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RHAMNOGALACTURONAN II FROM CELL WALLS OF *CRYPTOMERIA JAPONICA*

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Key Word Index—*Cryptomeria japononica*; Taxodiaceae; sugi; cell walls; polysaccharide; pectin; rhamnogalacturonan II

Abstract—The pectic polysaccharides isolated from cell walls of xylem-differentiating zones of sugi (*Cryptomeria japononica*) were degraded with *endo*-α-(1→4)-polygalacturonase and the polysaccharides, composed mainly of rhamnogalacturonan II (RG-II), were obtained from the degradation products. These polysaccharides consisted of rhamnosyl, fucosyl, arabinosyl, xylosyl, galactosyl, glucosyl, galacturonic acid, glucuronic acid and the characteristic sugars of RG-II, namely, 2-*O*-methylfucose, 2-*O*-methylxylose, apiose, aceric acid and thiobarbituric acid assay-positive glycosyl {probably, 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and 3-deoxy-D-*lyxo*-heptulosaric acid (Dha)}. The polysaccharides contained the glycosyl residues of RG-II, besides small amounts of the glycosyl linkages of RG-I. The RG-II was structurally analysed by partial acid hydrolysis and lithium treatment in ethylenediamine. The glycosyl sequences of three compounds generated by partial hydrolysis were not identical to the partial structure of the sycamore (*Acer pseudoplatanus*) RG-I and RG-II structures previously proposed by Albersheim *et al.* Furthermore, five novel glycosyl sequences were detected in the products from lithium treatment. The results suggest that the structure of sugi RG-II is somewhat different from that of sycamore RG-II.

INTRODUCTION

Primary cell walls consist of pectin, xyloglucan and cellulose [1]. Pectin is composed of three polysaccharide components, namely homogalacturonan, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) [2]. Pectin has attracted the attention of plant physiologists, because it is not only structurally complicated but also the source of bioactive oligosaccharides. For example, the oligogalacturonides from homogalacturonan were reported to play important roles in the regulation of plant cell growth and induction of phytoalexins [3]. However, bioactive oligosaccharides have not yet been obtained from RG-I and RG-II. RG-II reportedly inhibited the uptake of [^{14}C] leucine into suspension-cultured tomato cells [4]. Therefore, bioactive oligosaccharides could be prepared from RG-I and RG-II. Both RG-I and RG-II have complicated structures, which would make it difficult to purify the oligosaccharides sufficiently for bio-assay. Consequently, structural characterization will play an important role in the detection of bioactive oligosaccharides from RG-I and RG-II.

According to the structural analysis of sycamore RG-II, it consists of more than 12 sugar residues containing rare sugars, namely, 2-*O*-methylfucose, 2-*O*-methylxylose, apiose, aceric acid, 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and 3-deoxy-D-*lyxo*-heptulosaric acid (Dha); these glycosyl residues were connected by different glycosyl linkages [2]. Later, RG-II was isolated from the cell-walls of suspension-cultured Douglas fir (*Pseudotsuga menziesii*), maize (*Zea mays*) and rice (*Oryza sativa*) cells and the structural features of these RG-IIs were discussed [5, 6].

In our previous paper, we reported that pectin from cell walls of the xylem differentiating zones of sugi (*Cryptomeria japonica*) contained homogalacturonan, RG-I and RG-II [7]. In the present paper, the isolation and structural characterization of RG-II from the xylem-differentiating zones of sugi is reported.

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RESULTS AND DISCUSSION

All crude pectin fractions, namely, CDTA-I, CDTA-II, Na_2CO_3-I and Na_2CO_3-II, contained sugar

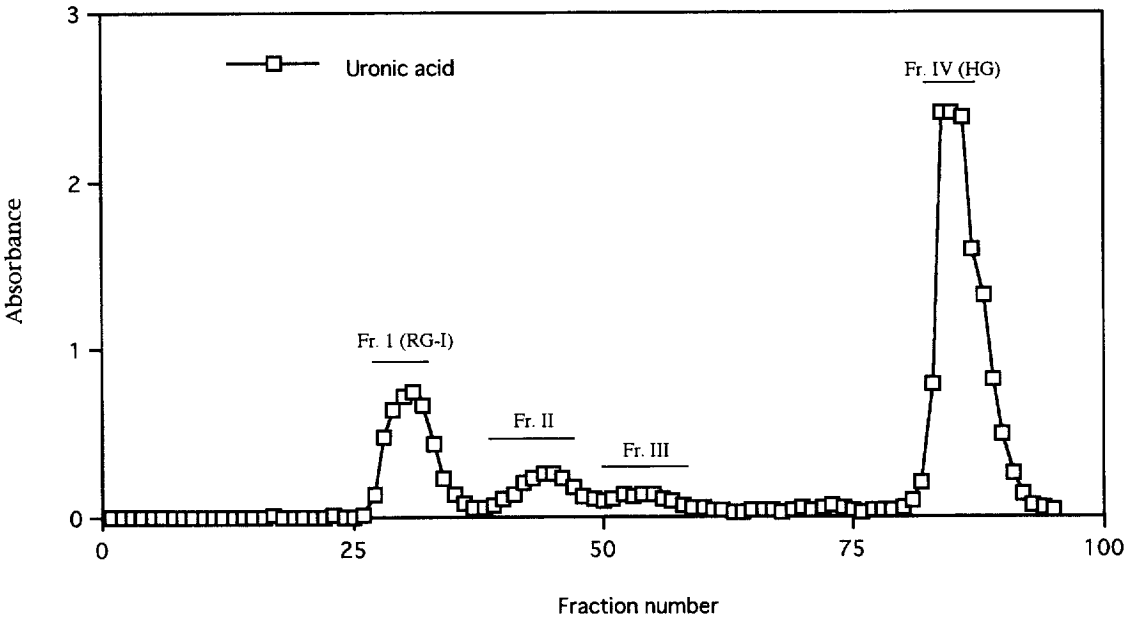

Fig. 1. Bio-Gel P-30 fractionation of degradation products of pectic polysaccharides from the Na_2CO_3-I extract with *endo*-α-(1→4)-polygalacturonase. Uronic acid was assayed by the *m*-hydroxybiphenyl method [21]. Notes: RG-I = rhamnogalacturonan I; RG-II = rhamnogalacturonan II; HG = oligogalacturonides derived from homogalacturonan by the action of *endo*-α-(1→4)-polygalacturonase.

characteristics of RG-II, such as 2-*O*-methylfucose, 2-*O*-methylxylose, apiose, aceric acid (3-*C*-carboxy-5-deoxy-L-xylose) (data not shown). These results confirmed that the fractions contained RG-II. Pectic polysaccharides purified from the Na_2CO_3-I extract were utilized for the isolation of RG-II. Purified pectic polysaccharides were digested with purified *endo*-polygalacturonase at 30° for 48 hr, and the products fractionated into four fractions on a Bio-Gel P-30 column (Figure 1). Glycosyl composition and glycosyl linkage analyses indicated that Frs. I and IV were RG-I and oligogalacturonides derived from homogalacturonan, respectively (data not shown). Both Frs. II and III contained sugar characteristics of RG-II, revealing that these fractions contained RG-II (Table 1). The contents of sugar characteristics of RG-II, such as apiose, aceric acid and the thiobarbituric acid assay positive sugar of Fr. II and III were less than those of sycamore RG-II. The glycosyl linkage composition of Frs. II, III and sycamore RG-II are shown in Table 2. Terminal- and 4-linked xylosyl residues were probably derived from 2-*O*-methyl-D-xylosyl residues. Because the contaminants from Super-Deuteride used for reduction of uronic acid residues made it difficult to precisely quantify some glycosyl residues, namely, 2-, 3- and 2,3-linked rhamnosyl, terminal fucosyl, 2- and 5-linked arabinosyl and terminal galactosyl. the glycosyl linkage compositions of Frs. II and III will be a different from the original compositions. The glycosyl linkages of RG-I, such as 2- and 2,4-linked arabinosyl residues, were found in Frs. II and III. Therefore, the RG-I fragments would also exist in these fraction. However, the glycosyl linakge composition of Fr. III was different from that of RG-I.

Further structural characterization was performed on Fr. III. This was degraded by partial acid hydrolysis and the released oligosaccharides obtained were analyzed. Prior to partial acid hydrolysis, the carboxyl groups of Fr. III were reduced with sodium borodeuteride to convert uronic acid residues into their corresponding 6,6-dideuteriohexosyl residues. The products were per-*O*-methylated, hydrolyzed by with 88% formic acid, reduced and per-*O*-pentadeuterioethylated, as described in the Experimental. The glycosyl sequences of the partially *O*-methylated, partially *O*-pentadeuterioethylated mono- and oligoglycosyl alditols were determined by GC-mass spectrometry. The determined glycosyl sequences are shown in Table 3. Diagnostic EI- and CI-mass spectral ions of the products are shown in Tables 4 and 5, respectively. The glycosyl sequences of seven oligosaccharides were determined from partial acid hydrolysis products. These oligosaccharides could not be purified because not enough Fr. III was available for partial acid hydrolysis. Therefore, constituents glycosyl residues of the oligosaccharides could not be identified. Hexosyl residues reduced with deuterides at *O*-6 positions were derived from uronic acid residues. The positions of pentadeuterioethyl groups were the sites cleaved by partial acid hydrolysis. Compounds I, II and VII can be generated from the structure of sycamore RG-II [8]. Compound V can be obtained from RG-I [2]. The origin of the other compounds cannot be speculated from their structures.

Table 1. Glycosyl compositions of RG-II isolated from the Na_2CO_3-I extract of sugi cell-walls and sycamore RG-II

Fraction	Glycosyl composition (*mol* %)											
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	2-*O*-MeFuc	2-*O*-MeXyl	Api	Aceric acid	3-deoxy sugar*	Uron. Acid§
Fr.II†	14.4	1.3	32.4	0.0	0.0	18.0	1.7	2.1	3.0	5.3	0.8	0.9	20.9
Fr.III†	23.0	3.7	22.1	0.0	0.0	20.4	1.7	4.1	5.3	7.7	2.0	0.8	10.0
Sycamore‖	12.4	2.8	10.0	0.0	0.0	9.0	0.0	3.5	4.8	12.2	3.5	7.0	34.4

2-*O*-MeXyl = 2-*O*-Methyl-D-Xylose; Api = Apiose; Aceric acid = 3-*C*-carboxy-5-deoxyl-L-xylose.
* 3-deoxysugar (Kdo and Dha) content determined by thiobarbituric acid assay [21].
§ Uronic acid content determined by *m*-hydroxybiphenyl assay [21].
† Fractions II and III shown in Fig. 1.
‖ Ref. [22].
n.d. = determined.

To obtain further information on the structure of RG-II, the oligosaccharides generated by Li-treatment in ethylenediamine were analyzed structurally. Because this treatment cleaved uronic acid residues, oligosaccharides composed only of neutral glycosyl residues were released. The glycosyl sequences of 17 oligosaccharides were determined as per-*O*-methylated mono-, di- and triglycosyl alditols by GC-mass spectrometry (EI and CI) analysis (Table 6). The diagnostic ions of the EI- and CI-mass spectra of these compounds are shown in Tables 7–10, respectively. Because not enough Fr. III was obtained, constituent glycosyl residues could not be identified. Although the parent ions of compounds 15-17, per-*O*-methylated triglycosyl alditols, could not be found by CI-mass spectrometry, they could be detected by FAB. The glycosyl sequences of compounds **1–4**, **7**, **10–12**, **15** and **16** can be derived from the structure of sycamore RG-II [8]. The oligosaccharide structures proposed from sycamore RG-II are shown in Table 11. Compounds, **8** and **13**, can be obtained from RG-I [2]. The other structures were probably produced from unknown partial structures of sugi RG-II.

Our results indicate that some oligosaccharides released by partial hydrolysis and Li-treatment in ethylenediamine were not compatible with the partial structure of sycamore RG-I and RG-II. As the major polysaccharide of Fr. III was RG-II, the novel oligosaccharides were probably produced from RG-II. Therefore, we assume that the structure of sugi RG-II differed in some respects from that of sycamore RG-II.

EXPERIMENTAL

Preparation of pectin

Cell walls of xylem-differentiating zones of sugi (*C. Japonica* D. Don.) were prepared as described in ref. [9]. Cell walls were treated with 0.05 M *trans*-1,2-cyclohexamediamine-N,N,N′,N′-tetra-acetic acid (pH 6.5) and 0.05 M Na_2CO_3 soln. Cell wall (*ca* 1 g) was treated with 100 ml of 0.05 M CDTA soln at 20° for 6 hr (CDTA-I) and, then, at 20° for 2 hr (CDTA-II). Residual cell wall was then treated with 50 mM Na_2CO_3 containing 20 mM $NaBH_4$ under N_2 at 1° for 16 hr (Na_2CO_3-I) and, then, with 0.05 M N_2CO_3 soln under N_2 at 20° for 3 hr (Na_2CO_3-II).

Purification of pectic polysaccharides

Each extract was de-esterified at pH 12 and 4° for 2 hr [5]. De-esterified pectin was purified on a DEAE-Sepharose Fast Flow column (80 cm × 1.5 cm i.d.) [9].

Separation of RG-I, RG-II and oligogalacturonides.

Purified pectin was dissolved in 50 mM NaOAc buffer (pH 5.2) and *endo*-α-(1→4)-polygalacturonase (EPGase) purified from pectinase of *Aspergillus niger*

Table 2. Glycosly linkage compositions of RG-IIs from cell walls of sugi and sycamore

Glycosyl Residue	Glycosyl linkage	Fr.II*	Fr.III*	Sycamore †
		Mol %		
Rhamnosyl (p)	terminal	3.7	4.5	5.2
	2-linked	+	1.3	1.8
	3-	+	1.2	6.0
	2,3-	+	3.3	0.0
	2,4-	5.6	3.3	0.0
	2,3,4-	+	+	5.5
Fucosyl (p)	terminal-	0.0	4.0	4.6
	3,4-linked	0.0	0.0	2.3
Apiosyl (f)	3′-linked	nd	nd	7.8
	terminal	8.1	6.0	7.7
	2-linked	0.0	0.0	5.0
	5-	21.2	9.3	0.0
	2,5-	0.0	1.8	0.0
Xylosyl (p) §	terminal-	0.8	1.9	4.6
	4-linked	+	4.9	0.0
Galactosyl (p)	terminal	8.1	7.7	6.8
	3-linked	0.0	1.2	0.0
	4-	3.9	3.0	0.0
	2,4-	7.3	5.8	5.7
	2,4,6-	0.0	3.0	0.0
Glucosyl (p)	4-linked	2.9	0.0	0.0
Galacturonic acid (p)	terminal	6.6	7.3	11.1
	2-linked	0.0	4.3	0.0
	3-	0.0	+	0.0
	4-	14.6	18.4	5.6
	2,4-	4.2	2.4	6.4
	3,4-	1.5	0.0	9.1
Glucuronic acid (p)	terminal	9.6	6.1	4.6

* RG-II fractions shown in Fig. 1.

§ Xylosyl residues derived from 2-*O*-methyl-D-xylosyl residues (Table 1). † Ref. [22]. determined, + = detected, but not quantitatively determined, f = furanose, p = pyranose.

(Sigma, EC 3.2.1.15, 5 units mg^{-1} of sample) [10] was added. The soln was incubated at 35° for 48 hr. The EPGase-treated pectin fr. was separated into RG-I, RG-II and oligogalacturonides on a Bio-Gel P-30 column (80 cm × 1.5 cm i.d.) [11].

Glycosyl composition

Glycosyl composition was determined as alditol acetate derivatives after hydrolysis with 2 M (TFA) at 121° for 1 hr. Alditol acetates were prepared [11] and analysed by GC on a SP-2330 capillary column (30 m × 0.25 mm i.d.) [11]. Characteristic sugars of RG-II, such as 2-*O*-methylfucose, 2-*O*-methylxylose, apiose and aceric acid, were detected by GC-MS analysis as described under mass spectrometry.

Glycosyl linkage analysis

Glycosyl linkage composition was determined by GC and GC-MS analyses of partially *O*-methylated alditol acetates. Per-*O*-methylation was performed by a modification of ref [12] of the Hakomori procedure [13]. Per-*O*-methylated polysaccharides were purified using a Sep-Pak C_{18} cartridge [14]. Me esters of per-*O*-methylated galacturonic acid residues in pectic polysaccharides were reduced with a 1 M soln of Li-triethylborodeuteride in THF (Super-Deuteride, Aldrich) at room temp. for 1 hr, after per-*O*-methylation [11]. The per-*O*-methylated, carboxyl-reduced polysaccharides were hydrolysed with 2 M TFA at 121° for 1 hr and converted into their alditol acetate derivatives [11]. GC-MS analysis was carried out as described under mass spectrometry.

Partial acid hydrolysis and preparation of per-O-alkylated oligoglycosyl alditols

The procedure was performed according to ref. [15]. Uronic acid residues were reduced to 6,6′-dideutriohexosyl residues by an improved method [11] of ref. [16]. Reduced RG-II was per-*O*-methylated by a

Table 3. Structures of partially *O*-methylated, partially *O*-deuterioethylated mono- and diglycosyl alditols generated by partial acid hydrolysis of sugi RG-II

Numerical designation	Rt[a] (min)	Partially *O*-methylated, partially *O*-deuterioethylated mono- and digylcosyl alditols
I*	13.04	hex,6,6-d_2-(1→4)-deoxyhexol-1-d 5 3 1 ↑ ↑ ↑ Et Et Et
II*	13.22	Et→4)-hex-6,6-d_2-(1→4)-deoxyhexol-1-d ? 5 3 1 ↑ ↑ ↑ ↑ Et Et Et Et
III	14.06	Et→4)-hex-6,6-d_2-(1→4)-hexol-1-d 5 1 ↑ ↑ Et Et
IV	14.28	Et→4)-hex-6,6-d_2-(1→4)-hexol-1-d ? 5 1 ↑ ↑ ↑ Et Et Et
V§	14.33	Et→4)-hex-(1→4)-hexol-1-d 5 1 ↑ ↑ Et Et
VI	14.40	Et→4)-hex-6,6-d_2-(1→4)-hexol-1-d ? 5 2 1 ↑ ↑ ↑ ↑ Et Et Et Et
VII*	21.59	hex→hex-6,6-d_2→deoxyhexol-1-d 5 ? 1 ↑ ↑ ↑ Et Et Et

[a] Eluted from DB-1 column hex-6,6-d_2 = GalA or Glc A; deoxyhex = Fuc or Rha; hex = Gal or Glc; Et = pentadeuterioethyl * Compounds could be derived from the structure of sycamore RG-II [8]. The original structure was proposed as:

$$\alpha\text{-D-Gal}p\text{-}(1\rightarrow2)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow4)\text{-}\beta\text{-L-Fuc}p\text{-}(1\rightarrow$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad 3$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \uparrow$$

§ Compound could be derived from RG-I, for example, Gal-(1→4)-Gal.

modification of the Hakomori procedure, as described above. The per-*O*-methylated sample obtained was hydrolyzed with 16 ml of 88% HCO_2H at 70° for 80 min; the HCO_2H was co-evapd with toluene. The partially *O*-methylated oligosaccharides obtained were reduced with $NaBD_4$. The resulting soln was passed through a Dowex 50W column (1 ml, H^+ form) and was evaporated to dryness. The reduced sample was per-*O*-pentadeuterioethylated by a modification of the Hakomori procedure, as described above. The partially *O*-pentadeuterioethylated, partially *O*-methylated oligoglycosyl alditols were analysed by GC-MS.

Lithium treatment of RG-II

The procedure was performed according to ref. [17–19]. Dried RG-I (20 mg) was dissolved in 2 ml of ethylenediamine and a small piece of Li wire (Aldrich) was added. The soln was stirred for 1 hr and kept blue by suppling additional pieces of Li wire. Cold H_2O (5 ml) was added and the soln evapd with toluene. The products were dissolved in H_2O and adjusted to pH 4.5 with HOAc. The soln was passed through a Dowex 50W column (H^+ form), concd to small vol. under red. pres. and lyophilized. The Li-treated products were reduced with $NaBH_4$ for 3 hr. After a few drops

Table 4. Diagnostic fragment ions for partially *O*-methylated, partially *O*-deuterioethylated mono- and diglycosyl alditols obtained by partial acid hydrolysis of sugi RG-II

Per-*O*-alkylated mono- and diglycosyl alditols*	*EI-MS ions* *aaldJ*1	*aldA*1	*aA*1	*aA*2	*Alditols*			
I	323 [25]	263 [21]	221 [32]	189 [100]	391 [3]	172 [2]		
II	323 [24]	263 [73]	240 [33]	208 [100]	410 [3]	172 [14]		
III	336 [20]	276 [41]	221 [36]	189 [100]	404 [3]			
IV	336 [45]	276 [84]	259 [55]	227 [100]	442 [7]	398 [2]		
V	334 [10]	274 [51]	238 [28]	206 [100]	395 [2]			
VI	355 [33]	295 [35]	259 [6]	227 [100]	442 [4]			
	*baaldJ*1	*aaldA*1	*aaldJ*1	*aldA*1	*abA*1	*abA*2	*bA*1	*bA*2
VII	529 [4]	469 [3]	323 [6]	263 [37]	425 [18]	393 [42]	219 [25]	187 [100]

* Glycosyl sequences of compounds shown in Table 3. The nomenclature is based on refs. [23] [24]. Numbers in square brackets indicate ion intensities.

Table 5. Diagnostic ions for partially *O*-methylated, partially *O*-deuterioethylated mono- and diglycosyl alditols obtained by partial acid hydrolysis of RG-II

Per-*O*-alkylated mono- and diglycosyl alditols*	*CI-MS ions* $(M+1)^+$	*aldA*1	$aldA1+H_2O$	*aA*1	*aA*2			
I	501 [4]	263 [-]	281 [-]	221 [-]	172 [1]			
II	521 [64]	263 [6]	281 [100]	240 [5]	208 [48]			
III	514 [32]	276 [4]	294 [100]	221 [16]	189 [61]			
IV	552 [20]	276 [8]	294 [100]	259 [15]	227 [48]			
V	529 [11]	274 [3]	292 [100]	238 [6]	206 [48]			
VI	571 [27]	295 [12]	313 [15]	259 [-]	227 [65]			
	$(M+1)^+$	*Elim*1	*aldA*1	$aldA1+H_2O$	*abA*1	*abA*2	*bA*1	*bA*2
VII	705 [-]	499 [4]	263 [3]	281 [100]	425 [-]	393 [9]	219 [14]	187 [80]

* Glycosyl sequences of compounds shown in Table 3. The nomenclature is based on ref. [15] [23] [24]. Numbers in square brackets indicate ion intensities.

Table 6. Structures of mono-, di- and trigylcosylalditols generated by lithium treatment of sugi RG-II in ethylenediamine

Numeral designation	Rt (min)*	per-*O*-methylated mono- and oligoglycosyl alditols
1§	11.5	Pent→Deoxyhexol
2§	11.5	Deoxyhex→Pentol
3§	12.2	Deoxyhex→Deoxyhexol
4§	12.4	Pent→Deoxyhexol
5	12.5	Hex→Pentol
6	13.3	Hex-(→4)-Deoxyhexol
7§	14.1	Pent→Hexol
8†	14.5	Hex→Hexol
9	20.1	Deoxyhex→Pent→Deoxyhexol
10§	20.5	Pent→Deoxyhex→Deoxyhexol
11§	21.0	Deoxyhex→Pent→Hexol
12§	21.1	Pent→Hexol→Deoxyhexol
13†	21.3	Pent→Hex→Deoxyhexol
14	22.0	Hex→Pent→Pentol
15§	27.3	Deoxyhex→Pent→Hexol→Deoxyhexol
16§	28.3	Pent→Deoxyhex→Deoxyhex→Pentol
17	29.4	Pent→Hex→Deoxyhex→Pentol

* On a DB-1 column. § Compounds can be derived from sycamore RG-II (Table 7).
† Compounds can be derived from RG-I.

Table 7. Diagnostic ions for per-*O*-methylated monoglycosyl alditols generated by lithium treatment of sugi RG-II in ethylenediamine

	Diagnostic ions							
Per-*O*-methylated monoglycosyl alditols*	*CI-MS* $(M+1)^+$	$aldA1+H_2O$	*aA*1	*EI-MS* *aaldJ*1	*aldA*1	*aA*1	*aA*2	*Alditol*
1	397	209	189	251	191	189	157	
	[74]	[25]	[100]	[18]	[90]	[100]	[29]	
2	397	223	175	265	205	175	143	
	[100]	[92]	[89]	[21]	[8]	[94]	[100]	
3	411	223	189	265	205	189	157	
	[100]	[22]	[12]	[10]	[78]	[100]	[65]	
4	397	223	175	265	205	175	143	
	[100]	[-]	[73]	[16]	[24]	[39]	[100]	
5	427	209	219	251	191	219	189	
	[100]	[14]	[64]	[74]	[35]	[100]	[26]	
6	441	223	219	265	205	219	187	307
	[95]	[86]	[100]	[33]	[100]	[38]	[87]	[4]
7	427	253	175	295	235	175	143	
	[46]	[100]	[48]	[17]	[22]	[100]	[94]	
8	471	253	219	295	235	219	187	
	[100]	[-]	[46]	[29]	[100]	[97]	[84]	

* Glycosyl sequences of compounds shown in Table 6. Numbers in square brackets indicate ion intensities. [-] not detected.

Table 8. Diagnostic ions of per-*O*-methylated diglycosyl alditols generated by lithium treatment of sugi RG-II in ethylenediamine

Per-*O*-methylated diglycosyl alditols*	*EI-MS ions* *abaldJ*1	*baldA*1	*baldJ*1	*aldA*1	*baA*1	*baA*2	*aA*1	*aA*2
9	426	366	265	205	349	317	189	157
	[10]	[1]	[19]	[36]	[37]	[24]	[100]	[60]
10	439	379	265	205	349	317	175	143
	[1]	[1]	[-]	[14]	[9]	[12]	[100]	[91]
11	455	395	295	235	349	295	189	157
	[4]	[-]	[8]	[40]	[13]	[8]	[100]	[35]
12	469	409	249	189	395	363	175	143
	[-]	[14]	[28]	[79]	[9]	[2]	[100]	[98]
13	469	409	265	205	379	347	175	143
	[3]	[1]	[1]	[24]	[12]	[7]	[91]	[100]
14	425	365	251	191	379	347	219	187
	[9]	[11]	[9]	[43]	[11]	[6]	[17]	[100]

* Glycosyl sequences of compounds shown in Table 6. Numbers in square brackets indicate ion intensities. [-] not detected.

Table 9. Diagnostic ions for per-*O*-methylated diglycosyl alditols obtained by lithium treatment of sugi RG-II in ethylenediamine

Per-*O*-methylated diglycosyl alditols*	*CI-MS ions* $(M+1)^+$	*baldA*1 + H_2O	*aldA*1 + H_2O	*Elim* 1	*aA*1
9	571	384	223	411	189
	[5]	[5]	[91]	[2]	[100]
10	571	397	223	298	175
	[69]	[11]	[81]	[13]	[100]
11	601	413	253	441	189
	[3]	[2]	[40]	[1]	[16]
12	601	427	223	397	175
	[3]	[3]	[23]	[2]	[80]
13	601	427	223	397	175
	[2]	[1]	[18]	[2]	[13]
14	601	383	209	425	219
	[5]	[2]	[31]	[4]	[20]

* Glycosyl sequences of compounds shown in Table 6. Numbers in square brackets indicate ion intensities.

of HOAc were added, the soln was passed through a Dowex 50W column (H^+ form) and lyophilized. MeOH was added and evapd to remove borate. The sample obtained was per-*O*-methylated by a modification of the Hakomori procedure. per-*O*-Methylated samples were analysed by GC-MS.

Mass spectrometry

Quantification of alditol acetates and partially *O*-methylated alditol acetates were performed using a SP-2330 capillary column (Supelco, 30 m × 0.25 mm i.d.). An analysis was carried out at 220° in the split mode (split ratio, 50:1). GC-MS of partially *O*-methylated alditol acetates was carried out as follows. The temp. prog. started at 50° for 2 min, increased to 170° at 30° min^{-1}, then to 235° at 4° min^{-1} and then kept at 235° for 10 min. Response factors [11] were used to caluculate glycosyl linkage composition. Glycosyl sequences of per-*O*-alkylated mono- and oligoglycosyl alditols were determined by GC-MS using a DB-1 capillary column (30 m × 0.25 mm i.d.) in the splitless mode with a temp. prog. starting at 50° for 2 min, to 190° at 30° min^{-1}, followed 6° min^{-1} to 340°. The

Table 10. Diagnostic ions for per-*O*-methylated triglycosyl alditols obtained by lithium treatment of sugi RG-II in ethylenediamine

Per-*O*-methylated triglycosyl alditols*	*EI-MS ions*											*FAB-ions*
	abcaldJ1	*bcaldA1*	*bcaldJ1*	*caldA1*	*caldJ1*	*aldA1*	*cbaA1*	*cbaA2*	*baA1*	*baA2*	*aA1*	*aA2*	$(M+Na)^+$
15	629	569	469	409	265	205	569	537	349	317	189	157	797
	[1]	[3]	[1]	[7]	[1]	[6]	[3]	[1]	[15]	[12]	[100]	[52]	
16	599	539	425	365	251	191	523	473	349	317	175	143	753
	[-]	[-]	[1]	[1]	[6]	[29]	[1]	[-]	[2]	[5]	[100]	[99]	
17	629	569	425	365	251	191	553	521	379	355	175	143	783
	[-]	[-]	[2]	[7]	[16]	[72]	[2]	[-]	[6]	[8]	[72]	[100]	

* Glycosly sequences of compounds shown in Table 6.
Numbers in square brackets indicate ion intensities.
[-] not detected.

ionization voltage was 70 eV and the source temp. 180°. FAB-MS were measured using an emission current of 10 mA and an acceleration voltage of 3 kV. Argon was used as bombardment gas, with glycerol as matrix.

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Table 11. Structures of compounds generated by lithium treatment in ethylenediamine from sugi RG-II, proposed from the structure of sycamore RG-II

Numeral designation*	Structures from sycamore RG-II
1	α-L-Ara*f*-(1→2)-α-L-Rha*p*
2	α-L-Rha*p*-(1→2)-α-L-Ara*p*
3	α-L-Fuc*p*-(1→4)-β-L-Rha*p*
4	2Me α-D-Xyl*p*-(1→3)-α-L-Fuc*p*
7	α-L-Ara*p*-(1→4)-α-D-Gal*p*
10	2Me α-D-Xyl*p*-(1→3)-α-L-Fuc*p*-(1→4)-β-L-Rha*p*
11	α-L-Rha*p*-(1→2)-α-L-Ara*p*-(1→4)-α-D-Gal*p*
12	α-L-Ara*p*-(1→4)-α-D-Gal*p* 2 ↑ 2Me α-L-Fuc*p*
15	α-L-Rha*p*-(1→2)-α-L-Ara*p*-(1→4)-α-D-Gal*p* 2 ↑ 2Me α-L-Fuc*p*
16	2Me α-D-Xyl*p*-(1→3)-α-L-Fuc*p*-(1→4)-β-L-Rha*p*-(1→3)-D-Api*f*

* Numeral designations correspond to those of Table 6. Some compounds were excluded because they were not applicable to the sycamore RG-II structure [8].