdot+}$(of $CH_3^{\cdot}$, $COOCH_3$, and TMSiOH, respectively and provide additional evidence for the molecular weight of the derivative. The ion at *m/z* 495 results from the elimination of the $COOCH_3$ group on C-2 and is a characteristic fragment ion in the EI mass spectra of the Kdo derivatives. In addition, the absence of the C series of ions in the EI mass spectrum of the Kdo derivatives is characteristic of 3-deoxy sugars (24). The elimination of radicals from the first ion of the B series (*m/z* 320) gives fragment ions at *m/z* 305 ($B_1 - CH_3^{\cdot}$), *m/z* 217 ($B_1 - CH_2OSiTM^{\cdot}$ or $B_1 - CH(COOCH_3)OCH_3^{\cdot}$, 100%), and *m/z* 129 ($B_1 - [TMSiO]_2CH^{\cdot}$). The ions at *m/z* 451 and *m/z* 361 (451 − TMSiOH) may originate by the formation of a bond between C-1 and C-8 followed by the elimination of $CH_2OSiTM^{\cdot}$.

3.9. The EI mass spectra of the TMS methyl glycoside derivatives of apiose

Apiose is present in pectic polysaccharides (Hart & Kindel, 1970; O'Neill et al., 1990) and plant aroma precursors (Günata, Dugelay, Sapis, Baumes & Bayonove, 1992), although the monosaccharide itself is not commerically available. Three peaks (R_t 11.84, 12.40, and 13.82) were detected by GC-MS analysis of the TMS methyl glycosides generated from red wine RG-II and from *Lemna* cells walls that correspond to apiose derivatives. No specific fragments

ions were present that distinguished apiose from other pentoses. Indeed, the EI mass spectra of the apiose derivatives contained ions (*m/z* 380, 365, 349, 333, 290, 275, 259 and 245) that are also present in the spectra of the arabinose and xylose derivatives. However, the ion at *m/z* 204 was not present in the EI mass spectrum of the apiose derivatives and this may be attributed to the fact that apiose is branched at C-3.

3.10. Determination of the glycosyl-residue compositions of plant polysaccharides using GC-EI-MS analysis of the TMS methyl glycoside derivatives

Red wine polysaccharides were treated with methanolic-HCl. The liberated methyl glycosides were converted to their TMS derivatives and then analyzed by GC-MS. The components in each peak (Fig. 2 and Table 3) were identified from their retention times (relative to meso-inositol) and by the characteristic fragment ions in their EI mass spectra. Three peaks (labeled 18, 19, and 43 in Fig. 2) were detected that originate from trace amounts of contaminants in the solvents used to prepare the derivatives. The response factors (average ± standard deviation) of the commercially available monosaccharides are: 2-*O*-methyl xylose 0.19 ± 0.04; rhamnose, 0.78 ± 0.04; fucose, 0.44 ± 0.04; arabinose, 0.51 ± 0.04; xylose, 0.50 ± 0.02; Kdo, 0.24 ± 0.08; galactose, 0.50 ± 0.03; galacturonic acid, 0.43 ± 0.04; and glucuronic acid, 0.29 ± 0.03. The glycosyl-residue composition of red wine polysaccharides (Table 4) is consistent with previous studies (Brillouet et al., 1990; Doco & Brillouet, 1993; Doco, Lecas, Pellerin, Brillouet & Moutounet, 1995; Dubourdieu et al., 1981; Pellerin et al., 1996; Pellerin et al., 1995; Pellerin et al., 1993; Waters, Pellerin & Brillouet, 1994) and confirms that wine contains a mixture of pectic polysaccharides, arabinogalactans, mannans, and glucans.

The glycosyl-residue compositions of the water-soluble polysaccharides isolated from apple and carrot (Doco et al., 1997), and of grape cell walls are also indicated in Table 4 as examples of identification and quantification of complex polysaccharides by GC-MS analysis of their TMS methyl glycoside derivatives.

4. Discussion

We have shown that GC with a DB-1 capillary column resolves all the TMS methyl glycoside derivatives generated from the polysaccharides present in plant cell walls and in red wine. Each monosaccharide gives a characteristic pattern of multiple peaks that are identified by a combination of their retention times and by their EI mass spectra.

The TMS procedure we have described is rapid and facile, and may be used for determining the neutral and acidic glycosyl-residue compositions of polysaccharides that are available in limited amounts (10 μg). In contrast, the alditol acetate procedure typically requires larger amounts (100 μg) of polysaccharide, since it involves several steps (acid hydrolysis, $NaBH_4$ reduction of the released monosaccharides to their corresponding alditols, and *O*-acetylation) (Albersheim et al., 1967; Fox et al., 1990; Jansson, Kenne, Liedgren, Lindberg & Lönngren, 1976). Moreover, hexuronic acid residues can only be quantified as their alditol acetates after their carboxyl groups have been quantitatively reduced to primary alcohols (Taylor & Conrad, 1972). The alditol acetate procedure must also be modified to quantify Kdo and Dha, because both these sugars are degraded by treatment with hot aqueous acids (Stevenson et al., 1988; York et al., 1985).

The recoveries of monosaccharides are likely to be higher when a polysaccharide is converted to its constituent methyl glycosides by treatment with methanolic-HCl, since methyl glycosides are more resistant than monosaccharides to acid-catalyzed degradation (Selvendran & Ryden, 1990). The release of hexuronic acid residues is also increased by methanolic-HCl treatment since the carboxyl groups of acid glycosyl residues are converted to methyl esters. The glycosidic bonds of methyl-esterified hexuronic acids are more readily cleaved than the glycosidic bonds of the free acid (Selvendran, March & Ring, 1979).

We conclude that GC-EI-MS analysis of the TMS methyl glycoside derivatives generated after methanolic-HCl treatment of complex acidic polysaccharides gives glycosyl-residue compositions. All the glycosyl residues that are constituents of plant cell wall polysaccharides and of red wine polysaccharides are identified by this procedure. The TMS procedure described will be of value for determining the glycosyl-residue compositions of the polysaccharides present in plant-derived foods and beverages.

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