Carbohydrate Research, 243 (1993) 333–343
Elsevier Science Publishers B.V., Amsterdam

Isolation and characterisation of a rhamnogalacturonan II from red wine

Thierry Doco and Jean-Marc Brillouet

Institut National de la Recherche Agronomique, Institut des Produits de la Vigne, Laboratoire des Polymères et des Techniques Physico-Chimiques, 2 place Viala, 34060 Montpellier (France)

(Received July 23rd, 1992; accepted November 23rd, 1992)

ABSTRACT

A complex polysaccharide originating from grape was purified from a red wine by size-exclusion and ion-exchange chromatography. This polysaccharide contained 2-*O*-methylfucose, rhamnose, fucose, 2-*O*-methylxylose, arabinose, apiose, galactose, galacturonic and glucuronic acids, and thiobarbituric acid assay-positive material, presumably 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and 3-deoxy-D-*lyxo*-2-heptulosaric acid (Dha). Methylation structural analysis showed that this polysaccharide is a typical rhamnogalacturonan II (RG-II), a structurally complex pectic polysaccharide that has been isolated previously from the primary cell walls of sycamore. Red wine RG-II presented discrete differences from previously described RG-II's, a greater richness in uronic acids and some additional glycosyl linkages, which might be related to the pattern of degradation of grape-wall pectic substances by endogenous pectinolytic enzymes after pressing the berries and subsequent fermentation to wine.

INTRODUCTION

Rhamnogalacturonan II (RG-II) is a pectic polysaccharide occurring in the primary cell walls of higher plants and was first isolated from suspension-cultured sycamore (*Acer pseudoplatanus*) cells[1] after treatment of walls by a fungal endopolygalacturonase. Since then, RG-II's have been obtained by similar treatments of walls from suspension-cultured cells of Douglas fir (*Pseudotsuga menziesii*)[2] and rice (*Oryza sativa*)[3], and found in Pectinol AC[4,5], cell walls of onion (*Allium cepa*)[6] and kiwi fruit (*Actinidia deliciosa*)[7,8], and recently in *Bupleurum falcatum* roots[9]. RG-II is a complex polysaccharide containing L-rhamnose, L-arabinose, L-fucose, D-galactose, D-galacturonic and D-glucuronic acids, and rare monosaccharides "specifically" found in RG-II's, i.e., 2-*O*-methyl-L-fucose[1], 2-*O*-methyl-D-xylose[1], D-apiose[1], aceric acid (3-*C*-carboxy-5-deoxy-L-xylose)[4], 3-deoxy-D-*manno*-2-octulosonic acid (Kdo)[5], and 3-deoxy-D-*lyxo*-2-heptulosaric acid (Dha)[10]. The backbone of RG-II is built of galacturonosyl residues, some of which are methyl-esterified, and to which a variety of oligosaccharidic side-chains are attached[11,12].

Polysaccharides in wine originate mainly from grape and yeast, and generally play a detrimental role in wine-making technology[13,14]. Complex pectic polysaccha-

rides occurring in the pulp of grape berries are now recognised[15–18], but their degraded forms in wine are poorly understood[19]. We describe herein the purification, identification, and characterisation of a rhamnogalacturonan II from red wine.

RESULTS AND DISCUSSION

Isolation of RG-II.—Purification of RG-II from red wine to homogeneity required several steps due to the presence in fraction C_4 of several polysaccharidic populations originating from both grape and yeast[14]. Material collected after size-exclusion chromatography on Ultrogel AcA 44 (Fig. 1A) was separated into neutral (unbound) and acidic (bound) fractions by ion-exchange chromatography on DEAE-Sephacel (Fig. 1B). The acidic fraction was further purified on Sephadex G-75 (Fig. 1C), which proved insufficient to separate RG-II from lower molecular weight material. Thus, an additional pass on Bio-Gel P-10 (Fig. 1D) was necessary to attain apparent homogeneity, the ratio of neutral to acidic sugars being fairly constant (~ 1) along the peak. The purified polysaccharide gave a sharp symmetrical peak by HPSEC (Fig. 2), which indicated low polydispersity, and contained co-eluting UV-absorbing material.

Composition and molecular weight of RG-II.—The composition of red wine RG-II is given in Table I, showing that only $\sim 73\%$ of the dry matter could be determined. Thus, about a quarter of the molecule remained unknown, including inorganic material which was not determined in the present study. Uronic acids and neutral sugars were in similar amounts (see also Fig. 1D), each corresponding to about one-third of the molecule.

Kdo (3-deoxy-D-*manno*-2-octulosonic acid) and Dha (3-deoxy-D-*lyxo*-2-heptulosaric acid), which were not formally identified in this study, were however measured in admixture by a modified thiobarbituric acid assay and accounted for $\sim 1.6\%$. Aceric acid (3-*C*-carboxy-5-deoxy-L-xylose) has not been identified in our study. Methyl ester groups were detected and assigned to uronosyl residues, giving a degree of methylation (dm) of 32%; however, depending on the actual relative proportions of galacturonic and glucuronic acids, the dm could be higher. Acetyl groups were present, but could not be assigned to a particular sugar type, since Spellman et al.[20] detected *O*-acetyl groups in the heptasaccharidic portion of sycamore RG-II, and because acetyl groups could also be carried by galactopyranosyluronic acid residues[21]. Contrary to the sycamore RG-II[1], traces of protein were detected, corresponding perhaps to the UV-absorbing coeluting material.

Gas–liquid chromatography of polyol acetates obtained after acid hydrolysis, reduction, and peracetylation indicated the presence of 2-*O*-methylfucose (*m/z* 117, 215, 275)[22], rhamnose, fucose, 2-*O*-methylxylose (*m/z* 117, 261)[22], arabinose, apiose (*m/z* 145, 187, 302, 362)[23], and galactose; rhamnose and arabinose were predominant. RG-II was xylose-free.

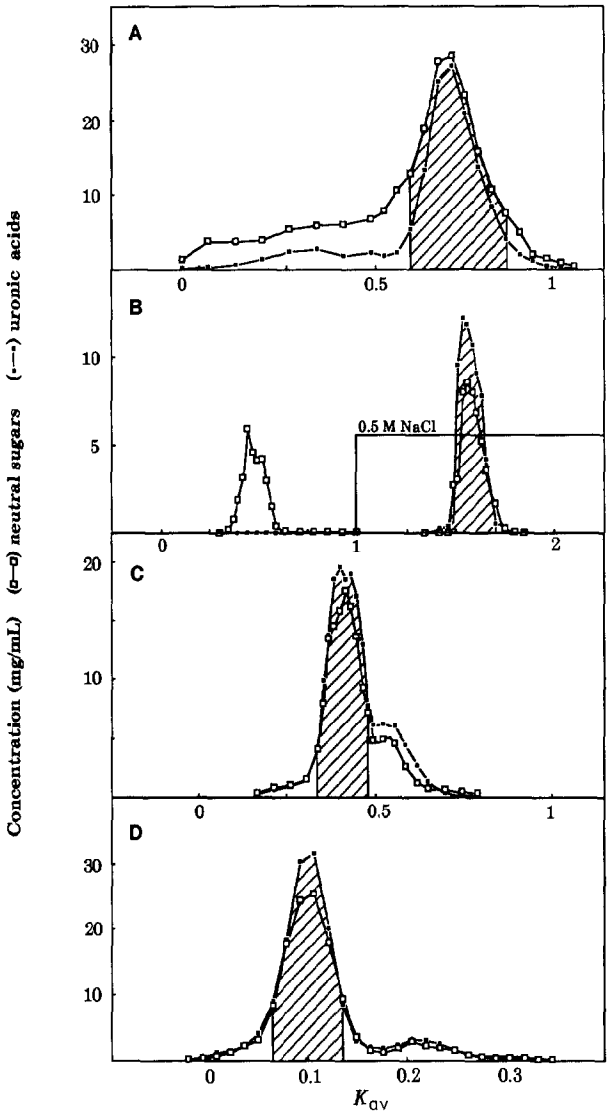

Fig. 1. Purification of red wine RG-II by (A) size-exclusion chromatography on Ultrogel AcA 44, (B) ion-exchange chromatography on DEAE-Sephacel, and successive size-exclusion chromatography on (C) Sephadex G-75 and (D) Bio-Gel P-10. Fractions were assayed for neutral and acidic sugars by automated orcinol and *m*-phenylphenol methods, respectively. Abcissae are in K_{av}, except for (B) which is in volume (L). Hatched areas were pooled for further purification. Concentrations were normalised to 6 g of starting material (Fraction C_4).

The weight-average molecular weight (M_w) of RG-II (Na^+ form) was determined by two methods: calibration of our HPSEC system with narrow pullulan molecular-weight standards and low-angle laser-light-scattering (LALLS). The calibration equation was: $\log M_w = 10.9 - 0.381 \times t_R$ (t_R = column retention-time

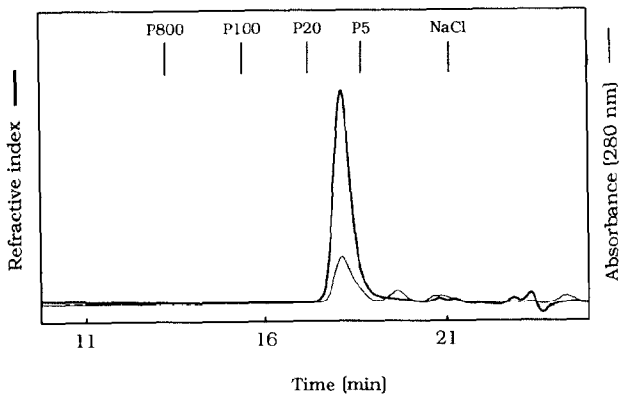

Fig. 2. HPSEC profile of red wine RG-II on Shodex OH-pack columns (KB-803, KB-805) in 0.1 M $LiNO_3$. Elution times of pullulan standards (P5 → P800) and NaCl are also shown.

at peak maximum; $r^2 = -0.998$) giving M_w of $9.8 \cdot 10^3$. LALLS allowed an absolute determination of M_w of $5.3 \cdot 10^3$ according to the following equation: $Kc/\Delta R_\theta = 2.326 \cdot 10^{-3} \times c + 1.898 \cdot 10^{-4}$ ($r^2 = 0.862$), which would correspond to a degree of polymerisation (dp) of 33 (average anhydrous molecular weight of constituent monosaccharides ~ 160). Discrepancies between HPSEC and LALLS M_w determinations are not yet understood, although the HPSEC calibration we performed does not account for the charge, conformation, and intrinsic viscosity of the molecule as in universal calibration[24]. Stevenson et al.[11] found, by reducing-end

TABLE I

Composition [a] of native and carboxyl-reduced rhamnogalacturonans II (RG-II) isolated from red wine

	Red wine RG-II	
	Native	Carboxyl-reduced
Uronic acids	31.8	11.9
Total neutral sugars [b]	34.5	52.8
Methanol	1.8 (32.1) [c]	
Acetic acid	1.7	
Kdo–Dha	1.6	
Protein	1.5	
2-*O*-CH_3-Fucose	5.7 [d]	4.0 [d]
Rhamnose	33.3	25.8
Fucose	3.7	3.6
2-*O*-CH_3-Xylose	4.8	3.4
Arabinose	27.9	19.6
Apiose	12.0	7.8
Galactose	12.6	24.9
Glucose	–	10.9

[a] % (w/w). [b] Neutral sugars determined by GLC of the alditol acetates, and expressed as "anhydro sugars". [c] Value in parentheses is the degree of methylation (dm) calculated as molar ratio of methanol to "anhydrogalacturonic acid" × 100. [d] Anhydromole %.

measurement, ~30 sugar residues (~$5 \cdot 10^3$) for RG-II isolated from the cell walls of suspension-cultured sycamore cells. However, previously characterised RG-II's[1,25] had higher (neutral–acidic) sugar ratios averaging ~2, but they were obtained by degradation of cell wall galacturonans by a fungal endopolygalacturonase from *Colletotrichum lindemuthianum*. Since the red wine RG-II is richer in uronic acids, one could have expected a higher molecular weight. This RG-II eluted at K_{av} 0.08–0.12 in 0.05 M acetate buffer (pH 4.8) containing 0.2 M NaCl (see Fig. 1D), and at 0.19–0.23 in the same buffer without added NaCl. Sycamore RG-II eluted, under very similar conditions [Bio-Gel P-10, 0.05 M acetate buffer (pH 5.2)], at K_{av} 0.33–0.38[1,5]. This tremendous difference in elution K_{av} on the same gel and with a similar buffer shows that red wine RG-II has a bigger hydrodynamic volume (higher M_w) than the sycamore one. In fact, measurement of RG-II M_w proved quite difficult[11,26], and none of our M_w figures could be considered as representative. Red wine RG-II originates from heterogeneous grape tissues (pulp and epiderm cell walls) through degradation of pectic cell-wall material by grape endogenous pectinolytic enzymes (endopolygalacturonases and pectinmethylesterases)[27,28] when berries are pressed prior to fermentation. Indeed, the fermentation yeast, *Saccharomyces cerevisiae*, has no pectin depolymerases[29]. Thus, it is possible that grape enzymes had a different mode of action on wall galacturonans or that they are progressively inhibited in the fermentation medium by grape polyphenols, leaving undegraded portions of the acidic backbone of RG-II.

Structure of RG-II.—Native and carboxyl-reduced RG-II's have been methylated according to Hakomori and results are displayed in Table II. The relative proportions of the sugars calculated either from the analyses of the alditol acetates or partially methylated alditol acetates were in good agreement and the (terminal–branched) ratio was close to one for the native RG-II; thus, the methylation was assumed to be complete. In order to improve the reduction efficiency, RG-II was de-esterified in the cold[17] prior to reduction by the carbodiimide method[30], and no losses in neutral sugars and uronic acids were observed after neutralisation and dialysis. However, only ~64% of the uronosyl residues were converted, after two reduction steps, into 6,6′-dideuterio-labeled hexosyl residues, contrary to higher conversions consistently observed in similar cases[17,26,30]. De-esterification of RG-II at ambient temperature (0.05 M KOH, 20°C, 30 min), followed by neutralisation and extensive dialysis, resulted in a 50% loss of uronic acids by β-elimination.

Methylation of native RG-II with trideuteriomethyl iodide gave two peaks of partially trideuteriomethylated and partially methylated alditol acetates corresponding to terminal non-reducing 2-*O*-methylfucosyl (*m/z* 117, 134, 164, 181) and 2-*O*-methylxylosyl (*m/z* 117, 120, 164, 167) residues[22]. Darvill et al.[1] reported for the first time the presence in sycamore RG-II of these rarely observed sugars. Equivalent molar ratios of 2,3,4-linked rhamnosyl, 3,4-linked fucosyl, and terminal non-reducing 2-*O*-methylxylosyl residues were found in red wine RG-II, which

TABLE II

Methylation analysis of native and carboxyl-reduced RG-II's from red wine

Glycosyl residue	Methyl ether	Linkage	Red wine RG-II	
			Native	Carboxyl-reduced
Rhamnosyl	2,3,4-Me_3-Rha [a]	Rha*p* [b]-(1 →	13.7 [c]	10.5
	3,4-Me_2-Rha	→ 2)-Rha*p*-(1 →	6.4	4.9
	2,4-Me_2-Rha	→ 3)-Rha*p*-(1 →	9.1	6.9
	3-Me-Rha	→ 2,4)-Rha*p*-(1 →	0.8	0.9
	4-Me-Rha	→ 2,3)-Rha*p*-(1 →	0.6	0.7
	Rha	→ 2,3,4)-Rha*p*-(1 →	5.7	4.4
		Total	36.3 [d]	
			(33.3) [c]	
2-*O*-CH_3-Fucosyl	2,3,4-Me_3-Fuc	2-*O*-CH_3-Fuc*p*-(1 →	5.3	4.1
			(5.7)	
Fucosyl	2-Me-Fuc	→ 3,4)-Fuc*p*-(1 →	5.6	4.4
			(3.7)	
Apiosyl	2,3-Me_2-Api	→ 3′)-Apif-(1 →	10.5	8.1
	Api	→ 2,3,3′)-Apif-(1 →	3.8	2.9
		Total	14.3	
			(12.0)	
Arabinosyl	2,3,5-Me_3-Ara	Ara*f* [b]-(1 →	12.7	9.8
	2,3,4-Me_3-Ara	Ara*p*-(1 →	0.4	0.4
	2,3-Me_2-Ara	→ 4 or 5)-Ara-(1 →	1.3	1.1
	4-Me-Ara	→ 2,3)-Ara*p*-(1 →	6.1	4.7
		Total	20.5	
			(27.9)	
2-*O*-CH_3-Xylosyl	2,3,4-Me_3-Xyl	2-*O*-CH_3-Xyl*p*-(1 →	4.9	3.8
			(4.8)	
Galactosyl	2,3,4,6-Me_4-Gal	Gal*p*-(1 →	1.7	1.5
	2,4,6-Me_3-Gal	→ 3)-Gal*p*-(1 →	1.0	0.7
	2,3,6-Me_3-Gal	→ 4)-Gal*p*-(1 →	1.2	0.8
	2,6-Me_2-Gal	→ 3,4)-Gal*p*-(1 →	2.3	1.7
	2,4-Me_2-Gal	→ 3,6)-Gal*p*-(1 →	1.2	0.8
	3,6-Me_2-Gal	→ 2,4)-Gal*p*-(1 →	5.7	4.4
		Total	13.1	
			(12.6)	
Galactosyluronic acid	2,3,4,6-Me_4-Gal [f]	GalA*p*-(1 →		7.2
	2,3,6-Me_3-Gal [f]	→ 4)-GalA*p*-(1 →		7.5
	2,6-Me_2-Gal [f]	→ 3,4)-GalA*p*-(1 →		2.1
Glucosyluronic acid	2,3,4,6-Me_4-Glc [f]	GlcA*p*-(1 →		5.7

[a] 2,3,4-Me_3-Rha = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-rhamnitol, etc. [b] *p* = pyranose, *f* = furanose. [c] Relative mole percent of each methyl ether within total neutral sugars. [d] Relative mole percent of each parent sugar family (sum of ethers from one sugar type) within total sugars. [e] Values in parentheses correspond to the compositional analysis (Table I). [f] 6,6′-dideuterated ethers.

might correspond to a portion of the previously described octasaccharidic side-chain of sycamore RG-II[11]. Similarly, the presence of equivalent molar proportions of 2,4-linked galactosyl and terminal non-reducing 2-*O*-methylfucosyl residues indicated that red wine RG-II might likely contain an oligosaccharidic structure resembling the heptasaccharidic side-chain from sycamore RG-II[20]. If the latest RG-II model[12] has some general validity in the plant kingdom, the same number of hepta- and octa-saccharidic side-chains would have to be found, which is corroborated by the equivalent molar proportions of the five above-mentioned glycosyl residues. Moreover, a 3′-linked apiosyl residue (*m/z* 117, 189, 247) was found in a 2-molar amount, which might correspond to the two attachment sites of the hepta- and octa-saccharidic chains on the acidic backbone[12].

Terminal non-reducing rhamnosyl and arabinofuranosyl groups, and 4-linked, 3,4-linked, and terminal non-reducing galactopyranosyluronic acid residues were also found in red wine RG-II, as in RG-II's from other sources[1–3]. However, no 2,4-linked galactopyranosyluronic acid residues were detected.

Apart from these already known structural features[1–5,10–12,20,26], we found additional glycosyl linkages, i.e., 2-linked rhamnosyl, 2,3-linked arabinopyranosyl (*m/z* 117, 261, 275)[22], 2,3,3′-linked apiosyl[23], 3,4-linked galactosyl, and terminal non-reducing glucopyranosyluronic acid residues; 2-linked rhamnosyl and 3,4-linked galactosyl residues have also been found in noticeable amounts in Pectinol AC[11], but were assumed to arise from a contaminant. Puvanesarajah et al.[12] showed that glucuronic acid was 2-substituted by a terminal non-reducing galactosyl group, whereas in our case it was terminally linked, which could be related to the low amount of terminal non-reducing galactosyl groups. The molar proportion of 2,3-linked arabinopyranosyl residue was equivalent to the 2,4-linked galactosyl and terminal non-reducing 2-*O*-methylfucosyl residues, but in sycamore RG-II heptasaccharidic side-chain, arabinopyranose was found only 2-substituted by a terminal non-reducing rhamnosyl group[20].

CONCLUSIONS

According to our experience on wines from different origins (various red and white grape cultivars), it is now possible to claim that wine-making technology (pressing and further maceration–fermentation) generates, in derived wines, RG-II-like structures. The particular RG-II described in this study resembles, in many respects, sycamore and related RG-II's[1–4]. This is the first time, to our knowledge, that an RG-II is reported to be released from mother wall pectic molecules by plant enzymes, i.e., grape, instead of fungal enzymes. Discrete differences with regard to sycamore and related RG-II's, i.e., a higher proportion of uronic acids and additional glycosyl linkages, could have arisen from different patterns of action and/or limitation of grape pectinolytic activities during wine making. More work is needed to ascertain if these differences are intrinsic to grape cell-wall pectic substances or if they arise from grape enzyme action.

EXPERIMENTAL

Wine sample.—A traditional red wine was prepared, as formerly described (wine A)[13], from mature grapes of the Carignan noir cultivar harvested from an experimental vineyard in 1989 at the INRA-Pech Rouge/Narbonne Experimental Station (Gruissan, France).

Isolation of rhamnogalacturonan II (RG-II).—Depigmented colloids were isolated as described[14], and four fractions ($C_1 \rightarrow C_4$) were separated by preparative size-exclusion chromatography on a column of Ultrogel AcA 34[14].

(a) Size-exclusion chromatography on Ultrogel AcA 44. Fraction C_4 from Ultrogel AcA 34 (500 mg) was dissolved in 0.05 M acetate buffer (10 mL, pH 4.8) containing 0.2 M NaCl and applied to a column (90 × 0.6 cm) of Ultrogel AcA 44 (Sepracor, France), equilibrated with the same buffer. Fractions (6 mL) were assayed for uronic acids and neutral sugars as described below. Material eluting in the K_{av} range $0.65 \rightarrow 0.85$ was pooled, dialysed against distilled water, and freeze-dried.

(b) Ion-exchange chromatography on DEAE-Sephacel. Material obtained from several passes on Ultrogel AcA 44 (3.76 g) was dissolved in 0.05 M acetate buffer (pH 4.8) and loaded onto a DEAE-Sephacel (Pharmacia Fine Chemicals) column (30 × 5 cm), and elution was performed with the same buffer at 120 mL/h until negative response for carbohydrate (orcinol). Bound acidic polymers were then eluted by 0.5 M NaCl in the same buffer (pH 4.8), and the eluate was dialysed, concentrated under reduced pressure (< 40°C), and then freeze-dried.

(c) Size-exclusion chromatography on Sephadex G-75 and Bio-Gel P-10. A solution of the acidic fraction (100 mg) in 0.05 M sodium acetate buffer (5 mL, pH 4.8) containing 0.2 M NaCl was applied to a column (90 × 2.2 cm) of Sephadex G-75 equilibrated with the same buffer. Fractions (4 mL) were analysed as described below, and material eluting in the K_{av} range $0.35 \rightarrow 0.45$ was pooled, dialysed, and freeze-dried. Finally, homogeneity was attained by passing 30 mg of the latter fraction (2 mL) on a thermostated (40°C) column (200 × 2 cm) of Bio-Gel P-10 (BioRad) eluted with 0.05 M sodium acetate buffer (pH 4.8) containing 0.2 M NaCl. Material centered on K_{av} 0.1 (RG-II) was collected and further analysed.

Analytical methods.—Protein was determined by the method of Lowry et al.[31]. Uronic acids and neutral sugars were measured in column effluents by automated *m*-phenylphenol[32,33] and orcinol[34] methods, respectively. The results were corrected for mutual interferences and expressed as "anhydro sugars" with galacturonic acid and arabinose as the respective standards. Uronic acids of RG-II were measured manually[35], the same data being obtained with or without preliminary de-esterification. Kdo (3-deoxy-D-*manno*-2-octulosonic acid) and Dha (3-deoxy-D-*lyxo*-2-heptulosaric acid) were measured in admixture by the thiobarbituric acid assay[36] as modified by York et al.[5] for Kdo and Stevenson et al.[10] for Dha. Methyl groups were measured after de-esterification of RG-II[37], by enzymic oxidation of methanol by alcohol oxidase followed by colorimetric determination of formalde-

hyde[38], and acetic acid was determined by the enzymic–UV method[39]. Neutral sugars were determined, after hydrolysis by 2 M trifluoroacetic acid (120°C, 1.15 h)[40], by GLC of the alditol acetate derivatives[41] at 210°C on a fused-silica DB-225 capillary column (30 m × 0.32 mm i.d., 0.25-μm film; J&W Scientific) with H_2 as the carrier gas. Separation of apiose from xylose was achieved on the DB-225 column using temperature programming: 180°C for 25 min, then 5°C/min to 210°C.

Methylation analysis.—The polysaccharide was methylated by the Hakomori method[42] as described by Jansson et al.[22]. Carboxyl groups of uronosyl residues of RG-II were preliminarily reduced, after de-esterification (2 mg/mL), by the carbodiimide method[30]. Prior to methylation, carboxyl groups of RG-II were converted into their H^+ form[43] in order to ensure solubility in Me_2SO. After hydrolysis, the partially methylated sugars were converted into alditol acetates and analysed on DB-1 and DB-225 capillary columns[17]. Identifications were based on retention times and confirmed by GLC–MS, using the DB-225 column (on-column injection at 50°C; injector, 50 → 250°C at 180°C/min; oven, 150 → 180°C at 50°C/min for 15 min, then 5°C/min to 210°C; He as carrier gas at 2 mL/min) coupled to a Finnigan Mat ITD 700 mass spectrometer. For identification of the glycosyl linkage of 2-*O*-methylfucose and 2-*O*-methylxylose, RG-II was methylated with trideuteriomethyl iodide.

Molecular weight determination.—The weight-average molecular weight (M_w) of the polysaccharide was determined by high-performance size-exclusion chromatography (HPSEC) using 2 Shodex OH-pack columns (KB-803, KB-805), eluted with 0.1 M $LiNO_3$ at 1 mL/min from a Waters 510 pump, with on-line refractive index (Erma-ERC 7512 detector thermostated at 40°C) and UV (Waters 440) detection. Calibration was performed with narrow pullulan molecular-weight standards (P-5, M_w = 5 800; P-10, M_w = 12 200; P-20, M_w = 23 700; P-50, M_w = 48 000; P-100, M_w = 100 000; P-200, M_w = 186 000; P-400, M_w = 380 000; P-800, M_w = 853 000; Showa Denko K.K.). M_w was also determined by low-angle laser-light-scattering (LALLS) with a laser photometer Chromatix KMX-6 (λ = 633 nm)[44]. At a low angle of observation (θ = 4.88°), M_w can be obtained as the (intercept)$^{-1}$ from a plot of $Kc/\Delta R_\theta$ vs. $2A_2c + 1/M_w$, where A_2 is the second virial coefficient, c is the RG-II concentration (g/mL), ΔR_θ is the measured excess Rayleigh factor, and K is an optical constant depending on geometrical parameters of the photometer, the refractive index of the solvent (0.1 M NaCl), and the refractive index increment (dn/dc) of the RG-II solution [(dn/dc) measured value = 0.165 mL/g]. Under our experimental conditions, $K = 7.2 \cdot 10^{-6} \times [dn/dc]^2$ expressed in (mL^2/g^2).

ACKNOWLEDGMENTS

We thank Dr. R. Baumes (I.N.R.A.-I.P.V., Laboratoire des Arômes et des Substances Naturelles, Montpellier) for the GLC–MS facilities, and Dr. G. Müller

(URA-CNRS 500 "Polymères, Biopolymères, Membranes", Faculté des Sciences, Rouen) for the low-angle laser-light-scattering experiment.

REFERENCES

1 A.G. Darvill, M. McNeil, and P. Albersheim, *Plant Physiol.*, 62 (1978) 418–422.
2 J.R. Thomas, M. McNeil, A.G. Darvill, and P. Albersheim, *Plant Physiol.*, 83 (1987) 659–671.
3 J.R. Thomas, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 185 (1989) 261–277.
4 M.W. Spellman, M. McNeil, A.G. Darvill, P. Albersheim, and K. Henrick, *Carbohydr. Res.*, 122 (1983) 115–129.
5 W.S. York, A.G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 138 (1985) 109–126.
6 S. Ishii, *Phytochemistry*, 21 (1982) 778–780.
7 R.J. Redgwell, L.D. Melton, and D.J. Brasch, *Carbohydr. Res.*, 182 (1988) 241–258.
8 R.J. Redgwell, L.D. Melton, D.J. Brasch, and J.M. Coddington, *Carbohydr. Res.*, 226 (1992) 287–302.
9 H. Yamada, M. Hirano, and H. Kiyohara, *Carbohydr. Res.*, 219 (1991) 173–192.
10 T.T. Stevenson, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 179 (1988) 269–288.
11 T.T. Stevenson, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 182 (1988) 207–226.
12 V. Puvanesarajah, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 218 (1991) 211–222.
13 M.-P. Belleville, J.-M. Brillouet, B. Tarodo de la Fuente, and M. Moutounet, *J. Food Sci.*, 55 (1990) 1598–1602.
14 M.-P. Belleville, J.-M. Brillouet, B. Tarodo de la Fuente, L. Saulnier, and M. Moutounet, *Vitic. Enol. Sci.*, 46 (1991) 100–107.
15 J.-C. Villettaz, R. Amado, and H. Neukom, *Carbohydr. Polym.*, 1 (1981) 101–105.
16 J.-M. Brillouet, *Biochimie*, 69 (1987) 713–721.
17 L. Saulnier, J.-M. Brillouet, and J.-P. Joseleau, *Carbohydr. Res.*, 182 (1988) 63–78.
18 L. Saulnier, J.-M. Brillouet, M. Moutounet, C. Hervé du Penhoat, and V. Michon, *Carbohydr. Res.*, 224 (1992) 219–235.
19 J.-M. Brillouet, C. Bosso, and M. Moutounet, *Am. J. Enol. Vitic.*, 41 (1990) 29–36.
20 M.W. Spellman, M. McNeil, A.G. Darvill, P. Albersheim, and A. Dell, *Carbohydr. Res.*, 122 (1983) 131–153.
21 M.C. Jarvis, *Plant Cell Environ.*, 7 (1984) 153–164.
22 P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–20.
23 J.-M. Brillouet, Z. Gunata, S. Bitteur, R. Cordonnier, and C. Bosso, *J. Agric. Food Chem.*, 37 (1989) 910–912.
24 Z. Grubisic, P. Rempp, and H. Benoit, *Polym. Lett.*, 5 (1967) 753–759.
25 M. O'Neill, P. Albersheim, and A.G. Darvill, *Methods Plant Biochem.*, 2 (1990) 415–441.
26 L.D. Melton, M. McNeil, A.G. Darvill, P. Albersheim, and A. Dell, *Carbohydr. Res.*, 146 (1986) 279–305.
27 G. Marteau, J. Scheur, and C. Olivieri, *Ann. Technol. Agric.*, 10 (1961) 161–183.
28 G. Marteau, J. Scheur, and C. Olivieri, *Ann. Technol. Agric.*, 12 (1963) 155–176.
29 I.S. Pretorius and T.J. van der Westhuizen, *S. Afr. J. Enol. Vitic.*, 12 (1991) 3–31.
30 R.L. Taylor and H.E. Conrad, *Biochemistry*, 11 (1972) 1383–1388.
31 O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.
32 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.
33 J.-F. Thibault, *Lebensm.-Wiss. Technol.*, 12 (1979) 247–251.
34 M.T. Tollier and J.P. Robin, *Ann. Technol. Agric.*, 28 (1979) 1–15.
35 A.E.R. Ahmed and J.M. Labavitch, *J. Food Biochem.*, 1 (1977) 361–365.
36 Y.D. Karkhanis, J.Y. Zeltner, J.J. Jackson, and D.J. Carlo, *Anal. Biochem.*, 85 (1978) 595–601.
37 O. Marcelin, P. Williams, and J.-M. Brillouet, *Carbohydr. Res.*, 240 (1993) 233–243.
38 J.A. Klavons and R.D. Bennett, *J. Agric. Food Chem.*, 34 (1986) 597–599.
39 Boehringer Mannheim GMBH Biochemica, Acetic acid–UV method, Technical leaflet, 589.3. 248.878. 1989.

40 P. Albersheim, D.J. Nevins, P.D. English, and A. Karr, *Carbohydr. Res.*, 5 (1967) 340–345.
41 P.J. Harris, R.J. Henry, A.B. Blakeney, and B.A. Stone, *Carbohydr. Res.*, 127 (1984) 59–73.
42 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
43 T.J. Waeghe, A.G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.
44 D. Hourdet and G. Muller, *Carbohydr. Polym.*, 16 (1991) 113–135.